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*A Study of Hydrogen – Deuterium Exchange on the
Photophysical Properties of Ruthenium (II)
Polypyridyl Complexes.*

BY

MICHELLE E. HIGGINS



A THESIS SUBMITTED TO THE UNIVERSITY OF DUBLIN FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF DUBLIN,
TRINITY COLLEGE.

SEPTEMBER 2001

Declaration

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Michelle Higgins
Michelle E. B. Higgins

I dedicate this thesis to my parents

*Thank You,
With All my Love.*

A Study of Hydrogen – Deuterium Exchange on the Photophysical Properties of Ruthenium (II) Polypyridyl Complexes.

MICHELLE ELLEN BERNADETTE HIGGINS.

This main aim of the present study is to investigate the effect of protium–deuterium exchange on the photophysical and excited state energetics of Ru(II) complexes by UV-Vis absorption, steady-state and time-resolved emission spectroscopies. The three main complexes synthesised, characterised and studied are $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ **8**, and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9**. For each of these complexes, a series of partially deuterated analogues were successfully synthesised. For example, for **4**, a series of complexes of the form, $[\text{Ru}(\text{d}_x\text{-phen})_2\text{d}_y\text{-dppz}]^{2+}$, where $x = 0$ and 8 , and $y = 0, 4, 6$, and 10 were prepared. These species could be used to probe: (i) the nature of the excited state and (ii) in which part of the dppz ligand (*i.e.* 1,10-phenanthroline (phen) or phenazine (phz)) the excited state electron is located.

Two methodologies of H-D exchange were utilized for the preparation of the desired deuterated ligands. The first method, Pd/C in the presence of D_2O , is a modification of a literature method, promoted efficient H-D exchange for a wide range of polypyridyl ligands. The second method, of DCl in the presence of D_2O , is a novel method devised to perdeuterate diaminobenzene (dab) and its derivatives. Furthermore, systematic studies were performed to optimise the rate and efficiency of H-D exchange for these methods. On preparation of the deuterated ligands and complexes their characterisation was carried out by the methods of UV-Vis absorption, ^1H NMR and mass spectroscopies¹.

Absorption spectra, in a range of solvents, indicate that shifts in the absorption peaks of both the free and complexed ligands are observed upon substitution of the dppz ligand. Therefore, substitution effects the electron distribution in the excited states, most notably in the case of the diMedppz ligand **13** and complex **8**. A detailed photophysical study of complexes, **4**, **8**, and **9**, revealed that the emission parameters are highly sensitive to their immediate environment, most notably on the polarity of the medium, and they are all non-emissive in water. In organic solvents the emission quantum yields and lifetime decays are of the order: $\mathbf{8} > \mathbf{4} > \mathbf{9}$. No significant differences were found in the absorption and emission energies on deuteration of the complexes. In contrast, the emission quantum yields and lifetimes are strongly affected and the magnitude of the effect was found to be dependent on the site of deuteration. For all three series of complexes, similar trends were observed. For both emission parameters there is a marked increase in cases where the vibrational modes are affected by deuteration of the phen moiety of the dppz ligand (*i.e.* $\text{d}_6\text{-dppz}$). On this basis, we propose that in the excited state the promoted electron is preferentially localised on the phen moiety of dppz.

A number of routes were tried to isolate the enantiomerically pure complexes of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**. The enantiomers of **4** and its deuterated analogues **4a** and **4d** were successfully synthesised and characterised by CD spectroscopy. In the presence of CT-DNA, the changes in the absorption spectra are consistent with previously reported values. Similar effects were noted for the diMedppz complex **8**, but not for the corresponding diFdppz complex **9**. Steady-state emission studies show that these complexes behave as molecular light switches, as they do not emit in water but display emission when in the presence of DNA, in the order $\mathbf{8} > \mathbf{4} > \mathbf{9}$. Time-resolved luminescence studies for the racemic, enantiomeric and deuterated complexes display a biexponential decay on binding to DNA. For the enantiomeric complexes of **4**, the lifetime components and pre-exponential factors correspond to the Δ -enantiomer, attributed to the preferential emission by the Δ -enantiomer in DNA. However, on D-incorporation into these complexes, we observe that although there is a greater abundance of the long-lived lifetime component there is essentially no difference between the Δ and Λ -enantiomers of complexes, **4a** and **4d**, upon binding to the DNA helix.

Abbreviations.

A	Absorbance
Δ	Delta (right-handed)
Λ	Lamda (left-handed)
$B_\tau / \Sigma B$	Pre-exponential factor of the lifetime τ / Sum of all pre-exponential factors
Bpy	2,2'-bipyridine
C	Concentration
CD	Circular dichroism
CT-DNA	Calf thymus deoxyribonucleic acid
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
Dppz	[dipyrido[2,3-a:2',3'-c]phenazine]
DiFdppz	12,13 – difluoro [dipyrido[2,3-a:2',3'-c]phenazine]
DiMedppz	12,13 – dimethyl [dipyrido[2,3-a:2',3'-c]phenazine]
ϵ	Extinction coefficient ($M^{-1} cm^{-1}$)
E_{em}	Energy of emission
Φ	Quantum Yield
HOMO	Highest occupied molecular orbital
ic	Internal conversion
isc	Intersystem crossing
IL	Intraligand
k_{nr}	Non-radiative rate constant
k_r	Radiative rate constant
k_q^{O2}	Quenching Rate constant
λ	Wavelength (nm)
LUMO	Lowest unoccupied molecular orbital
MLCT	Metal-to-ligand charge transfer
NMR	Nuclear magnetic resonance
Phen	1,10-Phenanthroline
τ	Lifetime (ns)
UV/Vis	Ultra-violet/Visible
SPC	Single photon counting

Summary

This main aim of the present study is to investigate the effect of protium–deuterium exchange on the photophysical and excited state energetics of Ru(II) complexes by UV-Vis absorption, both steady-state and time-resolved emission spectroscopies. The three main complexes synthesised, characterised and studied are $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ **8**, and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9**. For each of these complexes, a series of partially deuterated complexes were successfully synthesised. For example, for **4**, a series of complexes of the form, $[\text{Ru}(\text{d}_x\text{-phen})_2\text{d}_y\text{-dppz}]^{2+}$, where $x = 0$ and 8 , and $y = 0, 4, 6$, and 10 were prepared. These species could be used to probe: (i) the nature of the excited state and (ii) in which part of the dppz ligand (*i.e.* 1,10-phenanthroline (phen) or phenazine (phz)) the excited state electron is located.

Two methodologies of H-D exchange were utilized for the preparation of the desired deuterated ligands. The first method, Pd/C in the presence of D_2O , is a modification of a literature method, promoted efficient H-D exchange for a wide range of polypyridyl ligands. The second method of DCl in the presence of D_2O , is a novel method devised to perdeuterate diaminobenzene (dab) and its derivatives. Furthermore, systematic studies were performed to optimise the rate and efficiency of H-D exchange for these methods. On preparation of the deuterated ligands and complexes their characterisation was carried out by the methods of UV-Vis absorption, ^1H NMR and mass spectroscopies¹.

A series of deuterated complexes of $[\text{Ru}(\text{L})_x(\text{L}')_{3-x}]^{2+}$ (where $\text{L} = \text{bpy}$ **1** and phen **6**, $\text{L}' = \text{d}_8\text{-bpy}$ and $\text{d}_8\text{-phen}$, and $x = 0, 1, 2$, and 3) were also prepared, and examined by both steady-state and lifetime measurements over a range of temperatures. For complexes of **1**, the emission lifetime increased with the extent of deuteration at all temperatures. For **6**, the behaviour is more complex.

Absorption spectra, in a range of solvents, indicate that shifts in the absorption peaks of both the free and complexed ligands are observed upon substitution of the dppz ligand. Therefore, substitution effects the electron distribution in the excited states, most notably in the case of the diMedppz ligand and complex **8**. A detailed photophysical study of complexes **4**, **8**, and **9**, revealed that the emission parameters are highly sensitive to their immediate environment, most notably on the polarity of the medium, and they are all non-emissive in water. In organic solvents the emission quantum yields and lifetime decays are of the order: **8** > **4** > **9**.

No significant differences were found in the absorption and emission energies on deuteration of the complexes. In contrast, the emission quantum yields and lifetimes are strongly affected and the magnitude of the effect was found to be dependent on the site of deuteration. For all three series of complexes, similar trends were observed. For both emission parameters there is a marked increase in cases where the vibrational modes are affected by deuteration of the phen moiety of the dppz ligand (*i.e.* d₆-dppz). On this basis, we propose that in the excited state the promoted electron is preferentially localised on the phen moiety of dppz.

A number of routes were tried to isolate the enantiomerically pure complexes of [Ru(phen)₂dppz]²⁺ **4**. The enantiomers of **4** and its deuterated analogues **4a** and **4d** were successfully synthesised and characterised by CD spectroscopy. In the presence of CT-DNA, the changes in the absorption spectra are consistent with previously reported values. Similar effects were noted for the diMedppz complex **8**, but not for the corresponding diFdppz complex **9**. Steady-state emission studies show that these complexes behave as molecular light switches, as they do not emit in water but display emission when in the presence of DNA, in the order **8** > **4** > **9**. Time-resolved luminescence studies for the racemic, enantiomeric and deuterated complexes display a biexponential decay on binding to DNA. For the enantiomeric complexes of **4**, the lifetime components and pre-exponential factors correspond to the 4Δ-enantiomer, attributed to the preferential emission by the Δ- enantiomer in DNA. However, on D-incorporation into these complexes, we observe that although there is a greater abundance of the long-lived lifetime component there is essentially no difference between the Δ and Λ-enantiomers of complexes, **4a** and **4d**, upon binding to the DNA helix.

¹ The natural abundance ligands and complexes are referred to as "protiated" and not "protonated".

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Chapter One

1.0 Introduction

The best known natural photochemical processes, photosynthesis and vision, are nature's ways to convert light energy into chemical energy and to capture, store and process optical information. Recent vigorous research in photochemistry and photophysics has essentially the same ultimate goals. The understanding of chemistry and dynamics of electronic excited states is of great importance for the rational design and development of new photonic materials and photo-chemical reactions. Importantly, for polypyridyl complexes of d^6 transition metals the simultaneous existence of distinct reactivity and relaxation pathways, together with the presence of closely spaced disparate excited states provides the possibility to control the photo-behaviour of these compounds by external stimuli. This can be especially useful in the development of photochemical molecular devices and light sensitive probes in biological systems^{[1][2]}. The excited states of these complexes have provided fundamental information on the effects that the structural change in the acceptor ligand, the energy gap, and the medium have on nonradiative decay. The dynamic spectroscopies used to probe these phenomena have been largely based on emission and absorption measurements. Herein, the basic principles will be explained and illustrated utilizing the model $[\text{Ru}(\text{bpy})_3]^{2+}$ complex **1**, where bpy = 2,2'-bipyridine.

Variation of the electronic environment around the central ion always brings about corresponding variations in the spectral properties of the complex. For Ru(II) polypyridyl complexes, it has been well documented that their photoluminescence properties are extremely sensitive to their immediate environment. In the present study we have chosen dipyrido[3,2-a:2',3'-c]phenazine, dppz **2** as the principal ligand. To satisfy the remaining coordination sites we have used 1,10-phenanthroline, phen **3**, to give the $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4**. The reason behind this choice of ligands is three-fold: (i) **3** is known to stabilise Ru(II) due to the extended aromaticity relative to bpy **5**; (ii) complexes containing the dppz ligand have played an important role in the study of DNA intercalation, as they display a high binding affinity $K_b \geq 10^6 \text{ M}^{-1}$ for DNA^[3] and; (iii) $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** is a light sensitive compound. Furthermore, **4** is

known as a ‘molecular light switch’, whereby the short-lived (~ 250 ps) emission is undetectably small in water^[4], but displays moderately long-lived emission (400 ns) in nonaqueous solvents such as acetonitrile or ethanol and when intercalated in DNA^{[5][6]}. However, the photophysical reasons for this phenomenon are not yet fully understood. Essentially, the important questions are:

- How does the local environment modulate the spectroscopic properties and;
- What is the precise nature of the excited state and where on the dppz ligand does the excited electron reside?

The behaviour denoted as the “molecular light switch effect”^[3] has been attributed to the special nature of the dppz ligand. The heterocyclic π system of **2** combines the chelating function of the α -diimines^[7] (*i.e.* phen or bpy moiety) with the electron/proton transfer capacity of the 1,4-diazine (phenazine (phz) or quinoline (quin))^[8], as illustrated in Figure 1.1.

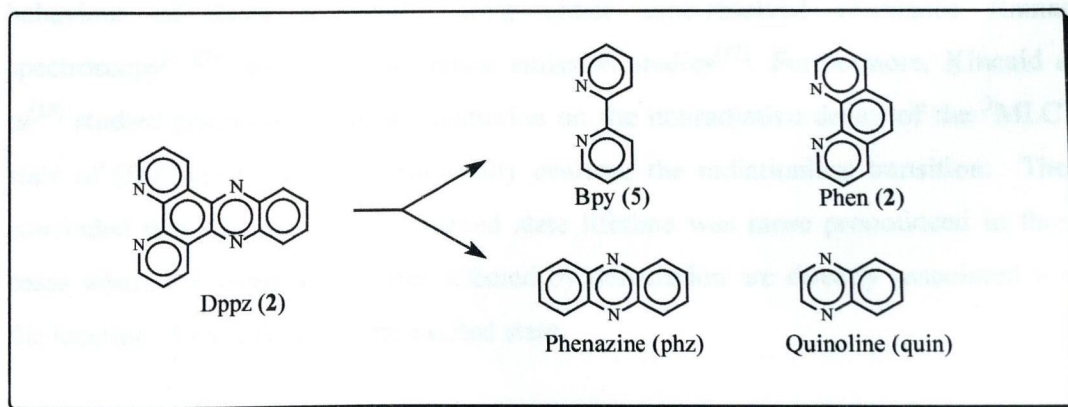


Figure 1.1: The phen/bpy and quinoline/phenazine components of the dppz Ligand.

Despite a substantial body of research^{[2][8]} there still remains considerable controversy regarding the location of the excited state electron in $[\text{Ru}(\text{L})_2\text{dppz}]^{2+}$ complexes, and continuing debate exists as to whether the charge is:

- localised on specific areas of the dppz ligand upon excitation or;
- distributed over the entire dppz skeleton?

Given the unique luminescent properties observed with Ru(II) complexes of dppz, it becomes important to characterise further their luminescent characteristics and to explore the generality of the observation.

A simple method to determine the emitting ligand in heteroleptic Ru(II) complexes is based on the effect of D-incorporation on the emission parameters of these complexes^[9]. It has been reported that the location of the excited state electron density is dependent on the effect of deuteration on radiationless deactivation process^[10]. Deuterium incorporation decreases the contribution of the nonradiative decay (k_{nr}) pathway, resulting in increased lifetimes of the excited state for these complexes^{[11][12]}. In the literature, a number of studies have employed the phenomena of the ‘deuterium isotope effect’ to simplify the ¹H NMR spectra of ruthenium complexes in the absence^[13] and presence^[14] of DNA, or to obtain information about the excited-state behaviour of these complexes using either time-resolved resonance Raman spectroscopy^{[15][16]} and low temperature emission studies^[17]. Furthermore, Kincaid *et al*^[18] studied position-dependent deuteration on the nonradiative decay of the ³MLCT state of [Ru(bpy)₃]²⁺ **1** to experimentally evaluate the radiationless transition. They concluded that the effect on the excited state lifetime was more pronounced in those cases where the vibrational modes affected by deuteration are directly associated with the location of the electron in the excited state.

For the present study, a series of four families, based on [Ru(phen)₂dppz]²⁺ complex **4** were synthesised. Variation of substituents at the periphery of the dppz ligand has been made in order to probe the location of the excited state electron. The structures and abbreviations are shown in Figure 1.2. To assist in our understanding of these systems, extensive studies were initially performed on the model tris-chelated [Ru(L)₃]²⁺ complexes (where L = bpy **1** and phen **6**).

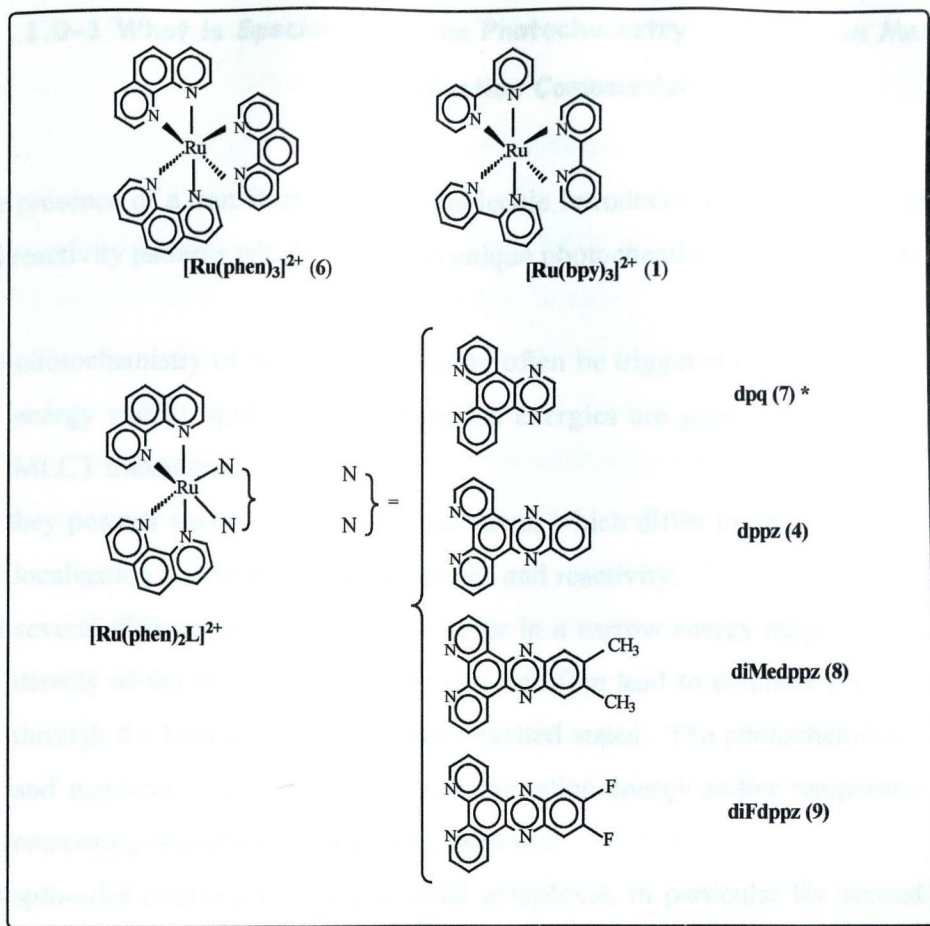


Figure 1.2 : Systematic illustration of the series of Ru(II) complexes prepared in the current study.
 *This family of complexes are not examined in the current study.

The focus of the present study has been to probe the exact location of the excited state electron using, as a tool, the selective deuteration of different constituents of the mixed-ligand complex 4, and related complexes, 8 and 9, respectively. To date, a systematic study of this phenomenon has not been undertaken. In order to appreciate the different aspects of the research presented in this thesis, it is necessary to have an overview of the differing topics of photoluminescent properties of these Ru(II) polypyridyl complexes, the nature of the dppz ligand, and the deuterium isotope effect in the absence and in the presence of DNA. Accordingly, the topics are reviewed in the following sections.

1.0-1 What is Special About the Photochemistry of Transition Metal Coordination Compounds?

The presence of a transition metal in a molecule introduces new types of excited states and reactivity patterns which give rise to unique photochemistry and photophysics:

- (i) photochemistry of these complexes can often be triggered by irradiation with low-energy visible light since the excitation energies are generally low-lying allowed MLCT transitions;
- (ii) they possess various types of excited states, which differ in their orbital parentage, localisation within the molecule, energy, and reactivity;
- (iii) several different excited states can occur in a narrow energy range due to the high density of the excited states. This can therefore lead to complex photo-behaviour through the interaction of the various excited states. The photochemical dynamics and quantum yields often depend on excitation energy and/or temperature due to competing relaxation and reactivity pathways;
- (iv) spin-orbit coupling is strong in metal complexes, in particular for second or third row transition metals. This results in fast intersystem crossing for these complexes;
- (v) as transition metals display redox-active electronic ground states, electron transfer reactions can involve a change in the oxidation state of the metal atom, ligand(s) or both. Furthermore, this redox activity is retained in the excited state and finally;
- (vi) great synthetic versatility allows fine-tuning of the excited state character and energy ordering by structural variations.

All of these aspects make the study of photochemical mechanisms and dynamics of coordination compounds very intriguing and challenging.

1.1 The Structure and Physical Properties of Ru(II) Polypyridyl Complexes.

1.1-1 Introduction.

By far, the best known and most studied^[3] tris-chelate complex is that of $[\text{Ru}(\text{bpy})_3]^{2+}$ **1**, see Figure 1.2, which was first synthesised by Burstall^[19] and used as the basis for the study of more complex systems. $[\text{Ru}(\text{L})_3]^{2+}$ (where $\text{L} = \text{bpy}$ **1** and phen **6**) are low-field d^6 systems in which the heavy transition metal Ru(II) core co-ordinates with three bidentate polypyridine ligands yielding an octahedral configuration. The polypyridyl ligands are usually colourless molecules possessing σ -donor orbitals localised on the N atom and π -donor and π^* -acceptor orbitals delocalised on the aromatic ring. A great deal of photophysical and photochemical behaviour can be explained with the use of a localised molecular orbital (MO) approach and simple one electron excitations, Figure 1.3. Excited states are often named according to their initial and final orbitals, respectively. Promotion of an electron from a π_{M} metal orbital to a π^*_{L} ligand orbital gives rise to a metal-to-ligand charge transfer (MLCT) excited state, whereas promotion of an electron from π_{M} to σ^*_{M} orbitals gives rise to metal centred (MC) excited states. Ligand centred (LC) excited states can be obtained by promotion of an electron from π_{L} to π^*_{L} ligand orbitals. All these excited states may have singlet or triplet multiplicity, although spin-orbit coupling causes singlet-triplet mixing in the MC and MLCT excited states.

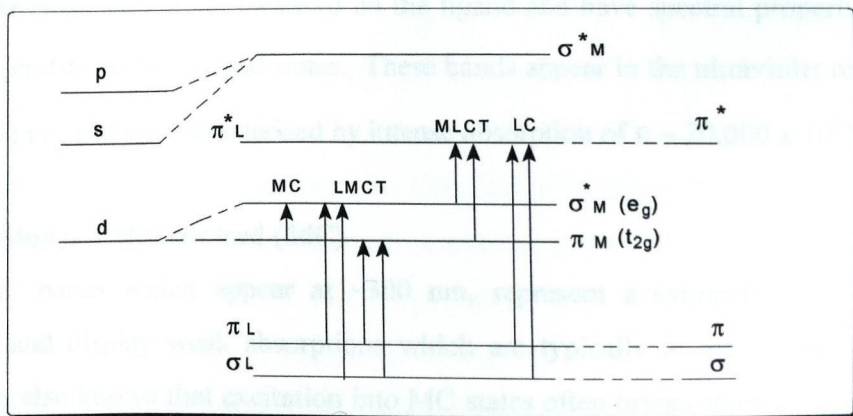


Figure 1.3: Molecular orbital diagram of the energy levels of the principal orbitals and transitions which occur for an octahedral complex of Ru(II) ($\text{M} = \text{metal}$, $\text{L} = \text{ligand}$).

$[\text{Ru}(\text{bpy})_3]^{2+}$ **1**, as well as other $[\text{Ru}(\text{L})_3]^{2+}$ complexes, (where L = bidentate polypyridine ligand), exhibit a D_3 symmetry. The proton ^1H NMR can be interpreted in terms of four spin coupled spins in each of six equivalent pyridine rings. The solution NMR is thus consistent with retention of D_3 symmetry for solvated species. X-ray structures of **1** show that the $[\text{Ru}-\text{N}]$ bond lengths are short indicating a degree of overlap of the t_{2g} orbitals of the metal with the π^* orbitals in the ligands. The bipyridyl ligands are not quite planar, the rings being twisted by a few degrees. Ruthenium complexes with mixed ligand systems, allow the photophysical and photochemical properties of the complex to be varied in an ordered fashion. Compounds of the form, $[\text{Ru}(\text{L})_2(\text{L}')^{2+}]$, exhibit C_2 symmetry. All of these complexes are present as two enantiomeric (Δ and Λ) forms, respectively.

1.1-2 Absorption.

The absorption spectra of polypyridyl complexes of Ru(II) are dominated by MLCT transitions. The results of a series of spectroscopic and theoretical studies suggest that absorption is attributed to an MLCT transition that is largely singlet in character, $S^0 \rightarrow {}^1\text{MLCT}(\text{d}\pi \rightarrow \pi^*)$, and ligand localised on the IR-Raman time scale^[20]. The UV/Vis absorption spectrum of **1** is shown in Figure 1.4, and consists of three fundamental types of electronic transitions:

(i) $(\pi \rightarrow \pi^*)$; Ligand-centred (LC)

These transitions are mainly localised on the ligand and have spectral properties which closely resemble the free ligand states. These bands appear in the ultraviolet region $\lambda = (200 - 400)$ nm and are characterised by intense absorption of $\epsilon \sim 20,000 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

(ii) $(\text{d}\pi \rightarrow \text{d}\sigma^*)$; Metal-centred (MC)

These 'dd' bands which appear at ~ 300 nm, represent a symmetrically forbidden transition and display weak absorptions which are typically in the 10^1 - $10^2 \text{ M}^{-1}\text{cm}^{-1}$ range. It is also known that excitation into MC states often brings about efficient ligand dissociation^[21].

(iii) ($d\pi \rightarrow \pi^*$); metal-to-ligand charge transfer (MLCT)

These transitions of lowest excited state energy between a metal centred $d\pi$ (t_{2g}) ground state and ligand π^* states, are observed in the visible region $\lambda_{\max} = (400 - 500)$ nm with intense absorptions of $\epsilon \sim 14,000 \text{ M}^{-1}\text{cm}^{-1}$ and are both solvent and substituent dependent. Thus, in a formal sense this excitation results in metal oxidation and ligand reduction^[22].

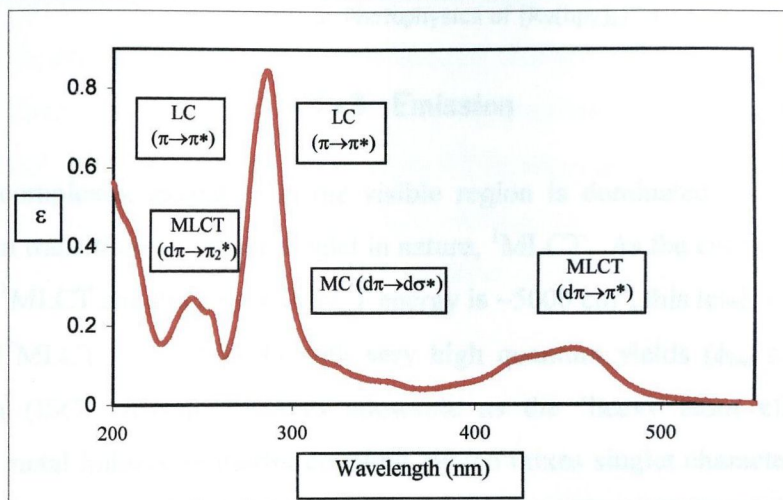


Figure 1.4: Absorption spectrum of $[\text{Ru}(\text{bpy})_3]^{2+}$ 1 in aqueous solution with assignments for the various bands.

The photophysics of **1**, is well known, and the pertinent details are shown in Figure 1.5. It has been well established that photoexcitation of **1**, results in absorption of a photon of light (1fs) and leads to the population of the initially formed MLCT excited state of predominantly singlet character. For most Ru(II) complexes, this is followed by rapid conversion via intersystem crossing with unit efficiency^[23] to a lower lying state which has been identified as an $^3\text{MLCT}$ state, is largely triplet in nature and responsible for emission^{[20][23]}. In spite of the presence of the heavy Ru atom, it has been established that it is reasonable to assign the electronic transitions of **1** as being “singlet” or “triplet” states. The nature of the MLCT state is determined by the lowest π^* orbitals and the metal. Generally, a spectrochemical correlation is observed for most polypyridyl complexes, and the excited state can act both as powerful oxidising and reducing agents, giving rise to two distinct redox processes.

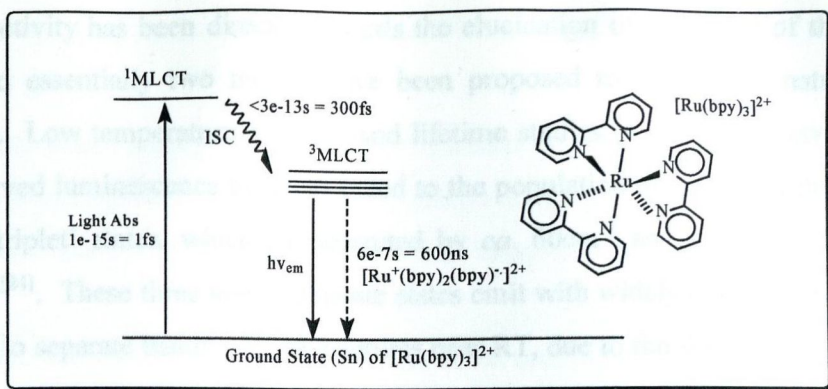


Figure 1.5: Photophysics of $[\text{Ru}(\text{bpy})_3]^{2+}$ 1^[1a].

1.1-3 Emission

For these complexes, excitation in the visible region is dominated by intense bands arising from transitions which are singlet in nature, $^1\text{MLCT}$. As the energy gap between the lowest $^1\text{MLCT}$ and the lowest $^3\text{MLCT}$ energy is $\sim 5000 \text{ cm}^{-1}$, this leads to intersystem crossing ($^1\text{MLCT} \longrightarrow ^3\text{MLCT}$) with very high quantum yields ($\phi_{\text{isc}} \cong 1$)^[23]. The intersystem (ISC) crossing becomes allowable as the ‘heavy atom effect’ of the ruthenium metal induces spin-orbit coupling, which mixes singlet character into triplet states. The emission of **1** originates from an $^3\text{MLCT}$ state which is reasonably long-lived and the possible deactivation pathways for the excited states of **1** and related systems, as proposed by Crosby^[24] and Meyer^[25], are shown in Figure 1.6.

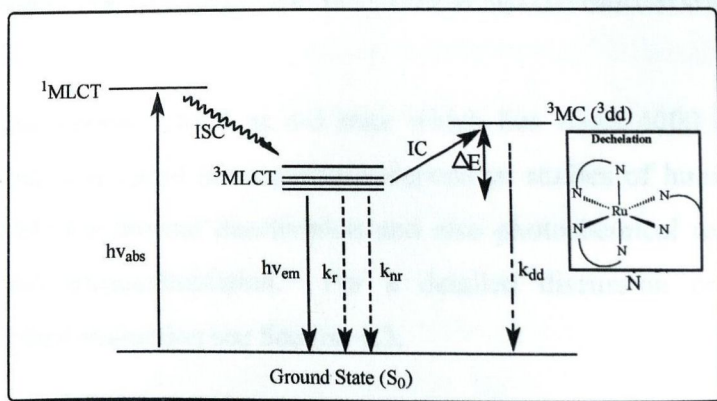


Figure 1.6 : Diagram of the excited state levels of $[\text{Ru}(\text{L})_3]^{2+}$ complexes; $h\nu_{\text{abs}}$ = absorption, $h\nu_{\text{em}}$ = emission, ISC = intersystem crossing, IC = internal conversion. ΔE represents the energy gap between the ^3dd and the manifold of $^3\text{MLCT}$ emitting states.

Intense activity has been directed towards the elucidation of the nature of the $^3\text{MLCT}$ state, and essentially two models have been proposed to explain the nature of the emission. Low temperature emission and lifetime studies (4.2 to 77 K) have revealed the observed luminescence to be attributed to the population of the lowest three closely spaced 'triplet' states, which are separated by *ca.* 60cm^{-1} and possess <11% singlet character^[24]. These three non-degenerate states emit with widely different lifetimes and give rise to separate bands. At temperatures near RT, due to the Boltzmann distribution all three states exist in thermal equilibrium, and hence, the emission is considered to arise from an average emitting state with average characteristics. This state exhibits slow nonradiative decay, with characteristic long luminescence lifetimes (μsec) and broad emission bands. At temperatures above 77 K, there is evidence of an additional thermally accessible $^3\text{MLCT}$ state^[26], which is found approximately 800 cm^{-1} above the lowest lying MLCT state, and can make a significant contribution to nonradiative decay see Figure 1.7.

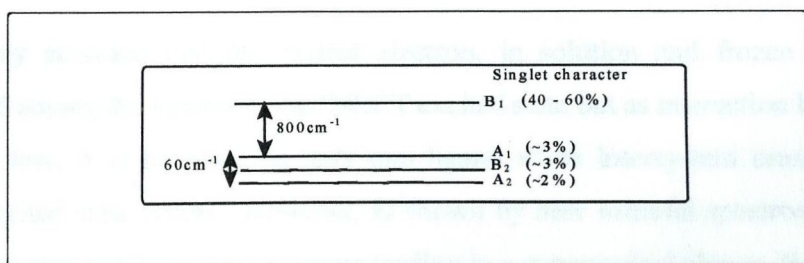


Figure 1.7: A schematic representation of the $^3\text{MLCT}$ state of Ru(II) Polypyridyl complexes^[24]

An excited metal-centred (^3MC) or d-d state which lies about 4000 cm^{-1} above the $^3\text{MLCT}$ manifold is revealed in temperature-dependent studies of luminescence^{[27][28]}, and is responsible for thermal deactivation and also photochemical reactions such as racemisation and photosubstitution. For a detailed discussion on the emission parameters and their evaluation see Section 1.3.

1.1.1-4 The Nature of the $^3\text{MLCT}$ State.

In the past, many absorption studies have focused on the position of the 'promoted electron' in the excited state of Ru(II) polypyridyl complexes. However, for complex **1**, an element of controversy surrounds the level of interaction between the ligands.

It is agreed, that there is a transient charge separation at the molecular level in the metal **1**, which display a light-induced metal-to-ligand charge transfer excited state^[29]. Magnetic circular polarised luminescence studies^[31] and low temperature emission studies^[30] of a matrix-isolated site suggest a D_3 symmetric system – $[\text{Ru}(\text{bpy})_3]^{2+}$ – seemed to favour the delocalisation of the electron over all three bipyridines ligands. In contrast, electronic absorption^[21], laser spectroscopies^[32], and resonance Raman measurements^[21], in aqueous solution, at ambient temperatures supported the localisation on one of the bpy ligands, suggesting that the light-induced electron transfer is vectorial in nature, and represented as $[\text{Ru}(\text{bpy})_2(\text{bpy}^{\bullet-})]^{2+}$ – a C_2 symmetric system.

It is widely accepted that the excited electron, in solution and frozen glasses, is delocalised among the ligands in the $^1\text{MLCT}$ excited state but as interaction between the ligands is low, it is localised on only one ligand when intersystem crossing to the $^3\text{MLCT}$ excited state occurs. However, as shown by near infrared spectroscopy, rapid electron-hopping between ligands occurs leading to a symmetrical charge distribution in the excited state^[33]. Thus the excited state of **1** has the disadvantage of a lack of directed charge transfer character. According to Meyer *et al*^[25], this problem may be overcome by replacing one of the bpy ligands with stronger π -accepting groups.

In the case of mixed ligand complexes, the electron is expected to be localised on the ligand that is most easily reduced^[34] (*i.e.* the lowest π^* orbital), forming a radical anion species of the ligand site. An example of such a complex is $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ **10**. As the dppz ligand is more readily reduced than bpy ligand by 1V, due to the lower energy π^* -orbital of the phenazine moiety, the electron is localised on the electron-

withdrawing dppz ligand, the relaxed $^3\text{MLCT}$ excited state may be formulated as $[\text{Ru}^{\text{III}}(\text{bpy})_2(\text{dppz}^{\bullet-})]^{2+}$ [34].

1.2 The Dppz Ligand.

1.2-1 $^3\text{MLCT}$ or $^3\pi-\pi^*$ Excited State Ru(II) Complexes.

To date, much work has been done on the photophysical properties of Ru(II) complexes which give an insight into the electronic properties of ruthenium, especially in photoexcited forms. Our interest lies mainly in Ru(II) complexes containing the dppz 2 and related substituted ligands. However, before the interactions of these complexes could be fully comprehended, the photophysical properties have to be definitely established. Although a substantial body of research has been undertaken, several key areas remain largely unresolved.

Resonance Raman and time-resolved resonance Raman (TR^3) methods are particularly effective for probing the vibrational structure of the excited states of transition metal complexes. One of the most important contributions was made by Dallinger, Woodruff and co-workers^[21], who demonstrated the utility of TR^3 spectroscopy for interrogation of $^3\text{MLCT}$ state vibrational modes. These techniques are versatile, enabling studies in both homogeneous and heterogeneous media, reflecting in the latter instance in their ability to provide valuable insight on the effect of microheterogeneous environments such as DNA.^{[35][36][37]} The nature of the excited states of these complexes has been the subject of some discussion. There are two (related) points to be considered. (1) The general nature of the excited state – charge transfer or ligand centred? (2) If the former, where does the excited electron reside?

(1) Schoonover *et al*^[38], compared 355 nm-generated TR^2 spectra of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ 10 with the electrochemically generated $\text{dppz}^{\bullet-}$ radical anion, and reported that the spectra are markedly similar. This was confirmed by McGarvey and co-workers^{[37][39]} who reported the presence of a radical-like $\text{dppz}^{\bullet-}$ ligand which carries the electron

density in the MLCT excited states. In contrast, it has been suggested by Chen *et al*^[36], that the TR² species probed in a 355 nm single colour is the ³ π - π^* state of dppz and not the dppz-localised MLCT consisting of the dppz^{•-} radical anion. But the spectra, proved, on closer scrutiny to be comparable with the electronically generated dppz radical anion^[35].

(2) Sauvage *et al*^[34] proposed from studies involving **10** and [Ru(dppz)₃]²⁺ **15**, that the MLCT excited state is expressed as [Ru^{III}(bpy)₂dppz^{•-}]²⁺, and the dppz ligand acts as though it were composed of two electronically independent units – bpy and phenazine. They concluded from photophysical and electrochemical measurements that following excitation of **10**, with dppz as the acceptor ligand pattern (similar pattern to electrochemically prepared dppz^{•-}) that the excited electron was localised on the phenazine portion of the dppz ligand. Another possibility is that a dppz based MLCT excited state is reached. This is supported by resonance Raman studies^{[37][39]}, which suggests that in the ³MLCT excited state of **10**, localisation of the “transferred electron” is concentrated on the phen portion of the dppz ligand, but the state also showed some delocalised character. Furthermore, evidence suggests that a more complex process occurs than that described by Sauvage’s model.

In summary, the TR² spectrum of **4** are best described as being MLCT in character, with the excited electron occupying orbitals associated with the dppz ligand.

1.2-2 Molecular Orbital (MO) Calculations for Ru(II) Complexes.

Extended Huckel calculations have been performed by a number of research groups^{[40][41]}, in order to obtain compositions and energies of the dppz ligand **2** orbitals and to rationalise the spectroscopic results. The dppz ligand combines features of α -diimine (bpy or phen)^[7] and of 1,4-diazine (phz or quin)^[8] moieties, as shown in Figure 1.1. Thus, it is not immediately obvious what MLCT excited states are available for photoemission. Theoretical treatments have established that the dppz π -system exhibits three relatively low lying unoccupied π^* molecular orbitals (LUMO). A graphical

representation of the squared molecular orbital (MO) coefficients is shown in Figure 1.8. They comprise

- (i) the $b_1(\text{phz})$ orbital, centred on the phenazine (phz) portion of **2**,
- (ii) the $b_1(\psi)$ orbital, localised on the bpy moiety of **2**, and
- (iii) the $a_2(\chi)$ orbital also localised on the bpy part of **2**.

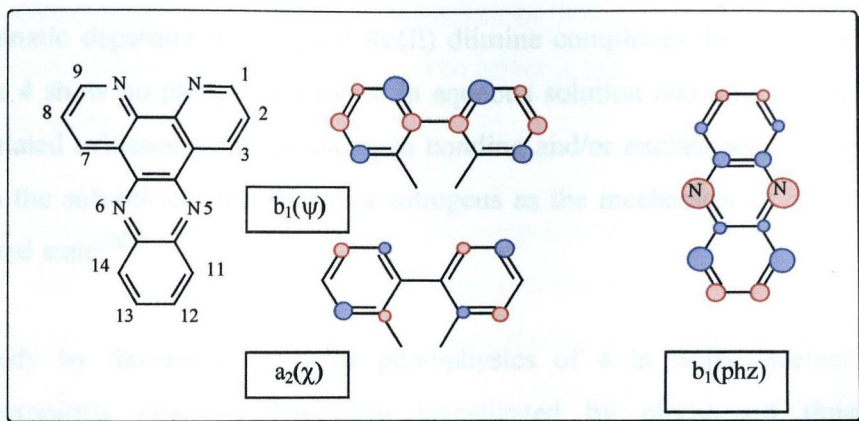


Figure 1.8: Schematic representation of Molecular Orbitals (MO) for dppz **2**, according to ref^[40]

These MO's lie close in energy. The latter two α -diimine acceptor orbitals, $b_1(\psi)$ and $a_2(\chi)$, are particularly close, and can interact with the π -electron rich Ru(II) centre in an established manner. However they were shown to lie *above* the phenazine-based π^* MO, $b_1(\text{phz})$. The HOMO (highest occupied molecular orbital) is mainly localised on the metal, the phen ligands and the phen part of the dppz ligand.

According to electrochemical and spectroscopic studies^[40] the lowest lying π^* orbital of dppz is localised almost exclusively in the phenazine part of the ligand, with very little contribution and effects from the α -diimine chelating sites. These calculations show that there are high MO coefficients on the non-coordinating phenazine N atoms. It has shown by Kaim *et al*^[7], that the extinction coefficients are directly correlated to the molecular orbitals coefficients through small oscillator strength. Therefore, as the amplitude of the wavefunction for the LUMO $b_1(\text{phz})$ is very small at the chelating

nitrogens, the MLCT transitions of complexes containing the dppz ligand **2** would be expected to be small. This is a very schematic representation of the main features of the absorption spectrum.

1.2-3 The Phenomenon of the 'Molecular Light Switch' Effect in Solution.

In a dramatic departure from typical Ru(II) diimine complexes the $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4** show no photoluminescence in aqueous solution and other protic solvents. Accumulated evidence points to hydrogen bonding and/or excited state proton transfer between the solvent and the phenazine nitrogens as the mechanism of deactivation of the excited state^{[3][5]}.

In a study by Barbara *et al*^[4], the photophysics of **4** in water, acetonitrile, and water/acetonitrile mixtures, has been investigated by picosecond time-resolved absorption and emission spectroscopy. The photoluminescence quantum yields of these complexes are found to be extraordinarily sensitive to their environment. They proposed that the 'light switch' behaviour of **4** is due to the involvement of not one, but two MLCT excited states, as outlined in the proposed mechanism in Figure 1.9.

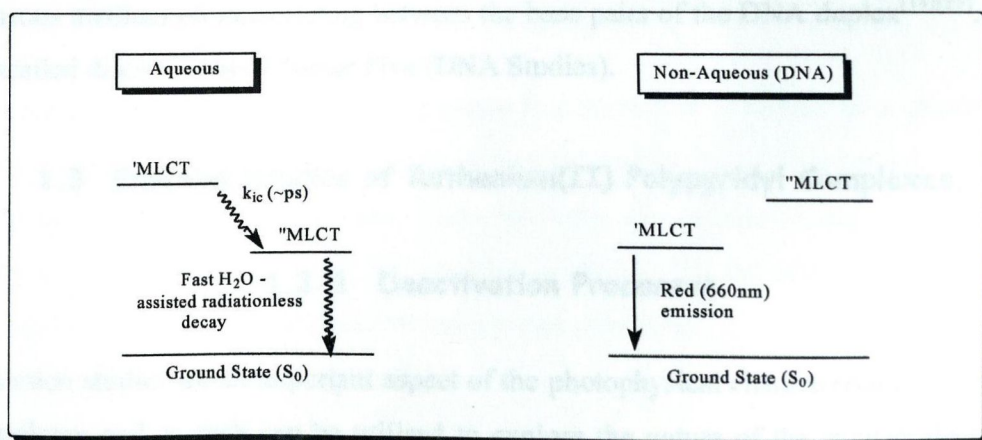


Figure 1.9: MLCT excited states of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** and proposed 'light switch' mechanism according to Barbara *et al*^[4].

In aqueous media: The suggested mechanism involves population via intersystem crossing to an initial $^3\text{MLCT}$ excited state, referred to as $^1\text{MLCT}$. It is proposed that this $^1\text{MLCT}$ state undergoes interconversion (IC) on a picosecond timescale to a second lower lying short lived MLCT state – denoted as $''\text{MLCT}$. It has been suggested that this $^1\text{MLCT}$ state has increased charge density on the phenazine portion of the dppz ligand and therefore undergoes rapid radiationless decay via water induced quenching of the MLCT state. The interconversion of $^1\text{MLCT} \rightarrow ''\text{MLCT}$ is assigned to an intramolecular charge-transfer process, dependent on both the solvent polarity (E_T) and proton-donating ability (α) of the solvent. The effect is more significant on considering the polarity of the solvent.

In a non-aqueous environment: The $^1\text{MLCT}$ is proposed to be slightly higher in energy than the $''\text{MLCT}$ state, effectively preventing conversion from the initially formed $^1\text{MLCT}$ state to the $''\text{MLCT}$ state, thus preventing the rapid radiationless decay observed in water. This $^1\text{MLCT}$ state is thought to produce the red emission ($\lambda_{\text{max}} = 610 \text{ nm}$) observed in acetonitrile (and in the presence of DNA).

In the presence of DNA: Polymers in aqueous solution including DNA, can also provide local hydrophobic pockets for these complexes. Hence, luminescence is observed for these systems but only when the phenazine nitrogens are protected from the surrounding aqueous medium on intercalating between the base pairs of the DNA duplex^{[14][65]}. For a detailed discussion see Chapter Five (DNA Studies).

1.3 Emission Studies of Ruthenium(II) Polypyridyl Complexes.

1.3-1 Deactivation Processes.

Emission studies are an important aspect of the photophysical characterisation of Ru(II) complexes and as such can be utilised to explore the nature of the excited electronic state. The possible deactivation pathways for the excited states of $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and related systems are shown in Figure 1.6. The energy gap between the excited state and

ground state, structural rigidity and the extent of delocalisation of the excited electron over the acceptor ligand are the most important factors affecting the excited state lifetimes.

The processes involved in the deactivation of the excited state may be formulated as,

$$k_{tot} = k_r + k_{nr} + k_{dd} + k_q^{O_2} [O_2] \quad 1.1$$

$$\text{where } \frac{1}{\tau_{meas}} = k_{tot} \quad 1.2$$

For the purposes of interpretation of our results it is essential that we elaborate further on these parameters.

k_r is the rate constant for *radiative decay* and can be accounted for quantitatively by the Einstein coefficient for spontaneous emission^[28] by

$$k_r = \left(\frac{4E_{em}^3}{3\hbar^4} \right) \cdot \left| \left\langle \psi_e \left| \vec{d} \right| \psi_g \right\rangle \right|^2 \quad 1.3$$

where : E_{em} is the emission energy; ψ_e and ψ_g are the excited and ground state electronic wave functions and $\vec{d}$ is the electron transition dipole moment operator.

In a radiative transition energy conservation is achieved by emission of a photon and from the above equation we see that k_r is proportional to E_{em}^3 , if the transition dipole moment remains relatively constant. Thus, the higher the emission energy the lower the value of k_r and the more intense the resultant emission. For the sake of simplicity, we assume that the transition is insensitive to solvent variations.

While k_r is important, it is the rate constant for *intramolecular non-radiative* decay, k_{nr} , which shows the greater variation with structure and appears to dominate the emission behaviour. Decay of the excited states of polypyridyl complexes, at room temperature, in fluid solution are typically dominated by these k_{nr} processes.

In order for radiationless decay processes to occur with energy conservation, the energy change associated with the change in the electronic configuration must appear in the surrounding vibrations. k_{nr} is the product of two factors: (i) vibrationally induced electronic coupling term and, (b) vibrational overlap or Franck-Condon integral, $F(E)$, between the two states. k_{nr} may be considered on the basis of the 'energy-gap law'^{[42][43]} for radiationless transitions, equation 1.4, assuming the approximations of high temperature ($\hbar\omega_M \ll k_B T$) and weak-vibrational coupling (*i.e.* small excited state distortions) ($|\Delta E_{em}| / \hbar\omega_M S_M \gg 1$)

$$k_{nr} = (C^2 \omega_{em}) \cdot \left(\frac{\pi}{2\hbar\omega|\Delta E_{em}|} \right)^{1/2} \cdot \exp(-S) \cdot \exp\left(\frac{-\gamma|\Delta E_{em}|}{\hbar\omega_m}\right) \quad 1.4$$

where: $|\Delta E_{em}| = (|\Delta E| - \hbar\omega_k)$, where ΔE is the energy gap between the thermally equilibrated ground and excited states; ω_m is the angular frequency for the promoting vibrations; C^2 is the nuclear momentum matrix element for ω_{em} which leads to transitions between states; $S (= \frac{1}{2} \sum_j \Delta_j^2)$ is a measure of the extent of excited state distortion in the acceptor vibration; Δ_j is the dimensionless fractional displacement of normal mode j between the equilibrium configurations of the ground and excited states, $\gamma = [\ln(|\Delta E_{em}| \hbar\omega_m S_m) - 1]$. k_B is the Boltzmann distribution

This equation may be simplified to give:

$$\ln k_{nr} \propto (E_{em}) \quad 1.5$$

Typically, increases in the nonradiative k_{nr} processes are facilitated by a lowering of the energy gap between the ground and excited states, as predicted by the energy-gap law. This term is also dependent on the solvent reorganisational trapping energy (χ_0) associated with the transfer of an electron from the ligand to the metal. In organic

solvents, the predicted linear relationship between $\ln k_{nr}$ and E_{em} is observed because variations in χ_0 are relatively small^[44]. However, it is found that this approximation does not hold for hydroxylic solvents, like CH_3OH and H_2O , as the non-radiative decay is always larger than expected. The term k_{nr} has been shown to be temperature dependent but the effect is sufficiently small as to be negligible here.

k_{dd} relates to a *temperature - dependent term* and constitutes an additional nonradiative deactivation pathway which involves deactivation of the thermally populated metal-localised “dd” (3MC) states. The 3MC state lies *ca.* 4000 cm^{-1} above the 3MLCT manifold and subsequently undergoes very rapid radiationless decay ($10^9 - 10^{10}\text{ s}^{-1}$) to the ground state or photodecomposition of the complex via ligand loss. k_{dd} is a solvent dependent kinetic term and may be derived as,

$$k_{dd} = k^0 \cdot \exp \left[\frac{-\Delta E}{RT} \right] \quad 1.6$$

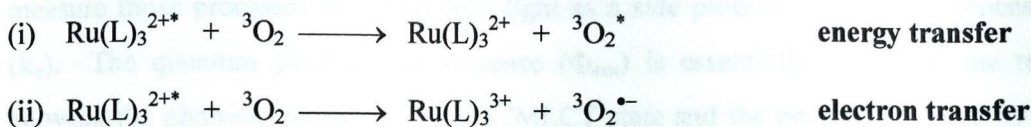
where: k^0 is the pre-exponential factor for $^3MLCT \rightarrow ^3MC$ and ΔE is the energy difference between the 3dd states and the manifold of 3MLCT emitting states. R is the gas constant.

The appearance of these relatively low lying dd states in $Ru(II)$ polypyridyl complexes, although of fundamental interest, can represent a major drawback to their use as photosensitisers. At low temperatures, thermal population of the 3MC state is not possible and assuming that $\Sigma k_{nr} (= k_{dd} + k_{nr})$, we observe an increase in k_r resulting in a more intense emission. At room temperature, thermal population of the 3MC state is accessible and k_r will decrease as the emission intensity decreases. Extensive studies have focused on the preparation of MLCT excited states where the dd states do not interfere with the desired photochemical reaction pathways.

$k_q^{O_2}$ is the rate constant for *bimolecular quenching process* involving species ‘Q’. For the majority of ruthenium polypyridyl complexes their lowest excited 3MLCT states are long-lived and are quenched by molecular oxygen (3O_2). Oxygen possesses a triplet ground state $O_2 (^3\Sigma_g^-)$. Upon quenching, two different species are formed $O_2^*(^1\Delta_g)$ and $O_2^*(^1\Sigma_g^+)$, which lie 94 and 157 kJ mol^{-1} , respectively above the ground state. The

efficiency of production of each of these excited species depends on the energy of the triplet, which is being quenched. The higher energy species rapidly decays to the lower one so when we speak of 'singlet oxygen' we refer invariably to contributions arising from the lower $O_2^*(^1\Delta_g)$ species.

The most important bimolecular processes are;



where the latter process may involve either oxidation or reduction of the excited state. These three quenching processes may occur in parallel so that the observed quenching rate $k_q^{O_2}$ may contain contributions from all three processes. The efficiency of the luminescence quenching process can be correlated with the redox potentials for the excited state and is solvent dependent^[7]

While there has been extensive development of the theoretical framework for dealing with deactivation of the electronic excited state, it is difficult to devise effective experimental approaches to this issue in as much as the actual processes are not conveniently observed. The excited state lifetime (τ) being the only direct parameter that is experimentally accessible through time-correlated single photon counting proves to be an invaluable parameter. The mean lifetime ($\tau = 1/\Sigma k$) is a measure of all the radiative (k_r), non-radiative (k_{nr}), thermal-related (k_{dd}) and quenching ($k_q^{O_2}$) processes which account for the relaxation of the excited state to the ground state.

Although the processes proceed in parallel it is possible to deduce the observed quenching rate constant from the Stern -Volmer equation,

$$\frac{1}{\tau_{meas}} = \frac{1}{\tau_o} + k_q^{O_2} [O_2] \quad \tau_o = \left(\frac{1}{k_r + k_{nr} + k_{dd}} \right) \quad 1.7$$

where: τ_{meas} and τ_o are the observed lifetimes in the presence and absence of the quencher respectively, $[O_2]$ is the concentration of quencher i.e. triplet oxygen and $k_q^{O_2}$ is the luminescence quenching rate constant.

From equation 1.7, we observe that by varying the concentration of the quencher (oxygen) in solution, a Stern-Volmer plot of $1/\tau_{meas}$ - vs - $[O_2]$ will lead to $k_q^{O_2}$ as the slope.

In order to examine the other decay constants we used steady-state emission studies to measure those processes which produce light as a side product *i.e.* radiative processes (k_r). The quantum yield of luminescence (Φ_{em}) is essentially defined as the ratio between the photons emitted (k_r) by the 3MLCT state and the photons absorbed by the fundamental ground state S^0 , as represented in equation 1.8. Assuming that the above mathematical analysis (1.7) is correct, the value of k_r and k_{nr} , respectively, may be determined at any temperature, according to equation 1.8,

$$\Phi_{em} = \Phi_{ISC} \cdot \left(\frac{k_r}{k_r + \sum_i k_{nr}} \right) = \Phi_{ISC} \cdot k_r \cdot \tau_{meas} \quad 1.8$$

where: Φ_{ISC} (assumed to be unity) relates to the process $^1MLCT \rightarrow ^3MLCT$, $\sum_i k_{nr}$ represents the terms ($k_{nr} + k_{dd}$).

The recorded emission spectrum gives E_{em} (energy of maximum emission), and the quantum yield of luminescence (Φ_{em}) which may be calculated. These parameters, in conjunction with the rate decay constants permit an understanding of these ruthenium complexes in solution. A more detailed discussion is given in Chapter Four to the evaluation of the emission parameters.

1.4 Deuterium Isotope Effect on the Emission Studies of Ru(II)

Polypyridyl Complexes

1.4-1 Introduction

Many processes are involved in the deactivation of the excited state, but the nonradiative rate constants, k_{nr} , determined largely by vibrational overlap between the ground and excited states, dominate the luminescence behaviour of Ru(II) complexes. Therefore, a reduction in the value of the nonradiative rate constant, k_{nr} , will result in increased emission efficiency^[27]. This has been achieved by incorporation of deuterium into a wide diversity of complexes. The effect of substituting deuterium for hydrogen in the local environment of Ru(II) complexes, enhances the phosphorescence yield without concomitant changes in the radiative lifetime and the structure of the emission spectrum^{[45][46]}.

The earliest application of the deuterium isotope effect can be found in the work of Hutchison^[47] and Wright^[48] who investigated the effects of deuterium incorporation on the photophysical properties of naphthalene and benzene. Since then, numerous detailed studies have been undertaken for a wide range of systems, which include poly-aromatic hydrocarbons^[49] and rare earth ions and their complexes^[54], for a review on this subject see Vos^[46]. Watts^[11] extended this phenomena to transition metal systems by examining the effects of deuteration on the lifetime of the lowest ³MLCT excited state of 1.

Upon isotopic exchange, a number of changes in the nature of the photophysical properties of these systems were observed. In general, deuteration has been applied predominantly to increase emission lifetimes and quantum yields in an attempt to probe the structure of the free ions and their complexes in solution. Applications of this phenomenon were extended to include a wide range of compounds and demonstrated^{[20][22]} a lifetime dependence not only on the number of deuterium substituents but also on the position of substitution^[50].

1.4-2 The Effect of Deuteration on Excited State Lifetime Decay - Theory.

In 1960, Hutchison^[45] examined the phosphorescence lifetimes of naphthalene in a durene matrix. At 77 K, the perdeuteration of naphthalene leads to a dramatic increase in the triplet state lifetime, from 2.1 sec (for C₁₀H₈) to 16.9 sec (for C₁₀D₈), respectively, while matrix deuteration had no effect on the decay lifetimes. At temperatures above 100 K, significant temperature dependence was observed for the excited state lifetimes^[51]. These results provided evidence for the importance of Franck-Condon factors for C-H vibrations, and the involvement of the vibrations of both the complex and the lattice in radiationless decay processes. These observations stimulated studies to develop an understanding of the isotope effect.

A detailed discussion of the mathematical basis of the theory of nonradiative electronic transitions and the origins of the effect of deuteration is beyond the scope of this study and the reader is referred to the work of Siebrand^[52], Robinson and Frosch^[49], and Jortner^[42]. The observed excited state lifetime,

$$\tau_{meas} = \frac{1}{k_r + \sum k_{nr}} \quad 1.9$$

is given by the sum of a radiative rate constant (k_r) and the nonradiative rate constants ($\sum k_{nr}$). In as much as the only excited state energy dissipation channel which is expected to be influenced by deuteration is the nonradiative decay occurring from the ³MLCT states of Ru(II) polypyridyl complexes^[53], further discussion will concentrate on the k_{nr} term in equation 1.9. It has been suggested that an important factor to the overall rate of nonradiative decay is the vibrational stretching modes. As a result, the $\sum k_{nr}$ includes the term k_{X-H} , where X = C, N or O, the rate of radiationless deactivation due to the X-H vibrational coupling. This term can be expressed by equation 1.10

$$k_{X-H} = \left(\frac{2\pi}{\hbar} \right) \rho J F(E) \quad 1.10$$

Here ρ is the density of the final vibrationally excited state and J is the electronic coupling between the two states, and the Franck-Condon factor $F(E)$ is the sum of the products of the vibrational overlap integrals.

In aromatic hydrocarbons, it is the highest frequency vibrational modes, C-H stretch at *ca.* 3000 cm⁻¹, which are primarily responsible for dissipating the electronic excitation energy^[51]. Substitution of hydrogen by deuterium will lead to a reduction in the C-H out-of-plane bending amplitudes and thus will reduce the rate of radiationless transition k_{X-H} . According to Robinson^[68], the rate of radiationless transitions depends mainly on the magnitude of the product of the vibrational overlap integrals between the initial and final states. The deuterium effect arises because of the reduced amplitude and frequency of the heavier C-D vibrations relative to equivalent C-H vibrations^[67]. This leads to a reduction of the vibrational overlap products for the same energy gap, as illustrated in Figure 1.10.

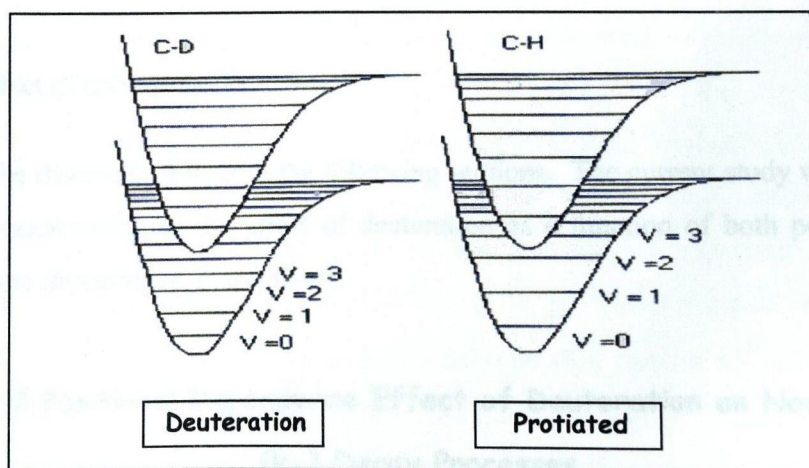


Figure 1.10: Changes in the vibrational levels and overlap which occur upon deuteration.

Since the Franck-Condon factor $F(E)$ and the nonradiative rate are reduced we observe an increase in the observed lifetime upon substituting deuterium for hydrogen. This effect is now well established experimentally and the Franck-Condon factor has received extensive theoretical verification from the calculations of Siebrand^{[52][55]}. There is a large deuterium effect on k_{nr} values for organic systems, with a much smaller effect observed for inorganic (rare earth metal) systems.

1.4-3 The Deuterium Isotope Effect.

1.4.3-1 Introduction.

Studies into the effect of deuteration on the excited state lifetime of Ru(II) complexes revealed that a number of important contributing factors need to be considered. These may be categorised according to:

- Deuteration (*i.e.* full or partial H-D exchange) of the complex
- The effect of temperature variation.
- The effect of solvent deuteration.

and will be discussed further in the following sections. The current study will include a detailed examination of the effect of deuteration as a function of both positional and temperature dependence, respectively.

1.4.3-2 Positional Dependence Effect of Deuteration on Nonradiative (k_{nr}) Decay Processes.

More recently, a different type of deuterium effect on triplet lifetimes was observed, whereby the excited state lifetime showed a dependence on the position of substitution^{[10][51]}. Watts and Strickler^[10] compared the triplet lifetimes of a series of naphthalene isomers having numerically the same but positionally different deuterium

substituents. If the deuterium effect was due solely to an effect on the Franck-Condon factor associated with the vibrational modes, then in principle, all positions should have equal probability for accepting electronic energy. Hence, two isomers should essentially show the same triplet decay rate. It was constitutently found that the introduction of deuterons in the α -positions of naphthalene induces a larger increase in the observed lifetime (by a factor of 1.6) than does replacement of a proton in the β -position.

According to the "active H atom" theory of Robbins and Thomson^[56], this lifetime effect will be largest for those protons with increased electron density, leading to greater efficiency in radiationless decay relaxation. This is in agreement with results observed for naphthalene, where the electron coefficient at the α carbons is greater than at the β carbons. Burr *et al*^[50] also have observed similar effects in biphenyls whereby H-D exchange at the *meta* positions induced longer-lived lifetimes than substituents at the *ortho* or *para* positions.

• $[\text{Ru}(\text{bpy})_3]^{2+}$ **1**

Although the photophysics and photochemistry of **1** has been extensively studied and has accumulated a large body of information the nature of these decay processes remain uncertain. For inorganic systems, although the effect of ligand substitution is relatively small, it is appropriate to observe the effects of selective deuteration. Initially, the work by Watts^[57] reported that deuteration of the free bpy **5** substrate doubled the fluorescence lifetime, from 0.9 sec to 2.2 sec, at 77 K. Perdeuteration of **1**, causes a more modest 20% increase in the emission lifetime (5.1 μsec $\rightarrow$ 6.1 μsec at 77 K in EtOH/MeOH and 580 ns $\rightarrow$ 690 ns at 298 K in H₂O, respectively). Further investigation by Kincaid and co-workers^[22] have examined the positional dependence deuterium effects of isotopically substituted analogues of **1**. These studies showed that deuteration of the 3,3' or 4,4' positions (regions of low electron density) had little effect on the emission lifetimes (610 and 650 ns, respectively), and no measurable effect on the nonradiative decay constants. Whereas deuteration of the 5,5' or 6,6' positions exhibited significantly longer lifetimes of 635 and 645 ns respectively, compared with $\tau = 590$ ns

for protiated **1**, and a relatively large effect on nonradiative deactivation processes. Similar trends were observed for di- and trideuterated complexes. These studies are consistent with the "active H atom" theory of Robbins and Thomson^[56], whereby increased electron densities in the regions of the atoms, which is of the order $3,3' < 4,4' < 5,5' < 6,6'$, for **1** lead to greater efficiency of radiationless decay pathways and shorter emission lifetimes for the complexes on moving across the series^[58]. These studies suggest that the ability of X-H vibrational modes to deactivate the excited state is dependent on the electron density contribution in the excited state.

For mixed ligand complexes, a more recent approach has investigated the application of partial deuteration of the ligands to locate the ligand which contains the emitting ³MLCT state. This was illustrated by Vos and co-workers^[9], who examined the effect of partial deuteration for Ru(II) complexes of the form, $[\text{Ru}(\text{bpy})_2\text{L}]^{2+}$.

1.4.3-3 The Effect of Solvent Deuteration on the Excited State

Lifetime of Ru(II) Polypyridyl Complexes.

An important contribution to the deactivation of the excited state lifetime is the surrounding medium. As excess energy is transferred to the solvent bath, the connection between solvation dynamics and excited state relaxation needs to be addressed. van Houten and co-workers^[27] reported that for **1**, solvent deuteration (H_2O to D_2O), caused the observed excited state lifetime to almost double ($0.58 \rightarrow 1.02 \mu\text{sec}$ at 298 K). Whereas, perdeuteration of the complex only resulted in a 20-25% increase in the triplet lifetime (5.1 to 6.1 μsec at 77 K and $0.58 \rightarrow 0.69 \mu\text{sec}$ at 298 K respectively). If as assumed, the excited state were purely MLCT in nature then we would expect a larger increase in the lifetime decay upon deuteration of complex **1**. Based on nonradiative theories for organic compounds^{[42][43]}, the difference between the ligand C-H and the solvent O-H vibrational modes' ability to deactivate the lowest ³MLCT states may be attributed to the partial charge to solvent character (CTTS) of the excited state. It has been suggested that increased electron density is distributed over the solvent cage, facilitating transfer of electronic energy to solvent vibrational modes. Furthermore, it

was observed that as the energy gap (between the ground and excited states) increases, the contribution of the O-H vibrational modes to the overall rate of radiationless deactivation diminishes because of the reduced overlap. This results in smaller deuteration effects. The observation that the most pronounced effect is observed upon deuteration of the solvent is strong evidence for increased electron density in the region of the solvent.

1.4.3-4 The Effect of Temperature on the Excited State Lifetime of Deuterated Ru(II) Polypyridyl Complexes.

Several studies into the effect of deuteration on the photophysical properties of **1** at elevated temperatures have been reported^[49]. These studies reveal the importance of deactivation processes from the excited state and suggest the thermal population of a set of excited 'dd' states (³MC) which lie higher than the lowest excited ³MLCT states, as shown in Figure 1.6. Deactivation via these ³MC states was found to be independent of both the effects of ligand and solvent deuteration. As both these effects are reduced there is a reduction of the triplet lifetime on increasing the temperature^[12]. This strongly suggests that in order for a deuteration effect to be observed the X-H vibrational coupling must make a significant contribution to the overall radiationless decay rate constant of the excited state.

1.4.3-5 Concluding Remarks.

Although, the effects of deuteration are not as large as those observed for organic systems, the application of the deuterium isotope effect on inorganic photophysics is fast becoming a well defined area of research. It provides a simple yet effective method for the elucidation of the electronic structure and nature of the excited states of these complexes.

1.5 The Interaction of Ru(II) Complexes with DNA.

1.5-1 Introduction.

Deoxyribonucleic acid, DNA, holds the key to the biology of all living organisms.

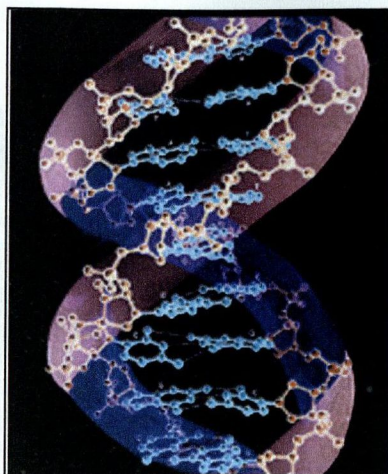


Figure 1.11: Representation of double helical DNA.

In Chapter Five (DNA Studies) a more detailed discussion is given to the structure and chemistry of DNA. There is considerable interest in the DNA binding properties of inert transition metal complexes^{[3][59][60]}, in particular, in the study and possible application of small, functionally active molecules which can interact in a site-specific manner with DNA. As transition metals have a wide range of applications that are dependent upon their ability to bind to DNA, it is important that a detailed understanding of the metal complex-DNA association be obtained. Herein, we briefly review the developments in the area of metallointercalators that bind and react with DNA.

1.5-2 The Binding of Ru(II) Polypyridyl Complexes with DNA.

The first experiments describing the interaction of coordinatively saturated octahedral transition-metal complexes with DNA focused on tris(phen) complexes of zinc, cobalt and ruthenium to DNA. On the basis of photophysical and NMR studies, it was

proposed that these complexes bound through noncovalent binding modes to DNA^{[61][64][65]}. A model of the binding modes of the enantiomers of **6** is illustrated in Figure 1.12. The interaction with DNA is enantioselective, and despite extensive research, the binding modes of **6** (whether intercalative or surface bound in the major/minor grooves) remain an area of vigorous controversy.

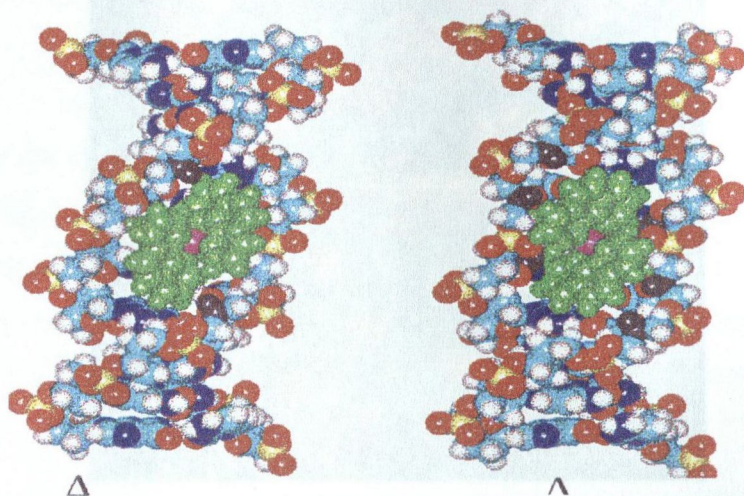


Figure 1.12: Schematic model of the binding of $[\text{Ru}(\text{phen})_3]^{2+}$ **6**. (Courtesy of Norden *et al.*^[59])

To increase the intercalative binding affinities metalintercalators with extended aromatic surface area heterocyclic ligands were synthesised, and have become immensely powerful tools in probing nucleic acids. One of the most interesting classes are those complexes containing dppz, namely $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** and its cousin $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ **10**. In combination, with the potentially intercalating behaviour of dppz (*i.e.* planar aromatic heterocyclic functionality can insert and stack between the base pairs of the double helix), this special electronic configuration can be exploited by using these complexes as ‘light switches’^[3]. Their MLCT luminescence is quenched by water^[4] but not in micelles of water or DNA. In the presence of DNA they display enhanced luminescence, with binding constants $K_b \geq 10^6 \text{ M}^{-1}$ ^[3], due to possible specific intercalation of the phenazine portion of the dppz into a hydrophobic pocket of the double helical DNA structure^{[5][6]}. Additionally, the Δ -enantiomer shows greater luminescence than the Λ -isomer when bound to DNA.

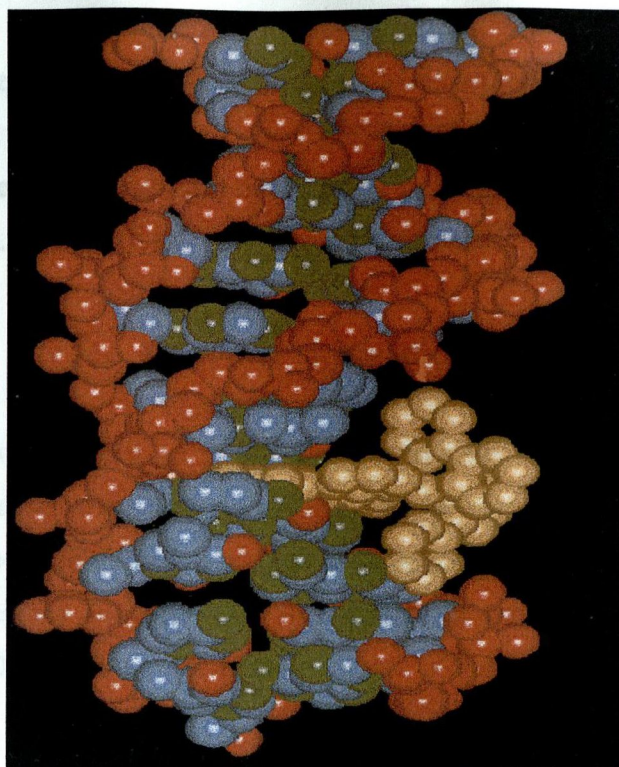


Figure 1.13: Model of DNA intercalated with $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ $10^{[65]}$.

