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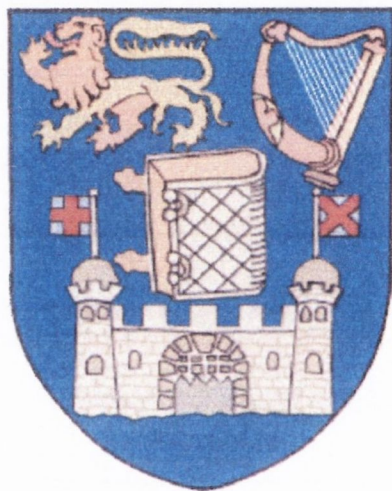
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The Synthesis, Photophysical and Biological Evaluation of Novel Bis(1,8-naphthalimides)

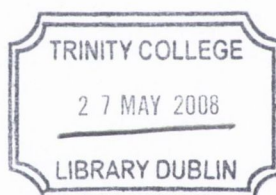
**By Lisa Jane Gillespie,
2006**



University of Dublin, Trinity College

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

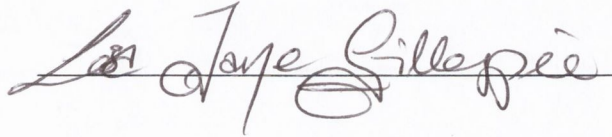
*A thesis submitted to the Department of Chemistry,
University of Dublin, Trinity College for the degree
of Doctor of Philosophy.*



THESIS
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Declaration

This thesis is submitted for the degree of Doctor of Philosophy to the University of Dublin, Trinity College and has not been submitted for any degree or examination to this or any other University. Other than where acknowledged, all work described herein is original and carried out by the author.

A handwritten signature in dark ink, reading "Lisa Jane Gillespie". The signature is written in a cursive style with a horizontal line drawn through the middle of the text.

Lisa Jane Gillespie

For Mum and Pops

"Quod Scripsi, Scripsi"

from John 19 : 22

*"It matters not how strait the gate,
How charged with punishments the scroll,
I am the master of my fate;
I am the captain of my soul."*

from 'Invictus' by W.E. Henley, 1849-1903

Abstract

This thesis is entitled “The Synthesis, Photophysical and Biological Evaluation of Novel Bis(1,8-naphthalimides)” and is divided into six chapters.

Chapter One provides a review of 1,8-naphthalimide research and, in particular, the exploration of such compounds as anti-cancer agents. Advances in anti-cancer therapeutics and targeting of DNA are also discussed. A description of the recent developments in bisnaphthalimide and naphthalene diimide structures as potential anti-tumour agents, DNA binders and DNA probes is provided. The background for this research project is discussed in detail. This project sought to design and create two novel families of bisnaphthalimides along with the necessary mono-peptide and dipeptide functionalised mononaphthalimides and spacer systems *en route*. Conventional and new synthetic ideas and syntheses would be employed. These systems are intended as DNA binders and potential anti-cancer agents. The design and synthetic plan for the development of these novel families of naphthalene diimide linked bisnaphthalimides is illustrated. A ‘tail-to-tail’ bisnaphthalimide family was planned by tethering two compounds *via* the 4-position through a naphthalene diimide spacer. A ‘head-to-head’ bisnaphthalimide family was designed by tethering two compounds *via* a peptide functionalisation possessing a free terminal amine through a naphthalene diimide spacer. All these bisnaphthalimides would be symmetrical. The aims of this project are discussed and include the achievement of some degree of sequence selectivity in DNA binding, the observation of the effect on binding of altering the length of a spacer, the enhancement of binding over that of mononaphthalimides and the demonstration of anti-cancer activity.

Chapter Two describes the initial syntheses of a panel of peptide functionalised mononaphthalimides and (amino)alkyl functionalised naphthalene diimide spacer systems. These compounds were then combined to provide three families of novel ‘tail-to-tail’ bisnaphthalimides – these employed PheAla, Cbz(Lys)Ala and LeuAla as the dipeptide functionalities and spacers with (amino)propyl, (amino)pentyl and (amino)hexyl arms. This provided nine novel bisnaphthalimides. Hydrolysis of two of the Cbz(Lys)Ala systems provided two more examples of these systems. The synthesis of five compounds incorporating mono-peptide functionalised naphthalimides is detailed. Alternative synthetic conditions and purification methods are discussed. The development of the ‘head-to-head’ bisnaphthalimide family by reacting lysine, ornithine and 2,4-diamino butyric acid functionalised 4-nitro-1,8-naphthalimides with 1,4,5,8-tetracarboxylic naphthalene diimide is also described. These three 4-nitro-1,8-naphthalimides were also incorporated into

bisnaphthalimides with dicarboxyl bridges. In total, the successful synthesis of seventeen novel bisnaphthalimides is illustrated.

Chapter Three details a series of photophysical investigations of these bisnaphthalimides that were undertaken. A comprehensive solvent study of the 'tail-to-tail' bisnaphthalimides and their constituent units was conducted. The sensitivity of these compounds to their solvent environment proposed that their spectroscopic behaviour in the presence of DNA might be employed to probe any interactions that may occur. Each analogue of each family of the 'tail-to-tail' bisnaphthalimides was then studied by titration with *ct*-DNA, poly(dA-dT)poly(dA-dT) and poly(dG-dC)poly(dG-dC). These titrations were monitored by absorbance and emission spectroscopy. The intrinsic binding plots from these studies and the calculation of intrinsic binding constants, K , are described. The dipeptide functionality seems to dictate the sequence selectivity of the system (LeuAla K > PheAla K > Cbz(Lys)Ala K) and the spacer length tunes the strength of the binding that occurs (hexyl $\approx$ pentyl > propyl). Similar studies for five of the six novel 'head-to-head' bisnaphthalimides are described and these systems appear to bind more weakly than the 'tail-to-tail' systems. This may be due to the retention of the 4-nitro substituents and the use of dicarboxyl linkers. Circular dichroism, thermal denaturation and viscosity studies are detailed although these did not provide definitive conclusions on the nature of the DNA interactions. Possible modes of interaction are suggested in *Chapter Three*.

Chapter Four discusses the biological assays conducted on fifteen of the novel bisnaphthalimides conducted by our collaborators in St James' Hospital, Dublin. These compounds were studied in HL-60 cell lines, an acute promyelocytic leukaemia, and K562 cell lines, a much more chemo-resistant chronic myeloid leukaemia cell line. These compounds show promise as potential anti-cancer agents and some are active against both these cancer cell lines. The results are detailed and reflect the trends in the binding constants, K , calculated in *Chapter Three*. In comparison, the 'head-to-head' bisnaphthalimides are not as active. This chapter also describes some recent studies conducted on a very active monoepitope bisnaphthalimide. Preliminary fluorescent microscopy studies suggest that the dipeptide functionalised bisnaphthalimides penetrate the nuclei and thereby achieve their anti-cancer activity.

Chapter Five details the experimental techniques employed and presents the synthesis and characterisation of the compounds discussed within the thesis. *Chapter Six* cites the literature, articles and texts referenced.

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Abbreviations

α	alpha
β	beta
γ	gamma
Δ	delta, change
ϵ	molar extinction coefficient
λ	wavelength
λ_{max}	wavelength of maximum absorbance
μ	micro ($\times 10^{-6}$)
ν	frequency
∞	infinity
A	adenine
Abs	absorbance
Ala	alanine
a.u.	arbitrary units
bp	base pairs
BOC	<i>tert</i> -butoxycarbonyl
br.	broad
C	cytosine
CD	circular dichroism
$^{\circ}\text{C}$	degrees celcius
^{13}C	carbon 13 isotope
Cbz	carboxybenzyl
CDCl_3	deuterated chloroform
$(\text{CD}_3)_2\text{SO}$	deuterated dimethyl solfoxide
CH_2Cl_2	dichloromethane
CHCl_3	chloroform
CH_3CN	acetonitrile
CML	Chronic Myeloid Leukaemia
<i>ct</i> -DNA	calf thymus deoxyribonucleic acid
d	doublet
dd	double doublet
D	dextrorotatory

DCM	dichloromethane
DMA	dimethyl acetamide
DMAP	dimethyl aminopyridine
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDCI	ethylene diaminecarbodiimide
ESMS	electrospray mass spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
Et ₃ N	triethyl amine
G	guanine
GI ₅₀	growth inhibition
Gly	glycine
h	hour
¹ H	proton-1 isotope
HBr	hydrobromic acid
HCl	hydrochloric acid
His	histidine
HOBt	hydroxybenzotriazol
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IC ₅₀	inhibition
ICT	internal charge transfer
i-PrOH	iso-propanol
IR	infrared
ISC	inter system crossing
K	binding constant
L	levorotatory
LD ₅₀	lethal dose
Leu	leucine
LUMO	lowest unoccupied molecular orbital
Lys	lysine
m	multiplet

MeCN	acetonitrile
MeOH	methanol
Met	methionine
MHz	megahertz
min.	minutes
MRI	magnetic resonance imagery
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide
mRNA	messenger RNA
nm	nanometers
NMR	nuclear magnetic resonance
Orn	ornithine
PBS	phosphate buffered solution
PET	photoinduced electron transfer
Ph	phenyl
pH	$-\log[\text{H}_3\text{O}^+]$
PI	propidium iodide
pK _a	$-\log[\text{K}_a]$ where K_a is the acidity constant
Phe	phenylalanine
ppm	parts per million
RNA	ribonucleic acid
s	singlet
S ₀	singlet ground state
S ₁	first excited singlet state
SDS	sodium dodecyl sulfate
Ser	serine
T	thymine
T ₁	first excited triplet state
T _m	thermal denaturation temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane
q	quartet
UV	ultra violet

UV-vis

ultra violet-visible

Val

valine

Z

carboxybenzyl

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Chapter One

Introduction



1.1 Cancer

‘Cancer’ is derived from the Latin for canker or tumour. It is a term that encompasses a multitude of diseases; *e.g.* carcinoma, sarcoma, lymphoma, leukaemia, myeloma, brain tumour, cancer of the mouth, throat, oesophagus, stomach, gastrointestinal, breast, prostate, ovary, bladder, *etc.*¹ Recent statistics reveal that *ca.* seven thousand people in Ireland die annually as a result of malignancies.² Of these deaths the most common include lung, colorectal, breast and prostate cancer. Over the past three decades many advances have been made, converting once fatal diseases into foes that we now better understand and are better equipped to battle against. This quest, however, is far from over since the need for still better treatments and drugs persists.

Cancer is a difficult disease to treat, due to its complexity.³⁻⁵ Cancerous cells occur when regulation of the cell life cycle fails and “immortal” cells are produced without control, in an unregimented and mutinous fashion. Cells become cancerous when fundamental mutations or changes occur in the genes that govern growth and necrosis (natural cell death). These abnormal cells continue to divide creating a mass of tissue known as a tumour. From a malignant tumour, cancerous cells can then invade and damage local tissues and organs and spread into the bloodstream (leukaemia) and lymphatic system (lymphoma). Such cells do not undergo natural cell death (necrosis) but continue to grow until the tumour leads to illness and possible death. The aim of cancer treatments is to artificially induce the death of these rampant cells.⁶

Essentially, cancer is a genetic disease. The mutations, including the failure of oncogenes, tumour suppressor genes and mismatch-repair genes, that lead to tumours can be due to a number of factors which may relate to environment, lifestyle or heredity. Environmental causes include exposure to radiation, chemical carcinogens and asbestos. Lifestyle habits such as high intake of alcohol, an unhealthy diet and tobacco consumption (which leads to 85 % of all lung cancer deaths) are other major triggers of cancer. Certain types of cancer are known to be inherent, particularly breast, colon, ovarian and prostate cancers.

A variety of cancer treatments currently exist, including surgery, radiotherapy, hormone therapy and biological therapy.^{7,5} Chemotherapy is the use of drugs to kill cancer cells.⁷ This kind of therapy can be used prior to surgery to shrink tumours, afterwards to prevent the recurrence of the tumour or independent of any invasive measures. A combination of anti-cancer drugs is frequently used. In general, such drugs affect all rapidly dividing cells, so that, as well as targeting the cancer cells, other healthy cells

(such as blood cells, cells in the digestive tract, follicles *etc.*) are damaged. Current anti-cancer agents attack the replication processes of the cells or interact with Deoxyribose Nucleic Acid (DNA) in such a manner so as to disrupt the transcription and kill the cells. This programmed death is known as apoptosis. These drugs may also target certain pathways of proteins, receptors and anti-bodies that are responsible for the growth and development of the tumour itself and thereby achieve apoptosis.

If not detected in ample time, two-thirds of advanced cancers are incurable and, ultimately, fatal. Furthermore, these treatments can damage healthy cells and tissues resulting in illness and side effects. There persists an overwhelming need to develop discrete wonderdrugs to treat different cancers. A specific and targeted approach to cancer seems to be the light at the end of a very long tunnel. This challenge is considerable, ideally such a wonderdrug ought to:

- be capable of selectively targeting cancer cells and not damaging healthy cells and tissue,
- destroy the tumour, the cancerous cells and the possibility of recurrence,
- block the pathways that the cancer exploits, *i.e.* block hormone and/or enzymes used in transcription and translation,
- be viable at different stages of the cancer's progression,
- be non-toxic and limit side-effects.

Therefore, this approach to cancer treatment involves choosing a target, such as hormones, receptors, enzymes and DNA, for which the drug is then designed. Cancers that are fuelled by hormones, such as breast and prostate cancers, can be treated by blocking the action of oestrogen and aromatase, and androgen, respectively. The spread of the tumour may be prevented by stopping the hormone from triggering the response which is responsible for enhancing its growth. This can be achieved by blocking the receptors, eliminating the receptors completely or suppressing the hormone production, so a drug might be fashioned that targets the receptor, destroys the receptor completely or targets the generation of the hormone. Equally, an enzyme, such as topoisomerases and those involved in transcription and translation, might be targeted by the drug. This prevents the reproduction of the cancerous DNA and causes the cells to die.

An elegant target is DNA. A cancerous mutation is characterised by a certain genetic code, for example, the Philadelphia chromosome is responsible for Chronic Myeloid Leukaemia (CML), so a drug that possesses a complimentary charge,

functionality, topology *etc.*, might effectively target cells that display that mutation and by interacting with that region disrupt the replication processes of the DNA. The following section details the structure of DNA and how it can be effectively targeted in the treatment of cancer.

1.2 Anti-Cancer Drugs & DNA Interaction

1.2.1 Deoxyribose Nucleic Acid

DNA is the chemical encryption of the code of life. This genetic material and its structure was elucidated by Watson, Crick and Franklin in 1953. Their discovery began the genetic age and we now possess an understanding of molecular damage and repair, transcription and translation, replication and inheritance. The past twenty years has seen researchers attempt to cure genetic diseases by targeting the responsible mutations in the DNA.⁸

In vivo DNA exists primarily in the B-form and it consists of nucleotides linked together into chains. Each nucleotide contains a base (a nucleoside), a sugar and a phosphate group. There are four bases involved in any DNA sequence – Adenine (A) and Guanine (G) (both purines), Cytosine (C) and Thymine (T) (both pyrimidines), **Figure 1.1.**⁹ Adenine pairs readily with Thymine through two hydrogen bonds, while Guanine and Cytosine are also complimentary and pair through three such bonds. The sugar is 2'-deoxyribose and together the base and the sugar compose a deoxyribonucleoside unit. Each of these units is linked to the next through 3'- to 5'-phosphodiester bonds, providing the strong and stable backbone of DNA. Two strands of complimentary base chains coil about each other to give DNA its double helical structure.⁸

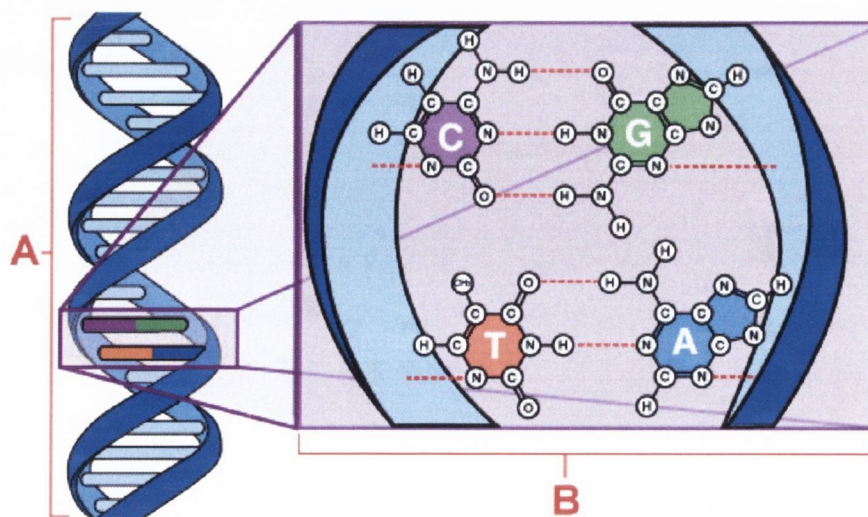


Figure 1.1: B-form DNA and its base pairs⁹

The B-form of DNA exists as a right-handed, double-stranded helix, with major and minor grooves on the outer edge of the structure. Ten nucleosides occupy each turn of the helix with distances of 3.4 Å between adjacent base pairs. The bases point towards the centre of the helix and the spine of the structure consists of an alternating sugar-phosphate system. The A-form of DNA resembles the B-form in that it is a right-handed double helix, however, it contains eleven base pairs in each turn of the helix. The Z-form of DNA is a left-handed double helix with twelve base pairs per turn. **Figure 1.2** depicts these three forms.⁹

In all forms of DNA two glycosidic bonds connect the bases to the sugar rings. These bonds are not parallel and this causes the sugar-phosphate skeleton to have unequally sized grooves; *i.e.* the helix has both deep, major and shallow, minor grooves. Different regions of the purines and pyrimidines are exposed depending on the type of groove. This leads to substrates preferring to bind at one groove over the other since different interactions are proffered by the exposed section of the bases. Thus, a compound may selectively interact at the minor or major groove depending upon what electrostatic and hydrogen bonding options are available and what bases are accessible.

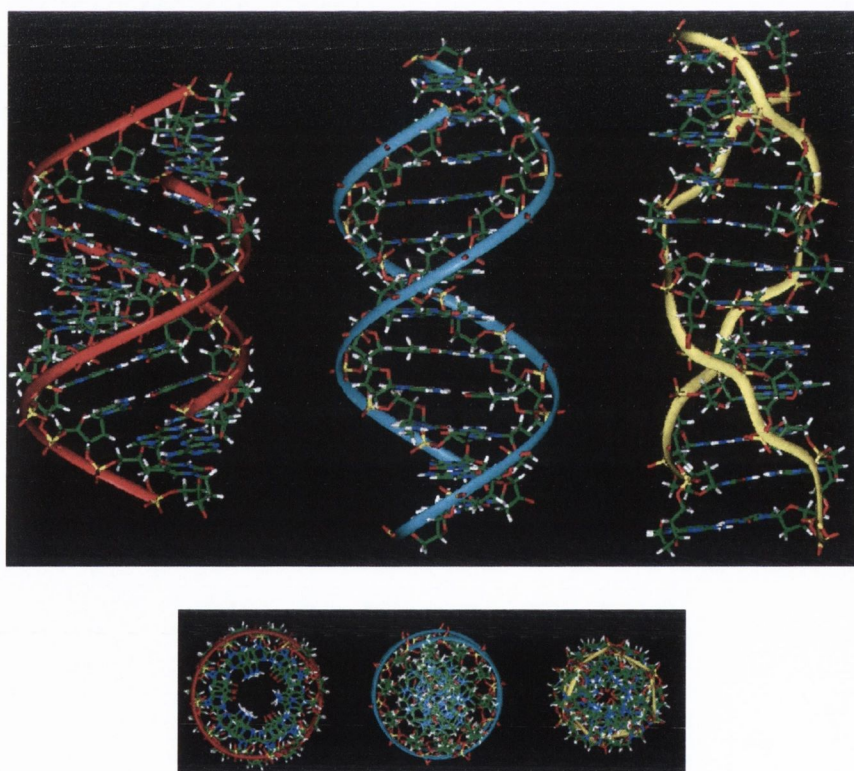


Figure 1.2: A-, B- and Z-forms DNA,
profile and apex view⁹

1.2.2 Targeting DNA

All biological information is stored by DNA and is manifested by proteins, where messenger RNA (mRNA) acts as the conduit of the genetic code. Developments in selectively preventing this translation, and in particular preventing that of cancerous cells, by hindering DNA hybridisation have led to the successful design of potential tailor-made anti-cancer agents. The strategy of targeting DNA as a basis for treating diseases has come to prominence in the past twenty years. One can design a molecule that interacts with DNA and also incorporates structural and electrostatic regions that are preferentially attracted to certain base pairs sequences. However, since DNA is secreted within the nuclei of cells, behind two membranes, it is a much more difficult target than receptors or enzymes.⁸

DNA contains four different bases but fifteen bases are needed to define a unique DNA sequence in humans *via* base specificity. One turn of a helix contains ten base pairs and the possible combinations of A, T, C and G give rise to 524,800 different permutations. For this reason, the task of directly and specifically targeting cancerous cell DNA is a challenge. If a molecule is to be sequence specific it must have a span and shape that permits it to read the fifteen bases that define a certain sequence. A novel compound with a unique, complimentary shape may be capable of topological recognition. Thus by reading many bases, it may interact with only certain DNA sequences and provide a selective and efficient means of dealing with cancer at a cellular level. One may choose from large proteins and synthetic strands of DNA and RNA that will interact with the DNA helix in a complimentary fashion. Simple molecules cannot achieve such specificity though they interact easily, thus they can be very toxic since they bind and damage a variety of sites.⁸

Molecules typically interact with DNA in one, or a combination, of three modes which are defined as; a) covalent binding, b) intercalation and c) groove binding, **Figure 1.3.**¹⁰ Covalent binding requires a region of the molecule to be electron rich or poor and to

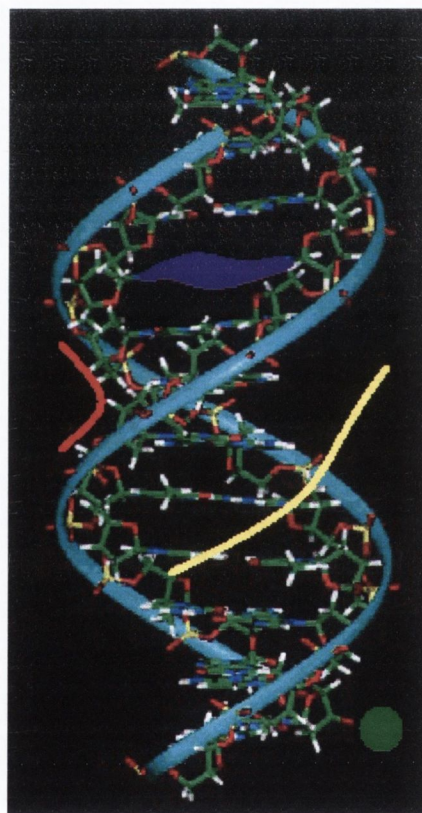
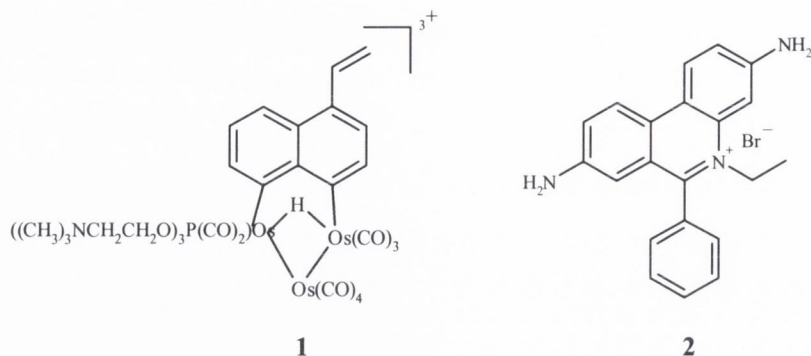


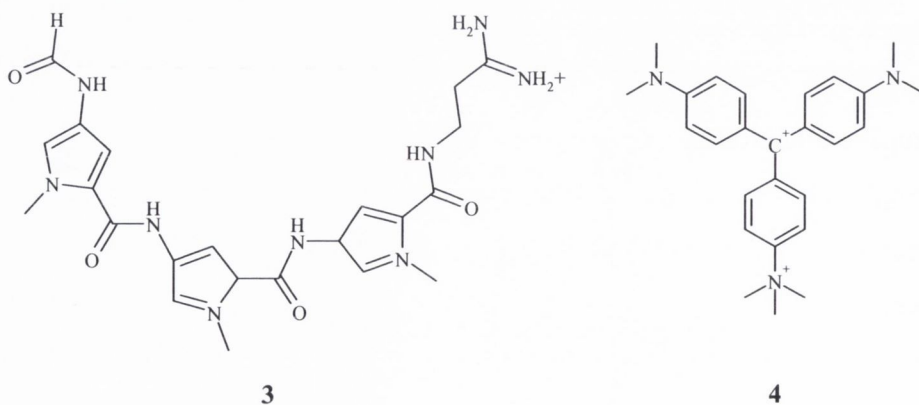
Figure 1. 3: DNA - Drug Interaction;
Green = Covalent Bonding,
Purple = Intercalation,
Red = Minor Groove Binding,
Yellow = Major Groove Binding¹⁰

complimentarily bind to a region of an exposed base pair (*e.g.* a benzoheterocycle triosmium cluster, **1**).¹¹ This causes disruption to the helix and consequently perturbs transcription leading to cell death.

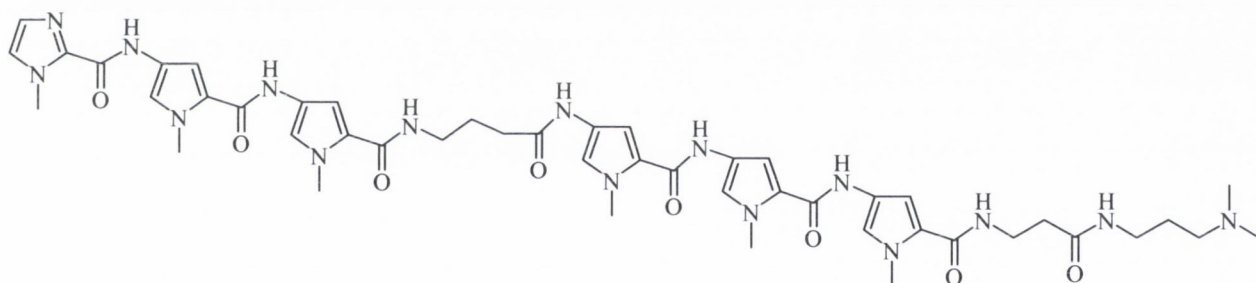


Intercalation involves a planar, π -deficient, aromatic molecule, of suitable size and stereochemistry, often positively charged, which is capable of 'slotting' in between the base pairs and interrupting their hydrogen bonding (*e.g.* ethidium bromide, **2**). Intercalation increases the distance between the paired bases and the distance between adjacent base pairs. Van der Waals forces and π - π stacking also favour intercalation. Therefore, an intercalator causes the overall architecture of the helix to kink and thus impairs translation and transcription which ultimately leads to cell death. Intercalators can also interact with topoisomerase enzymes, inhibiting their action and prevent replication in that way.

Groove binders entwine into a minor (*e.g.* Disamycin A, **3**¹²) or major groove (*e.g.* methyl green, **4**¹³) because they possess a complimentary charge and shape to that exterior wall of the helix. The presence of these molecules can further twist the conformation of the DNA and disrupt the helix. These adduct structures interfere with the mRNA when transcription is attempted and the cell cannot replicate, thus a groove binder can affect cell death.



It is feasible to design a molecule that fulfils one, or more, of these binding criteria in an effort to affect disruption of the helix's conformation and hamper its replication. This is a challenging and potentially efficient means of treating genetic diseases such as cancer. It is therefore clear that if a molecule incorporates interaction with special sequence preferences one might achieve a specific targeting of the desired region of DNA and leave normal areas unaffected. This is a more efficient use of the drug and involves less side-effects for the patient. An example of such an approach is the work of Dervan *et al.* into the development of potential anti-cancer and anti-infective agents. His group has sought to create 'four chemical keys' capable of differentiating between the Watson-Crick base pairs A•T, T•A, G•C, C•G. Linking such keys together might permit a particular strand sequence to be selectively targeted, through H-bonding recognition at the minor and major grooves.¹⁴ This strategy has led to the development of small molecules, helical and, especially, hairpin polyamides,¹⁵ as sequence specific groove binders (*e.g.* **5**). Such compounds can inhibit and compete with transcription factor binding and interfere with gene expression, typically at the minor groove, although their pharmacokinetics, bioavailability and toxicity remain to be established.¹⁵



5

1.2.3 Classic Anti-Cancer Drugs : History of Drug Design

An agent can target DNA as previously explained and upon interaction it may cause further damage to the helix by alkylation, free radical production, electron transfer *etc.* The molecule may indirectly destroy the cancerous cell DNA by inhibiting the enzymes that are involved in DNA replication, such as: topoisomerase II, m-RNA, *etc.* The mitotic, replication, processes of the cell may also be a target for the drug.

Cis-diaminedichloroplatinum (Cisplatin®, **6**), an early anti-cancer chemotherapeutic, dates from the 1960's although its action was elucidated long after.¹⁶ It acts by coordinating irreversibly with DNA, thus impeding its replication by blocking the action of DNA polymerase and leads to apoptosis.⁴ *In vitro* one of the chlorine ligands is

hydrolysed, **Figure 1.4**. This positively charged form binds readily to guanine units through a covalent bond at the N7 atom. When both chlorine ligands are removed the molecule can intrastrand bind to two adjacent bases. This adduct leads to double alkylation which disrupts π - π stacking of the helix causing it to become kinked.⁴

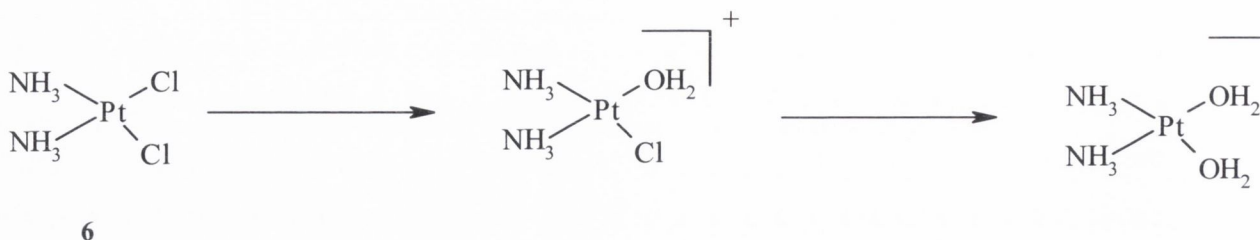
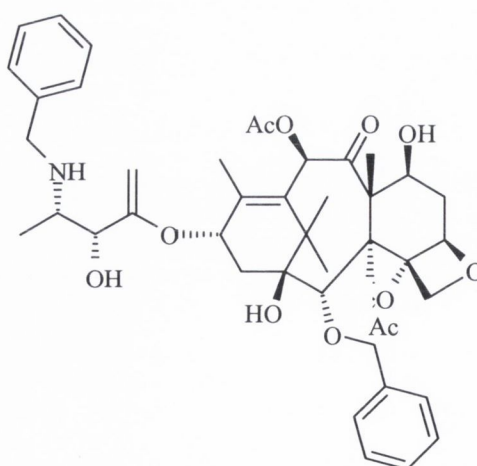


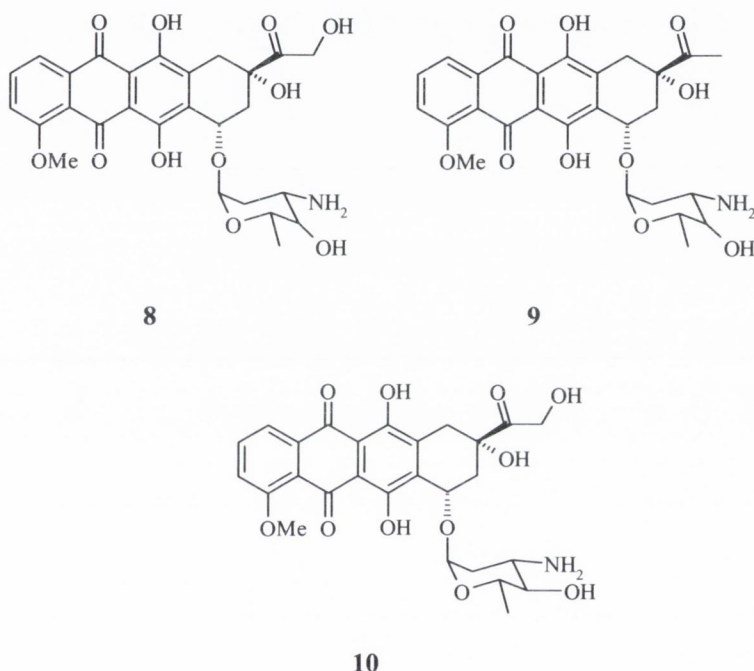
Figure 1.4: The biological hydrolysis of Cisplatin

Taxol®, 7, is another successful anti-cancer drug and was derived in the 1970's from a natural product of the bark of the Pacific Yew.⁴ This compound attacks the mitotic operation of the cell. Mitosis is the basis of cell replication and is the process whereby a parent nucleus divides to produce two identical daughter nuclei. Microtubules are small tubular organelles constituted of protein tubulin dimers. Taxol® enhances the assembly of these microtubules. It then stabilises the tubules to such a degree that the mitosis process is totally hindered. To a normal, healthy cell this could be harmful, but to a rapidly dividing cancer cell this is fatal. Taxol® is also relatively non-toxic and has limited side-effects which are factors that contribute to its widespread use against breast and ovarian cancer.⁴



7

Anthracyclines are also natural product derivatives, from strains of *Streptomyces*, and include the anti-tumour agents Adriamycin (Doxorubicin®), **8**, Daunomycin (Daunorubicin®), **9**, and Epirubicin (Epidoxorubicin®), **10**.^{17,18} Their planar architecture permits them to easily intercalate, causing the helix to buckle out of shape.¹⁹ The ring of the anthracycline slots in between the base pairs and the substituent slides into the minor groove as well as forming hydrogen bonds with the nearby base.²⁰ These combined interactions make for a very stable DNA-drug adduct and structure and replication of the helix is damaged leading to cell death. These compounds are used in the treatment of leukaemia.

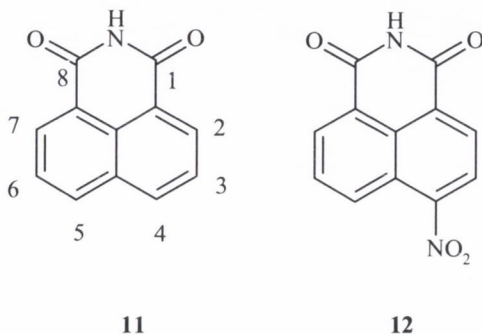


The origin, design and mode of action with DNA of a range of anti-cancer agents has been discussed, a more specific family of anti-cancer compounds will now be addressed, those with structures based upon 1,8-naphthalimides.

1.3 Naphthalimides

1.3.1 Introduction

1,8-Naphthalimide or benzo[*de*]isoquinolin-1,3-dione, **11**, is a planar molecule composed of two conjugated aromatic rings fused to a third ring



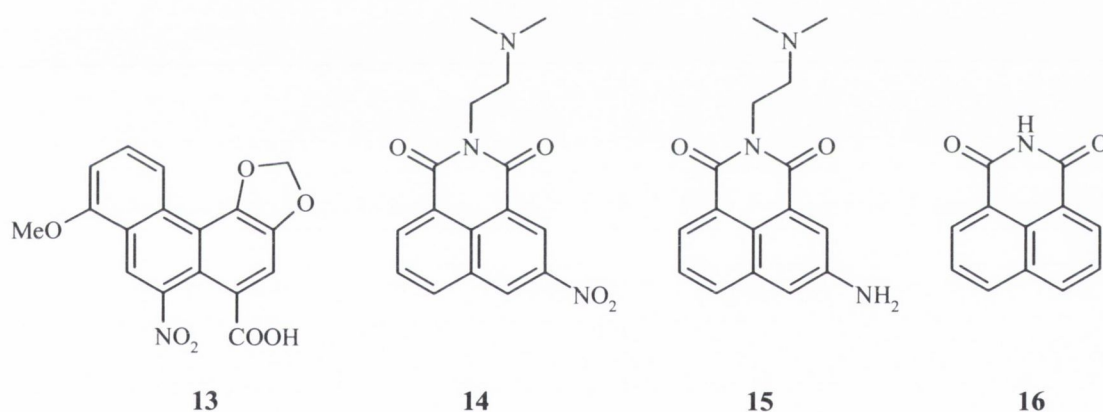
which includes an azole dione functionality. Derivatives such as 4-nitro-1,8-naphthalimide, **12**, have many practical applications. They are widely used as electro-optically sensitive and laser activating materials,²¹ as solar energy collectors,²² and when amino-substituted at the 3- or 4-positions they have strong fluorescent properties making excellent dyes because of their internal charge transfer abilities, protein cross-linking agents and models for PET events.²³

In the 1990's Grabchev *et al.* investigated the impact that 1,8-naphthalimide dyes had on copolymerisation when the imide nitrogen was bound to different polymerising groups or when different amino groups were placed at the 4-position.^{24,25} They found that photophysical properties, polymerisation rate and viscosity of the polymers were all effected by the nature of the amino group in the dye. More recently they have studied a range of poly(amidoamine) dendrimers which elegantly incorporate 1,8-naphthalimides at the peripheral amines.^{26,27} The nature of these 1,8-naphthalimides influence the photostability and photophysical characteristics of the dendrimers, typically enhancing the fluorescence and these macromolecules hold promise as sensors for metal cations and protons. Whilst Konstantinova *et al.* have examined the photostability of a range of 1,8-naphthalimides which entail a heteroaromatic ring connected to the imide nitrogen and how these dyes might be commercially used for the mass colouration of wool and polyamide fibres.^{28,22}

1.3.2 Mononaphthalimides as Anti-Cancer Agents

Naphthalimides have also been employed in medicinal chemistry and medicine. Substituted 1,8-naphthalimides have demonstrated dramatic anti-cancer activity and are in clinical trials.²⁵ The extended planarity and aromatic character of derivatives of 1,8-naphthalimide potentially makes them powerful intercalators.²⁹⁻³³ The degree of intercalation is a result of the substituent electronic effects, the hydrogen bonding and the steric effects of the compounds. A nitro group at the four position is thought to rotate significantly into the mono-imide plane, perturbing the stacking and leading to greater viscosity and stronger binding. However, there may be many other mechanisms by which these compounds might achieve apoptosis in cancerous cells. The naphthalimides may also be capable of inducing cell death by free radical generation,³⁴ photogeneration of carbocations,³⁴ electron transfer from oxidisable guanine or proton abstraction from thymine.³⁵ It is also possible that the plane of the naphthalimide might intercalate whilst an imide or 4-position substituent may itself wind around and groove bind.²⁹⁻³³

The application of 1,8-naphthalimide derivatives as anti-cancer agents was pioneered by Braña *et al.* in the 1970's.²⁹⁻³² Since aristolochic acid, **13**, was known to exhibit anti-cancer effects, most likely due to its β -nitro naphthalene moiety, a range of imide derivatives of 3-nitro-1,8-naphthalic anhydride were designed and synthesised. Braña *et al.* incorporated other structural motifs to assist intercalation including glutarimide rings and positively charged amine substituent chains.²⁵ Initial derivatives such as mitonafide, **14**, showed activity against HeLa and KB cells *in vitro* as well as against murine Ehrlich ascites and rat Yoshida carcinoma.²⁵ Since the cytotoxicity of this compound was found to be reversible it was deduced that no covalent cellular binding was involved in its mode of action.²⁵ Mitonafide causes both the unwinding of closed circular DNA and increased DNA viscosity, these are indicative of intercalative behaviour.²⁵ Further investigations showed that this DNA interaction was accompanied by an inhibition of topoisomerase II.²⁵ Mitonafide and its amino analogue, amonafide, **15**, entered clinical trials in the 1990's. Unfortunately, mitonafide damaged the central nervous system and caused memory loss and both it and amonafide showed low clinical anti-tumour activity.



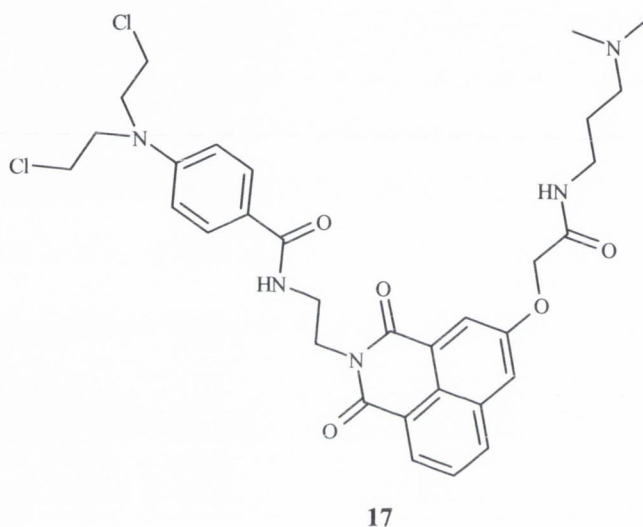
Nafidimide, **16**, was also synthesised, this lacks any functionalisation at the 4-position and is the simplest 1,8-naphthalimide.²⁹⁻³² This displayed considerable activity in animal tumours, both *in vitro* and *in vivo*, and entered clinical testing. Andersson *et al.* studied its antileukaemic viability, interaction with DNA and its cellular transport.³³ This was also a powerful intercalating agent and topoisomerase II inhibitor. The drug was found to be successful against myeloid leukaemia cells (KBM-3, HL-60), but unfortunately destroyed healthy bone marrow cells.

Other naphthalimide derivatives from the Braña group have also entered clinical trials and his group has built an impressive body of work including the development of acridine based anti-cancer motifs,³⁶ anti-tumour dendritic imides^{37,38} and

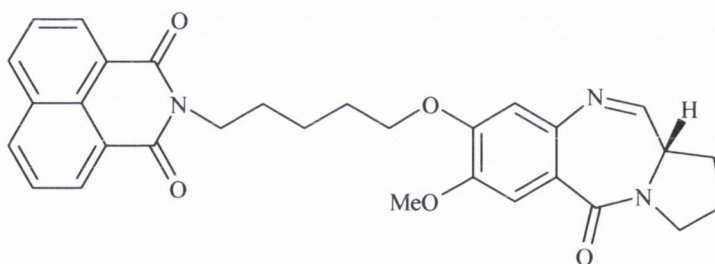
bisnaphthalimides.²⁵ Braña *et al.* remain the authority on the design, synthesis and biological investigation of derivatives of 1,8-naphthalimides.^{29-32, 36-37,38}

1.3.3 Second Generation Mononaphthalimides

Gupta *et al.* have developed a range of 1,8-naphthalimide derivatives with nitrogen mustard functionalities at the imine position in an effort to combine their individual anti-tumour activities, *e.g.* **17**.³⁹ These compounds were found to achieve sequence specific alkylation at the guanine N7 position and show considerable anti-cancer activities. This is a prime example of how it is possible to manipulate 1,8-naphthalimide moieties by incorporating other structural motifs to enhance their activities and specificities.

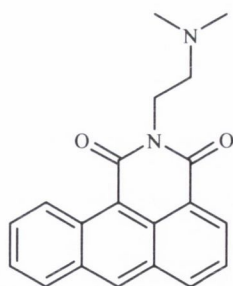


A range of imide functionalised naphthalimides, *e.g.* **18**, have been developed by Kamal *et al.*, these were based on a group of pyrrolobenzodiazepines (PBDs) which are anti-tumour natural products produced by *Streptomyces*.⁴⁰ These compounds form covalent adducts in the minor groove of DNA, the amine at position 2 of guanine nucleophilically (S_N2) attacks the C-11 position of the PBD. Attaching a naphthalimide at the C-8 position of the PBD could combine the groove binding of the latter and the intercalative properties of the former. Thus, these PBD-naphthalimide hybrids are double-edged swords in their interaction with DNA. Compound **18** is an example of such a compound. A linker chain length of four or five carbons achieved the optimum fit in the minor groove. These compounds have displayed promising activity against leukaemia, colon, prostate and ovarian cancers cell lines.⁴⁰

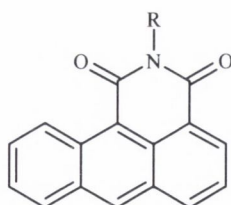


18

Sami *et al.* have developed a range of amonafide analogues using the anthracence moiety instead of the naphthalene moiety.⁴¹⁻⁴³ A family of nineteen compounds, including compounds **19** and **19 a-i**, was designed and synthesised with a variety of substituents, on the imide nitrogen, containing a basic nitrogen. These substituents ranged from aliphatic amino alkanes to aliphatic chains with amine ring systems at the termini. These compounds exhibited greater potency against murine and human tumours than amonafide itself. The 2-(dimethylamino)ethyl side chain, compound **19**, was most promising and this was further examined against colon cancer, leukaemia and melanoma cell lines and exhibited a high degree of cytotoxicity.⁴¹⁻⁴³ These compounds are DNA intercalating agents and topoisomerase II inhibitors. Structure-activity correlations have suggested that the basicity, length, steric hindrance and rigidity of the side chain may influence the ultimate efficacy of the compound.⁴¹⁻⁴³

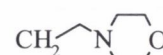
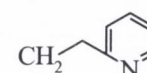
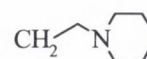
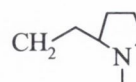
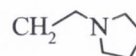


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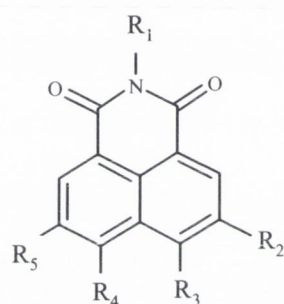


19 a-i

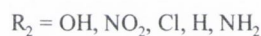
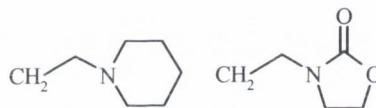
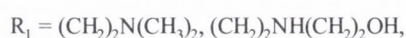
R = (CH₂)₂NHCH₃
 (CH₂)₂NH(CH₂)₂OH
 (CH₂)₃N(CH₂CH₂OH)₂
 (CH₂)₂N



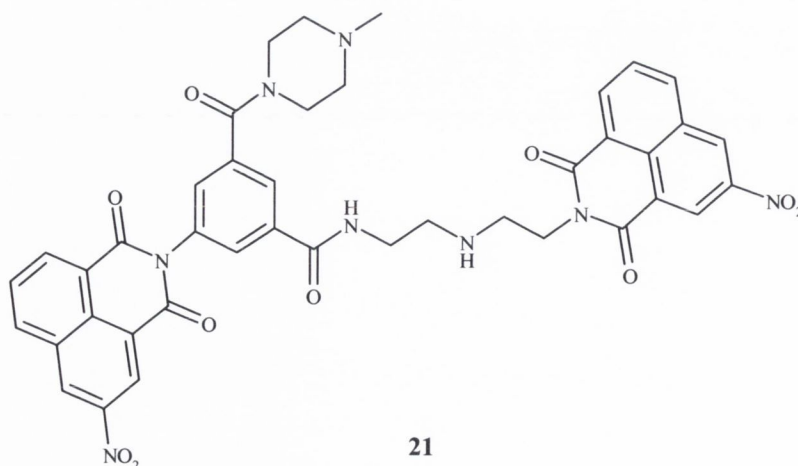
Zee-Cheng and Cheng launched a systematic study of *N*-(aminoalkyl)-substituted derivatives of a variety of imides, **20** and its derivatives.^{44,45} They investigated the type of ring system, the modification of the side chain and the ring substituents. These compounds were tested against leukaemia (P388) and melanoma (B16) *in vivo* and against leukaemia (L1210) and colon adenocarcinoma *in vitro*. They found that a prominent cytotoxic role is played by the [2-(substituted amino)-ethyl]amino fragment when attached to naphthalimides or anthraquinones.^{44,45} Substitution at the 3 position or the 6 position with electron-withdrawing groups like NO₂ or with electron-donating groups like NH₂ or OH was found to enhance the potency of the compounds. Dinitro and diamino substituted derivatives also displayed viable anti-cancer activities, whilst the introduction of an extra methylene unit in the side chain reduced the anti-cancer activities.



20

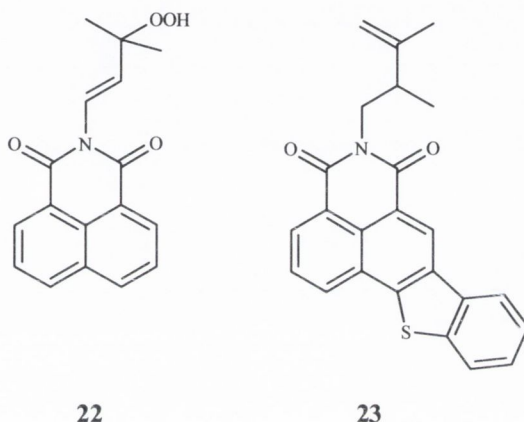


Novel naphthalimidobenzamide derivatives were synthesised and studied by Suzuki *et al.*, e.g. **21**.⁴⁶ These compounds were found to intercalate with DNA and showed a preference for GC and AA sequences. Compound **21** was found to inhibit melanoma and gastric cancer cell lines at nanomolar concentrations.



21

Qian *et al.* have designed a series of 1,8-naphthalimide hydroperoxides, *e.g.* **22**, as intercalating DNA cleavers. These compounds interact with DNA in a two step process.⁴⁷ Firstly by intercalation, then upon irradiation the hydroperoxides generate hydroxyl radicals which affect the cleavage of DNA.⁴⁷ When a second ring was conjugated to the naphthalene ring, as in **23**, greater cleavage was achieved. It was surmised that the larger planar system enhances the intercalation and that this contributes to the effective cleaving.⁴⁷

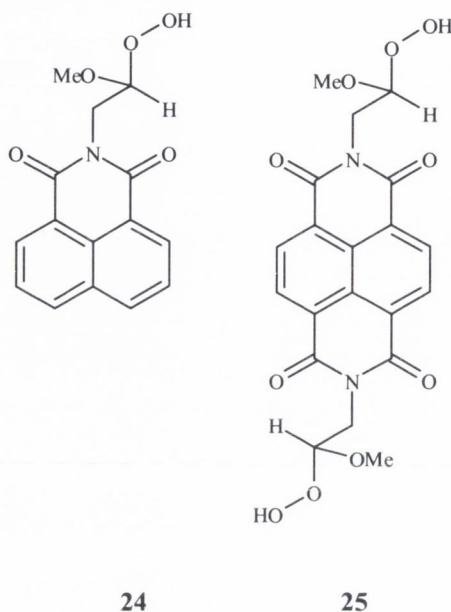


The range of 1,8-naphthalimides developed during the 1970's and 1980's provided a spectrum of derivatives and conjugates that produced varying degrees of anti-cancer activity. The past two decades have seen the probing of the various possible modes of action employed by such compounds *in vivo* and *in vitro*. The following passages highlight the work of some groups in investigating these mechanisms.

1.3.4 Modes of Action of Naphthalimides

In the late 1990's Aveline *et al.* conducted a comprehensive investigation into the photochemical mechanisms available to naphthalimides and their derivatives.³⁴ Several *N*-substituted 1,8-naphthalimides, *e.g.* **24**, and 1,4,5,8-naphthalene diimides, *e.g.* **25**, were photophysically studied and found to be capable of undergoing intramolecular γ hydrogen abstraction, oxygen-centred radical formation, hydroxyl radical release (provided by the O-OH functionalisation), singlet oxygen formation, and electron transfer (to give the radical anion and the radical cation of the corresponding naphthalenic derivative) upon excitation.³⁴ For these compounds hydroxyl radical formation and electron-transfer from guanine sites have been proposed and 5'G and 5'-GG-3' selective cleavage has been observed.³⁴ Singlet oxygen formation may cause Type II biological damage in the presence

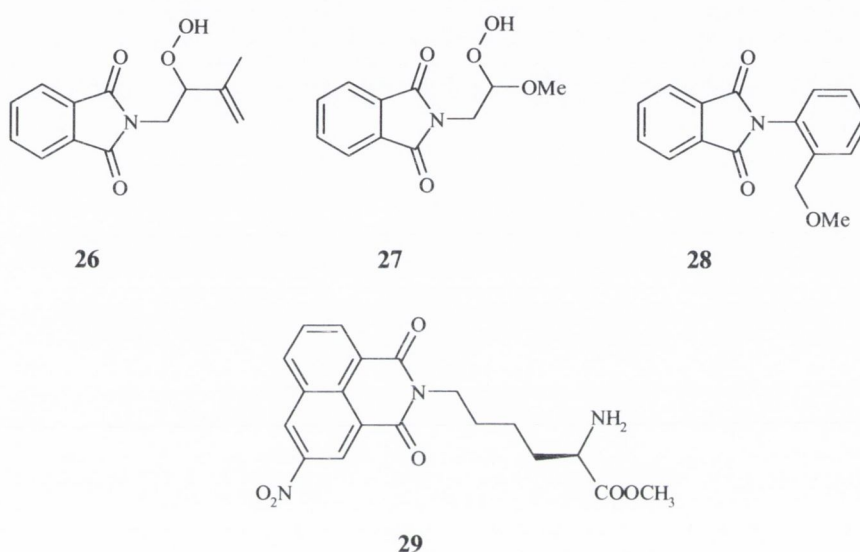
of suitable substrates and electron transfer reactions are known to cause Type I biological damage.³⁴ These mechanisms may be part of the versatile arsenal employed by naphthalimides under physiological conditions against cancer and give one an idea of how complicated the mechanisms of such species are.



Sami *et al.* have investigated the structure-activity relationships of amonafide and azonifide derivatives to elucidate their behaviour with cancerous cell DNA.⁴¹⁻⁴³ They found that the *in vitro* anti-tumour activity showed a significant dependence on the position of substitution. Nitro and amino substituents at 4-position of the amonifides and 5-position of the azonafides endow the compounds with added potency over similar substitution at other positions and substitution in any position with the less effective acetylamino and aliphatic groups. This structure-activity correlation is indicative of intercalation being enhanced or hampered by the pendant groups on the planar ring system of the molecules. Thus, it seems that ring substitutions of small, rigid, basic nitrogen groups, which can orientate themselves to facilitate maximum intercalation, result in significant anti-tumour potency.

In the 1990's Saito, Takayama and Matsuura investigated the photochemical hydroxyl radical generation of phthalimide hydroperoxides and photoactivated cleaving ability of lysine derivatives of naphthalimides.^{48,35} Long wavelength irradiation of hydroperoxide derivatives of phthalimides, **26**, **27** and **28**, lead to the unimolecular generation of radicals which in turn can cleave DNA. More recently, these compounds have been tethered to DNA binding components in order to combine cleavage with sequence specificity. *N*-Lysine derivatives of 1,8-naphthalimide, *e.g.* **29**, have been shown

to permit the selective cleavage at the 5' side of 5'-GG-3'.⁴⁹ A nitro group at the 3-position alters this preference to thymine residues at 5'-GT-3' steps. Thus, substituents can be used to tune the selectivity of naphthalimides. This process it thought to involve the oxidative transformation of the thymine's methyl to a formyl group, caused by the initial abstraction of a hydrogen from the methyl group by the photoexcited nitro group. The naphthalimide that lacks a nitro group obviously cannot achieve this and it has been proposed that it displays GG preference due to electron transfer from the electron rich GG residue to the naphthalimide moiety.⁴⁹



The screening programme that led to the development of mitonafide, **14**, and amonafide, **15**, provided Braña *et al.* with valuable insights into the action of these naphthalimide derivatives.³² They found that a basic terminal group on the *N*-position side chain is necessary for the cytotoxic activity. In this regard, a nitrogen is the most effective heteroatom and substitution of this by a carbon, sulphur or oxygen atom results in inactive compounds. If the basicity of the nitrogen is reduced, by acetylation or quaternisation, the potency also decreases. As for mitonafide and amonafide, the optimum structure for activity was a dimethyl terminal nitrogen, the activity decreases with a decrease in the number of substituents (*e.g.* mitonafide $IC_{50} = 0.47 \mu M$, monomethylamine analogue $IC_{50} = 13 \mu M$, an IC_{50} value quantifies the anti-cancer activity of an agent by quoting the concentration required to reduce the number of cells by 50 %). When the nitrogen atom in the basic side chain is separated from the naphthalene imide nitrogen by two methylene units the activity was also optimised.

A range of aromatic substituents were also investigated such as nitro, amino, chloride, hydroxyl, methoxy, bromide and sulphoxide groups.²⁹⁻³² Substitution at the 2, 3 and 4-positions were all studied. Placing the ring substituent nitro group at the 3-position was more effective than at the 2 or 4 positions and nitro substituted compounds are more potent than their amino analogues. Mitonafide possesses an electron-withdrawing nitro group, whilst amonafide has an electron-donating amino group. It has been suggested that the nitro group in mitonafide encourages the formation of a charge-transfer complex with DNA base pairs.²⁹⁻³² In the case of amonafide the amino substituent may favour hydrogen bonding with the DNA backbone and thus stabilise the drug-DNA complex.

Amonafide and mitonafide were both studied at the National Cancer Institute and found to be very active *in vitro* and *in vivo* against murine and human tumour cell lines. They both bind with DNA by intercalation and Waring *et al.* discovered that they cause unwinding of closed circular supercoiled DNA and increase the viscosity of rod-like DNA fragments.⁵⁰ These compounds show a preference for GC rich regions.

Nishio and Uyeki noted that mitonafide inhibits DNA synthesis and induces strand breakage in Hamster ovarian cells.⁵¹ Amonafide produces single and double strand breakage and DNA-protein cross-links in CML cell lines.³³ These compounds are topoisomerase inhibitors and poisons and are known to form ternary complexes with DNA and topoisomerase II. This stable complex hinders DNA replication and it is thought that the side chain sterically interacts with the enzyme active site. In this way specific cleavage is achieved at nucleotide number 1830 on pBR322 DNA.⁵² The Capranico and Liu groups have both studied the complicated specificity behaviour of these compounds and have found inverted repeat sequences and GC rich areas are commonly favoured.^{53,52}

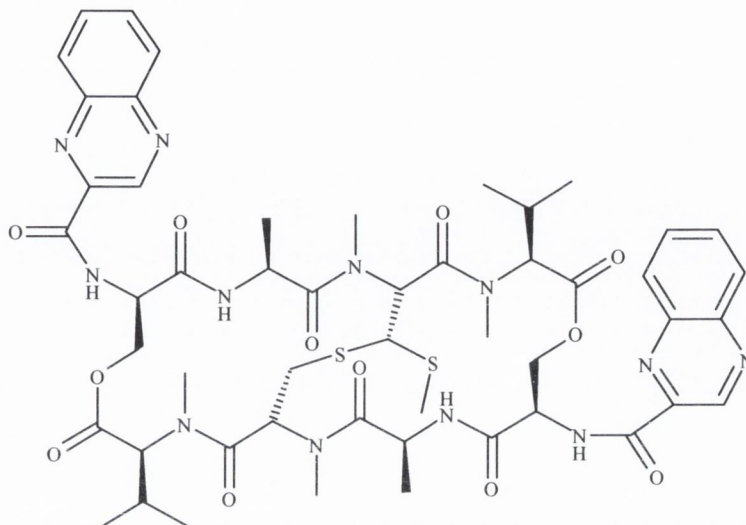
1.4 Bisnaphthalimides

1.4.1 Development from Mononaphthalimides

Since one naphthalimide is capable of anti-cancer activity incorporating two naphthalimides into one larger structure might possibly enhance the potency. The binding behaviour of such a molecule may involve bisintercalation or intercalation by one moiety and groove binding by the other. This was the fundamental concept that spurred the work of many groups in the 1980's and 1990's.^{32,54,55}

The first report of a bisintercalator was by Waring *et al.* in the mid 1970's.⁵⁶ The natural product antibiotic echinomycin, **30**, possesses two chromophores, capable of

simultaneous intercalation, linked by a cyclic octapeptide bridge which winds into the minor groove between the two intercalation sites.⁵⁶ This bridge hydrogen bonds to the exposed base pairs in the groove and in this way achieve a selectivity for poly(dG-dC) sites.⁵⁶

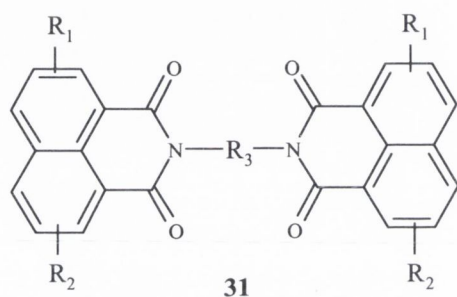


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Braña *et al.* sought to improve the therapeutic properties of their lead compounds, mitonifide and amonifide, by designing novel bisnaphthalimide intercalating agents.^{57-59,32} A series of compounds, all with the general structure of **31**, with amine and nitro groups as ring substituents (since these were the most effective in the mononaphthalimides), were designed and synthesised. A variety of bridges were employed and these all contained at least one amine functionality in an effort to enhance interaction between the bridge and the grooves of the DNA helix. These compounds are linked *via* their imide nitrogens. In general, this series was found to have higher cytotoxic activities than their parent mononaphthalimides compounds. Certain aromatic substituents increase this activity and the order $\text{CH}_3\text{CONH} < \text{NH}_2 < \text{H} < \text{NO}_2$ was found for analogues with the same bridge. The length and nature of the bridge itself was also effected the activity, the optimum being $(\text{CH}_2)_2\text{NH}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_2$. Elinafide, **32**, has progressed to clinical trials and is curative against breast cancer and melanoma and shows promising potency against lung, colon and ovarian cancers.⁵⁹

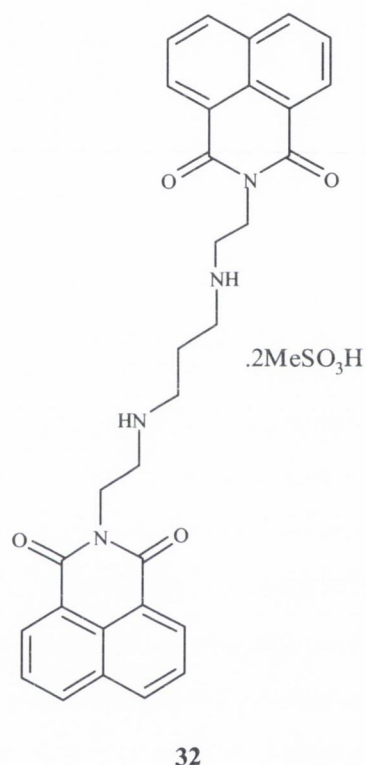
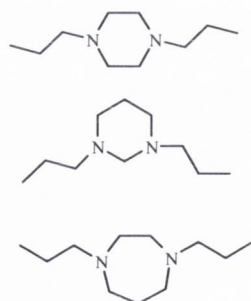
Bailly *et al.* have studied elinafide, **32**, and its 4-nitro and 4-amino substituted analogues.⁶⁰ All these compounds bisintercalate with DNA, increasing the overall length of linear DNA, elinafide causes a steep rise, and subsequent fall, in the viscosity of closed circular DNA. DNA-footprinting experiments have shown that elinafide has a sequence specific interaction with alternating purine-pyrimidine regions that occurs *via* the major

groove.⁶⁰ Gallego *et al.* have further elucidated this binding using ¹H NMR and molecular dynamics simulations.⁶¹ They suggest that the molecule binds with the hexanucleotide d(ATGCAT)₂ whereby the two naphthalimide cores intercalate at the TpG and CpA steps, stacking primarily with G and A bases. The linker most likely lies in the major groove and this would appear to be the main governing influence over the groove recognition and specificity of the molecule.

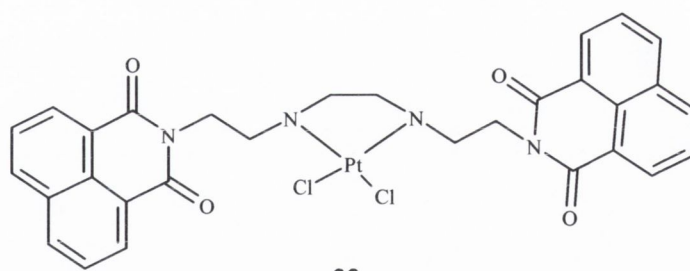


R_1 or R_2 = NO₂, NH₂, NHCOCH₃,
H, Br, OH,

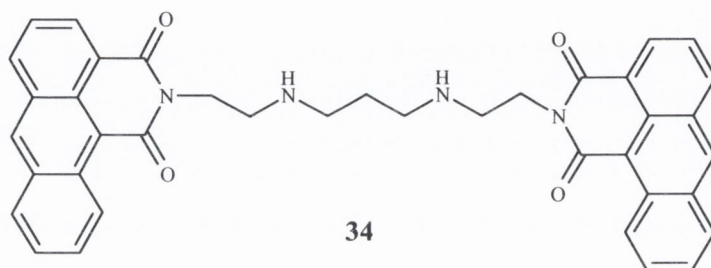
R_3 = (CH₂)_nNH(CH₂)_n,
(CH₂)_nNH(CH₂)NH(CH₂)_n,



Braña *et al.* have also investigated the complexation of bisnaphthalimides with platinum, **33**, and novel bisnaphthalimides with anthracence rings instead of naphthalimides. The platinum complexes are potential anti-cancer agents since they show promising activity and are capable of circumventing resistance to cisplatin.³² The anthracence derivatives inhibit topoisomerases I and II and are active against colon carcinoma. Bibenoline, **34**, is currently in clinical testing.^{62,32}

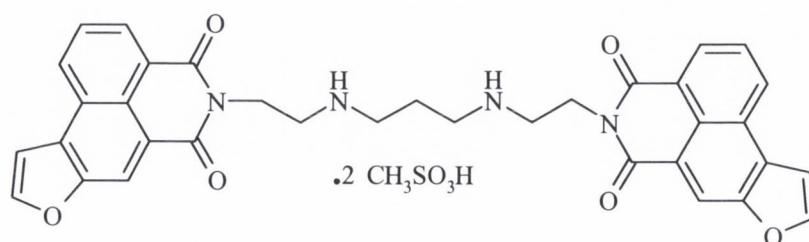


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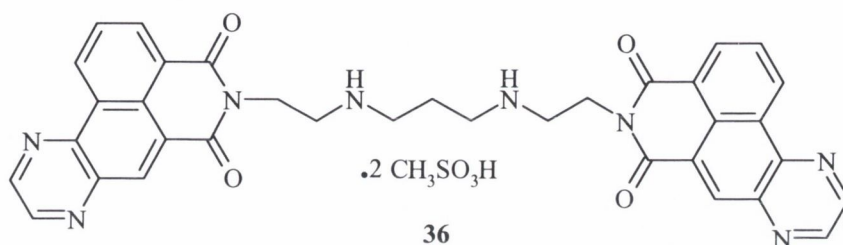


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More recently, Braña *et al.* have designed a series of bisfuronaphthalimides. This fusing of the naphthalimide ring to a furan ring works in the same way as the previously demonstrated anthracence motif *i.e.* extending the planar surface enhances its potential to stack with other planar molecules such as nucleotides.⁵⁴ Compound **35** binds more strongly than its parent monofuronaphthalimide and has a greater affinity for intercalation at GC rich sequences. This compound is active against CEM leukaemia cell lines and is three times more potent ($IC_{50} = 4.9$ nM) than elinafide ($IC_{50} = 16.5$ nM).⁵⁴ A further development of this construction was a series of pyrazinonaphthalimides, *e.g.* **36**. These have considerable antiproliferative activities against colon (HT-29), cervical (HeLa) and prostate (PC-3) carcinomas showing IC_{50} values of 0.02, 0.20 and 0.53 μ M, respectively, compared to 0.02, 0.07 and 0.32 μ M for elinafide.⁵⁴ This series of compounds also bisintercalate, although they do not inhibit topoisomerase II. Further *in vivo* investigations and exploration of the selectivity for the colon strain are currently being conducted.



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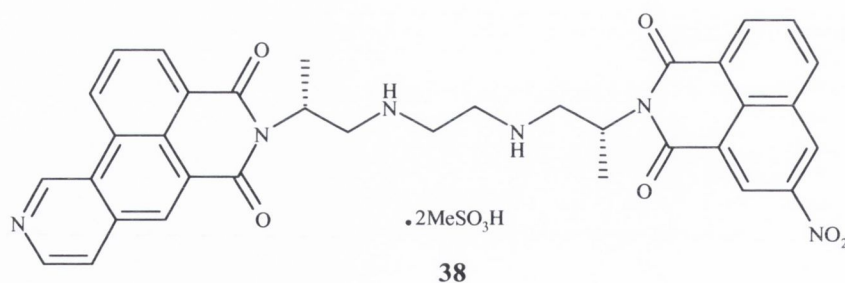
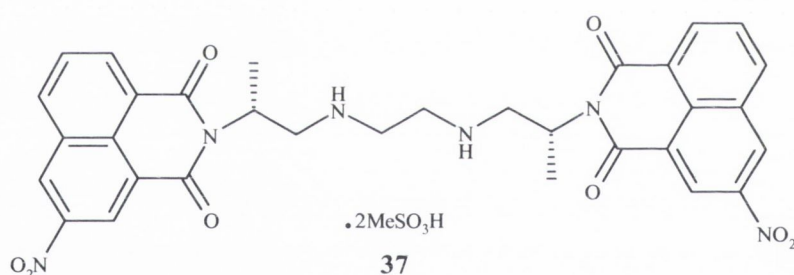


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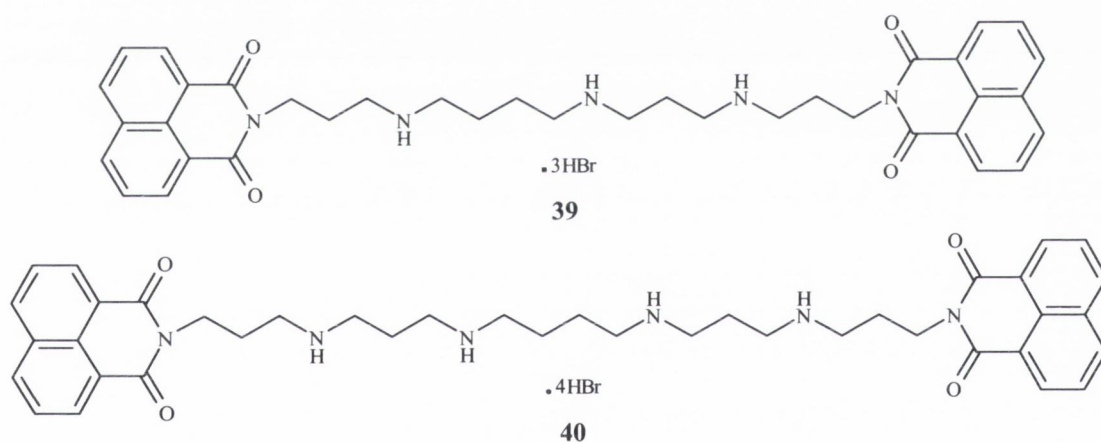
1.4.2 Second Generation Bisnaphthalimides

In the wake of initial advances by Braña, Bailly, Waring and Gallego many other investigators developed their own bisnaphthalimides. McRipley *et al.* designed DMP 840, **37**.⁵⁵ This compound was effective against several human tumour xenographs in mice and went on to Phase II clinical trials. It was found to be a monointercalator with a preference for GC rich fragments. Nitiss *et al.* studied the mode of action of this compound in detail and found that it acts against eukaryotic topoisomerase II (but not topoisomerase I) and kills cells by converting this enzyme into a poison.⁶³

Cherney *et al.* hypothesised that since only one chromophore intercalated these two units serve different functions when the molecule interacts with DNA and a new series of unsymmetrical bisnaphthalimides was designed.⁶⁴ DMP 315, **38**, came from this synthetic programme and was water soluble. It had considerable potency against murine leukaemia with an *in vitro* $IC_{50} = 0.035 \mu M$ compared to the IC_{50} value of the parent DMP 840 of $0.034 \mu M$. It was surmised that the cytotoxic effects of DMP 840 are not dependant on its symmetry and altering the nature of one chromophore can tune the activity of the compound.

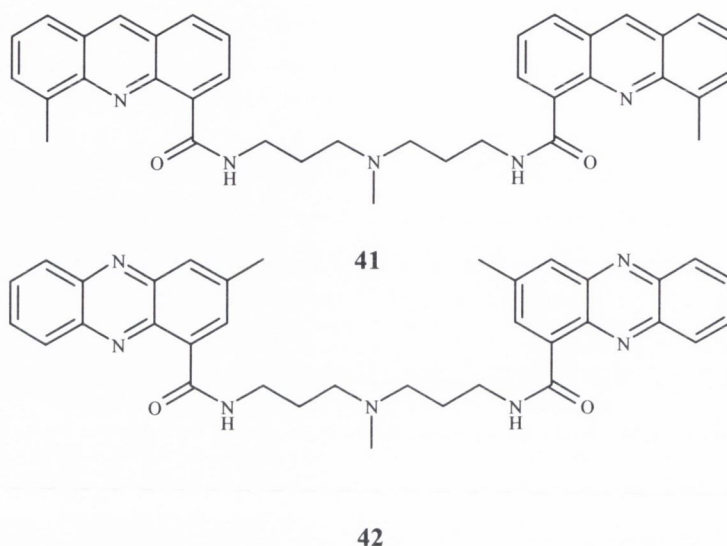


Since the late 1990's Lin *et al.* have developed a series of bisnaphthalimides based on the structure of elinafide with polyamino alkyl chains to improve the solubility of the compounds.^{65,66} By synthesising aliphatic polyamino bridges based on putrescine, oxaputrescine, spermidine and spermine they hoped to counter the insolubility of the two naphthalimide units. Whilst the former two derivatives showed excellent growth inhibition of a panel of cancer cell lines they were not very soluble. The spermidine, **39**, and spermine, **40**, bridged analogues were much more soluble due to their respective one and two extra nitrogens. These compounds also retained high growth inhibition and as such are promising anti-cancer agents.^{65,66} More recent examination has found that these compounds bisintercalate and the compounds were found to increase the melting temperature of calf thymus DNA (*ct*-DNA) and IC₅₀ values of 13.50 and 13.32 μ M respectively were found for breast cancer (MCF) cell lines. These derivatives were discovered to have migrated inside the cell nucleus after 6 h. of drug exposure and this rate of uptake is related to their cytotoxic effects.



Spicer and Gamage *et al.* have combined many of these concepts in a synthetic programme developing bis(acridine-4-carboxamides) with tertiary nitrogen containing bridges, *e.g.* **41** and **42**.⁶⁷⁻⁶⁹ They were designed as combined bisintercalators and possible topoisomerase II inhibitors and with the view of avoiding the central nervous system toxicity exhibited by bis(acridine). These novel compounds have shown promising activity, with nanomolar IC₅₀ values against murine Lewis lung carcinoma and human Jurkat leukaemia and inhibited topoisomerase I and are currently being examined as topoisomerase targeted anti-cancer drugs. Structure-activity relationship studies implied that large substituents at any position on the ring decreased the potency and small lipophilic substituents *peri* to the ring nitrogen enhanced the potency. They then went on to develop phenazine derivatives with similar features.⁷⁰ These behaved in the same fashion

and were dual topoisomerase I and II inhibitors and poisons. These compounds showed considerable growth inhibition of human colon tumour lines and overall an improvement of the inhibition achieved by the bis(acridine) analogues.



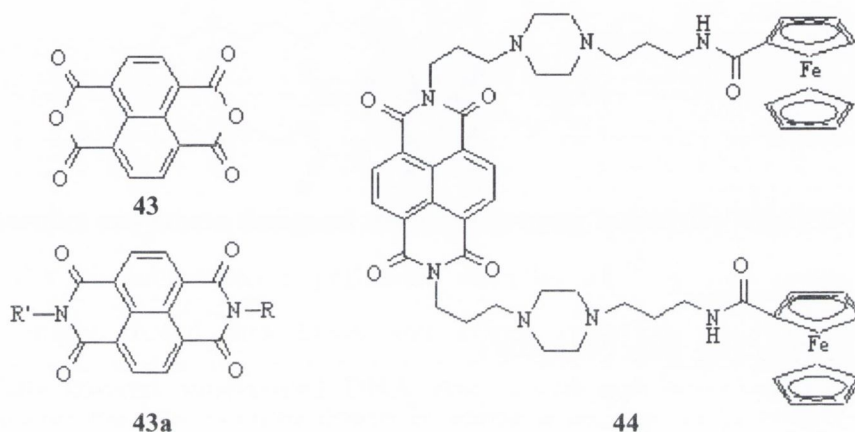
The advances and anti-tumour success of naphthalimide synthesis has been discussed. Naphthalene diimides are well known intercalators. Whilst most examples to date have detailed linearly linking two naphthalimides, such diimides were considered interesting as potential spacer scaffolds for the development of novel bisnaphthalimides within the Gunnlaugsson group. The following passages introduce naphthalene diimides and their uses in various fields of research.

1.5 Naphthalene Diimides

1.5.1 Introduction

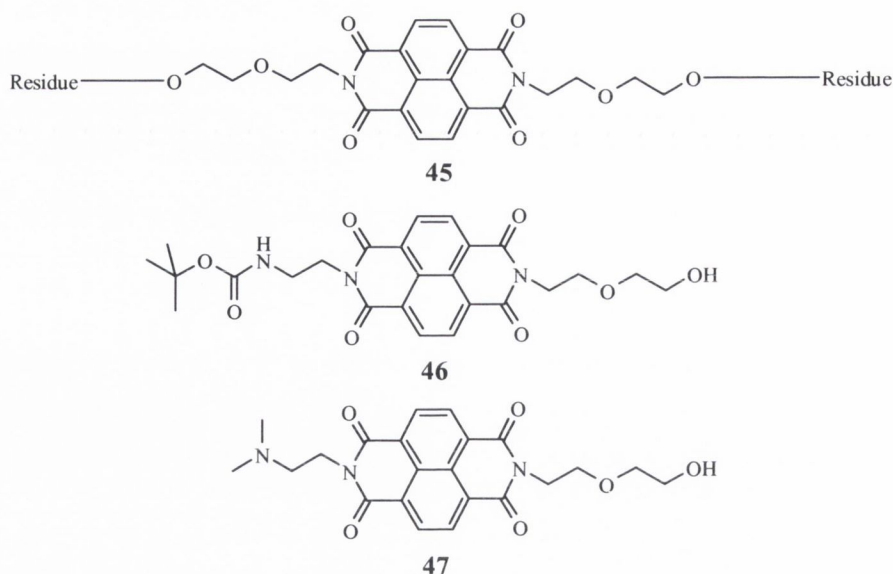
Derivatives of naphthalene tetracarboxylic dianhydride, **43**, known as naphthalene diimides, such as **43a**, are employed in many branches of science and technology. They are commonly used as optical brighteners,⁷¹ laser dyes,⁷¹ fluorescent labelling systems,⁷² conducting materials^{73,74} and, importantly, are widely reported as intercalators of DNA.⁷⁵ The extended planar, aromatic nature of naphthalene diimides means they can readily slot between base pairs and partake in π - π interactions with the nucleotides above and below. The fact that naphthalene diimides may be derivatised to offer novel intercalating species, which also happen to display interesting photophysical characteristics, makes these candidates for incorporation into DNA probes and possibly DNA targeting drugs. For

example, Takenaka *et al.* have developed a naphthalene diimide derivative bound to a gold electrode, **44**.⁷⁶ When this is washed with solutions of *ct*-DNA interaction of the diimide produces an electrochemical signal and in this way the system allows subpicomolar detection of DNA.



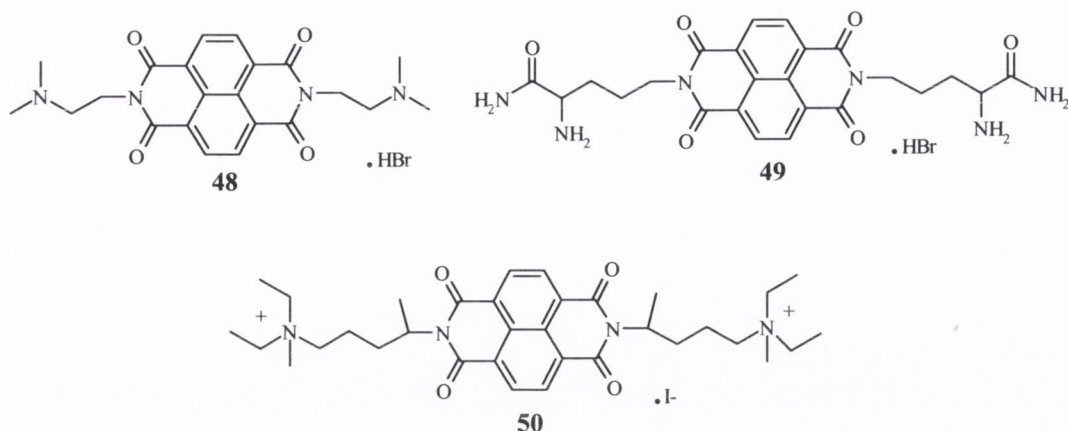
1.5.2 DNA Stabilisers

McLaughlin *et al.* have spent the past decade developing naphthalene diimide systems as DNA stabilisers.⁷⁷⁻⁸⁰ A series of naphthalene and perylene based diimide derivatives were designed as spacers to bridge the terminal base triplet of a DNA triplex and provide base-stacking.⁷⁷⁻⁸⁰ Compound **45** is an example of such a compound which is capable of targeting single-stranded nucleic acids with the formation of hairpin triplexes. The desired stabilisation was evident from the enhancement of the melting temperature (T_m) of the DNA by a significant 28 °C and from the hypochromicity in the fluorescence emission. This stabilisation of the triplex structure may be advantageous in that it increases the lifetime of the 'antisense complex' of the triplex and the linker, which interferes with replication and other biological processes. They have also shown that naphthalene diimide intercalators, *e.g.* **46** and **47** are very efficient long-range DNA photooxidants.⁸¹ When covalently tethered to a triplex-forming oligonucleotide it was observed that charges can migrate over 25-34 base pairs in both directions along the helix and along both duplex strands generating base lesions. This long-range oxidation process results in specific damage to the 5' guanine of 5'-GG-3' sites.

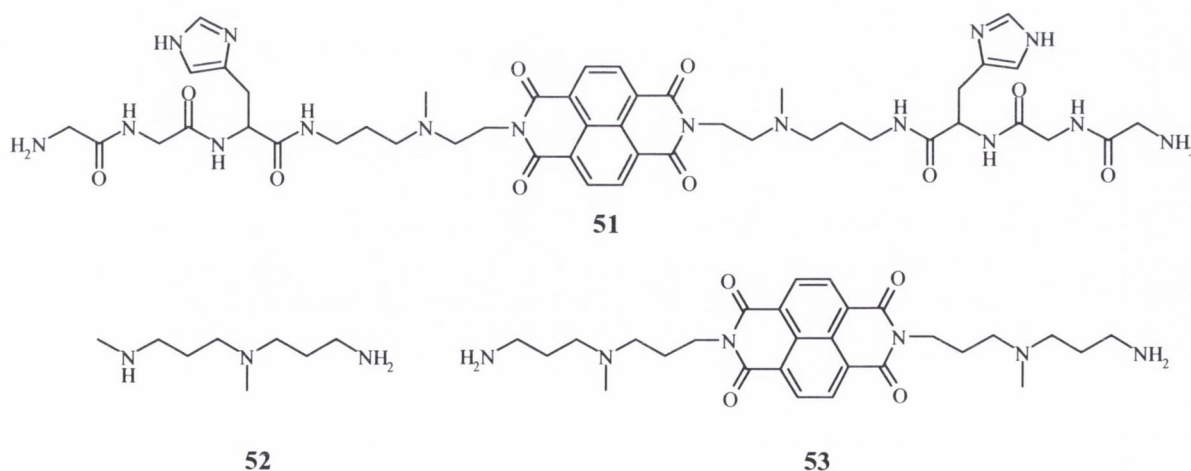


1.5.3 Threading Intercalators

Wilson *et al.* studied a series of naphthalimides and corresponding naphthalene diimides as probes for groove interactions and intercalation, *e.g.* **48**, **49** and **50**.⁷⁵ They found that naphthalene diimides 'thread' into DNA, that is, the aromatic core slots into the helix and intercalates whilst the bulky substituents wind into opposite grooves of the DNA at the intercalation site. Thus, the aromatic core threads the whole, larger molecule through the DNA with pendant arms extending into the grooves. This added factor of groove binding is thought to enhance the binding affinity and 'lock' the molecule into the helix leading to added disruption of the DNA and of replication processes. Changes evident in viscometric studies, circular dichroism and absorption spectroscopy all supported intercalation and docking modelling together with kinetics experiments implied major groove binding. This was one of the earliest reports of major groove binding molecules since most DNA intercalators were found to exhibit only minor groove binding. It is possible that the intercalation of the large extended area of the diimide core is optimised when one side chain is positioned in the major groove and the other is positioned in the minor groove, whilst for smaller intercalators positioning of both in the minor groove is sufficient. More recently they found that such compounds bind preferentially to GC sequences and that the association and dissociation of the diimide-DNA complex is much slower than that of classical intercalators and this supports the threading intercalation model.⁸²

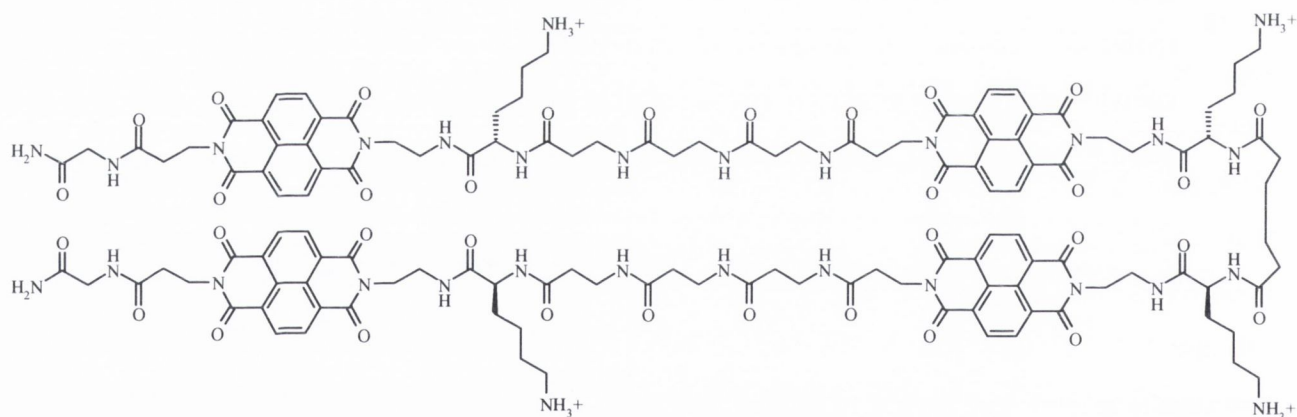


Steullet and Dixon designed a Ni(II) chelating system by attaching the peptide to a Gly-Gly-His bis-substituted naphthalene diimide, **51**.⁸³ It was hoped that the metal complex might thread into DNA and affect oxidative cleavage. It was found to successfully convert supercoiled DNA into nicked and linear forms. The naphthalene diimide derivative was a hundred times more effective in cleaving DNA than solely the Ni(II) complex of the GGH peptide. They also studied a variety of anthraquinone and naphthalene diimide intercalating derivatives with amine-containing side chains such as **52** and its naphthalene diimide derivative **53**.⁸⁴ These cleaved plasmid DNA at abasic sites more effectively than the amine would itself. It was proposed that the threading of these molecules positions the amino functionalities in the grooves and thus they can achieve cleavage more readily than they would alone. Those intercalators with two amine moieties were more successful than those with only one amine.



1.5.4 Naphthalene Diimides as Polyintercalators and Anti-Microbials

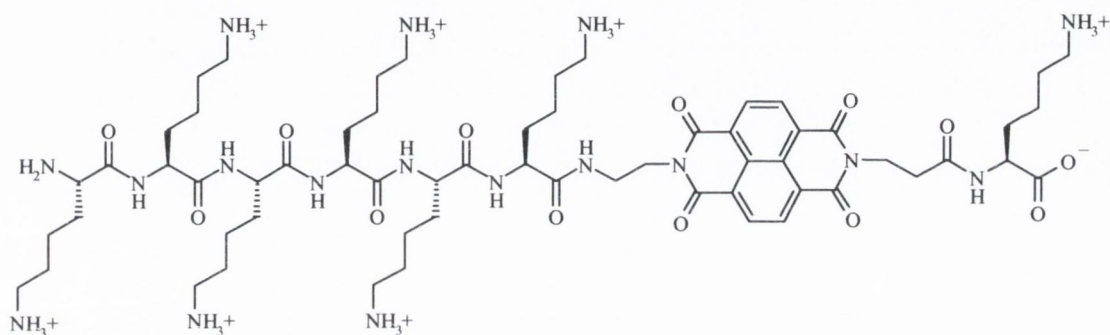
Iverson *et al.* at the University of Texas have developed elegant threading polyintercalators.⁸⁵⁻⁸⁹ Compound **54**, which incorporates the amino acids lysine, alanine and glycine, was designed as a molecular scaffold for exploring sequence specific DNA recognition, using four naphthalene diimide units linked by flexible peptide bridges. This molecule was found to tetraintercalate with the linkers positioning themselves alternately in the minor and major grooves, a topology which Iverson *et al.* has compared with a snake winding its way along a ladder.⁸⁸ There are possible advantages to a polyintercalating scheme over that of simple naphthalene diimides such as **51**. The interactions with functional groups in both grooves would confer enhanced sequence specificity and increased disruption to protein-DNA interactions since both grooves are obscured.⁸⁷ Moreover, slow dissociation of the intercalator-helix complex and such a long architecture may permit recognition of relatively long DNA patterns. They have since developed octakisintercalators with a motif similar to that of **54**.⁸⁵ Iverson hypothesised that the lack of functionality of this molecule combined with its flexibility would limit the sequence selectivity and therefore a new generation of polyintercalators are currently in development with more rigid linkers and more potential hydrogen bonding sites.^{85,87}



54

Before exploiting naphthalene diimides as polyintercalators Iverson *et al.* investigated their application in antimicrobial systems.⁹⁰ Sequences of amino acids, particularly L-lysine and glycine, were attached to the 1,4,5,8-naphthalene tetracarboxylic diimide. Structure-activity studies showed that increasing number of positively charged lysine motifs (at least seven such residues), such as in **55**, provided the optimum activity against Gram-negative (*e.g. E. coli*) and Gram-positive (*e.g. B. subtilis*) bacteria. One naphthalene diimide unit (which is hydrophobic and electron deficient) was sufficient to

confer this activity, even though its position in the sequence of peptides did not seem important to the anti-bacterial activity.⁹⁰ It is thought that since these molecules are unlikely to penetrate into the interior of bacterial cells the mode of action probably involves disruption of the bacterial membrane. D-lysine was found to be as effective as L-lysine and this negates a specific receptor mediated process and instead the physical properties of the molecule are more crucial.

