PREPARATION, OPTIMISATION AND CHARACTERISATION

OF SEQUENCE SELECTIVE COMPOUNDS

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STATEMENT OF AUTHENTICATION

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, Medical Science, at the University of Western Sydney; College of Health and Science; School of Biomedical and Health Sciences; Department of Chemistry. I here admit that the work presented in this thesis is original except as acknowledged in the text. I declare that I have not previously submitted this work, either in whole or in part, for a degree at this or any other institution.

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ABSTRACT

DNA is the pharmacological target for most platinum drugs; however, the majority of these drugs show little or no specificity for particular base pairs. Considerable progress has been made in the design of sequence selective compounds, such that an antiparallel association of a polyamide can have high affinity for selected DNA base pairs. Hairpin polyamides have distinct advantages as they achieve affinities and specificities that are comparable to that of DNA-binding proteins. Platinum(II) hairpin polyamides are expected to display antitumour activity and target specific sequences of DNA.

Five DNA-sequence-selective hairpin polyamide platinum(II) complexes, containing pyrrole (Py) and imidazole (Im) heterocyclic rings, have been synthesised using a combination of solid and solution phase chemistry. One mononuclear sequence selective complex, $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_4$-Pt (HLSP-6) [$\beta$-Ala is $\beta$-alanine, L$_4$ is 4-(Fmoc-amino)butyric acid and Pt is transplatin], and two dinuclear sequence selective complexes, $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6$'-Pt-(Pt) (DNHLSP-6) [L$_6'$ is 2,6-Fmoc-Lysine-(Fmoc)-OH] and $\beta$-Ala-PyPyImImIm-L$_4$'-PyPyPyPy-L$_6$'-Pt-(Pt) (DNHLSP-10) (L$_4'$ is 2-Boc-4-Fmoc-L-diaminobutyric acid), were synthesised entirely using solid phase chemistry. Two mononuclear sequence selective complexes, Pt-L$_6$-$\beta$-Ala-Py-L$_4$-Im (HSP-2) and Pt-L$_6$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm (HSP-6), were synthesised using a combination of solid and solution phase chemistry. The synthesis of a trinuclear sequence selective polyamide was also attempted using a combination of solid and solution phase chemistry.

The polyamides were synthesised in a series of reaction steps. Each heterocyclic ring and linker was coupled through solid phase chemistry using 2-(1H-benzo[d]triazole-1-y1)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). Once the organic components were assembled, the platinum(II) group/s was/were added using either solid or solution phase chemistry.
The polyamide sequence of PyPyPy-L4-ImImIm was designed to target the guanine rich telomere region of DNA. The metal complexes reported in this study will span sequences between 2, 5 or 7 DNA base pairs (depending on their length), which include 5'- (A/T)GGG(A/T)-3' and 5'-(A/T)(A/T)(A/T)GGG(A/T)-3'.

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ABBREVIATIONS

A Adenine
Ac₂O Acetic anhydride
ACN Acetonitrile
β-Ala β-Alanine
Boc Di-tert-butylidicarbonate
cisplatin cis-Diaminedichloroplatinum(II)
C Cytosine
CL Cross-link
DMF Dimethylformamide
DMSO Dimethylsulfoxide
DNA Deoxyribonucleic acid
E. coli Escherichia coli
eq. Equivalence
Fmoc 9-Fluorenylmethyl chloroformate
G Guanine
h Hour/hours
HBTU 2-(1H-benzotriazole-1-y1)-1,1,3,3-tetramethyluronium hexafluorophosphate
Hp N-Methyl-3-hydroxy-2-pyrrolecarboxylic acid
Im N-Methyl-1H-imidazole
Im' N-Methyl-1H-imidazole-2-carboxylic acid
Im A 2-Trichloroacetyl-N-methyl-1H-imidazole
Im B 4-Nitro-2-trichloroacetyl-N-methyl-1H-imidazole
Im C tert-Butyl-4-Nitro-N-methyl-1H-imidazole-2-carboxylate
Im D tert-Butyl-4-[(9-fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylate
Im E 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylic acid
Im-L₄-COOEt Ethyl γ-(N-methyl-1H-imidazole-2-carboxamido)butyricate
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RESEARCH PUBLICATIONS

Publications contributing to this thesis:


Conference papers contributing to this thesis:


CHAPTER 1: INTRODUCTION

1.1 Deoxyribonucleic acid (DNA)

DNA was first isolated in the late 1800s from salmon sperm by German biochemist Friedrich Miescher.\textsuperscript{1,2} Further studies revealed that DNA consisted of four repeated bases. Scientists couldn’t believe that DNA, which is so simple, could carry our entire genetic code. Instead, proteins, that appeared to be very complex molecules, were credited with carrying the genetic information.\textsuperscript{1,2}

It was not until halfway through the 20\textsuperscript{th} century that DNA was confirmed as the carrier of the genetic code, after Avery, Macleod and McCarty transferred pathogenic Pneumococcus DNA into non-pathogenic strains.\textsuperscript{1,2} The mutated strains were genetically stable and succeeding generations retained the pathogenic characteristics. Scientists were finally convinced when Hershey and Chase studied the infection of Escherichia coli (E. coli) by a bacterial virus, the bacteriophage T2. Knowing that the cytoplasm of the T2 consists mainly of sulphur and traces of phosphorus, while its DNA contains only phosphorus, scientists labelled the T2 bacteriophage with radioactive $^{35}$S and $^{32}$P and then attached it to E. coli. It was observed that only the $^{32}$P, hence the bacteriophage DNA, was transferred to the bacteria.\textsuperscript{1,2}

DNA is also an important target for drugs. Scientists believe that by creating drugs that target specific sequences of DNA, it is possible to manipulate gene regulation with therapeutic results. Possible applications for this technology include the treatment of HIV and cancer cells, whose growth in human beings is a cause of illness and suffering worldwide.

1.1.1 Structure of DNA

It was not until 1953 that the current structure of DNA was first proposed by James Watson and Francis Crick.\textsuperscript{3} They found that DNA consists of a double helix, composed of two complementary strands of unbranched polynucleotides. Each
nucleotide consists of a carbohydrate (2-deoxy-D-ribose), a phosphate group and a heterocyclic aromatic amine base. The bases are divided into two groups, the purines, adenine (A) and guanine (G), and the pyrimidines, cytosine (C) and thymine (T) (Figure 1. 1). The bases from the two strands are paired in a specific and complementary manner, where A binds to T and G binds to C through a series of Hydrogen-bonding (H-bonding) interactions (Figure 1. 2).\(^3\) Even though there are only four bases, variation in the structure of DNA is more diverse than one would first assume, mainly due to the different primary, secondary and tertiary structures that are adopted by the DNA macromolecules.

Figure 1. 1: The four nitrogenous bases A, G, C and T. A and G are purine derivatives, while C and T are pyrimidine derivatives.\(^3\)

Figure 1. 2: The base pairing and H-bonding that exists between A-T and G-C nucleotide base pairs.\(^4\)
Each strand of the DNA double helix binds its complementary strand in an antiparallel fashion. This is due to the complementary binding association of the nitrogenous bases. The helix is stabilised by H-bonding between the base pairs, which are stacked on top of each other in a manner that makes their planes perpendicular to the central axis of a right-handed helix.

A-, B- and Z- are the three most common types of DNA (Figure 1.3). A-DNA, being the shortest of the three, creates a kink in the double helix. A- and B-DNA were first crystallised at Kings College in London by R. Franklin. X-ray diffraction studies showed that the structure of B-DNA has an X-pattern (Figure 1.4), whereas the structure of A-DNA could not be determined and further investigation is required in order to understand its structural implications. These two types of DNA differ mainly in their conditions of crystallisation.
Figure 1. 3: A schematic of A-, B- and Z-DNA (top). The bottom is a diagram of a DNA double helix consisting of A-, B- and Z-stretches. Each stretch consists of 12 base pairs where the sequence is d(TTAGGG)$_2$. 
The tertiary structure of DNA differs depending on conditions (ionic concentration, temperature and pH) and base sequence.\textsuperscript{6} A- and B-type DNA are right-handed helices while Z-DNA is left-handed. B-DNA, the type reported by Watson and Crick, is the most common form of DNA. Its base pairs are stacked 3.4 Å (0.34 nm) apart with 10 base pair residues required to equate for a full rotation of the helix. This makes the length of a full rotation 34 Å while the diameter is only 20 Å. The two grooves are considerably different, with the minor groove being narrow (4.8 Å) and the major groove wider (10.5 Å); however, their depths are identical at ~ 8 Å.\textsuperscript{7,8} A-DNA, on the other hand, consists of 11 base pair residues per turn with a shorter distance (2.25 Å) between the bases. The major groove is deep while the minor groove is wide and shallow.\textsuperscript{1,9} A-DNA, being the shortest of the three, creates a kink in the double helix (Figure 1. 3).\textsuperscript{4} The third type of DNA, Z-DNA, has 12 base pair residues per turn with a distance of 3.7 Å separating the adjacent base pairs from one another. The major groove is shallow compared to the minor groove, which is deep and narrow.\textsuperscript{4} There are other DNA structures that exist which are not as common as the three already mentioned; they include C-, D-, E- and T-type DNA.\textsuperscript{4}
It is important to note the differences in the major and minor grooves for each DNA structure, as they are potential sites where proteins and other substrates can interact with DNA bases from outside the helix. Moreover, the phosphates of the nucleotides make the DNA negatively charged. This may be considered when designing drugs that could potentially interact with DNA, as positively charged drugs may display more affinity to the DNA molecule.

1.1.2 Modes of DNA binding

Drugs can interact with DNA through electrostatic binding, groove binding, intercalation and/or covalent binding. The first three modes of binding are reversible non-covalent intermolecular associations. Different compounds usually show preference to a particular type of binding; however, multiple binding modes (non-exclusive), where one mode can be replaced by another over a period of time, also exist.\textsuperscript{10}

Electrostatic binding occurs when cations, such as\textsuperscript{11} \(\text{Ca}^{2+}\), \(\text{Na}^{+}\) and \([\text{Mg(H}_2\text{O)}_6]^{2+}\) interact with the negatively charged phosphates at the exterior of the DNA helix. This results in a conformational change in the helix accompanied with a reduction in net charge.\textsuperscript{12}

Groove binding involves interaction between a molecule and the major or minor groove of DNA. Due to the differences between the two grooves in size, hydration and electrostatic potential, the position of H-bonding sites may vary. As a result, different complexes can show preference to either one of the two grooves. Large proteins (GCN-4) which are capable of H-bonding favour the major groove, while square planar complexes and other small molecules show preference for AT sequences in the minor groove.\textsuperscript{13-15} The minor groove is also preferred by most organic groove binding molecules, such as distamycin, Hoechst 33258 and \((\text{ImHpPyPy})_2\) (where \textbf{Im} is \(N\)-methyl-1H-imidazole, \textbf{Hp} is 3-hydroxy-\(N\)-methyl-1H-pyrrole and \textbf{Py} is \(N\)-methyl-1H-pyrrole) (Figure 1. 5)\textsuperscript{16}, due to the stronger van der Waals interactions with the groove’s narrower regions, especially at AT sequences. Molecules that are capable of H-bonding to the \(\text{O}_2\) of thymine (oxygen at the 2-position) and \(\text{N}_3\) of adenine (nitrogen at the 3-position) also favour minor groove
binding. Octahedral complexes, such as $[\text{Ru(DIP)}_3]^{2+}$ and $[\text{Ru(TMP)}_3]^{2+}$ (DIP is 4,7-diphenyl-1,10-phenanthroline and TMP is 3,4,7,8-tetramethyl-1,10-phenanthroline), have been shown to interact with both the major and minor groove.\textsuperscript{17}

The third type of non-covalent reversible interaction is intercalation. Lerman first proposed intercalation in the 1960s when he described the process by which proflavine inserts itself between DNA base pairs.\textsuperscript{18} Intercalators are generally planar aromatic polycyclic cations that can be inserted between DNA base pairs (Figure 1. 6). Upon intercalation, adjacent base pairs separate by \(\sim 7.8 \text{ Å}\) to accommodate the aromatic rings.\textsuperscript{18} Unlike electrostatic and groove binding, which only causes minor changes to DNA upon binding, intercalation causes an increase in the length of the nucleic acids that can be readily detected through an increase in viscosity. In comparison to DNA, intercalated DNA displays reduced mobility on an electrophoresis plate as a result of the increased length. Circular dichroism (CD), linear dichroism (LD), high resolution structural NMR and X-ray crystal diffraction can also be used to detect intercalating drugs.
Intercalation is stabilised by the overlapping π-clouds of the intercalator and the adjacent nitrogenous bases. Some complexes, such as ethidium, proflavine, acridine orange and 3,5,6,8-tetramethyl-N-methylphenanthroline, can intercalate through either the major or minor groove (Figure 1. 7). Transition metal complexes with flat ligands, such as [Pt(trpy)HAT]$^{2+}$ (trpy is 2,2':6',2"-terpyridine and HAT is 1,4,5,8,9,12-hexaazatriphenylene) and [Pt(en)phen]$^{2+}$ (en is 1,2-diaminoethane) can also act as intercalators. However, intercalation is not restricted to planar and flat complexes, as octahedral and tetrahedral complexes, such as [Co(phen)$_2$]$^{2+}$, [Ni(bpy)$_2$dpdz]$^{2+}$ (bpy is 2,2'-bipyridine and dpdz is dipyrido[3,2-a:2'3'-c]phenazine), [Ru(phen)(dpdz)]$^{2+}$, [Ru(phen)(dpq)]$^{2+}$ (dpq is dipyrido[3,2-d:2',3'-f]quinoxaline), [Ru(phen)(dpqC)]$^{2+}$ (dpqC is dipyrido[3,2-a:2'3'-c](6,7,8,9-tetrahydro)phenazine) and [Rh(phi)(NH$_3$)$_4$]$^{3+}$ (phi is 9,10-phenanthrenenequinone diimine) have also been shown to intercalate.

Even though intercalators are not specific in binding DNA, many show slight selectivity for GC base pairs because they have a larger inherent dipole than AT base pairs.
Some drugs are capable of intercalating DNA in more than one site or groove. Bis-intercalators, which have two positively charged fused rings that are linked together by flexible chains, are capable of such intercalation. Echinomycin and triostin\textsuperscript{25} are two drugs that have been found to bind DNA via the bis-intercalator mode. They exhibit higher affinity for DNA and a slower dissociation rate compared to mono-intercalators as a result of their two positively charged fused rings.\textsuperscript{25}

The last type of DNA-drug interaction is non-reversible covalent binding. Unsaturated metal complexes such as \([\text{PtCl}_4]^{2-}\) and \(\text{cis-}[\text{Pt(NH}_3)_2\text{Cl}_2]\) (cisplatin) bind covalently to DNA by interstrand or intrastrand cross-linking, through coordination with the \(\text{N}_7\) of guanine (Figure 1. 8).\textsuperscript{10,24}

It is also important to note that some drugs have more than one exclusive mode of interaction. For example, complexes like \([\text{Co(phen)}_2]^{2+}\) can either intercalate or covalently bind DNA;\textsuperscript{10} however, covalent binding can only occur when the metal complex loses one phenanthroline ligand.
1.2 History of cancer

Cancer is a disease of impaired genome stability, which is characterised by the uncontrollable and unregulated growth of cells within the body. This unregulated growth can be caused by a series of acquired or inherited mutations to DNA within cells. Such growth, which is the result of damage to the genetic information that defines cell functions, may hinder cell division. Cancer develops as a result of unreppaired damage to DNA. In many cases, when DNA is damaged, the body is able to repair the damage. It is only when the damage is beyond repair that the cells can become cancerous.

The most insidious aspect of cancer is the way it spreads. Once established in one part of the body, pieces of malignant tissue break off and are transported to other organs through the blood stream or lymph vessels. The cancer cells will then attach themselves to other body organs and form new tumours. This process is known as metastasis and eventually leads to systemic organ failure and death. Cancer can also spread into the adjacent tissue by direct growth, a process known as invasion. Unlike normal cells that grow, divide and die in an orderly fashion, cancer cells outlive normal cells and continue unregulated mitotic divisions.

There are many types of abnormal cells from benign tumours that engage in symbiotic relations with their hosts to extremely malignant and high energy cancers that exterminate their host and themselves. Cancer can manifest in many parts of the body, such as the lungs, blood (leukemia), liver, brain, skin and breasts. Breast cancer, predominant in women, and lung cancer are considered the top two most prevalent cancers in the world. Breast cancer is the leading cause of death for women between the age of 35 and 54 years. It also accounts for more than 75% of all cancer related deaths in women 55 years and above. No cure against breast cancer has been found. The prevalence of cancer drives the search for new therapeutic drugs and treatments. Development of better anticancer agents would have enormous benefits in chemotherapy. This, however, is predicated on an improved understanding of the mechanism of action of newly developed drugs.

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4 When cancer cells travel from one part of the body (such as the lungs) to another area (such as the breasts), the cancer is called lung cancer and not breast cancer.
There are five main therapeutic strategies that can be used to treat cancer: surgery, chemotherapy, radiotherapy, hormone therapy and biotherapy. Combinations of more than one type of therapy can also be used to increase the odds of successful treatment. Surgery may be used to remove cancerous tissues from the affected organ. In some cases the surrounding tissue is also removed. Surgical removal can lead to a permanent recovery, but undetected malignant cells may have already metastasised to other organs. Chemotherapy involves the use of drugs, many of which are intended to attack cancer cells and destroy them. However, the drugs can also attack healthy cells resulting in serious side effects such as hair loss, nausea and irritation of the stomach. Radiation therapy uses large doses of X-ray energy to kill cancer cells, but may also destroy healthy tissue. The X-ray source can be external, known as external beam radiation, or it can be internal, in the form of radioactive seeds or pellets and is known as brachytherapy. Hormone therapy and biotherapy are not as common, but are still used against certain cancers. Hormone therapy is primarily used for the treatment of prostate cancer, while biotherapy involves the use of biological agents that mimic certain natural signals that the body uses to regulate growth. Each of these treatments entails a certain degree of risk and suffering for the patient, but cancerous cells that are left untreated will almost inevitably cause the failure of vital organs and stop blood circulation eventuating in the patient’s death.

Finding a cure for cancer has proven to be a difficult test for medical researchers over the decades, and the development of new treatments requires substantial investments in both time and money. However, there are some forms of cancer which are no longer considered untreatable. One such cancer is leukaemia, which can suddenly stop growing. This is known as remission, and is seen as a blessing for patients who have been battling their conditions for years. For those many unlucky patients who will not experience remission, science may yet develop cures and safer methods for the treatment of cancer.

One of the most effective procedures in cancer treatment is chemotherapy. Cancer chemotherapy refers to the administration of cytotoxic chemicals with the aim of

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b Cytotoxic chemicals are chemicals with cell killing properties.
destroying the tumour or reducing its size, thereby prolonging the life of the patient. The treatment of cancer with chemicals goes back several hundred years, but it wasn’t until the 1940s that the first successful drug therapy, using nitrogen mustard, took place.\textsuperscript{29} The 1960s and 1970s saw the arrival of natural anticancer agents such as the antibiotics actinomycin D and doxorubicin.\textsuperscript{30} The first milestone in modern inorganic chemotherapy occurred in the late 1970s, with the introduction of the drug cis-diaminedichloroplatinum(II) (cisplatin). Cisplatin was initially used for the treatment of testicular cancer, but was later found to be useful in suppressing other solid tumours.\textsuperscript{31} By the year 2000, 44 cytotoxic drugs had been developed and approved in Sweden. The list of drugs includes alkylators and alkylator-like drugs (cisplatin, carboplatin), antimetabolites (fluorouracil and methotrexate), DNA interacting drugs (bleomycin), membrane perturbation drugs (miltefosine), topoisomerase inhibitors (actinomycin D and doxorubicin), microtubule interacting agents (docetaxel and vindesin) and amino acid depletion drugs (asparaginase).\textsuperscript{32} However, the process of drug development is very slow and only a few drugs per year are added to the list of therapeutic drugs.

In the modern era, chemotherapy is used in four different settings: induction therapy, adjuvant therapy, preoperative therapy and neoadjuvant therapy.\textsuperscript{33} Induction therapy is required for advanced and localised disease with the aim of reducing the tumour size to achieve clinical effects ranging from symptom relief to cure, depending on the tumour type. Adjuvant therapy, which is instigated after controlling the main tumour using surgery or radiotherapy, is required to eliminate any tumour cells that may have metastasised to other parts of the body. The aim of this therapy is to decrease the possibility of the cancer from reappearing. Preoperative therapy is required for inoperable tumour cells prior to surgery in order to increase the chance of achieving local tumour control. Neoadjuvant therapy, which is also given preoperatively, is carried out to achieve a higher long-term tumour control on tumours that are operable by effects on microscopic disease.\textsuperscript{33}

It has been three decades since the introduction of the drug cisplatin, and the need for new and more active drugs in cancer chemotherapy is now greater than ever before. So far, the strategies used for the design of cytotoxic drugs have been based on empirical observations, synthesis of analogues to already known drugs, the use of
laboratory systems for screening of cytotoxic properties among available chemicals and/or rational drug design based on research in cellular biochemistry.\textsuperscript{32} Drug development has relied heavily on \textit{in vitro} testing against tumour cells in the laboratory. The drugs are then subjected to evaluation in tumour bearing mice followed by tumour bearing mammals, such as monkeys. The final step involves testing the drugs at increasing doses in cancer patients in order to define the optimal dose, and eventually define the drugs’ antitumour activity.

Within the process of developing new cytotoxic drugs, the major strategy is based on the principle that there may be special targets that exist only in tumour cells and not in normal cells. The exploitation of such molecular targets may yet prove to be a useful strategy in chemotherapy. The findings of such targets may be related to the study of the progression of the cell cycle.\textsuperscript{32,34,35} Possible drug targets include tumour-specific properties in the cell signal transmission system, composition of the cell membrane, angiogenesis\textsuperscript{c}, telomerase\textsuperscript{d} and matrix interaction.

### 1.3 Telomeres as a drug target

Telomeres are physical caps at the ends of linear eukaryotic chromosomes. They are specialised nucleoprotein complexes with important functions, such as replication, protection (protects the chromosomes from recombination, exonuclease degradation and end to end fusion) and stabilisation of the chromosomal ends.\textsuperscript{36} Human telomeres are not linear and, for most of the cell cycle, are maintained in a loop structure, which serves to protect the vulnerable ends of the chromosome.\textsuperscript{37,38} In most eukaryotes, telomeric DNA consists of tracts of tandemly repeated sequences running in a 5' to 3' direction towards the distal ends of the chromosome.\textsuperscript{39,40} Composed of DNA and proteins, human telomeres consist of a guanine-rich DNA sequence (TTAGGG)\textsubscript{n} that is regularly repeated.\textsuperscript{41,42} These terminal repeats are highly conserved, so much that all vertebrates have the same simple repeat sequence in their telomeres. DNA sequences that are adjacent to these telomeric repeats have been found to be highly polymorphic.

\textsuperscript{c} Angiogenesis is the process by which new blood vessels are formed.

\textsuperscript{d} Telomerase will be discussed in Chapter 1.3.
rich in DNA repetitive elements and in some cases, contain genes in their proterminal regions.\cite{41}

Telomerase is a highly specialised enzyme which is responsible for the extension of the telomeric repeats in most studied species.\cite{41,43} In order for telomeres to protect chromosomes, they have to be maintained above a critical length (“a capped status”), otherwise cellular death is inevitable. The telomerase enzyme maintains the critical length of telomeres.