The luminescent characteristics of these dppz complexes display biexponential decays in the presence of DNA compared to single exponentials in the absence of DNA. It is agreed, that these complexes bind intercalative with the extended dppz ligand inserting between the base pairs of the double helix and that two binding modes exist. However, the exact geometry and orientation of their binding modes is not yet well understood.

The Binding mode - Major vs. Minor. Norden and coworkers^{[59][63][66]}, on the basis of the similarity of the binding geometry to that of actinomycin D and photophysical studies using T4-DNA, have proposed that both Δ - and Λ - $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** intercalate from the **minor** groove. Alternatively, on the basis of both photophysical and NMR studies, Barton and coworkers^{[5][60][62]} have proposed that the Δ - and Λ -enantiomer of **4** intercalate from the DNA **major** groove.

The current study employs protium-deuterium exchange techniques in an effort to investigate these Ru(II) complexes. Steady state and time-resolved luminescence studies of the racemic and enantiomeric protiated and deuterated complexes of **4**, have been examined in the absence and presence of DNA. The complexes in this thesis have also been examined by the technique of resonance Raman spectroscopy in collaboration with Professor J.J. McGarvey, Queens University, Belfast^[63].

1.6 Aims of the Work.

The aim of the work carried out in this thesis is to study the transition metal Ru(II) complexes in the absence and presence of DNA. At present, there is great interest in the site and sequence-specific reaction of these complexes with DNA. Much research in this area is concerned with the study of the binding and photocleavage of Ru(II) complexes with DNA. We are interested in the use of Ru(II) complexes ultimately for this purpose.

Chapter Two describes the preparation and properties (their characterisation by ¹H NMR, UV-Visible absorption, and ES⁺-mass spectroscopies) of the protiated, predeuterated and selectively deuterated ligands and their corresponding complexes.

Chapter Three details the scope and limitations of the methods of deuteration employed in the current study. It details the controls experiments (*i.e.* catalyst, solvent, temperature and time) performed to access and optimise protium-deuterium exchange

Chapter Four looks at the influence of solvent, ligand deuteration and temperature on the photophysical properties (emission quantum yield, lifetime, and rate decay constants) of the ruthenium complexes; [Ru(bpy)₃]²⁺ **1** and [Ru(phen)₃]²⁺ **6**. A systematic study of the effects of solvent, ligand substitution, and selective deuteration, for the parent [Ru(phen)₂dppz]²⁺ complex **4** has also been investigated.

Chapter Five describes work carried out with the racemic, Δ- and Λ-enantiomers of the protiated and deuterated Ru(II) complexes of **4** in the presence and absence of CT-DNA, by UV/Vis absorption, steady state, and time correlated lifetime spectroscopy studies.

Chapter Six details the materials and experimental methods used in the current study

Chapter Seven details possible future work.

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Chapter Two

2.0 Introduction.

This chapter addresses aspects of the preparation and characterization of the deuterated ligands, their racemic and enantiomeric Ru(II) polypyridyl complexes and examines their photophysical and photochemical properties. The focus is on complexes containing the dppz 2 moiety because due to the extended planar aromatic nature of this ligand it has the ability to insert itself in between the base pairs of double helical DNA. In an attempt to acquire a more comprehensive understanding of the excited states of these systems a vast array of Ru(II) complexes have been prepared, characterised and subsequently studied. For the sake of clarity, the synthesis of the complexes used in this study are structurally divided into four categories;

- (i) The methods of deuteration involved in the preparation of partially and fully deuterated dppz ligands. The techniques of NMR and mass spectroscopies are discussed in relation to their assessment.
- (ii) Ru(II) complexes of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, which incorporate substituents at the distal 12 and 13 positions of the dppz ligand; $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ **8** and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9** complexes have been prepared. The results of ^1H NMR, Electrospray ionization mass spectroscopy ES^+ -MS, and UV-Vis absorption studies are discussed.
- (iii) The Ru(II) complexes **4**, **8**, and **9** in (ii) have undergone selective deuteration of different constituents (*i.e.* phenanthroline (phen) and /or the dppz ligand (all or part thereof)) in an attempt to determine the nature of the excited state of such systems. For the parent complex **4**, a series of eight complexes were prepared and characterised.

(iv) The isolation of the Δ - and Λ -enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** and its deuterio analogues; $[\text{Ru}(\text{phen})_2\text{d}_4\text{-dppz}]^{2+}$ (**4a**) and $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$ (**4d**), is described (see Chapter Five). NMR and circular dichroism (CD) spectroscopies were used to assess the purity and enantiomeric chirality of these complexes.

2.1 Aims of Research

2.1-1 Introduction.

With the aim of probing the excited state properties of these dppz containing Ru(II) complexes, we require a series of partially deuterated ligands. The derivatives studied comprise the 11,12,13,14 - d_4 (**2a**); the 2,3,4,7,8,9, - d_6 (**2b**), and the d_{10} (**2c**) dppz ligand, as illustrated in Figure 2.1.

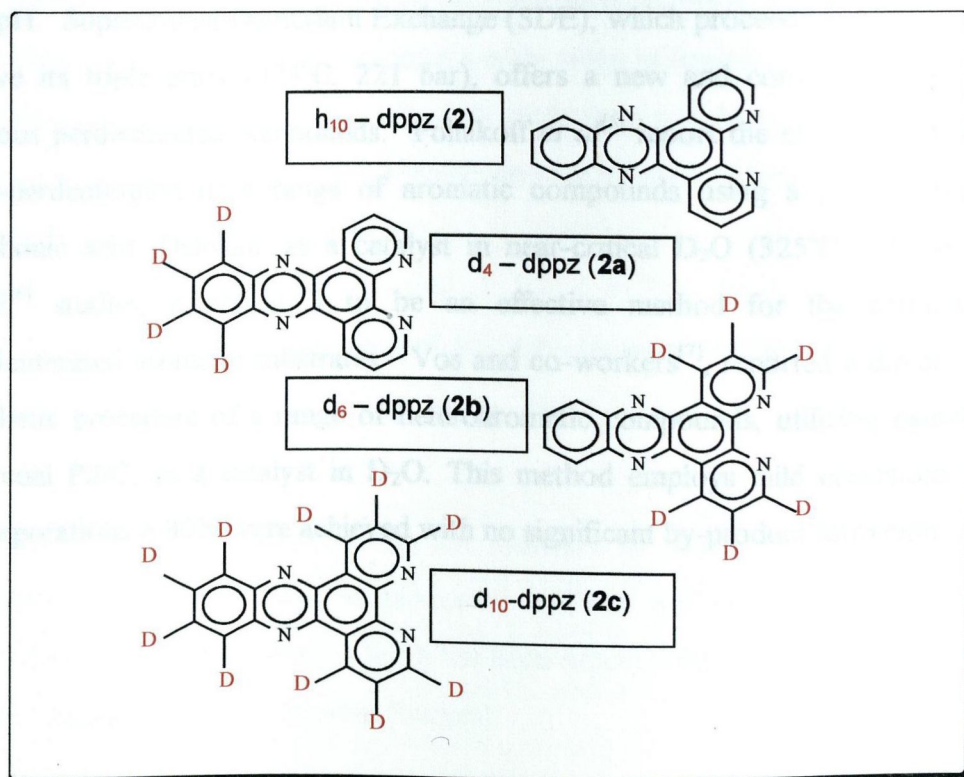


Figure 2.1: Protiated and deuterated analogues of dppz (2 - 2c).

2.1-2 Methods of Deuteration.

Protium-Deuterium exchange is a relatively under-exploited approach, mainly due to the synthetic difficulties in obtaining deuterated compounds. Common deuteration methods include exchange in the presence of weak or strong acids^[1], bases^[2], or noble metal catalysts^[3] under heterogeneous or homogeneous conditions. However, all of these methods suffer inconveniences which include skeletal rearrangements, expensive or relatively in-accessible reagents, incomplete isotopic exchange, and low yields.

Current research on protium-deuterium exchange involves the use of deuterium oxide, D₂O. Recently, several groups have reported various examples of the nondestructive exchange of a variety of organic molecules in superheated (200-300°C, 75 bar) water^{[4][5]}. Its potential for protium-deuterium exchange has been demonstrated at high temperatures and pressures, displaying different reactivity and selectivity according to the pH. Supercritical Deuterium Exchange (SDE), which proceeds in deuterium oxide above its triple point (374°C, 221 bar), offers a new and convenient approach to various perdeuterated compounds. Poliakoff *et al*^[5] report the efficient and selective ring-perdeuteraion of a range of aromatic compounds using a polymer-supported sulphonic acid, Deloxan, as a catalyst in near-critical D₂O (325°C). Base-induced SDE^[6] studies, displayed it to be an effective method for the preparation of perdeuterated aromatic substrates. Vos and co-workers^[7], reported a direct one-step synthetic procedure of a range of heteroaromatic compounds, utilizing palladium on charcoal Pd/C, as a catalyst in D₂O. This method employs mild conditions and D-incorporations > 80% were achieved with no significant by-product formation.

In the literature, a number of studies have focused attention on the deuteration of polypyridyl ligands:

- **Cook** - d_8 -2,2'-bipyridine prepared by treatment of 2,2'-bpy-1,1'-dioxide with D_2O -NaOD and then reduced with PCl_3 .^[8]
- **Poliakoff** - Deuteration of range of compounds using polymer supported sulphonic acid, Deloxan in near critical D_2O ($325^\circ C$)^[5]
- **Junk** - Base-induced Supercritical Deuterium Exchange (SDE) at $374^\circ C$, 221 bar^[6]
- **Vos** - d_{10} -phenanthroline/ d_8 -bipyridine prepared in presence of D_2O and Pd/C at $200^\circ C$ for 8 days^[7].

Due to its direct synthesis, relatively inexpensive materials and quantitative yields the method of Vos and co-workers^[7] was initially investigated. This approach has been used to perdeuterate heteroaromatic substrates such as triazoles, phen and bpy, respectively, but we are unaware of its application to other reagents and therefore examined the effect upon application to a wide range of ligands. Whilst it proved to be a highly efficient H-D exchange method its application was found to be limited to non-functional heteroaromatics. So a novel and alternative approach was devised for deuteration of the desired ligands.

2.1-3 General Experimental H-D Exchange Methods used in the Current Study

The first key reaction employed in the synthesis of the deuterated ligands, was a modification of the method as pioneered by Vos and co-workers^[7]. A general procedure is detailed for phen **3**, but it has been successfully applied to bpy **5** and dppz **2**, see Chapter Three (Deuteration Studies).

In a general procedure, phen **3** in D₂O was reacted in the presence of palladium on activated charcoal, Pd/C in a teflon coated steel high pressure reactor for 2 days at 190°C. The contents of the reactor were cooled to room temperature, and filtered to remove the Pd/C catalyst. The D₂O was removed under vacuum, and the product obtained in this manner, was found to be >95% perdeuterated as determined by ES⁺-MS and ¹H NMR spectroscopy. Treatment of this partially deuterated phen with fresh D₂O for a further 2 days at 190°C resulted in complete D-incorporation in the isolated product in yields of 80-90%. Heating and cooling times are not reported in the reaction times. Isotopic purities were characterized by ES⁺-MS, by comparison between observed and calculated isotope clusters, and by ¹H NMR spectroscopy.

However, the above procedure failed to introduce deuterium into diaminobenzene (dab) **11** and its derivatives. This led to the development of a protocol, as described below, to convert the readily available parent ligands, namely dab and derivatives, to their corresponding perdeuterated derivatives. In a typical experiment, dab **11** was reacted in D₂O and DCl (35% in D₂O) in a teflon coated steel high pressure reactor for 1 day at 190°C. The contents of the reactor were cooled to room temperature, and the D₂O, DCl were removed under vacuum, to obtain the d₄-dab product, isolated as a dichloride salt. In a single run, protium-deuterium exchange >85% was achieved with no side-product formation. Isotopic purities were characterized by ES⁺-MS and by ¹H NMR spectroscopy.

These methods allowed for the preparation of the perdeuterated ligands; d₈-phen (**3a**) and d₄-dab (**11a**), from which the selectively deuterated dppz ligands could be derived by subsequent reaction. Control experiments were performed to examine the effect of both the external (*i.e.* time and temperature) and internal (*i.e.* catalyst and solvent) variables on the rate of protium-deuterium exchange and the results are detailed in Chapter Three (Deuteration Studies).

2.2 Preparation of Selectively Deuterated Dppz Ligands.

2.2-1 H₁₀-Dppz (Parent Ligand 2)

The synthesis and characterisation of the parent (h₁₀) dppz ligand **2** has been included to serve as a comparison with the isotopically labeled analogues of the dppz ligand and to complete the series of ligands. Repetition of experiments were performed to ensure that ES⁺-Mass spectra and ¹H NMR analysis gave reliably reproducible results.

The parent dppz ligand **2** was prepared following the method of Summers *et al* ^[9], as illustrated in Figure 2.2. 1,10-phenanthroline-5,6-dione, phendione **12** and diaminobenzene, dab **11** were reacted in refluxing ethanol for 30 minutes. The brown solution was slowly cooled to room temperature upon which straw-like needles were isolated. Subsequent recrystallisation from aqueous ethanol gave dppz as brown-orange needles in quantitative yields.

In this study, ¹H NMR and ES⁺-mass spectroscopy were used extensively and proved to be very useful characterisation tools to identify and assess the purity of the ligands. Due to the C₂ axis of dppz five distinct sets of aromatic signals are recorded in deuteriochloroform, and are displayed and assigned in Figure 2.3.

2.2-2 D₈-dppz (2a)

Electrospray ionization mass spectrometry (ES⁺-MS) has proved to be a mild ionization method for the characterization of coordination compounds. The isotopic composition of dppz revealed a strong molecular ion peak at $m/z = 283$ (MH⁺), and is accompanied by peaks at 305 (MNa⁺) and 587 (2MNa⁺), respectively. The isotopic patterns observed for the species are in good agreement with the calculated isotopic compositions.

on C₂D₈N₂D₈ 2DCH₃ species. This was subsequently added to phendione 12 and refluxed in ethanol for 30 minutes. The resulting yellow-brown solution was cooled to room temperature and filtered, yielding a brown solid.

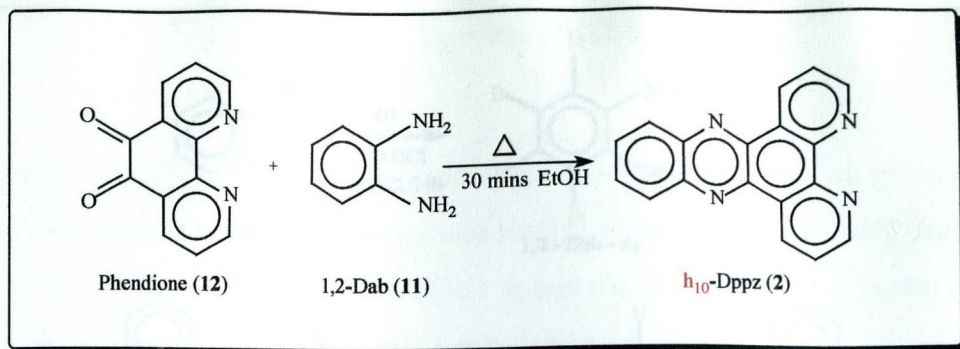


Figure 2.2: Preparation of h₁₀ - dppz 2.

Structure of Dppz Ligand (2)	Proton	Chemical Shift (δ , ppm)
C_2 symmetry	2,9	9.29 (dd)
	3,8	7.82 (m)
	4,7	9.68 (dd)
	11,14	7.92 (m)
	12,13	8.35(m)

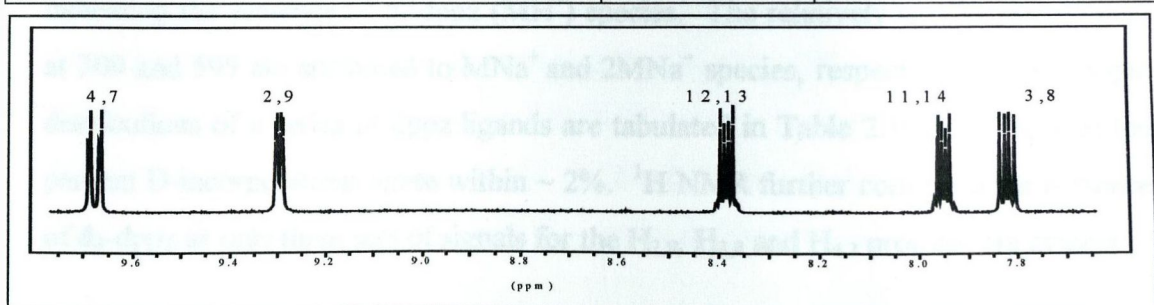


Figure 2.3: ¹H NMR spectrum (CDCl₃) of h₁₀-dppz and assignment of its proton signals.

2.2-2 D₄ - dppz (2a)

D₄-dppz was synthesised according to a two step reaction, as detailed in Figure 2.4. Firstly, dab 11 was deuterated in the presence of D₂O, DCl in a teflon coated steel high pressure reactor vessel for 24 hours at 190°C, to yield d₄-dab 11a. A positive ES⁺-MS displayed a dominant ion peak at $m/z = 190$, attributed to the formation of the dihydrochloride salt, C₆D₄N₂D₄.2DCIH⁺ species. This was subsequently added to phendione 12 and refluxed in ethanol for 30 minutes. The resulting yellow-brown solution was cooled to room temperature and filtered, yielding a brown solid.

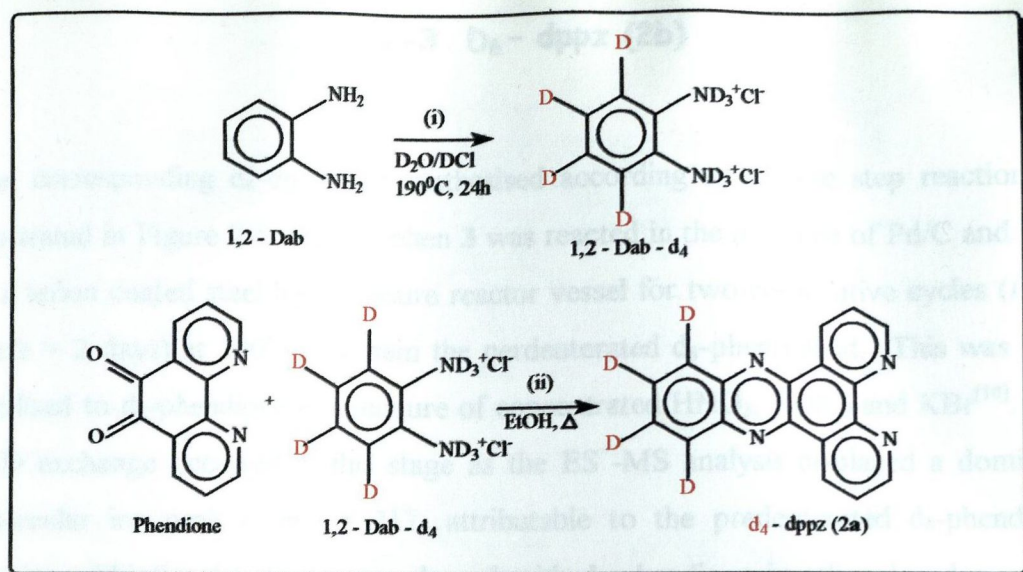


Figure 2.4 : Preparation of partially deuterated dppz : d₄- dppz, 2a .

A positive ES⁺ mass spectrum displayed a dominant molecular ion peak at $m/z = 287$ indicating the presence of d₄-dppz (MH⁺) species. The relatively weaker peaks found at 309 and 595 are attributed to MNa⁺ and 2MNa⁺ species, respectively. The isotopic distributions of a series of dppz ligands are tabulated in Table 2.1, indicating that the percent D-incorporations agree within ~ 2%. ¹H NMR further confirmed the presence of d₄-dppz as only three sets of signals for the H_{2,9}, H_{3,8} and H_{4,7} protons, are evident.

d ₄ -dppz	Mass Spectra Mr = 286 g/mol ^a					(%) H-D exchange ^b
	285	286	287 (H ⁺)	288 (D ⁺)	289 (D ⁺ , ¹³ C)	
Batch 1	1.5	22.7	50.5	23.2	2.0	98
Batch 2	3.1	27.6	51.0	17.3	1.0	96
Batch 3	3.7	27.8	46.3	18.5	3.7	96
Batch 4	4.7	23.1	46.3	22.2	3.7	95

Table 2.1 : ES⁺-Mass Spectra of partially deuterated dppz: d₄- dppz (2a). ^aRelative percent (%) abundance of peaks. ^b Total H-D exchange as measured by m/z peaks greater than 285 (i.e. d₄-dppz Mr = 286 g/mol)

2.2-3 D₆ - dppz (2b)

The corresponding d₆-dppz was synthesised according to a three step reaction, as illustrated in Figure 2.5. Firstly, phen **3** was reacted in the presence of Pd/C and D₂O in a teflon coated steel high pressure reactor vessel for two consecutive cycles (*i.e.* 1 cycle = 2 days) at 190°C to obtain the perdeuterated d₈-phen ligand. This was then oxidised to d₆-phendione in a mixture of concentrated HNO₃, H₂SO₄ and KBr^[10]. No H-D exchange occurred at this stage as the ES⁺-MS analysis displayed a dominant molecular ion peak at $m/z = 217$, attributable to the predeuterated d₆-phendione species. Thirdly, dab **11** was condensed with d₆-phendione in ethanol under reflux. The resulting dark brown solution was cooled to room temperature and filtered, yielding a brown solid.

The isotopic compositions of d₆-dppz were determined by ES⁺-MS and are tabulated in Table 2.2, and reveal that D-incorporation differs by only ~3%. The spectrum revealed a strong molecular ion peak at $m/z = 289$ (MH⁺), and is accompanied by peaks at 311 (MNa⁺) and 699 (2MNa⁺), respectively. ¹H NMR further confirms the presence of d₆-dppz, as only signals for the H_{11,14} and H_{12,13} protons, are evident.

d ₆ -dppz	Mass Spectra Mr = 288 g/mol ^a					(%)H-D exchange ^b
	287	288	289	290	291	
			(H ⁺)	(D ⁺)	(D ⁺ , ¹³ C)	
Batch 1	3.2	26.6	53.2	15.96	1.1	97
Batch 2	2.6	22.2	53.0	20.1	2.1	97
Batch 3	1.1	21.4	53.0	19.1	4.3	99
Batch 4	3.8	24.5	54.3	15.2	2.2	96

Table 2.2 : ES⁺-Mass Spectra of partially deuterated dppz: d₆-dppz (2b). ^aRelative percent (%) abundance of peaks. ^b Total H-D exchange as measured by m/z peaks greater than 287 (*i.e.* d₆-dppz Mr = 288 g/mol)

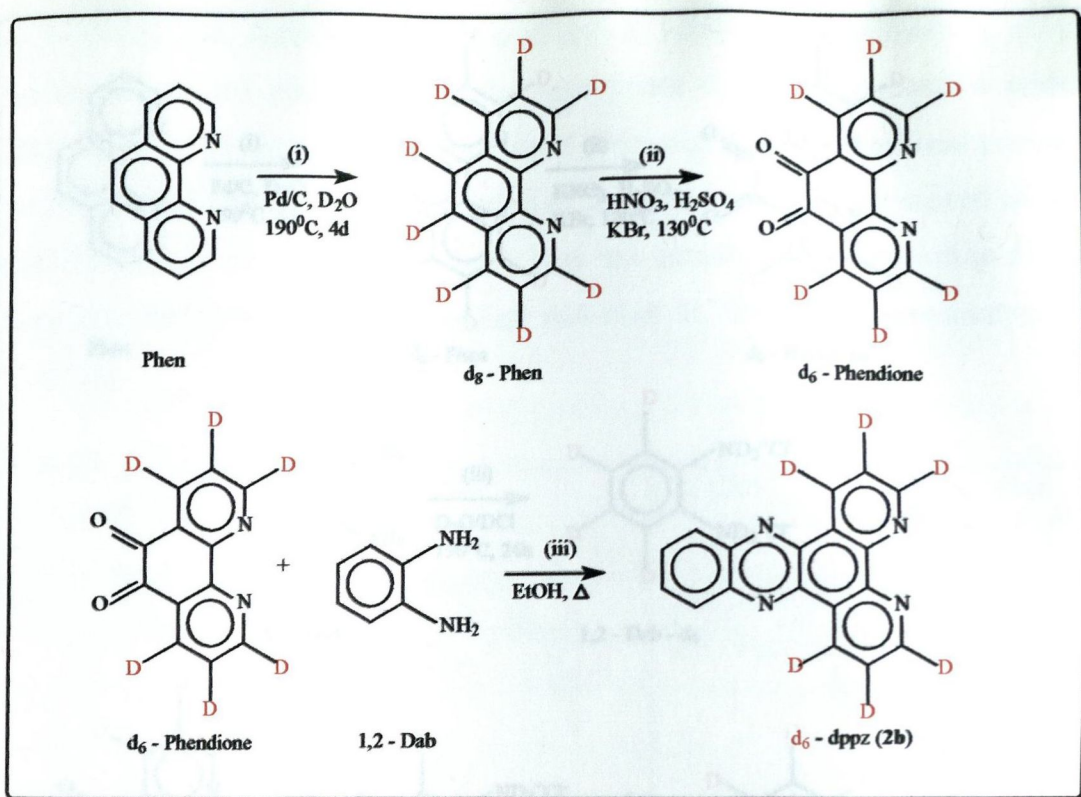


Figure 2.5: Preparation of partially deuterated dppz: d_6 -dppz (2b).

2.2-4 D_{10} - dppz (2c)

The final member of the dppz series, d_{10} -dppz, was prepared using a combination of the synthetic conditions described previously, see Figure 2.6. The ES^+ -MS isotopic patterns show the formation of d_{10} -dppz at $m/z = 293$ (MH^+), with >95% perdeuteration, as shown in Table 2.3. Similarly, additional peaks were observed at 311 (MNa^+) and 606 (2MNa^+). The ^1H NMR spectrum was silent, indicating that the ligand had undergone extensive H-D exchange and confirms the presence of fully perdeuterated d_{10} -dppz.

Table 2.3: ES^+ -Mass Spectra of perdeuterated dppz: d_6 -dppz (2b). * Relative percent (%) abundance of peaks. ** Total H-D exchange as measured by m/z peaks greater than 291 (i.e. d_{10} -dppz $\text{M}^+ = 292$ g/mol).

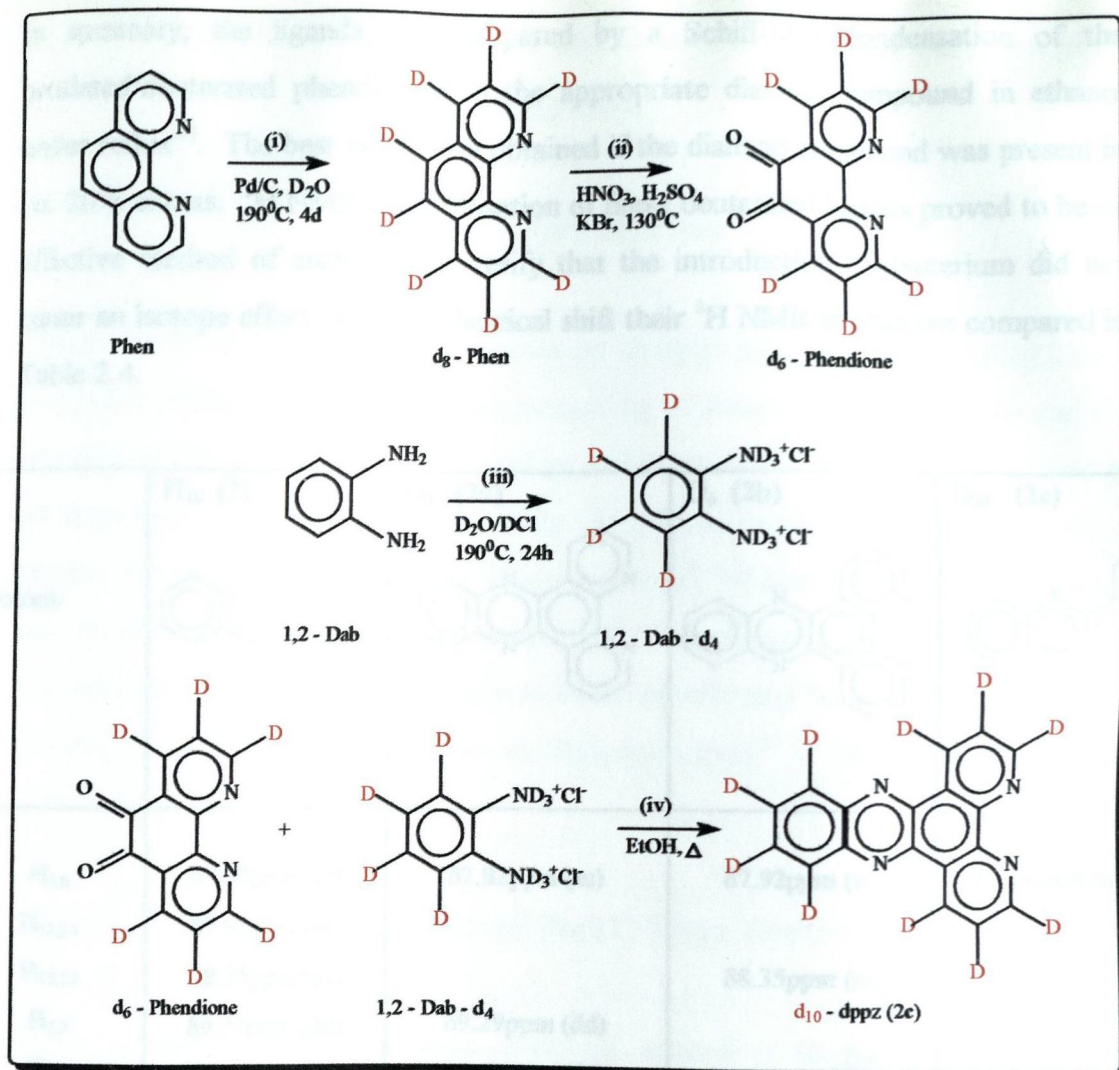


Figure 2.6: Preparation of perdeuterated dppz ligands: $\text{d}_{10}\text{-dppz (2c)}$.

$\text{d}_{10}\text{-dppz}$	Mass Spectra $\text{Mr} = 292 \text{ g/mol}^a$					(% H-D exchange) ^b
	291	292	293 (H^+)	294 (D^+)	295 (D^+ , ^{13}C)	
Batch 1	3.3	30.8	36.6	18.3	4.7	97
Batch 2	6.1	34.6	46.3	11.1	1.9	94
Batch 3	5.7	26.0	47.1	16.6	4.6	94
Batch 4	3.6	30.8	42.4	17.4	5.7	96

Table 2.3: ES^+ -Mass Spectra of perdeuterated dppz: $\text{d}_{10}\text{-dppz (2c)}$. ^a Relative percent (%) abundance of peaks. ^b Total H-D exchange as measured by m/z peaks greater than 291 (*i.e.* $\text{d}_{10}\text{-dppz}$ $\text{Mr} = 292 \text{ g/mol}$)

In summary, the ligands were prepared by a Schiff-base condensation of the protiated/deuterated phendione with the appropriate diamino compound in ethanol under reflux^[9]. The best yields were obtained if the diamino compound was present in *ca.* 20% excess. ¹H NMR characterisation of these deuterated ligands proved to be an effective method of analysis. To verify that the introduction of deuterium did not cause an isotope effect of the ¹H chemical shift their ¹H NMR spectra are compared in Table 2.4.

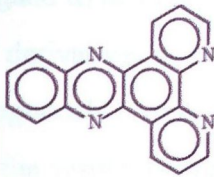
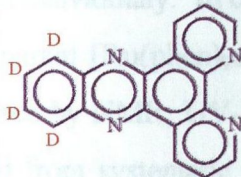
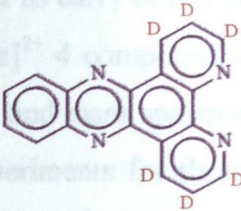
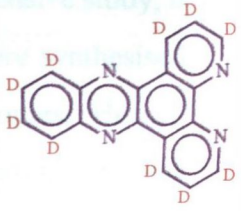
Proton ^v	H ₁₀ (2)	D ₄ (2a)	D ₆ (2b)	D ₁₀ (2c)
				
H _{3,8}	δ7.82ppm (m)	δ7.82ppm (m)	δ7.92ppm (m)	No proton signals
H _{11,14}	δ7.92ppm (m)			
H _{12,13}	δ8.35ppm (m)		δ8.35ppm (m)	
H _{2,9}	δ9.29ppm (dd)	δ9.29ppm (dd)		
H _{4,7}	δ9.68ppm (dd)	δ9.68ppm (dd)		
Mr	Mr = 282g/mol	Mr = 286g/mol	Mr = 288g/mol	Mr = 292g/mol
ES ⁺ -MS ^a	m/z = 283	m/z = 287	m/z = 289	m/z = 293
Total H-D exchange ^a (%)	0.0	96.7	97.3	96.4

Table 2.4: ¹H NMR (400MHz, CDCl₃) data (chemical shifts (δ, ppm)) for the d_x-dppz ligands (where x = 0, 4, 6 and 10). ^a Average mass spectrum (m/z) value of four batches.

2.3 Preparation of Protiated and Deuterated Ru(II) Polypyridyl Complexes.

2.3-1 Introduction

These selectively deuterated dppz ligands on complexation to $[\text{Ru}(\text{phen})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$, were then utilized to gain a further understanding of these systems in solution and the presence of DNA, by allowing the phen/bpy and phenazine/quinoxaline components of the dppz ligand to be examined individually. In order to carry out an extensive study, a number of derivatives of the parent $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** compound were synthesised and unambiguously characterised by NMR, UV-Vis and mass spectroscopies. Herein, we report the results obtained from systematic experiments for these complexes and investigate the effect of deuteration on $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** series of complexes in further detail.

2.3-2 Synthesis of Ru(II) Dppz Complexes

These ruthenium complexes were prepared according to the literature^[11] and characterised by NMR, UV-vis and mass spectroscopies. The general synthetic route involved the preparation and isolation of the free dppz ligand^[10]. The ligand itself was prepared, by condensation of phendione **12** with the appropriate σ -phenylenediamine:

- (a) 1,2-diaminobenzene (dab) – to produce complex **4**
- (b) 4,5-dimethyl-1,2-diaminobenzene – to produce complex **8**
- (c) 2-amino-4,5-difluoroaniline – this was synthesized by reduction of 2-nitro-4,5-difluoroaniline – to produce complex **9**,

as shown in Figure 2.7. Typically, yields of 75-85% were obtained for these ligands.

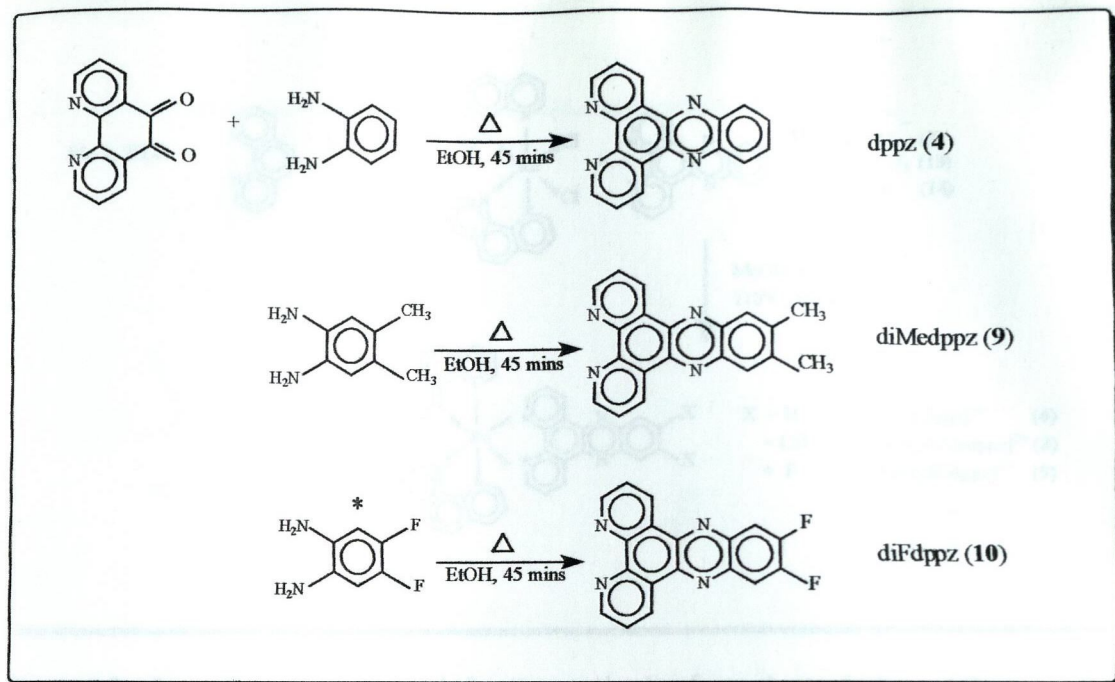


Figure 2.7: Shown (from left to right) are the orthodiamines used in constructing dppz derivatives and the ligand abbreviations. * Not commercially available, see experimental section Chapter Six.

The appropriate dppz ligand was subsequently reacted with $\text{Ru(phen)}_2\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ in refluxing aqueous methanol for 10 hours. Although, the latter complex is extremely stable with respect to the two phen ligands it may be successively and variously substituted in the two remaining positions. The solution was filtered and the complex was precipitated as its chloride or hexafluorophosphate salt upon addition of either a saturated solution of LiCl or NH_4PF_6 . The complex was filtered and further dried under vacuum, and did not require further purification. The synthetic approach for the preparation of these mixed-ligand Ru(II) complexes is shown in Figure 2.8. By employing this method, a number of substituted $[\text{Ru(phen)}_2\text{dppz}]^{2+}$ complexes have been prepared, namely $[\text{Ru(phen)}_2\text{diMedppz}]^{2+}$ 8 and $[\text{Ru(phen)}_2\text{diFdppz}]^{2+}$ 9 complexes.

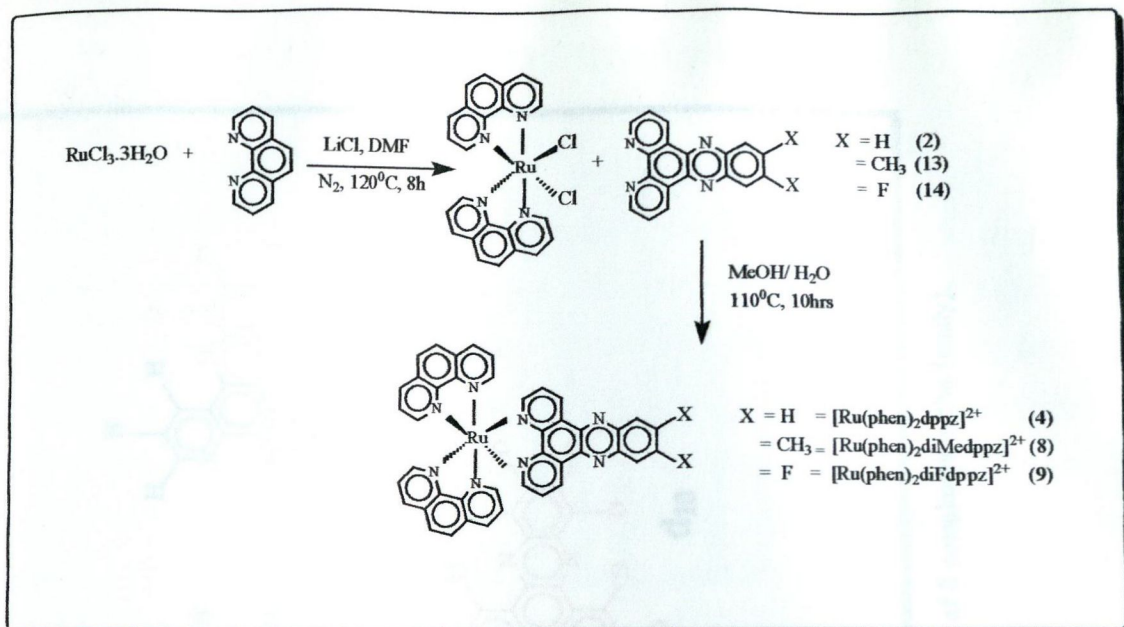


Figure 2.8: General synthetic approach for the synthesis of complexes **4**, **8**, and **9** used in this study^[11].

For complex **4**, the corresponding deuterated complexes of the general formula; $[\text{Ru}(\text{d}_x\text{-phen})_2\text{d}_y\text{-dppz}]^{2+}$ (where $x = 0$ or 8 ; $y = 0, 4, 6$ and 10); have been prepared in good yield in an essentially similar manner. Reaction of $[\text{Ru(phen)}_2\text{Cl}_2]$ or its deuterated $[\text{Ru}(\text{d}_8\text{-phen})_2\text{Cl}_2]$ analog with the protiated or selectively deuterated dppz ligands (*i.e.* $\text{d}_y\text{-dppz}$, where $y = 0, 4, 6$ and 10) allowed for the preparation of a wide range of deuterated complexes of **4**, see Figure 2.9. In the current study, a series of six families have been prepared and are shown in Table 2.5 whereby the $[\text{Ru(phen)}_3]^{2+}$ **6** and $[\text{Ru(bpy)}_3]^{2+}$ **1** families serve as model systems. All of these complexes are readily soluble in organic solvents and appear stable in solution after prolonged periods. These complexes allow the effect of D-incorporation into both the ancillary phen ligands and more importantly the effect of deuteration on the dppz ligand to be investigated. Furthermore, the $[\text{Ru(phen)}_2(\text{diXdpz})]^{2+}$ complexes, (**4**, **8** and **9**), have allowed for a more comprehensive study of the nature of the dppz ligand.

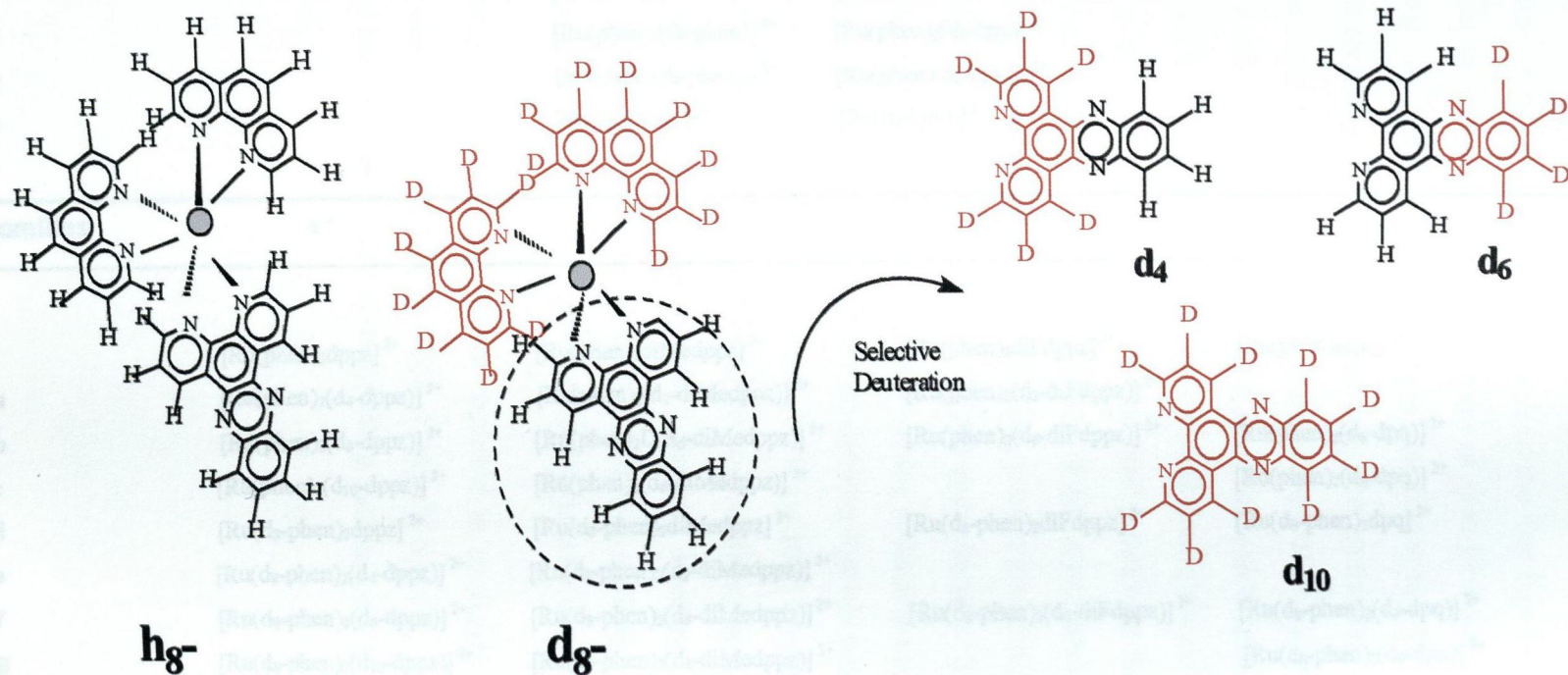


Figure 2.9: Synthetic preparation of $[Ru(phen)_2dppz]^{2+}$ series of deuterated complexes (4-4g) (Total of 8 complexes in the family).

Table 2.5: Families of Deuterated Ru(II) Complexes used in this study.

Model Families		6 ^a	1 ^a
		$[\text{Ru}(\text{phen})_3]^{2+}$	$[\text{Ru}(\text{bpy})_3]^{2+}$
a		$[\text{Ru}(\text{phen})_2(\text{d}_8\text{-phen})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_8\text{-bpy})]^{2+}$
b		$[\text{Ru}(\text{phen})(\text{d}_8\text{-phen})_2]^{2+}$	$[\text{Ru}(\text{phen})(\text{d}_8\text{-bpy})_2]^{2+}$
c		$[\text{Ru}(\text{d}_8\text{-phen})_3]^{2+}$	$[\text{Ru}(\text{d}_8\text{-bpy})_3]^{2+}$

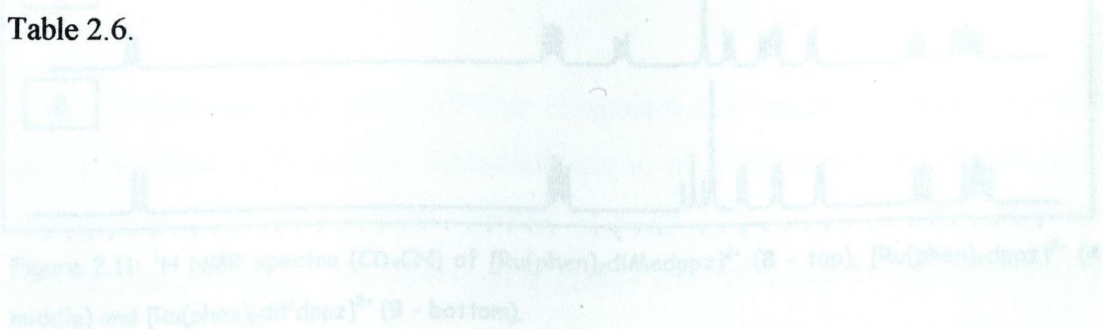
Dppz Families		4 ^a	8 ^a	9 ^b	6 ^c
		$[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$	$[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$	$[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$	$[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$
a		$[\text{Ru}(\text{phen})_2(\text{d}_4\text{-dppz})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_2\text{-diMedppz})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_2\text{-diFdppz})]^{2+}$	
b		$[\text{Ru}(\text{phen})_2(\text{d}_6\text{-dppz})]^{2+}$	$[\text{Ru}(\text{phen})_2\text{D}(\text{d}_6\text{-diMedppz})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_6\text{-diFdppz})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_6\text{-dpq})]^{2+}$
c		$[\text{Ru}(\text{phen})_2(\text{d}_{10}\text{-dppz})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{d}_6\text{-diMedppz})]^{2+}$		$[\text{Ru}(\text{phen})_2(\text{d}_8\text{-dpq})]^{2+}$
d		$[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2\text{diMedppz}]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2\text{diFdppz}]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2\text{dpq}]^{2+}$
e		$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_4\text{-dppz})]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_2\text{-diMedppz})]^{2+}$		
f		$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_6\text{-dppz})]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_6\text{-diMedppz})]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_6\text{-diFdppz})]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_6\text{-dpq})]^{2+}$
g		$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_{10}\text{-dppz})]^{2+}$	$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_8\text{-diMedppz})]^{2+}$		$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_8\text{-dpq})]^{2+}$

Table 2.5: List of homoleptic $[\text{Ru}(\text{L})_3]^{2+}$ (where L = phen and bpy) and heteroleptic $[\text{Ru}(\text{L})_2\text{L}']^{2+}$ (where L = phen and L' = dppz, diMedppz, diFdppz, dpq) derivatives prepared in this study and the numbering scheme for these complexes. ^a Cl⁻ salts, ^b PF₆⁻ salts ^c This family is not considered in any detail in this study.

2.3-3 ^1H NMR Spectroscopy

Upon complexation of dppz to the Ru(II) centre, the ^1H NMR of the $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4** is relatively simple, as expected, displaying nine sharp resonances in the aromatic region. Each of the free ligands (*i.e.* phen and dppz) have a C_2 axis, so the mixed ligand complexes of type $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ also display a C_2 symmetry. As a consequence, two phen ligands and two halves of dppz are chemically and magnetically equivalent. Therefore, the resulting signals in the spectrum corresponding to one full phen ligand and half of the dppz ligand, as illustrated in Figure 2.10. The octahedral structure of **4** leads to anisotropic (intraligand) effects. This is attributed to the ring current of one aromatic ligand exerting a shielding effect on the protons of another ligand, and is most prominent for the protons close to the complexing site. The $\text{H}_{2,9}$ phen ligands in **4** are asymmetric (*i.e.* non-equivalent) and show interesting NMR behaviour. The H_2 proton *cis* to the dppz ligand lies over the dppz ring and is shifted downfield by 0.2 ppm relative (8.24 ppm) to the *trans* H_9 proton which lies over a phen ring (8.04 ppm), reflecting the relative deshielding effect induced by the electron-ring current of the two ligands when a H atom is orientated directly over the aromatic ring system. H_9 is assigned on the basis of its chemical shift (8.04 ppm) compared to the related protons in $[\text{Ru}(\text{phen})_3]^{2+}$ **6** which are found at 8.05 ppm. The remaining phen protons are not resolved at fields up to 400 MHz. Since the deshielding effect of the dppz ligand is markedly greater than that of phen, the chemical shifts of the latter protons are downfield from those of the former.

Representative ^1H NMR data were recorded in deuterated acetonitrile for all three Ru(II) complexes; diMedppz **8**, dppz **4** and diFdppz **9**, respectively. The chemical shifts of the free ligands and corresponding ruthenium(II) complexes are tabulated in Table 2.6.



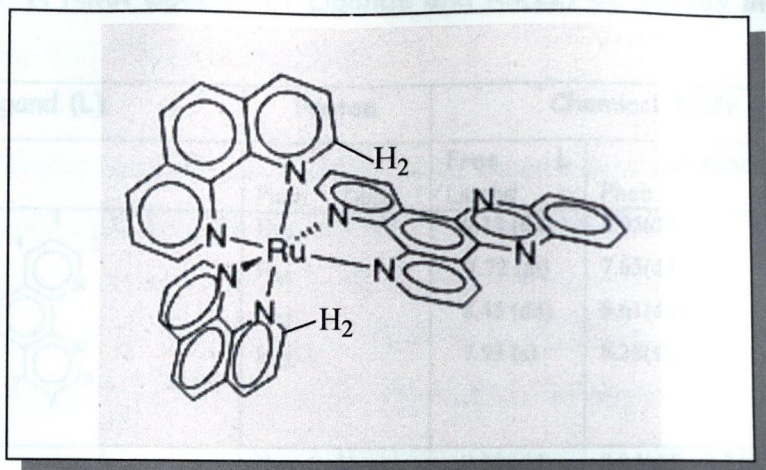


Figure 2.10: Structure of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**4**), view showing the position of the H_2 atoms above the dppz ligand.

Complexation of the ligands to the metal centre and the fact that Ru(II) has low energy orbitals with small back-donation from the Ru(II) center reduces the residual electron density on the ligands resulting in a deshielding effect on the resonance field. On comparison of the ^1H NMR spectra of the complexes, small shifts were observed in the positions of the $\text{H}_{2,9}$ and $\text{H}_{4,7}$ dppz protons with substitution at the 12 and 13 positions. The expected changes at ~ 8.13 ppm are seen, and the signal from the protons in positions 11 and 14 change from a single methyl proton resonance in complex **8**, to a doublet in complex **4**, to an apparent 'triplet' (is actually a doublet doublet) proton resonance in complex **9**, as illustrated in Figure 2.11.

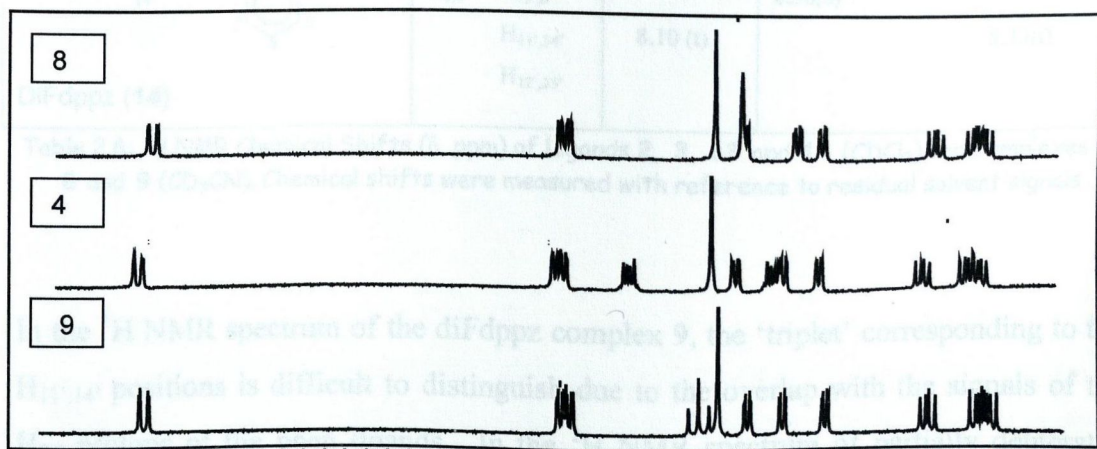


Figure 2.11: ^1H NMR spectra (CD_3CN) of $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ (**8** - top), $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**4** - middle) and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ (**9** - bottom).

Table 2.6: ^1H NMR Spectra of Ligands and Ru(II) Complexes in this study.

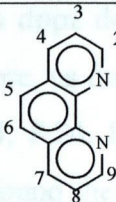
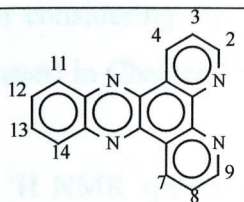
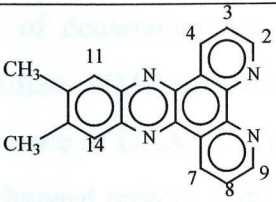
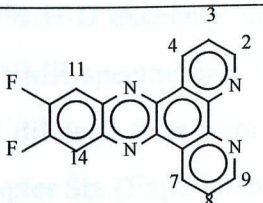
Ligand (L)	Proton		Chemical Shift (δ , ppm)		
	Phen	Dppz	Free L	[Ru(phen) $_2$ L] $^{2+}$	
 Phen(3)	H $_{2,9}$ H $_{3,8}$ H $_{4,7}$ H $_{5,6}$		9.13 (dd) 7.72 (m) 8.45 (dd) 7.93 (s)	8.05(dd) 7.65(dd) 8.61(dd) 8.28(s)	
 Dppz (2)	H $_{2,9}$ H $_{3,8}$ H $_{4,7}$ H $_{5,6}$	H $_{2',9'}$ H $_{3',8'}$ H $_{4',7'}$ H $_{5',6'}$ H $_{11',14'}$ H $_{12',13'}$	9.29 (dd) 7.82 (m) 9.69 (dd) 7.96 (m) 8.39 (m)	8.04(dd) 8.24(dd) 7.67(m) 8.64(m) 8.30(s)	8.13(dd) 7.80(m) 9.67(m) 8.17(m) 8.50(m)
 DiMedppz (13)	H $_{2,9}$ H $_{3,8}$ H $_{4,7}$ H $_{5,6}$	H $_{2',9'}$ H $_{3',8'}$ H $_{4',7'}$ H $_{5',6'}$ H $_{11',14'}$ H $_{12',13'}$ CH $_3$	9.28 (d) 7.81 (m) 9.66 (d) 8.13 (s) 2.64 (s)	8.04(dd) 8.24(dd) 7.67(m) 8.64(m) 8.30(s)	8.10(dd) 7.78(m) 9.63(m) 8.24(s) 2.69(s)
 DiFdppz (14)	H $_{2,9}$ H $_{3,8}$ H $_{4,7}$ H $_{5,6}$	H $_{2',9'}$ H $_{3',8'}$ H $_{4',7'}$ H $_{5',6'}$ H $_{11',14'}$ H $_{12',13'}$	9.30 (dd) 7.81 (dd) 9.58 (dd) 8.10 (t)	8.04(dd) 8.22(dd) 7.67(m) 8.64(m) 8.30(s)	8.14(dd) 7.80(m) 9.62(m) 8.33(t)

Table 2.6: ^1H NMR Chemical Shifts (δ , ppm) of Ligands **2**, **3**, **13** and **14** (CDCl_3) and Complexes **4**, **8** and **9** (CD_3CN). Chemical shifts were measured with reference to residual solvent signals

In the ^1H NMR spectrum of the diFdppz complex **9**, the ‘triplet’ corresponding to the H $_{11',14'}$ positions is difficult to distinguish due to the overlap with the signals of the H $_{2,9}$ protons of the phen ligands. In the ^1H NMR spectrum of partially deuterated $[\text{Ru}(\text{d}_8\text{-phen})_2\text{diFdppz}]^{2+}$, the spectrum is greatly simplified due to the removal of the

phen protons and the multiplicity of this signal is much clearer. For complex **9**, a ^{19}F NMR spectrum in deuterated acetonitrile was obtained which showed a single peak at approximately -127.33ppm as expected.

The lack of noticeable shifts implies, that, in the ground state, substitution at the distal protons in dppz does not significantly alter the electron density in the dppz ligand. Furthermore, as there are no shifts in the signals corresponding to the phen protons – 7.67, 8.04, 8.24, 8.30, and 8.64 ppm, there is no dramatic change in the electron density around the Ru(II) metal center. The location of electron density is important when considering the rate of protium-deuterium exchange for these complexes (as discussed in Chapter Three).

The ^1H NMR spectra of the protiated parent $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4** and its deuterated analogues (**4a-4g**) were examined and are illustrated in Figure 2.12. The use of deuteration has been reported as a simple and straightforward solution to facilitate NMR structural assignments of complexes both in the absence^[12] and presence of DNA^[11]. Comparison of the spectra and the removal of the signals of the exchanged protons from the spectrum allow for unambiguous assignments to be made. This compares well with mass spectral analysis in aqueous solution which indicated >97% H-D exchange for these complexes, as shown in Table 2.7. The ES^+ -MS and ^1H NMR spectral data for the analogous series of complexes, containing diMedppz **8** and diFdppz **9** as terminal ligands are given in Tables 6.8 and 6.9, respectively in Chapter Six (Experimental).

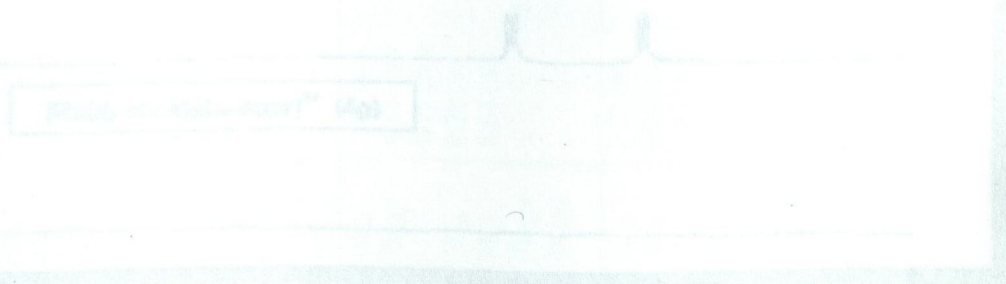


Figure 2.12. ^1H NMR spectra (CD_3CN) of the series of deuterated $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**4-4g**) complexes, for assignments see Table 2.7.

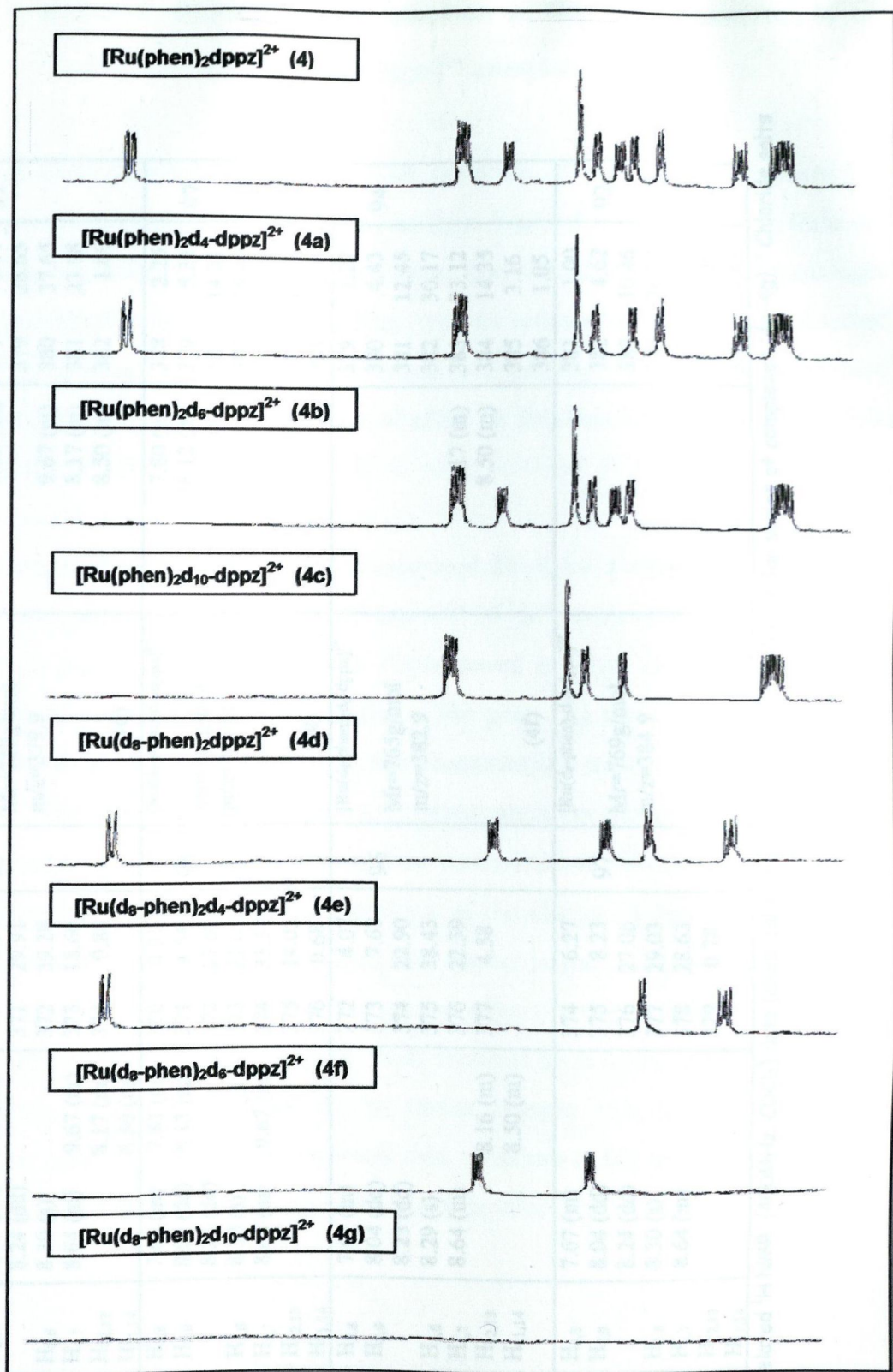


Figure 2.12: ¹H NMR spectra (CD₃CN) of the series of deuterated [Ru(phen)₂dppz]²⁺ (4-4g) complexes, for assignments see Table 2.7.

Complex ^a	Proton	Chemical Shift (δ , ppm)		Mass	Spectra	% D	Complex	Chemical Shift (δ , ppm)		Mass	Spectra	%D
		Phen	Dppz	(m/z)	(%)			Phen	Dppz	(m/z)	(%)	
[Ru(phen) ₂ dppz] ²⁺ Mr=743g/mol m/z=371.9 (4)	H _{3,8}	7.67 (m)	7.80 (m)	369	5.52	95	[Ru(d ₈ -phen) ₂ dppz] ²⁺ Mr=759g/mol m/z=379.9 (4d)		7.80 (m)	377	3.92	97
	H _{2,9}	8.04 (dd)	8.13 (dd)	370	5.81				8.13 (dd)	378	4.11	
		8.24 (dd)		371	29.91					379	28.65	
	H _{5,6}	8.30 (s)		372	39.28				9.67 (m)	380	37.65	
	H _{4,7}	8.64 (m)	9.67 (m)	373	18.60				8.17 (m)	381	23.98	
	H _{12,13}		8.17 (m)	374	0.88				8.50 (m)	382	1.69	
	H _{11,14}		8.50 (m)									
[Ru(phen) ₂ d ₄ -dppz] ²⁺ Mr=747g/mol m/z=373.9 (4a)	H _{3,8}	7.66 (m)	7.81 (m)	370	0.91	98	[Ru(d ₈ -phen) ₂ d ₄ -dppz] ²⁺ Mr=763g/mol m/z=381.91 (4e)		7.80 (m)	378	2.25	97
	H _{2,9}	8.04 (dd)	8.13 (dd)	371	4.54				8.12 (d)	379	5.31	
		8.24 (dd)		372	13.60					380	14.31	
	H _{5,6}	8.30 (s)		373	32.65					381	35.40	
	H _{4,7}	8.65 (m)	9.67 (m)	374	33.57					382	28.01	
	H _{12,13}			375	14.05					383	13.29	
	H _{11,14}			376	0.68					384	1.43	
[Ru(phen) ₂ d ₆ -dppz] ²⁺ Mr=749g/mol m/z=374.9 (4b)	H _{3,8}	7.67 (m)		372	4.07	96	[Ru(d ₈ -phen) ₂ d ₆ -dppz] ²⁺ Mr=765g/mol m/z=382.9 (4f)			379	1.27	94
	H _{2,9}	8.04 (dd)		373	7.63					380	4.43	
		8.23 (dd)		374	22.90					381	12.45	
	H _{5,6}	8.29 (s)		375	38.43					382	30.17	
	H _{4,7}	8.64 (m)		376	22.39			8.17 (m)		383	33.12	
	H _{12,13}		8.16 (m)	377	4.58			8.50 (m)		384	14.35	
	H _{11,14}		8.50 (m)							385	3.16	
[Ru(phen) ₂ d ₁₀ -dppz] ²⁺ Mr=753g/mol m/z=376.9 (4c)	H _{3,8}	7.67 (m)		374	6.27	97	[Ru(d ₈ -phen) ₂ d ₁₀ -dppz] ²⁺ Mr=769g/mol m/z=384.9 (4g)			381	1.00	92
	H _{2,9}	8.04 (dd)		375	8.23					382	4.62	
		8.24 (dd)		376	27.06					383	10.46	
	H _{5,6}	8.30 (s)		377	29.03					384	26.28	
	H _{4,7}	8.64 (m)		378	28.63					385	36.98	
	H _{12,13}			379	0.78					386	18.25	
	H _{11,14}									387	2.47	

Table 2.7: Correlated ¹H NMR (400MHz, CDCl₃) data (chemical shifts (δ , ppm)) and ES⁺-MS data for series of complexes (4a-4g) ^a Chloride salts

2.3.3-1 Effect of Concentration on the NMR Spectrum of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex (4).

It has been reported that the ^1H NMR spectrum of polypyridyl complexes is very sensitive to concentration^[13]. For the free dppz ligand **2**, in deuteriochloroform, all the protons are shifted downfield with increasing concentration, using the solvent signal as an internal reference. The largest effect, with an increase of ~ 0.2 ppm, is observed for the $\text{H}_{2,9}$ protons while the smallest effect is noted for the 4 and 7 positions. On increasing the concentration the order of effect on the dppz ligand protons is as follows; $2,9 > 11, 14 > 3, 8 > 12, 13 > 4, 7$.

The effect of concentration was also examined for **4**, by comparison of two spectra at different concentrations, $2.0 \times 10^{-3} \text{ M}^{-1}$ and $6.0 \times 10^{-3} \text{ M}^{-1}$, respectively. The most affected protons are the dppz protons (with displacement as large as 0.2 ppm) which move downfield with increasing concentration. The phen $\text{H}_{2,9}$ move upfield under the same conditions, as they are located above the phenanthroline part of the dppz ligand (Figure 2.10). The other phen protons, are not concentration dependent as their chemical shifts are unaltered. We have attributed this concentration effect to an aggregation of mononuclear species through π - π stacking of the elongated dppz portion in solution. Aggregation, which must be very rapid with respect to the NMR time scale, modifies the local electron density and/or the ring current effects in the vicinity of the dppz ligand. Consistent with this hypothesis, it has been reported by Bolger *et al*^[13] that the ^1H NMR spectrum of Ru(II) complexes display similar changes as a function of temperature. Further interpretation of these chemical shift variations is not possible without accurate knowledge of the aggregation geometry and dynamics.

2.3-4 Absorption Spectroscopy.

UV-Vis absorption spectroscopy is an extremely useful technique which gives valuable information regarding the nature of the excited state initially formed when the complex is excited at a particular wavelength. Much has been published in relation to the absorption spectral properties of ruthenium polypyridyl complexes^[14], and the dependence of charge-transfer on solvent polarity is a well known phenomenon^[15]. To assess the effect, if any, of the solvent medium, counter-ion induced changes, and substituents, namely F and CH₃, in the 12 and 13 positions of the dppz ligand, the UV-Visible electronic spectra of the free ligands and their complexes were recorded in a variety of solvents. The extinction coefficients (ϵ_{max}) were obtained from Beer's law and determined for at least two dilution factors at the wavelength corresponding to the maximum absorption (λ_{max}) for each peak in the spectrum. Where data was available from the literature, the values were in good agreement.

2.3.4-1 Absorption Spectra for the Model Tris-chelated $[\text{Ru}(\text{L})_3]^{2+}$ Complexes.

The UV-Visible electronic spectra of the homoleptic complexes, **1** and **6**, were recorded in a variety of solvents to examine the effect of the solvent media, and the results are tabulated in Table 2.8. For these complexes, two maxima are seen. These intense absorption bands occur at 340 nm and 450 nm for **1** and at 420 nm and 447 nm for **6**, respectively, and have been assigned to ³MLCT transitions.

Table 2.8 : UV-Visible spectra of $[\text{Ru}(\text{L})_3]^{2+}$ complexes^a

Complex	Solvent	Absorption peaks (ϵ , $10^{-3} \text{ M}^{-1} \text{ cm}^{-1}$) nm ^b
1 $[\text{Ru}(\text{bpy})_3]^{2+}$	CH_3CN	244 (28.2), 287 (86.8), 323 (65.3), 345 (65.0) 451 (14.2)
	H_2O^c	238 (28.9), 250 (25.2), 285 (86.3), 323 (63.4), 453 (14.6)
	EtOH	207 (46.8), 244 (29.9), 286 (86.7), 450 (14.2)
	MeOH	209 (42.3), 244 (28.9), 287 (83.5), 452 (14.6)
6 $[\text{Ru}(\text{phen})_3]^{2+}$	CH_3CN	223 (98.4), 260 (118), 420 (19.2), 445 (20.1)
	H_2O^c	223 (101), 262 (133), 420 (19.6), 447 (20.3)
	EtOH	223 (89.7), 262 (121), 421 (19.4), 445 (20.2)
	MeOH	223 (89.0), 263 (120), 422 (18.6), 446 (20.1)

Table 2.8: ^a Measurements were performed using PF_6^- salts (unless otherwise stated), $T = 23^\circ \pm 1^\circ\text{C}$

^b Errors: $\lambda_{\text{max}} \pm 2 \text{ nm}$, $\epsilon \pm 10\%$, $0.05\text{--}0.1 \times 10^{-4} \text{ M}^{-1} \text{ cm}^{-1}$. ^c Cl^- salts

For each complex, the similarities in the position and extinction coefficient (λ_{max}) values observed for the MLCT, LC, and IL bands in differing environments suggests that these complexes are not solvent dependent. It was noted, that for all solvents, the phen complex **6** has an extinction coefficient value *ca.* 25% higher than that of the corresponding bpy compound **1**.

There is a more striking result on comparing the complexes, in aqueous solution. Figure 2.13 displays large differences in the peak shape of the bpy and phen species. On increasing the aromaticity of the complex, going from complex 1 (blue line) to 6 (red line), the highest energy MLCT peak at 451 nm undergoes a small red shift to 447 nm (~ 4 nm) while the less intense band found at 345 nm is dramatically blue-shifted (~ 75 nm) to 420 nm. These changes may be attributed to the greater rigidity and reduced rotation about the central metal ion for 6. These small but obvious differences may be important for the elucidation of their photochemical processes.

