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Synthesis, Photophysical and DNA Binding Studies of Novel Pyridinium Based 1,8-Naphthalimide Derivatives

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October 2012

University of Dublin
Trinity College

Based on research carried out under the direction of Prof. Thorfinnur Gunnlaugsson and Prof. John M. Kelly

A thesis submitted to the School of Chemistry, University of Dublin, Trinity College for the degree of Doctor of Philosophy
Declaration

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Summary

This thesis entitled “Synthesis, Photophysical and DNA Binding Studies of Novel Pyridinium Based 1,8-Naphthalimide Derivatives” is divided into six chapters. Chapter 1 is the introductory chapter, which provides a brief introduction to the cell cycle and how misregulation of cell cycle can lead to cancer development. This is followed by a brief description of the DNA structure, different binding modes by which small molecules can interact with DNA and a summary of various methods widely used to probe drug-DNA interactions. The development of various antitumour agents based on the 1,8-naphthalimide structure is presented citing relevant examples from the literature and also work performed within the Gunnlaugsson group. The photophysical properties of various 1,8-naphthalimide derivatives are also discussed. The chapter also provides an introduction to the $C_2$ symmetric Troger’s base structure and its applications in supramolecular chemistry, with special focus to the development of $C_2$ symmetric chiral DNA binding agents.

In Chapter 2, the synthesis, X-ray crystal structure and the photophysical properties of a series of 4-amino-1,8-naphthalimide derivatives containing the pyridinium side chain are discussed. The effect of $N,N'$-dimethyl amino substitution on the photophysical properties of 1,8-naphthalimide derivatives is also presented. The second part of this chapter focuses on the interactions of these naphthalimide derivatives with mononucleotides, $st$-DNA and synthetic polynucleotides using UV/vis absorption, steady-state and time-resolved fluorescence spectroscopy, which shows that these naphthalimide derivatives form $\pi$-stacked complex with DNA bases. The fluorescence quantum yields of these derivatives show significant enhancement in the presence of DNA and adenine rich sequences, which is attributed to the decrease in non-radiative decay processes in the nonpolar and rigid environment. On the contrary, the fluorescence emission is partly quenched in the presence of guanine rich sequences, due to photoinduced electron transfer from guanine to the photoexcited naphthalimide moiety.

In Chapter 3, the synthesis, and photophysical studies of two chiral Troger’s base derived bis-naphthalimides are presented. The enantiomers of both compounds are separated by cation exchange chromatography using a chiral anion as the eluent. The interaction of the enantiomers with DNA and synthetic polynucleotides are studied by a variety of spectroscopic methods including UV/vis absorbance, circular dichroism, linear dichroism, thermal melting and ethidium bromide displacement assays, which show that these compounds bind to the DNA grooves with very high affinity ($ca. K = 10^6 M^{-1}$),
where the (-)-enantiomer shows preferential binding. These compounds also exhibit significant photocleavage of plasmid DNA.

The development of a bifunctional Pt(II) complex incorporating the 2,2′:6′,2″-terpyridine moiety and a 4,N,N'-dimethylamino-1,8-naphthalimide unit is described in Chapter 4. The solid state structure and the photophysical properties of the Pt(II) complex is also presented. The DNA binding studies of the Pt(II) complex is performed using various spectroscopic techniques, which show the possible displacement of the naphthalimide ligand in the presence of guanosine and coordination of the [Pt(II)terpy]^2+ unit to the DNA base. The Pt(II) complex shows high cytotoxicity and induces apoptosis in malignant cells compared to the corresponding 4,N,N'-dimethylamino-1,8-naphthalimide ligand.

Chapter 5 describes the investigation of the excited state properties of a 4-amino-1,8-naphthalimide derivative and a Tröger’s base derived bis-naphthalimide using the ps-transient absorption and the ps-time-resolved infrared spectroscopies. At higher concentrations (> 0.1 mM), both of the compounds undergo significant aggregation, resulting in biexponential decays of the excited states. Further, ps-TA measurements at lower concentrations of the 4-amino-1,8-naphthalimide and the Tröger’s base derivative indicated that the excited state of the monomeric Tröger’s base derivative is very short-lived (ca. 30 ps) in water compared to the 4-amino-1,8-naphthalimide precursor (ca. 2.2 ns).

Finally in Chapter 6, general experimental procedure, the synthesis and characterisation of each of the compounds are presented, followed by a list of literature references and appendices providing the spectroscopic data supporting the work described in the main text.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>Å</td>
<td>ångström ($1 \times 10^{-10}$ m)</td>
</tr>
<tr>
<td>A</td>
<td>adenine</td>
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<tr>
<td>AMP</td>
<td>adenosine-5'-monophosphate</td>
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<tr>
<td>ATR</td>
<td>attenuated total reflectance</td>
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<td>a.u.</td>
<td>arbitrary units</td>
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<td>BET</td>
<td>back electron transfer</td>
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<td>broad</td>
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<td>cytosine</td>
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<td>N,N-dimethylformamide</td>
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<td>deoxyribonucleic acid</td>
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<td>dppz</td>
<td>dipyrido[3,2-a:2',3'-c]phenazine</td>
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<td>intraligand</td>
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<td>IR</td>
<td>infra red</td>
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<td>intersystem crossing</td>
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<td>J</td>
<td>coupling constant (expressed in Hz)</td>
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<td>$k_{nr}$</td>
<td>rate constant of nonradiative decay</td>
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<tr>
<td>$k_r$</td>
<td>rate constant of radiative decay</td>
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<td>Abbreviation</td>
<td>Definition</td>
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<td>------------</td>
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<tr>
<td>λ</td>
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<td>multiplet</td>
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<td>naphthalimide</td>
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<td>nanometre</td>
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<td>NMR</td>
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<td>nuclear overhauser effect spectroscopy</td>
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<td>P/D</td>
<td>phosphate to drug</td>
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<td>Pd/C</td>
<td>palladium on carbon catalyst</td>
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<td>PET</td>
<td>photoinduced electron transfer</td>
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<td>PF₆⁻</td>
<td>hexafluorophosphate anion</td>
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<td>phen</td>
<td>1,10-phenanthroline</td>
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<tr>
<td>pKₐ</td>
<td>-log[Kₐ] where Kₐ is the acidity constant</td>
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<td>PNA</td>
<td>peptide nucleic acid</td>
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<td>ppm</td>
<td>parts per million</td>
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<td>platinum</td>
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<td>SAR</td>
<td>structure activity relationship</td>
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<td>salmon testes DNA</td>
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<td>t</td>
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<td>transient absorption</td>
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<td>Tröger's base</td>
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<tr>
<td>TCSPC</td>
<td>time correlated single photon counting</td>
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<td>TFA</td>
<td>trifluoroacetic acid</td>
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<td>tetramethylsilane</td>
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<td>time-resolved infrared</td>
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<td>UV/vis</td>
<td>ultraviolet/visible</td>
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<td>v</td>
<td>frequency</td>
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Introduction
1.1 Preface

In the area of anticancer research, the development of small molecules capable of binding to deoxyribonucleic acid (DNA) and exhibiting anticancer activity has received enormous attention in recent times.\(^1\) In this context, 1,8-naphthalimide derivatives represent an important family of DNA binders that exhibit their antitumor effects both \textit{in vitro} and \textit{in vivo}.\(^2\) Additionally, the scope of functionalisation at either the aromatic "naphthalene" ring or at the \textit{N}-imide position and the tunable photophysical properties of the 1,8-naphthalimide derivatives depending on the aryl substituent, results in the extensive use of these derivatives in the field of supramolecular and medicinal chemistry.\(^2^3\) The work described in this thesis presents various approaches to further extend the utility of the 1,8-naphthalimide derivatives as DNA binders. The aim of this introductory chapter is to discuss the development of various 1,8-naphthalimide derivatives as potential anticancer agents. The chapter begins with a short discussion on the link between cell cycle misregulation and cancer, followed by a description of DNA as a promising target for anticancer agents.

1.2 Cancer and Cell Cycle

According to a survey by the World Health Organisation (WHO) in 2007 cancer is a major cause of death in the current century, accounting for \textit{ca.} 13\% of total death worldwide.\(^4\) Cancer is caused by a complex combination of several factors leading to uncontrolled proliferation of cells due to failure in the control mechanisms that regulate cell division and growth.\(^5\) Cell proliferation is highly regulated by several control mechanisms that determine whether a cell should go through a cell division cycle. The term "cell cycle" represents the general sequence of events that occur during the lifetime of a cell. For a eukaryotic cell, this is divided into four distinct phases as described below (Figure 1.1).\(^6\)

1. Mitosis and cell division take place during the M phase.
2. This is followed by the G1 phase (gap phase), which covers the longest period of the cell cycle. Cell growth occurs mainly during this phase.
3. G1 phase is followed by S phase (for synthesis) during which DNA synthesis takes place.
4. This is then followed by the relatively short G2 phase, during which the tetraploid cell prepares to enter into mitosis phase, thereby commencing a new round of the cell cycle.
For a multicellular organism, cell cycle duration can vary from several hours to 100 days for different types of cells. Importantly, terminally differentiated cells such as neurons or muscle cells never divide; they assume a quiescent state known as the G0 phase.

![Cell cycle and cyclin dependent regulation of protein kinase activity (cdk) in animal cell.](image)

Normally the events of the cell cycle are highly regulated by a series of phosphorylation and dephosphorylation of a variety of nuclear proteins. Studies of marine invertebrate embryos revealed that a class of protein known as cyclins accumulate steadily during the cell cycle and abruptly disappears before the anaphase portion of mitosis. Homologs of these cyclin proteins have also been discovered in many eukaryotes from sea urchins to human. The second family of proteins, known as cyclin dependent protein kinases (CdK) can bind to cyclins and induce various downstream processes through phosphorylation on serine/threonine amino acids on specific proteins. There are two major types of cyclins: (a) mitotic cyclins that bind to CdK during G2 to form a complex known as M-phase promoting factor (MPF). Activation of MPF occurs through a series of phosphorylations and dephosphorylations at the G2/M boundary, which triggers mitosis. At the metaphase-anaphase boundary, MPF is deactivated due to degradation of mitotic cyclin in a ubiquitin mediated...
pathway and the cells exit from M phase and (b) G1 cyclins that bind to CdK molecules during G1 phase and control the entry into S phase.

In general any genetic change that causes disruption in the cell cycle regulation can result in an uncontrolled cellular proliferation. Cancer cells differ in a number of ways from the normal cells. For example the plasma membrane of malignant cells have a more fluid character and altered ratios of many of the cell surface components such as glycoproteins and glycolipids compared to normal cells. This presumably results in a more rounded appearance of cancer cells than the corresponding normal cells. Moreover, cancer cells have very high metabolic rates compared to the normal cells. The conversion of normal cells to cancerous cell is caused by genetic changes accompanied by a series structural and biochemical changes as described below:

1. **Altered Proteins**: Simple point mutation in a protein-encoding gene (proto-oncogene) can result in an oncogene (Greek: oncos-mass or tumour), which may give rise to a protein with anomalous activity. For example the *ras* oncogene, isolated from human bladder carcinomas, differs from the corresponding proto-oncogene by a point mutation of the Gly 12 codon (GGC) to a Val codon (GTC). The resulting amino acid change attenuates the GTPase activity and results in the prolonged phosphorylation activity of the ras G-protein.

2. **Altered Regulatory Sequence**: Uncontrolled high expression of a normal cellular protein can result in malignant transformation. For example the retroviral oncogene v-*fos* and its corresponding proto-oncogene (c-*fos*) encode similar proteins but they differ in their regulatory sequences. Therefore, c-*fos* can be converted into an oncogene by adding the v-*fos* enhancer sequence.

3. **Loss of degradation signal**: A slower rate of degradation of an oncogene protein compared to a normal cellular protein can cause malignant transformation. For example the oncogene *v-jun* lacks a 27-residue long amino acid segment essential for efficient ubiquination and fails to undergo efficient proteolytic degradation.

4. **Chromosomal Rearrangement**: Chromosomal rearrangement can bring an oncogene under the control of a foreign regulatory sequence and results in inappropriate expressions of the gene. In Burkitt’s lymphoma, proto-oncogene *c-myc* is translocated from its normal location, one end of chromosome 8, to the end of chromosome 14, adjacent to a highly active immunoglobin regulatory sequence and results in over expression of *c-myc* gene product.

5. **Loss or Inactivation of tumour suppressor gene**: Oncogenes can cause inactivation of the products of normal cellular tumour suppressor genes and results in malignant
transformation. In almost 50 % of human cancers, the tumour suppressor gene \( p53 \) has emerged as the most commonly altered gene. The \( p53 \) gene encodes a 53 kD nuclear phosphoprotein that acts as a transcriptional activator. If the cellular DNA is damaged, cellular level of \( p53 \) protein increases, which in turn activates the transcription of the gene \( Picl \). This encodes a 21 kD protein capable of binding to, as well as inhibiting, various Cdkxs and eventually leads to cell cycle arrest at the G1 phase. When cellular repair enzymes fail to repair the DNA damage, \( p53 \) triggers apoptosis to prevent proliferation of genetically damaged cells.\(^{10}\)

### 1.3 DNA as a Therapeutic Target

An understanding of the DNA structure and its function is imperative for the successful development of anti-cancer agents. Naturally occurring DNA (B-DNA) is a ~20 Å–diameter right handed double helix, consisting of two types of nitrogenous bases, purines, namely adenine (A) and guanine (G) and pyrimidines, namely thymine (T) and cytosine (C) (Figure 1.2).\(^{11}\) The ribose sugar derivatives of the bases are called nucleosides and the corresponding phosphates (mono-, di- and tri-) are called nucleotides. The two polynucleotide strands are held together by adjacent base pairs through hydrogen bonding. Among the four bases, G pairs with C and A pairs with T (Figure 1.3). The planes of the bases are nearly perpendicular to the helical axis of DNA. In the ideal B-DNA structure, there are 10 base pairs (bp) per turn of the helix of B-DNA resulting in a helical twist of 36° per bp. The bases are partially stacked on each other with the distance between each basepair is 3.4 Å. The sugar-phosphate backbone of the polynucleotide chain is located on the outside of the helix and thus the phosphate groups are free to interact with the polar environment. The nitrogen containing bases are located inside the helix in a stacking arrangement that is perpendicular to the helix axis. Due to this structural feature, the strand backbones are closer together on one side of the helix than the other, forming two types of grooves in the helical structure. In the major groove, the phosphate backbones are far apart and in minor groove the backbones are close together. These grooves play vital roles in the interactions of DNA with different small molecules and important biomolecules such as proteins.
1.3.1 Other double stranded DNA helices

Double stranded DNA can assume various conformations depending on the environments, which are described below.

A DNA: If the relative humidity of DNA solution is reduced to 75%, B-DNA undergoes a reversible conformation change to the A-form. A-DNA has a wider and flatter right-handed helical structure than B-DNA. Most of the self-complementary oligonucleotides (length <10 bp) such as d(GGCCGGCC) and d(GGTATACC) have been found to crystallise in A-DNA conformation. Structural features of this form are summarised in Table 1.1.\textsuperscript{12}
Z-DNA: Fibre diffraction and NMR studies revealed that complementary polynucleotides with alternating purines and pyrimidines such as poly d(GC).poly d(GC) or poly d(AC).poly d(GT) can adopt a left-handed double helical conformation in the presence of high salt concentrations. Methylation of cytosine residues at C5 position can also promote Z-DNA conformation because in this conformation a hydrophobic methyl group is less exposed to solvent molecules than in the B-DNA conformation. Structural features of Z-DNA are summarised in Table 1.1.

**Table 1.1: Structural features of ideal A-, B- and Z-DNA.**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Helical sense</strong></td>
<td>Right handed</td>
<td>Right handed</td>
<td>Left handed</td>
</tr>
<tr>
<td>Diameter (Å)</td>
<td>26</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>bp /helical turn</td>
<td>11</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Helical twist /bp (°)</td>
<td>33</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>Helix pitch (Å)</td>
<td>28</td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td>Helix rise/bp (Å)</td>
<td>2.6</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Base tilt normal to the helix axis (°)</td>
<td>20</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td><strong>Major Groove</strong></td>
<td>Narrow and deep</td>
<td>Wide and deep</td>
<td>Flat</td>
</tr>
<tr>
<td><strong>Minor Groove</strong></td>
<td>Wide and shallow</td>
<td>Narrow and deep</td>
<td>Narrow and deep</td>
</tr>
<tr>
<td><strong>Sugar pucker</strong></td>
<td>C3'-endo</td>
<td>C2'-endo</td>
<td>C2'-endo for Py</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C3'-endo for Pu</td>
</tr>
<tr>
<td><strong>Glycosidic bond</strong></td>
<td>Anti</td>
<td>Anti</td>
<td>Anti for Py</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Syn for Pu</td>
</tr>
</tbody>
</table>

Owing to its central role in replication, transcription and recombination, DNA is considered to be a major drug target because binding of small molecules can interfere with DNA mediated processes and can result in cell cycle arrest and apoptosis. As a consequence, development of small molecules capable of sequence specific DNA recognition has received enormous attention over past couple of decades.

In general, the DNA-ligand interactions can be classified into two major types: (i) covalent and (ii) noncovalent. A covalent interaction usually involves alkylation or coordination of the drug to DNA. One of the earliest developments in this field has been the discovery of the drug cisplatin, which was found to covalently bind with the guanine bases of the major groove - thus bending the DNA in the direction of the major groove. This
distortion favours binding of several proteins such as TATA binding protein (TBP), human upstream binding factor (hUBP), etc. in the minor groove. These proteins can mask the formation of bulky cisplatin–DNA adducts from the Nucleotide Excision repair machinery and results in cytotoxicity.\textsuperscript{15} Temozolomide 1 represents another important alkylating agent, which can methylate guanine residues of DNA.\textsuperscript{16} However, alkylating agents such as chlorambucil 2, was found to be more efficient because of their ability to crosslink two complementary strands of DNA.\textsuperscript{17}

Noncovalent interactions can be of three types (a) intercalation, (b) electrostatic, and (c) groove binding. The intercalation process occurs when small planar polyaromatic molecules bind between the stacked DNA bases and distort the DNA backbone. Simple intercalators like proflavine,\textsuperscript{3,18} and ethidium bromide,\textsuperscript{4,19} have a positive charge on their ring system to enhance the interaction with the negative phosphate backbone of DNA. More complex intercalators like nogalamycin,\textsuperscript{20} actinomycin D,\textsuperscript{21} and doxorubicin,\textsuperscript{22} consist of side chains, sugar and peptide moieties. In the case of actinomycin D, the side chain interacts with DNA bases in the minor groove by van der Waals forces and hydrogen bonding, whereas for nogalamycin, the nogalose ring interacts with the minor groove while the other amino sugar group binds to the major groove. Polyamines such as spermine\textsuperscript{5,23} and spermidine\textsuperscript{24} being positively charged at physiological pH, electrostatically interact with the DNA backbone. Natural products like netropsin,\textsuperscript{6,25} and distamycin\textsuperscript{7,26} with heteroaromatic ring structures possess crescent elongated structures that can fit in the minor groove of DNA, exhibiting a preference towards A/T base pairs. Another important class of DNA binders are polyamides-
incorporating pairs of pyrrole-imidazole rings as in the case of 8. These compounds bind to the minor groove of DNA and recognise a 6 bp sequence with subnanomolar affinity comparable with DNA binding proteins.\(^{27}\)

The interaction of nucleic acids with small molecules can be studied by various techniques, which will be described in the following section.

1.4 Methods to Study DNA-Ligand Interactions

A wide variety of methods have been employed to study the interactions of small molecules with nucleic acids. A combination of these methods can provide useful information about the binding affinity, kinetics, thermodynamics of the binding interaction, mode of binding and the conformational changes of macromolecules upon ligand binding. Some of the widely used techniques are described below.

1.4.1 Optical Spectroscopy

Electronic spectroscopic techniques such as UV/visible (UV/vis), fluorescence and circular dichroism (CD) spectroscopy and linear dichroism spectroscopy are routinely used to characterise nucleic acid-ligand interactions since these methods provide rapid information about the mode of binding, conformational changes, binding affinity and are preferred as they require small amounts of material and are non-destructive.
1.4.1.1 UV/vis absorption Spectroscopy: DNA exhibits a characteristic absorption band centred at ca. 260 nm. The concentration of DNA can thus be calculated from the absorbance at ca. 260 nm. Drug-DNA interactions can be monitored by following the changes in the absorbance of DNA or the drug molecules. Since most of the ligand molecules display strong π-π* transition bands in the UV region, which overlaps with the absorption band of DNA, usually the longer wavelength absorption bands characteristic of the ligand molecule are used to probe the binding interactions. Frequently binding of a ligand molecule to nucleic acid results in a hypochromism (decrease in absorbance) or hyperchromism (increase in absorbance) associated with a shift in the λ_{max} of the absorption band. The binding constants for the association can be determined from the changes in the absorption spectrum of the ligand in the presence of increasing amount of DNA by fitting the data to various binding models, which will be further discussed in Chapter 2.

1.4.1.2 Thermal denaturation measurements: Thermal denaturation study is an optical technique that determines the stability of double stranded DNA against heat-induced denaturation. This technique relies on the use of absorption spectroscopy of DNA and measures the absorbance of a DNA sample at 260 nm as a function of temperature. In the double helical structure, the nucleobases are held together by hydrogen bonding between the complementary strands. When a solution of double stranded DNA is gradually heated above a characteristic temperature, the native duplex structure collapses and the individual strands assume flexible single stranded conformations. The denaturation process is characterised by a sharp increase in the absorbance of nucleobases at 260 nm due to the disruption of the stacking interactions between the nearby bases. The temperature at which half of the maximum absorbance increase is attained (i.e. DNA strands are 50% denatured) is known as the melting temperature (T_m). The stability of double stranded DNA depends on several factors such as the ionic strength, pH of the solution, base composition etc. A typical melting curve for double stranded DNA is shown in Figure 1.4. The T_m value is taken as the midpoint of the transition and is usually determined from the maximum of the first derivative plot of absorbance vs. temperature. When a small molecule binds to DNA, it can either stabilise or destabilise double stranded DNA against heat denaturation and therefore modulates the T_m.
Chapter 1: Introduction

Figure 1.4: The typical melting curve for double stranded DNA in aqueous solution showing the changes in the absorbance at 260 nm as a function of temperature (modified from Ref 11c).

1.4.1.3 Circular Dichroism (CD) Spectroscopy: CD spectroscopy is based on the differential absorption of left and right handed circularly polarised light by an optically active molecule as demonstrated in Figure 1.5. CD spectroscopy is largely used to study the changes in conformation of macromolecules due to their interaction with small molecules.

![Diagram of CD spectroscopy](image)

\[ CD = \begin{bmatrix} \text{left polarized light} & - \text{right polarized light} \end{bmatrix} + \text{unoriented chiral molecule} \]

The nucleobases in DNA are achiral. However, in the polynucleotide chain, nucleobases acquire asymmetry due to the presence of the chiral deoxyribose sugar units. The CD spectrum of B-DNA shows a positive band centred at ca. 275 nm and a negative band centred at ca. 240 nm, with the zero cross-over at ca. 258 nm. These two bands result from the coupling of all electronic transitions of all nucleobases and depend on the skewed orientation of the nucleobases. The CD signal can become positive or negative at 220 nm depending on the polynucleotide sequence and ionic strength of the medium. In the presence of ligand molecules the CD spectrum of DNA changes due to the coupling between the electronic transitions of the DNA bases and that of the ligand molecule. Often an induced CD (ICD) band is also observed for bound achiral ligands due to its localisation in a chiral environment. Usually groove binders tend to result in positive ICD signals, whereas a negative ICD is
characteristic of an intercalative mode of binding, however the magnitude of ICD for an intercalator is usually very small and also depends on the orientation of the transition dipoles within the binding pocket.\textsuperscript{30}

### 1.4.1.4 Linear Dichroism (LD) Spectroscopy:

For an oriented sample, linear dichroism is defined as the differential absorption of light polarised parallel ($A_\parallel$) and perpendicular ($A_\perp$) to a reference axis (equation 1.1, Figure 1.6).\textsuperscript{30}

\[
LD = A_\parallel - A_\perp
\]

(1.1)

![Figure 1.6: Schematic illustration of Linear Dichroism spectroscopy showing the differential absorption of parallel and perpendicular polarised light by an oriented sample (taken from Ref 30).](image)

Long flexible polymers such as peptides or DNA are usually oriented by the technique of flow orientation. In this technique, the molecules are oriented by shear force generated by a flow gradient in the solution flowing between the narrow gap between a spinning cell and a stationary cylindrical rod (Figure 1.7).

Quantitative information about the orientation of a chromophore with respect to the reference axis can be obtained from reduced LD ($LD'$) as described in equation 1.2 below,

\[
LD' = \frac{LD}{A} = \frac{A_\parallel - A_\perp}{A} = \frac{3}{2} S (3 \cos^2 \alpha - 1)
\]

(1.2)

where, $A$ is the absorbance of the sample under isotopic condition (i.e. no orientation), $S$ refers to the orientation factor ($S = 1$ for a perfectly oriented sample, $S = 0$ for a random orientation) and $\alpha$ is the angle between the transition dipole of the chromophore and the reference axis. The magnitude of $S$ provides information about structural changes in the macromolecule such as lengthening, stiffening, bending etc.
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Figure 1.7: Schematic diagram showing flow orientation in a Couette cell (taken from Ref 30).

B-DNA displays a strong negative signal around 260 nm arising from the nearly perpendicular orientation of the transition moments of DNA bases relative to DNA helical axis. LD spectroscopy can be used to study the mode of binding of ligands to DNA.

Electrostatic binding: Ligands that bind mainly to the phosphate backbone usually do not give significant LD signals due to improper orientation.

Intercalation: In this mode of binding the planar aromatic molecules like ethidium bromide lie in the same plane of the nucleobases and usually display negative LD signals.

Minor groove binders: Molecules like netropsin and DAPI can fit in the minor groove of DNA such that their long axis is oriented at about 45° relative to the DNA helical axis and give rise to positive LD signals.

1.4.1.5 Fluorescence Spectroscopy: The interaction of small molecules with nucleic acids can be studied using fluorescence emission spectroscopy by following the changes in the intrinsic fluorescence of the nucleic acid binding molecules. Binding of small molecules to nucleic acids often results in a large shift in the fluorescence spectrum and significant enhancement in the fluorescence intensity as a consequence of several factors. For example, the fluorescence of the ruthenium (II) polypyridyl complex [Ru(phen)$_2$dppz]$^{2+}$ is quenched in aqueous solution but significantly increases when bound to DNA due to the shielding of the nitrogen atoms from the aqueous environment. Similarly the cyanine dyes also exhibit significant fluorescence enhancement in the presence of DNA, which has been attributed to the decrease in the rate of nonradiative decay processes in the rigid environment. In contrast to this, the fluorescence intensity of some photosensitizers are significantly quenched in the presence of DNA and especially guanine rich nucleic acids due to photoinduced electron transfer (PET) from guanine to the excited chromophore. The binding constants can also be determined from the fit of the changes in the fluorescence of the ligand in the presence of increasing
amount of nucleic acid using various binding models.

For nonfluorescent ligands the binding with nucleic acids can be monitored by the displacement assays using a fluorescent intercalator or groove binding molecule.\textsuperscript{34} For example, ethidium bromide is weakly emissive in aqueous solution but becomes highly emissive, when bound to DNA.\textsuperscript{35} The principle of an ethidium bromide displacement assay is based on the loss of fluorescence, resulting from the displacement of the bound ethidium bromide from DNA by a competitive DNA binding agent. The percentage of fluorescence decrease is directly proportional to the extent of DNA binding and therefore can be used to determine binding affinities of various ligands relative to ethidium bromide. Boger \textit{et al.}, demonstrated that this technique can be used to determine the DNA binding affinities and sequence selectivity of both intercalative and non-intercalative DNA binders.\textsuperscript{34}

Fluorescence polarization/anisotropy measurements provide valuable information regarding the mobility, size, shape and flexibility of the molecules, and also on the fluidity and viscosity of the surroundings of the fluorescent molecules. In this technique, a fluorophore is excited using a polarized light and the polarized emission is then recorded at the same direction and at an orientation perpendicular to the polarized excitation. The binding of a ligand to nucleic acid can result in an enhancement in the fluorescence anisotropy due to restricted vibrational and rotational motions.\textsuperscript{36}

\textbf{1.4.2 Equilibrium Dialysis}

In a simple equilibrium dialysis assay, a known concentration of the nucleic acid is placed in one of the chambers and a ligand solution of known concentration is placed in the second chamber separated by a semi-permeable membrane. The membrane is chosen such that the ligand can pass through freely from one chamber to the other, but the nucleic acid is retained in one chamber. Finally, the ligand concentration in each chamber is measured using UV/vis absorption spectroscopy after equilibrium is reached. Müller and Crothers demonstrated the first use of the equilibrium dialysis method to determine the sequence specific binding of proflavin and acridine analogues.\textsuperscript{37} This method has been further extended to determine the preferential binding of small molecules to various nucleic acid conformations such as triple-stranded or quadruplex nucleic acids.\textsuperscript{38} Although this method is widely used to study the interaction of small molecules with nucleic acids, the technique has several disadvantages in terms of long equilibration time, requirement of large volume and particularly the adhesion of molecules to the membrane.
1.4.3 Viscosity Measurements

Binding of a ligand via intercalation leads to a significant increase in the length and viscosity of linear DNA fragments.\textsuperscript{39} Cohen and Eisenberg demonstrated that for an intercalative mode of binding the plot of the relative increase in the viscosity of DNA fragments \((\eta/\eta_0)^{1/3}\) varies proportionally to the binding ratio, \(r\) (where \(r = \text{moles of ligand bound per mole of DNA base pairs}\)), \(\eta_0\) and \(\eta\) represent the viscosity of DNA alone and in the presence of the ligand.\textsuperscript{40} Usually, for most of the classical intercalators the plot of \((\eta/\eta_0)^{1/3}\) vs. \(r\) gives a straight line with a slope between 0.5 and 1, while for the groove binders such as Hoechst 33258 a slope of zero is observed.\textsuperscript{41} In the case of plasmid DNA, initial binding of an intercalator results in an increase in the viscosity, which reaches a maximum at a certain concentration of the intercalator as a consequence of conversion from the negative supercoiled DNA to the fully relaxed form. Further increase in the concentration of intercalator results in a decrease in the viscosity due to introduction of positive supercoils.\textsuperscript{42}

1.4.4 Isothermal Calorimetric Titration (ITC)

Calorimetric measurements not only provide the direct measurement of binding affinity of a ligand but also give a measurement of the thermodynamic parameters such as total heat change (\(\Delta H\)) and total entropy change (\(\Delta S\)) associated with the process and stoichiometry of the interactions\textsuperscript{43} and are widely used to study nucleic acid-ligands interactions.\textsuperscript{44} In a typical ITC experiment, the total heat change of a reaction is measured as a function of the concentration of ligand added. Chaires and co-workers initially proposed the relationship between the thermodynamic profile of binding and the various noncovalent modes of interactions.\textsuperscript{45} In general an intercalation process is enthalpy driven, while the binding of small molecules to the DNA grooves is entropy driven process. Usually binding of charged molecules is entropically favoured due to the release of cations from the DNA structure regardless of their mode of binding.\textsuperscript{46} In recent times, ITC measurements have became an essential tool to complement spectroscopic data as these measurements can provide a direct measure of the binding affinity. However, proper care should be taken in selecting the buffer in order to avoid degradation and volatility of the buffer.

1.4.5 DNA Unwinding Assay

Binding of small molecules to closed circular supercoiled DNA via intercalation results in an unwinding of DNA, which consequently changes the electrophoretic mobility of DNA. The principle of the \textit{in situ} unwinding assay is based on the changes in electrophoretic
mobility of negatively supercoiled DNA in the presence of varying concentration of the ligand. Intercalation of ligands initially causes a decrease in the mobility caused by helical unwinding. However, as the concentration of the intercalating agent is increased, the mobility reaches a minimum value and then further addition of ligand causes an increase in the electrophoretic mobility due to introduction of positive supercoils. This assay can provide an estimate of the degree of unwinding per intercalated ligand molecule if the binding constant is known.\[^{47}\]

This method provides an accurate estimation of unwinding angle for low to moderate affinity ligands.\[^{48}\] However, for highly charged molecules and high affinity ligands this method is not very useful.\[^{49}\]

Keller developed a more accurate method to determine the unwinding angle based on the changes in the topological properties of closed circular DNA in the presence of DNA relaxing enzymes and intercalating agents.\[^{50}\] The topological properties of closed circular supercoiled DNA are described by the following equation,

\[
L_k = T_w + W_r
\]

(1.3)

where \(T_w\) is the twist about the helical axis and \(W_r\) represents the writhe or superhelicity of the helical axis through three dimensional space. Although \(T_w\) and \(W_r\) can vary with environmental conditions, the linking number \(L_k\) is a topological property that remains constant in the absence of any single stranded nick. Topoisomerase I enzymes can generate transient single stranded nicks and facilitate the removal of superhelical strain. In the presence of bound intercalators, supercoiled closed circular DNA becomes unwound (\(\Delta T_w < 0\)) so that \(W_r\) becomes less negative to keep the value of \(L_k\) constant. Treatment of the supercoiled DNA containing bound intercalators with topoisomerase I enzymes generates fully relaxed circular DNA, where \(W_r = 0\). When the intercalating agent is removed the change in \(T_w\) is compensated by the changes in \(W_r\) by the reappearance of superhelical turns to keep \(L_k\) unchanged. The extent of such induced superhelical turns is related to the amount of intercalators bound at the time of enzymatic religation. In practice, the thermal fluctuations at the time of ring closure results in a set of closed circular DNA samples differing in their linking numbers. Those topoisomers can be readily separated by gel electrophoresis. The difference in the linking number between the DNA samples topoisomerised in the presence of varying amount of intercalating agents can be determined by simply counting the number of DNA bands, which can be used to determine the degrees of unwinding per intercalating agent from the known ratio of [ligand]/[DNA] concentrations. This method has been successfully utilised for various intercalating agents including ethidium bromide, methylene blue, several ruthenium polypyridyl complexes etc.\[^{51}\] A modification of this assay was developed by
Crothers and co-workers involving two dimensional gel electrophoresis, which can be used to determine the association constant and the unwinding angle simultaneously for various intercalating agents.\textsuperscript{52}

1.4.6 NMR Spectroscopy

In recent years, Nuclear Magnetic Resonance (NMR) has emerged as an important tool to study nucleic acid-ligand interactions. This method allows structural determination of a molecule in solution and also enables one to study the noncovalent interactions. The combination of several two dimensional NMR techniques allows the study of nucleic acid-ligand interaction on the molecular scale. However, complete structural determination is time consuming and work intensive. Additionally for long oligonucleotides, the resonances can significantly overlap thereby making the determination of drug binding site and changes in nucleic acid conformations difficult. The assignment of the aromatic base protons can be done using one dimensional spectrum combined with Nuclear Overhauser Effect Spectroscopy (NOESY) and Correlation Spectroscopy (COSY).\textsuperscript{53} NOESY spectroscopy is a two dimensional NMR spectroscopy that can determine the through space interactions between the protons lying within 5Å distance, where the intensity of the NOE signal is proportion to \( r^6 \) (\( r \) is the distance between the coupled nuclei) and therefore can provide information regarding the spatial arrangements of atoms relative to one another. On the other hand COSY experiments can provide information about the nuclei coupled through covalent bonds. NMR spectroscopy can be used to monitor the binding of small molecules to nucleic acids. For example, covalent binding of Pt(II) centres to the N7 position of a guanine base in an oligonucleotide sequence results in a downfield shift in the resonance of the adjacent H8 proton from \textit{ca.} 8 ppm to \textit{ca.} 8.5-9.0 ppm depending on the solvent, temperature and salt concentration of the medium.\textsuperscript{54} Additionally for Pt(II) based drugs, formation of covalent adducts with DNA bases can also be monitored by \(^{195}\text{Pt} \) NMR spectroscopy. However, the main disadvantage of \(^{195}\text{Pt} \) NMR spectroscopy is the requirement of several thousands of scans to get a good signal to noise ratio.\textsuperscript{55} One dimensional \(^{31}\text{P} \) NMR spectroscopy can also provide useful information about binding of ligands, which bind \textit{via} intercalation or form covalent adducts with nucleic acids thereby causing significant changes in the torsion of the phosphate backbone.\textsuperscript{56}

1.4.7 X-ray Crystallography

X-ray crystallography enables the structural determination at the atomic level using a crystal of the nucleic acid-ligand complex and provides the direct visualisation of the binding
interactions, structure and function of the molecules. However the success of this method depends on the availability of suitable crystals. Usually the conditions for favourable crystal growth are optimised by varying multiple parameters such as i) metal ions and polyamines, ii) type and concentration of the ligands, iii) buffer, iv) pH, v) temperature vi) choice of precipitant etc. Recently Hall et al., demonstrated the high resolution crystal structure of the \([\text{Ru}(\text{TAP})_2\text{dppz}]^{2+}\) complex with the oligonucleotide \(d(TCGCGCCGA)\) in the presence of \(\text{Ba}^{2+}\) ion, where the dppz ligand binds via intercalation while the orthogonal TAP ligand binds to a second symmetrically equivalent duplex via a semi-intercalation mode resulting in noncovalent crosslinking of the adjacent duplex molecules and a \(51°\) kink in the structure. The overall curvature of the duplex DNA was similar to that observed for oligonucleotide bound to cisplatin and such distorted DNA structures are anticipated to modulate the DNA transcription process by interfering with the recognition of the DNA template by transcription machinery.

Although single crystal X-ray diffraction data provides the detailed structural information, however those structures are governed by crystal packing forces and therefore may or may not accurately represent the solution structure.

The techniques described represent some of the widely used tools to study nucleic acid-ligand interactions. A combination of several of these techniques can provide a comprehensive understanding of the mode of interaction of small molecules with nucleic acid.

The 1,8-naphthalimides belong to a class of intercalators, which showed high antitumour activity towards various human and murine cells. The development of potential anticancer agents based on the 1,8-naphthalimide structure will be elaborated in the following sections.

1.5 1,8-Naphthalimides as Anticancer Agents

The 1,8-naphthalimides constitute a class of DNA-binding agents developed initially by Braña and co-workers. Fabrication of these early naphthalimide derivatives were achieved by the incorporation of the structural elements from various known anticancer agents into a single structure for e.g. the \(\beta\)-nitronaphthalene of aristocholic acid, the basic side chain from tilorone and morpholine-\(\beta\)-thalidomide and the glutarimide unit of cycloheximide (Figure 1.8).
Two leading members of this family amonafide, 9, and mitonafide, 10, have entered into phase II clinical trials. Clinical studies showed that both 9 and 10 exhibit high antitumour activity with IC\textsubscript{50} values (the concentration of a drug required to inhibit viral replication by 50\%) of 0.47 \textmu M and 8.80 \textmu M, respectively, against HeLa cell lines.\textsuperscript{2,60} The dihydrochloride salt of 9 developed by ChemGenex Pharmaceuticals has successfully entered into phase II clinical trials for prostate cancer under the generic name of Quinamed\textsuperscript{®}.\textsuperscript{61} These compounds have also been found to stabilise double stranded DNA against heat denaturation.\textsuperscript{62} The naphthalimide derivative 9 has been found to induce DNA strand breaks and protein–DNA crosslinking in cultured mammalian cells\textsuperscript{53} and can inhibit nucleic acid synthesis at a concentration where protein synthesis is generally unaffected.\textsuperscript{60} The 3-nitro substituted derivative 10 can cause unwinding of closed circular DNA and increases the viscosity of sonicated DNA.\textsuperscript{64} Both 9 and 10 can inhibit topoisomerase II activity by interfering with the breakage-rejoining step of the enzymatic cycle and stabilise the enzyme-DNA cleavage complex.\textsuperscript{65}

Structure-activity studies have pointed out some crucial parameters, which influence the anticancer property of the naphthalimide series. The presence of a basic terminal group in
the side chain and the presence of two or three methylene units separating the terminal nitrogen of the side chain from the naphthalene ring play a key role in their anti-cancer activity. The valency of the terminal amino nitrogen also plays an important role in determining the anticancer activity. The 3-nitro substituted 1,8-naphthalimides have been found to exhibit better anti-tumour activity compared to the 4-nitro analogues. This is presumably because of better stacking interactions between the 3-nitro-1,8-naphthalimides and DNA, where the nitro group can assume a coplanar orientation with the imide ring. However, for the 4-nitro derivative, the angular orientation of the nitro group with respect to the imide plane destabilises the stacking interaction.

Zee-Cheng and Cheng reported the development of N-(dialkylaminoethyl)-derivatives of 3,6-dinitro and 3,6-diamino-1,8-naphthalimides as potential DNA binders. These derivatives showed high anticancer activity against leukemia with IC$_{50}$ values of 0.036 and 0.33 μM, respectively, and colon adenocarcinoma cell lines (IC$_{50}$ values of 0.041 and 0.68 μM respectively). These compounds also exhibited high anticancer activity in vivo against the P388 leukemia model. Subsequently, Braña and co-workers reported the development of a series of 3-amino-6-nitro-1,8-naphthalimide derivatives. These compounds exhibited very high cytotoxicity compared to amonafide and mitonafide against human CX-1 colon carcinoma and LX-1 lung carcinoma cell lines. However, the presence of alkyne substitution at the 3- and 4-position of the naphthalene ring was found to decrease the cytotoxic activity of these compound.

Azonafide, 11 represents another important member, where an anthracene moiety is introduced in place of the naphthalene ring. The derivative 11 showed significantly enhanced antiumour activity in vitro compared to 9. Among various derivatives of 11, basicity of the side chain nitrogen, length of side chain and size of the substituent on the anthracene moiety were found to be important in determining the anti tumour activity. It has also been shown that the 4-, 5-, 7- and 9-amino derivatives exhibited significantly higher potency than the unsubstituted compound 11 against leukemia cell lines. In order to achieve improved
affinity for DNA and increase the cytotoxic potential several bis-1,8-naphthalimide derivatives have been developed, which will be discussed in the following section.

1.6 Bis-1,8-naphthalimide Based Anticancer Agents

Bis-naphthalimides were initially developed by Braña and co-workers, where two 1,8-naphthalimide moieties are connected by a polyamine spacer to enhance the DNA binding and antitumour activity. Generally the nitro/amino substituted molecules exhibited better antitumour activity. However, the bis-naphthalimide, elinafide (LU79553), developed by Braña et al., lacks any aromatic substitution and exhibits high activity against a variety of human xenograft models such as LX-1 (lung), CX-1 (colon), LOX (melanoma). The bis-naphthalimide, 12 has been found to be a bisintercalator, which binds to DNA along the major groove and interacts with DNA in a sequence specific manner, exhibiting a preference for mixed purine-pyrimidine dinucleotide steps. Gallego et al., showed that two naphthalimide chromophores of 12 bisintercalate at TpG and CpA steps in the hexameric d(ATGCAT)₂ sequence.

Binding of 12 with DNA involves a two step interaction with dissociation rates of $10^{-2}$ s⁻¹ and 1-4 s⁻¹, respectively. The sequences flanking the tetra nucleotide binding site have been found to influence the overall binding, particularly the intercalation step. The interaction is strongly disfavoured in the presence of A-rich tracts at the 3’-end of the tetranucleotide motif due to poor stacking interaction between the naphthalimide-DNA and among the DNA basepairs. Chen and co-workers reported the development of another bis-naphthalimide DMP 840, 13, that exhibited potent antiproliferative activity against leukemia and various solid tumours in vitro. Mechanistic studies showed that 13 inhibits DNA and RNA biosynthesis by interfering with the incorporation of thymidine and uridine respectively and induced DNA single strand breaks. Moreover, 13 can also act as a eukaryotic Topoisomerase II
poison and stabilises the cleavage complex of topoisomerase II with DNA and causes cell death.\(^7\)

In spite of their potent cytotoxic activities, clinical evaluation of most of the naphthalimides is limited because of the associated adverse side effects such as central neurotoxicity. Clinical studies suggested that 9 is converted to \(N\)-acetyl amonafide by \(N\)-acetyltransferase 2 enzyme during metabolism in human.\(^8\) This metabolite can result in various unpredictable toxicities. Moreover, the differential extent of \(N\)-acetylation between individuals also causes obstruction in clinical development. Several strategies have been developed to modify the naphthalimide chromophore to improve its potency and lower the side effects. The next section describes the development of different structurally modified naphthalimides.

### 1.7 Structural Modifications to Improve the Antitumour Activity of the 1,8-Naphthalimides

#### 1.7.1 Modified 3-Substituted 1,8-Naphthalimide Derivatives

To improve the antitumour effects of amonafide 9, as well as to minimise its toxic side effects, Quaquebeke \etal., developed several amonafide analogues by incorporating amide, urea, imine, amine and thiourea functional groups at the 3-position of the naphthalene ring.\(^8\) Structure–activity relationship (SAR) studies showed that the urea derivatives exhibited improved anticancer activity both \textit{in vitro} and \textit{in vivo} compared to the amide, amine, imine and thiourea derivatives. Notably, compound 14 (UNBS3157) was found to have a ca. 4-fold higher maximum tolerated dose compared to 9 and did not induce hematotoxicity in mice models at a dose that causes significant antitumour effects. However, intercalation efficiency of compound 14 was found to be much weaker than 9. The antiproliferative effect exerted by 14 has been attributed to its ability to induce autophagy and senescence in cancer cells.
As a strategy to modify the 3-amino substituent of 9, a series of naphthalimides, 15a-g containing a phenyl moiety at the 3-position have been synthesised. These compounds bind to calf thymus DNA (ct-DNA) via intercalation and displayed high cytotoxicity against HeLa and P388D1 (murine lymphoid neoplasm) cell lines compared to 9, suggesting the importance of the phenyl ring in improving the activity of these derivatives.

1.7.2 Naphthalimide-Polyamine Conjugates

In order to achieve tumour cell specific entry of naphthalimide derivatives, several research groups developed naphthalimide-polyamine conjugates. Recently, Tian et al., reported the synthesis and cytotoxic activity of a series of 1,8-naphthalimide-polyamine conjugates. The presence of the triamine moiety and spermine/homospermine skeleton was found to be crucial for their anticancer activity. The derivative 16 bearing a spermine side chain was shown to cause caspase activation and induces apoptosis by lowering the expression of the Bel2 protein and releasing cytochrome c from mitochondria. Derivative 16 was also found to up-regulate the expression of polyamine oxidase and lead to accumulation of reactive oxygen species through the depletion of the reduced glutathione (GSH) pool. In a related study, Xie et al., demonstrated that the 3-nitro-naphthalimide-norspermine conjugate, (NPC-16) can be taken up by tumour cells via the active polyamine transporter (PAT). NPC-16 displays high cytotoxicity against Bel-7402 and HepG3 cells and induces apoptosis. However, the mechanism of apoptosis was different in the two cell lines. In the Bel7402 cell lines, NPC-16 induced caspase activation and apoptosis via the mitochondrial pathway, whereas in HepG2 cell lines, it induced formation of autophagosome and increased lysosomal activity followed by cell death.
Lin and co-workers have designed a series of bis-naphthalimide conjugates using spermine and spermidine as the linker. The bis-naphthalimide-spermidine conjugate 17 exhibits high cytotoxicity against the colon adenocarcinoma cell lines Caco-2 and HT29. The polyamine conjugate was found to induce apoptosis through DNA fragmentation, chromatin condensation and caspase activation. This study was further extended to synthesise bisoxynaphthalimidopolyamine 18, by incorporating oxygen atoms at the α position with respect to the naphthalimide ring. Thermal melting and agarose gel electrophoretic mobility assays suggested that 18 behaves as an intercalator. However, this was found to be less cytotoxic than 17. The reduced cytotoxicity of 18 is thought to be due to poor cellular uptake by the polyamine transporter due to presence of heteroatoms in the conjugates.

\[
\begin{align*}
19, 20a: & \quad R = n-C_8H_{17} \quad X = \begin{array}{c}
N \end{array} \\
19, 20b: & \quad R = n-C_{12}H_{25} \quad X = \begin{array}{c}
N \end{array} \\
19, 20c: & \quad R = n-C_8H_{17} \quad X = \begin{array}{c}
N \end{array} \\
19, 20d: & \quad R = n-C_{12}H_{25} \quad X = \begin{array}{c}
N \end{array}
\end{align*}
\]

To avoid the \textit{in vivo} acetylation of amonafide, Chen et al., developed a novel class of naphthalimide derivatives functionalized at the imide N-and the 4-position of naphthalene ring with polyamines and long alkyl chains 19a-d and 20a-d. These derivatives show moderately high affinity for ct-DNA (ca. \(10^5\) M\(^{-1}\)) and inhibit topoisomerase II activity. Linear and flexible polyamine conjugates (19c,d and 20c,d) displayed higher inhibitory activity. The majority of these derivatives show high antiproliferative activity compared to 9 against a variety of human cancer cell lines. These derivatives were found to induce lysosomal membrane permeabilisation, which releases several proteases (cathepsin) in the cytosol.

1.7.3 Naphthalimide-Amino Acid Conjugates

In order to overcome the poor aqueous solubility and to achieve enhanced cellular uptake, Qian and co-workers have developed naphthalimide derivatives conjugated to a leucine amino acid, 21-22. These were shown to possess moderately high affinity (ca. \(10^4\) M\(^{-1}\)) towards ct-DNA and bind to DNA via intercalation. These naphthalimide-leucine conjugates showed significant cytotoxicity against a wide range of tumour cells. Moreover, these molecules exhibited strong interaction with the bovine serum albumin protein (BSA),
which is the bovine analogue of the most abundant transport protein of blood plasma. Qian and co-workers have also developed naphthalimide derivatives based on 23-25 having chiral amino side chains at the imide position. Among these chiral intercalators, S-enantiomers showed higher affinity for DNA and greater photocleavage ability than the corresponding R-enantiomers.

1.7.4 Heterocyclic Fused Naphthalimide Derivatives

Braña et al., reported the synthesis of a series of mono naphthalimides, where the naphthalene ring was fused to a furan or thiophene ring, 26. The naphthalimide derivatives containing the furan ring oriented towards the outside of the naphthalimide moiety were found to be the most active. The dimerization of this moiety using a polyamine linker (MCI3335), 27, has been found to increase its affinity for DNA. Dimerisation of these furanonaphthalimides also enhanced the cytotoxicity more than 100 times compared to the corresponding mononaphthalimides against CEM leukemia cell lines.

Bailly et al., showed that the bis-naphthalimide 27 exhibits different sequence selectivity with a marked preference for GC steps compared to 12, which suggests that the furan ring plays a crucial role in determining the sequence selectivity. It has been suggested that the drug-DNA complex is stabilised by stacking and H-bonding interaction between the
furan ring and the amino group of guanine. Moreover, the H-bonding interactions between the protonated side chain of the ligand and O6 and N7 atoms of the guanine base in the major groove act as an anchor and maintain the stability of the drug-DNA complex.

Subsequent molecular modelling studies by Braña and co-workers on monomer 26 and d(TG)\textsubscript{2} suggested that in the most stable conformation, the furan ring is stacked between AC, while the side chain is located between O6 and N7 of guanine, stabilised by H-bonding in the major groove.\textsuperscript{91} Molecular modelling studies of 27 in the presence of d(ATGCAT)\textsubscript{2} revealed that in the most stable conformation, the naphthalimide chromophores adopt relative anti-parallel orientations and are located in the major groove.\textsuperscript{91} However, this group did not report any H-bonding interaction between furan-oxygen and amino group of guanine in the minor groove, which was postulated to be crucial for the GC selectivity by Bailly and co-workers.\textsuperscript{92} In contrast to the furanonaphthalimide, dimerisation of the imidazonaphthalimides using polyamine linkers did not improve their cytotoxic activity.\textsuperscript{93} Molecular modelling studies suggested that the linker length is not sufficiently long in these conjugates to form stable complex with DNA. However, the corresponding mono-imidazonaphthalimide, 28 showed enhanced cytotoxic activity compared to 9 against human colon carcinoma cell lines. This increased affinity towards DNA may be attributed to the presence of an additional heterocyclic ring that increases the stacking interaction. Subsequently Qian and co-workers
developed a series of “4-1” pentacyclic naphthalimides, where the naphthalimide ring is fused with an imidazole ring bearing an unfused aryl (29) or heteroaryl ring (30). These derivatives bind to DNA via intercalation and stabilised DNA against heat denaturation to a great extent.\(^4\)

Replacement of the imidazole moiety with a π-deficient pyrazine ring was found to increase the DNA binding and cytotoxic activity of the naphthalimide derivatives, 31 and 32 presumably due to enhanced stacking interaction with the DNA bases, which favoured intercalation.\(^5\) Additionally derivatives 31a and 32a were also found to inhibit topoisomerase I activity \textit{in vitro}. In contrast the bis-naphthalimide derivative, 32b bearing two trifluoromethyl groups did not exhibit any improved activity. This has been rationalised in terms of steric hindrance resulting form the presence of two trifluoromethyl groups interfering with the bis-intercalation. Monomer 31a and bis-naphthalimide 32a containing pyrazine moieties were suggested to bind along the minor groove of DNA and showed strong preference for GC rich DNA sequences.\(^6\) It should be mentioned here, none of the heterocyclic naphthalimide or bis naphthalimide derivatives was found to inhibit Topoisomerase II activity as observed for amonafide 9 and elinafide 12. The mechanisms for the high cytotoxicity of most of these compounds have not been reported.

1.7.5 Thio-heterocycle Fused Naphthalimide Derivatives:

Thiazole or polythiazole moiety has been exploited in the design of several photonucleases and anticancer antibiotics.\(^{1b,97}\) Qian and co-workers reported the synthesis of a series of photonucleases, 33a-d, combining a thiazole moiety with the intercalating 1,8-naphthalimide unit.\(^{98}\) These derivatives bind to \textit{ct}-DNA with a significantly high affinity (ca. \(10^5\) M\(^{-1}\)) presumably by intercalation. And showed high antitumour activities against A549 (human lung cancer cell) and P388 (murine leukemia cell). The cytotoxic potency of the derivatives was found to be highly dependent on the structure of the aminoalkyl side chain.
Compound \(33a\) (with IC\(_{50}\) values of 82.8 and 31 nM against A549 and P388 cell lines, respectively) and \(33c\) (with an IC\(_{50}\) 20.8 nM against A549 cell lines) showed about 6-fold and 50 fold higher antitumour activity respectively compared to \(9\) (IC\(_{50}\) 1100 and 200 nM against A549 and P388 cell lines respectively). The high cytotoxicity of these derivatives compared to \(9\) has been attributed to the presence of the methylthiazole ring. Moreover, these derivatives were also found to induce strand cleavage of closed circular pBR322 plasmid DNA under photoirradiated conditions (\(\lambda = 360 \text{ nm}\)) via the formation of superoxide anion generated through electron transfer from the naphthalimide chromophore to O\(_2\). The order of photocleavage activity was found to parallel their DNA binding ability \(33b > 33a > 33c > 33d\). However, \(33b\) exhibited the weakest cytotoxicity among all the derivatives.

Cytotoxicity of a molecule depends on both its DNA binding ability as well as its ability to penetrate the cell membrane. Depending on the basicity of the side chain N-atoms, derivatives \(33a-d\) will be protonated to different extents at physiological pH. A high degree of protonation of the side chain will favour DNA binding. However, cellular uptake of highly charged molecules will be less efficient. Therefore, a balance between these two factors determines the cytotoxicity. This balance is presumably lowest for derivative \(33b\) resulting in weak cytotoxicity.

Qian and co-workers have also reported the synthesis of the 1,8-naphthalimide derivatives \(34a-f\), containing a phenyl substituted thiazole moiety. All of these derivatives were found to induce strand cleavage under photoirradiated conditions with the order of photocleavage being \(34a\) (H) > \(34d\) (o-Cl) > \(34b\) (p- Me) > \(34f\) (m-NO\(_2\)) > \(34c\) (p-OMe) > \(34e\) (o-OH). It has been suggested that photoirradiation produces a naphthalimide-thiazole radical.
through the excited triplet state of the chromophore, which in turn causes DNA damage due to hydrogen abstraction. Semiemperical calculations indicated that in the triplet state the electron clouds are mainly localized on the thiazole ring and the electron density on the C-N is highest in compound 34a, which probably accounts for its higher activity. Li et al., reported the development of another new naphthalimide series, 35-36, containing the 2-aminothiazole moiety. These derivatives show moderate affinity towards ct-DNA (10^4 M^-1). The intercalating ability of 36, with its linear heterocyclic fused chromophore, was found to be higher than 35, with an angular chromophore. These compounds also exhibit photocleavage activity. Liang et al., demonstrated that 36 can induce expression of tumour suppressor gene p53 in Hela Cells and MCF7 (human breast cancer) cell lines. The gene product p53 can interact with the promoter region of Bcl2, an important regulator of apoptosis and can also down regulate the expression of Bcl2 and induces apoptosis in a caspase independent manner.

\[ \text{Qian et al., reported that an } N-[\beta-(N',N'-dimethyl amino) ethyl] \text{ dithiono-1,8-naphthalimide can cause DNA photocleavage more efficiently than its oxo counterpart.} \]

This led to the further development of a series of naphthalimides fused with a thioheterocyclic ring 37a-d. These molecules were found to absorb visible light and demonstrated efficient photocleavage of closed supercoiled pBR322 DNA after photoirradiation with visible light (\( \lambda = 450 \text{ nm} \)). The presence of \( N,N' \)-dimethyl aminoethyl or analogous group on the imide-\( N \) was found to be important for the photocleaving ability of 37a-c, while 37d did not exhibit significant photocleavage. The order of photocleavage activity of 37a-d parallels the order of their fluorescence quantum yield under physiological pH. Under such condition, the \( N,N' \)-dimethyl aminoethyl group, or analogues, exists in its protonated form, thereby inhibiting the intramolecular photoinduced electron transfer (PET) process. This results in an enhanced emission of these derivatives under physiological pH. The photocleavage reaction has been speculated to involve superoxide ion. These derivatives
were also found to degrade maize genomic DNA under photoirradiated conditions without impairing the activity of biologically significant enzymes such as trypsin suggesting their potential use in the removal of transgenic materials during biochemical preparation of proteins and enzymes.\textsuperscript{105}

These authors also reported the development of structural isomers 38a-d bearing a five membered thio-heterocyclic ring. These compounds exhibit very high affinity for ct-DNA (ca. $10^5$ M$^{-1}$). However, the six membered heterocyclic isomers were found to have higher photocleavage activity than the five membered heterocyclic compounds. Derivative 38a displayed very high cytotoxicity against a number of human cancer lines compared to 9. Compound 38a can bind to the ATPase domain of topoisomerase II and function as a topoisomerase poison. It can also trigger cell cycle arrest and apoptosis by inducing double strand DNA break by stabilising the Topo II-DNA cleavage complex.\textsuperscript{106} Based on the structure of 38a, several non-ring fused naphthalimide derivatives, 39a-d have been developed containing sulphur substitution at the 4-position of the naphthalene ring.\textsuperscript{107} These compounds were taken up into human mammary cancer cell lines and showed efficient photocleavage of pBR322 closed supercoiled DNA. The antiproliferative activity of these compounds has been assigned to their ability to inhibit the expression of Topoisomerase II.

Xu et al., reported the development of novel N-aryloxythioxo-naphthalimide based photonucleases 40a-c.\textsuperscript{108} Semi-empirical calculations suggested that the photocleaving activities of these derivatives are correlated with the electron density on the N-O bond in the triplet state. For the $m$-dichloro analogue 40a, the electron cloud density on the N-O bond is very low resulting in the easy cleavage of the N-O bond and generation of an aryloyloxyl radical upon photoirradiation, which in turn induces DNA cleavage.
1.7.6 Naphthalimide Derivatives Conjugated with Triazol

Qian and co-workers also developed a series of 1,8-naphthalimide derivatives incorporating a triazol moiety at the 3- and 4-position of the naphthalene ring (41a-e and 42a-e). These derivatives possess high affinity towards ct-DNA (ca. $10^5$ M$^{-1}$) and exhibit high cytotoxicity against a variety of human cancer cell lines compared to amonafide 9. The enhanced cytotoxicity has been attributed to the presence of the triazol ring. Moreover, the presence of a basic side chain was found to be crucial for the cytotoxic activity. All of the triazol-substituted derivatives were found to induce DNA cleavage under photoirradiated conditions. In order to elucidate the mechanism of photonuclease activity, a detailed understanding of the excited state behaviour of the naphthalimide derivatives is necessary, which will be described in Section 1.7.

1.7.7 Naphthalimide Based Dyad Systems

Recently, Kamal and co-workers have reported the synthesis and cytotoxicity study of a series of hybrid molecules, where the naphthalimide unit was conjugated with pyrrolo[2,1-c][1,4]benzodiazepines 43a-e through a pyrazine moiety with an alkane spacer. Among this series, the hybrid molecules 43b-c, having three or four ethylene units, between the pyrolobenzodiazepine and piperazine ring showed the highest DNA binding affinity and substantial increase in cytotoxicity.

![Diagram of 43a-e](image)

1.7.8 Naphthalimide Derived Prodrugs

To reduce the toxicity of naphthalimide derivatives and improve their therapeutic index, several naphthalimide based prodrugs (44-45) have been synthesised by incorporating the tertiary amine N-oxide moiety in the side chain. Derivatives 44 and 45 showed much lower affinity towards ds-DNA compared to the corresponding amine. This could be due to lack of the protonated side chain in the ligand, which favour electrostatic binding with DNA.
Importantly, the N-oxide derivatives exhibit less cytotoxicity in oxic A375 cell lines compared to the corresponding amine, while they are found to be highly cytotoxic in hypoxic tumour cell lines. This selectivity arises from the bioreduction of the N-oxides to the corresponding amines by the CYP3A isozyme of NADPH:cytochrome C (P-450). The resulting amines can therefore bind to DNA and inhibit topoisomerase function. Recently, Yin et al., demonstrated that these N-oxides of naphthalimides could also be used as fluorescent markers for hypoxic cells.\textsuperscript{112} Under physiological condition the fluorescence intensity of N-oxide derivatives is quite low due to aggregation and H-bonding interactions with polar water molecules. In contrast, the corresponding amines show higher fluorescence quantum yields. Therefore, obvious differential fluorescence response can be observed from hypoxic and oxic cells due to preferential reduction of the N-oxides under hypoxic conditions.

Photo-Fenton reagents based on the 1,8-naphthalimide-hydroperoxide derivatives 46-47 have been designed by Tao et al.\textsuperscript{113} These molecules were demonstrated to intercalate into DNA and generate OH\textsuperscript{-} radicals upon photoactivation, which in turn causes DNA cleavage.

1.7.9 Other Modified Naphthalimides

A nitrogen mustard derivative has been developed by combing the naphthalimide unit with the N,N\texttextsuperscript{-}bis(2-chloroethyl)amino group, 48a which showed high cytotoxicity against murine carcinoma cell lines.\textsuperscript{114} Recent studies on 3-nitro-1,8-naphthalimide conjugated with nitrogen mustard 48b showed significantly high antitumour activity and low systemic toxicity against hepatocellular carcinoma compared to 9.\textsuperscript{115}
Recent studies demonstrated that the naphthalimide-benzoic acid conjugates, 49a-b, exhibit cytotoxic activity comparable to that of 9 in a variety of cancer cell lines. These compounds were found to induce cell cycle arrest at the G2/M boundary and trigger apoptosis.

In recent times, several research groups have coupled the intercalating 1,8-naphthalimide moieties with peptide or oligonucleotide sequences to construct potential candidates for photodynamic therapy. These examples are illustrated in the following section.

1.7.10 1,8-Naphthalimide-Oligonucleotide/Peptide Nucleotide Conjugates

Peptide nucleic acids (PNA) have received much attention in recent time due to their high affinity and selective recognition of nucleic acid sequences, stability towards cellular nucleases, proteases and easy synthetic pathways using solid state peptide chemistry. Ikeda et al., reported the synthesis of PNA incorporating the 1,8-naphthalimide unit. The structure of naphthalimide containing PNA monomer is shown in structure 50. PNA oligomers containing a naphthalimide moiety at the amino terminus was found to be more stable against heat denaturation presumably due to an extended π-π interaction by the naphthalimide moieties. This study illustrates that the naphthalimide photosensitiser can be selectively incorporated at a predetermined site on PNA oligomers and represents potential candidates for photodynamic therapy.

Wamberg et al., reported the synthesis of a series of intercalating nucleic acids incorporating 1,8-naphthalimides 51a-b. Thermal melting studies demonstrated that the intercalators with short linker lengths (< five carbon atoms) were unable to stabilise DNA/DNA duplexes because the chain length is probably not optimal to position the intercalator for base stacking without disturbing the DNA backbone. Compound 51b with a five-carbon linker length has been shown to discriminate between DNA and RNA. It can
stabilise DNA/DNA duplexes while destabilising DNA/RNA duplexes. Intercalator 51b was found to be a sensitive reporter of nucleotide mismatch in an oligonucleotide sequence, showing a decrease in the melting temperature of the oligonucleotide up to 29°C in some cases, especially if the naphthalimide units were separated by two or four base pairs in-between.

Majima and co-workers have demonstrated the incorporation of 4-amino-1,8-naphthalimide and pyrene chromophore into oligonucleotide sequences to construct FRET donor-acceptor systems, which can be useful to study structure and conformational dynamics of nucleic acids. Incorporation of dye molecules did not interfere with duplex stability and the quantum yield of emission of the fluorophore. This group have also illustrated the use of oligonucleotides modified with naphthalimide photosensitiser to study charge transport in DNA.

In order to understand the photosensitising properties of naphthalimide derivatives, detailed understanding of the photophysical properties of these systems is necessary, which will be described in Section 1.8.