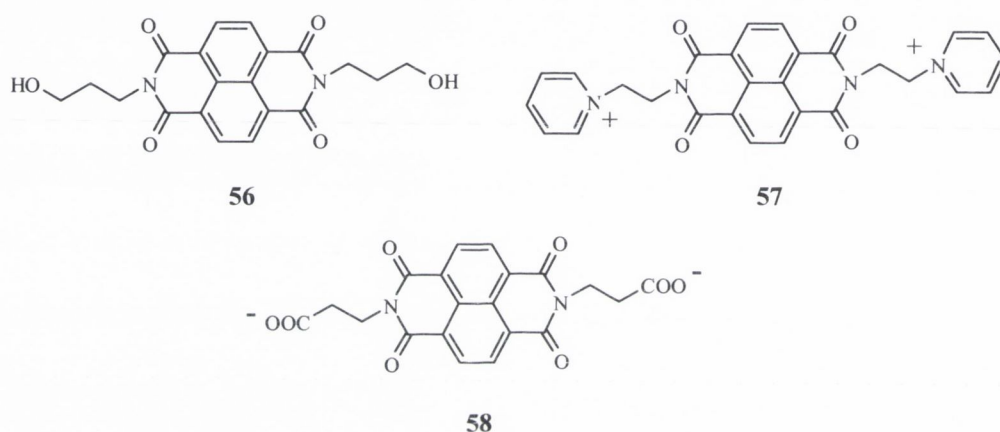


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1.5.5 Photophysics of Naphthalene Diimides

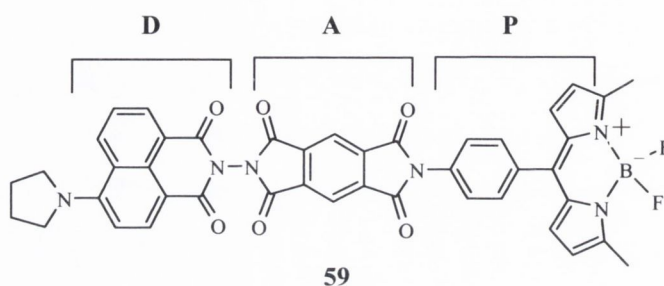
Kelly *et al.* have spent much of the past decade investigating the interesting photophysics associated with naphthalene diimides.⁹¹ For example, they have demonstrated that compounds such as **56** and **57** can undergo photoinduced electron transfer from guanosine 5'-monophosphate to the lowest electronically excited triplet state.⁹¹ These compounds can be said to achieve the photooxidation of the nucleic acids *via* their triplet state and as such may be termed redox-active photonucleases. Although the site of base oxidation depends upon the thermodynamic properties of the diimide employed, a general kinetic trend has been observed, mostly likely due to the planar extent of the structures. Diimide electron acceptors can be used to conduct selective and guanine-specific oxidative damage or non-selective single-electron oxidation. The correlation between the extent of diimide-calf thymus DNA complexation and the triplet state and radical production after pulse photolysis has also been investigated.⁹² It was found that yield of the long-lived triplet state decreased linearly as the degree of complexation increased.⁹² The radical anion observed by transient absorption spectroscopy can be accounted for by self-quenching interactions. The fact that no additional radical anions

were produced by the DNA-bound molecules is consistent with very rapid electron transfer and charge recombination processes initiated by the electronically excited singlet state. By comparison, when water soluble carboxyl-substituted naphthalene diimides such as **58**, were examined, it was found that the one-electron-reduced naphthalimide is produced, after pulsed excitation at 355 nm, possibly *via* intramolecular electron transfer from the covalently attached carboxylate group.⁹³ These intermediates therefore react with electron acceptors as opposed to electron donors as previously seen. Kelly *et al.* suggest that rapid homolytic bond cleavage occurs yielding a reactive carbon-centred radical.⁹³ Such carboxyl functionalised naphthalene diimides are known to display anti-tumour activity and interact readily with DNA and proteins.

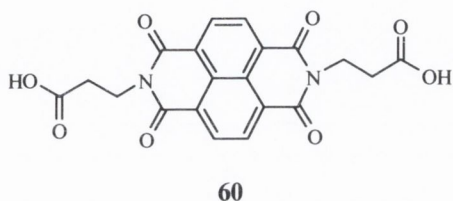


1.5.6 Supramolecular Uses of Diimides

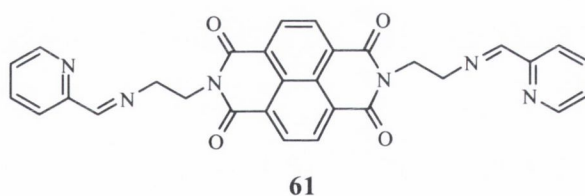
Wasielewski *et al.* have studied the photophysics of extended aromatic diimides in detail and in the 1990's incorporated a derivative into a molecular system for probing an electric field, **59**.⁹⁴ The electron deficient pyromellitic diimide (**A**) acts as an electron acceptor when attached to naphthalene-1,8-dicarboximide (an electron donor, **D**) and to an indacene derivative (a molecular probe, **P**). Excitation of **D** leads to an electron transfer from **D*** to **A** in **59** and this subsequently generates an effective electric field at **P** of 6 ± 1 MV/cm. The magnitude of this field implies the possibility for the creation of a molecular switch in which the photogenerated electric field is utilised to switch 'on' or 'off' a secondary electron transfer reaction.⁹⁴



Tomasulo *et al.* have recently developed self-assembling naphthalene diimides with two terminal carboxylic acids, *e.g.* **60**.⁹⁵ Aggregation in aqueous solution spontaneously forms cylindrical, rod-like microstructures on a solid surface. In these supramolecular arrays the chromophoric cores allow examination of the structure by absorption and fluorescence spectroscopy. However, the mechanism that results in this elegant self-assembly has yet to be understood.



The work of Lichelli's group has used diimides, such as **61**, as bischelating ligands.⁹⁶ In the presence of stoichiometric amounts of Zn (II), Cu (I) and Cd (II), in chloroform (CHCl₃) and acetonitrile (CH₃CN), these ligands form nonhelical [2 + 2] adducts. This metal induced self-assembly is signalled by excimer-type emissions due to the intramolecular interaction of the two naphthalene diimide subunits. When an excess of the metal ion is used a disassembling occurs and a 2:1 metal:ligand complex is formed. The excimer can no longer form since the interaction between the diimides has been lost. This means that the assembly/disassembly process can be monitored by the appearance and disappearance of the excimer band in the emission spectrum.



1.6 Selectivity and Peptides

1.6.1 Sequence Selectivity

A compound is sequence selective if it seeks out certain patterns in DNA over others and should recognise and efficiently bind such patterns. Examples of such selectivity include the preference for AT regions over GC or AA over GG and a preference for a certain gene code such as binding the Philadelphia chromosome (which results from

the reciprocal translocation between a chromosome 9 and a chromosome 22 and is involved in leukaemia) over all others. Selectivity is related to bulk, steric arrangement, electrostatic configuration or stereochemistry leading to preferred binding modes with specific regions of the DNA helix.

Interaction with this sequence will lead to disruption of the replication process and damage the cell. Therefore, one can see that the mere interaction with DNA is not the end of the story and one can further enhance and attenuate the binding by redesigning the molecule itself so that it seeks out particular patterns and sequences and does not simply and randomly attack all rapidly dividing cells.

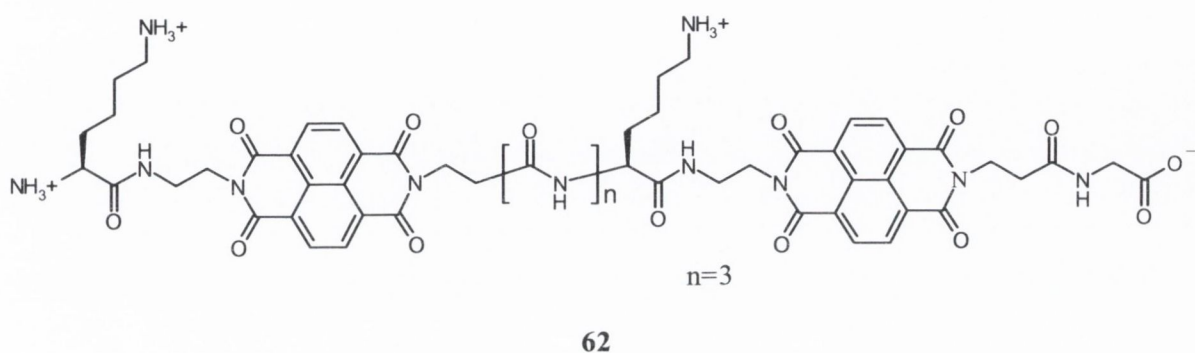
The challenge is therefore to specifically and efficiently target a particular sequence of DNA. While derivatives of 1,8-naphthalimide readily bind DNA, incorporating recognition moieties will combine specificity with anti-tumour activity.

Small molecules, such as naphthalimides *e.g.* **14**, **15** and **16**, readily interact with DNA, but in a promiscuous manner. A larger molecule can be designed to achieve selective interaction because of its size, steric hindrance, stereochemistry and, importantly, its preferred binding modes. While amino acids are small structures they can easily be incorporated into larger sequences of amino acids or peptides. Such molecules have inherent shapes and have hydrogen binding possibilities, this imbues them with great potential for selective interaction with certain, targeted patterns of base pairs. The following section will discuss how other groups have achieved site discernment.

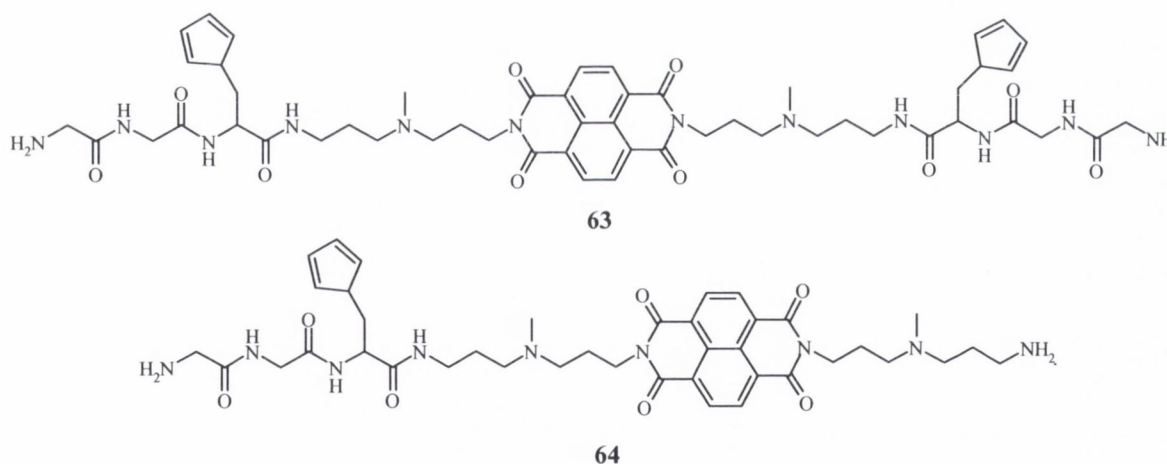
1.6.2 DNA Binders attached to Amino Acids and Peptides

Iverson *et al.* have focused on the design of polyamides and naphthalene diimides in search of sequence selective DNA-binding agents.⁹⁷ The polyamides developed by his group achieve a one-to-one correspondence, recognising DNA base pairs *via* specific hydrogen bonding patterns in the minor groove. They have developed over three hundred polyintercalating molecules employing the 1,4,5,8-naphthalene tetracarboxylic diimide structure which is known to thread and groove bind. Most intercalators have a simple, inbuilt preference for certain base pair steps (*e.g.* 5'-Py-Pu-3') but such specificity can be overridden with noncovalent contacts with the helix.⁹⁷ These can be achieved by complex, rigid sidechains. Iverson *et al.* hypothesised that the inclusion of amino acid linkers between naphthalene diimide cores might render the polyintercalator pattern selective.⁹⁷ The resulting combinatorially synthesised molecules contain naphthalene diimides, amides and lysine motifs, such as in **62**.⁹⁷ These compounds were screened for sequence

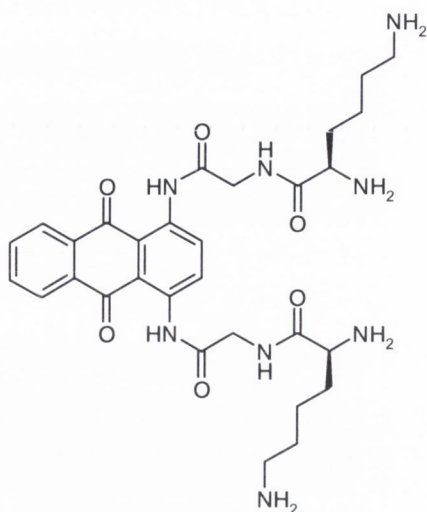
selectivity using DNase I footprinting with a 231 base-pair restriction fragment and were found to exhibit preference within a 15 base pair GC rich repeat. In particular, a lysine-tris- β -alanine derivative was found to preferentially bind within a 19 base pair palindrome.⁹⁷ Thus, subtle changes in the amino acids employed can tune the binding of naphthalene diimides.



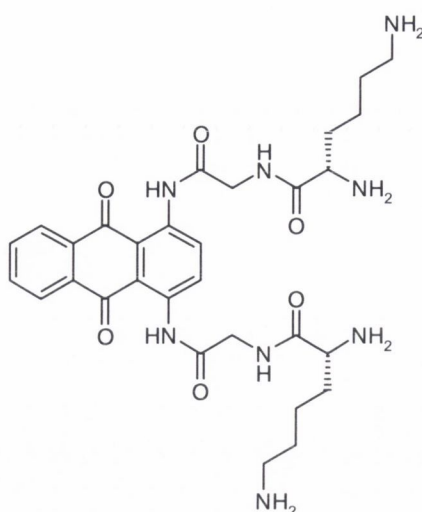
Steullet and Dixon have developed two Gly-Gly-His based naphthalene diimides, **63** and **64**.⁹⁸ Both of these compounds demonstrated single-strand cleavage of pBR322 DNA in the presence of nickel acetate and oxone. This effect was thought to be a result of the high concentration of the metal chelating conjugate in the groove enhanced by the intercalation of the naphthalene diimide unit.



A range of novel peptide functionalised anthraquinones, such as **65** and **66**, have been synthesised by Palumbo *et al.*⁹⁹ A strong DNA binding constant (comparable with that of the anti-cancer agent mitoxantrone) was witnessed for compounds containing glycine and L-lysine side chains. These compounds were threading binders and preferred GC sequences. The enantiomer containing D-lysine had a stronger binding constant.¹⁰⁰

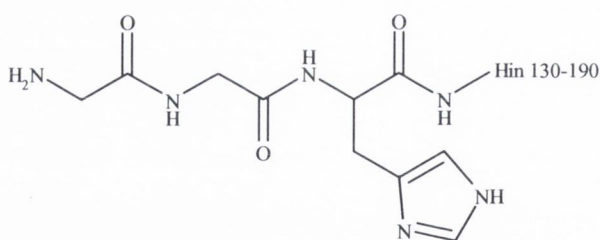


65



66

Dervan *et al.* have employed amino acids in the design of sequence selective DNA-cleaving proteins.^{101,102} The tripeptide H-Gly-Gly-His-OH was bound to the amine functionality of the DNA-binding domain of histidine recombinase. This provided a new protein, Gly-Gly-His(Hin 139-190), **67**, synthesised by solid phase methods. Gly-Gly-His is the copper or nickel binding domain for human serum albumin and Hin 139-190 binds DNA. This novel protein can bind to DNA at four sites comprised of thirteen base pairs. In the presence of sodium ascorbate, hydrogen peroxide along with less than one equivalent of Cu (II) the protein was found to cleave predominantly at one of the Hin sites.

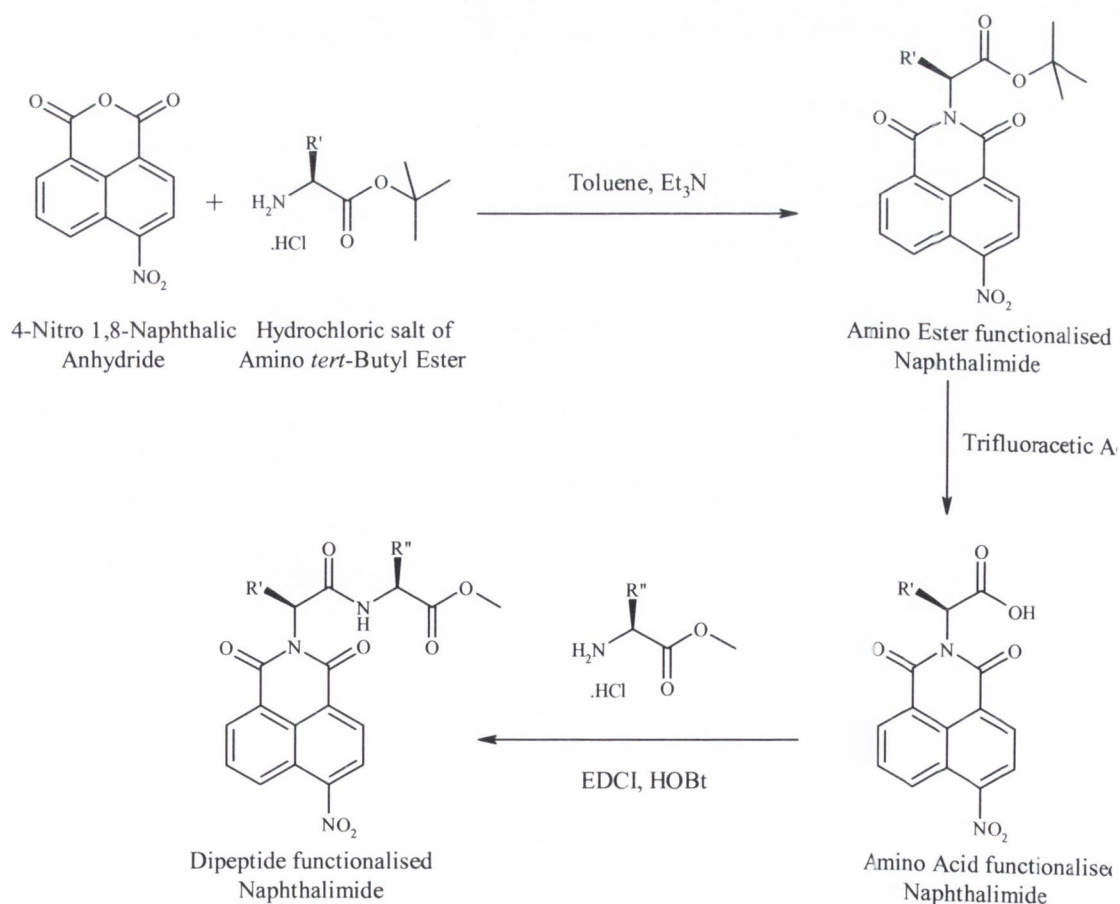


67

1.7 Naphthalimide Advances in the Gunnlaugsson Group

Over the past eight years, the Gunnlaugsson group has been interested in derivatives of 1,8-naphthalimides. This research has focused on the incorporation of amino acid, ester and peptide moieties into the naphthalimide structure with a view to accomplishing nucleic acid recognition. This project was developed with the intention of achieving a targeted treatment for cancers such as chronic myeloid leukaemia and prostate cancer. To date, this programme has amassed *ca.* three hundred novel mono-peptide, dipeptide and bis-system conjugates of 1,8-naphthalimide, in collaboration with Prof. Mark Lawler, Department of Haematology, St. James' Hospital, Dublin, the anti-tumour activity of this library against several cell lines has been investigated.

The synthetic strategies developed for the functionalisation of the naphthalimide nitrogen is shown in **Scheme 1.1**. In this way, tertiary butyl esters of a range of amino acids were added to the naphthalimide scaffold. These could then be converted to their corresponding acids and subsequently coupled to other amino methyl esters produced peptide functionalised naphthalimides using standard peptide chemistry. Phelan focused on dipeptides involving L-alanine and L-leucine and varied the substituents at the 3 and 4 positions and the order of the amino acids themselves in the dipeptide chain.

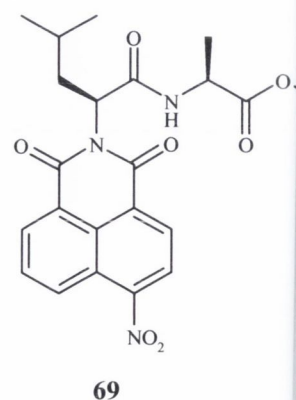
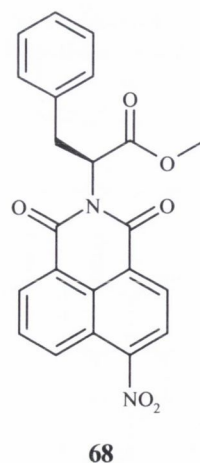


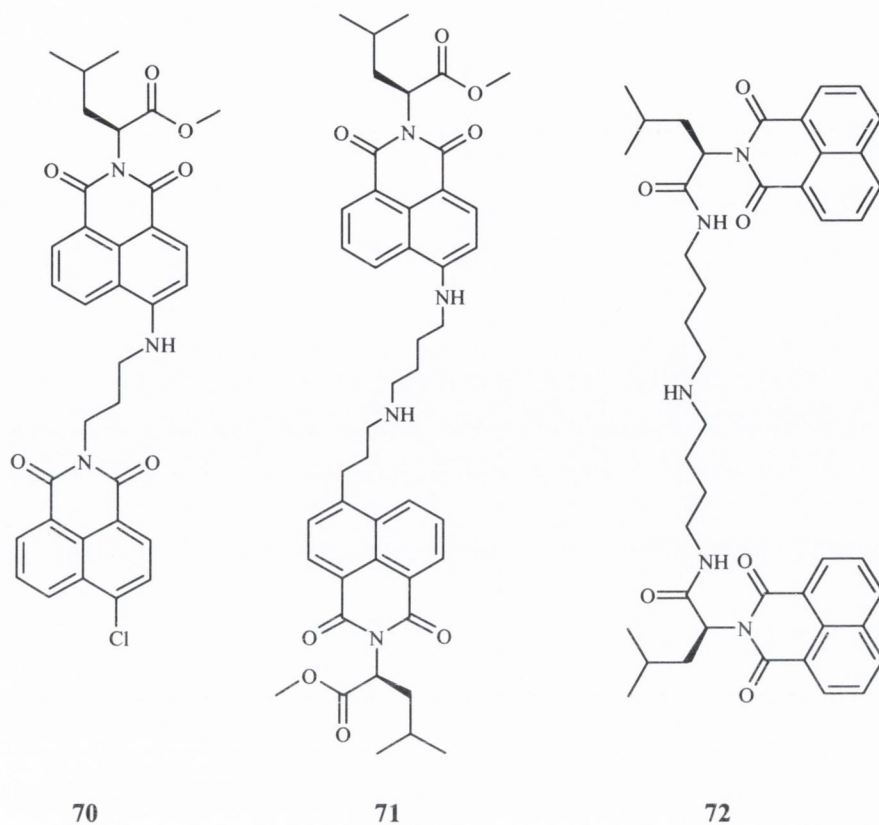
Scheme 1.1 General synthetic route to peptide functionalised 4-nitro 1,8-naphthalimides

The earliest compounds designed during this research were mono-peptide derivatives of 3- and 4-nitro and 3- and 4-amino 1,8-naphthalimides. These involved the methyl and tertiary butyl esters of glycine, L-alanine, L-leucine and L-phenylalanine. Of these, the 4-nitro substituted variations exhibited the highest activity against HL-60, a CML cell line. IC₅₀ values such as 1.95 μ M for **68** were obtained compared with that of mitonafide of 0.23 μ M.¹⁰³ Of Phelan's families of compounds **69** was the most potent against HL-60 cell lines with an IC₅₀ of 1.57 μ M and against K562, a more chemoresistant CML cell line, with an IC₅₀ of 3.49 μ M.¹⁰³

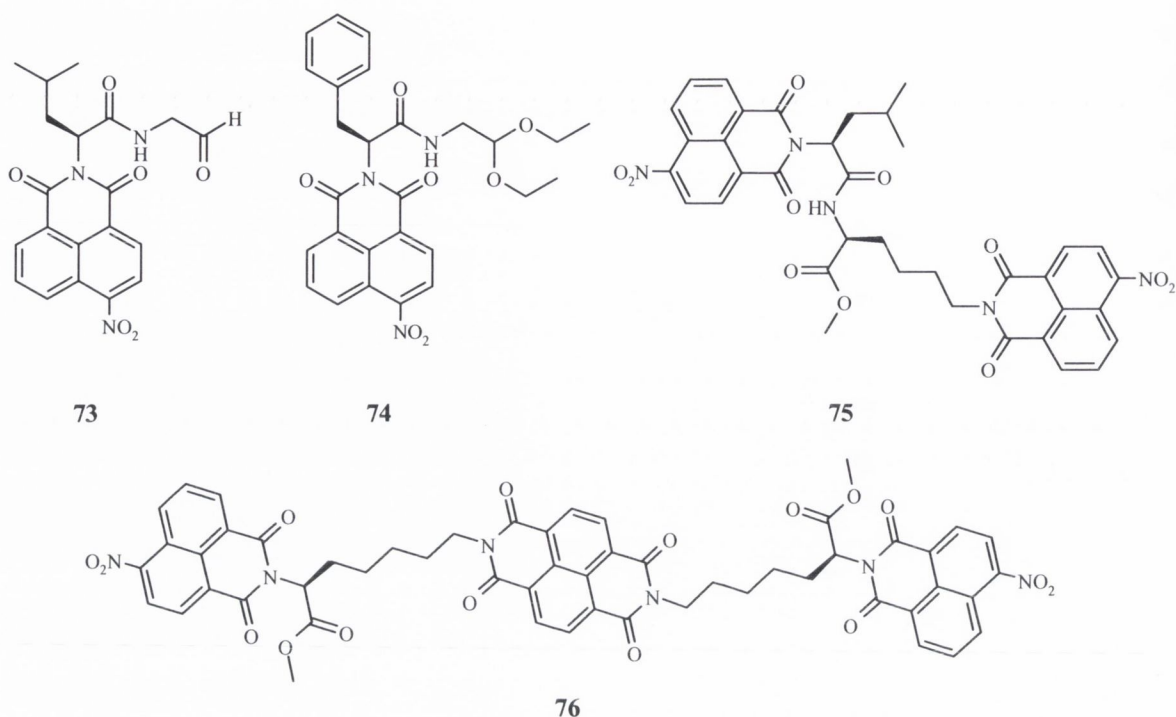
This early research helped to establish that for the mono-peptide derivatives the larger the *N*-substituted amino acid function, *i.e.* L-leucine and L-phenylalanine, were more effective than the smaller glycine and L-alanine and that the size of the ester at the second position, methyl or tertiary butyl, seemed to have little impact on the potency of the compound. The biological studies of the Gunnlaugsson compounds have consistently found the 4-nitro compounds to be more active than the 3-nitro, this is in contradiction of observations made by Braña *et al.*

This work was further developed by Blais by the incorporation of functionalised derivatives into 'head-to-tail' bisnaphthalimides (so-called since the units are linked from the 4-position of one unit to the imide position of the other) by reacting the 4-amino derivative with 4-nitro-1,8-naphthalic anhydride.¹⁰⁴ The activities for these compounds were improvements on the mononaphthalimide molecules, *e.g.* **70** has an IC₅₀ value of 0.81 μ M compared with a value > 100 μ M for the parent molecule. Some 'tail-to-tail' bisnaphthalimides (so-called since the units are linked from the 4-position of both naphthalimides) with aliphatic aminoalkane linkers were also synthesised as part of this programme, *e.g.* **71** which has an IC₅₀ value of 2.70 μ M. A family of 'head-to-head' (so-called since the units are linked through the imide position of both naphthalimides) bisnaphthalimides were also produced such as **72**. These involved the condensation of aliphatic aminoalkanes with an amino acid functionalised naphthalimide. These were interesting compounds and the spermidine linked derivative was very active in HL-60 cell lines with an IC₅₀ of 3.46 μ M.





This work was further extended by Hussey who developed various dipeptide naphthalimides along with naphthalimide based colorimetric sensors, exploiting the photochemical potential of these compounds. As potential anti-cancer agents a series of 4-nitro derivatives that included L-phenylalanine and L-lysine, again varying the sequence of the amino acid motifs and peptide functionalised aldehyde, thiazolidine and thiazole derivatives of 1,8-naphthalimides were also developed.¹⁰⁵ These naphthalimide families synthesised by Hussey highlighted some interesting trends. It was observed that monopeptide methyl esters are more cytotoxic than their tertiary butyl esters which in turn are more cytotoxic than the corresponding acids. Also, the introduction of aldehydes (*e.g.* **73**, $IC_{50} = 4.50 \mu M$), acetals (*e.g.* **74**, $IC_{50} = 2.00 \mu M$) and olefins at the β -amino functionality showed promising activity.¹⁰⁵ Hussey made some ‘head-to-head’ L-lysine (*e.g.* **75**, $IC_{50} = 3.20 \mu M$) and L-ornithine based bisnaphthalimides as well as some examples of ‘tail-to-tail’ and ‘head-to-tail’ systems. In the latter stages of the project a novel bisnaphthalimide, **76**, was synthesised that involved the ‘head-to-head’ linkage of two lysine functionalised naphthalimides *via* a 1,4,5,8-naphthalene tetracarboxylic dianhydride core.¹⁰⁵ Although this compound was not biologically tested due to solubility issues it did inspire an interest in the use of naphthalene diimide cores in Gunnlaugsson bisnaphthalimides.



This collection of work combines to provide an overview of the general trends exhibited by the 1,8-naphthalimide derivatives designed by the Gunnlaugsson group which can be summarised as follows;

- methyl esters are typically more potent than tertiary butyl esters
- esters are more potent than their corresponding acids
- nitrogen containing substituents on the aromatic ring are more active than less basic oxygen or sulphur containing groups
- 4-substituted naphthalimides are more active than their 3-substituted analogues
- 4-nitro substituted naphthalimides are more cytotoxic than the 4-amino analogues
- bulkier amino ester functionalisation at the first position leads to more potent compounds
- dipeptide conjugates are more cytotoxic than monopeptides
- bisnaphthalimides tend to be more cytotoxic than their mononaphthalimide parent compounds

This research programme has, therefore, successfully produced a range of potent naphthalimides with various functionalities and motifs. Many of these compounds have promising activities in HL-60 and K562 cell lines and are potential anti-cancer agents. The

breadth of possible application and the mode of action of these families have yet to be fully elucidated. The ongoing nature of this project proves how such versatile and interesting naphthalimides provide an intriguing challenge and endless possibilities for further diversification and adaptation.

This chapter has introduced 1,8-naphthalimide derivatives and discussed their merits as anti-cancer agents. The possibilities of developing novel naphthalimides and bisnaphthalimides and tuning their potency to target cancerous DNA has also been described. The many directions available to an organic chemist in the design and development of original naphthalimides make this a challenging and interesting field of synthesis and design. To this end, the research detailed within this thesis sought to incorporate the DNA binding prowess of naphthalene diimides with the well documented anti-tumour activity of 1,8-naphthalimides. It was hypothesised that the melding of two such motifs might result in a dually active molecule with enhanced potency, this will be detailed in the following section.

1.8 Project Objectives

1.8.1 Rationale Towards Novel Bisnaphthalimides

The ability to render a naphthalimide derivative sequence selective by the introduction of amino acids functionalities has been explained in this introductory chapter. The possibility that bisnaphthalimides may interact with the macromolecule in a number of different ways, including one terminal intercalating while the other binds in the major or minor groove has also been highlighted. The interaction of naphthalene diimides with DNA by intercalation or a 'threading' mode is widely reported in the literature.

If these three motifs and their characteristics were combined in one larger molecule a novel bisnaphthalimide with the potential for greater binding affinity and selectivity might result. This fundamental rationale developed into the basis for this research project, the design and synthesis of families of novel bisnaphthalimides using the potent dipeptide naphthalimide conjugates developed in the Gunnlaugsson laboratory and incorporating naphthalene diimide cores as linkers. Using this strategy 'tail-to-tail' and 'head-to-head' bisnaphthalimides could both be constructed according to this general template *via* different synthetic routes. Each of these routes would involve the construction and subsequent incorporation of two different structural motifs.

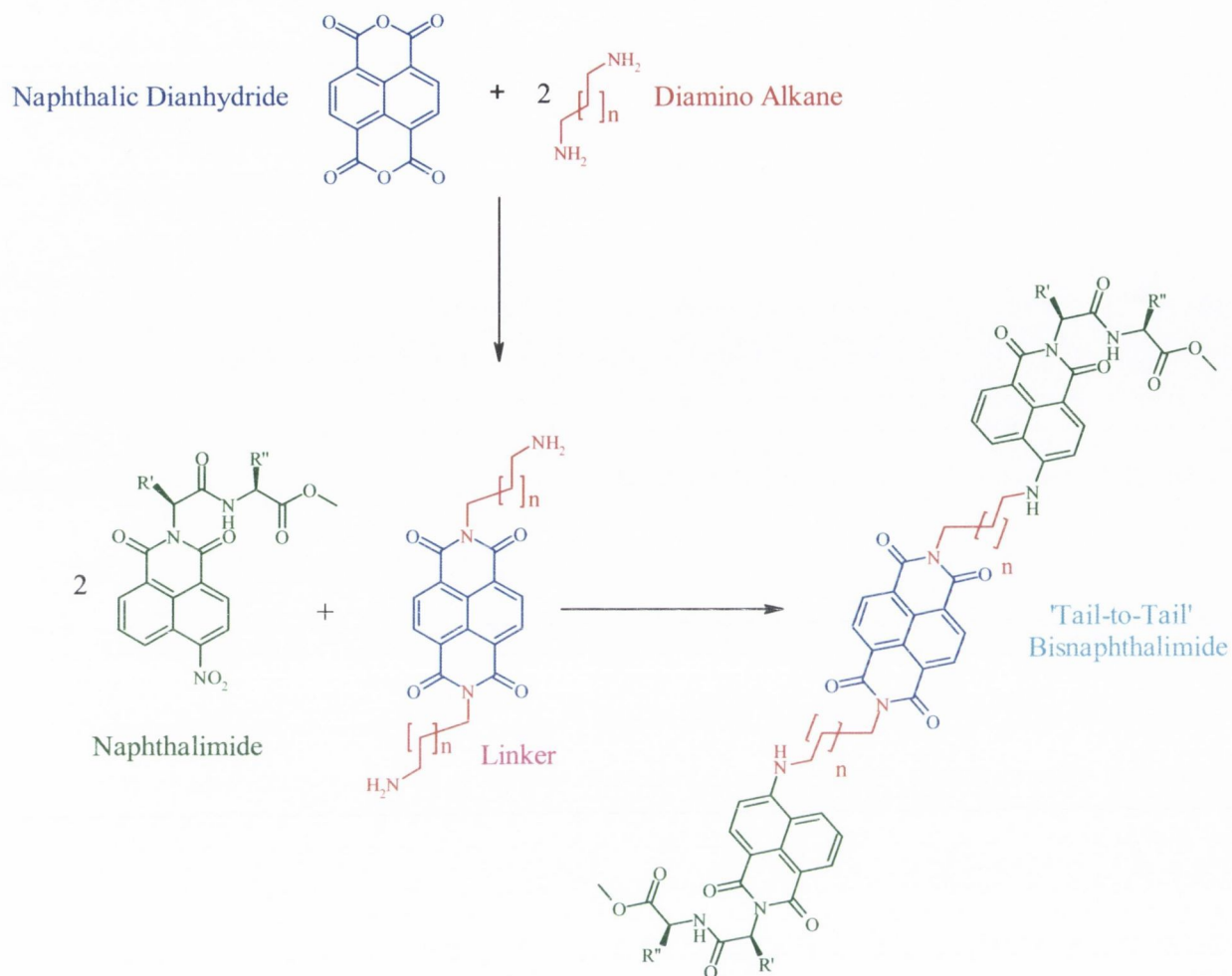
1.8.2 'Tail-to-Tail' Bisnaphthalimides

Two principal building blocks would be used to construct these novel 'tail-to-tail' bisnaphthalimides, dipeptide functionalised naphthalimides and diimide spacer moieties, **Scheme 1.2.**

It was decided to build on the earlier success within the Gunnlaugsson group by focussing on a group of amino acids that previously showed promise in biological assays in other dipeptide mono and bisnaphthalimides. L-phenylalanine, L-lysine, L-leucine and L-alanine would be the candidates of choice. The 4-nitro-1,8-naphthalimides with L-phenylalanine, L-lysine (protected and unprotected) and L-leucine at the first position with L-alanine methyl ester at the second would be synthesised for use in 'tail-to-tail' bisnaphthalimides

1,4,5,8-Naphthalene tetracarboxylic dianhydride would be used as the skeleton from which to form the 'spacer' moiety to bridge two naphthalimide units. This planar aromatic core has great potential for slotting in between the base pairs in DNA and for π - π stacking. By itself intercalating, this core would 'thread' the larger bisnaphthalimides molecule through the DNA. In order to connect the core with the naphthalimides a diamino alkane would be used. Reacting this with the dianhydride would produce a naphthalene diimide with two aliphatic amine chains extending from both imide nitrogens. These amines could then each substitute the 4-nitro group on each naphthalimide moiety and ultimately give the desired bisnaphthalimide.

By using different diamino alkanes the length of the spacer unit could be readily altered. It can be reasoned that the binding of such a bisnaphthalimide would involve the intercalation of the diimide and the groove binding of the naphthalimides or the groove binding of the diimide and the intercalation of both naphthalimides or the intercalation or groove binding of all three units. Whichever of these modes of interaction is dominant the length of the spacer could be expected to impact on the ease and degree of binding and there is most likely an optimum distance between the three extended aromatic regions. Thus, there would be merit in varying the length of the linker itself as well as changing the dipeptide functionalities.



Scheme 1.2: The proposed synthetic route to 'tail-to-tail' bisnaphthalimides

1.8.3 'Head-to-Head' Bisnaphthalimides

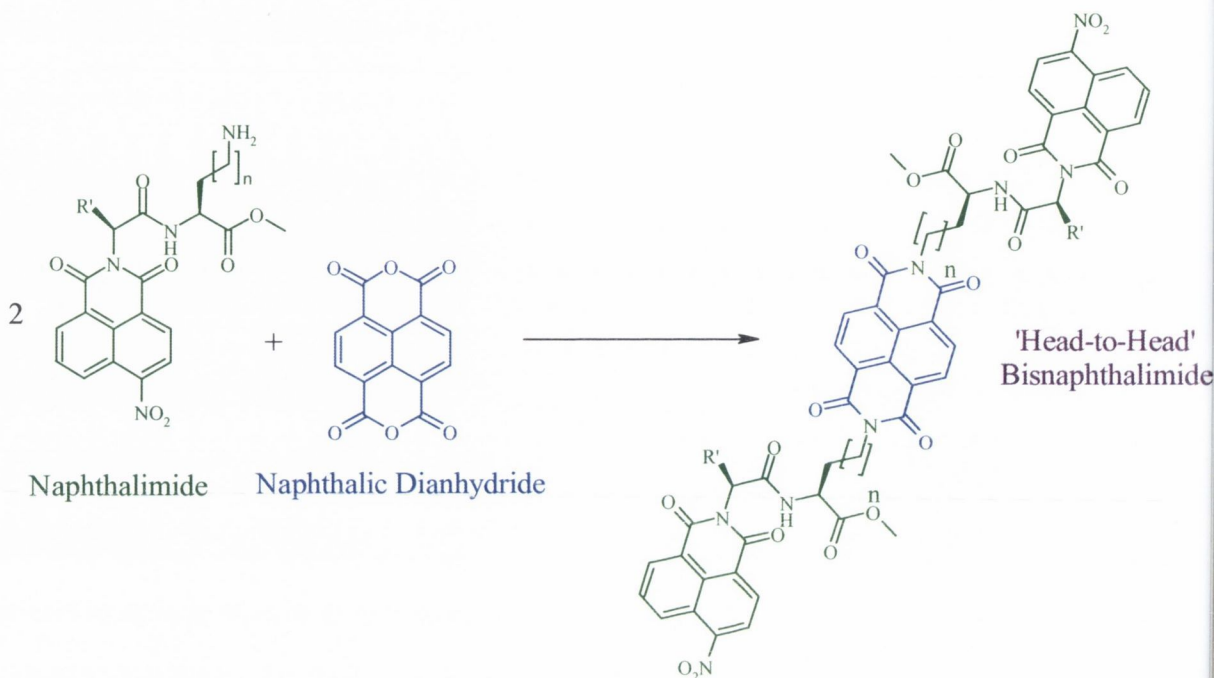
Similarly, two building blocks would be used to construct a new family of 'head-to-head' bisnaphthalimides, dipeptide functionalised naphthalimides with pendant amines and the spacer core moiety, **Scheme 1.3**.

The 4-nitro-1,8-naphthalimides with L-phenylalanine at the first position and L-lysine, L-ornithine and L-2,4-diamino butyric methyl esters at the second position would be synthesised for use in a series of 'head-to-head' bisnaphthalimides.

Since these naphthalimides contain free terminal amines no amine functionalised naphthalene diimides would be necessary. It would simply be a matter of reacting the naphthalimide with 1,4,5,8-naphthalene tetracarboxylic dianhydride to produce the desired bisnaphthalimides. A dicarboxyl linker motif would also be explored using oxalyl chloride.

Such bisnaphthalimides could probably operate in the same fashion as the 'tail-to-tail' family although since the 4-nitro group is maintained the cytotoxic effect of this could

be examined. By employing L-lysine, L-ornithine and L-2,4-diamino butyric methyl esters in this family the length of the linker chain would be varied and the effect of so doing could also be investigated.



Scheme 1.3 ; The proposed synthetic route to 'head-to-head' bisnaphthalimides

Therefore, the design and synthesis of two families of bisnaphthalimides was originally planned and would involve several synthetic steps. Firstly, the naphthalimide mono-peptides, acids and dipeptides were to be synthesised. The construction of the naphthalene linkers would then be addressed, the propyl, pentyl and hexyl analogues were all to be synthesised and optimised. These would be employed in the formation of some twenty 'tail-to-tail' bisnaphthalimides. Six 'head-to-head' bisnaphthalimides would be constructed from the phenylalanine-lysine dipeptide and its analogues reacting with the naphthalic dianhydride and an alternative dicarboxyl linker motif. This design and synthesis programme is discussed in detail in *Chapter Two*.

1.8.4 Investigating Novel Bisnaphthalimides

A selection of these 'tail-to-tail' bisnaphthalimides and 'head-to-head' bisnaphthalimides would be chosen for a series of photophysical and physical investigations in an effort to probe their interactions with DNA.

The effect of successive additions of *ct*-DNA to a cuvette solution of the compound would be monitored by absorption (ultraviolet-visible, UV-visible) and

fluorescence spectroscopy. Changes in the spectra would suggest interaction and possible binding between the compound and the *ct*-DNA, these changes could take the form of red or blue shifts and increases or decreases in band intensity. Such studies, involving titrations against the homopolymers poly(dG-dC) and poly(dA-dT), would hopefully provide insight into the degree of sequence selectivity, if any, achieved by these bisnaphthalimides.

Circular dichroism (CD) is the difference in absorption of left and right circularly polarised light. Measuring the difference over the UV-visible spectrum yields a plot characteristic of the chiral nature of a compound. CD is very sensitive to structural changes and is convenient when examining interactions that might alter the conformational environment. When the CD spectrum of *ct*-DNA is measured an induced CD signal results from the transitions of the purine bases (negative, minimum at 240 nm) and pyrimidine bases (positive, maximum at 275 nm). When another moiety intercalates or binds with the helix the CD spectrum is disrupted, just as the helix is perturbed. Titrating a solution of the bisnaphthalimide against *ct*-DNA would be monitored by CD. The resulting spectrum would be examined for any changes in the characteristic signal of *ct*-DNA and any indication of binding between the helix and the molecule.

Thermal denaturation studies would also be conducted. When a compound binds to DNA it typically affects the melting point of the macromolecule by either stabilising (and so increasing the melting point) or destabilising (and so decreasing the melting point). The melting point can be observed by UV spectroscopy. A significant change in the melting point or T_m could be correlated to an interaction with the bisnaphthalimide. Viscosity investigations would also be performed. Intercalation results in an unwinding of the double helix and this perturbation leads to an increase in the viscosity of the macromolecule. So the viscosity of a solution of *ct*-DNA in a buffered solution could be compared with that of a solution of *ct*-DNA with the bisnaphthalimide. All of these studies were conducted for nine ‘tail-to-tail’ bisnaphthalimides and six ‘head-to-head’ bisnaphthalimides and are detailed in *Chapter Three*.

The MTT assay is an indicative cell viability test often quoted in literature to establish how potent a potential anti-cancer agent might be. The assay quantifies cell death by the IC_{50} value, this is the concentration of compound required to induce apoptosis in half the cells. The activity of the compounds may best be depicted by dose vs. response curves, and the IC_{50} values (after twenty-four or forty-eight hours of incubation) are typically recorded as lying in the micromolar (μM) range. Such assays would be conducted in collaboration with Prof. Mark Lawler, Dr. Tony McElligott and Daniel Frimmansson,

Department of Haematology, St. James' Hospital. The cytotoxicity of the two families of bisnaphthalimides against HL-60, K562 and PC-3 cell lines would be established. The findings of these assays are presented in *Chapter Four*.

1.9 Conclusion

This project sought to design and create two novel families of bisnaphthalimides along with the necessary mono-peptide and di-peptide mononaphthalimides and naphthalene and alternative linker systems *en route*. Conventional and new synthetic ideas and syntheses were employed. Fifteen of these novel bisnaphthalimides, which were the principal products of this synthetic programme, were selected for examination in detail wherein their photophysical, physical and biological behaviours were investigated. These bisnaphthalimides have proven themselves interesting, elegant macromolecules as well as potent potential anti-cancer agents. This thesis discusses all the facets of this research. *Chapter Two* is concerned with the design and synthetic strategy that allowed these bisnaphthalimides to be constructed. *Chapter Three* deals with the photophysical and physical studies and *Chapter Four* with the biological investigations. *Chapter Five* details the experimental techniques employed and *Chapter Six* cites the literature, articles and texts referenced.



Chapter Two

Synthesis of Novel Bisnaphthalimides



2.1 Introduction

The principal aim of this project was the synthesis of novel bisnaphthalimides. These were designed as potential nucleic acid binders, incorporating amino acid functionalities in an effort to achieve sequence selectivity, which could function as potential anti-cancer agents. Since one major failing of many chemotherapeutic agents is the lack of specificity, leading to debilitating side effects, a more targeted approach would be of considerable value.⁷ It was hoped that a novel design would achieve this objective, thus these naphthalimides incorporate several distinct structural motifs. Two naphthalimides, which themselves are functionalised with amino acids or dipeptides, would each be tethered to a core moiety *via* a 'bridge'. This core and bridge together would form the linker or 'spacer' moiety. An amine functionalised planar, aromatic moiety, a naphthalene diimide, was chosen to link the two terminal naphthalimide chromophores. This spacer is itself capable of interacting with DNA, most likely by intercalating and thereby enhancing the binding (intercalation or groove binding) of the naphthalimide units. It was hoped that this dual action might result in very efficient binding with DNA and subsequently hinder its replication and damage a cancerous cell. The structures of the proposed 'tail-to-tail' and 'head-to-head' bisnaphthalimides are depicted in **Figure 2.1** and **Figure 2.2**, respectively.

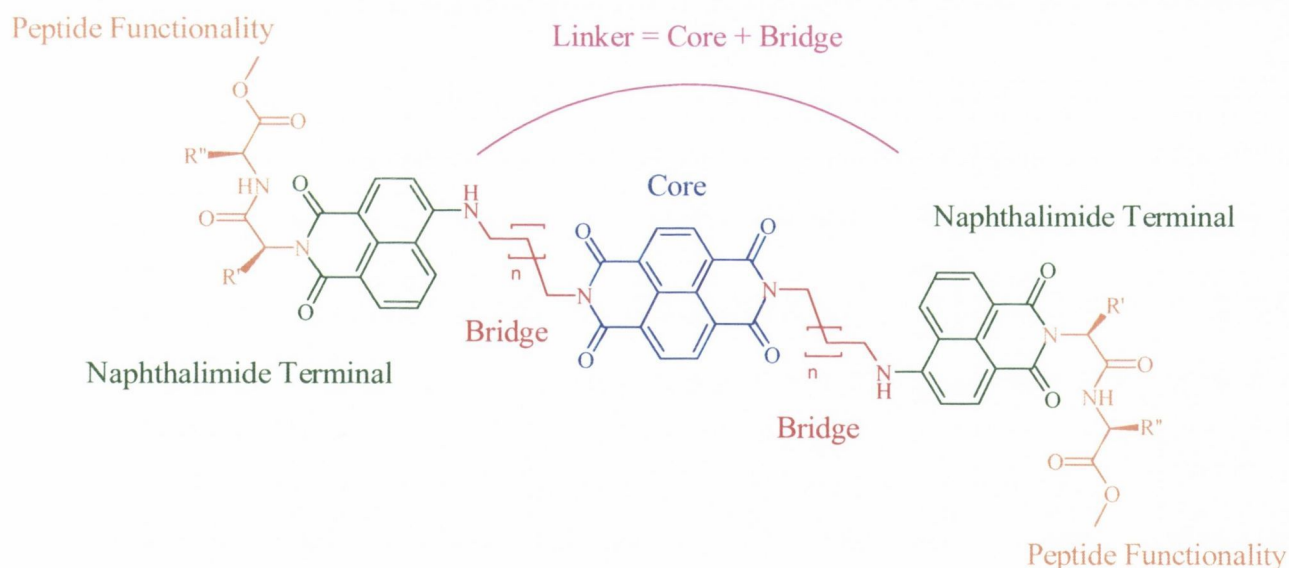


Figure 2.1: The general architecture of a 'tail-to-tail' bisnaphthalimide, highlighting the different structural motifs

Naphthalimide Terminal

Linker = Core + Bridge

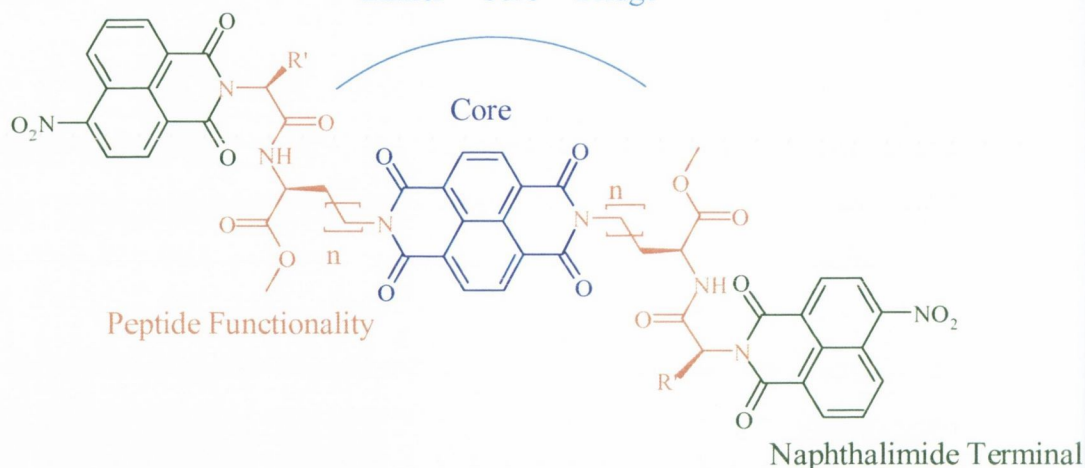


Figure 2.2: The general architecture of a ‘head-to-head’ bisnaphthalimide, highlighting the different structural motifs

Each of these functionalities and their individual syntheses had to be considered when originally designing the target bisnaphthalimides. Whilst the contribution of each component to the overall photophysical and biological behaviour of the larger bisnaphthalimide structure could not be envisaged, the results would themselves be as interesting and challenging as the synthesis. The results of these investigations will be discussed in *Chapter Three* and *Chapter Four*, respectively.

The initial architecture, **Figure 2.1** and **Figure 2.2**, has features and functionalities that can be manipulated. The effects of altering different areas of this design would be of great interest during this research. For example, by varying the length or nature of the bridge between the aromatic core and the naphthalimides one might attain an optimum span for DNA interaction. The amino acid functionality (be it ester, acid or dipeptide, the presence of protecting groups, *etc.*) itself is also available for modification. By systematically examining the differing behaviour and activity resulting from such modifications, it was hoped that some structure : activity relationships and trends would emerge.

This chapter seeks to introduce the synthetic methodologies already employed in the Gunnlaugsson laboratory in peptide and naphthalimide chemistry, to detail the synthesis of naphthalene diimide spacers and, finally, to describe the task of combining these facets into novel bisnaphthalimides. This involved various synthetic steps and different approaches, conditions and procedures were explored and evaluated during this endeavour, and these will also be discussed.

2.2 Functionalising Naphthalimides with Monopeptides

2.2.1 Amino Acids and Peptides

Amino acids are fundamental biological building blocks. These residues are the components of proteins and they possess amino and carboxylic functional groups, hence the term amino acids,

Figure 2.3.^{106, 107} There are twenty naturally occurring α -amino acids, which can be neutral, acidic or basic depending upon the nature of the substituent side chain. They can assemble into long chains known as peptides linked by amide bonds, this is a very inert bond and explains why it is ubiquitous in Nature. A compound involving two linked amino acids can be referred to as a dipeptide and one with only one amino acid as a monoamine.

^{106, 107}

Since all amino acids, other than glycine, are chiral at their α -carbons two enantiomers of each are possible, these are *L* (*levorotatory*, clockwise optical rotation) and a *D* (*dextrorotatory*, anticlockwise optical rotation). Nature employs *L* enantiomers exclusively when building protein *etc.*, so as to mimic biological systems this research focussed on *L*- α -amino acids.

Phelan used glycine (Gly), alanine (Ala), leucine (Leu), phenylalanine (Phe), cysteine (Cys) and histidine (His) during her postgraduate work which concentrated on peptide derivatives of 3- and 4-nitro-1,8-naphthalimide, **Figure 2.4.**¹⁰³ Hussey also employed Ala, Leu, Phe and Cys in her research and extended this pool to include lysine (Lys) and the synthetic amino acids ornithine (Orn, which is derived from arginine) and 2,4-diamino butyric acid.¹⁰⁵ These were incorporated into 'monoamines', dipeptides and linearly linked bisnaphthalimides. This project builds on the biological results of the compounds developed during these projects. This would permit direct comparisons to be drawn from the biological and physical behaviour of the intended bisnaphthalimides with the mononaphthalimides and simple bisnaphthalimides already developed within the Gunnlaugsson group.

It was noted that dipeptides were usually more potent in cancer cell lines than the monoamines and so this project focuses on dipeptides. In these functionalities it was found that a bulky amino acid at the first position achieved anti-cancer activity and that

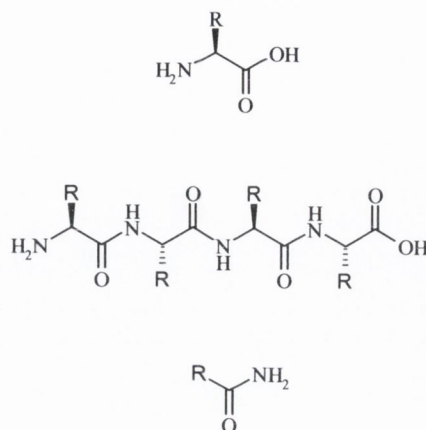


Figure 2.3: General structures of (i) an amino acid, (ii) a tetrapeptide and (iii) an amide bond

varying the second amino acid had little effect. Thus, for this project Phe, Lys and Leu were chosen at the first position and Ala and Lys analogues at the second position. Orn and 2,4-diamino butyric acid also provide useful analogues to Lys since the number of methylene groups between the two amine groups are stepped four, three, two within this family of amino acids. These amino acids, with their free ϵ -amines, were selected for use in the 'head-to-head' bisnaphthalimide architectures.

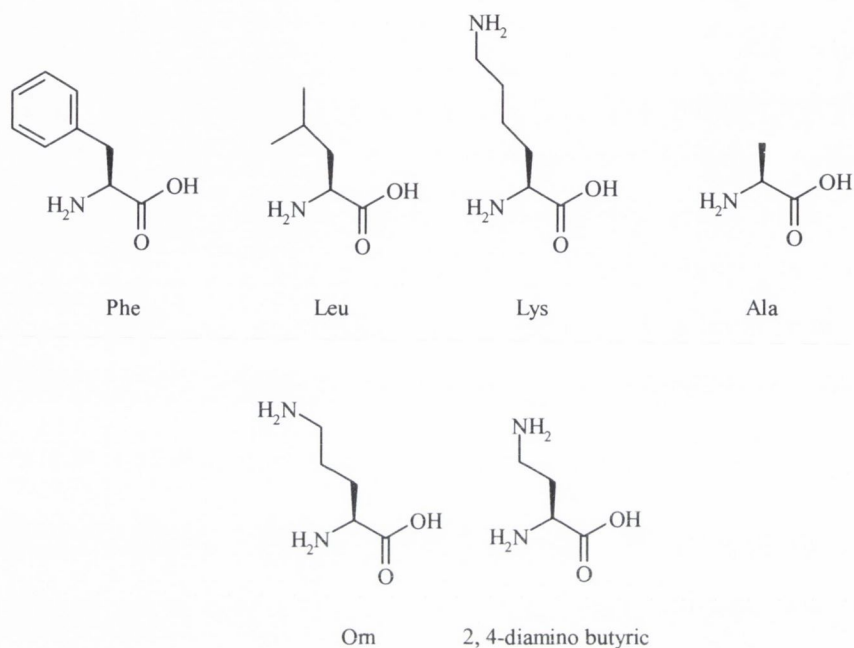


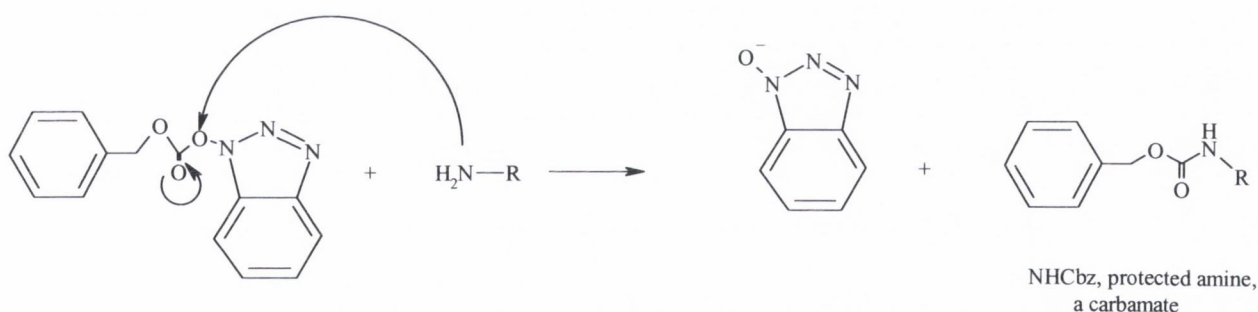
Figure 2.4: The pool of amino acids employed in current bisnaphthalimide synthesis

The initial synthetic strategy in the Gunnlaugsson naphthalimide projects was the reaction of the amino group of an amino acid with 1,8-naphthalic anhydride to form a 'mono-peptide' naphthalimide, this could then be extended to a dipeptide. This extension can be achieved in two different ways. A stepwise approach whereby the amino acids were linked together before finally reacting with the 1,8-naphthalic anhydride was originally attempted by Phelan. However, an anchored technique, involving reacting the chosen amino acid with the anhydride and then conducting any peptide reactions on the naphthalimide derivative as a whole, was more successful. This method gave the desired products in higher yields and greater purity than the stepwise process and has been the procedure of choice since and was exclusively used during this project. However, because three of the intended amino acids, Lys, Orn and 2,4-diaminobutyric acid, each possess an α -amine and ϵ -amine this project would also rely on those stalwarts of peptide chemistry, protecting groups.

2.2.2 Protecting Groups in Peptide Chemistry

Lysine, Ornithine and 2,4-diaminobutyric acid were selected for use in the synthesis of bisnaphthalimides because they offer a second amine, the distal amine, which is available for further functionalisation. These amino acids are basic and may favour enhanced electrostatic interaction with DNA due to protonation of the side chain. When reacting such an amino acid at the α -amino group, protection of the distal amine is necessary. Many protecting groups are available, when choosing from these the conditions under which it is required to be stable as well as the deprotection methods needed (which should not affect any other region of the molecule) must be considered.^{107, 108} Two commercially available amine protecting groups were deemed suitable for the compounds and reactions involved in this synthetic strategy, carboxybenzyl (Cbz, **Scheme 2.1**) and *tert*-butoxycarbonyl (Boc, **Scheme 2.2**).

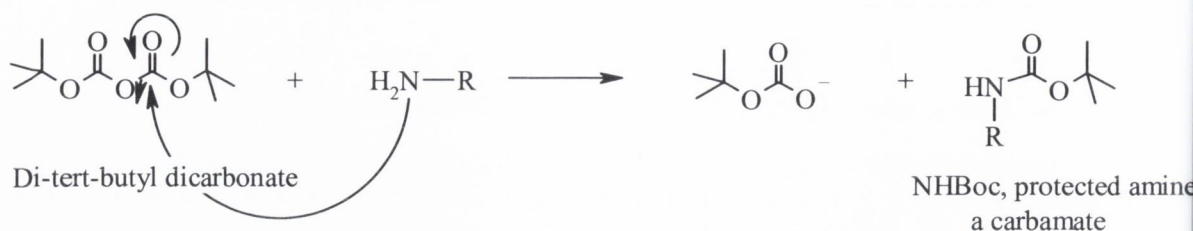
When using Lys in the synthesis of the proposed ‘tail-to-tail’ bisnaphthalimides, the ϵ -amino group needed to be protected during reactions with 4-nitro-1,8-naphthalic anhydride, coupling reactions and, finally, reactions with the spacer species. Since Cbz-*N*-Lys *tert*-butyl ester is commercially available and the protecting group is stable to trifluoroacetic acid (TFA) and base this seemed a logical choice. If desired, this protecting group could be cleaved using hydrobromic (HBr) acid in acetic acid (2M solution) to yield the HBr salt of the bisnaphthalimide, this will be detailed in *Section 2.5.3*.



Scheme 2.1: Introduction of a Cbz protection to an amine, forming a carbamate

For the use of Orn and 2,4-diamino butyric acid as the second amino acid in the dipeptides of the ‘head-to-head’ bisnaphthalimides, the methyl esters were not commercially available. Cbz and Boc protecting groups were both employed in the syntheses of these methyl esters and their coupling with Phe functionalised naphthalimides. Boc is easily introduced by reacting the amino acid with di-*tert*-butyl

dicarbonate in a nucleophilic acyl substitution reaction giving a carbamate, which is stable during coupling reactions. Selective, monoprotection of 2,4-diamino butyric acid is achieved using copper complexation and dropwise addition of a dioxane solution of the di-*tert*-butyl dicarbonate. The Boc protecting group can be easily removed by hydrolysis



Scheme 2.2: Introduction of a Boc protection to an amine, forming a carbamate

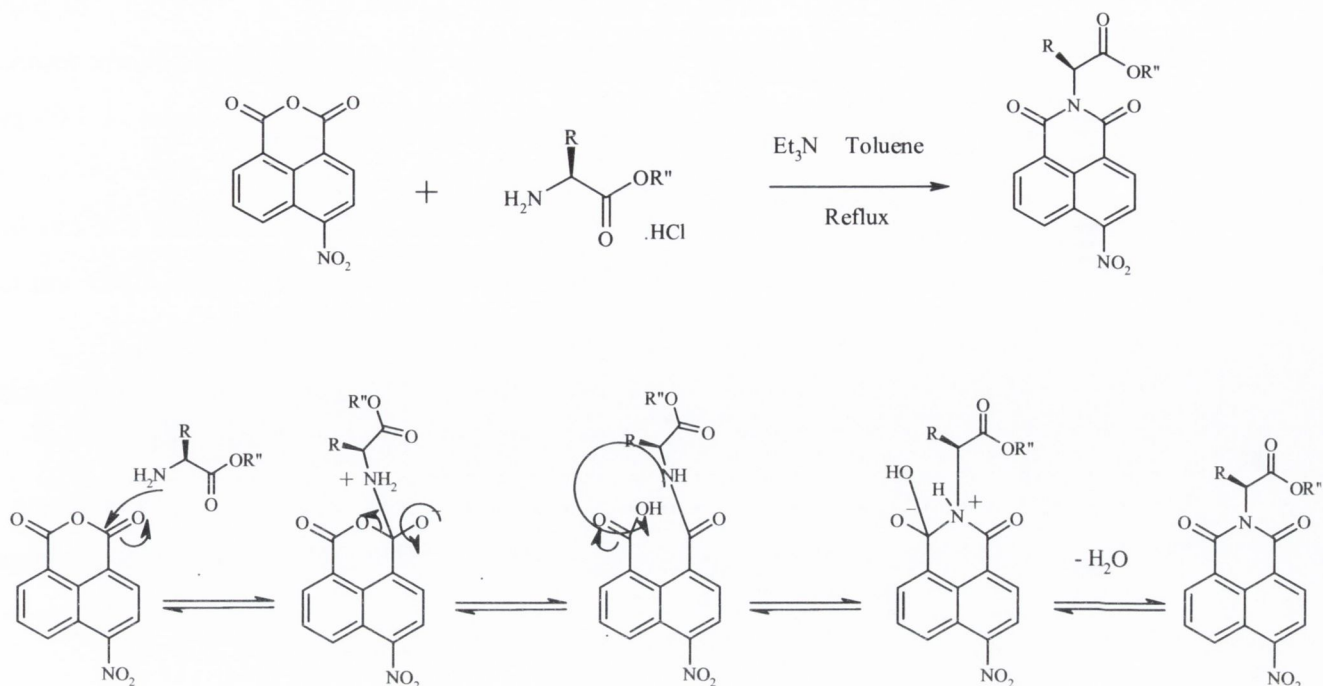
using TFA.

2.2.3 Synthesis of Amino Acid and Ester Functionalised

1,8-Naphthalimides

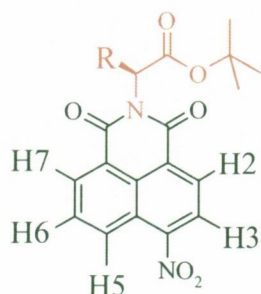
As previously mentioned, the first step in the anchored approach to functionalised naphthalimides is the peptide coupling of an amino acid, or amino ester, with naphthalic anhydride. This project dealt solely with the 4-nitro-1,8-naphthalic anhydride reagent since biological assays on previous families of naphthalimides had found these more potent than the 3-nitro analogues. The direct synthesis of amino acid functionalised naphthalimides was previously investigated within the group but these proved poor yielding reactions and the product were impure.¹⁰³ However, the method could efficiently be employed for methyl esters and *tert*-butyl esters of a wide range of amino acids and conversion to the corresponding amino acid would then be possible.

Typically, 4-nitro-1,8-naphthalic anhydride and the hydrochloric salt of the amino ester, were refluxed together overnight in anhydrous toluene, in the presence of triethylamine (Et_3N), **Scheme 2.3**. The amino group of the free α -amino ester is a nucleophile and proceeds to attack one of the carbonyl groups of the anhydride resulting in the formation of a dipolar tetrahedral intermediate. An intramolecular nucleophilic attack by the amine occurs, water is expelled and the *N*-functionalised naphthalimide is produced.¹⁰³ This mechanism is depicted in **Scheme 2.3**.



Scheme 2.3 ; Condensation reaction and reaction mechanism of an amino ester and 4-nitro-1,8-naphthalic anhydride to produce an amino ester functionalised naphthalimide.

L-Phenylalanine *tert*-butyl ester hydrochloride was reacted with 4-nitro-1,8-naphthalic anhydride to give *N*-[(1*S*)-*tert*-butoxycarbonyl-2-ethylphenyl]-4-nitro-1,8-naphthalimide, **73**, as a rich brown solid after purification by recrystallisation from dichloromethane (DCM, CH_2Cl_2) and isolated in a yield of 90 %. This product was fully characterised by ^1H and ^{13}C nuclear magnetic resonance (NMR) and by high resolution mass spectrometry (HRMS), electrospray mass spectrometry (ESMS) and infrared (IR) spectroscopy and this evidence agreed with that previously reported by Hussey.¹⁰⁵



73, $\text{R} = \text{CH}_2\text{Ph}$, 90 %

74, $\text{R} = (\text{CH}_2)_4\text{NHCOOCHPh}$, 52 %

75, $\text{R} = \text{CH}_2\text{CH}(\text{CH}_3)_2$, 89 %

Figure 2.5 shows the ^1H (400MHz, CDCl_3) NMR spectrum obtained for **73**. The aromatic naphthalimide signals, appear as four doublets, H7 at 8.82 ppm, H5 at 8.66 ppm,

H2 at 8.61 ppm and H3 at 8.37 ppm; and one double doublet, H6 at 7.96 ppm. The phenyl signal is a multiplet resonating at 7.13 ppm. The proton at the stereogenic centre resonates upfield at 5.94 ppm, whereas the signal of the benzyl protons is a multiplet at 3.65 ppm. The *tert*-butyl group occurs as a singlet at 1.45 ppm. The ^{13}C NMR spectrum contains twenty carbon signals, including characteristic signals at 167.6 ppm for the *tert*-butyl group and at 162.6 ppm and 161.6 ppm for the two naphthalimide C=O carbons. Experimental and characterisation information can be found in *Chapter Five*.

Similarly, Cbz-*L*-lysine *tert*-butyl ester hydrochloride was used in the synthesis of *N*-(1*S*)-*tert*-butoxycarbonyl-6-benzyloxycarbonylaminoethyl]-4-nitro-1,8-naphthalimide, **74**, in a yield of 52 %. This compound was fully characterised using the conventional techniques. In particular, the ^1H (400MHz, CDCl_3) NMR spectrum depicts the lysine CH_2 protons as multiplets over a range of 3.16 – 1.28 ppm and the *tert*-butyl singlet is found at 1.36 ppm.

The final α -amino ester chosen was *L*-leucine *tert*-butyl ester and the hydrochloric salt was reacted in the usual manner with 4-nitro-1,8-naphthalic anhydride and gave the desired product *N*-[(1*S*)-*tert*-butoxycarbonyl-3-methylbutyl]-4-nitro-1,8-naphthalimide, **75**, in a yield of 89 %. Full characterisation was obtained and the ^1H (400MHz, CDCl_3) NMR spectrum depicts the two methyl groups of the leucine chain have their signal at 1.01 ppm and 0.90 ppm.

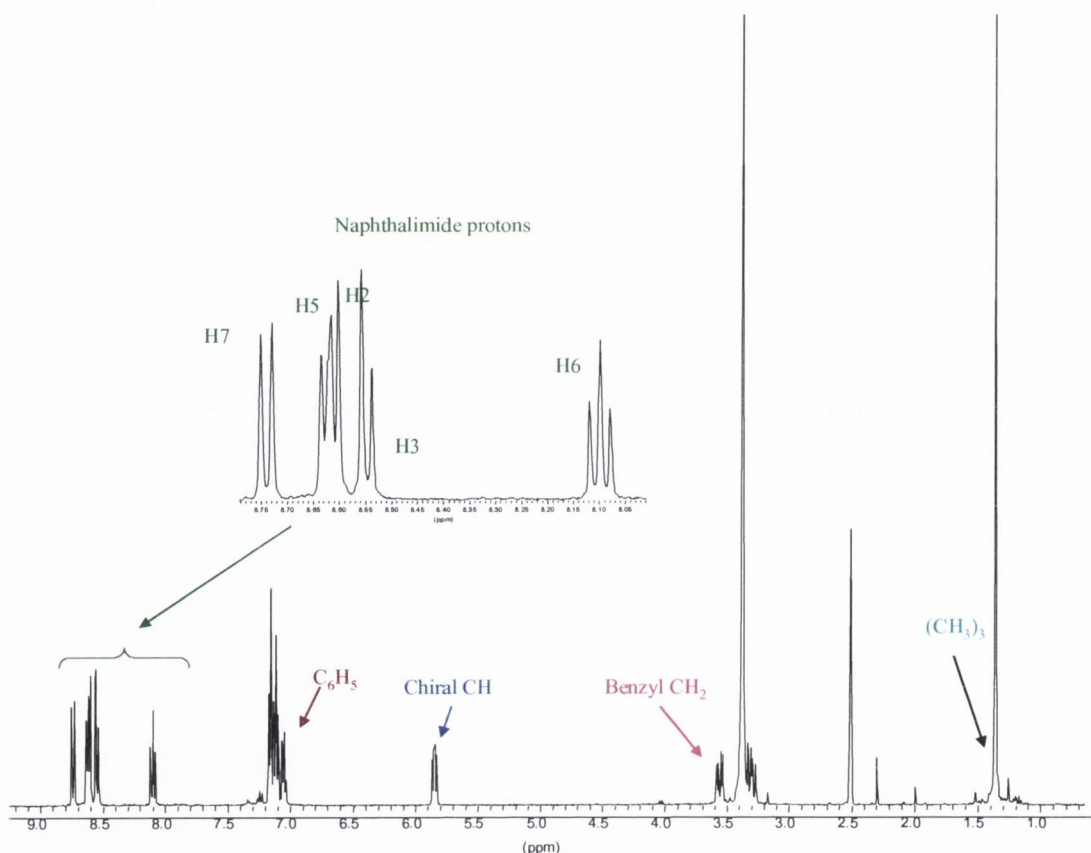


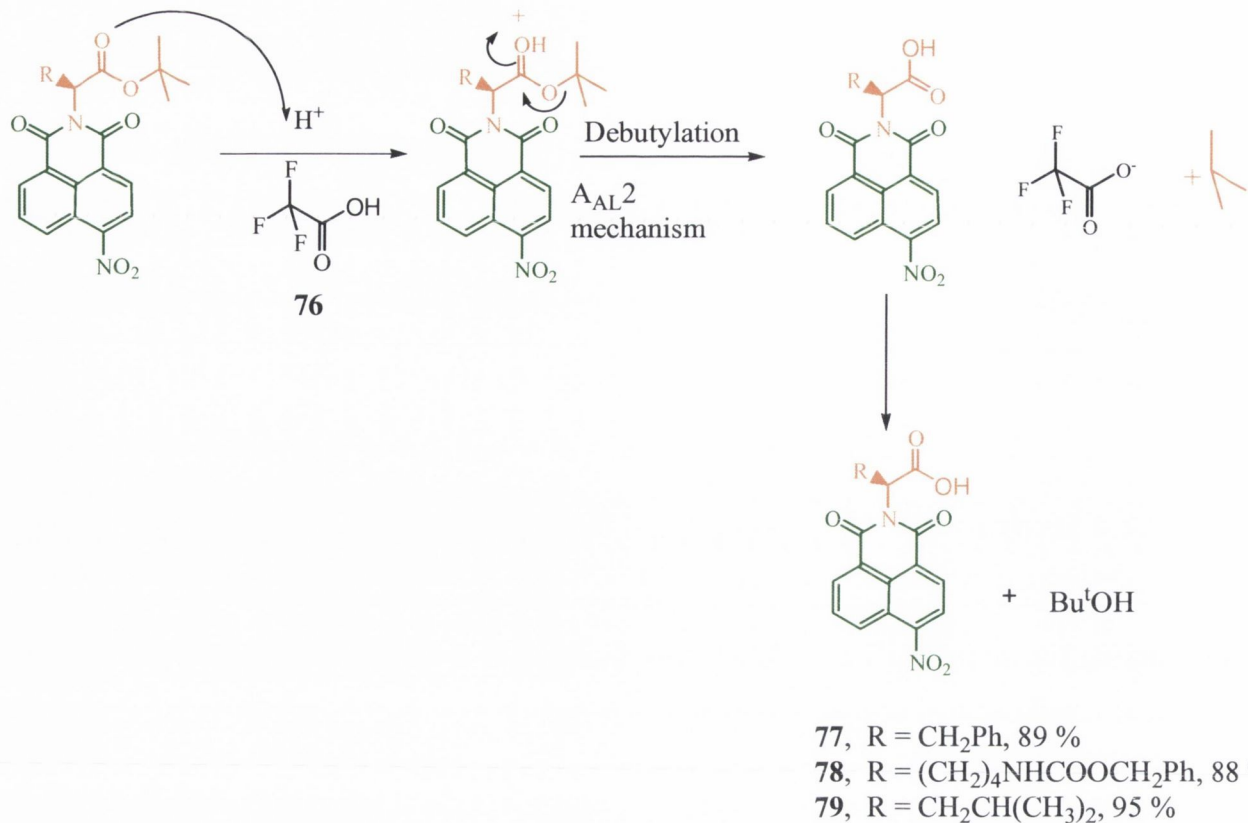
Figure 2.5: ^1H NMR Spectrum of Compound 73

The three intended α -amino ester functionalised 4-nitro-1,8-naphthalimides were all successfully synthesised, purified and characterised. These ester naphthalimides now provide three basic structural motifs which could be elaborated into different families of dipeptidic naphthalimides. The next step was to remove the ester groups so that coupling reactions could be pursued.

2.2.4 Hydrolysis of Functionalised Naphthalimides

The *tert*-butyl esters of the α -amino acids were favoured over the methyl esters during this project since the latter were found to be difficult to cleave *via* either acid or base mediated hydrolysis.¹⁰³ However, a *tert*-butyl ester can be treated with TFA, **76**, in CH_2Cl_2 . This mild acid hydrolyses the carboxy group, **Scheme 2.4**.^{103, 108}

Compounds **73**, **74** and **75** were all treated with TFA in DCM for 2 h. under an inert atmosphere. The solvent was then removed under reduced pressure and coevaporation with diethyl ether ensured the removal of any residual TFA. These reactions successfully yielded *N*-[(1*S*)-carboxy-2-ethylphenyl]-4-nitro-1,8-naphthalimide, **77**, *N*-[(1*S*)-carboxy-6-benzyloxycarbonylaminoethyl]-4-nitro-1,8-naphthalimide, **78**, and *N*-[(1*S*)-carboxy-3-methylbutyl]-4-nitro-1,8-naphthalimide, **79**, respectively. These were high yielding reactions, 89 %, 88 % and 95 %, respectively, and the products were all characterised by conventional techniques. The absence of the *tert*-butyl singlets from the ^1H NMR spectra and of the *tert*-butyl carbon signal in the ^{13}C NMR spectra was indicative that the ester had been successfully hydrolysed. These compounds were now available for coupling with other amino acids to form dipeptidic naphthalimides.



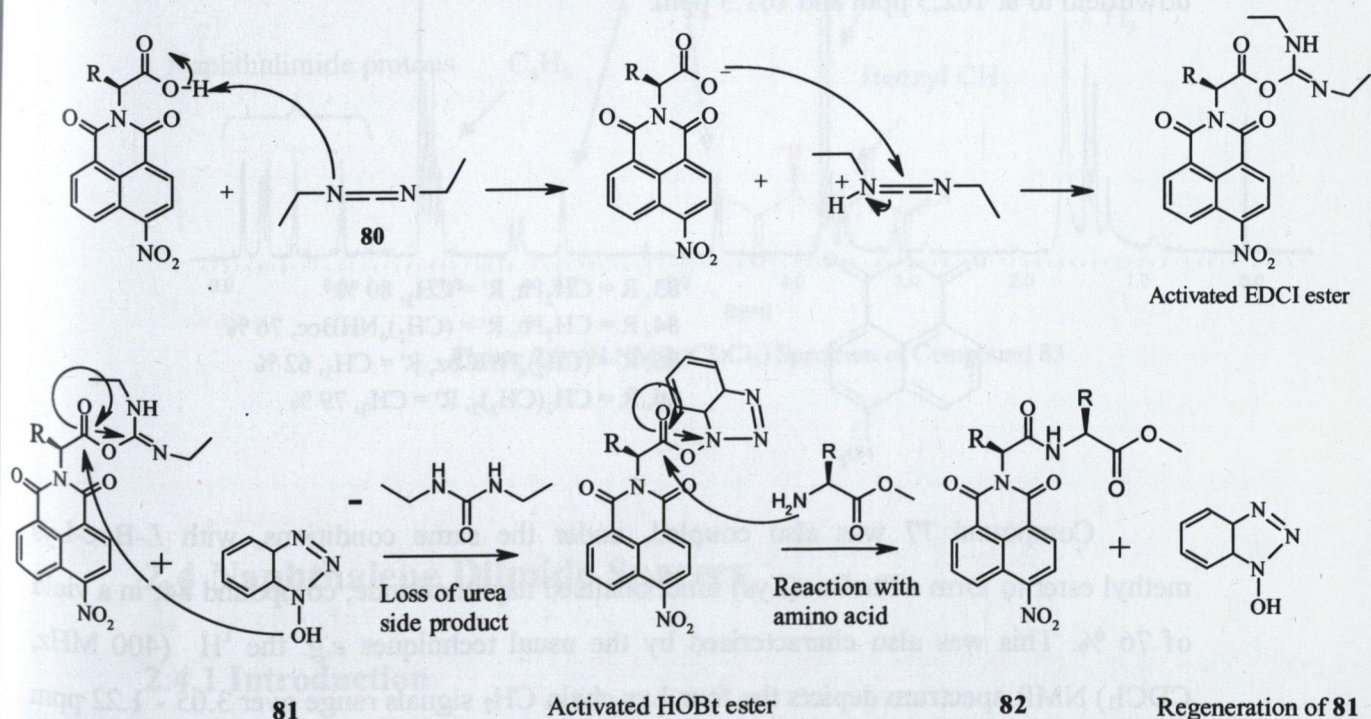
Scheme 2.4: The reaction mechanism of acid mediated hydrolysis using TFA, cleaving *tert*-butyl esters to amino acids

2.3 Synthesis of Dipeptide Functionalised Naphthalimides

2.3.1 Use of Coupling Agents in Peptide Chemistry

Two coupling agents have been investigated in the Gunnlaugsson group for use in the formation of dipeptide functionalised naphthalimides; dicyclohexylcarbodiimide (DCC) and ethylene diaminecarbodiimide (EDCI), **80**. The latter of these was found to permit higher yields and greater purity in the resulting products and was thus the agent of choice. EDCI reacts with the carboxylic acid to form an activated ester. The urea formed by this carbodiimide is water soluble and this side product can be easily removed during the work-up by washing with sodium hydrogen carbonate (NaHCO₃). **Scheme 2.5** depicts the reaction mechanism.

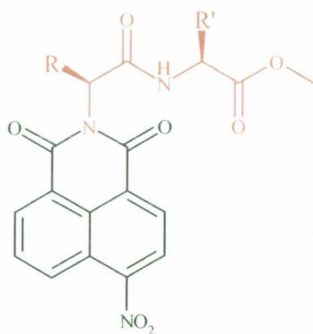
An additional coupling reagent, 1-hydroxybenzotriazole (HOBt), **81**, is used in tandem with EDCI. HOBt intercepts the activated ester forming a new and more reactive ester, this new compound prevents any possible racemisation (since the bulk of the triazole blocks one side of the new intermediate ester from substitution attack). This HOBt ester is, in turn, attacked by the amino acid which one wishes to couple to the acid and the desired dipeptide is yielded, **82**. This reaction is conducted by adding HOBt and the α -amino acid methyl ester hydrochloride of choice to the amino acid 4-nitro-1,8-naphthalimide in anhydrous tetrahydrofuran (THF). This solution is cooled in an ice-bath and stirred for fifteen minutes, after which EDCI.HCl and Et₃N are introduced and the reaction is stirred at 0 °C for a further thirty minutes. Once removed from the ice-bath the reaction mixture is stirred at room temperature overnight, under an inert atmosphere. The solvent is then evaporated under reduced pressure and the remaining residue is dissolved in CH₂Cl₂ and washing with acid and base is conducted. The product can be purified by recrystallisation from CH₂Cl₂ or by preparative silica plate chromatography using MeOH as the eluent. This procedure produces the desired dipeptide functionalised naphthalimides in quantitative yields and great purity.^{103, 105}



Scheme 2.5: The reaction mechanism of an EDCI/HOBt mediated peptide coupling

2.3.2 Coupling Amino Ester Naphthalimides with Amino Acids

This peptide coupling methodology was employed for the synthesis of the dipeptides used as building blocks for the designed bisnaphthalimides. The *L*-phenylalanine functionalised naphthalimide, **77**, was successfully coupled with *L*-alanine methyl ester to give the PheAla functionalised naphthalimide, **83**, as a brown crystalline solid, in a yield of 80 %, and was characterised by ^1H and ^{13}C NMR and by HRMS, ESMS and IR. In the ^1H (400 MHz, CDCl_3) NMR spectrum, **Figure 2.6**, all the naphthalimide proton signals are present, though are somewhat shifted by the coupling compared with those of **77**, H7 at 8.83 ppm, H5 at 8.67 ppm, H2 at 8.61 ppm, H3 at 8.38 ppm and the double doublet of H6 at 7.97 ppm. The phenyl multiplet is found at 7.17 ppm. The dipeptide contains two stereogenic centres and their resonances are multiplets at 6.05 ppm (CHCH_2Ph) and 4.68 ppm (CHCH_3). The *tert*-butyl ester singlet is absent, however the methyl ester signal can be seen at 3.73 ppm. The remaining benzyl and methyl peaks are found at 3.58 ppm and 1.36 ppm, respectively. The ^{13}C (400 MHz, CDCl_3) NMR spectrum contains nineteen signals, notably the *tert*-butyl ester carbon is absent, whilst the methyl ester carbon lies at 173.9 ppm, the two naphthalimide $\text{C}=\text{O}$ carbons have shifted slightly downfield to at 162.3 ppm and 161.5 ppm.



- 83**, R = CH_2Ph , R' = CH_3 , 80 %
84, R = CH_2Ph , R' = $(\text{CH}_2)_4\text{NHBoc}$, 76 %
85, R = $(\text{CH}_2)_4\text{NHCbz}$, R' = CH_3 , 62 %
86, R = $\text{CH}_2(\text{CH}_3)_2$, R' = CH_3 , 79 %

Compound **77** was also coupled, under the same conditions, with *L*-Boc-Lys methyl ester to form a PheBoc(Lys) functionalised naphthalimide, compound **84**, in a yield of 76 %. This was also characterised by the usual techniques *e.g.* the ^1H (400 MHz, CDCl_3) NMR spectrum depicts the four Lys chain CH_2 signals range over 3.05 - 1.22 ppm and the Boc protecting group singlet appears at 1.45 ppm. This dipeptide was intended for incorporation in the 'head-to-head' bisnaphthalimides and to this end the Boc protecting would have to be removed using TFA, this will be discussed in *Section 2.7*. Two further dipeptide derivatives were synthesised. *L*-Cbz-Lys functionalised naphthalimide, **78**, and *L*-leucine functionalised naphthalimide, **79**, were each coupled with *L*-Ala methyl ester

giving **85** and **86**, respectively. Compound **85** was obtained as a brown hygroscopic solid, in a yield of 62 % and identified by HRMS (613.1921 a.u. corresponding to $[M + Na]^+$). Compound **86** was a brown powder, produced in a yield 79 %. The ^1H (600 MHz, CDCl_3) NMR spectrum of this compound contains resonances due to the two Leu methyl groups, the methyl group, methyl ester and the three chiral protons. Complete characterisation information for these compounds can be found in *Chapter Five*.

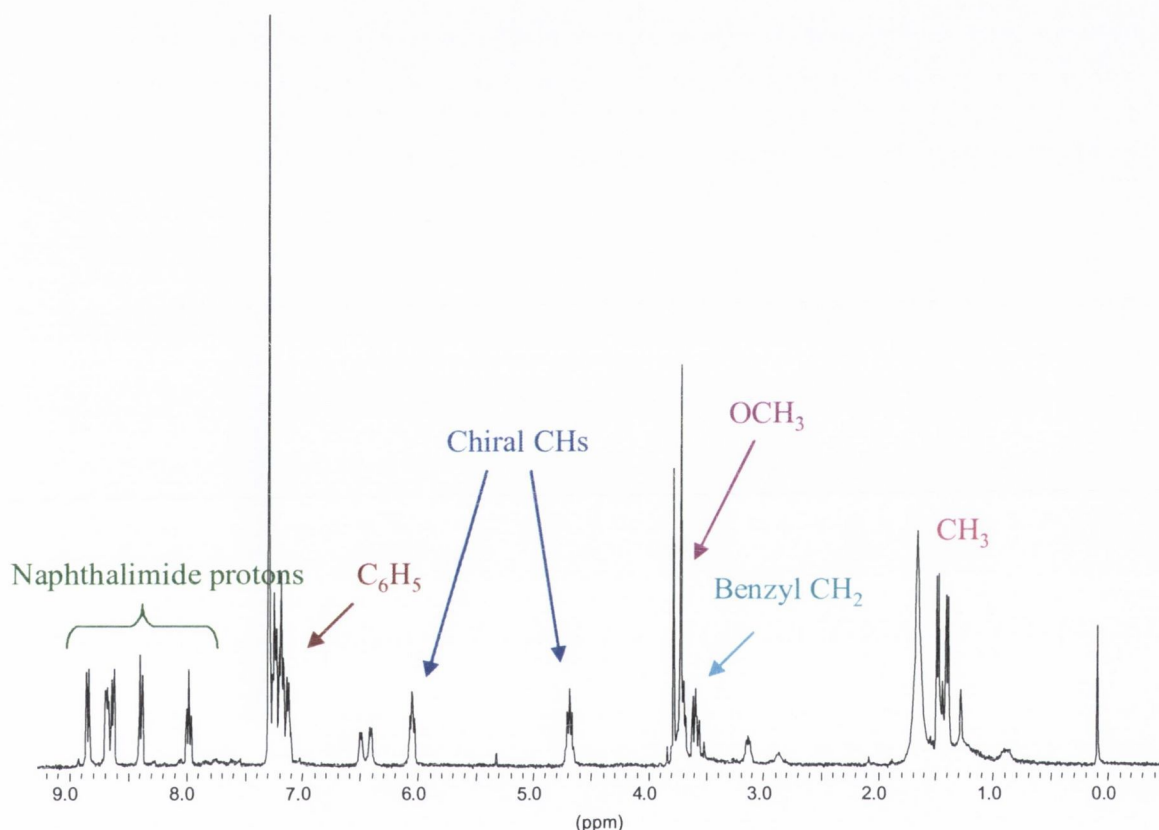


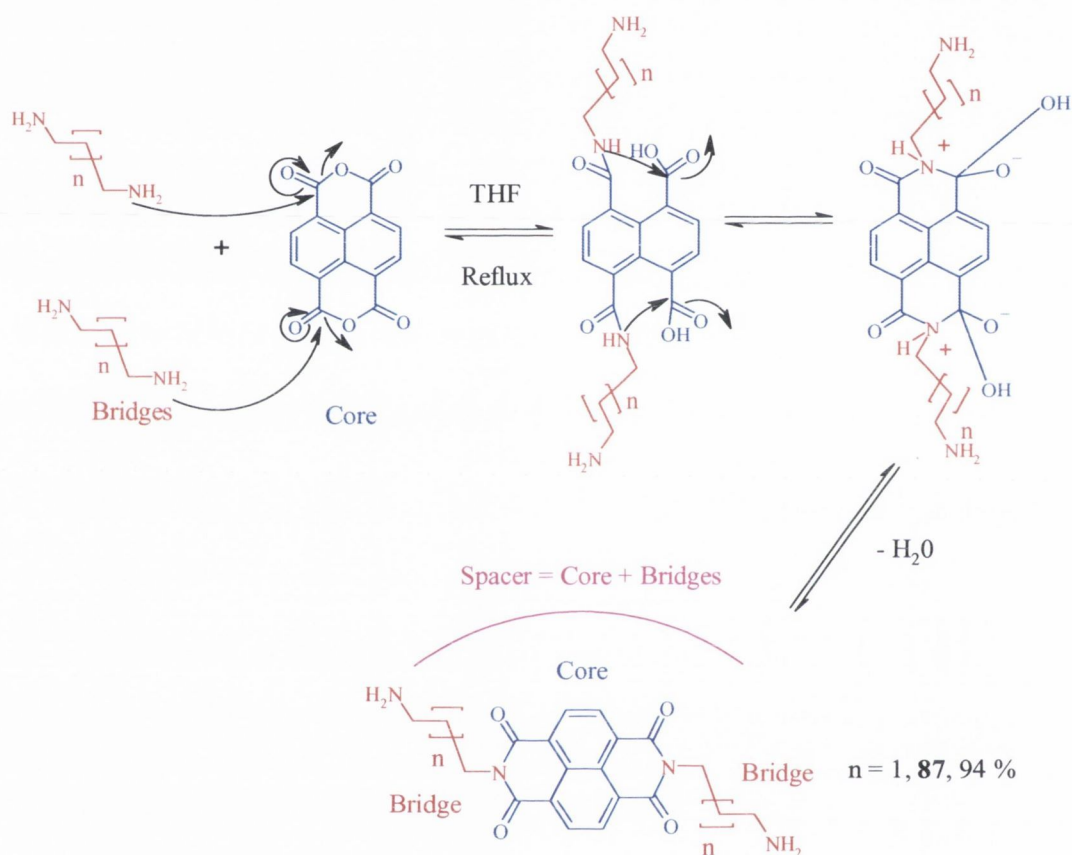
Figure 2.6: ^1H NMR (CDCl_3) Spectrum of Compound **83**

2.4 Naphthalene Diimide Spacers

2.4.1 Introduction

The next task was the development of a comprehensive approach to the synthesis of a family of spacers. The planar, extended aromatic character of naphthalene diimides endows them with a tendency to slide between base pairs and interact with the neighbouring nucleotides *via* π - π interactions. 1,4,5,8-Naphthalene tetracarboxylic dianhydride, **43**, can be converted to the corresponding diimide, functionalised with

substituent pendant chains. The possibility for further derivatisation to form novel intercalating species is evident. To this naphthalene core would first be added the pendant chains, or bridges, these would together form the desired spacer, **Scheme 2.6**. Varying the lengths of these bridges would allow a family of novel naphthalene diimide spacers to be synthesised, which might then be reacted with the peptide functionalised naphthalimides to finally yield the proposed bisnaphthalimides. The following section describes the syntheses explored for these spacers, the methodology chosen and the family of spacers prepared.



