

Spectroscopic and Crystallographic Evaluation of Ruthenium(II) Complexes Bearing Phenanthroline-Naphthalimide Ligands

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

Declaration

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Abstract

This Thesis predominantly focusses on the design, synthesis, and characterisation of novel, luminescent, Ru(II)-polypyridyl complexes bearing 1,8-naphthalimide (**Nap**) ligands, for use as DNA probes. In addition, a large X-ray crystallography sub-theme underpins this Thesis, where throughout the Chapters significant aspects are devoted to crystallographic studies of ligands and complexes and, where appropriate, relating these studies to broader themes such as their photophysical and biological properties.

Chapter 1 provides a broad overview of anti-cancer therapies including chemotherapeutics, photodynamic therapy (PDT), and, in particular, DNA targeting agents, before extensively exploring the photophysics underpinning ruthenium(II) polypyridyl complexes and the use of such complexes in a broad array of biomedical applications; particular emphasis here is placed on the interactions of these complexes with DNA. Following this, **Naps** are introduced and their photophysical and anti-cancer properties are described, before concluding the Chapter by broadening out the discussion to cover examples of photophysical and biological studies of metal-**Nap** conjugates.

Chapter 2 explores the synthesis and structural characterisation of two novel Ru(II)-polypyridyl complexes, bearing either two 1,10-phenanthroline ligands (**phen**) or two 1,4,5,8-tetraazaphenanthrene (**TAP**) ligands. Their coordination spheres are completed by *N*-(1,10-phenanthroline)-naphthalene-1,8-dicarboximide (**PhenNap**), **80**, giving the complexes [Ru(**phen**)₂(**PhenNap**)]²⁺ (**75**) and [Ru(**TAP**)₂(**PhenNap**)]²⁺ (**76**). A detailed crystallographic study is undertaken which includes the structurally similar **bpy** complex, [Ru(**bpy**)₂(**PhenNap**)]²⁺ (**89**), where **bpy** = 2,2'-bipyridine, demonstrating the role of the **PhenNap** in directing the crystallographic packing of the three complexes. The photophysical (UV-visible absorption spectroscopy; steady-state and time-resolved emission spectroscopy) and electrochemical (cyclic voltammetry) properties are described, followed by extensive DNA binding studies (monitored *via* UV-visible absorption, emission, linear dichroism, and circular dichroism spectroscopies, and thermal denaturation experiments) to determine the effect of **Nap** conjugation on the DNA binding properties of complexes **75** and **76**.

Chapter 3 develops the chemistry of the **PhenNaps**, incorporating different functional groups on the 4-position of the **Nap** (nitro, **91**, bromo, **92**, and chloro, **93**), and complexing them to generate a new family of Ru(II)-polypyridyl complexes (**94-96**). Additional efforts toward the synthesis of the

amino substituted **PhenNap** ligand (97) and the corresponding complex (99) are described. Extensive crystallographic studies, as well as theoretical calculations, electrochemical and photophysical measurements on the free ligands and on the complexes reveal the effects of substitution of these ligands. Analogous DNA binding studies to those described in Chapter 2 are also carried out.

Chapter 4 broadens out the chemistry of the **Nap**-based ligands, discussing attempts to generate **Nap** ligands with extended conjugation (106-108) or ditopic ligands capable of coordinating two metal centres (98 and 109), as well as detailing efforts to coordinate ligand 98 to Ru(II). The chapter concludes by exploring some Ir(III)-based chemistry analogous to that discussed for the Ru(II)-polypyridyl complexes in previous Chapters.

Chapter 5 builds on the crystallographic sub-themes of previous Chapters by discussing two dedicated crystallographic studies. The first study involves a large family of **Nap** molecules (C1-C8) bearing different substitutions on the imide *N*-terminus and/or the 4-position of the **Nap**. Particular care is given to highlighting trends in packing arrangements. The second study details efforts undertaken to resolve the structures of two enantiomers of a dinuclear, triple-stranded Eu(III)-based helicate (L1₃.Eu₂⁶⁺, C9, *S,S*, and L2₃.Eu₂⁶⁺, C10, *R,R*). The role of the counterions (CF₃SO₃⁻ and ClO₄⁻) in crystallisation is discussed, followed by an in-depth analysis of the molecular structure and extended packing of the *S,S* helicate C9.

Chapter 6 concludes the Thesis, and provides a summary of the overall conclusions for each of the Chapters contained in this body of research, with specific reference to the potential of Ru(II)-**Nap** conjugates as photoreactive nucleic acid targeting agents.

Lastly, Chapter 7 summarises the experimental methods and techniques employed in this thesis, as well as detailed synthetic protocols and structural characterisation of the molecules synthesised as part of this thesis. An additional electronic document containing relevant experimental and crystallographic data is also included.

Abbreviations

A	adenine or hydrogen bond acceptor
AIE	aggregation induced emission
APCI	atmospheric pressure chemical ionization
app	apparent
aq.	aqueous
atm	atmospheric pressure
BET	back electron transfer
Boc	tert-butyloxycarbonyl
bp	base pairs
bpy	bipyridine
C	cytosine
<i>ca.</i>	circa
calc.	calculated
CCC	covalently closed circular [DNA]
CDT	chemodynamic therapy
COD	cyclooctadiene
COSY	correlation spectroscopy
CPL	circularly polarised luminescence
CT	charge transfer
CV	cyclic voltammetry
d	doublet
D	hydrogen bond donor or electron donor
DCM	dichloromethane
dd	double doublet
decomp.	decomposition
DFT	density functional theory
DIP	direct insertion probe
DMF	dimethylformamide
DNA	deoxyribonucleic acid
DNP	dinitrophenylhydrazine
DPE	dipicolinic acid
dppz	[2,3-h]dipyrido[3,2-a:2',3'-c]phenazine
dt	doublet of triplets

EDTA	ethylenediaminetetraacetic acid
EGEE	2-ethoxyethanol
ESI	electrospray ionisation
EtOAc	ethyl acetate
eq.	equivalent(s)
ESI	electrospray ionisation
ET	electron transfer
Fc/Fc ⁺	ferrocene/ferrocenium
FMO	frontier molecular orbital
FRET	Förster resonance energy transfer
frag.	fragmentation
G	guanine
GPR	general purpose grade
GS	ground state
GMP	guanosine-5'-monophosphate
G4	guanosine quadruplex
h	hour
HAT	histone acetyltransferase or 1,4,5,8,9,12-hexaazatriphenylene
HDAC	histone deacetylase
HeLa	cervical cancer cell line
HMBC	heteronuclear multiple bond correlation
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence spectroscopy
HSV	herpes simplex virus
IC	internal conversion
IC ₅₀	half maximal inhibitory concentration
ICT	intramolecular charge transfer
IL	intraligand
IPA	isopropyl alcohol
IR	infrared
ISC	intersystem crossing
L	ligand
LED	light emitting diode

LC	ligand centred
LMCT	ligand-to-metal charge transfer
LUMO	lowest unoccupied molecular orbital
m	multiplet
MC	metal centred
min	minute
MLCT	metal-to-ligand charge transfer
m.p.	melting point
MS	Mass Spectrometry
m/z	mass-to-charge ratio
NACs	nitroaromatic compounds
Nap	1,8-naphthalimide
NBI	1,8-naphthalenebenzimidazole
NDI	naphthalene diimide
NMR	nuclear magnetic resonance spectroscopy
NTR	nitroreductase
OC	open circular [DNA]
PA	picric acid
PB	phosphate buffer solution
PCET	proton coupled electron transfer
PES	polyethersulfone
PET	photoinduced electron transfer
PDT	photodynamic therapy
phen	1,10-phenanthroline
PI	photocytotoxicity index
PL	photoluminescence
ppb	parts per billion
ppm	parts per million
q	quaternary
red.	reduction
ROS	reactive oxygen species
rpm	revolutions per minute
s	singlet
S	singlet state
SAR	structure activity relationship

sat.	saturated
SCE	saturated calomel electrode
SDT	sonodynamic therapy
SOC	spin-orbit coupling
SPS	solvent purification system
t	triplet
T	thymine or triplet state
TA	transient absorption
TAP	1,4,5,8-tetraazaphenanthrene
TB	Tröger's base
TCSPC	time correlated single photon counting
TEM	transmission electron microscopy
TFA	trifluoroacetic acid
TLC	thin layer chromatography
TME	tumour microenvironment
TOP2	topoisomerase II
TPA	triphenylamine
tpy	terpyridine
UV	ultraviolet
vis	visible

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Chapter 1:

Introduction

1.1: Introduction

Since their discovery in 1959, heavy luminescent d^6 transition metal complexes comprised of Ru(II), Rh(III), Re(I), Os(II), Ir(III), or Pt(IV) ions have been investigated for applications across many different areas of science ranging in optoelectronics, therapeutics and imaging.¹⁻⁴ Their diverse range of applications are attributed to their readily tuneable photophysical properties. Examples of d^6 transition metal complexes include $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Ir}(\text{ppy})_3]$ (where **bpy** = 2,2'-bipyridine and **ppy** = 2-phenylpyridine) which display deep red and green luminescence, respectively. While d^6 transition metal complexes may vary chromatically, they share many attractive photophysical features such as long-lived luminescent excited state lifetimes due to efficient spin-orbit coupling (SOC). Such properties can be readily tuned by careful selection and design of the coordinating ligands. Among these complexes, the family that has gained probably the most attention in the field of medicinal chemistry is the Ru(II) polypyridyl complexes, based in large part on the work by Barton, Kelly and others in the late 1980s.⁵⁻¹¹ This has been driven by a combination of structural factors that characterise these complexes: the octahedral geometry about the metal centre and (often extended) aromaticity of the surrounding ligand frameworks allow for complex interactions with large biomolecules, and this can be enhanced particularly with DNA, where the doubly charged, cationic complexes interact *via* favourable electrostatic interactions with anionic DNA.

Separately, the 1,8-naphthalimide (**Nap**) is an organic, planar, aromatic moiety that has also been studied extensively within many research groups, including our research group, because of their wide applicability in many different applications. Their inherent luminescence, which can be greatly enhanced and tuned upon introducing electron donating groups such as amines into the 3- and/or 4-positions of these systems, renders them highly attractive for applications such as sensing or organic devices, while their planarity and ease of functionalisation has made them popular candidates for therapeutic purposes, particularly for treating cancer.¹²⁻²²

This PhD thesis focuses on the development of spectroscopically active Ru(II)-polypyridyl-**Nap** conjugates as DNA targeting agents and luminescent probes with potential anticancer activity. In the current Chapter the aim is to introduce literature relevant to the scope of the thesis. First, an introduction to cancer and current cancer therapies is provided, followed by a discussion of the structure of DNA and the major types of DNA-drug binding. The medicinal and therapeutic applications of both the **Nap** motif and Ru(II)-polypyridyl complexes is then provided followed by details of the work carried out in this thesis.

1.2: Anticancer Therapies – An Overview

Unfortunately, cancer as a group of diseases needs little introduction and has been common throughout history, with the first reports recorded in ancient scripts.²³ Cancer was the leading cause of death in 2020, accounting for almost ten million deaths globally. In Ireland, skin cancer is the most common type of cancer with over twelve thousand cases diagnosed annually.²⁴ Cancer cells bypass internal regulatory checks and are categorised by uncontrolled growth, often resulting in a mass or solid tumour. This mass can impact a patient directly by suppressing or damaging surrounding organs and tissues or indirectly, by spreading to other parts of the body through the lymphatic system, blood stream, or by direct extension of the mass.²⁴ The treatment of cancer is a highly complex process as each type of cancer is unique. Conventional cancer therapy employs surgical procedures, radiotherapy and chemotherapy as single treatments or in combination.²⁵ Radiotherapy and surgery are examples of targeted and invasive procedures, while chemotherapy is systemic, non-selective, and can produce adverse side-effects such as the destruction of non-cancerous cells and the development of drug resistance.²³⁻²⁶ Chemotherapy utilises the high proliferation rate of cancer cells and employs drugs which interact with DNA to produce a genotoxic effect on the cells. Chemotherapeutic drugs may be cytostatic or cytotoxic and are further classified based on their interaction with DNA. Examples of chemotherapy drugs used in clinical applications are alkylating agents (cyclophosphamide (Cytosan®), **1**), topoisomerase inhibitors (Topotecan, **2**), anthracyclines (Doxorubicin (Adriamycin®), **3**) and antimetabolites (5-Fluorouracil (5-FU), **4**), among many others [Figure 1.1]. Further details regarding DNA-drug interactions are provided (*vide infra*, Section 1.3.2).²⁶

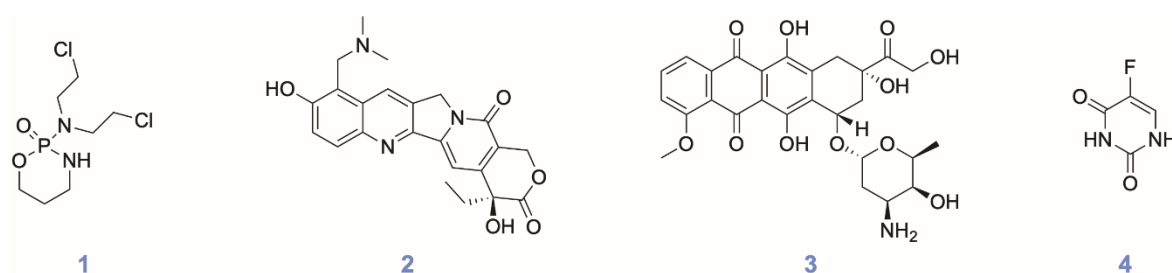


Figure 1.1: Chemical structures of chemotherapeutic drugs **1–4** used in clinical applications.

In recent years, advancements have been made toward more innovative cancer therapy with an aim to develop targeted treatments with less side-effects.²⁷ The fields of stem cell therapy, photodynamic therapy (PDT), sonodynamic therapy (SDT), and chemodynamic therapy (CDT) are among some that have received great attention in recent research.²⁷⁻²⁹ PDT and SDT employ light and ultrasound, respectively, as external stimuli to generate cytotoxicity within cancer cells. PDT and SDT use a sensitising drug, which upon excitation by the external stimuli, transfer energy to

molecular oxygen generating reactive oxygen species (ROS) (radicals, singlet oxygen and peroxides), which in turn, achieve cytotoxicity through subsequent oxidation of cellular components. These two therapies hold distinct advantages over conventional cancer therapies as they are minimally invasive and more selective. The advantage of SDT over PDT is the increased penetration of ultrasound compared to light, through mammalian tissue, thus allowing it to reach a wider range of areas.^{30,31} CDT considers the tumour microenvironment (TME) for cancer therapy and employs chemodynamic agents, typically Fenton agents, to generate cell death. The TME includes hypoxia and the overexpression of H_2O_2 which upon reaction with the chemodynamic agent, generates ROS (mainly hydroxyl radicals) which induce cell death.^{27,28} Many of the sensitisers detailed above contain transition metal ion complexes and corresponding nanomaterials to achieve the desired therapeutic effect. As such, a comprehensive understanding of the photophysical and photochemical processes of these metal complexes is vital before investigating the therapeutic properties of the systems.

1.3: DNA as a Molecular Target for Anticancer Drugs

DNA (deoxyribonucleic acid) is a biomolecule that regulates all the actions within a cell, including gene expression and its own replication. In living systems, structure and function are highly correlated at all levels of the biological hierarchy, perhaps most significantly at the molecular level. Examples of correlation between structure and function in biology include enzyme–substrate interactions, cell–cell recognition processes and cell replication processes. The structure of DNA is highly conserved, and its intrinsic functions can become impaired or obsolete upon minor changes to its environment. Targeting of DNA with small molecules within cells often results in a disruption to regular cellular processes which is an attractive feature for the treatment and destruction of cancer cells. All cancer cells share the same characteristic feature of excessive, uncontrolled growth and bypassing of regulatory cellular check-points.²⁴ Targeting cancer cells in a selective manner offers potential for the development of anticancer agents which do not display the same drawbacks of conventional cancer chemotherapy and is an active area of ongoing research.³² Understanding the structure and function of DNA within cells is critical to developing selective cancer treatments. As such, DNA as a molecular target and current cancer treatment processes applicable to the work carried out in this thesis will be introduced in the following sections.

1.3.1: Molecular Structure of DNA

DNA is a complex biomolecule that has many different chemical environments throughout its structure. The design of small molecules capable of selectively binding to different regions with the DNA molecule is an attractive field of research for anticancer therapies. Following observations of

X-ray crystallographic data obtained by Franklin, Wilkins and Gosling,^{33,34} Watson and Crick famously unveiled the structure of DNA in 1953 which subsequently ignited the field of research into DNA targeting and the understanding of the fundamental processes of gene expression and cell replication.^{35,36} It should be noted, however; that DNA (known at the time to be a phosphorus rich substance) was first isolated much earlier in 1869 by Swiss chemist, Friedrich Miescher. Many other scientists were responsible for gathering and analysing data pertaining to the chemical composition of DNA which led to the deciphering of its structure by Watson and Crick.³⁷ DNA exists in multiple forms throughout nature, the most common form being that of the canonical B-DNA form. Other double-stranded forms that are observed in nature include the A- and Z-DNA forms which can be isolated by varying salt and water concentrations.^{33,38}

DNA is one of the most common types of nucleic acids found in nature alongside RNA, which lacks the 2'-OH group found in DNA. DNA and RNA are polymeric structures that are made up of small repeating mononucleotides. Figure 1.2 displays a graphical representation of the B-DNA polymer and some characteristic structural features of the biomolecule.³⁹

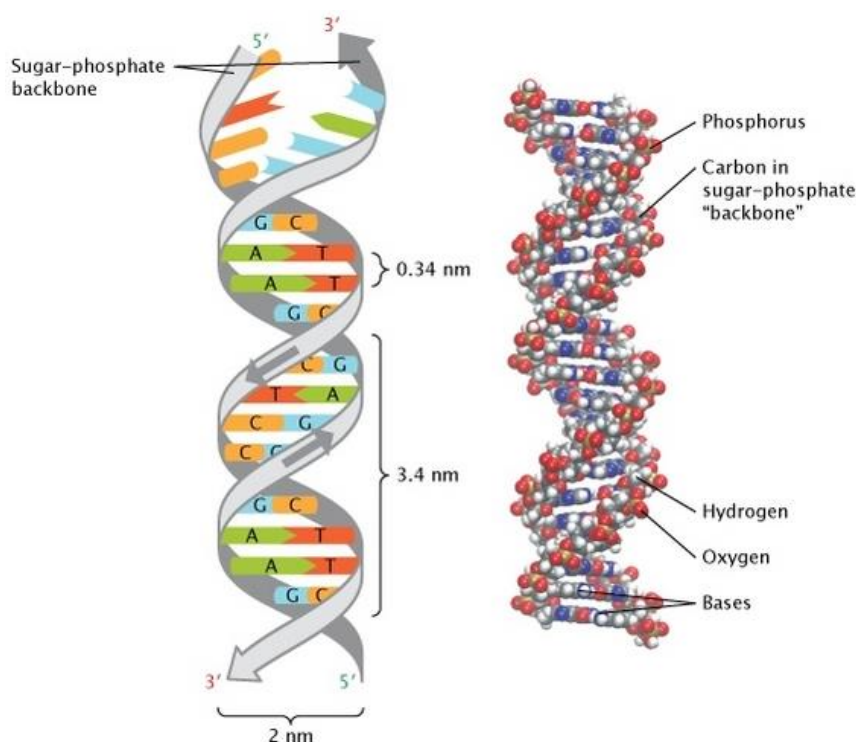


Figure 1.2: Graphical representation of the structure of DNA. A, T, G and C represent the four nucleobases found in DNA. Image from ref.³⁹

B-DNA consists of two anti-parallel strands which run in opposite directions, arranged in a right-handed, double helical structure. Each strand consists of a repeating monomeric unit known as a nucleotide which contains a phosphate group and a single sugar unit (deoxyribose), which is bonded to a nucleobase (one of adenine (A), thymine (T), cytosine (C) and guanine (G)). The sugar

and phosphate groups alternate in sequence to make up the backbone of each strand and the nucleobases protrude into the centre of the helical structure. The presence of the phosphate group yields a polyanionic backbone throughout the structure which is stabilised by water molecules and Na^+ and K^+ cations. Structurally, B-DNA is 20 Å wide in diameter and contains approximately 10 base pairs per helical turn. The bases found in nucleic acids are either pyrimidines, characterised by a single nitrogen containing six-membered ring, or purines characterised by fused five- and six-membered nitrogen containing rings. Adenine and guanine constitute the purine bases while thymine and cytosine are the pyrimidine bases. The chemical structure of the nucleobases found in DNA and hydrogen bonding interactions between complementary bases on opposite strands are shown in Figure 1.3.⁴⁰

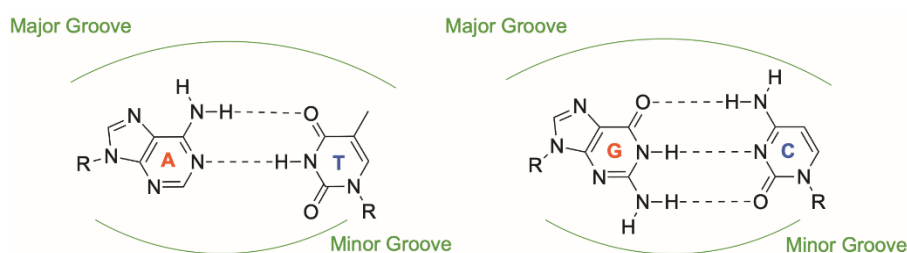


Figure 1.3: Chemical structure and hydrogen bonding interactions of purine (A and G, red) and pyrimidine (T and C, blue) bases found in DNA. R group denotes glycosidic linkage to deoxyribose moiety of DNA.

The two strands within the DNA molecule are held together by hydrogen bonds between complementary hydrogen bond donor and acceptor regions of the nucleobases as depicted above [Figure 1.3]. The complementary hydrogen bonding patterns dictate Watson-Crick base pairing in B-DNA, whereby adenine bonds with thymine and cytosine bonds with guanine *via* two and three hydrogen bonds, respectively. These pairing patterns were unveiled following previous studies conducted by Chargaff who determined the 1:1 ratio of purines bases to pyrimidine bases in DNA.^{41,42} Other types of non-conventional base pairing can occur, such as Hoogsteen base pairing which, in guanine rich sequences of DNA gives rise to G-quadruplex secondary structures. These structures are shown to play an important role in gene expression and the longevity of cells and targeting these structures is an active area of research.⁴³ The hydrogen bonding interactions between bases on opposite strands and more importantly, stacking interactions between adjacent nucleobases on the same strand embark stability upon the DNA molecule. Furthermore, due to the stacking interactions and asymmetric nature by which the nucleobase is attached to the sugar, the DNA molecule contains major and minor groove regions with discrete chemical environments. The major groove is considerably wider and chemically more complex than the minor groove. For the A-T base pair above, different functional groups are exposed on the major groove and minor groove sites. Accessing this chemical information within the grooves allows for the design of molecules

capable of binding to DNA in a selective manner and also provides insight into the protein binding interactions at these sites which direct fundamental cellular processes.⁴⁰

In summary, B-DNA is a complex yet highly conserved structure that has been the focus of academic research following its isolation over 150 years ago. B-DNA has many discrete physiochemical environments throughout its structure including, but not limited to, the polyanionic backbone, hydrophobic core, major and minor groove sites with distinct shapes, sizes and functional groups and secondary structures which can be accessed. Equipped with this knowledge, many molecules have been designed to specifically target different regions within DNA with the primary aim of disrupting its structure and thus function. There are many ways that small molecules can interact with DNA, some examples of which will be discussed in the next section.

1.3.2: Drug-DNA Binding Modes

The interaction of small organic or inorganic molecules with DNA depends on the chemical nature of the small molecule. Broadly, DNA-drug interactions can be of a covalent or non-covalent nature and there are four main types of non-covalent interactions that can occur, namely: intercalation, electrostatic interactions, major and minor groove binding, as well as covalent binding [Figure 1.4].⁴⁴

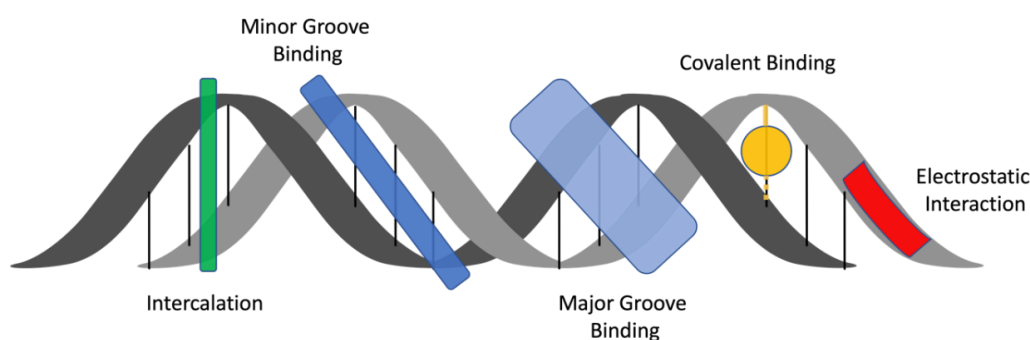


Figure 1.4: Graphical representation of the five major types of DNA-drug binding modes.

Interactions of metal complexes and other cationic units with DNA dominate the non-covalent binding modes and such systems have shown effects in anticancer, antimicrobial and antiparasitic therapies.¹ As DNA is the template for binding, the major structural factors that allow for DNA targeting include the polyanionic phosphate backbone, the shape and size of the grooves and the spacing between the base pairs. Non-covalent binding modes are commonly non-specific in nature, whereby the ligand can bind to DNA *via* multiple binding modes. Ligands that bind in the grooves, termed groove binders, often share some structural features and are designed to recognise sequences of DNA in the groove region based on hydrogen bonding capabilities. For example, the hydrogen bonding pattern present in the minor groove for a G-C base pair is donor,

acceptor, donor (D–A–D) [Figure 1.3]. Thus, molecules with complementary hydrogen bonding patterns (acceptor, donor, acceptor (A–D–A)) and similar groove curvatures would bind to this specific region of DNA. Moreover, introducing one or more positive charge in the design would increase interaction and allow for electrostatic contributions from the small molecule to the anionic backbone of DNA. Examples of some typical minor groove binders (MGBs) that display research and clinical applications and that favour A-T rich sequences of DNA are presented in Figure 1.5 alongside a crystal structure of DNA-MGB interaction.^{40, 45, 46}

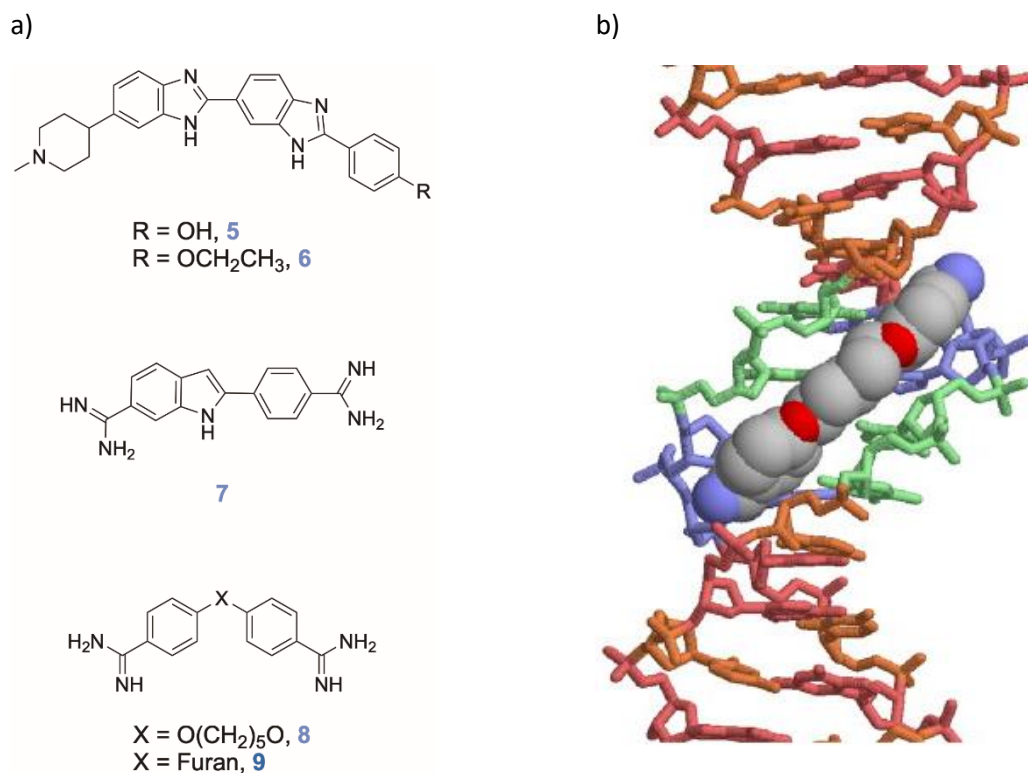


Figure 1.5: a) Structures of some MGBs used in clinical applications: Hoechst 33258, **5**, Hoechst 33342, **6**, DAPI, **7**, pentamidine, **8** and furamidine, **9**. b) Crystal structure of **8** bound to the minor groove of DNA. Crystal structure obtained by Neidle and co-workers.⁴⁷ Image from ref.⁴⁸

Common features among MGBs include an overall concave 3D shape complementary to the minor groove, positive charges, linked aromatic/heteroaromatic rings and moieties capable of Hydrogen bonding. Compounds **5** and **6**, containing bis-benzimidazole groups, have primary uses as blue-fluorescent dyes in cellular imaging techniques, but were also shown to have anti-tumour activity towards leukaemia cell lines.⁴⁵ Binding of **5** to the minor groove has been determined through single crystal X-ray diffractometry⁴⁹ and NMR solution studies.⁵⁰ DAPI, **7**, represents another common nuclear stain which binds preferentially to A-T rich sequences of chromosomes in the typical fashion. This compound also possesses antiprotozoal properties,⁵¹ and along with other diamidine derivatives such as Pentamidine, **8**, and Furamidine, **9**, which have been used in the treatment of Leishmania and Trypanosoma infections; although **9**, is the only one to display

significant human use. The mechanism of action of these MGBs toward parasites is in part due to the enhanced retention of the drug in the parasite, and interaction with parasitic DNA-dependent enzymes. Minor groove binders, **5-9**, have been shown to bind DNA with binding affinities in the 10^6 M^{-1} range without distorting the overall structure.^{40,46,48,52,53}

Intercalation is a non-covalent binding mode which results in an overall distortion and lengthening of the DNA-double helix, through insertion of a planar aromatic ligand between the base pairs of DNA, and inhibition of DNA-templated cellular functions. Many intercalators exert their anticancer activity by synergistic effects of: i) direct interaction with the DNA molecule, and ii) inhibition or poisoning of topoisomerase II (TOP2), which is an enzyme that controls the unwinding processes of DNA during replication and transcription.⁵⁴ Lerman originally proposed the concept of intercalation in the early 1960s upon observation of changes to viscosity and sedimentation coefficients following a series of acridine-DNA interactions.⁵⁵ TOP2 constitutes two enzymes (TOP2 α and TOP2 β) that play regulatory roles in DNA directed syntheses. TOP2 enzymes are generally overexpressed in many cancer cells due to their high proliferation rate and as such, are targets for many chemotherapeutic drugs.^{56,57,58} Examples of simple and complex intercalators are provided in Figure 1.6.⁴⁰

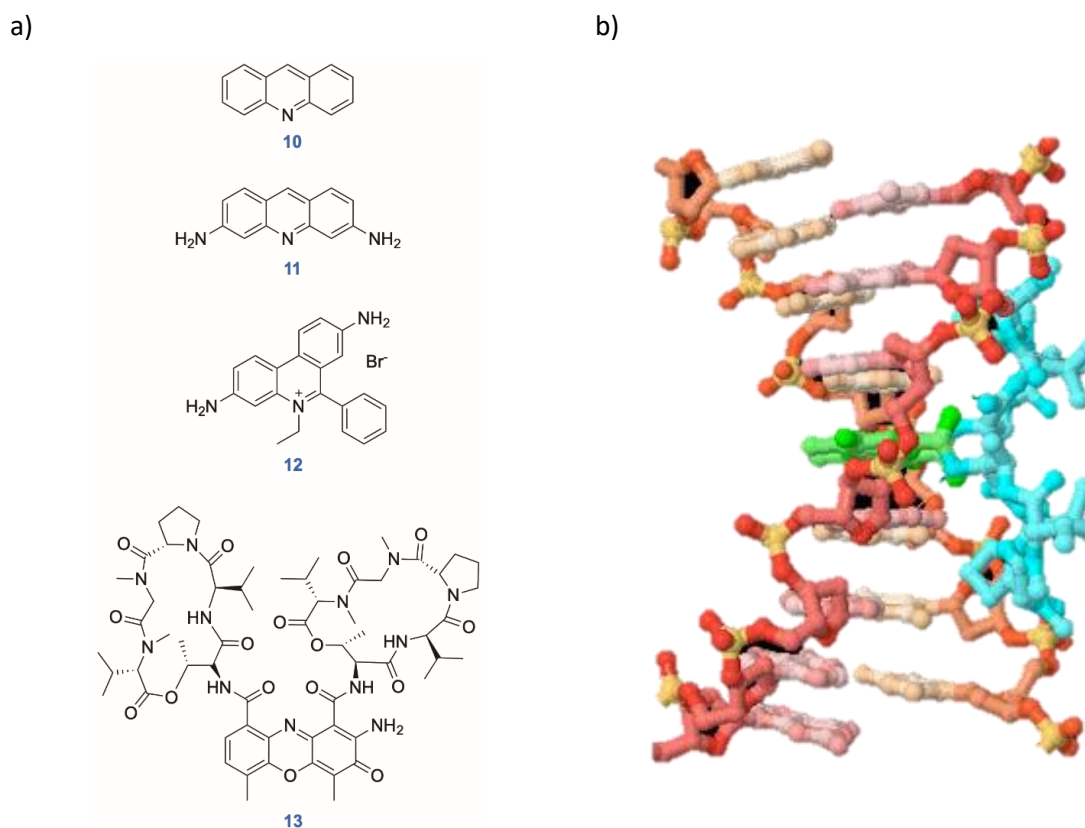


Figure 1.6: a) Chemical structure of intercalators used in clinical applications, acridine, **10**; proflavine, **11**; ethidium bromide, **12**; and actinomycin, **13**. b) Crystal structure of **13**-DNA interaction from PDB entry 173d. Image from ref.⁵⁹

General structural characteristics of intercalators include planar, fused ring systems, typically three six-membered fused rings containing at least one heterocycle. This structural property is responsible for the intercalative activity of the chromophore as it allows for insertion of the small molecule between adjacent base-pairs of DNA. The intercalator can span the length of a base-pair or insert just partially, the latter termed semi-intercalators. To accommodate the ligand, the DNA molecule becomes partially unwound, the distance between adjacent base pairs increases and the sugar-phosphate backbone changes conformation. This process is aided by many driving forces such as reduction of the repulsive negative charges associated with the DNA backbone and π -stacking interactions.⁵⁴ Acridine, **10**, proflavin, **11** and ethidium bromide, **12**, are among the simplest intercalators used in clinical applications and their DNA-intercalator recognition process is non-selective (sequence neutral) in nature. Historically, this family of compounds have found uses as antibiotics⁴⁰ and recently, efforts have been made to further understand the excited-state interaction between **11** and DNA using ultrafast time resolved absorption spectroscopy.⁶⁰ While the planar chromophore of intercalators exert π -stacking and Van der Waals interactions with adjacent base-pairs, the overall DNA-drug complex can be further stabilised by the inclusion of ionisable groups such as for **8**, whereby the amino-groups bond ionically with the phosphate backbone.⁵⁴

Complex intercalators can be considered as hybrid molecules that may display multiple binding modes, such as for the cyclic peptide functionalised intercalator, actinomycin D, **13**. Herein, the peptide side chains of **13** reside in the minor-groove of DNA and allow for Hydrogen bonding interactions between the threonine residue and the N2 and N3 atoms of guanine. Intercalation of the planar phenoxazine moiety and Hydrogen bonding interactions between the chromophore and the DNA-backbone further stabilises the structure. This overall drug-DNA interaction was confirmed by X-ray crystallography and displays sequence specificity for the 5'-GC-3' sequences. The stable complex that is formed inhibits the unwinding of DNA and transcription processes, ultimately leading to cytotoxicity.^{54,61,62} The multiple binding modes displayed by **13** reinforces the importance of the design of molecules with complementary structural properties to DNA. This is one of the main research aims of this thesis. Another class of nucleic acid intercalators include the 1,8-naphthalimide (**Nap**) structural motif, which has been studied extensively within the Gunnlaugsson group. **Naps** display a wealth of biological properties, including acting as cytotoxic agents and this will be discussed Section 1.5.^{63, 64, 12}

The final class of DNA-targeting drugs discussed in this section are those that interact covalently with DNA. Covalent DNA-ligand interactions result in severe damage to DNA and thus, inhibition of DNA templated functions. Within the field of medicinal chemistry, DNA alkylating

agents were the first effective and clinically used chemotherapeutics. Simple alkylating agents, such as **14** were derived from the chemical warfare agent mustard gas, **15**, employed during World War I.²³ Generally, covalent interactions can result in several changes to the DNA molecule including single or double strand breaks, inter or intra-strand cross-linking and/or the formation of DNA-drug adducts through generation of ROS from light. Structural details on these covalent adducts have been obtained by chemical probe experiments, NMR solution studies and crystallographic analysis.⁴⁰ Historically, Platinum drugs have dominated this class of molecules, and recently within the Gunnlauugsson group, efforts have been made to study photoadduct formation between small-molecules and DNA.⁶⁵ Figure 1.7 displays examples of common alkylating agents, and an X-ray crystal structure depicting a covalent drug–DNA interaction.

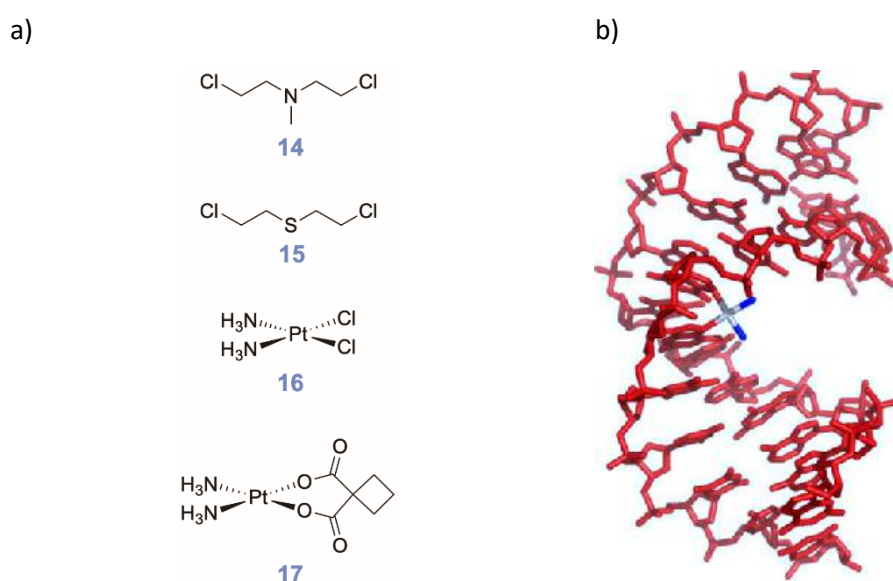


Figure 1.7: a) Chemical structures of typical alkylating agents, chloromethine, **14**; mustard gas, **15**; cis-platin, **16**; and carboplatin, **17**. b) Image of crystal structure of **16** and DNA displaying the intra-stand G-G cross-linking from PDB entry 1AIO.^{65, 66} Image from ref.⁶⁷

As purine nucleotides are most susceptible to nucleophilic attack by drug molecules, alkylating agents **14** and **15** damage DNA by adding alkylating the guanine N7 position, causing strand breakages or cross-linking and ultimately cell death. The antineoplastic activity of cisplatin, **16**, and carboplatin, **17**, occurs after activation *in cellulo* and crosslinking of adjacent guanine molecules either on the same strand, or on opposite strands leading to cell death.⁶⁸ Both families of molecules, the mustard gases and platinum-based drugs were discovered serendipitously as anticancer agents and paved the way for breakthrough cancer treatments.^{40,69,70}

1.4: Ru(II)-Polypyridyl Complexes

Ru(II)-polypyridyl complexes have long been studied for their applications across a large range of disciplines. Their popularity is attributed to their attractive photophysical properties, which will be discussed herein. Ru(II)-polypyridyl complexes display favourable, structure and site-specific binding to DNA, allowing for applications in cellular imaging and therapeutics.¹ In chemotherapeutics, genome disruption by small molecule interaction is responsible for the antiproliferative outcome of the therapeutic and can occur in many different ways (*vide supra*, Section 1.3.2). Ru(II)-polypyridyl complexes typically exert their mode of action by a combination of binding modes which can be modulated by the coordinating ligands. These interactions include electrostatic interaction of the metal with the anionic backbone of DNA, partial or full intercalation of the ligands achievable through rational ligand design, and association of parts of the molecules with the grooves (*vide infra*, Section 1.4.2). This thesis focuses on the design of spectroscopically active Ru(II)-polypyridyl complexes possessing functionalised **Nap** moieties for DNA interaction and thus, an understanding of the photophysical properties of such complexes will be next explained following by relevant literature examples.

1.4.1: Photophysical Properties of Ru(II)-Polypyridyl Complexes

The reactivity of Ru(II) complexes can be tuned based on design of the coordinating ligands and thus, it is necessary to understand the photophysical transitions that occur in such systems. Figure 1.8 displays a general Jabłoński diagram depicting the photophysical properties of a chromophore and the major electronic transitions that can occur upon photoexcitation of a molecule. Upon photoexcitation (absorption of a photon), the molecule is promoted from the ground state to the excited state which is typically a short-lived state characterised by containing excess energy. This excess energy can subsequently be dissipated from the lowest energy vibrational level of the excited state to the ground state (Kasha's Rule) *via* different mechanisms, including photoluminescence, to return to the ground state. Internal conversion (IC) and intersystem crossing (ISC) also describe deactivation pathways, however, these processes describe isoenergetic crossovers which form part of the dark processes and usually result in vibrational and solvent relaxation, rather than direct deactivation processes of the excited chromophore. Photoluminescence is a type of radiative deactivation pathway that occurs when a molecule emits a photon. Fluorescence occurs when the emission of the photon occurs from an excited state of the same multiplicity as the ground state ($S_1 \rightarrow S_0 + h\nu$) while phosphorescence describes the spin-forbidden transition between states of different multiplicity ($T_1 \rightarrow S_0 + h\nu$). The physical deactivation of excited states contains competing processes which are modulated by the nature of the atoms present.⁷¹

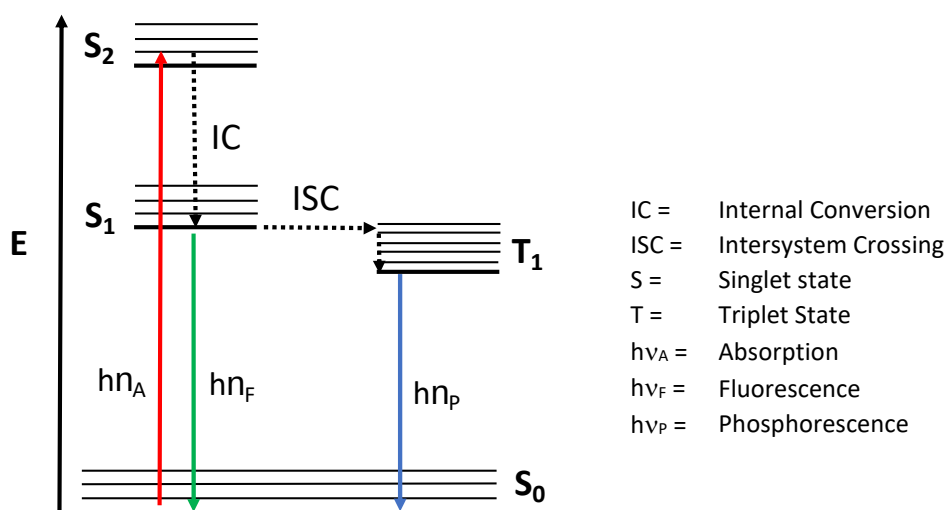


Figure 1.8: Representation of the excited states of a molecule. Red arrow depicts absorption. Solid downward arrows represent radiative transitions. Dotted arrows represent non-radiative transitions.⁷¹

Ru(II)-polypyridyls are d^6 metal ion complexes typically comprised of multidentate ligands that form an octahedral geometry around the metal ion centre. A molecular orbital (MO) diagram for a typical octahedral ML_6 type complex is shown in Figure 1.9. This MO diagram shows the general electronic transitions that can occur after photoexcitation. The ligands represented here possess σ and π -donor as well as π^* -acceptor orbitals. Photoexcitation can promote an electron from an occupied orbital to a vacant orbital by different means and are termed metal centred (MC), ligand centred (LC), metal-to-ligand charge transfer (MLCT) and ligand-to-metal charge transfer (LMCT) transitions.⁷¹

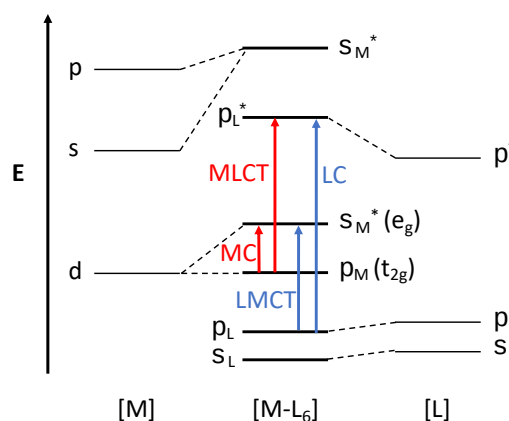


Figure 1.9: Molecular orbital diagram of ML_6 depicting the typical electronic transitions upon photoexcitation: LMCT, LC, MLCT and MC.⁷¹

Of these systems, the photophysical properties of simple, symmetric complexes such as $[Ru(bpy)_3]^{2+}$, **18** and $[Ru(phen)_3]^{2+}$, **19**, have arguably been the most studied of any other complexes since the photoluminescence properties of **18** were discovered in 1959.⁴ The intensive studies of this complex have led to a generally accepted photophysical schematic shown in Figure 1.10. The

transitions depicted in Figure 1.10 are described as follows: absorption of a photon results in the population of a $^1\text{MLCT}^*$ state which quickly undergoes ISC to generate the lower energy $^3\text{MLCT}^*$ state. At low temperatures, this state was shown to actually consist of three lower lying MLCT states ($\Delta E = 10\text{-}90\text{ cm}^{-1}$) which are in rapid equilibrium as well as a fourth state which is higher in energy.⁷²⁻
⁷⁴ This formally spin-forbidden process occurs partially due to the heavy atom effect of the Ru(II) centre which relaxes the spin selection rule. From here, multiple competing deactivation processes can occur.

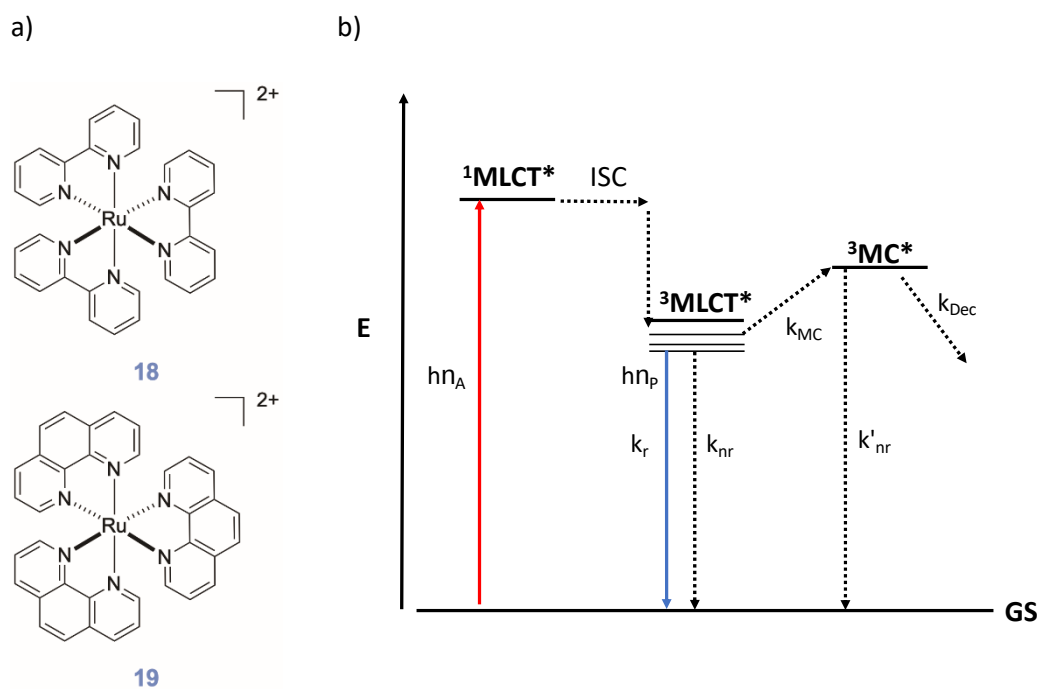


Figure 1.10: a) Chemical structures of **18** and **19**; b) energy level diagram for **18** and **19**. Solid arrows represent radiative transitions. Dotted arrows represent non-radiative transitions.⁷⁵

Firstly, relaxation back to the GS by radiative decay from the $^3\text{MLCT}^*$ occurs, emitting photon, which is described by the radiative rate constant (k_r). This deactivation pathway is responsible for the luminescence attributed to **18** and **19**. Secondly, a non-radiative decay process described by the non-radiative decay constant (k_{nr}) can occur from the $^3\text{MLCT}^*$ which primarily dissipates the excess energy in the form of heat. Finally, a third deactivation pathway described by the dechelation constant (k_{Dec}) can occur which involves the loss of a ligand due to the population of the $^3\text{MC}^*$ state *via* thermal activation. This state is quite unstable due to the strong antibonding character of the newly occupied orbital and distortion of the Ru-N bonds can result in ejection of the ligand.^{71,75} Interestingly, the instability of $^3\text{MC}^*$ states of Ru(II)-polypyridyls has been investigated for use in biological systems, where upon photorelease of a ligand, the complex exhibits a therapeutic effect.⁷⁶⁻⁷⁸

Solvent effects can play a huge role in the deactivation pathways of Ru(II)-polypyridyls. Polar protic solvents exert a stabilisation effect on the $^3\text{MLCT}^*$ and thus increase the energy gap between the triplet excited states. Furthermore, the deactivation rate constants for **18**, k_r and k_{nr} are influenced by the nature of the solvent where k_r is only slightly affected by a change of solvent while k_{nr} increases upon increasing solvent polarity, as expected from the energy gap law.^{75,79} Replacing one of the **bpy** ligands in the **18** with dipyrdo[3,2-a:2',3'-c]phenazine (**dppz**) yields $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}, **20** [Figure 1.11]. This complex and the corresponding **phen** analogue, **21**, displayed strikingly different luminescence properties in polar versus non-polar solvents. **20** and **21** are referred to as the “DNA light-switch” complexes as their luminescence properties are enhanced in non-polar environments and in polar, protic solvents the luminescence is “switched-off” (*i.e.* non-emissive in water). As such, an alternative photophysical model can be used to depict these systems which display an excited state equilibrium between bright and dark charge transfer states [Figure 1.11].$

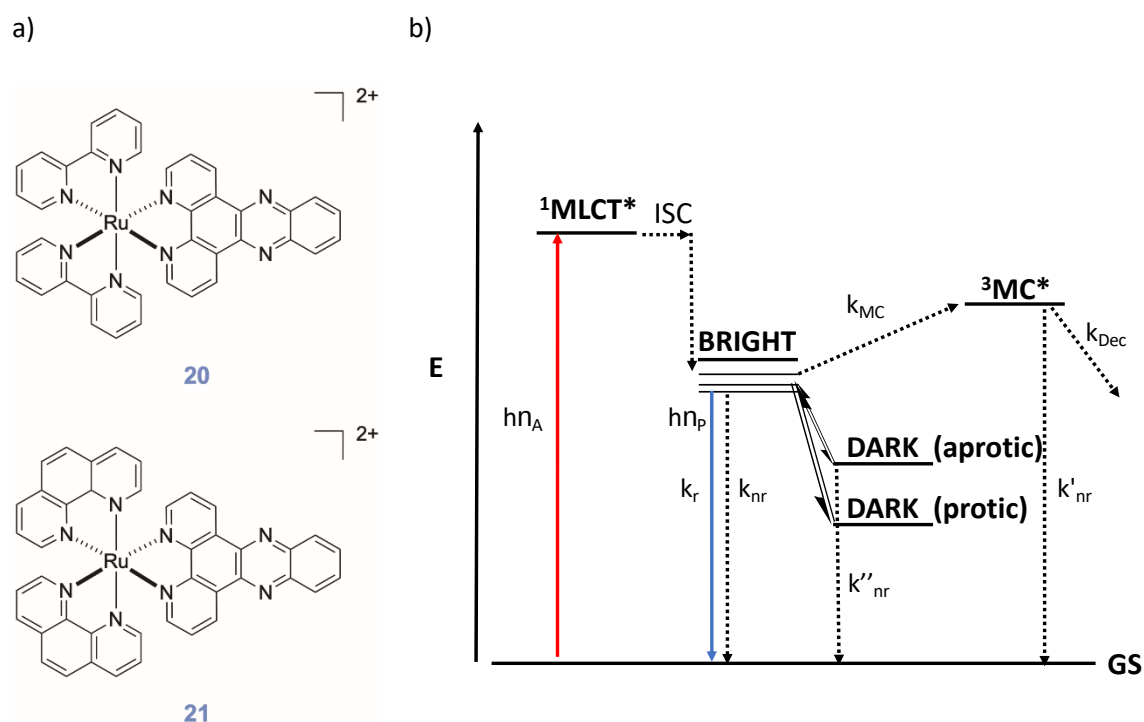


Figure 1.11: a) Chemical structures of **20** and **21**; b) representation of photophysical scheme of **20** and **21** depicting the bright and dark $^3\text{MLCT}^*$ states. Solid arrows represent radiative transitions. Dotted arrows represent non-radiative transitions.⁷⁵

The two distinct MLCTs occur as a result of a charge transfer to different parts of the **dppz** ligand. The bright MLCT excited state forms due to an electron transfer from the metal to the phenanthroline portion of the **dppz** ligand whereas the dark state forms from a MLCT transition from the metal to the phenazine portion of the **dppz** ligand. In polar protic solvents, this dark $^3\text{MLCT}^*$ is stabilised by hydrogen bonding interactions to the phenazine nitrogen atoms, thus

lowering the energy of this state allowing for higher population of it in these conditions.⁷⁵ The deactivation of **21** was found to occur from three excited states, each unique due to its number of hydrogen bonding interactions between the solvent and the phenazine nitrogen atom(s) (none, one or two).⁸⁰ By contrast, the bright state is more favourably populated in aprotic conditions as the bright and dark states are closer in energy and are in equilibrium.^{8,75,80-83} Our research group, in collaboration with the groups of Cardin, Quinn, Long and Kelly have achieved a direct observation of the bright and dark states associated with **21** by time-resolved infrared spectroscopy providing a clearer understanding of the observed light-switch mechanism.⁸⁴ Recent experiments and Density Functional Theory (DFT) calculations by Cullen, Long and Pryce shed further light on unique photophysical processes of **21**, where they found that the presence of water directly influenced nature of the Frank-Condon state, and the subsequent population of the different competing triplet excited states thereafter.⁸⁵

Considerable research has gone into elucidating the photophysical properties of Ru(II)-polypyridyl complexes which has shown them to be excellent agents for biological reactivity. Their attractive photophysical properties (absorption in the visible region and long luminescence lifetimes), water solubility, ease of functionalisation and sensitivity to their environments are all factors that continue to generate interest into these system as both imaging agents and as therapeutics, details of which will be discussed in the next section.

1.4.2: Medicinal Chemistry Applications of Ru(II)-Polypyridyl Complexes

Transition metal ion complexes such as the Pt based drugs **16** and **17**, and multiple derivatives thereof, have received considerable attention in the field of chemotherapeutics after the serendipitous discovery of the anticancer effects of **16**.⁸⁶ The toxicities toward non-cancer cells and emerging resistances are major drawbacks of this class and as such, advancements have been made toward the use of Ru(II)-polypyridyls for more targeted anticancer applications. Ru(II)-polypyridyls can exert therapeutic effects by a number of mechanisms including electrostatic interactions, groove binding mechanisms and ligand intercalation. Interaction of small molecules with DNA can cause disruption to the central dogma of molecular biology and inhibit cell division/replicative processes. Additionally, Ru(II)-polypyridyls can also act as non-toxic cellular imaging agents and as photosensitisers for PDT. In this section, the interaction of relatively simple Ru(II)-polypyridyls such as the tris-homoleptic complexes $[\text{Ru}(\text{bpy})_3]^{2+}$, **18**, $[\text{Ru}(\text{phen})_3]^{2+}$, **19**, and the **dppz** analogues **20** and **21** will be described followed by a description of more specialised systems such as binuclear complexes, systems capable of selective binding and targeted therapies.

The interest in Ru(II)-polypyridyl complexes for biological applications evolved in the 1980s after many studies were performed elucidating their photophysical properties for different applications.^{5,9,87} Early studies on the interactions of **18** and **19** with DNA found that **19** associates more strongly than **18**, as the latter primarily interacts through electrostatic interactions with the anionic backbone, proven by facile displacement of the complex upon increasing ionic strengths.⁹ At low ionic strength, both complexes displayed emission enhancement when bound to DNA, with marked enhancement observed for poly[d(A-T)] rich sequences, while no enhancement of emission was observed for **18** at high ionic strengths. On the other hand, **19** was found to associate with DNA *via* surface binding in the minor groove and contributions from partial intercalation of one of the **phen** ligands between the DNA base pairs as described by the unwinding of DNA by $22 \pm 1^\circ$ per acid residue in the topoisomerisation assay.^{5,9,88} Thus, it seemed reasonable that to increase the binding affinities of the Ru(II)-polypyridyls with DNA, an extended aromatic ligand capable of full intercalation should be employed.⁸⁹ As such, the **dppz** ligand and other structurally similar analogues were developed [Figure 1.12] and will be discussed. The following section will provide an overview of the interactions of **dppz** and **dppz**-like Ru(II) complexes such as those bearing benzo[*i*]dipyrido[3,2-*a*:2',3'-*c*]phenazine (**dppn**),⁹⁰ dipyrido[3,2-*a*:2',3'-*c*][1,2,5]thiadiazolo[3,4-4,3]phenazine (**dtp**),⁹¹ 2-phenyl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline (**pip**)⁹² and tetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-*h*:2''',3'''-*j*]phenazine (**tpphz**)⁹³ ligands.

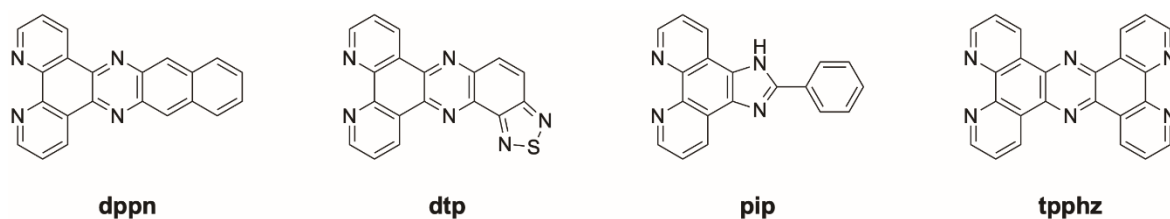


Figure 1.12: Chemical structures of polypyridyl ligands developed from **dppz**.

As mentioned, the **dppz** complexes **20** and **21** bearing **bpy** and **phen** as the auxiliary ligands, respectively, act as “DNA light switches” which show little to no luminescence in aqueous media but strikingly, when bound to DNA, their emission switches on. The photophysical behaviour of the complexes in their bound forms is analogous to their characteristics in organic solvents. The emissive nature of **20** and **21** when bound to DNA is attributed to protection of the phenazine nitrogen atoms from hydrogen bonding interactions upon intercalation into the hydrophobic cavity between the base pairs (*vide supra*, Section 1.4.1) which circumvents non-radiative relaxation to the ground state *via* transfer of energy to the bulk water.^{8,81,84}

Hall *et al.* reported the first crystal structure reflecting the interaction between *rac*-**21** and DNA, a couple of decades after it was first synthesised.^{83,94} Indeed, both complexes Λ -**21** and Δ -**21**

were found to bind DNA in different orientations. Figure 1.13 displays the binding interactions of the enantiomeric pair with a short oligonucleotide sequence and the differences in the luminescence intensities of each enantiomer when fully bound to DNA. This study revealed several interesting observations: i) the Δ -enantiomer is indeed a better fit for the right-handed DNA double helix, as was proposed by numerous other studies prior.^{95,96} The Ru–P distance was shorter in the case of Δ -**21**, resulting in greater electrostatic contributions modulated by steric influence when compared to Λ -**21**. ii) Both enantiomers display a binding preference for a pyrimidine-purine base step. iii) The **dppz** moiety of both enantiomers intercalates from the minor groove. iv) Δ -**21** displayed greater luminescence enhancement upon binding with DNA than Λ -**21** [Figure 1.13 (b)], a property which is influenced by coordinating water molecules to the phenazine nitrogen atoms. The crystal structure revealed one fully occupied water molecule engaged in a hydrogen bonding interaction with one of the phenazine nitrogen atoms of Δ -**21**. In the case of Λ -**21**, two water molecules participate in hydrogen bonding interactions with the phenazine nitrogen atoms. This observation is in agreement with the three-state light switch character of **21** proposed by Lincoln and co-workers who assigned distinct excited states of **21** when it has two, one or no hydrogen bonds from the solvent.⁸⁰

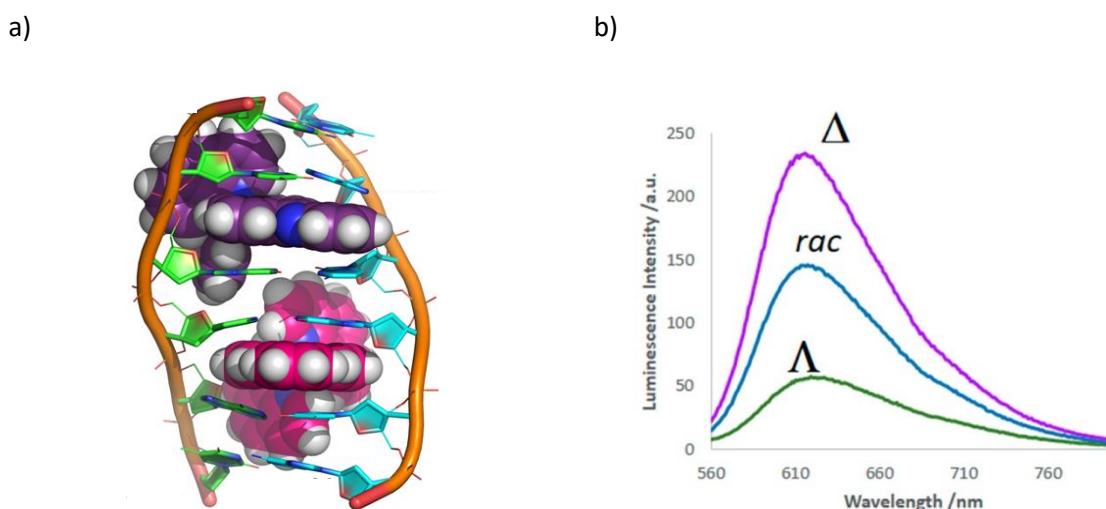


Figure 1.13: a) Crystal structure of the interaction between Δ - and Λ -**21** with d(ATGCAT)₂ viewed into the major groove. Purple structure denotes Δ -**21** and pink represent Λ -**21**. b) Luminescence intensities of Δ -, Λ - and *rac*-**21** when bound to DNA. Images reproduced from ref.⁹⁴

The light switch complexes **20** and **21**, which displayed intriguing photophysical properties upon interaction with DNA ignited the field of research into Ru(II)-polypyridyls as medicinal chemistry agents. Research efforts have focused on increasing the selectivity and sensitivity of the therapeutic agents. The next section aims to introduce this class of molecules with reference to **dppz**-like structures presented in Figure 1.12.

Aromatic extension of the intercalative motif of **dppz** by one phenyl group results in the **dppn** ligand [Figure 1.12], also capable of DNA intercalation. Turro and co-workers developed a Ru(II)-**dppn** complex, **22**, which exerts a dual therapeutic action with applications in PDT [Figure 1.14]. The dual reactivity involves singlet oxygen ($^1\text{O}_2$) sensitisation with $^1\text{O}_2$ quantum yield, $\Phi = 0.72$ and ligand exchange with the solvent upon photoexcitation. The distinct advantage of **22** over the archetypal **20** and **21** is attributed to selectivity through photoinduced cell death. In conditions with no light, **22** was found to be non-reactive toward DNA while it cleaves the DNA in the presence of light. **22** displays greater controlled cytotoxicity than $[\text{Ru}(\text{bpy})_2(\text{dppn})]^{2+}$ analogue with three times greater photocytotoxicity index (PI), where $\text{PI} = \text{IC}_{50}^{\text{dark}}/\text{IC}_{50}^{\text{irradiation}}$. These results indicate that the lowest lying excited state is $^3\text{LC}^*$ in character, localised on the **dppn**. Thus, **22** displays the potential of this class of complexes as dual action therapeutic agents, modulated by the coordinating ligands.⁹⁰

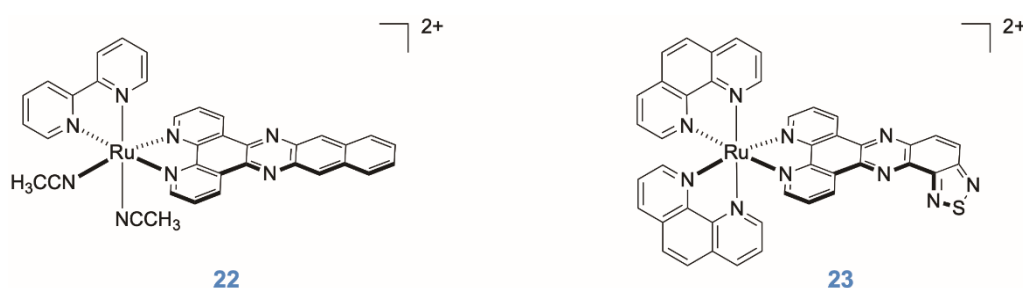


Figure 1.14: Chemical structure of Ru(II)-polypyridyl complexes **22** and **23** with **dppz**-like extended aromatic ligands.

Separately, the Gunnlaugsson research group have recently developed a **dtp** functionalised Ru(II) complex, **23**, which also displayed the light-switch effect upon binding with DNA. Furthermore, **23** displayed strong DNA binding constants (10^7 M^{-1} in 10 mM phosphate-buffered solution), cellular localisation in the cytoplasm, and moderate cellular toxicity which, unlike **22**, was not modulated or enhanced after light irradiation.⁹⁷ The presence of the sulphur may provide a handle for further functionalisation of the complexes to enhance their therapeutic properties, such as through surface functionalisation of gold nanoparticles, an area of research also pursued by our group.^{98,99} An advantage of employing Ru(II)-functionalised nanoparticles is to allow access to the nucleus where the complexes alone could not access.⁹⁸

The imidazole fused phenanthroline ligand, **pip**, which retains the coordination motif of **dppz** but contains an element of free rotation in the intercalative unit, has been incorporated into complexes such as $[\text{Ru}(\text{bpy})_2(\text{pip})]^{2+}$, **24**, which have shown strong DNA binding affinities [Figure 1.15].^{92,100} Furthermore, **24** has been shown to stabilise triple stranded RNA, which is of great biological importance for the understanding of gene regulation and for potential gene-targeting

therapies.¹⁰¹ As mentioned, one caveat to the use of Ru(II)-polypyridyls as cellular probes is their poor uptake efficiencies. Keyes and co-workers have overcome this challenge by synthesising a Ru(II)-polypyridyl peptide conjugate, $[\text{Ru}(\text{bpy})_2(\text{pic-Arg}_8)]^{2+}$, (**pic** = 2-(4-carboxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline) with appended arginine (Arg) functionalities, **25**. The Ru-peptide conjugate, **25** was the first of its kind to display rapid and passive transport across the cell membrane of myeloma cells highlighting the versatility of Ru(II)-complexes as membrane permeable cellular imaging probes.¹⁰²

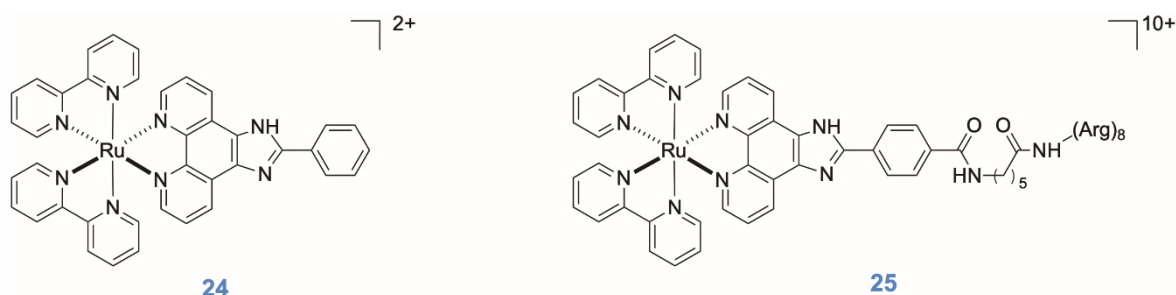


Figure 1.15: Chemical structure of Ru(II)-polypyridyl complexes **24** and **25** with **pic** auxiliary ligands.

The final class of Ru(II)-polypyridyl complexes discussed in this section are binuclear Ru(II) systems containing **phen** or **bpy** as the ancillary ligands which display enhanced cellular uptake and DNA binding affinities compared to their mono-nuclear analogues. Early work by O'Reilly *et al.* described binuclear Ru(II)-derivatives of $[\text{Ru}(\text{bpy})_2(\text{Me}_2\text{bpy})]^{2+}$ linked by alkyl chains which displayed 100-fold improved DNA binding affinities and greater resistance to high ionic strength when compared to the mononuclear complex.¹⁰³ More recent studies on flexibly linked binuclear complexes containing **bpy** or **phen** as the ancillary ligands showed that their cellular localisation was modulated by the length of the alkyl linker, with longer chains increasing their uptake efficiencies attributed to increased lipophilic character of the complexes. Furthermore, it was revealed that the DNA binding affinities of the series of complexes (varying in the length of the alkyl linker) was not correlated with cell death and that cell localisation (primarily in the mitochondria) was highly dependent on the cell line employed.¹⁰⁴

Thomas and co-workers developed binuclear complexes with fused rigidity between the cations through use of the **tpphz** ligand [Figure 1.16 (a)]. The Ru(II) binuclear complexes **26** and **27**, of the general formula $[(\text{L})_2\text{Ru}(\text{tpphz})\text{Ru}(\text{L})_2]^{4+}$, where L = **phen** or **bpy** respectively, exhibited the well-known “light switch” effect upon DNA binding.

Cytotoxicity investigations displayed low IC_{50} values ($> 100 \mu\text{M}$) against human breast cancer cell lines (MCF-7). Of the two complexes, **27** successfully localised in the nucleus of living cells, overcoming one of the major drawbacks of the development of mono-nuclear Ru(II)-

polypyridyls for live cell staining. **26** on the other hand, stained the nucleus of fixed cells and displayed the distinct metal-based advantages of conventional probes. The mechanism of cellular uptake of **27** was found to be non-endocytotic, but temperature-dependent as shown by using luminescence- and transmission electron microscopy (TEM) studies. Co-staining experiments revealed that **27** binds exclusively to DNA, as demonstrated by the co-staining with SYTO 9 [Figure 1.16 (b), *bottom*] which preferentially binds RNA.

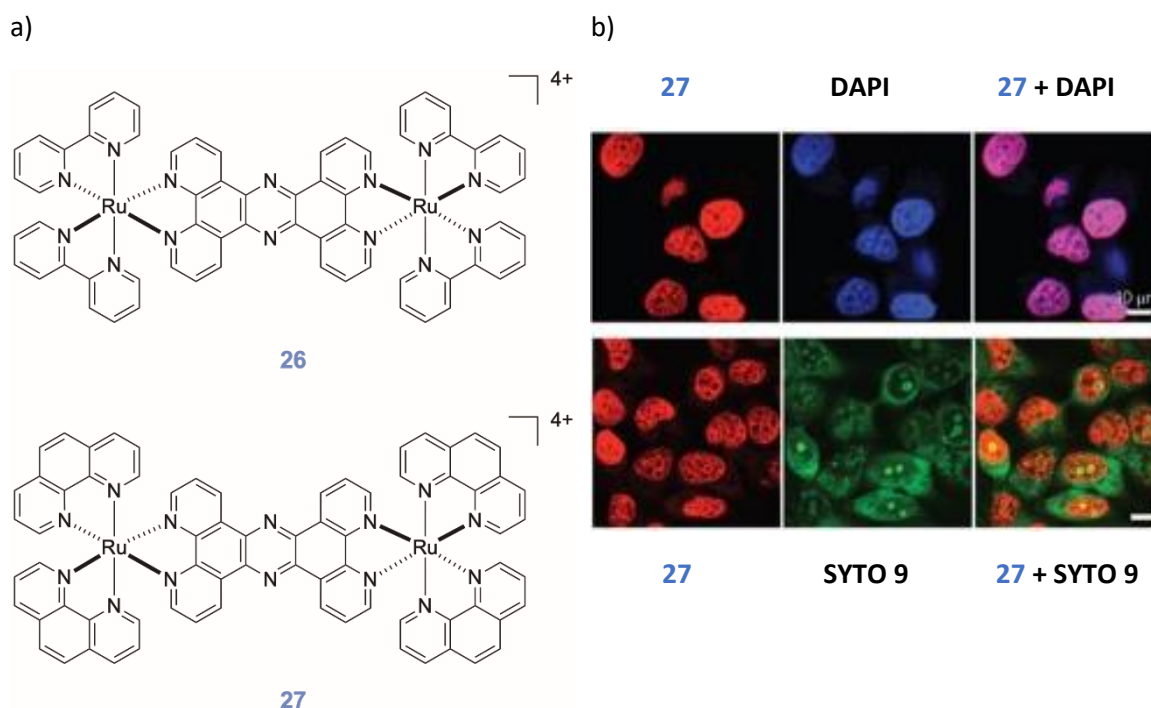


Figure 1.16: a) Chemical structures of binuclear Ru(II)-complexes **26** and **27**. b) Co-staining of MCF-7 cells with **27** and DAPI (top) and **27** and SYTO 9 (bottom). Images reproduced from ref.⁹³

Most interesting from this study, **27** displayed the ability to differentiate between different secondary structures of DNA, namely duplex and quadruplex DNA. A blue shifted emission profile when bound to quadruplex DNA ($\lambda_{em} = 630$ nm) over duplex DNA ($\lambda_{em} = 680$ nm) was observed *in vitro* which prompted further investigation into **27** as a DNA quadruplex probe. Staining of a cell line rich in quadruplex DNA confirmed the hypothesis that **27** acts as a probe for different secondary structures of DNA within live cells.⁹³

More recently, Thomas and co-workers resolved the three individual isomers of **26** ($\Lambda, \Delta, \Delta, \Delta, \Lambda, \Delta$) and studied their interactions with short oligonucleotides.¹⁰⁵ This revealed, for the first time, a threaded interaction between **26** and DNA. Unsurprisingly, the different conformations displayed different binding modes, further highlighting the importance of enantiomeric resolution. The kinetics of the threading interactions was found to be much faster in unstable DNA forms compared to natural B-DNA, which may lead to the use of these complexes as probes for

mismatched DNA.¹⁰⁶ Recent cellular studies on **27** revealed that the binuclear complex not only interacts with DNA, but also interacts with the processes involving the cytoskeleton. Specifically, **27** displayed an inhibitory effect on the polymerisation of G-actin to F-actin, affecting the late stages of cytokinesis. Errors at this stage of the cell cycle can lead to cancer and as such, there is major interest in the development of cytokinesis inhibitors for therapeutic applications.¹⁰⁵ The preceding section aimed to provide an overview for the wide array of Ru(II)-polypyridyl complexes bearing **phen** or **bpy** ancillary ligands that have been studied in the context of chemical biology ever since the pioneering work on the light-switch archetype by Barton, Kelly and co-workers. The previous section was not intended to be comprehensive, indeed this feat would be near impossible. Instead, it was designed to provide the reader with a description of the photophysical processes of these systems and how they have been exploited for use in many areas of biology. The next section aims to introduce a class of Ru(II)-polypyridyl complexes which contain π -deficient ancillary ligands. Such complexes display discrete photophysical properties and controlled photoreactivity with DNA, ultimately inducing cell death in a more selective manner.

1.4.3: Photophysical properties of Ru(II)-Polypyridyls containing π -deficient ligands

The introduction of π -deficient ligands for Ru(II) complexation results in complexes with very different properties compared to those described above. Complexes containing at least two ligands of π -deficient character, such as 1,4,5,8-tetraazaphenanthrene (**TAP**) or 1,4,5,8,9,12-hexaazatriphenylene (**HAT**), display extremely oxidising triplet excited states allowing for applications as DNA photo-oxidisers [Figure 1.17].¹⁰⁷ The research group of Kirsch-De Mesmaeker originally reported the unusual photophysical behaviour of $[\text{Ru}(\text{TAP})_3]^{2+}$, **28**, namely concerning the lifetime values which i) were shorter in MeCN compared to H_2O , ii) displayed temperature dependence, iii) oxygen insensitivity, and iv) no solvent isotope effects. Furthermore, a dynamic equilibrium was observed between the $^3\text{MLCT}$ and ^3MC states in organic solvents.¹⁰⁸ Since then, much research has explored the photoreactivity of this complex and its derivatives with DNA.^{107,109}

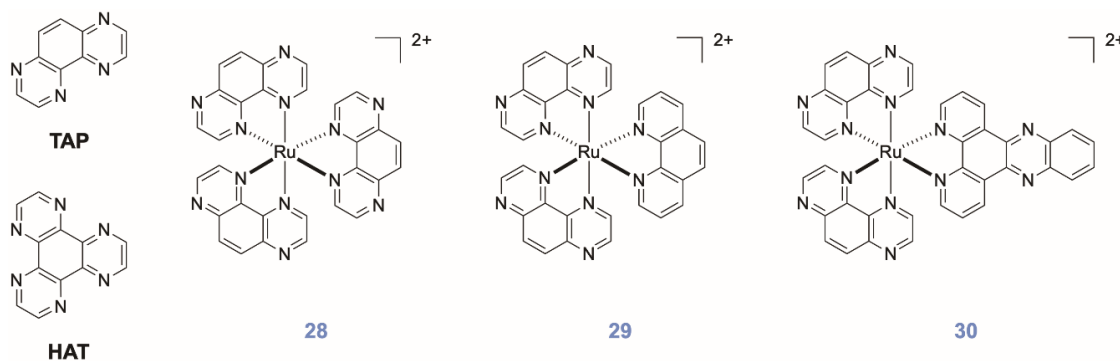


Figure 1.17: Chemical structures of π -deficient ligands, **TAP** and **HAT**, and Ru(II)-polypyridyl complexes **28-30** containing at least two **TAP** ligands.

As mentioned, complexes containing at least two π -deficient ligands displayed unusual photophysical behaviour with excited states of complexes **28-30** being extremely oxidising in comparison to the **bpy** or **phen** analogues. Table 1.1 summarises the potentials of the first reduction wave (vs. SCE) for both **TAP** (highly oxidising) and **bpy/phen** (non-oxidising) Ru(II)-complexes in their different electronic states. This property allows complexes **28-30** to photo-oxidise (*i.e.* abstract an electron) from biomolecules with appropriate potentials, ultimately resulting in the formation of photoadduct products. Of the nucleobases, guanine is the most easily oxidised ($E_{\text{ox}}(G^+/G) = 1.07 \text{ V vs. SCE}$),¹¹⁰ meaning that the excited states of the **TAP** complexes are sufficiently oxidising to abstract an electron from the weakly reducing guanosine-5'-monophosphate (GMP) [Table 1.1]. This is evident through examination of the change in free energy of the system after the photoinduced electron transfer (PET). Note that the oxidation potentials of (GMP/GMP $^{\cdot+}$) varies between experiments (CV vs. pulsed radiolysis),^{110,111} and that those values listed here do not consider the proton transfer step. Thus, the PET reaction was found to be exergonic for the **28-30** and endergonic for **18** and **19**.

The excited state of Ru(II)-polypyridyl complexes resides on the ligand with the most positive reduction potentials, *i.e.* the **TAP** ligands for complexes **28-30**. Kelly *et al.* originally described the two distinct types of photophysical behaviours between the tris-homoleptic complex **28** and DNA. In this case, the emission of **28** was quenched in the presence of ctDNA and in polynucleotides containing only G–C residues. This contrasted with the enhancement of emission displayed by **18** and **19** in the same conditions. Furthermore, the emission of **28** was enhanced in the presence of poly[d(A–T)] sequences. Emission lifetimes confirmed the luminescence intensity observations with reduced lifetimes observed when **28** was bound to G–C residues. This reduced lifetime was attributed to the photooxidation of guanine residues by **28** upon photoexcitation.¹⁰

Table 1.1: Reduction potentials of the first reduction wave, E_{red} (vs. SCE) and corresponding excited state reduction potentials E_{red}^* (vs. SCE) for oxidising (**28-30**) and non-oxidising (**18** and **19**) Ru(II)-polypyridyl complexes in MeCN at room temperature. Data compiled from ref.¹⁰⁹

Complex	$E_{\text{red}} / \text{V}$	$E_{\text{red}}^* / \text{V}^{[a]}$	$\Delta G^0 / \text{eV (GMP)}^{[b]}$
28	-0.75	+1.30	-0.23
29	-0.83	+1.15	-0.08
30	-0.80	+1.20	-0.13
18	-1.35	+0.65	+0.42
19	-1.35	+0.70	+0.37

^[a] Excited state reduction potentials (E_{red}^*) estimated from: $E_{\text{red}}^* = E_{\text{red}} + \Delta E_{00} \approx E_{\text{red}} + \Delta E_{\lambda}^{\text{max}}$.¹⁰⁹

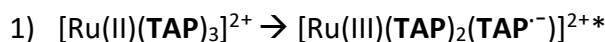
^[b] $\Delta G_{\text{PET}}^0 = -nF[E_{\text{ox}}(\text{D}) - E_{\text{red}}(\text{RuL}_3^{2+*}/\text{RuL}_3^{2+})]$, where D = electron donor, GMP and L = ligand.¹⁰⁹

The photophysical properties of **30** does not display the same light-switch behaviour as the **phen** and **bpy** analogues, **20** and **21**, when bound to DNA. Interestingly, **30** was found to be emissive in aqueous solution, a property that is quenched when bound to DNA sequences containing guanine.¹¹² Insights into these peculiarities are provided in the next section.

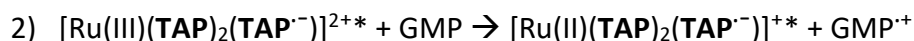
1.4.4: Photochemical Reactivity of Ru(II)-Polypyridyls Containing π -deficient Ligands

Some years following the observation of the peculiar photophysical properties of **28**, the covalent photoadduct between **28** and GMP was described, its formation attributed to a PET from the high oxidising power of the excited state of **28**.¹¹³⁻¹¹⁵ Dialysis experiments confirmed the covalent nature of the adduct between **28** and DNA, and further early studies suggested a reaction mechanism involving its formation.¹¹⁵ The reaction mechanism is proposed to proceed as follows, as summarised in Scheme 1.1.

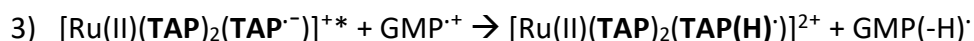
First, photoexcitation of **28** promotes an electron from the t_{2g} metal-based orbital to the ligand centred π^* orbital resulting in the generation of the highly oxidising $^3\text{MLCT}$ excited state (Step 1):



In the presence of GMP, two radical cations are formed from an electron transfer (ET) process from the weakly reducing guanine to the excited state of the complex, proven by laser flash photolysis experiments which detected monoreduced **28** and monooxidised GMP (Step 2).¹¹⁵

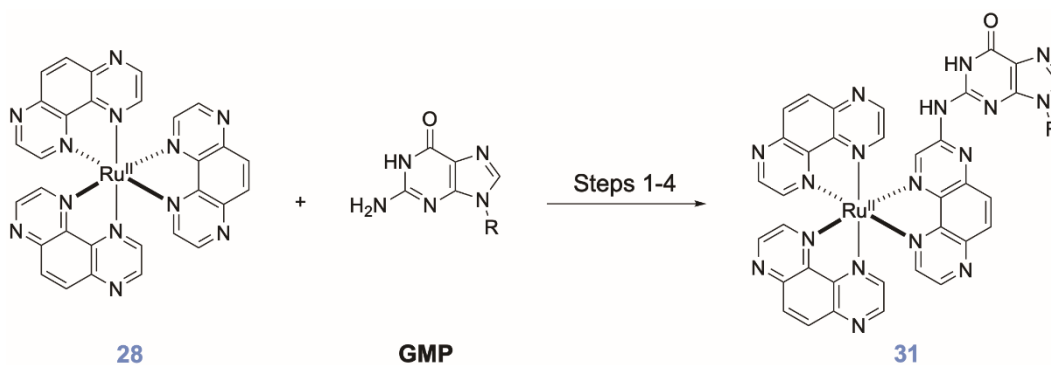
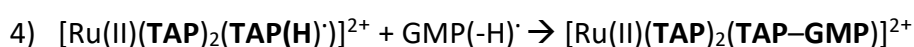


The resulting guanine radical can combine with the monoreduced complex;¹¹³ however, this guanine radical is quite acidic ($\text{pK}_a = 3.9$ vs 9.4 for guanosine) and as such, is anticipated to deprotonated within the physiological pH window.¹¹⁶ Furthermore, a pH dependence of the PET has also been studied which revealed that at pH 5 and above, the PET occurs with the non-protonated form of the excited complex, as the pK_a of this reduced complex was found to be 7.6 .¹¹⁷ Thus, the PET can be considered a photo-induced proton coupled electron transfer (PCET) reaction as the proton can be exchanged between the two species. It is unclear when this proton transfer occurs; before, after or simultaneously to the ET (Step 3).



From here, three different reactions may occur. i) A back electron process (BET) may occur, as was detected by transient absorption measurements which results in the regeneration of the starting

species. ii) Radical combination may occur between the two species resulting in the formation of the covalent photoadduct, **31**, after rearomatisation (Step 4). iii) Finally, DNA photocleavage may occur due to the reactivity of the guanine radical formed in Step 2. The photoinduced damage was shown through conversion of covalently closed circular (CCC) plasmid DNA into its open circular (OC) form. It is believed that this process occurs following a “leak reaction” from the previous step where the guanine (in its protonated or deprotonated form) can abstract an electron from a sugar moiety.¹¹⁸ Thus, the resulting photoadduct exists as Ru(II)-polypyridyl complex with no change to the coordination sphere around the metal. The covalent bond is formed between the carbon in the α -position relative to one of the non-coordinating nitrogen atoms of **TAP** and the exocyclic amine of GMP.¹¹⁸



Scheme 1.1: Schematic representation of the photoadduct formation between **28** and GMP where R = sugar-phosphate.¹¹⁸

The research groups of Kelly, Cardin, and Quinn, in collaboration with Gunnlaugsson, recently investigated the photooxidation reaction between Λ -**30** and guanine in DNA crystals by employing powerful techniques in tandem. X-ray crystallography and ultrafast time-resolved infrared spectroscopy techniques allowed for the direct monitoring of the ET process including the reaction site and rates of the individual PET steps listed above.¹¹⁹ The preceding section aimed to explore the photophysical and photochemical reactivities of Ru(II)-polypyridyl complexes containing at least two π -deficient ligands, namely **TAP** as this ligand has been subject to investigation in this thesis. The formation of a photoadduct has also been observed when the **TAP** ligand is substituted with a **HAT** ligand, which induces and provides an even more oxidising excited state within the complex due to its increased π -deficient character.¹²⁰ The formation of such photoadducts is not limited to a mono-linked product. Indeed, **29** and the bis-**HAT** analogue have been shown to crosslink DNA through the formation of a bi-adduct, leading to irreversible damage to DNA in a similar manner to the mechanism of action of the Pt based chemotherapeutics **16** and **17**.¹⁰⁹ As such, the properties of Ru(II)-polyazaaromatic complexes may lead to uses as controlled,

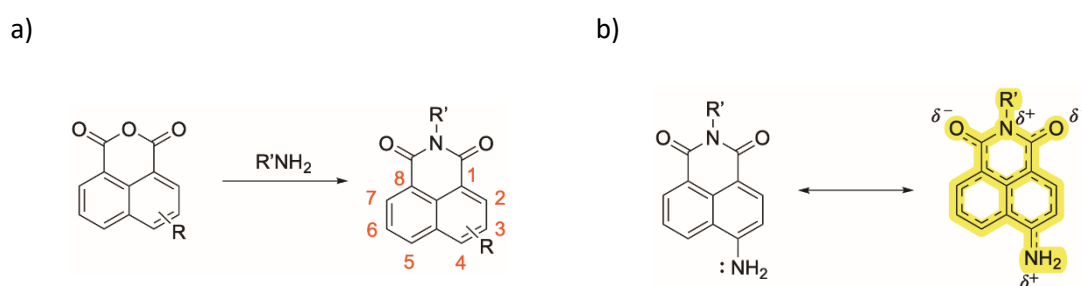
photoactivated chemotherapeutics. The next section aims to introduce the structural and photophysical properties of the 1,8-naphthamide (**Nap**) motif.

1.5: 1,8-Naphthalimide

The 1,8-naphthalimide (**Nap**), formally: benzo[*de*]isoquinoline-1,3-dione, is a heterocyclic unit that has been investigated for its DNA binding and photocleavage properties, making it an attractive chemotherapeutic agent as introduced in Section 1.1.⁶⁴ This project outlines the synthesis and characterisation of 1,8-**Naps** with functionalisation in for 4-position as ligands for transition metal complexes which bind DNA. In this section, the structural properties of **Naps** are outlined, followed by an account of their medicinal chemistry properties, and uses as ligand scaffolds, with reference to appropriate literature examples.

1.5.1: Structural Properties of Naps

The development of the chemistry underpinning **Naps** has led to their use in many biological applications in recent years, such as in therapeutics, as fluorescent imaging agents and fluorescent sensors.⁶⁴ The wide array of applications is attributed to the high versatility of the **Nap** scaffold which can be easily functionalised at either the *N*-imide site and/or at the 3/4-position on the naphthalene ring [Scheme 1.2 (a)]. **Naps** possess many attractive photophysical and photochemical properties as a result of their functionalisation.¹²¹ Braña *et al.* first described the synthesis of *N*-substituted **Naps** *via* a condensation reaction between the corresponding 1,8-naphthalic anhydride precursor and various primary amines for their use as anticancer agents.¹²² Since then, it has become the focus of much academic research, both in medicinal and supramolecular chemistry, and within the dye industry.^{64,123-125} In fact, since 2017, the number of publications focusing on the 1,8-**Nap** approached 500 annually.



Scheme 1.2: a) General synthetic route to – and numbering of – a generic *N*-substituted 1,8-**Nap**. b) Internal charge transfer (ICT) character within 4-amino-**Nap**.

The nature of the substituent on the naphthalene ring greatly affects the electronic properties of the **Nap**. Substitution of a nitro group in the 3- or 4- position of the **Nap** results in molecules that possess low energy HOMOs and high energy LUMOs, due to the presence of the electron withdrawing imide moiety and nitro group and have found uses as enzyme activated

probes and therapeutic agents.^{125,126} Molecules with these substitution patterns typically emit at short wavelengths, and induce broad, unstructured absorption bands at 350 nm. By contrast, when the **Nap** is substituted in the 3- or 4- position with an electron-donating group such as an amine an alternative transition is observed, resulting in red-shifted broad absorption and emission bands centred at 450 and 550 nm, respectively. In the case of the amine functionalised **Naps**, a highly solvent dependent, intramolecular charge transfer (ICT) excited state is observed due to the electron donating amino group, and the electron withdrawing imide moiety [Scheme 1.2 (b)].^{15,125,127} In non-polar aprotic solvents, amino-functionalised **Naps** are highly emissive and possess high photoluminescence quantum yields (Φ_{PL}), up to 96% in some cases.¹²⁸ The ICT is stabilised in polar protic solvents by hydrogen bonding interactions between the amino moiety and solvent thus, quenching the emission and shifting the absorption to higher wavelengths. These photophysical properties make them attractive candidates in the fields of colorimetric and fluorescent anion sensing,^{18,121,126} cellular imaging,⁶⁴ theranostics,²¹ and display technologies.¹²⁸ Additionally, the planar structure of the **Nap** contributes to its uses as a theranostic agent, particularly with respect to DNA binding, where it has been shown to be effective at undergoing DNA intercalation.^{12,19,20,63,129-132} Early structure-activity relationship studies led by Braña *et al.* resulted in extensive research of this class of compounds in the field of medicinal chemistry,¹³³ some examples of which are discussed next.

1.5.2: Medicinal Chemistry Properties of Naps

1.5.2.1: Naps as Anticancer Agents

A series of 3-nitro-**Naps** [Figure 1.18: **32-37**] synthesised by Braña *et al.* initiated investigations into the **Nap** motif for biological applications.¹²² All four compounds exhibited strong cytostatic activity toward HeLa and KB cancer cell lines and moderate toxicity to animal models. Of the four **Naps** investigated, **32** and **34** were the most biologically active and displayed high cytotoxicity (IC_{50} values $< 0.5 \mu\text{g/mL}$), inhibition of nucleic acid synthesis and DNA stabilisation effects. Mitonafide, **32**, and pinafide, **34** were also shown to stop the development and advancement of tumours in rat animal models.¹²² These results triggered further studies into the pharmacokinetics, biotransformation, tissue distribution and protein binding of **32** and **34**.¹³⁴ Waring *et al.* showed that **32** interacts with DNA *via* intercalation ($K_b = 1.45 \times 10^5 \text{ M}^{-1}$), perturbing 3-4 nucleobases per bound ligand. Viscosity studies showed that each bound drug molecule produced an increase in the length of DNA fragments by 2.2-2.5 Å. Furthermore, **32** induced DNA strand breakage by inhibition of TOP2.¹³⁵ Ultimately, neurotoxicity and undesirable side effects halted the clinical progression of **32**;¹³⁶ however, the discovery of the biological activity of the **Nap** paved the way for intense investigation

of structurally similar compounds, varying both the substituents on the imide and naphthalene rings to enhance desired therapeutic effects.

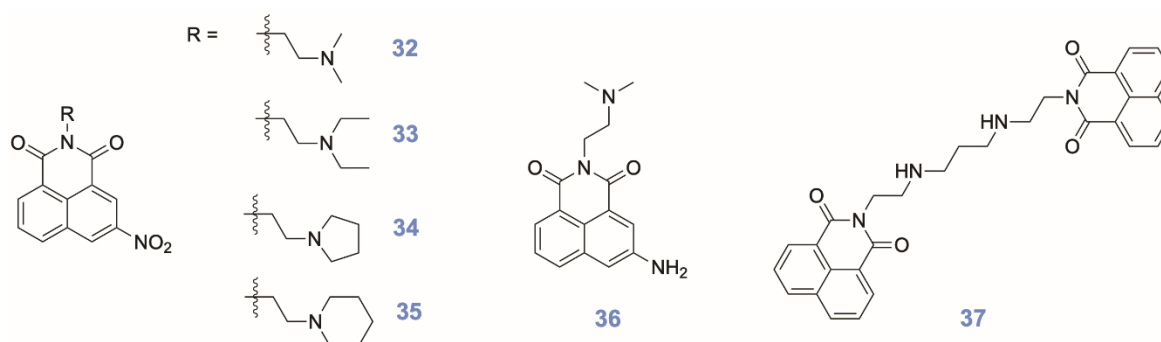


Figure 1.18: Chemical structures of four early analogues of 3-nitro-**Naps** (**32-35**), amonafide, **36**, and elinafide, **37**.

Braña and co-workers synthesised a large library of some 70 structurally similar mono-**Naps**, varying the substituents in the 3/4-positions and at the *N*-imide site. Each candidate was screened for its anticancer activity against HeLa cell lines. Structure activity relationships (SAR) revealed that a basic terminal group on the *N*-imide position was necessary to achieve optimum biological activity. The study also showed that substitution in the 3-position of the **Nap** ring was favoured for potent anticancer activity, attributed to the substituent more readily adopting a coplanar conformation with the **Nap** ring, assisting intercalation with DNA. The cytotoxic profiles were inferred to result from a cooperative binding mode with the genetic material involving intercalation of the **Nap** core and association of the basic side chain with TOP2; thus, the rich biological activities of these small molecules had been unveiled.¹⁴ Amonafide, **36**, the amine derivative of mitonafide, **32** was among other samples from the series sent to the National Cancer Institute for screening where its activity toward leukaemia models was confirmed. Since then, **36** has been the subject of much academic and clinical research.^{12,132,137,138} Its biological activity encompassed cell viability, cell toxicity and virucidal activity. Cell viability studies showed that **36** was toxic toward HeLa cell lines (IC₅₀ = 8.80 µg/mL). **36** also displayed toxicity against the herpes simplex (HSV-2) virus, while no activity was observed against RNA-type viruses.^{14,139} The dihydrochloride salt of **36**, in conjunction with cytarabine, progressed to phase III clinical trials by Antisoma Research Ltd. for treatment of secondary acute myeloid leukaemia (sAML), however it failed to display any distinct advantages over benchmark care in randomised studies.¹³⁷ Of all the mono-**Naps**, **32** and **36** were found to be the most cytotoxic and have made the most progress toward commercial clinical use. The major clinical drawback of **36** is due to the unpredictable side effects caused from toxic metabolites, and as such, research has also focused administering **36** in a prodrug approach in tandem with triggered enzymatic release *in situ*.^{132,138}

The bis-**Naps** constitute another interesting class of **Naps**. Elinafide, **37**, an example of a bis-**Nap** is comprised of two non-substituted **Naps** covalently connected by a diamine spacer. This inclusion of a second potential intercalative unit was found to greatly enhance the DNA binding affinity of **37** compared with that of the mono-**Nap** derivatives **32** and **36**. Compound **37** was found to display excellent activities against a wide range of tumour types, with either partial or complete regression of a range of solid tumour models observed.¹⁴⁰ The mode of action of **37** was found to be as a DNA *bis*-intercalator whereby both **Nap** functionalities intercalate with sequence specificity between base pairs, while the polyamine linker lies within the major groove of DNA.¹³⁰ These major advances in the area have presented an exciting field of research for the specific design and analysis of small molecules capable of interacting with DNA in a controlled manner.¹³²

1.5.2.2: **Naps** as Enzymatic Inhibitors and Fluorescent Probes

The medicinal chemistry properties of the **Nap** motif are not restricted to interactions with nucleic acids. As outlined in the previous section, **Naps** interact with TOP2 enzyme during cell replicative processes inhibiting its function which can lead to enhanced enzyme mediated cleavage of DNA.^{12,136} **Naps** have been designed to interact with a range of enzymes including histone deacetylases (HDACs),¹⁴¹ glycosidases,¹³⁸ and nitroreductases (NTRs),¹⁴² to elicit a variety of biological responses. Enzymes are extremely important biological targets in the field of medicinal chemistry as they are involved in all biochemical pathways and typically are expressed in different amounts depending on cell type. The nucleosome describes the basic structural packing unit of DNA and histone proteins in eukaryotic organisms where the DNA is wrapped around eight histone proteins.¹⁴³ The acetylation of histone proteins by histone acetyltransferases (HATs) regulated gene expression by removing the positive charge on conserved lysine residues of the histones thus, decreasing the DNA-protein association.¹⁴⁴ This causes the chromatin, (a combination of DNA and proteins) to adopt a more relaxed structural conformation that is transcriptionally active. HDAC enzymes reverses this action and leads to the formation of a transcriptionally silenced, condensed form of chromatin. Therefore the development of HDAC inhibitors is important as it have been found to affect tumour cells, with both cytostatic and cytotoxic effects.¹⁴⁴

As an example, HDAC inhibitors may consist of a zinc-binding domain connected *via* a spacer to a capping group. Scriptaid, **38**, was identified as a HDAC inhibitor by high-throughput transcriptional screening [Figure 1.19]. It is an example of a non-emissive **Nap** which shows modest selectivity towards HDAC6, an enzyme linked to cancer cell progression.¹⁴¹

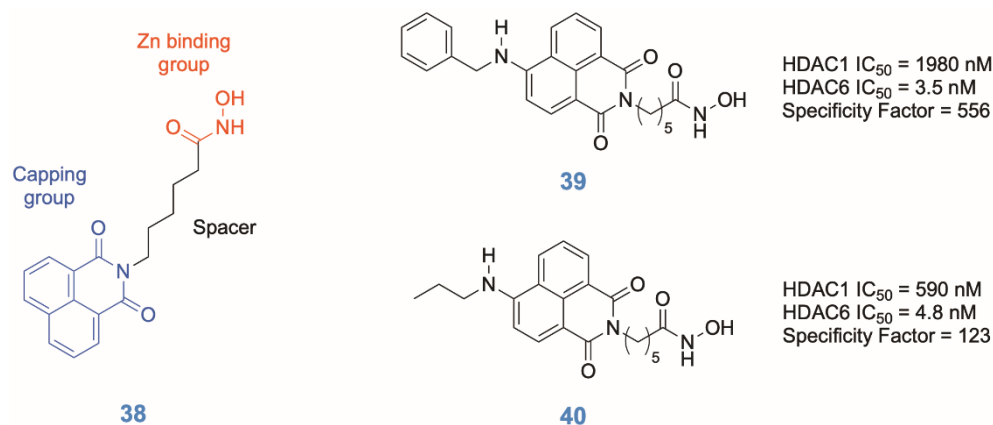


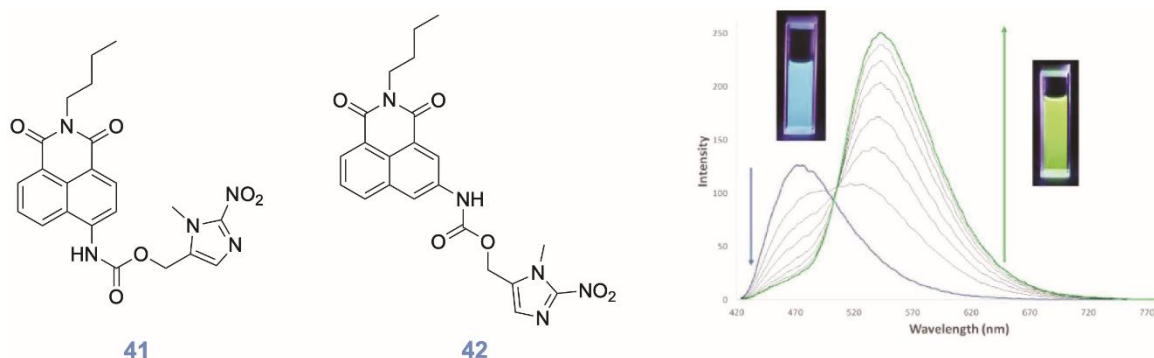
Figure 1.19: Chemical structures of scriptaid, **38**, selective fluorescent HDAC6 inhibitors **39** and **40** and associated specificity factors for different HDAC enzymes.^{22,141}

Recent research from Pfeffer and co-workers has focused on the development of a library of fluorescent analogues of **38** with specificity toward HDAC6 inhibition [Figure 1.19].^{22,145} Functionalisation of **38** in the 4-position of the **Nap** with different amines was performed, which generated ICT character resulting in a series of potent, fluorescent HDAC inhibitors. Of the series, **39** and **40** displayed the most attractive results with Φ_{PL} values of 83 and 81% in DMSO, respectively. Furthermore selectivity enhancement toward HDAC6 *versus* HDAC1, and reduced damage to the whole organism was observed for **39** and **40** when compared to **38**. Fluorescent imaging studies displayed cellular uptake of **39** and **40** into the cytoplasm and membrane blebbing, consistent with cellular toxicity studies. Finally, whole organism imaging on Zebrafish embryos confirmed uptake and localisation of the compounds in sites rich in HDAC6.²² As observed for **39** and **40**, a relatively minor change in the substitution of the capping group results in appreciable changes in the biological activities of the systems.

Another important family of enzymes for monitoring reductive stress *in cellulo* is the nitroreductases (NTRs) and indeed, research is ongoing into the design of new **Naps** that are responsive to these enzymes. The enzymes act as biological markers in certain cell types where they are over expressed and have been linked to many diseases such as heart failure,¹⁴⁶ solid cancers¹⁴⁷ and Alzheimer's disease.¹⁴⁸ NTRs reduce nitroaromatics to the corresponding amino- or nitroso compounds *in situ*. As mentioned previously, the 4-nitro and 4-amino-**Naps** display distinctive optical properties as a result of their substitution patterns and exploiting this feature can lead to the development of systems for non-invasive imaging of tumour hypoxia, subsequently allowing for better prediction of drug efficacies in treating tumours.¹⁴⁷ Research is emerging in this field and many groups have focused on the development of nitrobenzene and nitrothiophene triggers for NTR.¹²⁵

Elmes and co-workers recently developed highly sensitive nitroimidazole functionalised **Naps**, **41** and **42** which produced an “off-on” fluorescence response upon reaction with NTRs due to the differing optical properties of the nitro ($\lambda_{\text{em}} = 470 \text{ nm}$) and amino ($\lambda_{\text{em}} = 570 \text{ nm}$) derivatives [Figure 1.20(a)]. The proposed reaction mechanism proceeds through a reduction-fragmentation approach [Figure 1.20(b)]. **41** was found to be more sensitive than **42** or indeed any nitrobenzene derivative at detecting reductive stress in HeLa cell and was non-toxic to cells.¹⁴²

a)



b)

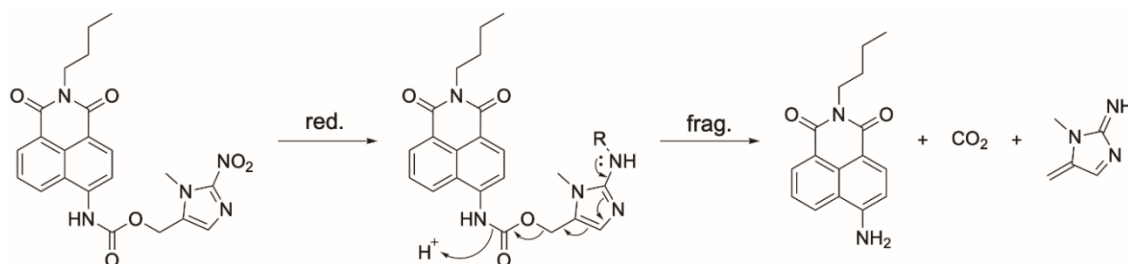


Figure 1.20: a) Fluorescent NRT probes **41** and **42** and changes in the fluorescence spectra of **41** ($\lambda_{\text{ex}} = 345 \text{ nm}$) over 10 minutes after addition of NTR ($1 \mu\text{g/mL}$). b) Proposed reduction-fragmentation mechanism for fluorescence switching upon interaction with NTR.¹⁴²

As demonstrated, the **Nap** is a highly versatile motif for medicinal chemistry purposes. The core aromatic skeleton of the **Nap** is capable of intercalative interactions with DNA and has been explored for such anticancer purposes for decades. The **Nap** can be functionalised at either the *N*-imide or naphthalene site to increase binding affinities to certain enzymes as was the case for the TOP2-**36** interactions and HDAC-**38** interactions.¹³² The difference in the fluorescence intensities and emission wavelengths of the nitro vs amino **Naps** has been a valuable property for the design of fluorescent sensors or probes enzymes which can deduce information about the local environments of cells, as observed for NRT-**42** interaction.¹²⁵ The following section will introduce the use of **Naps** as ligand scaffolds for metal complexation and discuss the intriguing photophysical properties of these systems.

1.5.3: **Naps** as Ligand Scaffolds

The photophysical properties of **Naps** and their corresponding coordination complexes have also been explored for uses outside the field of medicinal chemistry including in anion sensing,¹²¹ energy transfer and luminescence based technologies.¹⁴⁹ Castellano and co-workers have developed a series of transition metal complexes decorated with **Naps** [Figure 1.21: **43-49**] and **Nap** derivatives, such as 1,8-naphthalenebenzimidazole (**NBI**) ligand [Figure 1.21: **50**] capable of harvesting visible light and which display extended excited state lifetimes.¹⁵⁰⁻¹⁵³

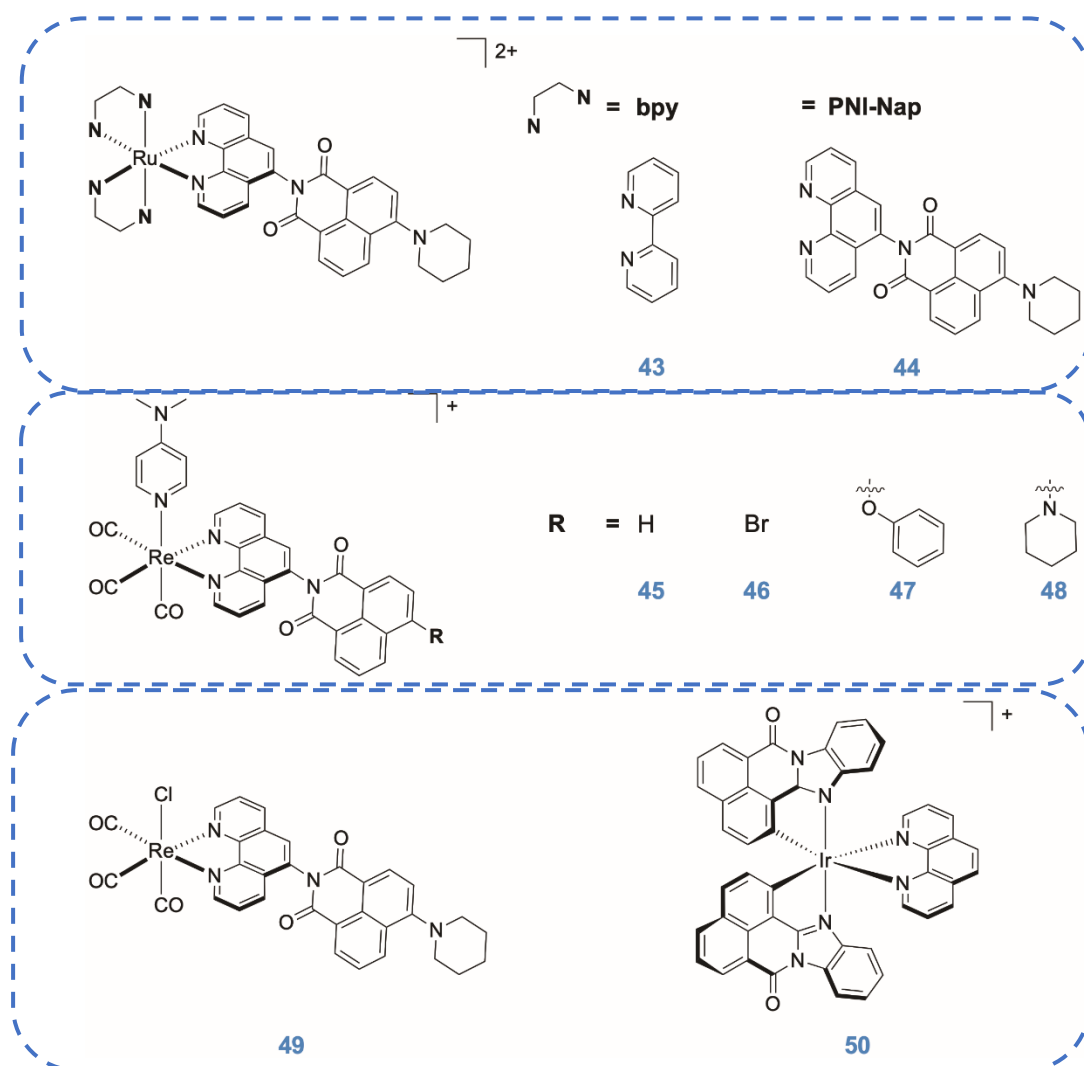


Figure 1.21: Chemical structures of metal complexes bearing **Nap** and/or **NBI** ligand systems developed by Castellano and co-workers.

Ru(II)-**Nap** complexes **43** and **44** bearing one and three **PNI-Nap** ligands, respectively were shown to display greatly enhanced room temperature luminescence lifetimes (up to 114 μs in DMSO) as a result of the intervening intraligand (IL) excited states. The **Nap** chromophore was used to sensitize the $^3\text{MLCT}$ excited state which displays thermal equilibrium with ^3IL at room temperature. DFT calculations and time resolved spectroscopy reveal almost quantitative quenching of the singlet fluorescence of the **Nap** chromophore in both complexes by Förster-like

energy transfer. Complex **44** displayed longer luminescence lifetime values than **43**, consistent with more efficient energy transfer from three **Nap** ligands compared to one. Dual emission from both complexes was observed at low temperatures (77 K) using time resolved spectroscopy, attributed to $^3\text{MLCT}$ and ^3IL phosphorescence which independently decay to the ground state (GS). This study highlighted the emission lifetime tuning effects of the **Nap** ligands on the emitting excited state and provided a fundamental insight into the singlet-triplet partitioning [Figure 1.22], instrumental to the generation of new technologies, such as optoelectronic devices.¹⁵⁰

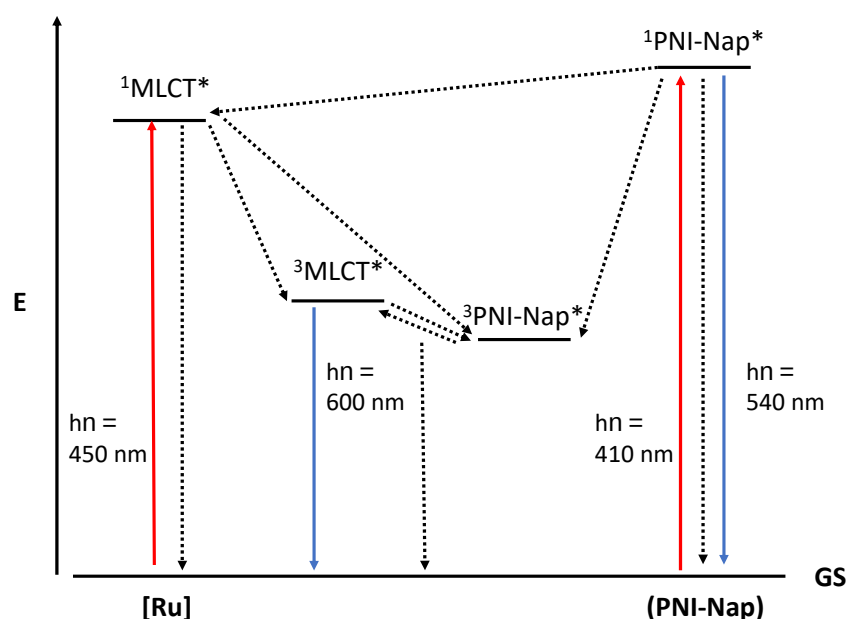


Figure 1.22: Energy level diagram for **43** and **44**. Adapted from ref.¹⁵⁰ Red lines depict absorption. Solid blue lines depict radiative transitions. Dotted lines depict non-radiative transition.

In a further attempt to tune the excited state equilibria, a series of Re(I) bichromophoric complexes containing carbonyl diimine ligands **45-48** were prepared where **Naps** with differing substitution patterns in the 4-position were used, thus increasing the visible light absorbing properties of the complexes. The emission arising from excitation of **45-48** at different wavelengths was primarily MLCT in character at room temperature where the **Nap** fluorescence was quantitatively quenched for **45** and **46**, and predominately quenched for **47**, bearing -H, -Br and -phenoxy groups in the 4-position, respectively. **Nap** based emission appeared as the primary luminescence in **48**, bearing piperidine in the 4-position of the **Nap** which implies that the efficiency of energy transfer from the **Nap** to the MLCT decreases with increasing electron donating properties in the 4-position. This observation can be explained by more red-shifted emission profiles of the **Nap** ligands and less spectral overlap between the donor **Nap** fluorescence and the acceptor Re(I) absorbance for the latter, assuming Förster-like resonance energy transfer is responsible for the **Nap** fluorescence quenching. Thus, the primary luminescence observed for the bichromophoric complexes **45-47** is derived from a triplet excited state equilibrium formed

between competing $^3\text{MLCT}$ and ^3LC states, whereas for **48**, it is mainly fluorescence from the unquenched **Nap** emission. The decay kinetics further corroborate this result. At room temperature, **45-47** display biexponential decay lifetimes in the microsecond regime [Table 1.2] which is much longer than that observed for a purely $^3\text{MLCT}$ decay profile often observed in the nanosecond timescale.

Table 1.2: Photophysical properties and time resolved photoluminescence data for complexes **43-50** in acetonitrile (black), THF (blue) and DCM (green). Data compiled from refs.¹⁵⁰⁻¹⁵³

Complex	$\lambda_{\text{abs max}} / \text{nm}$ ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) ^[a]	$\lambda_{\text{em max}} / \text{nm}$ (RT) ^[a, b]	$\lambda_{\text{em max}} / \text{nm}$ (77 K) ^[c, d]	τ_{PL} (RT) ^[e]	τ_{PL} (77 K) ^[f]
43	430	606	--	27.6 μs	--
44	427	595	--	60.8 μs	--
45	334 (20400) 334 (20200)	630 614	536, 512	6.5 μs , 27.0 μs	11.1 μs , 544 ms
46	343 (23200) 343 (23900)	626 617	555, 516	9.5 μs , 60.7 μs	10.6 μs , 8.6 ms
47	364 (21600) 363 (24000)	435, 628 611	546, 514	16 μs , 64.6 μs	12.6 μs , 612 ms
48	415 (15800) 410 (15400)	548 523	595, 512	< 15 ns	9.2 μs , 288 ms
49	408 (11980)	509, 593	524	30 ps, 651 μs	5.8 μs , 270 ms
50	484 (13150), 608 (120)	624	618	3.34 μs	4.59 μs

^[a] Room temperature measurements were made with ACN or THF as a solvent.

^[b] Samples deaerated using the freeze-pump-thaw method.

^[c, d] Measurements were made with 2-methyl-THF. ^[c] Emission maximum determined from time-gated PL spectra using the LP 920.

^[e] Decay of **45-48** collected at 600 nm, 650 nm for **49** and in deaerated acetonitrile at room temperature.

^[f] Decay of **45-48** measured at 535 nm (**45**), 555 nm (**46**), 550 nm (**47**), 600 nm (**48**), 540 and 650 nm (**49**) in 2-methyl-THF.

This observation suggests that the decays are as a result of competing $^3\text{MLCT}$ and ^3LC states. No delayed photoluminescence was observed for **48** whose ^3LC states are low in energy therefore, making it less likely to thermally populate the $^3\text{MLCT}$ states. At low temperatures, time resolved PL spectra were obtained which featured an initial broad luminescence attributed to $^3\text{MLCT}$ emission followed by vibronic, structured bands attributed to emission from the **Nap**. The τ_{PL} values were fit to a biexponential decay, assigned as a shorter lived $^3\text{MLCT}$ excited state decay and an extremely long lived ^3LC excited state decay in the millisecond regime [Figure 1.23 and Table 1.2].¹⁵¹

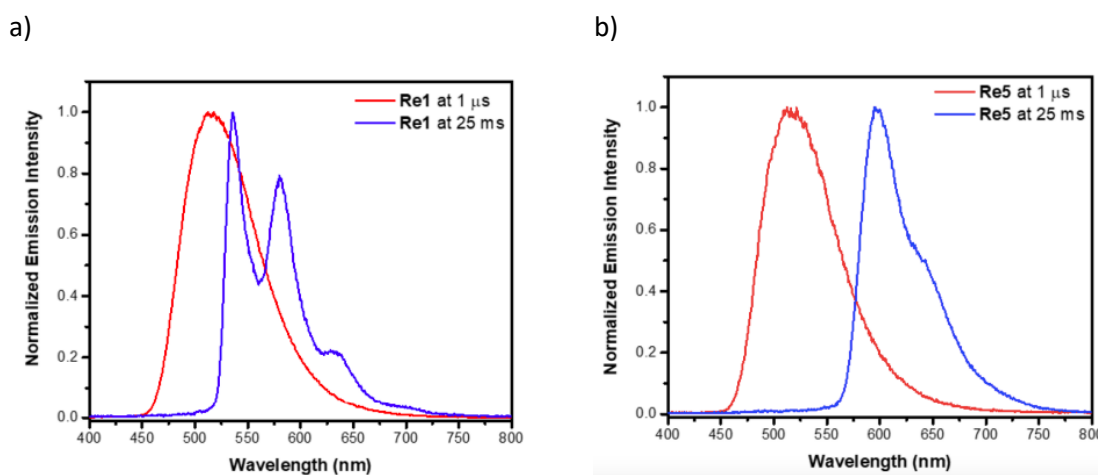


Figure 1.23: Time resolved photoluminescence emission spectra at 77 K and at 1 μ s (red) and 25 ms (blue) delay times of a) **45** and b) **48** in 2-MeTHF. Spectra reproduced from ref.¹⁵¹

This thorough photophysical study also employed transient absorption (TA) spectroscopy to further understand the kinetics of the intriguing photophysical properties of the complexes, and revealed critical evidence of the “ping-pong” like energy transfer of **45-47** where the initially excited **Nap** chromophore quickly (femto-to-picosecond timescales) sensitizes the $^3\text{MLCT}$ excited state which in turn, returns the energy to the **Nap** in the form of a long-lived ^3LC excited state. Both excited states then attained thermal equilibrium. In **48**, the thermal equilibrium favours the lower lying ^3LC state which dissipates energy through non-radiative relaxation from the ^3LC state with an average time constant greater than 100 μ s at room temperature [Figure 1.24].¹⁵¹ This “ping-pong” like energy transfer was originally observed for **49**, which displayed a 3000-fold lifetime enhancement compared to its non-**Nap** substituted analogue $[\text{Re}(\text{phen})(\text{CO})_3\text{Cl}]$ (where **phen** = 1,10-phenanthroline), at room temperature while maintaining the same Φ_{PL} .¹⁵²

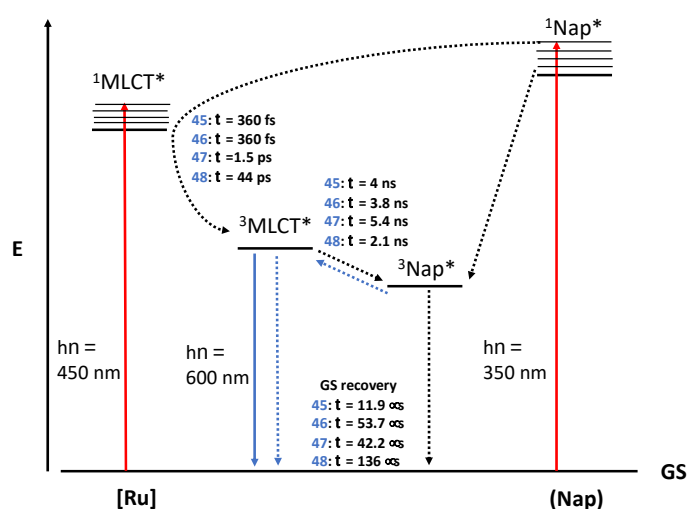


Figure 1.24: Energy level diagram for **45-48** at room temperature. Red lines depict absorption. Solid blue lines depict radiative transitions. Dotted lines depict non-radiative transitions. Blue lines denote transitions reserved for systems **45-47**. Adapted from ref.¹⁵¹

The final complex discussed in this series is **50**, a bis-cyclometalated iridium(III) complex. It contains two rigid, conjugated **NBI** ligands as the cyclometalating ligands. In general, cyclometalating ligands are strong σ -donors due to the formal anionic charge on the carbon atoms and this has significant effects on the optical properties. The strong ligand field destabilises non-emissive MC states, conferring these complexes with high quantum yields, while the high metal-ligand covalency allows for facile tuning of the emission colour by simple modification of the aryl rings. Thus, this family of luminescent metal complexes differ substantially to other classes of d^6 transition metal complexes, such as $[\text{Ru}(\text{N}^{\wedge}\text{N})_3]^{2+}$ or $[\text{Re}(\text{CO})_3(\text{N}^{\wedge}\text{N})\text{L}]$, which are often poorly emissive and/or difficult to colour tune. Complex **50** and its tris-**NBI** homoleptic analogue displayed desirable photophysical properties such as high photoluminescence quantum yields approaching 30% as a result of high SOC and subsequent efficient ISC. This molecule, and the homoleptic tris-**NBI**-cyclometalate, displayed long-lived excited states in the micro second regime [Table 1.2], arising from ^3LC excited states localised on the **NBI** ligands which are attractive for use in photochemical reactions. The enhancement of the excited-state lifetimes and visible light absorption properties can be particularly useful for the photochemical activation of small molecules using benign visible light.¹⁵³ Table 1.2 summarises the absorption, emission and luminescence lifetime values for complexes **43-50** at room temperature and 77 K.