1.7.11 Naphthalimide-Metal Complex

Over the past couple of decades, transition metal complexes have received a great deal of interest for developing cancer chemotherapeutic agents. In order to achieve enhanced DNA binding and cytotoxicity Pérez et al., developed novel Pt-bis-naphthalimide complexes 52a-b, which overcame the cellular resistance to cisplatin, a common problem encountered in cisplatin based chemotherapeutics. The high cytotoxicity exerted by these derivatives results from the intercalation of the bis-naphthalimide unit combined with the platination of DNA bases. Development of gold (I) phosphine complexes bearing thio-naphthalimide ligands such as 53 was found to be an important strategy to enhance the antiproliferative activity of gold(I)-complexes. The gold(I)-complex 53 displayed significant growth inhibitory effects in MCF-7 breast cancer and HT-29 colon carcinoma cells. The presence of
thio-naphthalimide ligand also increased the cellular uptake and accumulation of gold in the nuclei of tumour cells compared to the naphthalimide free analogue. In a recent study, Chakravarty and co-workers reported development of the “3d-metal Scorpionates”-bearing 1,8-naphthalimide chromophore (54a-c), which exhibit moderately high affinity towards ct-DNA (ca.10^5 M^{-1}). Additionally, the Co(II) and Cu(II) complexes 54a and 54b were found to induce DNA cleavage and also exhibited significantly high cytotoxicity against HeLa cervical cancer cells upon UV irradiation.

Several Ru(II)-naphthalimide conjugates have been developed within the Gunnlaugsson research group as potential DNA binders and photocleaving agents. These will be described in Section 1.9.

1.8 Photophysical Properties of 1,8-Naphthalimide Derivatives

In addition to the wide range of biological activities, the 1,8-naphthalimide derivatives also possess various interesting photophysical properties. Wintgens et al., have shown that in the case of unsubstituted 1,8-naphthalimide derivatives, the lowest excited electronic state (S1) belongs to the \( \pi-\pi^* \) category (Figure 1.9). Due to the presence of an isoenergetic n-\( \pi^* \) triplet state (T2), these chromophores exhibit efficient intersystem crossing (ISC).
The reactivity of the excited triplet state of the 1,8-naphthalimides ($^3\text{NI}^*$) has been studied by several groups.\textsuperscript{127} Demeter et al., showed that the $^3\text{NI}^*$ undergoes photoreduction in the presence of aliphatic amines (AH$_2$) to form the contact ion pair (CIP) (Scheme 1.1; equation i).\textsuperscript{127a} In polar solvents such as acetonitrile, this CIP dissociates to form the solvent separated ion pair (SSIP) (equation ii) and subsequently free solvated ions (equation iii). However, in nonpolar solvents such as dichloromethane, the CIP can dissociate to form the naphthalimide ketyl radical (NIH) and the α-aminoalkyl radical (AH) (equation vi), which then decay by second order kinetics by reaction with self or cross radical species (equation vii). Step (vi) competes efficiently with the back electron transfer process (equation v).

\textbf{Scheme 1.1: Reaction of $^3\text{NI}^*$ with an aliphatic amine.}
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The excited state properties of the naphthalimides are largely influenced by the nature of the substituent on the naphthalene ring. The presence of an electron donating substituent at the 4-position induces a polar charge transfer (CT) excited state.\textsuperscript{128} Recently, Glusac and co-workers have reported the electronic properties of various 4-substituted 1,8-naphthalimide derivatives, 55a-e.\textsuperscript{129} Contrary to the previous reports, these workers have reported that the initial photoexcitation produces the nπ* excited state (S\textsubscript{2}) for all naphthalimide derivatives. However, this nπ* excited state is deactivated to S\textsubscript{1} by fast internal conversion. For derivatives, 55c-e, the S\textsubscript{1} state has polar CT character, while for 55a and 55b, S\textsubscript{1} state is ππ* in nature, which undergoes ISC to produce the triplet state.

Due to their excellent electron donor-acceptor properties, naphthalimide derivatives have been extensively used to construct artificial donor-acceptor electronic dyads in order to mimic the electron transfer processes in natural photosynthetic systems. For example Cho \textit{et al.}, demonstrated the photoinduced electron transfer processes in 1,8-naphthalimide-phenothiazine dyads and in bis-naphthalimides using transient absorption spectroscopy.\textsuperscript{130} In naphthalimide-phenothiazine dyads (56-58), photoexcitation produced a naphthalimide radical anion (NI\textsuperscript{-}) and phenothiazine radical cation (PTZ\textsuperscript{+}) through electron transfer from PTZ to the 1NI* as well as the 3NI* in acetonitrile medium.\textsuperscript{130b} In the NI-C11-PTZ (56) dyad, the long linker results in formation of a long-lived charge separated state due to less efficient coupling between the donor and the acceptor. The presence of a poly-ether linker between the donor and the acceptor in dyad 58 was found to enhance the charge transfer and charge recombination process through a superexchange mechanism. With the bis-naphthalimide dyads, photoexcitation of the naphthalimide chromophore generated NI\textsuperscript{-} through
intramolecular quenching of $^{3}\text{N}^\text{I}^*\text{-L-NI}$ ($L =$ linker). The rate of charge recombination was found to decrease with increasing the linker length from $\text{C}_3\text{H}_6$ to $\text{C}_8\text{H}_{16}$.$^{130a}$

In order to achieve long-lived charge separated state, recently Glusac and co-workers described the interaction between 4-thiomethyl-1,8-naphthalimide $55e$ and 2-cyano-4-nitro pyridine.$^{131}$ Photoirradiation of $55e$ in the presence of pyridine produces the naphthalimide radical cation ($\text{NI}^+$) and pyridine radical anion ($\text{PYR}^-$). The initially formed $\text{PYR}^-$ can abstract a proton from $\text{NI}^+$ to form neutral radicals as demonstrated in Scheme 1.2. As a consequence, the charge recombination process is slowed down between the neutral radicals resulting in long-lived excited states.

![Scheme 1.2: Schematic representation of proton coupled electron transfer (PCET) between $55e$ and 2-cyano-4-nitro pyridine.](image)

Photoexcitation of various naphthalimide derivatives have been shown to induce sequence selective DNA strand cleavage.$^{132}$ The oxidative nature of the naphthalimide excited states was initially demonstrated by Saito and co-workers, where the 1,8-naphthalimide conjugated with $L$-lysine, $59a$, can cause photoinduced sequence selective cleavage at the 5'-G residue of 5'-GG sequences.$^{132a}$ The 5'G specific cleavage has been explained by the PET from the most oxidizable DNA base guanine, to the triplet excited state of the naphthalimide. The site of photocleavage was found to be influenced by the substitution pattern of the naphthalimide ring. In contrast to $59a$, the 3-nitro derivative $59c$, was shown to photocleave DNA preferentially at T residues.$^{133}$ The thymine specific cleavage is thought to be initiated by H-abstraction from the methyl group of thymine by the photoexcited $59c$. However, with the 4-nitro derivative $59b$, both 5'-GG and T specific cleavages were observed.
Kelly and co-workers have reported the interaction of mononucleotides and DNA with the cationic naphthalimide, 60, and its corresponding naphthalene diimide.\textsuperscript{33,132c,134} These derivatives showed a preference for binding to purine nucleotides even in the ground electronic state. Laser flash photolysis showed that in the presence of mononucleotides and DNA, the triplet state of 60 is quenched with concomitant growth of the imide radical anion (NI·).\textsuperscript{33} As DNA concentration increased, the fraction of DNA bound chromophore increased and the relative yield of the triplet excited state (\( ^3\text{NI}^* \)) was found to decrease. This has been explained in terms of increased singlet state quenching within the DNA bound imide complex (Path a, Scheme 1.3), which in turn decreased the efficiency of ISC.

\[
\text{NI} + \text{DNA} \rightleftharpoons K \text{NI...DNA}
\]

\textbf{Path a}

(i) \[ \text{[NI...DNA]} \xrightarrow{\text{hv}} [^1\text{NI}^*_\text{DNA}] \]

(ii) \[ [^1\text{NI}^*_\text{DNA}] \xrightarrow{\text{PET}} [^1\text{NI}^*_{\text{DNA}^+}] \]

(iii) \[ [^1\text{NI}^*_{\text{DNA}^+}] \xrightarrow{\text{Charge Recombination}} [\text{NI...DNA}] \]

\textbf{Path b}

(i) \[ \text{NI} \xrightarrow{\text{hv}} [^1\text{NI}^*_\text{DNA}] \]

(ii) \[ [^3\text{NI}^* + \text{DNA}] \xrightarrow{\text{ISC}} [^3\text{NI}^*_{\text{DNA}^+}] \]

(iii) \[ [^1\text{NI}^*_{\text{DNA}^+}] \xrightarrow{\text{ISC}} [^3\text{NI}^*_{\text{DNA}^+}] \]

(iv) \[ [^3\text{NI}^*_{\text{DNA}^+}] \xrightarrow{\text{PET}} [^3\text{NI}^*_{\text{DNA}^+}] \]

(v) \[ [^3\text{NI}^*_{\text{DNA}^+}] \xrightarrow{\text{PET}} [^3\text{NI}^*_{\text{DNA}^+}] \]

\textit{Scheme 1.3:} PET scheme from DNA bases to \(^1\text{NI}^*\) or \(^3\text{NI}^*\).
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Kelly and co-workers have shown that the ISC efficiency decreased from 0.71, for free 60, to 0.08, when 60 was fully bound to DNA. This nonzero ISC value indicated a finite amount of NI' was produced from the DNA bound chromophore in competition with efficient singlet state quenching (Path b, Scheme 1.3). The cationic naphthalimide 60 has also been shown to cause photocleavage of supercoiled DNA in the absence of oxygen. The radicals produced from the quenching of the triplet 60 by ground state chromophores are proposed to be effective for the cleavage.

Takada et al., have also investigated the interaction of cationic, anionic and neutral naphthalimides, 61a-c, with oligonucleotide sequences. Cationic naphthalimide 61a exhibited strong association with oligonucleotides. For 61a bound to the oligonucleotide, the one-electron-reduced form of the naphthalimide (NI') was not observed in nano-second transient absorption measurements. This suggested an occurrence of rapid charge transfer from the nucleobase to the singlet excited naphthalimide followed by rapid charge recombination in the singlet excited state (Path a, Scheme 1.3). However, the one electron reduced form was observed for the anionic naphthalimide 61c, which does not associate with negatively charged oligonucleotides (Path b, Scheme 1.3). Additionally, the charge separation efficiency was found to increase when the oligonucleotide contained sequential G's, as the oxidation potential of G has been shown to decrease by stacking interactions.

Majima and co-workers have investigated the photosensitised damage in DNA using various oligonucleotide sequences covalently attached to naphthalimide chromophores. Photoirradiation of naphthalimide promotes electron transfer from the adjacent nucleobase to \( \text{NI}^* \) generating \( \text{NI}^- \) and radical cation of nucleobase, also known as hole. Presence of a stretch of adenine bases between the naphthalimide sensitizer and guanine results in rapid hole transfer and efficient separation of \( \text{NI}^- \) and \( \text{G}^+ \). Lifetime of the charge-separated state was found to increase with number of intervening A-T base pairs. The resulting \( \text{NI}^- \) and \( \text{G}^+ \) can promote DNA damage by reacting with molecular \( \text{O}_2 \) and water respectively, before the charge recombination can take place.

1.9 Recent Advances within the Gunnlaugsson Group

Over the past decade, Gunnlaugsson et al., have been involved in the design and synthesis of various 1,8-naphthalimide derivatives capable of binding to DNA. A large number of mono and bis-naphthalimides have been developed so far, where the intercalating naphthalimide moiety is coupled with \( \alpha \)-amino acids or esters, such as glycine, L-alanine, L-lysine, leucine and phenylalanine or short peptides. Biological studies of these naphthalimide-
peptide conjugates, carried out at St. James Hospital in collaboration with Prof. Mark Lawler, indicated that these molecules showed significant cytotoxicity against K562 and HL 60 leukemia cell lines. Of these, the leucine and phenylalanine conjugates were found to be more potent than the glycine and alanine derivatives. Among the dipeptide derivatives, naphthalimides containing either leucine or Boc-lysine exhibited higher cytotoxicity. However, the lysine-naphthalimide conjugate without any Boc-protection showed a tenfold reduction in activity, presumably because of the failure of the charged molecules to cross the cell membrane. Various peptide-based bis-naphthalimides have also been developed within the Gunnlaugsson group. The first series involved the synthesis of the “Side on Side” derivatives such as 62, developed by Dr. Phelan and Dr. Blais. In this family, the two naphthalimide chromophores were coupled through either a 1,3 propane or a spermidine linkage. However, these derivatives showed significantly less activity compared to the parent mononaphthalimides. “Top to Tail” bis-naphthalimide derivatives were obtained by linking the 4-position of one naphthalimide chromophore to the imide position of the other. The second family of bis-naphthalimide derivatives were synthesised by Hussey by linking L-lysine naphthalimide conjugates in “Head to Head”, “Tail to Tail” (as in 63) and “Head to Tail” arrangements. Among these, the 4-nitro derivatives were found to be more potent than the 3-nitro and 3-amino derivatives. As an extension to bis-naphthalimide derivatives, Dr. Veale and Dr. Murphy developed a series of bis-naphthalimides linked by the Tröger’s base moiety. These compounds will be discussed in Section 1.10.

Within the Gunnlaugsson group, Ryan et al., developed ruthenium(II) polypyrindyl complexes conjugated with 1,8-naphthalimide derivatives using flexible and rigid linkers. The use of a rigid aromatic linker as in 64, was found to provide control over the relative
orientation of the two chromophores, placing the naphthalimide in close proximity to the metal complex. These complexes showed a significant hypochromism and red shift in $\lambda_{\text{max}}$ of the MLCT band in the presence of DNA, with binding constants $ca. 10^6 \text{ M}^{-1}$ in 10 mM phosphate buffer (pH 7.0). Moreover, these conjugates caused DNA cleavage upon photoirradiation in aerated solution ($\lambda_{\text{ex}} > 390 \text{ nm}$). The 4-aminonaphthalimide conjugate was found to be a more active photocleaving agent than the 4-nitro analogue, presumably due to the higher quantum yield of emission of the former. As a further extension to this work, Dr. Elmes designed Ru(II)-1,8-naphthalimide conjugates $65a-b$ incorporating $\pi$-deficient TAP ligands. These derivatives exhibit significant modulation in their photophysical properties upon binding to ss-DNA, with a binding constant $ca. 10^6 \text{ M}^{-1}$ in 10 mM phosphate buffer (pH 7.4). Confocal laser scanning microscopy indicated that these compounds localise in the cell nucleus in HeLa cells and also behave as efficient DNA cleaving agents under photoirradiated condition ($\lambda_{\text{ex}} > 390 \text{ nm}$).

1.10 Tröger's base

Tröger’s base (TB) refers to a methano-1,5-diazocine ring containing cleft like structure $66$. The compound is chiral with a $C_2$ axis of symmetry due to the presence of two stereogenic nitrogen centres. The Tröger’s base $66$ was originally synthesised in the year 1887 by Julius Tröger by the reaction of $p$-toluidine with formaldehyde in the presence of HCl. Spielman and co-workers first established the structure of the compound in 1935. The X-ray structure of $66$ reported by Wilcox et al., showed that the molecule has a rigid cleft like structure with the two aromatic rings nearly at...
right angle to each.\textsuperscript{144} The dihedral angle between the aromatic rings can vary form \textit{ca}. \textdegree{81-104} depending on the substituent present on the ring.

General synthesis of TB derivatives involves reaction of an aromatic amine with HCHO,\textsuperscript{145} or HCHO equivalents such as paraformaldehyde, or hexamethylenetetraamine\textsuperscript{146} or dimethoxymethane\textsuperscript{147} in the presence of an acid. Wagner and co-workers demonstrated that the mechanism for the formation of 66 involves aromatic electrophilic substitution and proceeds through multiple intermediates, which will be discussed in chapter 3.\textsuperscript{148}

\textbf{1.10.1 Enantiomeric Resolution}

Prelog and Wieland reported the successful resolution of (±)-66 using α-D-lactose as the enantioselective stationary phase.\textsuperscript{149} In fact, this was the first example of resolution of a chiral tertiary amine, where the chirality is solely due to nitrogen stereocentres. Their initial attempts to resolve (±)-66 using 10-camphorsulfonic acid as the resolving agent led to partial resolution due to acid promoted racemisation. The racemisation was postulated to occur through the iminium ion formation as shown in Scheme 1.4. Greenberg and co-workers have investigated the acid induced racemisation of (±)-66 by NMR and UV-vis spectroscopy.\textsuperscript{150} They have reported the presence of monocationic and dicationic forms of 66 with \textit{C}_{2} symmetry in dilute and concentrated acidic conditions respectively. However, an iminium ion was not detected in measurable amount for 66. In contrast, for the 13,13-dimethyl analogue, iminium ion was observed under concentrated acidic condition. This has been attributed to the additional stability of the tertiary iminium ion of dimethyl derivative compared to 66. Lenev et al., demonstrated that the presence of a methyl substituent at the ortho positions relative to the stereogenic N-atoms raises the energy barrier for racemisation.\textsuperscript{151} For the racemisation process the methylene group of the iminium intermediate needs to pass through the plane of the aromatic ring followed by ring closure on the opposite side. This process becomes sterically unfavorable in the presence of bulky ortho substituents, thereby increasing the energy barrier for the racemisation process.
For a long period of time, enantiomeric resolution of (±)-66 was believed not to be feasible by diastereomeric salt formation using chiral acids because of the fast racemisation of the partially resolved enantiomers under acidic condition. However, Wilen and co-workers reported the successful resolution of (±)-66 by formation of diastereomeric salts using (-)-1,1'-binaphthalene-2,2'-dihydrogen phosphate (67), from which (+)-66 was recovered in high enantiomeric purity. This finding suggested that the resolution was achieved by crystallisation induced asymmetric transformation (CIAT), where the (-)-enantiomer racemises and is eventually converted to (+)-66 in the diastereomeric salt. CIAT of Tröger’s base analogues can also be achieved in the presence of homochiral (S) or (R)-1-phenylethyl substitution in the molecule. Enantiomeric separation via formation of diastereomeric salts have also been achieved with the ethano Tröger’s base and naphthyl TB using di-p-toluoyl tartaric acid 68a. Recently, Periasamy and co-workers have reported the successful resolution of (±)-66 through the formation of diastereomeric aggregate formation using dibenzoyl-L-tartaric acid 68b. Resolution of a proflavine-TB analogue has been achieved by crystallisation in the presence of 68b. In recent years, enantiomer separation of various Tröger’s base derivatives have been achieved using polysaccharide based chiral stationary phase and commercially available chiral stationary phase for example, Whelk O1.
1.10.2 Absolute Configuration of the Tröger’s Base

The absolute configuration of 66 was initially assigned by comparing the chiroptical properties of 66 with an alkaloid argemonine, which has structural similarity with 66. By this method the configuration of (+)-66 was determined to be (S,S). Later, Mason and co-worker reassigned the configuration of (+)-66 to be (R,R), from the analysis of circular dichroism data. This assignments have been cited in the literature for a long period of time until Wilen et al., reported the single crystal X-ray diffraction data (XRD) of the diastereomeric salt (+)-66.(-)-67 and established that the absolute configuration of (+)-66 is (S,S) and that of (-)-66 is (R,R). This assignment has also been reconfirmed from the XRD data of (-)-66.(-)-68b by Periasamy and co-workers and also in accordance with the calculated and measured vibrational-circular dichroism spectra of 66. Subsequently absolute configuration of several TB-derivatives has been assigned by comparing their CD spectra with that of 66. However, this should be done with caution as the magnitude and sign of the cotton effect may change depending on the substituent present on the aromatic ring.

1.10.3 Application of the Tröger’s Base Analogues

The rigid “V”-shaped geometry and chirality of TB derivatives have been exploited in recent times in the field of supramolecular chemistry in the design of molecular torsion balances, water-soluble cyclophanes, receptors for cations, dicarboxylic acids, metal-mediated self assembly systems, molecular tweezer, optoelectronic devices as well as DNA binders. TB-analogues have also been used to develop catalysts, sensors for C60 and fluorescence imaging agents. Some of these examples will be discussed in the following subsections.

1.10.3.1 Molecular Torsion balance: Wilcox and co-workers have developed molecular torsional balances based on TB derivatives such as 69. The energy barrier between the open and folded conformations has been calculated using NMR spectroscopy to study the role of edge-to-face aromatic interactions, which play important roles in protein folding and various molecular recognition processes.
1.10.3.2 **Design of H-bonding receptors:** Based on the “V”-shaped structure of 66, several receptors have been designed for the recognition of cyclic ureas and adenine derivatives, carboxylic acids. For example, the thiophene based TB derivative 70, containing a terminal pyridyl amino group, has been designed as a receptor for dicarboxylic acids. Receptor 70 showed high selectivity towards malonic and phthalic acids with a 1:2 stoichiometry of binding.

1.10.3.3 **Optoelectronic devices:** In recent times TB-derivatives have been largely exploited to develop organic light emitting diodes (OLEDs). Their “V”-shaped structures often result in poor π-π stacking between the neighbouring molecules in solid phase, which commonly results in fluorescence quenching. For example, the bispyridinium derivative 71 shows very weak emission in solution, which has been explained in terms of various nonradiative processes such as intramolecular vibrations, enantiomerisation etc., operating in solution phase. However, in the solid state the molecules are loosely packed due to their shape, which reduces the extent of distance dependent intermolecular fluorescence quenching and results in enhanced emission in the solid state. The aggregation induced emission (AIE) properties of 71 has been used for the sensing of proteins such as BSA. In the protein-bound form, the nonradiative processes that are responsible for the weak emission of 71 in solution are suppressed resulting in a fluorescence “turn on” sensor for proteins. Sergeyev and
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Co-workers have also developed similar bichromophoric “donor-π-acceptor” systems incorporating p-nitrophenyl and benzothiazolium acceptors that display interesting nonlinear optical properties.\textsuperscript{176}

1.10.3.4 Tröger’s base derivatives as imaging agents: Recently Wu et al., developed a novel TB-derivative 72 that has been used for \textit{in vivo} imaging of lysosomes with high selectivity and sensitivity using two-photon fluorescence microscopy.\textsuperscript{172} In designing compound 72, two benzyl-dimethylaminomethyl moieties were combined using a Tröger’s base core to construct a donor-π-acceptor-π-donor scaffold suitable for two-photon microscopy. Moreover, the basic nature of dimethylaminomethyl functionality also found to facilitate the lysosomal uptake of 72.

\begin{center}
\includegraphics[width=0.5\textwidth]{72.png}
\end{center}

1.10.3.5 Tröger’s base derived DNA binders: In recent times, many Tröger’s base-containing small molecules have been designed as chiral probes for nucleic acids. The combination of a polyaromatic intercalating unit with the cleft like structure of a TB can result in important DNA probes because of their various possible mode of interactions. Additionally, introduction of the TB structure can result in a helical structure, which can be similar or opposite to the helicity of double stranded DNA and can therefore result in an enantioselective binding.

Yashima \textit{et al.}, described for the first time the preparation of such a TB derivative 73 by the acid-induced reaction of 5-amino-1,10-phenanthroline with HCHO.\textsuperscript{169a} Compound 73 can induce significant alteration in the secondary structure of DNA compared to the parent 1,10-
phenanthroline. Additionally the Cu(I) complex of 73 was found to be an efficient DNA cleaving agent. Demeunynck and co-workers have demonstrated the synthesis and DNA binding properties of the acridine substituted TB derivative 74. Resolution of 74 was achieved by the crystallisation of its dibenzoyl tartarate salt. UV/vis and CD titration studies indicated that the (-)-enantiomer of 74 binds selectively to *ct*-DNA. This was also evidenced from the ability of (-)-enantiomer to stabilise double stranded DNA against heat-induced denaturation to a greater extent compared to the (+)-enantiomer. Moreover, the (-)-7R, 17R enantiomer of 74 binds selectively to DNA sequences containing both A.T and G.C base pairs, while the (+)-7S, 17S enantiomer did not exhibit any such sequence selectivity. Compound 74 displayed a very weak signal in the electric linear dichroism (ELD) measurements compared to other known intercalators such as proflavin. Therefore any information about the mode of binding of 74 could not be conclusively obtained from ELD measurements. Moreover compound 74 also failed to promote DNA unwinding mediated by topoisomerase I or II, unlike other intercalators, and did not interfere with the methylation of N-7 of guanine residues by dimethylsulfate. These findings suggest that 74 presumably binds in the minor groove of DNA. The mode of binding of 74 has also been addressed by molecular modelling studies. Two possible modes have been considered as shown in Figure 1.10: i) One acridine ring intercalates between adjacent base pairs, while the second acridine binds to the minor or major groove; ii) both of the acridine rings bind to groove. The difficulty to assign the mode of interaction arises due to the symmetric structure of 74. To overcome this problem, Demeunynck and co-workers developed the asymmetric proflavine-phenanthroline-Tröger’s base, 75. This hybrid molecule was found to bind selectively to GTC.GAC rich DNA sequences. A combination of CD and ELD measurements showed that in the hybrid molecule, the proflavine moiety intercalates into DNA base pairs and the phenanthroline unit occupies the minor groove.

Figure: 1.10: Mode of binding of 74 with DNA: i) intercalation with minor groove binding; ii) binding to major groove.
Valik et al., reported the synthesis of bisdistamycin derivatives containing the TB-unit. Binding of these bisdistamycin analogues with ct-DNA and homopolymeric nucleic acids have been investigated by spectroscopic and biophysical techniques. These derivatives also bind to the minor groove of DNA in a manner similar to distamycin A. However, in contrast to distamycin A, these derivatives display significantly higher affinity towards GC rich sequences. Moreover, the \((R,R)\) enantiomer was found to display higher affinity towards ct-DNA.

Ru(II)-complexes have achieved much attention for a long time due to their interesting photophysical properties and potential applications in various fields. Ru(II) complex bearing bisphenanthroline TB-derivative 76 has been synthesised starting from enantiomerically pure \(\Delta/\Lambda\)-cis-[Ru(phen)\(_2\)py\(_2\)]\(^{2+}\) with (±)-73. Interaction of various diastereomers of 76 with ct-DNA has been investigated by various photophysical measurements, which showed the in the diastereomers with \(\Delta\)-Ru(II) center, phenanthroline chromophore is in a more protected environment compared to the \(\Lambda\)-(R/S)-diastereomers. Moreover, binding affinity of \(\Delta\)-S-76 was found to be much higher than \(\Lambda\)-S-76 and rac-Ru(phen)\(_3\)^{2+}.

1.10.4 TB-Derivatives Developed within the Gunnlaugsson Group

Bis-naphthalimides (77a-c) containing the Tröger’s base moiety for DNA targeting have been developed by Veale et al. The molecules were designed such that the terminal nitrogen atom in the side chain of all three bis-naphthalimides are protonated at physiological pH, thereby increasing their water solubility and favouring electrostatic interactions with the negatively charged phosphate backbone of DNA. These bis-naphthalimides were found to bind to ct-DNA with significantly high affinity (ca. \(10^6\) M\(^{-1}\)) and stabilise ct-DNA against thermal denaturation to a great extent (\(\Delta T_m > 15^\circ C\)). Biological activities of these molecules have been accessed by Dr. Frimannsson and suggested that the molecules are taken up readily by HL-60 cells (leukemia cell line) and localised within the nucleus. Compound 77a and
77b were found to have significantly high cytotoxicity (LD₅₀ 5.21 and 5.5 μM, respectively) compared to their 4-amino substituted mononaphthalimide precursors (27.7 and 80.9 μM, respectively).¹³⁸

As an extension to this work, Dr. Murphy developed the TB-derivatives 78a-e derived from 3-amino-1,8-naphthalimide.¹³⁹ These derivatives were found to have weaker fluorescence quantum yields of emission compared to their 4-substituted analogues 77a-c. Photophysical measurements showed that they bind to DNA with similar affinity and display dual modes of binding with one naphthalimide ring intercalated between the DNA basepairs while the second one binding to the groove. Furthermore, Dr. Murphy also developed several TB-derivatives incorporating various amino acids and peptide side chains as shown in compound 79. These compounds can be used as potential chiral solvatochromic probes.¹³⁹

Dr. Elmes has developed several 1,8-naphthalimide based TB derivatives conjugated with Ru(II)-polypyridyl complexes (80-81).¹⁴¹¹⁸³ These complexes were found to have weak affinity towards st-DNA compared to the Ru(II)-naphthalimide conjugate. Interestingly, both of these conjugates were readily taken up by cervical cancer cell lines and were found to cause membrane blebbing, which opens up the scope of using these complexes for imaging and therapeutic purposes.¹⁴¹¹⁸³
Chapter 1: Introduction

1.11 Conclusion

The interaction of small molecules with DNA has been a topic of intense research in recent times because binding of small molecules can interfere with replication and cause cell cycle arrest and cell death. This chapter gave a brief overview of the cell cycle, structure of DNA and various mechanisms by which small molecules can interact with DNA. In this context, 1,8-naphthalimide derivatives represent an important family of DNA binders, which mainly bind to DNA via intercalation and showed anticancer activity both in vitro and in vivo. However, the first series of naphthalimide derivatives showed associated side effects in animal models. Therefore, several modifications have been discussed in order to overcome these toxic effects. Many of these modified naphthalimide derivatives present potential candidates for imaging and phototherapeutic applications. Brief discussions on the photophysical properties of naphthalimide derivatives and their excited state reactivity have also been presented. Moreover, recent developments within the Gunnlaugsson group in designing naphthalimide based therapeutic and imaging agents have been presented. The use of the Tröger’s base moiety in developing bis-naphthalimides as DNA binders have also been discussed. Introduction of the TB-moiety was found to enhance the DNA binding affinities of the derivatives significantly.
1.12 Work Described in this Thesis

Building on previous work carried out in the Gunnlaugsson group, the aim of the work discussed in this thesis involves the photophysical studies of various naphthalimide derivatives with particular emphasis on the bis-naphthalimides containing the Tröger's base moiety such as (77a-c) developed initially by Veale et al.\textsuperscript{138} The excited state interaction of these bis-naphthalimides with DNA will be studied by transient spectroscopy to understand their mode of action in detail.

In Chapter 2, the synthesis and photophysical evaluations of a series of 4-amino substituted 1,8-naphthalimide derivatives 82-83 containing pyridinium side chains are discussed. The pyridinium side chain was incorporated to provide high water solubility and favour electrostatic interaction with DNA. Interactions of these derivatives with nucleotide-5'-monophosphate GMP and AMP, st-DNA and synthetic polynucleotides have been studied with various photophysical techniques. Derivative 84 was synthesized to investigate the effect of N,N'-dimethyl group on the photophysics and the DNA binding properties and compare with the 4-amino analogues 82 and 83.

\[ \text{82, 87: } R = \]
\[ \text{83, 84, 88: } R = \]

In Chapter 3, the synthesis of two novel TB-derivatives 87-88 is discussed. Both of the TB-derivatives have been resolved into enantiomers and the interaction of racemic, (+) and (−)-enantiomers with st-DNA and synthetic polynucleotides have been studied by various photophysical techniques.
In chapter 4, the development of a [Pt(II)terpyridyl] complex, 109, conjugated with a 1,8-naphthalimide ligand is discussed. The interaction of 109 with ss-DNA has been investigated using various spectroscopic techniques.

![Chemical structure of 109]

In chapter 5: The excited state properties of 4-amino-1,8-naphthalimide derivative 83 and the corresponding TB-derivative 87 have been investigated using picosecond-transient infrared and picosecond-transient absorption spectroscopy. Additionally, their interactions with GMP and AMP have also been studied using these ultrafast spectroscopic techniques.
Chapter 2

Photophysical and DNA Binding Studies of Novel 1,8-naphthalimide Derivatives
Chapter 2: Photophysical and DNA Binding Studies of Novel 1,8-naphthalimide Derivatives

2.1 Introduction

The development of small molecules capable of binding to DNA and exhibiting anticancer activities is currently an active area of research. In this context, 1,8-naphthalimide derivatives, originally developed by Braña and co-workers represent an important family of DNA binders that exhibit significant anti-tumour activities \textit{in vitro} and \textit{in vivo}. As described in Chapter 1, amonafide, 9, and mitonafide, 10 were found to be the most potent mononaphthalimide derivatives, which entered into phase II clinical trials. The naphthalimide derivative 9 was found to induce DNA strand breaks and protein–DNA crosslinking in cultured mammalian cells and can inhibit nucleic acid synthesis. The 3-nitro substituted derivative 10 can cause unwinding of closed circular DNA and increases the viscosity of sonicated DNA. Both 9 and 10 can also inhibit topoisomerase II activity by interfering with the breakage-rejoining step of the enzymatic cycle and stabilise the enzyme-DNA cleavage complex.

As mentioned in Chapter 1, in addition to their excellent DNA binding abilities, 1,8-naphthalimide derivatives display interesting photophysical properties, which are dependent on the nature, as well as the position, of any aryl substituents. Photo-excitation of various naphthalimide derivatives has been also shown to cause sequence selective DNA strand cleavage. For example, Saito et al. showed that the unsubstituted L-lysine derived 1,8-naphthalimide 59a and the 4-nitro analogue 59b can cause sequence selective cleavage at the 5'-G residue of 5'-GG sequence, upon UV-irradiation and treatment with hot piperidine. The 5'-G specific cleavage has been explained by the photoinduced electron transfer (PET) from the most oxidisable DNA base guanine, to the triplet excited state of naphthalimide. The site of photocleavage was found to be influenced by the substitution pattern of the naphthalimide ring. In contrast to 59a, the 3-nitro derivative 59c, was shown to photocleave DNA preferentially at the T residues, possibly, through H-abstraction from the methyl group of thymine by the photoexcited 59c. In a related study, Kelly and co-workers have reported the interaction of the cationic naphthalimide 60, functionalised with an \textit{N}-ethyl pyridinium group, with DNA and mononucleotides using steady-state and time-resolved
fluorescence spectroscopy and laser flash photolysis technique.\textsuperscript{33,132c,134,185} The cationic naphthalimide 60 has also been shown to cause photocleavage of supercoiled DNA in the absence of oxygen.\textsuperscript{132c} The radicals produced from the quenching of the triplet excited state of 60 by ground state chromophores are proposed to be effective for the cleavage.

In contrast to the unsubstituted and 3 and 4-nitro substituted naphthalimides, which possess high energy excited states absorbing in the short wavelength region, the 3- and 4-amino-1,8-naphthalimide derivatives absorb in the visible region ($\lambda_{\text{max}}$ ca. 450 nm) and emit at longer ($\lambda_{\text{max}}$ ca. 550 nm) wavelengths, with reasonably high quantum yields of emission in various organic and aqueous solvents.\textsuperscript{5} Their excited states are characterised by a “push–pull” internal charge transfer (ICT) character, arising from the presence of the electron-withdrawing imide and the electron donating amino moiety. This “push-pull” character becomes more efficient, when the electron donating group is in the 4-position of the naphthalene ring due to resonance stability. Due to their polarity sensitive emission properties and tunable redox properties, 4-amino-1,8-naphthalimide derivatives have been extensively explored for the development of sensors for biologically relevant ions,\textsuperscript{186} molecules,\textsuperscript{187} potential cellular imaging agents\textsuperscript{188} and DNA binding molecules.\textsuperscript{138}

The objective of the work described in this chapter will be the development and study of a series of solvatochromic fluorophores 82-84 based on the 4-amino-1,8-naphthalimide structure and to study the interaction of these ligands with DNA by following the changes in the photophysical properties of the naphthalimide derivatives in the presence of DNA. In the design of 82-84, it was anticipated that the incorporation of the pyridinium side chain would impart water solubility as well as favour electrostatic interaction with the negative phosphate backbone of DNA. Additionally, due to the presence of a quaternarized nitrogen atom on the side chain, the photophysical properties should be independent of the pH of the medium. Moreover, introduction of alkyl, especially methyl substituents have been shown to improve the binding affinity of intercalating metal complexes especially those containing the dppz ligand for DNA due to favourable hydrophobic and van der Waals interactions.\textsuperscript{189}
Compounds 83-84 were therefore designed to study the effect of methyl group substitution on the pyridinium side chain (as in 83) as well as on the 4-amino nitrogen atom (as in 84).

The overall aims of the work presented in this chapter were to evaluate the interactions of 82-84 with DNA using various spectroscopic techniques and also to evaluate their potential use as solvatochromic probes to monitor DNA binding. Firstly, the synthesis and characterisation of 82-84 will be described followed by a discussion on their photophysical properties. Finally, the interaction of these ligands with DNA will be studied using various spectroscopic techniques.

2.2 Design, Synthesis and Solid State Characterisation of 82-84

Compounds 82-84 were synthesised from commercially available starting materials. Detailed synthetic procedure and characterisation are provided in the experimental section (Chapter 6).

2.2.1 Synthesis and Solid State Structural Analysis of 82

Compound 82 was synthesised in three steps as shown in Scheme 2.1. The first step involved formation of 86, which was achieved through a condensation reaction between the commercially available 4-nitro-1,8-naphthalic anhydride and ethanolamine in dry ethanol under an inert atmosphere and reflux. Product 86 was isolated as a brown solid in 75% yield and fully characterised by $^1$H NMR, $^{13}$C NMR, HRMS, IR and elemental analysis. Compound 85 was then formed, as an orange solid in 90% yield by catalytic hydrogenation of 86 using 10% Pd/C in DMF at 3 atm H$_2$. Refluxing 85 in the presence of 4-toluene sulfonyl chloride in anhydrous pyridine, gave the desired product 82 as the tosylate salt. The crude product was dissolved in water, washed with CH$_2$Cl$_2$ and purified using silica flash chromatography using a mixture of CH$_3$CN:H$_2$O:NaCl (88:11:1). The product was then
precipitated as its PF$_6^-$ salt using NH$_4$PF$_6$ and converted to the chloride salt of 82 by the treatment with DOWEX-1 × 8–200 ion exchange resin in methanol. Compound 82 was obtained as an orange solid after removal of excess methanol under reduced pressure in 60% yield and was characterised by conventional $^1$H, $^{13}$C NMR, HRMS, IR techniques and elemental analysis.

Scheme 2.1: Synthetic pathway for 82.

The $^1$H NMR of 82 (600 MHz, DMSO-$d_6$)(Figure 2.1) showed that the aromatic region is composed of one triplet and three doublets, for the naphthalimide ring, a doublet and a triplet for pyridine ring and a singlet for the amino protons. Assignment of the NMR peaks was achieved by H-H COSY, HSQC and HMBC experiments. The resonances for H2 and H16 were found to be overlapped, giving a multiplet signal at 8.07 ppm. The methylene protons appeared as triplets at 4.94 and 4.57 ppm.

Figure 2.1: The $^1$H NMR spectrum of 82 (600 MHz, DMSO-$d_6$) as its Cl$^-$ salt.
Successful formation of 82 was further confirmed from mass spectrometry, where 82 displayed a peak at \( m/z = 319.1233 \) corresponding to the \( M^+ \) ion, \( (M^+ \cdot C_{19}H_{16}N_2O_3 \text{ requires } 319.1243) \).

Large orange, single crystals of 82 suitable for X-ray diffraction were obtained as the PF\(_6^-\) salt by slow evaporation of acetone. The X-ray diffraction analysis was carried out by Dr. Jonathan A. Kitchen, within the Gunnlaugsson group. The low temperature (112 K) structure of 82 is shown in Figure 2.2, where it can be seen that the pyridinium ring is oriented towards the top face of the naphthalimide ring as the ethylene linker adopts a gauche conformation, placing the pyridinium moiety over one of the oxygen atoms of the imide structure. This is probably a consequence of the electron deficient nature of the pyridinium ring and the electron rich imide portion of the naphthalimide ring. Indeed, Gao and Marcus showed that this ‘push–pull’ character places a partial positive charge on the 4-amino moiety and a partial negative charge at the imide site causing the charge to be distributed on the carbonyl portions of the imide, making the central nitrogen moiety electron deficient. This might explain the orientation of the pyridinium ring in Figure 2.2.

![Figure 2.2](image)

**Figure 2.2:** (a) The X-ray crystal structure of 82 as its PF\(_6^-\) salt with thermal ellipsoids shown at 50% probability. (b) The packing of 82 when viewed down the crystallographic a-axis, showing the \( \pi-\pi \) interactions between the stacked naphthalimide rings which adopt a ‘head-to-tail’ type orientation.

The packing interactions of 82 were found to be governed by N–H hydrogen, anion...\( \pi \) interactions and \( \pi...\pi \) stacking. H-bonding interactions between the amino proton on one molecule and the carbonyl oxygen atom on an adjacent molecule give rise to a 1D polymeric chain in the crystallographic \( a \)-direction \([\text{N(1)...O(1)} = 2.919(3) \, \text{Å} \text{ and } \angle(\text{N(1)–H(1X)–O(1)}) = 154^\circ]\). \( \pi...\pi \) stacking \([\text{centroid...centroid } = 3.595 \, \text{Å}] \) between naphthalimide moieties on an
adjacent chain links the chains in an anti-parallel (head-to-tail) fashion (as shown in Figure 2.2b). The PF$_6^-$ counter anion is also involved in H-bonding [$N(1)-F(5) = 3.304(3)$ Å and $<(N(1)-H(1Y)-F(5)) = 139^\circ$] and anion...π interactions (F...centroid = 3.241 Å) between a fluorine atom and the imide ring further extending the packing.

### 2.2.2 Synthesis and Solid State Structural Analysis of 83

Compound 83 was synthesised in three steps as shown in Scheme 2.2. The first step involved condensation of 4-nitro-1,8-naphthalic anhydride with 4-(2-aminoethyl)pyridine in anhydrous toluene in the presence of triethylamine to yield 90. After completion of the reaction, toluene was removed under reduced pressure and the crude residue was dissolved in CH$_2$Cl$_2$ and washed with saturated NaHCO$_3$ solution, followed by washing with water. Finally compound 90 was obtained in 47% yield as a brown solid after recrystallisation from hot ethanol. Compound 90 was fully characterised by conventional $^1$H, $^{13}$C NMR, HRMS, IR techniques and elemental analysis. Reduction of 90 was carried out using hydrazine in methanol in the presence of a catalytic amount of 10% Pd/C. After removal of excess methanol under reduced pressure, 89 was obtained as an yellow solid in 94% yield. The final compound 83 was obtained as an iodide salt by reacting 89 with excess methyl iodide in refluxing anhydrous acetone. The resulting precipitate was isolated by filtration, washed with CH$_2$Cl$_2$ and purified by silica flash chromatography using a mixture of CH$_3$CN:H$_2$O:NaCl (saturated) (88:11:1) as eluent. Finally compound 83 was converted to its PF$_6^-$ salt using an aqueous solution of NH$_4$PF$_6$ to conveniently remove the excess NaCl before being converted to the Cl$^-$ salt using amberlite IRA 400 (Cl$^-$) ion exchange resin in methanol. Excess methanol was removed under reduced pressure and the product was obtained as a bright yellow solid in 93% yield. Compound 83 was fully characterised by conventional $^1$H NMR, $^{13}$C NMR, HRMS, IR techniques and elemental analysis. The $^1$H NMR assignment of 83 is shown in Appendix II (Figure A2.1).

![Scheme 2.2: Synthetic pathway for 83.](image)
Small yellow plate shaped crystals of \(83\) as the chloride salt suitable for X-diffraction were grown by slow evaporation of a methanolic solution and analysed by Dr. Jonathan A. Kitchen. The low temperature (108 K) X-ray crystal structure shows that \(83\) crystallised in the triclinic space group P-1 (Figure 2.3a) and contained one molecule of \(83\) with two interstitial water molecules. Unlike that observed for \(82\), the ethylene linker between the naphthalimide and pyridinium moieties adopts an anti-conformation. The pyridinium ring is not co-planar with the naphthalimide mean plane, instead it is angled at ca. 35° out of the expected coplanar arrangement. This is possibly a direct consequence of the weak, non-classical hydrogen bonding interactions occurring between the four pyridinium protons and chloride counter anions or interstitial water molecules (Appendix II, Figure A2.2). Extensive \(\pi...\pi\) stacking interactions were observed between adjacent naphthalimide groups resulting in the head to tail arrangement of molecules (Figure 2.3b).

**Figure 2.3:** (a) The X-ray crystal structure of \(83\) as its Cl salt with thermal ellipsoids shown at 50% probability. (b) The packing of \(83\) showing the \(\pi...\pi\) interactions. (c) H-bonding network, where (......) shows classical NH or OH hydrogen bonding interactions and (........) denotes non-classical CH based hydrogen bonding.
Packing interactions are primarily dominated by an extensive hydrogen-bonding network involving the amino group and interstitial water protons as donors, and chloride, carbonyl oxygen atoms and water oxygen atoms as acceptors (Figure 2.3c). The complete list of H bonding parameters observed in the crystal structure of 83 is detailed in Table 2.1.

Table 2.1: Hydrogen bonds for 83 [Å and °].

<table>
<thead>
<tr>
<th>D-H...A</th>
<th>d(D-H)</th>
<th>d(H...A)</th>
<th>d(D...A)</th>
<th>&lt;(DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(1)-H(1X)...O(10)(^a)</td>
<td>0.85</td>
<td>2.05</td>
<td>2.882(4)</td>
<td>166.2</td>
</tr>
<tr>
<td>N(1)-H(1Y)...O(20)(^b)</td>
<td>0.85</td>
<td>2.17</td>
<td>2.978(4)</td>
<td>158.2</td>
</tr>
<tr>
<td>O(10)-H(10X)...O(1)(^c)</td>
<td>0.85</td>
<td>2.00</td>
<td>2.838(3)</td>
<td>170.0</td>
</tr>
<tr>
<td>O(10)-H(10Y)...Cl(1)</td>
<td>0.99</td>
<td>2.19</td>
<td>3.168(3)</td>
<td>166.9</td>
</tr>
<tr>
<td>O(20)-H(20X)...Cl(1)</td>
<td>0.83</td>
<td>2.33</td>
<td>3.150(3)</td>
<td>170.6</td>
</tr>
<tr>
<td>O(20)-H(20Y)...Cl(1)(^d)</td>
<td>0.97</td>
<td>2.21</td>
<td>3.167(3)</td>
<td>168.2</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms:
\(^a\) \(-x,-y,-z\)  \(^b\) \(x-1,y-1,z\)  \(^c\) \(x,y+1,z\)  \(^d\) \(-x+2,-y+2,-z+1\)

2.2.3 Synthesis and Solid State Structural Analysis of 84

Compound 84 was synthesised in a three step synthetic pathway as shown in Scheme 2.3. The first step involved the conversion of 4-bromo-1,8-naphthalic anhydride into the 4-N,N'-dimethyl analogue 92, using a modified literature procedure in the presence of CuSO\(_4\) in DMF in 78% yield as a yellow solid.\(^{192}\)

![Scheme 2.3: Synthetic pathway for 84.](image)

The next step involved the condensation of 92 with 4-(2-aminoethyl)pyridine in anhydrous toluene in the presence of triethylamine to yield 91. Excess toluene was removed under reduced pressure and the resulting solid was dissolved in CH\(_2\)Cl\(_2\) and washed with saturated NaHCO\(_3\) solution and followed by washing with water. Finally 91 was obtained as a bright yellow solid after recrystallisation from methanol in 56% yield. Compound 91 and 92
have been fully characterised by conventional NMR, HRMS and IR analysis. The final step of
the synthesis involved reaction of 91 with excess methyl iodide in refluxing acetone. The
product was isolated by suction filtration and purified by silica flash column chromatography
using a gradient of CH_2Cl_2:methanol (85:15) to give 84 as an orange solid in 40% yield and
finally converted to its Cl^- salt using IRA(400) Cl ion exchange resin. Compound 84 was
characterised by ^1H, ^13C NMR, HRMS, IR techniques and elemental analysis. The ^1H NMR
(600 MHz) of 84 in DMSO-d_6 is shown in Figure 2.4. The aromatic region is composed of
two doublet signals from the pyridinium ring, four doublets and one triplet from the
naphthalimide ring. The protons from the CH_3 group on pyridyl nitrogen appear as a singlet at
4.29 ppm and the CH_3 protons from N,N'-dimethyl group appear more upfield at 3.11 ppm
also as a singlet.

![The 'H NMR spectrum of 84 (600 MHz, DMSO-d_6) as its Cl^- salt.

Yellow coloured single crystals suitable for X-ray diffraction studies were obtained by
the slow evaporation of an ethanolic solution of 84 as the PF_6^- salt. X-ray diffraction analysis
was carried out by Dr. J A. Kitchen and the low temperature (100 K) crystal structure is
shown in Figure 2.5a. As is the case for 83 the ethylene linker in 84 adopts the anti
conformation. The pyridinium ring and the naphthalimide ring assume almost coplanar
orientation in the crystal structure. The NMe_2 group was found to be in a twisted
conformation and lies slightly out of plane of the naphthalene ring. The packing is governed
by strong anion...π interactions between the fluorine atom of the PF_6^- ion and the imide ring

Figure 2.4: The 'H NMR spectrum of 84 (600 MHz, DMSO-d_6) as its Cl^- salt.
(F3...centroid distance 2.875 Å) and π...π stacking between the naphthalene rings on adjacent molecules (centroid...centroid distance 3.521 Å) interactions resulting in head-to-tail organisation of the naphthalimide molecules (Figure 2.5b).

![Image](a) ![Image](b)

**Figure 2.5:** (a) The X-ray crystal structure of 84 as its PF₆⁻ salt with thermal ellipsoids shown at 50% probability. (b) The packing of 84 showing the π...π interaction and anion-π between the stacked naphthalimide rings which adopt a 'head-to-tail' type orientation.

Compounds 82-84 were converted to their Cl⁻ salt using IRA (400) Cl ion exchange resin for the photophysical and DNA binding studies described in the following sections.

### 2.3 Photophysical Studies of 82-84

The UV/vis absorption and fluorescence emission spectra of 82 (8.3 μM) in 10 mM phosphate buffer solution (pH 7.0) are shown in Figure 2.6a. In aqueous solution, 82 exhibits a broad absorption band 436 nm (ε = 13,200 M⁻¹ cm⁻¹), arising due to the intramolecular charge transfer (ICT) between the electron rich amino group at the 4-position of naphthalene ring and the electron deficient carbonyl group, which gives rise to a large excited state dipole moment (Figure 2.6b). Similar ICT bands have been previously reported for other naphthalimide derivatives bearing an electron donating group at the 4-position of the naphthalene ring. The ICT bands are characterised by (i) broad shapes, (ii) high molar extinction coefficients (typically ε in the range of 10,000-15,000 M⁻¹ cm⁻¹) and (iii) the sensitivity of the band towards solvent polarity. Higher energy π-π* transitions were also observed at ca. 250 nm (ε = 21,800 M⁻¹ cm⁻¹). Excitation of 82 at 436 nm resulted in a broad emission band centred at 552 nm, which has been observed for similar 4-amino-1,8-naphthalimides and
assigned to the ICT emission band.\textsuperscript{16} Compound \textit{82} shows good water solubility, and obeys the Beer–Lambert law in aqueous solution at concentrations up to 100 \( \mu \text{M} \) as expected if the molecule exists in its monomeric form under these conditions. However, in experiments carried out at higher concentrations (ca. 10 \( \text{mM} \)), it was observed that \textit{82} could undergo aggregation, which will be described in Chapter 5.

![Figure 2.6](image)

\textbf{Figure 2.6}: (a) Normalised UV/vis absorption spectrum, fluorescence excitation and emission spectra of \textit{82} (8.3 \( \mu \text{M} \)) in 10 mM phosphate buffer (pH 7.0); \( \lambda_{\text{ex}} = 435\text{nm} \); (b) Schematic representation showing the effect of ICT on the excited state of 4-amino-1,8-naphthalimide derivatives.

Compound \textit{83} also displayed a broad ICT absorption band centred at 435 nm (\( \varepsilon = \)13, 800 \( \text{M}^{-1} \text{cm}^{-1} \)) similar to \textit{82} and high energy \( \pi-\pi^* \) transitions ca. 250 nm (26,150 \( \text{M}^{-1} \text{cm}^{-1} \)). Excitation of \textit{83} at 435 nm resulted in broad emission band centred at 550 nm (Figure 2.7a). In contrast to \textit{82} and \textit{83}, the absorption band for the \( N,N' \)-dimethyl analogue \textit{84} was found to be shifted to a longer wavelength (\( \lambda_{\text{max}} = 450 \text{nm} \), \( \varepsilon = \)11, 290 \( \text{M}^{-1} \text{cm}^{-1} \) ) with a shoulder at ca. 400 nm, which was not observed for the 4-amino analogues (Figure 2.7b) and this feature was also absent in the UV/vis absorption spectrum of \textit{84} in relatively less polar solvents such as various alcohols. The shoulder centred around 400 nm observed in the absorption spectrum of \textit{84} was found to be present in a range of concentrations studied (2-100 \( \mu \text{M} \)) as shown in Figure 2.7c. It should be noted that \textit{84} was found to obey the Beer-Lambert law in this concentration range, however, \( ^1\text{H} \) NMR analysis of \textit{84} in \( \text{D}_2\text{O} \) (Figure 2.7d) showed that the protons from the naphthalene ring exhibited significant downfield shift on changing the concentration from 1 \( \text{mM} \) to 0.01 \( \text{mM} \), indicative of aggregation at higher concentration. However, the UV/vis spectrum of \textit{84} remained practically unchanged in this concentration range. Based on the \( ^1\text{H} \) NMR data (600 MHz, \( \text{D}_2\text{O} \)), the possibility of some aggregation could not be completely ruled out even at 10 \( \mu \text{M} \) concentration. A summary of the absorption
properties of these systems along with their respective molar extinction coefficients is given in Table 2.2. Excitation of 84 at 450 nm resulted in a broad emission band centred at 555 nm. The emission band was not affected by changing the excitation wavelength from 450 nm to 400 nm. The shoulder at 400 nm observed in the UV/vis absorption spectrum of 84 was not present in the excitation spectrum recorded in aqueous solution using $\lambda_{em} = 555$ nm, suggesting that the emission is arising from the monomeric species in solution. However, the intensity of emission of 84 was found to be much weaker compared to that seen for 82 and 83.

**Figure 2.7:** Normalised UV/vis absorption spectrum, fluorescence excitation and emission spectra of (a) 83 (7.1 \( \mu \)M) and (b) 84 (7.0 \( \mu \)M) in 10 mM phosphate buffer (pH 7.0), (c) Normalised UV/vis absorption spectrum of 84 at various concentrations in water, (d) \(^1\)H NMR spectrum of 84 in D\(_2\)O at various concentrations.

**Table 2.2:** Extinction coefficients of 82-84 in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>[( \varepsilon ) (M(^{-1})cm(^{-1})) $\pm$ 10%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICT</td>
<td>$\pi-\pi^*$</td>
</tr>
<tr>
<td>82</td>
<td>436 (13,200)</td>
<td>250 (21,800)</td>
</tr>
<tr>
<td>83</td>
<td>435 (13,800)</td>
<td>255 (26,150)</td>
</tr>
<tr>
<td>84</td>
<td>450 (11,290) 400 (shoulder)</td>
<td>255 (19,700)</td>
</tr>
</tbody>
</table>
2.3.1 Effect of Solvent Polarity on the Photophysical Properties of 82-84

To investigate the effect of varying solvent polarity on the absorption and emission spectra of 82-84, the spectra were recorded in different solvents of varying polarity. All measurements were carried out such that the naphthalimide solution had an optical density (O.D) of ca. 0.1 at the excitation wavelength, which was chosen as the $\lambda_{\text{max}}$ observed in the absorption spectrum of each ligand in the respective solvent. Some representative spectra for 82 and 84 are shown in Figure 2.8 a and b, respectively. The changes in the UV/vis spectra of 83 with increase in solvent polarity were similar to 82 and are shown in Appendix II (Figure A2.3). In all the solvents studied, 82 and 83 exhibited a broad absorption band, which showed a slight blue shift (ca. 8–9 nm) on moving from the less polar butanol to highly polar water. The absorption and emission data for 82-84 are summarised in Table 2.3. In the case of 84, the absorption maxima remained relatively unchanged between various alcohols but showed a ca. 23 nm red shift on changing solvent from ethanol to water. Additionally, in all alcoholic solvents, the absorption maxima of the dimethyl analogue 84 is blue shifted compared to 82 and 83, however, the absorption band for 84 is significantly red shifted in water relative to the other systems. The anomalous behaviour of 84 in all alcoholic solvents can be explained if the dimethylamino (NMe$_2$) moiety is twisted relative to the naphthalimide ring such that the amino nitrogen cannot participate in the ICT process as efficiently as in the 4-amino analogues. The solid-state structure of 84 also revealed that the NMe$_2$ group is slightly twisted relative to the naphthalene ring thereby supporting the spectroscopic data. The considerably red shifted absorption band in aqueous media suggests the possibility of aggregation in the case of 84, which is possibly a consequence of the presence of two additional hydrophobic methyl groups.

Figure 2.8: UV/vis absorption spectra of (a) 82 and (b) 84 in various solvents.
Table 2.3: Absorption and Emission data of 82-84 in different solvents.

<table>
<thead>
<tr>
<th></th>
<th>BuOH (nm)</th>
<th>i-PrOH (nm)</th>
<th>n-PrOH (nm)</th>
<th>EtOH (nm)</th>
<th>H$_2$O (nm)</th>
<th>BuOH (nm)</th>
<th>i-PrOH (nm)</th>
<th>n-PrOH (nm)</th>
<th>EtOH (nm)</th>
<th>H$_2$O (nm)</th>
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</tbody>
</table>

BuOH = Butanol, i-PrOH = Propan-2-ol, n-PrOH = Propan-1-ol, EtOH = Ethanol.

In contrast to the absorption spectra, an increase in the polarity of the media resulted in significantly large red shifts in the fluorescence band for all of the derivatives. For instance, changing solvent from the less polar butanol to highly polar water resulted in ca. 9 nm blue shift in the absorption spectra of 82, while the corresponding emission maxima showed ca. 22 nm red shift on moving from butanol to water. The representative spectra for 82 and 84 are shown in Figure 2.9a and b, respectively and the fluorescence maxima in each solvent are summarised in Table 2.3. These changes suggest that the excited state in these compounds has a more polar character than the ground state.

Figure 2.9: Steady-state fluorescence spectra of (a) 82 and (b) 84 in various solvents.

The quantum yields of emission ($\Phi_F$) of 82-84 have been measured in all the solvents using fluorescein in 0.1 N NaOH ($\Phi_F = 0.92$, $\lambda_{ex} = 436$ nm) as the reference and presented in Table 2.4. For all the derivatives, the fluorescence quantum yield ($\Phi_F$) was found to decrease with an increase in solvent polarity. Such decrease in fluorescence quantum yield and red shift in the emission maxima with increase in the solvent polarity is characteristic of an ICT excited state.

Additionally, dimethyl substitution on the 4-amino group was found to have significant effect on the fluorescence quantum yield. The fluorescence quantum yield of 84
decreased significantly in all the solvents studied compared to its 4-amino analogue 83. This drop was much more pronounced in aqueous media than in less polar butanol (cf. Table 2.4).

The fluorescence decay of 82-84 was measured in various solvents using time correlated single photon counting (TCSPC) technique and is summarised in Table 2.4. The decay profiles of 82 in various solvents are shown in Figure 2.10 a. Compounds 82 and 83 followed single exponential decay kinetics in all the solvents studied. However, the fluorescence lifetime was found to decrease with increasing solvent polarity. The dimethyl analogue 84 was found to have much shorter lifetimes compared to 82 and 83 in all the solvents studied. In less polar solvents such as butanol and propan-2-ol, 84 was found to follow biexponential decay kinetics with the major components having a lifetime of $\tau_1 = 0.28$ ns (93%) and 0.20 ns (90%) respectively and are shown in Figure 2.10b. The magnitude of the major component was below the pulse width of the excitation source (1.2 ns). Therefore, fluorescence decay measurements in highly polar solvents could not be carried out reliably due to instrumental limitations. The radiative and non-radiative rate constants of 82-84 in various solvents were calculated from the measured value of $\phi_F$ and $\tau_F$ using equation 2.1 and 2.2 respectively and are presented in Table 2.5.

$$k_r = \frac{\phi_F}{\tau_F}$$  \hspace{1cm} (2.1)

$$k_{nr} = \frac{1 - \phi_F}{\tau_F}$$  \hspace{1cm} (2.2)

Figure 2.10: (a) Fluorescence decay kinetics of 82 in different solvents, (b) Fluorescence decay of 84 in butanol. IRF represents the instrument response function.
Chapter 2: Photophysical and DNA Binding Studies of Novel 1,8-naphthalimide Derivatives

**Table 2.4:** Fluorescence Quantum Yield ($\phi_F$) and Fluorescence Lifetime ($\tau_F$) of 82-84 in various solvents ($\lambda_{ex} = 458$ nm for fluorescence lifetime measurements).

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
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<tbody>
<tr>
<td>BuOH</td>
<td>0.40</td>
<td>0.51</td>
<td>0.015</td>
</tr>
<tr>
<td>i-PrOH</td>
<td>0.29</td>
<td>0.50</td>
<td>0.013</td>
</tr>
<tr>
<td>n-PrOH</td>
<td>0.23</td>
<td>0.47</td>
<td>0.012</td>
</tr>
<tr>
<td>EtOH</td>
<td>0.16</td>
<td>0.37</td>
<td>0.007</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>0.08</td>
<td>0.11</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuOH</td>
<td>5.1</td>
<td>8.4</td>
<td>0.28</td>
</tr>
<tr>
<td>i-PrOH</td>
<td>3.9</td>
<td>8.2</td>
<td>0.20</td>
</tr>
<tr>
<td>n-PrOH</td>
<td>3.2</td>
<td>7.8</td>
<td>8.2</td>
</tr>
<tr>
<td>EtOH</td>
<td>2.6</td>
<td>7.3</td>
<td>-</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>2.2</td>
<td>3.1</td>
<td>-</td>
</tr>
</tbody>
</table>

a Fluorescence decay could not be measured.

**Table 2.5:** Radiative ($k_r$) and non-radiative decay constants ($k_m$) of 82-84 in various solvents.

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>BuOH</td>
<td>7.8</td>
<td>6.0</td>
<td>6.4</td>
</tr>
<tr>
<td>i-PrOH</td>
<td>7.4</td>
<td>6.1</td>
<td>6.5</td>
</tr>
<tr>
<td>n-PrOH</td>
<td>7.2</td>
<td>6.0</td>
<td>-</td>
</tr>
<tr>
<td>EtOH</td>
<td>6.1</td>
<td>5.1</td>
<td>-</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>3.6</td>
<td>3.6</td>
<td>-</td>
</tr>
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</table>

<table>
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<tr>
<th></th>
<th>82</th>
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<tbody>
<tr>
<td>BuOH</td>
<td>12</td>
<td>5.8</td>
<td>352</td>
</tr>
<tr>
<td>i-PrOH</td>
<td>18</td>
<td>6.1</td>
<td>494</td>
</tr>
<tr>
<td>n-PrOH</td>
<td>24</td>
<td>6.8</td>
<td>-</td>
</tr>
<tr>
<td>EtOH</td>
<td>32</td>
<td>8.6</td>
<td>-</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>40</td>
<td>29</td>
<td>-</td>
</tr>
</tbody>
</table>

a Fluorescence decay could not be measured.

As can be seen from Table 2.5, for both 82 and 83 the magnitude of the radiative decay constant ($k_r$) is not significantly altered with increase in polarity of the alcohol, while the non-radiative decay constant ($k_m$) increased substantially between butanol and ethanol. The increase in $k_m$ on moving towards more polar and H-bonded solvents accounts for the decrease in $\phi_F$. For 84, $k_r$ and $k_m$ could be calculated only for the relatively nonpolar solvents butanol and propan-2-ol using the major component of the decay. Comparison of 84 with the 4-amino analogues 82-83 revealed that the radiative decay constants ($k_r$) did not vary much with the dimethyl substitution and also remained relatively constant between the two alcoholic systems studied. However, the magnitude of the non-radiative decay constant ($k_m$) for 84 increased on moving from butanol to propan-2-ol. Additionally, in both butanol and propan-2-ol, the $k_m$ value was found to be two orders of magnitude higher for the dimethyl derivative 84 compared to the simple 4-amino analogues 82 and 83. The large difference in the $k_m$ values of 84 compared to 82-83 in the solvents studied here, possibly arises from the difference in the rate of internal molecular motions in the excited state such as rotation or twisting about the C–N bond that connects the dimethylamino group to the naphthalimide ring. In a related study, Saha et al., proposed that an increased rate inversion of the amino nitrogen can also influence the $k_m$ value in a series of 4-amino-1,8-naphthalimide and 4-amino nitrobenzoxadiazole derivatives. The increased value of $k_m$ in the system studied here might be caused by a similar mechanism. Glusac and co-workers reported that in the optimised excited state geometry of 4-N,N'-dimethylamino-1,8-naphthalimide, the 4-
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substituent is at a 90° angle relative to the naphthalene ring. Such twisted excited states are more stabilised in highly polar solvents. These authors also reported the shortening of fluorescence lifetime of 4-\(N,N'\)-dimethylamino-1,8-naphthalimide with increasing solvent polarity in a manner similar to that observed in the case of 84. It has been proposed that the rate of twisting process is enhanced with an increase in solvent polarity and consequently results in a decrease in the quantum yield of emission and lifetime of the excited state. A similar mechanism might be responsible for the observed photophysical behaviour of 84. However, the absence of dual emission in this case suggests that the twisted excited state is presumably non-fluorescent as observed previously for the 4-\(N,N'\)-dimethylaminophthalimide derivatives. Additionally, as discussed before, the possibility of a small extent of aggregation in aqueous medium could not be ruled out completely in the case of 84, which can also result in a very weak emission of 84 in aqueous media.

Since 82-84 exhibit significant solvatochromism and as their emission quantum yield is strongly dependent on solvent polarity, the binding of these derivatives to nucleotide monophosphates and DNA is expected to cause substantial changes in the photophysical properties of 82-84. This will be described in the following sections.

2.4 Interaction of 82-84 with Mononucleotides

The interactions of 82-84 with mononucleotides such as guanosine-5'-monophosphate (GMP) and adenosine-5'-monophosphate (AMP) were investigated using UV/vis absorption, steady-state and time resolved fluorescence spectroscopy, the results of which will be described in the following subsections.