Scheme 2.6: The reaction mechanism of diamino alkanes with naphthalene tetracarboxylic dianhydride to form aminoalkyl functionalised naphthalene diimide linkers

2.4.2 Direct Synthesis of Functionalised Naphthalene Diimides

Diamino alkanes were chosen as the bridging chains in the spacer systems since one of the terminal amines can nucleophilically attack the dianhydride to form the bridge functionalised naphthalene diimide spacer, **Scheme 2.6**. The remaining terminal amine can then be used to conjugate the spacer to the naphthalimide moieties, (**83** - **86**). Varying the

lengths of the spacer structures would allow any relationship between overall naphthalimide-core-naphthalimide distance and the strength and nature of DNA binding to be explored. Three diamino alkanes were selected; 1,3-diamino propane, 1,5-diamino pentane and 1,6-diamino hexane, providing bridging chains consisting of three, five and six methylene groups, respectively.

Preliminary reactions were explored using 1,3-diamino propane by direct reaction with half an equivalent of 1,4,5,8-naphthalene tetracarboxylic dianhydride, under reflux in dimethyl acetamide (DMA) overnight, under an inert atmosphere. A lengthy precipitation from MeOH isolated a viscous black oil, this was identified as an oligomeric product by ¹H NMR and ESMS. Similar reactions in propan-2-ol and using neat 1,3-diamino propane, as well as attempts at a variety of work-ups, also failed to successfully produce the desired spacer, these are summarised in **Table 2.1**.

Table 2.1: A summary of attempted spacer methodologies

Solvent System	Reaction Conditions	Work-Up	Product
DMA	24 h, reflux	Methanol ppt	Black, oligomeric product
Propan-2-ol	24 h, reflux	Hexane : ethyl acetate ppt	Black, oligomeric product
1,3-Diamino propane	4 h, reflux	Acetonitrile ppt	Dark red, oligomeric hydroscopic product
1,3-Diamino propane	24 h, reflux	Acetonitrile ppt	Dark red, oligomeric hydroscopic product
THF	24 h, reflux	Diethyl ether ppt, Recrystallisation from MeOH	<i>N,N'</i> -bis (aminopropyl) naphthalene diimide

However, an overnight reflux in anhydrous THF followed by a slow precipitation from diethyl ether and recrystallisation from MeOH gave a red powder, in a yield of 94 %. The identity of this solid, **87**, was confirmed by ¹H (400 MHz, (CD₃)₂SO) NMR. The spectrum, **Figure 2.7**, reflects the C₂ symmetry of the molecule since the naphthalene diimide protons appear as a singlet at 8.69 ppm, a broad NH₂ singlet is found at 4.94 ppm and three propyl CH₂ resonances appear over the range 4.14 - 2.00 ppm.

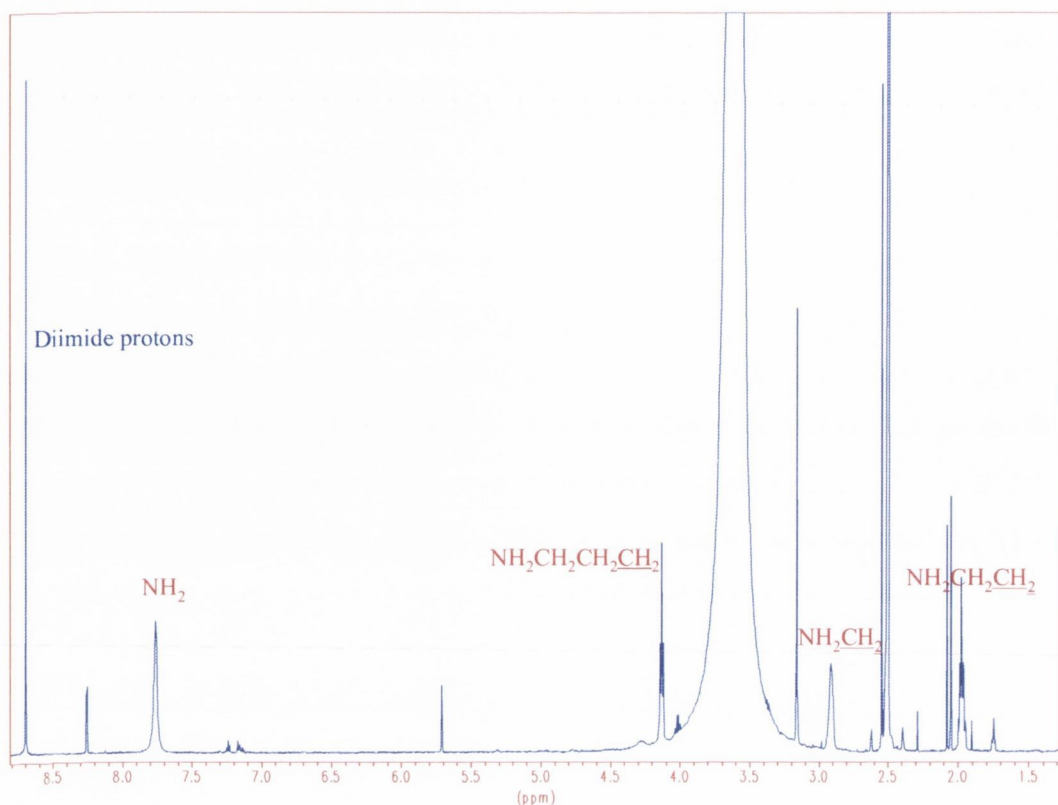


Figure 2.7: ^1H NMR spectrum of **87**

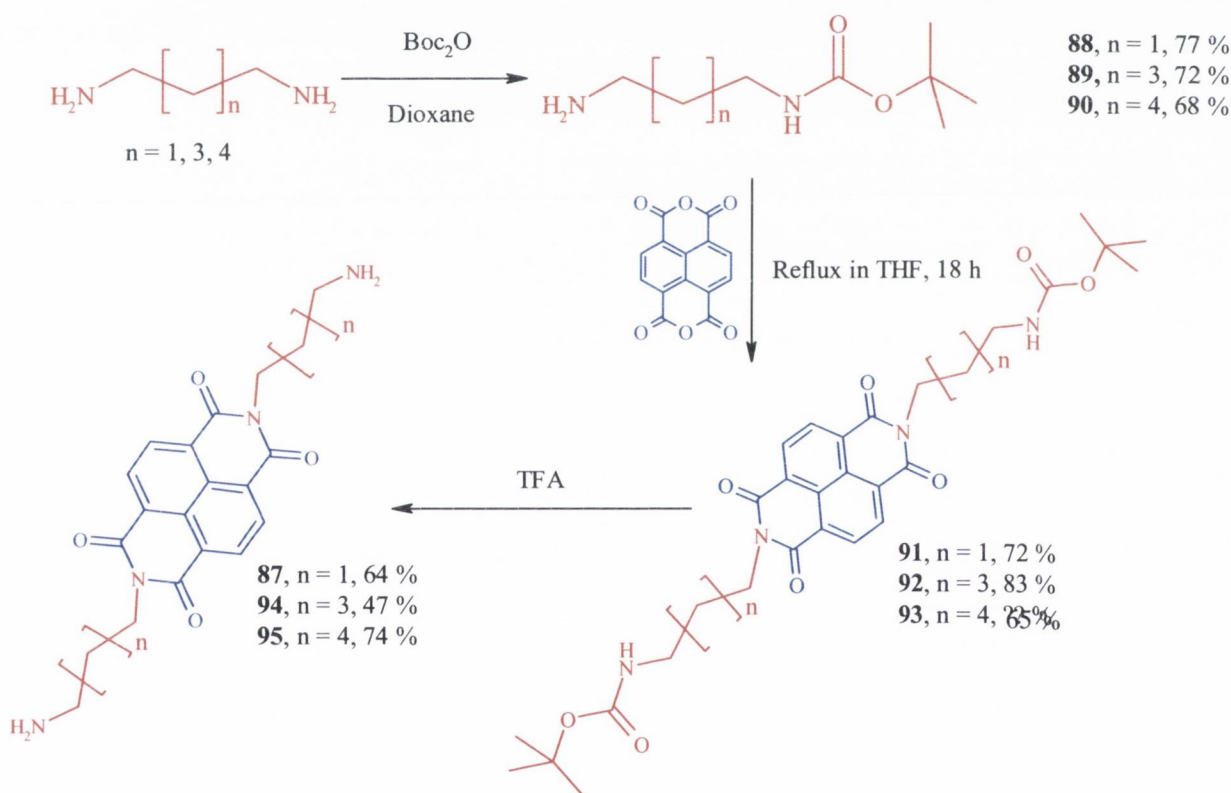
This reaction procedure was subsequently attempted using 1,5-diamino pentane and 1,6-diamino hexane but these reactions were both unsuccessful, even after 72 h. at reflux no spacer product was identified. The reaction mixtures consisted of unreacted starting material and there was evidence (broad NMR signals and numerous ESMS peaks) of the presence of oligomeric compounds. Changing the reaction solvent to DMA and dimethylformamide (DMF) did not lead to the formation of the desired products. The difficulty in producing these longer spacers may be due to the fact that the longer diamines are more reactive and proceed to form oligomers with the dianhydride and no monomer of the spacers can be isolated under these conditions. Since this method was successful only for obtaining the propyl naphthalene diimide spacer an alternative approach was needed that would successfully synthesise all three intended spacers, this will be discussed in the following section.

2.4.3 Protected & Free Aminoalkyl Naphthalene Diimides

In an effort to constrain the reactivity of the diamino alkanes it was decided that a mono-protection using di-*tert*-butyl dicarbonate, (Boc_2O), would facilitate the introduction of the diamine to the naphthalene dianhydride core. This was chosen as the most suitable

protecting group since it works well with secondary amines, can be introduced under mild conditions and can later be easily removed using TFA.^{107, 108}

The synthesis of the desired monoprotected amines (**88** - **90**) was achieved by dropwise addition a solution of di-*tert*-butyl dicarbonate in dioxane to a stirring solution of 4 equivalents of the diamino alkane in dioxane, **Scheme 2.7**. The solution was then stirred overnight, under an inert atmosphere. Deionised water was then added to the solution and the bis-*tert*-butyl dicarbonate precipitated out and was removed by filtration. The product was then extracted from the filtrate using CH₂Cl₂ and the solvent was removed. For 1,3-diamino alkane a viscous oil was obtained which solidified to an off-white semi-solid, in a yield of 77 %. This product was characterised by the conventional methods and identified as the desired *N*-Boc-1,3-diamino propane, **88**. HRMS showed a *m/z* peak at 175.1424 a.u., corresponding to the protonated amine.



Scheme 2.7: The synthesis of the mono-Boc protected diamine and the subsequent reaction with the 1,4,5,8-naphthalic dianhydride followed by the removal of this protecting group by acid mediated hydrolysis

This method was repeated using 1,5-diamino pentane and 1,6-diamino hexane. The desired diimides were obtained in great purity and in yields of 72 %, **89**, and 68 %, **90**, respectively. These products, *N*-Boc-1,5-diamino pentane and *N*-Boc-1,6-diamino hexane, **89** and **90**, respectively, were characterised by the usual techniques.

Having successfully formed the desired monoprotected diamines the next step was to introduce these to the naphthalene core. This was successfully achieved by reacting these products with 1,4,5,8-naphthalene tetracarboxylic dianhydride under reflux for three nights in anhydrous THF, under an inert atmosphere, **Scheme 2.7**. A slow precipitation from diethyl ether and recrystallisation from MeOH produced orange, pink and red solids, respectively, in yields of 72 %, 83 %, and 65 %, respectively. These products were fully characterised and were identified as the *N*-Boc-3-amino propyl naphthalene diimide, **91**, the *N*-Boc-5-amino pentyl naphthalene diimide, **92**, and the *N*-Boc-6-amino hexyl naphthalene diimide, **93**, respectively. The ^1H NMR (400 MHz, $(\text{CD}_3)_2\text{SO}$) spectra of these compounds are related to the Boc-protected amines with some shifting due to the effect of the naphthalene ring and the presence of the diimide proton peaks typically located at *ca.* 8.80 ppm. This signal appears as a singlet since the molecule possesses a C_2 symmetry. **Figure 2.8** shows the ^1H (400 MHz, $(\text{CD}_3)_2\text{SO}$) NMR spectrum of **93** as an example. Elemental analysis and ESMS further verified these compounds as the desired protected spacers.

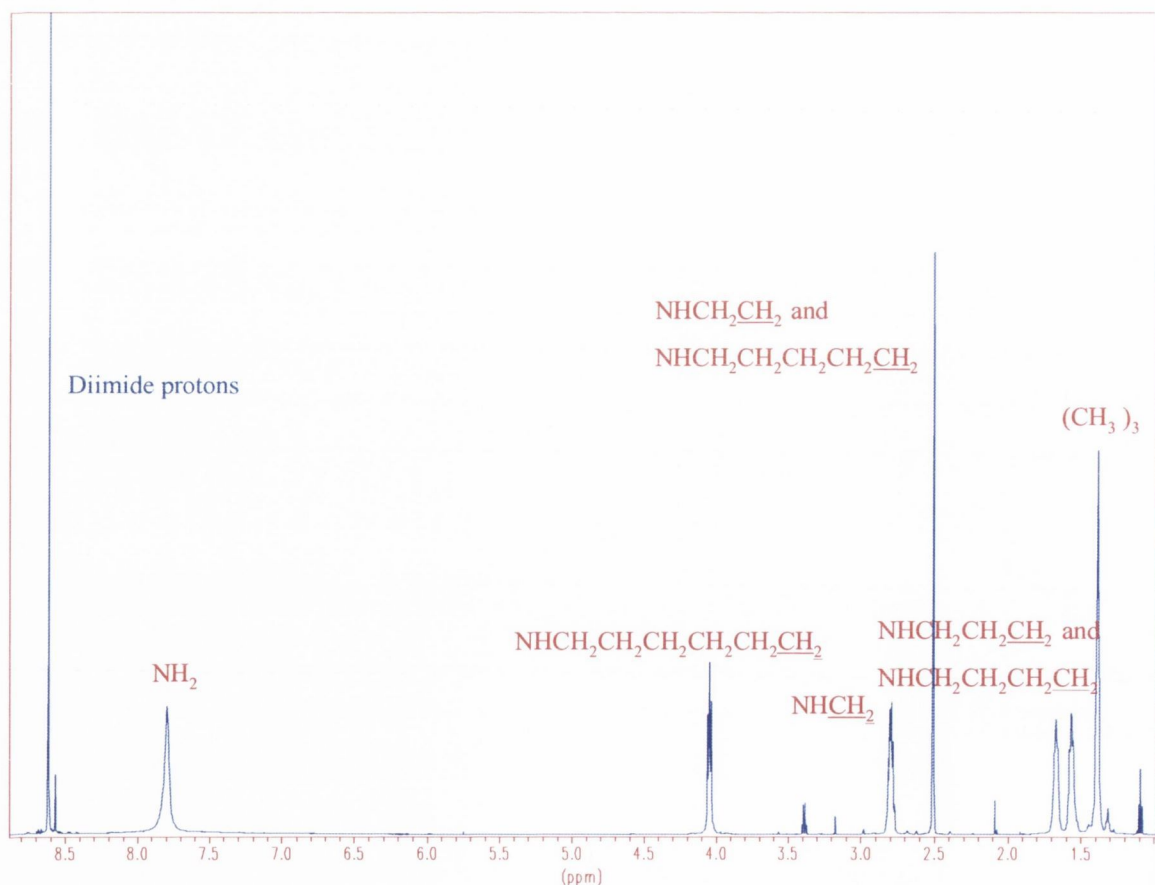


Figure 2.8: ^1H NMR spectrum of **93**

It therefore remained for these protected spacers to be converted to their corresponding free amines by treating them with TFA in CH₂Cl₂, **Scheme 2.7**. The same method employed in *Section 2.3.2* for the hydrolysis of the *tert*-butyl groups esters was used for this transformation. The resulting solids were coevaporated with diethyl ether to remove any remaining TFA. The deprotected products were isolated as orange, pink and red solids, with respective yields of 64 % for the **87**, 47 % for **94**, and 74 % for **95**. These compounds did not require any further purification. The absence of the *tert*-butyl singlet from the ¹H NMR spectra of these products confirmed that the deprotection was successful. The analysis of the propyl spacer so obtained agreed with that described in *Section 2.4.1* via the direct functionalisation of the naphthalene dianhydride. Thus, this method is applicable for synthesising pure aminoalkyl naphthalene diimide spacers in high yields and although this method does involve more synthetic steps it does succeed for the pentyl and hexyl analogues where the direct method faltered.

Recently, Sanders *et al.* at Cambridge have developed a novel approach to the synthesis of amino acid functionalised naphthalene diimides.¹⁰⁹ They have synthesised a range of symmetric and asymmetric diimides in quantitative yields by sequentially reacting the desired amino acids with 1,4,5,8-naphthalene tetracarboxylic dianhydride. The reactions were conducted in DMF, in the presence of Et₃N and were heated in a microwave reactor to 140 °C for 5 min. This approach will be investigated in future as a means of synthesising these spacer systems.¹⁰⁹

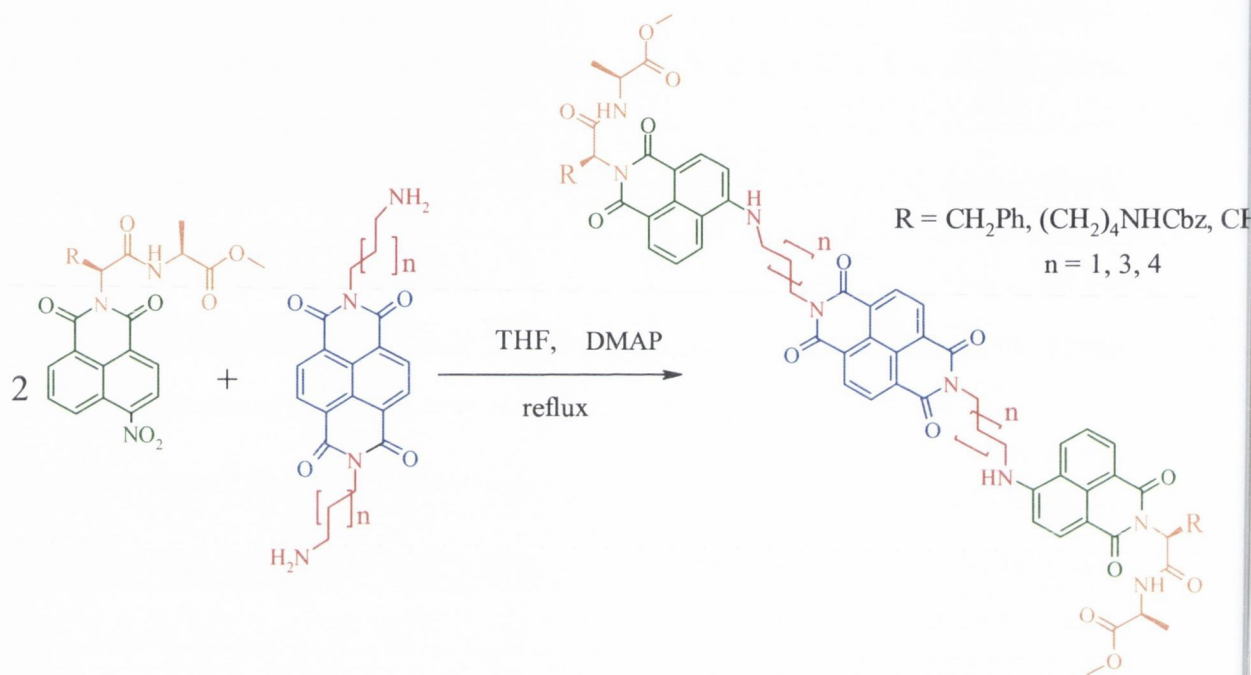
Section 2.4 has detailed the design and synthesis of a family of novel spacer systems based upon a naphthalene skeleton. These were now available for incorporation into a range of ‘tail-to-tail’ bisnaphthalimides.

2.5 ‘Tail-to-Tail’ Bisnaphthalimides

2.5.1 Introduction

The final stage in the bisnaphthalimide strategy was the amalgamation of the chosen naphthalimides (**83**, **85** and **86**) with the novel spacer systems (**87**, **94** and **95**). It was proposed that each of the terminal amines of these spacers would accomplish simultaneous nucleophilic aromatic substitution (NAS) at the 4-position of two naphthalimides, as had already been seen within the group, **Scheme 2.8**.^{103, 104, 105} These would substitute the nitro groups from the 4-positions and result in unifying of three units to create one whole, a novel bisnaphthalimide conjugate. Each naphthalimide component

would then effectively be 4-amino substituted and would be tethered to another naphthalimide unit through the naphthalene diimide spacer. The synthesis detailed in *Section 2.2 – Section 2.4* shows that there are many avenues of modification of the components and, thus, many possible bisnaphthalimides. The following section discusses the construction of eleven novel bisnaphthalimides that were synthesised from the building blocks described in the preceding sections.

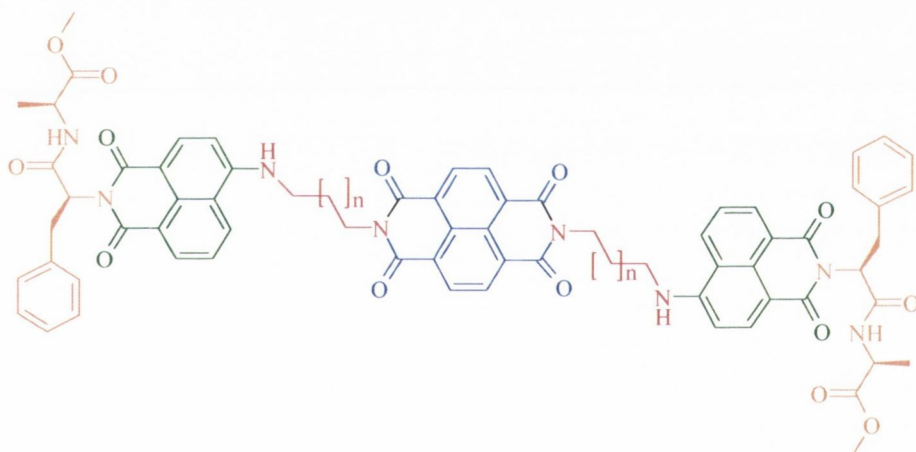


Scheme 2.8: Two dipeptide functionalised naphthalimides and one *N, N'*-bis-diaminoalkyl-naphthalene diimide linker combine to yield a novel bisnaphthalimide

2.5.2 Dipeptide Bisnaphthalimides featuring *L*-Phenylalanine

Compound **83**, the PheAla functionalised 4-nitro-1,8-naphthalimide, was selected as the initial naphthalimide for incorporation in a bisnaphthalimide. It was dissolved in anhydrous THF and to this solution was added half an equivalent of the propyl naphthalene diimide spacer, **87**, along with four equivalents of dimethyl aminopyridine (DMAP). THF was the solvent of choice since the spacer systems were found to be very insoluble in a range of other solvents, such as DMA and DMF. It is worth noting that although not totally soluble in THF as the reaction mixture is heated the reagents becomes more solubilised and whilst the bisnaphthalimide produced is totally soluble in THF any unreacted spacer

will precipitate once the reaction is removed from reflux. The reaction mixture was stirred at reflux, under an inert atmosphere, for 72 h. The reaction was monitored by thin layer chromatography (tlc), using MeOH as the eluent, and the appearance of a luminescent amine spot ($R_f = 0.72$) was noted. No evidence of amine attack at the ester function was observed. However, the reaction did not go to completion and remaining starting material was removed by hot filtration through a celite plug. The solvent was then removed under reduced pressure and the resulting residue was dissolved in CH_2Cl_2 and a wash with 0.1 M HCl and 0.1 M NaOH was conducted before removing the organic solvent under reduced pressure. The product was recrystallised from CH_2Cl_2 and dried under vacuum to give **96** as a brown solid, in a yield of 18 %. This product was further purified by chromatographic preparative plate using MeOH as the eluent, from which a fluorescent yellow band ($R_f = 0.72$) was isolated, recrystallised from hexane : EtOAc, 6 : 1 and dried under vacuum to proffer a brown crystalline solid in an overall yield of 11 %. This product was characterised by ^1H and ^{13}C NMR and the usual techniques.



96, $n = 1$, 18 %

97, $n = 3$, 23 %

98, $n = 4$, 19 %

The ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum obtained for **96**, 2,7-bis- $\{N-[N'-\{(1S)-[(2S)\text{-propionic acid methyl ester}]\text{-carboxyamido-2-ethylphenyl}\}-1,8\text{-naphthalimido}]\text{-3-aminopropyl}\}$ -benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone is shown in **Figure 2.9**. The aromatic naphthalimide signal for H7 is a doublet at 8.72 ppm. However, that of H5 and H2 are found as a multiplet along with the diimide protons, Ar-H, at 8.56 ppm, integrating to a total of eight protons. The signals for H3 and H6 are found at 8.34 ppm and 8.09 ppm, respectively. The phenyl group occurs as a multiplet at 7.07 ppm. The stereogenic centres appear at 5.73 ppm and 4.42 ppm. The methyl ester singlet

resonates at 3.63 ppm, the benzyl protons are a multiplet at 4.10 ppm. The methyl ester singlet resonates at 3.68 ppm and the alanine methyl protons at 1.25 ppm. The propyl bridge is evidenced by the three methylene multiplets seen over the range of 2.62 – 1.15 ppm. The ^{13}C NMR spectrum also supports this identification with additional diimide carbonyl signals at 173.4 ppm and a total of twenty-four signals. The methyl ester carbonyl is seen at 168.0 ppm and the naphthalimide carbonyls appear at 168.3 ppm and 162.8 ppm.

The complicated nature of this molecule called for additional NMR investigations to be conducted. Two dimensional experiments were conducted, including a ^1H - ^1H COSY, **Figure 2.10**, and a ^{13}C - ^1H COSY, **Figure 2.11**, in order to clarify the identity and coupling of the three methylene signals and the diimide protons themselves. Together these allowed identification of all the protons and carbons and their inter-relationships. Importantly, these confirm that the diimide protons are those found in the broad multiplet at 8.56 ppm. Each of the propyl bridge CH_2 protons were also identified.

The fact that this product was indeed the desired bisnaphthalimide was corroborated by spectroscopic methods, **Figure 2.12**. The UV-visible spectrum of **96** in EtOAc shows a maximum at 355 nm, due to the diimide core, and a second band at 450 nm, due to the 4-amino-1,8-naphthalimides. The fluorescence spectrum when excited at 355 nm shows intense diimide bands at 412 nm and 495 nm and when excited at 440 nm emission assigned to the 4-amino-1,8-naphthalimide was observed at 505 nm. The dipeptide and the spacer themselves display different behaviour in this solvent, this will be examined in depth in *Chapter Three*.

A solution of **96** in CH_2Cl_2 and MeOH was submitted for mass spectrometry analysis. ESMS showed a peak at 1237.30 a.u. and elemental analysis found C, 62.87; H, 4.67; N, 8.46 %, which corresponds to $\text{C}_{70}\text{H}_{60}\text{N}_8\text{O}_{14} \cdot \text{CH}_2\text{Cl}_2 \cdot \text{MeOH} \cdot 2\text{H}_2\text{O}$ confirming the identity of the product.

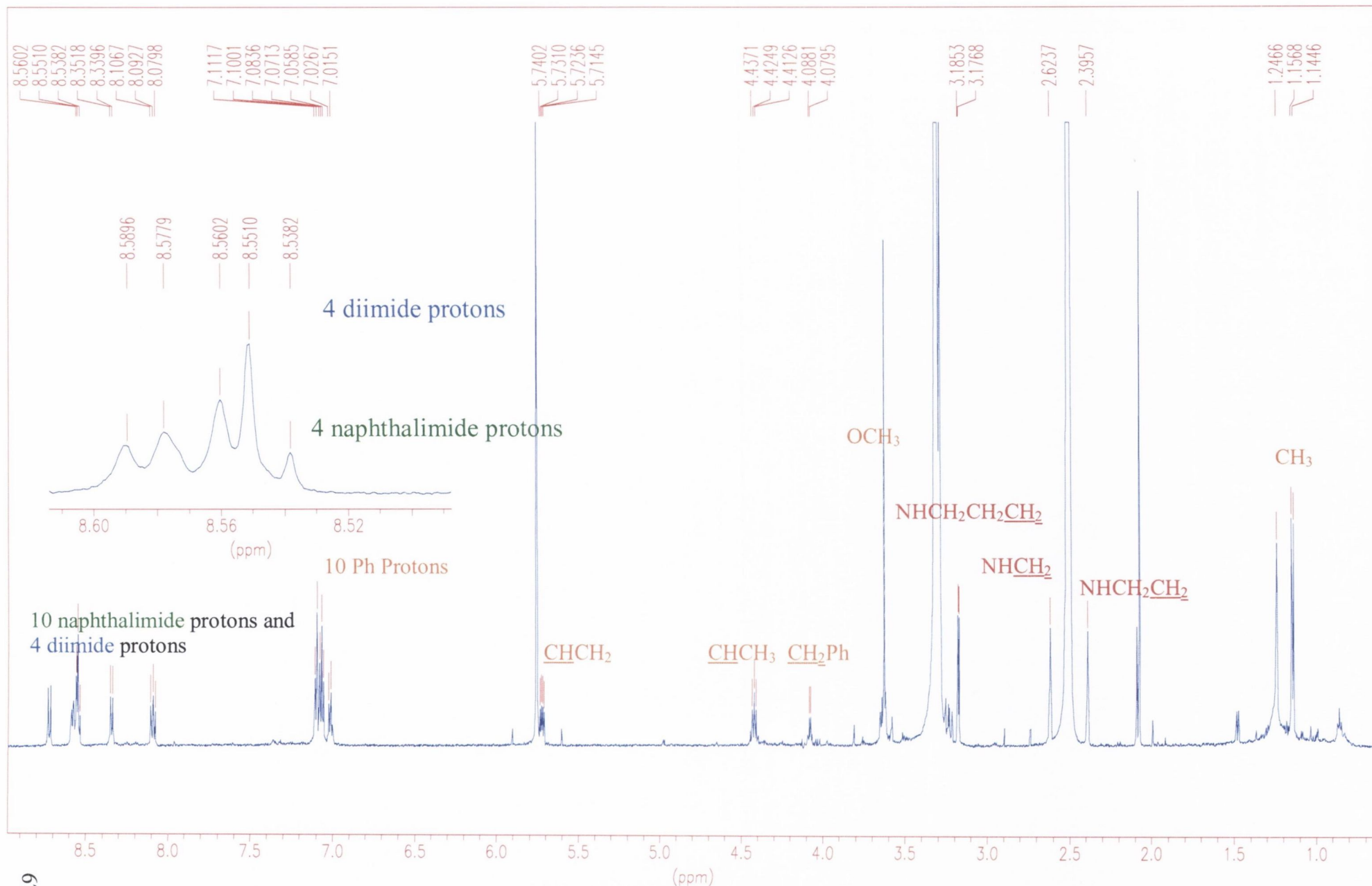


Figure 2.9: ¹H NMR spectrum of 96

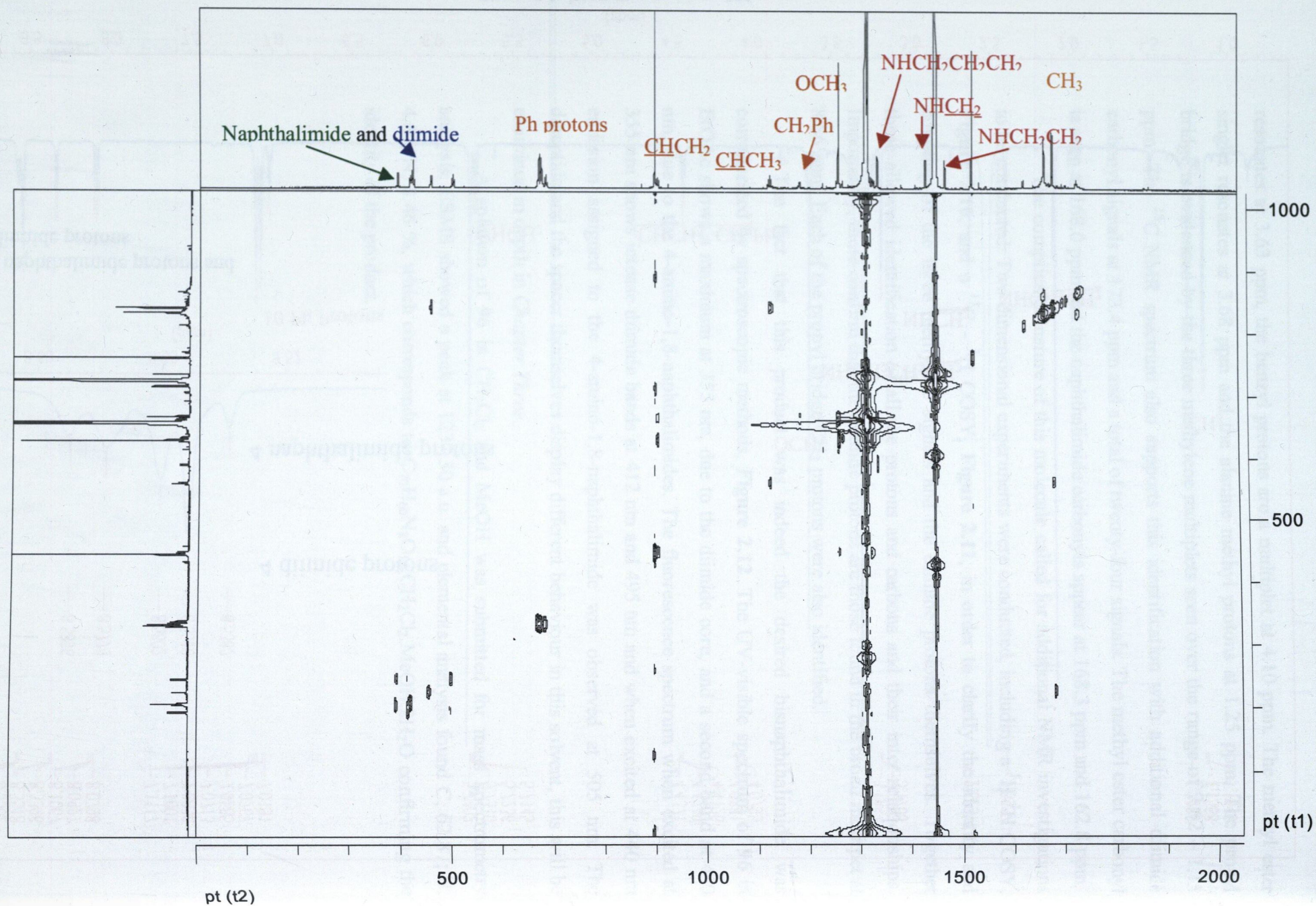


Figure 2.10: ^1H - ^1H COSY NMR spectrum of 96

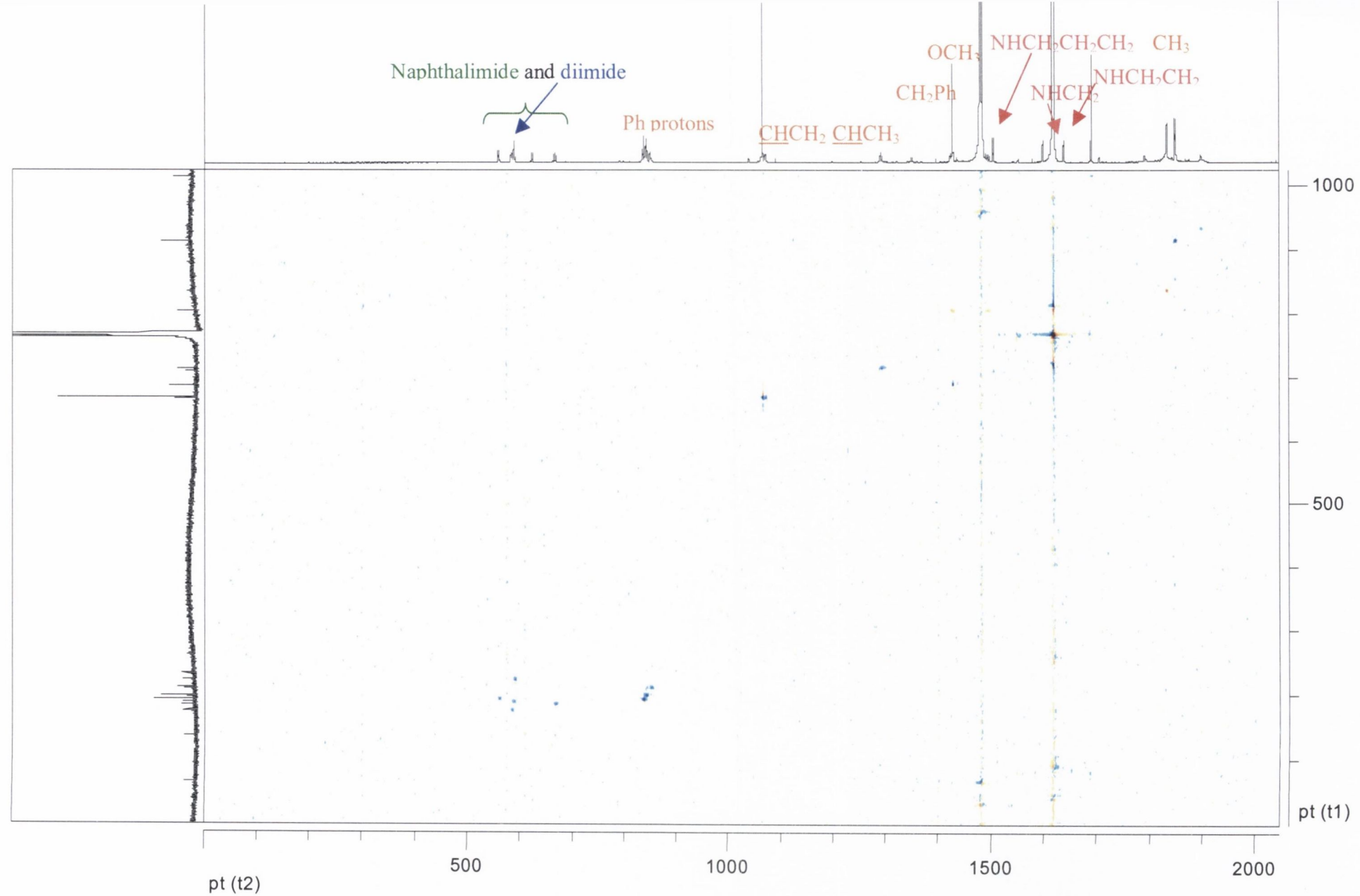


Figure 2.11: ^{13}C - ^1H COSY NMR Spectrum of 96

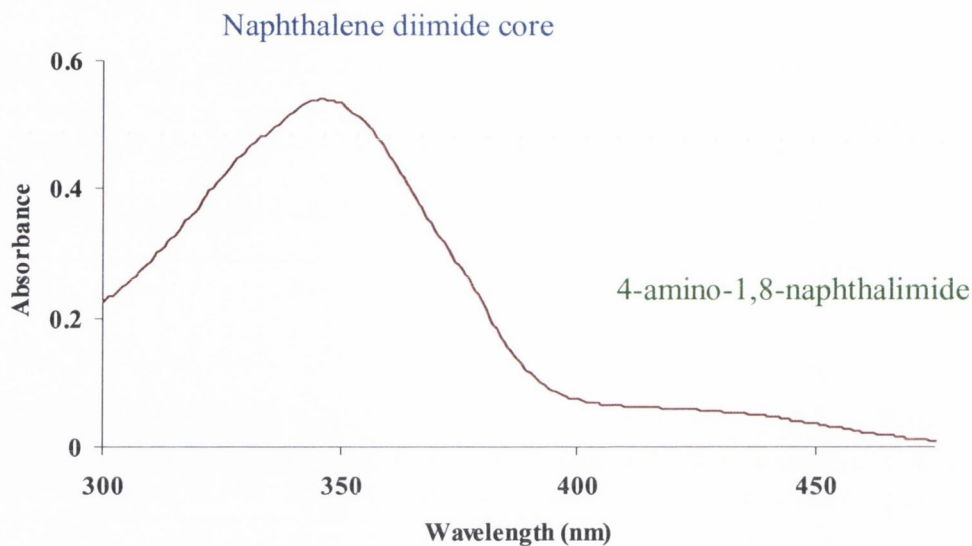


Figure 2.12: UV-visible absorbance spectrum of **96**

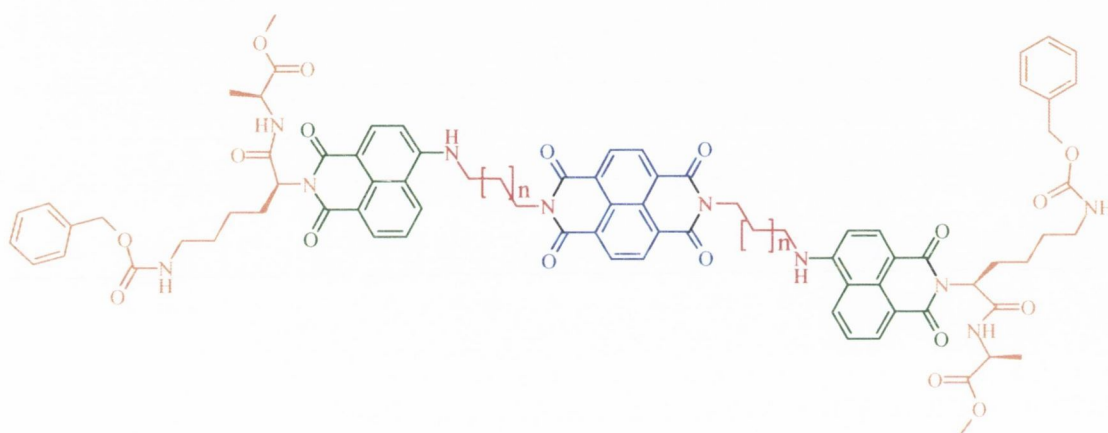
This synthetic methodology was also utilised to produce the pentyl and hexyl linked analogues, **97** and **98**. Compound **97**, 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-1,8-naphthalimido]-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, was synthesised using **83**, the pentyl spacer, **94**, and DMAP in THF as previously described. The product obtained by recrystallisation from CH₂Cl₂ was a fine brown in a yield of 23 %. Further purification by preparative plate chromatography gave a yield of 15 %. Conventional characterisation techniques were employed and the ¹H NMR (600 MHz, (CD₃)₂SO) spectrum shows all the indicative signals and a total of five methylene multiplets are located over the range 2.62 ppm – 1.01 ppm.

The final analogue in this family, **98**, 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, was likewise synthesised, using the hexyl spacer, **95**. This crude product was purified by recrystallisation from CH₂Cl₂ since the hot filtration had succeeded in removing most of the unreacted reagents and the washings had eliminated any byproducts. After recrystallisation the desired compound was a pale brown solid, in a yield of 19 %, further purified by preparative plate chromatography gave a yield of 15 %. Two dimensional NMR experiments were employed along with the conventional ¹H and ¹³C NMR spectra to fully characterise **98**. The ¹H NMR (600 MHz, (CD₃)₂SO) spectrum includes all the expected resonances,

including the six alkyl chain CH₂ signals which are located over the range 2.64 ppm – 1.01 ppm. Characterisation was completed *via* the conventional techniques.

2.5.3 Dipeptide Bisnaphthalimides featuring *L*-Cbz(Lysine)

The same synthetic method employed for the syntheses of **96**, **97** and **98** was adopted for the synthesis of *L*-Cbz(Lys)Ala using **85**. Firstly, this was reacted with the propyl spacer, **87**. The work-up methodology previously optimised was again utilised giving a brown hygroscopic solid, **99**, in a yield of 18 %. Further purification by preparative plate chromatography produced the desired bisnaphthalimide in an overall yield of 12 %.



99, *n* = 1, 18 %

100, *n* = 3, 16 %

101, *n* = 4, 23 %

Figure 2.13 shows the ¹H NMR (600 MHz, (CD₃)₂SO) spectrum obtained for **99**, 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic-acid-methyl-ester]-carboxyamido-6-benzyloxy-carbonylaminohexyl}-1,8-naphthalimido}]-3-aminopropyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone. The aromatic naphthalimide appear as four multiplets at 8.74 ppm, 8.60 ppm, 8.13 ppm, 7.80 ppm. The multiplet at 8.60 ppm integrates for eight protons and corresponds to H5, the diimide Ar-H protons and H2. The phenyl resonance occurs as a multiplet at 7.29 ppm, while the protons of the stereogenic centres appear at 5.65 ppm and 4.38 ppm. The benzyl protons resonate at 5.37 ppm. The methyl ester singlet appears at 3.62 ppm and the alanine methyl protons are seen at 1.26 ppm. The lysine chain

methylene multiplets are located at 3.23 ppm, 2.64 ppm, 2.08 ppm and 1.65 ppm. The propyl bridge is evidenced by the three methylene multiplets at 2.92 ppm, 1.92 ppm and 1.48 ppm. The ^{13}C NMR spectrum also supports this identification with the additional diimide carbonyl signals at 173.5 ppm.

Elemental analysis found C, 61.15; H, 5.21; N, 9.20 %, corresponding to $\text{C}_{80}\text{H}_{78}\text{N}_{10}\text{O}_{18} \cdot 2\text{CH}_2\text{Cl}_2$. The UV-visible and fluorescence spectra produced by **99** agree with those of **96**. Full experimental and characterisation information can be found in Chapter Five.

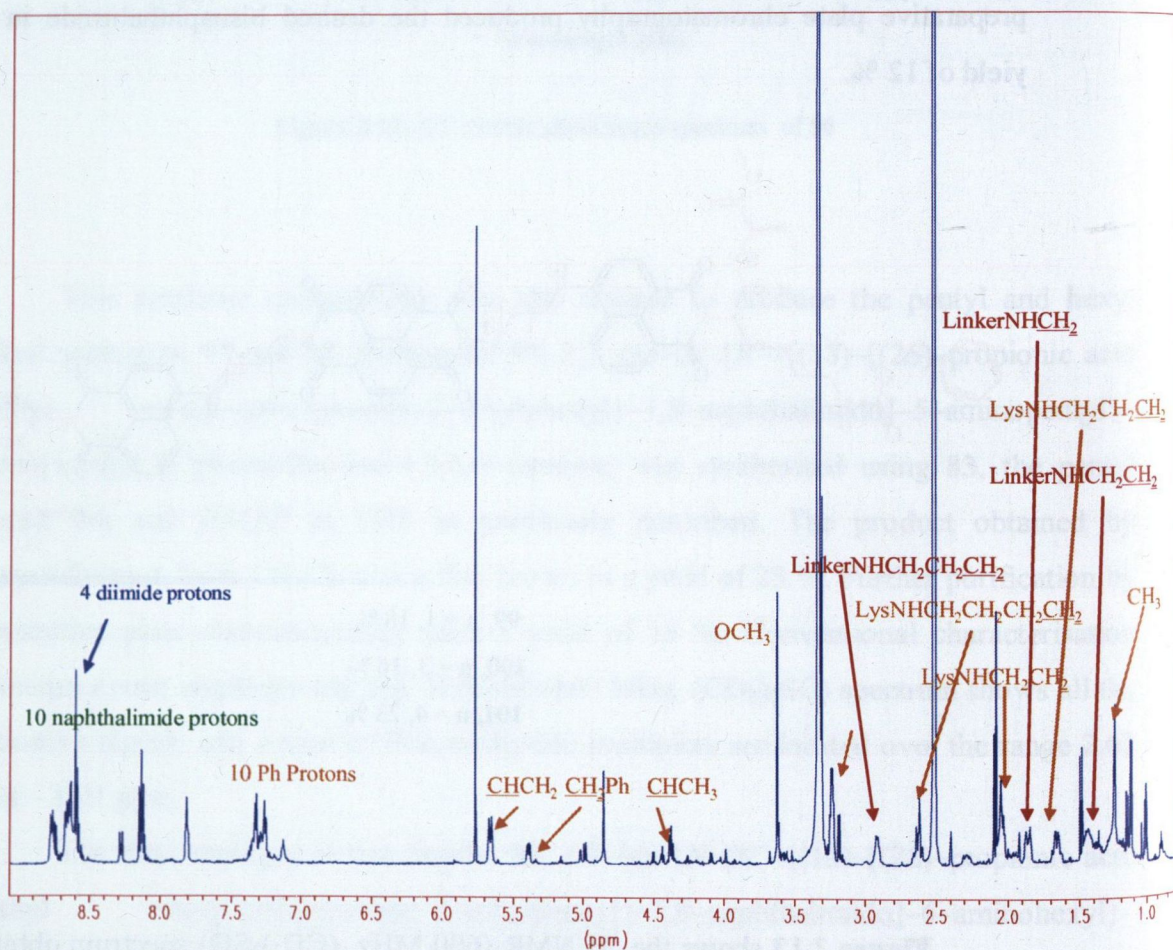


Figure 2.13: ^1H NMR spectrum of **99**

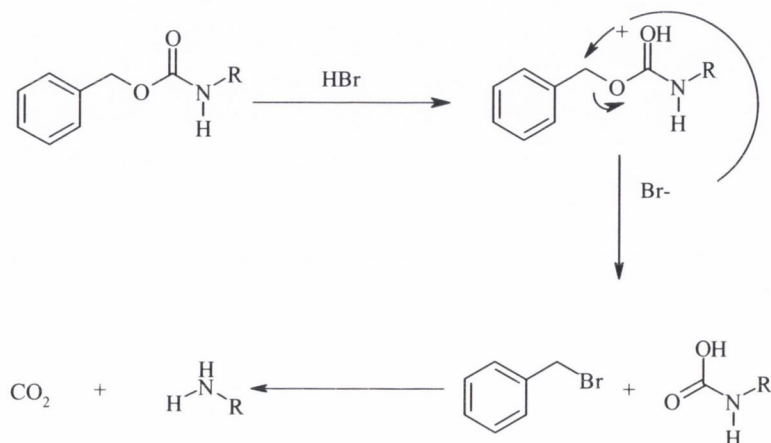
The pentyl linked analogue, **100**, 2,7-bis- $\{N-[N'-\{(1S)-[(2S)\text{-propionic-acid-methyl ester}]\text{-carboxyamido-6-benzyloxycarbonylaminohexyl}\}-1,8\text{-naphthalimido}]\}-5\text{-amino pentyl}\}$ -benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, was also synthesised using **85**, the pentyl spacer, **94**, and DMAP. The crude product was a brown solid in a yield of 16%. The product obtained was further purified by recrystallisation from CH_2Cl_2 , purified by preparative plate chromatography and was isolated as a dark brown solid, in a

yield of 9 %. Again, the ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum along with two dimensional experiments were necessary for the identification of all the signals. All the dipeptide and naphthalimide signals are present and a total of five alkyl chain CH_2 signals, these are located at 3.62 ppm, 3.22 ppm, 2.91 ppm, 1.39 ppm and 1.15 ppm.

Finally, the hexyl analogue in this *L*-Cbz-LysAla family, **101**, 2,7-bis- $\{N-[N'-((1S)-[(2S)\text{-propionic acid methyl ester}]\text{-carboxyamido-6-benzyloxycarbonylaminohexyl})-1,8\text{-naphthalimido}]\}-6\text{-aminohexyl}\}$ -benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, was similarly synthesised, by reacting **85** and **95** in the presence of DMAP. The product was also purified by recrystallisation from hexane: EtOAc, 6:1 and was as a fine dark brown powder, in a yield of 23 %, and in a yield of 12 % following preparative plate chromatography. The ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum contains the dipeptide and naphthalimide signals and a total of five alkyl chain CH_2 signals corresponding to the six methylene groups of the spacer, these are located at 3.17 ppm, 2.63 ppm, 1.49 ppm, 1.25 ppm and 1.02 ppm, this last signal integrates for eight protons where the other integrate for four protons each.

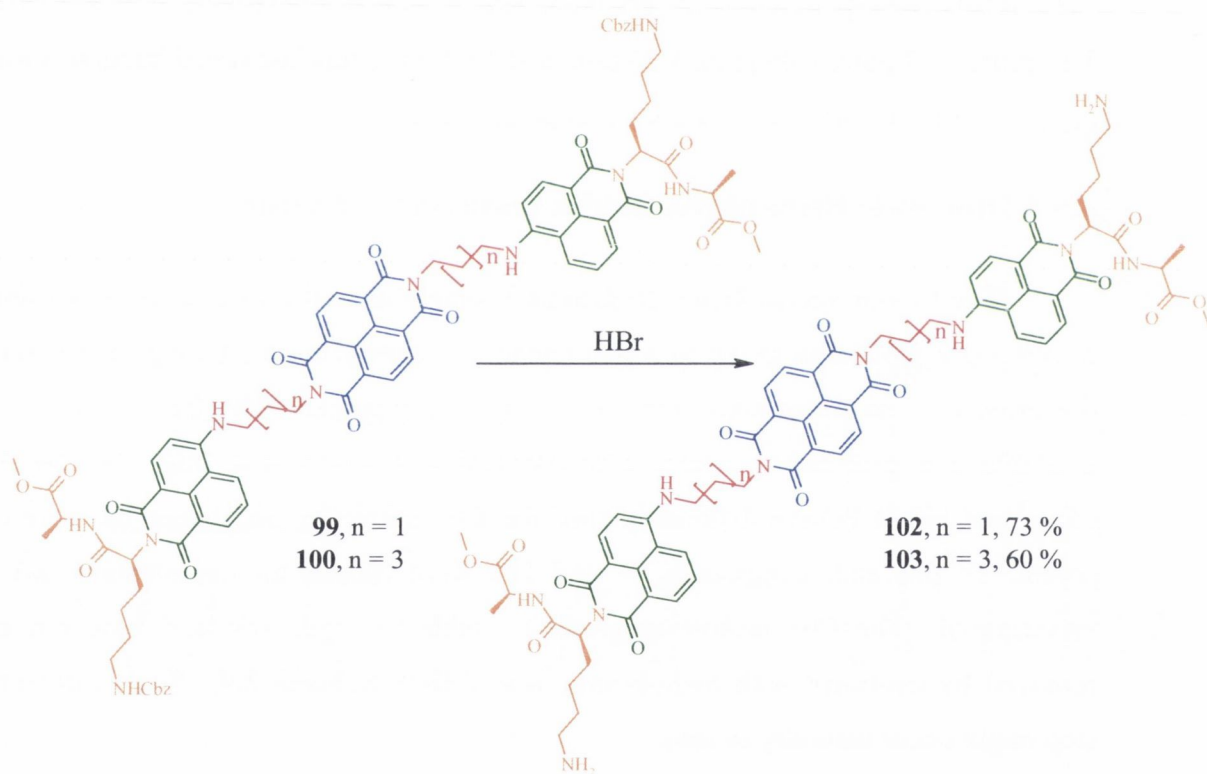
2.5.4 Dipeptide Bisnaphthalimides featuring *L*-Lysine

The Cbz-protected lysine containing bisnaphthalimides offered an opportunity for converting a protecting group to a free amine and comparing the biological behaviour of the protected and deprotected versions of these compounds. The free amine would be available for protonation under physiological conditions and thus the deprotected compound might behave differently than the Cbz derivative and hence be more or less potent. To this end, compounds **99** and **100** were chosen for deprotection and further investigation. The Cbz protecting group is stable to weak acid and base but can be removed by treatment with hydrobromic acid (HBr), **Scheme 2.9**. Such a deprotection step might occur naturally *in vivo*.



Scheme 2.9: The acid mediated cleavage of a Cbz protecting group

Compound **99** was dissolved in an excess of a solution of 30 % HBr / acetic acid, **Scheme 2.10**. This reaction mixture was stirred at room temperature for 1 h. while the reaction vessel was fitted with a calcium carbonate (CaCO_3) drying tube. The advised work-up for such an acid mediated hydrolysis is the addition of EtOAc to induce precipitation of the hydrolysed product. This was attempted but no precipitate was formed. Instead, the solvent was removed under reduced pressure and the product was washed twice with K_2CO_3 (10 %, w/v) and twice with deionised water. The crude product was dissolved in CH_2Cl_2 and dried under vacuum. The desired product, **102**, was isolated as a viscous oil, in a yield of 73 %. The ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum obtained is similar to that for compound **99** except for the absence of the Cbz signal indicating that the protecting group was successfully removed.



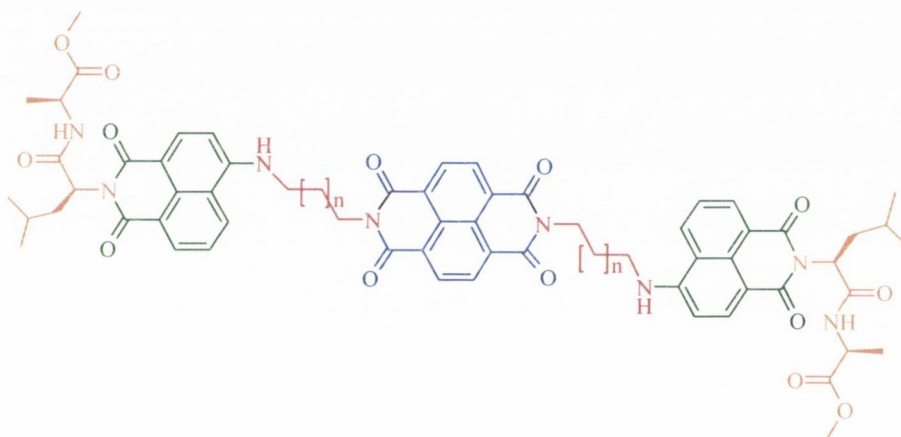
Scheme 2.10: The synthesis of compounds **102** and **103**

Compound **100** was treated with HBr acid under identical conditions. The desired product, compound **103**, was also isolated as a viscous oil after drying under vacuum, in a yield of 60 %. Again, the ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum showed the absence of the Cbz group. These compounds were principally of interest in the biological assays that were conducted by our collaborators in St James' Hospital, Dublin and the details of this can be found in *Chapter Four*. In theory, these compounds might be further reacted with

other functionalities such as 1,4,5,8-naphthalene tetracarboxylic dianhydride through their free terminal amines available to elongate the systems to polynaphthalimides, complimenting those developed by Iverson *et al.*⁹⁷ However, this is beyond the scope of this thesis and shall not be discussed here.

2.5.5 Dipeptide Bisnaphthalimides featuring *L*-Leucine

The final family for incorporation into the bisnaphthalimide design was the LeuAla functionalised 4-nitro-1,8-naphthalimide. This was achieved by reacting **86** and the propyl spacer, **87**, in the presence of DMAP under the usual reaction conditions. The usual work-up followed by recrystallisation from CH₂Cl₂ gave a brown solid, in a yield of 22 %, compound **104**. Further purification by chromatography resulted in an ultimate yield of 13 %.



104, $n = 1$, 22 %

105, $n = 3$, 19 %

106, $n = 4$, 22 %

The ¹H NMR (600 MHz, (CD₃)₂SO) spectrum obtained for **104** confirmed its identity as 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic acid methyl ester]-carboxamido-3-methylbutyl}-1,8-naphthalimido]-5-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone. The aromatic naphthalene diimide and naphthalimide signals are apparent as two doublets multiplets, H7 at 8.75 and H3 at 8.29 ppm, one multiplet over the range 8.67 – 8.57 ppm, corresponds to H5, the diimide protons, Ar-H, and H2, and H6 resonates as a triplet at 8.13 ppm. The stereogenic centre protons appear at 5.45 ppm and 4.36 ppm. The methyl ester resonates as a singlet at 3.62 ppm and the alanine methyl protons occur at 1.13 ppm. The CH₂ of the Leu group appear as two multiplets at 2.16 and 1.88 and

the methyl groups appear as two doublets at 0.91 and 0.84 ppm. The propyl bridge is evidenced by the three methylene multiplets at 3.29 ppm, 3.06 ppm and 1.47 ppm. The ^{13}C NMR spectrum also supports this identification with additional diimide carbonyl signals at *ca.* 173.4 ppm. Elemental analysis found C, 55.30; H, 4.96; N, 8.47 %, corresponding to $\text{C}_{64}\text{H}_{64}\text{N}_8\text{O}_{14}\cdot 4\text{CH}_2\text{Cl}_2\cdot \text{DMAP}$ which requires C, 55.23; H, 5.07; N, 8.59 %.

The pentyl linked analogue, **105**, 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic acid methyl ester]-carboxyamido-3-methylbutyl}-1,8-naphthalimido]-5-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, was synthesised using **86**, the pentyl spacer, **94**, and DMAP. The product obtained was further purified by recrystallisation from CH_2Cl_2 and was isolated as a dark brown solid, in a yield of 19 % and in a yield of 14 % following chromatography. The ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum depicts all the expected dipeptide and naphthalimide signals. The naphthalene diimide singlet is located among the naphthalimide multiplet over the range 8.68 - 8.58 ppm. The five methylene multiplets of the pentyl spacer are seen at 2.63 ppm, 2.40 ppm, 1.48 ppm, 1.27 ppm, and 1.01 ppm.

Lastly, the hexyl linked analogue, **106**, 2,7-*bis*-{*N*-[*N'*-{(1*S*)-[(2*S*)-propionic acid methyl ester]-carboxyamido-3-methylbutyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, synthesised using **87**, the hexyl spacer, **95**, and DMAP. The product obtained was purified by recrystallisation from DCM and was isolated as a dark brown solid, in a yield of 22 % and in a yield of 11 % following preparative plate chromatography. The ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{SO}$) spectrum shows all the expected dipeptide and naphthalimide signals are present. The naphthalene diimide singlet is located among the naphthalimide multiplet over the range 8.69 - 8.65 ppm. The six methylene group of the hexyl spacer appear as five multiplets at 3.30 ppm, 2.52 - 2.48 ppm, 2.09 ppm, 1.48 ppm and 1.25 ppm.

2.5.6 Optimising the Syntheses of Bisnaphthalimides

Over the course of the synthetic section of this project, a number of alternative conditions were explored in an effort to optimise the percentage yields of the bisnaphthalimide reactions, since these failed to go to completion and the crude yields were rather low, *ca.* 16 % - 23 %, *cf.* Sections 2.5.2-2.5.5. The incompleteness of the reactions is most likely due to the insolubility of the spacer systems. As mentioned previously, a range of solvents were tested for their ability to dissolve the family of

naphthalene diimides and none (including DMA and DMF) were more successful than THF and so this was exclusively employed as the reaction solvent.

Towards the latter part of the syntheses two other alterations were investigated. Repeat syntheses of **97**, **100** and **105** were kept under reflux for two weeks. This was found to provide the desired bisnaphthalimides in yields of 29 %, 22 % and 35 %. All the syntheses were repeated by placing the reagents in sealable pressure tubes. These allow a reaction to be conducted under pressure and the solvent to be heated to higher temperatures. The reactions were heated at 120 °C for 72 h and the products were obtained in yields of 24 % - 38 %, respectively. When repeating the syntheses of any of the bisnaphthalimides the pressure tube procedure was adopted since it typically permitted a *ca.* 30 % yield over the same reaction duration. These syntheses are summarised in **Table 2.2**.

One further alternative means of synthesis was investigated. This is detailed in the following section.

Table 2.2: A summary of percentage yields obtained *via* different conditions of bisnaphthalimide synthesis

Compound	72 h Reflux	2 Wk Reflux	Pressure Tubes
96	18 %		24 %
97	23 %	29 %	30 %
98	19 %		29 %
99	18 %		36 %
100	16 %	22 %	37 %
101	23 %		38 %
104	22 %		28 %
105	19 %	25 %	30 %
106	22 %		35 %

2.5.7 An Alternative Synthesis of ‘Tail-to-Tail’ Dipeptide

Bisnaphthalimides

With the aim of improving the yield obtained from the bisnaphthalimide syntheses another synthetic route was investigated. While the naphthalene core may be functionalised to an alkyl naphthalene diimide which can tether two naphthalimides

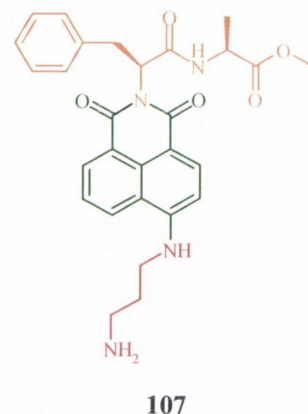
together, one might equally functionalise the 4-position of a naphthalimide with the intended bridge and subsequently react this with the dianhydride and so construct a bisnaphthalimide from alternative means.

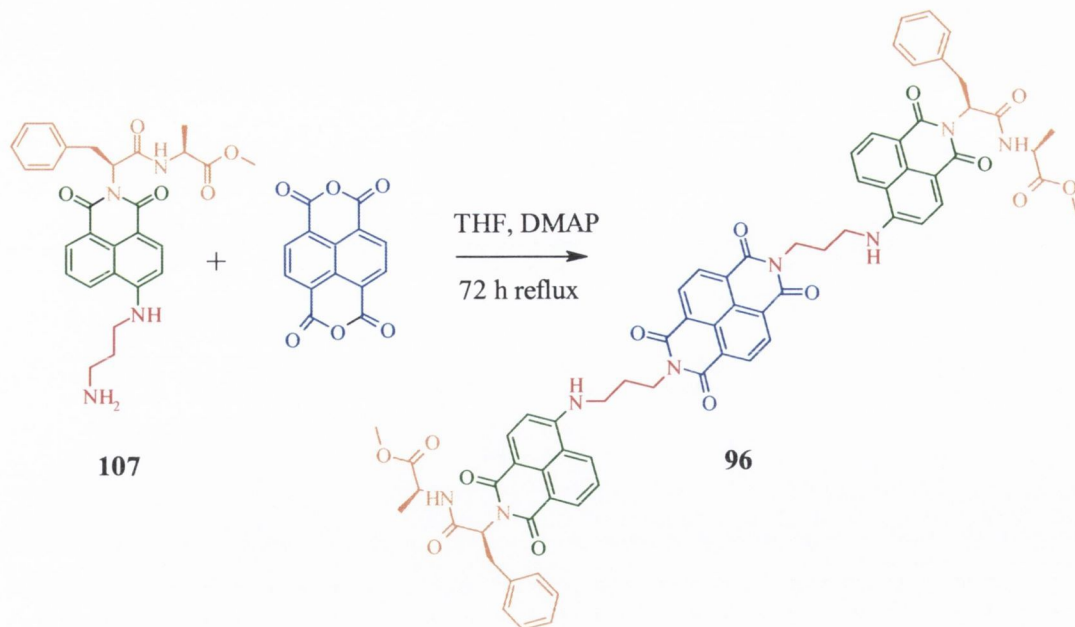
The synthesis of 4-substituted 1,8-naphthalimides^{103, 104, 105, 108} has been developed within the Gunnlaugsson group, in particular alkyl amino substituted naphthalimides have been formed by reacting an amine or diamine with the naphthalimide and refluxing for 72 h in THF.^{104, 108}

This methodology was employed to produce some 1,3-diamino propane, 1,5-diamino pentane and 1,6-diamino hexane functionalised analogues of some of the mono-peptide and dipeptide 1,8-naphthalimides described in *Section 2.3.2*. This will be further discussed in *Section 2.6*.

Compound **83** was dissolved in anhydrous THF and to this was added an excess of 1,3-diamino propane. This reaction mixture was refluxed, under argon, for 72 h. The solvent and remaining 1,3-diamino propane were then removed under reduced pressure and the resulting fluorescent residue was dissolved in CH₂Cl₂ and washed with HCl (1 M), saturated sodium hydrogencarbonate (NaHCO₃) and deionised water. After removing the solvent under reduced pressure the crude product was crystallised from CH₂Cl₂ and dried under high vacuum. The brown crystalline solid, compound **107**, was isolated in a yield of 25 % and was characterised by the conventional methods. The ¹H NMR (400MHz, CDCl₃) spectrum includes all the expected dipeptide and naphthalimide signals along with the new bridge methylene resonances at 1.76 ppm, 1.45 ppm and 1.21 ppm. A broad NH singlet appears at 3.50 ppm. ESMS and elemental analysis also confirmed the identity of the product which also had a UV-visible maximum centred at 450 nm characteristic of a 4-amino substituted naphthalimide structure.

Subsequently, compound **107** was dissolved in anhydrous THF with 0.5 equivalents of 1,4,5,8-naphthalene tetracarboxylic dianhydride, in the presence of DMAP, **Scheme 2.11**. This reaction mixture was heated at reflux, under argon, for 72 h, following the same work-up employed in *Sections 2.5.2-2.5.5*, the product was isolated and purified by preparative plate chromatography giving a brown crystalline solid, in a yield of 17 %. Conventional characterisation techniques supported the identification of this product as **96**, the initial bisnaphthalimide synthesised in *Section 2.5.1*. Therefore, this alternative avenue can equally be used to synthesise the desired bisnaphthalimide, although this method does not improve the percentage yield obtainable.





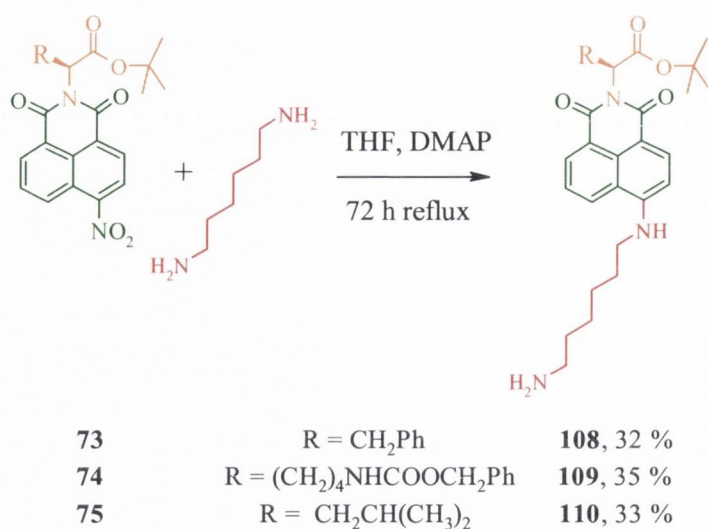
Scheme 2.11: The alternative bisnaphthalimide synthesis of **96**

The Sanders *et al.* approach to the synthesis of amino acid functionalised naphthalene diimides might be a viable option optimising the yields of this synthesis and definitely warrants investigation since the 4-amino functionalised naphthalimides are comparable to the amino acids employed by Sanders *et al.*¹⁰⁹

2.6 ‘Tail-to-Tail’ Monopeptide Bisnaphthalimides

The synthesis of a range of monopeptide functionalised bisnaphthalimides was also undertaken to provide a sample of such compounds for biologically testing. This would allow comparison with the activity of their corresponding mononaphthalimide and dipeptide bisnaphthalimides. This plan also provided an opportunity for further investigating the merits of the alternative synthesis developed in *Section 2.5.6*. The deprotection of the hexyl spacer prior to its incorporation in a bisnaphthalimide had proved temperamental for a period of this project, *cf. Section 2.4.3*. Therefore if this alternative method could successfully produce hexyl linked bisnaphthalimides it would provide an alternative to the spacer route.

Each of the *tert*-butyl esters of Phe, **73**, Cbz-Lys, **74**, and Leu, **75**, were reacted with one equivalent of 1,6-diamino hexane, **Scheme 2.12**, according to the method detailed in *Section 2.5.7*. The desired 4-(1,6-diamino hexane) substituted products were successfully isolated, compounds **108**, **109** and **110**, in yields of 32 %, 35 % and 33 %, respectively. These were oils that solidified from evaporation of hexane : EtOAc, 6 : 1 to give hygroscopic solids and were easily characterised by ¹H NMR (400 MHz, CDCl₃).



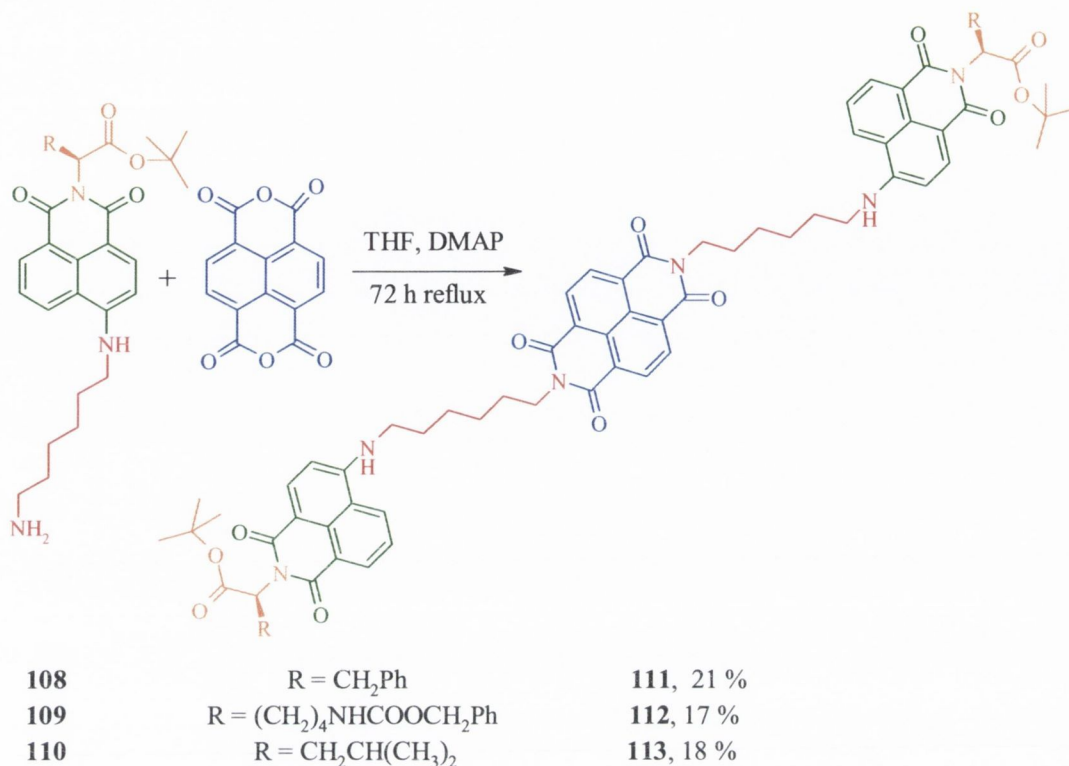
Scheme 2.12: The syntheses of 4-diamino-1,8-naphthalimides

Subsequently, compound **108** was dissolved in anhydrous THF with 0.5 equivalents of 1,4,5,8-naphthalene tetracarboxylic dianhydride and this was heated under reflux, under argon, for 72 h, in the presence of Et_3N , **Scheme 2.13**. Similar work-up and washings were conducted as for the original bisnaphthalimide syntheses in *Sections 2.5.2-2.5.5*. A fluorescent yellow oil was isolated, in a yield of 21 %. The ^1H NMR (400 MHz, CDCl_3) spectrum includes all the expected resonances for the naphthalimides, phenyl group, benzyl protons, the chiral proton and the *tert*-butyl singlet. The methylene groups of the diamino hexyl chain spacer appear over a range of 3.68 ppm – 1.26 ppm. The confirmation that this product was the desired bisnaphthalimide, **111**, was supplied by the appearance of the naphthalene diimide signal among the naphthalimide multiplet at 8.66 – 8.59 ppm.

The Cbz-Lys *tert*-butyl ester analogue, **112**, was similarly synthesised from compound **109** and 1,4,5,8-naphthalene tetracarboxylic dianhydride. The intended monopeptide hexyl bridged bisnaphthalimide was obtained as a fluorescent yellow oil in a yield of 17 %. The ^1H NMR (600 MHz, CDCl_3) spectrum of this compound shows the characteristic signals of the monopeptide as for **109** together with the naphthalene diimide signal amongst the naphthalimides multiplet over the range 8.76 – 8.68 ppm.

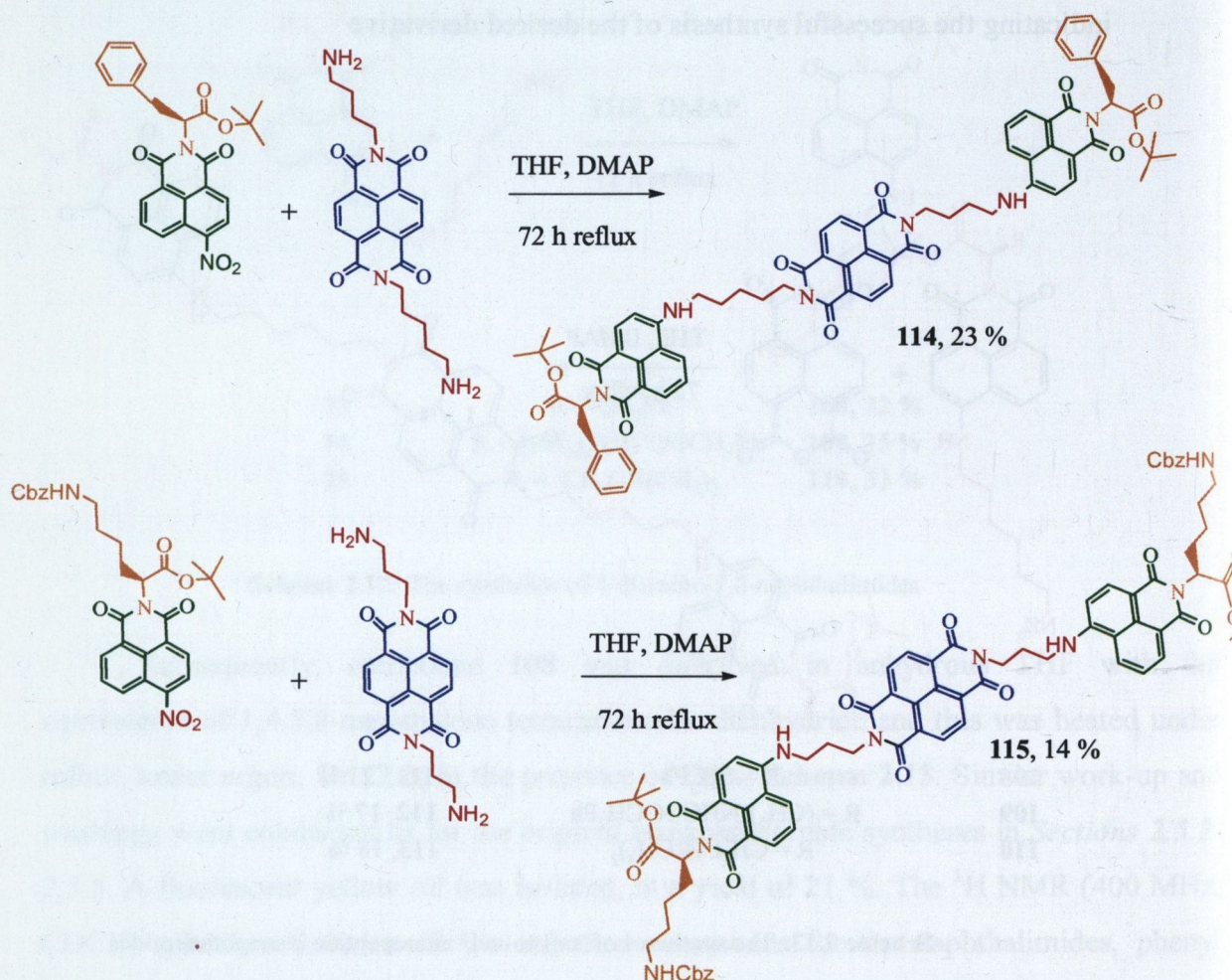
Finally, the Leu *tert*-butyl ester analogue was synthesised using compound **110** and 1,4,5,8-naphthalene tetracarboxylic dianhydride. The desired monopeptide hexyl bridged bisnaphthalimide, **113**, was isolated as a fluorescent yellow oil, in a yield of 18 %. As for **111** and **112**, the ^1H NMR (600 MHz, CDCl_3) spectrum of this compound depicts the characteristic resonances of the monopeptide resonances (as for **110**) and the naphthalene

diimide signal amongst the naphthalimides multiplet over the range 8.65 - 8.42 ppm., indicating the successful synthesis of the desired derivative



Scheme 2.13: The syntheses of ‘tail-to-tail’ mono-peptide bisnaphthalimides

These reactions proved that one might approach the bisnaphthalimide synthesis from an alternative direction and that mono-peptide bisnaphthalimides may be constructed in a similar manner to dipeptide bisnaphthalimides. This method may be especially preferred when using longer spacers since the deprotection step for the hexyl naphthalene diimide spacer was not very high yielding. The yields obtained for these bisnaphthalimides are comparable to those found for the analogous dipeptide syntheses already described. The solubility of these products, however, was an issue and it was not always possible to obtain high-quality ^{13}C NMR spectra. Two additional mono-peptide functionalised bisnaphthalimides, compounds **114** and **115**, were synthesised for biological testing, these are also summarised in **Scheme 2.14**. These were both successfully obtained, in a yield of 23 % and 14 %, respectively, using the original synthetic strategy.



Scheme 2.14: The syntheses of ‘tail-to-tail’ mono-peptide bisnaphthalimides **114** and **115**

This section has detailed the synthesis of five novel mono-peptide functionalised bisnaphthalimides which are linked ‘tail-to-tail’ *via* amino alkyl substituted naphthalene diimides. The biological investigation of these compounds will be discussed in *Chapter Four*.

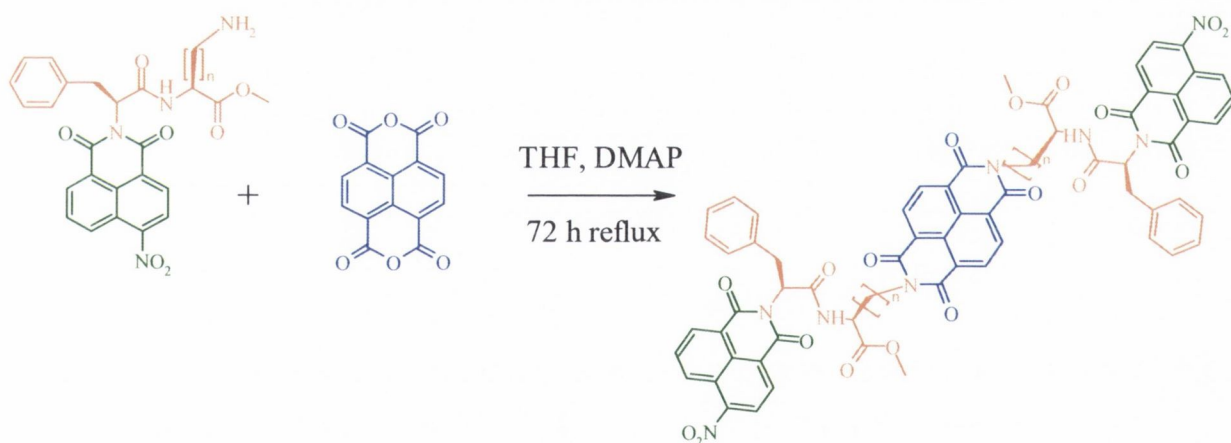
2.7 ‘Head-to-Head’ Dipeptide Bisnaphthalimides with Naphthalene Diimide Spacers

2.7.1 Introduction

The bisnaphthalimides discussed to date have been exclusively ‘tail-to-tail’ bisnaphthalimides, *i.e.* the two naphthalimides units are tethered to each other *via* a spacer

which is attached to the 4-position of the naphthalimides. However, as described in *Section 1.8.2*, another type of bisnaphthalimide is possible, *i.e.* a ‘head-to-head’ bisnaphthalimide, where a substituent at the imide nitrogen of the naphthalimide terminates in a free amine which is available for tethering that naphthalimide to a core moiety. This means that a functional chain of the naphthalimide unit itself acts as the bridge and there is no need for a functionalised spacer; the core would react with two such functionalised naphthalimides to form a bisnaphthalimide, **Scheme 2.15**. Such compounds would provide a variation on the bisnaphthalimide theme, retaining the 4-nitro group where this functional group is lost in the ‘tail-to-tail’ syntheses and allowing any consequential potency to be explored in biological investigations.

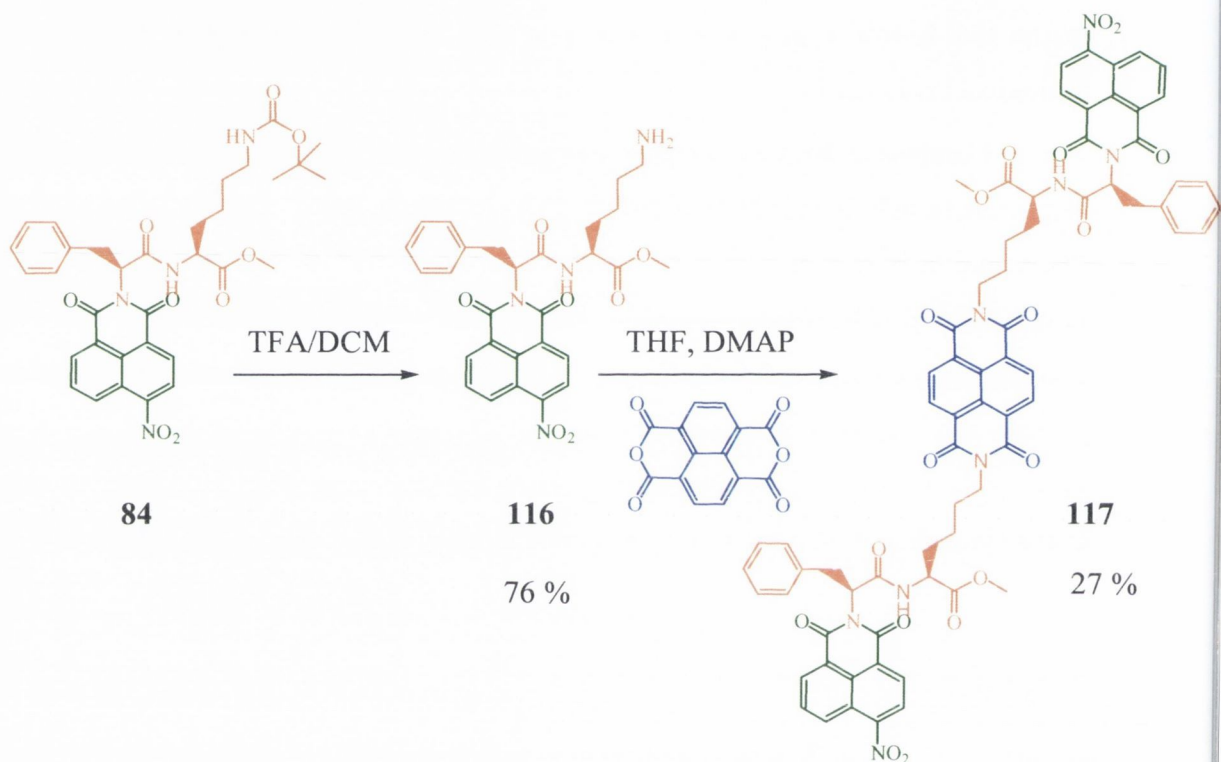
Compound **84** was selected as the archetypal structure for the necessary amine chain functionalised naphthalimides.¹⁰⁵ This is a PheBoc(Lys) dipeptide and offers a bulky Phe group in the first position and a Lys chain at the second position. The Boc protecting group can be hydrolysed to provide the required free amine necessary for incorporation into the central spacer. The analogues of lysine methyl ester, *L*-ornithine (with three CH₂ groups) and *L*-2,4-diamino butyric (with two CH₂ groups) methyl esters, supply a means of varying the length of the bridge, and so the overall spacer length, just as was accomplished in the synthesis of functionalised naphthalene diimide spacers in *Section 2.4*.



Scheme 2.15: The synthesis of ‘head-to-head’ bisnaphthalimides, $n = 2, 3, 4$

2.7.2 Dipeptide Bisnaphthalimide featuring *L*-Lysine

Compound **84** was treated with TFA/CH₂Cl₂ and coevapoation using diethyl ether and recrystallisation from CH₂Cl₂ allowed the isolation of the desired hydrolysed product, **116**, a brown solid, in a yield of 76 %, **Scheme 2.16**. The ¹H NMR (400MHz, CDCl₃) spectrum no longer depicts a *tert*-butyl signal and it was deduced that the Boc group had been successfully removed.