Telomerase is inactive in most human somatic cells, but is active in highly proliferative cells such as skin cells, intestinal crypt cells, germ cells and lymphoid cells.\cite{43} Human telomerase contains two essential components (Figure 1. 9); a telomerase reverse transcriptase catalytic subunit (hTERT)\cite{44} and a functional telomerase RNA (hTR or TERC).\cite{45} The active component is the telomerase reverse transcriptase (hTERT) and is responsible for the extension of the telomeric repeats. Over time, only the highly proliferative cells will maintain a capped status, while others will lose ~ 100 base pairs per end, per division. The reason cells possessing inactive telomerase lose their base pairs is due to the inability of DNA polymerase to replicate the ends of the chromosomes; the regions where telomeres are located. This is known as the “end replication” problem.\cite{8} Telomerase stabilises the telomeres’ length by adding TTAGGG repeats onto the ends of the chromosomes, thereby compensating for the continued degradation of telomeres that occurs in its absence.\cite{44,45}

Figure 1. 9: The telomerase components: telomerase reverse transcriptase catalytic subunit (hTERT) and functional telomerase RNA (hTR).\cite{45}
According to Blackburn, the discoverer of telomerase, telomeres exist to solve the “end replication” problem. Since DNA polymerases are unable to duplicate the ends of linear DNA, the presence of telomeric repeats at those ends prevents the loss of critical genetic information, which otherwise would have been stored at the chromosomal ends. Telomeres are therefore a long stretch of non-coding sequences that cap the chromosomal ends. In humans, they are ~ 10-20 kilo bases (kb) in length.

Another major function of telomeres is to help the cell distinguish between the normal end of a linear chromosome and a DNA breakpoint. Blackburn reports that when DNA breaks, “alarm bells go off”. The cell stops dividing and stimulates its DNA damage repair machinery into action. Normal function will be resumed only when the break is completely mended. If a telomere is recognised as a breakpoint by the cell, severe genetic problems will arise as the chromosomes split apart during anaphase. This misinterpretation can be avoided through proteins that bind to the telomeres and help the cell to distinguish them from DNA damage break points.

As mentioned earlier, DNA is the main molecular target for many anticancer drugs. Alkylating agents, such as nitrogen mustard, generally interact non-specifically with DNA by attaching an alkyl group and forming cross-links (CL). Antitumour antibiotics, such as actinomycin and bleomycin, are more specific in their interactions and are often associated with modest sequence selectivity. Molecules that specifically target DNA either from the major or minor groove are of great interest to researchers. In particular, molecules that can bind specific sequences, such as polyamides (target the minor groove) and nucleic bases (form triplex structures in the major groove). These molecules are likely agents to exert a desired biological response through binding to specific sequences of DNA.

1.3.1 Formation of cancer cells

In vitro studies on primary human cells show that cells divide approximately fifty times before cellular replication ceases. After the cells stop dividing, they are still alive in a state referred to as senescence. This threshold is known as the Hayflick
limit; the telomere length beneath which the cell will stop dividing, and is the result of
the gradual decrease in telomere length that arises during every cell division. In 1989,
Shay and Woodring-Wright proposed the M1/M2 model (Figure 1.10), which is a
two-stage mechanism for cellular senescence.48

During the M1 stage (mortality 1), the cell is in a normal replicative state of
senescence with the Hayflick limit. M1 occurs when a few short telomeres elicit a
DNA-damage signal resulting in growth arrest. According to Shay, “No human cells
in vitro have ever been shown to spontaneously escape the blockade”.48 For M1 to be
bypassed, the cell has to accumulate mutations in a critical tumour suppressor gene
such as p53 or Rb. Despite bypassing M1, the telomeres continue to shrink in length
due to the lack of active telomerase. The more the telomeres’ length shrinks, the more
unstable the cell genomes become, yet the cell remains alive due to the accumulated
mutations. When the telomeres become so short that they fail to protect the ends of
the chromosomes, the ends become ligated to produce dicentric chromosomes. The
cell will finally enter M2 (mortality 2) or “crisis”, where the telomeres begin to join
end to end. DePinho believes that whenever there is a massive cell death and a large
genomic instability, only a few cancer cells will rise and survive. The vast majority of
cells will die when they enter M2. Those cells that survive, which is one or two in ten
million, will do so by reactivating telomerase.48 This leads to an immortal cell and
cancer cell progression. Both M1 and M2 can be thought of as potent initial barriers to
continued cell division even though, at crisis, the end fusions and ensuing
chromosome rearrangements might contribute to the genomic instability that
characterises most cancer cells.49-51 This means that telomeres can be considered as
molecular clocks that count the number of times a cell has divided and determine
when cellular senescence (M1) and crisis (M2) occurs.52,53
In order for cancer to develop, several events need to occur. In 1998, Shay and associates introduced the hTERT gene into normal human cells. As expected, the cells bypassed the Hayflick limit and proceeded into the immortality zone. From the study, it was found that cancerous cells and immortal cells are not the same and although telomerase plays an important role in establishing cancer, it is not the cause.\textsuperscript{54}

A year later, a direct relationship between cancer and telomerase was established through studies undertaken by Weinberg. The studies involved the ectopic expression of three genes: hTERT, a mutant allele of ras and the simian virus 40 large T antigens.\textsuperscript{55} This proved to be sufficient for the conversion of normal human cells into cancerous cells\textsuperscript{55} and showed that while telomerase is inactive in most normal human somatic cells, the reverse transcriptase enzyme is active in over 80\% of tumorous
cells. As mentioned earlier, telomerase catalyses the synthesis of the telomeric DNA repeats d(TTAGGG)$_2$. In contrast to normal cells, telomeres of tumorous cells are therefore stabilised in length and immortalised by hTERT.

Cancers that do not consist of activated telomerase (<15%) normally produce weak and non-progressing tumours such as stage four neuroblastoma. Reynolds, from the Children’s Hospital in Los Angeles, studied stage four neuroblastoma in newborns and infants. The children did not show the true characteristics of stage four neuroblastoma, such as bone lesions; instead, they suffered from metastases in the skin and bone marrow in addition to diffused infiltration of the liver. Remarkably, the neuroblastoma was found to regress in 85% of children from the age of three months without any intervention. This lead Shay and colleague Hiyama to study the telomerase activity in stage four neuroblastoma patients in Japan. As predicted, biopsies performed on children who recovered from neuroblastoma displayed very low telomerase activity. By studying the patients over a period of time, it was observed that a decrease in telomeres’ length coincided with the regression of the tumour. This suggested that the regression of stage four neuroblastoma is dependant on the absence of telomerase and the resultant shortening of telomeres. This discovery shows that telomerase is not required for the initiation of the cancer, but it is important for its development as it stabilises the associated unstable genomes.

### 1.3.2 Inhibition of telomeres/telomerase

The associated activity of telomerase in a variety of tumours has attracted much attention in medical research. Also of interest is the G-rich sequence contained within the telomeres, as some metal complexes have been developed with the purpose of binding to this sequence, rather than interacting with the enzyme itself. This may provide a pathway to the inhibition of telomerase and consequently tumour regression.

Disruption of telomere maintenance, which leads to eventual cell death, may be exploited for therapeutic intervention in cancer. Telomeric DNA has the ability to fold into a four-stranded guanine quadruplex (G4) structure held together by H-bonding between the guanine bases. The G-quadruplexes are composed of two or
more G-tetrads assembled into either intermolecular or intramolecular structures (Figure 1.11).\textsuperscript{60} When this quadruplex structure is formed at the 3' end of the telomeric DNA, the telomerase is prevented from adding further repeated telomeric sequences due to steric hinderence.\textsuperscript{57}

![Image of G-tetrad and G-quadruplexes](image)

**Figure 1.11: The G-tetrad and G-quadruplexes:** (a) four guanine residues form a planar G-tetrad through H-bonding, (b) an intermolecular parallel G-quadruplex model, (c) an intermolecular antiparallel G-quadruplex model, (d) an intermolecular basket G-quadruplex model and (e) an intramolecular chair G-quadruplex model.\textsuperscript{60}

The guanine-rich DNA sequence of the G4-quadruplexes is a potential target for molecules with high affinity for guanine residues. This information has led scientists to search for smaller drugs capable of selectively binding and stabilising the G4-quadruplexes. By stabilising the G4-quadruplexes, which is a more favourable conformation than the linear structure, the telomeres become less vulnerable to additions by the telomerase. Currently, molecules that intercalate are being used to target these structures. This illustrates the potential of telomeres as molecular targets.
and how drugs can be directed to a certain region of DNA for the purpose of achieving selectivity.

There exist a few drugs capable of interacting with G4-quadruplex structures, such as the tricyclic anthraquinone-based G-quadruplex-interactive telomerase inhibitors, fluorenones, bisubstituted acridines, cationic porphyrins, a perylenetetracarboxylic diimide derivative, indolo-quinolines and a benzonaphthofuranandione tetracyclic compound. Unfortunately, these compounds displayed poor selectivity for quadruplex structures in comparison with duplex DNA and poor cytotoxicity (IC$_{50}$ in the micromolar range), thus making them weak telomerase inhibitors. This was reflected in studies performed at concentrations similar to that required for telomerase inhibition which resulted in acute cell death. Platinum(II) antitumour complexes, which include both cis- and trans-isomers, have been reported to display therapeutic activity against telomerase inhibitors (Figure 1.12). Other potential telomerase inhibitors based on ethidium dibenzophenanthrolines, pentacyclic acridines, 9-anilinoproflavin, fluoroquinophenoxazines and the microbial agent telomestatin (Figure 1.13) have been reported though the results of cellular assays are yet to be presented.

![Chemical Structures](image)

**Figure 1.12:** The chemical structures of (a) cis-[Pt(Py$_2$)Cl$_2$], (b) trans-[Pt(Py$_2$)Cl$_2$] and (c) [Pt(phen)en]$^{2+}$. 
In contrast to normal cells, tumour cells consist of shorter telomeres and show no net loss of average telomeres’ length despite successive cell divisions. This suggests that stabilising the telomeres may be essential for cells to bypass replicative senescence and proliferate indefinitely. Most malignant cells need to become immortal in order to sustain their growth. Telomerase activity could therefore be the rate-limiting step required for the continuous proliferation of advanced cancers.\textsuperscript{66,67} The telomere/telomerase hypothesis of ageing and cancer is based on the fact that most human tumours express telomerase activity while normal human somatic cells do not.\textsuperscript{68} This opens a large window in which cancer cells can be efficiently targeted by
telomerase inhibitors, while normal proliferative male germline and embryonic stem (ES) cells remain unaffected due to their longer telomeres’ length and slower rates of cell division (Figure 1. 14). 69

Figure 1. 14: A comparison of telomerase inhibition in normal versus cancer cells.

As depicted in Figure 1. 15, most, but not necessarily all, anti-telomerase therapies require a substantial period of time to drive already short telomeres into a state of “crisis” and subsequently apoptotic cell death. During the treatment period, telomeres would gradually shorten while the tumour size would continue to increase. This will eventually lead to cell death but only after several cell divisions. Therefore, telomere inhibitors might not be effective without the combination of a second type of therapy.

a- Telomerase inhibitor: cancer cells with short telomeres die, others continue to proliferate

Telomere length: 7 6 5 4 3.5 3

Tumour size:

b- Chemotherapy: produces a setting of minimal residual disease, but might not affect telomeres

Telomere length: 7 7 7 7 7 7 7

Tumour size: 1st treatment 2nd treatment

Figure 1. 15: The predicted outcomes of telomerase therapy.

In contrast, chemotherapy can cause an immediate reduction in the size of the tumour, but continued treatment will result in therapy resistance and tumour relapse. However, using chemotherapy/radiation therapy to reduce the tumour size, and combining this approach with telomerase inhibitors could improve overall survival, or at least delay tumour regrowth. The development of combination therapy may yet prove to be

70,71
essential in enhancing telomere reduction and causing a faster decrease in cancer cell proliferation without affecting the telomeres of normal cells.

1.3.3 Perspectives on telomeres and cancer

Over the past two years, there have been many significant developments in the telomere/telomerase field of research. Despite the need for additional clinical trials and proof of efficacy studies, there is a general consensus that telomere/telomerase therapy is a promising and novel approach in cancer chemotherapy, which may lead to effective interventions in the treatment of cancer with minimal side effects. The emerging preclinical evidence for telomerase as a target for cancer chemotherapy is very encouraging and worthy of future investigation.

1.4 Platinum(II) anticancer complexes

The discovery of cisplatin’s antitumour activity thirty years ago has had a major contribution in the treatment of several cancers. Cisplatin, cis-[Pt(NH$_3$)$_2$Cl$_2$], (Figure 1. 16) is known to exhibit or induce cumulative and irreversible toxicities, such as nephrotoxicity, ototoxicity and peripheral neuropathy. It is believed that the complex induces a cytostatic action because it forms one or more bifunctional adducts with DNA. Cisplatin was first synthesised in 1845, yet it wasn’t until the 1970’s that it’s cytostatic activity as an antitumour drug was discovered. Cisplatin is successfully used in the treatment of both testicular and ovarian cancer and is increasingly used against cervical, bladder and head/neck tumours. Doses of 100 mg/day are administered to patients for five consecutive days. The key to the mechanism of action of cisplatin is its ability to bind cellular DNA, preventing it from replication. The disadvantage of cisplatin is that it not only targets DNA of cancerous cells, but also normal cells and many other molecules in the body. The non-selective nature of cisplatin causes the apoptosis of both cancerous and healthy cells. The result is several toxic side effects that include hair loss, kidney and liver toxicity, nausea, vomiting and loss of sensation in the hands. Resistance against cisplatin develops after initial doses. For this reason, carboplatin, oxaliplatin and many other analogues

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*e* Nephrotoxicity is the property of being toxic or destructive to the kidney cells.

*f* Ototoxicity is damage to the hearing or balance functions of the ear by drugs or chemicals.

*g* Peripheral neuropathy describes damage to the peripheral nervous system.

(Figure 1.16) have been synthesised. Their lower toxicity levels permits the administration of much higher doses (up to 2000 mg/dose). Unfortunately they also show cross resistance with cisplatin.\textsuperscript{80,81}

![Chemical structures](image)

Figure 1.16: The chemical structures of (a) cisplatin, (b) carboplatin and (c) oxaliplatin.\textsuperscript{48}

The cytotoxicity of cisplatin is thought to be the result of the different adducts which are formed when the platinum(II) complex binds DNA (Figure 1.17).\textsuperscript{78,82,83} Cisplatin forms bifunctional adducts with purine residues including intrastrand and interstrand cross-links (CL).\textsuperscript{82,84} The major intrastrand adducts formed when cisplatin binds DNA are at GG and AG sites and they represent 65 and 25\% of bound platinum(II) respectively. It has been argued that the mechanism of action of cisplatin could be related to the recognition of these adducts by proteins involved in various cellular processes.\textsuperscript{82}

![Adducts](image)

Figure 1.17: The different types of adducts that are formed when cisplatin binds DNA.\textsuperscript{82}
1.4.1 Transport of cisplatin

There is evidence that cisplatin, when administered either by injection/infusion or by oral administration, circulates in the blood mainly as the chloride complex due to the high chloride concentration (100 mM). After entering the cytoplasm, where the chloride concentration drops to 4 mM, it loses one of its chloride ligands before interacting with the peptides, proteins and DNA within the cell (Figure 1.18).

Inside the cell: \([\text{Cl}^-] = 4 \text{ mM}\)

Figure 1.18: A schematic diagram of the cell-wall transport of cisplatin and the reactions in the cell prior to binding DNA in the cellular nucleus.\(^{48}\)

1.4.2 Mechanism of action of cisplatin

Cisplatin consists of two labile chloride ligands and two inert amine ligands in the cis configuration. When present in an aqueous medium such as the cell’s cytoplasm, the chlorides are displaced in a step-wise fashion by water molecules (Scheme 1),\(^{85,86}\) which themselves are good leaving groups. This allows the platinum(II) centre to bind in the major groove of DNA via the \(N_7\)\(^h\) of guanine or adenine residues (Figure 1.19). The cross-linking prevents DNA replication and therefore inhibits cell growth.\(^{85,86}\) The attack occurs only at \(N_7\) and not \(N_1\) because the pKa value of \(N_1\) is too high (9.2) in guanine residues. This causes the amine to be protonated under acid or neutral conditions, preventing the attack from \(\text{cis-}[\text{Pt}(\text{NH}_3)_2(\text{OH}_2)_2]^{2+}\).\(^{87}\) For adenine, the pKa

\(^{85}\) \(N_7\) is the nitrogen at the 7-position on guanine or adenine in this case.

of N1 is lower (3.6), therefore at pH 4 the attack from cis-[Pt(NH3)2(OH2)2]2+ may occur at both N1 and N7 sites in the ratio of 0.66 to 1.00 respectively. Even though adenine can react through both N1 and N7, the N7 position is predominantly susceptible under most conditions. Cytosine, on the other hand, is attacked at N3, while thymine hardly reacts under acidic or neutral conditions because its N3 will be protonated.

**Scheme 1: In an aqueous medium, the chloride ligands of cisplatin are displaced in a step-wise fashion (steps 1-7) by water molecules.**

\[\text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH}_2\text{)]}^+ \xrightarrow{-\text{Cl}^- + \text{H}_2\text{O}} \text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} + \text{H}^+\]

\[\text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} \xrightarrow{\text{Cl}^- + \text{H}_2\text{O}} \text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} + \text{H}^+\]

\[\text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} \xrightarrow{\text{Cl}^- + \text{OH}^-} \text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} + \text{H}^+\]

\[\text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} \xrightarrow{-\text{H}^+} \text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} + \text{H}^+\]

\[\text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} \xrightarrow{-\text{H}^+} \text{cis-[Pt(NH}_3\text{)}_2\text{Cl(OH)}_2\text{]} + \text{H}^+\]

In cancer chemotherapy, cisplatin is administered in a saline solution. In its neutral form, cisplatin is relatively inert to substitution, but capable of cell wall penetration. When administered as the drug cis-[Pt(NH3)2(OH2)2]2+, it is reported to be inactive due to its inability to cross cell boundaries.
The reaction with DNA yields monofunctional adducts, intrastrand cross-links and interstrand cross-links with the platinum(II) centre coordinated to the N\textsubscript{7} of guanine or adenine. Adduct formation results in the inhibition of DNA replication, RNA transcription, arrest at the G2 phase of the cell cycle and/or programmed cell death.\cite{90}

### 1.4.3 The distorted DNA structure and body response

When cisplatin binds in the major groove, the DNA structure becomes kinked and slightly contracted (Figure 1.20), with bending of the helix at an angle of 45°.\textsuperscript{91-93} The DNA conformation at the 5\textsuperscript{'} end from the bound complex is also converted from B- to A-DNA. The helix creates a small angle between the planes of the chelating guanine residues, while the platinum(II) centre lies ~1 Å out of the plane (Figure 1.20).
Figure 1. 20: The DNA distortions caused by a 1, 2-d(GG)-cisplatin intrastrand adduct in a double-stranded deoxyoligonucleotide, with the sequence $d(CCTCTG^*G*TCTCC)_2$: (a) major groove of normal B-DNA and (b) DNA bending caused by the cisplatin adducts as determined by X-ray crystallography.$^{91-93}$

Lippard and co-workers$^{94}$ have shown that when DNA becomes kinked the body activates DNA-repair systems, such as the *Uvr* systems (in prokaryotes) and the high mobility group (HMG) proteins (in eukaryotes). These systems recognise bent and unwound DNA and counteract the bending effect such that the DNA returns to its original shape.$^{95}$ Later studies revealed that the effect of these repair systems may ensure cell survival after the first cisplatin administration to the human body.$^{95}$ If the cell survives the initial cisplatin treatment, it develops resistance to the drug. When resistance develops, cisplatin becomes ineffective against the resistant cells. This makes oxaliplatin and other cisplatin analogues that bind DNA using the same mechanism ineffective against the resistant cell lines.$^{94}$ Several mechanisms that explain how resistance is developed have been proposed; yet none so far have been supported, although the theory of inactivation of the platinum(II) complexes by sulphur compounds is currently under investigation.$^{95}$

### 1.4.4 Transplatin

Early evidence that only the *cis*-configuration of platinum(II) complexes exhibit antitumour activity has been recently challenged with the discovery of active antitumour *trans*-platinum(II) complexes.$^{96}$ For several years, *trans*-platinum(II) complexes such as transplatin have been known to exhibit less antitumour activity, even though they bind DNA by the same mechanism as the geometric isomer cisplatin. Like cisplatin, the *trans*-isomer creates 1,3-intrastrand adducts when it binds the $N_7$ of guanine or adenine and 1,4-intrastrand adducts when it binds the $N_3$ of
cytosine.\textsuperscript{97,98} Later studies revealed that in order for \textit{trans}-platinum(II) complexes to exhibit antitumour activity, the inert groups should be planar heterocyclic ligands or imino ethers,\textsuperscript{63,99} or the complexes should contain bridging diamine linkers of carbon chain lengths between 2 and 6.\textsuperscript{100,101}

Structure-activity relationships for platinum(II) compounds have been formulated. For platinum(II) compounds to exhibit antitumour activity, the two amines must be in the \textit{cis}-configuration, whether they are symmetric, asymmetric or chelating. Leaving groups with a weaker \textit{trans}-effect than that of the amines were found to form the most potent analogues.\textsuperscript{78,102} Other favourable characteristics include higher water solubility and suppressed reactions with sulphur-containing ligands. Most importantly, compounds should have minimal toxic side effects and should not display cross-resistance. This may be achieved by using ligands other than amines. Thus by changing: (a) the position of the amine ligand on the platinum(II) centre (chelating or not, hydrogen-bond donor, steric effects, side arms for secondary DNA interactions) and (b) the leaving groups on the platinum(II) centre (non-toxic, optimal ligand exchange kinetics, possibility of acting as a pro-drug), new drugs can be created (Figure 1.21).\textsuperscript{103}

Figure 1.21: The chemical structures of some platinum compounds that are at an advanced stage of testing and clinical application.\textsuperscript{48}
1.4.5 Polynuclear platinum(II) complexes

Cisplatin has been widely used for the treatment of various solid tumours. Although some response has been observed in previously untreated patients, subsequent salvage therapy is most often ineffective. For this reason, the development of other anticancer agents that do not display cisplatin cross-resistance and have a wider range of antitumour activity has been initiated.