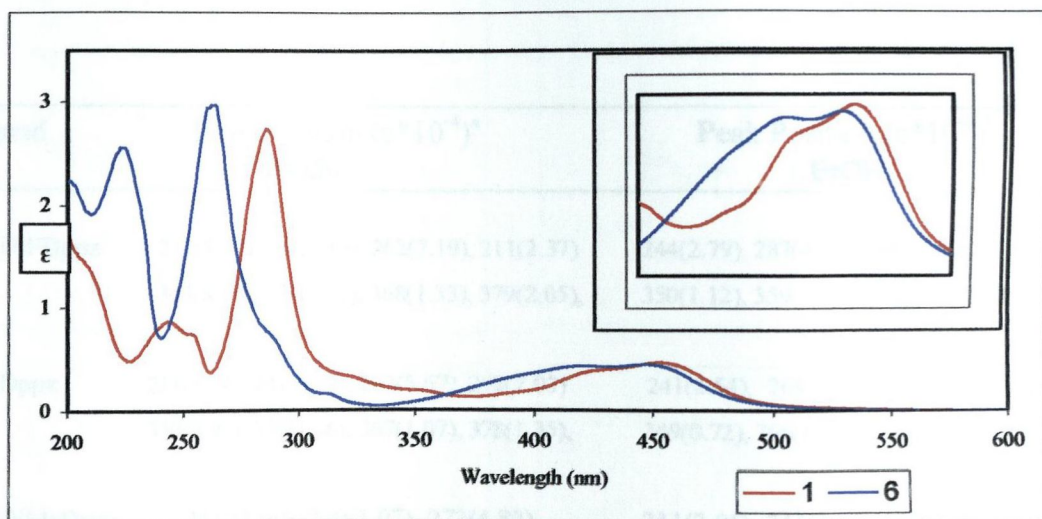


Figure 2.13: Spectra of $[\text{Ru}(\text{bpy})_3]^{2+}$ (1) and $[\text{Ru}(\text{phen})_3]^{2+}$ (6) complexes in aqueous solution. See inset for MLCT region 350 - 550 nm.

The electronic absorption spectra were also recorded for the selectively deuterated analogues of the parent complexes, 1 and 6. For these complexes, the position and intensities of the absorption energies were unchanged for the protiated, partially deuterated and perdeuterated complexes, the spectra being superimposable on each other.

2.3.4-2 Effect of Substituents on Absorption Spectra of Free Ligands and Complexes.

The UV-Vis absorption spectra for the free ligands; dppz **2**, diMedppz **13**, and diFdppz **14**, were measured in acetonitrile and ethanol at room temperature, and are presented in Figure 2.14. The absorption data listing energy maxima and absorption coefficients are summarized in Table 2.9.

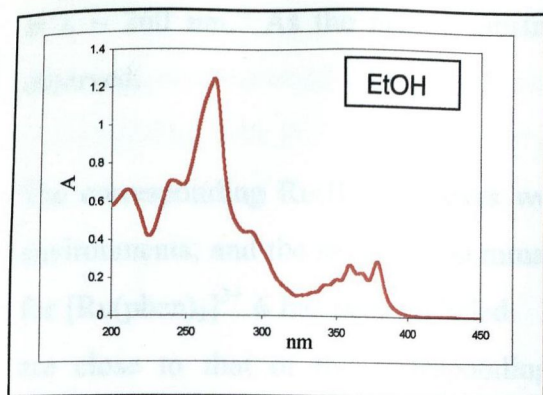
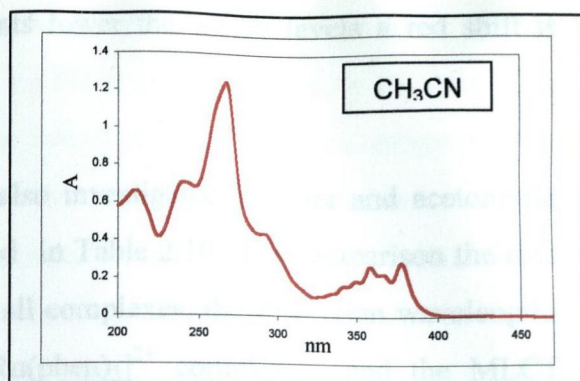
Ligand	Peak Position ($\epsilon \cdot 10^{-4}$) ^a CH₃CN	Peak Position ($\epsilon \cdot 10^{-4}$) ^b EtOH
14 DiFDppz	211(2.37), 262(7.19), 262(7.19), 211(2.37) 343(8.77), 359(1.62), 368(1.33), 379(2.05),	244(2.79), 287(4.97), 294(2.15), 342(1.01) 350(1.12), 359(1.85), 367(1.55), 378(2.03)
2 Dppz	211(3.24), 241(3.28), 263(5.67), 292(2.03) 350(0.90), 359(1.16), 367(1.07), 378(1.35),	241(2.54), 268(4.67), 294(1.64), 342(0.62) 349(0.72), 366(1.01), 358(0.88), 377(1.07)
13 DiMeDppz	211 (2.09), 240(1.97), 272(4.82), 366(1.16), 375(0.97), 386(1.50)	211(2.05), 241(1.80), 274(4.73), 365(1.10), 374(0.96), 385(1.47),

Table 2.9: UV-Visible Absorption Spectral Data for Free ligands measured in ^aCH₃CN and ^bEtOH at T = 23°C ± 1°C. Errors: $\lambda_{\max} \pm 2\text{nm}$; $\epsilon \pm 10\%$, $0.05\text{--}0.1 \times 10^{-4} \text{M}^{-1}\text{cm}^{-1}$.

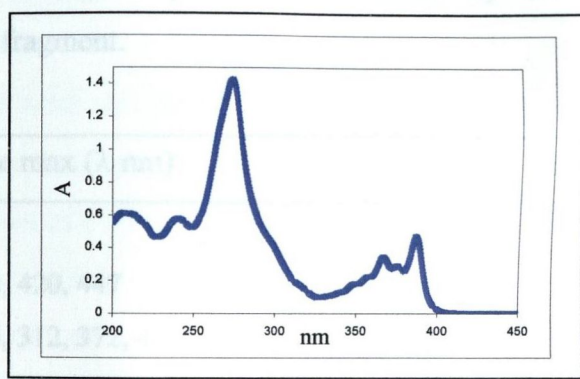
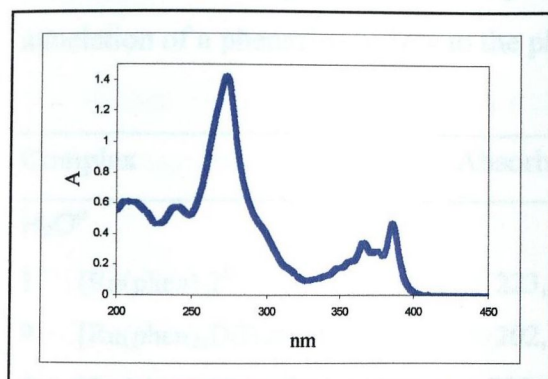
Figure 2.14: Comparison of the UV-Vis absorption spectra of the free dppz ligands **2**, **13** and **14** in EtOH and CH₃CN (absorption of complex = 0.2 at lowest energy UV maximum, 0.01% molarities of highest energy UV max. = 0.01 mol). All data were recorded at 298K using 1cm path length quartz cells.

As can be seen in Figure 2.14, the substituents on dppz, namely **1**, **11**, and **13** at the 12 and 13 positions have an effect on the absorption spectra. The electron-withdrawing fluorine substituents cause the spectrum to undergo a blue shift as they increase the energy levels of the ligand, the effect being more noticeable for the higher energy peaks.

2

CH₃CN

13



14

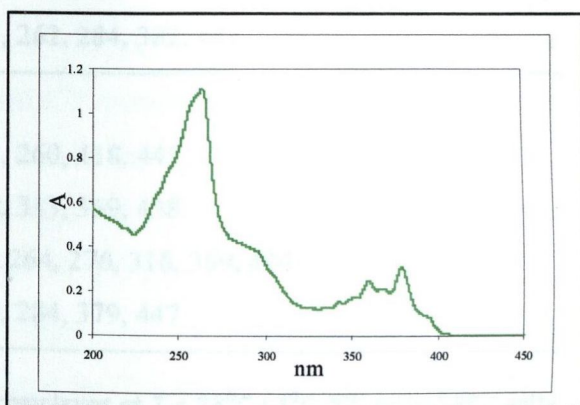
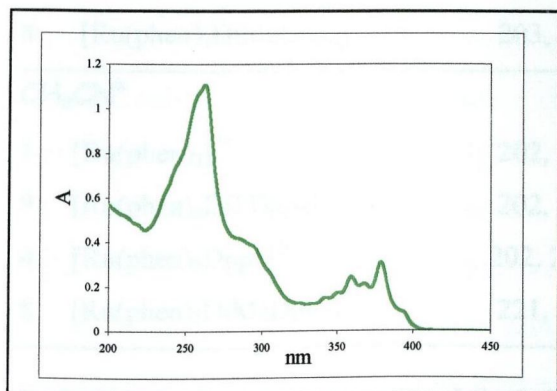


Figure 2.14: Comparison of the UV-Vis absorption spectra of the free dppz ligands 2, 13, and 14 in EtOH and CH₃CN (absorption of samples = 0.2 at lowest energy UV maximum, spectra normalized at highest energy UV max, ~270nm). All data were recorded at 298K using 1cm path length quartz cells.

As can be seen in Figure 2.14, the substituents on dppz, namely F, H, and CH₃, in the 12 and 13 positions have an effect on the absorption spectra. The electron-withdrawing fluorine substituents cause the spectrum to undergo a blue shift as they increase the energy levels of the ligand, the effect being more noticeable for the higher energy peaks,

at $\lambda \sim 260$ nm. As the methyl substituents lower the energy levels a red shift is observed.

The corresponding Ru(II) complexes were also investigated in water and acetonitrile environments, and the results are summarized in Table 2.10. For comparison the data for $[\text{Ru}(\text{phen})_3]^{2+}$ **6** has been included. For all complexes, the absorption wavelengths are close to that of the corresponding $[\text{Ru}(\text{phen})_3]^{2+}$ complex **6**, and the MLCT absorption wavelengths do not vary substantially in position or intensity by the annelation of a phenazine moiety to the phen fragment.

Complex	Absorbance max (λ nm)
H₂O^a	
1 $[\text{Ru}(\text{phen})_3]^{2+}$	223, 262, 420, 447
9 $[\text{Ru}(\text{phen})_2\text{DiFDppz}]^{2+}$	202, 264, 312, 372, 439 ^b
4 $[\text{Ru}(\text{phen})_2\text{Dppz}]^{2+}$	220, 264, 278, 318, 358, 373, 441
8 $[\text{Ru}(\text{phen})_2\text{DiMeDppz}]^{2+}$	203, 221, 262, 284, 382, 442
CH₃CN^b	
1 $[\text{Ru}(\text{phen})_3]^{2+}$	202, 223, 260, 418, 445
9 $[\text{Ru}(\text{phen})_2\text{DiFDppz}]^{2+}$	202, 262, 313, 369, 438
4 $[\text{Ru}(\text{phen})_2\text{Dppz}]^{2+}$	202, 223, 264, 276, 316, 369, 444
8 $[\text{Ru}(\text{phen})_2\text{DiMeDppz}]^{2+}$	221, 263, 284, 379, 447

Table 2.10: Spectroscopic properties of the Ru(II) complexes at $T = 23^\circ\text{C} \pm 1^\circ\text{C}$. ^aCl⁻ salts. ^bPF₆⁻ salts
Concen: $[\text{Ru}] \sim 10^{-5}\text{M}$. Errors: $\lambda_{\text{max}} \pm 2\text{nm}$

For **4** we observe that the lowest absorption band in the visible region at $\lambda_{\text{max}} = 441$ nm, is characteristic of, and has been unambiguously assigned to, MLCT transitions which are largely singlet in nature. This broad band is actually composed of multiple absorptions attributed to the overlap of $\text{Ru} \rightarrow \text{phen} (\pi^*)$ and $\text{Ru} \rightarrow \text{dppz} (\pi^*)$ metal-to-ligand charge-transfer bands^[16]. Inspection of the electronic absorption spectra of complex **4** and free dppz **2** reveal a dppz ligand centred (LC) π - π^* transition in the 370-

390 nm region. In addition, the absorption band of the phen experiences a red shift as the ligand is complexed to the Ru(II) centre *e.g* for phen $\lambda = 260 \text{ nm} \rightarrow 263 \text{ nm}$. The corresponding peak at 378 nm observed in the free dppz ligand has been assigned to the $n \rightarrow \pi^*$ transition of the phenazine (phz) part of the dppz ligand. A slight shoulder which appears at $\sim 315 \text{ nm}$ has been assigned as a spin-forbidden MC ($d\pi \rightarrow d\pi^*$) transition^[15]. Below 350 nm, the strong bands can be attributed to intraligand (IL) absorption bands from both the phen ($\pi \rightarrow \pi^*$) and dppz ($\pi \rightarrow \pi^*$) transitions, appearing as a shoulder at $\lambda \sim 220 \text{ nm}$.

The absorption spectra of complexes **8** and **9** were also examined and typically display two maxima with little variation in the ϵ_{max} for these bands. The UV-Vis spectra for all three complexes, in aqueous solution, is shown in Figure 2.15. Systematically varying the substituent at the 12 and 13 protons in the dppz ligand results in little change in the energy of the MLCT bands, while larger changes are observed in the intraligand bands of these complexes. The latter band is red-shifted ($\sim 10 \text{ nm}$) in the case of the diMedppz compound **8** (blue line), and undergoes a very slight blue shift ($\sim 1\text{-}2 \text{ nm}$) for the diFdppz complex **9** (see green line). The MLCT transitions were found to undergo small bathochromic shifts as the ligand was varied from CH_3 to H to F, respectively. The most notable effects were found for diMedppz compound **8** with a red-shift in the MLCT band. The positions and shapes of the MLCT transitions do not differ greatly between the complexes but all are blue shifted $\sim 5\text{-}8 \text{ nm}$ compared to that of the $[\text{Ru}(\text{phen})_3]^{2+}$ complex **1**. This may be explained by (i) a reduction in symmetry ($D_3 \rightarrow C_2$) of the mixed ligand complexes and (ii) cumulative inductive effects of the σ -donating and π -withdrawing ligands of the LUMO-HOMO levels.

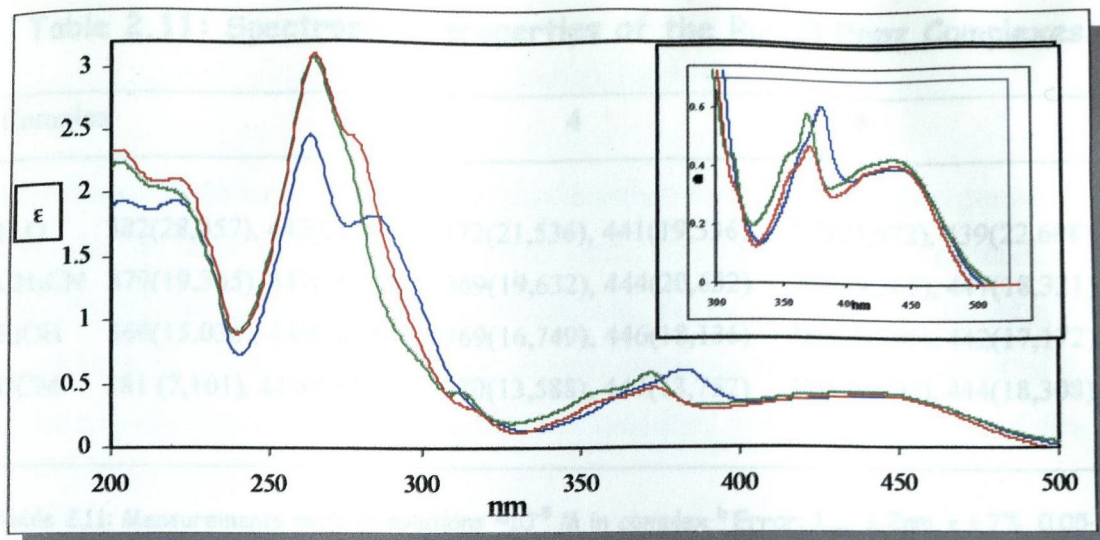


Figure 2.15: Comparison of the UV absorption spectra of the Ru(II) complexes in aqueous solution, where (8) - Blue line, (4) - Red line, and (9) - Green line. Absorption of samples = 0.4 at lowest energy UV maximum, spectra normalized at lowest energy UV max, ~440 nm. All data were recorded at 298K using 1cm path length. See inset for region 300-550 nm.

Comparing the absorption spectra of each complex in acetonitrile and water, a shift (4nm) to shorter wavelengths was observed for complex **8**, whereas for **9** a slight red shift (~1 nm) is noticeable. Spectral peaks of 1-2 nm can not be accurately detected on the Shimadzu UV-2401 PC spectrometer. We observe that the values of the λ_{max} in aqueous solution are consistently lower than in CH_3CN . In water, there is a modest flattening and broadening of the MLCT band which may be attributed to the strong H-bonding properties of water, which may lead to aggregation of the complex.

The similarity of the absorption spectra in the all four solvents strongly suggests that the electronic character and energies of the $^1\text{MLCT}$ ($\lambda_{\text{max}} \cong 440 \text{ nm}$) and $\pi\pi^*$ ligand centred excited states ($\lambda_{\text{max}} \cong 380 \text{ nm}$) are not significantly solvent dependent. As can be seen from the data in Table 2.11, the absorption spectra undergo a minimal shift on moving from EtOH to CH_3CN , but on moving from DCM to EtOH or CH_3CN more noticeable shifts with all three complexes are observed, most distinctly for complex **8**.

Table 2.11: Spectroscopic properties of the Ru(II) Dppz Complexes.

Complex	8	4	9
H ₂ O	382(28,457), 442(21,030)	372(21,536), 441(19,536)	372(23,972), 439(22,608)
CH ₃ CN	379(19,365), 447(15,024)	369(19,632), 444(20,632)	369(19,365), 447(18,321)
EtOH	369(15,036), 446(16,280)	369(16,749), 446(18,136)	369(20,200), 442(17,172)
DCM	381 (7,101), 442(5,533) ^c	370(13,588), 447(13,752)	370(21,604), 444(18,308)

Table 2.11: Measurements made on solutions $\sim 10^{-5}$ M in complex ^b Error: $\lambda_{\max} \pm 2\text{nm}$, $\epsilon \pm 7\%$, 0.05-0.1 $\times 10^{-4} \text{M}^{-1}\text{cm}^{-1}$ ^c Low ϵ value to poor solubility.

UV-visible electronic absorption spectroscopic measurements were carried out to ascertain the effect of deuterium on the spectral properties of complexes, **8**, **4**, and **9**. While slight differences were evident in the UV region 200-300 nm there was no effect on the IL and MLCT band energies. The absorption spectra of the deuterated species agree with those of the corresponding protiated complexes. A full characterisation of the enantiomers of **4** and its deuterated analogues in the absence and presence of DNA is presented in Chapter Five.

2.3.4-3 Conclusions from Absorption Studies.

A comparison of the electronic absorption spectra of the dppz species; **2**, **13** and **14**, with the spectra of the Ru(II) complexes; **8**, **4** and **9**, under similar conditions, suggests that the transitions involved are a superposition of an IL ($^1\pi\pi^*$) and an $^1\text{MLCT}$ band. This is supported by the broadening of the absorption peaks of the free ligands upon complexation to the Ru(II) metal center, especially around 350 nm. The substituents on the dppz ligand have an effect on the absorption spectra in both the metal-free form and when complexed to Ru(II). Varying the solvent polarities results in small shifts in the absorption peaks, most noticeably in the case of the diMedppz complex **8**.

2.4 Concluding Remarks

This chapter gives an overview of protium-deuterium exchange methods reported in the literature and those employed in the current study. Application of these methodologies has allowed for the preparation of perdeuterated; d_8 -phen (**3a**) and d_4 -dab **11a**, from which a series of deuterated dppz ligands were synthesised. The preparation of these ligands, d_x -dppz, where $x = 4, 6$, and 10 , was described and characterised according to ^1H NMR and mass spectroscopies.

Subsequent reaction of these deuterated dppz ligands afforded a whole series of complexes of the form; $[\text{Ru}(d_x\text{-phen})_2(d_y\text{-dppz})]^{2+}$, where $x = 0, 8$, and $y = 0, 4, 6$, and 10 . In addition, families of $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$ (**7-7g**), $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ (**8-8g**) and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ (**9-9g**), were also successfully synthesized.

^1H NMR studies show small differences between the three ligands and complexes, implying that substitution in the distal 12 and 13 positions of the dppz ligand causes few changes in the electron density distributions in the ground state. D-incorporation into the ligands and complexes simplified the ^1H NMR spectra and facilitated NMR structural assignments. Furthermore, the NMR spectrum of Ru(II) complexes was found to be sensitive to concentration. For complex **4**, the dppz protons are the most affected and are shifted downfield while the $\text{H}_{2,9}$ phen protons move upfield with increasing metal concentrations.

The UV-Vis absorption spectra for the model tris-chelated complexes, $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and $[\text{Ru}(\text{phen})_3]^{2+}$ **6**, display no significant differences in the absorption bands in different solvents. However, shifts in the positions of the absorption bands of both the free ligands and metal complexes of **4** were observed upon substitution of the dppz ligand. Therefore, substituents on the dppz ligand have an effect on the electron distribution in the higher excited states. The most noticeable changes are observed for

the diMedppz ligand **13** and complex **8**, respectively. Furthermore, the UV-Vis absorption spectra of the partially and fully deuterated complexes agree with those of the protiated parent complexes. The positions and intensities of the absorption energies remain relatively unchanged upon D-incorporation into these systems.

During the course of research, a systematic study was carried out to acquire an understanding of the mechanism and optimize the conditions of H-D exchange. These developments are detailed in Chapter Three.

2.5 References.

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The importance of the deuterium isotope effect as a probe to study the photophysical properties of organic systems has been studied as early as 1933^{10a} in the work of Hachison¹⁰ and Wright^{10b}. Since then, this phenomena has been related to a host of aromatic and heterocyclic systems. Some common H-D exchange methods involve water¹¹, base¹², or metal ion catalyst¹³. In this study, enriched deuterium compounds were prepared by the reaction of the corresponding compound 1, via an intermediate (see Scheme 1), with deuterium gas (D₂) in the presence of a catalyst (PtCl₂) or an oxide (D₂O) under similar conditions.

Chapter Three

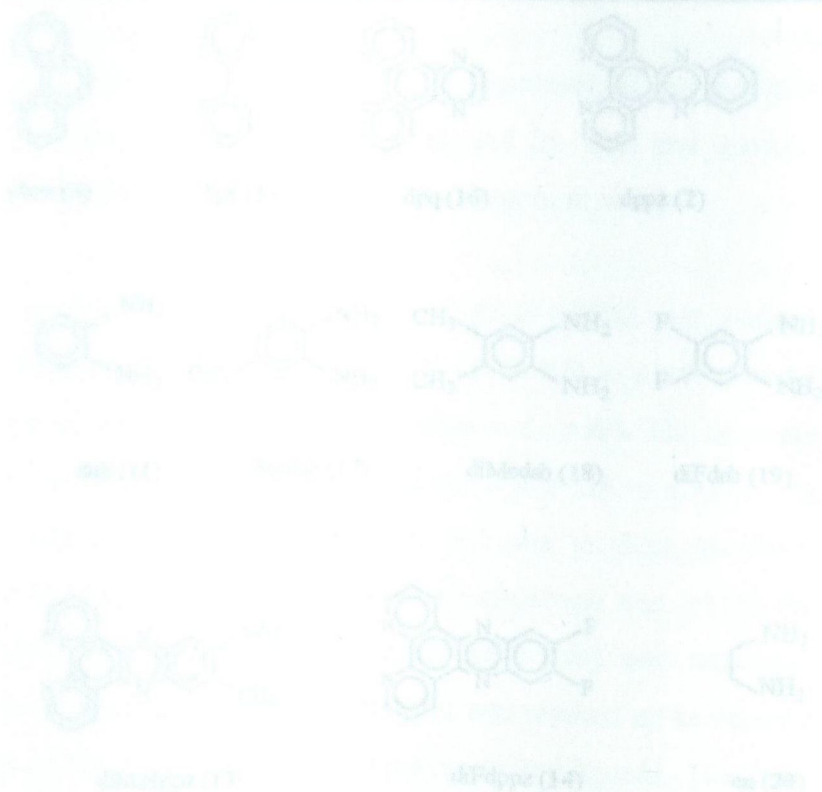


Figure 3.1: Ligands used in the current study.

3.0 Introduction

The importance of the deuterium isotope effect as a probe to examine the photophysical properties of organic systems has been studied as early as the 1960's, in the work of Hutchison^[1] and Wright^[2]. To date, this phenomena has been applied to a host of aromatic and heteroaromatic substrates. Some common H-D exchange methods involve acids^[3], bases^[4], or noble metal catalysts^[5]. In this study, successful deuterium incorporation has been demonstrated for a range of ligands, as shown in Figure 3.1, via reaction with (i) Palladium on activated charcoal (Pd/C) or (ii) Deuterium Chloride (DCl), in the presence of deuterium oxide (D₂O) under similar conditions.

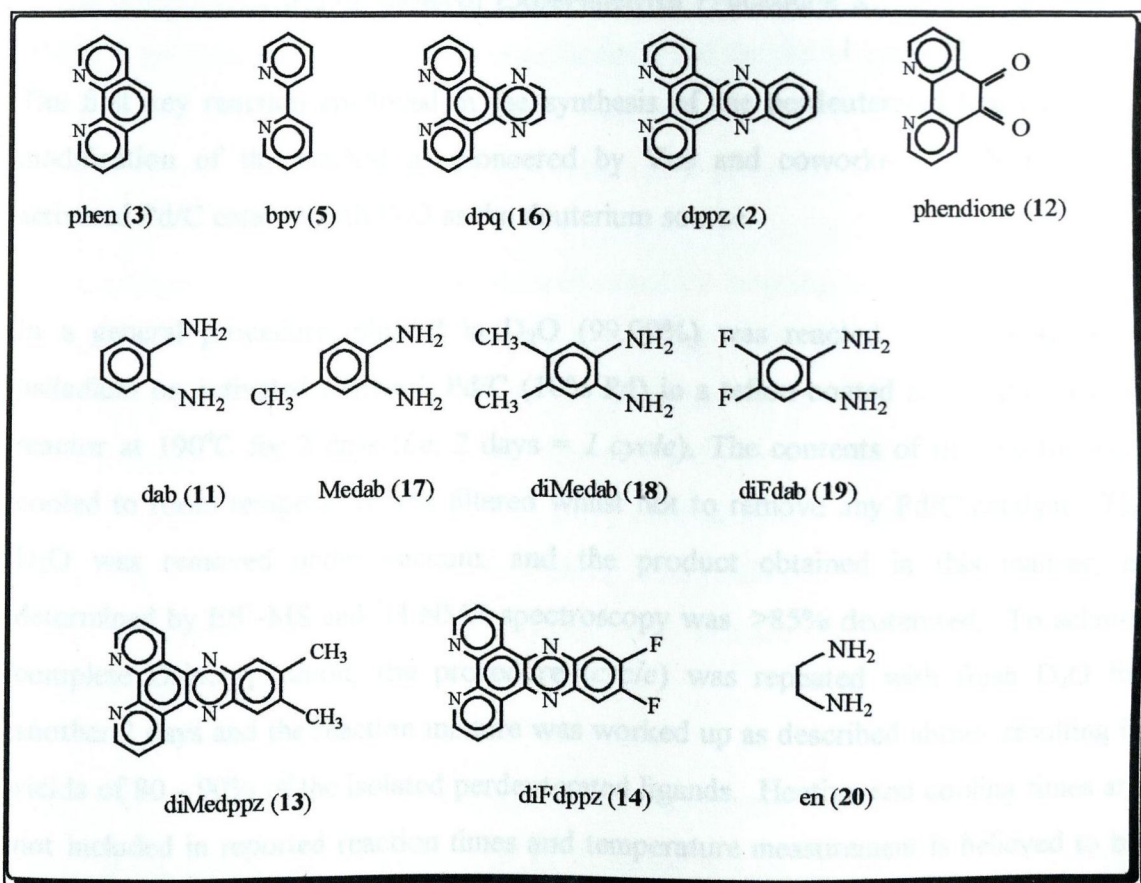


Figure 3.1: Ligands used in the current study.

Herein, we report the scope and limitations of these two methods for the preparation of deuterated ligands. Further investigation was undertaken to optimise these H-D exchange procedures (*i.e.* minimum reaction times required to attain equilibria). In addition, carefully controlled experiments were performed to assess the effectiveness of the catalyst; palladium on activated charcoal (Pd/C) on the ligands, and to ascertain the effect, if any, of variable environmental factors (*e.g.* temperature and solvent) on the efficiency of isotopic labeling.

3.1 Deuteration Method I - Pd/C catalyst in the Presence of D₂O

3.1-1 General Experimental Procedure I.

The first key reaction employed in the synthesis of the perdeuterated ligands, was a modification of the method as pioneered by Vos and coworkers^[6], which utilizes activated Pd/C catalyst with D₂O as the deuterium source.

In a general procedure, phen **3** in D₂O (99.99%) was reacted in the presence of palladium on activated charcoal, Pd/C (10% Pd) in a teflon coated steel high pressure reactor at 190°C for 2 days (*i.e.* 2 days = 1 cycle). The contents of the reactor were cooled to room temperature and filtered whilst hot to remove any Pd/C catalyst. The D₂O was removed under vacuum, and the product obtained in this manner, as determined by ES⁺-MS and ¹H NMR spectroscopy was >85% deuterated. To achieve complete D-incorporation, the procedure (*cycle*) was repeated with fresh D₂O for another 2 days and the reaction mixture was worked up as described above, resulting in yields of 80 - 90% of the isolated perdeuterated ligands. Heating and cooling times are not included in reported reaction times and temperature measurement is believed to be accurate to approximately $\pm 5^{\circ}\text{C}$ (obviously correction would be necessary if accurate kinetic information was desired). Following cool down, isotopic purities were characterised unambiguously by ES⁺-MS, by comparison between observed and calculated isotope clusters, and by NMR spectroscopy, as appropriate.

Generally, this procedure proves to be very efficient for promoting H-D exchange, and involves the use of mild conditions and requires relatively short reaction times (approximately 4 days (2 cycles)). Therefore, it has considerable potential for the preparation of deuterated aromatic ligands.

3.1-2 The Achievements and Limitations of Pd/C as a Catalyst.

Studies reveal this method to be efficient for the direct deuteration of polypyridyl bipyridine ligands^{[6][7]}. We have investigated the generality of this simple method when applied to a wider range of ligands. To this end, several ligands were reacted according to the general procedure as detailed above in section 3.1-1 (*i.e.* D₂O, Pd/C for 4 days at 190°C). Subsequently, the products were isolated and the techniques of ES⁺-MS and ¹H NMR were used to spectroscopically identify and assess the isotopic purities incorporated into the ligands in comparison with their protiated samples.

As is evident, the method is particularly well suited for labelling polypyridyl ligands; with phen, bpy, dpq and dppz (**3**, **5**, **16**, and **2**) being perlabelled under these reaction conditions, as indicated in Table 3.1. In a typical run the reaction was performed in a neutral medium with a non-deuterated catalyst (Pd/C) for 4 days (2 cycles) at 190°C ± 5°C. It was found that isotopic purities >90% were achieved in reasonable yields of 55-92% with no significant by-product formation. The yields could be increased upon (10-15%) by soxhlet extraction of the residual Pd/C into acetone (for ligands **3**, **5**) and ethanol (for ligands **2**, **16**).

The effects of Pd/C in D₂O were examined for dab **11** and substituted derivatives (**17-19**). It was noted that the parent dab **11** substrate was not perlabelled by this method, although protium-deuterium exchange was observed for the diaminobenzene derivatives; Medab **17** and diMedab **18**, respectively. In these cases, reaction (2 days at 190°C) resulted in perdeuterated ligands, which extended to the

Table 3.1: H-D exchange of ligands in D₂O, Pd/C at 190°C

Ref ^a	Substrate ^b	Product ^c	%H-D exchange ^e	Isolated Yield
3	phen <i>m/z</i> = 180	D ₈ - phen <i>m/z</i> = 189	90**	85%
5	bpy <i>m/z</i> = 156	D ₈ - bpy <i>m/z</i> = 164	90*	80%
16	dpq <i>m/z</i> = 232	D ₈ - dpq <i>m/z</i> = 240	90**	57%
2	dppz <i>m/z</i> = 282	D ₁₀ - dppz <i>m/z</i> = 292	90**	92%
11	dab ^f <i>m/z</i> = 108		0	
17	Medab <i>m/z</i> = 122	D ₃ - Medab ^d <i>m/z</i> = 128	ring: 90, Me:90*	90%
18	diMedab <i>m/z</i> = 136	D ₂ - diMedab ^d <i>m/z</i> = 142	ring: 90, Me:90*	90%
19	diFdab ^f <i>m/z</i> = 144		0	oily product
14	diFdppz ^g <i>m/z</i> = 318	D ₂ - diFdppz ⁱ <i>m/z</i> = 321	H _{2,9} : 100, H _{3,8} : 5, H _{11,14} : 16 ^{h*}	10%
20	en ^f <i>m/z</i> = 58		0	oily product

^a See Figure 3.1 for structure ^b Reactions performed in high T/P batch reactor, and unless otherwise stated exchange was approximately equal at all positions ^c D₂O, Pd/C at 190°C for 4 days (2 cycles), unless stated otherwise. ^d D₂O, Pd/C at 190°C for 2 days (1 cycle); ^e Determined by mass spectrometry. ^f No H-D exchange, ^g Partial H-D exchange. ^h H_{4,7} slowest exchangeable proton used as internal reference. ⁱ D₂O, Pd/C at 190°C for 6 days (3 cycles). * Average value (2 batches), ** Average Value (> 4 batches).

methyl moieties yielding CH₃ → CD₃ transformations, step 1 in Figure 3.2, and represents one of the few examples of CH₃ → CD₃ exchange reported in the literature^{[8][9]}. Subsequent reaction with phendione 12 in refluxing ethanol for 30 minutes yielded (CD₃)-d₃-dppz 21a and (CD₃)₂-d₂-dppz 13a respectively, see step 2 of Figure 3.2. The ¹H NMR spectra confirmed the presence of the selectively deuterated dppz derivatives relative to that of the protiated ligands. It was also noted, in both cases, that the methyl peak observed at δ2.64 ppm in the corresponding protiated substrates is absent due to H-D exchange.

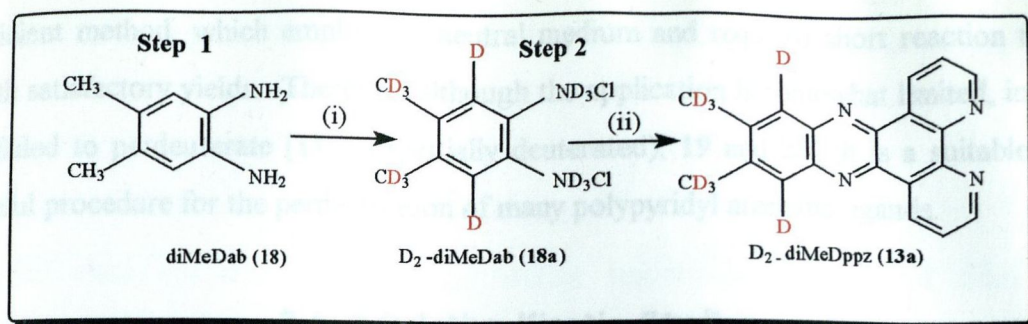
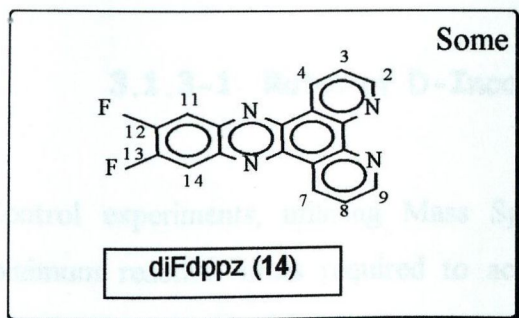


Figure 3.2: Preparation of d₂-DiMedab (18a) and d₂-diMedppz (13a) in the presence of Pd/C and D₂O; (i) Pd/C, D₂O at 190°C for 2 days, (ii) phendione, refluxing EtOH, 30 mins.

In contrast to the complete exchange observed for the ligands, 2, 3, 5, and 16, the procedure is not efficient for labeling strongly electron-withdrawing aromatic ligands; for instance diFdab 19 gave no isotopic exchange, even after prolonged periods (6 days (3 cycles)). Similarly, ethylenediamine (en) 20 underwent no H-D exchange under the same conditions.



Some of the ligands, however only underwent partial H-D exchange. For instance, D-incorporation into diFdppz 14 after 4 days (2 cycles) at 190°C, was less than 30%, allowing for the preparation of d₂-diFdppz with near selective (>95%) deuteration of

the H_{2,9} aromatic ring protons. Subsequent deuteration, even on prolonged periods (3 cycles) was limited; H_{2,9} position (D > 95%), H_{11,12} (D > 16%) and H_{3,8} (D > 5%), as calculated by ES⁺-MS and ¹H NMR spectroscopy, using the slowest exchangeable protons (H_{4,7}) as an internal standard. An overall 37% H-D exchange was achieved under these reaction conditions.

Although the reaction conditions employed in this study are vigorous, the post heating NMR and mass spectra did not show any indication of any products other than the starting material and its deuterated analogues. In conclusion, this proved to be an

efficient method, which employed a neutral medium and required short reaction times with satisfactory yields. Therefore, although the application is somewhat limited, in that it failed to perdeuterate [11, 14 (partially deuterated), 19 and 20], it is a suitable and useful procedure for the perdeuteration of many polypyridyl aromatic ligands.

3.1-3 Relative Kinetic Studies.

Although a number of deuteration methods have been reported in the literature^{[8][10]} a systematic investigation of the kinetics involved in the exchange mechanism has not been carried out. To this end, a detailed study of method I has been undertaken which addresses several aspects; the rate of H-D and reversible D-H exchange, calculation of the percent (%) deuterium at specific proton sites, and the positional preference of deuteration, therefore allowing for a more comprehensive understanding of the reaction mechanisms.

3.1.3-1 Rates of D-Incorporation into Phen and Dppz.

Control experiments, utilizing Mass Spectroscopy, were performed to assess the minimum reaction times required to achieve >95% deuterium exchange. In these experiments, phen **3** and dppz **2** were reacted in the presence of Pd/C in D₂O at 190°C. The reaction was monitored, at three hour intervals for **3** and at 24 hour intervals for **2**, whereby the reactor was removed and the contents were rapidly cooled to room temperature by immersing the reactor vessel in a water-bath. Aliquots were drawn and the chemical and isotopic composition of the reaction was analyzed by MS to monitor the progress of exchange and attain a better understanding of the contents of the reactor vessel. Fresh D₂O was added, after 18 hours for **3** and 48 hours for **2**, and the reaction was continually monitored until the desired isotopic purities (>95%) were reached. ES⁺-mass spectrometry proved to be an extremely useful tool in monitoring the rate of protium-deuterium exchange, and provided an accurate determination of the minimum

reaction times required to achieve completely perdeuterated d_8 -phen (**3a**) and d_{10} -dppz (**2c**).

• Phen

In ES^+ -mass spectroscopy, molecular weight information is obtained from the *protonated*¹ phen ligand; $[h_8\text{-phen-H}^+]$, and the observed m/z value of 181 is one unit greater than the true molecular weight of 180.21 g/mol. In addition, the shoulder peak observed at $m/z = 182$ is attributed to the natural abundance of ^{13}C (1.1%). For the perdeuterated phen ligand; d_8 -phen (**3a**), in the presence of D_2O the peaks at $m/z = 189$ and 190 correspond to the *protonated* $[d_8\text{-phen-H}^+]$ species and the ^{13}C species respectively, while the observed peak at $m/z = 191$ is attributed to the phen “solvated D^+ ” species, $[d_8\text{-phen-D}^+]$, see Figure 3.3.

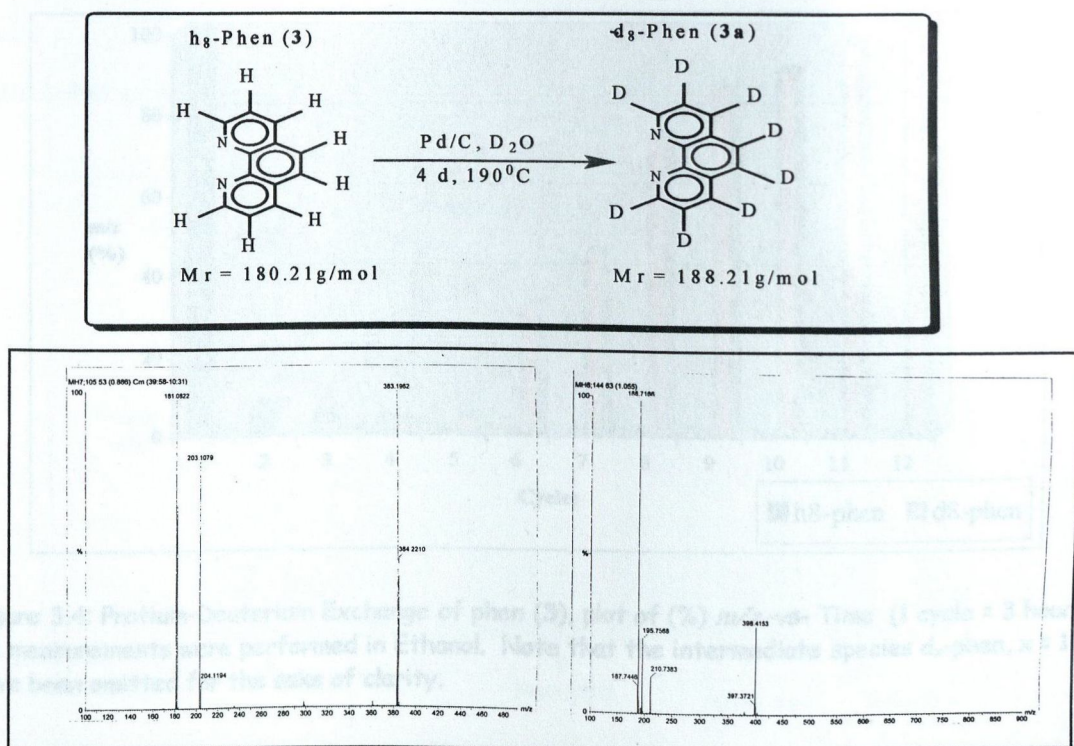


Figure 3.3: Preparation of deuterated d_8 -phen (**3a**). Mass Spectra (m/z) of isotopic abundances of protiated h_8 -phen (**3**) and perdeuterated d_8 -phen (**3a**).

¹ The natural abundance ligands and complexes are referred to as “protiated” and not protonated for the purpose of mass spectral analysis; $[h_8\text{-phen}]$ (protiated) and $[d_8\text{-phen-H}^+]$ (protiated).

For the series of deuteration experiments on phen **3**, the rate of deuterium exchange may be best illustrated by means of a graphical presentation of the percent m/z abundance of the individual isotopic compositions for each three-hour sample plotted as a function of time. Several distinct features are observed on monitoring the progression of H-D exchange for this reaction, as shown in Figure 3.4. The most prominent features are; (i) the **rapid** decrease in the isotopic abundance of the fully protiated phen **3** [$\text{h}_8\text{-phen}$] species, m/z 181, (see blue columns). This species has been completely removed from the reaction mixture after 12 hours at 190°C , (ii) The **gradual** introduction of deuterated phen (**3a**) [$\text{d}_8\text{-phen-H}^+$] species, m/z 189, which reaches a maximum value on reacting for 33 hours (see red columns), and (iii) the **presence** of a series of short lived partially deuterated species (*i.e.* [$\text{d}_x\text{-phen}$], where $x = 1-7$), which are not included in the plot for the sake of clarity.

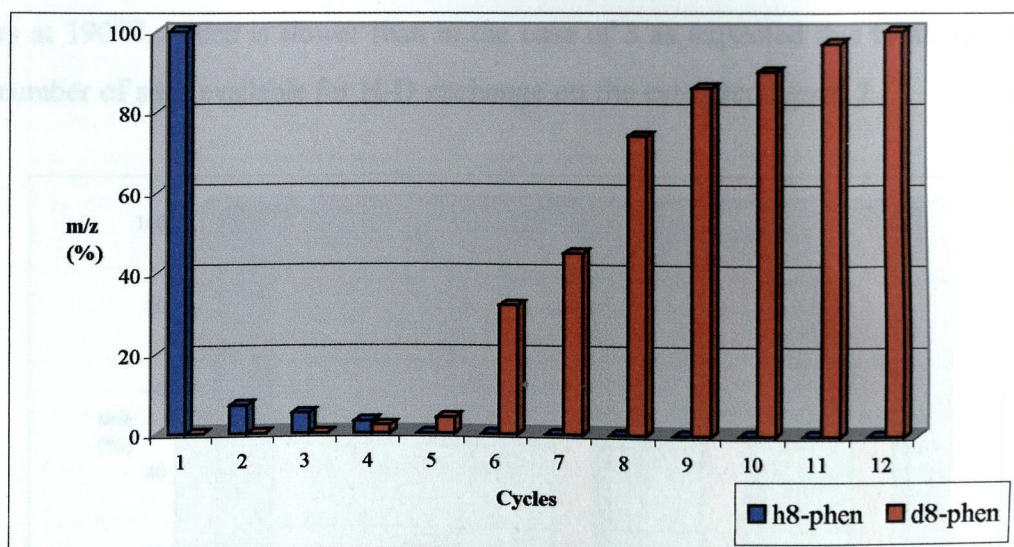


Figure 3.4: Protium-Deuterium Exchange of phen (**3**), plot of (%) m/z -vs- Time (1 cycle = 3 hours). All measurements were performed in Ethanol. Note that the intermediate species $\text{d}_x\text{-phen}$, $x = 1-7$, have been omitted for the sake of clarity.

Complete isotopic exchange was determined by the relative abundance of the peaks greater than m/z 187, as these are attributed to the [$\text{d}_8\text{-phen}$], [$\text{d}_8\text{-phen-H}^+$] and [$\text{d}_8\text{-phen-D}^+$] species, respectively. Thus, mass spectrometry allows for the determination of the end-point (minimum reaction time) of the reaction. For **3**, complete isotopic labeling

>95% occurred after 33 hours at 190°C. After such time, H-D exchange reached a maximum value and no subsequent detectable change in the isotopic abundances of the perdeuterated species was noted.

• Dppz

Application of this method was extended to access the rate of D-incorporation into the larger dppz ligand **2**. Monitoring the reaction at 24 hour intervals, with mass spectroscopy resulted in a similar trend to that observed for **3**, as illustrated in Figure 3.5. Again we observe that the $[h_{10}\text{-dppz-H}^+]$ substrate at $m/z = 283$ is quickly depleted while the $[d_{10}\text{-dppz-H}^+]$ species found at $m/z = 293$ is gradually introduced into the system (the range of intermediate partially deuterated species $[d_x\text{-dppz}]$, where $x = 1-9$, are omitted for clarity). The desired isotopic exchange of >95% was attained after 96 hours at 190°C, which is slower than in the case of **3** as expected due to an increase in the number of sites available for H-D exchange on the extended ligand **2**.

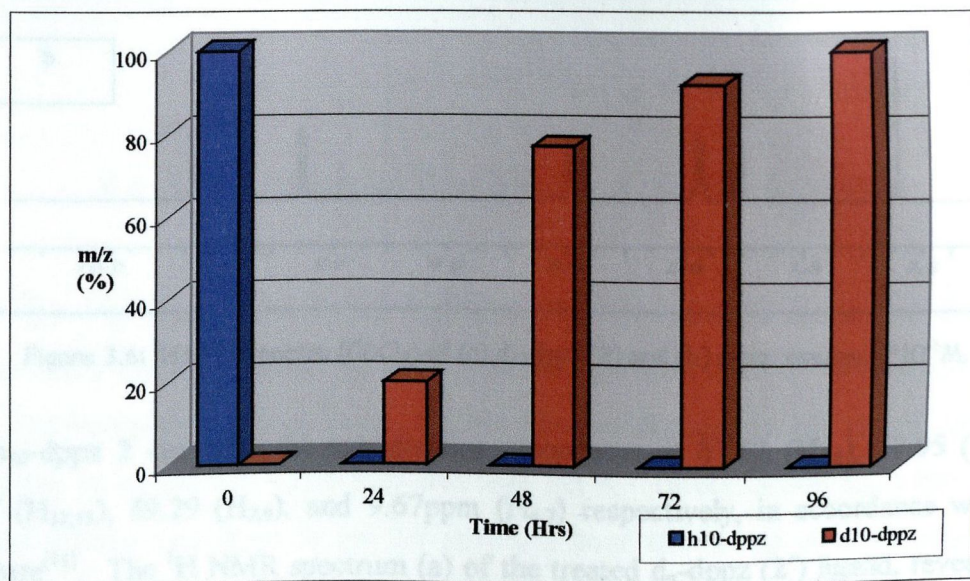


Figure 3.5: Protium-Deuterium Exchange for dppz (**2**), plot of (%) m/z -vs- Time (hrs). All measurements were performed in acetonitrile. Note that the intermediate species $d_x\text{-phen}$, $x = 1-9$, have been omitted for the sake of clarity.

3.1.3-2 ^1H NMR Studies to Examine the Extent of Deuteration into Dppz (2).

To assess the potential of Pd/C catalysed D-incorporation, the percentage amount of deuterium into the various positions of a test molecule (*i.e.* dppz) was determined. The protiated h_{10} -dppz **2** was treated with Pd/C and D_2O for 4 days (2 cycles) at 190°C , resulting in deuterated d_n -dppz (**2'**), where n denotes the number of deuteriums present in the ligand. The amount of H-D exchange that occurred (*i.e.* n) was estimated by comparing the ^1H NMR spectra (CDCl_3 , *ca.* $1 \times 10^{-6}\text{M}$) of untreated h_{10} -dppz **2** and the deuterated d_n -dppz **2'** ligands. The spectra are illustrated in Figure 3.6, the solvent CDCl_3 peak at $\delta 7.2$ ppm being used as an internal standard.

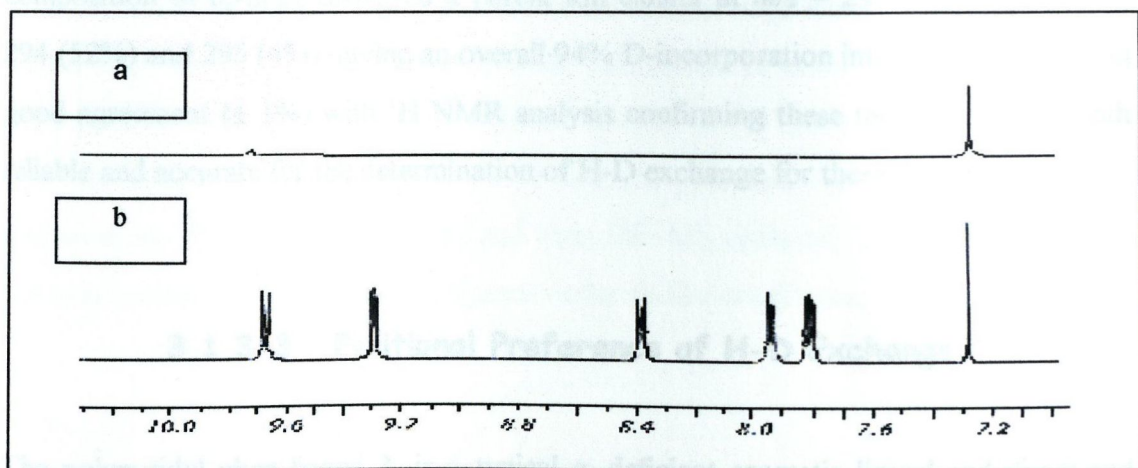


Figure 3.6: ^1H NMR spectra (CDCl_3) of (a) d_n -dppz (**2'**) and (b) dppz; concn. $1 \times 10^{-6}\text{M}$.

The h_{10} -dppz **2** spectrum (b) exhibits five resonances at $\delta 7.82$ ($\text{H}_{3,8}$), $\delta 7.95$ ($\text{H}_{11,14}$), $\delta 8.37$ ($\text{H}_{12,13}$), $\delta 8.29$ ($\text{H}_{2,9}$), and 9.67ppm ($\text{H}_{4,7}$) respectively, in accordance with the literature^[11]. The ^1H NMR spectrum (a) of the treated d_n -dppz (**2'**) ligand, reveals that dppz has undergone complete deuteration at the $\text{H}_{2,9}$, $\text{H}_{3,8}$, $\text{H}_{11,14}$ and $\text{H}_{12,13}$ positions, these signals being removed from the spectrum. However, perdeuteration did not occur at all of the available proton sites and the peak in spectrum (a) at $\delta 9.69$ ppm is attributed to the protons at the $\text{H}_{4,7}$ position. Extensive deuteration at the latter position has

resulted in a downfield shift (~ 0.07 ppm) and a significant reduction in the intensity of the signal. Assuming that the intensities are not affected by relaxation time differences, the percentage deuteration at this site may be estimated by comparing the relative peak heights (I) of the protiated (I_{Ha}) and deuterated (I_{Hb}) dppz ligands. Essentially, the amount of deuterium present at the 4,7 position is estimated by the difference of these resonance peak heights, ($I_{\text{Ha}} - I_{\text{Hb}}$), as measured from the spectra, which equates to 95% exchange at the latter site. As the $\text{H}_{2,9}$, $\text{H}_{3,8}$, $\text{H}_{11,14}$ and $\text{H}_{12,13}$ positions have been removed from spectrum (b) we assume that 100% exchange (within $\pm 2\%$) has occurred at these sites. Therefore, there is an overall 95% H-D exchange for the dppz ligand.

Furthermore, ES^+ -MS data was recorded for both ligands. The untreated h_{10} -dppz **2** gave a molecular ion peak at $m/z = 283$ (94%), 284 (6%) as expected. The isotopic composition of d_n -dppz displayed a parent ion cluster at $m/z = 292$ (6%), 293 (38%), 294 (52%) and 295 (4%), giving an overall 94% D-incorporation into **2**. This result is in good agreement ($\pm 1\%$) with ^1H NMR analysis confirming these techniques to be both reliable and accurate for the determination of H-D exchange for these systems.

3.1.3-3 Positional Preference of H-D Exchange.

The polypyridyl phen ligand **3**, is a typical π -deficient aromatic ligand and direct and selective H-D exchange have been little explored. Stewart *et al*^[12], have examined the AMI Hamiltonian calculations for **3**, and the effective charges for the relative positions are shown in Figure 3.7. This indicates that the 4,7 carbon atoms have the highest electron density, and those in the 2,9 positions have the lowest electron population. They reported that the relatively small difference in the electron population between the various positions seem to lead to preferential substitution in the order of $\text{H}_{2,9} > \text{H}_{5,6} > \text{H}_{3,8} > \text{H}_{4,7}$.

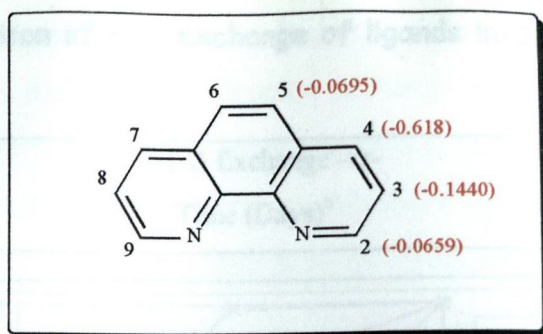


Figure 3.7: Measured Electron Density ^[12], for Phen 3.

To experimentally assess the positional preference for Pd/C-catalysed deuteration the amount of D-incorporation into various locations of the ligands; phen **3**, dpq **16** and dppz **2**, was determined. In a typical experiment, the ligands were treated with Pd/C and D₂O for 4 days (2 cycles) at 190⁰C, and the products isolated after each cycle were analysed by NMR spectroscopy. Because of the relative large experimental error in both temperature and heating time measurements, the amount of deuteration was determined semi-quantitatively. The percent deuteration at each site in these ligands was estimated from the relative integration intensities of both the protiated and deuterated ligands as measured by ¹H NMR spectroscopy and from ES⁺-MS analysis. Table 3.2 summarizes the results obtained for the various ligands under similar conditions.

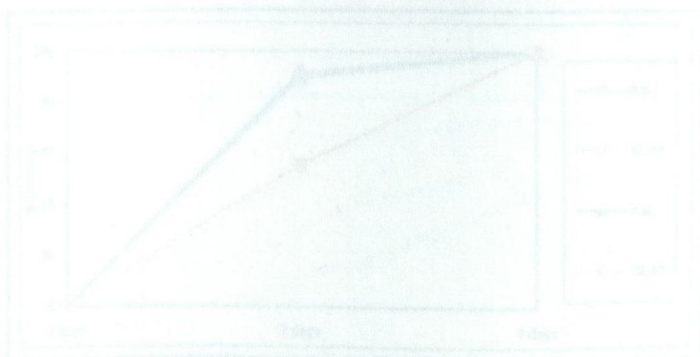


Table 3.2: * D₂O, Pd/C at 190⁰C for 4 days (2 cycles). H₂, strongest exchangeable proton is used as internal standard. After 2 cycles >97% exchange occurred at all positions

Table 3.2: Comparison of H-D exchange of ligands in D₂O, Pd/C at 190°C

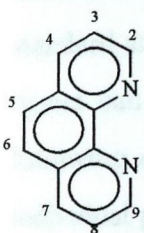
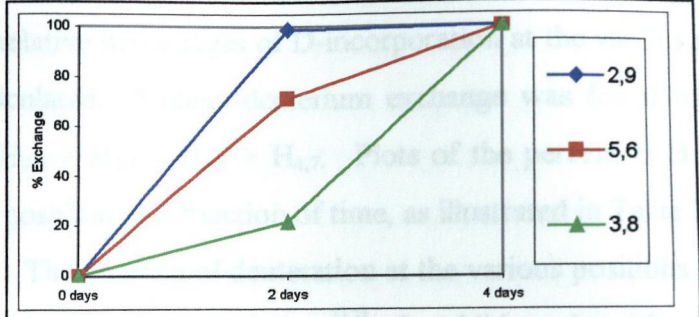
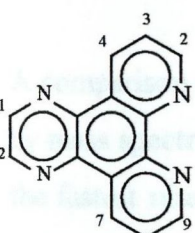
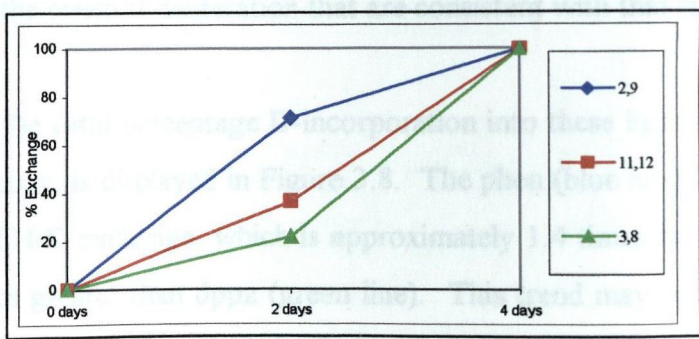
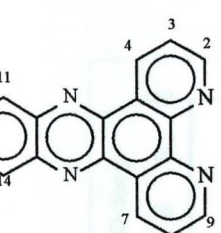
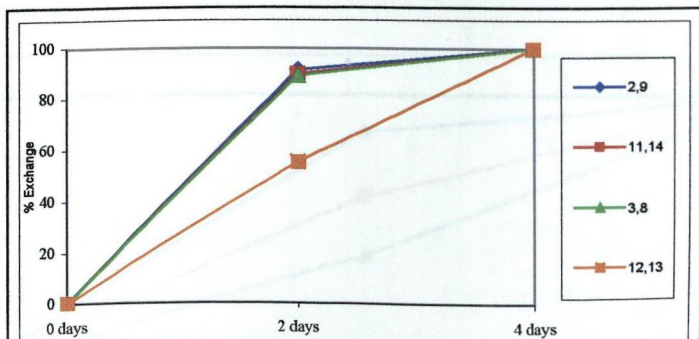
Ligand ^a	% H-D Exchange -vs- Time (Days) ^b	Positional Preference of H-D Exchange
 phen 3		H_{2,9} H_{5,6} H_{3,8} H_{4,7}
 dpq 16		H_{2,9} H_{11,12} H_{3,8} H_{4,7}
 dppz 2		H_{2,9} H_{11,14} H_{3,8} H_{12,13} H_{4,7}

Table 3.2: ^a D₂O, Pd/C at 190°C for 4 days (2 cycles). H_{4,7}, slowest exchangeable proton is used as internal standard. After 2 cycles >97% exchange occurred at all positions

In the case of **3**, the H_{2,9} positions being adjacent to the nitrogen atoms are regions of low electron density, and therefore readily exchange their protons. NMR data confirmed that greater than 90% deuteration occurred at these positions on reacting for 2 days at 190°C. In contrast, the H_{4,7} positions which are high electron density areas are the slowest to facilitate exchange of their protons. This provides us with an internal standard and the relative percentages of D-incorporation at the various sites of the phen ligand can be calculated. Protium-deuterium exchange was found to proceed in the following order; H_{2,9} > H_{5,6} > H_{3,8} > H_{4,7}. Plots of the percent H-D exchange at the individual proton positions as a function of time, as illustrated in Table 3.2, clearly show this general trend. The ordering of deuteration at the various positions are in agreement with the proposed Hamiltonian calculations^[12]. In addition, dpq **16** and dppz **2** display similar trends for the order of deuteration that are consistent with that found for phen **3**.

A comparison of the **total** percentage D-incorporation into these ligands, as determined by mass spectroscopy, is displayed in Figure 3.8. The phen (blue line) ligand undergoes the fastest rate of H-D exchange, which is approximately 1.4 times faster than dpq (red line) and 1.5 times greater than dppz (green line). This trend may be attributed to the increasing size and number of available exchange sites for this series of ligands.

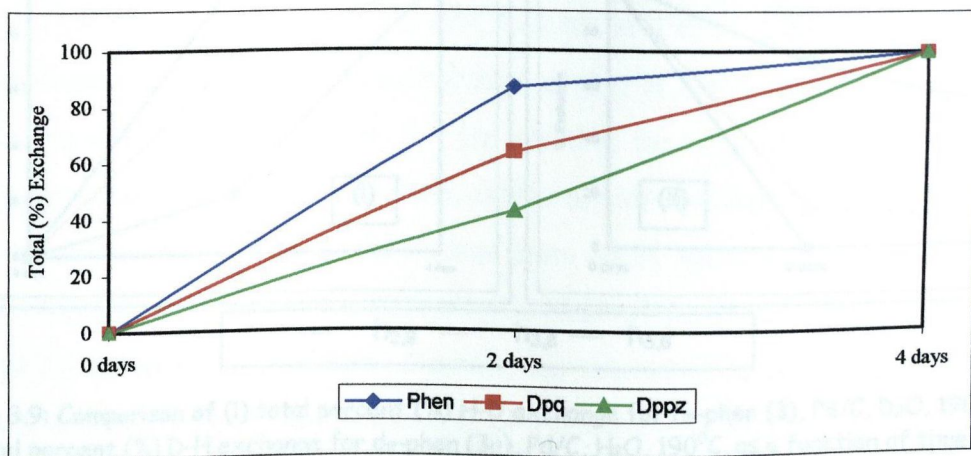


Figure 3.8: Comparison of total percent (%) exchange of phen (**3**), dpq (**16**) and dppz (**2**) as a function of time (days).

3.1.3-4 Rate of Reversible D-H Exchange into Phen (3).

It has been shown previously that application of the method of Pd/C in D_2O promotes efficient H-D exchange for a whole range of ligands. But is this a reversible process and if so, are the rates of H-D and D-H exchange equivalent. To investigate this effect, protiated phen **3** was treated (a) with Pd/C and D_2O for 4 days (2 cycles) at $190^\circ C$, resulting in the preparation of perdeuterated d_8 -phen (**3a**). This was subsequently treated, (b) with Pd/C in the presence of H_2O for 4 days (2 cycles) at $190^\circ C$. The reaction scheme is shown in Figure 3.9. The percent H- and D-incorporation into the various positions of **3** and **3a** were monitored at 24 hour intervals by 1H NMR ($H_{4,7}$ positions used as internal reference) and mass spectroscopies.

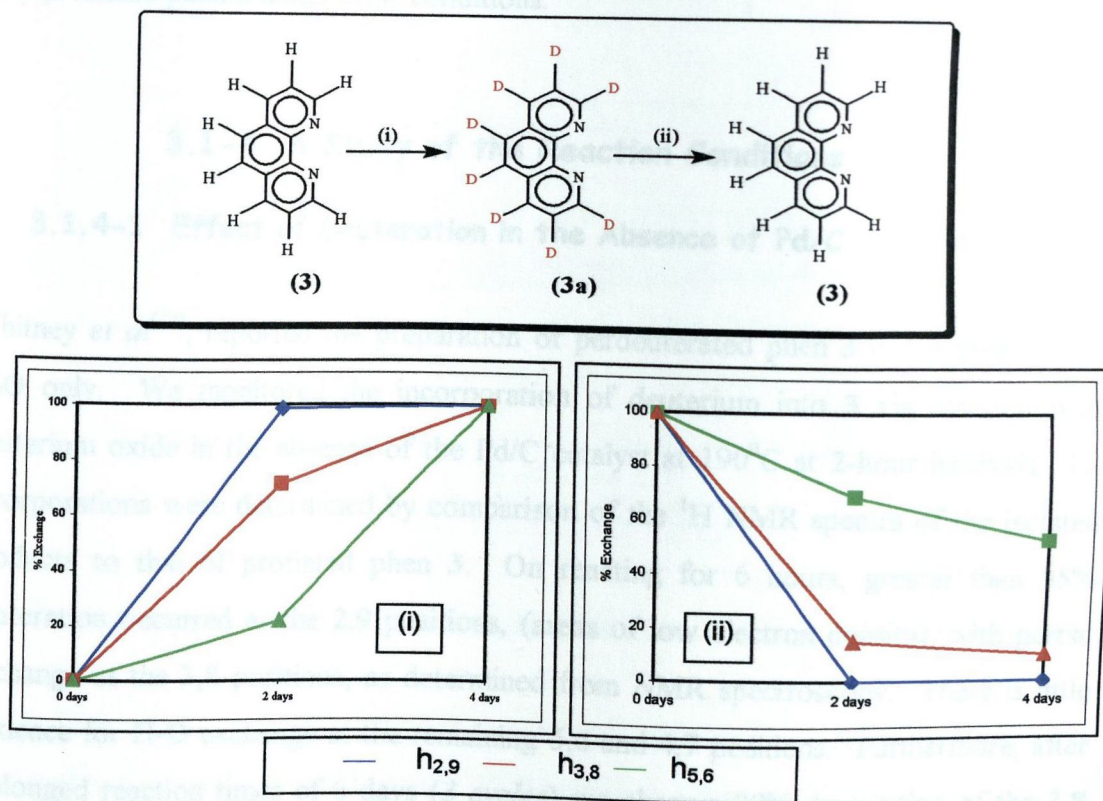


Figure 3.9: Comparison of (i) total percent (%) H-D exchange for h_8 -phen (**3**), Pd/C, D_2O , $190^\circ C$ and (ii) total percent (%) D-H exchange for d_8 -phen (**3a**), Pd/C, H_2O , $190^\circ C$, as a function of time.

For reaction (i), h₈-phen **3** → d₈-phen **3a**, on reacting for 2 days in Pd/C, D₂O at 190°C H-D exchange rates of 90% (H_{2,9}), 76% (H_{5,6}) and 18% (H_{3,8}), were observed. Complete deuteration (>95%) is obtained on reacting for 4 days (2 cycles). For the reverse reaction (ii), d₈-phen **3a** → h₈-phen **3**. D-H exchange occurred on reacting for 2 days at 190°C with H-incorporations of 90% (H_{2,9}), 53% (H_{5,6}) and 10% (H_{3,8}) for the phen positions. On subsequent reaction for an additional 2 days, we note that complete exchange occurred at the 2,9 positions with only partial exchange at the remaining positions, an overall 75% exchange for **3a**. Even after prolonged periods (6 days (3 cycles)) no further exchange was observed. For both reactions, the rate of exchange is of the same ordering; H_{2,9} > H_{5,6} > H_{3,8} > H_{4,7}. Although the process is reversible it was found that D-H exchange is less efficient and occurs at a slower rate failing to produce fully protiated phen **3** under these conditions.

3.1-4 A Study of the Reaction Conditions

3.1.4-1 Effect of Deuteration in the Absence of Pd/C Catalyst

Whitney *et al*^[13], reported the preparation of perdeuterated phen **3** in the presence of D₂O only. We monitored the incorporation of deuterium into **3** via reaction with deuterium oxide in the absence of the Pd/C catalyst at 190°C at 2-hour intervals. D-incorporations were determined by comparison of the ¹H NMR spectra of the isolated products to that of protiated phen **3**. On reacting for 6 hours, greater than 95% deuteration occurred at the 2,9 positions, (areas of low electron density), with partial exchange at the 3,8 positions, as determined from NMR spectroscopy. There is little evidence for H-D exchange at the remaining 5,6 and 4,7 positions. Furthermore, after prolonged reaction times of 6 days (3 cycles) we observe 29% deuteration of the 3,8 positions, and exchange occurring although to a lesser extent for the 5,6 (9%) and 4,7 (8%) positions. The positional preference of deuteration; H_{2,9} > H_{3,8} > H_{5,6} > H_{4,7}; is of the same order as **3** when treated with Pd/C in the presence of D₂O, under identical conditions.

Protium-deuterium exchange for **3** in both the presence of (i) Pd/C, D₂O and (ii) DCl, D₂O at 190°C has been examined in further detail. A comparison of the two methodologies, are shown in Figure 3.10, which displays the extent of D-incorporation into **3** as a function of time, as monitored by NMR and ES⁺-MS spectrometry. Initially, the extent of H-D exchange is similar, for both methods. In the absence of the Pd/C catalyst (blue line), extensive exchange >95% occurred at the 2,9 positions, with evidence of little exchange in the remaining sites on reacting for 6 hours at 190°C. However, these reaction conditions are not efficient at promoting total H-D exchange into **3** and even on prolonged reaction times of 125 hours only a 56% exchange rate is observed for **3**. In contrast, in the presence of the Pd/C catalyst (red line), H-D exchange increases steadily and produces >95% isotopic purities on reacting for 44 hours at 190°C.

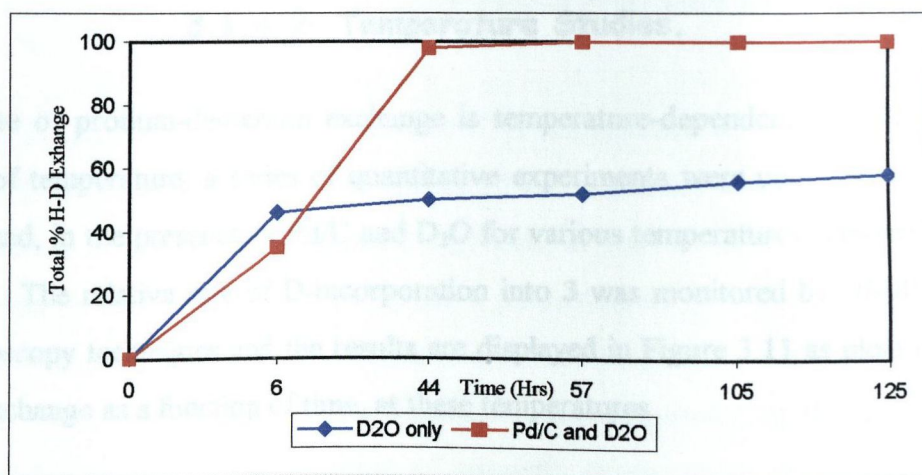


Figure 3.10: Comparison of total percent (%) exchange into phen (**3**) in D₂O, Pd/C and D₂O at 190°C.

Application of this method, to dppz **2** and dab **11**, was also examined and the results tabulated in Table 3.3. In these studies, H-D exchange either did not occur, or occurred to a lesser extent and at fewer sites when Pd/C was absent. Under these conditions, only 46% D-incorporation was noted for **2**, while D₂O under neutral conditions was not efficient for the promotion of H-D exchange for **11**.

Table 3.3: H-D exchange of ligands in D₂O only at 190°C

Entry ^a	Substrate ^b	Product ^c	%H-D exchange ^e	Isolated Yield
3	phen <i>m/z</i> = 180	D ₅ - phen ^d <i>m/z</i> = 186	54*	85%
2	dppz <i>m/z</i> = 282	D ₄ - dppz ^e <i>m/z</i> = 287	46*	65%
11	dab ^f <i>m/z</i> = 108		0	

^a See Figure 3.1 for structure * Average value (> 4 batches) ^b Reactions performed in high T/P batch reactor ^c Determined by mass spectrometry. ^d D₂O at 190°C for 4 days (2 cycles), ^e D₂O at 190°C for 6 days (3 cycles). **No H-D exchange.** **Partial H-D exchange**

3.1.4-2 Temperature Studies.

The rate of protium-deuterium exchange is temperature-dependent. To illustrate the effect of temperature, a series of quantitative experiments were undertaken using **3** as the ligand, in the presence of Pd/C and D₂O for various temperatures between 25°C and 190°C. The relative rate of D-incorporation into **3** was monitored by NMR and mass spectroscopy techniques and the results are displayed in Figure 3.11 as plots of percent H-D exchange as a function of time, at these temperatures.