2.4.1 Ground State Interaction of 82-84 with GMP

The interactions of 82-84 with GMP were investigated by adding small aliquots of the latter to a solution of the ligand in 10 mM phosphate buffer (pH 7.0). All the titrations were repeated three times to ensure reproducibility. The addition of GMP to the solution of 82 in 10 mM phosphate buffer (pH 7.0) resulted in a decrease in the long-wavelength absorption band at 436 nm and ca. 6 nm red shift in the \(\lambda_{\text{max}}\) (Figure 2.11a) with formation of a single isosbestic point at 470 nm. The absorption spectrum of 83 in the presence of GMP was affected in a similar manner (Figure A2.4a, Appendix II). These observations suggest the formation of spectroscopically distinct free and GMP bound naphthalimide species in solution. Similar behaviour has been previously observed for the unsubstituted naphthalimide 60 and has been attributed to naphthalimide–nucleotide stacking interactions. The changes
in the UV/vis absorption spectra of 84 in the presence of GMP were significantly different than those observed for 82 and 83. With increasing GMP concentration, the band centred at 450 nm showed a \textit{ca}. 8 nm red shift. However, the absorbance at 450 nm did not decrease in the presence of GMP (Figure 2.11b). Additionally, the absorbance at 400 nm corresponding to the shoulder decreased significantly and the shoulder feature disappeared at high GMP concentrations. However, the decrease in absorbance at 400 nm did not reach a plateau even at very high concentrations of GMP (50 mM). The decrease in absorbance at 400 nm in the presence of GMP is indicative of naphthalimide-GMP stacking interaction. However, lack of proper saturation in the binding isotherm suggests that 84 is interacting very weakly with GMP compared to the 4-amino analogues and therefore, complete binding could not be achieved, even at very high concentrations of GMP. Such a weak interaction of 84 with GMP possibly results from the presence of the twisted dimethylamino group, which can interfere with the binding of GMP to the planar surface of the naphthalimide ring. The relative changes in absorbance for the three derivatives 82-84 are shown in Figure 2.11c and the spectral changes are summarised in Table 2.6.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure2_11.png}
\caption{Changes in the absorption spectrum of (a) 82 (7.2 \mu{}M) and (b) 84 (6.8 \mu{}M) in the presence of increasing concentration of GMP (0-50 mM) in 10 mM phosphate buffer pH 7.0. (c) Plot of the relative changes in absorbance (A/A_0) vs. concentration of GMP.}
\end{figure}
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2.4.1.1 Determination of Binding constant for GMP from UV/vis Absorption: The ground state association constants for the interaction of 82-83 with GMP were evaluated from the changes in absorbance at 435 nm in the presence of GMP. The absorbance changes for 84 could not be fitted to any binding model due to lack of proper end point. Assuming a 1:1 complex between the naphthalimide (NI) derivative and GMP, the interaction can be represented by the following equilibrium: where, K is the equilibrium constant for the association of NI with GMP, and is defined by equation 2.3;

\[ \text{NI} + \text{GMP} \underset{K}{\overset{\text{NI:GMP}}{\rightleftharpoons}} \]

\[ \frac{[\text{NI:GMP}]}{[\text{NI}][\text{GMP}]} \]  \hspace{1cm} (2.3)

where, [NI], [GMP] and [NI:GMP] refer to the concentration of free naphthalimide, free GMP and naphthalimide-GMP complex in equilibrium, respectively. If \( \Delta A \) is the change in absorbance of naphthalimide at 435 nm in the presence of GMP, then \( \Delta A \) is proportional to the concentration of NI:GMP complex, where, \( \Delta A = [\text{NI:GMP}] (\varepsilon_f - \varepsilon_b) \), \( \varepsilon_f \) and \( \varepsilon_b \) are the molar extinction coefficients of free and bound naphthalimide, respectively. The association constant \( K \) can be determined from the Scatchard plot (equation 2.4),\(^{29} \) where, \( \Delta \varepsilon = (\varepsilon_f - \varepsilon_b) \). (The derivations of binding equations are shown in Appendix I).

\[ \frac{\Delta A}{[\text{NI}][\text{GMP}]} = K \Delta \varepsilon = \frac{K \Delta A}{[\text{NI}]} \]  \hspace{1cm} (2.4)

The binding constant (K) of 82 for the association with GMP was determined from the slope of the Scatchard plot and was found to be 117 (± 11) M\(^{-1} \) (Figure 2.12a). The association constant for 83 was found to be 100 (± 5) M\(^{-1} \), comparable to that obtained for 82. This suggests that the methyl group on the pyridyl nitrogen does not have any effect on the interaction of the naphthalimide derivatives with GMP. Binding data were also analysed using the non-linear Deranleau model (equation 2.5) assuming 1:1 binding stoichiometry,\(^{197} \) The fit of binding data to equation 2.5 for 82 is shown in Figure 2.12b.

\[ \frac{\Delta A}{[\text{NI}]} = \frac{K \Delta \varepsilon [\text{GMP}]}{1 + K[\text{GMP}]} \]  \hspace{1cm} (2.5)

71
The association constants for the binding of 82 and 83 to GMP obtained from the non-linear fit were found to be 135 ± 5 M⁻¹ and 125 ± 8 M⁻¹, respectively. These values are in good agreement with those obtained from the linear Scatchard plots. Additionally, the binding constants of 82 and 83 for GMP were found to be almost two times higher than the unsubstituted naphthalimide derivative 60. The increased affinity of 82-83 towards GMP can possibly be due to the presence of the electron donating 4-amino moiety, which results in a highly polar structure capable of π-π stacking with the mononucleotides.

Table 2.6: Summary of absorption parameters for the interaction of 82-84 with GMP.

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_max (Abs) (Free) (nm)</td>
<td>436</td>
<td>435</td>
<td>450</td>
</tr>
<tr>
<td>λ_max (Abs) (Bound) (nm)</td>
<td>442</td>
<td>442</td>
<td>458</td>
</tr>
<tr>
<td>Δλ (nm)</td>
<td>+6</td>
<td>+7</td>
<td>+8</td>
</tr>
<tr>
<td>% hypochromism</td>
<td>12</td>
<td>11</td>
<td>a, 20b</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>470</td>
<td>460</td>
<td>455 (not well defined)</td>
</tr>
<tr>
<td>K (M⁻¹)</td>
<td>Scatchard</td>
<td>117 ± 11</td>
<td>100 ± 5</td>
</tr>
<tr>
<td></td>
<td>Deranleau</td>
<td>135 ± 5</td>
<td>125 ± 8</td>
</tr>
</tbody>
</table>

a no decrease in absorbance at λ_max = 450 nm.

b hypochromism at 400 nm.

c data could not be fitted due to lack of end point.

2.4.2 Excited State Interaction of 82-84 with GMP

The excited state interaction of the naphthalimide derivatives with GMP was investigated using both steady-state and time-resolved fluorescence spectroscopy as described below.
2.4.2.1 Steady-State Emission Measurements with GMP: The excited state interaction of 82-84 with GMP was studied by monitoring the changes in the fluorescence intensity of the ligand in the presence of an increasing concentration of GMP until a plateau was observed. In 10 mM phosphate buffer (pH 7.0) solution, 82 showed a broad emission band centred at ca. 552 nm when excited at the isosbestic point at 470 nm. With addition of GMP, the fluorescence intensity of 82 was quenched significantly (65%) without any significant shift in the $\lambda_{\text{max}}$ (Figure 2.13). Compound 83 showed similar behaviour in the presence of GMP, where addition of GMP (up to 50 mM) caused ca. 75% quenching of the emission intensity of 83 when excited at the isosbestic point (i.e. 460 nm) accompanied by a ca. 2 nm blue shift in the peak position (Figure A2.4b Appendix II). The relative changes in the fluorescence intensity of both 82 and 83 are presented in the inset of Figure 2.13. Quenching of fluorescence of the unsubstituted naphthalimide 60 by GMP has been observed previously and has been attributed to photoinduced electron transfer (PET) from guanine to the photoexcited naphthalimide moiety. Therefore, it is likely that PET is also occurring from guanine to the excited 4-amino naphthalimide derivatives 82 and 83. Quenching of the emission intensity of 82-83 at a lower concentration of GMP occurs in a 1:1 complex. The residual fluorescence observed for both of the ligands at very high concentrations of GMP, where the ligands should be mostly bound can be explained by considering fluorescence emission from the N1:GMP complexes. At very high concentration of GMP, possibility of collisional quenching cannot be ruled out.

Figure 2.13: Changes in the steady-state emission spectra of 82 (7.2 $\mu$M) in the presence of increasing concentration of GMP (0–50 mM) ($\lambda_{\text{ex}} = 470$ nm) in 10 mM phosphate buffer (pH 7.0). Inset: plot of $I/I_0$ vs. concentration of GMP for 82 and 83.
In contrast to that observed for 82-83, the emission intensity of 84 was enhanced almost linearly with increasing GMP concentration (0-50 mM) and did not reach a plateau at high concentration of GMP (Figure 2.14). As already discussed in Section 2.3.1, 84 has a very low quantum yield of emission in aqueous media compared to the 4-amino analogues 82-83. It has been proposed that the presence of dialkyl group on the amino nitrogen enhances non-radiative deactivation of the excited singlet state due to internal motions such as rotation/twisting of C-N bonds and nitrogen inversion.\textsuperscript{128g,195} Stacking interactions of 84 with mononucleotides like GMP can provide some extent of a rigid environment, which reduces the extent of non-radiative deactivations operating in the aqueous solution and therefore can enhance the fluorescence quantum yield. The absence of any plateau in the binding isotherm indicates weak interaction between 84 and GMP, in agreement with the ground state absorption data.

![Figure 2.14: Changes in the steady-state emission spectra of 84 (6.8 μM) in the presence of increasing concentration of GMP (0–50 mM) (λ\textsubscript{ex} = 450 nm) in 10 mM phosphate buffer (pH 7.0). Inset: plot of I/I\textsubscript{o} vs. concentration of GMP for 84.](image)

2.4.2.2 Time-Resolved Emission Measurements with GMP: To understand the mechanism of quenching, fluorescence lifetime measurements of 82-83 were carried out in the presence of varying concentrations of GMP (0-60 mM). In the absence of GMP, 82 and 83 displayed monoexponential fluorescence decay in 10 mM phosphate buffer (pH 7.0) with fluorescence lifetimes of 2.2 ns and 3.1 ns, respectively (cf. Table 2.4, Section 2.3.1). Addition of a low concentration of GMP (ca. 5 mM) resulted in biexponential decay kinetics (Table 2.7) for both of the naphthalimides. A short-lived component of ca. (0.7 ± 0.1) ns was observed in the presence of GMP for both 82-83, and the relative contribution of this component increases
with an increase in the GMP concentration and remained almost constant at higher concentrations of GMP (>40 mM) (cf. Table 2.7). The short-lived component in both cases presumably arises from a (1:1) excited state complex between the naphthalimide derivative (NI) and GMP.

Table 2.7: Fluorescence lifetime of 82-83 at different concentrations of GMP. ($\lambda_{ex} = 458$ nm).

<table>
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<th>[GMP] mM</th>
<th>82</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\tau_1$ (ns) (%)*</td>
<td>$\tau_2$ (ns) (%)*</td>
</tr>
<tr>
<td>0</td>
<td>2.20 (100)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2.18 (84)</td>
<td>0.79 (16)</td>
</tr>
<tr>
<td>20</td>
<td>2.01 (64)</td>
<td>0.74 (36)</td>
</tr>
<tr>
<td>40</td>
<td>1.90 (45)</td>
<td>0.72 (55)</td>
</tr>
<tr>
<td>60</td>
<td>1.86 (34)</td>
<td>0.73 (66)</td>
</tr>
</tbody>
</table>

* the quantities within brackets represent the relative proportion of each kinetic component.

The lifetime for the (1:1) NI: GMP complex can be roughly estimated from the steady-state fluorescence. If the relative quantum yields of free NI and NI:GMP are proportional to the steady state fluorescence intensity of free NI and fully bound NI respectively, then the lifetime of NI:GMP complex can be estimated from equation 2.6:

$$\frac{I}{I_0} \propto \frac{\phi}{\phi_0} \propto \frac{\tau}{\tau_0}$$  \hspace{1cm} (2.6)

where, $I_0$, $\phi_0$ and $\tau_0$ represent the fluorescence intensity, quantum yield and singlet state lifetime of free NI, respectively, and $I$, $\phi$ and $\tau$ represent the steady state fluorescence intensity, quantum yield and lifetime of NI fully bound to GMP, respectively. For 82, the value of $\tau$ can be estimated as ca. 0.77 ns (where $I/I_0 = 0.35$ and $\tau_0 = 2.20$ ns), which is in good agreement with the experimentally observed value of ca. (0.7 ± 0.1) ns. Similarly, for 83, the value of $\tau$ estimated using equation 2.6 (where $I/I_0 = 0.25$ and $\tau_0 = 3.10$ ns) was found to be ca. 0.78 ns, which is in agreement with the observed value. Here, it should be mentioned that the values of the short-lived component were within the pulse duration of the Nano-LED excitation source (1.2 ns) and were derived by deconvoluting the instrument response. Therefore, the lifetime values are expected to be associated with relatively large errors. An accurate determination of this short component requires excitation with a picosecond pulse, which will be described in Chapter 5. Moreover, it should be noted that at the highest concentration used, the long-lived component still represents 34% and 47% of the emitting species for 82 and 83 respectively, even though UV/vis titrations suggested that the ligands
should be mostly bound at such high concentrations of GMP. A possible explanation could be that the long-lived species is $\text{NI}:(\text{GMP})_2^*$. A longer lifetime for $\text{NI}:(\text{GMP})_2^*$ would be similar to the behaviour recently reported for Pt(II)(meso-tetrakis(4-\text{N}-\text{methylpyridyl}))porphyrin (Por), where the Por(GMP)$_2^*$ has a lifetime approximately five times longer than the (1:1) Por(GMP)* complex.$^{198}$

The above study showed that 82-83 can form a ground state complex with GMP similar to the unsubstituted naphthalimide 60. The binding constants of 82 and 83 for the association with GMP were comparable. However, these values were much higher than that reported for the unsubstituted compound, suggesting that the presence of the 4-amino group makes the molecules more polar and more capable of being involved in stacking interactions. The dimethylamino analogue 84 interacts very weakly compared to 82-83. Fluorescence intensity of 82-83 was quenched in the presence of GMP presumably due to PET from GMP to the photoexcited chromophores. This was further supported by fluorescence lifetime measurements, which revealed that in the presence of GMP, a short-lived component was observed, which was assigned to the (1:1) NI:GMP complex. However, a long-lived component was observed at higher concentrations of GMP, where most of the ligands should be bound to GMP, which presumably represents the NI:(GMP)$_2$ complex. Scheme 2.4 below summarises the possible mode of interactions of 82-83 with GMP. The possibility of PET from guanine to the excited singlet state of naphthalimide was also investigated by transient absorption spectroscopy, which will be described in Chapter 5.

\[
\text{hv} \quad \text{NI} \quad + \quad \text{GMP} \quad \overset{\text{Fast}}{\longrightarrow} \quad \text{NI:GMP} \quad \overset{\text{Fast}}{\longrightarrow} \quad \text{GMP} \quad \overset{\text{Fast}}{\longrightarrow} \quad \text{NI:(GMP)$_2$}
\]

**Scheme 2.4:** Proposed scheme showing possible interactions of 82-83 with GMP.

The interaction of 82-84 with AMP was investigated in a manner similar to GMP using UV/vis absorption, steady-state and time-resolved fluorescence spectroscopy, which will be described in the following sections.
2.4.3 Ground State Interaction of 82-84 with AMP

The addition of AMP to a solution of 82 in 10 mM phosphate buffer (pH 7.0) resulted in a decrease in the absorbance of the ICT absorption band centred at 436 nm with a ca. 10 nm red shift in $\lambda_{\text{max}}$ (Figure 2.15a). An isosbestic point was observed at ca. 463 nm at all concentrations of AMP. These observations suggest that AMP also forms a ground state complex with 82 similar to that seen for GMP. Similar changes were observed in the absorption spectra of 83 in the presence of AMP and those are shown in Appendix II (Figure A2.5). The UV/vis absorption spectra of 84 in the presence of AMP were altered in a different manner than 82-83. The changes in the absorption spectra of 84 in the presence of AMP were similar to that observed with GMP (Figure 2.15b). The absorption band at 450 nm showed a ca. 10 nm red shift in the presence of AMP. However, the absorbance at 450 nm did not decrease significantly; while the absorbance at 400 nm (corresponding to the shoulder) decreased significantly in the presence of AMP and reached a plateau at 40 mM in contrast to GMP, where the decrease in absorbance did not level off at high GMP concentrations (60 mM).

![Figure 2.15: The UV/vis absorption spectra of (a) 82 (7.2 μM) and (b) 84 (7.9 μM) in the presence of increasing concentration of AMP (0-100 mM) in 10 mM phosphate buffer pH 7.0. (c) Plot of relative changes in absorbance ($A/A_0$) vs. concentration of AMP at the $\lambda_{\text{max}}$.](image-url)
The relative changes in absorbance for the three naphthalimide ligands in the presence of increasing concentration of AMP are shown in Figure 2.15c and the absorption parameters are summarised in Table 2.8.

2.4.3.1 Determination of Binding constant for AMP from UV/vis Absorption: The ground state association constants for the interaction of 82-84 with AMP were evaluated from the changes in absorbance in the presence of AMP using the Scatchard Plot (equation 2.4). Good linearity was observed for the association of 83 and 84 with AMP over the entire concentration range. Representative plots are shown in Appendix II (Figure A2.5). The binding constants for the interaction of 83 and 84 with AMP were found to be 100 (± 5) M⁻¹ and 70 (± 5) M⁻¹ respectively. However, for the interaction of 82 with AMP, an attempt to fit the data to the Scatchard plot showed deviation from linearity at higher nucleotide concentration, which possibly indicates that higher order complexes also exist under these conditions. Therefore, data points corresponding to higher and lower concentrations of AMP were fitted separately to the Scatchard equation (equation 2.4). Absorbance data were also fitted to the nonlinear model of Deranleau (equation 2.7) taking both 1:1 and 1:2 stoichiometry of interaction into account.¹⁹⁷

\[
\frac{\Delta A}{[NI]} = \frac{K_1\Delta \varepsilon_1[AMP] + K_1K_2\Delta \varepsilon_2[AMP]^2}{1 + K_1[AMP] + K_1K_2[AMP]^2}
\]  

(2.7)

K₁ and K₂ refer to the binding constants for the (1:1) and (1:2) complexes, respectively. Δε₁ and Δε₂ are the changes in molar extinction coefficients for (1:1) and (1:2) complex formation. The binding constants are shown in Table 2.8.

| Table 2.8: Summary of the absorption parameters for the interaction of 82-84 with AMP. |
|----------------------------------|---|---|---|
|                                | 82 | 83 | 84 |
| \(\lambda_{\text{max}}\) (Abs) (Free) (nm) | 436 | 435 | 450 |
| \(\lambda_{\text{max}}\) (Abs) (Bound) (nm) | 445 | 446 | 460 |
| \(\Delta \lambda\) (nm) | +10 | +11 | +10 |
| % hypochromism | 19 | 13 | -a, 27b |
| Isosbestic Point (nm) | 463 | 460 | 455 (not very clear) |
| K (M⁻¹) | Scatchard | \(K_1 = 138 \pm 10\) | 100 \± 5 | 70 \± 3 |
| | | \(K_2 = 37 \pm 5\) | | |
| | Deranleau | \(K_1 = 155 \pm 7\) | 125 \± 8 | 66 \± 4 |
| | | \(K_2 = 35 \pm 5\) | | |

a no decrease in absorbance at \(\lambda_{\text{max}} = 450\) nm.
b hypochromism at 400 nm.
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Comparison of the binding constants values (cf. Table 2.6 and 2.8) showed 82 has a slightly higher affinity towards AMP than GMP, while 83 did not exhibit any such preference. The dimethyl analogue 84 also appeared to have higher affinity for AMP though the absolute binding constants for GMP could not be estimated. It should be noted that the binding constants obtained for both the amino derivatives towards AMP and GMP were substantially higher than that reported for the unsubstituted compound 60 by Kelly and co-workers. However, the affinity of the dimethyl derivative 84 towards AMP and GMP was comparable to the unsubstituted compound suggesting that the 4-amino group is possibly involved in the interaction with mononucleotides. Additionally, the slightly twisted structure of the N,N'-dimethyl amino group in 84 might also affect the interaction with the mononucleotides.

2.4.4 Excited State Interaction of 82-84 with AMP

The interaction of 82-84 with AMP was investigated by both steady-state and time-resolved fluorescence spectroscopy described in the following sections.

2.4.4.1 Steady-State Emission Measurements with AMP: In contrast to what was observed for GMP, the addition of AMP to the solution of 82-84 resulted in significant enhancement in the emission intensity accompanied with a blue shift in $\lambda_{\text{max}}$. However, the extent of fluorescence enhancement and shift in the $\lambda_{\text{max}}$ were different for each of the naphthalimide derivatives. For 82, the fluorescence intensity was enhanced by a factor of ca. 2 accompanied by a ca. 8 nm blue shift in the $\lambda_{\text{max}}$ (Figure 2.16). In the case of 83, the fluorescence intensity initially decreased at low concentrations of AMP (0-0.9 mM) and then was enhanced by a

![Figure 2.16](image-url)
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factor of 1.5 with further addition of AMP (Figure 2.16 inset). The initial decrease in the fluorescence observed in the case of 83 can result from the stacking interaction with AMP. The fluorescence intensity for 84 increased by a factor of four in the presence of increasing concentrations of AMP (0-100 mM) (Figure 2.16 inset and Figure A2.6, Appendix II). However, the changes in fluorescence did not reach a plateau at higher concentration of AMP unlike that observed for the interaction of 82 and 83. This could be the consequence of the weaker affinity of 84 towards AMP as determined by the UV/vis absorption titration. Additionally, as shown in the inset of Figure 2.16, the changes in the fluorescence intensity of 84 in the presence of AMP followed a biphasic pattern indicating possible formation of 1:1 and 1:2 complex with AMP. The increase in relative quantum yield of emission of all the three derivatives in the presence of AMP can be attributed primarily to a decrease of the rate of non-radiative decay processes in the complex, where the ligand is less accessible to solvent molecules and also is in a relatively more rigid orientation. Similar changes in non-radiative decay processes have been reported for the interaction of various porphyrin and ethidium bromide derivatives with nucleobases and DNA. This enhancement effect was significantly greater for 84 than that observed for 82-83 despite of its weaker affinity towards AMP. The higher enhancement factor can be attributed mainly to the extremely low emission quantum yield of 84 in aqueous medium compared to the other two derivatives. Such an environment sensitive fluorescence “switch on” effect displayed by the 4-N,N'-dimethyl 1,8-naphthalimide has been demonstrated previously by Imperiali and co-workers to study protein-protein interactions.

2.4.4.2 Time-Resolved Emission Measurements with AMP: The increase in the quantum yields of emission of the naphthalimide derivatives in the presence of AMP were also supported by fluorescence lifetime measurements. Due to very weak quantum efficiency of emission of 84 in aqueous media, reliable lifetime measurements could not be obtained. In the presence of AMP, biexponential fluorescence decay was observed for both 82 and 83 (Table 2.9). The kinetic analysis was performed by fixing the value of the short-lived component at 2.2 and 3.1 ns for 82 and 83, respectively, which represents the fluorescence lifetime of the respective free ligand in aqueous media. With increase in the AMP concentration, the relative contribution of the long-lived component increases in both cases at the expense of the short-lived component (Figure 2.17), which presumably represents a (1:1) complex of the naphthalimide with AMP. The average lifetime calculation showed that when fully bound to AMP, the average fluorescence lifetime of 82 increased from 2.2 ns to 4.5 ns, which agrees well with an almost two fold increase in the relative quantum yield of emission of 82.
Similarly for 83 the average fluorescence lifetime increased from 3.1 ns to 5.7 ns in its AMP bound form, which is proportional to the increase in fluorescence quantum yield of 83 in the presence of AMP. As observed for both 82-83, in the presence of very high concentrations of AMP (100 mM), where most of the ligands are expected to be bound, the lifetime decay was still biexponential. This could be due to some (1:2) complex formation at higher AMP concentration.

![Figure 2.17: Fluorescence lifetime decay of 83 (8.4 μM) in the presence of varying concentration of AMP, (b) Plot of relative contribution of short-lived (τ1) and long-lived (τ2) component vs. AMP concentration for 82 and 83.](image)

<table>
<thead>
<tr>
<th>[AMP] mM</th>
<th>82</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>τ1 (ns) (%)*</td>
<td>τ2 (ns) (%)*</td>
</tr>
<tr>
<td>0</td>
<td>2.20 (100)</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>2.20 (43)</td>
<td>3.85 (57)</td>
</tr>
<tr>
<td>20</td>
<td>2.20 (35)</td>
<td>4.22 (65)</td>
</tr>
<tr>
<td>40</td>
<td>2.20 (24)</td>
<td>4.40 (76)</td>
</tr>
<tr>
<td>60</td>
<td>2.20 (19)</td>
<td>4.67 (81)</td>
</tr>
<tr>
<td>100</td>
<td>2.20 (13)</td>
<td>4.84 (87)</td>
</tr>
</tbody>
</table>

* the quantities within brackets represent the relative proportion of each kinetic component.

The photophysical study described above showed that the naphthalimide derivatives 82-84 form a ground state complex with AMP through π-π stacking interactions. UV/vis titrations showed the presence of a single isosbestic point over the entire concentrations of AMP in the case of all the three ligands studies suggesting formation of (1:1) complexes between the naphthalimide derivative and AMP. However, binding constant analysis suggested the possible existence of higher order complexes at higher concentrations of AMP in 10 mM buffer. The dimethylamino analogue 84 interacts very weakly compared to 82-83. This could
be a consequence of the slightly twisted conformation of the dimethyl amino ring relative to the plane of naphthalimide ring, which can interfere with the stacking of AMP. In the presence of AMP the fluorescence quantum yield and the fluorescence lifetime increased for all systems studied, indicating that the ligand was located in a relatively nonpolar and rigid environment, where the rate of non-radiative processes primarily responsible for the deactivation of the excited singlet state are reduced. Based on the ground state absorption and excited state measurements, the following scheme (Scheme 2.5) is proposed summarising the possible mode of interaction of 82-83 with AMP.

![Scheme 2.5: Proposed scheme showing possible interactions of NI with AMP.](image-url)

After investigating the interaction of 82-84 with mononucleotides, detailed investigations of the interactions between these ligands with double stranded DNA and synthetic polynucleotides were performed. These results will be described in the following section.

### 2.5 Interaction of 82-84 with Salmon-Testes (st)-DNA

The interaction of 82-84 with st-DNA was studied using UV/vis, fluorescence spectroscopy, circular and linear dichroism spectroscopy and thermal denaturation techniques, which will be presented and discussed in the following subsections.

#### 2.5.1 Ground State Interaction of 82-84 with st-DNA

The interaction of 82-84 with st-DNA was initially examined using UV/vis absorption spectroscopy. The titrations were carried out in a manner similar to that described for AMP and GMP by adding small aliquots of st-DNA to a solution of each ligand in 10 mM phosphate buffer (pH 7.0) until the changes in absorbance reached a plateau. All the titrations were repeated three times to ensure reproducibility. The overall changes in the UV/vis absorption spectrum of 82 is shown in Figure 2.18a. In the presence of st-DNA, a large
hypochromicity and a ca. 11 nm red shift was observed in the ICT absorption band, accompanied by a single isosbestic point at ca. 480 nm for all DNA/ligand (P/D) concentrations suggesting the presence of only two spectroscopically distinct species i.e. the DNA bound and the free ligand. In the case of 83, similar changes in the absorption spectra were observed (Figure A2.7, Appendix II). For 84, absorbance corresponding to the $\lambda_{\text{max}}$ (450 nm) decreased by 22% accompanied with a ca. 15 nm red shift in $\lambda_{\text{max}}$ (Figure 2.18b). However, the absorbance at 400 nm corresponding to the shoulder decreased by ca. 62%. An isosbestic point was observed at 480 nm at all P/D ratios similar to 82-83. Similar changes in the absorption spectra have been observed for various unsubstituted and substituted 1,8-naphthalimides, where the hypochromicity of the naphthalimide absorption band has been suggested due to intercalation of the planar naphthalimide ring between the stacked DNA bases. 64,66,185,201

![Figure 2.18](image)

**Figure 2.18:** Changes in the UV/vis absorption spectra of (a) 82 (7.2 $\mu$M), (b) 84 (7.8 $\mu$M) in the presence of increasing concentration of st-DNA (0-438 $\mu$M) in 10 mM phosphate buffer (pH 7.0). (c) The plot of $A/A_0$ vs. DNA nucleotide phosphate/ligand (P/D) for 82-84.
2.5.1.1 Determination of Binding Constant from UV/vis Titration: The determination of intrinsic binding constants for the association of 82-84 with st-DNA would allow the evaluation and comparison of their binding affinities under the same experimental conditions. In general, the intrinsic binding constant $K$ is defined as,

$$K = \frac{L_b}{L_f S_f}$$

(2.8)

where, $L_b$ is the concentration of the bound ligand, $L_f$ is the concentration of free ligand and $S_f$ is the free site concentration, which is usually taken as the DNA base concentration divided by the number of DNA bases required for the ligand binding site.\(^{202}\)

Binding constants for the association of 82-84 with st-DNA were determined by analysing the changes in absorbance at the respective $\lambda_{\text{max}}$ using the non-cooperative model of McGhee and von Hippel (equation 2.9), where $r = C_b/DNA$ and $n$ is the binding site size.\(^{202}\) Concentration of bound ligand ($C_b$) and concentration of free ligand $C_f$ can be calculated using equation 2.10 and 2.11 respectively, where, $A_r$ and $A_b$ are the absorbance values corresponding to free and completely bound ligand. $A$ is the absorbance of the solution at any point during titration and $C$ is the total concentration of the ligand.

$$\frac{r}{C_f} = K(1 - nr)\left(\frac{1 - nr}{1 - (n - 1)r}\right)^{n-1}$$

(2.9)

$$C_b = \frac{A_r - A}{A_f - A_b} C$$

(2.10)

$$C_f = C - C_b$$

(2.11)

According to the non-cooperative model of McGhee and von Hippel, the binding constant, $K$ can be determined using the Scatchard plot. Binding data were plotted in terms of $r$ vs. $r/C_f$ and fitted to the non-cooperative model of McGhee and von Hippel (equation 2.9) using Origin Pro8 to determine the binding constant ($K$) and the binding site size ($n$). The representative plots of $r$ vs. $r/C_f$ and the corresponding fit to the non-cooperative binding model of McGhee and von Hippel for 82 and 84 are shown in Figure 2.19 a and b, respectively and the binding parameters are summarised in Table 2.10. For 84, the changes at 400 nm were also analysed since, larger hypochromism was observed for this shoulder.
compared to the $\lambda_{\text{max}}$ and the binding constants obtained from the changes in absorbance at 450 nm and 400 nm were comparable. In 10 mM phosphate buffer solution the intrinsic binding constants for the association of 82-84 with st-DNA were found to be of the order of $10^5$ M$^{-1}$, which is comparable to the affinity of phenothiazine dyes for DNA.\textsuperscript{203} Notably this is an order of magnitude higher than that seen for the unsubstituted analogue 60, demonstrating once more the importance of the 4-amino moiety of 82-84.\textsuperscript{134,185b} Similar DNA binding affinities have recently been reported by Qian and co-workers for thio-heterocyclic rings fused with naphthalimides, where the enhanced binding affinity was attributed to the presence of an additional heterocyclic ring fused with the naphthalimide.\textsuperscript{99,104,107} The values of the binding site size "$n$" determined for 82-84 were comparable with the value expected for an intercalative mode of binding ($n = 2-2.5$).\textsuperscript{204}

![Figure 2.19: The plot of $r$ vs. $r/C_f$ (■) and the best fit (—) to the non-cooperative McGhee and von Hippel model for (a) 82 and (b) 84.](image)

**Table 2.10: Summary of binding parameters obtained from the absorption spectra of 82-84 in the presence of st-DNA in 10 mM phosphate buffer solution.**

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Free)</td>
<td>436</td>
<td>435</td>
<td>450</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Bound)</td>
<td>447</td>
<td>445</td>
<td>465</td>
</tr>
<tr>
<td>$\Delta\lambda$ (nm)</td>
<td>+11</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>44</td>
<td>44</td>
<td>22\textsuperscript{a}, 62\textsuperscript{b}</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>480</td>
<td>480</td>
<td>480</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>20</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>$K \times 10^5$ M$^{-1}$</td>
<td>$(1.85 \pm 0.05)$</td>
<td>$(1.38 \pm 0.05)$</td>
<td>$(1.49 \pm 0.10)$\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$(1.55 \pm 0.05)$\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$(3.05 \pm 0.25)$\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$(3.40 \pm 0.08)$\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Measured using absorbance at 450 nm.

\textsuperscript{b} Measured using absorbance at 400 nm.
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Interestingly, the presence of the dimethyl substitution on the 4-amino nitrogen did not appear to have any significant effect on the DNA binding affinity of 84, in contrast to that observed for the mononucleotides.

2.5.2 Excited State interaction of 82-84 with st-DNA

Fluorescence titrations were carried out in a similar manner by adding small aliquots of st-DNA to a solution of each ligand in 10 mM phosphate buffer (pH 7.0) and measuring the changes in fluorescence intensity of the ligand until the changes reached a plateau. Both the steady-state fluorescence and the lifetimes of the ligands were measured as a function of st-DNA concentration as described below.

2.5.2.1 Steady-State Emission Measurements with st-DNA: In the presence of st-DNA the fluorescence intensity of all the three naphthalimide derivatives 82-84 was enhanced concomitant with a blue shift in the $\lambda_{\max}$. However, the extent of the fluorescence enhancement and shift in $\lambda_{\max}$ were different for each naphthalimide-DNA pair (Table 2.11). Upon titration of 82 with st-DNA, the fluorescence intensity of the ICT emission band centred at 552 nm was almost doubled accompanied by a ca. 5 nm blue shift in the $\lambda_{\max}$. (Figure 2.20a) The changes in emission reached a plateau at a P/D ratio of 20. In contrast, for 83 the emission intensity of the ICT band ($\lambda_{\max} = 550$ nm) initially increased by a factor of 1.6, reaching a maximum at a P/D ratio ca.17 accompanied by a ca.11 nm blue shift in the $\lambda_{\max}$. Further increase in DNA concentration (P/D 20→55) resulted in a decrease of the emission intensity. This was associated with a further 3 nm blue shift in the $\lambda_{\max}$. The fluorescence enhancement was much more pronounced with 84, showing ca.16 fold increase in the emission intensity, when fully bound to st-DNA.

| Table 2.11: Summary of fluorescence parameters for the binding of 82-84 with st-DNA in 10 mM phosphate buffer (pH 7.0) ($\lambda_{ex} = 480$ nm). |
|---|---|---|
| $\lambda_{max}$ (Flu (nm) (Free)) | 82 | 83 | 84 |
| 552 | 550 | 555 |
| $\lambda_{max}$ (Flu) (nm) (Bound) | 547 | 539 (P/D=0→17) | 536 (P/D=20→55) |
| -5 | -11 (P/D=0→17) | -3 (P/D=20→55) |
| $\Delta \lambda$ (nm) | 2.0 | 1.6 (P/D=0→17) | 1.2 (P/D=20→55) |
| $I/I_0$ | 1.6 (P/D=0→17) | 1.2 (P/D=20→55) |
| $\Phi/\Phi_0$ | 2.0 | 15 |
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Figure 2.20: Changes in the steady state emission spectra of (a) 82 (7.2 μM), (b) 83 (7.0 μM) (c) 84 (7.8 μM) in the presence of increasing concentration of st-DNA (0–438 μM) in 10 mM phosphate buffer (pH 7.0) (λ_ex = 480 nm). (d) Plot of I/I_0 vs. DNA nucleotide phosphate/ligand (P/D) for 82-84; Inset: Plot of I/I_0 vs. P/D for 82-83.

The fluorescence enhancement observed for 82-84 in the presence of DNA presumably results from the localisation of the ligand in a nonpolar hydrophobic site, where the chromophore is less accessible to solvent molecules. As a consequence, the chromophore can no longer experience the relaxation processes induced by solvent molecules, which results in an increase in the fluorescence quantum yield. This is in parallel with the higher quantum yield of 82-84 in less polar solvents such as in ethanol compared to water (Table 2.4, Section 2.3.1). Additionally, the rigid microenvironment of the binding site in DNA also reduces the non-radiative decay processes by restricting various internal motions, which promote radiationless decay of the excited state as previously discussed.\textsuperscript{32,200a} The fluorescence enhancement effect was found to be much more pronounced in 84, which shows very low quantum yield of emission in water compared to that observed for 82-83. The presence of the
dimethyl substituent on the 4-amino nitrogen increases the extent of non-radiative
deactivation of the excited singlet state in 84 (Table 2.4 and 2.5, Section 2.3.1) facilitated by
rotation/twisting of C-N bonds and inversion of amino nitrogen compared to 82-83. These internal motions are expected to be significantly reduced, when bound to macromolecules such as DNA, resulting in enhancement of fluorescence quantum yield. The biphasic fluorescence change observed for 83 suggests different distribution of the ligand on the polynucleotide. The increase in the first phase can result from the localisation of 83 in a rigid, nonpolar environment. The fluorescence decrease observed in the second phase can arise, if the ligand redistributes to a GC rich binding site resulting in partial fluorescence quenching. Similar biphasic fluorescence behaviour has been previously reported for various ruthenium complexes, where the enhancement in the first phase arises from the closely bound intercalators on adjacent DNA sequences, which provides protection of the metal complexes from solvent molecules, while the decrease in the fluorescence in the second phase represent the emission from the isolated complex bound to DNA. However, these authors also observed the second mode of binding from the UV/vis absorption spectra, which was not the case with 83.

2.5.2.2 Time-Resolved Fluorescence Measurements with st-DNA: To complement the steady-state measurement data, fluorescence lifetime measurements of 82-83 were carried out as a function of st-DNA concentration. Lifetime measurement of 84 could not be carried out as mentioned before due to its very short fluorescence lifetime. In the presence of st-DNA, the excited state decay followed biexponential kinetics for both 82-83 (Table 2.12).

<table>
<thead>
<tr>
<th>P/D</th>
<th>82</th>
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<tr>
<td></td>
<td>$\tau_1$ (ns) (%)*</td>
<td>$\tau_2$ (ns) (%)*</td>
</tr>
<tr>
<td>0</td>
<td>2.20 (100)</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>2.50 (37)</td>
<td>7.88 (63)</td>
</tr>
<tr>
<td>20</td>
<td>3.13 (27)</td>
<td>8.69 (73)</td>
</tr>
<tr>
<td>40</td>
<td>3.27 (26)</td>
<td>8.86 (74)</td>
</tr>
<tr>
<td>60</td>
<td>3.40 (27)</td>
<td>8.90 (73)</td>
</tr>
<tr>
<td>100</td>
<td>3.73 (30)</td>
<td>9.23 (70)</td>
</tr>
</tbody>
</table>

* the quantities within brackets represent the relative proportion of each kinetic component.

$\tau_{av} = \frac{\sum a_i \tau_i}{\sum a_i}$. 

Table 2.12: Fluorescence lifetime of 82-83 in the presence of st-DNA in 10 mM phosphate buffer (pH 7.0). $\lambda_{ex} = 458$ nm.
Average lifetime calculations showed that in the presence of st-DNA, the excited state lifetime of 82 was increased consistently. However, for 83, steady state fluorescence measurements showed that the emission intensity initially increased by a factor of 1.6 up to a P/D = 17, followed by ca.12% decrease in the emission intensity at higher P/D. However, the average fluorescence lifetime did not exhibit such a biphasic trend (Table 2.12). The reason for such discrepancy between the steady-state and time-resolved data is currently not understood.

2.6 Effect of Ionic Strength on the Interaction of 82-84 with st-DNA

As mentioned before, while designing 82-84, it was anticipated that the incorporation of the pyridinium side chain would provide water solubility and allow electrostatic interaction with double stranded DNA. Therefore, the ionic strength of the medium was expected to play a vital role in the binding of 82-84 with st-DNA. Usually, an increase in the ionic strength of the buffer results in the shrinking of a DNA double helix as large numbers of cations can shield the negative phosphate backbone more efficiently and thereby reduce phosphate-phosphate repulsion. Therefore, the binding affinities of ligands usually decrease at higher ionic strength. To investigate the effect of ionic strength on the binding, the UV/vis absorption and fluorescence titrations were carried out by adding small aliquots of NaCl (5 M) to a solution of the ligand bound to st-DNA (P/D = 40). Figure 2.21a shows the UV/vis spectra of 82 in the absence of DNA (P/D = 0), in the presence of st-DNA (P/D = 40) and the changes upon addition of small aliquots of NaCl. With increase in NaCl concentration (0→450 mM), the absorbance of the ICT band at 436 nm increased by 36% accompanied with a ca. 6 nm blue shift in the λmax. These changes indicate partial reversal of the binding at higher ionic strength. Importantly, even at very high ionic strength complete recovery of the absorbance was not observed (Figure 2.21b). Similar changes were also observed for 83 and 84 (Figure A2.8 in Appendix II).

To verify whether the lack of recovery in the absorbance was due to aggregation of 82 at higher ionic strength, UV/vis spectra of 82 were recorded at various ionic strengths. The results however showed that the addition of NaCl did not affect the UV/vis absorption spectrum of 82 significantly (Figure A2.8, Appendix II), indicating that no such aggregation occurred at the concentration of the ligand used in these measurements. Taken together, these results suggest strong binding of 82-84 with st-DNA at higher ionic strengths.

The effect of increasing ionic strength of the medium on the ligand binding was also followed by fluorescence spectroscopy. When fully bound to st-DNA (P/D = 40), emission intensity of the ligands 82-84 increased. Addition of NaCl (0→450 mM) resulted in a
decrease in the steady-state emission intensity for all the three systems (Figure 2.21c), suggesting dissociation of ligands from DNA at elevated ionic strength. However, as observed from ground state spectroscopy, complete reversal of binding was not observed for any ligand (Figure 2.21d). Therefore it can be concluded from these NaCl reverse salt titrations that although electrostatic interactions play an important role in the binding of these ligands, the overall association is governed by the intercalating ability of the planar naphthalimide ring as well.

**Figure 2.21:** (a) The UV/vis absorption spectra of 82 (8.4 μM) bound to st-DNA (P/D = 40) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-450 mM), (b) The fraction of ligand liberated for 82-84 as a function of ionic strength measured from the absorption spectra, (c) The fluorescence spectra of 82 (8.4 μM) bound to st-DNA (P/D = 40) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-450 mM) (λex = 480 nm), (d) The fraction of ligand liberated 82-84 as a function of ionic strength measured from the fluorescence spectra.
As reverse salt titration demonstrated the contribution of electrostatic interaction in the binding process, UV/vis absorption and fluorescence titrations were carried out at a higher ionic strength to quantitate the DNA binding affinity of 82-84.

2.6.1 Ground-state Interaction of 82-84 with st-DNA at 100 mM NaCl Concentrations

The binding of 82-84 to st-DNA was studied at a higher ionic strength in a way similar to that described in Section 2.5, by addition of small aliquots of st-DNA to a solution of each ligand in 10 mM phosphate buffer containing 100 mM NaCl, until the changes in absorbance reached a plateau. The changes in the UV/vis absorption spectra of 82 at higher ionic strength are shown in Figure 2.22. Similar to what was observed at low ionic strength, an increase in st-DNA concentration resulted in ca. 36% hypochromism of the ICT absorption band at 436 nm associated with a ca. 11 nm red shift in \( \lambda_{\text{max}} \). An isosbestic point was observed at 480 nm at all P/D ratios suggesting the presence of two spectroscopically distinct species in solution. However, in the presence of 100 mM NaCl, changes in absorbance reached a plateau at much higher P/D compared to 10 mM phosphate buffer, which suggests that at higher ionic strength the affinity of 82 towards st-DNA was markedly reduced implying significant contribution of electrostatic interaction in the binding process.

![Figure 2.22: Changes in the UV/vis absorption spectra of 82 (7.3 \( \mu \text{M} \)) in the presence of increasing concentration of st-DNA (0–1168 \( \mu \text{M} \)) in 10 mM phosphate buffer containing 100 mM NaCl (pH 7.0). Inset: Plot of \( A/A_0 \) vs. DNA nucleotide phosphate/ligand (P/D) for 82-84 in 10 mM phosphate buffer containing 100 mM NaCl.](image-url)
Similar changes were observed for 83 and 84 in the presence of st-DNA at a higher ionic strength (Figure A2.9a and A2.9b respectively in Appendix II). The relative changes in absorbance for the three ligands are shown in Figure 2.22 inset, which showed that at higher ionic strength 82 and 83 have comparable binding affinities. However, 84 appeared to have much reduced affinity, with the changes in absorbance reaching a plateau at higher P/D compared to 82-83, which can presumably result from the presence of the twisted NMe$_2$ group on the naphthalimide ring that can interfere with the intercalative binding. The summary of the binding data for 82-84 is presented in Table 2.13.

The binding constant for the association of 82-84 with st-DNA at higher ionic strength was estimated by analysing the changes in the absorbance at the respective $\lambda_{\text{max}}$ using the non-cooperative model of McGhee and von Hippel (equation 2.9). Representative binding curves are shown in Appendix II (Figure A2.9 insets) and the binding constants are presented in Table 2.13. For 84, the changes at 400 nm could not be fitted to the binding model, as this resulted in an unrealistic binding site value ($n > 8$) and was not therefore included in the Table 2.13.

**Table 2.13:** Summary of binding parameters obtained from the absorption spectra of 82-84 in the presence of st-DNA in 10 mM phosphate buffer containing 100 mM NaCl solution.

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Free)</td>
<td>436</td>
<td>435</td>
<td>450</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Bound)</td>
<td>447</td>
<td>445</td>
<td>462</td>
</tr>
<tr>
<td>$\Delta \lambda$ (nm)</td>
<td>+11</td>
<td>+10</td>
<td>+12</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>36</td>
<td>36</td>
<td>18$^a$, 47$^b$</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>480</td>
<td>480</td>
<td>480</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>70</td>
<td>70</td>
<td>95</td>
</tr>
<tr>
<td>$K \times 10^5$ M$^{-1}$</td>
<td>0.26 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>(0.14 ± 0.01)$^c$</td>
</tr>
<tr>
<td>n (bp)</td>
<td>4.49 ± 0.12</td>
<td>4.81 ± 0.20</td>
<td>(4.41 ± 0.47)$^a$</td>
</tr>
</tbody>
</table>

$^a$ Measured using absorbance at 450 nm.
$^b$ Measured using absorbance at 400 nm.
$^c$ Absorbance data at 400 could not fitted to McGhee von Hippel binding model.

The results obtained from the UV/vis absorption data in the presence of 100 mM NaCl showed that the ligands 82-84 can bind to st-DNA, even at higher ionic strength. However, the magnitude of the binding constants were significantly reduced compared to the 10 mM buffer titration data showing an obvious effect of ionic strength on the binding interaction. These results were also confirmed from the steady-state fluorescence measurements, described in the following section.
2.6.2 Excited State Interaction of 82-84 with st-DNA at 100 mM NaCl Concentrations

The changes in the emission intensity of 82-84 in the presence of increasing concentration of st-DNA were recorded in 10 mM phosphate buffer containing 100 mM NaCl. The overall changes in the ICT band in the fluorescence spectra of 82 at higher ionic strength are described in Figure 2.23, which shows that upon addition of st-DNA, the intensity of the ICT emission band for 82 centred at 552 nm increased by a factor of ca. 2 accompanied by a ca. 12 nm blue shift in the $\lambda_{\text{max}}$. Similar behaviour was observed for 84, which showed ca. 13 fold fluorescence enhancement in the presence of st-DNA concomitant with a ca. 9 nm blue shift in the emission band (Figure 2.23 inset and Figure A2.10, Appendix II). However, for both 82 and 84, the extent of this emission enhancement was somewhat lower than that observed at low ionic strength.

![Figure 2.23](image-url)

*Figure 2.23:* Overall changes in the steady-state emission spectra of 82 (7.3 $\mu$M) in the presence of increasing concentration of st-DNA (0–1168 $\mu$M) in 10 mM phosphate buffer containing 100 mM NaCl (pH 7.0) ($\lambda_{\text{ex}} = 480$ nm). Inset: Plot of $I/I_0$ vs. DNA nucleotide phosphate/ligand (P/D) for 82-84.

As observed at lower ionic strength, compound 83 exhibited a biphasic fluorescence response in the presence of increasing st-DNA concentration at higher ionic strength (Figure 2.24). However, in the first phase, the fluorescence intensity increased by a factor of 1.2 associated with a ca. 7 nm blue shift in the $\lambda_{\text{max}}$ upto P/D 0→18. Further addition of st-DNA resulted in a decrease in the fluorescence intensity, which reached a plateau at a P/D = 120,
with the fluorescence intensity being almost the same as in the free ligand. The relative changes in emission intensity of 83 in the presence of st-DNA at low and high ionic strengths are shown as an inset in Figure 2.24. It is interesting to note that in both cases the maximum emission intensity was observed at a P/D ratio \( \text{ca.} \ 17-18 \), followed by a decrease in emission quantum yield. The decrease in emission intensity in the second phase beyond P/D suggests that a second mode of binding is prevalent at higher P/D ratio possibly arising from a redistribution of ligand molecules to GC rich sites at both low and high ionic strengths. The changes in the photophysical properties of 82-84 in the presence of 100 mM NaCl are summarised in Table 2.14.

**Figure 2.24:** Overall changes in the steady state emission spectra of 83 (7.0 \( \mu \text{M} \)) in the presence of increasing concentration of st-DNA (0–1120 \( \mu \text{M} \)) in 10 mM phosphate buffer containing 100 mM NaCl (pH 7.0) \( (\lambda_{\text{ex}} = 480 \text{ nm}) \). Inset: Plot of \( I/I_0 \) vs. P/D for 83 in 10 mM phosphate (■) and 10 mM phosphate buffer containing 100 mM NaCl (○).

**Table 2.14:** Summary of fluorescence parameters for the binding of 82-84 with st-DNA in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl.

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
<th>84</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (Flu (nm) (Free))</td>
<td>552</td>
<td>550</td>
<td>555</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (Flu) (nm) (Bound)</td>
<td>540</td>
<td>543 (P/D=0→18) 539 (P/D=20→160)</td>
<td>546</td>
</tr>
<tr>
<td>( \Delta \lambda ) (nm)</td>
<td>-12</td>
<td>-7 (P/D=0→17) -4 (P/D=20→55)</td>
<td>-9</td>
</tr>
<tr>
<td>( I/I_0 )</td>
<td>1.85</td>
<td>1.2 (P/D=0→18) 0.98 (P/D=20→160)</td>
<td>13</td>
</tr>
<tr>
<td>( \Phi/\Phi_0 )</td>
<td>1.80</td>
<td>1.2 (P/D=0→18) 0.99 (P/D=20→160)</td>
<td>12</td>
</tr>
</tbody>
</table>
Chapter 2: Photophysical and DNA Binding Studies of Novel 1,8-naphthalimide Derivatives

It has been demonstrated above that the compounds 82-84 have high affinities for DNA and that the binding causes significant changes in the photophysical properties of these compounds. Moreover, as described in Section 2.4, binding of 82-83 to AMP leads to increased emission intensities and excited state, lifetimes while GMP causes a partial quenching of fluorescence intensity. It was therefore of interest to study the behaviour of these ligands in the presence of the synthetic polynucleotides poly(dA–dT)$_2$ and poly(dG–dC)$_2$, the results of which will be described in the following sections. Additionally, as described before compound 83 showed biphasic fluorescence changes in the presence of st-DNA at both low and high ionic strength, it was also necessary to investigate if 83 displayed sequence selective binding to the polynucleotides.

2.7 Interaction of 82-83 with Synthetic Polynucleotides

To investigate the sequence specific interaction of compounds 82-83 with double stranded DNA, the interactions with poly(dA-dT)$_2$ and poly(dG-dC)$_2$ were investigated using UV/vis absorption and fluorescence spectroscopy in a manner similar to that described earlier.

2.7.1 Ground State Interaction of 82-83 with poly(dA-dT)$_2$

The changes in the UV/vis absorption spectra of 82-83 were initially investigated in the presence of increasing concentration of poly(dA-dT)$_2$ in 10 mM phosphate buffer (pH 7.0). As stated before, all the measurements were repeated three times to ensure reproducibility. As shown in Figure 2.25a, the addition of poly(dA-dT)$_2$ to a solution of 82 in 10 mM phosphate buffer resulted in ca. 44% hypochromism in the ICT absorption band centred at 436 nm accompanied by a ca. 12 nm red shift of the band. An isosbestic point was observed at 480 nm at all P/D ratios. In the case of 83, addition of poly(dA-dT)$_2$ resulted in much larger degree of hypochromism (ca. 57%) in the ICT absorption band and a ca.12 nm red shift (Figure 2.25b). However, no clear isosbestic point was observed over the course of the titration of 83 with poly(dA-dT)$_2$, which suggested the possible existence of more than one form of DNA bound ligands. The relative changes in absorbance for 82-83 with increasing poly(dA-dT)$_2$ concentration are shown in Figure 2.25c, which shows that in the case of 82 the changes reached a plateau at P/D = 11, while for 83, higher concentrations of poly(dA-dT)$_2$ were required for the complete binding. This suggests that 82 has a slightly higher affinity towards AT rich sequences compared to 83. The changes in the absorption spectrum of 82 and 83 in the presence of poly(dA-dT)$_2$ are summarised in Table 2.15. The binding constants for
the interaction of these ligands with poly(dA-dT)₂ were estimated from the changes in absorbance at 435 nm in the presence of increasing concentrations of polynucleotide using the non-cooperative model of McGhee and von Hippel (equation 2.9). The representative plots are shown as an inset in Figure 2.25a and the binding constants are presented in Table 2.15, which showed that both 82 and 83 possess high affinity for AT rich sequence, however, 82 exhibits a slightly higher affinity towards poly(dA-dT)₂ compared to that of 83.

Figure 2.25: The UV-vis absorption spectra of (a) 82 (7.0 μM) in the presence of increasing concentration of poly(dA-dT)₂ (0-265 μM); (b) 83 (4.0 μM) in the presence of increasing concentration of poly(dA-dT)₂ (0-140 μM) in 10 mM Phosphate buffer (pH 7.0). Inset a and b: The plot of r/C₇ vs. r (■) and best fit (—) to the McGhee–von Hippel model for 82 and 83 respectively. (c) Plot of A/A₀ vs. P/D for 82 and 83 at 435 nm.
Table 2.15: Summary of binding parameters obtained from the absorption spectra of 82-83 in the presence of poly(dA-dT)$_2$ in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Free)</td>
<td>436</td>
<td>435</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (Abs) (nm) (Bound)</td>
<td>447</td>
<td>447</td>
</tr>
<tr>
<td>$\Delta \lambda$ (nm)</td>
<td>+11</td>
<td>+12</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>44</td>
<td>57</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>480</td>
<td>No IP</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>$K$ ($\times 10^5$ M$^{-1}$)</td>
<td>5.95 ± 0.07</td>
<td>4.35 ± 0.01</td>
</tr>
<tr>
<td>$n$ (bp)</td>
<td>2.54 ± 0.12</td>
<td>1.36 ± 0.02</td>
</tr>
</tbody>
</table>

2.7.2 Excited State Interaction of 82-83 with Poly(dA-dT)$_2$

Fluorescence emission measurements were carried out in the presence of increasing concentrations of poly(dA-dT)$_2$ for both 82-83 in 10 mM phosphate buffer (Figure 2.26a and b). Addition of poly(dA-dT)$_2$ to the ligand solution in both cases resulted in an emission enhancement accompanied by a blue shift in the $\lambda_{\text{max}}$. Since no isosbestic point was observed during the titration of 83 with poly(dA-dT)$_2$, emission measurements were carried out using $\lambda_{\text{ex}} = 435$ nm and the emission intensity was corrected for the decrease in the absorbance at the excitation wavelength. In the case of 82, all fluorescence measurements were carried out by exciting the solution at 480 nm. The enhancement effect was much more pronounced in the case of 83, showing an about 3.5 fold increase in the emission quantum yield concomitant with a ca. 15 nm blue shift in the emission maxima. In contrast, the emission intensity of 82 was enhanced by a factor of two associated with a ca. 5 nm blue shift in the $\lambda_{\text{max}}$. These changes are summarised in Table 2.16.

Table 2.16: Summary of the various fluorescence parameters obtained from the binding of 82-83 with poly(dA-dT)$_2$ in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (Flu) (nm) (Free)</td>
<td>552</td>
<td>550</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (Flu) (nm) (Bound)</td>
<td>547</td>
<td>535</td>
</tr>
<tr>
<td>$\Delta \lambda$ (nm)</td>
<td>-5</td>
<td>-15</td>
</tr>
<tr>
<td>$I/I_0$</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>$\Phi/\Phi_0$</td>
<td>2.2</td>
<td>3.8</td>
</tr>
<tr>
<td>$\tau_1$ (ns) (P/D = 30)</td>
<td>3.1 (6%)*</td>
<td>5.8 (9%)*</td>
</tr>
<tr>
<td>$\tau_2$ (ns)</td>
<td>9.9 (94%)*</td>
<td>11.3 (91%)*</td>
</tr>
</tbody>
</table>

* The quantities in bracket next to lifetime values represent the relative contribution of each component.
Chapter 2: Photophysical and DNA Binding Studies of Novel 1,8-naphthalimide Derivatives

Figure 2.26: The Fluorescence spectra of (a) 82 (7.0 \( \mu \)M) in the presence of increasing concentration of poly(dA-dT)\(_2\) (0–265 \( \mu \)M) (\( \lambda_{ex} = 480 \) nm); (b) 83 (4.0 \( \mu \)M) in the presence of increasing concentration of poly(dA-dT)\(_2\) (0–140 \( \mu \)M) (\( \lambda_{ex} = 435 \) nm) in 10 mM phosphate buffer (pH 7.0). Inset a and b: Plot of \( l/I_0 \) vs. P/D for 82 and 83 respectively.

Fluorescence lifetime measurements were carried out to complement the steady-state fluorescence data. In the case of 82, when fully bound to DNA, the fluorescence lifetime increased from 2.2 ns for the free ligand to 9.9 ns (94%), which is consistent with the enhancement observed in the quantum yield of 82 (Table 2.16). For 83, the fluorescence lifetime increased from 3.1 ns to 11.3 ns (91%). In both cases a comparatively short-lived component comprising of less than 10% of the decay was also observed. Two different kinetic components presumably represent two different localisation of the bound ligand.

2.7.3 Ground-State Interaction of 82-83 with Poly(dG-dC)\(_2\)

The addition of poly(dG-dC)\(_2\) to the solution of each ligand in 10 mM phosphate buffer (pH 7.0) resulted in significant hypochromism and red shift in the ICT absorption bands. In the case of 82, an isosbestic point was observed (at 480 nm) at low concentration of the polynucleotide (P/D \( \approx 3 \)), which disappears at higher concentration of the polynucleotide, indicating the possible existence of more than one bound form of ligand at higher concentration. In contrast, no isosbestic point was observed during the titration of 83 with poly(dG-dC)\(_2\) over the entire concentration range of the polynucleotide. The overall changes in the UV/vis absorption spectra of 82 in the presence of poly(dG-dC)\(_2\) are shown in Figure 2.27a and that of 83 are shown in the Appendix II (Figure A2.11). Comparison of the changes observed for 82 and 83 (Figure 2.27 b) reveals that for 83 the changes in absorbance reached a plateau at a lower P/D (ca. P/D = 25), while the changes for 82 levelled off at much higher P/D (ca. P/D = 50), suggesting that 83 has a higher affinity for poly(dG-dC)\(_2\) compared to that of 82. The overall changes in absorbance are summarised in Table 2.17.

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Figure 2.27: (a) Overall changes in the UV/vis absorption spectra of 82 (4.0 µM) in the presence of increasing concentration of poly(dG–dC)$_2$ (0–410 µM); Inset (a): plot of $r/C_f$ vs. $r$ (□) and best fit (−) to McGhee–von Hippel model (b) Plot of $A/A_0$ vs. P/D for 82 and 83 respectively.

The changes in absorbance at the respective $\lambda_{max}$ were analysed in terms of the non-cooperative McGhee and von Hippel model and the binding constants (Table 2.17) showed that 83 has a higher affinity towards GC rich sequence ($K = 2.87 \times 10^5$ M$^{-1}$) compared to that of 82 ($K = 0.82 \times 10^5$ M$^{-1}$). Interestingly, for 82 the binding affinity for the AT rich sequences ($K = 5.95 \times 10^5$ M$^{-1}$) was ca.7 times higher than that observed for the GC rich sequences ($K = 0.82 \times 10^5$ M$^{-1}$). This sequence selectivity contrasts the behaviour of the unsubstituted compound, which did not exhibit any sequence selectivity. However, 83 showed much less sequence selectivity, where the binding affinity for AT rich sequences ($K = 4.35 \times 10^5$ M$^{-1}$) was only 1.5 times higher than the affinity for GC rich sequences ($K = 2.87 \times 10^5$ M$^{-1}$). The preferential binding of 82 towards AT rich sequences might originate from the differences between the electrostatic potentials of AT and GC rich segments, where AT rich sequences
have been shown to have higher negative electrostatic potential thereby favouring binding of cationic molecules. In contrast, the comparable binding affinity of 83 toward AT and GC rich sequences possibly originates from the presence of additional methyl group on the pyridyl nitrogen, which might result in better binding to both AT and GC sequences due to hydrophobic interaction. However, these are only speculations, detailed structure-activity analysis and molecular modelling studies would be required to provide a complete insight.

Table 2.17: Summary of various binding parameters obtained from the changes in the absorption spectra of 82-83 in the presence of poly(dG-dC)2 in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (Abs) (nm) (Free)</td>
<td>436</td>
<td>435</td>
</tr>
<tr>
<td>λmax (Abs) (nm) (Bound)</td>
<td>445</td>
<td>447</td>
</tr>
<tr>
<td>Δλ (nm)</td>
<td>+9</td>
<td>+12</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>52</td>
<td>57</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>480 (P/D 0-3)</td>
<td>No IP (P/D &gt;3)</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>K (× 10^5 M⁻¹)</td>
<td>0.82 ± 0.02</td>
<td>2.87 ± 0.10</td>
</tr>
<tr>
<td>n (bp)</td>
<td>3.62 ± 0.09</td>
<td>2.20 ± 0.07</td>
</tr>
</tbody>
</table>

2.7.4 Excited State Interaction of 82-83 with Poly(dG-dC)2

The fluorescence emission spectra of 82-83 were recorded as a function of increasing concentration of poly(dG-dC)2 in 10 mM phosphate buffer (pH 7.0). Since no isosbestic point was observed in the UV/vis absorption spectra of either of the ligands in the presence of poly(dG-dC)2, emission spectra were recorded using 435 nm excitation wavelength and the emission intensity values were therefore corrected for the absorbance decrease at the excitation wavelength. The excited state behaviours of 82 and 83 with poly(dG–dC)2 were strikingly different from that seen for poly (dA–dT)2 or st-DNA; as the fluorescence was partially quenched rather than being enhanced. Thus in the presence of poly(dG–dC)2, the emission intensity of 82 was reduced by ca. 30% after correcting for the absorbance decrease at 435 nm (Figure 2.28). This was associated with a ca. 8 nm blue shift in the λmax. Similar behaviour was exhibited by 83, which showed about 25% fluorescence quenching in the presence of poly(dG-dC)2. The weaker fluorescence quenching of 82 and 83 in the presence of GC rich sequences compared to the unsubstituted compound 60 presumably arise from the less oxidising nature of the amino derivatives. The fluorescence intensity plot (Inset in Figure 2.28) again revealed the marked difference in the binding affinities of 82 and 83 for
poly(dG-dC)_2 and also demonstrated much lower affinity of 82 towards GC rich compared to AT rich sequence.

**Figure 2.28**: Overall changes in the steady-state fluorescence emission spectra of 82 (4.0 μM) in the presence of increasing concentration of poly(dG-dC)_2 (0–410 μM). (λ_ex = 435 nm) in 10 mM phosphate buffer (pH 7.0). Inset: plot of I/I_0 vs. P/D for 82 and 83, where (■) and (▲) represent the uncorrected fluorescence intensities of 82 and 83, respectively, while (■) and (▼) represent the fluorescence intensities of 82 and 83, respectively after correcting for the absorbance decrease at 435 nm.

The fluorescence lifetime measurements showed that in the presence of excess poly(dG-dC)_2, the singlet excited state decay of 82 and 83 could be best described by biexponential kinetics. The excited singlet state of 82 (in the presence of poly(dG-dC)_2, P/D = 105) decayed by a biexponential process with τ_1 = 1.5 ns (51%) and τ_2 = 3.8 ns (49%) (Table 2.18). Similarly the excited state decay of 83 in the presence of excess poly(dG-dC)_2 (P/D = 60) followed a biexponential kinetic profile with τ_1 = 2.6 ns (68%) and τ_2 = 5.8 ns (32%) (Table 2.18). In both of the cases the calculated average singlet state lifetime of 82 (τ_{av} = 2.6 ns at P/D = 105) and 83 (τ_{av} = 3.6 ns at P/D = 60) bound to poly(dG-dC)_2 remained practically unchanged from the free ligand. This behaviour may be contrasted with what was found for 5'-GMP, where no longer-lived emission was observed and with that of 5'-AMP, where a much longer-lived component was detected. One possible explanation might be that two conflicting trends are operative for the singlet excited state of the amino derivatives 82-83 in the presence of poly(dG-dC)_2. It is expected that binding to the polynucleotide should result in an increased lifetime due to location within the non-aqueous and more rigid environment of the polynucleotide, which will be counterbalanced by a reduction in the excited state lifetime.
because of PET from guanine to the excited naphthalimide, as found with 5'-GMP, resulting in almost no net change in fluorescence lifetime.