Scheme 2.16: The synthesis of lysine based 'head-to-head' bisnaphthalimide, **117**

Compound **116** was refluxed in anhydrous THF with 0.5 equivalent of 1,4,5,8-naphthalene tetracarboxylic dianhydride, under argon, for 72 h. The product was worked-up in the same manner as for the 'tail-to-tail' bisnaphthalimide in *Sections 2.5.2-2.5.5*. A dark brown solid was isolated, in a yield of 27 %. Further purification was achieved by chromatography, giving **117** in a final yield of 17 %. The conventional methods of characterisation confirmed that this was the intended bisnaphthalimide, compound **117**. The ¹H NMR (600 MHz, (CD₃)₂SO) spectrum depicts the naphthalimide, phenylalanine and the chiral protons signals. The peaks due to the bridge methylene signals of the lysine chain, at 3.96 ppm, 3.19 ppm, 2.63 ppm and 2.40 ppm, and the naphthalene core, at 8.52 ppm, protons constituting the spacer moiety are evident. ESMS also corroborated this characterisation. The solubility of this product, however, was an issue and it was not possible to obtain a high-quality ¹³C NMR spectrum.

The synthesis of this bisnaphthalimide was also attempted using the pressure tube procedure detailed in *Section 2.5.5*. This was successful and compound **117** was obtained in a yield of 31 %. This would imply that the favourability of the reaction to form a ‘head-to-head’ bisnaphthalimide is similar to that to form a ‘tail-to-tail’ bisnaphthalimide.

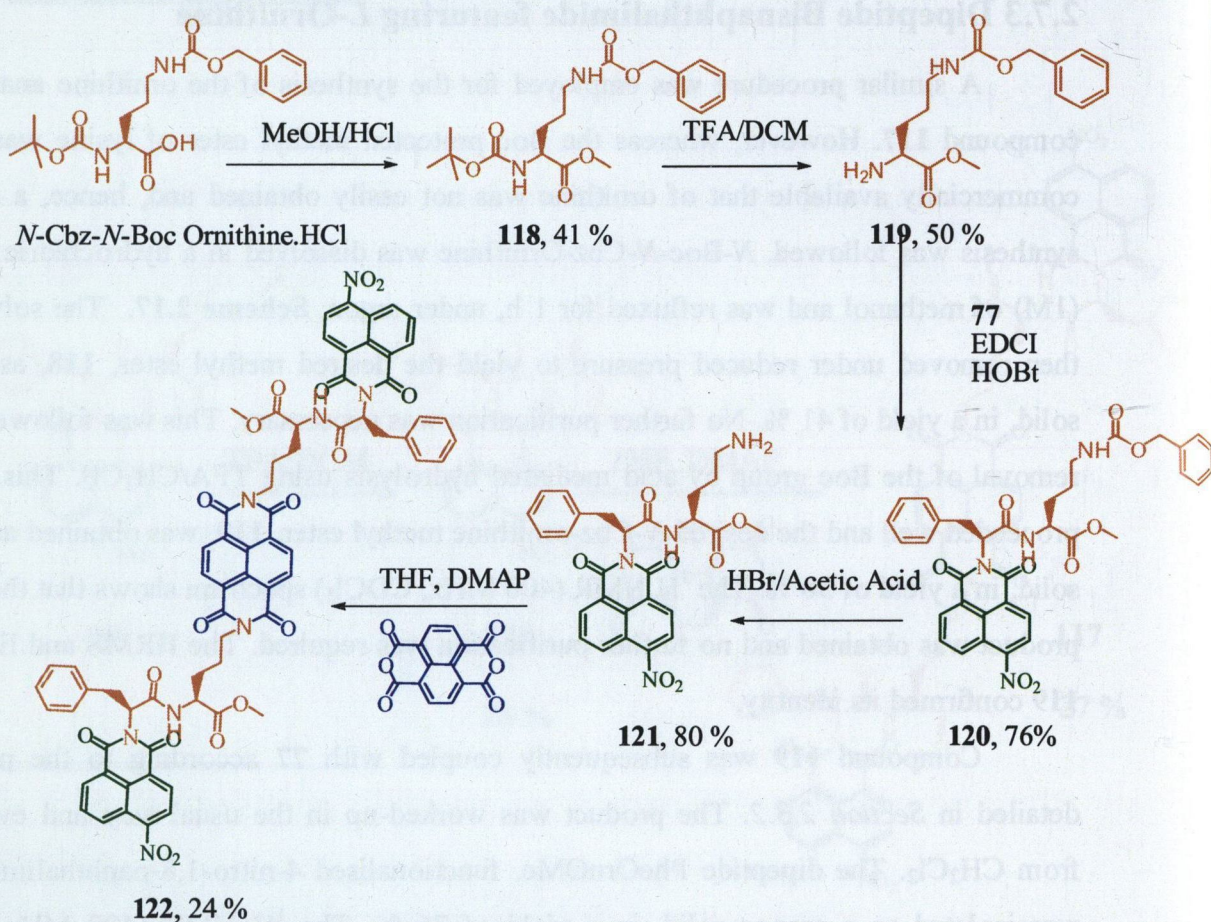
2.7.3 Dipeptide Bisnaphthalimide featuring *L*-Ornithine

A similar procedure was employed for the synthesis of the ornithine analogue of compound **117**. However, whereas the Boc protected methyl ester of lysine was readily commercially available that of ornithine was not easily obtained and, hence, a stepwise synthesis was followed. *N*-Boc-*N*-Cbz-Ornithine was dissolved in a hydrochloric solution (1M) of methanol and was refluxed for 1 h, under argon, **Scheme 2.17**. The solvent was then removed under reduced pressure to yield the desired methyl ester, **118**, as a white solid, in a yield of 41 %. No further purification was necessary. This was followed by the removal of the Boc group by acid mediated hydrolysis using TFA/CH₂Cl₂. This reaction proceeded well and the desired *N*-Cbz-ornithine methyl ester, **119**, was obtained as a white solid, in a yield of 50 %. The ¹H NMR (400 MHz, CDCl₃) spectrum shows that the desired product was obtained and no further purification was required. The HRMS and ESMS for **119** confirmed its identity.

Compound **119** was subsequently coupled with **77** according to the procedure detailed in *Section 2.3.2*. The product was worked-up in the usual way and evaporated from CH₂Cl₂. The dipeptide PheOrnOMe, functionalised 4-nitro-1,8-naphthalimide, **120**, was isolated as a orange solid, in a yield of 76 %. The ¹H NMR (400 MHz, CDCl₃) spectrum shows the phenylalanine, naphthalimide, chiral protons signals along with the ornithine chain methylene peaks and the methyl ester signal. The next step involved the deprotection of the primary amine, this was achieved by dissolving **120** in a solution of 30 % HBr/acetic acid, *cf. Section 2.5.3*. The solvent was removed under reduced pressure and the product was washed with K₂CO₃ (10 %, w/v) and deionised water. The deprotected product, **121**, was isolated as an orange solid, in a yield of 80 %. The ¹H NMR (400 MHz, CDCl₃) spectrum is similar to that of **120**, except that the Cbz functional group is no longer present, confirming the successful removal of the protecting group.

Compound **121** could now be incorporated into the designed ‘head-to-head’ bisnaphthalimide. This was achieved by reacting it with 0.5 equivalents of 1,4,5,8-naphthalene tetracarboxylic dianhydride, under argon, for 72 h. After the usual work-up, *cf. Section 2.5*, a brown oily residue was obtained, in a yield of 24 %. The ¹H NMR (600

MHz, CDCl₃) spectrum shows the presence of the naphthalimide and dipeptide signals. The resonances due to the bridge methylene signals of the ornithine chain are located at 3.57 ppm, 2.91 ppm and 2.74 ppm and the naphthalene diimide protons appear among the naphthalimide multiplet at 8.55 ppm, confirming that the intended ornithine based ‘head-to-head’ bisnaphthalimide, **122**, was successfully isolated.



Scheme 2.17: The synthesis of ornithine based ‘head-to-head’ bisnaphthalimide, **122**

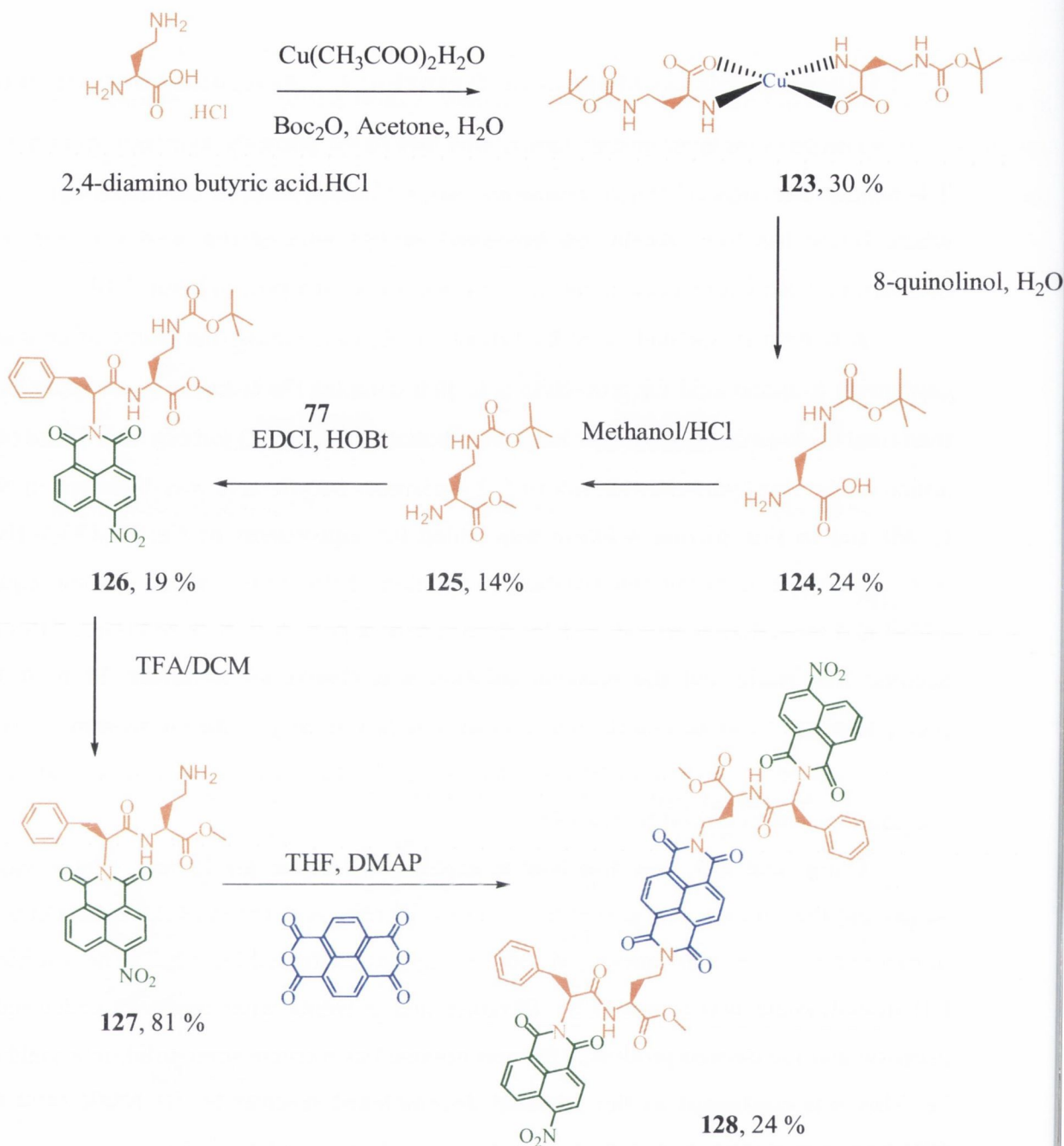
2.7.4 Dipeptide Bisnaphthalimide featuring *L*-2,4-diamino butyric acid

The final analogue in this family was that based upon the synthetic α -amino acid, 2,4-diamino butyric acid which possesses two methylene units in its distal amino chain where lysine has four. Again, the protected methyl ester of this acid was not readily commercially available and a stepwise synthesis was developed, **Scheme 2.18**.

A traditional method, used by Wiejak *et al.*, of isolating one amine of an ε -amino containing α -amino acid for protection is to first complex the α -amine to a copper ion and then treat the ε -amine with di-*tert*-butyl dicarbonate.¹¹⁰ A Cu(II) ion can coordinate two α -amino acids. The hydrochloride salt of *L*-2,4-diamino butyric acid was dissolved in NaOH (2 M) and to this stirring solution was added 0.5 equivalents of Cu(CH₃CO₂)₂·H₂O in water, giving an intensely blue solution. A solution of Boc₂O in acetone was subsequently added and the solution was stirred for 24 h, under argon. A further addition of Boc₂O in acetone was made and the reaction solution was stirred for a further 24 h. A black precipitate was removed and the solvents were removed under reduced pressure.¹¹⁰ A light blue waxy solid, [2, 4-diaminobutyric acid(Boc)]₂Cu, **123**, was isolated in a yield of 30 % and this was characterised by HRMS.

Compound **123**, was dissolved in acetone and stirred for 15 min. Water was then added and the solution was stirred for a further 10 min. A solution of 2.5 equivalents of 8-quinolinol, in water, was introduced and stirring was continued for 4 h.¹¹⁰ The resulting Cu (II) quinolinolate was removed by filtration and solvents were removed under reduced pressure and the desired product, **124**, was obtained as a cream semi-solid, in a yield of 24 %. This was confirmed as the intended decomplexed product by ¹H NMR (400 MHz, CDCl₃).

Compound **124** was subsequently esterified by the same method used in the ornithine synthesis employing hydrochloric methanol. The desired methyl ester, **125**, was obtained as a viscous brown semi-solid, in a yield of 14 %. The ¹H NMR (400 MHz, CDCl₃) spectrum shows the phenylalanine, naphthalimide, chiral protons signals along with the butyric chain methylene peaks and the methyl ester signal. Compound **125** was then coupled with **77** according to the conventional procedure and work-up. Recrystallisation from CH₂Cl₂ proffered the desired dipeptide, **126**, in a yield of 19 %. The Boc group of **126** was removed using TFA/CH₂Cl₂. The deprotected product, **127**, was isolated as a viscous yellow oil, in a yield of 81 %. The ¹H NMR (400 MHz, CDCl₃) spectrum is similar to that of **126**, however, the Boc *tert*-butyl group is no longer present indicating the success of the synthesis.



Scheme 2.18: The synthesis of 2,4-diamino butyric acid based 'head-to-head' bisnaphthalimide, **128**

Compound **127** could now be incorporated into the designed 'head-to-head' bisnaphthalimide, it was reacted with 0.5 equivalents of 1,4,5,8-naphthalene tetracarboxylic dianhydride, under argon, for 72 h. After work-up, a yellow oil, which solidified to give a gel when dried under high vacuum, was obtained in a yield of 24 %. This was characterised by ^1H NMR and ESMS. The ^1H NMR (600 MHz, CDCl_3) spectrum shows the expected naphthalimide and dipeptide signals. The resonances due to the bridge methylene signals of the butyric chains are located at 1.71 ppm and 1.46 ppm and the signal of the naphthalene diimide protons occur among the naphthalimide multiplet at 8.74

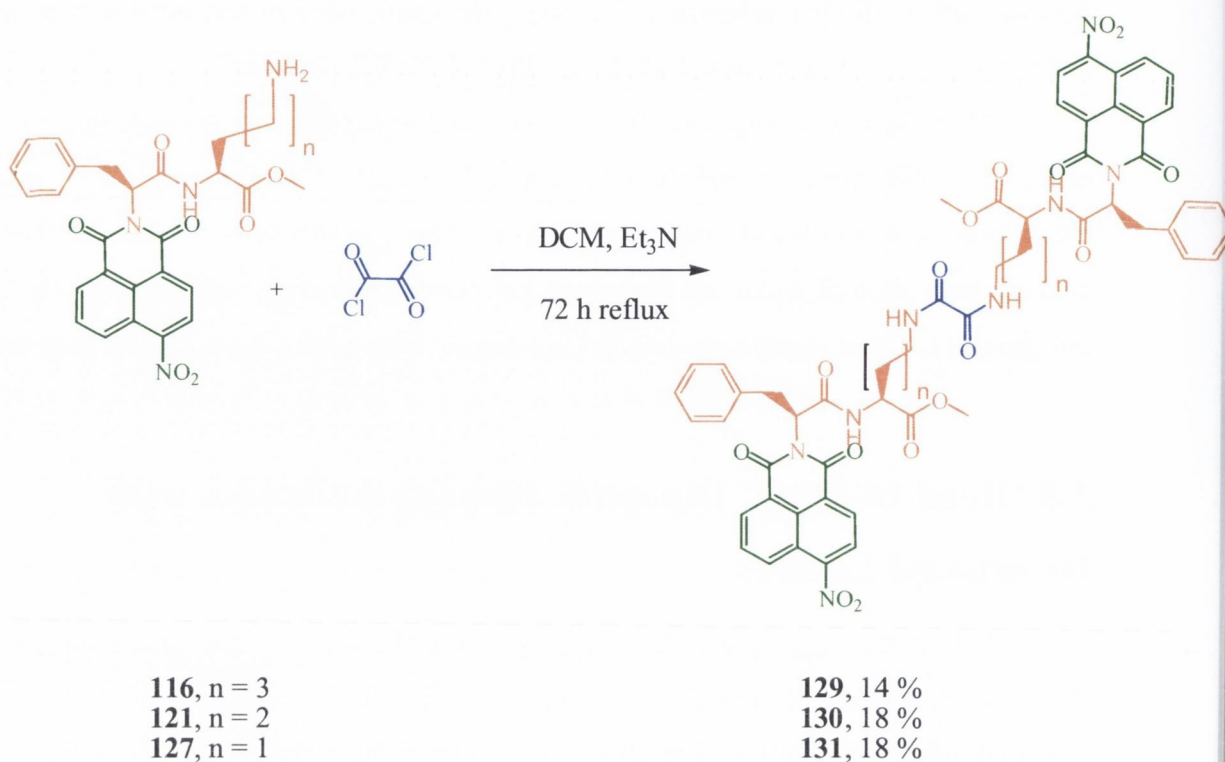
ppm. This confirms that the intended butyric based ‘head-to-head’ bisnaphthalimide **128**, was also successfully synthesised. However, the insolubility of this product was an issue and it was not possible to obtain a high-quality ^{13}C NMR spectrum.

These syntheses supplied three novel ‘head-to-head’ bisnaphthalimides, linked *via* naphthalene diimide cores and the variation in the length of the bridging chain by altering the amino acid employed proved successful. These compounds would be thoroughly studied, both photophysical and biological, providing interesting contrasts with the ‘tail-to-tail’ families. These studies are detailed in *Chapter Three* and *Chapter Four*, respectively.

2.8 ‘Head-to-Head’ Dipeptide Bisnaphthalimides with Dicarboxyl Linkers

Whilst the naphthalene diimide cores were the focus of the spacer design during this project, the distal amine containing dipeptide functionalised naphthalimides also provided interesting compounds that could react with carbonyl functionalities to give alternative spacer moieties. Polyamide linkers have been used previously by Iverson *et al.* (e.g. **54**) for DNA targeting drugs, so it was thought that reacting these naphthalimides with an activated dicarboxyl compound could produce a more flexible diamide linked bisnaphthalimide.⁹⁷ As this would result in the formation of amide bonds linking two naphthalimides enhanced electrostatic interaction with the phosphate backbone of the DNA helix might occur leading to disruption of replication processes.

To this end, **116** was dissolved in CH_2Cl_2 and to this stirring solution 0.5 equivalents of oxalyl chloride (2 M solution in CH_2Cl_2) was added dropwise, **Scheme 2.19**. This reaction was then refluxed, under argon, for 72 h, in the presence of Et_3N . The solvent along with any unreacted oxalyl chloride was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 . The solution was washed with HCl (1 M), saturated potassium carbonate (K_2CO_3) and deionised water. The organic layer was then dried over magnesium sulphate (MgSO_4) and the solvent was removed under reduced pressure. Attempts at recrystallisation from a range of solvents failed and an orange oil was isolated after drying under reduced pressure, in a yield of 14 %. The ^1H NMR (600 MHz, DMSO) spectrum of this product is similar to that of compound **116** with slight shifting of some signals and the appearance of a broad NH peak at 6.57 ppm identifying this as the desired PheLys functionalised bisnaphthalimide with a dicarboxyl centre bridged by the lysine chains, **129**. However, the insolubility of this product was an issue and it was not possible to obtain a high-quality ^{13}C NMR spectrum.



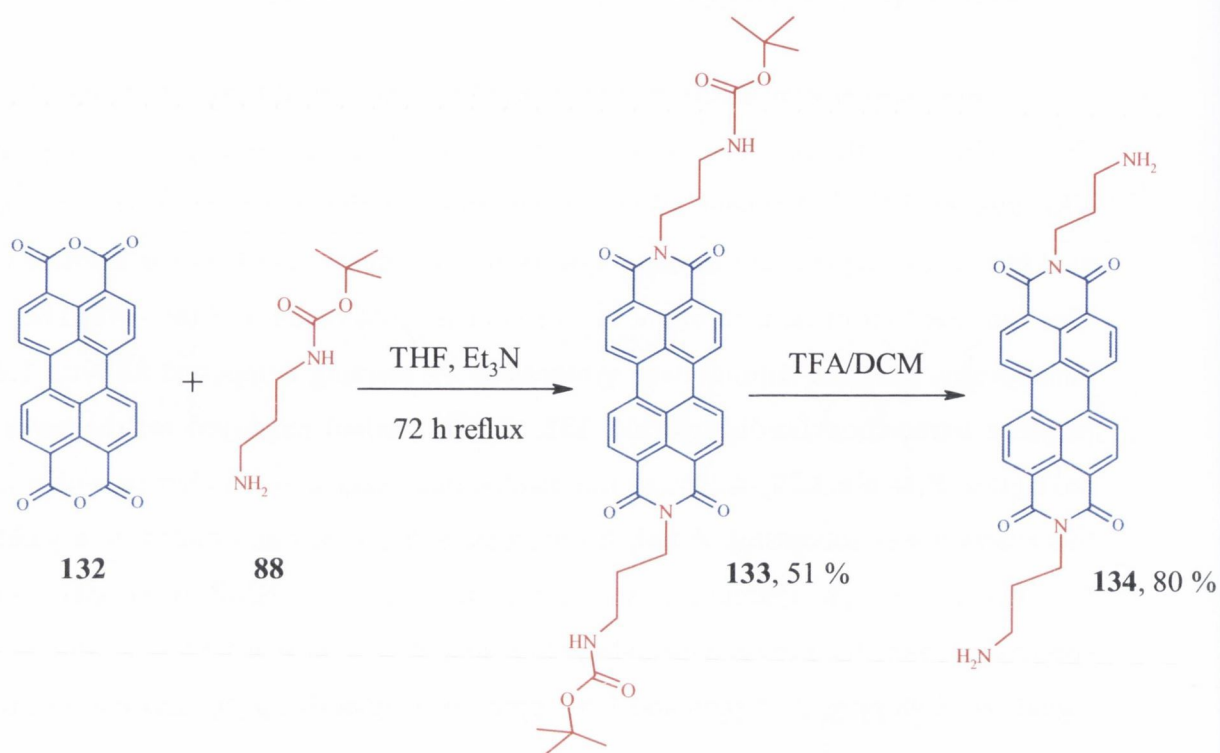
Scheme 2.19: The synthesis of dicarboxyl linked ‘head-to-head’ bisnaphthalimides

Compound **121**, the ornithine analogue of **116**, was also reacted with oxalyl chloride in this fashion. A brown viscous oil, **130**, was obtained in a yield of 18 %. This product was characterised in the manner described for **129** and was the successfully synthesised analogous ornithine dicarboxyl linked bisnaphthalimide. Lastly, to complete this family of ‘head-to-head’ dicarboxyl linked bisnaphthalimides, compound **127** was reacted with oxalyl chloride. The viscous brown oil that was isolated, in a yield of 18 %, was indeed characterised as the desired bisnaphthalimide, **131**, with the ^{13}C NMR (600 MHz, CDCl_3) spectrum depicting the additional amide carbonyl signal at 173.3 ppm.

This provided a second novel group of ‘head-to-head’ bisnaphthalimides. These compounds also retained the 4-nitro substituent group and would prove an interesting companion group to those detailed in *Section 2.7*. These compounds were also investigated photophysically and were biologically examined against cancerous cell lines, these studies are discussed in *Chapter Three* and *Chapter Four*, respectively.

2.9 Attempted Synthesis Other Spacer Moieties

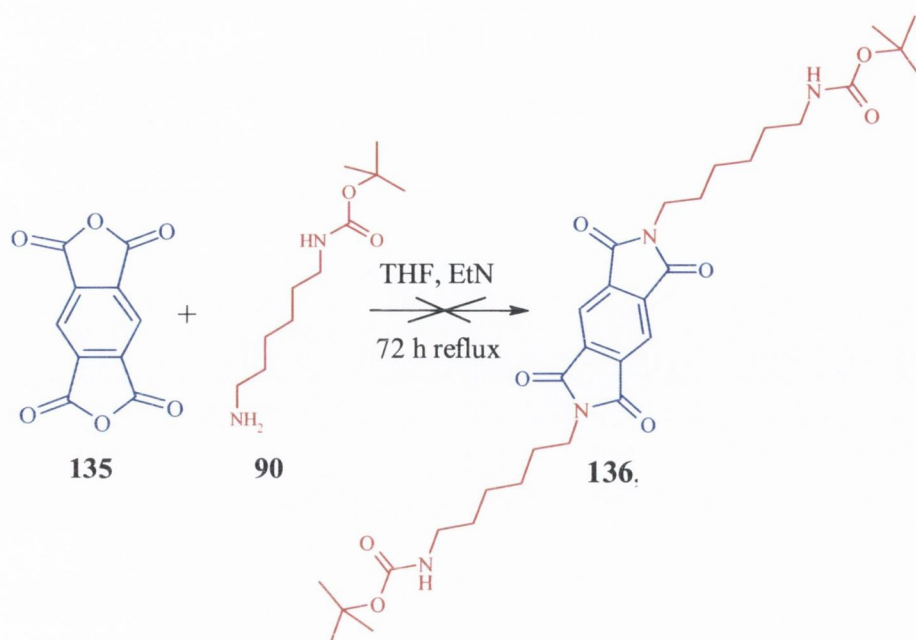
Other spacer core structures were briefly considered during this research project. The synthesis of the perylene analogue of the propyl naphthalene spacer, compound **87**, was attempted.^{77, 78} Perylene offers an extension of the planar aromatic core already exploited in the naphthalene diimide spacers and is similarly available for functionalisation. This increased aromatic area might further enhance intercalation of the core. The *N*-Boc-aminopropyl perylene diimide was synthesised by reacting compound **88** with 1,6,7,12-perylene tetracarboxylic dianhydride, **132**, *via* the method employed for the naphthalene analogues, **Scheme 2.20**. Although the dianhydride reagent was rather insoluble in THF this reaction was successful. A red, hygroscopic solid, **133**, was isolated, in a yield of 51 %, after a slow precipitation from diethyl ether. The ¹H NMR (600 MHz, DMSO) spectrum depicts the perylene diimide proton singlet is located at 8.95 ppm, the three CH₂ signals at 2.98 ppm, 2.75 ppm and 1.63 ppm, the singlet due to the *tert*-butyl of the Boc group is seen at 1.14 ppm. Compound **133** was subsequently treated with TFA/ CH₂Cl₂ and was successfully hydrolysed to **134**, a red solid, in a yield of 80 %. The absence of the *tert*-butyl signal from the ¹H NMR (600 MHz, (CD₃)₂SO) spectrum of this product confirms that the deprotection was successful. This product was very insoluble in most solvents and ¹³C NMR experiments were unsuccessful.



Scheme 2.20: The synthesis of perylene diimide spacer

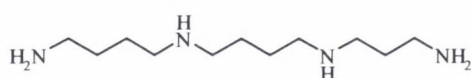
When the bisnaphthalimide synthesis was attempted by reacting **134** and **83** (refluxing in THF for 72 h, under argon, in the presence of DMAP) the reaction was unsuccessful. Changing the solvent to DMF or dioxane proved in vain. It is possible that the perylene diimide aggregates in solution making it very insoluble and unreactive. Since **132** is highly insoluble itself, it is unlikely that the alternative synthesis outlined in *Section 2.5.6* would be advantageous in this situation. Therefore this synthetic route was suspended.

Similarly, the synthesis of another planar, aromatic spacer was investigated.⁹⁴ 1,3,4,6-Pyrometallitic dianhydride, **135**, was reacted with the mono-Boc protected hexyl bridge, **90**, **Scheme 2.21** and desired product was the protected pyrometallitic diimide product, **136**. The product obtained was a highly insoluble powder and attempts at purification failed due these solubility issues. ESMS suggested that the product was oligomeric. It was therefore not possible to proceed with the synthesis of the intended pyrometallitic diimide linked bisnaphthalimide. However, the analogous reaction using the propyl bridge, **88**, and **135** should be investigated, as should the alternative synthesis of a bisnaphthalimide *via* the reaction of compound **107** with 1,3,4,6-pyrometallitic dianhydride.

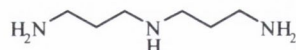


Scheme 2.21: The attempted synthesis of pyrometallitic diimide spacer

Another concept explored was the use of polyamino alkanes as bridging moieties. Additional amines along the arms of the spacer unit were hypothesised to enhance attraction between the corresponding bisnaphthalimide and the phosphate backbone of the DNA helix.⁹⁷ To this end, spermine and spermidine, **137** and **138**, respectively, were selected as trial reagents.

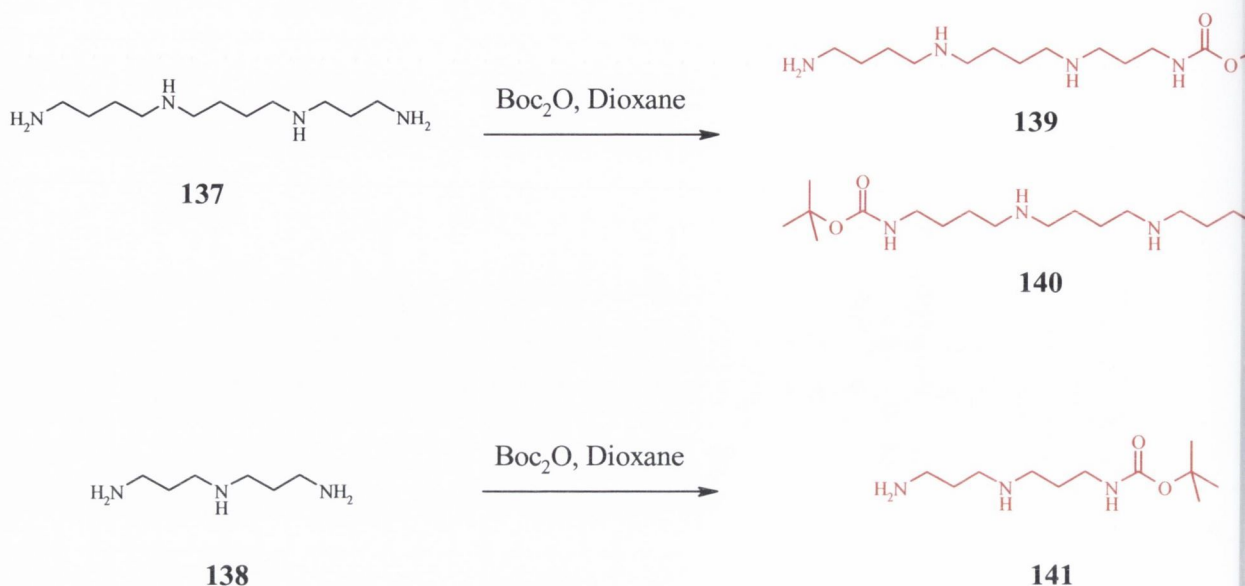


137



138

Spermine is asymmetric and the attempted mono-Boc protection of this compound would lead to a mixture of products, **139** and **140**. However, spermidine is a symmetric compound and the attempted mono-Boc protection ought to have provided, **141**. However, the product was an inseparable mixture. Full characterisation was not successful and it may have been the case that a mixture of the chain amines were protected as well as the terminal amines. By ESMS the product also appeared to be oligomeric in nature.



2.10 Conclusions

This chapter has described the design and synthetic elements of this research project. In all, thirty-six compounds were successfully synthesised and their reactions were optimised. A new family of naphthalene diimide spacers were successfully produced. The 1,4,5,8-naphthalene tetracarboxylic dianhydride core was functionalised by reaction with 1,3-diamino propane, 1,5-diamino pentane and 1,6-diamino hexane resulting in amino propyl, (87), pentyl, (94), and hexyl, (95), naphthalene diimide spacers. These were then incorporated into eleven novel ‘tail-to-tail’ bisnaphthalimide systems, involving four different dipeptide motifs ; PheAla, (96, 97 and 98), CbzLysAla, (99, 100 and 101), LysAla, (102 and 103), and LeuAla, (104, 105 and 106). Various means of optimising these syntheses were explored and an alternative route using 4-amino functionalisation of the dipeptide naphthalimide was successfully pursued. Five other ‘tail-to-tail’ bisnaphthalimides were synthesised incorporating mono-peptide functionalised naphthalimides (111, 112, 113, 114 and 115).

In addition, a family of three ‘head-to-head’ bisnaphthalimides has been accomplished. These involve lysine, ornithine and 2,4-diamino butyric acid methyl esters at the second peptide position of the 4-nitro-1,8-naphthalimides. The free distal amines of these moieties reacted with 1,4,5,8-naphthalene tetracarboxylic dianhydride to produce three bisnaphthalimides each of which is tethered through the naphthalene core by the bridge provided by its own ϵ -amine chain. Thus, this gave three bisnaphthalimides with

three different spacer spans of a naphthalene diimide core and four, three and two methylene units, respectively (**117**, **122** and **128**). Analogues of these compounds were formed by reacting these dipeptide 4-nitro-1,8-naphthalimides with oxalyl chloride, these bisnaphthalimides (**129**, **130** and **131**) also have different spacer spans being tethered through two adjacent amide bonds, *i.e.* the distal amines are bonded to the dicarboxyl core.

Finally a number of attempted reactions and explorations of varying the bisnaphthalimide skeleton were described and while these were not wholly successful they do suggest means of elaborating these interesting structures in the future.

The synthesis of these novel systems was one facet of this research. Since these bisnaphthalimides involve distinctly different structural motifs it is natural that the regions would endow the compound as a whole with different features. It was a principal objective to explore these features and attempt to grasp the character of these compounds. These compounds might be considered supramolecular in stature and from this point of view their photophysics and physical behaviour might prove interesting. Originally designed as a new generation of Gunnlaugsson potential anti-cancer naphthalimides, these systems would hopefully interact with DNA. There are various ways to probe the interaction between compounds and DNA and among these photophysics and cell assays are some of the most elucidating.

The next two chapters seek to complete the story of the bisnaphthalimides depicted in this chapter. *Chapter Three* will describe their photophysical characteristics before moving on to detail the range of photophysical and physical experiments employed to examine how these compounds behave in the presence of DNA as well as whether they show any sequence selectivity. *Chapter Four* will discuss the biological assays undertaken to investigate how effective these novel bisnaphthalimides are against different cell lines and explore how they might behave *in vivo*.

Chapter Three

Photophysical Investigations of

Bisnaphthalimides

3.1 Introduction

As discussed in *Chapter One*, 1,8-naphthalimides exhibit anti-cancer potency and their capacity to interact with DNA is speculated to contribute to this activity.³² Diverse modes of action with DNA have been observed for different derivatives including intercalation,²⁹ groove binding,⁴⁰ cleavage by hydroxyl radicals⁴⁷ and acylation.³⁹ These interactions damage the DNA structure ultimately leading to cell death. Probing the interactions between 1,8-naphthalimides and DNA is important for the elucidation of possible mechanisms of anti-cancer action. In an effort to propose the binding modes and method of action at work, this chapter details investigations into the photophysical characteristics of a panel of novel bisnaphthalimides and into their interactions with DNA.

The bisnaphthalimides designed and synthesised (*Chapter Two*) during this project comprise several structural motifs whose binding to DNA may be through complicated, possibly co-operative, processes. **Figure 3.1** and **Figure 3.2** show the key ‘tail-to-tail’ and ‘head-to-head’ bisnaphthalimide architectures employed. The nature of these molecules, incorporating different structural motifs, means that multiple binding modes are possible.

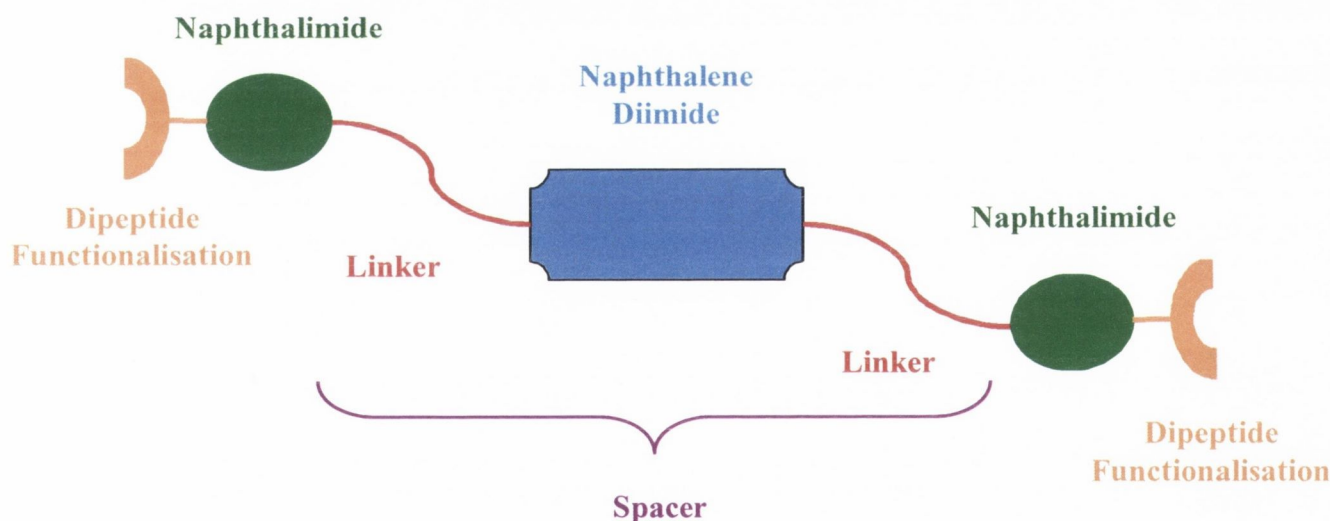


Figure 3.1: Key to ‘Tail-to-Tail’ Bisnaphthalimide Structure

The naphthalene diimide and the 1,8-naphthalimide motifs are constant in all the ‘tail-to-tail’ bisnaphthalimide structures. The naphthalene diimide core is capable of threading the DNA helix, of intercalating, and of groove binding.⁷⁵ The 1,8-naphthalimide

units themselves may intercalate or groove bind.^{29, 40} Both the naphthalene diimide core and the flanking 1,8-naphthalimides, being extended aromatic moieties, are chromophores. Their electronic absorbance and emission spectroscopic behaviours are sensitive to changes in the local environment experienced by these moieties. Therefore, the chromophores can report on changes in the binding environment of these regions of the bisnaphthalimide. Hence, it may be possible to observe the binding modes for both these structural motifs independently, elucidating the overall binding behaviour as well as any co-operative interactions that exist.

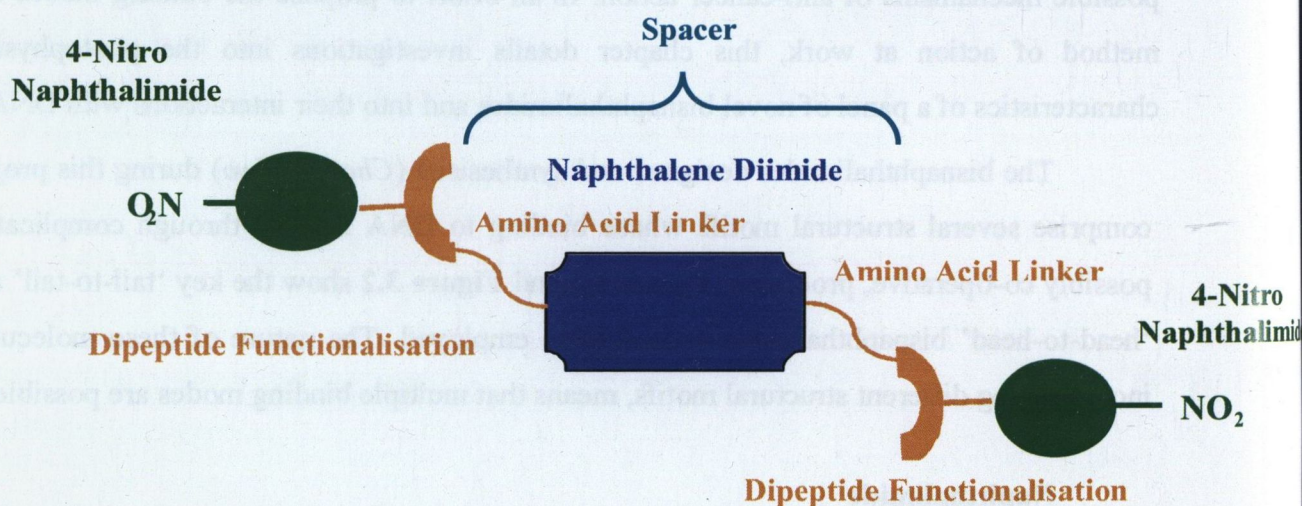


Figure 3.2: Key to 'Head-to-Head' Bisnaphthalimide Structure

The extent to which these chromophores might bind to DNA might be expected to be influenced by the length of the spacer and the bulk and nature of the chiral dipeptide functionality. The spacer length dictates the distance between the chromophores and the overall span of the molecule, *e.g.* if the core threads through the molecule and the 1,8-naphthalimide units tend to either intercalate or groove bind there ought to be adequate length between the units to allow this to easily occur. Whether the 1,8-naphthalimide units intercalate or bind in the minor and major grooves, the strength of this binding interaction is expected to depend upon the bulk and stereochemistry of the chiral functionality. Since these factors may well govern the extent of bisnaphthalimide : DNA binding, studies of bisnaphthalimide systems with varying spacers and functionalisations were undertaken to establish a dependence relationship between these structural aspects and the resultant binding.

Any interaction might involve a multi-step process since the four different structural motifs within each bisnaphthalimide might interact in different ways, at different stages, to different extents. A series of photophysical and physical investigations were conducted to understand how the DNA binding of these novel bisnaphthalimides may contribute to their function as anti-cancer agents or, alternatively, as DNA probes. DNA-drug titrations are effective tools for probing such interactions. Monitoring the electronic spectroscopy of the agent or of the DNA provides such information since electronic transitions of the chromophore are sensitive to subtle changes in the binding environment.¹¹¹ Hypochromicity, hyperchromicity and spectral shifts can all indicate alterations in the local environment as can any loss of fine structure and the appearance or disappearance of absorbance or emission bands. With this in mind, the effect of successive additions of *ct*-DNA to a solution of the chosen bisnaphthalimide was monitored simultaneously by absorption (UV-visible) and emission (fluorescence) spectroscopy. The selected compounds were also studied in the presence of the homopolymers poly(dA-dT)poly(dA-dT) and poly(dG-dC)poly(dG-dC) to investigate whether any sequence selectivity existed.

Another UV-visible absorbance technique employed was circular dichroism (CD) and this examines the difference in absorption of left and right circularly polarised light.¹¹² CD is very sensitive to structural changes and is convenient when examining interactions that might alter the conformational environment of a chiral molecule such as DNA. Thermal denaturation studies were also conducted using UV-visible spectroscopy. A significant change in the melting point (ΔT_m) could be correlated with an interaction with the bisnaphthalimide.¹¹³ These latter two techniques can be used as indicators of intercalation or groove binding.¹¹⁴ Finally, one further technique, viscosity, was used to examine binding modes. Intercalation results in an unwinding of the double helix and this perturbation leads to an increase in the viscosity of the macromolecule.^{75, 115, 116} Decreases in viscosity are indicative of partial intercalation or of groove binding. Comparisons of the viscosity of a solution of *ct*-DNA in the presence and absence of bisnaphthalimides in a buffered solution were conducted, *i.e.* the resultant intrinsic viscosities were compared with initial intrinsic viscosities. The effect of the interaction upon the turn angle of the helix can be extracted and the 'unwinding angle' deduced. A large unwinding angle would suggest a threading intercalation behaviour.^{115, 116}

The bisnaphthalimides are partially soluble (possibly due to aggregation) in aqueous media, so solutions were prepared in a 2 : 98, DMSO : phosphate buffer solution at pH 7. The panel of compounds selected for thorough investigation included the nine

principal ‘tail-to-tail’ dipeptide bisnaphthalimides and the three ‘head-to-head’ bisnaphthalimides since these twelve compounds reflect alterations in dipeptide families, spacer lengths and the ring substitution. The ‘tail-to-tail’ panel provided the propyl, pentyl and hexyl linked analogues of each of the PheAla (**96**, **97** and **98**), CbzLysAla (**99**, **100** and **101**) and LeuAla (**104**, **105** and **106**) families. Unfortunately, the deprotected LysAla family (**102** and **103**) precipitated from the DMSO : phosphate buffer solution and were not included in these studies. The mono-peptide bisnaphthalimides were excluded since it is probable that the overall binding behaviour of such structures is unlikely to differ very much from those displayed by the dipeptide analogues. Of the ‘head-to-head’ bisnaphthalimides, **116**, **122** and **128** were chosen for investigation since they reflect analogues of spacer length provided by the different amino esters employed and serve as an interesting contrast to the ‘tail-to-tail’ panel since these compounds retain their 4-nitro substituents.

It was hoped that collating the data from these studies might elucidate the behaviour of these novel bisnaphthalimides, highlight any differences imparted by altering functionalities and portray binding trends. This chapter describes the experimental procedures and details the results obtained.

3.2 Photophysical Characteristics of 1,8-Naphthalimides, Diimides and Bisnaphthalimides

3.2.1 Introduction

The spectroscopic properties of 1,8-naphthalimides and 1,4,5,8-naphthalene diimides have been investigated by a number of research groups.^{34, 92}

Naphthalimides, depending upon the substituents upon the ring, typically absorb in the blue. 4-Nitro-1,8-naphthalimides display absorbance maxima at *ca.* 350 nm, while 4-amine functionalised analogues tend to absorb at longer wavelengths *ca.* 450 nm. Aveline *et al.* and Kelly *et al.* have shown that when the polarity of the solvent is increased the absorption maxima are bathochromically shifted, indicating a $\pi\pi^*$ character of the first singlet excited state S_1 .^{34, 92} Their fluorescence behaviour has been studied by such groups as Middleton and Clarke and by Vasquez *et al.*^{34, 117-121} The emission spectra are mirror images of their absorbance spectra, at longer wavelengths (typically *ca.* 410 nm and *ca.* 520 nm for 4-nitro and 4-amino-1,8-naphthalimides, respectively), with strong intensities and are due to the loss of radiation from this excited singlet state, $S_1^0 \rightarrow S_0^0$, **Figure 3.3**.

Naphthalene diimides have been studied by Erten *et al.* who showed that these exhibit more complicated absorbance behaviour, with several local maxima.¹¹⁷ As seen by Aveline *et al.* and by Kelly and co-workers naphthalene diimides exhibit interesting spectral behaviour with three absorption maxima at *ca.* 340 nm, 355 nm and 370 nm.^{34, 92} When the polarity of the solvent is increased these maxima are hypsochromically shifted, indicating an $n\pi^*$ character of the first singlet excited state S_1^0 .¹³ Chen *et al.* and Aveline *et al.* have explored the fluorescence of naphthalene diimides.^{34, 118-121} Aveline *et al.* comprehensively investigated the photophysical behaviour of several *N*-substituted 1,8-naphthalimides and 1,4,5,8-naphthalene diimides, including **24** and **25**.³⁴ These were studied by absorption and fluorescence spectroscopy and by flash photolysis. The absorption spectra of the chosen 1,8-naphthalimides, which were unsubstituted at the 4 position, were characterised by a broad band with a $\lambda_{\text{max}} = 330$ nm. Those of the studied naphthalene diimides depicted a band with fine structure. Three maxima were seen at 340 nm, 355 nm and 375 nm. Again, the emission spectra were mirror images of their absorbance spectra, at *ca.* 390 nm, 410 nm and 560 nm, respectively.

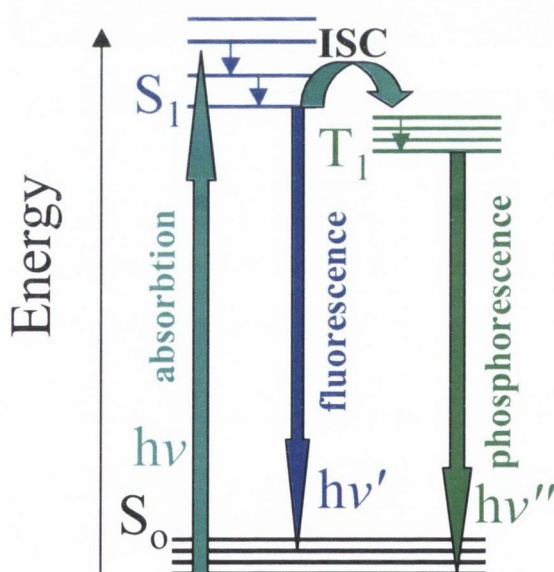


Figure 3.3: Jablonski Diagram, simplified representation of the relative positions of the electronic energy levels of a molecule. Vibrational levels of states of a given electronic state lie above each other. The diagram depicts stimulation by an external radiation source, absorption of energy at the ground state, S_0^0 , and promotion to an excited state, S_1^0 . Radiative loss of energy by fluorescence results in a return to S_0^0 . Non-radiative loss of energy by intersystem crossing (ISC) from the singlet excited state, S_1^0 , to a triplet excited state, T_1 , ultimately leads to a radiative loss of energy by phosphorescence.

3.2.2 Solvent Studies

As already stated, the characteristic bands of naphthalimides and naphthalene diimides are known to alter in intensity and position depending upon the solvent employed.¹²² Therefore, a solvent dependency study was undertaken to investigate the character and relation of these compounds. Solutions of **83** at a concentration of 20 μM were prepared in toluene, EtOAc, CH_2Cl_2 , acetone, DMF, acetonitrile (MeCN) and MeOH. Compound **83** possess one strong absorbance band in all solvents. The intensity and position of this band was found to be sensitive to the solvent. **Table 3.1** summarises the changes and the extinction coefficients (ϵ) observed and **Figure 3.4** depicts the spectrum. As reported in the literature these maxima are usually bathochromically shifted with increasing solvent polarity indicating a $\pi\pi^*$ character of the first singlet excited state S_1^0 .^{34, 92} A bathochromic shift in the position of the absorbance maximum (λ_{max}) of up to 5 nm is generally observed upon increasing the polarity of the solvent employed (although toluene and CH_2Cl_2 do not follow this trend). This reflects the general behaviour seen by Zhang *et al.* for the $\pi\pi^*$ S_1^0 .¹²² The emission spectra upon excitation at λ_{max} display one intense band with maxima centred at *ca.* 550 nm, these were also found to be solvent dependant. The emission spectra upon excitation at the maximum of absorbance involve one intense band with maxima centred at *ca.* 550 nm, these were also found to be solvent dependant. The bathochromic shift reflects the fact that the fluorophore is stabilised in more polar solvents.

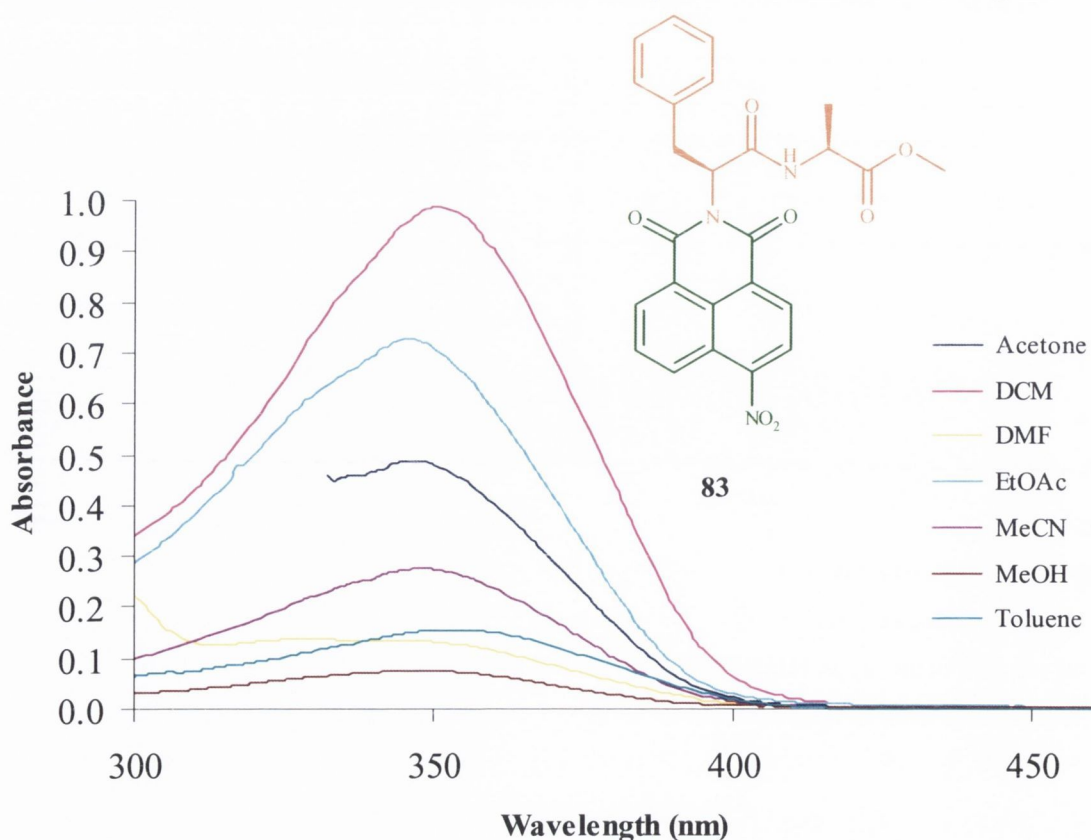


Figure 3.4: Comparison plot of absorbances of **83**, 20 μM in different solvents

Table 3.1: Summary of results observed for the ground state investigation of absorbance maxima

Solvent	Relative Polarity	83			107			95			97		
		λ_{max} (nm)	Abs	ϵ	λ_{max} (nm)	Abs	ϵ	λ_{max} (nm)	Abs	ϵ	λ_{max} (nm)	Abs	ϵ
Toluene	0.099	354	0.16	8,000	463	0.09	4,500						
EtOAc	0.228	345	0.73	36,500	436	0.08	4,000				345	0.54	27,000
											430	0.06	3,000
DCM	0.309	350	0.99	49,500	460	0.09	4,500				351	0.33	16,500
											428	0.04	2,000
Acetone	0.355	346	0.49	24,500	458	0.01	500	340	0.46	23,000	346	0.69	34,500
								357	0.77	38,500	428	0.08	4,000
								377	0.95	47,500			
DMF	0.404	343	0.14	7,000	454	0.10	5,000	342	0.48	24,000	349	0.35	17,500
								360	0.77	38,500	426	0.04	2,000
								381	0.87	43,500			
MeCN	0.460	347	0.28	14,000	443	0.03	1,500				348	0.27	13,500
											428	0.03	1,500
MeOH	0.762	348	0.08	4,000	467	0.17	8,500	339	0.22	11,000	346	0.29	14,500
								358	0.37	18,500	436	0.03	1,500
								378	0.44	22,000			

As a reference study, **107**, a 4-diamino alkyl 1,8-naphthalimide was studied in the same range of solvents, since it replicates the spacer and 1,8-naphthalimide section of the ‘tail-to-tail’ bisnaphthalimides. As for 4-amino-1,8-naphthalimides in general, this depicts a λ_{max} at *ca.* 450 nm, **Figure 3.5** and excitation at λ_{max} results in an emission centred at *ca.* 520 nm, **Figure 3.6**.^{123, 124} The maxima are bathochromically shifted with increasing solvent polarity, the results are summarised in **Table 3.1**, indicating a $\pi\pi^*$ character of the first singlet excited state S_1^0 .^{34, 92} The solvent dependency and intensities reflect that such compounds are capable of internal charge transfer (ICT) from the nitrogen atom of the amine group at the 4-position to the oxygen atoms in the carbonyl groups.¹²²⁻¹²⁸

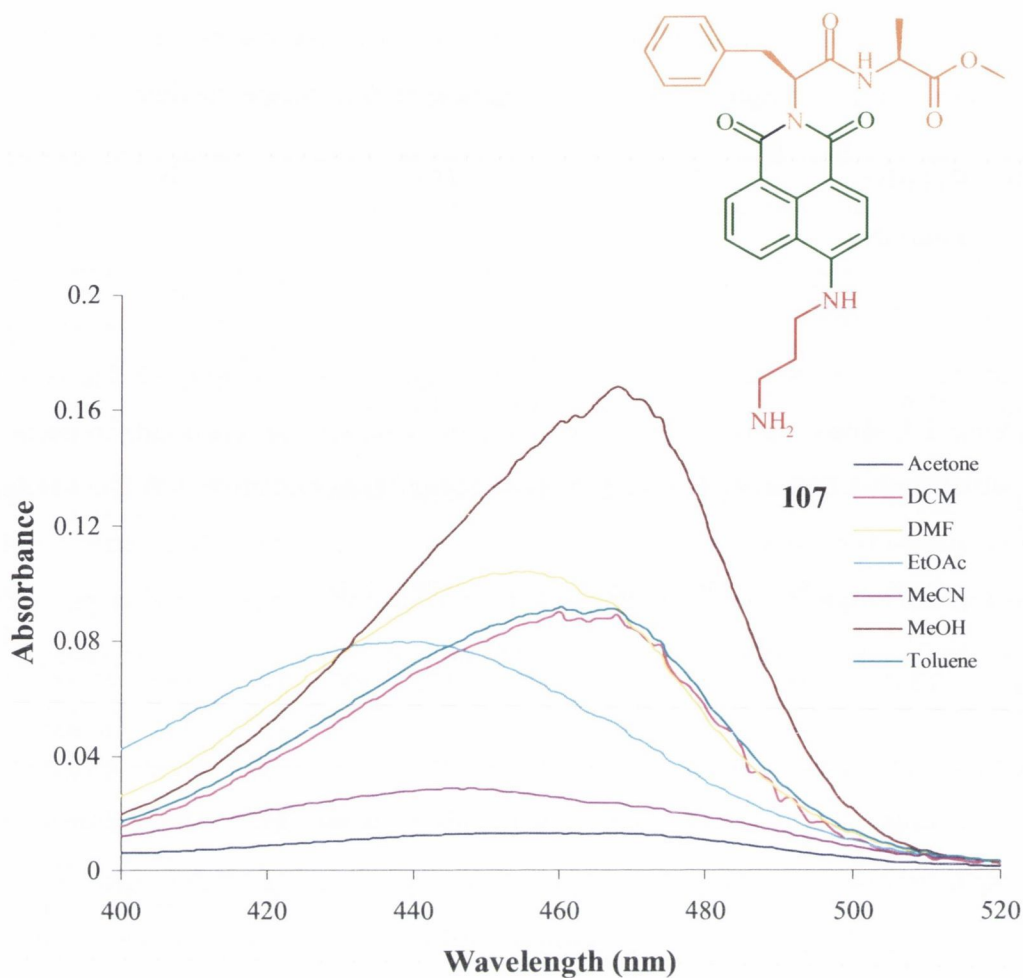


Figure 3.5: Plot of absorbance of **107**, 20 μ M in various solvents

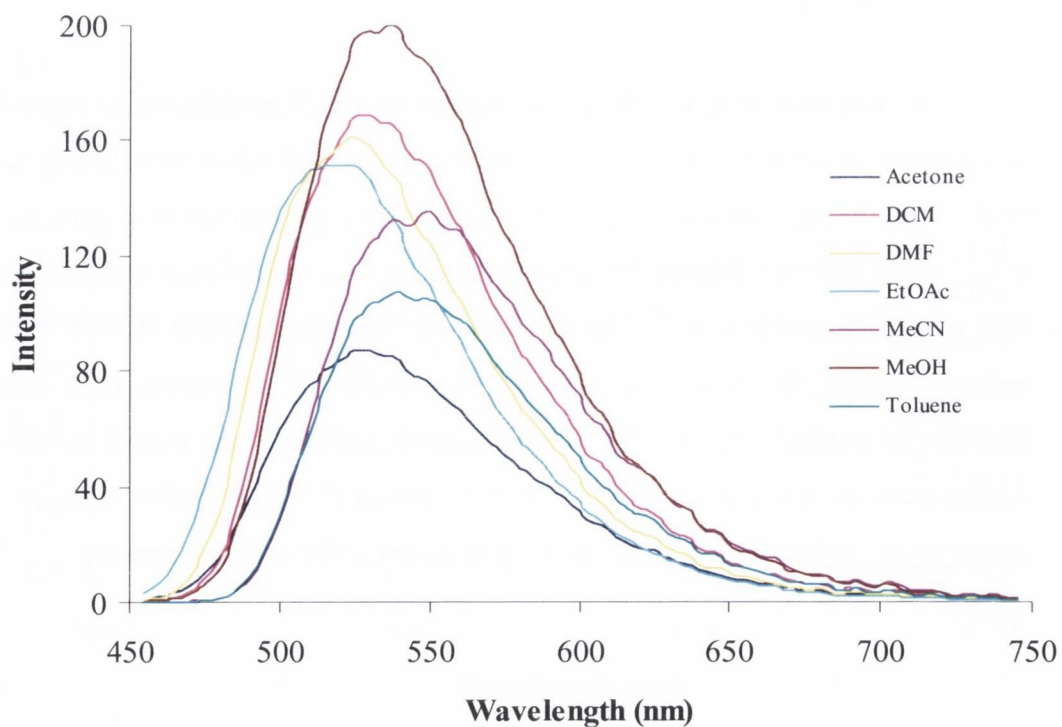


Figure 3.6: Plot of fluorescence of **107**, 20 μ M in various solvents

The naphthalene diimide spacers were also studied as 20 μM solutions in different solvents, although solubility issues restricted those available to acetone, DMF and MeOH, the results are summarised in **Table 3.1**. As reported in the literature for other functionalised naphthalene diimides, the absorbance spectra of these compounds display three maxima over a broad range.^{34, 92} **Figure 3.7** shows the absorption spectrum of **95**, the hexyl spacer, with three bands at *ca.* 340 nm, 355 nm and 380 nm. Similar spectra were also seen for the propyl and pentyl spacer analogues. The emission spectra of these compounds were studied upon excitation at 355 nm. The fluorescence spectra of these compounds are mirror images of their absorbance spectra and display three maxima over a wide range.^{47, 112, 113} An additional band is also seen at longer wavelength, *ca.* 560 nm, and this was also observed recently by Cho *et al.*¹²⁹ They have identified this as an intramolecular exiplex band. This is further supported by the fact that DMF, a polar solvent, reduces the relative intensity of the three monomer bands and degrades their fine structure while enhancing the exiplex band, *i.e.* in the polar solvent there may be stacking or aggregating of the exiplex system strengthening this band. **Figure 3.8** shows the example spectrum of **95**, the hexyl spacer, with three peaks at *ca.* 392 nm, 410 nm and 430 nm. Similar spectra are also seen for the propyl and pentyl spacers, in all cases these maxima were hypsochromically shifted with increasing solvent polarity, from DMF to MeOH, indicating a $\pi\pi^*$ character of the first singlet excited state S_1^0 .^{34, 92}

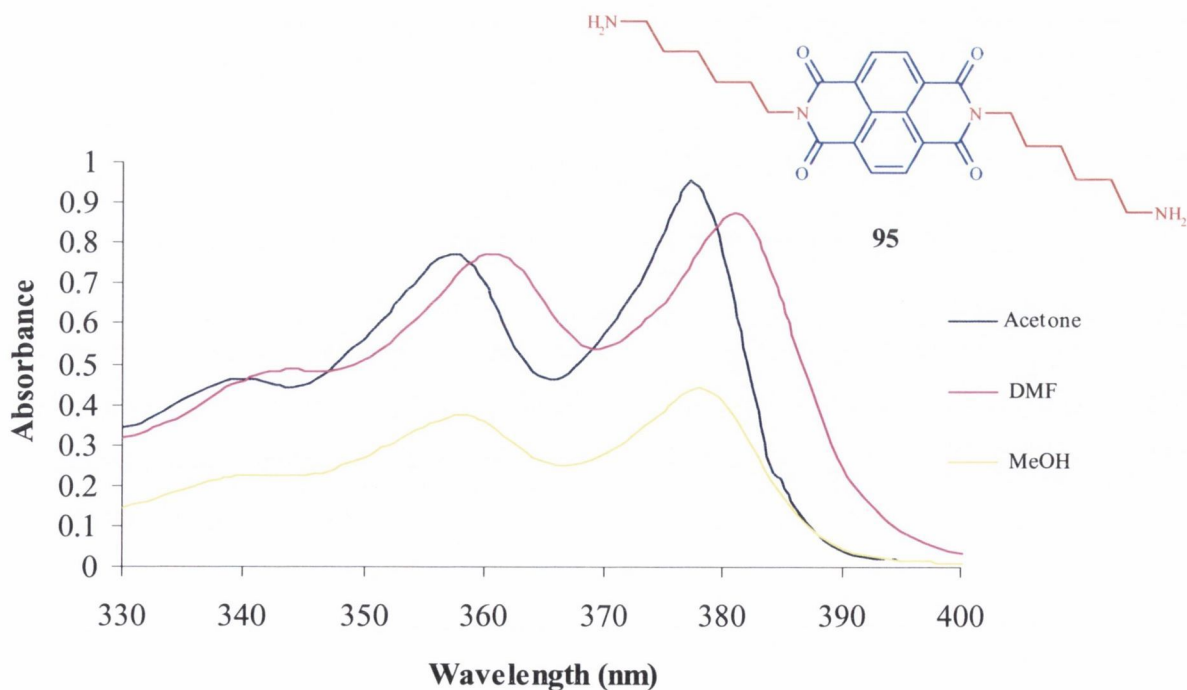


Figure 3.7: Comparison plot of absorbance of **95**, 20 μM in different solvents

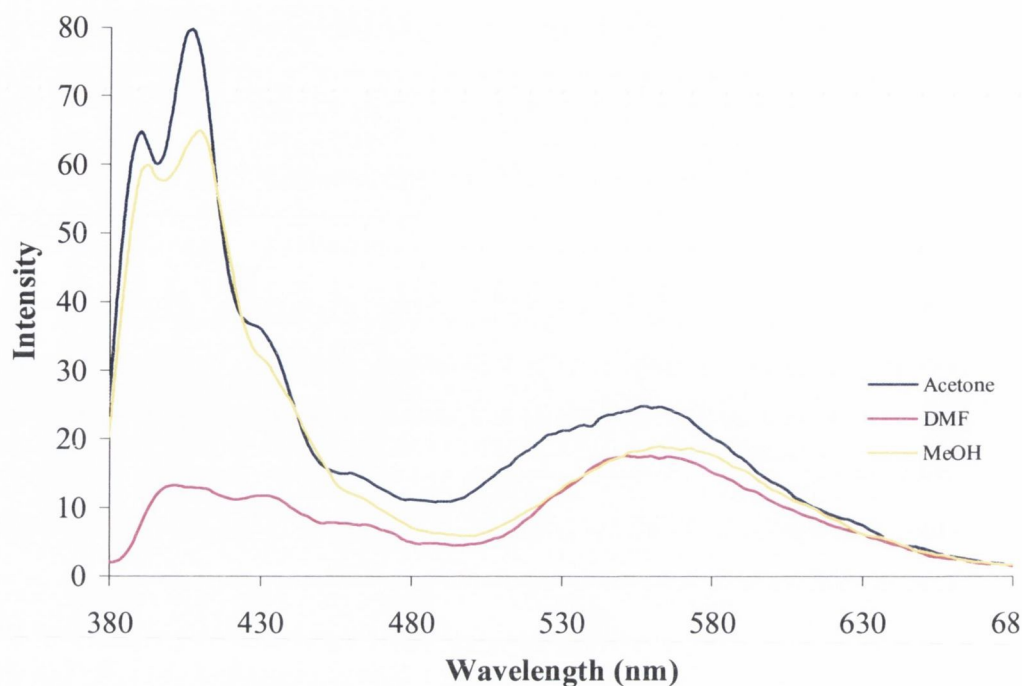


Figure 3.8: Comparison plot of fluorescence of **95**,
20 μM in different solvents, $\lambda_{\text{excitation}} = 355 \text{ nm}$

Finally, **97** was examined as an exemplary ‘tail-to-tail’ bisnaphthalimide as a 20 μM solution in EtOAc, CH_2Cl_2 , acetone, DMF, MeCN and MeOH. The compound displayed an absorption spectrum containing two bands, the results are summarised in **Table 3.1**. An intense band centred at *ca.* 355 nm and a minor band centred at *ca.* 440 nm, **Figure 3.9**. This spectrum therefore incorporates the principal characteristics of the component units in the bisnaphthalimide. The dominant band at *ca.* 355 nm corresponds to the naphthalene diimide core. It is important to note that the fine structure of the spacer spectrum has been replaced by a broad band with a single maximum. This broadening is expected to be a result of stacking interaction between the diimide and with either other diimide cores or with terminal 1,8-naphthalimide units. The minor band is due to the 1,8-naphthalimide and reflects the pendant amino functionality (resembling **Figure 3.5**). As for the diimide, when the polarity of the solvent is increased these maxima are hypsochromically shifted, indicating an $n\pi^*$ character of the first singlet excited state S_1 .³⁴

Figure 3.10 depicts the absorbance bands of a bisnaphthalimide and its constituent moieties. This shows that the bisnaphthalimide band combines the spacer signature with

the 4-amino-1,8-naphthalimide band and shows that the relative intensities and ϵ values are reflected in the bisnaphthalimide system.

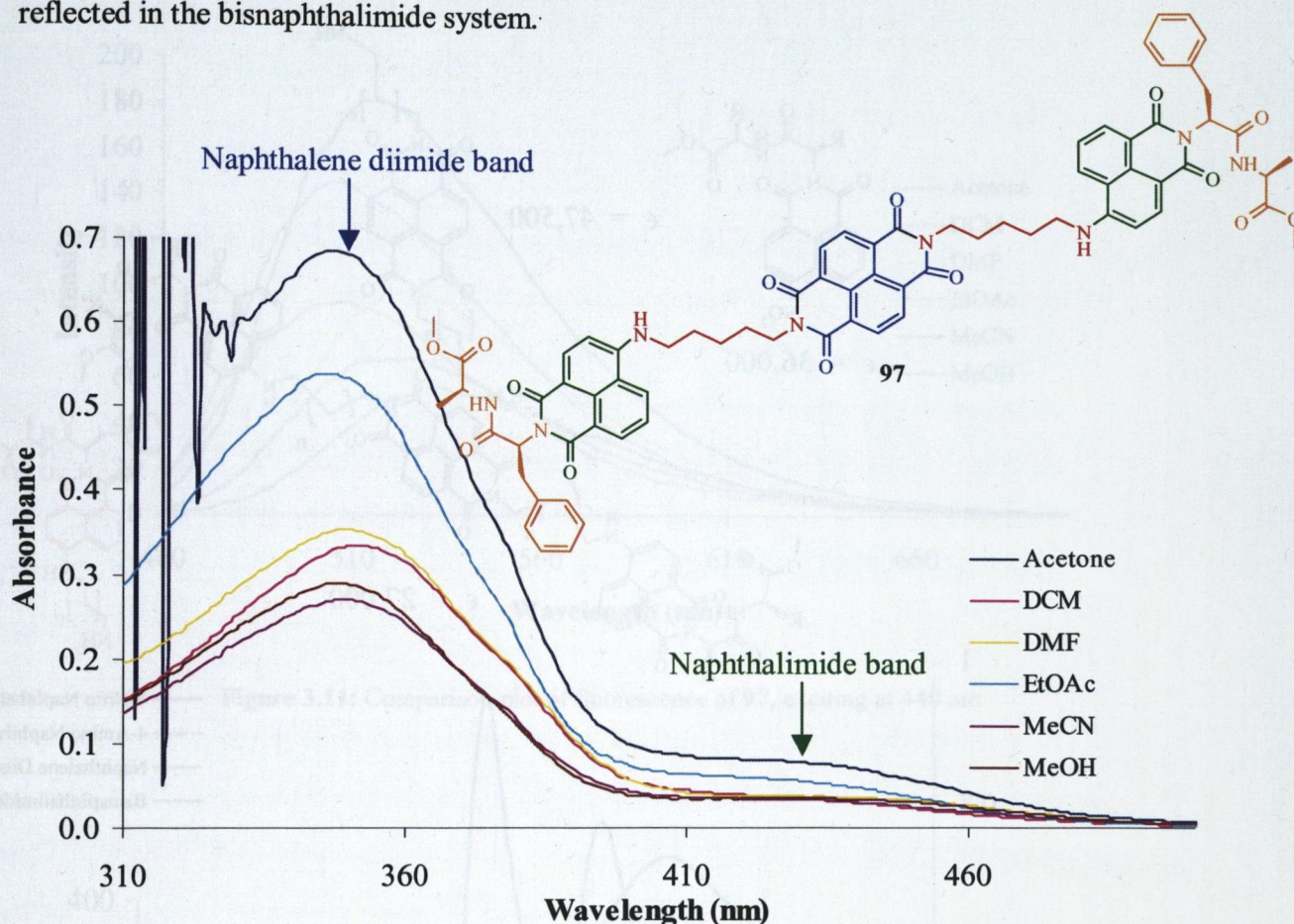


Figure 3.9: Comparison plot of absorbances of 97, 20 μ M in different solvents

Examining the 4-amino-1,8-naphthalimide unit by exciting at 440 nm gives an intense fluorescence band centred at *ca.* 525 nm, is solvent dependant and reflects the ICT nature of the moiety, **Figure 3.11**. As expected, this resembles the emission obtained for 107. When the naphthalene diimide unit was probed by exciting at *ca.* 355 nm the nature of the emission was found to be solvent dependant, **Figure 3.12**. In acetone, CH_2Cl_2 , EtOAc and MeCN there is a minor band at *ca.* 410 nm and a major band at *ca.* 510 nm. In DMF the band at *ca.* 410 nm is reduced and a maximum band was seen at *ca.* 510 nm, this is the exiplex band and in this polar solvent the relative intensities of the band are inverted. However, in MeOH the band at *ca.* 410 nm is the major band and that at *ca.* 510 nm is a minor band, this solvent does not favour the exiplex formation and only the monomer band is seen. As in the absorbance spectra, the overall fine structure that is observed for the free spacer system is no longer seen when the naphthalene diimide is tethered to two 1,8-naphthalimides.

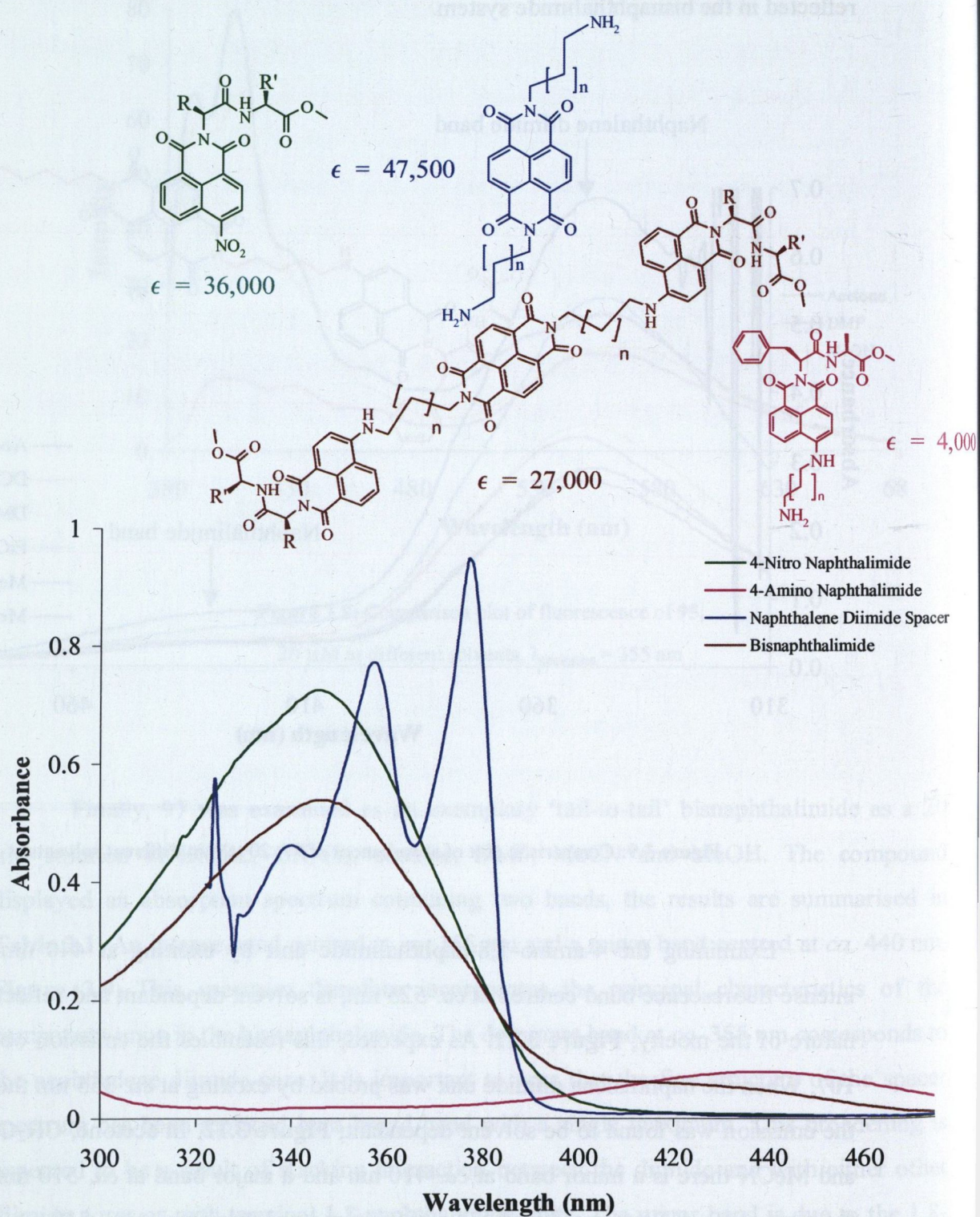


Figure 3.10: Comparison plot of absorbance bands of bisnaphthalimide and constituent units, 20 μ M in EtOAc

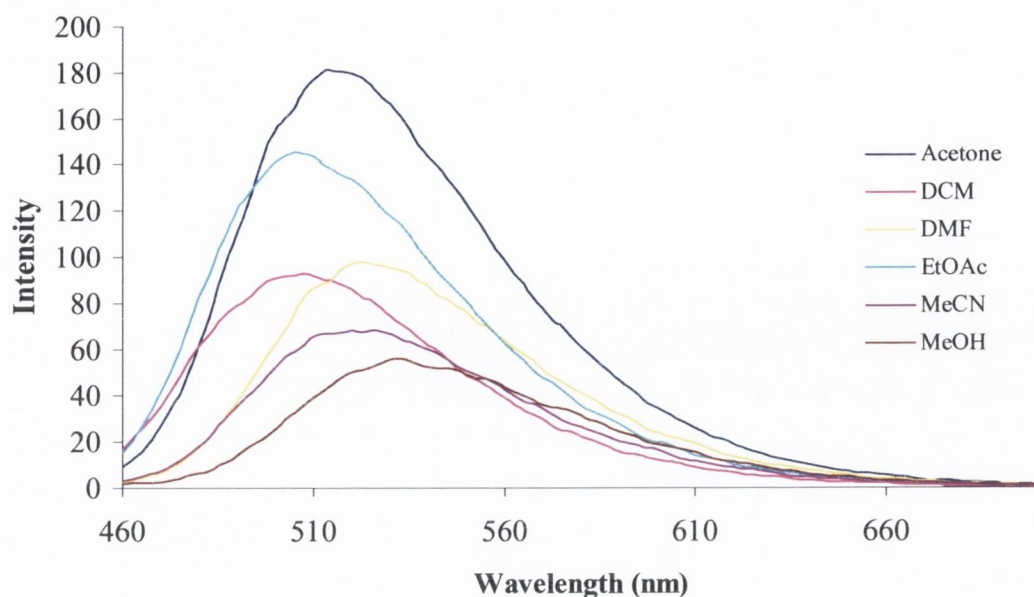


Figure 3.11: Comparison plot of fluorescence of **97**, exciting at 440 nm

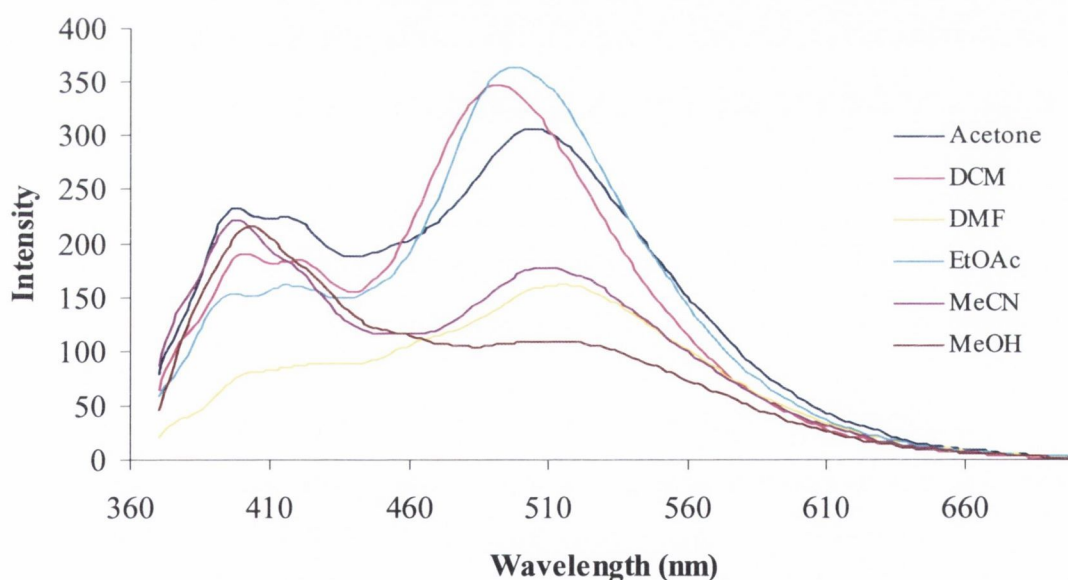


Figure 3.12: Comparison plot of fluorescence of **97**, exciting at 355 nm

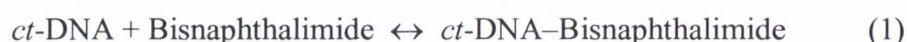
Since these bisnaphthalimides exhibit solvent dependency they may be equally sensitive to their local bonding environment and their interaction with DNA, since this may itself be thought of as a hydrophobic solvent. The naphthalene diimide band is the dominant spectroscopic feature and this will provide a means of investigating the DNA interactions and examining the strength of any binding.

3.3 Investigating the Interaction of ‘Tail-to-Tail’ Bisnaphthalimides with DNA by Ground State and Excited State Spectroscopy

3.3.1 Introduction

Since changes in the environment of a molecule effect its spectroscopic signal interaction between a compound and DNA can be observed by monitoring the resulting changes in the absorbance and emission spectra upon the addition of DNA. Changes in these spectra, in the form of bathochromic (to longer wavelengths) or hypsochromic (to shorter wavelengths) shifts and increases or decreases in band intensity, provide indications about the nature of such interactions. To investigate the binding of bisnaphthalimides to DNA solutions of bisnaphthalimides were prepared in 2 : 98, DMSO : phosphate buffer (10 mM, pH 7). Aliquots of a stock solution of *ct*-DNA were introduced so that successive additions provided certain molar ratios of *ct*-DNA (phosphate, P) to compound (drug, D), P/D ratios. The absorption and emission spectra were concurrently recorded after each addition and these titrations were conducted at 25 °C. Each titration was continued until the changes in the spectra plateaued. Each *ct*-DNA titration was repeated three times, the homopolymer titrations were conducted once. The nature of these changes would suggest the strength of interactions. The absorbance data was then used to plot several graphs;

- i. A vs. $1/[ct\text{-DNA}]$; this would depict the change in absorbance over the course of the titration.
- ii. $[bp\ ct\text{-DNA}] / (\epsilon_A - \epsilon_F)$ vs. $[bp\ ct\text{-DNA}]$; The slope of this graph divided by the intercept would provide the binding constant, K . This quantifies the strength of the interaction between the compound and *ct*-DNA. This graphical deduction of K is widely quoted in literature, particularly by Kumar,¹³⁰ Wilson¹³¹ and Suh¹¹¹. This plot is known as the intrinsic binding plot and is derived as follows;



The formation constant is defined as:

$$K = [ct\text{-DNA-Bisnaphthalimide}] / [ct\text{-DNA}][\text{Bisnaphthalimide}] \quad (2)$$

To calculate K one must know:

- (i) [bp *ct*-DNA] concentration of calf thymus DNA in base pairs
- (ii) [*ct*-DNA–Ligand]
- (iii) [Ligand]

$$A = \epsilon cl \text{ or } [ct\text{-DNA– Bisnaphthalimide}] = (\Delta A / \Delta \epsilon l) \quad (3)$$

This can be expressed as a double reciprocal form (Hildebrand-Benesi equation)

$$1/(\epsilon_A - \epsilon_F) = 1/(\epsilon_B - \epsilon_F) + 1/K(\epsilon_B - \epsilon_F) [\text{bp } ct\text{-DNA}]$$

ϵ_A = apparent extinction coefficient of Bisnaphthalimide at each addition of *ct*-DNA

ϵ_F = extinction coefficient of Bisnaphthalimide when free

ϵ_B = effective extinction coefficient of Bisnaphthalimide when completely bound

[bp *ct*-DNA] = concentration of *ct*-DNA in basepairs

Where $\Delta\epsilon_{ap} = [\epsilon_A - \epsilon_F]$ and $\Delta\epsilon = [\epsilon_B - \epsilon_F]$ and

Equation becomes:

$$1/\Delta\epsilon_{ap} = 1/\Delta\epsilon + 1/K\Delta\epsilon [\text{bp } ct\text{-DNA}]$$

Multiplying by [*ct*-DNA] gives:

$$[\text{bp } ct\text{-DNA}] / \Delta\epsilon_{ap} = [\text{bp } ct\text{-DNA}] / \Delta\epsilon + 1/K\Delta\epsilon$$

Plotting:

$$[\text{bp } ct\text{-DNA}] / \Delta\epsilon_{ap} \text{ vs. } [\text{bp } ct\text{-DNA}]$$

Gives a slope of:

$$1 / \Delta\epsilon \quad (4)$$

With an intercept of:

$$1/ K\Delta\epsilon \quad (5)$$

The intrinsic binding constant K is

$$K = \text{Slope} / \text{Intercept} \quad (6)$$

The fluorescence data allowed the plotting of an additional graph;

- iii. I/I_{initial} vs. P/D ratio; this would depict the overall change in fluorescence over the course of the titration.

The nine 'tail-to-tail' bisnaphthalimides were soluble in the 2 : 98, DMSO : phosphate buffer solution and these compounds were all investigated by titration with *ct*-DNA. Subsequently, titrations were conducted with homopolymers to investigate whether any of these bisnaphthalimides exhibit sequence selectivity, preferentially interacting with certain base pair sequences over others. Poly(dG-dC)poly(dG-dC) and poly(dA-dT)poly(dA-dT) were employed in these studies.

3.3.2 DNA Titrations of Bisnaphthalimides featuring L-Phenylalanine

Compound **96**, the 'tail-to-tail' PheAla functionalised bisnaphthalimide linked by the (amino)propyl-naphthalimide diimide spacer, was the first conjugate to be investigated. The absorption spectrum has λ_{max} at *ca.* 355 nm due to the diimide and another band of lower intensity at *ca.* 440 nm due to the 4-amino-1,8-naphthalimide units. A solution (3 μM) in 2 : 98, DMSO : phosphate buffer was prepared, this compound having $\epsilon = 19,192 \text{ M}^{-1} \text{ cm}^{-1}$ at 355 nm, and was titrated with a stock solution of *ct*-DNA (4.9 mM). Successive additions resulted in a hypochroism of 17 % of the band at 355 nm, plateauing at P/D = 80, **Figure 3.13**. No spectral shift occurred in λ_{max} at 355 nm.

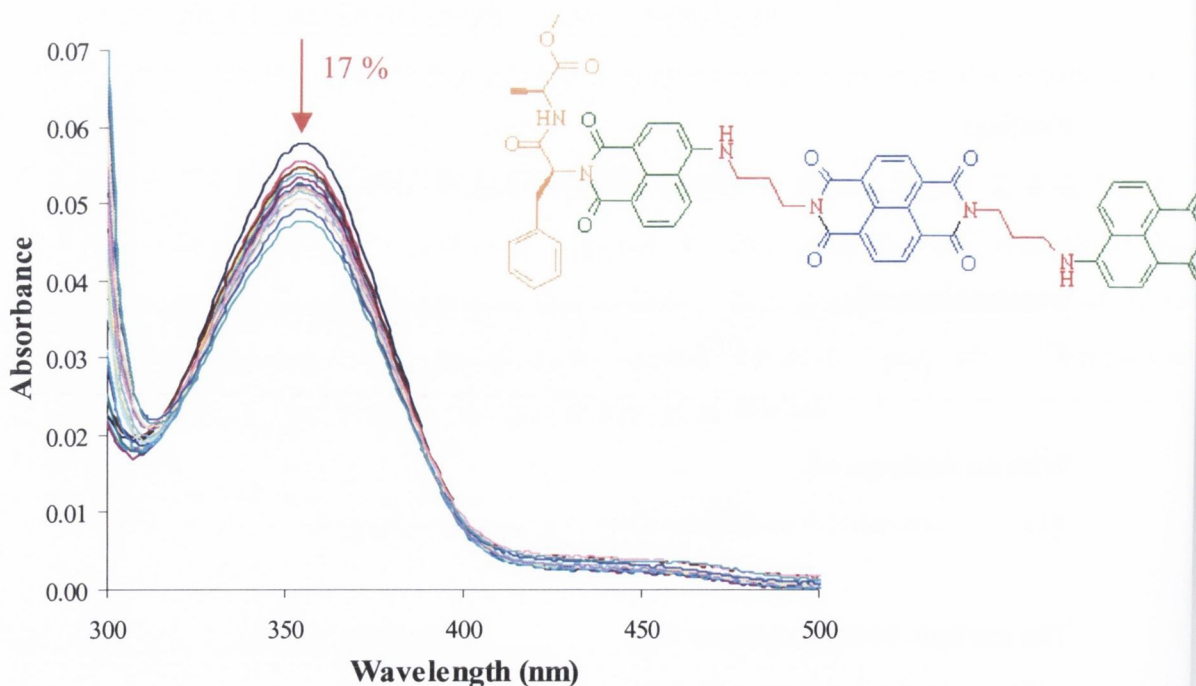


Figure 3.13: Absorption spectrum of titration of **96** (*ca.* 3 μM) with increasing concentration of *ct*-DNA (0-360 μM , P/D = 0-80) in 2:98, DMSO:phosphate buffer

When the diimide band at 353 nm is excited the resulting emission spectrum has $\lambda_{\text{max}} = 430$ nm, by a P/D = 140 this has shifted to 412 nm and the band has undergone a hypochroism of 37 %, **Figure 3.14**. Exciting the 1,8-naphthalimide band at 440 nm yields a spectrum with a λ_{max} at 545 nm, this does not shift throughout the titration, but is reduced in intensity by 19 %, **Figure 3.15**. (The erratic appearance of these spectra is due to the fact that fluorimeter lamp was approaching the end of it lifespan.) This fluorescence behaviour implies that both regions of the molecule are interacting with the double helix.