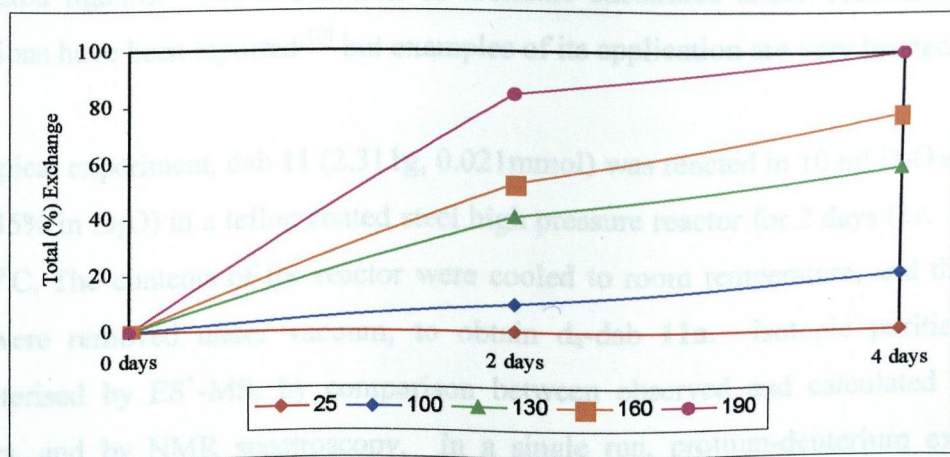


Figure 3.11: Comparison of total percent (%) exchange for (**3**) at temperatures 25-190°C.

At room temperature, no H-D exchange occurred over a time period of 4 days (2 cycles). Predictably, as the temperature increases so too does the rate of D-incorporation into the various phen positions. There is a linear relationship between the rate of exchange and the temperature. The optimum temperature for efficient protium-deuterium exchange into **3** involves heating to *ca.* 190°C, under these reaction conditions. Experiments were not performed at temperatures exceeding this temperature (due to safety limitations of the reactor vessel) although it has been reported that at elevated temperatures shorter reaction times are observed for the production of perdeuterated ligands^{[8][14]}.

3-2 Deuteration Method II - DCl Catalyst in the Presence of D₂O

3.2-1 General Experimental Procedure II

Despite the generality of the Pd/C method, it is not efficient for perdeuteration of dab **11**, which is necessary for the preparation of the selectivity deuterated dppz ligands. This lead us to develop an alternative approach, to convert the readily available dab ligand and its derivatives to their respective perdeuterated derivatives. This synthetic procedure employs D₂O in the presence of an acidic medium by addition of deuterium chloride, DCl, which catalyses the isotopic exchange producing the corresponding deuterated ligands. The deuteration of aromatic substrates under subcritical acidic conditions have been reported^[10] but examples of its application are very limited.

In a typical experiment, dab **11** (2.311g, 0.021mmol) was reacted in 10 ml D₂O and 2ml DCl (35% in D₂O) in a teflon coated steel high pressure reactor for 2 days (*i.e.* 1 cycle) at 190°C. The contents of the reactor were cooled to room temperature, and the D₂O, DCl were removed under vacuum, to obtain d₄-dab **11a**. Isotopic purities were characterised by ES⁺-MS, by comparison between observed and calculated isotope clusters, and by NMR spectroscopy. In a single run, protium-deuterium exchange

>85% was achieved with no side-product formation. Heating and cooling times are not included in reported reaction times.

This 'novel' methodology permits direct and efficient deuteration for a number of aromatic ligands. The distinct advantages of short reaction times and cheap reagents make this a convenient approach for H-D exchange. Furthermore, control experiments were performed to assess the effect of internal and external variables on the rate of H-D exchange.

3.2-2 The Achievements and Limitations of DCl in D₂O.

Our present work involved a detailed study of the applicability of this method on a range of aromatic ligands. The respective ligands were reacted according to the general procedure as detailed in section 3.2-1 (*i.e.* D₂O, DCl for 2 days at 190⁰C), and the isotopic purities of their isolated products were analysed by mass spectroscopy and by NMR spectroscopy techniques, as appropriate.

Application of this procedure to perlabel phen **2** and bpy **5** proved successful, for results see Table 3.4. In a single reaction, 2 days (*1 cycle*), at 190⁰C, D-incorporations of >90% was achieved. No subsequent reaction or purification was required and excellent yields typically in the range of 82-85% were produced.

Table 3.4: H-D exchange of ligands in D₂O, DCl at 190°C

Ref ^a	Substrate ^b	Product ^g	%H-D exchange ^g	Yield
Isolated				
3	phen <i>m/z</i> = 180	D ₈ - phen ^c <i>m/z</i> = 189	90*	82%
5	bpy ^g <i>m/z</i> = 156	D ₈ - bpy ^c <i>m/z</i> = 164	91**	92%
2	dppz <i>m/z</i> = 282	D ₄ - dppz ^c <i>m/z</i> = 286	90**	92%
		D ₁₀ - dppz ^c <i>m/z</i> = 292	90**	92%
11	dab ^f <i>m/z</i> = 108	D ₄ - dab ^c <i>m/z</i> = 190	90**	95%
17	Medab <i>m/z</i> = 122	D ₃ - Medab ^d <i>m/z</i> = 187	ring: 90, Me:0*	87%
18	diMedab <i>m/z</i> = 136	D ₂ - diMedab ^d <i>m/z</i> = 210	ring: 90, Me:0**	86%
19	diFdab ^f <i>m/z</i> = 144		Extensive Tarring	
14	diFdppz ^f <i>m/z</i> = 318		Extensive Tarring	
20	en ^f <i>m/z</i> = 58		Extensive Tarring	

^a See Figure 3.1 for structure ^b Reactions performed in high T/P batch reactor ^c D₂O, DCl at 190°C for 2 days (1 cycle) ^d D₂O, DCl at 190°C for 4 days (2 cycles) ^e D₂O, DCl at 190°C for 24 hours ^f No H-D exchange occurred. ^g Determined by mass spectrometry. *Average value (2 batches), ** Average Value (> 4 batches). Unless stated otherwise approximately equal exchange at all positions.

The effect of DCl in D₂O was examined for **2** and some interesting results were obtained, as illustrated in Figure 3.12. It was noted that reaction of **2** at 190°C for 24 hours in the presence of D₂O, DCl promoted selective H-D exchange at the H_{11,14} and H_{12,13} positions respectively, with insignificant exchange (<10%) in the remaining proton sites, thus facilitating the preparation of selectively deuterated d₄-dppz **2a**. The presence of this species was confirmed unambiguously by ES⁺-mass spectroscopy which displayed a parent ion cluster at *m/z* = 287 attributed to the [d₄-dppz-H⁺] species and by the removal of the H_{11,14} and H_{12,13} proton signals at 87.92 ppm and 88.35ppm in the ¹H NMR spectrum, respectively.

D-incorporation into the remaining phen positions; H_{2,9}, H_{3,8} and H_{4,7} protons, was achieved after prolonged reaction times of 4 days (2 cycles) at 190°C. This resulted in the formation of perdeuterated d₁₀-dppz **2c**, which displayed a parent ion cluster at $m/z = 293$ due to the [d₁₀-dppz-H⁺] species. A silent ¹H NMR spectrum further confirmed the presence of perdeuterated d₁₀-dppz.

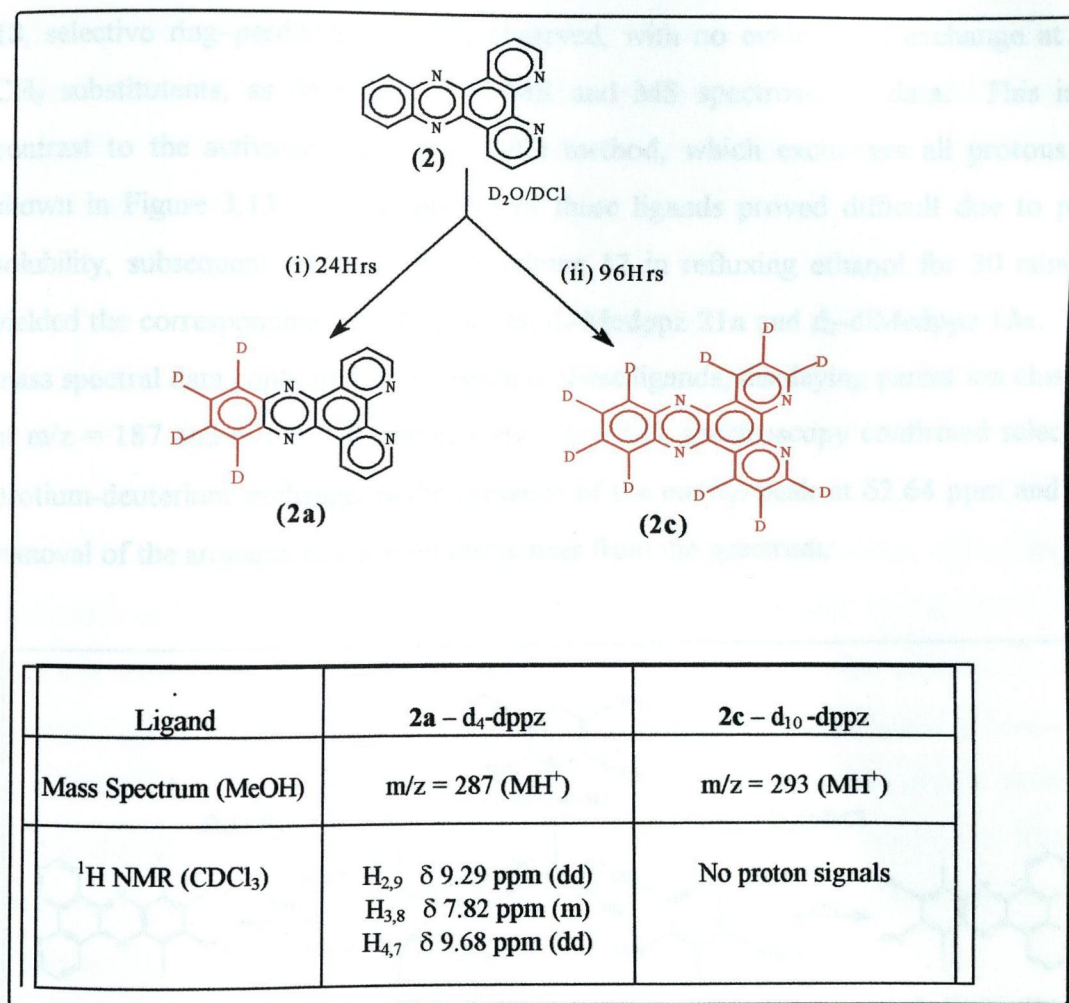


Figure 3.12: Structure and characterisation of partially (**2a**) and fully deuterated (**2c**) dppz prepared from D₂O, DCl method.

This D₂O, DCl procedure is also well suited to introduce deuterium into non-functionalised and functionalised dab derivatives. Treatment of **11**, in D₂O and DCl for 2 days at 190°C, lead to the formation of perdeuterated d₄-dab **11a**. The presence of this species was confirmed by ES⁺-MS which displayed a dominant molecular ion peak

at $m/z = 190$, attributed to the dihydrochloride $C_6D_4N_2 \cdot 2DCl_2H^+$ salt, as determined by X-Ray Crystallography. Additional evidence was attained on reacting deuterated d_4 -dab with phendione **12** in boiling ethanol for 35 minutes, producing the desired d_4 -dppz **2a** product, as characterised by both NMR and mass spectroscopy.

As a corollary, for the dab derivatives bearing methyl groups; Medab **17** and diMedab **18**, selective ring-perdeuteration was observed, with no evidence of exchange at the CH_3 substituents, as determined by NMR and MS spectroscopic data. This is in contrast to the activated palladium (Pd/C) method, which exchanges all protons, as shown in Figure 3.13. As the analysis of these ligands proved difficult due to poor solubility, subsequent reaction with phendione **12** in refluxing ethanol for 30 minutes yielded the corresponding dppz derivatives; d_3 -Medppz **21a** and d_2 -diMedppz **13a**. The mass spectral data confirmed the presence of these ligands, displaying parent ion clusters at $m/z = 187$ and $m/z = 210$, respectively. 1H NMR spectroscopy confirmed selective protium-deuterium exchange by the presence of the methyl peak at $\delta 2.64$ ppm and the removal of the aromatic ring proton resonances from the spectrum.

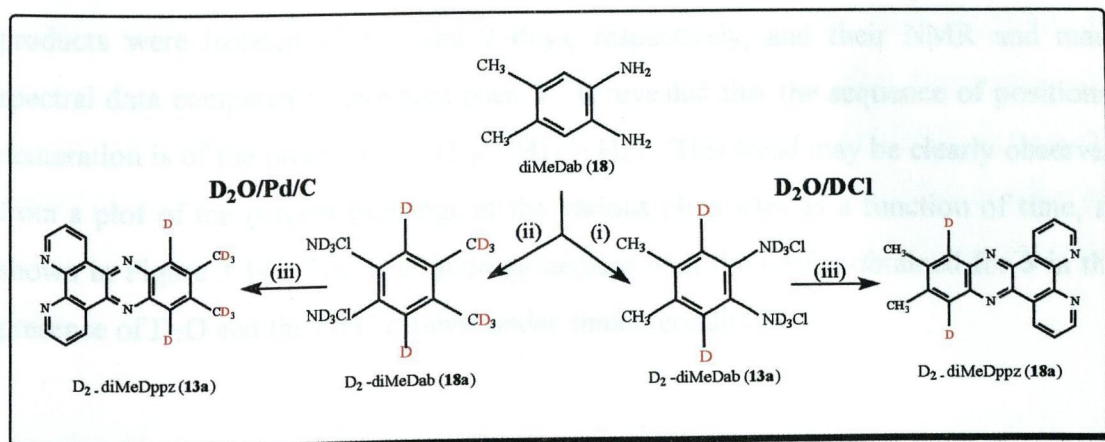


Figure 3.13: Comparison of products obtained from diMedab (**18**), where (i) D_2O/DCl at $190^\circ C$ for 2 days; (ii) Pd/C , D_2O at $190^\circ C$ for 2 days; (iii) phendione, EtOH, 30 mins.

However some of the ligands, namely diFdab **19**, diFdppz **14** and en **20**, underwent considerable decomposition (resulting in tarred black products), and gave no isotopic exchange on heating to 190°C in D₂O and DCl.

The method of deuterium chloride (DCl) in D₂O proved to be a useful tool for the preparation of deuterated ligands. It provides an alternative method of converting dab derivatives into their corresponding deuterated ligands and provides an additional synthetic route for perdeuteration of aromatic ligands (**3**, **5** and **2**). Furthermore, the production of maximum yields in minimum reaction times make this a very effective and attractive procedure.

3.2-3 Relative Kinetic Studies

3.2.3-1 Rate of Positional Preference of H-D exchange into Phen (**3**).

A detailed ¹H NMR study has been undertaken to assess both the extent and positional preference of H-D exchange for phen **3** in the presence of D₂O and DCl at 190°C. The products were isolated after 1 and 2 days, respectively, and their NMR and mass spectral data compared to protiated phen **3**. It revealed that the sequence of positional deuteration is of the order; H_{2,9} > H_{5,6} > H_{3,8} > H_{4,7}. This trend may be clearly observed from a plot of the percent exchange at the various phen sites as a function of time, as shown in Figure 3.14. This is in good agreement with the results obtained for **3** in the presence of D₂O and the Pd/C catalyst under similar conditions.

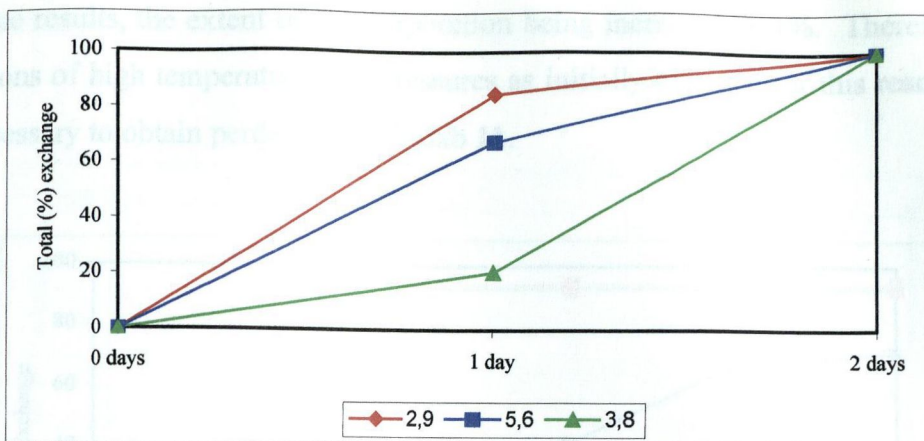


Figure 3.14: Protium-Deuterium exchange for phen (**3**) in the presence of D_2O/DCI for 2 days at $190^{\circ}C$. $H_{4,7}$ protons used as an internal standard.

3.2-4 A Study of the Reaction Conditions.

3.2.4-1 Temperature Studies.

Controlled quantitative experiments were performed to ascertain the effect, of temperature on protium-deuterium exchange in the presence of this D_2O , DCI medium. For dab **11**, a series of simultaneous reactions at room temperature, and at temperatures of $60^{\circ}C$, $100^{\circ}C$, and $190^{\circ}C$ were analysed at 24 hour intervals. D-incorporation into **11** was monitored by ES^+ -MS and 1H NMR spectroscopy. The percent deuteration was determined from the relative abundance of the peaks greater than m/z 190, as these are attributed to the $[d_4-dab]$, $[d_4-dab-H^+]$ and $[d_4-dab-D^+]$ species. The results are presented as the extent of D-incorporation into the ligand as a function of time (hrs), see Figure 3.15.

For dab **11**, it was found that on stirring at room temperature for 2 weeks in the presence of D_2O , DCI no isotopic exchange occurred. At $T = 60^{\circ}C$, limited exchange of $\sim 68\%$ was obtained for **11** on reacting for 5 days. The rate of H-D exchange increased as the temperature was increased, and the desired isotopic purity ($\sim 93\%$) was obtained on refluxing **11** in D_2O , DCI for 48 hours at $100^{\circ}C$. Alternatively, treating **11** at $190^{\circ}C$ in a Teflon coated steel high pressure reactor for 48 hours produces essentially

the same results, the extent of D-incorporation being increased by 1%. Therefore, the conditions of high temperatures and pressures as initially expected for this reaction are not necessary to obtain perdeuterated d₄-dab **11**.

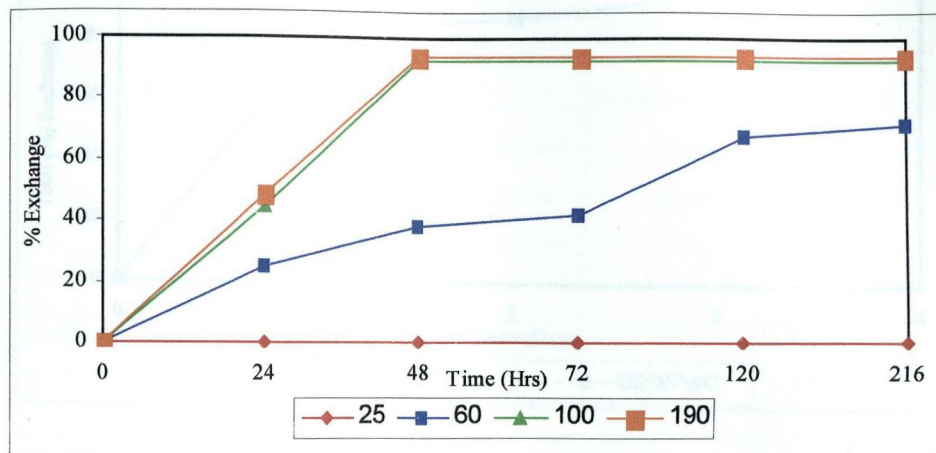


Figure 3.15: Comparison of percent (%) deuteration into dab (**11**) at 25°C, 60°C, 100°C and at elevated temperatures of 190°C.

3.3 Conclusions.

3.3-1 Comparison of Pd/C, D₂O and DCl, D₂O Methods on H-D Exchange.

To assess the relative rate of H-D exchange for both methods phen **3** was treated in the presence (i) Pd/C, D₂O and (i) DCl, D₂O at 190°C for 1,2,3 and 4 days, respectively. The products were isolated, analysed and the extent of D-incorporation into **3** is shown in Figure 3.16.

For Pd/C, D₂O method (blue line), we observe that after a single run (1 day at 190°C), D-incorporations of 84% were found for **3**. On subsequent reaction, the extent of H-D exchange steadily increases yielding the desired isotopically labeled product **3a** after 4 days at 190°C. Alternatively, for the DCl, D₂O procedure (red line) under identical conditions, the data revealed that the isotopic exchange was considerably faster

(approximately 1.2 times greater) producing perdeuterated phen **3a** after 2 days at 190°C.

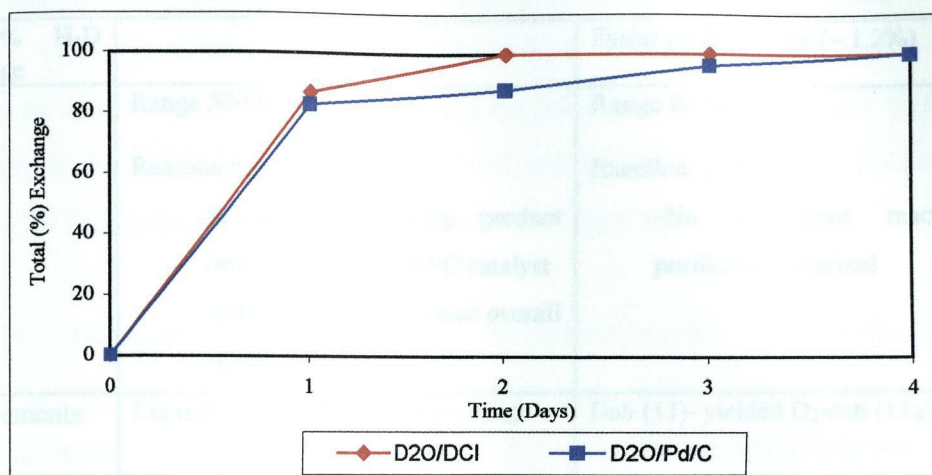


Figure 3.16: Comparison of percent (%) deuteration into phen (**3**) in the presence of Pd/C, D₂O and DCI, D₂O at 190°C.

A comparison of the rates, yields, scope and limitations of the two methods used to incorporate deuterium into a range of ligands is summarised in Table 3.5.

Table 3.5: Comparison of the methods of Deuteration used in this study.

Pd/C - Method I		DCI - Method II
Rate % H-D Exchange		Faster exchange rate (~ 1.2%)
Yields	Range 50-90% Reasonable yields -difficulty in isolating product from finely divided Pd/C catalyst -consecutive cycles reduce overall yields.	Range 80-90% Excellent yields -No subsequent reaction or purification required
Achievements	Ligands (2, 3, 5, 16)- >95% exchange diFdppz (14) – selective ring deuteration lead to exchange of 2,9 psns exclusively. Medab(17) and diMedab(18) – deuteration extended to the methyl substituents, CH₃→CD₃ formations.	Dab (11)- yielded D₄-dab (11a) dppz(2) – both selective (d₄-dppz) after 24 hrs and perdeuterated (d₁₀-dppz) after 4 days. Medab (17) and diMedab (18) – selective ring exchange to give D₃-Medab and D₂-DiMedab
Limitations	Dab (11) – no H-D exchange Fluorinated ligands 1. diFdab(19) unstable loss of fluorines lead to d₁₀-dppz 2. diFdppz(14) near selective exchange at H_{2,9} protons only. No further exchange after prolonged period. (8 days)	Fluorinated ligands 1. diFdab (19) 2. diFdppz (14) 3. en (20)
Advantages	1. Short reaction times (2 cycles) 2. Reasonable yields 3. Excellent solubility (facile to acquire NMR and MS analysis) 4. Cheap reagents	1. Shorter reaction times (1 cycle) 2. Excellent yields 3. Poor solubility (difficult to acquire NMR and MS analysis) 4. Reasonably cheap reagents

3.3-2 Concluding Remarks

In the current study, two methodologies of H-D exchange, namely (i) Pd/C, D₂O and (ii) DCl, D₂O, have been employed. While these experimental techniques are not sufficiently controllable to selectively deuterate sites, one or both of these methods in combination, as detailed in Chapter Two (Synthesis and Characterization Studies), have allowed for the preparation of a series of deuterated dppz ligands.

The Pd/C, D₂O method permits direct and efficient deuteration of polypyridyl ligands; phen **3**, bpy **5**, dppz **2**, and dpq **16**, in optimum yield with no by-product formation. However, it was found that this procedure failed to introduce any labels into dab **11**. Therefore an alternative route was developed to successfully perdeuterate the dab ligand and its derivatives. This protocol of DCl in the presence of D₂O provides a new and versatile route to attain perdeuterated (and in some cases selectively deuterated) ligands; **3**, **5**, diMedab **17**, and diMedppz **18**, in near quantitative yields. However, under the conditions studied, no H-D exchange was observed for the fluorinated ligands, diFdab **19** and diFdppz **14**, and for ethylenediamine **20**.

Both these methods proved very efficient, and reproducibly afforded the required derivatives in high yields and purity. It was found that once prepared these ligands are thermally robust and exhibit no reversible D-H exchange, even in the presence of extreme alkaline and acidic environments. Generally, the ligands are soluble in polar organic solvents such as acetonitrile and acetone and less soluble in ethanol, dichloromethane, and water.

In addition to the practical application of ligand deuteration, these exchange reactions can provide important information about the relative kinetics and ordering of deuteration within a molecule. For Pd/C, D₂O method a detailed systematic study was undertaken to ascertain the effects of the catalyst, temperature, time, and solvent on the rate and efficiency of protium-deuterium exchange. Similar studies, although to a lesser

extent, were performed for DCl, D₂O method. This allowed a comparison to be made between these methods as detailed in Table 3.5.

In general these methods are powerful, selective and have several advantages over more classical methods and the full scope of the preparative approach to deuterated aromatic substrates remains to be explored in greater detail.

3.4 References

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Chapter Four

4.0 Introduction

There continues to be great interest in the physical properties of transition metal polypyridyl compounds, due to the possibility of producing compounds that are capable of performing light-induced functions for application in photochemically driven molecular devices. Investigations in this area have focused on Ru(II) complexes as much is known about their photophysical properties^[1]. For these complexes, the single most important question that must be answered before a proper analysis of these states can be undertaken is

“Are the excited states localised or delocalised.....and, if localized, what is the precise location of the excited state electron?”.

Their excited states have been investigated by several methods. One such method, is the ‘deuterium isotope effect’, and many studies have examined this phenomenon for Ru(II) complexes^{[2][3][4]}. It is well established that the emission lifetime increases upon incorporation of deuterium into these systems^{[3][4]}. Indeed, partial deuteration, in combination with emission lifetime studies have been used to identify the location of the emitting state in Ru(II) complexes^[5]. However, the effect of deuteration on the emission lifetime of mixed ligand complexes has so far been relatively unexplored.

4.1 Aims of Research

The main aim of the present work is to put forth an experimental relationship between the excited state decay lifetime of Ru(II) dppz complexes and the position of deuterium substituents, in an attempt to locate the position of the excited state electron on the dppz ligand. In order to understand the complex photophysics of mixed ligand complexes we must first turn our attention to the parent homoleptic complexes. The effects of solvent, protium-deuterium exchange, ligand substituents and temperature on the nature of the excited state properties of these complexes have been studied.

This study is divided into three main categories;

- (i) the tris-chelated $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and $[\text{Ru}(\text{phen})_3]^{2+}$ **6** complexes and their deuterated analogues;
- (ii) the heteroleptic $[\text{Ru}(\text{phen})_2(\text{L})]^{2+}$ complexes, where $\text{L} = \text{diMedppz}$ **8**, dppz **4** and diFdppz **9**,
- (iii) the protiated complexes, **4**, **8**, and **9**, in (ii) and their corresponding selectively deuterated complexes.

In the present study, the electronic structures of several families of complexes; (**1** and **6**) and (**8**, **4**, and **9**), have been compared, based on spectroscopic studies of their protiated and selectively deuterated analogues. This protium-deuterium exchange study is hoped to provide us with some insight into the nature and deactivation of the excited states involved.

4.2 Photophysics of $[\text{Ru}(\text{L})_3]^{2+}$ Complexes

4.2-1 Introduction

A general introduction to Ru(II) complexes was reviewed in Chapter One (Introduction), herein we discuss the $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and $[\text{Ru}(\text{phen})_3]^{2+}$ **6** complexes specifically. There has been considerable interest in the nature of the lowest excited state of **1**, and to a lesser extent, in **6**. In this context, a large number of investigations^{[1][3]} have been carried out to determine whether for **1**, the promoted electron is **localised** in the π^* orbital of a single bpy ligand, as $[\text{Ru}^{\text{III}}(\text{bpy})_2(\text{bpy}^{\bullet-})]^{2+*}$, with possible rapid exchange occurring between the ligands, or if the excited electron may occupy a molecular orbital which is **delocalised** over all three ligands, as $[\text{Ru}^{\text{III}}(\text{bpy}^{-1/3\bullet-})]^{2+*}$, see Figure 4.1.

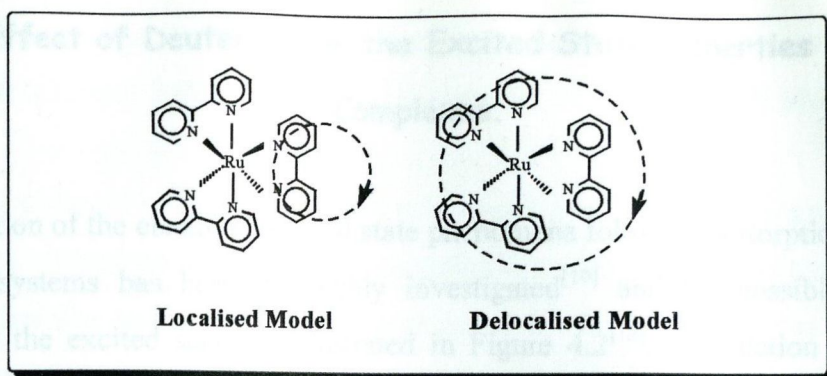


Figure 4.1: Schematic representation of localised and delocalised models of $[\text{Ru}(\text{bpy})_3]^{2+}$ complex **1**.

For complex **1**, over a wide range of temperatures, there is good spectroscopic evidence that the excited electron in the MLCT excited state is localized on a single bpy ligand^{[6][7]}. Thus, as a consequence of charge localisation, an appreciable intramolecular charge transfer component exists for the excited state decay, $[\text{Ru}^{\text{III}}(\text{bpy})_2(\text{bpy}^{\bullet-})]^{2+*} \longrightarrow [\text{Ru}^{\text{II}}(\text{bpy})_3]^{2+}$, which provides a basis for solvent effects.

Due to the structural and electronic similarities between phen and bpy, it has been assumed that these complexes are essentially the same. However, the photophysics of **6**, is unclear. As **6** is structurally more rigid, complexes whose reactivity is influenced by the ligands ability to distort could provide a situation where marked differences are observed in the chemistry of these complexes. Furthermore, the excited state properties of these complexes are very sensitive to environmental conditions, such as solvent and temperature, making evaluation difficult. It has been suggested from resonance Raman and TR³ studies^[8] of **6** that the lowest lying MLCT excited state is delocalised, $[\text{Ru}^{\text{III}}(\text{phen}^{-1/2*})]^{2+*}$, or hopping among the ligands at a rate comparable to that of vibrational frequencies ($\sim 10^{13} \text{ s}^{-1}$). However this interpretation has been debated^[9], with time-resolved infrared (TRIR) studies supporting a localized description for **6**. Despite extensive study the nature of the excited states and the debate as to whether systems **1** and **6** behave in a similar manner remains controversial.

4.2-2 Effect of Deuterium on the Excited State Properties of Ru(II) Complexes.

The deactivation of the electronic excited state phenomena following absorption of light by **1** and related systems has been thoroughly investigated^[10] and the possible deactivation pathways for the excited state are illustrated in Figure 4.2^[12]. Population of the closely spaced triplet ³MLCT states follows excitation in the visible region (¹MLCT) and occurs with high quantum yield ($\Phi_{isc} \cong 1$)^[11]. Deactivation of the excited state can occur through radiative (k_r), direct nonradiative (k_{nr}) and nonradiative via crossover into the thermally accessible d-d excited states (k_{dd}), respectively. For a more detailed discussion of the theoretical background of these deactivation processes, see Chapter One (Introduction).

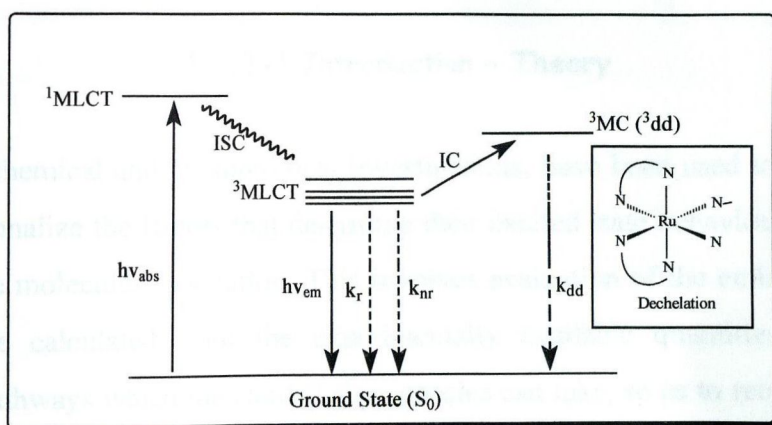


Figure 4.2: Energy level diagram showing the excited state levels of [Ru(L)₃]²⁺ complexes; hν_{abs} = absorption, hν_{em} = emission, ISC = intersystem crossing, IC = internal conversion^[12]

Of these processes, the nonradiative decay k_{nr} is of fundamental importance, and it has been reported^[10] that for **1** approximately 70% of the excited state energy is dissipated via k_{nr} at room temperature in aqueous solution. However, elucidation is difficult as the only direct measurable parameter is the excited state lifetime (τ), expressed as, $\tau^{-1} = k_r + k_{nr} + k_{dd} + k_q O_2$, and representing the sum of all the deactivation processes from the excited states back to the ground state. Generally the approach is to acquire lifetime data as a function of variable environmental factors (e.g. solvent and temperature)^{[10][13][14]}. In addition to the

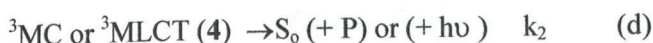
'external' variations, the study of D-incorporation on the measured lifetimes can potentially provide valuable insight into the precise mechanisms of these nonradiative processes^{[15][16]}.

According to Siebrand's theory for nonradiative transitions^{[15][16]}, high energy, anharmonic C-H stretching vibrations are important promotional modes in nonradiative decay. Upon deuteration there is a decrease in the overlap of (lower frequency) C-D modes relative to C-H modes for the same energy gap (see Figure 1.14), which decreases the nonradiative decay leading to longer excited state lifetimes. Furthermore, extended studies have revealed that there is a lifetime dependence not only on the number of deuterium substituents but also on the position^{[14][17]}.

4.2-3 Emission Studies.

4.2.3-1 Introduction - Theory

Parallel photochemical and photophysical investigations, have been used to establish and to attempt to rationalize the factors that determine their excited state behaviour so as to form a 'picture' of the molecules in solution. This involves evaluation of the emission parameters, which may be calculated from the experimentally available quantities. The various deactivation pathways which the excited state species can take, so as to return to the ground state are presented in Equations 4.1 (a) → (d):



(4.1): The decay pathways via which the excited state can return to the ground state.

We will briefly describe the methods involved in the elucidation of the emission parameters, as they will offer an insight into the behaviour of the excited states of these Ru(II) complexes, both in solution, and in the absence and presence of DNA.

Quantum yield: The fluorescence efficiency for a given molecular system may be defined and calculated from the quantum yield, Φ . This is given by the ratio of the number of photons emitted (*i.e.* k_r) by the $^3\text{MLCT}$ state relative to the number of photons absorbed by the ground state (S_0). The quantum yield (Φ_{em}), is calculated from the area under the curve of the recorded spectra relative to $[\text{Ru}(\text{bpy})_3]^{2+}$ **1**, where $[\Phi_{\text{em}} = 0.028]$ in aerated aqueous solution and $[\Phi_{\text{em}} = 0.042]$ in degassed aqueous solution at room temperature^[18]. Corrections were also made for the differing refractive indices of the solvents^[18] (see Chapter Six).

Lifetime: The ‘mean lifetime’ measured is a reflection of all the possible deactivation processes of the excited state back to the ground state, see equation 4.2.

$$\frac{1}{\tau} = k_r + k_{nr} + k_{dd} + k_q^{O_2} [O_2] \quad 4.2$$

The lifetime can then be subsequently ‘separated’ into its ‘individual deactivation components’ – k_r , k_{nr} and $k_q^{O_2}$, respectively. k_r and k_{nr} : These values are determined experimentally from the luminescent lifetime τ_{meas} and quantum yield of emission Φ_{em} , using equations 4.3(a) and 4.3(b).

$$k_r = \frac{\Phi_{\text{em}}}{\tau_{\text{meas}}} \quad 4.3 \text{ (a)}$$

$$\sum k_{nr} = \frac{1}{\tau_{\text{meas}}} - \frac{1}{\tau_r} \quad \left(\text{where } \tau_r = \frac{1}{k_r} \right) \quad 4.3 \text{ (b)}$$

$k_q^{O_2}$: For all complexes, the emission lifetimes were measured under three conditions; *aerated*, *degassed*, and *oxygenated*. For *aerated* measurements, no attempt was made to eliminate oxygen from the experiments, since the focus of the study was with a view of assessing their utility as probes for DNA. As molecular oxygen (3O_2) can quench the excited states of these complexes^[19], measurements were performed under *degassed* conditions, to ascertain additional information regarding these molecules in solution, as we observe a dramatic increase in the $\tau_{degassed}$ values. Solutions were degassed using a freeze-thaw-pump mechanism, with at least four cycles, to a pressure of 4×10^{-3} mbar, prior to use. Finally, *oxygenated* samples were prepared by bubbling a stream of O_2 , via a syringe, through the sample in a septum-covered cuvette for a minimum of 45 mins prior to use. By varying the concentration of oxygen in solution we can deduce the luminescence quenching rate constant, $k_q^{O_2}$, from the slope of a Stern-Volmer plot of $1/\tau_{meas}$ versus $[O_2]$.

4.2.3-2 Results

A study of the emission energies and lifetimes for the excited states of **1** and **6**, in a variety of solvents was investigated to attempt to rationalize the solvent dependence of their excited state properties. Steady-state and time-resolved luminescence studies for complexes **1** and **6**, typically of concentration $\sim 10^{-5}$ M, were measured at room temperature, in (a) aqueous and organic (acetonitrile, methanol, and ethanol) solutions and (b) at varying concentrations of molecular oxygen (3O_2). The luminescence data is presented in Table 4.1, and discussed according to energy of emission, quantum yield, emission lifetime and decay rate constants.

Table 4.1: Room Temperature Luminescence Maxima and Emission Quantum Yields, and Lifetime Data for complexes 1 and 6^a

Complex	$\lambda_{\text{max, em}}^b$	$\Phi_{\text{em}}^{b,d}$		$\tau_{(\text{ns})}^{c,d}$			$k_q \text{O}_2 \text{s}^{-1}$	k_r	Σk_{nr}^e
	$E_{\text{em}} (\text{cm}^{-1})$	Degass	Air	Degass	Air	Oxy ^e	$(10^9 \text{M}^{-1}\text{s}^{-1})$	(10^4s^{-1})	(10^5s^{-1})
[Ru(bpy)₃]²⁺ 1									
H ₂ O	612 (16400)	0.042 ^f	0.028	609	392	166	3.70	0.68	1.57
CH ₃ CN	608 (16450)	0.068	0.0058	764	169	42	2.48	0.89	1.21
MeOH	607 (16420)	0.030	0.0067	690	222	59	1.51	0.44	1.41
EtOH	604 (16560)	0.048	0.0077	653	238	69	1.31	0.74	1.45
[Ru(phen)₃]²⁺ 6									
H ₂ O	596 (16800)	0.058	0.032	996	497	174	4.40	0.58	0.94
CH ₃ CN	592 (16890)	0.049	0.0061	406	296	107	3.51	0.96	2.36
MeOH	590 (16950)	0.019	0.0064	300	115	72	2.09	0.63	3.26
EtOH	585 (17090)	0.002	0.0071	205	119	38	2.19	0.83	4.78

^a Measurements were performed on Cl⁻ salts (water) and PF₆⁻ salts (non-aqueous solvents). Errors: $E_{\text{em}} \pm 2\text{nm}$, $\Phi_{\text{em}} \pm 10\%$, $\tau \pm 5\%$, and $k_q \text{O}_2 \pm 7\%$. ^b $\lambda_{\text{ex}} = 440\text{nm}$. ^c $\lambda_{\text{ex}} = 337\text{nm}$, $\lambda_{\text{em}} = 590\text{nm}$ ^d Concentration of oxygen in (i) CH₃CN; (degassed – 0.0mM, air-saturated – 1.90mM, oxygenated – 9.10mM O₂) (ii) H₂O; (degassed – 0.0mM, air-saturated – 0.27mM, degassed – 1.27mM O₂), for MeOH and EtOH values see experimental section Chapter Six^[21] ^e Used as a standard for calculation of Φ_{em} values. ^f $\Sigma k_{\text{nr}} = k_{\text{nr}} + k_{\text{dd}}$.

Energy of Emission: From the recorded spectra, it is clear that the actual band shape of the spectrum is insensitive to the solvent. The major effect of solvent variation is to change the energy of the emission maximum (E_{em}). In more polar solvents, water, the maximum energy is slightly red-shifted ($\sim 4\text{ nm}$) for both complexes. This behaviour is characteristic of the expected charge-transfer nature of the excited state involved in the emission process, and may be attributed to an increased stabilization of the excited state by the re-orientation of the surrounding polar water molecules. Furthermore, as bpy has a greater σ -donor strength and a small dipole moment relative to phen^[22], the maximum energy of emission (E_{em}) should be less intense and relatively less sensitive to changes in the solvent polarity. This is observed in the change in energy, ΔE_{em} , which is noticeably larger for 6, with a value of $\Delta E_{\text{em}} \sim 290$

cm^{-1} compared to $\Delta E_{\text{em}} \sim 160 \text{ cm}^{-1}$ for **1**. We observe that the emission energies, E_{em} , in the different solvents, do not coincide well with the emission quantum yields and lifetime decays.

Quantum Yields: Comparison of the data show that the quantum yields for the bpy and phen complexes in water are similar (0.042 vs 0.058). In non-aqueous environments the bpy chelate complex **1** displays slightly higher quantum yields relative to complex **6**.

Luminescence lifetime decays. For all solvents, the emission lifetimes were recorded for degassed, aerated and oxygenated solutions and were found to display single exponential decays. On examination of the data, contrasting behaviour for the “similar” phen and bpy complexes was observed. Deactivation of the excited states to the ground state is complex and many contributing factors need to be considered, which include;

- Solvent – Polarity - OH vibrations – dielectric constant – dissociation constant
- Conjugation – π -delocalisation
- Flexibility – bpy is more flexible relative to phen
- Nature of the excited states – delocalised or localised model – MLCT/LMCT/ ^3MC
- Temperature – population of the higher lying $^3\text{MLCT}$ (4) and thermally activated ^3MC states

We will concentrate our discussion mainly on the effects of the position of the $^3\text{MLCT}$ excited state and the solvent ($\nu(\text{OH})$ vibrations) on the nature of the excited states of these complexes.

$[\text{Ru}(\text{phen})_3]^{2+}$ In water, an important factor to consider are the contributions from OH vibrations, for as they play the role of energy acceptors the nonradiative decay is increased thereby resulting in shorter emission lifetimes. It has been reported that the $^3\text{MLCT}$ states are very sensitive to their environment and due to the stabilizing effect of the more polar surroundings on the charge separated state, these $^3\text{MLCT}$ states are found to be lower in water than in acetonitrile. Typically, the lower the energy of the $^3\text{MLCT}$ state the more coupled it will be to the ground state^[23], thereby increasing k_{nr} decay to give lower emission

lifetimes. However, for complex **6**, this is not observed. As a longer emission lifetime of $\tau = 996$ ns is observed in water compared to $\tau = 406$ ns in acetonitrile. How do we account for this unusual behaviour?. It may be explained according to the position of the $^3\text{MLCT}$ excited states and the size of the energy gap, ΔE , activation barrier for the $^3\text{MLCT} \rightarrow ^3\text{MC (dd)}$ state conversion. In water, as ΔE is large (due to the lower lying $^3\text{MLCT}$ states) the crossover to the thermally activated higher lying ^3MC states is reduced so longer emission lifetimes are observed, under these conditions. Whereas in acetonitrile ΔE is reduced so population of the higher lying states is more accessible leading to greater contributions from these states and smaller emission lifetimes. In addition, the solvent isotope effect has been studied for **6**, and showed that $\nu(\text{OH})$ vibrations are of little importance, with emission lifetimes of $\tau = 990$ ns in H_2O and $\tau = 1012$ ns D_2O , respectively^[24]. For **6**, in the presence of ethanol and methanol solvents, the decreased excited state lifetimes are related to an increase in the emission energies, E_{em} .

$[\text{Ru}(\text{bpy}_3)]^{2+}$ As the longest emission lifetime is observed in acetonitrile with $\tau = 764$ ns (compared to $\tau = 609$ ns in water), the proposed model for **6** does not hold here. Furthermore, in contrast to the phen complex there is a large isotope effect observed in water with $\tau = 619$ ns in water compared to $\tau = 1090$ ns in heavy water^[17]. We propose that for **1**, as observed for complex **6**, that the $^3\text{MLCT}$ state has a lower energy in water than in acetonitrile, and that the shorter emission lifetime observed in water is attributed to the greater importance of the isotope effect. In addition, the more open structure and flexible nature of the bpy relative to phen complex may also be of importance. For **1** decreased excited state lifetimes were observed in polar solvents, which is related to a very small increase in the emission energies, E_{em} .

In general, the bpy complexes exhibit longer emission lifetimes compared to the phen complexes, under the conditions studied. Clearly, the subtle differences between these complexes is much more complex than originally conjured, and may imply that their emission is controlled by different mechanisms. A full accounting of the effect of solvent on the emission lifetime of these complexes requires extensive analysis.

Rate constants: The calculated radiative and nonradiative rate constants for **1** and **6** are presented in Table 4.1. For these complexes the emitting MLCT species are predominantly triplet in character and exhibit radiative rate constants, $k_r \sim 10^5 \text{ s}^{-1}$, which reflect the formally spin-forbidden character of the emission. There is little significant change in k_r which is relatively solvent independent and displays no apparent vibrations with emission energy. For **1**, in aqueous solution our value of $k_r = 6.79 \times 10^5 \text{ s}^{-1}$ compares with the reported value of $k_r = 6.9 \times 10^5 \text{ s}^{-1}$ [3]. The nonradiative decay constants $\sum k_{nr}$, where $\sum k_{nr} = k_{nr} + k_{dd}$, vary and were found to increase as the emission energy decreases. Furthermore, the nonradiative process is less important for **6** in water, on comparing $\sum k_{nr} = 0.94$ and $1.55 \times 10^6 \text{ s}^{-1}$ for Ru(phen)_3 and Ru(bpy)_3 , respectively. A plot of $\ln k_{nr}$ -vs- E_{em} is shown in Figure 4.3(A) for complex **1** and in Figure 4.3(B) for complex **6**. It reveals that the energy-gap law appears to hold for complex **6**, but does not hold for the related bpy complex **1**. In addition, the $\sum k_{nr}$ values are larger for **6** (with the exception of water), which may reflect a smaller energy gap between the $^3\text{MLCT}$ and $^3\text{MLCT} (4)$ and/or ^3MC states and greater solvent effects for this complex.

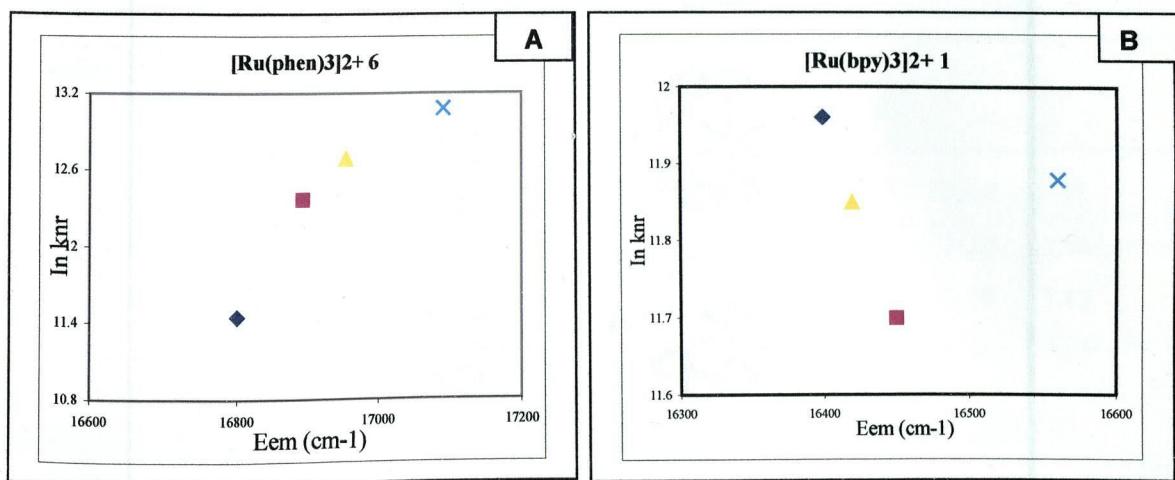


Figure 4.3: Plots of $\ln k_{nr}$ - vs - E_{em} for complexes (A) $[\text{Ru(phen)}_3]^{2+}$ **1** and (B) $[\text{Ru(bpy)}_3]^{2+}$ **6** in a range of solvents (where $\blacklozenge$ = H_2O , $\blacksquare$ = CH_3CN , $\blacktriangle$ = MeOH , and $\times$ = EtOH) at $T = 23^\circ\text{C} \pm 2^\circ\text{C}$

Rate constants for oxygen quenching $k_q^{\text{O}_2}$, for **1** and **6**, were evaluated from the slope of a Stern-Volmer plot of $1/\tau$ -vs- $[\text{O}_2]$. They were found to vary with the oxidation potential of

the ligands *i.e.* phen < bpy, with the more oxidizing ligand (bpy) exhibiting lower quenching constants, for the solvents examined. We found that E_{em} , Φ_{em} , τ_{meas} and Σk_{nr} which account for > 90% of the decay process, vary with solvent, while there is little significant change in k_r , respectively.

4.2-4 Effect of Ligand Deuteration on the Excited State Properties of $[Ru(L)_3]^{2+}$ Complexes.

4.2.4-1 The Current Study.

A systematic investigation of the parent complexes, **1** and **6**, with varying degrees of deuterium incorporation, $[Ru(L)_{3-x}(d_8-L)_x]^{2+}$, where L = bpy / phen and x = 0 - 3 (*i.e.* none, one, two and three deuterated ligands), has been undertaken as illustrated in Figure 4.4.

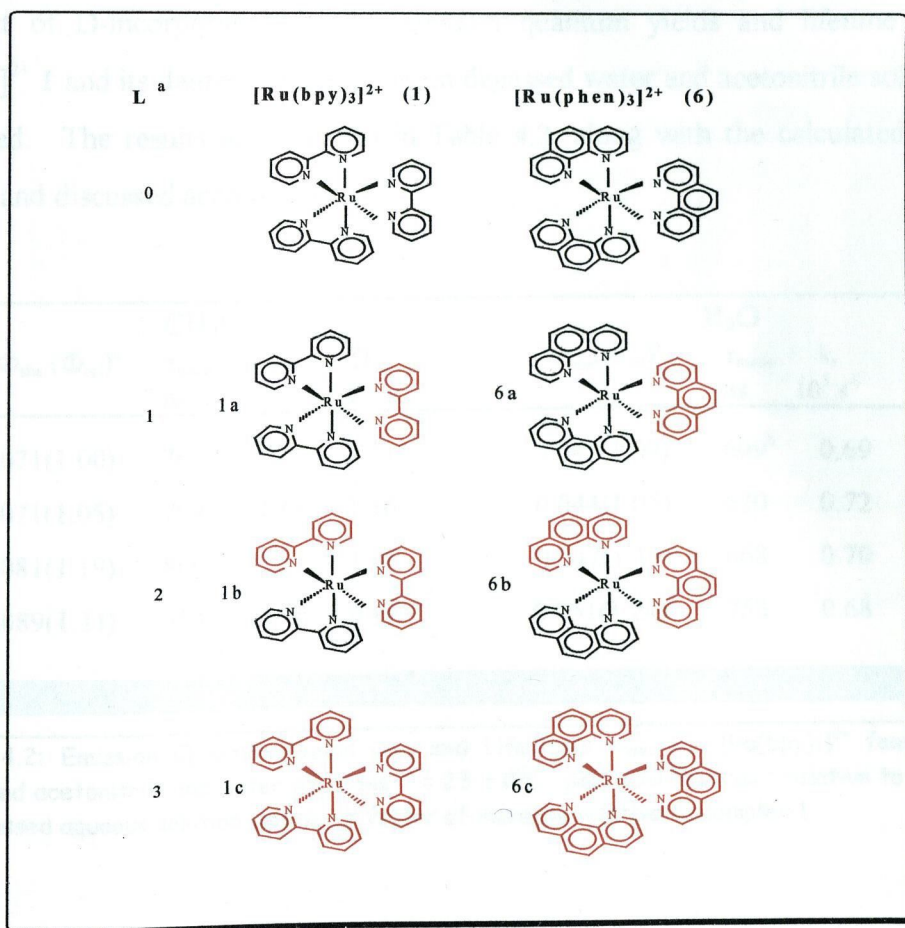


Figure 4.4: Schematic illustration for $[Ru(bpy)_3]^{2+}$ (**1**) and $[Ru(phen)_3]^{2+}$ (**6**) complexes and their deuterated analogues. L^a corresponds to the number of deuterated bpy/phen ligands in the complex

Herein, the effect of ligand deuteration on the photophysical behaviour of complexes **1** and **6**, in both aqueous and in acetonitrile solutions, was measured by steady-state, time-resolved luminescence, and temperature-dependent lifetime emission studies.

4.2.4-2 Luminescence Lifetime Studies.

Variations in the position and extent of deuterium incorporation on the excited state lifetime and quantum yields for complexes, (**1-1c**) and (**6-6c**), were investigated. Although, the effects of ligand deuteration are relatively small, it is appropriate to investigate the issue of selective deuteration effects for these systems.

• Bpy (**1**)

The effect of D-incorporation on the emission quantum yields and lifetime decays for $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and its deuterated analogues, in degassed water and acetonitrile solutions were investigated. The results are tabulated in Table 4.2, along with the calculated decay rate constants and discussed accordingly.

Complex	$\Phi_{\text{abs}}(\Phi_{\text{rel}})^a$	CH ₃ CN			$\Phi_{\text{abs}}(\Phi_{\text{rel}})^a$	H ₂ O		
		τ_{meas} ns	k_r 10^5 s^{-1}	Σk_{nr} 10^5 s^{-1}		τ_{meas} ns	k_r 10^5 s^{-1}	Σk_{nr} 10^5 s^{-1}
1	0.071(1.00)	764	0.89	1.21	0.042(1.00)	609 ^b	0.69	1.57
1a	0.071(1.05)	798	0.89	1.16	0.044(1.05)	620	0.72	1.54
1b	0.081(1.19)	866	0.94	1.06	0.047(1.12)	668	0.70	1.43
1c	0.089(1.31)	953	0.94	0.96	0.051(1.24)	753	0.68	1.26
% Increase	31	25			24	24		

Table 4.2: Emission Quantum Yields Φ_{abs} and Lifetimes τ_{meas} for $[\text{Ru}(\text{bpy})_3]^{2+}$ family (**1-1c**) in degassed acetonitrile and water solutions $T = 23 \pm 2^\circ\text{C}$. $[\text{Ru}] \sim 10^{-5}\text{M}$ ^a Φ_{abs} = relative to $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** in degassed aqueous solution, and Φ_{rel} = factor of increase relative to complex **1**

Quantum Yields. Two quantum yield values are reported in Table 4.2. Φ_{abs} is the calculated quantum yield by comparison to $[\text{Ru}(\text{bpy})_3]^{2+}$ **1**, with $[\Phi = 0.042]$ in degassed aqueous solutions, and Φ_{rel} represents the factor of increase with regard to the value obtained for the protiated complex **1**. The emission quantum yields were found to increase on increasing deuteration of **1**, with a relative increase of 31% in acetonitrile and 24% in water, respectively. Figure 4.5, displays plots of Φ_{rel} as a function of deuteration for the series of complexes (**1-1c**), in water and acetonitrile solutions. The percent increase in Φ_{rel} is also included in the plot. On progressive D-incorporation on moving across the series, it was found that the rate of increase in Φ_{rel} is not linear, as shown by the line of best fit for both solvents.

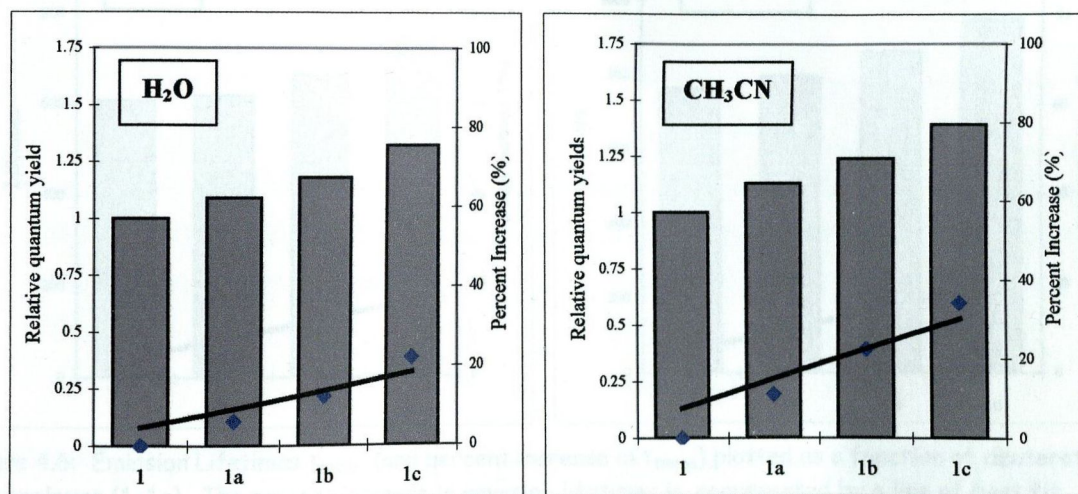


Figure 4.5: Relative quantum yields Φ_{rel} plotted as a function of deuteration of complexes (**1-1c**), in degassed water and acetonitrile solutions. The percent increase in Φ_{rel} represented by a line of best fit.

Lifetimes. The protiated complex **1** in degassed aqueous solution, at room temperature, gave an emission lifetime of $\tau = 609$ ns, while the perdeuterated complex **1c** gave a value of $\tau = 753$ ns. These values are in agreement with Kincaid *et al*^[2], who reported that perdeuteration of **1** increases the lifetime of the ³MLCT state from 600 ns to 790 ns. The partially deuterated analogues, **1a** and **1b** gave intermediate excited state lifetimes of $\tau = 620$ ns and 668 ns, respectively. A similar trend was observed upon dissolution in acetonitrile, whereby

perdeuteration of **1** increased the lifetime from 764 ns to 953 ns, with intermediate lifetime values of $\tau = 798$ and 866 ns, respectively, for the partially deuterated complexes.

For complexes (**1-1c**), the emission lifetimes, τ_{meas} were plotted as a function of H-D exchange and are illustrated in Figure 4.6. The percent increase in τ_{meas} (relative to the protiated complex **1**), is also included in the plot. On moving across the series there is an increase in τ_{meas} , with an average value of $\sim 24\%$ in water and acetonitrile solutions. However, the rate of increase is non-linear as shown by the line of best fit for both plots.

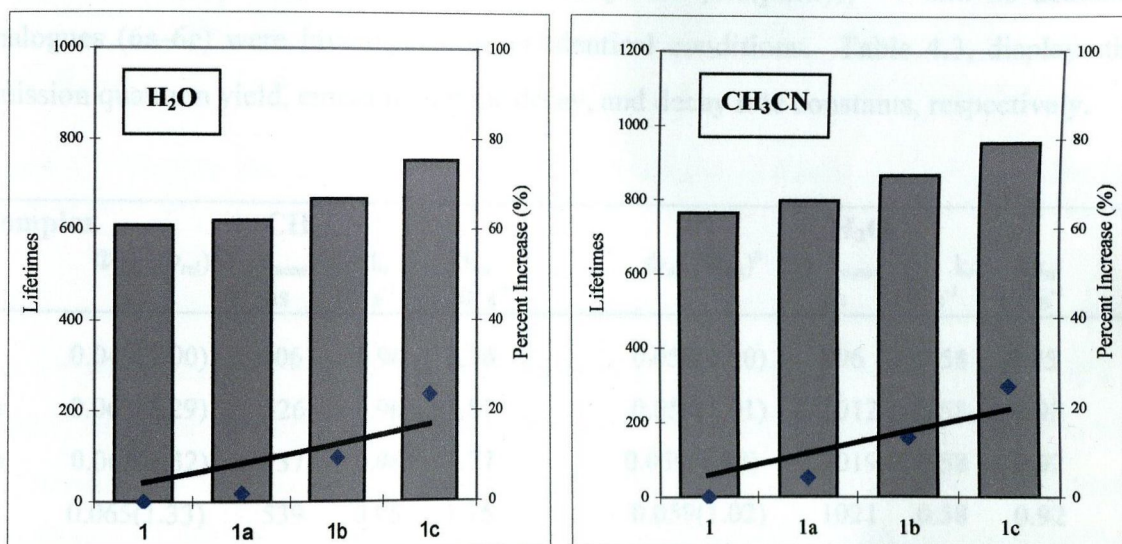


Figure 4.6: Emission Lifetimes τ_{meas} (and percent increase in τ_{meas}) plotted as a function of deuteration of complexes (**1-1c**). The percent increase in emission lifetimes is represented by a line of best fit.

Decay Rate Constants: The calculated radiative and nonradiative decay rate constants for complexes (**1-1c**) are presented in Table 4.2. They display small and invariant radiative constants, $k_r \sim 10^4 \text{ s}^{-1}$, which are independent of H-D exchange. In contrast, the nonradiative decay constants $\sum k_{\text{nr}}$ vary and were found to decrease as the extent of deuteration was increased. The values are slightly larger under aqueous conditions. These results confirm that deuteration only affects the nonradiative decay processes for these complexes.

On comparing the two solvents, there appears to be no significant differences in the relative increases in the quantum yields Φ_{em} (24% - H₂O and 31% - CH₃CN) and emission lifetimes τ_{meas} (24% - H₂O and CH₃CN) on D-incorporation into complex 1. The observed behaviour is consistent with a model where the electron is located on either a h₈-bpy 5 or d₈-bpy 5a ligand in an approximately statistical fashion.

• Phen

The emission quantum yields and lifetime decays for [Ru(phen)₃]²⁺ 6 and its deuterated analogues (6a-6c) were investigated, under identical conditions. Table 4.3, displays their emission quantum yield, emission lifetime decay, and decay rate constants, respectively.

Complex	CH ₃ CN				H ₂ O			
	$\Phi_{abs}(\Phi_{rel})^a$	τ_{meas} ns	k_r 10 ⁵ s ⁻¹	Σk_{nr} 10 ⁵ s ⁻¹	$\Phi_{abs}(\Phi_{rel})^a$	τ_{meas} ns	k_r 10 ⁵ s ⁻¹	Σk_{nr} 10 ⁵ s ⁻¹
6	0.049(1.00)	406	0.96	2.36	0.058(1.00)	996	0.58	0.95
6a	0.063(1.29)	526	0.96	1.81	0.059(1.01)	1012	0.58	0.93
6b	0.065(1.32)	537	0.96	1.77	0.059(1.02)	1019	0.58	0.92
6c	0.065(1.33)	539	0.96	1.75	0.059(1.02)	1021	0.58	0.92
% Increase	33	33			2	2		

Table 4.3: Emission Quantum Yields Φ_{abs} and Lifetimes τ_{meas} for [Ru(phen)₃]²⁺ family (6-6c) in degassed acetonitrile and water solutions T = 23 ± 2°C. [Ru] ~ 10⁻⁵M ^a Φ_{abs} = relative to [Ru(bpy)₃]²⁺ 1 in degassed aqueous solution, and Φ_{rel} = factor of increase relative to complex 6

Quantum Yields. For the series of complexes (6-6c), the emission quantum yield Φ_{rel} increases on increasing deuteration of the complexes. Figure 4.7, displays plots of Φ_{rel} (and percent increase in Φ_{rel}) as a function of deuteration of 6, under water and acetonitrile conditions. On moving across the series the emission quantum yield increases but the percent increase is not linear. Furthermore, there is a marked difference in the percent increase in Φ_{rel} , with a 33% increase found in acetonitrile and only a 3% increase observed in water.

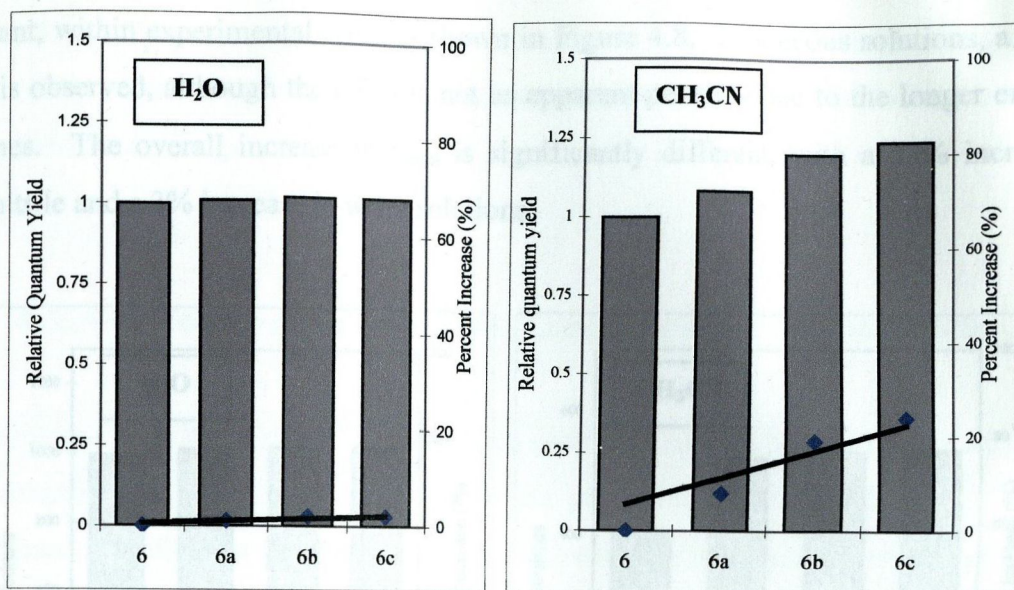


Figure 4.7: Relative quantum yields Φ_{rel} plotted as a function of deuteration of complexes (6-6c), in degassed water and acetonitrile solutions. The percent increase in Φ_{rel} represented by a line of best fit.

Lifetimes. In aqueous solutions, the emission lifetime τ_{meas} of the natural abundance complex **6** is $\tau = 996$ ns and is in agreement with O'Reilly *et al*^[26], who reported a value of $\tau = 990$ ns. Perdeuteration of complex **6** increases the lifetime of the 3MLCT state, from 990 ns to 1021 ns, respectively in aqueous solution at room temperature. The selectively deuterated complexes, **6a** and **6b**, display lifetime values of $\tau = 620$ and 668 ns, as tabulated in Table 4.3. In acetonitrile, the emission lifetimes are much shorter (factor of ~ 2), compared to the values obtained in water. The protiated complex **6** gave the smallest emission lifetime of $\tau = 406$ ns, with the partially deuterated complexes, **6a** and **6b** displaying longer lifetimes of $\tau = 526$ and 537 ns. Finally, the largest emission lifetime with a value of $\tau = 539$ ns was observed for the perdeuterated complex **6c**.

In Figure 4.8, the emission lifetime τ_{meas} (and percent increase in τ_{meas}) was plotted as a function of H-D exchange, for complexes (6a-6c) in water and acetonitrile conditions. In acetonitrile, there is a marked initial increase in τ_{meas} on H-D exchange of one ligand in the complex, **6a**. However, subsequent deuteration appears to have no significant effect on the

emission. It was found that the values of τ_{meas} for the three deuterated complexes are invariant, within experimental error, as shown in Figure 4.8. In aqueous solutions, a similar trend is observed, although the effect is not as apparent possibly due to the longer emission lifetimes. The overall increase in τ_{meas} is significantly different, with a 33% increase in acetonitrile and a 3% increase in water solutions.

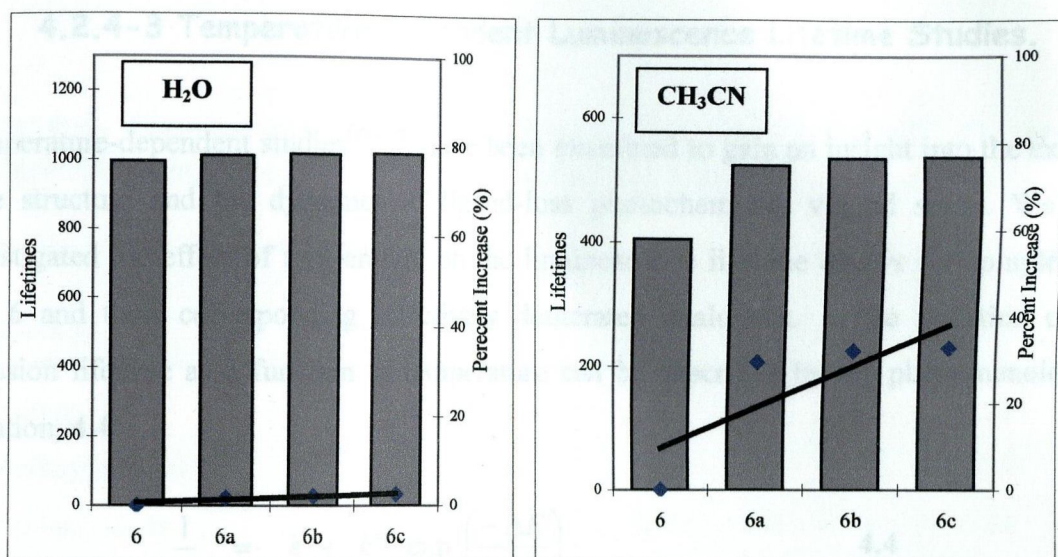


Figure 4.8: Emission Lifetimes τ_{meas} (and percent increase in τ_{meas}) was plotted as a function of deuteration of complexes (6–6c). The percent increase in emission lifetimes is represented by a line of best fit.

Decay Rate Constants: These complexes display small radiative constants, $k_r \sim 10^5 \text{ s}^{-1}$, which show no variation as a function of D-incorporation into the system. In contrast, the nonradiative decay $\sum k_{nr}$ decreases as the extent of deuterium is increased for **6**. In water, the decrease in k_{nr} is very small and appears to be almost constant. In acetonitrile, the nonradiative decays rates are greater by a factor of ~ 2 than those displayed in water, reflecting the smaller emission lifetimes observed for **6** in acetonitrile.

In summary, the emission lifetimes τ_{meas} and quantum yields Φ_{abs} for the selectively deuterated analogues of **1** and **6** have been examined. For these complexes, there is an increase in the values of Φ_{rel} and τ_{meas} on progressive deuteration into these systems. For **1**, the relative quantum yields and the emission lifetime decays are closely related, with a 28% increase for Φ_{rel} compared to 27% for τ_{meas} , in water and acetonitrile solutions. However, the

situation is more complex for **6**. In acetonitrile there is a 33% increase for Φ_{rel} and τ_{meas} compared to a 3% increase in both emission parameters in aqueous solutions. This suggests that for **6** the nature of the deactivation of the excited states occurs via different mechanisms for the solvents investigated.

4.2.4-3 Temperature Dependent Luminescence Lifetime Studies.

Temperature-dependent studies^{[27][28]} have been examined to gain an insight into the excited-state structure and the dynamics of ligand-loss photochemistry via dd states. We have investigated the effect of temperature on the luminescence lifetime decays for complexes **1** and **6** and their corresponding selectively deuterated analogues. The variation of the emission lifetime as a function of temperature can be described by the phenomenological equation, 4.4

$$\frac{1}{\tau} = k + k' \exp \left(\frac{-\Delta E}{RT} \right) \quad 4.4$$

This equation contains a temperature-dependent term ($k' \exp (-\Delta E / RT)$) and a temperature-independent (k) term. The latter term is assumed to be equal to the sum of the radiative and nonradiative constants ($k_r + k_{nr}$) associated with the average $^3\text{MLCT}$ state. The temperature-dependent term(s) correspond(s) to the thermally activated crossing to the ^3MC state. The energy gap ΔE , has been interpreted as the activation barrier for the $^3\text{MLCT} \rightarrow ^3\text{MC}$ (dd) state conversion and k' as the rate of barrier crossing.

• Bpy

Luminescence lifetime studies for **1** and its deuterated analogues (**1a-1c**) were performed in degassed aqueous solutions over the temperature range 4⁰C to 44⁰C. The results are tabulated in Table 4.4, also included are;

(i) % increase in τ_{meas} on moving down the table (blue values)

e.g. At $T = 4^{\circ}\text{C}$, $\left(\frac{1c-1}{1}\right) = \left(\frac{896-723}{723}\right)\% = 23\%$ and

(ii) % decrease in τ_{meas} on moving across the table (yellow values)

e.g. For complex **1**, $\left(\frac{4^{\circ}\text{C}-44^{\circ}\text{C}}{4^{\circ}\text{C}}\right) = \left(\frac{723-504}{723}\right)\% = 30\%$.