Table 2.18: Summary of the various fluorescence parameters obtained for the binding of 82 and 83 with poly(dG-dC)₂ in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>82</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (Flu (nm) (Free))</td>
<td>552</td>
<td>550</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (Flu) (nm) (Bound)</td>
<td>544</td>
<td>543</td>
</tr>
<tr>
<td>( \Delta \lambda ) (nm)</td>
<td>-8</td>
<td>-7</td>
</tr>
<tr>
<td>( I/I_0 )</td>
<td>0.70</td>
<td>0.74</td>
</tr>
<tr>
<td>( \Phi/\Phi_0 )</td>
<td>0.71</td>
<td>0.80</td>
</tr>
<tr>
<td>( \tau_1 ) (ns)</td>
<td>1.5 (51%)*</td>
<td>2.6 (68%)*</td>
</tr>
<tr>
<td>( \tau_2 ) (ns)</td>
<td>3.8 (49%)*</td>
<td>5.8 (32%)*</td>
</tr>
</tbody>
</table>

* The quantities in bracket next to the lifetime values represent the relative contribution of each component.

In summary, the above studies showed 82 and 83 bind to synthetic homopolymeric sequences with high affinity. Compound 82 showed high preference for AT rich sequences compared to GC rich sequences \( (K_{AT}/K_{GC} \approx 7) \), while this sequence preference was significantly reduced for 83 \( (K_{AT}/K_{GC} \approx 1.5) \). Interestingly, the affinities of 82-83 for AT rich sequences are comparable, the difference arises because of comparatively higher affinity of 83 for GC rich sequences than 82. This difference has been tentatively attributed to the presence of an additional methyl group in 83, which can possibly engage in favourable hydrophobic interactions with both AT and GC rich sequences. For both of the ligands, emission intensities and fluorescence lifetimes were substantially enhanced in the presence of poly(dA-dT)₂ consistent with their localisation in a rigid nonpolar environment, while in the presence of poly(dG-dC)₂ partial emission quenching was for both 82 and 83, presumably due to PET from guanine to the photoexcited naphthalimide ligand.

The following sections will describe the mode of interaction of 82-84 with st-DNA using various spectroscopic techniques.

2.8 Thermal Denaturation Studies of st-DNA in the presence of 82-84

As discussed in Chapter 1, thermal denaturation study is an optical technique that determines the stability of double stranded DNA against heat-induced denaturation.\(^\text{11c}\) The thermal denaturation studies of st-DNA (150 μM) alone and in the presence of 82-84 (P/D =10) were carried out in 10 mM phosphate buffer (pH 7.0). The solutions were degassed prior to heating. The thermal denaturation was monitored by measuring the absorbance of the DNA
solution at 260 nm as the temperature was gradually increased from 30°→90°C at a rate of 1°C per minute.

The thermal melting curves in the presence of 82-84 are shown in Figure 2.29. In the absence of any ligand, the Tm value for st-DNA was found to be (68 ± 0.5)°C. In the presence of ligand 82-84 (P/D = 10), stabilisation of st-DNA was observed, indicated by a ca. 5-6° increase in the DNA melting temperature of st-DNA in 10 mM buffer solution (Figure 2.29 and Table 2.19). It is perhaps significant that the extent of stabilisation is smaller than the effect observed for well-established intercalators such as ethidium bromide (ΔTm > 10°).

However such moderate stabilisation has been observed for other mononaphthalimide based DNA intercalators previously and perhaps suggests a weaker intercalation efficiency of 82-84.

Since 82 displayed strong preference for AT rich sequence, the thermal denaturation profile of poly(dA–dT)2 was also recorded in the presence of 82 at a P/D = 5 and P/D = 10. In the absence of any ligand, poly(dA–dT)2 was found to have a melting temperature of 46 ± 0.5°C. As shown in Figure 2.29b, 82 was found to stabilise poly(dA–dT)2 (ΔTm = 12°C) to a

---

**Table 2.19:** Summary of melting temperature of st-DNA in the presence of 82-84 in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th>Species</th>
<th>P/D = 0</th>
<th>P/D = 5</th>
<th>P/D = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tm (°C)</td>
<td>ΔTm (°C)</td>
<td>Tm (°C)</td>
</tr>
<tr>
<td>st-DNA</td>
<td>68.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 82</td>
<td>75</td>
<td>6.5</td>
<td>72</td>
</tr>
<tr>
<td>+ 83</td>
<td>76</td>
<td>7.5</td>
<td>73</td>
</tr>
<tr>
<td>+ 84</td>
<td>74</td>
<td>5.5</td>
<td>72</td>
</tr>
</tbody>
</table>
much greater extent than it did for \( st \)-DNA, which contains 58% AT basepairs. This is in agreement with the higher affinity of 82 towards AT rich sequences as concluded from the spectroscopic titrations. Unfortunately due to the high melting temperature of poly(dG–dC)_2 (ca. 90°C) in 10 mM phosphate buffer, thermal denaturation profile of the polynucleotide could not be measured in the presence of 82.

The results obtained from thermal denaturation studies further support the binding interaction of 82-84 with \( st \)-DNA. However, this technique does not provide any evidence for the binding mode as substantial changes in the melting temperature of DNA can result from both groove binding and intercalative binding. Therefore the mode of binding of 82-84 with \( st \)-DNA was further investigated using Circular Dichroism (CD) and Linear Dichroism (LD) spectroscopy as described in the following two sections.

### 2.9 Circular Dichroism Spectroscopy of \( st \)-DNA in the Presence of 82-84

As discussed in Chapter 1, CD spectroscopy is largely used to study the changes in conformation of macromolecules upon binding to small molecules. The CD titrations were carried out by monitoring the conformational changes of \( st \)-DNA (150 \( \mu \)M) in the presence of increasing concentrations of the ligand of interest. To avoid dilution effects, a range of solutions were prepared in 10 mM phosphate buffer (pH 7.0) containing fixed concentration of \( st \)-DNA (150 \( \mu \)M) while varying the ligand concentrations. The CD spectra of \( st \)-DNA in the presence of varying concentrations of 82 are shown in Figure 2.30 with those of 83 and 84 are shown in Figure A2.12, Appendix II.

**Figure 2.30:** The CD spectra of \( st \)-DNA (150 \( \mu \)M) in the presence of varying concentration of 82 (P/D 0-20) in 10 mM phosphate buffer (pH 7.0).
In the presence of increasing concentration of 82, the ellipticity of the negative peak at 245 nm increased from -14 to -11.0, while that of the positive peak at 275 nm increased from +12 to +22 mdegree and both of the peaks showed ca. 3 nm red shifts. Significantly, no induced CD signal was observed for the bound naphthalimide in any case. An induced signal would be expected if it were groove-bound to the DNA as has previously been reported for its unsubstituted analogue 60. Unfortunately, the CD titration data did not show any evidence for intercalative binding of 82-84, however these results demonstrated that the interaction of 82-84 alters the secondary structure of DNA significantly. In order to investigate the mode of interaction of 82-84, the linear dichroism measurements of st-DNA were carried out as described below.

2.10 Linear Dichroism Spectroscopy of st-DNA in the Presence of 82-84

As shown in Figure 2.31, in the absence of any ligand, a negative LD signal is observed for st-DNA at λ = 260 nm, characteristic of B-DNA, arising from the nearly perpendicular orientation of the transition moments of DNA bases relative to DNA helical axis. In the presence of ligand 83, in addition to the negative LD signal at 260 nm for DNA, another negative LD signal was observed at around 440 nm corresponding to the ICT absorption band of 83.

![Figure 2.31](image.png)

**Figure 2.31:** The LD spectra of st-DNA (400 μM) in the presence of varying concentration of 83 (P/D 0-30) in 10 mM phosphate buffer (pH 7.0).
Due to significant overlap of the DNA absorption band and the absorption band of 83 below 300 nm, a complete quantitative interpretation of the flow linear dichroism data was not possible. However, the presence of a negative LD signal centred at ca. 440 nm in the presence of 83 suggests that the transition dipoles of the chromophore are oriented at an angle greater than 54.7° relative to the helical axis (According to equation 1.2, Chapter 1, Section 1.4.1.4) indicative of an intercalative binding mode of 83 to st-DNA. The LD spectra of st-DNA in the presence of 82 and 84 (Figure A2.13, Appendix II) also showed appearance of a negative LD band at ca. 440 nm, showing these derivatives also bind to DNA via intercalation. Table 2.20 summarises the LD values for the absorption bands at 260 nm and 440 nm at a P/D ratio = 10 for the three ligands 82-84. For the 260 nm band corresponding to the transitions of DNA bases, reduced linear dichroism (LD') was calculated to be \((-0.027 \pm 0.003\) ), a value similar to that obtained for B-DNA samples by other research groups. In the presence of 82-84, the LD' values calculated for the 440 nm band and the nucleobase region were found to be similar (Table 2.19), supporting the conclusion that the naphthalimide ligands lie in the same plane as the DNA bases.

**Table 2.20:** LD' data for 82-84 in the presence of st-DNA. The values represent mean of three measurements (error ± 10%).

<table>
<thead>
<tr>
<th></th>
<th>LD' 260 nm</th>
<th>LD' 440 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>st-DNA</td>
<td>-0.027</td>
<td>-</td>
</tr>
<tr>
<td>st-DNA + 82</td>
<td>-0.026</td>
<td>-0.034</td>
</tr>
<tr>
<td>st-DNA + 83</td>
<td>-0.031</td>
<td>-0.032</td>
</tr>
<tr>
<td>st-DNA + 84</td>
<td>-0.026</td>
<td>-0.034</td>
</tr>
</tbody>
</table>

2.11 Conclusions

In this chapter, the synthesis, photophysical and DNA binding studies of three novel naphthalimide derivatives 82-84 have been presented. These compounds were synthesised in reasonably high yield starting from commercially available precursors. The photophysical properties of these derivatives have been studied in a variety of alcohols and water, which differ in their H-bonding abilities and polarities. These studies showed that the excited state properties (such as \(\lambda_{\text{max}}\) of emission, fluorescence quantum yield, fluorescence lifetime) of these molecules depend strongly on the polarity of the medium. All three derivatives 82-84 exhibited ICT emission, characterised by high fluorescence quantum yields in relatively nonpolar solvents such as butanol and much weaker emission in water. Dimethyl substitution on the amino nitrogen in 84 was found to have a major effect on the fluorescence quantum yield of the system. The weak quantum yield of 84 compared to that observed for the amino analogues 82 and 83 has been rationalised in terms of increased non-radiative deactivation...
pathways compared to the 4-amino derivatives induced by the presence of twisted NMe₂ group in 84.

The interactions of 82-84 with mononucleotides and DNA have been studied by various spectroscopic techniques. All three naphthalimide derivatives were found to form 1:1 stacked complexes with AMP and GMP; however, ground state and excited state studies also suggested the possible existence of the higher order complexes at high concentration of the mononucleotides. Binding constant analysis suggested that the amino derivatives have higher affinities towards the mononucleotides compared to the previously reported unsubstituted compound 60. This might be caused by the presence of electron donating 4-amino group, which increases the electron density on the naphthalimide ring thereby favouring stronger π...π stacking interactions. In contrast, the binding affinity of the dimethyl amino derivative 84 towards AMP and GMP were comparable with the reported values for unsubstituted derivative 60, implying the 4-amino group might be involved in the interaction.

Linear dichroism and other spectroscopic studies showed that the derivatives bind to st-DNA via intercalation with significantly high affinity (ca. 10⁵ M⁻¹). The emission quantum yields of 82-84 were strongly enhanced in the presence of st-DNA presumably due to a decreased rate of non-radiative processes in the rigid nonpolar environment. In the case of 83, a biphasic fluorescence response was observed, where initial binding to st-DNA resulted in an increase in fluorescence quantum yield of 83, followed by partial quenching of emission at higher concentration of DNA. This could result from the redistribution of 83 towards GC rich intercalation sites as DNA concentration is raised. This was further supported from the comparable binding affinity of 83 towards poly(dA-dT)₂ and poly(dG-dC)₂. In contrast, 82 showed strong preference for AT rich sequences. The similar emission enhancement observed in the case of 82 with both st-DNA and poly(dA–dT)₂ strongly suggests that in the natural DNA the intercalator preferentially binds to AT sequences. The stronger interaction of 82 for AT rich sequences was further confirmed from thermal denaturation experiments, which showed that the binding of 82 to poly(dA–dT)₂ caused a significantly higher stabilisation of the double-stranded form than that found for st-DNA. The reason behind the differential sequence preference between 82 and 83 is not yet clear. It has been speculated that the presence of the methyl group on the pyridyl nitrogen in 83 can be involved in hydrophobic and van der Waals interactions, thereby favouring intercalation of 83 at both AT and GC rich sites. However, further studies are required to completely understand this behaviour.
Chapter 3

Photophysical and DNA Binding Studies of the Tröger’s Base Containing Bis-1,8-naphthalamides
3.1 Introduction

Over the past few decades, the interaction of small molecules with DNA has received great attention particularly within the field of medicinal chemistry since these interactions can interfere with replication and transcription and ultimately leads to cell death. These small molecules can bind to DNA through various modes, some of which have been already discussed in Chapter 1 (Section 1.3.1). Planar heteroaromatic molecules, such as ethidium bromide and proflavine bind to DNA via intercalation, where the planar aromatic rings stack between the DNA base pairs and these complexes are mainly stabilised by π-π stacking interactions. In contrast molecules like netropsin and distamycin bind to the minor groove of DNA and the binding is governed by H-bonding and van der Waals interactions between the ligand and DNA grooves. In this context, 1,8-naphthalimide derivatives represent an important family of DNA binders, which were shown to bind to DNA mainly by intercalation as discussed in Chapter 1 and 2. Bis-naphthalimide derivatives in particular have received much attention since they can potentially bind via bis-intercalation depending on the nature of the spacer present and exhibit higher affinities for DNA. Recently, the development of bis-naphthalimide derivatives containing the Tröger’s base unit has been reported from our group. Introduction of the Tröger’s base moiety resulted in enhanced DNA binding affinity (ca. 10^6 M^-1) and high cytotoxicity of the bis-naphthalimide derivatives compared to their mononaphthalimide precursors.

As discussed in Chapter 1, “Tröger’s base” (TB) refers to the methano-1,5-diazocine ring containing structures similar to 66 (Figure 3.1a). TB-derivatives are chiral compounds due to the presence of two stereogenic nitrogen centres with a C2 axis of symmetry placing the two aromatic rings at 80-104° angle to each other (Figure 3.1b). As discussed in Chapter 1, the “V”-shaped structure the Tröger’s base motif has received great attention in the area of supramolecular chemistry, to develop molecular torsion balances, water-soluble cyclophanes, receptors for cations, dicarboxylic acids, metal-mediated self assembly systems, molecular tweezers, optoelectronic devices, and fluorescence imaging agents. In recent times the chiral cleft-like structure of Tröger’s base has been incorporated in designing chiral probes for nucleic acids. Incorporation of the TB-unit resulted in helical structured DNA binding ligands, which can exhibit enantioselective binding with DNA.
3.2 Design and Synthesis of Bis-1,8-naphthalimide Derivatives Containing the Troger’s Base Moiety

As discussed in Chapter 1, several 4-amino substituted bis-naphthalimide derivatives incorporating the TB unit have been developed within the Gunnlaugsson group over the past few years.\textsuperscript{138,210} Previous studies with the racemic TB-derivatives showed that they possess high affinity for ct-DNA (ca. 10\textsuperscript{6} M\textsuperscript{-1}) and were readily taken up by leukemia cell lines (HL 60), where the derivatives localise in the nucleus. These TB-derivatives showed higher cytotoxicity against leukaemia cell lines compared to their 4-amino-1,8-naphthalimide precursors. This study was further extended by developing 3-amino-1,8-naphthalimide derived TB-derivatives.\textsuperscript{139} Recently as part of developing novel naphthalimide based TB-derivatives as supramolecular clefts for MOF frameworks, the first X-ray crystal structure of a pyridyl-naphthalimide TB derivative was characterised by Dr. J. A. Kitchen (Figure 3.1c), which showed that the two naphthalimide mean-planes intersected at 82°.\textsuperscript{217} As discussed before, such cleft like structure has significant potential in the field of supramolecular chemistry as well as medicinal chemistry. Building on the previous work within the Gunnlaugsson group, the aim of the work described in the chapter herein, was to develop novel $C_2$-symmetric chiral bis-1,8-naphthalimide derivatives $87$ and $88$ by incorporating the TB-motif. Moreover, it was aimed to resolve the enantiomers of $87$ and $88$ and to investigate the interaction of these enantiomers with DNA with the view to determine if there was any stereoselectivity of one enantiomer over the other. In designing $87$ and $88$, the ethyl pyridinium side chain was incorporated in the naphthalimide structure to provide high water
solubility and to favour electrostatic interactions with the negatively charged phosphate backbone of DNA. The synthesis and resolution of the TB-derivatives will be discussed in the following section, followed by discussion on the interactions of these derivatives with st-DNA and synthetic polynucleotides.

3.2.1 General Mechanism of Synthesis of the TB-derivatives

TB-derivative (±)-66 was originally synthesised by the reaction of p-toluidine and formaldehyde in the presence of HCl. Wagner initially investigated the mechanism of the formation of TB-derivatives. This study suggested that the reaction proceeds through several intermediates as shown in Scheme 3.1. The first step involves reaction of p-toluidine 93 with paraformaldehyde under acid catalysed conditions to give the imine 94 as an intermediate. Intermediate 94 then undergoes protonation and subsequently reacts with a second molecule of an aromatic amine to form aminobenzylarylamine 95. Intermediate 95 then undergoes condensation with excess formaldehyde to form the tetrahydroquinazoline 96; followed by condensation of 96 with another molecule of formaldehyde, which generates 1-hydroxymethyl tetrahydroquinazoline 97. Subsequent rearrangement of 97 and elimination of a water molecule under acid catalysed conditions finally generates the TB-derivative 66. Coelho and co-workers investigated the mechanism of formation of (±)-62 by reacting p-toluidine with paraformaldehyde in the presence of TFA by direct infusion electrospray ionisation mass and tandem mass spectrometric experiments and were able to characterise three key intermediates 94, 96 and 97.
3.2.2 Synthesis of Bis-1,8-naphthalimides containing the Tröger’s base

The bis-naphthalimide derivatives 87 and 88 containing the Tröger’s base unit were synthesised as racemic mixtures by reacting the 4-amino-1,8-naphthalimide precursors 81 and 82 with paraformaldehyde in neat TFA as shown in Scheme 3.2. The synthesis of the 4-amino precursors have been discussed in Chapter 2 (Section 2.2). Upon completion, excess TFA was removed under reduced pressure. The resulting residue was dissolved in minimum volume of methanol and the product was precipitated from diethyl ether to give 87 and 88 in 60% and 65% yields, respectively. Previously Dr. Veale demonstrated that the use of paraformaldehyde and TFA was more efficient than condensation of the 4-amino-1,8-naphthalimide with excess of formaldehyde in the presence of concentrated HCl, which yielded a mixture of products that were difficult to separate and furthermore the yield of the reaction was found to be low.\textsuperscript{138b}
The Tröger’s base derivative 87 and 88 were formed as racemic mixtures and were fully characterised by $^1$H, $^{13}$NMR, HRMS and IR analysis. The $^1$H-NMR spectra of 87 (600 MHz, DMSO-$d_6$) and 88 (600 MHz, CD$_3$CN) are shown in Figure 3.2 and Figure 3.3, respectively. In both cases, the presence of the diazocine ring was confirmed by the appearance of a well-separated doublet of doublets between 4.64 and 5.17 ppm, assigned to the methylene protons of the diazocine ring, which also reflect the $C_2$ symmetry of the molecule. In both cases, the aromatic region was composed of protons from both naphthalimide and pyridine rings. A detailed assignment of all the protons was performed by H-H COSY, HSQC and HMBC experiments.

![Figure 3.2: The $^1$H-NMR spectrum of 87 (DMSO-$d_6$, 600 MHz).]
Formation of the desired TB-derivatives was also confirmed from mass spectrometry, where 87 displayed a peak at \( m/z = 707.2169 \) corresponding to the \([\text{M}+\text{Cl}]^+\) ion (\([\text{M}+\text{Cl}]^+\cdot \text{C}_{41}\text{H}_{32}\text{N}_{6}\text{O}_{4}\text{Cl} \) requires 707.2174) and 88 displayed a peak at \( m/z = 700.2794 \) corresponding to the \( \text{M}^+ \) ion (\([\text{M}]^+\cdot \text{C}_{43}\text{H}_{36}\text{N}_{6}\text{O}_{4} \) requires 700.2798).

It should be mentioned here that even though 87 and 88 appeared pure from NMR and HRMS analysis, further spectroscopic studies suggested the presence of a minor strongly luminescent impurity, which could not be detected in the aforementioned analysis. The UV/vis absorption spectrum of 87 in 10 mM phosphate buffer (pH 7.0) is shown in Figure 3.4a, which displayed a broad absorption band centred at 382 nm and a shoulder at 350 nm, with high energy \( \pi-\pi^* \) transition bands were observed at shorter wavelength. In the same solvent, 87 displayed a broad fluorescence emission band with \( \lambda_{\text{max}} \) at 498 nm, upon excitation at \( \lambda_{\text{ex}} = 382 \) nm (Figure 3.4 b). However, when the excitation wavelength was changed to 410 nm, the emission band showed a slight shift with \( \lambda_{\text{max}} = 508 \) nm. Moreover, the excitation spectrum was found to be quite different than the UV/vis absorption spectrum, exhibiting two bands centred at \( \lambda_{\text{max}} = 365 \) nm and \( \lambda_{\text{max}} = 420 \) nm, indicating the possible presence of a luminescent impurity as mentioned above.

Figure 3.3: The \(^1\text{H}-\text{NMR spectrum of 88 (CD}_3\text{CN, 600 MHz).} \)
Several purification processes were attempted to remove this luminescent impurity, which are summarised in Table 3.1. Due to the presence of dicaticonic side chains, compounds 87 was initially attempted to be purified by cation exchange chromatography using C25 sephadex as the stationary phase, which is often used for the purification of bivalent metal complexes. Various mobile phases such as aqueous NaCl and p-toluene sulfonate solutions were used however successful purification of 87 could not be achieved. Crude 87 was also tried to be purified on silica gel using a mixture of CH$_3$CN:H$_2$O:NaCl (88:11:1), which was found to be efficient for the purification of the cationic 4-amino-1,80naphthalimides 82-83. However, this solvent system did not result in a satisfactory separation of the bands on either silica or alumina in the case of 87. Finally, compound 87 was obtained in high purity using silica gel column and a mixture of CH$_3$CN:H$_2$O:NaNO$_3$ (saturated) (88:10:2) as the eluent system following a modified literature procedure. This eventually resulted in a separation of two bands; of which the first band was found to be the highly fluorescent impurity and the second band being the actual Tröger’s base product. Compound 87 was precipitated as its PF$_6^-$ salt to remove the excess NaNO$_3$, before being converted to the Cl$^-$ salt using amberlite-IRA-400(Cl) in methanol. Excess methanol was removed under reduced pressure and 87 was obtained as a bright yellow solid in 57% yield. Due to very low concentration, it was not possible to identify the highly luminescent impurity present in the sample.
Chapter 3: Photophysical and DNA Binding Studies of the Tröger’s Base Containing Bis-1,8-naphthalimides

Table 3.1: Summary of various purification attempts for 87.

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Stationary phase</th>
<th>Mobile phase</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C25 sephadex</td>
<td>NaCl (0-0.5 M)</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>C25 sephadex</td>
<td>Sodium p-toluene sulfonate (0.1 M)</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>Silica</td>
<td>CH$_3$CN:H$_2$O:NaCl (saturated) 88:11:1</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td>Alumina</td>
<td>CH$_3$CN:H$_2$O:NaCl (saturated) 88:11:1</td>
<td>x</td>
</tr>
<tr>
<td>5</td>
<td>Silica</td>
<td>CH$_3$CN: H$_2$O: NaNO$_3$ (saturated) = 88: 10: 2</td>
<td>✓</td>
</tr>
</tbody>
</table>

The UV/vis absorption, emission and excitation spectra of 87 after column chromatography are shown in Figure 3.5. Emission of 87 in aqueous media was found to be very weak with the emission band centred at 560 nm. Since the emission intensity was found to be very low, all the measurements were carried out using 20 nm and 10 nm slits for excitation and emission bandpasses, respectively. As seen from Figure 3.5b, two bands were observed at ca. 340 nm and 380 nm in the excitation spectrum of 87, which were similar to the UV/vis spectrum though they were found to be very weak and noisy. Following the successful purification of 87, compound 88 was also obtained as a yellow solid in 53% yield after purification by column chromatography on silica gel using a mixture of CH$_3$CN:H$_2$O:NaNO$_3$ (80:18:2) as the eluent. Fluorescence measurements also showed that 88 was also weakly emissive in aqueous media similar to 87. The emission and the excitation spectra of 88 are shown in Appendix 3 (Figure A3.1).

![Figure 3.5: (a) The fluorescence emission spectra with λ$_{ex}$ = 380 nm (—), 400 nm (—) and (b) the excitation spectra with λ$_{em}$ = 520 nm (—), 540 nm (—), 560 nm (—) of 87 (12 μM) in 10 mM phosphate buffer (pH 7.0).](image-url)
3.3 Enantiomeric Resolution of TB Derivatives

As discussed in Section 3.1, TB-derivatives are chiral molecules with two bridgehead nitrogen centers. However, most of the applications exploited the “V” shaped geometry of (±)-66 and deal with racemates. Studies with enantiomerically pure TB-analouges are very limited largely because of the poor availability of the pure enantiomers. In acidic medium TB-derivatives have been reported to undergo racemisation through the formation of iminium intermediate, which suggests that the use of acidic resolving agent such as 10-camphorsulfonic acid is not suitable for the separation of enantiomers. In recent times, enantiomeric separation of several TB-derivatives has been achieved through diastereomeric salt formation using di-p-toluoyl tartaric acid, dibenzoyl-L-tartaric acid. These examples have been discussed in Chapter 1.

With the advent of chiral stationary phase (CSP), high performance liquid chromatography using CSP appears to be an attractive method for enantiomeric resolution. Prelog and Wieland demonstrated the partial separation of enantiomers of (±)-66 using α-D-lactose. Separation of enantiomers of TB-derivatives on microcrystalline cellulose triacetate has been studied in detail. Efficient separation of the enantiomers of (±)-66 on cellulose triacetate stationary phase has been used as a model system for simulated moving bed chromatography. Among the polysaccharide based CSPs, phenylcarbonate, benzoyleformate, benzoylecarbamate functionalised cellulose and amylose have been used for the resolution of (±)-66. Methods for the separation of (±)-66 have also been developed using a steroid glycoside and chiral poly(diphenyl-2-pyridylmethyl methacrylate) as chiral selectors in HPLC and in capillary electrochromatography. Sergeyev et al., reported the analytical separation of enantiomers for several functionalised TB-derivatives using commercially available Chiralcel OJ that contains cellulose tris-(4-methyl benzoate) as the chiral selector. Use of “brush type” CSP WhelkO1 with covalently bound 3,4-disubstituted 1,2,3,4-tetrahydrophenanthrene as the chiral selector was found to be a superior choice over Chiralcel OJ for TB-derivatives that show strong retention on cellulose. Covalently bonded CSPs are more advantageous compared to polysaccharide coated CSPS, which can leach off with use. Moreover, covalently bonded CSPs are compatible with a wide range of solvents including highly polar and chlorinated ones.

In this study, the separation of the enantiomers of the cationic TB-derivatives 87 and 88 was achieved by cation exchange chromatography using C25 sephadex as the stationary phase and a chiral anion as the eluent, which will be described in the following section.
3.3.1 Separation of Enantiomers of (+)-87 and (−)-88

The TB-derivatives 87 and 88 described in this work have been synthesised as racemates as described in Section 3.2.2 (Scheme 3.2). The separation of (+)- and (−)-enantiomers of TB-derivatives (+)-87 and (−)-88 in this work have been achieved using a column chromatographic technique developed by Keene and co-workers using SP Sephadex C25 as the stationary phase and a chiral eluent sodium (−)-dibenzoyl-L-tartarate as the mobile phase. In this technique, the cationic analyte mixtures to be separated are adsorbed on the anionic stationary phase and eluted with suitable eluent (usually as aqueous solution of their Na+ salt). The separation is achieved as a result of differential ion-exchange interactions between the components of the analyte mixture and the eluent electrolyte solution. SP-sephadex consists of a cross-linked α-D-glucopyranoside functionalised with propane sulfonate, which provides a chiral environment. Cation exchange chromatography is mainly driven by coulombic forces, however, the charge densities or polarities of the cations can also influence the separation process. In the case of the enantiomers, separation on SP-Sephadex stationary phase using an achiral eluent arises as a consequence of differential interaction of the matrix with two enantiomers on the basis of their shape. Since the sephadex matrix itself has a significant effect on the separation of two enantiomers, use of suitable chiral counter anion in the eluent solution can significantly improve the resolution. Chiral anions in the eluent solution can display differential association with the enantiomers thereby affecting the overall separation process.

For the resolution of (+)-87, the compound was dissolved in a minimum amount of water and adsorbed onto the Sephadex stationary phase in a 1 m long Perspex column (diameter 2 cm) and an aqueous solution of sodium (−)-dibenzoyl-L-tartarate (0.05 M) neutralised to pH 7.0 was employed as the mobile phase. Compound (+)-87 was allowed to recycle through the column for three times to facilitate the complete resolution into two distinct bands. Both the bands were collected into several fractions and combined accordingly. The enantiomers were precipitated as PF6 salts from the collected fractions and converted into Cl salts by treatment with Amberlite-IRA-400(Cl) in methanol. The enantiomeric purity was confirmed by circular dichroism analysis (Figure 3.6a).

In the case of resolution of (±)-88, the compound was found to stick on the sephadex matrix in the presence of 0.05 M solution of sodium (−)-dibenzoyl-L-tartarate (pH 7.0) and did not resolve. Therefore, the concentration of the eluent solution was raised to 0.07 M and like compound (±)-87, effective separation of two enantiomers was achieved after recycling (±)-88 three times through the column (Figure 3.6b).
Importantly, enantiomers of both compounds 87 and 88 were found to be stable in 10 mM phosphate buffer (pH 7.0) and did not undergo racemisation over a period of 6 months. This is probably due to the mild separation conditions used here (aqueous eluent solution at pH 7.0), which does not promote protonation on the bridgehead nitrogen atoms and induce racemisation, since TB-derivatives are prone to undergo racemisation under acidic condition.

![Figure 3.6: Circular dichroism spectra of (+) and (-) enantiomers of (a) 87 (10 μM) and (b) 88 (14 μM) in 10 mM phosphate buffer (pH 7.0).](image)

3.4 Effect of Solvent Polarity on the Photophysical Properties of (±)-87 and (±)-88

The excited state properties of 1,8-naphthalimide derivatives largely depend on the nature of substituent on the naphthalene ring as was discussed in Chapter 1 and 2. The presence of an electron donating substituent at the 4-position results in a polar internal charge transfer (ICT) excited state, due to the "push-pull" nature of the chromophore, originating from the electronic conjugation between the electron donating substituent at the 4-position and electron accepting imide functional groups. The photophysical properties of the 4-amino precursors 82 and 83 in different solvents have been discussed in Chapter 2, which showed that the ground state UV/vis spectra, quantum yield of fluorescence and the fluorescence lifetime of these derivatives depend strongly on the polarity and H-bonding ability of solvents. In order to investigate the effect of the Tröger's base moiety on the photophysical properties, the UV/vis absorption and fluorescence spectra of (±)-87 and (±)-88 were recorded in different solvents of varying polarity, which will be described in the following sections.
3.4.1 Ground State Studies of 87-88 in Various Solvents

The UV/vis absorption spectra of (±)-87 in different solvents are shown in Figure 3.7a. Both of the TB-derivatives 87 and 88 exhibited much smaller shift in the $\lambda_{\text{max}}$ of absorption band with increasing solvent polarity than that observed with the 4-amino-1,8-naphthalimides. In dichloromethane (CH$_2$Cl$_2$), (±)-87 displayed a broad band at 388 nm with a sharp shoulder at 347 nm. On changing the solvent from nonpolar CH$_2$Cl$_2$ to water, the absorption band at 388 nm showed a ca. 8 nm blue shift. The effect of solvent polarity on the UV/vis absorption spectrum of (±)-88 was also investigated in the same set of solvents and a few representative spectra are shown in Figure 3.7 b. Similar to (±)-87, compound (±)-88 also displayed a broad absorption band at 390 nm with a sharp shoulder at 348 nm in CH$_2$Cl$_2$. A change in solvent from CH$_2$Cl$_2$ to water resulted in a ca. 10 nm blue shift in the $\lambda_{\text{max}}$ of the absorption band.

![Figure 3.7: Effect of solvent polarity on the UV/vis spectra of (a) (±)-87 and (b) (±)-88.](image)

Previously Deprez et al.,$^{229}$ and Veale et al.,$^{138,210}$ have also reported similar insensitivity of the UV/vis spectra of TB-derivatives towards solvent polarity. The small shift observed in the UV/vis absorption spectra of the TB-derivatives might be due to its “V”-shaped structure imposed by the Tröger’s base motif, which affects the ability of the 4-amino group to donate its lone pair of electrons to the naphthalene ring. Though the absorption spectra of (±)-87 and (±)-88 were not significantly affected by the polarity and H-bonding ability of the solvents, the ICT nature of the absorption band was evident from the following observations: i) a fairly broad shape of the absorption band and ii) an intense nature with high molar extinction coefficient in the range 10,000-15,000 M$^{-1}$cm$^{-1}$.
3.4.2 Steady-State Fluorescence Spectra and Quantum Yield of Emission

Unlike the ground state UV/vis spectra, the effect of varying solvent polarity was much more pronounced in the emission spectra of (±)-87 and (±)-88 as shown in Figure 3.8a and 3.8b, respectively. The fluorescence excitation spectra of (±)-87 and (±)-88 are shown in Appendix 3 (Figure A3.2). All the fluorescence measurements were carried out such that the compounds had an absorbance of ca. 0.1 at the excitation wavelength, which was chosen as the \( \lambda_{\text{max}} \) observed in the absorption spectrum of (±)-87 and (±)-88 in each solvent. In CH\(_2\)Cl\(_2\), (±)-87 displayed a broad emission band centred at 520 nm. Changing the solvent from CH\(_2\)Cl\(_2\) to water caused a ca. 40 nm red shift in the \( \lambda_{\text{max}} \) of the emission band. The emission parameters for (±)-87 are summarised in Table 3.2.

![Figure 3.8: Effect of solvent polarity on the fluorescence spectra of (a) 87 and (b) 88.](image)

Compound (±)-88 also displayed a similar broad emission band centred at 512 nm in CH\(_2\)Cl\(_2\), which was found to show a ca. 48 nm red shift on changing the solvent from CH\(_2\)Cl\(_2\) to water. The fluorescence spectral data for (±)-87 and (±)-88 are summarised in Table 3.3. Such significantly large shift in \( \lambda_{\text{max}} \) of emission with increasing solvent is characteristic of an ICT excited state, where polar and H-bonding solvents stabilise the polar excited state to a greater extent.\(^3\) This consequently decreases the energy gap between \( S_0 \) and \( S_1 \) and results in red shifted emission band in highly polar solvents.\(^3\)^

The fluorescence quantum yields (\( \Phi_F \)) of 87 and 88 were measured in different solvents using quinine sulfate (\( \Phi_F = 0.546 \) in 1 N H\(_2\)SO\(_4\), \( \lambda_{\text{ex}} = 365 \) nm) as the reference.\(^1\) The method for determination of the quantum yield of emission is detailed in the Experimental Chapter. Both the TB-derivatives 87 and 88 were found to have high \( \Phi_F \) in non-polar solvents such as CH\(_2\)Cl\(_2\). However, with an increase in solvent polarity and H-bonding ability the \( \Phi_F \) values were found to decrease significantly, and the compounds were nearly non-emissive in water, where the fluorescence intensities of 87 and 88 were even weaker than the Raman scattering band of water and therefore accurate \( \Phi_F \) values could not be determined.
for these compounds in water. The $\Phi_F$ values for 87 and 88 in different solvents are summarised in Table 3.2 and Table 3.3, respectively.

Deprez et al.,$^{229}$ and Veale et al.,$^{138,210}$ have also reported similar trends in $\Phi_F$ as a function of solvent polarity for various TB-derivatives. As the TB-derivatives 87 and 88 display a significant red shift of emission band and marked decrease in $\Phi_F$ of emission with an increase in solvent polarity similar to the 4-amino-1,8-naphthalimide precursors, it can be concluded that they also possess ICT excited state.

**Table 3.2: Summary of various UV/vis and emission spectral data for 87 in different solvents.**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (Abs) nm</th>
<th>$\lambda_{\text{max}}$ (Flu) nm</th>
<th>$\Phi_F^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>388</td>
<td>520</td>
<td>0.089</td>
</tr>
<tr>
<td>Acetone</td>
<td>387</td>
<td>530</td>
<td>0.013</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>388</td>
<td>545</td>
<td>0.009</td>
</tr>
<tr>
<td>DMF</td>
<td>387</td>
<td>550</td>
<td>0.007</td>
</tr>
<tr>
<td>Ethanol</td>
<td>385</td>
<td>552</td>
<td>0.003</td>
</tr>
<tr>
<td>Water</td>
<td>382</td>
<td>560</td>
<td>$&lt;0.001^b$</td>
</tr>
</tbody>
</table>

$^a$Error $\pm$ 10%.

$^b$An accurate measurement of $\phi_F$ in water could not be done due to interference from Raman Scattering band.

**Table 3.3: Summary of various UV/vis and emission spectral data for 88 in different solvents.**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (Abs) nm</th>
<th>$\lambda_{\text{max}}$ (Flu) nm</th>
<th>$\phi_F^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>390</td>
<td>512</td>
<td>0.098</td>
</tr>
<tr>
<td>Acetone</td>
<td>386</td>
<td>538</td>
<td>0.017</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>385</td>
<td>546</td>
<td>0.011</td>
</tr>
<tr>
<td>DMF</td>
<td>389</td>
<td>550</td>
<td>0.008</td>
</tr>
<tr>
<td>Ethanol</td>
<td>387</td>
<td>552</td>
<td>0.004</td>
</tr>
<tr>
<td>Water</td>
<td>380</td>
<td>560</td>
<td>$&lt;0.004^b$</td>
</tr>
</tbody>
</table>

$^a$Error $\pm$ 10%.

$^b$An accurate measurement of $\phi_F$ in water could not be done due to interference from Raman Scattering band.

The significant decrease in $\Phi_F$ on going from nonpolar to polar and H-bonding solvent is presumably due to increased non-radiative decay mechanisms in the polar solvents as suggested by Deprez et al.$^{229}$ Yuan et al., have also explained the very weak emission of TB-derivative such as 71 (discussed in Chapter 1) in aqueous solution in terms of various non-radiative processes such as intramolecular vibrations, enantiomerisation, etc.$^{174-175}$ In the solid phase, molecules are loosely packed due to their "V"-shaped structure, which decreases the extent of distance dependent intermolecular quenching and results in enhanced emission in the case of 71. In a recent communication, Parise and co-workers demonstrated that in addition to the geometrical connecting role, the
methano diazocine ring can also induce electronic coupling between the adjacent aryl rings and cause charge delocalisation, which may also be responsible for the weak emission efficiency in high polar solvents.\textsuperscript{230}

As described above, the photophysical properties of 87 and 88 depend strongly on the polarity and H-bonding ability of the solvent, it was therefore anticipated that the photophysical properties of these molecules could also be affected if they bind to DNA as DNA has widely varying polarity along the grooves. The changes in the photophysical behaviour of 87 and 88 in the presence of DNA can be monitored via spectroscopic titrations, which can also provide information on the equilibrium binding constant and binding site size. These measurements will be described in the following sections.

3.5 Ground State Interaction Studies of 87-88 with st-DNA in 10 mM Phosphate Buffer

It was anticipated while designing the bis-naphthalimide derivatives 87 and 88 that incorporation of the TB-motif would result in a "V" shaped structure that can position the two naphthalimide chromophores into the major or minor groove of DNA and the cationic pyridinium side chain would be able to interact with the negatively charged phosphate backbone. Such mode of binding has been observed previously for acridine based Tröger's base derivatives.\textsuperscript{157} Alternatively, one of the naphthalimide chromophores can potentially intercalate between the DNA basepairs while the other naphthalimide unit can bind to the groove. Such mixed mode of binding has been previously reported for assymetric acridine-phenanthroline Tröger's base derivative\textsuperscript{179} as well as for the 3-amino-1,8-naphthalimide derived TB derivatives.\textsuperscript{139} All these possible modes of interactions are shown in Figure 3.9.

\textbf{Figure 3.9:} Possible modes of interaction of 87-88 to double stranded DNA.
3.5.1 Interaction of (±)-87 with st-DNA

To evaluate the ability of (±)-87 to bind to st-DNA, UV/vis absorption titrations were carried out in 10 mM phosphate buffer (pH 7.0) by adding small aliquots of st-DNA to a buffered solution of (±)-87. The titrations were repeated three times to ensure reproducibility and the binding parameters presented in this chapter are the average of three measurements. In 10 mM phosphate buffer (pH 7.0), (±)-87 displayed a broad band at 382 nm (ε = 12,000 M⁻¹ cm⁻¹) with a shoulder at 350 nm (Figure 3.10). Moreover, high-energy π-π* transitions were observed at 230 nm. The interaction of (±)-87 with st-DNA was followed by recording the changes in the absorption band at 382 nm, which is far beyond the absorption band of nucleobases at 260 nm. In the presence of st-DNA, the absorbance of the band at 382 nm was found to decrease by about 34% (hypochromism) accompanied with a ca. 10 nm red shift in the λ_max. An isosbestic point was observed at 412 nm for all DNA/ligand concentrations suggesting the presence of two distinct species i.e. free and bound ligand. These changes are shown in Figure 3.10 Inset and suggest strong ground state interaction of (±)-87 with st-DNA. Similar changes in absorption spectrum have been previously reported for several naphthalimide derivatives 134,185b,201 as well as the 1,8-naphthalimide based TB-derivatives.138,139,210

Figure 3.10: The UV/vis absorption spectra of (±)-87 (10.4 μM) in the presence of increasing concentration of st-DNA (0-146 μM) in 10 mM phosphate buffer (pH 7.0). Inset: Plot of relative changes absorbance at 382 nm (A/A₀) vs. [DNA base]/[87] i.e. P/D.
3.5.2 Ground State Interaction Studies of (+) and (-) enantiomers of 87 with \( st\)-DNA

The UV/vis absorption spectra of the (+) and (-)-enantiomers of 87 were recorded in 10 mM phosphate buffer (pH 7.0) in the presence of increasing concentration of \( st\)-DNA. In the presence of \( st\)-DNA, the UV/vis spectra of both of the enantiomers were affected in a similar manner to that observed for (±)-87 as shown in Figure 3.11a and 3.11b, respectively. The absorbance of the band at 382 nm decreased significantly in the presence of \( st\)-DNA accompanied with a red shift in the \( \lambda_{\text{max}} \). However, subtle differences were observed in the magnitude of these changes for the two enantiomers as in the case of (-)-87, slightly higher degree of hypochromism (36\%) was observed for the 382 nm absorption band than that seen for (+)-87 (ca. 34\%). Importantly, in the case of (-)-87 a clear isosbestic point was observed at 410 nm in the presence of \( st\)-DNA, however, the isosbestic point was not so clear in the case of (+)-87. This could be due to more than one mode of binding of the (+)-enantiomer with \( st\)-DNA. These changes are summarised in Table 3.4. The plots of \( A/A_0 \) vs. nucleotide phosphate/drug (P/D) for (±), (+) and (-)-87 are shown in Figure 3.11c, which showed that for both the enantiomers and (±)-87, the changes in absorbance reached a plateau at P/D of 2.5.

The high degree of hypochromism and bathochromic shifts observed in the UV/vis spectra of both (+) and (-)-87 and the (±)-87 is usually observed for ligands binding to \( st\)-DNA via intercalation. However, such hypochromic effect and bathochromic shift in the UV/vis absorption spectra are not exclusively observed for the intercalators; several groove binders for example Hoechst 33258 and its analogues also displayed similar hypochromism and red shift in the \( \lambda_{\text{max}} \) upon binding to DNA.\(^{41,231}\) Therefore, several other measurements are required to determine the mode of binding, which will be described in Section 3.10.

\textbf{Table 3.4: Summary of various binding parameters obtained from the UV/vis titration for (±)-87, (+)-87, (-)-87 in 10 mM phosphate buffer (pH 7.0).}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(±)-87</th>
<th>(+)-87</th>
<th>(-)-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (free) (nm)</td>
<td>382</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (bound) (nm)</td>
<td>392</td>
<td>390</td>
<td>392</td>
</tr>
<tr>
<td>( \Delta \lambda_{\text{max}} ) (nm)</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>35</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>412</td>
<td>418</td>
<td>410</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>2.5 → 15</td>
<td>2.5 → 15</td>
<td>2.5 → 15</td>
</tr>
</tbody>
</table>
3.5.3 Interaction of (±)-88 with st-DNA

Introduction of alkyl, especially methyl substituent on the nucleobase or ligand has been shown to improve the stacking interaction and binding affinity of ligands for the target nucleotide sequences. This has often been explained in terms of favourable hydrophobic interactions. Additionally, the presence of methyl groups has also been shown to increase the molecular polarisability, thereby allowing more favourable van der Waals interactions between the neighbouring molecules. Taking these points into consideration, it was anticipated that the introduction of methyl substituent on the pyridine ring might result in improved binding of the resulting TB-derivative 88 with DNA.

The TB-derivative (±)-88 displayed a broad band at 380 nm \( (\varepsilon = 10,700 \text{ M}^{-1} \text{ cm}^{-1}) \) with a shoulder at 350 nm similar to that previously observed for (±)-87 in 10 mM phosphate buffer (pH 7.0). Moreover, a high-energy \( \pi-\pi^* \) transition was also observed at 230 nm. The
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interaction of (±)-88 with st-DNA was monitored by recording the changes in the absorption spectra after adding small aliquots of st-DNA to a buffered solution of (±)-88 in 10 mM phosphate buffer (pH 7.0) until a plateau was reached (Figure 3.12) in a manner similar to that described above.

The changes in the UV/vis spectrum of (±)-88 in the presence of st-DNA were strikingly different from the behaviour of (±)-87. In case of (±)-88, with increasing concentration of st-DNA, the absorbance of ICT absorption band centred at 380 nm initially decreased by ca. 40% upto P/D of 2 accompanied with a 10 nm bathochromic shift in the λ_{max}. However, with further increase in DNA concentrations (P/D 2-15), the absorbance of this band increased by ca. 15% without any further shift in the λ_{max}. Veale et al., have also reported similar biphasic binding patterns for other 4-amino-1,8-naphthalimide based TB derivatives.\textsuperscript{138,210}

![Figure 3.12: (a) The UV/vis absorption spectra of (±)-88 (9.6 μM) in the presence of increasing concentration of st-DNA (0-150 μM) in 10 mM phosphate buffer (pH 7.0); Inset: Plot of A/A₀ (at 380 nm) vs. P/D for (±)-87 (●), (±)-88 (■).](image)

Analogous biphasic behaviour has previously been seen with the binding of [Ru(phen)₂dppz]\textsuperscript{2+} complex 89\textsuperscript{31} and binding of pyropheophorbide-spermine conjugate 90\textsuperscript{233} with ct-DNA. It has been suggested that for 89, the initial decrease in absorbance at lower DNA concentration results from the stacking interaction of the planar dppz moiety with DNA bases,\textsuperscript{31} while the subsequent increase in absorbance in the “second phase” results from the redistribution of the complex, resulting in increased distances between the closely bound metal complexes, which partly reverses the hypochromic effect. On the other hand, 90 has been shown to form aggregates in phosphate buffer. Addition of ct-DNA to a solution of 90 in
phosphate buffer results in a biphasic binding interaction, depending on the [90]/[DNA] molar ratio. In the presence of lower concentrations of DNA, the aggregated dye molecules bind to DNA resulting in the initial hypochromism in the absorbance. In the presence of excess DNA, the molecules can redistribute themselves and the subsequent destacking event results in an increase in absorbance at high DNA concentrations.

TB derivative (+)-88 was found to obey the Beer-Lambert law up to a concentration of 100 μM in 10 mM phosphate buffer (pH 7.0) and no additional band was observed in the UV/vis spectrum of (+)-88 even at very high concentration. These suggest that at the concentration (ca. 10 μM) employed in the titrations, (+)-88 predominantly exists as a monomer. Therefore, the biphasic binding observed for (+)-88 can possibly be explained considering DNA-induced aggregation at low DNA concentrations, where the molecules stack along the polyanion backbone, possibly due to strong electrostatic interactions and results in a sharp decrease in absorbance up to a P/D of 2. However, as the DNA concentration is raised, molecules reorganise themselves along the DNA backbone and bind to their preferred sites, which consequently increases the absorbance due to destacking.

Comparison of the binding of (+)-87 and (+)-88 (Figure 3.12 Inset) with ±-DNA reveals that for (+)-88 the extent of hypochromism was significantly higher and the changes in the absorbance reached a plateau at lower P/D (1.6) compared to (+)-87 (plateau at P/D 2.5). The substantially stronger binding observed for (+)-88 presumably results from the presence of the additional methyl group, which favours the binding to DNA due to the hydrophobic effect. Similar high affinity binding has been previously observed for several other DNA binders such as Cr(III) complex bearing a dimethyl dppz ligand, 208 dimethyl-pteridine 91 and 2-amino-1,8-naphthyridine 92 derivatives. Moreover, the presence of methyl groups can also increase the polarisability of (+)-88 and consequently allows better stacking along DNA due to favourable van der Waals interaction as described earlier. 189a,232
3.5.4 Ground State Interaction Studies of (+) and (-) Enantiomers of 88 with st-DNA

The interaction of the two enantiomers with st-DNA was investigated in a manner similar to that described above by adding small aliquots of st-DNA to a buffered solution of an enantiomerically pure compound until the changes in absorbance reached a plateau. The changes in the UV/vis absorption spectra of the (+) and (-)-88 in the presence of st-DNA are shown in Figure 3.13. In the presence of an increasing concentration of st-DNA, the absorbance of the band centred at 380 nm decreased by ca. 40% initially for both of the enantiomers up to a P/D of 1.6 accompanied with a bathochromic shift in the $\lambda_{\text{max}}$, similar to what was observed for (±)-88. Upon further increasing the DNA concentrations, the absorbance of the band centred at 380 nm increased by ca. 15%. An isosbestic point was observed at ca. 418 nm for both of the enantiomers up to a P/D of 1.6. The spectral changes of (±)-88, (+)-88 and (-)-88 in the presence of st-DNA are summarised in Table 3.5.

Table 3.5: Summary of various binding parameters obtained from the UV/vis titration for (±)-88, (+)-88, (-)-88 in 10 mM phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-88</th>
<th>(+)-88</th>
<th>(-)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ (free) (nm)</td>
<td>380</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (bound) (nm)</td>
<td>390</td>
<td>388</td>
<td>390</td>
</tr>
<tr>
<td>$\Delta \lambda_{\text{max}}$ (nm)</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>420</td>
<td>418</td>
<td>418</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>40 (P/D = 0→1.6)</td>
<td>40 (P/D = 0→1.6)</td>
<td>41 (P/D = 0→1.6)</td>
</tr>
<tr>
<td>% Hyperchromism</td>
<td>15 (P/D = 2.0→15)</td>
<td>14 (P/D = 2.0→15)</td>
<td>15 (P/D = 2.0→15)</td>
</tr>
</tbody>
</table>

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Figure 3.13: The UV/vis absorption spectra of (a) (+)-88 (9.1 μM) in the presence of increasing concentration of st-DNA (0-136 μM); (b) (-)-88 (8.8 μM) in the presence of increasing concentration of st-DNA (0-132 μM in 10 mM phosphate buffer (pH 7.0); (c) Plot of $A/A_0$ vs. P/D for (+)-88 (●) and (-)-88 (■) at 380 nm.

The binding constants for the association of (±)-87 and (±)-88 and their enantiomers were also evaluated from the changes in the UV/vis absorption spectra, which will be discussed in the following section.

3.5.5 Binding Constants Determined from UV/vis Titrations

The binding constants for the association of (±)-87, (±)-88 and their enantiomers with st-DNA were determined by analysing the changes in absorbance at 382 and 380 nm respectively in the presence of st-DNA using the non-cooperative model of Mcghee and von Hippel (equation 2.9 in Chapter 2).

Assuming non-cooperative and binding to one type of discrete site, binding constants were also determined from the Bard model using equation 3.1 and 3.2, where, $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ correspond to the apparent extinction coefficient, extinction coefficient for the free compound and extinction coefficient for the bound ligand. $C$ is the total concentration of the ligand, [DNA] is the concentration of DNA in terms of base pair and $n$ is number of nucleotides occupied by the bound ligand.
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\[
\frac{\varepsilon_a - \varepsilon_f}{\varepsilon_b - \varepsilon_f} = \frac{b - (b^2 - \frac{2K^2bC[DNA]}{n})^{1/2}}{2KbC}
\]

(3.1)

\[
b = 1 + K_bC + K_b[\text{DNA}]/2n
\]

(3.2)

Representative binding plots for (±)-87 are shown in Figure 3.14 and the binding constants are summarised in Table 3.6. The plots for (+)-87, (-)-87 are shown in Appendix (Figure A3.3 and A 3.4).

\(\text{Figure 3.14: (a) Plot of } r vs. r/C_f \text{ and fit } (\rightarrow) \text{ to equation 2.9; (b) Plot of } (\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) \text{ vs. } [\text{DNA bp}] \text{ and fit } (\rightarrow) \text{ to equation 3.1 for (±)-87.}\)

As shown in Figure 3.12 and 3.13, (±)-88 and its enantiomers exhibited biphasic-binding interaction with st-DNA at 10 mm phosphate buffer (pH 7.0). Therefore, the binding constant for the association of (±)-88 and its enantiomers could not be determined using the non-cooperative model of McGhee and von Hippel due to a lack of defined end points in the titration. However, the changes in the absorption spectra in the first phase of the binding process could be satisfactorily fitted to the Bard model (equation 3.1) to determine the binding constants for the first binding phase. It should be mentioned here, due to an insufficient number of points in the plateau region, the binding constants values determined from the Bard model (equation 3.1) were found to be associated with large errors. The representative plots for the two enantiomers of (±)-88 are shown in Figure 3.15.
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Figure 3.15: Plot of \((c_a - c_d)/(c_d - c_a)\) vs. [DNA bp] using the data points between P/D 0→2.0 and fit (—) to equation 3.1 for (a) (+)-88 and (+)-88.

Table 3.6: Summary of binding constants for (±)-87, (±)-88 and their enantiomers with st-DNA in 10 mM Phosphate buffer (determined from the changes in absorbance at 382 nm).

<table>
<thead>
<tr>
<th></th>
<th>McGhee-von Hippel Model</th>
<th>Bard Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(K_b (\times 10^6 \text{ M}^{-1}))</td>
<td>(n \text{ (base pair)})</td>
</tr>
<tr>
<td>(±)-87</td>
<td>1.18 ± 0.08</td>
<td>0.69 ± 0.01</td>
</tr>
<tr>
<td>(+)-87</td>
<td>1.01 ± 0.06</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>(-)-87</td>
<td>1.67 ± 0.09</td>
<td>0.71 ± 0.01</td>
</tr>
<tr>
<td>(±)-88</td>
<td>[a]</td>
<td>[a]</td>
</tr>
<tr>
<td>(+)-88</td>
<td>[a]</td>
<td>[a]</td>
</tr>
<tr>
<td>(-)-88</td>
<td>[a]</td>
<td>[a]</td>
</tr>
</tbody>
</table>

[a] Data could not be fitted due to lack of proper end point. [b] Data upto P/D = 2 were fitted to the Bard model.

In general the binding constant analysis from both the McGhee von Hippel and the Bard model suggests that the TB-derivatives 87 and 88 have substantially higher affinity (ca. \(10^6 \text{ M}^{-1}\)) for st-DNA compared to their 4-amino precursors (ca. \(10^5 \text{ M}^{-1}\)). The binding constant values obtained for 87 and 88 are significantly higher than several commonly used as acridine based antitumour agents, such as 9-aminoacridine (5.3\(\times 10^5 \text{ M}^{-1}\)), \(m\)-AMSA and \(\alpha\)-AMSA (AMSA: 4′-(9-acridinyl-amino)methanesulfonanilide) 1.8 \(\times 10^5 \text{ M}^{-1}\) and 3.2 \(\times 10^5 \text{ M}^{-1}\) respectively,²³⁵ 9-anilinoacridine derivatives²³⁶ at an ionic strength of 0.01 M. Similar binding affinity has also been reported for other naphthalimide based TB-derivatives.¹³⁸,¹³⁹,²¹⁰

Interestingly, the binding site size (n) was found to be less than unity for both of the TB-derivatives and their enantiomers (Table 3.6). According to the neighbour-exclusion principle, for simple intercalators such as proflavin, value of n should be ca. 2 basepairs. The significantly lower values of n observed here suggest that the TB-derivatives probably bind to the DNA grooves rather than being intercalated. The mode of binding of these derivatives will
be described in Section 3.10. Comparison of the two TB-derivatives showed that TB-derivative 88 binds about 4.5 times stronger to st-DNA than 87. This is presumably due to the presence of a methyl group on the pyridinium nitrogen, which allows favourable hydrophobic and van der Waals interaction with DNA as explained above. Additionally, as seen from Table 3.5, the (-)-enantiomers of both 87 and 88 showed slightly higher extent of hypochromism and larger binding constant than the corresponding (+)-enantiomers for st-DNA in 10 mM phosphate buffer, however, the magnitudes of the binding constants are not significantly different at 10 mM phosphate buffer. Therefore, the measurements were also carried out at higher ionic strengths (in the presence of 50 mM and 150 mM NaCl) to investigate any sort of enantiomeric preference, which will be discussed in the following section.

3.6 Effect of Ionic Strength on the Interaction of 87-88 with st-DNA

As previously mentioned, the pyridinium side chain was incorporated in designing 87 and 88 to provide high water solubility and allow improved electrostatic interaction with the DNA polyanionic backbone. Therefore, the ionic strength of the medium is expected to play a crucial role in the interaction of 87 and 88 with DNA. As discussed in Chapter 2, usually, an increase in the ionic strength of the solution results in the shrinking of a DNA helix as large numbers of cations can shield the negative phosphate groups more efficiently and reduce the phosphate-phosphate repulsion. Therefore, at higher ionic strength, the binding affinity of ligands is expected to decrease. To investigate the effect of electrostatic interaction on the overall DNA binding of these compounds, the UV/vis absorption spectra of 87 and 88 were recorded at P/D 10 such that the ligands are completely bound to DNA and the ionic strength of the solution was gradually increased by adding small aliquots of NaCl from a stock solution of 5 M. The UV/vis spectra of (±)-87 in the absence of DNA (P/D = 0), in the presence of st-DNA (P/D = 10) and the changes observed upon increasing NaCl concentration are shown in Figure 3.16. With increase in NaCl concentration (0-485 mM), the absorbance of the band at 392 nm (corresponding to bound 87) was found to increase by ca. 29% accompanied by a 10 nm blue shift in the $\lambda_{\text{max}}$. These changes suggest partial reversal of the binding at higher ionic strength. However, even at very high ionic strength ([NaCl] = 485 mM), the absorbance of 87/st-DNA mixture was still lower than that of the free 87, which signifies that the binding process is mostly irreversible.
Chapter 3: Photophysical and DNA Binding Studies of the Tröger's Base Containing Bis-1,8-naphthalimides

Figure 3.16: (a) The UV/vis absorption spectra of (±)-87 (9.3 μM) bound to st-DNA (P/D = 10) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-485 mM), (b) fraction of the ligand liberated with increasing concentration of NaCl.

The effect of increasing ionic strength on the binding of (+) and (-)-87 to st-DNA was examined in a similar manner. The changes in the UV/vis spectra of the (+) and the (-) enantiomers are shown in Appendix 3 (Figure A3.6). For both, the absorbance was found to increase with a blue shift in the absorption maxima indicating partial reversal of binding for both of them as seen above. However, the fraction of bound ligand at higher ionic strength (ca. 485 mM NaCl) was found to be different for the two enantiomers; where the (-)-enantiomer remained more tightly bound compared to the (+)-enantiomer.

The reverse NaCl titration was carried out in a similar manner for (±)-88 and its enantiomers, which is shown in Figure 3.17. In the presence of increasing concentration of NaCl (0-485 mM), the absorbance of the band at 390 nm (corresponding to the bound form of 88) was found to increase accompanied by a ca. 8 nm blue shift in the λmax. However, the extent of hyperchromism observed for (±)-88 was found to be about 9%, and the fraction of bound (±)-88 was much higher compared to that seen for (±)-87 and its enantiomers at higher ionic strength. This is in accordance with the higher affinity of 88 for st-DNA compared to 87. Both of the enantiomers of 88 exhibited similar behaviour and remained quite strongly bound in the presence of increasing salt concentration (Figure A3.7). Moreover, the absorbance of (-)-88 recovered to a lesser extent than that of the (+)-88 (Figure 3.17b) suggesting the greater affinity of the (-)-88 towards st-DNA than its (+)-enantiomer.

In order to investigate the possibility of aggregation of the TB-derivatives at higher ionic strength, the UV/vis spectra of (±)-88 were recorded at various ionic strengths (Figure A3.8), which showed that an increase in the concentration of NaCl in the media (0-485 mM),
resulted only in a 6% decrease in the absorbance, thereby suggesting that (±)-88 exists predominantly as a monomeric species even at higher ionic strength.

**Figure 3.17:** (a) The UV/vis absorption spectra of (±)-88 (9.2 μM) bound to st-DNA (P/D = 10) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-485 mM), (b) fraction of the ligand liberated with increasing concentration of NaCl.

The reverse salt titration data showed that both of the TB-derivatives (±)-87 and (±)-88 and their corresponding enantiomers remained strongly bound to st-DNA even at higher ionic strengths. In fact the fraction of unbound ligand for (±)-88 was found to be lower than that of (±)-87 indicating higher binding affinity of the TB-derivative 88 for st-DNA. Additionally, for both of the TB-derivatives, the (-)-enantiomer remained more strongly bound than the corresponding (+)-enantiomer. To verify the enantioselective binding of the (-)-enantiomer, UV/vis titrations were carried out at higher ionic strength, the results of which will be detailed in the following section.

### 3.6.1 Interaction of (±)-87 with DNA at Higher Ionic Strength

The titrations at higher ionic strength were carried out using UV/vis absorption spectroscopy using a method similar to that described in section 3.5 by adding small aliquots of st-DNA to the buffered solution of the ligand in 10 mM phosphate buffer containing 50 mM and 150 mM NaCl respectively, until a plateau was reached in the absorbance. The changes in the absorption spectra for (±)-87 in the presence of st-DNA in 50 mM and 150 mM NaCl are shown in Figure 3.18a and 3.18b respectively. The overall changes in the absorption spectrum of (±)-87 were similar to those recorded at low ionic strength. However, the changes in absorbance reached plateau at higher P/D ratios (P/D = 10→30 for 50 mM NaCl and P/D = 35→100 for 150 mM NaCl, respectively). As shown in Figure 3.18 a, in the presence of 50 mM NaCl the degree of hypochromism was ca. 35% accompanied by a 8 nm bathochromic
shift for the absorption band at 382 nm. An isosbestic point was observed at 412 nm for all P/D ratios, suggesting the presence of (1:1) complex. In the presence of 150 mM of NaCl, the extent of hypochromism was found to be 29% for the absorption band at 382 nm accompanied by a bathochromic shift of 5 nm.

**Figure 3.18:** (a) The UV/vis absorption spectra of (±)-87 (9.5 µM) in the presence of increasing concentration of st-DNA (0-285 µM) in 10 mM phosphate buffer containing 50 mM NaCl; (b) UV/vis absorption spectra of (±)-87 (8.0 µM) in the presence of increasing concentration of st-DNA (0-480 µM) in 10 mM phosphate buffer containing 150 mM NaCl.

### 3.6.2 Interaction of (+)-87 and (-)-87 with DNA at Higher Ionic Strength

Interactions of (+) and (-)-87 with st-DNA were also investigated in the presence of 50 mM and 150 mM NaCl, respectively and the UV/vis spectra of both the enantiomers showed similar changes as shown in Figure A3.9 and A.3.13 in Appendix 3. As found previously, the absorption band at 382 nm experienced significant changes for both enantiomers in the presence of st-DNA, however, compared to previous titrations the changes in absorbance reached a plateau at much higher P/D ratios than that observed for the low ionic strength condition; levelling at P/D = 7 for (-)-87 and P/D = 12 for (+)-87, respectively (Figure 3.19a) in 50 mM NaCl. Moreover, the degree of hypochromism observed for the enantiomers differed significantly. In the presence of 50 mM NaCl, the hypochromism for the 382 nm absorption band was found to be greater for the (-)-enantiomer (38%) compared to the (+)-enantiomer (31%) upon addition of st-DNA, indicating the greater affinity of the (-)-87 towards st-DNA under high ionic strengths. These changes are summarised in Table 3.7.
Figure 3.19: Plot of the changes in the UV/vis absorption spectra of (+)-87 (▲) and (-)-87 (■) at 382 nm in the presence of st-DNA in (a) 10 mM phosphate buffer containing 50 mM NaCl and (b) 10 mM phosphate buffer containing 150 mM NaCl.