Thus, this considerable effort from Castellano and co-workers employed techniques such as static and dynamic PL spectroscopy, DFT calculations and TA spectroscopy to investigate the fundamental charge transfer processes of a series of TM complexes with intervening ligand centred triplet states from pendant **Nap** ligands and their derivatives. These techniques revealed the fundamental excited state dynamics of a series of d^6 transition metal complexes that possess tuneable properties attractive for use in a wide range of reactivities. The next section aims to introduce the chemistry and literature surrounding metal based-**Nap** conjugates with a focus on their applications in medicinal chemistry.

1.6: Ru(II) and Re(I)-**Nap** Conjugates for Medicinal Chemistry Applications

Section 1.4 provided an introduction to the **Nap** motif, including a description of the structural properties and medicinal chemistry applications of organic **Naps** as well as a detailed overview of the unique and tuneable photophysical properties of **Nap** when employed as ligand in various metal-based systems, *vide supra*. This section aims to introduce **Naps** as DNA targeting and bioimaging agents when conjugated to metal centres. Ryan *et al.* first demonstrated the DNA binding and photocleavage properties of Ru(II)-polypyridyl **Nap** derivatives, **51** and **52** toward

plasma DNA, where the **Nap** is conjugated through a rigid phenyl-**bpy** moiety to the metal centre [Figure 1.25].¹⁶

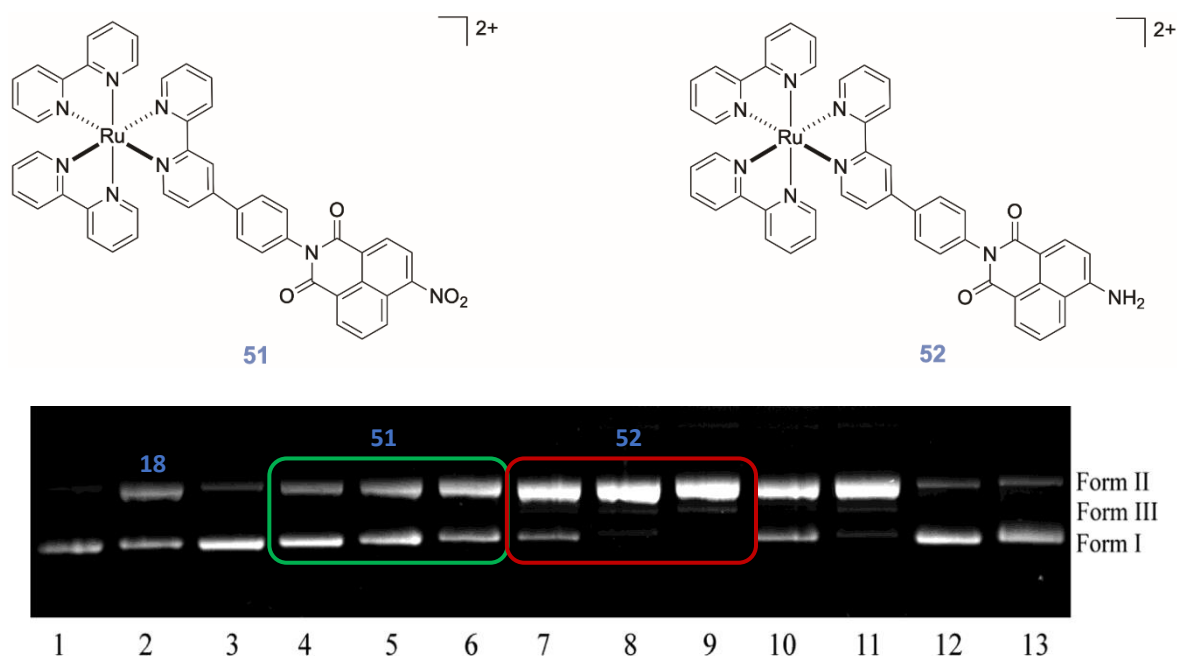


Figure 1.25: Top: Chemical structures of **Nap**-metal complexes **51** and **52** synthesised by Ryan *et al.* Bottom: Agarose gel electrophoresis of plasmid DNA upon exposure to **18** and increasing concentrations of **51** (green box) and **52** (red box). Form I represents supercoiled DNA, form II represents nicked DNA and Form III represents linear DNA.¹⁶

Nitro- and amino-functionalised **Naps** were employed in this study due to their well established and distinctive photophysical properties resulting in complexes **51** and **52**, respectively. Upon addition of stDNA, significant changes were observed in the absorption and emission profiles of the complex and binding constants in the order of 10^6 to 10^7 M⁻¹ were derived, which indicated a strong association of the complexes for DNA. Hypochromic shifts of 34% and 15% were observed for the **Nap**- and MLCT-centred absorption bands of **51**, respectively while for **52** a 30% hypochromic shift was observed for the broad MLCT based absorption band. These electronic changes were far more enhanced than for the model complex $[Ru(bpy)_3]^{2+}$, **18**, indicating the significance of the **Nap** unit for DNA binding, which was expected to occur through intercalation. While **51** displayed stronger binding affinities for DNA, only **52** was found to induce full photocleavage of plasmid DNA (Form I) into linear DNA (Form III) as described by gel electrophoresis experiments, further signifying the different manner in which the complexes bind to DNA.¹⁶

Elmes *et al.* extended the studies of **51** and **52** and developed the corresponding binuclear ruthenium complexes, connected *via* a **Nap** derived Tröger's base (**TB**) [Figure 1.26: **53** and **54**].¹⁵⁴ The **TB**, **55**, was originally synthesised by Julius Tröger and is characterised as a V-shaped molecule

which possesses chirality due to the stereogenic nitrogen atoms. Its structure was originally reported by Spielman, who concluded the C_2 axis of symmetry.^{155,156}

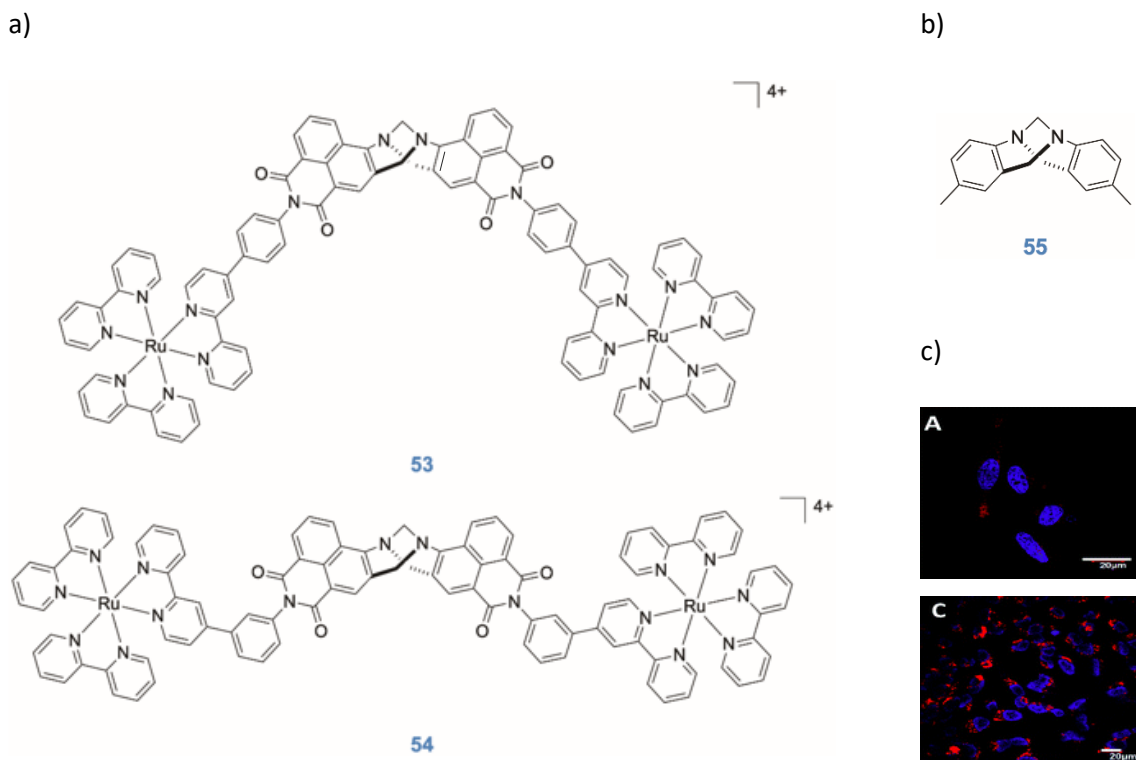


Figure 1.26: a) Binuclear Ru(II) complexes containing 4- (**53**) and 3-phenyl substituted (**54**) **Nap-TB** bridging ligands. The coordination spheres around the Ru(II)-centre are completed by **bpy** ligands. b) Original **TB** synthesised by Julius Tröger.¹⁵⁵ c) Red emission of **53** (top) and **54** (bottom) from HeLa cells after 4 and 24 h of incubation, respectively. Images from ref.¹⁵⁴

The first X-ray crystal structure was resolved by Wilcox who unveiled the orthogonal nature of the rings, respective to each other.¹⁵⁷ Due to its unique rigid structure, the **TB** has been employed in many areas of chemistry, including as a supramolecular scaffold.^{20,63,158} The **TBs** were synthesised by employing the 4-amino-**Naps** as a direct precursor which cyclises upon reaction with paraformaldehyde in acidic conditions. Both **53** and **54**, which differ in their connectivity on the phenyl group, act as efficient luminescent cellular probes with rapid cellular uptake, localising in the cytoplasm of HeLa cells. Initially, the complexes displayed no cellular toxicity, in contrast to other **Nap** derived **TBs** synthesised by Veale *et al.*, which were found to bind strongly to DNA in at high ionic strength and undergo cellular uptake.⁶³ However, later studies confirmed their cytotoxicity upon light irradiation (unpublished). Furthermore, **53** and **54** were shown to stabilise DNA using thermal denaturation experiments and **54** also promoted membrane blebbing in the cell line, highlighting potential therapeutic uses for these complexes.¹⁵⁴

Other research groups have also explored the use of metal based **Nap** systems for biological applications. Pope and co-workers have developed a series of biocompatible Re(I)-tricarbonyl

complexes **56-59** with fluorescent 4-amino-**Nap** ligands with structural variations in the *N*-imide position of the **Nap** [Figure 1.27]. These complexes emit in the green as their luminescence results from an ICT transitions arising from the 4-amino-**Nap**. The complexes and associated ligands were employed as cellular imaging agents in a range of cell lines where the cellular localisations were controlled by the nature of the **Nap**. Interestingly, the specific nature of the substituent on the imide moiety can drastically modulate the imaging and uptake properties, from poor uptake with imaging capabilities (**59**) to excellent uptake with distinct imaging in cellular grana (**57**). Of the series, **57** displayed the most attractive uptake and localisation.¹⁵⁹

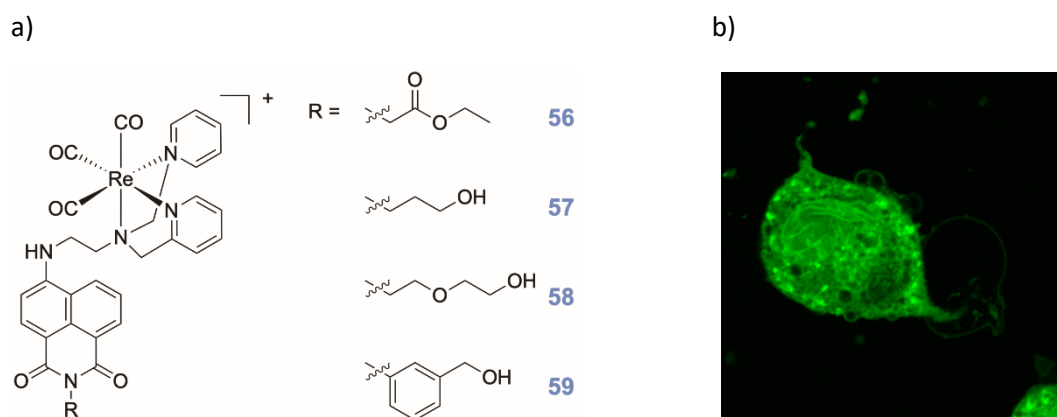


Figure 1.27: a) Chemical structures of Re(I)-Nap conjugates **56-59** by Pope and co-workers. Green luminescence of **57** from human osteoarthritic cell undergoing apoptosis. Images from ref.¹⁵⁹

All of the previous examples have explored systems containing monosubstituted **Nap** substituents. Incorporation of a bis-**Nap** motif as a ligand for Ru(II)-polypyridyl complexation was achieved by Ryan *et al.* who compared the effects of the mono- and bis-**Nap** complexes **60** and **61**, respectively on the DNA binding properties and cell viabilities upon photoexcitation [Figure 1.28].¹⁶⁰

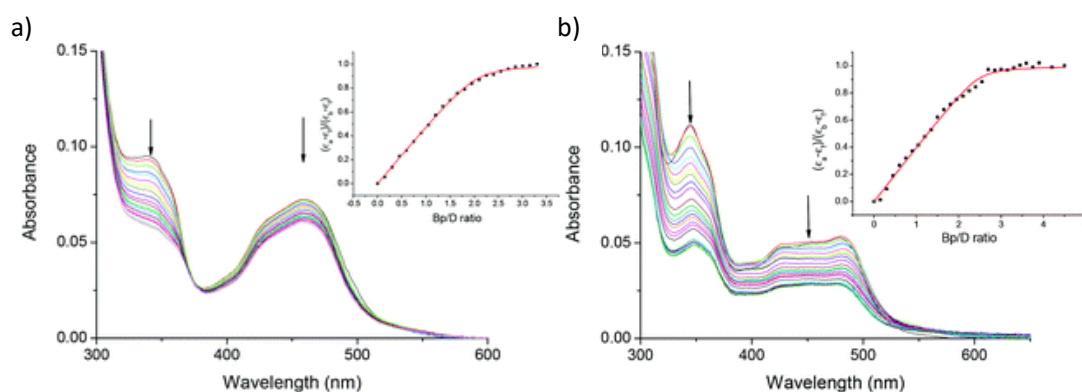
In this case, the **Nap** moieties are connected to the Ru(II)-centre *via* a flexible linker. Herein, an unexpected negative allosteric effect upon binding of the second **Nap** motif of **61** to DNA was observed where the Ru(II) centre was believed to be displaced from the DNA backbone. To the best of our knowledge, this is the only DNA binding study in the literature involving Ru(II)-**Nap** conjugates without any substitution in the 4-position of the **Nap**. Complex **60** displayed a primarily intercalative mode of binding with DNA, facilitated by electrostatic interactions between the Ru(II)-centre and anionic backbone with derived binding constants in the order of 10^6 M^{-1} . Furthermore, this complex exhibited efficient cleavage of the plasmid DNA upon photoexcitation.



60



61



f 160

Contrastingly, **61** displayed higher DNA binding constants in the order of 10^7 M^{-1} but only

As outlined, there exists a vast array of complexes which utilise the **Nap** motif for potential bioimaging and/or therapeutic applications. Understanding the fundamental photophysical properties of the systems is vital in determining potential applications. The Ru(II)-**Nap** conjugates display a distinct advantage over the more conventional Ru(II)-polypyridyl systems as their steady state absorption characterisation allow for individual resolution of both the **Nap** and Ru(II) MLCT-

centred transitions. The next section will outline recent advantages within the Gunnlaugsson research group that has inspired the work in this thesis.

1.7: Recent Advances in the Gunnlaugsson Group

Many collaborators have been involved in the research efforts focusing on Ru(II)-polypyridyl complexes within the Gunnlaugsson group, namely Prof. John M. Kelly (TCD), Prof. Susan Quinn (UCD), Prof. D. Clive Williams (TCD) and Prof. Robert B. P. Elmes (Maynooth University). The Gunnlaugsson supramolecular and medicinal chemistry research group is highly interdisciplinary in nature, with interests in organic fluorophores such as **Naps** and their corresponding higher-ordered materials for sensing and biological applications, transition metal complexes (*vide supra*), luminescent lanthanide-based materials and self-assembly processes. In this regard, recent advances within the Gunnlaugsson group from the past three years will next be presented.

Dr Delente *et al.* recently developed a first of its kind, aggregation induced emission (AIE) active **TB** derived from the 4-amino-**Nap** fluorophore, **62**, for sensing purposes [Figure 1.29]. The chemosensor, **62**, was designed for the detection of trace amounts of nitroaromatic compounds (NACs) in competitive media.¹⁶¹ This work was inspired by previous research outputs from our group which employed **TB**-coordination polymers for similar applications.^{162,163} The **TB-Nap**, adorned with terpyridine (**tpy**) groups at each end of the molecule displayed AIE properties in aqueous DMSO mixtures, resulting in an emission “switch-on” effect in these conditions. Its emissive state was attributed to the classic ICT transition typically observed for **Nap**-based systems containing the 4-amino functionality. Changes in the ICT based emission were monitored upon addition of a range of NACs where significant quenching of the emission was observed for select molecules, namely picric acid (PA) and 2,4-dinitrophenylhydrazine (2,4-DNP) [Figure 1.29]. Selectivity of **62** toward PA and 2,4-DNP was observed with AIE response being affected at concentrations of PA as low as 20 ppb.

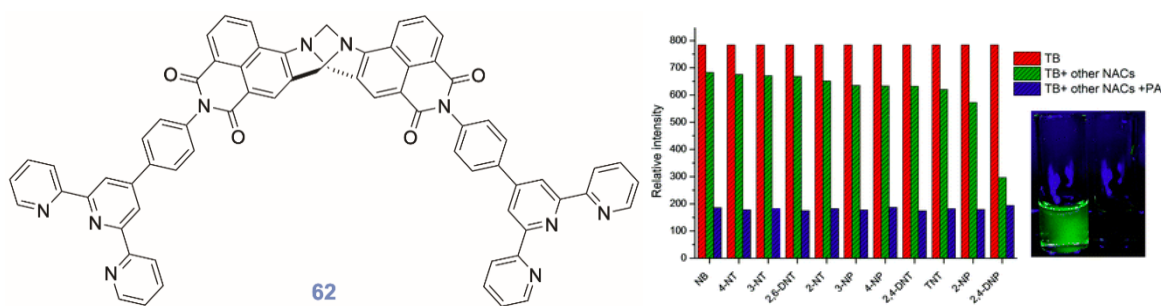


Figure 1.29: Chemical structure of chemosensor **62** and emission quenching selectivity toward different NACs in the presence and absence of PA. Far right: Emission quenching observed by eye under the UV light irradiation before (left) and after (right) addition of PA. Images from ref.¹⁶¹

Another interesting literature example from the Gunnlaugsson group came from Dr Poynton, who in collaboration with Kelly and others, have contributed to the understanding of the factors that control: i) the photooxidation of DNA by **TAP-dppz** complex, **30** and ii) the photoreactivity of **phen-dppz** complex, **21** with quadruplex DNA (*vide supra*), through the use of sophisticated transient absorption and transient resonance infrared spectroscopy, ultimately leading to knowledge of the respective fundamental processes for the global community.^{164,165} In this area, Dr Estalayo-Adrián recently explored the effect of alkyl chain length on the biological properties of a family of six Ru(II)-complexes, **63-68**, containing **phen** or **TAP** as the auxiliary ligands [Figure 1.30].^{166,167}

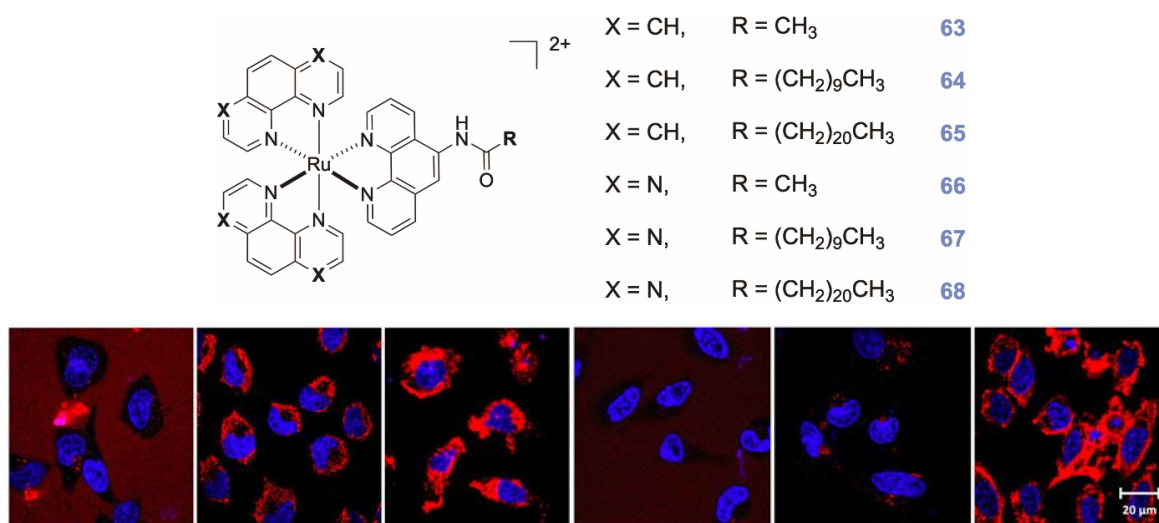


Figure 1.30: Chemical structures of Ru(II)-polypyridyl complexes **63-68** and confocal images of localisation in HeLa cells (sequential from left to right). Images from ref.¹⁶⁷

All complexes were found to bind DNA through steady state absorption and emission spectral changes. However, complexes **65** and **68** containing 21 carbon alkyl chain demonstrated unusual photophysical properties upon interaction with DNA. The reactivities of the remainder of the complexes could be rationalised in a similar manner to the [Ru(**phen**)₃]²⁺, **19**, and [Ru(**TAP**)₂**phen**]²⁺, **29**, parent complexes by classical fitting of the changes of MLCT absorption or emission. The cellular uptake of the complexes was found to be dependent on the length of the alkyl chain, with longer chains (increased lipophilicity) being more readily internalised into HeLa cells, specifically residing in the membrane. Complex **65** displayed the most attractive phototoxicity properties with a PI value of *ca.* 27, indicating its potential as a light-activated therapeutic agent.¹⁶⁷

A series of other **bpy** derived Ru(II)-**Nap** conjugates bearing nitro-groups in the 3- or 4-positions have been developed by Ryan *et al.* which were separated from the Ru(II)-centre by flexible alkyl chains of differing lengths. These complexes displayed the classical off-on light-switch behaviour when bound to DNA. The manuscript, titled “Hook, Line and Sinkers!...” analysed the

effect of the substitution pattern of the **Nap** (hook) and the length of the alkyl chain spacer (line) on the DNA binding properties. By careful choice of the “hook” and “line” the resulting complex (sinker) could easily report on the effectiveness of the interaction through changes in its luminescence properties.¹⁶⁸

The final class of Ru-**Nap** conjugates discussed in this section were developed by Elmes *et al.* who reported the biological profiling of a series of four complexes **69-72**, differing in their substitution in the 4-position of the **Nap** and bearing either **bpy** or **TAP** auxiliary ligands [Figure 1.31].

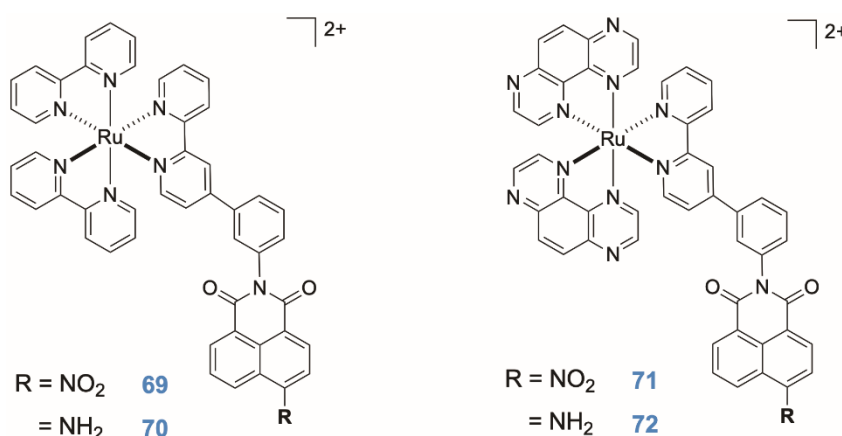


Figure 1.31: Chemical structures of Ru(II)-**Nap** conjugates **69-72** developed by Elmes *et al.*¹⁶⁹

This study positioned the **Nap** in a meta-arrangement around the rigid spacer and introduced the π -deficient **TAP** ligands as auxiliary ligands with an aim to improve the overall binding capabilities and phototoxicity. Interestingly, cellular localisation studies revealed that all complexes resided around the nucleus of HeLa cells while complex **69** was found in the cytoplasm and the **TAP** derivatives, **71** and **72** were localised in the nucleus. These **TAP** complexes were also found to be far more effective DNA photocleavage agents than their **bpy** counterparts, as expected due to their highly oxidising excited state character.¹⁶⁹

The final work highlighted in this section combines the biological and materials realms, detailing the development of a first of its kind, supramolecular hydrogel containing guanosine quadruplexes (G4) functionalised with chiral organic ligands, and lanthanide ions.¹⁷⁰ The G4 hydrogels were formed through hydrogen bonding interactions between four guanine units, which were arranged into a helical planar quartet and stabilized by group I cations. G4 were then functionalised with mannose-derived picolinic acid ligand (green) using boric acid chemistry [Figure 1.32]. The addition of Eu(III) to the developed hydrogels allowed for its binding to the picolinic acid unit of organic ligand where the metal acted as the cross-linker within the hydrogel. As a result the authors were able to observe the effect of the chiral helical arrangement of the G4 on the Eu(III)-

centred circularly polarised luminescence (CPL) response within the gels, **73**. The latter had self-healable properties and potential application as on-demand drug release systems.¹⁷⁰

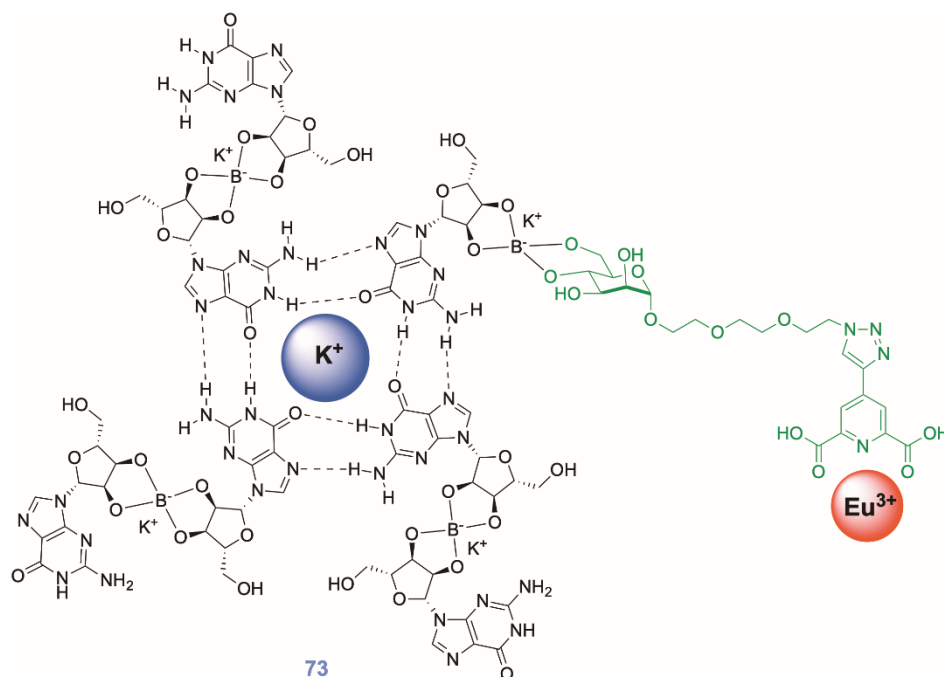


Figure 1.32: Multicomponent supramolecular Eu(III) gel, **73** developed by Kotova *et al.*¹⁷⁰

In addition to the research referenced in this section, the Gunnlaugsson group has also published several reviews for the scientific community in the preceding years.¹⁷¹⁻¹⁷³ The type of research conducted in the Gunnlaugsson group is high-impact, multidisciplinary and vast. The final section will describe the work carried out in this thesis.

1.8: Work Described in this Thesis

This Chapter has introduced a wealth of background literature relevant to the scope of this project, including current and potential new anti-cancer therapies and insights into the photophysical and photochemical processes of metal-based systems. This research project is concerned with the development of Ru(II)-polypyridyl complexes as DNA probes through conjugation of other biocompatible motifs to enhance therapeutic outcomes.

Chapter 2 describes the synthesis, photophysical, electrochemical and DNA binding properties of two new Ru(II)-complexes bearing **TAP** or **phen** auxiliary ligands and a **Nap**-chromophore attached directly to the metal through a coordinating phenanthroline moiety. No substitution pattern on the **Nap** was introduced for these systems, as it was anticipated that due to the spatial arrangement of the **Nap** in relation to the metal, the **Nap** would be well positioned to insert itself freely into the DNA cavity with a rotational degree of freedom between the intercalative **Nap** and coordinating **phen** moieties. Single crystal X-ray diffraction studies were

performed on the complexes and additionally, a third **bpy** analogous complex is included here for comparison purposes. These solid-state structure experiments were investigated to analyse the inter/intra-molecular interactions of the systems in the extended structure.

Chapter 3 details the synthesis and characterisation of a series of **Nap** ligands and the corresponding Ru(II)-**Nap** conjugates, differing in the substitution of the 4-position of the **Nap**. The photophysical, electrochemical and DNA binding properties of the ligands and metal complexes are presented, alongside a description of their solid-state structures, again, with emphasis on the interactions within the bulk. Some preliminary computational studies are provided which attempt to address the failed reaction series of a seemingly facile reduction of the 4-nitro substituted **Nap** both as the free ligand and when tethered to the Ru(II)-complex.

Chapter 4 details the attempted synthesis of binuclear Ru(II)-**TB** complex, as well as efforts toward the synthesis and development of extended aromatic **Nap**-like ligands, which were designed for metal complexations. The synthesis and photophysical characterisation of a cyclometallated Ir(III)-**Nap** complex is also provided, which is structurally related to the complexes presented in Chapter 2. This complex was designed with the aim of exploring the effect of metal substitution on the DNA binding parameters. However, due to the reduced overall charge of this complex, aqueous solubility was not observed and as such, the DNA binding studies were not performed.

Chapter 5 discusses the X-ray crystal structures of several **Nap** molecules resolved throughout the course of this research is presented with a summary of the overarching interactions shared among most **Naps**. Furthermore, the X-Ray crystal structures of enantiopure triple-stranded Eu(III)-helicate were solved and refined, and are presented here. These novel complexes are structurally related to other Eu(III)-helicates, previously developed, of which, the solid-state structures have proved elusive to our research group for the past 15 years. These structures are presented as connectivity models only, due to the weak diffraction observed.

Chapter 6 provides a summary of the overall conclusions for this body of research with specific reference to the potential of Ru(II)-**Nap** conjugates as photoreactive nucleic acid targeting agents.

Finally, Chapter 7 presents general experimental techniques and methods employed throughout the course of this research, alongside structural characterisation, and general procedures, for the compounds discussed in this thesis. Finally, an accompanying electronic document containing experimental and structural refinement details for the solid-state structures detailed in this thesis is submitted in addition to this main document.

Chapter 2:

Ruthenium(II)-Polypyridyl
Complexes Containing a Non-
Substituted **PhenNap** Ligand

2.1: Introduction

As was outlined in the introduction chapter, luminescent d^6 transition metal ion complexes display a wealth of biological applications, including acting as structural cellular probes, as well as providing theranostic and therapeutic benefits.^{2,3,67} One family of such complexes is the Ru(II)-polypyridyl complexes which offer a range of favourable optical, photophysical and chemical properties highly influenced by the design of the coordinating ligands.^{174,175} The attractive photochemical and photophysical properties of Ru(II)-polypyridyl complexes and their use as DNA-targeting agents has been discussed in detail in the preceding chapter.¹⁷⁶ This chapter aims to develop Ru(II)-polypyridyl-1,8-naphthalimide (Ru(II)-**Nap**) conjugates and explore their use as spectroscopic probes for DNA and their potential as DNA-reactive agents. Research into the DNA binding properties and biological profiling of Ru(II)-**Nap** conjugates has been explored previously by our group; however, in these cases, the **Nap** unit was coordinated to the Ru(II) centre either *via* long, flexible linkers or by rigid aromatic spacer groups.^{16,160,168,169} Furthermore, in all of these studies except one, the **Nap** unit was derivatised to its 4-nitro- or 4-amino-form.^{16,168,169} This study aims to delineate the effect of a simple, non-substituted **Nap** chromophore on the DNA binding properties, by directly attaching the **Nap** to the Ru(II) centre through 1,10-phenanthroline coordinating motif.

Ru(II)-polypyridyl complexes typically exert their anti-cancer activity *via* direct interaction with DNA and subsequent inhibition of DNA-templated cellular functions.² Often, an extended aromatic ligand is chosen to intercalate between the base pairs of DNA, while the Ru(II) centre facilitates this interaction through electrostatic attraction with the anionic back bone of DNA. This is the mode of action for the extensively studied archetypes, the “light-switch” complexes, which include $[\text{Ru}(\text{L})_2\text{dppz}]^{2+}$, where L = 2,2'-bipyridine (**bpy**), **20**, 1,10-phenanthroline (**phen**), **40**, or 1,4,5,8-tetraazaphenanthrene (**TAP**), **49**; **dppz** = dipyrido[3,2-a:2',3'-c]phenazine [Figure 2.1]. The attractive photophysical properties of Ru(II)-**dppz** complexes (*vide supra*, 1.4.1) were debuted almost 40 years ago,⁸² and the complexes were discovered as molecular probes for DNA shortly thereafter,⁶ due to their solvent dependent luminescence intensities, and have been subject to much investigation ever since.^{8,80,81,84,94,112,177-179}

The **dppz**-functionalised metal systems exhibit complex binding modes to DNA, unique for both the Λ - or Δ - isomers (*vide supra*, 1.4.2). The photophysical properties of **20** and **21** are specific to their environments and overall, they display poor luminescence in aqueous media. The “light-switch” effect arises upon intercalation of the **dppz** ligand between the DNA base pairs as significant π -overlap between the bases and ligand protects the phenazine portion of the ligand from the aqueous environment, minimising energy loss to through nonradiative pathways, and thus the MLCT centred luminescence is “switched-on”. An inverse behaviour is observed for the **dppz**-

analogue **30** which contains the π -deficient polyazaaromatic auxiliary ligands, **TAP**. Herein, the lowest lying excited state is localised on the **TAP** ligands, as opposed to the **dppz** ligands for **20** and **21**. As such, the luminescence of **30** decreases upon DNA binding as it is capable of photo-oxidation of DNA, ultimately resulting in covalent attachment.^{89,112,164,180}

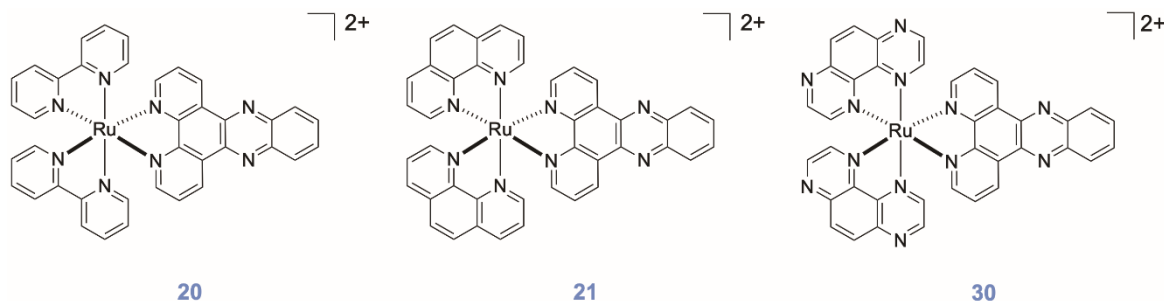


Figure 2.1: Chemical structures of “light-switch” compounds $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$, **20**, $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$, **21**, and $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$, **30**.

1,8-Naphthalimides (**Nap**) display many uses in medicinal chemistry, including acting as inhibitors of DNA-templated functions and associated enzymes, and as fluorescent imaging agents.^{64,181} Research is extremely topical in this area with publications approaching 500 annually for the previous five years. The flat, planar **Nap** moiety interacts with DNA *via* intercalation, as is the case for the mono- and bis-intercalators, Amonafide, **36** and Elinafide, **37** [Figure 2.2], which display excellent DNA binding affinities and toxicity toward a wide range of cancer cells.^{130,131} **37** bis-intercalates DNA with the diamine linker residing in the major groove and it had progressed to phase II clinical trials for the treatment of ovarian cancer. It was ultimately discontinued due to elevated neuromuscular toxicity effects.¹⁴⁰ As part of an ongoing investigation of **Nap**-based ligands, Ryan, Gunnlaugsson and Quinn have developed a series of bis-**Naps** conjugated to a Ru(II) metal centre and explored their use as DNA probes. The **Nap** moiety differed in its nitro-substitution position and aliphatic chain lengths. The most efficient DNA probe of the series was **74**, which displayed an 11-fold luminescence enhancement when bound to DNA *versus* in the free form. This study highlights the benefit of employing bichromophoric $[\text{Ru}(\text{L})_2(\text{Nap})]^{2+}$ systems as: i) sensitive probes for DNA which is reported by spectroscopic changes in characteristic photophysical properties, and, ii) therapeutic agents by combining known anticancer properties of **Naps**.¹⁶⁸

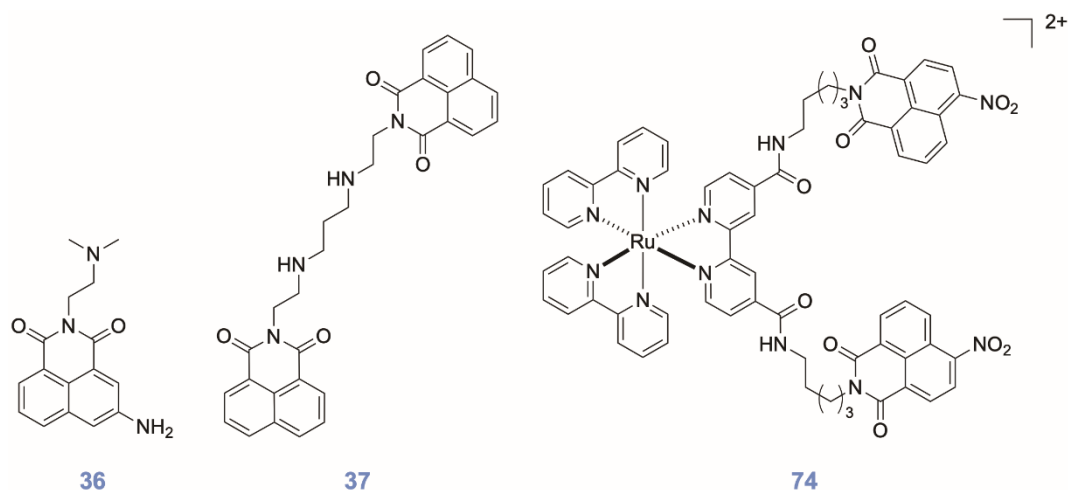


Figure 2.2: Chemical structures of Amonafide, **36**, Elinafide, **37**, and Ru-C₅-2**Nap**-4NO₂, **74**.

Herein, a discussion of the synthesis and the characterisation of two structurally related Ru(II)-polypyridyl complexes is presented, followed by a discussion of the steady state absorption and emission properties. The DNA binding behaviour was also evaluated through monitoring the changes in the photophysical properties of the complex, where the MLCT transition was probed, as well as by thermal denaturation, CD and LD experiments. The design of these complexes was inspired by previous work within the Gunnlaugsson group that was described in the preceding section, with structural modifications to enhance DNA binding and to analyse the direct effect of the **Nap**-based ligand on the binding properties. Compared with the “light-switch” complexes, **20** and **21**, an extra degree of rotational freedom was introduced in the anticipated intercalating moiety. The intercalator constituted a simple, non-substituted **Nap** which is affixed to the Ru(II) centre through a coordinating **phen** ligand. The two polypyridyl complexes were evaluated bearing **phen** or **TAP** ligands, respectively [Figure 2.3]. The **phen** derivative, **75**, was expected to interact with DNA by a combination of both intercalation and electrostatic interactions, and as such, it could be used as a luminescent DNA probe. The DNA binding properties of the π -deficient **TAP** derivative, **76**, were also investigated, as the complex was expected to display phototoxicity due to the presence of a strongly oxidising excited state as observed for **30**.

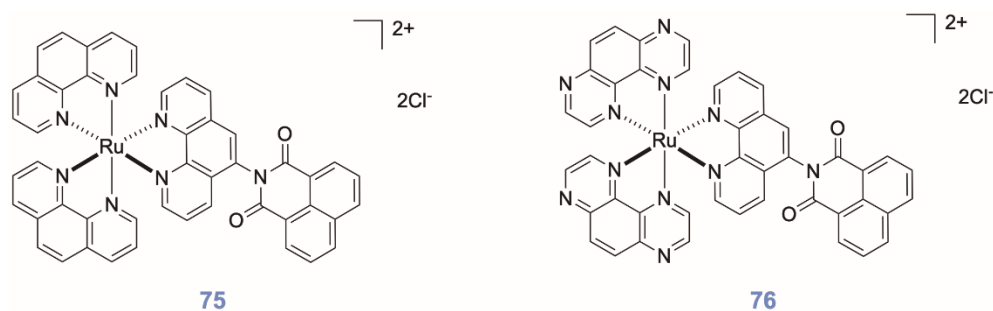
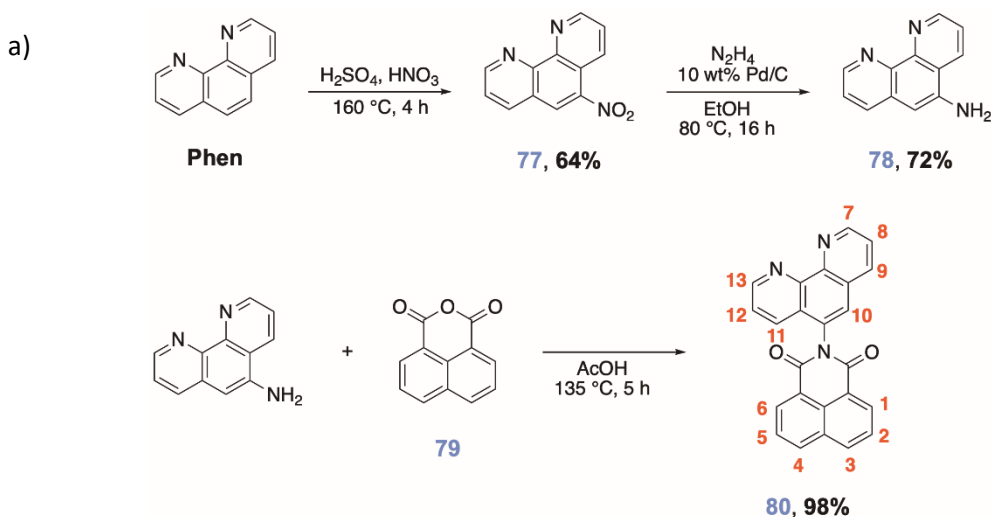


Figure 2.3: Chemical structures of **75** and **76**.

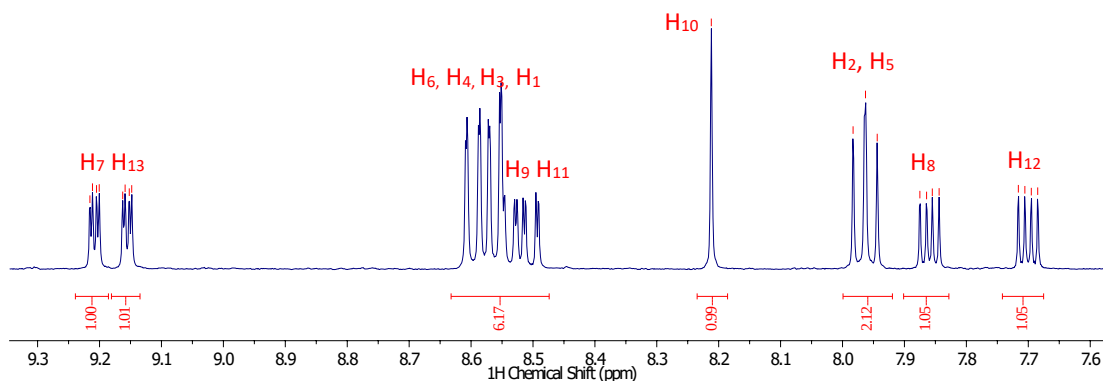
2.2: Synthesis and Characterisation of **75** and **76**

Ru(II)-polypyridyl complexes **75** and **76** were synthesised according to modified literature procedures for $[\text{Ru}(\text{L})_2(\text{L}')]]$ where $\text{L} = 1,10\text{-phenanthroline (phen)}$ or $1,4,5,8\text{-tetraazaphenanthrene (TAP)}$, and $\text{L}' = N\text{-(1,10-phenanthroline)-naphthalene-1,8-dicarboximide (PhenNap)}$.^{182,183} First, the **PhenNap** ligand, **80**, was obtained by a three step reaction sequence involving a condensation reaction between naphthalic anhydride and 5-aminophenanthroline [Figure 2.4 a)].^{184,185}

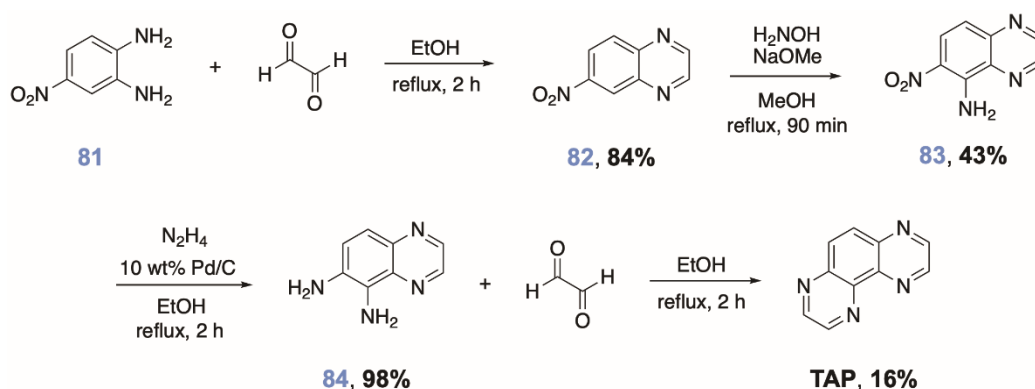
5-Aminophenanthroline, **78**, was obtained by functionalisation of **phen**. First, 1,10-phenanthroline was dissolved in sulfuric acid and nitrated *via* dropwise addition of nitric acid at 160 °C and the mixture was reacted for four hours. The solution was then neutralised using 6 M NaOH on ice. The resulting yellow precipitate was collected and washed several times with water until the washings ran colourless and 5-nitro-1,10-phenanthroline, **77**, was isolated in 64% yield. **77** was then reduced to the 5-amino-1,10-phenanthroline, **78**, *via* catalytic reduction employing 10 wt% Pd/C and hydrazine monohydrate. To achieve this, hydrazine monohydrate was added dropwise to a suspension of **77** and Pd/C in EtOH, and the resulting mixture was heated at 80 °C overnight. The mixture was cooled to room temperature and isolated *via* vacuum filtration through celite. The resulting residue was washed with EtOH and the solvent was removed under reduced pressure. The yellow/orange powder was washed with water to remove excess hydrazine and the desired product, **78**, was isolated in 72% yield. Subsequent reaction of **78** with naphthalic anhydride, **79**, was achieved *via* a condensation reaction in acetic acid at 135 °C for five hours in a pressure vessel to yield the target ligand, **PhenNap**, **80**. Water was added to aid precipitation and the product was isolated as a beige powder in 98% yield.



b)

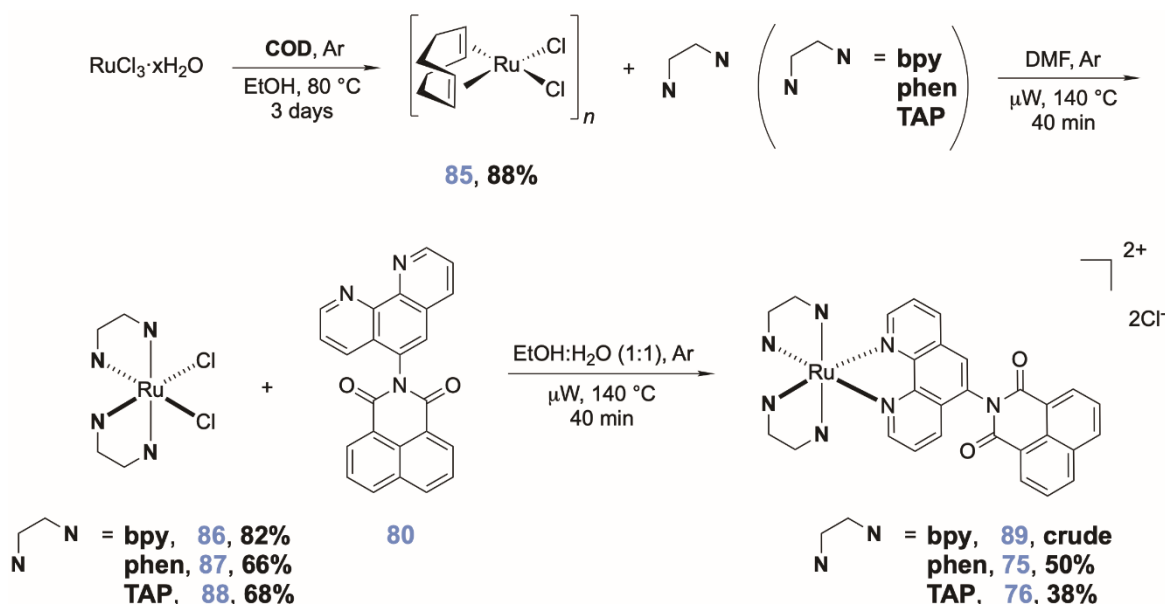
Figure 2.4: a) Synthetic pathway to-, and b) the ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$), of **80**.

The **TAP** auxiliary ligand was synthesised according to modified literature procedures, as summarised in Scheme 2.1.^{186,187} The first step involved a double condensation reaction between 4-nitro-1,2-phenylenediamine, **81**, and glyoxal (40 wt% in water) in refluxing ethanol to yield 6-nitroquinoxaline, **82**, in 84% yield. Using a mixture of hydroxylamine and freshly prepared sodium methoxide, direct amination of **82** in the 5-position was achieved *via* nucleophilic aromatic substitution to yield 5-amino-6-nitroquinoxaline, **83**, in 43% yield. This product was reduced to 5,6-diaminoquinoxaline, **84**, *via* catalytic reduction using Pd/C and hydrazine monohydrate in EtOH at reflux, in a similar manner described for the phenanthroline analogue **77** above, to yield the desired product **85** as a deep red solid in 98% yield. A double condensation reaction with glyoxal was performed to afford the target ligand, **TAP**, in 16% yield after purification by flash column chromatography on silica, eluting with DCM:acetone (8:2), and recrystallisation from EtOH.^{186,187} Full characterisation details of the targets **PhenNap**, **80** and **TAP** are available in the appendix [Figures A2.1–A2.7] and experimental section [Chapter 6].

Scheme 2.1: Synthetic pathway for the **TAP** ligand.^{186,187}

As previously mentioned, complexes **75** and **76** bearing **phen** and **TAP** ligands, respectively, and their interactions with DNA are the focus of investigation of this chapter.^{182,183} The structurally similar **bpy** complex, **89** was also synthesised, however, this complex is included for X-ray crystallographic comparison purposes only. As such, the synthesis of all three complexes (**75**, **76**

and **89**) is described in this section and the conditions for complexation to Ru(II) are summarised in Scheme 2.2. The precursor complex, $[\text{Ru}(\eta^4\text{-(COD)Cl}_2)]_n$, **85**, (where **COD** = 1,5-cyclooctadiene) was obtained in 88% yield from reaction of $\text{RuCl}_3 \cdot x(\text{H}_2\text{O})$ and **COD** (20 eq.) in refluxing EtOH for three days under an inert atmosphere. The polypyridyl ligands, **bpy**, **phen** and **TAP**, were complexed to Ru(II) in DMF under microwave irradiation at 140 °C for 40 min to yield the appropriate Ru(II)-precursor complexes, **86-88**, with the chloride coordinating ligands in a *cis*-orientation. All three complexes, **75**, **76** and **89** were synthesised from the appropriate precursor complexes, **86-88**, by displacement of the chloride ions with **PhenNap**, **80**, in aqueous ethanol under microwave irradiation. Further purification was necessary for complexes **75** and **76** and column chromatography on neutral alumina eluting with 0-4% H_2O in MeCN was performed to obtain the pure target complexes in yields of 38-50%. Complex **89** was synthesised on a small scale and crystals suitable for single crystal X-ray diffraction analysis were obtained by slow evaporation of deuterated MeCN directly from the NMR tube (*vide infra*, Section 2.3.3) [Scheme 2.2].



Scheme 2.2: Synthetic pathway for Ru(II)-polypyridyl complexes **75**, **76** and **89**.

Full characterisation was performed on both **75** and **76** including ^1H and ^{13}C NMR spectroscopy, elemental analysis, IR spectroscopy, HRMS and melting point analyses. The ^1H NMR spectra are displayed below [Figures: 2.5 and 2.6]. Complex splitting patterns were observed in the aromatic regions between 7.55-8.75 ppm with integration totalling 29 protons for **75** while **76** displayed 25 protons in the region of 7.60-9.10 ppm, consistent with the respective molecular formulae. Furthermore, the ^{13}C NMR spectrum of **75** revealed 48 carbon signals in the region of 122-165 ppm [Figure A2.8] while for **76**, 43 out of a total of 44 carbon signals were resolved [Figure A2.12]. The assignment of the metal ion complexes **75** and **76** were made through comparison of

the ligand ^1H NMR spectrum [Figure 2.4(b)] and through 2D homo- and heteronuclear NMR spectroscopy.

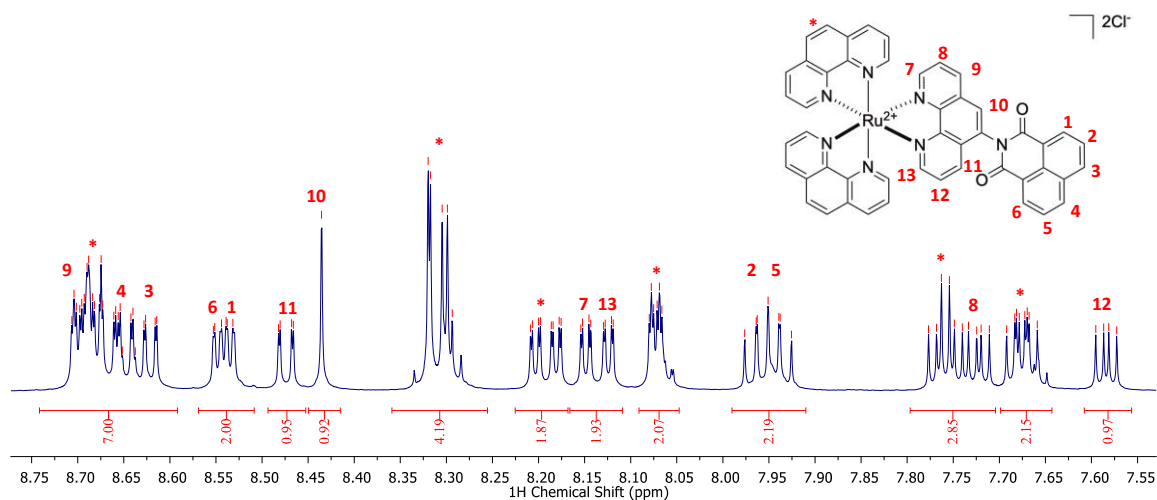


Figure 2.5: The ^1H NMR spectrum (600 MHz, CD_3CN) of **75**.

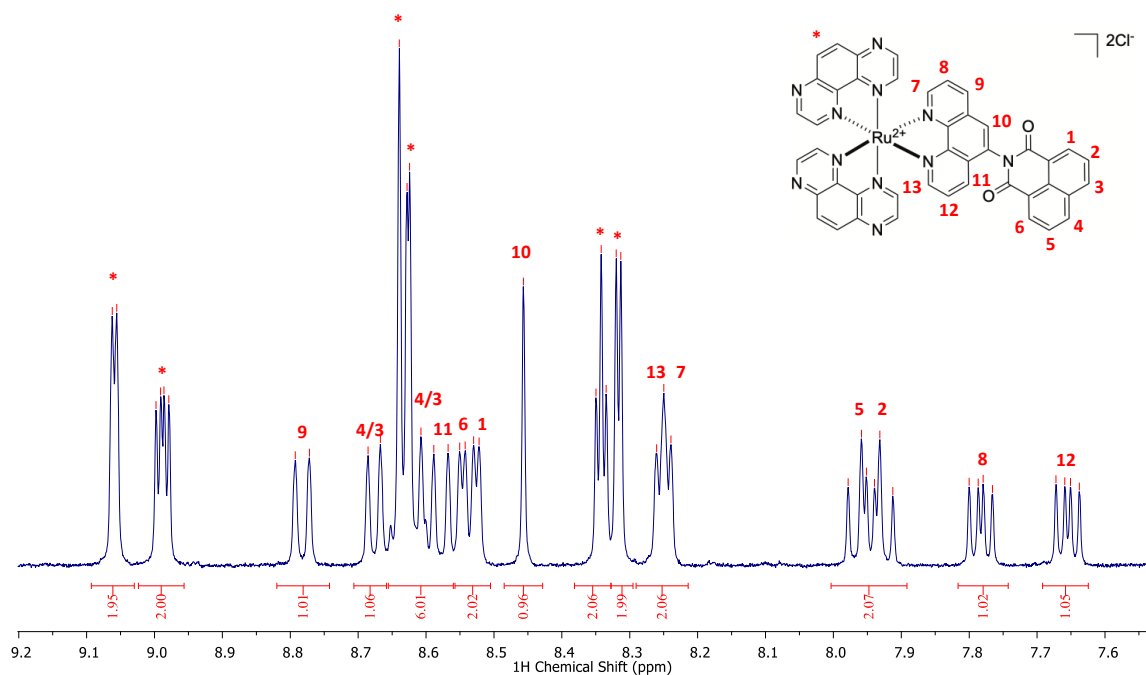


Figure 2.6: The ^1H NMR spectrum (400 MHz, CD_3CN) of **76**

Mass spectrometry analysis is an essential technique for the characterisation of products and ESI-HRMS of the two complexes further supported their formations. **75** and **76** displayed peaks at m/z of 865.7091 and 420.5618 corresponding to the $[\text{M}]^+$ and $[\text{M}]^{2+}$ ions, respectively. The overlap of the calculated and experimental isotopic distribution patterns for the Ru(II) complexes indicate the successful formation of the products [Figure 2.7].

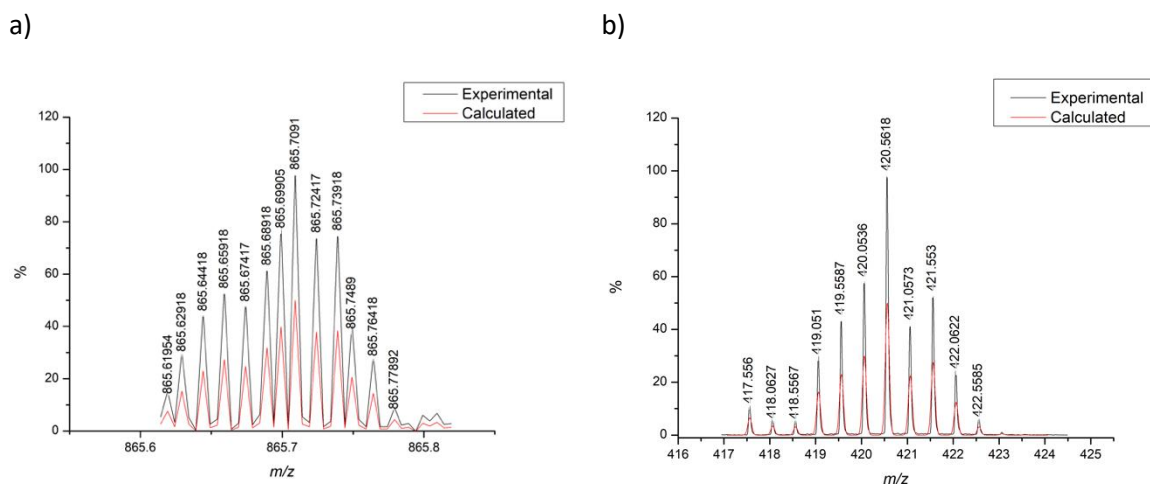


Figure 2.7: Experimental (black) and calculated (red) isotopic distribution patterns for a) **75** ($[M]^+$) and b) **76** ($[M]^{2+}$) from ESI-MS.

Full characterisation was also performed by other techniques including melting point analysis, IR and elemental analysis: the individual details of which can be viewed in the appendix [Figures A2.8–A2.14] and experimental sections.

2.3: Crystallographic Studies of **75**, **76** and **89**

Having successfully synthesised complexes **75** and **76**, investigation into the solid-state structure of complexes **75**, **76** as well as complex **89** were undertaken. As solution studies involved analyses of the complexes in their dichloride forms, attempts were made to crystallise both complexes as chloride salts, however, where necessary, anion exchange techniques were employed to allow for successful crystallisations with alternative counterions. All crystal data sets were collected independently on either a Bruker APEX DUO (MoK α or CuK α source) or D8 Quest ECO (MoK α source) instrument. Details of experiments are provided in the Experimental Section [Chapter 6]. Full data corresponding to the collection of reflections and structure refinement from all three crystal structure data sets is presented in Table A2.15 in the appendix.

2.3.1: Crystallographic Studies of **75**

Many attempts were made to crystallize **75** as the chloride salt, including the use of slow evaporation from a solution of MeCN and slow diffusion of multiple antisolvents into solutions of **75** in MeCN and MeOH. Unfortunately, all these attempts proved unsuccessful. These slow diffusion crystallisation attempts yielded only amorphous or polycrystalline material upon diffusion into MeCN solution, while the complex remained in solution upon diffusion into MeOH. As such, the complex was converted to the PF₆[−] salt *via* anion exchange as it was speculated that the chloride anion did not facilitate adequate packing arrangements in the bulk structure. To accomplish this,

the chloride salt was dissolved in water and precipitated as the PF_6^- salt by addition of a saturated aqueous NH_4PF_6 solution. The resulting precipitate was washed three times through the use of centrifugation with water and dried *in vacuo* to yield **75**(PF_6)₂ as an orange powder.

75(PF_6)₂ (10 mg) was dissolved in minimal MeCN and single crystals were obtained by slow diffusion of Et_2O into the mother liquor. The crystal structure was solved in the triclinic space group $P\bar{1}$ and the asymmetric unit contains one unique molecule of **75**, two PF_6^- counterions, and 3.5 MeCN molecules. A thermal ellipsoid representation of the major occupied cationic Ru(II) species is shown here for simplicity with heteroatoms labelled [Figure 2.8].

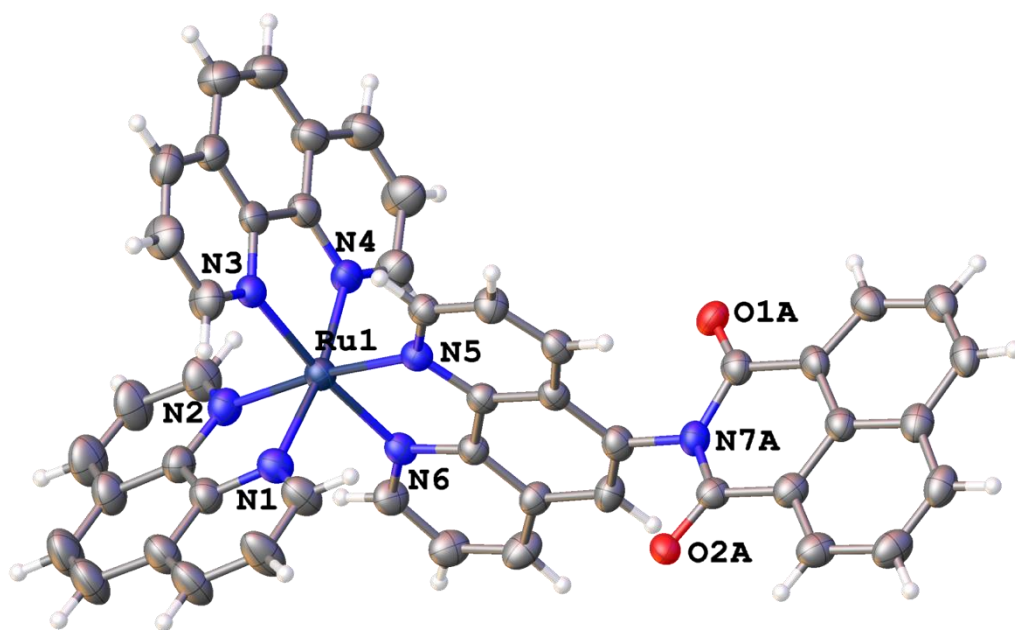


Figure 2.8: ORTEP style representation of the complex cation in the single crystal X-ray structure of {[**75**](PF_6)₂·3.5(CH_3CN)}, including labels for heteroatoms. Disorder, counterions and solvent molecules in the structure are omitted for clarity. Thermal ellipsoids are shown at 50% probability. Colour code: Ru – Navy, N – Blue, O – Red, C – Grey, H – White.

The full asymmetric unit can be viewed in Figure A2.16 (a). Positional disorder was observed over the **Nap** moiety with 63% and 34% occupancies. Further positional disorder was found within one of the counterions which was disordered over two positions with 58% and 42% occupancies; such disorder is typical for PF_6^- . The solvent molecules were modelled as one fully occupied and six partially occupied MeCN molecules with occupancies (4 molecules at 25% occupancy, one at 50% occupancy and occupancy 75%). The solid-state structure analysis of **75** revealed an octahedral geometry around the Ru(II) centre typical for tris(bidentate) transition metal complexes. This geometry is formed through coordination of six nitrogen atoms to the Ru(II) centre: four nitrogen atoms from coordinating **phen** auxiliary ligands and two nitrogen atoms from the **phen**-functionalised **Nap**. The Ru-N bond lengths range from 2.048(6) to 2.069(6) Å and the N–Ru–N bond

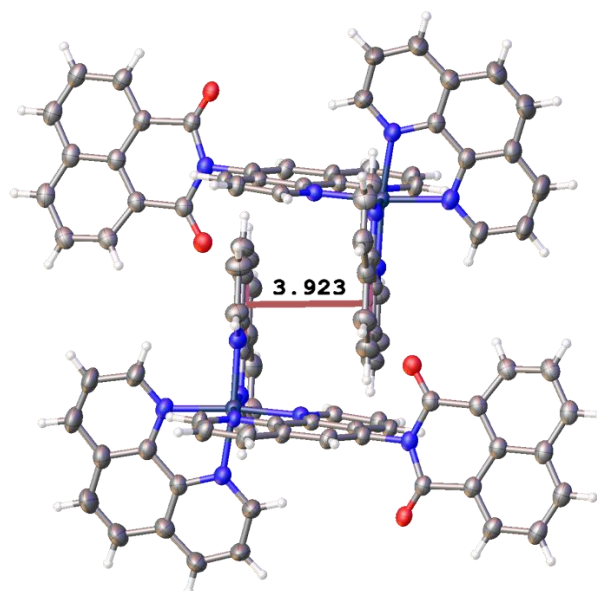
angles range from 79.5(2) to 96.5(2)° [Table 2.1]. These bond lengths are in agreement with recent Ru(II) polypyridyl complexes in the literature.^{188,97}

Table 2.1: Summary of bond lengths and bond angles for selected region of **75**.

Atoms			Length/Å	Atoms				Angle/°	Atoms				Angle/°
Ru1	N5		2.050(6)	N5	Ru1	N1		94.8(2)	N6	Ru1	N1		91.0(2)
Ru1	N2		2.069(6)	N5	Ru1	N3		94.3(2)	N6	Ru1	N4		96.5(2)
Ru1	N1		2.062(6)	N5	Ru1	N4		91.8(2)	N3	Ru1	N2		90.1(2)
Ru1	N6		2.048(6)	N1	Ru1	N2		79.8(2)	N3	Ru1	N1		92.6(2)
Ru1	N3		2.056(6)	N6	Ru1	N5		79.5(2)	N4	Ru1	N2		94.0(2)
Ru1	N4		2.051(6)	N6	Ru1	N2		96.4(2)	N4	Ru1	N3		80.5(2)

Inter- and intramolecular interactions are useful to analyse in the bulk structure as they may provide an explanation as to possible observed phenomena such as luminescence quenching pathways. Hydrogen bonding interactions were observed in **75** between the PF₆[−] counterions and C–H donor atoms of a **phen** ligand as well as between O2 and H49b, which corresponds to a hydrogen atom of a neighbouring MeCN solvent molecule [Figure A2.16 b) and Table A2.17]. The presence of a hydrogen bonding interaction between the imide oxygen atom and solvent may suggest a pathway to the luminescence quenching observed in aqueous media which will be further discussed in Section 2.5.2. Interestingly in this system, two distinct intermolecular π – π stacking interactions were observed. The stacking distances were obtained by analysing the distance between the mean planes of the central rings of the stacking motifs. Firstly, π – π stacking interactions between the flat, planar auxiliary **phen** ligands of two symmetry related molecules was observed. The closest centroid-centroid distance between two **phen** moieties (C16–C21 to C16i–C21i, where i is the symmetry transformation: 1-x, 1-y, -z) is 3.923 Å, with an angle of 0.0(4)° between the planes and a slippage of 1.620 Å [Figure 2.9(a)]. Secondly, π – π stacking interactions among the flat, planar **Nap** moieties (C38–42,47 to C38–42,47i where i is the symmetry transformation: 1-x, -y, 1-z) is 3.683 Å, with an angle of 0.0(9)° and a slippage of 1.208 Å between the planes [Figure 2.9(b)].

a)



b)

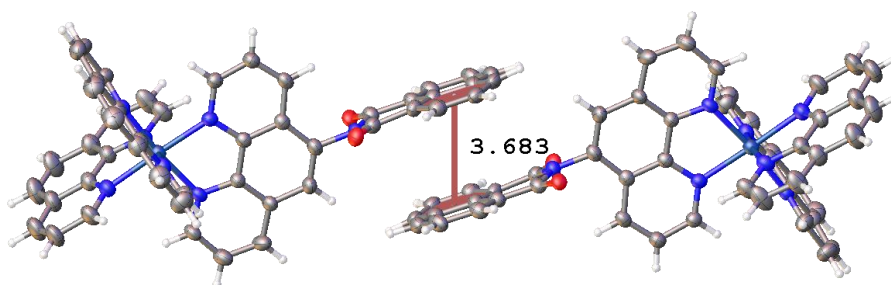


Figure 2.9: ORTEP style representation of the paired structure of **75** showing a) π - π stacking between **phen** auxiliary ligands and b) π - π stacking between the **Nap** moieties. Positional disorder, solvent molecules and counterions are omitted for clarity. Thermal ellipsoids are shown at 50% probability. Distances between the planes denoted by red line / Å.

Another interesting observation (most noticeable from the images presented in Figure 2.9) is the twisted nature between the planes of the **Nap** and the coordinating **phen** moiety within the non-auxiliary ligand. This twisted orientation is induced by a large steric clash between the imide oxygen and adjacent C-H of the **phen** moiety. The plane-to-plane twist angle was found to be 76.3(8) and 83.4(13)° for the major and minor occupied species, respectively. As will be discussed in Section 2.5.2, this can indicate an electronic decoupling between these two moieties and can aid the understanding of the electronic transitions between the two systems.

2.3.2: Crystallographic Studies of **76**

Purple block crystals of **76**(Cl)₂ were obtained from diffusion of Et₂O into a solution of the complex in MeOH. The crystal was solved in the monoclinic space group C2/c and the asymmetric unit contains one unique molecule of **76**, two Cl⁻ counterions, 3.5 water molecules, a MeOH molecule (occupancy 0.5) and a Et₂O molecule (occupancy 0.5). A thermal ellipsoid representation

of the major occupied cationic Ru(II) species is shown for simplicity with heteroatoms labelled [Figure 2.10]. The full asymmetric unit can be viewed in Figure A2.18.

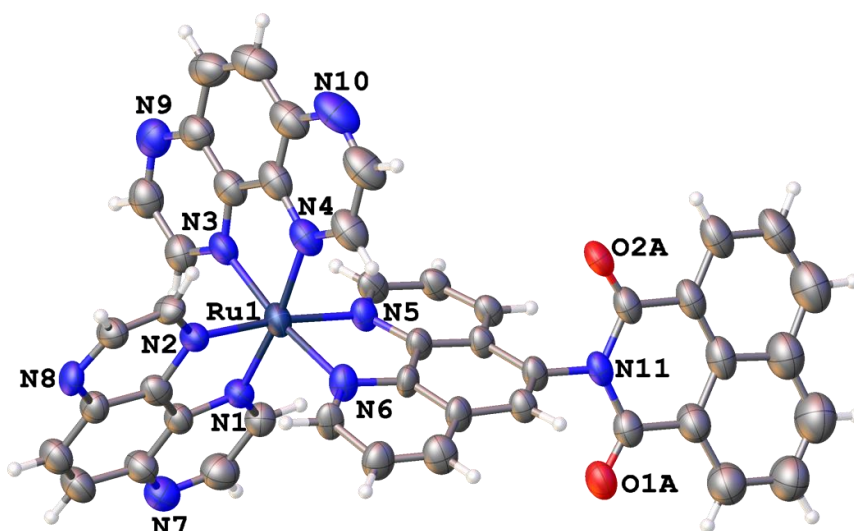


Figure 2.10: ORTEP style representation of the complex cation in the single crystal X-ray structure of {[76]Cl₂·3.5(H₂O)·0.5(CH₃OH)·0.5(C₄H₁₀O)}, including labels for heteroatoms. Positional disorder in the complex, counterions and solvents are omitted for clarity. Thermal ellipsoids are shown at 50% probability.

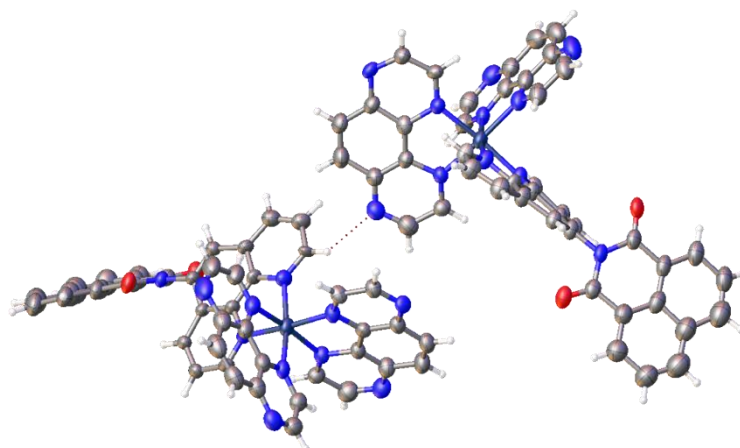
Positional disorder was observed over the **Nap** moiety with 51% and 49% occupancies. Further positional disorder was found within one of the auxiliary **TAP** ligands, which was disordered over two positions with 51% and 49% occupancies. All solvent molecules and counterions displayed occupancy factors of less than unity. The octahedral geometry of **76**(Cl)₂ around the Ru(II) centre was again found to be typical for tris(bidentate) transition metal complexes, formed through coordination of the Ru(II) ion to six nitrogen atoms in total: four from coordinating **TAP** auxiliary ligands and two from the **phen**-functionalised **Nap**. The Ru–N bond lengths ranged from 2.02(3) to 2.17(3) Å and the N–Ru–N bond angles ranged from 76.9(10) to 101.2(7)° [Table 2.2]. These bond lengths are in agreement with recent Ru(II) polypyridyl complexes in the literature,^{188,97} but have a larger spread than those found in **75**, possibly due to the presence of more thermal and positional disorder in this crystal compared to the **phen** analogue.

Table 2.2: Summary of bond lengths and bond angles for selected region of **76**.

Atoms	Length/Å	Atoms	Angle/°	Atoms	Angle/°
Ru1 N5	2.02(3)	N1 Ru1 N2	83.9(15)	N6 Ru1 N1	97.2(11)
Ru1 N2	2.066(8)	N4 Ru1 N3	79.8(2)	N6 Ru1 N4	91.3(8)
Ru1 N1	2.084(8)	N6 Ru1 N5	76.9(10)	N3 Ru1 N2	86.0(10)
Ru1 N6	2.17(3)	N5 Ru1 N4	89.1(9)	N3 Ru1 N1	91.7(8)
Ru1 N3	2.048(5)	N5 Ru1 N1	93.8(13)	N4 Ru1 N2	94.2(11)
Ru1 N4	2.0(6)	N6 Ru1 N2	96.3(13)	N5 Ru1 N3	101.2(7)

Within the extended structure, hydrogen bonding interactions between solvent molecules and counterions dominate. Hydrogen bonding interactions among neighbouring water molecules result in a networked aqueous channel throughout the structure forming water pockets [Figure A2.19]. The solvent molecules exhibit thermal disorder and were refined using suitable ISOR and DFIX restraints. Hydrogen atoms of water molecules were placed using AFIX instructions and positions of hydrogen atoms were optimised to maximise realistic hydrogen bonding networks. Full details of hydrogen bonding interactions within the bulk structure can be viewed in the appendices [Table A2.20]. Notable intermolecular interactions include a hydrogen bonding interaction ($d = 3.189 \text{ \AA}$) between the external N7 of a **TAP** ligand and a C-H donor atom of the **phen** moiety of the **PhenNap** coordinating species [Figure 2.11(a)]. Furthermore, π - π stacking interactions exist between two planar **Nap** moieties on neighbouring structures in a head-to-tail orientation, typical for the **Nap** motif.¹⁸⁹ The closest centroid-centroid distance (C44A–C48A,C50A to C44A–C48A,C50Ai, where *i* is the symmetry transformation: 1-x, 1-y, -z) is $3.050(13) \text{ \AA}$, with an angle of 0° between the planes and a slippage distance of $1.250 \text{ \AA}$.

a)



b)

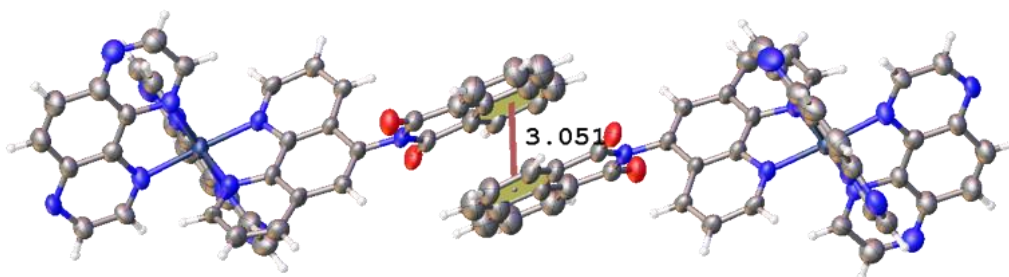


Figure 2.11: ORTEP representation of intermolecular interactions involving **76** in the solid state. Thermal ellipsoids are shown at 50% probability. A) Hydrogen bonding interaction between N7 of one complex and H26a of a neighbouring complex of **76**, represented by red dotted line. Structures shown normal to the O11 plane. B) π - π stacking interaction between **Nap** moieties of neighbouring complexes. Distances between the planes denoted by red line / $\text{\AA}$. Positional disorder in the complex, counterions and solvents are omitted for clarity.

The plane-to-plane twist angle between the planes of the **Nap** and the coordinating **phen** moiety within the **PhenNap** ligand were found to be 62.9(7) and 60.3(8)° for the major and minor occupied species. As in **75**, this can be indicative of electronic decoupling between the two chromophores, which will be further discussed in Section 2.5.2.

2.3.3: Crystallographic Studies of **89**

The structurally similar bipyridine (**bpy**) complex, **89** was also successfully synthesised, crystallised, and the solid-state structure was investigated in the chloride salt form [Scheme 2.2]. Pink block crystals were obtained by slow evaporation of a solution of the complex in MeCN. The crystal was solved in the monoclinic space group $P2_1/c$ and the asymmetric unit contained one unique molecule of **89**, two Cl^- counterions, one water molecule, and three MeCN molecules. A thermal ellipsoid representation of the major occupied cationic Ru(II) species is shown with heteroatoms labelled [Figure 2.12].

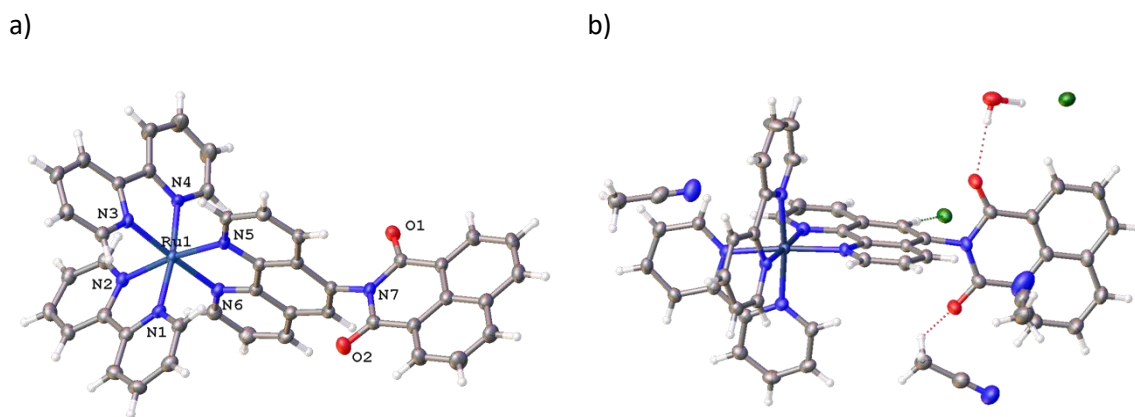


Figure 2.12: ORTEP style representation of (a) the complex cation in the single crystal X-ray structure including labels for heteroatoms and (b) the asymmetric unit of $\{[\mathbf{89}]\text{Cl}_2\cdot\text{H}_2\text{O}\cdot 3(\text{CH}_3\text{CN})\}$. Hydrogen bonding and halogen-H bonding interactions are represented by red and green dotted lines, respectively. Thermal ellipsoids are shown at 50% probability. Selected counterions and solvents are omitted for clarity.

The full asymmetric unit can be viewed in Figure 2.12 (b). The complex did not display any positional disorder. All solvent guest molecules and counterions displayed occupancy factors of unity within the asymmetric unit. As in the cases of the previous two complexes, the solid-state structure analysis of $\mathbf{89}(\text{Cl})_2$ revealed an octahedral geometry around the Ru(II) centre typical of (tris)bidentate transition metal complexes, formed through coordination of the Ru(II) ion to six nitrogen atoms of the ligands. As above, this is through two coordinating **bpy** ligands and the two nitrogen atoms from the **phen**-functionalised **Nap**. The Ru-N bond lengths range from 2.054(2) to 2.072(2) Å and the N–Ru–N bond angles range from 78.95(9) to 100.15(9)° [Table 2.3]. These bond lengths are in agreement with recent Ru(II) polypyridyl complexes in the literature.^{188,97}

Table 2.3: Summary of bond lengths and bond angles for selected region of **89**.

Atoms			Length/Å	Atoms			Angle/°	Atoms			Angle/°
Ru1	N3		2.060(2)	N3	Ru1	N5	100.15(9)	N1	Ru1	N6	88.11(9)
Ru1	N5		2.072(2)	N3	Ru1	N4	78.95(9)	N1	Ru1	N2	78.98(10)
Ru1	N4		2.062(2)	N4	Ru1	N5	91.04(9)	N6	Ru1	N5	79.53(10)
Ru1	N1		2.054(2)	N4	Ru1	N6	97.55(9)	N2	Ru1	N3	84.60(9)
Ru1	N6		2.063(2)	N1	Ru1	N3	95.40(9)	N2	Ru1	N4	96.07(10)
Ru1	N2		2.056(3)	N1	Ru1	N5	94.23(9)	N2	Ru1	N6	96.11(10)

Within the extended structure, both oxygen atoms of the **Nap** moiety participate in hydrogen bonding interactions. The O1 atom participates in a hydrogen bonding interaction ($d = 2.872(3)$ Å) with a hydrogen atom of a water molecule while O2 interacts ($d = 3.198(4)$ Å) with a C-H donor from an MeCN molecule. These interactions can provide an understanding of the essentially non-emissive nature of complexes **75** and **89** in aqueous media, as observed for the structurally similar “light-switch” complexes, **20** and **21**, that undergo rapid radiationless decay in aqueous media.^{2,83,84} The full extended structure can be viewed in Figure A2.21. Hydrogen atoms of the water molecule were placed using AFIX instructions and positions of hydrogen atoms were fit to maximise the hydrogen bonding network. Full details of hydrogen bonding interactions within the bulk structure can be viewed in the appendices [Table A2.22]. Furthermore, π – π stacking interactions exist between two planar **Nap** moieties on neighbouring structures in a head-to-tail orientation as observed for **76** and is typical for the **Nap** motif [Figure 2.13].¹⁸⁹ The closest centroid-centroid distance between the mean planes of the 10-membered naphthene moiety of the **Nap** ligand (C34–C43 to C34–C43i, where i is the symmetry transformation: 1-x, y, 1-z) is 3.7846(19) Å, with an angle of 0.00(9)° between the planes and a slippage of 1.435 Å.

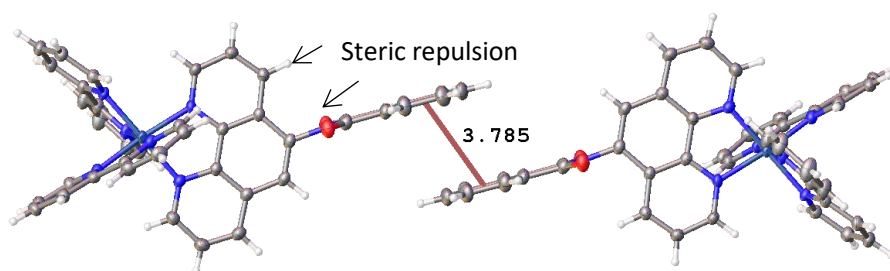


Figure 2.13: ORTEP representation of the intermolecular interactions of $\{[\mathbf{89}]\text{Cl}_2 \cdot \text{H}_2\text{O} \cdot 3(\text{CH}_3\text{CN})\}$, showing π – π stacking between **Nap** moieties of **PhenNap** ligand. Structures shown normal to the O11 plane. Thermal ellipsoids are shown at 50% probability. Distances between the planes denoted by red line / Å. Counterions and solvents are omitted for clarity.

The orientation of the **Nap** with respect to **phen** was found to be almost perfectly orthogonal with a plane-to-plane twist angle between the two respective planes of the coordinating **PhenNap** ligand of 89.26(9)°. For all three complexes, **75**, **76** and **89**, this significant twist was observed between the **Phen** and **Nap** motifs of **80**, and is attributed to a large steric interference between the imide oxygen atom and the hydrogen atom of the coordinating **Phen** portion of the **PhenNap** ligand [Figure 2.13].

2.3.4: Summary and Implications for Photophysical Properties

In summary, the X-ray crystal structures of complexes **75**, **76** and **89** were solved and refined to final R indexes [(R_1, wR_2) , $I \geq 2\sigma(I)$] of **75**(0.0893, 0.2064), **76**(0.0887, 0.2501) and **89**(0.0420, 0.1141). Structural similarities between the major cationic units of **75**, **76** and **89** were observed, such as a substantial degree of rotation between the two ring moieties of the coordinating **PhenNap** ligand, which ranged from 62-90°. Additionally, the extended structure of all three species displayed π stacking interactions between the **Nap** moieties. The base pairs of DNA participate in π - π stacking interactions with intercalators, which was discussed in detail in Section 1.3.2. Intercalators and semi-intercalators are typically planar, aromatic moieties capable of inserting between the base pairs of DNA. Complexes **75**, **76** and **89** have an extra degree of rotation between the coordinating **Phen** group and the anticipated intercalating **Nap** moiety of the **PhenNap** ligand when compared to classic intercalators such as **dppz**, which may allow for more dynamic binding. Furthermore, complex **75** displayed secondary π - π stacking interactions between the auxiliary **phen** ligands. Finally, where guest water molecules occupied the crystal lattice, hydrogen bonding interactions between these water molecules and the oxygen atoms of the **Nap** species were observed, as depicted in Figure 2.12 (b). This may provide an explanation of the environment dependent photophysical properties of these systems in solvents of differing polarities, the details of which will be discussed in Section 2.5.

2.4: Electrochemical Properties

Ru(II)-polypyridyl complexes are extremely versatile biological agents due to their rich photophysical, photochemical and electrochemical properties.¹⁹⁰ As such, understanding of the electrochemical properties of redox active systems is important and this was investigated for both **75** and **76**. Furthermore, electrochemical measurements may be used to provide an insight into the photochemical processes of complexes with DNA, as was the case for $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$, which was found to become reduced by a direct electron transfer (ET) process by spectro-electrochemical investigation.¹⁹¹

Electrochemical measurements of the complexes were performed in degassed MeCN with NBu₄PF₆ as the electrolyte. These measurements were performed to determine the oxidation and reduction potentials of the complexes which are relevant to understanding electron transfer processes that underpin, for example, the excited states of the complexes, or in their interactions with DNA. The complexes were first converted to the PF₆ salts form to eliminate artefacts from the chloride form, as it is also redox active. To achieve this, precipitation of the complexes from aqueous solution *via* addition of a saturated solution of NH₄PF₆ to the chloride anion forms was performed and the resulting precipitates were collected by filtration.

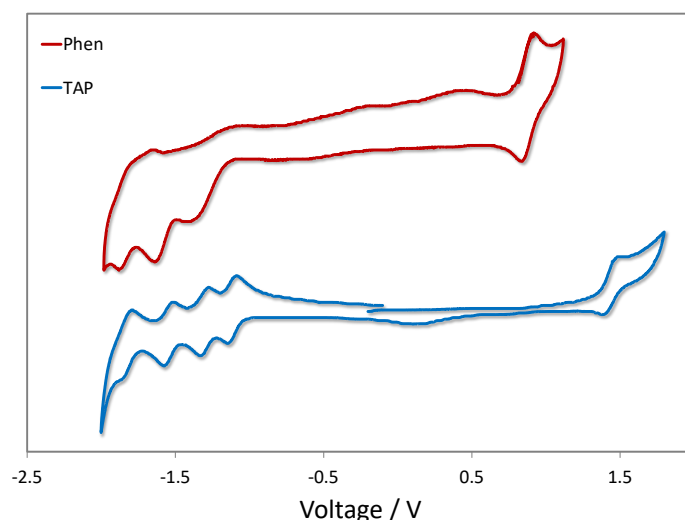


Figure 2.14: The CV traces of **75** (red; top) and **76** (blue; bottom) in deaerated MeCN, referenced with respect to the Fc/Fc⁺ redox couple. Deaeration achieved by vigorous bubbling of N₂ for 15 min immediately before measurement. Scan rate = 100 mV/s in the positive scan direction. Electrolyte = NBu₄PF₆.

The cyclic voltammetry (CV) traces for the two complexes are shown above in Figure 2.14. The ferrocene/ferrocenium (Fc/Fc⁺) redox couple was employed as the internal standard and the data is referenced *versus* Fc/Fc⁺.¹⁹² **75**, containing **phen** as the auxiliary ligands, displayed one reversible oxidation and three irreversible reductions, while **76**, containing **TAP** as the auxiliary ligands, displayed one reversible oxidation wave and four well-defined reversible reduction waves. The oxidations located at +0.91 and +1.48 V for **75** and **76**, respectively, are attributed to their respective Ru(III)/Ru(II) redox couples [Figure 2.14].

75 displays a similar oxidation potential ($E_{1/2} = +0.91$ V) for the Ru(III)/Ru(II) redox couple to that of the tris-homoleptic complex, [Ru(**phen**)₃]²⁺ ($E_{1/2} = +0.90$ V *versus* Fc/Fc⁺).¹⁹³ Compared to [Ru(**phen**)₂dppz]²⁺ ($E_{1/2} = +0.92$ V *versus* Fc/Fc⁺),¹¹² **75** displays an analogous oxidation potential for the Ru(III)/Ru(II) redox couple. These strikingly similar values may indicate an electronic decoupling between the **Phen** and **Nap** moieties of **75**, as the presence of the **Nap** chromophore has little effect on the oxidation potential of the metal. The electronic decoupling could arise from the orthogonal

structural arrangement of the **Nap** with respect to the coordinating **Phen** group of the ligand, as clearly observed from the solid-state analysis (*vide supra*). The oxidation potentials of the tris-homoleptic and parent complexes were calculated using conversion constants from Pavlishchuk and Addison, as the given potentials were referenced *versus* Ag/AgCl reference electrode.¹⁹² In this study, the oxidation potential of the metal was not drastically affected by the presence of the **Nap** moiety when compared to $[\text{Ru}(\text{phen})_3]^{2+}$ and $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ complexes, however, this is not always the case. A recent electrochemical study of the redox potentials of a series of electronically altered $[\text{Ru}(\text{phen})_3]^{2+}$ complexes displays the contrary.¹⁹⁴ The $[\text{Ru}(\text{phen})_3]^{2+}$ series were functionalised with different electron withdrawing and donating groups on the **phen** ligands which resulted in positive shifts of more than 1.4 V for the Ru(III)/Ru(II) redox wave. This displays the dramatic effect ligand substitution can have on the excitation of an electron from the metal based HOMO to the LUMO.¹⁹⁴

The oxidation potential for the Ru(III)/Ru(II) redox couple of **76** occurred at +1.48 V *versus* Fc/Fc⁺ which again is similar to the tris-homoleptic analogue $[\text{Ru}(\text{TAP})_3]^{2+}$ ($E_{1/2} = +1.51$ V *versus* Fc/Fc⁺) and is anodically shifted compared to $[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}$ ($E_{1/2} = +1.39$ V *versus* Fc/Fc⁺).^{193,195} Notably, the oxidation potential of the of **76** is significantly more positive than **75** (+570 mV) which conforms with the literature trends [Table 2.4].

Table 2.4: Oxidation $E_{(\text{ox})}$ and reduction $E_{(\text{red})}$ potentials of complexes **75** and **76** vs Fc/Fc⁺ in degassed MeCN at room temperature with NBu₄PF₆ as the electrolyte.

Complex	$E_{(\text{ox})} / \text{V}$	$E_{(\text{red})} / \text{V}$
75	+0.91	-1.39 ^[c] , -1.62 ^[c] , -1.87 ^[c]
$[\text{Ru}(\text{phen})_3]^{2+[\text{a}]}$	+0.90	-1.79, -1.89, -2.10
$[\text{Ru}(\text{phen})_2\text{dppz}]^{2+[\text{b}]}$	+0.92	-1.38, -1.82 ^[c, d]
76	+1.48	-1.10, -1.23, -1.53, -1.82
$[\text{Ru}(\text{TAP})_3]^{2+[\text{a}]}$	+1.51	-1.13, -1.27, -1.57
$[\text{Ru}(\text{TAP})_2\text{dppz}]^{2+[\text{b}]}$	+1.39	-1.24, -1.36, -1.45, -2.00

^[a] Values obtained from ref.¹⁹³ Potentials were converted from the Ag/AgCl to the Fc/Fc⁺ scale using conversion constants from ref.¹⁹²

^[b] Values obtained from ref.¹¹² Potentials were converted from the Ag/AgCl to the Fc/Fc⁺ scale using conversion constants from ref.¹⁹²

^[c] Irreversible wave.

^[d] Third and fourth reduction waves are poorly resolved.

The ligands of the **TAP** complex **76** also display more positive reduction potentials than **75**. The first reduction wave found at -1.10 V is attributed to a reduction on the **TAP** ligand. This wave was found to be well defined and reversible, and is 290 mV anodically shifted compared to the first reduction of the **phen** complex **75**. **75** displays a less resolved, irreversible reduction at -1.39 V

attributed to reduction of the **Nap** and occurs at a similar potential as the second reduction of **76** ($E_{1/2(\text{red})} = -1.23 \text{ V}$). The electrochemical measurements provide an insight to the excited state properties of these systems, as MLCT-based excited states typically localise on the ligand with the most positive reduction potential.¹⁹⁶ Table 2.4 displays the oxidation and reduction potentials of the complexes in MeCN as well as values for the reference compounds.

Overall, it was observed that the **phen** complex, **75**, was easier to oxidise than the **TAP** derivative, **76**, and this is consistent with the presented literature. By contrast, the reduction of **76** occurs more readily due to the presence of the π -deficient **TAP** ligands. A comparison of the electrochemical gap can be drawn between the complexes by analysing the difference between the peak potentials ($\Delta E_{\text{redox}} = [E_{\text{red}} - E_{\text{ox}}], \text{ V}$). The $\Delta E_{\text{redox}} = 2.3 \text{ versus } 2.6 \text{ V}$ for **75** and **76**, respectively. The similar ΔE_{redox} values are corroborated by a similar onsets of absorption values and emission wavelengths (*vide infra*) in the photophysical analyses, which will be discussed in the next section.

2.5: Photophysical Properties of **75** and **76**

Full photophysical characterisation of **75** and **76** was performed in both aqueous buffer solution (10mM aqueous sodium phosphate buffer, pH = 7.4) and MeCN at room temperature. Overall, the results demonstrate that the emission intensities of the complexes are greatly affected by the nature of the solvent, commonly observed for systems such as **20** and **21** in the past. As such, it was anticipated that **75**, containing **phen** as the auxiliary ligands, would be relatively non-emissive in aqueous media as the ³MLCT is centred on the **PhenNap** ligand. This facilitates a non-radiative relaxation pathway through hydrogen bonding interactions with the carbonyls of the **Nap** and the protic solvent, thus quenching the emission. Conversely, **76** containing **TAP** as the auxiliary ligand was anticipated to be emissive in aqueous media as the excited state is localised on the **TAP** ligand.^{81,80}

2.5.1: Absorption Properties

The UV-vis absorption and the excitation and emission spectra for both complexes in MeCN and in 10 mM aqueous phosphate buffer (PB) solutions at pH 7.4 and are shown in Figure 2.15. The molar absorptivity values, calculated using the Beer–Lambert Law, for the major absorption bands of **75** and **76** are given in Table 2.5.

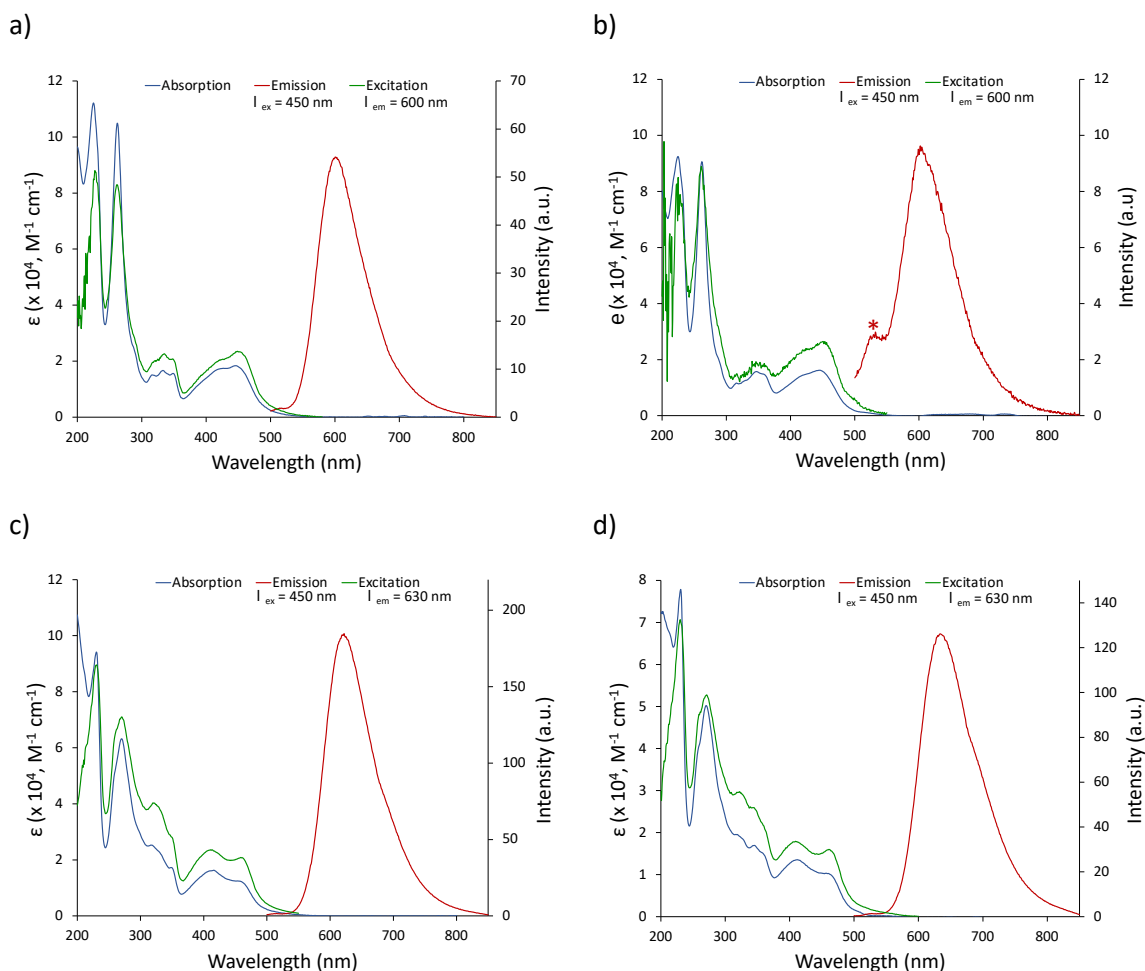


Figure 2.15: Absorption, excitation and emission spectra of **75** ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 600 \text{ nm}$, $3.5 \times 10^{-6} \text{ M}$) in (a) MeCN and (b) PB and **76** ($\lambda_{\text{ex}} = 450 \text{ nm}$, $\lambda_{\text{em}} = 630 \text{ nm}$, $4.4 \times 10^{-6} \text{ M}$) in (c) MeCN and (d) PB at 298 K. Water Raman band is denoted by *.

In MeCN solution, complexes **75** and **76** displayed similar absorption and displayed bands typical of Ru(II) centred MLCT with **Nap** chromophores.¹⁶⁸ **75** shows a high energy absorption band at 264 nm with a high molar absorptivity ($9.4 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$) which is attributed to $\pi-\pi^*$ intra-ligand (IL) transitions of the **phen** ligands. Three less intense ($1.5 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$) bands were present at 320-350 nm which are attributed to $\pi-\pi^*$ transitions within the **Nap** moiety. Lastly, two further broad bands centred at 420 and 450 nm correspond to the MLCT transitions [Figure 2.15 (a)] between the Ru(II) ion and the ligands. **76** displayed analogous, albeit slightly red-shifted absorption transitions [$\epsilon = 6 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$ ($\lambda = 273 \text{ nm}$), $1.6 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$ ($\lambda = 346 \text{ nm}$) and $1.2 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$ ($\lambda = 462 \text{ nm}$)], in the same solvent [Figure 2.15 (c)], which is ascribed to the presence of the more π -deficient **TAP** ligands.¹⁰⁸ Importantly, the bands corresponding to the absorption centred at both the **Nap** and Ru(II)-polypyridyl centres can be individually resolved allowing for specific and selective probing of the interactions of these parts of the complexes with DNA.

Table 2.5: Absorption properties of **75** and **76** at 298 K.

Absorption λ_{max} / nm (ϵ / $10^4 \text{ M}^{-1} \text{ cm}^{-1}$) ($\pm 10\%$) ^[a]			
Organic Media (MeCN)			
Complex	$\pi-\pi^*$ IL	$\pi-\pi^*$ IL	MLCT
75	264 (9.4 $\pm$ 0.3)	331 (1.5 $\pm$ 0.05)	450 (1.6 $\pm$ 0.05)
[Ru(phen) ₂ dppz] ²⁺ [c]	264	352	440
[Ru(phen) ₃] ²⁺ [d]	262	---	446
76	273 (6.0 $\pm$ 0.2)	346 (1.6 $\pm$ 0.06)	462 (1.2 $\pm$ 0.04)
[Ru(TAP) ₂ dppz] ²⁺ [c]	278	362	452
[Ru(TAP) ₃] ²⁺ [e]	276	---	408, 437
Aqueous Media (10 mM Sodium-phosphate Buffer)			
75	262 (9.6 $\pm$ 0.9)	347 (1.7 $\pm$ 0.2)	446 (1.6 $\pm$ 0.2)
[Ru(phen) ₂ dppz] ²⁺ [b, c]	264	358	440 (2.34)
[Ru(phen) ₃] ²⁺ [d]	262	---	421, 447
76	273 (5.1 $\pm$ 0.1)	348 (1.8 $\pm$ 0.06)	464 (1.1 $\pm$ 0.05)
[Ru(TAP) ₂ dppz] ²⁺ [b, c]	278 (10.41)	366 (1.45)	454 (1.58)
[Ru(TAP) ₃] ²⁺ [e]	276	---	408, 437
[Ru(TAP) ₂ phen] ²⁺ [b, f]	275	---	405, 470

^[a] Molar absorptivity values correspond to the mean $\pm$ SEM^[b] Values displayed from measurement in H₂O^[c] Values from ref.¹¹²^[d] Values from ref.⁸⁴^[e] Values from ref.¹⁰⁸^[f] Values from ref.¹⁸⁴

All of the absorption bands are present in the excitation spectra for both complexes (λ_{ex} = 600, 630 nm for **75** and **76**, respectively), showing that the photophysics of the complexes obey Kasha's rule [Figure 2.15]. As described above, the $\pi-\pi^*$ IL transitions of the **Nap** moiety (320-350 nm) are of particular interest to this study, as this region of the electronic spectra in both complexes undergoes the most appreciable changes upon interaction with DNA (*vide infra*, Section 2.6). Notably, these absorption bands are absent from the tris-homoleptic systems [Ru(**phen**)₃]²⁺ and

[Ru(**TAP**)₃]²⁺ complexes^{84,108} [Table 2.5] making **PhenNap** Ru(II) bichromophoric species, **75** and **76**, an interesting class of molecules for photophysical investigation upon DNA binding.

2.5.2: Emission Properties

As above, the emission characteristics of **75** and **76** were analysed in both 10 mM aqueous sodium-phosphate buffer and MeCN solutions. Excitation of both the **Nap**-centred ($\lambda = 347$ nm) and MLCT-based ($\lambda = 450$ nm) absorption bands resulted in a weak, broad and unstructured ³MLCT-based luminescence band centred at 609 and 603 nm for **75** in buffered water and MeCN, respectively. No emission arising from the **Nap** moiety was observed upon excitation of the **Nap** localised absorption band. The **Nap**-centred emission within these systems is likely being quenched due to efficient Förster resonance energy transfer (FRET) to the Ru(II)-polypyridyl centre, as described by Castellano and co-workers for related metal-**PhenNap** bichromophoric species.^{150,151} The emission of **75** was substantially quenched in aqueous media, showing sensitivity of this complex to its environment which is indicative of excited state quenching by non-radiative relaxation pathways, as observed for [Ru(**phen**)₂**dppz**]²⁺.^{81,84} The emission properties were also greatly affected by the presence of dissolved oxygen and the intensity of emission of **75** was enhanced (*ca.* 5-fold increase) in deaerated solutions [Figure 2.16 (a)]. Since only ³MLCT-based emission is observed upon excitation of the different chromophores, this indicates that the MLCT-based excited state may arise from an efficient singlet-singlet energy transfer process (¹Nap*⁻¹MLCT*), followed by intersystem crossing to generate the ³MLCT state.¹⁶⁰

Unlike **75**, **76** displayed luminescence in both buffered water and MeCN solutions. Upon excitation, mixed ligand systems of the form [Ru(L)₂L']²⁺ generate MLCT excited states where the electron is localised on the ligand with the most positive reduction potential; in this case, the **TAP** ligand.¹⁹⁶ Indeed, this was observed in the CV measurements of the complexes (*vide supra*, Section 2.4). Excitation of the **Nap**-centred and MLCT-based absorption bands of **76** both resulted in similarly broad and unstructured emission bands, centred at 636 and 624 nm in aqueous buffer solution and MeCN, respectively. These were modestly redshifted by 27 nm and 697 cm⁻¹ for buffer, and by 21 nm and 558 cm⁻¹ for MeCN, in comparison to **75**. This result is explained by a different MLCT excited state: for **75**, the MLCT state is a metal-to-**phen/PhenNap** transition, while for **76**, it is a metal-to-**TAP** transition, due to the lower lying π -orbitals localised on the **TAP** ligands. Literature reports of the [Ru(**phen**)₂**dppz**]²⁺ and [Ru(**TAP**)₂**dppz**]²⁺ reference complexes corroborate these photophysical observations, as well as the sensitivities of **75** and **76** to the solvent nature and to the presence of dissolved oxygen.^{80,112,150,177}

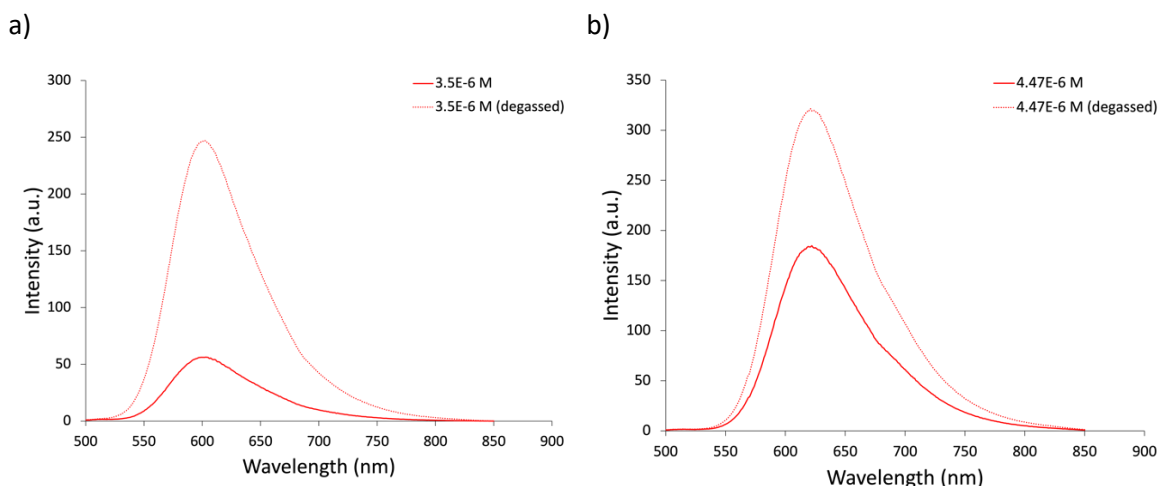


Figure 2.16: Emission enhancement under absorbance matched excitation conditions of aerated and deaerated MeCN solutions of (a) **75** ($\lambda_{\text{ex}} = 450 \text{ nm}$, $3.5 \times 10^{-6} \text{ M}$) and (b) **76** ($\lambda_{\text{ex}} = 450 \text{ nm}$, $4.5 \times 10^{-6} \text{ M}$).

Dilution studies were performed to determine the relationship between emission intensity and concentration, and can be viewed in Figures A2.23 and A2.24 in the Appendix for **75** and **76**, respectively. Unsurprisingly, the intensity of emission for both complexes were found to be proportional to concentration, following the Beer-Lambert Law.

Next, the photoluminescence quantum yield (Φ_{PL}) of **75** and **76** were determined using the optically dilute solution method ($\lambda_{\text{ex}} = 450 \text{ nm}$, $A_{\text{MLCT}} = 0.1, 0.075, 0.05, 0.025$) using an non-degassed aqueous solution of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ as the reference and the reported values correspond to the average of two independent measurements.¹⁹⁷⁻²⁰⁰ The Φ_{PL} is defined as the ratio between the number of photons emitted and the total number of photons absorbed by the sample. Linear regression analysis between the absorption and emission intensity verified the linear relationship with R^2 values surpassing 0.99 in all cases. Individual relative quantum yield values were calculated for each solution and reported values represent the slope value. Quantum yield values of each sample were determined according to Equation 2.1:

$$\varphi_s = \varphi_r \left(\frac{A_r}{A_s} \right) \left(\frac{I_s}{I_r} \right) \left(\frac{n_s}{n_r} \right)^2 \quad [2.1]$$

where Φ_r is the absolute quantum yield of the reference (in this case: $\text{Ru}(\text{bpy})_3\text{Cl}_2$ in aerated water), A is the absorbance at the excitation wavelength ($\lambda_{\text{ex}} = 450 \text{ nm}$), I is the integrated emission and η is the refractive index of the solvent. The r and s subscripts refer to those properties of the reference and sample, respectively.

Photoluminescence quantum yields were recorded in aerated and degassed conditions. To obtain deaerated samples, the mother solvent was first degassed *via* sparging with argon for 30 minutes and was kept under inert conditions. Each sample was further degassed by gentle bubbling of N_2 inside the optical cuvette for 1 min immediately prior to obtaining the measurements with no

or minimal loss of solvent. Overall, **75** was found to be essentially non-emissive ($\Phi_{\text{PL}} \leq 0.2\%$) in both aerated and degassed aqueous phosphate buffered solutions and displayed a Φ_{PL} value of 1% in air-saturated MeCN. This value increased to 4% in degassed MeCN as expected, due to the reduction of excited state quenching processes by molecular oxygen and by elimination of non-radiative hydrogen bonding facilitated relaxation pathways.

76 displayed higher Φ_{PL} values of 3% in PB, 4% in MeCN and notably, 8% in degassed MeCN. Table 2.6 summarises the results. The Φ_{PL} values for **75** are in agreement with recently reported values of 0–1% in aqueous media for structurally similar bichromophoric $[\text{Ru}(\text{L})_2(\text{Nap})]^{2+}$ systems, and display comparable values to the **dppz** analogues in MeCN.^{168,177} **76** displayed higher quantum yields values (4–8%) than **75** (0–4%) which is expected due to the difference in their excited state characters. The low Φ_{PL} values may be due to population of low-lying metal-based states which would quench the emission. The emission properties of the complexes vary greatly depending on the solvent environment, particularly for **75**. It is anticipated that **75** bearing **phen** auxiliary ligands will become more emissive when bound to DNA as the local environment of the **Nap** will be more hydrophobic, generating a potential “off-on” switch for DNA. Conversely, the emissive nature of the **TAP** analogue, **76**, is expected to decrease when bound to DNA, if electron transfer takes place. As such, it is the change of the Φ_{PL} in the DNA-bound *versus* unbound states that is of particular interest for these complexes as their potential as luminescent DNA sensors and DNA-binding agents are explored.

Table 2.6. Emission properties of **75** and **76** in 10 mM aqueous sodium phosphate-buffered solution at pH 7.4 and MeCN, at 298 K.

	$\lambda_{\text{max}}^{\text{em}}$ buffer /nm	$\lambda_{\text{max}}^{\text{em}}$ MeCN /nm	$\Phi_{\text{PL}}^{[\text{a}]}$ buffer, air /%	$\Phi_{\text{PL}}^{[\text{a}]}$ buffer, N ₂ /%	$\Phi_{\text{PL}}^{[\text{a}]}$ MeCN, air /%	$\Phi_{\text{PL}}^{[\text{a}]}$ MeCN, N ₂ /%
75	607	603	--	--	1.0	4.0
$\text{Ru}(\text{phen})_2\text{dppz}^{[\text{b}]}$	--	607	--	--	0.73	3.3
76	636	624	3.1	4.0	3.8	8.1
$\text{Ru}(\text{TAP})_2\text{dppz}^{[\text{c}]}$	636 ^[\text{d}]	621	3.5 ^[\text{c}]	--	--	--

Estimated errors $\pm 10\%$.

^[\text{a}] An aerated aqueous solution of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ ($\Phi_{\text{r}} = 2.8\%$) was used as the external reference.^{198,200}

^[\text{b}] Values from ref.¹⁷⁷

^[\text{c}] Values from ref.¹¹²

^[\text{d}] Measured in aqueous solution.

Luminescence emission lifetime values (τ_{em}) of **75** and **76** were recorded in aqueous buffer and MeCN solutions, using a time correlated single-photon counting (TCSPC) detector at room temperature. In all cases, the data was fit to single exponential decay functions with χ^2 values in

the range of 1–1.2. The pulse length for the 458 nm NanoLED is 1.3 ns and is therefore not expected to influence the lifetime measurements, which are expected to be at least two orders of magnitude larger. As such, the prompt measurements were omitted from the fitting. The measurement was performed at 600 nm where the MLCT emission is observed. For reference, an example of the data and the aforementioned fitting for **76** in deaerated MeCN is shown in Figure 2.17, with further details available in [Figures A2.25 and A2.26].

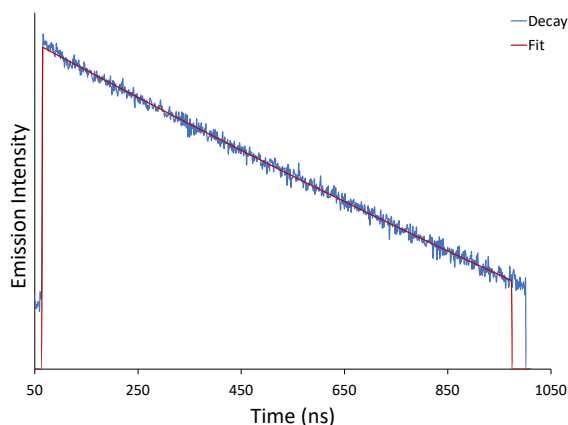


Figure 2.17: Time-resolved emission intensity decay (blue) and monoexponential fit (red) of the data for **76** (OD at 450 nm = 0.1) in deaerated MeCN.

The lifetime values for both complexes were higher in deaerated conditions due to the absence of quenching processes associated with molecular oxygen as discussed above. The τ_1 values for **75** in aqueous media were extremely short (approximately 20 ns) for Ru(II)-polypyridyl containing extended systems, which typically display values in the hundreds of nanoseconds to microseconds range.²⁰¹ This result is in agreement with the low Φ_{PL} value measured for **75** in aqueous media (< 0.2%) and indicates that efficient emission quenching processes are occurring. The nature of the quenching process is hypothesised to be due to hydrogen bonding interactions with the oxygen atoms of the **Nap** carbonyl groups and the solvent – similar to that hypothesised for the phenazine nitrogen atoms of the **dppz** analogue – and indeed, the complex displayed much higher τ_1 values in aprotic MeCN solution (139 and 304 ns in aerated and deaerated conditions, respectively). $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ displayed τ_{em} values of 177 and 663 ns in aerated and deaerated MeCN, respectively, while showing no luminescence in aqueous media due to hydrogen bond induced non-radiative quenching.¹⁷⁷

76 displayed greatly enhanced luminescence lifetime values of 622 and 1121 ns in aerated and deaerated MeCN solutions, respectively, typical for emission from $^3\text{MLCT}$. In aqueous media, **76** bearing the **TAP** ligands, overcomes the quenching observed for **75** as the nature of the excited state is localised on a different area of the complex. This provides further evidence that the excited state is deactivated by hydrogen-bonding processes facilitated by the imide carbonyl atoms of the

Nap substituent. The τ_{em} values were found to be 630 and 762 ns in aerated and deaerated aqueous sodium phosphate buffer solutions, respectively. The $[Ru(\text{TAP})_2(\text{phen})]^{2+}$ complex, which differs from **76** by the absence of the **Nap** unit, displays analogous values of 690 and 835 ns, which are lower than the **dppz** analogue which ranges from 820-1090 ns in water.¹¹² This provides further evidence for an electronic decoupling between the **Phen** and **Nap** moieties of the **PhenNap** ligand. As observed in the steady state emission spectra [Figure 2.16], the luminescence decreases in the presence of dissolved oxygen in solution due to quenching. Full details for the luminescent lifetime values are displayed in Table 2.7.

Table 2.7: Luminescent lifetime values of **75** and **76** in 10 mM aqueous sodium phosphate-buffered solution at pH 7.4, and MeCN, at 298 K vs $[Ru(\text{phen})_2(\text{dppz})]^{2+}$ and $[Ru(\text{TAP})_2(\text{dppz})]^{2+}$.

Complex	τ_{em} buffer, air /ns	τ_{em} buffer, N ₂ /ns	τ_{em} MeCN, air /ns	τ_{em} MeCN, N ₂ /ns
75	--	--	139	304
$[Ru(\text{phen})_2(\text{dppz})]^{2+}$ ^[177]	--	--	177	663
76	630	762	622	1121
$[Ru(\text{TAP})_2(\text{dppz})]^{2+}$	820 ^[a]	1090 ^[a]	1280 ^[b]	1785 ^[b]

Estimated errors $\pm 5\%$.

^[a] Measured in aqueous solution. Values from ref.¹¹²

^[b] Measured in D₂O. Values from ref.¹¹²

In summary, both **75** and **76** display photophysical properties that are highly sensitive to dissolved oxygen and to solvents with hydrogen bonding capabilities. The photophysical properties highlight the bifunctional design of the complexes, whereby they display characteristic absorption bands attributed to different parts of the molecule, namely the Ru(II)-centred MLCT and the **Nap** chromophore. This property will allow for the sensitive monitoring of these parts upon interaction with DNA. The absorption and emission characteristics were proportional to concentration for both complexes. **75** was found to be weakly emissive in aqueous solution ($\Phi_{PL} < 0.2\%$) and showed moderate emission in deaerated MeCN ($\Phi_{PL} = 4\%$), which is attributed to phosphorescence from the triplet excited state and is typical of Ru(II)-polypyridyl complexes. Interestingly, **75** showed remarkably different Φ_{PL} values in buffered water *versus* MeCN solutions. The low Φ_{PL} and lifetime values of **75** in aqueous media are attributed to a non-radiative quenching pathway introduced through hydrogen bonding interactions between the solvent and complex. It has been shown by Poynton and co-workers that solvent molecules hydrogen bonded to the phenazine moiety of the

dppz ligand of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ deactivate the excited state by efficient energy transfer to bulk water resulting in efficient emission quenching.⁹⁷ This property has been exploited, for example, in fluorescent lifetime imaging microscopy (FLIM) in biological media, as DNA light-switch probes, and as potential therapeutic agents. **76** displayed moderate emission quantum yields (maximum of 8% in deaerated MeCN) and long-lived excited state lifetimes ranging between 600–1100 ns in aerated *versus* deaerated media. These emission properties and luminescence lifetime values were very similar to the structural analogue, $[\text{Ru}(\text{TAP})_2(\text{phen})]^{2+}$, indicating little consequence upon addition of the **Nap** chromophore on the lifetime and Φ_{PL} values for the **TAP** complexes. The luminescence lifetime experiments will be repeated by extending the acquisition time to a minimum of 4-fold of the expected lifetime. Next, the ability of **75** and **76** to recognise and sensitise DNA will be investigated and the effect of the auxiliary ligands on the DNA binding properties will be explored.