Table 4.4: Luminescence measured Lifetimes (ns) for $[\text{Ru}(\text{bpy})_3]^{2+}$ (1**) and its Deuterated analogues (**1a-1c**) in Degassed Aqueous solution^a.**

Compound	τ_{meas} (ns)					% Decrease ^c
	$T = 4^{\circ}\text{C}$	14°C	24°C	34°C	44°C	
1 $[\text{Ru}(\text{bpy})_3]^{2+}$	723	678	619	563	504	30
1a $[\text{Ru}(\text{bpy})_2(\text{d}_8\text{-bpy})]^{2+}$	754	716	646	600	540	28
1b $[\text{Ru}(\text{bpy})(\text{d}_8\text{-bpy})_2]^{2+}$	810	757	682	633	564	30
1c $[\text{Ru}(\text{d}_8\text{-bpy})_3]^{2+}$	896	831	753	674	604	33
% Increase ^b	23	22	21	20	19	

^a $\lambda_{\text{ex}} = 337\text{nm}$, $\lambda_{\text{em}} = 590\text{nm}$. ^b Percent (%) increase relative to the protiated complex at that temperature.

^c Percent (%) decrease relative to the same complex at 4°C

Over the temperature range monitored, the protiated complex **1** displays the smallest emission lifetime values, while the perdeuterated complex **1c** results in the largest observed lifetime values as expected. The effects of temperature and deuteration on the emission lifetimes of **1** are illustrated in the form of a three dimensional barchart, see Figure 4.9. For these complexes, two trends are observed for τ_{meas} ;

(1) A decrease on moving from $4^{\circ}\text{C} \rightarrow 44^{\circ}\text{C}$, and

(2) An increase in going from complexes **1** $\rightarrow$ **1c**.

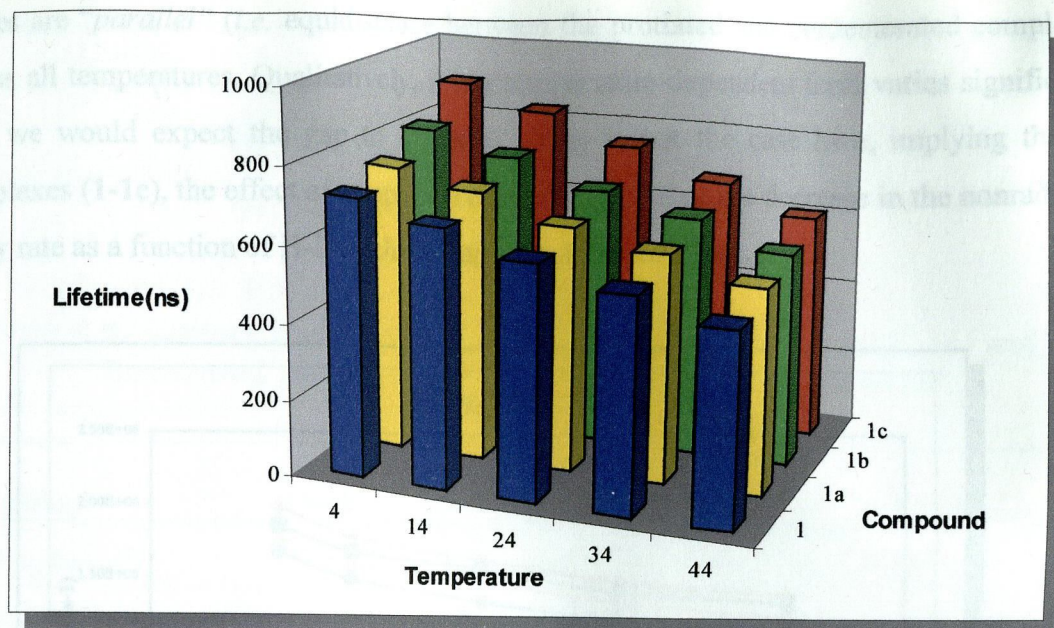


Figure 4.9: Emission lifetimes (τ_{meas} , ns) at different temperatures for **1** and the deuterated derivatives (compound **1**: protiated, **1a**: one ligand deuterated, **1b**: two ligands deuterated, **1c**: perdeuterated)

- (1) There is a gradual **decrease** in τ_{meas} as the temperature is increased from 4°C to 44°C. For all complexes the decrease is essentially equal, with an average value of *ca.* 30% for the temperature range monitored. This may be attributed to the thermal population of the higher lying ^3MC ('dd') and/or $^3\text{MLCT}$ (4) states which undergo rapid radiationless decay resulting in reduced emission lifetimes τ_{meas} at higher temperatures.
- (2) The value of τ_{meas} **increases** on increasing the extent of deuteration into these complexes, on going from **1** to **1c**. Across all temperatures, there is little variation in the percent increase in τ_{meas} for all four complexes, with an average increase of 21% respectively.

According to equation 4.4, a plot of $1/\tau$ -vs- $1/T$ allows the determination of the three variables; k , k^0 , and ΔE , respectively. Figure 4.10 displays a series of plots for complexes (**1**-**1c**). However these plots are nonlinear, indicating that the data are not fit by a simple Arrhenius type equation. Although, we were unsuccessful in fitting the curves a number of observations could be deduced from the data. On D-incorporation into **1**, we observe that the

curves are “parallel” (i.e. equidistance between the protiated and perdeuterated complexes) across all temperatures. Qualitatively, if the temperature dependent term varies significantly then we would expect the gap to increase. This is not the case here, implying that for complexes (1-1c), the effect of temperature is small, and that the decrease in the nonradiative decay rate as a function of H-D exchange appears to be constant.

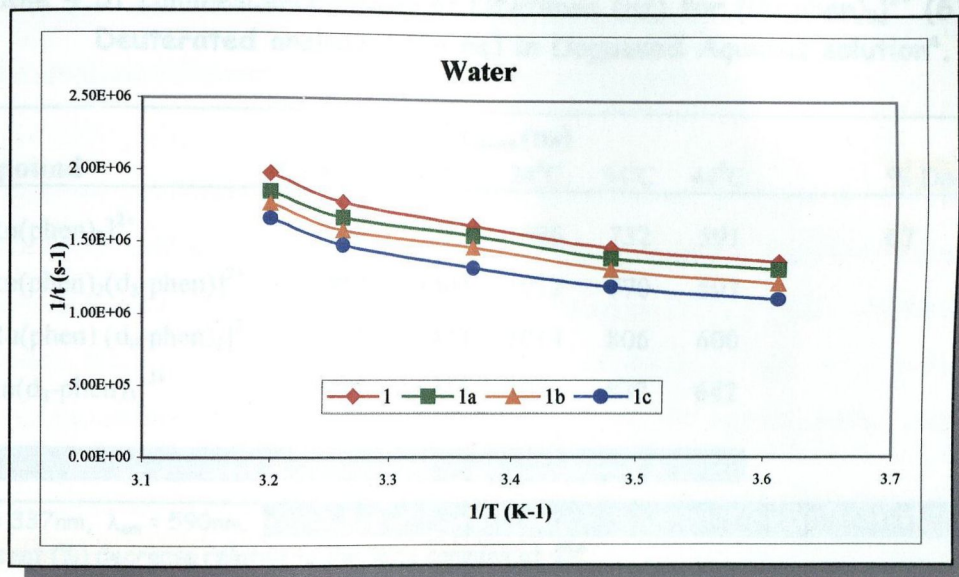


Figure 4.10: Plot of $1/\tau$ vs $1/T$ for the series of complexes (1-1c) at different temperatures in degassed aqueous solutions.

• Phen

The luminescence emission lifetimes for the protiated $[\text{Ru}(\text{phen})_3]^{2+}$ **6** and its deuterated analogues, (**6a-6c**) have also been examined, under identical conditions. The results in degassed aqueous solution, monitored between 4°C and 44°C, are compiled in Table 4.5, along with the relative percent increase and decrease in the values of the emission lifetimes τ_{meas} .

As can be seen, the smallest lifetimes are displayed for the protiated complex **6**, while the perdeuterated complex **6c** gives the largest emission lifetimes, for the range of temperatures monitored. The effects of temperature and extent of deuteration on the emission parameters

are presented as a three dimensional barchart as shown in Figure 4.11. As for bpy, two trends are observed for τ_{meas} :

- (1) A decrease on moving from 4^oC → 44^oC
- (2) An increase in going from complex 6 → 6c

Table 4.5: Luminescence measured Lifetimes (ns) for [Ru(phen)₃]²⁺ (6) and its Deuterated analogues (6a-6c) in Degassed Aqueous solution^a.

Compound	T = 4 ^o C	τ_{meas} (ns)				% Decrease ^c
		14 ^o C	24 ^o C	34 ^o C	44 ^o C	
6 [Ru(phen) ₃] ²⁺	1768	1355	996	732	591	67
6a [Ru(phen) ₂ (d ₈ -phen)] ²⁺	1853	1403	1012	770	597	68
6b [Ru(phen)(d ₈ -phen) ₂] ²⁺	1901	1423	1019	806	600	67
6c [Ru(d ₈ -phen) ₃] ²⁺	1948	1436	1021	832	642	68
% Increase ^b	10	6	3	14	9	

^a $\lambda_{\text{ex}} = 337\text{nm}$, $\lambda_{\text{em}} = 590\text{nm}$. ^b Percent (%) increase relative to the protiated complex at that temperature.
^c Percent (%) decrease relative to the same complex at 4^oC

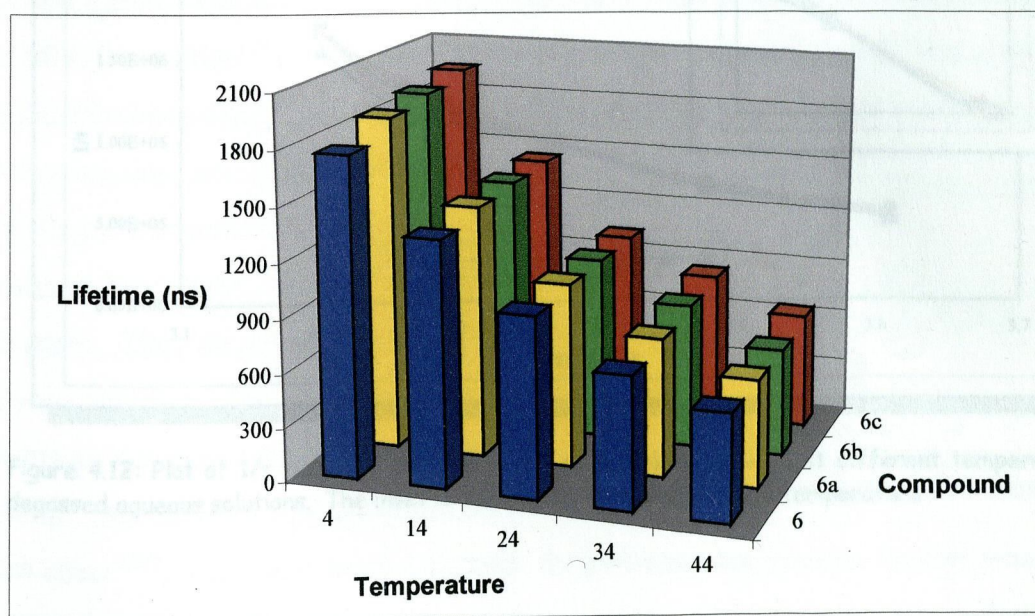


Figure 4.11: Emission lifetimes (τ_{meas} , ns) at different temperatures for 6 and the deuterated derivatives (compound 6: protiated, 6a: one ligand deuterated, 6b: two ligands deuterated, 6c: perdeuterated)

- (1) There is a significant **decrease** in τ_{meas} as the temperature is increased from 4⁰C through to 44⁰C. For all complexes, the decrease in the emission lifetimes is invariant, with an average value of 67% across the temperature range monitored.
- (2) On increased ligand deuteration of **6**, τ_{meas} exhibits a small **increase** for all temperatures. However, the relative increase in τ_{meas} across the temperature range is not constant with the smallest difference (~2%) observed at room temperature.

For the series of complexes, plots of $1/\tau$ -vs- $1/T$ are shown in Figure 4.12 and display non-uniform behaviour across the series, most notably at $T = 24^{\circ}\text{C}$. This is in direct contrast to the plots obtained for the corresponding bpy complexes (**1-1c**), under identical conditions. At present, the unusual behaviour of the phen complexes as a function of both temperature and extent of deuteration cannot be readily explained and will require further study.

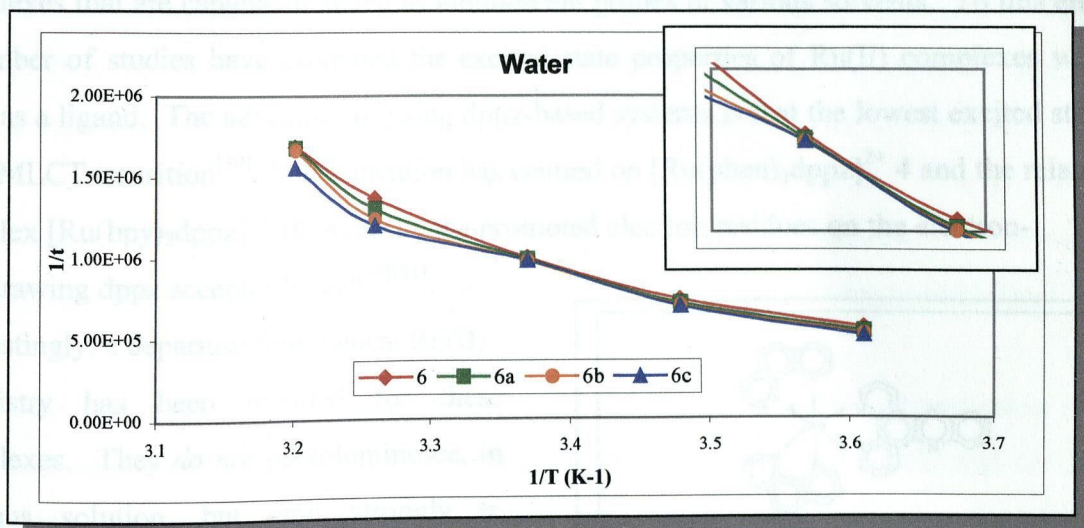


Figure 4.12: Plot of $1/\tau$ -vs- $1/T$ for the series of complexes (**6-6c**) at different temperatures in degassed aqueous solutions. The inset is the results obtained at room temperature

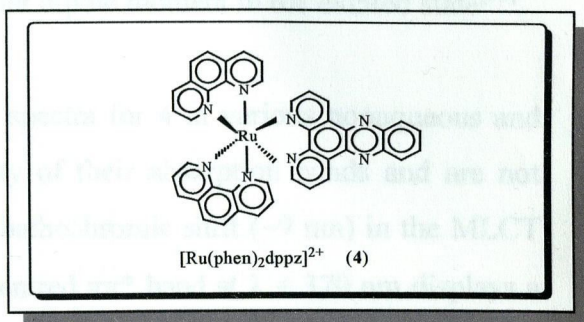
In summary, the quantum yields Φ_{abs} and excited state lifetimes τ_{meas} for both series of complexes, (1-1a) and (6-6a), were found to decrease as the temperature is increased from 4⁰C to 44⁰C, which is in accordance with the literature^[29]. The effect was more pronounced for the phen complexes which displayed a 66% decrease compared to 33% for the bpy complexes. In addition, Φ_{abs} , and τ_{meas} were found to increase as the extent of deuteration into these complexes was increased. A greater enhancement of 22% was displayed for the bpy series while only a 2% increase was noted for the phen complexes.

4.3 Optical Properties of [Ru(phen)₂dppz]²⁺ 4 and Related Complexes.

4.3-1 Introduction.

There has been an increasing demand for the design and development of transition metal complexes that are capable of acting as luminescent probes in various solvents. To this end, a number of studies have examined the excited state properties of Ru(II) complexes with dppz as a ligand. The advantage of using dppz-based systems is that the lowest excited state is an MLCT transition^[30]. Much attention has centred on [Ru(phen)₂dppz]²⁺ 4 and the related complex [Ru(bpy)₂dppz]²⁺ 10, whereby the promoted electron resides on the electron-withdrawing dppz acceptor ligand^{[30][31]}.

Interestingly, a departure from typical Ru(II) chemistry has been reported for these complexes. They *do not* photoluminesce, in aqueous solution, but emit strongly in nonaqueous solvents, such as acetonitrile and alcohols. This has been denoted as the “light switch effect”^[32]. Despite, extensive research the photophysical reasons for this behaviour is not yet fully understood. Accumulated evidence points to hydrogen bonding and/or excited state proton transfer between the solvent and the phenazine nitrogens as the mechanism of deactivation of the excited state of these ruthenium complexes^{[32][33][34]}.



In principle, the local environment surrounding the phenazine nitrogen portion of **4** should directly influence the spectroscopic properties of the complex, in terms of the hydrogen-bonding ability and polarity of the medium. Upon excitation, the excited electron is localised on the dppz ligand to form, $[\text{Ru}^{\text{III}}(\text{phen})_2(\text{dppz}^{\bullet-})]^{2+*}$. In this state, it has been postulated^[32] that the electron on the reduced dppz ligand is located on the non-coordinating phenazine nitrogen atoms, which would significantly increase their basicity and allow proton transfer or H-bond formation.

4.3-2 Photophysical Study of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**

To examine the unique luminescent characteristics of **4** we must first understand how the local environment modulates the spectroscopic properties. As a preliminary to understanding this phenomena, a detailed photophysical study of **4**, in a wide range of solvents has been examined to probe the local environment. The results are tabulated in Table 4.6, and are discussed according to their absorption, emission, and lifetime decay parameters. Also considered in the discussion are the solvents parameters, E_{T} and α . α , corresponds solely to the hydrogen bonding ability of the solvent, and E_{T} , is a measure of the solvents ability to solvate and stabilize charged molecules and to donate hydrogen bonds. The E_{T} value corresponds to the maximum energy absorption for a solvatochromic organic dye that has a large dipole moment in the ground state and a smaller dipole moment in the excited state^[35].

Absorption spectroscopy: The UV/Vis absorption spectra for **4** in various nonaqueous and aqueous media display similar shape and intensity of their absorption bands and are not significantly solvent dependent. There is a small bathochromic shift (~ 9 nm) in the MLCT absorption band at $\lambda = 440$ nm, while the ligand centred $\pi\pi^*$ band at $\lambda = 370$ nm displays a small shift of ~ 4 nm. For a more detailed study see Chapter Two (Section 2.3.4-2).

Table 4.6: Emission Characteristics of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (4) in Nonaqueous Solvents at $T = 25^\circ\text{C}$.

Solvent	E_T^a	α^a	$\lambda_{\text{max}}^{\text{abs}}$	$E_{\text{em}} (\text{cm}^{-1})$	Φ_{abs}^b		$\tau_{\text{meas}} (\text{ns})^c$			$\tau (\text{ns})^e$		k_r	Σk_{nr}	$k_q^{\text{O}_2}$
	$k_{\text{cal}}/\text{mol}$		nm	(λ_{max} nm)	degas	air	degas	air	oxy	N_2	air	(10^4s^{-1})	(10^6s^{-1})	($10^9 \text{M}^{-1} \text{s}^{-1}$)
Pyridine	40.2	0.00	372, 448	16340 (612)	0.075	0.017	755	235	113	752	315	9.93	1.21	1.25
DMF	43.8	0.00	372, 447	15800 (633)	0.025	0.0096	374	212	101	399	207	6.68	2.60	1.39
DMSO	45.0	0.00	372, 448	16000 (625)	0.014	0.0071	263	211	120	330	232	5.32	3.75	2.07
CH_3CN	46.0	0.15	372, 448	16260 (615)	0.033	0.0059	651	183	49	663	177	5.07	1.48	2.07
EtOH	51.9	0.86	368, 446	16500 (606)	0.027	0.0064	246	162	69	91	93	0.11	2.97	1.06
MeOH	55.5	0.98	368, 447	16610 (602)	0.003	0.0022	34	25	24	45	45	8.80	28.31	0.92
TFE ^d	59.5	1.35		0	0	0	0	0	0	0	0	-	-	-
Water ^d	63.1	1.13	372, 440	0	0	0	0	0	0	0	0	-	-	-

Table 4.6: ^a The E_T value, is a measure of solvent polarity, and α value, is a measure of the ability of the medium to donate hydrogen bonds, are from ref 35. ^b Relative Quantum yield compared to $[\text{Ru}(\text{bpy})_3]^{2+}$ ($\phi = 0.042$ in deaerated aqueous solution and $\phi = 0.028$ in aerated aqueous solution) $[\text{Ru}] \sim 0.1$ at lowest UV peak. ^c Error: $\tau \pm 5\%$, average of three measurements and have an estimated error of $\pm 5\%$. ^d There is no emission observed in water or TFE. ^e From Ref 35

Emission spectroscopy: The emission spectra were found to be somewhat solvatochromic, the emission energy (E_{em}) being solvent sensitive. The recorded spectra, in fluid aerated and degassed solvents, are illustrated in Figure 4.13. They were found to be broad, featureless and centred in the region 550 - 640 nm. The emission maxima exhibit a shift of ~ 30 nm around the generic ~ 610 nm peak, as the MLCT bands are very sensitive to their environment. There is an observed red-shift in the λ_{max} of emission as the polarity of the solvent is increased due to the stabilizing effect of the more polar surroundings on a charge-separated state.

We have concentrated on the results obtained in water and acetonitrile as much of the data on this complex and related complexes have been reported in these solvents. In water (and TFE (trifluoroethanol)) no emission was evident. The lack of emission in aqueous medium may be due to rapid, water-induced quenching of the MLCT state. In contrast, in acetonitrile moderately strong emission centred at ~ 615 nm is observed. Acetonitrile/water mixtures were found to be intermediate in behaviour, showing significantly less emission than in pure acetonitrile (omitted from Figure 4.13 for the sake of clarity).

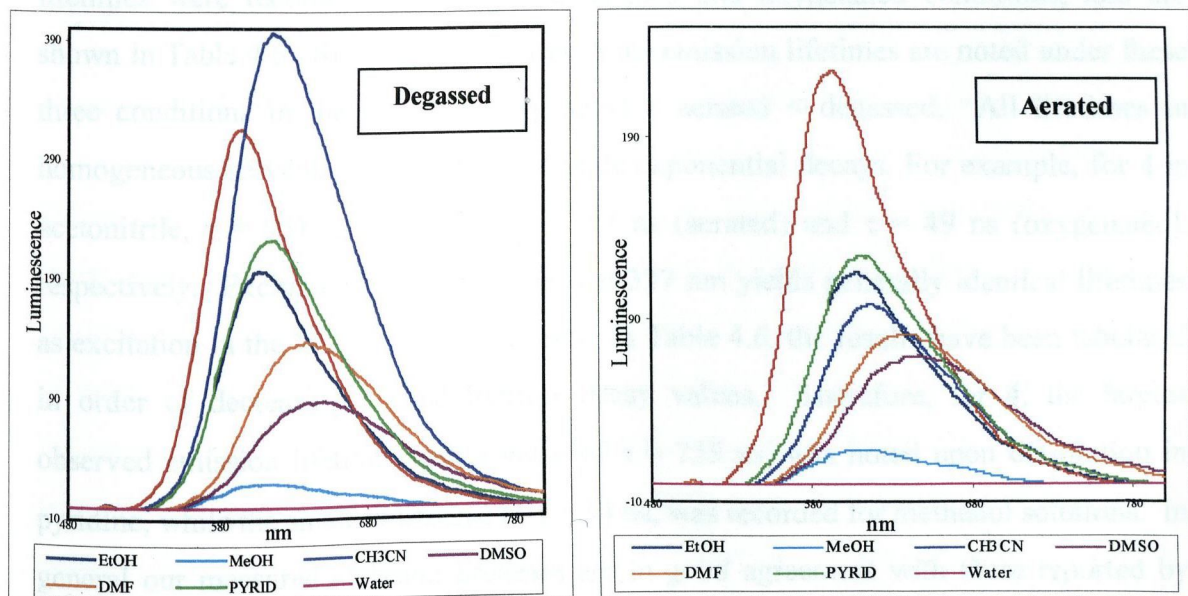


Figure 4.13: Photoluminescence spectra of $[Ru(phen)_2dppz]^{2+}$ 4 in several solvents in aerated and degassed conditions. The scales are not equivalent for both diagrams.

The emission quantum yields Φ_{abs} for **4**, under aerated and degassed conditions have been included in Table 4.6. Figure 4.14 displays plots of the emission quantum yields for the various solvents as a function of the solvent parameters, E_T and α . These plots display poor linear relationships between the parameters, with correlation coefficients of $R^2 = 0.58$ and 0.49 , respectively.

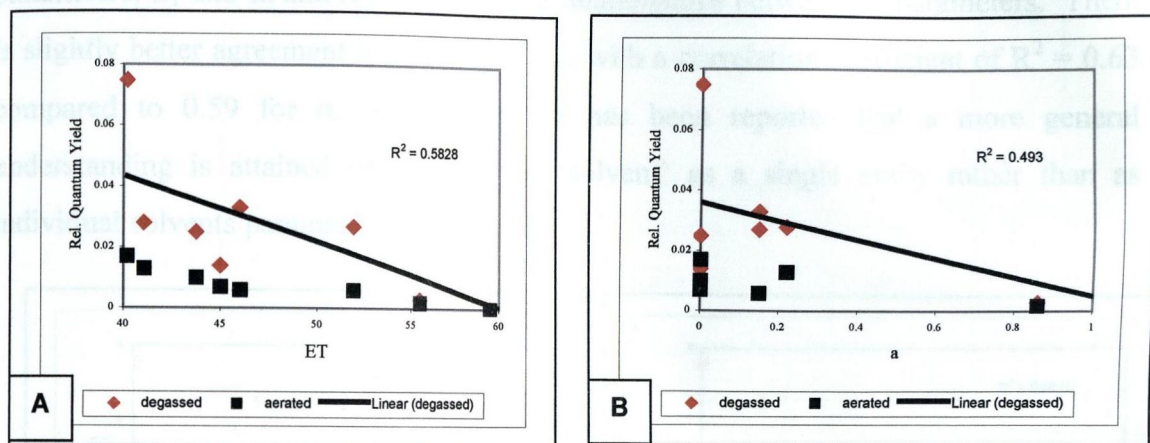


Figure 4.14: Plot of emission quantum yield Φ_{abs} as a function of E_T (A) and α (B) for **4**, in a wide range of aerated and degassed solvents.

Luminescence lifetime decays: Upon dissolution in an array of solvents, the emission lifetimes were recorded under degassed, aerated and oxygenated conditions, and are shown in Table 4.6. Significant decreases in the emission lifetimes are noted under these three conditions in the order of, oxygenated < aerated < degassed. All lifetimes in homogeneous solvents were well-fit by single exponential decays. For example, for **4** in acetonitrile, $\tau = 951$ ns (degassed), $\tau = 183$ ns (aerated) and $\tau = 49$ ns (oxygenated), respectively. Excitation in the dppz bands at 337 nm yields generally identical lifetimes as excitation in the MLCT band at 532 nm. In Table 4.6, the results have been tabulated in order of decreasing aerated lifetime decay values. Therefore, for **4**, the largest observed emission lifetime, with a value of $\tau = 755$ ns, was noted upon dissolution in pyridine, while the smallest lifetime of $\tau = 34$ ns, was recorded for methanol solutions. In general our measured emission lifetimes are in good agreement with those reported by Murphy *et al*^[35], which have been included in Table 4.6 for the sake of comparison. There is a discrepancy in the value obtained in ethanol, for which we obtained a longer

emission lifetime of $\tau = 264$ ns (compared to 91 ns^[35]) which could be explained by using a higher spectroscopic grade ethanol (99.9% as opposed 99.5%).

Is there a trend? The results have been examined according to the solvent parameters, E_T and α . Figure 4.15 displays plots of the emission lifetimes τ_{meas} for **4** and the solvent parameters, E_T and α , and reveal poor linear relationships between the parameters. There is slightly better agreement in the case of E_T , with a correlation coefficient of $R^2 = 0.63$ compared to 0.59 for α , respectively. It has been reported that a more general understanding is attained on viewing the ‘solvent’ as a single entity rather than as individual solvents parameters.

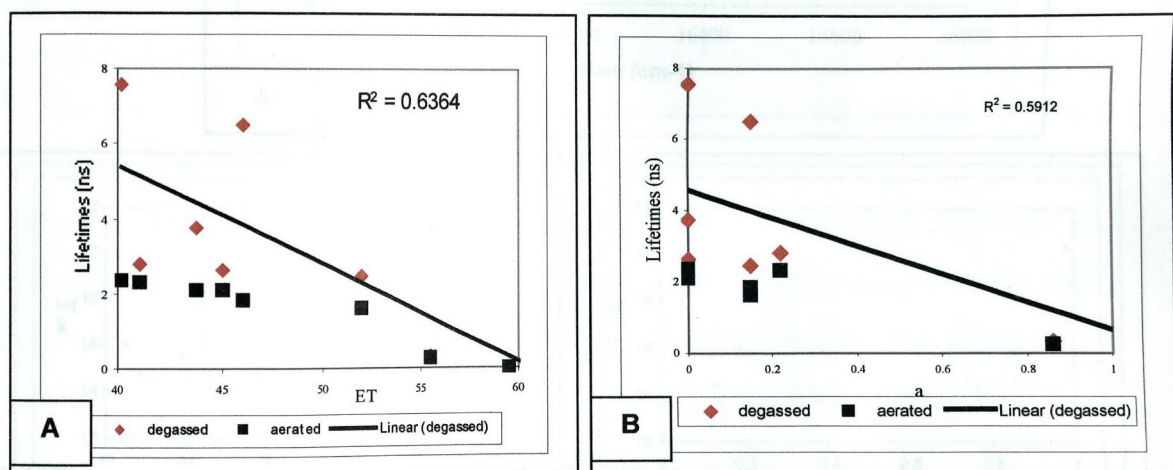


Figure 4.15: Plot of emission lifetime τ - vs - E_T (A) and α (B) for **4**, in aerated and degassed solvents.

Decay Rate Constants: The decay rate constants, k_r , k_{nr} , and k_qO_2 , have been calculated (from equations 4.3(a) and 4.3 (b)) and included in Table 4.6. We have implicitly assumed that, like complex **1**, population of the ³MLCT state occurs with a quantum yield of unity, $\phi_{isc} \cong 1$ for complex **4**. The small radiative rates, $k_r \sim 10^5$ s⁻¹, are essentially independent of the emission energy, and vary little as a function of solvent. The nonradiative decay constants, Σk_{nr} , were found to vary as a function of the solvent polarity and increase on moving to more polar solvents. A plot of $\ln \Sigma k_{nr}$ - vs- E_{em} examines the correlation between the rates of nonradiative decay and the energy gap between the ground and excited states as measured by the emission maximum energy,

E_{em} , and is shown in Figure 4.16. In addition, plots of $\ln \Sigma k_{nr}$ -vs- E_T and $\ln \Sigma k_{nr}$ - vs- α , are also displayed. However, these plots show poor linear correlations between the nonradiative rate of decay and E_{em} , E_T and α parameters, respectively.

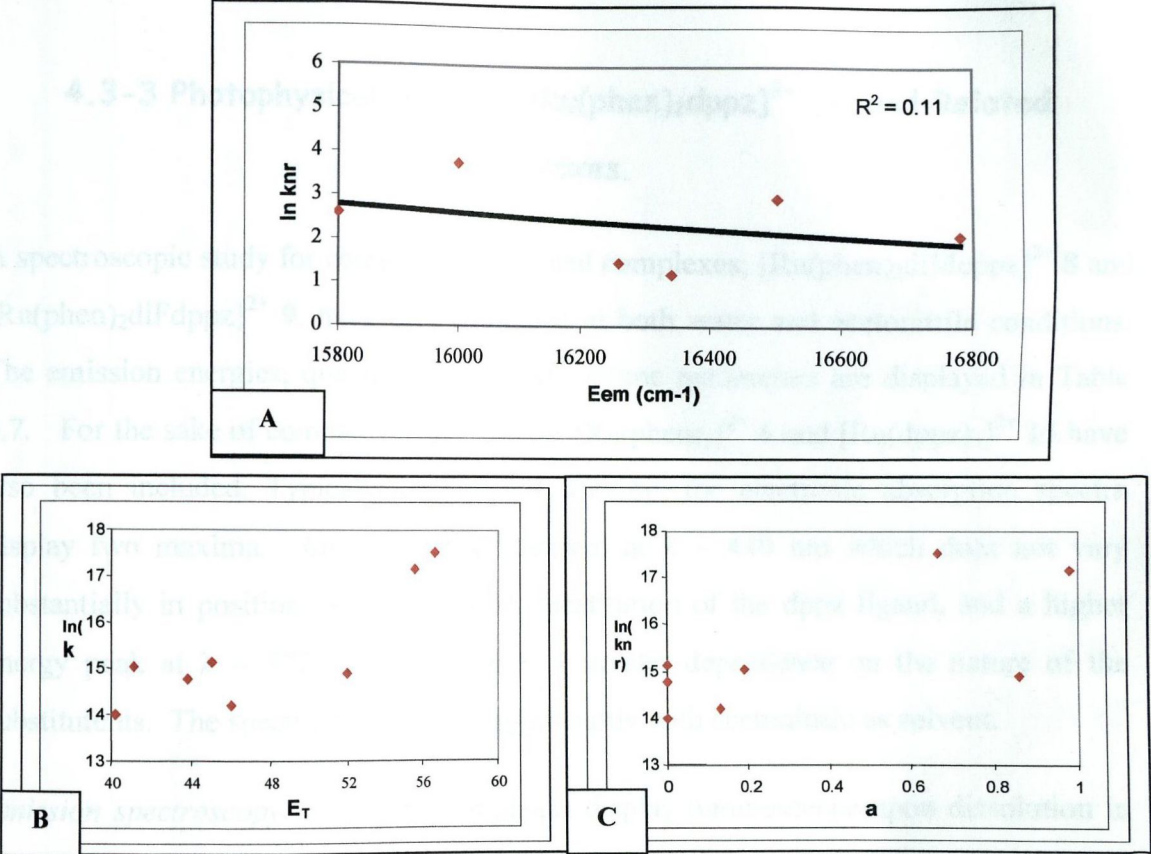


Figure 4.16: Plot of $\ln k_{nr}$ -vs- the emission parameters (A) E_{em} , (B) E_T and (C) α , for complex 4 for a broad range of solvents.

The rate constant of oxygen quenching, $k_q^{O_2}$, was found to vary, although not to a great extent for the solvents examined. Stern-Volmer plots of the emission intensity of 4 as a function of oxygen quenching in nonaqueous solvents were generally linear, and demonstrate correlation between the luminescence oxygen quenching rate constant, $k_q^{O_2}$, and the various solvents. The most notable effect was found for acetonitrile, while the polar solvents, MeOH and EtOH produced the smallest effects.

From our study, the emission maxima, lifetimes, and intensities of **4** were found to be highly sensitive to their local environment. This complex was also found to be applicable for optical probing of nonaqueous environments. However, we propose that the emission quantum yields and lifetimes, prove to be somewhat better indicators of the polarity of the immediate environment for complex **4**.

4.3-3 Photophysical Study of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (**4**) and Related Complexes.

A spectroscopic study for complex **4** and related complexes; $[\text{Ru}(\text{phen})_2\text{diMeppz}]^{2+}$ **8** and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9**, have been examined in both water and acetonitrile conditions. The emission energies, quantum yields, and lifetime parameters are displayed in Table 4.7. For the sake of comparison, the data for $[\text{Ru}(\text{phen})_3]^{2+}$ **6** and $[\text{Ru}(\text{dppz})_3]^{2+}$ **15** have also been included. Typically, in aqueous solution, the electronic absorption spectra display two maxima. An MLCT band present at $\lambda \sim 440$ nm which does not vary substantially in position or intensity with substitution of the dppz ligand, and a higher energy peak at $\lambda \sim 372$ nm, which show a greater dependence on the nature of the substituents. The spectra do not differ significantly with acetonitrile as solvent.

Emission spectroscopy: All of the complexes display luminescence upon dissolution in acetonitrile, and the emission spectra under aerated and degassed conditions are displayed in Figure 4.17. The emission maxima vary, as does the intensity, but all are in the range of 590 - 620 nm. On examination of the data, we observe a shift to the blue (~ 8 nm) in the emission maximum of the methyl complex **8**. While for **9**, the diFdppz ligand necessitates a lower MLCT state, resulting in a large red-shift (~ 20 nm) of the emission maximum. The emission quantum yield and lifetimes follow the sequence **8** > **4** > **9**.

	$E_{em} (cm^{-1})^a$	Φ_{abs}^c	$\tau_{meas} (ns)^e$	$E_{em} (cm^{-1})$	Φ_{abs}^c	$\tau_{meas} (ns)^e$
	($\lambda_{max} (nm)$)	Degas Air	Degas Air Oxy	($\lambda_{max} (nm)$)	Degas Air	Degas Air Oxy
	H₂O			CH₃CN		
1 [Ru(phen) ₃] ²⁺	16750 (597) ^a	0.058 0.032	996 497 174	16890 (592) ^a	0.049 0.051	406 107 30
8 [Ru(phen) ₂ DiMeDppz] ²⁺	d	d	d	16470 (607) ^b	0.095 0.060	821 170 45
4 [Ru(phen) ₂ Dppz] ²⁺	d	d	d	16260 (615) ^b	0.033 0.006	651 183 49
9 [Ru(phen) ₂ DiFDppz] ²⁺	d	d	d	15900 (635) ^b	0.011 0.005	202 126 48
15 [Ru(dppz) ₃] ²⁺	d	d	d	16610 (604) ^a	0.103 0.002	737 214 59

Complex	$k_r (10^4 s^{-1})$	$\sum k_{nr} (10^6 s^{-1})$	$k_q^{O_2} (s^{-1})$	$k_r (10^4 s^{-1})$	$\sum k_{nr} (10^6 s^{-1})$	$k_q^{O_2} (10^9 s^{-1})$
	H₂O			CH₃CN		
1	1.21	1.46	4.40	1.21	2.34	3.51
8	d	d	d	1.16	1.10	2.30
4	d	d	d	0.51	1.49	2.07
9	d	d	d	0.54	4.89	1.75
15	d	d	d	1.40	1.22	1.71

Table 4.7: Spectroscopic properties of Ru(II) complexes in water and acetonitrile solutions. Measurements made on solutions $\sim 10^{-5} M$ in complex at 25°C Relative to [Ru(bpy)₃]²⁺ in degassed aqueous solutions [$\Phi = 0.042$]. ^a $\lambda_{ex} = 372$ nm, ^b $\lambda_{ex} = 440$ nm. ^c Error: $\Phi \pm 10\%$, $0.05-0.1 \cdot 10^{-4}$ ^d No detectable emission was observed ^e $\lambda_{ex} = 337$ nm, $\lambda_{em} = 610$ nm

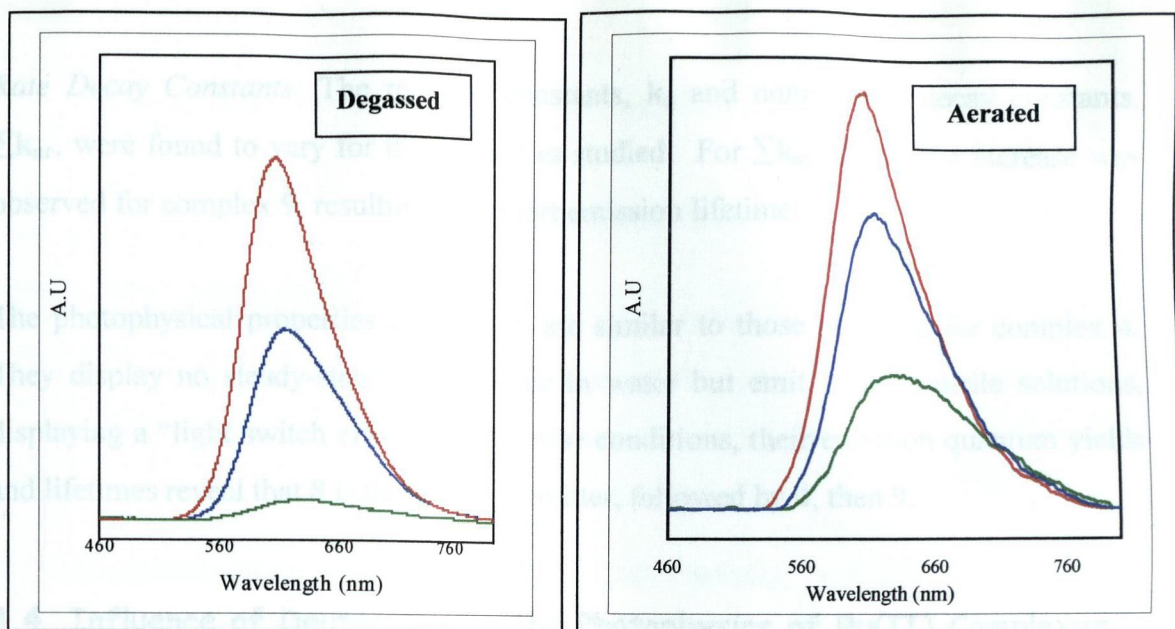


Figure 4.17: Normalised Emission Spectra of diMedppz (**8** - red line), dppz (**4** - blue line) and diFdppz (**9** - green line) Ruthenium (II) complexes in acetonitrile (degassed solutions - left), (aerated solutions - right). $\lambda_{\text{ex}} = 440\text{nm}$.

In contrast, no luminescence was detected for these complexes (on a nanosecond timescale) in aqueous solution when irradiated in the MLCT transition. This is in contrast, to Barton *et al*^[36] who reported that **8**, displayed emission centred $\sim 620\text{ nm}$ with a lifetime of *ca.* 50 ns, in aqueous solution at room temperature.

Luminescence Lifetime Decays: The emission lifetime measurements for these complexes were recorded for both solvents, and when luminescent, exhibited monoexponential luminescent decays. The complexes **4**, **8**, and **9** (and **15**), do not photoluminescence in water but display emission upon dissolution in acetonitrile. The longest emission lifetime was observed for **8**, with $\tau = 821\text{ ns}$, and attributed to both the electron-donating nature of the methyl substituents and the additional protection of the phenazine nitrogens by the bulky substituents. The shortest lifetime, with a value of $\tau = 140\text{ ns}$, was observed for **9**, due to the electron-withdrawing nature of the fluorine substituents and increased susceptibility of deactivation of the excited state $^3\text{MLCT}$ back to the ground state. For the parent complex **4**, an intermediate value of $\tau = 651\text{ ns}$ was observed.

Rate Decay Constants: The radiative constants, k_r and nonradiative decay constants, Σk_{nr} , were found to vary for the complexes studied. For Σk_{nr} , the largest increase was observed for complex **9**, resulting in its short emission lifetime.

The photophysical properties of **8** and **9**, are similar to those observed for complex **4**. They display no steady-state luminescence in water but emit in acetonitrile solutions, displaying a “light switch effect”. Under these conditions, their emission quantum yields and lifetimes reveal that **8** is the strongest emitter, followed by **4**, then **9**.

4.4 Influence of Deuteration on the Photophysics of Ru(II) Complexes.

4.4-1 Introduction.

For $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** and related complexes, it has been well established that the excited electron is localized on the most easily reduced ligand, which in this case is the dppz ligand^[30]. But the precise location of the promoted electron remains a matter of debate. To this end, the ‘deuterium isotope effect’ has been employed in the current study. Perdeuteration of these systems, affects a relatively small, but distinct, increase in the values of the emission quantum yields and lifetime decays. Of particular interest, is the selective deuteration of a ligand (or part thereof), as the emission lifetime will only be affected (*i.e.* increased) by H-D exchange when the excited state is located on the ligand and is directly involved in the emission process. Therefore, deuteration of spectator ligands should not influence the emission lifetime. So, in effect, selective deuteration of different components of the dppz ligand and/or the ancillary phen ligands, should provide a very effective and direct tool to acquire information about the relative excited state energies of these complexes.

4.4-2 Steady-State and Time-Resolved Luminescence Studies.

4.4.2-1 Introduction.

This study examines the effect of deuteration on the emission properties of a series of complexes, namely **4**, **8**, and **9**, using steady-state and time-resolved luminescence studies. The systematic deuteration of these complexes resulted in the formation of the following families;

- (i) $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ - **4** (**4-4g** $\rightarrow$ 8 complexes),
- (ii) $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ - **8** (**8-8g** $\rightarrow$ 8 complexes),
- (iii) $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ - **9** (**9-9f** $\rightarrow$ 5 complexes).

For the sake of simplicity, we will refer to $[\text{Ru}(\text{h}_8\text{-phen})_2(\text{h}_{10}\text{-dppz})]^{2+}$ as **h₈h₁₀**, and $[\text{Ru}(\text{d}_8\text{-phen})_2(\text{d}_6\text{-dppz})]^{2+}$ as **d₈d₆**, and so on. Furthermore, the complexes have been grouped according to the location of deuterium substitution in the complex, so as to allow for a more comprehensive analysis of the effects of H-D exchange on the complexes studied. The three categories are as follows;

Group A. **h₈h₁₀**, **d₈h₁₀**, and **d₈d₁₀**.

- (i) **h₈h₁₀** and **d₈d₁₀** – effect of perdeuteration of the complex
- (ii) **h₈d₁₀** and **d₈h₁₀** – effect of deuteration of the ancillary phen ligands and
- (iii) **d₈h₁₀** and **d₈d₁₀** – effect of perdeuteration of the dppz ligand

Group B. **h₈d₄**, **h₈d₆**, and **h₈d₁₀** – the effect of selective deuteration of the dppz ligand.

Group C. **d₈d₄**, **d₈d₆**, and **d₈d₁₀** – effect of selective deuteration of the dppz ligand (when the ancillary phen ligands are perdeuterated).

4.4.2-2 [Ru(phen)₂dppz]²⁺ (4)

The parent family [Ru(phen)₂dppz]²⁺ **4** contains eight complexes: the protiated, perdeuterated and six selectively deuterated complexes. For these complexes, there are no significant differences observed in the absorption and emission energies. As the complexes do not luminescence in water, the steady-state and lifetime measurements were performed under degassed acetonitrile conditions. The effect of H-D exchange on the emission quantum yields and lifetimes are presented separately and discussed according to the position of deuteration of the complex, as denoted by groups A, B, and C.

Figure 4.18: Luminescence spectra of complexes (4a-4g) under 10% D₂O.

Quantum Yields: The emission spectra for the series of complexes (**4-4g**) were recorded under acetonitrile conditions and are displayed in Figure 4.18. We observe that the shape and position of the emission maximum (λ_{max}) do not vary. However, the relative intensity of λ_{max} varies according to the extent of deuteration in the complex, and clearly demonstrates experimentally distinguishable quantum yields differences for the six deuterated analogues of **4**. The protiated complex **4**, is used as an internal standard for the series of complexes, where $\Phi_{\text{rel}} \cong 1$. The relative quantum yields, Φ_{rel} for the series of complexes (**4-4g**) are compiled in Table 4.8. Upon progressive deuteration of the complexes there is an increase in the values of emission quantum yields Φ_{rel} . A plot of Φ_{rel} as a function of increasing H-D exchange is illustrated in the form a bar-chart in Figure 4.19. The relative percent increase in Φ_{rel} has also been included in the plot (see white line).

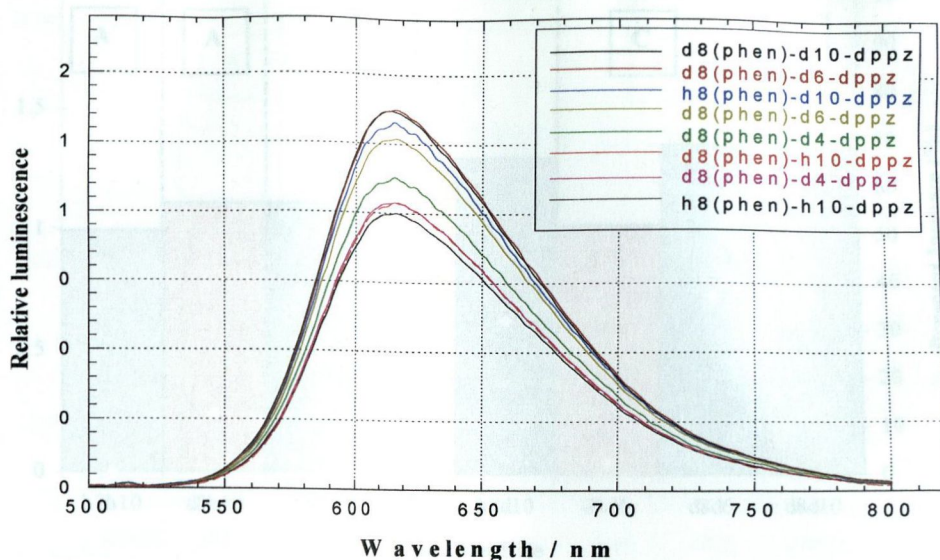


Figure 4.18: Luminescence emission spectra, in degassed acetonitrile, for 4 and its deuterated analogues (4a-4g), under identical conditions. Errors: $\Phi_{em} \pm 10\%$. $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (4) is used as the internal standard

Relative Quantum Yields		
Complex	Group No. ^b	Φ_{rel}
h_8h_{10} ^a	A	1.00
d_8h_{10}	A	1.12
h_8d_4	B	1.12
h_8d_6	B	1.27
h_8d_{10}	B	1.32
d_8d_4	C	1.03
d_8d_6	C	1.37
d_8d_{10}	A,C	1.37

Table 4.8: Relative emission quantum yields Φ_{rel} for complexes (4-4f) complexes, ^a Fully protiated complex 4 is used as an internal reference. All measurements in degassed acetonitrile, at $T = 23^\circ\text{C} \pm 2^\circ\text{C}$. Errors : $\Phi_{em} \pm 10\%$ ^b For A, B, and C, see text.

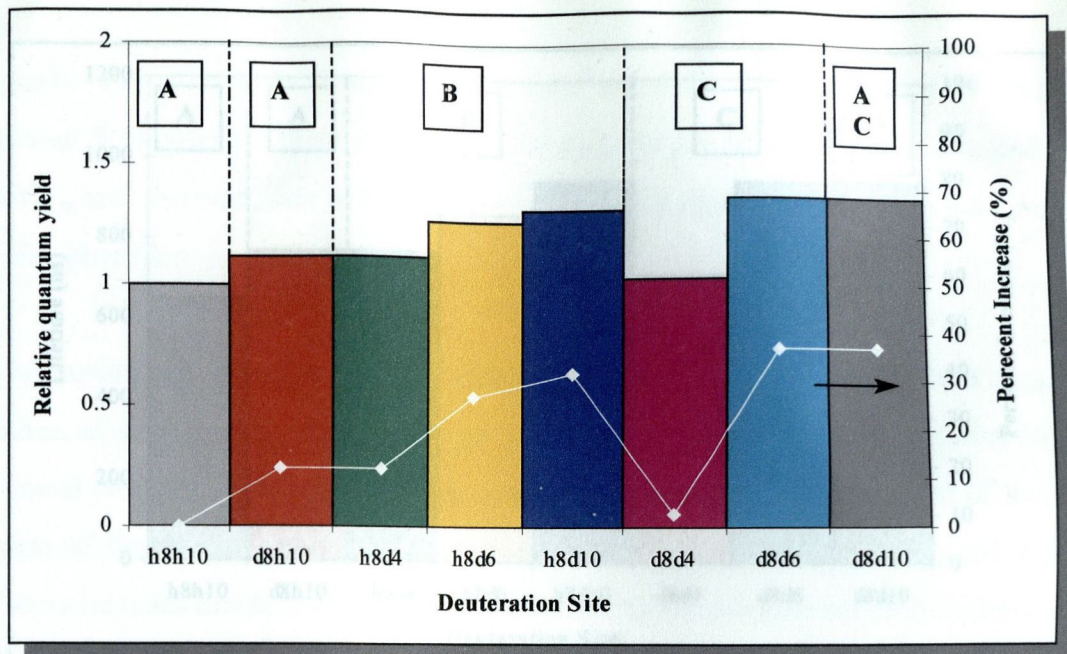


Figure 4.19: Emission quantum yields Φ_{rel} for the series of complexes (4-4g) complexes ^a Fully protiated complex 4 is used as an internal reference. The emission lifetimes and relative percent increase are also tabulated. ^c For A, B, and C, see text. Errors: $\Phi_{em} \pm 10\%$.

Luminescence Lifetime Decays: For the series of complexes (4-4g) the emission lifetimes, τ_{meas} are tabulated in Table 4.9. The percent increase in τ_{meas} relative to the protiated complex 4 has also been included. The data is presented in Figure 4.20, as a plot of τ_{meas} (and percent increase in τ_{meas}) as a function of D-incorporation into these complexes.

Complex	Group No.	τ_{meas} (ns)	% Increase τ_{meas}
h₈h₁₀ ^a	A	651	
d₈h₁₀	A	770	18.3
h₈d₄	B	770	18.3
h₈d₆	B	867	33.2
h₈d₁₀	B	952	46.2
d₈d₄	C	714	9.68
d₈d₆	C	946	45.3
d₈d₁₀	A,C	952	46.2

Table 4.9: Emission lifetimes τ_{meas} for complexes (4-4f) complexes, ^a Fully protiated complex 4 is used as an internal reference. All measurements in degassed acetonitrile, at $T = 23^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Errors : $\tau_{meas} \pm 5\%$

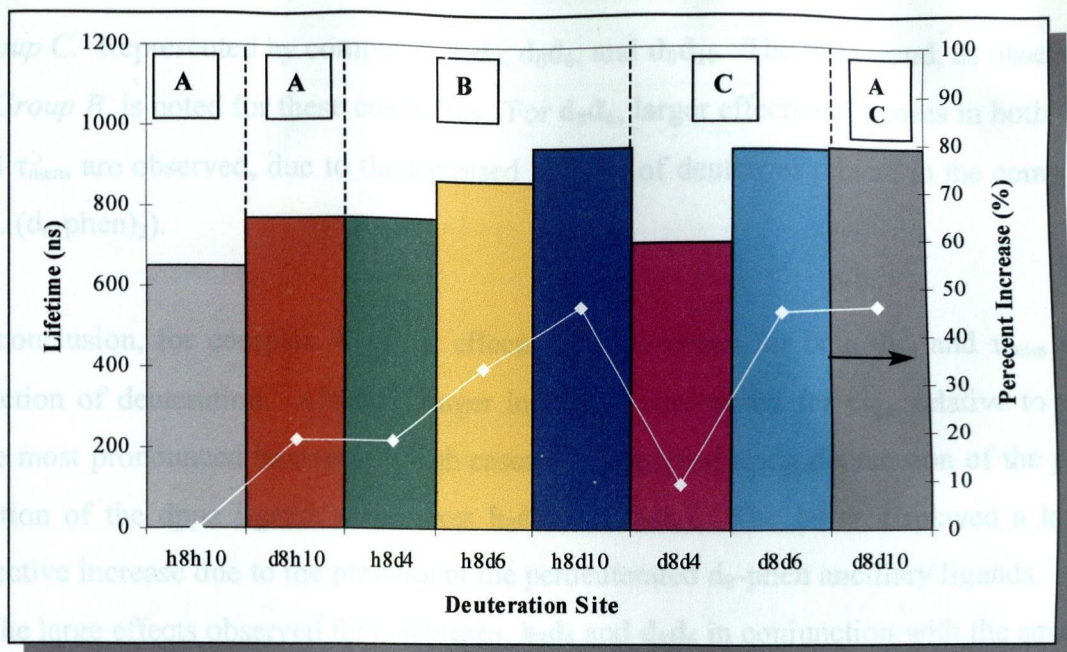


Figure 4.20: Luminescence emission lifetimes, as a function of deuteration for the series of complexes (4-4g) complexes ^a Fully protiated complex 4 is used as an internal reference. The relative percent increase are also tabulated. ^c For A, B, and C, see text. Errors: $\tau_{\text{meas}} \pm 5\%$

From the plots in Figures 4.19 and 4.20, it is evident that the rate of increase in Φ_{rel} and τ_{meas} (see white lines) across the series of complexes display similar trends on H-D exchange of complex 4. The emission parameters are discussed in relation to the position of deuteration within the complex as described by groups A, B, and C, respectively

Group A. The protiated **h8h10** complex 4, is used as an internal standard for the series of complexes. Deuteration of the ancillary phen ligands, **d8h10**, results in a small effect of 12% for Φ_{rel} and 18% for τ_{meas} , respectively. Perdeuteration of the complex, **d8d10**, effects an increase of 37% in Φ_{rel} and 46% in τ_{meas} .

Group B. Consists of **h8d4**, **h8d6**, and **h8d10** complexes. We observe that deuteration of the phenazine portion of the dppz ligand, **h8d4**, results in small increases of 12% for Φ_{rel} and 18% for τ_{meas} . In contrast, H-D exchange of the phen component of the dppz ligand, **h8d6**, results in large increases of 27% for Φ_{rel} and 31% for τ_{meas} . Perdeuteration of the dppz ligand, **h8d10**, causes an increase of 32% in both Φ_{rel} and τ_{meas} parameters.

Group C. Represented by complexes **d₈d₄**, **d₈d₆**, and **d₈d₁₀**. The same trend, as observed in *Group B*, is noted for these complexes. For **d₈d₆**, larger effective increases in both Φ_{rel} and τ_{meas} are observed, due to the increased number of deuterons present in the complex (*i.e.* (d₈-phen)₂).

In conclusion, for complex **4** similar effects were observed for both Φ_{rel} and τ_{meas} as a function of deuteration. Although larger increases were noted for τ_{meas} relative to Φ_{rel} . The most pronounced increase, in both cases was observed upon deuteration of the phen portion of the dppz ligand, (complexes **h₈d₆**, and **d₈d₆**). The latter displayed a larger effective increase due to the presence of the perdeuterated d₈-phen ancillary ligands. Due to the large effects observed for complexes, **h₈d₆** and **d₈d₆** in conjunction with the smaller effects noted for the **h₈d₄** and **d₈d₄** complexes, we propose that for the excited states of the [Ru(phen)₂dppz]²⁺ **4** complex, the *promoted electron is located on the phen portion of the dppz ligand*.

4.4.2-3 [Ru(phen)₂diMedppz]²⁺ (**8**)

The second family, the DiMedppz series of complexes also contains eight complexes; the protiated, perdeuterated and six selectively deuterated analogues (**8a** - **8g**), respectively. The effect of H-D exchange on the emission quantum yields and lifetimes were examined, under conditions as stated previously. The Φ_{rel} and τ_{meas} are tabulated and plotted as a function of D-incorporation into the complexes, and are shown in Figures 4.21 and 4.22. Note that the nomenclature for this series of complexes is slightly different to that used for the dppz series of complexes.

Relative Quantum Yields		
Complex	Group No.	Φ_{rel}
$\text{h}_8\text{h}_8^{\text{a}}$	A	1.00
d_8h_8	A	1.03
h_8d_2	B	1.03
h_8d_6	B	1.16
h_8d_8	B	1.22
d_8d_2	C	1.02
d_8d_6	C	1.21
d_8d_8	A,C	1.20

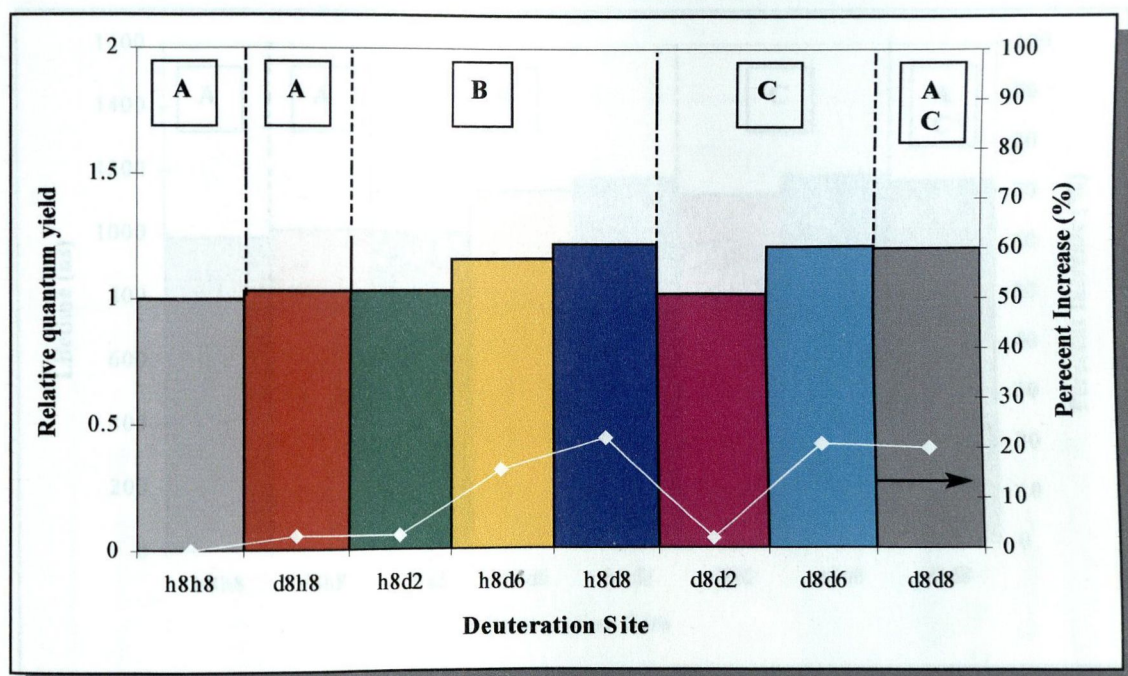


Figure 4.21: Relative emission quantum yields, Φ_{rel} for complexes (8-8g) complexes, ^a Fully protiated complex 8 is used as an internal reference. All measurements in degassed acetonitrile, at room temperature. Errors: $\Phi_{\text{em}} \pm 10\%$.

Lifetime Decays			
Complex	Group No.	τ_{meas} (ns)	% Increase τ_{meas}
h₈h₈^a	A	990	
d₈h₈	A	1012	2.2
h₈d₂	B	1010	2.0
h₈d₆	B	1131	14.2
h₈d₈	B	1172	18.4
d₈d₂	C	1117	12.8
d₈d₆	C	1180	19.2
d₈d₈	A,C	1162	18.2

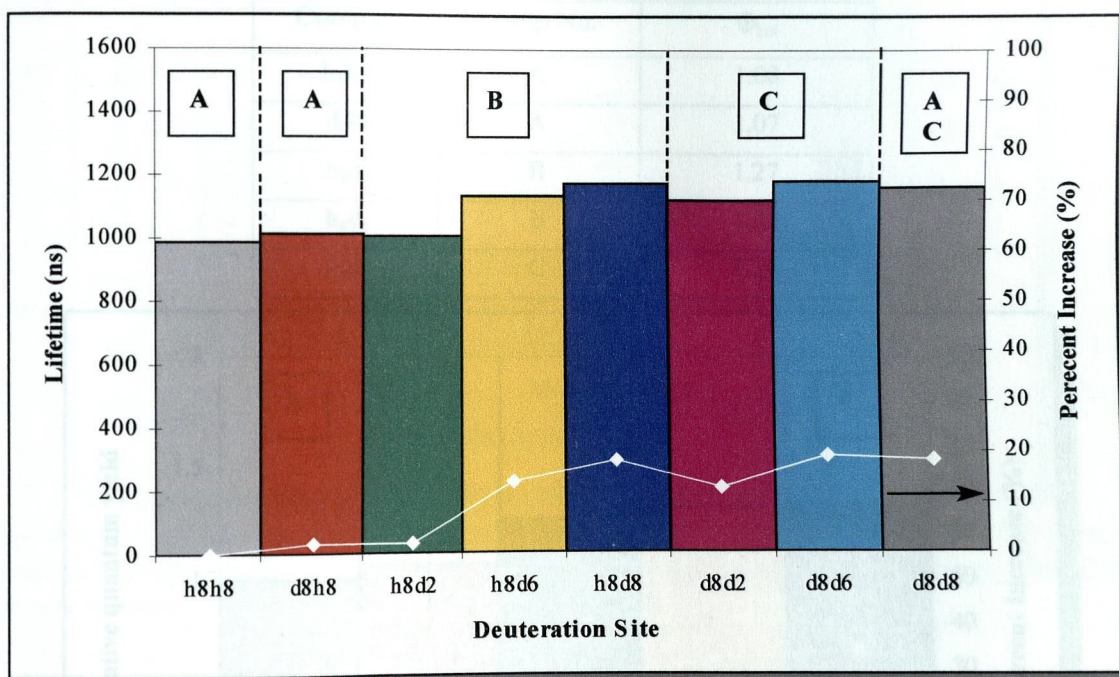


Figure 4.22: Luminescence emission lifetimes, τ_{meas} as a function of deuteration for the series of complexes (8-8g) complexes ^a Fully protiated complex 8 is used as an internal reference. The relative percent increase are also tabulated. Errors: $\tau_{\text{meas}} \pm 5\%$

On D-incorporation into **8**, there is an increase in both the emission quantum yield and emission lifetime values. Examination of the data, indicates that similar trends, as observed for complexes (**4-4g**), are displayed for the diMedppz family of complexes. However, the relative changes are somewhat smaller. For complexes (**8-8g**), there is an increase of ~20% for Φ_{rel} and 18% for τ_{meas} , compared to 37% and 46% for the corresponding dppz family.

4.4.2-3 [Ru(phen)₂diFdppz]²⁺ (9)

The third, and final family, is the diFdppz series of complexes (9, 9a, 9b, 9c, and 9f) which contains a total of five complexes; the protatiated and four deuterated complexes. The nomenclature is similar to that used for the diMedppz family in the previous section. The Φ_{rel} and τ_{meas} are tabulated and plotted as a function of H-D exchange into the complex, as shown in Figures 4.23 and 4.24.

Relative Quantum Yields		
Complex	Group No.	Φ_{rel}
h₈h₈^a	A	1.00
d₈h₈	A	1.07
h₈d₂	B	1.27
h₈d₆	B	1.32
d₈d₆	C	1.34

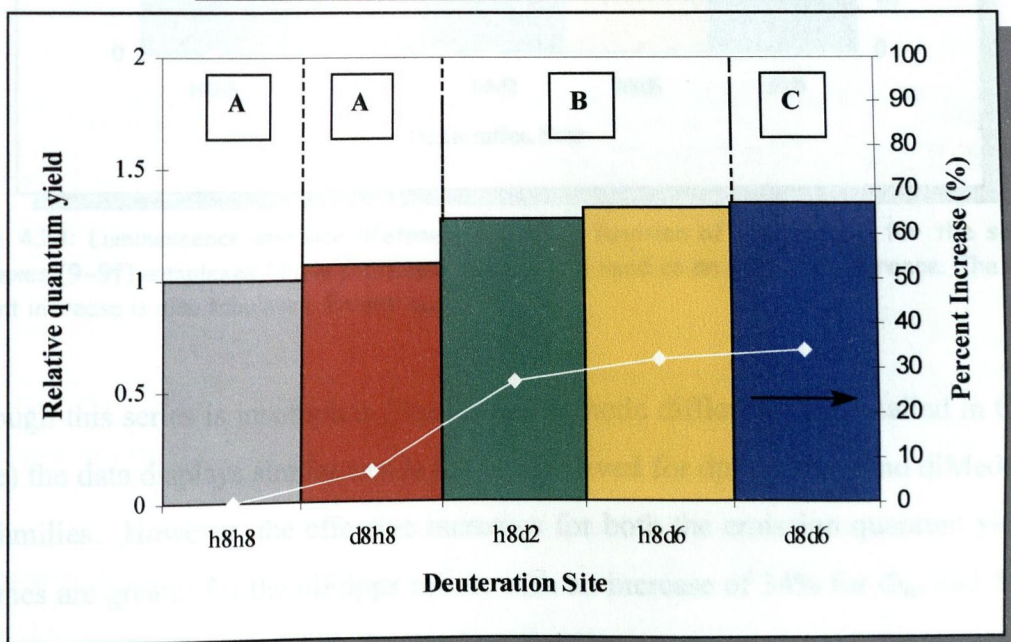


Figure 4.23: Relative emission quantum yields, Φ_{rel} for complexes (9-9f) complexes,^a Fully protiated complex 9 is used as an internal reference. The percent increase in Φ_{rel} is also included. Errors: $\Phi_{\text{em}} \pm 10\%$

Lifetime Decays			
Complex	Group No.	τ_{meas} (ns)	% Increase τ_{meas}
$\text{h}_8\text{h}_8^{\text{a}}$	A	203	
d_8h_8	A	213	4.9
h_8d_2	B	256	26.1
h_8d_6	B	301	48.3
d_8d_6	C	307	51.2

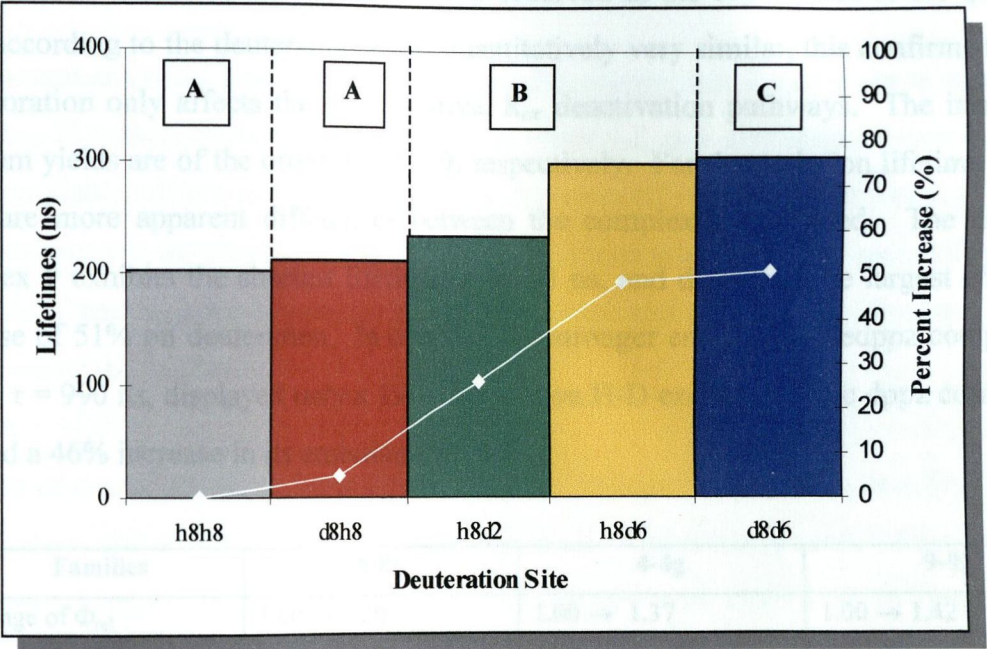


Figure 4.24: Luminescence emission lifetimes, τ_{meas} as a function of deuteration for the series of complexes (9-9f) complexes ^aFully protiated complex 9 is used as an internal reference. The relative percent increase is also tabulated. Errors: $\tau_{\text{meas}} \pm 5\%$

Although this series is incomplete (due to the synthetic difficulties as detailed in Chapter Three) the data displays similar trends to that observed for dppz (4-4g) and diMedppz (8-8g) families. However, the effective increases for both the emission quantum yield and lifetimes are greater for the diFdppz series, with an increase of 34% for Φ_{rel} and 51% for τ_{meas} , compared to 37% and 46% for the dppz family.

4.4-3 Conclusions from Emission Studies.

On H-D exchange, the emission quantum yields and luminescence lifetimes are strongly affected and the magnitude of this effect was found to be dependent on the site of deuteration. For all three families, similar trends are observed for Φ_{rel} and τ_{meas} on progressive deuteration of the parent complexes. Table 4.11, summarises the results obtained for these complexes. As the trend observed in the dependence of the quantum yield according to the deuteration site is quantitatively very similar, this confirms that D-incorporation only affects the non-radiative, k_{nr} deactivation pathways. The increasing quantum yields are of the order, $8 > 4 > 9$, respectively. For the emission lifetime decays there are more apparent differences between the complexes examined. The diFdppz complex **9** exhibits the shortest lifetime, $\tau = 203$ ns, and displayed the largest effective increase of 51% on deuteration. In contrast, the stronger emitting diMedppz complex **9**, with a $\tau = 990$ ns, displayed only a 19% increase on H-D exchange. The dppz complex **4** showed a 46% increase in its emission lifetime.

Families	8-8g	4-4g	9-9g
Range of Φ_{rel}	1.00 $\rightarrow$ 1.20	1.00 $\rightarrow$ 1.37	1.00 $\rightarrow$ 1.42
% Increase in Φ_{rel}	20%	37%	42%
Range of τ_{meas}	990 ns $\rightarrow$ 1180 ns	651 ns $\rightarrow$ 952 ns	203 ns $\rightarrow$ 307 ns
% Increase in τ_{meas}	19%	46%	51%
Largest effect in Φ_{rel}	h_8d_6 – 16 %	h_8d_6 – 27 %	h_8d_6 – 32 %
Largest effect in τ_{meas}	h_8d_6 – 14 %	h_8d_6 – 33 %	h_8d_6 – 48 %

Table 4.11: A comparison of the changes in the relative quantum yields and lifetimes for **4**, **8**, and **9** complexes as a function of deuteration.

For all three families, the results confirms that the acceptor ligand is the dppz ligand. On examination of the data, we propose that the excited electron is most probably localised on the phen moiety of the dppz ligand, as deuteration of these protons incurred the most prominent increases in the luminescence parameters. The electron is unlikely to be based on the phenazine end of the dppz ligand, as the vibrational modes affected by deuteration are not available for deactivation of the excited state.

4.5 Concluding Remarks.

To investigate the nature of the excited states of these Ru(II) complexes we have studied the influence of solvent, selective protium-deuterium exchange, and temperature on the emission parameters.

Steady-state emission studies were performed for complexes, **1** and **6**, in a number of solvents. However, contrasting behaviour was observed, as **6** displayed a lower emission quantum yield and smaller emission lifetime in acetonitrile compared to water. This has been attributed to the position of the $^3\text{MLCT}$ state, the size of the energy gap, ΔE between the $^3\text{MLCT}$ and higher lying energy states ^3MC states, and contributions from the $\nu(\text{OH})$ vibrations of the solvent.