Table 3.7: Summary of various binding parameters obtained from the UV/vis titration for (+)-87, (-)-87, in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(+)-87</th>
<th>(-)-87</th>
<th>(-)-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (free) (nm)</td>
<td>382</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (bound) (nm)</td>
<td>390</td>
<td>388</td>
<td>392</td>
</tr>
<tr>
<td>( \Delta \lambda_{\text{max}} ) (nm)</td>
<td>8</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>412</td>
<td>418</td>
<td>410</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>35</td>
<td>31</td>
<td>38</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>10→30</td>
<td>12→30</td>
<td>7→30</td>
</tr>
</tbody>
</table>

In a similar manner, in the presence of 150 mM NaCl, the absorbance at 382 nm experienced a greater degree of hypochromism for (-)-87 upon addition of st-DNA compared to the (+)-enantiomer (Figure 3.19b). Moreover, the changes in absorbance were found to reach a plateau at a P/D = 30 for (-)-87, whereas for the (+)-enantiomer, the changes levelled off at a much higher P/D of 45. The binding parameters in the presence of 150 mM NaCl are summarised in Table 3.8. These changes again suggest the ability of the (-)-87 to bind strongly to st-DNA compared to its (+)-enantiomer.
Table 3.8: Summary of various binding parameters obtained from the UV/vis titration for (±)-87, (+)-87, (-)-87 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-87</th>
<th>(+)-87</th>
<th>(-)-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_{max} (free) (nm)</td>
<td>382</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>λ_{max} (bound) (nm)</td>
<td>387</td>
<td>386</td>
<td>390</td>
</tr>
<tr>
<td>Δλ_{max} (nm)</td>
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<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>412</td>
<td>418</td>
<td>410</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>29</td>
<td>26</td>
<td>36</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>35→100</td>
<td>45→100</td>
<td>30→100</td>
</tr>
<tr>
<td>McGhee - von Hippel model</td>
<td>K (×10^6 M⁻¹)</td>
<td>0.10 ± 0.004</td>
<td>0.03 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>1.05 ± 0.02</td>
<td>1.19 ± 0.06</td>
</tr>
<tr>
<td>Bard Model</td>
<td>K (×10^6 M⁻¹)</td>
<td>0.20 ± 0.01</td>
<td>0.07 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>1.12 ± 0.12</td>
<td>0.99 ± 0.10</td>
</tr>
</tbody>
</table>

The binding constant for the interaction of the (±)-87, (+) and (-)-87 with st-DNA in the presence of 50 mM and 150 mM NaCl were determined by analysing the changes in absorbance at 382 nm using the McGhee von Hippel and Bard models. Representative binding curves are shown in Appendix 3 (Figures A.3.10-A.3.16). At higher ionic strengths, the ligand (±)-87 and its enantiomers became completely bound at higher P/D ratios compared to 10 mM phosphate buffer and the magnitudes of the binding constants decreased considerably, which suggests that the higher salt concentration disrupts the binding ability of these ligands, implying significant contribution of the electrostatic interactions in the binding process. In the presence of 50 mM NaCl (Table 3.7), the binding constant of (-)-87 with st-DNA (0.64×10^6 M⁻¹) calculated using the McGhee von Hippel model was found be about 1.7 times higher than that of the (+)-enantiomer (0.36 ×10^6 M⁻¹). The enantiomeric preference was more pronounced at 150 mM NaCl concentration, where the binding constant of (-)-87 was found to be about four times higher than that of (+)-enantiomer (Table 3.8). This behaviour was also observed in the reverse salt titration of the two enantiomers (Figure 3.16), where the fraction of (-)-87 remained bound at physiological concentration of Na⁺ (ca. 150 mM) was higher than that of (+)-87. Similar enantiomeric preference has previously been observed for the proflavine-TB analogue, where the (-)-enantiomer was found to bind preferentially to ds-DNA.¹⁷⁷

3.6.3 Interaction of (±)-88 with st-DNA at Higher Ionic Strength

Figure 3.20a and 3.20b show the UV/vis absorption spectra of (±)-88 in the presence of increasing concentration of st-DNA in 10 mM phosphate buffer containing 50 mM and 150
mM NaCl, respectively. As observed previously at low ionic strengths, the absorption band at 380 nm experienced significant hypochromism and bathochromic shift in the presence of st-DNA at higher ionic strength. However, the extents of these changes were smaller than that observed in low salt concentration. Most strikingly, while $(\pm)-88$ was found to exhibit biphasic-binding behaviour under low salt concentration, a single mode of binding was observed at higher ionic strengths (50 mM and 150 mM NaCl). This would imply the strong association governed by electrostatic interaction between $88$ and st-DNA is minimised at higher ionic strength. A single isosbestic point was observed at 416 nm for all P/D ratios in the presence of both 50 mM and 150 mM NaCl, suggesting the presence of two spectroscopically distinct species under all DNA/ligand ratios. The spectral parameters in the presence of 50 mM and 150 mM NaCl are summarised in Table 3.9 and 3.10, respectively. Comparison of $(\pm)-87$ and $(\pm)-88$ reveals that the extent of hypochromism at the $\lambda_{\text{max}}$ of the ICT absorption band is higher in case of $(\pm)-88$ under both 50 mM and 150 mM NaCl concentrations. Moreover, a higher concentration of DNA was required for the complete binding of $(\pm)-87$. For example, in 50 mM NaCl the changes in absorbance reached plateau at P/D 3.5 for $(\pm)-88$, while $(\pm)-87$ was fully bound at P/D 10. These changes suggest that $(\pm)-88$ binds much more strongly to st-DNA. In fact, binding constant analysis showed that the binding affinity of $(\pm)-88$ towards st-DNA was nearly 3-4 times higher than that of $(\pm)-87$ in the presence of both 50 mM and 150 mM NaCl.

Figure 3.20: (a) The UV/vis absorption spectra of $(\pm)-88$ (9.0 $\mu$M) in the presence of increasing concentration of st-DNA (0-135 $\mu$M) in 10 mM phosphate buffer containing 50 mM NaCl; (b) the UV/vis absorption spectra of $(\pm)-88$ (9.2 $\mu$M) in the presence of increasing concentration of st-DNA (0-280 $\mu$M) in 10 mM phosphate buffer containing 150 mM NaCl.
3.6.4 Interaction of (+)-88 and (-)-88 with st-DNA at Higher Ionic Strength

Interactions of (+) and (-)-88 with st-DNA were also investigated in the presence of 50 mM and 150 mM NaCl, respectively. For both enantiomers, the changes in the UV/vis absorption spectra were similar to that of (±)-88 (Figures A3.17 and A3.21 in Appendix 3). However, in the presence of st-DNA, the enantiomers exhibited a significantly different extent of hypochromism and bathochromic shift under these conditions. The changes in absorbance reached a plateau at a P/D = 3 for (-)-88 and a P/D = 5 for (+)-88, respectively in the presence of 50 mM NaCl (Figure 3.21a). Additionally, the degree of hypochromism at 380 nm was also found to be greater for the (-)-enantiomer (39%) compared to the (+)-enantiomer (35%) upon addition of st-DNA, indicating the greater affinity of the (-)-88 towards st-DNA under these conditions. These changes are summarised in Table 3.9. A similar trend was also observed in the presence of 150 mM NaCl, with a greater extent of hypochromism observed for (-)-88 upon addition of st-DNA compared to the (+)-enantiomer (Figure 3.21b). Moreover, the changes in absorbance were found to reach a plateau at a P/D = 10 for (-)-88, whereas for the (+)-enantiomer, the changes levelled off at a P/D = 15. The spectral changes in the presence of 150 mM NaCl are summarised in Table 3.10.

![Figure 3.21: Plot of the changes in the UV/vis absorption spectra of (+)-88 (▲) and (-)-88 (■) at 380 nm in the presence of st-DNA in (a) 10 mM phosphate buffer containing 50 mM NaCl and (b) 10 mM phosphate buffer containing 150 mM NaCl.](image)

The binding constants of (±)-88, (+)-88 and (-)-88 for the association with st-DNA in the presence of 50 mM and 150 mM NaCl were estimated from the absorbance changes at 380 nm using the McGhee von Hippel and the Bard models. The binding curves are shown in Appendix 3 (Figure A3.18-A3.20 and A3.22-A3.24) and the binding constants are summarised in Table 3.9 and 3.10, respectively, which again showed the ability of the (-)-88 to bind more strongly to st-DNA than the (+)-enantiomer. However, due to much higher
affinity of 88 towards st-DNA, the difference in the binding constants between the two enantiomers is much less prominent compared to that observed for (+) and (-)-enantiomers of 87.

**Table 3.9:** Summary of various binding parameters from the UV/vis titration for (+)-87, (±)-88, (+)-88, (-)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

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<th>(+)-88</th>
<th>(-)-88</th>
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<tr>
<td>Xmax (free) (nm)</td>
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</tr>
<tr>
<td>Xmax (bound) (nm)</td>
<td>390</td>
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<td>386</td>
</tr>
<tr>
<td>A Xmax (nm)</td>
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<td>6</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>412</td>
<td>416</td>
<td>418</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>35</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>10→30</td>
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McGhee von Hippel model

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<tr>
<td>K (×10^6 M⁻¹)</td>
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<td>n (bp)</td>
<td>1.12 ± 0.02</td>
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<td>0.72 ± 0.03</td>
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Bard Model

<table>
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<tr>
<td>K (×10^6 M⁻¹)</td>
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<td>0.81 ± 0.07</td>
<td>1.12 ± 0.09</td>
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<tr>
<td>n (bp)</td>
<td>1.03 ± 0.12</td>
<td>0.32 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
</tbody>
</table>

- [a] Data could not be fitted to the McGhee von Hippel model.

**Table 3.10:** Summary of various binding parameters from the UV/vis titration for (+)-87, (±)-88, (+)-88, (-)-88 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).

<table>
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<tr>
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<tr>
<td>Xmax (free) (nm)</td>
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<td>Xmax (bound) (nm)</td>
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<td>Isosbestic Point (nm)</td>
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<td>418</td>
</tr>
<tr>
<td>% Hypochromism</td>
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<td>34</td>
<td>24</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>35→100</td>
<td>12→30</td>
<td>15→30</td>
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McGhee von Hippel model

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<th>(+)-88</th>
<th>(-)-88</th>
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<tr>
<td>K (×10^6 M⁻¹)</td>
<td>0.10 ± 0.004</td>
<td>0.75 ± 0.01</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>n (bp)</td>
<td>1.05 ± 0.02</td>
<td>1.04 ± 0.01</td>
<td>0.98 ± 0.06</td>
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Bard Model

<table>
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<th>(-)-88</th>
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<tbody>
<tr>
<td>K (×10^6 M⁻¹)</td>
<td>0.20 ± 0.01</td>
<td>0.74 ± 0.02</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>n (bp)</td>
<td>1.12 ± 0.12</td>
<td>0.52 ± 0.01</td>
<td>0.64 ± 0.02</td>
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</table>

As observed previously for TB-derivative (±)-87 and its enantiomers, a higher concentration of DNA was required for the complete binding of (±)-88 and its enantiomers at higher ionic strength compared to 10 mM phosphate buffer. This suggests that at higher salt concentration the binding abilities of these ligands are considerably reduced indicating a significant contribution of the electrostatic interactions in binding of these ligands to st-DNA. In order to investigate if 87 and 88 show any sequence selective interaction with DNA, their interaction with homopolymeric sequences poly(dA-dT)₂ and poly(dG-dC)₂ was studied, which will be described in the following section.
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3.7 Interaction of 87-88 with Synthetic Polynucleotides

The ground state interaction of (±)-87 and (±)-88 and their enantiomers with poly(dA-dT)_2 and poly(dG-dC)_2 was studied in a manner similar to that described in section 3.5. Since both (±)-87, (±)-88 and their enantiomers displayed very high binding affinity (ca. 10^6 M^-1) towards st-DNA under low ionic strengths and no significant difference was observed between the affinity of the enantiomers, the titrations with polynucleotides were carried out in 10 mM phosphate buffer containing 50 mM NaCl. All the titrations were repeated at least three times to ensure reproducibility.

3.7.1 Ground-State Interaction of (±)-87-88 and Their Enantiomers with Poly(dA-dT)_2

The interaction of individual TB-derivative with poly(dA-dT)_2 is described in the following subsections.

3.7.1.1 Interaction of (±)-87 and its enantiomers with poly (dA-dT)_2: In a manner similar to that described in Section 3.5, the interaction of (±)-87 and its enantiomers with poly(dA-dT)_2 was followed by monitoring the changes in absorbance at 382 nm. The changes in the UV/vis absorption spectra of (±)-87 in the presence of increasing concentration of poly(dA-dT)_2 is presented in Figure 3.22a, which shows that the addition of poly(dA-dT)_2 resulted in a marked decrease in absorbance (47%) of the band centred at 382 nm associated with a ca. 9 nm red shift in the λ_max. An isosbestic point was observed at 415 nm during the titration of (±)-87 with poly(dA-dT)_2 at lower concentrations of the polynucleotide (P/D 0→3.4). However, the isosbestic point disappeared at higher concentrations of the polynucleotide, indicating possible presence of more than one DNA bound form. Titration of (+)-87 and (-)-87 with poly(dA-dT)_2 showed similar changes (Figures A3.25, Appendix 3). However, the extent of hypochromism and red shift was found to be different for the two enantiomers. The relative changes in absorbance at 382 nm for the two enantiomers in the presence of poly(dA-dT)_2 are shown in Figure 3.22b, which shows that the (-)-87 exhibited a greater degree of hypochromism in the presence of the polynucleotide. The changes in absorbance reached a plateau at a P/D of 6 for (-)-87, and at a P/D of 12 for the (+)-enantiomer, suggesting a higher binding affinity of (-)-87 towards poly(dA-dT)_2. Additionally, in the case of (+)-87, no isosbestic point was observed during the titration, while for (-)-87, an isobestic point was observed at 420 nm, suggesting a further difference in the binding of the enantiomers to
poly(dA-dT)$_2$. The binding parameters for (±)-87 and the enantiomers are summarised in Table 3.11.

**Figure 3.22:** (a) The UV/vis absorption spectra of (±)-87 (7.3 μM) in the presence of increasing concentration of poly(dA-dT)$_2$ (0-189 μM) in 10 mM phosphate buffer containing 50 mM NaCl, Inset: Plot of $r/C_f$ (■) for (±)-87 and (—) fit to the McGhee von Hippel model. (b) Plot of $A/A_0$ at 382 nm vs. $P/D$ for (+) and (-)-87.

The binding constants for the association of (±)-87 and the enantiomers with poly(dA-dT)$_2$ were estimated by analysing the changes in absorbance at 382 nm using the non-cooperative McGhee von Hippel and the Bard models and the binding constants are presented in Table 3.11. The representative binding curve for (±)-87 fitted to the McGhee von Hippel model is shown as an inset of Figure 3.22a while those for the (+) and (-)-87 are shown in Figures A3.26-A3.27 in Appendix 3. As can be seen from Table 3.11, (±)-87 and the enantiomers exhibited high binding affinity towards poly(dA-dT)$_2$, however the affinity of (-)-87 was almost three times than that observed for the (+)-enantiomer.
Table 3.11: Summary of the various binding parameters from UV/vis titration for (±)-87, (+)-87, (-)-87 with poly(dA-dT)_2 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(±)-87</th>
<th>(+)-87</th>
<th>(-)-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (free) (nm)</td>
<td>382</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>( \Delta \lambda_{\text{max}} ) (nm)</td>
<td>391</td>
<td>388</td>
<td>393</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>415 (upto P/D = 3.4)</td>
<td>-</td>
<td>420</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>47</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>8.5 → 30</td>
<td>12 → 30</td>
<td>6 → 30</td>
</tr>
</tbody>
</table>

McGhee von Hippel model

- \( K \times 10^b \text{ M}^{-1} \)
  - (±)-87: 0.97 ± 0.06
  - (+)-87: 0.50 ± 0.01
  - (-)-87: 1.66 ± 0.15

- \( n \) (bp)
  - (±)-87: 1.73 ± 0.07
  - (+)-87: 1.26 ± 0.02
  - (-)-87: 1.38 ± 0.06

Bard Model

- \( K \times 10^b \text{ M}^{-1} \)
  - [a]
    - (±)-87: 0.79 ± 0.10
    - (+)-87: 0.79 ± 0.10
    - (-)-87: 1.76 ± 0.10

- \( n \) (bp)
  - [a]
    - (±)-87: 1.08 ± 0.03
    - (+)-87: 1.08 ± 0.03
    - (-)-87: 0.99 ± 0.02

[a] could not be fitted to the Bard Model.

3.7.1.2 Interaction of (±)-88 and its enantiomers with poly (dA-dT)_2: Interaction of (±)-88 and its enantiomers with poly(dA-dT)_2 was also investigated in a manner similar to that described above for 87 by monitoring the changes in the absorbance at 380 nm in the presence of increasing concentration of poly(dA-dT)_2. The changes in the UV/vis spectra of (±)-88 as a function of poly(dA-dT)_2 concentrations are shown in Figure 3.23a. As observed previously, for (±)-87 and its enantiomers, addition of poly(dA-dT)_2 resulted in a significant decrease in the absorbance of the band at 380 nm accompanied by a ca. 9 nm red shift in the \( \lambda_{\text{max}} \). Additionally, comparison of the binding parameters of (±)-87 and (±)-88 for poly(dA-dT)_2 showed that (±)-88 had a higher affinity towards the polynucleotide (Figure 3.23b), which was also observed in the titration of 87 and 88 with st-DNA (Section 3.5).

The UV/vis absorption spectra of (+) and (-)-88 in the presence of an increasing concentration of the polynucleotide were affected in a manner similar to that observed in the case of (±)-88 (Figure A3.28, Appendix 3). However, the extent of hypochromism was found to be higher for the (-)-88 enantiomer, similar to that observed for 87 and the changes in absorbance reached a plateau at a P/D ratio of 2 in the case of (-)-88 compared to P/D ratio of 3.5 for the (+)-enantiomer, again indicating the higher binding affinity of the (-)-enantiomer towards double stranded polynucleotide sequence.

The changes in absorbance at 380 nm in the presence of poly(dA-dT)_2 were analysed as before using the McGhee von Hippel and Bard models and the binding constants are summarised in Table 3.12 showing that (-)-88 displayed almost three times higher binding affinity towards poly(dA-dT)_2 than the (+)-enantiomer, similar to the situation observed for 87.
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Figure 3.23: (a) The UV/vis absorption spectra of (±)-88 (9.0 μM) in the presence of increasing concentration of poly(dA-dT)2 (0-112 μM) in 10 mM phosphate buffer containing 50 mM NaCl. Inset: Plot of r vs. r/Δr for (±)-88 and (-) fit to the McGhee von Hippel model. (b) Comparison of the relative changes in absorbance at the λmax of (±)-87 and (±)-88 with increasing poly(dA-dT)2 concentration (c) Plot of A/A₀ at 380 nm vs. P/D for (+) and (-)-88.

Table 3.12: Summary of binding parameters from UV/vis titration for (±)-88, (+)-88, (-)-88 with poly(dA-dT)2 in 10 mM phosphate buffer + 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-88</th>
<th>(+)-88</th>
<th>(-)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (free) (nm)</td>
<td>380</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>λmax (bound) (nm)</td>
<td>389</td>
<td>387</td>
<td>391</td>
</tr>
<tr>
<td>Δλmax (nm)</td>
<td>9</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>48</td>
<td>44</td>
<td>51</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>2.5→12</td>
<td>3.5→12</td>
<td>2→12</td>
</tr>
<tr>
<td>McGhee von Hippel model</td>
<td>K (x10^6 M⁻¹)</td>
<td>3.06±0.02</td>
<td>1.30±0.02</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>0.95±0.01</td>
<td>0.77±0.01</td>
</tr>
<tr>
<td>Bard Model</td>
<td>K (x10^6 M⁻¹)</td>
<td>_[a]</td>
<td>_[a]</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>_[a]</td>
<td>_[a]</td>
</tr>
</tbody>
</table>

[a] could not be fitted to the Bard Model.
3.7.2 Ground-State Interaction of (±)-87-88 and Their Enantiomers with Poly(dG-dC)₂

The interaction of (±)-87 and (±)-88 and their enantiomers with poly(dG-dC)₂ was also carried out as described above. The interaction of individual TB-derivative with poly(dG-dC)₂ is described in the following subsections.

3.7.2.1 Interaction of (±)-87 and its enantiomers with poly(dG-dC)₂: The interaction of (±)-87 and its enantiomers with poly(dG-dC)₂ was monitored by following the absorbance change at 382 nm. In all cases, the addition of poly(dG-dC)₂ resulted in significant changes in the absorption spectra as shown in Figure 3.24a for (±)-87. The changes in the UV/vis absorption spectra of the (+) and (-)-87 are shown in Appendix 3 (Figure A.3.30). As observed previously with the titrations with poly(dA-dT)₂, the absorbance of the band centred at 382 nm decreased significantly concomitant with a red shift in the λ_max for all the compounds. These spectral changes are summarised in Table 3.13.

The relative changes in the absorbance of the two enantiomers at 382 nm are shown in Figure 3.24b demonstrating a significant difference between the binding affinities of (+) and (-)-enantiomers of 87 towards poly(dG-dC)₂, where the absorbance change for (-)-87 reached a plateau at a P/D = 12.5 while for the (+)-87-enantiomer the plateau was observed at a P/D ratio of ca. 20, suggesting stronger interaction of the (-)-enantiomer with the polynucleotide.

Table 3.13: Summary of the various binding parameters from UV/vis titration for (±)-87, (+)-87, (-)-87 with poly(dG-dC)₂ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-87</th>
<th>(+)-87</th>
<th>(-)-87</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ_max (free) (nm)</td>
<td>382</td>
<td>382</td>
<td>382</td>
</tr>
<tr>
<td>λ_max (bound) (nm)</td>
<td>389</td>
<td>387</td>
<td>391</td>
</tr>
<tr>
<td>Δ λ_max (nm)</td>
<td>7</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>54</td>
<td>51</td>
<td>58</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>16→30</td>
<td>20→30</td>
<td>12.5→30</td>
</tr>
<tr>
<td>McGhee von Hippel model</td>
<td>K (×10⁶ M⁻¹)</td>
<td>0.38 ± 0.01</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>2.01 ± 0.02</td>
<td>1.42 ± 0.02</td>
</tr>
<tr>
<td>Bard Model</td>
<td>K (×10⁶ M⁻¹)</td>
<td>0.90 ± 0.01</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>1.60 ± 0.04</td>
<td>1.20 ± 0.02</td>
</tr>
</tbody>
</table>
Chapter 3: Photophysical and DNA Binding Studies of the Tröger’s Base Containing Bis-1,8-naphthalimides

Figure 3.24: (a) The UV/vis absorption spectra of (±)-87 (6.5 μM) in the presence of increasing concentration of poly(dG-dC)_2 (0-195 μM) in 10 mM phosphate buffer containing 50 mM NaCl. Inset: Plot of r vs. r/C_f for (±)-87 and (-) fit to the McGhee von Hippel model. (b) Plot of A/A_0 at 382 nm vs. P/D for (+) and (-)-87. (c) Comparison of the relative changes in absorbance at 382 nm for (±)-87 in the presence of poly(dA-dT)_2 and poly(dG-dC)_2.

Comparison of the relative changes in the absorbance of (±)-87 and its enantiomers in the presence of poly(dG-dC)_2 and poly(dA-dT)_2 revealed that these compounds displayed high preference for the AT rich sequences. The binding constants of (±)-87 and its enantiomers for poly(dG-dC)_2 were estimated from the absorbance change at 382 using the McGhee von Hippel and Bard models. The fit of the absorbance data to the McGhee von Hippel model for (±)-87 is shown in the inset of Figure 3.24a, while those for (+) and (-)-87 are shown in Appendix 3 (Figure A3.31) and the values are summarised in Table 3.13.
3.7.2.2 **Interaction of (±)-88 and its enantiomers with poly(dG-dC)₂:** The interaction of (±)-88 and its enantiomers with poly(dG-dC)₂ was also studied by following the changes in absorbance at 380 nm. Figure 3.25 shows the changes in the UV/vis spectra of (±)-88 in the presence of increasing concentration of poly(dG-dC)₂, while those for the enantiomers are shown in Appendix 3 (Figure A3.32). As observed previously for 87, the addition of poly(dG-dC)₂ to a solution of (±)-88 and its enantiomers resulted in significant hypochromism of the absorption band at 380 nm associated with a substantial red shift in the peak position. The spectral changes for (±)-88, (+) and (-)-enantiomers are summarised in Table 3.14. Similar to what was observed for 87, (-)-88 was found to have higher affinity for the duplex, where the changes in absorbance reached a plateau at a P/D = 6, while a much higher concentration of poly(dG-dC)₂ was required for the complete binding of (+)-88 (plateau from a P/D = 12). This differential affinity of the enantiomers was further confirmed from the binding constant analysis using the McGhee von Hippel and Bard models, which are also summarised in Table 3.14.

![Figure 3.25](image-url)

**Figure 3.25:** (a) The UV/vis absorption spectra of (±)-88 (6.5 μM) in the presence of increasing concentration of poly(dG-dC)₂ (0-195 μM) in 10 mM phosphate buffer containing 50 mM NaCl. Inset: Plot of r vs. r/Cf (■) for (±)-88 and (–) fit to the McGhee von Hippel model. (b) Plot of A/A₀ at 380 nm vs. P/D for (+) and (-)-88. (c) Comparison of the relative changes in absorbance at 380 nm for (±)-88 in the presence of poly(dA-dT)₂ and poly(dG-dC)₂.
Table 3.14: Summary of the various binding parameters obtained from the UV/vis titration for (±)-88, (+)-88, (-)-88 with poly(dG-dC)₂ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-88</th>
<th>(+)-88</th>
<th>(-)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{max} ) (free) (nm)</td>
<td>380</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>( \lambda_{max} ) (bound) (nm)</td>
<td>389</td>
<td>387</td>
<td>391</td>
</tr>
<tr>
<td>( \Delta \lambda_{max} ) (nm)</td>
<td>9</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>57</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>10→20</td>
<td>12→20</td>
<td>6→20</td>
</tr>
</tbody>
</table>

McGhee von Hippel model

<table>
<thead>
<tr>
<th></th>
<th>(±)-88</th>
<th>(+)-88</th>
<th>(-)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K \times 10^6 ) M⁻¹</td>
<td>1.05 ± 0.03</td>
<td>0.48 ± 0.02</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>n (bp)</td>
<td>1.57 ± 0.03</td>
<td>1.10 ± 0.02</td>
<td>1.44 ± 0.02</td>
</tr>
</tbody>
</table>

Bard Model

<table>
<thead>
<tr>
<th></th>
<th>(±)-88</th>
<th>(+)-88</th>
<th>(-)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K \times 10^6 ) M⁻¹</td>
<td>1.11 ± 0.10</td>
<td>0.85 ± 0.10</td>
<td>1.37 ± 0.28</td>
</tr>
<tr>
<td>n (bp)</td>
<td>1.05 ± 0.20</td>
<td>1.06 ± 0.04</td>
<td>1.08 ± 0.02</td>
</tr>
</tbody>
</table>

Additionally as was the case with 87, (±)-88 and both of the enantiomers of 88 exhibited strong preference for AT rich sequences. As shown in Figure 3.25c, (±)-88 was found to be fully bound to poly(dA-dT)₂ at a P/D = 2.5, while in the case of poly(dG-dC)₂ a complete binding of (±)-88 was observed at a P/D of 10. The summary of the binding data of (±)-87 and (±)-88 in the presence of st-DNA, poly(dA-dT)₂ and poly(dG-dC)₂ are shown in Table 3.15, which shows that both (±)-87 and (±)-88 displayed strong preference for AT rich sequences. The enantiomers of the both TB-derivatives also displayed similar preference for AT rich sequences. This is perhaps correlated with the higher negative electrostatic potential of the AT rich minor grooves, which facilitates binding of cationic molecules as discussed previously in Chapter 2.²⁰⁷ In addition to this, the minor grooves in the AT rich sequences are narrower than those of GC rich regions, which can in turn allow optimal hydrophobic

Table 3.15: Summary of the various binding parameters obtained from the UV/vis titration for (±)-87 and (±)-88 with poly(dG-dC)₂ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>(±)-87</th>
<th>(±)-88</th>
</tr>
</thead>
<tbody>
<tr>
<td>( % \text{Hypochromism} )</td>
<td>35</td>
<td>37</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>10→30</td>
<td>2.5→12</td>
</tr>
<tr>
<td>( K^* \ (\times 10^6 ) M⁻¹</td>
<td>0.56 ± 0.02</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>n (bp)*</td>
<td>1.12 ± 0.02</td>
<td>2.01 ± 0.02</td>
</tr>
</tbody>
</table>

* Determined from the fit of UV/vis absorption data to the non-cooperative model of McGhee and von Hippel.
interaction between the ligand and the grooves and favour binding of these “V”-shaped TB-derivatives along the minor groove of DNA.

3.8 Displacement Assays Using Ethidium Bromide

The DNA binding affinity of (±)-87, (±)-88 and their enantiomers were further investigated using ethidium bromide (EtBr) displacement assay. As mentioned in Chapter 1, EtBr interacts with DNA via intercalation. Free EtBr is weakly emissive in aqueous solution but becomes highly emissive, when bound to DNA. The principle of EtBr displacement assay is based on the loss of fluorescence, resulting from the displacement of bound EtBr from the DNA by a competitive DNA binding agent. The percentage of fluorescence decrease is directly proportional to the extent of DNA binding and therefore can be used to determine binding affinities of various ligands relative to EtBr. The EtBr displacement assays for 87 and 88 were performed according to the methodologies developed by Boger et al., to determine the DNA binding affinities of these compounds.

Since no noticeable difference was observed between the binding affinities of (+) and (-)-enantiomers of 87 and 88 in 10 mM phosphate buffer, the displacement assays were performed at higher ionic strength phosphate buffer (10 mM) containing 50 mM NaCl. The fluorescence of EtBr (5 μM) was initially recorded in 10 mM phosphate buffer containing 50 mM NaCl (λ Exc = 545 nm) and normalised to 0% of relative intensity. To this an appropriate concentration of st-DNA was added such that the ratio of EtBr to DNA base pairs was 1:2 ([EtBr]/[DNA bp] = 1:2). At this concentration of DNA, all the intercalation sites can be assumed to be saturated. The mixture was then allowed to be equilibrated for ca. 20 minutes and the fluorescence was measured and was normalised to 100% of relative intensity. Small aliquots of the ligand solution (ca. 400 μM stock) was then added to the solution of EtBr bound to st-DNA, equilibrated for 2 minutes after each addition and the fluorescence was recorded. The titration was continued until the decrease in fluorescence intensity reached a plateau.

The emission spectrum for the titration of EtBr bound to st-DNA with (±)-87 is shown in Figure 3.26a. In general, the addition of (±)-87 and (or) (±)-88 and their enantiomers resulted in the decrease in the fluorescence of EtBr, demonstrating that these TB-derivatives displaced ethidium bromide efficiently.
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Figure 3.26: (a) The changes in the emission spectra of EtBr (5 μM) bound to st-DNA (10 μM) in the presence of increasing concentration of (±)-87 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0). (b) Normalised fluorescence intensity of EtBr at 605 nm upon addition of (±)-87 (■), (+)-87 (○), (-)-87 (▲), 82 (▼).

The concentration of a ligand required to cause a 50% reduction in the fluorescence intensity of ethidium bromide is known as \( C_{50} \). This \( C_{50} \) value can be used to determine the binding affinity (\( K_{\text{app}} \)) of a ligand relative to ethidium bromide according to equation 3.3,

\[
K_{\text{app}} = K_{\text{EtBr}} \frac{[\text{EtBr}]}{C_{50}}
\]

The \( C_{50} \) values obtained from the titration of EtBr bound to st-DNA with (±)-87, (±)-88 and their enantiomers are summarised in Table 3.15. For comparison, the \( C_{50} \) values determined for the 4-amino-1,8-naphthalimide precursors 82 and 83 are also included in Table 3.16., which shows that 87, 88 and their corresponding enantiomers as well as and the 4-amino -1,8-naphthalimide precursors 81 and 82 can displace EtBr from st-DNA. However, the the \( K_{\text{app}} \) values for 87 and 88 derived from the corresponding \( C_{50} \) values were found to be an order of magnitude higher than the values obtained for the corresponding 4-amino-1,8-naphthalimide precursors 81 and 82. This trend is in agreement with the higher binding affinity of 87 and 88 determined from the UV/vis titration compared to their precursors 81 and 82 and emphasises the role of the Tröger's base moiety in improving the binding affinity.

Among the TB derivatives, (±)-88 was found to be more capable of displacing bound EtBr from st-DNA than compound (±)-87, which follows the order of their binding affinity for st-DNA as determined previously in Section 3.5 and 3.6. Moreover, for both of the TB-
derivatives the (-)-enantiomer was found to displace EtBr more strongly than the (+)-enantiomer. This is in accordance with the higher binding affinity of the (-)-enantiomer for both of the TB derivatives.

**Table 3.16:** $C_{50}$ values and $K_{app}$ from the ethidium bromide displacement assays in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0). [EtBr] = 5 μM.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$C_{50}$ (μM)</th>
<th>$K_{app}$ ($\times 10^6$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-87</td>
<td>7.89</td>
<td>0.76</td>
</tr>
<tr>
<td>(+)-87</td>
<td>9.45</td>
<td>0.63</td>
</tr>
<tr>
<td>(-)-87</td>
<td>5.50</td>
<td>1.09</td>
</tr>
<tr>
<td>(±)-88</td>
<td>6.00</td>
<td>1.00</td>
</tr>
<tr>
<td>(+)-88</td>
<td>6.80</td>
<td>0.88</td>
</tr>
<tr>
<td>(-)-88</td>
<td>5.10</td>
<td>1.18</td>
</tr>
<tr>
<td>82</td>
<td>79.00</td>
<td>0.08</td>
</tr>
<tr>
<td>83</td>
<td>90.00</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**3.9 Thermal Denaturation Studies of st-DNA in the Presence of 87 and 88**

As described in Chapter 1, thermal denaturation studies can be used to investigate the ability of a ligand molecule to stabilise double stranded DNA against heat denaturation. The thermal denaturation studies of st-DNA (150 μM) alone and in the presence of (±)-87, (±)-88 and their enantiomers were carried out in 10 mM phosphate buffer (pH 7.0). All measurements were carried out at a P/D ratio of 10, at which all the ligands are assumed to be completely bound to DNA. The solutions were degassed prior to heating. The thermal denaturation was monitored by measuring the absorbance of the DNA solution at 260 nm as the temperature was gradually increased from 30°-90°C. The thermal melting curves in the presence of (±)-87, (+)-87 and (-)-87 are shown in Figure 3.27. The melting curves for (±)-88, (+) and (-)-88 are shown in Figure 3.28. For comparison the melting curves for the respective precursors 82 and 83 are also included. In the absence of any ligand, the $T_m$ value for st-DNA was found to be (68 ± 0.5)°C. In the presence of the TB-derivatives (±)-87 and (±)-88 significant stabilisation of the double stranded DNA was observed ($\Delta T_m > 7$°C) (Figure 3.27 b and 3.28 b). In fact, the denaturation process was found to be still incomplete at 90°C. In contrast, moderate stabilisation was observed for the 4-amino-1,8-naphthalimide precursors, 82 and 83 ($\Delta T_m = 4-5$°C). The high stabilisation of DNA in the presence of both TB-derivatives correlate with their high binding affinity for st-DNA as observed in the UV/vis titrations (Section 3.5). Moreover such higher extent of stabilisation observed with the TB-derivatives compared to the 4-amino-1,8-naphthalimide precursors points out the importance of rigid “V”-shaped structure of these TB-derivatives in stabilising st-DNA and has been observed for other TB-derivatives previously.¹³⁸,¹³⁹,²¹⁰
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Figure 3.27: (a) Thermal denaturation curves of st-DNA (150 μM) in 10 mM phosphate buffer alone (■) and in the presence of 82 (■), (±)-87 (■), (+)-87 (■), (-)-87 (■), (b) the $T_m$ values from the first derivative plot of absorbance vs. temperature.

Figure 3.28: (a) Thermal denaturation curves of st-DNA (150 μM) in 10 mM phosphate buffer alone (■) and in the presence of 83(*) , (±)-88 (▲), (+)-88(▲), (-)-88 (#), (b) the $T_m$ values from the first derivative plot of absorbance vs. temperature.

The thermal denaturation measurements of st-DNA carried out in the presence of the enantiomers of (±)-87 and (±)-88 showed that both of the (+) and (-)-enantiomer stabilised st-DNA to an extent similar to that observed for the racemic mixtures and no significant difference was observed between the enantiomers. This agrees well with the fact that both (+) and (-) enantiomers have a comparable high affinity for st-DNA in 10 mM phosphate buffer as discussed in Section 3.5.

As pointed out in Chapter 2 that thermal denaturation studies are used to monitor ligand binding but these measurements do not provide any information about the mode of
binding of small molecules. Since both groove binders\textsuperscript{211} as well as intercalators\textsuperscript{212} can result in a significant stabilisation of double helical DNA structures, the mode of binding of the TB-derivatives was investigated using CD and LD spectroscopy, which will be described in the following two sections.

3.10 Circular Dichroism Spectroscopy of \(st\)-DNA in the Presence of \((\pm)-87\)-(\pm)-88

CD spectroscopy can be used to monitor the changes in DNA conformation upon binding of small molecules to DNA as described in Chapter 1 and 2.\textsuperscript{30} The CD titrations were carried out by monitoring the conformational changes of \(st\)-DNA (150 \(\mu\)M) in the presence of increasing concentration of 87 and/or 88. To avoid dilution effects, a range of solutions were prepared in 10 mM phosphate buffer (pH 7.0) containing a fixed concentration of \(st\)-DNA (150 \(\mu\)M) while varying the ligand concentrations. The CD spectra of \(st\)-DNA in the presence of varying concentrations of \((\pm)-87\) are shown in Figure 3.29a. The ellipticity of the negative peak centred at 245 nm increased from -14 to -9.0, while that of the positive peak centred at 275 nm increased from +12 to +16 mdegree. These changes suggest the \((\pm)-87\) interacts strongly with \(st\)-DNA and have been observed previously for other naphthalimide based TB-derivatives.\textsuperscript{138} More importantly, a weak negative CD signal was observed at \(ca.\) 420 nm as the concentration of \((\pm)-87\) was raised. Similar behaviour has been reported for previously reported for Ru(II) and Cr(III) complexes, where such appearance of such ICD signal has been explained due to enantio-preferential binding of one of the enantiomers to DNA.\textsuperscript{31,208} In 10 mM phosphate buffer as both (+) and (-) 87 display very high affinity for \(st\)-DNA therefore the ICD signal arising from the binding of two enantiomers in a racemic mixture would almost cancel out. Therefore, the weak intensity of the ICD signal observed in this case is presumably due to poor enantioselective binding of (-)-87.
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Figure 3.29: The CD spectrum of st-DNA (150 µM) alone and in the presence of (a)(±)-87 and (b)(±)-88 at varying P/D in 10 mM phosphate buffer (pH 7.0). Insets: ICD signals for (±)-87 and (±)-88, respectively.

The effect of (±)-88 on the CD spectrum of st-DNA is shown in 3.29b. As observed previously, in the presence of increasing concentration of (±)-88, the molar ellipticity of the band at 245 nm changed from -14 to -12 mdegree and that of the positive band at 275 nm changed from +12 to +19 mdegree. A negative induced CD signal was observed again at 420 nm. However, the magnitude of this induced CD signal was even weaker than that observed for (±)-87, which is probably due to the comparable high affinity of both of the enantiomers under low ionic strength.

The CD spectra of st-DNA in the presence of increasing concentration of (+) and (-)-87 were also recorded (Figure 3.30a and b). However, since the enantiomers themselves displayed strong CD band above 300 nm in absence of any DNA, the binding could not be followed in a manner similar to the (±)-87, as the CD band of the enantiomer grows steadily with successive additions of (+) or (-)-87 to the DNA solution. Figure 3.30c shows the composite spectrum of the enantiomers of 87 obtained by summation of the spectra of the (+) and (-)-87 in the presence of st-DNA at a P/D ratio of 10. The composite spectrum was found to be similar to the CD spectra observed for that of (±)-87 in the presence of st-DNA, thereby further confirming the preferential DNA binding of the (-)-enantiomer. The induced CD spectra of the (+) and (-)-87 obtained are shown in Figure 3.30d, which were obtained by subtracting the spectrum of st-DNA from the spectrum of the individual enantiomers in the presence of DNA at a P/D ratio of 10. The overall ICD spectra for the two enantiomers were found to be similar. Since the CD titration of (+) or (-)-87 with st-DNA did not provide any additional information about the binding, the titrations with (+) and (-)-88 with st-DNA was not carried out.
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Figure 3.30: The CD spectrum of free st-DNA (150 μM) and st-DNA in the presence of (a) (+)-87 and (b)(-)-87 at varying P/D in 10 mM Phosphate buffer (pH 7.0). (c) Sum of CD spectra of (+)- and (-)-87 at P/D=10 and comparison with the CD spectra of the enantiomers with st-DNA (P/D = 10) (d) ICD spectra of (+)- and (-)-87 obtained by subtracting the CD spectrum of st-DNA (150 μM) from the CD spectra of the enantiomers in the presence of st-DNA (P/D = 10) in 10 mM sodium phosphate buffer (pH = 7).

3.11 Linear Dichroism Spectroscopy of st-DNA in the Presence of (±)-87-(±)-88

The LD spectra of st-DNA (400 μM) in the absence and in the presence of (±)-87 and (±)-88 (P/D = 10 →30 are shown in Figure 3.31a and 3.31b, respectively. In the absence of any ligand, a negative LD signal was observed at λ = 260 nm arising from the nearly perpendicular orientation of the transition moments of the DNA bases relative to the DNA helical axis. In the presence of ligand (±)-87 and/or (±)-88, the LD signal for the 260 nm band was still negative, however a positive LD signal was observed in both cases around 380 nm corresponding to the ICT absorption band of the compounds. Table 3.17 summarises the LD values for the absorption bands at 260 nm and 380 nm for (±)-87 and (±)-88 at P/D ratio of 10, where the ligands should be completely bound to st-DNA. As shown in Table 3.17 the
magnitudes of LD value for the DNA absorption region was found to increase in the presence of both 87 and 88, which is consistent with the lengthening and stiffening of DNA. Additionally the appearance of positive LD signals in the presence of both (±)-87 and (±)-88 suggest that the transition dipoles of these ligands are oriented at angles less than 54.7° relative to the helical axis (according to equation 1.2 in Chapter 1, Section 1.4.1.4), indicative of binding of 87 and 88 to the DNA groove. A complete quantitative determination of the orientation of these compounds along the groove of DNA could not be obtained due to the overlap of the absorption bands of the DNA bases and the TB-derivatives 87 and 88 below 300 nm.

Figure 3.31: The LD spectrum of st-DNA (400 μM) alone and in the presence of (a) (±)-87 and (b) (±)-88 at varying P/D in 10 mM phosphate buffer (pH 7.0).

Table 3.17: Summary of the LD data for (±)-87-88 in the presence of st-DNA (error ± 10%).

<table>
<thead>
<tr>
<th></th>
<th>LD 260 nm</th>
<th>LD 380 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>st-DNA</td>
<td>-0.022</td>
<td>-</td>
</tr>
<tr>
<td>st-DNA + 87</td>
<td>-0.024</td>
<td>+ 0.026</td>
</tr>
<tr>
<td>st-DNA + 88</td>
<td>-0.027</td>
<td>+ 0.021</td>
</tr>
</tbody>
</table>

Previously Bailly et al., reported the minor groove binding for the acridine based TB-derivative. This assignment was derived from the fact that the acridine based TB-derivative did not show DNA unwinding activity like classical intercalators and did not interfere with the methylation induced by dimethyl sulfate, which is usually observed for the major groove binders.
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To investigate the effect of ionic strength on the binding, the LD spectrum of st-DNA was recorded in the presence of ligand 87 and 88 in 10 mM phosphate buffer containing 100 mM NaCl. The LD spectra of st-DNA in the presence of 87 (P/D = 10) in 10 mM phosphate buffer containing 100 mM NaCl are shown in Figure 3.32, which showed that (±)-87 remained bound to st-DNA at such high ionic strength, however the magnitude of the positive LD signal at 380 nm is reduced due to decreased binding affinity in the presence of 100 mM NaCl.

Figure 3.32: The LD spectrum of free st-DNA (400 µM) alone and in the presence of (a)(±)-87 at P/D= 10 in 10 mM phosphate buffer (pH 7.0) in the absence and in the presence of 100 mM NaCl.

For the TB-derivative, (±)-88 the magnitude of positive LD signal was also found to decrease at higher ionic strength (Appendix 3), suggesting the significant contribution of electrostatic interaction in the binding. However, the positive signal did not disappear completely for any of the TB-derivatives at higher ionic strength, which is usually seen for groove binders. These results again show the very high affinity of these ligands compared to the simple 4-amino-1,8-naphthalimide precursors.

Since various 1,8-naphthalimide derivatives displayed DNA photocleavage activities, the abilities of (±)-87 and 88 to induce DNA cleavage under photoirradiated conditions were examined, which can be useful to develop molecules for photodynamic therapy. The results of these studies will be described in the following section.

3.12 DNA Photocleavage Studies of 87-88

Photooxidative DNA damage can result either from the direct UV irradiation of DNA bases or through the excitation of a photosensitiser molecule. The nucleic acid fragments resulting from the photocleavage of DNA can be separated using gel electrophoresis.
technique according to their ability to migrate through the gel. In its normal form, the supercoiled plasmid DNA (known as Form I) readily moves through the gel. Presence of single strand nicking generates the relaxed circular form (Form II), which has a much reduced mobility compared to the supercoiled form. However, double strand DNA breaks generates the linear DNA (Form III), which has mobility in between that of supercoiled (Form I) and relaxed circular DNA (Form II). Therefore, these various forms can be readily separated on an agarose gel. The cleavage processes and the difference in the migration of various forms are illustrated in Figure 3.33.

Figure 3.33: Schematic representation of the generation of single strand nick (Form II) and double strand break (Form III) and their electrophoretic mobility pattern on agarose gel (modified from ref 237).

The initial DNA photocleavage studies were performed by treating pBr322 DNA (1mg/ml) with (+)-87 at a P/D ratio of 10 in 10 mM phosphate buffer solution. UV/vis absorption titrations showed at this P/D, all the ligands should be completely bound to DNA. The samples were then irradiated using a Hg-Xe lamp (2J/cm²) using a combination of a green glass filter (>350 nm) and water filter (IR filter). The samples were irradiated for a varying amount of time (5 min, 20 min and 60 min) and the results are shown in Figure 3.34a. The band densities were also analysed using Image J software to estimate the relative proportion of each form, as shown in the bar plot in Figure 3.34b. When incubated with (+)-87 (P/D = 10) in the absence of light, significant retardation in the mobility of supercoiled DNA was observed (lane 2). This is perhaps due to the dicationic nature of the TB-derivative, binding of which results in reduction in the overall negative charge of plasmid DNA and hence caused reduced mobility. To verify this, pBR322 incubated with (+)-87 was subjected to phenol: CHCl₃ extraction prior to electrophoresis, which was found to remove the bound ligand and restores the electrophoretic mobility of the supercoiled DNA (lane 3). As can be seen from lanes 4 and 6, irradiation for 5 minutes and 20 minutes resulted in significant dragging of the band, a situation observed in the non-irradiated sample. However, in both
cases extracting the irradiated samples with phenol:CHCl\textsubscript{3} resulted in an efficient removal of the ligand (lanes 5 and 7). Moreover, this also showed that after 5 minutes of irradiation, the amount of Form II \textit{(i.e.} closed circular relaxed DNA\textit{)} did not increase significantly (lane 5). However, irradiating the samples for 20 minutes and subsequent extraction resulted in generation of Form II in a significant amount \textit{(ca. 60\%)} (lane 7). The highest amount of Form II was observed, when the samples were irradiated for 60 min (lane 8) and extracted prior to electrophoresis.

**Figure 3.34:** (a) Agarose gel electrophoresis of pBR322 DNA (1 mg/ml) after irradiation at $\lambda>$ 350 nm in 10 mM phosphate buffer (pH 7.0). **Lane 1:** pBR322 control, **Lane 2:** (±)-87 (P/D 10) non-irradiated, **Lane 3:** (±)-87 (P/D 10) non-irradiated + extracted, **Lane 4:** (±)-87 (P/D 10) 5 min. Irradiation, **Lane 5:** (±)-87 (P/D 10) 5 min irradiation followed by extraction, **Lane 6:** (±)-87 (P/D 10) 20 min irradiation, **Lane 7:** (±)-87 (P/D 10) 20 min irradiation followed by extraction, **Lane 8:** (±)-87 (P/D 10) 60 min irradiation followed by extraction (b) Relative proportions of Form I and Form II as calculated using the Image J software.

The results described above showed (±)-87 is capable of generating the closed circular DNA (Form II) upon photoirradiation using $\lambda$ > 350 nm. The extent of DNA damage mediated by (±)-87 was found to depend strongly on the irradiation time.

Having optimised the irradiation time of 60 minutes, the photocleavage studies were performed on (±)-87 and (±)-88 in detail. For comparison the 4-amino-1,8-naphthalimide precursors 82 and 83 were also included. The unsubstituted 1,8-naphthalimide analogue 60 was chosen as the reference compound, which is known to induce photocleavage of DNA.\textsuperscript{132c} As described before, pBR322 DNA (1 mg/ml) was treated with the TB-derivatives and their
precursors and irradiated for 60 minutes. The samples containing TB-derivatives 87 and 88 were extracted with phenol:CHCl₃ prior to electrophoresis. The results are shown in Figure 3.35.

![Image of gel electrophoresis](image_url)

**Figure 3.35:** (a) Agarose gel electrophoresis of pBR322 DNA (1mg/ml) after irradiation at λ >350 nm in 10 mM phosphate buffer (pH 7.0). Lane 1: pBR322 control, Lane 2: 82 (P/D 20) non-irradiated, Lane 3: (±)-87 (P/D 10) non-irradiated Lane 4: (±)-87 (P/D 10) non-irradiated + extracted, Lane 5: 82 (P/D 10) irradiated Lane 6: (±)-87 (P/D 10) irradiated, Lane 7: (±)-87 (P/D 10) irradiated followed by extracted, Lane 8: 83 (P/D 20) non-irradiated, Lane 9: (±)-88 (P/D 10) non-irradiated, Lane 10: (±)-88 (P/D 10) non-irradiated + extracted, Lane 11: 83 (P/D 20) irradiated, Lane 12: (±)-88 irradiated, Lane 13: (±)-88 irradiated and extracted, Lane 14: 60 (P/D 20) irradiated.

In the absence of light, the 4-amino precursors 82 and 83 did not exhibit any photocleavage activity (lane 2 and 8). In the absence of irradiation, the electrophoretic mobility of pBR322 was reduced in the presence of either of the TB-derivatives 87 and 88 (lanes 3 and 9). However, treatment of these samples with phenol:CHCl₃ was found to remove the bound ligands and restored the electrophoretic mobility of supercoiled DNA (Lanes 4 and 10). The 4-amino precursors 82 and 83 did not induce any significant photocleavage upon photoirradiation, with ca. (17-18)% cleavage being observed (Lane 5 and 11). The failure to observe significant photocleavage activity with the 4-amino analogues is perhaps related to their lower oxidising potential as already discussed in Chapter 2, as a consequence 82 and 83 did not show significant fluorescence quenching in the presence of GC rich sequences. In the presence of the unsubstituted analogue 60, ca. 70% photocleavage of the pBR322 DNA was observed (Lane 14). In contrast to the 4-amino precursors, the TB-derivatives 87 and 88 were found to cause significant (85-90%) photocleavage of the plasmid upon irradiation (Lane 6, 7 and Lane 12, 13 respectively). The picosecond transient absorption measurements (Described in Chapter 5) studies showed that the excited state of the TB-derivatives is very short-lived; suggesting that the triplet excited state cannot be involved in the photocleavage reaction.
To verify the possibility of covalent adduct formation between the TB-derivatives and DNA upon photoirradiation, a solution of st-DNA containing (±)-87 at a P/D = 10 was irradiated for one hour and subjected to the phenol: CHCl₃ extraction, which can remove the ligands bound by non-covalent interactions. Figure 3.36 shows the UV/vis absorption spectra of the organic and aqueous layer of the photoirradiated and the non-irradiated samples. In order to avoid interference of the phenol absorption the UV/vis absorption spectra were recorded from 300-600 nm.

As shown in Figure 3.36, the UV/vis absorption spectra of the organic layers of both the irradiated and non-irradiated samples showed the presence of the TB-derivative (±)-87. This would suggest that the extraction of the samples with phenol: CHCl₃ mixture can efficiently remove most of the bound ligand from st-DNA, which would not be the case if (±)-87 was irreversibly bound to DNA.

Having established the strong DNA binding affinity and ability to induce DNA photocleave upon photoirradiation, the cytotoxicity and the cellular uptake studies of (±)-87 and (±)-88 were performed in collaboration with Prof. Clive Williams in the School of Biochemistry, Trinity College Dublin. The studies described here, were carried out by Dr. Sandra Bright in the Williams research group.

### 3.13 Preliminary Cytotoxicity and Cellular Uptake Studies

The cytotoxicity properties of (±)-87 and (±)-88 were evaluated against HeLa cell lines using an alamar blue cytotoxicity assay. This assay determines the cell viability by utilising a non-fluorescent dye resazurin, which can be converted into a fluorescent dye resorufin in response to chemical reduction of the growth medium resulting from cell growth. Reduction related to growth causes the redox indicator to change from the oxidised (non-
fluorescent, blue) form to the reduced (fluorescent, red) form. The fluorescent signal was monitored using excitation and emission wavelengths of 544 nm and 590 nm respectively. Experiments were performed in triplicate on three independent days with activity expressed as percentage cell viability compared to vehicle treated controls. As shown in Figure 3.37, (±)-87 and (±)-88 did not reduce the viability of the HeLa cells significantly upto concentrations of 100 µM both in dark and under light-irradiated conditions. The lack of cytotoxicity of (±)-87 and (±)-88 against HeLa cells strongly contrasts the strong DNA binding and significantly high photocleavage activities exhibited by these compounds. It was therefore, necessary to investigate the cellular uptake of these compounds.

![Graph showing % Viability vs [Compound, µM] (log10)](image)

**Figure 3.37:** The antiproliferative effect of (±)-87 and (±)-88 on HeLa cell lines. Samples were irradiated for one hour.

Populations of HeLa cells (1×10^5 cells/well) were treated with the compounds for up to 48 hrs, followed by the addition of nuclear staining dye DRAQ5 and were viewed using an Olympus FV1000 confocal microscope. Figure 3.38 shows the fluorescence confocal laser scanning microscopy images of HeLa cells treated with (±)-87, which demonstrates rapid cellular uptake within 30 minutes of incubation and apparent localisation of the compound within the cytoplasm (Figure 3.38a). The nuclear staining is shown using DRAQ5 (red) in Figure 3.38b and 3.38e. Compound (±)-87 was not found to localise within the nucleus as demonstrated in the overlaid emission of (±)-87 and nuclear stain DRAQ5 as shown in Figure 3.38c and 3.38f. Compound (±)-88 also showed similar behaviour, with predominant localisation within the cytoplasm (Figure A3.40, Appendix 3). The failure to localise within the nucleus of HeLa cells presumably results in lower cytotoxicity of these molecules in spite of their high DNA binding and photocleavage activities. Further studies are currently being undertaken to investigate their uptake and cytotoxic properties in other malignant cell lines.
Chapter 3: Photophysical and DNA Binding Studies of the Tröger’s Base Containing Bis-1,8-naphthalimides

Figure 3.38: Confocal laser scanning microscopy images of HeLa cells treated with (a) (±)-87 (20 μM) (b) nuclear stain DRAQ5 and (c) overlay of (±)-87 and DRAQ5 (phase contrast) after 30 minutes of incubation. (d) (±)-87 (20 μM) (b) nuclear stain DRAQ5 and (c) overlay of (±)-87 and DRAQ5 (phase contrast) after 5 hrs of incubation.

3.14 Conclusions and Future Studies

The work presented in this chapter describes the synthesis of two novel bis-1,8-naphthalimide based Tröger’s base derivatives 87 and 88 in reasonable yields. This study also showed the first successful resolution of the Tröger’s base containing bis-1,8-naphthalimide derivatives by cation exchange column chromatography using a chiral eluent. Due to nonavailability of suitable single crystal and X-ray crystallographic data, the absolute configurations of the enantiomers could not be assigned. Determination of the absolute configuration of these enantiomers using various techniques including quantum chemical calculations remains a subject of future studies. The photophysical properties of (±)-87 and (±)-88 have been studied in a range of solvents of varying polarity and H-bonding ability using both UV/vis and fluorescence spectroscopic techniques. These studies highlighted that these TB-derivatives exhibit an ICT excited state similar to their 4-amino-1,8-naphthalimide precursors. However, the quantum yield of emission was found to be very weak compared to the 4-amino-1,8-naphthalimides and both of the derivatives (±)-87 and (±)-88 were nearly non-fluorescent in aqueous medium. The weak quantum yield of emission was most likely to be due to the enhanced rate of non-radiative decay processes, which deactivate the excited singlet state. Various internal motions can act as the non-radiative decay pathways for the
deactivation of the excited singlet state. Alternatively the possible electronic coupling between the aryl rings facilitated by the Tröger’s base moiety can also result in weak emission of these compounds.

Following the initial photophysical characterisation of (±)-87 and (±)-88, the interaction of these TB-derivatives and their enantiomers with st-DNA in aqueous solution has been studied using UV/vis titration, ethidium bromide displacement assays, thermal denaturation assay, circular dichroism and linear dichroism techniques.

Both of the TB-derivatives (±)-87, (±)-88 and their enantiomers showed strong affinity for st-DNA (ca. $10^6$ M$^{-1}$) in 10 mM phosphate buffer. Moreover, the binding site size was found to be less than unity in all cases, suggesting that these molecules presumably bind to the DNA groove. Additionally, in 10 mM phosphate buffer a biphasic binding pattern was observed for (±)-88 and its enantiomers. This has been attributed to the DNA-mediated stacking along the polyanion backbone at lower concentrations of DNA followed by redistribution of the ligands at higher DNA concentrations. The TB-derivative (±)-88 also displayed significantly higher binding affinity for st-DNA compared to that of (±)-87. This could be due to the presence of the methyl group, which allows favourable hydrophobic and van der Waals interactions with DNA. Additionally, in the case of 88 the close proximity of the positively charged pyridinium group to the DNA phosphate backbone might also result in a stronger binding interaction. The high DNA binding affinities of both of the TB-derivatives and their enantiomers were also confirmed from their ability to displace ethidium bromide from st-DNA. The binding constants calculated from ethidium bromide displacement assay followed the order (±)-88 > (±)-87.

At low ionic strength buffer, the (+) and (-) enantiomers of both 87 and 88 showed comparable DNA binding affinity presumably due to very strong association of the compounds under such conditions. However, at higher ionic strength, the binding affinity of the (-)-enantiomer was found to be higher than the (+)-enantiomer for both of the TB-derivatives 87 and 88. This enantioselectivity was significantly pronounced in the presence of 150 mM NaCl, which closely resembles the physiological Na$^+$ concentration. The enantioselectivity was also reflected in the reverse NaCl titration. As the ionic strength of the media is gradually raised, the (-) enantiomer remained more strongly bound for both 87 and 88. During reverse salt titrations none of the TB-derivatives were completely displaced from st-DNA at higher ionic strength (ca. 485 mM), indicating very high affinity of these molecules under high ionic strengths. This enantioselectivity probably results from the different three-dimensional shape of the enantiomers, which prefers binding of the (-)-enantiomer to the right-handed B-DNA over the (+)-enantiomer.
Both of the TB-derivatives (±)-87 and (±)-88 and their enantiomers exhibited strong preference for AT rich sequences. In all cases the binding affinity follows the order (-)-enantiomer > (±)>(+)-enantiomer.

Circular dichroism studies of sT-DNA in the presence of (±)-87 and (±)-88 showed the appearance of a weak negative induced CD signal ca. 420 nm, which again indicated the preferential binding of the (-)-enantiomer with sT-DNA.

Linear Dichroism spectroscopy revealed that both (±)-87 and (±)-88 bind to DNA groove and caused significant stiffening of DNA structure. The “V”-shaped structure of these TB-derivatives presumably exerts steric constrains to allow intercalation of the planar naphthalimide ring. Although LD spectroscopy could not provide information regarding the major groove or minor groove binding, the strong preference of (±)-87 and (±)-88 and their enantiomers for the AT-rich polynucleotide suggests that these TB-derivatives possibly bind to the minor groove of DNA. However, this requires further investigation.

Both (±)-87 and (±)-88 can induce significant photocleavage of pBR322 DNA upon light irradiation (>350 nm). The absence of any long-lived excited state for these derivatives suggests that the photocleavage reaction is probably not mediated by the triplet-excited state. The photoinduced electron transfer from DNA base to the excited state of (±)-87 and (±)-88 is presumably responsible for the photocleavage activity. The mechanism of these photoreactions needs to be investigated in detail. Though these compounds are rapidly taken up by HeLa cells, they failed to localise within the cell nucleus and exert cytotoxic activities. Further studies are required to investigate nuclear localisation of these compounds in other malignant cell lines.

The unique “V”-shaped structure of these TB-derivatives combined with their strong affinity for DNA and ability to induce DNA photocleavage can be used to develop probes for various DNA secondary structures such as “hairpin loop”, “bulged nucleotide sequences”. These secondary nucleic acid structures play important roles during key physiological events like translational initiation, protein cleavage, and mutagenesis and are considered as important drug targeting sites. Therefore, the ability of TB-derivatives to recognise specific various secondary nucleic acid structures needs to be investigated in future.
Chapter 4

Synthesis, Photophysical and DNA Binding Studies of [Pt(terpy)naph]^{2+} Complex
4.1 Introduction

Studying the interaction of transition metal complexes with biomolecules such as DNA and proteins comprises a wide area of research in medicinal chemistry over several decades. In this context, platinum (II) based coordination compound cisplatin (93) and its structural congeners such as carboplatin (94) and oxaliplatin (95) represent a small selection of the leading chemotherapeutic agents that are currently being used in clinical treatment worldwide for more than three decades for treating head and neck cancers, lung, testicular, ovarian and cervical cancers. However the cellular resistance (inherent/acquired after the initial treatment) has been found to hamper the efficacy of these chemotherapeutic agents leading to the continuing need to develop new platinum based drugs.

Due to their unique photophysical properties and biological activities a large number of square planar Pt(II) complexes containing 2,2':6',2''-terpyridine (abbreviated to [Pt(terpy)X]n+, where X represents ligands occupying the fourth coordination site and “n” is the charge of the complex) have been extensively investigated.

Lippard and co-workers provided the first experimental evidence that [Pt(terpy)Cl]n+ 96 and [Pt(terpy)HET]n+ 97 (where HET represents 2-hydroxyethanethiol) bind to ct-DNA via intercalation. The addition of 97 to a solution of ct-DNA caused a significant lengthening of DNA, an increase in the viscosity of DNA as well as an increase in the melting temperature of ct-DNA. Moreover 97 displayed a binding constant ca. 1.2 ± (0.2)×10^6, which was comparable to the association constant of the classical intercalator ethidium bromide and also competitively inhibited the binding of ethidium bromide to DNA. The intercalative mode of binding was also evident from the abilty of 97 to unwind ct-DNA demonstrated by the ultracentrifugation studies. Subsequent X-ray diffraction studies revealed that 97 unwound ct-DNA by 22 ± (6)°. In a related study, Lippard and co-workers examined the effect of charge of the metal complex and the nature of the coordinating ligands on the DNA binding affinity by comparing a series of [Pt(terpy)X]n+ complexes (96-102). This study showed that each of these complexes caused unwinding of DNA and competitively inhibited the binding of ethidium bromide to ct-DNA, indicating their ability to bind via intercalation.
Among these, the AET complex $98$, displayed the highest DNA binding affinity, which was suggested to be due to the dicationic nature of the complex at physiological pH. Moreover, this study also revealed that the affinity of the metal complexes increased with increasing the number of coplanar aromatic ligands as was evident from the higher affinity of $[\text{Pt(terpy)}X]^{n+}$ systems $96-100$ compared to the analogues containing bipyridyl units $101-102$.

The chloro complex $96$ was also found to form covalent adducts with $E\ coli$ t-RNA, which disrupts the three dimensional structure of the biomolecule. The ability of $96$ to form covalent adducts with the DNA bases such as adenosine-5'-monophosphate (AMP) and uridine-5'-monophosphate (UMP) was initially investigated using UV/vis spectroscopy, which suggested formation of a (1:2) complex between AMP and $96$.

McMillin and co-workers reported the interaction of the $[\text{Pt(terpy)}\text{OH}]^+$ complex $103a$ with DNA, which binds via intercalation as evident from the changes in the ground state UV/vis absorption spectrum of $103a$ and viscosity measurements. However, over a longer
time scale (overnight equilibration) a second mode of binding was observed, where 103a was found to lose the labile hydroxide ligand and formed covalent adducts with DNA. This binding mode was observed at higher [DNA]/[metal complex] ratios, where long stretches of the duplex DNA become available for covalent binding. Moreover binding of 103a with st-DNA or poly(dA-dT)$_2$ resulted in significant enhancement in the luminescence of the complex due to protection of the chromophore from solvent molecules and molecular oxygen. This study was further extended to develop [Pt(4'-NMe$_2$-terpy)CN]$^{2+}$ 103b UV/vis absorption, emission, CD spectroscopy along with viscosity measurements studies were used to show that 103b exhibited biphasic-binding interactions in the presence of double stranded DNA. At low concentrations of DNA, 103b was found to aggregate on the DNA surface, however, at high concentrations of DNA 103b showed intercalative binding with a preference for GC rich sites. Similar to the hydroxo analogue 103a, intercalation of 103b resulted in an enhanced luminescence intensity in the presence of poly(dI-dC)$_2$ (I = inosine) and poly(dA-dT)$_2$; whereas binding to poly(dG-dC)$_2$ resulted in the quenching of emission due to the strong reducing nature of guanine. However, in contrast to 103a, no covalent adduct formation was observed due to the higher strength of the Pt-C bond, such that CN$^-$ can not act as a good leaving group.