2.6: DNA Binding Interactions

Ru(II)-polypyridyl complexes have been shown to interact with DNA enantioselectively *via* a number of mechanisms depending on the nature of the ligands, the base sequences and the secondary structures of DNA. In general, the cationic metal centres of such complexes associate strongly with the anionic DNA backbone *via* electrostatic interactions, while the π -conjugated ligands are designed to interact with other functionalities of the biomolecule, such as through intercalation between the base pairs and/or groove binding mechanisms, as is the case for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.⁸¹ $[\text{Ru}(\text{TAP})_2(\text{dppz})]^{2+}$ can bind in the same manner, however, it also possesses a highly oxidising excited state which can undergo photoinduced electron transfer (PET) with DNA causing irreversible DNA or base pair damage. Furthermore, the long-lived triplet excited state of this family of complexes makes them capable of generating singlet oxygen, which is highly reactive and can induce cell death in selective conditions.^{2,112} **75** and **76** were designed with similar binding modes in mind, however, since the **Nap** unit is singly bonded to the **phen** moiety, this introduces an extra degree of rotational freedom between the **Nap** unit and the complex when compared to the **dppz** analogues. This was expected to aid intercalation of the **Nap** into the DNA, due to the capacity of the **Nap** unit to freely adjust itself to fit within the DNA cavity and undergo additional secondary interactions with the DNA. In addition, intercalation of the planar **Nap** unit may be facilitated by interactions of the conjugated **phen** ligand, as has been shown for the homoleptic analogue $[\text{Ru}(\text{phen})_3]^{2+}$,⁹⁵ and its structural analogue $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.^{97,202} Several techniques were employed to determine the strength and nature of the interactions of **75** and **76** with DNA. Firstly, changes in the ground and excited state photophysical properties of the complexes were monitored upon increasing additions of DNA to determine the binding affinity of the complexes to

DNA. Secondly, the structural environment of the DNA was probed upon additions of complexes **75** and **76** *via* thermal denaturation, and circular (CD) and linear dichroism (LD) spectroscopy.

2.6.1: Spectroscopic Titrations

UV-Visible absorption and emission spectroscopy can provide both qualitative and quantitative information about binding events. DNA titration experiments were performed by monitoring any changes in the absorbance and emission properties of the complexes upon increasing additions of natural ctDNA in aqueous 10 mM sodium-phosphate buffer at a pH of 7.4. The spectra were collected 1 min post DNA addition and no further controls were used to ensure equilibrium was reached. The photophysical properties were monitored until negligible changes were observed, even at high DNA loading. All measurements were performed at minimum, in triplicate, and the temperature was kept stable at 298 K to ensure reproducibility.

2.6.1.1: Absorption Titrations

The low energy absorption bands of the **75** and **76** can be assigned to specific transitions within the complexes, namely $\lambda_{\text{Abs}} \approx 350$ nm and 450 nm correspond to $\pi-\pi^*$ **PhenNap** and MLCT transitions, respectively [Section 2.4]. Both complexes experience degrees of changes to these local environments but the degree of hypochromicity is more appreciable for the **Nap** localised transition at 347 nm for **75** and **76**. Figure 2.18 displays the changes in the absorbance profiles ($\lambda = 300-600$ nm) for the two complexes upon addition of aliquots of DNA. The DNA titrations were also performed in the presence of high ionic strength (160 mM NaCl) which has a stabilisation effect on the DNA. Performing the titrations at high ionic strength also allows for an approximation of the electrostatic contributions to DNA binding from the complex.¹⁶⁸

The tris-homoleptic complex $[\text{Ru}(\text{bpy})_3]^{2+}$ does not undergo DNA intercalation. Instead, it associates with DNA *via* strong electrostatic interactions which result in an approximate 10% hypochromic shift in the MLCT transition upon interaction with DNA.^{164,203} The broad absorption regions between 400–550 nm [Figure 2.18] account for two discrete MLCT transitions, each of which displayed the same decrease in intensity for both complexes. **75** and **76** displayed a 7% and 13% hypochromic shift for the $d\pi(\text{Ru(II)})-\pi^*(\text{N}^{\wedge}\text{N})$ MLCT transition, respectively, which is indicative of a primarily electrostatic mode of binding. No shift to different wavelengths was observed for this transition for both complexes, indicating the electrostatic nature of the binding event. The **Nap** centred transitions experience the largest degree of change: a 48% hypochromic shift was observed in both cases, accompanied by bathochromic shifts (7 nm, 569 cm^{-1} and 5 nm, 328 cm^{-1}) in the bands corresponding to the $\pi-\pi^*$ **PhenNap** transitions at 347 nm for **75** and **76**, respectively. This observation suggests that the **Nap** moiety of the complex undergoes the most significant

interaction with DNA, most likely through an intercalative mode of binding where the relative absorbance of the **Nap** chromophore decreases upon π - π interactions with the DNA bases.^{168,204} Furthermore, when compared to the mono-unsubstituted Nap-Ru(II) conjugate, **60** (*vide supra*, 1.6), which displays a 37% hypochromic shift in the **Nap**-centred absorption upon DNA binding, complexes **75** and **76** were found to be more spectroscopically sensitive to this binding event. Overall, the absorption spectroscopic changes of both complexes upon addition of DNA were observed to be closely similar. As such, it is hypothesised that the binding mode(s) of both complexes with DNA is also similar and is dominated by intercalative interactions with the **PhenNap** ligand. At high ionic strength (160 mM NaCl), the decrease in the **Nap** centred absorption bands was less than at low ionic strength (**75**: 40%, **76**: 21%), indicating an important electrostatic contribution to the binding mode; details of which can be viewed in Figure A2.27. Importantly, monitoring the changes in the regions attributed to **Nap** localised transitions allows for direct reporting on the interaction of the **Nap** with DNA.

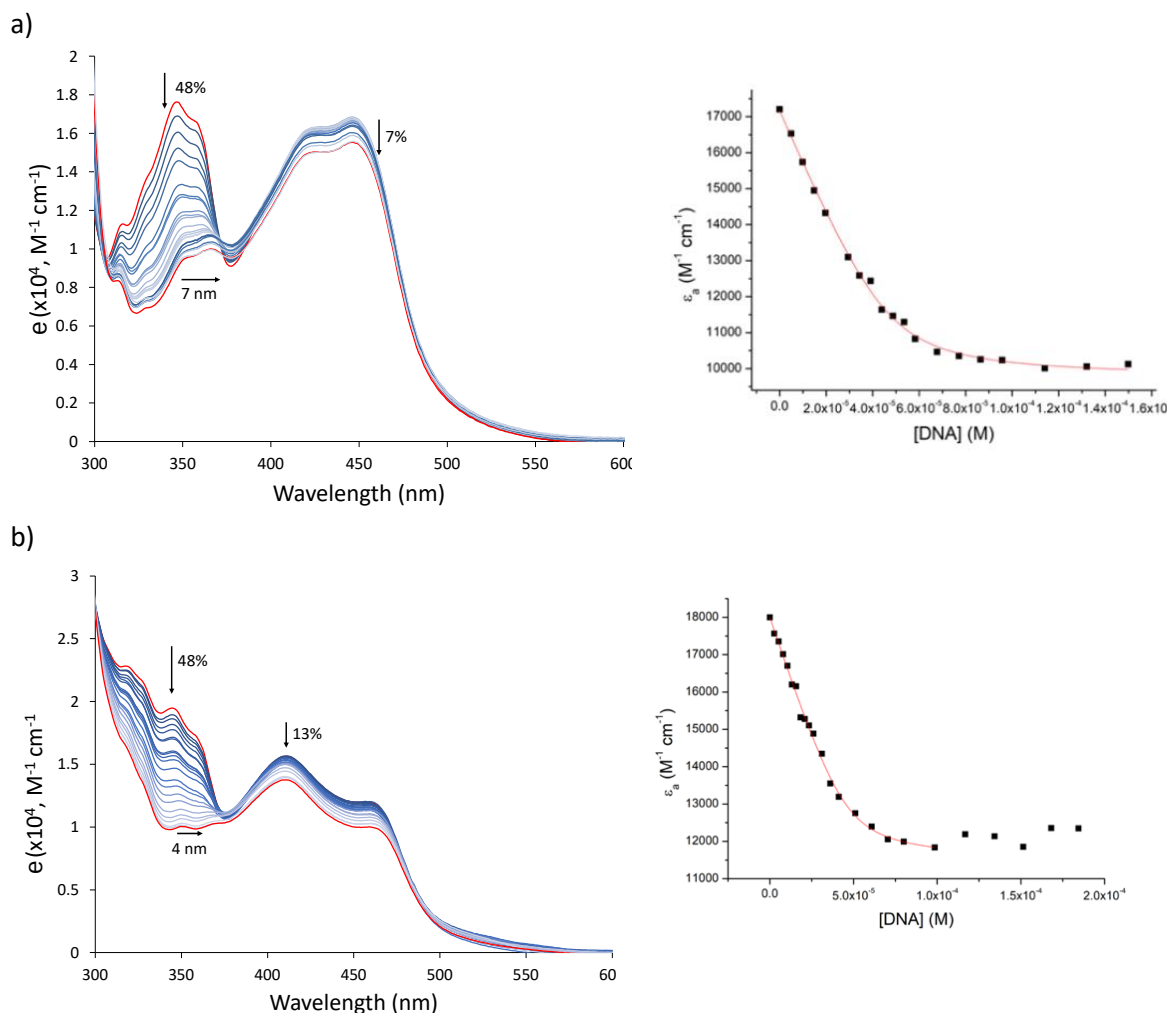


Figure 2.18: Changes of UV-vis absorbance profile and fit of the data to the Bard equation at $\lambda = 347$ nm for a) **75** (1×10^{-5} M, buffered water) and b) **76** (1×10^{-5} M, buffered water) upon addition of aliquots of ctDNA (0–0.2 mM).

The intrinsic binding constant (K_b) and binding site size in base pairs (n) were determined for both complexes by plotting the changes in the molar absorbance values (ε_a) as a function of the concentration of DNA and fitting the data. Recently, our group published a practical modification of the method developed by Carter, Rodriguez and Bard for calculating these parameters.^{205,97} The original equation [Equation 2.2] involved expressing the total fraction of complex bound to DNA $[(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f)]$ as the dependant variable, which in turn, required an approximation for ε_b , where ε_a , ε_f and ε_b represent the apparent molar absorptivity, and the molar absorption of free and bound complex, respectively.

$$\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{b - \left(b^2 - \frac{4K_b^2 C_t [DNA]}{n} \right)^{\frac{1}{2}}}{2K_b C_t} \quad [2.2]$$

C_t represents the total concentration of the complex and $b = 1 + K_b C_t + K_b [DNA]/n$. Using Equation 2.2, it is possible to derive the binding constant (K_b) and binding site size (n) of the complex, however, as mentioned, an estimation of ε_b is necessary and is usually derived by determining the molar absorptivity of the complex at high DNA concentrations. A small error in the estimation of ε_b can result in a large change in the derived K_b value and as such, Equation 2.2 can be re-written as Equation 2.3. The advantage of this rearrangement is that no estimation for ε_b is necessary and both ε_b and ε_f can be derived alongside K_b and n for a more accurate representation of the derived parameters.⁹⁷

$$\varepsilon_a = \frac{b - \left(b^2 - \frac{4K_b^2 C_t [DNA]}{n} \right)^{\frac{1}{2}}}{2K_b C_t} (\varepsilon_b - \varepsilon_f) + \varepsilon_f \quad [2.3]$$

The binding constants and binding site sizes were derived from the fit of the data to Equation 2.3. The binding constants, K_b , were determined to be $1.36 \times 10^6 (\pm 0.4) \text{ M}^{-1}$ and $1.54 \times 10^6 (\pm 0.7) \text{ M}^{-1}$ for **75** and **76**, respectively. In both cases, the binding site size was approximately two base pairs [Table 2.8].

Table 2.8: DNA binding parameters from fits to absorbance data using Equation 2.3 ($\lambda = 347 \text{ nm}$)^[a].

Complex	Binding constant, $K_b / 10^6 \text{ M}^{-1}$	Binding site size, $n/\text{base pairs}$	R^2
75	1.36 (± 0.39)	2.36 (± 0.1)	> 0.99
76	1.54 (± 0.73)	2.29 (± 0.11)	> 0.99

^[a] Results correspond to the mean $\pm$ SEM

The large decrease in the **Nap** localised absorbance bands, coupled with the strong K_b values of 10^6 M^{-1} and the binding site size of approximately two base pairs, strongly indicates that the complexes bind to DNA through an intercalative mode of binding, although this does not preclude other secondary or additional binding modes occurring also. To further probe the interaction of the complexes with DNA, changes in the emission properties and corresponding fit to Equation 2.3 upon addition of ctDNA were determined and will be discussed in the following section.

2.6.1.2: Emission Titrations

While the changes in the absorption properties followed a similar trend for both complexes, fundamental differences in the emission properties of the two systems were observed upon the addition of DNA. For **75**, the emission was enhanced, while it was quenched for **76**. The behaviour of **75** is characteristic of the “light-switch” DNA binding effect described for complexes such as $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$.^{8,95} In its free form, **75** shows essentially no emission in aqueous media ($\Phi_{\text{PL}} < 0.2\%$), however, upon addition of DNA, a 30-fold emission enhancement for the complex was observed in the fully bound form. This value is significantly greater than recently published values of intercalating Ru-**Nap** species, which display 0.25-to-10-fold increases in emission intensity upon DNA binding.^{168,169} This makes complex **75** of particular interest as a highly sensitive luminescent probe for DNA. Analogous to the phenazine nitrogen atoms in $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$, the emission enhancement is attributed to shielding of the **Nap** imide oxygen atoms from the aqueous solvent environment upon intercalation between the base pairs, which otherwise would undergo efficient quenching of the MLCT emission *via* hydrogen bonding interactions with the bulk water.⁸⁴ This is further supported by the X-Ray crystal packing of **75** in aqueous media [Figure 2.12(b)], which displays hydrogen bonding networks through the **Nap** oxygen atoms. In contrast, the emission properties of **76** were quenched by 42% upon the addition of DNA. It is known that in the excited state, complexes containing **TAP** as the auxiliary ligand can undergo PET with guanine residues, which ultimately results in quenching of the emission [Figure 2.19].^{112,206}

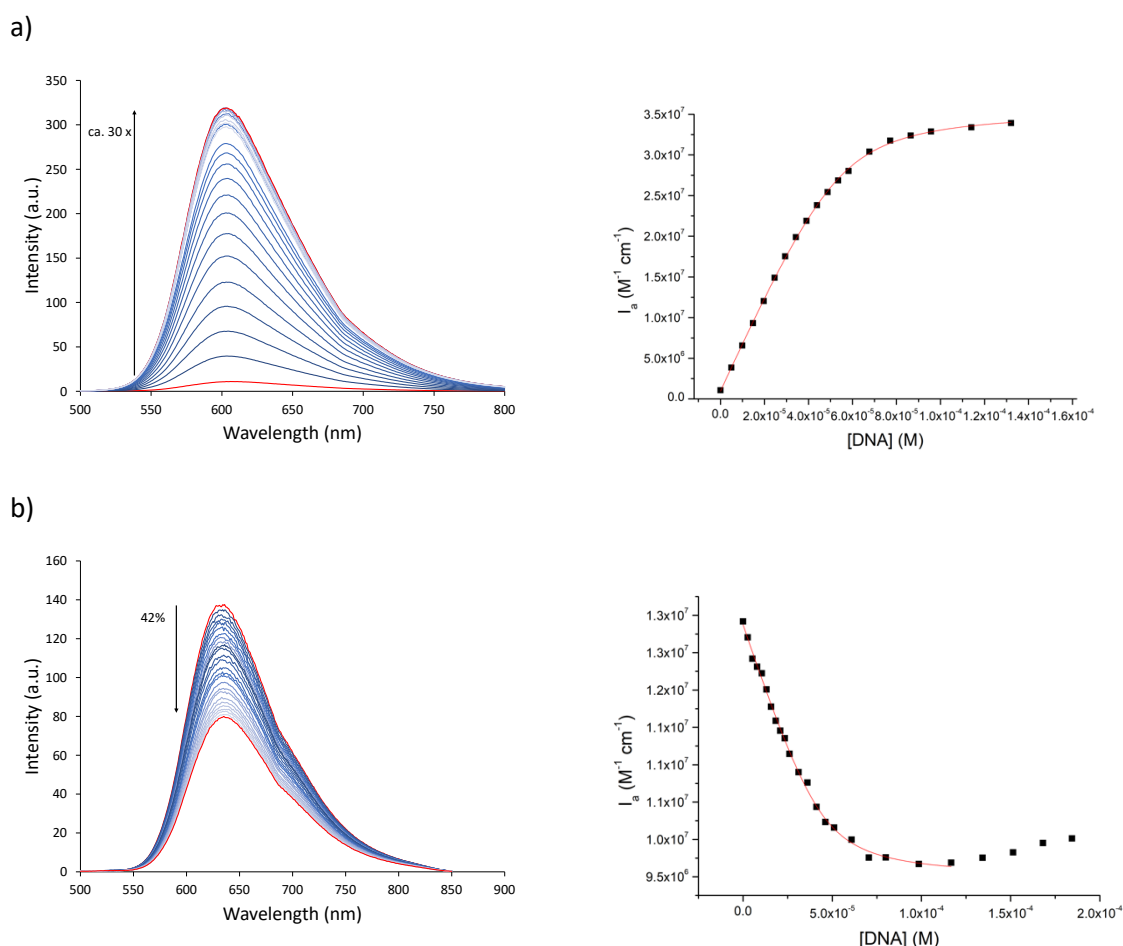


Figure 2.19: Changes of emission profile and fit of the data to the Bard equation,²⁰⁵ $\lambda_{\text{ex}} = 450 \text{ nm}$ for a) **75** ($1 \times 10^{-5} \text{ M}$, buffered water) b) **76** ($1 \times 10^{-5} \text{ M}$, buffered water) upon addition of aliquots of ctDNA (0–0.2 mM).

The data was fit to Equation 2.3 where the apparent molar absorptivity, ε_a , was replaced by the apparent molar emission, I_a where $I_a = \text{Emission}/C_t$. Importantly, the K_b and n values obtained from fitting of the emission data closely mirrored the values obtained from the absorbance data, which is interesting as different parts of the molecule are probed in the respective experiments. The K_b values obtained from the fitting of the emission titration data were again determined to be in the order of 10^6 M^{-1} for **75** and **76**. The binding site size was found to be closer to three base pairs for **75** and two base pairs for **76**. Table 2.9 summarises the results.

Table 2.9: DNA binding parameters from fits to emission data ($\lambda_{\text{ex}} = 450 \text{ nm}$)^[a].

Complex	Binding constant, $K_b / 10^6 \text{ M}^{-1}$	Binding site size, $n/\text{base pairs}$	R^2
75	1.40 (0.15)	2.75 (0.04)	> 0.99
76	1.23 (0.36)	2.24 (0.09)	> 0.99

^[a] Results correspond to the mean $\pm$ SEM

The standard error of the parameters derived from the emission data was less than those obtained from the absorption data indicating a better fit of the data and more accurate values. It should be noted that there are important caveats to these values that must be considered. Firstly, the complexes studied herein are racemic mixtures of enantiomers. DNA is chiral and indeed it is well-established that the Λ - and Δ - enantiomers of Ru(II)-polypyridyl complexes interact differently with DNA. Furthermore, multiple binding modes of enantiopure complexes to DNA are often observed.^{94,95,164,202,206,207} One such example is Λ -[Ru(**TAP**)₂**dppz**]²⁺, which, through single crystal X-Ray structure analysis, was found to bind to duplex DNA i) through intercalation of the **dppz** ligand, and ii) through semi-intercalation of one of the **TAP** ligands, which resulted in marked DNA kinking and non-covalent crosslinking between DNA duplexes between symmetry-generated molecules.²⁰⁸ As the individual enantiomers were not resolved in this study, it was not possible to attribute preferential binding of the Λ - or Δ - forms with DNA, and thus the measurements presented represent a combination of both binding modes.

As mentioned, the titrations were also performed in high ionic strength conditions and the changes in the emission can be viewed in the appendix Figure A2.28. Figure 2.20 displays the enhancement (75) and quenching (76) of luminescence upon increasing concentration of ctDNA in both low and high ionic strength conditions. From these trends, it is clear that the electrostatic component of binding is significant for these complexes. Binding is greatly altered in the presence of high ionic strength, where the relative enhancement or quenching of the emission continued beyond the titration points. The errors from the attempted fit of the data were quite high and, as such, are not reported herein. However, the K_b values are estimated to be at least an order of magnitude lower (*ca.* 10^5 M^{-1}) than the complexes in the absence of 160 mM NaCl. More experiments are necessary at lower ionic strengths of NaCl to further analyse the effect of salt concentration on the overall strength of binding of complexes 75 and 76 to DNA.

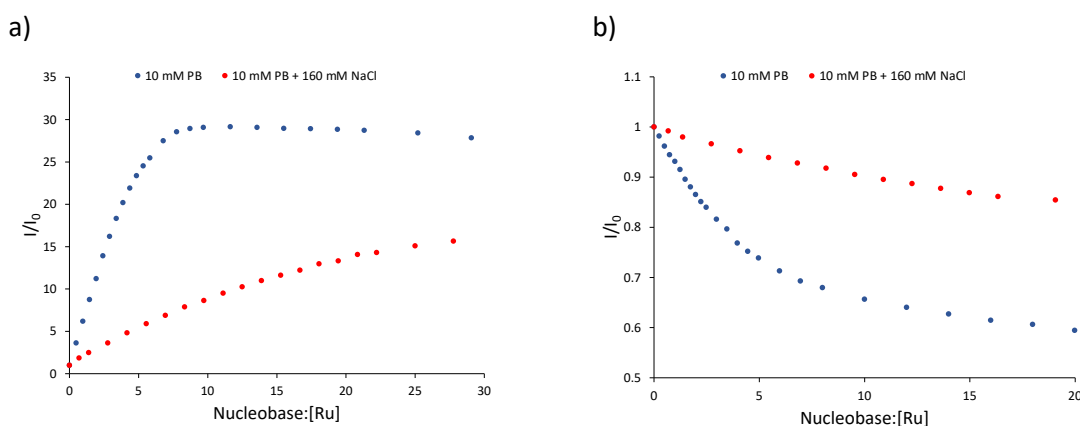


Figure 2.20: Changes in the luminescence intensities for a) 75 ($1 \times 10^{-5} \text{ M}$, $\lambda_{\text{ex}} = 450 \text{ nm}$) and b) 76 ($1 \times 10^{-5} \text{ M}$, $\lambda_{\text{ex}} = 450 \text{ nm}$) upon addition of ctDNA (0–0.2 mM) in 10 mM aqueous sodium-phosphate buffer with (red) and without (blue) the presence of 160 mM NaCl at pH 7.4.

The excitation spectra of the complexes were also analysed at increasing DNA concentration [Figure A2.29 (a)]. The **TAP** functionalised complex, **76** showed an overall decrease in emission intensity [Figure A2.29 (b)] upon increasing DNA concentration, as expected. The excitation spectra of **75** was more complex, particularly at high DNA concentrations [Figure A2.29 (a)]. For **75**, an initial increase in emission intensity upon MLCT excitation ($\lambda_{\text{em}} = 623 \text{ nm}$) was observed until the complex reached the fully bound form ($[\text{DNA}] = 1 \times 10^{-4} \text{ M}$). After this point, large changes in the DNA excitation region are observed, while the MLCT emission remains constant. This phenomenon may be due to an inner filter effect as the experiment was performed in a 3.2 mL cuvette, far from optically dilute conditions. However, in stating that, the same phenomenon is not observed with the **TAP** analogue, **76**, at similar DNA concentrations. It is hypothesised that an energy transfer process from **75** to DNA at high DNA concentrations may be occurring although more experiments are necessary to establish this. The decrease of the **PhenNap** contribution ($\lambda = 350 \text{ nm}$) to the Ru(II) MLCT-based emission in the presence of DNA is evident in Figure 2.21 (b). The disappearance of the **Nap** centred band is attributed to protection of the **Nap** unit upon DNA intercalation, which is presumably rapidly quenched. The raw absorption data through to 200 nm is also presented for comparison of the absorption and excitation data in the DNA region. The blue traces in Figure 2.21 denote the absorbance and emission ($\lambda_{\text{em}} = 623 \text{ nm}$) of the free complex, **75**, before the addition of DNA. As **75** is essentially non-emissive in aqueous media (*vide supra*, 2.5), the emission spectrum of the **75** is normalised [Figure 2.21 (b)] to overlay the MLCT-centred transitions for comparative purposes.

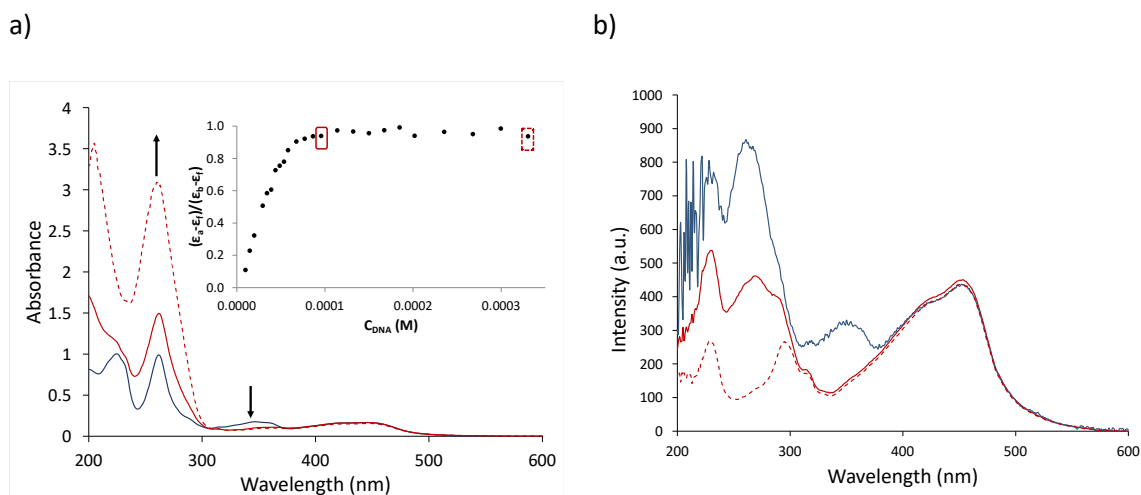


Figure 2.21: a) The raw absorption data of **75** at the selected titration points in the inset. B) The excitation spectra of **75** (normalised to MLCT emission, $1 \times 10^{-5} \text{ M}$, $\lambda_{\text{em}} = 623 \text{ nm}$, (blue) in the presence of $1 \times 10^{-4} \text{ M}$ ctDNA (red solid line) and $3 \times 10^{-4} \text{ M}$ ctDNA (red dashed line) in 10 mM aqueous sodium phosphate buffer at pH 7.4.

Overall, the described photophysical analysis of **75** and **76** upon DNA binding suggest a strong association of the complexes with DNA, with binding constants in the order of 10^6 M^{-1} . A binding site size of either two to three base pairs was determined for the complexes with natural ctDNA, indicating an intercalative mode of binding. The binding was found to be sensitive to increasing ionic strength, indicating an important electrostatic component to the overall DNA-complex association, as was outlined above. The results show that the **Nap** unit undergoes the most substantial changes to the absorption and emission properties at increasing DNA concentrations, possibly due to exposure to a new chemical environment upon intercalation between the DNA bases. Importantly, the derived parameters (binding constants and binding site sizes) from the absorption and emission titrations agree, with the latter being more accurate. The electronic coupling between the metal and the **Nap** at high DNA concentrations is disrupted, as shown in the excitation spectra where the **Nap** contribution to the emission is substantially decreased. Further experiments are necessary to determine whether a second energy transfer process is occurring at high DNA loading or if this observation is attributed to an inner filter effect of the instrumentation. Next, thermal denaturation experiments were performed to determine the effect of the complexes on the overall stability of the DNA molecule.

2.6.2: Thermal Denaturation Studies

To further probe the nature of the interaction of the complexes with double stranded ctDNA, thermal denaturation studies were performed. Thermal denaturation studies are used to analyse a substrate's ability to stabilise or destabilise DNA by monitoring changes in the melting temperature (T_m) of DNA.⁹ Denaturation of DNA is a process of unwinding and separating the two strands *via* breaking of the hydrogen bonds between complementary nucleobases, and can be initiated by many factors including, but not limited to, high temperatures, low pH and low salt concentrations.^{209,210} The T_m of DNA is defined as the temperature at which half of the DNA molecule is in the denatured state and can be examined by monitoring the change of the absorption band at 260 nm with increasing temperature. As the DNA unwinds, the absorption from the nucleobases increases.²¹¹ Since binding of small molecules to DNA typically results in a stabilisation effect, the change in T_m of the DNA can be correlated to the binding strength of the substrate.^{97,2,166}

Thermal denaturation studies [Figure 2.22] were performed by monitoring hyperchromic changes in the absorption at 260 nm of ctDNA (150 μM) at increasing temperatures (35-90 $^{\circ}\text{C}$ / 1 $^{\circ}\text{C}$ per minute) at different phosphate-to-dye ratios (P:D = 50, 20, 10) for both complexes [Figure 2.22]. All experiments were conducted twice for reproducibility.

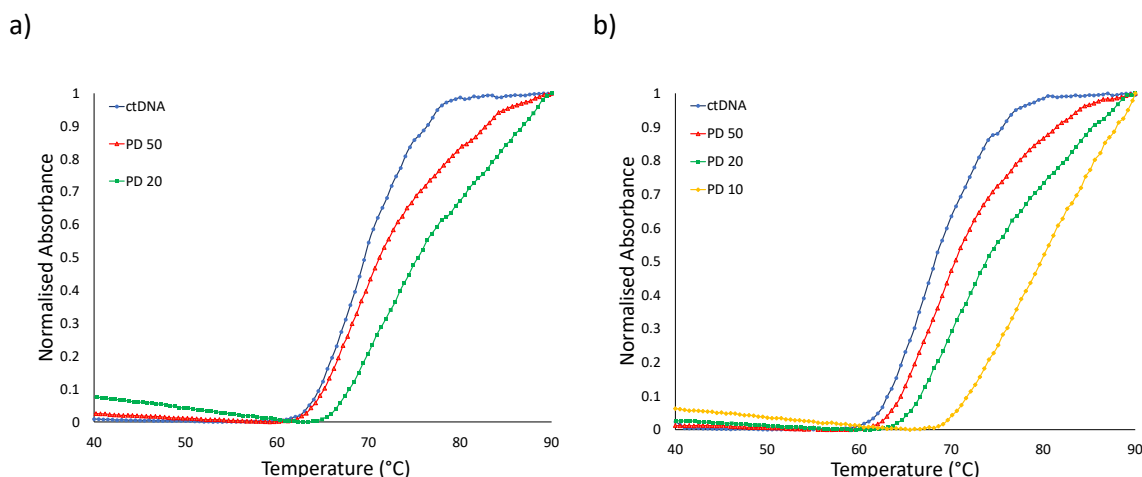


Figure 2.22: Thermal denaturation curves of ctDNA (150 μM) in the presence of (a) **75** and (b) **76** at different nuclear phosphate:dye (P:D) ratios. Absorbance was monitored at 260 nm.

The T_m for ctDNA in the absence of any Ru(II) complex was found to be 68.5 °C. Upon addition of small amounts of complexes (P:D=50), increases in the T_m were observed. Greater increases in the T_m of DNA were observed for **75** compared to **76** at the same P:D ($\Delta T_m = 6$ versus 2.5 °C, respectively at P:D = 50), indicating a greater stabilisation effect on the double helix for **75**. As an overall trend, the melting temperature of DNA increased with increasing concentration of Ru(II) complex, as observed for the $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{TAP})_2(\text{dppz})]^{2+}$ analogues, which interact with DNA primarily by intercalation. The greatest T_m of 80.5 °C was observed for **75** at a P:D of 10. Overall, at all P:D ratios, **75** was found to have a slightly greater stabilisation effect on the complex compared to **76**. When compared to complex **60**, the stabilisation effect of **75** and **76** on duplex DNA is far greater. Complex **60** exhibited a maximum shift of 6.8 °C at high dye loading concentrations (P:D = 5).¹⁶⁰ Changes in the T_m of ctDNA at different complex concentrations are presented in Table 2.10.

Table 2.10: Thermal denaturation data displaying changes in the melting temperature of ctDNA at differing P:D ratios in 10 mM aqueous phosphate buffer at pH = 7.4. The T_m of ctDNA alone is 68.5 °C.

Complex	ΔT_m (°C)		
	P:D = 50	P:D = 20	P:D = 10
75	6.0	8.0	12
76	2.5	6.5	---

Thus, it can be concluded from the thermal denaturation experiments that both complexes were found to greatly stabilise the DNA structure as a function of the complex concentration. It is important to note that while thermal denaturation experiments proved useful in determining a stabilisation effect of the complexes on DNA, the exact mode of binding cannot be discerned by

this technique. It has been shown that addition of simple cations, such as Mg^{2+} or purely electrostatic DNA binders, such as $[\text{Ru}(\text{bpy})_3]^{2+}$, to DNA exerts an overall stabilisation effect on the double helix, resulting in a shift in the melting temperature but preservation of the overall sigmoidal curve shape.¹¹ Thus, it can be inferred from the shape of the melting curves upon addition of **75** and **76** to DNA, that the binding mode is not purely electrostatic in nature. Further studies are necessary to elucidate the binding mode(s) which will be discussed in the following sections.

2.6.3: Circular Dichroism in Presence of DNA

Circular dichroism (CD) is a spectroscopic property displayed by chiral molecules and can be a useful probe in analysing DNA-drug interactions.²¹² CD spectroscopy measures the difference of absorption of left- and right-handed circularly polarised light by chiral molecules. Typically, B-DNA is characterised by a negative CD band at 248 nm, corresponding to the right-handed helicity of this form, and a positive CD band at 260 nm which is indicative of the $\pi-\pi^*$ transitions between the stacked nitrogenous bases.²¹³ While racemic mixtures do not elicit CD responses, upon binding to DNA, an induced circular dichroism (ICD) response was observed for both **75** and **76**. ICD signals between *rac*-Ru(II) complexes and DNA are therefore a direct indicator of a change of structure of the DNA and indicate an interaction between the complex and DNA.²¹⁴

To probe these interactions, CD studies [Figure 2.23] were performed in buffered water at constant DNA concentration (150 μM , $A_{260} \approx 1$) and P:D ratios of 50, 20, 10 and 5 for both complex **75** and **76** [Figure 2.23]. Titrations were performed twice to ensure reproducibility and each trace is an average of three measurements.

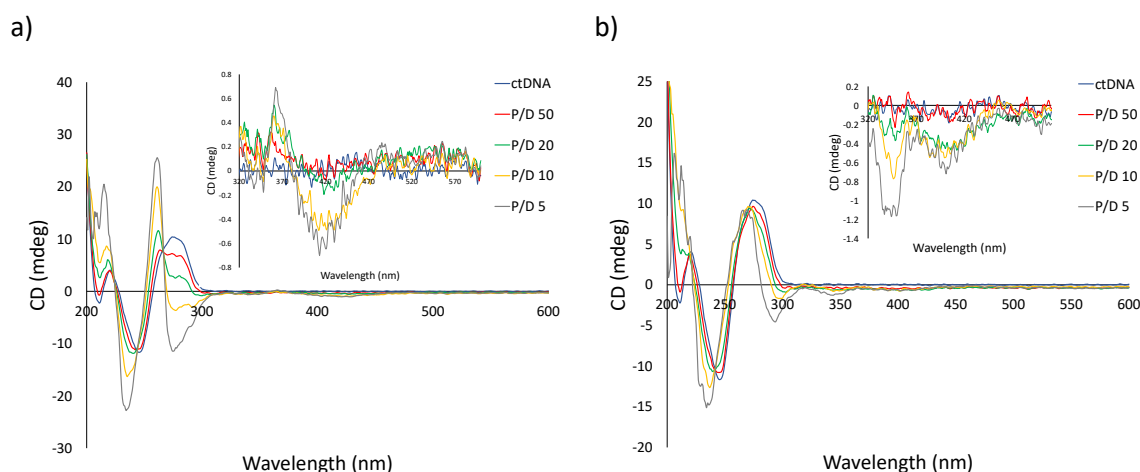


Figure 2.23: CD spectra of ctDNA (150 μM) in buffered water upon addition of a) **75** and b) **76** at different P:D ratios. Inset: region between 320-500 nm displaying an ICD signal of the **Nap**- and MLCT- centred bands of each respective complex.

75 was found to elicit more drastic changes in the CD response of ctDNA than **76**, particularly in the DNA absorption window (200–300 nm). The characteristic positive DNA CD band centred at 260 nm is significantly altered in the presence of complex and may even shift to lower wavelength as a result of conformational changes of the DNA molecule. At high complex loadings (P:D = 5), **75** induces a strongly negative band centred at 278 nm. This induced CD signal is attributed to π – π^* IL transitions of the auxiliary **phen** ligands. **76** results in less prominent changes of the DNA CD spectrum, however, a negative ICD band is observed at 295 nm which is attributed to the **TAP** π – π^* IL transitions. The changes in the signals from the DNA absorption envelope are significantly greater for **75** than **76**, potentially indicating an association of the auxiliary **phen** ligands in the DNA grooves.²¹² Both complexes induce a slight bathochromic shift in the band centred at 248 nm which indicates structural changes to the DNA molecule. Furthermore, the appearance of ICD bands at longer wavelengths (outside the DNA absorption window) can be directly assigned to an association of the complex with DNA. ICD signals in the regions of 350–500 nm are observed at high concentrations of complex and correspond to the absorption bands of the **Nap** IL and MLCT transitions. These signals indicate a direct interaction of the metal complexes with DNA and/or structural changes within the DNA molecule.²¹⁴ The difference in the ICD signals outside of the DNA absorption window may be due to preferential binding of the of the enantiomers. **75** displays a stronger response in the MLCT region, while **76** displays greater ICD signals in the **Nap** region.

To summarise, the CD titrations provide an excellent indicator that significant interactions are present between both complexes and DNA, although the exact nature of binding cannot be determined from these experiments. It appears that **75** induces greater changes than **76** in the overall structure of DNA and that the auxiliary **phen** ligands may be interacting with DNA in a groove-binding mechanism. The appearance of ICD bands at long wavelength (320–450 nm) shows that the metal centre is tightly associated with the DNA. **76**, on the other hand, evoked less changes in the overall DNA structure DNA as a function of concentration, suggesting that it is possibly interacting with DNA in an intercalative manner, as indicated by the greater ICD signal at 350 nm. To further investigate the mechanism of binding, linear dichroism studies were performed and are discussed in the next section.

2.6.4: Linear Dichroism

Solution phase flow linear dichroism (LD) spectroscopy is a technique that has received great attention in nucleic acid research since it can probe the orientation of molecules in solution. LD describes the difference between the absorbance signals of two linearly polarised lights: one parallel and one perpendicular to the reference orientation axis of the molecules in solution, *i.e* the

DNA helical axis. DNA is ideally suited to LD detection as the molecules are long, thin and align well in flow. B-DNA displays a characteristic negative band centred at 260 nm, which corresponds to the electronic $\pi-\pi^*$ transitions of the aromatic nucleobases and are localised perpendicular to the DNA-axis. Thus, LD is a powerful technique to understand DNA-drug binding modes as molecules that intercalate between the base pairs will result in a negative LD response as they are orientated in the same plane as the nucleobases, i.e. perpendicular to the DNA helical axis. Conversely, molecules that interact with DNA along the orientation axis (such as in the minor groove of B-DNA) will result in a positive LD response.²¹⁵ Furthermore, it can be assumed that any appearance of a signal outside of the 240-280 nm DNA envelope can be recognised as the interaction of a substrate with DNA.²¹²

LD titrations were performed on ctDNA at differing P:D ratios in a similar manner to the CD titrations and full details can be viewed in the Appendix [Figure S.21]. The magnitude of the LD response is greatly affected by the sample concentration and spin/flow rate, among other parameters. While every effort was made to produce accurate responses, this technique is very sensitive and multiple measurements were necessary to obtain comparable results for both complexes. As such, the LD response of B-DNA (300 μ M) and changes from both complexes are displayed in Figure 2.24 at a P:D ratio of 5. In both cases, a change in the DNA region (240-280 nm) is observed, however, both **75** and **76** also absorb in this region, so it is difficult to draw a definitive conclusion as to the reason of the splitting of this signal. The appearance of a peak at 270 nm may be attributed to exciton coupling between the auxiliary **phen** or **TAP** ligands, as is the case for the $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$ analogue.⁹⁶ Titrations at higher dye loading are necessary to confirm this hypothesis. Both complexes elicit negative LD responses in the region attributed to the $\pi-\pi^*$ transitions of the **Nap**, which further supports the suggestion that there is an intercalative binding mode of the **Nap** unit between the base pairs. The MLCT absorption regions also possess structured LD bands indicating that the metal centres are in proximity to the DNA axis, however, their negative signals indicate that no moieties are bound within the grooves of the DNA. Notably, the long wavelength MLCT band of **76** has an LD response of 0, indicating preferential binding of one of the ligands to DNA, which is most likely the **PhenNap** ligand.

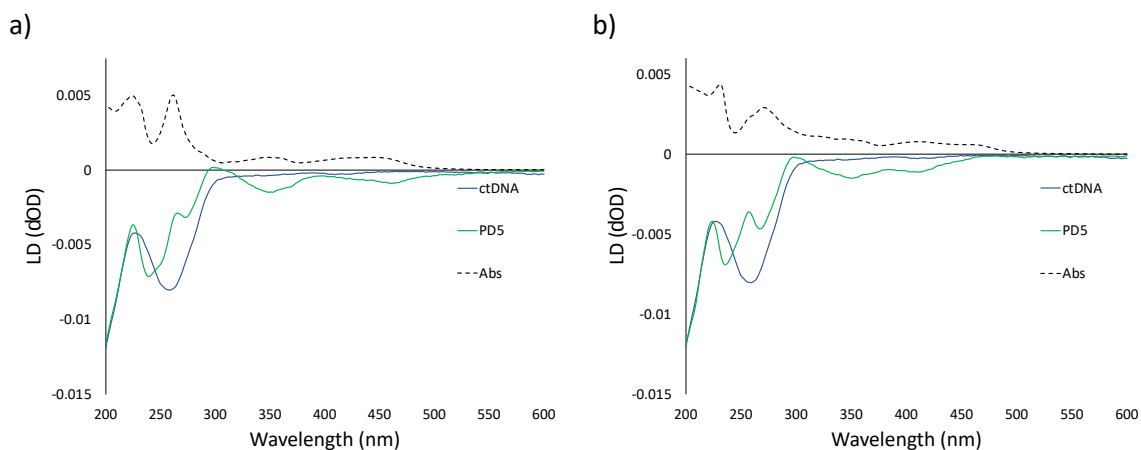


Figure 2.24: LD spectra of ctDNA (300 μ M) in 10 mM aqueous sodium-phosphate buffered water at pH = 7.4 upon addition of a) **75** and b) **76** at P:D = 5.

The above experiments confirm that the binding modes of the complexes to DNA involve interactions that are perpendicular to the helical axis and strongly suggest that the **Nap** moiety intercalates between the base pairs, with some interaction also from the Ru(II)-polypyridyl species. The results should again be taken as an approximation due to the presence of racemates in solution. Further experiments such as viscosity measurements are necessary to assign the exact binding mode of the complexes to DNA. Preliminary viscosity measurements were performed which indicate an overall positive relationship between the concentration of the complexes and the viscosity of the DNA-drug solutions, however these results are preliminary and the results of which can be viewed in the Figure A2.30.

2.6.5: Viscosity Studies

Preliminary viscosity measurements were performed and yielded inconclusive results, as the specific size of the DNA fragments upon shearing was not determined. Instead, a method previously employed in our group was followed and assumed to hold accurate.⁹⁷ The viscosity of the DNA solution initially decreased when **75** was added, which may be attributed to kinking of the double helix, thus shortening its length and lowering the relative viscosity of the DNA-**75** solution in comparison to the DNA alone. Preliminary viscosity measurements of the **TAP** complex, **76** and DNA seemed to indicate a positive relationship between the concentration of the drug and viscosity of the DNA solution. The DNA employed in this experiment was only subject to ultrasound irradiation for 30 s and thus, the length of the DNA fragments was also not known [Figure A2.31].

2.7: Conclusions and Future Perspectives

This chapter has outlined the synthesis, photophysical, electrochemical and solid state properties of two new bichromophoric Ru(II)-polypyridyl complexes containing appendaged **Nap** ligands. The complexes differ in their auxiliary ligand substituents: **Phen** versus **TAP**. The complexes were

designed to combine the attractive and tuneable photophysical properties of the Ru(II)-centre and the known anticancer properties of the **Nap** unit. The complexes were found to bind strongly to DNA in the order of 10^6 M^{-1} , as observed from the large changes in the absorption and emission properties arising from the MLCT emission and subsequent fitting of the data to a modification of the Bard equation. The binding site sizes were found to be approximately two base pairs, suggesting an intercalative mode of binding.

75 displayed the characteristic “light-switch” effect upon binding to DNA with a substantial 30-fold increase in emission intensity in the bound *versus* free form, attributed to protection of the metal centre from singlet oxygen and a different chemical environment experienced by the **Nap** unit upon intercalation. Additionally, solid state structure analysis of the complexes in aqueous media displayed hydrogen bonding interactions between the solvent and imide oxygen atoms of the appended **Nap** ligand. These interactions could account for the non-emissive nature of this complex in aqueous media as energy transfer to the bulk water can be facilitated through the vibronic modes. **76** conversely displayed emission quenching upon DNA binding, attributed to a PET process to the π -deficient **TAP** ligands from the DNA. Binding was also found to be sensitive to the ionic strength on the media. Separation of the Λ - and Δ -enantiomers is necessary to provide a full understanding of the mechanism of binding, but it is hypothesised that binding occurs in a cooperative manner, with multiple binding modes present. The changes in the CD spectra for both complexes differed in the DNA absorption region, however, both complexes elicited ICD responses in the complex absorption regions, indicating direct association of the complexes with DNA. Complimentary results were obtained by analysis of the LD spectra. The complexes were also found to have an overall stabilisation effect on the DNA structure as displayed by thermal denaturation experiments.

Overall, the complexes discussed herein display a range of attractive properties that make them excellent candidates for DNA probes and DNA-reactive agents, which was the main aim of this chapter. When compared to the other non-substituted Ru(II)-**Nap** conjugate in the literature, **60**, complexes **75** and **76** elicited greater stabilisation effects on duplex DNA, as shown by thermal melting experiments. Their binding constants are comparative, however, the absorption and emission responses are more sensitive for **75** and **76** toward the binding event, when compared to **60**. This supports the effective design of **75** and **76** which placed the **Nap** in close proximity to the Ru(II) metal centre, as opposed to being separated by an aliphatic spacer, as is the case for **60**. **75** may display uses as a luminescent probe for nucleic acids as it displays marked emission enhancement (30-fold increase in emission intensity) in the bound form with natural ctDNA (G-C content of 42%). Future work on this complex includes enantiomer resolution and sequence specific

studies to analyse the effect of nucleobase sequence on the emission properties. Further investigation of a potential secondary interaction with DNA is also warranted from the unusual observations in the excitation spectra at high DNA concentrations. **76** may display uses as a photoreactive agent in photodynamic therapy (PDT), as has been demonstrated for other Ru(II)-complexes containing polyazaaromatic ligands. As such, both complexes warrant further investigation into their i) abilities to sensitise $^1\text{O}_2$, ii) cellular localisations and iii) cytotoxicity profiles for the potential use as PDT and/or theranostic agents.

Chapter 3:

Ruthenium(II)-Polypyridyl

Complexes Containing

Substituted **PhenNap** Ligands

3.1: Introduction

As was previously discussed, some examples of Ru(II)-Polypyridyl-**Nap** complexes have been previously explored within the Gunnlaugsson group and have displayed attractive sensing, bioimaging and therapeutic applications, modulated by: i) the position of the **Nap** with respect to the Ru(II)-centre, and ii) the substitution pattern on the **Nap** chromophore (*vide supra*, Sections 1.6 and 1.7).^{16,160,168,169,216,217} In this regard, Dr(s). Gary Ryan and Robert Elmes have studied spectroscopically active Ru(II)-**Nap** conjugates which displayed attractive photophysical properties, and furthermore, allowed for the individual spectral resolution of the interaction of different parts of the molecules with DNA.^{168,169} Some examples of previously designed structures are presented in Figure 3.1, where variation in the linker motifs between the **Nap** and Ru(II)-centre is displayed. This structural change was found to modulate the photoreactivity properties of the complexes, where the complexes with a rigid aromatic arrangement of the **Nap** around the Ru(II)-centre (**69** and **70**), displayed enhanced photoreactivity toward DNA compared to the flexible, alkyl-linked analogues, **90**.^{168,169} To date, no examples of Ru(II)-polypyridyls containing the **Nap** motif directly coordinated to the Ru(II)-metal centre through a single bond have been explored for biological utility.

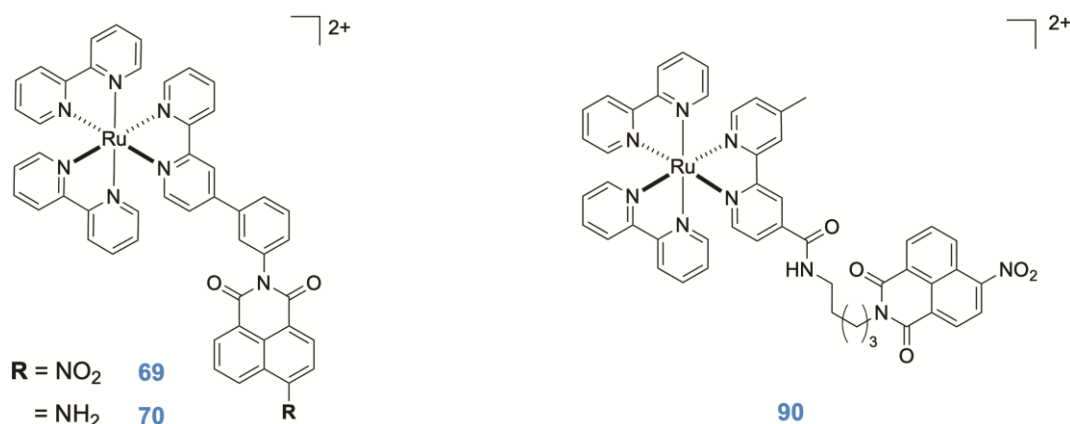


Figure 3.1: Chemical structures of Ru(II)-Nap conjugates developed in the Gunnlaugsson group.^{168,169}

Chapter 2 described the photophysical and DNA binding properties of a bis-**phen** and bis-**TAP** Ru(II)-polypyridyl complex, bearing an unsubstituted **PhenNap** ligand, **80**. Following the observed emission intensity sensitivity of the **phen** analogue, **75**, upon DNA binding (*ca.* 30-fold increase, *vide supra*, Chapter 2), functionalisation in the 4-position of ligand **80** with a variety of functional groups was introduced. The photophysical properties of the ligands, and their corresponding Ru(II)-polypyridyl complexes were analysed, as well as changes in these properties upon DNA binding of the complexes. It was anticipated that functionalisation in the 4-position with hydrogen bond donor or acceptor groups may give rise to the greater association of the complexes

with the biomolecule than the non-substituted derivative. Furthermore, halogen-substituted systems were also designed to tune the photophysical properties of the systems, as incorporation of halogens to transition metal complexes has been shown to elicit more red shifted photoluminescence properties, which is an attractive outcome for cellular biology.¹⁷⁶ Following on from Chapter 2, the aim of this chapter was to design and explore the properties of *N*-(1,10-phenanthrolin-5-yl)-1,8-naphthalimide ligands **91-93**, and their corresponding [Ru(**phen**)₂]²⁺ complexes, **94-96**, which potentially should be capable of targeting DNA and acting as cellular therapeutics and luminescent imaging agents [Figure 3.2]. Thus, while the preceding chapter explored the photophysical and the DNA binding of Ru(II)-polypyridyl-**Nap** complexes displaying no substitution on the **Nap** moiety (complexes **75** and **76**), this chapter aims to build upon that work by developing complexes with functionalisation in the 4-position of ligand **80** with a variety of groups, such as nitro, bromo and chloro groups, **91-93**, and explore the subsequent chemistry associated with the systems in a similar manner. These will be the first examples of such halide based **Nap** complexes studied within the Gunnlauugsson group as DNA targeting Ru(II)-polypyridyl-**Nap** complexes (**94-96**).

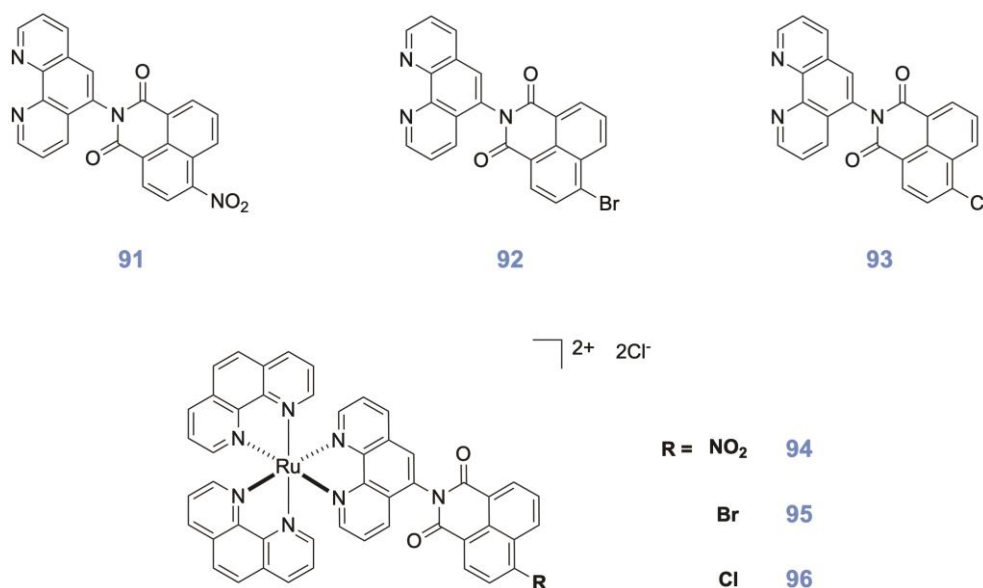


Figure 3.2: Chemical structures of ligands **91-93** and complexes **94-96**.

Substitution of the 1,8-**Nap** chromophore with amines has been shown to give rise to a highly efficient internal charge transfer (ICT) excited states generated from the presence of the electron donating amino group and electron withdrawing imide moieties. This ICT character is most pronounced in the case of substitution in the 4-position of the **Nap** chromophore.^{18,218} As such, ligand **97** was also designed which was expected to form easily under standard reducing conditions from the corresponding nitro- precursor, **91**. As will be discussed, this was not the case, and **97** was only obtained in trace quantities, which was quite surprising, given that other related 4-amino **Nap**

systems are normally easily obtained using classical reduction methods from their corresponding nitro- analogues. This amino derivative was designed to act as a structural handle to increase access to higher ordered structures such as the derived Tröger's base (**TB**), **98**, as well as the corresponding mono- and dinuclear ruthenium polypyridyl complex, **99** and **100** [Figure 3.3]. Preliminary theoretical calculations are presented to attempt to explain the failed reduction series of **91**. Efforts made toward the synthesis of the higher ordered structures **98** and **100** is reserved for Chapter 4.

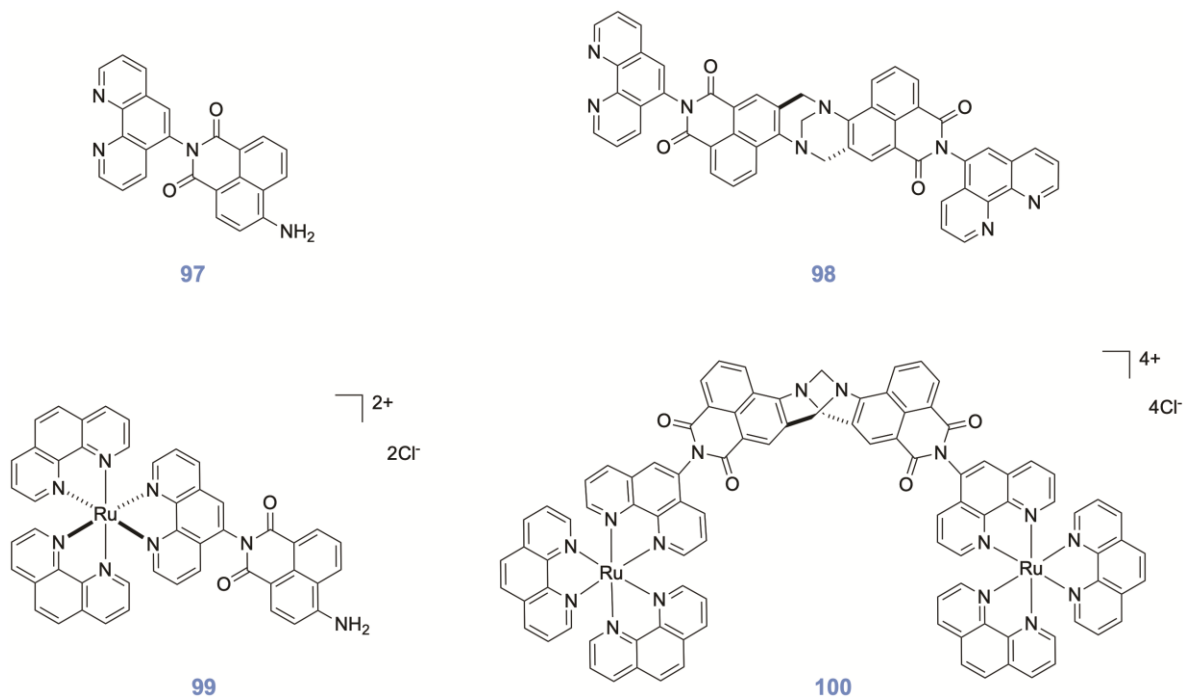
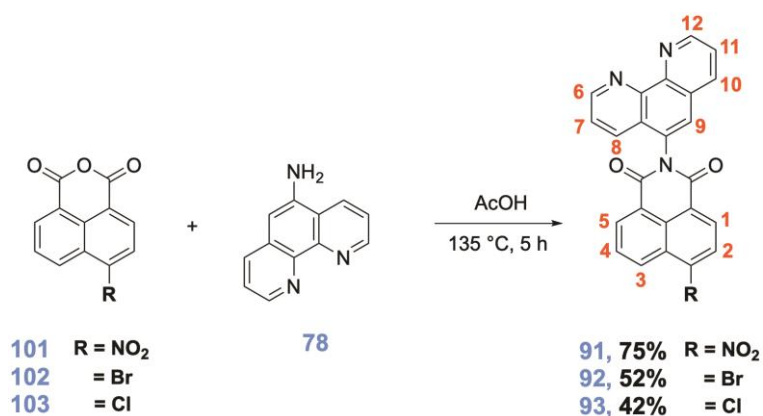


Figure 3.3: Chemical structures of ligands **97** and **98** and desired complexes **99** and **100**.

Thus, the work presented in this chapter was inspired by previous work within the Gunnlaugsson group, results from the preceding chapter, and structures developed by Thomas and co-workers such as the work on dinuclear complex **46** (*vide supra*, 1.5.2).^{1,168,169} This chapter details the synthesis, characterisation and photophysical properties of the series of **Nap** ligands **91**, **92**, **93** and **97** and corresponding Ru(II)-**Nap** conjugates **94**, **95**, **96** and **99**. The photophysical, electrochemical and DNA binding properties of the metal complexes are presented alongside a description of their solid-state structures with emphasis on the intermolecular interactions expected to be prevalent upon interaction with DNA. Attempts toward the synthesis of a 4-amino derived **TB**, **98**, and the corresponding dinuclear Ru(II)-**TB** complex **100** is also presented along with some preliminary computational studies which attempts to address the failed reaction series involving the seemingly facile reduction of an aromatic nitro- group to its free amine form.

3.2: Synthesis and Characterisation of Ligands **91**, **92**, **93** and **97**3.2.1: Synthesis and Characterisation of **91**, **92** and **93**

The synthesis and characterisation of the structurally related 4-substituted-1,8-naphthalimide ligands were performed. The **PhenNap** derivatives, *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91**, *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92**, *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93** were synthesised in 75, 52 and 42% yields, and isolated as beige, pink and white powders, respectively. The reaction proceeded *via* condensation reaction of the appropriate 1,8-naphthalic anhydride (4-NO₂/Br/Cl-naphthalic anhydride; **101–103**) with 5-amino-1,10-phenanthroline, **78**, in acetic acid in a pressure vessel at 135 °C for 5 hours [Scheme 3.1]. Details of the synthesis of **78** was presented previously (*vide supra*, Figure 2.4).^{184,219} The **PhenNap(NO₂)** derivative, **91** was originally synthesised by Castellano and co-workers¹⁵⁰ employing EtOH and the solvent; however, improved yields and shorter reaction times were obtained by modifying the procedure to that developed by Franco *et al.* for the **PhenNap(Br)** analogue, **92**.²²⁰ The same conditions were also employed for the **PhenNap(Cl)** derivative, **93**.²²⁰ Ligands **91** and **92** have been previously studied by Castellano, Franco and Doyle for transition metal photocatalysis, namely CO₂ reduction and have displayed interesting excited state properties that was previously discussed (*vide supra*, Section 1.4.3), while ligand **93** has not yet been reported in the literature.^{150,151,185,220,221} To the best of our knowledge, the biological activity of this series of ligands have not been investigated.

Scheme 3.1: Synthesis and proton numbering of substituted **PhenNap** ligands **91**, **92** and **93**.^{185,220}

The ligands were characterised by multiple techniques including ¹H, ¹³C NMR, HRMS and IR spectroscopy, the details of which can be viewed in the appendix A3.1-A3.12 and experimental sections. The ¹H NMR spectra of the three ligands are presented in Figure 3.4, where the electronic effect of the substituent in the 4-position of the **Nap** is observed from the relative chemical shifts of protons 1-5, while the phenanthroline protons (6-12) are essentially unaffected by the substitution pattern. In particular, the roofing doublets observed for the **PhenNap(Br)** species, **92**,

at 8.34 and 8.42 ppm corresponding to H₁ and H₂, respectively, undergo the greatest chemical shifts when the electronegative nitro- or chloro- groups are introduced in the 4-position of the **Nap**.

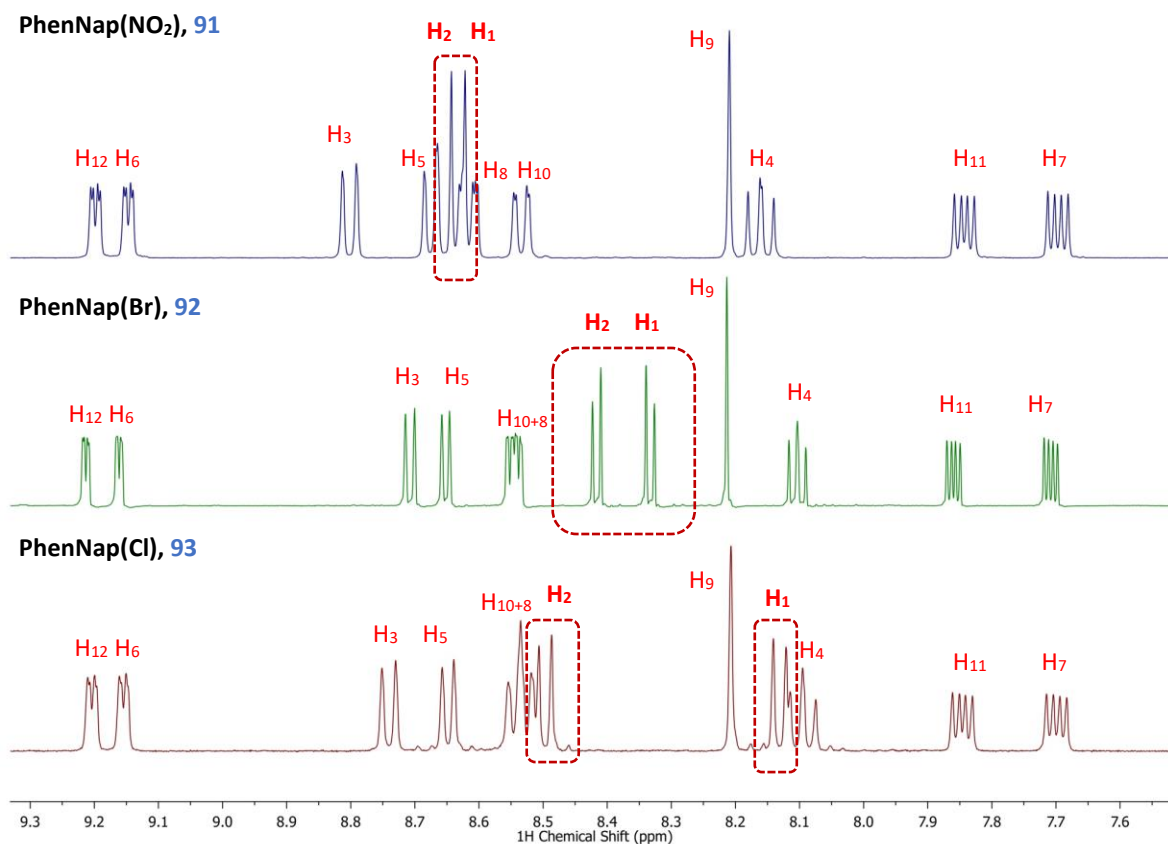


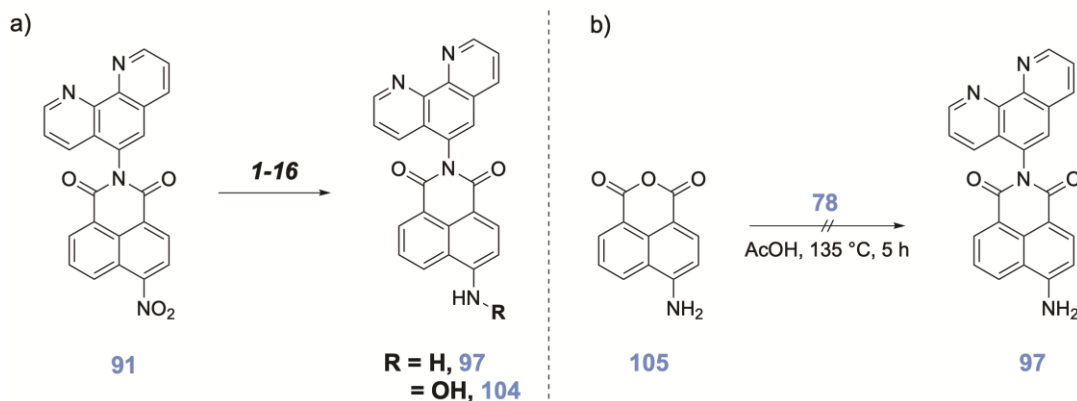
Figure 3.4: The ¹H NMR spectra (400 MHz, DMSO-*d*₆) of ligands **91** (top, blue), **92** (middle, green) and **93** (bottom, red).

Having obtained **PhenNap(NO₂)**, **91**, reduction to the corresponding amine, **97**, was next attempted which will be discussed in the next section.

3.2.2: Synthesis and Characterisation of **97**

While seemingly trivial, the synthesis of **PhenNap(NH₂)**, **97** from the corresponding nitro-precursor, **91** was extremely challenging. This strenuous synthetic endeavour ultimately yielded the product on a minute scale and some of the unexpected challenges will be discussed herein. As discussed in Chapter 1, the 4-amino-1,8-**Naps** possess interesting photophysical properties due to the internal charge transfer (ICT) excited states arising from the electron donating amine and electron withdrawing imide groups. **PhenNap(NH₂)**, **97**, was anticipated to be highly luminescent due to the unique ICT character, and furthermore, would act as a direct synthetic precursor for the synthesis of the **Phen**-functionalised Tröger's Base (**TB**), **TBPhen**, **98**. Many synthetic conditions were employed in an attempt to reduce the aromatic nitro group to the primary amine [Scheme 3.2 (a), Table 3.1], all of which were unsuccessful in yielding the product in any reasonable amounts. This

was surprising observation as the structurally similar *N*-aryl- and terpyridine- substituted **Nap(NO₂)** ligands proceeded reliably.^{161,222}



Scheme 3.2: a) Attempted reduction of **91** to **97**. b) Attempted synthesis of **97** from the 4-aminonaphthalic anhydride precursor, **105**.

Table 3.1: Reaction conditions for the reduction of **91** to **97**

Entry	Reducing Agent	Catalyst	Catalyst amount / w/w	Solvent(s)	Product
1 2	H ₂ (3 atm)	Pd/C (10 wt%)	10% 20%	EtOH	--- Partial reduction
3 4	H ₂ (3 atm)	Pd/C (10 wt%)	10% 20%	DMF	--- ---
5 6	H ₂ (3 atm)	Pd/C (10 wt%)	10% 20%	EtOH:DMF (9:1)	--- Partial reduction
7 8	H ₂ (3 atm)	Pd(OH) ₂ /C (20 wt%)	20%	EtOH:DMF (9:1)	Partial reduction
9 10	H ₂ (3 atm)	Pd(OH) ₂ /C (20 wt%)	40%	EtOH:EtOAc (1:1)	Partial reduction
11	N ₂ H ₄	Pd/C (10 wt%)	10% 20%	EtOH	---
12 13	SnCl ₂	HCl	xs	EtOH IPA	Trace quantity
14	SnCl ₂	---	---	EtOH	Trace quantity
15	Na ₂ SO ₄	---	---	EtOH:H ₂ O	Starting material
16	NH ₄ HCO ₂	Pd/C (10 wt%)	10%	EtOH	Starting material

The reduction of **91** was first attempted by employing standard conditions used within our group for the reduction of related 4-nitro substituted **Naps**.^{17,20,63,223} The attempted reduction conditions are summarised in Entries **1-10** [Table 3.1]. In all these cases, **91** was subject to catalytic hydrogenation at 3 atm of H₂ using a Parr hydrogenation shaker apparatus. Throughout these attempts, the catalyst, catalyst loading, solvent systems and reaction times were varied to optimise the synthesis. After hydrogenation, the resulting reaction mixtures were filtered through Celite and

washed with the respective solvent until the washings ran clear. The solvents were removed under reduced pressure and the products were isolated as red/brown solids. The ^1H NMR spectra indicated the presence of **91** as a minor product (< 25%) and a major product corresponding to the partially reduced hydroxylamine, **104** [Figure 3.5], as evident from the significant chemical shift of proton number 2 (H2) to lower ppm due to shielding from the external magnetic field upon reduction of the electron withdrawing NO_2 group to an electron donating species. The presence of hydroxylamine intermediate, **104**, was confirmed by mass spectrometry analysis and NMR spectroscopy which identified labile -OH and -NH protons at 10.7 and 9.5 ppm, respectively, that underwent deuterium-hydrogen exchange upon addition of D_2O to the NMR vessel. Attempts were made to purify this mixture by recrystallisation, and by washing and trituration with various solvents, all of which were unsuccessful, attributed to the extremely poor solubility of the crude product.

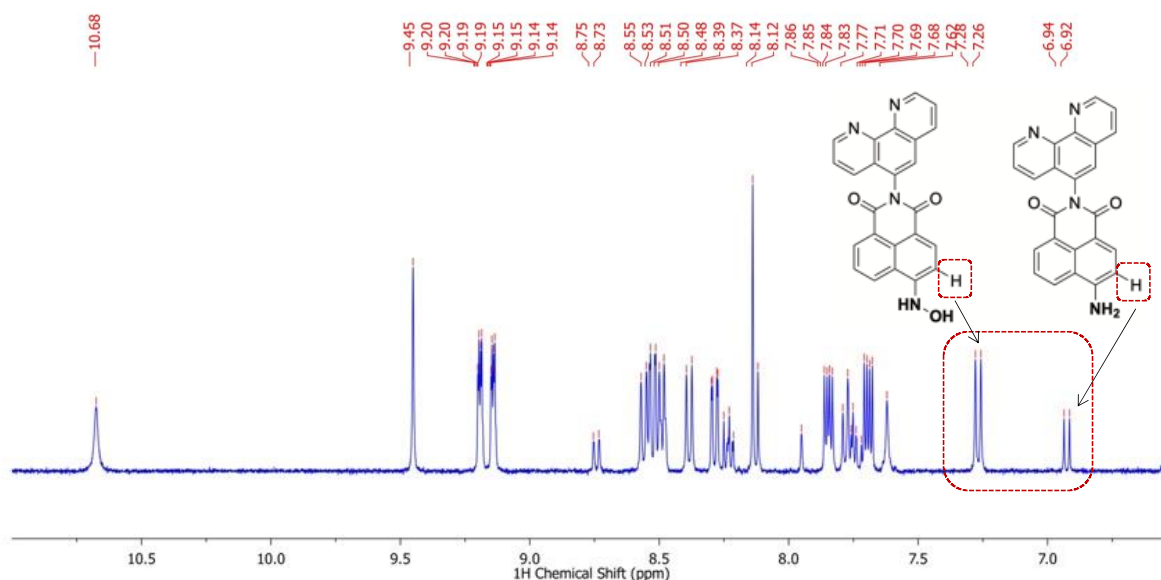


Figure 3.5: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of mixed products **97** and **104** upon reduction of **91** employing conditions from Table 3.1, Entry 6.

Thus, different reducing agents were investigated, such as hydrazine monohydrate [Entry 4, Table 3.1]. To a mixture of **91** and Pd/C (10 wt%, 20% w/w), hydrazine monohydrate was added, and the reaction heated at reflux and was monitored by TLC until full consumption of the starting material was observed. The resulting reaction mixture was filtered hot through Celite, and the solid product was washed with ethanol. As above, the solvent was removed under reduced pressure and the resulting solid product was further washed with water and dried *in vacuo*. Upon analysis of the product by ^1H NMR, apparent decomposition was observed and as such, these conditions were not pursued any further [Figure A3.13].

Progress was made toward the synthesis of **97** by employing tin(II) chloride as the reducing agent in the presence of an acid catalyst [Entry **12**, Table 3.1].²²⁴ To a mixture of **91** and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in EtOH or IPA, concentrated HCl was added dropwise and precipitation of a yellow solid was observed, which formed at a much faster rate in IPA, attributed to poorer solubility in this solvent. Insolubility of the product in all conventional solvents and solvent mixtures proved troublesome in isolating the product in pure form. The products from this reaction again produced a mixture of starting material, **91**, reduced species, **97**, and the partially reduced hydroxyl amine intermediate, **104**. The reduction of **91** was further optimised using a method developed by Young *et. al* [Entry **13**, Table 3.1] for the reduction of a highly insoluble nitro-substituted macrocycle to the corresponding amine, employing similar conditions to those described above, but without the presence of acid.²²⁵ Following this method, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (10 eq.) and **91** were reacted at reflux under Ar in EtOH. Precipitation of an orange solid was observed, and the reaction mixture was basified using 6 M NaOH to pH 12. The aqueous solution was extracted into CHCl_3 and the organic layer was washed multiple times with water, EDTA, and brine, and finally, dried over MgSO_4 . Proton NMR analysis of the product revealed highly deshielded signals at 9.89 ppm, assigned to the alpha protons of the phenanthroline moiety, indicating that complexation the tin metal from the stannous chloride reducing agent had occurred. Subsequently, the product was washed several more times with an aqueous solution of EDTA, a strong chelating competitor which reduced the intensity of these signals [Figure A3.14], and finally the product was isolated in the pure form by Soxhlet extraction for 24 h with CHCl_3 . Figure 3.6 displays the ^1H NMR of the novel ligand, **97** in the pure form. Indeed, this material was found to be highly luminescent, as anticipated, and as indicated by the photograph [inset: Figure 3.6], which displays less than 5 mg of **97** in 40 mL CHCl_3 .

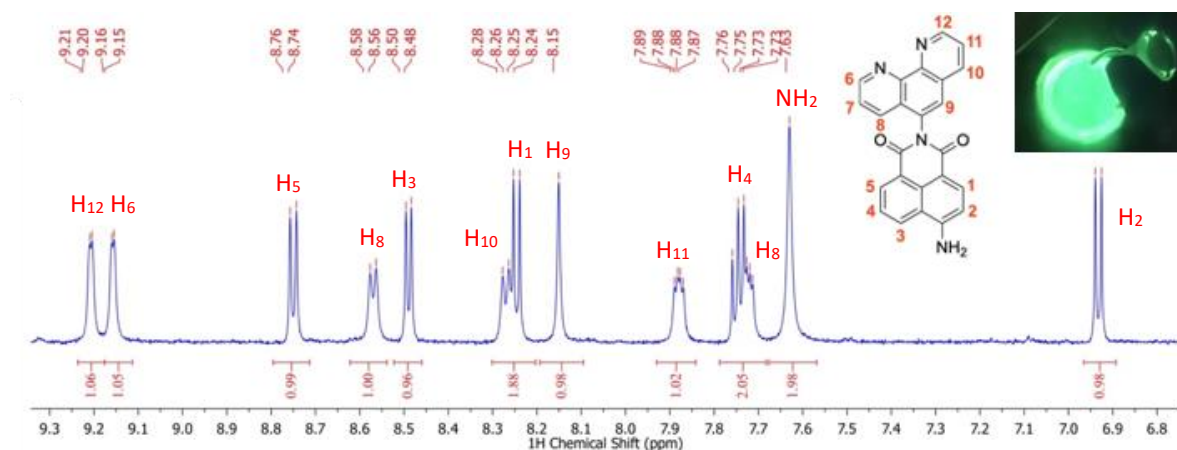


Figure 3.6: The ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$) of **97**. Inset: Observed fluorescence of **97** in CHCl_3 solution under the UV lamp ($\lambda_{\text{ex}} = 254 \text{ nm}$).

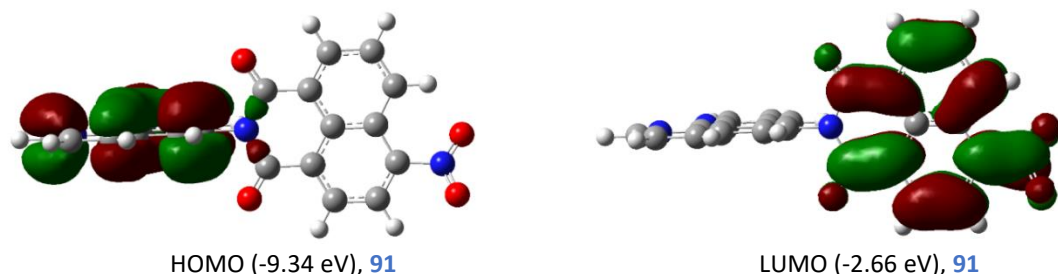
Finally, other conditions were employed to either, i) reduce **91** to **97** using sodium thiosulfate or ammonium formate [Table 3.1, entries **15** and **16**] or, ii) to synthesise **97** from

alternative starting materials such as in Scheme 3.2 (b), however these attempts returned only starting materials. Thus, while the synthesis of **97** was ultimately achieved, poor solubility and difficult synthetic endeavours proved to be major obstacles and returned the product only on a small scale. Further synthetic investigation is required to fully utilise this ligand for further studies, and may involve the use of high energy synthesis, such as through microwave irradiation, or the presence of different metal based catalysts, such as oxo-rhenium complexes recently developed by Grieco and Blacque to yield the product on an acceptable scale.²²⁶ Full characterisation of the ligand can be viewed in the experimental and appendix section [Figures A3.15-A3.18]. As this reaction proved so troublesome, preliminary theoretical calculations of **91** and **97**, were performed to investigate their electronic properties and are presented in the next section.

3.3: Theoretical Calculations of **91** and **97**

Theoretical calculations for ligands **91** and **92** were performed by Dr Deivasigamani Umadevi at The Indian Institute of Technology Palakkad (IITPKD), Kerala, India. The structures were optimised by using the semiempirical PM6 method and were characterised as minima after verifying the presence of all real frequencies. Frontier molecular orbital (FMO) density diagrams were constructed to investigate the electronic properties and orbital distributions of the ligands. All the calculations were done using the Gaussian 16 programme [Figure 3.7].²²⁷ The crystal structure data of **91** (*vide infra*, Section 3.5), was provided to aid the calculations.

a)



b)

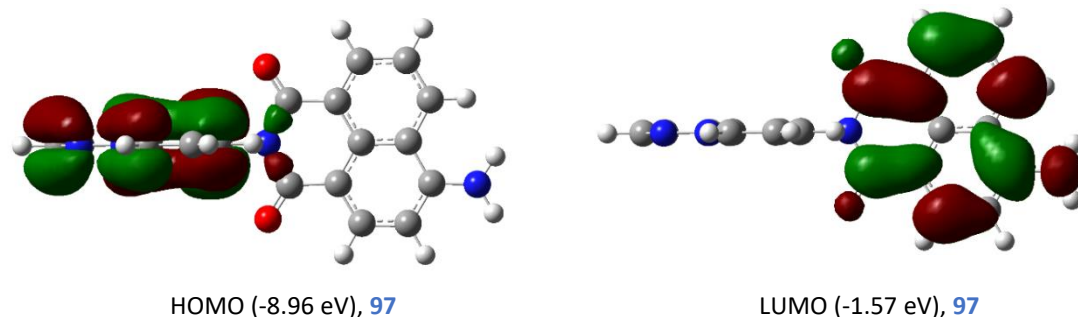


Figure 3.7: FMO density distribution diagrams and associated energies (eV) of a) **91** and b) **97**.

Interestingly, the HOMO and LUMO orbital distributions were found to be extremely similar for both ligands. In both cases, the HOMOs were found to be localised on the **phen** moiety and the LUMOs found distributed on the **Nap** moiety. This indicates the possibility for photo-induced electron transfer (PET) from **phen** to **Nap** unit. Importantly for **97**, it was anticipated that the HOMO would be localised on the amino-**Nap** while the LUMO was expected to reside on the imide moiety of the **Nap**, which would support the typical ICT transition observed for such systems.^{64,158,228,229} These calculations instead indicated that for **97**, a PET process from the **phen** to the **Nap** would prevail over the ICT. Further calculations are necessary in order to fully understand the chemical reactivity of the ligands, however it is noted that the electron donating amino group has a destabilising effect on the energy of the LUMO, resulting in a greater HOMO-LUMO gap for **97** compared to **91**, as expected. To further probe the electronic properties of the ligands presented, the solid state structures of **91**, **92** and **93** were investigated and are presented in the next section.

As was mentioned in the introduction, Dr Robert Elmes at Maynooth University, has been developing enzymatic probes for NTR activity in real time. Having discussed our work with him using complex **94**, we entered collaboration where this nitro complex was subjected to the enzyme. Indeed, this collaboration showed that the corresponding amino complex **99** could be generated *in situ* with large luminescent enhancement being observed in the MLCT emission. Concomitantly, the Elmes group also tried to reduce **91** to give **97** using non-enzymatic reactions. However, as above, they also encountered difficulties in doing so. While we did not consider the formation of the 4-azide analogue of **91**, they did, and have recently communicated to us that they have now been able to generate **97** in that manner by using the Staudinger Reaction.

3.4: Crystallographic Studies of **91**, **92** and **93**

Having successfully synthesised ligands **91**, **92**, and **93** investigation into their solid state structures was undertaken by single crystal X-Ray diffractometry. All crystal structure data sets were collected on a Bruker APEX DUO (CuK α source) instrument. Details of experiments are provided in the appendix section. Herein, the novel crystal structures of the ligands are described alongside their and major intermolecular and intramolecular interactions in the extended structure, with specific reference to interactions which are expected to affect their DNA binding properties.

3.4.1: X-Ray Crystal Structure of **91**

Colourless needles of **91** were obtained by slow evaporation of a solution of **91** in toluene and the crystal structure was solved in the monoclinic space group *C2/c*. The asymmetric unit contained one molecule of **91**, one water molecule, and one toluene molecule. A table summarising the refinement and experimental parameters is displayed in Table A3.1 and the full asymmetric unit

can be viewed in the appendices Figure A3.19. The **Nap(NO₂)** moiety of **91** was disordered over two positions with 81% and 19% occupancies, while the toluene solvent molecule displayed positional and thermal disorder over three positions with 59%, 31% and 10% occupancies. The solvent was refined using suitable SIMU and SUMP restraints. The disorder present in the **Nap(NO₂)** moiety required an EADP constraint to fix the imide-O positions and a SIMU restraint to model the nitro-group. A thermal ellipsoid representation of the major occupied species with heteroatoms labelled is shown [Figure 3.8 (a)].

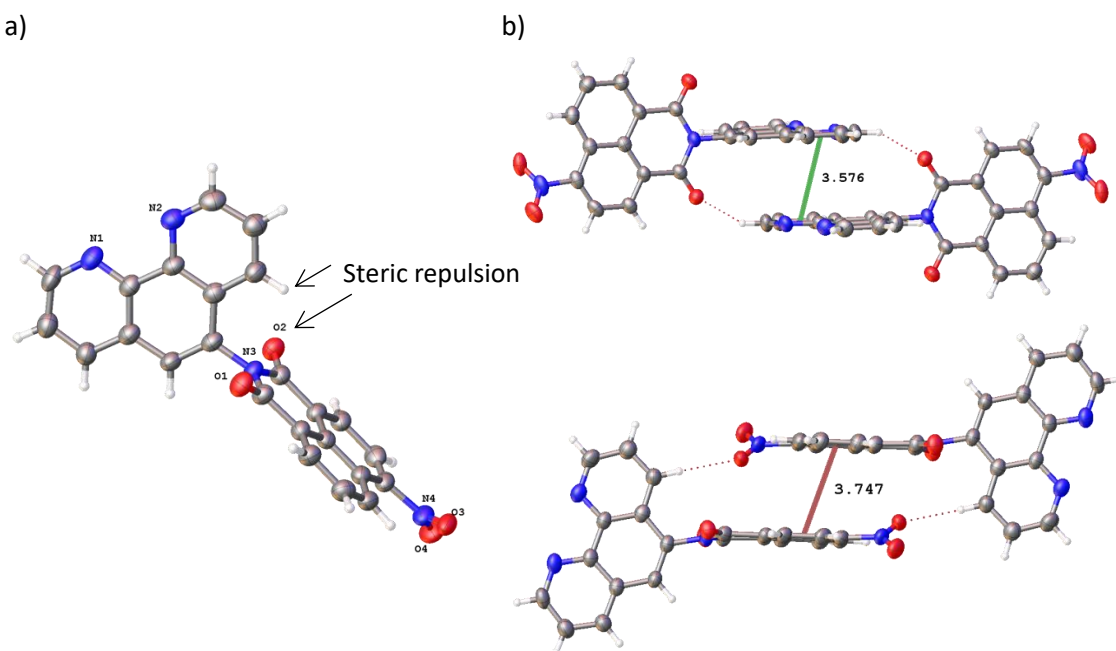


Figure 3.8: a) Representation of the major occupied species in the single crystal X-ray structure of **[(PhenNap(NO₂))(0.5)H₂OC₇H₈]**, including labels for heteroatoms. b) Representation of a paired structure showing π - π stacking between (top) **phen** motifs, and (bottom) **Nap** motifs. H-bonds are represented as red dotted lines. Disorder in the structures and solvent molecules are omitted for clarity. Thermal ellipsoids are shown at 50% probability. Distances between the planes / Å.

The angle between the planes of the **phen** and **Nap** motifs of **91** was 87.66(3)°. The near orthogonal nature of the angle between the two motifs occurs as a result of two factors: i) a large solvent void from the disordered toluene molecules in the extended structure, and ii) the steric repulsion between the imide oxygen atom of the **Nap** and C-H of the **phen** [Figure A3.20]. As was observed for systems presented in Chapter 2, this may indicate electronic decoupling between the **Phen** and **Nap** functionalities and may give rise to interesting charge transfer properties between the two substituents.

The packing of the bulk structure is facilitated by a number of intermolecular interactions, namely hydrogen bonding and π - π stacking interactions [Figure 3.8 (b)]. Hydrogen bonding interactions between i) the imide oxygen and C-H donor atom of **Phen** moiety and ii) the oxygen atom of the appended nitro- group and C-H donor atom of **Phen** are present and evoke further

conformational constraints on the molecule. Offset head-to-tail π - π stacking interactions are observed between **Nap** moieties on adjacent molecules and between the corresponding **phen** moieties, which run through the crystal lattice in orthogonal directions. The stacking distances were analysed by measuring the distance between mean planes of central stacking motifs using the Platon software.²³⁰ The distance between the closest centroid-centroid of neighbouring **Phen** functionalities (N1,C14–C18 to N1,C14–C18*i*, where *i* is the symmetry transformation: 1-*x*, 1-*y*, 1-*z*) was computed as 3.576(3) Å with an angle of 0.0(2)° and a slippage of 0.966 Å between the planes [Figure 3.8(b), top], while that between neighbouring **Nap** functionalities (C2–C6 to C6–C11*i*, where *i* is the symmetry operation: 3/2-*x*, -1/2+*y*, 3/2-*z*) was 3.747(3) Å, with an angle of 0.03(2)° and slippage of 1.452 Å between the planes [Figure 3.8(b), bottom]. This chapter aims to utilise the π - π stacking feature of these ligands for interaction with DNA bases in an intercalative manner. Furthermore, the hydrogen bonding features of the nitro- group may facilitate further interaction with the biomolecule through interactions with hydrogen bond acceptor units in the grooves.

3.4.2: X-Ray Crystal Structure of **92**

Pink block crystals of the bromo- functionalised derivative, **PhenNap(Br)**, **92** were obtained by crystallisation from acetic acid. The crystal structure was solved in the monoclinic space group $P\bar{1}$ and the asymmetric unit contained one molecule of **92** and two acetic acid solvent molecules. The full asymmetric unit can be viewed in the Figure A3.21 and a representation of the major occupied ligand species is shown in Figure 3.9 (a).

The extended structure featured an overall head-to-tail orientation of homo- π -stacked arrays of **92**, sandwiched between acetic acid solvent channels. Hydrogen bonding interactions between the solvent channel and *N*- acceptor atom of the **Phen** motif were observed at one end of the array while the moderately electronegative bromine facilitated hydrogen bonding interactions at the other [Figure A3.22].

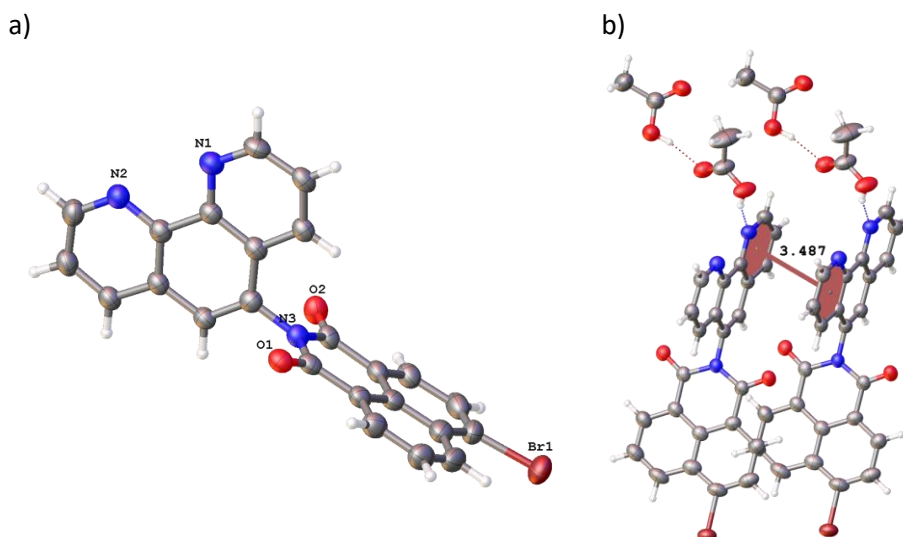


Figure 3.9: a) Representation of the major occupied species in the single crystal X-ray structure of $[(\text{PhenNap}(\text{Br})\cdot 2(\text{CH}_3\text{COOH}))]$, **92**, including labels for heteroatoms. b) Representation of a paired structure showing π - π stacking between **Phen** motifs. Hydrogen bonding interactions are denoted by red and blue dotted lines. Disorder in the structure is omitted for clarity. Thermal ellipsoids are shown at 50% probability. Distances between the planes / Å.

The closest centroid-centroid distance between the planes of offset **Phen** functionalities (N2,C14-C17,C22 to N1,C21-C25i, where *i* is the symmetry transformation: $-1+x, y, z$) was 3.488(2) Å with an angle of $1.30(19)^\circ$ and a slippage of 1.148 Å between the planes [Figure 3.9 (b)]. Additionally, the twist angle between the planes of the **Nap** and **Phen** moieties was found to be $79.72(12)^\circ$ which is smaller than that of **91**, possibly due to less intermolecular interactions within the structure. The overall twist between the two chromophores and the presence of the heavy Br atom may give rise to electronic decoupling between the two motifs and induce interesting charge transfer properties on the ligand (*vide infra*, Section 3.6).

3.4.3: X-Ray Crystal Structure of **93**

Finally, the X-ray crystal structure of the chloro-derivative, **93**, was also solved. Thin, colourless crystals were obtained by slow evaporation of a solution of **93** in CHCl_3 . The crystal structure was solved in the monoclinic space group $P2_1/c$ and the asymmetric unit contained one molecule of **93** and one water molecule. The **Nap** unit was disordered over two positions with 77% and 23% occupancies and the full asymmetric unit can be viewed in the appendix Figure A3.23. An image of the major occupied species is presented in Figure 3.10 (a).

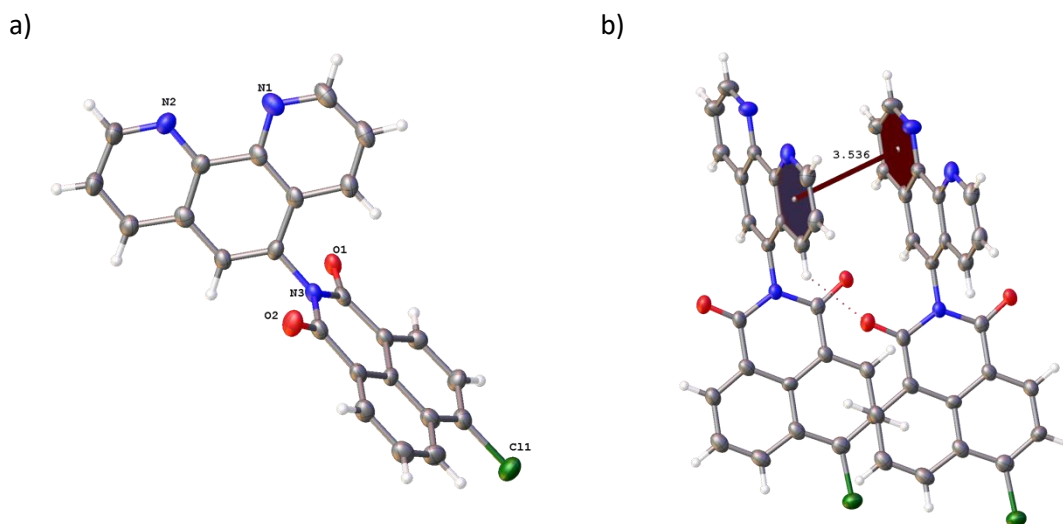


Figure 3.10: a) Representation of the major occupied species in the single crystal X-ray structure of $[(\text{PhenNap}(\text{Cl}))(\text{H}_2\text{O})]$, **93**, including labels for heteroatoms. b) Representation of a paired structure showing π - π stacking between **Phen** motifs. Hydrogen bonding interactions are denoted by red dotted lines. Disorder in the structure is omitted for clarity. Thermal ellipsoids are shown at 50% probability. Distances between the planes / Å.

Hydrogen bonding interactions between the water molecule and **phen** nitrogen atom were observed [Figure A3.23], as well as between the imide oxygen atom and CH donor atom of the **phen** ligand [Figure 3.10 (b)]. Similar π - π stacking features were observed for **93** when compared to **92** and occurred between neighbouring **phen** motifs, highlighted in Figure 3.10 (b), and are characterised by a distance of 3.536(3) Å, a slippage of 1.218 Å and an angle of 1.2(2)° between the centroids of the highlighted planes. Indeed, the extended structures of the halogen functionalised **Naps** were also comparable. **93** featured an overall head-to-tail orientation of homo- π -stacked arrays, sandwiched between water solvent channels. The twist angle between the central rings of the **phen** and **Nap** motifs was found to be 74.47(15)°. Table 3.2 summarises the crystallographic features highlighted in the previous sections.

Table 3.2: Summary of crystallographic parameters described for ligands **91**, **92** and **93**

Ligand	Chemical Occupancies / %	Phen-Nap twist / °	π - π Stacking Motifs	Hydrogen Bonding Interactions
91	81, 19	87.66(3)	Phen-Phen Nap-Nap	O _{nitro} - CH _{phen} O _{imide} - CH _{phen}
92	82, 18	79.72(12)	Phen-Phen	N _{phen} - OH _{solvent}
93	77, 23	74.47(15)	Phen-Phen	O _{imide} - CH _{phen} N _{phen} - OH _{solvent}

In summary, the structural properties of ligands **91**, **92** and **93** were investigated in their solid states and were solved and refined to final R indexes [$I \geq 2\sigma(I)$, (R_1 , wR_2)] of **91**(0.0837, 0.2330), **92**(0.0663, 0.1819) and **93**(0.0901, 0.2415). All three ligands were found to display i) hydrogen bonding interactions, ii) intermolecular π - π stacking interactions, and iii) positional

disorder over the **Nap** unit [Table 3.2]. The solid-state structures also unveiled a near orthogonal nature between the **Nap** and **phen** motifs with angles ranging from 75–88°, induced by the steric repulsion between the hydrogen atom of **phen** and imide oxygen atom of **Nap** in a co-planar conformation [Figure 3.8 (a)]. The steric repulsion between these two atoms induces rotational restriction on the ligands, as free rotation about the single bond connecting the **phen** and **Nap** motifs is energetically unfavourable. As such, the **Nap** unit may become rotationally “locked” with respect to the **phen** ligand, whereby the substituent in the 4-position of the **Nap** is located either above or below the plane of the **phen**. This may have a pronounced effect on the structural properties of the associated Ru(II)-complexes, which will be discussed (*vide infra*, Section 3.8). Full reflection parameters and data corresponding to the crystal structure refinement can be viewed in Table A3.1. To further probe the electronic properties of the systems, the electrochemical properties of ligands **91** and **92** were investigated and are presented in the next section.

3.5: Electrochemical Measurements of Ligands **91** and **92**

Cyclic voltammetry (CV) measurements were performed on ligands **91** and **92** in deaerated MeCN solution to discern their electrochemical properties. Figure 3.11 displays the CV traces of the ligands referenced with respect to the Fc/Fc⁺ redox couple internal standard.¹⁹² **PhenNap(NO₂)**, **91** displayed two reversible reductions ($E_{1/2(\text{red})} = -1.07$ and -1.35 V) and one irreversible reduction wave close to the solvent window at -2.42 V. The bromo-analogue, **92**, displayed two less resolved, *quazi*-irreversible reductions at ($E_{1/2(\text{red})} = -1.36$ and -1.50 V).

The observed reductions are ascribed to individual reductions on the **Phen** and **Nap** moieties, respectively, due to their structurally decoupled states (*vide supra*, Section 3.4.2), each of which can act as an electron sink. The presence of the electron withdrawing nitro- group of **91** results in the most positive reduction potential of the ligands due to a stabilisation effect, and occurs at -1.07 V. This assignment of the reductions is supported by CV experiments and DFT calculations from Doyle and co-workers on a Re(I)-**Nap** complex employing **91** as a ligand for the photocatalytic reduction of CO₂.²²¹

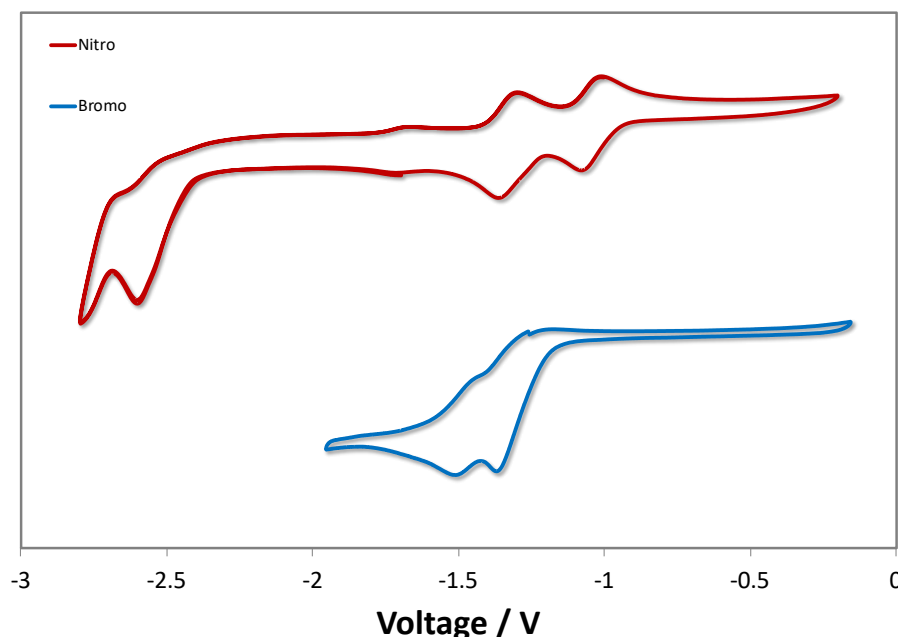


Figure 3.11: CV traces of **91** (red) and **92** (blue) in deaerated MeCN, referenced with respect to the Fc/Fc⁺ redox couple. Scan rate 100 mV/s in the negative direction.

The reductions at -1.35 and -1.36 V for **91** and **92**, respectively, are ascribed to reduction of the **phen** moiety of each structures while the remaining reduction ($E_{1/2(\text{red})} = -1.50$ V) observed for **92** is assigned to a reduction on the **Nap(Br)** functionality. Overall, the presence of the electron withdrawing nitro- group of **91** results in a more positive reduction potential for this ligand compared to the bromo-analogue, **92**. Thus, the electrochemical analysis supported by the torsional twist from the solid state analysis between the **Phen** and **Nap** moieties indicates significant electronic decoupling between the two chromophores of the ligands. The CV measurements of the ligands will be used to inform the electrochemical assignment of the associated Ru(II)-polypyridyl complexes, **94** and **95** and will be discussed in later sections (*vide supra*, Section 3.10). Next, investigation into the photophysical properties of the ligands will be discussed.

3.6: Photophysical Characterisation of **91**, **92**, **93** and **97**

The photophysical properties of ligands **91**, **92**, **93** and **97** were explored in MeCN solution at room temperature. Full photophysical characterisation of each of the individual ligands (their absorption, fluorescence emission and excitation spectra) is presented in the appendix alongside the effects of dilution on their emission properties [Figures A3.25-A3.28]. The normalised absorption spectra (non-normalised available in the Appendix) of each of the ligands is presented in Figure 3.12.

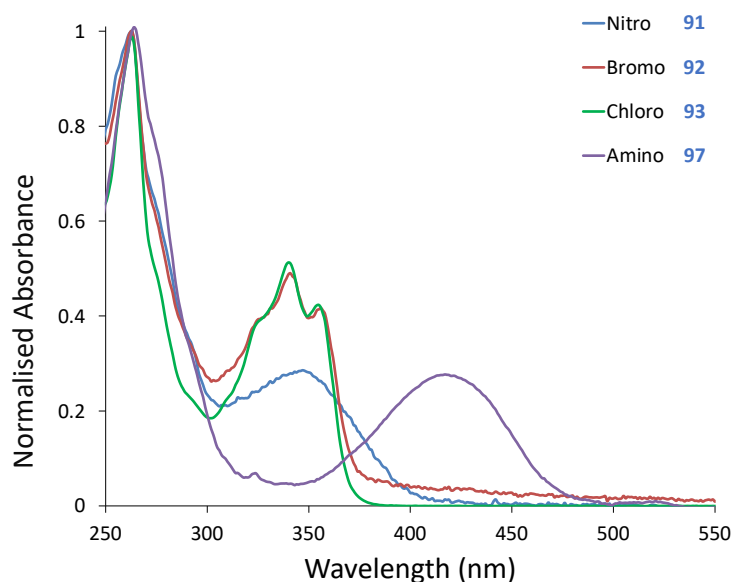


Figure 3.12: Normalised absorption spectra of **91** (blue, 1.5×10^{-5} M) and **92** (red, 1.5×10^{-5} M), **93** (green, 1.5×10^{-5} M) and **97** (purple, $< 1.5 \times 10^{-5}$ M) in MeCN.

The ligands each displayed: i) high energy absorption bands centred at 264 nm attributed to $\pi-\pi^*$ intraligand (IL) transitions of the **phen** moiety and ii) lower intensity absorption bands at longer wavelengths corresponding to **Nap** localised transitions or charge transfer transitions. Similarities in the photophysical absorption properties were observed between the two halogen substituted **Naps**, **92** and **93** and between the nitro- and amino substituted systems, **91** and **97**.

The bromo- and chloro- derivatives, **92** and **93**, respectively, each displayed structured lowest energy absorption transitions at 356 nm, ascribed to the $\pi-\pi^*$ transitions of the **Nap**. In the case of the nitro analogue, **91**, this transition appears as an unstructured, featureless, broad absorption band centred at 347 nm and is again attributed to $\pi-\pi^*$ IL **Nap** transition. This transition becomes significantly red-shifted for the amine derivative, **97**, consistent with charge transfer character, arising from the internal charge transfer (ICT) excited states of 4-amino-**Naps** (*vide supra*, 1.4.1).⁶⁴ The assignment of these transitions is further supported by electronic and solution studies performed by Castellano and co-workers on similar *N*-aryl substituted **Nap** ligands with functionalisation in the 4-position who obtained the same findings.¹⁵¹ As mentioned previously, the insolubility of **97** produced a major drawback to its practicality. Regrettably, exact concentrations of **97** in solution could not be determined as precipitation occurred in the stock solutions after short period. Nevertheless, the steady state absorption and emission properties were analysed by dilution studies at low concentrations (maximum concentration = 1.5×10^{-5} M, as prepared).

The emission properties of the ligands at 1.5×10^{-5} M concentrations in MeCN solution were next investigated [Figure 3.13] by excitation into the **Nap** centred absorption bands. The **PhenNap(NO₂) 91** featured a broad and unstructured emission band centred at 450 nm attributed

to the **Phen**_{HOMO} to **Nap(NO₂)**_{LUMO} $\pi-\pi^*$ transition, which is supported by the theoretical calculations (*vide supra*, 3.3). The amino derivative, **97**, displayed a similarly unstructured emission band that was red-shifted ($\lambda_{\text{em}} = 520$ nm) in comparison to **91**, indicative of ICT character.

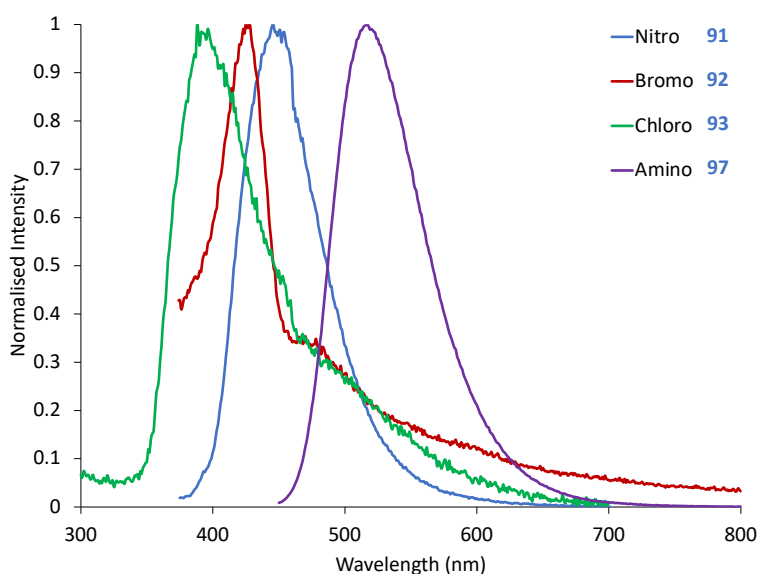


Figure 3.13: Normalised emission spectra of **91** (blue, 1.5×10^{-5} M, $\lambda_{\text{ex}} = 350$ nm) and **92** (red, 1.5×10^{-5} M, $\lambda_{\text{ex}} = 356$ nm), **93** (green, 1.5×10^{-5} M, $\lambda_{\text{ex}} = 356$ nm) and **97** (purple, $< 1.5 \times 10^{-5}$ M, $\lambda_{\text{ex}} = 450$ nm) in acetonitrile at room temperature.

The excited states of the bromo- and chloro- derivatives **92** and **93**, respectively, were more structured and complex in nature and are indicative of dual emission from these chromophores. The high energy structured emission bands at 420 and 394 nm for **92** and **93**, respectively, were attributed to fluorescence from the S_1 $\pi-\pi^*$ excited state. While the lower energy emission bands ($\lambda_{\text{em}} = 481$ and 492 nm for **92** and **93** in MeCN, respectively, [Figure 3.13]) were broader in nature and displayed sensitivity to solvent polarity.

In DMSO, ligand **92** exhibited two localised emission bands at $\lambda_{\text{em}} = 420$ and 495 nm [Figure 3.14]. The dual emission for these systems could arise from differences in the geometries of the ligand in solution.¹⁵¹ The excitation spectra of **92** were recorded at both wavelengths ($\lambda_{\text{em}} = 420$ and 495 nm) and the arising emission was broad and unstructured, thus mimicking the lower energy emission profile. Separately, a third emission band at 625 nm was observed for **92**, appearing as a shoulder upon excitation of **92** at 327 nm and is attributed to emission due to excimer formation.²³¹

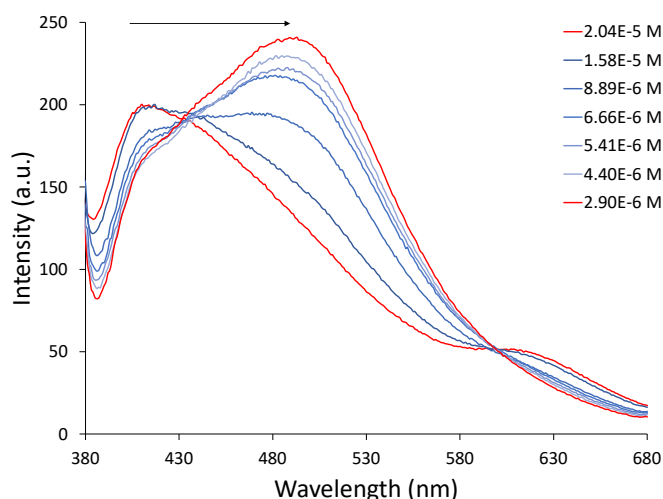


Figure 3.14: Emission response of **92** in DMSO upon dilution.

Ligand **93** also exhibited structured emission, most noticeable at lower concentrations [Figure A3.27 (b)]. This dual fluorescence of these systems may be attributed to relaxation from distinct excited states, which differ significantly in their charge distribution and conformational properties. This observation is supported by comprehensive literature investigations into the excited states of *N*-Aryl substituted **Naps** which display excited states sensitive to solvent polarity and importantly, rotational relaxation pathways.^{151,232,233} Thus, the dual emission observed for the halogen substituted ligands may be explained as follows: the lower energy emission may have originated from a conformational change in the chromophores allowing for the formation of a planar ICT excited state. This phenomenon has been reported previously for *N*-aryl substituted **Naps** bearing electron donating groups in the para position of the *N*-aryl ring. Herein, a pseudo-Jahn-Teller coupling of the two lowest lying excited states occur and allows for crossing to the S_2 surface. Once here, the molecule can undergo rotation where the **Nap** and aryl groups are in a co-planar configuration, thus stabilising the charge transfer state.^{232,233} In the case of **92** and **93**, rotational transitions may occur between the **Phen** and **Nap** moieties allowing for the two distinct fluorescence observations. Thus, the higher energy emission of **92** and **93** is ascribed to fluorescence from the S_1 excited state while the lower energy emission may arise from relaxation from the S_2 CT excited states. The photophysical characterisation of the ligands are summarised in Table 3.3.

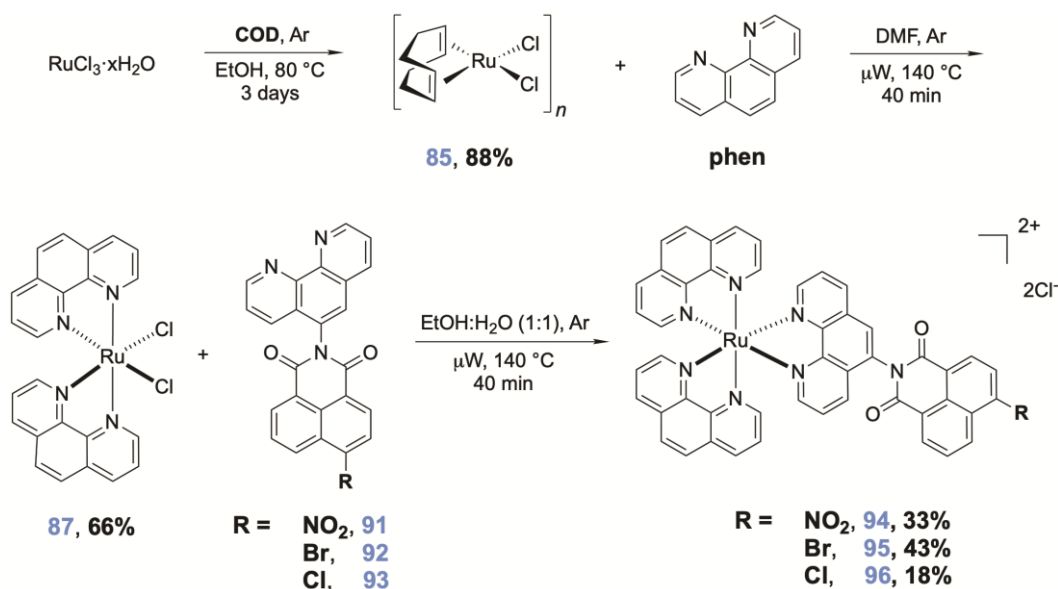
Table 3.3: Absorption and emission characterisation of **91**, **92**, **93** and **97** in MeCN at 298 K

	Absorption (λ_{max} / nm)		Emission (λ_{max} / nm)	
	$\pi\text{-}\pi^*$ (Phen)	$\pi\text{-}\pi^*$ (Nap)	$S_1 - S_0$	$S_2 - S_0$
PhenNap(NO₂) , 91	264	347	450	---
PhenNap(Br) , 92	264	326, 342, 356	420	481
PhenNap(Cl) , 93	264	326, 342, 356	398	492
PhenNap(NH₂) , 99	264	420	521	---

To test whether the structured emission from these systems arose due to de-excitation from the S_1 state to individual vibronic modes of the S_0 state from the presence of the heavy and electronegative halogen atoms, the emission properties of the non-substituted **Nap**, **80** were investigated. While **80** was quite poorly emissive, Figure A3.29 clearly shows the presence of structured emission bands upon excitation at 338 nm, further supporting the hypothesis that some degree of pseudo-Jahn Teller coupling is present in these systems, arising from electron donation from the **Phen** moiety, which allows for dual fluorescence. In conclusion, the photophysical properties of the ligands **91**, **92**, **93** and **97** were analysed which will provide insights when analysing the analogous properties of the corresponding metal complexes. The synthesis and characterisation of the Ru(II)-metal complexes **94**, **95**, **96** and **99** will thus be introduced in the next section.

3.7: Synthesis and Characterisation of Complexes **94**, **95** and **96**

Having explored the structural and electronic properties of ligands **91**, **92** and **93** in detail, their corresponding ruthenium polypyridyl complexes **94**, **95** and **96** were next synthesised. The precursor complexes, $[\text{Ru}(\eta^4\text{-(COD)}\text{Cl}_2)]_n$, **85**, and $\text{Ru(phen)}_2\text{Cl}_2$, **87**, were synthesised according to modified literature procedures and the details can be viewed in the General Experimental Procedure 2 [Chapter 6].^{182,234} The synthesis of the desired complexes was performed according to General Synthetic Procedure 3 [Chapter 6] and involved reaction of the appropriate ligand, **91**, **92** or **93** (1.1 eq.) with **87** (1.0 eq.) in EtOH:H₂O (1:1) under microwave irradiation at 140 °C for 40 min [Scheme 3.3].¹⁸³

Scheme 3.3: Synthesis of complexes **94**, **95** and **96**

The crude complexes obtained after removal of the solvents under reduced pressure were purified by flash column chromatography: **94** (SiO₂, MeCN:H₂O:NaNO_{3(aq.)} 40:10:1), **95** (SiO₂, MeCN:H₂O:NaNO_{3(aq.)} 40:10:1) and **96** (Al₂O₃, MeCN:H₂O 10:0 – 9:1) and isolated as red powders. In order to remove excess salt present after purification of complexes **94** and **95**, the samples were first dissolved in dry acetonitrile solution and passed through an Acrodisc® syringe filter (0.45 μm) and the solvents were removed under reduced pressure. Complexes **94** and **95** were then converted to their dichloride forms by swirling solutions of the complex and Amberlite chloride resin in MeOH for 1 h. The products were isolated by filtration and the solvents were removed under reduced pressure. Complexes **94**, **95** and **96** were obtained as red powders in yields of 33%, 43% and 18%, respectively.

Apparent successful formation of the products was indicated by: i) TLC analysis, which displayed the presence of only one product, ii) ¹H NMR spectroscopy, which displayed integration totalling 28 protons for each complex, as expected, and iii) ESI-HRMS, which indicated the presence of the [M⁺] ion in each case [Figures A3.30 – A3.47]. The ¹H NMR spectrum of complex **94** after purification by column chromatography is presented in Figure 3.15.

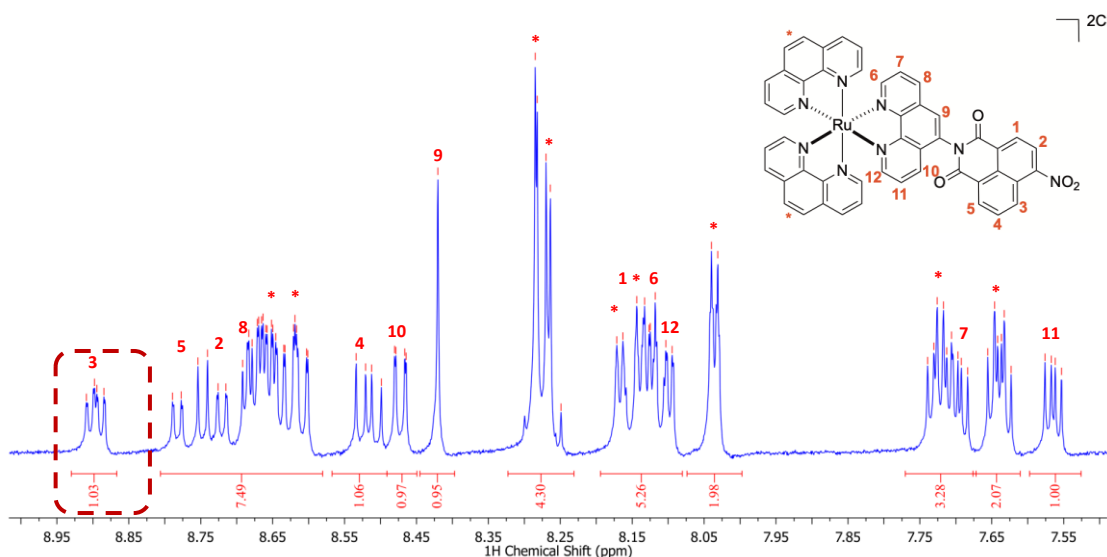
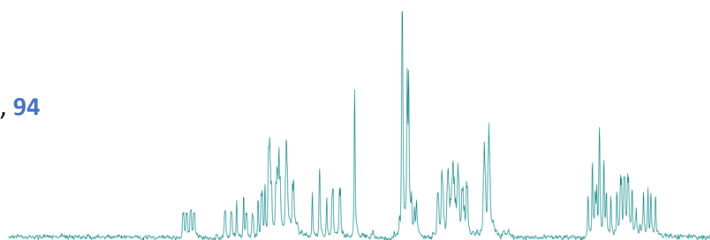


Figure 3.15: The ^1H NMR spectrum (600 MHz, CD_3CN) of **94**.

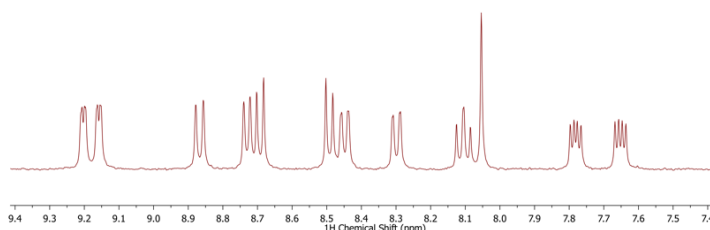
Upon analysis of the H-H COSY and HSQC 2D NMR spectra of **94** [Figures A3.43 and A3.44], it was observed that the signal present at highest chemical shift (8.80 ppm) appeared to correspond to two proton signals. As such, investigation into this peculiar observation was then carried out. First, to investigate whether there was a presence of **Nap** based impurity, the ^1H NMR spectra of the ligand, **91** and complex in CDCl_3 were compared [Figure 3.16 (a)] which did not indicate the presence of free **Nap** in solution. Next, analytical HPLC analysis was carried out to test the purity of the sample by PhD student Mark Nolan from the research group of Prof. Eoin Scanlan at TCD. The sample was analysed on a C18 column eluting with $\text{H}_2\text{O}:\text{MeCN}$ (1:1 + 0.1% formic acid) and the results indicated that only one species was present [Figure 3.16 (b) and Figure A3.48].

a)

Metal complex, **94**



Ligand, **91**



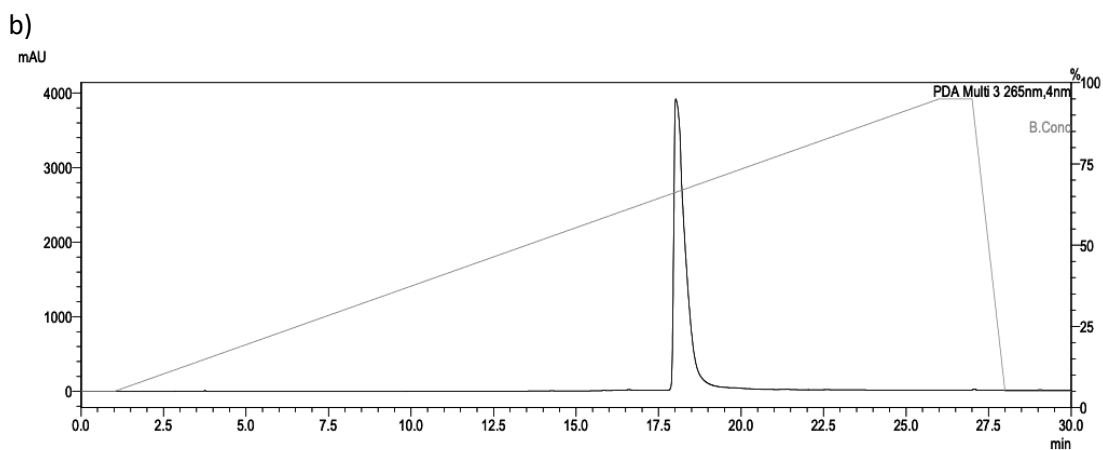


Figure 3.16: a) The ^1H NMR spectra of **94** (top) and **91** (bottom) (400 MHz, CDCl_3). b) Analytical HPLC analysis of **94** monitored at 265 nm displaying the collection of one species.

A comparison of the ^1H NMR spectra of the structurally similar complexes, **95** and **96** was next performed, as each of the complexes displayed substitution in the 4-position of the **Nap**, and indeed, the same anomaly was observed [Figures 3.17 and 3.18].