A series of deuterated complexes; $[\text{Ru}(\text{L})_x(\text{L}')_{3-x}]^{2+}$, where $\text{L} = \text{bpy}$ and phen , $\text{L}' = \text{d}_8\text{-bpy}$ and $\text{d}_8\text{-phen}$, and $x = 0, 1, 2$, and 3 , were prepared. A systematic study of the effect of progressive ligand deuteration into these complexes was examined by steady-state emission and time-correlated lifetime measurements, under aqueous and acetonitrile conditions. In general, there is an increase in both the emission quantum yield ($\sim 28\%$) and lifetime decays ($\sim 27\%$) for complex **1** (in H_2O and CH_3CN) and for **6** (in CH_3CN). For the latter complex, there is only a 2% increase in the emission parameters in aqueous solution.

Temperature dependent studies for families **1** and **6** were performed in degassed aqueous solutions in the range 4°C to 44°C . For both complexes, the value of the emission lifetime was found to decrease on moving to higher temperatures, while an increase was noted on increasing the extent of deuterium in the complex. For the temperatures monitored these changes are uniform for the bpy series (**1-1c**), but were found to vary for the corresponding phen family (**6-6c**), most notably at $T = 24^\circ\text{C}$.

A detailed photophysical study of **4**, in a wide range of solvents was examined. The emission maxima, lifetimes and decay rate constants of **4** were found to be highly sensitive to their environment and may be best described by the polarity of the immediate environment. The study was extended to include $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ **8** and $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9**. All three complexes display a "light switch effect". In water they do not photoluminescence but display emission in non-aqueous environments. The emission quantum yields and lifetime decays follow the sequence $8 > 4 > 9$.

The parent complexes, **4**, **8**, and **9**, were fully and partially deuterated to give three sets of families. The quantum yields and emission lifetimes are strongly affected and the magnitude of the effect was found to be dependent on the site of deuteration. For all three families, similar trends were observed. The most pronounced effect was the marked increase in the emission quantum yield and lifetime decays upon deuteration of the phen positions of the dppz ligand (*i.e.* $\text{d}_6\text{-dppz}$) and the small increase observed for H-D exchange of the phenazine moiety (*i.e.* $\text{d}_4\text{-dppz}$). On the basis of these results, we propose that the "*promoted electron is located on the phen portion of the dppz ligand*" for the complexes studied.

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Chapter Five

Chapter Five

5.1 Aims of Research.

Up until now, we have been concerned with the spectroscopic properties of Ru(II) complexes with dppz and substituted ligands, in an attempt to understand their internal electronic properties and use them as molecular probes for DNA. The current study has emerged from our interest in the binding interactions of the Δ - and Λ -enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** with DNA. Although the photophysical properties of these complexes bound to DNA have been extensively studied^{[1][2][3]}, the questions involving the DNA site-specificity and intercalative geometries (whether major or minor groove binding) of these complexes remain an extremely controversial issue^{[1][4]}. To enable an understanding the structural features of nucleic acids are described in Section 5.2 and the physical and chemical properties of Ru(II) dye/nucleic acid binding are examined and discussed in Section 5.3. In the present study, the effects of (i) substitution on the dppz ligand and (ii) deuteration on the DNA binding properties of the parent complex **4** have been investigated by UV-Vis absorption, steady-state and time correlated lifetime studies.

5.2 The Structure of DNA.

On a primary level, DNA is composed of two counter-propagated polymeric strands wrapped around each other in a double helical formation. The basic repeating unit of a heterocyclic base, sugar and phosphate made up a monomer, commonly known as a nucleotide. The four possible bases are the bicyclic purines; adenine (A) and guanine (G) and the monocyclic pyrimidines, thymine (T) and cyostine (C). The base is attached to 2-deoxy-D-ribose sugar by a covalent bond at the C1' position through the ring nitrogen of the NH group forming a nucleoside. This β -glycosidic linkage is on the same side of the sugar ring as the C5' hydroxyl group and the bases can adopt either a syn- or anti-conformation relative to the sugar ring. The furanose rings are twisted out of the plane in order to minimise non-bonding interactions between the substituents. This leads to C2' endo and C3' endo conformations, that are in rapid equilibrium in solution. The sugar is bound to a negatively charged phosphate

via the C5' and C3' hydroxyl groups, which when linked constitutes a polynucleotide chain that runs in the direction of the '5 to the '3 sugar carbons.

A number of researchers contributed information which subsequently lead to solving the structure of nucleic acids. By x-ray diffraction studies carried out on DNA, Watson and Crick proposed that DNA consists of two polynucleotide chains that are coiled around a common axis in an antiparallel manner to form a right handed double helix^[5]. This produces a structure with a largely hydrophobic interior comprising the DNA bases, and a hydrophilic exterior comprising the sugar-phosphate backbone. The strands run in opposite directions and are bound through an ensemble of hydrogen bonds formed between complementary base pairing. The H-bonds are selective, allowing only A and T, or C and G base pairs to be formed. The winding of the structure creates two distinct helical grooves, the *minor* and the *major*, which spiral around the surface of the DNA.

Molecular studies have revealed that DNA can adopt a wide variety of helical secondary conformations which include A-, B- and Z-DNA, as shown in Figure 5.1. Some of the structural details of these families have been summarized in Table 5.1^[6]. Apart from these regular double helix structures, DNA may also exist in single stranded form (ss), double stranded closed circular form (ccc), *etc.*

As shown, DNA can exist in many different polymorphic forms but this work will only consider the most predominant B-form of DNA, which the polymer assumes both *in vitro* in neutral solution and *in vivo*. At normal conditions of high humidity and low salt concentration B-DNA is favoured. The bases on each strand are stacked directly above each other and are near perpendicular to the centre of the helix resulting in major and minor grooves of different widths but approximately equal depth. The walls of the grooves are formed by the nucleotide base-pairs viewed edge-on. The vertical distance between the base-pairs is 3.4 Å and successive base-pairs are rotated at an angle of 36° about the helical axis. Thus, there are ten base pairs per helical turn and the sugars are B-D-2-deoxy-furanose species in the C2'-endo conformation.

DNA Duplex	Helix handedness	Bp/Turn	Helix Diameter (nm)	Major Groove	Minor Groove	Sugar Pucker Conformation
B	Right	10.0	~2.0	Wide + Deep 11.6 8.5	Narrow + Deep 6.0 8.0	C2'-endo
A	Right	11.0	~2.6	Wide + Narrow 3.0 13.0	Wide + Shallow 11.1 2.6	C3'-endo
Z	Left	12.0	~1.8	Flat 8.8 3.7	Narrow + Deep 2.0 13.8	C3'-exo (syn)

Table 5.1: DNA parameters of A-, B-, and Z-forms of DNA^[6]

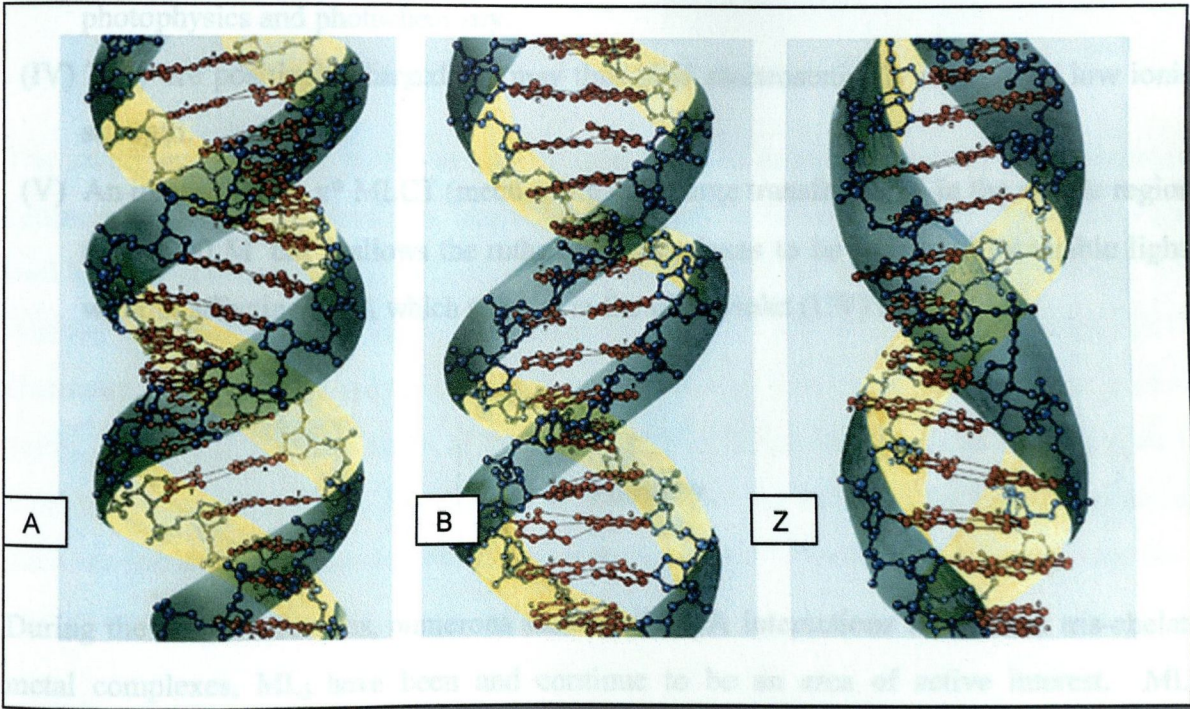


Figure 5.1: DNA structures of A-, B-, and Z-forms of DNA^[6].

5.3 The Interactions of Ruthenium(II) Polypyridyl Complexes with DNA.

5.3-1 Why do we study Ruthenium(II) complexes with DNA?

Due to a number of favourable characteristics, the present study has focused on the interactions of ruthenium complexes to DNA.

- (I) Due to d^6 nature, they are kinetically inert.
- (II) The thermodynamic stability of these molecules make them inert in the absence of light.
- (III) They have easily controlled properties: size, shape, spectroscopic characteristics, photophysics and photochemistry.
- (IV) They are positively charged and may thus bind electrostatically to DNA at low ionic strength.
- (V) An intense $d\pi \rightarrow \pi^*$ MLCT (metal-to-ligand charge transfer) band in the visible region ($\epsilon \sim 20,000 \text{ M}^{-1}\text{cm}^{-1}$) allows the ruthenium complexes to be irradiated by visible light, without affecting DNA which absorbs in the ultra-violet (UV) region.

5.3-2 History.

During the last two decades, numerous studies of DNA interactions with chiral tris-chelate metal complexes, ML_3 have been and continue to be an area of active interest. ML_3 complexes are shaped like three-bladed propellers and have two enantiomeric forms, corresponding to right-(Δ) and left-(Λ) handed screws. In 1976 Norden *et al.*^[7] suggested possible enantioselective binding of the chiral molecule $[\text{Fe}(\text{bpy})_3]^{2+}$ to B-DNA. It has been proposed^[7] that the ML_3 complexes bind in the *major* groove of DNA. This proposal is supported by the fact that the size of ML_3 complex ($\sim 10 \text{ \AA}$ across) precludes it from adopting a fully intercalated site and also suggests that the minor groove would be

unfavourable. This induced extensive studies on $[\text{Ru}(\text{phen})_3]^{2+}$ **6** (model system), which appears in two inversion-stable forms the Δ and Λ enantiomers, as depicted in Figure 5.3. Despite much work, the modes of interaction of **6** with DNA remain an intensely controversial issue^{[8][9]}.

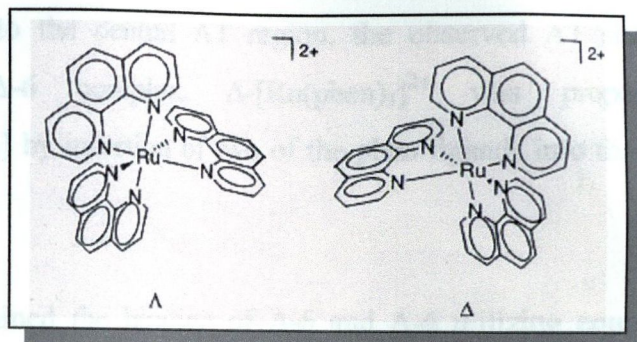


Figure 5.3: Systematic representation of Δ - and Λ -enantiomers of $[\text{Ru}(\text{phen})_3]^{2+}$ complex **6**.

The major questions for both enantiomers have been to determine the DNA intercalative geometry, (*i.e.* where does the binding take place, externally at the phosphate-sugar backbone or at the surface in either of the grooves), what if any site-specificity there is, and whether one of the phen ligands intercalates or not. Our review of this rich Ru(II)/DNA chemistry is limited as it only highlights the developments that are of interest in the current study. Table 5.2, contains some of the experimental techniques that have been applied to study the interactions of **6** in the presence of DNA. While the nature of the binding mode has been the focus of many investigations, many other aspects of the Ru/DNA interactions have also received much attention.

Ericksson *et al*^[10], examined the binding of Δ - and Λ - $[\text{Ru}(\text{phen})_3]^{2+}$ enantiomers of **6** utilising two-dimensional NMR results. They reported that the exchange rate between the bound and free states is rapid on the NMR timescale at temperatures above 20°C, yielding sharp peaks for all exchangeable protons in the NMR spectrum. This is in contrast, to most intercalators which exhibit broad peaks for protons affected by DNA. Strong indications for non-intercalative binding was found, and it was suggested that both the Δ -**6** and Λ -**6** enantiomers bind in the *minor* groove of the oligonucleotide $[\text{d}(\text{CGCGATCGCG})_2]$.

Ericksson and co-workers^[11] also studied the interactions of Δ -6 and Λ -6 with the oligonucleotide duplex $[d(CGCGATCGCG)]_2$ with NMR and CD spectroscopy. From NOESY data, it was shown that the interaction primarily takes place in the minor groove of the oligonucleotide (which remains in a B-like conformation). Furthermore, the metal preferentially binds to the central AT region, the observed AT specificity being more pronounced for Δ -6 complex. Δ -[Ru(phen)₃]²⁺ was proposed to bind to $[d(CGCGATCGCG)]_2$ by insertion of two of the phen ligands into the *minor* groove of the oligonucleotide.

Barton *et al*^[9] examined the binding of Δ -6 and Λ -6 utilizing equilibrium analysis and photophysical methods. They postulated two non-covalent binding modes of [Ru(phen)₃]²⁺ to the DNA helix. One intercalating bound mode in the *major* groove which favoured Δ -[Ru(phen)₃]²⁺ and the other, a surface bound mode along the DNA minor groove, showing a weak preference for Λ -[Ru(phen)₃]²⁺. Luminescence studies of 6 bound to double-helical DNA displayed a biexponential decay; with one lifetime of 2 μ s attributed to the intercalative form, and a second lifetime of 550 ns assigned to the surface-bound form (or to an alternate binding mode in which the dppz is less shielded than if tightly intercalated). Furthermore, on the basis of polarization data, it was suggested that the enantiomers bound *via* an intercalative mode, and consistently displayed a greater polarisation for Δ -6 with DNA.

Technique used	Complexes Studied	Ref
1D ¹ HNMR	Δ/Λ -[Ru(phen) ₃] ²⁺ with [d(GTGCAC)] ₂ and [d(CGCGCG)] ₂	39,40
2D ¹ HNMR	Δ/Λ -[Ru(phen) ₃] ²⁺ / [d(CGCGATCGCG)] ₂	10,11
Steady-state & Time Resolved luminescence	Λ -[Ru(phen) ₃] ²⁺	38
Electric linear dichroism	Δ/Λ - [Ru(phen) ₃] ²⁺	36,37
Linear dichroism	Δ/Λ - [Ru(phen) ₃] ²⁺	12
Topoisomerase unwinding	rac -[Ru(phen) ₃] ²⁺	4
Viscometry	Δ/Λ - [Ru(phen) ₃] ²⁺	13
Molecular Modelling & Energy minimization studies	Δ/Λ -[Ru(phen) ₃] ²⁺	35
Equilibrium analysis & Photophysical methods	Δ/Λ -[Ru(phen) ₃] ²⁺	9

Table 5.2: Experimental techniques used to probe the nature of the binding modes for complex 6

Since 1990, Hiort and co-workers have argued from linear and circular dichroism studies, in favour of *major* groove binding for both enantiomers^[12]. An earlier speculation, that Δ -6 should bind with one of its phenanthroline wings partially intercalated between the base pairs, is inconsistent with an angle of approximately 70° between the 3-fold axis of the complex and the DNA helix axis, as confirmed by linear dichroism (LD) spectra. It was proposed, that for Δ -6 complex that two chelates sit in the groove. A corresponding angle of 50° was obtained for the complex, which suggested that one chelate wing points into the middle of the major groove. However, major groove binding of drug molecules is rarely reported, and its nature is still poorly understood.

As the binding of complex 6 is relatively weak, Sactyanarayana *et al*^[13] have utilized viscosity measurements to examine the effects of binding on the hydrodynamic properties of the duplex. They argued that since neither enantiomer of 6 lengthened short, rod-like DNA, classical intercalation could not be the binding mode. In addition, molecular modeling and energy minimization calculations confirmed that there is, at best, only partial insertion of the phen ring between the base pairs.

However, the background luminescence of the free $[\text{Ru}(\text{phen})_3]^{2+}$ form, the relatively weak binding, and the extent of enhancement on binding proved insignificant for the broader applications of this ruthenium complex as a nonradioactive nucleic acid probe. Despite a considerable amount of published material, our knowledge of the nature of the binding geometries of 6, intercalative, or not, is still a controversial issue^{[10][12]}.

• $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ 4

Many new structural analogs based on the parent compound 6, have been synthesised, and these include the second generation complexes, namely $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ 10 and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ 4, respectively. The ligand dppz, was synthesized in 1970^[14], while the first complex with ruthenium, $[\text{Ru}(\text{dppz})_3]^{2+}$ 15, was reported in 1984^[15], soon to be followed by Sauvage and coworkers^[16] with 10 in 1985. Friedman *et al*^[17], reported that

these complexes act as molecular “light switches” for DNA. A negligible background emission from the triplet metal-to-ligand charge transfer (MLCT) excited state of **4** in aqueous solution display an intense photoluminescence in the presence of double-helical DNA^{[17][18][19]}. The quantum yield in aqueous buffer is increased by a factor of $>10^4$ upon addition of DNA, which the complex binds with an equilibrium binding constant, $K_b > 10^6 \text{ M}^{-1}$ in 50 mM NaCl buffer^[17]. Based on an observed DNA unwinding angle of $30 \pm 11^\circ$ per ruthenium bound, and considering the extended planar structure of the dppz ligand an intercalative binding mode for the complex has been proposed. The complex therefore provides an unique spectroscopic probe for DNA.

The electronic structure of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$, has been described in terms of a coupling of a $[\text{Ru}(\text{bpy})_3]^{2+}$ chromophore to a phenazine electron acceptor^[19]. The MLCT excited state of Ru(II) complexes is characterised by a strong long-lived luminescence from a state from which the promoted electron is expected to be localised on the most easily reduced ligand^[16]. Therefore, the lowest energy MLCT transition is localised on the dppz ligand as determined from emission and absorption studies. This excited state $[\text{Ru}^{\text{III}}(\text{L})_2(\text{dppz}^{\bullet-})]^{2+*}$ structure, suggests that the reactivity of this intercalating reduced ligand may be monitored specifically, and the luminescence of the bound complex can therefore be used as a sensitive reporter of its helical environment.

Unlike $[\text{Ru}(\text{phen})_3]^{2+}$ **6**, for which the exact binding mode remains a matter of debate^{[8][9]}, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** undoubtedly binds through intercalation of the extended dppz ligand between the base pairs. The emission decays of the enantiomers bound to nucleic acids are biexponential, and both their lifetimes and relative contributions have been found to vary with binding ratio and DNA sequence. Accumulated evidence suggests that intercalation of the nitrogens of the dppz ligand are located in a hydrophobic area of the double helix, protecting the excited state from a deactivation solvent protonation process, and resulting in emission. Nevertheless, the origin of these biexponential decay curves remains unsolved and two very different binding modes have been proposed.

"The Debate of Major vs Minor Binding Modes"

There is a vast amount of research on this topic. A brief review of what is known concerning the DNA binding mode(s) of **6** is detailed here, and Table 5.3 contains some of the techniques employed to study their nucleic-acid binding properties.

The interaction of Δ -**4** with the oligonucleotide [CGCGATCGCG]₂ using NMR spectroscopy^[11] indicated that the binding kinetics are in the intermediate exchange range and are thus slower than the parent compound [Ru(phen)₃]²⁺. This observation is consistent with stronger stacking interactions of the dppz ligand with the base pairs of DNA, and with intercalation. Barton and Dupureur^[20] utilised perdeuterated phen in the assembly of Δ -[Ru(phen-d₈)₂dppz]²⁺ (**4d**), which simplified the ¹H NMR spectra and permitted the focus to be centred on the activities of the dppz ligand. One-dimensional spectra of this complex bound to the oligonucleotide d(GTCGAC)₂ indicated that at least two binding modes existed for the complex. This was confirmed by two-dimensional NOESY spectra of Δ -[Ru(d₈-phen)₂d₆-dppz]²⁺ (**4f**) in the presence of d(GTCGAC)₂. Furthermore, they detected an NOE between protons on the dppz ligand, located close to the centre of the complex and an aromatic proton facing the *major* groove. This approach yielded results which establish the interaction of Δ **4** into DNA from the *major* groove.

Emission decay studies^[18] for aqueous solutions of Δ -**4** and Λ -**4** which contain DNA, displayed a biexponential decay with lifetimes, $\tau = 93$ ns (63%) and $\tau = 512$ ns (37%), in 5 mM tris, 50 mM NaCl (pH = 6.9). It was found that there was static quenching only of the long component by the hydrophobic quencher. It was argued, that if the short component was indeed due to an isolated molecule that it should also have been subjected to static quenching. Therefore, the long and short components have been assigned to two different binding modes as they are quenched independently.

Technique used	Complexes Studied	Ref
1D ¹ H NMR	Δ/Λ -4 / [d(GTGCAC)] ₂	11
	Δ/Λ -4 / Actinomycin D	
2D ¹ H NMR	Δ/Λ - [Ru(d ₈ -phen) ₂ dppz] ²⁺	20
	Δ/Λ - [Ru(d ₈ -phen) ₂ d ₆ -dppz] ²⁺	
¹ H NMR	Δ/Λ -4 / CT-DNA	
Linear Dichroism	Δ/Λ -4 / CT-DNA	25
Resonance Raman	Δ/Λ -4 / CT-DNA	23
Resonance Raman	Rac - 4 / CT-DNA	24
	Rac - [Ru(phen) ₂ diFdpz] ²⁺	
Viscometry & Fluorescence	Rac/ Δ/Λ -4 / CT-DNA	43
Energy Transfer		
Luminescence Studies	Δ/Λ -4 / CT-DNA	18,4
	Δ/Λ -4 / T4-DNA	22
Time Resolved Luminescence	Δ -4 / poly d(AT)	21
	poly d(GC)	
	CT-DNA	
	Δ/Λ -4 / CT-DNA	18
Anionic Quenching Studies	Rac-4 / CT-DNA	1

Table 5.3: Experimental techniques used to probe the nature of the binding modes for complex 4

Holmlin *et al*^[21], investigated the binding of Δ -4 and Λ -4 to poly d(AT), poly d(GC) and CT-DNA by time-resolved luminescence spectroscopy. The effect of major and minor groove DNA binding agents on the luminescence profile of the complex were examined. They found that, on titrating the major groove binding agent, Λ - α -[Rh[(R,R)-Me₂trien]phi]³⁺, to complex 4 bound to [d(5'-GAGTGCCTC-3')₂], that 4 was displaced from the major groove by the Rhodium complex.

Hiort *et al*^[4] synthesised enantiomerically pure Δ -4 and Λ -4 in order to study their interactions with calf-thymus DNA. Equilibrium binding constants for both enantiomers were found to be around 10⁸ M⁻¹ in solutions containing 10 mM NaCl. However, the relative luminescence yield of the Δ -4 bound complex was found to be 6-10 times greater than the Λ -4 DNA complex. An intercalative binding mode for both enantiomers was further supported by a negative linear dichroism of the dppz ligand transition at 380 nm. Furthermore, for each enantiomer two distinct excited state lifetimes were found on binding to DNA. The longer lifetime was found to increase with increasing binding ratio. Hence the existence of the two lifetimes was explained not as two separate binding modes, but as being due to a "dye distribution effect" of intercalating complexes along the DNA helix. So ligands bound contiguously have longer lifetimes, while isolated complexes are more accessible to solvent and therefore have shorter lifetimes, respectively.

Recently, spectroscopic studies^[22] of 4 were performed in the presence of a variety of nucleotides of different conformation and composition. These included T4-DNA, which is 100% glycosylated at the cytosine 5-CH₂-OH position in the major groove. Hindered interaction with T4-DNA has previously been used to support major groove binding of 6, and no effect of glycosylation used to support minor groove binding. As both enantiomers of 4 emit strongly in both CT-DNA and T4-DNA, they proposed that complex 4 interacts with the *minor* groove of DNA helix.

The understanding of the electronic structure of the free and DNA-bound rac- $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complexes is vital in determining the factors that govern the nature of the binding mode(s) with DNA. Resonance Raman (rR) spectroscopy is a useful tool for the investigation of the molecular conformation of both the ground and excited states in homogeneous and microheterogeneous environments. The π - π interaction of the dppz ligand and DNA bases may be probed by monitoring the changes in the spectral profiles of **4** upon addition of DNA. Resonance Raman reports how binding to DNA affects the energy levels of the metal complexes.

Coates *et al*^[23] reported the use of rR spectroscopy as a useful probe of the interactions between the $[\text{Ru}(\text{L})_2\text{dppz}]^{2+}$ complexes and DNA. Excited state rR spectra, in the presence of DNA, resulted in the appearance of a prominent new feature at 1526 cm^{-1} , which has been attributed to intercalative interactions between the radical like $\text{dppz}^{\bullet-}$ ligand and the DNA binding sites.

Chen *et al*^[24] have utilized rR spectroscopy to gain an insight into the electronic structure of dppz, which is separated into contributions from the phen and the phz (phenazine) parts of the ligand. Time-resolved rR (TR^3) spectrum of **4** and $[\text{Ru}(\text{phen})_2(\text{diFdppz})]^{2+}$ **9**, in the presence of DNA both lead to a decrease in the intensity of the peaks associated with the intercalating phenazine part of the dppz ligand. This is explained in terms of the stronger π - π interactions between the intercalating dppz ligand and the DNA bases.

Anionic quenching experiments were utilized by Hartshorn *et al*^[1] which demonstrated that upon binding to B-form DNA, both racemic complexes, **4** and **10**, exhibit a biexponential decay in emission. This was interpreted to result from two orientations of the dppz ligand in the intercalative pocket, as shown in Figure 5.4. The first, is a perpendicular mode whereby the dppz ligand intercalates from the major groove such that the metal-phenazine axis lies along the DNA dyad axis, while the second is a side-on mode where the metal-phenazine axis lies along the long axis of the base pairs. In the former case, it was suggested that both the phenazine nitrogen atoms are largely protected from the solvent which yield a longer

lifetime ($\tau = 750$ ns). The latter model resulted in only one of the phenazine nitrogens being protected and the other being partially exposed to solvent thus resulting in a shorter lifetime ($\tau = 120$ ns) on the basis of water quenching.

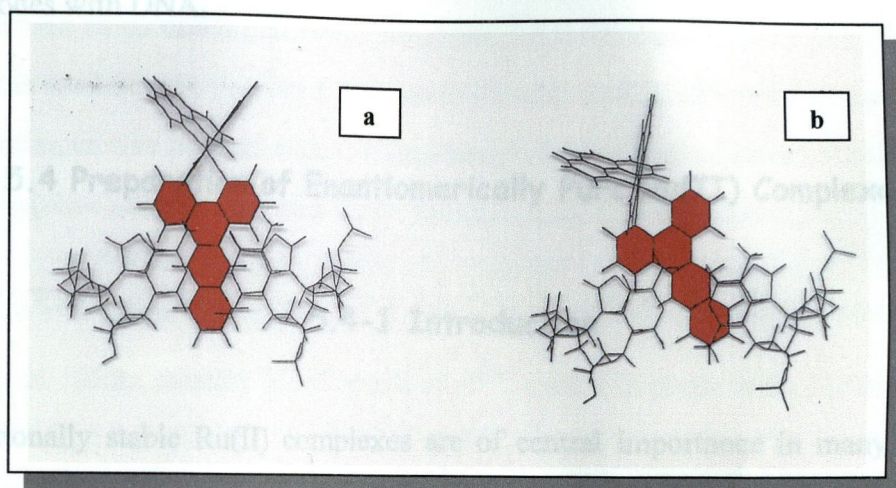


Figure 5.4: Schematic representation of DNA binding modes for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ (4) upon addition of CT-DNA, whereby (a) perpendicular mode (long-lived lifetime) and (b) side-on-mode (short lived lifetime).

Lincoln *et al*^[25] utilized linear dichroism (LD) to probe the binding geometries between DNA and Δ -4 and Λ -4 complexes. They reported that the markedly different LD spectra observed was attributed to a small but distinct clockwise inclination (“roll”) of the complex around the long axis of the intercalating dppz wing. Interestingly, they reported that the ‘roll’ is in the same direction for both enantiomers. Furthermore, complex 4 shows a very similar structure to the chiral intercalator actinomycin D, which has been crystallized with a oligonucleotide^[26]. Actinomycin D has one planar aromatic three ring system which is intercalated and two peptide propellor wings that correspond to the symmetric (dppz) and disymmetric (the two phen) moieties of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ and is considered as a model for the structure of a bulky intercalator compound. Actinomycin binds from the *minor* groove, and the steric interference of the chelate wings gives rise to a distinct bend of the helix. It has been suggested that the inclination may reflect a property intrinsic of DNA (tilt of bases) or be a result of steric interference of the two phen “propellor blades” with a groove.

Given these two very different proposed models, we have performed photophysical studies on the racemic and enantiomeric mixtures of the parent protiated complex **4** and its selectively deuterated analogues, **4a** and **4d**, respectively, to elucidate the nature of their binding modes with DNA.

5.4 Preparation of Enantiomerically Pure Ru(II) Complexes

5.4-1 Introduction

Configurationally stable Ru(II) complexes are of central importance in many domains of chemistry and have been extensively studied in the fields of photochemistry, photophysics and as probes for DNA^{[2][3][8]}. As the individual centers are tris(bidentate) in nature, each may inherently possess right- or left- handed chirality (designated Δ or Λ , respectively), see Figure 5.5. Many applications require these compounds in an enantio-enriched form and thus the preparation and determination of their optical purity is a necessity. To this end, we have briefly reviewed some of the methods available in the literature for resolution of these complexes. Herein, we wish to describe the preparation, resolution and properties of a series of monosubstituted complexes, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complexes.

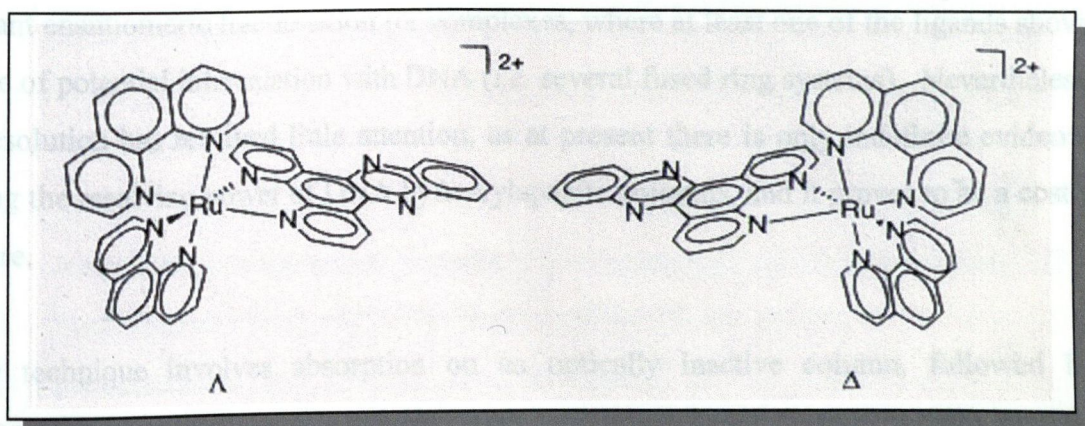
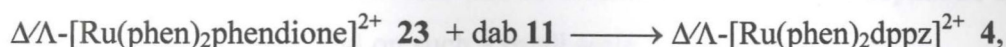


Figure 5.5: Systematic representation of the enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4**, denoted as **4 Δ** and **4 Λ** , respectively

Lincoln *et al*^[4] utilized the classical method of fractional crystallization of diastereomeric salts of the ruthenium racemate with a homochiral anion. This is a modification of the method devised by Bosnich and Dwyer in 1966^[27] for the resolution of $[\text{Ru}(\text{phen})_2(\text{py})_2]^{2+}$ **23**. For this method, the Λ -isomer was resolved with sodium-arsenyl-L-(+)-tartrate, while the Δ - enantiomer was isolated with sodium arsenyl-D-(-)-tartrate. The enantiomerically pure Δ and Λ - $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ isomers were synthesized by condensation of,



in high yields. More recently MacDonnell *et al*^[28] used this preparation for the isolation of various ruthenium complexes.

Many coordination compounds are absorbed on surfaces and this property has been used in two different ways to separate optical isomers. Firstly, if the absorbing surface is optically active, one of the isomers may be absorbed more strongly than the other. Strekas *et al*^[29] reported a novel application of separating the enantiomers of **6** by immobilizing double-stranded calf-thymus (CT) DNA on a column of hydroxylapatite and passing a racemic solution of **6** through the column. Circular dichroism spectra showed that the fractions eluting first were enriched in Λ -**6**, and that the tailing fractions were enriched in Λ -**6**. Both bands were ~95% or higher enantiomerically pure. This method is useful in accomplishing significant enantiomeric fractionation for complexes, where at least one of the ligands shows evidence of potential intercalation with DNA (*i.e.* several fused ring systems). Nevertheless, DNA resolution has received little attention, as at present there is only indefinite evidence regarding the resolving power of DNA-hydroxylapatite columns, and it proves to be a costly procedure.

Another technique involves absorption on an optically inactive column, followed by extraction with an optically active solvent. This cationic exchange chromatography method developed for the resolution of tris(bidentate) Ru(II) complexes is based on the differential

association of the enantiomeric forms with a chiral organic counteranion. This method called preferential absorption, has been improved upon^[30], and to date a wide range of optically active solvents have been reported. According to Barton and coworkers, enantiomers were resolved by passing potassium antimonyl tartrate through a sephadex SP C-25 cation exchange column^[18]. The isomers separated in this manner contain ~75% of the desired enantiomer. This preparation has numerous shortcomings, in that the desired product could not be produced in quantitative yield due to the significant loss of material upon three to four passes through a large column. Furthermore, the separated isomers only contain ~75% of the desired enantiomer and as commercially available sephadex is rather expensive, it proves uneconomical on such a small scale.

There are a number of other approaches to the isolation of individual stereoisomeric forms of ligand-bridged species. von Zelewsky and coworkers^[32] have reported the use of ligands, 'chiragens', which impose a particular stereochemistry on the monomer precursors (stereospecificity).

5.4-2 Methods used in the Current Study for the Chiral Resolution of $[\text{Ru}(\text{phen})_2\text{L}]^{2+}$ Complexes.

A number of synthetic routes were attempted in the current study for the preparation of these optical active complexes, as outlined in the Flowchart in Figure 5.6. Studies involved the isolation of individual stereoisomers of these complexes using both stereoselective synthetic procedures through precursors with predetermined chiralities and/or chromatographic techniques. They include;

- (i) Assembling enantiomerically pure complexes using $[\text{Ru}(\text{phen})_2\text{phendione}]^{2+}$ **23**
- (ii) Stereoretentive reactions using $[\text{Ru}(\text{phen})_2(\text{py})_2]^{2+}$ **24**
- (iii) Stereoretentive reactions using $[\text{Ru}(\text{bpy})_2(\text{py})_2]^{2+}$ **25**
- (iv) Cation exchange chromatographic techniques for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**

Route (i) Initially, we followed the method of assembling enantiomerically pure complexes from chiral precursors. This method involved the condensation reaction of chiral $[\text{Ru}(\text{phen})_2\text{phendione}]^{2+}$ **23** with dab **11**, to give the enantiomers of **4**. Despite extensive studies, we were unsuccessful in isolating the desired products.

Parameter	Experimental Conditions						
Temperature (°C)	80	100	120	140	160	180 reduced to 160	180 reduced to 130
Rxn Time (Hrs)	8	12	24	36	48	96	120
Solvent System	MeOH/H ₂ O	EtOH/H ₂ O	Ethylen e glycol	Ethylene glycol/H ₂ O			
Ligands	Dppz 2	Phendione 12	Bpy 5	Phen 3			

Table 5.4: Experimental conditions employed for the preparation of the enantiomers of complex **4**. The experimental parameters were interchanged. All reactions were performed in the dark to avoid photoracemisation

Routes (ii) and (iii). The stereoretentive reactions of von Zelewsky *et al*^[32] were attempted. The resolution of $[\text{Ru}(\text{L})_2(\text{py})_2]^{2+}$ complexes (where L = bpy **25** and phen **24**) were carried out with Dr. A. von Zelewsky, University of Fribourg. They were conveniently resolved by conventional diastereoisomer formation using chiral arsenyl-(+)-tartrate (for **24**) and O,O'-dibenzoyltartrate anions (for **25**), respectively. Reaction of these chiral precursors with dppz (under both aqueous methanol and ethylene-glycol mixtures) was hoped to provide the anticipated Δ - and Λ -**4** enantiomers. To increase the solubility of the free dppz ligand in these solvents a range of (i) temperatures and (ii) reaction times, were introduced into the reaction. In addition, an intermediate step was employed using phendione **12** in place of dppz **2**, the product of which was subsequently reacted with dab **11**. Table 5.4, summarises the range of experimental conditions employed. However, these reactions proved to be

unsuccessful, yielding only partially resolved enantiomerically pure samples, as determined from CD analysis.

Route (iv) The cation-exchange chromatographic technique^[33], allowed for the efficient resolution of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex and its deuterated analogs. These experiments were carried out with Dr. N. Fletcher, Queens University, Belfast. This method is based on the differential association of the enantiomeric forms with a chiral organic counteranion, and discriminates according to (i) charge and (ii) size.

In a typical experiment, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** was resolved on a column of sephadex SP C-25 cation exchanger using 0.2 mol dm⁻³/ 0.09 M (-)-O,O'-dibenzoyl-L-tartrate solution as the eluent, which contained 5% acetone to maintain complex solubility. The column was sealed, enabling the complex to be recycled several times (if necessary) down its length with the aid of a peristaltic pump. The "effective column length" (ECL) for the separation represents the total length of support travelled by the sample. Two distinct bands were observed on passing the complex down the column (Δ -**4** being eluted faster), with an effective column length (ECL) of ~3 m, see Figure 5.7. Following precipitation as the PF₆⁻ salts they were converted to their corresponding chloride salts and further purified by gel permeation chromatography on sephadex LH20 support (acetone/MeOH eluent) to remove inorganic impurities. The purity and identity of the complexes were verified by circular dichroism (CD), UV-Vis absorption, ¹H NMR, and electrospray mass (ES⁺-MS) spectroscopy. The experiment was carried out in the absence of light as a precautionary measure, although no photoracemisation of these complexes were observed under normal laboratory lighting conditions. Recoveries after chromatography were in general only about 50%.

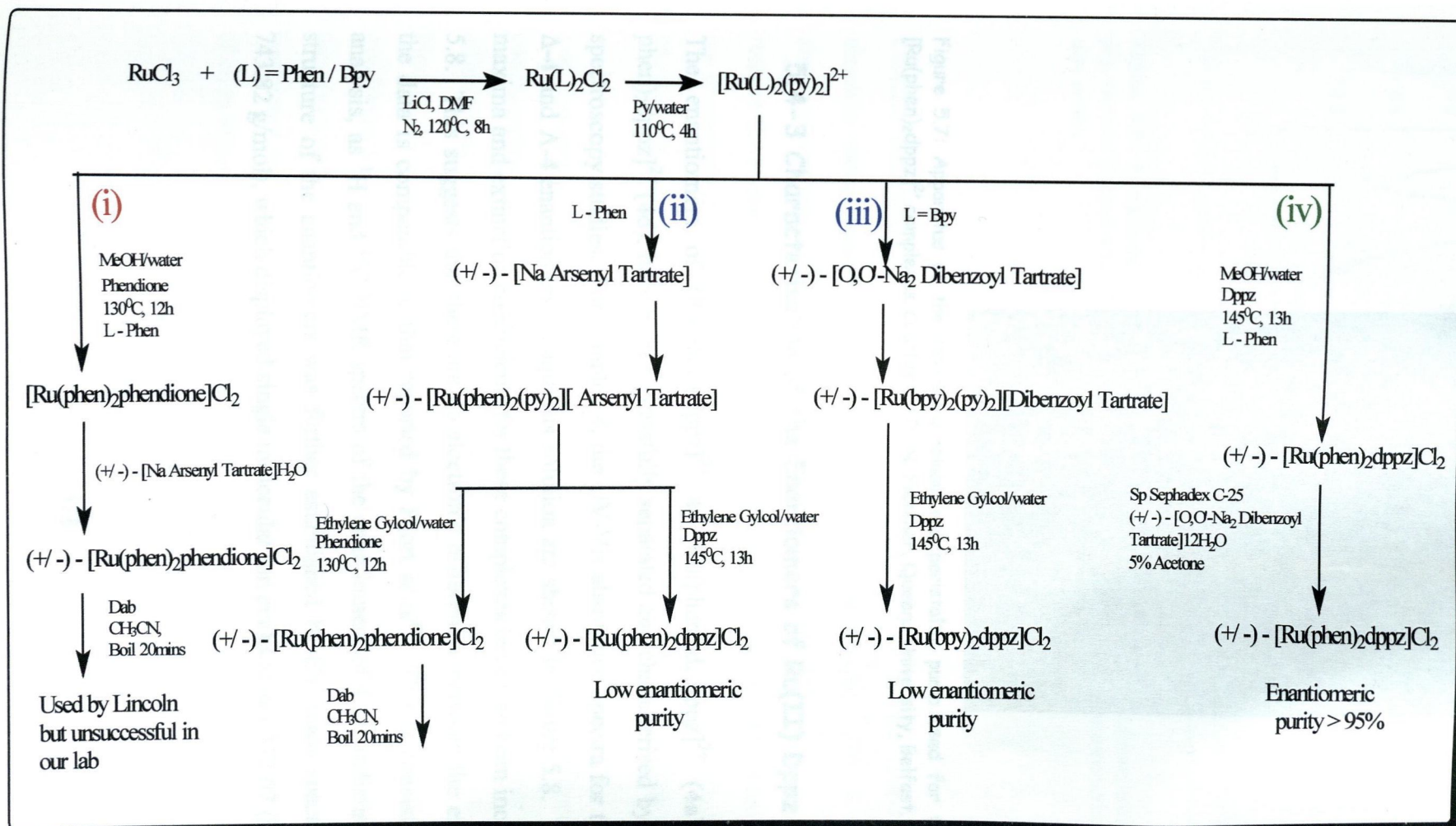


Figure 5.6: Flowchart representing the synthetic procedures for the resolution of the $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex 4 into its Δ - and Λ -enantiomers.

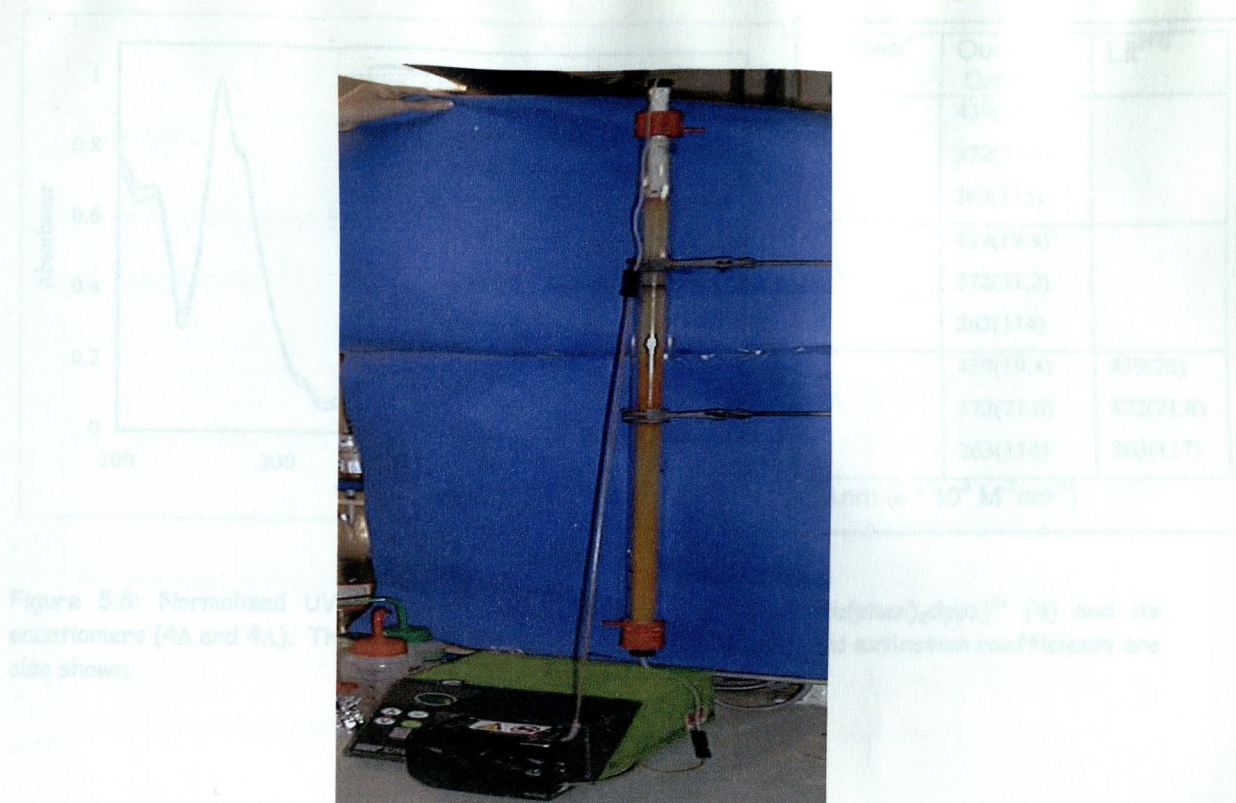


Figure 5.7: Apparatus of the separating column and peristaltic pump used for the resolution of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complexes. Courtesy of Dr N. Fletcher, Queens University, Belfast.

5.4-3 Characterisation of the Enantiomers of Ru(II) Dppz Complexes

The enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, $[\text{Ru}(\text{phen})_2\text{d}_4\text{-dppz}]^{2+}$ (**4a**) and $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$ (**4d**), have been successfully separated and characterised by NMR and CD spectroscopy studies. For complex **4**, the UV-Vis absorption spectra for the racemic and Δ -**4** and Λ -**4** enantiomers, in aqueous solution are shown in Figure 5.8. The absorption maxima and extinction coefficients for these complexes have also been included in Figure 5.8. This suggests that there are no electronic differences between the enantiomers and the data is comparable to that reported by Hiort *et al*^[4]. This is consistent with NMR analysis, as ^1H and ^{13}C NMR spectra of the enantiomers of **4** are indistinguishable. The structure of the enantiomers was further established by ES^+ -mass spectroscopy ($M_r = 743.82$ g/mol), which displayed single molecular ion peaks at m/z 372.07 (MH^+)²⁺.

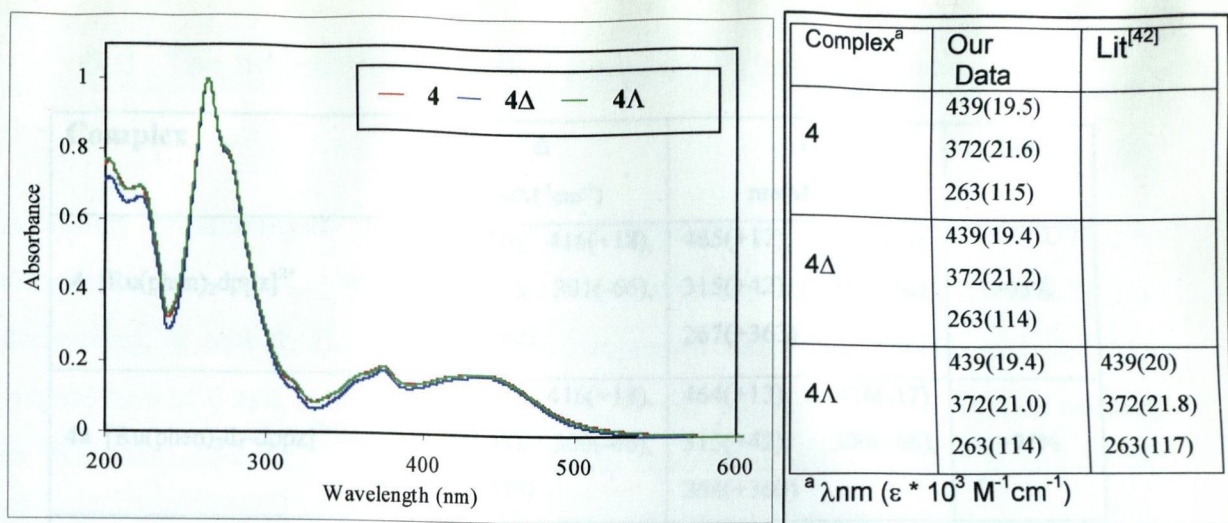


Figure 5.8: Normalised UV-Visible Absorption Spectra (H₂O) for [Ru(phen)₂dppz]²⁺ (**4**) and its enantiomers (**4Δ** and **4Λ**). The corresponding absorption energy maxima and extinction coefficients are also shown.

5.4.3-1 Circular Dichroism.

Circular dichroism (CD) spectroscopy is a powerful tool for the determination of the absolute stereochemistry and optical purity of a molecule. CD is the differential absorption of left and right-handed *circular* polarized light, $CD = a_{\text{left}} - a_{\text{right}}$, and does not require the molecules to be orientated or immobilized. As only a molecule that is chiral (or is perturbed by a chiral environment), shows CD and since the CD spectrum of one enantiomer is the negative of the other, racemic mixtures do not show any CD spectrum.

For complex **4**, comparison of the CD spectra of Δ-**4** and Λ-**4** confirmed the absolute configuration at the metal centers. The enantiomers of **4** show equal but opposite molar ellipticities, while no CD spectrum is observed for the racemic complex, as shown in Figure 5.9. The enantiomer with the negative Δε in the CD spectrum at the absorption maximum λ = 450 nm was assigned as the Λ-enantiomer. The results of CD analysis for the series of complexes (**4-4d**) are compiled in Table 5.5. They display similar absorption bands and are comparable to the literatures values which have been included for the sake of comparison.

Complex	Δ nm(M ⁻¹ cm ⁻¹)	Λ nm(M ⁻¹ cm ⁻¹)	%ee
4 [Ru(phen) ₂ dppz] ²⁺	465(-16), 416(+18), 315(-43), 301(-66), 267(-360)	465(+17), 416(-18), 315(+42), 301(+66), 267(+363)	>95%
4a [Ru(phen) ₂ d ₄ -dppz] ²⁺	463(-14), 416(+18), 315(-43), 300(-66), 267(-359)	464(+13), 416(-17), 315(+42), 300(+66), 268(+360)	>95%
4b [Ru(phen) ₂ d ₆ -dppz] ²⁺	463(-15), 415(+19), 315(-45), 299(-63), 268(-361)	*	>93%
4c [Ru(phen) ₂ d ₁₀ -dppz] ²⁺	*	463(+14), 416(-17), 315(+41), 299(+66), 266(+361)	>92%
4d [Ru(d ₈ -phen) ₂ dppz] ²⁺	464(-17), 416(+19), 315(-43), 300(-64), 267(-360)	464(+16), 416(-18), 315(+42), 300(+66), 267(+361)	>94%
Hiort <i>et al</i> ^[4] 4 [Ru(phen) ₂ dppz] ²⁺		465(+17), 416(-18), 301(+66), 267(+363) 254(-178)	>95%

Table 5.5: The Circular dichroism (CD) data for [Ru(phen)₂dppz]²⁺ (**4**) and its deuteriated complexes (**4a**, **4c** and **4f**) *Low enantiomeric purity samples

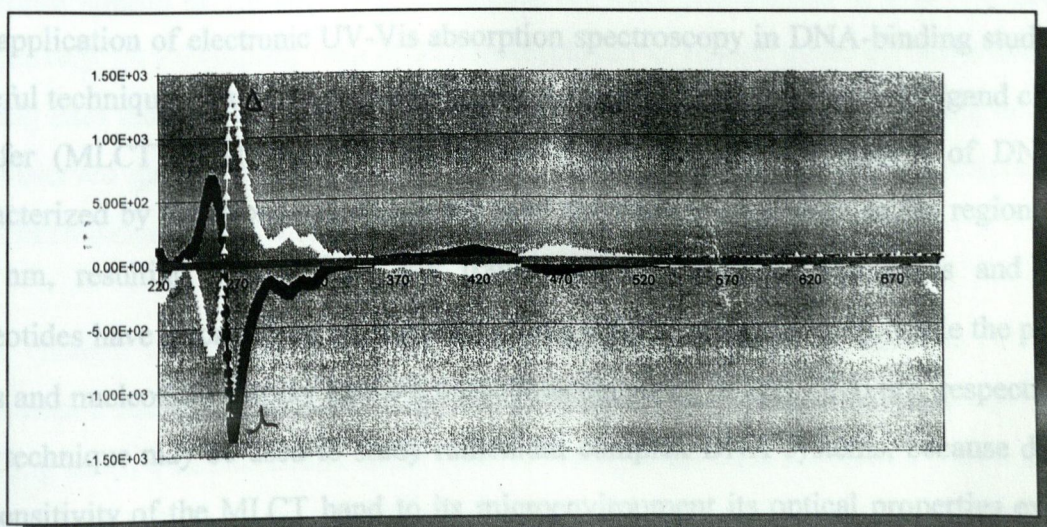


Figure 5.9: CD spectra of Δ - and Λ -enantiomers of [Ru(phen)₂dppz]²⁺ complex (**4**).

5.5 The interaction of Ru(II) Polypyridyl Complexes with DNA.

A variety of techniques have been used to examine the interactions of ruthenium complexes with DNA. We will consider (i) $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** and its substituted derivatives, **8** and **9**, (ii) the selectively deuterated analogues (**4a-4f**) and (iii) the enantiomers of **4** and its deuterated analogues, **4a** and **4d**, respectively. Despite interest in $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9** and the selectively deuterated complexes of **4** and **8**, no photophysical studies of these have yet been reported, although reports of ^1H NMR studies in the presence of DNA have appeared^[20]. The interaction of the dppz ligand with the DNA π -stack may be probed by monitoring the changes in the spectral profiles of these complexes upon addition of DNA. In the present study, the DNA-binding properties were investigated by UV-Vis absorption, steady-state, and time-resolved luminescence studies. These monitoring techniques by themselves, are not definitive, but used in conjunction with each other give an overall indication of the DNA interactions of these complexes. It was difficult to make direct comparisons with reported studies as many different conditions, techniques, and models of analysis have been used, even within single studies.

5.5-1 Electronic Absorption Spectroscopy.

The application of electronic UV-Vis absorption spectroscopy in DNA-binding studies is a useful technique for monitoring the changes in the visible region metal-to-ligand charge transfer (MLCT) band of Ru(II) complexes. The absorption spectrum of DNA is characterized by the strong absorption bands of the nucleic acid bases in the region 250-280 nm, resulting mainly from π - π transitions. The pyrimidine bases and their nucleotides have a corresponding band at 260 nm (due to π - π transition), while the purine bases and nucleotides display two π - π transitions occurring at 260 - 270 nm, respectively. This technique may be used to study ruthenium complex-DNA systems, because due to the sensitivity of the MLCT band to its microenvironment its optical properties exhibit changes (although small in magnitude) on binding to DNA. The spectral changes, in

particular the degree of hypochromicity, observed on addition of DNA to the complex solution can be analysed so as to obtain the thermodynamic parameters; binding constant (K_{app}) and the site size (B_{app}), respectively.

5.5.1-1 Effect of DNA on the UV-Vis Spectra of $[Ru(phen)_2dppzX_2]^{2+}$ Complexes

Absorption titrations of $[Ru(phen)_2dppz]^{2+}$ **4**, and related racemic complexes $[Ru(phen)_2diMedppz]^{2+}$ **8** and $[Ru(phen)_2diFdppz]^{2+}$ **9**, were performed with CT-DNA, in 10 mM Tris buffer, without salt NaCl, at pH = 7.1.

In the *absence of DNA*, the absorption spectra typically displayed two maxima. An MLCT band, is present at approximately 440 nm with little variation upon substitution. The remaining visible band common to these complexes, an IL band, lies at higher energy (370 - 385 nm) and tends to show a greater dependence on the nature of the substituents. In the presence of 10 mM tris buffer without DNA the spectra remain unchanged relative to the complexes in aqueous solution.

UV-Vis absorption spectra were obtained for aqueous solutions of **4**, **8**, and **9**, ($[Ru] = 5\mu M$), as a function of DNA concentration, for P/D values ranging from 0 $\rightarrow$ 100. For all three complexes, addition of CT-DNA induced spectral changes. Figure 5.10, illustrates spectra in the 300 - 600 nm region for these complexes, at P/D = 0 and 20, respectively. A P/D mixing ratio of 20 was employed to ensure, given the large excess of binding sites that most of the complex is expected to be bound, if binding were to occur. The observed spectral changes are tabulated in Table 5.6, the model system $[Ru(phen)_3]^{2+}$ **6** has been included for the sake of comparison.

What changes do we expect to see for these Ru(II) complexes on addition of DNA? Intercalation has a characteristic effect on the visible bands of the intercalator generally resulting in; (i) a bathochromic effect (*i.e.* red shift) of the band associated with a change in the solvent polarity as the Ru(II) center interacts with DNA, (ii) due to a stabilisation of

the excited state through strong π - π stacking interactions between an aromatic chromophore and the base pairs of DNA, there is a red shift and a pronounced hypochromism (a decrease in the absorbance intensity) of the maximum absorbance (λ_{max}) and finally (iii) the occurrence of an isobestic point *may* indicate a single mode of binding/orientation over the range of P/D ratios studied.

Complex	Absorption λ_{max} (nm)		$\Delta\lambda$ (nm) % Hypo	
	Free ^a	Bound ^b	(MLCT) ^c	(MLCT) ^d
6 [Ru(phen) ₃] ²⁺	447	430	17	12
4 [Ru(phen) ₂ dppz] ²⁺	372, 440	380, 442	2	18
9 [Ru(phen) ₂ diFppz] ²⁺	372, 439	370, 440	1	4
8 [Ru(phen) ₂ diMeppz] ²⁺	382, 442	387, 444	2	23

Table 5.6: Absorption Spectroscopic properties of the Ru(II) complexes on addition to DNA.

^a Wavelength of maximum absorption of MLCT(λ_{max}) when ruthenium complexes are 'free' (*i.e.* in 10mM tris buffer) - [Ru] = 5 μ M

^b Wavelength of absorbance (λ_{max}) when 100% of the ruthenium complexes are bound at P/D = 20

^c The change in λ_{max} (440nm) from conditions of 'free' to 'fully bound' ruthenium complex.

^d This is related to the observed overall decrease in absorbance intensity at λ_{free} (*see* ^a). The values have been corrected for dilution effects.

On addition of DNA to **4**, the most striking effect was observed in the IL absorbance band, which undergoes a marked decrease in the intensity coupled with a red shift (~ 8 nm) in the λ_{max} . In the visible region, the broad, poorly resolved MLCT transitions of both the dppz and the phen ligands, undergo a slight intensity decrease and further band broadening (~ 1 nm) in the presence of DNA. Addition of salt, does not appear to have an influence on hypochromicity of the absorption bands. For **4**, there is a greater hypochromicity (18%) of the MLCT band compared to that observed for complex **6** (12%). This enhancement is expected as the extent of hypochromism commonly parallels the intercalative binding strength, which is enhanced in complex **4** due to the extended planar dppz ligand.

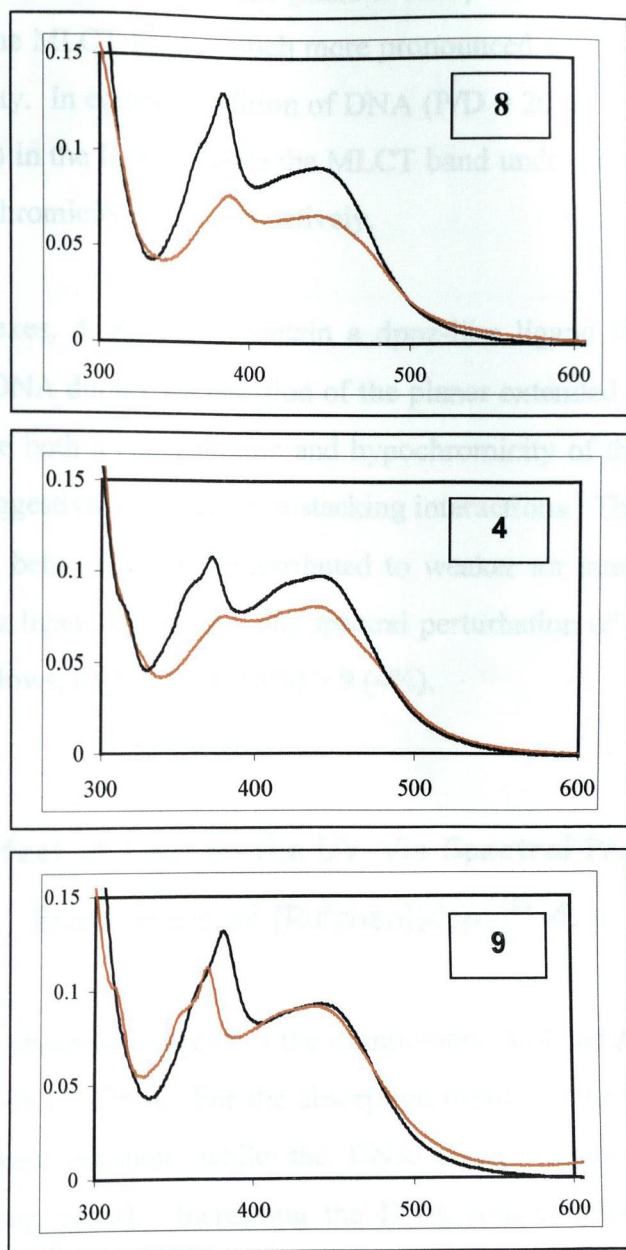


Figure 5.10: The UV-Vis absorption spectra of complexes, 8, 4, and 9 in aqueous 10 mM tris buffer solution (black line) and in the presence of CT-DNA at P/D = 20 (red line). The spectra were corrected for dilution, upon addition of DNA.

The diMedppz complex **8**, also displayed a bathochromic and hypochromicity for both the IL and MLCT bands in the presence of DNA ($P/D = 20$). On examination of the data, small shifts of 5 nm (IL band) and 2 nm (MLCT band) were observed. However, the hypochromicity of the MLCT band is much more pronounced displaying a 23% decrease in absorption intensity. In contrast, addition of DNA ($P/D = 20$) to complex **9** resulted in a blue-shift (~ 2 nm) in the IL band while the MLCT band undergoes a small red-shift of ~ 1 nm with a hypochromicity of 4%, respectively.

As all three complexes, **4**, **8**, and **9**, contain a dppz-like ligand they are expected to intercalate into the DNA duplex via insertion of the planar extended dppz π -system. For **4**, and **8**, we observe both a bathochromic and hypochromicity of the absorption bands, which is strongly suggestive of intercalative stacking interactions. This is not the case for **9**, and the observed behaviour may be attributed to weaker $\pi\pi$ interaction between the intercalating diFdppz ligand and DNA. The spectral perturbation of the complexes upon addition of DNA follows; **8** (23%) > **4** (18%) > **9** (4%).

5.5.1-2 Effect of DNA on the UV-Vis Spectral Properties of Enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**.

The behaviour of the absorption spectra of the enantiomers, Δ -**4** and Λ -**4**, were examined upon intercalation with CT-DNA. For the absorption titrations, the total metal complex concentration was kept constant, while the DNA concentration was increased (*i.e.* increasing P/D mixing ratios). Increasing the DNA concentration, resulted in three different phases of events for the complexes, the first two phases are illustrated in Figure 5.11. The observed spectral changes upon addition of DNA to complexes, **4**, Δ -**4** and Λ -**4**, are compiled in Table 5.7.

Complex	λ_{free} P/D = 0	λ_{DNA} P/D = 20	$\Delta\lambda(\text{nm})$ MLCT	% Hypo MLCT	Isobestic Point	K_B (M bp)
Rac-4	373, 440	381, 442	2	18	480	$5 \cdot 10^6$
Δ-4	373, 440	381, 443	3	32	482	$2.9 \cdot 10^6$
Λ-4	373, 440	381, 442	2	26	477	$1.5 \cdot 10^6$

Table 5.7: Absorption Spectroscopic properties of **Rac-4**, **Δ -4** and **Λ -4** complexes in the presence of DNA. $[\text{Ru}] = 5 \mu\text{M}$ at P/D = 20.

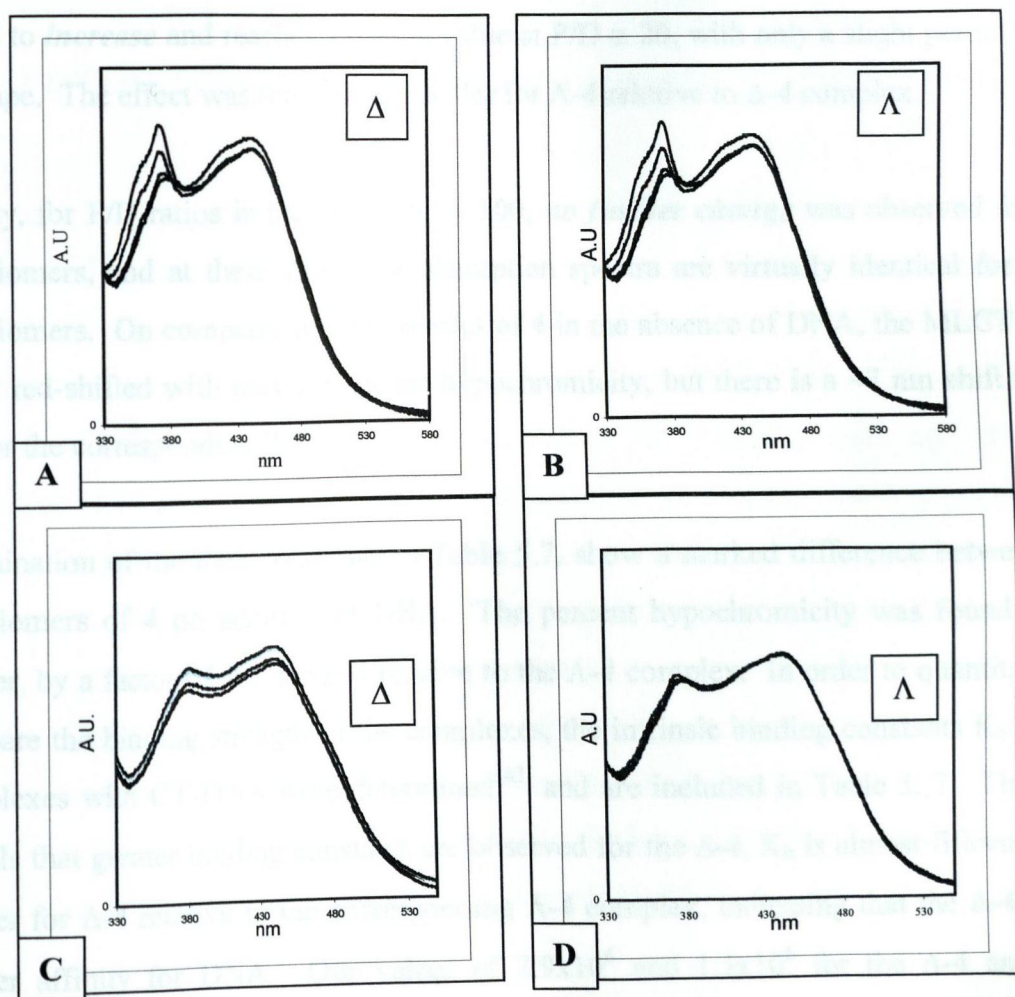


Figure 5.11: Absorption spectra (arbitrary units) of **Δ -4** and **Λ -4** in the presence of CT-DNA. The mixing P/D ratios are the following: (A and B) from top to bottom 0 (no DNA present), 1, 1.5, 2, 3 and 5 and (C and D) from bottom to top: 10, 20, 50. $[\text{Ru}] = 10^{-5} \text{ M}^{-1}$

The first phase is characterized by a hypochromic effect, as shown in Figure 5.11 [(A) for Δ and (B) for Λ], respectively. The absorption intensity at 440 nm (MLCT) and 372 nm (IL) bands **decrease** linearly on increasing the DNA concentration, from zero up to P/D = 4. The effect is more pronounced for the IL band, but occurs to different extents for the two enantiomers. An absorption decrease of 32% and 26% occurred for Δ -4 and Λ -4 at P/D = 4. An isobestic point was observed at 478 nm for Λ -4 at 481 nm for Δ -4, respectively. This is in accordance with the literature, with reported values of 477 and 482 nm for Λ - and Δ -complexes^[4].

The second phase of events occurs at higher P/D ratios, and is shown in Figure 5.11 [(C) for Δ and (D) for Λ]. For this phase, a different behaviour is observed, the absorption was found to **increase** and reaches a plateau value at P/D $\cong$ 20, with only a slight perturbation of shape. The effect was found to be smaller for Λ -4 relative to Δ -4 complex.

Finally, for P/D ratios in the range 16 $\rightarrow$ 100, **no further change** was observed for the enantiomers, and at these values the absorption spectra are virtually identical for both enantiomers. On comparison to the spectra of 4 in the absence of DNA, the MLCT band is not red-shifted with only a moderate hypochromicity, but there is a $\sim$ 7 nm shift to the red for the corresponding IL band.

Examination of the data, as shown in Table 5.7, show a marked difference between the enantiomers of 4 on addition of DNA. The percent hypochromicity was found to be greater, by a factor of 1.2, for Δ -4 relative to the Λ -4 complex. In order to quantitatively compare the binding strength of the complexes, the intrinsic binding constants K_b of the complexes with CT-DNA were determined^[43] and are included in Table 5. 7. The data reveals that greater binding constants are observed for the Δ -4, K_b is almost fifteen times greater for Δ -4 relative to the corresponding Λ -4 complex, indicating that the Δ -4 has a greater affinity for DNA. Our values of 2.9×10^6 and 1.5×10^6 for the Δ -4 and Λ -4 complexes compare well to reported values of 3.2×10^6 and 1.7×10^6 , respectively^[54].

5.5-2 Steady State Spectroscopy Studies in the presence of DNA.

5.5.2-1 Introduction.

Given the preferential charge transfer onto the intercalating dppz ligand, the luminescence of the bound complex provides a sensitive reporter of its environment. Furthermore, if the dppz ligand is intercalated as both photophysical and thermodynamical properties suggest, it is clear for steric reasons that the two ancillary phen ligands, should have quite different orientations relative to the DNA and therefore give rise to different properties for the Δ - and Λ -enantiomers. The extent of DNA binding, is perhaps, best illustrated by steady-state and emission lifetime studies (see section 5.5-3).

5.5.2-2 Effect of DNA on the Excited State properties of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ 4.

The steady state emission spectra of **4** was examined in aerated solution with 10 mM tris buffer (pH 7.1) in the absence and presence of CT-DNA, at ambient temperatures. In water solutions no emission was found. However, in the presence of DNA emission was observed, the complex behaving as a molecular 'light switch'^[17]. Figure 5.12, exhibits the luminescence from **4** by DNA addition for P/D = 20. Luminescence with emission centred at ~613 nm was found, which is comparable in intensity and is slightly blue-shifted to that found in ethanol (see Chapter Four). The spectrum appears broad and structureless which is indicative of an MLCT emissive state. In these photophysical studies an enhancement in the emission intensity is consistent with binding of the complex with CT-DNA, as described in the literature^[1]. It has been suggested that the DNA helix protects the excited state by surrounding the phenazine nitrogens. In effect, they are no longer able to interact with the water molecules which deactivate the MLCT excited state by proton transfer, or partial proton transfer^[18].

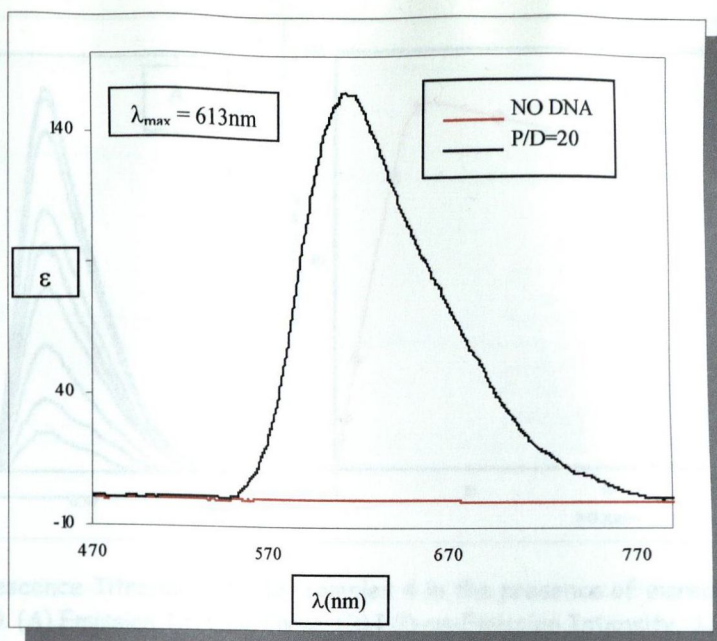


Figure 5.12: Emission enhancement of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ on addition of CT-DNA to an aqueous solution. $\text{P/D} = 20$, 10mM tris buffer, $\text{pH} = 7.1$.