Polynuclear platinum(II) complexes are among the most promising of the new anticancer agents. They act by a different mechanism to that of cisplatin and its analogues. The most frequently reported of these drugs is BBR3464 which has displayed activity against cisplatin resistant cell lines and toxicity up to 40-folds higher than cisplatin (Figure 1. 22). BBR3464 binds covalently and irreversibly to DNA. It makes contact at the minor groove, where the central platinum(II) unit rests, while the hydrocarbon arms are wrapped around the DNA, allowing the terminal platinum(II) centres to bind in the major groove. As the central platinum(II) unit does not cause conformational changes in the DNA, the mode of binding for BBR3464 is more closely related to that of dinuclear compounds than to that of cisplatin. The bifunctional binding of BBR3464 through the terminal platinum(II) centres is characterised by the rapid formation of long-range intra- and interstrand cross-links (CLs) in the major groove of DNA. The central platinum(II) centre pre-associates in the minor groove and does not contribute to the covalent binding. It contributes to the overall charge and hydrogen bonding, thus increasing the affinity of the compound for DNA. It is then not surprising that the complex BBR3464, with a charge of 4+, has a higher affinity for DNA than cisplatin. Despite the 4+ charge, BBR3464 is capable of crossing the cell membrane. Like cisplatin, when BBR3464 enters the cell’s cytoplasm, where the chloride concentration is low, the chlorides are displaced by water molecules to generate a more positively charged species that can form coordinate covalent bonds with purine bases on DNA.
It has been shown through phasing assay experiments based on gel electrophoresis that the drug-DNA adducts formed by BBR3464 and cisplatin are different. 108 This was further supported through chemical probes of DNA conformation. 108,109 BBR3464 bends the DNA helix by 32-34° towards the major groove and unwinds DNA by 13°. 109 This causes extreme perturbation of H-bonding within the 5'-coordinated GC base pair and spans between 4-5 base pairs at the site of the CL. 109 Cisplatin, on the other hand, bends the helix towards the major groove by 35° and unwinds the DNA by ~ 23°. 110,111 It can be seen that both cisplatin and BBR3464 bend the DNA helix and block DNA and RNA polymerases. It is hypothesised that the different binding angle formed by BBR3464 is not recognised by the HMG domain proteins, which repair the DNA damage. This difference might help explain why cells develop resistance to cisplatin but not to BBR3464. 107

BBR3464 forms 1,2- and 1,3-intrastrand CLs between guanine residues. It can also form 1,5-intrastrand CLs that isomerise into interstrand CLs. While cisplatin can form intrastrand CLs (Figure 1. 17) with higher frequency, the most common adduct for BBR3464 is the interstrand CL. 107 BBR3464 is even capable of forming 1,4- and 1,6-interstrand CLs (Figure 1. 23). 112 This is due to the long hydrocarbon chain that separates the platinum(II) centres in BBR3464. 112 In general, DNA interstrand adducts cause more cytotoxic lesions and inhibit more DNA and RNA polymerase activity than intrastrand adducts. Damage created by interstrand CLs is also harder to repair because it involves both strands of the helix. 113 This further explains the activity of BBR3464 against cisplatin resistant cell lines.
Figure 1. 23: A diagram of BBR3464 forming a 1,4-interstrand CL in (a) and a 1,6-interstrand CL in (b). In (a), the two -(CH$_2$)$_6$- chains of BBR3464 pass on either side of the C$_8$' phosphate, whereas in (b) there are two phosphate groups (C$_8$' and A$_7$') between the two aliphatic chains. The guanine residues are shown in yellow, platinum(II) centres in white, am(m)ines in blue (pale and dark) and the chlorides in green.\textsuperscript{112}

Although BBR3464 is effective against cisplatin resistant cell lines, it displays no selectivity for DNA; that is, it can bind to many other biological molecules in the body. The problem of cell line resistance is overcome, but selectivity has not been yet addressed.

1.4.6 Sequence selective compounds

Researchers have explored various methods in the search for an antitumour complex that would show selectivity for cancerous cells. One of the most promising strategies is the synthesis of sequence selective polyamides that exhibit high affinity for selected DNA sequences. By attaching a platinum(II) centre, such as cisplatin or transplatin, to a suitable polyamide carrier, a selected DNA sequence could be targeted and blocked. Of interest, is the TTAGGG sequence, which is highly repeated in the telomere region.
Over the last 10 years, polyamides that are capable of targeting specific DNA sequences have been synthesised. These compounds (Figure 1.24), containing N-methyl-1H-pyrrole (Py), N-methyl-1H-imidazole (Im) and 3-hydroxy-N-methyl-1H-pyrrole (Hp) heterocyclic rings, β-alanine (β-Ala) and γ-amino butyric acid residues can be assembled in an antiparallel association to target specific DNA sequences (Figure 1.25) in the DNA minor groove.\textsuperscript{114,115} Each pair of heterocyclic rings (every ring and its dimer) recognises a specific DNA base pair: Im/Py and Py/Im pairings distinguish between G·C and C·G base pairs respectively, while Hp/Py and Py/Hp recognise T·A and A·T base pairs. A combination of Py/Py is selective for A·T and T·A base pairs, but cannot distinguish between them.\textsuperscript{114,115}

![Figure 1.24: The chemical structures of (a) N-methyl-1H-pyrrole (Py), (b) N-methyl-1H-imidazole (Im) and (c) 3-hydroxy-N-methyl-1H-pyrrole (Hp).](image)

![Figure 1.25: Achieving selectivity using pyrrole and imidazole polyamides.](image)

The use of sequence selective polyamides as drugs is limited because they can only recognise 5-6 base pairs.\textsuperscript{116,117} To specifically discriminate different genes, it is
necessary to target longer DNA sequences. Studies suggest that beyond five consecutive rings, the polyamides curvature fails to match the pitch of the DNA double helix, thus disrupting the hydrogen bonds and van der Waals interactions necessary for specific DNA sequence recognition.$^{116-118}$

Second generation molecules have been based on benzimidazole architecture to provide greater DNA-minor groove recognition.$^{119-123}$ The new molecules, which consist of a 5-6 bicyclic-ring structure, preserve the same connectivity along the recognition scale as the first generation molecules. In order to achieve selective base pair recognition, different heteroatoms and substituents were introduced on the 6-membered rings. The imidazo-pyridine-pyrrole (IpPy) pair mimics the ImPy pair and distinguishes G·C from C·G, T·A and A·T base pairs, while the hydroxybenzimidazole-pyrrole pair (HzPy) mimics the HppPy pair and distinguishes T·A from A·T, G·C and C·G base pairs (Figure 1.26).$^{124}$

![Figure 1.26: The second generation 5-6 bicyclic rings on the left versus the first generation 5-membered rings on the right.$^{124}$](image-url)
Groove binders have the potential to be sequence selective because they bind regions of DNA that usually span several base pairs. Distamycin (Figure 1.27) is a groove binder made of first generation 5-membered heterocyclic rings connected by flexible amide bonds with rotating capabilities to allow the molecule to take the shape of the groove. This conformation is stabilised by van der Waals interactions between the heterocyclic rings and the DNA minor groove. Brabec and co-workers\textsuperscript{125} attached a platinum(II) complex to the end of distamycin. The molecule was expected to target the sequence 5'-TTTTAAAA-3'. Studies revealed that the mode of binding to DNA for distamycin-cisplatin is different to that of cisplatin alone. The major difference lies in the ability of the distamycin-cisplatin complex to form interstrand CLs with DNA. Distamycin-cisplatin can form three times more interstrand CLs with DNA than cisplatin, which can only form about 6% of interstrand CLs in linear DNA and slightly more in negatively supercoiled DNA.\textsuperscript{126-128} Despite the increase in the formation of interstrand CLs, the intrastrand CLs remain the major adduct.\textsuperscript{126} The attachment of the platinum(II) centre at the end of distamycin localises the molecule in the minor groove rather than the major groove where active platinum(II) compounds usually bind.\textsuperscript{125} This could account for the complexes low activity.\textsuperscript{126}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{distamycin.png}
\caption{The chemical structure of distamycin, which is expected to target the DNA sequence d(TTTTTAAA)\textsubscript{2}.\textsuperscript{125}}
\end{figure}

Sequence selective polyamides can be designed to target a selected DNA base sequence. A polyamide may bind in the DNA minor groove, while the linker of sufficient length allows the attached platinum(II) centre to bind at the major groove. It was found that the complex DJ1953-2 (Figure 1.28) binds its target sequence,

\[ d(CATTGTCAGAC)_2 \], with greater affinity than its mismatched sequence, \[ d(CATTGAGAGAC)_2 \] (Figure 1.29).\(^{129}\)

![Sequence selective compound DJ1953-2](image)

**Figure 1.28:** The sequence selective compound DJ1953-2, forming an informal dimer in solution.

![ICD graph](image)

**Figure 1.29:** The compound DJ1953-2 is shown to have higher affinity to the matched sequence, which is the intended target, rather than the undesired mismatched sequence containing 2 altered base pairs.

DJ1953-2 can form coordinate covalent bonds with DNA but appears to form dimers in the minor groove.\(^ {129}\) This problem may be overcome through the synthesis of a hairpin polyamide, which may be achieved by connecting two polyamides using a hydrocarbon linker that also shows selectivity for the desired sequence (Figure 1.30).
1.5 Aims

The initial aim of this project was to optimise the reaction conditions for the conversion of \( N \)-methyl-1H-imidazole (Im) into 4-[(9-fluorenylmethoxycarbonyl)-amino]-\( N \)-methyl-1H-imidazole-2-carboxylic acid (Im E) and \( N \)-methyl-1H-pyrrole (Py) into 4-[(9-fluorenylmethoxycarbonylamino]-\( N \)-methyl-1H-pyrrole-2-carboxylic acid (Py E). Im and Py were each subjected to five reaction steps in order to obtain Im E and Py E.

The subsequent aim of the project was to synthesise mono- and multinuclear platinum(II) sequence selective polyamides that consist of imidazole and pyrrole rings. The heterocyclic rings were connected in a step-wise fashion using solid phase chemistry to produce hairpin polyamides with one or two platinum(II) centres. These polyamides were designed to achieve selectivity based on Dervan’s pairing rules. In a similar fashion to Farrell’s platinum(II) complexes, it was expected that the 5-membered rings will target DNA sequences in the minor groove while the platinum(II) centres bind in the major groove.

The organic components of the desired polyamides were synthesised entirely using solid phase chemistry, while the coordination of the platinum(II) centres was carried...
out using either solid or solution phase chemistry. The chemical structures of the polyamides synthesised in this project are shown in Figure 1. 31.

Figure 1. 31: The chemical structures of mono- and dinuclear platinum(II) sequence selective polyamides. (I), (II) and (III) target an (A/T)GGG(A/T) sequence while (IV) targets an (A/T)(A/T)(A/T)GGG(A/T) sequence.
The polyamides were designed to bind covalently and irreversibly in the DNA minor groove, while the platinum(II) centres bind at the major groove. The complexes were expected to target the guanine rich area of DNA telomeres and the cytosine rich area on the complementary strand. Polyamides (I), (II) and (III) were expected to bind selectively to regions of DNA that are five bases in length and comprise (T/A)GGG(T/A) sequences. Polyamide (IV) spans seven base pairs comprising of (T/A)(T/A)(T/A)GGG(T/A), of which TTAGGGT is highly repeated in the telomere region. The platinum(II) sequence selective hairpin polyamides were expected to show higher DNA affinity than the previously reported single stranded polyamides.\textsuperscript{129}

The platinum(II) polyamides were characterised using $^1$H and $^{195}$Pt Nuclear Magnetic Resonance (NMR) spectroscopy, ESI mass spectrometry and elemental analysis.
CHAPTER 2: SOLUTION PHASE CHEMISTRY

2.1 Introduction

Polyamides based on $N$-methyl-1H-pyrrole and $N$-methyl-1H-imidazole are regarded as an important family of sequence specific DNA binders.\textsuperscript{131,132} These low molecular weight molecules have been shown to form dimers within the DNA minor groove with unprecedented affinity and selectivity. Base recognition rules, established by Dervan and co-workers over the past decade, allow researchers to target a specific DNA sequence by controlling the sequential formation of polyamides.\textsuperscript{123} The stability, strength and utility\textsuperscript{133} of these DNA recognising ligands have led to the synthesis of sequence specific polyamides through efficient solid phase techniques based on amino acid peptide coupling.\textsuperscript{134}

Unlike naturally occurring $\alpha$-amino acids, the preparation of these building blocks requires elaborate procedures and laborious purification techniques. While the introduction of the carboxylic acid group on the 2-position was straightforward, the inclusion of a nitrogen atom on the 4-position has proven to be more challenging. The nitration step was regarded as the “Achilles’ heel” of the synthetic procedure, resulting in low overall yields (10-20%).\textsuperscript{134,135} In an attempt to improve the overall yield, an alternative route (Figure 2.1) has been developed. The new procedure involved the addition of a trichloroacetyl group at the 2-position, which creates a low electron density around the 5-position, thus making it easier to nitrate at the 4-position. After nitration, the trichloroacetyl group was then converted to a tert-butyl ester followed by hydrogenation of the nitro group, protection of the amine and hydrolysis of the ester. The reaction procedures can be summarised as follows:

1. Friedel-Crafts acylation of the trichloroacetyl group at the 2-position;
2. nitration at the 4-position;
3. displacement of the acetyl group;
4. hydrogenation of the nitro group and protection of the amine;
5. hydrolysis of the ester using titanium tetrachloride.
A key requirement of solid phase peptide synthesis was that the substrate consists of a carboxylic acid group and a protected amine. The two most commonly used protecting groups are di-tert-butyldicarbonate (Boc$_2$O) and 9-fluorenymethylchloroformate (Fmoc-Cl). While the Boc-protecting group is stable under basic conditions (removed with acid), the Fmoc-protecting group is unstable under basic conditions and is commonly removed using piperidine.$^{136,137}$ The Fmoc-protecting group was therefore the preferred group to be used with the chlorotrityl resin which is stable under basic conditions. The Fmoc-protecting group was cleaved non-hydrolitically to provide the free amine for further coupling steps.

2.2 Experimental

The chemicals, reagents and instruments used for the synthesis and characterisation of the molecules described in this chapter are listed in Appendix I, Section A1.1 and Section A1.2.

2.2.1 Preparation of trichloroacetyl chloride

Anhydrous trichloroacetic acid (100 g, 0.55 mol), thionyl chloride (50 mL) and dimethylformamide (4 mL) were stirred at 80-85 °C for 2.5 h. The solution was distilled at 117 °C to obtain the product (98.1 g, 88%) as a clear and colourless liquid: b.p. 114.1-115.8 °C. IR (NaCl Disk, Nujol mull) 1,750 cm$^{-1}$ (C=O).
2.2.2 Synthesis of the imidazole monomer

2.2.2.1 2-Trichloroacetyl-N-methyl-1H-imidazole (Im A)

\[
\begin{align*}
\text{Im A} & \quad \text{N-Methyl-1H-imidazole (10.0 g, 0.122 mol) in DCM (80 mL) was added dropwise to} \\
& \quad \text{trichloroacetyl chloride (22.0 g, 0.122 mol) in DCM (80 mL) over a period of 2.5 h.} \\
& \quad \text{The mixture was stirred overnight, cooled to 0 °C and triethylamine (TEA, 17 mL)} \\
& \quad \text{was added over a period of 1 h. The salt was removed by filtration, the filtrate} \\
& \quad \text{evaporated and the residue purified by column chromatography}^i \text{ (silica gel, 1:4} \\
& \quad \text{hexane/DCM, } R_f \ 0.53) \text{ to yield } \text{Im A (16.0 g, 57%) as a light yellow solid: m.p. 76-78} \\
& \quad \degree \text{C. } ^1 \text{H NMR 300 MHz (d}_6\text{-DMSO): } \delta 7.71 \ (s, \ 1H), \ 7.31 \ (s, \ 1H), \ 3.98 \ (s, \ 3H).
\end{align*}
\]

2.2.2.2 4-Nitro-2-trichloroacetyl-N-methyl-1H-imidazole (Im B)

\[
\begin{align*}
\text{Im B} & \quad \text{To acetic anhydride (Ac}_2\text{O, 160 mL) at -5 \degree \text{C was added fuming nitric acid (14 mL,}} \\
& \quad \text{0.30 mol) and sulphuric acid (0.56 mL). \text{ Im A (20.0 g, 0.09 mol) was added slowly} \\
& \quad \text{over a period of 1 h. The solution was allowed to warm to room temperature (r.t.) and} \\
& \quad \text{stirred overnight. Chloroform (180 mL) was added and the solution extracted using} \\
& \quad \text{sodium bicarbonate (1 M, 3 × 50 mL) and brine (3 × 50 mL). The organic layers were} \\
& \quad \text{dried using anhydrous magnesium sulphate and evaporated under reduced pressure to} \\
& \quad \text{obtain a red/brown oil. The oil was mixed with hexane/ethyl acetate (1:1, 24 mL) and} \\
& \quad \text{sonicated.}^j \text{ The mixture was left at -20 °C}^k \text{ for 1 h and the precipitate was filtered to} \\
& \quad \text{obtain } \text{Im B (10.3 g, 64%) as a yellow powder: m.p. 139-141 °C. } ^1 \text{H NMR 300 MHz} \\
& \quad (\text{d}_6\text{-DMSO): } \delta 8.82 \ (s, \ 1H), \ 7.31 \ (s, \ 1H), \ 3.98 \ (s, \ 3H).
\end{align*}
\]

---

\(^i\) All columns were packed with Silica gel 60 (3-40 nm in diameter) for column chromatography.

\(^j\) Sonication causes the product to precipitate out of the solution.

\(^k\) Temperatures of -20 °C were obtained using acetone and dry ice.
To a mixture of Im B (5.0 g, 18 mmol) in tert-butyl alcohol (50 mL) was added sodium tert-butoxide (2.0 g, 21 mmol) over 1 h. The reaction was refluxed (83 ºC) under dried N$_2$(g) for 5 h, quenched with water (50 mL) and extracted with chloroform (3 × 50 mL). The solvent was evaporated to obtain Im C (2.3 g, 55%) as yellow/white crystals: m.p. 164-166 ºC. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 8.55 (s, 1H), 3.95 (s, 3H), 1.55 (s, 9H).

Im C (0.50 g, 2.2 mmol) was dissolved in DMF (15 mL) and 10% Pd/C (0.05 g) was added. The mixture was vigorously stirred for 20 h under H$_2$(g) (34 psi) and then filtered through celite and washed with DMF (20 mL). Fmoc-Cl (0.59 g, 2.3 mmol) and 4,4-dimethylaminopyridine (DMAP, 0.28 g, 2.3 mmol) were added and the suspension stirred overnight under N$_2$(g). DCM (100 mL) was added before the product was extracted using HCl (1 M, 2 × 40 mL), brine (30 mL) and water (30 mL). The organic layer was then dried using anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure to yield a green oil. The oil was purified by column chromatography (silica gel, 3:1 hexane/ethyl acetate, $R_f$ 0.16) to yield Im D (0.59 g, 67%) as a white solid: m.p. 105-107 ºC. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 10.39 (s, 1H), 7.89 (d, 2H, $J = 7.4$ Hz), 7.65 (d, 2H, $J = 7.6$ Hz), 7.41 (t, 2H, $J = 7.0$ Hz).  

---

1 Caution: the Pd/C catalyst is flammable and must not be allowed to dry out. It was suspended in DMF before it was disposed off.
2.2.2.5 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylic acid (Im E)

2.2.3 Synthesis of the pyrrole monomer

2.2.3.1 2-Trichloroacetyl-N-methyl-1H-pyrrole (Py A)

N-Methyl-1H-pyrrole (10.0 g, 0.12 mol) in DCM (40 mL) was added dropwise to trichloroacetyl chloride (22.0 g, 0.12 mol) in DCM (40 mL) over a period of 3 h. The mixture was stirred overnight and the product purified using column chromatography\(^a\) (silica gel, 7:3 hexane/DCM, \(R_f\) 0.68) to yield Py A (20.5 g, 73%) as a pale yellow solid: m.p. 71-73 °C. \(^1\)H NMR 300 MHz (d\(_6\)-DMSO): \(\delta\) 8.21 (d, 1H, \(J = 1.9\) Hz), 7.24 (d, 2H, \(J = 1.8\) Hz), 3.88 (s, 3H).

---

\(^a\) TiCl\(_4\) must be kept under N\(_2\)\(_{\text{g}}\) to avoid the formation of TiO\(_2\).

\(^a\) The mixture was not reduced in volume prior to purification using column chromatography.
2.2.3.2 4-Nitro-2-trichloroacetyl-N-methyl-1H-pyrrole (Py B)

![Chemical structure of Py B]

Nitric acid (25 mL, 70%) in Ac$_2$O (120 mL) was added dropwise over 1 h to a solution of Py A (30.0 g, 0.13 mol) in Ac$_2$O (75 mL) at -40 °C.° The mixture was warmed to r.t. and stirred for 2 h. The solution was then cooled to -20 °C, cold isopropanol (125 mL) added and left at -20 °C overnight in a low temperature freezer. The solid was filtered and washed with cold isopropanol to yield Py B (20 g, 57%) as a white powder: m.p. 134-136 °C. $^1$H NMR 300 MHz (d$_6$-DMSO): $\delta$ 8.54 (d, 1H, $J = 1.9$ Hz), 7.80 (d, 1H, $J = 1.9$ Hz), 3.99 (s, 3H).

2.2.3.3 tert-Butyl-4-nitro-N-methyl-1H-pyrrole-2-carboxylate (Py C)

To a mixture of Py B (19.70 g, 72 mmol) in tert-butyl alcohol (210 mL) was added sodium tert-butoxide (9.85 g, 110 mmol) over 1 h. The reaction was refluxed under dried N$_2$(g) for 5 h, quenched with water (200 mL) and extracted with chloroform (3 × 200 mL). The solvent was removed to obtain Py C (15.0 g, 91%) as yellow/brown crystals: m.p. 81-83 °C. $^1$H NMR 300 MHz (d$_6$-DMSO): $\delta$ 8.19 (d, 1H, $J = 1.8$ Hz), 7.22 (d, 1H, $J = 1.9$ Hz), 3.85 (s, 3H), 1.52 (s, 9H).

° Temperatures of -40 °C were obtained using acetone and dry ice.
2.2.3.4  
**tert-Butyl-4-[(9-fluorenlymethoxycarbonyl)amino]-N-methyl-1H-pyrrole-2-carboxylate (Py D)**

![Chemical structure of Py D](image)

To a solution of Py C (9.5 g, 42 mmol) in DMF (50 mL) was added 10% Pd/C (1.0 g) in DMF (10 mL). The mixture was vigorously stirred overnight under H$_2$(g) (36 psi), filtered through celite and washed with DMF (80 mL). Fmoc-Cl (11.9 g, 46 mmol) and DIEA (16.6 mL, 95 mmol) were added and the solution stirred overnight under N$_2$(g). The solvent was evaporated under reduced pressure and water (125 mL) added. The product was extracted with diethyl ether (2 × 200 mL), dried using anhydrous magnesium sulphate and the ether layer concentrated to ~ 150 mL. Hexane (200 mL) was added and the solution stored at 4 ºC for 3 h. The resulting brown precipitate was filtered and washed with cold methanol/water (1:1) to yield Py D (4.6 g, 26%) as a white solid: m.p. 176-178 ºC. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 9.34 (bs, 1H), 7.89 (d, 2H, $J = 7.4$ Hz), 7.69 (d, 2H, $J = 7.6$ Hz), 7.41 (t, 2H, $J = 7.0$ Hz), 7.32 (t, 2H, $J = 7.5$ Hz), 6.90 (s, 1H), 6.60 (s, 1H), 4.41 (d, 2H, $J = 6.9$ Hz), 4.28 (t, 1H, $J = 6.9$ Hz), 3.74 (s, 3H), 1.43 (s, 9H).

2.2.3.5  
**4-[(9-Fluorenlymethoxycarbonyl)amino]-N-methyl-1H-pyrrole-2-carboxylic acid (Py E)**

Py D (5.0 g, 12 mmol) was dissolved in DCM (100 mL) and cooled to 0 ºC. TiCl$_4$ (1 M in DCM, 25 mL) was added dropwise under N$_2$(g) and the mixture stirred for 30 min at 0 ºC. Cold HCl (250 mL) was added dropwise and the precipitate collected by vacuum filtration and washed with cold water to yield Py E (3.8 g, 88%) as a white solid: m.p. 208-209 ºC. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 12.02 (bs, 1H), 9.38 (bs, 1H), 7.89 (d, 2H, $J = 7.4$ Hz), 7.71 (d, 2H, $J = 7.6$ Hz), 7.41 (t, 2H, $J = 7.0$ Hz), 7.32 (t, 2H, $J = 7.5$ Hz), 6.90 (s, 1H), 6.60 (s, 1H), 4.41 (d, 2H, $J = 6.9$ Hz), 4.28 (t, 1H, $J = 6.9$ Hz), 3.74 (s, 3H), 1.43 (s, 9H).

(t, 2H, J = 7.5 Hz), 7.02 (s, 1H), 6.61 (s, 1H), 4.41 (d, 2H, J = 6.9 Hz), 4.28 (t, 1H, J = 6.9 Hz), 3.74 (s, 3H).