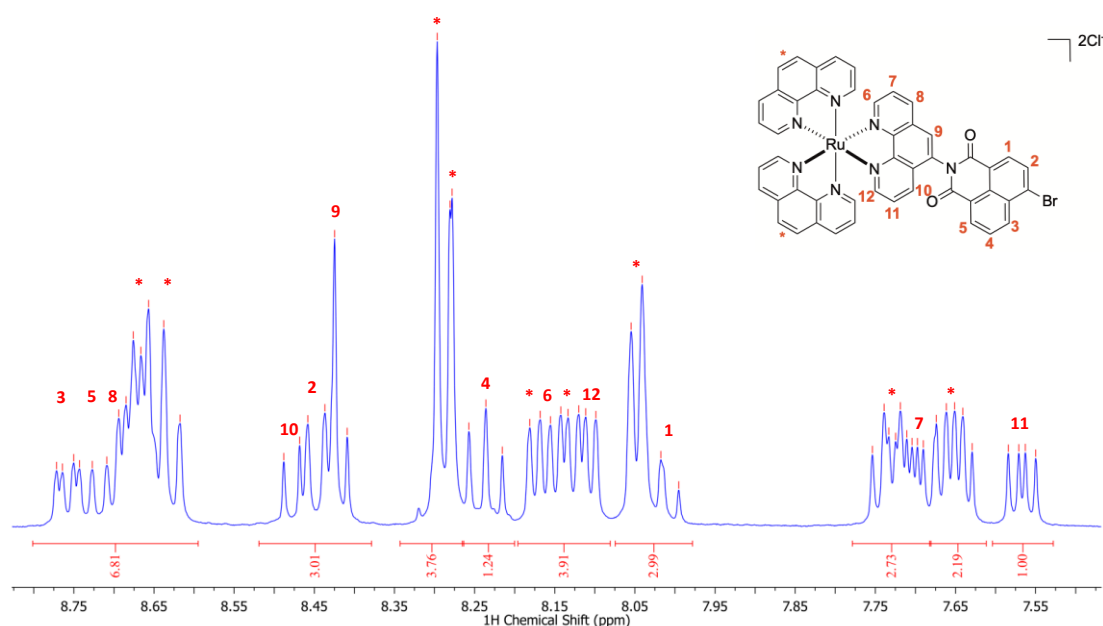


Figure 3.17: The ^1H NMR spectrum (400 MHz, CD_3CN) of **95**.

Upon analysis of the 1D and 2D NMR spectra of the chloro-substituted complex, **96**, a relationship between the signals that presented as two distinct protons became apparent. The signals highlighted in the ^1H NMR spectrum [Figure 3.18] are assigned to protons on the main body of the **Nap** motif and can be individually resolved in the 2D NMR spectra [Figure A3.49]. As per the crystal structure analyses (*vide supra*, Section 3.4), the **Nap** motif displayed disorder over two positions and, as previously mentioned, the steric repulsion between the imide oxygen and proton 10 (complex numbering scheme, insets: NMR spectra) confers a rotational restraint on the ligand.

Thus, once the **Nap** coordinates to the Ru(II)-centre, the **Nap** becomes conformationally “locked” in place, where the substituent in the 4-position (NO₂, Br or Cl) either resides above or below the plane of the coordinating **Phen**. This confers a different chemical environment for the protons of the **Nap** ligand and gives rise to diastereomers that can be individually resolved on the NMR timescale. The protons of the **Phen** motifs, which are in close proximity to the Ru(II)-centre did not appear chemically distinct with respect to their diastereomeric forms, but instead appeared as a single entity, or as superimposable signals.

Racemic mixtures of Ru(II)-polypyridyl complexes possessing C₂-symmetry, such as Ru(**phen**)₂Cl₂, display eight proton signals in ¹H NMR analysis, consistent with two non-interconverting enantiomers in solution [Figure A3.47]. The introduction of the asymmetric ligand coordinating in the *cis*-orientation, breaks the C₂ symmetry and gives rise to complicated NMR spectra, as has been reported previously by Recca and co-workers for a series of Ru(II)- and Os(II)-polypyridyl complexes.²³⁵ The ¹H NMR spectra obtained for the asymmetric complexes **94**, **95** and **96** therefore indicated *pseudo*-diastereomers in solution, which was not observed in the case of the non-substituted **Nap** Ru(II)-polypyridyl complexes **75** and **76** presented previously in Chapter 2.

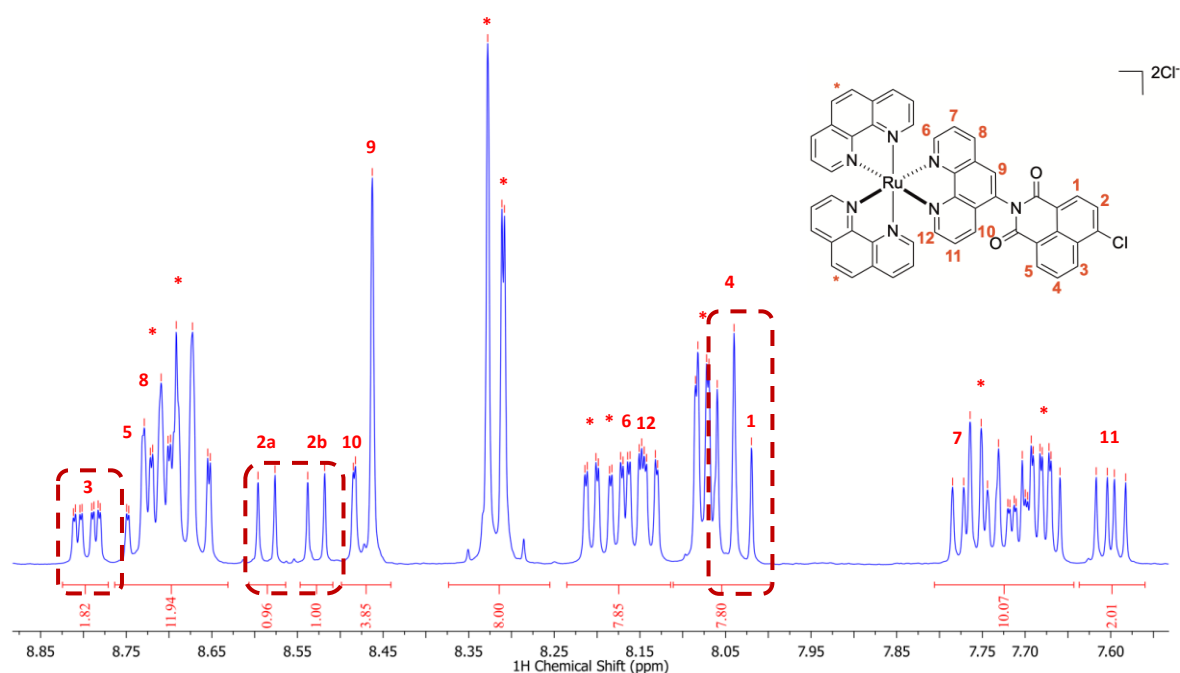


Figure 3.18: The ¹H NMR spectrum (600 MHz, CD₃CN) of **96**.

Thus, after thorough investigation into the structural characterisation of the complexes and satisfied that they were obtained in pure forms, the solid-state structures were next investigated.

3.8: Crystallographic Studies of **94**, **95** and **96**

Having successfully synthesised the Ru(II)-polypyridyl complexes, **94**, **95** and **96**, their solid-state structures were next investigated to determine the inter- and intramolecular interactions that would govern their interactions with DNA. As solution studies were performed on the complexes as their dichloride forms, attempts were made to crystallise the complexes as the dichloride salts, however; this was not always successful and as such, their solid-state investigations were performed with alternative counterions. In some cases, crystals of the complexes suitable for X-ray diffraction experiments were obtained after column chromatography, eluting with a mixture of H₂O:MeCN:NaNO_{3(aq)} and as such, the nitrate counterion was present in the structure solutions. All crystal data sets were collected independently on either a Bruker APEX DUO (MoK α or CuK α source) or D8 Quest ECO (MoK α source) instrument. Details of experiments are provided in the Experimental Section [Chapter 7]. Full data corresponding to the collection of reflections from the crystal structures of the three complexes are presented in Table A3.2.

3.8.1: Crystallographic Studies of **94**

Red block crystals of **94** suitable for X-ray diffraction experiments were obtained from crystallisation of the pure sample fraction after column chromatography. The crystal structure was solved in the monoclinic space group *C2/c* and the asymmetric unit contained one molecule of **94**, two NO₃[−] counterions, two acetonitrile solvent molecules, one water molecule, and a salt cluster comprised of hexaaqua-sodium(I) and a nitrate counterion. A thermal ellipsoid representation of the major cationic ruthenium species is presented in Figure 3.19 with heteroatoms labelled for simplicity. The full asymmetric unit can be viewed in appendix Figure A3.48.

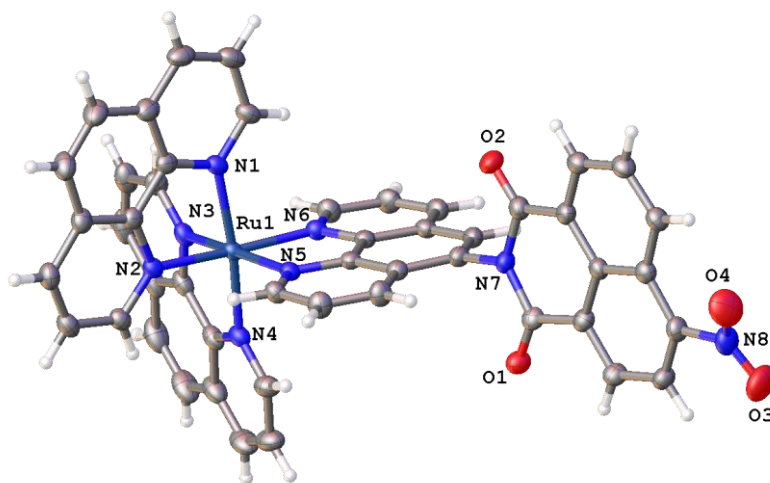


Figure 3.19: ORTEP style representation of the complex cation in the single crystal X-ray structure of {[**94**](NO₃)₂·(H₂O)·2(CH₃CN)·(Na(H₂O)₆(NO₃))}, including labels for heteroatoms. Positional disorder in the complex is omitted for clarity. Thermal ellipsoids are shown at 50% probability.

As was observed in the crystal structure of the nitro-substituted ligand, **91** (*vide supra*, Section 3.4.1), positional disorder was observed over the **Nap** unit in complex **94** with 76% and 24% occupancies. Further positional disorder was observed over one of the nitrate counterions with 72% and 28% occupancies. All other solvent and guest molecules displayed occupancy factors of unity. The octahedral geometry of **94**(NO₃)₂ around the ruthenium centre was found to be typical for tris(bidentate) coordination complexes, formed through coordination of the ruthenium to six nitrogen atoms in total: four from coordinating **Phen** auxiliary ligands and two from the **phen**-functionalised **Nap**(NO₂). The bond Ru–N bond lengths ranged from 79.85(9)–96.12(9) and the N–Ru–N bond angles ranged from 2.060–2.068 Å, consistent with recent literature values.^{97,106}

Table 3.4: Summary of bond lengths and bond angles for selected region of **94**

Atoms			Length/Å		Atoms				Angle/°	
Ru1	N2		2.067(2)		N1	Ru1	N2		79.85(9)	
Ru1	N4		2.068(2)		N3	Ru1	N4		80.20(9)	
Ru1	N5		2.067(2)		N6	Ru1	N5		80.05(9)	
Ru1	N6		2.061(2)		N5	Ru1	N4		95.63(9)	
Ru1	N3		2.060(2)		N1	Ru1	N5		89.97(9)	
Ru1	N1		2.067(2)		N3	Ru1	N6		94.44(9)	
					N6	Ru1	N1		96.12(9)	
					N6	Ru1	N4		87.96(9)	
					N3	Ru1	N2		90.96(9)	
					N3	Ru1	N1		94.50(9)	
					N2	Ru1	N4		96.51(9)	
					N2	Ru1	N5		94.79(8)	

Within the extended structure, the sodium salt cluster and solvent molecules exhibited a complex hydrogen bonding network which ran normal to the crystallographic *b*-axis [Figure 3.20 (a)]. Complex **94** was involved in this complex hydrogen bonding network through interaction between a CH donor atom of the **PhenNap** ligand proton and an acceptor oxygen atom from the nearest nitrate counter ion. Intermolecular hydrogen bonding interactions were also present in the extended structure and occurred between a CH-donor atom on the **PhenNap** moiety and the NO₂ group of a symmetry generated molecule [Figure 3.20 (b)]. Surprisingly, no π – π stacking interactions were observed within the extended structure, possibly due to the large voids exerted by the salt-solvent network. Along the *c*-axis, the **Naps** were arranged in an off-set head-to-tail configuration between the sodium-solvent voids, with a closest atom-atom distance of 3.255(8) Å and closest centroid-centroid distance between the two planes of 4.354 Å. The full extended structure and details of hydrogen bonding distances are provided in Figure A3.49 and Table A3.3, respectively.

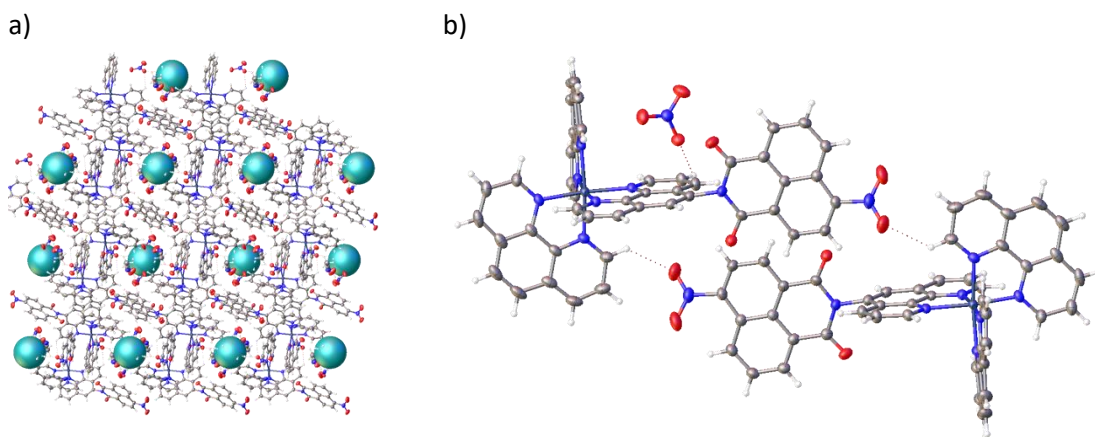


Figure 3.20: ORTEP style representation of a) the solvent-salt channel shown normal to the *b*-plane. Na(I) ions represented as turquoise spheres and b) hydrogen bonding interactions, represented by red dotted lines for the single crystal X-ray structure of {[**94**](NO₃)₂·(H₂O)·2(CH₃CN)·(Na(H₂O)₆(NO₃))}. Thermal ellipsoids are shown at 50% probability. Disorder in the structure omitted for clarity.

The plane-to-plane twist angles between the planes of the **Nap(NO₂)** and the coordinating **phen** moiety within the **PhenNap(NO₂)** ligand were found to be 88.2(3) and 90.2(12)° for the major and minor occupied species, respectively, further supporting the theory of significant electronic decoupling between the two chromophores.

3.8.2: Crystallographic Studies of **95**

Complex **95** (10 mg) was dissolved in minimal amount of methanol and red block crystals were obtained from slow evaporation of the methanolic solution. The crystal structure was solved in the triclinic space group $P\bar{1}$. The asymmetric unit contained one molecule of **95**, two Cl[−] counterions (occupancies 1 and 0.75), one NO₃[−] counterion (occupancy 0.25), and three water molecules. A thermal ellipsoid representation of the major occupied ruthenium cation is shown for simplicity with heteroatoms labelled [Figure 3.21]. The full asymmetric unit can be viewed in appendix Figure A3.50.

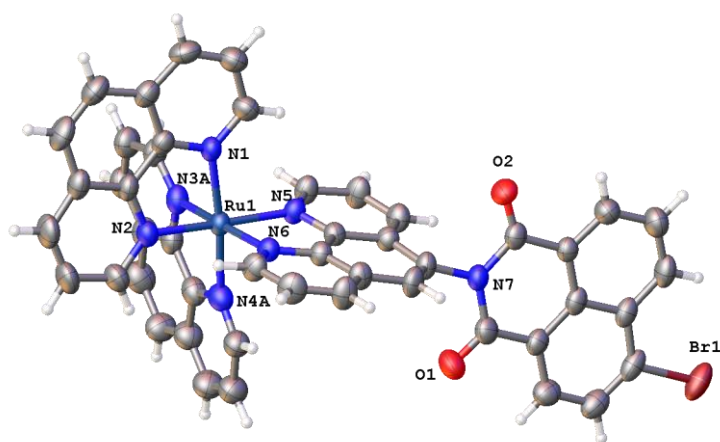


Figure 3.21: ORTEP style representation of the complex cation in the single crystal X-ray structure of {[**95**](Cl)_{1.75}(NO₃)_{0.25}·3(H₂O))}, including labels for heteroatoms. Positional disorder in the complex is omitted for clarity. Thermal ellipsoids are shown at 50% probability.

As found for **94**, positional disorder was observed over the **Nap(Br)** moiety with 88% and 12% occupancies, representing the major and minor occupied species, respectively. Further positional disorder was also observed over one of the coordinating **phen** ligands with occupancies of 51% and 49%. This disorder over the **phen** moiety was modelled using an EADP and EXYZ command to constrain one of the coordinating nitrogen atoms to the same site. The three water molecules displayed occupancy factors of unity, while a mixed counterion system was observed. Within the crystal structure, a trace of nitrate counterion was observed at 25% occupancy. This was surprising, as anion exchange techniques had been previously employed to convert **95** to the dichloride form. The two chloride counterions were therefore modelled with occupancies of one and 0.75 to charge balance the structure. The octahedral geometry around the ruthenium centre was again, found to be typical for tris(bidentate) coordination complexes, with N-Ru-N bond angles that ranged from 76.67(14) to 97.73(14)° and Ru-N bond lengths which ranged from 2.053(4) to 2.062(3) Å [Table 3.5], comparative to those observed for **94**.

Table 3.5: Summary of bond lengths and bond angles for selected region of **95**

Atoms			Length/Å	Atoms				Angle/°	Atoms				Angle/°
Ru1	N1		2.056(4)	N1	Ru1	N2		79.67(14)	N4A	Ru1	N2		96.30(15)
Ru1	N2		2.062(3)	N3A	Ru1	N2		87.9(9)	N5	Ru1	N4A		86.52(15)
Ru1	N3A		2.06(3)	N6	Ru1	N5		79.71(13)	N6	Ru1	N4A		92.83(16)
Ru1	N4A		2.061(4)	N1	Ru1	N3A		91.3(8)	N3A	Ru1	N5		95.1(9)
Ru1	N5		2.061(3)	N1	Ru1	N5		97.73(14)	N3A	Ru1	N4A		84.2(8)
Ru1	N6		2.053(4)	N6	Ru1	N1		92.03(14)	N6	Ru1	N2		97.36(14)

Within the extended structure, hydrogen bonding and π -stacking interactions stabilised the packing. Hydrogen bonding interactions were observed between the water solvent molecules and counterions, and from the water molecules to the imide oxygen acceptor atoms. Intramolecular hydrogen bonding interactions were also observed between imide oxygen atoms of the **Nap** and the CH acceptor atom of a neighbouring auxiliary **phen** ligand [Figure 3.22 (a)]. Hydrogen atoms of water molecules were placed using AFIX instructions and positions of hydrogen atoms were optimised to maximise realistic hydrogen bonding networks. The full extended structure can be viewed in the appendix [Figure A3.50] with details of the hydrogen bonding interactions displayed in Figure A3.51.

Two distinct types of π - π stacking interactions were observed: i) between the planes of two neighbouring **Phen** auxiliary ligands and ii) between two planar **Nap** moieties on neighbouring structures in a head-to-tail orientation, typically observed for the **Nap** motif.¹⁸⁹ The closest centroid-centroid distance (N2,C7-C11 to N2,C7-C11*i*, where *i* is the symmetry transformation: 1-

x, 2-y, 1-z) between the planes of the **phen** motifs was 3.899(3) Å, with an angle of 0.0(3)° and a slippage distance of 1.762 Å; while for the **Nap** motifs, the closest centroid-centroid (C39-43,49–C11 to C39-43,49*i*, where *i* is the symmetry transformation: 1-x, -y, -z) distance was 3.656(3) Å, with an angle of 0.0(3)° and a slippage of 1.219 Å between the planes [Figure 3.22 (b)].

As was observed previously, the plane-to-plane twist angle between the **Phen** and **Nap(Br)** was significant, and measured 78.74(14)° which inferred interesting photophysical properties on the ligands and is attributed to both steric repulsion between the **Nap** imide oxygen atom and **Phen** proton, and to intermolecular interactions, such as hydrogen bonding interactions observed above.

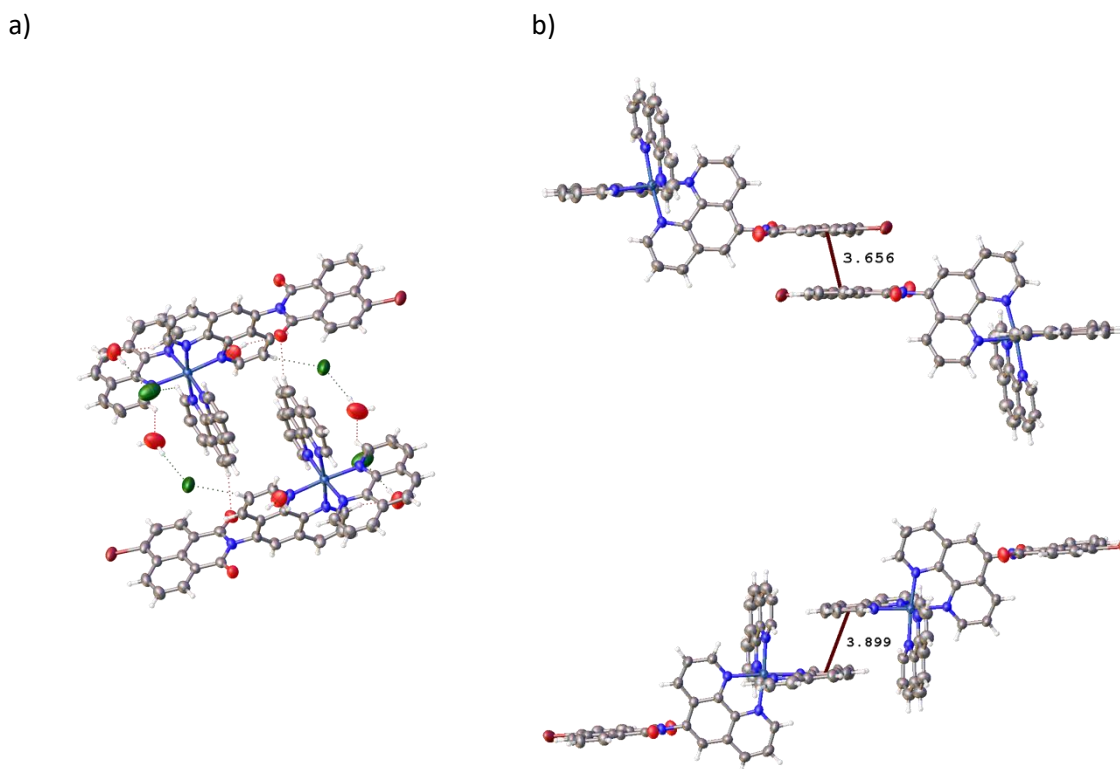


Figure 3.22: ORTEP style representation of **95** displaying a) hydrogen bonding interactions, represented by red and green dotted lines, and b) π - π stacking interaction between **Nap** moieties (top) and **phen** moieties (bottom) of neighbouring complexes. Distances between the planes denoted by red line / Å. Thermal ellipsoids are shown at 50% probability. Disorder in the structure omitted for clarity.

3.8.3: X-Ray Crystal Structure Analysis of **96**

Following the successful crystallisation of **94** and **95**, many attempts were made to crystallize **96**, including the use of slow evaporation and solvent diffusion techniques employing MeOH and MeCN as the mother solvents. Unfortunately, all of these attempts proved unsuccessful and the complex either remained in solution, or precipitated out of solution in polycrystalline or powder form. Complex **96** (10 mg) was next dissolved in water and red fragmented crystals were obtained after slow evaporation of H₂O over a three-day period. The red fragment crystals were solved in the

triclinic space group $P\bar{1}$ and the asymmetric unit contained two unique molecules of **96**, four Cl^- counterions and ten water molecules. The major occupied cationic species are shown in [Figure 3.23].

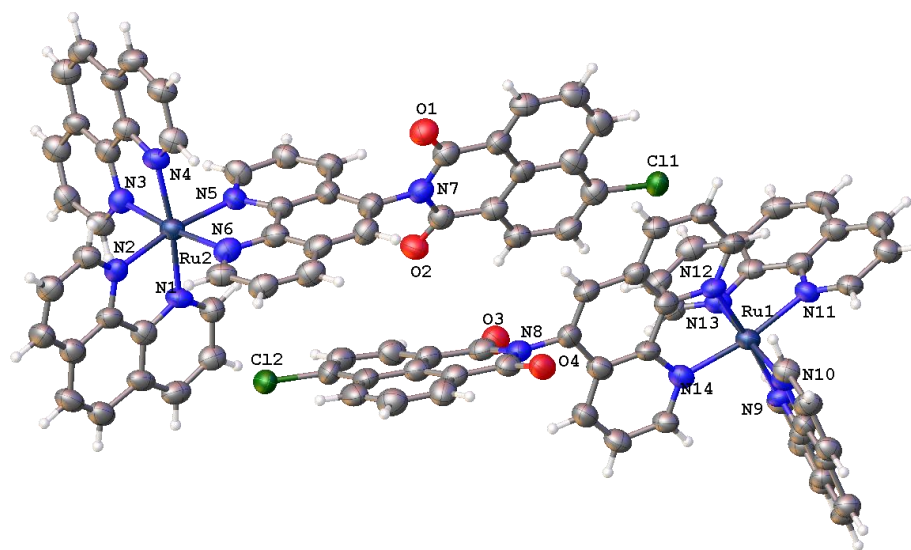


Figure 3.23: ORTEP style representation of the complex cations in the single crystal X-ray structure of **96**, including labels for heteroatoms. Positional disorder in the complex is omitted for clarity. Thermal ellipsoids are shown at 50% probability.

Positional disorder was observed over the **Nap** moieties 79% and 21%, and 72% and 28% occupancies for the two cationic species. The structure was solved and refined as a two-component inversion twin with scale factors 0.948(3) and 0.052(3). Following refinement of cationic units, the Fourier difference map revealed multiple regions of disordered electron density that surrounded the stacked layers of complexes and ran through the extended structure parallel to the crystallographic b -axis. Positional disorder was observed for the Cl^- counterions as well as the water molecules and as such, no reasonable refinement model could be obtained for these residuals. Thus, the SQUEEZE function on Platon software was employed to remove these areas of electron density in order to provide meaningful refinement statistics.²³⁶ This operation removed a total of 246 electrons, accounting for approximately four Cl^- counterions and ten water molecules.

The structure was found to display π -stacking interactions analogous to those observed for complexes **94**. Figure 3.24 displays the π -stacking interactions between adjacent **phen** motifs and adjacent **Nap** motifs present in the extended structure of **96**. Intermolecular hydrogen bonding interactions were observed between the chloro-substituent and the CH-acceptor atom of the **Phen**, as was also observed previously for the structurally related complexes. The plane-to-plane twist angles between the central planes of the **Phen** and **Nap** motifs ranged from 71.3(3)–72.1(3) for two species in the asymmetric unit, which was the smallest angle observed for these complexes.

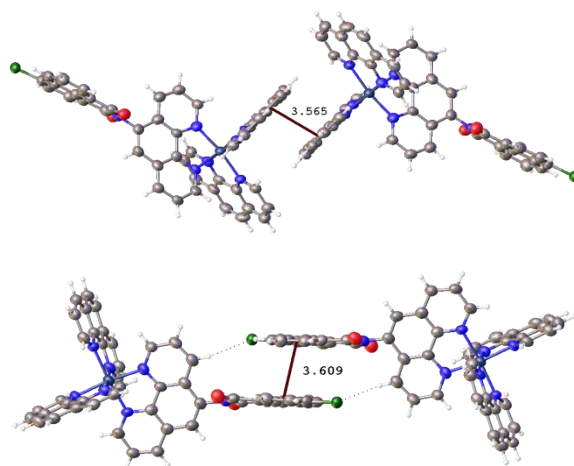


Figure 3.24: π - π Stacking interaction between **phen** moieties (left) and **Nap** moieties (right) of neighbouring complexes. Distances between the planes denoted by red line / Å. Thermal ellipsoids are shown at 50% probability. Disorder in the structure omitted for clarity.

In summary, the solid-state structures of the three novel complexes were solved and refined to final R indexes [$I \geq 2\sigma(I)$, (R_1 , wR_2)] of **94**(0.0428, 0.1141), **95**(0.0549, 0.1419) and **96**(0.0867, 0.2307). Common features were observed among the structures, such as a substantial degree of rotation (71–89°) between the central rings of the **PhenNap** ligand, similarly observed in the solid-state structure of the ligands. Their crystal structures provided an insight into electronic properties of the systems and unveiled multiple intermolecular interactions that were potentially suitable for DNA association, such as π -stacking interactions and hydrogen/halogen bonding, arising from multiple areas of the complexes. Thus, to further probe the electronic properties of the complexes, electrochemical analysis of complexes **94** and **95** was performed and will be discussed in the next section.

3.9: Electrochemical Properties of **94** and **95**

Ru(II)-polypyridyl complexes are known to display rich photophysical, photochemical and electrochemical properties which imparts a wealth of purposes for such complexes across many fields of science, including in photocatalysis and biological applications.^{1,2,118} As such, it is important to understand the electrochemical properties of these redox active systems as these properties, in turn, can provide insight into the relative energies of the molecular orbitals and, thus their reactivities. The electrochemical properties can be used to inform the electron transitions that occur upon optical absorption (*vide infra*, Section 3.12), which is crucial to understanding the reactivity of the different parts of the molecules with DNA.

The electrochemical properties of **94** and **95** were investigated to discern the effects of the substituent in the 4-position of the **Nap** on the redox potentials of the complexes. The complexes

were first converted to the PF_6^- salts by precipitation of the complexes from aqueous solution *via* addition of a saturated solution of NH_4PF_6 to the chloride anion forms. The resulting orange precipitates were collected by filtration and electrochemical measurements of **94** and **95** were carried out in degassed acetonitrile with tetrabutylammonium hexafluorophosphate as the electrolyte. The ferrocene/ferrocenium (Fc/Fc^+) redox couple was employed as the internal standard and the data is referenced *versus* Fc/Fc^+ .¹⁹² The electrochemical measurements were performed at multiple scan rates and in both the positive and negative directions. The experiments were performed twice to ensure reproducibility and the CV traces are presented in Figure 3.25.

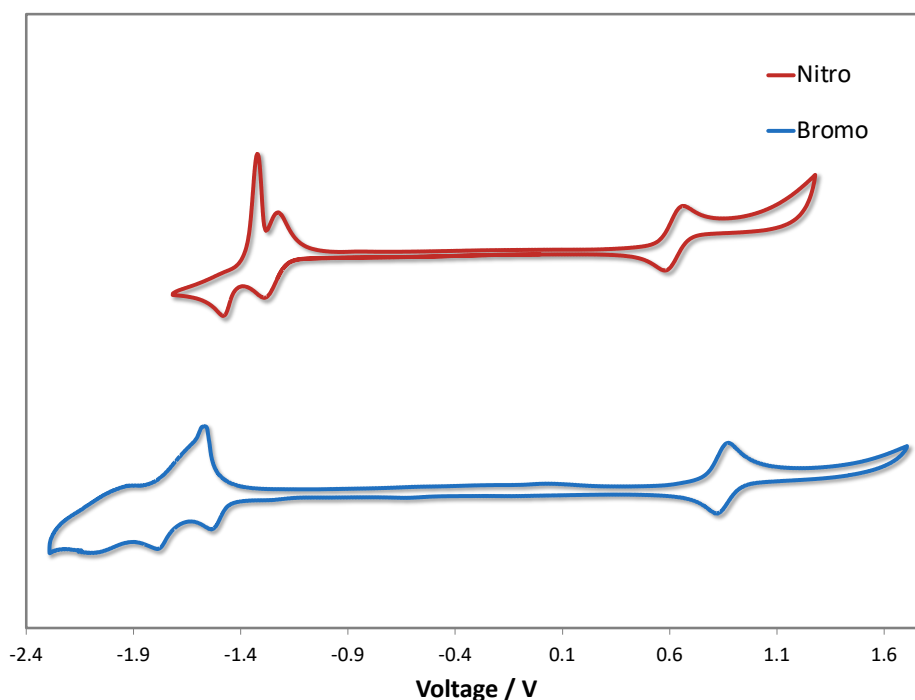


Figure 3.25: The CV traces of **94** and **95** in deaerated MeCN, referenced with respect to the Fc/Fc^+ redox couple. Deaeration achieved by vigorous bubbling of N_2 for 15 min immediately before measurement. Scan rate = 100 mV/s in the positive scan direction. Electrolyte = NBu_4PF_6 .

The nitro- and bromo-substituted complexes, **94** and **95** each displayed one reversible oxidation corresponding to the $\text{Ru(III)}/\text{Ru(II)}$ redox couple which occurred at +0.63 and +0.87 V, respectively. A comprehensive literature review from Troian-Gautier and Moucheron, focusing on the structural and electrochemical properties of Ru(II) -polypyridyl complexes indicates that the $\text{Ru(III)}/\text{Ru(II)}$ couple typically occurs in the range of 0.80 – 1.50 V, which are much higher than that observed for **94**.²³⁷ Recent electrochemical studies of a Ru(II) water oxidation catalyst by Oretaga and co-workers have shown pH and cross-linking dependent redox tuning properties over a range of 200 mV, for the $\text{Ru(III)}/\text{Ru(II)}$ couple. Compared to the unsubstituted Ru(II) **PhenNap** complex, **75** (+0.91 V), the bromo-analogue **95** (+0.87 V) and $[\text{Ru}(\text{phen})_3]^{2+}$ (+0.90 V) vs the Fc/Fc^+ internal standard, the oxidation potential for the $\text{Ru(III)}/\text{Ru(II)}$ couple of **94** occurred at a more negative

potential. While the presence of the electron withdrawing nitro group on the **Nap** may influence the electronic properties of the complex, it was not expected to exert such an extreme shift in the metal oxidation potential, as this moiety is both spatially and electronically separated from the ruthenium centre. The solid-state structure analysis of **94** (*vide supra*, Section 3.8.1) has shown crosslinking in the bulk structure through intermolecular hydrogen bonding and π -stacking interactions and as such, this may impact the value of the redox couple, but more experiments are necessary to discern the origin of this value. The oxidation potential for the Ru(III)/Ru(II) couple of **95** occurred at +0.87 V, which is consistent with other Ru(II)-polypyridyl complexes bearing bis-**phen** auxiliary ligands in the literature.²³⁷

In line with the CV analyses of the ligands **91** and **92**, the nitro-substituted complex, **94** displayed more positive reduction potentials than **95**. The first reduction wave of **94** occurred at -1.24 V and is attributed to reduction of the nitro group and a second reduction at -1.46 V attributed to reduction of the **Phen** ligand. The first reduction was well defined and reversible, however; when the scan range was increased to -2.0 V, the return wave for the two reductions converged [Figure A3.52]. This may suggest electrochemical degradation of the complex involving multielectron reduction processes under these conditions. The bromo-substituted complex, **95**, displayed three quazi-irreversible reductions waves at -1.50, -1.74 and -1.98 V. The first two reductions are assigned to reductions of the **Nap** ligand and occur at similar potentials as **92** and the third is assigned to a reduction on the **phen** ligands. Table 3.6 displays the oxidation and reduction potentials of the complexes in acetonitrile as well as values for the reference compounds.

Table 3.6: Oxidation $E_{(ox)}$ and reduction $E_{(red)}$ potentials of ligands **91** and **92**, and complexes **94** and **95** vs Fc/Fc⁺ in degassed acetonitrile at room temperature with tetrabutylammonium hexafluorophosphate as the electrolyte. The redox potentials for complexes **75** [Chapter 2] and [Ru(**phen**)₃]²⁺ are included for comparison

Compound	$E_{(ox)} / V$	$E_{(red)} / V$
Ligand 91	---	-1.07, -1.35, -2.42 ^[a]
Complex 94	+0.63	-1.24, -1.46
Ligand 92	---	-1.36 ^[a] , -1.50 ^[b]
Complex 95	+0.87	-1.50 ^[a] , -1.74, -1.98 ^[b]
Complex 75	+0.91	-1.39 ^[a] , -1.62 ^[a] , -1.87 ^[a]
[Ru(phen) ₃] ^{2+[c]}	+0.90	-1.79, -1.89, -2.10

^[a] Irreversible wave.

^[b] Waves are poorly resolved.

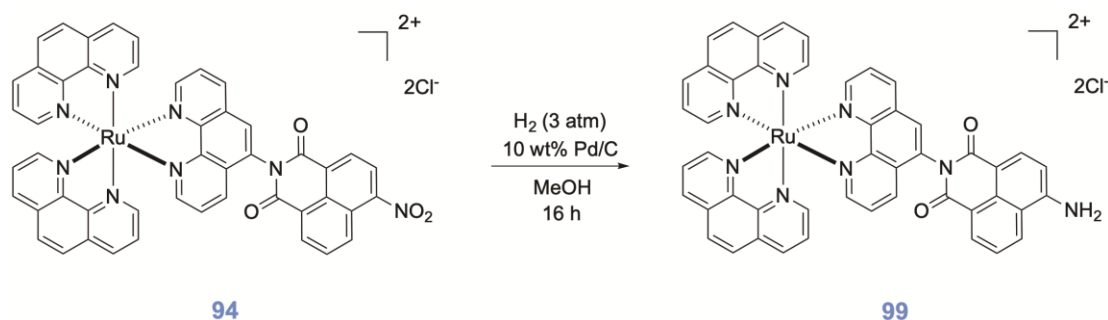
^[c] Values obtained from ref.¹⁹³ Potentials were converted from the Ag/AgCl to the Fc/Fc⁺ scale using conversion constants from ref.¹⁹²

Overall, the complexes **94** and **95** displayed complex redox behaviours with well defined oxidations. Complex **94** was found to be both simultaneously easier to reduce and oxidise than the bromo counterpart, **95**, the former being consistent with the presence of the electron withdrawing nitro group. The effect of substitution of the **Nap** ligands on the oxidation potential of the metal requires further investigation. Since both the nitro- and bromo- groups are electron withdrawing in nature, the investigation of a complex with an electron donating group, such as an amino group on the **Nap** was desired to probe the effect of this substitution pattern on the photophysical and electrochemical properties of the metal complexes. As mentioned previously, ligand **97** was not afforded in any appreciable quantity to utilise for metal complexation reactions and as such, the reduction of the nitro group of **94** to the amine derivative **99** was attempted and is presented in the next section.

3.10: Synthesis and Purification of $[\text{Ru}(\text{Phen})_2\text{PhenNap}(\text{NH}_2)]\text{Cl}_2$, **99**

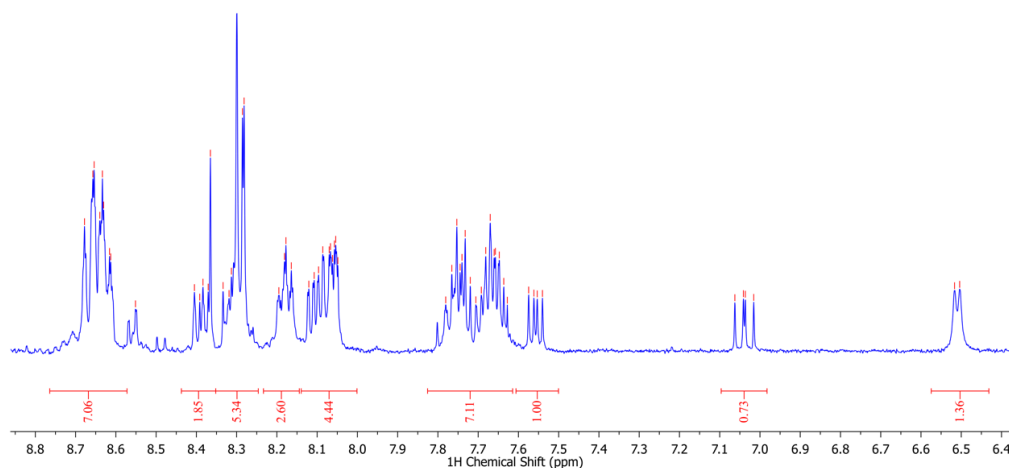
Section 3.2.2 (*vide supra*) detailed a series of failed reactions toward the synthesis of the amino substituted **Nap** ligand **97**, and the recent development from the Elmes group who have managed to form the desired ligand by alternative synthesis. As outlined, this ligand was designed with the ultimate aim to complex it to Ru(II)-polypyridyl system and explore the DNA binding affinities by placing the ligand in direct contact with the Ru(II)-centre *via* the **phen** coordinating motif. Indeed, our research group, among others, have previously explored the therapeutic properties of 4-nitro and 4-amino Ru-**Nap** conjugates, where the **Nap** ligand was coordinated to the metal *via* flexible aliphatic chains or rigid aromatic linkers.^{160,168,169,216,217} The therapeutic properties of Ru(II)-**Nap** conjugates wherein the **Nap** ligand is directly coordinated to the metal, however, has not yet been investigated. Furthermore, detailed photophysical studies by Castellano and co-workers on a series of Re(I)-complexes bearing similar 4-substituted **PhenNap** ligands, demonstrated that so-called “ping-pong” energy transfer between the triplet excited states of the ligand and the metal could be achieved by varying the substituent in the 4-position of the **Nap**.¹⁵¹ The excited state equilibria were found to favour the ligand centred excited state when electron donating species were present. These studies highly motivated the design of the complexes **94**, **95**, **96** and **99**, however, since complexes **94-96** all contained electron withdrawing substituents, much effort was placed on isolating complex **99** for comparative studies.

The synthesis of **99** was attempted using similar techniques to those described for the reduction of the ligand **91** and as such, these will not be discussed in detail. Scheme 3.4 summarises the reaction conditions for the synthesis of **99**.

Scheme 3.4: Synthesis of **99** from the precursor **94**.

Complex **94** was dissolved in MeOH and subject to catalytic hydrogenation using 10wt% Pd/C (1.0 eq.) at 3 atm of H₂ using a Parr hydrogenation shaker apparatus. The product was isolated as a red solution after filtration through a pad of Celite. The solvent was removed under reduced pressure, revealing a mixed product system containing reduced and partially reduced species. Purification was attempted by flash column chromatography, first performed on Al₂O₃, (MeCN:H₂O 10:0 – 9:1) and secondly, on SiO₂ (MeCN:H₂O:NaNO_{3(aq)} 40:10:1). The product was isolated as a red solid which still contained 15% baseline impurities by ¹H NMR. The product was then dissolved in minimal MeCN and following slow evaporation of the solvent, a red crystalline material began to form which was isolated in minor quantities by filtration and was washed with DCM and Et₂O.

Successful formation of the product was inferred from the ¹H NMR spectrum [Figure 3.26] which exhibited a proton that experienced an upfield chemical shift in comparison to the **94**, characteristic of a shielding affect from the adjacent amino group. However, as mentioned, some baseline impurity was still present. HRMS also confirmed formation of the product [Figure A3.53], where the observed isotopic distribution pattern matched that of the calculated one. Unfortunately, due to the low yield of material afforded, the complex was not characterised by ¹³C NMR, elemental analysis or other conventional techniques as the material was reserved for photophysical measurements and DNA binding studies (*vide infra*).

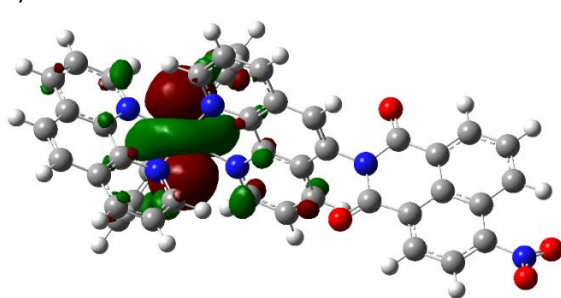
Figure 3.26: The ¹H NMR spectrum of **99** (400 MHz, CD₃CN).

The ^1H NMR spectrum above displayed are consistent with other amino-functionalised Ru(II)-**Naps** in the literature, such as complex **70** (*vide supra*, Section 3.1).¹⁶⁹ Furthermore, the photophysical evaluation of **99** (*vide infra*, Section 3.12), further indicated successful formation of the product and displayed charge transfer character in the **Nap** optical absorption window, typical for 4-amino functionalised **Naps**, as opposed to the structured absorption bands observed for **94**, **95** and **96**. The next section presents preliminary theoretical calculations that were obtained in order to investigate the electronic distributions of complexes **94** and **99**.

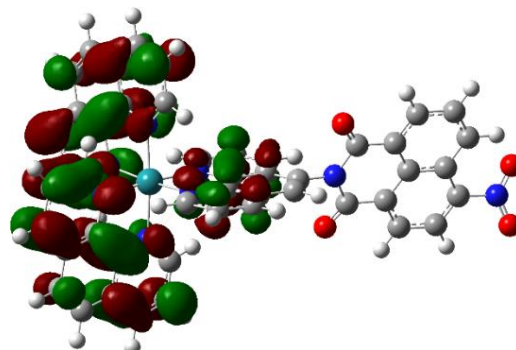
3.11: Theoretical Calculations of **94** and **99**

Theoretical calculations for complexes **94** and **99** were performed by Dr Deivasigamani Umadevi at The Indian Institute of Technology Palakkad (IITPKD), Kerala, India. The structures were optimised by using the semiempirical PM6 method and were characterised as minima after verifying the presence of all real frequencies. Frontier molecular orbital (FMO) density diagrams were constructed to investigate the electronic properties and orbital distributions of the complexes using the Gaussian 16 programme [Figure 3.27].²²⁷ The crystal structure data of **94** (*vide supra*, Section 3.8.1), was provided to aid the calculations.

a)

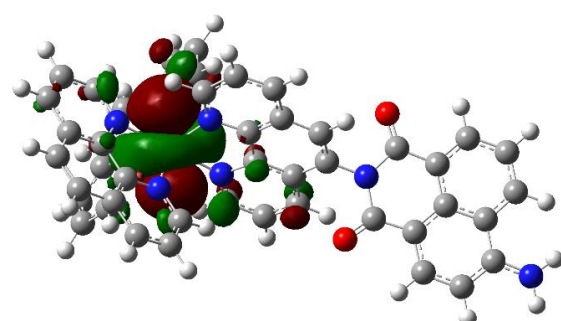


94, HOMO (-12.06 eV)

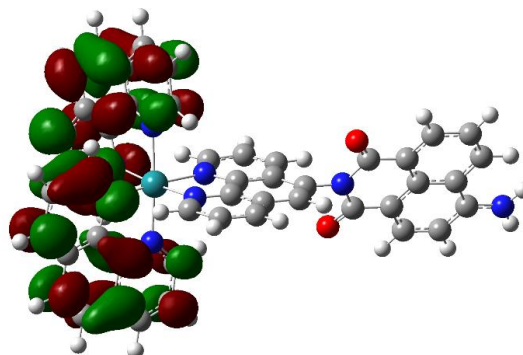


94, LUMO (-6.30 eV)

b)



99, HOMO (-11.83 eV)



99, LUMO (-6.10 eV)

Figure 3.27: FMO density distribution diagrams and associated energies (eV) of a) **94** and b) **99**.

Full geometry optimization and frequency calculations were performed at the semiempirical PM6 method using Gaussian 16.²²⁷ The calculations revealed that the HOMO of both complexes was localized on the Ru(II) metal centres, while the LUMO of both complexes was largely located on the **phen** ligands. For **94**, the LUMO was distributed over all three **phen** ligands, due to the electron withdrawing nitro group, while for **99**, the LUMO was localised only on the ancillary **phen** ligands. These results support the existence of MLCT process in both the complexes. Overall, these results confirm that although substantial geometric decoupling exists between the **phen** and **Nap** chromophores of the coordinating ligand, substitution in the 4-position of the **Nap** influences the LUMO orbital distribution on the complexes. This observation can be used to inform the optical properties of the complexes which are discussed in the next section.

3.12: Photophysical Properties of Complexes **94**, **95**, **96** and **99**

In line with studies carried out in Chapter 2, the spectroscopic properties of complexes **94**, **95**, **96** and **99** were investigated in 10 mM aqueous sodium phosphate buffer (pH = 7.4) at room temperature. The UV-Vis absorption, excitation and emission spectra of the complexes are shown in Figure 3.28 and display features characteristic of both ruthenium centred MLCT and **Nap** chromophores.^{168,169} The complexes each displayed high energy absorption bands centred at 262 nm attributed to $\pi-\pi^*$ IL transitions of the **phen** ligands with high molar absorptivity constants calculated from the Beer-Lambert Law. Complexes **94**, **95** and **96** displayed structured **Nap** localized absorption bands at 362, 354 and 350 nm, respectively, which mirrored the absorption bands of the free ligands (*vide supra*, Section 3.6). The amino-derivative, **99**, displayed the lowest molar absorptivity constant for the **phen** centred transition, and did not display the structured **Nap** absorption band. Finally, two broad bands centred at *ca.* 420 and 450 nm were observed for each complex, corresponding to the MLCT transitions between the Ru(II)-metal and the ligands. The molar absorptivity constant for the MLCT transitions was largest for the amine-derivative, attributed to overlapping ICT transitions localised on the **Nap** in this region, as was observed in the photophysical absorption analysis of the free ligand, **97**. The absorption properties of complexes **94**, **95**, **96** and **99** are summarized in Table 3.7 and are comparable to the Ru(II)-**Nap** complexes connected through the rigid aromatic spacers, **69** and **70**. The halogen substituted complexes displayed more structured **Nap** based absorption profiles, consistent with the free ligand analysis. Importantly, the bands corresponding to the absorption centred at both the **Nap** and Ru(II)-polypyridyl centres can be individually resolved allowing for specific and selective probing of the interactions of these parts of the complexes with DNA.

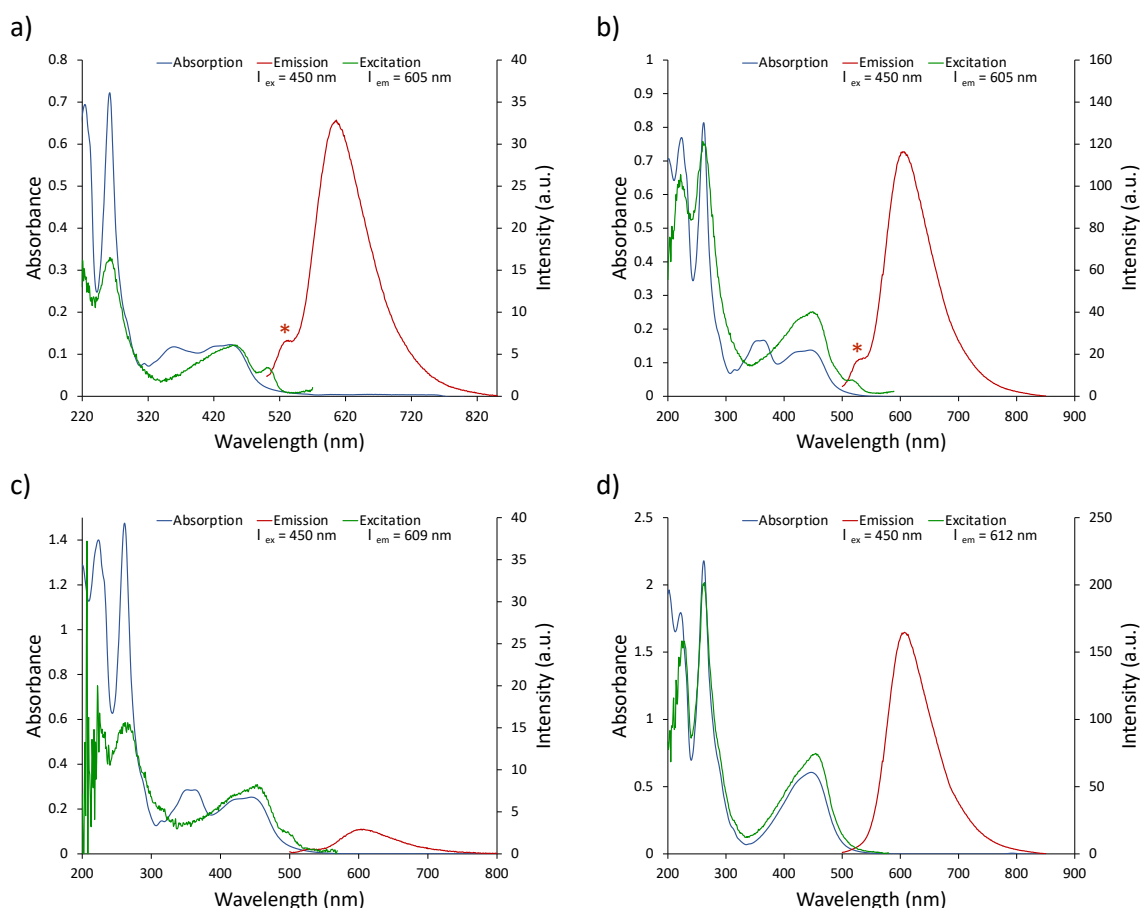


Figure 3.28: Absorption, excitation and emission spectra in aerated 10 mM sodium phosphate buffer solution at 298 K of a) **94** ($\lambda_{\text{ex}} = 450$ nm, 9×10^{-6} M; $\lambda_{\text{em}} = 605$ nm, 3×10^{-6} M), b) **95** ($\lambda_{\text{ex}} = 450$ nm, 9×10^{-6} M; $\lambda_{\text{em}} = 605$ nm, 3×10^{-6} M), c) **96** ($\lambda_{\text{ex}} = 450$ nm, $\lambda_{\text{em}} = 609$ nm, 9×10^{-6} M) and d) **99** ($\lambda_{\text{ex}} = 450$ nm, 2.5×10^{-5} M; $\lambda_{\text{em}} = 612$ nm, 3×10^{-6} M). Water Raman band is denoted by *.

For complexes **94**, **95** and **96**, excitation of both the **Nap**-centred (362, 354 and 350 nm, respectively) and MLCT-based ($\lambda = 450$ nm) absorption bands resulted in a weak, broad and unstructured $^3\text{MLCT}$ luminescence band centred at 610 nm. No emission arising from the **Nap** moiety was observed upon excitation of the **Nap** localised absorption band. The **Nap** centred emission within these systems is likely quenched due to efficient Förster resonance energy transfer (FRET) to the Ru(II)-polypyridyl centre, as described by Castellano and co-workers for related metal-**PhenNap** bichromophoric species, and in our own prior work where Ru(II)-polypyridyl complexes were employed.^{150,151} Unlike the non-substituted Ru(II)-**PhenNap** complexes, **75** and **76** studied in Chapter 2, which excitation spectra displayed all of the bands corresponding to the absorption spectra, the bands corresponding to **Nap** in **94**, **95** and **96** were absent from the excitation spectrum when monitoring the emission at $^3\text{MLCT}$ based band. For the amine derivative, **99**, the superimposable absorption and excitation spectra ($\lambda_{\text{em}} = 612$ nm) demonstrates efficient ($^1\text{Nap}^* \rightarrow ^1\text{MLCT}^*$) energy transfer followed by intersystem crossing to generate the $^3\text{MLCT}$ state. Table 3.7 summarises the absorption and emission properties of the complexes, including their

photoluminescence quantum yield values which were obtained using the optically dilute solution method with [Ru(**bpy**)₃]Cl₂ as the reference.¹⁹⁷⁻²⁰⁰

Table 3.7: Absorption and emission properties of **94**, **95**, **96** & **99** at 298 K. Complexes **69** and **70** are included for comparason.

	Absorption λ_{max} / nm [ϵ / M ⁻¹ cm ⁻¹] ($\pm$ 10%) ^[a]			Emission Properties	
	$\pi-\pi^*$ IL	$\pi-\pi^*$ Nap	MLCT	λ_{max} / nm	Φ_{PL} ($\pm$ 10%)
94	263 [82070]	362 [13560]	450 [13730]	609	< 0.006
95	263 [89300]	354 [18740]	447 [15700]	610	0.02
96	262 [99675]	350 [18975]	448 [16740]	610	< 0.001
99	262 [86635]	---	450 [23940]	612	0.70
69 ^[b]	287 [78159]	351 [18787]	457 [14765]	625	0.001
70 ^[b]	287 [53055]	---	451 [17502]	625	0.018

^[a] Data was reproducible over several measurements.

^[b] Data compiled from ref.¹⁶⁹

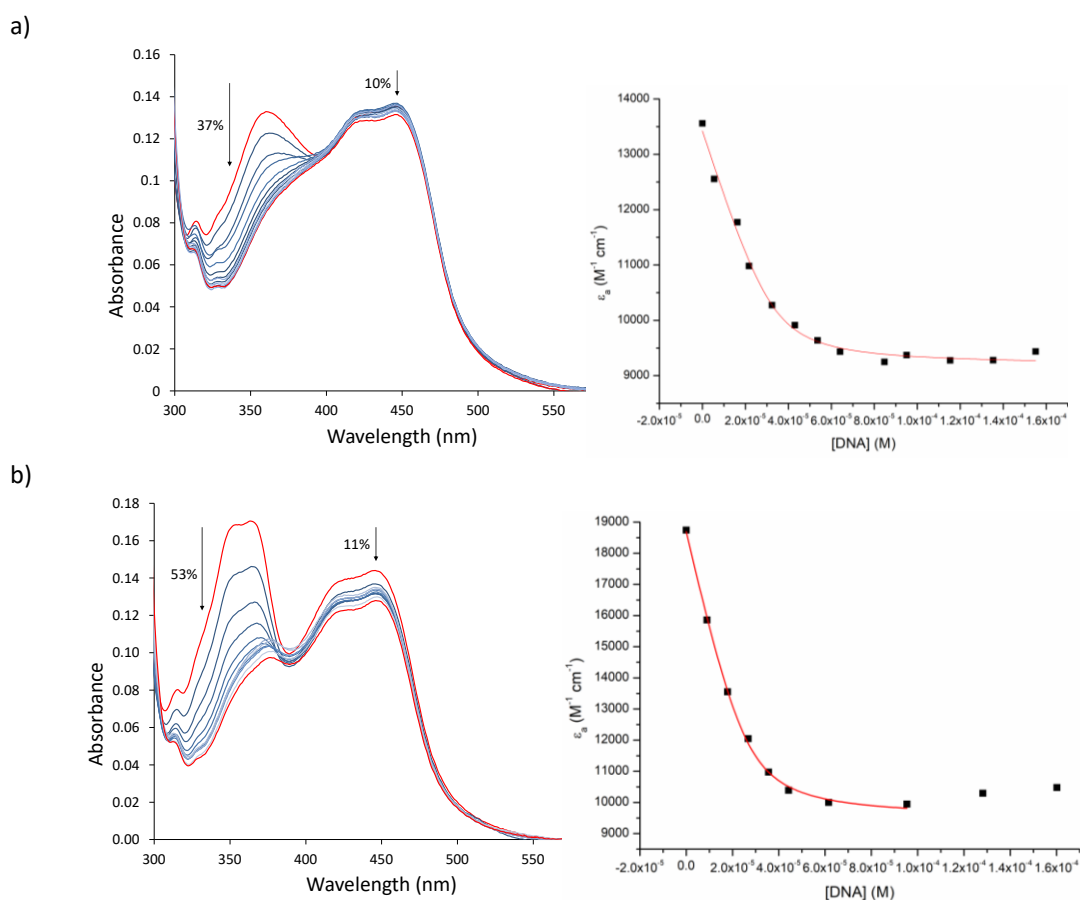
Dilution studies were performed to determine the relationship between emission intensity and concentration for all complexes and can be viewed in Figures A3.54 – A3.58. The intensity of emission for all complexes were found to be proportional to concentration, following the Beer-Lambert Law. Furthermore, the emission intensities were sensitive to the presence of molecular oxygen, supporting the ³MLCT based phosphorescence of the complexes. Interestingly, complex **94** displayed an increase in emission intensity upon dilution in acetonitrile. This may be explained by π -stacking phenomena in this aprotic solvent [Figure A3.54 (d)]. Thus, having analysed the photophysical properties of the complexes, their interactions with DNA was probed by spectroscopic techniques and will be presented in the next section.

3.13: DNA Binding Studies of **94**, **95**, **96** and **99**

As mentioned previously, the design of the complexes resulting in photophysical properties described above allow for direct monitoring of the different parts of the complexes interacting with DNA. The DNA binding properties of the complexes were analysed in a similar manner to the **Phen** and **TAP** Ru(II)-polypyridyl complexes discussed in Chapter 2 (*vide supra*, Section 2.6.1). DNA titration experiments were performed by monitoring changes in the absorbance and emission properties of the complexes upon increasing additions of natural ctDNA in 10 mM sodium-phosphate buffer at a pH of 7.4. The photophysical properties were monitored until negligible changes were observed. All measurements were performed at least twice and temperature was kept stable at 298 K to ensure reproducibility.

3.13.1: Absorption Titrations of **94**, **95**, **96** and **99** with ctDNA

For complexes **94**, **95** and **96**, the low energy absorption bands corresponding to the **Nap**-centred and Ru(II)-MLCT centred transitions underwent changes to their local environment upon addition of DNA to optically dilute solutions. These changes were more appreciable for the bands corresponding to the $\pi-\pi^*$ **PhenNap** transitions, ($\lambda_{\text{abs}} = 362$ (**94**), 354 (**95**) and 350 (**96**) nm), where the degree of hyperchromicity was 37%, 53% and 48%, respectively for the sequentially numbered complexes. These optical shifts most likely reflect intercalation of the planar **Nap** structure between the base pairs of DNA. The changes in the absorbance profiles for these three complexes is presented [Figure 3.29 (a), (b) and (c)] where the moderate hypochromic shifts in the $d\pi(\text{Ru(II)})-\pi^*(\text{N}^{\wedge}\text{N})$ MLCT transition (10% (**94**), 11% (**95**) and 12% (**96**), respectively) are attributed to an electrostatic interaction from the Ru(II)-metal centre, as observed for the non-intercalating Ru(II)-polypyridyl complex $[\text{Ru}(\text{bpy})_3]^{2+}$.^{11,100} The changes in the relative absorbance profiles indicate interaction with DNA both through the **Nap** motif as well as association of the metal centre. Similar changes were observed for $[\text{Ru}(\text{phen})_2\text{L}]^{2+}$ complexes **69**, **70** and **75**.¹⁶⁹ The interaction of the amino functionalised derivative, **99** was probed through monitoring changes in the low energy absorption transitions at $\lambda_{\text{abs}} = 420, 450$ nm [Figure 3.29 (d)] upon addition of DNA.



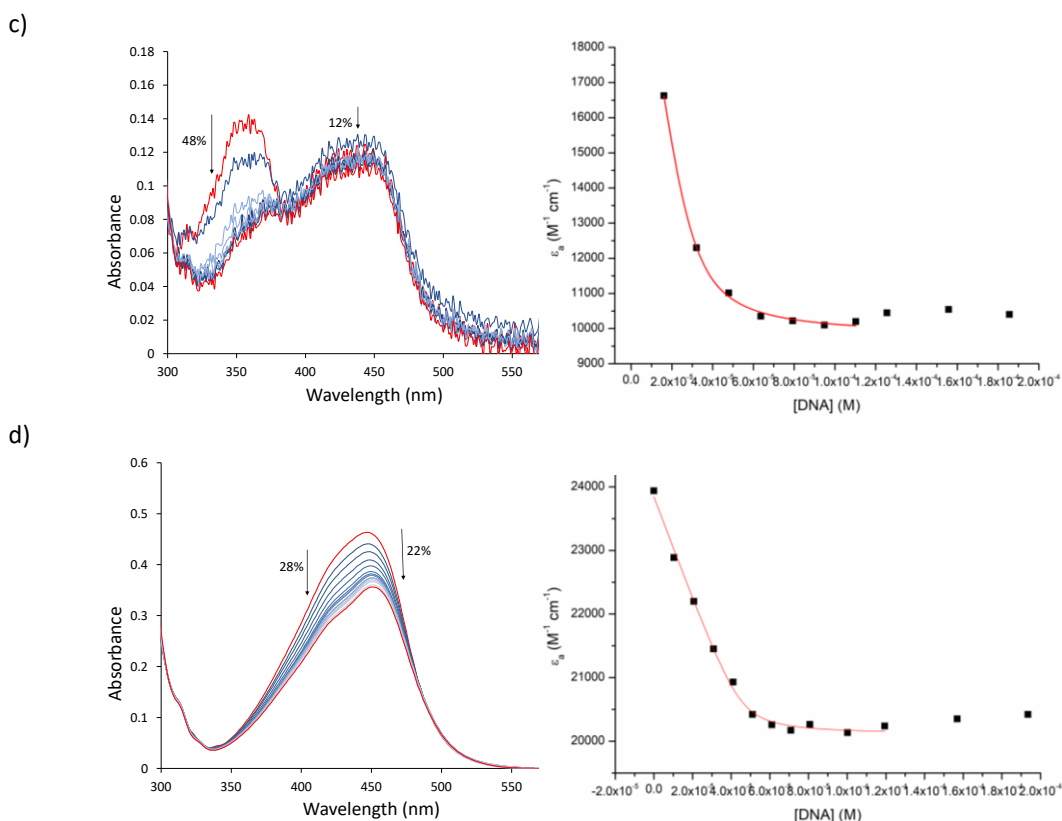


Figure 3.29: Changes of UV-vis absorbance profile and fit of the data to the Bard equation for a) **94** ($\lambda = 360$ nm; 1×10^{-5} M), b) **95** (1×10^{-5} M), c) **96** (1×10^{-5} M) and d) **99** (1×10^{-5} M) upon addition of aliquots of ctDNA (0–0.2 mM) in sodium phosphate buffered water at 298 K.

Unsurprisingly, this region experienced more appreciable changes at these wavelengths than **94**, **95** and **96** as for **99**, as it represents transitions from both $\pi-\pi^*$ IL **PhenNap** transitions as well as $d\pi(\text{Ru(II)})-\pi^*(\text{N}^{\wedge}\text{N})$ MLCT transition. The overall absorption spectroscopic changes for all four complexes upon addition of DNA were similar, indicating a common binding mode for the complexes, hypothesised to be multicomponent and consisting of intercalation and electrostatic binding.

The DNA binding parameters were obtained by plotting the changes in the molar absorbance values (ϵ_a) as a function of the concentration of DNA and fitting the data to the modified Bard equation in the same manner as the complexes described in Chapter 2.^{97,205} The intrinsic binding constants and binding site sizes were derived from the best fit of the data to Equation 2.3 (*vide supra*, Section 2.6.1). The binding constants, K_b , were determined to be **94**: 1.24×10^6 (± 0.7) M^{-1} , **95**: 1.13×10^6 (± 0.4) M^{-1} , **96**: 2.09×10^6 (± 1.1) M^{-1} , and **99**: 2.18×10^6 (± 1.4) M^{-1} , while the binding site size ranged from one to two base pairs [Table 3.8].

Table 3.8: DNA binding parameters from fits to absorbance data ($\lambda = 347$ nm)^[a]

Complex	% Hyperchromism (Nap)	Binding constant, $K_b / 10^6 \text{ M}^{-1}$	Binding site size, n/base pairs	R ²
94	37	1.24 (± 0.73)	1.70 (± 0.2)	> 0.99
95	53	1.13 (± 0.38)	1.47 (± 0.09)	> 0.99
96	48 ^[b]	2.09 (± 1.06)	2.08 (± 0.35)	> 0.99
97	---	2.18 (± 1.41)	1.13 (± 0.05)	> 0.99

^[a] Results correspond to the mean $\pm$ SEM^[b] Data poorly resolved.

The DNA absorption titrations provide an excellent indicator that strong interactions of the **Nap** motif with DNA is present, as depicted by the derived binding constants which were in the order of magnitude of 10^6 M^{-1} and the derived binding site sizes of approximately 1-2 base pairs, indicative of intercalative binding. Caution should be exercised when analysing these statistics however, as other binding modes, such as hydrogen bonding interactions, enantiospecific binding modes and electrostatic interactions are most likely also present. In order to further probe the interaction of the complexes with DNA, the emission responses of the complexes upon DNA titration were analysed.

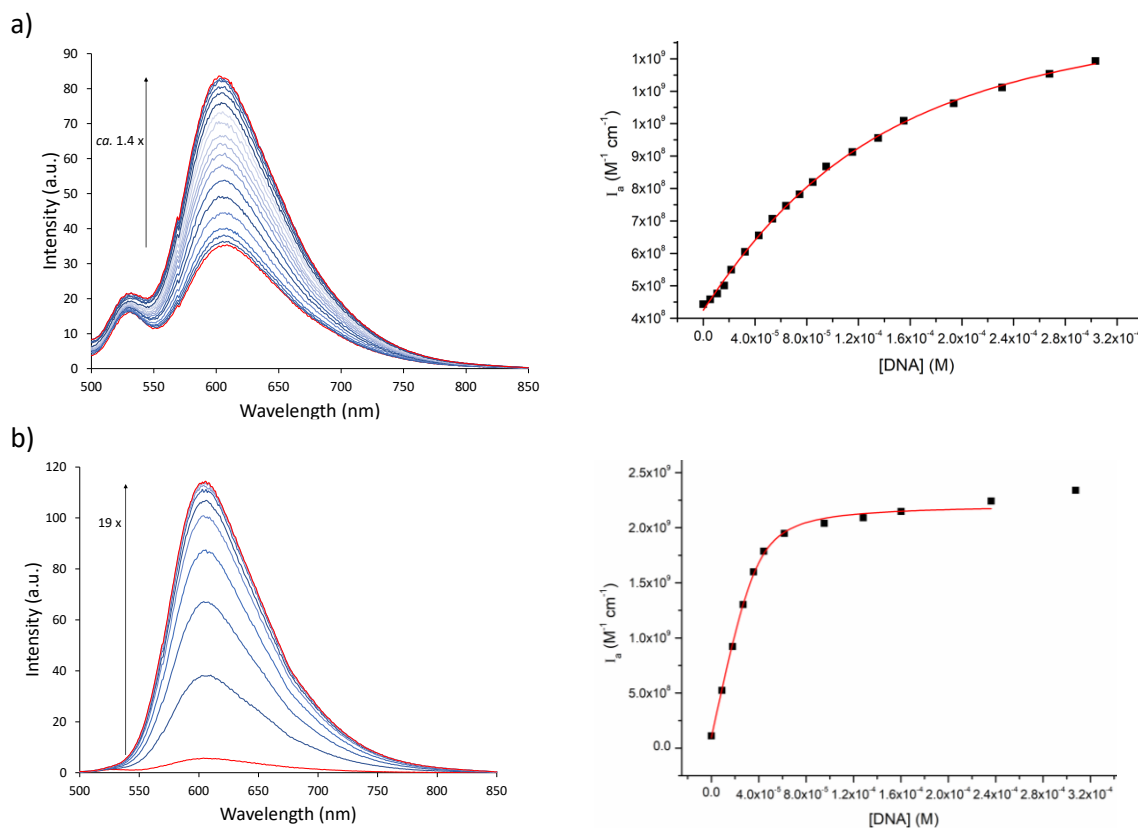
3.13.2: Emission Titrations of **94**, **95**, **96** and **99** with ctDNA

It is well known that Ru(II)-polypyridyl complexes containing **phen** or **bpy** ancillary ligands as well as the **dppz** ligand experience emission enhancement when bound to DNA due to protection of the phenazine nitrogen atoms from the aqueous water environment upon DNA intercalation (*vide supra*, Section 1.5).^{8,84,164,178,179,238} Though due to different phenomena, complex **75**, bearing **phen** ancillary ligands and the unsubstituted **PhenNap** ligand was also found to display an impressive 30-fold emission enhancement when bound to ctDNA versus in the free form. As such, complexes **94**, **95**, **96** and **99** were also expected to display similar emission intensity responses and the results are presented herein.

Unlike that seen for **dppz** systems, where hydrogen bonding plays a major role in the quenching of the MLCT emission in water, the observed emission enhancement in the complexes presented here, may occur due to similar protection mechanisms of the **Nap** imide oxygen atoms from the aqueous environment or as a result of protection of the Ru(II) centre from quenching through tight association with the DNA molecule upon binding of the **Nap** unit. In their free forms, complexes **94**, **95**, **96** and **99** displayed low photoluminescence quantum yield values of 0.006, 0.02, 0.001 and 0.7, respectively. However, when bound to DNA, each of the complexes experience

notable 1.4-, 19-, 34- and 3-fold emission enhancements, respectively, rendering the complexes as sensitive luminescent DNA probes. The changes of the DNA emission profiles of the complexes are displayed in Figure 3.30.

As described in Chapter 2, the apparent integrated emission intensity was best fit to Equation 2.3. Unlike the complexes studied in Chapter 2, where the K_b values and n values obtained from the fitting of the emission data closely mirrored the values obtained from the absorbance data, this was not observed in this case, which is unsurprising due to the pseudo diastereomers present in the case of these asymmetric complexes. Nonetheless, the K_b values obtained from the fitting of the emission titration data were again determined to be in the order of 10^6 M^{-1} for the complexes. The binding site size was found in the range of one to two base pairs for complexes **95**, **96** and **99** and four base pairs for **94**. The poor derived parameters for **94** is again, unsurprising, due to the effective quenching properties of the nitro group and the resulting weak emission profile. Due to the marked emission enhancement in luminescence upon DNA binding, the photoluminescence quantum yields of the complexes in their fully bound forms were determined using the optically dilute solution method, to represent the response more accurately. Table 3.9 summarises the results.



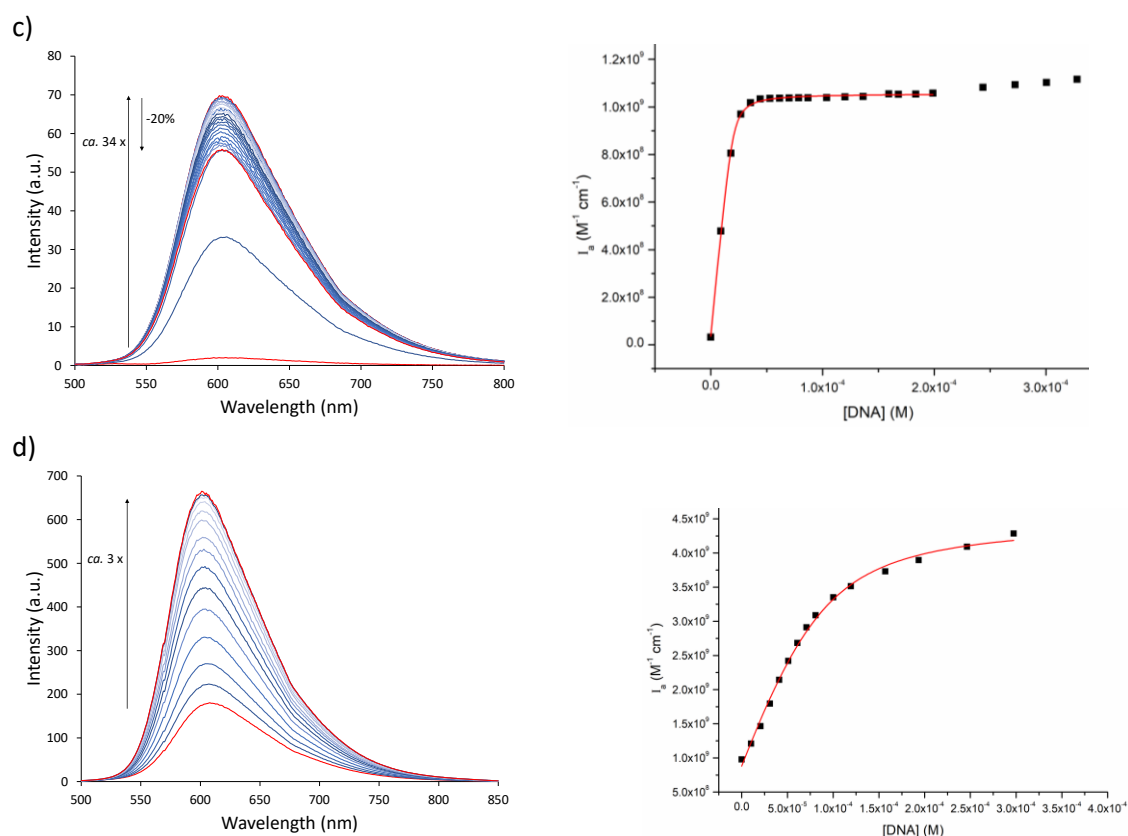


Figure 3.30: Changes of emission profiles and fit of the data to the Bard equation at $\lambda = 600$ nm for a) **94** (1×10^{-5} M), b) **95** (1×10^{-5} M), c) **96** (1×10^{-5} M) and d) **99** (1×10^{-5} M) upon addition of aliquots of ctDNA (0–0.2 mM).

Table 3.9: DNA binding parameters from fits to emission data ($\lambda = 605$ nm)^[a] and changes in the Φ_{PL} values in the free versus fully bound forms.

Complex	Binding constant, $K_b / 10^6 \text{ M}^{-1}$	Binding site size, n/base pairs	R^2	$\Phi_{\text{PL}} (\pm 10\%)$ (Complex)	$\Phi_{\text{PL}} (\pm 10\%)$ (Complex + DNA)
94	0.1 (± 0.06)	4.44 (± 1.07)	> 0.99	< 0.006	0.09
95	1.10 (± 0.38)	2.11 (± 0.16)	> 0.99	0.02	1.54
96	3.47 (± 0.50)	1.42 (± 0.03)	> 0.99	< 0.001	1.83
99	1.63 (± 0.75)	1.69 (± 0.22)	> 0.99	0.70	2.95

^[a] Results correspond to the mean $\pm$ SEM

Of the series, the halogen substituted complexes, **95** and **96** experience the greatest emission enhancement and the effects are comparable to non-substituted complex, **75** discussed in the previous chapter. This can be rationalised due to the smaller atomic radii of the halogen atoms when compared to the nitro or amino- substituted analogues and thus, the luminescence enhancement effect is in support of the theory of protection of the Ru(II)-centre due to tighter association of the metal with DNA.

Overall, the optical response of the complexes upon addition of small aliquots of DNA suggest a strong association of the complexes with DNA, with binding constants in the general order of 10^6 M^{-1} . The results show that the **Nap** unit undergoes the most substantial changes in the absorption profiles at increasing DNA concentrations, due to exposure to a new chemical environment upon intercalation between the DNA bases. The photoluminescence quantum yield values of the complexes drastically increased when bound to DNA, attributed to protection of the metal centre from quenching mechanisms upon tight association of the complex with DNA, with the largest emission enhancement response occurring with the halogen substituted complexes.

When compared to nitro- and amino- substituted Ru(II)-**Nap** conjugates, **69** and **70** from the literature, wherein the **Nap** is connected to the metal centre through a rigid aromatic spacer, complexes **94-96** and **99** display similar changes to their **Nap** localised optical absorption bands upon DNA binding. The derived binding constants for **69** and **70** were found to be at least an order of magnitude higher (1.9 and $1.1 \times 10^7 \text{ M}^{-1}$, respectively) than those presented herein, however, these were obtained from fits of the data to the original Bard equation, and not the rearranged form published by our group which is believed to provide a more accurate representation of the binding event.^{97,169,205} In contrast, the emission intensity responses upon DNA binding differed greatly. The maximum emission enhancement from the published systems was observed for complex **70**, which displayed a modest 2.6-fold enhancement of emission in the bound *versus* free form. The complexes presented herein displayed a striking 19- and 34-fold emission enhancement for the halogen-substituted complexes, **95** and **96** indicating the potential applications of these complexes as effective DNA luminescent probes.

The final spectroscopic investigation of the complexes was performed by CD titrations, which analysed the environment of the DNA upon increasing additions of complexes and is presented in the next section.

3.13.3: Circular Dichroism of DNA in the presence of **94**, **95**, and **99**

To probe the interactions of the complexes with DNA, CD studies were carried out in buffered water at constant DNA concentration ($150 \mu\text{M}$, $A_{260} \approx 1$) and P/D ratios of 50, 20, 10 and 5 for the nitro-, bromo- and amino- complexes. Titrations were carried out twice to ensure reproducibility and each trace is an average of three measurements. The results are presented in Figure 3.31, and demonstrate the binding to DNA by these family of complexes does give rise to changes in the chiroptical properties of the complexes, similar to that observed for other **Nap** based systems developed in our group.^{16,168,169}

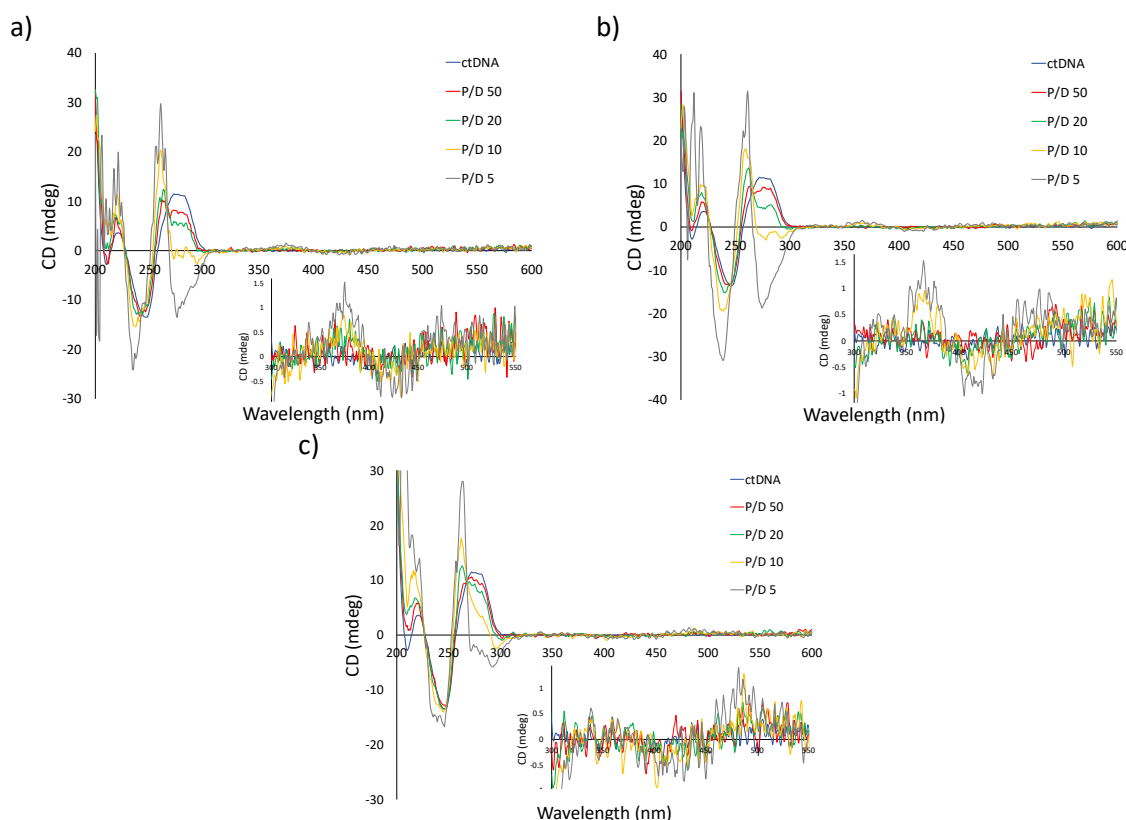


Figure 3.31: Circular dichroism spectra of ctDNA (150 μ M) in buffered water upon addition of a) **94**, b) **95**, c) **99** at different P/D ratios. Inset: region between 320-500 nm displaying an ICD signal of the **Nap**- and MLCT-centred bands of the complex.

All three complexes elicited similar effects on the DNA with the largest changes occurring in the DNA optical window and more minor changes observed at longer wavelength. The characteristic positive DNA centred band at 260 nm is significantly altered indicating both major changes in the conformation of DNA as well as possible induced CD signals from interaction of the ancillary **phen** ligands, which also absorb in this region. At high complex loading, the region where the **Nap** absorbs displays a positive induced CD signal, potentially indicating interaction in the grooves.^{212,213,239} While the exact mode of binding of the complexes with DNA cannot be discerned from this technique on account of a racemic mixture of complexes being present, the appearance of induced CD signals outside the DNA optical window (320-500 nm) provides an indicator that the complexes associate tightly with the DNA helix, as the chirality of the DNA is experienced by the complexes. The regions experiencing the chirality correspond to the absorption bands of the **Nap** IL and MLCT transitions.

3.14: Conclusions and Future Perspectives

This chapter has outlined the synthesis, structural and photophysical characterisation of a series of **PhenNap** ligands bearing substitution in the 4-position of the **Nap**, of which, two ligands **93** and **97** are novel to the literature. While the other two ligands, **91** and **92** had previously been

reported,^{151,221} their solid-state structures were previously unknown. The structural and photophysical properties of the ligands were thoroughly investigated, and preliminary theoretical calculations exposing the orbital distributions of **91** and **97** were provided. The ligands were designed to confer interesting charge transfer properties on four novel bis-phenanthroline Ru(II)-polypyridyl complexes which were also presented in this chapter: **94**, **95**, **96** and **99**. This in-depth study of the ligands provided insight into the associated properties of the complexes.

The bichromophoric Ru(II)-polypyridyl complexes were designed to display both the rich photophysical properties of the Ru(II)-polypyridyl motif, as well as the known anticancer properties of the **Naps** for the purpose of DNA binding experiments. The design placed the **Nap** in close proximity to the metal centre, in order to discern the effects of substitution in the 4-position on the overall properties of the complexes, an area which has not yet been explored for biological purposes. The synthesis, structural and photophysical properties of complexes were presented, as well as the solid-state structures of **94**, **95** and **96**. The structural characterisation revealed the presence of pseudo diastereomers for the complexes, due to asymmetry and rotational restriction between the **phen** and **Nap** motifs. The solid-state structures were useful to discern this observation and also revealed several intermolecular interactions suitable for association with DNA.

The complexes were found to bind DNA strongly, with derived binding constants from the fitting of the spectroscopic changes upon addition of DNA to a modification of the Bard equation, in the order of 10^6 M^{-1} in most cases and binding site sizes of approximately one to two base pairs. An exception to this trend was observed from the fitting of emission data of the nitro-substituted complex, **94** to the equation that return a weaker binding constant in the order of 10^5 M^{-1} (which is still considered moderate for such interactions) and a larger binding site size of four base pairs. The complexes were found to be sensitive luminescent DNA probes, and displayed emission enhancement upon binding DNA, most appreciable for the chloro-substituted complex, **96** which displayed a 34-fold emission intensity enhancement in the bound *versus* the free form.

When compared to similar Ru(II)-**Nap** conjugates bearing substitution in the 4-position of the **Nap** in the literature, the complexes presented herein displayed greater sensitivity to DNA, and greater emission enhancement. Furthermore, the halogen complexes are the first of their kind to be analysed for DNA binding. Overall, the complexes discussed herein display a range of attractive properties that make them excellent candidates for DNA probes and DNA-reactive agents. Future work on the ligands and complexes includes optimisation of the synthesis of the amino-derivatives **97** and **99**. Further structural data, electrochemical and DNA binding studies should be performed

on the complexes, where necessary, and include electrochemical investigation of **96** and **99** and full structural characterisation of **99** should be obtained.

Due to the asymmetry of the complexes, enantiomer resolution is highly desirable. The effect on the stabilisation on different DNA sequences should be explored by thermal denaturation experiments. Furthermore, cellular uptake and toxicity studies are warranted for the complexes due to these high binding affinities and their potential as photoreactive agents should be explored.

Chapter 4:

Toward the Development of
Conjugated **Nap** Derivatives
and Associated Metal Ion
Complexes

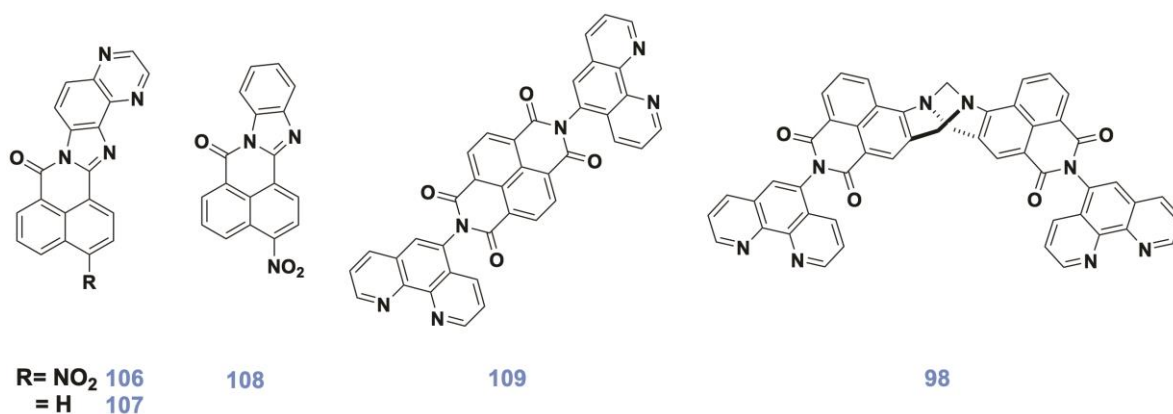
4.1: Introduction

The design and synthesis of small molecules capable of selectively targeting, and binding to nucleic acids, remains an important focus in the field of medicinal chemistry. One of the early aims of this research project was the development of conjugated benzimidazole-**Nap** and **TB** derivatives, primarily for use as ligands for Ru(II)-polypyridyl complexes, which were envisaged to bind to DNA. The synthesis toward the development of novel ligands performed during this research is presented in this Chapter. Furthermore, efforts towards the development of a dinuclear Ru(II)-complex, wherein the metal centres are connected by **TB-Nap** linking motifs is furthermore presented. Finally, this chapter also presents the synthesis and photophysical characterisation of a novel Ir(III)-**Nap** complex, which is structurally similar to the Ru(II) complexes discussed in Chapter 2. This metal complex, substituted with ligand **80**, was designed to analyse the effect of metal substitution on the overall DNA binding and luminescence sensing properties observed for complexes **75** and **76**. Unfortunately, due to the reduced overall charge of the complex (+1) (*versus* that seen in Chapter 2 and 3), good water solubility was not observed, and as such, these studies were not performed. The synthesis toward a bis-**NBI** cyclometalated Ir(III)-**dppz** analogue is also presented, which was designed to analyse the effect of the extended aromatic ligands on the overall photophysical properties of the system.

4.1.1: **Naps** and **Nap**-Derivatives as Ligand Scaffolds

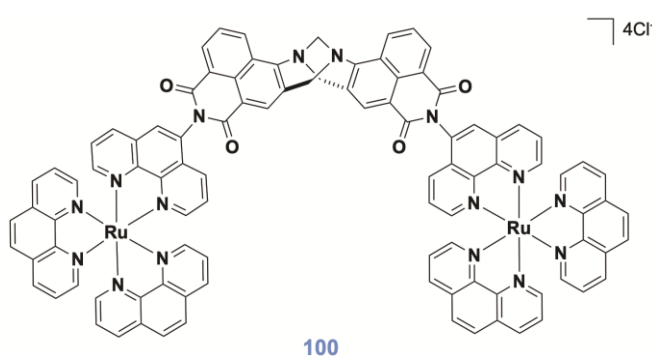
The use of **Nap** derivatives, including the 1,8-naphthalenebenzimidazole (**NBI**) and naphthalenediimide (**NDI**) motifs as ligand scaffolds was introduced in the Chapter 1, (*vide supra*, Section 1.5.3). Derivatisation of these **NBI** and **NDI** structures allow for many attractive photophysical outcomes of their associated metal complexes, such as fine-tuning of the emission energy maxima, excited-state lifetime and quantum yield values, as well as redox potentials.¹⁵³ As such, the development of functionalisable novel organic molecules, capable of coordinating metal ions, is an important and fast growing field of research.

An introduction to the **TB** motif was also provided in Chapter 1 (*vide supra*, Section 1.6) which, due to its intrinsic chirality, can be employed in the field of molecular recognition and sensing, making it an attractive motif in the field of supramolecular chemistry (*vide infra*, Chapter 5).^{157,161,163,229} In this Chapter, the synthesis toward the development of novel organic **NBI**, **NDI** and **TBNap** structures is presented, which were designed with the aim of i) investigating the associated photophysical characteristics of the ligands, and ii) developing metal-based complexes of these systems and investigating the interactions of these complexes with DNA. Examples of some of the ligands designed in this regard are presented in Figure 4.1.

Figure 4.1: Chemical structures of ligands **98**, **106-109**.

4.1.2: Dinuclear Ru(II)-Polypyridyl Complexes

The development of Ru(II)-polypyridyl complexes for DNA binding and anticancer therapies has been discussed in detail in the preceding chapters. In addition to the work presented in this thesis thus far, which primarily focused on the development of Ru(II)-**Nap** conjugates, another area of research that our group has explored is the development of **TB** functionalised Ru(II)-polypyridyl complexes for anticancer therapies.^{154,240} As such, efforts towards the synthesis of complex **100** was undertaken and the results are presented herein. Previously, the Gunnlaugsson group has developed examples of such Ru(II)-**TBNap** conjugates, using **bpy** coordinating motifs and has shown them to be excellent light activated therapeutics (unpublished). The group also extended this work towards the formation of **tpy** derivatives.¹⁶¹ However, these examples all contained an orthogonal aryl spacer between the **TBNap** unit and the polypyridyl structure. Hence, it was anticipated that **100**, which lacks that aryl spacer, would provide an alternative means of binding such dinuclear Ru(II) systems to nucleic acids.

Figure 4.2: Chemical structure of dinuclear Ru(II)-complex **100**.

4.1.3: Cyclometalated-Ir(III) Complexes

The final class of compounds investigated in this Chapter are cyclometalated Ir(III)-complexes, bearing **Nap** and **NBI** ligands. Complexes of this kind, including **50** (*vide supra*, Section 1.5.3), have been shown to display high Φ_{PL} values approaching 30% and long-lived excited state lifetime values in the microsecond regime, making them attractive candidates for use in, for example,

photochemical reactions.¹⁵³ Traditionally, the applications of these complexes have been explored primarily in the fields of optoelectronics and photocatalysis, due to their remarkable colour tunability (from near-IR to near-UV phosphorescence) and other attractive luminescence properties.^{153,241-244} More recently, research has shifted toward expanding the applications of these complexes, such as by improving their water solubility for aqueous electron transfer chemistry,²⁴⁵ and by exploring the biological applications as luminescent anticancer agents.¹⁷⁶ In this regard, complexes **110** and **111** were envisaged as potential targets of interest.

Efforts toward the development of **110** are first presented herein, followed by a discussion of the synthesis and the preliminary photophysical evaluations of **111**. Figure 4.3 displays the chemical structures of the Ir(III)-complexes investigated in this chapter.

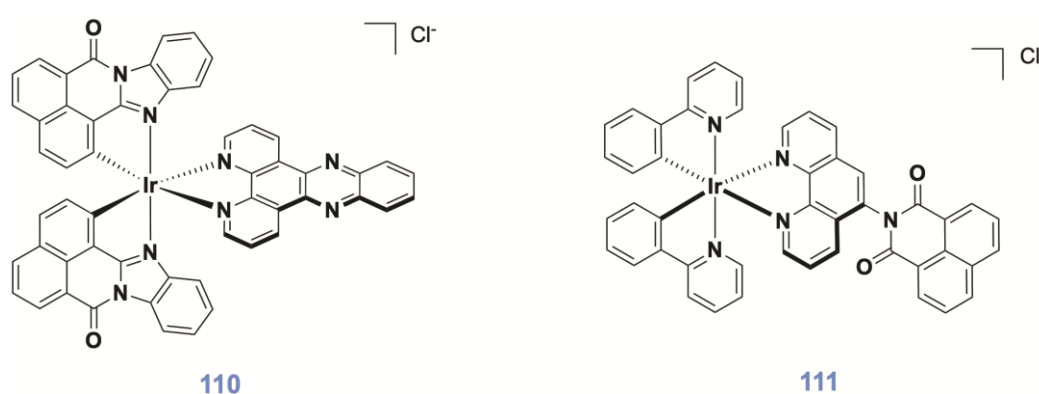


Figure 4.3: Chemical structures Ir(III)-complexes **110** and **111**.

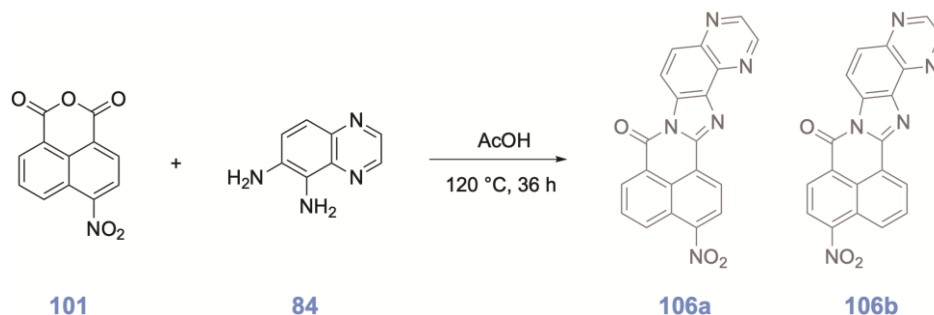
4.2: Attempted Synthesis of **Nap** Derivatives with Extended Conjugation

The benzimidazole-**Nap** derivatives, **106** and **107**, were designed with the nitro-group in the 4-position of the naphthalene ring. It was anticipated that the electron withdrawing nature of this group would confer its own set of electronic properties on the ligands, and furthermore, the nitro-group could act as a functional handle for the development of other derivatives of the compounds, such as their corresponding amino-forms, which would display distinct properties from their nitro-analogues. Other benzimidazole-**Nap** derivatives, such as the use of 4-chloro- or 4-bromo-1,8-naphthalic anhydrides (employed in Chapter 3) as starting materials, were also considered due to their ability to induce ISC due to heavy atom affect.

4.2.1: Attempted Synthesis of Benzimidazole-**Nap** Derivatives, **106**, **107** and **108**

Compound **106** was designed as a ligand for Ru(II)-complexes, which was anticipated to bind in a bidentate fashion, through coordination the pyrazinyl- and benzimidazole nitrogen atoms. The synthesis of **106** was attempted according to a modified literature procedure reported by Dai *et al.* which described the synthesis of a series of nitro-substituted asymmetric **NBIs** as effective sensors

for hypoxic tumour cells.²⁴⁶ The reaction involved the condensation of 4-nitro-1,8-naphthalic anhydride, **101**, with 5,6-diaminoquinoxaline, **84** in acetic acid in a pressure vessel at 120 °C [Scheme 4.1]. The reaction was monitored by TLC where complete consumption of the starting material was observed after 3 days.



Scheme 4.1: Synthesis of quinoxaline-**Nap** **106**.

The solvent was removed under reduced pressure and the resulting solid product was neutralised by washing with sodium bicarbonate and water, before subsequently drying *in vacuo*. The brown solid was suspended in DCM, where partial solubility of half of the mass was observed. Flash column chromatography was performed on the fraction which displayed solubility in DCM, (SiO₂, DCM:MeOH 0–10%). APCI-DIP HRMS confirmed the presence of **106** in both- the insoluble material, and in the major fraction isolated by chromatography [Figure A4.1]. ¹H NMR analysis (600 MHz, DMSO-*d*₆) of the insoluble fraction displayed a clean product, with broadened signals [Figure 4.4] and subsequent analysis of the HSQC revealed 18 CH signals [Figure A4.2], corresponding to twice the number expected. As such, it is hypothesised that two isomers were present, **106a** and **106b**, where the condensation on the external imide sites by **84**, results in the formation of these isomers. At this stage, it was thought to be unlikely that other isomers could be formed, such as where the quinoxaline was ‘faced inwards’ (see next discussion) due to electronic repulsion between the nitrogen moiety and the carbonyl. Nevertheless, this could not be fully excluded.

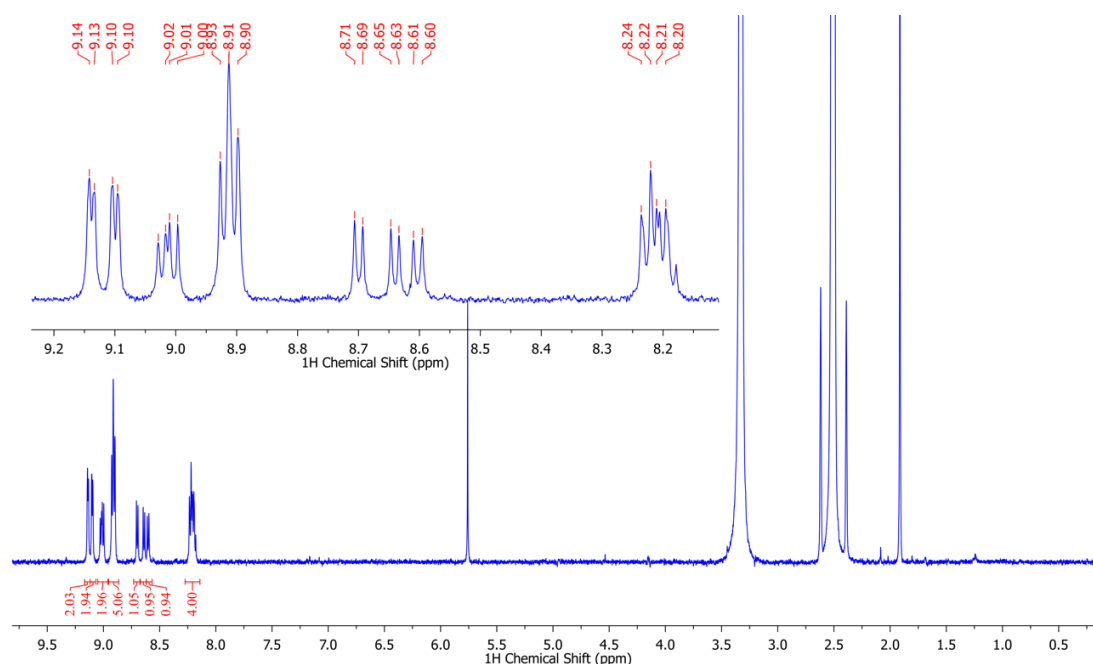
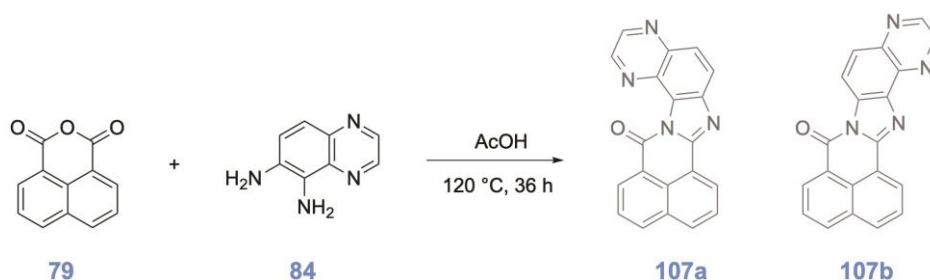


Figure 4.4: The ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$) **106**.

Regrettably, the synthetic paper published by Dai *et al.* did not describe the purification steps of any of the **NBI** derivatives, nor did it mention the formation or separation of any potential isomers.²⁴⁶ The synthesis of **106** was therefore investigated using alternative methods employed for the synthesis of **NBI** derivatives in the literature, including mechanical solid-state synthesis, and direct sublimation, which only returned starting material and decomposed products, respectively.^{247,248}

As mentioned above, and from the reaction outlined in Scheme 4.1, four structural isomers of **106** could theoretically be formed; these being **106a**, **106b**, and the two isomers wherein the pyrazine nitrogen and imide oxygen atom are in close proximity (*i.e.* ‘faced inwards’). Subsequently, the synthesis of the non-substituted analogue, **107** was investigated [Scheme 4.2] to probe if such isomers could indeed be generated. This reaction was performed under analogous conditions to those investigated for the 4-nitro derivative above, and ^1H NMR analysis indeed returned similarly broad peaks, displaying overlapping multiplet signals, again, potentially indicating the formation structural isomers, **107a** and **107b**.



Scheme 4.2: Synthesis of quinoxaline-**Nap** **107**.

In order to investigate the source of this issue, complexation reactions were performed with Cd(II) and Zn(II) salts, as it was anticipated that the **107b** isomer would bind preferentially, and that the structures could be investigated by single crystal X-Ray diffraction. To achieve this, **107** (10.0 mg, 31 μmol) and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (9.6 mg, 31 μmol) were added to DMF:H₂O (2:1, 3 mL) and were placed on a heating block element at 80 °C for 14 h. Concurrently, **107** (10.0 mg, 31 μmol) and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (9.2 mg, 31 μmol) was subject to the same synthetic conditions. In both cases, a crystalline material was observed after cooling the mixture to room temperature; however, the crystals were not of adequate size for X-ray diffraction experiments and instead, the crystalline material was isolated by filtration and analysed by ^1H NMR [Figure 4.5].

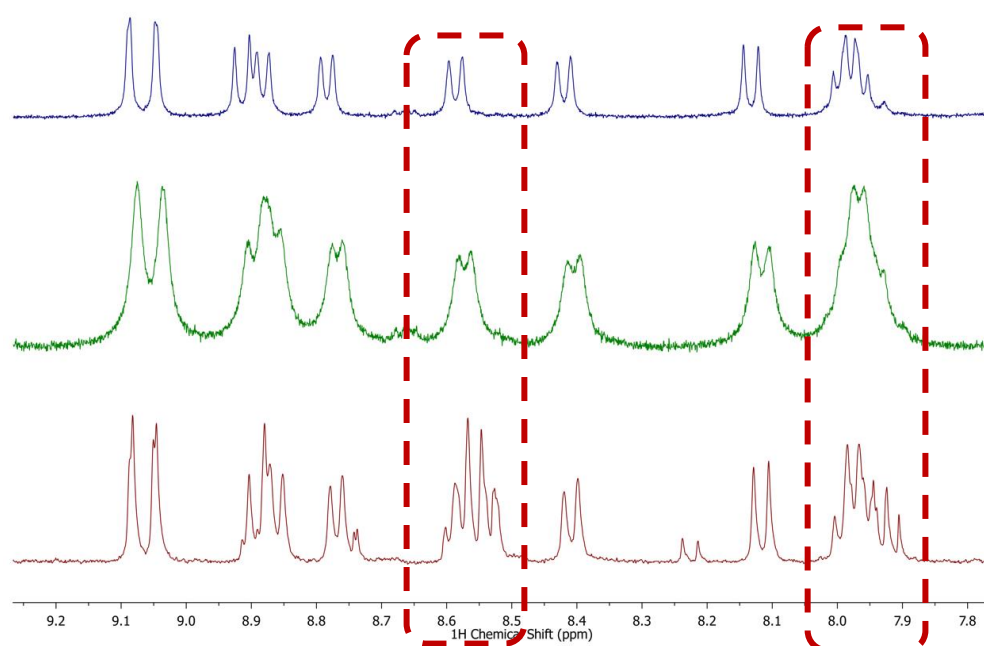


Figure 4.5: The ^1H NMR spectrum (400 MHz, DMSO- d_6) of **107** (red, bottom), **107** + $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (green, middle) and **107** + $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (blue, top).

The ^1H NMR spectra of **107** obtained after the crystallisations displayed minor changes, mainly in the regions where substantial overlap occurred in the crude spectra. Furthermore, the integration of the spectra after crystallisation returned 10 protons, as expected from compound **107**. Similar experiments were also performed for **106** and the results can be viewed in the appendix [Figure A4.4].

Based on the above outcomes, the synthesis of the simpler **NBI** derivative, 4-nitro-**NBI**, **108** was also investigated; but related 4-chloro systems are known in the literature as intermediates in the formation of 4-amino derivatives, which have been employed, for instance in pH **PET** sensing by de Silva *et al.*^{249,250} While compounds **106** and **107** were designed to coordinate Ru(II)-metal ion complexes in a typical **N^N** bidentate fashion, complex **108** was designed to chelate Ir(III)-metal ions in a **N^C** bidentate cyclometallating mode. The attempted synthesis of **108** was performed by

condensation of *o*-phenylenediamine, **112**, and 4-nitro-1,8-naphthalic anhydride, **101** and is summarised in Scheme 4.3.

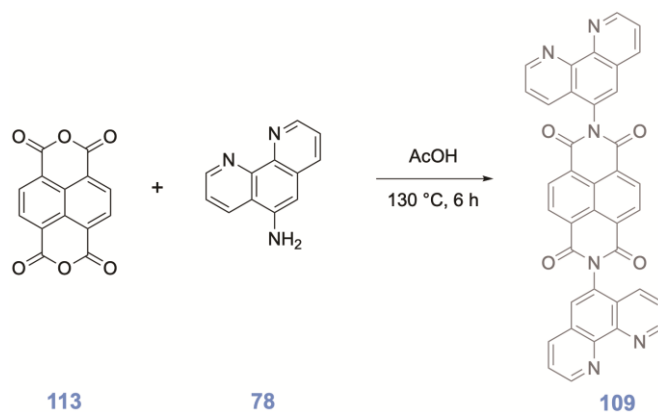


Scheme 4.3: Attempted synthesis of 4-nitro-**NBI** **108**.

Unfortunately, the structural analysis again, proved inconclusive, and the ^1H NMR spectrum displayed many broad and overlapping peaks, indicating a mixture of products [Figure A4.5]. As such, the pursuit of the **NBI** derivatives substituted in the 4-position of the **Nap** unit was not continued, and known structures, such as **NBI** and the **dppz** ligands were instead synthesised for metal complexations (*vide infra*, Section 4.4).

4.2.2: Attempted Synthesis of **Phen-NDI** Ligand, **109**

As mentioned in the introduction, the development of ligands displaying a bis-coordination motif was a desirable outcome of this project. In this regard, the **NDI** ligand, **109** was designed, utilising the **phen** coordination motif, which was employed in previous studies **Nap** structures in this research. The synthesis of **109** was attempted by reaction of naphthalenetetracarboxylic dianhydride, **113**, and 5-aminophenanthroline, **78** in CHCl_3 using a method previously developed by our group for the for the condensation of aromatic amines with the 1,8-**Nap**.¹⁶³ However, this proved unsuccessful and returned a red/brown oil after removal of the solvent, which was subsequently recrystallised from acetone and a yellow precipitate was isolated which contained a mixture of products [Figure A4.6]. The reaction conditions were changed to employ acetic acid as the solvent, as was previously successful for the synthesis of **PhenNap** ligands in previous chapters [Scheme 4.4].



Scheme 4.4: Attempted synthesis of **NDI** **109**

While the successful formation of the product **109** was again, inconclusive, the ^1H NMR analysis did indicate the formation of a clean product with proton signals integrating to 18, consistent with the chemical formula [Figure 4.6]. HSQC analysis displayed a total of 9 CH signals, indicating the C_2 symmetry of the molecule; however, the unique proton signals may appear due to the asymmetric nature of the coordinating **phen** motif, which, as was observed in the solid state analysis of the structurally related **Naps**, becomes “locked” in place due to the steric clash induced by the imide oxygen atoms. The ^{13}C NMR spectrum is also included [Figure A4.8] which displayed many overlapping signals.

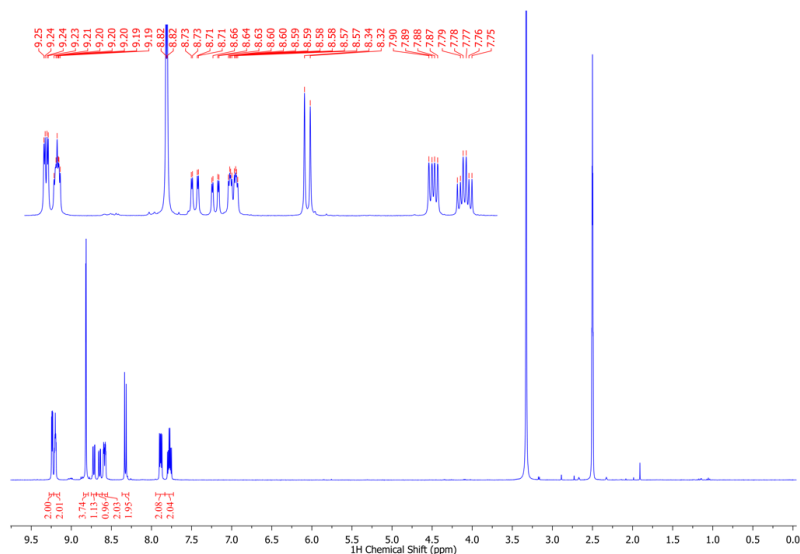
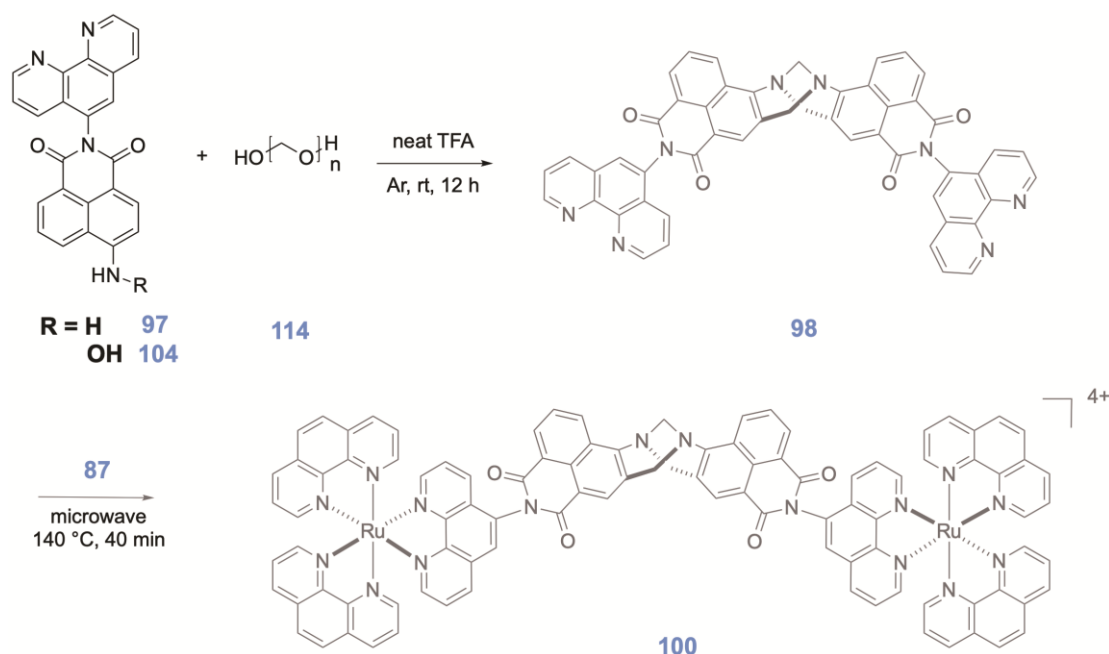


Figure 4.6: The ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$) of **109**.

This observation further highlights the synthetic difficulty of employing the coordinating **phen** motif in such close proximity to the body of the structural unit, in this case, the **NDI**. Indeed, synthetic struggles were also encountered when attempting the synthesis of the **TB** compound, **98**, containing the **phen** coordinating unit at the *N*-imide position, which will be discussed in the next section.

4.3: Attempted Synthesis of **TB**, **98** and Dinuclear Ru(II)-Polypyridyl Complex, **100**

Chapter 3 outlined the difficulties encountered upon attempts to reduce the nitro- substituted **PhenNap** ligand, **91** to the corresponding amine, **97** (vide supra, [Scheme 3.2]). Since a large amount of material was generated from these multiple attempts, the products from the reactions that produced a mixture of the reduced, and partially reduced species, **97** and **104**, were retained and the material was combined. This material was then employed for the attempted synthesis of the desired **TBNap** **98**, as shown in Scheme 4.5.

Scheme 4.5: Attempted synthesis of **98** and **100**.

The synthesis of **98** was attempted by reacting the crude mixtures of **97/104** with paraformaldehyde, **114**, in neat TFA (5 mL) for 12 h under inert atmosphere. The solution was then basified to pH 9 using 6 M NaOH upon which a colour change from yellow to dark orange was observed. Brine was added (20 mL) to dilute the product before extraction into EtOAc was attempted; however, the product remained in the aqueous phase. The mixture was then subject to centrifugation (4000 rpm for 15 min) and the organic layer was decanted. The aqueous solvent was removed under reduced pressure and a hygroscopic yellow crystalline material was obtained which became viscous upon exposure to the atmosphere. The hygroscopic material was dried in a desiccator containing P_2O_5 and was then stirred in dry MeCN to remove excess salt, after which, the solution was passed through a syringe filter (0.45 μm) and the solvent removed under reduced pressure. The resulting material retained the hygroscopic properties and as such was kept under inert atmosphere.

Typically, successful formation of **TBs** is determined by the presence of six protons, which appear as two doublets, and one singlet, in the region of 4.60–5.30 ppm, corresponding to the protons of the methanodiazocine ring.²²² This splitting pattern was not observed in this case and instead, the appearance of one doublet and one multiplet signal were observed in the same region [Figure 4.7].

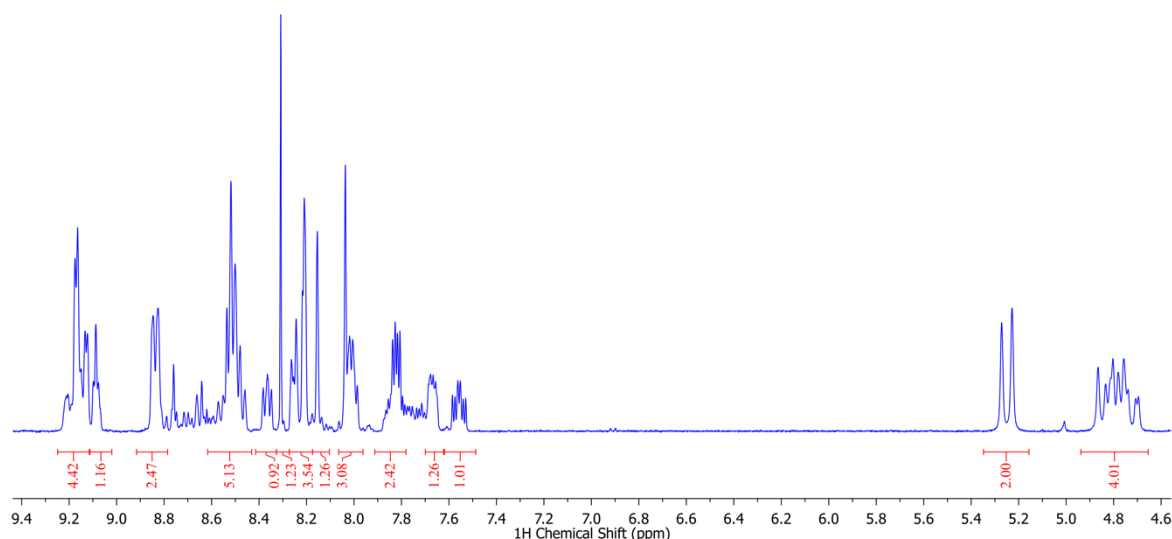


Figure 4.7: The ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$) of **109**.

HRMS analysis confirmed the presence of **98** [Figure A4.9] with detection of the $[\text{M}+\text{H}]^+$ ion and as such, the crude product was reacted in excess with $[\text{Ru}(\text{phen})_2\text{Cl}_2]$, **87** according to the conditions described in General Experimental Procedure 3 in an attempt to isolate the Ru(II)-complex, **100**. Following the reaction [Scheme 4.5], a deep-red solution was obtained and the solvents were removed under reduced pressure. The crude product was subject to column chromatography (SiO_2 , $\text{MeCN}:\text{H}_2\text{O}:\text{NaNO}_3(\text{aq.})$ 40:4:1) and three major fractions were isolated. The ^1H NMR spectra of these fractions [**100(F1)**, **100(F2)** and **100(F3)**] were obtained [Figure A4.10], each of which displayed multiple aromatic signals. The samples were subsequently converted to the PF_6^- salts by precipitation from a saturated aqueous solution of NH_4PF_6 , before isolating the solids by filtration.

In order to discern the structures of the isolated fractions, single crystals suitable for X-ray diffraction experiments were obtained by slow diffusion of antisolvents into solutions of the complexes in MeOH or MeCN. Unfortunately, none of the structures represented the desired product and, instead, solvated $[\text{Ru}(\text{phen})_2\text{L}_2]^{2+}$ side products were observed [Figure 4.8]. Interestingly, the solid-state structure of **100(F1)** was found to be a *trans*- $[\text{Ru}(\text{phen})_2]^{2+}$ isomer, indicating that some form of ligand rearrangement occurred during reaction. The crystal structure analysis of **100(F2)** and **100(F3)** displayed *cis*- $[\text{Ru}(\text{phen})_2]^{2+}$ complexes, with solvents and counterions completing the coordination environment. A ball and stick model of the major occupied cationic species within the asymmetric unit for each of the fractions is displayed [Figure 4.8].

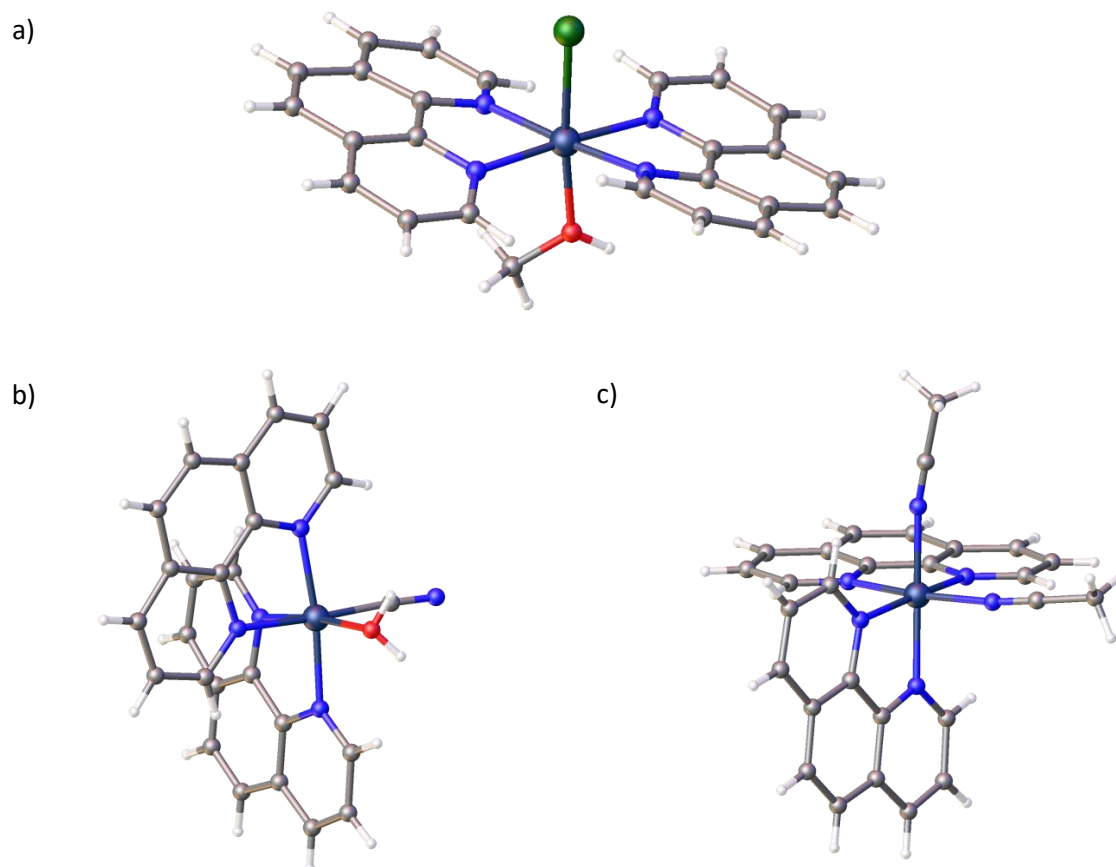
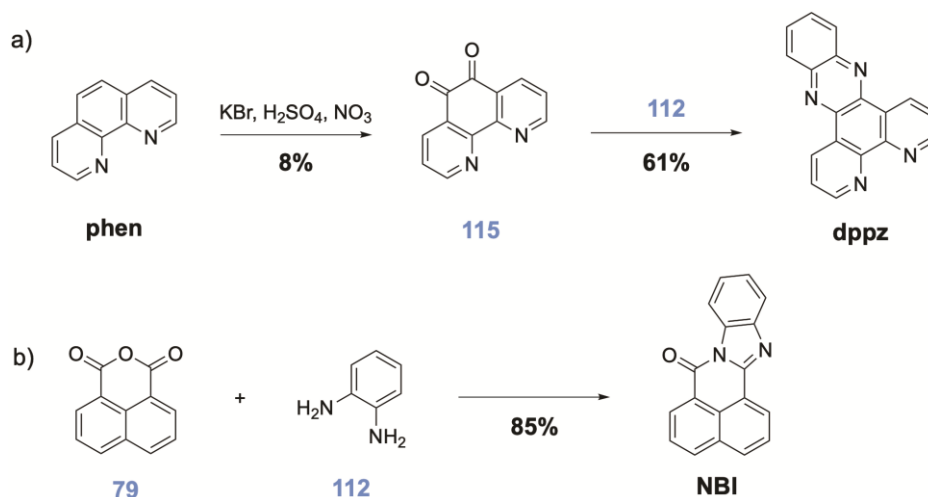


Figure 4.8: Ball and stick model of the major occupied cationic species present in the asymmetric unit of (a) **100(F1)**, (b) **100(F2)** and (c) **100(F3)**. Full details corresponding to the structural and experimental data can be found in the supplementary appendix section accompanying this thesis.