For **4**, the occurrence of luminescence, at constant concentration, was monitored as a function of increasing amounts of DNA. Figure 5.13 displays a plot of intensity of emission as a function of increasing P/D ratios in the range $0 \rightarrow 100$, the emission titration curve has also been included.

The maximum wavelength of emission, at $\lambda_{\text{max}} = 613 \text{ nm}$ remains unchanged with increasing P/D ratios. At low P/D ratios the emission centred at $\lambda = 613 \text{ nm}$, increases linearly up to P/D ratio ~ 20 (maximum emission point) where the complex is expected to be fully bound. A second phase was observed where the emission decreases upon further addition of DNA to a P/D ~ 60 , possibly due to the dilution of the sample at high concentrations of DNA. After this point, no further changes were observed. It has been reported^[4] that complex **4** displays similar effects in the presence of $[\text{poly}-(\text{dA-dT})]_2$.

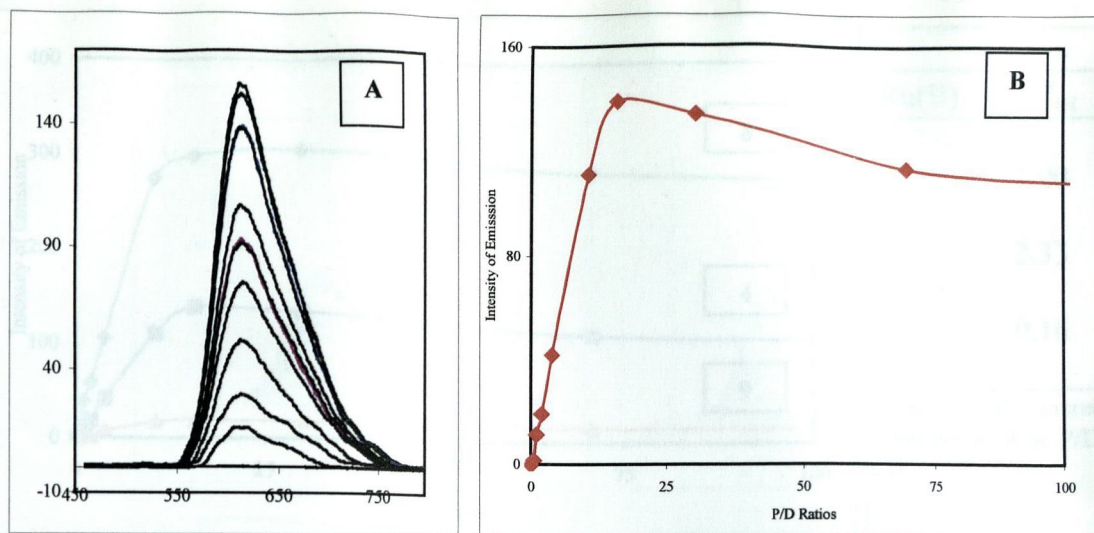


Figure 5.13: Luminescence Titration Curve for complex **4** in the presence of increasing amounts of CT-DNA, P/D = 0 - 100. (A) Emission Titration Curve, (B) P/D-vs-Emission Intensity. $\lambda_{\text{ex}} = 440 \text{ nm}$

The effect of DNA on the excited state properties of the related complexes, **8** and **9**, were investigated, under identical conditions. In aqueous solution, no luminescence was found for these complexes. This is in contrast to Barton *et al*^[41] who reported that complex **8** displays weak emission in aqueous solution, while complex **9** has not been previously examined in this context.

Addition of DNA to complexes, **4**, **8**, and **9**, resulted in an enhancement in the emission intensity, as the complexes interact with DNA by insertion of their extended dppz ligand between the base-pairs of the DNA helix. Therefore these complexes can act as molecular “light switches. Plots of the intensity of emission as a function of increasing P/D ratios is shown in Figure 5.14. They reveal that the strongest emission was observed for diMeppz **8** (green line), followed by dppz **4**, and then the diFdppz **9** complex in the presence of DNA. The relative quantum yields, Φ_{rel} , (relative to **4** at a P/D value of 20) were calculated and show that **8** was found to be approximately 2 times greater than complex **4**, and 15 times greater than **9**. This trend is also observed for the emission quantum yields in acetonitrile in the absence of DNA, as detailed in Chapter Four.

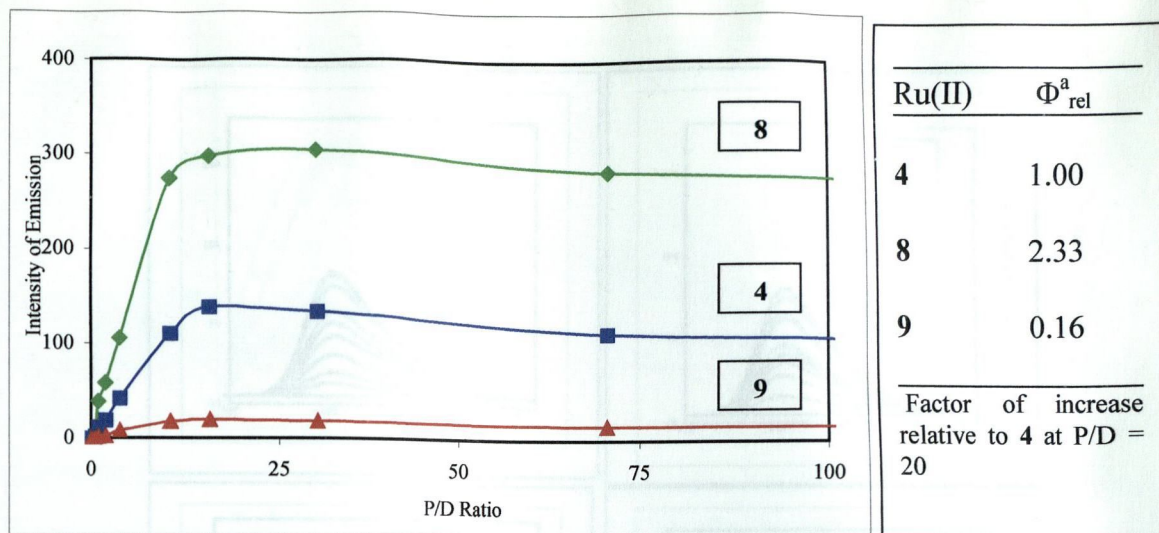


Figure 5.14: Emission of complexes, 8, 4, and 9, versus increasing P/D ratios, P/D = 0 - 100. In all cases, the complex concentration is constant and the experimental set-up is comparable.^a Factor of increase in the quantum yield relative to complex 4 at P/D = 20

5.5.2-3 The Effect of DNA on the Deuterated Complexes of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ 4.

Emission titration studies for the protiated 4, perdeuterated (4g) and selectively deuterated analogues (4a-4f), were examined upon addition of increasing amounts of CT-DNA, under identical conditions. The emission titration curves, for the series of complexes (4-4g) are shown in Figure 5.15. These plots display an increase in the intensity of emission on increasing deuteration of the complexes. From the luminescence titration curves, the largest emission enhancement was observed for the perdeuterated complex 4g, while 4 exhibits the smallest emission intensity, respectively. The luminescence intensity for complexes (4-4g) were also plotted as a function of increasing concentrations of DNA, with P/D = 0 - 100, as shown in Figure 5.16. The emission quantum yield, Φ_{rel} (relative to the protiated complex 4) at P/D = 20 is also included in Figure 5.16.

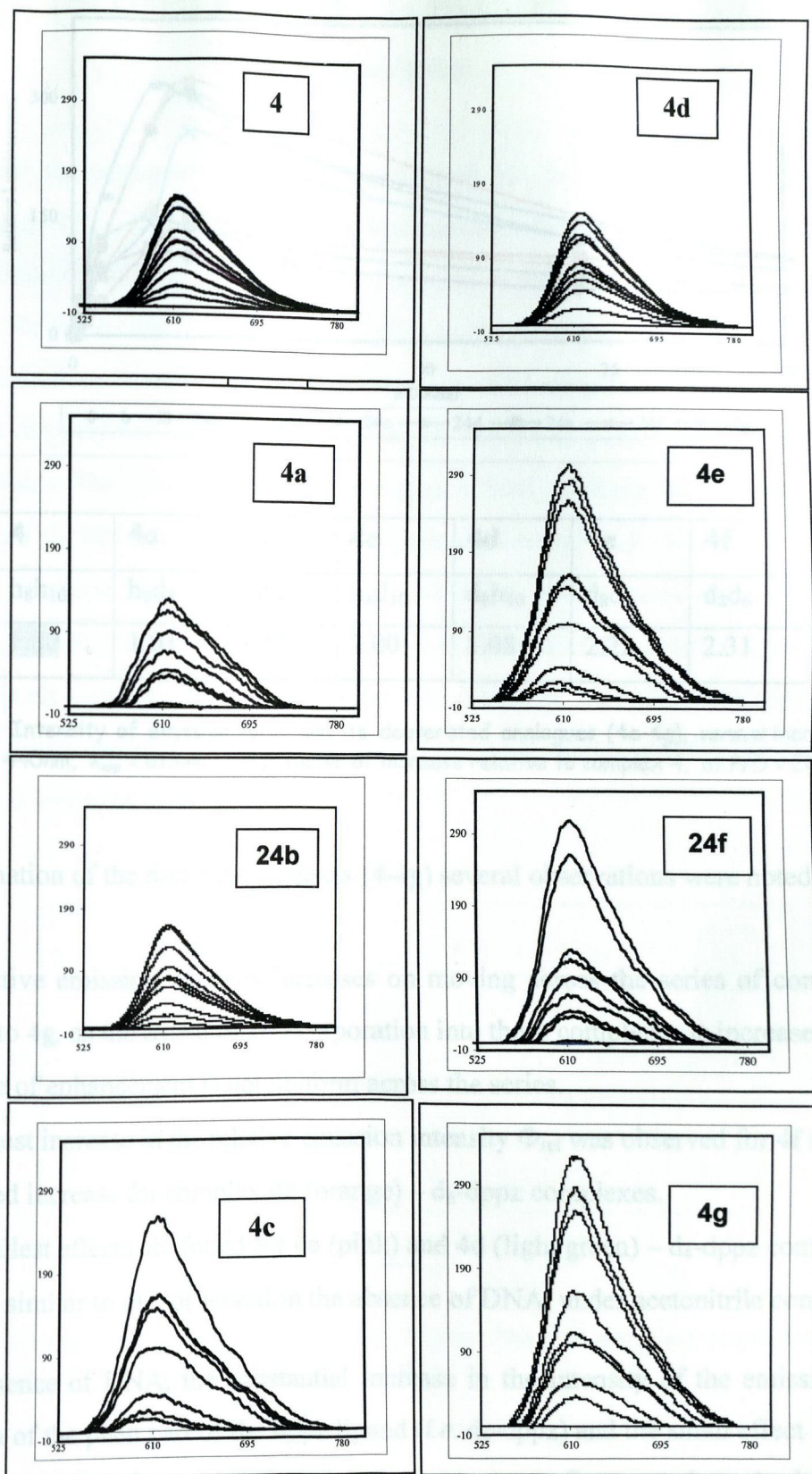
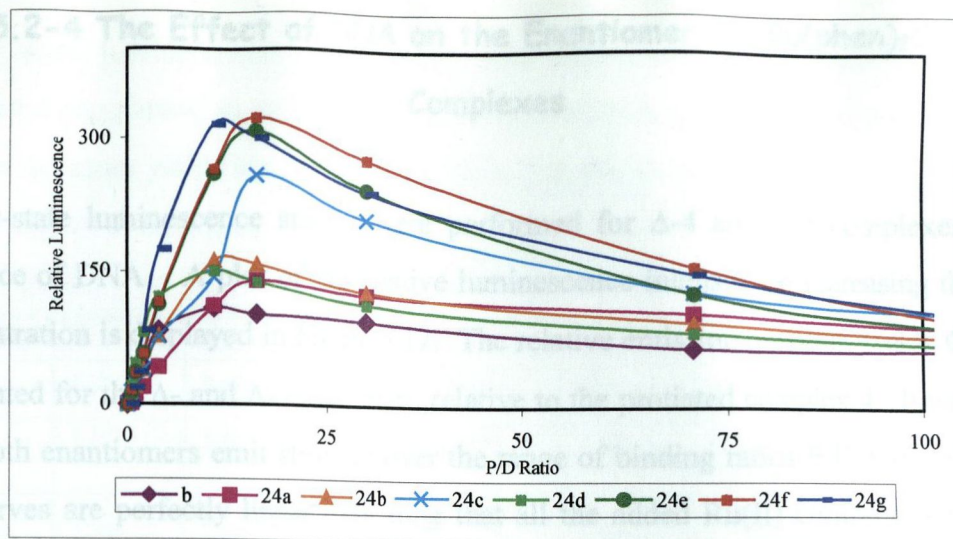


Figure 5.15: Luminescence Titration Curves for series of compounds (4-4g) in the presence of DNA.



Complex	4	4a	4b	4c	4d	4e	4f	4g
	hgh ₁₀	hgd ₄	hgd ₆	hgd ₁₀	dgh ₁₀	dgd ₄	dgd ₆	dgd ₁₀
Φ_{rel}^a	1.00	1.03	1.17	2.00	1.08	2.22	2.31	2.48

Figure 5.16: Intensity of emission for **4** and its deuterated analogues (**4a-4g**), versus increasing P/D ratios. $\lambda_{ex} = 440\text{nm}$, $\lambda_{em} = 613\text{nm}$. ^a Φ_{rel} , factor of increase relative to complex **4**, at P/D = 20.

On examination of the data for complexes (**4-4g**) several observations were noted.

- The relative emission intensity increases on moving across the series of compounds, from **4** to **4g**, as the extent of D-incorporation into these complexes is increased.
- The rate of enhancement is not uniform across the series.
- The largest increase in the relative emission intensity Φ_{rel} was observed for **4f** (red)
- A marked increase for complex **4b** (orange) – d₆-dppz complexes.
- The smallest effects are found for **4a** (pink) and **4d** (light green) – d₄-dppz complexes.
- Trend is similar to that observed in the absence of DNA, under acetonitrile conditions.

In the presence of DNA, the substantial increase in the intensity of the emission upon deuteration of the phen part of the dppz ligand (*i.e.* d₆-dppz) and the small effect observed on exchange of the phenazine protons (*i.e.* d₄-dppz), confirms our hypothesis that the ‘promoted’ electron is most likely to be located on the phen moiety of the dppz ligand.

5.5.2-4 The Effect of DNA on the Enantiomers of $\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$

Complexes

Steady-state luminescence studies were performed for Δ -4 and Λ -4 complexes in the presence of DNA. A plot of the relative luminescence intensity on increasing the DNA concentration is displayed in Figure 5.17. The relative emission quantum yields Φ_{rel} were calculated for the Δ - and Λ - complexes, relative to the protiated complex 4. It was found that both enantiomers emit strongly over the range of binding ratios $P/D = 0 - 17$ where the curves are perfectly linear indicating that all the added $\text{Ru}(\text{II})$ complex is bound at constant yields. The plot shows that the Δ -4 (green line), displays the strongest emission when bound to DNA with a maximum emission point of $P/D \sim 21$. In contrast, the maximum emission point for Λ -4 was not so clear-cut, but the curve *appears* to change slope at similar P/D ratios of Δ -4 complex. It was found that the emission quantum yield for Δ -4 was six times greater than that of the corresponding Λ -4. Owing to the difference in their quantum yields, the emission of 4 Λ may be considered negligible compared to Δ -4 in racemic mixtures.

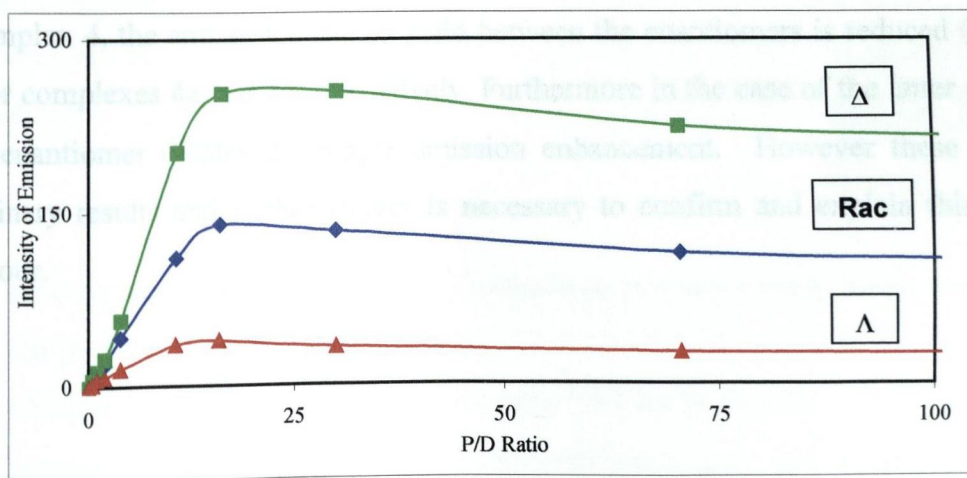


Figure 5.17: Emission titration of Δ - and Λ -4 with CT-DNA. The DNA was added to complex, $[\text{Ru}] = 5\mu\text{M}$, in tris buffer (pH 7.1) and equilibrated for 10 mins.

Steady state luminescence studies were also performed for the enantiomers of the deuterated complexes, 4a and 4d, in the presence of DNA, under identical conditions. The relative quantum yields are compiled in Table 5.8, the values for complex 4 are included for the sake of comparison.

Complex	4	4a	4b	4c	4d	4e	4f	4g
	h ₈ h ₁₀	h ₈ d ₄	h ₈ d ₆	h ₈ d ₁₀	d ₈ h ₁₀	d ₈ d ₄	d ₈ d ₆	d ₈ d ₁₀
Φ_{rel}^a	1.00	1.03	1.17	2.00	1.08	2.22	2.31	2.48
$\Delta \Phi_{rel}$	1.80	2.40	2.25	*	1.21	*	*	*
$\Lambda \Phi_{rel}$	0.27	1.55	*	0.92	2.68	*	*	*

Table 5.8: A comparison of the emission intensity enhancement relative to the racemic and enantiomeric protiated complexes of Ru(II) complexes in the presence of DNA. ^a Measured at λ_{ex} = 440nm, λ_{em} = 613nm. ^b Tris buffer (10mM), [Ru] = 5 μ M. ^c P/D = 20. * Not successfully resolved *via* chromatography techniques

For the enantiomers of the protiated complex 4, the Δ - enantiomer displays the greater intensity of emission (factor of 6) compared to the Λ -enantiomer. On D-incorporation into the complex 4, the emission quantum yield between the enantiomers is reduced (factor of 2-3) for complexes 4a and 4d, respectively. Furthermore in the case of the latter complex, the Λ -enantiomer displayed stronger emission enhancement. However these are only preliminary results and further studies is necessary to confirm and explain this unusual behaviour.

5.5-3 Time Correlated Lifetime Measurements in the Presence of DNA

5.5.3-1 Introduction

Single photon counting (SPC) spectroscopy is a powerful technique in monitoring changes in the photophysical behaviour of Ru(II) polypyridyl complexes on binding to DNA. The emission lifetime decays of (i) racemic complexes **4** and **4a** (ii) enantiomers of **4** and (iii) deuterated enantiomers of complexes of **4a** and **4d**, were investigated in the presence of increasing concentrations of DNA. As these Ru(II) complexes bind to DNA with at least two different orientations, they exhibit biexponential decays. All experiments containing DNA were performed in 10mM tris buffer and 50mM NaCl, with a pH of 7.1. The emission lifetimes were measured by excitation at λ_{ex} 337 nm and emission at λ_{em} 610nm and were monitored over a P/D range of 0 to 140, respectively.

Table 5.9: Luminescence decay parameters for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, in the presence of CT-DNA.

5.5.3-2 Racemic $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ Complex in the presence of DNA.

The emission lifetimes of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **rac-4** and $[\text{Ru}(\text{phen})_2\text{d}_4\text{-dppz}]^{2+}$ **rac-4a**, were monitored as a function of increasing concentrations of DNA. The results obtained are tabulated in Tables 5.9 (**rac-4**) and Table 5.10 (**rac-4a**), respectively. Also included are;

- Relative abundancy (α_1 and α_2) - Normalised pre-exponential factor. α_i reflects the proportion of *i* present
- Chi-squared (χ^2) - goodness of fit, where $\chi^2 = 1$ for perfect fit
- Intensity of emission, I_{cal} - calculated according to $I_{\text{cal}} \propto \alpha_1\tau_1 + \alpha_2\tau_2$,

Table 5.10: Luminescence decay parameters for $[\text{Ru}(\text{phen})_2\text{d}_4\text{-dppz}]^{2+}$ **4a**, in the presence of CT-DNA.

* Mixing P/D ratio. The concentration of the metal complex was 5 μM . * Normalized pre-exponential factors obtained from biexponential decay analysis. α_i reflects the proportion of component *i* in the luminescence at $t = 0$ (e.g. directly after the excitation). * Goodness-of-fit parameter, $\chi^2 = 1$ for a perfect fit. * Standard deviation for the lifetime decay constant. * Relative abundancy of 610 nm calculated according to $I_{\text{cal}} \propto \alpha_1\tau_1 + \alpha_2\tau_2$.

Rac - [Ru(phen) ₂ dppz] ²⁺ 4						
P/D Ratio ^a	τ ₁	α ₁ ^b	τ ₂	α ₂	χ ² ^c	I _{calc} ^e
1.36	260 ± 8 ^d	50	911 ± 9	50	1.049	5.86 *10 ⁴
2.00	341 ± 7	41	977 ± 11	59	1.009	7.16
2.72	248 ± 6	48	913 ± 7	52	1.102	5.94
8.84	244 ± 6	51	932 ± 7	49	1.072	5.81
17.04	215 ± 5	54	950 ± 6	46	1.119	5.53
29.24	252 ± 8	54	978 ± 8	46	1.193	5.86
59.84	205 ± 7	49	956 ± 8	51	1.116	5.88
70.04	187 ± 6	42	948 ± 10	58	1.054	6.29
100	179 ± 5	34	927 ± 10	66	1.193	6.73
>100	179 ± 5	33	916 ± 10	67	1.046	6.73

Table 5.9 : Luminescence decay parameters for Rac-[Ru(phen)₂dppz]²⁺, 4, in the presence of CT-DNA.

Rac - [Ru(phen) ₂ d ₄ -dppz] ²⁺ 4a						
P/D Ratio ^a	τ ₁	α ₁ ^b	τ ₂	α ₂	χ ² ^c	I _{calc} ^e
1.36	257 ± 9 ^d	44	936 ± 8	56	1.015	6.37 *10 ⁴
2.00	278 ± 9	44	957 ± 7	56	1.098	6.58
2.72	271 ± 8	46	964 ± 6	54	1.026	6.46
8.84	290 ± 12	50	970 ± 8	50	1.122	7.30
17.04	212 ± 5	52	990 ± 6	48	1.031	5.85
29.24	260 ± 10	45	1012 ± 8	55	1.067	6.74
59.84	254 ± 18	39	1050 ± 12	61	1.084	7.40
70.04	246 ± 17	38	1011 ± 13	62	1.133	7.10
100	174 ± 4	30	973 ± 9	70	1.001	7.33
>100	184 ± 5	31	961 ± 11	69	1.026	7.20

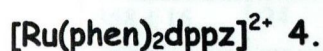
Table 5.10: Luminescence decay parameters for Rac-[Ru(phen)₂d₄-dppz]²⁺, 4a, in the presence of CT-DNA.

^a Mixing P/D ratio. The concentration of the metal complex was 5 μM. ^b Normalized preexponential factors obtained from biexponential decay curves. α_i reflects the proportion of component α_i in the luminescence at t = 0 (e.g. directly after the illumination). ^c Goodness-of-fit parameter, χ² = 1 for a perfect fit. ^d Standard deviation for the lifetime decay constant. ^e Relative intensities at 610 nm calculated according to I_{calc} proportional to α₁τ₁ + α₂τ₂

On examination of the data, similar trends were observed for **rac-4** and **rac-4a** complexes. In the presence of DNA two lifetimes were observed, which comprise a short-lived τ_1 (sh) and a long-lived τ_2 (lo) lifetime component. In both cases, the longer-lived component, τ_2 has a longer emission lifetime and higher relative abundancy. The magnitudes of the lifetimes do not vary significantly over the range of P/D concentrations investigated. However, there was a general trend found for these complexes on binding to DNA. The emission lifetime increased up to P/D ~ 29 (maximum emission point), whereby further additions of DNA resulted in a slight decrease in the emission lifetime. The relative abundances (α_1 and α_2), are approximately equal $\sim 50\%$ at low P/D ratios. On reaching the maximum emission point there is a greater percentage of τ_2 present, which increases on increasing additions of DNA to the complex solutions. The calculated intensity of emission, I_{cal} was found to be greater by *ca.* 24% for **rac-4a** complex, attributed to the longer emission lifetime values of τ_2 as a result of D-incorporation into the complex.

Interpretation of the data is difficult as the racemic compounds consist of a mixture of binding modes. Indeed, little attention has focused on the decays of these complexes.

5.5.3-3 Luminescence Lifetime Studies for Enantiomers of



Extensive research has focused on the elucidation of the nature of the binding modes of the Δ - and Λ -enantiomers of Ru(II) complexes in the presence of a wide range of DNA conformations. We wish to examine the emission lifetimes of the enantiomers, Δ -4 and Λ -4, as a function of increasing amounts of CT-DNA, under conditions as previously described.

a. Δ - [Ru(phen)₂dppz]²⁺ with CT-DNA. 4Δ						
P/D Ratio ^a	τ_1^b	α_1	τ_2	α_2	χ^2 ^c	I_{calc} ^e
1.36	209 $\pm$ 15 ^d	60	770 $\pm$ 10	40	1.059	4.34 *10 ⁴
2.00	279 $\pm$ 11	43	922 $\pm$ 7	57	1.039	6.45
2.72	289 $\pm$ 10	38	940 $\pm$ 9	62	0.991	6.93
8.84	314 $\pm$ 10	42	955 $\pm$ 8	58	1.059	6.86
17.04	308 $\pm$ 10	40	950 $\pm$ 7	60	1.185	6.93
29.24	321 $\pm$ 9	24	969 $\pm$ 8	76	0.973	8.13
59.84	283 $\pm$ 8	43	984 $\pm$ 7	57	1.158	6.82
70.04	236 $\pm$ 7	41	966 $\pm$ 5	59	1.036	6.64
100	217 $\pm$ 9	42	957 $\pm$ 6	58	1.000	6.46
>100	195 $\pm$ 10	42	924 $\pm$ 8	58	1.012	6.18

b. Λ - [Ru(phen)₂dppz]²⁺ with CT-DNA. 4Λ						
P/D Ratio ^a	τ_1^d	α_1	τ_2	α_2	χ^2 ^c	I_{calc} ^e
1.36	91 $\pm$ 4	73	445 $\pm$ 10	27	1.293	1.86 *10 ⁴
2.00	119 $\pm$ 8	69	494 $\pm$ 21	31	1.129	2.35
2.72	118 $\pm$ 8	68	461 $\pm$ 19	32	1.180	2.27
8.84	115 $\pm$ 7	69	464 $\pm$ 20	31	1.168	2.22
17.04	90 $\pm$ 14	76	426 $\pm$ 14	24	1.098	1.70
29.24	89 $\pm$ 5	77	398 $\pm$ 22	23	1.128	1.60
59.84	79 $\pm$ 4	76	368 $\pm$ 16	24	1.127	1.48
70.04	66 $\pm$ 4	77	348 $\pm$ 14	23	1.028	1.31
100	58 $\pm$ 5	71	273 $\pm$ 11	29	1.046	1.11

Table 5.11: Luminescence decay parameters for enantiomers of [Ru(phen)₂dppz]²⁺, 4 Δ and 4 Λ , in the presence of CT-DNA.

^b Mixing P/D ratio. The concentration of the metal complex was 5 μ M. ^c Normalized preexponential factors obtained from biexponential decay curves. α_i reflects the proportion of component i in the luminescence at $t = 0$ (e.g. directly after the illumination). ^d Goodness-of-fit parameter, $\chi^2 = 1$ for a perfect fit. ^e Standard deviation for the lifetime decay constant. ^f Relative intensities at 610 nm calculated according to I_{calc} proportional to $\alpha_1\tau_1 + \alpha_2\tau_2$

The lifetimes τ_1 and τ_2 do not significantly alter over the DNA-titration range monitored. The emission lifetimes of Δ -4 and Λ -4, were plotted as a function of increasing DNA concentrations, and are shown in Figure 5.18. They reveal distinct differences between the two enantiomers.

- The long-lived lifetime component, τ_2 has a substantially longer lifetime, $\tau_2 = 969$ ns (76%) for Δ -4 compared to $\tau_2 = 398$ ns (23%) for the corresponding Λ -4 complex.
- The short-lived lifetime component, τ_1 is also enhanced for Δ -4 although to a much lesser extent.
- There is general increase in τ_1 and τ_2 at low P/D values up to P/D ~ 29 (Δ -4) and P/D (Λ -4). At higher P/D values there is a decrease in both emission lifetimes, respectively

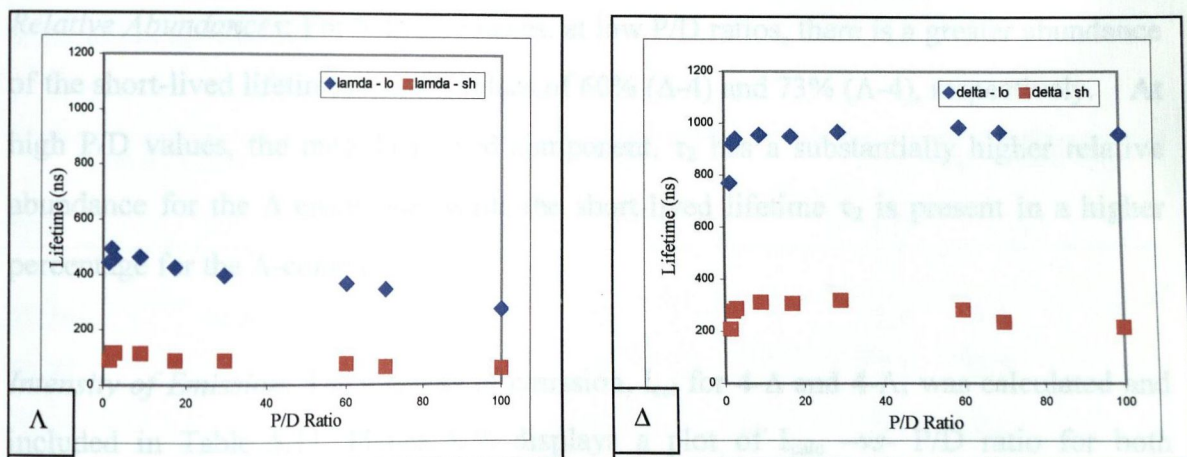


Figure 5.18: The emission lifetime decay constants for enantiomers, 4 Δ and 4 Λ , complexes as a function of increasing DNA concentration. Where lamda-lo denotes the long-lived lifetime, τ_1 for the Λ -enantiomer and delta-sh corresponds to the short lived lifetime, τ_2 for the Δ -complex.

For **rac-4**, Δ -4, and Λ -4, a plot of τ_2 , as a function of increasing DNA concentration, is shown in Figure 5.19. It displays that the emission lifetime of the racemic (blue) and Δ -4 (red) complexes are very similar, while the emission from Λ -4 (green) is small. This confirms that the lifetime component of the racemic mixture corresponds mainly to contributions from Δ -4 complex. Furthermore, this supports our conclusion from steady-state measurements (see Section 5.5.2-4) that the luminescence intensity is mainly attributed to contributions from Δ -DNA mixtures.

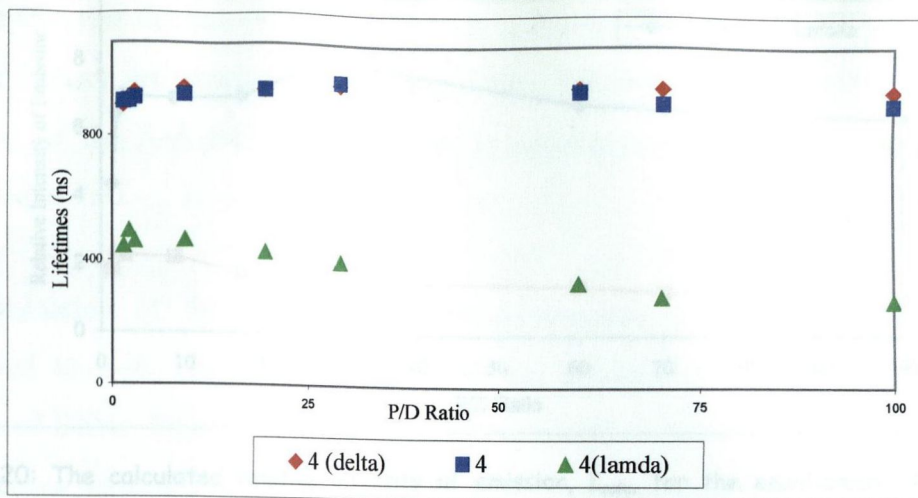


Figure 5.19: The emission lifetime decay constant, τ_2 , for rac-4, and the enantiomers, 4 Δ (delta) and 4 Λ (lamda), complexes as a function of increasing DNA concentration.

Relative Abundances: For both complexes, at low P/D ratios, there is a greater abundance of the short-lived lifetime, τ_1 , with values of 60% (Δ -4) and 73% (Λ -4), respectively. At high P/D values, the most long-lived component, τ_2 has a substantially higher relative abundance for the Δ enantiomer while the short-lived lifetime τ_2 is present in a higher percentage for the Λ -complex.

Intensity of Emission: The intensity of emission, I_{cal} for 4- Δ and 4- Λ , was calculated and included in Table 5.11. Figure 5.20 displays a plot of I_{calc} -vs- P/D ratio for both enantiomers over the range of DNA concentrations investigated. For Δ -4 the intensity of emission is significantly greater (factor of 5), relative to the Λ -4 complex. Owing to the difference in the intensities of emission, contributions from the Λ -enantiomer are expected to be small compared to those of the Δ -complex in racemic mixtures.

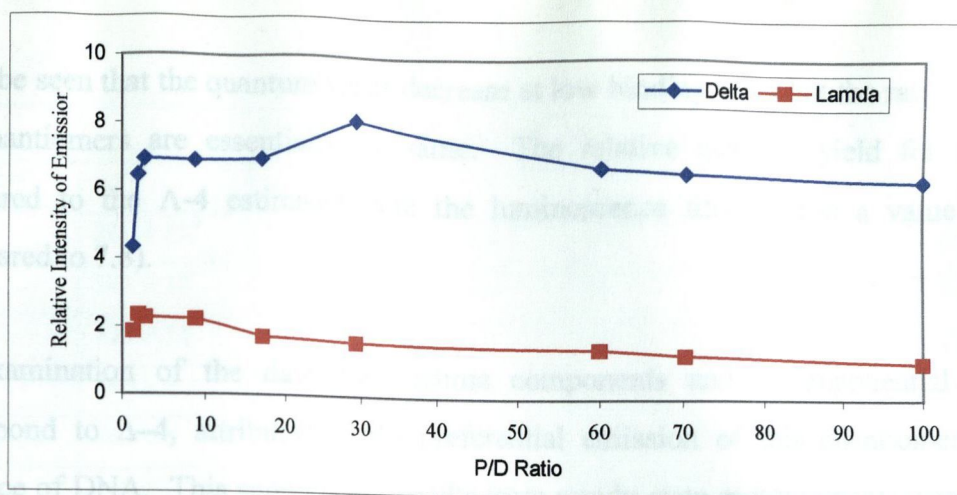


Figure 5.20: The calculated relative intensity of emission, I_{calc} , for the enantiomers, 4Δ and 4Λ , complexes as a function of increasing DNA concentration.

Table 5.12 provides a comparison between the steady-state emission intensities and time-resolved data for $\Delta-4$ and $\Lambda-4$ at $P/D = 29$, under which all the metal complex is expected to be bound.

<u>Δ-enantiomer</u>			<u>Λ-enantiomer</u>		<u>Δ/Λ-enantiomer ratio</u>	
P/D	RI ^b	calc ^c	RI ^b	calc ^c	RI ^d	calc ^c
29.24	=100	=100	17	20	5.9	5.0
59.84	86	84	12	18	7.2	4.7
70.04	82	81	10	16	8.2	5.1
100	81	79	9	14	9.0	5.6
>100	73	71	8	13	9.1	5.4
					av = 7.88	av = 5.16

Table 5.12: The relative intensities determined from steady-state and time-resolved measurements

b Relative intensities at 610 nm, determined from steady-state emission spectra, normalized (=100) to the CT-DNA complex ($P/D = 29$)

c Relative intensities at 610 nm, calculated from time-resolved data in Table 1, according to I_{calc} proportional to $\alpha_1\tau_1 + \alpha_2\tau_2$ normalised as in footnote b.

d Calculated intensity ratio of Δ to Λ enantiomers at 610 nm determined as in footnote c.

It can be seen that the quantum yields decrease at low binding ratios but the ratio between the enantiomers are essentially the same. The relative quantum yield for the Δ -4 compared to the Λ -4 estimated from the luminescence titration has a value of 5.2 (compared to 7.8).

On examination of the data, the lifetime components and pre-exponential factors correspond to Δ -4, attributed to the preferential emission of this enantiomer in the presence of DNA. This supports our results from steady-state measurements where higher luminescence intensities were observed for Δ -4/DNA mixtures, compared to Λ -DNA. This is attributed to a correspondingly higher intrinsic quantum yield of the bound Δ -4 complex. In conclusion, there is good agreement in the data obtained from both steady-state and time-correlated studies.

5.5.3-4 Deuterated $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ Complexes in the presence of DNA.

The technique of H-D exchange of these Ru(II) complexes has been employed as it hoped that the increased excited state lifetime decays will provide us with a useful tool to probe the nature of the binding modes with DNA. To this end, the emission lifetime decays as a function of increasing P/D values in the range 0 to 100 for (i) $[\text{Ru}(\text{phen})_4\text{-dppz}]^{2+}$ - Δ -4a and Λ -4a; (ii) $[\text{Ru}(\text{phen})_6\text{-dppz}]^{2+}$ - Δ -4d; and (iii) $[\text{Ru}(\text{d}_8\text{-phen})\text{dppz}]^{2+}$ - Δ -4d and Λ -4d complexes were investigated, under the conditions as previously stated. The results of these studies are tabulated in Tables 5.13 (4a), 5.14 (4d), and 5.15 (4b), respectively.

Lifetimes: In the presence of DNA, the value of the emission lifetimes increase on increasing the extent of deuteration into the complex in the order; $4 < 4a < 4b < 4d$. Figure 5.21 displays plots of the emission lifetimes of the enantiomers of all four complexes as a function of increasing P/D ratios. As all the plots are relative, a direct comparison between the series of complexes can be made.

a. Δ - [Ru(phen)₂d₄-dppz]²⁺ with CT-DNA. Δ - 4a						
P/D Ratio ^a	τ_1	α_1^b	τ_2	α_2	χ^2^c	I_{calc}^d
1.36	302 ± 13 ^d	36	952 ± 8	64	1.031	7.18 *10 ⁴
2.00	343 ± 12	42	991 ± 9	58	1.019	7.19
2.72	331 ± 11	42	984 ± 9	58	1.048	7.11
8.84	298 ± 9	43	974 ± 6	57	1.006	6.83
17.04	283 ± 8	43	989 ± 6	57	1.077	6.86
29.24	265 ± 7	44	997 ± 6	56	1.133	6.74
59.84	231 ± 6	47	998 ± 6	53	1.069	6.38
70.04	217 ± 4	55	984 ± 6	45	1.029	5.62
100	194 ± 4	60	948 ± 6	40	0.971	4.95
>100	179 ± 3	68	921 ± 7	32	1.073	4.16

b. Λ - [Ru(phen)₂d₄-dppz]²⁺ with CT-DNA Λ-4a						
P/D Ratio ^a	τ_1	α_1^b	τ_2	α_2	χ^2^c	I_{calc}^d
1.36	228 ± 11 ^d	43	900 ± 9	57	1.113	6.11 *10 ⁴
2.00	248 ± 7	42	930 ± 6	58	1.121	6.43
2.72	267 ± 8	44	937 ± 6	56	1.108	6.42
8.84	272 ± 8	45	942 ± 6	55	1.078	7.40
17.04	229 ± 6	49	920 ± 6	51	1.035	5.91
29.24	228 ± 6	50	935 ± 6	50	1.112	5.82
59.84	213 ± 5	53	966 ± 6	47	1.117	5.67
70.04	178 ± 4	61	952 ± 6	39	1.147	4.80
100	154 ± 4	64	872 ± 10	36	1.023	4.13
>100	153 ± 3	75	863 ± 17	25	1.043	3.30

Table 5.13: Luminescence decay parameters for enantiomers of [Ru(phen)₂d₄-dppz]²⁺, Δ -4a and Λ -4a, in the presence of CT-DNA.

^b Mixing P/D ratio. The concentration of the metal complex was 5 μ M. ^c Normalized preexponential factors obtained from biexponential decay curves. α_i reflects the proportion of component α_i in the luminescence at $t = 0$ (e.g. directly after the illumination).^d Goodness-of-fit parameter, $\chi^2 = 1$ for a perfect fit. ^e Standard deviation for the lifetime decay constant. ^f Relative intensities at 610 nm calculated according to I_{calc} proportional to $\alpha_1\tau_1 + \alpha_2\tau_2$

Δ - $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$ with CT-DNA. Δ -4c						
P/D Ratio ^a	τ_1	α_1^b	τ_2	α_2	χ^2 ^c	I_{calc} ^d
1.36	346 ± 9^d	53	1148 ± 12	47	1.156	$7.23 * 10^4$
2.00	382 ± 10	51	1216 ± 11	49	1.136	7.91
2.72	354 ± 9	50	1167 ± 10	50	1.142	7.61
8.84	344 ± 8	49	1164 ± 89	51	1.193	7.63
17.04	412 ± 15	43	1266 ± 11	57	1.028	8.95
29.24	413 ± 15	43	1275 ± 12	57	1.093	9.04
59.84	372 ± 14	42	1259 ± 12	58	1.090	8.87
70.04	345 ± 14	48	1219 ± 14	53	0.964	8.12
100	306 ± 10	53	1226 ± 13	48	1.018	7.50
>100	256 ± 9	57	1219 ± 13	48	1.174	6.70

Λ - $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$ with CT-DNA. Λ -4d						
P/D Ratio ^a	τ_1	α_1^b	τ_2	α_2	χ^2 ^c	I_{calc} ^d
1.36	336 ± 10^d	58	1000 ± 17	42	1.068	$6.15 * 10^4$
2.00	348 ± 11	55	1106 ± 15	45	1.092	6.89
2.72	320 ± 10	53	1102 ± 13	47	1.047	6.88
8.84	306 ± 9	54	1142 ± 11	46	1.039	6.90
17.04	297 ± 9	54	1194 ± 12	46	1.075	7.09
29.24	291 ± 8	55	1198 ± 12	45	1.098	6.99
59.84	325 ± 11	55	1276 ± 16	45	1.078	7.53
70.04	304 ± 19	51	1257 ± 24	49	1.099	7.71
100	244 ± 7	64	1230 ± 14	36	1.083	5.99
>100	239 ± 6	67	1218 ± 13	33	1.176	5.61

Table 5.14: Luminescence decay parameters for enantiomers of $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$, $4d\Delta$ and $4d\Lambda$, in the presence of CT-DNA.

^b Mixing P/D ratio. The concentration of the metal complex was $5 \mu\text{M}$. ^c Normalized preexponential factors obtained from biexponential decay curves. α_i reflects the proportion of component i in the luminescence at $t = 0$ (e.g. directly after the illumination).^d Goodness-of-fit parameter, $\chi^2 = 1$ for a perfect fit. ^e Standard deviation for the lifetime decay constant. ^f Relative intensities at 610 nm calculated according to I_{calc} proportional to $\alpha_1\tau_1 + \alpha_2\tau_2$

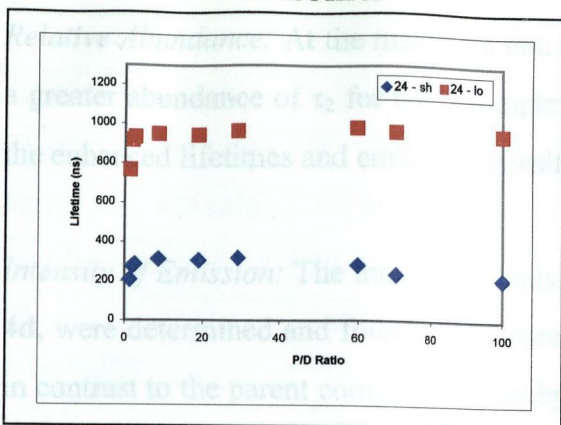
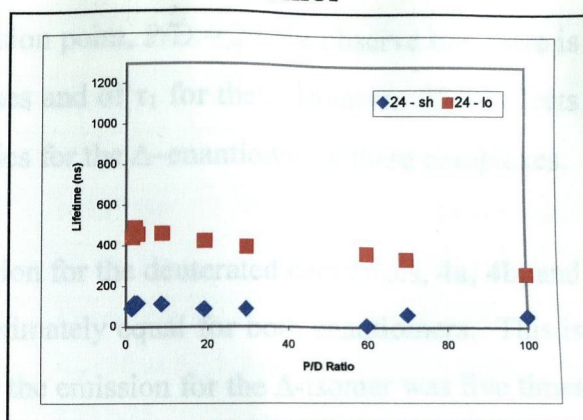
Δ - $[\text{Ru}(\text{phen})_2\text{d}_6\text{-dppz}]^{2+}$ with CT-DNA. Δ -4b						
P/D Ratio ^a	τ_1	α_1 ^b	τ_2	α_2	χ^2 ^c	I_{calc} ^e
1.36	388 ± 11^d	53	1045 ± 13	47	1.019	$7.01 \cdot 10^4$
2.00	382 ± 10	50	1096 ± 11	50	1.058	7.39
2.72	375 ± 10	48	1095 ± 10	52	1.039	7.49
8.84	376 ± 10	47	1116 ± 9	53	0.987	7.68
17.04	424 ± 11	40	1171 ± 13	60	1.102	8.73
29.24	349 ± 9	42	1172 ± 12	58	1.088	8.27
59.84	280 ± 7	42	1173 ± 9	58	1.003	7.93
70.04	242 ± 7	49	1156 ± 7	51	1.062	7.04
100	217 ± 6	54	1139 ± 7	46	1.128	6.41
>100	194 ± 5	60	1121 ± 7	40	1.103	5.64

Table 5.15: Luminescence decay parameters for enantiomers of $[\text{Ru}(\text{phen})_2\text{d}_6\text{-dppz}]^{2+}$, **4b** Δ , in the presence of CT-DNA.

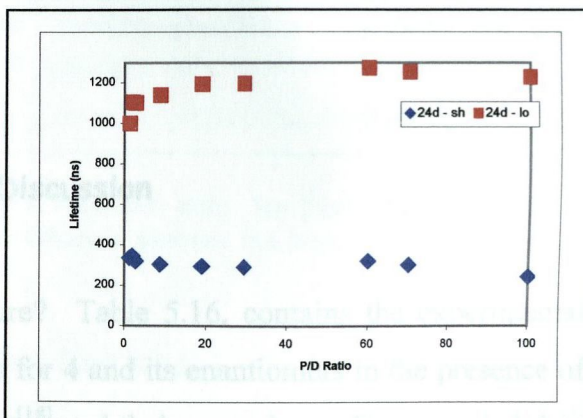
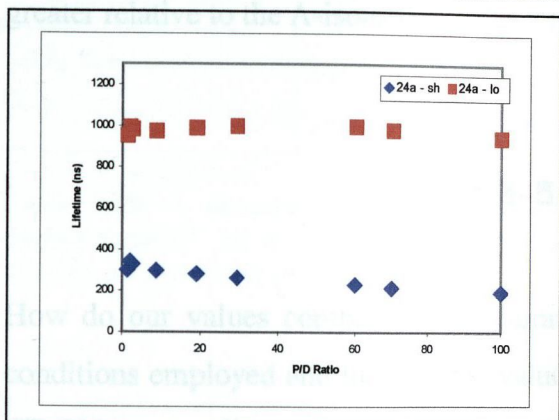
^b Mixing P/D ratio. The concentration of the metal complex was 5 μM . ^c Normalized pre-exponential factors obtained from biexponential decay curves. α_i reflects the proportion of component α_i in the luminescence at $t = 0$ (e.g. directly after the illumination).^d Goodness-of-fit parameter, $\chi^2 = 1$ for a perfect fit. ^e Standard deviation for the lifetime decay constant. ^f Relative intensities at 610 nm calculated according to I_{calc} proportional to $\alpha_1\tau_1 + \alpha_2\tau_2$

Emission Lifetimes: For the Δ -complexes, there is a significant difference between the contributions of the two lifetimes, where τ_2 displays a greater emission lifetime. On D-incorporation into these complexes we observe that the Δ -complexes for **4a**, **4b**, and **4d**, are similar. For the Λ -complexes, there is a much smaller difference between the contributions of the two lifetimes. However, D-incorporation into the complexes results in marked differences. Firstly, there is a significant difference between the values of the two lifetimes, with τ_2 displaying the greater abundance of the two lifetime components. Secondly, the difference between the enantiomers is not as apparent and the lifetime decays are almost equivalent for complexes **4a** and **4d**, as shown in Figure 5.21

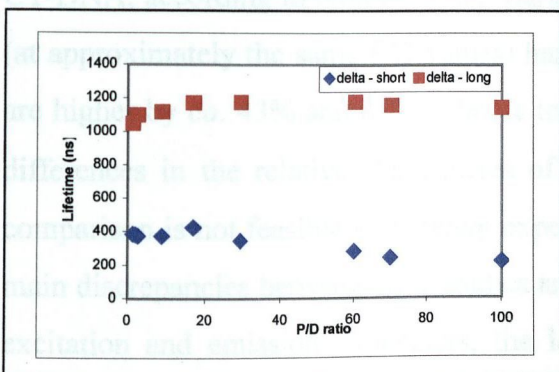
4

 Δ -isomer Λ -isomer

4a



4b



4d

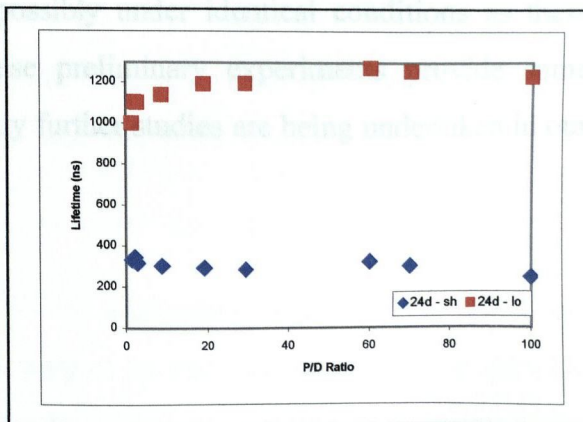
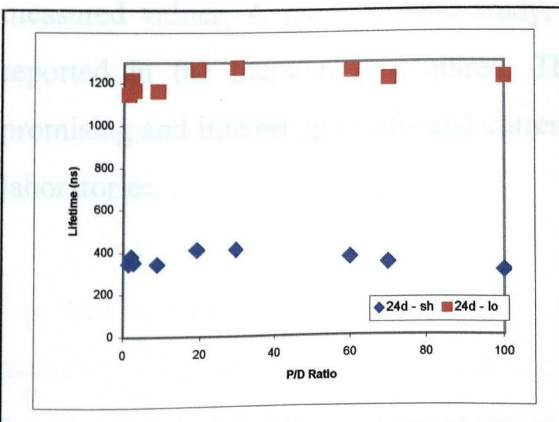


Figure 5.21: Lifetime decays of the enantiomers for Ru(II) complexes as a function of CT-DNA.

Relative Abundance: At the maximum emission point, $P/D = 29$, we observe that there is a greater abundance of τ_2 for the Δ -complexes and of τ_1 for the Λ -isomers. This reflects the enhanced lifetimes and emission intensities for the Δ -enantiomer of these complexes.

Intensity of Emission: The intensity of emission for the deuterated complexes, **4a**, **4b**, and **4d**, were determined and found to be approximately equal for both enantiomers. This is in contrast to the parent complex **4**, whereby the emission for the Δ -isomer was five times greater relative to the Λ -isomer.

5.5.3-5 Discussion

How do our values compare to the literature? Table 5.16, contains the experimental conditions employed and the reported values for **4** and its enantiomers in the presence of CT-DNA, according to Norden^[22] and Barton^[18] and their coworkers. Our recorded data (at approximately the same P/D values) has also been included. Our measured lifetimes are higher by *ca.* 43% and 82%, relative to those reported in the literature, and there are differences in the relative abundancies of the emission lifetimes, τ_1 and τ_2 . A direct comparison is not feasible as different experimental conditions have been employed. The main discrepancies between these studies are the type of buffer (tris or phosphate) and the excitation and emission parameters, the latter should have little, if any effect on the measured values. A more detailed study, possibly under identical conditions as these reported in the literature is required. These preliminary experiments provide some promising and interesting results and currently further studies are being undertaken in our laboratories.

Barton ^[18]			Our recorded data		Tuite ^[22]		Our recorded data	
	τ_1 (ns)	τ_2 (ns)	τ_1 (ns)	τ_2 (ns)	τ_1 (ns)	τ_2 (ns)	τ_1 (ns)	τ_2 (ns)
Rac	93 (63) •	512 (37)	205 (49)	956 (51) •			252 (54) •	978 (46)
Δ	97 (57) •	473 (43)	283 (43)	794 (57) •	132 (74) •	794 (26)	321 (24)	969 (76) •
Λ	61 (43)	346(57) •	79 (76) •	262 (23)	37 (94) •	262 (6)	89 (77) •	398 (23)
Conditions: λ_{ex} 480nm λ_{em} 617nm P/D = 50 5mM Tris buffer, 50mM NaCl pH 8.5			Conditions: λ_{ex} 440nm λ_{em} 610nm P/D = 59 10mM Tris buffer, 50mM NaCl pH 7.1		Conditions: λ_{ex} 440nm λ_{em} >580nm P/D = 25 5mM Phosphate buffer pH 6.9		Conditions: λ_{ex} 440nm λ_{em} 610nm P/D = 29 10mM Tris buffer, 50mM NaCl pH 7.1	

Table 5.16: A comparison of the reported and recorded data for the lifetime decay of [Ru(phen)₂dppz]²⁺ (**4**) in the presence of CT-DNA. Where • denotes the higher relative abundance lifetime present in the complex.

5.6 Concluding Remarks.

The interaction of the parent complex **4**, with nucleic acids is much more complicated than the binding of a simple intercalator, and extensive research is needed to unravel the remaining mysteries. To this end, the present study involved a detailed investigation of the interactions of the racemic, protiated and deuterated, and enantiomeric complexes of [Ru(phen)₂dppz]²⁺ **4** in the presence of CT-DNA.

A number of synthetic routes were attempted for the preparation of the enantiomerically pure complexes of **4**. After much time and effort, the enantiomers of the parent protiated complex **4** and its deuterated analogues, **4a** and **4d**, were successfully separated, as determined by CD spectroscopy.

The intensity changes and shifts in the UV-Vis absorption band maxima for **4** are consistent with previous reported values and support an intercalative binding mode with the DNA helix. Similar effects were found for the diMedppz complex **8**. In contrast, for the diFdppz complex **9** shifts to shorter wavelengths of the MLCT band were observed.

Steady-state emission studies, reveal that $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ and related complexes are highly spectroscopic probes for DNA, behaving as molecular “light switches”. No detectable luminescence was observed for all three complexes in aqueous solution, however, its luminescence is switched on by interaction with DNA. This is in contrast to Barton *et al*^[41], who reported detectable emission for complex **8** in aqueous solution. Comparison of the emission intensities of the three complexes show that **8** exhibits the strongest emission. Complex **9** is the weakest emitter, which is intrinsic to the complex and not necessarily caused by a weak interaction with the polynucleotide. It was noted that, upon binding, the magnitude of change observed for the absorption intensity (10-20%) is much less than that for emission intensity (60-150%). So the error is greater in the case of the latter measurements.

For complex **4**, the enhanced hypochromicity in the MLCT absorption band and the higher emission quantum yield of the Δ -enantiomer relative to the Λ -enantiomer, suggest that it is primarily responsible for the luminescence of the racemic complex upon binding to DNA.

Time-correlated lifetime studies were performed for the racemic, Δ - and Λ -enantiomers of **4** as a function of increasing concentrations of CT-DNA. Each of the complexes displayed a biexponential luminescence decay upon binding to DNA, displaying a short (τ_1) and long (τ_2) lifetime component, implying at least two binding modes upon interaction with DNA. Both the lifetime components and pre-exponential factors correspond to the 4Δ -enantiomer, attributed to the preferential emission by the 4Δ -isomer in the presence of right-handed double helical DNA. Furthermore, this supports our results from steady-state measurements that the higher luminescence intensity obtained for the 4Δ -DNA mixtures, compared to 4Λ -DNA, is attributed to a correspondingly higher intrinsic quantum yield of the bound Δ -complex.

A number of interesting and promising preliminary results were obtained upon D-incorporation into the dppz ligand (*i.e.* d₄-dppz – phenazine protons) and the ancillary phen ligands (*i.e.* d₈-phen) of these systems. It was expected that the properties of the deuterated enantiomeric complexes, **4a** and **4d**, would display similar results to the parent complex **4**. This however was not found. For both complexes, the emission lifetime decays and pre-exponential factors of the Δ- and Λ-enantiomers display no preference, the enantiomers being essentially equal. At present, we can offer no explanation for this unusual observation, but have found it to be reproducible.

5.7 References.

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Chapter Six

Chapter Six

6.0 Introduction

This chapter describes the procedures used during the course of this work in their most general forms. Exact conditions for each experiment are given within the section of this thesis where the results are reported.

6.1 Materials

6.1-1 Reagents

Starting materials for synthetic work were obtained from Sigma or Fluka and used without further purification. Hydrated ruthenium trichloride was purchased from Johnson Matthey Materials Technology, UK, and used as received. SP Sephadex C-25 and LH-20, in anhydrous forms were from Aldrich Chem. Co. Ltd.

6.1.1-1 Solutions for DNA Studies

High Molecular weight calf thymus DNA was obtained from Sigma and purified as follows. Approximately 1 gram of DNA was allowed to dissolve overnight in sterile water (500 ml) and then sodium dodecylsulphonate and NaCl added to make it 1% sodium dodecylsulphonate and 1M NaCl. An equal volume of phenol/chloroform/isoamylalcohol (25:24:1) was added 0.5% w/v of 8-hydroxyquinoline. This mixture was saturated by standing over an aqueous 100 mM tris/HCl buffer (pH 7.1). The mixture was shaken for 1 hour at 4°C, and separated by centrifugation at 10,000 rpm. Protein impurities were trapped as a white film between the aqueous and organic layers. The aqueous DNA solution was removed, and the process repeated until it was protein free, usually 1 or 2 times. The DNA was then precipitated using two volumes of ice-cold ethanol and spooled onto a glass rod. It was re-dissolved in water to a final concentration of about 3×10^{-3} M phosphate and dialysed extensively against water and then 10 mM phosphate buffer to remove low molecular weight DNA. The DNA was free of protein if the absorption ratio A_{260}/A_{280} nm was greater than 1.9. The CT-DNA was stored at -20°C , in sterile Eppendorf tubes to prevent bacterial growth^[1].

All the experiments involving the interaction of the complexes with DNA, were prepared directly in the cuvette using doubly distilled water, with 10 mM Tris Buffer (pH 7.1) and 50 mM NaCl. The DNA concentration per nucleotide was determined by spectrophotometrically using the molar absorption coefficient, $6600 \text{ M}^{-1}\text{cm}^{-1}$ at 260 nm ^[2]. Metal complex stock solutions, prepared by dissolving the chloride salts in water, were kept in the dark to avoid photodegradation.

6.1-2 Solutions

6.1.2-1 Solutions

Metal complex stock solutions, prepared by dissolving and sonicating the chloride salts in water and the ammonium hexafluorophosphate salts in acetonitrile were kept in the dark to avoid degradation. Experimental samples were freshly prepared before each experiment from stock solutions of accurately determined concentration using accurate calibrated micropipettes (Gilson P20, P200, and P1000). Typically, concentrations $((1-6) \times 10^{-5} \text{ M})$ were low enough to avoid the necessity for re-adsorption corrections. In experiments involving spectroscopy the nucleic acid concentrate was added stepwise in small volumes allowing time for equilibrium and corrections were made for the dilution of the sample.

6.1.2-2 Solvents

For spectroscopic measurements, all solvents were obtained from Aldrich and were of the highest purity available. Deuterium Oxide (D_2O) in 99.9% isotopic purity and Deuterium Chloride (DCl) were obtained from Apollo Scientific Ltd., Derbyshire, UK.

6.1.2-3 Buffers.

Tris Buffer; 10 M Tris Buffer (pH 7.1) was prepared by dissolving 1.21 g of Trizma base (tris[hydroxymethyl]amino methane, Sigma, 99.9%) in 80 ml water and adding 1ml concentrated HCl, as specified in Maniatis^[3].

6.1.2-4 Solution Degassing and Oxygenating.

Studies, as a function of the triplet-state quencher $^3\text{O}_2$, were performed both in the absence and the presence of DNA. The concentration of oxygen in a number of systems is tabulated in Table 6.1^[4]. The samples were oxygenated by bubbling water-saturated oxygen, through a syringe, into a septum-covered cuvette for a minimum of 30 minutes. When degassing of samples was necessary, solutions were degassed on a high vacuum pump by a freeze-thaw-pump mechanism with at least four cycles to a pressure of $\sim 4.5 \times 10^{-3}$ mbar, prior to measurements. These were found to give reproducible results and were considered reliable.

	[O ₂] mM/L		
	Degassed	Aerated	Oxygenated
Atmosphere	0.21 atm O ₂		1 atm O ₂
Water	0.00	0.27	1.27
Acetonitrile	0.00	1.90	9.10
CH ₂ Cl ₂	0.00	2.20	10.70
EtOH	0.00	2.10	9.92
MeOH	0.00	2.10	10.20
DMSO	0.00	0.46	2.20
THF	0.00	2.10	10.0
Pyridine	0.00	1.20	5.66

Table 6.1 : Concentration of O₂ in various solvents at 25°C^[4].

6.2 Experimental Apparatus and Methods

UV Absorption spectra, emission spectra and lifetimes were acquired at concentrations of samples in the range (3×10^{-6} M – 5×10^{-5} M), at ambient temperature, in 1 cm quartz cuvettes. The concentration of the Ru(II) complexes were determined spectroscopically using molar absorption of Ru(II) ion, $\epsilon = 21,800 \text{ M}^{-1} \text{ cm}^{-1}$ at 373 nm^[5]

6.2-1 Absorption (UV-vis) Spectroscopy.

Absorption Spectra and optical density were recorded on a Cary 4UV spectrophotometer or a Shimadzu UV-2401 PC UV-vis spectrophotometer in the range 100-900 nm. Data were stored, manipulated and analysed using the IBM 386. Solutions were measured in 1 cm curvette cells. Concentration of ruthenium complexes were in the order of $\sim 10^{-5}$ M so as to achieve a maximum absorption in the range of 0-2 in the visible range of the spectrum. Extinction coefficients are accurate to 5%.

The extinction coefficients ϵ were calculated using the Beer-Lambert Law (1),

$$A = -\log(I/I_0) = \epsilon \cdot c \cdot l \quad (1)$$

The value of ϵ is usually reported at a wavelength of a maximum absorption peak and is denoted as $\epsilon_{\max}$. In order to determine $\epsilon_{\max}$ for ligands and ruthenium(II) complexes synthesised here, solutions of known concentrations were prepared and their absorption spectra were obtained.

6.2-2 Steady State Emission and Excitation Spectroscopy.

Steady state emission and excitation spectra, and fluorescence intensity data were recorded using a Perkin-Elmer LS-50B luminescence spectrofluorimeter, with a 150W Xenon lamp as the light source. Fluorescence was detected at right angles to excitation with a Hamamatsu R928 red-sensitive photomultiplier. Spectra were not corrected for photomultiplier response. All spectra were recorded in a 1 cm cuvette with excitation and emission monochromators set to 6 nm slit-widths.

• Quantum Yields.

Quantum Yields of fluorescence in various solvents were determined from fluorescence spectra recorded at 298 K using $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** ($\Phi = 0.042$ for degassed and $\Phi = 0.028$ for aerated) in aqueous solution as a standard^{[6][7][8]}, and were calculated using equation 6.1^[9]. Corrections were made for the refractive indices of

the solvents used, see Table 6.2. Spectra were uncorrected for variations in lamp intensity and photomultiplier response

$$\Phi_{em} = \Phi'_{em} \left(\frac{I}{I'} \right) \left(\frac{A}{A'} \right) \left(\frac{n}{n'} \right)^2 \quad 6.1$$

Where I (sample) and I' (standard) are the integrated emission intensities, A and A' the absorbances at the excitation wavelength, and n and n' the refractive indices of the solvents, and Φ_{em} is the uncorrected emission quantum yield.

Solvent	n_D^{20}	Solvent	n_D^{20}
CH ₃ CN	1.34423	Pyridine	1.5100
DCM	1.4242	DMSO	1.4790
EtOH	1.3611	MeOH	1.3618
H ₂ O	1.33299	DMF	1.4310

Table 6.2: Refractive Indices of Solvents^[8].

6.2-3 ¹H NMR Nuclear Magnetic Resonance

The ¹H NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer operating at 400.13 MHz for ¹H NMR, 100.14 MHz for ¹³C and 376.45 MHz for ¹⁹F NMR, respectively. All measurements were carried out at room temperature, using CDCl₂ and CD₃CN as the solvents, with the chemical shifts being reported in ppm downfield from TMS.

6.2-4 Circular Dichroism (CD) Spectroscopy.

Circular Dichroism (CD) spectra were recorded in aqueous solution at concentrations of 1 –3 x10⁻⁵M at Queen's University, Belfast using a Jasco Spectrometer. All CD spectra are presented as $\Delta\epsilon$ vs λ (nm).

6.2-5 Time Correlated Single Photon Counting (SPC)

The emission lifetimes were determined from decays following single short pulsed-laser excitation, and also time-resolved single photon counting (SPC). The use of the two different techniques confirmed the reproducibility of the measurements and reliability of the data treatment. Under pulsed-laser excitation, the emission lifetimes were measured by excitation at 440 nm using a Nd:YAG laser (pulse duration 15 ns).

following standard conditions

Time correlated single photon counting was used for the measurement of the luminescence decays in the absence and in the presence of DNA. The photomultiplier triggers the excitation source and generates an electronic and optical pulse simultaneously. The electronic pulse reaches the START input of the time-to-amplitude converter (TAC) and initiates the charging of the capacitor. The optical pulse excites the sample, which begins to luminesce. The system is fixed so that only one photon may be detected for each exciting event. The signal from the resulting photon stops the charging in the TAC. The amplitude of the released pulse is proportional to the charge in the capacitor, so that there is a time difference between the START and STOP pulses. A 'count' relating to this is subsequently stored in the data storage device. This is repeated until enough counts are present to represent a decay curve of the sample. All measurements were deconvoluted, whereby a time profile of the excitation pulse was also recorded (the sample being replaced by a light scattering solution) and subtracted from the observed decay.

The data was fitted according to the equation

$$I(t) = A + B_1 \exp\left(\frac{-t}{\tau_1}\right) + B_2 \exp\left(\frac{-t}{\tau_2}\right) + \dots \quad 6.2$$

Where I relates to the emission intensity. A relates to the background, B_1 and B_2 relates to the relative contribution from each emitting species to the process, and τ_1 , τ_2 , are lifetimes of each species.

General Experimental Conditions for SPC spectroscopy.

In the studies presented in this thesis, analysis of the results revealed that in homogeneous solvents the decays of the excited states of all the complexes were monoexponential in behaviour. In the presence of DNA, biexponential models were necessary to perform a satisfactory fit. All experiments were performed under the following standard conditions.

(i) instrumental	instrument	Edinburgh Analytical Instruments
	hardware	FLA – 900 Spectrophotometer
	lamp	nF 900 ns Flash Lamp

(ii) Lamp	filling gas	99.999% nitrogen
	gas pressure (lamp off)	0.29 bar
	electrode gap	0.3 mm
	EHT	3.4 KHz
	Frequency (pulse rate)	20.0 KHz
	intensity	approx. 6.0 – 10.0
	time before first measurement	at least one hour

The electrode gap of 0.3 mm was essential since the gas pressure is 0.29 bar. An increase in the electrode gap gives a higher intensity but requires a much longer time to equilibrate the spark (misfiring and free runs are more likely to occur). An EHT of 3.4 KHz was utilized for all experiments. It was observed that daily cleaning of the electrodes was essential. Dirty electrodes resulted in increased lifetime decay values (and a large deviation of the chi squared value, χ^2 from 1). The frequency was chosen by the expected lifetime, 20.0 KHz was used for all compounds investigated.

(iii) monochromator

[Ru(L) ₃] ²⁺	excitation	337 nm
	emission	590 nm
[Ru(phen) ₂ L'] ²⁺	excitation	337 nm
	emission	610 nm

The λ_{em} was chosen so that it was situated close to the maximum wavelength in the emission band of each complex. Solutions of ruthenium complexes of optical density 0.1 A at $\lambda = 440$ nm were used for all of the experiments. Changing the excitation wavelength did not significantly affect the results, however 337nm was preferred since longer wavelengths required longer counting times.

(iv) recording mode	gain	1023 channel <i>i.e.</i> 1-1023
	temperature	25°C $\pm$ 1°C
	counting rate	approx. 200-250 per second
	number of counts	5000
	cell	1 cm fluorescence quartz cell

The cell was thermostated at 25°C $\pm$ 2°C during all the measurements, unless stated otherwise (*e.g.* temperature dependent studies for [Ru(bpy)₃]²⁺ **1** and [Ru(phen)₃]²⁺ **6** complexes). The start/stop ratio (often called the F/D value) corresponds to a ratio of the frequency (*i.e.* 20 KHz) to the counting rate. It has been proposed^[10], that a F/D value of < 2% should be used. However, Edinburgh Instruments perform experiments with F/D values of up to 8%. Above this value there is a problem of pile-up (this will cause non-exponential fits, possibly one will see an additional lifetime). A F/D value of $\leq$ 4% should be sufficient in both mono- and bi-exponential decays. For experiments using a double exponential, the F/D value *i.e.* counting rate/frequency could be as high as 0.05, since the accuracy is not high enough to detect the difference. [Ru(bpy)₃]²⁺ was used as a standard for single exponentials measurements, whereby an aerated aqueous solution, [Ru] = $\sim 10^{-5}$ M at 25°C has a lifetime decay $\tau = 402 \pm 4$ ns. Our value of $\tau = 406 \pm 4$ ns agrees within 2 % of the reported value^[11].

For the determination of goodness of fit, usually two parameters are considered.

(i) Inspection of the residuals is very informative and as such inspected carefully. Ideally, most of the points should be within three standard deviations ($\pm 3\sigma$) which corresponds to a 99.9% confidence. (ii) The Chi square value χ^2 is very useful. It is a normalised factor and a good fit will give a value of approximately 1. Usually a range

of 1.0 $\rightarrow$ 1.3 is considered acceptable. Typical profiles of an monoexponential and biexponential decay for $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex **4** are displayed in Figure 6.1.