2.2.4 Preparation of the Boc mono-substituted linkers

**General Method:** A solution of di-tert-butyl dicarbonate (2.0 g, 9 mmol) in DCM (24 mL) was added over a period of 2.5 h to a solution of 1,2-diaminoethane (4.2 g, 70 mmol), 1,3-diaminopropane (5.2 g, 70 mmol) or 1,6-diaminohexane (8.1 g, 70 mmol) in DCM (24 mL), which was cooled in an ice bath. The mixture was allowed to stir at r.t. for 24 h before the solvent was removed under reduced pressure. Water (40 mL) was added and the mixture filtered. The filtrate was extracted with DCM\(^9\) (3 × 30 mL), the organic layer dried using anhydrous magnesium sulphate and the solvent evaporated to yield the product.

2.2.4.1 tert-Butyl-2-aminoethylcarbamate (L\(_2\))

\[
\text{H}_2\text{N} \quad \text{NHBOc}
\]

The product was isolated as a clear oil (0.96 g, 65%). \(^1\)H NMR 300 MHz (CDCl\(_3\)): \(\delta\)

5.16 (bs, 1H), 3.04 (q, 2H, \(J = 6.0\) Hz, \(J = 10.8\) Hz), 2.66 (t, 2H, \(J = 6.0\) Hz), 1.42 (s, 2H), 1.39 (s, 9H).

2.2.4.2 tert-Butyl-3-aminopropylcarbamate (L\(_3\))

\[
\text{H}_2\text{N} \quad \text{NHBOc}
\]

The product was isolated as a clear oil (1.46 g, 92%). \(^1\)H NMR 300 MHz (CDCl\(_3\)): \(\delta\)

5.16 (bs, 1H), 3.19 (q, 2H, \(J = 6.0\) Hz, \(J = 12.3\) Hz), 2.76 (t, 2H, \(J = 6.3\) Hz), 1.51 (m, 2H), 1.43 (s, 2H), 1.37 (s, 9H).

\(^9\) In the case of 1,6-diaminohexane, excess diamine was removed by backwashing the organic layer with water (2 × 20 mL).
2.2.4.3 tert-Butyl-6-aminohexylcarbamate (L₆)

\[
\begin{align*}
\text{H₂N} & \quad \text{NHBoc} \\
\end{align*}
\]

The product was isolated as a clear oil (1.49 g, 75%). ¹H NMR 300 MHz (CDCl₃): δ 4.58 (bs, 1H), 3.09 (q, 2H, J = 6.0 Hz, J = 12.3 Hz), 2.66 (t, 2H, J = 6.3 Hz), 1.41 (bm, 13H), 1.31 (m, 6H).

2.2.5 Synthesis of (N-methyl-1H-imidazole-2-carboxamido) butyric acid (Im-L₄-COOH)

(N-Methyl-1H-imidazole-2-carboxamido) butyric acid was synthesised using two methods:

2.2.5.1 Method 1: coupling 2-trichloroacetyl-N-methyl-1H-imidazole (Im A) to ethyl 4-aminobutyrate hydrochloride to produce ethyl \( \gamma \)-((N-methyl-1H-imidazole-2-carboxamido)butyricate (Im-L₄-COOEt)

\[
\begin{align*}
\text{Im A} \quad + \quad \text{HClH₂N} & \quad \text{COOEt} \\
\end{align*}
\]

Im-L₄-COOEt
To a cold mixture of Im A (3.0 g, 13.0 mmol) and ethyl-4-aminobutyrate hydrochloride (2.3 g, 14.0 mmol) in ethyl acetate (30 mL) was added TEA (6 mL, 42.0 mmol) and the mixture stirred at r.t. for 1-2 h (TLC\textsuperscript{a}, 5% MeOH/DCM). DCM (50 mL) was added and the product washed with water (3 × 50 mL). The solvent was evaporated to obtain Im-L_4-COOEt as a brown oil (3.1 g, 98%). The mixture was used in the next step without any further purification. \textsuperscript{1}H NMR 300 MHz (d\textsubscript{6}-DMSO): \(\delta\) 8.37 (t, 1H, \(J = 5.8\) Hz), 7.28 (d, 1H, \(J = 1.5\) Hz), 6.94 (d, 1H, \(J = 1.4\) Hz), 4.02 (q, 2H, \(J = 7.2\) Hz), 3.92 (s, 3H), 3.22 (q, 2H, \(J = 7.3\) Hz), 2.29 (t, 2H, \(J = 7.4\) Hz), 1.75 (m, 2H, \(J = 7.1\) Hz), 1.16 (t, 3H, \(J = 7.2\) Hz).

2.2.5.2 Method 2: coupling \(N\)-methyl-1H-imidazole-2-carboxylic acid (Im') to ethyl 4-aminobutyrate hydrochloride to produce ethyl \(\gamma\)-(\(N\)-methyl-1H-imidazole-2-carboxamido)butyricate (Im-L_4-COOEt)

\[
\begin{array}{c}
\text{Im'} \\
\text{HCl} \quad \text{H}_2\text{N} \\
\text{OH} \quad \text{CH}_2\text{N} \\
\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO} \\
\end{array}
\]

\[
\begin{array}{c}
\text{Im-L_4-COOEt} \\
\text{OEt} \\
\end{array}
\]

To Im' (1.0 g, 8.0 mmol) in DMF (60 mL) was added HBTU (2.7 g, 7.0 mmol) and DIEA (1.4 mL, 8.0 mmol) and the solution stirred for 5 min. Ethyl 4-aminobutyrate hydrochloride (1.5 g, 9.0 mmol) and DIEA (1.5 mL, 9.0 mmol) were added and the mixture stirred overnight under \(N_2\)(g). DMF was removed under reduced pressure and the mixture purified by column chromatography (silica gel, 5% MeOH/DCM, \(R_f\)

\textsuperscript{a}All TLC were carried out using Silica gel F\textsubscript{254} aluminium backed sheets.
0.58) to yield the product (\text{Im-L}_4\text{-COOEt}, 1.7 \text{ g}, 91\%) as a brown oil. The $^1$H NMR spectrum was identical to that described in Chapter 2.1.5.1.

\subsection*{2.2.5.3 (N-Methylimidazole-2-carboxamido)butyric acid (Im-L}_4\text{-COOH)}

![Chemical Structures](image)

To ethyl $\gamma$-(N-methyl-1H-imidazole-2-carboxamido)butyrate (1.8 g, 11.0 mmol) in ethanol (40 mL) was added NaOH (0.6 M, 23 mL) and the mixture stirred overnight (TLC 5\% MeOH/DCM, $R_f$ 0.06). DCM (45 mL) was added to the solution and the product extracted with water (50 mL). The aqueous layer was cooled to 0 ºC and acidified with concentrated HCl (1 mL, pH 2-3). The aqueous layer was then washed with DCM (2 × 20 mL) and lyophilised to yield (N-methyl-1H-imidazole-2-carboxamido)butyric acid (1.4 g, 88\%) as a pale yellow solid. $^1$H NMR 300 MHz (d$_6$-DMSO): $\delta$ 9.23 (t, 1H, $J = 5.8$ Hz), 7.68 (d, 1H, $J = 1.5$ Hz), 7.58 (d, 1H, $J = 1.4$ Hz), 3.99 (s, 3H), 3.29 (q, 2H, $J = 7.3$ Hz), 2.32 (t, 2H, $J = 7.4$ Hz), 1.78 (m, 2H, $J = 7.1$ Hz).

\section*{2.3 Discussion}

\subsection*{2.3.1 Preparation of trichloroacetyl chloride}

The synthesis of the monomers (Im E and Py E) was initiated with the preparation of trichloroacetyl chloride. All solvents were dried prior to use; trichloroacetic acid was dried by coevaporating the water with benzene under reduced pressure and the solid stored under vacuum; thionyl chloride was dried via distillation from linseed oil and the liquid stored in a vacuum desiccator. Trichloroacetyl chloride was prepared immediately before use via acyl halide conversion of the carboxylic acid using thionyl chloride, with DMF as the catalyst (Figure 2.2). The reaction was allowed to proceed for 2 h before the clear product was distilled. The first and last 10 mL of distillation were discarded to ensure purity. In the Infra-red analysis of the distilled product the
2.3.2 Synthesis of the imidazole monomer

Production of the imidazole monomeric precursor required for solid phase synthesis was carried out from \(N\)-methyl-1H-imidazole (Im, Figure 2.3) using adaptations of various literature methods.\(^{135,138-141}\) Trichloroacetylation of Im afforded Im A, which was then nitrated at the 4-position to produce Im B. Conversion to the \(t\)-butyl ester yielded Im C, which was reduced before Fmoc-Cl and DMAP were added to produce Im D. The \(t\)-butyl group was removed with TiCl\(_4\) and the product, Im E, precipitated with HCl.

2.3.2.1 2-Trichloroacetyl-\(N\)-methyl-1H-imidazole (Im A)

The conversion of Im to Im A (Figure 2.4) required the dropwise addition of Im into a solution of trichloroacetyl chloride in DCM, which was dried with basic alumina.

\[
\text{Im} \xrightarrow{1, \text{57\%}} \text{Im A} \quad \text{Im A} \xrightarrow{2, \text{64\%}} \text{Im B} \quad \text{Im B} \xrightarrow{3, \text{55\%}} \text{Im E} \quad \text{Im E} \xrightarrow{5, \text{35\%}} \text{Im D} \quad \text{Im D} \xrightarrow{4, \text{67\%}} \text{Im C} \quad \text{Im C} \xrightarrow{\text{FmocHN}} \text{Im D} \xrightarrow{\text{FmocHN}} \text{Im E}
\]

Figure 2.4: The conversion of \(N\)-methyl-1H-imidazole (Im) to 4-\{9-fluorenylmethoxycarbonyl\}amino\-\(N\)-methyl-1H-imidazole-2-carboxylic acid (Im E).
before use. These conditions limit the substitution of the trichloro group to the 2-position. The resulting purple mixture was allowed to stir overnight before TEA was added and the mixture stirred for a further 1 h. The product was purified on a silica gel column without reducing the solvent volume. The compound was loaded slowly with the column unpressurised to avoid compacting the reaction mixture. Purification with hexane/DCM (1:4) using column chromatography saw the product elute first as a pale yellow band ($R_f$ 0.53), while a purple band (impurities) migrated at approximately half the rate of the product. The product was obtained as a light yellow solid (57%). In previous reactions, the 2-position was occupied by either an ethyl or tert-butyl ester group; however, the acetyl group was preferred to the ester group in this case due to its stronger 4-position directing effect which is necessary for the ensuing nitration of the heterocyclic ring. The presence of the trichloroacetyl group creates a lower electron density around the 4-position due to the high electron withdrawing effects of the chlorine atoms.

In the $^1$H NMR spectrum of Im A (Figure 2.5), dissolved in d$_6$-DMSO, the expected proton resonances were observed. The two singlets at 7.71 and 7.31 ppm were assigned as the two aromatic hydrogens on the imidazole ring. The proton at the 5-position (H$_5$) was further downfield (7.71 ppm) compared to the proton at the 4-position (H$_4$, 7.31 ppm). The large singlet at 3.98 ppm was assigned as the protons of the N-CH$_3$ group.$^5$

---

$^5$ For future reference, “the protons of the N-CH$_3$ group” will be reffered to as “the N-CH$_3$ group”. This also applies for CH, CH$_2$, CH$_3$, NH, NH$_2$, NH$_3$, tert-butyl groups and Boc groups.
2.3.2.2 4-Nitro-2-trichloroacetyl-N-methyl-1H-imidazole (Im B)

Nitration at the 4-position of Im A was achieved through aromatic electrophilic addition by stirring Im A with sulphuric acid and fuming nitric acid (Figure 2.6 and Figure 2.7).

---

The peak for d$_6$-DMSO was observed as a multiplet at ~ 2.49 ppm in all spectrums. The peak, arising from residual water in d$_6$-DMSO, was observed as a broad singlet at ~ 3.20 ppm.
Ac₂O was used fresh from sealed bottles to ensure dryness. The nitronium ion (NO₂⁺) was obtained by drawing water from the solution (forming acetic acid from Ac₂O). It was essential that the nitric acid/Ac₂O mixture was prepared by dropwise addition of the acid to a solution of Ac₂O, cooled to -5 °C, in order to control the formation of NO₂⁺. Addition at r.t. results in a violent reaction in which the rate of formation of NO₂⁺ is fast. The condensation that occurs as a result of the freezing conditions limits the formation of the NO₂⁺ and increases the concentration of NO₃⁻ which would cause a reduction in the yield. Problems with water condensation inside the flask at low temperatures were reduced by purging the flask with N₂(g). Once all the nitric acid has been added to the Ac₂O, the temperature was maintained at -5 °C and Im A was added in a dropwise fashion over 1 h. The mixture was allowed to react overnight before the product was extracted to obtain a yellow powder (64%). In the ¹H NMR spectrum of Im B (Figure 2. 8) one aromatic proton (H₅) was observed at 8.82 ppm. The disappearance of the second aromatic proton (H₄) was a clear indication that the nitration was carried out successfully. The singlet at 4.06 ppm was assigned as the N-CH₃ group. Unreacted starting material, which constitutes 4.6% of the crude product, was also observed. However, Im B was not subjected to any further purification procedures and was carried through to the next step, which involved the formation of the tert-butyl ester group.
2.3.2.3 tert-Butyl-4-nitro-N-methyl-1H-imidazole-2-carboxylate (Im C)

The formation of Im C was obtained by refluxing Im B (with minor impurities) and sodium tert-butoxide in tert-butyl alcohol (Figure 2.9). All reagents were kept in a dry environment prior to use because the reaction is water sensitive. The alcohol was stored under Ar(g) while sodium tert-butoxide powder was dried overnight, under reduced pressure, at 50 °C in a drying oven. After 5 h of reflux, the reaction was quenched with water and the product extracted with chloroform. The solvent was evaporated to obtain the product as yellow crystals (55%).

In the $^1$H NMR spectrum of Im C (Figure 2.10) the H$_5$ singlet was observed at 8.55 ppm and the N-CH$_3$ singlet at 3.95 ppm. The appearance of a large singlet at 1.55 ppm, which was assigned as the nine methyl protons (3 × CH$_3$) of the tert-butyl group, was a clear indication that the reaction was carried out successfully. It was also
observed that the impurities, which were carried over from the nitration step, were removed during the purification of Im C.

Figure 2.10: The $^1$H NMR spectrum of Im C (Figure 2.9) in d$_6$-DMSO at 35°C.

2.3.2.4 tert-Butyl-4-[(9-fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylate (Im D)

The hydrogenation of Im C (Figure 2.11) was achieved in DMF using 30-50 psi of H$_2$(g) and 10% Pd/C catalyst over a period of 20 h. The solution was filtered over celite, to remove the palladium catalyst, and washed with DMF. The product was quickly filtered, with Fmoc-Cl and DMAP added without delay as the free amine (Figure 2.12) is unstable. The suspension was stirred overnight before the product was extracted with diethyl ether and purified using column chromatography (silica gel, 3:1 hexane/ethyl acetate, $R_f$ 0.16, Figure 2.13) to obtain the product as a white solid (67%).
The hydrogenation of the nitro group and the addition of the Fmoc-protecting group were initially carried out in DMF according to previously published methods. The hydrogenation was conducted at 50 psi and allowed to react for 20 h instead of 5 h at 500 psi. The reaction was monitored by TLC (5% MeOH/DCM) and the intermediate product was successfully characterised using $^1$H NMR. The
hydrogenation was also carried out successfully in EtOH/EtOAc (1:1); however, DMF was the preferred solvent because it was also used in the following step. In the process of determining the optimal hydrogenation conditions (Table 1) different solvents were used, hydrogen pressure varied (atmospheric pressure-50 psi) and reaction time varied (2-20 h). The highest yields were obtained when the reaction was carried out using DMF at 30-50 psi of H$_2$ over a period of 20 h.

The addition of the Fmoc-protecting group was initially carried out exactly as reported in the literature; however, the product was only obtained in a mere 1-15% yield. In an attempt to increase the yield, the reaction was then repeated several times varying the reaction conditions. The protection of the amine was carried out using either Fmoc-chloride (Fmoc-Cl) or Fmoc-succinate (Fmoc-Su). Fmoc-Cl resulted in better yields and was therefore the preferred choice. The reaction was also carried out using different bases, including DIEA, NaHCO$_3$ and DMAP, and in some cases without base. DMAP acts as a nucleophilic catalyst which can be used to activate the carbonyl of the Fmoc-protecting group (Figure 2.14) and deprotonate the free amine. The optimum results were obtained when the reaction was carried out in DMF using Fmoc-Cl (1.1 eq.) and DMAP (1.1 eq.) over a period of 15 h to obtain the product at a 67% yield. The reaction conditions are described in Table 1.

![Figure 2.14: The mechanism for the activation of the Fmoc carbonyl using DMAP.](image-url)
Table 1: The reaction conditions for the hydrogenation reaction (red) and the addition of the Fmoc-protecting group (blue) during the conversion of Im C to Im D.

<table>
<thead>
<tr>
<th>Rxn</th>
<th>Im C (g)</th>
<th>Solvent</th>
<th>$H_2$ (psi)</th>
<th>T (h)</th>
<th>Solvent</th>
<th>Fmoc (g)</th>
<th>Base</th>
<th>Amount</th>
<th>T (h)</th>
<th>Yield (%)</th>
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<td>0.28</td>
<td>DMF</td>
<td>atm 11</td>
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<td>-</td>
<td>-</td>
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<td>&lt; 15</td>
</tr>
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<td>2</td>
<td>4.65</td>
<td>DMF</td>
<td>atm 5</td>
<td></td>
<td>DMF</td>
<td>-Cl 5.19</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>0</td>
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<td>EtOH-EtOAc</td>
<td>atm 3</td>
<td></td>
<td>DMF</td>
<td>-Cl 0.33</td>
<td>DIEA 0.420 mL</td>
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<td>0.20</td>
<td>DMF</td>
<td>50</td>
<td>7</td>
<td>DMF</td>
<td>-Cl 0.29</td>
<td>DIEA 0.390 mL</td>
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<tr>
<td>5</td>
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<td></td>
<td>DMF</td>
<td>-Cl 0.67</td>
<td>DIEA 0.320 mL</td>
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<td>-Cl 0.67</td>
<td>DIEA 0.150 mL</td>
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<td>DMF</td>
<td>-Cl 0.67</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>0</td>
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<td>8</td>
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<td>atm 3</td>
<td></td>
<td>DMF</td>
<td>-Su 0.54</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>0</td>
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<td>9</td>
<td>0.10</td>
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<td></td>
<td>Acetone/Water</td>
<td>-Su 0.16</td>
<td>NaHCO$_3$ 0.038 g</td>
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<td>DMF</td>
<td>-Cl 0.12</td>
<td>DMAP 0.056 g</td>
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<tr>
<td>11</td>
<td>0.10</td>
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<td></td>
<td>DMF</td>
<td>-Cl 0.12</td>
<td>-</td>
<td>-</td>
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<td>0</td>
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<td>atm 17</td>
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<td>DMF</td>
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<td>-</td>
<td>-</td>
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<td>0</td>
</tr>
<tr>
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<td>0.10</td>
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<td>atm 2</td>
<td></td>
<td>DMF</td>
<td>-Cl 0.12</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>0</td>
</tr>
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<td>DMF</td>
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<td>18</td>
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<td>NaHCO$_3$ 0.039 g</td>
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<td>45</td>
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<td>DMF</td>
<td>32</td>
<td>16</td>
<td>DMF</td>
<td>-Cl 0.23</td>
<td>-</td>
<td>-</td>
<td>15.0</td>
<td>0</td>
</tr>
<tr>
<td>17</td>
<td>0.20</td>
<td>EtOH-EtOAc</td>
<td>atm 3</td>
<td></td>
<td>DMF</td>
<td>-Cl 0.24</td>
<td>DMAP 0.112 g</td>
<td>15.0</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.20</td>
<td>EtOH-EtOAc</td>
<td>atm 3</td>
<td></td>
<td>DMF</td>
<td>-Cl 0.24</td>
<td>DMAP 0.112 g</td>
<td>15.0</td>
<td>65</td>
<td></td>
</tr>
<tr>
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<td>0.50</td>
<td>DMF</td>
<td>34</td>
<td>20</td>
<td>DMF</td>
<td>-Cl 0.59</td>
<td>DMAP 0.281 g</td>
<td>15.0</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

- a one pot reaction
- b amine was added after 3 h
- c hydrogenation did not work

In the $^1$H NMR spectrum of the intermediate (Im D-amino), formed during the conversion of Im C to Im D (Figure 2.15), the aromatic hydrogen (H$_5$) was observed at 6.39 ppm. The free amine (NH$_2$) was observed as a broad peak at 4.43 ppm, and upon addition of D$_2$O, the peak disappeared as the amine exchanged with the deuterated solvent. The singlet at 3.76 ppm was assigned as the N-CH$_3$ group, while the singlet at 1.42 ppm was assigned as the tert-butyl group.

![Figure 2.15: The $^1$H NMR spectrum of Im D-NH$_2$ (Figure 2.12) in d$_6$-DMSO at 35°C.](image_url)
In the $^1$H NMR spectrum of Im D (Figure 2. 16) the singlet at 10.39 ppm was assigned as the amide proton (NH), which characteristically occurs downfield of 8.00 ppm. The four signals at 7.89, 7.65, 7.41 and 7.30 ppm were assigned as the aromatic Fmoc protons as each integrates to 2H and have similar $J$-coupling values. The two peaks at 7.89 and 7.65 ppm were observed as doublets (H$_a$ and H$_d$), while the peaks at 7.41 and 7.30 ppm were observed as triplets (H$_b$ and H$_c$). The aromatic proton (H$_5$) on the imidazole ring was observed as a singlet at 7.06 ppm. The two peaks at 4.40 and 4.28 ppm were observed as a doublet and triplet and correspond to the CH$_2$ and CH of the Fmoc-protecting group respectively. The N-CH$_3$ singlet was observed at 3.85 ppm, while the tert-butyl group was observed as a singlet at 1.58 ppm.

![NMR spectrum of Im D](image)

**Figure 2. 16: The $^1$H NMR spectrum of Im D (Figure 2.11) in d$_6$-DMSO at 35°C.**

### 2.3.2.5 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylic acid (Im E)

The hydrolysis of Im D was carried out via the dropwise addition of TiCl$_4$ followed by acidification with HCl (Figure 2. 17). TiCl$_4$ reacts immediately upon exposure to air to form HCl$_{(g)}$ and TiO$_2$, so it was important to keep the reaction vessel under N$_2$(g)
during the addition. Once the product was collected it was left to dry under reduced pressure for 2 h, then overnight in a drying oven at 40 °C. The use of strong bases (such as NaOH and LiOH) for the hydrolysis of the ester was avoided as that would cause the deprotection of the amine, as the Fmoc-protecting group is labile under basic conditions. The product was obtained as a yellow solid (35%).

![Diagram](attachment:image.png)

**Figure 2.17: The hydrolysis of Im D to Im E using TiCl₄.**

The $^1$H NMR assignment of **Im E** (Figure 2.18) is provided in Figure 2.19. The amide proton was observed as a singlet at 10.39 ppm. The resonances at 7.89, 7.65, 7.41 and 7.30 ppm each integrates to 2H, indicative of the Fmoc protons. H₅ and H₆ were observed as doublets, while H₇ and H₈ were observed as triplets. The singlet at 7.06 ppm was assigned as the aromatic hydrogen on the imidazole ring (H₅), while the doublet and triplet at 4.40 and 4.24 ppm were assigned as the CH₂ and CH of the Fmoc-protecting group respectively. The singlet at 3.85 ppm was assigned as the N-CH₃ group, while the OH proton was thought to resonate at a similar frequency as the water peak and as a consequence could not be observed.