Over the years, numerous DNA binders based on Pt(II)-terpyridine complexes have been developed. For example the dicationic [Pt(terpy)(4-picoline)]$^{2+}$ complex 104a was shown to unwind DNA and stabilised DNA against heat denaturation, which suggested that 104a can bind to DNA via intercalation. Lowe and co-workers presented the detailed characterisation of the covalent binding of 104a with guanine, adenine and cytosine derivatives using NMR spectroscopy. This study showed that the incubation of 104a with guanosine or deoxyguanosine at 37°C for several days resulted in the displacement of the 4-picoline ligand and coordination of the [Pt(terpy)$^{2+}$ unit to the N$^7$-position of guanine (as
shown in Figure 4.1a. Since this site is not involved in Watson-Crick base pairing in standard B-DNA structure, this site may be available for covalent binding. Additionally, 104a was also found to form a tricationic species with adenosine by coordinating to the N$^1$ and N$^6$ amino group (Figure 4.1b). The tendency of the [Pt(terpy)$^{2+}$ to form π-stacked structures was proposed to be the driving force to form such binuclear complexes.

In a related study Cusamano et al., compared the steric and electronic effect on the binding affinity of a series of pyridine ligands 104a-c, where the binding affinity was found to follow the order 104a>104c>104b. The increased affinity of 104a compared to the unsubstituted pyridine analogue 104c has been attributed to the presence of the electron donating nature of the methyl group at the 4-position, which can enhance the electron density on the intercalating terpyridine moiety. However, in 104b, the presence of the methyl group in the 2-position presumably caused a steric obstacle for intercalation to occur resulting in a lower DNA binding affinity of the complex.

In order to improve the binding affinity as well as the binding specificity, several binuclear Pt(II)terpy complexes involving various flexible and rigid spacers have been developed. The DNA binding ability of a series of dinuclear complexes 105(a-g) containing alkane dithiol linkers were studied using viscometry and helix unwinding assays, which showed that complexes 105b-d, where the number of intervening methylene units varied from five to seven, behaved as bisintercalators. However, with the derivatives with longer spacer lengths 105e-g, a mixed monofunctional and bifunctional modes were observed.

Goto and co-workers reported the interaction of ct-DNA with binuclear complexes 106a-b incorporating the rigid 1,3- or 1,4-benzenedimethanethiol bridging units. The CD titrations of ct-DNA with 106a-b showed a marked difference, which suggested that 106a
with the 1,3- benzenedimethanethiol linker binds via bisintercalation causing significant distortion in the DNA structure, while 106b with the 1,4-benzenedimethanethiol spacer functions as a monointercalator.\textsuperscript{252c}

In recent times, Pikranenou and co-workers reported the development of a “hair-pin” shaped binuclear complex 107, in which two [Pt(terpy)(SPh)]\textsuperscript{+} units are connected via a neodymium(III) aminocarboxylate bridge.\textsuperscript{253} In this structure, the two [Pt(terpySPh)]\textsuperscript{+} intercalating units are separated by 10.5\AA, which is ideal for bisintercalation. Flow linear dichroism measurements and molecular modelling data provided further evidence for binding of 107 via bisintercalation.

![Image of complex 107]

In spite of the extensive use of the 1,8-naphthalimide derivatives in developing DNA targeted antitumour agents (as detailed in Chapter 1), there are only a few examples of DNA binders based on Pt(II) complexes incorporating the naphthalimide unit. As described in Chapter 1 (Section 1.6.11), Pérez \textit{et al.}, developed novel Pt-bis(1,8-naphthalimide) complexes 52a-b, which exhibited high cytotoxicity against various cisplatin sensitive and resistant cell lines.\textsuperscript{123} DNA unwinding studies suggested that the cytotoxicity of these derivatives largely arise from the combination of intercalation and platination.

Taking these examples into account, the novel Pt(II) metal complexes 108-109 were designed by combining the intercalating Pt(terpy)\textsuperscript{2+} unit with the intercalating 4-amino-1,8-naphthalimide chromophore. Such bifunctional metal complexes were anticipated to have high DNA binding affinities compared to monofunctional [Pt(terpy)X]\textsuperscript{n+} derivatives with further applications in cellular imaging and therapeutics. Additionally, the incorporation of the naphthalimide unit is also expected to influence the photophysical properties of the [Pt(terpy)X]\textsuperscript{2+} complex. The work presented in this chapter was carried out in collaboration with Dr. Jonathan A. Kitchen in the Gunnlaugsson group and describes the successful synthesis, characterisation and X-ray crystal structure of 109. The photophysical properties of 109 were studied in a range of solvents and compared with the free ligand. The DNA binding
ability of 109 was studied using UV/vis absorption, fluorescence spectroscopy, circular dichroism, linear dichroism and thermal melting assay. The antitumour activity of 109 against HeLa was also evaluated in collaboration with Prof. Clive Williams in the school of Biochemistry, Trinity College Dublin.

4.2 Design and Synthesis of 108-109

The first step involved the synthesis of the [Pt(terpy)Cl]Cl complex 110 following a literature procedure, which involved reaction of the commercially available potassium tetrachloroplatinate (K₂PtCl₄) with 2,2':6',2''-terpyridine in water under refluxing conditions at 110°C until a clear solution was obtained (Scheme 4.1). The desired product 110 was obtained as a dark orange solid in 89% yield after acidification of the solution using concentrated HCl. Complex 110 was fully characterised by ¹H NMR, ¹³C NMR, HRMS and IR analysis. Mass spectrometric analysis further confirmed the successful formation of 110 indicated by the presence of a peak at 463.0292 corresponding to the M⁺ ion (M⁺·C₁₃H₁₁N₃ClPt requires 463.0289).

The ligands 89 and 91 were synthesised in reasonable yields starting from the commercially available precursors as discussed in Chapter 2 (Section 2.2.2 and 2.2.3 respectively).

The complexation of 89 and 91 with 110 was attempted following a modified literature procedure that involved stirring a suspension of 110 in DMF in the presence of an excess of AgNO₃ at room temperature in the absence of light for 48 hours, Scheme 4.1. The resulting mixture was then filtered through celite to remove AgCl precipitate and the solutions of 89 or 91 in DMF were added to the filtrate and stirred for 2-3 hours. In the case of 89, this procedure did not result in successful formation of the desired product 108, as ¹H NMR and TLC analysis of the resulting mixture showed the presence of the unreacted starting materials.
Heating the reaction mixture at 90°C for 3-4 days was also found to be unsuccessful, as were the attempts using microwave assisted heating. Further modification of the synthetic procedure was attempted; where a suspension of 110 was stirred in the presence of AgPF₆ in acetone followed by the addition of 89 after the removal of AgCl precipitate as before. However, as above this modification also did not result in the formation of the target complex.

A summary of the various attempts for the synthesis of 108 is shown in Table 4.1. In spite of these repetitive attempts, the failure to obtain 108 was thought to be due to the presence of the 4-amino group, which can possibly compete with the pyridine donor site for the coordination to Pt(II).

\[
\text{Scheme 4.1: Synthetic pathway for 108-109.}
\]

\[
\text{Table 4.1: Summary of attempts for the synthesis of 108.}
\]

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stirring at room temperature in DMF</td>
<td>X</td>
</tr>
<tr>
<td>2</td>
<td>Heating at 90°C for 4 days</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>Heating in microwave for 2 hrs.</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>Reaction in microwave using AgPF₆ in acetone</td>
<td>X</td>
</tr>
</tbody>
</table>
In contrast to these results the treatment of the labile Pt(II) complex \([\text{Pt(terpy)Cl}]^+\) with one equivalent of \(\text{91}\) following the aforementioned literature procedure resulted in the successful formation of the target complex \(\text{109}\) as a dark red solid after precipitation from diethyl ether (Scheme 4.1). The crude complex \(\text{109}\) was purified by repetitive trituration with methanol at room temperature to give the target complex in \(ca.\) 82\% yield. The complex \(\text{109}\) was fully characterised using \(^1\text{H}\) NMR, \(^{13}\text{C}\) NMR, IR and HRMS analysis. The \(^1\text{H}\)-NMR resonances were assigned using H-H COSY and C-H COSY experiments. The \(^1\text{H}\) NMR spectrum of \(\text{109}\) in CD\(_3\)OD is shown in Figure 4.2, which showed that the aromatic region was composed of multiple peaks from the naphthalimide ring, pyridyl ring of the ligand and the terpyridine ring. The NMR resonances corresponding to the terpyridine ring were found to lie very close to each other and the assignments were done by a combination of HSQC and HMBC techniques and are shown in Figure 4.2. The successful formation of \(\text{109}\) was clearly evident from the HRMS analysis, where \(\text{109}\) displayed a peak at 386.6043 corresponding to the \(\text{M}^{2+}\) ion (\(\text{M}^{2+}\text{\cdotC}_{36}\text{H}_{36}\text{N}_6\text{O}_2\text{Pt}\) requires 386.6039). A comparison of the calculated and obtained isotopic distribution pattern is shown in Figure 4.3, which further confirms the identity of the product.

\[\text{Figure 4.2: } ^1\text{H NMR spectrum of 109 (600 MHz, CD}_3\text{OD).}\]
In order to investigate the role of the naphthalimide chromophore on the photophysical properties of the metal complex, a model [Pt(terpy)(4-picoline)]^{2+} complex 104a was also synthesised using the above mentioned procedure (Scheme 4.2) as a pink solid in 41% yield. Complex 104a was fully characterised by ^1H NMR, ^13C NMR, HRMS, and IR analysis.

4.2.1 Structural Analysis of 91 and 109

Small plate shaped yellow coloured crystals of 91 were grown by the slow evaporation of ethanol. The X-ray crystal structure analysis was carried out by Dr. J. A. Kitchen. The low temperature (118 K) crystal data showed that 91 crystallised in the monoclinic space group P2(1)/n with one molecule of 91 in the asymmetric unit (Figure 4.4 a).

All the bond lengths and angles were consistent with the naphthalimide derivatives reported in Chapter 2. As shown in Figure 4.4a, the ethylene linker in 91 assumes an anti-
conformation placing the pyridyl and the naphthalimide rings in almost coplanar orientation, similar to that observed in naphthalimides 83-84 described in Chapter 2. The NMe₂ moiety was slightly twisted out of the naphthalimide plane and the nitrogen atom adopts a tetrahedral like geometry. The packing is governed by off-set face-to-face π...π interactions between the adjacent naphthalimide rings, resulting in a head-to-tail arrangement of the molecules with the average distances between the mean planes ranging from 3.3Å-3.4 Å as shown in Figure 4.4b.

![Figure 4.4:](image)

**Figure 4.4:** (a) The X-ray crystal structure of 91 with thermal ellipsoids shown at 50% probability. (b) The packing of 91 showing the π...π interactions between the stacked naphthalimide rings, which adopt a ‘head-to-tail’ type orientation, naphthalimide mean planes are shown as red sheets.

Small dark red coloured plate shaped crystals of 109 as its NO₃⁻ salt suitable for X-ray diffraction analyses were also grown by the slow evaporation of acetonitrile. The X-ray crystal structure analysis was performed by Dr. J. A. Kitchen. The low temperature (100 K) crystal structure showed that 109 crystallises in the monoclinic space group C2/C, with one molecule of [Pt(terpy)(L)](NO₃)₂ and two interstitial CH₃CN molecules in the asymmetric unit (Figure 4.5a). One molecule of CH₃CN was removed by the SQUEEZE routine in Platon. The Pt(II) is in the expected square planar geometry with the cis N-Pt-N angles in the range 81.1°(2)-99.9°(2) as described in Table 4.2. The Pt...N bond lengths ranged from 1.9 Å-2.03
Å, consistent with the literature values. The structure of the naphthalimide ligand was similar to that of the free ligand 91 described above. Strong π...π stacking interactions were observed between the terpyridine moieties and the naphthalimide rings from the neighbouring coplanar complexes with centroid...centroid distances of 3.644 Å and 3.532 Å respectively as shown in Figure 4.5b. They were repeated throughout the complex to give a twisted head-to-tail arrangement.

**Figure 4.5:** (a) The X-ray crystal structure of 109 with thermal ellipsoids shown at 30% probability. (b) The packing of 109 showing the π–π interactions between the stacked naphthalimide and terpyridine rings.
Chapter 4: Synthesis, Photophysical and DNA Binding Studies of \([\text{Pt}(\text{terpy})\text{naph}]^{2+}\) Complex

Table 4.2: Selected bond lengths [Å] and angles [°] for 109.

<table>
<thead>
<tr>
<th>Bond lengths/ angles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt(1)-N(5)</td>
<td>1.945(7)</td>
</tr>
<tr>
<td>Pt(1)-N(4)</td>
<td>2.028(5)</td>
</tr>
<tr>
<td>Pt(1)-N(3)</td>
<td>2.034(7)</td>
</tr>
<tr>
<td>Pt(1)-N(6)</td>
<td>2.035(6)</td>
</tr>
<tr>
<td>N(5)-Pt(1)-N(4)</td>
<td>81.4(2)</td>
</tr>
<tr>
<td>N(5)-Pt(1)-N(3)</td>
<td>177.9(2)</td>
</tr>
<tr>
<td>N(4)-Pt(1)-N(3)</td>
<td>97.5(2)</td>
</tr>
<tr>
<td>N(5)-Pt(1)-N(6)</td>
<td>81.1(2)</td>
</tr>
<tr>
<td>N(4)-Pt(1)-N(6)</td>
<td>162.5(2)</td>
</tr>
<tr>
<td>N(3)-Pt(1)-N(6)</td>
<td>99.9(2)</td>
</tr>
</tbody>
</table>

The X-ray crystal structure of 109 showed that both of the planar terpyridyl moiety and the naphthalimide unit were involved in extensive stacking interactions, which is an important criterion for intercalation into DNA. The following sections will describe the photophysical studies and DNA binding studies of 109 using various spectroscopic techniques.

4.3 Photophysical Studies of 109

Square planar Pt(II) complexes with $d^9$ electronic configuration are “low spin” complexes due to the large energy gap between the highest ($d_{x^2-y^2}$) and the second highest ($d_e$) orbitals, where the $x$-$y$ plane is considered to be the plane of the metal complex. On photoexcitation, if an electron is promoted to this $d_{x^2-y^2}$ orbital, the molecule will undergo significant distortion as the Pt-L bond length increases in the excited state. Consequently, the potential energy surface of the excited state is significantly displaced relative to the ground state (Figure 4.6a). This leads to an unfavourable situation for emission. Under such condition the molecules can revert back to the ground state through a thermally accessible isoenergetic crossing point of ground state and excited state potential energy surfaces by non-radiative internal conversion or intersystem crossing mechanisms as shown in Figure 4.6a. Additionally, due to the relatively small values for the radiative decay rate constants for the Laporte-forbidden $d$-$d$ transitions, Pt(II) complexes are usually very weakly luminescent or non-luminescent in fluid state at room temperature. In the presence of aromatic ligands around the metal ion, ligand centred transitions ($\pi$-$\pi^*$ or n-$\pi^*$) or charge transfer transitions from the $d$ orbital on the metal ion to the $\pi^*$ orbital centred on the ligand can take place and often for Pt(II) complexes, emission occurs from either the MLCT or the LC excited states, which lie at lower energies than the metal centred $d$-$d$ energy levels. Due to the high spin-orbit coupling constant of platinum, the luminescence is usually observed from the excited triplet state. However, the
close proximity of the thermally accessible d-d states can still provide a pathway for non-radiative deactivation and affect the luminescence quantum yield at room temperature (Figure 4.6b). The presence of the terdentate terpyridyl ligand imparts additional rigidity to the system and favours the square planar geometry. However, due to the structural rigidity imposed by the ligand, the lateral M-N bond lengths are usually lengthened beyond the ideal values, which results in less ligand field effects and consequently the energy of the d-d excited state is lowered. A similar situation is encountered in the case of [Ru(terpy)2]2+ complex, which is nearly non-luminescent at room temperature compared to [Ru(bpy)3]2+. However, the presence of a strong field ancillary ligand in the fourth coordination site can influence the photophysical properties of these complexes. The work described in the following sections will investigate the effect of the naphthalimide chromophore coordinated to [Pt(terpy)]2+ in complex 109 through a moderately strongly field pyridyl ligand and will be compared with the [Pt(terpy)(4-picoline)]2+ complex 104a.

4.3.1 Ground State Studies

The UV/vis absorption spectra of 104a, 109 and 110 in water at room temperature are shown in Figure 4.7. The absorption spectrum of [Pt(terpy)Cl]1+ 110 in water shows two structured bands at around 329 nm and 344 nm, attributed to the intraligand π-π* transitions of the terpyridyl ligand. The weak shoulder in the wavelength region 350-450 nm has been assigned to the 1MLCT transition from the comparison with the Zn(terpy)Cl2 complex, which does not exhibit this band as Zn2+ can not be further oxidized and thus d-π* excited states do
not exist. The [Pt(terpy)4-picoline]$^{2+}$ complex 104a and the [Pt(terpy)naph]$^{2+}$ complex 109 also exhibited two sets of structured bands at 340 nm and 324 nm, which can be assigned to the intraligand $\pi$-$\pi^*$ transitions of the terpyridyl ligand by comparison with the [Pt(terpy)Cl]$^{+}$ system. For the picoline system, 104a, weak bands were observed at wavelengths greater than 350 nm (370 nm, 390 nm), which presumably represents the $^1$MLCT transition from Pt($d$)$\rightarrow$terpy($\pi^*$). These features were also observed in the absorption spectrum of the [Pt(terpy)naph]$^{2+}$ complex, but overlapped with the strong absorption band at 450 nm assigned to the intraligand charge transfer (ICT) transition of the naphthalimide ligand. The molar extinction coefficients for the various bands for complex 109 are listed in Table 4.3. It should be mentioned here, though square planar [Pt(terpy)X]$^+$ complexes are known to undergo aggregation in aqueous solution, 109 was found to follow Beer Lambert’s law up to a concentration of 100 $\mu$M, which could be due to the dicationic nature of the complex, which prevents aggregation. Therefore, at the concentrations used for the photophysical measurements in this study, 109 predominantly exists in its monomeric form.

![Figure 4.7: Normalised UV/vis absorption spectra of 104a, 109 and 110 in water.](image)

**Table 4.3: Summary of the molar extinction coefficients for the various bands of 109 in water (Error ± 10%).**

<table>
<thead>
<tr>
<th></th>
<th>324 nm ($\pi$-$\pi^*$ terpy)</th>
<th>340 nm ($\pi$-$\pi^*$ terpy)</th>
<th>450 nm (ICT naphthalimide)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(M$^{-1}$ cm$^{-1}$)</td>
<td>(M$^{-1}$ cm$^{-1}$)</td>
<td>(M$^{-1}$ cm$^{-1}$)</td>
</tr>
<tr>
<td>104a</td>
<td>9660</td>
<td>13500</td>
<td>7800</td>
</tr>
<tr>
<td>109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>110</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.2 Solid State Emission Measurements

The [Pt(terpy)Cl]^+ complex was found to be non-emissive in water at room temperature due to fast non-radiative deactivation of the low lying d-d excited states as discussed earlier. In contrast it displayed strong luminescence in the solid state. However, the emission behaviour is strongly influenced by the nature of the counterion. The red ClO_4^- salt displayed a relatively narrow shaped emission band centred at ca. 720 nm, which has been assigned to the metal-metal to ligand charge transfer (MMLCT) excited state (dσ*(Pt)-π*(terpy)). Such MMLCT-excited states originate from the strong d^2-d^2 interaction between the metal atoms from neighbouring molecules. In contrast, the Cl-, CF_3COO^- and PF_6^- salts exhibited an extremely broad emission band centred at ca. 640 nm, which has been assigned to the luminescence arising from an eximeric intraligand transition resulting from various π...π interactions between the closely spaced metal complexes.

In the solid state both of the complexes studied here, 104a and 109 exhibited an intense broad emission band centred at ca. 680 nm, when excited at 390 nm, as shown in Figure 4.8. By comparison with the literature, this broad band was assigned to the aggregation-induced emission involving π...π and metal-metal dσ*-π* interactions as observed previously for 110. The emission band of 109 and 104a were found to be quite shifted compared to the literature values, which could be due to the presence of the NO_3^- counter ion, resulting in a different packing arrangement in the solid state.

![Figure 4.8: Aggregation induced emission from solid 109 and 104a as their NO_3^- salts at 298 K. λ_ex = 390 nm.](image-url)
4.3.3 Emission Studies in Solution

In aqueous solution, the [Pt(terpy)(4-picoline)]\(^{2+}\) complex 104a displayed an extremely weak emission band at ca. 540 nm, when excited at \(\lambda_{ex} = 390\) nm (Figure 4.9a). This has been tentatively assigned to the \(^3\)MLCT [Pt (d\(\rightarrow\)terpy (\(\pi^*\))] excited state.\(^{260}\) The intensity of this band did not increase upon degassing using N\(_2\), suggesting that the excited triplet state is presumably short-lived, possibly due to the presence of thermally accessible close lying d-d excited states, which can cause deactivation of excited states through non-radiative pathways.

Figure 4.9: (a) Steady-state emission spectrum of 104a in water with \(\lambda_{ex} = 390\) nm (b) UV/vis absorption (—), Emission (—) with \(\lambda_{ex} = 450\) nm and excitation (—) spectra with \(\lambda_{em} = 550\) nm of 109 in water.

In aqueous solution 109, when excited at \(\lambda_{ex} \geq 390\) nm displayed a broad structureless emission band at 550 nm. Excitation spectrum recorded with \(\lambda_{em} = 550\) nm resembles the naphthalimide ICT absorption band (Figure 4.9b). Therefore, the emission band at 550 nm observed for 109 presumably corresponds to the ICT emission band centred on the ancillary naphthalimide ligand but this band possibly also has some contribution from the \(^3\)MLCT [Pt (d\(\rightarrow\)terpy (\(\pi^*\))] transition, which has been observed for the corresponding picoline complex 104a.

4.3.4 Effect of Solvent Polarity on the Photophysical Properties of 109

To investigate the effect of solvent polarity on the photophysical properties of complex 109, the UV/vis absorption spectra of 109 were recorded in water and various alcohols possessing different polarities. The UV/vis spectra and emission spectra of 109 are shown in Figure 4.10a below, which shows that the UV/vis absorption spectrum of 109 was
significantly altered upon moving from water to a methanolic solution. The ICT absorption band centred on the naphthalimide ligand showed a ca. 27 nm blue shift. Moreover, the weak shoulders at 370 nm and 390 nm observed in aqueous solution disappeared in methanolic solution. The absorption bands at 324 nm and 340 nm corresponding to the intraligand transitions of the terpyridyl ligand did not show any significant shift, however, the absorbance of the corresponding bands decreased markedly. The UV/vis absorption spectrum of 109 did not show any significant change, when the solvent was changed from methanol to less polar butanol.

**Figure 4.10:** UV/vis absorption spectra of (a) 109, (b) 91 and (c) 104a in various solvents.

For comparison, the UV/vis absorption spectra of the free naphthalimide ligand 91 were recorded in the same set of solvents. Due to insolubility of 91 in an aqueous media, a spectrum of 91 could not be recorded in water. As shown in Figure 4.10b, changing the solvent from methanol to butanol caused a ca. 4 nm blue shift in the \( \lambda_{\text{max}} \) in the case of 91. The changes in the UV/vis spectra of 109 in various alcohols closely resembled the spectra of the free ligand 91. The additional 27 nm red shift on changing the solvent from methanol to water suggests that the ground state of 109 has substantial polar character. For further comparative studies, the UV/vis absorption spectra of the corresponding picoline complex 104a were also recorded in water and ethanol. However, no such solvent dependence was
observed for the terpy centred band at 340 nm and 320 nm (Figure 4.10c). Therefore, the reason for the hypochromism observed for 109 on moving from water to alcoholic solvents remains unknown at the moment.

![Figure 4.11: Steady-state emission spectra of (a) 109, (b) 91 in various solvents.](image)

It is worth pointing out that, the steady-state emission spectrum of 109 showed significant changes on changing the solvent polarity. As discussed earlier, 109 displays a weak emission band centred at 550 nm in aqueous solution upon excitation at 450 nm. On changing the solvent from water to methanol, this band showed a ca. 17 nm blue shift and a significant enhancement in the emission quantum yield. On moving from methanol to butanol, this band showed a further ca. 9 nm blue shift and a further increase in the emission intensity (Table 4.4). The quantum yields of emission of 109 in various solvents were calculated using [Ru(bpy)_3]^2+ as the reference (\(\Phi_F = 0.028\) in aerated aqueous solution, \(\lambda_{ex} = 436\) nm), which showed that the increasing solvent polarity resulted in significant decrease in the quantum yield of emission for 109. These changes are summarised in Table 4.4. Lifetime measurements in various solvents showed that increasing polarity of the solvent resulted in the shortening of the emission lifetime (Table 4.4) (Figure A4.1 in Appendix 4). In water, due to a very low quantum yield of emission, reliable lifetime measurements could not be carried out. Such a decrease in fluorescence quantum yield accompanied with a red shift in the emission maxima as a function of solvent polarity is characteristic of an ICT excited state.

The emission spectra of the corresponding naphthalimide ligand 91 were altered in a similar manner as a function of solvent polarity (Figure 4.11b). On increasing the solvent polarity from butanol to methanol, the emission band showed a ca. 12 nm red shift, associated with significant decrease in quantum yields of emission in highly polar solvents (Table 4.5).
Table 4.4: Photophysical parameters of 109 in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric constant (20°C)</th>
<th>(\lambda_{\text{max}}) (Abs) nm</th>
<th>(\lambda_{\text{max}}) (Em) nm</th>
<th>(\Phi_F)</th>
<th>(\tau) ns ((\lambda_{\text{ex}} = 458) nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol</td>
<td>17.9</td>
<td>419, 343, 329</td>
<td>524</td>
<td>0.127</td>
<td>0.6 (4%), 9.55 (96%)</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>19.5</td>
<td>420, 343, 329</td>
<td>522</td>
<td>0.122</td>
<td>0.8 (5%), 9.24 (95%)</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>21.7</td>
<td>422, 343, 329</td>
<td>525</td>
<td>0.100</td>
<td>0.8 (4%), 9.10 (96%)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>25.0</td>
<td>423, 342, 328</td>
<td>527</td>
<td>0.091</td>
<td>0.8 (1%), 8.90 (99%)</td>
</tr>
<tr>
<td>Methanol</td>
<td>33.0</td>
<td>423, 340, 327</td>
<td>533</td>
<td>0.067</td>
<td>7.85 (100%)</td>
</tr>
<tr>
<td>Water</td>
<td>80.0</td>
<td>450, 340, 324</td>
<td>555</td>
<td>0.006</td>
<td>--c</td>
</tr>
</tbody>
</table>

\(a\) excitation of the solution at the naphthalimide centred band.

\(b\) measured w.r.t Ru(bpy)_3 standard in aerated aqueous solution \(\Phi_F = 0.028, (\lambda_{\text{ex}} = 436\) nm).

\(c\) Fluorescence decay could not be measured.

Table 4.5: Photophysical parameters of 91 in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(\lambda_{\text{max}}) (Abs) nm</th>
<th>(\lambda_{\text{max}}) (Em) nm</th>
<th>(\Phi_F)</th>
<th>(\tau) ((\lambda_{\text{ex}} = 458) nm) ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanol</td>
<td>421</td>
<td>525</td>
<td>0.023</td>
<td>0.32 (93%), 8.90 (7%)</td>
</tr>
<tr>
<td>Propan-2-ol</td>
<td>421</td>
<td>526</td>
<td>0.019</td>
<td>0.30 (91%), 9.00 (9%)</td>
</tr>
<tr>
<td>Propan-1-ol</td>
<td>421</td>
<td>527</td>
<td>0.015</td>
<td>0.26 (91%), 9.10 (9%)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>422</td>
<td>530</td>
<td>0.008</td>
<td>--c</td>
</tr>
<tr>
<td>Methanol</td>
<td>425</td>
<td>537</td>
<td>0.004</td>
<td>--c</td>
</tr>
<tr>
<td>Water</td>
<td>--a</td>
<td>--a</td>
<td>--a</td>
<td>--c</td>
</tr>
</tbody>
</table>

\(a\) Could not be measured due to insolubility.

\(b\) measured w.r.t fluorescein in 0.1 N NaOH (\(\Phi_F = 0.92, (\lambda_{\text{ex}} = 436\) nm).

\(c\) Fluorescence decay could not be measured.

It should be noted here that in all of the solvents studied, the quantum yields of emission of 91 were found to be much lower than that recorded for 109. This was also reflected in the fluorescence lifetime measurements of 91, which was found to have very short excited state lifetimes compared to 109 in all the solvents studied. In less polar solvents such as butanol and propan-2-ol, 91 was found to follow a biexponential decay with the major components having a decay constant \(\tau_1 = 0.32\) ns (93 %) and 0.30 ns (91%), respectively. The magnitude of the major component was below the pulse width of the excitation source (1.2 ns). Therefore, fluorescence decay measurements in highly polar solvents could not be carried out reliably due to instrumental limitations. The decrease in quantum yield of emission and fluorescence lifetime along with red shifted emission maxima suggested that the emission of 91 arises from the ICT excited singlet state. As described in Chapter 2, the low quantum yield of emission has been explained in terms of increased non-radiative decay mechanisms arising
from the C-N rotation and pyramidal $N$-inversion.\textsuperscript{128,195} Comparison of the data in Table 4.4 and 4.5 showed that with an increase in the solvent polarity, the quantum yield of emission and the excited state lifetimes of 109 changed in a manner similar to that seen for the free ligand 91. This suggested that the excited state of 109 also has significant internal charge transfer character. The quantum yield of emission and lifetime of this naphthalimide centred band was found to be much higher in the metal complex 109 compared to the free naphthalimide ligand 91, (cf. Table 4.4 and 4.5). The stronger emission band centred at 550 nm observed for 109 suggests that the emissive state has substantial triplet intraligand ($^3$IL) character, which would be induced by the strong spin-orbit coupling constant of the Pt(II) centre. However, in all solvents tested here the lifetime values obtained for 109 are much shorter than that usually observed for the excited triple states and the triplet state appears at longer wavelength compared to the corresponding singlet state. One possible explanation for this could be that the triplet and the singlet excited states have similar energy and the triplet state also has ICT character. The relatively short lived $^3$IL excited state can arise from the fast deactivation through various non-radiative pathways involving internal molecular motions such as rotation/twisting of C-N bonds or inversion of the amino nitrogen. The rate of these non-radiative deactivation pathways is enhanced with increasing solvent polarity, resulting in weak emission of 109 in highly polar solvents such as water. However, in long chain alcohols, due to the comparatively higher viscosity of the media, these molecular motions are restricted and the emission from $^3$IL state occurs efficiently.

4.3.5 Summary and Conclusions from the Photophysical Studies

The study discussed above showed that the [Pt(terpy)Cl]$^+$ complex is completely non-luminescent in solution, which is in agreement with previous reports. Introduction of the moderately strong field pyridine ligand in the fourth coordination site in the [Pt(terpy)4-picoline]$^{2+}$ complex 104a results in weak emission centred at 540 nm, which has been assigned to the $^3$MLCT [Pt ($d$)$\rightarrow$terpy ($\pi^*$)] transition. In the [Pt(terpy)naph]$^{2+}$ complex 109, the predominant emission band was observed around 550 nm in aqueous solution, when excited at 450 nm. The quantum yields of emission and the excited state lifetimes corresponding to this emission band were found to be sensitive to solvent polarity; characteristic of the ICT nature of the excited state. However, for complex 109, the quantum yield of emission and the excited state lifetime of the ICT emission band were higher in all the solvents studied here compared to the free ligand 91, suggesting that the excited state has significant triplet state character.
Having established the basic photophysical properties, we next investigated the interaction of 109 with st-DNA using various spectroscopic techniques. The results of these studies will be described in the following sections.

4.4 Interaction of 109 with st-DNA

Based on the results presented in the previous chapters, it was anticipated in designing 109 that the presence of the 1,8-naphthalamide chromophore would modulate the interaction of the [Pt(terpy)X]^{2-} system with DNA due to the bifunctional nature of the resulting complex. Moreover, the presence of the naphthalamide chromophore would also allow monitoring of the binding process using fluorescence spectroscopy. The interaction of 109 with st-DNA was studied by a number of spectroscopic methods, the results of which are described in the following sections.

4.4.1 UV/Vis Absorption Titrations of 109 with st-DNA

The interaction of 109 with st-DNA was firstly investigated using UV/vis absorption spectroscopy in a manner similar to that described in the previous chapters, by adding small aliquots of st-DNA solution to a solution of the metal complex in 10 mM phosphate buffer (pH 7.0), until the changes in absorbance reached a plateau. The titrations were repeated three times to ensure reproducibility of the data. Changes in the ICT absorption band of naphthalamide at 450 nm and in the intraligand π-π* transition bands of terpyridine at 325 nm and 340 nm were monitored in the presence of increasing concentration of st-DNA. The changes in the ground state UV/vis spectra of 109 are shown in Figure 4.12, which showed that 109 exhibited a ca. 22% decrease in the ICT absorption band at 450 nm associated with a ca. 8 nm red shift in the λ_{max}, while the absorbance decreased by 37% and 48% for the 324 nm and 340 nm bands, respectively, centred on terpyridine associated with a ca. 4 nm red shift in both cases. Additionally, isosbestic points were observed at 480 nm and 345 nm at low P/D ratios (P/D 0→3), however, slight deviations were observed at higher P/D ratios, suggesting the possibility of more than one mode of binding of 109 at higher DNA concentrations. These changes are summarised in Table 4.6. Strong hypochromism accompanied with red shifts of all the absorption bands suggested strong interactions between the electronic states of the bound chromophore and the DNA bases, which has been previously observed for other intercalating [Pt(terpy)X]^{n+} based systems.\textsuperscript{241,246-247,249,251}
Figure 4.12: The UV/vis absorption spectra of 109 (14.3 μM) in the presence of increasing concentration of st-DNA (0-162 μM) in 10 mM phosphate buffer (pH 7.0). Inset: Plot of relative changes in absorbance for the various bands vs. P/D.

The changes in absorbance at both 450 nm and 340 nm were analysed using the non-cooperative model of McGhee and von Hippel as described in the previous chapters.202 The representative binding curves are shown in Appendix 4 (Figure A4.2) and the binding parameters are presented in Table 4.6. Binding constant analysis showed that in 10 mM phosphate buffer, 109 exhibited high affinity (ca. 1.6 ± 10^6 M\(^{-1}\)) for st-DNA. However, the extent of hypochromism for the terpyridine centred absorption band at 340 nm and the binding constant estimated for this band were significantly higher than that of the naphthalimide band, suggesting higher intercalation affinity of the planar terpyridine ring compared to the naphthalimide moiety. This is presumably due to the presence of the slightly twisted dimethyl amino group on the 4-position of the naphthalimide ring, which can interfere with the intercalation process. It should be mentioned here that a similar effect was observed for the interaction of the 4-N,N-dimethyl amino analogue 84 with st-DNA, when compared to the 4-amino derivatives 82 and 83, which exhibited a much greater extent of hypochromism (Chapter 2). The binding constant estimated for 109 in 10 mM phosphate buffer was comparable to the association constant of the [Pt(terpy)HET]\(^+\) complex (97) for ct-DNA by Lippard and co-workers (in 1 mM phosphate buffer containing 3 mM NaCl)\(^{241}\) and higher than the value reported for the [Pt(terpy)SPh]\(^+\) complex (0.01 M ionic strength).\(^{248b}\) The higher affinity of 109 compared to [Pt(terpy)HET]\(^+\) and [Pt(terpy)SPh]\(^+\) complexes is
Chapter 4: Synthesis, Photophysical and DNA Binding Studies of [Pt(terpy)naph]^2+ Complex

presumably due to the dicationic nature of 109, which can facilitate closer association with the negative phosphate backbone of DNA. Importantly, for the [Pt(terpy)(4-picoline)]^2+ complex 104a, Lowe and co-workers reported two different modes of binding based on the CD spectroscopic data, with equilibrium binding constants of 2×10^7 M^-1 (n = 4 bp) and 1×10^6 M^-1 (n = 2 bp), respectively. The binding constant obtained for 109 was similar to the second binding constant of 104a at higher DNA concentrations reported by Lowe and co-workers. The interaction of 109 with st-DNA was further investigated using fluorescence spectroscopy, which will be discussed in the following section.

Table 4.6: Summary of various DNA binding parameters obtained from the UV/vis titration in 10 mM Phosphate buffer (pH 7.0).

<table>
<thead>
<tr>
<th></th>
<th>324 nm</th>
<th>340 nm</th>
<th>450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δλ (nm)</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>% Hypochromism</td>
<td>37</td>
<td>48</td>
<td>22</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>-</td>
<td>345</td>
<td>480</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>3→11</td>
<td>3→11</td>
<td>3→11</td>
</tr>
<tr>
<td>K (× 10^6 M^-1)*</td>
<td>-</td>
<td>1.67 ± 0.05</td>
<td>1.33 ± 0.03</td>
</tr>
<tr>
<td>n (bp)*</td>
<td>-</td>
<td>1.21 ± 0.01</td>
<td>1.23 ± 0.02</td>
</tr>
</tbody>
</table>

* K and n were determined from the fit of the absorbance data to the non-cooperative model of McGhee and von Hippel.

4.4.2 Fluorescence Titrations of 109 with st-DNA

As described before, the excited state properties of 109 were found to be strongly dependent on the polarity of the media, it was therefore anticipated that the emission properties would be significantly altered in the presence of st-DNA due to binding of 109 in a hydrophobic environment. The fluorescence titration of 109 with st-DNA was carried out in parallel with the UV/vis measurements using λ_ex = 450 nm and the changes in the emission spectra of 109 in the presence of increasing concentration of st-DNA are shown in Figure 4.13, which showed that the changes in fluorescence followed a biphasic behaviour with an increase in DNA concentration. At lower P/D ratio (0→1.1), the addition of st-DNA resulted in a ca. 28% decrease in the emission intensity associated with a ca. 8 nm blue shift in the λ_max. Further increases in DNA concentration (P/D 1.3 →11) resulted in an enhancement of emission of 109. However, this increase never reached a plateau upto a P/D =11. Similar biphasic binding isotherms have been previously reported for the binding of various pyropheophorbides,233 metal complexes including bapositive platinum polypyridine,247 platinum-proflavine263 and zinc proflavine complexes.264 In these systems, the fluorescence
decrease in the first phase (lower P/D) has been assigned to the aggregation of the metal complex at the outer surface of DNA polyanion, followed by intercalation at the higher P/D, resulting in an emission enhancement due to the localisation of the chromophore in a rigid environment less accessible for quenching by solvent \(O_2\) molecules.

Due to the dicationic nature of 109, electrostatic interaction presumably plays a significant role in the DNA binding. In order to investigate the effect of ionic strength on the binding of 109 with st-DNA, UV/vis and fluorescence titrations were carried out at higher ionic strength, which will be described in the following section.

**Figure 4.13:** The steady-state fluorescence spectra of 109 (14.3 \(\mu\)M) in the presence of increasing concentration of st-DNA (0-162 \(\mu\)M) in 10 mM phosphate buffer (pH 7.0) \(\lambda_{\text{ex}} = 450\) nm. Inset: Plot of the relative changes in emission intensity at 550 nm vs. P/D.

**4.5 Interaction of 109 with st-DNA at Higher Ionic Strength**

Cusumano *et al.*, previously demonstrated that in the case of [Pt(terpy)pyridine]\(^{2+}\) complexes, the coordinated pyridine ligand can undergo slow substitution reactions in the presence of Cl\(^-\).\(^{265}\) In order to investigate the effect of Cl\(^-\) on 109, the UV/vis spectrum of 109 was recorded in the presence of an increasing concentration of NaCl as shown in Figure 4.14a. In the presence of NaCl the absorption band at 340 nm corresponding to the intraligand transition of the terpyridine showed significant hypochromism and was red shifted. The band at 325 nm showed relatively small hypochromism and a red shifted. The naphthalimide centred absorption band at 450 nm also showed relatively small changes. These changes can arise either from the aggregation of 109 induced at higher ionic strength or can result from the substitution of the pyridyl based naphthalimide ligand by the Cl\(^-\) anion. In contrast to this,
when the ionic strength of the media was increased by the gradual addition of NaNO₃, the UV/vis spectrum of 109 remained relatively unchanged (Figure 4.14b) suggesting that the changes in the presence of NaCl were most likely due to substitution of the pyridyl based naphthalimide ligand by the Cl⁻ anion. The changes of the absorption band at 325 nm could not be monitored in the presence of NaNO₃ due to the strong absorption band of NO₃⁻ at 300 nm. Since, 109 appeared to be stable in the presence of NaNO₃, the ionic strength of the media was adjusted using NaNO₃. The results of the titrations of 109 with st-DNA carried out in 10 mM phosphate buffer containing 100 mM NaNO₃ (pH 7.0) are described in the following section.

![Figure 4.14: The UV/vis absorption spectra of 109 (13.6 µM) in 10 mM phosphate buffer (pH 7.0) in the presence of (a) increasing concentration of NaCl (0-315 mM) and (b) increasing concentration of NaNO₃ (0-315 mM).](image)

### 4.5.1 UV/Vis Absorption Titrations of 109 with st-DNA at Higher Ionic Strength

The interaction of 109 with st-DNA at higher ionic strength was investigated in a manner similar to that described previously by adding small aliquots of st-DNA to a solution of 109 in 10 mM phosphate buffer containing 100 mM NaNO₃ until a plateau was reached in the absorbance. The changes in the UV/vis absorption spectra of 109 in 10 mM phosphate buffer containing 100 mM NaNO₃ are shown in Figure 4.15. In the presence of st-DNA the absorbance at 450 nm decreased by a ca. 20% accompanied with a ca. 7 nm red shift in the λ_max. The absorbance of the terpyridine centred bands at 325 nm and 340 nm decreased by ca. 35% and 48% respectively concomitant with a ca. 4 nm red shift in both cases. Isosbestic points were observed at 480 nm and 345 nm at low P/D ratios (0 →2.0), however, deviations
were observed at higher P/D ratio suggesting the possibility of more than one mode of binding to DNA. The overall changes in the UV/vis absorption spectra in 10 mM phosphate buffer containing 100 mM NaN$_3$ were similar to that observed at the lower ionic strengths with only minor changes in the extent of hypochromism. However, the changes in absorbance reached a plateau at a higher P/D ratio at higher ionic strength compared to that observed in 10 mM phosphate buffer (Figure 4.15 Inset), suggesting reduced binding affinity of 109 towards st-DNA at higher ionic strengths. The changes in absorbance at 340 nm and 450 nm were analysed using the non-cooperative McGhee von Hippel model. A summary of the binding parameters are presented in Table 4.7 and the binding curves are shown in Figure A4.3, Appendix 4. The binding constant analysis showed that 109 exhibited high affinity towards st-DNA with $K = 0.84 \times 10^6$ M$^{-1}$ at higher ionic strength. Importantly, this value was significantly higher than the corresponding values reported for [Pt(terpy)HET]$^{2+}$, [Pt(phen)ethylenediammine]$^{2+}$ and [Pt(terpy)(4-picoline)]$^{2+}$ complexes under similar ionic strength conditions, which suggest that although electrostatic interactions have a significant role in the binding of 109 to DNA, the bifunctional nature of 109 accounts for the enhanced affinity compared to the related complexes.

Figure 4.15: The UV/vis absorption spectra of 109 (13.5 $\mu$M) in the presence of increasing concentration of st-DNA (0-387 $\mu$M) in 10 mM phosphate buffer containing 100 mM NaNO$_3$ (pH 7.0). Inset: Plot of relative changes in absorbance for the various bands vs. P/D.
Table 4.7: Summary of the various DNA binding parameters obtained from the UV/vis titration of 109 with st-DNA in 10 mM phosphate buffer containing 100 mM NaNO₃ (pH 7.0).

<table>
<thead>
<tr>
<th>Δλ (nm)</th>
<th>324 nm</th>
<th>340 nm</th>
<th>450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Hypochromism</td>
<td>35</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>Isosbestic Point (nm)</td>
<td>-</td>
<td>346</td>
<td>480</td>
</tr>
<tr>
<td>Bound P/D</td>
<td>6→28</td>
<td>6→28</td>
<td>6→28</td>
</tr>
<tr>
<td>McGhee von Hippel</td>
<td>K (× 10⁶ M⁻¹)</td>
<td>0.84 ± 0.04</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>n (bp)</td>
<td>3.12 ± 0.10</td>
<td>2.46 ± 0.06</td>
</tr>
</tbody>
</table>

4.5.2 Fluorescence Titrations of 109 with st-DNA at Higher Ionic Strength

The interaction of 109 with st-DNA in 10 mM phosphate buffer containing 100 mM NaNO₃ was also studied by fluorescence spectroscopy. The changes in the emission spectra of 109 in the presence of increasing concentration of st-DNA are shown in Figure 4.16. Similar to that observed in 10 mM phosphate buffer, the changes in the fluorescence emission of 109 followed a biphasic behaviour at higher ionic strength in the presence st-DNA. The initial addition of st-DNA (P/D = 0→1.2) resulted in a sharp decrease in the emission intensity accompanied with a ca. 3 nm blue shift in the λ_max. Further addition of st-DNA caused an enhancement in the fluorescence intensity associated with a further ca. 6 nm blue shift.

Figure 4.16: The steady-state emission spectra of 109 (13.5 μM) in the presence of increasing concentration of st-DNA (0-387 μM) in 10 mM phosphate buffer containing 100 mM NaNO₃ (pH 7.0) λ_ex = 450 nm. Inset: Plot of relative changes in emission intensity at 550 nm vs. P/D.
As shown in Figure 4.16, the fluorescence titrations at higher ionic strength also suggested two binding modes for the interaction of 109 with st-DNA similar to that observed in low ionic strength. Similar behaviours have been previously reported for Pt-proflavine\textsuperscript{263} and Zn-proflavine\textsuperscript{264} derivatives, where the biphasic binding was observed at high ionic strength (~ 0.11 M). In these cases, the first binding mode was attributed to the external binding of the metal complex along the DNA backbone, causing a decrease in fluorescence intensity followed by binding via intercalation at the higher concentrations of DNA, resulting in an enhanced emission of the chromophore and blue shift in the $\lambda_{\text{max}}$\textsuperscript{263-264}. Usually increasing the ionic strength of the media results in a decrease in external binding; however, the very strong affinity of 109 even at higher ionic strength might be responsible for such biphasic behaviour. Alternatively the biphasic behaviour can result from two different modes of binding, where in the first phase, binding of 109 to GC rich sequences on DNA can result in the quenching of emission intensity presumably due to PET from guanine to the photoexcited naphthalimide, however, at higher concentrations of st-DNA the ligand molecules can redistribute to AT rich sites resulting in an enhancement of fluorescence due to protection of the chromophore from solvent molecules. Moreover, since many Pt(II) complexes are known to form covalent adducts with DNA as mentioned in Section 4.1, the possibility of some irreversible interactions between 109 and DNA also needs to be verified.

In order to investigate the possibility of some irreversible interaction between 109 and DNA bases, the interaction of 109 with DNA bases was studied by NMR spectroscopy with the help of Dr. John O’Brien, School of Chemistry, Trinity College Dublin, which will be described in the following section.

\subsection*{4.6 NMR Studies of the Interaction of 109 with Guanosine}

To study the interaction of 109 with DNA bases by NMR spectroscopy guanosine 5'-monophosphate (GMP) was initially used as the model compound. However, addition of GMP to a solution of 109 in D\textsubscript{2}O resulted in precipitation of the complex from solution. Therefore, further NMR studies were carried out using guanosine. The addition of 1.5 equivalents of guanosine to the solution of 109 (0.5 mM) in D\textsubscript{2}O did not cause any immediate change in the $^1$H NMR spectrum (Figure 4.17b, 600 MHz, D\textsubscript{2}O). However, the $^1$H NMR spectrum of the mixture changed significantly over a longer time scale. As shown in Figure 4.17c, after 24 hours of equilibration, a new doublet appeared at \textit{ca}. 6.1 ppm corresponding to the H1' of guanosine, which showed a 0.2 ppm downfield shift compared to the corresponding peak in the free guanosine. The H8 atom of guanosine appeared as a singlet at \textit{ca}. 7.9 ppm, which did
not show any significant shift. This is in contrast to previous reports, where the H8 peak of guanosine showed a significant downfield shift, when coordinated to [Pt(terpy)]$^{2+}$. Additionally, as shown in Figure 4.17, the doublet at ca. 8.95 ppm corresponding to the pyridyl protons (H17) of the coordinating naphthalimide ligand showed a 0.1 ppm upfield shift in the presence of guanosine after 24 hours. Several new peaks also appeared in the aromatic region, which are presumably arising from the displaced naphthalimide ligand. The $^1$H NMR spectrum of the mixture recorded after 4 days (Figure 4.17d) showed the presence of two additional doublets at ca. 6.0 ppm and 6.2 ppm corresponding to the resonance of H1’ of guanosine, which are significantly shifted compared to the H1’ of free guanosine. The presence of multiple peaks for the H1’ of guanosine suggested the possible coordination of [Pt(terpy)]$^{2+}$ to the various possible nitrogen donor sites of guanosine (shown by red arrows in the top panel Figure 4.17). The shift in the H8 resonance of guanosine cannot be followed due to significant interference from the overlapping peaks in that region.

![Figure 4.17: Top panel represents the structure and numbering of 109 and guanosine and the bottom panel represents the $^1$H NMR spectra of (a) 109 (0.5 mM), (b) 109 (0.5 mM) in the presence of guanosine (0.75 mM) immediately after addition, (c) $^1$H NMR spectrum of the mixture after 1 day and (d) $^1$H NMR spectrum of the mixture after 4 days in D$_2$O (600 MHz).](image)
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It should be noted here that the displacement of the naphthalimide ligand by guanosine was not completed even after 10 days of equilibration period, which suggests much slower displacement kinetics than was observed for the [Pt(terpy)Cl)]\(^+\) complex 110 and the picoline complex 104a.\(^{250,266}\) The complex 109 was found to be stable in D\(_2\)O in the absence of guanosine over a period of two weeks (\(^1\)H NMR of 109 is shown in Appendix 4, Figure A4.4).

4.7 Ethidium Bromide Displacement Assays for 109

The binding affinity of 109 for \(\phi\)-DNA was also evaluated from its ability to displace bound EtBr from \(\phi\)-DNA in 10 mM phosphate buffer. EtBr is weakly luminescent in an aqueous media, while the emission was significantly enhanced in the presence of \(\phi\)-DNA as described earlier in Chapter 3. Gradual addition of 109 resulted in a decrease in the fluorescence of EtBr (Figure 4.18) showing the ability of 109 to displace EtBr from DNA in a manner similar to that observed for various monovalent and divalent Pt complexes.\(^{241,243a,244}\)

The apparent binding constant (\(K_{\text{app}}\)) for the association of 109 with \(\phi\)-DNA was calculated according to the competitive binding model described by Boger and co-workers\(^{34}\) and was found to be \(2.41 \times 10^6 \text{ M}^{-1}\) in 10 mM phosphate buffer, which is in good agreement with the binding affinity of 109 determined from the UV/vis absorption titration under similar conditions.

![Figure 4.18: Changes in the fluorescence spectrum of EtBr (4.75 \(\mu\)M) bound to \(\phi\)-DNA (9.5 \(\mu\)M) \(\phi\)-DNA in the presence of increasing concentration of 109 in 10 mM phosphate buffer. Inset: Normalised fluorescence intensity of EtBr at 600 nm upon addition of 109.](image-url)

\(K = 2.41 \times 10^6 \text{ M}^{-1}\)
4.8 Thermal Denaturation Studies of 109

The strong association of 109 with st-DNA was further verified by thermal denaturation of st-DNA in the presence of varying concentrations of 109. In the absence of any ligand, the melting temperature (Tm) of st-DNA was found to be 68.5 (± 0.5)°C. In the presence of 109, a significant increase in the Tm value of st-DNA was observed (Figure 4.19). However, exact values of Tm could not be determined at P/D ratios of 5 and 10 as the denaturation process was not complete until 90 °C in both cases. This clearly showed that the presence of 109 strongly stabilised st-DNA against heat denaturation. Additionally, the melting profile in the presence of 109, showed a biphasic nature, which is more prominent at P/D = 10. Similar biphasic denaturation processes have also been reported for the [Pt(terpy)(4-picoline)]^{2+} derivative 104a,249 where the first phase was assigned to the melting of the st-DNA region not bound to the Pt(II) complex, while the DNA region containing bound intercalator melted at higher temperatures. Alternatively, Cusumano et al., assigned the first phase to the transition from double stranded structure to single stranded DNA, while the second phase was attributed to the presence of a new species formed at higher temperatures due to prolonged heating.251 These could be the possible explanations for the biphasic melting profile observed with 109, alternatively the two phases might represent melting of different regions of double stranded DNA bound to naphthalimide and terpy units respectively.

<table>
<thead>
<tr>
<th></th>
<th>Tm (°C)</th>
<th>ΔTm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>st-DNA</td>
<td>68.5</td>
<td>-</td>
</tr>
<tr>
<td>109 (P/D = 10)</td>
<td>&gt;76</td>
<td>&gt;7.5</td>
</tr>
<tr>
<td>109 (P/D = 5)</td>
<td>&gt;83</td>
<td>&gt;14.5</td>
</tr>
</tbody>
</table>

Figure 4.19: Thermal denaturation profile of st-DNA (150 μM) in 10 mM phosphate buffer (pH 7.0) in the presence of varying concentrations of 109.
4.9 Circular Dichroism Studies of 109

In order to investigate the mode of binding of 109 with st-DNA, the CD spectrum of st-DNA was recorded in the presence of varying concentrations of 109 as shown in Figure 4.20. The CD spectra revealed that in the presence of increasing concentrations of 109, a weak induced negative CD band appeared in the wavelength region 300-400 nm, corresponding to the absorption band of the terpyridine. The presence of the induced negative CD (ICD) signal suggested the binding of terpyridine via intercalation. In contrast, no induced CD signal was observed for the naphthalimide chromophore suggesting weaker intercalation efficiency, which was also observed for other naphthalimide derivatives 82-84, discussed in Chapter 2. Moreover, the intensity of the positive CD band of st-DNA at 275 nm showed a ca. 5 nm red shift accompanied with an increase in molar ellipticity from +9.3 millidegree to +13.7 millidegree, while that of the negative band at 240 nm changed from -13.3 to -19.6 millidegree, suggesting strong interaction of the metal complex with DNA, however these changes are difficult to interpret as the metal complex 109 also absorbs in this spectral region.

To obtain further information about the mode of binding, the interaction of 109 with st-DNA was investigated using LD spectroscopy, which will be described in the following section.

Figure 4.20: The CD spectra of st-DNA (150 µM) in 10 mM phosphate buffer (pH 7.0) in the presence of varying concentrations of 109. Inset: ICD band in the spectral region 300-400 nm in the presence of increasing concentrations of 109.
4.10 Linear Dichroism Studies of 109

As shown in Figure 4.21, in the absence of any ligand a negative LD signal is observed for st-DNA at \( \lambda = 260 \text{ nm} \) characteristic of B-DNA, arising from the nearly perpendicular orientation of the transition moments of DNA bases relative to the DNA helical axis. In the presence of 109, in addition to the negative LD signal observed at 260 nm for DNA, negative LD signals were observed in the wavelength regions 400-500 nm and 300-400 nm, which correspond to the ICT absorption band of the naphthalimide chromophore and the absorption band of the terpyridine moiety respectively. Due to significant overlap of the DNA absorption band and absorption band of 109 below 300 nm, a complete quantitative interpretation of the flow linear dichroism data was not possible. However, the presence of negative LD signals centred at ca. 458 nm and ca. 350 nm in the presence of 109 suggests that the transition dipoles of both of the chromophores are oriented at angles greater than 54.7° relative to the helical axis (according to equation 1.2, Chapter 1, Section 1.4.1.4) indicative of an intercalative mode of binding for both the naphthalimide and terpyridine moieties.

**Figure 4.21:** The LD spectra of st-DNA (400 \( \mu \text{M} \)) in 10 mM phosphate buffer (pH 7.0) in the presence of varying concentrations of 109.

Based on these results, several possibilities of binding can be proposed for this bifunctional metal complex. The overall binding profile may reflect a heterogeneous equilibrium mixture of various bound populations, in some of which the terpyridine chromophore is intercalated, while the naphthalimide moiety is externally bound and vice versa (mode a, Figure 4.22). Alternatively, a dual binding mode cannot be ruled out, where
two chromophores can potentially intercalate into adjacent DNA helices, thereby crosslinking two DNA molecules (mode b in Figure 4.22). Crystal structure data showed that the inter-chromophoric distance between the terpyridine and naphthalimide unit is 8.45Å, which is sufficiently long for bisintercalation, however due to the presence of the rigid pyridyl group in the linker region bisintercalation geometry would possibly need distortion of the double helical structure (mode c in Figure 4.22), which was not seen in the CD titration. However, further investigations are necessary to confirm the mode of binding of this bifunctional intercalator.

*Figure 4.22: Schematic illustration showing possible modes of intercalation for the bifunctional metal complex 109.*

### 4.11 Preliminary Cytotoxicity Studies of 109

The Pt(II) complex 109 was tested for its *in vitro* antiproliferative activity against the cervical cancer cell line (HeLa) in collaboration with Prof. Clive Williams research group in the School of Biochemistry and Immunology at Trinity College Dublin. The cytotoxicity work presented here was carried out by Dr. Sandra Bright in the Williams research group.

For the biological studies, the stock solution of 109 and 91 were prepared in a mixture of water:DMSO (v/v 75:25) and diluted such that the final concentration of DMSO in the cells did not exceed 0.5%. The cytotoxicity properties of 109 and the corresponding naphthalimide
ligand 91 were evaluated using an alamar blue cytotoxicity assay as described in Chapter 3. Experiments were performed in triplicate on three independent days with activity expressed as percentage cell viability compared to vehicle treated controls. As shown in Figure 4.23a, 109 displayed high cytotoxicity with the IC\textsubscript{50} (the half maximal inhibitory concentration) value of 12.8 \mu M while the IC\textsubscript{50} value of the ligand 91 was found to be 33.6 \mu M.

Further biological studies on 109 were carried out to investigate its ability to induce apoptosis in HeLa cells using propidium iodide FACS (fluorescent activated cell sorter) analysis, which can determine the percentage of apoptotic nuclei after propidium iodide staining in the pre-G1 phase of the cell cycle. As shown in Figure 4.23b 109 was found to induce significant apoptosis in HeLa cells. The ability of the corresponding ligand 91 to induce apoptosis could not be determined in a similar manner due to precipitation of the compound from the cell culture media. These preliminary studies suggested that the Pt(II) complex 109 possesses significantly high cytotoxicity, which is presumably due to its strong affinity for DNA and possible formation of a covalent adduct with DNA bases as suggested from the NMR experiments. The formation of covalent adducts with DNA is considered to be largely responsible for the cytotoxic activities of many Pt(II) complexes.\textsuperscript{267} The failure to repair the DNA damage by the intrinsic cellular repair mechanism can lead to programmed cell death.

\[\text{IC}_{50}\]

4.12 Conclusions and Future Studies

The work described in this chapter showed the successful synthesis and characterisation of a bifunctional [Pt(terpy)naphthalimide]\textsuperscript{2+} complex 109 in a reasonable yield (82\%). X-ray diffraction studies showed that Pt(II) assumes a square planar geometry,
with the four nitrogen donors defining the plane. Both the terpyridine and the naphthalimide units were involved in extensive π...π stacking interactions.

In an aqueous media [Pt(terpy)naph]^{2+} complex 109 displayed a weak emission band, centred at 550 nm, when excited at 450 nm. The quantum yield of emission and the excited state lifetime corresponding to this emission band were found to be sensitive to solvent polarity, characteristic of the ICT nature of the excited state. Additionally, the quantum yield of emission and the excited state lifetime of the ICT emission band in 109 was found to be higher in all the solvents compared to the free ligand 91, suggesting that the excited state has significant triplet character, presumably induced by the high spin orbit coupling constant of platinum. However, the excited state lifetime of 109 appeared much shorter (ca. 10 ns) than the expected triplet lifetime values and the emission spectra appeared in the same region as that of the free ligand 91, which can possibly arise if the triplet excited state has similar energy as that of the ICT singlet state and has an ICT character, which can be shortened by various non-radiative decay processes such as internal motion, twisting/rotation around the C-N bond.

DNA binding studies showed that 109 has a substantially high binding affinity for st-DNA compared to [Pt(terpy)Cl]^{+}, which was also verified by its strong ability to displace ethidium bromide from DNA. The thermal melting studies along with CD and LD measurements suggested a biphasic mode of binding of 109 with st-DNA. Based on these spectroscopic data, several binding modes have been proposed, where naphthalimide and terpyridine moieties can potentially intercalate in the same or adjacent DNA molecules. However, further investigations such as DNA unwinding assays, viscosity measurements and molecular modelling studies are required to validate the binding mode. Emission measurements at both low and high ionic strengths indicated a biphasic binding pattern for 109, where the first phase presumably arises from the external binding of the complex at low P/D, while at higher concentrations of DNA, the molecules can redistribute themselves and bind via intercalation resulting in an enhancement in emission intensity. Alternatively, the luminescence quenching at lower P/D ratio can result from the binding of 109 to GC rich sites, however at higher concentrations of st-DNA the ligands can redistribute and bind to AT rich sites resulting in an enhanced emission due to shielding from the solvent molecules. The \(^{1}\text{H NMR}\) studies with guanosine also suggested the possible displacement of the coordinating naphthalimide ligand by the nucleophilic guanosine. However, the kinetics of such nucleophilic displacement reactions were found to be slow. The cytotoxicity assay suggested that the Pt(II) complex 109 exhibited significant cytotoxicity and induced apoptosis in HeLa cells. Additionally, the cytotoxic effect of 109 was significantly higher than that of the free
ligand 91, which suggests that the high cytotoxicity of 109 possibly results from the combination of higher intercalation efficiency and platination compared to free ligand. However this requires further investigation. Future work involves determination of the cellular localisation of 109. Additionally the cytotoxic activity of 109 needs to be compared with cisplatin and the effect of 109 has to be checked on various cisplatin resistant cell lines.

Moreover, this study showed that the pyridyl based naphthalimide ligand in the Pt(II) complex 109 can undergo slow substitution in the presence of other nucleophiles such Cl or DNA bases. Therefore further synthetic variation of the naphthalimide ligands using an alkyne group as shown in structures 111 and 112 might be useful to enhance the stability of the metal complexes. Additionally such cyclometalated Pt(II) complexes are also expected to have long-lived MLCT/3IL emission, which can be useful to study their interactions with DNA.
Chapter 5

Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger's Base Derivatives

5.1 Introduction

As described in Chapter 1 and 2, the excited state properties of 1,8-naphthalimide derivatives can be readily tuned by the nature of the substituent present on the naphthalene ring.\textsuperscript{126,128,268} For example the lowest excited singlet state for the unsubstituted 1,8-naphthalimide has $\pi-\pi^*$ character, while the introduction of an electron donating (such as amino) or electron accepting (such as nitro) substituent results in a charge transfer excited state. Additionally, the redox potential of the naphthalimide derivatives can also be modulated depending on the aryl substituent present. Majima and co-workers have developed various donor-acceptor electronic dyads based on 1,8-naphthalimide, which can mimic the electron transfer processes in natural photosynthetic system.\textsuperscript{130} The oxidative nature of the unsubstituted as well as 3- and 4-nitro substituted 1,8-naphthalimide derivatives has been utilised to develop DNA photocleaving agents.\textsuperscript{33,121a,132a,132c,133-134,185b} The CT excited states of 4-amino-1,8-naphthalimide derivatives have also been used to develop the donor-acceptor systems for solar cells,\textsuperscript{269} supramolecular systems mimicking bacterial photosynthetic reaction centres,\textsuperscript{270} organic light-emitting diodes (OLEDs),\textsuperscript{271} and sensors for metal ions and anions.\textsuperscript{3} Despite their usefulness in a number of areas little is known about the femtosecond/picosecond behaviour of 4-amino-1,8-naphthalimide excited states and particularly about their vibrational spectra. The work presented in this chapter described the picosecond UV/visible Transient absorption and Transient Infrared studies of the 4-amino-1,8-naphthalimide derivative 82 following 400 nm excitation using the high-sensitivity ULTRA apparatus at the Central Laser Facility of the Science & Technology Facilities Council in the Rutherford Appleton Laboratory, UK. A key point of interest would be to identify unambiguously the excited states involved (such as the $\pi\pi^*$, $n\pi^*$ and ICT states), which are expected to have quite distinct IR signatures. Additionally, as demonstrated in Chapter 2, compound 82 can form 1:1 complexes with the mononucleotides AMP or GMP, where the excited state of 82 was quenched in the presence of GMP presumably due to photoinduced electron transfer. The aim of this study was to use picosecond transient absorption (ps-TA) and picosecond transient infrared (ps-TRIR) spectroscopy to get evidence for the formation of NI radical anion and the guanine radical cation, the latter shows distinct band centred at ca.1700 cm\textsuperscript{-1} in TRIR.\textsuperscript{272} Moreover, as discussed in Chapter 3, the Tröger's base derivative 87 also displayed an ICT excited state similar to the 4-amino-1,8-naphthalimide, however the emission quantum yield was very much lower than the 4-amino precursor 82. We have also studied the excited state behaviour of 87 using ps-TA and ps-
TRIR to compare with the simple-amino derivative 82 and to determine the excited state lifetime of 87 in aqueous solution.