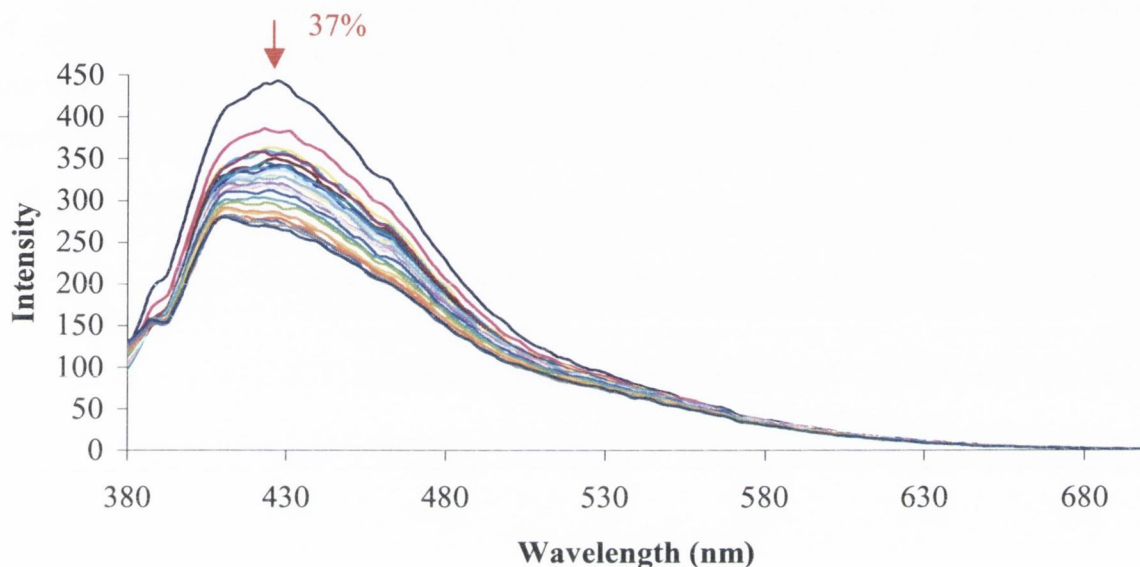


Figure 3.14: Emission spectrum of titration of **96** (ca. 3 μM) with increasing concentration of *ct*-DNA (0-360 μM , P/D = 0 - 140) in 2:98 DMSO:phosphate buffer, exciting at the diimide maximum at 355 nm

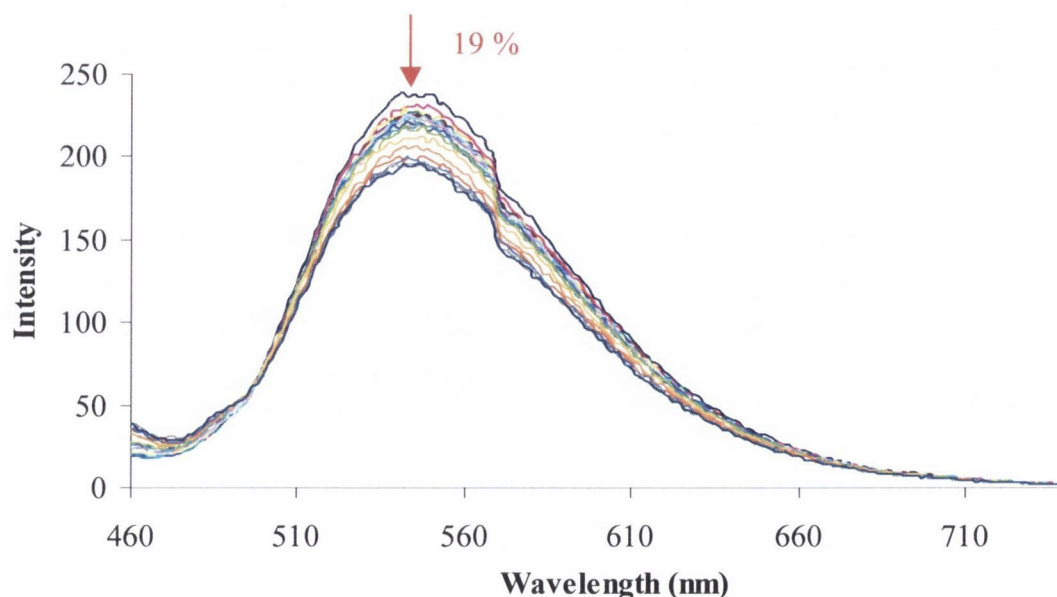


Figure 3.15: Emission spectrum of titration of **96** (ca. 3 μM) with increasing concentration of *ct*-DNA (0-360 μM , P/D = 0 - 140) in 2:98 DMSO:phosphate buffer, exciting at the 4-aminonaphthalimide maximum at 440 nm

The intrinsic binding plot, **Figure 3.16**, of $[\text{bp } ct\text{-DNA}] / \Delta\epsilon_{\text{ap}}$ vs. $[\text{bp } ct\text{-DNA}]$ yielded a linear trend graph, of slope $m = 0.0003$, from which a binding constant of $K = 5.0 \times 10^4 \text{ M}^{-1}$ was deduced.^{111, 130} Upon closer examination this graph has two regions, one until $P/D = 40$ and another until $P/D = 80$. If these are examined separately they give binding constants of $K = 2.0 \times 10^5 \text{ M}^{-1}$ and $K = 3.0 \times 10^3 \text{ M}^{-1}$, respectively. This may reflect a two step binding process, there appears to be stronger binding at lower concentrations of *ct*-DNA and this weakens at increased concentrations. It may be that at increased *ct*-DNA concentration that there is some degree of intermolecular binding, *i.e.* that one bisnaphthalimide straddles two strands of DNA. However, this may be an oversimplification of the mode of interaction. Since the magnitude of the overall binding constant is the average of those of the individual treatments the original binding constant is most likely a sufficient approximation of the strength of the binding of the molecule to *ct*-DNA.

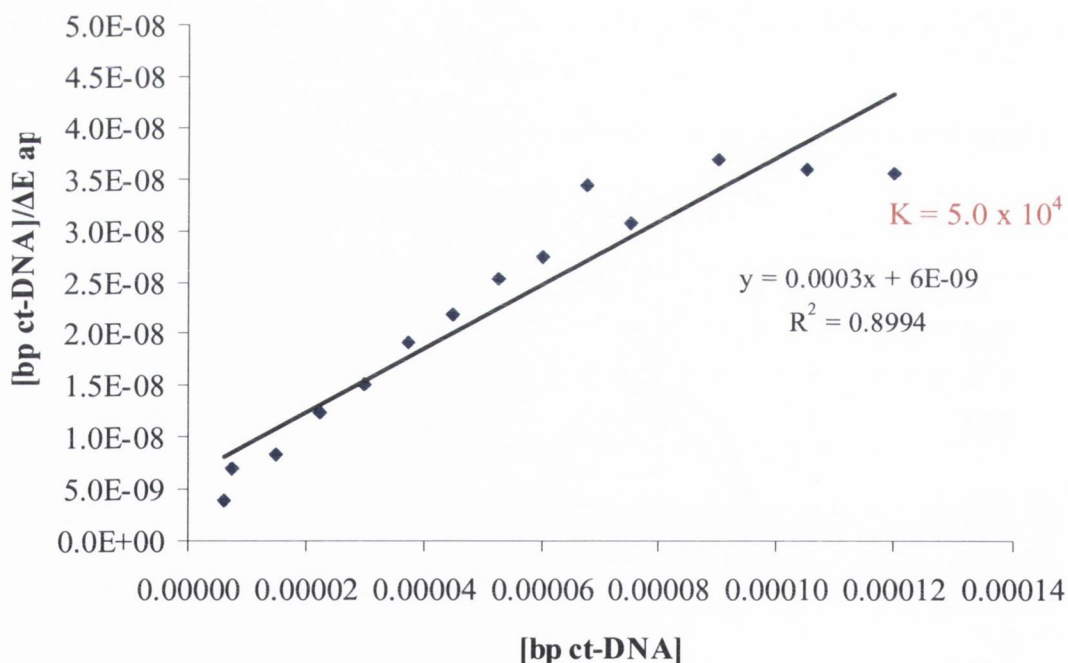


Figure 3.16: Intrinsic binding curve of **96** vs. *ct*-DNA from absorbance at 355 nm

Figure 3.17 depicts another interpretation of the binding by plotting absorbance vs. $1/[ct\text{-DNA}]$. This reflects the decreasing absorbance as the concentration of *ct*-DNA is increased over the course of the titration.

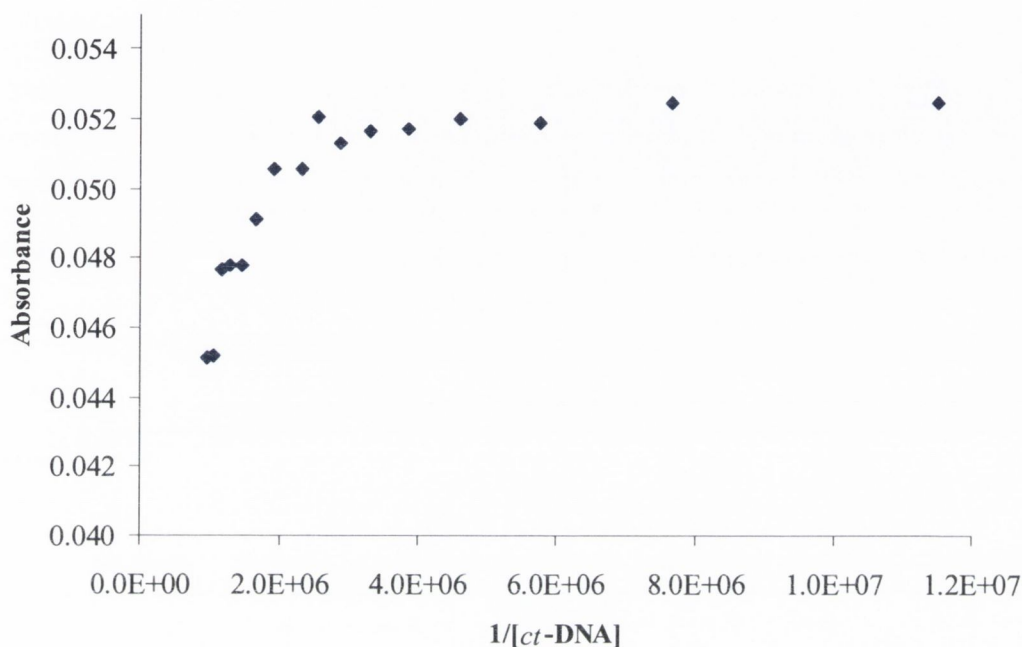


Figure 3.17: Binding curve of **96** vs. *ct*-DNA from absorbance at 355 nm

A solution of **96** ($3.10\ \mu\text{M}$) in 2 : 98 DMSO : phosphate buffer was prepared and was titrated with a stock solution of the homopolymer poly(dA-dT)poly(dA-dT) ($2.00\ \text{mM}$). The same behaviour was observed as seen for the *ct*-DNA titration, **Figure 3.18**. The band at 350 nm sequentially decreased, plateauing at a P/D = 120, with an overall hypochromicity of 35 %. There was no spectral shift of λ_{max} at 350 nm. The band at 440 nm decreases in by 70 %.

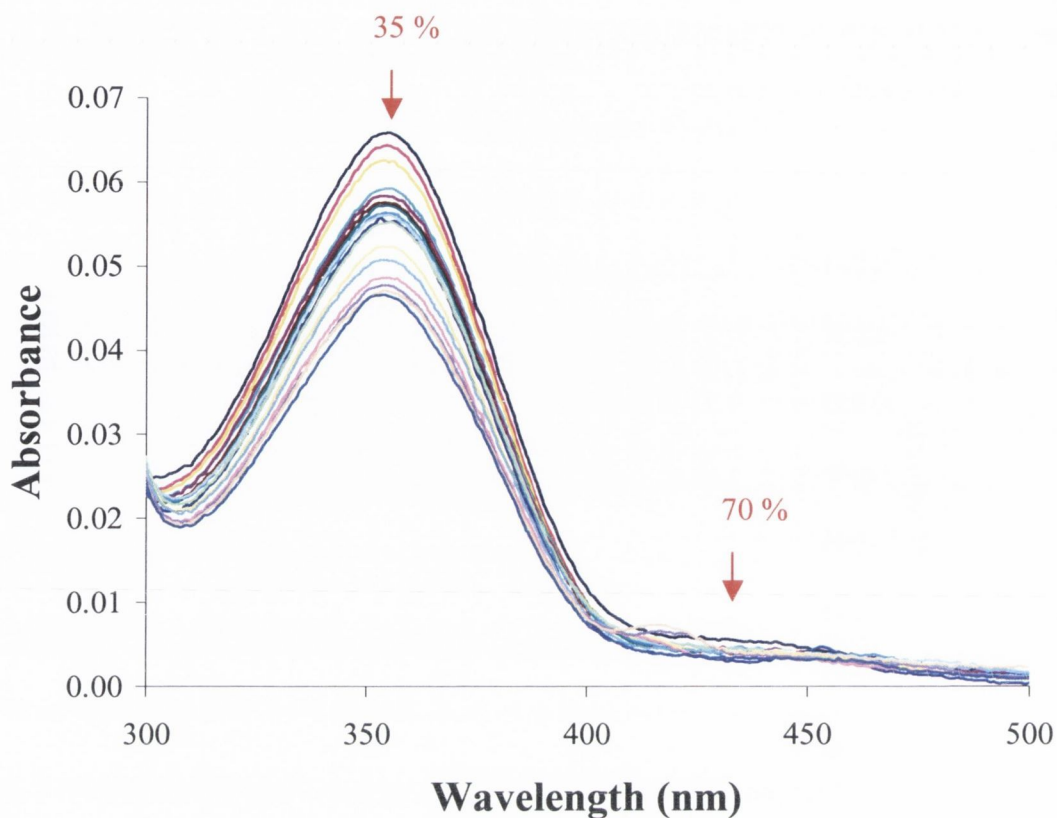


Figure 3.18: Absorption spectrum of titration of **96** (ca. 3.10 μM) with increasing concentration of poly(dA-dT)poly(dA-dT) (0-372 μM , P/D = 0 -120) in 2:98 DMSO:phosphate buffer

When the diimide transition at 355 nm was excited the resulting emission spectrum had a λ_{max} at 430 nm, and this band was decreased in intensity by 26 % over the course of the titration, **Figure 3.19**. Exciting the 1,8-naphthalimide band at 440 nm yields an emission spectrum with a λ_{max} at 545 nm and is likewise decreased in intensity by 19%, **Figure 3.20**.

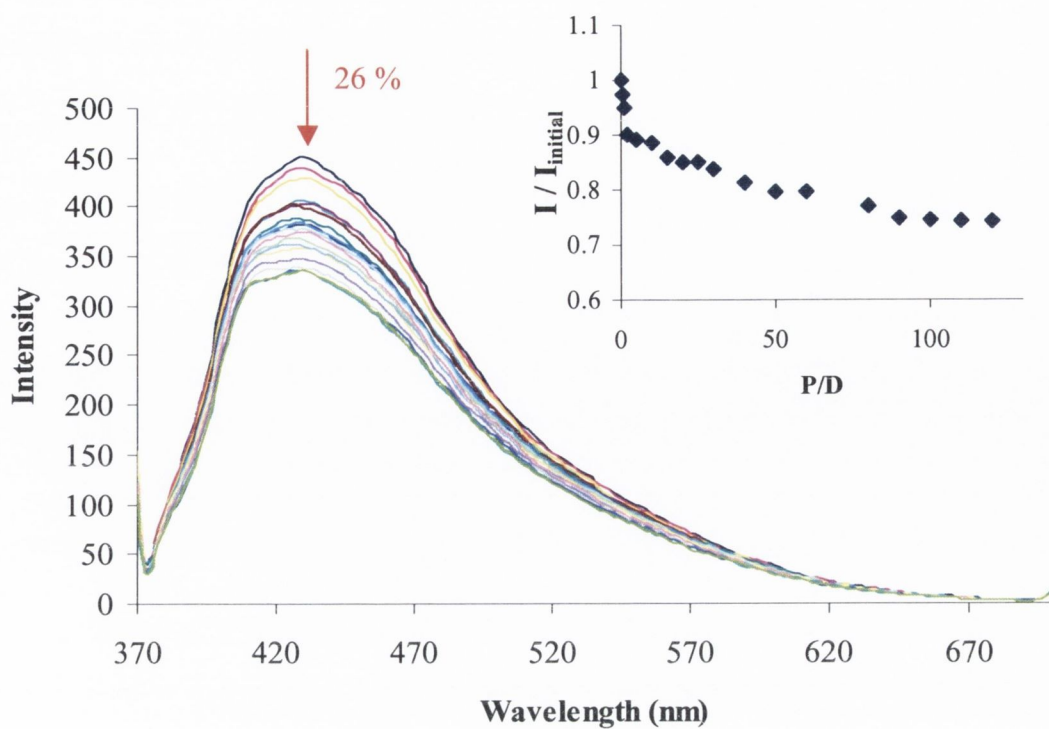


Figure 3.19: Emission spectrum of titration of **96** (ca. 3.10 μM) with increasing concentration of poly(dA-dT)poly(dA-dT) (0-372 μM , P/D = 0-120) in 2:98 DMSO:phosphate buffer exciting at the diimide maximum at 355 nm

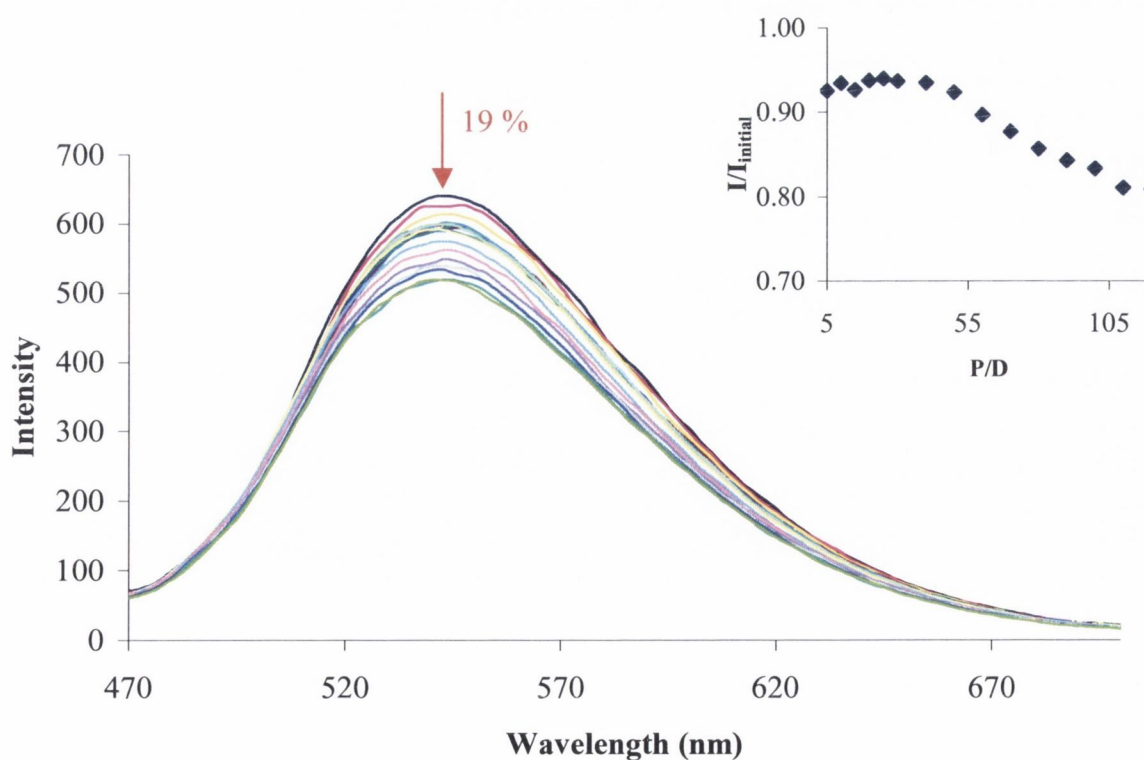


Figure 3.20: Emission spectrum of titration of **96** (ca. 3.10 μM) with increasing concentration of poly(dA-dT)poly(dA-dT) (0-372 μM , P/D = 0-120) in 2:98 DMSO:phosphate buffer exciting at the 4-amino naphthalimide maximum at 440 nm

The intrinsic binding plot, **Figure 3.21**, yielded a binding constant of $K = 2.0 \times 10^4 \text{ M}^{-1}$. This binding constant is of the same order magnitude as seen from the *ct*-DNA study. From **Figure 3.21** the two regions are more obvious than seen in the *ct*-DNA study. The first region, $P/D = 0 - 40$, gives a binding constant of $K = 3.0 \times 10^5 \text{ M}^{-1}$, while the second region, $P/D = 40 - 120$, gives a binding constant of $K = 7.5 \times 10^3 \text{ M}^{-1}$. These average at a binding constant of $K = 1.8 \times 10^4 \text{ M}^{-1}$. While the two regions differ, the original overall binding constant again approximates the binding of the molecule to poly(dA-dT)poly(dA-dT) to provide an estimate of the strength of the interaction. Henceforth, the binding constant, K , quoted will refer to the value calculated using the titration data from initiation to plateau.

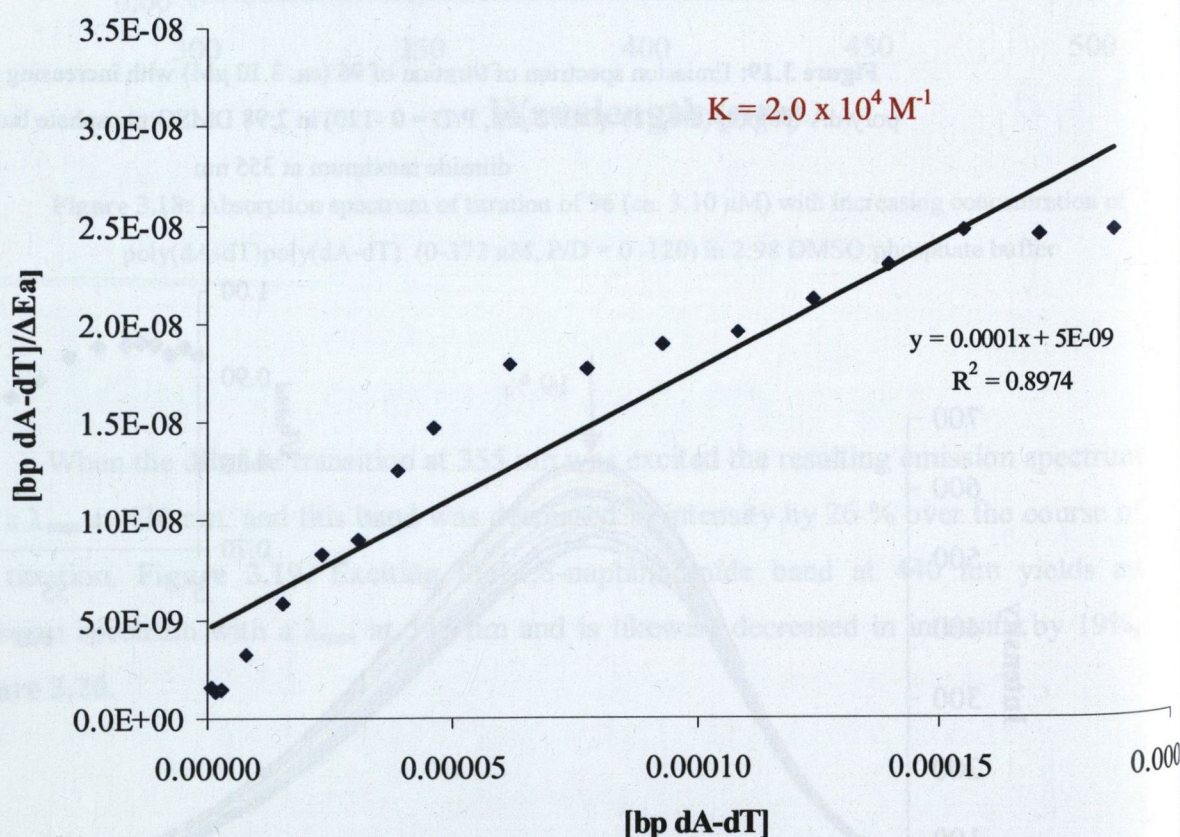


Figure 3.21: Intrinsic binding curve of **96** vs. poly(dA-dT)poly(dA-dT) from absorbance at 355 nm

A solution of **96** (2.70 μM) in 2 : 98 DMSO : phosphate buffer was prepared and was titrated with a stock solution of the homopolymer poly(dG-dC)poly(dG-dC) (13.00 mM). The band at 350 nm sequentially decreased, plateauing at a P/D = 70, **Figure 3.22**. An overall hypochromicity of 16 % was observed at λ_{max} . There was no shift in the λ_{max} from its initial position at 351 nm, although an isosbestic point can be seen at 312 nm.

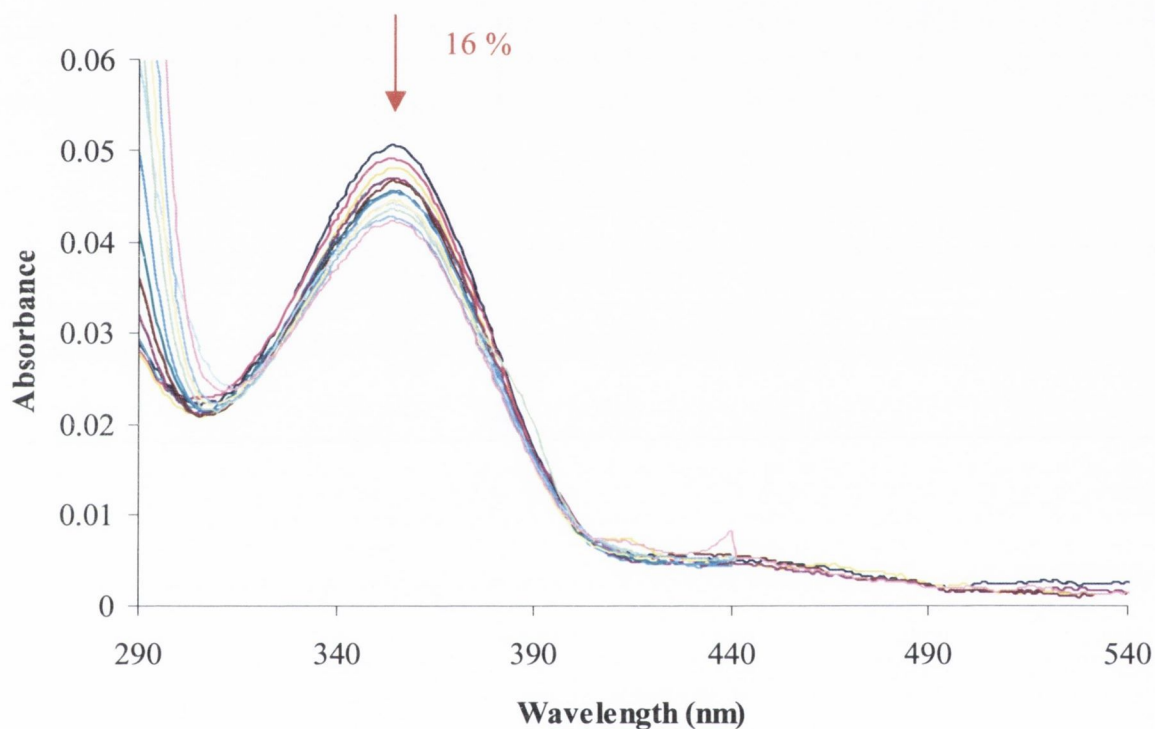


Figure 3.22: Absorption spectrum of titration of **96** (ca. 2.70 μM) with increasing concentration of poly(dG-dC)poly(dG-dC) (0-257 μM , P/D = 0 - 70) in 2:98 DMSO:phosphate buffer

When the diimide band at 355 nm is excited the resulting fluorescence spectrum has a λ_{max} at 427 nm, and the band has been diminished by *ca.* 6 %, **Figure 3.23**, this is a minor change and may indicate a limited amount of interaction between the diimide core and DNA. Such a minor change is within the instrumental error of the spectrometer. Exciting the 4-amino-1,8-naphthalimide band at 440 nm yields a fluorescence spectrum with a λ_{max} at 545 nm, this does not shift throughout the titration, but is likewise decreased by 14 %, **Figure 3.24**.

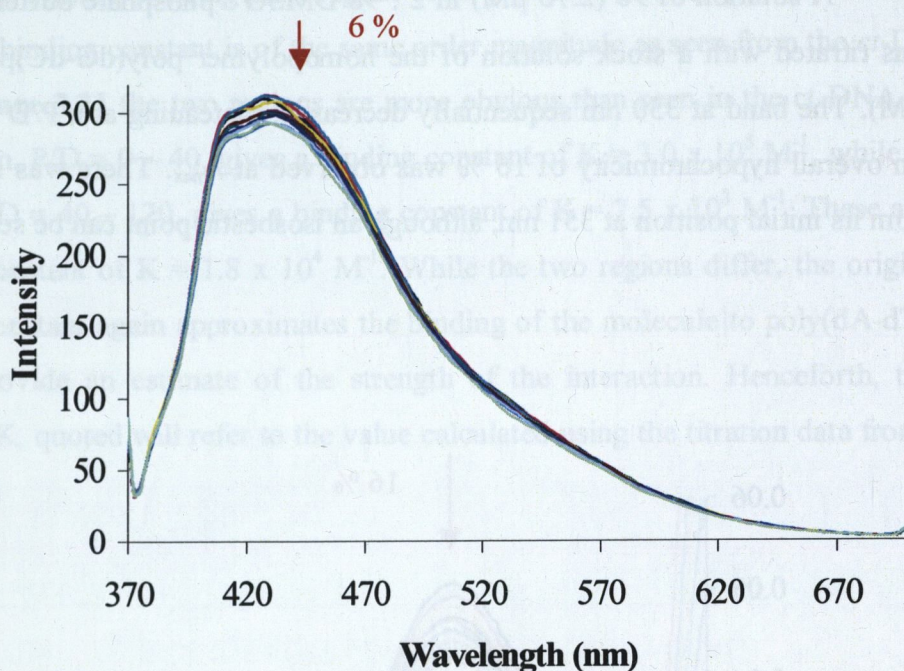


Figure 3.23: Emission spectrum of titration of **96** (ca. 2.70 μM) with increasing concentration of poly(dG-dC)poly(dG-dC) (0-257 μM, P/D = 0 - 31) in 2:98 DMSO:phosphate buffer exciting at the diimide maximum at 355 nm

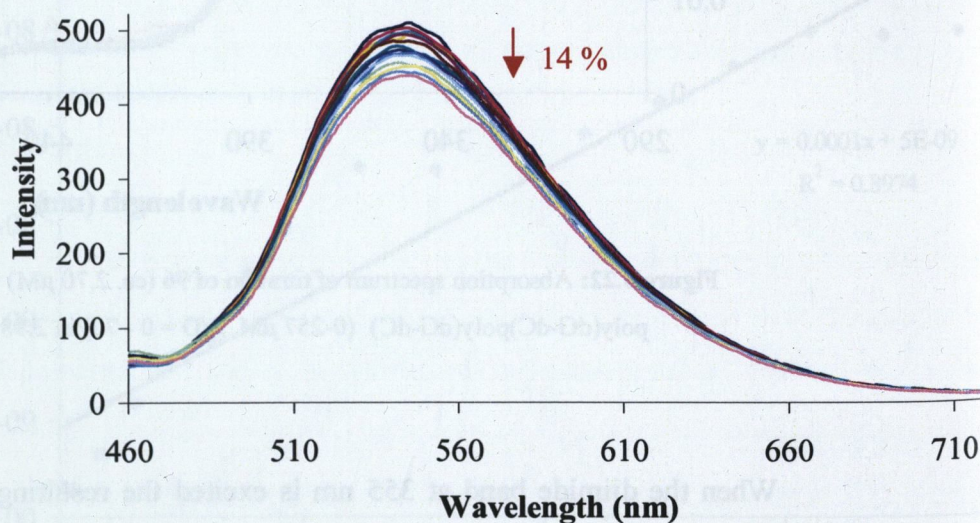


Figure 3.24: Emission spectrum of titration of **96** (ca. 2.70 μM) with increasing concentration of poly(dG-dC)poly(dG-dC) (0-257 μM, P/D = 0 - 70) in 2:98 DMSO:phosphate buffer exciting at the amino naphthalimide maximum at 440 nm

The intrinsic binding plot yielded a linear trend graph, **Figure 3.25**, with slope $m = 0.0004$, from which a binding constant of $K = 1.3 \times 10^5 \text{ M}^{-1}$ was deduced. This binding constant is an order of magnitude greater than seen from the *ct*-DNA and poly(dA-dT)poly(dA-dT) studies, this suggests that **96** exhibits a preference for GC rich sequences, *i.e.* it displays a degree of selectivity.

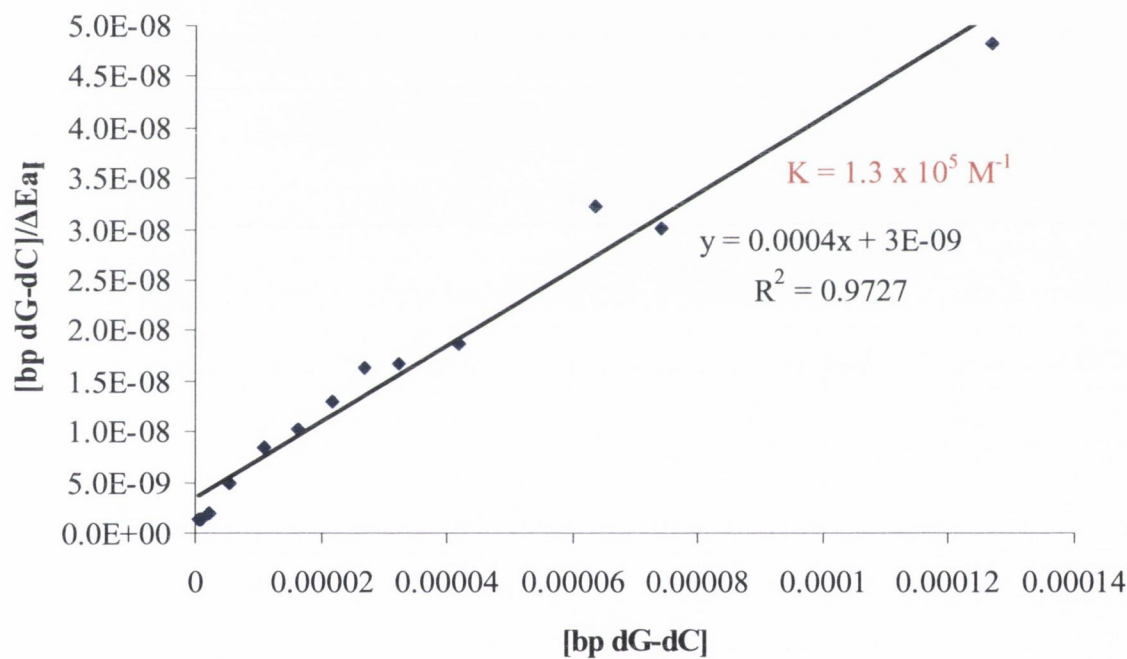


Table 3.2 summaries the results of these investigations of **96**. The increasing concentration of each type of DNA causes hypochromicity in the absorbance spectra. Both the diimide and 4-amino-1,8-naphthalimide emission bands are effected over the course of the titration implying that both structural motifs of the bisnaphthalimide interact with DNA to some degree. Thus, the fact that there are significant changes in the absorbance and emission signatures of this compound in the presence of DNA suggests that some binding is at play, although this does not define the nature of the binding. Compound **96** binds to all three types of DNA with a binding constant, K , in the order of $10^4 - 10^5 \text{ M}^{-1}$ and seems to display a preference for poly(dG-dC)poly(dG-dC) sequences.

Table 3.2: Summary of Titration Data for **96**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex}} 355 \text{ nm}$	Δ_{Emission} $\lambda_{\text{ex}} 440 \text{ nm}$	Final P/D Ratio	$K \text{ (M}^{-1}\text{)}$	R^2
<i>ct</i> -DNA	17 %	37 %	19 %	80	5.0×10^4	0.8994
poly(dA-dT) poly(dA-dT)	35 %	26 %	19 %	120	2.0×10^4	0.8974
poly(dG-dC)poly(dG-dC)	16 %	6 %	14 %	70	1.3×10^5	0.9727

The pentyl linked analogue of the PheAla family of bisnaphthalimides, **97**, was studied in the same manner and similar behaviour was observed as for **96**. **Table 3.3** summaries these results. Of particular interest is the fact that the binding constant, $K = 2.5 \times 10^5 \text{ M}^{-1}$, for *ct*-DNA binding is an order of magnitude greater than that for **96**. The binding for poly(dA-dT)poly(dA-dT) is also greater and saturation occurs earlier at a P/D = 30 and suggests that a strong interaction or fast binding kinetics are at play. Although the small decrease in the emission of the diimide is unusual and may suggest that merely the naphthalimide termini interact with DNA. Compound **97** displays a preference for *ct*-DNA over the two homopolymers and the binding constants, K , of **97** to *ct*-DNA and poly(dA-dT)poly(dA-dT) is an order of magnitude greater than that seen for **96**, *i.e.* 10^5 M^{-1} as compared to 10^4 M^{-1} .

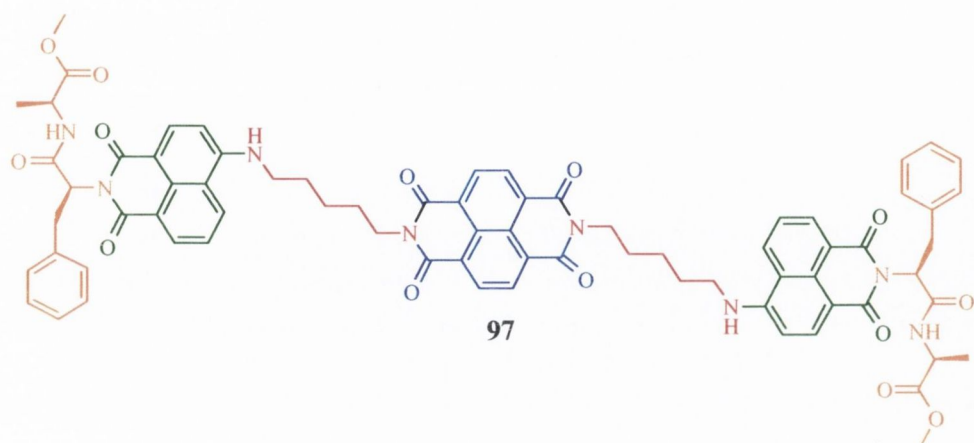


Table 3.3: Summary of Titration Data for **97**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex}} 355\text{nm}$	Δ_{Emission} $\lambda_{\text{ex}} 440\text{nm}$	Final P/D Ratio	$K \text{ (M}^{-1}\text{)}$	R^2
<i>ct</i> -DNA	11 %	7 %	18 %	120	2.5×10^5	0.9144
poly(dA-dT)poly(dA-dT)	16 %	<i>ca.</i> 1 %	<i>ca.</i> 5 %	30	1.3×10^5	0.7522
poly(dG-dC)poly(dG-dC)	33 %	11 %	25 %	95	1.0×10^5	0.9555

Finally, investigations were conducted for **98**, the hexyl linked analogue of the PheAla family of bisnaphthalimides. **Table 3.4** summaries these investigations and shows that increasing the spacer length has significant effects in this case. For *ct*-DNA the same order of magnitude of 10^5 M^{-1} for the binding constant, K , is deduced as for **97**. For poly(dA-dT)poly(dA-dT) an increase in the binding constant to 10^6 M^{-1} is observed. This is larger than any binding constant observed for PheAla mononaphthalimides within the Gunnlaugsson group¹⁹. Not only does this analogue display a preference for poly(dA-dT)poly(dA-dT) sequences but the magnitude of the binding itself is considerable and since saturation is reached at P/D = 50 is may

be that fast binding kinetics operate. For poly(dG-dC)poly(dG-dC) a binding constant of $K = 5.0 \times 10^4 \text{ M}^{-1}$ is calculated and is lower than those deduced for **96** and **97** which were of the order of 10^5 M^{-1} . It is important to note that the decrease of the diimide emission band is only *ca.* 2 - 4 % by the end of each of these three titrations and this may indicate that the span of the spacer is so long that it does not facilitate optimum binding. However, this compound offers the best selectivity between the three types of DNA; *ct*-DNA $10^4 \text{ M}^{-1} < \text{poly(dG-dC)poly(dG-dC)} 10^5 \text{ M}^{-1} < \text{poly(dA-dT) poly(dA-dT)} 10^6 \text{ M}^{-1}$.

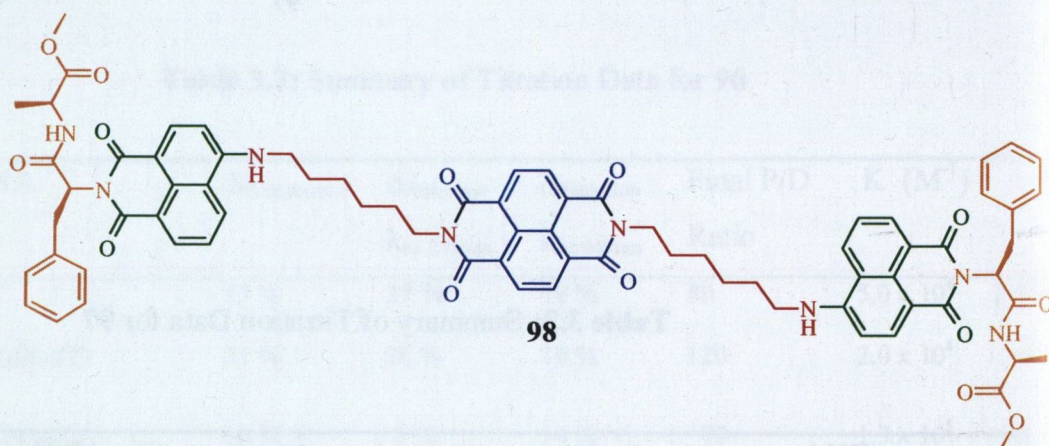


Table 3.4: Summary of Titration Data for **98**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex}} 355\text{nm}$	Δ_{Emission} $\lambda_{\text{ex}} 440\text{nm}$	Final P/D Ratio	$K (\text{M}^{-1})$	R^2
<i>ct</i> -DNA	21 %	<i>ca.</i> 2 %	19 %	150	1.5×10^5	0.9846
poly(dA-dT)poly(dA-dT)	9 %	<i>ca.</i> 3 %	<i>ca.</i> 1 %	50	5.7×10^6	0.8010
poly(dG-dC)poly(dG-dC)	24 %	<i>ca.</i> 4 %	22 %	157	5.0×10^4	0.9706

3.3.3 DNA Titrations of Bisnaphthalimides featuring L-Cbz(Lysine)

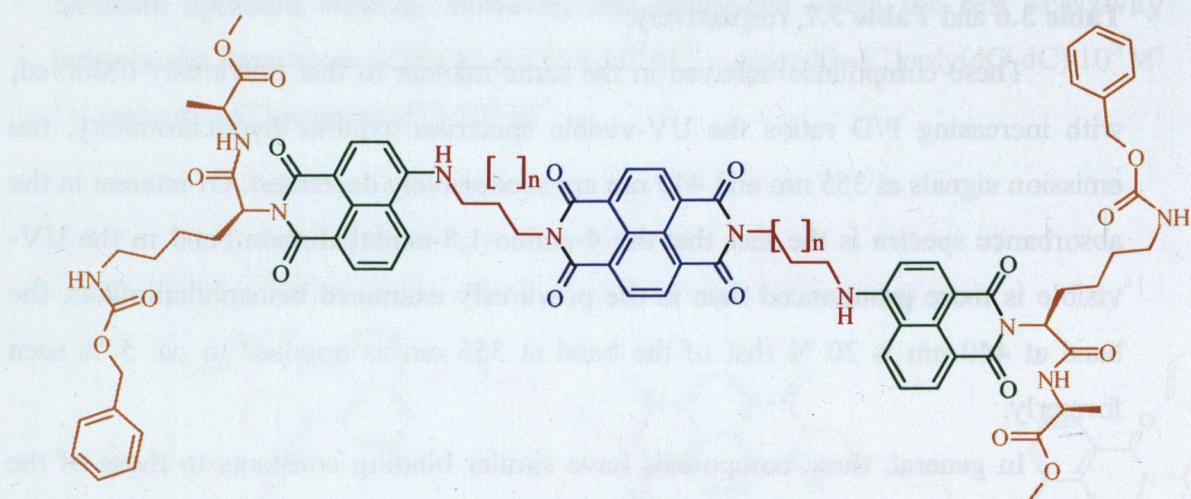
The three analogous bisnaphthalimides incorporating Cbz(Lys)Ala dipeptide motifs, **99**, **100** and **101** (all at *ca.* 2.00 μM), were also similarly studied in the presence of *ct*-DNA and the homopolymers poly(dA-dT)poly(dA-dT) and poly(dG-dC)poly(dG-dC). The results of these investigations are summarised in **Table 3.5**, **Table 3.6** and **Table 3.7**, respectively.

These compounds behaved in the same manner to that previously observed, with increasing P/D ratios the UV-visible spectrum exhibits hypochromicity, the emission signals at 355 nm and 440 nm are successively decreased. Of interest in the absorbance spectra is the fact that the 4-amino-1,8-naphthalimide band in the UV-visible is more pronounced than in the previously examined bisnaphthalimides, the band at 440 nm is 20 % that of the band at 355 nm as opposed to *ca.* 5 % seen formerly.

In general, these compounds have similar binding constants to those of the PheAla bisnaphthalimides, being $10^4 - 10^5 \text{ M}^{-1}$ in magnitude. Compound **99**, **Table 3.5**, has a deduced binding constant, K , with *ct*-DNA of $5.0 \times 10^4 \text{ M}^{-1}$ and for both homopolymers a binding constant, K , of $1.0 \times 10^5 \text{ M}^{-1}$ but no distinction is made between them. Compound **100**, **Table 3.6**, binds *ct*-DNA and poly(dG-dC)poly(dG-dC) more strongly than poly(dA-dT)poly(dA-dT), with binding constants, K , of the order of 10^5 M^{-1} compared to 10^4 M^{-1} . In contrast, **101**, **Table 3.7**, seems to bind weakly to *ct*-DNA since the absorbance is only slightly affected by $P/D = 200$. The minor hypochromicity of *ca.* 4 % is within the instrumental error of the spectrometer and is not significant enough to merit an intrinsic binding plot to deduce binding constant, K . Likewise, the interaction with poly(dA-dT)poly(dA-dT) has little effect on the absorbance or emission spectra. However, binding to poly(dG-dC)poly(dG-dC) is comparably noteworthy with a binding constant, $K = 1.0 \times 10^5 \text{ M}^{-1}$. Whereas the PheAla analogue **98** preferentially bound poly(dA-dT)poly(dA-dT), the Cbz(Lys)Ala functionalised version displays a specificity for poly(dG-dC)poly(dG-dC).

It is important to note that the decrease of the diimide emission band is only *ca.* 4 % by the end of the titration and this may indicate that the span of the spacer is so long that it does not facilitate optimum binding, *i.e.* perhaps it is not suitable for the intercalation of the diimide accompanied by groove binding of the naphthalimide termini, instead perhaps the termini themselves either intercalate or groove bind, singly or dually, and this results in the lower binding constant. If only one terminal

intercalates it may be that the remainder of the molecule dangles from the new DNA-bisnaphthalimide complex. The fact that some of the changes in the emission are lower than those observed for the PheAla family may be because the extra bulk of the termini is prohibitive to many molecules associating with the DNA.



99, n = 1; 100, n = 3; 101, n = 4

Table 3.5: Summary of Titration Data for 99

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex}} 355\text{nm}$	Δ_{Emission} $\lambda_{\text{ex}} 440\text{nm}$	Final P/D Ratio	K (M^{-1})	R^2
ct-DNA	27 %	19 %	28 %	130	5.0×10^4	0.9372
poly(dA-dT)poly(dA-dT)	17 %	46 %	ca. 5 %	150	1.0×10^5	0.9669
poly(dG-dC)poly(dG-dC)	19 %	ca. 6 %	12 %	78	1.0×10^5	0.9760

Table 3.6: Summary of Titration Data for **100**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission}	Δ_{Emission}	Final P/D Ratio	K (M^{-1})	R^2
		$\lambda_{\text{ex } 355\text{nm}}$	$\lambda_{\text{ex } 440\text{nm}}$			
<i>ct</i> -DNA	16%	22 %	22 %	150	4.0×10^5	0.9929
poly(dA-dT)poly(dA-dT)	16 %	14 %	13 %	60	3.3×10^4	0.6052
poly(dG-dC)poly(dG-dC)	19 %	13 %	15 %	110	2.0×10^5	0.9829

Table 3.7: Summary of Titration Data for **101**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission}	Δ_{Emission}	Final P/D Ratio	K (M^{-1})	R^2
		$\lambda_{\text{ex } 355\text{nm}}$	$\lambda_{\text{ex } 440\text{nm}}$			
<i>ct</i> -DNA	<i>ca.</i> 4 %	<i>ca.</i> 3 %	19 %	200	n/a	
poly(dA-dT)poly(dA-dT)	<i>ca.</i> 7 %	<i>ca.</i> 3 %	<i>ca.</i> 6 %	50	n/a	
poly(dG-dC)poly(dG-dC)	26 %	<i>ca.</i> 4 %	12 %	198	1.0×10^5	0.9825

3.3.4 DNA Titrations of Bisnaphthalimides featuring L-Leucine

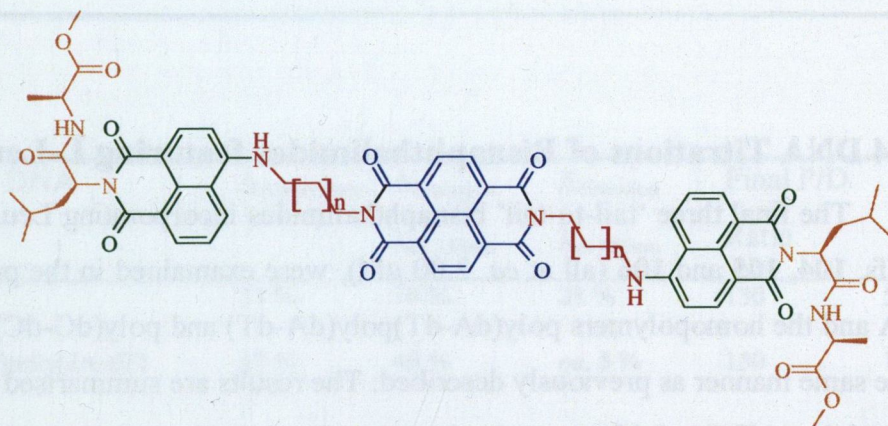
The final three ‘tail-to-tail’ bisnaphthalimides incorporating LeuAla dipeptide motifs, **104**, **105** and **106** (all at *ca.* 2.00 μM), were examined in the presence of *ct*-DNA and the homopolymers poly(dA-dT)poly(dA-dT) and poly(dG-dC)poly(dG-dC) in the same manner as previously described. The results are summarised in **Table 3.8**, **Table 3.9** and **Table 3.10**.

These compounds adopt the same behaviour as the previous two families and have similar binding constants, K, being $10^4 - 10^5 \text{ M}^{-1}$ in magnitude. Compound **104**, **Table 3.8**, binds to each of the three DNA sequences with binding constants, K, of the order of magnitude of 10^5 M^{-1} . Compound **105**, **Table 3.9**, binds to *ct*-DNA with a binding constant of $K = 4.5 \times 10^5 \text{ M}^{-1}$ while the homopolymer binding constants, K, are of the order 10^4 M^{-1} and there is little distinction between them. In contrast, the hexyl linked bisnaphthalimide, **106**, **Table 3.10**, displays such weak interaction with both *ct*-DNA and poly(dG-dC)poly(dG-dC) that binding constants could not be

calculated. However, for poly(dA-dT)poly(dA-dT) a significant hypochroism in the absorbance spectra of 23 % is observed by P/D = 50 and a binding constant of $K = 1.3 \times 10^5 \text{ M}^{-1}$ can be deduced. This low P/D may be the result of fast binding kinetics. This reflects the selectivity of the Cbz(Lys)Ala analogue, **101**. Again, this longer spacer length results in a minor change in the diimide emission band of *ca.* 3 %, suggesting that the hexyl analogues cannot achieve optimum interaction.

Table 3.8: Summary of Titration Data for **104**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex}} 355\text{nm}$	Δ_{Emission} $\lambda_{\text{ex}} 440\text{nm}$	Final P/D Ratio	$K \text{ (M}^{-1}\text{)}$	R^2
ct-DNA	10 %	<i>ca.</i> 4 %	11 %	100	3.3×10^5	0.9502
poly(dA-dT)poly(dA-dT)	16 %	9 %	<i>ca.</i> 2 %	80	1.0×10^5	0.9007
poly(dG-dC)poly(dG-dC)	19 %	12 %	19 %	78	1.7×10^5	0.9489



104, $n = 1$; **105**, $n = 3$; **106**, $n = 3$

Table 3.9: Summary of Titration Data for **105**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} λ_{ex} 355nm	Δ_{Emission} λ_{ex} 440nm	Final P/D Ratio	K (M ⁻¹)	R ²
<i>ct</i> -DNA	9 %	9 %	17 %	100	4.5 x 10 ⁵	0.9529
poly(dA-dT)poly(dA-dT)	15 %	16 %	10 %	150	6.7 x 10 ⁴	0.6537
poly(dG-dC)poly(dG-dC)	23 %	13 %	20 %	120	3.3 x 10 ⁴	0.8970

Table 3.10: Summary of Titration Data for **106**

DNA	$\Delta_{\text{Absorbance}}$	Δ_{Emission} λ_{ex} 355 nm	Δ_{Emission} λ_{ex} 440nm	Final P/D Ratio	K (M ⁻¹)	R ²
<i>ct</i> -DNA	<i>ca.</i> 2 %	18 %	13 %	80	n/a	n/a
poly(dA-dT)poly(dA-dT)	23 %	<i>ca.</i> 3 %	9 %	50	1.3 x 10 ⁵	0.9463
poly(dG-dC)poly(dG-dC)	<i>ca.</i> 1 %	<i>ca.</i> 5 %	15 %	40	n/a	n/a

3.3.5 Conclusion

In summary, all of the bisnaphthalimides interact with *ct*-DNA and with the homopolymers and trends are observed. In the propyl and pentyl analogues the changes in both the diimide and 4-amino-1,8-naphthalimide emission bands suggest that both these regions of the compounds interact with DNA. A possible mode of dual interaction may be the intercalation of the central diimide region along with the intercalation or, more likely, groove binding of the naphthalimide termini. In the hexyl analogues the minor changes in the diimide emission band may reflect that this spacer span is too long to facilitate optimum binding. A simpler binding mode may be at play here, it is possible that both or only one of the naphthalimide termini are intercalating or groove binding. So, the spacer length seems to dictate the strength of binding.

Sequence preferences can be achieved depending upon the functional groups and are tuned by the spacer length.

The propyl analogues of PheAla, **96**, binds poly(dG-dC)poly(dG-dC) > ct-DNA > poly(dA-dT)poly(dA-dT) as does the LeuAla analogue, **104**. The Cbz(Lys)Ala analogue, **99**, binds ct-DNA > homopolymers but there is no distinction between the two homopolymers. The pentyl analogues of each family preferentially bind ct-DNA over the homopolymers. Finally, the hexyl analogue of the PheAla and LeuAla families seem to achieve sequence selectivity for poly(dA-dT)poly(dA-dT), while that of the Cbz(Lys)Ala family is sequence selective for poly(dG-dC)poly(dG-dC).

Overall, the strongest binding to ct-DNA is found for the pentyl analogue of the LeuAla family with a binding constant of $K = 4.5 \times 10^5 \text{ M}^{-1}$, to poly(dA-dT)poly(dA-dT) is found for the hexyl analogue of the PheAla family with a binding constant of $K = 5.7 \times 10^6 \text{ M}^{-1}$ and to poly(dG-dC)poly(dG-dC) for pentyl analogue of the Cbz(Lys)Ala with a binding constant of $K = 2.0 \times 10^5 \text{ M}^{-1}$. Overall the pentyl analogues seems to bind most strongly and perhaps this length of spacer facilitates optimum DNA interactions and this may very well involve the intercalation of the naphthalene core and the winding around of the naphthalimides termini into the helix's grooves. The propyl spacer may be too short and the hexyl spacer may be too long to allow for this optimisation

While the PheAla and LeuAla families typically display binding constants of $K = 10^4 - 10^6 \text{ M}^{-1}$, the Cbz(Lys)Ala family exhibit binding constants of $K = 10^4 - 10^5 \text{ M}^{-1}$. This difference may be due to the bulk of the functional groups on the naphthalimide termini which may hamper binding of these units and may be important for effective binding with the DNA. The smaller the functional groups the stronger the binding, *i.e.* LeuAla $K >$ PheAla $K >$ Cbz(Lys)Ala K .

The largest binding constant $K = 5.7 \times 10^6 \text{ M}^{-1}$ which was calculated for **96** binding to poly(dA-dT)poly(dA-dT) is considerable and compares favourably with binding studies of mononaphthalimides conducted by Hussey.¹⁰⁵ The typical binding constant K deduced was of the order of $K = 10^3 - 10^4 \text{ M}^{-1}$. This stronger binding of the bisnaphthalimides may the result of a complicated, co-operative mode of binding that has been hypothesised previously.

It would be interesting to see whether these trends are reflected in the biological activity of there families when tested against different cancer cell lines.

3.4 Investigating the Interaction of ‘Head-to-Head’ Bisnaphthalimides with DNA by Ground State and Excited State Spectroscopy

3.4.1 Introduction

Comparable studies were conducted for the ‘head-to-head’ family of bisnaphthalimides in an analogous way to those detailed for the ‘tail-to-tail’ bisnaphthalimides. However, these titrations were restricted to those with *ct*-DNA since these compounds were found to interact more weakly with DNA than the ‘tail-to-tail’ bisnaphthalimides. Compounds **117**, **122**, **128**, **130** and **131** were all studied. Unfortunately, **129** was excluded from these investigations since it precipitated from the 2 : 98 DMSO : phosphate buffer (10 mM, pH 7) solution.

3.4.2 DNA Titrations of Bisnaphthalimides with Naphthalene Diimide Spacers

The behaviour of the panel of ‘head-to-head’ bisnaphthalimides upon successive additions of *ct*-DNA followed the same pattern as observed for the ‘tail-to-tail’ systems in *Section 3.4*. The absorbance spectra of these compounds display a λ_{max} *ca.* 350 nm and this is due to the superimposition of the diimide band at 355 nm and the 4-nitro-1,8-naphthalimide band at 345 nm. The emission spectra resulting from excitation at this wavelength had λ_{max} at *ca.* 470 nm. The absorbance and the emission bands underwent hypochromicity over the course of the titrations. However, the degree of this hypochromicity was not comparable with that seen previously for ‘tail-to-tail’ bisnaphthalimides.

Compound **117**, a PheLys ‘head-to-head’ bisnaphthalimide featuring a naphthalene diimide core and a bridge provided by the lysine chain, was titrated with *ct*-DNA. A hypochromicity of 9 % was witnessed over the course of the titration, plateauing at P/D = 150, **Figure 3.26**. There was no spectral shift from its initial position at *ca.* 355 nm. The emission spectrum, with a λ_{max} at *ca.* 405 nm, was decreased by 20 %, **Figure 3.27**. This titration data was then used to explore the binding behaviour *via* the usual parameters. The intrinsic binding plot, **Figure 3.28**, allowed a binding constant of $K = 6.5 \times 10^4 \text{ M}^{-1}$ to be calculated. **Table 3.11** summarises the titration data and binding evaluation for these bisnaphthalimides.

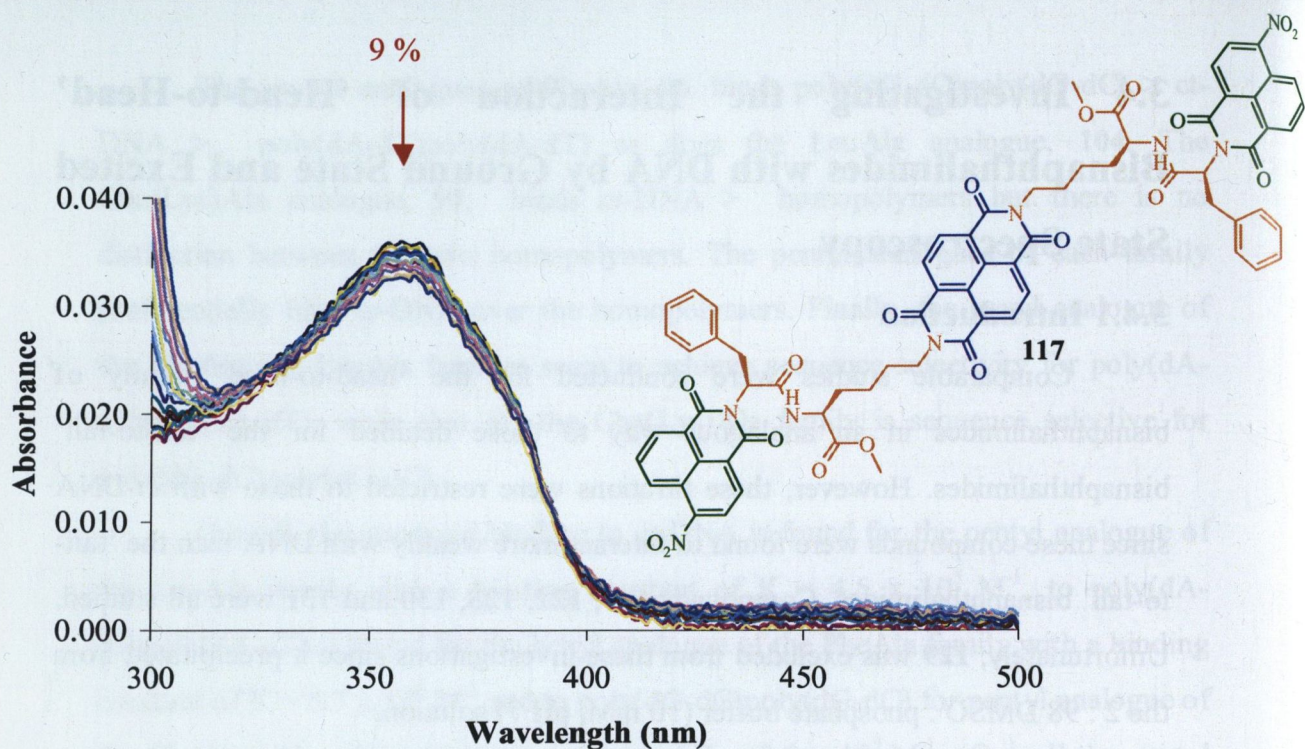


Figure 3.26: Absorption spectrum of titration of **117** (ca. 1.45 μM) with increasing concentration of *ct*-DNA (0–218 μM , P/D = 0–150) in 2:98 DMSO:phosphate buffer

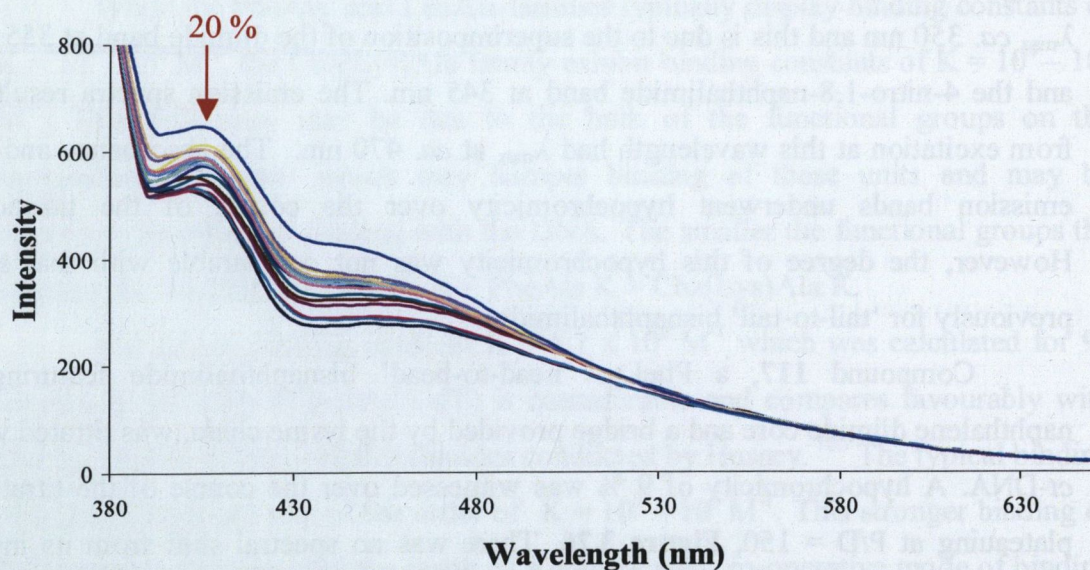


Figure 3.27: Emission spectrum of titration of **117** (ca. 1.45 μM) with increasing concentration of *ct*-DNA (0–218 μM , P/D = 0–150) in 2:98 DMSO:phosphate buffer

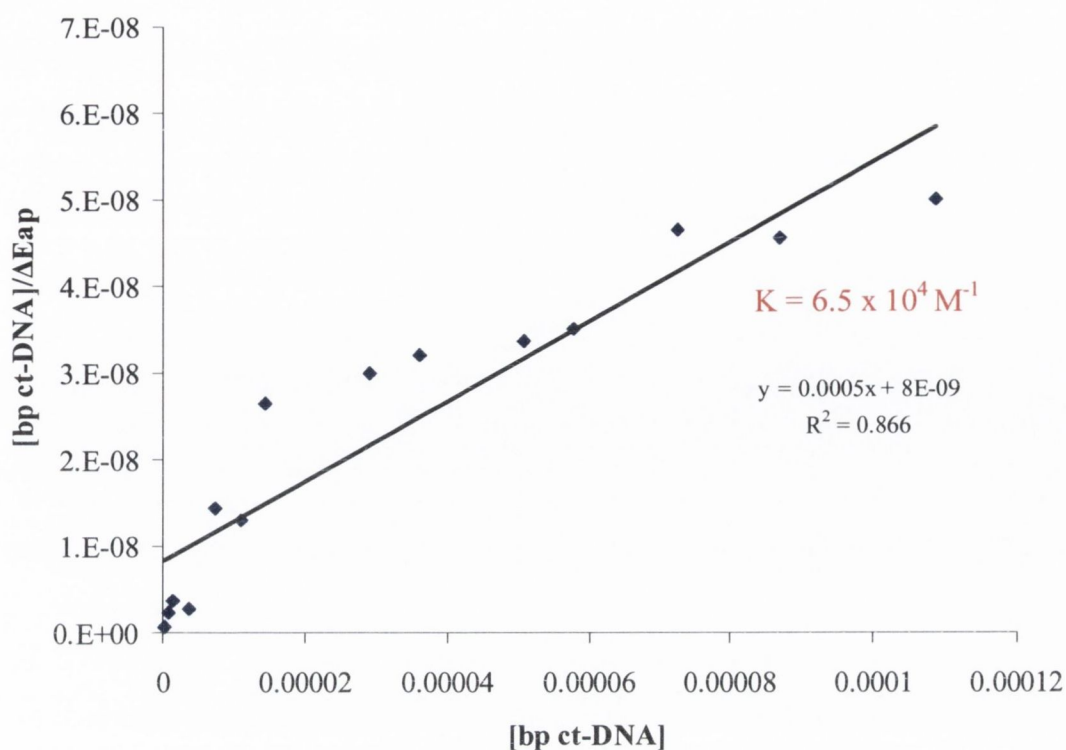


Figure 3.28: Intrinsic binding curve of **117** vs. *ct*-DNA from absorbance at 355 nm

Table 3.11: Summary of Titration Data for ‘Head-to-Head’ Bisnaphthalimides

Compound	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex 355 nm}}$	Final P/D Ratio	$K \text{ (M}^{-1}\text{)}$	R^2
117	9 %	20 %	150	6.5×10^4	0.8660
122	8 %	27 %	20	1.5×10^4	0.9879
128	11 %	44 %	30	1.7×10^5	0.9994

Compound **122**, the PheOrn ‘head-to-head’ bisnaphthalimide analogue was similarly studied, using a $4 \times 10^{-5} \text{ M}$ solution. Hypochromicity of 8 % was achieved before plateauing at $\text{P/D} = 20$. There was no shift in the λ_{max} from its initial position at 350 nm. This intrinsic binding plot permitted a binding constant of $K = 1.5 \times 10^4 \text{ M}^{-1}$ to be calculated. The emission spectrum, with a λ_{max} at 470 nm, was decreased by 27 %. **Table 3.11** summarises the titration data and resulting binding evaluation. The plateau P/D ratio is very low and suggests that binding kinetics are fast by comparison with **117**.

Compound **128**, the PheBut ‘head-to-head’ bisnaphthalimide analogue was similarly studied, using a $6.30 \times 10^{-5} \text{ M}$ solution. A decrease in intensity of 11 % was

observed, plateauing at $P/D = 30$. There was no shift in the λ_{max} at *ca.* 350 nm and no isosbestic point was seen. The intrinsic binding plot provided a binding constant of $K = 1.7 \times 10^5 \text{ M}^{-1}$. The emission spectrum, with a λ_{max} at *ca.* 470 nm, was quenched by 44 %. **Table 3.11** summarises the titration data and resulting binding evaluation. The P/D ratio being greater than that of **122** and smaller than that of **117** suggests intermediate binding kinetics.

The small changes witnessed in the absorbance spectra coupled with the significant decreases in the emission spectra for these compounds might suggest that some form of photoelectron transfer is occurring.

These ‘head-to-head’ bisnaphthalimides were not titrated against homopolymers, merely a comparison with the binding to *ct*-DNA achieved by ‘tail-to-tail’ bisnaphthalimides was desired. These binding constants compare favourably with those of $K = 10^4 - 10^6 \text{ M}^{-1}$ calculated in *Section 3.4*. Of interest is the fact that smaller hypochromicity is witnessed in the absorbance spectra than seen for the ‘tail-to-tail’ bisnaphthalimides, although for **122** and **128** the plateau is reached by $P/D = 20 - 30$ suggesting stronger interaction or faster kinetics of interaction than for **117**. The decreases in the emission spectra are considerable but since this is a combination of the diimide and 4-nitro-1,8-naphthalimide bands it is not possible to suggest whether both regions of the compound interact with the DNA and whether a dual mode of binding is in operation or whether the 4-nitro-1,8-naphthalimide units alone bind. However, the fact that the shorter butyric acid analogue, with the shorter spacer and shorter overall span of the compound, has the largest binding constant of $K = 1.7 \times 10^5 \text{ M}^{-1}$. This is probably too short to allow simultaneous intercalation of the naphthalene core and the winding around of the termini to intercalate or groove bind. This may mean that the core itself merely intercalates or that both or one of the 4-nitro-1,8-naphthalimide units intercalate or groove bind. Just as the hexyl spacer in the ‘tail-to-tail’ bisnaphthalimides was too long for dual binding this is probably too short for a dual binding mode.

3.4.3 DNA Titrations of Bisnaphthalimides with Carboxyl Linkers

The behaviour of the dicarboxyl linked bisnaphthalimides, **130** and **131**, upon successive additions of *ct*-DNA followed the same trend as observed for the ‘tail-to-tail’ systems. The absorbance spectra of these compounds display a λ_{max} *ca.* 350 nm assigned to the 4-nitro-1,8-naphthalimide band. The emission spectra resulting from excitation at this maximum gave a λ_{max} at *ca.* 470 nm. The absorbance and the

emission bands both underwent hypochromicity over the course of the titrations. However, the degree of these changes was not comparable with that seen previously for the ‘tail-to-tail’ bisnaphthalimides. The PheLys dicarboxyl linked bisnaphthalimide, **129**, could not be studied in this manner since it precipitated from the 2 : 98 DMSO : phosphate buffer solution.

A solution of **130**, the PheOrn ‘head-to-head’ bisnaphthalimide featuring a dicarboxyl core and a bridge provided by the ornithine chain, 2.80 μM , was titrated with *ct*-DNA. A hypochromicity of 8 % was witnessed over the course of the titration, plateauing at P/D = 150. There was no spectral shift in λ_{max} from its initial position at 350 nm. The high P/D may be indicative of slow interaction kinetics. The emission spectrum, with λ_{max} at *ca.* 470 nm, was diminished by 6 %. **Table 3.12** summarises the titration data and resulting binding evaluation. The intrinsic binding plot, **Figure 3.30**, gives a binding constant of $K = 1.3 \times 10^4 \text{ M}^{-1}$ was calculated for binding to *ct*-DNA.

Table 3.12: Summary of Titration Data for Carboxyl Linked Bisnaphthalimides

Compound	$\Delta_{\text{Absorbance}}$	Δ_{Emission} $\lambda_{\text{ex 355 nm}}$	Final P/D Ratio	$K \text{ (M}^{-1}\text{)}$	R^2
130	8 %	6 %	150	1.3×10^4	0.9076
131	21 %	16 %	150	5.00×10^3	0.8229

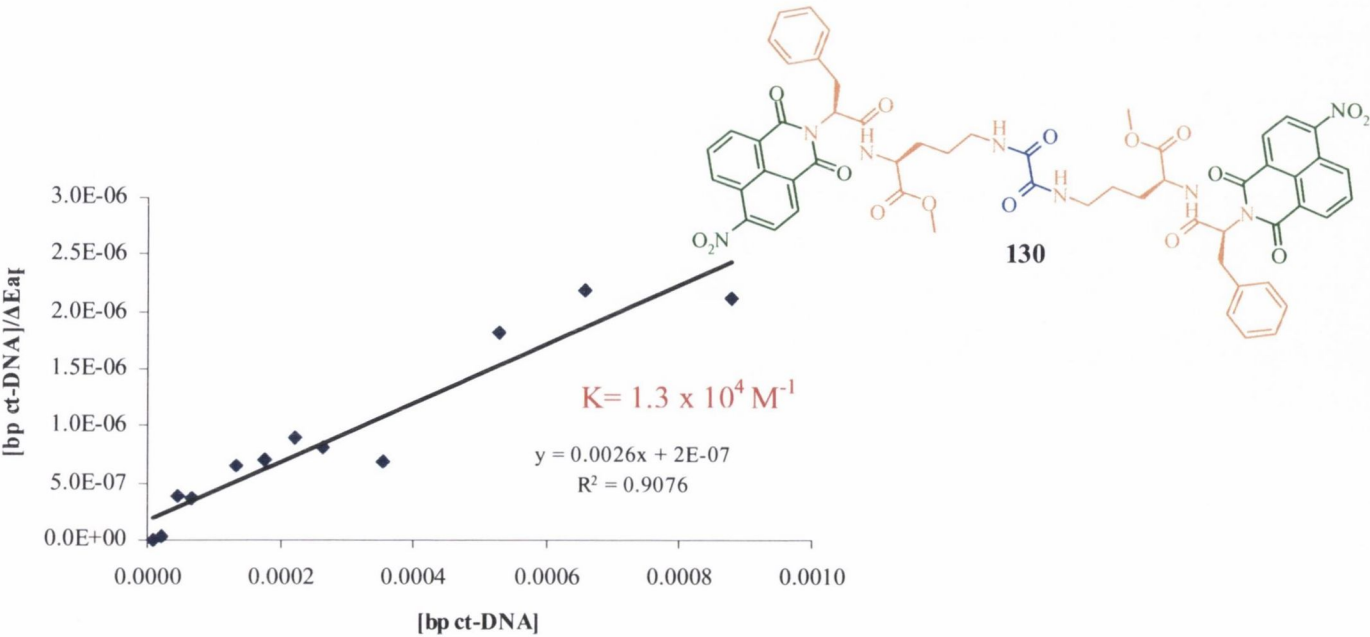


Figure 3.30: Intrinsic binding curve of **130** vs. *ct*-DNA from absorbance at 355 nm

Compound **131**, the PheBut analogue was also titrated with *ct*-DNA. A hypochromicity of 21 % was observed over the course of the titration, plateauing at $P/D = 150$. There was no shift in the λ_{max} from its initial position at 350 nm, although an isosbestic point can be seen at 330 nm. The emission spectrum, with a λ_{max} at 470 nm, was decreased by 16 % and an isosbestic point is found at 452 nm. The intrinsic binding plot gave a binding constant of $K = 5.0 \times 10^3 \text{ M}^{-1}$.

These dicarboxyl linked ‘tail-to-tail’ bisnaphthalimides interact with *ct*-DNA though the lower binding constants, K , of the order of $10^3 - 10^4 \text{ M}^{-1}$, reflect a weaker binding mode than the naphthalene diimide bridged analogues, as one might expect. Since these two compounds have quite short linker lengths it is unlikely that both naphthalimide termini intercalate. It is possible that one intercalates while the other groove binds or that merely one unit intercalates or binds.

3.5 Investigating the Interaction of Bisnaphthalimides with DNA by Circular Dichroism

3.5.1 Introduction

A chiral molecule will preferentially absorb either left-handed or right-handed circularly polarised light depending upon its stereochemistry. Circular dichroism (CD) is the difference in absorption of left-handed and right-handed circularly polarised light, **Figure 3.31**,¹³² measuring this difference in absorption over the ultraviolet spectrum yields a plot characteristic of the chiral nature of a compound.¹¹² If another molecule binds with this chiral molecule, causing changes in its immediate environment, the absorption of circularly polarised light may change and effect the resulting circular dichroism spectrum. By the same token, an achiral molecule that binds with a chiral molecule can be endowed with an intrinsic chirality and this yields an individual intrinsic circular dichroism plot.¹¹²

Circular dichroism is very sensitive to structural changes and is widely employed when probing interactions that might alter the conformational environment. When the circular dichroism spectrum of DNA is measured an induced circular dichroism signal results from the transitions between the purine bases (negative, minimum at 240 nm) and between the pyrimidine bases (positive, maximum at 275 nm). When another moiety intercalates or binds with the DNA the circular dichroism spectrum is disrupted as the helix is perturbed. Typically, when a molecule

intercalates with its long axis parallel to the long axes of the base pairs it causes a negative shift in a circular dichroism spectrum, whilst one that intercalates perpendicularly results in a positive shift.¹¹²

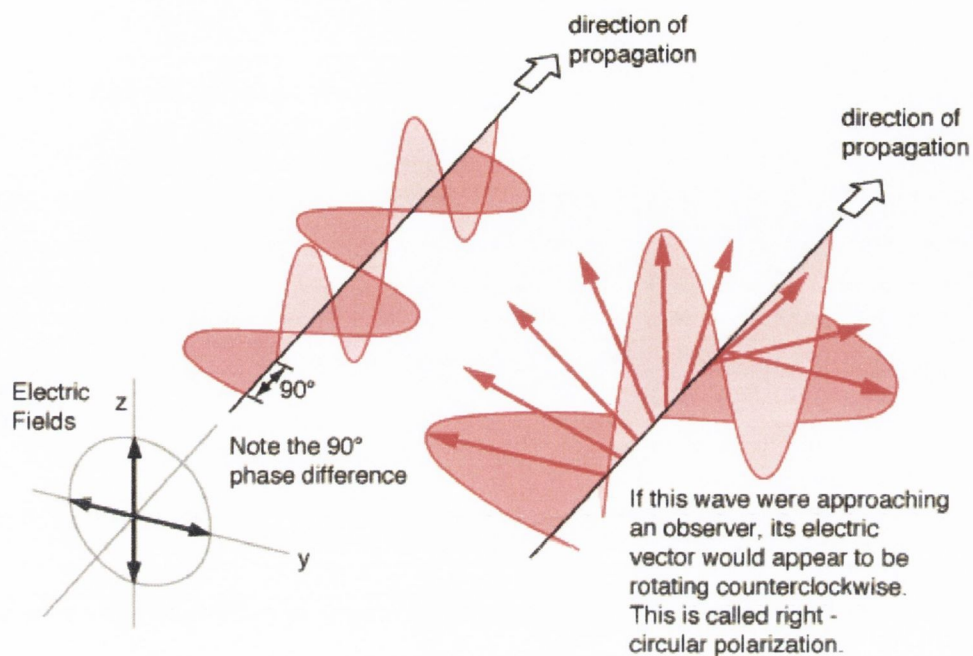


Figure 3.31: Circular Polarisation of Light¹³²

A series of studies were conducted in an effort to probe the interaction between a panel of bisnaphthalimides detailed in *Chapter Two* and *ct*-DNA. Initially, a background spectrum of 10mM phosphate buffer solution and the spectrum of *ct*-DNA (10^4 M) in buffered solution were recorded. Since the total volume of the circular dichroism spectrometer cuvette was 1 mL, individual *ct*-DNA-bisnaphthalimide solutions with decreasing P/D ratios *i.e.* increasing bisnaphthalimide concentration, and constant volume were prepared and their spectra recorded, instead of introducing aliquots to the original *ct*-DNA solution. The nine 'tail-to-tail' bisnaphthalimides were investigated using P/D ratios of 100, 50, 25, 10 and 5. Finally, for the 'head-to-head' bisnaphthalimides, **117**, **122**, **128**, **130** and **131**, P/D ratios of 100, 50, 25, 10 and 5 were also used. This section details the results obtained from these spectroscopic studies.

3.5.2 CD Behaviour of *ct*-DNA with 'Tail-to-Tail' Bisnaphthalimides

The CD studies of all the 'tail-to-tail' bisnaphthalimides were recorded. The circular dichroism spectra for the pentyl bridged bisnaphthalimide of the PheAla family, **97**, is shown in **Figures 3.32** and **3.33**, as exemplary of the data obtained.

Although each scan was conducted to 600nm the expected chromophore signal at 355 nm was not observed.

Typically, the CD signal changes by less than 5 millidegrees from the signal of the *ct*-DNA signal to that of the various P/D ratios of the bisnaphthalimide and *ct*-DNA. These are minor changes and this shift was usually positive, although a negative shift was observed for **96** and no shift was seen for **105**. In all cases, there is little perturbation of the *ct*-DNA signature between 230 nm and 300 nm. Unfortunately, these changes are not significant enough to deduce anything definite about the nature of the binding between these compounds and DNA although such small shifts have been displayed by intercalators in the literature.

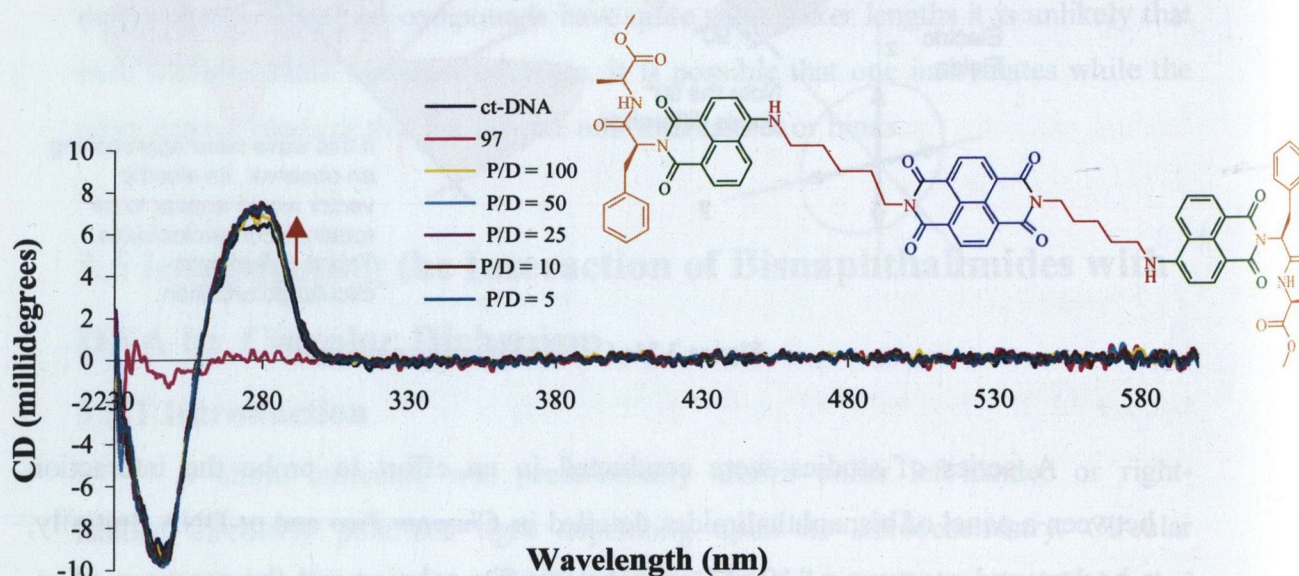


Figure 3.32: CD study of compound 97 with *ct*-DNA

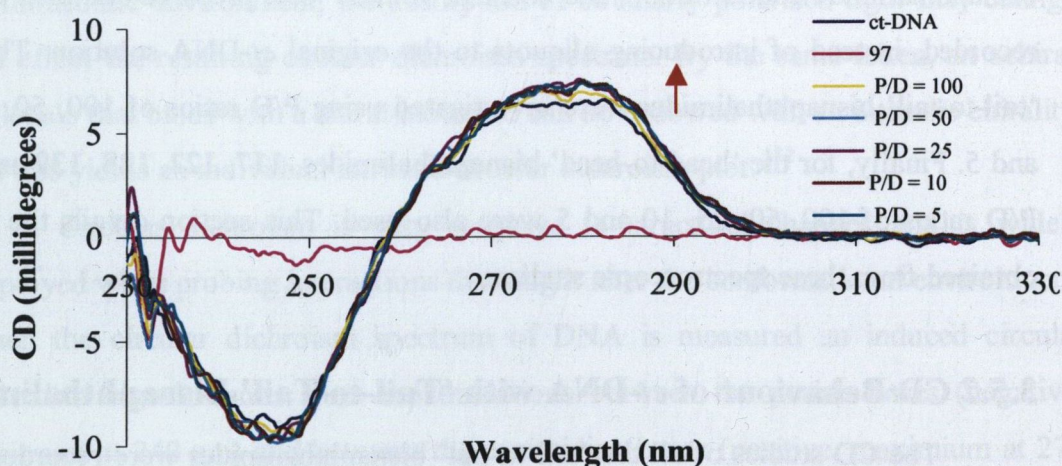


Figure 3.33: Detail of CD study of 97 with *ct*-DNA

3.5.3 CD Behaviour of *ct*-DNA with ‘Head-to-Head’ Bisnaphthalimides.

Similarly minor changes were observed during the studies of the naphthalene diimide, **117**, **122** and **128**, and dicarboxyl, **130** and **131**, linked bisnaphthalimides. The spectra for **117** and **130** are provided in **Figures 3.34** and **Figure 3.35**, respectively, as examples of the typical behaviour observed. As for the ‘tail-to-tail’ bisnaphthalimides, the changes that occurred over the course of the studies were minor and for each compound these were negative shifts in the CD signal. This behaviour of *ct*-DNA in the presence of these novel ‘head-to-head’ bisnaphthalimides is not considerable enough to infer the nature of the interaction that occurs.

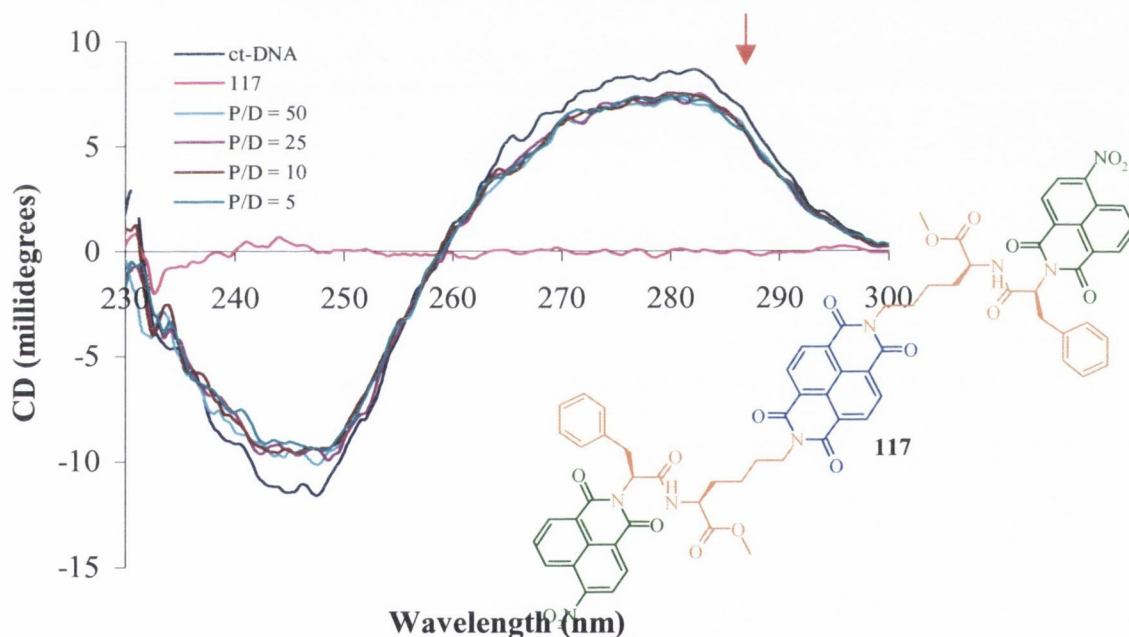
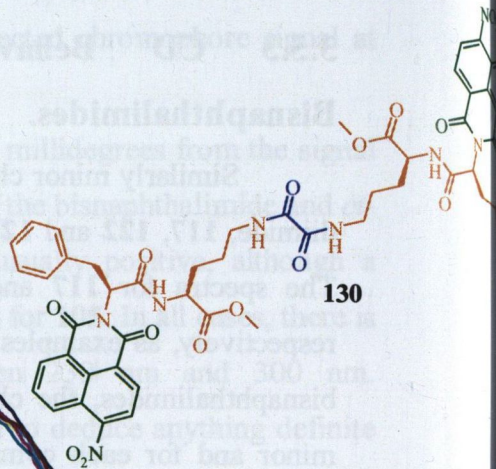


Figure 3.34: CD study of **117** with *ct*-DNA



3.6 Investigating the Interaction of Bisnaphthalimides with DNA by Thermal Denaturation Studies

As well as studying the interactions between a compound and DNA by observing changes in photophysical behaviour, one can also monitor changes in physical characteristics. The temperature at which 50 % of double helical DNA separates into two strands is known as the thermal denaturation temperature, T_m . B-form DNA typically has a T_m of *ca.* 70 ° C, the double stranded helix is denatured and degrades into single strands and smaller fragments.^{111, 113} However, should a molecule bind with the helix it may stabilise or destabilise the helix macromolecule. Stabilisation makes the DNA more resistant to thermal denaturation and a higher temperature is required to degrade the DNA. Destabilisation has the opposite effect, and the helix fragments at low temperatures. Intercalators tend to stabilise the helix

and, therefore, DNA:intercalator complexes display thermal denaturation temperatures, T_m , greater than *ca.* 70 °C. Groove binders may increase or decrease the T_m but these changes are smaller than those observed for intercalators.