![Diagram](attachment:image1.png)

**Figure 2.18: The chemical structure of Im E.**
To distinguish between Hₐ/Hₜ and Hₘ/Hₜ protons a Nuclear Overhauser Effect Spectroscopy (NOESY) experiment was performed. In the NOESY spectrum of Im E (Figure 2.20) an NOE cross peak was observed between the doublet at 7.65 ppm (Hₐ or Hₜ) and the triplet at 4.24 ppm, which corresponds to the CH of the Fmoc-protecting group. As a result the doublet at 7.65 ppm was assigned as Hₜ due to its close proximity to the CH group and by elimination the doublet at 7.89 ppm was assigned as Hₐ. The triplet at 7.30 ppm (Hₘ or Hₜ) shares cross peaks with the CH₂ and CH of the Fmoc-protecting group and was therefore assigned as Hₘ. The remaining triplet at 7.41 ppm was assigned, by elimination, as Hₙ. The aromatic region of the NOESY spectrum (Figure 2.21) was inconclusive as cross peaks were observed between all four peaks. Upon varying the intensity of the spectrum, the most prominent cross peak for Hₜ was observed with the triplet at 7.30 ppm, which corresponds to Hₘ, while the most prominent cross peak for Hₐ was observed with the triplet at 7.41 ppm, which corresponds to Hₙ. These results are consistent with the conclusions determined from Figure 2.20.
Figure 2.20: The NOESY spectrum of Im E.

Figure 2.21: The aromatic region of the NOESY spectrum of Im E.
Looking back at the $^1$H NMR spectrum of Im A, Im B, Im C, Im D and Im E it was observed that the chemical shift of H$_5$ varied significantly with the substitution of the group at the 4-position. When a proton occupied the 4-position, H$_5$ appeared at 7.71 ppm (Im A). However, when the compound was nitrated, H$_5$ was shifted further downfield to 8.82 ppm (Im B) and 8.55 ppm (Im C). In Im D and Im E where the nitro group was reduced and then protected, H$_5$ was shifted slightly upfield to 6.19 ppm (with the free amine) and 7.06 ppm (with the protected amine). In conclusion H$_5$ was furthest downfield when the nitro group occupied the 4-position and furthest upfield when a free amine occupied the 4-position. The variation in the $^1$H NMR chemical shifts of the H$_5$ and N-CH$_3$ protons is presented in Table 2.

Table 2: The $^1$H NMR chemical shifts of the H$_5$ and N-CH$_3$ protons as the group occupying the 4-position on the imidazole ring was varied.

<table>
<thead>
<tr>
<th></th>
<th>H (Im A)</th>
<th>NO$_2$ (Im B/Im C)</th>
<th>NH$_2$ (Im D-amino)</th>
<th>NHFmoc (Im D/Im E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_5$ (ppm)</td>
<td>7.71</td>
<td>8.82/8.55</td>
<td>6.19</td>
<td>7.06/7.06</td>
</tr>
<tr>
<td>N-CH$_3$ (ppm)</td>
<td>3.98</td>
<td>4.06/3.95</td>
<td>3.95</td>
<td>3.85/3.85</td>
</tr>
</tbody>
</table>

### 2.3.3 Synthesis of the pyrrole monomer

Production of the pyrrole monomer precursor required for solid phase synthesis was achieved using adaptations of various literature methods (Figure 2.22).$^{135,138-141}$ Py A was afforded by trichloroacetylation of N-methyl-1H-pyrrole (Py), which was followed by nitration at the 4-position to provide Py B. Conversion to the tert-butyl ester yielded Py C, which was reduced before Fmoc-Cl and DIEA were added to produce Py D. The tert-butyl group was removed with TiCl$_4$ and the product, Py E, precipitated with HCl.
2.3.3.1 2-Trichloroacetyl-N-methyl-1H-pyrrole (Py A)

The conversion of Py to Py A (Figure 2. 23) was carried out using similar reaction conditions described for the synthesis of Im A. Unlike the imidazole monomer, the pyrrole monomer did not require the addition of TEA after being stirred overnight in DCM. Purification with hexane/DCM (7:3) using column chromatography saw the product elute first as a pale yellow band ($R_f$ 0.68), while a purple band (impurities) migrated at approximately half the rate of the product. The product was obtained as a pale yellow solid (73%) at a higher yield than that of Im A. Like the imidazole monomer, however, the trichloroacetyl group was incorporated instead of the tert-butyl ester group due to its stronger 4-directing effect, which is necessary for the ensuing nitration of the heterocyclic ring. \(^{142}\)

In the $^1$H NMR spectrum of Py A (Figure 2.24) the expected proton resonances were observed. The two doublets at 8.21 and 7.24 ppm were assigned as the three aromatic hydrogens on the pyrrole ring. The proton at the 5-position ($H_5$) was further downfield (8.21 ppm) compared to the protons at the 3- and 4-positions ($H_3$ and $H_4$ at 7.24 ppm). The large singlet at 3.88 ppm was assigned as the $N$-CH$_3$ group.

![Figure 2.24: The $^1$H NMR spectrum of Py A (Figure 2.23) in d$_6$-DMSO, containing TMS, at 35 °C.](image)

### 2.3.3.2 4-Nitro-2-trichloroacetyl-$N$-methyl-1H-pyrrole (Py B)

The nitration at the 4-position of Py A was achieved through aromatic electrophilic addition by stirring Py A with 70% nitric acid (Figure 2.25). The mechanism of nitration is similar to that described for the nitration of the imidazole ring in Chapter 2.3.2.2.
Like the imidazole monomer, it was essential that the nitric acid/Ac$_2$O mixture was prepared by dropwise addition of the acid to a solution of Ac$_2$O, cooled to -5 °C, in order to control the formation of NO$_2$$. Once all the nitric acid had been added to the Ac$_2$O, the acidic mixture was added dropwise to a solution of Py A in Ac$_2$O at -40 °C. The mixture was allowed to react for 2 h at r.t. and then stored at -20 °C for 2-3 h before the product was precipitated with isopropanol to obtain a white solid (57%).

In the $^1$H NMR spectrum of Py B (Figure 2. 26) the two aromatic protons, H$_5$ and H$_3$, were observed at 8.54 and 7.80 ppm respectively. H$_5$ was further downfield than H$_3$ due to its proximity to the nitrogen atom at the N-position (N$_1$). The disappearance of the third aromatic proton (H$_4$) was a clear indication that the nitration was carried out successfully. The singlet at 3.99 ppm was assigned as the N-CH$_3$ group.

Unlike the imidazole monomer, the nitration of the pyrrole ring did not require fuming nitric acid; instead the reaction was achieved using 70% nitric acid. The N$_3$ of the imidazole ring creates a higher electron density around the 3-position and
subsequently a low electron density around the 4-position. The imidazole ring is therefore harder to nitrate thus fuming nitric acid was required. In the case of the pyrrole ring, the 3-position is occupied by a carbon which makes the 4-position more electron dense and therefore easier to nitrate. As a result, Py B was obtained in a higher degree of purity (> 99%) compared to Im B (95% purity).

2.3.3.3 tert-Butyl-4-nitro-N-methyl-1H-pyrrole-2-carboxylate (Py C)

The formation of Py C was achieved by refluxing Py B and sodium tert-butoxide in tert-butyl alcohol (Figure 2.27). These were the same reaction conditions used for the conversion of Im B to Im C. All reagents were dried prior to use as described for the synthesis of the corresponding imidazole monomer. The solution was refluxed for 5 h, after which the reaction was quenched with water, the product extracted with chloroform and the solvent evaporated to obtain Py C as yellow crystals (91%).

![Figure 2.27: The conversion of Py B to Py C using tert-butyl alcohol.](image)

In the $^1$H NMR spectrum of Py C (Figure 2.28) H$_5$ and H$_3$ were observed as two doublets at 8.19 and 7.22 ppm respectively, while the N-CH$_3$ group was observed as a singlet at 3.85 ppm. The appearance of a large singlet at 1.52 ppm, which corresponds to the tert-butyl group, was a clear indication that the reaction was carried out successfully.
The hydrogenation and protection of Py C (Figure 2.29) was achieved using similar experimental conditions described for the imidazole monomer (Im D) in Chapter 2.3.2.4. The major exception was that the addition of the Fmoc-protecting group onto the intermediate formed, Py D-amino (Figure 2.30), was carried out using a stronger base (DIEA) instead of DMAP. This is because the 3-position on the pyrrole ring is occupied by a carbon atom instead of a nitrogen atom, thus making it less susceptible for the addition of an Fmoc-protecting group. The base was required to deprotonate the free amine which could then attack the carbonyl of the Fmoc-protecting group, with the chloride ligand acting as a good leaving group. The reaction mixture was stirred overnight before the product was extracted using diethyl ether and precipitated with hexane as a white solid (26%).
Figure 2. 29: The conversion of Py C to Py D. Step 1 involves the formation of the free amine, which was protected using an Fmoc-protecting group in the second step.

Figure 2. 30: The chemical structure of the intermediate (Py D-amino) formed during the conversion of Py C to Py D.

In the $^1$H NMR spectrum of Py D (Figure 2. 31) the amide proton (NH) was observed as a broad singlet at 9.34 ppm. The four signals at 7.89, 7.69, 7.41 and 7.32 ppm were assigned as the aromatic Fmoc protons as each integrates to 2H and have similar $J$-coupling values. The two peaks at 7.89 and 7.69 ppm were observed as doublets (H$_a$ and H$_d$), while the peaks at 7.41 and 7.32 ppm were observed as triplets (H$_b$ and H$_c$). The two aromatic protons on the pyrrole ring (H$_5$ and H$_3$) were observed as two singlets at 6.90 and 6.60 ppm. The two peaks at 4.41 and 4.28 ppm were observed as a doublet and triplet and correspond to the CH$_2$ and CH of the Fmoc-protecting group respectively. The N-CH$_3$ and tert-butyl groups were observed as two singlets at 3.74 and 1.43 ppm respectively.
2.3.3.5 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-pyrrole-2-carboxylic acid (Py E)

The hydrolysis of Py D was achieved using the same experimental procedures described for the conversion of Im D to Im E in Chapter 2.3.2.5 (Figure 2.32). The product was obtained as a white solid (88%).

The \( ^1H \) NMR assignment of Py E (Figure 2.33) is provided in Figure 2.34. The amide proton (NH) was observed as a singlet at 9.38 ppm. The resonances at 7.89, 7.71, 7.41 and 7.32 ppm each integrates to 2H, indicative of the Fmoc protons. H_a and H_d were observed as doublets, while H_b and H_c were observed as triplets. The singlets
at 7.02 and 6.61 ppm were assigned as the aromatic hydrogens on the pyrrole ring (H₅ and H₃), while the doublet and triplet at 4.41 and 4.28 ppm were assigned as the CH₂ and CH of the Fmoc-protecting group respectively. The singlet at 3.74 ppm was assigned as the N-CH₃ group, while the OH proton (which was expected to resonate at a similar frequency to the water peak) was observed at 12.02 ppm. It is unclear at this stage what causes the acid peak to shift downfield from ~ 3.3 ppm to ~ 12 ppm.

Figure 2.33: The chemical structure of Py E.

To distinguish between Hₐ/Hₖ, Hₐ/Hₜ and H₃/H₅ protons a NOESY experiment was performed (Figure 2.35). NOE cross peaks were observed between the doublet at 7.71 ppm (Hₐ or Hₖ) and the CH₂ and CH of the Fmoc-protecting group. The doublet

Figure 2.34: The ¹H NMR spectrum of Py E (Figure 2.33) in d₆-DMSO at 35°C.
at 7.71 ppm was therefore assigned as $H_d$ due to its close proximity to the $CH_2$ and $CH$ groups and by elimination the doublet at 7.89 ppm was assigned as $H_a$. The triplet at 7.32 ppm shares a cross peak with $H_d$ and was therefore assigned as $H_c$ (Figure 2.36). By elimination, $H_b$ was determined as the triplet at 7.41 ppm, which also shares a cross peak with $H_c$. $H_5$ was assigned as the singlet at 7.02 ppm as it shares an NOE with the N-CH$_3$ group which resonates at 3.74 ppm (Figure 2.35). This suggests that the second aromatic singlet at 6.61 ppm corresponds to $H_3$ as it displays no cross peaks with the N-CH$_3$ group.

Figure 2.35: The NOESY spectrum of Py E.
The chemical shifts of the aromatic hydrogens (H$_5$ and H$_3$) on the pyrrole ring vary significantly when the group at the 4-position was substituted, as was seen for the imidazole monomer. When a proton occupied the 4-position (Py A), H$_5$ and H$_3$ were observed at 7.43 and 7.42 ppm respectively. However, when the compound was nitrated, H$_5$ and H$_3$ shifted further downfield to 8.54/7.80 ppm (Py B) and 8.19/7.22 ppm (Py C) respectively. In Py D, where the nitro group was converted to the protected amine, H$_5$ and H$_3$ shifted slightly upfield to 6.90 and 6.60 ppm respectively. In conclusion H$_5$ and H$_3$ were furthest downfield when the nitro group occupied the 4-position and furthest upfield when an amine group occupied the 4-position. This is consistent with the results obtained for the imidazole monomer, even though the chemical shifts of the aromatic protons on the pyrrole ring were not studied when the 4-position was occupied by a free amine. The variation in the $^1$H NMR chemical shifts of the H$_5$, H$_3$ and N-CH$_3$ protons is presented in Table 3.

Table 3: The $^1$H NMR chemical shifts of the H$_3$, H$_5$ and N-CH$_3$ protons as the group occupying the 4-position on the pyrrole ring was varied.

<table>
<thead>
<tr>
<th></th>
<th>H (Py A)</th>
<th>NO$_2$ (Py B/Py C)</th>
<th>NHFmoc (Py D/Py E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_3$ (ppm)</td>
<td>7.42</td>
<td>7.80/7.22</td>
<td>6.6/6.6</td>
</tr>
<tr>
<td>H$_5$ (ppm)</td>
<td>7.43</td>
<td>8.54/8.19</td>
<td>6.9/6.9</td>
</tr>
<tr>
<td>N-CH$_3$ (ppm)</td>
<td>3.88</td>
<td>3.99/3.85</td>
<td>3.74/3.74</td>
</tr>
</tbody>
</table>

2.3.4 Preparation of the Boc-mono-substituted linkers

The mono-protection of 1,2-diaminoethane, 1,3-diaminopropane and 1,6-diaminohexane was carried out according to published methods, although some changes were made in order to separate the minor di-substituted from the desired mono-substituted product (Figure 2.37). This was achieved by dissolving the mono-substituted product in chloroform and then filtering the di-substituted compound. In all three cases, the monoprotected amine was obtained as a slightly coloured oil. In the case of 1,6-diaminohexane, excess diamine was removed by backwashing the organic layer with water.

In the $^1$H NMR spectrum of L$_2$ (Figure 2.38) the Boc-protected amine (NH) was observed as a broad singlet at 5.16 ppm. The two CH$_2$ groups were observed as a quartet and a triplet at 3.04 and 2.66 ppm respectively. The quartet corresponds to the CH$_2$ at the 2-position, while the triplet corresponds to the CH$_2$ at the 1-position. The two singlets at 1.42 and 1.39 ppm were assigned as the NH$_2$ group and the Boc group ($3 \times$ CH$_3$) respectively.
In the $^1$H NMR spectrum of $L_3$ (Figure 2. 39) the Boc-protected amine was observed as a broad singlet at 5.16 ppm. The three CH$_2$ groups were observed as a quartet (3-position), a triplet (1-position) and a multiplet (2-position) at 3.19, 2.67 and 1.51 ppm respectively. The two singlets at 1.43 and 1.37 were assigned as the NH$_2$ group and the Boc group respectively.

\[ \text{Figure 2. 38: The } ^1 \text{H NMR spectrum of } L_2 \text{ in CDCl}_3 \text{ at } 25^\circ \text{C.} \]

\[ \text{Figure 2. 39: The } ^1 \text{H NMR spectrum of } L_3 \text{ in CDCl}_3 \text{ at } 25^\circ \text{C.} \]

\[ ^1 \text{The peak for CHCl}_3 \text{ was observed as a singlet around 7.26 ppm in all spectrums.} \]
In the $^1$H NMR spectrum of $L_6$ (Figure 2. 40) the Boc-protected amine was observed as a broad singlet at 4.58 ppm, which is ~ 0.5 ppm lower than that of $L_2$ and $L_3$. This upfield shift is a result of the longer aliphatic chain of the hexane linker. Two CH$_2$ groups were observed as a quartet (6-position) and a triplet (1-position) at 3.09 and 2.66 ppm respectively. The two multiplets at 1.41 and 1.31 were assigned as the NH$_2$ group, four CH$_2$ (2-, 3-, 4- and 5-position) groups and the Boc group.

![Figure 2. 40: The $^1$H NMR spectrum of $L_6$ in CDCl$_3$ at 25°C.](image)

The Boc-mono-substituted linkers ($L_2$, $L_3$ and $L_6$) were synthesised to provide a ligand with which to connect a platinum(II) centre to the carboxylic acid terminal of a sequence selective polyamide. The platinum(II) centre would be initially coordinated to the free amine before the NHBoc is deprotected to provide a free amine for incorporation using peptide coupling techniques. This will be further discussed in Chapter 4.3.1. Table 4 compares the chemical shifts of the common constituents of the three aliphatic linkers. This would be useful for identifying the chemical shifts of protons after the linker is attached to a polyamide.
Table 4: A comparison between the chemical shifts of the major constituents of L₂, L₃ and L₆.

<table>
<thead>
<tr>
<th></th>
<th>NH</th>
<th>CH₂-(NHBOC)</th>
<th>CH₂-(NH₂)</th>
<th>NH₂</th>
<th>Boc</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₂ (ppm)</td>
<td>5.16</td>
<td>3.04</td>
<td>2.66</td>
<td>1.42</td>
<td>1.39</td>
</tr>
<tr>
<td>L₃ (ppm)</td>
<td>5.16</td>
<td>3.19</td>
<td>2.76</td>
<td>1.43</td>
<td>1.37</td>
</tr>
<tr>
<td>L₆ (ppm)</td>
<td>4.58</td>
<td>3.09</td>
<td>2.66</td>
<td>1.41</td>
<td>1.41</td>
</tr>
</tbody>
</table>

2.3.5 Synthesis of (N-methyl-1H-imidazole-2-carboxamido) butyric acid (Im-L₄-COOH)

The synthesis of (N-methyl-1H-imidazole-2-carboxamido) butyric acid was carried out in two steps. The first involves the coupling of an imidazole ring to the butyrate linker and the second involves the hydrolysis of the ester group to form the acid.

The first step was carried out successfully using two different methods. In Method I, the trichloroacetyl group of Im A was utilised to form an amide bond with the amine of ethyl 4-aminobutyrate hydrochloride (Figure 2.41). TEA was used to activate the amine on the butyrate linker, while no coupling agent was required due to the highly reactive nature of the trichloroacetyl group. The product was obtained as a brown oil (98%).

![Figure 2.41: The synthesis of ethyl γ-(N-methyl-1H-imidazole-2-carboxamido)butyric acid using Im A and ethyl 4-aminobutyrate hydrochloride.](image-url)
In Method II, the butyrate linker was coupled to the carboxylic acid group of Im’ (Figure 2. 42). HBTU was used as a coupling agent while DIEA was used to activate the amine group on the butyrate linker and to deprotonate the carboxylic acid group on the imidazole ring. The mixture was purified using column chromatography (silica gel, 5% MeOH/DCM, \( R_f \) 0.58) to yield the product as a brown oil at a yield of 91%. Both methods produced the product in over 90% yields; however, Method II was preferred as it produced Im-L4-COOEt of higher purity, as determined by the \(^1\)H NMR spectra.

![Figure 2. 42: The synthesis of ethyl \( \gamma \)-(N-methyl-1H-imidazole-2-carboxamido)butyricate using Im’ and ethyl 4-aminobutyrate hydrochloride.](image)

The hydrolysis of the ester was carried out overnight at r.t. using NaOH in ethanol (Figure 2. 43). The product was acidified using concentrated HCl and obtained as a yellow solid (88%). The hydrolysis was also attempted using LiOH at 60 °C for 1.5 h; however, the desired product could not be isolated and was obtained as a brown oil consisting of unreacted starting material, as observed in the \(^1\)H NMR spectrum. A comparison between the \(^1\)H NMR spectra of the products obtained via both methods suggests that NaOH in methanol affords the product in a higher degree of purity.
In the $^1$H NMR spectrum of Im-$L_4$-COOH (Figure 2. 44) the amide proton was observed as a broad triplet at 9.23 ppm. The two aromatic hydrogens on the imidazole ring were observed as doublets at 7.68 ($H_5$) and 7.58 ($H_4$) ppm. The $N$-CH$_3$ group was assigned as the singlet at 3.99 ppm, while the peaks for the three CH$_2$ groups were observed as a quartet (8-position, 3.29 ppm), a triplet (10-position, 2.32 ppm) and a multiplet (9-position, 1.78 ppm).

**Im-$L_4$-COOH** was synthesised for use in solid phase chemistry. The molecule consists of a carboxylic acid group which can be coupled to free amines on the solid phase resin. This synthesis was undertaken because coupling imidazole rings to other heterocyclic rings/linkers had initially proven to be difficult and required coupling times in excess of 10 h. As discussed in Chapter 3, Im-$L_4$-COOH requires only 3-4 h to couple to the resin because the carboxylic acid group is on the butyrate linker and not on the imidazole ring. After several experiments, Im $E$ was successfully coupled to the resin (using solid phase chemistry) in reasonable reaction times (4-5 h), and Im-$L_4$-COOH was no longer required.
2.4 Conclusion

The Fmoc-protected pyrrole and imidazole monomers, Py E and Im E, were successfully synthesised in reasonable yields for use in solid phase chemistry. The synthesis was achieved by subjecting Py and Im to a series of reactions which included trichloroacetylation, nitration, esterification, hydrogenation/Fmoc-protection and hydrolysis using TiCl$_4$. The monomers, obtained with a protected amino group and a free carboxylic acid component, are suitable for peptide synthesis using solid phase supports. The protection of the amine was afforded by an Fmoc-protecting group, which prevents polymerisation during coupling and remains stable in acidic conditions but can be removed under basic conditions. The free acid enables the monomer to be coupled to a free amine on the resin. Once the monomer has been coupled to the peptide chain, the Fmoc-protecting group can be removed using base and the next monomer can be added, providing a simple and sequential method for peptide chain synthesis.
CHAPTER 3: SOLID PHASE CHEMISTRY

3.1 Introduction

The preparation of sequence selective polyamides was initially carried out using solution phase chemistry techniques, which were regarded as inefficient with respect to time, yield and purity. In solid phase chemistry, the polyamides can be synthesised in a higher degree of purity and yield within a shorter period of time compared to solution phase chemistry. The resin (~0.1-0.2 mm in diameter) simplifies the purification procedure by allowing any unreacted starting material to be removed in a simple washing step, thus eliminating the need for column chromatography.

Solid phase chemistry provides a simple and sequential method for the preparation of polyamides by adding one amino acid-like substrate at a time. The coupling times for each monomer were determined using the ninhydrin test, which is a rapid and sensitive method for the quantitative determination of free amino groups during solid-phase peptide synthesis.145,146

The potential of self-associating and self-complementary single stranded polyamides, that are linked to a platinum(II) centre and capable of coordinate covalent bond formation with DNA, has been previously examined.129 Higher DNA affinity and the ability to prevent DNA transcription may be imparted by using a γ-turn hairpin polyamide linked to a platinum(II) centre. The γ-turn linker allows hairpin polyamides to target selected sequences and their complementary strands.