5.2 Picosecond Transient Absorption and Transient Infrared Studies of the 4-amino-1,8-naphthalimide 82

The samples for ps-TA and ps-TRIR measurements were prepared in 10 mM phosphate buffer in D$_2$O (pH 7.0) by dropping a known volume of solution of 82 (typically 70 μL) between two CaF$_2$ (25 mm diameter) windows (Crystan Ltd., UK), separated by a Teflon spacer of known length (typically 56 μm) in a demountable solution IR cell (Harrick Scientific Products Inc., New York). The ps-TA and ps-TRIR pump-probe experiments were carried out using the high-sensitivity ULTRA apparatus ($\lambda_{\text{excitation}} = 400$ nm, 150 fs laser pulse) at the Central Laser Facility of the Science & Technology Facilities Council in the Rutherford Appleton Laboratory (the detailed description is given in the Experimental chapter). It is important to mention here, that the naphthalimide concentrations used in these study were in the mM range, considerably higher than those used in the ground state absorption and emission studies described in Chapter 2.

5.2.1 Transient Absorption Data for Concentrated 4-amino-1,8-naphthalimide derivative

The UV/vis absorption spectrum of 82 in phosphate buffer made in D$_2$O is shown in Figure 5.1a, where the broad peak centred at ca. 440 nm was assigned to the ICT absorption band. The ps-TA spectra of 82 (10 mM) in sodium phosphate buffer (D$_2$O) is shown in Figure 5.1b. Upon 400 nm excitation, a negative band was observed at 440 nm corresponding to the depletion of the ground state following photoexcitation along with the formation of weak transient bands in the wavelength region 650-750 nm. The kinetics of ground state recovery
was found to exhibit a biexponential behaviour with a short-lived component $\tau_1 = 75 \pm 9$ ps (60%) and a long-lived component $\tau_2 = 352 \pm 85$ ps (27%) (Figure 5.1c and Table 5.1). The decay was not completed within 3 ns, suggesting the presence of a long-lived component (ca. 13%). The decay profile of the transient band at 720 nm (Figure 5.1d) was also found to follow a biexponential kinetics, with a short-lived component $\tau_1 = 64 \pm 10$ ps (65%) and a long-lived component $\tau_2 = 328 \pm 80$ ps (28%).

Figure 5.1: (a) The UV/vis absorption spectrum of 82 (10 mM) in Na-phosphate buffer in D$_2$O, (b) The ps-TA spectra of 82 (10 mM) in Na-phosphate buffer in D$_2$O. $\lambda_{ex} = 400$ nm, (excitation energy 500 nJ), Kinetics at (c) 440 nm and (d) 720 nm and (--) fit to biexponential decay.

In addition to the ground state bleach band and weak transient bands, a stimulated emission peak centred at ca. 560 nm was also observed in the transient spectra of 82 upon excitation at 400 nm. The stimulated emission peak at 560 nm showed complex kinetics (Figure 5.2a); initially, a decay phase was observed upt0 first 20 ps ($\tau = 2.3 \pm 0.04$ ps) (Figure 5.2b), followed by a rise phase (upto 500 ps) with lifetime of 76 $\pm$ 3 ps (Figure 5.2c). This was finally followed a decay at longer delays (Figure 5.2d). It should be mentioned here,
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger's Base Derivatives

the stimulated emission peak appeared at 550 nm immediately after excitation and showed ca. 10 nm red shifts at longer delays. This can result from the solvent dynamics since excited state of 82 has a charge transfer character and significant redistribution of solvent dipoles can occur within this time.

Figure 5.2: (a) Kinetics of stimulated emission at 560 nm, (b) kinetics upto first 20 ps, (c) kinetics from 20-550 ps and (d) kinetics at delays longer than 550 ps.

Recently Glusac and co-workers reported that the photo-excitation of the 4-N,N′-dimethylamino-1,8-naphthalimide initially produces a nπ* (S2) excited state, which within 40 ps, undergoes internal conversion to form the ICT excited state (S1) in dichloromethane and acetonitrile. Therefore, the short-lived component ca. 75 ps observed in the kinetics of ground state recovery at 440 nm and the decay of transient band can possibly arise from the nπ* excited state as observed by these authors. However, the long-lived component observed in the case of 82 was found to be much shorter than the fluorescence lifetime of 82 (ca. 2.2 ns) in water. To verify the formation of two different excited states following the excitation of 82,
picosecond transient infrared measurements were also carried out, which will be described in Section 5.2.2.

5.2.1.1 Transient absorption data for concentrated 4-amino-1,8-naphthalimide derivative in the presence of GMP

In the presence of 20 mM GMP, no detectable change was observed in the ps-transient absorption spectra of 82 (Figure 5.3 a) and the excited state lifetimes for the ground state bleach band at 440 nm or the weak transient band at 720 nm (see Figure 5.3 b and c) remained almost unaffected. The decay at 440 nm was found to follow biexponential kinetics (Table 5.1) with a short-lived component $100 \pm 11$ ps and a long-lived component $514 \pm 72$ ps. The stimulated emission peak was also observed at ca. 560 nm, which was found to follow a biexponential kinetics with a fast component of $3.9 \pm 0.12$ ps (57%) and a slow component of $1684 \pm 118$ ps (38%). The failure to observe any quenching in the presence of GMP was surprising as according to the UV/vis titration data described in Chapter 2 (Section 2.4.1), the fraction of bound naphthalimide should be above 0.7 ($K_b = 125 \text{ M}^{-1}, [\text{GMP}] = 20 \text{ mM}$), and both steady state and time resolved measurements showed significant quenching of the singlet excited state of 82 in the presence of GMP under the experimental condition employed in the ultrafast measurements (Chapter 2, Section 2.4.2).

5.2.1.2 Transient absorption data for concentrated 4-amino-1,8-naphthalimide derivative in the presence of AMP

The ps-transient absorption spectra of 82 (10 mM) were also recorded in the presence of 20 mM AMP (Figure A5.1, Appendix 5), however, no new band was observed in the presence of AMP. Additionally, the ground state recovery at 440 nm (Figure A5.2a in Appendix 5) and the transient decay at 720 nm (Figure A5.2b in Appendix 5) followed biexponential kinetics, with the lifetimes comparable to that observed for 82 (Table 5.1). This behaviour was contrary to that observed in the steady-state and the time–resolved fluorescence measurements described in Chapter 2 (Section 2.4.4), where the steady-state fluorescence emission and the singlet state lifetime of 82 was found to be almost doubled in the presence of AMP.
Figure 5.3: (a) The ps-TA spectra of 82 (10 mM) in the presence of GMP (20 mM) in Na-phosphate buffer in D$_2$O. $\lambda_{ex}$ = 400 nm, Excitation energy 500 nJ. Comparison of kinetics at (b) 440 nm (c) 560 nm and (d) 720 nm of 82 in the absence and in the presence of GMP and AMP.

Table 5.1: Summary of various kinetic parameters obtained from the ps-TA data of 82 (10 mM) alone, in the presence of GMP (20 mM) and AMP (20 mM).

<table>
<thead>
<tr>
<th></th>
<th>$\lambda$ (nm)</th>
<th>$Y_0$ (%)</th>
<th>$A1$ (%)</th>
<th>$\tau_1$ (ps)</th>
<th>$A2$ (%)</th>
<th>$\tau_2$ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>82 (10 mM)</td>
<td>440</td>
<td>13</td>
<td>60</td>
<td>75 ± 9</td>
<td>27</td>
<td>352 ± 85</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>7</td>
<td>65</td>
<td>64 ± 10</td>
<td>28</td>
<td>328 ± 80</td>
</tr>
<tr>
<td>82 (10 mM) + GMP (20 mM)</td>
<td>440</td>
<td>10</td>
<td>50</td>
<td>100 ± 11</td>
<td>40</td>
<td>514 ± 72</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>6</td>
<td>51</td>
<td>64 ± 6</td>
<td>43</td>
<td>485 ± 52</td>
</tr>
<tr>
<td>82 (10 mM) + AMP (20 mM)</td>
<td>440</td>
<td>22</td>
<td>43</td>
<td>85 ± 7</td>
<td>35</td>
<td>412 ± 36</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>18</td>
<td>50</td>
<td>80 ± 11</td>
<td>32</td>
<td>485 ± 80</td>
</tr>
</tbody>
</table>

* Kinetics were fitted to the equation $y = Y_0 + A1 \exp\left(-\frac{x}{\tau_1}\right) + A2 \exp\left(-\frac{x}{\tau_2}\right)$

The ps-TA studies of 82 described here showed the presence of two excited state processes with lifetimes ca. 75 ps and 350 ps, respectively. The magnitude of the short-lived component was comparable to the lifetime of nπ* state as proposed by Glusac and co-workers. However, the long-lived lifetime value was found to be significantly shorter than the fluorescence lifetime of 82 in water (2.2 ns). Additionally, the excited state decay kinetics...
of 82 remained largely unaffected in the presence of GMP; while steady-state and time-resolved emission measurements suggested significant shortening of excited state lifetime in the presence of GMP. To understand the excited state behaviour of 82, further, ps-transient infrared measurements (TRIR) were carried out under similar conditions, which will be described in the following section.

5.2.2 Picosecond Transient Infrared Study of the 4-amino-1,8-naphthalimide 82

The ps-TRIR spectra of 82 (10 mM) recorded in D2O-phosphate buffer are shown in Figure 5.4. The bottom panel in Figure 5.4 represents the FTIR spectrum of 82 in phosphate buffer made in D2O. The assignments of various IR bands of 82 are described in Table 5.2.

Excitation of 82 (10 mM) with 400 nm laser pulse in D2O-phosphate buffer resulted in the bleaching of the ground states at 1670, 1629, 1579 and 1372 cm⁻¹ wavenumbers (Figure 5.4 a), which was accompanied by the formation of several transient bands at 1587, 1542, 1496 and 1444 cm⁻¹. Representative kinetic traces for the ground state bleaching at 1670 cm⁻¹ and the transient decay at 1496 cm⁻¹ are shown in Figure 5.4 b and c, respectively. The decay kinetics for the various other bleaching and transient bands are shown in Appendix 5 (Figures A5.3 and A5.4). For all the bleach bands and transients (except 1496 cm⁻¹) a fast component was observed at early delays (completed within 8.0 ps), which probably represents vibrational relaxation. All the data, therefore, have been fitted from delays greater than 8 ps. At all wavenumbers, the recovery of the ground state was found to follow a biexponential kinetics with a short-lived component of ca. 45 ± 7 ps (44%) and a long-lived component of ca. 223 ± 20 ps (42 %). The ground state bleach did not recover within 3 ns time scale. The transient bands were also found to exhibit biexponential decay kinetics with a short-lived component of ca. 56 ± 16 ps (33 %) and a long-lived component of ca. 259 ± 28 ps (64 %). All the kinetic parameters are summarised in Table 5.3.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

Figure 5.4: (a) The ps-TRIR spectra of 82 (10 mM) in 10 mM phosphate buffer (D_2O), \( \lambda_{\text{ex}} = 400 \text{ nm} \), (—) represents spectra at delays shorter than 7.5 ps and (—) represents spectra at longer delays (> 7.5 ps). Bottom panel: the FTIR spectrum of 82 (10 mM) in 10 mM phosphate buffer (D_2O) (b) Decay kinetics at 1670 cm\(^{-1}\) and (—) fit to the biexponential decay function, (c) Decay kinetics at 1496 cm\(^{-1}\) and (—) fit to the biexponential decay function.

Table 5.3: Summary of the kinetic parameters obtained from the biexponential fit of TRIR data of 82 (10 mM).

<table>
<thead>
<tr>
<th>Band (cm(^{-1}))</th>
<th>Y0 (%)</th>
<th>A_1 (%)</th>
<th>( \tau_1 ) (ps)</th>
<th>A_2 (%)</th>
<th>( \tau_2 ) (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1670</td>
<td>19</td>
<td>39</td>
<td>38 ± 13</td>
<td>42</td>
<td>223 ± 65</td>
</tr>
<tr>
<td>1629</td>
<td>18</td>
<td>41</td>
<td>40 ± 11</td>
<td>41</td>
<td>203 ± 47</td>
</tr>
<tr>
<td>1580</td>
<td>12</td>
<td>47</td>
<td>42 ± 10</td>
<td>41</td>
<td>215 ± 45</td>
</tr>
<tr>
<td>1372</td>
<td>8</td>
<td>50</td>
<td>55 ± 10</td>
<td>42</td>
<td>251 ± 53</td>
</tr>
<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1591</td>
<td>1</td>
<td>18</td>
<td>38 ± 8</td>
<td>81</td>
<td>239 ± 12</td>
</tr>
<tr>
<td>1542</td>
<td>9</td>
<td>37</td>
<td>70 ± 25</td>
<td>54</td>
<td>281 ± 76</td>
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<tr>
<td>1496</td>
<td>1</td>
<td>47</td>
<td>69 ± 12</td>
<td>52</td>
<td>285 ± 50</td>
</tr>
<tr>
<td>1444</td>
<td>2</td>
<td>29</td>
<td>48 ± 14</td>
<td>69</td>
<td>231 ± 28</td>
</tr>
</tbody>
</table>
As mentioned in Section 5.2.1, Glusac and co-workers reported that the photoexcitation of the dimethyl-4-amino substituted 1,8-naphthalimide initially produces $n\pi^*$ ($S_2$) excited state, which within 40 ps forms ICT excited state ($S_1$) via internal conversion.\[^{129}\] To validate whether the two excited state processes observed in the case of \(82\) were arising from the two different excited states, the TRIR spectra of \(82\) were compared at early (10 ps) and longer time delays (200 ps) (Figure 5.5a). However, this did not reveal any difference between the two spectra. Therefore, the short-lived component observed in our measurements is probably not arising from the $n\pi^*$ excited state, because $n\pi^*$ and $\pi\pi^*$ excited states should have clearly different spectroscopic signature in IR, which is not the case here.

\[\text{Figure 5.5: (a) Normalised TRIR of 82 (10 mM) in Na-phosphate buffer (D}_2\text{O) (pH 7.0) at 10 ps and 200 ps delays after excitation (} \lambda_{\text{ex}} = 400 \text{ nm). (b) Normalised kinetics of ground state recovery at 1670 cm}^{-1} \text{ of 82 at excitation energies 110 nJ and 500 nJ.}\]

Additionally, to verify the possibility of energy dependent annihilation processes, which can also result in a biexponential kinetics, TRIR spectra of \(82\) were recorded using 500 nJ and 110 nJ excitation energies. However, no significant difference was observed between the TRIR spectra recorded at different energies, as shown by the almost perfect overlap between the kinetics at 1670 cm\(^{-1}\) in Figure 5.5b. Based on the TRIR results, the possibility of two different excited states can be ruled out. Since these ultrafast measurements were carried out at significantly higher concentration than those described in Chapter 2, the possibility of aggregation of naphthalimide molecules exists and this will be described in Section 5.3.

### 5.2.2.1 Transient Infrared data for concentrated 4-amino-1,8-naphthalimide derivative in the presence of GMP

The ps-TRIR spectra of \(82\) (10 mM) recorded in the presence of GMP (20 mM) are shown in Figure 5.6a. Comparison of the normalised ps-TRIR spectra of \(82\) alone, and in the presence of GMP (Figure 5.6b) showed that no new band appeared in the TRIR spectra in the
presence of GMP. However, the bleach bands at 1579, 1629 and 1670 cm$^{-1}$ and the transient bands at 1496, 1542, 1590 cm$^{-1}$ showed _ca._ 4-5 nm red shifts in the presence of GMP probably due to interaction between 82 and GMP. Kinetic analysis (Figure A5.5, Appendix 5) showed that all the bleach bands can be fitted to a biexponential model (at delays $> 7.5$ ps) with average lifetime of _ca._ $270 \pm 27$ ps (42.5 %) and $56 \pm 8$ ps (42.5%), respectively. The decay was not completed within 1.2 ns similar to that observed in the ps-TA measurements ($Y_0 = 15 \%$). The decay of the transients were also found to follow biexponential kinetics (Figure A5.6, Appendix 5) with a short component of $32 \pm 28$ ps (29 %) and a long-lived component of _ca._ $275 \pm 33$ ps (68%). The lifetime values are summarised in Table 5.4. The normalised kinetics at 1670 cm$^{-1}$ and 1496 cm$^{-1}$ in the absence and in the presence of GMP are shown in Figure 5.6c and d, respectively, which showed that the excited state lifetime of 82 remained almost unchanged in the presence of GMP (20 mM) similar to that observed in the ps-TA measurements previously.

**Figure 5.6:** (a) The ps-TRIR spectra of 82 (10 mM) in the presence of GMP (20 mM) in 10 mM phosphate buffer (D$_2$O), $\lambda_{ex} = 400$ nm, (——) represents spectra at delays shorter than 7.5 ps and (—) represents spectra at longer delays. Bottom panel: FTIR spectrum of 82 (10 mM) + GMP (20 mM) in 10 mM phosphate buffer (D$_2$O) (b) Normalised ps-TRIR of 82 (10 mM) alone and in the presence of GMP (20 mM) at 10 ps delay after excitation. Normalised kinetics of 82 alone and in the presence of GMP and AMP (c) at 1670 cm$^{-1}$ and (d) at 1496 cm$^{-1}$.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

Table 5.4: Summary of the kinetic parameters obtained from the biexponential fit of TRIR data of 82 (10 mM) in the presence of GMP (20 mM).

<table>
<thead>
<tr>
<th>Band (cm⁻¹)</th>
<th>Y₀ (%)</th>
<th>A₁ (%)</th>
<th>τ₁ (ps)</th>
<th>A₂ (%)</th>
<th>τ₂ (ps)</th>
</tr>
</thead>
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<td><strong>Bleach</strong></td>
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<td></td>
</tr>
<tr>
<td>1670</td>
<td>17</td>
<td>34</td>
<td>45 ± 12</td>
<td>49</td>
<td>273 ± 42</td>
</tr>
<tr>
<td>1629</td>
<td>16</td>
<td>46</td>
<td>60 ± 14</td>
<td>38</td>
<td>288 ± 55</td>
</tr>
<tr>
<td>1579</td>
<td>14</td>
<td>49</td>
<td>55 ± 11</td>
<td>37</td>
<td>231 ± 55</td>
</tr>
<tr>
<td>1372</td>
<td>14</td>
<td>42</td>
<td>62 ± 16</td>
<td>44</td>
<td>286 ± 74</td>
</tr>
<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1591</td>
<td>1</td>
<td>35</td>
<td>19 ± 5</td>
<td>64</td>
<td>298 ± 27</td>
</tr>
<tr>
<td>1542</td>
<td>0.3</td>
<td>25</td>
<td>14 ± 4</td>
<td>74.7</td>
<td>249 ± 14</td>
</tr>
<tr>
<td>1496</td>
<td>3</td>
<td>39</td>
<td>74 ± 28</td>
<td>58</td>
<td>309 ± 94</td>
</tr>
<tr>
<td>1444</td>
<td>4</td>
<td>18</td>
<td>21 ± 14</td>
<td>78</td>
<td>244 ± 23</td>
</tr>
</tbody>
</table>

5.2.2.2 Transient Infrared data for concentrated 4-amino-1,8-naphthalimide derivative in the presence of AMP

The ps-TRIR spectra of 82 (10 mM) were also recorded in the presence of 20 mM AMP (Figure A5.7, Appendix 5), which did not show appearance of any new band. Additionally, the kinetics of ground state recovery at all the wavenumbers were found to follow biexponential behaviour with a short-lived component of τ₁ = 57 ± 8 (36 %) and a long-lived component of τ₂ = 264 ± 9 ps (40 %) (Figure A5.8, Appendix 5). The transient bands at 1496, 1542, 1590 cm⁻¹ also exhibited biexponential kinetics with τ₁ = 59 ± 6 (46 %) and τ₂ = 311 ± 31 ps (49 %) (Figure A5.9, Appendix 5). The average lifetime values obtained for the various bands are summarised in Table 5.5. Comparison of the excited state kinetics of 82 in the presence and in the absence of AMP (Figure 5.6 c and d, respectively) showed that the excited state lifetime of 82 was not significantly enhanced in the presence of AMP, which is in contrast to the results obtained from the steady-state and time-resolved fluorescence measurements of 82 as described in Chapter 2.

Table 5.5: Summary of the kinetic parameters obtained from the biexponential fit of TRIR data of 82 (10 mM) in the presence of AMP (20 mM).

<table>
<thead>
<tr>
<th>Band (cm⁻¹)</th>
<th>Y₀ (%)</th>
<th>A₁ (%)</th>
<th>τ₁ (ps)</th>
<th>A₂ (%)</th>
<th>τ₂ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1670</td>
<td>30.99</td>
<td>31.52</td>
<td>53 ± 11</td>
<td>37.49</td>
<td>253 ± 53</td>
</tr>
<tr>
<td>1629</td>
<td>22.23</td>
<td>39.34</td>
<td>65 ± 20</td>
<td>38.42</td>
<td>266 ± 89</td>
</tr>
<tr>
<td>1579</td>
<td>23.26</td>
<td>37.21</td>
<td>47 ± 6</td>
<td>39.53</td>
<td>261 ± 32</td>
</tr>
<tr>
<td>1372</td>
<td>19.07</td>
<td>38.03</td>
<td>61 ± 12</td>
<td>42.90</td>
<td>275 ± 53</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1591</td>
<td>4.35</td>
<td>80.75</td>
<td>67 ± 15</td>
<td>14.91</td>
<td>340 ± 50</td>
</tr>
<tr>
<td>1542</td>
<td>2.49</td>
<td>37.04</td>
<td>57 ± 15</td>
<td>60.47</td>
<td>327 ± 59</td>
</tr>
<tr>
<td>1496</td>
<td>9.30</td>
<td>32.87</td>
<td>53 ± 14</td>
<td>57.83</td>
<td>270 ± 37</td>
</tr>
<tr>
<td>1444</td>
<td>6.57</td>
<td>30.99</td>
<td>59 ± 20</td>
<td>62.43</td>
<td>306 ± 50</td>
</tr>
</tbody>
</table>
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger's Base Derivatives

5.2.3 Conclusions from ps-TA and TRIR of a Concentrated Solution of 4-amino-1,8-naphthalimide 82 (10 mM)

Both the ps-TA and TRIR data obtained for 82 suggested the presence of two processes following excitation at 400 nm; a fast one with a lifetime of ca. 56 ps, and a relatively slow one with a lifetime of ca. 259 ps, suggesting the presence of two excited state processes presumably arising from the excited singlet state. However, both of the lifetimes are significantly shorter than the fluorescence lifetime of 82 in water. Additionally, the kinetic measurements suggested the presence of a long-lived state, which did not decay within 3 ns indicated by the non-zero $Y_0$ value. This is presumably the triplet state of 82 as observed previously by Castellano and co-workers for the 4-piperidinyl-1,8-naphthalimide. But we were unable to identify the other components of the decay. Since all the measurements have been carried out at sufficiently high concentration of 82 (10 mM), it was necessary to investigate if the ligand is undergoing any aggregation under the experimental condition, which has been observed for several naphthalimide derivatives recently. This will be discussed in the following section.

5.3 Aggregation Studies of 82

The concentration dependent NMR studies of 82 in D$_2$O were carried out with the assistance of Dr. John O’Brien in the School of Chemistry, Trinity College Dublin. The $^1$H NMR analysis of 82 in D$_2$O (Figure 5.7a) revealed that the aromatic protons showed significant downfield shift with dilution (10 mM $\rightarrow$ 1 μM), although the UV/vis absorption spectrum of 82 recorded at 10 mM concentrations showed only a small red shift (ca. 4 nm) compared to that at 10 μM concentrations and no new band appeared in the UV/vis absorption spectrum at higher concentrations (Figure 5.7c). The chemical shift values of 82 (D$_2$O, 600 MHz) at different concentrations are summarised in Table 5.6. Since no further downfield shift was observed on going from 10 μM $\rightarrow$ 1 μM, it can be assumed that 82 exists predominantly as a monomeric species at concentration 10 μM and lower (i.e. the concentrations used in the measurements described in Chapter 2). The chemical shift for each proton has been normalised using equation 5.1

$$\alpha = \frac{\delta - \delta_{0.001\,mM}}{\delta_{0.001\,mM}}$$  (5.1)
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The plot of $\alpha$ vs. log[concentration of 82] is shown in Figure 5.7b. This showed that the naphthalimide protons exhibited significant downfield shift with dilution indicative of intermolecular stacking interaction between the planar naphthalimide moiety of 82, which has been observed for various other naphthalimide derivatives.

Table 5.6: Summary of chemical shift ($\delta$) values of 82 at different concentrations.

<table>
<thead>
<tr>
<th>H</th>
<th>10 mM</th>
<th>1 mM</th>
<th>0.1 mM</th>
<th>0.01 mM</th>
<th>0.001 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.56</td>
<td>7.88</td>
<td>8.05</td>
<td>8.18</td>
<td>8.18</td>
</tr>
<tr>
<td>3</td>
<td>6.46</td>
<td>6.72</td>
<td>6.85</td>
<td>6.93</td>
<td>6.93</td>
</tr>
<tr>
<td>5</td>
<td>7.91</td>
<td>8.23</td>
<td>8.37</td>
<td>8.42</td>
<td>8.43</td>
</tr>
<tr>
<td>6</td>
<td>7.26</td>
<td>7.50</td>
<td>7.61</td>
<td>7.68</td>
<td>7.68</td>
</tr>
<tr>
<td>7</td>
<td>7.84</td>
<td>8.14</td>
<td>8.31</td>
<td>8.42</td>
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<tr>
<td>9</td>
<td>4.43</td>
<td>4.59</td>
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<td>-</td>
</tr>
<tr>
<td>10</td>
<td>4.80</td>
<td>4.88</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>8.74</td>
<td>8.76</td>
<td>8.79</td>
<td>8.82</td>
<td>8.82</td>
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<tr>
<td>12</td>
<td>7.89</td>
<td>7.88</td>
<td>7.90</td>
<td>7.92</td>
<td>7.92</td>
</tr>
<tr>
<td>13</td>
<td>8.46</td>
<td>8.46</td>
<td>8.48</td>
<td>8.47</td>
<td>8.48</td>
</tr>
</tbody>
</table>

Figure 5.7: (a) The $^1$H NMR of 82 in D$_2$O at different concentrations (600 MHz), (b) the plot of normalised chemical shift ($\alpha$) vs. log([82]) in D$_2$O, (c) the UV/vis absorption spectra of 82 at 10 mM and 10 $\mu$M concentrations in phosphate buffer (D$_2$O).
We have also investigated the interaction of 82 with GMP in D$_2$O using $^1$H-NMR spectroscopy. The $^1$H NMR spectra of 82 (at concentrations 1 mM and 10 mM) in the presence of GMP (20 mM) are shown in Figure 5.8 a and b, respectively. At lower concentration of 82 (1 mM), both naphthalimide and pyridinium protons showed significant shift in the presence of GMP. However, at higher concentrations of 82 (10 mM), addition of GMP (20 mM) has only a small effect on the naphthalimide ring protons. Under all conditions the pyridinium protons (H11) showed downfield shift (8.74 to 8.81) in presence of GMP. This could be due to interaction of positive charged pyridinium moiety with the phosphate group of GMP.

![Figure 5.8](image_url)

**Figure 5.8:** The $^1$H NMR spectra of 82 in D$_2$O (600 MHz) in the presence of GMP (20 mM). (a) [82] = 1 mM, (b) [82] = 10 mM.
5.3.1 Conclusions from the NMR Analysis

The $^1$H NMR dilution studies suggested that at high concentration (10 mM) compound 82 exists in the form of a dimer or higher order aggregates (as shown in the equilibrium i, in the Scheme 5.1). The complex kinetic behaviour observed in the ps-TA and TRIR measurements presumably represents the excited states arising due to intermolecular stacking interactions between the ligands.

In the presence of GMP, the overall interaction can be presented as a combination of two equilibria (i and ii) as shown in the Scheme 5.1. At high naphthalimide concentration (10 mM), where aggregation of 82 is prevailing, addition of GMP (20 mM) possibly cannot perturb equilibrium (i) and does not result in the formation of appreciable amount of the NI:GMP complex. Consequently, no major shift was observed in the naphthalimide ring protons under these condition. This possibly explains the failure to observe any quenching in the presence of GMP in the ps-TA and TRIR measurements described in Section 5.2.

\[
\begin{align*}
(i) & \quad n \text{NI} \quad \xrightarrow{\text{GMP}} \quad (\text{NI})_n \\
(ii) & \quad n \text{NI} \quad \xrightarrow{\text{GMP}} \quad \text{NI: GMP}
\end{align*}
\]

**Scheme 5.1:** Schematic representation of concentration dependent aggregation equilibrium for a naphthalimide derivative (NI) and the interaction with GMP.

Since we could not gain any information about the excited state of 82 due to aggregation at higher concentration, in a later visit to the Rutherford Appleton Laboratory, Prof. J M. Kelly kindly carried out further ps-TA measurements using dilute solutions of 82 in D$_2$O and water. These results will be discussed in the following section. Due to time constrains, ps-TRIR measurements of these samples could not be performed.

5.4 Picosecond Transient Absorption Spectra of 82 at Low Concentrations

The ps-TA of the 4-amino-naphthalimide derivative 82 (32 µM) in water (energy 400 nJ) are shown in Figure 5.9a. The transient spectra of 82 showed a strong transient band centred at 380 nm and a ground state bleach band at 440 nm. Both of these bands were found to follow biexponential kinetics (Figure 5.9b and c) with a short-lived component $\tau_1 = 22.5 \pm 13$ ps (10%) and a long-lived component $\tau_2 = 2295 \pm 85$ ps (84%), which are summarised in Table 5.7. Weak transients were also observed at longer wavelength region (> 700 nm). Additionally, stimulated emission peak was observed at ca. 550 nm immediately after excitation, which was found to be red shifted at longer delays as observed previously for the
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

concentrated solution of 82 (Figure 5.9d). The stimulated emission peak also followed a biexponential kinetics with $\tau_1 = 17 \pm 13$ ps (46%) and $\tau_2 = 2598 \pm 400$ ps (46%). The magnitude of the long-lived component was consistent with the fluorescence lifetime of 82 in water (2.2 ns), suggesting that the initial excited state is ICT in nature, which has been discussed earlier in Chapter 2. However, the short-lived component could not be assigned to any transition. To verify the effect of aggregation, ps-TA spectra of 82 were also recorded at very dilute concentration (8 µM) (Figure A5.10a in Appendix 5). At very dilute concentrations of 82, the transient band centred at 380 nm and the recovery of the ground state still exhibited biexponential kinetics. Due to high noise the kinetic parameters were associated with very large errors. The lack of baseline recovery (as indicated by large Y0 values, Table 5.7) for both the transient band centred at 380 nm and the bleach band centred at 440 nm suggested formation of long-lived triplet state. Further ps-TRIR measurements are required to verify the possible formation of two different excited states as proposed by Glusac and co-workers.129

![Figure 5.9: (a) The ps-TA spectra of 82 (32 µM) in 10 mM phosphate buffer (H2O), $\lambda_{ex} = 400$ nm (b) the kinetics of recovery of ground state bleach at 440 nm, (c) the decay kinetics of transient at 380 nm, (d) the kinetics of stimulated emission band at 560 nm and (— ) fit to the biexponential decay function.](image)
5.4.1 Picosecond Transient Absorption Spectra of \(82\) at Low Concentration in the Presence of GMP

In the presence of 20 mM GMP no notable changes were observed in the shape of the transient spectrum of \(82\) (Figure A5.10b, Appendix 5). However, in the presence of GMP, decay of the transient band at 380 nm and the ground state bleaching, were found to be biexponential with a short-lived component of \(\text{ca.} 10\ \text{ps}\) and a long component of \(\text{ca.} 700\ \text{ps}\) (Figure 5.10 and b), which are summarised in Table 5.7. It should be mentioned here, that a \(\text{ca.} 700\ \text{ps}\) component was also observed in the fluorescence lifetime measurement of \(82\) in the presence of GMP and the relative proportion of this component was found to increase with the concentration of GMP (Chapter 2, Section 2.4.2.2). From the time-resolved emission measurements, this component has been assigned to the \((1:1)\) complex of \(82\) with GMP as shown in Scheme 5.2. However, no new transient species was observed in the ps-TA, suggesting that the back electron transfer (BET) is very rapid (Scheme 5.2).

![Graphs](image-url)  

**Table 5.7:** Kinetic parameters from the fit of ps-TA data of \(82\) in the absence and in the presence of 20 mM GMP in Na-phosphate buffer (\(H_2O\)).

<table>
<thead>
<tr>
<th>(\lambda) (nm)</th>
<th>(Y0) (%)</th>
<th>(A1) (%)</th>
<th>(\tau_1) (ps)</th>
<th>(A2) (%)</th>
<th>(\tau_2) (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(82) (32 (\mu)M)</td>
<td>380</td>
<td>5</td>
<td>5</td>
<td>13 ± 10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>7</td>
<td>15</td>
<td>32 ± 9</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>20</td>
<td>34</td>
<td>17 ± 13</td>
<td>46</td>
</tr>
<tr>
<td>(82) (8 (\mu)M)</td>
<td>380</td>
<td>20</td>
<td>32</td>
<td>9 ± 6</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>39</td>
<td>19</td>
<td>51 ± 45</td>
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<tr>
<td></td>
<td>560</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
</tr>
<tr>
<td>(82) (8 (\mu)M) + GMP (20 (\mu)M)</td>
<td>380</td>
<td>15</td>
<td>29</td>
<td>11 ± 9</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>440</td>
<td>15</td>
<td>17</td>
<td>15 ± 8</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>560</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
<td>([\text{a}])</td>
</tr>
</tbody>
</table>

\([\text{a}]\) data could not be fitted.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

5.4.2 Conclusions from the ps-TA Measurements of the Dilute Sample of 82

The ps-TA measurements of 82 suggested that at lower concentration (< 0.1 mM), ICT excited state forms very rapidly (< 20 ps) upon excitation at 400 nm, which then predominantly decays by fluorescence and non-radiative processes. However, the lack of baseline recovery over 3 ns suggests formation of long-lived triplet state. In the presence of GMP, much shorter lifetime (ca. 700 ps) was recorded, consistent with the PET from guanine to the $S_1$ state of 82. However, due to rapid back electron transfer (BET), no new transient band was observed.

5.5 Picosecond Transient Absorption and Transient Infrared Studies of the Tröger’s Base derivative 87

The samples of 87 for the UV-Vis transient absorption and transient infrared measurements were prepared in 10 mM phosphate buffer in D$_2$O (pH 7.0) the ps-TA and TRIR pump-probe experiments were carried in a manner similar to that described in Section 5.2 using the high-sensitivity ULTRA apparatus ($\lambda_{\text{excitation}} = 400$ nm, 150 fs laser pulse) at the Central Laser Facility of the Science & Technology Facilities Council in the Rutherford Appleton Laboratory.

5.5.1 Transient Absorption Data for Concentrated Tröger’s Base Derivative 87

The UV/vis absorption spectrum of 87 (10 mM) in phosphate buffer (D$_2$O) is shown in Figure 5.11a, where the band centred at 380 nm was assigned to the ICT absorption band. The ps-TA spectra of 87 (10 mM) in 10 mM phosphate buffer prepared in D$_2$O (pH 7.0) are shown in Figure 5.11b. Upon excitation at 400 nm, a broad transient band was observed centred at ca. 410 nm, along with weak transient bands at the longer wavelength region (>700 nm). As shown in Figure 5.11c, the decay of the transient at 410 nm was fitted to biexponential kinetics with a short-lived component of $74 \pm 10$ ps (67 %) and a long-lived component of $266 \pm 62$ ps (33%). The kinetic analysis of the transients at 720 nm and 490 nm also showed similar lifetime values, which are summarised in Table 5.8. The ps-TA spectra at early time
delays time (2 ps, 10 ps) were compared with that at longer delay (100 ps) to verify the possible formation of different excited states (Figure 5.11d), however, no significant difference was observed between the spectrum recorded at various time delays, suggesting the presence of a single excited state. The lifetime values obtained from the biexponential fit of transient decay were in the picosecond region, suggesting that the excited state is arising from singlet state. The interaction of 87 with GMP was also investigated using ps-TA, which will be described below.

![Figure 5.11](image)

**Figure 5.11:** (a) The UV/vis absorption spectrum of 87 (10 mM) in phosphate buffer (D$_2$O), (b) the ps-TA spectra of 87 (10 mM) in 10 mM phosphate buffer (D$_2$O), $\lambda_{ex} = 400$ nm (c) Decay of transient at 410 nm and (—) fit to the biexponential decay function, (d) Normalised TA spectra at various time delays.

### 5.5.1.1 ps-TA of concentrated sample of 87 with GMP

In the presence of 20 mM GMP, no detectable change was observed in the ps-TA spectrum of 87 (Figure A5.11, Appendix 5). The transient bands at 410 nm and 720 nm still exhibited biexponential kinetics, however, both the lifetimes being significantly shorter than that recorded for 87 (Figure 5.12 and Table 5.8) in the absence of GMP.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

Figure 5.12: Comparative plot of transient decay at (a) 410 nm and (b) 720 nm for 87 in the absence and in the presence of GMP.

Table 5.8: Kinetic parameters from the fit of ps-TA data of 87 (10 mM) in the absence and in the presence of 20 mM GMP in Na-phosphate buffer (D₂O).

<table>
<thead>
<tr>
<th></th>
<th>λ (nm)</th>
<th>Y₀(%)</th>
<th>A₁(%)</th>
<th>τ₁ (ps)</th>
<th>A₂ (%)</th>
<th>τ₂ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>87 (10 mM)</td>
<td>410</td>
<td>1</td>
<td>67</td>
<td>73 ± 9</td>
<td>32</td>
<td>265 ± 62</td>
</tr>
<tr>
<td></td>
<td>490</td>
<td>4</td>
<td>54</td>
<td>69 ± 10</td>
<td>42</td>
<td>280 ± 33</td>
</tr>
<tr>
<td></td>
<td>720</td>
<td>2</td>
<td>62</td>
<td>75 ± 9</td>
<td>36</td>
<td>303 ± 61</td>
</tr>
<tr>
<td>87 (10 mM) + GMP (20 mM)</td>
<td>410</td>
<td>0</td>
<td>64</td>
<td>38 ± 6</td>
<td>36</td>
<td>153 ± 36</td>
</tr>
<tr>
<td></td>
<td>490</td>
<td>3</td>
<td>55</td>
<td>34 ± 4</td>
<td>42</td>
<td>161 ± 22</td>
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<tr>
<td></td>
<td>720</td>
<td>6</td>
<td>55</td>
<td>38 ± 5</td>
<td>39</td>
<td>186 ± 43</td>
</tr>
</tbody>
</table>

To understand the excited state properties of 87, further ps-TRIR measurements were also carried out in parallel to the ps-TA measurements, the results of which will be described in the following section.

5.5.2 ps-Transient Infrared Data for Concentrated Tröger’s Base Derivative 87

Figure 5.13 represents the ps-TRIR spectra of 87 (10 mM) upon 400 nm pulsed excitation, where intense bleach signals were observed at ca. 1692 cm⁻¹ and 1653 cm⁻¹, assigned to the asymmetric and symmetric stretching of the C=O moieties, respectively.²⁷⁴ Bleach bands at 1404, 1382 and 1360 cm⁻¹ possibly originate either from the vibration of the diazocine ring or from the naphthalimide unit.²⁷⁷ Several transient bands were also observed at 1609, 1589, 1552 and 1516 cm⁻¹. Kinetic analysis of all the bleach bands exhibited
biexponential behaviour with a short-lived component of $\tau_1 = 59 \pm 8$ ps (48%) and a long-lived component of $\tau_2 = 233 \pm 45$ (49%) (Figure 5.13b and A5.12 in Appendix 5).

The decay constants for various transient bands shown in Figure 5.13 are summarised in Table 5.9, which shows that the decay of the various transient bands also followed biexponential kinetics with $\tau_1 = 54 \pm 16$ ps (46 %) and a long-lived component of $\tau_2 = 214 \pm 4$ (53%) (Figure 5.13 b and A5.12, in Appendix 5), similar to that observed for the bleach bands. To investigate whether these two kinetic components are arising from different excited states, the TRIR spectrum of 87 at various time delays were also compared (Figure 5.13c). However, no significant difference was observed between the spectrum recorded at early and longer delays. Both ps-TA and TRIR measurements of 87 at high concentration showed that the kinetics of ground states recovery and the excited state decay followed biexponential behaviours, however, the lifetime values were in the picosecond time scale; suggesting that the transients are arising from an excited singlet state. However, the origin of such
biexponential kinetics observed in the case of 87 could not be explained from these ultrafast measurements. Since these measurements were carried out at very high concentrations of 87, it was necessary to investigate the possibility of aggregation under these conditions, which was also observed to be the case with 82 as discussed in Section 5.3. The results of the aggregation studies of 87 will be described in Section 5.6.

5.5.2.1 ps-TRIR of concentrated sample of 87 with GMP

In the presence of 20 mM GMP, the overall nature of the ps-TRIR spectra of 87 (10 mM) remained unchanged and no new band was observed. However, kinetics of the ground state recovery and the transient decay were found to be much faster in the presence of GMP compared to that observed for 87 (Figure 5.14 b and c). In the presence of GMP, the recovery of ground state was found to follow monoexponential kinetics with $\tau = 76 \pm 3$ ps (Figure A5.13, Appendix 5), which is much shorter than the excited state lifetime observed for 87, previously (Table 5.9 and 5.10). The decay of the transient bands could be fitted to biexponential model with $\tau_1 = 28 \pm 9$ ps (42%) and $\tau_2 = 142 \pm 26$ ps (46%) (Figure A5.13, Appendix 5), which were similar to the values obtained from ps-TA measurements of 87 in the presence of GMP. The shortening of the excited state lifetime of 87 in the presence of GMP can possibly result from PET occurring from guanine to the excited singlet state of 87. However, the absence of any new peak in the TRIR spectrum is indicative of rapid back electron transfer (BET) process in this case.

Table 5.9: Summary of the kinetic parameters obtained from the biexponential fit of TRIR data of 87 (10 mM).

<table>
<thead>
<tr>
<th>Band (cm$^{-1}$)</th>
<th>$Y_0$ (%)</th>
<th>$A_1$ (%)</th>
<th>$\tau_1$ (ps)</th>
<th>$A_2$ (%)</th>
<th>$\tau_2$ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1692</td>
<td>2</td>
<td>49</td>
<td>60 ± 12</td>
<td>49</td>
<td>273 ± 42</td>
</tr>
<tr>
<td>1653</td>
<td>7</td>
<td>51</td>
<td>68 ± 25</td>
<td>42</td>
<td>268 ± 110</td>
</tr>
<tr>
<td>1404</td>
<td>0.3</td>
<td>50</td>
<td>59 ± 14</td>
<td>49.7</td>
<td>211 ± 43</td>
</tr>
<tr>
<td>1382</td>
<td>3</td>
<td>41</td>
<td>48 ± 17</td>
<td>56</td>
<td>180 ± 38</td>
</tr>
<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1609</td>
<td>1</td>
<td>67</td>
<td>76 ± 20</td>
<td>32</td>
<td>293 ± 100</td>
</tr>
<tr>
<td>1589</td>
<td>4</td>
<td>54</td>
<td>42 ± 14</td>
<td>42</td>
<td>212 ± 73</td>
</tr>
<tr>
<td>1552</td>
<td>1</td>
<td>26</td>
<td>43 ± 20</td>
<td>73</td>
<td>166 ± 21</td>
</tr>
<tr>
<td>1516</td>
<td>1</td>
<td>36</td>
<td>55 ± 10</td>
<td>63</td>
<td>183 ± 20</td>
</tr>
</tbody>
</table>
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger's Base Derivatives

Figure 5.14: (a) The ps-TRIR spectra of 87 (10 mM) in the presence of GMP (20 mM) in 10 mM phosphate buffer (D$_2$O), $\lambda_{ex} = 400$ nm, (—) represents spectra at delays shorter than 7.5 ps and (—) represents spectra at longer delays (> 7.5 ps), (b) Normalised kinetics of ground state recovery at 1653 cm$^{-1}$ (c) Normalised kinetics of transient decay 1609 cm$^{-1}$ of 87 in the absence and in the presence of GMP.

Table 5.10: Summary of the kinetic parameters obtained from the biexponential fit of TRIR data of 87 (10 mM) in the presence of 20 mM GMP.

<table>
<thead>
<tr>
<th>Band (cm$^{-1}$)</th>
<th>Y0 (%)</th>
<th>$A_1$ (%)</th>
<th>$\tau_1$ (ps)</th>
<th>$A_2$ (%)</th>
<th>$\tau_2$ (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1692</td>
<td>1</td>
<td>99</td>
<td>74 ± 4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1653</td>
<td>2</td>
<td>98</td>
<td>75 ± 5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1404</td>
<td>7</td>
<td>93</td>
<td>79 ± 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1382</td>
<td>[a]</td>
<td>[a]</td>
<td>[a]</td>
<td>[a]</td>
<td>[a]</td>
</tr>
<tr>
<td><strong>Transient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1609</td>
<td>1</td>
<td>46</td>
<td>15 ± 3</td>
<td>53</td>
<td>112 ± 15</td>
</tr>
<tr>
<td>1589</td>
<td>4</td>
<td>64</td>
<td>37 ± 10</td>
<td>32</td>
<td>173 ± 84</td>
</tr>
<tr>
<td>1552</td>
<td>1</td>
<td>50</td>
<td>28 ± 8</td>
<td>49</td>
<td>152 ± 55</td>
</tr>
<tr>
<td>1516</td>
<td>1</td>
<td>40</td>
<td>31 ± 6</td>
<td>59</td>
<td>131 ± 25</td>
</tr>
</tbody>
</table>

[a] could not be fitted due to high noise.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger's Base Derivatives

5.6 Aggregation Studies of 87

As discussed previously for 82 in Section 5.3, the concentration dependent $^1$H NMR (600 MHz) studies of 87 in D$_2$O also demonstrated that the aromatic protons showed significant downfield shift upon changing the concentration from 10 mM to 0.01 mM (Figure 5.15a). However, UV/vis absorption spectrum remained almost same at higher concentrations (Figure 5.15b). The chemical shift values (600 MHz, D$_2$O) at different concentrations of 87 are summarised in Table 5.11. Assuming that 87 exists as a monomer at 10 μM concentration, the chemical shift values for 87 were normalised using equation 5.1. The plots of $\alpha$ vs. log[concentration of 87] are shown in Figure 5.15c, which shows that the naphthalene ring protons exhibited significant downfield shift upon decreasing the concentrations of 87 in a manner similar to that observed for the 4-amino-1,8-naphthalimide derivative 82. This would suggest that at higher concentrations used for the ps-TRIR and ps-TA studies 87 exists in the form of a dimer or higher order aggregates. Therefore, further ultrafast measurements using dilute samples of 87 were carried out as described in the following section.

Table 5.11: Summary of chemical shift values of 87 at different concentrations.

<table>
<thead>
<tr>
<th></th>
<th>10 mM</th>
<th>1 mM</th>
<th>0.1 mM</th>
<th>0.01 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7.49</td>
<td>7.73</td>
<td>7.87</td>
<td>7.9</td>
</tr>
<tr>
<td>5</td>
<td>8.37</td>
<td>8.59</td>
<td>8.73</td>
<td>8.78</td>
</tr>
<tr>
<td>6</td>
<td>7.71</td>
<td>7.85</td>
<td>7.89</td>
<td>7.90</td>
</tr>
<tr>
<td>7</td>
<td>8.10</td>
<td>8.29</td>
<td>8.39</td>
<td>8.43</td>
</tr>
<tr>
<td>9</td>
<td>-[a]</td>
<td>4.54</td>
<td>-[a]</td>
<td>-[a]</td>
</tr>
<tr>
<td>10</td>
<td>-[a]</td>
<td>4.84</td>
<td>-[a]</td>
<td>-[a]</td>
</tr>
<tr>
<td>11</td>
<td>8.72</td>
<td>8.75</td>
<td>8.79</td>
<td>8.80</td>
</tr>
<tr>
<td>12</td>
<td>7.83</td>
<td>7.86</td>
<td>7.90</td>
<td>7.88</td>
</tr>
<tr>
<td>13</td>
<td>8.39</td>
<td>8.42</td>
<td>8.45</td>
<td>8.46</td>
</tr>
</tbody>
</table>

[a] Chemical shifts could not be determined due to overlap with D$_2$O peak.
Chapter 5: Transient Absorption and Transient Infrared Spectroscopic Studies of 4-amino-1,8-Naphthalimide and Tröger’s Base Derivatives

Figure 5.15: (a) The $^1$H NMR of 87 in D$_2$O at different concentrations (600 MHz). (b) UV/vis absorption spectra of 87 at 10 mM and 10 μM concentrations in phosphate buffer (D$_2$O). (b) (c) The plot of normalised chemical shift (a) vs. log(Tröger’s base) concentrations for 87 in D$_2$O.
5.7 ps Transient Absorption Studies of 87 at Low Concentration

The ps-transient absorption spectra of 87 (13 μM) are shown in Figure 5.16a. Upon 400 nm pulsed excitation of 87 (13 μM) in water; ground state bleaching was observed at 380 nm simultaneous with the formation of a transient band centred at ca. 415 nm. The recovery of the ground state and the decay of the transient were found to have similar monoexponential kinetics with a decay constant of ca. 28 ps (Figure 5.16 b). Kinetic analysis was also carried out at several other wavelengths, such as, 490 nm and 725 nm. The decay at 725 nm was consistent with a monoexponential function with decay constant of ca. 33 ± 2 ps (99.6%). However, the decay at 490 nm could not be fitted to a single exponential function but biexponential fit showed that the decay was dominated by a short-lived component of 25 ± 1 ps (82%). However, a long-lived component of 248 ± 58 (9%) was also observed. This may arise also from the fluctuations in data points at longer delays. All the kinetic parameters are summarised in Table 5.12. Moreover, the kinetic parameters were found to be unaffected with further dilution of the solution, suggesting the presence of monomeric species under all of the experimental condition employed.

Figure 5.16: (a) The ps Transient absorption of 87 (13 μM) in water; (b) kinetics of bleach at 380 nm and transient decay at 415 nm and monoexponential fit (—). λex = 400 nm.

Table 5.12: Summary of the kinetic parameters obtained from the monoexponential fit of ps-TA data of 87 (13 μM).

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>Y0(%)</th>
<th>A1(%)</th>
<th>τ1 (ps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>380</td>
<td>1.0</td>
<td>99.0</td>
<td>28 ± 0.6</td>
</tr>
<tr>
<td>415</td>
<td>0.4</td>
<td>99.6</td>
<td>28 ± 0.5</td>
</tr>
<tr>
<td>490*</td>
<td>10</td>
<td>90</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>725</td>
<td>5</td>
<td>95</td>
<td>33 ± 2</td>
</tr>
</tbody>
</table>

* biexponential fit of the kinetics at 490 nm gave τ1 = 25 ± 1 ps (82 %) and τ2 = 248 ± 58 (9 %).
5.8 Conclusions

Picosecond TA and TRIR data obtained using a 10 mM solution of the 4-amino-1,8-naphthalimide derivative 82 indicated the presence of two fast processes with lifetimes of ca. 50 ps and ca. 300 ps respectively. The transient bands in both the ps-TA and the ps-TRIR spectra were formed immediately after excitation. In all cases, the ground state recovery and the transient decay were not complete within 3 ns suggesting the formation of triplet state. However, no new band appeared at longer delays, which suggests that either the triplet state was formed very rapidly or the singlet and the triplet excited states have similar spectra. In the presence of GMP, the excited state lifetime of 82 was not shortened in contrast to the data obtained from the steady-state and time-resolved fluorescence performed at lower concentrations of 82. Although UV/vis absorption spectroscopy did not provide any evidence of aggregation, subsequent 1H NMR studies (600 MHz, D₂O) showed that 82 could potentially exist as a dimer or higher order aggregates at the concentrations employed in the measurements. Further ps-TA measurements using a dilute sample of 82 (32 μM), showed that the ICT excited state was formed very rapidly (< 20 ps) upon photoexcitation and decayed by fluorescence and non-radiative decays. The lack of baseline recovery over 3 ns suggests formation of long-lived triplet state. Additionally, for the dilute sample of 82 (8 μM) much shorter lifetime (ca. 700 ps) was recorded in the presence of GMP, which is consistent with PET from guanine to the S₁ state of 82. However, presumably due to rapid back electron transfer, no new transient band was observed in the spectrum. Further ps-TRIR studies are required on a dilute solution of 82 to understand the nature of the excited states.

Picosecond TA and TRIR of the Tröger’s base derivative 87 (10 mM) were also undertaken, which showed the presence of two excited state processes with lifetimes of ca. 65 ps and ca. 250 ps, respectively. The excited state was formed immediately after excitation and decayed completely within 500 ps, which suggests that the excited state has singlet character. Picosecond TA and TRIR measurements at various time delays did not provide evidence for the formation of any new band, suggesting the presence of one species in solution. The 1H NMR (600 MHz, D₂O) dilution studies showed that 87 possibly exists as a dimer or in the form of aggregates at higher concentrations (> 0.1 mM). Subsequent, ps-TA measurements at lower concentrations of 87 (13 μM) revealed that the excited state of 87 was very short-lived (ca. 30 ps) in water, consistent with the non-emissive singlet state observed in the steady-state measurements as described in Chapter 3. Picosecond-TA and TRIR measurements of the concentrated samples of 87 (10 mM) in the presence of GMP showed significant shortening of excited state lifetime. Since 87 possibly exists in aggregated form at such high concentration,
the shortening of the excited state lifetime can therefore presumably result from PET from guanine to the excited state of the aggregated species. It should be noted here that the short-lived component (ca. 35 ps, 55 %) for the 10 mM solution of 87 in the presence of GMP, was found to be similar to the excited state lifetime recorded for the dilute sample of 87 (30 ps). The short-lived component of 87 (10 mM) in the presence of GMP can also represent the monomeric form of 87 resulting from the GMP induced dissociation of the higher order aggregates. Due to time limitations, the interaction of GMP with dilute sample of 87 could not be carried out. Therefore, future ps-TA and TRIR studies are required to elucidate the excited state interaction of GMP with monomeric samples of 87.

5.9 Overall Conclusions and Future Perspectives

The work described in this thesis discussed the development of several pyridinium based 4-amino-1,8-naphthalimide derivatives as potential DNA binders. The 4-amino-1,8-naphthalimides 82-84, discussed in Chapter 2 showed polarity sensitive ICT emission and bind to DNA via intercalation, resulting in significant changes in their photophysical properties. Several strategies have been discussed in the subsequent chapters to enhance the DNA binding properties of these compounds, for example, in Chapter 3, the development of C2-symmetric chiral bis-naphthalimides 87 and 88 incorporating the Tröger’s base unit was described. These compounds were found to bind to the DNA grooves with significantly higher affinity compared to the corresponding 4-amino-1,8-naphthalimide precursors. The enantiomers of these chiral bis-naphthalimides were successfully separated, however, the absolute configurations of the enantiomers need to be assigned in future. Additionally, DNA DNA binding studies indicated the enantioselective binding of the (-)-enantiomer to the double stranded DNA. Further investigations are required to elucidate their DNA binding mode in detail. Preliminary biological studies demonstrated that 87 and 88 were rapidly taken up by HeLa cells and the compounds were localised in the cytoplasm. Future studies will involve evaluation of the cytotoxic activities of these compounds against other malignant cell lines and also investigate their binding affinities for various nucleic acid secondary structures, such as “hair-pin loop”, “bulged-sequences” etc. These studies can be useful to develop potential DNA targeted therapeutic agents based on these compounds.

In Chapter 4, the development of a bifunctional Pt(II) complex incorporating both the 1,8-naphthalimide and the terpyridyl unit is addressed. Preliminary studies showed that the Pt(II) complex showed significantly high DNA binding affinity and enhanced cytotoxicity against HeLa cells compared to the corresponding 1,8-naphthalimide ligand. Future studies
will involve investigation of the mode of DNA binding and cytotoxic activity of this complex against cisplatin resistant cell lines. Moreover, the complex was found to be unstable in the presence of other nucleophilic ligands such as chloride anion and DNA bases, therefore further synthetic modifications will be required to modulate their photophysical and DNA binding properties and also to increase the stability of the resulting complex.

The work presented in Chapter 5 demonstrated that both the simple 4-amino-1,8-naphthalimide 82 and the Tröger’s base derived bis-naphthalimide 87 were found to undergo aggregation at higher concentrations (> 0.1 mM), which resulted in biexponential decay of the excited states. The ps-TA and TRIR studies at lower concentrations are also required to understand their photophysical properties in detail.
Chapter 6

Experimental
6.1 General Experimental Details

All NMR spectra were recorded using either a Bruker DPX-400 or AV-600 spectrometer, operating at 400/600 MHz for $^1$H NMR and 100/150 MHz for $^{13}$C NMR, respectively. Chemical shifts were referenced relative to the internal solvent signals. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), double triplet (dt). Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer equipped with a Universal ATR sampling accessory. Electrospray 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 using HPLC grade methanol, water or acetonitrile as carrier solvents. High resolution mass spectra were obtained by a peak matching method using leucine enkephaline (Tyr-Gly-Gly-Phe-Leu) as the reference (m/z = 556.2771). All accurate masses were quoted to ≤5 ppm. Melting points were determined using an Electrothermal 1A9000 digital melting point apparatus. Elemental analysis of all compounds was carried out at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University of Dublin.

6.1.1 Enantiomeric Resolution Chromatography

The enantiomers of the TB-derivatives were resolved by cation exchange chromatography on CM Sephadex C25 (Sigma Aldrich) as the stationary phase and an aqueous solution of (-)-(9,0'-dibenzoyl-L-tartaric acid (as its sodium salt) as the chiral mobile phase. The concentration of the eluent was adjusted at 0.05 M or 0.07 M to achieve better resolution. In each case, the successful resolution of the enantiomers was achieved after three times recycling through a 1m long Perspex column fitted to a peristaltic pump and in each case the (-)-enantiomer eluted before the (+)-enantiomer.

6.1.2 UV/vis Absorption Measurements

UV/vis absorption spectra were recorded on a Shimadzu UV-2401PC spectrophotometer or on a Varian CARY 50 spectrophotometer. All the spectroscopic measurements were carried out in quartz cuvettes (10 mm × 10 mm). The wavelength range was 200-800 nm with a scan rate of 600 nm/min. MilliQ water was used in DNA related work. Phosphate buffer: two 1 M stock solutions of Na$_2$HPO$_4$ and NaH$_2$PO$_4$ were made up with MilliQ water. Portions of each solution were diluted together to achieve 10 mM phosphate buffer of pH 7.0, which was then filtered using a 0.45 μM syringe filter. Baseline
corrections were performed for all spectral measurements. All solutions were prepared fresh prior to measurement. The UV/vis titrations were carried out by monitoring the changes in the absorption spectra of the ligand of interest in 10 mM phosphate buffer (pH 7.0) upon gradual addition of mononucleotides/st-DNA/polynucleotides. All the titrations were repeated at least three times to ensure reproducibility.

6.1.3 Fluorescence Measurements

Steady-state fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorimeter. Fluorescence titrations were carried out using optically dilute solutions (absorbance < 0.1) following the same procedure as described for UV/vis titrations. Fluorescence quantum yields of the 4-amino-1,8-naphthalimide derivatives were calculated using fluorescein in 0.1 N NaOH ($\phi_F = 0.92, \lambda_{ex} = 436$ nm) as the reference, while the quantum yield of the Tröger's base derivatives and the [Pt(terpy)naph]$^{2+}$ complexes were calculated by comparison with quinine sulfate ($\phi_F = 0.546, \lambda_{ex} = 365$ nm) and [Ru(bpy)$_3$]$^{2+}$ ($\phi_F = 0.028, \lambda_{ex} = 436$ nm), respectively. For determination of the quantum yields, a number of solutions of the ligand with absorbance ranging from 0.02 to 0.1 were used. Optically matched solutions of the samples and reference were used. The fluorescence emission spectra of the samples and the standard were measured under same experimental settings. The integrated areas under the emission spectra measured using the in-built software of the spectrofluorimeter. The quantum yield of a sample is proportional to the gradient of the straight line obtained by plotting the integrated fluorescence intensity vs. absorbance of the sample. The quantum yield was then calculated using equation 6.1.

$$\phi_X = \phi_{ref} \left( \frac{\text{Grad}_X}{\text{Grad}_{ref}} \right) \left( \frac{n_X^2}{n_{ref}^2} \right)$$  \hspace{1cm} (6.1)

In equation 6.1, subscripts $ref$ and $X$ denote the reference compound and the sample respectively; $\phi$ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs. absorbance, and $n$ the refractive index of the solvent. All the quantum yield values reported are within 10% error. Fluorescence lifetimes were measured using a Horiba Jobin Yvon Fluorolog FL 3-22 equipped with a FluoroHub v2.0 single photon controller using the time-correlated single photon counting method (TCSPC), run in reverse mode. The samples were excited with a pulsed nanosecond light-emitting diode (NanoLED®, $\lambda_{ex} = 458$ nm). All the decay traces were corrected for the instrument response by recording the scatter signal at identical excitation/emission wavelength using a dilute
solution of colloidal silica (Ludox®). All the measurements were performed at 298 K. The decay traces were analysed using the IBH DAS6 software built in the Fluorolog 3 instrument using a nonlinear least-squares error minimisation analysis (equation 6.2):

\[ I(t) = \sum_{i=1}^{n} \alpha_i \cdot \exp \left( \frac{-t}{\tau_i} \right) \]  

(6.2)

6.1.4 Circular Dichroism and Linear Dichroism

CD spectra were recorded on a JASCO J810 spectropolarimeter. Each CD trace represents the average of three scans. Linear dichroism spectra were recorded on a JASCO J-815 CD spectropolarimeter equipped with a Diacopica Scientific Ltd. linear dichroism accessory. The LD spectra were presented as the average of there scans.

6.1.5 Thermal Denaturation Assays

Thermal denaturation experiments were conducted on a Perkin-Elmer35 UV/vis spectrophotometer coupled to a Peltier temperature controller. The temperature was ramped from 30°C-90°C at a rate of 1°C/min rate and the absorbance at 260 nm was measured at every 0.2°C interval. All the solutions were thoroughly degassed prior to measurement.

6.1.6 Time-Resolved Infrared and UV/vis Absorption ULTRA

The samples for UV-Vis transient absorption or infrared measurements were prepared in D_2O by dropping ca. 70 μL of the solutions between two CaF_2 (25 mm diameter) windows (Crystan Ltd., UK), separated by a Teflon spacer of known length (typically 56 μm), in a demountable solution IR cell (Harrick Scientific Products Inc., New York). The picosecond transient absorption pump-probe experiments were carried out by using the high-sensitivity ULTRA apparatus at the Central Laser Facility of the Science & Technology Facilities Council in the Rutherford Appleton Laboratory.

Briefly, the time-resolved infrared spectrometer (TRIR) consists of a 10 kHz titanium sapphire chirped pulse amplifier (developed by Thales Laser) generating two synchronised outputs (0.8 mJ) at 800 nm with 40 fs and 2 ps pulse durations, respectively. The second harmonic upconversion of the 800 nm laser generated the 400 nm pump excitation used in the study of the naphthalimides. The IR probe beam was split to generate the reference and the
probe beams, which were passed through spectrographs to MCT array detectors (IR Associates). A pump-on-pump-off infrared absorption difference signals were acquired and processed by the high-speed data acquisition systems (Quantum detector). The samples were rastered in x- and y- directions to minimise photodecomposition effects and to avoid eventual re-excitation during pump-probe experiments. The difference signal was calibrated using cis-stilbene absorption lines in TRIR measurements. UV/vis absorption and IR spectra were recorded before and after each measurement to check for decomposition.

\[ \text{Scheme 6.1: Schematic illustration showing the generation of various pump-probe beams in the ULTRA apparatus.}^{273} \]

### 6.1.7 Plasmid DNA Photocleavage Studies

The DNA photocleavage studies were conducted by treating the pBR322 plasmid DNA (1 mg/mL) with the ligand of interest at varying P/D ratios. The samples were then irradiated for one hour with a Hg-Xe lamp using a green glass filter and water IR filter ($\lambda > 350$ nm). An equal volume of the buffer saturated phenol:CHCl$_3$:isoamyl alcohol mixture was added to the irradiated plasmid DNA samples and mixed gently using a micropipette. The mixtures were centrifuged for 2 min and the aqueous layer was carefully removed. The extracted DNA samples were then separated using horizontal agarose gel electrophoresis in a TBE (8.9 mM Tris-HCl, 8.9 mM boric acid and 1 mM EDTA, pH 8.0) buffer.

A 0.8\% (w/v) agarose solution was prepared by dissolving 0.8 g of agarose in 100 mL of TBE buffer and melted by boiling. The gel was poured while warm in a gel-casting tray and allowed to solidify. The plasmid DNA samples were mixed a loading dye solution composed of sucrose (40\%), xylene-cyanol (0.25\%) and bromophenol blue (0.25\%) and loaded into the wells. Electrophoresis was carried out at ca. 5 V/cm (40 mA, 90 V) to separate the covalently
closed circular (Form I), open circular (Form II) and linear (Form III) forms of plasmid DNA. The DNA samples were stained using an aqueous solution of ethidium bromide for 90 minutes, destained with MilliQ water and visualised using a transilluminator (Bioblock 254UV illuminator) equipped with a camera. The ratio of the various DNA forms was estimated using the ImageJ Gel analysis software.

6.1.7.1 Preparation of Phenol: CHCl3: Isoamyl Alcohol (25:24:1): 20 gm phenol was taken into ca. 20 mL 50 mM Tris-HCl (pH 8.0) and shaken well and left until the phenol completely liquefies and the phases separated. The top phase was carefully removed with a glass pipette and another 20 mL 50 mM Tris-HCl (pH 8.0) was added and shaken well. This step was repeated until the pH of the top phase was between 7 and 8. The organic layer was then collected in Falcon tubes. To 5 mL of this buffer saturated phenol layer, another 5 mL of CHCl3:isoamylalcohol mixture (24:1) was added and mixed gently. This mixture was overlaid with 10 mL Tris-HCl (pH 8.0) and stored in the freezer at -20°C in an upright position.