Throughout the course of this research project, a number of attempts were made to expand knowledge in the fields of chemistry outlined above. Unfortunately, many of the synthetic projects proved difficult to achieve, due to poor solubility of the products in conventional solvents, resulting in difficult purification steps and challenging analyses of the products, potentially due to the possible structural isomers formed. As such, efforts toward the development of novel Ir(III)-complexes bearing known aromatic ligands were next investigated.

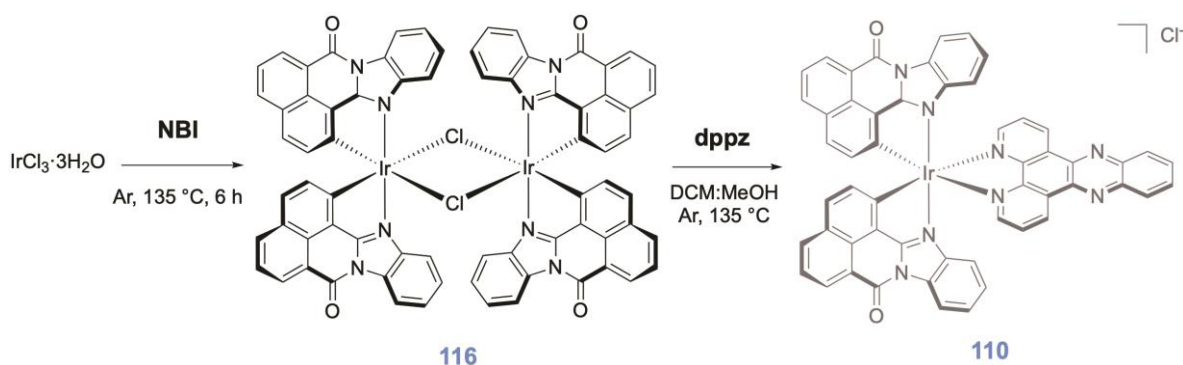
4.4: Synthesis and Photophysical Evaluation of Ir(III)-Complexes

This section first introduces the attempted synthesis of a bis-**NBI**-cyclometalated Ir(III)-**dppz** complex, **110** and is followed by a discussion of the synthesis and photophysical characterisation of the Ir(III)-**Nap** complex, **111**. The **NBI** and **dppz** ligands were obtained by following a multistep synthesis published in the literature and are summarised in Scheme 4.6.

Scheme 4.6: a) Synthesis of **dppz** and b) **NBI** ligands.

First, 1,10-phenanthroline-5,6-dione, **115**, was obtained from functionalisation of **Phen** according to literature procedures and was obtained in 8% yield.^{251,252} Compound **115** was then subject to condensation reaction with *o*-phenylenediamine, **112**, to yield the **dppz** ligand in 61% yield, again, according to known literature procedures.^{253,254} The **NBI** ligand was obtained from condensation of **112** with naphthalic anhydride, **79**, in acetic acid and was obtained in 85% yield.^{153,247} Full experimental details and structural characterisation is presented in Chapter 7 with the ^1H NMR spectra presented in the appendix [Figures A4.11 and A4.12].

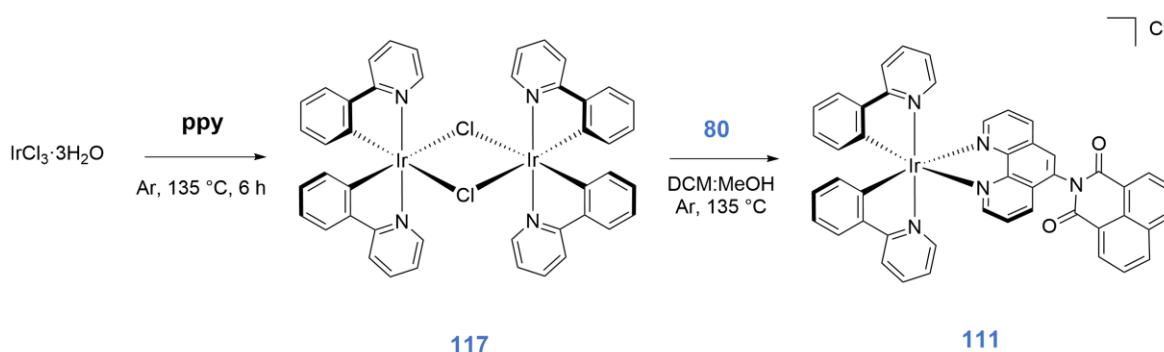
Having synthesised the **dppz** and **NBI** ligands, next an investigation into the synthesis of the corresponding Ir(III)-complex, **110** was attempted [Scheme 4.7].

Scheme 4.7: Attempted Synthesis of Ir(III)-complex **110**.

The Ir(III)-dimer, **116**, was synthesised according to General Procedure 4 by reacting the **NBI** ligand (169 mg, 0.6 mmol, 2.2 eq.) with $\text{IrCl}_3 \cdot 3\text{H}_2\text{O}$ (100 mg, 0.3 mmol, 1.0 eq.) in 2-ethoxyethanol: H_2O (3:1, 12 mL) at 135 °C for 6 h under inert atmosphere. The resulting mixture was diluted with water (15 mL) and collected by filtration. The red precipitate was subsequently washed with H_2O :EtOH (1:1, 10 mL), acetone (30 mL), and hexanes:Et₂O (1:1, 20 mL), before drying *in vacuo*. The dimer was then used directly in the next step, as is common for the syntheses of these types

of complexes.^{243,255} Complex **116** (50 mg, 0.03 mmol, 1.0 eq.) and **dppz** (20 mg, 0.07 mmol, 2.2 eq.) were added to DCM:MeOH (1:1, 10 mL) and the mixture was flushed with Ar for 10 min. The mixture was heated at reflux overnight and upon completion of the reaction, was cooled to room temperature. The solvent was removed under reduced pressure and the resulting crude product was subjected to column chromatography SiO₂, DCM:MeOH (0–5% MeOH)] and the product was isolated as a yellow powder. The ¹H NMR and HRMS analyses suggested formation of the product; however, the ¹H NMR spectrum indicated the presence of baseline impurities and the need for further purification [Figure A4.13 and A4.14]. This reaction was carried out on a small scale and it was therefore not practical to perform another purification attempt. However, concurrently to this reaction, the synthesis of the Ir(III)-**Nap** derivative was also being performed and will be discussed next.

As was mentioned, the Ir(III)-**Nap** complex, **111**, was designed as a structural analogue to complexes **75** and **76** discussed in Chapter 2. The synthesis of complex **111** was performed as outlined in Scheme 4.8.



Scheme 4.8: Synthesis of Ir(III)-**Nap** **111**.

First, the Ir(III)-dimer, **117**, was synthesised according to General Procedure 4, employing 2-phenylpyridine, **ppyH**, as the cyclometalating agent. To achieve this, IrCl₃·3H₂O (162 mg, 0.5 mmol, 1.0 eq.) and **ppyH** (157 mg, 1.0 mmol, 2.2 eq.) were added to 2-ethoxyethanol:H₂O (3:1, 12 mL) and the mixture was degassed for 15 min. The mixture was heated at reflux for 6 h after which a yellow precipitate was observed. The precipitate was collected by filtration, washed with H₂O:EtOH (1:1, 50 mL), hexanes:Et₂O (1:1, 50 mL) and dried *in vacuo*. Compounds **117** (100 mg, 0.1 mmol, 1.0 eq.) and **80** (77 mg, 0.2 mmol, 2.2 eq.) were then added to degassed DCM:MeOH (1:1, 10 mL) and the mixture was heated at reflux under inert atmosphere overnight. The mixture was cooled to room temperature and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (SiO₂, DCM:MeOH (0–2% MeOH)) and isolated as an orange crystalline material (154 mg, 0.2 mmol, 93%). NMR analysis confirmed the product in high purity and further confirmation of the desired product was obtained by HRMS analysis and other techniques [Figures A4.15–18].

Preliminary photophysical measurements were performed on complex **111** and the UV-vis absorption, and the excitation and emission spectra for the complex in MeCN are shown in Figure 4.9. The molar absorptivity values, calculated using the Beer–Lambert Law, for the major absorption bands of **111** are given in Table 4.1 with those values obtained for complexes **75** and **76** included for comparison.

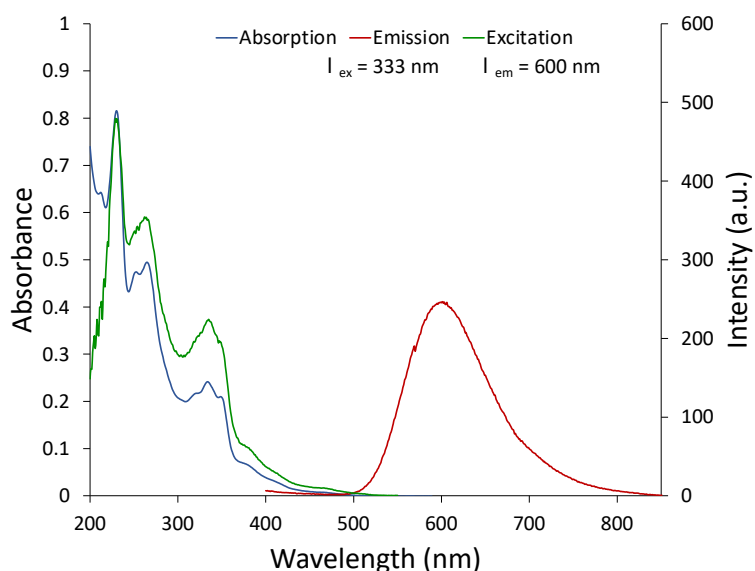


Figure 4.9: Absorption, excitation and emission spectra of **111** ($\lambda_{\text{ex}} = 333$ nm, $\lambda_{\text{em}} = 600$ nm, 8.9×10^{-6} M) in MeCN at 298 K.

Complex **111** showed similar absorption bands in MeCN when compared to complexes **75** and **76**. The molar absorptivity constants for the π – π^* centred transitions of the **ppy** and **Nap** were comparable; however, complex **111** showed much weaker MLCT based transitions which is common for Ir(III) complexes [Table 4.1]. As was observed for the Ru(II)-complexes, the structured bands corresponding the **Nap** based transitions are present. The excitation spectrum also mirrors the absorption spectrum, which shows that the photophysical properties of the complex obey Kasha's Rule.

Table 4.1: Absorption and emission properties of **111**, **75** and **76** at 298 K in MeCN.

Absorption $\lambda_{\text{max}} / \text{nm}$ ($\epsilon / 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) ($\pm 10\%$)				Emission	
Complex	π – π^* IL	π – π^* IL	MLCT	$\lambda_{\text{max}}^{\text{em}} / \text{nm}$	$\Phi_{\text{PL}}^{[\text{a}]}$ air / %
116	230 (9.1), 262 (5.5)	334 (2.7)	380 (0.7)	609	2.1
75	264 (9.4)	331 (1.5)	450 (1.6)	603	1.0
76	273 (6.0)	346 (1.6)	462 (1.2)	624	3.8

^[a] An aerated aqueous solution of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ ($\Phi_{\text{r}} = 2.8\%$) was used as the external reference.^{198,200}

The emission characteristics of the complex were also analysed and the complex was found to display a broad and unstructured emission band centred at 609 nm in MeCN. Excitation into the absorption bands centred at 230, 262 and 333 nm resulted in the same emission centred at 609 nm; however, the later displayed slightly more intense emission than those at higher energy. This was somewhat counterintuitive, as the higher energy bands displayed higher molar absorptivity constants and as such, it was expected the excitation into those bands would result in more intense emission [Figure 4.10].

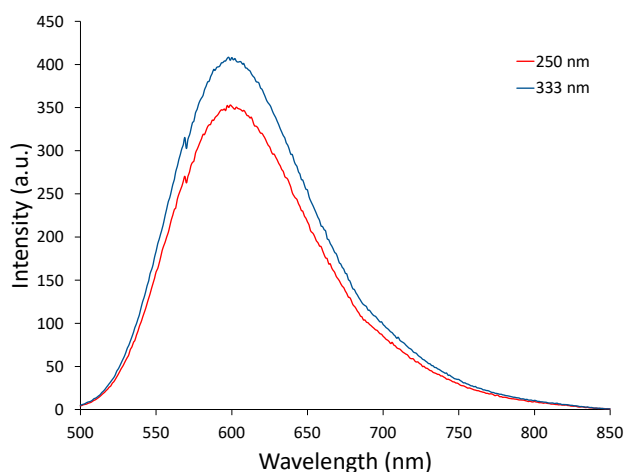


Figure 4.10: Emission enhancement from excitation of **111** at 333 nm when compared to 250 nm (1.5×10^{-5} M).

Furthermore, the Φ_{PL} value was determined in aerated MeCN using the optically dilute method as described in Chapter 2 and was again found to be comparable to the complexes **75** and **76**.¹⁹⁷⁻²⁰⁰

4.5: Summary

Overall, this chapter outlined a range of syntheses attempted during the initial stages of the project. Many of these syntheses proved challenging, with the structures presented in grey scale for those products whose formation was inconclusive. It is hoped that this report will assist in other researchers' pursuit of structurally similar ligands in the future. Furthermore, the development of two Ir(III)-complexes was presented, which warrant further investigation into their photophysical properties, such as their photoluminescence lifetime values and the general properties in different solvents. It would also be desirable to functionalise the ligands on these complexes with polar groups to increase water solubility for biological applications, which is an emerging field of research for the Ir(III)-cyclometalated complexes.²⁴⁵

Chapter 5:
X-Ray Crystal Structure
Analysis of **Nap** Derivatives
and Eu(III)-Helicates

5.1: Introduction

The field of supramolecular chemistry seeks to understand the governing forces at play between molecular assemblies, which are controlled by intermolecular bonds.²⁵⁶ Also described as the “chemistry beyond the molecule,” supramolecular chemistry exists as an established field of research that focuses on understanding and controlling the forces which govern the assembly of highly ordered structures.²⁵⁷ The term was modernised by Jean-Marie Lehn, who alongside Pederson and Cram were awarded the Nobel Prize for their roles in recognising the importance of the chemistry which exists outside of the covalent bond.²⁵⁸ Currently, research in this field is plentiful and includes host-guest chemistry, self-assembly interactions, templated syntheses, and solid-state studies.²⁵⁸ Applications of the field span many areas of chemistry and chemical biology, and are observed throughout the natural world. This Chapter seeks to utilise the powerful technique of single crystal X-Ray diffraction experiments to understand some of the supramolecular interactions between systems studied within the Gunnlaugsson group. A brief context of the work presented in this Chapter is next outlined, followed by an introduction to relevant literature and finally, a discussion of the supramolecular interactions analysed by this technique.

5.1.1: Context of the Work Presented in this Chapter

The research presented in this thesis thus far was highly focused on the development of **Nap** ligands and corresponding Ru(II)-polypyridyl complexes as DNA targeting agents and luminescent probes, which indeed, are examples of some supramolecular interactions. The Gunnlaugsson research group is highly interdisciplinary in nature, with major research focuses in the fields of medicinal chemistry,² metal-templated synthesis,²⁵⁹ aggregation induced emission (AIE),¹⁶¹ and materials chemistry.²⁶⁰ Through collaboration with other Gunnlaugsson group members whose main research goals reside in these fields, many side projects have evolved over the past number of years, some of which are presented in this Chapter.

Following training in the field of single crystal X-ray diffraction by Dr Brendan Twamley in TCD, several crystal structures were solved for multiple group members. The structures which are related to the **Nap** motif (**C1-C8**, [Figures 5.1 and 5.2]) are presented in this Chapter, with other service structures presented as Figures in the Appendix section [Figures 5.9 – 5.16]. Furthermore, the solid-state structures of two novel enantiopure Eu(III)-triple stranded helicates were resolved, which will also be presented. Due credit and acknowledgements are provided below with further mention in the relevant sections to those researchers who contributed toward the results. The synthesis and crystallisation of **C1-C10** were performed by the following Gunnlaugsson group members:

C1 and **C4**: PhD student Laura Ramírez Lázaro.

C2, **C7** and **C8**: Dr Adam Henwood.

C3: Senior Sophister student Caoimhe Beirne.

C5: PhD student Louisa Sigurvinnsson.

C6: Visiting PhD student Alyssa Spear, Doyle group (Syracuse University, New York).

C9 and **C10**: Dr Oxana Kotova.

Additionally:

Figure 5.19 was provided by Dr Oxana Kotova.

Structure refinement of the helicate data obtained from single crystal X-Ray diffraction experiments of **C9** and **C10** were assisted by Dr Brendan Twamley.

Several other structures were collected and solved for multiple members of the Gunnlaugsson group, which will not be discussed in this Chapter. Details of these experiments and graphical representations of the structures are presented in the appendix Figures A5.10–A5.17.

Overall, the research presented in this Chapter seeks to contribute toward the understanding of the relevant forces that account for supramolecular interactions of i) **Naps** and ii) chiral lanthanide complexes by employing single crystal X-Ray diffraction techniques. This technique is a powerful tool that can provide structural information about the chemical make-up of a molecule, including bond lengths and bond angles, but also about the interactions that govern their formation into highly ordered, crystal lattices. Specific analysis of the interactions between molecules in their extended structures can provide a wealth of information about their supramolecular properties.²⁶¹

5.1.2: Supramolecular Chemistry of **Naps**

Solid-state structural analysis of **Naps** can provide a wealth of information about their electronic and photophysical properties, such as their sensing and fluorescence properties.^{20,189,262} For example, the ICT emission property displayed by 4-amino **Naps** has recently been observed by single crystal X-ray diffraction experiments. Herein, two major resonance forms of the 4-amino **Nap** was observed in the solid state, which displayed different N–C bond lengths between the amine donor and **Nap** core of 1.357(2) and 1.460(2) Å, corresponding to double- and single-bond character, induced by the electron donating amine and electron withdrawing imide.²⁶³ Furthermore, the solid-state analysis of **Naps** bearing large, bulky, aromatic groups can provide direct information about other fluorescent phenomena, such as aggregation induced emission (AIE) properties.^{189,264}

As such, a series of compounds (**C1–C8**) containing the **Nap** motif with differing functionalities in the *N*-imide and 4-positions are presented in this Chapter. In the first series, a

selection of **Naps** bearing substituents suitable for hydrogen bonding interactions are presented [Figure 5.1: **C1-C3**]. A simple **ABC** labelling scheme for the **Nap** core is shown in Figure 5.1 to aid discussion throughout the Chapter, where **A** = imide plane, **B** = non-substituted naphthalene plane and **C** = 4-substituted naphthalene plane.

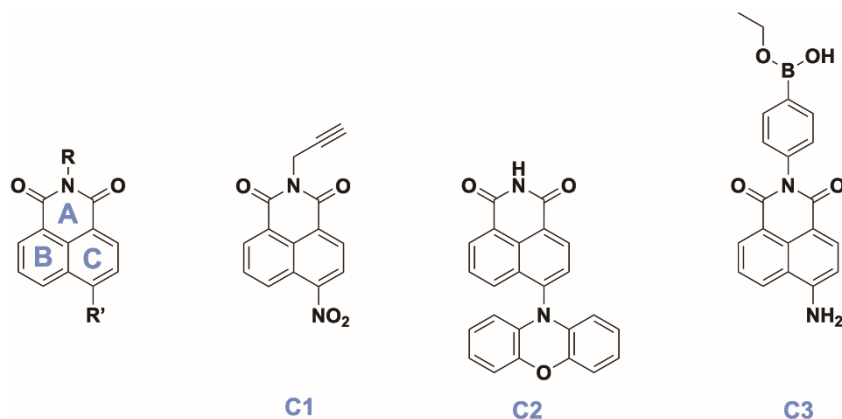


Figure 5.1: Ring numbering scheme and substitution patterns of 1,8-**Nap** and structures **C1**, **C2** and **C3** discussed in this chapter.

The second series presented in this Chapter is comprised of **Nap** compounds bearing the triphenylamine (**TPA**) motif (**C4-C7**), at either the *N*-imide, or 4-position of the **Nap**. These molecules are currently under investigation within our research group for their various AIE responses in different media.

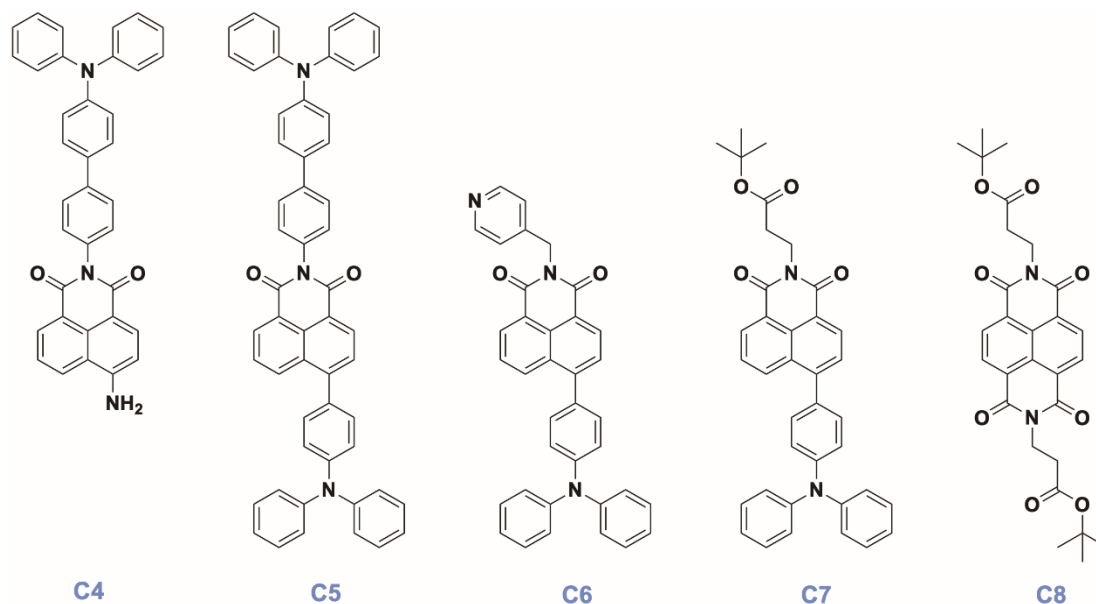


Figure 5.2: Chemical structure of compounds **C4**, **C5**, **C6**, **C7** and **C8**.

Compound **C8** is the exception among the series and is structurally related to **C7**, but is comprised of the naphthalene diimide core, **NDI**, in place of the **Nap** and lacks the **TPA** group. This compound is included in the series for its role in the effect of the alkyl-**Boc** spacer on overall packing arrangements.

5.1.3: Supramolecular Chemistry of Eu(III) Triple Stranded Helicates

Helical structures are found in abundance in nature with the most famous example being DNA. The X-Ray crystal structure of DNA revealed by efforts piloted by Wilkins and Franklin in the early 1950s,^{33,34} ultimately led to the correct inference of its molecular structure by Watson and Crick.³⁵ Our research group has long been invested in the metal templated synthesis of highly ordered materials, with a particular focus on lanthanide templated synthesis of helicates and other hierarchical materials.^{171,259,265,266} The first X-ray crystal structure of a dinuclear triple-stranded helical complex was reported by Piguet and co-workers in the early 1990s,²⁶⁷ and since then, much effort has been spent into controlling and directing the synthesis of such complexes.^{171,257,258}

The Gunnlaugsson group has contributed heavily to this field, in particular to the design of ligands and formation of associated complexes with lanthanide ions.^{121,170,171,265,268,269} The crystal structure of two enantiopure Eu(III)-triple stranded helicates is presented in this chapter, employing the ligands **L1** and **L2** which correspond the *S,S* and *R,R*- enantiomers [Figure 5.3].

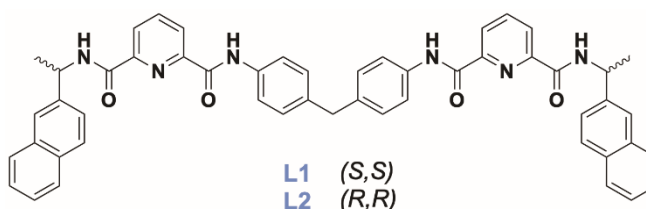


Figure 5.3: Chemical structure of ligands, **L1** and **L2**.

The formation of the helical structures were confirmed by Dr Kotova using various techniques including spectroscopic titrations, where the changes in absorbance, fluorescence, and Eu(III)-centred emission of the ligand were monitored upon gradual metal addition (0 – 4.0 eq.). These changes were further analysed and the derived binding constants confirmed the formation of 2:3 (M:L) species in the solution. Furthermore, the determination of *q* values (the number of water molecules directly bound to the Eu(III) centre) been determined for these complexes, and the values found were equal to 0 which indicates saturation of Eu(III) coordination environment with the donor atoms of the ligand, which could be achieved in the case of the helicate formation. The formation of 2:3 (M:L) was also confirmed using MALDI and ESI mass spectrometry analyses, as well as by changes in CD spectra of the ligand upon addition of Eu(III). As will be discussed, the solid state structure of this complex was extremely desirable and major contributions toward the resolution of complexes **C9** and **C10** were provided by multiple group members throughout the years.

5.2: Crystallographic Analysis of **Nap** Series 1: **C1-C3**5.2.1: X-Ray Crystal structure of 4-Nitro**Nap**-*N*-(CH₂CCH) **Nap**, **C1**

Compound **C1** is composed of a 4-nitro-*N*-alkyne **Nap**. The crystal structure of compound **C1** was solved in the orthorhombic space group *Pca*2₁ and the asymmetric unit contains two independent molecules of **C1**. A thermal ellipsoid representation of the one of the molecules of **C1** is shown in Figure 5.4 (a). Data corresponding to the collection of reflections and structure refinement are presented in Table A5.1 and the full asymmetric unit can be viewed in the appendix [Figure A5.2]

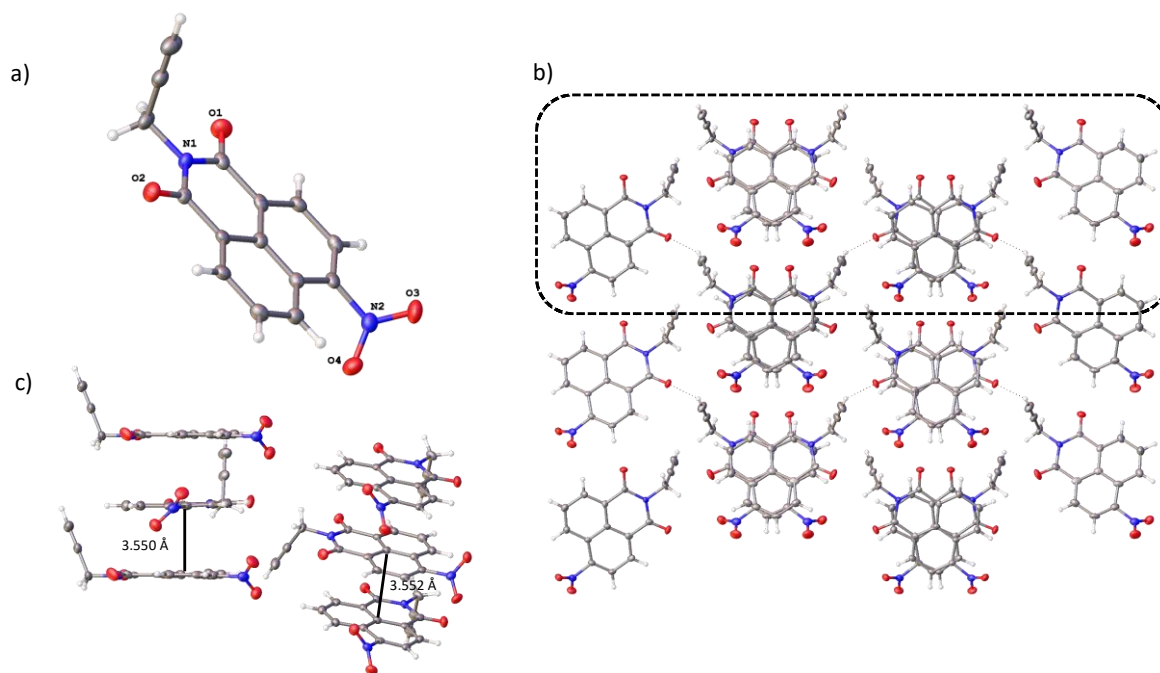


Figure 5.4: a) Representation of one molecule of **C1** present in the asymmetric unit with heteroatoms labelled. b) Intermolecular interactions in the extended structure shown normal to the *b*-axis. Red dotted lines denote CH...O interactions. c) π -Stacking interactions between **Nap** cores.

Two types of intermolecular interactions are present within the extended structure, namely: i) C-H...O hydrogen bonding interactions and ii) π -stacking interactions. The C-H...O intermolecular interaction is a weak hydrogen bonding interaction²⁷⁰ that occurs between the terminal hydrogen atom of the alkyne, and a neighbouring imide oxygen atom ($d(\text{C-H}\cdots\text{O}) = 3.175(8)$ Å) [Table 5.1]. This intermolecular interaction arranges the molecules in a zig-zag orientation along the crystallographic *b*-axis [Figure 5.4 (b)]. Two distinct types of π -stacking interactions occur between homo- π -stacked arrays of each of the individual molecules of **C1** in the asymmetric unit [Figure 5.4 (c)]. The **Nap** cores of these interactions are arranged in a twisted, but cofacial orientation, induced by the steric interference of the *N*-substituted alkyne. The distance between the central carbon atoms of the **ABC** ring system [Figure 5.1] are 3.5502(4) and 3.5519(5) Å for the two unique molecules in the asymmetric unit, respectively.

5.2.2: X-Ray Crystal Structure of 4-Phenoxazine-*N*-(H) **Nap**, **C2**

Compound **C2** is composed of 4-phenoxazine naphthalic anhydride. The crystal structure of compound **C2** was solved in the triclinic space group $P\bar{1}$ and the asymmetric unit contains two molecules of **C2** and one dichloromethane solvent molecule. A thermal ellipsoid representation of the one of the molecules of **C2** is shown in Figure 5.5 (a). The full asymmetric unit can be viewed in the appendix [Figure A5.3 (a)] and full structure data and structure refinement details are presented in Table A5.1.

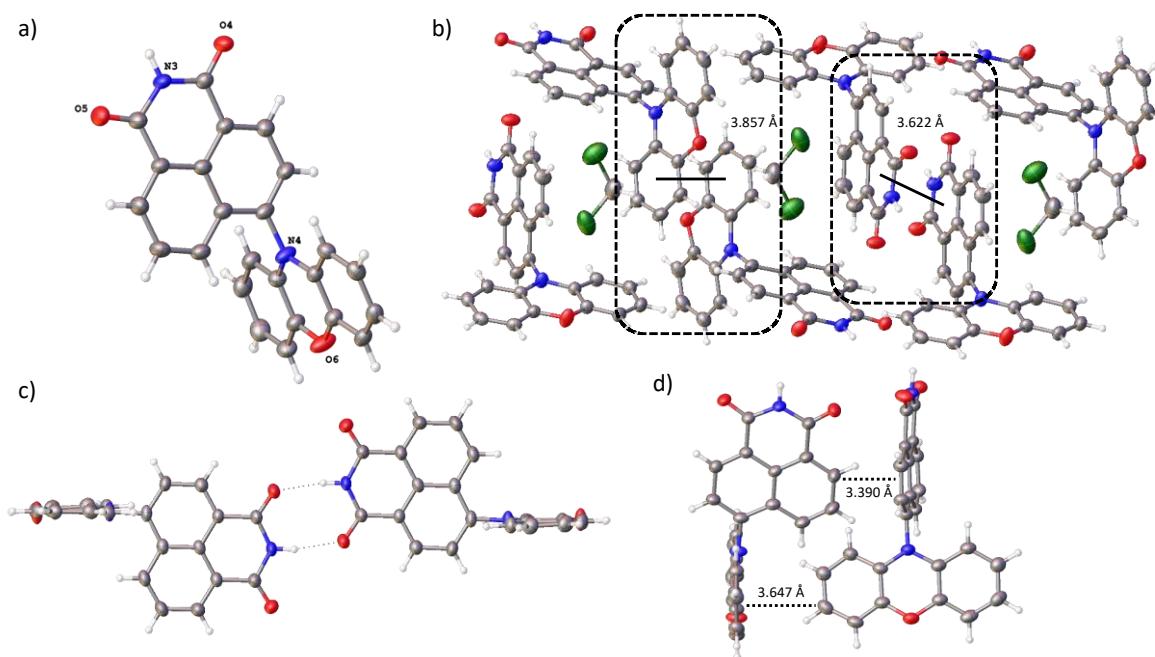


Figure 5.5: a) Representation of one molecule of **C2** present in the asymmetric unit with heteroatoms labelled. b) π -Stacking interactions between phenoxazine moieties and between **Nap** cores. c) Intermolecular hydrogen bonding interactions denoted by red dotted lines. d) Edge-to-face $\text{CH}\cdots\pi$ interactions.

The most notable feature of the structure of **C2** is the orthogonal arrangement of the **Nap** and phenoxazine moieties. The two molecules within the asymmetric unit display plane-to-plane-dihedral angles of 87.34(11) and 90.80(11)°, respectively. The aromaticity of and orthogonal relationship between the two chromophores gives rise to many intermolecular interactions within the extended structure [Figure 5.5].

The extended structure exhibits an overall columnar stacking behaviour [Figure A5.3 (b)] facilitated by head-to-tail interactions between pairs of **Naps** and pairs of phenoxazine moieties. The phenoxazine pairs exhibit slipped π -stacking interactions where the terminal rings are stacked cofacially, while the **Nap** dimers exhibit head-to-tail π -stacking between their **A**-rings [Figure 5.5 (b)], with distances of 3.857(3) and 3.662(2) Å, respectively. Hydrogen bonding interactions further stabilise the structure and occur between $\text{N-H}\cdots\text{O}$ which results in a crosslinking of the columns along the *a*- and *c*-axes. These interactions are supplemented by weak edge-to-face $\text{C-H}\cdots\pi$

interactions between the **Nap** C-H groups of each pair ($d = 3.472(3) \text{ \AA}$), as well as between the phenoxazine moieties ($d = 3.710(6) \text{ \AA}$). The closest distance was observed between C34 and ring **B** mean plane ($d = 3.390(4) \text{ \AA}$) indicating a moderate degree of electrostatic interaction.²⁷¹

5.2.3: X-Ray Crystal Structure of 4-Amino-*N*-(B(OH)(OCH₂CH₃)) **Nap**, **C3**

Compound **C3** is composed of a 4-amino-*N*-(B(OH)(OCH₂CH₃)) **Nap**. The crystal structure of compound **C3** was solved in the monoclinic space group C2/c and the asymmetric unit contains one molecule of **C3**, with the **Nap** displaying positional disorder over two sites with 90 and 10% occupancies. A thermal ellipsoid representation of the major occupied species of **C3** is shown in Figure 5.5 (a). The full asymmetric unit can be viewed in the appendix [Figure A5.4 (a)] and full structure data and structure refinement details are presented in Table A5.1.

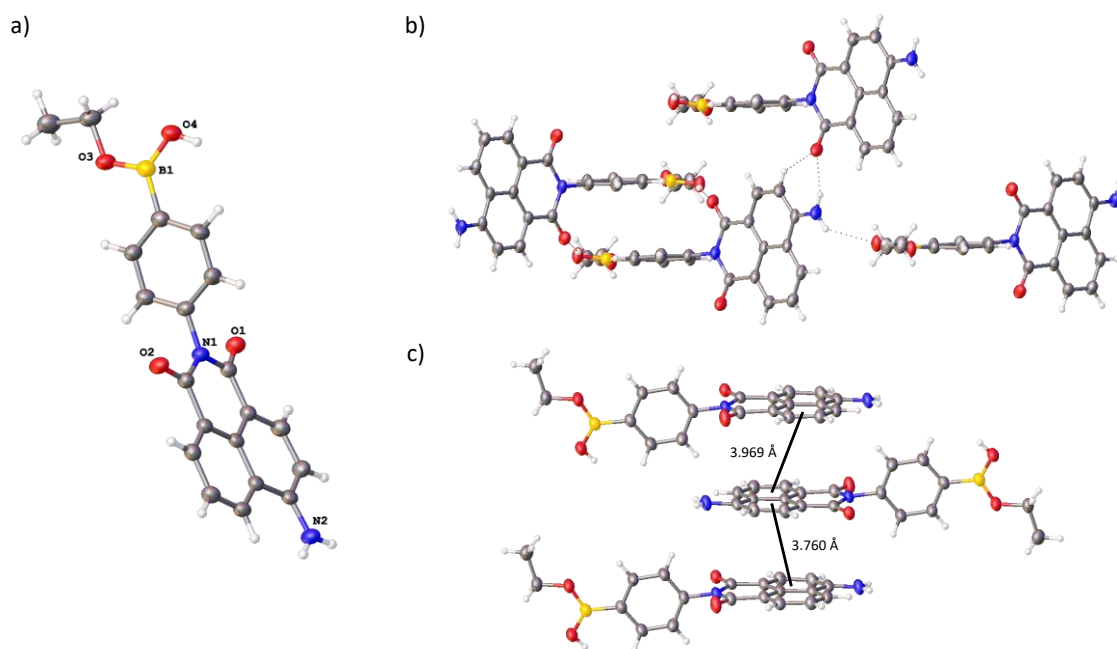


Figure 5.6: a) Representation of the major occupied species of **C3** present in the asymmetric unit with heteroatoms labelled. b) Intermolecular hydrogen bonding interactions denoted by red dotted lines. c) π -Stacking interactions between **Nap** cores.

For the major occupied species, hydrogen bonding interactions and head-to-tail π -stacking interactions lead to an overall columnar extended structure of compound **C3**. Hydrogen bonding interactions between i) the boronic acid OH donor and imide oxygen acceptor atoms (O-H $\cdots$ O, $d = 2.791(3)$), ii) an amino proton of the **Nap** and neighbouring oxygen imide atoms (N-H $\cdots$ O, $d = 2.850(3) \text{ \AA}$) and, iii) an amino proton of the **Nap** and boronic acid oxygen acceptor (N-H $\cdots$ O, $d = 3.0264(19) \text{ \AA}$) crosslink the structure along the *c*-axis [Figure 5.5 (b)]. The structure is also stabilised by offset π -stacking interactions between respective symmetry generated **B** rings ($d(\pi-\pi) = 3.969(7) \text{ \AA}$) and **C** rings ($d(\pi-\pi) = 3.760(4) \text{ \AA}$) of the **Nap** motif, which give rise to both brick layer

and slipped stacking systems throughout the structure. Table 5.1 summarises the hydrogen bonding distances and geometries for the interactions in the extended structures of **C1-C3**.

Table 5.1: Hydrogen Bonds for **C1-C3**. D = donor atom, H = hydrogen atom, A = acceptor atom.

	D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C1	C30	H30	O1 ¹	0.95	2.36	3.175(8)	143.1
C2	N3	H3A	O4 ²	0.88	1.93	2.804(3)	169.4
C3	O3	H3	O1 ³	0.84	1.99	2.791(3)	160.3
	O3	H3	O1A ³	0.84	1.84	2.63(4)	157.9
	N2	H2A	O2 ⁵	0.88	2.01	2.850(3)	159.2
	N2	H2B	O3 ⁶	0.88	2.42	3.0264(19)	126.1
	C4	H4	O2 ⁵	0.95	2.43	3.199(3)	137.9

Symmetry transformations needed to generate equivalent atoms: ¹3/2-X,+Y,1/2+Z; ²2-X,-Y,1-Z; ³1-X,+Y,1/2-Z; ⁴1/2+X,1/2+Y,+Z; ⁵1/2+X,3/2-Y,1/2+Z

In summary, the X-ray crystal structures of compounds **C1**, **C2** and **C3** were obtained and resolved to final R indexes (R_1 , wR_2) for **C1**(0.0935, 0.2483), **C2**(0.0602, 0.1545) and **C3**(0.0480, 0.1367). Common features were observed in the packing arrangements of all three crystal structure systems which include hydrogen bonding interactions and π - π stacking interactions between the **Nap** and, where present, between the other aromatic substituents present in the structures. This can have important implications for the overall charge transfer properties of the molecules.²⁷²

5.3: Crystallographic Analysis of **Nap** Series 2: **C4-C8**

The next section focuses the packing arrangements of **Naps** that are substituted in either the 4- or *N*-imide positions with bulky, triphenylamine (**TPA**) groups.

5.3.1: X-Ray Crystal Structure of 4-Amino-*N*-(Ph(**TPA**)) **Nap**, **C4**

Compound **C4** is composed of a 4-amino-*N*-phenyl(**TPA**) **Nap**. The crystal structure was solved in the triclinic space group $P\bar{1}$ and the asymmetric unit contains one molecule of **C4** and an unknown solvent site. A chemically acceptable model for this solvent cluster could not be determined and the diffracting component of this area, consisting of 53 electrons, was removed with the use of the SQUEEZE function in the Platon software.²³⁶ A disordered orientation of the **Nap** moiety was observed, which is typically observed for 4-monosubstituted **Naps**.^{13,273} The disorder ratios displayed occupancies of 93 and 7%, which were obtained from free variable refinement. A thermal ellipsoid representation of the major occupied species is displayed in Figure 5.7 (a). The full asymmetric unit can be viewed in the appendix [Figure A5.6 (a)] and full structure data and structure refinement details are presented in Table A5.5.

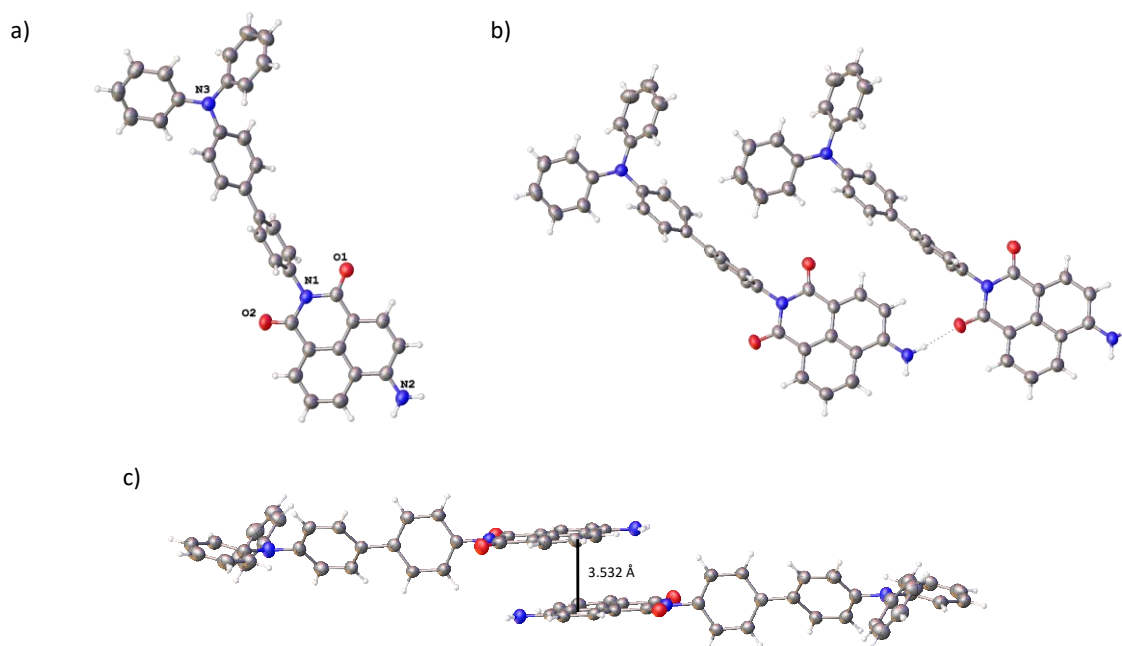


Figure 5.7: a) Representation of the major occupied species of **C4** present in the asymmetric unit with heteroatoms labelled. b) Intermolecular hydrogen bonding interactions denoted by red dotted lines. c) π -Stacking interactions between **Nap** cores.

The extended structure of **C4** features hydrogen bonding interactions between the amino donor hydrogen and imide oxygen acceptor atoms (N-H $\cdots$ O, $d = 2.870(6)$ Å), which connects the molecules in an offset, sheet-like array, parallel to the b -axis [Figure 5.7 (b)]. Pairs of **C4** also display offset, head-to-tail π -stacking interactions ($d = 3.532(5)$ Å) between the **C** planes of the **Nap** ring, facilitated by the large dipole moment which arises from the electron donating amine and electron withdrawing imide moieties. A near orthogonal arrangement between the **A** plane of the **Nap** and the N -phenyl rings is observed and displays plane-to-plane twist angles of $85.27(17)^\circ$ for the major occupied species, while the adjacent phenyl rings adopt a far more coplanar orientation, with an angle of $31.54(16)^\circ$ between the mean planes. Figure A5.6 (b) displays the extended structure of **C4**.

5.3.2: X-Ray Crystal Structure of 4-TPA- N -(Ph(TPA)) **Nap**, **C5**

Compound **C5** is composed of a 4-TPA- N -phenyl(TPA) **Nap**. The crystal structure was solved in the triclinic space group $P\bar{1}$ and the asymmetric unit contains one molecule of **C5**. Additional data is presented in Table A5.5 and Figure A5.7. Unlike any of the previous structures, no immediately obvious interactions within the bulk are noticeable. The structure does not display any π -stacking interactions due to the presence of two bulky **TPA** substituents on each end of the molecule. Instead, the packing of this structure shows weak CH $\cdots$ π and dipole interactions.

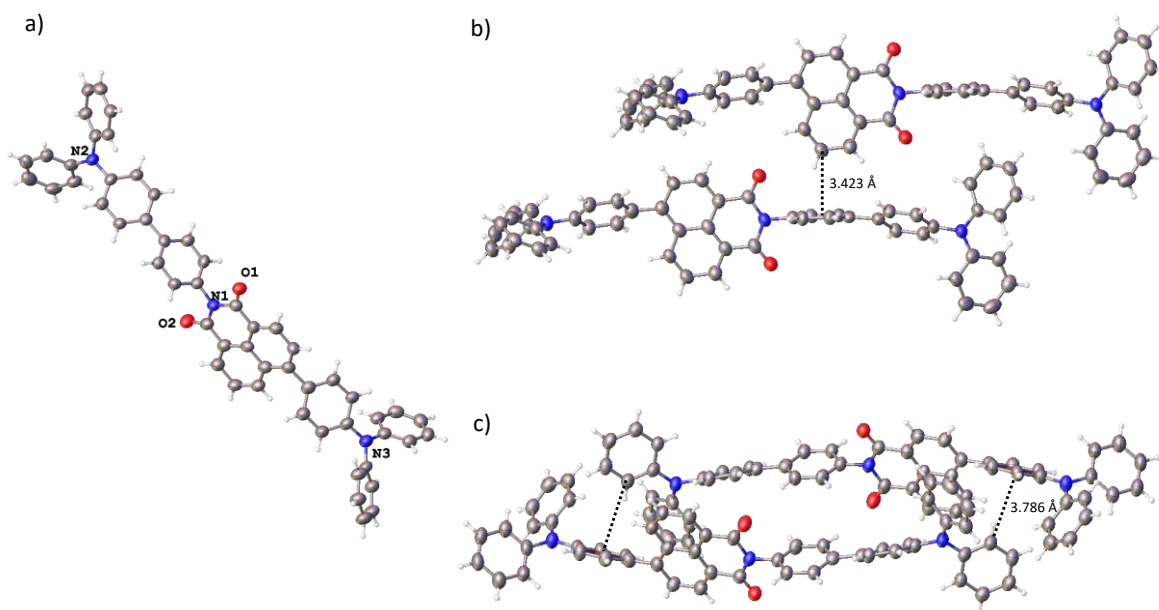


Figure 5.8: a) Asymmetric unit of **C5** unit with heteroatoms labelled. b) Edge-to-face CH... π interactions between molecules of **C5** in a head-to-head orientation and c) Edge-to-face CH... π interactions between two **C5** molecules in a head-to-tail orientation.

Unsurprisingly, the addition of the second **TPA** group has major effects on the crystal packing of **C5** in comparison to the other structures discussed. Weak hydrogen bonding interactions between an imide oxygen and para-hydrogen atom of an *N*-substituted phenyl group ($d(\text{C-H}\cdots\text{O}) = 3.181(7) \text{ \AA}$) arrange the molecules in an offset, face to face orientation parallel to the *b*-axis. Face-to-face and head-to-tail packing arrangements of **C5** are observed throughout the structure, supplemented by C-H... π interactions. Figure 5.8 (a) displays the closest of these interactions, which occurs between the mean plane of the *N*-phenyl ring and closest C-H of the **Nap B** ring. The distance between the closest carbon atom of the **Nap B** ring to the *N*-phenyl ring mean plane is $3.423(6) \text{ \AA}$ and occurs at an angle of 63° between the C-H donor projection and phenyl plane. This C-H... π interaction results in offset face-to-face packing in the structure. Figure 5.8 (b) displays a second C-H... π interaction between C-H donor of the *N*-substituted **TPA** and the mean plane of the 4-substituted phenyl ring, where the distance between closest carbon and phenyl ring is $3.786(5) \text{ \AA}$. The projection between the C-H bond and phenyl plane is 81° and this interaction results in an overall head to tail arrangement of the molecules of **C5** in the extended structure.

Furthermore, the angles between the planes of the **Nap** chromophore phenyl substituents were analysed. The plane-to-plane twist angle between the **C** ring of the **Nap** and 4-substituted phenyl group is significantly more acute ($49.973(3)^\circ$) than that between the **A** ring and *N*-substituted phenyl ring (80.57°), presumably due to the electron donating properties of the former ring. As was observed for **C4**, the Ph-Ph twist angle was almost coplanar ($26.17(17)^\circ$) induced by

the large dipole moment from the electron donating **TPA** groups located at the terminus of the molecule and electron withdrawing imide core of **C5**.

5.3.3: X-Ray Crystal Structure of 4-**TPA**-*N*-(4-picolyl) **Nap**, **C6**

The coordination behaviour and ligand packing structures of a series of non-substituted or 4-nitro-substituted *N*-(2- or 3-picolyl) **Naps** has previously been described by our group.²⁶⁰ These ligands display interesting coordination behaviours with *d*-block metal ions, modulated by the position of pyridyl nitrogen atoms. The 2-picolyl derivatives were found to display unique coordination behaviour with the metal ions, chelating in a bidentate fashion through coordination of the pyridyl nitrogen and imide oxygen atoms. Conversely, the 3-picolyl derivatives varied in their respective coordination behaviour, with the nitro-derivative yielding a series of low molecular weight metallogels with Mn(II) and Co(II) metal ions.²⁶⁰ As such, this family of ligands was further developed by exploring the 4-picolyl substituent in the *N*-imide position and by functionalising the **Nap** in the 4-position with the **TPA** motif, with the aim of exploring the chemical and photophysical behaviour of the ligands bearing different electron donating properties.

Compound **C6** is composed of a 4-**TPA**-*N*-(4-picolyl) **Nap**. The crystal structure of compound **C6** was solved in the monoclinic space group $P2_1$ and the asymmetric unit contains two independent molecules of **C6**. The molecule is achiral, but crystallised as a chiral mixture in the noncentrosymmetric Sohncke space group, $P2_1$. This phenomenon has been previously observed for other achiral structures like benzene and phenol, among others.^{274,275} The error in the Flack parameter was significant -0.0(5), but refinement as an inversion twin shows a refined Flack parameter of -0.2(7), indicating that the chirality is indeterminate using the given data. Furthermore, one of the **Nap** cores of **C6** is disordered and modelled over two positions with occupancy factors of 72 and 28%, obtained from free variable refinement [Figure A5.8 (a)]. A thermal ellipsoid representation of (a) one of the molecules with heteroatom labelling scheme, and (b) a graphical overlay of the two unique molecules in the asymmetric unit is presented [Figure 5.9]. Additional data is provided in Table A5.5.

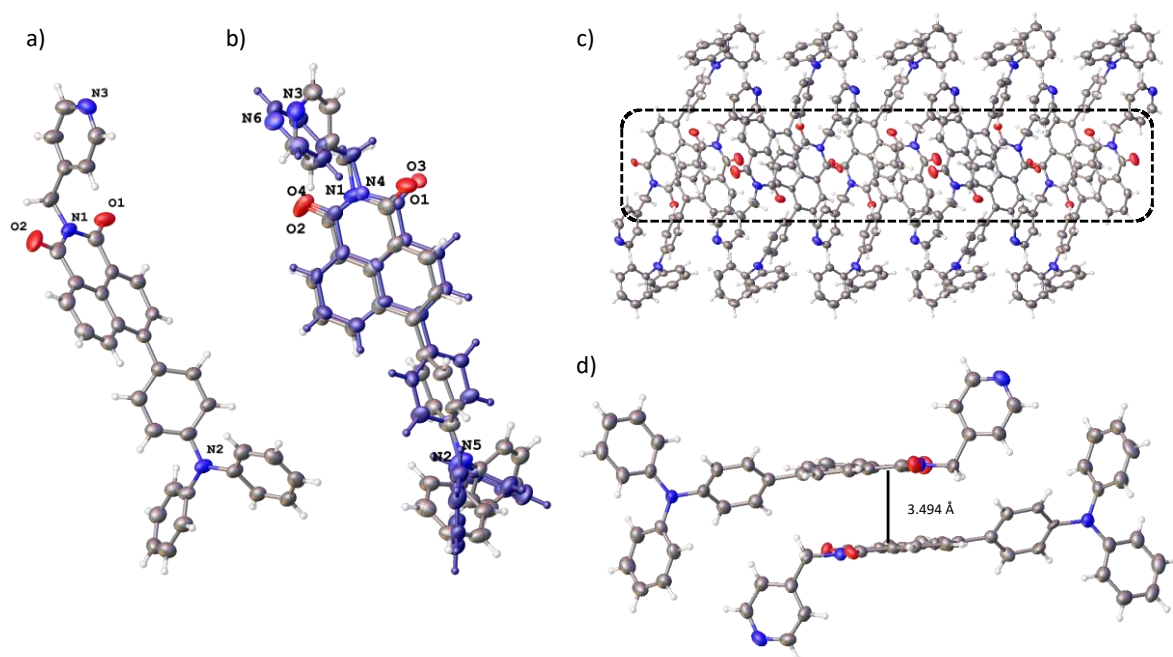


Figure 5.9: a) Representation of one molecule of **C6** with heteroatoms labelled. b) Graphical overlay of the two unique molecules of **C6** in the asymmetric unit. c) Head-to-tail packing of extended structure of **C6**. d) π -stacking interactions.

As observed in Figure 5.9 (b), the two unique molecules of **C6** adopt very different orientations within the crystal lattice, particularly with respect to the relative twists of the **TPA** phenyl groups. In this case, the presence of the **TPA** group in the 4-position does not perturb the π -stacking arrangements, as was observed for **C5**. The two unique molecules in the asymmetric unit participate in head-to-tail π -stacking interactions, typically observed for the **Nap** core with a distance of 3.494(8) Å between the planes of the **Nap A** and **B** rings.

Edge-to-face interactions further supplement the extended structure, and interestingly, strong C=O $\cdots\pi$ attractive interactions were observed for the first time in the series, giving rise to an overall herringbone-like overall structure [Figure A5.9 (c)]. A recent study by Li, Shimizu and co-workers analysed the stability trends of a series of CO $\cdots\pi$ aromatic interactions and found that these interactions were repulsive for non-substituted aromatic surfaces and became increasingly attractive when electron withdrawing groups were present on the π -surface.²⁷⁶ A distance of 2.992(9) Å between imide oxygen atom and the central carbon of the **ABC Nap** ring system was observed, indicating that the carbonyl group displays an overall stabilising effect on the aromatic surface. This overall stabilising effect is further supported by the position of the oxygen atom, which was found to be centred with respect to the **ABC** plane, as opposed to being an edge interaction which is observed for repulsive forces.²⁷⁷ Although a solid-state phenomenon, this interaction could have a large effect on the supramolecular properties of higher ordered structures containing the **C6** motif and warrants further development within our group.

5.3.4: X-Ray Crystal Structure of 4-**TPA**-*N*-(CH₂CH₂Boc) **Nap**, **C7** and **NDI** derivative, **C8**

The next compound discussed in the series, **C7** is composed of 4-**TPA**-*N*-(CH₂CH₂Boc) **Nap**, where **Boc** = tert-butyloxycarbonyl. The crystal structure of compound **C7** was solved in the monoclinic space group *P*2₁/*c* and the asymmetric unit contains one molecule of **C7**. A thermal ellipsoid representation of **C7** is shown in Figure 5.10 (a). Additional data is presented in Table A5.9.

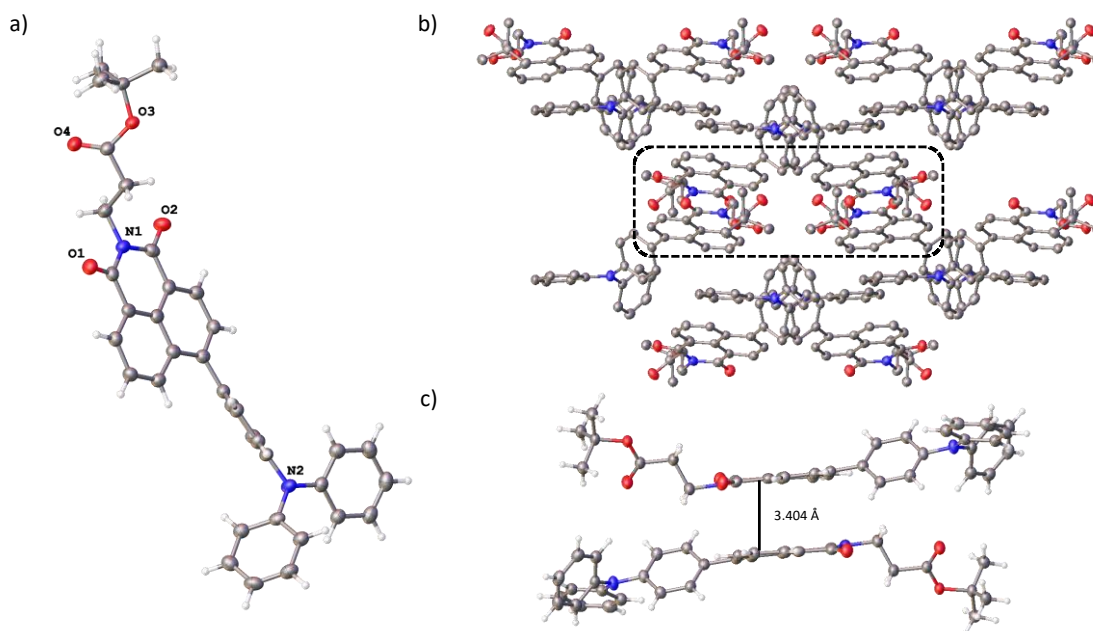


Figure 5.10: a) Asymmetric unit of **C7** with heteroatoms labelled. b) Extended structure of **C7** viewed normal to the *c*-axis. Hydrogen atoms omitted for clarity. c) Head-to-tail π -stacking interactions of the **Nap** core.

The extended structure is dominated by distinct local regions of π -stacking interactions [Figure 5.10 (b)]. As was observed previously, the **Nap** core of **C7** participates in offset, head-to-tail π -stacking interactions between sets of dimeric pairs dispersed throughout the structure. These pairs are crosslinked by weaker π -stacking interactions between the phenyl rings of the **TPA** and the **A** ring of an adjacent **Nap**. This interaction deviates substantially from the coplanarity observed in all of the previous systems, with an angle of 18.18(2)° observed between the planes. Overall, the packing in **C7** is the least regular of the series, which is perhaps not surprising given the flexibility of the CH₂CH₂Boc group.

5.3.5: X-Ray Crystal Structure of *N*-(CH₂CH₂Boc) **NDI**, **C8**

The next molecule discussed is an **NDI** derivative of **C7**, where **NDI** = naphthalene diimide and is included in this series for comparison. Compound **C8** was synthesised and crystallised by Dr Adam Henwood of the Gunnlaugsson group and is composed of an *N*-(CH₂CH₂Boc)-**NDI** which lacks the bulky, rotating aromatic **TPA** substituent of **C7**. The crystal structure was solved in the triclinic space group *P* $\bar{1}$ and the asymmetric unit contains one molecule of **C8**. Figure 5.11 (a) displays the asymmetric unit of **C8** and additional data is presented in Table A5.9.

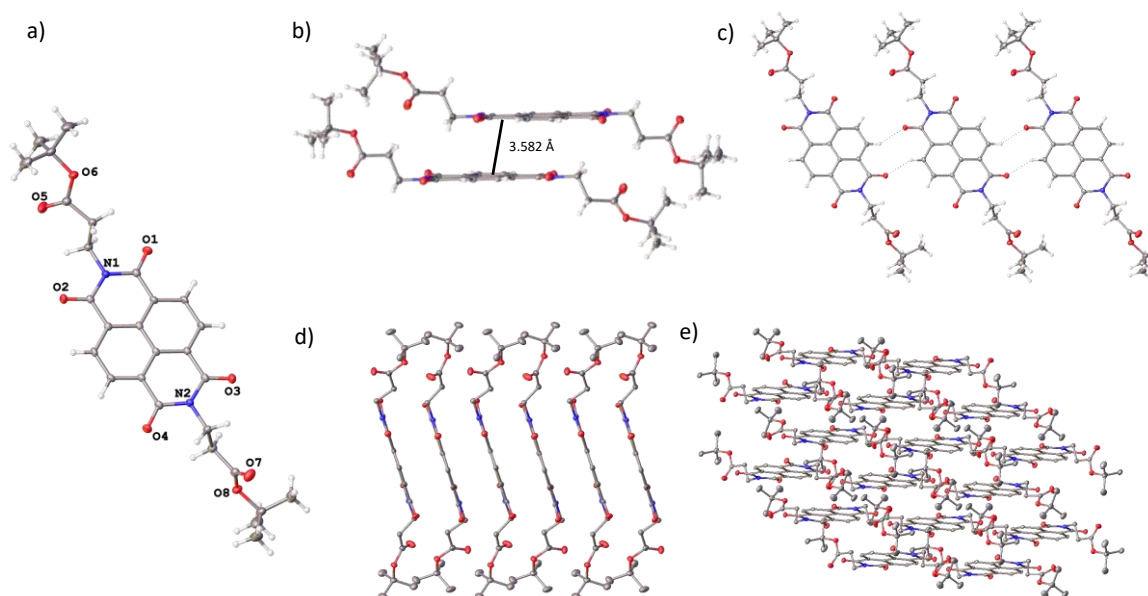


Figure 5.11: a) Asymmetric unit of **C8** with heteroatoms labelled. b) Offset, head-to-tail π -stacking interactions of the **NDI** core. c) Hydrogen bonding interactions showing head-to-head arrangement. d) Extended structure of **C8** viewed normal to the *b*-axis showing head-to-tail π -stacking interactions. e) Extended structure of **C8** viewed normal to the *c*-axis showing brick-like layering system. Hydrogen atoms in d) and e) are omitted for clarity.

The extended structure of **C8** was found to be highly ordered, as expected due to the planar nature of the **NDI** motif. Hydrogen bonding interactions between the C-H donor atoms on the **NDI** core and imide oxygen acceptor atoms facilitate a sheet-like arrangement parallel to the *b*-axis [Figure 5.10 (c and d)]. Similar to the **Nap** examples presented, π -stacking interactions between the **NDI** cores in a head-to-tail orientation are observed, with the distance between the centroids of offset imide and naphthalene planes 3.582(2) Å. Table 5.2 summarises the hydrogen bonding interactions observed for crystal structures **C4-C8**.

Table 5.2: Hydrogen Bonds for **C4-C8**. D = donor atom, H = hydrogen atom, A = acceptor atom.

	D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C4	N2	H2A	O2 ¹	0.88	2.02	2.870(6)	163.0
C5	C56	H56	O1 ²	0.95	2.47	3.181(7)	131.6
C6	C51A	H51A	O1 ³	0.95	2.22	3.041(13)	143.5
C8	C3	H3	O3 ⁴	0.95	2.31	3.162(3)	148.4
	C9	H9	O2 ⁵	0.95	2.31	3.159(3)	148.4
	C16	H16A	O4 ⁶	0.99	2.40	3.185(3)	135.5

Symmetry transformations needed to generate equivalent atoms: ¹-1+*X*,+*Y*,+*Z*; ²-1+*X*,1+*Y*,+*Z*; ³1-*X*,1/2+*Y*,1-*Z*; ⁴+*X*,1+*Y*,+*Z*; ⁵+*X*,-1+*Y*,+*Z*; ⁶2-*X*,2-*Y*,1-*Z*

5.4: Summary of X-Ray Crystal Structure Analyses from Sections 5.2 and 5.3

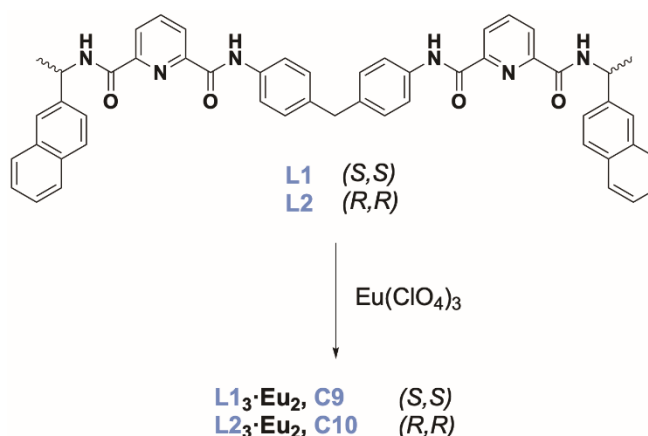
The crystal structures of eight **Naps** (**C1-C8**) were presented in a two-part series. These compounds were synthesised by individual group members who are credited in the relevant sections. The **Naps** presented in Series 1, **C1-C3**, constitute small molecules which display functionalisation at either the *N*-imide site or 4-position of the **Nap** core with substituents capable of classic hydrogen bonding interactions. The extended structures of these compounds were dominated by hydrogen bonding interactions. Different types of π -stacking interactions between the **Nap** cores were observed for the series, influenced by the relative substituents. Compound **C2**, which contains a rigid 4-substituted phenoxazine moiety additionally displayed interesting edge-to-face C-H $\cdots\pi$ interactions between the phenoxazine and **Nap** chromophores. These small molecules display similarities in their packing arrangements to ligands **91-93** (*vide supra*, Chapter 3), namely, hydrogen bonding interactions which crosslink columnar layers in the extended structures.

Conversely, the **Naps** described in Series 2 (**C4-C8**), which were designed primarily for AIE studies within the Gunnlaugsson group, mainly contain bulky **TPA** substituents, which drastically alter their packing arrangements. Each of these structures display offset head-to-tail π -stacking interactions between the **Nap** motifs, with **C5** being the only exception. Compound **C5** is functionalised at both ends of the molecule with the **TPA** group and its packing interactions are dominated by weak Van der Waals and dipole-dipole interactions.

5.5: X-Ray Crystal Structure of Chiral Eu(III)-Helicates

The crystals of the two Eu(III)-triple stranded helicates **C9** and **C10** were grown by Dr Oxana Kotova. A special mention is to be given to Dr Brendan Twamley and Dr Chris Hawes (Keele University) for their help and advice in the mounting, solving, and refining of these crystal structures.

As mentioned previously, extensive solution studies and molecular modelling techniques amongst other, have confirmed a 2:3 (M:L) helicate structure for related systems.²⁶⁵ However, the solid-state structural characterisation of these systems has proved elusive to our group for the past 14 years since their initial development.^{265,266} A scheme outlining the formation of the crystalline samples **C9** and **C10** is presented below.



Scheme 5.12: Schematic representation of the formation of **C9** and **C10**.

Optimisation of crystal growth techniques by Dr Kotova resulted in crystals suitable and stable enough for single crystal X-ray diffraction. All crystalline samples produced prior to this point deteriorated almost immediately upon exposure to atmospheric and experimental conditions. On initial exposure to these crystals, multiple diffraction experiments were attempted, but unfortunately, the intensity of the scattering was too low to obtain a suitable structural model. In these cases, measurable diffraction was not observed at a resolution past 1.2 Å. Following this feedback, Dr Kotova further stabilised the crystals of the perchlorate complex by addition of sodium perchlorate, which resulted in marked improvement toward the handling of these crystals.

5.5.2: Selection of Crystals Suitable for Single Crystal X-Ray Analysis

Crystals of differing morphologies were observed when Eu(III) triflate or perchlorate salts were employed. Upon initial assessment, the crystalline samples obtained upon complexation of the ligands, **L1** (*S,S*-enantiomer), and **L2** (*R,R*-enantiomer), with $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ appeared to yield crystals more suitable for single crystal X-ray diffraction than $\text{Eu}(\text{ClO}_4)_3$. Figure 5.13 displays the optical microscopy images of **C9**(CF_3SO_3)₃ and **C10**(CF_3SO_3)₃ resulting from **L1** and **L2**, respectively.

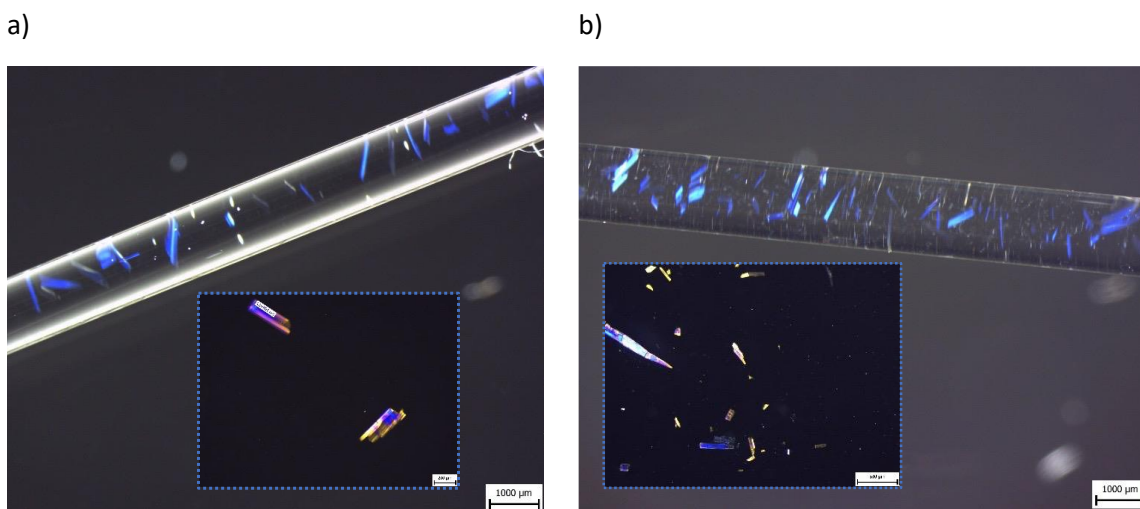


Figure 5.13: Optical microscopy images of a) **C9**(CF_3SO_3)₃ and b) **C10**(CF_3SO_3)₃. The crystals were placed into a capillary tube in order to stabilise and stop the desolvation of the complexes.

The main images in Figure 5.13 display the crystals in the mother liquor in a capillary tube and the insets display the crystals in immersion oil after five minutes exposure time. These crystals appeared as blue rods under the polarised light, with the colour changing upon decomposition from blue to pink to yellow in the oil before dissolving. Such extreme degradation of the crystalline samples in oil did not occur when $\text{Eu}(\text{ClO}_4)_3$ was used, showing the stabilisation effect of the excess perchlorate counterions. The optical microscopy images of these samples are presented in Figure 5.14.

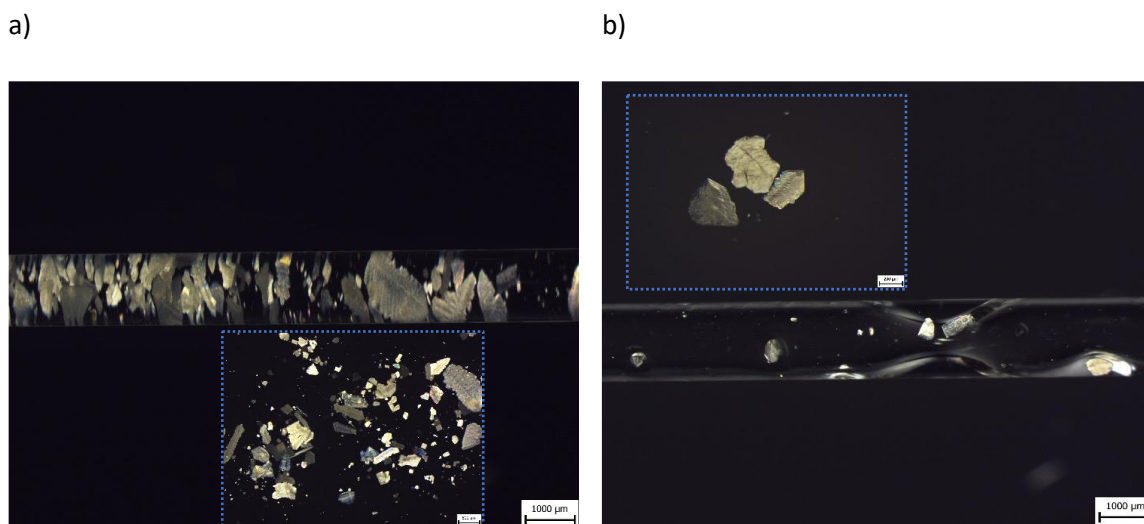


Figure 5.14: Optical microscopy images of a) **C9**(ClO_4)₃ and b) **C10**(ClO_4)₃. The crystals were placed into a capillary tube in order to stabilise and stop the desolvation of the complexes.

Multiple crystals from these samples were screened, which produced the same unit cell, but as before, diffraction was limited to a resolution of 1.2 or 1.0 Å in these cases. Until this point, all samples were screened on the Bruker Apex Duo instrument available at TCD using microfocus Cu Kα ($\lambda = 1.5405$ Å) radiation. For this sample, the Bruker D8 Quest ECO diffractometer, using 1kW graphite-monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation, was used due to the other instrument being made unavailable. To our surprise, the selection of a crystal of **C9**(ClO_4)₃ yielded diffraction past a resolution of 1.0 Å and a structural model was determined *ab initio*. This solid-state structure confirmed the 2:3 M:L stoichiometry, which supported the previously published solution studies of structurally similar complexes.²⁶⁵

5.5.3: X-Ray Crystal Structure Analysis of **C9**

The crystal structure of **C9** was solved in the rhombohedral space group, R3 which displays threefold rotational symmetry. The asymmetric unit contains two unique one third molecules of **L1**, four Eu(III) ions located at special positions, four fully occupied perchlorate ions, and partially

occupied solvent molecules over four sites. The model was constructed by first identifying the Eu(III)-ion centres from the electron density map and then by building the model of **L1** around the metal ion centres, given the predicted structure. Following the successful building of a sensible connectivity map of the triple-stranded helicate, many regions of disordered electron density were observed both within the helical cavities and surrounding the structure. These regions could not be modelled accurately, and as such, were removed with the SQUEEZE function using the Platon software.²³⁶ The final R1 value is 10.76 at a resolution of 0.98 Å, with a final formula of $C_{306.5}H_{252}Cl_{17}Eu_4N_{36}O_{72}$ and a refined Flack parameter of 0.13(5). Figure 5.15 displays the asymmetric unit and symmetry generated asymmetric unit of **C9**.

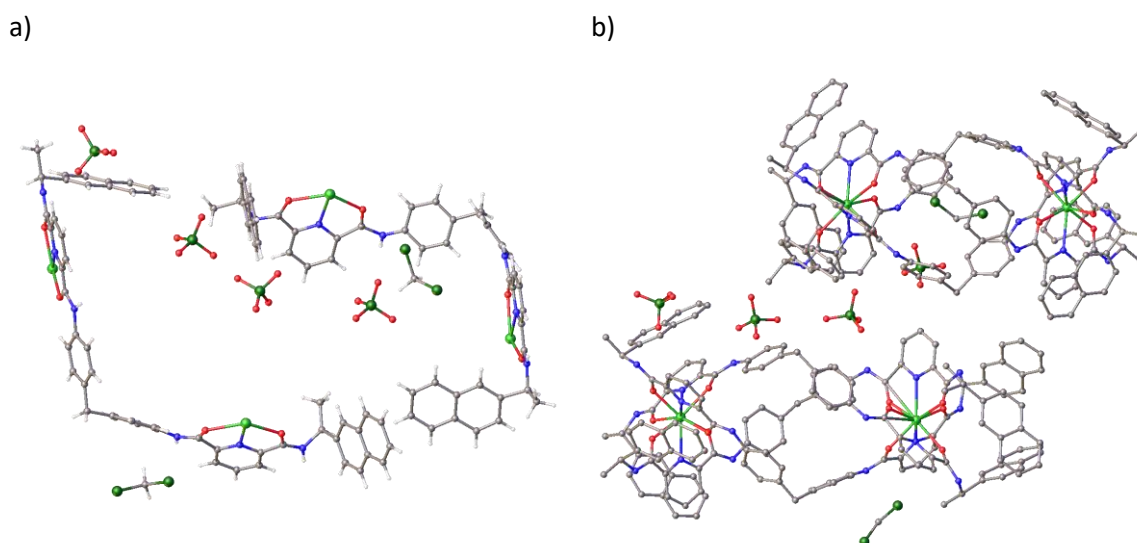


Figure 5.15: Ball and stick model of a) asymmetric unit of **C9** and b) symmetry generated structure of the asymmetric unit with hydrogen atoms omitted for clarity.

The two unique molecules in the asymmetric unit display the same connectivity around the Eu(III)-centres, characterised by coordination of O-N-O donor atoms of the dipicolonic acid (**DPA**) motifs of **L1**. The coordination environment of the Eu(III)-centres are saturated by the donor atoms of three **DPA** ligands, which supports the calculation of the q values from the solution studies. Figure 5.16 displays the symmetry generated asymmetric unit of **C9** and the extended structure, viewed normal to the c -axis.

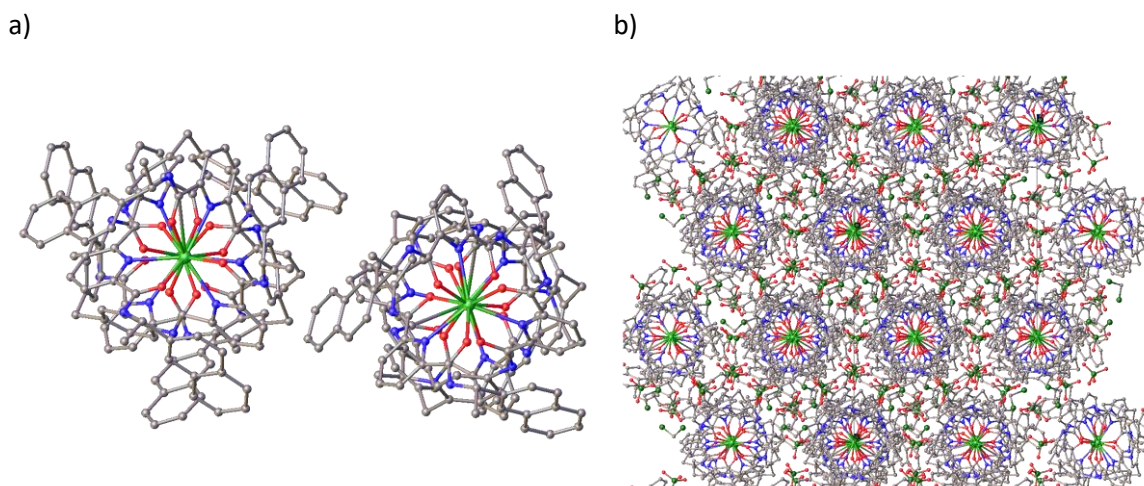


Figure 5.16: Ball and stick model of a) symmetry generated asymmetric unit and b) packed structure of **C9** viewed down the c-axis, with hydrogen atoms omitted for clarity.

The two triple-stranded helicate structures within the asymmetric unit display similar helical arrangements around the two metal centres. The structures mainly differ in the positions of the terminal naphthalene groups which can be seen in Figure 5.16 (a). These two species display C-N-C-C torsional angles between the planes of the **DPA** coordinating units and naphthalene groups of $[73.8784(11)^\circ, 66.2824(6)^\circ]$ and $[84.402(2)^\circ, 60.0937(12)^\circ]$. Furthermore, the distances between the two Eu(II)-centres of each helicate differs, with Eu(III)-Eu(III) distances of 14.037(5) and 13.108(6) Å for each individual helicate. This also indicates the fluctuational nature of the helicate structure and the incredible serendipity of obtaining this structure. The absolute configuration of the chiral centres present in **L1** was confirmed by the refined Flack parameter value of 0.13(5) which determined each centre as the *S*-enantiomer, which is concurrent with the respective enantiomer of ligand used.^{278,279} The compact packing arrangements of the helicate structures are shown in Figure 5.16 (b), with the extended structure stabilised by counterion and solvents channel networks.

5.5.4: X-Ray Crystal Structure Analysis of **C10**

The crystal structure of **C10** was also solved in the rhombohedral space group R3 and initial analysis of the unit cell parameters, including the cell lengths and cell volume were almost identical to that of **C9**. Furthermore, an initial structure solution using the ShelXT solution program returned an electron density map with a pattern analogous to that of **C9**. As such, the structure obtained from **C9** was imported with inverted chirality and used as a rigid model to aid in the refinement of the *R*-enantiomer, **C10**. The asymmetric unit contains two unique one third molecules of **L2**, four Eu(III) ions located at special positions, four perchlorate ions, one partially occupied dichloromethane solvent molecule and three partially occupied chloroform solvent molecules. The structure was refined to a final R_1 value of 9.42. Figure 5.17 displays the asymmetric unit and symmetry generated asymmetric unit of **C10**.

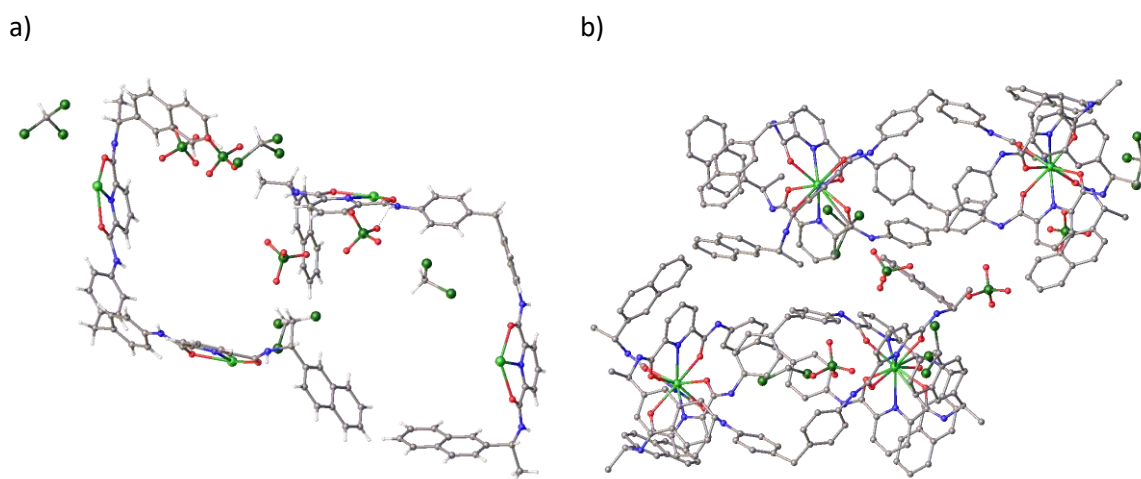


Figure 5.17: Ball and stick model of the a) asymmetric unit of **C10** and b) symmetry generated structure of the asymmetric unit with hydrogen atoms omitted for clarity.

As was observed for **C9**, the Eu(III)-coordination environment is saturated by O-N-O donor atoms from three **DPA** motifs of the **L2** ligands. This further supports the calculation of the q value of zero from solution studies of this complex. Similarly, the interatomic distances between the Eu(III)-ions within individual helicate structures are 13.198(5) and 14.846(4) Å, with the former displaying a solvent molecule in the cavity. Figure 5.18 displays the symmetry generated asymmetric unit of **C10** and the packed structure, viewed normal to the c -axis.

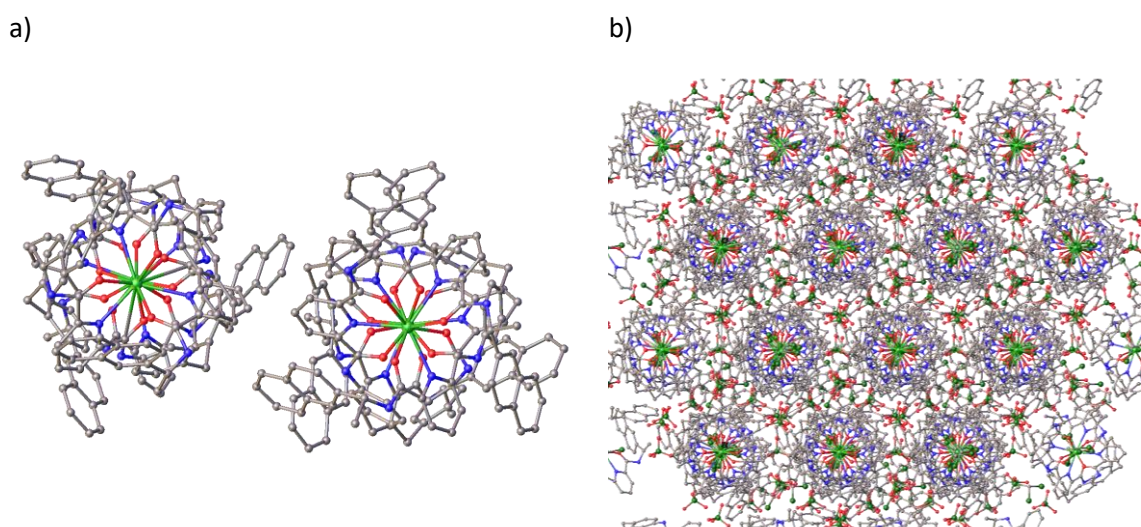


Figure 5.18: Ball and stick model of a) symmetry generated asymmetric unit and b) packed structure of **C10** as viewed down the c -axis, with hydrogen atoms omitted for clarity.

As was observed for the previous structure, the arrangements of the terminal naphthalene rings deviate between the two helicate structures and again, the Flack parameter confirmed the absolute configuration of the structure as the R -enantiomer with a value of 0.009(8).^{278,279}

In summary, the X-Ray crystal structures of two novel enantiopure Eu(III)-triple-stranded helicate complexes bearing ligands **L1** and **L2** with S,S - and R,R - chiral configurations, respectively,

are provided. A space-filling model which displays the helical arrangements of the complexes viewed normal to the *c*-axis is provided in Figure 5.19.

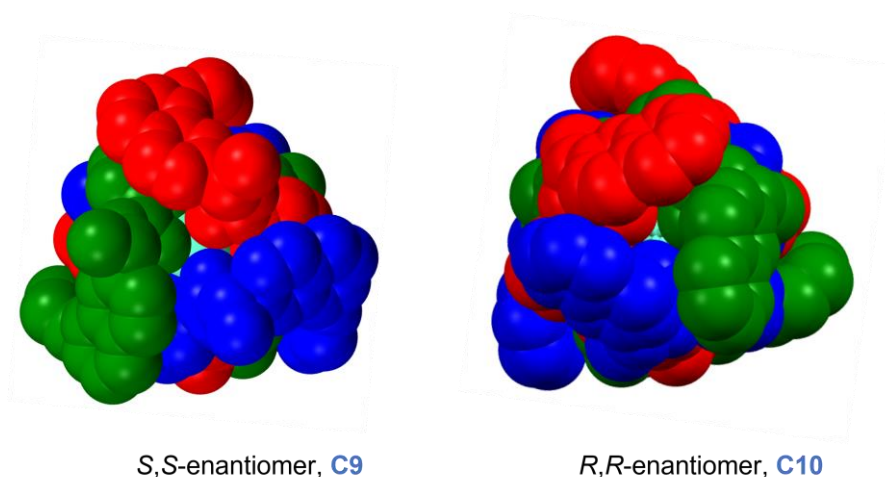


Figure 5.19: Spaced filled representation of ligand arrangement around the Eu(III)-metal centre as viewed normal to the *c*-axis.

Thus, the X-ray crystal structure analysis of a series of compounds, **C1-C10** discussed in this chapter have provided an insight into the packing arrangements of the molecules in their solid states. A number of similarities between the packing arrangements of the structures containing the **Nap** core have been revealed, which will contribute to our overall understanding of the preferred supramolecular interactions of the systems. A structural connectivity model was also provided for the enantiopure Eu(III)-triple stranded helicates which has been sought after from our group for many years. Overall, this chapter contributes to the field of supramolecular chemistry and provides insights into the “chemistry beyond the molecule”.

Chapter 6:

Conclusions

The main research focus of this thesis explored the development of **Nap**-based ligands and their corresponding luminescent Ru(II)-polypyridyl complexes, and probed their interaction with DNA. The design of the molecules was based upon the versatile photophysical properties of Ru(II)-polypyridyl complexes and known anticancer properties of the **Nap**. The **Nap** systems were employed as ligands in Ru(II)-polypyridyl complexes, which allowed for further expansion of their properties. The synthesis of the selected organic **Nap** ligands and corresponding complexes were performed, and in many cases, their single crystal X-ray structures were solved and refined. The photophysical properties of the complexes in solution were first investigated, followed by DNA titration studies and derivation of their binding constants, with a secondary aim of understanding the binding mode through these experiments. The potential for some of these complexes as highly sensitive luminescent nucleic acid probes was also investigated. The attempted synthesis of a conjugated-**Nap** derivatives and a dinuclear Ru(II)-**TB** complex was also described, as well as a novel Ir(III)-**Nap** complex. The final results Chapter in this thesis focused on the crystallographic analysis of a series of **Nap** derivatives developed by multiple members of Gunnlaugsson group, with a total of ten structures discussed in this Chapter and a further six presented in the appendix.

In Chapter 2, the synthesis, characterisation, structural evaluation, photophysical and DNA binding properties of two novel Ru(II)-polypyridyl complexes bearing the non-substituted **PhenNap** ligand, **80**, and either **phen** (**75**) or **TAP** (**76**), as auxiliary ligands were described and discussed. The complexes **75**, **76** and **89** (where **89** contained **bpy** auxiliary ligands), were found to display major structural similarities between their their ligand arrangements, with a substantial degree of rotation between the two ring moieties of the coordinating **PhenNap** ligand, ranging between 62-90°. The complexes also displayed π -stacking interactions between the **Nap** moieties which was believed to facilitate the DNA interaction through intercalation. The structure of complex **75** displayed secondary π - π stacking interactions between the auxiliary **phen** ligands compared to other complexes studied in this Chapter. The hydrogen bonding interactions within the crystal lattice between the guest water molecules and the oxygen atoms of the **Nap** were observed which may provide an explanation of the environment dependent photophysical properties of these systems in solvents of differing polarities.

The CV studies of the complexes conformed with literature trends, and revealed that the **phen** complex, **75**, was easier to oxidise than the **TAP** derivative, **76**, due to the presence of the π -deficient **TAP** ligands on **76**. The photophysical properties of both complexes were evaluated in MeCN and 10 mM aqueous phosphate buffer solutions at pH 7.4, and were found to be highly sensitive to dissolved oxygen and to solvents with hydrogen bonding capabilities. Their relative absorption bands were assigned to distinct transitions of the molecules, namely the Ru(II)-centred MLCT and the **Nap** chromophore allowing for the sensitive monitoring of these parts upon

interaction with DNA. Molar extinction coefficients for the major absorption bands were also calculated. Their luminescent properties were also analysed, where **75** was found to be weakly emissive in aqueous solution ($\Phi_{\text{PL}} < 0.2\%$) and showed moderate emission in deaerated MeCN ($\Phi_{\text{PL}} = 4\%$). The low Φ_{PL} and lifetime values of **75** in aqueous media are attributed to a non-radiative quenching pathway introduced through hydrogen bonding interactions between the solvent and complex. **76** displayed moderate emission quantum yields (maximum of 8% in deaerated MeCN) and long-lived excited state lifetimes ranging between 600–1100 ns in aerated *versus* deaerated media.

The complexes were found to bind strongly to DNA in order of 10^6 M^{-1} and elicited major stabilisation effects on duplex DNA, as shown by thermal melting experiments. This supports the effective design of **75** and **76** which placed the **Nap** in close proximity to the Ru(II) metal centre, as opposed to being separated by an aliphatic spacer. Furthermore, **75** displayed the potential for application as a luminescent probe for nucleic acids, with marked emission enhancement (30-fold increase in emission intensity) in the bound form with natural ctDNA (G-C content of 42%). It was hypothesised that binding of **75** and **76** to DNA occurs in a cooperative manner, with multiple binding modes present.

In Chapter 3, nitro-, bromo- and chloro- derivatives, **91-93**, of **PhenNap** ligand, **80** were synthesised and characterised along with their corresponding Ru(II) complexes, **94-96**. The reduction of the nitro- (**91**) to amino derivative (**97**) was found to be challenging with various reduction methods attempted, with the compound afforded in minor yields. The X-ray crystal structure analysis of **91-93** was performed, where all the structures were found to display i) hydrogen bonding interactions, ii) intermolecular π – π stacking interactions, and iii) positional disorder over the **Nap** unit. Furthermore, a near orthogonal nature between the **Nap** and **Phen** motifs (angles ranging from 75–88°), induced by the steric repulsion between the hydrogen atom of **Phen** and imide oxygen atom of **Nap** in a co-planar conformation was observed. It was shown that the **Nap** unit may become conformationally “locked” with respect to the **Phen** ligand, whereby the substituent in the 4-position of the **Nap** is located either above or below the plane of the **Phen**, with this property becoming pronounced in the associated Ru(II)-polypyridyl complexes bearing these ligands.

The evaluation of electrochemical properties for **91** and **92** suggested that the presence of the electron withdrawing nitro- group of **91** results in a more positive reduction potential for this ligand compared to the bromo-analogue, **92**. The photophysical properties of ligands **91**, **92**, **93** and **97** were explored in MeCN solution at room temperature, where the ligands each displayed: i) high energy absorption bands centred at 264 nm attributed to π – π^* intraligand (IL) transitions of the **phen** moiety and ii) lower intensity absorption bands at longer wavelengths corresponding to **Nap**

localised transitions or charge transfer transitions. The emission properties of the ligands were described and displayed emission maxima centred at 398 nm (**93**, **PhenNap(Cl)**), 420 nm (**92**, **PhenNap(Br)**), 450 nm (**91**, **PhenNap(NO₂)**) and 521 nm (**99**, **PhenNap(NH₂)**).

Similarly, the Ru(II)-polypyridyl complexes, **94–96**, bearing these ligands were obtained and their structural properties were discussed where again, common features such as the substantial degree of rotation (71–89°) between the central rings of the **PhenNap** ligands were observed. The amino derivatised complex was also obtained [Ru(**Phen**)₂**97**]Cl₂, **99**, being obtained, but again, as was observed from the reduction of the nitro ligand, this synthesis proved challenging. The structural properties of the series identified interactions potentially suitable for DNA association, such as π -stacking interactions and hydrogen/halogen bonding, arising from multiple areas of the complexes were also observed.

The cyclic voltammetry of **94** and **95** displayed complex redox behaviours with well-defined oxidations. Complex **94** was found to be both simultaneously easier to reduce and oxidise than the bromo counterpart, **95**, the former being consistent with the presence of the electron withdrawing nitro group. The spectroscopic properties of complexes **94**, **95**, **96** and **99** were investigated in 10 mM aqueous sodium phosphate buffer (pH = 7.4) at room temperature and their UV-Vis absorption, excitation and emission spectra displayed features characteristic of both ruthenium-centred-MLCT and **Nap** chromophores. For complexes **94**, **95** and **96**, excitation of both the **Nap**-centred and MLCT-based absorption bands resulted in a weak, broad and unstructured ³MLCT luminescence band centred at 610 nm with Φ_{PL} within the range of <0.001–0.02 %. For the amine derivative, **99**, the superimposable absorption and excitation spectra demonstrates efficient (¹Nap*⁻¹MLCT*) energy transfer followed by intersystem crossing to generate the ³MLCT state with emission maximum centred at 612 nm and Φ_{PL} yield at 0.70 %. The complexes were found to bind DNA strongly, with binding constants values in the order of 10⁶ M⁻¹ and binding sizes of approximately one to two base pairs except for the nitro-substituted complex, **94**, where a weaker binding constant of 10⁵ M⁻¹ and a larger binding site size of four base pairs was observed. The complexes displayed luminescence enhancement upon binding to DNA, where the highest luminescence enhancement of 34-fold was observed for chloro-substituted complex **96** which was substantially greater than the literature values for structurally similar systems which displayed a maximum of 2.6-fold emission enhancement in the free form.

In Chapter 4, the synthetic investigation toward a series of asymmetric **Nap**-like ligands which displayed aromatic conjugation was described. Many challenges were faced when attempting these studies, with appropriate discussion therein. The synthesis toward the **TB**, **98**, and its dinuclear Ru(II) complex, **100**, was also presented. Due to the synthetic difficulties experienced for the extended aromatic ligands, which was believed to involve the formation of structural

isomers, known **Nap**-like structures, such as **NBI** and the extended aromatic ligand **dppz** were synthesised for use as ligands for the development of novel Ir(III)-complexes. Furthermore, the synthesis and photophysical evaluation of an Ir(III)-**Nap** complex bearing ligand **80** was also described.

In Chapter 5 the structural analysis of ten crystal structures (**C1–C10**) from the single crystals X-ray diffraction experiments was discussed. As this chapter includes various classes of compounds the conclusions regarding each individual structures were included within the results and discussion part of this chapter and will not be included here.

In conclusion, the work presented in this thesis was highly interdisciplinary, and connected many fields of research including organic, inorganic, and supramolecular chemistry. The research displayed the powerful advantage of combining different structural techniques to control and understand relevant interactions between luminescent Ru(II)-polypyridyl complexes and nucleic acid, through effective design of the ligands.

Chapter 7:

Experimental

7.1: General Experimental Techniques & Apparatus

Cyclic voltammetry (CV): Electrochemical measurements were performed on an Electrochemical Analyzer potentiostat model 600D from CH Instruments with a PC controlled electrochemical workstation. Solutions for cyclic voltammetry were prepared in dry MeCN obtained from an in-house SPS system. A minimum volume of solvent (*ca.* 2–3 mL) was used. Pt wires were used as the pseudo-reference electrode and the counter electrode, while a Pt disk electrode was used for the working electrode. Both before and after the experiments, the Pt disk electrode was cleaned by polishing the surface in an ethanol slurry of abrasive powder (gamma alumina powder, 0.05 μm , CH Instruments), before sonicating in ethanol, washing with acetone, water, and finally ethanol, and then drying. The electrochemical cell consisted of a glass vessel (*ca.* 40 mL) covered with a plastic lid and drilled with four holes: one for each electrode, and one for nitrogen purging. The vessel was sealed with parafilm to ensure minimal oxygen contamination. Tetra(*n*-butyl)ammonium hexafluorophosphate (TBAPF₆; *ca.* 0.1 M) was used as the supporting electrolyte, with the solution degassed by vigorous bubbling for 10 min prior to scanning the blank sample to ensure a clean background. Small quantities of sample were then added in portions, and then the mixture was degassed and scanned. This process was repeated until an adequate signal-to-background ratio became apparent. The measurements in the appropriate electrochemical windows were then carried out, at scan rates of 0.1 and 1 V s⁻¹. At the end of each experiment, ferrocene was added as an internal reference, and then the scans were repeated, again at 0.1 and 1 V s⁻¹. Estimated error: ± 30 mV. The redox potentials are reported relative to the ferrocenium/ferrocene (Fc⁺ /Fc) redox couple.

Elemental Analysis: Elemental analyses were conducted at the Microanalytical Laboratory, School of Chemistry, University College Dublin (UCD).

High-Performance Liquid Chromatography (HPLC) – Reverse phase HPLC was performed on a Shimadzu Prominence system by PhD student Mark Nolan on complex [Ru(phen)₂PhenNap(NO₂)]Cl₂, [94](#). Ascentis Express C18 columns (90 Å, C18, 100 x 4.6 mm, 5 μm) were used. UV absorption signals were detected with a Photo Diode Array (PDA) detector at wavelengths of 200–400 nm eluting with H₂O:MeCN (1:1, 0.1% formic acid).

Infrared (IR) Spectroscopy: Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a Universal ATR Sampling Accessory for solid samples.

The baseline was subtracted from the FT-IR spectra for clear visualisation of the bands associated with the molecules under investigation.

Mass Spectrometry (MS): Electrospray ionisation (ESI) mass spectra were recorded on a Micromass LCT spectrometer or a MALDI QToF Premier, running Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with HPLC-grade methanol or acetonitrile. High resolution mass spectra were determined by a peak matching method, using leucine enkephalin (Tyr-Gly-Gly-Phe-Leu) as the standard reference ($m/z = 556.2771$). All accurate mass were reported within ± 5 ppm.

Melting Point (mp): Melting points were determined using an IA9000 digital melting point apparatus.

Microwave reactions: Reactions requiring microwave irradiation were carried out using a Biotage® Initiator microwave synthesizer.

Nuclear Magnetic Resonance Spectroscopy (NMR): All NMR spectra were recorded using either a 400 MHz Bruker Spectrospin DPX-400 or 600 MHz AV-600 spectrometer, operating at 400/600 MHz for ^1H NMR, 101/151 MHz for ^{13}C NMR and 162 MHz for ^{31}P NMR. Chemical shifts (δ) are referenced relative to the internal solvent signals. All NMR spectra were carried out at *ca.* 22 °C except for those performed at variable temperatures, all of which are stated in the relevant sections.

X-Ray Crystallography: Single crystals were grown and obtained by individual methods discussed in the relevant sections. X-ray data were collected on a (1) Bruker APEX-II DUO diffractometer using microfocus Cu K α ($\lambda = 1.5405 \text{ \AA}$) or graphite-monochromated Mo K α ($\lambda = 0.71073 \text{ \AA}$) radiation or (2) a Bruker D8 Quest using graphite-monochromated Mo K α ($\lambda = 0.71073 \text{ \AA}$) radiation. Single crystals were mounted on MiTeGen microloop in NVH immersion oil and all data collections were carried out using standard ω and ϕ scans at 100 K with temperature control using an Oxford Cryosystem Cobra cryostream or Cryostream, respectively. The data were reduced²⁸⁰ and multi-scan absorption corrections applied using SADABS²⁸¹ within the Bruker APEX3 software suite.²⁸² Datasets were solved using the intrinsic phasing routine within XT²⁷⁸ and refined on F^2 using least squares techniques with XL²⁸³ within the OLEX-2 software.²⁸⁴ Non-hydrogen atoms were located from their residuals on the Fourier difference map, and hydrogen atoms were either placed

in calculated positions with U_{iso} dependencies derived from their carrier atoms, or manually located on the Fourier map and refined subject to a distance restraint/constraint.

7.2: Photophysical Characterisation

All samples were prepared in spectroscopic grade acetonitrile (MeCN), DMSO or in freshly prepared sodium phosphate buffer solution (pH = 7.4) in varying μ M concentrations. Degassed samples were prepared by vigorous bubbling of Ar through the mother solvent for 30 min followed by gentle bubbling with N_2 *in situ* for 2 min directly before obtaining each measurement.

Luminescence Spectroscopy: Luminescence measurements were performed in an optical path 1 cm quartz cuvette (3 mL) on a Varian Cary Eclipse Fluorimeter. Emission spectra and excitation spectra were obtained with varying slit widths. Individual parameters are provided where appropriate.

Luminescence Quantum Yields (Φ_{em}): Emission quantum yields were determined using the optically dilute method.^{285,286} Stock solutions of absorbance values of *ca.* 0.3 at λ_{ex} = 450 nm were prepared and diluted to yield solutions with absorbances of *ca.* 0.1, 0.075, 0.05 and 0.025. The Beer-Lambert Law was found to be linear at these concentrations. Emission spectra were obtained in aerated and deaerated conditions (the latter conditions specified in the relevant sections). Linear regression analysis between the absorption and emission intensity verified the relationship with R^2 values surpassing 0.99 in all cases. Individual relative quantum yield values were calculated for each solution and reported values represent the slope value [Equation 6.1]:

$$\varphi_s = \varphi_r \left(\frac{A_r}{A_s} \right) \left(\frac{I_s}{I_r} \right) \left(\frac{n_s}{n_r} \right)^2 \quad [6.1]$$

where Φ_r is the absolute quantum yield of the reference and n is the refractive index of the solvent. A and I describe the absorbance values at the excitation wavelength and integrated area under the emission curves, respectively while r and s subscripts denote the reference and sample values, respectively. A solution of Ru(**bpy**)₃Cl₂ (Φ_r = 2.8%) was used as the external reference.^{198,197}

Time Resolved Luminescence: Luminescence lifetime (τ_{PL}) values were determined by time correlated single-photon timing (TCSPC), recorded on a Horiba Scientific Fluorolog FL 3-22 equipped with a FluoroHub v2.0 single photon timing module by using a 458 nm pulsed

nano-LED (Horiba N-460) as the excitation source. The emission lifetimes represent an average of two replicate measurements with 10,000 counts at the peak channel. The decays were analysed using the Horiba DAS6 software and the data was fit to a sum of the minimum number of exponentials, n [Equation 6.2], employing a proprietary grid-search error minimisation algorithm.

$$I_{em}(t) = \sum_{i=1}^n a_i \exp\left(-\frac{t}{\tau_i}\right) \quad [6.2]$$

where $I_{em}(t)$ is the intensity of luminescence at time, t , a_i represents the i^{th} pre-exponential factor and τ_i is the i^{th} emission lifetime. The goodness-of-fit was assessed by the χ^2 value which was less than 1.2 in all cases.

UV-Vis Absorption Spectroscopy: UV-vis absorption spectra were recorded in an optical path 1 cm quartz cuvette (3 mL) on a Varian CARY 50 spectrophotometer with a wavelength range of 200–900 nm and a scan rate of 600 nm min⁻¹. Baseline correction measurements were used for all spectra. **Molar absorption coefficients (ϵ)** were determined by measuring the absorption spectra of the metal complex at different concentrations. The absorption maxima were plotted versus concentration of complex and absorption coefficients were calculated from the slope of the best linear regression according to the Bouguer-Lambert-Beer law [Equation 6.3]:

$$A(\lambda) = \log \frac{I_0}{I} = \epsilon \times c \times l \quad [6.3]$$

where A is the absorbance, I_0 and I are the intensity of light passing through the reference and the sample cells, respectively, ϵ is the molar absorption coefficient (M⁻¹ cm⁻¹), c is the concentration (M) and l is the path length (cm).

7.3: DNA Binding Techniques

Circular Dichroism Spectroscopy (CD): CD studies were performed in an optical path 1 cm quartz cuvette (3 mL) on a JASCO J-815 circular and linear spectropolarimeter with a wavelength range of 163–900 nm. Spectral results represent an average of three scans and were acquired between 600–200 nm at a scan rate of 200 nm min⁻¹, response of 1 s, data pitch of 1 nm, and bandwidth of 1 nm. CD studies were carried out in sodium phosphate buffered solutions (10 mM) at constant DNA concentration (150 μ M, $A_{260} \approx 1$) and P/D ratios of 50, 20, 10 and 5.

Linear Dichroism Spectroscopy (LD):²⁸⁷ LD studies were performed in a quartz Couette flow LC cell with outer and inner diameters of 5.0 and 2.9 mm, respectively on a JASCO J-815 circular and linear spectropolarimeter equipped with a Couette flow cell system with a wavelength range of 163–900 nm. Spectral results represent an average of three scans and were acquired between 600–200 nm. LD studies were carried out in sodium phosphate buffered solutions (10 mM) at constant DNA concentration (300 μ M, $A_{260} \approx 2$) and P/D ratios of 15, 5 and 2.5 using 40 μ L volumes of sample and the spin rate was 6000 rpm.

Spectroscopic DNA Titrations: Steady state absorption and emission titrations were performed on Ru(II)- samples (dye) at differing concentrations (individual details are listed in the appropriate section) with concentrations not exceeding 1×10^{-5} M. Titrations were performed at constant temperature (298 K) by monitoring changes in the absorption, emission and excitation spectra of the Ru(II)-complex (phosphate buffer, pH = 7.4) upon addition of aliquots of ctDNA. Data is expressed as nucleotide phosphate-to-dye ratio (P/D) and results represent a minimum of two independent titrations. Derived parameters: DNA binding constants, K_b and binding site size, n , were determined according to the modified Bard equation [Equation 2.3]:^{97,205}

$$\varepsilon_a = \frac{b - \left(b^2 - \frac{4K_b^2 C_t [DNA]}{n} \right)^{\frac{1}{2}}}{2K_b C_t} (\varepsilon_b - \varepsilon_f) + \varepsilon_f \quad [2.3]$$

where $b = 1 + K_b C_t + K_b [DNA]/n$, and C_t represents the total concentration of the complex.

Thermal Denaturation: Thermal denaturation studies were performed on a thermoelectrically coupled Perkin Elmer LAMBDA 35 UV-Vis Spectrophotometer. Buffered solutions containing 150 μ M of ctDNA (optical density of approximately 1.0 for the DNA absorption at 260 nm) were used. The temperature in the cell was ramped from 30 to 90 $^{\circ}$ C, at a rate of 1 $^{\circ}$ C min⁻¹ and the absorbance at 260 nm was measured every 0.2 $^{\circ}$ C. The melting temperature value was obtained from the maximum of the first derivative of the absorbance ($\delta A/\delta T$).

Viscometry Measurements:²⁸⁸ Relative viscosity was measured with a Cannon-Manning semimicro viscometer at 295 K. Constant ctDNA concentration of 1.2 mM and a total sample volume of 1 mL were used. DNA was sheared with ultrasounds (JENCONS Ultrasonic Processor, 100% power, in 1 min intervals (30 s ON/30 s OFF) on ice for a total time of 20

min) to obtain fragments of *ca.* 200 bp; however, the DNA fragment lengths were not confirmed *via* agarose gel electrophoresis. Dye/phosphate concentration ratios (D/P) ranging from 0.02 to 0.25 were measured. Viscosity (η) was calculated as sample flow time minus buffer flow time. $(\eta/\eta_0)^{1/3}$ values (where η_0 is the viscosity of untreated ctDNA) were plotted against D/P.

7.4: Materials

Anion Exchange: The Amberlite IRA-400 (chloride form) resin was prepared according to a modification of the manufacturer protocol before use.²⁸⁹ The resin was soaked in HPLC grade methanol for 12 h and the solvent was removed by filtration. This procedure was repeated twice more. The beads were then washed and suspended in water overnight. The resin was exercised by washing the beads with an aqueous, dilute solution of NaOH (0.1 M, 300 mL), followed by water until the washings ran neutral. The beads were washed with a dilute solution of HCl (0.1 M, 500 mL) and with water, again, until the washings ran neutral.

Buffered Water Solutions: Sodium phosphate buffered water (10 mM) was prepared by diluting appropriate amounts of solutions of Na₂HPO₄ and NaH₂PO₄ with distilled water. The pH was adjusted to 7.4 *via* addition of aqueous NaOH (1 M) and was monitored using a pH meter.

Chemicals and Solvents: All chemicals were obtained from Sigma-Aldrich, TCI, Alfa Aesar, Fluorochem Ireland or Precious Metals Online and unless specified, were used without further purification. Chromatographic columns were run using either Silica gel 60 Å (230–400 mesh) or Aluminium oxide 60 Å (Brockmann Grade I) (50–200 µm). Analytical thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F₂₅₄ silica gel plates or aluminium oxide neutral plates. Solvents for synthetic purposes were used at general purpose reagent (GPR) or HPLC grade. Dry solvents for electrochemical measurements were obtained from Innovative Technology Incorporated solvent purification system (SPS). Deuterated solvents for NMR use were purchased from Apollo Ltd. Deionised water was obtained from PURELAB Flex water purifier. ctDNA was obtained from Sigma Aldrich and was stored at 2 °C as its sodium salt. [Ru(COD)Cl₂]_n, **85**, *cis*-[Ru(**bpy**)₂Cl₂], **86**, *cis*-[Ru(**phen**)₂Cl₂], **87** and *cis*-[Ru(**TAP**)₂Cl₂], **88** were prepared according to the literature procedures.^{182,234}

DNA Solution Preparation: Solutions of DNA were prepared by dissolving *ca.* 10-20 mg of ctDNA in freshly prepared sodium phosphate buffer (10 mM, pH = 7.4, 10 mL). The mixture was agitated on a shaker for 1 h and refrigerated overnight at 4 °C. The solution was then passed through a syringe filter (0.45 µm polyethersulfone (PES) membrane, PALL Acrodisc®) and stored at 4 °C for no more than four days. Sample purity was determined from the UV absorbance ratio at 260:280 nm for each sample prior to use and was found to be ≥ 1.8 as per manufacturer specification sheet. The concentration of each DNA sample was determined spectrophotometrically using the molar absorptivity value of $6600 \text{ M}^{-1} \text{ cm}^{-1}$ (nucleotide) at 260 nm.

7.5: General Synthetic Procedures

7.5.1: General Procedure 1: Synthesis of PhenNap Ligands¹⁸⁵

The appropriate naphthalic anhydride (1,8-naphthalic anhydride, **79**, 4-nitro-1,8-naphthalic anhydride, **101**, 4-bromo-1,8-naphthalic anhydride, **102**, or 4-chloro-1,8-naphthalic anhydride, **103**) (1 eq.) and 5-amino-1,10-phenanthroline, **78**, (1 eq.) were added to glacial acetic acid (8 mL) in a pressure vessel and heated to 125 °C for 5 h. The solutions were cooled to room temperature and water was added to promote crystallisation. Products were collected *via* vacuum filtration, washed with a sat. solution of NaHCO_3 (4 × 50 mL), water (4 × 50 mL), cold EtOH (50 mL) and Et_2O (30 mL). The desired solids were isolated as powders. Recrystallisation was performed where necessary and is stated in the relevant section.

7.5.2: General Procedure 2: Synthesis of $[\text{Ru}(\text{L})_2\text{Cl}_2]$ ^{182,234}

$\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, (1.00 g, 3.82 mmol, 1.0 eq.) and cyclooctadiene (**COD**) (9.38 mL, 76.49 mmol, 20.0 eq.) were added to degassed EtOH (25 mL). The mixture was heated at 80 °C under Ar for 72 h. The resulting mixture was cooled and the insoluble brown solid was collected *via* vacuum filtration, washed with EtOH (5 × 100 mL) and dried *in vacuo* to yield $[\text{Ru}(\eta\text{-COD})\text{Cl}_2]_n$, **85**, (935 mg, 3.35 mmol, 88%). The appropriate polypyridyl ligand (**bpy**, **phen** or **TAP**) (2.0 eq.) and **85** (1.0 eq.) were added to DMF (12 mL) and the mixture was degassed *via* vigorous bubbling with Ar for 15 min. The mixture was subject to microwave irradiation for 40 min at 140 °C and then cooled to room temperature. Acetone (20 mL) was added to the resulting purple suspension and the mixture was cooled to -15 °C for 72 h. The purple powder was isolated via centrifugation after washing with acetone, Et_2O , cold EtOH and H_2O several times until the aqueous washings ran clear. The respective products *cis*-

[Ru(**bpy**)₂Cl₂], **86**, *cis*-[Ru(**phen**)₂Cl₂], **87**, and *cis*-[Ru(**TAP**)₂Cl₂], **88**, were isolated as purple powders in 82%, 66% and 68%, respectively.

7.5.3: General Procedure 3: Synthesis of [Ru(L)₂L']Cl₂¹⁸³

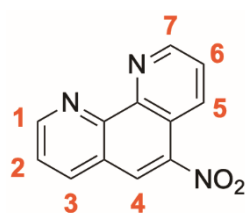
The appropriate *cis*-[Ru(L)₂Cl₂] precursor, **86**, **87**, or **88** (1.0 eq.) and the appropriate **PhenNap** ligand, **80**, **91**, **92** and **93** (1.1 eq.) were added to EtOH:H₂O (7 mL, 1:1 v/v) and the mixture was degassed by vigorous bubbling with Ar for 15 min. The mixture was subject to microwave irradiation for 40 min at 140 °C and was cooled to room temperature. The solvent was evaporated under reduced pressure and the red solid products were dried *in vacuo*. The crude products were purified by chromatography (SiO₂, MeCN:H₂O:NaNO_{3(aq.)} 40:10:1) or (Al₂O₃, MeCN:H₂O 10:0 to 9:1) yielding a red solid which was dried *in vacuo*. Individual further purification details are provided in the relevant sections.

7.5.4: General Procedure 4: Synthesis of Iridium Dimers²⁴³

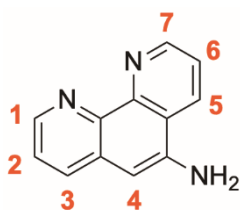
The synthesis of both Iridium dimers, **116** and **117**, was carried out by reacting the appropriate cyclometallating ligand **NBI** or **ppy**, where **NBI** = naphthalenebenzimidazole and **ppy** = 2-phenylpyridine (2.2 eq.) with IrCl₃·3H₂O (1.0 eq.) in 2-ethoxyethanol:H₂O (3:1) at 135 °C for 6 h under inert atmosphere. The dimers were isolated by filtration and used without further purification.

7.6: Synthesis and Characterisation of Compounds Described in Chapter 2

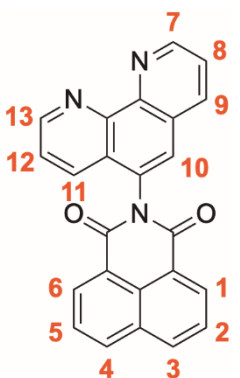
5-Nitro-1,10-phenanthroline, **77**²¹⁹



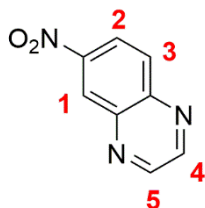
1,10-phenanthroline, **phen** (5.0 g, 27.75 mmol, 1.0 eq.) was dissolved in H₂SO_{4(conc.)} (30 mL) and the solution was heated to 160 °C. Fuming HNO₃ (15 mL) was added and the solution was stirred at 160 °C for 4 h. The solution was cooled to 0 °C and was neutralised with aqueous NaOH (6 M, 200 mL). The yellow precipitate was isolated *via* vacuum filtration and washed with H₂O (3 × 200 mL) until the washings ran clear and dried *in vacuo* (4.02 g, 17.9 mmol, 64%). δ_{H} (400 MHz, DMSO-*d*₆): 9.28 (d, 1H, ³*J* = 4.3 Hz, ⁴*J* = 1.5 Hz, H₁), 9.23 (d, 1H, ³*J* = 4.2 Hz, ⁴*J* = 1.3 Hz, H₇), 9.03 (s, 1H, H₄), 8.88 (d, 1H, ³*J* = 8.6, ⁴*J* = 1.5 Hz, H₃), 8.78 (d, 1H, ³*J* = 8.1 Hz, ⁴*J* = 1.3 Hz, H₅), 8.02-7.89 (m, 2H, H₂₊₆). δ_{C} (101 MHz, DMSO-*d*₆): 153.7, 151.6, 147.1 (q), 145.7 (q), 144.3 (q), 139.0, 132.6, 126.4, 126.0 (q), 125.04, 124.98, 120.8 (q).

5-Amino-1,10-phenanthroline, 78¹⁸⁴

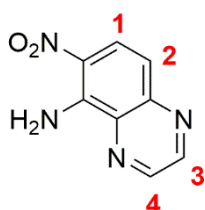
5-Nitro-1,10-phenanthroline, **77** (4.00 g, 17.8 mmol, 1.0 eq.) and 10 wt% Pd/C (0.95 g, 8.9 mmol, 0.5 eq.) were suspended in EtOH (100 mL) and the mixture was heated to 80 °C. Hydrazine monohydrate (64 – 65%, 4.41 mL, 5.1 eq.) was added dropwise and the mixture was heated at reflux overnight. The mixture was cooled to room temperature and filtered through a pad of Celite. The solid residue was washed with EtOH and the solvent was removed under reduced pressure. The resulting yellow/orange powder was washed with H₂O (*ca.* 600 mL) until the washings ran clear and the solid was isolated as a yellow powder (2.50 g, 12.8 mmol, 72%). δ_{H} (400 MHz, DMSO-*d*₆): 9.05 (d, 1H, $^3J = 4.2$ Hz, $^4J = 1.5$ Hz, H₇), 8.69 (m, 2H, H₁₊₅), 8.04 (d, 1H, $^3J = 8.1$ Hz, $^4J = 1.6$ Hz, H₃), 7.73 (dd, 1H, $J = 8.3, 4.2$ Hz, H₆), 7.50 (dd, 1H, $J = 8.1, 4.2$ Hz, H₂), 6.88 (s, 1H, H₄) 6.16 (s, 2H, NH₂). ESI⁺-HRMS (*m/z*): [M + H]⁺ calcd. for C₂₄H₁₄N₃O₂, 196.0869; found, 196.0872.

N-(1,10-Phenanthroline)-naphthalene-1,8-dicarboximide, 80¹⁵¹

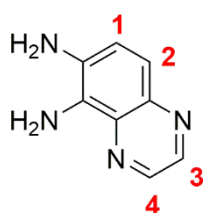
Compound **80** was synthesised according to General Synthetic Procedure 1 using 1,8-naphthalic anhydride, **79** (100 mg, 0.5 mmol, 1 eq.) and 5-amino-1,10-phenanthroline, **78** (99 mg, 0.5 mmol, 1 eq.) yielding the product as a beige powder (184 mg, 0.5 mmol, 98%). δ_{H} (400 MHz, DMSO-*d*₆): 9.21 (d, 1H, $^3J = 4.3$ Hz, $^4J = 1.7$ Hz, H₇), 9.16 (d, 1H, $^3J = 4.2$ Hz, $^4J = 1.6$ Hz, H₁₃), 8.60 (d, 2H, $^3J = 8.3$ Hz, $^4J = 0.8$ Hz, H₆₊₁₁), 8.56 (d, 2H, $^3J = 7.4$ Hz, $^4J = 0.9$ Hz, H₄₊₃), 8.54 (d, 1H, $^3J = 8.1$ Hz, $^4J = 1.7$ Hz, H₉), 8.50 (d, 1H, $^3J = 8.3$ Hz, $^4J = 1.6$ Hz, H₁₁), 8.21 (s, 1H, H₁₀), 7.96 (m, 2H, H₅₊₂), 7.86 (dd, 1H, $J = 8.1, 4.3$ Hz, H₈), 7.70 (dd, 1H, $J = 8.3, 4.2$ Hz, H₁₂). δ_{C} (151 MHz, DMSO-*d*₆): 174.5 (q), 164.6 (q), 151.3, 150.7, 146.4 (q), 145.9 (q), 137.0, 135.3 (2C), 132.6, 132.1 (q), 131.9 (q), 131.5 (2C), 128.9 (q), 128.2 (q), 128.0, 127.8 (2C), 126.9 (q), 124.24, 124.15, 123.12 (2C, q). ν_{max} (ATR/cm⁻¹): 3057, 1771, 1743, 1707, 1663, 1584, 1510, 1419, 1348, 1304, 1234, 1186, 1117, 1020, 882, 845, 801, 776, 739, 702. ESI⁺-HRMS (*m/z*): [M + H]⁺ calcd. for C₂₄H₁₄N₃O₂, 376.1081; found, 376.1082.