The covalent linking of two antiparallel polyamides results in molecules with increased DNA affinity and sequence specificity.147 γ-Amino butyric acid connects the carboxylic terminus of one polyamide to the amino terminus of another. Polyamides with γ-turns have displayed ~100 fold increase in DNA affinity compared to self-associating and self-complementary single stranded polyamides. In fact, eight-ring hairpins display affinity and sequence specificity similar to DNA-binding proteins (Kd < 1 nM).147 The γ-turn linker locks the register of the ring pairings, preventing the possibility of slipped dimer formation (i.e. polyamide binding to DNA in either a 2:1 or 1:1 ligand-DNA binding mode).148 Hairpin compounds
The successful synthesis of mononuclear and dinuclear platinum(II) complexes via solid phase chemistry depends largely on the type of linker used for the coordination of the platinum(II) centre. 4-(Fmoc-amino)butyric acid (L₄) allows the formation of mononuclear complexes due to the presence of one site suitable for coordination, while 2,6-Fmoc-lysine-(Fmoc)-OH (L₆') allows the formation of dinuclear complexes (Figure 3. 1a and Figure 3. 1b). L₆' consists of two Fmoc-protected amines, which upon deprotection using piperidine, can coordinate to two platinum(II) centres. The resulting dinuclear platinum(II) complexes will contain a BBR3005-like component (a drug already shown to have cytotoxicity better than cisplatin in sensitive and cisplatin-resistant cell lines), with two trans-chloroplatinum(II) centres separated by a 1,5-diaminopentane chain (Figure 3. 1c and Figure 3. 1d). Like BBR3005, the dinuclear complexes may also be able to form flexible, long range, DNA interstrand adducts, which would be able to bypass removal by DNA repair proteins and thereby display higher cytotoxicity than cisplatin.

Telomeres are specialised structures, at the end of eukaryotic chromosomes, that modulate chromosome replication. They comprise of a G-rich repeat sequence (TTAGGG in humans). During cell division in normal cells, telomeres shorten in length due to the inability of DNA polymerase to replicate the ends of linear
In contrast, many cancerous cells show no net loss of telomere length upon cell division. Accordingly, drugs, which bind to telomere sequences in DNA or block telomerase activity, could preferentially target cancer cells.

### 3.2 Experimental

The chemicals, reagents and instruments used for the synthesis and characterisation of the molecules described in this chapter are listed in Appendix I, Section A1.1 and Section A1.2. The resins used were Fmoc-β-alanine-OH-WANG resin and Fmoc-β-alanine-chlorotrityl resin. Fmoc-β-alanine-OH-WANG resin is commercially available while Fmoc-β-alanine-chlorotrityl resin was prepared from 2-chlorochlorotrityl resin (Figure 3.2).

![Chemical structures](image)

**Figure 3.2:** The chemical structures of (a) Fmoc-β-alanine-OH-WANG resin, (b) 2-chlorochlorotrityl resin and (c) Fmoc-β-alanine-chlorotrityl resin.

#### 3.2.1 Preparation of Fmoc-β-alanine-chlorotrityl resin

2-Chloro-chlorotrityl resin (500 mg, 0.5 mmol) was suspended in anhydrous DCM (5 mL). Separately, Fmoc-β-alanine-OH (312 mg, 1.0 mmol) and DIEA (257 mg, 348 µL, 2 mmol) were stirred in anhydrous DCM (4 mL) for 5 min. The Fmoc-β-alanine-OH solution was added to the resin and the suspension shaken for 5 h. Methanol (2.5 mL) was then added and the mixture shaken for a further 30 min. The resin was filtered, washed with DCM and dried. Yield (603 mg, 0.622 mmol/g).
3.2.2 Activation of the heterocyclic monomers and linkers

3.2.2.1 Monomer/linker activation for synthesis on WANG resin

**General procedures:** Py E (0.31 mmol) was dissolved in DMF (1 mL) and 1-methyl-2-pyrrolidinone (NMP, 3 mL). HBTU (0.28 mmol) was added followed by DIEA (0.31 mL, 1.78 mmol) and the solution stirred for 10 min before it was added to the resin (0.28 mmol). The activation conditions for each monomer are described in Table 5.

<table>
<thead>
<tr>
<th></th>
<th>Mass (g)</th>
<th>Moles (mmol)</th>
<th>DMF (mL)</th>
<th>NMP (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py E</td>
<td>0.11</td>
<td>0.31</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>L₄</td>
<td>0.10</td>
<td>0.31</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Im'</td>
<td>0.04</td>
<td>0.31</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Im' is N-methyl-1H-imidazole-2-carboxylic acid

3.2.2.2 Monomer/linker activation for synthesis on chlorotrityl resin

**General procedures:** Py E (0.62 mmol) was dissolved in DMF (2 mL) and NMP (5 mL). HBTU (0.56 mmol) was added followed by DIEA (0.31 mL, 1.78 mmol) and the solution stirred for 10 min before it was added to the resin (0.28 mmol). The activation conditions for each monomer are described in Table 6.
Table 6: The activation of the monomers for use with chlorotrityl resin.

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Moles (mmol)</th>
<th>DMF (mL)</th>
<th>NMP (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Py E</td>
<td>0.23</td>
<td>0.62</td>
<td>2</td>
</tr>
<tr>
<td>Im-L₄-COOH</td>
<td>0.13</td>
<td>0.62</td>
<td>5</td>
</tr>
<tr>
<td>Im E</td>
<td>0.23</td>
<td>0.62</td>
<td>4</td>
</tr>
<tr>
<td>Im'</td>
<td>0.08</td>
<td>0.62</td>
<td>5</td>
</tr>
<tr>
<td>L₄'</td>
<td>0.27</td>
<td>0.62</td>
<td>5</td>
</tr>
<tr>
<td>L₆'</td>
<td>0.37</td>
<td>0.62</td>
<td>7</td>
</tr>
<tr>
<td>β-Ala</td>
<td>0.19</td>
<td>0.62</td>
<td>5</td>
</tr>
</tbody>
</table>

L₄' is 2-Boc-4-Fmoc-L-diaminobutyric acid
β-Ala is β-alanine

Activation of transplatin, trans-[Pt(NH₃)₂Cl₂] (2.5 eq. with respect to starting resin): AgNO₃ (0.11 g, 0.63 mmol) was added to transplatin (0.21 g, 0.70 mmol) in DMF (4.2 mL) and the solution stirred in the dark under N₂(g) for 15 h. The mixture was passed through a 0.45 µm cartridge filter to obtain trans-[Pt(NH₃)₂(NO₃)Cl]⁺ in DMF.

3.2.3 General procedures used for solid phase synthesis

The synthesis of sequence selective polyamides was performed using solid phase chemistry on a 0.28 mmol scale with respect to the starting resin. Each cycle of monomer addition involved a DCM wash (7 units), a DMF wash (7 units), deprotection with 20% piperidine/DMF (7 units) for 3 min, draining the reaction vessel, a DMF wash (7 units), deprotection for 17 min (7 units), a DMF wash (7 units), a DCM wash (7 units), a DMF wash (2 × 7 units), draining the reaction vessel, coupling for 3.5-5 h (10 h when coupling to an imidazole ring) and finally draining the reaction vessel. The activated acids (0.62 mmol) were added manually to the reaction vessel at the end of every deprotection cycle. The cycle was interrupted, reaction vessel vented, the activated acid added and the cycle was resumed. Once the polyamide was assembled, the resin was washed with DMF (2 × 7 units) and DCM (3 × 7 units) before it was dried for 1 h under N₂(g). In the case of platinum(II) polyamides, the resin was washed with DMF (2 × 7 units), brine (2 × 7 units), water (2 × 7 units), DMF (2 × 7 units) and DCM (3 × 7 units). The general methods are simplified in the flow chart shown on the following page, where each volume unit represents 1.15 mL.
I. Resin preparative: (washing and deprotecting the resin)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resin-(\beta)-Ala.NHFmoc (0.28 mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DCM wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
<tr>
<td>3</td>
<td>DMF wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
<tr>
<td>4</td>
<td>20% piperidine/DMF</td>
<td>Fill 7 units</td>
<td>mix 3 min</td>
</tr>
<tr>
<td>5</td>
<td>DMF wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
<tr>
<td>6</td>
<td>20% piperidine/DMF</td>
<td>Fill 7 units</td>
<td>mix 17 min</td>
</tr>
<tr>
<td>7</td>
<td>Begin ring/linker activation on the bench</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>DMF wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
<tr>
<td>9</td>
<td>DCM wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
<tr>
<td>10</td>
<td>DMF wash</td>
<td>Fill 7 units</td>
<td>mix 0.5 min</td>
</tr>
</tbody>
</table>

II. Coupling

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Add the activated ring to the resin</td>
<td>mix 3.5-5 h or 10 h</td>
</tr>
<tr>
<td>2</td>
<td>Drain, wash and dry the reaction vessel</td>
<td></td>
</tr>
</tbody>
</table>

III. Repeat steps I and II for the coupling of each of the remaining monomers/linkers

General procedure for cleaving the polyamides from chlorotrityl resin: Once prepared, the polyamides were cleaved from the resin by adding a solution of DCM (10 mL), trifluoroethanol (TFE, 2.4 mL) and acetic acid (1.2 mL) to the resin-polyamide and shaking gently for 1.5 h. The resin was removed by filtration and washed with TFE:DCM (1:4 v/v, 6 mL). In some cases, the polyamides were subjected to the cleaving conditions twice more. The filtrate was collected and the solvent concentrated under reduced pressure to ~ 2 mL. Cold diethyl ether (4 mL at 4 °C) was added and the mixture maintained at 4 °C for 1.5 h to induce precipitation of the product. The solvent was decanted and water (3 mL) was added to the solid before the solution was lyophilised to yield the desired polyamide.
3.2.4 **Synthesis of β-Ala-Py-L₄-Im**

### 3.2.4.1 Method (1) using WANG resin

![Resin-β-Ala-Py-L₄-Im](image)

The synthesis of **β-Ala-Py-L₄-Im** was initially performed on WANG resin using the general procedure described for solid phase chemistry with two variations. The first was the addition of dimethylsulphoxide (1 mL) at the end of every coupling step, with mixing for a further 1 h. The second was that the coupling step for each monomer/linker was performed twice before the resin was deprotected and the following monomer/linker was coupled. The coupling procedures are described in Table 7.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Add</th>
<th>Coupling time (h)</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Drain</td>
</tr>
<tr>
<td>2</td>
<td>Py E</td>
<td>3.5</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>L₄</td>
<td>3.5</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Drain</td>
</tr>
<tr>
<td>4</td>
<td>L₄</td>
<td>3.5</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>Im'</td>
<td>4</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Drain</td>
</tr>
<tr>
<td>6</td>
<td>Im'</td>
<td>4</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

**Cleaving the polyamide from WANG resin via aminolysis**

(Dimethylamino)propylamine (L₃', 3-4 mL) was added to the resin-polyamide (0.38 g) in a scintillation vial and the solution heated at 55 °C for 24 h. The resin was removed by filtration and the excess amine evaporated under reduced pressure to obtain a yellow oil. ACN (2 mL) was added to induce the precipitation of a white
solid, which was collected by filtration. The white solid could not be isolated from (dimethylamino)propylamine, which complicated characterisation by $^1$H NMR. The $^1$H NMR spectrum of the crude product did not match the predicted spectrum of the desired product (shown below).

\[
\begin{align*}
\text{L}_{3}{'}\text{-}\beta\text{-Ala-Py-L}_{4}\text{-Im}
\end{align*}
\]

**Cleaving the polyamide from WANG resin using TFA**

To a sample of resin-polyamide (0.38 g), 95% TFA/DCM (6 mL) was added and the mixture shaken gently for 2 h. The resin was removed by filtration and the solvent evaporated under reduced pressure to obtain a brown oil. Unfortunately, the $^1$H NMR spectrum of the crude product did not match the predicted spectrum of the desired product (shown below).

\[
\begin{align*}
\beta\text{-Ala-Py-L}_{4}\text{-Im}
\end{align*}
\]
3.2.4.2 Method (II) using chlorotrityl resin

The synthesis of $\beta$-Ala-Py-L$_4$-Im was performed on chlorotrityl resin using the general procedure described for solid phase chemistry, with one variation. DMSO (1 mL) was added at the end of the coupling step of Im-L$_4$-COOH and mixing for a further 1 h was required. The coupling procedures are summarised in Table 8.

Table 8: The coupling procedures used for the preparation of $\beta$-Ala-Py-L$_4$-Im using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Activity</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Add</th>
<th>Coupling time (h)</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>-</td>
<td>-</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Im-L$_4$-COOH</td>
<td>3.5</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin, using the general procedure described for chlorotrityl resin polyamide cleavage, to obtain a pale yellow powder (0.08 g, 67%). $^1$H NMR 300 MHz (d$_6$-DMSO): δ 9.74 (s, 1H), 8.41 (t, 1H, J = 5.8 Hz), 7.99 (t, 1H, J = 5.5 Hz), 7.31 (s, 1H), 7.07 (s, 1H), 6.95 (s, 1H), 6.62 (s, 1H), 3.92 (s, 3H), 3.75 (s, 3H), 3.34 (q, 2H, J = 7.0 Hz), 3.24 (q, 2H, J = 7.2 Hz), 2.42 (t, 2H, J = 7.2 Hz), 2.22 (t, 2H, J = 7.4 Hz), 1.77 (m, 2H, J = 7.1 Hz).
3.2.5 Synthesis of β-Ala-ImIm

The synthesis of β-Ala-ImIm was performed on chlorotrityl resin using the general procedure described for solid phase chemistry, with one variation. The second imidazole ring (Im') was coupled for 9 h before DMSO (1 mL) was added and the coupling was allowed to proceed for a further 1 h. The coupling procedures are summarised in Table 9.

<table>
<thead>
<tr>
<th>Step</th>
<th>Activity</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Add</th>
<th>Coupling time (h)</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Im E</td>
<td>5</td>
<td>On</td>
<td>-</td>
<td>-</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Im'</td>
<td>9</td>
<td>Off</td>
<td>DMSO</td>
<td>1</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin, using the general procedure described for chlorotrityl resin polyamide cleavage, to obtain a pale yellow powder (0.07 g, 78%).

$^1$H NMR 300 MHz (d$_6$-DMSO): δ 9.71 (s, 1H), 8.16 (t, 3H, $J = 5.9$ Hz), 7.49 (s, 1H), 7.41 (d, 1H, $J = 0.9$ Hz), 7.04 (d, 1H, $J = 0.9$ Hz), 3.98 (s, 3H), 3.94 (s, 3H), 3.42 (q, 2H, $J = 6.9$ Hz), 2.48 (q, 2H, $J = 7.0$ Hz).

3.2.6 Synthesis of $\beta$-Ala-PyPyPy-L$_4$-ImImIm

The synthesis of $\beta$-Ala-PyPyPy-L$_4$-ImImIm was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. The coupling procedures are summarised in Table 10.

Table 10: The coupling procedures used for the preparation of $\beta$-Ala-PyPyPy-L$_4$-ImImIm using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>4</td>
<td>L$_4$</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>im E</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>6</td>
<td>im E</td>
<td>9</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>7</td>
<td>Im’</td>
<td>9</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

**Cleaving the polyamide from chlorotrityl resin**

The polyamide was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out twice to obtain a pale yellow powder (0.20 g, 80%). $^1$H NMR 300 MHz (d$_6$-DMSO): $\delta$ 10.00 (s, 1H), 9.84 (s, 1H), 9.83 (s, 1H), 9.78 (s, 1H), 9.57 (s, 1H), 8.30 (t, 1H, $J = 5.8$ Hz), 7.96 (t, 1H, $J = 5.4$ Hz), 7.63 (s, 1H), 7.51 (s, 1H), 7.43 (s, 1H), 7.21 (d, 1H, $J = 1.4$ Hz), 7.17 (d, 1H, $J = 1.4$ Hz), 7.15 (d, 1H, $J = 1.4$ Hz), 7.07 (s, 1H), 7.03 (d, 1H, $J = 1.4$ Hz), 6.88 (d, 1H, $J = 1.6$ Hz), 6.85 (d, 1H, $J = 1.6$ Hz), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.39 (t, 2H, $J = 7.0$ Hz), 3.37 (q, 2H, $J = 7.3$ Hz), 2.47 (t, 2H, $J = 7.1$ Hz), 2.29 (t, 2H, $J = 7.4$ Hz), 1.82 (m, 2H, $J = 7.1$ Hz).

![β-Ala-PyPyPy-L$_4$-ImImIm](image.png)

3.2.7 *Synthesis of β-Ala-PyPyPy-L$_4$'-ImImIm*

![Resin-β-Ala-PyPyPy-L$_4$'-ImImIm](image.png)
The synthesis of $\beta$-Ala-PyPyPy-$L_4'$-ImImIm was performed on chlorotrityl resin using the general procedure described for solid phase chemistry and the same procedure as described in Chapter 3.2.6 for $\beta$-Ala-PyPyPy-$L_4$-ImImIm. The only variation was the use of $L_4'$ instead of $L_4$ to connect the triple-pyrrole polyamide to the triple-imidazole polyamide. The coupling procedures are summarised in Table 11.

Table 11: The coupling procedures used for the preparation of $\beta$-Ala-PyPyPy-$L_4'$-ImImIm using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Py</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Py</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>4</td>
<td>$L_4'$</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>Im</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>6</td>
<td>Im</td>
<td>9</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>7</td>
<td>Im'</td>
<td>9</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out twice to obtain a pale yellow powder (0.27 g, 96%). $^1$H NMR 300 MHz (d$_6$-DMSO): δ 10.07 (s, 1H), 9.87 (s, 1H), 9.85 (s, 1H), 9.79 (s, 1H), 9.58 (s, 1H), 8.33 (t, 1H, $J$ = 5.8 Hz), 7.98 (t, 1H, $J$ = 5.4 Hz), 7.65 (s, 1H), 7.54 (s, 1H), 7.47 (s, 1H), 7.23 (d, 1H, $J$ = 1.4 Hz), 7.19 (d, 1H, $J$ = 1.4 Hz), 7.17 (d, 1H, $J$ = 1.4 Hz), 7.09 (s, 1H), 7.06 (d, 1H, $J$ = 1.4 Hz), 6.92 (d, 1H, $J$ = 5.8 Hz), 6.90 (d, 1H, $J$ = 1.6 Hz), 6.82 (bd, 2H), 4.03 (s, 3H), 3.97 (s, 3H), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.40 (t, 2H, $J$ = 7.0 Hz), 3.37 (q, 2H, $J$ = 7.3 Hz), 2.48 (t, 2H, $J$ = 7.1 Hz), 2.37 (t, 2H, $J$ = 7.4 Hz), 1.85 (m, 2H, $J$ = 7.1 Hz), 1.39 (s, 9H).
3.2.8 Synthesis of β-Ala-Py-L₄-Pt

The synthesis of β-Ala-Py-L₄-Pt was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. Activated transplatin (2.5 eq.) was coupled to the resin in the presence of TEA (0.14 mL, 0.98 mmol) for 20 h in the dark. The coupling procedures are summarised in Table 12.

Table 12: The coupling procedures used for the preparation of β-Ala-Py-L₄-Pt using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>L₄</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Transplatin</td>
<td>20 (dark)</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin, using the general procedure described for chlorotrityl resin polyamide cleavage, to obtain a pale yellow powder (0.14 g, 90%).

$^1$H NMR 300 MHz (d₆-DMSO): δ 9.78 (s, 1H), 7.98 (t, 1H, J = 5.4 Hz), 7.09 (s, 1H), 6.62 (s, 1H), 5.04 (bs, 2H), 3.91 (s, 6H), 3.79 (s, 3H), 3.43 (t, 2H, J = 7.4 Hz), 2.60

(m, 2H), 2.44 (t, 2H, J = 7.6 Hz), 2.35 (t, 2H, J = 7.5 Hz), 1.92 (m, 2H).$^{195}$Pt NMR 85 MHz (d$_7$-DMF): -2415 (bs). ESI-MS calc’d for C$_{13}$H$_{26}$ClN$_6$O$_4$Pt [M – Cl]$^+$ 560.91 m/z; found 560.2 m/z. Anal. Calc. for C$_{13}$H$_{26}$ClN$_6$O$_4$Pt.H$_2$O.0.5Cl.0.5NO$_3$: C, 24.88; H, 4.50; N, 14.51%; found: C, 25.11; H, 4.40; N, 14.90%.

β-Ala-Py-L$_4$-Pt

3.2.9 Synthesis of β-Ala-Im-L$_4$-Pt

The synthesis of β-Ala-Im-L$_4$-Pt was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. Activated transplatin (2.5 eq.) was coupled to the resin in the presence of TEA (0.14 mL, 0.98 mmol) for 12 h in the dark. The coupling procedures are summarised in Table 13.

Table 13: The coupling procedures used for the preparation of β-Ala-Im-L$_4$-Pt using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Im E</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>L$_4$</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Transplatin</td>
<td>12 (dark)</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin, using the general procedure described for chlorotrityl resin polyamide cleavage, to obtain a pale yellow powder (0.14 g, 89%).$^1$H NMR 300 MHz (d$_6$-DMSO): δ 10.33 (s, 1H), 7.80 (t, 1H, J = 5.3 Hz), 7.37 (s, 1H), 7.27-7.05 (m, 5H), 5.56 (s, 1H), 4.77 (s, 2H), 4.46 (s, 2H), 4.07 (s, 2H), 3.37 (s, 2H), 2.44 (t, 2H, J = 7.6 Hz), 2.35 (t, 2H, J = 7.5 Hz), 1.92 (m, 2H).

5.42 (bs, 2H), 3.97 (s, 6H), 3.84 (s, 3H), 3.46 (t, 2H, $J = 7.4$ Hz), 2.64 (m, 2H), 2.48 (t, 2H, $J = 7.6$ Hz), 2.36 (t, 2H, $J = 7.5$ Hz), 1.92 (m, 2H). $^{195}$Pt NMR 85 MHz (d7-DMF): -2414 (bs). ESI-MS calc’d for C$_{12}$H$_{25}$ClN$_{7}$O$_{4}$Pt [M – Cl]$^+$ 561.9 m/z; found 562.2. Anal. Calc. for C$_{12}$H$_{25}$Cl$_2$N$_7$O$_4$Pt.2H$_2$O: C, 22.76; H, 4.61; N, 15.48%; found: C, 22.72; H, 4.33; N, 15.40%.