3.6.2 Results of Thermal Denaturation Studies of *ct*-DNA with ‘Tail-to-Tail’ Bisnaphthalimides

A series of thermal denaturation studies were conducted using P/D = 10 of millimolar buffered solutions of *ct*-DNA and the ‘tail-to-tail’ bisnaphthalimides, **96**, **97**, **98**, **99**, **100**, **101**, **104**, **105** and **106**. A dual beam Varian UV-visible spectrometer and 1 mL quartz cuvettes were used. The absorbance of DNA at 260 nm was set at *ca.* 1 and this was monitored as the temperature was ramped from 20 °C to 90 °C and reversed back to 20 °C at a rate of 2 °C per minute. The resulting melting curve was compared to that of the *ct*-DNA solution alone and the point of inflection was taken as the thermal denaturation temperature, T_m .

The thermal denaturation curves for the PheAla family of compounds at P/D = 10 are shown in **Figure 3.38** as exemplary T_m graphs. Whereas the *ct*-DNA sample alone has a melting point of 70.02 °C, solutions of *ct*-DNA with **96**, **97** and **98** have T_m values of 69.22 °C, 69.10 °C and 70.02 °C, respectively. The pentyl analogue has the greatest effect, with a $\Delta T_m = 0.92$ °C. While these changes in T_m are minor it does appear that **96** and **97** do interact with DNA to such an extent that they effect the T_m .

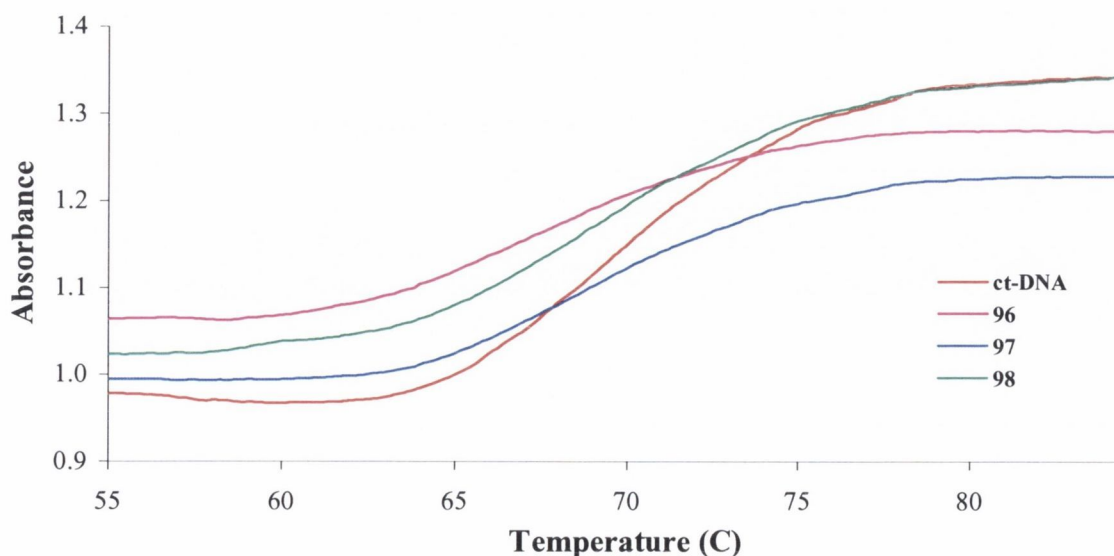


Figure 3.38: Thermal Denaturation Curves of *ct*-DNA and PheAla Bisnaphthalimides at P/D = 10

The other bisnaphthalimides studied behaved in a similar manner, not greatly impacting on the original T_m of the *ct*-DNA sample, these studies are summarised in **Table 3.13**. In the CbzLysAla family, the propyl analogue, **99**, gives a $\Delta T_m = -0.20$ °C and as for the PheAla family the pentyl analogue, **100**, has the greatest effect, producing a $\Delta T_m = -0.42$ °C. The hexyl analogue, **101**, does not affect the T_m . However, within the LeuAla family, the propyl analogue, **104** does not affect the T_m . Compounds **105** and **106** both produce a $\Delta T_m \approx \pm 0.20$ °C.

Table 3.13: Summary of thermal denaturation values obtained for studies of ‘tail-to-tail’ bisnaphthalimides

Compound	$T_m \pm 0.05$ °C
<i>ct</i> -DNA	70.02 °C
96	69.22 °C
97	69.10 °C
98	70.02 °C
99	69.82 °C
100	69.60 °C
101	70.02 °C
104	70.02 °C
105	70.21 °C
106	69.82 °C

The ΔT_m observed during these studies are not large enough to confirm that intercalation is at work. It may be that groove binding is occurring since smaller ΔT_m tends to suggest subtle interactions. These increases and decreases in T_m suggest that the double helix is being stabilised and destabilised by the different bisnaphthalimides.

The pentyl analogues seem to have the greatest effects upon the T_m and they may interact to a greater degree with the DNA helix than the other analogues, this reflects the binding constants observed during the DNA titrations discussed in *Section 3.4*. In the PheAla and Cbz(Lys)Ala families the propyl, **96** and **99**, and pentyl, **97** and **100**, linked compounds decrease the T_m , *i.e.* their interaction destabilises the DNA helix and denaturation occurs at lower temperatures. The hexyl linked compounds, **98** and **101**, do not effect the T_m and may not interact

effectly with the DNA. This also mirrors the trends seen in the DNA binding studies, the hexyl linked analogues bind more weakly to DNA.

The LeuAla family behaves somewhat differently in that the propyl linked version, **104**, does not affect the T_m , whilst the other two analogues have the same magnitude of ΔT_m . The pentyl linked analogue, **105**, actually increasing the T_m , *i.e.* stabilising the DNA helix, none of the other bisnaphthalimides displays this behaviour. The hexyl linked compound, **106**, decreases the T_m where the other families with this spacer length had no thermal denaturation effect. It may be that this family of compounds operate in a different manner to the former two families.

3.6.3 Results of Thermal Denaturation Studies of *ct*-DNA with ‘Head-to-Head’ Bisnaphthalimides

The thermal denaturation curves for the five ‘head-to-head’ bisnaphthalimides, **117**, **122**, **128**, **130** and **131**, are shown in **Figure 3.39** and these results are summarised in **Table 3.14**. Of the naphthalene diimide linked systems, **117**, **122** and **128**, the lysine bridged analogue, **117**, has the greatest destabilising effect on the *ct*-DNA, possibly by intercalation. The ornithine bridged version, **122**, has the next greatest destabilising effect, whilst the 2,4-diamino butyric acid bridged compound, **128**, does not effect the T_m . This trend suggests that progressively shortening the bridge decreases the degree of the ΔT_m . This does not agree with the DNA binding studies and binding constants observed in *Section 3.5*.

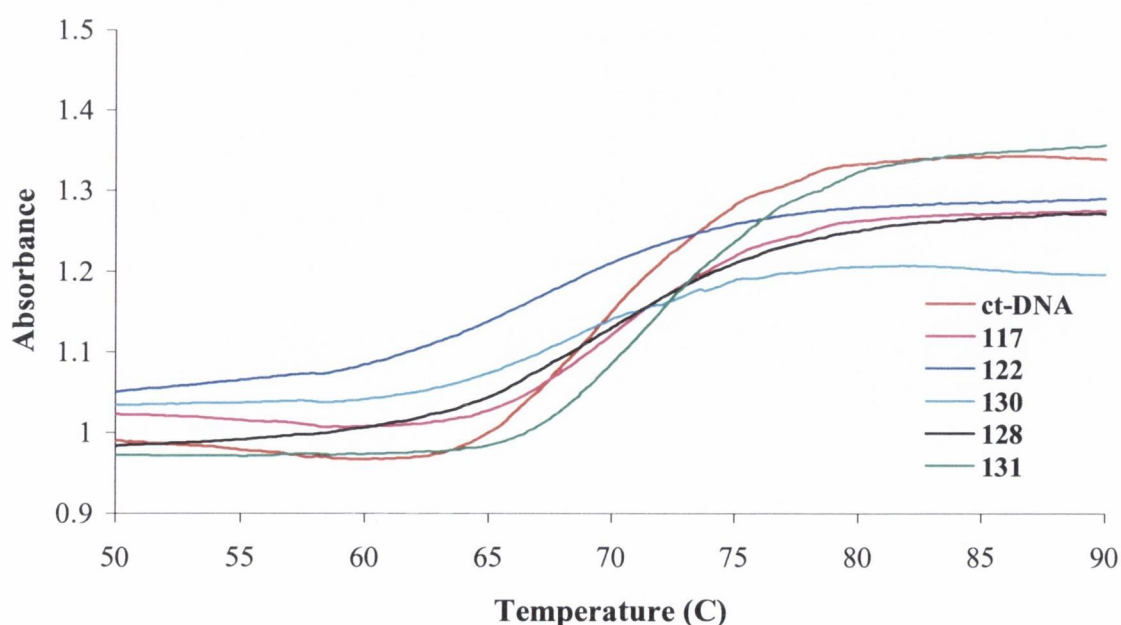


Figure 3.39: Thermal Denaturation Curves of ‘Head-to-Head’ Bisnaphthalimides at P/D = 10

The two dicarboxyl linked bisnaphthalimides effect the thermal denaturation of the *ct*-DNA. Compounds **130**, the ornithine analogue gives a $\Delta T_m = - 0.61$, *i.e.* it destabilises the DNA helix, whilst **131**, the 2,4-diamino butyric acid analogue gives a $\Delta T_m = + 0.81$, *i.e.* it stabilises the DNA helix. These different effects suggest that these analogues, though only different by one methylene group in the bridging chain, interact with DNA in different fashions. In particular, the magnitude of the ΔT_m produced by **131** might suggest that intercalation is occurring.

Table 3.14: Summary of thermal denaturation values obtained for studies of ‘head-to-head’ bisnaphthalimides

Compound	T_m
<i>ct</i> -DNA	70.02 °C
117	68.22 °C
122	69.22 °C
128	70.02 °C
130	69.41 °C
131	70.83 °C

3.7 Conclusions

This chapter has described the investigations conducted into the photophysical characteristics and behaviour of the novel bisnaphthalimides whose syntheses were detailed in *Chapter Two*. Both the ‘tail-to-tail’ and ‘head-to-head’ structures were examined. Of the latter, those linked by naphthalene diimide and dicarboxyl motifs were studied. A number of techniques were employed in an effort to examine how these systems behave.

A comprehensive solvent study of the ‘tail-to-tail’ bisnaphthalimides and their constituent units was conducted. The observations for 4-nitro-1,8-naphthalimides, 4-amino-1,8-naphthalimides and for the naphthalene diimide spacer systems agreed with those reported in the literature. The bisnaphthalimides exhibit absorption spectra containing a dominant band centred at *ca.* 355 nm and a minor band centred at *ca.* 440 nm. This incorporates the principal characteristics of the

component units, the band at *ca.* 355 nm corresponds to the naphthalene diimide core while the minor band is due to the 1,8-naphthalimide and reflects the pendant amino functionality. These compounds display solvent dependency and increasing polarity causes λ_{max} to be hypsochromically shifted, indicating an $n\pi^*$ character of the first singlet excited state S^1_0 . The emission of each component can be independently examined by exciting at the appropriate λ_{max} and these emission signatures are also solvent dependant. The sensitivity of these compounds to their solvent environment proposed that their spectroscopic behaviour in the presence of DNA might be employed to probe any interactions that may occur.

Each analogue of each family of the ‘tail-to-tail’ bisnaphthalimides was then studied by titration with *ct*-DNA, poly(dA-dT)poly(dA-dT) and poly(dG-dC)poly(dG-dC). These titrations were monitored by absorbance and emission spectroscopy. Intrinsic binding plots from these titrations allowed intrinsic binding constants, K , to be calculated for all but four of the studies, where the interactions were too weak. Several trends were observed as a result of these binding calculations. Most notably, the dipeptide functionality seems to dictate the sequence selectivity of the system and the spacer length tunes the strength of the binding that occurs. For example, the hexyl linked analogue of the LeuAla family, **106**, is selective for poly(dA-dT)poly(dA-dT) and the hexyl linked analogue of the Cbz(Lys)Ala family, **101**, is selective for poly(dG-dC)poly(dG-dC). For the PheAla family altering the spacer from the propyl to the pentyl to the hexyl analogues increases the binding constants, K , for binding to poly(dA-dT)poly(dA-dT) from 10^4 M^{-1} to 10^5 M^{-1} and ultimately to 10^6 M^{-1} . While the PheAla and LeuAla families typically exhibit binding constants of $K = 10^4 - 10^6 \text{ M}^{-1}$, the Cbz(Lys)Ala family display binding constants of $K = 10^4 - 10^5 \text{ M}^{-1}$. This difference may be due to the steric bulk of the functional groups on the naphthalimide termini which might hamper binding of these units and may be crucial for effective binding with DNA. The smaller the functional groups, the stronger the binding, *i.e.* LeuAla $K >$ PheAla $K >$ Cbz(Lys)Ala K . The magnitude of these binding constants compare favourably with those reported by Hussey,¹⁰⁵ showing enhancement over mononaphthalides binding.

Similar studies were conducted for five of the six novel ‘head-to-head’ bisnaphthalimides. The binding constants, K , calculated for the naphthalene diimide linked structures were of the order of $10^4 - 10^5 \text{ M}^{-1}$ and those calculated for the dicarboxyl linked structures were of the order of $10^3 - 10^4 \text{ M}^{-1}$. The former may bind

to DNA in a similar manner to the 'tail-to-tail' bisnaphthalimides, although the 4-nitro substituent would most probably affect the manner of the naphthalimide units binding. The weaker binding deduced for the latter is unsurprising since the dicarboxyl linker does not facilitate a threading intercalating behaviour. These compounds may mono- or bis-intercalate or groove bind.

While these investigations provided information on the existence and strength of interaction between the novel bisnaphthalimides and DNA the nature of this binding is still unclear, although extensive CD and T_m studies were conducted in an effort to probe these interactions they were inconclusive as to whether intercalation and/or groove binding are in operation. Viscometry investigations were also undertaken. Whilst increases in viscosity of *ct*-DNA were observed with increasing equivalents of bisnaphthalimide, no conclusions could be drawn since there was little linearity to these results. Unfortunately, the nature of the bisnaphthalimide : DNA interactions has yet to be elucidated.

Amongst the original aims of this research project was the achievement of some degree of sequence selectivity, the observation of the effect on binding of altering the length of a spacer and the achievement of enhanced binding over mononaphthalimides. This chapter has highlighted the realisation of these objectives. *Chapter Four* will now detail how effective these compounds are as anti-cancer agents and how this reflects their photophysical behaviour in the presence of DNA as described in *Sections 3.4 - 3.5*.



Chapter Four

Biological Investigations



4.1 Introduction

The final aspect of this research project was the biological assessment of the bisnaphthalimides. The design and synthesis of these novel compounds was challenging and the physical exploration revealed much about how they might behave and bind with DNA. The ultimate question lay in whether these systems might display *in vivo* prowess against cancerous cell lines and, whether, there would be any enhancement in the activity of mononaphthalimides. The effects of the different spacer lengths and the varying amino acids functional groups were also of interest.

Biological testing was conducted in collaboration with Prof. Mark Lawler (Department of Haematology, St. James' Hospital, Dublin) and Daniel Frimmansson (School of Chemistry, Trinity College, Dublin). A panel of fifteen bisnaphthalimides were examined and these results were compiled and collated with previously assessed naphthalimides synthesised within the Gunnlaugsson group. In this way general trends in the potency of a range of naphthalimides could be appreciated.^{103, 105}

The panel was first tested against the HL-60 cell line which is a moderately resistant strain of acute promyelocytic leukaemia. This cancer tends to affect children but can be successfully tackled since it is rather susceptible to a range of chemotherapies. This study is therefore a primary indication of the activity of a compound; a novel compound that shows promising potency against this cell line, comparable to that of drugs in clinical trials, is a candidate for further trials. However, if a compound displays limited activity against HL-60 it is unlikely to be effective against harder cancers. The bisnaphthalimides were then examined against K562, a much more chemo-resistant chronic myeloid leukaemia (CML) cell line. These studies would reflect whether these compounds are active against more resilient cancers and suggest whether such bisnaphthalimides are promising anti-cancer agents.

4.2 The MTT Assay

A cell viability evaluation is a standard assay for investigating the effect of a drug or compound on a healthy or cancerous cell line. The MTT assay is a quantitative colourimetric test and is widely employed in preclinical trials. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) is a yellow dye, which is cleaved by viable cells to form water-soluble, purple formazan dye, **Figure 4.1**. In living cells this cleavage is achieved by the mitochondrial enzyme succinate dehydrogenase. This colourimetric assay can be followed and quantified by UV-visible spectroscopy of the

conversion of the tetrazolium salt to the formazen, which absorbs at 595 nm. This absorbance is directly proportional to the amount of purple formazen produced by the sample and this is proportional to the amount of metabolically active cells. The desired effect of the test compound is to decrease the number of living cells (cancerous cells), thus lowering the metabolic rate and generating less formazen. Thus, a sample that is purple due to the presence of formazen possesses metabolically active cells and the compound has not killed many cells whereas a sample that is yellow due to the uncleaved MTT would suggest that most of the cells present are necrotic and that the compound has had an effect.

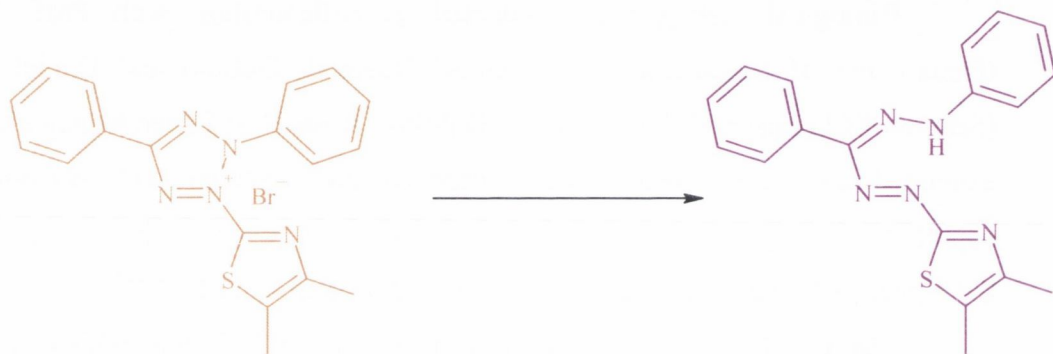


Figure 4.1: Conversion of tetrazolium salt to formazen

The assay is performed by preparing a 5 mM stock solution of the test compound in DMSO. Additions of this solution are made to a multiwell microtitre plate containing the cancerous test cells in a media and nutrient solution where each well contains approximately 6×10^4 cells for HL-60 or 4×10^4 cells for K562. These additions are such that eight different concentrations, 100 μM , 30 μM , 10 μM , 3 μM , 1 μM , 0.3 μM , 0.1 μM , 0.03 μM , of the compound, in triplicate, are employed, so an average viability can be obtained. These samples are then incubated for 24 h, 48 h and 72 h, respectively for the HL-60, K562 and PC3 cell line tests. The MTT dye (10 μL) is then introduced and the samples are incubated for a further 4 h before a solubilisation solution (100 μL) is added to dissolve the formazen crystals into solution and a further overnight incubation is conducted. The plate is placed in an ELISA plate reader and the absorbance of each well at 595 nm is recorded. A software program ‘Prism’ is employed to convert the data obtained into a cell viability graph of dose vs. response from which cell death can be quantified. This quantification is quoted in terms of an IC_{50} value (inhibitory concentration, sometimes known as an EC_{50}), this is the concentration of the compound required to achieve 50 % inhibition of the cancerous cell line.

4.3 Results for ‘Tail-to-Tail’ Bisnaphthalimides Against HL-60 Cell Lines

The three families of ‘tail-to-tail’ bisnaphthalimides were tested against the HL-60 myeloid leukaemia cell line for an incubation period of 24 h. These compounds showed promising activity. The dose vs. response curves for tests are shown in **Figures 4.2 – 4.10** and **Table 4.1** summarises the IC₅₀ evaluations.

The PheAla functionalised family, **96**, **97** and **98**, exhibited IC₅₀ values of 4.30 μ M, 1.70 μ M and 1.25 μ M, respectively, and are depicted in **Figures 4.2 - 4.4**. These results reflect the general trends observed in the binding behaviour studies detailed in *Chapter Three*. The amino-hexyl naphthalene linked species is most potent, followed by the pentyl and propyl analogues.

The Cbz(Lys)Ala functionalised family, **99**, **100** and **101**, displayed activities of infinity (*i.e.* it showed no activity over the incubation period), 3.50 μ M and 1.51 μ M, respectively, and are shown in **Figures 4.5 - 4.7**. This trend of the longer spacer corresponding to enhanced anti-tumour activity does not agree wholly with the binding studies since the propyl linked version does not achieve anti-tumour activity. This may be due to the bulk of the substituents coupled with the shortness of the spacer hampers effective interaction with DNA. This bulky Cbz(Lys)Ala functionalisation at the first substitution position also has an effect on the activity of this family in comparison to the PheAla family. This may be due to the increased steric hindrance of the Cbz(Lys) group restricting the efficiency of the groove binding of the naphthalimide units.

Although **102** and **103**, the hydrolysed versions of **99** and **100**, were not soluble enough for DNA titrations, as a comparative study the former was tested against the HL-60 cell line. This showed an IC₅₀ value of 8.14 μ M, **Figure 4.8**, suggesting that the removal of the Cbz protecting group enhances the potency of the compound. This may simply be due to the reduction in the bulk of the naphthalimide termini or that the Cbz group itself interferes with processes *in vivo*, *e.g.* limiting the transport of **102** across cell walls *etc.*, hampering its anti-cancer activity.

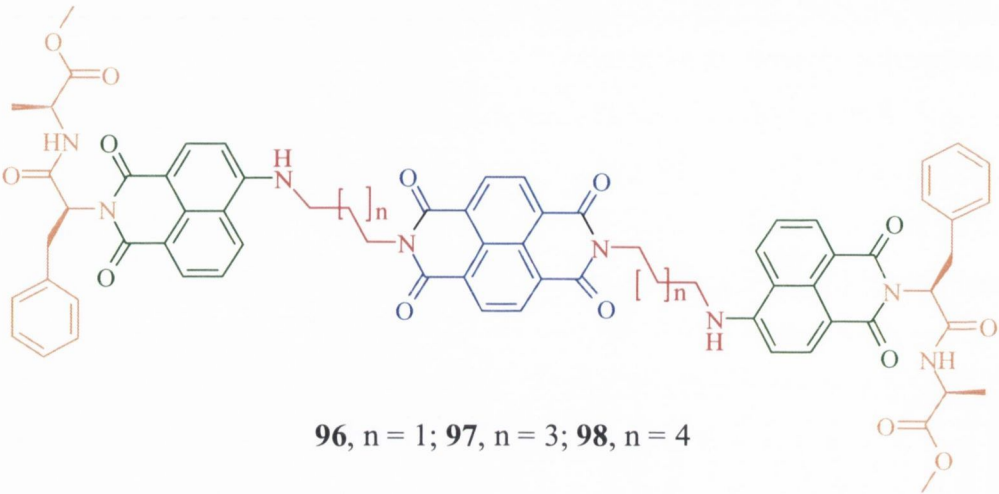
Finally, of the LeuAla functionalised family, compounds **104** and **106**, displayed activities of 3.20 μ M and 0.47 μ M, respectively, and are depicted in **Figures 4.9** and **4.10**. The pentyl linked analogue is currently in testing. These also follow the general trend witnessed for the Phe family and seen during the photophysical investigations. This family is the most potent of the ‘tail-to-tail’ bisnaphthalimides. Thus, another trend may be at work; that the less bulky the amino acid functionalisation, the more active the analogue.

This suggests that the smaller termini interact more effectively and perhaps groove bind more strongly leading the LeuAla family to be more potent than the PheAla family and this in turn to be more potent than the Cbz(Lys)Ala family. The typical reference compound, mitonifide, has an IC_{50} value of $0.23\ \mu\text{M}$ against this cell line and so the values compare very favourably

These trends agree with those seen during the calculations of binding constants, K , in *Chapter Three*, for the interaction of these compounds with *ct*-DNA. The spacer length effects the anti-cancer activity according to the trend hexyl > pentyl > propyl. The dipeptide functionality employed also effects the potency whereby LeuAla > PheAla > Cbz(Lys)Ala.

Table 4.1: Summary of IC_{50} values for ‘tail-to-tail’ bisnaphthalimides in HL-60 cell lines

Compound	Family	Spacer Length	IC_{50}
96	Phe-Ala	Propyl	$4.30\ \mu\text{M}$
97	Phe-Ala	Pentyl	$1.70\ \mu\text{M}$
98	Phe-Ala	Hexyl	$1.25\ \mu\text{M}$
99	Cbz(Lys)Ala	Propyl	$\infty\ \mu\text{M}$
100	Cbz(Lys)Ala	Pentyl	$3.50\ \mu\text{M}$
101	Cbz(Lys)Ala	Hexyl	$1.51\ \mu\text{M}$
102	Lys-Ala	Propyl	$8.14\ \mu\text{M}$
104	Leu-Ala	Propyl	$3.20\ \mu\text{M}$
106	Leu-Ala	Hexyl	$0.47\ \mu\text{M}$



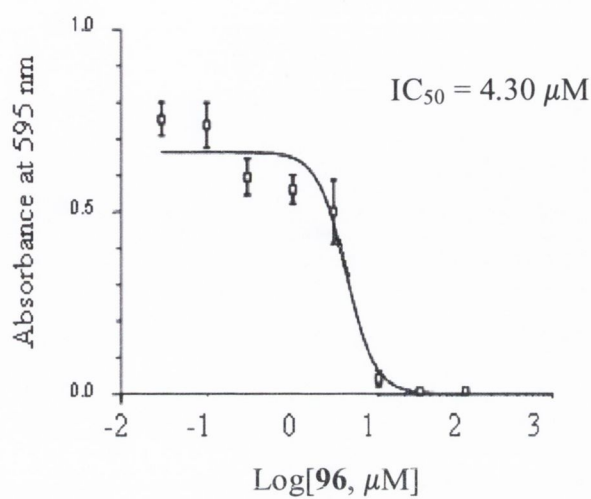


Figure 4.2: Dose vs. response plot of 96 in HL-60 cell line

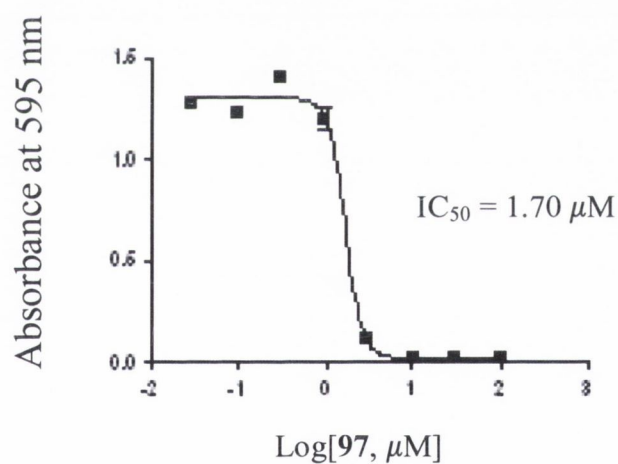


Figure 4.3: Dose vs. response plot of 97 in HL-60 cell line

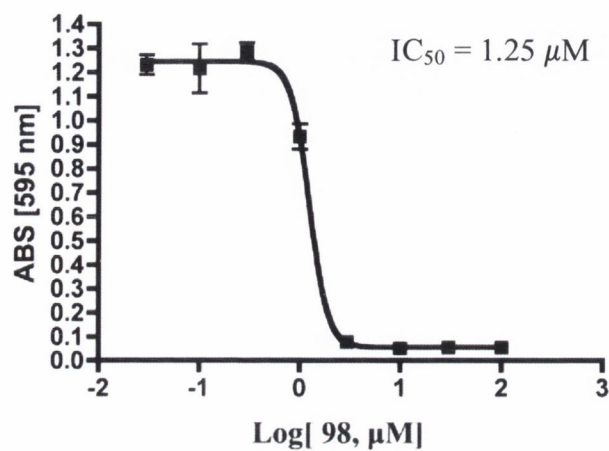


Figure 4.4: Dose vs. response plot of 98 in HL-60 cell line

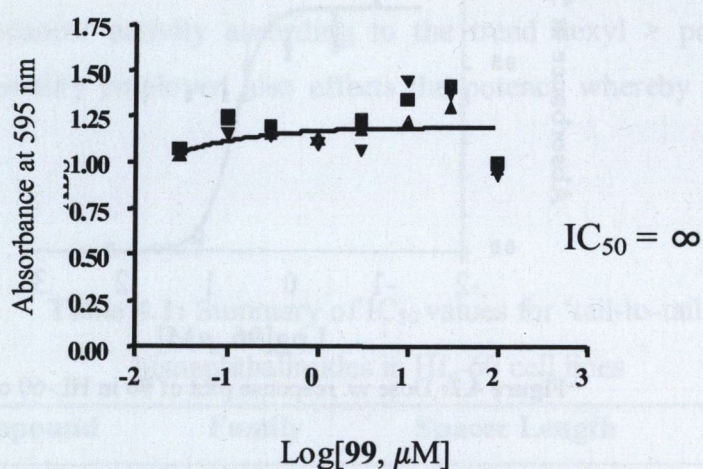
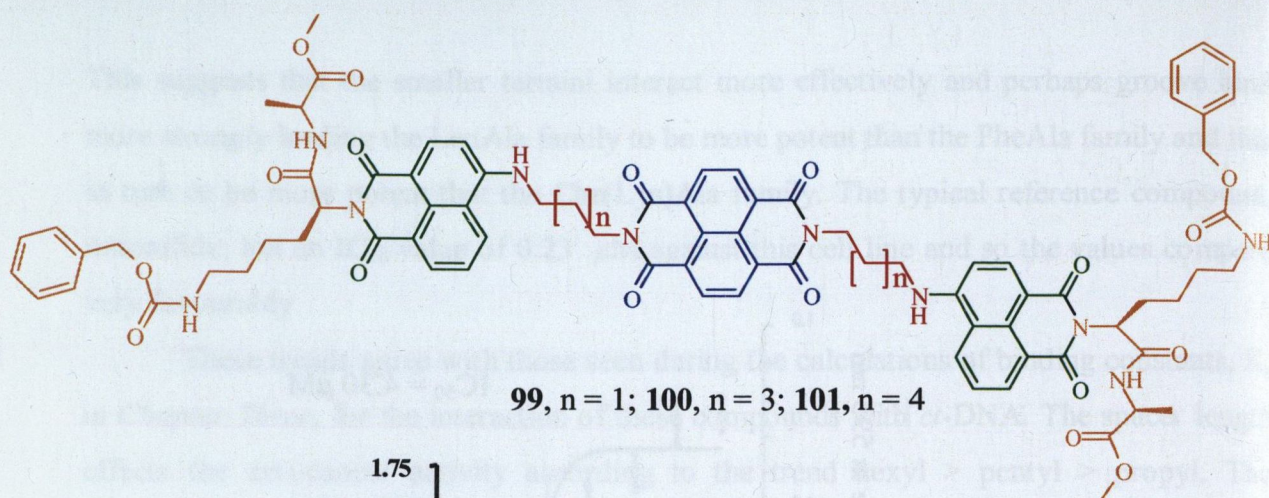


Figure 4.5: Dose vs. response plot of **99** in HL-60 cell line

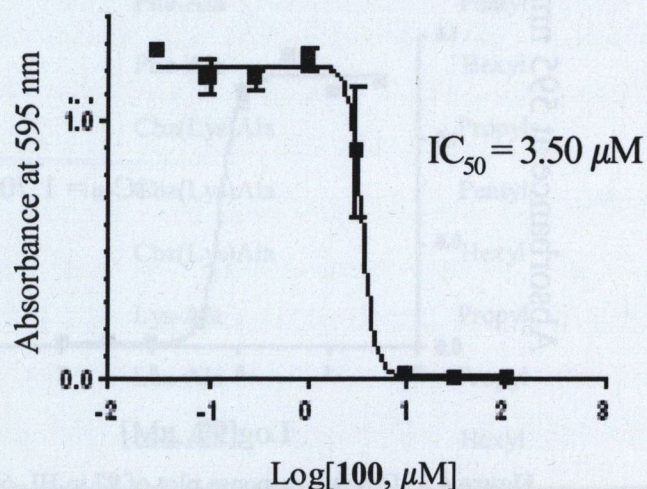


Figure 4.6: Dose vs. response plot of **100** in HL-60 cell line

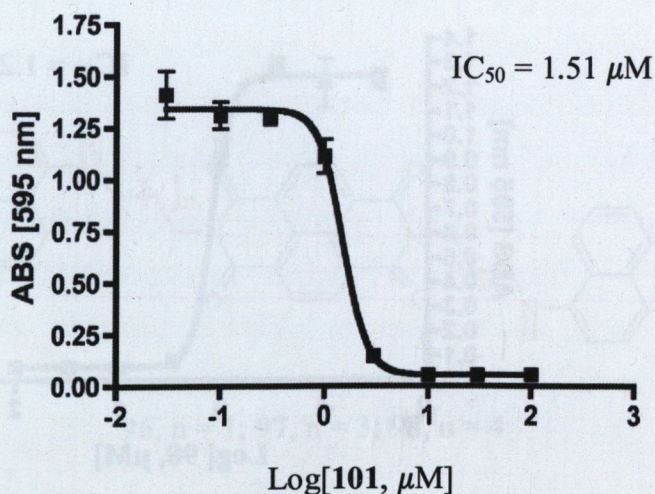


Figure 4.7: Dose vs. response plot of **101** in HL-60 cell

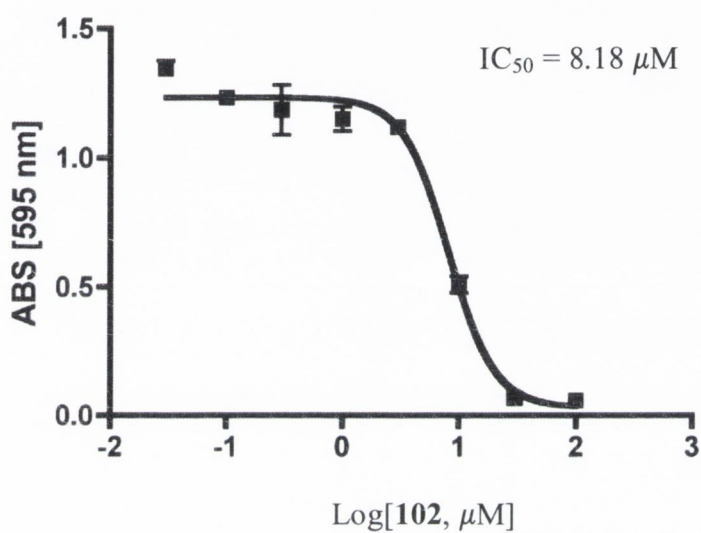
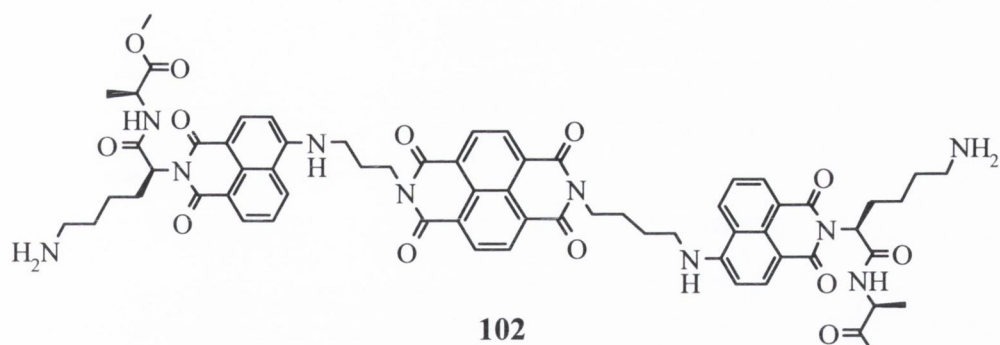
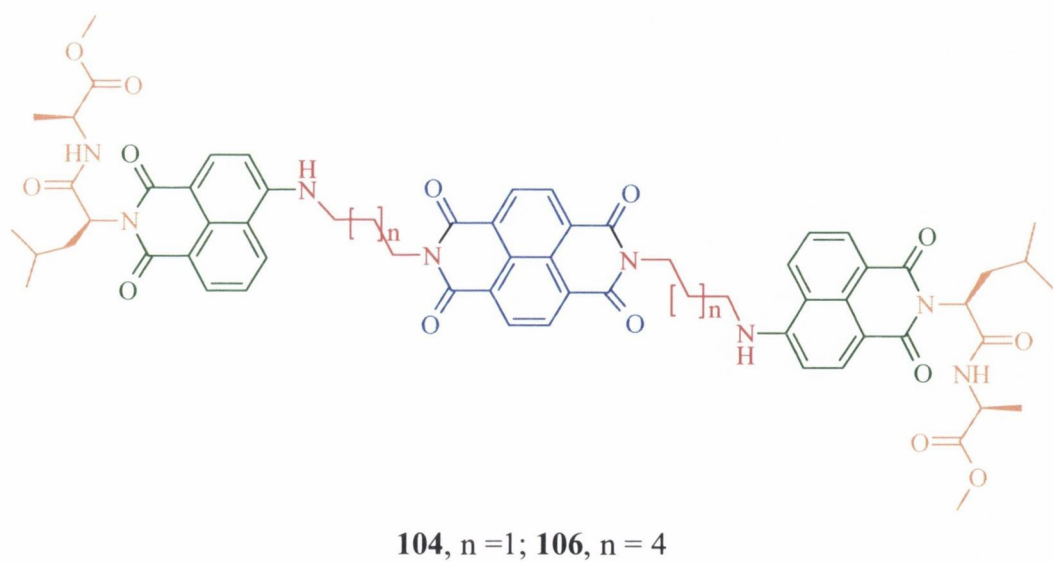


Figure 4.8: Dose vs. response plot of **102** in HL-60 cell



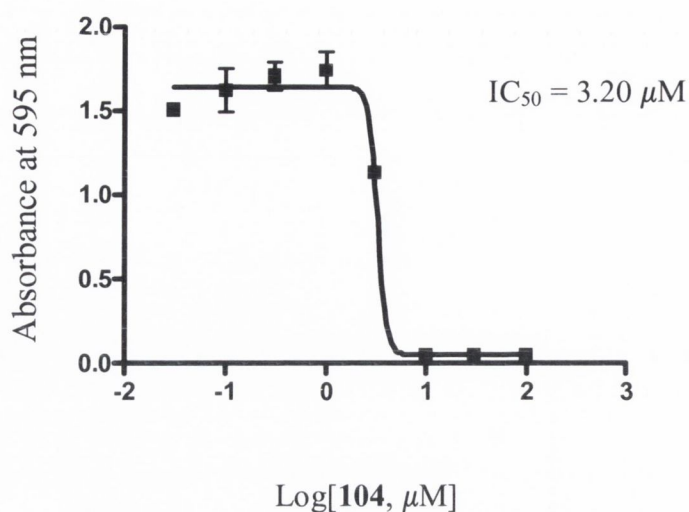


Figure 4.9: Dose vs. response plot of 104 in HL-60 cell

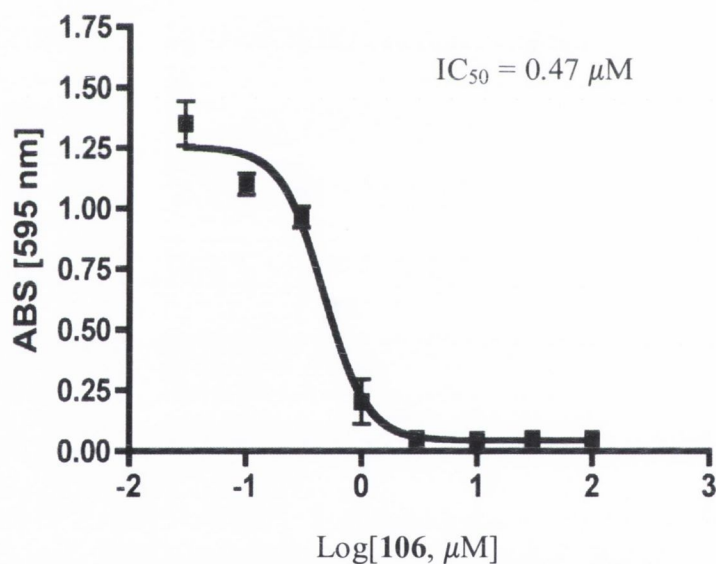


Figure 4.10: Dose vs. response plot of 106 in HL-60 cell

4.4 Results for ‘Head-to-Head’ Bisnaphthalimides Against HL-60 Cell Lines

Both varieties of ‘head-to-head’, naphthalene diimide and dicarboxyl linked, bisnaphthalimides were also tested against HL-60 myeloid leukaemia cell lines for an incubation period of 24 h, The dose vs. response curves for tests are shown in **Figures 4.11**

– **4.14** and **Table 4.2** summarises the IC₅₀ evaluations. While these compounds did show some DNA binding during the photophysical studies their *in vivo* activity is less promising. The naphthalene linked bisnaphthalimides involve lysine, ornithine and butyric acid motifs. The analogue with the longest span, the lysine compound, **117**, **Figure 4.11**, displayed no activity over the incubation period and so is quoted as having an IC₅₀ value of infinity. The ornithine analogue has an IC₅₀ value of 15.00 μ M and seems to imply that shortening the bridge enhances the interaction with the double helix. Finally, the butyric acid analogue also displayed an IC₅₀ value of infinity. This would suggest that the ornithine linker optimising the action of this type of bisnaphthalimide.

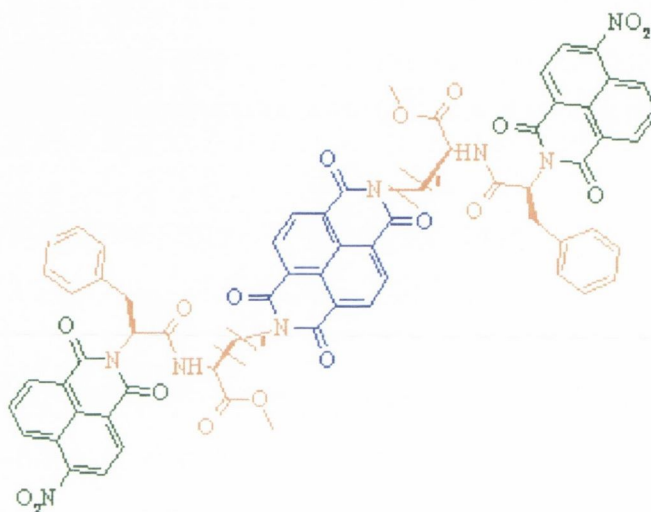
Table 4.2: Summary of IC₅₀ values for ‘head-to-head’
bisnaphthalimides in HL-60 cell lines

Compound	Family	Spacer or Linker	IC ₅₀
117	PheLys	Naphthalene Diimide	∞
122	PheOrn	Naphthalene Diimide	15.00 μ M
128	PheBut	Naphthalene Diimide	∞
129	PheLys	Dicarboxyl	15.00 μ M
130	PheOrn	Dicarboxyl	16.01 μ M
131	PheBut	Dicarboxyl	4.82 μ M

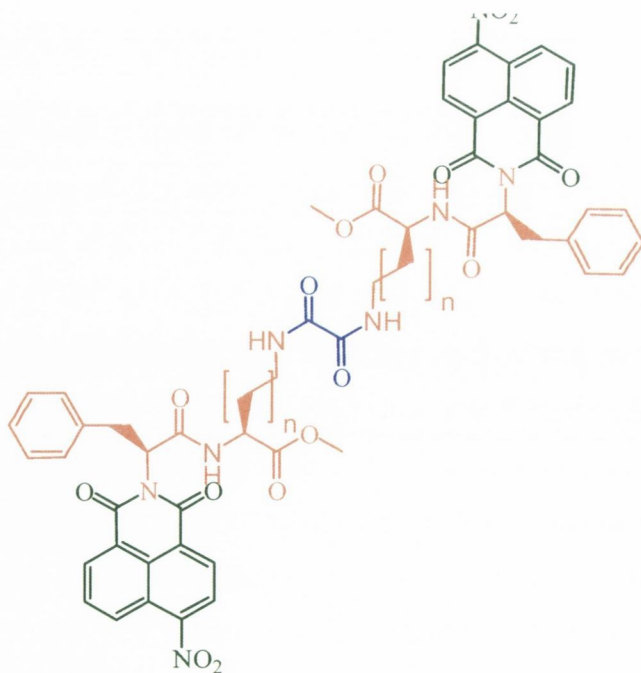
If these compounds behave in the same manner as the ‘tail-to-tail’ systems as hypothesised in *Chapter Three*, it may be that this length of bridge provides span enough for a two-step binding interaction of the naphthalene core intercalating before the naphthalimide termini groove bind. In comparison, the lysine bridge is too long and the termini do not groove bind and the butyric acid bridge is too short too allow for winding around of the termini. The lower potency of these compounds may be attributable to the fact that being ‘head-to-head’ bisnaphthalimides they retain the 4-nitro functionality. In previous evaluation of mononaphthalimides within the Gunnlaugsson group these have typically been found to be more potent than those with 4-amino functionalisations,^{103, 105} in contrast to the trend observed by Braña *et al.*^{29, 32}

The dicarboxyl linked bisnaphthalimides were not found to be very potent against the HL-60 cell lines. This is most likely because a different mode of binding is at work since the intercalative potential of the naphthalene diimide is lacking. The length of the

bridge seems to have a slight effect, here the butyric bridged species is the most successful, with an IC_{50} value of $4.82\ \mu\text{M}$, **Figure 4.14**. It may be that these compounds merely intercalate or groove bind since they have no aromatic core capable of threading through the DNA and intercalating itself. Again, the 4-nitro groups are probably an additional contributing factor to the poor activity observed.



117, $n = 4$; **122**, $n = 3$; **128**, $n = 2$



129, $n = 4$; **130**, $n = 3$; **131**, $n = 2$

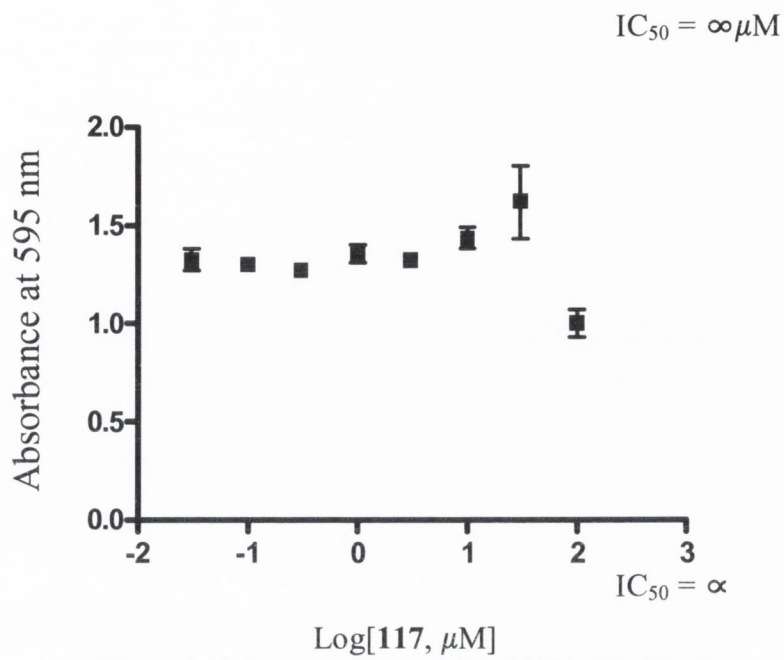


Figure 4.11: Dose vs. response plot of 117 in HL-60 cell

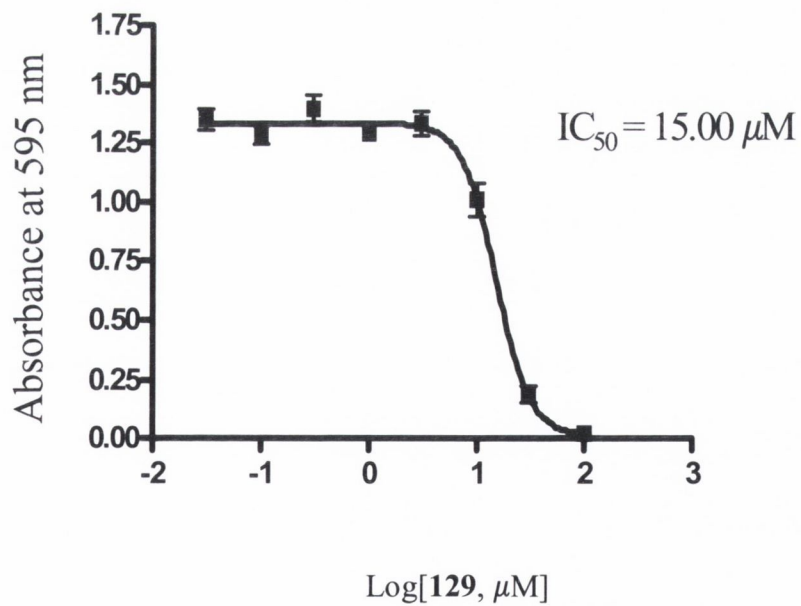


Figure 4.12: Dose vs. response plot of 129 in HL-60 cell

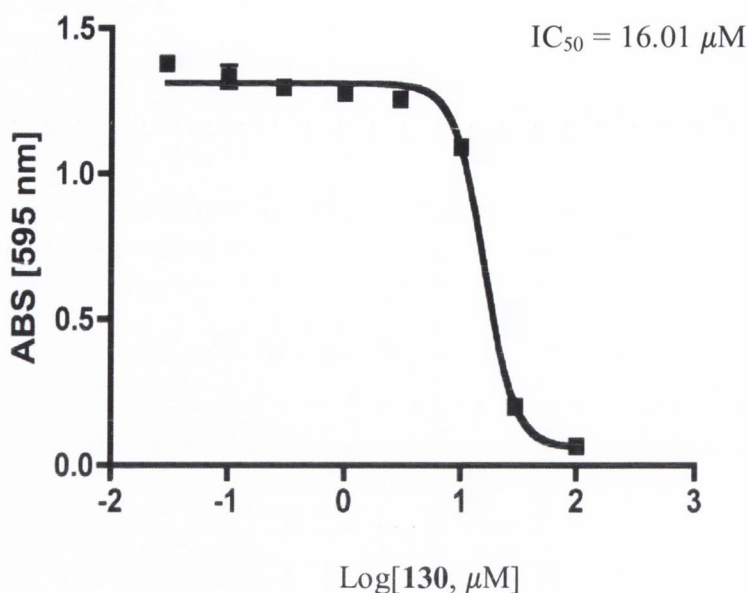


Figure 4.13: Dose vs. response plot of 130 in HL-60 cell

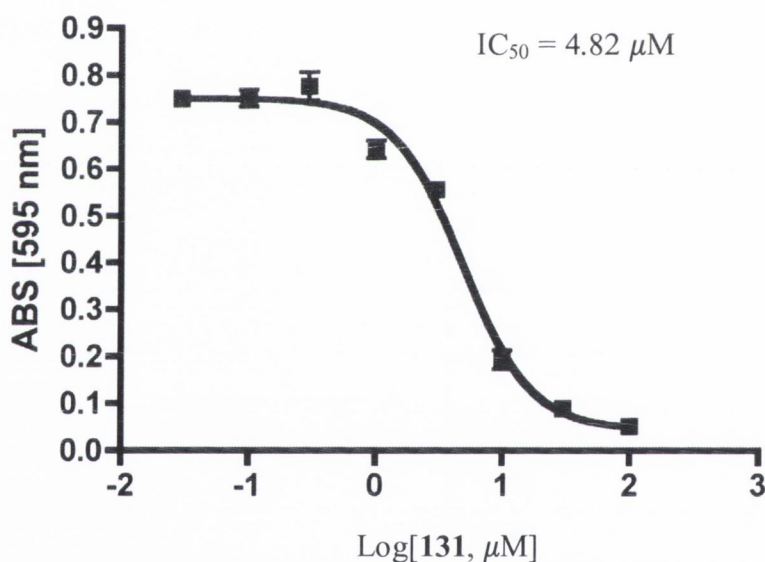


Figure 4.14: Dose vs. response plot of 131 in HL-60 cell

4.5 Results for ‘Tail-to-Tail’ Bisnaphthalimides Against K562 Cell Lines

The most promising of the three families of ‘tail-to-tail’ bisnaphthalimides were subsequently tested against the more chemo-resistant, K562 leukaemia cell line, this meant that **99** and **102** were omitted. The results from these tests were promising and generally reflected the general trends of potency observed in the HL-60 evaluation. The hexyl and pentyl linked analogues are more active than the propyl versions and LeuAla > PheAla > Cbz(Lys)Ala. The typical reference compound, mitonifide, has an IC_{50} value of $2.75 \mu\text{M}$

against this cell line and so the values shown for these bisnaphthalimides in **Table 4.3** compare very favourably. Currently, **105** and **106** are undergoing testing.

Table 4.3: Summary of IC₅₀ values for ‘tail-to-tail’
bisenaphthalimides in K562 cell lines

Compound	Family	Spacer Length	IC ₅₀
96	PheAla	Propyl	10.54 μM
97	PheAla	Pentyl	2.59 μM
98	PheAla	Hexyl	2.70 μM
100	Cbz(Lys)Ala	Pentyl	6.15 μM
101	Cbz(Lys)Ala	Hexyl	3.91 μM
104	LeuAla	Propyl	10.42 μM

4.6 Results for ‘Head-to-Head’ Bisnaphthalimides Against K562 Cell Lines

Although the ‘head-to-head’ bisnaphthalimides showed little effect against the HL-60 cell line they were tested against the K562 cell line for the sake of completion. Indeed, they showed little effect against this resilient cancer cell line and can be ruled out as potential anti-cancer agents since their activities in both these evaluations were so poor. Whilst these compounds seemed promising at the stage of photophysical probing of DNA interactions, their studies *in vivo* ultimately disappointed. This reaffirms that the elegance of a drug design, architecture and synthesis far from guarantees its future applications and although photophysical techniques may present interesting data the proof of the compound’s potential lies in biological investigation.

Table 4.4: Summary of IC₅₀ values for ‘head-to-head’
bisnaphthalimides in K562 cell lines

Compound	Family	Spacer or Linker	IC ₅₀
117	PheLys	Naphthalene Diimide	∞
122	Phe-Orn	Naphthalene Diimide	22.92 μM
128	Phe-But	Naphthalene Diimide	∞
129	PheLys	Dicarboxyl	22.46 μM
130	Phe-Orn	Dicarboxyl	2413 μM
131	Phe-But	Dicarboxyl	12.91 μM

4.7 Comparison of MTT Assay Results for a Range of Naphthalimide Derivatives

The motivation for the design of these novel bisnaphthalimides was the incorporation of two distinct motifs, each with an affinity for DNA, in an effort to enhance their individual activity and achieve an augmented, dual effect, anti-cancer activity. Therefore, to better comprehend the improvement of these new compounds over their constituent units and other mono and bisnaphthalimides already tested within the Gunnlaugsson group some comparison of IC₅₀ values would be helpful. The MTT results against the HL-60 cell line has been chosen for these comparisons. The general trends agree with those seen by Phelan¹⁰³ and Hussey¹⁰⁵ and extend their findings to confirm the achievement of the enhancement of activity from mononaphthalimides to the corresponding naphthalene diimide linked bisnaphthalimides.

Table 4.5 summarise the MTT results for examples of mono-peptide naphthalimides, dipeptide naphthalimides, 4-amino substituted naphthalimides, mono-peptide bisnaphthalimides with naphthalene spacers and a lone naphthalene spacer against the HL-60 cell line, respectively. Trends can be observed;

- the mono-peptide functionalised mononaphthalimides are less potent than the corresponding dipeptide analogues, this is accordance with findings for compounds of Phelan¹ and Hussey², *e.g.* **75** IC₅₀ = 8.90 μM *vs.* **86** IC₅₀ = 1.62 μM

- the introduction of a 4-amino substituent reduces the activity of the compound, be it a mono-peptide or dipeptide functionalised naphthalimide, *e.g.* **110** IC₅₀ = 46.15 µM vs. **75** IC₅₀ = 8.90 µM
- a naphthalene diimide spacer is less potent than its corresponding novel dipeptidic bisnaphthalimide, *e.g.* **87** IC₅₀ = 13.03 µM vs. **96** IC₅₀ = 4.30 µM
- the activity of mono-peptide functionalised bisnaphthalimides is not as great as that of their dipeptide analogues, following the trend seen for their mono-peptide counterparts, *e.g.* **113** IC₅₀ = 31.58 µM vs. **101** IC₅₀ = 1.51 µM
- the mononaphthalimides parent compounds are less potent than their naphthalene diimide linked bisnaphthalimides, *e.g.* **86** IC₅₀ = 1.62 µM vs. **106** IC₅₀ = 0.47 µM

Table 4.5: Summary of IC₅₀ values for the range of naphthalimides in HL-60 cell lines

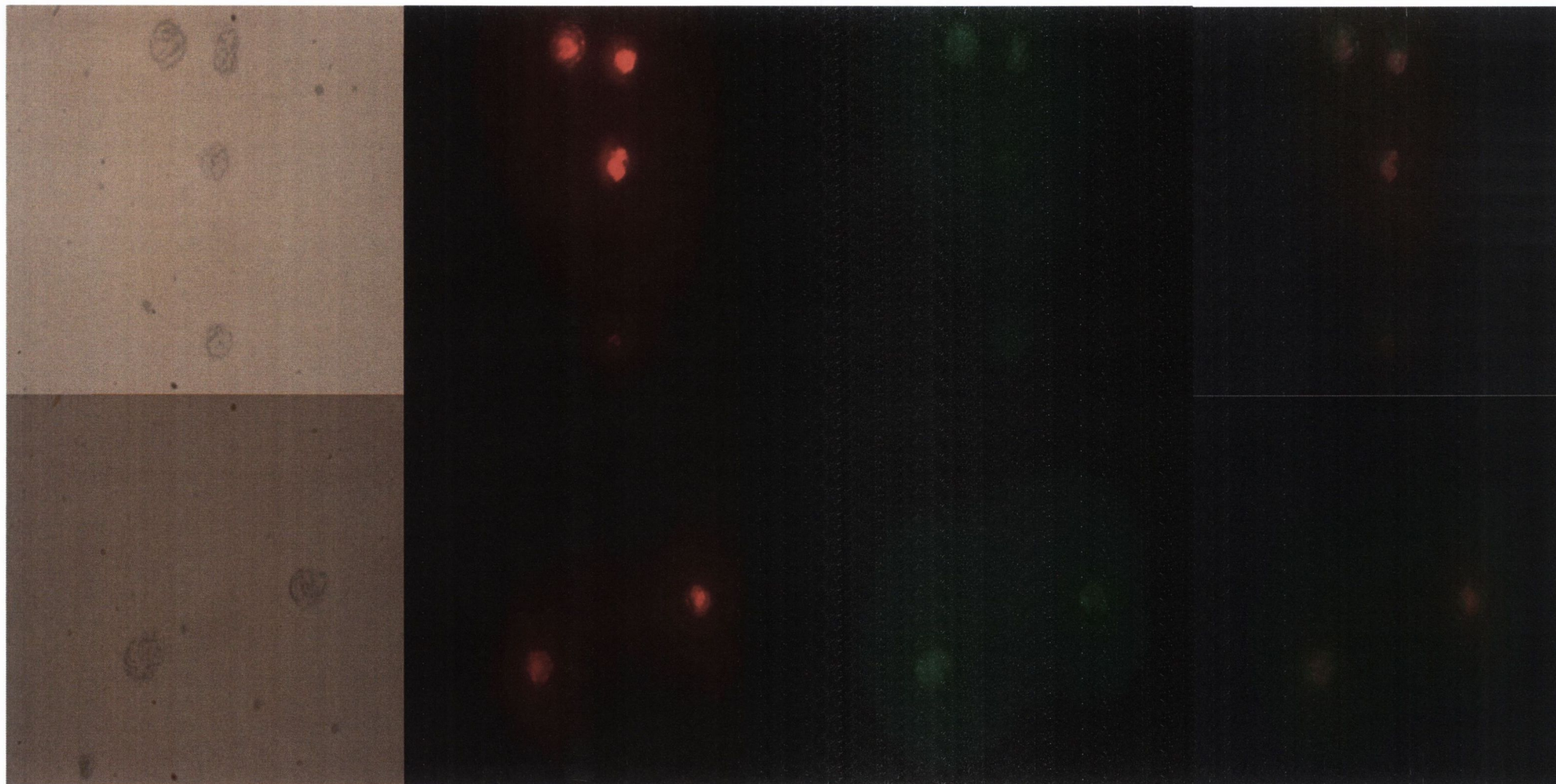
Type of Naphthalimide	Compound	4-Substituent	Family	Spacer Bridge	IC ₅₀
Mononaphthalimide <i>tert</i> -butyl ester	75	Nitro	Leu		8.90 µM
Naphthalene Diimide Spacer	87			Propyl	13.03 µM
Mononaphthalimide dipeptide	86	Nitro	LeuAla		1.62 µM
4-Amino Naphthalimide <i>tert</i> -butyl ester	108	1,5 Diamino pentane	Phe		16.84 µM
	109	1,6 Diamino hexane	CbzLys		52.07 µM
	110	1,6 Diamino hexane	Leu		46.15 µM
Mono-peptide Bisnaphthalimide	115		CbzLys	Propyl	3.37 µM
	113		CbzLys	Hexyl	31.58 µM
Dipeptide	96		PheAla	Propyl	4.30 µM
Bisnaphthalimide	97		PheAla	Pentyl	1.70 µM
	98		PheAla	Hexyl	1.25 µM
	100		Cbz(Lys)Ala	Pentyl	3.50 µM
	101		Cbz(Lys)Ala	Hexyl	1.51 µM
	104		LeuAla	Propyl	3.20 µM
	106		LeuAla	Hexyl	0.47 µM

4.8 Fluorescent Imaging of Cells Treated with Bisnaphthalimides

In an effort to explore how these bisnaphthalimides achieve their anti-cancer activity preliminary fluorescent microscopy studies were undertaken. Two of the most potent compounds, the PheAla pentyl linked analogue, **97**, and the LeuAla hexyl linked analogue, **106**, were selected for investigation in the HL-60 cell line.

A sample of HL-60 cells were treated with 10 μ M of the chosen bisnaphthalimide and incubated for 24 h. These cells were then collected and centrifuged to a glass slide. They were fixed with 90 % EtOH and stained with 100 μ L of the dye propidium iodide (PI) for 30 min. in darkness. PI is known as a DNA binder. Finally, they were washed with a phosphate buffered solution (PBS) solution and sealed with a coverslip. The fluorescent microscopic images were then recorded while exciting at different wavelengths. **Figures 4.15** and **4.16** depict the images recorded for **97** and **106**, respectively.

The first image is the cells under natural light, **(i)**. Under a lamp emitting in the red the localisation of the PI is evident by the red fluorescence, **(ii)**. Under a lamp emitting in the green there is a strong green fluorescence also localised in the cells, **(iii)**, since PI does not emit in this region of the electromagnetic spectrum this is due to the bisnaphthalimide. Finally, **(ii)** and **(iii)** were superimposed upon each other and this shows that the PI and the bisnaphthalimide localisation occur within the same region of the cells, **(iv)**. Both the dye and the compound appear to have penetrated the cell wall and entered the nuclei. Although more detailed mechanistic and pathway studies have yet to be conducted this suggests that these novel bisnaphthalimides affect their toxicity by nuclear means.



(i)

(ii)

(iii)

(iv)

Figure 4.15: Fluorescent microscopy images of cells treated with PI and 97

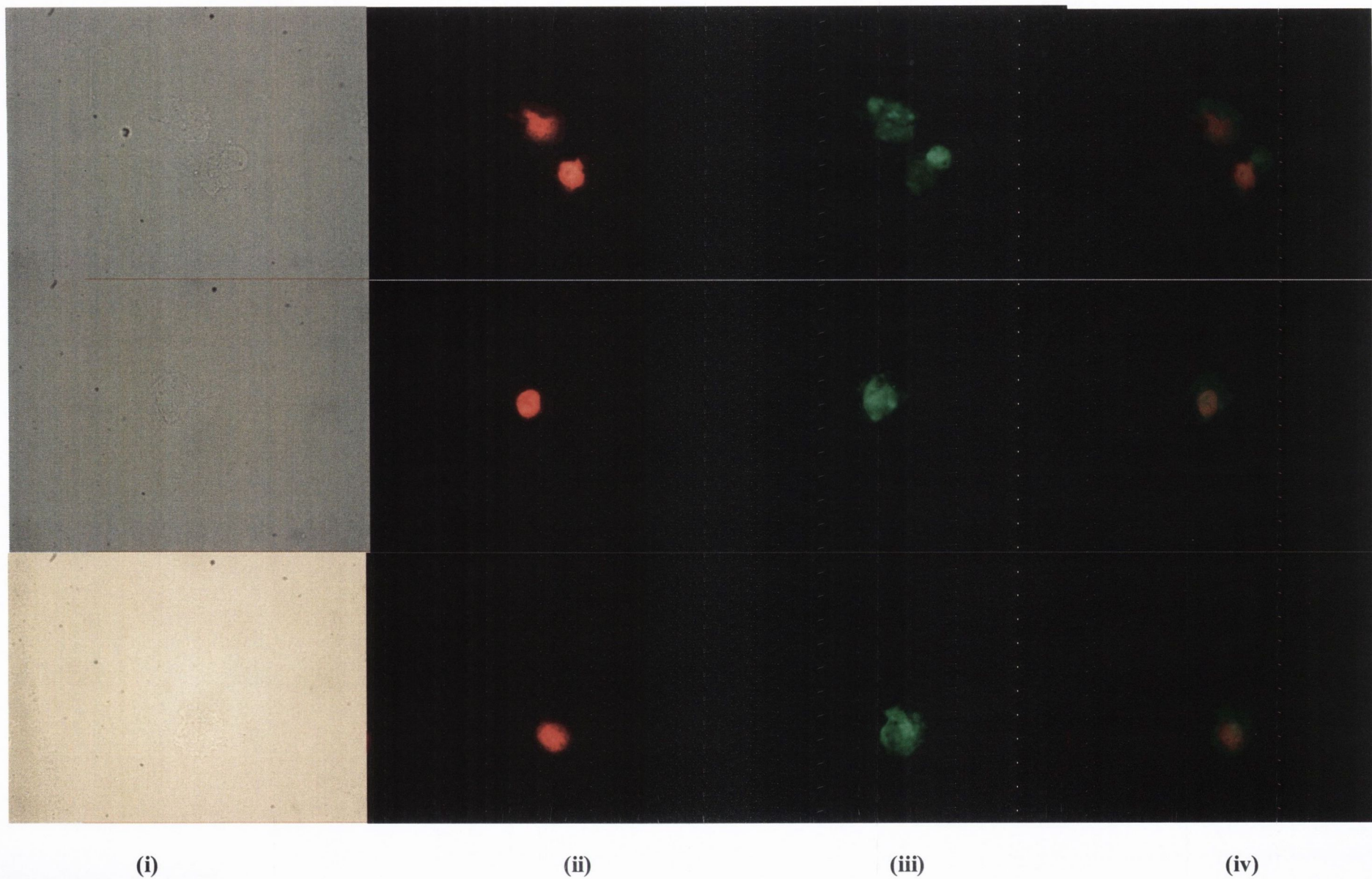
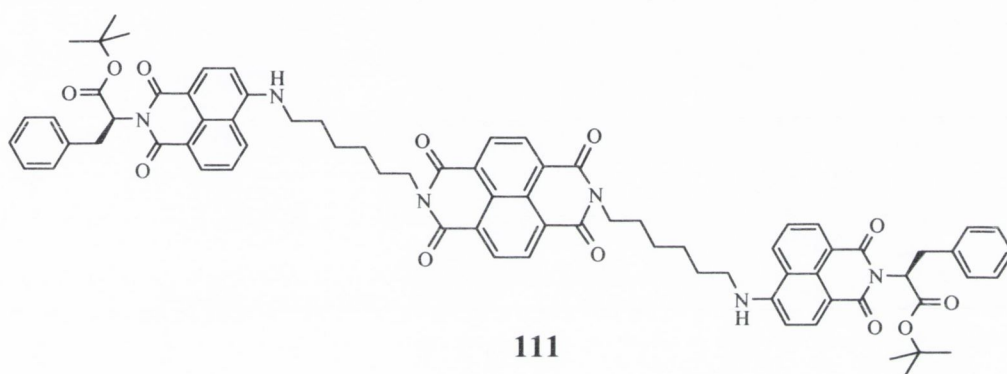


Figure 4.16: Fluorescent microscopy images of cells treated with PI and 106

4.9 Detailed Cell Viability Studies of 111

The most unexpected result observed during the MTT studies was the fact that **111** displayed an IC_{50} value of $0.11\ \mu\text{M}$ against HL-60, **Figure 4.17**, and of $0.12\ \mu\text{M}$ against K562, **Figure 4.18**. This is in the nanomolar range and is the most potent naphthalimides derivative sent for testing in St James' from the Gunnlaugsson group.^{103, 105} These also compare favourably with findings of Braña *et al.* This is an impressive potency made all the more interesting by the fact that it is a mono-peptide functionalised compound, when it might be expected to be less potent than the dipeptide analogue, as seen in the mononaphthalimides and confirmed by examples such as **113**. The dipeptide analogue of this compound is **98** which has an IC_{50} value of $1.25\ \mu\text{M}$.



Since the activity of **111** is so interesting, more extensive studies have recently been conducted. A range of investigations were conducted at lower concentrations of **111** by our collaborators in St James' and these are depicted in **Figure 4.19**. The results are shown in terms of statistical error (St. Error) and standard deviation (SD). These detail the growth inhibition achieved by **111** against both HL-60 and K562 cell lines after 24 h and 48 h. GI_{50} is the concentration of the compound required to inhibit the cancer cell growth by 50 %. After 24 h the GI_{50} values are $0.011\ \mu\text{M}$ and $0.009\ \mu\text{M}$ against HL-60 and K562, respectively. After 48 h the GI_{50} values are $0.013\ \mu\text{M}$ and $0.009\ \mu\text{M}$ against HL-60 and K562. Figures **4.20** and **4.21** summarise the inhibition studies of HL-60 and K562, respectively. After 24 h the GI_{50} values are $0.011\ \mu\text{M}$ and $0.009\ \mu\text{M}$ against HL-60 and K562, respectively. After 48 h the GI_{50} values are $0.013\ \mu\text{M}$ and $0.009\ \mu\text{M}$ against HL-60 and K562..

These results also provide an LD_{50} value, this quantifies the 50 % lethal dose, the concentration required to kill 50 % of the cell population. The LD_{50} values are $0.75\ \mu\text{M}$ and $0.05\ \mu\text{M}$ against HL-60 and K562, respectively. Not only does **111** achieve growth

inhibition of the cell proliferation at such low concentrations but the potency against the more virulent cancer cell line K562 is greater than that against HL-60, which is unusual. These investigations and studies of possible modes of action are ongoing.

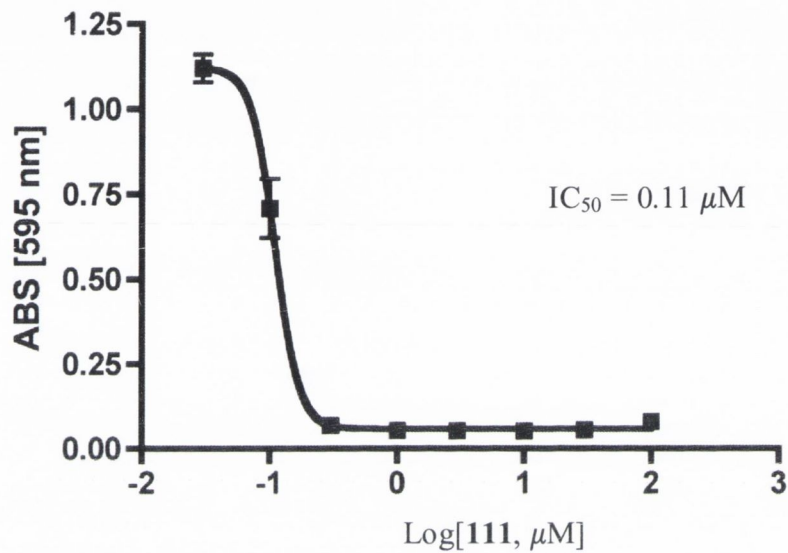


Figure 4.17: Dose vs. response plot of 111 in HL-60 cell line

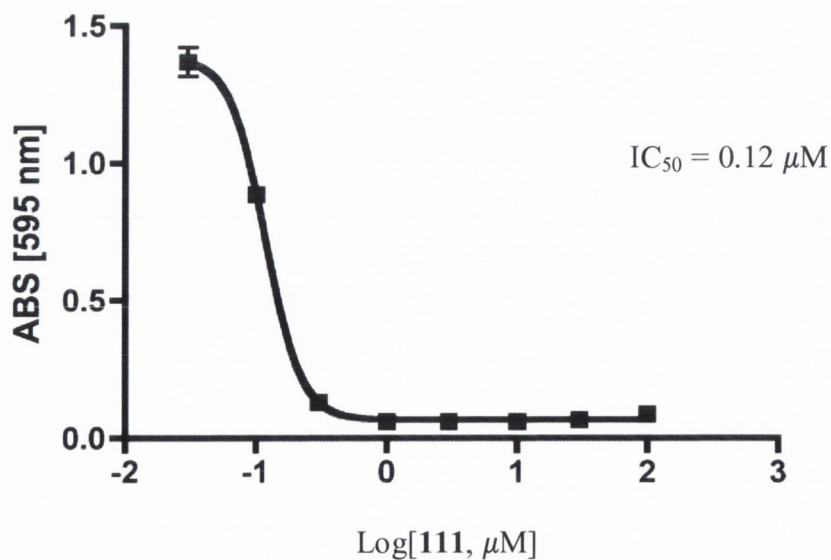


Figure 4.18: Dose vs. response plot of 111 in K562 cell line

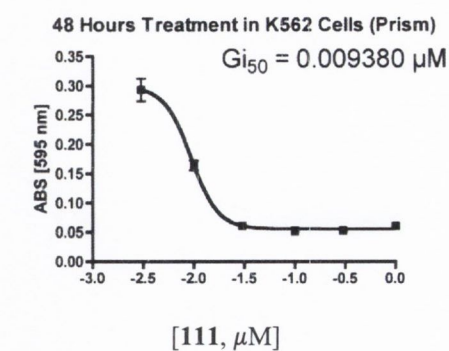
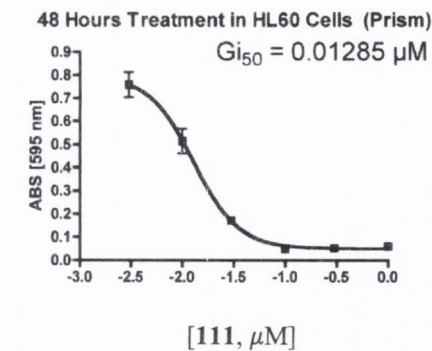
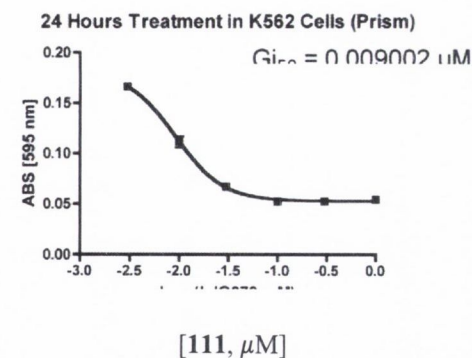
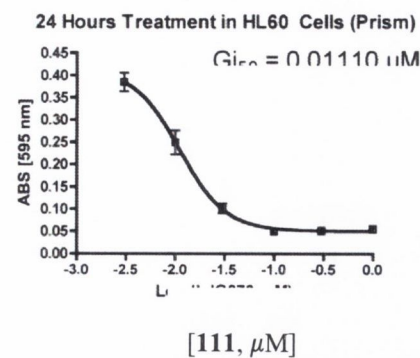
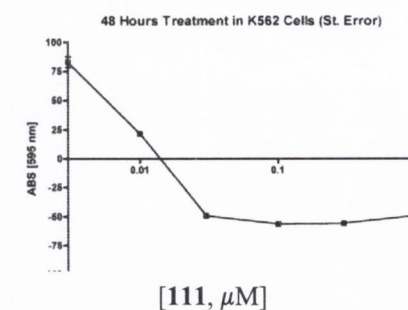
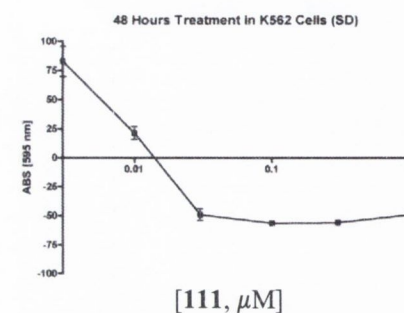
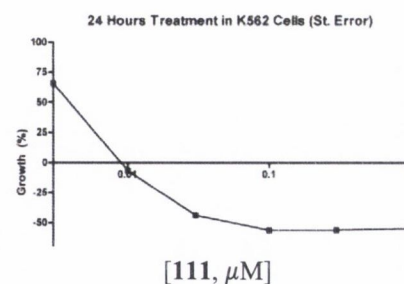
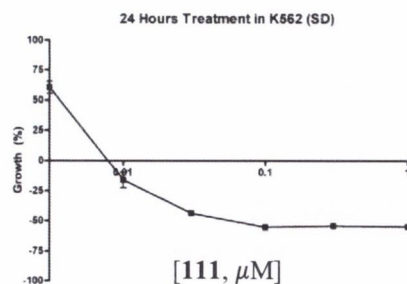
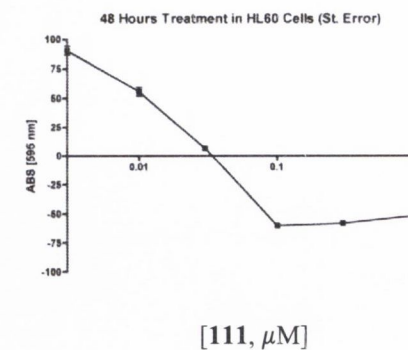
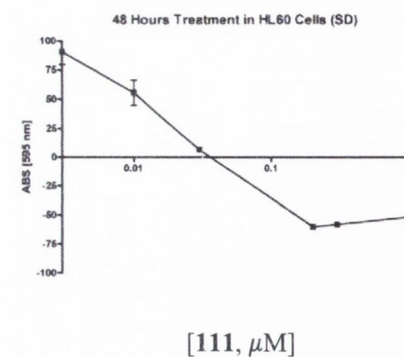
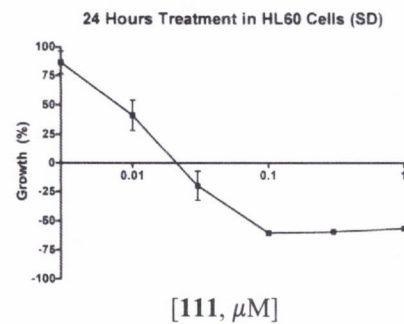
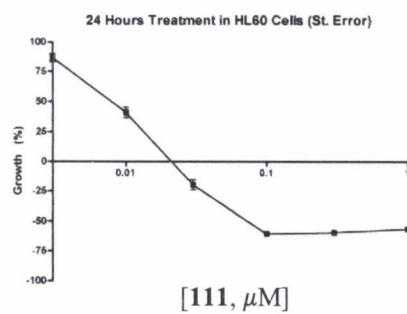


Figure 4.19: Detailed growth inhibition studies of 111 in HL-60 and K562 cell lines at lower concentration

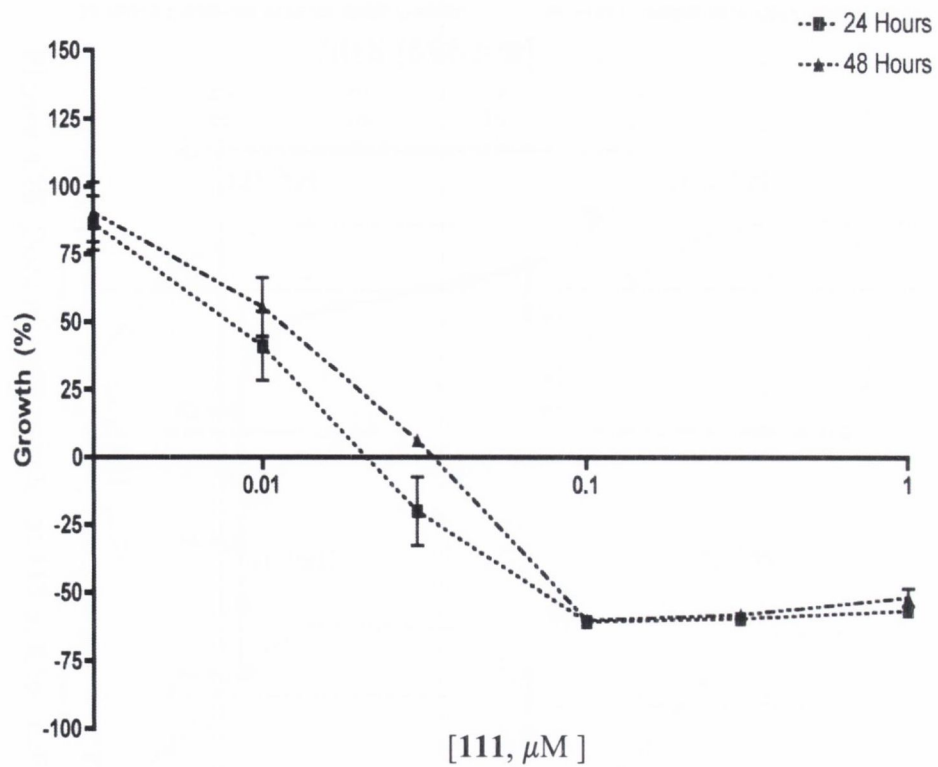


Figure 4.20: Summary of HL-60 studies (SD) of growth inhibition caused by 111

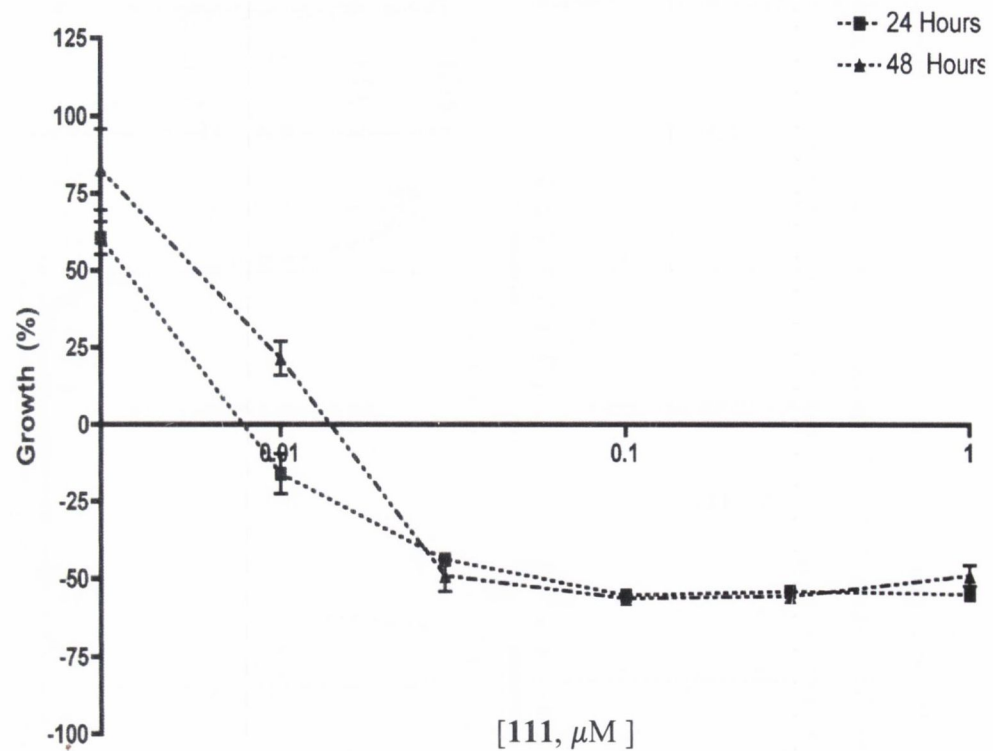


Figure 4.21: Summary of K562 studies (SD) of growth inhibition caused by 111

4.10 Conclusions

This chapter has detailed the biological assays conducted on fifteen novel bisnaphthalimides. These compounds were studied in HL-60 cell lines, an acute promyelocytic leukaemia, and K562 cell lines, a much more chemo-resistant chronic myeloid leukaemia cell line. The typical reference compound, mitonifide, has an IC_{50} value of 0.23 μ M and 2.75 μ M against these cell lines, respectively, and so the results obtained for many of these bisnaphthalimides compare favourably

Of the fifteen bisnaphthalimides studied, three candidates showed the most potential as anti-cancer agents. The hexyl bridged naphthalene diimide linked analogues of the PheAla, CbzLysAla and LeuAla bisnaphthalimides displayed the best IC_{50} values of the panel against HL-60. These also showed promising activity against K562. The pentyl analogues are also potent, whereas the propyl analogues display the lowest relative activity. There is a general correlation of the binding constants, K , deduced in *Chapter Three*, with the results of the cell viability assays. This suggests that, as hypothesised from the photophysical investigations of DNA binding, these longer analogues possess the appropriate span for interaction with DNA. Perhaps this span permits the diimide to intercalate and the naphthalimide units to slot into the minor and major grooves; this two-step, dual effect interaction is indeed what was desired when the compounds were initially designed. The fact that the IC_{50} results for the diimide linked bisnaphthalimides are not additive when compared with the mono-peptide parent molecules is likely due to differing modes of action. The mononaphthalimide most likely intercalates whereas the bisnaphthalimide may not bisintercalate but thread intercalate and groove bind, leading to the resulting co-operative potency. The results obtained for the propyl analogues are the poorest of the three analogues for each of the families. They may operate *via* an alternative binding mode and perhaps the spacer chain is too short and that the diimide simply intercalates or that merely groove binding of the naphthalimide units is at play.

The binding studies detailed in *Chapter Three* seemed to suggest that the hexyl analogues interacted less powerfully with the DNA since the emission signature was not dramatically altered. It may well be that *in vivo* this limitation is muted and that in fact this longest spacer allows optimum interaction, disruption and damage to the DNA.

In comparison, the diimide linked ‘head-to-head’ bisnaphthalimides showed poor IC_{50} values and little potential as anti-cancer agents, even against the least resistant HL-60 cell line. The retention of the 4-nitro substituent in these compounds and lack of the terminal dipeptide functionalisation would naturally render the action of these bisnaphthalimides different to that of the ‘tail-to-tail’ systems. It is possible that these

compounds bisintercalate or, indeed, that only one unit intercalates and the rest of the compound groove binds or fails to interact at all. This tallies with the reduced magnitude of the binding constants calculated in *Chapter Three* in comparison with the ‘tail-to-tail’ bisnaphthalimides.

Whilst the dicarboxyl linked compounds were assayed against HL-60 and K562 they too showed little activity, as one would expect since they do not possess the naphthalene core and it’s inherent potential to intercalate. This limited activity reflects the low binding constants deduced from the photophysical studies detailed in *Chapter Three*.

Although attempts were made to probe the nature of the interaction with DNA by thermal denaturation, CD and viscosity studies, these were not conclusive. Whether these compounds operate by groove binding or intercalation or by a complicated dual mode involving both these interactions has not been defined. The mechanistic pathways adopted by these novel bisnaphthalimides *in vivo* is also a matter for future investigation.

The recent findings for **111** are surprising since the mono-peptide bisnaphthalimides would be expected to display lower anti-cancer activity compared with dipeptide derivatives. The nanomolar range of the IC₅₀, the GI₅₀ and the LD₅₀ values in both the HL-60 and K562 cell lines after 24 h and 48 h incubation period is the lowest concentration range observed to date for such systems synthesised within the Gunnlaugsson group.^{103, 105} This compound and other related analogues will now be studied in more detail to explore how this anti-cancer activity is achieved.

The preliminary fluorescence microscopy findings for **97** and **106** show that these novel bisnaphthalimides behave interestingly *in vivo* and that they penetrate the cell nucleus. While this indicates a means of their anti-cancer activity further investigations are necessary since the complete mode of action of these systems is likely to be a complicated system. The nature of this cellular activity and possible mechanistic pathways must now be pursued.

More than twenty novel naphthalimides have been designed and synthesised as part of this project. Fifteen of these have been thoroughly investigated, photophysically and physically, in an effort to probe the nature of their interaction with DNA. They have also been biologically studied in cell viability assays to quantify their anti-cancer activity and gauge their potential as anti-cancer agents. The possibilities for developing, elaborating and exploring the modes of actions of these compounds are boundless and their elegant photophysics are also of interest. The remaining chapters in this Thesis detail the experimental procedures and characterisation of the synthesised compounds and catalogue the cited literature.

The original objectives of this research project included the establishment of some degree of sequence selectivity, the observation of the effect on binding of altering the length of a spacer and the improvement of binding over that of mononaphthalimides. The achievement of these aims was discussed in *Chapter Three*. Ultimately, these novel compounds were designed and synthesised with the intention of their application as potential anti-cancer agents. Unfortunately, the ‘head-to-head’ bisnaphthalimides showed low activity during cell viability assays. However, the ‘tail-to-tail’ bisnaphthalimides have proved themselves as promising potential anti-cancer systems, being potent against both the HL-60 and K562 cell lines. The merits of the design and investigation of these architectures has been demonstrated and such bisnaphthalimides warrant more extensive development.