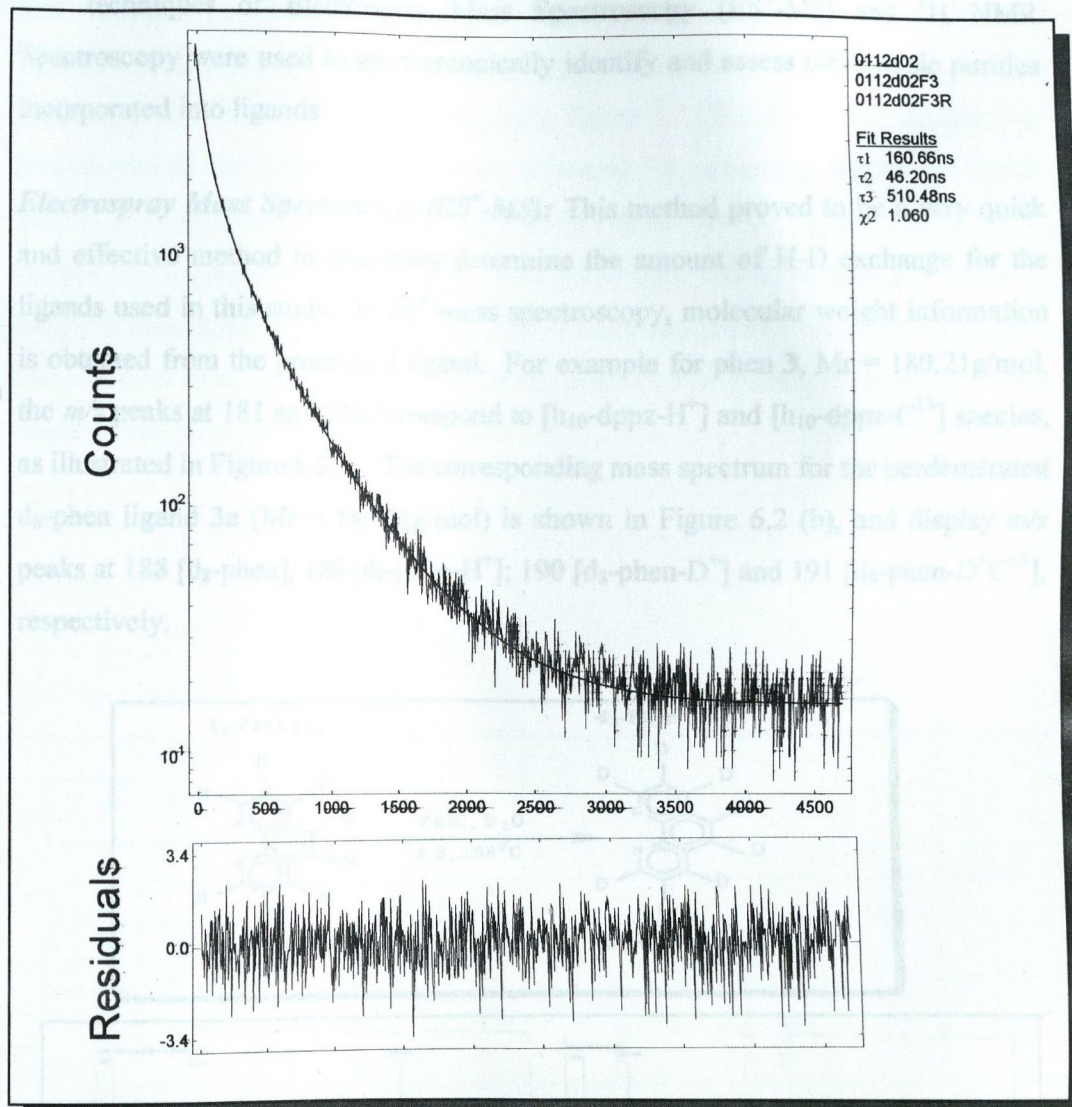


Figure 6.1: Decay Profile, of biexponential decay in aqueous DNA solution for $[\text{Ru}(\text{phen})_2\text{dppz}]_2$, **4**.

6.2-6 Microanalysis.

Microanalysis was acquired from the microanalysis laboratory, University College Dublin.

6.2-7 Mass Spectroscopy.

Mass spectra were recorded on an Electron Spray Micromass LCT instrument.

6.2-8 Studies to Calculate the Extent of Protium-Deuterium

Exchange into Ligands used in this Study.

The techniques of Electrospray Mass Spectroscopy (ES^+ -MS) and 1H NMR Spectroscopy were used to spectroscopically identify and assess the isotopic purities incorporated into ligands.

Electrospray Mass Spectroscopy (ES^+ -MS): This method proved to be a very quick and effective method to accurately determine the amount of H-D exchange for the ligands used in this study. In ES^+ -mass spectroscopy, molecular weight information is obtained from the protonated ligand. For example for phen **3**, $Mr = 180.21g/mol$, the m/z peaks at 181 and 182 correspond to $[h_{10}\text{-dppz-H}^+]$ and $[h_{10}\text{-dppz-C}^{13}]$ species, as illustrated in Figure 6.2(a). The corresponding mass spectrum for the perdeuterated d_8 -phen ligand **3a** ($Mr = 188.21g/mol$) is shown in Figure 6.2 (b), and display m/z peaks at 188 [d_8 -phen], 189 [d_8 -phen- H^+]; 190 [d_8 -phen- D^+] and 191 [d_8 -phen- D^+C^{13}], respectively.

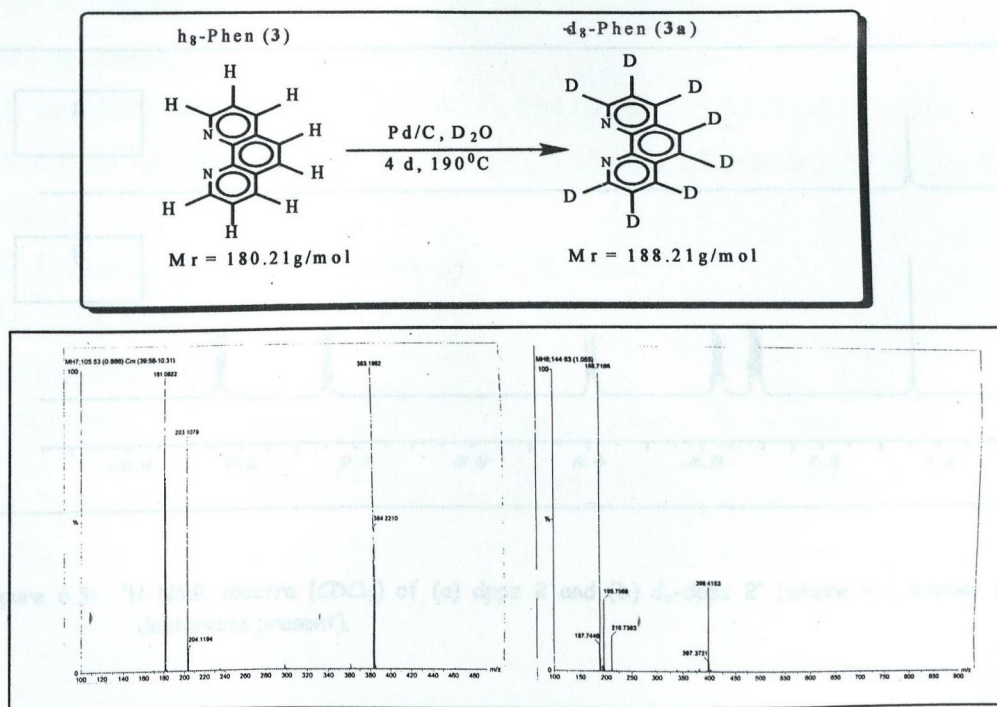


Figure 6.2: ES^+ - Mass spectra of (a) h_8 -phen **3** (b) d_8 -phen **3a**

^1H NMR Spectroscopy: To calculate the extent of H-D exchange using ^1H NMR spectroscopy, protiated $\text{h}_{10}\text{-dppz}$ **2** was treated with Pd/C and D_2O for 4 days (2 cycles) at 190°C , yielding $\text{d}_n\text{-dppz}$ **2'** (where n = number of deuteriums present). Equal amounts of **2** and **2'** were weighed out accurately using an analytical balance, dissolved in 1ml deuteriochloroform (CDCl_3) and sonicated for 5-10 mins. The ^1H NMR spectra were recorded and are shown in Figure 6.3. The percentage deuteration at each site in the dppz ligand may be estimated by comparing the relative peak heights (I) of the protiated (**2**) and deuterated (**2'**) dppz ligands. From Figure 6.3, the amount of H-D exchange at resonance peak $\delta 9.69$ ppm was calculated according to the difference in the peak heights, $(I_2 - I_{2'})$, and equates to 95% exchange at this site. The removal of a signal from the spectrum suggests that complete deuteration has occurred at that particular proton position.

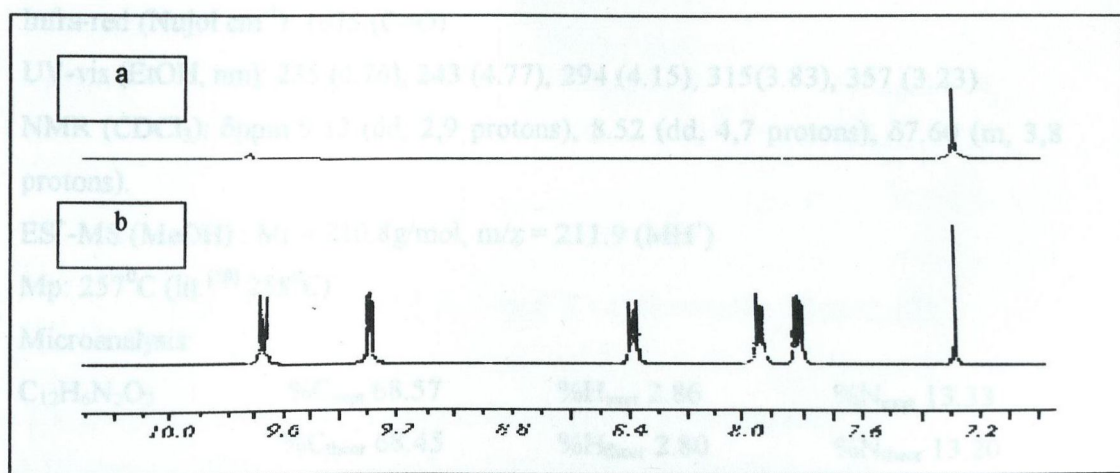


Figure 6.3: ^1H NMR spectra (CDCl_3) of (a) dppz **2** and (b) $\text{d}_n\text{-dppz}$ **2'** (where n = number of deuteriums present).

These techniques proved to be reliable and accurate for determination of the extent of H-D exchange and agree within $\pm 1\%$ for the systems used in this study.

6.3 Preparation of Ligands and Complexes used in this Study

6.3-1 Ligands

6.3.1-1 1,10-phenanthroline-5,6-dione (phendione) (12)

The following procedure is based on report by Paw^[12] and it yields the product almost quantitatively.

An ice-cold mixture of concentrated H_2SO_4 (40 ml) and HNO_3 (20 ml) was added to 1,10-phenanthroline (4.0 g, 0.022 mol) and KBr (4.0 g, 0.034 mol). The resulting red mixture was heated at reflux (with two condensers) for four hours. The hot yellow solution was poured onto 500 ml of ice and carefully neutralised with 10 M NaOH until neutral to slightly acidic pH was achieved. The yellow solution was extracted with dichloromethane, and dried with MgSO_4 . The filtered yellow solution was reduced to dryness. The crude extract was further purified by crystallisation from methanol.

Yield = 3.8g, 81.2%

Infra-red (Nujol cm^{-1}): 1675 (C=O)

UV-vis (EtOH, nm): 235 (4.76), 243 (4.77), 294 (4.15), 315(3.83), 357 (3.23)

NMR (CDCl_3): δ ppm 9.12 (dd, 2,9 protons), 8.52 (dd, 4,7 protons), δ 7.60 (m, 3,8 protons).

ES^+ -MS (MeOH) : Mr = 210.8g/mol, m/z = 211.9 (MH^+)

Mp: 257 $^{\circ}\text{C}$ (lit.^[10] 258 $^{\circ}\text{C}$)

Microanalysis:

$\text{C}_{12}\text{H}_6\text{N}_2\text{O}_2$	%C _{expt} 68.57	%H _{expt} 2.86	%N _{expt} 13.33
	%C _{theor} 68.45	%H _{theor} 2.80	%N _{theor} 13.20

6.3.1-2 Dipyrido[3,2-d:2'3'-f]quinoxaline (Dpq) (16)

This ligand was prepared via the method by Collins *et al*^[13]

A mixture of 1,10-phenanthroline-5,6-dione (1.0 g, 4.8 mmol) and ethylenediamine (0.44 ml) in ethanol (350 ml) was stirred for two hours at 40°C. During this time the reaction mixture darkened in colour. Subsequently, it was stirred overnight at room temperature. The resulting red-orange solution was reduced in volume (25 ml) to yield a yellow product. The crude product was left to stand for three hours, 90% methanol was added and a cream product was filtered. Recrystallisation from methanol yielded a white product.

Yield = 0.57g, 52%

UV-vis (CH₂Cl₂, nm) : 218(sh), 235, 253, 299, 311(sh), 340.

¹H NMR (d₆-DMSO) : δppm, 7.39 (3,8 protons), 8.61 (11,12 protons), 8.68 (4,7 protons), 8.90 (2,9 protons).

ES⁺-MS (CH₂Cl₂) : Mr = 232.2g/mol, m/z = 233 (MH⁺), 487 (2M + Na)

Mp: 254-255⁰C (lit.^[28] 250⁰C)

Microanalysis:

C ₁₄ H ₈ N ₄	%C _{expt} 72.42	%H _{expt} 3.44	%N _{expt} 24.14
	%C _{theor} 72.53	%H _{theor} 3.60	%N _{theor} 24.19

6.3.1-3 Dipyrido[3,2-a:2'3'-c]phenazine (Dppz) (2)

The complex was prepared following the method of Summers *et al*^[14]

1,10-phenanthroline-5,6-dione (0.5 g, 2.39 mmol) was dissolved in ethanol (20 ml) on heating. To the clear yellow solution was added 1,2-diaminobenzene (dab) (0.5 g, 4.58 mmol) and the resultant solution was boiled over a water bath for 30 minutes. On cooling the resulting brown crystals were consequently collected and recrystallized from aqueous ethanol to give dppz as straw-like needles.

Yield = 0.38g, 76%.

UV-vis (EtOH nm): 241(2.54), 268(4.67), 294(1.94), 342(0.62), 349(0.72), 366(1.01), 358(0.88), 377(1.07)

^1H NMR (400MHz, CDCl_3): δ ppm; 9.69 (dd, 4,7 protons), 9.30 (dd, 2,9 protons), 8.40 (m, 11,14 protons), 7.95 (m, 12,13 protons), 7.82 (m, 3,8 protons).

ES^+ -MS (MeOH) : Mr = 282.3g/mol, m/z = 283 (MH^+), 305 ($\text{M} + \text{Na}$)

Mp: 251 $^\circ\text{C}$ (lit.^[10] 250 $^\circ\text{C}$)

Microanalysis:

$\text{C}_{18}\text{H}_{10}\text{N}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$	%C _{expt} 74.22	%H _{expt} 3.78	%N _{expt} 19.24
	%C _{theor} 74.38	%H _{theor} 3.70	%N _{theor} 19.26

6.3.1-4 12,13-Difluoro-dppz (diFdppz) (14)

This ligand was synthesised according to a two-step reaction, the first was a modified procedure from Vogel, Practical Organic Synthesis^[20] for the reduction of a nitro to an amino group.

2-Amino-4,5-difluoroaniline

2-Nitro-4,5-difluoroaniline (2 g, 11.36 mmol) was boiled gently in ethanol (200 ml) to produce a clear bright yellow solution. To this, tin(II) chloride (20 g) dissolved in concentrated HCl (60 ml) was added. The solution was refluxed at 100 $^\circ\text{C}$ for 30 mins and a series of colour changes was observed, yellow→cream→white-grey→white→cream→pale yellow. The resulting solution was stirred at room temperature for a further 45 min, reduced in volume (50ml), and cooled overnight. The yellow solution was neutralised with 2 M NaOH to a slightly basic pH (8-9). The addition of ethanol resulted in the precipitation of NaCl, which was filtered off. The filtered solution was reduced to dryness and the brown product was recrystallised from ethanol / diethyl ether.

Yield = 1.36g, 82%

UV-vis (EtOH, nm) : 207, 258(sh), 308, 465(sh)

^1H NMR (400MHz, CDCl_3) : δ ppm; 6.53 (t, 3,6 protons), 5.31 (s, NH_2).

^{12}C NMR (400MHz, CDCl_3) : δ 76.52ppm (t).

^{20}F NMR (400MHz, CDCl_3) : δ 149.92ppm (t).

ES^+ -MS (EtOH) : Mr = 144.1g/mol, m/z = 288 (2M)

12,13-Difluorodipyridophenazine (diFdppz)

This desired 12,13-difluorodipyridophenazine was then prepared following slight modifications of the method of Summers *et al*^[14].

1,10-phenanthroline-5,6-dione (120 mg, 0.57 mmol) in (10 ml) ethanol was stirred with slight heat to produce a clear yellow solution. To this 2-amino-4,5-difluoroaniline (120 mg, 0.83 mmol) in ethanol was added and the solution was boiled for 30 minutes, and then allowed to stir for an addition 15 minutes at room temperature. The resulting yellow solution was cooled to room temperature and then filtered to yield a cream product. Subsequent recrystallisation from aqueous ethanol gave 7,8-difluoro-(dppz) as a cream-white product.

Yield = 0.48g, 57%.

UV-vis (EtOH nm): 244(2.79), 287(4.97), 294(2.15), 342(1.01), 350(1.12), 359(1.85), 367(1.55), 378(2.03).

^1H NMR (CDCl_3): δ ppm; 9.60 (dd, 4,7 protons), 9.31 (dd, 2,9 protons), 8.10 (t, 11,14 protons), 7.82 (m, 3,8 protons).

^{19}F NMR (400MHz, CDCl_3) : δ 131.47ppm (t).

^{20}F NMR (400MHz, CDCl_3) : δ 127.21ppm (t).

ES^+ -MS (EtOH) : Mr = 318.8g/mol, m/z = 320 (MH^+)

6.3.1-5 7, 8- DiMethyl-dppz (diMedppz) (13)

This was synthesised as above for Dppz (4) by substituting 1,2-diamino-4,5-dimethylbenzene (DiMedab) for 1,2-diaminobenzene (dab)

Yield = 1.06g, 71%

UV-vis (EtOH, nm): 211(2.05), 241(1.80), 274(4.73), 365(1.10), 374(0.96), 385(1.47).

$^1\text{H NMR}$ (CDCl_3) : δ ppm; 9.64 (dd, 4,7 protons), 9.29 (d, 2,9 protons), 8.12 (s, 11,14 protons), 7.80 (m, 3,8 protons), 2.63 (CH_3 , s).

ES^+ -MS (EtOH) : Mr = 310.3g/mol, m/z = 311(MH^+), 338, 621 (2M), 643 (2M+ Na^+)

Microanalysis:

$\text{C}_{20}\text{H}_{14}\text{N}_4 \cdot 2\text{H}_2\text{O}$	%C _{expt} 69.36	%H _{expt} 5.20	%N _{expt} 16.18
	%C _{theor} 77.01	%H _{theor} 5.29	%N _{theor} 16.20

6.3.1-6 Methyl-dppz (Medppz) (21)

This ligand was isolated following the preparation according to Summers *et al*^[14], replacing dab with methyl-dianinobenzene (Medab)

Yield = 56%

UV-vis (EtOH, nm) : 210, 241, 276, 342, 351, 366, 379.

$^1\text{H NMR}$ (CD_3CN): δ ppm; 9.70 and 9.62 (dd, 4,7 protons), 9.22 (d, 2,9 protons), 8.22 (d, 12 proton), 7.89 (m, 11,14 protons), 6.53 (m, 3,8 protons), 3.05 (CH_3 , s).

ES^+ -MS (EtOH) : Mr = 296.3g/mol, m/z = 297(MH^+), 615 (2M + Na).

6.4 Preparation of Ru(II) Polypyridyl Complexes

6.4-1 Synthesis of Precursor complexes of the type $[\text{Ru}(\text{L})_2]\text{Cl}_2$

The two bis-complexes cis- $[\text{Ru}(\text{bpy})_2]\text{Cl}_2$ and $[\text{Ru}(\text{phen})_2]\text{Cl}_2$, were synthesised according to Meyer *et al*^[15]

A solution of $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (7.8 g, 29.8 mmol), 2,2'-bipyridine (9.36 g, 60.0 mmol) and LiCl (8.4 g, 2 mmol) were heated at reflux in reagent grade DMF (50 ml) for 8 hours. The reaction was stirred magnetically and carried out in the presence of nitrogen throughout this period. After the reaction had cooled to room temperature, reagent grade acetone (250 ml) was added and the resultant mixture cooled to 0°C overnight. Filtering yielded a red-violet solution and a dark green-black

microcrystalline product. The solid was washed with water until the filtrate was colourless and dried with diethyl ether.

-[Ru(phen)₂]Cl₂.2H₂O (25)

Yield = 11.47 g, 58%.

UV-vis (CH₂Cl₂, nm) : 231, 268, 356, 473(sh), 543

¹H NMR (400MHz, CDCl₃): δppm; 9.69 (dd, 4,7 protons), 9.30 (dd, 2,9 protons), 8.40 (m, 11,14 protons), 7.95 (m, 12,13 protons), 7.82 (m, 3,8 protons).

ES⁺-MS (MeOH) : Mr = 568.52g/mol, m/z = 496 (M + Cl), 532 (M + 2Cl), 555 (M + 2Cl⁻ + Na)

Thin Layer Chromatography (DMF/H₂O (1:1)/1M NH₄Cl): R_f value = 0.78

Microanalysis: RuC₂₄H₁₆N₄Cl₂.2H₂O

%C _{expt} 50.70	%H _{expt} 3.52	%N _{expt} 9.86
%C _{theor} 49.85	%H _{theor} 3.32	%N _{theor} 9.54

-[Ru(bpy)₂]Cl₂.2H₂O (24)

Yield = 13.64 g, 73.9%.

UV-vis (CH₂Cl₂, nm) : 243, 299, 382, 556.

¹H NMR (400MHz, CDCl₃) : δppm; 9.69ppm (dd, 4,7 protons), 89.30ppm (dd, 2,9 protons), 88.40ppm (m, 11,14 protons), 87.95ppm (m, 12,13 protons), 87.82ppm (m, 3,8 protons).

ES⁺-MS (MeOH) : Mr = 520.5g/mol, m/z = 448 (M + Cl), 484 (M + 2Cl), 507 (M + 2Cl⁻ + Na⁺)

Thin Layer Chromatography (DMF/H₂O (1:1)/1M NH₄Cl): R_f value = 0.80

Microanalysis: RuC₂₀H₁₆N₄Cl₂.2H₂O

%C _{expt} 46.15	%H _{expt} 3.85	%N _{expt} 10.77
%C _{theor} 46.93	%H _{theor} 3.91	%N _{theor} 10.82

6.4-2 cis - [Ru(phen)₂phendione](PF₆)₂·5H₂O (26)

This complex was synthesised according to Goss and Abruna^[16]

[Ru(phen)₂Cl₂].2H₂O (0.502 g, 0.88 mmol) was heated at reflux under N₂ with 1.2 molar equiv of 1,10-phenanthroline-5,6-dione (0.21 g, 1.00 mmol) in thoroughly deaired 50/50 ethanol/water for a period of 3h. After this time, the reaction mixture was allowed to cool, and the complex was precipitated as a green solid by the addition of saturated aqueous NH₄PF₆. The complex was collected, washed with water, and dried with diethyl ether. Recrystallisation was achieved by acetronitrile/diethyl ether. The resulting black crystalline product was filtered and dried *in vacuo* for 24 hours.

Yield = 0.746 g, 85%.

UV-vis (CH₂Cl₂, nm) : 230, 264, 437

¹H NMR (400MHz, CD₃CN): δppm; 8.66 and 8.57 (d, 4,7 protons,P), 8.47 (d, 4,7 protons, p), 8.30 (d, 2,9 protons, P), 8.25 and 7.88 (d, 5,6 protons, P), 7.80 and 7.57 (m, 3,8 protons, P), 7.47 (m, 3,8 protons, p). [where P = phen and p = phendione]

ES⁺-MS (CH₃CN) : Mr = 1049.72g/mol, m/z = 335 (MH⁺), 254, 358 (MH⁺ + Na)

Microanalysis: RuC₃₆H₂₂N₆P₂F₁₂·5H₂O

%C _{expt}	41.18	%H _{expt}	3.05	%N _{expt}	8.00
%C _{theor}	41.26	%H _{theor}	3.15	%N _{theor}	8.09

6.4-3 cis - [Ru(phen)₃](PF₆)₂·1½H₂O (6)

The same procedure as described above^[16], for cis-[Ru(phen)₂phendione](PF₆)₂, gave this complex by using phen in place of phendione. Addition of a saturated NH₄PF₆ solution precipitated the complex as a bright orange powder.

Yield = 1.53 g, 93%.

UV-vis (H₂O, nm): 223(101), 262(133), 420(19.6), 447(20.3)

¹H NMR (400MHz, CD₃CN): δppm; 8.61 (dd, 4,7 protons), 8.27 (dd, 5,6 protons), 8.05 (m, 2,9 protons), 7.64 (m, 3,8 protons).

ES⁺-MS (MeOH) : Mr = 956.73g/mol, m/z = 321 (MH⁺), 787 (M-PF₆⁻)

Thin Layer Chromatography (10H₂O/3MeCN/0.1M KNO₃): R_f value = 0.69

Microanalysis: $\text{RuC}_{36}\text{H}_{24}\text{N}_6\text{P}_2\text{F}_{12} \cdot 1\frac{1}{2}\text{H}_2\text{O}$

UV-vis (CH_3CN , nm)	%C _{expt} 45.19	%H _{expt} 2.92	%N _{expt} 8.78
^1H NMR (400MHz, CD_3CN)	%C _{theor} 45.29	%H _{theor} 3.01	%N _{theor} 8.69

6.4-4 cis - $[\text{Ru}(\text{bpy})_3](\text{PF}_6)_2 \cdot 2\text{H}_2\text{O}$ (1)

The same procedure as described above, for cis- $[\text{Ru}(\text{phen})_2\text{phendione}](\text{PF}_6)_2$, gave this complex. Addition of a saturated NH_4PF_6 solution yielded the product as bright red.

Yield = 1.67 g, 86%.

UV-vis (H_2O , nm): 238(28.9), 250(25.2), 285(86.3), 323(63.4), 453(14.6)

^1H NMR (400MHz, CD_3CN): δ ppm; 8.42 (dt, 3,3' protons), 8.76 (d, 5,5' protons), 9.09 (dt, 4,4' protons), 9.56 (d, 2,2' protons).

ES⁺-MS (MeOH) : Mr = 893.52g/mol, m/z = 285 (MH^+)

Thin Layer Chromatography (10 H_2O /3 MeCN/0.1M KNO_3): R_f value = 0.75

Microanalysis: $\text{RuC}_{30}\text{H}_{24}\text{N}_6\text{P}_2\text{F}_{12} \cdot 2\text{H}_2\text{O}$

%C _{expt} 40.31	%H _{expt} 3.13	%N _{expt} 9.41
%C _{theor} 40.42	%H _{theor} 3.04	%N _{theor} 9.45

6.4-5 cis - $[\text{Ru}(\text{phendione})_3](\text{PF}_6)_2$ (22)

The compound was prepared according to a modified procedure by Gillard *et al* ^[17]

An ice-cold mixture of concentrated H_2SO_4 (10 ml) and HNO_3 (5 ml) was added to $\text{Ru}(\text{phen})_3\text{Cl}_2$ (0.162 g, 0.025 mmol) and NaBr (0.301 g, 2.9 mmol). The resulting blue-green mixture was heated to reflux at 100°C for 3 hours. The solution was allowed to cool to room temperature, and the complex was precipitated by addition of NaPF_6 . The green product was collected, washed with diethyl ether and dried under vacuum.

Yield = 0.211 g, 98%.

UV-vis (CH_3CN , nm) : 343, 414

^1H NMR (400MHz, CD_3CN): δ ppm; 8.62 (dd, 2,9 protons), 8.13 (dd, 4,7 protons), 7.71 (dd, 3,8 protons).

ES^+ -MS (MeOH) : Mr = 1019.52g/mol, m/z = 367 (MH^+)

Thin Layer Chromatography (10 H_2O /3 MeCN/0.1M KNO_3): R_f value = 0.58

6.4-6 $[\text{Ru}(\text{dppz})_3](\text{PF}_6)_2$ (15)

Yield = 0.035 g, 10%.

UV-vis (H_2O , nm) : 208, 279, 361, 369, 433

^1H NMR (400MHz, DMSO): δ ppm; 8.73 (dd, 4,7 protons), 8.51 (dd, 11,14 protons), 8.34 (d, 2,9 protons), 8.19 (dd, 12,13), 7.87 (dd, 3,8 protons).

ES^+ -MS (H_2O) : Mr = 1235.3g/mol, m/z = 475 (MH^+)

Thin Layer Chromatography (10 H_2O /3 MeCN/0.1M KNO_3): R_f value = 0.72

6.4-7 cis - $[\text{Ru}(\text{phen})_2\text{dppz}](\text{PF}_6)_2 \cdot 1\frac{1}{2}\text{H}_2\text{O}$ (4)

This complex was prepared according to the procedure devised by Barton *et al* ^[18].

$[\text{Ru}(\text{phen})_2\text{dppz}](\text{PF}_6)_2$ was assembled by refluxing dppz (0.268 g, 1.36 mmol) in (20 ml) 50% methanol. To this $[\text{Ru}(\text{phen})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ (0.25 g, 0.60 mmol) was added and the reaction mixture was refluxed at 110°C for 10hours. After this time the reaction was allowed to cool to room temperature, diluted with water (20 ml) and filtered to remove any impurities. The complex was then separated from insoluble impurities by precipitation with NH_4PF_6 . The brown-red solid was collected and washed with diethyl ether.

Yield = 0.84g, 83%.

UV-vis (H_2O , nm) : 220(sh), 263(115), 274(sh), 372(21.59), 440(19.5)

^1H NMR (400MHz, CD_3CN): δ ppm; 8.61 (dd, 4,7 protons), 8.27 (dd, 5,6 protons), 8.05 (m, 2,9 protons), 7.64 (m, 3,8 protons).

ES^+ -MS (H_2O): Mr = 1058.3g/mol, m/z = 372 (MH^+)

Thin Layer Chromatography (10 H_2O /3MeCN/0.1M KNO_3): R_f value = 0.85

Microanalysis:

$\text{RuC}_{42}\text{H}_{26}\text{N}_8\text{P}_2\text{F}_{12}\cdot 1\frac{1}{2}\text{H}_2\text{O}$	%C _{expt} 47.64	%H _{expt} 2.74	%N _{expt} 10.59
	%C _{theor} 47.50	%H _{theor} 2.85	%N _{theor} 10.51

6.4-8 $[\text{Ru}(\text{phen})_2\text{diMeppz}]\text{](PF}_6)_2\cdot 3\text{H}_2\text{O}$ (8)

This was prepared by a slight modification to that of Barton ^[18] by replacing dppz (2) with diMedppz (13).

Yield = 0.84 g, 83%.

UV-vis (H_2O , nm) : 205, 220, 263, 284, 383(28.5), 442(21.0)

^1H NMR (400MHz, CD_3CN): δ ppm; 8.61 (dd, 4,7 protons), δ 8.27ppm (dd, 5,6 protons), δ 8.05ppm (m, 2,9 protons), δ 7.64ppm (m, 3,8 protons).

ES^+ -MS (MeOH) : Mr = 1113.91g/mol, m/z = 236, 387 (MH^+)

Thin Layer Chromatography (10 H_2O /3 MeCN/0.1M KNO_3): R_f value = 0.53

Microanalysis:

$\text{RuC}_{44}\text{H}_{30}\text{N}_8\text{P}_2\text{F}_{12}\cdot 3\text{H}_2\text{O}$	%C _{expt} 47.44	%H _{expt} 3.23	%N _{expt} 10.06
	%C _{theor} 47.60	%H _{theor} 3.06	%N _{theor} 9.98

6.4-9 $[\text{Ru}(\text{phen})_2\text{diFppz}]\text{](PF}_6)_2\cdot 2\text{H}_2\text{O}$ (9)

This complex was prepared according to modifications of the above method as devised by Barton and coworkers ^[18].

Yield = 0.202 g, 48%.

UV-vis (H_2O , nm) : 202, 264(117), 312(sh), 372(24.0), 439(22.6)

^1H NMR (400MHz, CD_3CN): δ 8.61ppm (dd, 4,7 protons), δ 8.27ppm (dd, 5,6 protons), δ 8.05ppm (m, 2,9 protons), δ 7.64ppm (m, 3,8 protons).

ES^+ -MS (MeOH) : Mr = 1103.3g/mol, m/z = 780 (M^+), 923($\text{M} + \text{PF}_6^-$)

Thin Layer Chromatography (10 H_2O /3MeCN/0.1M KNO_3): R_f value = 0.78

Microanalysis:

$\text{RuC}_{42}\text{H}_{24}\text{N}_8\text{F}_2\text{P}_2\text{F}_{12}\cdot 2\text{H}_2\text{O}$	%C _{expt} 45.69	%H _{expt} 2.53	%N _{expt} 10.15
	%C _{theor} 45.58	%H _{theor} 2.41	%N _{theor} 10.04

6.4-10 [Ru(phen)₂dppq](PF₆)₂·2H₂O (7)

This was prepared by a slight modification to that of Barton ^[18] by replacing dppz (2) with dpq (16).

Yield = 0.202 g, 48%.

UV-vis (CH₂Cl₂, nm) : 217, 235, 253, 299, 311(sh), 447

¹H NMR (400MHz, CD₃CN): δ 8.61ppm (dd, 4,7 protons), δ 8.27ppm (dd, 5,6 protons), δ 8.05ppm (m, 2,9 protons), δ 7.64ppm (m, 3,8 protons).

ES⁺-MS (MeOH) : Mr = 1017.3g/mol, m/z = 347 (M⁺), 839 (M + PF₆⁻)

Thin Layer Chromatography (10H₂O/3 MeCN/0.1M KNO₃): R_f value = 0.63

6.5 Synthesis of Deuterated Ligands

The perdeuterated ligands described in this section were prepared according to slight modifications of the general experimental procedure. The partially deuterated ligands

6.5-1 d₈-phenanthroline (3a).

This complex was isolated following slight modifications of the preparation according to Vos *et al* ^[19].

1,10-Phenanthroline (phen) (2.31 g, 0.19 mol) was added to (20 ml) D₂O (deuteration 99.9%) and the reaction mixture was allowed to react in the presence of H-D exchange catalyst Pd/C (10% Pd) (0.33 g) in a Teflon-coated steel high Pressure reactor at 190 ± 5°C for 2 days. The contents of the reactor were collected, filtered and D₂O was removed under vacuum to obtain the product. The Pd/C was washed with acetone to remove any product present on its surface. One consecutive exchange cycle resulted in >80% purity, as evaluated by ES⁺-MS. The procedure was repeated until the desired isotopic purities (> 90%) was achieved.

Yield : 78%, 1.706g

^1H NMR (CDCl_3): NMR silent.

ES^+ -MS (MeOH): Mr = 188.25g/mol; m/z = 189 (MH^+ , d_8), 399 ($2\text{M} + \text{Na}$).

UV- Vis (MeOH, λ_{nm}) : 239, 264

TLC ($3\text{H}_2\text{O}/10\text{MeCN}/0.1\text{M KNO}_3$) : R_f = 0.78

6.5-2 d_8 -Bipyridine (5a).

Yield : 0.736 g, 35%.

ES^+ - MS (MeOH) : Mr = 164.19g/mol; m/z = 165 (MH^+ , d_8).

TLC ($3\text{H}_2\text{O}/10\text{MeCN}/0.1\text{M KNO}_3$) : R_f = 0.73

6.5-3 d_8 - Dipyrido[3,2-d:2'3'-f]quinoxaline (Dpq) (16a).

Yield : 0.162 g, 21.5%

ES^+ -MS (CH_2Cl_2) : Mr = 240.12g/mol; m/z = 241(MH^+ , d_8), 263($\text{M} + \text{Na}$), 503($2\text{M} + \text{Na}$).

UV- Vis (CH_2Cl_2 , λ_{nm}) : 216, 225, 253, 340.

TLC ($3\text{H}_2\text{O}/10\text{MeCN}/0.1\text{M KNO}_3$) : R_f = 0.92

6.5-4 d_{10} -Dyrido[3,2-a:2',3'-c]phenazine (2c).

Yield : 0.066 g, 22%.

ES^+ -Mass Spectrum (MeOH) : Mr = 292.10g/mol; m/z = 293 (MH^+ , d_{10}), 356, 585,

UV- Vis (MeOH, λ_{nm}) : 205, 241, 268, 349, 358, 366, 377.

TLC ($3\text{H}_2\text{O}/10\text{MeCN}/0.1\text{M KNO}_3$) : R_f = 0.95

6.5-5 d_6 -phendione (12a).

Yield : 0.072 g, 52%.

ES^+ -MS (MeOH) : Mr = 216.10g/mol; m/z = 217(MH^+ , d_6).

UV- Vis (MeOH, λ_{nm}) : 205, 241, 268, 349, 358, 366, 377.

TLC ($3\text{H}_2\text{O}/10\text{MeCN}/0.1\text{M KNO}_3$) : R_f = 0.95

KBr : 2278 cm^{-1} ($\nu_{\text{C-D}}$ vibration)

6.5-6 d₄-1,2-Diaminobenzene (11a).

This ligand was prepared according to the following procedure.

1,2-Diaminobenzene (dab) (1.533 g, 14.1 mmol) was added to (10 ml) D₂O (deuteration 99.9%) and sonicated for 10 minutes. DCl (15 ml) was added to the reaction mixture and allowed to react in a Teflon-coated steel high Pressure reactor at $190 \pm 5^\circ\text{C}$ for 24 hours. The contents of the reactor were cooled, and D₂O/DCl was removed under vacuum to obtain a green product.

Yield : 1.74 g, 22%

ES⁺- Mass Spectrum (MeOH) : Mr = 108.19g/mol; m/z = 178, 180, 191 (MH⁺, d₄ as DCl₂ salt).

UV- Vis (EtOH) : 206, 237, 280.

TLC (3H₂O/10MeCN/0.1M KNO₃) : R_f = 0.95

6.5-7 D₂-DiMediaminobenzene (18a)

This was prepared according to the procedure described above for preparation, with diaminobenzene in place of 1,2-dab **11**.

Yield : 0.289 g, 97%

ES⁺-MS (EtOH) : Mr = 136.2g/mol; m/z = 205, 217(MH⁺, d₂ as DCl₂ salt), 261, 276, 346.

UV- Vis (EtOH, λnm) : 284, 356, 373

TLC (3H₂O/10MeCN/0.1M KNO₃) : R_f = 0.84

6.6 Synthesis of Partially Deuterated Ligands.

The partially deuterated used in this study were prepared according to a modified procedure devised by Summers *et al.*^[12]. The general procedure is given in Section 6.3.1-3 for the protiated h_{10} -dppz **2**. The results are compiled in Table 6.3, respectively. For a full characterisation of the dppz series of ligands see Chapter Two.

Ligand	Yield (%, mg)	UV-vis	Mass Spectrum (EtOH)	TLC	¹ H NMR (CDCl ₃)
d ₄ -dppz ^a 2a	56%, 25mg	271 360 379	Mr = 286.30g/mol 287.10m/z (MH ⁺)	R _f = 0.82	δ7.82ppm (H _{3,8} m) δ9.30ppm (H _{2,9} dd) δ9.69ppm (H _{4,7} dd)
D ₂ -diMedppz ^a 13a	76%, 39mg	(EtOH) 210 242 272 365 385	Mr=312.2g/mol 297.22m/z (M ⁺) 615.45m/z	R _f = 0.78	δ7.78ppm (H _{3,8} m) δ9.29ppm (H _{2,9} dd) δ9.67ppm (H _{4,7} dt)
D ₂ -diMedppz ^b 13	32%, 26mg	(EtOH) 210 242 263 366 374 385	Mr=312.20g/mol 313.05m/z (MH ⁺) 338.29m/z 493.88m/z 621.16m/z 643.13m/z	R _f = 0.79	δ7.80ppm (H _{3,8} m) δ9.35ppm (H _{2,9} d) δ9.72ppm (H _{4,7} dd) δ2.65ppm (CH ₃ s)
D ₃ -Medppz ^a 21a	57%, 48mg	(EtOH) 204 264 359 367 378	Mr=338.87g/mol 339.87m/z (M ⁺)	R _f = 0.80	δ7.82ppm (H _{3,8} m) δ8.10ppm (H _{11,14} t) δ9.31ppm (H _{2,9} d) δ9.60ppm (H _{4,7} dd)

Table 6.3: Characterisation of some of the partially ligands used in this study. ^a D₂O/Pd/C method, and ^b D₂O/DCl procedure.

6.7 Preparation of Deuterated Ru(II) Complexes.

These Ru(II) complexes were prepared in an analogous manner to that for the parent $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complex 4. A full characterisation for each family of complexes has been carried out using UV-Vis, thin layer chromatography, and NMR and Mass spectroscopy, respectively. The results are tabulated in Tables 6.5 - 6.8, as explained below.

Table No	Parent Complex	Series of Compounds
6.5	$[\text{Ru}(\text{phen})_3]\text{Cl}_2$	6 - 6c
6.6	$[\text{Ru}(\text{bpy})_3]\text{Cl}_2$	1 - 1c
6.7	$[\text{Ru}(\text{phen})_2\text{dppz}]\text{Cl}_2$	4 - 4g
6.8	$[\text{Ru}(\text{phen})_2\text{diMedppz}]\text{Cl}_2$	8 - 8g
6.9	$[\text{Ru}(\text{phen})_2\text{dpq}]\text{Cl}_2$	7 - 7g
6.10	$[\text{Ru}(\text{phen})_2\text{diFdppz}]\text{Cl}_2$	9 - 9f

Table 6.4: Series of families synthesised in the present study.

Table 6.5

Compound (as Cl ⁻ salts)	UV-vis (H ₂ O, λ)	Mass Spectrum (H ₂ O)	TLC (10MeCN/3H ₂ O/ 0.1MKNO ₃)	Yield (%, mg)	¹ H NMR (CD ₃ CN)
[Ru(phen) ₃] ²⁺ (6)	420 448	Mr = 641.73g/mol 321.09m/z (MH ⁺)	R _f = 0.65	70%, 44mg	phen; δ 7.64ppm (H _{3,8} m) δ 8.04ppm (H _{2,9} d) δ 8.28ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(phen) ₂ (d ₈ -phen)] ²⁺ (6a)	420 448	Mr = 649.73g/mol 325.00m/z (MH ⁺)	R _f = 0.45	40%, 23mg	phen; δ 7.63ppm (H _{3,8} m) δ 8.04ppm (H _{2,9} d) δ 8.27ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(d ₈ -phen) ₂ phen] ²⁺ (6b)	421 448	Mr = 657.73g/mol 329.86m/z (MH ⁺)	R _f = 0.47	80%, 54mg	phen; δ 7.64ppm (H _{3,8} m) δ 8.05ppm (H _{2,9} d) δ 8.27ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(d ₈ - phen) ₃] ²⁺ (6c)	422 447	Mr = 665.73g/mol 333.05m/z (MH ⁺)	R _f = 0.68	57%, 48mg	phen; ¹ H NMR silent

Table 6.6

Compound (as Cl ⁻ salts)	UV-vis (H ₂ O, λ)	Mass Spectrum (H ₂ O)	TLC (10MeCN/3H ₂ O/ 0.1MKNO ₃)	Yield (%, mg)	¹ H NMR (CD ₃ CN)
[Ru(bpy) ₃] ²⁺ (1)	431 454	Mr = 569.73g/mol 285.2m/z (MH ⁺)	R _f = 0.52	52%, 40mg	bpy; δ 7.64ppm (H _{3,8} m) δ 8.04ppm (H _{2,9} d) δ 8.28ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(bpy) ₂ (d ₈ -bpy)] ²⁺ (1a)	431 454	Mr = 577.73g/mol 289.2m/z (MH ⁺)	R _f = 0.59	48%, 39mg	bpy; δ 7.63ppm (H _{3,8} m) δ 8.04ppm (H _{2,9} d) δ 8.27ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(d ₈ -bpy) ₂ bpy] ²⁺ (1b)	431 454	Mr = 585.73g/mol 293.2m/z (MH ⁺)	R _f = 0.56	65%, 57mg	bpy; δ 7.64ppm (H _{3,8} m) δ 8.05ppm (H _{2,9} d) δ 8.27ppm (H _{5,6} s) δ 8.62ppm (H _{4,7} m)
[Ru(d ₈ - bpy) ₃] ²⁺ (1c)	431 454	Mr = 593.73g/mol 297.2m/z (MH ⁺)	R _f = 0.54	61%, 43mg	bpy; ¹ H NMR silent

**Table
6.7**

Compound	UV-vis (ϵ)	Mass Spectrum	TLC	Yield	$^1\text{H NMR}$ (CD_3CN)
$[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ 4	263 (104.1) 373 (21.8) 439 (19.6)	$M_r = 371.91\text{g/mol}$ 372.07m/z (M^+)	$R_f = 0.71$	74%, 116mg	phen: $\delta 7.65\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.04\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.24\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.29\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.62\text{ppm}$ ($\text{H}_{4,7}$ m) dppz: $\delta 7.79\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.13\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.18\text{ppm}$ ($\text{H}_{12,13}$ d) $\delta 8.49\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 9.62\text{ppm}$ ($\text{H}_{4,7}$ d)
$[\text{Ru}(\text{phen})_2(\text{d}_4\text{-dppz})]^{2+}$ 4a	440 (14.35)	$M_r = 373.91\text{g/mol}$ 374.13m/z (M^+)	$R_f = 0.48$	43%, 43mg	phen: $\delta 7.68\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.05\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.24\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.30\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.66\text{ppm}$ ($\text{H}_{4,7}$ m) dppz: $\delta 7.71\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.14\text{ppm}$ ($\text{H}_{2,9}$ d)
$[\text{Ru}(\text{phen})_2(\text{d}_6\text{-dppz})]^{2+}$ 4b	440 (15.07)	$M_r = 374.91\text{g/mol}$ 375.20m/z (M^+)	$R_f = 0.75$	53%, 42 mg	phen: $\delta 7.67\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.05\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.24\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.29\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.65\text{ppm}$ ($\text{H}_{4,7}$ m) dppz: $\delta 8.17\text{ppm}$ ($\text{H}_{12,13}$ d) $\delta 8.50\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 9.67\text{ppm}$ ($\text{H}_{4,7}$ dd)
$[\text{Ru}(\text{phen})_2(\text{d}_{10}\text{-dppz})]$ 4c	440 (14.18)	$M_r = 376.33\text{g/mol}$ 283.12m/z 377.10m/z (M)	$R_f = 0.68$	57%, 69 mg	phen: $\delta 7.62\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.05\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.25\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.30\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.63\text{ppm}$ ($\text{H}_{4,7}$ m) dppz:
$[\text{Ru}(\text{d}_8\text{-phen})_2(\text{dppz})]^{2+}$ 4d	440 (14.35)	$M_r = 379.22\text{g/mol}$ 181.12m/z (phen) 253.06m/z 380.22m/z (M^+)	$R_f = 0.71$	58%, 48 mg	phen: $\delta 7.80\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.13\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.16\text{ppm}$ ($\text{H}_{12,13}$ d) $\delta 8.50\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 9.67\text{ppm}$ ($\text{H}_{4,7}$ d) dppz:
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_4\text{-dppz}]$ 4e	440 (14.17)	$M_r = 381.61\text{g/mol}$ 143.04m/z 208.04m/z 259.02m/z 381.61m/z (M)	$R_f = 0.69$	84%, 54mg	phen: $\delta 7.80\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.13\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 9.67\text{ppm}$ ($\text{H}_{4,7}$ d) dppz:
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_6\text{-dppz}]$ 4f	440 (14.12)	$M_r = 382.19\text{g/mol}$ 143.04m/z 208.04m/z 259.02m/z 383.13m/z (M^+)	$R_f = 0.81$	72%, 46 mg	phen: $\delta 8.18\text{ppm}$ ($\text{H}_{12,13}$ d) $\delta 8.49\text{ppm}$ ($\text{H}_{11,14}$ d) dppz:
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_{10}\text{-dppz}]$ 4g	440 (13.57)	$M_r = 384.91\text{g/mol}$ 253.06m/z 385.27m/z (M^+)	$R_f = 0.82$	38%, 32mg	phen: $\delta 8.18\text{ppm}$ ($\text{H}_{12,13}$ d) $\delta 8.49\text{ppm}$ ($\text{H}_{11,14}$ d) dppz:

**Table
6.8**

Compound	UV-vis λ_{nm}	Mass Spectrum	TLC (R_f)	Yield	^1H NMR (CD_3CN)
$[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ 8	203 264 382 442	$\text{Mr}=386.76\text{g/mol}$ 386.11m/z (M^+)	0.79	39%, 31mg	phen: $\delta 7.67\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.04\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.24\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.30\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.64\text{ppm}$ ($\text{H}_{4,7}$ m) diMedppz: $\delta 7.79\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.11\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.49\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 9.64\text{ppm}$ ($\text{H}_{4,7}$ d) $\delta 2.69\text{ppm}$ (CH_3 s)
$[\text{Ru}(\text{phen})_2(\text{d}_2\text{-diMedppz})]^{2+}$ 8a	203 263 383 425	$\text{Mr}= 373.91\text{g/mol}$ 387.00m/z (M^+)	0.62	95% 36mg	phen: $\delta 7.66\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.03\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.22\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.29\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.64\text{ppm}$ ($\text{H}_{4,7}$ m) $\delta 2.69\text{ppm}$ (CH_3 s) diMedppz: $\delta 7.78\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.10\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 9.64\text{ppm}$ ($\text{H}_{4,7}$ d)
$[\text{Ru}(\text{phen})_2\text{d}_6\text{-dimedppz}]^{2+}$ 8b	204 222 263 383 442	$\text{Mr}= 388.87\text{g/mol}$ 379.20m/z (M^+)	0.61	48% 32 mg	phen: $\delta 7.67\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.05\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.24\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.29\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.65\text{ppm}$ ($\text{H}_{4,7}$ m) $\delta 2.69\text{ppm}$ (CH_3 s) diMedppz: $\delta 8.24\text{ppm}$ ($\text{H}_{11,14}$ d)
$[\text{Ru}(\text{phen})_2\text{d}_8\text{-diMedppz}]^{2+}$ 8c	221 263 383 444	$\text{Mr}= 389.91\text{g/mol}$ 390.20m/z (M)	0.71	48%, 32 mg	phen: $\delta 7.62\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.05\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.25\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.30\text{ppm}$ ($\text{H}_{5,6}$ s) $\delta 8.63\text{ppm}$ ($\text{H}_{4,7}$ m) diMedppz:
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{diMedppz}]^{2+}$ 8d	204 222 263 383 442	$\text{Mr}= 393.76\text{g/mol}$ 253.03m/z 394.12m/z (M^+)	0.71	58%, 48 mg	phen: $\delta 8.13\text{ppm}$ ($\text{H}_{2,9}$ d) $\delta 8.50\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 9.67\text{ppm}$ ($\text{H}_{4,7}$ d) $\delta 2.69\text{ppm}$ (CH_3 s) dppz: $\delta 7.80\text{ppm}$ ($\text{H}_{3,8}$ m)
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_6\text{-diMedppz}]^{2+}$ 8e	262 284 382 443	$\text{Mr} = 396.91\text{g/mol}$ 397.03m/z (M)	0.34	>100%, 27mg	phen; diMedppz: $\delta 8.50\text{ppm}$ ($\text{H}_{11,14}$ d) $\delta 2.69\text{ppm}$ (CH_3 s)
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_2\text{-diMedppz}]^{2+}$ 8f	220 262 383 442	$\text{Mr} = 394.76\text{g/mol}$ 395.26m/z (M^+)	0.74	45%, 17 mg	phen; diMedppz: $\delta 7.78\text{ppm}$ ($\text{H}_{3,8}$ m) $\delta 8.10\text{ppm}$ ($\text{H}_{2,9}$ dd) $\delta 9.63\text{ppm}$ ($\text{H}_{4,7}$ dd) $\delta 2.68\text{ppm}$ (CH_3 s)
$[\text{Ru}(\text{d}_8\text{-phen})_2\text{d}_8\text{-diMedppz}]^{2+}$ 8g	220 382 262 432	$\text{Mr} = 397.75\text{g/mol}$ 398.24m/z (M^+)	0.80	27%, 18mg	phen; diMedppz:

**Table
6.9**

Compound	UV-vis	Mass Spectrum (MeCN)	TLC (R _f)	Yield (% , mg)	¹ H NMR (CD ₃ CN)
[Ru(phen) ₂ dpq] ²⁺ 7	222 254 423 446	Mr = 346.87g/mol 191.05m/z 233.17m/z (dpq) 347.30m/z (M ⁺ -PF ₆ ²⁻)	0.66	87%,44mg	phen: δ7.65ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.14ppm (H _{2,9} dd) δ8.29ppm (H _{6,6} s) δ8.63ppm (H _{4,7} d) dpq: δ7.79ppm (H _{3,8} m) δ8.14ppm (H _{2,9} d) δ9.25ppm (H _{11,12} s) δ9.54ppm (H _{4,7} d)
[Ru(phen) ₂ d ₆ -dpq] ²⁺ 7b	222 262 422 447	Mr = 349.76g/mol 338.47m/z 350.21m/z (M ⁺) 360.46m/z	0.75	93%,34mg	phen: δ7.65ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.14ppm (H _{2,9} dd) δ8.29ppm (H _{6,6} s) δ8.63ppm (H _{4,7} d) dpq: δ9.25ppm (H _{11,12} s)
[Ru(phen) ₂ d ₈ -dpq] ²⁺ 7c	204 222 262 422 447	Mr = 350.86g/mol 351.21m/z (M ⁺) 847.46m/z (M ⁺ +PF ₆ ²⁻)	0.70	93%,17mg	phen: δ7.64ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.15ppm (H _{2,9} dd) δ8.28ppm (H _{6,6} s) δ8.63ppm (H _{4,7} d) dpq:
[Ru(d ₈ -phen) ₂ dpq] ²⁺ 7d	221 262 423 446 448	Mr=354.88g/mol 354.51m/z (M)	0.36	77%,71mg	phen; dpq: δ7.79ppm (H _{3,8} m) δ8.14ppm (H _{2,9} d) δ9.25ppm (H _{11,12} s) δ9.54ppm (H _{4,7} d)
[Ru(d ₈ -phen) ₂ d ₆ -dpq] ²⁺ 7f	222 262 424 447	Mr=357.87g/mol 358m/z (M ⁺) 860.86m/z (M ⁺ +PF ₆ ²⁻)	0.65	29%,13mg	phen; dpq: δ9.25ppm (H _{11,12} s)
[Ru(d ₈ -phen) ₂ d ₈ -dpq] ²⁺ 7g	217 234 262 424 447	Mr=354.87g/mol m/z (M ⁺)	0.63	29%,17mg	phen; dpq:

**Table
6.9**

Compound	UV-vis	Mass Spectrum (MeCN)	TLC (R _f)	Yield (% , mg)	¹ H NMR (CD ₃ CN)
[Ru(phen) ₂ dpq] ²⁺ 7	222 254 423 446	Mr = 346.87g/mol 191.05m/z 233.17m/z (dpq) 347.30m/z (M ⁺ -PF ₆ ²⁻)	0.66	87%,44mg	phen: δ7.65ppm (H _{3,8} m) dpq: δ7.79ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.14ppm (H _{2,9} d) δ8.14ppm (H _{2,9} dd) δ8.29ppm (H _{5,6} s) δ9.25ppm (H _{11,12} s) δ8.63ppm (H _{4,7} d) δ9.54ppm (H _{4,7} d)
[Ru(phen) ₂ d ₆ -dpq] ²⁺ 7b	222 262 422 447	Mr = 349.76g/mol 338.47m/z 350.21m/z (M ⁺) 360.46m/z	0.75	93%,34mg	phen: δ7.65ppm (H _{3,8} m) dpq: δ8.04ppm (H _{2,9} dd) δ8.14ppm (H _{2,9} dd) δ8.29ppm (H _{5,6} s) δ9.25ppm (H _{11,12} s) δ8.63ppm (H _{4,7} d)
[Ru(phen) ₂ d ₈ -dpq] ²⁺ 7c	204 222 262 422 447	Mr = 350.86g/mol 351.21m/z (M ⁺) 847.46m/z (M ⁺ +PF ₆ ²⁻)	0.70	93%,17mg	phen: δ7.64ppm (H _{3,8} m) dpq: δ8.04ppm (H _{2,9} dd) δ8.15ppm (H _{2,9} dd) δ8.28ppm (H _{5,6} s) δ8.63ppm (H _{4,7} d)
[Ru(d ₈ -phen) ₂ dpq] ²⁺ 7d	221 262 423 446 448	Mr=354.88g/mol 354.51m/z (M)	0.36	77%,71mg	phen: dpq: δ7.79ppm (H _{3,8} m) δ8.14ppm (H _{2,9} d) δ9.25ppm (H _{11,12} s) δ9.54ppm (H _{4,7} d)
[Ru(d ₈ -phen) ₂ d ₆ -dpq] ²⁺ 7f	222 262 424 447	Mr=357.87g/mol 358m/z (M ⁺) 860.86m/z (M ⁺ +PF ₆ ²⁻)	0.65	29%,13mg	phen: dpq: δ9.25ppm (H _{11,12} s)
[Ru(d ₈ -phen) ₂ d ₈ -dpq] ²⁺ 7g	217 234 262 424 447	Mr=354.87g/mol m/z (M ⁺)	0.63	29%,17mg	phen: dpq:

**Table
6.10**

Compound	UV-vis	Mass Spectrum	TLC	Yield	¹ H NMR (CD ₃ CN)
[Ru(phen)diFdppz)] ²⁺ 9	204 264 372 (9.7) 440 (8.5)	Mr = 389.91g/mol 390.36m/z (M ⁺) 398.92m/z	R _f = 0.73	24%, 15mg	phen: δ7.65ppm (H _{3,8} m) δ8.04ppm (H _{2,9} d) δ8.24ppm (H _{2,9} d) δ8.29ppm (H _{5,6} s) δ8.62ppm (H _{4,7} m) diFdppz: δ7.79ppm (H _{3,8} m) δ8.13ppm (H _{2,9} d) δ8.34ppm (H _{11,14} t) δ9.62ppm (H _{4,7} d)
[Ru(phen) ₂ d ₂ -diFdppz)] ²⁺ 9a	225 234 276 372 442	Mr = 390.70g/mol 259.12m/z 335.15m/z 391.10m/z (M ⁺)	R _f = 0.80	59%, 19mg	phen: δ7.65ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.22ppm (H _{2,9} dd) δ8.30ppm (H _{5,6} s) δ8.65ppm (H _{4,7} m) diFdppz: δ7.80ppm (H _{3,8} d) δ8.34ppm (H _{11,12} t) δ9.62ppm (H _{4,7} d)
[Ru(phen) ₂ d ₆ -diFdppz)] ²⁺ 9b	203 264 372 (1.9) 439 (1.5)	Mr = 392.88g/mol 209.12m/z 223.10m/z 393.24m/z (M ⁺)	R _f = 0.32	70%, 21mg	phen: δ7.67ppm (H _{3,8} m) δ8.04ppm (H _{2,9} dd) δ8.22ppm (H _{2,9} dd) δ8.29ppm (H _{5,6} s) δ8.64ppm (H _{4,7} m) diFdppz: δ8.33ppm (H _{11,14} t)
[Ru(d ₈ -phen) ₂ diFdppz)] ²⁺ 9d	203 265 371 (11.3) 439 (8.6)	Mr = 397.98g/mol 398.32m/z	R _f = 0.75	58%, 50mg	phen: diFdppz: δ7.80ppm (H _{3,8} m) δ8.14ppm (H _{2,9} dd) δ8.33ppm (H _{11,14} t) δ8.62ppm (H _{4,7} dd)
[Ru(d ₈ -phen) ₂ d ₆ -diFdppz)] ²⁺ 9f	226 260 273 371 (9.6) 439 (7.4)	Mr = 400.91g/mol 401.32m/z (M ⁺)	R _f = 0.68	64%, 50mg	phen: diFdppz: δ8.39ppm (H _{11,14} t)

6.8 References

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Chapter Seven - Possible Future Work

The aim of the present study was to examine the photophysical properties of Ru(II) complexes, of the form $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4**, both in the absence and in the presence of DNA. Three main research areas have been detailed below along with some future possible work.

Firstly, the effect of protium-deuterium exchange on the excited state energies of complex **4** and its deuterated derivatives were investigated. It was postulated from this study that the excited state electron is located on the *phen* portion of the *dppz* ligand. This result was confirmed on examination of $[\text{Ru}(\text{phen})_2\text{diMedppz}]^{2+}$ **8** complex and its deuterated analogues. The study was also extended to include the complex $[\text{Ru}(\text{phen})_2\text{diFdppz}]^{2+}$ **9**, however, we were unsuccessful in preparing the complete series of deuterated complexes. Therefore, a protium-deuterium exchange route for the perdeuteration of the fluorinated ligands; diFdab and diFdppz, would permit the completion of this series of complexes. Thus allowing a more detailed study of the effect of deuterium on the location of the electron density in the excited state and on the nonradiative (k_{nr}) decay processes.

Secondly, a systematic temperature dependent study of the effect of progressive ligand deuteration into $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** and $[\text{Ru}(\text{phen})_3]^{2+}$ **6**, was performed in degassed aqueous solutions in the range 4°C to 44°C. As expected the value of the emission lifetime was found to decrease on moving to higher temperatures, while an increase was noted on increasing the extent of deuteration in these complexes. However, although uniform changes were noted for the bpy series (**1-1c**), these changes were found to vary for the corresponding phen series (**6-6c**). A more extensive study is needed which includes (i) a range of solvents (*e.g.* acetonitrile, methanol and ethanol) and (ii) related complexes such as $[\text{Ru}(\text{tap})_3]^{2+}$, $[\text{Ru}(\text{dpq})_3]^{2+}$ and $[\text{Ru}(\text{dppz})_3]^{2+}$ respectively. Subsequently, to the best of our knowledge, temperature dependent studies for the parent complex **4**, have not been previously studied and may provide useful information about the $^3\text{MLCT}$ and higher lying ^3MC and/or $^3\text{MLCT}$ (**4**) excited states.

Finally, the interactions of the enantiomers of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ **4** with CT-DNA were investigated. The introduction of deuterium into these systems provided a number of interesting and promising results. In contrast to the protiated parent complex there are no apparent difference in the emission lifetimes for the Δ - and Λ -enantiomers and the intensity of the emission is similar for the deuterated complexes; $[\text{Ru}(\text{phen})_2\text{d}_4\text{-dppz}]^{2+}$ **4a** and $[\text{Ru}(\text{d}_8\text{-phen})_2\text{dppz}]^{2+}$ **4d** respectively. The resolution of the entire dppz family of complexes, **4-4g** inclusive, may provide an explanation for the unusual behaviour exhibited for the Δ - and Λ -enantiomers of **4a** and **4d**, in the presence of DNA. Furthermore, this study could be extended to include alternative ligands into these complexes. The $[\text{Ru}(\text{phen})_2\text{dpq}]^{2+}$ series of complexes have been prepared (as detailed in Chapter Six), and already preliminary steady-state studies have been performed in our laboratories in the absence and presence of DNA.

Appendix

In order to study the photophysics of deoxygenated water, the variation of the temperature of lifetime (as detailed in Ch.

the complexes; [Ru(phen)₃]²⁺ and [Ru(bpy)₃]²⁺ lifetimes were measured as a function of the extent of deuteration of the solvent. The variation of τ can be described by the equation

which contains a temperature independent (a) term and a temperature dependent term ($k'' \exp(\Delta E/RT)$). The activation energy ΔE is the radiative and non-radiative energy gap between the ¹MC state and the ³MC state. The temperature dependent term(s) correspond to the thermal energy required for crossing to the ³MC state from the ¹MC state, or to the ³MLCT state.

However, for complexes monitored did not give ΔE difficult. As a result, Mike Lyons to plot $\ln \tau$ vs $1/T$ plot replicates the Arrhenius values for the activation energy for these Ru(II) complexes include: (i) a plot

plots of $\ln \tau$ vs $1/T$ for the complexes (1-4e) were making the evaluation of ΔE difficult. In a discussion on this topic, a plot of $\ln \tau$ vs $1/T$ for complexes (1-4e) and the complexes with higher ΔE term and a rapid to give experimental energy gap between the ¹MLCT and ³MC states. The activation plots are shown in Figure 1, and also include the Arrhenius plots of $\ln \tau$ vs $1/T$.

For the sake of comparison between the complexes with the increased temperature dependence. The plots of $\ln \tau$ vs $1/T$ for complexes (1-4e) are shown in Figure 1(A). The plots with good correlation coefficient and slope, the activation energy were calculated as 1867 cm^{-1} (a), 1074 cm^{-1} (b), 1074 cm^{-1} (c), and 1074 cm^{-1} (d), respectively.

Appendix

In order to study the photophysics of the complexes; $[\text{Ru}(\text{phen})_3]^{2+}$ **6** and $[\text{Ru}(\text{bpy})_3]^{2+}$ **1** in deoxygenated water, the excited state lifetimes were examined as a function of the variation of the temperature and the extent of deuteration of the complex. The variation of lifetime (as detailed in Chapter Four) can be described by the equation

which contains a temperature independent (k) term and a temperature dependent term ($k^0 \exp(\Delta E/RT)$). The former temperature independent term is assumed to be equal to the radiative and nonradiative ($k_r + k_{nr}$) transitions associated with the average $^3\text{MLCT}$ state. The temperature dependent term(s) correspond to the thermally activated crossing to the ^3MC and/or to a fourth $^3\text{MLCT}$ state.

However, for complexes **1** and **6**, plots of $1/\tau$ -vs- $1/T$ for the range of temperatures monitored did not give straight line plots making the evaluation of parameters k , k^0 and ΔE difficult. As a result of further discussion on this topic, it was suggested by Dr. Mike Lyons to plot $\ln 1/\tau$ -vs- $1/T$ for complexes (**1-1c**) and (**6-6c**) respectively. Such a plot neglects the temperature independent (k) term and is hoped to give appropriate values for the activation energy ΔE (energy gap between the $^3\text{MLCT}$ and ^3MC states) for these Ru(II) complexes. These Arrhenius plots are shown in Figure 1, and also include; (i) a fitted line ($y = mx + c$) and, (ii) the correlation coefficient (R^2).

For the sake of clarity, the complexes will be discussed individually before a comparison between the two is made. The phen series of complexes (**6-6c**), displayed in Figure 1(A), display straight line plots with good correlation coefficients. From the slope, the activation energies were calculated as 1867 cm^{-1} (**6**), 1895 cm^{-1} (**6a**), 1911 cm^{-1} (**6b**), and 1834 cm^{-1} (**6c**), respectively.

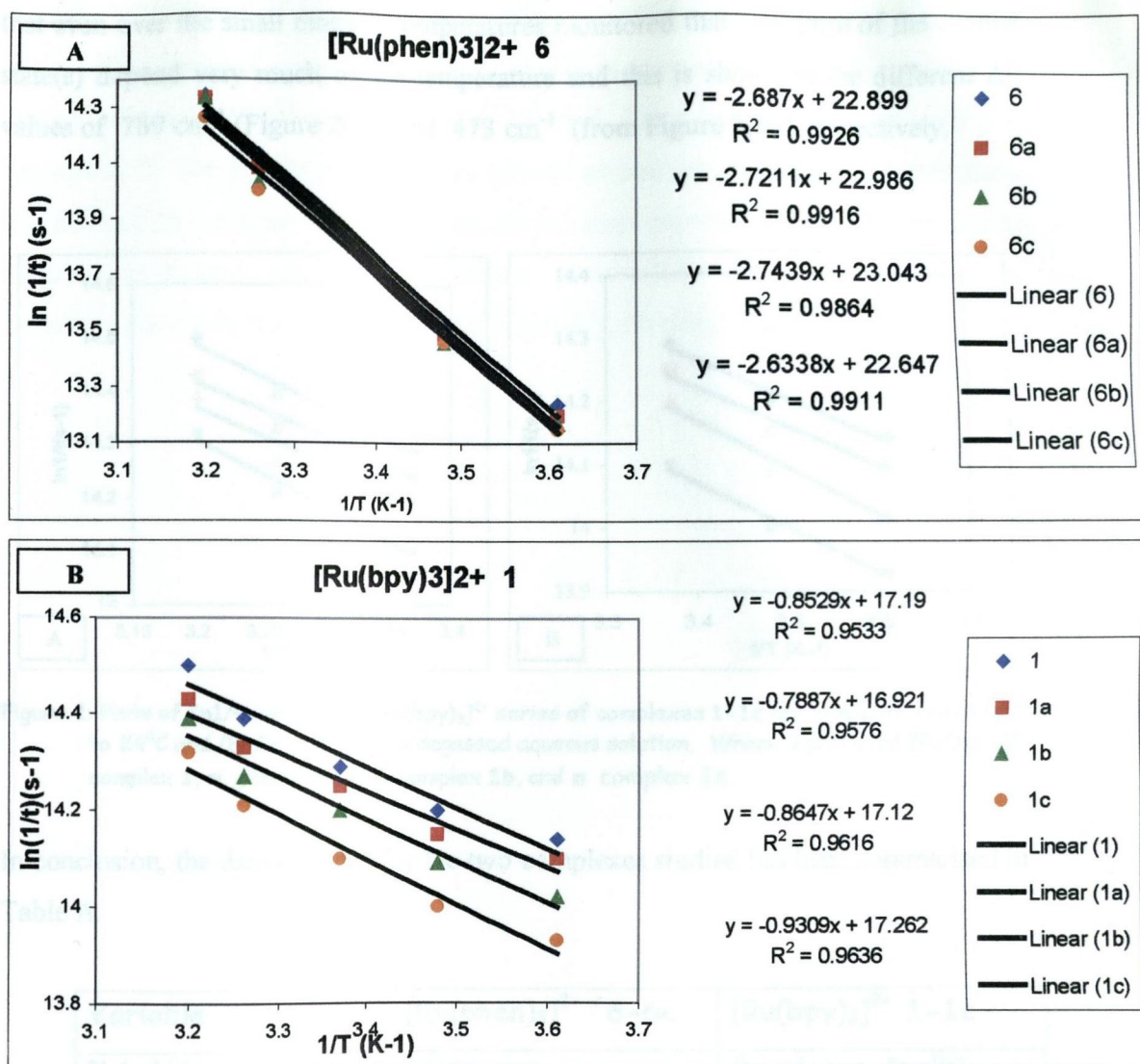


Figure 1: Plots of $\ln 1/\tau$ as a function of $1/T$ for (A) $[\text{Ru}(\text{phen})_3]^{2+}$ 6 and its deuterated analogues 6a-6c (B) $[\text{Ru}(\text{bpy})_3]^{2+}$ 1 and its deuterated complexes 1a-1c in degassed aqueous solutions

For the corresponding bpy series of complexes (1-1c), the plots of $\ln 1/\tau$ as a function of $1/T$ are displayed in Figure 1(B) and are not as clear cut as the plots appear to be curved lines. This may account for the smaller correlation coefficients obtained for bpy, with an average value of R^2 , $R^2_{av} = 0.960$ compared to 0.990 for the phen complexes. Furthermore, an activation energy ΔE of 594 cm^{-1} was obtained for complex 1, with values of 549 cm^{-1} (1a), 603 cm^{-1} (1b) and 648 cm^{-1} (1c), respectively. The data for complexes 1-1c was also fit for the range of temperatures from 4°C to 24°C and for the temperatures from 24°C to 44°C , as illustrated in Figure 2. It is evident from the plots

that even over the small range of temperatures monitored that the nature of the excited state(s) depend very much on the temperature and this is shown by the different ΔE values of 789 cm^{-1} (Figure 2(A)) and 473 cm^{-1} (from Figure 2(B)), respectively.

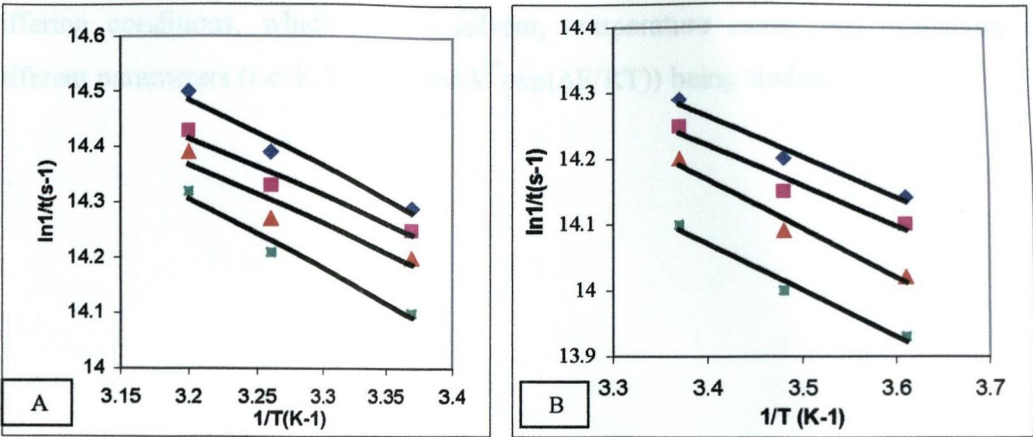


Figure 2: Plots of $\ln 1/\tau$ -vs- $1/T$ for $[\text{Ru}(\text{bpy})_3]^{2+}$ series of complexes 1-1c for temperatures (A) 4°C to 24°C and (B) 24°C to 44°C in degassed aqueous solution. Where $\diamond$ protiated $[\text{Ru}(\text{bpy})_3]^{2+}$ complex 1, $\blacksquare$ complex 1a, $\blacktriangle$ complex 1b, and $\blacksquare$ complex 1c.

In conclusion, the data obtained for the two complexes studied has been summarised in Table A.

Variable	$[\text{Ru}(\text{phen})_3]^{2+}$ 6-6c	$[\text{Ru}(\text{bpy})_3]^{2+}$ 1-1c
Plot of $1/\tau$ -vs- $1/T$	Curved Lines-	Curved Lines - Parallel
Plot of $\ln 1/\tau$ -vs- $1/T$	Straight line plots	Curved line plots
R^2 (Correlation Coeff.)	$R^2_{av} = 0.990$ Better regression for phen complexes	$R^2_{av} = 0.960$
ΔE (cm^{-1})	6 1867 6a 1895 6b 1911 6c 1834	1 594 1a 549 1b 603 1c 648

Table A : A comparison of the data obtained from the plots for series of complexes $[\text{Ru}(\text{phen})_3]^{2+}$ 6-6c and $[\text{Ru}(\text{bpy})_3]^{2+}$ 1-1c.

It was observed that straight line plots with better correlation coefficients and larger ΔE values were obtained for the phen series of complexes which may account for the larger

emission lifetime values for the protiated phen complex **6** and its deuterated analogues **6a-6c** under degassed aqueous conditions. However, it must be noted that these results and observations are only approximations. Indeed, it has been shown previously that parameters k^0 and ΔE display significant covariance and are not reliable. Furthermore, it is difficult to make any direct comparisons to values reported in the literature, due to differing conditions, which include solvent, temperature range, and evaluation of different parameters (*i.e.* k , k^0 , ΔE , and $k^0 \exp(\Delta E/RT)$) being studied.