3.2.10 Synthesis of β-Ala-PyPyPy-L$_4$ImImIm-L$_4$-Pt (HLSP-6)

The synthesis of β-Ala-PyPyPy-L$_4$ImImIm-L$_4$-Pt was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. The activated transplatin (2.5 eq.) was coupled to the resin using the same procedure described in Chapter 3.2.9 for β-Ala-Im-L$_4$-Pt. The coupling procedures are summarised in Table 14.
Table 14: The coupling procedures used for the preparation of β-Ala-PyPyPy-L4-ImImIm-L4-Pt using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>4</td>
<td>L4</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>Im E</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>6</td>
<td>Im E</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>7</td>
<td>Im E</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>8</td>
<td>L4</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>9</td>
<td>Transplatín</td>
<td>12 (dark)</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

trans-Chlorodiammino-{4-(butyrylamido)amino-4-[-2-2]-2-4-2-(-3-(propanamido)-N-methyl-1H-pyrrole-2-carboxamido)-N-methyl-1H-pyrrole-2-carboxamido}-N-methyl-1H-pyrrole-2-carboxamido-butyrylamino}-N-methyl-1H-imidazole-2-carboxamido|-N-methyl-1H-imidazole-2-carboxamido)-N-methyl-1H-imidazole-2-carboxamido}]] platinum(II) nitrate (HLSP-6): The polyamide was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out twice to obtain a brown/yellow solid (0.27 g, 77%). $^1H$ NMR 300 MHz (d$_6$-DMSO): $\delta$ 10.38 (s, 1H), 9.83 (s, 1H), 9.81 (s, 1H), 9.76 (s, 1H), 9.55 (bs, 2H), 8.21 (t, 1H, $J = 5.8$ Hz), 7.96 (t, 1H, $J = 5.4$ Hz), 7.63 (s, 1H), 7.51 (s, 2H), 7.21 (d, 1H, $J = 1.4$ Hz), 7.17 (d, 1H, $J = 1.4$ Hz), 7.15 (d, 1H, $J = 1.4$ Hz), 7.01 (d, 1H, $J = 1.4$ Hz), 6.86 (d, 1H, $J = 1.4$ Hz), 6.83 (d, 1H, $J = 1.6$ Hz), 5.18 (t, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.33 (m, 6H), 2.47 (t, 2H, $J = 7.1$ Hz), 2.38 (t, 2H, $J = 7.5$ Hz), 2.29 (t, 2H, $J = 7.4$ Hz), 1.82 (m, 4H, $J = 7.1$ Hz). $^{195}$Pt NMR 85 MHz (d$_7$-DMF): -2420 (bs). ESI-MS calc'd for C$_{44}$H$_{60}$ClN$_{20}$O$_{10}$Pt [M – Cl]$^+$ 1259.4 m/z found 1259.4 m/z. Anal. Calc. for C$_{44}$H$_{60}$Cl$_2$N$_{20}$O$_{10}$Pt.H$_2$O.2.5HCl: C, 37.18; H, 4.46; N, 20.17%; found: C, 37.04; H, 4.61; N, 20.18%.
3.2.11 Synthesis of $\beta$-Ala-PyPyPy-$L_4$-ImImIm-$L_6'$.Pt-(Pt) (DNHLSP-6)

The synthesis of $\beta$-Ala-PyPyPy-$L_4$-ImImIm-$L_6'$.Pt-(Pt) was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. Activated transplatin (5 eq.) was coupled to the resin in the presence of TEA (0.28 mL, 1.96 mmol) for 15 h in the dark. The coupling procedures are summarised in Table 15.

Table 15: The coupling procedures used for the preparation of β-Ala-PyPyPy-L₄-ImImIm-L₆'-Pt-(Pt) using chlorotrityl resin.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
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<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>4</td>
<td>L₄</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>im E</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>6</td>
<td>im E</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>7</td>
<td>im E</td>
<td>10</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
<tr>
<td>8</td>
<td>L₆'</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>9</td>
<td>Transplatin</td>
<td>15 (dark)</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>

Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out twice to obtain a brown/yellow solid (0.27 g, 77%). ¹H NMR 300 MHz (d₆-DMSO): δ 10.80 (s, 1H), 9.83 (s, 1H), 9.81 (s, 1H), 9.76 (s, 1H), 9.66 (s, 1H), 9.54 (s, 1H), 8.21 (t, 1H, J = 5.8 Hz), 7.96 (t, 1H, J = 5.4 Hz), 7.64 (s, 1H), 7.62 (s,1H), 7.51 (s, 2H), 7.21 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.15 (d, 1H, J = 1.4 Hz), 7.01 (d, 1H, J = 1.4 Hz), 6.85 (d, 1H, J = 1.4 Hz), 6.83 (d, 1H, J = 1.5 Hz), 5.79 (t, 2H), 5.62 (t, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.33 (m, 6H), 2.47 (t, 2H, J = 7.1 Hz), 2.38 (t, 2H, J = 7.5 Hz), 2.29 (t, 2H, J = 7.4 Hz), 1.82 (m, 4H, J = 7.1 Hz), 1.58 (bm, 2H). ¹⁹⁵Pt NMR 85 MHz (d₇-DMF): -2424 (bs). ESI-MS calc’d for C₄₆H₇₁Cl₂N₂₃O₁₀Pt₂ [M – 2Cl⁺]²⁺ 784.4 m/z; found 783.7 m/z, [M – H⁺ – 2Cl⁺]²⁺ 1566.4 m/z; found 1566.2 m/z. Anal. Calc. for C₄₆H₇₁Cl₂N₂₃O₁₀Pt₂.5H₂O: C, 31.97; H, 4.72; N, 18.64%; found: C, 31.65; H, 4.65; N, 18.97%.
3.2.12 Synthesis of $\beta$-Ala-PyPyImImIm-L$_4$'-PyPyPyPyPy-$\beta$-Ala-L$_6$'-Pt-(Pt) ($\text{DNHLSP-10}$)

The synthesis of $\beta$-Ala-PyPyImImIm-L$_4$'-PyPyPyPyPy-$\beta$-Ala-L$_6$'-Pt-(Pt) was performed on chlorotrityl resin using the general procedure described for solid phase chemistry. Activated transplatin (5 eq.) was coupled to the resin using the same procedure described in Chapter 3.2.11 for $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6$'-Pt-(Pt). The coupling procedures are summarised in Table 16.

<table>
<thead>
<tr>
<th>Step</th>
<th>Monomer</th>
<th>Coupling time (h)</th>
<th>Drain</th>
<th>Final activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>2</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>3</td>
<td>Im E</td>
<td>5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>4</td>
<td>Im E</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>5</td>
<td>Im E</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>6</td>
<td>L$_4$'</td>
<td>10</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>7</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>8</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>9</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>10</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>11</td>
<td>Py E</td>
<td>3.5</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>12</td>
<td>$\beta$-Ala</td>
<td>4</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>13</td>
<td>L$_6$'</td>
<td>4</td>
<td>On</td>
<td>Deprotect</td>
</tr>
<tr>
<td>14</td>
<td>Transplatin</td>
<td>15 (dark)</td>
<td>On</td>
<td>Wash/dry</td>
</tr>
</tbody>
</table>
Cleaving the polyamide from chlorotrityl resin

The polyamide was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out three times to obtain a brown yellow/solid (0.31 g, 48%). $^1$H NMR 300 MHz (d$_6$-DMSO): δ 10.32 (s, 1H), 10.24 (s, 1H), 9.94 (s, 1H), 9.87 (bs, 5H), 9.72 (s, 1H), 9.58 (s, 1H), 8.57 (t, 1H, $J = 5.6$ Hz), 7.82-8.02 (bt, 2H), 7.64 (s, 1H), 7.56 (s, 1H), 7.52 (s, 2H), 7.00-7.26 (bs, 11H), 6.88 (s, 2H), 6.86 (d, 1H), 5.79 (bt, 2H), 5.62 (bt, 2H), 3.71-4.12 (10s, 30H), 3.10-3.50 (bm, 6H), 2.56 (bm, 4H), 2.45 (bm, 3H), 1.80 (bm, 2H), 1.58 (bm, 5H), 1.28 (bm, 2H), 1.26 (s, 9H). $^{195}$Pt NMR 85 MHz (d$_7$-DMF): -2424 (bs), -2426 (sh). ESI-MS calc'd for C$_{78}$H$_{109}$Cl$_2$N$_{33}$O$_{17}$Pt$_2$ [M – 2Cl]$^{2+}$ 1121.0 m/z; found 1120.9 m/z, [M – H$^+$ – 2Cl]$^+$ 2241.9 m/z; found 2241.6 m/z. Anal. Calc. for C$_{78}$H$_{109}$Cl$_4$N$_{33}$O$_{19}$Pt$_2$.13H$_2$O: C, 36.78; H, 5.34; N, 18.15%; found: C, 36.97; H, 5.20; N, 17.84%.

β-Ala-PyPyImImIm-L$_4'$-PyPyPyPyPyPy-β-Ala-L$_6'$-Pt-(Pt)

3.3 Discussion

Prior to solid phase chemistry, the synthesis of polyamides was carried out using solution phase chemistry techniques, which proved to be effective but inefficient in terms of time and yield (<5%). The negligible yields and lengthy time frames were a consequence of the multiple steps required to synthesise and purify the polyamides. Solid phase chemistry produces the polyamides in a shorter time with higher yields.
and purity. This is possible because, in solid phase chemistry, the polyamides are attached to a resin; making column chromatography after each coupling step unnecessary. Solid phase chemistry is also automated to deliver the required reagents.

The solid phase methods employed in this work were initially adapted from those described by Dervan et al.\textsuperscript{135} Despite multiple attempts utilising Fmoc-\(\beta\)-alanine-OH-WANG resin, the synthetic methods were unsuccessful. It is hypothesised that the free aromatic amines on the heterocyclic rings interact with the WANG resin,\textsuperscript{160} although characterisation of the intermediates was not attempted. Successful synthesis of the desired polyamides was instead achieved using Fmoc-\(\beta\)-alanine-chlorotrityl resin, which yielded the products in high yields.

In solid phase chemistry, the resin required washing with DMF and DCM prior to the synthesis of the desired molecules in order to swell and increase its reaction-surface area. The Fmoc-protecting group, utilised by the monomers, is base labile and was removed using piperidine\textsuperscript{\textnormal{a}} to obtain the free amine. The monomers also consist of a carboxylic acid group which was activated using HBTU and then coupled to the free amine on the resin. The coupling time required was dependant on the two monomers to be coupled. DMSO was also used to help solubilise the HBTU-activated acids. Whilst it was possible to automatically add the activated acids, they were added manually to the reaction vessel to ensure stability through fresh preparation. Activated transplatin was coupled to the polyamides in the presence of TEA for 12-20 h in the dark. After which, the resin was washed with brine, to avoid having mixed counter ions, and then with DMF and DCM.

The polyamide synthesis was based on the formation of amide bonds between the free amine of one molecule and the activated acid of another. The mechanism for amide bond formation is described in Scheme 2. The base (DIEA) deprotonates the acid, allowing it to attack the carbocation of the activating molecule (HBTU) to create a good leaving group. The amine then attacks the acid to form an amide bond.

\textsuperscript{a} Piperidine is a mild base.
Scheme 2: The proposed mechanism for amide bond formation via activation using HBTU.

\[
\begin{align*}
R\text{-}O\text{H} & \xrightarrow{\text{DIEA, HBTU}} R\text{-}C\text{O} \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
& \quad \text{N(CH}_3\text{)}_2 \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R'\text{-NH}_2 + R\text{-C}\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}O\text{H} & \xrightarrow{\text{DIEA, HBTU}} R\text{-}C\text{O} \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
& \quad \text{N(CH}_3\text{)}_2 \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]

\[
\begin{align*}
R\text{-}C\text{O} & \xrightarrow{\text{N(CH}_3\text{)}_2} R\text{-}N\text{(CH}_3\text{)}_2 \\
& \quad \text{N(CH}_3\text{)}_2 \\
& \quad \text{(PF}_6\text{)}^- \\
& \quad \text{HBTU} \\
\end{align*}
\]
3.3.1 Preparation of Fmoc-β-alanine-chlorotrityl resin

Fmoc-β-alanine-chlorotrityl resin was prepared from 2-chloro-chlorotrityl resin and Fmoc-β-alanine-OH in the presence of DIEA (Figure 3.3). After stirring the mixture for 5 h, methanol was added to cap and block any unreacted resin sites. The loading of Fmoc-β-alanine-chlorotrityl resin was determined using the method described in Appendix II, Section A2.6.

![Figure 3.3: The preparation of Fmoc-β-alanine-chlorotrityl resin.](image)

3.3.2 Synthesis of β-Ala-Py-L₄-Im and β-Ala-ImIm

β-Ala-Py-L₄-Im and β-Ala-ImIm (Figure 3.4) were synthesised in order to determine the optimal conditions for the coupling of pyrrole and imidazole monomers using solid phase chemistry.

![Figure 3.4: The chemical structures of (a) β-Ala-Py-L₄-Im and (b) β-Ala-ImIm.](image)

The synthesis of β-Ala-Py-L₄-Im was initially attempted using Fmoc-β-alanine-OH-WANG resin as described in Chapter 3.2.4.1. The heterocyclic rings and L₄ were each coupled for 4.5-5 h, during which DMSO was used to help solubilise the activated acids. Acetic anhydride was also added after each cycle to help cap the unreacted sites on the resin beads. The polyanide was then subjected to the cleaving

procedures using (dimethylamino)propylamine, which affords the product with a tert-amine group, or TFA, which affords the product with a carboxylic acid group. In both cases, the product could not be isolated due to the possible interaction between the heterocyclic rings and the WANG resin. The synthesis of $\beta$-Ala-Py-L$_4$-Im was then attempted using an alternate resin, Fmoc-$\beta$-alanine-chlorotrityl resin. The reaction coupling times for the addition of the monomers/linkers were determined using the ninhydrin test (Appendix II, Section A2.4). The results indicate that the coupling of Py E and Im-L$_4$-COOH was complete within 3.5-4.5 h. DMSO was also required to enhance the solubility of Im-L$_4$-COOH. The polyamide was cleaved from the resin using acetic acid and TFE before it was lyophilised to obtain a yellow powder (67%).

In the $^1$H NMR spectrum of $\beta$-Ala-Py-L$_4$-Im (Figure 3.5) the three amide protons were observed as a singlet and two triplets at 9.74 (s), 8.41 (t) and 7.99 (t) ppm. The four aromatic hydrogens were observed as singlets at 7.31, 7.07, 6.95 and 6.62 ppm. The two singlets at 3.92 and 3.75 ppm were assigned as the two $N$-CH$_3$ groups. The aliphatic peaks, which correspond to the five CH$_2$ groups, were observed between 1.77 and 3.34 ppm.

![Figure 3.5: The $^1$H NMR spectrum of $\beta$-Ala-Py-L$_4$-Im (Figure 3.4 a) in d$_6$-DMSO at 35°C.](image)

The synthesis of $\beta$-Ala-ImIm was carried out to determine the coupling time for the addition of a heterocyclic ring onto an imidazole ring. The addition of the first ring
was carried out successfully within 4 h; however, the second ring required up to 9-10 h (monitored using the ninhydrin test)\cite{160}. As expected the coupling of a 5-membered heterocyclic ring to an imidazole ring required a longer reaction time. The product was cleaved using acetic acid and TFA before it was lyophilised to obtain a yellow powder (78%).

In the $^1$H NMR spectrum of $\beta$-Ala-ImIm (Figure 3.6) the two amide protons were observed as a singlet and a triplet at 9.71 and 8.16 ppm respectively. The three aromatic hydrogens were observed as three singlets at 7.49, 7.41 and 7.04 ppm. The two singlets at 3.98 and 3.94 ppm were assigned as the two $N$-CH$_3$ groups. The aliphatic peaks for the two CH$_2$ groups were observed as two quartets at 3.42 and 2.48 ppm.

Figure 3.6: The $^1$H NMR spectrum of $\beta$-Ala-ImIm (Figure 3.4b) in d$_6$-DMSO at 35°C.

### 3.3.3 Synthesis of $\beta$-Ala-PyPyPy-L$_4$-ImImIm and $\beta$-Ala-PyPyPy-L$_4'$-ImImIm

$\beta$-Ala-PyPyPy-L$_4$-ImImIm (Figure 3.7) is a sequence selective polyamide that consists of a $\beta$-alanine residue, three pyrrole rings and three imidazole rings connected via a $\gamma$-aminobutyric acid linker. The molecule was designed to bind across five bases in length comprising (A/T)GGG(A/T) sequences.
Figure 3. 7: The chemical structure of $\beta$-Ala-PyPyPy-L$_4$-ImImIm, which is selective for (A/T)GGG(A/T) sequences.

$\beta$-Ala-PyPyPy-L$_4$-ImImIm was prepared on chlorotrityl resin using solid phase chemistry. The three pyrrole rings and L$_4$ were coupled for 3.5 h each. The first imidazole ring was coupled over a period of 4 h, while the second and third imidazole rings were allowed 9-10 h each. The polyamide was cleaved off the resin using acetic acid and TFE to afford the product as a yellow powder (80%).

In the $^1$H NMR spectrum of $\beta$-Ala-PyPyPy-L$_4$-ImImIm (Figure 3. 8) the five singlets between 9.57 and 10.00 ppm were assigned as five amide protons. The remaining two amide protons were observed as triplets at 8.30 and 7.96 ppm. The ten aromatic protons were observed as four singlets and six doublets between 6.85 and 7.63 ppm. The six $N$-CH$_3$ groups were observed as six singlets between 3.80 and 4.03 ppm. The five CH$_2$ groups were observed as triplets and multiplets in the aliphatic region between 1.82 and 3.39 ppm.
β-Ala-PyPyPy-L₄-ImImIm was prepared as a “proof of concept”. The attachment of the platinum(II) centre to the carboxylic acid terminal may be achieved through solution phase chemistry. This will be later discussed in Chapter 4.

The synthesis of β-Ala-PyPyPy-L₄'-ImImIm (Figure 3. 9) was achieved on chlorotrityl resin using a similar procedure to that described for the synthesis of β-Ala-PyPyPy-L₄-ImImIm. The only difference was the use of 2-Boc-4-Fmoc-L-diaminobutyric acid linker (L₄') instead of 4-(Fmoc-amino)butyric acid linker (L₄). L₄', which includes a Boc-protected amine, provides a site for the coordination of a platinum(II) centre upon the deprotection of the amine under acidic conditions. β-Ala-PyPyPy-L₄'-ImImIm therefore offers two sites where a platinum(II) centre can be coupled or coordinated; the carboxylic acid terminal and the amine terminal. The product was obtained as a yellow powder (96%). β-Ala-PyPyPy-L₄'-ImImIm was designed to bind across five bases and comprise (A/T)GGG(A/T) sequences.
Figure 3. 9: The chemical structure of $\beta$-Ala-PyPyPy-L$_4$-$'^{'}$-ImImIm.

The $^1$H NMR spectrum of $\beta$-Ala-PyPyPy-L$_4$-$'^{'}$-ImImIm was similar to that of $\beta$-Ala-PyPyPy-L$_4$-ImImIm. The protons on each compound that are assumed to be equivalent were observed to resonate within 0.14 ppm of each other. The major difference was the appearance of a large singlet at 1.39 ppm, which corresponds to the protons of the Boc group, and a broad doublet at 6.82 ppm, which corresponds to the Boc-protected amine (Table 17).

Table 17: A comparison between the $^1$H NMR chemical shifts of A ($\beta$-Ala-PyPyPy-L$_4$-ImImIm) and B ($\beta$-Ala-PyPyPy-L$_4$-$'^{'}$-ImImIm).

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<td>7.23</td>
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3.3.4 **Synthesis of $\beta$-Ala-Py-L$_4$-Pt and $\beta$-Ala-Im-L$_4$-Pt**

$\beta$-Ala-Py-L$_4$-Pt and $\beta$-Ala-Im-L$_4$-Pt (Figure 3. 10) were prepared on chlorotrityl resin via solid phase chemistry to determine the optimal conditions for the coordination of transplatin with polyamides. Prior to coordination, transplatin was activated with 0.9 eq. of silver nitrate, where one chloride is replaced with a nitro group, thus making the platinum(II) centre more receptive to coordination.
Activated transplatin (trans-[Pt(NH$_3$)$_2$(NO$_3$)Cl]$^+$, 2.5 eq.) was reacted with resin-$\beta$-Ala-Py-L$_4$-NH$_2$ in the presence of TEA (3.5 eq.) over a period of 20 h. In this initial experiment the resin was not subjected to a brine wash and the polyamide was cleaved from the resin using TFE and acetic acid. The product was obtained as a pale yellow powder (90%) with a single $^{195}$Pt NMR resonance observed at -2415 ppm (Appendix IV, Section A4.2.1). This chemical shift is consistent with similar metal complexes with PtN$_3$Cl coordination spheres, where N is two trans-ammine ligands and one primary amine ligand.$^{162,163}$

In the $^1$H NMR spectrum of $\beta$-Ala-Py-L$_4$-Pt (Figure 3.11) the two amide protons were observed as a singlet (NH$_b$) at 9.78 ppm and a triplet (NH$_a$) at 7.98 ppm. The two aromatic protons were observed as singlets at 7.09 (H$_5$) and 6.62 ppm (H$_3$). The broad peak at 5.04 ppm was assigned as the NH$_2$ directly coordinated to the platinum(II) centre, while the singlet at 3.91 ppm integrates to 6H and was assigned as the two platinum(II)-bound NH$_3$ groups. The large singlet at 3.79 ppm was assigned as the N-CH$_3$ group, while the five CH$_2$ groups were observed in the aliphatic region between 1.92 and 3.43 ppm as multiplets and triplets.

In the ESI mass spectrum of $\beta$-Ala-Py-L$_4$-Pt (Appendix IV, Section A4.5.1) the most prominent ion peak occurs at m/z 560.2, which is consistent with the theoretical pattern predicted for the metal complex. Elemental analysis of $\beta$-Ala-Py-L$_4$-Pt shows the presence of one water molecule along with half an atom of chlorine and half a molecule of NO$_3$, which denotes mixed counter ions. The presence of mixed counter
ions could be eliminated by subjecting the resin-polyamide complex to a brine wash prior to cleavage. However, further purification was not attempted as the molecule was synthesised for the purpose of method development.

Figure 3.11: The $^1$H NMR spectrum of $\beta$-Ala-Py-L$_4$-Pt (Figure 3.10a) in d$_6$-DMSO at 35°C.

Activated transplatin was then added to resin-$\beta$-Ala-Im-L$_4$-NH$_2$ and reacted using the same procedure described for the pyrrole monomer. The $^{195}$Pt NMR spectrum revealed the presence of two peaks at -2414 and -2436 ppm corresponding to 89% and 11% of the reaction products respectively. The major peak corresponds to the desired product, while the minor peak is thought to correspond to a complex in which the platinum(II) group was coordinated to the imidazole through the aromatic nitrogen (Figure 3.12). From these results, it appears that the coordination of platinum(II) to amine groups is more favourable than coordination to the imidazole nitrogen. In the second experiment, the activated transplatin was reacted with resin-$\beta$-Ala-Im-L$_4$-NH$_2$ over 12 h instead of 20 h and the resin-polyamide complex was then washed with brine and water to prevent the formation of mixed counter ions. The product was obtained as a pale yellow powder (89%) with a single $^{195}$Pt NMR resonance observed at -2414 ppm (Appendix IV, Section A4.2.2), a chemical shift consistent with similar metal complexes with PtN$_3$Cl coordination spheres.$^{162,163}$

Figure 3.12: The nitrogen on the imidazole ring provides a site for the coordination of activated transplatin.
In the $^1\text{H}$ NMR spectrum of $\beta$-Ala-Im-L$_4$-Pt (Figure 3.13) the two amide protons were observed as a singlet ($\text{NH}_b$) at 10.33 ppm and a triplet ($\text{NH}_a$) at 7.80 ppm. The aromatic proton ($\text{H}_5$) was observed as a singlet at 7.37 ppm. The broad peak at 5.42 ppm was assigned as the NH$_2$ directly coordinated to the platinum(II) centre, while the singlet at 3.97 ppm integrates to six protons and was assigned as the two platinum(II)-bound NH$_3$ groups. The large singlet at 3.84 ppm was assigned as the $N$-CH$_3$ group, while the five CH$_2$ groups were observed in the aliphatic region between 1.92 and 3.46 ppm as multiplets and triplets.

In the ESI mass spectrum of $\beta$-Ala-Im-L$_4$-Pt (Appendix IV, Section A4.5.2) the most prominent ion peak occurs at $m/z$ 562.2, which is consistent with the theoretical pattern predicted for the metal complex. Elemental analysis of $\beta$-Ala-Py-L$_4$-Pt indicates the presence of two water molecules and chloride counter ions. NO$_3$ counter ions were not observed as a consequence of the brine wash.

Figure 3.13: The $^1\text{H}$ NMR spectrum of $\beta$-Ala-Im-L$_4$-Pt (Figure 3.10b) in d$_6$-DMSO at 35°C.