Chapter Five

Experimental Details



5.1 General Experimental Details

All air and moisture sensitive reactions were conducted under an argon atmosphere. Air and moisture sensitive liquids and solutions were transferred to the sealed reaction vessel via a canula. All anhydrous solvents used were either purchased in sure-sealed Winchester bottles or were purified using a still according to the standard procedures. All untreated solvents used were of HPLC standard. The reagents were purchased from Sigma-Aldrich, Lancaster, Fluka and NovaBioChem, these were used without further purification measures.

^1H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument or at 600 MHz using a Bruker Spectrospin DPX-600 instrument. Chemical shifts were expressed in parts per million (ppm) downfield from the internal reference standard, tetramethylsilane (TMS), followed by the number of protons, splitting pattern, coupling constant (where applicable) and assignment of proton. A doublet splitting pattern is represented by d, a double doublet by dd, a quartet by q, a singlet by s, a broad singlet by br. s and a multiplet by m. The assignment of the 1,8-naphthalimide ring protons are made whereby Ar-H2 is the proton at position 2, and Ar-H3, Ar-H5, Ar-H6, Ar-H7 assign the remaining naphthalimide protons, respectively. The aromatic protons of the diimide are assigned as Ar-H. ^{13}C NMR were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument or a Bruker Spectrospin DPX-600 instrument.

Melting points were determined using an Electrothermal IA9000 digital melting point apparatus. Analytical thin layer chromatography (tlc) was performed on Merck Kieselgel 60 F254 plates and were visualised using an ultraviolet lamp. Preparative chromatography plates used in purification of products were Silica Gel GF preparative layer with UV254, 20 x 20 cm of 1000 microns. Infrared spectra were recorded on a Perkin Elmer precisely Spectrum One FT-IR Spectrometer using a Uni ATR Sampling accessory.

Mass spectroscopy was conducted in HPLC grade solvents. The spectra were determined by detection using electrospray on a Micromass LCT spectrometer, using a Shimadzu HPLC or Water's 9360 to pump solvent. The whole system was controlled by MassLynx 3.5 on a Compaq Deskpro workstation. Accurate molecular masses were determined by a peak matching method, using leucine Enkephalin, (Tyr-Gly-Gly-Phe-Leu), as the standard reference. Elemental analysis was conducted in the

Microanalytical Laboratory, Dept. of Chemistry, University College, Dublin. Absorption spectra were recorded on a Varian Cary 50 spectrophotometer.

5.2 Photophysical and Physical Examination of Compounds

5.2.1 UV-visible Titrations

Absorption spectra and optical density were recorded on Varian Cary 300 spectrophotometer in the range of 200 – 600 nm. Solutions were measured in 3.00 mL (10 mm x 10 mm) quartz cuvettes. Water used in DNA related work was Millipore, HV (0.45 μ M). Phosphate buffer was composed of two 1 M stock solutions of K_2HPO_4 and KH_2PO_4 prepared with Millipore water. Portions of each solution were diluted together to achieve 10 mM phosphate buffer, at pH 7.

The concentration of each compound in 2 : 98, DMSO : buffer solution was adjusted until an absorption of 0.1 was achieved from a stock solution of known concentration *ca.* 10 mM. Successive aliquots of 1, 5, 10 or 20 μ L of *ct*-DNA or homopolymers (which had been sonicated for 30 min.) were introduced and changes to the spectrum were monitored throughout the titration.

5.2.2 Fluorescence Titrations

Fluorescence studies were conducted on a Cary Eclipse Luminescence spectrometer. Concurrently with the UV-visible investigations as each successive aliquot of sonicated *ct*-DNA or homopolymers was introduced changes to the spectra were monitored.

5.2.3 Circular Dichroism Measurements

CD spectra were recorded at a concentration corresponding to an optical density of approximately 1.0 in 2 : 98, DMSO : buffer solution on a Jasco J-810-150S spectropolarimeter. A 1 mL (10 mm x 10 mm) quartz cuvette was used. All circular dichroism spectra are represented as mdeg vs. λ (nm). The baseline of the solvent was subtracted from the spectra. CD titrations were conducted by successive additions of aliquots of a buffered solution of the compound to a cuvette containing *ct*-DNA. The spectrum was examined for any changes in the characteristic signal of the *ct*-DNA.

5.2.4 Thermal Denaturation Studies

Thermal denaturation studies were conducted using a matched pair of 1 mL quartz cuvettes with a pathlength of 1 cm and window width of 4 mm, supplied by Starna. A Cary Temperature controller in conjunction with dual beam Varian 300 UV-visible spectrometer. All solutions used were P/D = 10 of millimolar buffered solutions of *ct*-DNA and bisnaphthalimide and were thoroughly degassed prior to the measurements. The absorbance of DNA at 260 nm was set at *ca.* 1 and this was monitored as the temperature was ramped from 20 ° C to 90 ° C and reversed back to 20 ° C at a rate of 2 ° C / min. The resulting melting curve was compared to that of the *ct*-DNA solution alone and the point of inflection was taken as the T_m .

5.2.5 Viscosity Studies

Viscometry measurements were conducted using a Cannon-Ubbelohde semi-micro viscometer with a capillary diameter of 0.54 mm (Model 75, Cannon Instrument Company). This was submerged in a thermostatically controlled water bath at 25 °C. The flow rates were recorded in triplicate for the duration required for (a) the 10 mM phosphate buffer solution (t_{buffer}), (b) the *ct*-DNA (mM) in 10 mM phosphate buffer solution (t_{DNA}) and (c) each of the individual *ct*-DNA-bisnaphthalimide solutions with varying P/D ratios (50-2), to flow between the indicated boundaries on the bulb of the viscometer. Solutions of 0.5 mL volume were used and allowed to thermally equilibrate before measurements were taken. The flow rates were accurate to ± 0.05 s.

5.3 Biological Examination of Compounds

Compounds to be assayed were dissolved in DMSO (Sigma Aldrich) to a concentration of 5 mM and were stored at -20 °C.

5.3.1 Cell Lines

HL-60, an acute promyelocytic leukaemia cell line, K562, a chronic myelogenous leukaemia (CML) cell line, and PC-3, a prostate cancer cell line, were used in this study. The cells were cultivated in RPMI media supplemented with 10 % (v/v) fetal bovine serum, $100 \mu\text{mL}^{-1}$ penicillin and $100 \mu\text{mL}^{-1}$ streptomycin (complete

media) and this was incubated at 37 °C in a 5 % CO₂, 95 % air, humidified atmosphere in a LEEC Mk II Proportional Temperature Controller Incubator. All tissue culture reagents were purchase from Life Technologies. Cells were grown in Nunc tissue culture flasks, plates and dishes.

5.3.2 Cell Viability Assay (MTT)

Approximately 6×10^4 cells / well for HL-60 and 4×10^4 for K562 were seeded into 96 microtitre plates, in 100 μ L of complete media containing 100 μ M, 30 μ M, 10 μ M, 3 μ M, 1 μ M, 0.30 μ M, 0.10 μ M and 0.03 μ M of compound in triplicate. The microtitre plate was incubated for 24 h. and 48 h. for HL-60 and K562 cell lines, respectively. After the appropriate incubation period, 10 μ L of a 5 mg/mL MTT solution in a phosphate buffered solution (PBS) was added to each well. The microtitre plate was then incubated for a further 4 h in a humidified atmosphere at 37 °C and 100 μ L of 10 % (w/v) solubilisation sodium dodecyl sulfate (SDS) solution in 0.1 M HCl was added. The plate was incubated overnight and the absorbance was recorded using an ELISA microtitre plate reader at 595 nm.

5.3.3 Fluorescence Microscopy Studies

A sample of HL-60 cells were treated with 10 μ M of the chosen bisnaphthalimide and incubated for 24 h. These cells were then collected and centrifuged to a glass slide. They were fixed with 90 % EtOH and stained with 100 μ L of the dye propidium iodide (PI) for 30 min. (using 2 ug/mL PI, 50 ug/mL RNase, 0.1 % Triton-X 100 in PBS) in darkness. Finally they were washed with PBS solution and sealed with a coverslip. The cells were then viewed under the microscope using different excitation wavelengths and the fluorescent images were recorded.

5.4 General Procedures

Procedure 1: Reaction of amino acid with 4-nitro-1,8-naphthalic anhydride^{103, 104, 105}

Et₃N (2 eq.) was added to a mixture of the relevant α -amino acid or α -amino ester hydrochloride salt (1 eq.) and 4-nitro-1,8-naphthalic anhydride (1 eq.) in anhydrous toluene. The reaction mixture was refluxed for 24 h. The solution was then filtered through celite before being allowed to cool. The solvent was evaporated under reduced pressure. CH₂Cl₂ (30 ml) was added to the residue and the organic solution was washed twice with HCl (0.1 M) and once with deionised water. The organic layer was dried over MgSO₄ and was then filtered and evaporated to dryness. Recrystallisation was achieved using MeOH or CH₂Cl₂.

Procedure 2: Deprotection of *tert*-butyl ester functionality using trifluoroacetic acid / Removal of Boc-protecting group using trifluoroacetic acid¹⁰⁸

A solution of naphthalimide *tert*-butyl ester (or other N-Boc protected compound) in TFA or TFA/CH₂Cl₂ was stirred at room temperature for 2 h. The solvent was then removed under reduced pressure and co-evaporations using CH₂Cl₂ and diethyl ether ensured the removal of all traces of TFA. The product was then dried further under high vacuum.

Procedure 3: Coupling of mono-peptide 1,8-naphthalimide with second amino acid moiety^{103, 105, 108}

A solution of mono-peptide functionalised 4-nitro-1,8-naphthalimide (1 eq.) in anhydrous THF was prepared and to this was added HOBt (1 eq.) and α -amino acid methyl ester hydrochloride (1 eq.). The solution was stirred continually and placed in a ice-bath for 15 min. EDCI.HCl (1.1 eq.) and Et₃N (1.1 eq.) were then added and the reaction was stirred at 0 °C for 30 min. Once removed from the ice-bath the reaction mixture was stirred at room temperature for 18 h. Following filtration to remove unreacted reagent, the solvent was evaporated under reduced pressure and the

remaining residue was dissolved in CH_2Cl_2 and then washed twice with HCl (1 M), twice with K_2CO_3 (10 %, w/v) and finally with deionised water. The organic layer was dried over MgSO_4 , filtered and evaporated to dryness. If necessary, purification was achieved by chromatography using a silica preparative plate and methanol as the eluent.

Procedure 4: Syntheses of Spacers

a) Naphthalene tetracarboxylic dianhydride was refluxed overnight with the 1,3-diaminopropane (an excess) in anhydrous THF. Diethyl ether was added to induce precipitation. Evaporation of methanol gave the desired product as a red or pink solid.

b) Naphthalene tetracarboxylic dianhydride (or perylene tetracarboxylic dianhydride) was refluxed in the presence of the chosen *N*-Boc diamino alkane (8 eq.) in anhydrous THF (or DMA) for 72 h. Diethyl ether was added to induce precipitation. Recrystallisation from methanol gave the desired product as a red or pink solid.

Procedure 5: Syntheses of Bisnaphthalimides^{103, 105, 108}

a) The relevant spacer species was refluxed with the naphthalimide dipeptide (2 eq.) of choice, in anhydrous THF, in the presence of Et_3N (4 eq.), for 72 h. The solution was filtered through celite while hot. The solvent was then removed under reduced pressure and the residue was dissolved in CH_2Cl_2 and this solution was washed twice with HCl (0.1 M) or citric acid (10 %, w/v), twice with NaHCO_3 and once with deionised water. If necessary, the product was purified by preparative plate chromatography using CH_2Cl_2 as the solvent and MeOH as the eluent. Recrystallisation from MeOH or CH_2Cl_2 allowed the isolation of the desired product as a brown / mustard crystalline solid.

b) Naphthalene tetracarboxylic dianhydride was refluxed with the desired 4-nitro naphthalimide (2 eq.) in anhydrous THF in the presence of Et_3N (4 eq.) for 72 h. The solution was filtered through celite while hot. The solvent was then removed under reduced pressure and the residue was dissolved in CH_2Cl_2 and this solution was washed as described in **Procedure 5a**. Purification if necessary was conducted by preparative plate chromatography using CH_2Cl_2 as the solvent and MeOH as the

eluent. Recrystallisation from MeOH or CH₂Cl₂ allowed the isolation of the desired product as a solid.

c) The relevant spacer species was refluxed with the naphthalimide dipeptide (2 eq.) of choice, in anhydrous THF, in the presence of Et₃N (4 eq.), for two weeks. Work-up as for **Procedure 5a**.

d) The relevant spacer species was dissolved in anhydrous THF and placed in a sealable pressure vessel. To this was added the naphthalimide dipeptide (2 eq.) of choice in and Et₃N (4 eq.). The vessel was sealed and the reaction mixture was heated at 120 °C for 172 h. Work-up as for **Procedure 5a**.

Procedure 6: Mono-Boc protection of diamino-alkanes¹⁰⁸

A solution of di-*tert*-butyl dicarbonate (Boc₂O, 0.25 eq.) in dioxane was added dropwise, with constant stirring, to a solution of the chosen diamino alkane in dioxane. The solution was stirred overnight in an inert atmosphere. Deionised water was then added to the solution and the undesired bis-*tert*-butyl dicarbonate solid product was removed by suction filtration. The filtrate was washed with CH₂Cl₂, extracting the product into the organic layer. The solvent was then removed under reduced pressure. The resulting product was a viscous oil, which ultimately, after a week of drying (to air or under high vacuum), became a brightly coloured semi-solid.

Procedure 7: Amino Functionalisation of 4-position of Naphthalimide

10, 1045

The 4-nitro naphthalimide was refluxed in anhydrous THF for 72 h. with the relevant diamino alkane (1 eq.) or *N*-Boc-protected diamino alkane (1 eq.). The solvent was then removed under reduced pressure and the residue was dissolved in CH₂Cl₂. The solution was then washed twice with HCl (1 M), twice with saturated NaHCO₃ and once with deionised water. The product was yielded as an oil. In some cases, purification by crystallisation from methanol or CH₂Cl₂ allowed the isolation of the desired product as a brown crystalline solid.

Procedure 8: Methyl Esterification of an Amino Acid¹³³

N-Boc-*N*-Cbz-Ornithine was dissolved in a hydrochloric solution (1M) of methanol and was refluxed for 1 h. The solvent was then removed under reduced pressure to yield the desired methyl ester product as a white solid. No purification steps were needed.

Procedure 9: Mono Boc protection of amino acids using Cu(II) chelation^{105, 110}

To a stirring solution of the hydrochloride salt of 2,4-diamino butyric acid in NaOH (2M), a solution of $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$ (0.5 eq.) in water was introduced. To this was added a solution of Boc_2O (1.3. eq.) in acetone. After 24 h another addition of acetone was made and the solution was stirred for a further 24 h. A black precipitate was filtered off and washed with acetone and water. The filtrate and washings were combined and the solvent was removed under reduced pressure to yield the product as a light blue waxy solid.

Procedure 10: Cleavage of the Cu(II) complex to obtain Mono Boc protected amino acid^{105, 110}

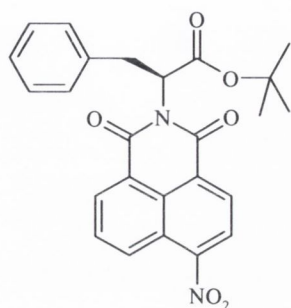
A suspension of $[2,4\text{-diaminobutyric acid}(\text{Boc})_2]\text{Cu}$ in acetone was stirred for 15 min., water was then added and the solution stirred for a further 10 min. Water and 8-quinolinol (2.5 eq.) were introduced and stirring was continued for 4 h. The copper(II) quinolinolate precipitate was filtered off and washed with water. The filtrate and washings were combined and acetone was evaporated. The residual aqueous solution was extracted with ethyl acetate and the water was then removed under reduced pressure to yield the desired product as a brown oil.

Procedure 11: Removal of Cbz-protecting group¹⁰⁸

The chosen Cbz-protected compound was dissolved in a solution of 30 % HBr / acetic acid. The reaction solution was stirred for 1 h whilst a CaCO_3 drying tube was attached to the flask throughout. Dry diethyl ether failed to induce precipitation of the de-protected product. Drying under high vacuum yielded a viscous, hygroscopic solid.

5.5 Experimental

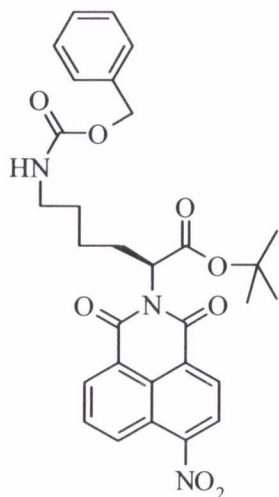
N-[(1*S*)-*tert*-Butoxycarbonyl-2-ethylphenyl]-4-nitro-1,8-naphthalimide, **73** ¹⁰⁵



73 was synthesised according to **Procedure 1** using L-phenylalanine *tert*-butyl ester hydrochloride (0.57 g, 2.20 mmol), and 4-nitro-1,8-naphthalic anhydride (0.54 g, 2.20 mmol, 1 eq.). This compound was yielded as a mustard-coloured solid (0.85 g, 2.00 mmol, 90 %). m.p.: 68 °C; HRMS: 469.45 ($[M + Na]^+$, $C_{25}H_{22}N_2O_6$ requires 446.46); Found: C, 67.11; H, 5.07; N, 6.07 %. $C_{25}H_{22}N_2O_6$ requires C, 67.26; H, 4.97; N, 6.27 % ; δ_H

(400 MHz, $CDCl_3$): 8.82 (1H, d, $J = 8.84$ Hz, Ar-H7), 8.66 (1H, d, $J = 7.52$ Hz, Ar-H5), 8.61 (1H, d, $J = 8.20$ Hz, Ar-H2), 8.37 (1H, d, $J = 7.52$ Hz, Ar-H3), 7.96 (1H, dd, $J = 6.84$ and 7.52 Hz, Ar-H6), 7.13 (5H, m, Ph), 5.94 (1H, m, $\underline{CHCH_2}$), 3.65 (2H, m, $\underline{PhCH_2}$), 1.45 (9H, s, $(CH_3)_3$) ; δ_C (100 MHz, $CDCl_3$): 167.6, 162.4, 161.6, 149.2, 136.9, 132.1, 129.6, 129.5, 129.0, 128.6, 128.5, 127.7, 126.1, 125.9, 124.8, 123.4, 123.1, 121.9, 81.9, 55.1, 34.4, 21.0; m/z : 469 ($M + Na$)⁺; ν_{max}/cm^{-1} : 3165, 3079, 3927, 2977, 2936, 2872, 1942, 1869, 1736, 1671, 1625, 1584, 1529, 1455, 1426, 1411, 1346, 1300, 1237, 1156, 931, 786, 755.

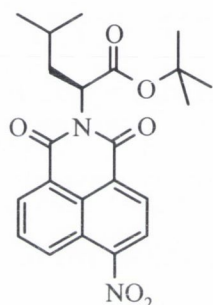
N-(1*S*)-*tert*-Butoxycarbonyl-6-benzyloxycarbonylaminoethyl]-4-nitro-1,8-naphthalimide, **74**



4-Nitro-1,8-naphthalic anhydride (0.66 g, 2.70 mmol) was reacted with Cbz - lysine *tert*-butyl ester hydrochloride (1.00 g, 2.70 mmol, 1 eq.) according to **Procedure 1**. The product was isolated as a yellow-brown solid (0.80 g, 1.40 mmol, 52 %). m.p.: 28 - 35 ° C; Found C, 63.68; H, 5.43; N, 6.84 %. $C_{30}H_{31}N_3O_8$ requires C, 64.16; H, 5.56; N, 7.48 %; δ_H (400 MHz, $CDCl_3$): 8.81 (1H, d, $J = 8.04$ Hz, Ar-H7), 8.77 (1H, d, $J = 7.53$ Hz, Ar-H5), 8.68 (1H, d, $J = 8.03$ Hz, Ar-H2), 8.48 (1H, d, $J = 8.03$ Hz, Ar-H3), 7.98 (1H, t, $J = 7.52$ and 8.04 Hz, Ar-H6), 7.31 (5H, m,

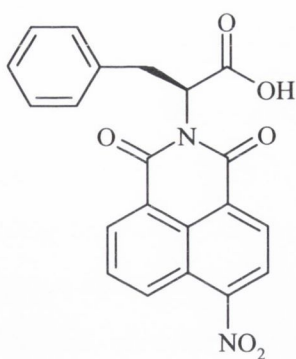
Ph), 5.97 (1H, m, $\underline{\text{CHCH}_2}$), 4.76 (1H, br. s, NH), 3.16 (2H, m, PhCH_2), 2.32 (2H, m, NHCH_2), 2.21 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.60 (2H, m, NHCH_2CH_2), 1.36 (9H, s, $(\text{CH}_3)_3$), 1.28 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$) ; δ_{C} (100 MHz, CDCl_3): 167.9, 162.6, 161.9, 155.9, 149.3, 146.6, 136.0, 134.3, 132.4, 131.7, 128.1, 127.6, 127.5, 123.8, 123.5, 123.2, 122.2, 76.8, 66.1, 54.0, 40.3, 28.9, 27.9, 27.5, 23.2 ; m/z : 281 ($\text{M} / 2$)⁺; $\nu_{\text{max}} / \text{cm}^{-1}$: 3765, 3670, 3567, 3466, 3329, 3218, 3109, 2975, 2936, 2893, 2836, 2791, 2680, 2607, 2546, 2406, 2345, 2274, 2028, 1918, 1869, 1801, 1779, 1720, 1648, 1596, 1455, 1431, 1394, 1342, 1302, 1191, 1102, 1040, 997, 972, 872, 755.

***N*–[(1*S*)–*tert*–Butoxycarbonyl–3–methylbutyl]–4–nitro–1,8–naphthalimide, 75** ¹⁰⁵



75 was synthesised according to **Procedure 1** using L-leucine *tert*-butyl ester hydrochloride (5 g, 22 mmol), and 4-nitro-1,8-naphthalic anhydride (5.35 g, 22 mmol, 1 eq.). This compound was yielded as a mustard-coloured solid (8.16 g, 19.6 mmol, 89 %). m.p.: 65 - 68 °C; Found C, 62.82; H, 6.03; N, 6.31 % $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_6 \cdot \text{C}_2\text{H}_6\text{O}$ requires C, 62.87; H, 6.59; N, 6.11 %; δ_{H} (400 MHz, CDCl_3): 8.98 (1H, d, $J = 8.64$ Hz, Ar-H7), 8.88 (1H, d, $J = 8.64$ Hz, Ar-H5), 8.78 (1H, d, $J = 7.92$ Hz, H2), 8.43 (1H, d, $J = 7.92$ Hz, H3), 8.03 (1H, t, $J = 7.50, 8.28$ Hz, Ar-H6), 5.68 (1H, m, $\underline{\text{CHCH}_2}$), 2.18 & 2.09 (1H + 1H, m, $(\text{CH}_3)_2\text{CHCH}_2$), 1.56 (1H, m, $\underline{\text{CH}}(\text{CH}_3)_2$), 1.40 (9H, s, $(\text{CH}_3)_3$), 1.01 (3H, d, $J = 6.78$ Hz, (CH_3)), 0.91 (3H, d, $J = 6.76$ Hz, (CH_3)); δ_{C} (100 MHz, CDCl_3): 168.4, 162.5, 161.8, 149.2, 134.6, 132.3, 130.3, 129.7, 129.5, 129.1, 128.8, 126.3, 124.1, 123.5, 123.2, 122.3, 81.6, 52.9, 37.5, 25.1, 23.0, 22.7, 21.6; m/z : 181.16 ($\text{M} + \text{K} + \text{H}_2\text{O} + \text{C}_7\text{H}_8$)³⁺, 534.52 ($\text{M} + \text{Et}_3\text{N} + \text{Na}$)⁺; $\nu_{\text{max}} / \text{cm}^{-1}$: 3070, 3004, 2973, 2947, 2866, 1735, 1709, 1626, 1594, 1582, 1531, 1471, 1451, 1425, 1406, 1394, 1368, 1344, 1272, 1256, 1230, 1188, 1159, 1147, 1129, 1100, 1075, 1039, 1007, 978, 956, 945, 934, 923, 901, 877, 851, 834, 787, 775.

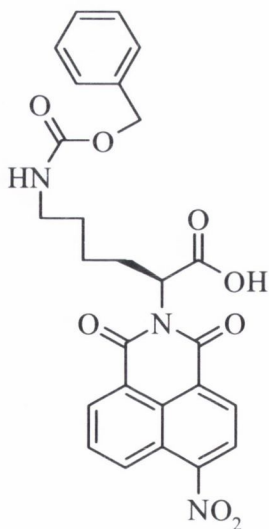
***N*–[(1*S*)–Carboxy–2–ethylphenyl]–4–nitro–1,8–naphthalimide, 77** ¹⁰⁵



73 (2.99 g, 6.70 mmol) was hydrolysed following **Procedure 2**. The product was obtained as a mustard-yellow crystalline solid (2.26 g, 6.00 mmol, 89 %) and was purified by titration in methanol. m.p.: 186 – 190 °C; Found: C, 60.17; H, 3.48; N, 6.28 %. $C_{21}H_{15}N_2O_6$.Mg requires C, 60.68; H, 3.64; N, 6.74 %; δ_H (400 MHz, $CDCl_3$): 8.82 (1H, d, $J = 8.52$ Hz,

Ar-H7), 8.67 (1H, d, $J = 7.52$ Hz, Ar-H5), 8.62 (1H, d, $J = 8.04$ Hz, Ar-H2), 8.38 (1H, d, $J = 8.04$ Hz, Ar-H3), 7.97 (1H, t, $J = 8.52$ and 7.52 Hz, Ar-H6), 7.12 (5H, m, Ph), 6.08 (1H, m, $\underline{CHCH_2}$), 3.69 (2H, m, $Ph\underline{CH_2}$); δ_C (100 MHz, $CDCl_3$): 174.3, 162.3, 161.5, 149.4, 136.1, 132.5, 129.9, 129.5, 129.3, 128.7, 128.6, 128.0, 126.4, 125.6, 123.4, 123.2, 121.7, 53.9, 34.1; m/z : 413 ($M + Na$)⁺; ν_{max} / cm^{-1} : 3656, 3629, , 3351, 3107, 3045, 2977, 2875, 2860, 2777, 2707, 2379, 2302,, 2226, 1910, 1856, 1791, 1704, 1624, 1607, 1551, 1450, 1394, 1299, 1179, 1140, 1063, 1029, 987, 905, 840, 802, 700, 683, 617.

***N*–[(1*S*)–Carboxy–6–benzyloxycarbonylaminohexyl]–4–nitro–1,8–naphthalimide, 78** ¹⁰⁵

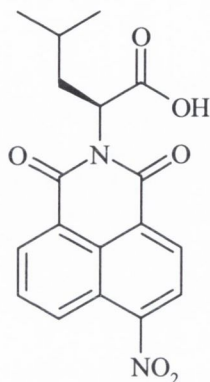


74 (0.25 g, 0.45 mmol) was hydrolysed according to **Procedure 2** yielding **78** as a brown hygroscopic solid (0.2 g, 0.40 mmol, 88 %). m.p.: 181 – 189 °C; δ_H (400 MHz, $CDCl_3$): 8.75 (1H, d, $J = 8.02$ Hz, Ar-H7), 8.67 (1H, d, $J = 7.53$ Hz, Ar-H5), 8.63 (1H, d, $J = 8.03$ Hz, Ar-H2), 8.57 (1H, d, $J = 8.03$ Hz, Ar-H3), 8.13 (1H, t, $J = 7.53$ and 8.02 Hz, Ar-H6), 7.63 (1H, br. s, NH), 7.32 (5H, m, Ph), 5.53 (1H, $\underline{CHCH_2}$, m), 4.48 (2H, s, $Ph\underline{CH_2}$), 2.73 (2H, m, $NH\underline{CH_2}$), 2.23 (2H, m, $NHCH_2CH_2CH_2\underline{CH_2}$), 1.56 (2H, m, $NHCH_2\underline{CH_2}$), 1.49 (2H, m, $NHCH_2CH_2\underline{CH_2}$) ; δ_C (100 MHz, $(CD_3)_2SO$: 162.9, 162.0, 156.0, 149.2, 137.3, 132.0, 130.3, 130.0, 129.0, 128.9, 128.4, 128.3, 127.7, 127.6,

126.5, 124.4, 122.8, 122.7, 65.0, 55.2, 40.0, 29.3, 28.6, 23.9; m/z : 506 ($M + H$)⁺; ν_{max} / cm^{-1} : 3567, 3466, 3218, 3109, 3019, 2893, 2836, 2680, 2607, 2546, 2406, 2345,

2274, 2028, 1918, 1869, 1801, 1779, 1648, 1596, 1431, 1394, 1342, 1303, 1250, 1191, 1159, 1102, 1040, 997, 972, 872, 755.

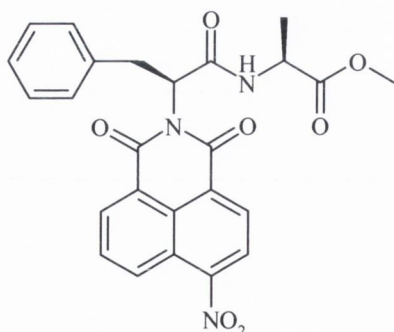
***N*–[(1*S*)–Carboxy–3–methylbutyl]–4–nitro–1,8–naphthalimide, 79** ¹⁰⁵



75 was hydrolysed to form **79** (6.74 g, 16.34 mmol) following **Procedure 2**. The product was obtained as a mustard-yellow crystalline solid (5.50 g, 15.44 mmol, 95 %) and was purified by titration in methanol. m.p.: 63 - 65 °C; HRMS: 379.0915 ($[M + Na]^+$, $C_{18}H_{16}N_2O_6$ requires 356.34); δ_H (400 MHz, $CDCl_3$): 8.88 (1H, d, $J = 8.64$ Hz, Ar-H7), 8.76 (1H, d, $J = 7.50$ Hz, Ar-H5), 8.72 (1H, d, $J = 7.92$ Hz, Ar-H2), 8.43 (1H, d, $J = 8.28$ Hz, Ar-H3), 8.02 (1H, t, $J = 7.56, 8.64$ Hz, Ar-H6), 5.84 (1H, m, $\underline{CHCH_2}$), 2.25 & 2.11 (1H + 1H, m, $(CH_3)_2CH\underline{CH_2}$),

1.55 (1H, m, $\underline{CH}(CH_3)_2$), 1.01 (3H, d, $J = 6.36$ Hz, (CH_3)), 0.95 (3H, d, $J = 6.78$ Hz, (CH_3)); δ_C (100 MHz, $CDCl_3$) : 175.4, 162.8, 162.0, 149.7, 146.1, 132.8, 132.0, 130.3, 129.9, 126.3, 123.8, 122.4, 51.9, 37.6, 25.2, 22.9, 21.8, 14.0; m/z : 379.09 ($M + Na$)⁺; ν_{max} / cm^{-1} : 3567, 3466, 3218, 3109, 3019, 2893, 2836, 2680, 2607, 2546, 2406, 2345, 2274, 2028, 1918, 1869, 1801, 1779, 1648, 1596, 1431, 1394, 1342, 1303, 1275, 1250, 1191, 1159, 1102, 1040, 997, 972, 955, 939, 872, 788, 775, 755.

***N*–{(1*S*)–[(2*S*)–Propionic acid methyl ester]–carboxyamido–2–ethylphenyl}–4–nitro–1,8–naphthalimide, 83** ¹⁰⁵

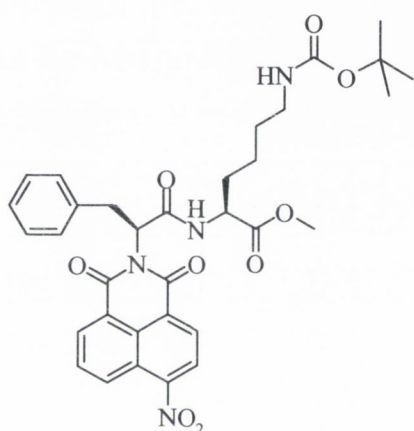


77 (0.2 g, 0.50 mmol) was reacted with L-alanine methyl ester hydrochloride (0.07 g, 0.50 mmol, 1 eq.) using HOBt (0.07 g, 0.50 mmol, 1 eq.) and EDCI (0.105 g, 0.55 mmol, 1.1 eq.), in the presence of Et_3N (0.0006 mL, 0.50 mmol, 1 eq.), according to **Procedure 3**. The desired compound was obtained as brown crystals (0.2 g, 0.42 mmol, 76 %) after purification by

recrystallisation from CH_2Cl_2 . m.p.: 89 °C; Found C, 62.23; H, 4.98; N, 9.57 %. $C_{25}H_{21}N_3O_7$ Na.DMAP requires C, 61.93; H, 5.03; N, 11.28 %; δ_H (400 MHz, $CDCl_3$): 8.83 (1H, d, $J = 8.20$ Hz, Ar-H7), 8.67 (1H, d, $J = 7.52$ Hz, Ar-H5), 8.61

(1H, d, $J = 8.16$ Hz, Ar-H2), 8.38 (1H, d, $J = 8.16$ Hz, Ar-H3), 7.97 (1H, t, $J = 8.88$ and 7.52 Hz, Ar-H6), 7.17 (5H, m, Ph), 6.05 (1H, m, $\underline{\text{CHCH}_2}$), 4.68 (1H, m, $\underline{\text{CHCH}_3}$), 3.73 (3H, s, OCH_3), 3.58 (2H, m, $\text{Ph}\underline{\text{CH}_2}$), 1.36 (3H, s, CH_3) ; δ_{C} (100 MHz, CDCl_3) : 173.9, 162.3, 161.5, 149.4, 136.1, 132.5, 129.9, 129.5, 129.3, 128.7, 128.6, 128.0, 126.4, 125.7, 123.4, 123.2, 121.8, 54.0, 34.1; m/z : 514.11 ($\text{M} + \text{K}$)⁺; ν_{max} / cm^{-1} : 3362, 3028, 2948, 1741, 1713, 1973, 1673, 1594, 1583, 1531, 1495, 1455, 1410, 1342, 1228, 1154, 1057, 984, 916, 865, 834, 786, 759, 716, 700, 657, 583.

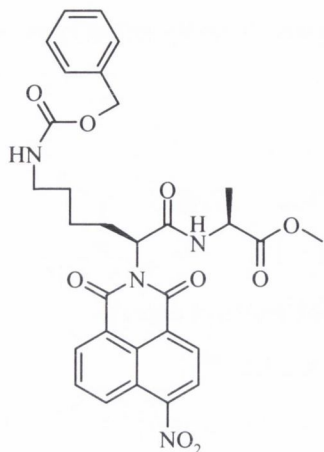
***N*-{(1*S*)-[(2*S*)-6-*tert*-Butoxycarbonylamino-hexanoic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimide, **84** ¹⁰⁵**



77 (1.00 g, 2.50 mmol) was coupled to Boc-L-lysine methyl ester hydrochloride (0.76 g, 2.50 mmol, 1 eq.) according to **Procedure 3**, using HOBT (0.35 g, 2.50 mmol, 1 eq.) and EDCI (0.26 g, mmol, 1.1 eq.), in the presence of Et_3N (0.40 ml, 2.75 mmol, 1 eq.). The desired product was isolated as a hygroscopic brown solid (1.15 g, 1.90 mmol, 76 %). m.p.: 74 °C; Found C, 62.00; H, 5.83; N, 9.15 %.

$\text{C}_{33}\text{H}_{37}\text{N}_4\text{O}_9$ requires C, 62.55 %; H, 5.89 %; N, 8.84 %; δ_{H} (400 MHz, CDCl_3): 8.85 (1H, d, $J = 8.04$ Hz, Ar-H7), 8.71 (1H, d, $J = 7.06$ Hz, Ar-H5), 8.62 (1H, d, $J = 7.52$ Hz, Ar-H2), 8.36 (1H, d, $J = 7.30$ Hz, Ar-H3), 7.98 (1H, t, $J = 10.04$, 8.06 Hz, Ar-H6), 7.17 (5H, m, Ph), 6.48 (1H, m, $\underline{\text{CHCH}_2\text{Ph}}$), 6.08 (1H, m, $\text{NH}\underline{\text{CHCH}_2}$), 4.67 (2H, br. s, NH), 3.78 (2H, m, $\text{Ph}\underline{\text{CH}_2}$), 3.57 (3H, s, OCH_3), 3.05 (2H, m, $\text{NH}\underline{\text{CH}_2}$), 1.84 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\underline{\text{CH}_2}$), 1.56 (2H, m, $\text{NHCH}_2\text{CH}_2\underline{\text{CH}_2}$), 1.45 (9H, s, $(\text{CH}_3)_3$), 1.22 (2H, m, $\text{NHCH}_2\underline{\text{CH}_2}$); δ_{C} (100 MHz, CDCl_3): 173.1, 168.1, 162.5, 155.5, 136.7, 136.6, 132.2, 129.7, 129.5, 128.8, 126.9, 126.3, 126.0, 125.5, 123.5, 122.9, 122.2, 78.5, 65.4, 55.3, 51.5, 39.9, 33.9, 31.4, 28.7, 27.9, 21.8; m/z : 655 ($\text{M} + \text{Na} + \text{H}$)²⁺ ; ν_{max} / cm^{-1} : 3601, 3191, 3110, 3065, 3001, 2973, 2865, 2410, 2331, 2197, 2018, 1869, 1792, 1724, 1627, 1584, 1473, 1410, 1343, 1241, 1165, 864, 810, 701.

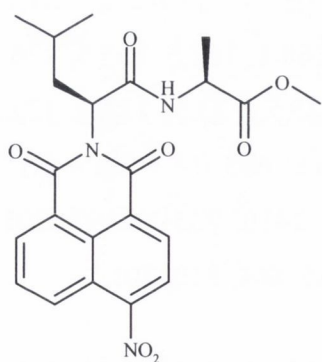
N*-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-6-benzylloxycarbonylaminohexyl}-4-nitro-1,8-naphthalimide, **85*



78 (0.5 g, 0.10 mmol) was reacted with L-alanine methyl ester hydrochloride (0.14 g, 0.10 mmol, 1 eq.) following **Procedure 3** using HOBt (0.14 g, 0.10 mmol, 1 eq.) and EDCI (0.2 g, 0.11 mmol, 1.1 equiv.) in the presence of Et₃N. **85** was yielded as a hygroscopic brown solid (0.036 g, 0.062 mmol, 62 %). m.p.: 58 °C; HRMS: 613.1921 ([M+Na]⁺, C₃₀H₃₀N₄O₉ requires 590.60); δ_H (400 MHz, CDCl₃): 8.85 (1H, d, J = 8.03 Hz, Ar-H7), 8.74 (1H, d, J = 7.51 Hz, Ar-H5), 8.65 (1H, d, J = 8.01 Hz, Ar-H2), 8.36 (1H, d, J = 8.02

Hz, Ar-H3), 7.97 (1H, m, Ar-H6), 7.30 (5H, m, Ph), 6.56 (1H, m, NH), 5.70 (2H, m, CHCH₂), 4.98 (2H, s, PhCH₂), 4.64 (1H, m, CHCH₃), 3.73 (3H, s, OCH₃), 3.16 (2H, m, NHCH₂), 2.29 (2H, m, NHCH₂CH₂CH₂CH₂), 1.48 (2H, m, NHCH₂CH₂), 1.29 (3H, s, CH₃), 0.89 (2H, m, NHCH₂CH₂CH₂); δ_C (100 MHz, CDCl₃) : 173.1, 167.8, 162.9, 162.1, 156.7, 149.3, 136.0, 132.5, 129.9, 129.5, 129.2, 128.8, 128.1, 128.0, 127.6, 127.5, 126.1, 123.4, 123.2, 122.2, 66.1, 54.4, 52.0, 47.9, 40.1, 28.9, 27.4, 23.0, 17.9; *m/z*: 613 (M + Na)⁺; ν_{max} / cm⁻¹: 3054, 2928, 2305, 1707, 1685, 1654, 1636, 1458, 1421, 1265, 896, 738, 705, 670.

***N*-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-3-methylbutyl}-4-nitro-1,8-naphthalimide, **86**¹⁰⁵**

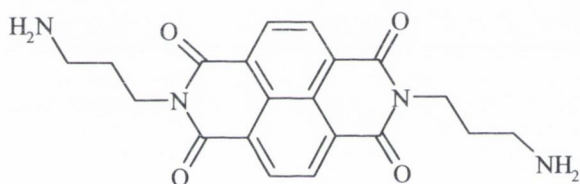


79 (5 g, 14 mmol) was reacted with L-alanine methyl ester hydrochloride (1.95 g, 14 mmol, 1 eq.) using HOBt (1.89 g, 14 mmol, 1 eq.) and EDCI (2.88 g, 15 mmol, 1.1 eq.), in the presence of Et₃N (1.72 mL, 15 mmol, 1 eq.), according to **Procedure 3**. The desired compound was obtained as brown crystals (5.30 g, 12 mmol, 86 %) after purification by recrystallisation from CH₂Cl₂. m.p.: 82 °C; Found C, 57.45; H, 5.65; N, 8.30 %. C₂₂H₂₃N₃O₇·Na·C₂H₆O requires C, 56.47;

H, 5.73; N, 8.23 %; δ_H (600 MHz, CDCl₃): 8.86 (1H, d, J = 8.70 Hz, Ar-H7), 8.75

(1H, d, J = 7.20 Hz, Ar-H5), 8.70 (1H, d, J = 7.92 Hz, Ar-H2), 8.41 (1H, d, J = 8.28 Hz, Ar-H3), 8.01 (1H, t, J = 7.50, 8.28 Hz, Ar-H6), 6.64 (1H, m, $\underline{\text{CHCH}_2}$), 4.65 (1H, m, $\underline{\text{CHCH}_3}$), 3.70 (3H, m, OCH₃), 2.36 & 2.07 (1H + 1H, m, (CH₃)₂CH $\underline{\text{CH}_2}$), 1.54 (1H, m, $\underline{\text{CH}}(\text{CH}_3)_2$), 1.45 (3H, m, CH₃), 1.03 (3H, d, J = 6.52 Hz, (CH₃)), 0.96 (3H, d, J = 6.52 Hz, (CH₃)); δ_{C} (100 MHz, CDCl₃): 173.5, 168.7, 162.7, 162.3, 149.5, 132.8, 132.2, 129.8, 129.4, 129.2, 126.6, 123.8, 123.5, 122.6, 53.5, 52.4, 48.3, 37.3, 25.5, 23.2, 21.8, 18.3; m/z: 534.49 (M + Na + C₄H₈O)⁺; ν_{max} / cm⁻¹: 3565, 3459, 3217, 3109, 3019, 2892, 2834, 2670, 2605, 2542, 2404, 2342, 2269, 2026, 1918, 1869, 1801, 1775, 1652, 1594, 1435, 1392, 1346, 1301, 1255, 1197, 1158, 1105, 1046, 999, 975, 870, 750.

2,7-bis-(3-aminopropyl)-benzo[1*mn*][3, 8] phenanthroline-1, 3, 6, 8-tetraone or *N,N*-bis-3-aminopropyl naphthalene diimide, 87



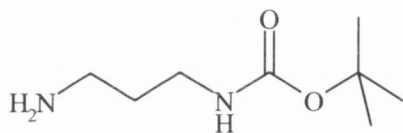
a) Naphthalene tetracarboxylic dianhydride (1.43 g, 5.30 mmol) was reacted with 1,3-diamino propane (22 ml, 0.27 mmol, in excess) in anhydrous THF according to

Procedure 4a. A lengthy precipitation from diethyl ether allowed the isolation of a red solid (1.99 g, 5.00 mmol, 94 %).

b) **91** (0.05 g, 86 mmol) was hydrolysed according to **Procedure 2**. The desired product was obtained as an orange-brown crystalline solid, by precipitation from diethyl ether (0.027 g, 55.00 mmol, 64 %). m.p.: 159 – 170 °C decomp.; HRMS : 381.1563 ($[M + H]^+$, $C_{20}H_{20}N_4O_4$ requires 380.1484); δ_H (400 MHz, $(CD_3)_2SO$): 8.69 (4H, s, Ar-H), 4.94 (4H, br. s, NH_2), 4.14 (4H, t, $J = 7.04$, 6.78 Hz, $NH_2CH_2CH_2CH_2$), 2.96 (4H, m, NH_2CH_2), 2.00 (4H, m, $NH_2CH_2CH_2$); δ_C (100 MHz, $(CD_3)_2SO$) : 163.3, 130.8, 126.6, 79.3, 49.1, 39.6, 25.4; m/z : 381.16 ($M + H$) $^+$; ν_{max} / cm^{-1} : 3691, 3536, 3356, 3413, 3335, 3132, 3005, 2974, 2869, 2744, 2664, 2579, 2345, 2105, 1993, 1960, 1871, 1810, 1757, 1701, 1627, 1603, 1529, 1500, 1410, 1378, 1345, 1294, 1154, 1020, 981, 883, 722, 664.

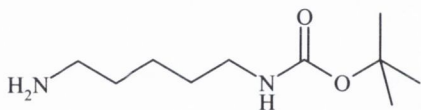
N-(*tert*-Butoxycarbonyl)-1,3-diaminopropane or *N*-Boc-1,3-diaminopropane,

88



1,3-diamino propane (1.08 ml, 13.00 mmol) was protected using Boc_2O (0.72 g, 3.30 mmol, 0.25 eq.) according to **Procedure 6**. The product was initially obtained as a cream oil, which solidified into a white-cream solid (1.59 g, 10.00 mmol, 77 %). m.p.: 82 – 94.4 °C; HRMS: 175.1411 ($[M + H]^+$, $C_8H_{18}N_2O_2$ requires 174.1424); δ_H (400 MHz, $CDCl_3$): 5.25 (1H, br. s, NH), 3.06 (2H, m, $NH_2CH_2CH_2CH_2$), 2.62 (2H, m, NH_2CH_2), 1.48 (2H, m, $NH_2CH_2CH_2$), 1.31 (9H, s, $(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) : 155.8, 78.7, 38.5, 37.6, 36.8, 27.6; m/z : 175.14 ($M + H$) $^+$; ν_{max} / cm^{-1} : 3154, 3112, 3863, 2746, 2701, 2420, 2345, 2205, 2078, 1958, 1618, 1481, 1335, 1213, 1061, 1003, 896, 782, 743, 561.

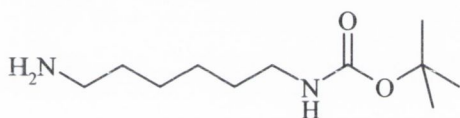
N-(*tert*-Butoxycarbonyl)-1,5-diaminopentane or *N*-Boc-1,5-diaminopentane, 89



1,5-diamino pentane (2.3 ml, 20.00 mmol) was protected using Boc_2O (1.09 g, 5.00 mmol, 0.25 eq.) following **Procedure 6**. The desired product was obtained firstly as an intensely yellow oil, then as a gel and finally as a strong coloured semi-solid (2.91 g, 14.00

mmol, 72 %). m.p.: 79 °C; HRMS: 203.1760 ($[M + H]^+$, $C_{10}H_{22}N_2O_2$ requires 202.3010); Found C, 56.79; H, 10.29; N, 11.55 %. $C_{10}H_{22}N_2O_2 \cdot H_2O \cdot C_3H_6O$ requires C, 55.97; H, 11.94; N, 11.85 %; δ_H (400 MHz, $CDCl_3$): 4.86 (2H, br. s, NH_2), 4.70 (1H, br. s, NH), 3.54 (2H, m, $NH_2CH_2CH_2CH_2CH_2CH_2$), 3.18 (2H, m, NH_2CH_2), 2.58 (4H, m, $NH_2CH_2CH_2$ and $NH_2CH_2CH_2CH_2CH_2$), 1.22 (9H, s, $(CH_3)_3$), 1.11 (2H, m, $NH_2CH_2CH_2CH_2$); δ_C (100 MHz, $CDCl_3$): 124.4, 73.1, 41.4, 40.2, 32.5, 31.4, 28.3, 25.1; m/z : 203 ($M + H$) $^+$; ν_{max} / cm^{-1} : 2926, 2256, 1925, 1689, 1456, 1379, 1328, 1275, 1174, 1090, 1050, 881, 804.

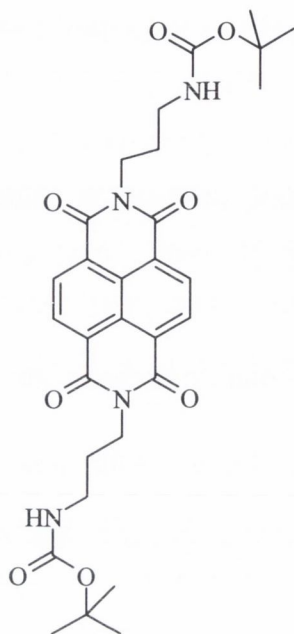
N-(*tert*-Butoxycarbonyl)-1,6-diaminohexane or *N*-Boc-1,6-diaminohexane, 90



1,6-diamino hexane (4.0 g, 34.00 mmol) was Boc-protected (1.87 g, 8.60 mmol, 0.25 equiv.) using **Procedure 6**. The product was successfully isolated as a

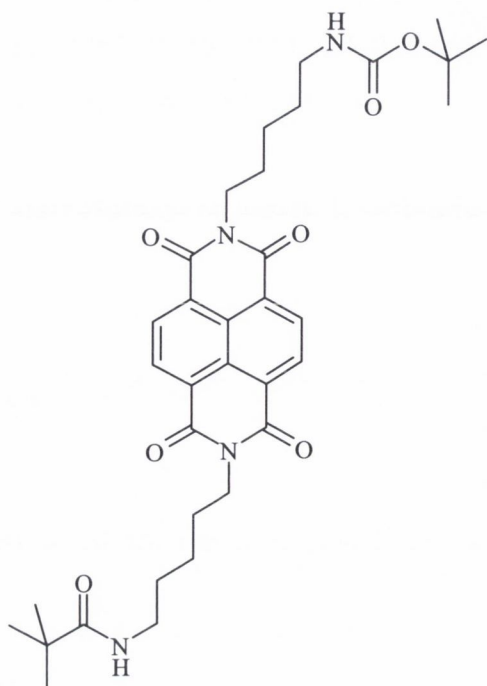
yellow oil, which in turn solidified to a gel and then to a white-cream semi-solid (5.00 g, 23.00 mmol, 68 %). m.p.: 83 °C; HRMS : 217.1916 ($[M + H]^+$, $C_{11}H_{24}N_2O_2$ requires 216.3311); δ_H (400 MHz, $CDCl_3$) : 4.91 (2H, br. s, NH_2), 2.97 (2H, m, $NH_2CH_2CH_2CH_2CH_2CH_2CH_2$), 2.57 (2H, m, NH_2CH_2), 1.47 (4H, m, $NH_2CH_2CH_2$ and $NH_2CH_2CH_2CH_2CH_2CH_2CH_2$), 1.33 (9H, s, $(CH_3)_3$), 1.22 (4H, m, $NH_2CH_2CH_2CH_2$ and $NH_2CH_2CH_2CH_2CH_2CH_2$); δ_C (100 MHz, $CDCl_3$): 162.2, 155.9, 77.6, 63.1, 41.7, 40.4, 33.3, 29.8, 26.4; m/z : 217.19 ($M + H$) $^+$; ν_{max} / cm^{-1} : 3370, 2982, 2939, 2869, 2284, 1687, 1522, 1481, 1389, 1365, 1341, 1276, 1251, 1222, 1173, 1137, 1049, 1016, 994, 977, 920, 870, 821, 780, 765, 728, 601.

2,7-bis-{N-[N'-(*tert*-Butoxycarbonyl)-3-aminopropyl]-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 91



Naphthalene tetracarboxylic dianhydride (0.057 g, 0.20 mmol.) was reacted with **88** (0.3 g, 1.70 mmol, 8 eq.) following **Procedure 4b**. The product was isolated by precipitation as a pink solid (0.08 g, 0.14 mmol, 72 %). m.p.: 210 – 215 °C; HRMS: 603.2431 ($[M + Na]^+$), $C_{30}H_{36}N_4O_8$ requires 580.6412; Found C, 61.87; H, 6.26; N, 9.44 %. $C_{30}H_{37}N_4O_8$ requires C, 61.95; H, 6.41; N, 9.63 %; δ_H (400 MHz, $CDCl_3$): 8.78 (8H, s, Ar-H), 5.12 (2H, br. s, NH), 4.31 (4H, t, $J = 6.53, 6.52$ Hz, $NHCH_2CH_2CH_2$), 3.21 (4H, m, $NHCH_2$), 1.97 (4H, m, $NHCH_2CH_2$), 1.45 (18H, s, $(CH_3)_3$); δ_C (100 MHz, $CDCl_3$) : 162.6, 155.5, 132.1, 126.3, 126.1, 78.8, 37.8, 37.2, 28.1, 27.9 ; m/z : 603.24 ($M + Na$) $^+$; ν_{max} / cm^{-1} : 3613, 3223, 3122, 3045, 3031, 3011, 2874, 2784, 2663, 2546, 2344, 2291, 1993, 1918, 1836, 1757, 1602, 1517, 1448, 1430, 1332, 1216, 1112, 1017, 927, 807, 756, 728.

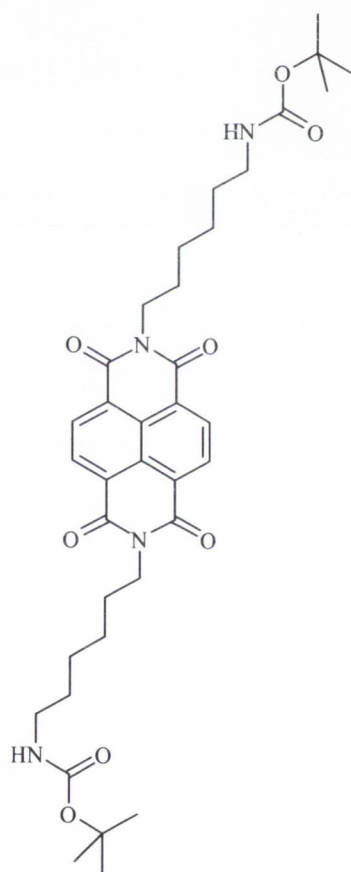
2,7-bis-{N-[N'-(*tert*-Butoxycarbonyl)-5-aminopentyl]-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 92



Naphthalene tetracarboxylic dianhydride (0.10 g, 0.38 mmol) was reacted with **89** (0.50 g, 2.60 mmol, 7 eq.) according to **Procedure 4b**. The product was isolated as a red-pink solid following a lengthy precipitation from diethyl ether (0.20 g, 0.32 mmol, 83 %). m.p.: 163 – 175 °C; HRMS : 659.3057 ($[M + Na]^+$, $C_{34}H_{44}N_4O_8$ requires 636.7512); Found C, 60.44; H, 6.71; N, 8.83 %. $C_{34}H_{44}N_4O_8 \cdot K$

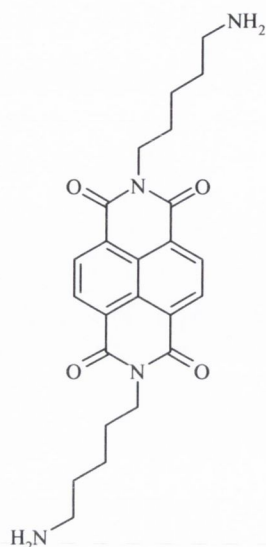
requires C, 61.89; H, 6.72; N, 8.49 %; δ_{H} (400 MHz, CDCl_3): 8.77 (4H, s, Ar-H), 4.59 (2H, br. s, NH), 4.22 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.14 (4H, br. m, NHCH_2), 2.18 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.55 (4H, m, NHCH_2CH_2), 1.44 (18H, s, $(\text{CH}_3)_3$), 1.36 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$); δ_{C} (100 MHz, CDCl_3): 162.8, 154.5, 126.6, 124.9, 117.0, 77.2, 40.6, 29.6, 28.1, 27.7, 24.1; m/z : 659.31 ($\text{M} + \text{Na}$)⁺; ν_{max} / cm^{-1} : 3701, 3509, 3201, 3086, 3039, 2967, 2870, 2760, 2683, 2479, 2363, 1975, 1923, 1792, 1686, 1603, 1581, 1483, 1405, 1365, 1307, 1221, 1097, 1043, 942, 870, 808, 735.

2,7-bis- $\{N-[N'-(tert\text{-}Butoxycarbonyl)-6\text{-aminohexyl}]\text{-benzo}[1mn][3,8]\text{-phenanthroline-1,3,6,8-tetraone, 93}$



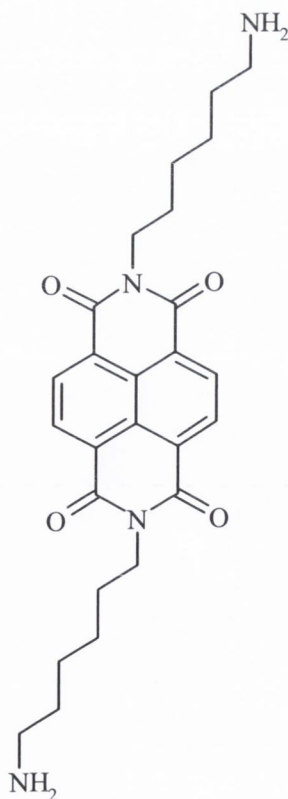
Naphthalene tetracarboxylic dianhydride (0.27 g, 1.00 mmol) and **90** (1.5 g, 7.00 mmol, 7 eq.) were reacted according to **Procedure 4b**, in the presence of DMAP (1 eq.). The product was obtained as a peach-pink solid (0.44 g, 0.65 mmol, 65 %). m.p.: 240 °C decomp.; HRMS: 687.3378 ($[\text{M} + \text{Na}]^+$ $\text{C}_{36}\text{H}_{48}\text{N}_4\text{O}_8$ requires 664.8123); Found C, 63.92; H, 7.29; N, 8.52 %. $\text{C}_{36}\text{H}_{48}\text{N}_4\text{O}_8 \cdot \text{MeOH}$ requires C, 63.77; H, 7.52; N, 8.04 %; δ_{H} (400 MHz, CDCl_3): 8.83 (4H, s, H), 3.75 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.52 (4H, m, NHCH_2), 1.91 (8H, m, NHCH_2CH_2 and $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.53 (8H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$ and $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.45 (18H, br. s, $(\text{CH}_3)_3$); δ_{C} (100 MHz, CDCl_3): 168.5, 160.5, 132.1, 129.6, 124.9, 122.2, 28.9, 28.6, 27.7, 26.7, 26.6; ν_{max} / cm^{-1} : 3052, 2925, 2854, 2305, 1462, 1377, 1264, 895, 742, 706.

***N,N'*-bis-5-aminopentyl] naphthalene diimide, 94**



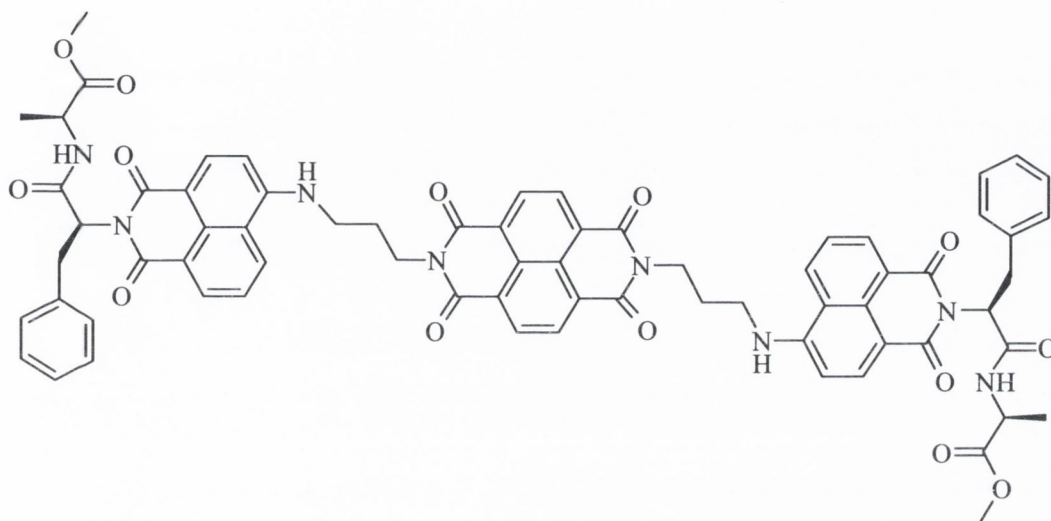
92 (0.165 g, 0.26 mmol) was converted to **94** according to **Procedure 2**, the product was obtained as a red hygroscopic solid (0.07 g, 0.12 mmol, 47 %). m.p.: 102 °C; HRMS : 437.2189 ($[M + H]^+$, $C_{24}H_{28}N_4O_4$ requires 436.5211); δ_H (400 MHz, $(CD_3)_2SO$): 8.67 (4H, s, Ar-H), 4.18 (2H, br. s, NH_2), 4.07 (4H, t, $J = 7.04, 7.04$ Hz, $NH_2CH_2CH_2CH_2CH_2CH_2$), 2.77 (4H, m, NH_2CH_2), 1.56 (4H, m, $NH_2CH_2CH_2CH_2CH_2$), 1.37 (4H, m, $NH_2CH_2CH_2$), 1.23 (4H, m, $NH_2CH_2CH_2CH_2$); δ_C (100 MHz, $CDCl_3$): 163.0, 158.1, 130.8, 126.7, 38.5, 27.3, 26.7, 23.7, 22.9; m/z : 437 ($M + H$) $^+$; ν_{max} / cm^{-1} : 3166, 2907, 2725, 2670, 2411, 2303, 2182, 2033, 1709, 1460, 1376, 1304, 1168, 1076, 966, 890, 844, 722.

***N,N'*-bis-6-aminohexyl naphthalene diimide, 95**



93 (0.1 g, 0.15 mmol) was hydrolysed following **Procedure 2**, and the desired product was isolated as a brown oil (0.064 g, 0.11 mmol, 74 %). m.p.: 110 °C decomp.; δ_H (400 MHz, $(CD_3)_2SO$): 8.69 (4H, s, Ar-H), 7.76 (4H, br. s, NH_2), 3.85-3.65 (8H, m, $NH_2CH_2CH_2CH_2CH_2CH_2CH_2$ and NH_2CH_2), 2.77 (4H, m, $NH_2CH_2CH_2CH_2CH_2CH_2$), 1.37 (4H, m, $NH_2CH_2CH_2$), 1.24 (8H, m, $NH_2CH_2CH_2CH_2$ and $NH_2CH_2CH_2CH_2CH_2$); δ_C (100 MHz, $CDCl_3$): 162.8, 158.8, 158.6, 130.7, 126.5, 126.4, 40.4, 40.2, 39.4, 39.0, 27.6, 27.2, 26.4; m/z : 578.26 ($M + DMAP$) $^+$; ν_{max} / cm^{-1} : 2923, 2853, 2725, 2669, 2399, 2181, 2033, 1864, 1712, 1631, 1461, 1376, 1304, 1207, 1154, 1075, 1029, 937, 890, 843, 721.

2,7-bis-{N-[N'-{(1*S*)-(2*S*)-Propionic acid methyl ester]-carboxymido-2-ethylphenyl}-1,8-naphthalimido]-3-aminopropyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 96



a) **96** was synthesised according to **Procedure 5a** by reacting **87** (0.076 g, 0.20 mmol) with **83** (0.19 g, 0.40 mmol, 2 eq.). The product was isolated, after recrystallisation from CH₂Cl₂, as a brown crystalline solid (0.045 g, 0.036 mmol, 18 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.023 g, 0.022 mmol, 11 %)

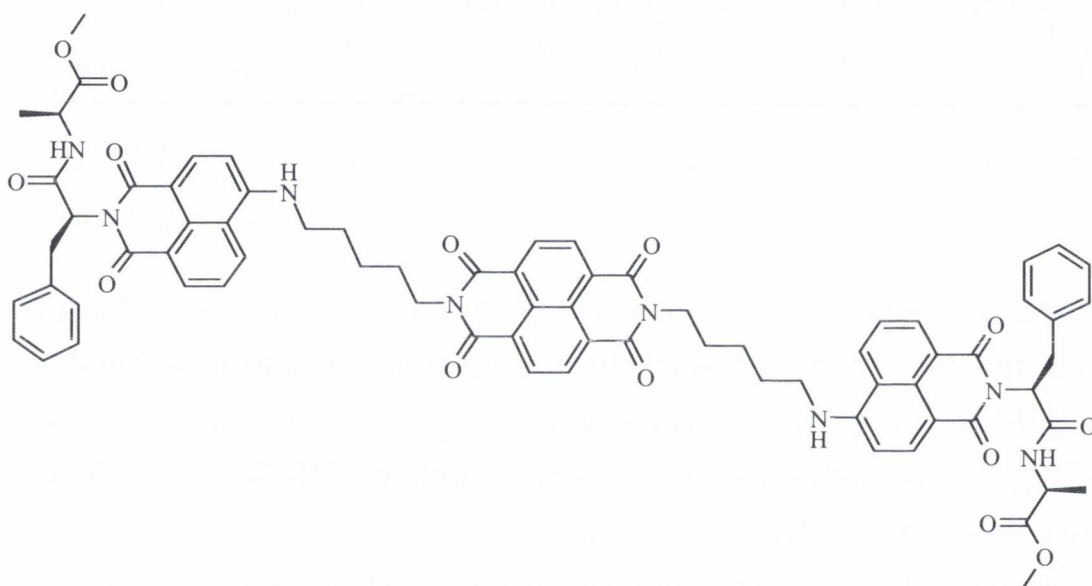
b) Naphthalene tetracarboxylic dianhydride (0.005 g, 0.02 mmol) was refluxed with **107** (0.02 g, 0.04 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a richly brown solid (0.0043 g, 0.0035 mmol, 17 %).

c) **96** was synthesised according to **Procedure 5d** by reacting **87** (0.14 g, 0.37 mmol) with **83** (0.35 g, 0.74 mmol, 2 eq.). The recrystallised product was isolated as a brown crystalline solid (0.17 g, 0.098 mmol, 24 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.12 g, 0.069 mmol, 17 %)

m.p.: 105 °C; Found C, 62.87; H, 4.67; N, 8.46 %. C₇₀H₆₀N₈O₁₄.CH₂Cl₂.MeOH.2H₂O requires C, 62.20; H, 5.07; N, 8.06 %; δ_H (600 MHz, (CD₃)₂SO): 8.72 (2H, d, J = 8.82 Hz, Ar-H7), 8.56 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.34 (2H, d, J = 7.32 Hz, Ar-H3), 8.09 (2H, t, J = 8.40 and 7.74 Hz, Ar-H6), 7.07 (10H, m, Ph), 5.73 (2H, m, CHCH₂), 4.42 (2H, m, CHCH₃), 3.63 (6H, s, OCH₃), 3.18 (4H, d, J = 5.10 Hz, PhCH₂), 2.62 (4H, m, NHCH₂CH₂CH₂), 2.40 (4H, m, NHCH₂), 1.25 (6H, s, CH₃),

1.15 (4H, m, NHCH₂CH₂); δ_C (100 MHz, CDCl₃): 173.4, 168.3, 162.8, 138.4, 132.1, 130.4, 130.0, 129.3, 128.4, 126.5, 124.6, 123.0, 123.1, 55.3, 55.2, 52.2, 48.1, 40.5, 34.2, 17.2 ; m/z : 1237.30 (M + H)⁺; $\nu_{\max}$ / cm⁻¹ 3362, 3065, 3027, 2852, 2361, 1942, 1869, 1744, 1711, 1671, 1584, 1453, 1351, 1245, 1160, 1126, 1056, 1028, 985, 904, 728.

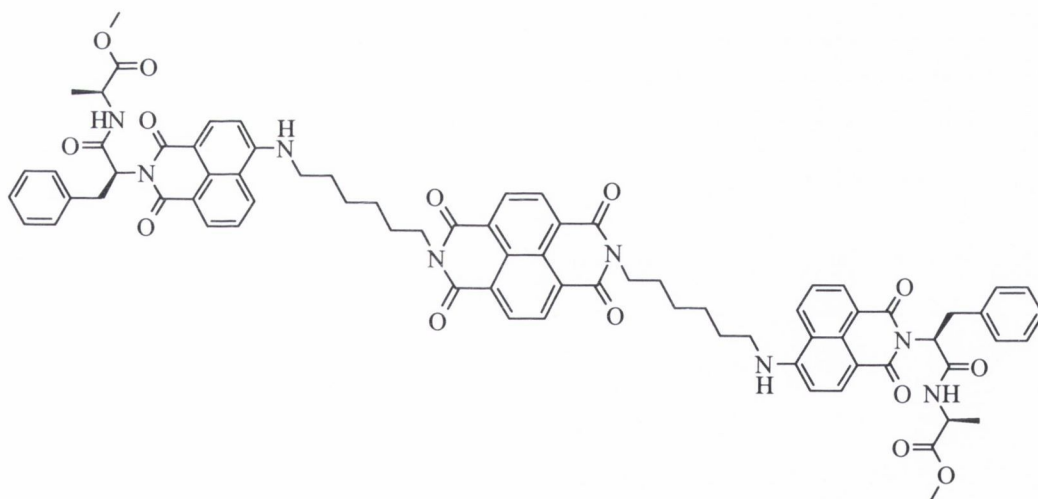
2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-1,8-naphthalimido]-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 97



- a) **94** (0.15 g, 0.28 mmol) was reacted with **83** (0.26 g, 0.55 mmol, 2 equiv.) according to **Procedure 5a**, in the presence of DMAP (0.017 g, 0.14 mmol, 0.5 eq.). The desired product was obtained as a mustard-brown solid (0.0845 g, 0.065 mmol, 23 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.031 g, 0.025 mmol, 15 %)
- b) **94** (0.30 g, 0.56 mmol) was reacted with **83** (0.52 g, 1.10 mmol, 2 equiv.) according to **Procedure 5c**, in the presence of DMAP (0.034 g, 0.28 mmol, 0.5 eq.). The desired product was obtained as a mustard-brown solid after recrystallisation (0.21 g, 0.16 mmol, 29 %)
- c) **94** (0.07 g, 0.16 mmol) was reacted with **83** (0.15 g, 0.32 mmol, 2 equiv.) according to **Procedure 5d**, in the presence of DMAP (0.0078 g, 0.06 mmol, 0.4 eq.). The product was obtained as a mustard-brown solid (0.062 g, 0.048 mmol, 30 %,

following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.043 g, 0.034 mmol, 21 %). m.p.: 85 °C; Found C, 62.31; H, 4.87; N, 7.96; C₇₄H₆₈N₈O₁₄·2CH₂Cl₂ requires C, 62.38 %, H, 4.96 %, N, 7.66 %; δ_{H} (600 MHz, (CD₃)₂SO): 8.72 (2H, d, J = 8.19 Hz, Ar-H7), 8.56 (8H, m, Ar-H5 and Ar-H2 and Ar-H), 8.35 (2H, d, J = 8.16 Hz, Ar-H3), 8.09 (2H, t, J = 8.84, 7.50 Hz, Ar-H6), 7.06 (10H, m, Ph), 5.72 (4H, m, CHCH₃), 4.42 (4H, m, CHCH₃), 3.63 (6H, s, OCH₃), 3.18 (4H, d, J = 4.38 Hz, PhCH₂), 2.62 (4H, m, NHCH₂CH₂CH₂CH₂CH₂), 2.40 (4H, m, NHCH₂), 1.25 (6H, s, CH₃), 1.15 (8H, m, NHCH₂CH₂CH₂CH₂ and NHCH₂CH₂), 1.01 (4H, m, NHCH₂CH₂CH₂); δ_{C} (100 MHz, (CD₃)₂SO): 173.12, 168.02, 162.79, 162.03, 149.22, 138.03, 131.77, 130.30, 130.27, 130.19, 129.67, 129.05, 128.99, 128.31, 128.08, 126.54, 126.24, 124.34, 122.75, 122.70, 55.04, 51.90, 48.80, 47.79, 33.86, 16.88, 14.25; ν_{max} / cm⁻¹: 3362, 3104, 3104, 3027, 2951, 2873, 2742, 2579, 2463, 2364, 1830, 1744, 1711, 1627, 1584, 1497, 1410, 1260, 1228, 1154, 1181, 1077, 1029, 970, 916, 905, 853, 811, 717, 669.

2,7-bis-{N-[N'-{(1S)-[(2S)-Propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1mn][3,8]-phenanthroline-1,3,6,8-tetraone, 98

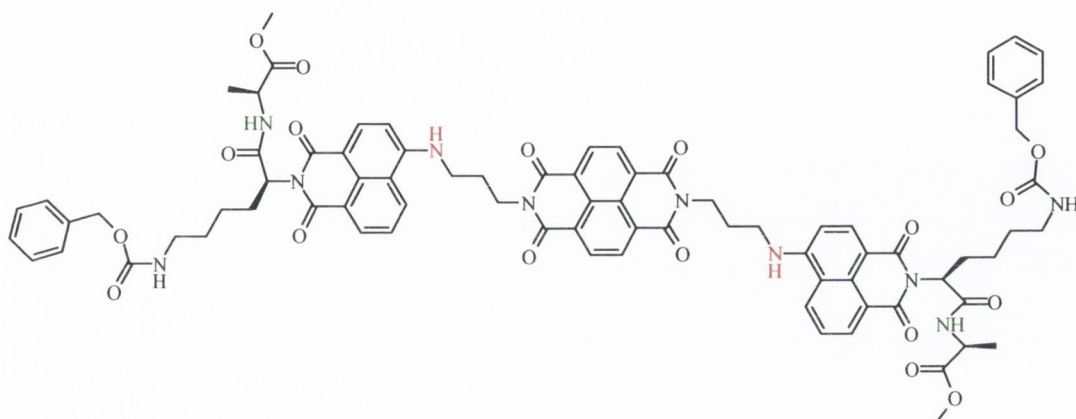


a) **95** (0.025g, 0.54 mmol) was reacted with **83** (0.52 g, 1.1 mmol, 2 equiv.) according to **Procedure 5a**, in the presence of DMAP (0.026 g, 0.25 mmol, 0.5 eq.). The desired product was obtained as a mustard-brown solid after recrystallisation (0.14 g, 0.10 mmol, 19 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.11 g, 0.08 mmol, 15 %)

b) Naphthalene tetracarboxylic dianhydride (0.027 g, 0.1 mmol) was refluxed with **108** (0.1 g, 0.2 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a richly brown solid (0.022 g, 0.017 mmol, 18 %).

a) **95** (0.025g, 0.54 mmol) was reacted with **83** (0.52 g, 1.1 mmol, 2 equiv.) according to **Procedure 5d**, in the presence of DMAP (0.026 g, 0.25 mmol, 0.5 eq.). The desired product was obtained as a mustard-brown solid (0.21 g, 0.15 mmol, 29 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.12 g, 0.085 mmol, 16 %). m.p.: 98 °C; δ_{H} (600 MHz, (CD₃)₂SO): 8.73 (2H, d, J = 8.70 Hz, Ar-H7), 8.57 (8H, m, Ar-H5 and Ar-H2 and Ar-H), 8.35 (2H, d, J = 7.14 Hz, Ar-H3) 8.09 (2H, t, J = 7.29, 7.86 Hz, Ar-H6), 7.07 (10H, m, Ph), 5.73 (4H, m, CHCH₃), 4.43 (4H, m, CHCH₃), 3.63 (6H, s, OCH₃), 3.25 (4H, m, PhCH₂), 2.64 (4H, m, NHCH₂CH₂CH₂CH₂CH₂CH₂), 2.40 (4H, m, NHCH₂), 2.09 (4H, m, NHCH₂CH₂CH₂CH₂CH₂), 1.48 (4H, m, NHCH₂CH₂), 1.25 (4H, m, NHCH₂CH₂CH₂CH₂), 1.15 (6H, s, CH₃), 1.01 (4H, m, NHCH₂CH₂CH₂); δ_{C} (100 MHz, (CD₃)₂SO): 173.5, 168.4, 149.1, 147.4, 138.4, 132.2, 130.6, 130.1, 129.4, 126.7, 124.8, 77.7, 70.5, 55.5, 52.7, 52.3, 40.7, 40.6, 40.4, 40.3, 40.1, 40.0, 39.9, 39.8, 39.6, 26.0, 17.3; m/z : 498.11 (M + 2C₄H₈O + Na)³⁺; ν_{max} / cm⁻¹: 3285, 3067, 3030, 2950, 1980, 1743, 1710, 1667, 1594, 1584, 1526, 1454, 1428, 1410, 1368, 1337, 1238, 1229, 1152, 1127, 1113, 1057, 1031, 986, 916, 864, 834, 785, 758, 729, 716, 699.

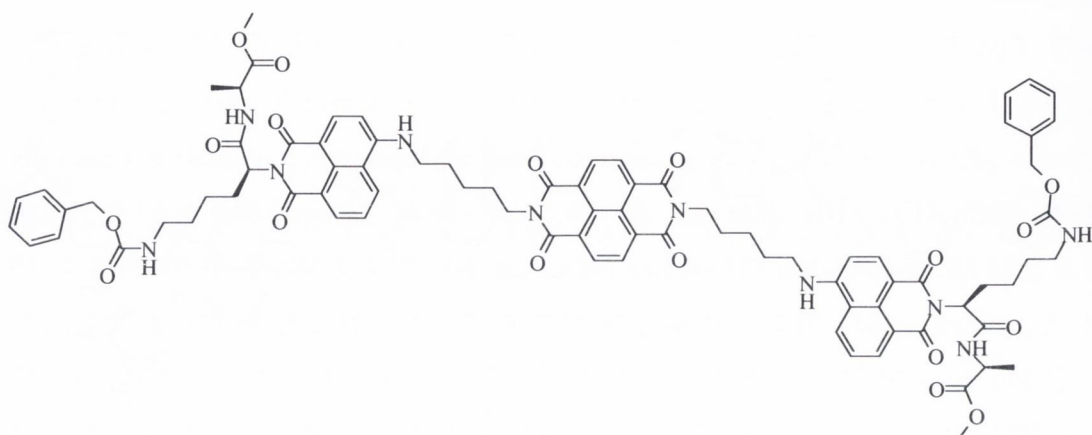
2,7-bis-{N-[N'-{(1S)-[(2S)-Propionic acid methyl ester]-carboxyamido-6-benzyloxycarbonylamino-hexyl}-1,8-naphthalimido}]-3-aminopropyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, **99**



a) **99** was synthesised according to **Procedure 5a** by reacting **87** (0.034 g, 0.09 mmol) and **85** (0.027 g, 0.045 mmol, 2 eq.). The desired product was obtained as a brown solid (0.024 g, 0.017 mmol, 18 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.016 g, 0.011 mmol, 12 %)

b) **99** was synthesised according to **Procedure 5d** by reacting **87** (0.71 g, 1.5 mmol) and **85** (1.98 g, 3 mmol, 2 eq.). The desired product was obtained as a brown solid (0.81 g, 0.55 mmol, 36 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.49 g, 0.34 mmol, 22 %). m.p.: 82 °C; Found C, 61.15; H, 5.21; N, 9.20 %. C₈₀H₇₈N₁₀O₁₈.CH₂Cl₂.MeCN..MeOH.H₂O requires C, 61.38; H, 5.46; N, 9.37; δ_H (600 MHz, (CD₃)₂SO): 8.74 (2H, m, Ar-H7), 8.60 (8H, m, Ar-H5, Ar-H2 and Ar-H), 8.13 (2H, m, Ar-H3), 7.80 (2H, m, Ar-H6), 7.29 (10H, m, Ph), 5.65 (2H, m, CHCH₂), 5.37 (4H, m, PhCH₂O), 4.38 (2H, m, CHCH₃), 3.62 (6H, s, OCH₃), 3.23 (4H, m, LysNHCH₂), 2.92 (4H, m, LinkerNHCH₂CH₂CH₂), 2.64 (4H, m, LysNHCH₂CH₂CH₂CH₂), 2.08 (4H, m, LysNHCH₂CH₂CH₂), 1.92 (4H, m, LinkerNHCH₂), 1.65 (4H, m, LysNHCH₂CH₂CH₂), 1.48 (4H, m, LinkerNHCH₂CH₂), 1.26 (6H, s, CH₃); δ_C (100 MHz, (CD₃)₂SO): 173.5, 171.8, 168.6, 149.5, 137.5, 132.1, 130.6, 130.5, 130.0, 129.1, 129.0, 128.6, 128.0, 127.9, 127.4, 126.7, 124.8, 124.5, 123.5, 123.1, 73.5, 66.4, 65.2, 56.2, 55.2, 52.1, 48.0, 41.6, 29.8, 29.6, 29.4, 29.1, 28.7, 28.0, 27.7, 23.5, 20.3, 18.6, 17.2; *m/z* : 613.19 (M + 3DMAP + 4H)³⁺; ν_{max} / cm⁻¹: 3345, 3066, 2934, 2860, 2185, 2161, 2148, 2017, 1966, 1708, 1660, 1626, 1583, 1526, 1454, 1434, 1409, 1368, 1339, 1240, 1228, 1181, 1154, 1096, 1022, 948, 908, 869, 845, 835, 785, 758, 731, 697.

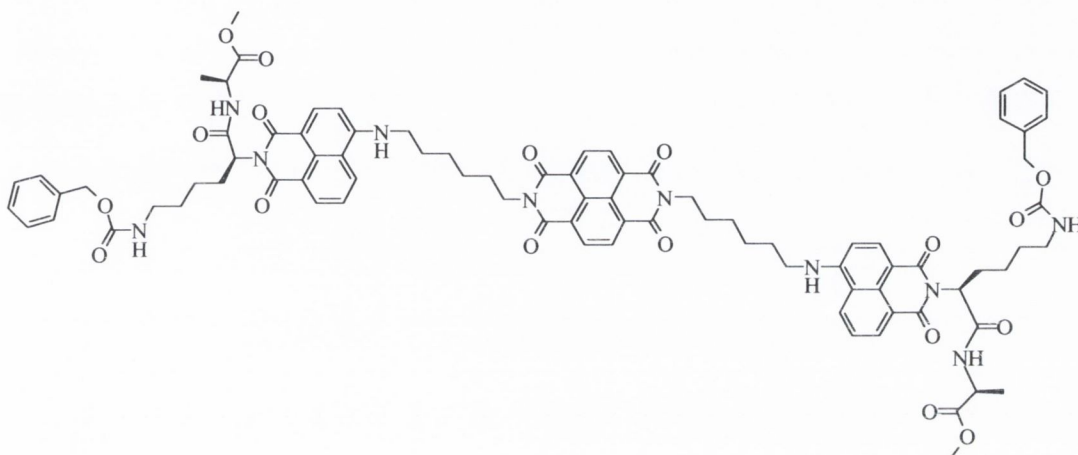
2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-6-benzoyloxycarbonylamino-hexyl}-1,8-naphthalimido}]-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 100



- a) **100** was synthesised according to **Procedure 5a** by reacting **94** (0.03 g, 0.06 mmol) with **85** (0.08 g, 0.12 mmol, 2 eq.) with. The desired product was obtained as a dark brown solid (0.017 g, 0.01 mmol, 16 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.01 g, 0.056 mmol, 9 %)
- b) **100** was synthesised according to **Procedure 5c** by reacting **94** (2.30 g, 4.60 mmol) with **85** (6.13 g, 9.20 mmol, 2 eq.). The desired product was obtained as a dark red solid after recrystallisation (1.83 g, 1.02 mmol, 22 %)
- c) **100** was synthesised according to **Procedure 5d** by reacting **94** (0.46, 0.91 mmol) with **85** (0.54 g, 0.81 mmol, 2 eq.). The desired product was obtained as a dark red solid (0.63 g, 0.37 mmol, 37 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.39 g, 0.22 mmol, 22 %). m.p.: 81 °C; Found C, 58.82; H, 4.82; N, 8.47 %. C₈₄H₈₆N₁₀O₁₈·3CH₂Cl₂·MeCN requires C, 58.75; H, 5.26; N, 8.47; δ_H (600 MHz, (CD₃)₂SO): 8.74 (2H, m, Ar-H7), 8.60 (8H, m, Ar-H5, Ar-H2 and Ar-H), 8.30 (2H, m, Ar-H3), 8.13 (2H, m, Ar-H6), 7.29 (10H, m, Ph), 5.65 (4H, s, PhCH₂O), 5.28 (2H, m, CHCH₂), 5.19 (2H, m, CHCH₃), 4.84 (6H, s, OCH₃), 3.67 (4H, m, LysNHCH₂), 3.62 (4H, m, LinkerNHCH₂CH₂CH₂CH₂CH₂), 3.42 (4H, m, LysNHCH₂CH₂CH₂CH₂), 3.31 (4H, m, LysNHCH₂CH₂), 3.22 (4H, m, LinkerNHCH₂), 2.91 (4H, m, LinkerNHCH₂CH₂CH₂CH₂CH₂), 2.04 (4H, m, LysNHCH₂CH₂CH₂CH₂), 1.39 (4H, m, LinkerNHCH₂CH₂), 1.15 (4H, m, LinkerNHCH₂CH₂CH₂CH₂), 1.12 (6H, s, CH₃); *m/z*: 534.5518 (M + Na + HCl + H)³⁺, 556.27 (M + C₄H₈O)³⁺; ν_{max} / cm⁻¹: 3356, 2076, 2932, 2861, 2284, 2051, 1979, 1956, 1819, 1708, 1661, 1626, 1584, 1526, 1454, 1429, 1409, 1369, 1340, 1295,

1242, 1230, 1185, 1077, 1025, 987, 970, 945, 907, 868, 846, 835, 784, 758, 731, 717, 697.

2,7-bis-{N-[N'-{(1S)-[(2S)-Propionic acid methyl ester]-carboxyamido-6-benzyloxycarbonylaminohexyl}-1,8-naphthalimido}] -6-aminohexyl}-benzo[1mn][3,8]-phenanthroline-1,3,6,8-tetraone, 101

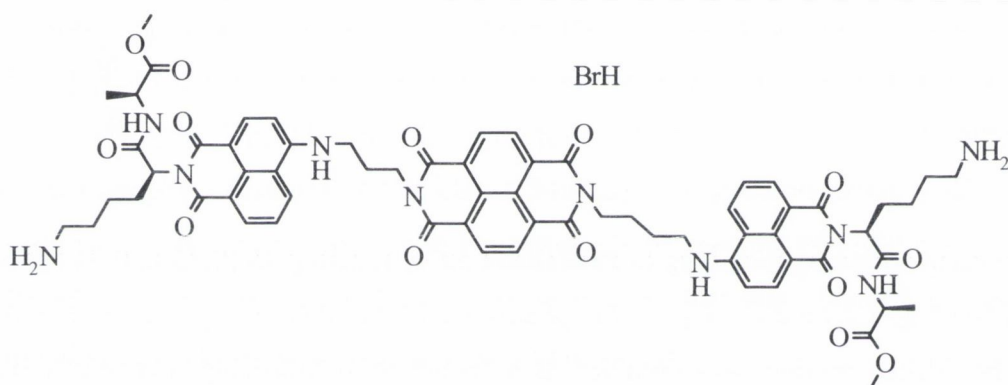


a) **100** was synthesised according to **Procedure 5a** by reacting **95** (0.25 g, 0.54 mmol) and **85** (0.65 g, 1.1 mmol, 2 eq.) in the presence of DMAP (0.026 g, 0.22 mmol, 0.4 eq.). The desired product was obtained as a brown solid following recrystallisation (0.19 g, 0.12 mmol, 23 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.10 g, 0.062 mmol, 12 %)

b) **100** was synthesised according to **Procedure 5d** by reacting **95** (0.25 g, 0.54 mmol) and **85** (0.65 g, 1.1 mmol, 2 eq.) in the presence of DMAP (0.026 g, 0.22 mmol, 0.4 eq.). The desired product was obtained as a brown solid (0.31 g, 0.20 mmol, 38 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.19 g, 0.12 mmol, 23 %). m.p.: 78 °C; δ_{H} (600 MHz, (CD₃)₂SO): 8.78 (2H, m, Ar-H7), 8.60 (8H, m, Ar-H5, Ar-H2 and Ar-H), 8.14 (2H, m, Ar-H3), 7.82 (2H, m, Ar-H6), 7.30 (10H, m, Ph), 5.65 (4H, s, PhCH₂O), 4.44 (2H, m, CHCH₂), 4.37 (2H, m, CHCH₃), 3.30 (6H, s, OCH₃), 3.24 (4H, m, LysNHCH₂), 3.17 (4H, m, LinkerNHCH₂CH₂CH₂CH₂CH₂CH₂), 2.63 (4H, m, LinkerNHCH₂), 2.05 (4H, m, LysNHCH₂CH₂CH₂CH₂), 1.85 (4H, m, LysNHCH₂CH₂), 1.49 (4H, m, LinkerNHCH₂CH₂CH₂CH₂CH₂CH₂), 1.25 (4H, m, LinkerNHCH₂CH₂CH₂CH₂), 1.16 (4H, m, LysNHCH₂CH₂CH₂), 1.02 (8H, m, LinkerNHCH₂CH₂CH₂CH₂ and

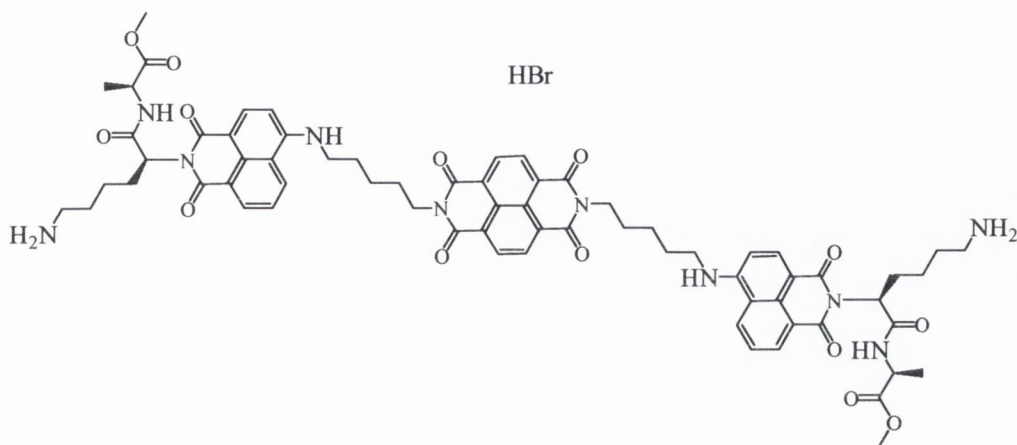
LinkerNHCH₂CH₂), 1.01 (6H, s, CH₃); δ_C (100 MHz, (CD₃)₂SO): 182.7, 132.3, 131.2, 130.7, 130.6, 130.1, 129.4, 129.2, 128.7, 128.1, 127.0, 125.1, 124.9, 124.7, 115.3, 112.6, 87.7, 73.6, 69.4, 67.7, 65.4, 57.5, 56.7, 48.2, 46.8, 41.2, 40.6, 39.9, 39.7, 39.6, 39.4, 39.1, 38.6, 30.8, 30.0, 20.5; m/z : 547.21 (M + 2Mg + K)³⁺, 614.17 (M + DMAP + 2C₄H₈O + Mg)³⁺; $\nu_{\max}$ / cm⁻¹: 3331, 3080, 2937, 2860, 2287, 2164, 2150, 2029, 1980, 1707, 1659, 1584, 1524, 1453, 1430, 1409, 1370, 1333, 1241, 1241, 1184, 1075, 1022, 945, 907, 835, 846, 783, 758, 730, 697.