6.1.8 General Biological Procedures

HeLa cells were grown in Dulbecco’s Modified Eagle Medium (Glutamax) supplemented with 10% fetal bovine serum and 50 μg/ml penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO2.

6.1.8.1 Alamar blue viability assay: HeLa cells were seeded at a density of 5×10^3 cells/well in a 96-well plate and treated with the indicated compounds for 48 hr. Alamar blue (20μl) was then added to each well and incubated at 37°C in the dark for 4 hr. Plates were then read on a fluorescent plate reader (SpectraMax Gemini, Molecular Devices) with excitation and emission wavelengths of 544 nm and 590 nm, respectively. Experiments were performed in triplicate on three independent days with activity expressed as percentage cell viability compared to vehicle treated controls. All data points (expressed as means ± S.E.M.) were analysed using GRAPHPAD Prism (Graphpad software Inc., San Diego, CA).

6.1.8.2 Flow Cytometry: HeLa cells were seeded at a density of 1×10^6 cells/well in a 6-well plate and treated with the indicated compounds for 48 hr. Samples were then centrifuged at 650xg for 5 min and resuspended in 100μl ice-cold PBS. Ice-cold 70% (v/v) ethanol (1ml) was then added to fix the samples overnight at 4°C. Samples were subsequently centrifuged at 800xg for 10 min; and resuspended in 200μl phosphate buffered saline. RNase A (12.5μl of 10 mg/ml) and propidium iodide (PI) (37.5μl of 1 mg/ml) were and samples were incubated for 30 min at 37°C. Cell cycle analysis was performed at 488 nm using a Becton Dickinson
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FACS Calibur flow cytometer. The Macintosh-based application CellQuest was then used to analyse the data of 10,000 gated cells once cell debris had been excluded. Data points represent the mean ± S.E.M of two independent measurements.

6.1.8.3 Confocal microscopy: HeLa cells were seeded at a density of $1 \times 10^5$ cells/well in glass bottom dish plates and treated with the indicated compounds for up to 48 hrs. Cells were washed followed by the addition of fresh media and DRAQ5 (red nuclear stain), followed by viewing using Olympus FV1000 confocal microscopy with a 60X oil immersion lens. Image analysis was performed using FluoView Version 7.1 Software. Compounds were excited by a 408nm argon laser, emission ca. 500nm, DRAQ5 was excited by a 633nm red helium-neon laser, emission >650nm.

6.2 Materials

All chemicals were obtained from Sigma-Aldrich, Fluka, TCI (Europe) and were used without further purification. Plasmid pBR322 was obtained from ISIS, Bray, Ireland. Deuterated solvents for NMR were purchased from Apollo Ltd. Dry solvents were prepared using standard procedures described in Vogel's Textbook of Practical Organic Chemistry (5th Ed.). The mononucleotides AMP, GMP, ss-DNA and the homopolymeric nucleotides poly(dA-dT)$_2$ and poly(dG-dC)$_2$ were obtained from Sigma Aldrich as their sodium salts. They were stored at -20 °C to prevent bacterial growth. The DNA concentration per nucleotide was determined spectrophotometrically using the molar extinction coefficients given in Table 6.1. The presence of contaminant proteins in ss-DNA was detected using UV/vis analysis, where the absorption ratio $A_{260\text{ nm}}/A_{280\text{ nm}}$ must be greater than 1.8 for protein-free DNA.

<table>
<thead>
<tr>
<th>Species</th>
<th>$\varepsilon$ (M$^{-1}$ cm$^{-1}$)</th>
<th>$\lambda$ (nm)</th>
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<td>260</td>
</tr>
<tr>
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<tr>
<td>ss-DNA</td>
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<td>260</td>
</tr>
<tr>
<td>poly(dG-dC)$_2$</td>
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<td>254</td>
</tr>
<tr>
<td>poly(dA-dT)$_2$</td>
<td>6,600</td>
<td>262</td>
</tr>
</tbody>
</table>
6.3 Synthesis and Characterisation of the Compounds Described in the Thesis

\textbf{N-(2-Hydroxy ethyl)-4-nitro-1,8-naphthalimide (86)}\textsuperscript{190}

4-Nitro-1,8-naphthalic anhydride (2.031 g, 8.35 mmol) was suspended in dry ethanol and ethanol amine (0.51 ml, 8.44 mmol) was added drop-wise. The mixture was refluxed for 24 hrs under an argon atmosphere and cooled to room temperature. The desired product was separated by filtration, washed with cold ethanol and obtained as a dark brown solid in 75% yield (1.8 g). m.p.157-160°C (Ref.,\textsuperscript{190} 157-160°C); Found: C, 58.55; H, 3.57; N, 9.64%. \(\text{C}_{14}\text{H}_{10}\text{N}_{2}\text{O}_{5}\) requires C, 58.74; H, 3.52; N, 9.79%. HRMS: 309.0488 ([M + Na]\textsuperscript{+}. \(\text{C}_{14}\text{H}_{10}\text{N}_{2}\text{O}_{5}\text{Na}\) requires 309.0487); \(\delta\text{H}\) (400 MHz, DMSO-\(\text{d}_{6}\)), 8.65 (1H, d, \(J = 8.5\) Hz, Ar-H7), 8.56 (1H, d, \(J = 8.0\) Hz, Ar-H5), 8.52 (1H, d, \(J = 8.0\) Hz, Ar-H2), 8.51 (1H, d, \(J = 8.0\) Hz, Ar-H3), 8.05 (1H, t, \(J = 8.0\) Hz, Ar-H6), 4.85 (1H, t, \(J = 6.0\) Hz, OH), 4.14 (2H, t, \(J = 6.5\) Hz, \(\text{CH}_{2}\)), 3.64 (2H, q, \(J = 6.0\) Hz, \(\text{CH}_{2}\)); \(\delta\text{C}\) (100 MHz, DMSO-\(\text{d}_{6}\)), 166.2 (C=O), 164.4 (C=O), 151.16 (C), 133.8 (CH), 132.2 (CH), 131.7 (CH), 130.8 (CH), 130.4 (C), 128.8 (C), 126.4 (CH), 124.9 (C), 124.8 (C), 59.8 (\(\text{CH}_{2}\)), 44.37 (\(\text{CH}_{2}\)).

\(\nu_{\text{max}}\) (neat sample)/ cm\(^{-1}\): 3516, 3079, 2943, 1692, 1649, 1619, 1580, 1520, 1322, 1052, 828, 786.

\textbf{N-(2-Hydroxy ethyl)-4-amino-1,8-naphthalimide (85)}

Compound 85 was synthesised by catalytic hydrogenation of 86 (1.00 g, 3.494 mmol) in DMF using a Parr Hydrogen shaker apparatus at 3 atm pressure in the presence of 10% Pd/C catalyst until the hydrogen consumption ceased. The reaction mixture was filtered through a plug of celite and washed with DMF. The excess solvent was removed under reduced pressure to obtain the desired product as a green solid in 90% yield (0.812 g). m.p. 260-262°C; Found: C, 65.00; H, 4.49; N, 10.83%. \(\text{C}_{14}\text{H}_{12}\text{N}_{2}\text{O}_{3}\cdot0.08\cdot\text{H}_{2}\text{O}\) requires C, 65.25; H, 4.76; N, 10.87%; HRMS: 279.0748 ([M + Na]\textsuperscript{+}. \(\text{C}_{14}\text{H}_{12}\text{N}_{2}\text{O}_{3}\text{Na}\) requires 279.0746); \(\delta\text{H}\) (400 MHz, DMSO-\(\text{d}_{6}\)), 8.63 (1H, d, \(J = 8.5\) Hz, Ar-H7), 8.43 (1H, d, \(J = 7.5\) Hz, Ar-H2), 8.20 (1H, d, \(J = 8.5\) Hz, Ar-H5), 7.65 (1H, dd, \(J = 7.5\) Hz and 8.0 Hz, Ar-H6), 7.44 (2H, s, NH\(_2\)), 6.85 (1H, d, \(J = 8.0\) Hz, Ar-H3), 4.80 (1H, t, \(J = 6.0\) Hz, OH), 4.11 (2H, t, \(J = 6.5\) Hz, \(\text{CH}_{2}\)), 3.57 (2H, q, \(J = 6.5\) Hz, \(\text{CH}_{2}\)); \(\delta\text{C}\) (100 MHz,
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DMSO-d$_6$, 163.9 (C=O), 163.0 (C=O), 152.6 (C), 133.9 (CH), 130.9 (CH), 129.7 (C), 129.2 (CH), 123.9 (CH), 121.8 (C), 119.3 (C), 108.1 (CH), 107.6 (C), 57.9 (CH$_2$), 41.3 (CH$_2$). $\nu_{\text{max}}$ (neat sample)/ cm$^{-1}$: 3438, 3349, 3251, 2925, 2896, 1668, 1630, 1608, 1587, 1379, 1326, 1247, 1050, 773.

2-(N-Pyridinium-ethyl)-4-amino-1,8-naphthalimide (82)

A mixture of 85 (0.8 g, 3.12 mmol) and $p$-toluene sulfonyl chloride (0.65 g, 3.91 mmol) were stirred in pyridine at 60°C for 20 hr and then refluxed for 4 days. The resulting precipitate was filtered and washed with CH$_2$Cl$_2$ followed by purification using silica flash column chromatography using CH$_3$CN:H$_2$O:NaCl (sat.) (88:11:1). The product was precipitated as PF$_6^-$ salt using a concentrated aqueous solution of ammonium hexafluorophosphate. The solid was dissolved in minimum amount of MeOH and treated with DOWEX-1×8-200 ion exchange resin to convert the product into its chloride form. The product was obtained as an orange-yellow solid after removal of excess MeOH under reduced pressure (0.60 g, 60%). m.p. degraded above 250°C; Found: C, 57.37; H, 4.35; N, 10.70%. C$_{19}$H$_{16}$ClN$_2$O$_3$$\cdot$0.5 NaCl $\cdot$ 0.8 H$_2$O requires C, 57.42; H, 4.46; N, 10.57%; HRMS: 319.1233 ([M$^+$]. C$_{19}$H$_{16}$N$_2$O$_3$ requires 319.1243); $\delta_{\text{H}}$ (600 MHz, DMSO-d$_6$), 9.13 (2H, d, $J$ = 6.0 Hz, Py-H15), 8.67 (1H, d, $J$ = 8.2 Hz, Ar-H5), 8.59 (1H t, $J$ = 6.0 Hz, Py-H17), 8.33 (1H, d, $J$ = 8.0 Hz, Ar-H7), 8.07 (2H, t, $J$ = 6.0 Hz, Py-H16), 8.07 (1H, d, $J$ = 8.0 Hz, Ar-H2), 7.63 (1H, t, $J$ = 8.0, Ar-H6), 7.59 (2H, s, NH$_2$), 6.83 (1H, d, $J$ = 8.6 Hz, Ar-H3), 4.94 (2H, t, $J$ = 4.9 Hz, CH$_2$), 4.57 (2H, t, $J$ = 4.5 Hz, CH$_2$); $\delta_C$ (150 MHz, DMSO-d$_6$), 164.4 (C=O), 163.3 (C=O), 153.5 (C), 146.2 (CH), 145.7 (CH), 134.5 (CH), 131.6 (CH), 130.3 (C), 130.0 (CH), 128.2 (CH), 124.3 (CH), 121.6 (C), 119.7 (C), 108.6 (CH), 107.0 (C), 60.2 (CH$_2$), 40.6 (CH$_2$). $\nu_{\text{max}}$(neat sample)/ cm$^{-1}$: 3375, 3136, 2920, 1680, 1632, 1576, 1638, 1254, 1028, 782, 756.
N-[2-(Pyridin-4-yl)ethyl]-4-nitro-1,8-naphthalimide (90)

4-Nitro-1,8-naphthalic anhydride (1.674 g, 6.88 mmol) was placed into a round bottom flask along with molecular sieves. To this 25 mL of anhydrous toluene was added using cannula, followed by the addition of 4-(2-aminoethyl)pyridine (0.83 mL, 6.88 mmol) and triethylamine (1.92 mL, 13.77 mmol) were added and the reaction mixture was stirred at reflux for 48 hrs under argon atmosphere. Upon completion, the mixture was filtered through celite while still hot and washed with toluene. The filtrate was removed under reduced pressure and the resultant brown solid was dissolved in CH₂Cl₂, washed once with saturated solution of NaHCO₃, followed by washing with water and brine, respectively. The organic layer was dried over MgSO₄ and excess DCM was removed under reduced pressure to give 90 as a shiny brown solid in 47% yield (1.13 g) after recrystallisation from ethanol. m.p. 163-163.8°C; Found: C, 62.81; H, 4.02; N, 11.32%. C₁₉H₁₃N₅O₄·0.9·H₂O requires C, 62.77; H, 4.10; N, 11.56%; HRMS Found 348.0980 ([M + H]⁺, C₁₉H₁₄N₅O₄ requires 348.0984). δH (400 MHz, CDCl₃), 8.84 (1H, d, J = 8.0 Hz, Ar-H₅), 8.72 (1H, d, J = 8.0 Hz, Ar-H₇), 8.67 (1H, d, J = 8.0 Hz, Ar-H₂), 8.52 (2H, d, J = 5.6 Hz, Py-H₁₆), 8.40 (1H, d, J = 8.0 Hz, Ar-H₃), 7.99 (t, 1H, J = 8.0 Hz, Ar-H₆), 7.28 (2H, d, J = 5.4 Hz, Py-H₁₇), 4.44 (2H, t, J = 8.0 Hz, CH₂), 3.06 (2H, t, J = 8.0 Hz, CH₂), δC (100 MHz, CDCl₃), 163.2 (C=O), 162.4 (C=O), 149.7 (C), 149.6 (CH), 147.6 (C), 132.6 (CH), 130.0 (CH), 129.8 (CH), 129.6 (CH), 129.1(C), 126.6 (C), 124.4 (CH), 123.9 (CH), 123.7 (C), 122.7 (C), 40.8 (CH₂), 33.8 (CH₂); vₘₐₓ (neat sample)/ cm⁻¹: 2938, 2852, 1702, 1657, 1625, 1595, 1520, 1438, 1410, 1338, 1272, 1233, 1007, 836, 784, 759.

N-[2-(Pyridin-4-yl)ethyl]-4-amino-1,8-naphthalimide (89)

Compound 90 (0.65 g, 1.87 mmol) was heated with 10% Pd/C in ethanol at 70°C for one hr. To this solution, an excess of hydrazine monohydrate (0.9 mL, 18.55 mmol) was added drop-wise. The colour of the reaction mixture changed from black to green within couple of minutes and the mixture was heated for further 6 hrs, filtered through celite while hot and washed with ethanol. The combined solvents were removed under reduced pressure and 89 was obtained as a bright yellow solid in 94% yield (0.56 g). m.p. decomposed above 289°C; HRMS: Found 318.1242 ([M + H]⁺, C₁₉H₁₆N₃O₂ requires 318.1243). δH (400 MHz, DMSO-d₆), 8.63 (1H, d, J = 8.0 Hz, Ar-H₅), 8.46 (2H, d, J = 5.5 Hz, Py-H₁₆), 8.42 (1H, d, J = 4.0 Hz, Ar-H₇), 8.19 (1H, d, J
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\[ \delta_c (100 \text{ MHz}, \text{DMSO-d}_6): 164.1 (C=O), 163.2 (C=O), 153.3 (C), 150.0 (CH), 148.3 (C), 134.5 (CH), 131.5 (CH), 130.2 (C), 129.9 (CH), 124.6 (CH), 124.5 (CH), 122.1 (C), 119.8 (C), 108.7 (CH), 107.8 (C), 39.9 (CH\_2), 33.4 (CH\_2). \]

\[ \nu_{\text{max}} \text{ (neat sample)/ cm}^{-1}: 3370, 2923, 2852, 1670, 1632, 1566, 1525, 1481, 1432, 1363, 1350, 1307, 1245, 1131, 826, 774, 759. \]

\[ \text{N-[2-(Methylpyridin-1-ium)ethyl]-4-amino-1,8-naphthalimide (83)} \]

Compound 90 (0.42 g, 1.33 mmol) was suspended in anhydrous acetone and CH\_3I (0.83 mL, 13.33 mmol) was added and the reaction mixture was then heated at reflux for 48 hrs. Upon completion, the resulting precipitate was collected by filtration and washed with CH\_2Cl\_2. The crude product was converted into its PF\_6 salt and purified using silica flash chromatography using CH\_3CN:H\_2O:NaCl (saturated) (88:11:1). The product was precipitated as its PF\_6 salt using a concentrated aqueous solution of ammonium hexafluorophosphate and finally converted to the Cl\^- salt using amberlite IRA 400 (Cl) ion exchange resin in methanol. Excess methanol was removed under reduced pressure and the product was obtained as a bright yellow solid in 93% yield (0.455 g). m.p. decomposed above 255°C; Found: C, 61.78; H, 4.68; N, 10.46%. C\_{20}H\_{18}ClN\_3O\_2ClNa requires C, 61.93; H, 4.84; N, 10.73%; HRMS: Found 333.1474 ([M + H]\^+), C\_{20}H\_{19}N\_3O\_2 requires 333.1477. \delta_d (600 \text{ MHz}, \text{DMSO-d}_6), 8.85 (2H, d, J = 6.0 Hz, Py-H17), 8.66 (1H, d, J = 8.6 Hz, Ar-H5), 8.41 (1H, d, J = 8.0 Hz, Ar-H7), 8.17 (1H, d, J = 8.4 Hz, Ar-H2), 8.04 (2H, d, J = 6.0 Hz, Py-H16), 7.66 (1H, t, J = 8.0 Hz, Ar-H6), 7.55 (2H, s, NH\_2), 6.87 (1H, d, J = 8.5 Hz, Ar-H3), 4.39 (2H, t, J = 7.0 Hz, CH\_2), 4.30 (3H, s, CH\_3), 3.27 (2H, t, J = 7.0 Hz, CH\_2). \delta_c (150 \text{ MHz}), 164.3 (C=O), 163.3 (C=O), 159.5 (C), 153.5 (C), 145.2 (CH), 134.6 (CH), 131.6 (CH), 130.3 (C), 130.2 (CH), 128.3 (CH), 124.5 (CH), 121.9 (C), 119.8 (C), 108.7 (CH), 107.5 (C), 47.7 (CH\_3), 39.2 (CH\_2), 34.2 (CH\_2). \nu_{\text{max}} \text{ (neat sample)/ cm}^{-1}: 3340, 2932, 2852, 1685, 1669, 1626, 1577, 1526, 1474, 1384, 1343, 1253, 1130, 841, 775, 757.

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4-N,N'-Dimethylamino-1,8-naphthalimide (92)\(^{192}\)

Dimethyamine (5 mL, 40% aqueous solution, excess) and CuSO\(_4\)-5H\(_2\)O (0.12 g, 0.48 mmol) were added to a suspension of 4-bromo-1,8-naphthalic anhydride (2.5 g, 9.00 mmol) in DMF (30 mL). The reaction mixture was stirred under reflux condition for 12 hrs under argon atmosphere. The solvent was removed under reduced pressure and the resulting yellow solid was purified by recrystallisation from hot methanol to yield the desired product as a bright yellow solid in 78% yield (1.70 g). m.p. (203-204)\(^\circ\)C (Ref.,\(^{278}\) 206\(^\circ\)C); Found: C, 68.63; H, 4.38; N, 5.89%. C\(_{14}\)H\(_{11}\)NO\(_3\) O.2 H\(_2\)O requires C, 68.68; H, 4.69; N, 5.72%; HRMS: Found 242.0829 ([M + H]\(^+\), C\(_{14}\)H\(_{12}\)NO\(_3\) requires 242.0817; \(\delta_H\) (400 MHz, CDCl\(_3\)), 8.54 (1H, d, \(J = 8.0\) Hz, Ar-H7), 8.50 (1H, d, \(J = 8.0\) Hz, Ar-H5), 8.42 (1H, d, \(J = 8.0\) Hz, Ar-H2), 7.68 (1H, t, \(J = 8.0\) Hz, Ar-H6), 7.10 (1H, d, \(J = 8.0\) Hz, Ar-H3), 3.21 (s, 6H, N(CH\(_3\))\(_2\)).

\(\nu_{\text{max}}\) (neat sample)/cm\(^{-1}\): 1753, 1720, 1582, 1568, 1522, 1495, 1393, 1339, 1307, 1186, 1117, 997, 927, 774, 747.

N-[2-(Pyridin-4-yl)ethyl]-4-N,N'-dimethylamino-1,8-naphthalimide (91)

4-(2-Aminoethyl)pyridine (0.70 mL, 5.87 mmol) and triethylamine (1.20 mL, 8.80 mmol) were added to the suspension of 92 (1.03g, 4.27 mmol) in anhydrous toluene and the mixture was stirred under reflux for 3 days under an argon atmosphere. The resulting mixture was filtered through celite while still hot and washed with toluene. The solvent was removed under reduced pressure and the resultant solid was dissolved in CH\(_2\)Cl\(_2\), washed once with saturated solution of NaHCO\(_3\), followed by washing with water and brine, respectively. The organic layer was dried over MgSO\(_4\) and the solvent was removed under reduced pressure. The resulting solid was purified by recrystallisation from hot methanol to yield the product as a bright yellow solid in 56% yield (0.83 g). m.p. (157.5-158)\(^\circ\)C; Found: C, 72.79; H, 5.43; N, 12.09%. C\(_{24}\)H\(_{21}\)N\(_3\)O\(_2\)-0.1 H\(_2\)O requires C, 72.65; H, 5.57; N, 12.10%; HRMS: Found 346.1551 ([M + H]\(^+\), C\(_{24}\)H\(_{20}\)N\(_3\)O\(_2\) requires 346.1551). \(\delta_H\) (400 MHz, CDCl\(_3\)), 8.57 (1H, d, \(J = 8.0\) Hz, Ar-H7), 8.53(2H, d, \(J = 4.0\) Hz, Py-H16), 8.48 (1H, d, \(J = 8.0\) Hz, Ar-H2), 8.46 (1H, d, \(J = 8.0\) Hz, Ar-H5), 7.68 (1H, t, \(J = 8.0\) Hz, Ar-H6), 7.31 (2H, d, \(J = 4.0\) Hz, Py-H17), 7.13 (1H, t, \(J = 8\) Hz, Ar-H3), 4.43 (2H, t, \(J = 8\) Hz, CH\(_2\)), 3.14 (s, 6H, N(CH\(_3\))\(_2\)), 3.06 (2H, t, \(J = 8\) Hz, CH\(_2\)). \(\delta_C\) (100 MHz):
164.5 (C=O), 163.9 (C=O), 157.2 (C), 149.6 (CH), 148.2 (C), 132.8 (CH), 131.4 (CH), 131.1 (CH), 130.3 (C), 125.3 (C), 124.9 (CH), 124.5 (CH), 122.8 (C), 114.5 (C), 113.3 (CH), 44.8 (CH3), 40.3 (CHj), 33.6 (CHj). \( \nu_{\text{max}} \) (neat sample)/ cm\(^{-1}\): 2965, 2843, 1685, 1645, 1583, 1569, 1560, 1415, 1383, 1349, 1267, 1241, 1022, 838, 756.

**\( N-[2-(\text{Methylpyridin-1-ium})\text{ethyl}]-4-N, N'\text{dimethylamino}-1,8-naphthalimide (84)\)**

Compound 91 (0.16 g, 0.46 mmol) was suspended in anhydrous acetone and CH\(_3\)I (0.30 mL, 4.82 mmol) was added and the reaction mixture was heated at reflux for 48 hrs. After completion of the reaction, the precipitate was collected by filtration, dissolved in DCM and purified using silica flash chromatography using DCM: Methanol (85:15) The iodide salt of 91 was obtained as an orange solid in 40% (0.09 g) yield after removal of excess solvent under reduced pressure and converted to the Cl salt using amberlite IRA 400 (Cl) ion exchange resin in methanol. m.p. (203.7-204)°C; Found: C, 61.73; H, 6.09; N, 9.69%. \( \text{C}_{22}\text{H}_{22}\text{ClN}_{3}\text{O}_{2} \cdot 1.8\cdot \text{H}_{2}\text{O} \) requires C, 61.69; H, 6.02; N, 9.81%; HRMS: Found 361.1794 ([M + H]\(^+\), \( \text{C}_{22}\text{H}_{23}\text{N}_{3}\text{O}_{2} \) requires 361.1790); \( \delta_{\text{H}} \) (600 MHz, DMSO-\( d_6 \)), 8.87 (2H, d, \( J = 6.0 \) Hz, Py-H17), 8.54 (1H, d, \( J = 8.5 \) Hz, Ar-H5), 8.45 (1H, d, \( J = 8.0 \) Hz, Ar-H7), 8.32 (1H, d, \( J = 8.5 \) Hz, Ar-H2), 8.07 (2H, d, \( J = 6.0 \) Hz, Py-H16), 7.77 (1H, t, \( J = 8.0 \) Hz, Ar-H6), 7.22 (1H, d, \( J = 8.0 \) Hz, Ar-H3), 4.41 (2H, t, \( J = 7.0 \) Hz, CH\(_2\)), 4.27 (3H, s, CH\(_3\)), 3.28 (2H, t, \( J = 7.0 \) Hz, CH\(_2\)), 3.10 (6H, s, N(CH\(_3\))\(_2\)). \( \delta_{\text{C}} \) (150 MHz), 164.0 (C=O), 163.2 (C=O), 159.2 (C), 156.9 (C), 144.8 (CH), 132.7 (CH), 132.0 (CH), 130.9 (CH), 130.0 (C), 128.0 (CH), 125.2 (CH), 124.2 (C), 122.2 (C), 113.0 (CH), 112.9 (C), 47.4 (CH\(_3\)), 44.5 (N(CH\(_3\))\(_2\)), 39.3 (CH\(_2\)), 33.6 (CH\(_2\)). \( \nu_{\text{max}} \) (neat sample)/ cm\(^{-1}\): 2913, 2834, 1693, 1638, 1589, 1574, 1518, 1449, 1395, 1371, 1347, 1264, 1234, 1124, 836, 779, 755.
Chapter 6: Experimental

Bis-\{[2-(N-Pyridinium)-ethyl]\}-9,18-methano-1,8-naphthalimido[\textit{b,f}][1,5]-diazocine (87)

Compound 82 (0.303 g, 0.856 mmol) and paraformaldehyde (0.057 g, 1.89 mmol) were stirred in trifluoroacetic acid (TFA) (6 mL) at 20°C for 12 hrs under an argon atmosphere. Excess TFA was removed under reduced pressure in the presence of an excess of CH$_2$Cl$_2$. The resulting yellow powder was dissolved in CH$_3$CN and purified on silica gel using a mixture of CH$_3$CN:H$_2$O:NaNO$_3$ saturated (88:10:2) as the eluent. The product was precipitated as its PF$_6^-$ salt using ammonium hexafluorophosphate. The PF$_6^-$ salt was dissolved in minimum amount of MeOH and treated with Amberlite IRA 400 (Cl) ion exchange resin to convert the product into the chloride form. The product was obtained as an yellow solid after removal of MeOH under reduced pressure in 57% yield (0.18 g) m.p. decomposed above 235°C; HRMS (MALDI): Found 707.2169 ([M+Cl]$^+$, C$_{41}$H$_{32}$N$_6$O$_4$Cl requires 707.2174); $\delta_{H}$ (600 MHz, DMSO-$d_6$): 9.15 (4H, d, $J$ = 6 Hz, Py-H16, Py-H16'), 8.73 (2H, d, $J$ = 8.4 Hz, Ar-H5, Ar-H5'), 8.57 (2H, t, $J$ = 7.9 Hz, Py-H17, Py-H17'), 8.40 (2H, d, $J$ = 8.0 Hz, Ar-H7, Ar-H7'), 8.95 (4H, d, $J$ = 6.0 Hz, Py-H16, Py-H16'), 8.00 (2H, s, Ar-H2, Ar-H2'), 7.96 (2H, t, $J$ = 8.0 Hz, Ar-H6, Ar-H6'), 5.16 (2H, d, $J$ = 17.5 Hz, H$_a$), 4.95 and 4.92 (4H, dt, $J$ = 14.0 Hz and $J$ = 7.5 Hz, CH$_2$, H14, H14'), 4.71 (2H, s, H$_c$), 4.62 (2H, d, $J$ = 17.5 Hz, H$_b$), 4.55 (4H, t, $J$ = 7.0 Hz, CH$_2$, H13, H13'). $\delta_{C}$ (150 MHz), 163.7 (C=O), 163.1 (C=O), 149.3 (C), 145.9 (CH), 145.4 (CH), 130.7 (CH), 130.4 (CH), 129.4 (CH), 127.8 (CH), 127.6 (C), 127.2 (CH), 126.7 (C), 126.1 (C), 122.2 (C), 117.3 (C), 65.9 (CH$_2$), 59.6 (CH$_2$), 56.7 (CH$_2$), 40.7 (CH$_2$). $\nu_{\text{max}}$ (neat sample)/ cm$^{-1}$: 3374, 1694, 1653, 1595, 1570, 1489, 1459, 1402, 1374, 1354, 1340, 1302, 1258, 1236, 1169, 925, 784.

Bis-\{[N-(2-(methylpyridin-1-ium)-ethyl)]-9,18-methano-1,8-
naphthalimido[\textit{b,f}][1,5]-diazocine (88)

Compound 83-PF$_6^-$ salt (0.3014 g, 0.631 mmols) and paraformaldehyde (0.054 g, 1.79 mmol) were suspended in TFA and stirred at 20°C for 12 hrs under an argon atmosphere. The excess TFA was then
removed under reduced pressure in the presence of an excess of DCM. The resulting yellow powder was dissolved in water and purified on silica gel using a mixture of CH$_3$CN:H$_2$O:NaNO$_3$ saturated (80:18:2) as the eluent. The product was precipitated as PF$_6^-$ salt using ammonium hexafluorophosphate. The PF$_6^-$ salt was dissolved in minimum amount of MeOH and treated with Amberlite IRA 400 (Cl) ion exchange resin to convert the product into the chloride form. The product was obtained as an yellow solid in 53 % yield (0.13 g) after removal of excess MeOH under reduced pressure. m.p. decomposed above 175°C; HRMS (MALDI): Found 700.2794 ([M]$^+$, C$_{43}$H$_{36}$N$_6$O$_4$ requires 700.2798); $\delta_{HH}$ (600 MHz, CD$_3$CN), 8.73 (2H, d, $J = 8.3$ Hz, Ar-H5, Ar-H5'), 8.47 (2H, d, $J = 8.0$ Hz, Ar-H7, Ar-H7'), 8.46 (4H, d, $J = 6.0$ Hz, Py-H17, Py-17'), 8.03 (2H, s, Ar-H2, Ar-H2'), 7.90 (4H, d, $J = 6$ Hz, Py-H16, H16'), 7.89 (2H, t, $J = 8.2$ Hz, Ar-H6, Ar-H6'), 5.14 (2H, d, $J = 17.4$ Hz, H$_a$), 4.67 (2H, s, H$_c$), 4.38 and 4.36 (4H, dt, $J = 17.4$ Hz and $J = 7.0$ Hz, Py-H14, H14'), 4.23 (6H, s, CH$_3$). $\delta_C$(150 MHz): 164.9 (C=O), 164.3 (C=O), 160.9 (C), 150.5 (C), 145.4 (CH), 131.5 (CH), 131.4 (CH), 130.3 (CH), 129.3 (CH), 128.9 (C), 128.3 (C), 128.0 (CH), 127.0 (C), 123.7 (C), 119.2 (C), 67.2 (CH$_2$), 57.7 (CH$_2$), 48.6 (CH$_3$), 40.2 (CH$_2$), 34.6 (CH$_3$). $\nu_{max}$(neat sample)/ cm$^{-1}$: 3376, 1692, 1647, 1595, 1571, 1459, 1402, 1372, 1339, 1302, 1257, 1231, 1187, 920, 786.

(2,2':6',2"'-Terpyridine)platinuni(II) chloride complex (110)$^{254}$

Potassium tetrachloroplatinate (K$_2$PtCl$_4$) (0.93 g, 2.24 mmol) was dissolved in minimum volume of water. To this 1 ml of dimethyl sulfoxide (DMSO) was added followed by the addition of 2,2',6',2"'-terpyridine (0.52 g, 2.24 mmol) and heated under refluxing condition at 110°C till the solution became clear. The solution was cooled to room temperature and acidified using concentrated HCl. The desired product was obtained as an orange-red precipitate in 89% yield (1 g). m.p. > 300°C; HRMS: Found 463.0292 ([M]$^+$, C$_{15}$H$_{11}$N$_3$ClPt requires 463.0289); $\delta_{HH}$(600 MHz, D$_2$O), 8.01 (1H, t, $J = 8.0$ Hz, Ar-H8), 7.98 (2H, t, $J = 8.0$ Hz, Ar-H3, Ar-H13), 7.75 (2H, d, $J = 8.0$ Hz, Ar-H4, Ar-H12), 7.75 (2H, d, $J = 8.0$ Hz, Ar-H7, Ar-H9), 7.62 (2H, d, $J = 4.0$ Hz, Ar-H1, Ar-H15), 7.25 (2H, t, $J = 8.0$ Hz, Ar-H2, Ar-H14), $\delta_C$(150 MHz), 157.0 (C), 153.4 (C), 150.4 (CH), 142.6 (CH), 142.3 (CH), 129.1(CH), 125.3(CH), 124.1 (CH). $\nu_{max}$(neat sample)/ cm$^{-1}$: 3311, 1605, 1475, 1452, 1439, 1401, 1315, 1285, 1031, 779, 722.
2,2':6',2''-Terpyridine(N-[2-(pyridin-4-yl)ethyl]-4-N,N-dimethylamino-1,8-naphthalimide)platinum(II) nitrate complex (109)

A solution of AgNO₃ in DMF (0.26 gm, 1.53 mmol) was added gradually to a suspension of 110 (0.40 g, 0.75 mmol) in DMF and the mixture was stirred at room temperature in the dark for 24 hrs. The resulting mixture was then filtered through celite to remove AgCl. To the resulting orange solution, a solution of 91 (0.26 gm, 0.75 mmol) in DMF was added and stirred at room temperature for 2-3 hrs. The product was precipitated from diethyl ether and obtained as a red powder after purification by trituration with methanol at room temperature in 82% yield (0.55 g). m.p. (278-279)°C; Found: C, 45.23; H, 2.97; N, 11.53%. C₃₆H₃₀N₈O₈·0.4·AgCl requires C, 45.27; H, 3.17; N, 11.73%; HRMS (ESI): Found 386.6043 (M²⁺, C₃₆H₃₀N₈O₈Pt requires 386.6039); δH (600 MHz, CD₃OD), 9.03 (2H, d, J = 6.0 Hz, Py-H17), 8.595 (1H, t, J = 8.0 Hz, Tpy-H8'), 8.59 (2H, d, J = 8.0 Hz, Tpy-H4', Tpy-H12'), 8.57 (1H, d, J = 8.0 Hz, Nap-H5), 8.567 (2H, t, J = 8 Hz, Tpy-H2', Tpy-H14'), 8.56 (2H, d, J = 8.0 Hz, Tpy-H7', Tpy-H9'), 8.518 (1H, d, J = 8.0 Hz, Nap-H7), 8.505 (2H, t, J = 8.0 Hz, Tpy-H3', Tpy-H13'), 8.41 (1H, d, J = 8 Hz, Nap-H2), 7.869 (2H, d, J = 4.0 Hz, Tpy-H1', Tpy-H15'), 7.866 (2H, d, J = 6.0 Hz, Py-H16), 7.72 (1H, t, J = 8.0 Hz, Nap-H6), 7.21 (1H, d, J = 8.0 Hz, Nap-H3), 4.57 (2H, t, J = 7.0 Hz, CH₂), 3.36 (2H, t, J = 7.0 Hz, CH₂), 3.14 (6H, s, N(CH₃)₂), δC (150 MHz), 165.1 (C=O), 164.6 (C=O), 158.9 (C), 158.2 (C), 156.6 (C),155.9 (C), 152.1 (CH), 151.6 (CH), 143.9 (CH), 143.5 (CH), 133.1 (CH), 132.5 (CH), 131.3 (CH), 130.7 (C), 129.3 (CH), 126.2 (CH), 125.3 (C), 124.9 (CH), 124.4 (CH), 122.7 (C), 113.6 (C), 113.3 (CH), 44.0 (CH₂), 40.3 (CH₂), 34.1 (N(CH₃)₂). νmax (neat sample)/ cm⁻¹: 3075, 1683, 1640, 1566, 1450, 1328, 1251, 827, 781.
A solution of AgNO$_3$ in DMF (0.064 gm, 0.376 mmol) was added gradually to a suspension of 110 (0.1 g, 0.188 mmol) in DMF and the mixture was stirred at room temperature in the dark for 24 hrs. The resulting mixture was then filtered through celite to remove the AgCl. To the resulting orange solution, 4-picoline (18.3 µl, 0.188 mmol) was added and stirred at room temperature for 2-3 hours. The product was precipitated from diethyl ether as a pink solid in 41% yield (0.05 g). m.p. $>$300°C (Ref. $^{249}$ >220°C); HRMS (MALDI): Found 521.1180 (M$^{2+}$, C$_{21}$H$_{14}$N$_{4}$Pt requires 521.1179); $\delta$$_H$ (600 MHz, CD$_3$OD), 9.06 (2H, d, $J$ = 6.0 Hz, Py-H2), 8.612 (2H, t, $J$ = 8.0 Hz, Tpy-H2', Tpy-H14'), 8.607 (1H, t, $J$ = 8.0 Hz, Tpy-H8'), 8.595 (2H, d, $J$ = 8.0 Hz, Tpy-H7', Tpy-H9'), 8.51 (2H, t, $J$ = 8.0 Hz, Tpy-H3', Tpy-13'), 7.89 (2H, d, $J$ = 8.0 Hz, Tpy-H1', Tpy-H15'), 7.825 (2H, d, $J$ = 6.0 Hz, Py-H3), 7.815 (2H, d, $J$ = 8.0 Hz, Tpy-H4', Tpy-H12'), 2.71 (3H, s, CH$_3$). $\delta$$_C$ (150 MHz): 158.8 (C), 156.0 (C), 155.5 (C), 151.9 (CH), 151.7 (CH), 144.0 (CH), 143.3 (CH), 129.6 (CH), 129.5 (CH), 126.1 (CH), 124.3 (CH), 20.5 (CH$_3$). $\nu_{max}$ (neat sample)/ cm$^{-1}$: 3044, 1610, 1482, 1450, 1350, 1330, 1290, 1131, 825, 789.
Chapter 7

References
Chapter 7: References


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Chapter 7: References


Appendices
Binding Equilibria between Ligand and Macromolecules

The 1:1 association of a naphthalimide molecule (NI) with a mononucleotide (XMP) can be represented by the following equilibrium:

\[ \text{NI} + \text{XMP} \xleftrightarrow{K} \text{NI:XM} \]

The association constant \( K \) is defined as

\[ K = \frac{[\text{NI}:\text{XMP}]}{[\text{XMP}][\text{NI}]} \]

Where, \([\text{XMP}], [\text{NI}], [\text{NI}:\text{XMP}]\) are the concentrations of XMP, NI and NI:XM, respectively, at the equilibrium.

The absorbance of a mixture of XMP and NI at any wavelength, where XMP does not absorb is defined as:

\[ A_{\text{Total}} = A_{\text{NI}} + A_{\text{NI:XM}} \]
\[ = \varepsilon_f[\text{NI}] + \varepsilon_b[\text{NI:XM}] \]
\[ = \varepsilon_f([\text{NI}]_0 - [\text{NI}:\text{XMP}]) + \varepsilon_b[\text{NI:XM}] \]
\[ = \varepsilon_f[\text{NI}]_0 + (\varepsilon_b - \varepsilon_f)[\text{NI:XM}] \]

where, \([\text{NI}]_0\) is the total concentration of NI, \( \varepsilon_f \) and \( \varepsilon_b \) are the extinction coefficients of free NI and bound NI respectively.

The absorbance of NI in the absence of any XMP is given by

\[ A_0 = \varepsilon_f[\text{NI}]_0 \]

The change in absorbance of NI on formation of the complex is given as:

\[ \Delta A = A_0 - A_{\text{Total}} \]
\[ = \varepsilon_f[\text{NI}]_0 - \varepsilon_f[\text{NI}]_0 - (\varepsilon_b - \varepsilon_f)[\text{NI:XM}] \]
If we assume \([XMP] \gg [NI]\), then \([XMP] \approx [XMP]_0\), therefore, from equation I.1, the fraction of bound NI (\(\vartheta\)) can be defined as:

\[
\vartheta = \frac{[NI:XMP]}{[NI]_0} = \frac{K[XMP]_0}{1 + K[XMP]_0}
\]

Equation (I.5) represents the binding isotherm. \(\vartheta\) can be related to the measured optical densities by combining equation I.4 and I.5

\[
\vartheta = \frac{[NI:XMP]}{[NI]_0} = \frac{\Delta A}{\Delta \varepsilon [NI]_0}
\]

From equations I.5 and I.6,

\[
\frac{\Delta A}{[NI]_0} = \frac{K\Delta \varepsilon [XMP]_0}{1 + K[XMP]_0}
\]

Equation 1.7 was developed by Deranleau for the 1:1 binding equilibria of small molecules, where \(\Delta A/[NI]_0\) can be plotted versus \([XMP]_0\) to give a nonlinear absorption isotherm.\(^1\)

Rearrangement of equation I.7 gives:

\[
\frac{\Delta A}{[XMP]_0[NI]_0} = K\Delta \varepsilon - K\frac{\Delta A}{[NI]_0}
\]

Equation 1.8 is known as the Scatchard Plot. A plot of \(\Delta A/[XMP]_0[NI]_0\) vs. \(\Delta A/[NI]_0\) will give a straight line with slope of \(-K\) and y-intercept \(K\Delta \varepsilon\).\(^1\)\(^2\)

Equations I.7 and I.8 can be used to determine binding constants for 1:1 associations. However, for complexes with stoichiometries other than 1:1, these simple methods are inadequate and do not give satisfactory fits to the experimental data points.

In the case of higher order complex formation, if one naphthalimide molecule binds to two mononucleotides, the binding equilibria can be represented by the following scheme,
This model assumes that XMP can bind to two distinct sites on NI and $k_1$ and $k_2$ are the microscopic association constants for the formation of two microscopically distinct species NI:XMP(1) and NI:XMP(2), respectively.

The association constants of the reactions are given by

$$k_1 = \frac{[\text{NI}: \text{XMP}(1)]}{[\text{NI}][\text{XMP}]}$$

$$k_2 = \frac{[\text{NI}: \text{XMP}(2)]}{[\text{NI}][\text{XMP}]}$$

$$k_{12} = \frac{[\text{NI}: (\text{XMP})_2]}{[\text{NI}: \text{XMP}(1)][\text{XMP}]} \text{ and } k_{21} = \frac{[\text{NI}: (\text{XMP})_2]}{[\text{NI}: \text{XMP}(2)][\text{XMP}]}$$

Therefore, the absorbance of the solution at any point can be represented by

$$A = \varepsilon_f[\text{NI}] + \varepsilon(1)[\text{NI}: \text{XMP}(1)] + \varepsilon(2)[\text{NI}: \text{XMP}(2)] + \varepsilon(1,2)[\text{NI}: (\text{XMP})_2]$$

The total concentration of NI is given by,

$$[\text{NI}]_0 = [\text{NI}] + [\text{NI}: \text{XMP}(1)] + [\text{NI}: \text{XMP}(2)] + [\text{NI}: (\text{XMP})_2]$$

Therefore, the change in absorbance $w.r.t$ the free NI is given by

$$\Delta A = A_0 - A = \varepsilon_f\{[\text{NI}] + [\text{NI}: \text{XMP}(1)] + [\text{NI}: \text{XMP}(2)] + [\text{NI}: (\text{XMP})_2]\}$$
From equation 1.9, 1.10 and 1.11

\[ [\text{NI}:\text{XMP}(1)] = k_1[\text{NI}][\text{XMP}] \text{ and } [\text{NI}:\text{XMP}(2)] = k_2[\text{NI}][\text{XMP}] \]

\[ [\text{NI}:(\text{XMP})_2] = k_{12}[\text{NI}]:\text{XMP}(1)][\text{XMP}] = k_1 k_{12}[\text{NI}][\text{XMP}]^2 \]

\[ \frac{\Delta A}{[\text{NI}]_0} = \frac{(\varepsilon_f - \varepsilon(1))k_1 + (\varepsilon_f - \varepsilon(2))k_2}{1 + k_1 k_2 [\text{XMP}] + k_1 k_2 [\text{XMP}]^2} \]

If \( K_1 \) and \( K_2 \) represent the macroscopic association constant such that

\[ K_1 = k_1 + k_2 \text{ and } K_1 K_2 = k_1 k_2 \]

and if \([\text{XMP}] >> [\text{NI}]\) such that \([\text{XMP}] \approx [\text{XMP}]_0\), the expression for the binding equilibrium becomes

\[ \frac{\Delta A}{[\text{NI}]_0} = \frac{K_1 \Delta \varepsilon_1 [\text{XMP}] + K_1 K_2 \Delta \varepsilon_2 [\text{XMP}]^2}{1 + K_1 [\text{XMP}] + K_1 K_2 [\text{XMP}]^2} \]

where \( \varepsilon_1 \) and \( \varepsilon_2 \) represent the weighted average macroscopic extinction coefficients such that

\[ \varepsilon_1 = \frac{k_1 \varepsilon(1) + k_2 \varepsilon(2)}{k_1 + k_2} \text{ and } \varepsilon_2 = \varepsilon(1,2) \]

Equation 1.18 was developed by Deranleau for 1:2 stoichiometry of binding.\(^3\)

For the interaction of a macromolecule with ‘n’ number of ligand molecules, the Scatchard equation for such equilibrium can be obtained by considering noncooperative binding \( \text{i.e.} \) when each of the n sites on the polymer has same affinity for ligand and binding to any site is independent of whether the other sites are occupied or not.\(^2\)
Where, $K$ is the association constant, $C_f$ is the concentration of free ligand. ‘$n$’ is the number of ligand molecules bound per polymer residue. Here, $r$ is defined as

$$\frac{r}{C_f} = K(n - r)$$ \hspace{1cm} I.20

$$r = \frac{C_b}{[M]}$$ \hspace{1cm} I.21

$C_b$ and $M$ are the concentrations of bound ligand and macromolecule respectively.

A Scatchard plot of $r/C_f$ vs. $r$ is expected to give a straight line with slope of $-K$ and intercept $nK$. However, a number of factors can cause deviations from linearity. Scatchard equation was originally developed for the interaction of small ligands to isolated binding sites. This situation is rarely observed for biopolymers. Binding of ligand to nonequivalent sites having different association constants and/or cooperative binding would lead to curved Scatchard plot.

McGhee and von Hippel derived the expressions for the interaction between small molecules to macromolecule using statistical mechanics. According to their model, if a bound ligand covers two or more repeat units of a polymer, potential binding sites overlap. Therefore, at any extent of binding, the number of free binding sites will depend on the number of bound ligands as well as on the distribution of these ligands along the polymer. These considerations leads to a curved Scatchard plot.

For non-cooperative binding, the binding equation derived by McGhee and von Hippel is given by equation I.22

$$\frac{r}{C_f} = K (1 - nr) \left[ \frac{1 - nr}{1 - (n - 1)r} \right]^{n-1}$$ \hspace{1cm} I.22

where, ‘$n$’ is the number of polymer residues covered by the ligand.
The binding curve is constructed from the plot of \( r/C_f \) vs. \( r \). The binding parameters, \( K \) and ‘\( n \)’ can be determined from the non-linear fit of the experimental points using equation 1.22.

It should be noted from equation, when \( n = 1 \), the equation becomes Scatchard equation 1.20. For \( n \geq 2 \), the plot of \( r/C_f \) vs. \( r \) will always be non-linear, convex downward.

For binding of ligands to DNA, usually the interaction is studied over a broad range of DNA concentrations. However, the binding data at the two extremes of the binding curves are often associated with fluctuations due to systematic errors in the measurements and sensitivity of the spectrophotometer. Therefore, for the determination of binding constant, it is recommended to use the data for which, the concentration of bound ligand lies between 20%-80% of the total ligand concentration. Additionally, the binding site heterogeneity and polyelectrolyte nature of DNA can also result in poor fit of the data at very low or very high ‘\( r \)’ values.

The DNA binding data were also fitted to the model derived by Bard et al (equation A1.23). The spectral data over the entire range of DNA concentrations can be fitted to this model and moreover the model requires lesser manipulation of the data compared to that required for the Scatchard plot.

\[
\frac{\epsilon_a - \epsilon_f}{\epsilon_b - \epsilon_f} = \frac{b - (b^2 - \frac{2K_b^2C[DNA]}{n})^{1/2}}{2K_bC} \tag{1.23}
\]

\[
b = 1 + K_bC + K_b[DNA]/2n \tag{1.24}
\]

\( \epsilon_a, \epsilon_f \) and \( \epsilon_b \) correspond to the apparent extinction coefficient, extinction coefficient for the free ligand and extinction coefficient for the bound ligand, respectively. \( C \) is the total concentration of the ligand, \([DNA]\) is the concentration of DNA in terms of base pair, ‘\( n \)’ is number of nucleotides occupied by the bound ligand.
References Cited in Appendix I

**Table A2.1.** Crystal data and structure refinement for **82**.

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**Table A2.2.** Crystal data and structure refinement for **83**.

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<tr>
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α = 90°, β = 98.63(3)°, γ = 96.32(3)°.
### Table A2.3: Crystal data and structure refinement for 84.

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|                     | b = 9.2489(4) Å \(\beta = 89.039(2)^\circ\)
|                     | c = 14.9085(6) Å \(\gamma = 62.137(2)^\circ\) |
| Volume              | 1070.27(8) Å\(^3\) |
| Z                   | 2 |
| Density (calculated)| 1.568 Mg/m\(^3\) |
| Absorption coefficient | 1.866 mm\(^{-1}\) |
| F(000)              | 520 |
| Crystal size        | 0.24 x 0.11 x 0.04 mm\(^3\) |
| Theta range for data collection | 8.34 to 63.95° |
| Index ranges        | -10 <= h <= 9, -10 <= k <= 10, -17 <= l <= 17 |
| Reflections collected | 7357 |
| Independent reflections | 3360 [R(int) = 0.0301] |

| c = 14.245(3) Å \(\gamma = 110.67(3)^\circ\) |
| 932.9(3) Å\(^3\) |
Appendix II

Completeness to theta = 63.95° 95.1 %
Absorption correction Semi-empirical from equivalents
Max. and min. transmission 0.7526 and 0.5895
Refinement method Full-matrix least-squares on F^2
Data / restraints / parameters 3360 / 0 / 310
Goodness-of-fit on F^2 1.074
Final R indices [I>2sigma(I)] R1 = 0.0496, wR2 = 0.1445
R indices (all data) R1 = 0.0521, wR2 = 0.1468
Largest diff. peak and hole 0.595 and -0.358 e.Å^-3

Figure A2.1: $^1$H-NMR of 83 as its Cl salt (DMSO-d$_6$, 600 MHz).

Figure A2.2: Demonstration of out of plane orientation of pyridyl ring in the solid-state structure of 83 due to non-classical H-bonding interactions.
Appendix II

Figure A2.3: UV/vis absorption and fluorescence of 83 in various solvents.

Figure A2.4: Changes in the (a) UV/vis absorption spectra (b) Fluorescence spectra of 83 (6.9 μM) in the presence of increasing concentration of GMP (0-60 mM). (C) Fit of absorbance data to the Scatchard Model and (d) to the nonlinear Deranleau model.
Figure A2.5: Changes in the (a) UV/vis absorption spectra (b) Fluorescence spectra of 83 (6.9 μM) in the presence of increasing concentration of AMP (0-100 mM). (C) Fit of absorbance data to Scatchard Model and (d) to nonlinear Deranleau model.

Figure A2.6: Fluorescence spectra of 84 (7.9 μM) in the presence of increasing concentration of AMP (0-100 mM).
Figure A2.7: UV/vis absorption spectra of 83 (7.0 μM) in the presence of increasing concentration of st-DNA (0-428 μM in 10 mM phosphate buffer (pH 7.0). Inset: Plot of $r/C_f$ vs. $r$ and fit to the non-cooperative model of Mcghee and von Hippel.

Figure A2.8: (a) The UV/vis absorption of 83 (8.6 μM) bound to st-DNA (P/D = 40) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-450 mM), (b) effect of addition of NaCl (0-450 mM) on 83 (8.6 μM) in 10 mM phosphate buffer (c) The UV/vis absorption of 84 (8.6 μM) bound to st-DNA (P/D = 40) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-450 mM), (d) effect of addition of NaCl (0-450 mM) on 84 (8.6 μM) in 10 mM phosphate buffer. Inset b and d, Inset: relative changes in absorbance at $\lambda_{max}$ for 83 and 84 nm as a function of NaCl.
Figure A2.9: UV/vis absorption spectra of (a) 83 (7.0 μM) and (b) 84 (8.2 μM) in the presence of increasing concentration of st-DNA in 10 mM phosphate buffer (pH 7.0) + 100 mM NaCl. Inset: Plot of r/Cf vs. r and fit to the non-cooperative model of Mcghee and von Hippel.

Figure A2.10: Changes in steady-state emission spectra of 84 (8.2 μM) in presence of increasing concentration of st-DNA in 10 mM phosphate buffer + 100 mM NaCl.
Figure A2.11: (a) UV/vis absorption and (b) Steady-state fluorescence spectra of 83 (4.0 μM) in the presence of increasing concentration of poly(dG-dC)₂ (0–410 μM). (λ_ex = 435 nm). Inset a: Plot of r/C_f vs. r and fit to McGhee von Hippel model.

Figure A2.12: CD spectra of st-DNA (150 μM) in presence of varying concentration of (a) 83 and (b) 84.
Figure A2.13: LD spectra of st-DNA (400 μM) in presence of varying concentration of (a) 82 and (b) 84.
Figure A3.1: (a) fluorescence emission, $\lambda_{ex} = 380$ nm, (b) excitation spectra, $\lambda_{em} = 560$ nm of 88 (12 $\mu$M) in 10 mM phosphate buffer (pH 7.0). (Slits: 20 nm/10 nm for emission and 10 nm/20 nm for excitation spectra). PMT = 750 Volts.

Figure A3.2: Effect of solvent polarity on the excitation spectra of (a) 87 and (b) 88.

Figure A3.3: The plot of $r$ vs $C_f$ (■) and the fit (——) to non-cooperative model of McGhee and von Hippel for (a) (+)-87 and (b)(-)-87 in 10 mM phosphate buffer (pH 7.0).
Appendix III

Figure A3.4: The plot of \((\varepsilon_a - \varepsilon_b)/(\varepsilon_a - \varepsilon_b)_{\text{vs}}[\text{DNA bp}]\) (■) and the fit (—) to the Bard model for (a) (+)-87 and (b) (-)-87 in 10 mM phosphate buffer (pH 7.0).

Figure A3.5: The plot of \((\varepsilon_a - \varepsilon_b)/(\varepsilon_a - \varepsilon_b)\) vs. [DNA bp] (■) and the fit (—) to the Bard model (±)-88 in 10 mM phosphate buffer (pH 7.0).

Figure A3.6: The UV/vis absorption spectra of (a) (+)-87 (9.3 µM) and (b) (-)-87 (9.3 µM) (bound to st-DNA (P/D = 10) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-485 mM).
Figure A3.7: The UV/vis absorption spectra of (a) (+)-88 (9.0 μM) and (b) (-)-88 (9.2 μM) bound to st-DNA (P/D = 10) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-485 mM).

Figure A3.8: The UV/vis absorption spectra of 88 (9.0 μM) in 10 mM phosphate buffer (pH 7.0) upon increasing concentration of NaCl (0-485 mM). Inset: The plot of A/A₀ vs. Concentration of NaCl.
Appendix III

Figure A3.9: The UV/vis absorption spectra of (a) (+)-87 (9.5μM) and (b) (-)-87 (9.5μM) in the presence of increasing concentration of st-DNA (0-285μM) in 10 mM phosphate buffer containing 50 mM NaCl.

Figure A3.10: (a) The plot of $r$ vs $C_f$ (■) and the fit (—) to non-cooperative model of McGhee and von Hippel and (b) The plot of $(e_r - e_d)/(e_b - e_d)$ vs [DNA bp] (■) and the fit (—) to the Bard model for (±)-87 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Appendix III

Figure A3.11: The plot of $r$ vs $C_f$ (■) and the fit (—) to non-cooperative model of McGhee and von Hippel for (a) (+)-87 and (b) (-)-87 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

Figure A3.12: The plot of $(\varepsilon_{r+c})/(\varepsilon_{b+c})$ vs [DNA bp] (■) and the fit (—) to the Bard model for (a) (+)-87 and (b) (-)-87 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Figure A3.13: UV/vis absorption spectra of (a) (+)-87 (7.3 µM) and (b)(-)-87 (7.1 µM) in the presence of increasing concentration of st-DNA (0-730 µM) in 10 mM phosphate buffer containing 150 mM NaCl.

Figure A3.14: (a) The plot of r vs C_f (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel and (b) the plot of (c_{c-DNA}/(c_{c-DNA}) vs [DNA bp] (■) and the fit (—) to the Bard model for (±)-87 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0)
Figure A3.15: The plot of $r$ vs $C_f$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-87 and (b) (-)-87 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).

Figure A3.16: The plot of $(\varepsilon_{r-c} - \varepsilon_{b-c})/[(\varepsilon_{b-c})] vs [DNA bp]$ (■) and the fit (—) to the Bard model for (a)(+)-87 and (b) (-)-87 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).
Figure A3.17: The UV/vis absorption spectra of (a) (+)-88 (7.3 µM) and (b) (-)-88 (7.1 µM) in the presence of increasing concentration of st-DNA (0-730µM) in 10 mM phosphate buffer containing 50 mM NaCl.

Figure A3.18: The plot of \((\varepsilon_{\text{c}-\varepsilon_{\text{b}}})/(\varepsilon_{\text{c}-\varepsilon_{\text{e}}}})\) vs [DNA bp] (■) and the fit (—) to the Bard model for (±)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Figure A3.19: The plot of $r$ vs $C_f$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-88 and (b) (-)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

Figure A3.20: The plot of $(\varepsilon_{r-Cf})/(\varepsilon_{3-DNA})$ vs [DNA bp] (■) and the fit (—) to the Bard model for (a) (+)-88 and (b) (-)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Figure A3.21: The UV/vis absorption spectra of (a) (+)-88 (9.8 μM) and (b) (-)-88 (9.5 μM) in the presence of increasing concentration of st-DNA (0-295 μM) in 10 mM phosphate buffer containing 150 mM NaCl.

Figure A3.22: (a) The plot of r vs C_f (%) and the fit (—) to the non-cooperative model of McGhee and von Hippel and (b) The plot of (ε_r-ε)/(ε_r+ε) vs [DNA bp] (■) and the fit (—) to the Bard model for (±)-88 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).
Figure A3.23: The plot of $r$ vs $C_f$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-88 and (b) (-)-88 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).

Figure A3.24: The plot of $(\epsilon_{b,\Delta f})/(\epsilon_{b,\Delta f})$ vs [DNA bp] (■) and the fit (—) to the Bard model for (a) (+)-88 and (b) (-)-88 in 10 mM phosphate buffer containing 150 mM NaCl (pH 7.0).
Figure A3.25: The UV/vis absorption spectra of (a) (+)-87 (7.5 μM) and (b) (-)-87 (7.3 μM) in the presence of increasing concentration of poly(dA-dT)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl.

Figure A3.26: The plot of $r$ vs $C_f$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-87 and (b) (-)-87 with poly(dA-dT)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0)
**Figure A3.27:** The plot of \((e_{a-e_g})/(e_{b-e_g})\) vs [DNA bp] (■) and the fit (—) to the Bard model for (a) (+)-87 and (b) (-)-87 with poly(dA-dT)\(_2\) in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

**Figure A3.28:** The UV/vis absorption spectra of (a) (+)-88 (9.5 µM) and (b) (-)-88 (9.0 µM) in the presence of increasing concentration of poly(dA-dT)\(_2\) in 10 mM phosphate buffer containing 50 mM NaCl.
Figure A3.29: The plot of $r$ vs $C_f$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-88 and (b) (-)-88 with poly(dA-dT)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0), (c) The plot of $(e_a-e_f)/(e_b-e_f)$ vs [DNA bp] (■) and the fit (—) to the Bard model for (-)-88 with poly(dA-dT)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

Figure A3.30: The UV/vis absorption spectra of (a) (+)-87 (6.7 μM) and (b) (-)-87 (6.5 μM) in the presence of increasing concentration of poly(dG-dC)$_2$ in 10 mM phosphate buffer + 50 mM NaCl.
Figure A3.31: The plot of $r$ vs $C_I$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-87 and (b) (-)-87 with poly(dG-dC)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

Figure A3.32: The plot of $(\epsilon_{\infty} - \epsilon_I)/(\epsilon_{\infty} - \epsilon_s)$ vs [DNA bp] (■) and the fit (—) to the Bard model for (a) (+)-87 and (b) (-)-87 with poly(dG-dC)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Appendix III

Figure A3.33: The UV/vis absorption spectra of (a) (+)-88 (8.8 μM) and (b) (-)-88 (8.5 μM) in the presence of increasing concentration of poly(dG-dC)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl.

Figure A3.34: The plot of $r$ vs $C_r$ (■) and the fit (—) to the non-cooperative model of McGhee and von Hippel for (a) (+)-88 and (b) (-)-88 with poly(dG-dC)$_2$ in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Figure A3.35: The plot of \( \frac{(\varepsilon_{a}-\varepsilon_{d})}{(\varepsilon_{b}-\varepsilon_{d})} \) vs [DNA bp] (■) and the fit (---) to the Bard model for (a) (+)-88 and (b) (-)-88 with poly(dG-dC)\(_{2}\) in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).

Figure A3.36: The changes in the emission spectra of ethidium bromide (5 \( \mu M \)) bound to st-DNA (10 \( \mu M \)) in the presence of increasing concentration of (a) (+)-87 and (b) (-)-87 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Figure A3.37: (a) The changes in the emission spectra of ethidium bromide (5 μM) bound to st-DNA (10 μM) in the presence of increasing concentration of (±)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0). (b) Normalised fluorescence intensity of ethidium bromide at 605 nm upon addition of (±)-88 (■), (+)-88 (□), (-)-88 (▲), 83(▼).

Figure A3.38: The changes in the emission spectra of ethidium bromide (5 μM) bound to st-DNA (10 μM) in the presence of increasing concentration of (a) (+)-88 and (b) (-)-88 in 10 mM phosphate buffer containing 50 mM NaCl (pH 7.0).
Appendix III

**Figure A3.39:** The LD spectrum of st-DNA (400 μM) containing (+)-88 at P/D = 10 in 10 mM Phosphate buffer (pH 7.0) in the absence and in the presence of 100 mM NaCl.

**Figure A3.40:** The confocal images of HeLa cells treated with (+)-88 at various concentrations after 5 hrs and 48 hrs of incubation.
### Table A4.1: Crystal data and structure refinement for 91.

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**Table A4.2:** Crystal data and structure refinement for 109.

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Appendix IV

Figure A4.1: Excited state lifetime of (a) 109 and (b) 91 in various alcohols.

Figure A4.2: Plot of $r/C_f$ vs $r$ (■) and fit (−) to the non-cooperative model of McGhee and von Hippel for the titration of 109 with st-DNA in 10 mM phosphate buffer analysing the changes at (a) 350 nm and (b) 450 nm absorption bands.
**Figure A4.3:** Plot of \( r/C_f \) vs \( r \) (□) and fit (−) to the non-cooperative model of McGhee and von Hippel for the titration of 109 with st-DNA in 10 mM phosphate buffer containing 100 mM NaNO₃ analysing the changes at (a) 350 nm and (b) 450 nm absorption bands.

**Figure A4.4:** \(^1\)H NMR of 109 in D₂O (600 MHz) over a period of time.
Figure A5.1: The ps-TA spectra of 82 (10 mM) in the presence of AMP (20 mM) in 10 mM phosphate buffer.

Figure A5.2: The kinetics at (a) 440 nm and (b) 720 nm and (—) biexponential fit for 82 in the presence of 20 mM AMP.
Figure A5.3: The kinetics of ground state recovery of $\text{O}_2$ for various bands and biexponential fit obtained from the ps-TRIR measurements.

Figure A5.4: Kinetics of transient decay of $\text{O}_2$ for various bands obtained from the ps-TRIR measurements and biexponential fit.
Figure A5.5: Kinetics of ground state recovery of 82 in the presence of GMP for various bands and biexponential fit.

Figure A5.6: Kinetics of transient decay of 82 in the presence of GMP for various bands and biexponential fit.
**Figure A5.7**: ps-TRIR of 82 (10 mM) in the presence of 20 mM AMP.

**Figure A5.8**: Kinetics of ground state recovery of 82 in the presence of AMP for various bands and biexponential fit.
Figure A5.9: Kinetics of transient decay of 82 in the presence of AMP for various bands and biexponential fit.

Figure A5.10: The ps-TA of (a) 82 (8 μM) and (b) 82 (8 μM) in the presence of 20 mM GMP in phosphate buffer.
Figure A5.11: The ps-TA spectra of 87 (10 mM) in the presence of GMP (20 mM) and kinetics of various transient bands.
Figure A5.12: The kinetics of ground state recovery and transient decays for the various bands of 87 (10 mM) obtained from the ps-TRIR measurements.
Figure A5.13: The kinetics of ground state recovery and transient decays for the various bands of 87 (10 mM) in the presence of 10 mM GMP obtained from the ps-TRIR measurements.
Publication