6-Nitroquinoxaline, 82¹⁸⁶

4-Nitro-*o*-phenylenediamine (4.00 g, 26.1 mmol, 1.0 eq.) was suspended in EtOH (100 mL). Oxalaldehyde aq. solution (40%, 6 mL, 82.8 mmol, 5.0 eq.) was added and the mixture was heated at reflux for 2 h. The solvent was removed under reduced pressure. The resulting dark orange/red solid was dissolved in water (200 mL) and the solution was extracted with DCM (4 x 50 mL). The organic solution was dried over MgSO₄ and the solvent was removed under reduced pressure to yield a yellow/orange solid, which was recrystallised from 2-propanol (300 mL). The product was isolated by vacuum filtration as golden crystalline needles (3.84 g, 21.94 mmol, 84%). δ_{H} (400 MHz, DMSO-*d*₆): 9.17 (s, 2H, H₄₊₅), 8.93 (s, 1H, ⁴*J* = 2.5 Hz, H₁), 8.58 (d, 1H, ³*J* = 9.1 Hz, ⁴*J* = 2.5 Hz, H₂), 8.36 (d, 1H, *J* = 9.1 Hz, H₃). δ_{C} (151 MHz, DMSO-*d*₆): 148.9, 148.1, 147.6 (q), 144.7 (q), 141.0 (q), 131.3, 125.3, 123.6. ESI⁺-HRMS (*m/z*): [M + H]⁺ calcd. for C₈H₆N₃O₂, 176.0455; found, 176.0482.

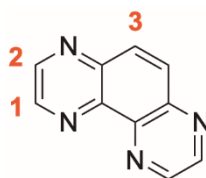
5-Amino-6-nitroquinoxaline, 83¹⁸⁷

Sodium metal (1.30 g, 56.5 mmol, 3.3 eq.) was added portion-wise to distilled MeOH (75 mL) and cooled to 0 °C. A solution of hydroxylamine hydrochloride (1.78 g, 25.6 mmol, 1.5 eq.) in distilled MeOH (30 mL) was added to the cold sodium methoxide solution. The resulting supernatant was added to a stirring suspension of 6-nitroquinoxaline, **82** (3.00 g, 17.1 mmol, 1.0 eq.) in dry boiling MeOH. The mixture was heated under reflux for 90 min. The crude product was recrystallised from a mixture of acetic acid and water (3:1) and was isolated as golden needles (1.40 g, 7.35 mmol, 43%). δ_{H} (400 MHz, DMSO-*d*₆): 9.10 (d, 1H, *J* = 1.9 Hz, H_{3/4}), 8.94 (d, 1H, *J* = 1.9 Hz, H_{3/4}), 8.51 (s, 2H, NH₂), 8.30 (d, 1H, *J* = 9.7 Hz, H₁), 7.18 (d, 1H, *J* = 9.7 Hz, H₂). δ_{C} (151 MHz, DMSO-*d*₆): 149.3, 146.3 (2C, q), 145.6 (q), 143.7, 134.6 (q), 126.6, 114.8.

5,6-Diaminoquinoxaline, [84](#)¹⁸⁷

5-Amino-6-nitroquinoxaline, [83](#) (1.50 g, 7.9 mmol, 1.0 eq.) and 10 wt% Pd/C (0.17 g, 1.6 mmol, 0.2 eq.) were suspended in EtOH (80 mL) and heated at reflux for 1 h. Hydrazine monohydrate (64 – 65%, 7.65 mL, 0.158 mol, 20.0 eq.) was added and the mixture was heated at reflux for

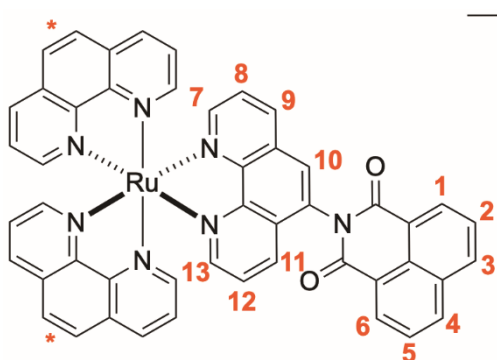
1 h. The mixture was filtered hot through a pad of Celite and washed with DCM (80 mL). The solvent was removed under reduced pressure to isolate a red powder which was dissolved in water and extracted into EtOAc. The organic layer was dried over MgSO₄ the solvent removed under reduced pressure and the solid product dried *in vacuo* (1.24 g, 7.7 mmol, 98%). δ_{H} (400 MHz, DMSO-*d*₆): 8.58 (d, 1H, *J* = 1.9 Hz, H_{3/4}), 8.50 (d, 1H, *J* = 1.9 Hz, H_{3/4}), 7.26 (d, 1H, *J* = 8.8 Hz, H_{1/2}), 7.19 (d, 1H, *J* = 8.8 Hz, H_{1/2}), 5.26 (s, 2H, NH₂), 5.14 (s, 2H, NH₂). δ_{C} (151 MHz, DMSO-*d*₆): 142.4, 140.6, 137.4 (q), 133.5 (q), 132.9 (q), 126.5 (q), 122.3, 116.8.

1,4,5,8-Tetraazaphenazine, TAP¹⁸⁶

5,6-Diaminoquinoxaline, [84](#) (1.00 g, 6.2 mmol, 1.0 eq.) was suspended in EtOH (100 mL) and oxalaldehyde aq. solution (40%, 5 mL, 17.0 eq.) was added and the solution was heated at reflux for 2 h. The solvent was removed under reduced pressure and the resulting solid was suspended

in water (100 mL) and extracted into DCM. The organic layer was dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (SiO₂, DCM/acetone 8:2) followed by recrystallisation from EtOH. The product was isolated as golden needles (185 mg, 1.0 mmol, 16%). m.p. 241 – 243 °C (lit.²⁹⁰ 244 °C). δ_{H} (400 MHz, DMSO-*d*₆): 9.23 (d, 2H, *J* = 1.9 Hz, H₁), 9.20 (d, 2H, *J* = 1.9 Hz, H₂), 8.37 (s, 2H, H₃). δ_{C} (101 MHz, DMSO-*d*₆): 147.4, 146.1, 144.0 (q), 140.7 (q), 132.0. ESI⁺-HRMS (*m/z*): calcd. for C₁₀H₆N₄Na 205.0485 found [M + Na]⁺ 205.0487.

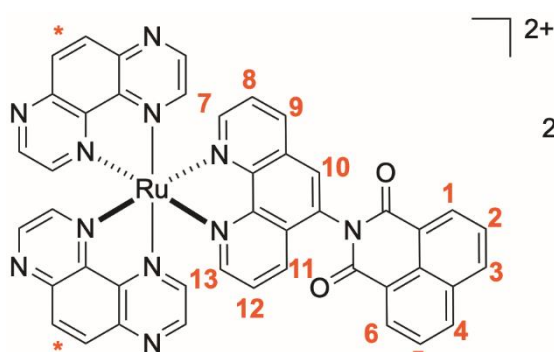
Bis(1,10-phenanthroline)(*N*-(1,10-phenanthroline)-naphthalene-1,8-dicarboximide)ruthenium(II) chloride, [Ru(phen)₂80**]Cl₂, **75**¹⁸³**



Complex **75** was prepared according to General Synthetic Procedure 3 employing precursors [Ru(phen)₂Cl₂], **87** (100 mg, 0.2 mmol, 1.0 eq.) and **PhenNap**, **80** (80 mg, 0.2 mmol, 1.1 eq.). The crude product was purified by column chromatography (Al₂O₃, MeCN:H₂O 10:0 to 9:1) to yield a red powder

(86 mg, 0.1 mmol, 50%). Calc. for C₄₈H₂₉N₇O₂Cl₂Ru + 5H₂O: C, 57.78; H, 3.94; N, 9.83. Found: C, 57.14; H, 3.61; N, 9.69. m.p._(decom.) > 212 °C. δ_H (600 MHz, CD₃CN): 8.74 – 8.59 (m, 7H, H_{9+4+3+phen}), 8.54 (m, 2H, H₆₊₁), 8.47 (d, 1H, ³J = 8.5 Hz, ⁴J = 1.1 Hz, H₁₁), 8.44 (s, 1H, H₁₀), 8.36 – 8.26 (m, 4H, H_{phen}), 8.20 (d, 1H, ³J = 5.2 Hz, ⁴J = 1.2 Hz, H_{phen}), 8.18 (d, 1H, ³J = 5.2 Hz, ⁴J = 1.2 Hz, H_{phen}), 8.15 (d, 1H, ³J = 5.3 Hz, ⁴J = 1.2 Hz, H₇), 8.12 (d, 1H, ³J = 5.2 Hz, ⁴J = 1.1 Hz, H₁₃), 8.09 – 8.05 (m, 2H, H_{phen}), 7.95 (m, 2H, H₅₊₂), 7.80 – 7.70 (m, 3H, H_{8+phen}), 7.68 (m, 2H, H_{phen}), 7.58 (dd, 1H, J = 8.5, 5.2 Hz, H₁₂). δ_C (600 MHz, CD₃CN): 164.53 (q), 164.50 (q), 153.9, 153.7, 153.1, 153.04, 153.02, 152.94, 148.4 (q), 148.01 (q), 147.94 (q, 2C), 147.91 (q), 147.89 (q), 137.1, 137.02, 136.95, 136.90, 136.88, 136.8 (q), 135.3 (2C), 133.5 (q), 132.5, 132.0 (q), 131.52, 131.48, 131.13 (q), 131.09 (q), 131.04 (q), 131.01 (q), 130.1 (q), 129.1 (q), 129.0, 128.8 (q), 128.12, 128.09 (2C), 128.0, 127.42, 127.38, 126.3, 126.2, 126.1, 126.0, 125.94 (2C), 125.89 (q). ν_{max} (ATR/cm⁻¹): 3647, 3091, 1709, 1668, 1630, 1589, 1514, 1428, 1351, 1237, 1191, 1153, 1118, 1046, 833, 778, 737, 720. ESI⁺-HRMS (*m/z*): [M]⁺ calcd. for C₄₈H₂₉N₇O₂Ru, 418.5708; found, 418.5716.

Bis(1,4,5,8-tetraazaphenanthrene)(*N*-(1,10-phenanthroline)-naphthalene-1,8-dicarboximide)ruthenium(II) chloride, [Ru(TAP)₂80**]Cl₂, **76**¹⁸³**

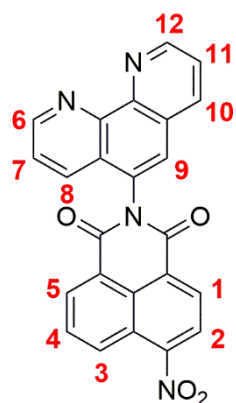


Complex **76** was prepared according to General Synthetic Procedure 3 employing precursors [Ru(TAP)₂Cl₂], **88** (132 mg, 0.25 mmol, 1 eq.) and **PhenNap**, **80** (95 mg, 0.21 mmol, 1.02 eq.). The crude product was purified by column chromatography (Al₂O₃, MeCN:H₂O 10:0 to 9:1) to yield a red

powder (87 mg, 0.1 mmol, 38%). m.p._(decom.) > 160 °C. δ_{H} (400 MHz, CD₃CN): 9.06 (d, 2H, J = 2.7 Hz, H_{TAP}), 8.99 (dd, 2H, J = 4.8 Hz, 2.7 Hz, H_{TAP}), 8.78 (d, 1H, J = 8.3 Hz, H₉), 8.68 (d, 1H, J = 7.3 Hz, H_{4/3}), 8.62 (m, 5H, H_{4/3+TAP}), 8.58 (d, 1H, J = 8.5 Hz, H₁₁), 8.54 (m, 2H, H₆₊₁), 8.46 (s, 1H, H₁₀), 8.34 (m, 2H, H_{TAP}), 8.32 (d, 2H, J = 2.7 Hz, H_{TAP}), 8.29 – 8.21 (m, 2H, H₁₃₊₇), 7.95 (m, 2H, H₅₊₂), 7.78 (dd, 1H, J = 8.3, 5.4 Hz, H₈), 7.65 (dd, 1H, J = 8.5, 5.2 Hz, H₁₂). δ_{C} (600 MHz, CD₃CN): 164.53 (q), 164.50 (q), 154.9, 154.7, 149.6, 149.2, 149.5 (2C), 149.4, 149.3, 148.9, 148.8, 147.5 (q), 147.0 (q), 145.6 (q), 145.52 (q), 145.47 (q), 145.4 (q), 142.5 (q), 142.4 (q, 2C), 138.6, 135.3 (2C), 134.1, 133.6 (q), 132.9, 132.84, 132.76, 132.7, 132.1 (q), 131.54, 131.51, 130.4 (q), 129.4 (q), 129.1, 128.8 (q), 127.43, 127.40, 126.6, 126.5, 122.69 (q), 122.67 (q). ν_{max} (ATR/cm⁻¹): 3350, 3053, 2163, 1705, 1665, 1589, 1487, 1427, 1407, 1373, 1349, 1273, 1235, 1189, 1154, 1101, 1047, 881, 849, 808, 780, 739, 723. ESI⁺-HRMS (m/z): [M]⁺ calcd. for C₄₄H₂₅N₁₁O₂Ru, 420.5613; found, 420.5614.

7.7: Synthesis and Characterisation of Compounds Described in Chapter 3

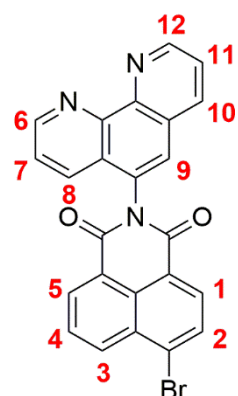
***N*-(1,10-Phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91**¹⁸⁵**



Compound **91** was synthesised according to General Synthetic Procedure 1 using 4-nitro-1,8-naphthalic anhydride, **101** (249 mg, 1.0 mmol, 1.0 eq.) and 5-amino-1,10-phenanthroline, **78** (200 mg, 1.0 mmol, 1.0 eq.) yielding the product as a beige powder (323 mg, 0.8 mmol, 75%). δ_{H} (400 MHz, CDCl₃): 9.31 (d, 1H, 3J = 4.3 Hz, 4J = 1.4 Hz, H₁₂), 9.27 (d, 1H, 3J = 4.2 Hz, 4J = 1.3 Hz, H₆), 8.97 (d, 1H, J = 8.7 Hz, H₃), 8.84 (d, 1H, J = 7.4 Hz, H₅), 8.79 (d, 1H, J = 8.0 Hz, H₁), 8.49 (d, 1H, J = 8.0 Hz, H₂), 8.32 (d, 3J = 8.1 Hz, 4J = 1.4 Hz, 1H, H₁₀), 8.10 (dd, 1H, J = 8.7, 7.4 Hz, H₄), 8.02

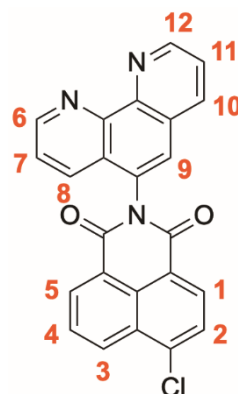
(d, 1H, $^3J = 8.3$ Hz, $^4J = 1.3$ Hz, H₈), 7.91 (s, 1H, H₉), 7.73 (dd, 1H, $J = 8.1, 4.3$ Hz, H₁₁), 7.61 (dd, 1H, $J = 8.3, 4.2$ Hz, H₇). δ_c (101 MHz, DMSO- d_6): 163.7 (q), 162.9 (q), 151.0, 150.3, 149.5 (q), 145.9 (q), 145.5 (q), 136.6, 132.1, 131.9, 130.9 (q), 130.1, 129.7, 129.4 (q), 129.1, 127.60 (q), 127.54 (q), 127.4, 126.3 (q), 124.2, 123.8, 123.6, 123.5 (q), 123.0 (q). $\nu_{\max}$ (ATR/cm⁻¹): 3368, 3073, 1713, 1673, 1624, 1591, 1523, 1510, 1463, 1407, 1368, 1344, 1330, 1236, 1190, 1146, 1107, 1084, 976, 897, 883, 844, 820, 787, 759, 739, 706. APCI⁺-HRMS (m/z): [M+H]⁺ calcd. for C₂₄H₁₃N₄O₄, 421.0931; found, 421.0938.

***N*-(1,10-Phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, 92**^{185,220}



Compound **92** was synthesised according to General Synthetic Procedure 1 using 4-bromo-1,8-naphthalic anhydride, **102** (287 mg, 1.0 mmol, 1.0 eq.) and 5-amino-1,10-phenanthroline, **78** (202 mg, 1.0 mmol, 1.0 eq.) yielding the product as a pink powder (247 mg, 0.5 mmol, 52%). δ_H (600 MHz, DMSO- d_6): 9.22 (d, 1H, $^3J = 4.3$ Hz, $^4J = 1.7$ Hz, H₁₂), 9.16 (d, 1H, $^3J = 4.2$ Hz, $^4J = 1.6$ Hz, H₆), 8.71 (d, 1H, $^3J = 8.5$ Hz, $^4J = 1.0$ Hz, H₃), 8.66 (d, 1H, $^3J = 7.4$ Hz, $^4J = 1.0$ Hz, H₅), 8.55 (m, 2H, H₁₀₊₈), 8.42 (d, 1H, $J = 7.9$ Hz, H₂), 8.34 (d, 1H, $J = 7.9$ Hz, H₁), 8.22 (s, 1H, H₉), 8.11 (dd, 1H, $J = 8.5, 7.4$ Hz, H₄), 7.86 (dd, 1H, $J = 8.1, 4.3$ Hz, H₁₁), 7.71 (dd, 1H, $J = 8.3, 4.2$ Hz, H₇). δ_c (101 MHz, DMSO- d_6): 163.63 (q), 163.57 (q), 150.9, 150.3, 145.9 (q), 145.5 (q), 136.5, 133.1, 132.1, 131.8, 131.5, 131.19, 131.15 (q), 130.2 (q), 129.6 (q), 129.4 (q), 129.0, 127.7 (q), 127.5, 126.3 (q), 123.8, 123.6, 123.5 (q), 122.7 (q). $\nu_{\max}$ (ATR/cm⁻¹): 3058, 1706, 1658, 1587, 1566, 1505, 1418, 1362, 1346, 1235, 1186, 1146, 1133, 1107, 1046, 954, 887, 849, 822, 802, 780, 741, 729, 707. ESI⁺-HRMS (m/z): [M + Na]⁺ calcd. for C₂₄H₁₂BrN₃NaO₂, 476.0005; found, 476.0005.

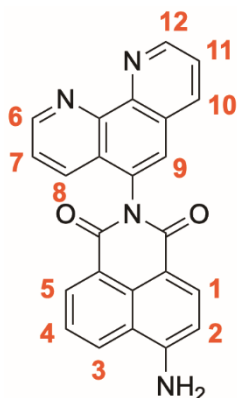
***N*-(1,10-Phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, 93**¹⁸⁵



Compound **93** was synthesised according to General Synthetic Procedure 1 using 4-chloro-1,8-naphthalic anhydride, **103** (249 mg, 1.1 mmol, 1.0 eq.) and 5-amino-1,10-phenanthroline, **78** (209 mg, 1.1 mmol, 1.0 eq.) yielding the product as a white crystalline powder (182 mg, 0.4 mmol, 42%). m.p._(decom.) > 180 °C. δ_H (400 MHz, DMSO- d_6): 9.21 (d, 1H, $^3J = 4.3$ Hz, $^4J = 1.3$ Hz, H₁₂), 9.16 (d, 1H, $^3J = 4.1$ Hz, $^4J = 1.2$ Hz, H₆), 8.75 (d, 1H, $J = 8.5$ Hz, H₃), 8.65 (d, 1H, $J = 7.3$ Hz, H₅), 8.54-8.49 (m, 3H, H₁₀₊₈₊₂), 8.21 (s, 1H, H₉), 8.17-8.07 (m, 2H, H₄₊₁), 7.85 (dd, 1H, $J = 8.1, 4.3$ Hz, H₁₁), 7.70

(dd, 1H, $J = 8.3, 4.1$ Hz, H_7). δ_c (151 MHz, DMSO- d_6): 163.7 (q), 163.4 (q), 150.9, 150.2, 145.9 (q), 145.5 (q), 137.9 (q), 136.5, 132.0, 131.8, 131.1 (q), 131.0, 130.5, 129.5 (q), 128.8 (q), 128.7, 127.8, 127.6 (q), 127.5, 126.3 (q), 123.7, 123.6, 123.5 (q), 122.2 (q). $\nu_{\max}$ (ATR/ cm^{-1}): 3413, 3058, 1707, 1662, 1588, 1572, 1607, 1422, 1364, 1347, 1237, 1188, 1144, 1107, 1051, 1025, 962, 890, 823, 805, 780, 749, 736, 707. ESI⁺-HRMS (m/z): $[M + H]^+$ calcd. for $C_{24}H_{13}ClN_3O_2$, 410.0691; found, 410.0691.

***N*-(1,10-Phenanthroline)-4-aminonaphthalene-1,8-dicarboximide, 97²²⁵**



The nitro precursor compound, **91** (100 mg, 0.2 mmol, 1.0 eq.) was dissolved in EtOH at 60 °C. $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (537 mg, 2.4 mmol, 10.0 eq.) was added the reaction mixture was heated at reflux for 6 h under Ar. The reaction mixture was cooled to room temperature, basified with 6 M NaOH (aq.) to pH 12 and the orange precipitate was extracted into CHCl_3 (50 mL $\times$ 4). The organic layers were combined and washed with water (100 mL $\times$ 4), EDTA aqueous solution (50 mL $\times$ 3), brine (100 mL)

and dried over MgSO_4 . The solvent was removed under reduced pressure and the orange solid product was washed with an aqueous solution of EDTA (300 mL). The product was isolated by Soxhlet extraction techniques for 24 h using CHCl_3 (4 mg, 0.01 mmol, 4%). δ_H (600 MHz, DMSO- d_6): 9.21 (d, 1H, $J = 4.2$ Hz, H_{12}), 9.16 (d, 1H, $J = 4.1$ Hz, H_6), 8.75 (d, 1H, $J = 8.4$ Hz, $H_{3/5}$), 8.57 (d, 1H, $J = 7.8$ Hz, H_8), 8.49 (d, 1H, $J = 7.2$ Hz, $H_{3/5}$), 8.28-8.24 (m, 2H, H_{1+10}), 8.15 (s, 1H, H_9), 7.88 (dd, 1H, $J = 7.7, 4.2$ Hz, H_{11}), 7.77-7.69 (m, 2H, H_{7+4}), 7.63 (s, 2H, NH_2), 6.93 (d, 1H, $J = 8.4$ Hz, H_2). δ_c (101 MHz, DMSO- d_6): 164.9 (q), 163.8 (q), 153.8 (q, 2C), 150.9, 150.5, 137.5, 134.9 (CH + q), 132.5, 132.0, 131.2 (q), 130.4, 128.4 (q), 127.9, 127.1 (q), 124.6, 124.29 (2C), 122.5 (q), 120.1 (q, 2C), 108.9, 107.9 (q). $\nu_{\max}$ (ATR/ cm^{-1}): 3125, 1692, 1648, 1578, 1511, 1359, 1237, 1119, 1161, 1145, 983, 893, 845, 770, 738, 625. ESI⁺-HRMS (m/z): $[M + \text{Na}]^+$ calcd. for $C_{24}H_{14}N_4\text{NaO}_2$, 413.1009 found, 413.1004.

4-Amino-1,8-Naphthalic Anhydride, 105¹²⁴

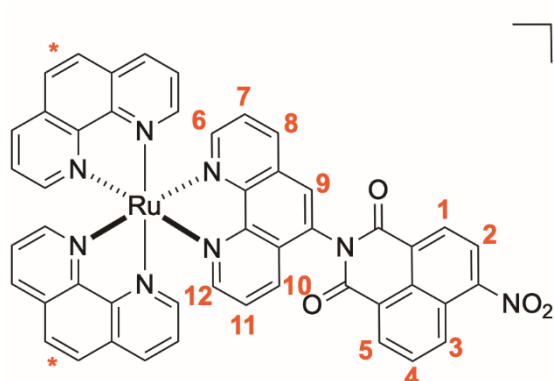


4-Nitro-naphthalic anhydride, **101** (300 mg, 1.2 mmol, 1.0 eq.) was dissolved in DMF (30 mL) and subject to catalytic hydrogenation using 3 atm H_2 in the presence of Pd/C (10 wt%, 30 mg, 0.2 eq.) for 24 h. The mixture was diluted with EtOH (200 mL) and the product was isolated by

filtration through a pad of Celite. The solid was washed with EtOH until the washings ran clear. The solvents were removed under reduced pressure and the product was dried *in*

vacuo yielding a red solid (176 mg, 0.8 mmol, 67%). δ_{H} (400 MHz, DMSO- d_6): 8.69 (d, $^3J = 8.4$ Hz, $^4J = 1.0$ Hz, 1H, H₅), 8.44 (d, $^3J = 7.3$ Hz, $^4J = 1.0$ Hz, 1H, H₃), 8.19 (d, 1H, $J = 8.5$ Hz, H₁), 7.78 (s, 2H, NH₂), 7.69 (dd, 1H, $J = 8.4, 7.3$ Hz, H₄), 6.88 (d, 1H, $J = 8.5$ Hz, H₂). δ_{C} (151 MHz, DMSO- d_6): 162.50 (q), 160.83 (q), 154.41, 136.41, 133.53, 133.10 (q), 131.19, 124.91, 119.82 (q), 118.79 (q), 109.23, 102.75 (q).

Bis(1,10-phenanthroline)(*N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide)ruthenium(II) chloride [Ru(phen)₂91**]Cl₂, **94****

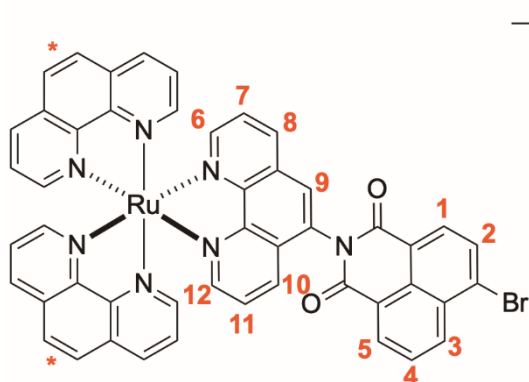


Complex **94** was synthesised according to General Synthetic Procedure 3 employing [Ru(phen)₂Cl₂], **87**, (101 mg, 0.2 mmol, 1.0 eq.) and *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91**, (88 mg, 0.2 mmol, 1.1 eq.). The crude product was purified by chromatography

(SiO₂, MeCN:H₂O:NaNO₃(aq.) 40:10:1) yielding a red solid which was reconverted to the chloride form by stirring with Amberlite chloride resin in MeOH for 1 h. The product was isolated by filtration and the solvent was removed under reduced pressure. The product was dissolved in dry MeCN and passed through a syringe filter (0.45 μm) to remove excess salt. The solvent was once again removed under reduced pressure and the product was dried *in vacuo* (60 mg, 0.1 mmol, 33%). Calc. for C₄₈H₂₉N₇O₂Cl₂Ru + 1.3NaNO₃ + 0.3NaCl: C, 53.34; H, 2.61; N, 12.05. Found: C, 52.48; H, 2.81; N, 11.92. m.p._(decom.) > 250 °C. δ_{H} (600 MHz, CD₃CN): 8.90 (d, 1H, $^3J = 8.3$ Hz, $^4J = 6.4$ Hz, H₃), 8.81 – 8.59 (m, 7H, H_{5+2+8+phen}), 8.52 (dd, 1H, $J = 13.1, 8.0$ Hz, H₄), 8.47 (d, $^3J = 8.6$ Hz, $^4J = 1.0$ Hz, H₁₀), 8.42 (s, 1H, H₉), 8.31 – 8.29 (m, 4H, H_{phen}), 8.20 – 8.07 (m, 5H, H_{phen+1+6+12}), 8.04 (m, 2H, H_{phen}), 7.76 – 7.68 (m, 3H, H_{phen+7}), 7.64 (m, 2H, H_{phen}), 7.56 (dd, 1H, $J = 8.5, 5.2$ Hz, H₁₁). δ_{C} (151 MHz, CD₃CN): 163.89 (q), 163.88 (q), 163.13 (q), 163.12 (q), 154.07 (q), 154.06 (CH), 153.81 (q), 153.79 (CH), 153.1 (CH), 153.0 (2CH), 152.9 (CH), 150.38 (q), 150.37 (q), 148.3 (q), 148.00 (q), 147.99 (q), 147.94 (2q), 147.91 (q), 137.2 (CH), 137.0 (CH), 136.94 (CH), 136.90 (CH), 136.88 (CH), 132.7 (q), 132.56 (CH), 132.5 (CH), 132.4 (CH), 131.13 (q), 131.09 (q), 131.05 (q), 131.02 (q), 130.37 (CH), 130.32 (CH), 130.16 (CH), 130.12 (CH), 130.07 (CH), 130.05 (CH), 129.8 (q), 129.05 (CH), 128.8 (q), 128.11 (CH), 128.08 (2CH), 128.05 (CH), 127.13 (q), 127.08 (q), 126.3 (CH), 126.2 (CH), 126.02 (CH), 125.97 (CH), 125.92 (2CH), 124.29 (CH), 124.27 (CH), 123.6

(2q), 123.30 (q), 123.26 (q). $\nu_{\max}$ (ATR/ cm^{-1}): 3347, 3055, 2163, 1710, 1667, 1588, 1425, 1356, 1349, 1238, 1191, 845, 781, 720. ESI⁺-HRMS (m/z): [M]⁺ calcd. for $\text{C}_{48}\text{H}_{28}\text{N}_8\text{O}_4\text{Ru}$, 441.0640; found 441.0644.

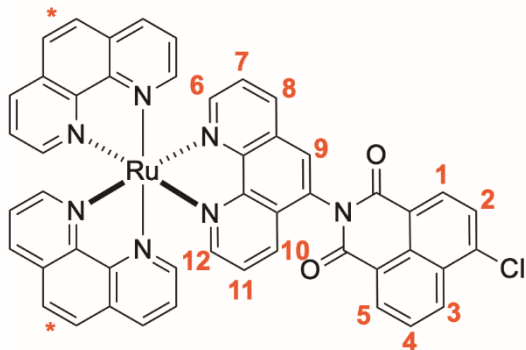
Bis(1,10-phenanthroline)(*N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide)ruthenium(II) chloride [Ru(phen)₂92**]Cl₂, **95****



Complex **95** was synthesised according to General Synthetic Procedure 3 employing [Ru(phen)₂Cl₂], **87**, (101 mg, 0.2 mmol, 1.0 eq.) and *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92**, (95 mg, 0.2 mmol, 1.1 eq.). The crude product was purified by chromatography

(SiO₂, MeCN:H₂O:NaNO_{3(aq.)} 40:10:1) yielding a red solid which was reconverted to the chloride form by stirring with Amberlite chloride resin in MeOH for 1 h. The product was isolated by filtration and the solvent was removed under reduced pressure. The product was dissolved in dry MeCN and passed through a syringe filter (0.45 μm) to remove excess salt. The solvent was once again removed under reduced pressure and the product was dried *in vacuo* (82 mg, 0.1 mmol, 43%). m.p._(decom.) > 250 °C. δ_{H} (400 MHz, CD₃CN): 8.80 – 8.60 (m, 7H, H_{3+5+8+phen}), 8.45 (m, 3H, H₁₀₊₉₊₂), 8.32 – 8.27 (m, 4H, H_{phen}), 8.23 (t, 1H, $J = 7.6$ Hz, H₄), 8.14 (m, 4H, H_{12+6+phen}), 8.03 (m, 3H, H_{1+phen}), 7.76 – 7.69 (m, 3H, H_{7+phen}), 7.68 – 7.61 (m, 2H, H_{phen}), 7.57 (dd, $J = 8.5, 5.2$ Hz, H₁₁). δ_{C} (151 MHz, CD₃CN): 164.91 (q), 164.87 (q), 164.86 (q), 164.83 (q), 154.95, 154.71, 154.06, 153.98, 153.95, 153.89, 149.26 (q), 148.90 (q), 148.84 (q), 148.83 (q), 148.82 (q), 148.80 (q), 138.04, 137.92, 137.86, 137.82, 137.80, 134.83, 133.97 (q), 133.37, 133.27, 133.25, 132.56, 132.54, 132.48, 132.45, 132.02 (q), 131.99 (q), 131.95 (q), 131.92 (q), 131.65 (q), 131.54 (q), 130.98 (q), 130.60 (q), 130.00, 129.86 (q), 129.68, 129.65, 129.01, 128.98, 127.2, 126.95, 126.90, 126.87, 124.27 (q), 124.23 (q), 123.48 (q), 123.45 (q). $\nu_{\max}$ (ATR/ cm^{-1}): 3354, 3057, 1981, 1708, 1666, 1586, 1507, 1425, 1344, 1237, 1190, 1046, 960, 892, 845, 779, 719. ESI⁺-HRMS (m/z): [M]⁺ calcd. for $\text{C}_{48}\text{H}_{28}\text{BrN}_7\text{O}_2\text{Ru}$, 457.5264; found, 457.5273.

Bis(1,10-phenanthroline)(*N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide)ruthenium(II) chloride [Ru(phen)₂93**]Cl₂, **96****

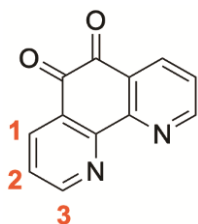


2Cl⁻ Complex **96** was synthesised according to General Synthetic Procedure 3 employing [Ru(phen)₂Cl₂], **87**, (150 mg, 0.3 mmol, 1.0 eq.) and *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93**, (127 mg, 0.3 mmol, 1.1 eq.). The crude product was purified by

chromatography (Al₂O₃, MeCN:H₂O 10:0 – 9:1) yielding a red solid which was dried *in vacuo* (47 mg, 0.083 mmol, 18%). m.p._(decom.) > 210 °C. δ_H (600 MHz, CD₃CN): 8.82 (m, 1H, H₃), 8.77 – 8.62 (m, 6H, H_{5+8+phen}), 8.60 – 8.54 (d, *J* = 7.9 Hz, 1H, H₂), 8.48 – 8.46 (m, 2H, H₁₀₊₉), 8.34 – 8.28 (m, 4H, H_{phen}), 8.21 – 8.14 (m, 4H, H_{12+6+phen}), 8.10 – 8.02 (m, 4H, H_{phen+4+1}), 7.80 – 7.66 (m, 5H, H_{7+phen}), 7.59 (dd, 1H, *J* = 8.5, 5.2 Hz, H₁₁). δ_C (151 MHz, CD₃CN): 164.11 (q), 164.09 (q), 163.82 (q), 163.80 (q), 153.9, 153.7, 153.1, 153.04, 153.03, 152.9, 148.3 (q), 148.0 (q), 147.93 (q), 147.91 (q), 139.5 (q), 137.2, 137.02, 136.95, 136.91, 136.90, 133.1 (q), 132.5, 132.4, 132.3, 131.6, 131.5, 131.4, 131.12 (q), 131.09 (q), 131.04 (q), 131.02 (q), 130.1 (q), 129.9 (q), 129.5 (q), 129.04, 128.98 (q), 128.59, 128.55, 128.11, 128.09 (3CH), 128.05, 127.94, 127.91, 126.29, 126.24, 126.04, 125.99, 125.94 (2CH), 123.4 (q), 123.3 (q), 121.99 (q), 121.95 (q). ν_{max} (ATR/cm⁻¹): 3347, 3051, 2163, 1708, 1665, 1588, 1574, 1509, 1424, 1365, 1349, 1237, 1190, 1051, 959, 894, 845, 780, 720. ESI⁺-HRMS (*m/z*): [M]⁺ calcd. for C₄₈H₂₈ClN₇O₂Ru, 435.5518; found, 435.5529.

7.8: Synthesis and Characterisation of Compounds Described in Chapter 4

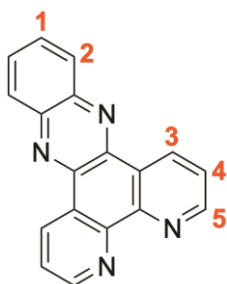
1,10-Phenanthroline-5,6-dione, **115**^{251,252}



Phenanthroline (1.00 g, 5.6 mmol, 1.0 eq.) and KBr (5.95 g, 50.0 mmol, 9.0 eq.) were cooled to -10 °C. Cold H₂SO₄ (98%, 20 mL) was added *via* addition down the cooled wall of the flask. Concentrated HNO₃ (68%, 10 mL) was cooled in an ice bath and added slowly to the reaction mixture. The solution was brought to room temperature and heated at 85 °C for 2 h. The solution was cooled to room temperature, poured onto an ice-water mixture (400 mL) and neutralised with NaHCO₃. The product was extracted into DCM and dried over Na₂SO₄. The

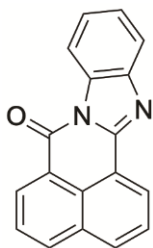
solvent was removed under reduced pressure and the crude product was recrystallised from MeOH to give the target solid as a yellow powder (0.09 g, 0.4 mmol, 8%). m.p. 259 °C (lit. 259 – 260 °C).²⁹¹ δ_{H} (400 MHz, DMSO- d_6): 8.99 (d, 2H, $^3J = 4.6$ Hz, $^4J = 1.0$ Hz, H₃), 8.39 (d, 2H, $^3J = 7.9$ Hz, $^4J = 1.0$ Hz, H₁), 7.67 (dd, 2H, $J = 7.9, 4.6$ Hz, 1H, H₂).

Dipyrido[3,2-a:2',3'-c]phenazine, dppz^{253,254}



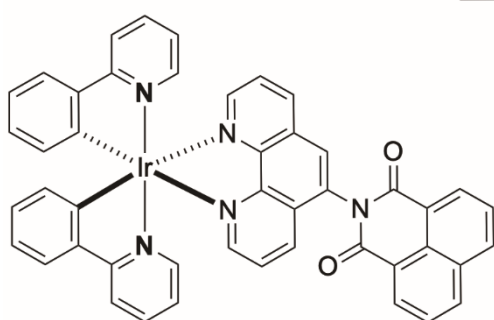
1,10-Phenanthroline-5,6-dione, **115** (80 mg, 0.4 mmol, 1.1 eq.) and *o*-phenylenediamine, **112** (37 mg, 0.4 mmol, 1.0 eq.) were dissolved in EtOH:H₂O (15 mL 1:1 v/v) and heated in a pressure vessel at 130 °C overnight. The reaction mixture was cooled to room temperature and the solid precipitate was isolated by filtration. The solid product was washed with water and ice cold EtOH, isolated as a yellow solid and dried *in vacuo* (60 mg, 0.2 mmol, 61%). m.p. 248 – 251 °C (lit. 251 – 252 °C).²⁵³ δ_{H} (400 MHz, DMSO- d_6): 9.57 (d, 2H, $^3J = 8.1$ Hz, $^4J = 1.8$ Hz, H₃), 9.23 (d, 2H, $^3J = 4.4$ Hz, $^4J = 1.8$ Hz, H₅), 8.42 (app. d, 2H, $^3J = 6.5$ Hz, $^4J = 3.4$ Hz, H₂), 8.08 (app. d, 2H, $^3J = 6.5$ Hz, $^4J = 3.4$ Hz, H₁), 7.97 (dd, 2H, $J = 8.1, 4.4$ Hz, H₄).

1,8-Naphthalenebenzimidazole, NBI^{153,247}



1,8-Naphthalic anhydride (500 mg, 2.5 mmol, 1.0 eq.) and *o*-phenylenediamine, **112** (300 mg, 2.8 mmol, 1.1 eq.) were heated at 125 °C in acetic acid for 36 h. The reaction mixture was cooled to room temperature and the product was isolated by filtration. The product was washed with sat. NaHCO_{3(aq.)} and water, dried *in vacuo* to yield a yellow powder (580 mg, 2.1 mmol, 85%). δ_{H} (400 MHz, CDCl₃): 8.86 (dd, 1H, $J = 7.3, 0.9$ Hz), 8.80 (dd, 1H, $J = 7.3, 1.1$ Hz), 8.57 (m, 1H), 8.29 (dd, 1H, $J = 8.2, 0.9$ Hz), 8.16 (m, 1H), 7.90 (m, 1H), 7.81 (dd, 2H, $J = 15.3, 7.2$ Hz), 7.49 (m, 2H).

Iridium(III)bis[1,8-naphthalenebenzimidazole]*N,N'*-(*N*-(1,10-Phenanthroline)-naphthalene-1,8-dicarboximide))chloride, [Ir(ppy)₂(68**)](Cl), **111**²⁴³**



Cl⁻ The dichloro-bridged iridium dimer, **117** (100 mg, 0.1 mmol, 1.0 eq.) and **PhenNap**, **80** (77 mg, 0.2 mmol, 2.2 eq.) were added to DCM:MeOH (1:1 v/v, 10 mL) and the mixture was degassed by vigorous bubbling of Ar for 15 min. The mixture was heated to 40 °C for 24 h under inert atmosphere. The mixture was

cooled to room temperature and the solvent was removed under reduced pressure. The product was purified by column chromatography (SiO₂, DCM:MeOH (0-2% MeOH)) yielding the product as a yellow powder (154 mg, 0.2 mmol, 93%). δ_{H} (600 MHz, CDCl₃): 9.23 (dd, 1H, $J = 8.3, 1.4$ Hz), 8.68 (dd, 1H, $J = 7.3, 1.1$ Hz), 8.64 (dd, 1H, $J = 7.3, 1.1$ Hz), 8.54 (s, 1H), 8.42 (t, 1H, $J = 1.3$ Hz), 8.40 (m, 2H), 8.36 (t, 1H, $J = 1.5$ Hz), 8.34 (dd, 1H, $J = 4.9, 1.4$ Hz), 8.32 (dd, 1H, $J = 5.0, 1.3$ Hz), 8.11 (dd, 1H, $J = 8.2, 5.1$ Hz), 7.89 (dd, 1H, $J = 8.4, 4.9$ Hz), 9.95 (appt, 2H, $J = 9.1$ Hz), 7.89 – 7.84 (m, 2H), 7.82 – 7.70 (m, 4H), 7.43 (m, 2H), 7.14 – 7.07 (m, 2H), 7.05 – 6.94 (m, 4H), 6.43 – 6.41 (appdt, 2H, $J = 8.0, 1.1$ Hz). δ_{C} (151 MHz, CDCl₃): 167.89 (q), 168.83 (q), 164.43 (q), 164.38 (q), 151.7, 151.3, 149.2 (q), 148.9 (q), 148.8, 148.7, 146.8, 143.61 (q), 143.54 (q), 140.34, 140.32, 138.3 (2C), 135.6 (2C), 134.1, 133.0 (q), 132.49, 132.47, 132.0 (q), 131.81, 131.78, 131.01, 130.98, 130.92 (q), 130.2, 129.5 (q), 128.9 (q), 127.7, 127.39 (2C), 127.34, 124.99, 124.95, 123.6, 123.4, 123.1, 123.0, 121.94 (q), 121.91 (q), 119.8 (q), 119.9 (q). ν_{max} (ATR/cm⁻¹): 3334, 3042, 2602, 2163, 1980, 1706, 1665, 1584, 1477, 1437, 1420, 1349, 1289, 1235, 1189, 1155, 1117, 1063, 1031, 885, 830, 779, 758, 729, 712, 702. ESI⁺-HRMS (m/z): [M]⁺ calcd. for C₄₆H₂₉N₅O₂Ir, 876.1947; found, 876.1949.

Chapter 8:

References

- (1) Gill, M. R.; Thomas, J. A. *Chem. Soc. Rev.* **2012**, *41*, 3179-3192.
- (2) Poynton, F. E.; Bright, S. A.; Blasco, S.; Williams, D. C.; Kelly, J. M.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2017**, *46*, 7706-7756.
- (3) Saeed, H. K.; Sreedharan, S.; Thomas, J. A. *Chem. Commun.* **2020**, *56*, 1464-1480.
- (4) Paris, J. P.; Brandt, W. W. *J. Am. Chem. Soc.* **1959**, *81*, 5001-5002.
- (5) Barton, J. K.; Danishefsky, A.; Goldberg, J. J. *Am. Chem. Soc.* **1984**, *106*, 2172-2176.
- (6) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518-5523.
- (7) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051-3058.
- (8) Friedman, A. E.; Chambron, J. C.; Sauvage, J. P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960-4962.
- (9) Kelly, J. M.; Tossi, A. B.; McConnell, D. J.; OhUigin, C. *Nucleic Acids Res.* **1985**, *13*, 6017-6034.
- (10) Kelly, J. M.; McConnell, D. J.; OhUigin, C.; Tossi, A. B.; Mesmaeker, A. K.-D.; Masschelein, A.; Nasielski, J. J. *Chem. Soc., Chem. Commun.* **1987**, 1821-1823.
- (11) Tossi, A. B.; Kelly, J. M. *Photochem. Photobiol.* **1989**, *49*, 545-556.
- (12) Tomczyk, M. D.; Walczak, K. Z. *Eur. J. Med. Chem.* **2018**, *159*, 393-422.
- (13) Calatrava-Pérez, E.; Delente, J. M.; Shanmugaraju, S.; Hawes, C. S.; Williams, C. D.; Gunnlaugsson, T.; Scanlan, E. M. *Org. Biomol. Chem.* **2019**, *17*, 2116-2125.
- (14) Braña, M. F.; Ramos, A. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 237-255.
- (15) Saha, S.; Samanta, A. J. *Phys. Chem. A* **2002**, *106*, 4763-4771.
- (16) Ryan, G. J.; Quinn, S.; Gunnlaugsson, T. *Inorg. Chem.* **2008**, *47*, 401-403.
- (17) Veale, E. B.; Gunnlaugsson, T. *J. Org. Chem.* **2008**, *73*, 8073-8076.
- (18) Duke, R. M.; Veale, E. B.; Pfeffer, F. M.; Kruger, P. E.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2010**, *39*, 3936-3953.
- (19) Li, X.; Lin, Y.; Wang, Q.; Yuan, Y.; Zhang, H.; Qian, X. *Eur. J. Med. Chem.* **2011**, *46*, 1274-1279.
- (20) Banerjee, S.; Kitchen, J. A.; Gunnlaugsson, T.; Kelly, J. M. *Org. Biomol. Chem.* **2012**, *10*, 3033-3043.
- (21) Lee, M. H.; Kim, J. Y.; Han, J. H.; Bhuniya, S.; Sessler, J. L.; Kang, C.; Kim, J. S. *J. Am. Chem. Soc.* **2012**, *134*, 12668-12674.
- (22) Fleming, C. L.; Natoli, A.; Schreuders, J.; Devlin, M.; Yoganantharajah, P.; Gibert, Y.; Leslie, K. G.; New, E. J.; Ashton, T. D.; Pfeffer, F. M. *Eur. J. Med. Chem.* **2019**, *162*, 321-333.
- (23) Papac, R. J. *Yale J. Biol. Med.* **2001**, *74*, 391-398.
- (24) Society, I. C. 2022. <https://www.cancer.ie> (accessed 2022 December).
- (25) Bayat Mokhtari, R.; Homayouni, T. S.; Baluch, N.; Morgatskaya, E.; Kumar, S.; Das, B.; Yeger, H. *Oncotarget* **2017**, *8*, 38022-38043.
- (26) Zaigham, A.; Sakina, R. An Overview of Cancer Treatment Modalities. In *Neoplasm*, Hafiz Naveed, S. Ed.; IntechOpen, 2018; pp 139-157.
- (27) Debela, D. T.; Muzazu, S. G.; Heraro, K. D.; Ndalama, M. T.; Mesele, B. W.; Haile, D. C.; Kitui, S. K.; Manyazewal, T. *SAGE Open Med.* **2021**, *9*, 20503121211034366.
- (28) El-Hussein, A.; Manoto, S. L.; Ombinda-Lemboumba, S.; Alrowaili, Z. A.; Mthunzi-Kufa, P. *Anticancer Agents Med. Chem.* **2021**, *21*, 149-161.
- (29) Cao, C.; Wang, X.; Yang, N.; Song, X.; Dong, X. *Chem. Sci.* **2022**, *13*, 863-889.
- (30) Arruebo, M.; Vilaboa, N.; Sáez-Gutierrez, B.; Lambea, J.; Tres, A.; Valladares, M.; González-Fernández, Á. *Cancers* **2011**, *3*, 3279-3330.
- (31) Costley, D.; McEwan, C.; Fowley, C.; McHale, A. P.; Atchison, J.; Nomikou, N.; Callan, J. F. *Int. J. Hyperthermia* **2015**, *31*, 107-117.
- (32) Rottenberg, S.; Disler, C.; Perego, P. *Nat. Rev. Cancer* **2021**, *21*, 37-50.

- (33) Franklin, R. E.; Gosling, R. G. *Nature* **1953**, *171*, 740-741.
- (34) Wilkins, M. H. F.; Stokes, A. R.; Wilson, H. R. *Nature* **1953**, *171*, 738-740.
- (35) Watson, J. D.; Crick, F. H. C. *Nature* **1953**, *171*, 737-738.
- (36) Hurley, L. H. *Nat. Rev. Cancer* **2002**, *2*, 188-200.
- (37) Dahm, R. *Dev. Biol.* **2005**, *278*, 274-288.
- (38) DNA: Alternative Conformations and Biology. *Madame Curie Bioscience Database [Internet]*. Landes Bioscience. <https://www.ncbi.nlm.nih.gov/books/NBK6545/#A40787> (accessed December 2022).
- (39) Pray, L. A. Discovery of DNA Structure and Function: Watson and Crick. Nature Education, 2008; Vol. 1.
- (40) Neidle, S. *Principles of Nucleic Acid Structure*; 1st ed. Academic Press: London, 2008.
- (41) Chargaff, E.; Lipshitz, R.; Green, C. J. *Biol. Chem.* **1952**, *195*, 155-160.
- (42) Chargaff, E. *Science* **1971**, *172*, 637-642.
- (43) Parkinson, G. N.; Berman, H. *Biorg. Med. Chem.* **2022**, *69*, 116887.
- (44) Kellett, A.; Molphy, Z.; Slator, C.; McKee, V.; Farrell, N. P. *Chem. Soc. Rev.* **2019**, *48*, 971-988.
- (45) Neidle, S. *Nat. Prod. Rep.* **2001**, *18*, 291-309.
- (46) Rahman, A.; O'Sullivan, P.; Rozas, I. *MedChemComm* **2019**, *10*, 26-40.
- (47) Edwards, K. J.; Jenkins, T. C.; Neidle, S. *Biochemistry* **1992**, *31*, 7104-7109.
- (48) Wilson, W. D.; Tanious, F. A.; Mathis, A.; Tevis, D.; Hall, J. E.; Boykin, D. W. *Biochimie* **2008**, *90*, 999-1014.
- (49) Quintana, J. R.; Lipanov, A. A.; Dickerson, R. E. *Biochemistry* **1991**, *30*, 10294-10306.
- (50) Gavathiotis, E.; Sharman, G. J.; Searle, M. S. *Nucleic Acids Res.* **2000**, *28*, 728-735.
- (51) Basselin, M.; Denise, H.; Coombs, G. H.; Barrett, M. P. *Antimicrob. Agents Chemother.* **2002**, *46*, 3731-3738.
- (52) Farahat, A. A.; Ismail, M. A.; Kumar, A.; Wenzler, T.; Brun, R.; Paul, A.; Wilson, W. D.; Boykin, D. W. *Eur. J. Med. Chem.* **2018**, *143*, 1590-1596.
- (53) Bray, P. G.; Barrett, M. P.; Ward, S. A.; de Koning, H. P. *Trends Parasitol.* **2003**, *19*, 232-239.
- (54) Avendaño, C.; Menéndez, J. C. Chapter 7 - Other Anticancer Drugs Targeting DNA and DNA-Associated Enzymes. In *Medicinal Chemistry of Anticancer Drugs (Second Edition)*, Avendaño, C., Menéndez, J. C. Eds.; Elsevier, 2015; pp 273-323.
- (55) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18-30.
- (56) Withoff, S.; De Jong, S.; De Vries, E. G.; Mulder, N. H. *Anticancer Res.* **1996**, *16*, 1867-1880.
- (57) Depowski, P. L.; Rosenthal, S. I.; Brien, T. P.; Stylos, S.; Johnson, R. L.; Ross, J. S. *Mod. Pathol.* **2000**, *13*, 542-547.
- (58) Nitiss, J. L. *Nat. Rev. Cancer* **2009**, *9*, 327-337.
- (59) Goodsell, D. 2013. http://dx.doi.org/10.2210/rcsb_pdb/mom_2013_4 (accessed 2023 January).
- (60) Zhou, J.; Jia, Y.; Wang, X.; Jia, M.; Pan, H.; Sun, Z.; Chen, J. *Molecules* **2022**, *27*, 8157.
- (61) Takusagawa, F.; Dabrow, M.; Neidle, S.; Berman, H. M. *Nature* **1982**, *296*, 466-469.
- (62) Kamitori, S.; Takusagawa, F. *J. Mol. Biol.* **1992**, *225*, 445-456.
- (63) Veale, E. B.; Frimannsson, D. O.; Lawler, M.; Gunnlaugsson, T. *Org. Lett.* **2009**, *11*, 4040-4043.
- (64) Banerjee, S.; Veale, E. B.; Phelan, C. M.; Murphy, S. A.; Tocci, G. M.; Gillespie, L. J.; Frimannsson, D. O.; Kelly, J. M.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2013**, *42*, 1601-1618.
- (65) Kelland, L. *Nat. Rev. Cancer* **2007**, *7*, 573-584.

- (66) Takahara, P. M.; Frederick, C. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1996**, *118*, 12309-12321.
- (67) Johnstone, T.; Suntharalingam, K.; Lippard, S. *Philos. Trans. Royal Soc.* **2015**, 373.
- (68) Dasari, S.; Bernard Tchounwou, P. *Eur. J. Pharmacol.* **2014**, *740*, 364-378.
- (69) Hargrave-Thomas, E.; Yu, B.; Reynisson, J. *World J. Clin. Oncol.* **2012**, *3*, 1-6.
- (70) Neidle, S. *Nucleic Acid Structure and Recognition*; Oxford University Press, 2002.
- (71) Wardle, B. *Principles and Applications of Photochemistry*; Wiley, 2009.
- (72) Harrigan, R. W.; Hager, G. D.; Crosby, G. A. *Chem. Phys. Lett.* **1973**, *21*, 487-490.
- (73) Durham, B.; Caspar, J. V.; Nagle, J. K.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4803-4810.
- (74) Barigelletti, F.; Juris, A.; Balzani, V.; Belser, P.; Von Zelewsky, A. *Inorg. Chem.* **1983**, *22*, 3335-3339.
- (75) Troian-Gautier, L.; Moucheron, C. *Molecules* **2014**, *19*, 5028-5087.
- (76) Soupard, A.; Alary, F.; Heully, J.-L.; Elliott, P. I. P.; Dixon, I. M. *Coord. Chem. Rev.* **2020**, *408*, 213184.
- (77) Schoonover, J. R.; Omberg, K. M.; Moss, J. A.; Bernhard, S.; Malueg, V. J.; Woodruff, W. H.; Meyer, T. J. *Inorg. Chem.* **1998**, *37*, 2585-2587.
- (78) Pourtois, G.; Beljonne, D.; Moucheron, C.; Schumm, S.; Kirsch-De Mesmaeker, A.; Lazzaroni, R.; Brédas, J.-L. *J. Am. Chem. Soc.* **2004**, *126*, 683-692.
- (79) Caspar, J. V.; Meyer, T. J. *J. Am. Chem. Soc.* **1983**, *105*, 5583-5590.
- (80) Olofsson, J.; Önfelt, B.; Lincoln, P. J. *Phys. Chem. A* **2004**, *108*, 4391-4398.
- (81) Olson, E. J. C.; Hu, D.; Hörmann, A.; Jonkman, A. M.; Arkin, M. R.; Stemp, E. D. A.; Barton, J. K.; Barbara, P. F. *J. Am. Chem. Soc.* **1997**, *119*, 11458-11467.
- (82) Chambron, J. C.; Sauvage, J. P.; Amouyal, E.; Koffi, P. *Nouv. J. Chim.* **1985**, *9*, 527-529.
- (83) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* **1992**, *31*, 10809-10816.
- (84) Poynton, F. E.; Hall, J. P.; Keane, P. M.; Schwarz, C.; Sazanovich, I. V.; Towrie, M.; Gunnlaugsson, T.; Cardin, C. J.; Cardin, D. J.; Quinn, S. J.; Long, C.; Kelly, J. M. *Chem. Sci.* **2016**, *7*, 3075-3084.
- (85) Cullen, A. A.; Long, C.; Pryce, M. T. *J. Photochem. Photobiol., A* **2021**, *410*, 113169.
- (86) Rosenberg, B.; Vancamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385-386.
- (87) Yamagishi, A. *J. Chem. Soc., Chem. Commun.* **1983**, 572-573.
- (88) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573-2584.
- (89) Cardin, C. J.; Kelly, J. M.; Quinn, S. J. *Chem. Sci.* **2017**, *8*, 4705-4723.
- (90) Albani, B. A.; Peña, B.; Leed, N. A.; de Paula, N. A. B. G.; Pavani, C.; Baptista, M. S.; Dunbar, K. R.; Turro, C. J. *J. Am. Chem. Soc.* **2014**, *136*, 17095-17101.
- (91) da Silva Miranda, F.; Signori, A. M.; Vicente, J.; de Souza, B.; Priebe, Jacks P.; Szpoganicz, B.; Gonçalves, N. S.; Neves, A. *Tetrahedron* **2008**, *64*, 5410-5415.
- (92) Wu, J.-Z.; Ye, B.-H.; Wang, L.; Ji, L.-N.; Zhou, J.-Y.; Li, R.-H.; Zhou, Z.-Y. *J. Chem. Soc., Dalton Trans.* **1997**, 1395-1402.
- (93) Gill, M. R.; Garcia-Lara, J.; Foster, S. J.; Smythe, C.; Battaglia, G.; Thomas, J. A. *Nat. Chem.* **2009**, *1*, 662-667.
- (94) Hall, J.; Keane, P.; Beer, H.; Buchner, K.; Winter, G.; Sørensen, T.; J. Cardin, D.; Brazier, J.; Cardin, C. *Nucleic Acids Res.* **2016**, *44*.
- (95) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* **1991**, *19*, 2595-2602.
- (96) Westerlund, F.; Pierard, F.; Eng, M. P.; Nordén, B.; Lincoln, P. J. *Phys. Chem. B* **2005**, *109*, 17327-17332.

- (97) Poulsen, B. C.; Estalayo-Adrián, S.; Blasco, S.; Bright, S. A.; Kelly, J. M.; Williams, D. C.; Gunnlaugsson, T. *Dalton Trans.* **2016**, 45, 18208-18220.
- (98) Elmes, R. B. P.; Orange, K. N.; Cloonan, S. M.; Williams, D. C.; Gunnlaugsson, T. *J. Am. Chem. Soc.* **2011**, 133, 15862-15865.
- (99) Estalayo-Adrián, S.; McManus, G. J.; Dalton, H. L.; Savyasachi, A. J.; Kelly, J. M.; Gunnlaugsson, T. *Dalton Trans* **2020**, 49, 14158-14168.
- (100) Zheng, K. C.; Deng, H.; Liu, X. W.; Li, H.; Chao, H.; Ji, L. N. *J. Mol. Struct.: THEOCHEM* **2004**, 682, 225-233.
- (101) Zhu, Z.; Peng, M.; Zhang, J.; Tan, L. *J. Inorg. Biochem.* **2017**, 169, 44-49.
- (102) Neugebauer, U.; Pellegrin, Y.; Devocelle, M.; Forster, R. J.; Signac, W.; Moran, N.; Keyes, T. E. *Chem. Commun.* **2008**, 5307-5309.
- (103) O'Reilly, F. M.; Kelly, J. M. *J. Phys. Chem. B* **2000**, 104, 7206-7213.
- (104) Pisani, M. J.; Fromm, P. D.; Mulyana, Y.; Clarke, R. J.; Körner, H.; Heimann, K.; Collins, J. G.; Keene, F. R. *ChemMedChem* **2011**, 6, 848-858.
- (105) Gill, M. R.; Jarman, P. J.; Hearnden, V.; Fairbanks, S. D.; Bassetto, M.; Maib, H.; Palmer, J.; Ayscough, K. R.; Thomas, J. A.; Smythe, C. *Angew. Chem. Int. Ed.* **2022**, 61, e202117449.
- (106) Fairbanks, S. D.; Robertson, C. C.; Keene, F. R.; Thomas, J. A.; Williamson, M. P. *J. Am. Chem. Soc.* **2019**, 141, 4644-4652.
- (107) Elias, B.; Kirsch-De Mesmaeker, A. *Coord. Chem. Rev.* **2006**, 250, 1627-1641.
- (108) Masschelein, A.; Jacquet, L.; Kirsch-De Mesmaeker, A.; Nasielski, J. *Inorg. Chem.* **1990**, 29, 855-860.
- (109) Marcélis, L.; Ghesquière, J.; Garnir, K.; Kirsch-De Mesmaeker, A.; Moucheron, C. *Coord. Chem. Rev.* **2012**, 256, 1569-1582.
- (110) Fukuzumi, S.; Miyao, H.; Ohkubo, K.; Suenobu, T. *J. Phys. Chem. A* **2005**, 109, 3285-3294.
- (111) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, 119, 617-618.
- (112) Ortmans, I.; Elias, B.; Kelly, J. M.; Moucheron, C.; Kirsch-DeMesmaeker, A. *Dalton Trans.* **2004**, 668-676.
- (113) Lecomte, J.-P.; Mesmaeker, A. K.-D.; Kelly, J. M.; Tossi, A. B.; Görner, H. *Photochem. Photobiol.* **1992**, 55, 681-689.
- (114) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Feeney, M. M.; Kelly, J. M. *Inorg. Chem.* **1995**, 34, 6481-6491.
- (115) Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. *J. Photochem. Photobiol., B* **1997**, 40, 91-106.
- (116) Reynisson, J.; Steenken, S. *Phys. Chem. Chem. Phys.* **2002**, 4, 527-532.
- (117) Troian-Gautier, L.; Mugeniwabagara, E.; Fusaro, L.; Moucheron, C.; Kirsch-De Mesmaeker, A.; Luhmer, M. *Inorg. Chem.* **2017**, 56, 1794-1803.
- (118) Herman, L.; Ghosh, S.; Defrancq, E.; Kirsch-DeMesmaeker, A. *Journal of Physical Organic Chemistry* **2008**, 21, 670-681.
- (119) Hall, J. P.; Poynton, F. E.; Keane, P. M.; Gurung, S. P.; Brazier, J. A.; Cardin, D. J.; Winter, G.; Gunnlaugsson, T.; Sazanovich, I. V.; Towrie, M.; Cardin, C. J.; Kelly, J. M.; Quinn, S. J. *Nat. Chem.* **2015**, 7, 961-967.
- (120) Jacquet, L.; Mesmaeker, A. K.-D. *J. Chem. Soc., Faraday Trans.* **1992**, 88, 2471-2480.
- (121) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, 250, 3094-3117.
- (122) Braña, M. F.; Castellano, J. M.; Roldán, C. M.; Santos, A.; Vázquez, D.; Jiménez, A. *Cancer Chemother. Pharmacol.* **1980**, 4, 61-66.
- (123) Grabchev, I.; Meallier, P.; Konstantinova, T.; Popova, M. *Dyes Pigm.* **1995**, 28, 41-46.
- (124) Veale, E. B.; Gunnlaugsson, T. *J. Org. Chem.* **2010**, 75, 5513-5525.

- (125) Geraghty, C.; Wynne, C.; Elmes, R. *Coord. Chem. Rev.* **2021**, *437*, 213713.
- (126) Du, W.; Xu, J.; Li, H.; Feng, C.; Yu, M.-M.; Li, Z.-X.; Wei, L. *RSC Adv.* **2015**, *5*, 15077–15083.
- (127) Poteau, X.; Brown, A. I.; Brown, R. G.; Holmes, C.; Matthew, D. *Dyes Pigm.* **2000**, *47*, 91-105.
- (128) Liu, J.; Tu, G.; Zhou, Q.; Cheng, Y.; Geng, Y.; Wang, L.; Ma, D.; Jing, X.; Wang, F. *J. Mater. Chem.* **2006**, *16*, 1431-1438.
- (129) Ryan, G. J.; Elmes, R. B. P.; Quinn, S. J.; Gunnlaugsson, T. *Supramol. Chem.* **2012**, *24*, 175-188.
- (130) Gallego, J.; Reid, B. R. *Biochemistry* **1999**, *38*, 15104-15115.
- (131) González-Bulnes, L.; Gallego, J. *Biopolymers* **2012**, *97*, 974-987.
- (132) Tandon, R.; Luxami, V.; Tandon, N.; Paul, K. *Bioorg. Chem.* **2022**, *121*, 105677.
- (133) Braña, M. F.; Sanz, A. M.; Castellano, J. M.; Roldan, C. M.; Roldan, C. *Eur. J. Med. Chem.* **1981**, *16*, 207-212.
- (134) Cid, P. R.; Fernandez, E. G.; Martin, F. R.; Braña, M. F. *Eur. J. Drug Metab. Pharmacokinet.* **1986**, *11*, 255-267.
- (135) Waring, M. J.; González, A.; Jiménez, A.; Vázquez, D. *Nucleic Acids Res.* **1979**, *7*, 217-230.
- (136) Ji, L.; Yang, S.; Li, S.; Liu, S.; Tang, S.; Liu, Z.; Meng, X.; Yu, S. *Oncotarget* **2017**, *8*, 37394-37408.
- (137) Stone, R. M.; Mazzola, E.; Neuberg, D.; Allen, S. L.; Pigneux, A.; Stuart, R. K.; Wetzler, M.; Rizzieri, D.; Erba, H. P.; Damon, L.; Jang, J. H.; Tallman, M. S.; Warzocha, K.; Masszi, T.; Sekeres, M. A.; Egyed, M.; Horst, H. A.; Selleslag, D.; Solomon, S. R.; Venugopal, P.; Lundberg, A. S.; Powell, B. J. *Clin. Oncol.* **2015**, *33*, 1252-1257.
- (138) Calatrava-Pérez, E.; Marchetti, L. A.; McManus, G. J.; Lynch, D. M.; Elmes, R. B. P.; Williams, D. C.; Gunnlaugsson, T.; Scanlan, E. M. *Chem. Eur. J.* **2022**, *28*, e202103858.
- (139) García Gancedo, A.; Gil-Fernández, C.; Vilas, P.; Perez, S.; Paez, E.; Rodriguez, F.; Braña, M. F.; Roldán, C. M. *Arch. Virol* **1982**, *74*, 157-165.
- (140) Bousquet, P. F.; Braña, M. F.; Conlon, D.; Fitzgerald, K. M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qian, X.-D.; Keilhauer, G.; Romerdahl, C. A. *Cancer Res.* **1995**, *55*, 1176-1180.
- (141) Su, G. H.; Sohn, T. A.; Ryu, B.; Kern, S. E. *Cancer Res.* **2000**, *60*, 3137-3142.
- (142) Ao, X.; Bright, S. A.; Taylor, N. C.; Elmes, R. B. P. *Org. Biomol. Chem.* **2017**, *15*, 6104-6108.
- (143) Neil A. Campbell, L. A. U., Michael L Cain, Steven A. Wasserman, Peter V. Minorsky. *Biology: A Global Approach, Global Edition*; Pearson, 2020.
- (144) Marks, P. A.; Rifkind, R. A.; Richon, V. M.; Breslow, R.; Miller, T.; Kelly, W. K. *Nat. Rev. Cancer* **2001**, *1*, 194-202.
- (145) Hearn, K. N.; Ashton, T. D.; Acharya, R.; Feng, Z.; Gueven, N.; Pfeffer, F. M. *Cells* **2021**, *10*.
- (146) Touyz, R. M.; Anagnostopoulou, A.; De Lucca Camargo, L.; Montezano, A. C. *Circ. Res.* **2016**, *119*, 969-971.
- (147) Chen, Q.; Chen, A. Z.; Jia, G.; Li, J.; Zheng, C.; Chen, K. *Hepatol. Commun.* **2022**, *6*, 652-664.
- (148) Lloret, A.; Fuchsberger, T.; Giraldo, E.; Vina, J. *Curr. Alzheimer Res.* **2016**, *13*, 206-211.
- (149) Mahato, P.; Saha, S.; Suresh, E.; Di Liddo, R.; Parnigotto, P. P.; Conconi, M. T.; Kesharwani, M. K.; Ganguly, B.; Das, A. *Inorg. Chem.* **2012**, *51*, 1769-1777.
- (150) Tyson, D. S.; Luman, C. R.; Zhou, X.; Castellano, F. N. *Inorg. Chem.* **2001**, *40*, 4063-4071.

- (151) Yarnell, J. E.; Wells, K. A.; Palmer, J. R.; Breaux, J. M.; Castellano, F. N. *J. Phys. Chem. B* **2019**, *123*, 7611-7627.
- (152) Yarnell, J. E.; Deaton, J. C.; McCusker, C. E.; Castellano, F. N. *Inorg. Chem.* **2011**, *50*, 7820-7830.
- (153) Yarnell, J. E.; De La Torre, P.; Castellano, F. N. *Eur. J. Inorg. Chem.* **2017**, *2017*, 5238-5245.
- (154) Elmes, R. B. P.; Erby, M.; Bright, S. A.; Williams, D. C.; Gunnlaugsson, T. *Chem. Commun.* **2012**, *48*, 2588-2590.
- (155) Tröger, J. *J. Prakt. Chem.* **1887**, *36*, 225-245.
- (156) Spielman, M. A. *J. Am. Chem. Soc.* **1935**, *57*, 583-585.
- (157) Wilcox, C. S. *Tetrahedron Lett.* **1985**, *26*, 5749-5752.
- (158) Lovitt, J. I.; Umadevi, D.; Raja Lakshmi, P.; Twamley, B.; Gunnlaugsson, T.; Shanmugaraju, S. *Supramol. Chem.* **2020**, *32*, 620-633.
- (159) Langdon-Jones, E. E.; Symonds, N. O.; Yates, S. E.; Hayes, A. J.; Lloyd, D.; Williams, R.; Coles, S. J.; Horton, P. N.; Pope, S. J. A. *Inorg. Chem.* **2014**, *53*, 3788-3797.
- (160) Ryan, G. J.; Poynton, F. E.; Elmes, R. B. P.; Erby, M.; Williams, D. C.; Quinn, S. J.; Gunnlaugsson, T. *Dalton Trans.* **2015**, *44*, 16332-16344.
- (161) Delente, J. M.; Umadevi, D.; Shanmugaraju, S.; Kotova, O.; Watson, G. W.; Gunnlaugsson, T. *Chem. Commun.* **2020**, *56*, 2562-2565.
- (162) Shanmugaraju, S.; Umadevi, D.; Savyasachi, A. J.; Byrne, K.; Ruether, M.; Schmitt, W.; Watson, G. W.; Gunnlaugsson, T. *J. Mater. Chem. A* **2017**, *5*, 25014-25024.
- (163) Shanmugaraju, S.; Dabadie, C.; Byrne, K.; Savyasachi, A. J.; Umadevi, D.; Schmitt, W.; Kitchen, J. A.; Gunnlaugsson, T. *Chem. Sci.* **2017**, *8*, 1535-1546.
- (164) Keane, P. M.; O'Sullivan, K.; Poynton, F. E.; Poulsen, B. C.; Sazanovich, I. V.; Towrie, M.; Cardin, C. J.; Sun, X.-Z.; George, M. W.; Gunnlaugsson, T.; Quinn, S. J.; Kelly, J. M. *Chem. Sci.* **2020**, *11*, 8600-8609.
- (165) Devereux, S. J.; Poynton, F. E.; Baptista, F. R.; Gunnlaugsson, T.; Cardin, C. J.; Sazanovich, I. V.; Towrie, M.; Kelly, J. M.; Quinn, S. J. *Chem. Eur. J.* **2020**, *26*, 17103-17109.
- (166) Estalayo-Adrián, S.; Blasco, S.; Bright, S. A.; McManus, G. J.; Orellana, G.; Williams, D. C.; Kelly, J. M.; Gunnlaugsson, T. *Chem. Commun.* **2020**, *56*, 9332-9335.
- (167) Estalayo-Adrián, S.; Blasco, S.; Bright, S. A.; McManus, G. J.; Orellana, G.; Williams, D. C.; Kelly, J. M.; Gunnlaugsson, T. *ACS Appl. Bio Mater.* **2021**, *4*, 6664-6681.
- (168) Ryan, G. J.; Gunnlaugsson, T.; Quinn, S. J. *Inorg. Chem.* **2022**, *61*, 12073-12086.
- (169) Elmes, R. B. P.; Ryan, G. J.; Erby, M. L.; Frimannsson, D. O.; Kitchen, J. A.; Lawler, M.; Williams, D. C.; Quinn, S. J.; Gunnlaugsson, T. *Inorg. Chem.* **2020**, *59*, 10874-10893.
- (170) Kotova, O.; O'Reilly, C.; Barwich, S. T.; Mackenzie, L. E.; Lynes, A. D.; Savyasachi, A. J.; Ruether, M.; Pal, R.; Möbius, M. E.; Gunnlaugsson, T. *Chem* **2022**, *8*, 1395-1414.
- (171) Henwood, A. F.; Hegarty, I. N.; McCarney, E. P.; Lovitt, J. I.; Donohoe, S.; Gunnlaugsson, T. *Coord. Chem. Rev.* **2021**, *449*, 214206.
- (172) Whelan, É.; Steuber, F. W.; Gunnlaugsson, T.; Schmitt, W. *Coord. Chem. Rev.* **2021**, *437*, 213757.
- (173) Martin, H.; Lázaro, L. R.; Gunnlaugsson, T.; Scanlan, E. M. *Chem. Soc. Rev.* **2022**, *51*, 9694-9716.
- (174) Coogan, M. Ligand Design in d-Block Optical Imaging Agents and Sensors. In *Ligand Design in Medicinal Inorganic Chemistry*, 2014; pp 81-111.
- (175) Elgar, C. E.; Yusoh, N. A.; Tiley, P. R.; Kolozsvári, N.; Bennett, L. G.; Gamble, A.; Péan, E. V.; Davies, M. L.; Staples, C. J.; Ahmad, H.; Gill, M. R. *J. Am. Chem. Soc.* **2023**, *145*, 1236-1246.
- (176) McGhie, B. S.; Aldrich-Wright, J. R. *Biomedicines* **2022**, *10*.

- (177) Nair, R. B.; Cullum, B. M.; Murphy, C. J. *Inorg. Chem.* **1997**, *36*, 962-965.
- (178) Chambron, J.-C.; Sauvage, J.-P. *Chem. Phys. Lett.* **1991**, *182*, 603-607.
- (179) Amouyal, E.; Homsy, A.; Chambron, J.-C.; Sauvage, J.-P. *J. Chem. Soc., Dalton Trans.* **1990**, 1841-1845.
- (180) Coates, C. G.; Callaghan, P.; McGarvey, J. J.; Kelly, J. M.; Jacquet, L.; Kirsch-De Mesmaeker, A. *J. Mol. Struct.* **2001**, *598*, 15-25.
- (181) Hu, W.; Huang, X.-S.; Wu, J.-F.; Yang, L.; Zheng, Y.-T.; Shen, Y.-M.; Li, Z.-Y.; Li, X. *J. Med. Chem.* **2018**, *61*, 8947-8980.
- (182) Doi, T.; Nagamiya, H.; Kokubo, M.; Hirabayashi, K.; Takahashi, T. *Tetrahedron* **2002**, *58*, 2957-2963.
- (183) Rau, S.; Schäfer, B.; Grüßing, A.; Schebesta, S.; Lamm, K.; Vieth, J.; Görls, H.; Walther, D.; Rudolph, M.; Grummt, U. W.; Birkner, E. *Inorg. Chim. Acta* **2004**, *357*, 4496-4503.
- (184) Lecomte, J. P.; Mesmaeker, A. K.-D.; Demeunynck, M.; Lhomme, J. *J. Chem. Soc., Faraday Trans.* **1993**, *89*, 3261-3269.
- (185) Yarnell, J. E.; McCusker, C. E.; Leeds, A. J.; Breaux, J. M.; Castellano, F. N. *Eur. J. Inorg. Chem.* **2016**, 1808-1818.
- (186) Nasielski-Hinkens, R.; Benedek-Vamos, M.; Maetens, D. *J. Heterocycl. Chem.* **1980**, *17*, 873-876.
- (187) Nasielski-hinkens, R.; Kotel, J.; Lecloux, T.; Nasielski, J. *Synth. Commun.* **1989**, *19*, 511-514.
- (188) Zou, X.-H.; Li, H.; Yang, G.; Deng, H.; Liu, J.; Li, R.-H.; Zhang, Q.-L.; Xiong, Y.; Ji, L.-N. *Inorg. Chem.* **2001**, *40*, 7091-7095.
- (189) Lovitt, J. I.; Gorai, T.; Cappello, E.; Delente, J. M.; Barwich, S. T.; Möbius, M. E.; Gunnlaugsson, T.; Hawes, C. S. *Mater. Chem. Front.* **2021**, *5*, 3458-3469.
- (190) Notaro, A.; Frei, A.; Rubbiani, R.; Jakubaszek, M.; Basu, U.; Koch, S.; Mari, C.; Dotou, M.; Blacque, O.; Gouyon, J.; Bedioui, F.; Rotthowe, N.; Winter, R. F.; Goud, B.; Ferrari, S.; Tharaud, M.; Řezáčová, M.; Humajová, J.; Tomšík, P.; Gasser, G. *J. Med. Chem.* **2020**, *63*, 5568-5584.
- (191) Keane, P. M.; Tory, J.; Towrie, M.; Sazanovich, I. V.; Cardin, C. J.; Quinn, S. J.; Hartl, F.; Kelly, J. M.; Long, C. *Inorg. Chem.* **2019**, *58*, 663-671.
- (192) Pavlishchuk, V. V.; Addison, A. W. *Inorg. Chim. Acta* **2000**, *298*, 97-102.
- (193) Gillard, M.; Piraux, G.; Daenen, M.; Abraham, M.; Troian-Gautier, L.; Bar, L.; Bonnet, H.; Loiseau, F.; Jamet, H.; Dejeu, J.; Defrancq, E.; Elias, B. *Chem. Eur. J.* **2022**, *28*, e202202251.
- (194) van der Westhuizen, D.; von Eschwege, K. G.; Conradie, J. *Electrochim. Acta* **2019**, *320*, 134540.
- (195) Kirsch-De Mesmaeker, A.; Nasielski-Hinkens, R.; Maetens, D.; Pauwels, D.; Nasielski, J. *Inorg. Chem.* **1984**, *23*, 377-379.
- (196) Mabrouk, P. A.; Wrighton, M. S. *Inorg. Chem.* **1986**, *25*, 526-531.
- (197) Ishida, H.; Tobita, S.; Hasegawa, Y.; Katoh, R.; Nozaki, K. *Coord. Chem. Rev.* **2010**, *254*, 2449-2458.
- (198) Katsumi, N. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2697-2705.
- (199) Suzuki, K.; Kobayashi, A.; Kaneko, S.; Takehira, K.; Yoshihara, T.; Ishida, H.; Shiina, Y.; Oishi, S.; Tobita, S. *Phys. Chem. Chem. Phys.* **2009**, *11*, 9850-9860.
- (200) Brouwer, A. M. *Pure Appl. Chem.* **2011**, *83*, 2213-2228.
- (201) Ji, S.; Wu, W.; Wu, W.; Song, P.; Han, K.; Wang, Z.; Liu, S.; Guo, H.; Zhao, J. *J. Mater. Chem.* **2010**, *20*, 1953-1963.
- (202) Hall, J. P.; Cook, D.; Morte, S. R.; McIntyre, P.; Buchner, K.; Beer, H.; Cardin, D. J.; Brazier, J. A.; Winter, G.; Kelly, J. M.; Cardin, C. J. *J. Am. Chem. Soc.* **2013**, *135*, 12652-12659.

- (203) Bouskila, A.; Amouyal, E.; Verchère-Béaur, C.; Sasaki, I.; Gaudemer, A. *J. Photochem. Photobiol., B* **2004**, *76*, 69-83.
- (204) Danilov, V. I.; Volkov, S. N. *Biopolymers* **1975**, *14*, 1205-1212.
- (205) Carter, M. T.; Rodriguez, M.; Bard, A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8901-8911.
- (206) Keane, P. M.; Poynton, F. E.; Hall, J. P.; Clark, I. P.; Sazanovich, I. V.; Towrie, M.; Gunnlaugsson, T.; Quinn, S. J.; Cardin, C. J.; Kelly, J. M. *J. Phys. Chem. Lett.* **2015**, *6*, 734-738.
- (207) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9319-9324.
- (208) Hall, J. P.; O'Sullivan, K.; Naseer, A.; Smith, J. A.; Kelly, J. M.; Cardin, C. J. *Proc. Natl. Acad. Sci.* **2011**, *108*, 17610-17614.
- (209) Schildkraut, C.; Lifson, S. *Biopolymers* **1965**, *3*, 195-208.
- (210) Thomas, R. *Gene* **1993**, *135*, 77-79.
- (211) Wartell, R. M.; Benight, A. S. *Phys. Rep.* **1985**, *126*, 67-107.
- (212) Nordén, B.; Kurucsev, T. *J. Mol. Recognit.* **1994**, *7*, 141-155.
- (213) Kypr, J.; Kejnovská, I.; Renciuik, D.; Vorlícková, M. *Nucleic Acids Res.* **2009**, *37*, 1713-1725.
- (214) Garbett, N. C.; Ragazzon, P. A.; Chaires, J. B. *Nat. Protoc.* **2007**, *2*, 3166-3172.
- (215) Rodger, A. *Sci. Prog.* **2008**, *91*, 377-396.
- (216) Elmes, R. B. P.; Gunnlaugsson, T. *Tetrahedron Lett.* **2010**, *51*, 4082-4087.
- (217) Mohan, B.; Estalayo-Adrián, S.; Umadevi, D.; la Cour Poulsen, B.; Blasco, S.; McManus, G. J.; Gunnlaugsson, T.; Shanmugaraju, S. *Inorg. Chem.* **2022**, *61*, 11592-11599.
- (218) Banerjee, S.; Bright, S. A.; Smith, J. A.; Burgeat, J.; Martinez-Calvo, M.; Williams, D. C.; Kelly, J. M.; Gunnlaugsson, T. *J. Org. Chem.* **2014**, *79*, 9272-9283.
- (219) Liu, D.; Yu, H.; Wang, Z.; Nie, Q. *Polym. Int.* **2010**, *59*, 937-944.
- (220) Franco, F.; Cometto, C.; Garino, C.; Minero, C.; Sordello, F.; Nervi, C.; Gobetto, R. *Eur. J. Inorg. Chem.* **2015**, 296-304.
- (221) Case, D. R.; Spear, A.; Henwood, A. F.; Nanao, M.; Dampf, S.; Korter, T. M.; Gunnlaugsson, T.; Zubietta, J.; Doyle, R. P. *Dalton Trans.* **2021**, *50*, 3479-3486.
- (222) Shanmugaraju, S.; McAdams, D.; Pancotti, F.; Hawes, C. S.; Veale, E. B.; Kitchen, J. A.; Gunnlaugsson, T. *Org. Biomol. Chem.* **2017**, *15*, 7321-7329.
- (223) Veale, E. B.; Kitchen, J. A.; Gunnlaugsson, T. *Supramol. Chem.* **2013**, *25*, 101-108.
- (224) Zhang, L.; Huang, Z.; Dai, D.; Xiao, Y.; Lei, K.; Tan, S.; Cheng, J.; Xu, Y.; Liu, J.; Qian, X. *Org. Lett.* **2016**, *18*, 5664-5667.
- (225) Young, M. J.; Akien, G. R.; Evans, N. H. *Org. Biomol. Chem.* **2020**, *18*, 5203-5209.
- (226) Grieco, G.; Blacque, O. *Appl. Organomet. Chem.* **2022**, *36*, e6452.
- (227) Frisch, M. J. T.; G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian Inc. Wallingford CT. 2016.
- (228) Wang, H.; Wu, H.; Xue, L.; Shi, Y.; Li, X. *Org. Biomol. Chem.* **2011**, *9*, 5436-5444.

- (229) Shanmugaraju, S.; Umadevi, D.; González-Barcia, L. M.; Delente, J. M.; Byrne, K.; Schmitt, W.; Watson, G. W.; Gunnlaugsson, T. *Chem. Commun.* **2019**, 55, 12140-12143.
- (230) Spek, A. *Acta Crystallogr. Sect. D* **2009**, 65, 148-155.
- (231) Cho, D. W.; Cho, D. W. *New J. Chem.* **2014**, 38, 2233-2236.
- (232) Nandhikonda, P.; Begaye, M. P.; Cao, Z.; Heagy, M. D. *Chem. Commun.* **2009**, 4941-4943.
- (233) Demeter, A.; Bérces, T.; Biczók, L.; Wintgens, V.; Valat, P.; Kossanyi, J. *The Journal of Physical Chemistry* **1996**, 100, 2001-2011.
- (234) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**, 17, 3334-3341.
- (235) Mamo, A.; Aureliano, A.; Recca, A. *Molecules* **2010**, 15, 1324-1339.
- (236) Spek, A. *Acta Crystallogr., Sect. C* **2015**, 71, 9-18.
- (237) Troian-Gautier, L.; Moucheron, C. *Molecules* **2014**, 19, 5028-5087.
- (238) Di Pietro, M. L.; La Ganga, G.; Nastasi, F.; Puntoriero, F. *Appl. Sci.* **2021**, 11, 3038.
- (239) Holmgaard List, N.; Knoops, J.; Rubio-Magnieto, J.; Idé, J.; Beljonne, D.; Norman, P.; Surin, M.; Linares, M. *J. Am. Chem. Soc.* **2017**, 139, 14947-14953.
- (240) Shanmugaraju, S.; la Cour Poulsen, B.; Arisa, T.; Umadevi, D.; Dalton, H. L.; Hawes, C. S.; Estalayo-Adrián, S.; Savyasachi, A. J.; Watson, G. W.; Williams, D. C.; Gunnlaugsson, T. *Chem. Commun.* **2018**, 54, 4120-4123.
- (241) Tamayo, A. B.; Alleyne, B. D.; Djurovich, P. I.; Lamansky, S.; Tsyba, I.; Ho, N. N.; Bau, R.; Thompson, M. E. *J. Am. Chem. Soc.* **2003**, 125, 7377-7387.
- (242) Lanoë, P.-H.; Chan, J.; Gontard, G.; Monti, F.; Armaroli, N.; Barbieri, A.; Amouri, H. *Eur. J. Inorg. Chem.* **2016**, 2016, 1631-1634.
- (243) Henwood, A. F.; Pal, A. K.; Cordes, D. B.; Slawin, A. M. Z.; Rees, T. W.; Momblona, C.; Babaei, A.; Pertegás, A.; Ortí, E.; Bolink, H. J.; Baranoff, E.; Zysman-Colman, E. *J. Mater. Chem. C* **2017**, 5, 9638-9650.
- (244) Sajoto, T.; Djurovich, P. I.; Tamayo, A.; Yousufuddin, M.; Bau, R.; Thompson, M. E.; Holmes, R. J.; Forrest, S. R. *Inorg. Chem.* **2005**, 44, 7992-8003.
- (245) Schreier, M. R.; Guo, X.; Pfund, B.; Okamoto, Y.; Ward, T. R.; Kerzig, C.; Wenger, O. S. *Acc. Chem. Res.* **2022**, 55, 1290-1300.
- (246) Dai, M.; Zhu, W.; Xu, Y.; Qian, X.; Liu, Y.; Xiao, Y.; You, Y. *J. Fluoresc.* **2008**, 18, 591-597.
- (247) Mamada, M.; Pérez-Bolívar, C.; Anzenbacher, P. *Org. Lett.* **2011**, 13, 4882-4885.
- (248) Mamada, M.; Pérez-Bolívar, C.; Kumaki, D.; Esipenko, N. A.; Tokito, S.; Anzenbacher Jr., P. *Chem. Eur. J.* **2014**, 20, 11835-11846.
- (249) Zheng, S.; Lynch, P. L. M.; Rice, T. E.; Moody, T. S.; Gunaratne, H. Q. N.; de Silva, A. P. *Photochem. Photobiol. Sci.* **2012**, 11, 1675-1681.
- (250) Daly, B.; Ling, J.; de Silva, A. P. *Chem. Soc. Rev.* **2015**, 44, 4203-4211.
- (251) Masaki, Y.; Yoshihito, T.; Yasuyuki, Y.; Shigeyasu, K.; Ichiro, S. *Bull. Chem. Soc. Jpn.* **1992**, 65, 1006-1011.
- (252) Ferretti, F.; Gallo, E.; Ragaini, F. *J. Organomet. Chem.* **2014**, 771, 59-67.
- (253) Dickeson, J. E.; Summers, L. A. *Aust. J. Chem.* **1970**, 23, 1023-1027.
- (254) Cloonan, S. M.; Elmes, R. B. P.; Erby, M.; Bright, S. A.; Poynton, F. E.; Nolan, D. E.; Quinn, S. J.; Gunnlaugsson, T.; Williams, D. C. *J. Med. Chem.* **2015**, 58, 4494-4505.
- (255) Li, L.; Zhang, S.; Xu, L.; Han, L.; Chen, Z.-N.; Luo, J. *Inorg. Chem.* **2013**, 52, 12323-12325.
- (256) Steed, J. W.; Turner, D. R.; Wallace, K. J. *Core concepts in supramolecular chemistry and nanochemistry*; John Wiley & Sons, 2007.
- (257) Desiraju, G. R. *Nature* **2001**, 412, 397-400.
- (258) Huang, F.; Anslyn, E. V. *Chem. Rev.* **2015**, 115, 6999-7000.

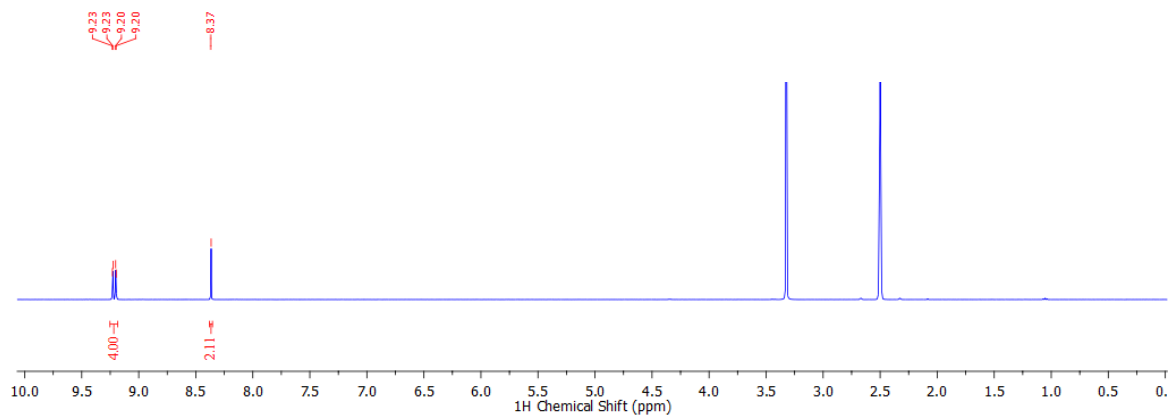
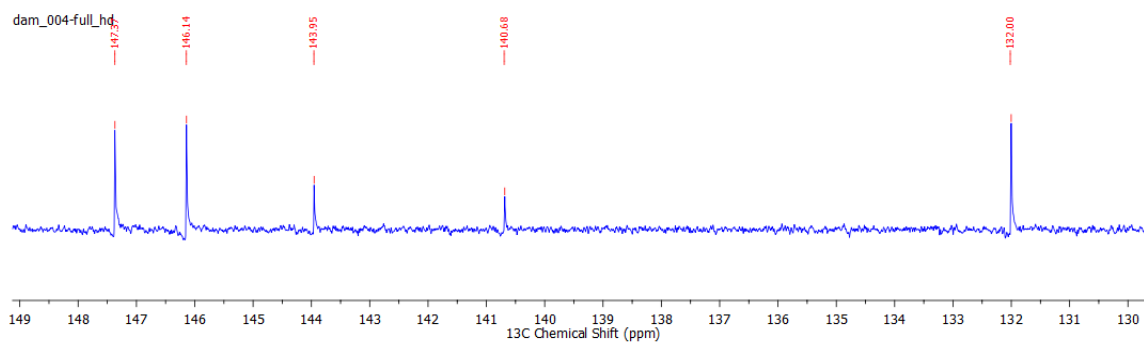
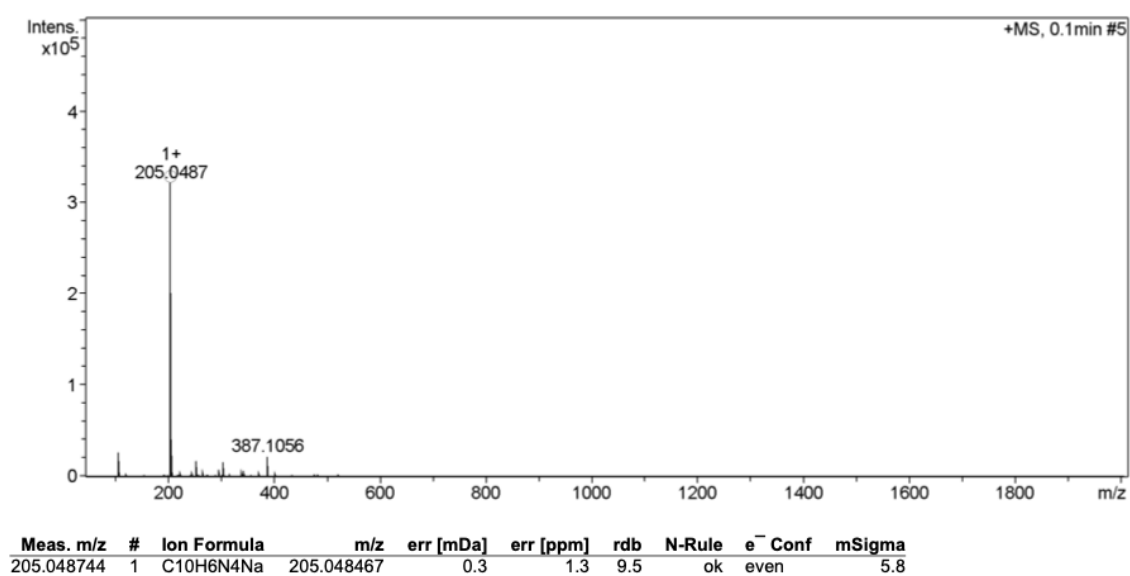
- (259) Barry, D. E.; Kitchen, J. A.; Pandurangan, K.; Savyasachi, A. J.; Peacock, R. D.; Gunnlaugsson, T. *Inorg. Chem.* **2020**, *59*, 2646-2650.
- (260) Lovitt, J. I.; Hawes, C. S.; Lynes, A. D.; Haffner, B.; Möbius, M. E.; Gunnlaugsson, T. *Inorg. Chem. Front.* **2017**, *4*, 296-308.
- (261) Powell, H. R. *The Biochemist* **2021**, *43*, 46-50.
- (262) Kannan, S.; Maayuri, R.; Shanmugaraju, S. *Inorg. Chim. Acta* **2023**, *550*, 121432.
- (263) Pollock, A. A.; Huther, H. A.; Birch, C. M.; Reinheimer, E. W.; Lewis, D. E.; Gerlach, D. L. *Acta Cryst. E* **2022**, *78*, 1161-1164.
- (264) Misra, S.; Singh, P.; Das, A.; Brandão, P.; Sahoo, P.; Sepay, N.; Bhattacharjee, G.; Datta, P.; Mahapatra, A. K.; Satpati, B.; Nanda, J. *Mater. Adv.* **2020**, *1*, 3532-3538.
- (265) Stomeo, F.; Lincheneau, C.; Leonard, J. P.; O'Brien, J. E.; Peacock, R. D.; McCoy, C. P.; Gunnlaugsson, T. *J. Am. Chem. Soc.* **2009**, *131*, 9636-9637.
- (266) Lincheneau, C.; Peacock, R. D.; Gunnlaugsson, T. *Chem. Asian J.* **2010**, *5*, 500-504.
- (267) Piguet, C.; Williams, A. F.; Bernardinelli, G. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1622-1624.
- (268) Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 12062-12063.
- (269) dos Santos, C. M. G.; Harte, A. J.; Quinn, S. J.; Gunnlaugsson, T. *Coord. Chem. Rev.* **2008**, *252*, 2512-2527.
- (270) Gu, Y.; Kar, T.; Scheiner, S. *J. Am. Chem. Soc.* **1999**, *121*, 9411-9422.
- (271) Tsuzuki, S.; Honda, K.; Uchamaru, T.; Mikami, M.; Tanabe, K. *J. Am. Chem. Soc.* **2000**, *122*, 3746-3753.
- (272) Yao, Z.-F.; Wang, J.-Y.; Pei, J. *Cryst. Growth Des.* **2018**, *18*, 7-15.
- (273) Calatrava-Pérez, E.; Acherman, S.; Stricker, L.; McManus, G.; Delente, J.; Lynes, A. D.; Henwood, A. F.; Lovitt, J. I.; Hawes, C. S.; Byrne, K.; Schmitt, W.; Kotova, O.; Gunnlaugsson, T.; Scanlan, E. M. *Org. Biomol. Chem.* **2020**, *18*, 3475-3480.
- (274) Matsuura, T.; Koshima, H. *J. Photochem. Photobiol., C* **2005**, *6*, 7-24.
- (275) Chulvi, K.; Costero, A. M.; Ochando, L. E.; Gaviña, P. *Cryst. Growth Des.* **2015**, *15*, 3452-3456.
- (276) Li, P.; Vik, E. C.; Maier, J. M.; Karki, I.; Strickland, S. M. S.; Umana, J. M.; Smith, M. D.; Pellechia, P. J.; Shimizu, K. D. *J. Am. Chem. Soc.* **2019**, *141*, 12513-12517.
- (277) Mooibroek, T. J.; Gamez, P.; Reedijk, J. *CrystEngComm* **2008**, *10*, 1501-1515.
- (278) Sheldrick, G. *Acta Crystallogr., Sect. A* **2015**, *71*, 3-8.
- (279) Watkin, D. J.; Cooper, R. I. *Chemistry* **2020**, *2*, 796-804.
- (280) Bruker. SAINT v8.38, Bruker AXS Inc., Madison, WI, USA. 2016.
- (281) Krause, L.; Herbst-Irmer, R.; Sheldrick, G. M.; Stalke, D. *J. Appl. Crystallogr.* **2015**, *48*, 3-10.
- (282) Bruker. APEX3 v2017.3-0, Bruker AXS Inc., Madison, WI, USA. 2017.
- (283) Sheldrick, G. *Acta Crystallogr., Sect. C* **2015**, *71*, 3-8.
- (284) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339-341.
- (285) Crosby, G. A.; Demas, J. N. *J. Phys. Chem.*; **1971**, *75*, 991-1024.
- (286) Fery-Forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76*, 1260.
- (287) Marrington, R.; Dafforn, T. R.; Halsall, D. J.; MacDonald, J. I.; Hicks, M.; Rodger, A. *Analyst* **2005**, *130*, 1608-1616.
- (288) Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45-55.
- (289) Moldes, A. B.; Alonso, J. L.; Parajó, J. C. *Bioprocess Biosyst. Eng.* **2003**, *25*, 357-363.
- (290) Shim, S. C.; Lee, S. K. *Synthesis* **1980**, *1980*, 116-116.

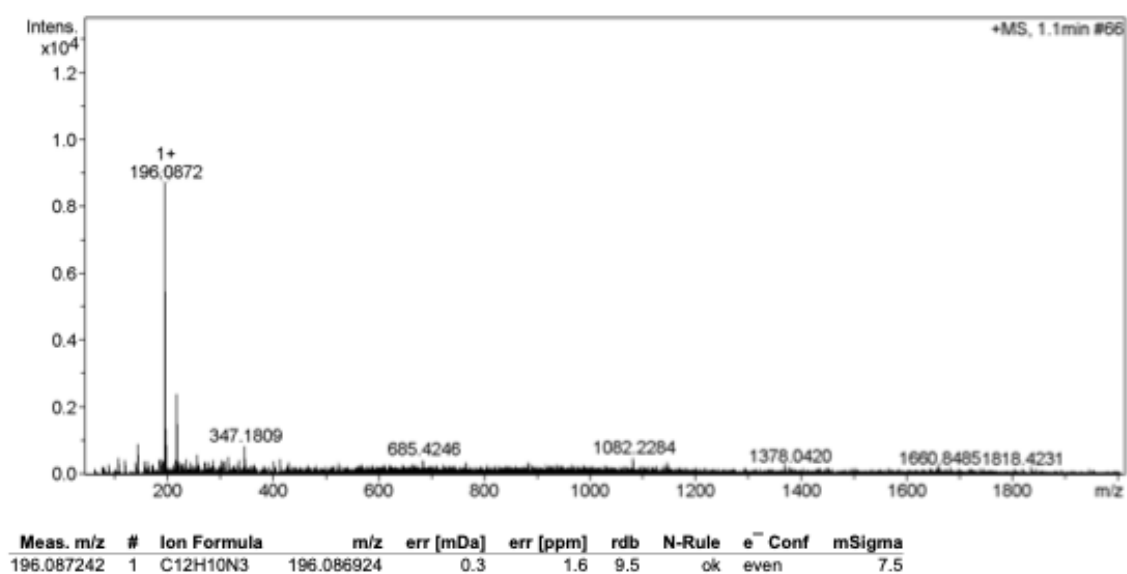
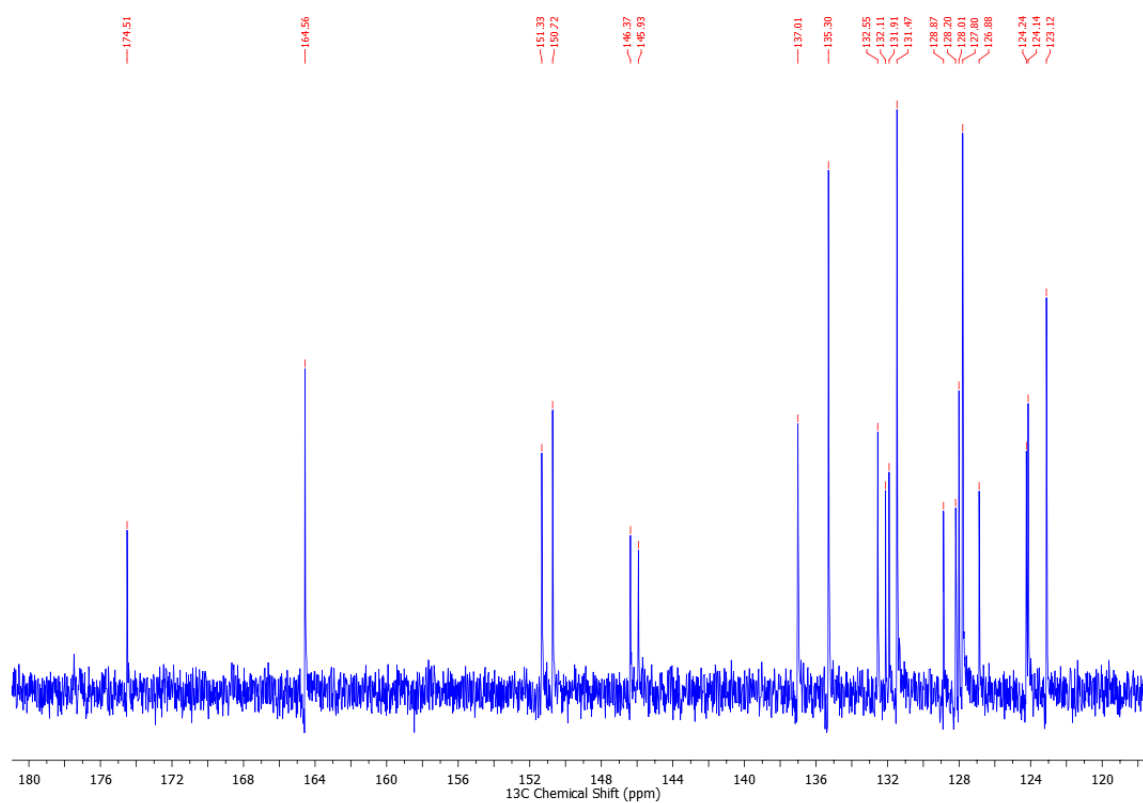
(291) Zheng, R. H.; Guo, H. C.; Jiang, H. J.; Xu, K. H.; Liu, B. B.; Sun, W. L.; Shen, Z. Q. *Chin. Chem. Lett.* **2010**, 21, 1270-1272.

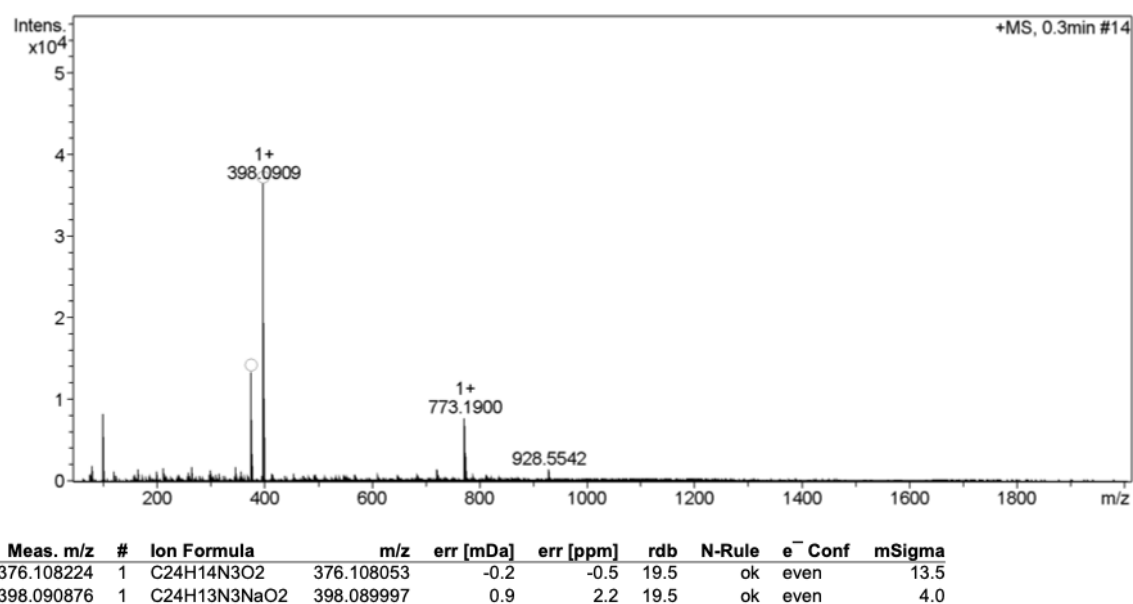
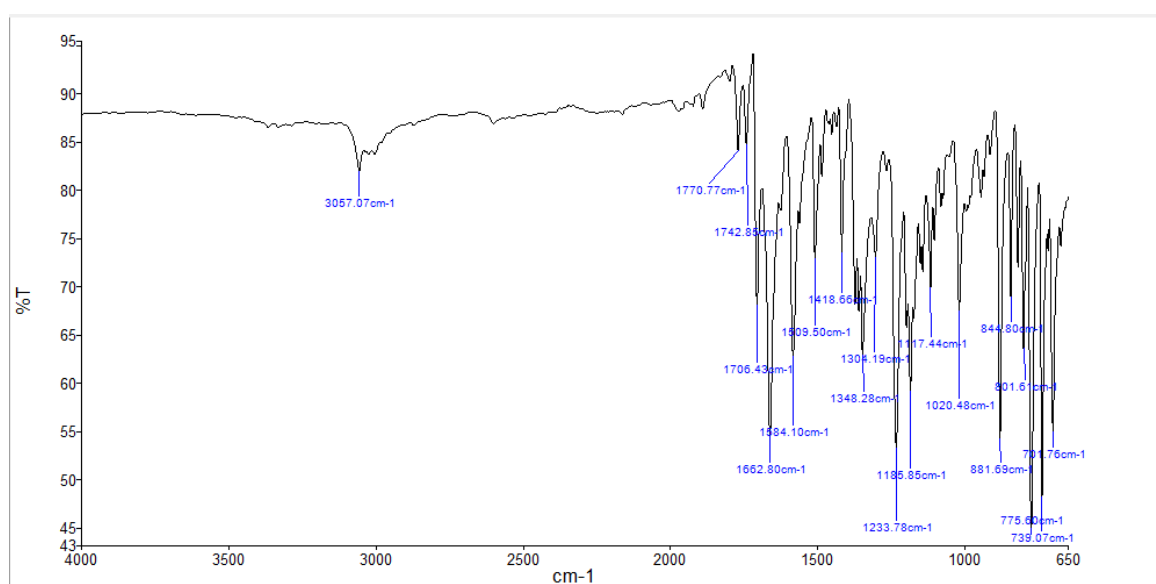
Chapter 9:

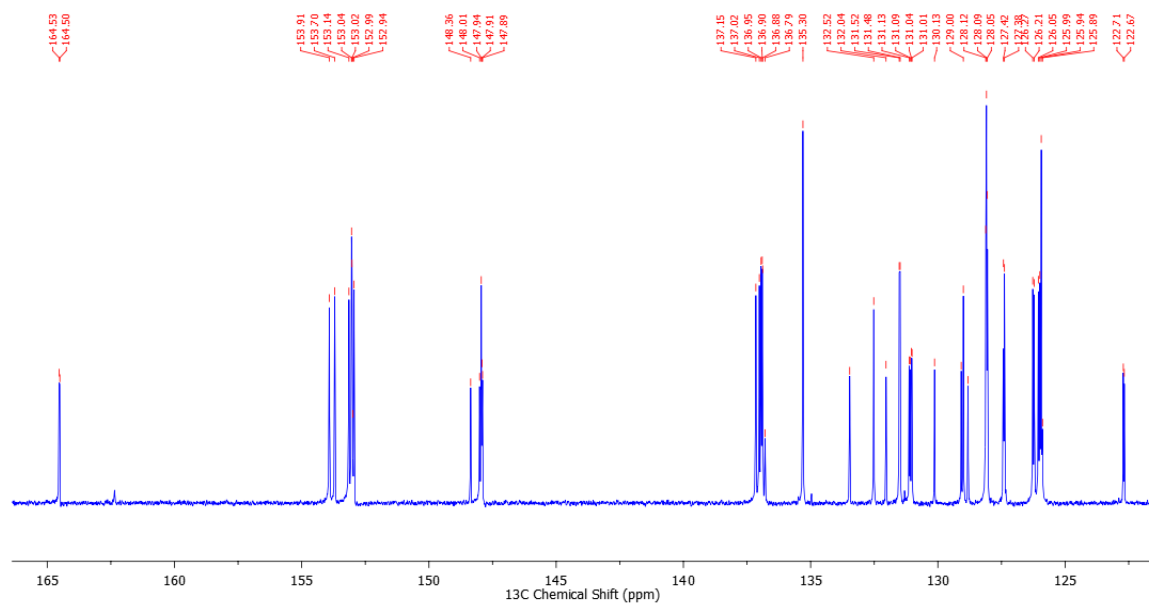
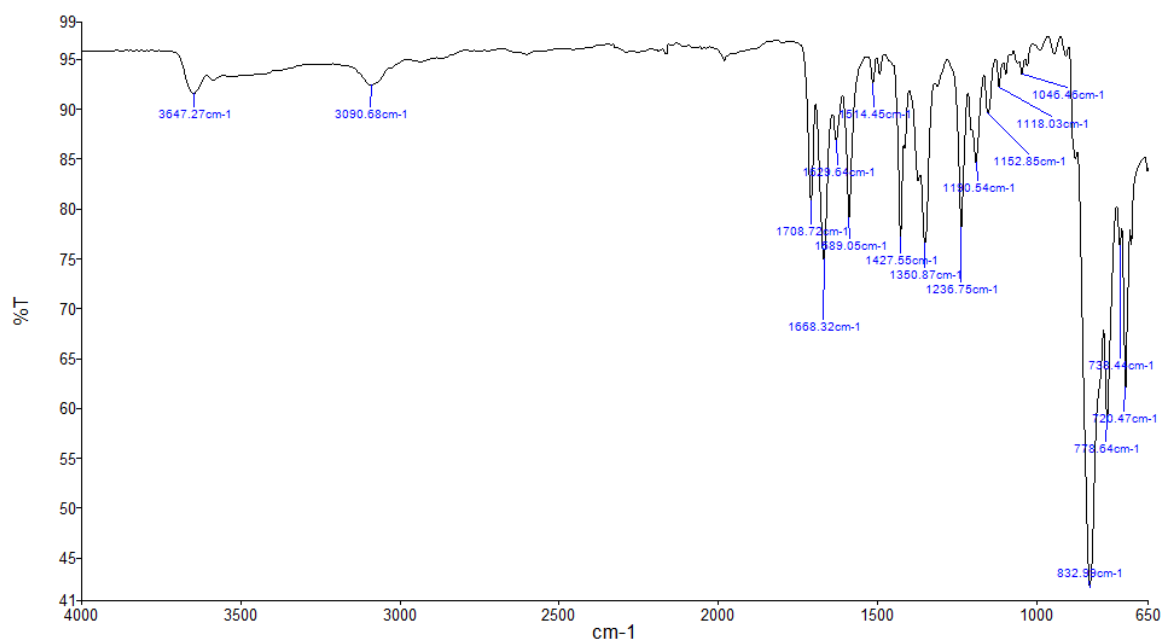
Appendices

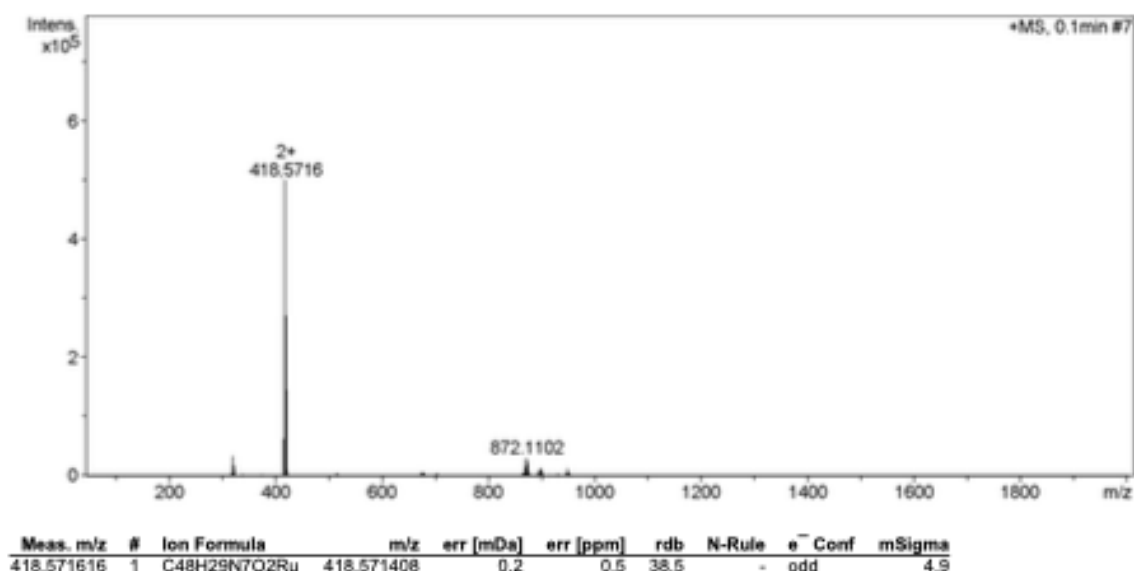
Appendix for Chapter 2

Figure A2.1: ¹H NMR spectrum of **TAP** (600 MHz, DMSO-*d*₆).Figure A2.2: ¹³C NMR spectrum of **TAP** (150 MHz, DMSO-*d*₆).Figure A2.3: Mass spectrum of **TAP**.

Figure A2.4: Mass spectrum of **78**.Figure A2.5: ¹³C NMR spectrum (150 MHz, DMSO-*d*₆) of **80**.

Figure A2.6: The mass spectrum of **80**.Figure A2.7: The IR spectrum of **80**.

Figure A2.8: ^{13}C NMR spectrum (150 MHz, CD_3CN) of **75**.Figure A2.9: The IR spectrum of **75**(PF_6)₂.

Figure A2.10: Mass spectrum of **75**.

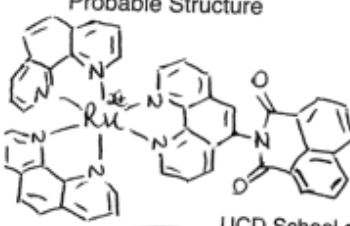
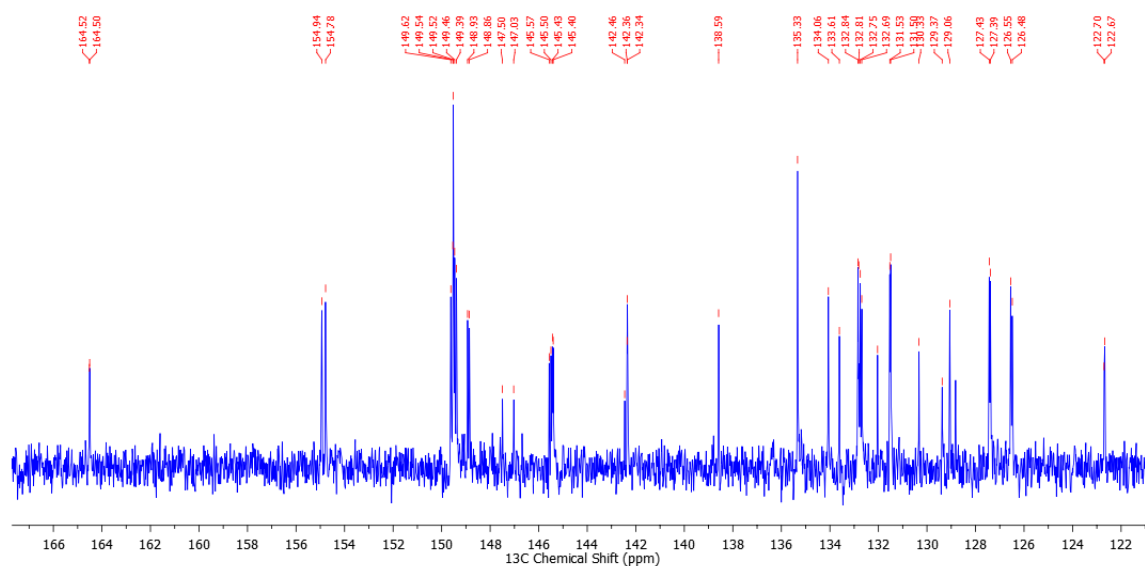
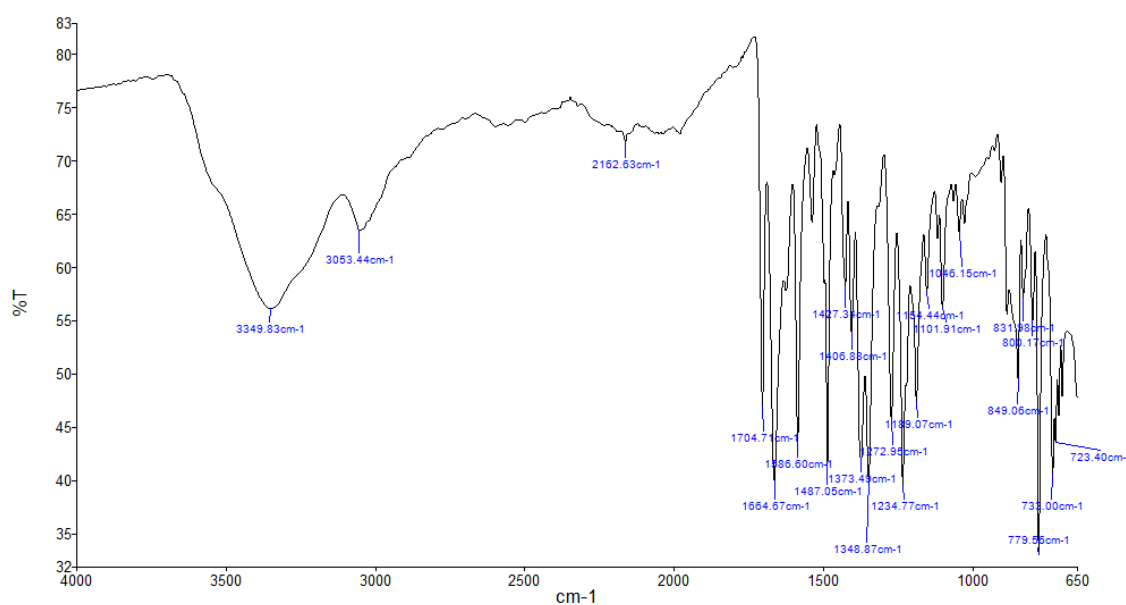
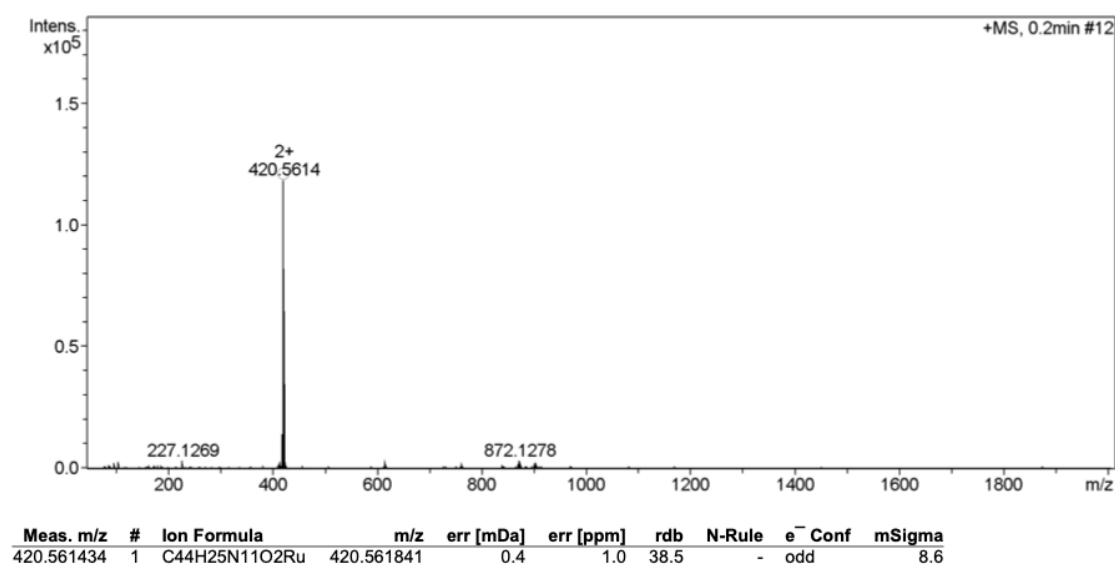
Submitted by DEIRDRE MCADAMS	Tel. No. 01-896 3928	Supervisor/Company Prof. T. Gunnlaugsson	M1 09-04-24 13039	Date in 09-04-11	Order No./Account No.			
SAMPLE INFORMATION			Analysis Req'd.	Theory	Found (A)%	Ref.	Found (B)%	Ref.
Sample No. DAMO41-r2								
Molecular Formula C₄₈H₂₉Cl₂N₇O₂Ru			1.C	63.51	57.14	67.99		
Tick if appropriate: Hazardous [] Air/Light Sensitive []			1.H	3.22	3.61			
Volatile [] Hygroscopic []			1.N	10.80	9.69			
Probable Structure 								
								
UCD School of Chemistry Microanalytical Laboratory University College Dublin Belfield, Dublin 4 Ireland T +353 1 716 2287 Email: microlab@ucd.ie								
comments: <i>Hi Ronan, can you please send me results via email - mcadamd@tcd.ie Apologies about the small amount</i>								

Figure A2.11: CHN Analysis for complex **75**.

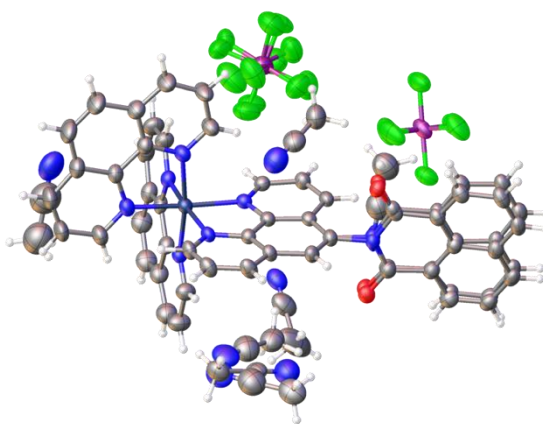
Figure A2.12: ¹³C NMR spectrum (150 MHz, CD₃CN) of **76**.Figure A2.13: The IR spectrum of **76**.