2,7-bis-{N-[N'-{(1*S*)-(2*S*)-Propionic acid methyl ester]-aminohexyl}-carboxyamido-6-aminoethyl}-1,8-naphthalimido}-[3-aminopropyl]-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 102



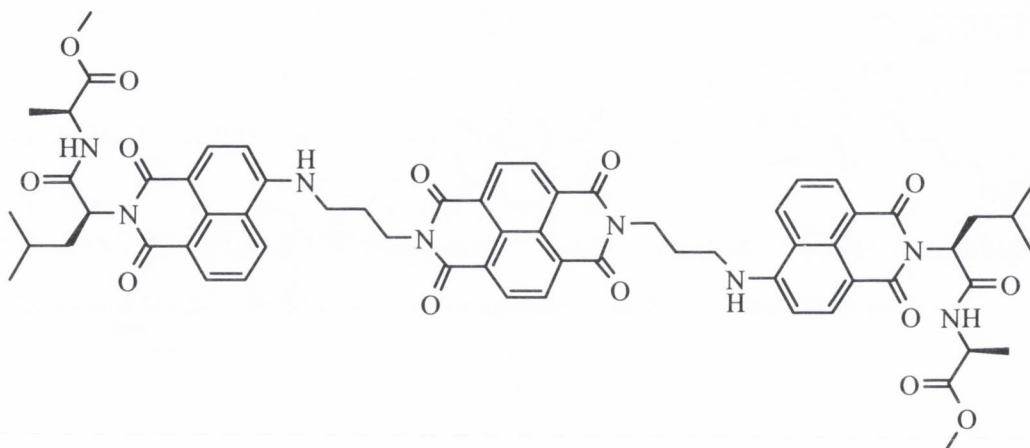
101 was synthesised according to **Procedure 11** by hydrolysing **99** (0.17 g, 0.12 mmol) with a solution of 30 % HBr / acetic acid. The desired product was obtained as a dark brown solid (0.1065g, 0.088 mmol, 73 %). m.p.: 93 °C decomp.; δ_H (600 MHz, (CD₃)₂SO): 8.75 (2H, m, Ar-H7), 8.59 (8H, m, Ar-H5, Ar-H2 and Ar-H), 8.13 (2H, m, Ar-H3), 7.57 (2H, m, Ar-H6), 5.38 (2H, m, CHCH₂), 4.84 (2H, m, CHCH₃), 4.17 (6H, s, OCH₃), 3.25 (4H, m, LysNHCH₂CH₂), 2.92 (4H, m, LinkerNHCH₂CH₂CH₂CH₂), 2.68 (4H, m, LysNHCH₂CH₂CH₂CH₂), 2.33 (4H, m, LysNHCH₂CH₂CH₂CH₂), 1.92 (4H, m, LinkerNHCH₂CH₂CH₂CH₂), 1.85 (4H, m, LysNHCH₂CH₂CH₂CH₂), 1.15 (4H, m, LinkerNHCH₂CH₂CH₂CH₂), 1.10 (6H, s, CH₃); m/z : 457.14 (M + 2Br)²⁺; $\nu_{\max}$ / cm⁻¹: 3354, 3072, 2942, 2351, 2162, 2031, 1984, 1702, 1658, 1652, 1581, 1526, 1455, 1408, 1338, 1244, 1186, 1056, 870, 836, 785, 769, 759, 713, 696, 672.

2,7-bis-{N-[N'-{(1S)-[(2S)-Propionic acid methyl ester]-aminohexyl}-carboxyamido-6-aminohexyl]-1,8-naphthalimido}]-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 103



103 was synthesised according to **Procedure 11** by hydrolysing **100** (0.18 g, 0.12 mmol) with a solution of 30 % HBr / acetic acid. The desired product was obtained as a dark brown solid (0.086g, 0.069 mmol, 58 %). m.p.: 76 °C ; δ_{H} (600 MHz, $(\text{CD}_3)_2\text{SO}$): 8.76 (2H, m, Ar-H7), 8.61 (8H, m, Ar-H5, Ar-H2 and Ar-H), 8.16 (2H, m, Ar-H3), 7.62 (2H, m, Ar-H6), 5.39 (2H, m, CHCH_2), 4.86 (2H, m, CHCH_3), 4.21 (6H, s, OCH_3), 3.31 (4H, m, LysNHCH_2), 2.94 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.72 (4H, m, $\text{LysNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.65 (4H, m, $\text{LysNHCH}_2\text{CH}_2$), 2.35 (4H, m, LinkerNHCH_2), 2.01 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.96 (4H, m, $\text{LysNHCH}_2\text{CH}_2\text{CH}_2$), 1.83 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2$), 1.17 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2\text{CH}_2$), 1.10 (6H, s, CH_3); m/z : 397.19 ($\text{M} + 4\text{Br} + \text{Na}$)⁴⁺; ν_{max} / cm^{-1} : 3329, 1627, 1583, 1529, 1460, 1364, 1242, 1189, 779, 711.

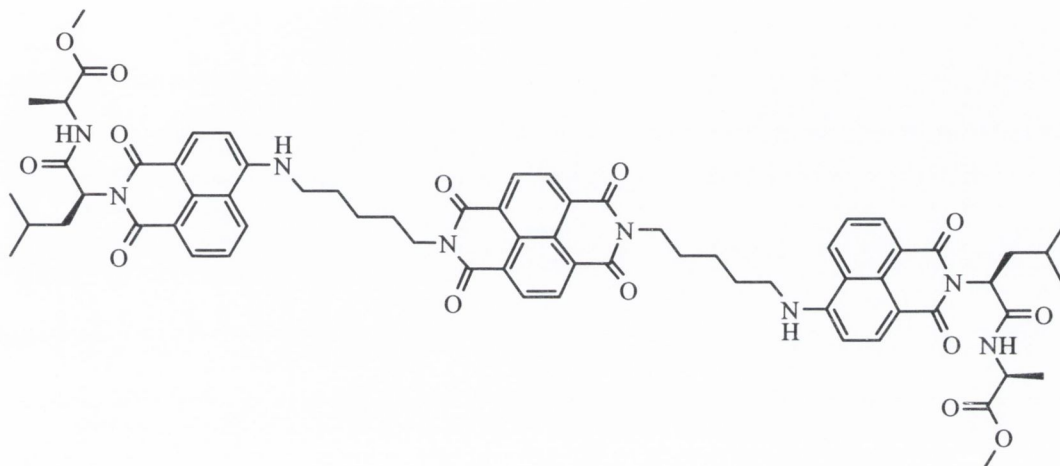
2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-3-methylbutyl}-1,8-naphthalimido]-3-aminopropyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 104



a) **102** was synthesised according to **Procedure 5a** by reacting **87** (0.32 g, 0.85 mmol) with **86** (0.75 g, 1.7 mmol, 2 eq.) in the presence of DMAP. The desired product was obtained as a brown solid (0.22 g, 0.19 mmol, 22 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.13 g, 0.011 mmol, 13 %)

b) **102** was synthesised according to **Procedure 5d** by reacting **87** (0.32 g, 0.85 mmol) with **86** (0.75 g, 1.7 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown solid following recrystallisation (0.277 g, 0.24 mmol, 28 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.178 g, 0.15 mmol, 18 %). m.p.: 89 °C; Found C, 55.30; H, 4.96; N, 8.47 %. C₆₄H₆₄N₈O₁₄·4CH₂Cl₂·DMAP requires C, 55.23; H, 5.07; N, 8.59 %; δ_{H} (600 MHz, (CD₃)₂SO): 8.75 (2H, d, *J* = 8.64 Hz, Ar-H7), 8.67-8.57 (8H, m, Ar-H5 and Ar-H2 and Ar-H), 8.29 (2H, d, *J* = 6.78 Hz, Ar-H3), 8.13 (2H, t, *J* = 8.28 and 7.50 Hz, Ar-H6), 5.45 (2H, m, CHCH₂), 4.36 (2H, m, CHCH₃), 3.62 (6H, s, OCH₃), 3.29 (4H, m, NHCH₂CH₂CH₂), 3.06 (4H, m, NHCH₂), 2.16 and 1.88 (2H+2H, 2m, CH₂CHCH₃), 1.47 (4H, m, NHCH₂CH₂), 1.13 (6H, d, *J* = 7.50 Hz, CH₃), 0.91 and 0.84 (6H + 6H, 2d, *J* = 6.78 Hz and *J* = 6.36 Hz, CH(CH₃)₂); δ_{C} (100 MHz, (CD₃)₂SO): 173.4, 168.9, 164.1, 163.2, 149.6, 132.1, 130.5, 130.0, 129.2, 124.6, 123.1, 67.8, 52.6, 52.1, 48.1, 40.4, 37.5, 25.1, 23.4, 22.3, 17.1; *m/z* : 579.34 (M + 4CH₂Cl₂ + DMAP)³⁺; ν_{max} / cm⁻¹: 2953, 1712, 1670, 1584, 1527, 1452, 1339, 1237, 1157, 852, 784, 760.

2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-3-methylbutyl}-1,8-naphthalimido]-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 105



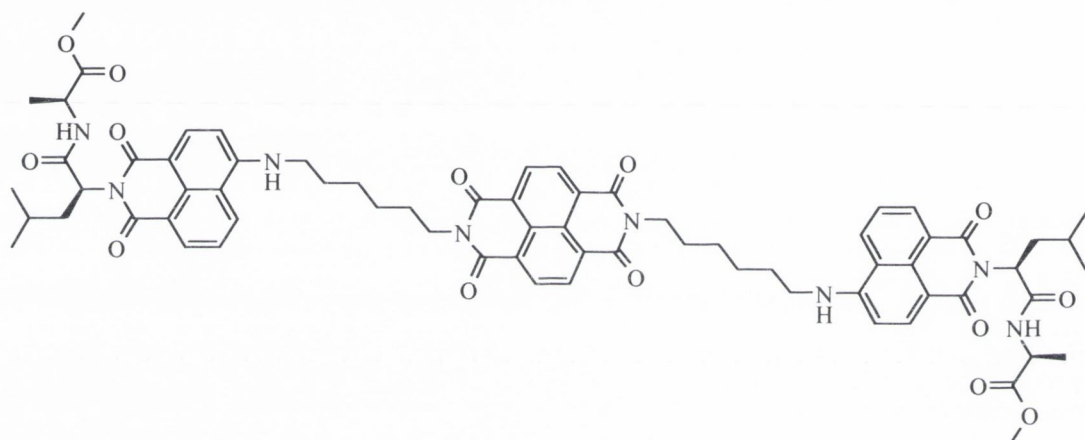
a) **105** was synthesised according to **Procedure 5a** by reacting **94** (0.075g, 0.17 mmol) with **86** (0.15 g, 0.34 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown solid after recrystallisation (0.037 g, 0.029 mmol, 19 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.027 g, 0.021 mmol, 14 %)

b) **105** was synthesised according to **Procedure 5c** by reacting **94** (0.075g, 0.17 mmol) with **86** (0.15 g, 0.34 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown crystalline solid after recrystallisation (0.052 g, 0.042 mmol, 25 %).

c) **105** was synthesised according to **Procedure 5d** by reacting **94** (0.075g, 0.17 mmol) with **86** (0.15 g, 0.34 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown crystalline solid (0.063 g, 0.05 mmol, 30 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.042 g, 0.033 mmol, 20 %). m.p.: 75 °C; Found C, 59.84; H, 5.64; N, 8.21%. C₆₈H₇₂N₈O₁₄.2CH₂Cl₂ requires C, 60.26; H, 5.49; N, 8.03; δ_H (600 MHz, (CD₃)₂SO): 8.75 (2H, d, J = 8.68 Hz, Ar-H7), 8.68-8.58 (8H, m, Ar-H5 and Ar-H2 and Ar-H), 8.27 (2H, m, Ar-H3), 8.14 (2H, m, Ar-H6), 5.44 (2H, m, CHCH₂), 4.37 (2H, m, CHCH₃), 3.62 (6H, s, OCH₃), 2.63 (4H, m, NHCH₂CH₂CH₂CH₂CH₂), 2.40 (4H, m, NHCH₂), 2.18 and 1.87 (2H+2H, 2m, CH₂CHCH₃), 1.48 (4H, m, NHCH₂CH₂CH₂CH₂), 1.27 (4H, m, NHCH₂CH₂), 1.14 (6H, s, CH₃), 1.01 (4H, m, NHCH₂CH₂CH₂), 0.91 and 0.85 (6H +

6H, 2m, CH(CH₃)₂); δ_C (100 MHz, (CD₃)₂SO): 168.9, 132.3, 130.6, 130.2, 130.0, 124.7, 81.5, 67.8, 65.5, 55.4, 52.8, 52.3, 48.3, 40.5, 40.4, 40.2, 40.1, 39.8, 39.6, 37.7, 29.5, 23.6, 22.4, 17.3; m/z : 578.26 (M + 4DMAP + Na)³⁺; $\nu_{\max}$ / cm⁻¹: 3358, 2954, 2871, 1710, 1664, 1583, 1527, 1452, 1410, 1366, 1338, 1228, 1187, 1156, 1054.78, 1036, 988, 905, 851, 832, 784, 759, 716.

2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-3-methylbutyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 106

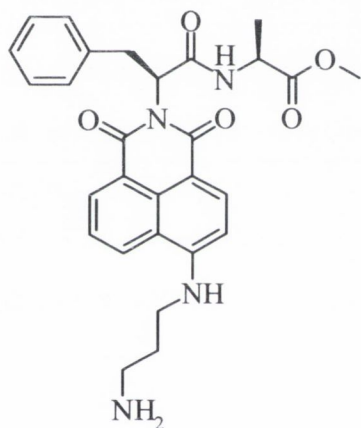


a) **106** was synthesised according to **Procedure 5a** by reacting **95** (0.25g, 0.54 mmol) with **86** (0.48 g, 1.1 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown crystalline solid (0.15 g, 0.12 mmol, 22 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH), 0.074 g, 0.061 mmol, 11 %)

b) **106** was synthesised according to **Procedure 5d** by reacting **95** (0.25g, 0.54 mmol) with **86** (0.48 g, 1.1 mmol, 2 eq.) in the presence of DMAP. The product was isolated as a brown solid after recrystallisation (0.20 g, 0.16 mmol, 29 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.12 g, 0.094 mmol, 17 %). m.p.: 79 °C; Found C, 59.72; H, 5.35; N, 9.27 %. C₇₀H₇₆N₈O₁₄·2CHCl₃·2DMAP requires C, 59.48; H, 5.69; N, 9.68; δ_H (600 MHz, (CD₃)₂SO): 8.75 (2H, d, J = 8.28 Hz, Ar-H7), 8.69-8.65 (6H, m, Ar-H5 and Ar-H), 8.59 (2H, d, J = 7.50 Hz, Ar-H2), 8.29 (2H, d, J = 6.78 Hz, Ar-H3), 8.14 (2H, t, J = 8.28, 8.28 Hz, Ar-H6), 5.46 (2H, m, CHCH₂), 4.37 (2H, m, CHCH₃), 3.63 (6H, s, OCH₃), 3.30 (4H, m, NH CH₂CH₂CH₂CH₂CH₂CH₂), 2.52 - 2.48 (obscured 4H, m,

NHCH₂ and NH CH₂CH₂CH₂CH₂CH₂), 2.18 and 1.88 (2H+2H, 2m, CH₂CHCH₃), 2.09 (4H, m, NH CH₂CH₂), 1.48 (4H, m, NHCH₂CH₂CH₂CH₂), 1.25 (4H, m, NH CH₂CH₂CH₂), 1.13 (6H, d, J = 7.50 Hz, CH₃), 0.92 and 0.85 (6H + 6H, 2d, J = 6.78 and 6.72 Hz, CH(CH₃)₂); δ_C (100 MHz, (CD₃)₂SO): 173.4, 168.9, 149.6, 132.1, 130.5, 130.0, 129.2, 129.0, 127.3, 124.6, 123.5, 123.1, 52.6, 52.2, 48.1, 37.5, 25.0, 23.4, 22.3, 17.2; m/z : 444.23 (M + 4DMAP + K)⁴⁺; $\nu_{\max}$ / cm⁻¹: 3367, 3082, 2955, 1729, 1712, 1671, 1585, 1525, 1454, 1411, 1337, 1237, 1207, 1189, 1159, 1125, 1062, 888, 852, 784, 760, 713, 686.

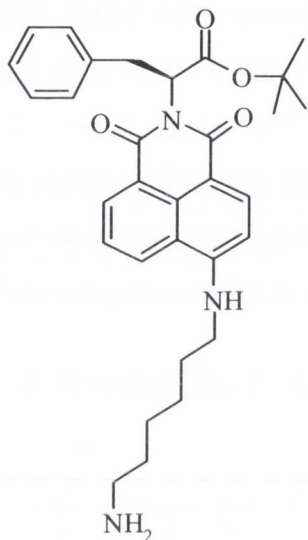
***N*-{(1*S*)-[(2*S*)-Propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-diaminopropane-1,8-naphthalimide, 107¹⁰⁴**



83 (0.06 g, 0.126 mmol) was reacted with 1,3-diamino propane (0.05 mL, 0.63 mmol, in excess) in anhydrous THF according to **Procedure 7**. Recrystallisation succeeded in isolating the desired product as a rusty-brown solid (0.016 g, 0.032 mmol, 25 %). m.p : 131 - 154 ° C; δ_H (400 MHz, CDCl₃): 8.50 (1H, d, J = 8.04 Hz, Ar-H7), 8.26 (1H, m, Ar-H5), 8.09 (1H, d, J = 8.04 Hz, Ar-H2), 7.42 (1H, d, J = 7.56 Hz, Ar-H3), 7.11 (5H, m, Ph), 6.79 (1H, m, Ar-H3), 4.23 (1H, m, CHCH₂), 3.90

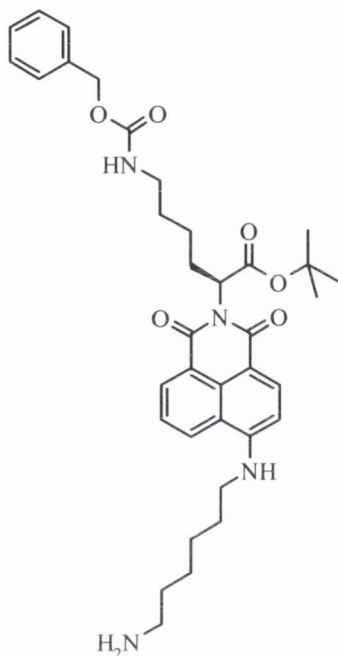
(1H, m, CHCH₃), 3.76 (2H, m, PhCH₂), 3.50 (3H, br. s, NH and NH₂), 2.55 (3H, s, OCH₃), 1.76 (2H, m, NH₂CH₂CH₂CH₂), 1.42 (3H, s, CH₃), 1.45 (2H, m, NH₂CH₂), 1.21 (2H, m, NH₂CH₂CH₂); δ_C (100 MHz, CDCl₃): 173.3, 162.3, 161.5, 149.4, 138.9, 133.8, 132.6, 132.2, 129.1, 128.8, 128.5, 127.9, 126.6, 126.4, 125.9, 121.6, 118.8, 54.2, 51.8, 48.8, 47.8, 34.0, 30.7, 16.8; m/z : 503.24 (M + H)⁺; $\nu_{\max}$ / cm⁻¹: 3436, 2252, 2126, 1655, 1056, 1028, 1008, 823, 761.

***N*-[(1*S*)-*tert*-Butoxycarbonyl-2-ethylphenyl]-4-diaminohexane-1,8-naphthalimide, 108**



73 (0.055 g, 0.12 mmol) was reacted with 1,6-diamino hexane (0.014 g, 0.12 mmol, 1 eq.) according to **Procedure 7**. The product was isolated as a brown oil after preparative plate chromatography, (solvent: CH₂Cl₂, eluent: MeOH), and later this dessicated to a hydropscopic orange residue (0.20 g, 0.038 mmol, 32 %). m.p.: 148 ° C decomp.; Found C, 49.05; H, 5.30; N, 5.66 %. C₃₁H₃₇N₃O₄·4CH₂Cl₂ requires C, 49.15; H, 5.30; N, 4.91 %; δ_H (400 MHz, CDCl₃): 8.83 (1H, d, J = 9.04 Hz, Ar-H7), 8.67 (1H, d, J = 7.04 Hz, Ar-H5), 8.61 (1H, d, J = 5.20 Hz, Ar-H2), 7.97 (1H, t, J = 7.56 and 8.52 Hz, Ar-H6), 7.11 (5H, m, Ph), 6.52 (1H, d, J = 5.40 Hz, Ar-H3), 5.97 (1H, m, CHCH₂), 3.67 (2H, d, J = 7.04 Hz, PhCH₂), 3.32 (2H, d, J = 7.04 Hz, NH₂CH₂CH₂CH₂CH₂CH₂CH₂), 2.21 (4H, br. s and m, NH₂ and NH₂CH₂), 1.37 (2H, m, NH₂CH₂CH₂), 1.32 (2H, m, NH₂CH₂CH₂CH₂CH₂CH₂), 1.22 (9H, s, (CH₃)₃), 1.07 (4H, m, NH₂CH₂CH₂CH₂ and NH₂CH₂CH₂CH₂CH₂CH₂); δ_C (100 MHz, CDCl₃): 169.4, 167.9, 163.3, 149.6, 139.1, 138.1, 137.2, 134.3, 132.4, 130.9, 129.7, 129.0, 128.9, 126.4, 124.3, 123.7, 119.8, 113.9, 107.1, 103.8, 82.3, 81.8, 66.9, 62.9, 60.2, 59.4, 55.4, 54.9; *m/z*: 578.34 (M + K + 2Na)⁺; ν_{max} / cm⁻¹: 3356, 2934, 1728, 1683, 1645, 1546, 1497, 1454, 1392, 1365, 1292, 1246, 1153, 1129, 1090, 916, 843, 774, 754, 733, 698.

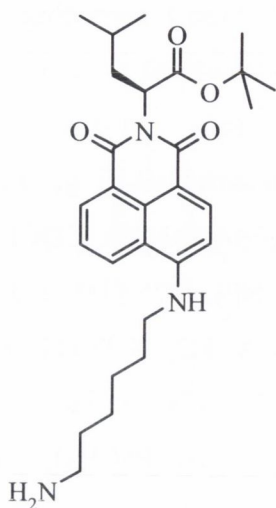
***N*-[(1*S*)-*tert*-Butoxycarbonyl-6-benzyloxycarbonylaminohexyl]-4-diaminohexane-1,8-naphthalimide, 109**



74 (0.3 g, 0.53 mmol) was reacted with 1,6-diamino hexane (0.062 g, 0.53 mmol, 1 eq.) according to **Procedure 7**. The product was isolated as a brown oil after prep-plate chromatography and later this became a hygroscopic brown solid (0.12 g, 0.19 mmol, 35 %). m.p.: 97 ° C; δ_{H} (400 MHz, CDCl_3): 8.85 (1H, d, $J = 8.64$ Hz, Ar-H7), 8.75 (1H, d, $J = 7.20$ Hz, Ar-H5), 8.47 (1H, m, Ar-H2), 8.38 (1H, m, Ar-H6), 8.10 (1H, m, Ar-H3), 7.32 (5H, m, Ph), 6.60 (1H, m, CHCH_2), 5.62 (2H, d, $J =$ Hz, PhCH_2), 3.40 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.15 (2H, m, NH_2CH_2), 2.30 (2H, m, NHCH_2), 2.18 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.87 (2H, m, NHCH_2CH_2), 1.62 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.57 (2H, m,

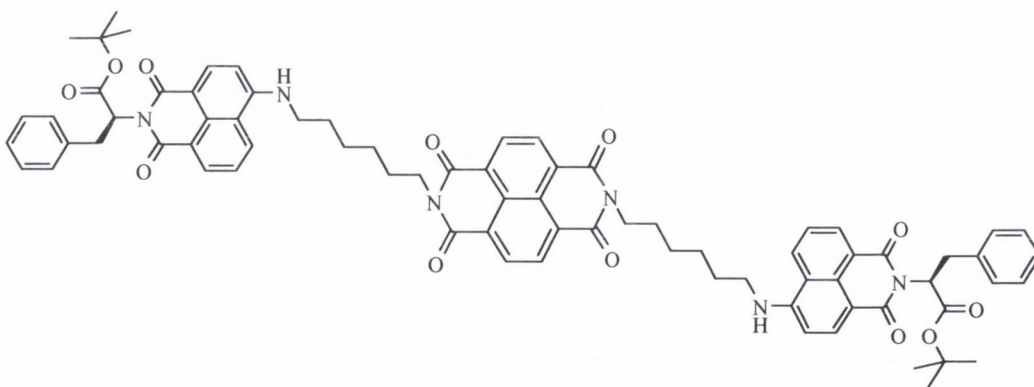
$\text{NH}_2\text{CH}_2\text{CH}_2$), 1.56 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$), 1.45 (9H, s, $(\text{CH}_3)_3$), 1.28 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ and (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$); δ_{C} (100 MHz, CDCl_3): 169.2, 163.7, 162.6, 155.8, 149.2, 130.9, 129.5, 128.0, 127.9, 127.6, 127.5, 127.4, 124.0, 123.4, 109.0, 103.7, 81.7, 65.9, 54.0, 53.2, 43.1, 40.3, 30.4, 29.2, 28.9, 28.2, 28.0, 27.9, 27.5, 26.5, 23.5; m/z : 321.18 ($\text{M} + \text{Na} + \text{H}$)²⁺; ν_{max} / cm^{-1} : 3357, 2930, 2861, 1688, 1643, 1578, 1531, 1454, 1392, 1365, 1286, 1244, 1155, 1023, 906, 845, 775, 757, 733, 696.

***N*–[(1*S*)–*tert*–Butoxycarbonyl–3–methylbutyl]–4–diaminohexane–1,8–naphthalimide, 110**



75 (0.055 g, 0.12 mmol) was reacted with 1,6-diaminohexane (0.014 g, 0.12 mmol, 1 eq.) according to **Procedure 7**. The product was isolated as a brown oil after prep-plate chromatography and later this became a hygroscopic orange residue (0.21 g, 0.039 mmol, 33 %). δ_{H} (400 MHz, CDCl_3): 8.73 (1H, d, $J = 8.24$ Hz, Ar-H7), 8.76 (1H, d, $J = 6.52$ Hz, Ar-H5), 8.72 (1H, d, $J = 8.04$ Hz, Ar-H2), 8.43 (1H, m, Ar-H6), 8.02 (1H, t, $J = 7.52, 7.56$ Hz, Ar-H3), 5.67 (1H, m, CHCH_2), 3.49 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.19 (2H, m, NH_2CH_2), 2.09 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$), 1.56 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$ and $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.43 (9H, s, $(\text{CH}_3)_3$), 1.27 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.02 (3H, d, $J = 6.52$ Hz, CHCH_3), 0.94 (3H, d, $J = 7.00$ Hz, CHCH_3); m/z : 482.28 ($\text{M} + \text{H}$)⁺; ν_{max} / cm^{-1} : 2929, 1671, 1585, 1534, 1369, 1348, 1270, 1239, 1160, 788.

2,7–bis–{*N*–[*N'*–{(1*S*)–*tert*–Butoxycarbonyl–2–ethylphenyl}–1,8–naphthalimido]–6–aminohexyl}–benzo[1*mn*][3,8]–phenanthroline–1,3,6,8–tetraone, 111

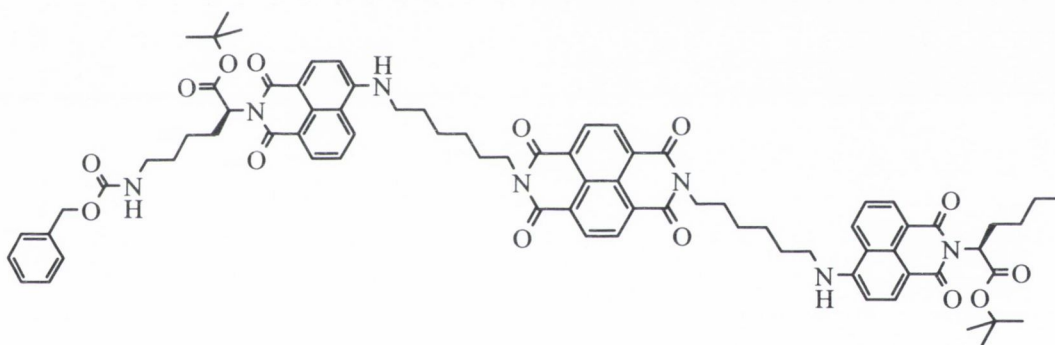


a) **111** was synthesised according to **Procedure 5a** by reacting **87** (0.076 g, 0.20 mmol) with **73** (0.19 g, 0.40 mmol, 2 eq.). The product was isolated after recrystallisation as an orange fluorescent oil (0.05 g, 0.042 mmol, 24 %).

b) Naphthalene tetracarboxylic dianhydride (0.005 g, 0.02 mmol) was refluxed with **108** (0.02 g, 0.04 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a fluorescent oil (0.005 g, 0.0041 mmol, 21 %). δ_{H} (400 MHz, CDCl_3):

8.80 (2H, d, $J = 9.04$ Hz, Ar-H7), 8.66-8.59 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.36 (2H, d, $J = 8.00$ Hz, Ar-H3) 7.96 (2H, t, $J = 7.52$, 7.04 Hz, Ar-H6), 7.12 (10H, m, Ph), 5.92 (4H, m, CHCH_3), 4.43 (4H, m, CHCH_3), 3.63 (6H, s, OCH_3), 3.74 (4H, m, PhCH_2), 3.68 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.65 (4H, m, NHCH_2), 3.48 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.86 (4H, m, NHCH_2CH_2), 1.44 (6H, s, CH_3), 1.30 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.26 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$); δ_{C} (100 MHz, CDCl_3): 167.6, 162.3, 161.5, 149.2, 136.9, 132.2, 129.6, 129.5, 129.0, 128.6, 128.5, 127.9, 126.1, 125.9, 123.4, 123.1, 122.0, 81.9, 76.8, 67.5, 55.1, 34.3, 30.5, 25.1; m/z : 439.16 ($\text{M} + 4\text{DMAP}$) $^{4+}$; ν_{max} / cm^{-1} : 2128, 1918, 943, 901, 775, 721

2,7-bis-{*N*-[*N'*-{(1*S*)-*tert*-Butoxycarbonyl-6-benzylloxycarbonylaminohexyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 112

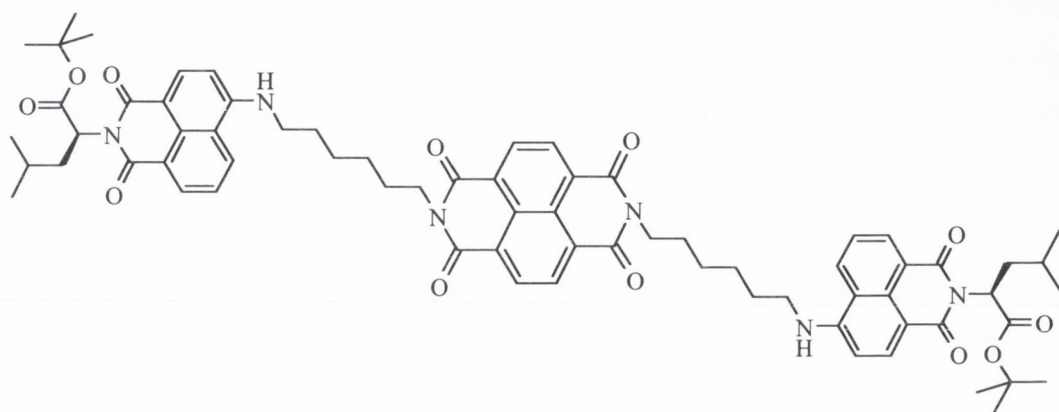


a) **112** was synthesised according to **Procedure 5a** by reacting **95** (0.076 g, 0.16 mmol) with **74** (0.18 g, 0.32 mmol, 2 eq.). The product was isolated after recrystallisation as a brown oil (0.06 g, 0.038 mmol, 24 %).

b) Naphthalene tetracarboxylic dianhydride (0.0054 g, 0.02 mmol) was refluxed with **109** (0.02 g, 0.04 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a brown oil (0.0047 g, 0.0033 mmol, 18 %). δ_{H} (400 MHz, CDCl_3): 8.87 (2H, d, $J = 8.52$ Hz, Ar-H7), 8.76-8.68 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.39 (2H, d, $J = 7.52$ Hz, Ar-H6), 7.99 (2H, m, Ar-H3), 7.31 (10H, m, Ph), 5.58 (4H, m, PhCH_2), 4.99 (2H, m, CHCH_2), 4.79 (4H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.75 (4H, m, NHCH_2), 3.16 (4H, m, LysNHCH_2), 2.31 (4H, m, $\text{LysNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.86 (12H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$, NHCH_2CH_2 and $\text{LysNHCH}_2\text{CH}_2$), 1.59 (4H, m,

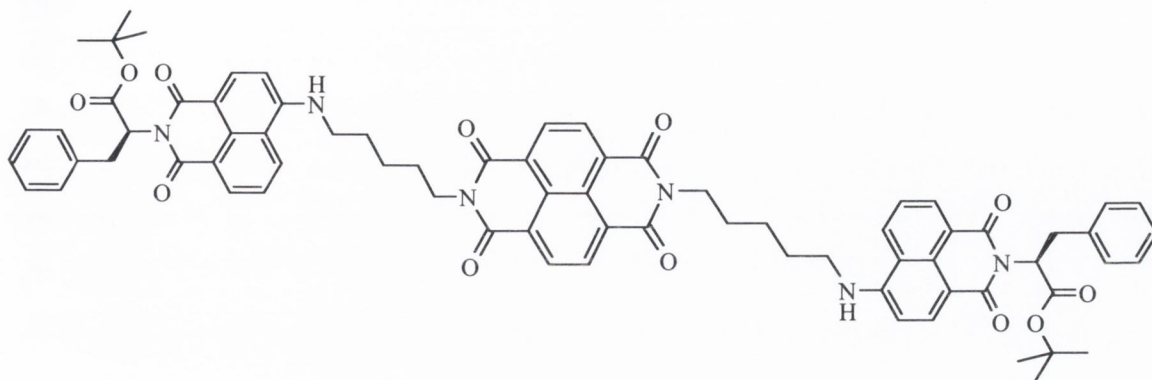
LysNHCH₂CH₂CH₂CH₂), 1.43 (18H, s, (CH₃)₃), 1.26 (8H, m, NHCH₂CH₂CH₂CH₂ and NHCH₂CH₂CH₂); *m/z*: 425.27 (M + 3C₄H₈O)⁴⁺; *ν*_{max} / cm⁻¹: 3748, 2369, 2267, 2231, 2207, 2175, 2161, 2138, 1988, 1968, 1947, 978, 914, 876, 747, 666.

2,7-bis-{N-[N'-{(1*S*)-tert-Butoxycarbonyl-3-methylbutyl}-1,8-naphthalimido]-6-aminohexyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 113



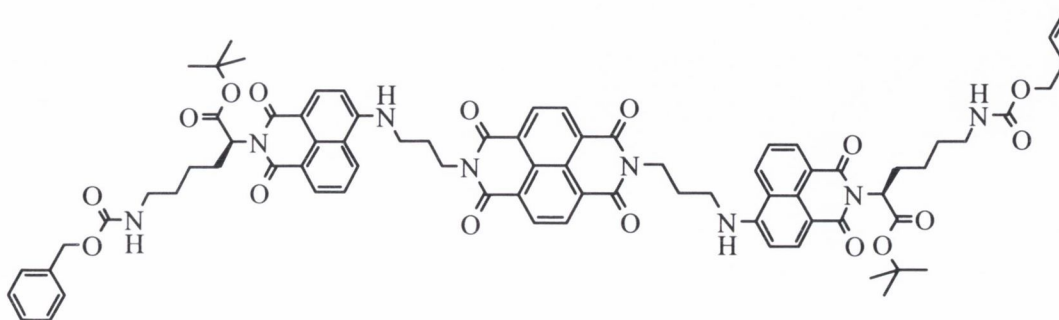
- a) **113** was synthesised according to **Procedure 5a** by reacting **95** (0.076 g, 0.16 mmol) with **75** (0.13 g, 0.32 mmol, 2 eq.). After recrystallisation the product was isolated as a brown crystalline solid (0.045 g, 0.038 mmol, 24 %).
- b) Naphthalene tetracarboxylic dianhydride (0.0027 g, 0.01 mmol) was refluxed with **110** (0.008 g, 0.02 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a richly brown solid (0.0022 g, 0.0018 mmol, 18 %). *m.p.*: 85 °C; Found C, 68.00; H, 7.94; N, 4.49 %. C₇₀H₇₈N₆O₁₂·7C₄H₈O·H₂O requires C, 68.51; H, 7.98; N, 4.89 %; *δ*_H (400 MHz, CDCl₃): 8.69 (2H, m, Ar-H7), 8.65-8.42 (8H, m, Ar-H5 and Ar-H and Ar-H2), 8.35 (2H, m, Ar-H3), 8.12 (2H, m, Ar-H6), 5.57 (2H, m, CHCH₂), 3.69 (4H, m, NHCH₂CH₂CH₂CH₂CH₂CH₂CH₂), 2.99 (2H, m, NHCH₂), 2.91 (2H, m, NHCH₂CH₂CH₂CH₂CH₂CH₂CH₂), 2.07 and 1.80 (obscured 2H+2H, 2m, CH₂CHCH₃), 1.71 (4H, m, NHCH₂CH₂), 0.24 (4H, m, NHCH₂CH₂CH₂CH₂CH₂CH₂CH₂), 0.17 (12H, s, CH(CH₃)₂), 0.07 (4H, m, NHCH₂CH₂CH₂CH₂CH₂CH₂CH₂); *m/z*: 534.7722 (M + 2DMAP + 2C₄H₈O + Na)³⁺; *ν*_{max} / cm⁻¹: 2956, 2926, 2873, 1748, 1462, 1373, 1236, 1048, 846, 784, 725.

2,7-bis-{N-[N'-(1*S*)-*tert*-Butoxycarbonyl-2-ethylphenyl]-1,8-naphthalimido}-5-aminopentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 114



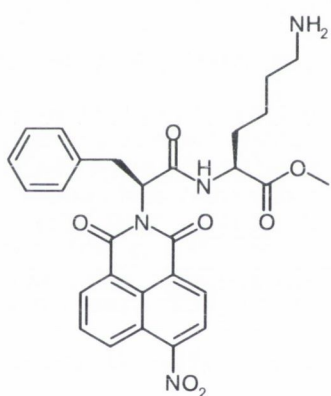
a) **114** was synthesised according to **Procedure 5a** by reacting **94** (0.076 g, 0.17 mmol) with **73** (0.16 g, 0.35 mmol, 2 eq.). After recrystallisation, the product was isolated as a fluorescent yellow oil (0.047 g, 0.038 mmol, 22 %). Found C, 62.97; H, 5.79; N, 4.48 %. $C_{75}H_{74}N_6O_{12} \cdot 4CH_2Cl_2$ requires C, 61.35; H, 5.80; N, 4.93 %; δ_H (400 MHz, $CDCl_3$): 8.92 (2H, m, Ar-H7), 8.84 - 8.67 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.40 (2H, m, Ar-H3), 7.97 (2H, t, $J = 8.00, 7.92$ Hz, Ar-H6), 7.18 (10H, m, Ph), 6.01 (2H, m, $\underline{CHCH_2}$), 5.32 (4H, m, $\underline{PhCH_2}$), 4.26 (4H, m, $\underline{NH_2CH_2CH_2CH_2CH_2CH_2}$), 3.52 (4H, m, $\underline{NH_2CH_2}$), 2.59 (2H, m, $\underline{NH_2CH_2CH_2CH_2CH_2}$), 2.39 (2H, m, $\underline{NH_2CH_2CH_2}$), 1.41 (18H, m, $(CH_3)_3$), 0.24 (2H, m, $\underline{NH_2CH_2CH_2CH_2}$); m/z : 623.11 (M)²⁺; ν_{max} / cm^{-1} : 2701, 2253, 1669, 902, 820, 720, 655.

2,7-bis-{N-[N'-(1*S*)-*tert*-Butoxycarbonyl-6-benzyloxycarbonylaminoethyl]-1,8-naphthalimido}-3-aminopropyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 115



115 was synthesised by reacting **87** (0.012 g, 0.03 mmol) with **74** (0.034 g, 0.061 mmol, 2 eq.) according to **Procedure 5a**. The desired product was isolated, after recrystallisation, as a brown oil (0.012 g, 0.0085 mmol, 14 %). δ_{H} (400 MHz, CDCl_3): 8.87 (2H, d, $J = 8.52$ Hz, Ar-H7), 8.76-8.68 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.39 (2H, d, $J = 8.04$ Hz, Ar-H3), 7.80 (2H, t, $J = 8.04$, 8.00 Hz, Ar-H6), 7.34 (10H, m, Ph), 6.95 (2H, br. s., NH), 5.58 (2H, m, CHCH_2), 5.32 (4H, s, PhCH_2O), 5.00 (4H, m, LysNHCH_2), 3.17 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2\text{CH}_2$), 2.33 (4H, m, $\text{LysNHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.17 (4H, m, $\text{LysNHCH}_2\text{CH}_2$), 1.73 (4H, m, LinkerNHCH_2), 1.55 (4H, m, $\text{LysNHCH}_2\text{CH}_2\text{CH}_2$), 1.52 (6H, s, CH_3), 1.26 (4H, m, $\text{LinkerNHCH}_2\text{CH}_2$); m/z : 534.43 ($\text{M} + \text{DMAP} + \text{C}_4\text{H}_8\text{O}$) $^{3+}$; ν_{max} / cm^{-1} : 2243, 1055, 1029, 1011, 923, 819, 759, 674, 666.

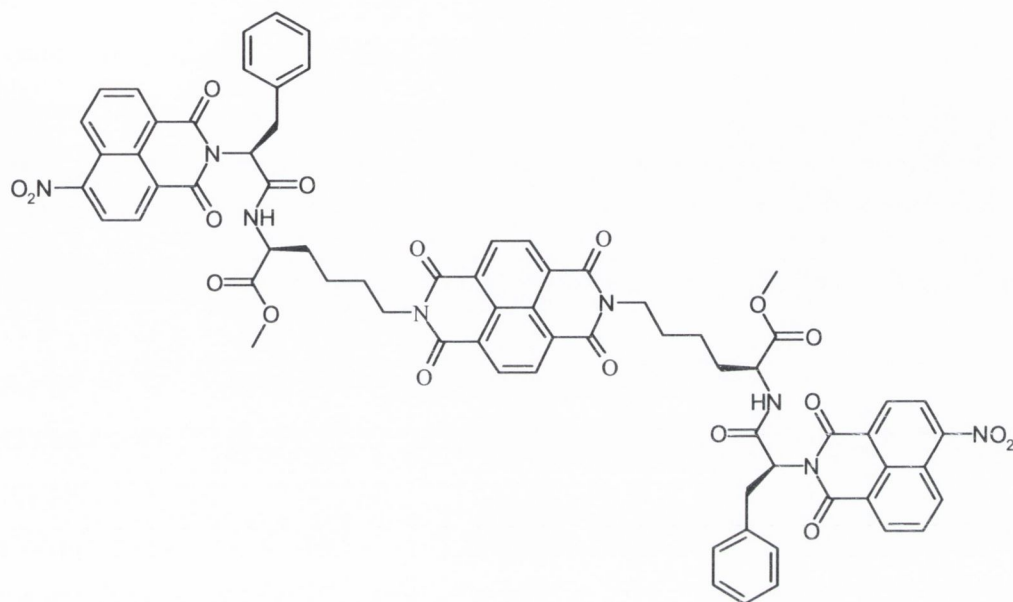
N*–{(1*S*)–[(2*S*)–6-amino-hexanoic acid methyl ester]–carboxyamido–2-ethylphenyl}–4-nitro–1,8-naphthalimide, **116*¹⁰⁵



84 (1.00 g, 2.50 mmol) was converted to **116** by acid mediated hydrolysis using $\text{CH}_2\text{Cl}_2/\text{TFA}$ according to **Procedure 2**. The desired product was isolated as a hygroscopic brown solid (1.15 g, 1.90 mmol, 76 %). m.p.: 84 °C; HRMS: 533.2036 ($[\text{M} + \text{H}]^+$, $\text{C}_{28}\text{H}_{28}\text{N}_4\text{O}_7$ requires 532.56); Found C, 49.96; H, 4.90; N, 7.20 %. $\text{C}_{28}\text{H}_{29}\text{N}_4\text{O}_7 \cdot 2\text{CH}_2\text{Cl}_2 \cdot 2\text{MeOH}$ requires C, 50.08; H, 5.38; N, 7.30 %; δ_{H} (400 MHz,

CDCl_3): 8.85 (1H, d, $J = 8.02$ Hz, Ar-H7), 8.71 (1H, d, $J = 7.04$ Hz, Ar-H5), 8.65 (1H, d, $J = 7.52$ Hz, Ar-H2), 8.40 (1H, d, $J = 7.30$ Hz, Ar-H3), 8.00 (1H, t, $J = 10.16$, 8.04 Hz, Ar-H6), 7.25 (5H, m, Ph), 6.45 (1H, m, CHCH_2Ph), 6.07 (1H, m, NHCHCH_2), 4.69 (2H, br. s, NH_2), 3.75 (3H, s, OCH_3), 3.55 (2H, m, PhCH_2), 3.04 (1H, br. s, NH), 1.87 (2H, m, NH_2CH_2), 1.70 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.61 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$), 1.45 (9H, s, $(\text{CH}_3)_3$), 1.221 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$); δ_{C} (100 MHz, CDCl_3): 172.6, 168.0, 163.0, 162.2, 155.9, 149.6, 136.7, 132.7, 130.1, 129.5, 128.1, 126.3, 123.8, 123.5, 122.3, 96.0, 79.0, 55.7, 52.0, 40.1, 34.5, 31.8, 29.6, 28.3, 22.1, 14.1; m/z : 533.17 ($\text{M} + \text{H}$) $^+$; ν_{max} / cm^{-1} : 3601, 3115, 3085, 3011, 2969, 2859, 2409, 2336, 2199, 2015, 1872, 1789, 1720, 1621, 1589, 1479, 1403, 1341, 1254, 1184, 869, 805, 715.

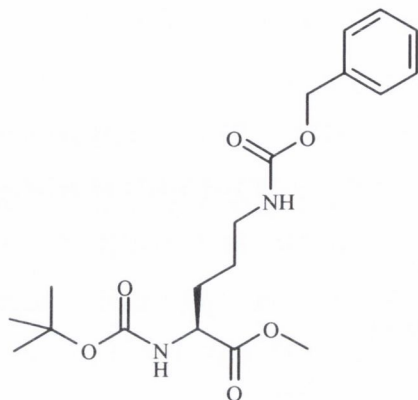
2,7-bis-{N-[N'-{(1S)-[(2S)-5-pentanoic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimido]-5-pentyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 117



a) Naphthalene tetracarboxylic dianhydride (0.005 g, 0.02 mmol) was refluxed with **116** (0.02 g, 0.04 mmol, 2 equiv.) according to **Procedure 5b**. The recrystallised product was a richly brown solid (0.007 g, 0.0054 mmol, 27 %, following purification by preparative plate chromatography (solvent: CH₂Cl₂, eluent: MeOH) 0.004 g, 0.0034 mmol, 17 %)

b) Naphthalene tetracarboxylic dianhydride (0.13 g, 0.50 mmol) was refluxed with **116** (0.5g, 1 mmol, 2 eq.) according to **Procedure 5d Pressure Tubes**. The recrystallised product was a richly brown solid (0.204 g, 0.16 mmol, 31 %). m.p.: 142 °C decomp.; Found C, 61.88; H, 4.21; N, 6.34 %. C₇₀H₅₆N₈O₁₈·C₄H₈O·2CH₂Cl₂·H₂O requires C, 59.56; H, 4.87; N, 6.95 %; δ_H (600 MHz, (CD₃)₂SO): 8.70 (2H, m, Ar-H7), 8.52 (8H, m, Ar-H5, Ar-H and Ar-H2), 8.07 (2H, d, J = 7.62 Hz, Ar-H3), 7.72 (2H, m, Ar-H6), 7.07 (10H, m, Ph), 5.71 (2H, m, CHCH₂), 4.44 (4H, m, PhCH₂), 3.96 (2H, m, NHCHCH₂), 3.62 (6H, s, OCH₃), 3.19 (4H, m, NHCHCH₂CH₂CH₂CH₂), 2.63 (4H, m, NHCHCH₂), 2.40 (4H, m, NHCHCH₂CH₂CH₂), 2.40 (4H, m, NHCHCH₂CH₂); δ_C (100 MHz, (CD₃)₂SO): 129.4, 128.1, 55.6, 55.2, 51.7, 37.4, 34.2, 31.3, 30.5, 29.4, 29.2, 26.5, 22.6, 22.4, 14.3; *m/z*: 578.27 (M + 3DMAP + K)³⁺; ν_{max} / cm⁻¹: 3362, 2930, 1783, 1742, 1707, 1661, 1583, 1526, 1453, 1370, 1336, 1282, 1234, 1167, 1152, 1123, 1027, 869, 834, 785, 757, 696.

(1S)-6-Butoxycarbonylamino-pentanoic acid methyl ester-*tert*-butyl-carboxyl,
118

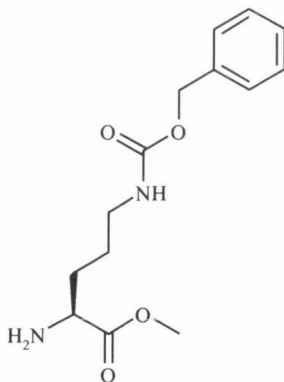


N-Boc-*N*-Cbz-Ornithine (10 g, 27 mmol) was dissolved in a hydrochloric solution (1M) of methanol and was refluxed for one hour according to **Procedure 8**. The solvent was then removed under reduced pressure to yield the desired methyl ester product as a white solid (4.17 g, 11 mmol, 41 %). m.p.: 103 °C Found C, 51.60; H, 6.59; N, 8.77 %.

$C_{19}H_{28}N_2O_6NaK$ requires C, 51.34; H, 6.80; N,

6.30 %; δ_H (600 MHz, $CDCl_3$): 7.30 (5H, m, Ph), 5.02 (2H, m, $\underline{CH_2}Ph$), 4.04 (1H, t, $J = 6.42, 6.36$ Hz, $\underline{CH}CH_2$), 3.70 (3H, s, OCH_3), 3.04 (2H, m, $NH\underline{CH_2}$), 1.84 (2H, m, $NHCH_2CH_2\underline{CH_2}$), 1.78 (2H, m, $NHCH_2CH_2$), 1.43 (9H, s, $(CH_3)_3$); δ_C (100 MHz, $CDCl_3$): 170.3, 158.2, 136.3, 128.1, 127.4, 81.2, 66.5, 53.3, 53.0, 52.9, 52.3, 52.0, 39.2, 38.5, 29.1, 26.9, 26.7, 24.3, 22.5; m/z : 220.13 ($M + Na + K$) $^{2+}$; ν_{max}/cm^{-1} : 3577, 3333, 2865, 2623, 2008, 1742, 1687, 1599, 1537, 1483, 1465, 1446, 1380, 1349, 1299, 1281, 1259, 1230, 1209, 1177, 1142, 1110, 1086, 1052, 1013, 938, 900, 886, 827, 797, 773, 736, 693.

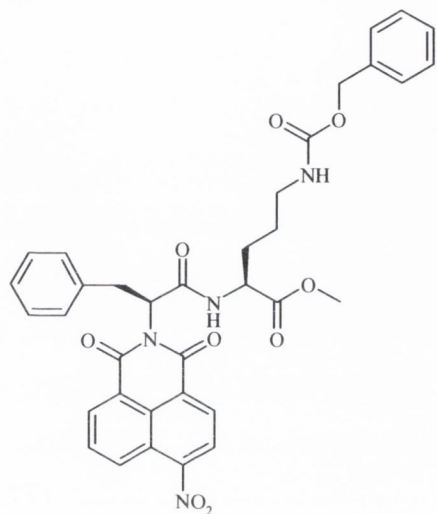
(1S)-6-Butoxycarbonylamino-pentanoic acid methyl ester, 119



118 (8 g, 0.02 mol) was hydrolysed to form **119** according to **Procedure 2**. The product was isolated as a viscous brown oil (3.08g, 0.01 moles, 50 %). HRMS: 281.1515 ($[M + H]^+$ $C_{14}H_{20}N_2O_4$ requires 280.33); δ_H (600 MHz, $CDCl_3$): 7.34 (5H, m, Ph), 5.08 (2H, m, $\underline{CH_2}Ph$), 4.09 (1H, m, $\underline{CH}CH_2$), 3.80 (3H, s, OCH_3), 3.22 (2H, m, $NH\underline{CH_2}$), 1.99 (2H, m, $NHCH_2CH_2\underline{CH_2}$), 1.67 (2H, m, $NHCH_2CH_2$); δ_C (100 MHz, $CDCl_3$):

170.3, 158.2, 136.3, 128.1, 127.4, 66.5, 53.3, 53.0, 52.9, 52.3, 52.0, 39.2, 38.5, 26.9, 26.7, 24.3, 22.5; m/z : 281.1448 ($M + H$) $^+$; ν_{max}/cm^{-1} : 3046, 2941, 2568, 2343, 1778, 1753, 1659, 1524, 1443, 1346, 1269, 1140, 1013, 841, 813, 797, 781, 759, 725, 698.

***N*-{(1*S*)-[(2*S*)-6-Butoxycarbonyl-amino-pentanoic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimide, 120¹⁰⁵**

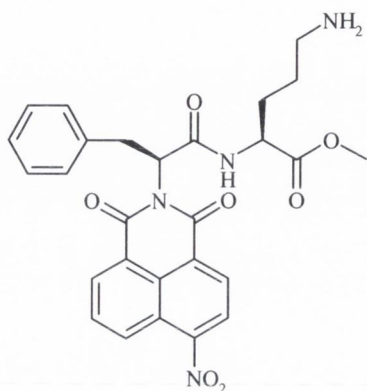


119 (3.90 g, 10 mmol) was coupled with **77** (3 g, 10 mmol, 1 eq.) according to **Procedure 3**, using HOBt (1.35 g, 10 mmol, 1 eq.), EDCI (2.11 g, 11 mmol, 1.1 eq.) and Et₃N (1.26 mL, 11 mmol, 1.1 eq.). The desired product was isolated as an orange solid (4.92g, 7.6 mmol, 76 %). m.p.: 81 °C; Found C, 63.27; H, 4.73; N, 9.14 %. C₃₅H₃₂N₄O₉·H₂O·Et₃N requires C, 63.80; H, 6.40; N, 9.07 %; δ_{H} (400 MHz, CDCl₃): 8.80 (1H, d, *J* = 7.92 Hz, Ar-H7), 8.65 (1H, d, *J* = 7.14 Hz, Ar-H5), 8.54 (1H, d,

J = 7.86 Hz, Ar-H2), 8.35 (1H, d, *J* = 7.86 Hz, Ar-H3), 7.97 (1H, m, Ar-H6), 7.14 (10H, m, Ph), 6.07 (1H, m, CHCH₂Ph), 4.89 (1H, m, NHCHCH₂), 3.75 (3H, s, OCH₃), 3.55 (2H, m, OCH₂Ph), 3.19 (2H, m, PhCH₂), 2.20 (2H, m, NHCH₂CH₂CH₂), 1.88 (2H, m, NHCH₂), 1.28 (2H, m, NHCH₂CH₂); δ_{C} (100 MHz, CDCl₃): 172.5, 172.4, 168.9, 168.4, 163.0, 162.6, 149.6, 136.7, 132.6, 129.9, 126.8, 122.2, 115.4, 96.0, 88.3, 69.6, 55.7, 54.1, 52.8, 45.7, 40.0, 38.8, 34.4, 30.8, 29.8, 28.8, 25.7, 22.1, 14.1, 8.5; ν_{max} / cm⁻¹: 3331, 3029, 2947, 1709, 1667, 1626, 1594, 1584, 1526, 1496, 1454, 1427, 1410, 1367, 1339, 1238, 1179, 1128, 1029, 989, 914, 864, 834, 784, 746, 698.

***N*-{(1*S*)-[(2*S*)-6-amino-pentanoic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimide, 121** ¹⁰⁵

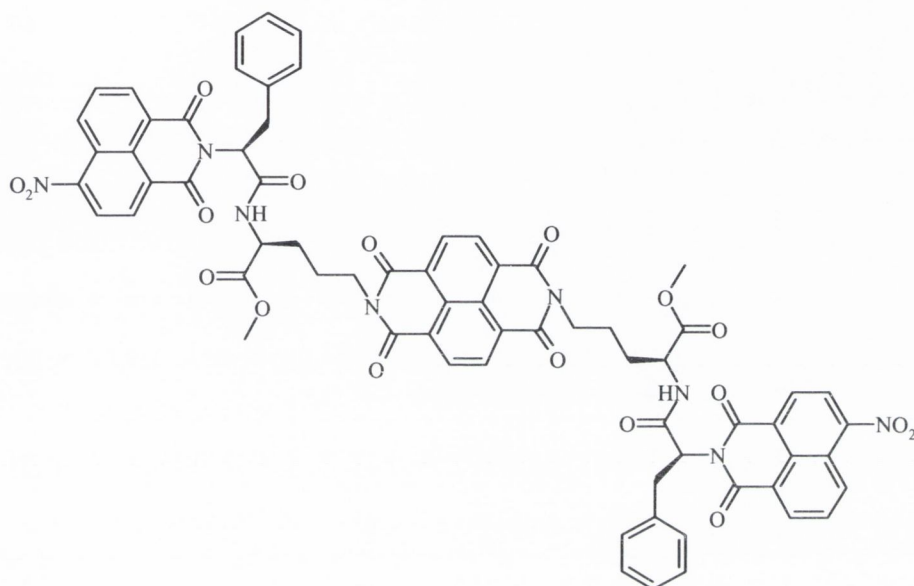
120 (1.66 g, 2.5 mmol) was treated with 30 % HBr / acetic Acid (25 mL, in excess) according to **Procedure 11**. The desired product was isolated as a an orange solid



from acetone (1.03 g, 2.0 mmol, 80 %). m.p.: 83 - 86 ° C; δ_{H} (600 MHz, CDCl_3): 8.81 (1H, d, $J = 7.98$ Hz, Ar-H7), 8.67 (1H, d, $J = 7.06$ Hz, Ar-H5), 8.56 (1H, d, $J = 7.54$ Hz, Ar-H2), 8.37 (1H, d, $J = 7.32$ Hz, Ar-H3), 7.94 (1H, m, Ar-H6), 7.10 (5H, m, Ph), 6.05 (1H, m, CHCH_2Ph), 4.91 (1H, m, NHCHCH_2), 3.72 (3H, s, OCH_3), 3.21 (2H, m, PhCH_2), 2.15 (2H, m, NH_2CH_2), 1.87 (2H, m,

$\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.26 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$); δ_{C} (100 MHz, CDCl_3): 172.3, 172.1, 168.4, 149.5, 136.3, 132.1, 129.7, 126.4, 122.1, 115.1, 95.5, 88.1, 69.4, 55.2, 54.0, 52.6, 46.6, 39.2, 37.8, 34.6, 31.8, 29.5, 28.2, 25.2, 22.0, 13.1; m/z : 259.21 ($\text{M} + 2\text{H}$)²⁺; ν_{max} / cm^{-1} : 3232, 2952, 1645, 1270, 1086, 876, 760, 738, 712.

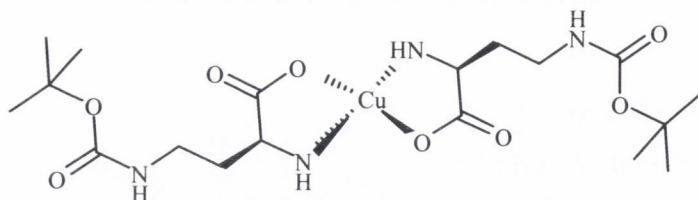
2,7-bis- $\{N-[N'-\{(1S)-[(2S)-4\text{-butanoic acid methyl ester}]\text{-carboxyamido-2-ethylphenyl}\}-4\text{-nitro-1,8-naphthalimido}]\text{-4-butyl}\}\text{-benzo}[1mn][3,8]\text{-phenanthroline-1,3,6,8-tetraone, 122}$



Naphthalene tetracarboxylic dianhydride (0.18 g, 0.66 mmol) was refluxed with **121** (0.34 g, 1.33 mmol, 2 eq.) according to **Procedure 5d**. Recrystallisation was unsuccessful, the organic layer was dried under vacuum and product was obtained as a brown oil/residue (0.20 g, 0.16 mmol, 24 %). δ_{H} (400 MHz, CDCl_3): 8.72 (2H, m, Ar-H7), 8.55 (6H, m, Ar-H5 and Ar-H), 8.30 (2H, m, Ar-H2), 8.05 (2H, m, Ar-H3), 7.33 (2H, m, Ar-H6), 7.09 (10H, m, Ph), 4.65 (NHCHCH₂), 4.19 (2H, m, CHCH₂Ph), 4.11 (6H, s, OCH₃), 4.04 (4H, m, PhCH₂), 3.57 (2H, m, NHCHCH₂CH₂CH₂), 2.91 (2H, m, NHCHCH₂), 2.74 (2H, m, NHCHCH₂CH₂); m/z : 425.20 ($\text{M} + 5\text{H}$)³⁺; ν_{max} / cm^{-1} : 3363, 2928, 1782, 1743, 1705, 1662, 1585, 1528, 1454, 1374, 1342, 1278, 1236, 1169, 1154, 1127, 1028, 870, 835, 786, 758, 697.

Bis-[(1*S*)-5-*tert*-butylcarboxylamino-butryic acid]-cuprate, 123 ¹⁰⁵

To a stirring solution of the 2,4-diamino butyric acid.HCl (10 g, 65 mmol) in NaOH (2M), a solution of Cu(CH₃COO)₂.H₂O (5.81 g, 32.5 mmol, 0.5 eq.) in water was

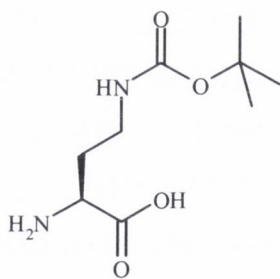


introduced, according to

Procedure 9. To this was added a solution of Boc₂O (18.33 g, 84.5 mmol, 1.3. eq.) in acetone. After 24 h a further addition of

acetone was made and the solution was stirred for a further 24 h. A black precipitate was filtered off and washed with acetone and water. The filtrate and washings were combined and the solvent was removed under reduced pressure to yield the product as a light blue waxy solid (10 g, 19 mmol, 30 %). Decomposition temperature: 194 °C; δ_{H} (400 MHz, CDCl₃): 3.77 (4H, m, NHCH₂CH₂CH₂), 3.02 (4H, m, NHCH₂), 1.60 (4H, m, NHCH₂CH₂), 1.32 (18H, s, (CH₃)₃); δ_{C} (100 MHz, CDCl₃) : 181.3, 175.1, 157.9, 157.4, 81.0, 80.7, 78.3, 71.8, 53.8, 36.9, 31.5, 27.7, 23.1; m/z : 263.09 (M + H)⁺; ν_{max} / cm⁻¹: 3286, 2192, 1560, 1435, 1402, 1339, 1167, 1049, 1019, 863.

(1*S*)-5-*tert*-butylcarboxylamino-butryic acid, 124 ¹⁰⁵

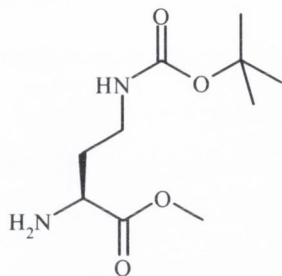


A suspension of [2,4-diaminobutyric acid(Boc)]₂Cu (10 g, 20 mmol) in acetone was stirred for 15 min, water was then added and the solution stirred for a further 10 min, according to **Procedure 10.** Water and 8-quinolinol (7.18 g, 50 mmol, 2.5 eq.) were introduced and stirring was continued for 4 h. The copper(II) quinolinolate precipitate was filtered off and washed with water. The filtrate and

washings were combined and acetone was evaporated. The residual aqueous solution was extracted with EtOAc and the water was then removed under reduced pressure to yield the desired product as a cream semi-solid. (1.04 g, 4.78 mmol, 24 %). δ_{H} (400 MHz, CDCl₃): 3.53 (2H, m, NHCH₂CH₂CH₂), 3.00 (2H, M, NHCH₂), 1.87 (2H, m, NHCH₂CH₂), 1.30 (9H, s, (CH₃)₃); δ_{C} (100 MHz, CDCl₃): 181.4, 157.5, 80.6, 57.4,

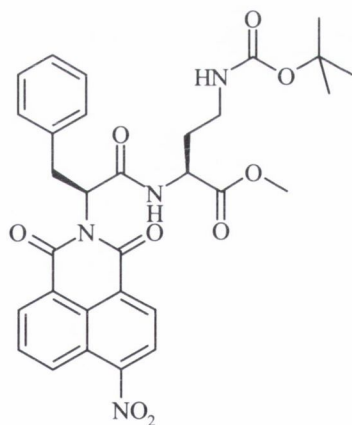
36.5, 30.0, 16.6; m/z : 241.1160 ($M + Na$)⁺; $\nu_{\max}/\text{cm}^{-1}$: 3371, 3277, 2227, 1681, 1636, 1551, 1404, 1338, 1270, 1245, 1166, 1050, 1019, 927, 783, 760.

(1S)-5-*tert*-butylcarboxylamino-butryic acid methyl ester, 125



124 (8 g, 36.67 mmol) was esterified using hydrochloric methanol according to **Procedure 8**. A brown semi-solid was isolated (1.20 g, 5 mmol, 14 %); δ_{H} (400 MHz, CDCl_3): 4.18 (2H, m, $\text{NHCH}_2\text{CH}_2\text{CH}_2$), 3.76 (3H, s, OCH_3), 3.16 (2H, m, NHCH_2), 3.10 (2H, m, NHCH_2CH_2), 2.15 (18H, s, $(\text{CH}_3)_3$); δ_{C} (100 MHz, CDCl_3): 171.1, 169.2, 53.8, 50.2, 48.8, 36.0, 35.8, 27.5, 27.3; m/z : 163.10 ($M + \text{H}_2\text{O} + \text{MeOH} + 2\text{H}$)²⁺; $\nu_{\max}/\text{cm}^{-1}$: 3343, 3211, 2907, 1978, 1741, 1613, 1508, 1467, 1346, 1286, 1238, 1199, 1143, 956, 779, 760.

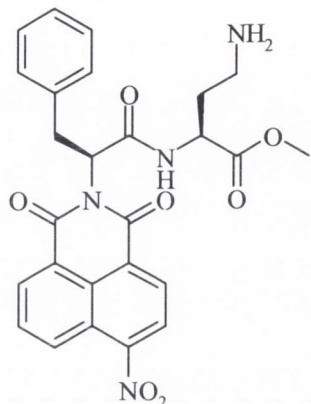
***N*-{[(1S)-[(2S)-5-*tert*-butylcarboxylamino-butryic acid methyl ester]-carboxyamido-2-ethylphenyl]-4-nitro-1,8-naphthalimide, 126¹⁰⁵**



115 (1.3 g, 0.64 mmol) was coupled with **77** (0.25 g, 0.64 mmol, 1 eq.) according to **Procedure 3**, using HOBt (0.086 g, 0.64 mmol, 1 eq.), EDCI (0.135 g, 0.70 mmol, 1.1 eq.) and Et_3N (0.08 mL, 0.70 mmol, 1.1 eq.). The desired product was obtained as a viscous brown oil (0.07 g, 0.12 mmol, 19 %). Found C, 65.98; H, 7.19; N, 10.17 %. $\text{C}_{31}\text{H}_{32}\text{N}_4\text{O}_9 \cdot 2\text{Et}_3\text{N}$ requires C, 64.00; H, 7.74; N, 10.24 %; δ_{H} (600 MHz, CDCl_3): 8.84 (1H, m, Ar-H7), 8.68 (1H, m, Ar-H5), 8.63 (1H, m, Ar-H2), 8.39 (1H, m, Ar-H3), 7.97 (1H, m, Ar-H6), 7.09 (5H, m, Ph), 6.05 (1H, m, CHCH_2Ph), 5.14 (1H, m, NHCHCH_2), 3.67 (3H, s, OCH_3), 3.31 (2H, m, PhCH_2), 2.56 (2H, m, NHCH_2CH_2), 2.34 (2H, m, NHCH_2); δ_{C} (100 MHz, CDCl_3): 163.0, 149.6, 142.2, 140.6, 136.4, 132.7, 130.1, 129.6, 128.7, 128.8, 126.8, 123.8, 122.3, 117.0, 56.8, 55.5, 45.7, 42.8, 42.2, 37.8, 34.6, 32.2, 29.5, 29.2, 26.2, 25.4, 22.6, 14.0; m/z : 546.21 ($M + \text{CH}_2\text{Cl}_2 + 3\text{Et}_3\text{N} + \text{C}_4\text{H}_8\text{O}$)²⁺; $\nu_{\max}/\text{cm}^{-1}$: 2932, 2190, 1672, 1533, 1344, 1270, 1204, 786, 760, 740, 715.

***N*-{(1*S*)-[(2*S*)-5-butryic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimide, 127**

126 (1.50 g, 2.5 mmol) was hydrolysed using TFA according to **Procedure 2**. The



desired product was obtained as a fluorescent yellow oil

(1.07 g, 2.0 mmol, 81 %). Decomposition temperature :

245 – 250 °C; δ_{H} (600 MHz, CDCl_3): 8.85 (1H, d, J =

8.70 Hz, Ar-H7), 8.68 (1H, d, J = 7.14 Hz, Ar-H5), 8.64

(1H, d, 7.92 Hz, Ar-H2), 8.40 (1H, d, J = 8.28 Hz, Ar-

H3), 7.99 (1H, t, J = 8.28, 7.56 Hz, Ar-H6), 7.14 (5H,

m, Ph), 6.11 (1H, m, CHCH_2Ph), 5.33 (1H, m,

NHCHCH_2), 4.11 (3H, s, OCH_3), 3.56 (2H, m, PhCH_2),

2.36 (2H, m, NH_2CH_2), 2.21 (2H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$); δ_{C}

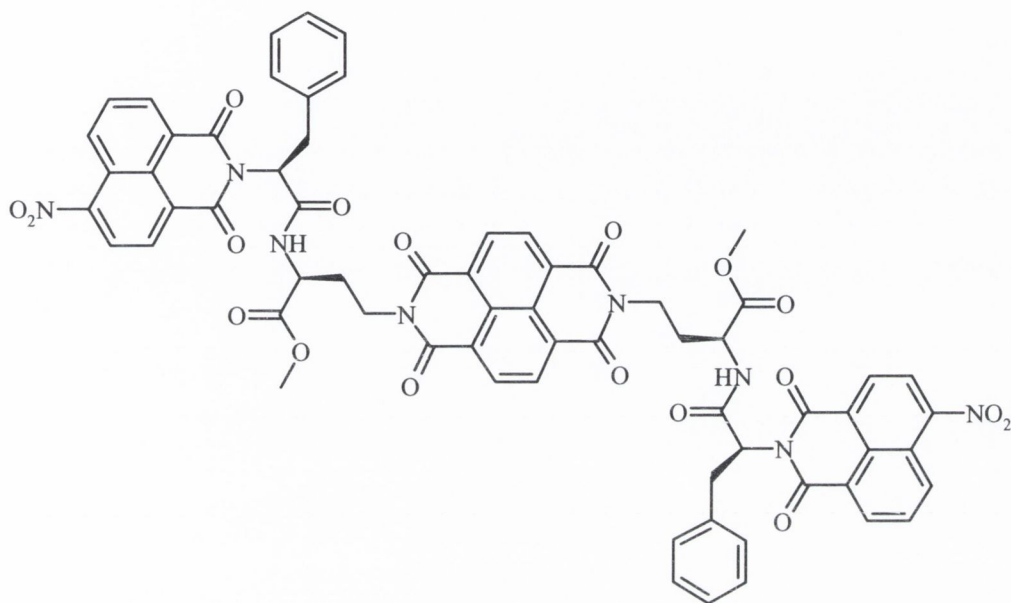
(100 MHz, CDCl_3): 162.6, 161.8, 149.7, 136.5, 132.7, 130.1, 129.6, 128.9, 126.7,

123.7, 122.1, 101.0, 98.8, 95.8, 89.6, 86.7, 84.1, 76.1, 60.3, 54.0, 48.6, 34.5, 30.8,

21.3; m/z : 578.24 ($\text{M} + \text{C}_4\text{H}_8\text{O} + \text{H}$)⁺; ν_{max} / cm^{-1} : 3216, 1668, 1531, 1348, 1270,

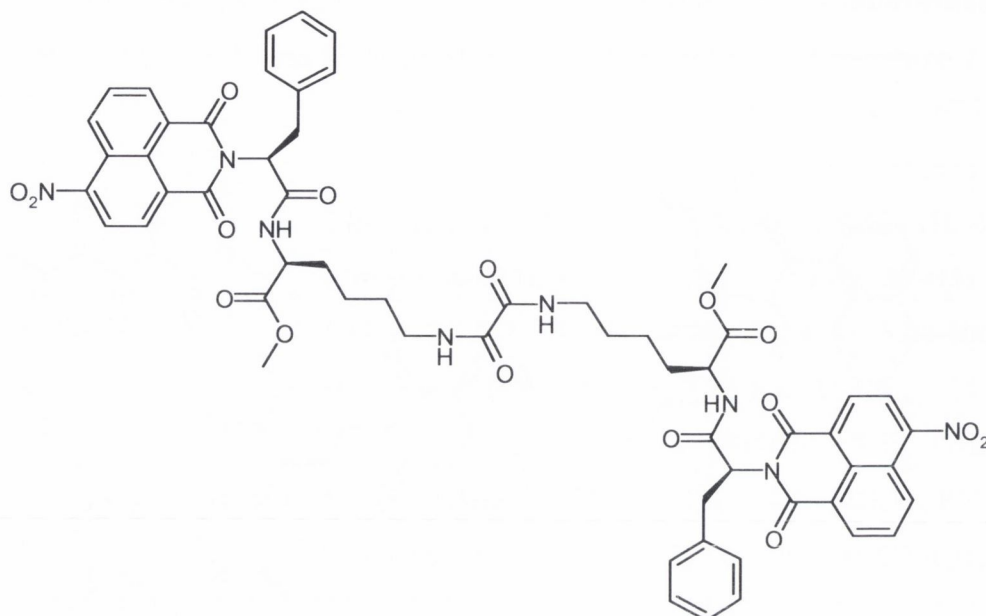
1112, 1047, 882, 783.

2,7-bis-{N-[N'-{(1*S*)-[(2*S*)-3-propionic acid methyl ester]-carboxyamido-2-ethylphenyl}-4-nitro-1,8-naphthalimido]-3-propyl}-benzo[1*mn*][3,8]-phenanthroline-1,3,6,8-tetraone, 128



Naphthalene tetracarboxylic dianhydride (0.11 g, 0.40 mmol) was refluxed with **127** (0.43 g, 0.80 mmol, 2 equiv.) according to **Procedure 5b**. Recrystallisation was unsuccessful, the organic layer was dried under vacuum and product was obtained as a yellow/white semi-solid (0.12 g, 0.094 mmol, 24 %). δ_{H} (400 MHz, CDCl_3): 8.90 (2H, d, $J = 6.52$ Hz, Ar-H7), 8.74 (6H, m, Ar-H5 and Ar-H), 8.43 (2H, d, $J = 7.52$ Hz, Ar-H2), 8.03 (2H, m, Ar-H3), 7.73 (2H, m, Ar-H6), 7.17 (10H, m, Ph), 6.05 (NHCHCH $\underline{\text{CH}_2}$), 5.43 (2H, m, CHCH $\underline{\text{CH}_2}$ Ph), 4.25 (6H, s, OCH_3), 3.67 (4H, m, PhCH $\underline{\text{CH}_2}$), 1.71 (2H, m, NHCHCH $\underline{\text{CH}_2}$ CH $\underline{\text{CH}_2}$), 1.46 (2H, m, NHCHCH $\underline{\text{CH}_2}$); m/z : 337.12 ($\text{M} + \text{C}_4\text{H}_8\text{O} + \text{K}$) $^{4+}$; ν_{max} / cm^{-1} : 3354, 2932, 1784, 1742, 1710, 1672, 1595, 1538, 1456, 1375, 1345, 1276, 1238, 1169, 1155, 1137, 1030, 875, 834, 782, 758, 695.

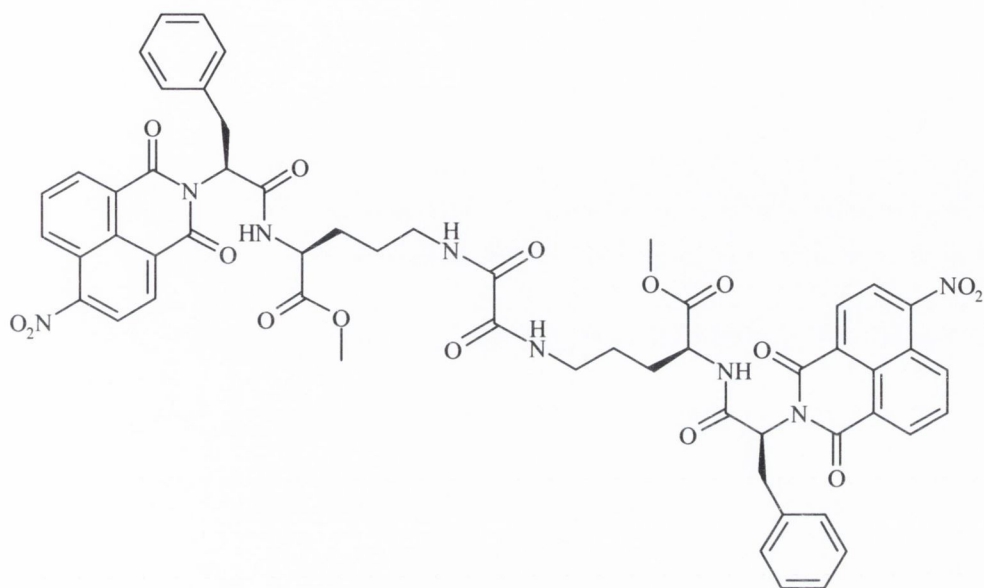
Bis- $\{N-[N-\{(1S)-(2S)\text{-pentanoic acid methyl ester}\}-\text{carboxyamido-2-ethylphenyl}\}-4\text{-nitro-naphthalimidyl}\}-\text{pentanamido}\}$ hexanoate, 129



116 (0.3 g, 0.56 mmol) was dissolved in CH_2Cl_2 and to this solution was added oxalyl chloride (2M solution in CH_2Cl_2 , 0.036 g, 0.027 mL, 0.28 mmol, 0.5 eq.). This reaction was refluxed, under argon, for 72 h, in the presence of Et_3N (0.042 g, 0.048 mL, 0.42 mmol, 0.75 eq.). The product mixture was coevapoarted with diethyl ether to remove any remaining oxalyl chloride. The solvent was removed under reduced pressure and resulting residue was dissolved CH_2Cl_2 . Precipitation from diethyl ether failed to isolate the product, as did attempts at re crystallisation from EtOAc / hexane 6:1. The residue was dissolved in acetone and dried under vacuum. The desired product was obtained as a viscous hyroscopic brown residue (0.043 g, 0.038 mmol, 14 %). δ_{H} (400 MHz, CDCl_3): 8.83 (2H, d, $J = 8.54$ Hz, Ar-H7), 8.66 (2H, d, $J = 7.03$ Hz, Ar-H5), 8.61 (2H, d, $J = 8.03$ Hz, Ar-H2), 8.39 (2H, d, $J = 8.03$ Hz, Ar-H3), 7.99 (2H, t, $J = 7.53$ and 8.53 Hz, Ar-H6), 7.17 (10H, m, Ph), 6.57 (2H, br. s, NH), 6.11 (2H, m, CHCH_2Ph), 4.69 (4H, m, PhCH_2), 4.25 (2H, m, NHCHCH_2), 3.74 (4H, m, $\text{NHCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 3.51 (6H, m, OCH_3), 3.35 (4H, m, NHCHCH_2), 1.56 (4H, m, $\text{NHCHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.32 (4H, m, $\text{NHCHCH}_2\text{CH}_2$); m/z : 511.88 ($\text{M} + 3\text{CH}_2\text{Cl}_2 + \text{C}_4\text{H}_8\text{O} + 3\text{H}$) $^{3+}$; ν_{max} / cm^{-1} : 3364, 2933, 1790, 1765, 1710, 1670, 1585, 1528, 1457, 1379, 1339, 1284, 1233, 1168, 1153, 1124, 1020, 865, 839, 781, 753, 697.

***Bis*–{*N*–[*N*–{(1*S*)–[(2*S*)–butannic acid methyl ester]–carboxymido–2–ethylphenyl}–4–nitro–naphthalimidyl]–butanamido}pentanoate**

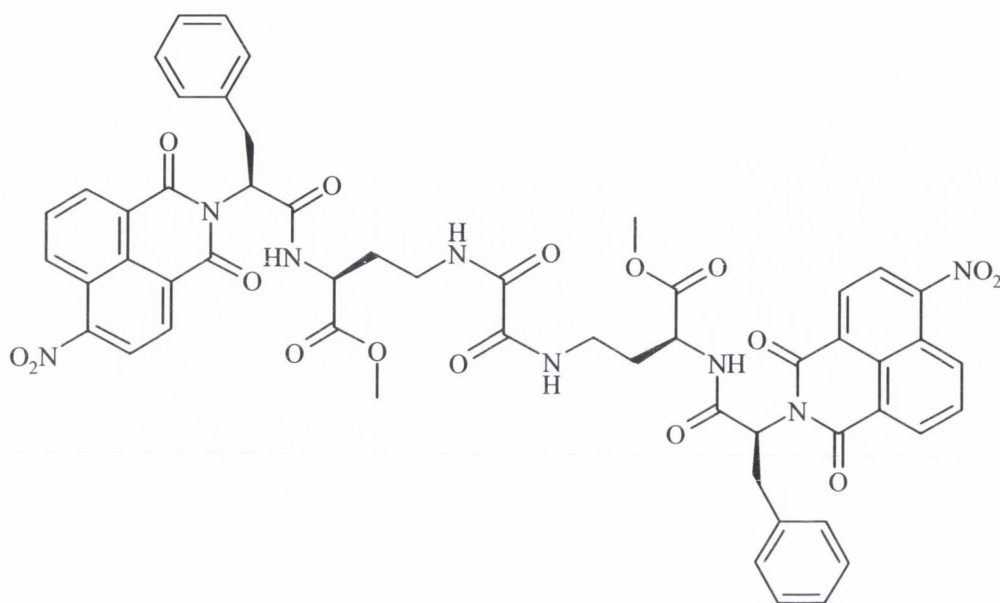
130



121 (0.90 g, 1.70 mmol) was dissolved in CH₂Cl₂ and to this solution was added oxalyl chloride (2M solution in CH₂Cl₂, 0.13 g, 0.00065 mL, 0.87 mmol, 0.5 eq.) This reaction was refluxed, under argon, for 72 h, in the presence of Et₃N (0.13 g, 0.15 mL, 1.28 mmol, 0.75 eq.). The product mixture was coevapoarted with diethyl ether to remove any remaining oxalyl chloride. The solvent was removed under reduced pressure and resulting residue was dissolved CH₂Cl₂. The residue was dissolved in acetone and dried under vacuum. The desired product was obtained as a hydropscopic brown residue (0.17 g, 0.16mmol, 18 %). δ_{H} (400 MHz, CDCl₃): 8.60 (2H, m, Ar-H7), 8.50 (2H, m, Ar-H5), 8.34 (2H, m, Ar-H2), 7.94 (2H, m, Ar-H3), 7.46 (2H, m, Ar-H6), 7.09 (10H, m, Ph), 4.10 (NHCHCH₂), 3.60 (2H, m, CHCH₂Ph), 3.29 (6H, s, OCH₃), 3.10 (4H, m, PhCH₂), 2.42 (2H, m, NHCHCH₂CH₂CH₂CH₂), 2.12 (2H, m, NHCHCH₂), 1.87 (2H, m, NH CHCH₂CH₂CH₂); m/z : 409.20 (M + 4CH₂Cl₂ + C₄H₈O + Na)⁺; ν_{max} / cm⁻¹: 3369, 2935, 1794, 1761, 1715, 1677, 1588, 1520, 1452, 1380, 1342, 1285, 1236, 1169, 1156, 1129, 1019, 849, 836, 782, 751, 696.

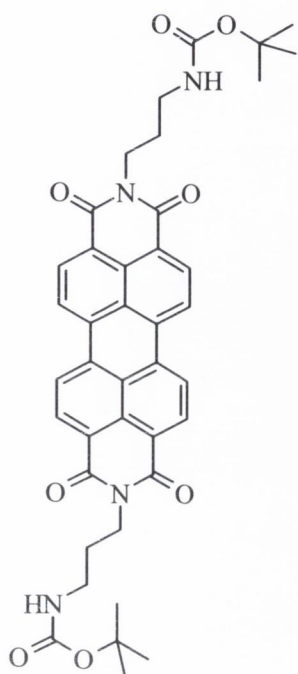
***Bis*–{*N*–[*N*–{(1*S*)–[(2*S*)–propionic acid methyl ester]–carboxymido–2–ethylphenyl}–4–nitro–naphthalimidyl]–propanamido}butanoate**

131



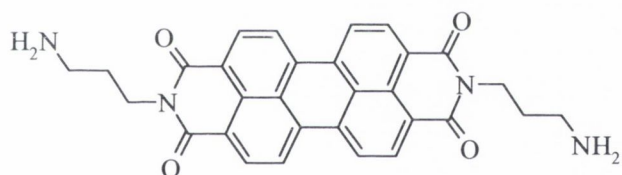
126 (0.4 g, 0.80 mmol) was dissolved in CH₂Cl₂ and to this solution was added oxalyl chloride (2M solution in CH₂Cl₂, 0.065 g, 0.0003 mL, 0.40 mmol, 0.5 eq.) with Et₃N (0.02 g, 0.023 mL, 0.75 eq.) This reaction was refluxed, under argon, for 72 h. The product mixture was coevapoarted with diethyl ether to remove any remaining oxalyl chloride. The solvent was removed under reduced pressure and resulting residue was dissolved CH₂Cl₂. The residue was dissolved in acetone and dried under vacuum. The desired product was obtained as a hydropscopic brown residue (0.08 g, 0.072 mmol, 18 %) Found C, 57.67; H, 4.45; N, 8.01 %. C₅₄H₆₄N₈O₁₆.CH₂Cl₂.C₄H₁₀O.Na requires C, 57.55; H, 4.91; N, 8.80 %; δ_{H} (400 MHz, CDCl₃): 8.79 (2H, d, J = 8.56 Hz, Ar-H7), 8.60 (2H, m, Ar-H5), 8.34 (2H, d, J = 8.04 Hz, Ar-H2), 7.95 (2H, m, Ar-H3), 7.60 (2H, m, Ar-H6), 7.15 (10H, m, Ph), 6.52 (NHCHCH₂), 6.07 (2H, m, CHCH₂Ph), 3.54 (2H, m, PhCH₂), 2.20 (6H, s, OCH₃), 1.27 (2H, m, NHCHCH₂CH₂), 0.90 (2H, m, NHCHCH₂); δ_{C} (100 MHz, CDCl₃): 173.3, 162.3 161.5, 149.3, 136.3, 134.6, 132.9, 132.4, 128.7, 128.0, 124.6, 123.5, 123.4, 121.8, 119.9, 108.5, 54.0, 52.4, 34.5, 34.1, 30.5, 29.3; m/z : 543.60 (M + 4CH₂Cl₂ + C₄H₈O + Na)³⁺; ν_{max} / cm⁻¹: 3372, 2938, 1796, 1768, 1719, 1671, 1582, 1524, 1455, 1388, 1343, 1286, 1239, 1174, 1158, 1130, 1021, 846, 832, 780, 755, 699.

[*N, N'*-bis-*N*-(*tert*-butoxycarbonyl)-3-aminopropyl]-perylene diimide, 133



Perylene tetracarboxylic dianhydride (0.00178 g, 0.178 mmol) and **88** (0.25 g, 1.43 mmol, 8 eq.) were reacted following **Procedure 4b**. The product was isolated by precipitation as a hygroscopic red solid (0.046 g, 0.09 mmol, 51 %). m.p.: 110 °C; δ_{H} (400 MHz, $(\text{CD}_3)_2\text{SO}$): 8.95 (4H, m, Ar-H), 8.55 (4H, m, Ar-H), 2.98 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 2.75 (4H, m, NH_2CH_2), 1.63 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$), 1.14 (18H, m, $(\text{CH}_3)_3$); m/z : 534.50 ($\text{M} + 2\text{Et}_3\text{N} + 2\text{C}_4\text{H}_8\text{O} + \text{H}_2\text{O}$) $^{2+}$; ν_{max} / cm^{-1} : 3119, 2967, 1756, 1742, 1730, 1692, 1595, 1507, 1407, 1366, 1301, 1235, 1151, 1130, 1122, 1025, 1017, 938, 860, 809, 793, 758, 733.

[*N, N'*-bis-3-aminopropyl]-perylene diimide, 134



133 (0.045 g, 0.60 mmol) was hydrolysed according to **Procedure 2**. The desired product was obtained as a hygroscopic wine red solid, by precipitation from diethyl ether (0.30 g, 0.48 mmol, 80 %). m.p.: 90 °C ; HRMS : 505.1876 ($[\text{M} + \text{H}]^+$, $\text{C}_{30}\text{H}_{24}\text{N}_4\text{O}_4$ requires 504.5512); δ_{H} (400 MHz, $(\text{CD}_3)_2\text{SO}$): 4.80 (4H, m, Ar-H), 4.75 (4H, m, Ar-H), 2.98 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2$), 1.94 (4H, m, NH_2CH_2), 1.06 (4H, m, $\text{NH}_2\text{CH}_2\text{CH}_2$); m/z : 253.32 (M) $^{2+}$, 505.61 ($\text{M} + \text{H}$) $^+$; ν_{max} / cm^{-1} : 3122, 2954, 2768, 2610, 2377, 2282, 2242, 2123, 2091, 1969, 1948, 1920, 1867, 1743, 1680, 1593, 1534, 1507, 1433, 1407, 1342, 1301, 1236, 1203, 1151, 1122, 1066, 1025, 972, 939, 861, 838, 809, 795, 759, 734, 641, 602.



Chapter Six

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