Figure A2.14: Mass spectrum of **76**.Table A2.15: Crystal data and structure refinement for **75**, **76** and **89**.

Identification code	75	76	89
Empirical formula	C _{54.5} H _{38.75} F ₁₂ N _{10.25} O ₂ P ₂ Ru	C _{46.5} H _{39.5} Cl ₂ N ₁₁ O _{6.75} Ru	C ₅₀ H ₄₀ Cl ₂ N ₁₀ O ₃ Ru
Formula weight	1260.22	1032.36	1000.89
Temperature/K	100(2)	100(2)	100(2)
Crystal system	triclinic	monoclinic	monoclinic
Space group	P-1	C2/c	P2 ₁ /c
a/Å	11.6049(7)	45.7041(14)	13.680(4)
b/Å	12.7065(8)	13.1974(4)	14.865(5)
c/Å	20.6041(13)	16.7441(6)	23.282(7)
α/°	82.135(3)	90	90
β/°	83.845(2)	92.5256(16)	97.267(9)
γ/°	66.801(2)	90	90
Volume/Å ³	2761.6(3)	10089.8(6)	4696(3)
Z	2	8	4
ρ _{calc} /cm ³	1.516	1.359	1.416
μ/mm ⁻¹	0.434	3.976	4.186
F(000)	1271.0	4220.0	2048.0

Crystal size/mm ³	0.227 × 0.134 × 0.058	0.408 × 0.125 × 0.072	0.08 × 0.04 × 0.03
Radiation	Mo Kα (λ = 0.71073)	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)
2θ range for data collection/°	3.998 to 52.522	3.87 to 140.034	6.514 to 140.162
Index ranges	-14 ≤ h ≤ 14, -15 ≤ k ≤ 15, -25 ≤ l ≤ 25	-55 ≤ h ≤ 54, -16 ≤ k ≤ 16, -20 ≤ l ≤ 20	-14 ≤ h ≤ 16, -17 ≤ k ≤ 18, -27 ≤ l ≤ 26
Reflections collected	43735	164551	19417
Independent reflections	11065 [R _{int} = 0.1126, R _{sigma} = 0.1060]	9507 [R _{int} = 0.0721, R _{sigma} = 0.0286]	8481 [R _{int} = 0.0439, R _{sigma} = 0.0571]
Data/restraints/parameters	11065/1628/988	9507/2015/1075	8481/0/598
Goodness-of-fit on F ²	1.062	1.078	1.086
Final R indexes [I > 2σ (I)]	R ₁ = 0.0893, wR ₂ = 0.2064	R ₁ = 0.0887, wR ₂ = 0.2501	R ₁ = 0.0420, wR ₂ = 0.1141
Final R indexes [all data]	R ₁ = 0.1540, wR ₂ = 0.2428	R ₁ = 0.1006, wR ₂ = 0.2657	R ₁ = 0.0466, wR ₂ = 0.1176
Largest diff. peak/hole / e Å ⁻³	0.91/-0.99	1.48/-1.04	1.35/-0.84

a)



b)

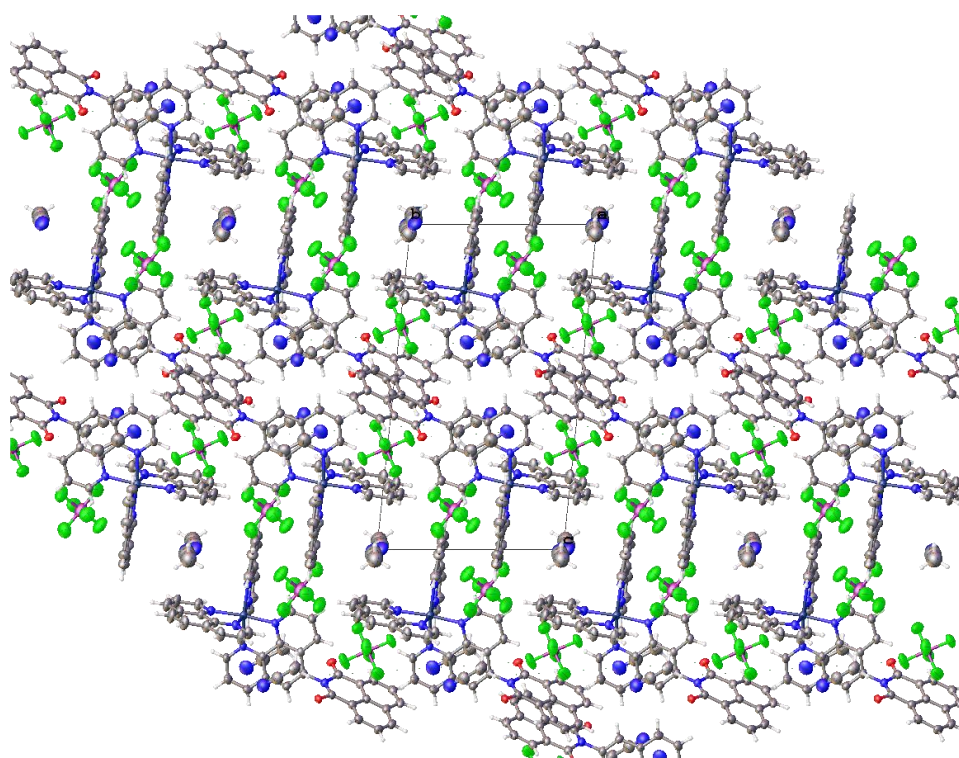


Figure A2.16: a) Asymmetric unit of **75** displaying Hydrogen bonding interactions. b) Packed structure of **75** shown normal to the 100 plane.

Table 2.17: Hydrogen Bonds for **75**. D = donor atom, H = hydrogen atom, A = acceptor atom.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C36	H36	F5 ¹	0.95	2.41	3.245(9)	146
C35	H35	O2 ²	0.95	2.62	3.22(5)	122
C35	H35	F3 ¹	0.95	2.56	3.170(10)	122
C9	H9	F2 ³	0.95	2.50	3.205(11)	131
C49	H49B	O2	0.98	2.37	3.12(4)	132
C49	H49B	F1	0.98	2.53	3.223(12)	128

¹-1+X,1+Y,+Z; ²1-X,1-Y,1-Z; ³+X,1+Y,+Z

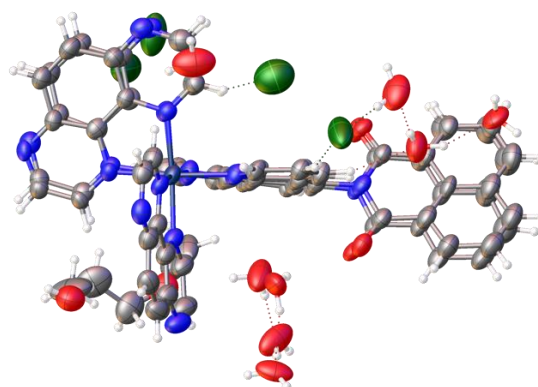


Figure A2.18: a) Asymmetric unit of **76**. Hydrogen bonding and halogen-H bonding interactions are represented by red and green dotted lines, respectively.

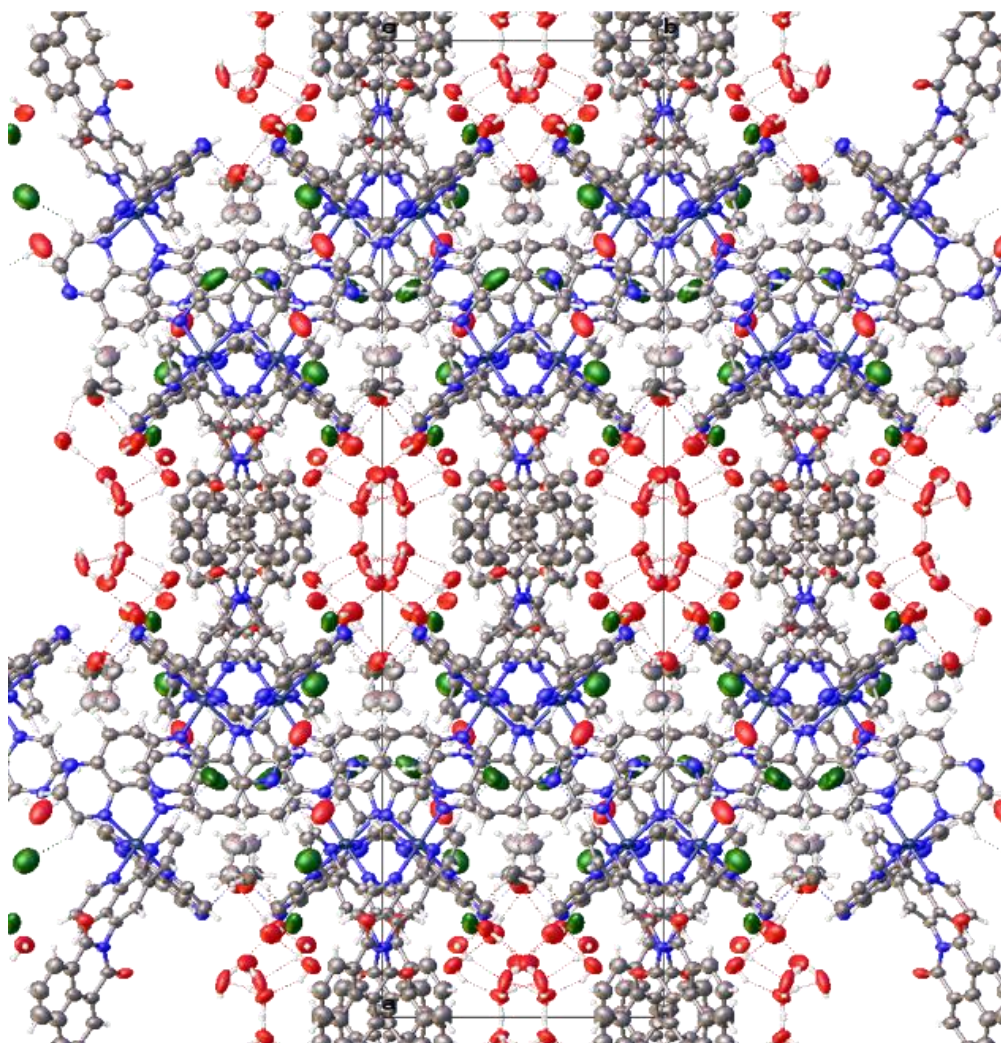
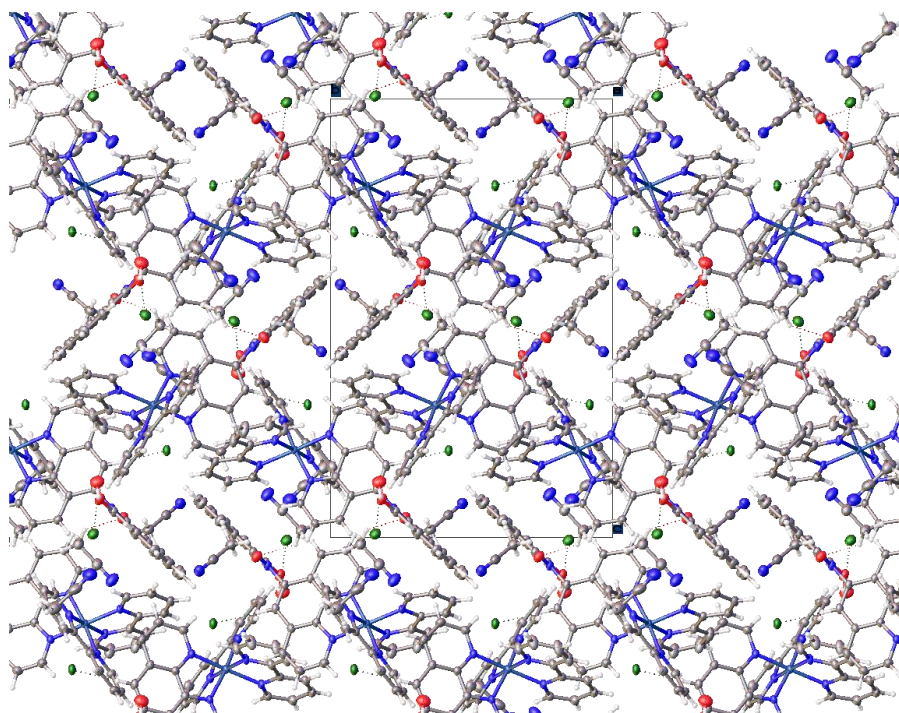


Figure A2.19: Packed structure of **76** shown normal to the 001 plane.

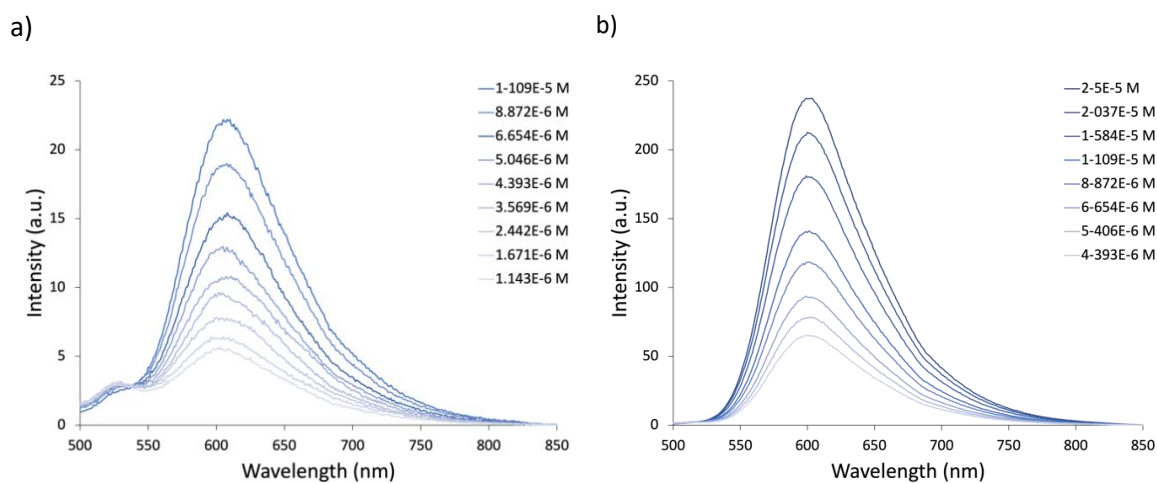
Table A2.20: Hydrogen Bonds for 76. D = donor atom, H = hydrogen atom, A = acceptor atom.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C14A	H14A	Cl2A	0.95	2.54	3.25(3)	132.0
C26A	H26A	N7 ¹	0.95	2.44	3.19(3)	135.2
C33B	H33B	O2	0.95	2.30	3.22(2)	162.4
O1	H1A	Cl2B	0.87	2.06	2.91(2)	166.1
O1	H1B	O53B ²	0.87	2.34	3.19(2)	165.1
C55B	H55A	N10 ³	0.98	2.08	3.024(18)	161.3
O4	H4A	O5	0.87	2.02	2.859(17)	161.0
O4	H4B	O6	0.87	2.18	2.81(3)	128.8
O5	H5B	O1 ⁴	0.87	2.36	3.18(2)	156.9
O2	H2A	O3	0.87	1.96	2.75(3)	151.9
O2	H2B	O1	0.87	2.01	2.86(2)	167.7
O7	H7B	O3 ⁴	0.87	1.86	2.66(3)	152.3
O3	H3B	O5 ²	0.87	2.11	2.91(3)	151.7
O51A	H51D	Cl1B ¹	0.84	2.64	3.280(18)	133.5
O8	H8A	Cl1B ⁵	0.87	2.25	3.117(19)	173.2
O8	H8B	Cl1B ⁶	0.87	1.94	2.73(3)	150.3

¹3/2-X,1/2+Y,1/2-Z; ²+X,-1+Y,+Z; ³+X,2-Y,-1/2+Z; ⁴+X,1+Y,+Z; ⁵3/2-X,-1/2+Y,1/2-Z; ⁶+X,1-Y,1/2+Z

Figure A2.21: Packed structure of **89** shown normal to the 100 plane.Table A2.22: Hydrogen Bonds for **89**. D = hydrogen bond donor atom, A = hydrogen bond acceptor atom.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C48	H48C	O2	0.98	2.41	3.198(4)	136.9
O3	H3A	O1	0.87	2.03	2.872(3)	162.7

Figure A2.23: Dilution studies of **75** in (a) MeCN and (b) PB at 298 K.

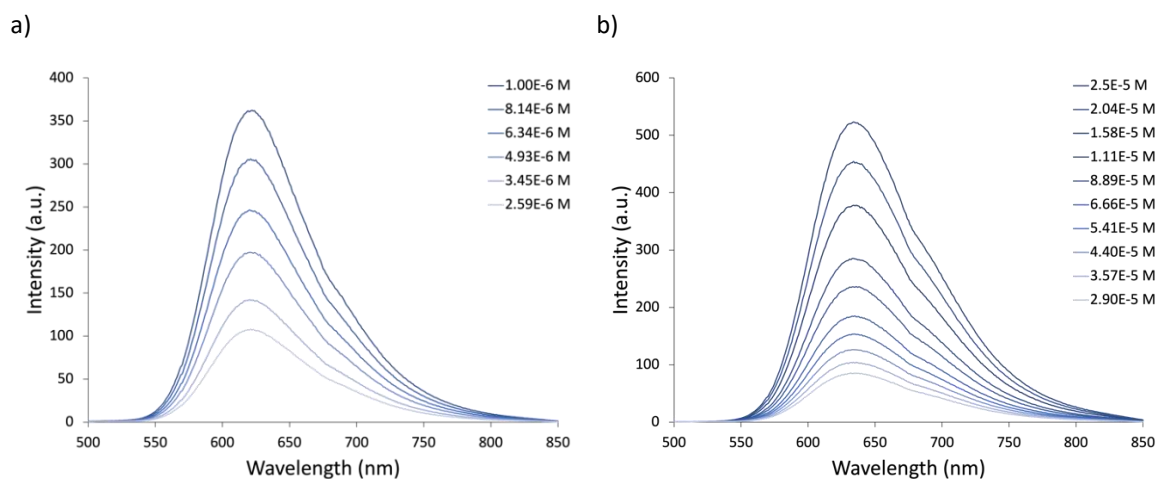


Figure A2.24: Dilution studies of **76** in (a) MeCN and (b) PB at 298 K.

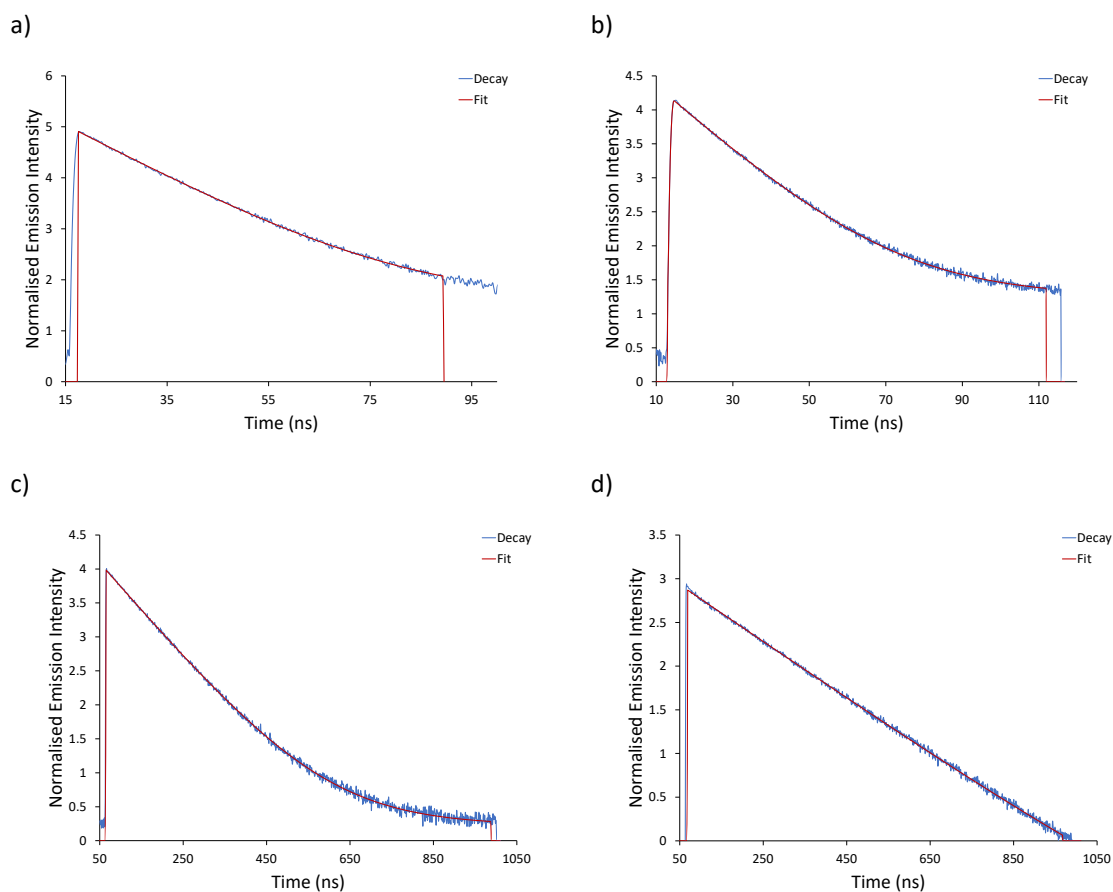


Figure A2.25: Time-resolved emission intensity and monoexponential fit of the data for **75** (OD at 450 nm = 0.1) in (a) buffered water, (b) deaerated buffered water, (c) MeCN and (d) deaerated MeCN

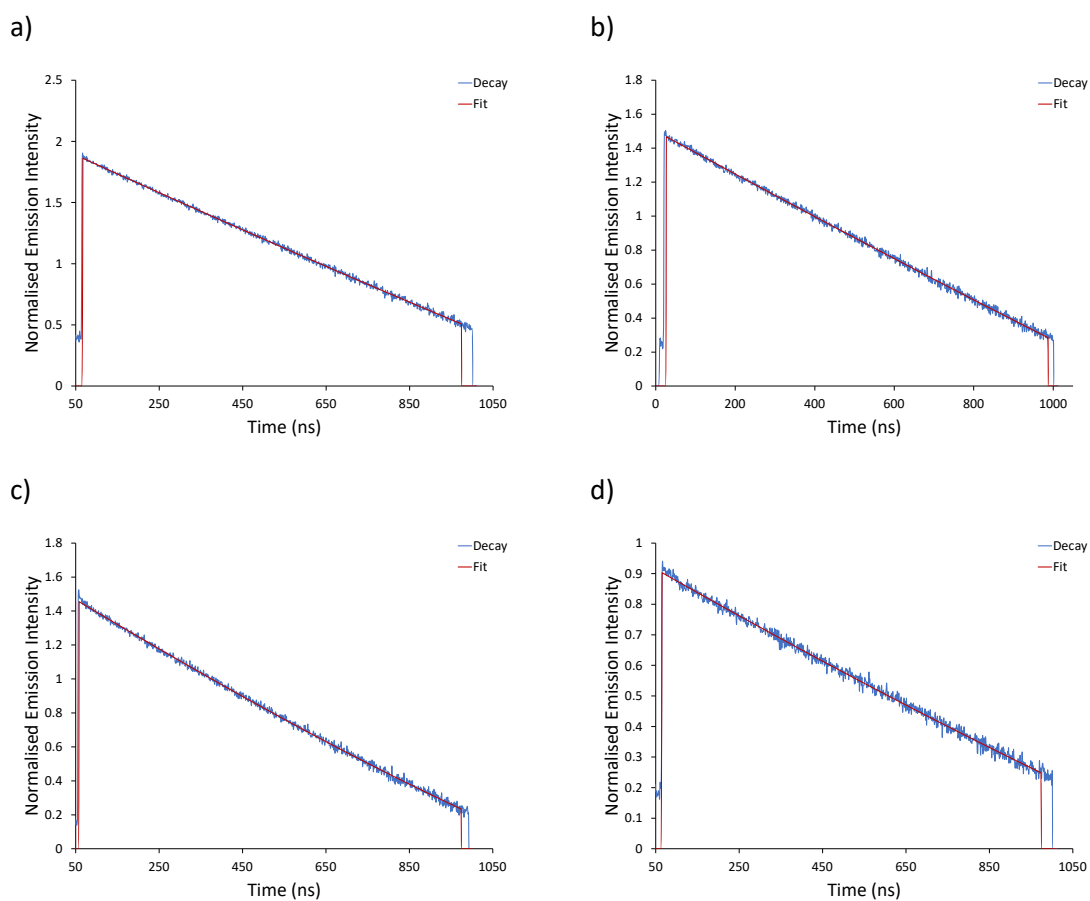


Figure A2.26: Time-resolved emission intensity and monoexponential fit of the data for **76** (OD at 450 nm = 0.1) in (a) buffered water, (b) deaerated buffered water, (c) MeCN and (d) deaerated MeCN.

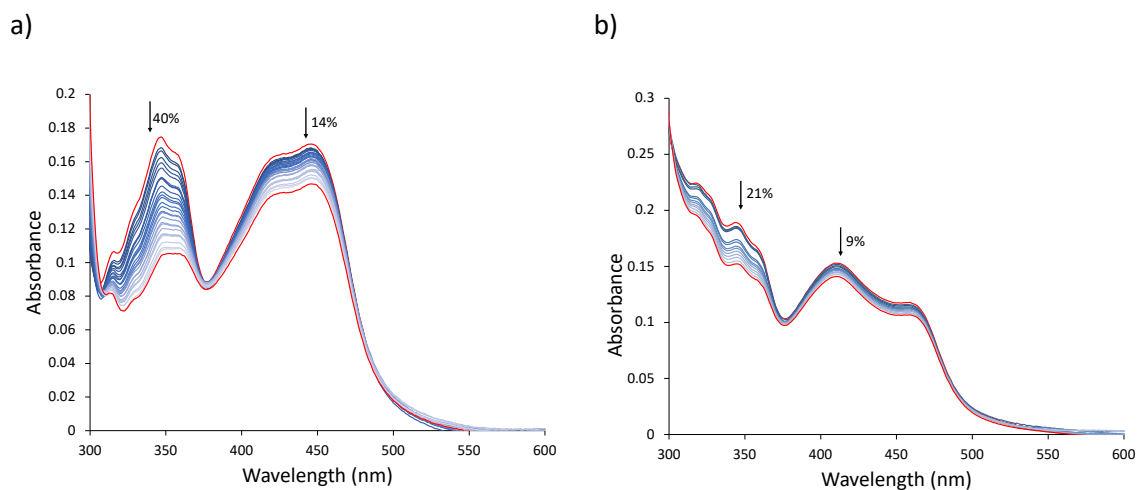


Figure A2.27: Changes in absorbance spectra for a) **75** (1x10⁻⁵ M, 10 mM PB + 160 mM NaCl), and b) **76** (1x10⁻⁵ M, 10 mM PB + 160 mM NaCl) upon addition of aliquots of ctDNA (0–0.6 mM)

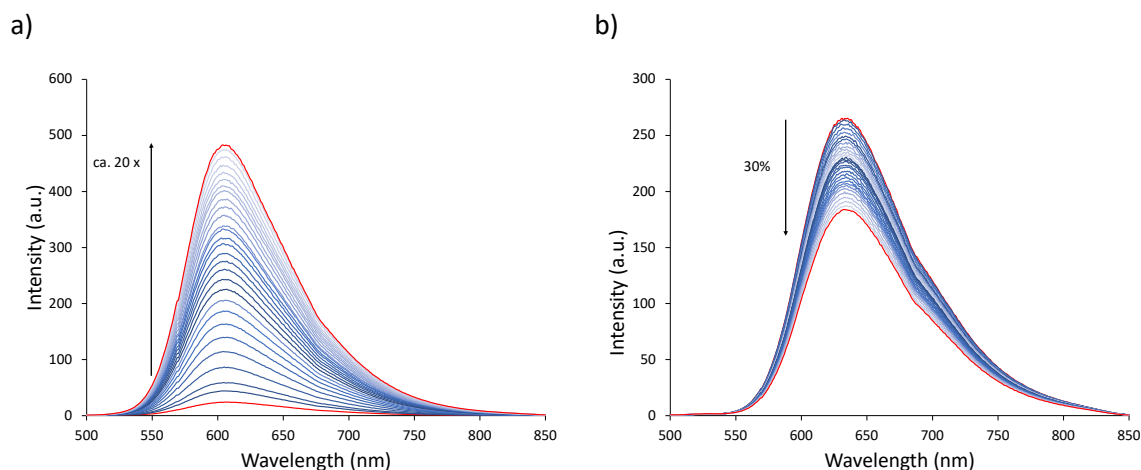


Figure A2.28: Changes in emission spectra for a) **75** and b) **76** (1x10⁻⁵ M in aqueous sodium phosphate-buffered solution + 160 mM NaCl) upon addition of aliquots of ctDNA (0–0.6 mM)

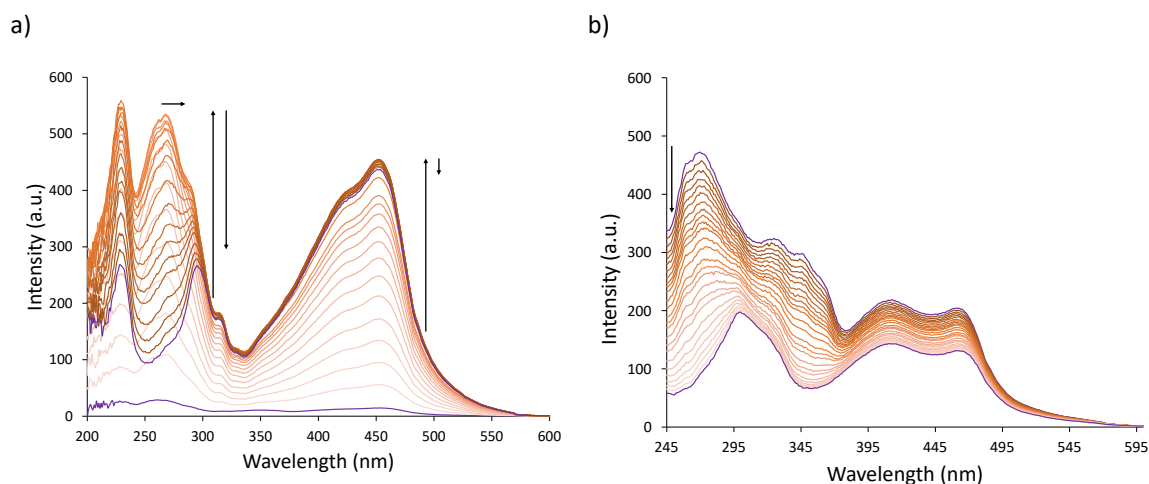


Figure A2.29: Excitation spectra for a) **75** (1x10⁻⁵ M, $\lambda_{em} = 600$ nm) and b) **76** (1x10⁻⁵ M, $\lambda_{em} = 630$ nm) in buffered water at 298 K.

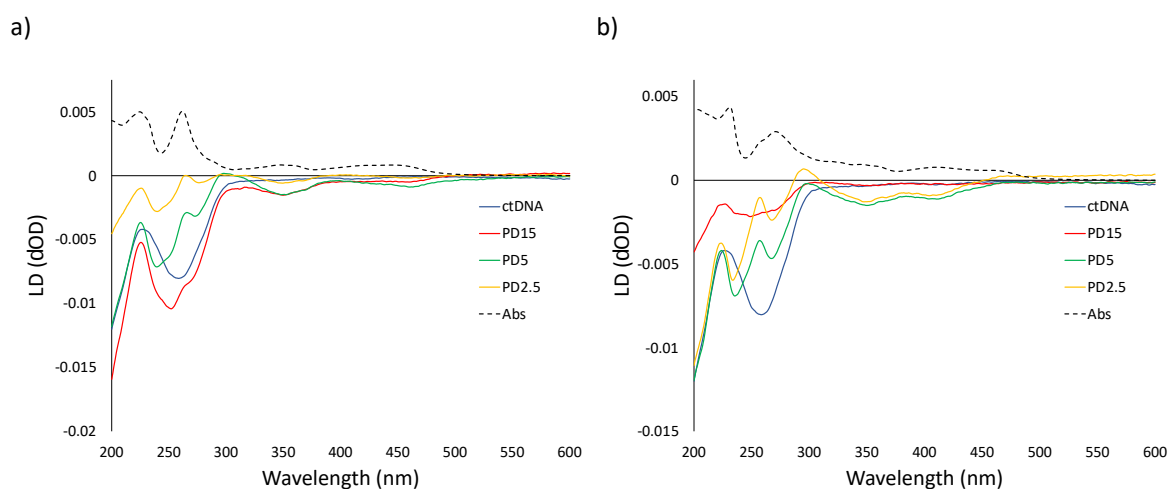


Figure A2.30: Linear dichroism spectra of ctDNA (300 μ M) in 10 mM sodium-phosphate buffered water at pH = 7.4 upon addition of (a) **75** and (b) **76** at different P/D ratios.

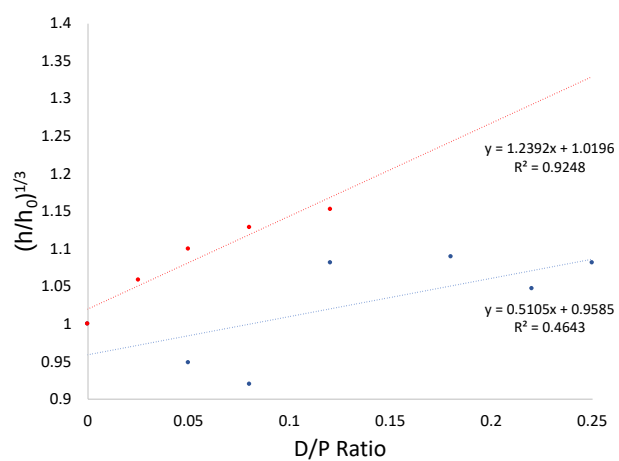


Figure A2.31: Viscosity measurements of **75** (blue) and **76** (red) at differing drug-to-phosphate ratios in aqueous sodium phosphate buffered solutions at pH = 7.4.

Appendix for Chapter 3:

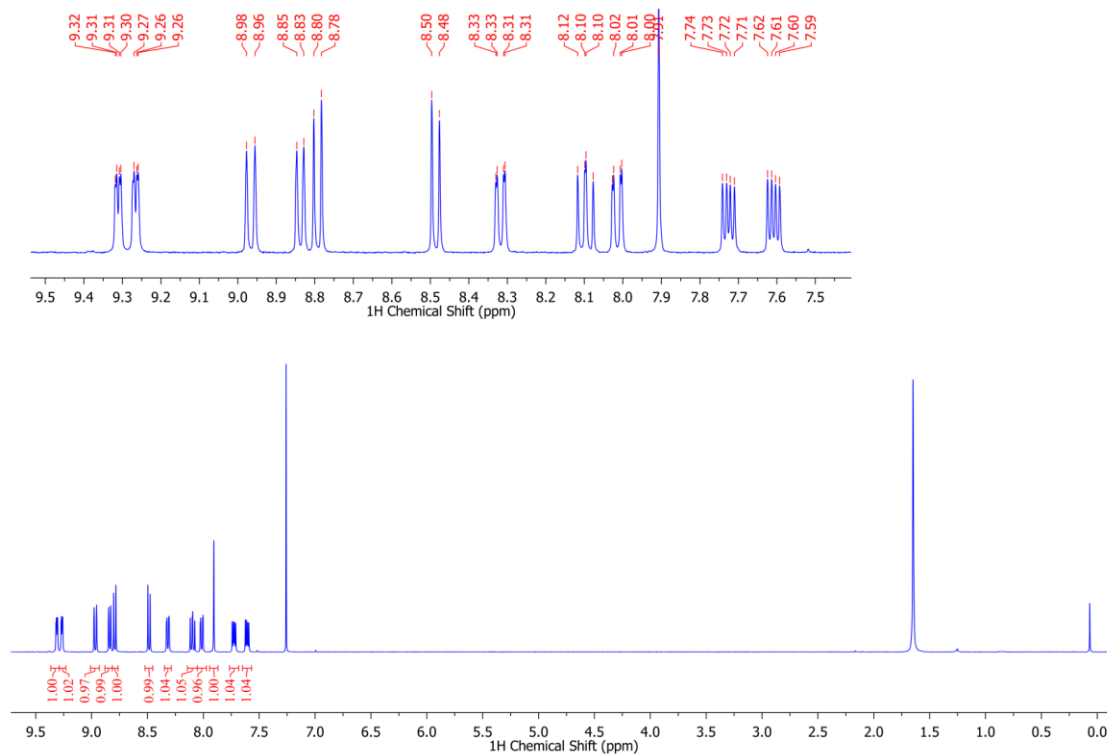


Figure A3.1: The ¹H NMR spectrum of *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91** (400 MHz, CDCl₃)

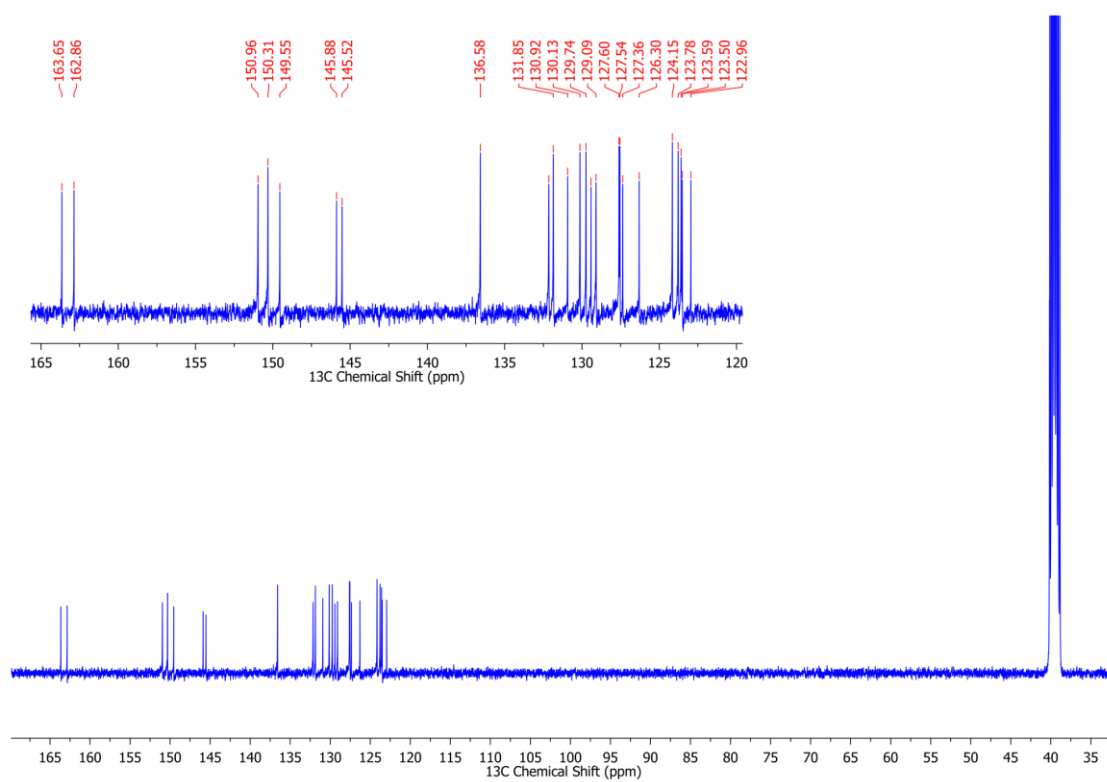


Figure A3.2: ¹³C NMR spectrum of *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91** (101 MHz, DMSO-*d*₆).

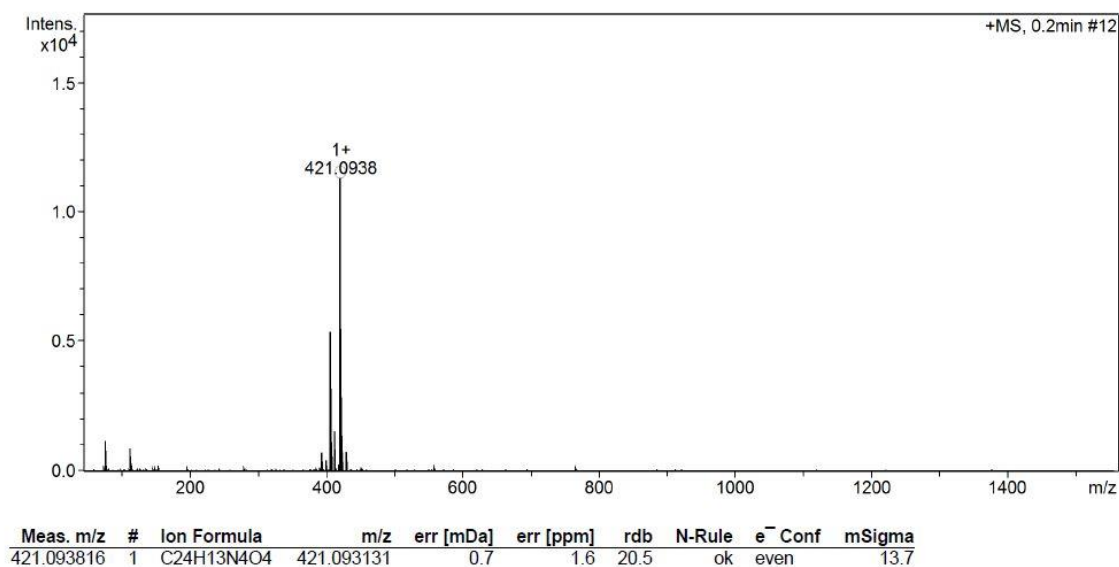


Figure A3.3: The ESI-HRMS spectrum of *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91** recorded in the ESI positive mode.

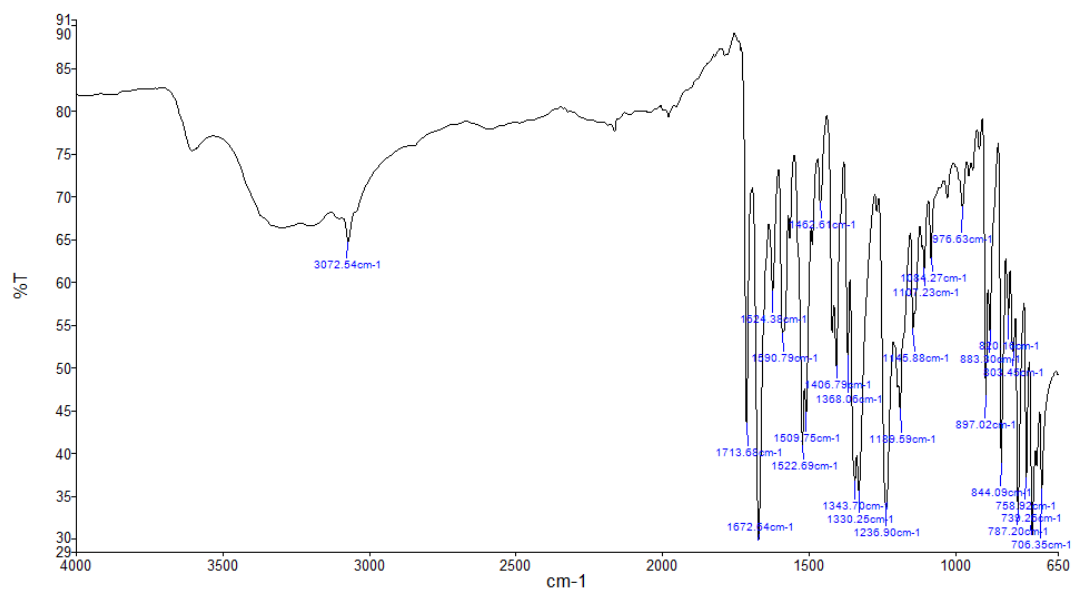


Figure A3.4: The IR spectrum of *N*-(1,10-phenanthroline)-4-nitronaphthalene-1,8-dicarboximide, **91**.

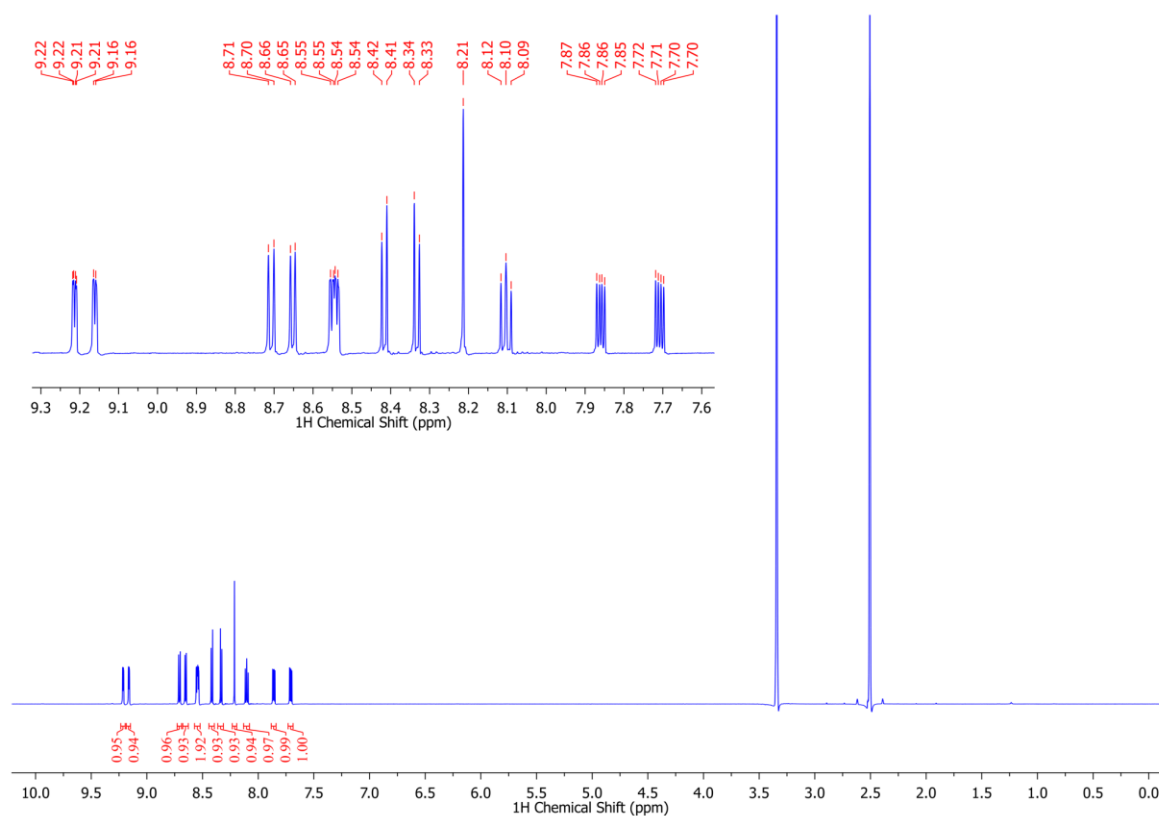


Figure A3.5: ¹H NMR spectrum of *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92** (400 MHz, DMSO-*d*₆).

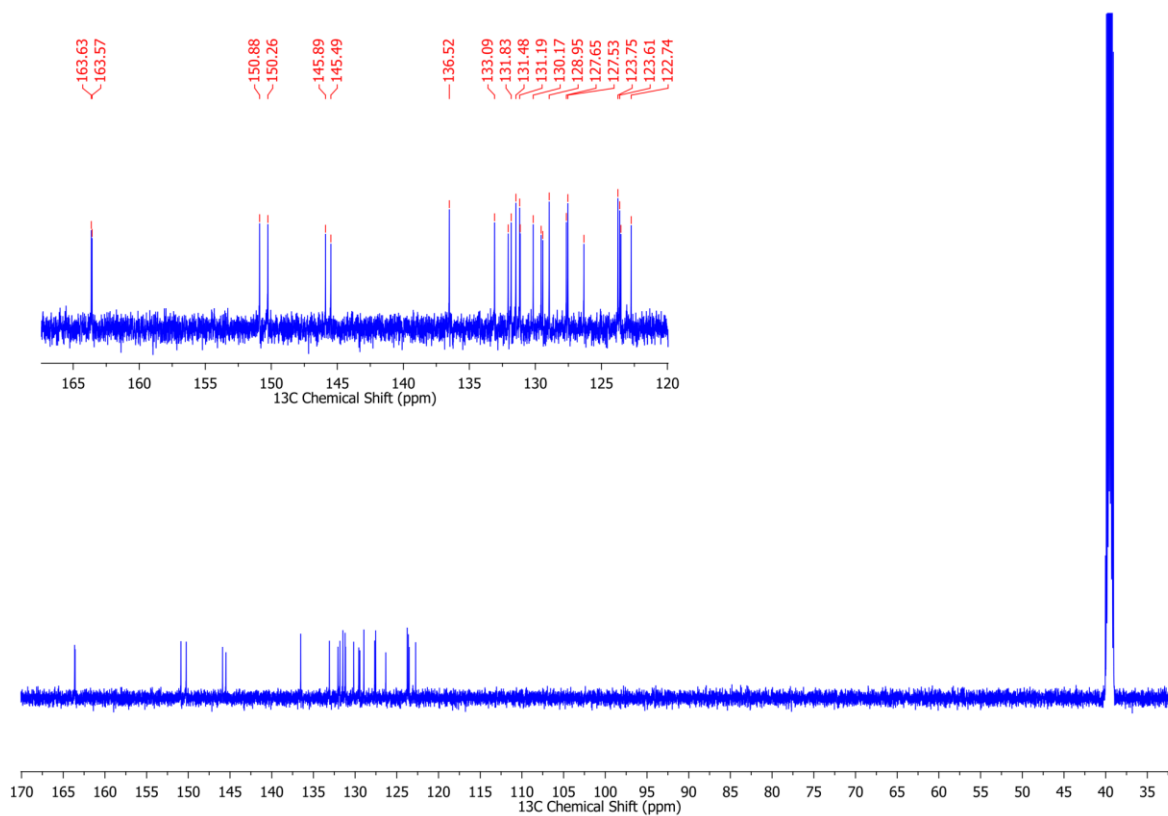


Figure A3.6: ¹³C NMR spectrum of *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92** (101 MHz, DMSO-*d*₆).

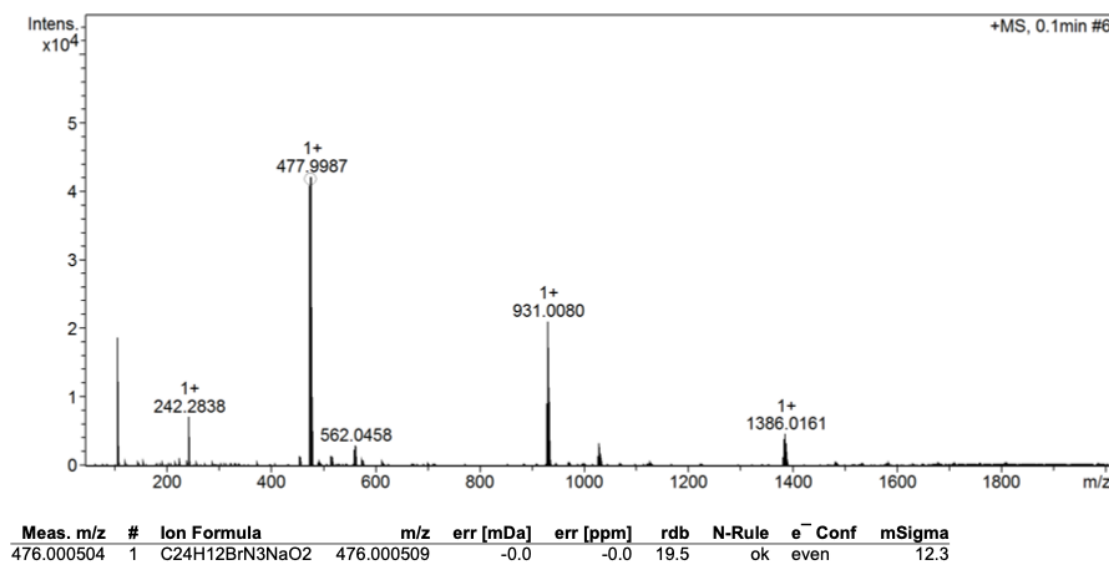


Figure A3.7: The HRMS spectrum of *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92** recorded in the ESI positive mode.

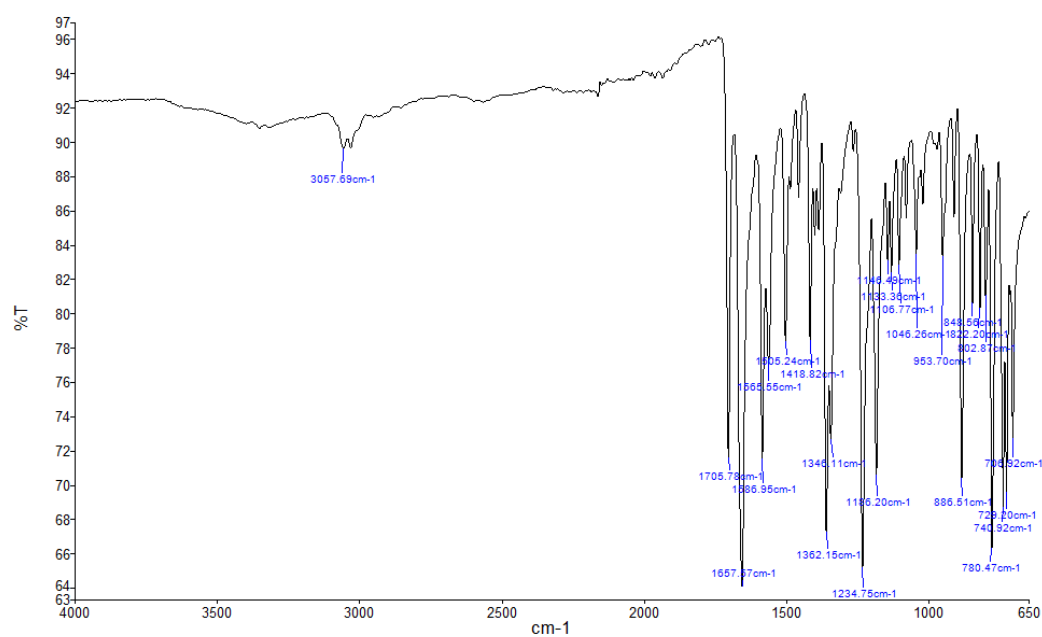


Figure A3.8: The IR spectrum of *N*-(1,10-phenanthroline)-4-bromonaphthalene-1,8-dicarboximide, **92**.

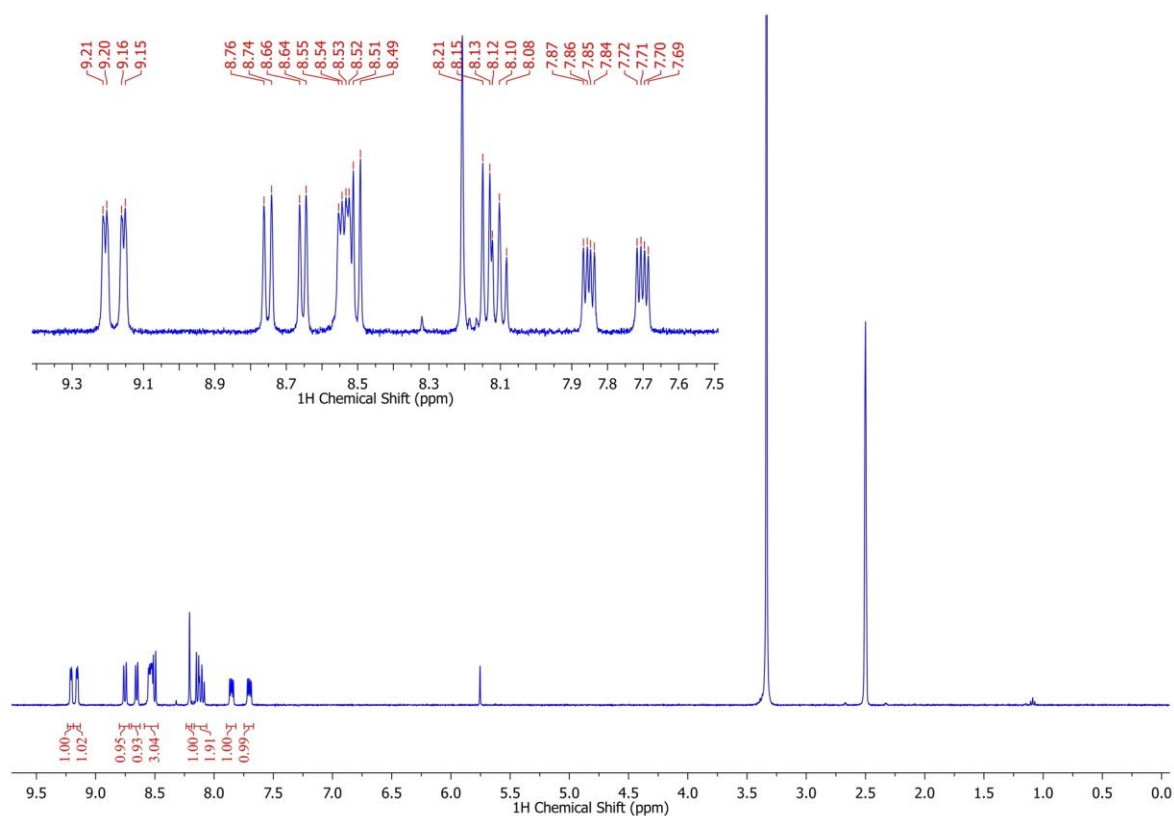


Figure A3.9: ¹H NMR spectrum of *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93** (400 MHz, DMSO-*d*₆).

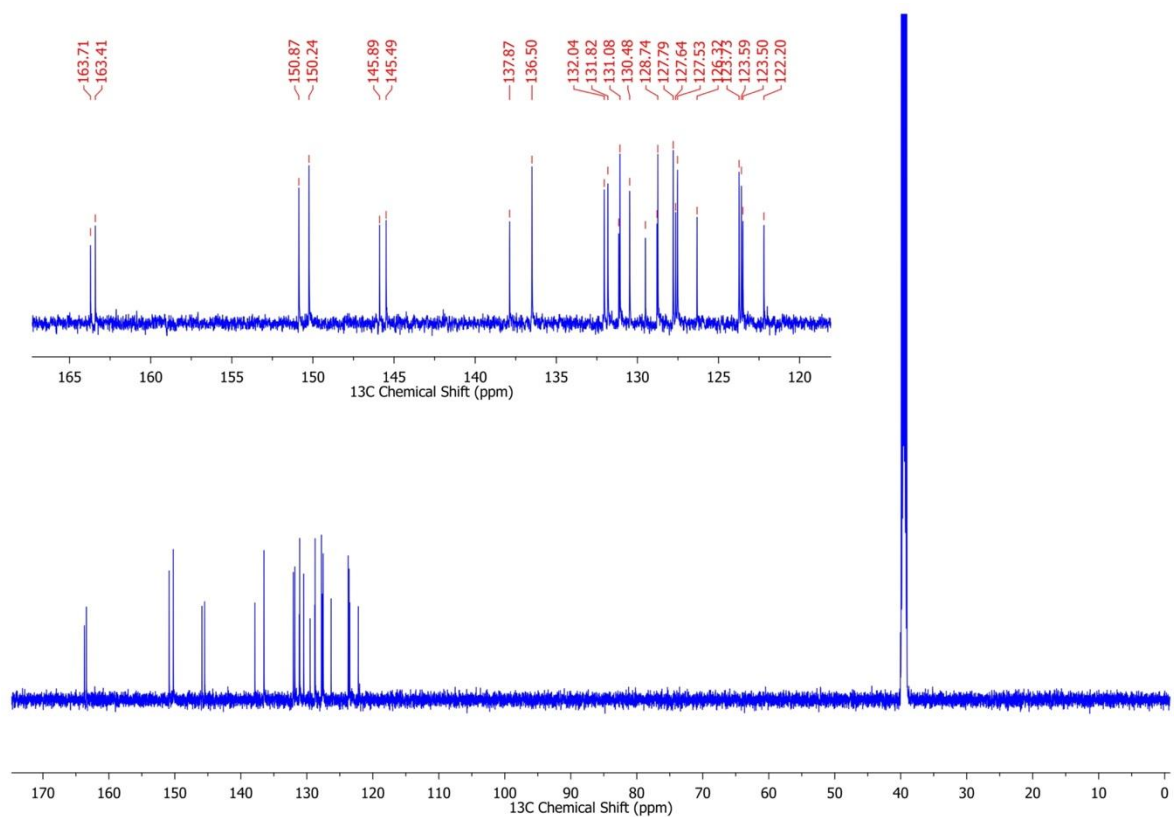


Figure A3.10: ¹³C NMR spectrum of *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93** (101 MHz, DMSO-*d*₆).

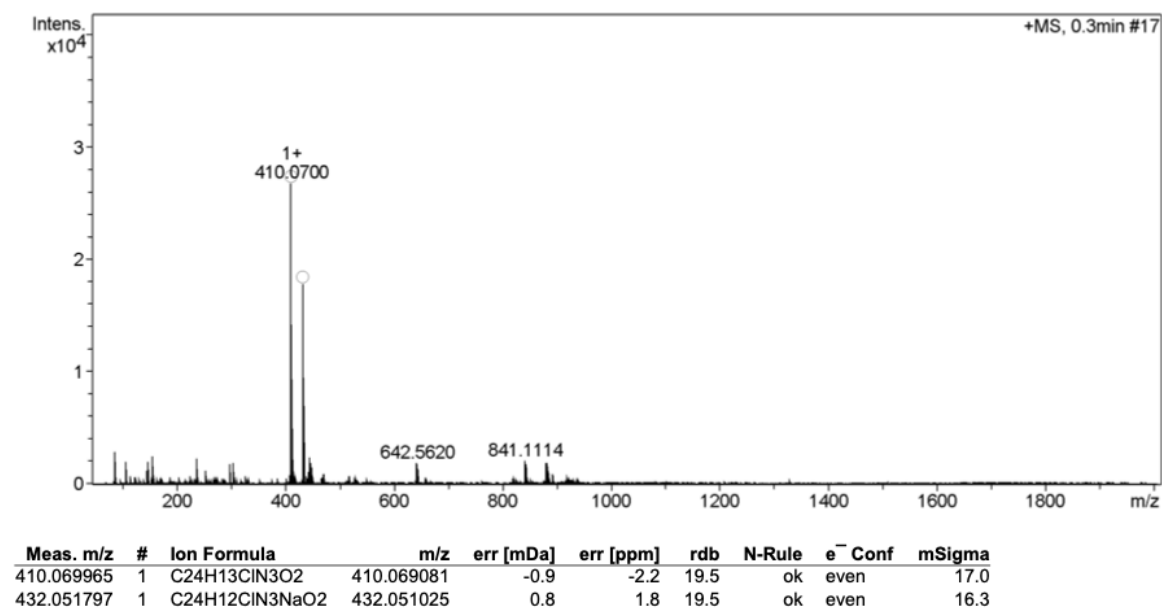


Figure A3.11: The ESI mass spectrum of *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93**.

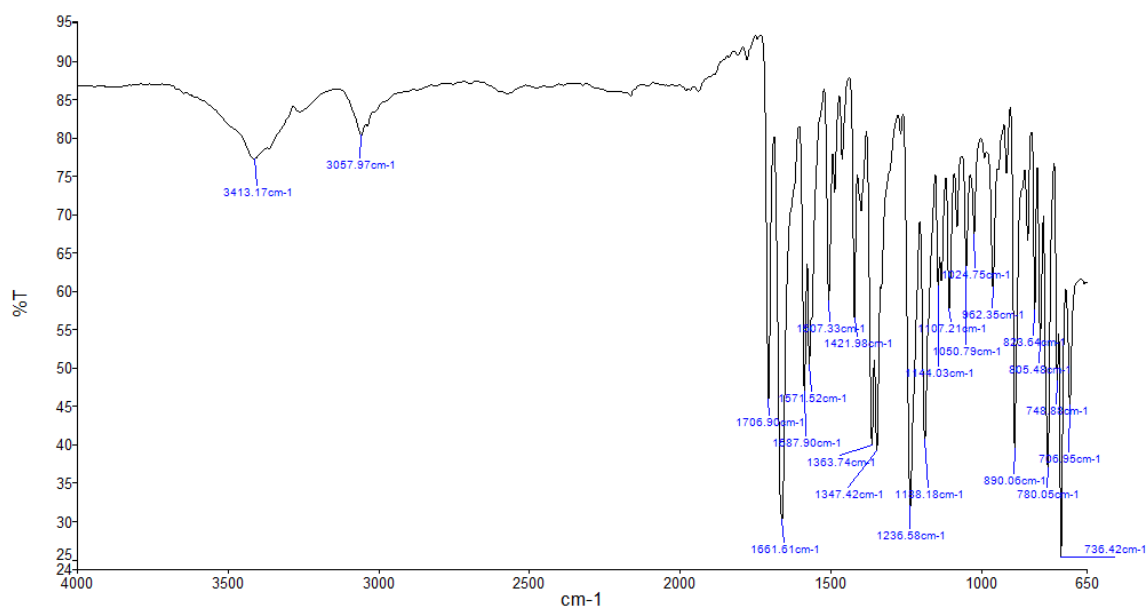


Figure A3.12: The IR spectrum of *N*-(1,10-phenanthroline)-4-chloronaphthalene-1,8-dicarboximide, **93**.

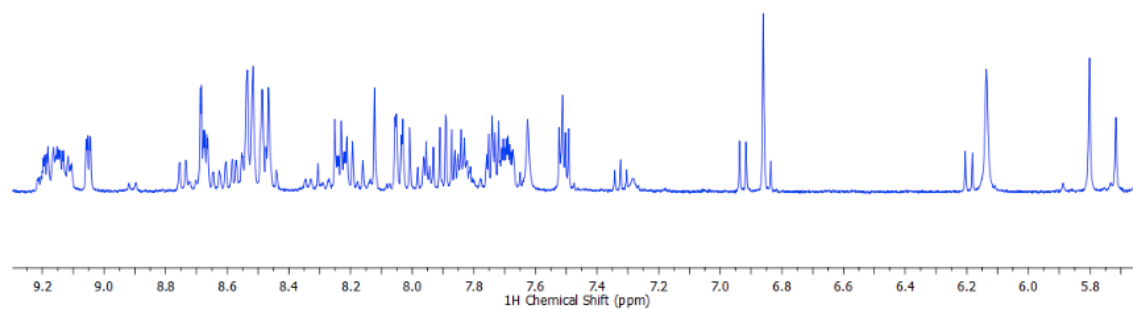


Figure A3.13: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of the crude product from [Table 3.1, Entry 5].

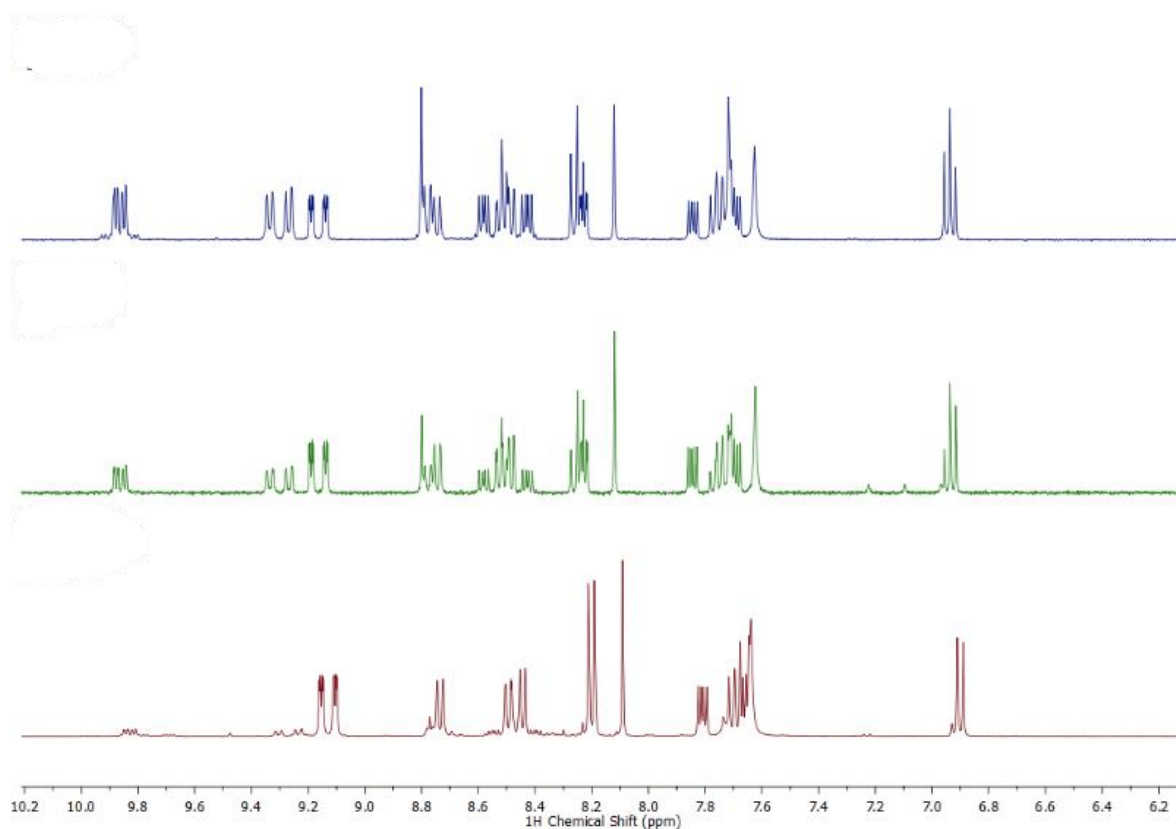


Figure A3.14: The ^1H NMR spectra (400 MHz, $\text{DMSO}-d_6$) of the crude product after workup (top), after multiple EDTA washes (middle and bottom) from conditions in Table 3.1, entry 14.

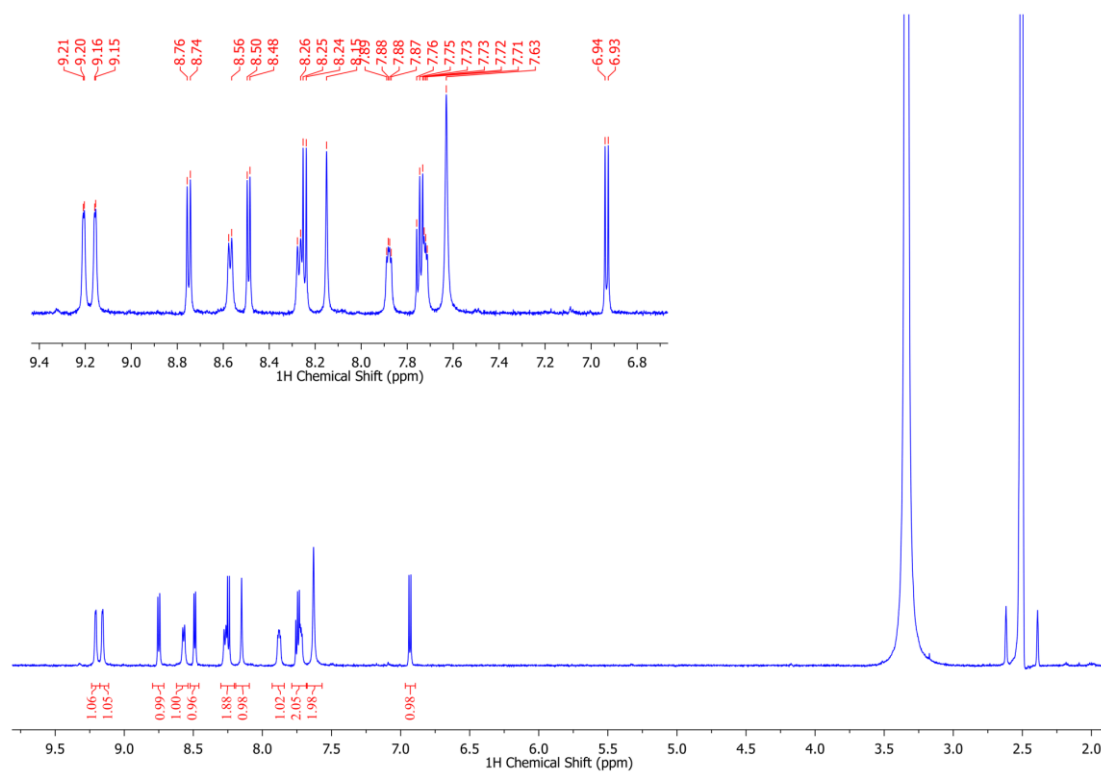


Figure A3.15: The ¹H NMR spectrum of *N*-(1,10-phenanthroline)-4-aminonaphthalene-1,8-dicarboximide, **97** (600 MHz, DMSO-*d*₆).

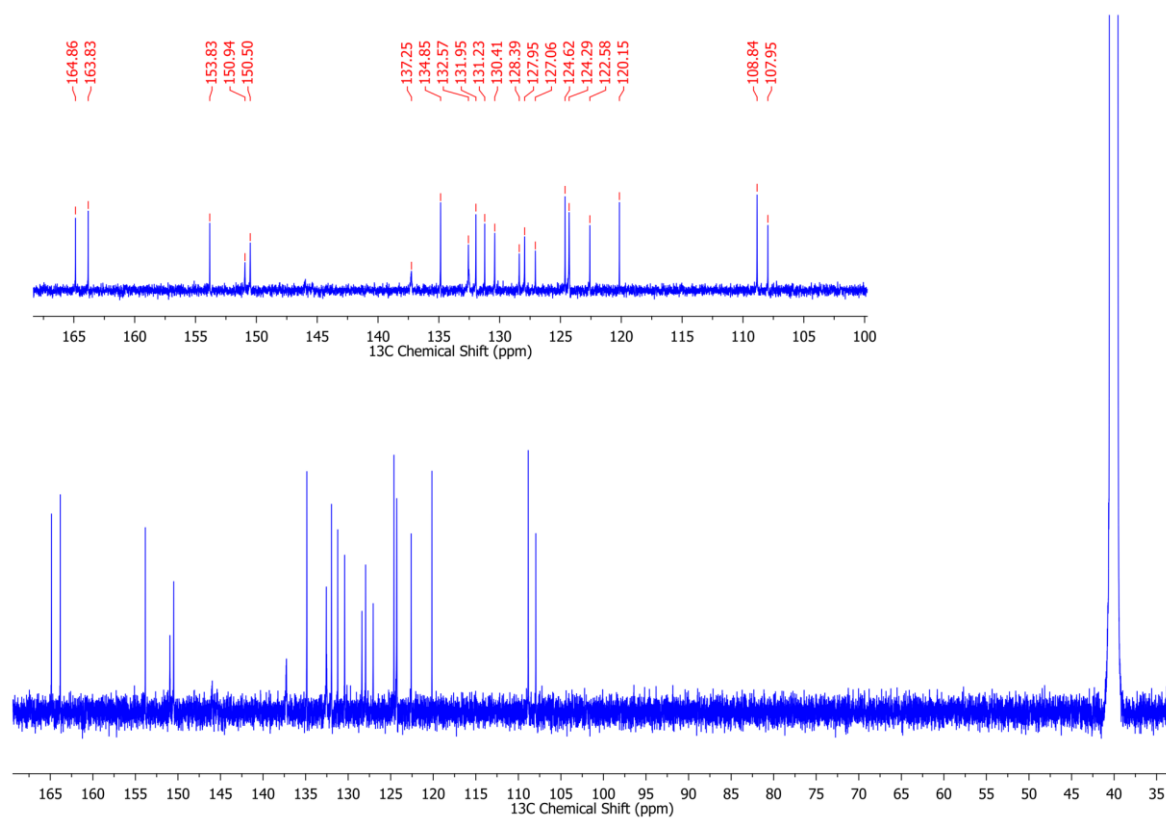


Figure A3.16: The ¹³C NMR spectrum of *N*-(1,10-phenanthroline)-4-aminonaphthalene-1,8-dicarboximide, **97** (101 MHz, DMSO-*d*₆).

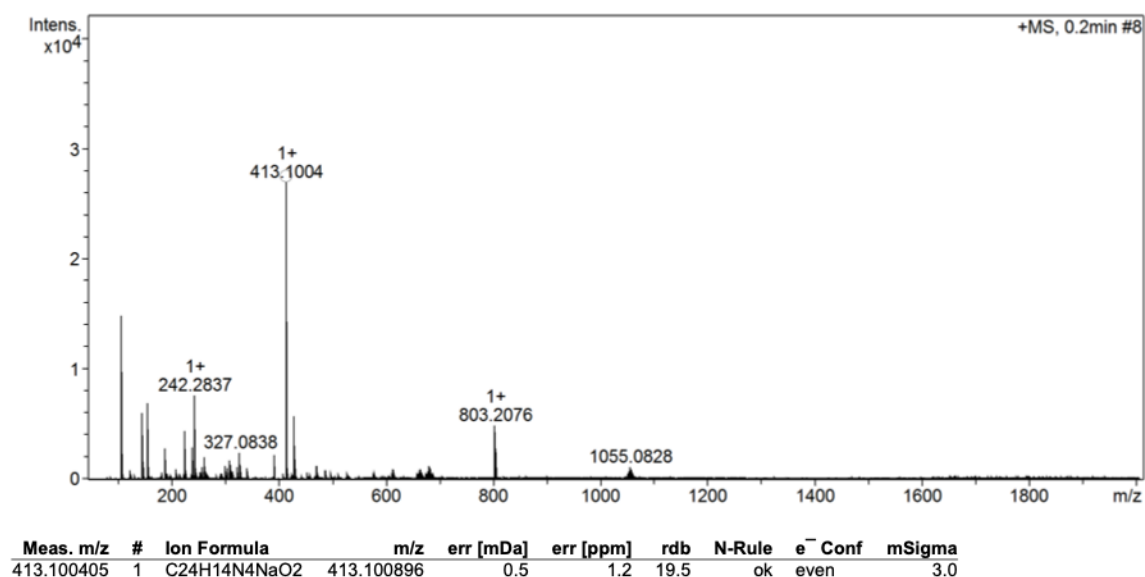


Figure A3.17: The ESI-mass spectrum of *N*-(1,10-phenanthroline)-4-aminonaphthalene-1,8-dicarboximide, **97**.

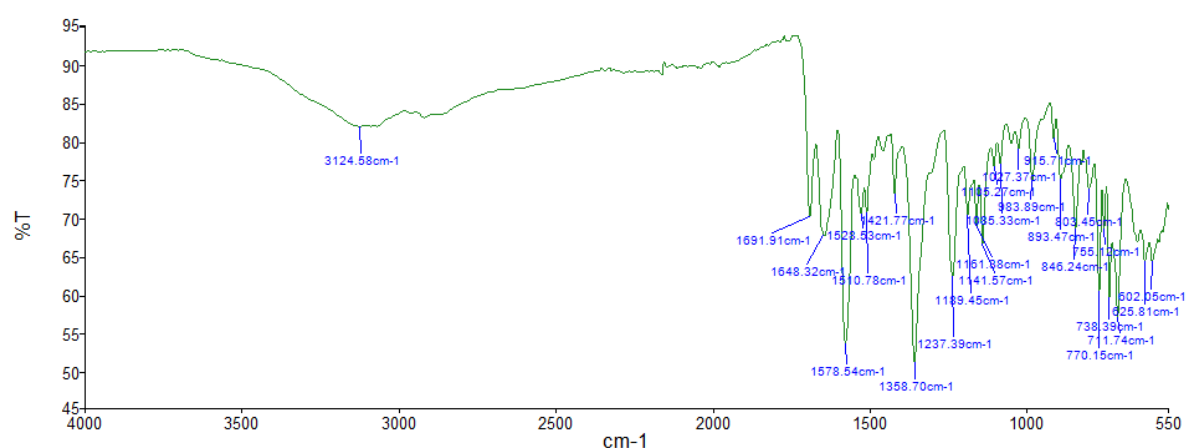


Figure A3.18: The IR spectrum of *N*-(1,10-phenanthroline)-4-aminonaphthalene-1,8-dicarboximide, **97**.

Table A3.1: Crystal data and structure refinement for ligands **91**, **92** and **93**

Identification code	91	92	93
Empirical formula	C ₃₁ H ₂₁ N ₄ O _{4.5}	C ₂₈ H ₂₀ BrN ₃ O ₆	C ₂₄ H ₁₄ ClN ₃ O ₃
Formula weight	521.52	574.38	427.82
Temperature/K	100(2)	100(2)	100(2)
Crystal system	monoclinic	triclinic	monoclinic
Space group	<i>C</i> 2/ <i>c</i>	<i>P</i> -1	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	27.472(12)	5.5841(4)	14.7806(10)
<i>b</i> /Å	13.047(6)	11.6540(8)	5.5867(4)

c/Å	14.649(6)	19.8909(12)	22.9131(16)
$\alpha/^\circ$	90	79.548(4)	90
$\beta/^\circ$	106.057(17)	86.556(4)	92.350(5)
$\gamma/^\circ$	90	83.372(5)	90
Volume/Å ³	5046(4)	1263.44(15)	1890.5(2)
Z	8	2	8
$\rho_{\text{calc}}/\text{g}/\text{cm}^3$	1.373	1.510	1.503
μ/mm^{-1}	0.771	2.639	2.082
F(000)	2168.0	584.0	880.0
Crystal size/mm ³	0.35 × 0.04 × 0.04	0.13 × 0.09 × 0.06	0.478 × 0.074 × 0.03
Radiation	CuK α (λ = 1.54178)	CuK α (λ = 1.54178)	Cu K α (λ = 1.54178)
2 θ range for data collection/ $^\circ$	9.218 to 130.14	4.52 to 136.552	5.984 to 130.428
Index ranges	-32 ≤ h ≤ 32, -14 ≤ k ≤ 15, -17 ≤ l ≤ 16	-5 ≤ h ≤ 6, -13 ≤ k ≤ 14, -23 ≤ l ≤ 23	-17 ≤ h ≤ 16, -6 ≤ k ≤ 6, -26 ≤ l ≤ 26
Reflections collected	13080	12316	16394
Independent reflections	4259 [R _{int} = 0.0719, R _{sigma} = 0.1023]	4442 [R _{int} = 0.0456, R _{sigma} = 0.0500]	3221 [R _{int} = 0.1283, R _{sigma} = 0.0968]
Data/restraints/parameters	4259/358/477	4442/0/360	3221/7/290
Goodness-of-fit on F ²	1.041	1.056	1.064
Final R indexes [$I \geq 2\sigma(I)$]	R ₁ = 0.0837, wR ₂ = 0.2330	R ₁ = 0.0663, wR ₂ = 0.1819	R ₁ = 0.0901, wR ₂ = 0.2415
Final R indexes [all data]	R ₁ = 0.1259, wR ₂ = 0.2722	R ₁ = 0.0758, wR ₂ = 0.1903	R ₁ = 0.1315, wR ₂ = 0.2764
Largest diff. peak/hole / e Å ⁻³	0.55/-0.46	0.51/-0.48	0.50/-0.48

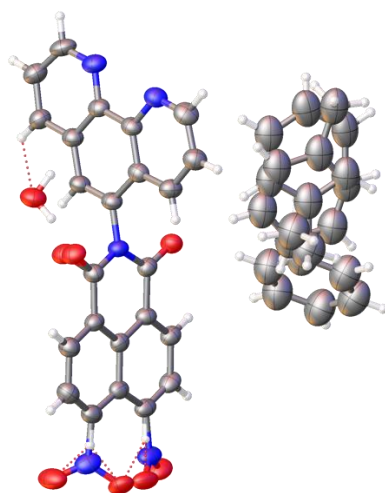


Figure A3.19: Asymmetric unit of **91**. Hydrogen bonding interactions are represented by red dotted line.

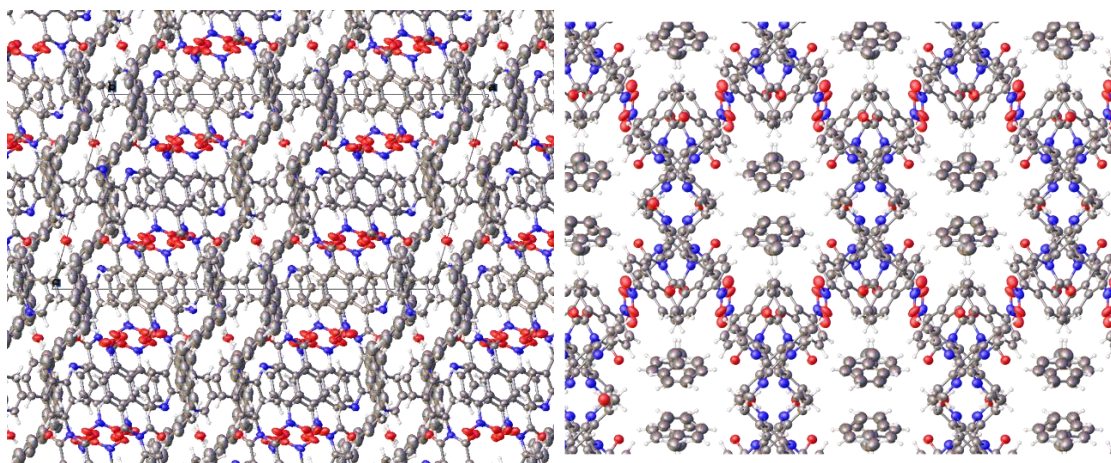


Figure A3.20: Extended structure of **91** displaying solvent voids from disordered toluene molecules shown normal to the 010 and 001 planes.

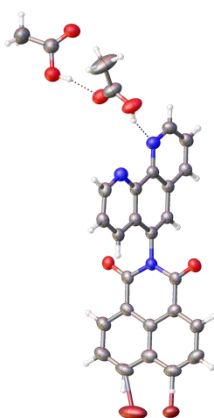


Figure A3.21: Asymmetric unit of **92**. H-bonding interactions are represented by red dotted line.

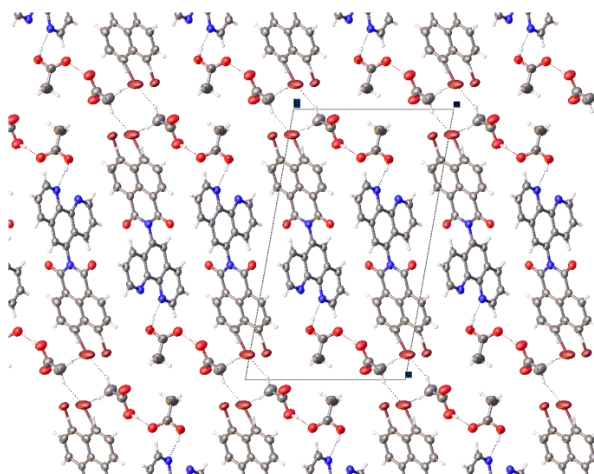


Figure A3.22: Extended structure of **92** displaying ordered head-to-tail interactions, homosorted arrays and acetic acid network shown normal to the crystallographic a-axis.

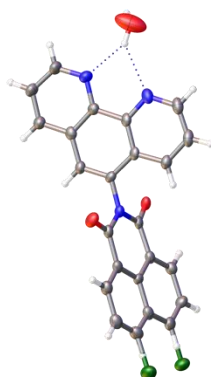


Figure A3.23: Asymmetric unit of **93**. H-bonding interactions are represented by blue dotted line.

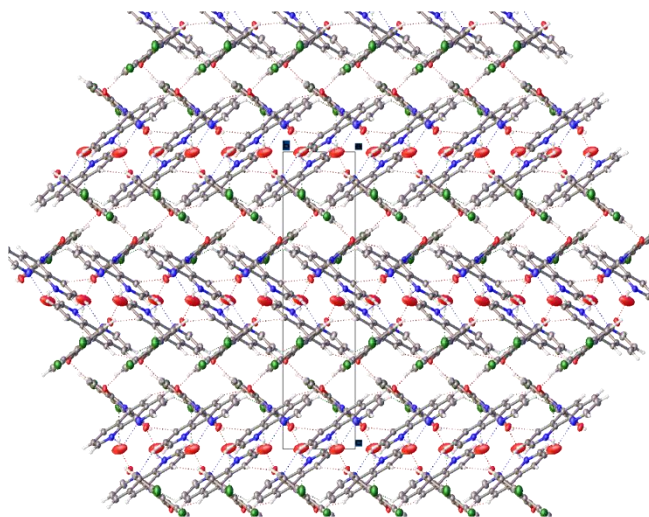


Figure A3.24: Extended structure of **93** displaying ordered off-set π – π stacking interactions and water channels shown normal to the crystallographic a-axis.

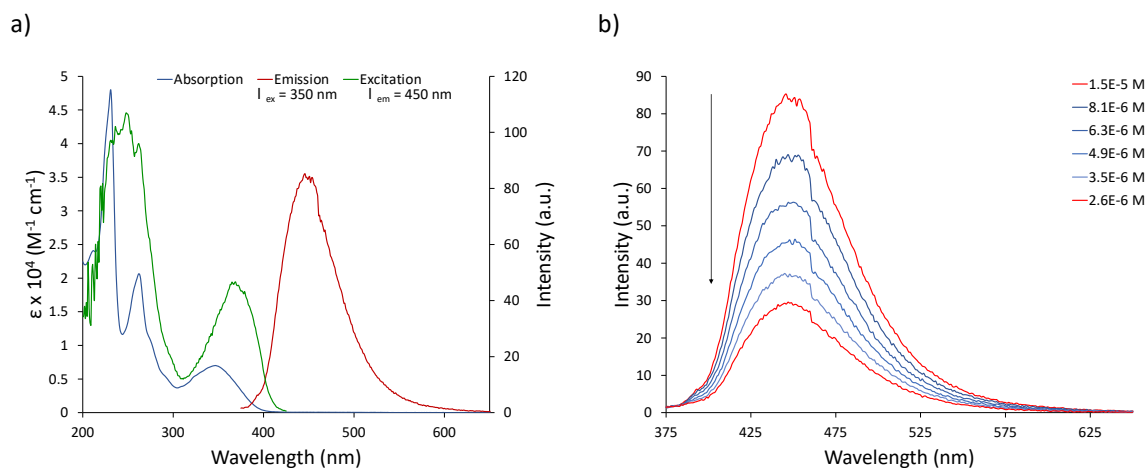


Figure A3.25: a) Photophysical characterisation of **PhenNap(NO₂)**, **91** ($\lambda_{\text{ex}} = 350 \text{ nm}$, $\lambda_{\text{em}} = 450 \text{ nm}$, $1.5 \times 10^{-5} \text{ M}$) and b) emission response upon dilution of **91** ($\lambda_{\text{ex}} = 350 \text{ nm}$) in MeCN.

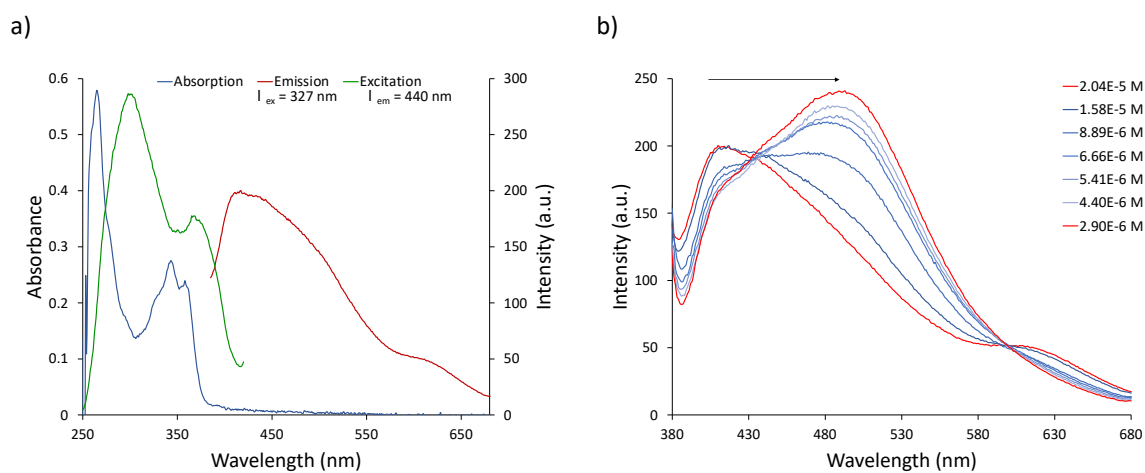


Figure A3.26: a) Photophysical characterisation of **PhenNap(Br)**, **92** ($\lambda_{\text{ex}} = 327 \text{ nm}$, $\lambda_{\text{em}} = 440 \text{ nm}$, $1.58 \times 10^{-5} \text{ M}$) and b) emission response upon dilution of **92** ($\lambda_{\text{ex}} = 327 \text{ nm}$) in DMSO.

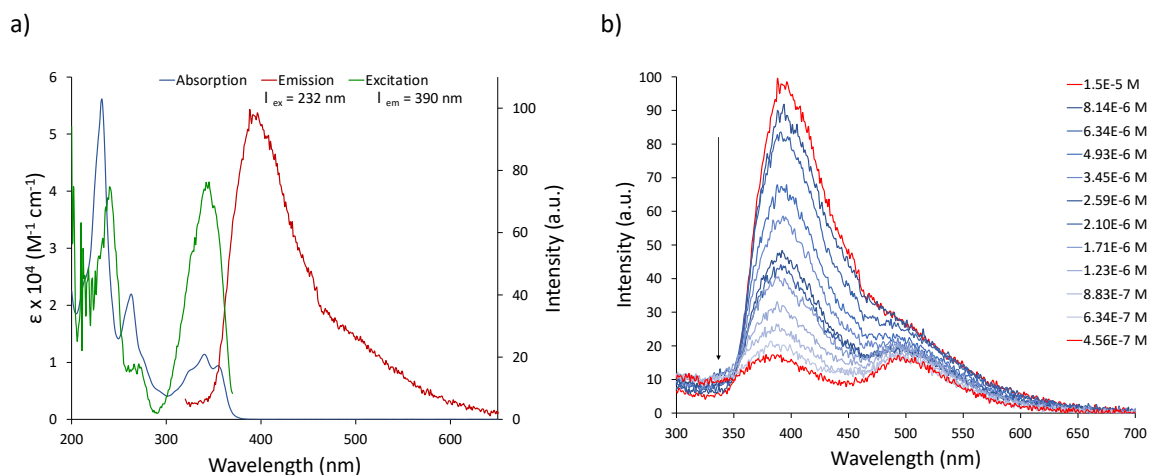


Figure A3.27: a) Photophysical characterisation of **PhenNap(Cl)**, **93** ($\lambda_{\text{ex}} = 232 \text{ nm}$, $\lambda_{\text{em}} = 390 \text{ nm}$, $1.5 \times 10^{-5} \text{ M}$) and b) emission response upon dilution of **93** ($\lambda_{\text{ex}} = 232 \text{ nm}$) in MeCN.

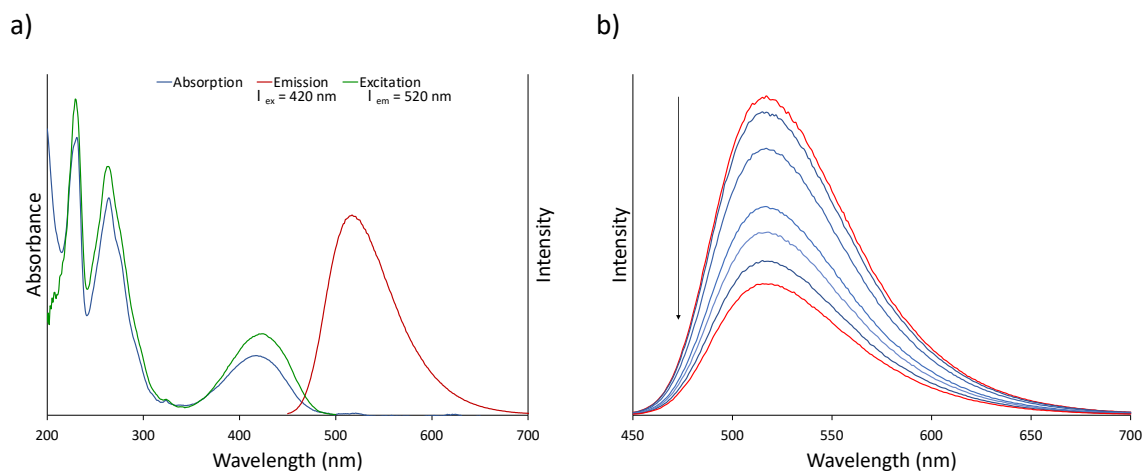


Figure A3.28: Photophysical characterisation of **PhenNap(NH₂)**, **97** ($\lambda_{\text{ex}} = 420 \text{ nm}$, $\lambda_{\text{em}} = 520 \text{ nm}$) and b) emission response upon dilution of **97** ($\lambda_{\text{ex}} = 450 \text{ nm}$) in MeCN. A slight precipitate was observed in the volumetric flask and thus, the exact concentrations are unknown.

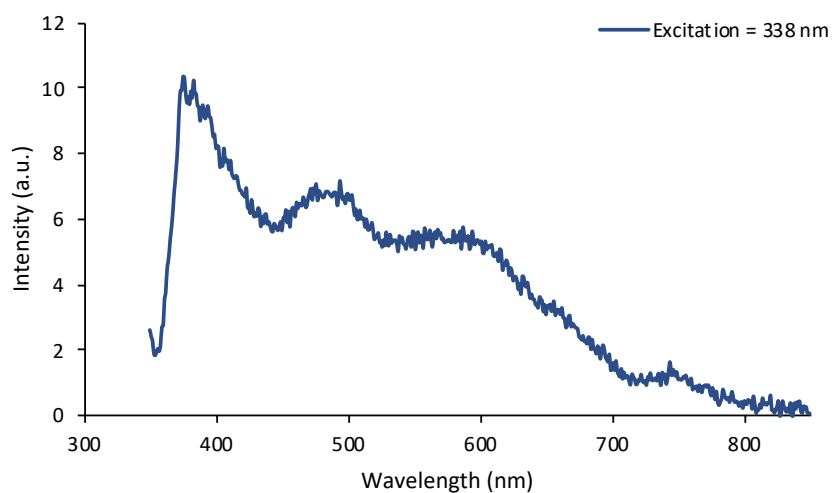


Figure A3.29: Emission of **PhenNap, 80** ($\lambda_{\text{em}} = 338 \text{ nm}$, $1.2 \times 10^{-4} \text{ M}$) in DMSO.

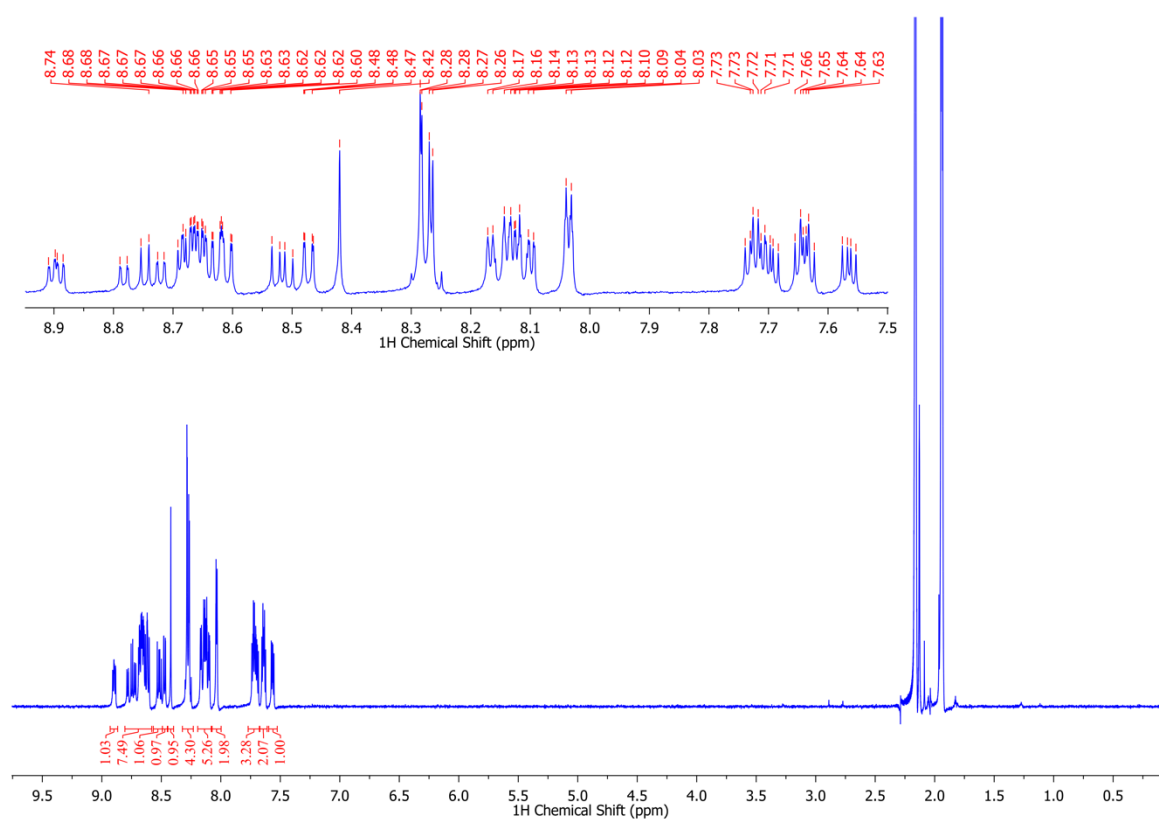
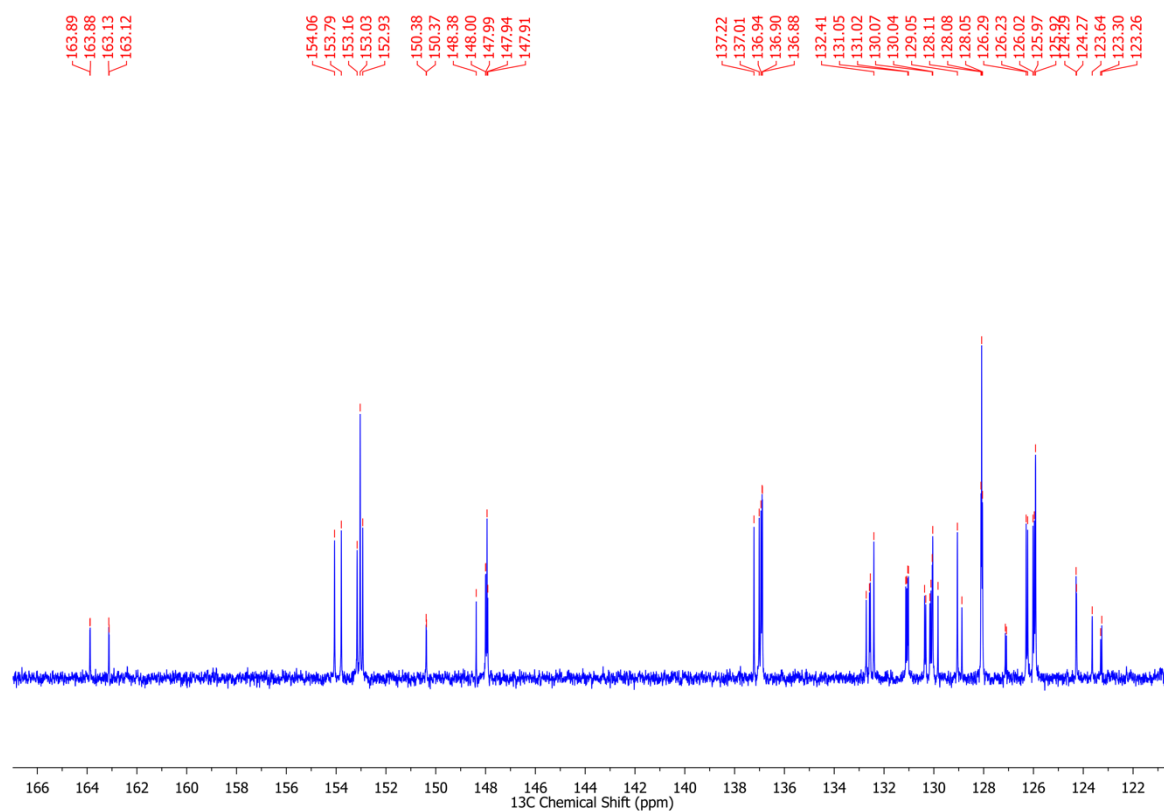
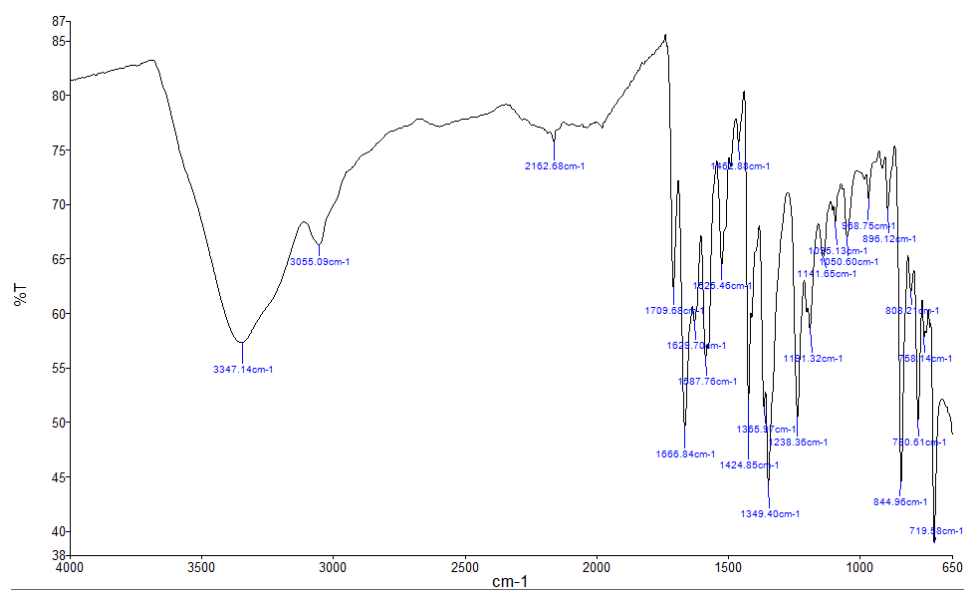


Figure A3.30: The ^1H NMR spectrum of **94** (600 MHz, CD_3CN).

Figure A3.31: The ^{13}C NMR spectrum of **94** (151 MHz, CD_3CN).Figure A3.32: The IR spectrum of **94**.

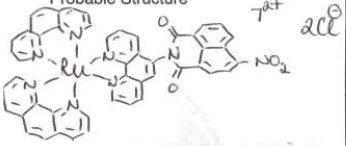
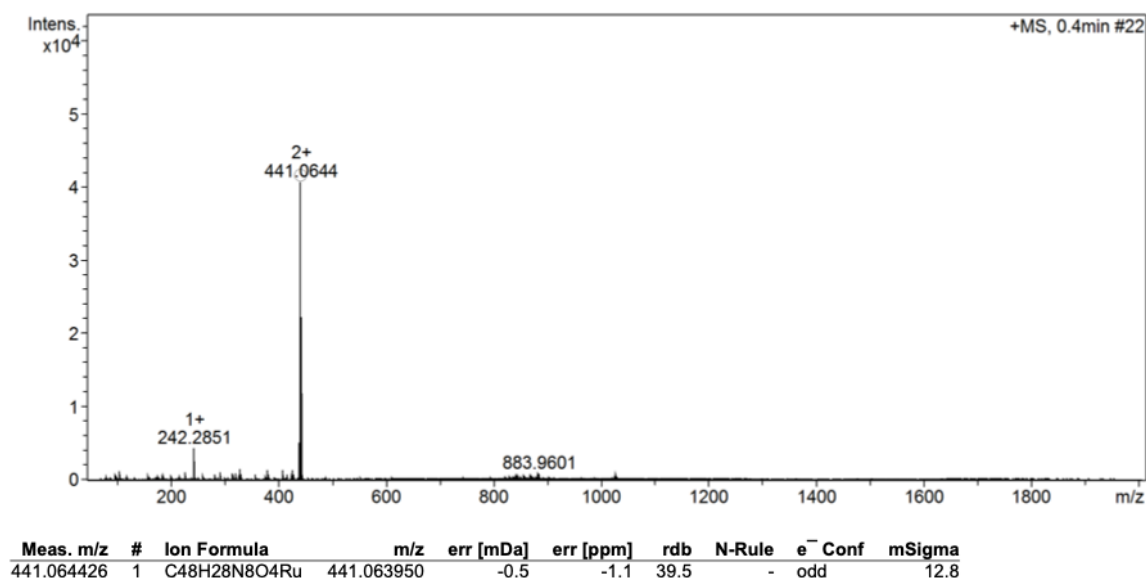
Submitted by DELEPRE WCADAMS	Tel. No. 3928	Supervisor/Company Prof. Gunnlaugsson	M1 A2762	Date in 25-11-20	Order No./Account No.	
SAMPLE INFORMATION			Analysis Reqd.	Theory	Found (A)%	Ref.
Sample No. 0AM036-r2						
Molecular Formula C₄₈H₂₈N₈O₄RuCl₂			%C	60.51	52.48	87722
Tick if appropriate: ☒ Air/Light Sensitive ☐			%H	2.96	2.81	
Hazardous ☐ Volatile ☐ Hygroscopic ☐			%N	11.76	11.92	
Probable Structure 						
 UCD School of Chemistry Microanalytical Laboratory University College Dublin Belfield, Dublin 4 Ireland T +353 1 716 2287 Email: microlab@ucd.ie						
			comments: <i>Hi Kieran, could you please send me the results via email to kieran.mcadams@tcd.ie. Thank you! -D</i>			

Figure A3.33: CHN analysis of **94**.Figure A3.34: The ESI mass spectrum of RuNap(NO₂), **94**.

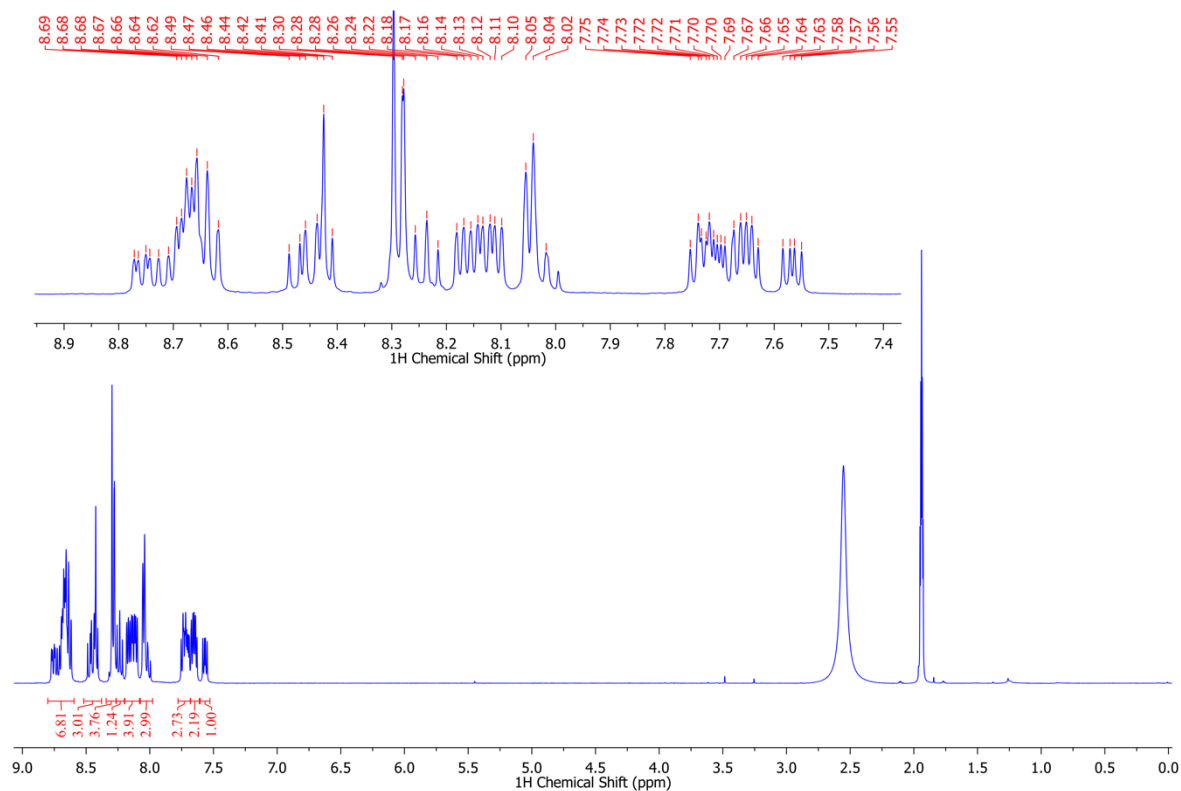


Figure A3.35: The ¹H NMR spectrum of **95** (400 MHz, CD₃CN).

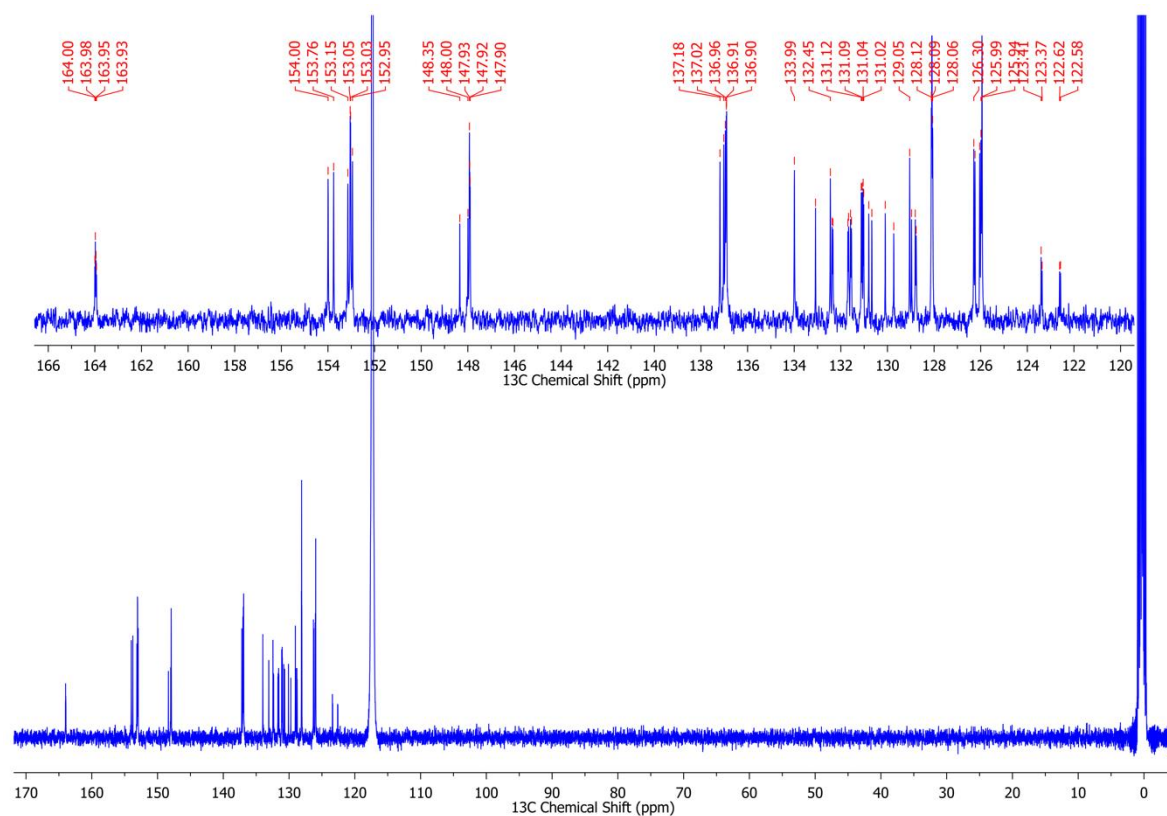
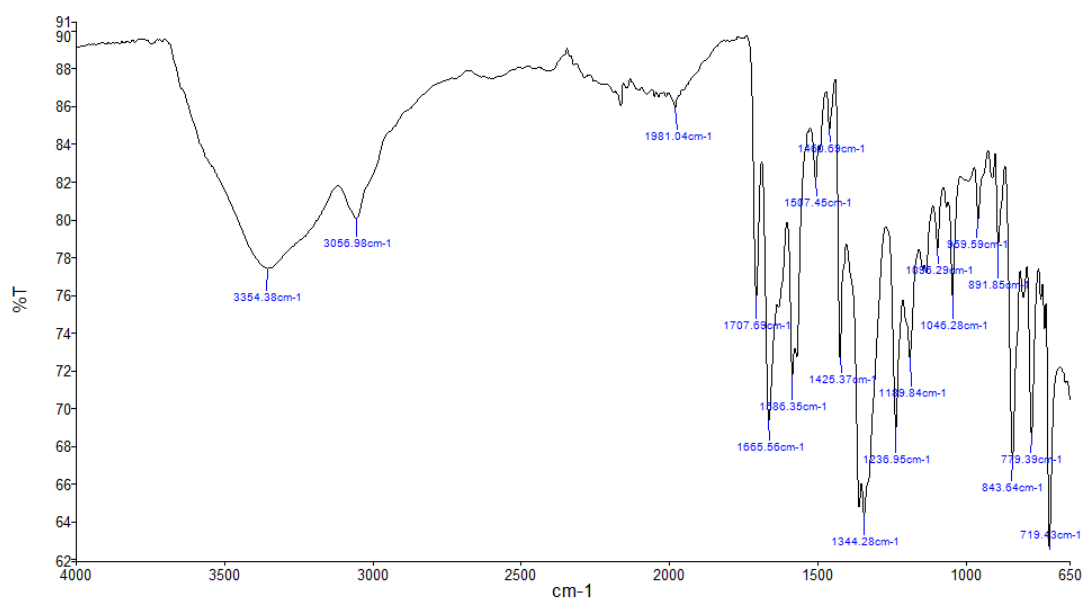
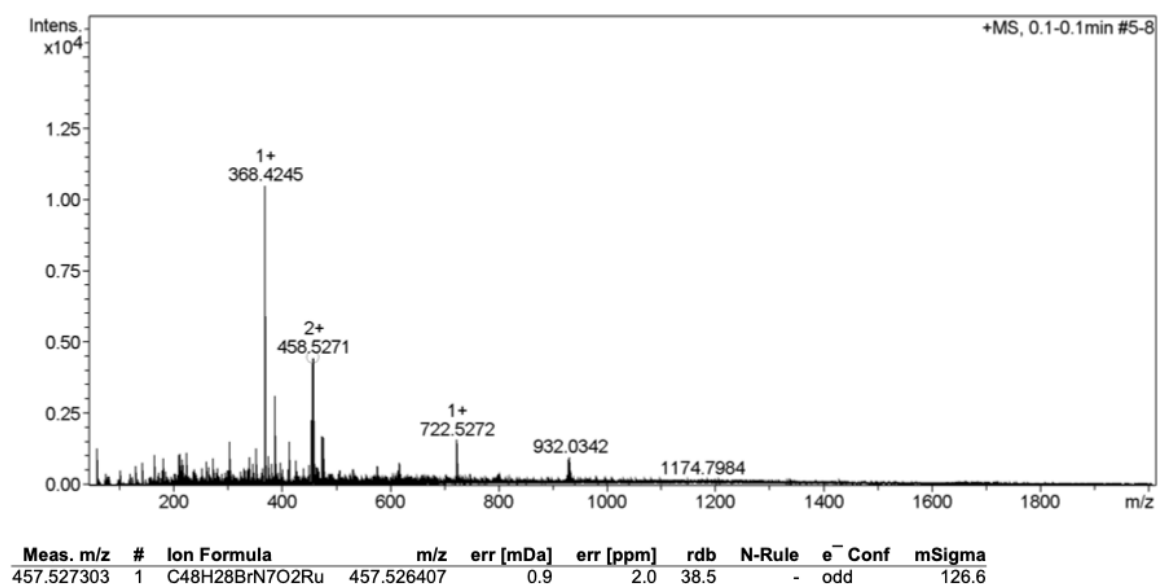
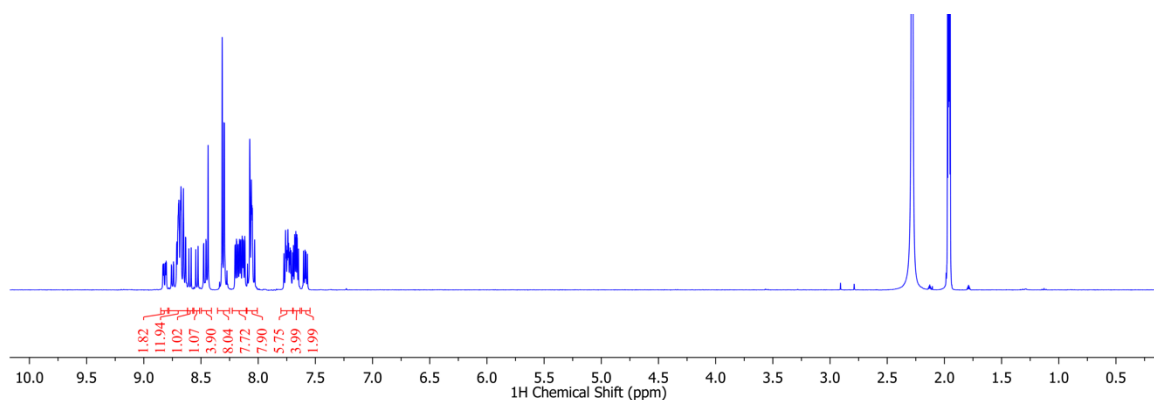


Figure A3.36: The ¹³C NMR spectrum of **95** (151 MHz, CD₃CN).

Figure A3.37: The IR spectrum of **95**.Figure A3.38: The ESI mass spectrum of RuNap(Br), **95**.Figure A3.39: The ¹H NMR spectrum of RuNap(Cl) **96** (600 MHz, CD₃CN).

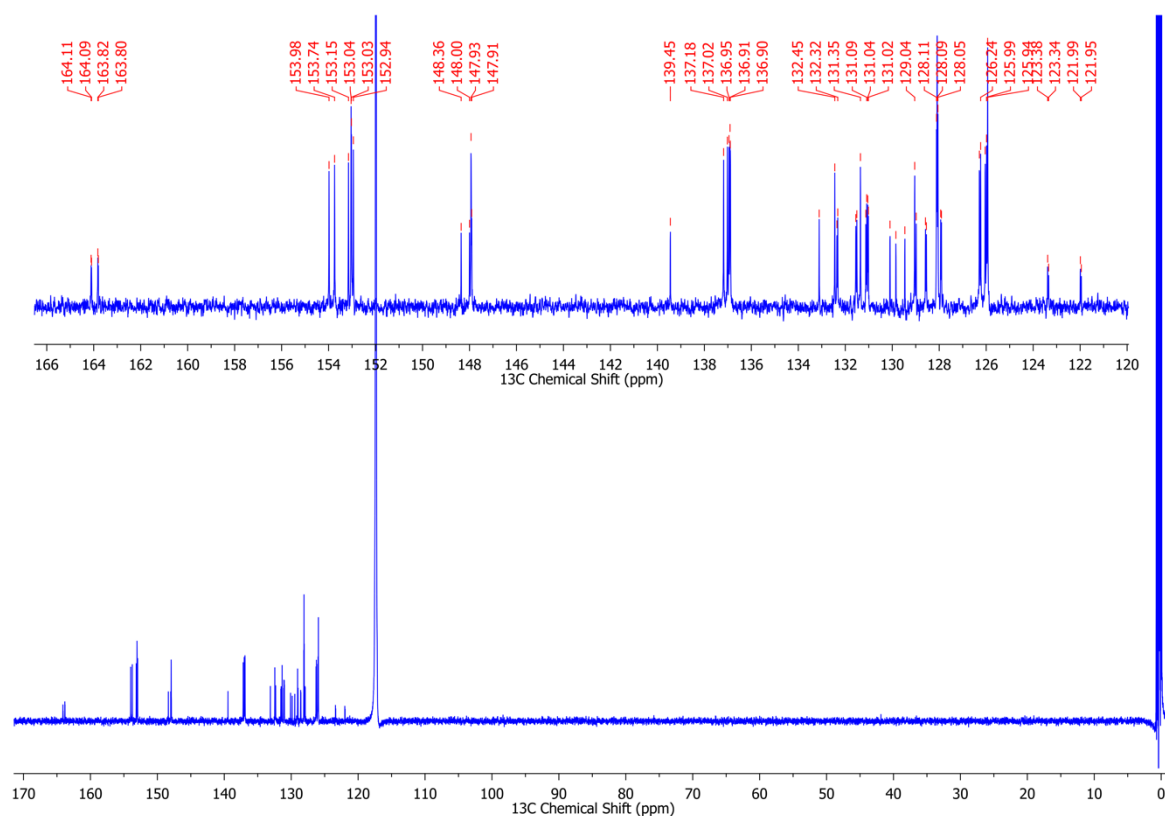


Figure A3.40: The ^{13}C NMR spectrum of **96** (151 MHz, CD_3CN).

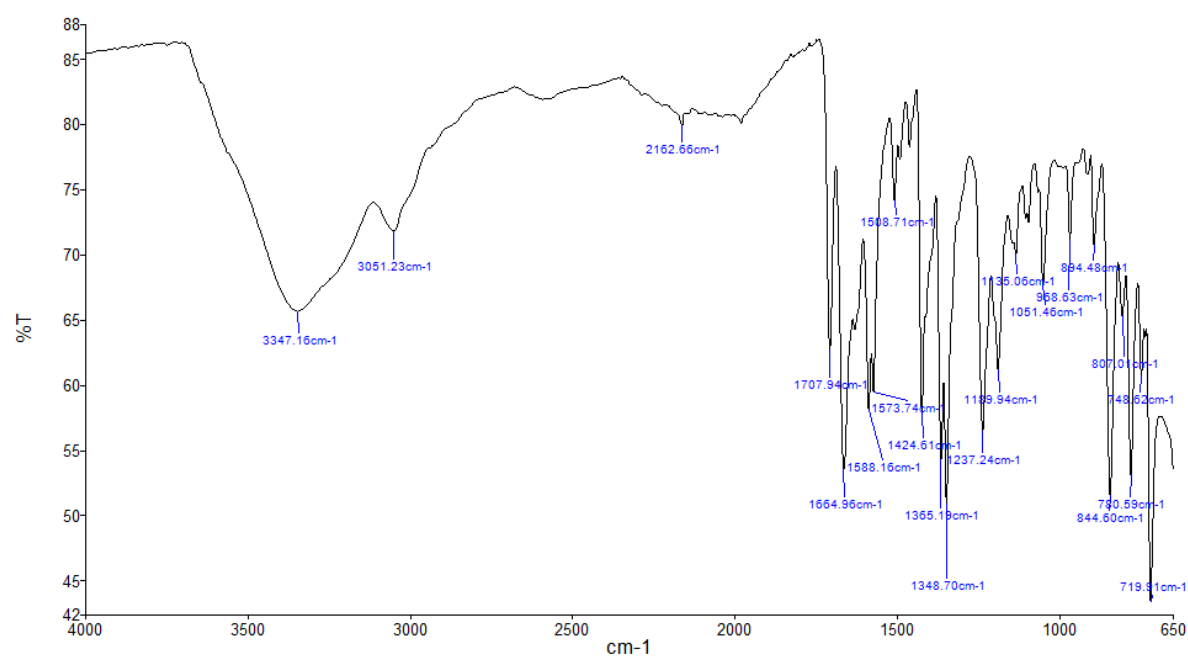
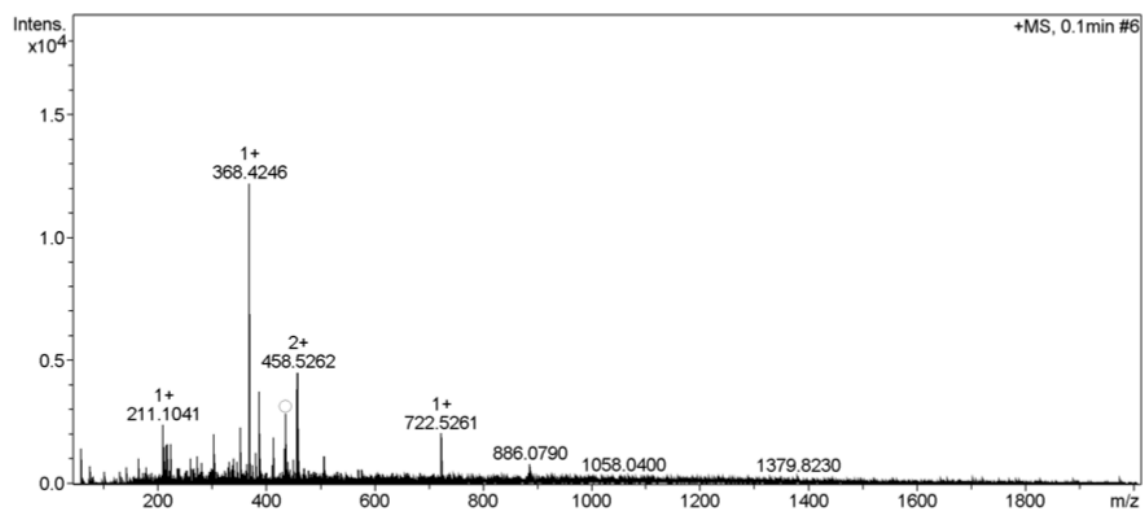
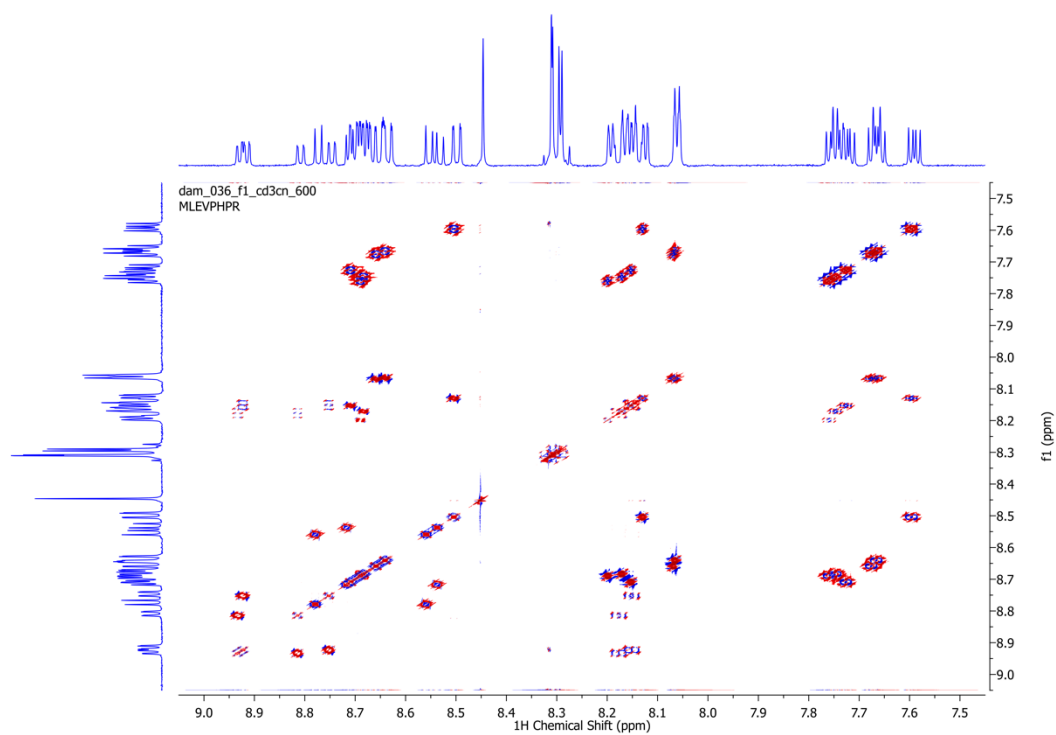


Figure A3.41: The IR spectrum of $\text{RuNap}(\text{Cl})$, **96**.



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
435.552880	1	C ₄₈ H ₂₈ ClN ₇ O ₂ Ru	435.551757	1.1	2.6	38.5	-	odd	53.0

Figure A3.42: The ESI mass spectrum of **RuNap(Cl)**, **96**.Figure A3.43: The H-H COSY NMR spectrum of **94** (600 MHz, CD₃CN).

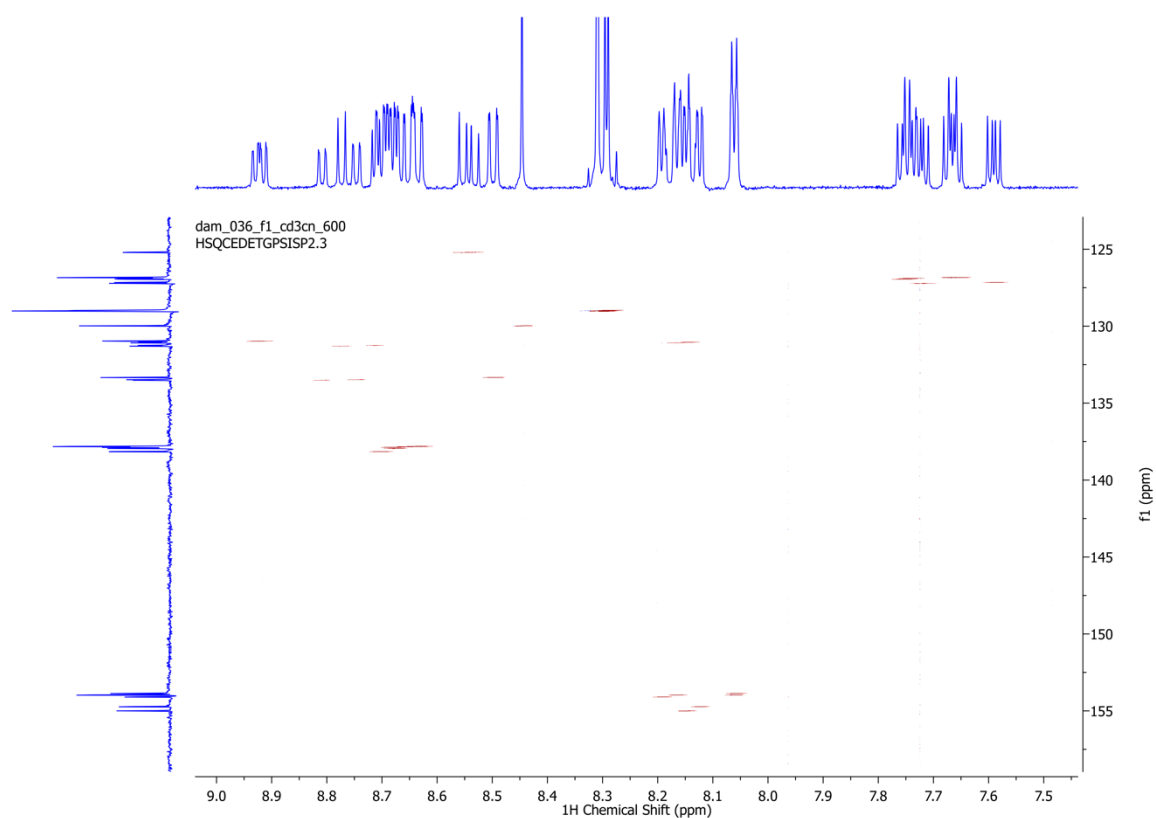


Figure A3.44: The HSQC NMR spectrum of **94** (600 MHz, 151 MHz, CD_3Cl).

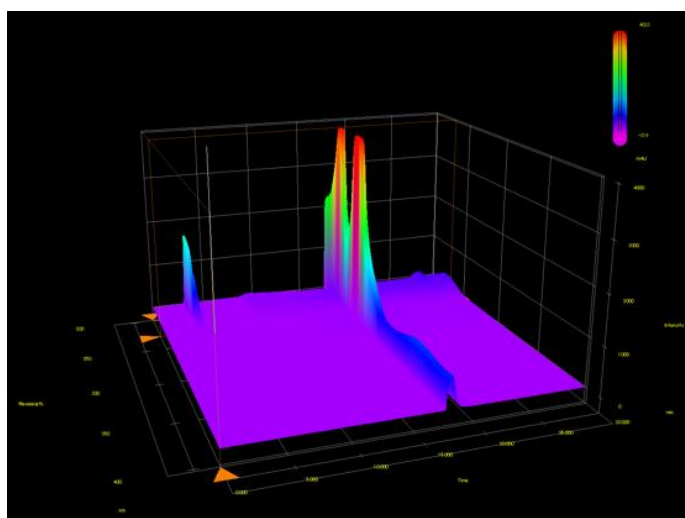
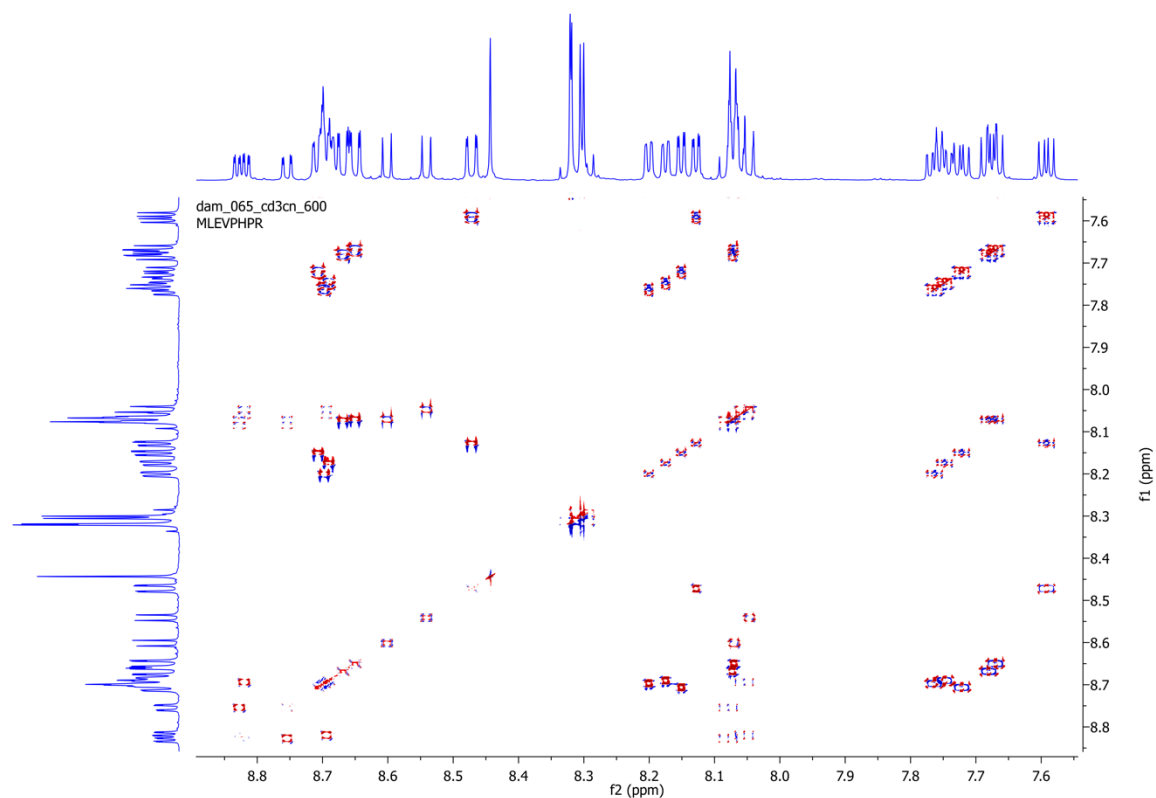
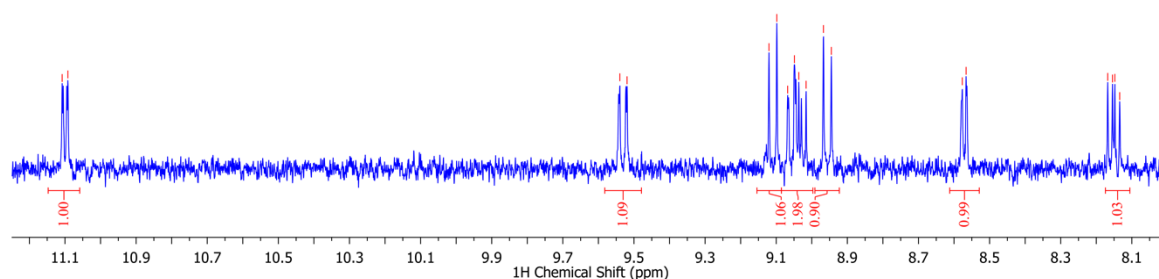


Figure A3.45: The 3D representation of the analytical HPLC spectrum of **94**.

Figure A3.46: HH COSY spectrum of **96** (600 MHz, CDCl₃).Figure A3.47: The ¹H NMR spectrum of Ru(**phen**)₂Cl₂ (400 MHz, DMSO-*d*₆).Table A3.2: Crystal data and structure refinement for complexes **94**, **95** and **96**

Identification code	94	95	96
Empirical formula	C ₅₂ H ₄₂ N ₁₃ NaO ₁₇ Ru	C ₄₈ H _{32.5} BrCl _{1.75} N _{7.25} O ₅ Ru	C ₄₈ H ₂₈ ClN ₇ O ₂ Ru
Formula weight	1233.55	1033.83	871.29
Temperature/K	100(2)	100(2)	100(2)
Crystal system	monoclinic	triclinic	triclinic
Space group	C2/c	P-1	P-1
a/Å	28.9188(13)	9.7229(5)	10.1864(3)
b/Å	13.4416(6)	14.9089(7)	19.4406(5)

c/Å	26.8927(12)	16.9111(8)	24.9208(7)
$\alpha/^\circ$	90	105.6082(19)	104.2035(16)
$\beta/^\circ$	98.6580(10)	97.0275(19)	90.466(2)
$\gamma/^\circ$	90	106.1209(18)	91.0613(19)
Volume/Å ³	10334.5(8)	2216.12(19)	4783.0(2)
Z	8	2	4
$\rho_{\text{calc}}/\text{cm}^3$	1.586	1.549	1.210
μ/mm^{-1}	0.397	1.417	3.503
F(000)	5044.0	1040.0	1768.0
Crystal size/mm ³	0.232 × 0.111 × 0.040	0.314 × 0.233 × 0.154	0.258 × 0.195 × 0.05
Radiation	Mo K α (λ = 0.71073)	Mo K α (λ = 0.71073)	Cu K α (λ = 1.54178)
2 θ range for data collection/ $^\circ$	5.24 to 53.82	4.464 to 56.08	3.658 to 140.074
Index ranges	-36 ≤ h ≤ 36, -17 ≤ k ≤ 17, -34 ≤ l ≤ 34	-12 ≤ h ≤ 12, -19 ≤ k ≤ 19, -22 ≤ l ≤ 22	-12 ≤ h ≤ 12, -23 ≤ k ≤ 22, 0 ≤ l ≤ 30
Reflections collected	91686	40625	17230
Independent reflections	11148 [R _{int} = 0.0351, R _{sigma} = 0.0189]	10687 [R _{int} = 0.0592, R _{sigma} = 0.0553]	17230 [R _{int} = N/A, R _{sigma} = 0.0838]
Data/restraints/259parameter	11148/1109/946	10687/571/721	17230/2/1078
Goodness-of-fit on F ²	1.074	1.034	1.062
Final R indexes [$I \geq 2\sigma(I)$]	R ₁ = 0.0428, wR ₂ = 0.1141	R ₁ = 0.0549, wR ₂ = 0.1419	R ₁ = 0.0867, wR ₂ = 0.2307
Final R indexes [all data]	R ₁ = 0.0497, wR ₂ = 0.1192	R ₁ = 0.0940, wR ₂ = 0.1651	R ₁ = 0.1205, wR ₂ = 0.2561
Largest diff. peak/hole / e Å ⁻³	0.90/-0.69	1.40/-0.64	2.60/-0.80

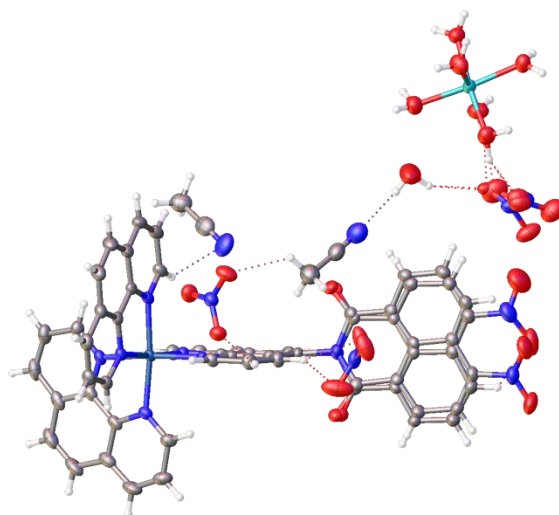


Figure A3.48: Asymmetric unit of **94**. Hydrogen bonding interactions are represented by red and blue dotted lines.

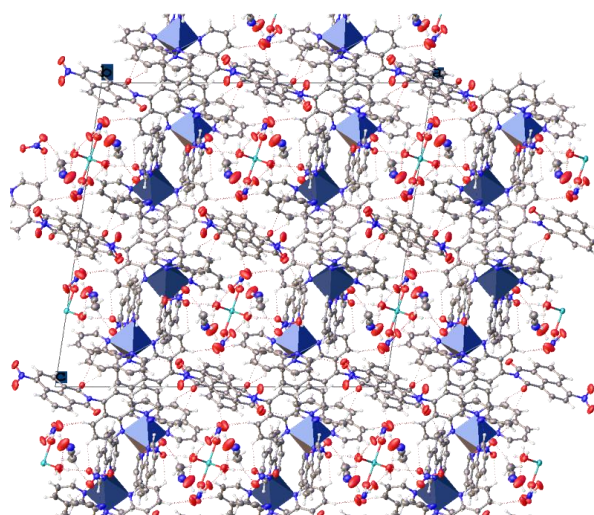


Figure A3.49: Extended structure of **94** displaying off set head-to-tail **Nap** interactions separated by sodium salt and solvent networks, shown normal to the crystallographic *b*-axis. Ruthenium centres represented by steel blue polyhedral for clearer visualisation of the metal centres.

Table A3.3: Hydrogen Bonds for **94**. D = donor atom, H = hydrogen atom, A = acceptor atom

D	H	A	$d(D-H)/\text{\AA}$	$d(H-A)/\text{\AA}$	$d(D-A)/\text{\AA}$	$D-H-A/^\circ$
C48	H48	O9 ¹	0.95	2.57	3.182(4)	122.3
C36	H36	O3 ²	0.95	2.47	3.184(5)	132.0
C47	H47	O9 ¹	0.95	2.60	3.187(4)	120.2
C23	H23	O4A ³	0.95	2.28	2.971(18)	129.4
C14	H14	O8	0.95	2.45	3.078(4)	123.6
C10	H10	O5 ²	0.95	2.45	3.127(6)	128.0
C31	H31	O1A ⁴	0.95	2.35	3.19(5)	147.6
O12	H12A	O8 ⁵	0.87	1.87	2.732(3)	169.9

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O12	H12B	O6 ⁶	0.87	1.94	2.791(4)	166.0
O13	H13A	O5 ⁵	0.87	1.80	2.666(4)	172.0
O13	H13B	O20	0.87	2.06	2.93(2)	178.5
O13	H13B	O17	0.87	1.92	2.783(5)	171.4
O11	H11A	O21 ⁷	0.89	2.13	2.93(5)	149.3
O11	H11A	O14 ⁷	0.89	1.93	2.743(7)	151.4
O1	H1A	N12	0.87	2.03	2.887(6)	168.3
O1	H1B	O20	0.87	2.41	3.188(14)	149.1
O1	H1B	O17	0.87	2.10	2.875(6)	147.3

¹3/2-X,1/2+Y,3/2-Z; ²1-X,1-Y,1-Z; ³1/2+X,1/2+Y,+Z; ⁴3/2-X,3/2-Y,1-Z; ⁵+X,-1+Y,+Z; ⁶1-X,-1+Y,3/2-Z; ⁷1-X,+Y,3/2-Z

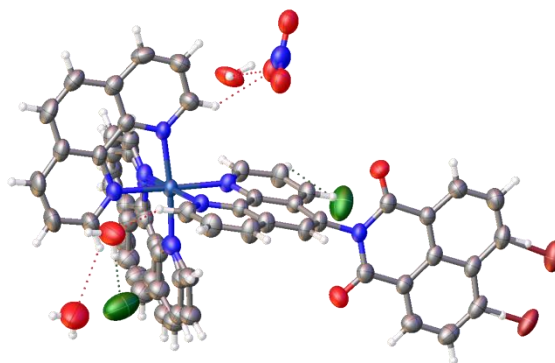


Figure A3.50: Asymmetric unit of **95**. Hydrogen bonding interactions are represented by red and green dotted lines.

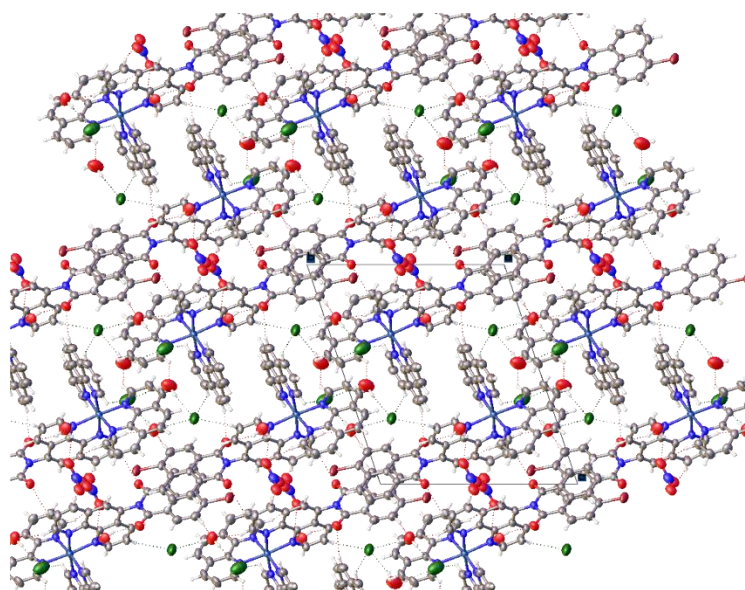
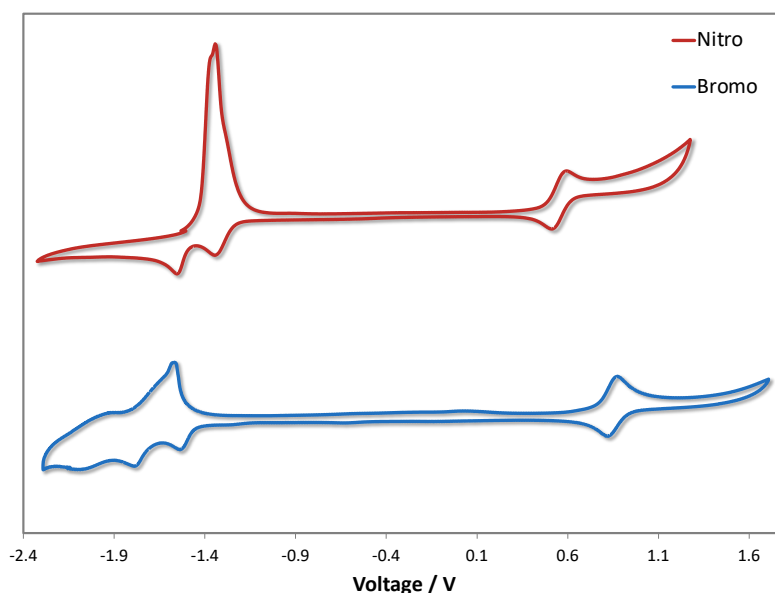


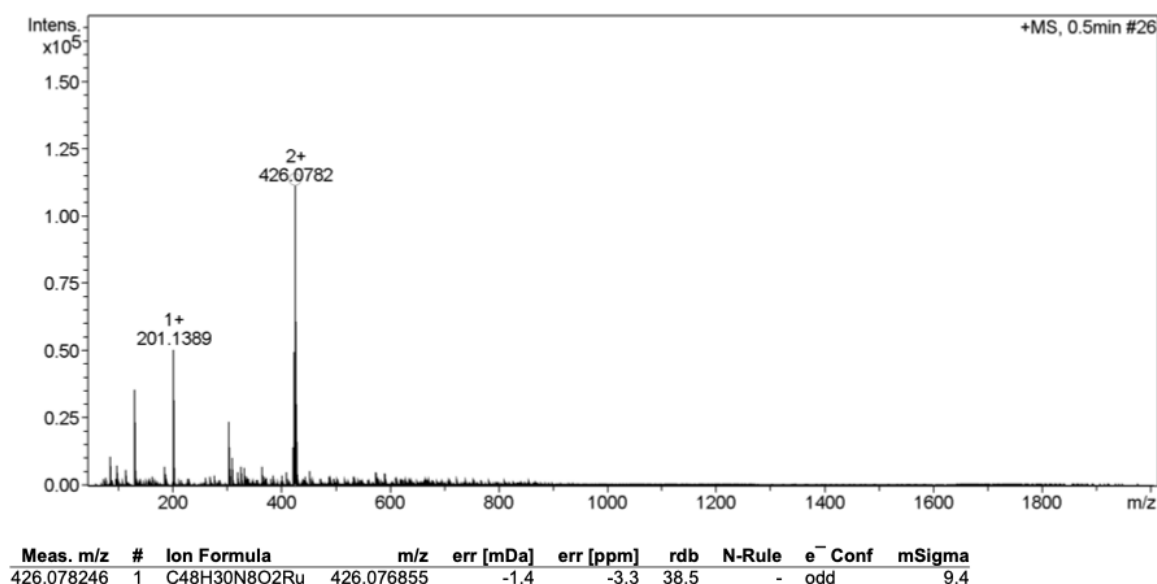
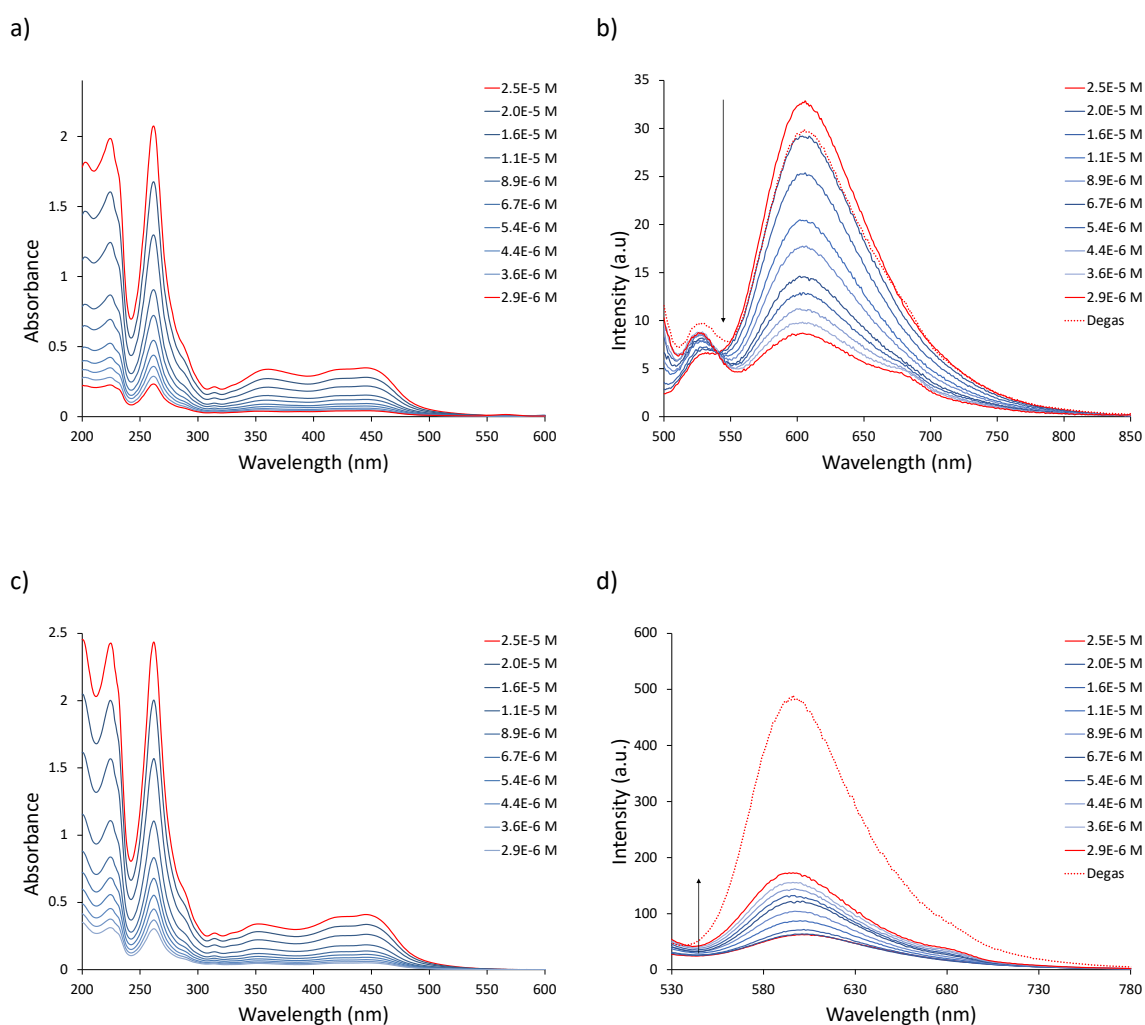
Figure A3.51: Extended structure of **95** shown normal to the crystallographic c-axis. Hydrogen bonding interactions are represented by red and green dotted lines.

Table A3.4: Hydrogen Bonds for **95**. D = donor atom, H = hydrogen atom, A = acceptor atom

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
C13A	H13A	Cl2 ¹	0.93	2.54	3.13(2)	121.7
C17A	H17A	O1 ²	0.93	2.38	3.078(15)	131.2
C17B	H17B	O1 ²	0.93	2.21	3.064(16)	152.7
O4	H4A	Cl1 ³	0.85	2.34	3.162(5)	162.9
O4	H4B	Cl2	0.85	2.31	3.133(5)	164.2
O3	H3B	Cl1 ²	0.85	2.32	3.132(6)	169.3
O5	H5A	O7	0.85	2.10	2.77(2)	135.7
O5	H5B	O1 ¹	0.84	2.20	3.002(17)	157.5

¹-1+X,+Y,+Z; ²1-X,1-Y,1-Z; ³1+X,1+Y,+Z

Figure A3.52: CV traces of **94** and **95** in degassed acetonitrile referenced with respect to Fc/Fc⁺ internal standard.

Figure A3.53: The ESI mass spectrum of RuPhenNap(NH₂), **99**.Figure A3.54: Photophysical characterisation of [Ru(phen)₂PhenNap(NO₂)], **94**: a) Absorption of **94** and b) decrease in emission intensity of **94** ($\lambda_{\text{ex}} = 450$ nm) upon dilution in 10 mM phosphate buffered solution. c) Absorption of and d) increase in emission intensity of **94** ($\lambda_{\text{ex}} = 450$ nm) upon dilution in MeCN solution. Degassed solutions denoted by dotted line and were measured at lowest concentration.

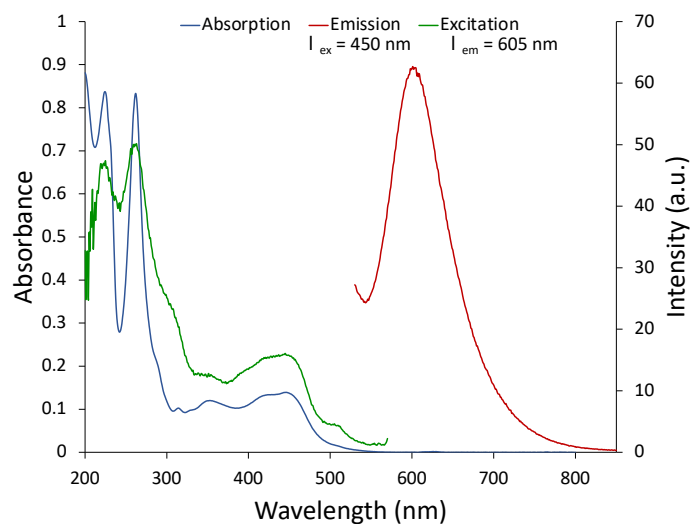


Figure A3.55: Absorption, emission and degassed excitation spectra of **94** ($\lambda_{\text{ex}} = 450 \text{ nm}$, $9 \times 10^{-6} \text{ M}$; $\lambda_{\text{em}} = 605 \text{ nm}$, $3 \times 10^{-6} \text{ M}$) in MeCN solution.

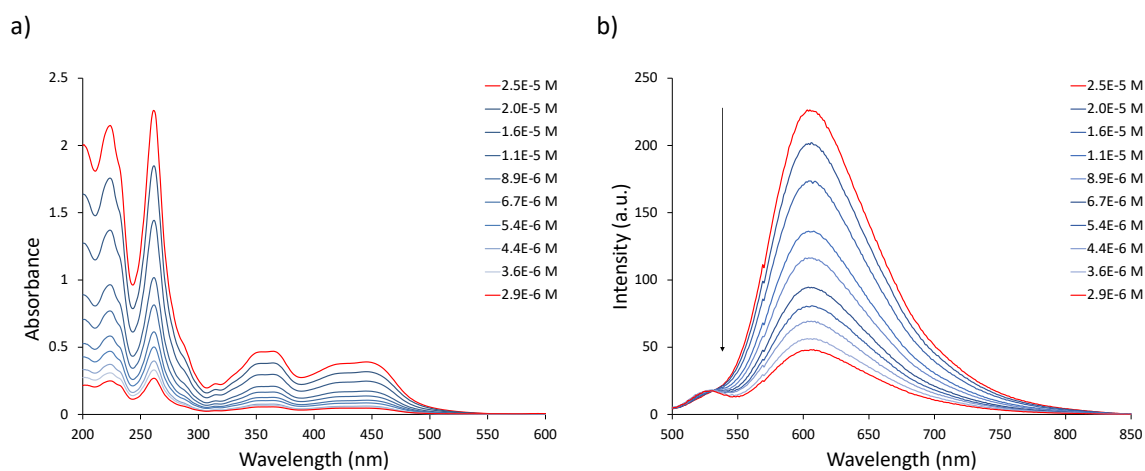


Figure A3.56: Photophysical characterisation of $[\text{Ru}(\text{phen})_2\text{PhenNap}(\text{Br})]$, **95**: a) Absorption of **95** and b) decrease in emission intensity of **95** ($\lambda_{\text{ex}} = 450 \text{ nm}$) upon dilution in 10 mM phosphate buffered solution.

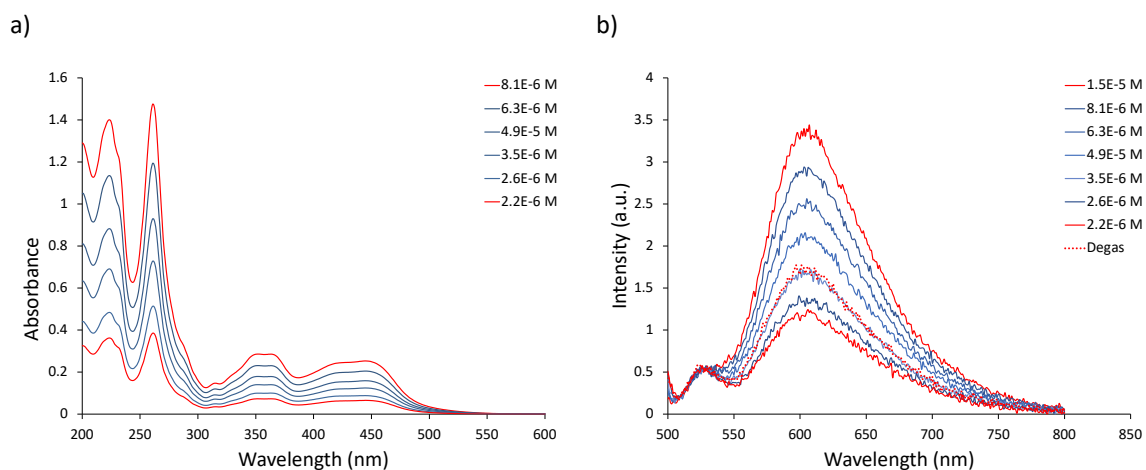


Figure A3.57: Photophysical characterisation of $[\text{Ru}(\text{phen})_2\text{PhenNap}(\text{Cl})]$, **96**: a) Absorption of **96** and b) decrease in emission intensity of **96** ($\lambda_{\text{ex}} = 450 \text{ nm}$) upon dilution in 10 mM phosphate buffered solution.

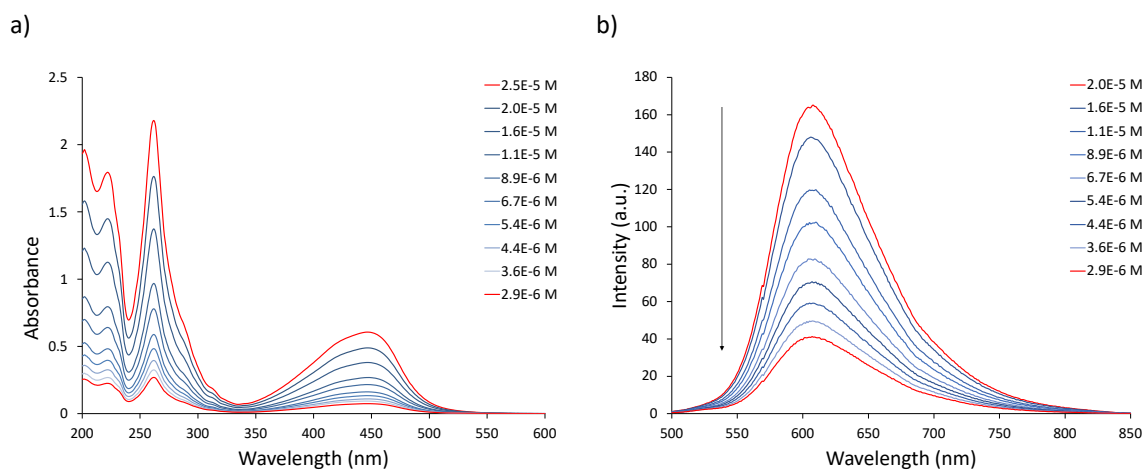
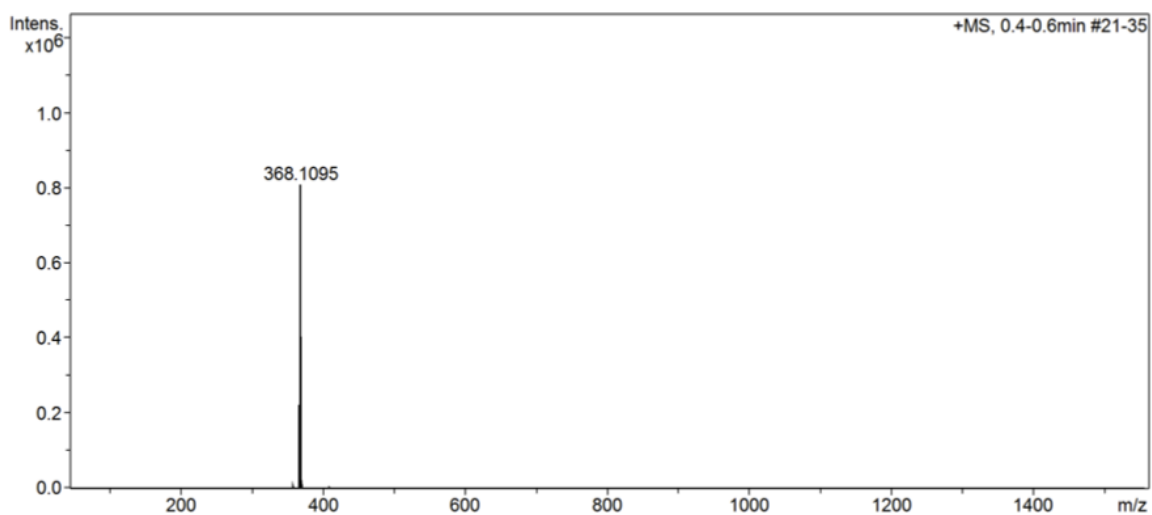
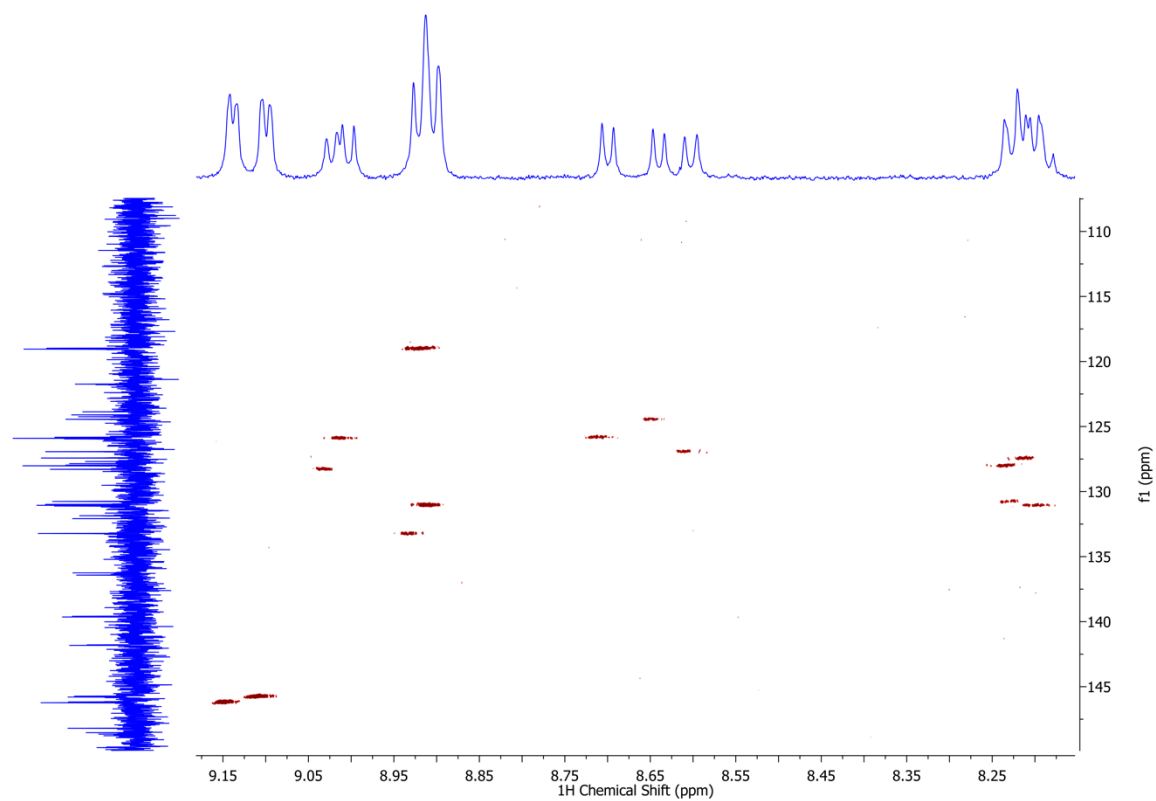


Figure A3.58: Photophysical characterisation of $[\text{Ru}(\text{phen})_2\text{PhenNap}(\text{NH}_2)]$, **99**: a) absorbance decrease of **99** and b) decrease in emission intensity of **99** ($\lambda_{\text{ex}} = 450 \text{ nm}$) upon dilution in 10 mM phosphate buffered solution.

Appendix for Chapter 4:

Figure A4.1: The mass spectrum (APCI-DIP) of **106**Figure A4.2: The HSQC spectrum (600, 151 MHz, DMSO- d_6) of **106**

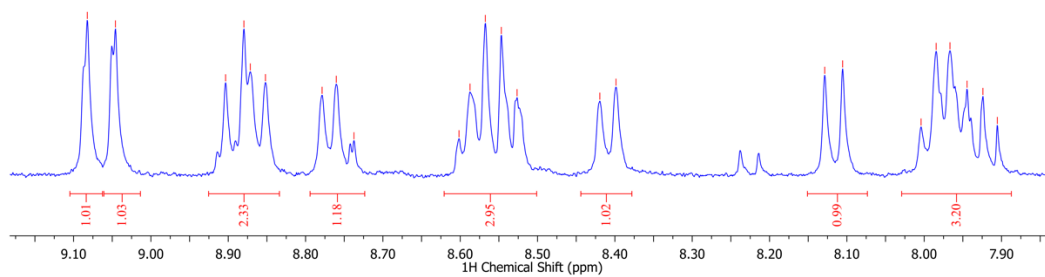


Figure A4.3: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of **107** of product isolated from Scheme 4.2

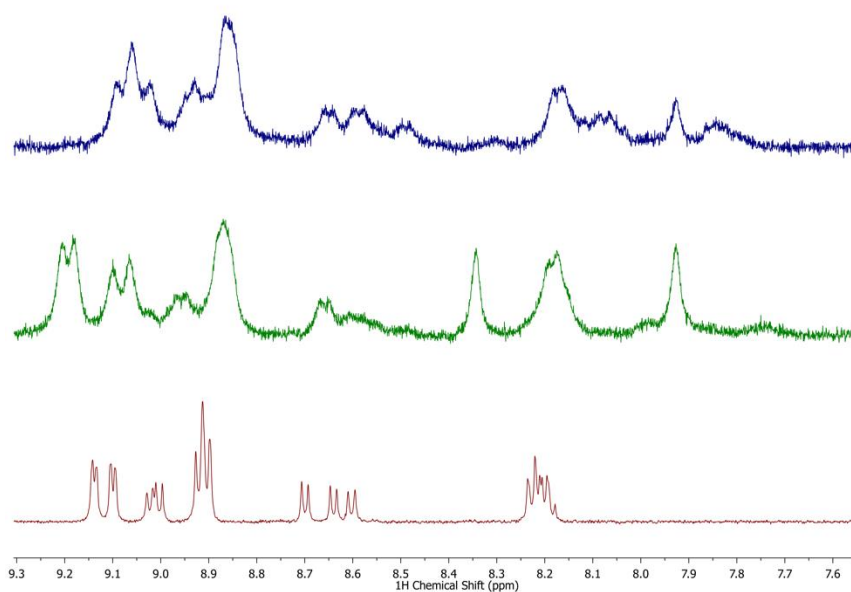


Figure A4.4: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of **106** (red, bottom), **106** + $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (green, middle) and **106** + $\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (blue, top).

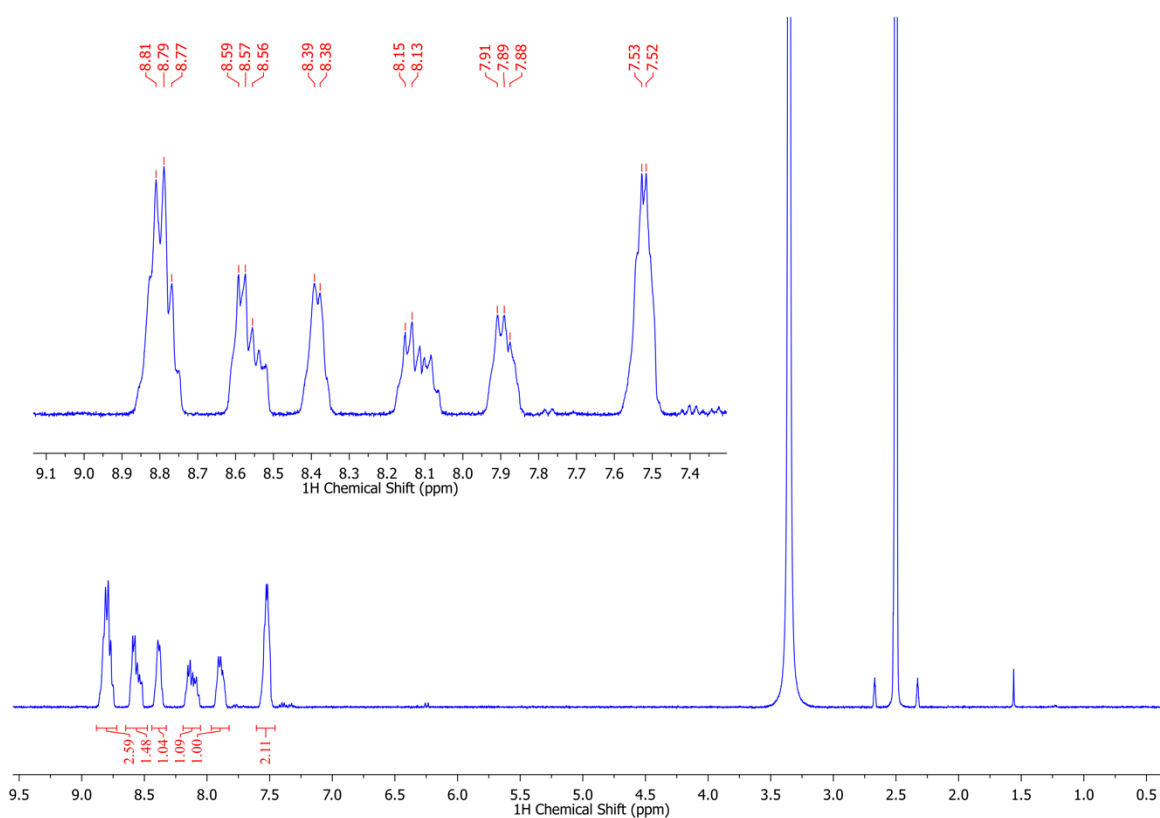


Figure A4.5: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of **108**

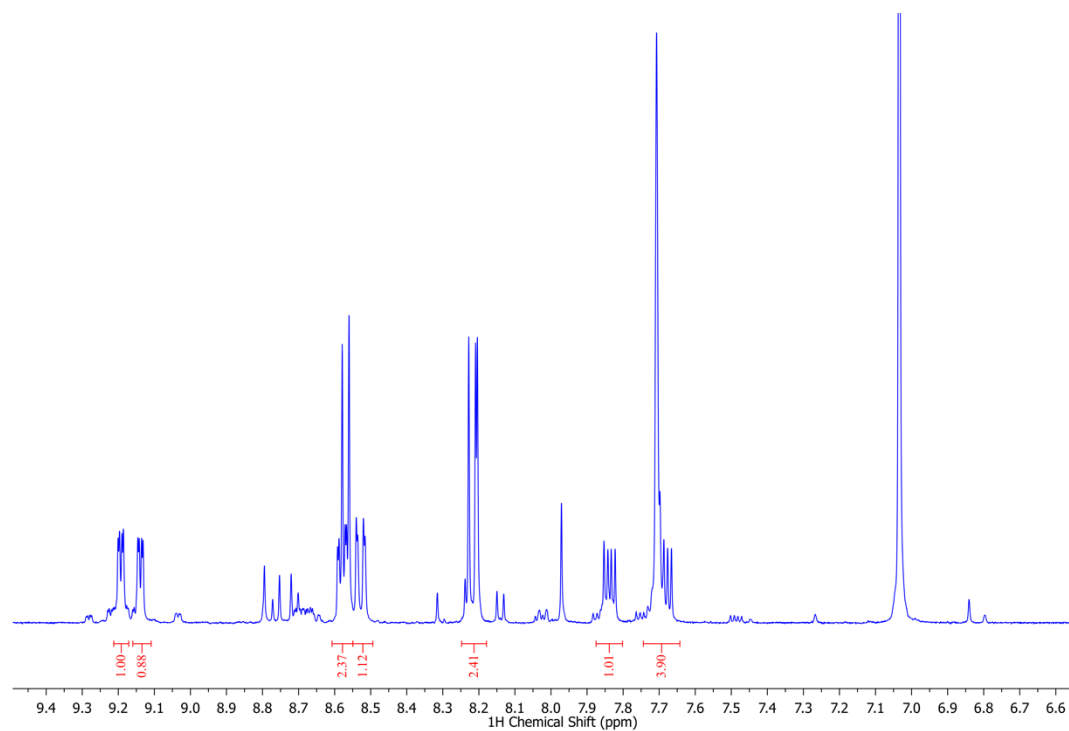
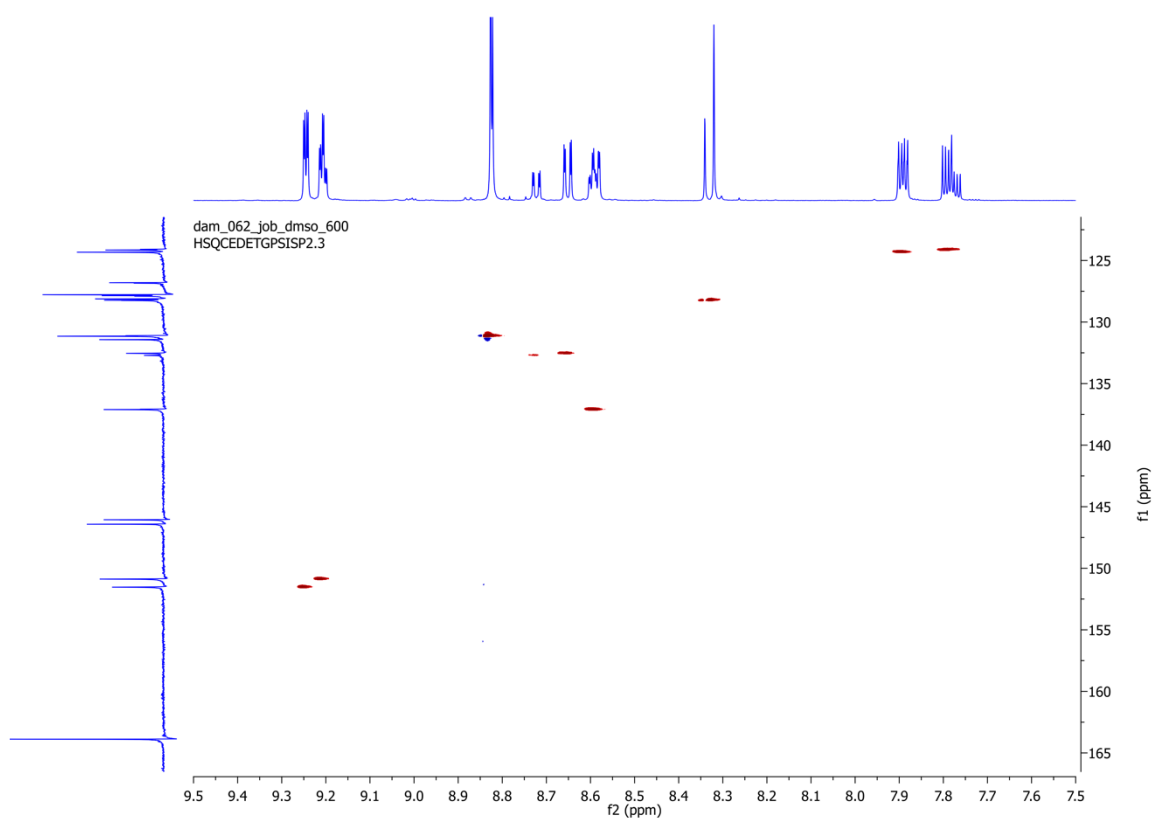
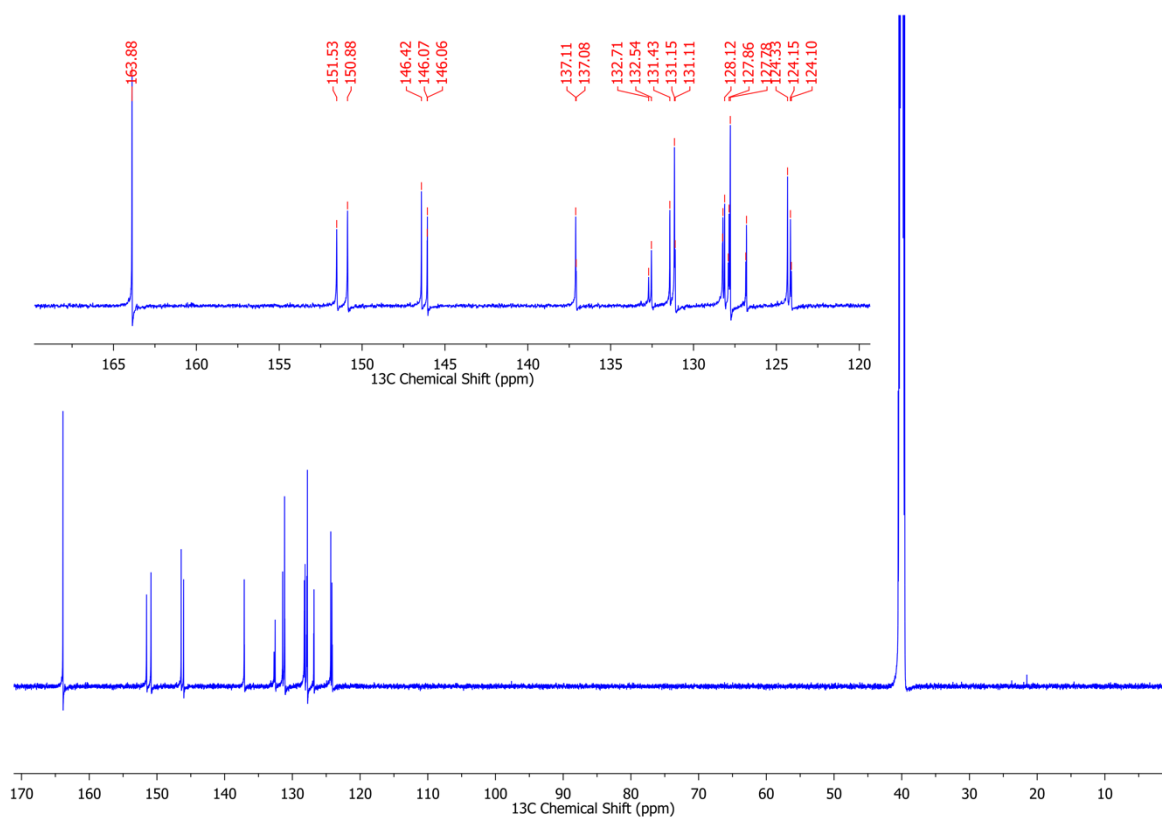
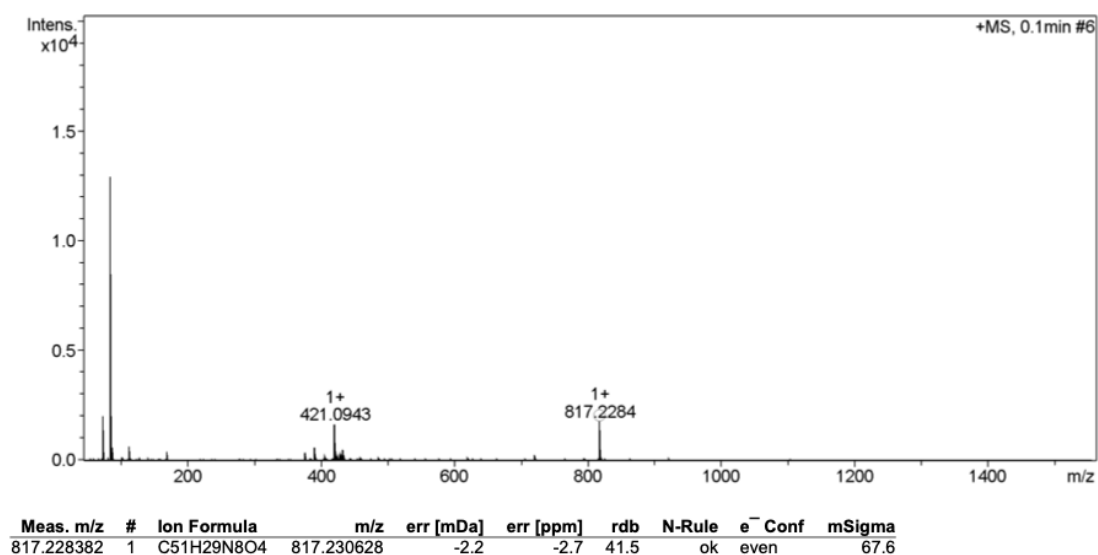
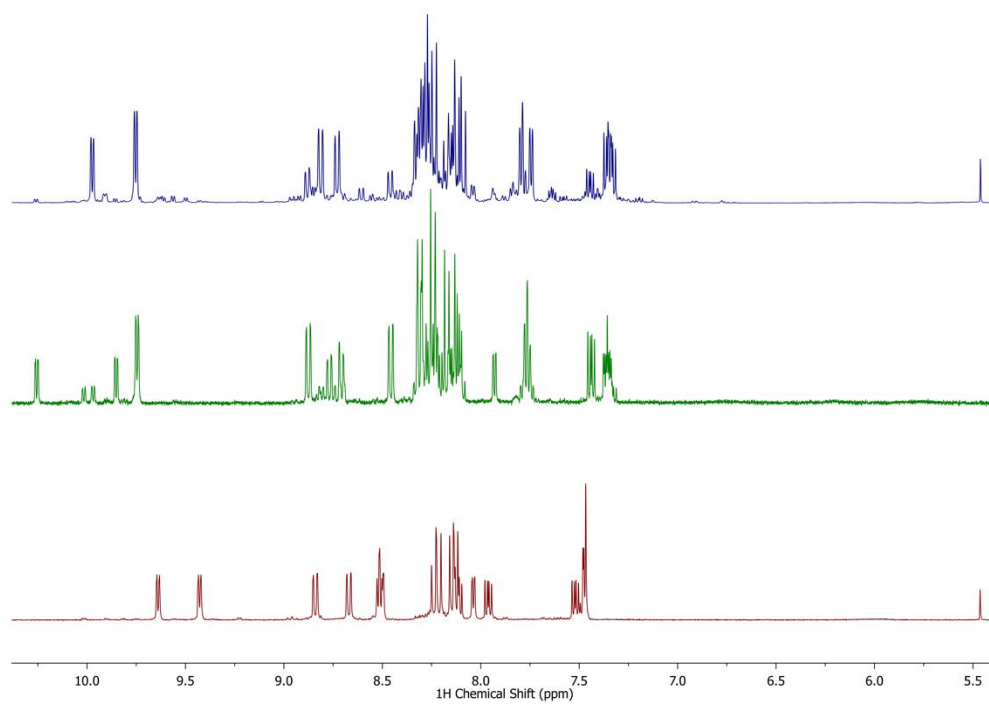
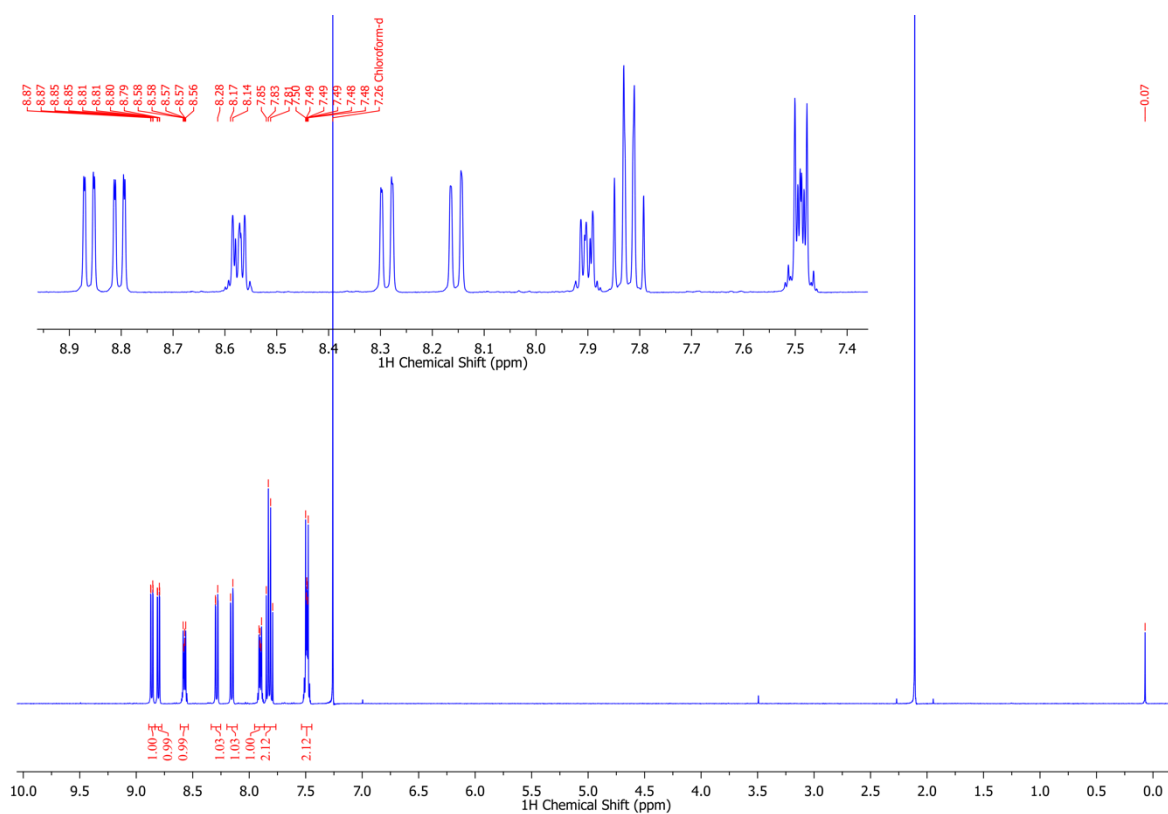
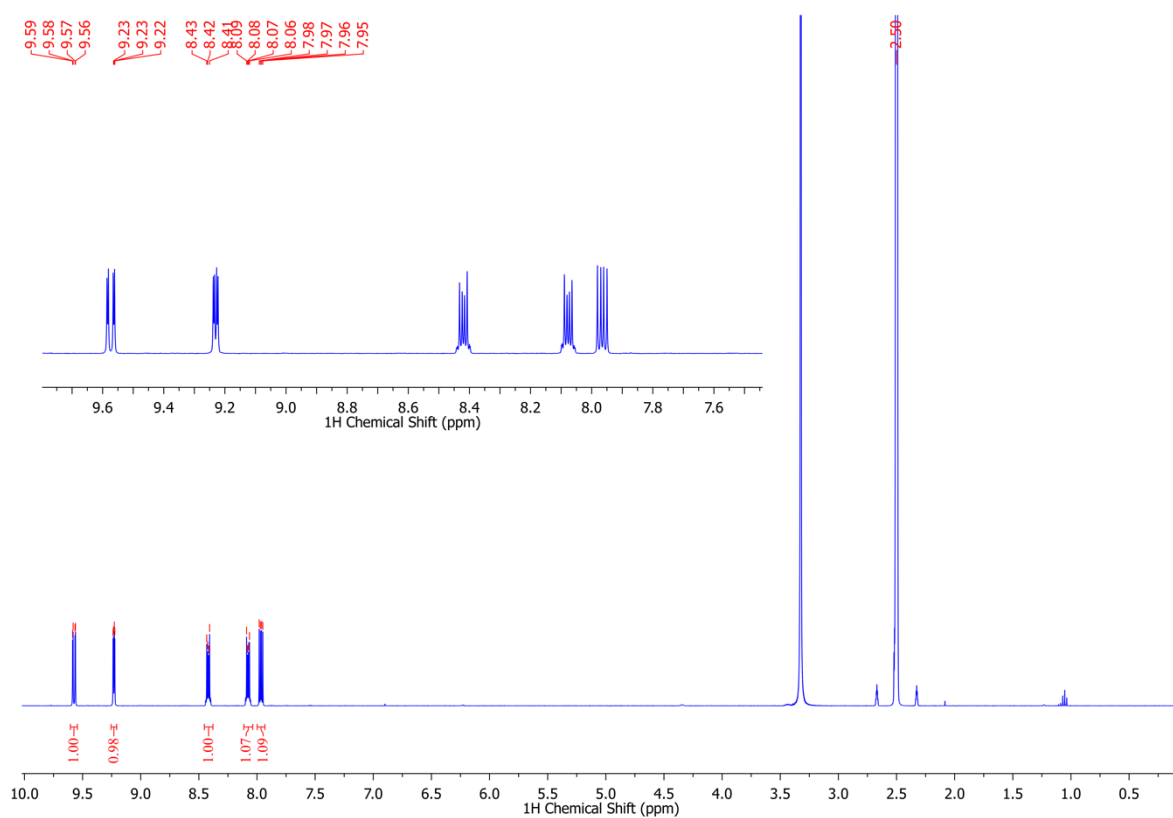
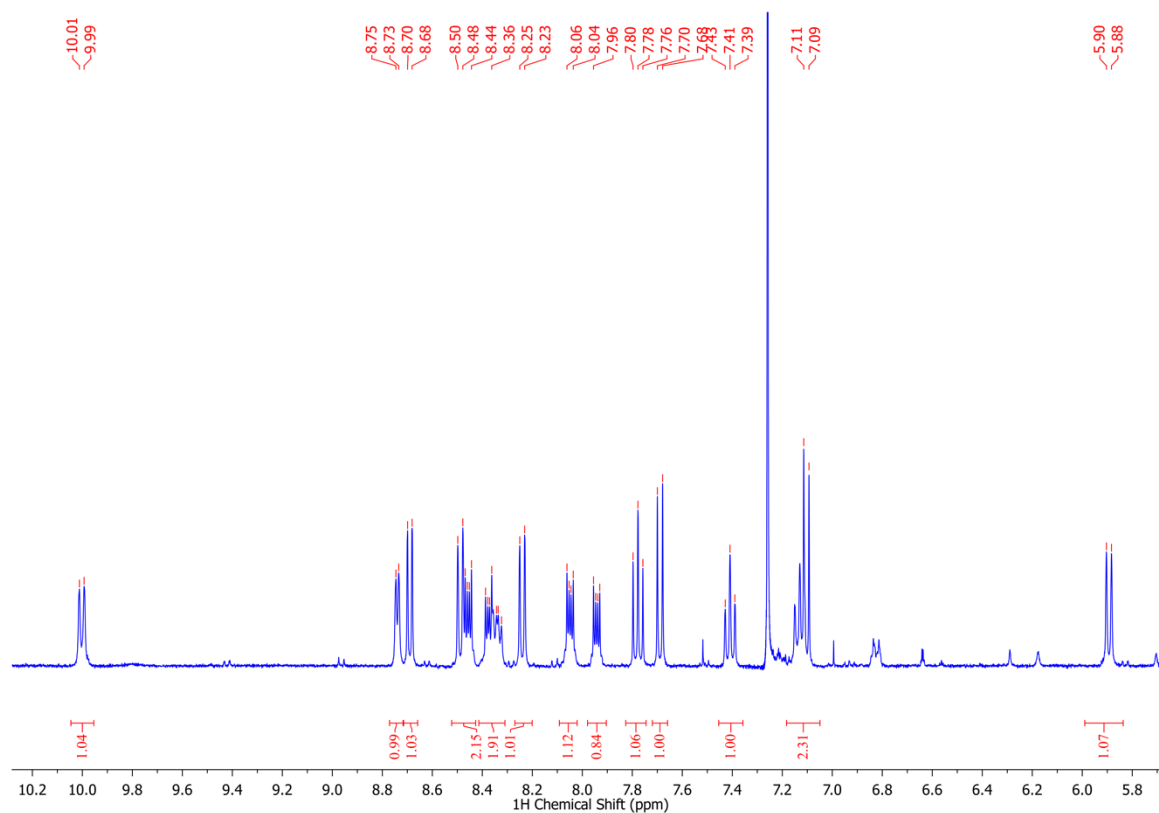
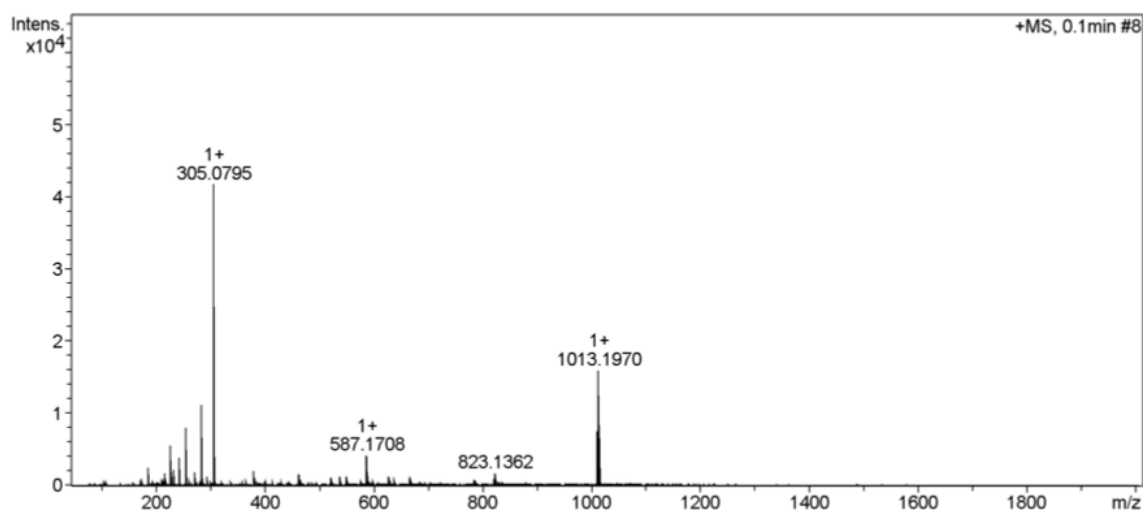


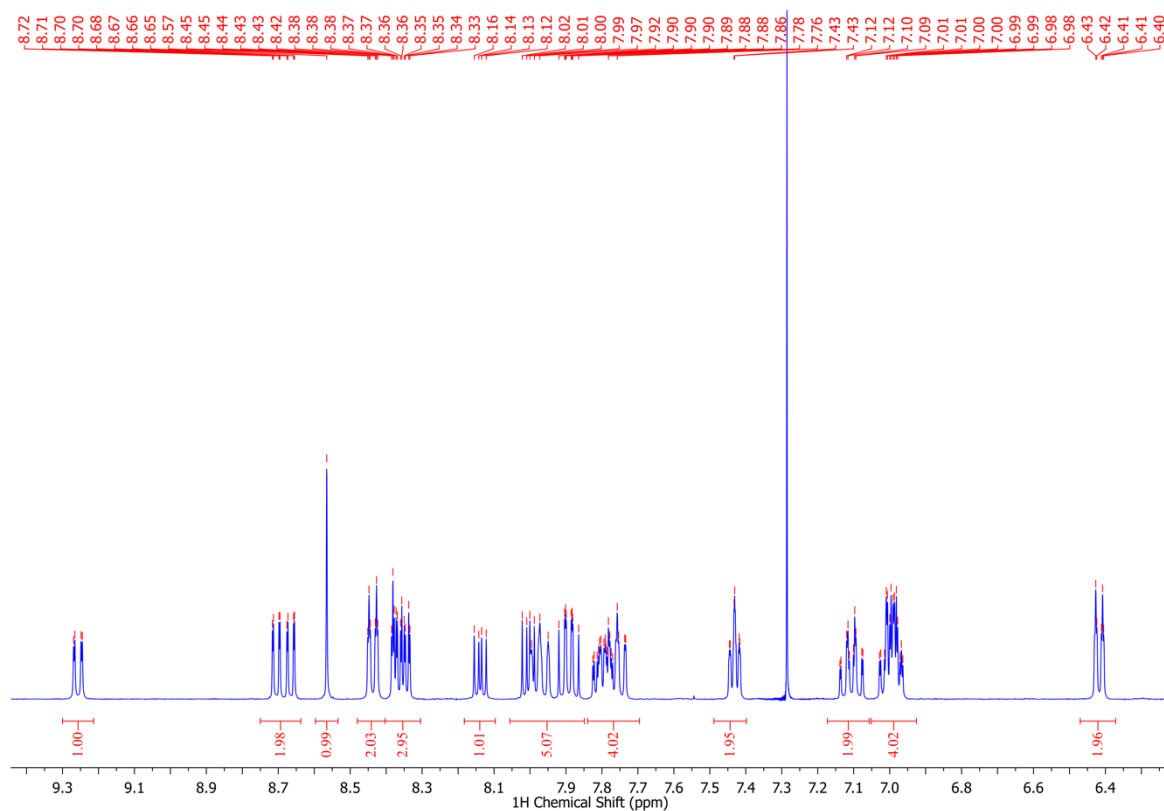
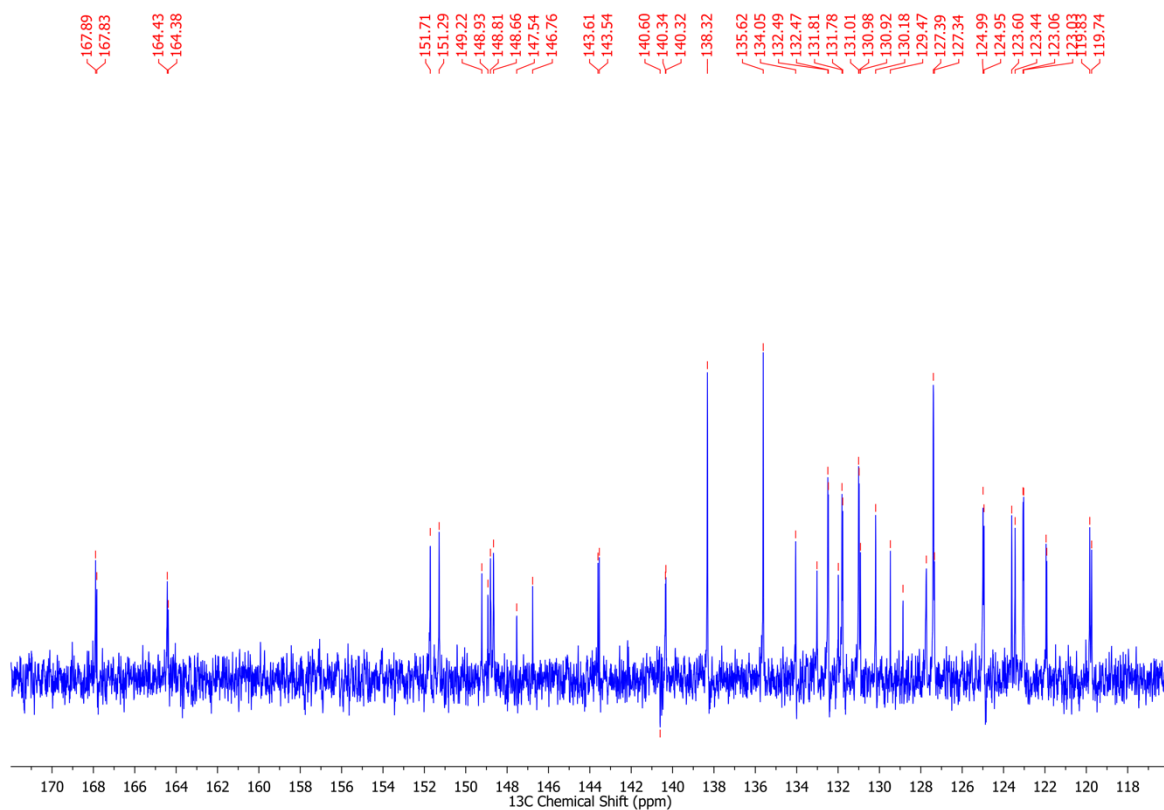
Figure A4.6: The ^1H NMR spectrum (400 MHz, $\text{DMSO}-d_6$) of **109** after recrystallisation from the first attempted synthesis.

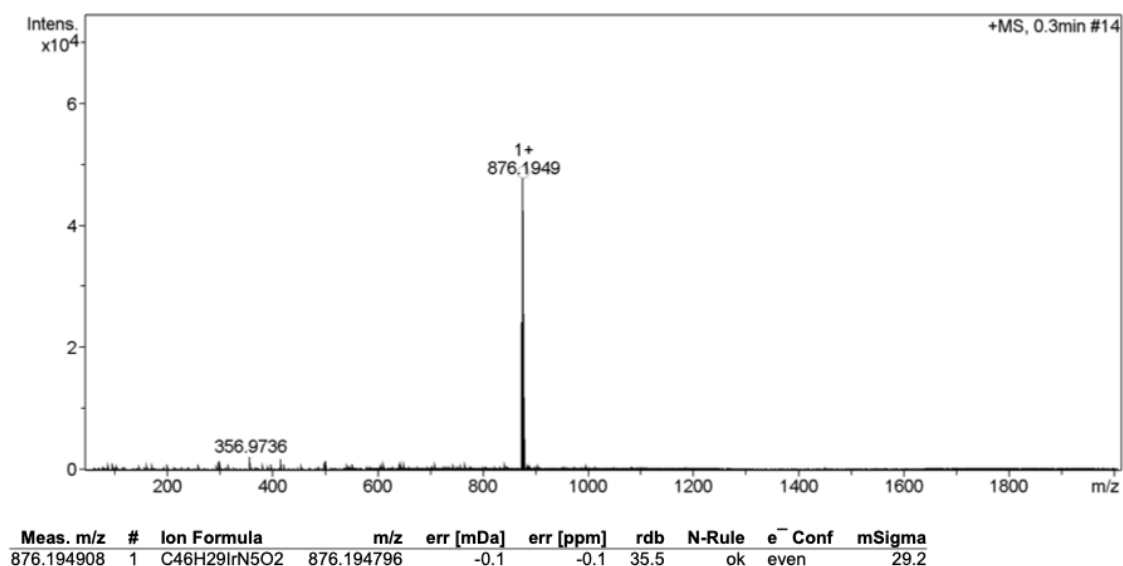
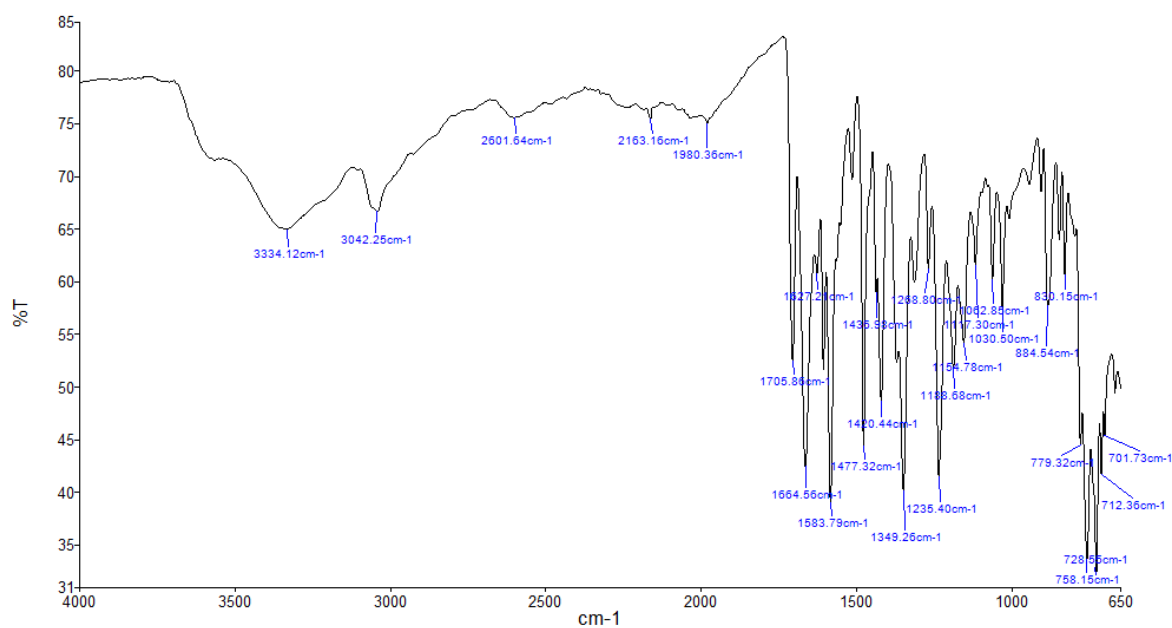
Figure A4.7: The HSQC spectrum (600, 151 MHz, DMSO- d_6) of **109**Figure A4.8: The ^{13}C spectrum (151 MHz, DMSO- d_6) of **109**

Figure A4.9: The mass spectrum (ESI⁺) of **98**.Figure A4.10: The ¹H NMR spectrum (400 MHz, DMSO-*d*₆) of first (red, bottom), second (green middle) and third (blue, top) fraction from column chromatography of **100**

Figure A4.11: The ^1H NMR spectrum (400 MHz, CDCl_3) of **NBI**Figure A4.12: The ^1H NMR spectrum (400 MHz, CDCl_3) of **dppz**

Figure A4.13: The ¹H NMR spectrum (600 MHz, CDCl₃) of **110**Figure A4.14: The mass spectrum (ESI⁺) of **110**.

Figure A4.15: The ¹H NMR spectrum (600 MHz, CDCl₃) of **111**

Figure A4.17: The mass spectrum (ESI⁺) of **111**.

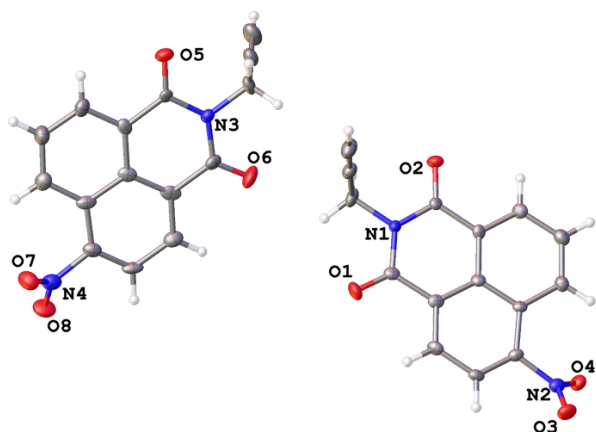
Appendix for Chapter 5:

Table A5.1: Crystal data and structure refinement for **C1-C3**

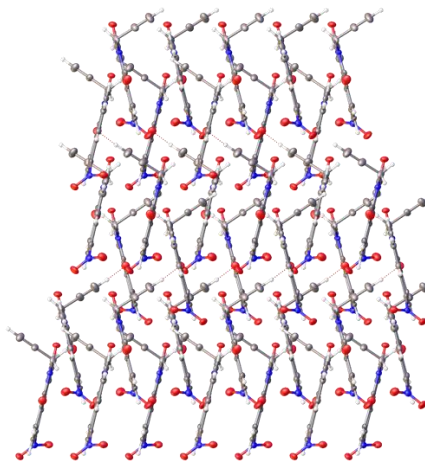
Identification code	C1	C2	C3
Empirical formula	C ₃₀ H ₁₆ N ₄ O ₈	C ₄₉ H ₃₀ Cl ₂ N ₄ O ₆	C ₂₀ H ₁₇ BN ₂ O ₄
Formula weight	560.47	843.68	360.16
Temperature/K	100(2)	100(2)	100(2)
Crystal system	orthorhombic	triclinic	monoclinic
Space group	Pca2 ₁	P-1	C2/c
a/Å	7.0981(5)	8.0005(2)	16.0498(5)
b/Å	19.5620(14)	13.7161(4)	9.7748(3)
c/Å	17.4861(12)	18.2601(5)	21.9261(7)
α/°	90	97.9432(19)	90
β/°	90	98.8369(19)	92.8074(16)
γ/°	90	99.0261(19)	90
Volume/Å ³	2428.0(3)	1928.25(9)	3435.72(19)
Z	4	2	8
ρ _{calc} /cm ³	1.533	1.453	1.393
μ/mm ⁻¹	0.961	2.014	0.795
F(000)	1152.0	868.0	1504.0
Crystal size/mm ³	0.7 × 0.22 × 0.04	0.161 × 0.062 × 0.032	0.19 × 0.17 × 0.1
Radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)
2θ range for data collection/°	4.516 to 139.976	6.616 to 130.266	8.074 to 139.702
Index ranges	-8 ≤ h ≤ 8, -23 ≤ k ≤ 23, -21 ≤ l ≤ 21	-9 ≤ h ≤ 9, -16 ≤ k ≤ 16, -21 ≤ l ≤ 21	-19 ≤ h ≤ 19, -11 ≤ k ≤ 9, -18 ≤ l ≤ 26
Reflections collected	21634	30572	10235
Independent reflections	4524 [R _{int} = 0.0556, R _{sigma} = 0.0452]	6477 [R _{int} = 0.0615, R _{sigma} = 0.0464]	3223 [R _{int} = 0.0344, R _{sigma} = 0.0303]
Data/restraints/parameters	4524/1/379	6477/0/550	3223/961/343
Goodness-of-fit on F ²	1.125	1.056	1.073
Final R indexes [I > 2σ (I)]	R ₁ = 0.0935, wR ₂ = 0.2483	R ₁ = 0.0602, wR ₂ = 0.1545	R ₁ = 0.0480, wR ₂ = 0.1367
Final R indexes [all data]	R ₁ = 0.1011, wR ₂ = 0.2610	R ₁ = 0.0872, wR ₂ = 0.1720	R ₁ = 0.0506, wR ₂ = 0.1392

Largest diff. peak/hole / $e \text{ \AA}^{-3}$	1.09/-0.42	0.86/-0.75	0.26/-0.21
Flack parameter	-0.02(17)		

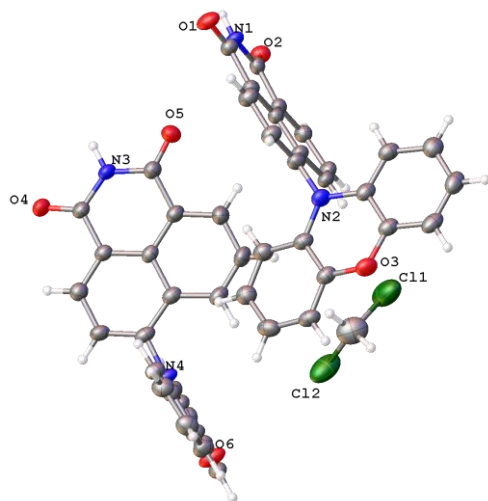
a)



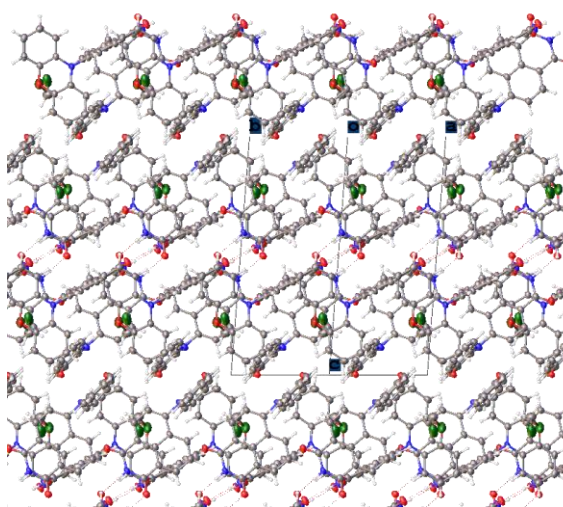
b)

Figure A5.2: a) Asymmetric unit of **C1** and b) packed structure shown normal to the *b*-axis.

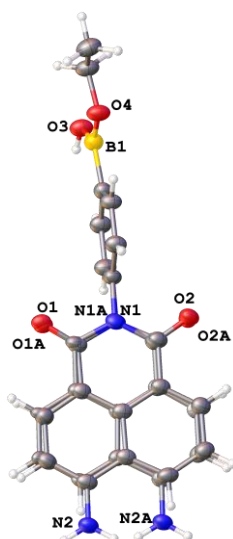
a)



b)

Figure A5.3: a) Asymmetric unit of **C2** and b) packed structure shown normal to the 110 plane

a)



b)

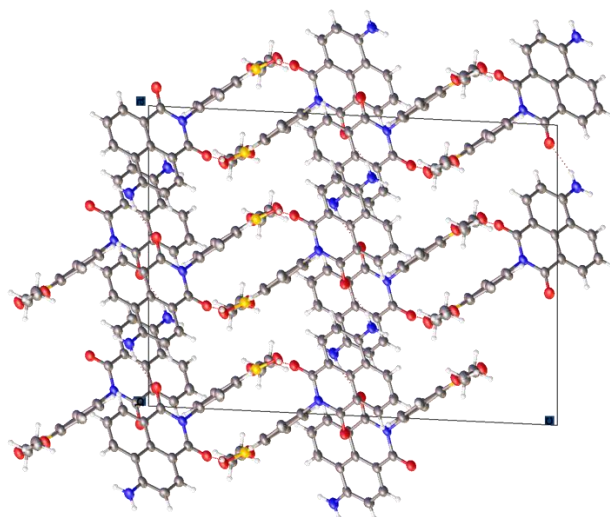


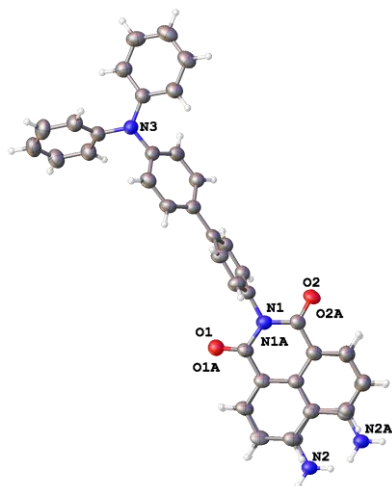
Figure A5.4: a) Asymmetric unit of **C3** and b) packed structure shown normal to the crystallographic *b*-axis.

Table A5.5: Crystal data and structure refinement for **C4-C6**

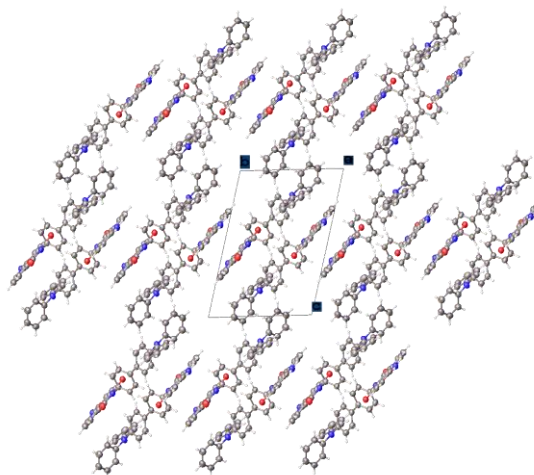
Identification code	C4	C5	C6
Empirical formula	C ₃₆ H ₂₅ N ₃ O ₂	C ₅₄ H ₃₇ N ₃ O ₂	C ₃₆ H ₂₅ N ₃ O ₂
Formula weight	531.59	1915	531.59
Temperature/K	100(2)	100(2)	100(2)
Crystal system	triclinic	triclinic	monoclinic
Space group	P-1	P-1	P2 ₁
a/Å	9.7325(3)	9.4102(4)	14.5594(7)
b/Å	11.2122(4)	9.8093(5)	8.6381(3)
c/Å	15.8447(6)	21.2869(12)	21.2930(8)
α/°	75.214(3)	90.731(4)	90
β/°	83.986(3)	100.745(4)	96.565(3)
γ/°	76.066(3)	96.990(4)	90
Volume/Å ³	1620.84(10)	1914.87(17)	2660.37(19)
Z	2	2	4
ρ _{calc} /g/cm ³	1.089	1.318	1.327
μ/mm ⁻¹	0.540	0.627	0.659
F(000)	556.0	796.0	1112.0
Crystal size/mm ³	0.159 × 0.084 × 0.038	0.15 × 0.08 × 0.02	0.155 × 0.058 × 0.052
Radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)
2θ range for data collection/°	5.774 to 130.59	4.228 to 136.572	4.176 to 136.798
Index ranges	-11 ≤ h ≤ 11, -13 ≤ k ≤ 13, -18 ≤ l ≤ 18	-11 ≤ h ≤ 10, -11 ≤ k ≤ 11, -25 ≤ l ≤ 25	-17 ≤ h ≤ 17, -10 ≤ k ≤ 10, -25 ≤ l ≤ 25
Reflections collected	27945	22232	30557
Independent reflections	5536 [R _{int} = 0.0830, R _{sigma} = 0.0878]	6974 [R _{int} = 0.0866, R _{sigma} = 0.1034]	9750 [R _{int} = 0.0951, R _{sigma} = 0.1512]
Data/restraints/parameters	5536/819/494	6974/0/533	9750/735/837
Goodness-of-fit on F ²	1.105	0.948	1.034
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0990, wR ₂ = 0.2733	R ₁ = 0.0906, wR ₂ = 0.2312	R ₁ = 0.0767, wR ₂ = 0.1935
Final R indexes [all data]	R ₁ = 0.1412, wR ₂ = 0.3199	R ₁ = 0.1641, wR ₂ = 0.2873	R ₁ = 0.1244, wR ₂ = 0.2298

Largest diff. peak/hole / $e \text{ \AA}^{-3}$	1.76/-0.40	0.47/-0.33	0.29/-0.26
Flack parameter			-0.2(7)

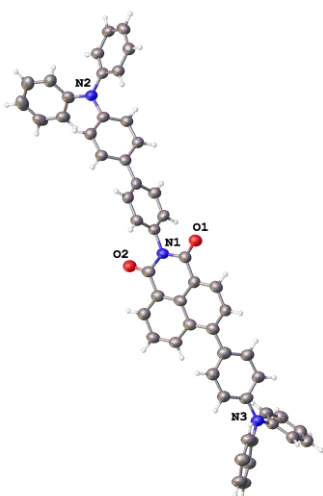
a)



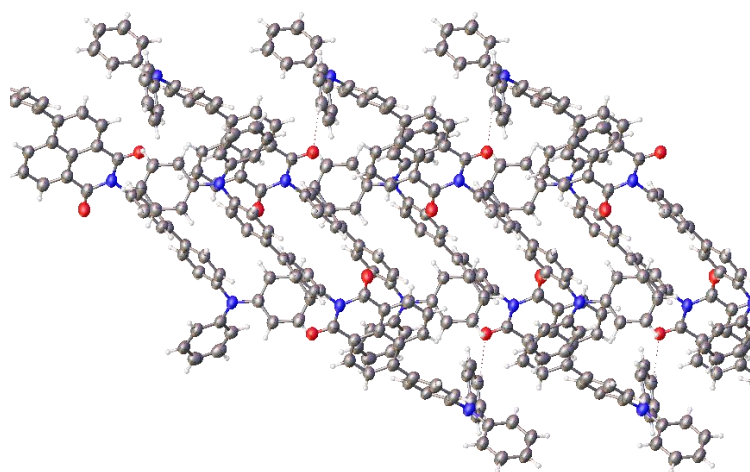
b)

Figure A5.6: a) Asymmetric unit of **C4** and b) packed structure shown normal to the *a*-axis.

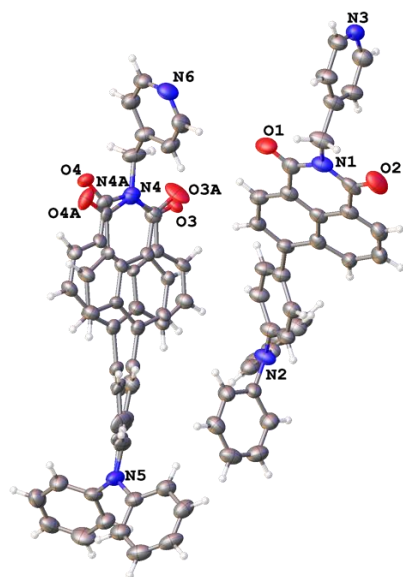
a)



b)

Figure A5.7: a) Asymmetric unit of **C5** and b) packed structure shown normal to the *b*-axis.

a)



b)

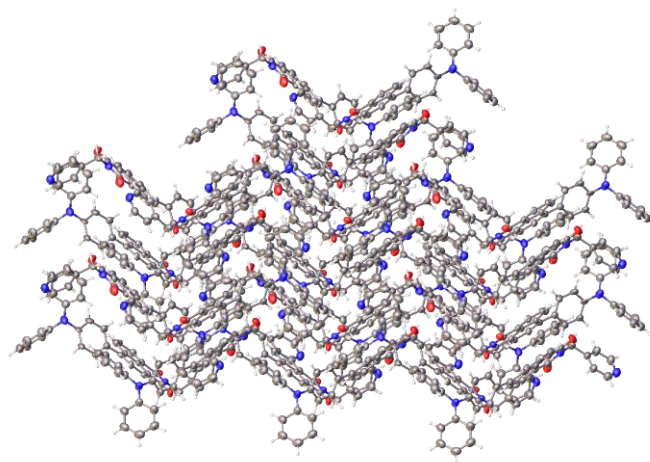
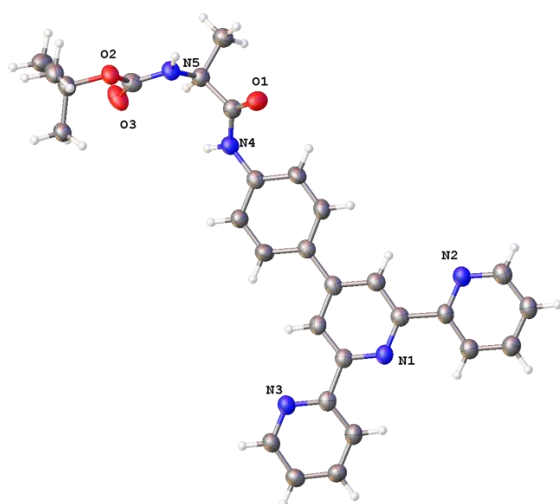


Figure A5.8: a) The asymmetric unit of **C6** and b) packed structure shown normal to the 101 plane.

Table A5.9: Crystal data and structure refinement for **C7** and **C8**

Identification code	C7	C8
Empirical formula	C ₃₇ H ₃₂ N ₂ O ₄	C ₂₈ H ₃₀ N ₂ O ₈
Formula weight	568.64	522.54
Temperature/K	100(2)	100(2)
Crystal system	monoclinic	triclinic
Space group	P2 ₁ /c	P-1
a/Å	9.7475(12)	6.797(5)
b/Å	18.370(2)	9.093(7)
c/Å	16.524(2)	21.156(16)
α/°	90	84.768(19)
β/°	103.965(3)	83.95(3)
γ/°	90	83.459(17)
Volume/Å ³	2871.2(6)	1287.6(17)
Z	4	2
ρ _{calc} /cm ³	1.315	1.348
μ/mm ⁻¹	0.684	0.099
F(000)	1200.0	552.0
Crystal size/mm ³	0.149 × 0.098 × 0.071	0.25 × 0.24 × 0.18
Radiation	Cu Kα (λ = 1.54178)	MoKα (λ = 0.71073)
2θ range for data collection/°	7.318 to 136.604	3.884 to 59.228
Index ranges	-11 ≤ h ≤ 11, -22 ≤ k ≤ 22, -19 ≤ l ≤ 17	-9 ≤ h ≤ 9, -12 ≤ k ≤ 12, -28 ≤ l ≤ 28
Reflections collected	24698	13246
Independent reflections	5254 [R _{int} = 0.0493, R _{sigma} = 0.0387]	6694 [R _{int} = 0.0290, R _{sigma} = 0.0490]
Data/restraints/parameters	5254/0/391	6694/0/349
Goodness-of-fit on F ²	1.063	1.050
Final R indexes [I ≥ 2σ (I)]	R ₁ = 0.0596, wR ₂ = 0.1635	R ₁ = 0.0589, wR ₂ = 0.1320
Final R indexes [all data]	R ₁ = 0.0717, wR ₂ = 0.1737	R ₁ = 0.0876, wR ₂ = 0.1462
Largest diff. peak/hole / e Å ⁻³	0.41/-0.30	0.46/-0.27

a)



b)

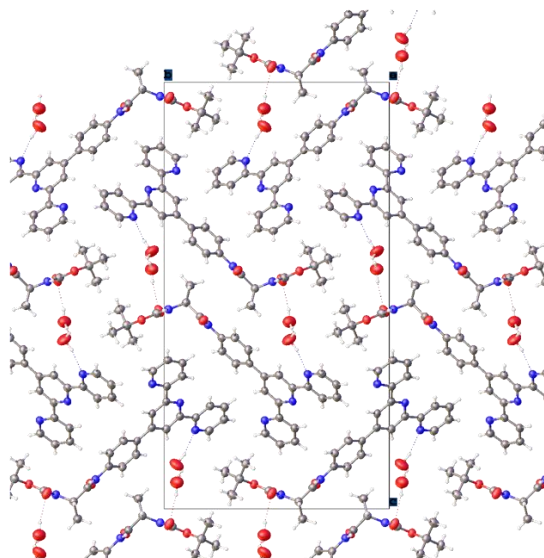
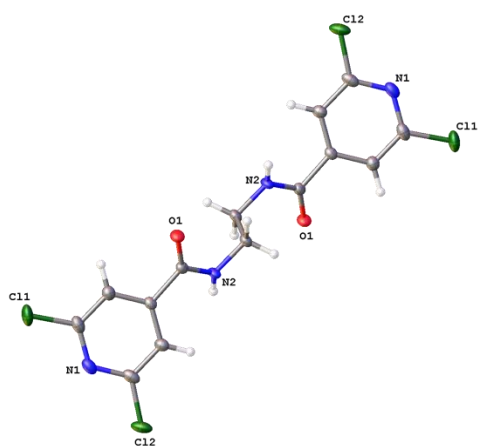


Figure A5.10: a) The asymmetric unit of **DAM018** and b) packed structure.

a)



b)

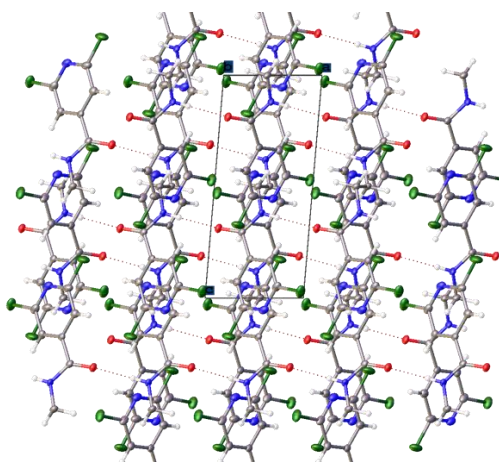


Figure A5.11: a) The grown asymmetric unit of **DAM021** and b) packed structure.

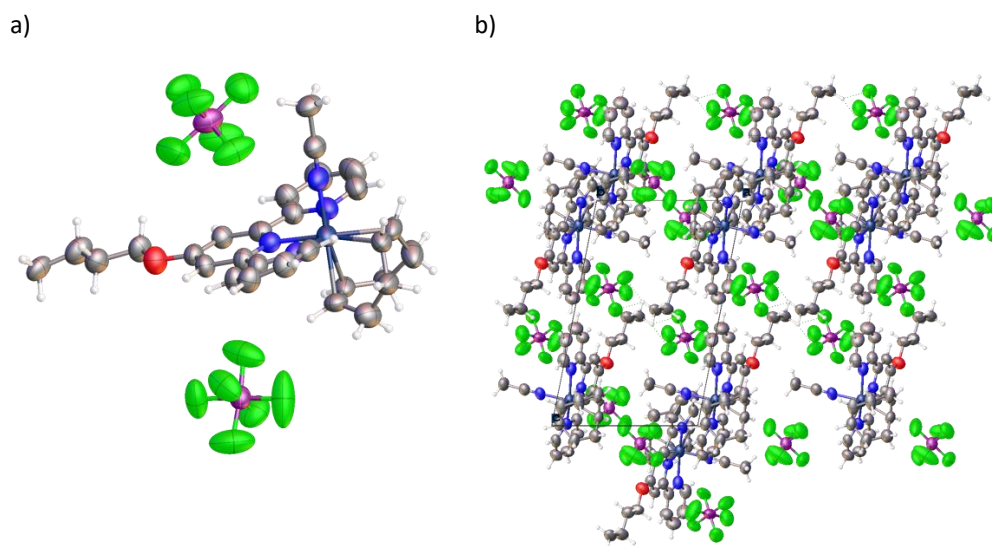


Figure A5.12: a) The asymmetric unit of **DAM027** and b) packed structure.

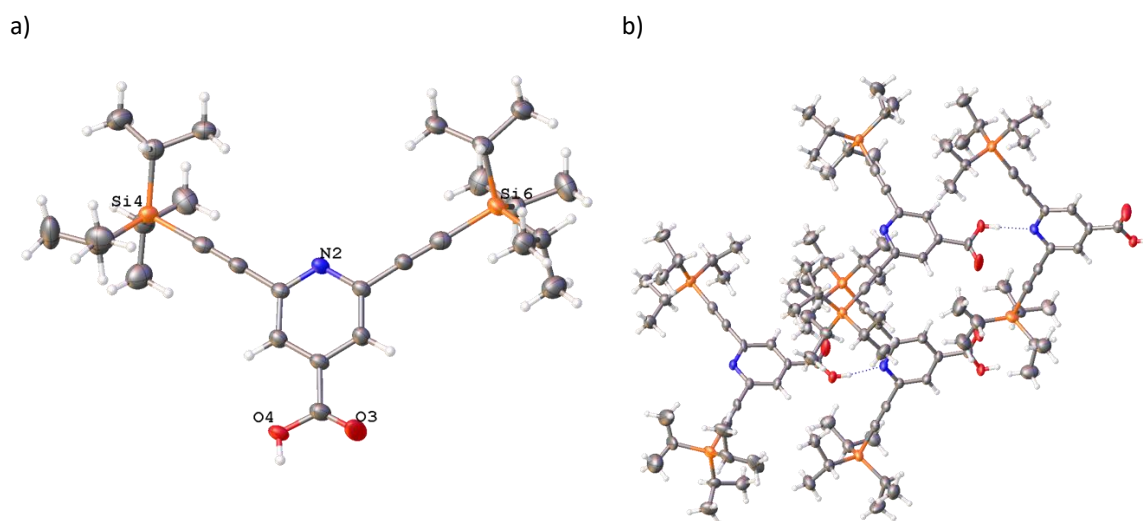


Figure A5.13: a) Representation of one molecule of **DAM030** and b) asymmetric unit.

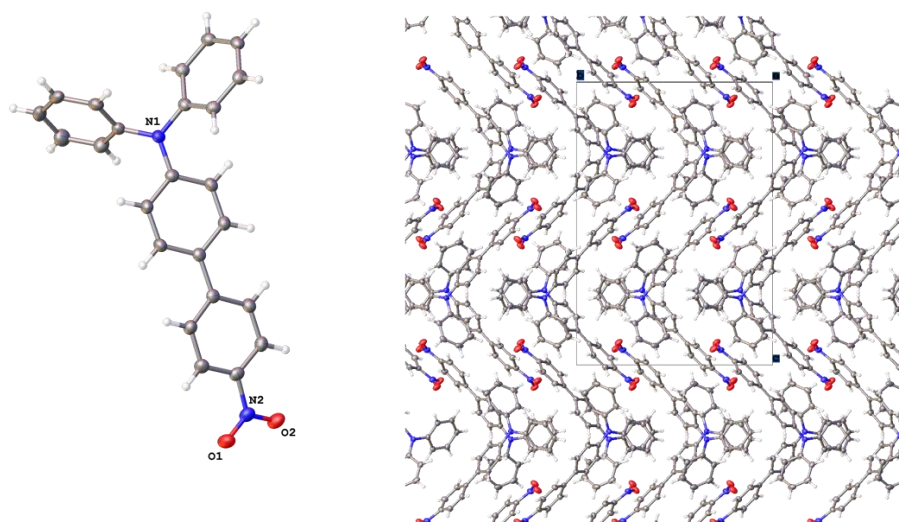
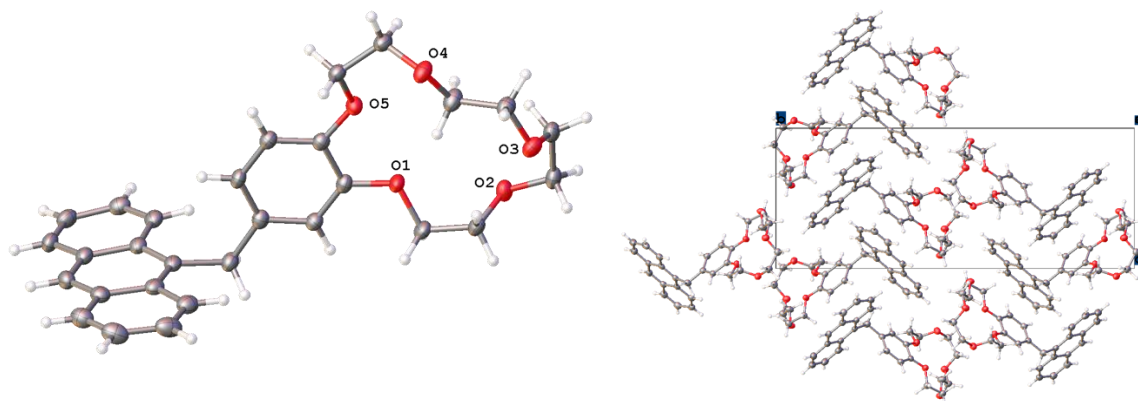


Figure A5.14: a) Asymmetric unit of **DAM031** and b) packed structure.

Figure A5.15: a) Asymmetric unit of **DAM047** and b) packed structure.Table A5.16: Crystal data and structure refinement for **DAM018**, **DAM021** and **DAM027**

Identification code	DAM018	DAM021	DAM027
Empirical formula	C ₂₉ H ₃₃ N ₅ O ₅	C ₁₄ H ₁₀ Cl ₄ N ₄ O ₂	C ₂₉ H ₃₄ F ₁₈ N ₄ OP ₃ Ru
Formula weight	531.60	408.06	990.58
Temperature/K	100(2)	100(2)	100(2)
Crystal system	orthorhombic	monoclinic	triclinic
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ /c	P-1
a/Å	5.0718(2)	4.8202(2)	10.1503(6)
b/Å	16.8555(6)	15.7478(6)	14.6716(9)
c/Å	31.9226(10)	10.9755(4)	15.3945(10)
α/°	90	90	96.382(5)
β/°	90	94.8040(10)	98.886(5)
γ/°	90	90	106.694(4)
Volume/Å ³	2728.99(17)	830.20(6)	2140.1(2)
Z	4	2	2
ρ _{calc} /g/cm ³	1.294	1.632	1.537
μ/mm ⁻¹	0.735	6.631	5.021
F(000)	1128.0	412.0	990.0
Crystal size/mm ³	0.265 × 0.079 × 0.032	0.2 × 0.12 × 0.07	0.278 × 0.065 × 0.033
Radiation	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)	Cu Kα (λ = 1.54178)
2θ range for data collection/°	5.536 to 140.67	9.846 to 136.436	5.89 to 118.564

Index ranges	$-5 \leq h \leq 6, -20 \leq k \leq 20, -38 \leq l \leq 38$	$-5 \leq h \leq 5, -18 \leq k \leq 18, -13 \leq l \leq 13$	$-11 \leq h \leq 11, -16 \leq k \leq 16, -17 \leq l \leq 17$
Reflections collected	27550	23094	19485
Independent reflections	5106 [$R_{\text{int}} = 0.0763, R_{\text{sigma}} = 0.0563$]	1517 [$R_{\text{int}} = 0.0402, R_{\text{sigma}} = 0.0146$]	6138 [$R_{\text{int}} = 0.0778, R_{\text{sigma}} = 0.0900$]
Data/restraints/parameters	5106/5/368	1517/0/101	6138/216/541
Goodness-of-fit on F^2	1.022	1.080	1.028
Final R indexes [$ I \geq 2\sigma(I)$]	$R_1 = 0.0668, wR_2 = 0.1751$	$R_1 = 0.0570, wR_2 = 0.1372$	$R_1 = 0.0933, wR_2 = 0.2480$
Final R indexes [all data]	$R_1 = 0.0785, wR_2 = 0.1845$	$R_1 = 0.0571, wR_2 = 0.1372$	$R_1 = 0.1200, wR_2 = 0.2748$
Largest diff. peak/hole / $e \text{ \AA}^{-3}$	0.40/-0.35	1.59/-1.47	2.36/-0.58
Flack parameter	-0.2(2)		

Table A5.17: Crystal data and structure refinement for **DAM030**, **DAM031** and **DAM047**

Identification code	DAM030	DAM031	DAM047
Empirical formula	$\text{C}_{56}\text{H}_{89.5}\text{N}_2\text{O}_4\text{Si}_4$	$\text{C}_{24}\text{H}_{18}\text{N}_2\text{O}_2$	$\text{C}_{29}\text{H}_{30}\text{O}_5$
Formula weight	967.15	366.40	458.53
Temperature/K	100(2)	100(2)	100(2)
Crystal system	monoclinic	orthorhombic	monoclinic
Space group	$P2_1$	$Pbca$	$P2_1/c$
$a/\text{\AA}$	13.4716(4)	10.3528(3)	7.9276(2)
$b/\text{\AA}$	29.9742(8)	15.7578(5)	27.1591(7)
$c/\text{\AA}$	15.0634(4)	22.7203(7)	10.6118(3)
$\alpha/^\circ$	90	90	90
$\beta/^\circ$	90.1177(18)	90	91.9989(17)
$\gamma/^\circ$	90	90	90
Volume/ $\text{\AA}^3$	6082.6(3)	3706.5(2)	2283.40(10)
Z	4	8	4
$\rho_{\text{calc}}/\text{g cm}^{-3}$	1.056	1.313	1.334
μ/mm^{-1}	1.219	0.674	0.726
$F(000)$	2110.0	1536.0	976.0
Crystal size/ mm^3	$0.36 \times 0.056 \times 0.048$	$0.41 \times 0.244 \times 0.155$	$0.216 \times 0.094 \times 0.023$

Radiation	Cu K α (λ = 1.54178)	Cu K α (λ = 1.54178)	Cu K α (λ = 1.54178)
2 θ range for data collection/ $^{\circ}$	5.866 to 137.476	7.782 to 137.386	6.508 to 136.688
Index ranges	$-15 \leq h \leq 16$, $-36 \leq k \leq 35$, $18 \leq l \leq 18$	$-11 \leq h \leq 12$, $-19 \leq k \leq 18$, $27 \leq l \leq 27$	$-9 \leq h \leq 9$, $-32 \leq k \leq 32$, $-12 \leq l \leq 12$
Reflections collected	86402	72236	35430
Independent reflections	22320 [R_{int} = 0.1240, R_{sigma} = 0.1133]	3419 [R_{int} = 0.0436, R_{sigma} = 0.0133]	4193 [R_{int} = 0.0714, R_{sigma} = 0.0388]
Data/restraints/parameters	22320/531/1426	3419/0/254	4193/0/307
Goodness-of-fit on F^2	1.049	1.032	1.083
Final R indexes [$I \geq 2\sigma(I)$]	R_1 = 0.0764, wR_2 = 0.1958	R_1 = 0.0362, wR_2 = 0.0970	R_1 = 0.0538, wR_2 = 0.1460
Final R indexes [all data]	R_1 = 0.1163, wR_2 = 0.2245	R_1 = 0.0373, wR_2 = 0.0982	R_1 = 0.0699, wR_2 = 0.1559
Largest diff. peak/hole / $e \text{ \AA}^{-3}$	0.43/-0.32	0.19/-0.20	0.28/-0.28
Flack parameter	0.06(3)		

Table A5.18: Crystal data and structure refinement for **C9**

Identification code	C9
Empirical formula	$\text{C}_{306.5}\text{H}_{252}\text{Cl}_{17}\text{Eu}_4\text{N}_{36}\text{O}_{72}$
Formula weight	6801.92
Temperature/K	100(2)
Crystal system	trigonal
Space group	R3
a/ $\text{\AA}$	22.1763(4)
b/ $\text{\AA}$	22.1763(4)
c/ $\text{\AA}$	57.6861(18)
$\alpha/^\circ$	90
$\beta/^\circ$	90
$\gamma/^\circ$	120
Volume/ $\text{\AA}^3$	24568.6(12)
Z	3

$\rho_{\text{calc}}/\text{cm}^3$	1.379
μ/mm^{-1}	0.975
F(000)	10380.0
Crystal size/ mm^3	$0.283 \times 0.152 \times 0.133$
Radiation	Mo K α ($\lambda = 0.71073$)
2 θ range for data collection/ $^\circ$	4.3 to 42.554
Index ranges	$-19 \leq h \leq 22$, $-22 \leq k \leq 22$, $-58 \leq l \leq 58$
Reflections collected	43988
Independent reflections	12169 [$R_{\text{int}} = 0.1028$, $R_{\text{sigma}} = 0.1074$]
Data/restraints/parameters	12169/1486/1126
Goodness-of-fit on F^2	1.043
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.1076$, $wR_2 = 0.2642$
Final R indexes [all data]	$R_1 = 0.1706$, $wR_2 = 0.3136$
Largest diff. peak/hole / $\text{e } \text{\AA}^{-3}$	0.97/-0.75
Flack parameter	0.13(5)