### 3.3.5 Synthesis of $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_4$-Pt (HLSP-6)

HLSP-6 is a hairpin polyamide platinum(II) complex that contains a $\beta$-alanine residue (selective for A or T DNA bases), three imidazole and three pyrrole rings (selective for GGG DNA sequences) and a $\gamma$-aminobutyric acid turn (selective for A or T DNA bases) (Figure 3.14). This compound was therefore designed to bind selectively to regions of DNA that are five bases in length comprising (A/T)GGG(A/T) sequences.
HLSP-6 consists of two terminal linking groups; a β-alanine on one end of the hairpin across from a γ-aminobutyric acid on the opposing end. It is not known if the presence of the γ-aminobutyric acid will affect the normal β-alanine selectivity for A/T DNA bases. If so, a longer linker such as γ-aminohexanoic acid or γ-aminoctanoic acid may be employed in the future to link the platinum(II) centre to the hairpin.

![Chemical structure of HLSP-6](image)

**Figure 3.14**: The chemical structure of HLSP-6, which is selective for (A/T)GGG(A/T) sequences.

HLSP-6 was the first Pt(II) sequence selective hairpin polyamide synthesised using solid phase chemistry. The synthesis of HLSP-6 was achieved through standard Fmoc deprotection and coupling through the activated acids (Scheme 3). The organic component of HLSP-6 was prepared on chlorotrityl resin using a similar procedure to that described in Chapter 3.2.3. Platination of the organic molecule was initially carried out with activated transplatin (1.1 eq.) in the presence of TEA for 20 h. It is believed that the reaction did not go to completion; leaving a considerable quantity of starting material (with the free amine) as the main impurity. A second attempt in which activated transplatin (2.5 eq.) was mixed with the resin in the presence of TEA for 12 h yielded the desired product in a high degree of purity. The product was cleaved from the resin using acetic acid and TFE to obtain a brown yellow solid (77%). Scheme 3 illustrates the synthesis of HLSP-6 and is shown as an example of how all polyamides were assembled in a stepwise fashion using solid phase chemistry.
Scheme 3: The solid phase synthesis of HLSP-6. Each step (except the addition of the platinum(II) centre) involves the removal of the Fmoc-protecting group using 20% piperidine/DMF followed by coupling of the activated ring or linker.
In the $^1$H NMR spectrum of **HLSP-6** (Figure 3.15) the five resonances between 9.4 and 10.4 ppm integrate for six protons and were assigned as six amide protons. The two broad triplets at 8.21 and 7.96 ppm were assigned as the two amide protons of the $\gamma$-aminobutyric acid and $\beta$-alanine residues. The nine singlets between 6.6 and 7.6 ppm were assigned as the nine aromatic protons of the heterocyclic rings. The broad resonance at 5.18 ppm was assigned as the NH$_2$ directly coordinated to the platinum(II) centre. This chemical shift is $\sim$ 0.6 ppm downfield of where the resonance was observed in the absence of a platinum(II) centre. The six singlets between 3.6 and 4.2 ppm were assigned as the $N$-CH$_3$ groups. The resonances between 1.6 and 3.6 ppm, which integrate for 16 protons, were assigned as the CH$_2$ protons of the $\gamma$-aminobutyric acid and $\beta$-alanine residues. Unfortunately, a lack of proton connectivity between the heterocyclic rings excludes assignment of the individual proton resonances by NOESY or g-DQCOSY.

![Figure 3.15: The $^1$H NMR spectrum of HLSP-6 (Figure 3.14) in d$_6$-DMSO at 35°C.](image)

In the $^{195}$Pt NMR spectrum of **HLSP-6** (Appendix IV, Section A4.2.3) a single platinum(II) resonance was observed at -2420 ppm. This chemical shift is consistent with similar metal complexes with PtN$_3$Cl coordination spheres.$^{162,163}$
HLSP-6 has displayed poor solubility in aqueous solutions; as a result DMSO was used to help solubilise the metal complex for the purposes of mass spectrometry. In the ESI mass spectrum of HLSP-6 (Appendix IV, Section A4.5.3), dissolved in 1% DMSO in 50% water/ACN, the most prominent ion peak occurs at \( m/z \) 1259.4, which corresponds to the metal complex with a 1+ charge. The isotopic pattern of this peak matches the theoretical pattern predicted for HLSP-6. Fragmentation ions were also observed. The peak at \( m/z \) 602.8 is consistent with the parent ion with a 2+ charge, resulting from the loss of the coordinated chloride and one ammine group. The peak at \( m/z \) 649.2 is consistent with the parent ion with a 2+ charge, in which the coordinated chloride was displaced by DMSO. The peak at \( m/z \) 995.4 is consistent with the parent ion minus the trans-chlorodiamine platinum(II) centre. As seen in the \(^1\)H NMR spectrum, the metal complex shows degradation after a short period of time (\( \sim 30 \) min) as DMSO displaces the coordinated chlorides. After incubating HLSP-6 for five days in DMSO, the ESI mass spectrum of the metal complex shows a 95% decrease in the intensity of the parent ion peak at \( m/z \) 1259.4. However, the peak at \( m/z \) 995.4, which corresponds to the parent ion minus the trans-chlorodiamine platinum(II) centre, remains at the same intensity. Three other peaks were also observed at \( m/z \) 595.2 (parent ion with a 2+ charge, resulting from the loss of the coordinated chloride and both ammine groups), 603.3 (parent ion with a 2+ charge, resulting from the loss of the coordinated chloride and one ammine group) and 611.7 (parent ion with a 2+ charge, resulting from the loss of the coordinated chloride). Elemental analysis of HLSP-6 supports the results observed in the mass spectrum and shows that the solid produced by lyophilisation contains one water of crystallisation along with 2.5 molecules of residual HCl.

The successful synthesis of HLSP-6 paved the way for the development of hairpin polyamides with multiple platinum(II) centres. Combining two or more platinum(II) centres with a hairpin polyamide was expected to improve solubility (by increasing the net charge of the molecule) and cytoxicity, whilst maintaining DNA sequence selectivity. True DNA binding specificity to mutant genes or telomere regions, however, will only be achieved when the polyamides are designed to recognise DNA sequences of 10-15 base pairs.\(^{164}\) Linking two 6-ring polyamide hairpins will create a molecule that will selectively bind 8-10 DNA base pairs. Additionally, it is generally acknowledged that for high cytoxicity, compounds require either one platinum(II)
centre with two DNA coordination sites or two platinum(II) centres with at least one DNA coordination site each.\textsuperscript{152,165} Attachment of two transplatin groups to the hairpin polyamides will produce multinuclear complexes that should be effective in preventing DNA transcription and replication.

### 3.3.6 Synthesis of $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6$'-Pt-(Pt) (DNHLSP-6) and $\beta$-Ala-PyPyImImIm-L$_4$'-PyPyPyPyPy-\(\beta\)-Ala-L$_6$'-Pt-(Pt) (DNHLSP-10)

DNHLSP-6 (Figure 3. 16) was synthesised to determine whether a dinuclear platinum(II) DNA sequence selective polyamide would demonstrate increased solubility and higher cytotoxicity compared to the mononuclear platinum(II) complex (HLSP-6). In this case, DNHLSP-6 contains a BBR3005-like component (Figure 3. 16). It is expected that like BBR3005, DNHLSP-6 would also form flexible and long range DNA interstrand adducts.\textsuperscript{152,165}

![Figure 3. 16: The chemical structure of the dinuclear complex, DNHLSP-6, which is selective for (A/T)GGG(A/T) sequences. The BBR3005-like component is shown in red.](image)

The synthesis of DNHLSP-6 was accomplished on chlorotrityl resin by the sequential coupling of $\beta$-alanine, pyrrole, imidazole and $\gamma$-aminobutyric acid residues, using the general procedure described for solid phase synthesis.\textsuperscript{160} The major synthetic difference to HLSP-6 was the use of 2,6-Fmoc-Lysine-(Fmoc)-OH linker ($L_4'$), which provides two sites for coordination, instead of 4-(Fmoc-amino)butyric acid ($L_4$). As a result, the coordination of transplatin to the polyamide was allowed to proceed for 15 h to ensure the successful coordination at both sites. The product was
obtained as a brown/yellow solid (77%). The molecule spans five bases in length and targets (A/T)GGG(A/T) sequences.

In the $^1$H NMR spectrum of DNHLSP-6 (Figure 3.17) six amide singlets were observed between 9.54 and 10.80 ppm. The two triplets at 8.21 and 7.96 ppm were assigned as the two amide protons on the aliphatic chains. The nine aromatic hydrogens were observed as three singlets and six doublets between 6.83 and 7.64 ppm. The two NH$_2$ groups directly coordinated to the platinum(II) centres were observed as two triplets at 5.79 and 5.62 ppm. The six N-CH$_3$ groups were observed as six singlets between 3.80 and 4.03 ppm. The four platinum(II)-bound NH$_3$ groups resonate at the same frequency as the six N-CH$_3$ groups and therefore could not be observed. The proton of the carboxylic acid is thought to exchange with the water present in solution and thus could not be individually observed. The remaining groups, which consist of nine CH$_2$ and a CH, were observed as triplets and multiplets between 1.82 and 3.50 ppm.

![Figure 3.17: The $^1$H NMR spectrum of DNHLSP-6 (Figure 3.16) in d$_6$-DMSO at 35°C. The impurities, which include acetic acid and ether, are denoted with a red arrow.](image)

In the $^{195}$Pt NMR spectrum of DNHLSP-6 (Appendix IV, Section A4.2.4) a single platinum(II) resonance was observed at -2424 ppm with a shoulder at a higher frequency (~ -2420 ppm), indicating there are two chemically similar, but separate,
platinum(II) groups. The two platinum(II) signals resonate at an almost identical chemical shift to that of similar metal complexes with PtN₃Cl coordination spheres.¹⁶⁰,¹⁶⁶

DNHLSP-6 has displayed poor solubility in aqueous mediums (soluble only in DMF and DMSO). In the ESI mass spectrum of DNHLSP-6 (Appendix IV, Section A4.5.4), dissolved in 1% DMSO in 50% water/ACN, the two most prominent ion peaks occur at \( m/z \) 783.7 and 1566.2. The peak at \( m/z \) 783.7 corresponds to the parent metal complex with a 2+ charge. The peak at \( m/z \) 1566.2 corresponds to the deprotonated metal complex (from the carboxylic acid terminal) with a 1+ charge. Fragmentation ions were also observed. The peak at \( m/z \) 1303.1 was assigned as the parent ion with a 1+ charge, resulting from the loss of one trans-chlorodiamine platinum(II) centre. Elemental analysis shows that the solid produced by lyophilisation contains five waters of crystallisation.

DNHLSP-10 (Figure 3. 18) comprises of β-alanine residues (selective for A or T DNA bases), pyrrole rings (selective for A or T DNA bases), three imidazole rings (selective for GGG DNA sequences) and a γ-aminobutyric acid turn (selective for A or T DNA bases). This compound is therefore expected to bind selectively to regions of DNA that are seven bases in length and comprise (A/T)(A/T)(A/T)GGG(A/T) sequences. Out of the sixteen different sequence combinations, the most relevant is TTAGGGT, which is a highly repeated sequence in the telomeres of DNA.

![Figure 3. 18: The chemical structure of the dinuclear complex, DNHLSP-10. The BBR3005-like component is shown in red.](image-url)
DNHLSP-10 contains a BBR3005-like component with two \textit{trans}-chloroplatinum(II) groups separated by a 1,5-diaminopentane chain. Like BBR3005, DNHLSP-10 may also be able to form flexible and long range DNA interstrand adducts, which would be able to bypass removal by DNA repair proteins and thereby display higher cytotoxicity than cisplatin.\textsuperscript{152,165}

The synthesis of DNHLSP-10 was achieved in a moderate yield (48.0\%) on chlorotrityl resin using solid phase chemistry. This is a large decrease compared HLSP-6 (77\%) and DNHLSP-6 (77\%), that were synthesised using the same procedure.\textsuperscript{151,160} It is possible that the yield for DNHLSP-10 may be increased by repeating the cleaving procedures. Based on HLSP-6, DNHLSP-6 and DNHLSP-10, it was observed that the higher the molecular weight of the platinum(II) complex, the more cleaving procedures were required to obtain a higher yield.

In the \textsuperscript{1}H NMR spectrum of DNHLSP-10 (Figure 3. 19) ten amide protons were observed as singlets between 9.58 and 10.32 ppm, while the remaining three amide protons were observed as broad triplets between 7.82 and 8.57 ppm. The seventeen aromatic hydrogens of the heterocyclic rings were observed as multiplets and overlapping singlets between 6.88 and 7.64 ppm. The Boc-protected amine was observed as a doublet at 6.86 ppm. The broad resonances at 5.79 and 5.62 ppm were assigned as the two NH\textsubscript{2} groups directly coordinated to the platinum(II) centre. This chemical shift is \textasciitilde 1 ppm downfield of where the resonance was observed in the absence of a platinum(II) centre. The ten N-CH\textsubscript{3} groups were observed as overlapping singlets between 3.71 and 4.12 ppm. The four platinum(II)-bound NH\textsubscript{3} groups resonate at the same frequency as the ten N-CH\textsubscript{3} groups and therefore could not be observed. The CH\textsubscript{2} and CH resonances were observed as broad multiplets between 1.28 and 3.50 ppm, while the Boc group was observed as a singlet at 1.26 ppm.

In the \textsuperscript{195}Pt NMR spectrum of DNHLSP-10 (Appendix IV, Section A4.2.5) a single platinum(II) resonance was observed at -2424 ppm with a shoulder at a lower frequency (\textasciitilde -2426 ppm). This indicates the presence of two platinum(II) centres in a similar chemical environment. The chemical shift is consistent with that of similar metal complexes with PtN\textsubscript{3}Cl coordination spheres.\textsuperscript{160,166}
DNHLSP-10 has displayed poor solubility in aqueous solutions. In the ESI mass spectrum of DNHLSP-10 (Appendix IV, Section A4.5.5), dissolved in 1% DMSO in 50% water/ACN, the two most prominent ion peaks occur at \( m/z \) 1120.9 and 2241.9. The peak at \( m/z \) 1120.9 corresponds to the parent metal complex with a 2+ charge. The peak at \( m/z \) 2241.9 corresponds to the deprotonated metal complex with a 1+ charge. Fragmentation ions were also observed. The peak at \( m/z \) 1975.6 was assigned as the parent ion with a 1+ charge, resulting from the loss of one \( \text{trans} \)-chlorodiamine platinum(II) centre and deprotonation at the carboxylic acid terminal. The peak at \( m/z \) 939.1 was assigned as the parent ion with a 2+ charge, resulting from the loss of one \( \text{trans} \)-chlorodiamine platinum(II) centre. Elemental analysis of DNHLSP-10 shows that the solid obtained by lyophilisation contains thirteen waters of crystallisation, which could be reduced by further drying under vacuum at 40 °C.

**Figure 3.19:** The \( ^1H \) NMR spectrum of DNHLSP-10 (Figure 3.18) in \( d_6 \)-DMSO at 35°C. The impurities, which include DMF and acetic acid, are denoted with a red arrow.
3.4 Conclusion

The preparation of sequence selective hairpin polyamides was achieved using solution and solid phase chemistry, through which pyrrole and imidazole rings were coupled together through a series of amide bonds. After analysis of both techniques it was determined that solid phase synthesis is more effective and efficient. Firstly, solid phase synthesis produces the desired product in a significantly higher yield. The total yield for the solid phase synthesis of HLSP-6 was 77% (based on the amount of starting resin), whereas the total yield for solution phase synthesis was ~4% (based on the amount of starting monomer).\textsuperscript{160} Secondly, while both methods require ~ the same number of synthetic steps (10-12), excluding the preparation of monomers, solid phase synthesis is far more time efficient. The total time required for the preparation of HLSP-6 using solid phase chemistry was just three days. In contrast, solution phase synthesis takes three to four months to achieve the final polyamide complex and includes laborious purification of each intermediate before proceeding to the next step. The main advantage of solution phase synthesis is that Boc-protecting groups (removed with acid) and/or Fmoc-protecting groups (removed with base) can be employed in the same reaction scheme. Only base labile protecting groups (or other protecting groups such as Cbz [benzyl carbamate]) can be used in solid phase chemistry when using chlorotrityl resin. Despite this, we believe that solid phase synthesis is still the preferred method. The cytotoxicity of HLSP-6, DNHLSP-6 and DNHLSP-10 was examined using growth inhibition assays (IC\textsubscript{50}) in L1210 murine cell lines; however, the results could not be determined accurately as the metal complexes (dissolved in DMSO) precipitated out of the aqueous medium used for the cell lines. The results of this study provide the synthetic protocols for assembling more complex molecules, such as single hairpins with multiple platinum(II) centres or multiple linked hairpins with one or more platinum(II) centres.
CHAPTER 4: COMBINING SOLUTION AND SOLID PHASE CHEMISTRY

4.1 Introduction

The preparation of platinum(II) polyamides using solid phase chemistry afforded the complexes with the platinum(II) centres on one terminal and a free carboxylic acid on the other. This allowed the complexes to be coupled, using solution phase chemistry, to a platinum(II)-coordinated linker that consists of a deprotected amine.

In an attempt to enhance the solubility of $\beta$-Ala-PyPyPy-L$_4$-ImImIm, HLSP-6, DNHLSP-6 and DNHLSP-10, the coupling of a platinum(II)-coordinated linker (Pt-L$_6$-NH$_2$) with each of the four polyamides was carried out using solution phase chemistry. The addition of Pt-L$_6$-NH$_2$ increases the overall charge of the polyamide complexes.

When coupled to Pt-L$_6$-NH$_2$, $\beta$-Ala-PyPyPy-L$_4$-ImImIm forms a mononuclear complex and HLSP-6 forms a dinuclear complex, whilst DNHLSP-6 and DNHLSP-10 form trinuclear complexes. The resulting complexes are expected to display increased solubility and cytotoxicity as a result of the additional platinum(II) centre.

4.2 Experimental

The chemicals, reagents and instruments used for the synthesis and characterisation of the molecules described in this chapter are listed in Appendix I, Section A1.1 and Section A1.2.

4.2.1 General procedure used for the removal of the Boc-protecting group

The Boc-protected compound (0.39 mmol) was dissolved in methanol (8 mL) before HCl (4 M, 7 mL) was added and the mixture stirred for 30 min. The reaction was monitored using thin layer chromatography (silica gel, 3% KNO$_3$ in 10% water/ACN). The solvent was coevaporated with absolute ethanol to obtain the
intermediate product as the amine salt. Dowex-OH anion exchange resin (0.34 g, 1.16 mmol) was activated by stirring with methanol (3 × 3 mL). Methanol was decanted from the Dowex resin and the intermediate product (amine salt), dissolved in the minimum amount of methanol (2 mL), was added and the mixture stirred for 1.5 h. The resin was removed by filtration and the filtrate evaporated under reduced pressure to yield the product.

4.2.2 General procedure used for the coordination of transplatin to free amines

Transplatin (2.50 g, 8.33 mmol, 1 eq.) and silver nitrate (1.27 g, 7.49 mmol, 0.9 eq.) in DMF (100 mL) were stirred in the dark under N₂(g) for 12 h. The mixture was passed through a 0.45 µm cartridge filter before the amino product (9.26 mmol, 1.1 eq.) in DMF (30 mL) was added and the solution stirred in the dark under N₂(g) for 18 h. The solvent was removed under reduced pressure before acetone (5 mL) was added to precipitate the product and the mixture kept at 4 ºC overnight. The product was filtered and dissolved in methanol (70 mL). Unreacted transplatin was removed by filtration and the filtrate evaporated under reduced pressure to obtain the platinum(II) complex.

4.2.3 Synthesis of Pt-L₆-NH₂

4.2.3.1 Pt-L₆-NHBoc

![Pt-L₆-NHBoc](image)

The synthesis of Pt-L₆-NHBoc was carried out according to the general procedure described for the coordination of transplatin to free amines (Chapter 4.2.2), by subjecting tert-butyl-6-aminohexylcarbamate (L₆, 1.1 eq.) to activated transplatin (1 eq.). Pt-L₆-NHBoc (2.7g, 76%) was obtained as a white powder. ³¹H NMR 300 MHz (d₆-DMSO): δ 6.68 (bt, 1H), 5.08 (bt, 2H), 3.96 (s, 6H), 2.91 (q, 2H, J = 6.1 Hz), 2.55

(bm, 2H), 1.57 (bm, 4H), 1.39 (s, 9H), 1.24 (bm, 4H). $^{195}$Pt NMR 85 MHz (d$_7$-DMF): -2417 (s).

4.2.3.2 Pt-L$_6$-NH$_2$

The conversion of Pt-L$_6$-NHBoc to Pt-L$_6$-NH$_2$ was carried out according to the general procedure described for the removal of the Boc-protecting group (Chapter 4.2.1). The only variation was that Pt-L$_6$-NHBoc was stirred with HCl for 48 h instead of 30 min. Pt-L$_6$-NH$_2$ (0.14 g, 87%) was obtained as a white solid. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 7.85 (s, 2H), 5.42 (s, 2H), 4.01 (s, 6H), 2.75 (t, 2H, $J = 6.1$ Hz), 2.56 (bm, 2H), 1.56 (bm, 4H), 1.30 (bm, 4H). $^{195}$Pt NMR 85 MHz (d$_7$-DMF): -2415 (bs).

4.2.4 Synthesis of Pt-L$_6$-β-Ala-Py-L$_4$-Im (HSP-2)

To β-Ala-Py-L$_4$-Im (50 mg, 0.12 mmol) in DMF (3 mL) was added HBTU (44 mg, 0.12 mmol) and DIEA (0.06 mL, 0.35 mmol). The resulting solution was stirred for 5 min before Pt-L$_6$-NH$_2$ (25 mg, 0.07 mmol) was added and the mixture stirred overnight under N$_2$(g). The solvent was removed under reduced pressure before acetone (4 mL) was added to precipitate the product. The solid was filtered to yield Pt-L$_6$-β-Ala-Py-L$_4$-Im (35 mg, 72%) as a pale yellow powder. $^1$H NMR 300 MHz (d$_6$-DMSO): δ 9.73 (s, 1H), 8.40 (t, 1H, $J = 5.8$ Hz), 7.92 (t, 1H, $J = 5.5$ Hz), 7.83 (t, 1H, $J = 5.6$ Hz), 7.31 (s, 1H), 7.07 (s, 1H), 6.96 (s, 1H), 6.61 (s, 1H), 5.41 (bt, 2H),

4.00 (s, 6H), 3.92 (s, 3H), 3.75 (s, 3H), 3.34 (q, 2H, J = 7.0, 14.1 Hz), 3.24 (q, 2H, J = 7.2 Hz), 3.02 (q, 2H, J = 6.7 Hz), 2.50 (bm, 2H), 2.42 (t, 2H, J = 7.2 Hz), 2.22 (t, 2H, J = 7.4 Hz), 1.77 (m, 2H, J = 7.1 Hz).

195Pt NMR 85 MHz (d7-DMF): -2425 (bs).

ESI-MS calc’d for C24H44ClN10O4Pt [M – Cl] 767.2 m/z; found 767.3 m/z. Anal. Calc. for C24H44Cl2N10O4Pt.H2O: C, 35.12; H, 5.65; N, 17.07%; found: C, 35.44; H, 5.64; N, 16.67%.

### 4.2.5 Synthesis of Pt-L6-β-Ala-PyPyPy-L4-ImImIm (HSP-6)

![Image of molecule](image)

#### 4.2.5.1 Method 1

To β-Ala-PyPyPy-L4-ImImIm (70 mg, 0.08 mmol) in DMF (3 mL) was added HBTU (28 mg, 0.07 mmol) and DIEA (0.04 mL, 0.22 mmol). The solution was stirred for 5 min before Pt-L6-NH2 (19 mg, 0.05 mmol) was added and the mixture stirred overnight under N2(g). The solvent was removed under reduced pressure before acetone (4 mL) was added to precipitate the product. The solid was filtered to yield HSP-6 (59 mg, 86%) as a pale yellow powder. 1H NMR 300 MHz (d6-DMSO): δ 9.98 (s, 1H), 9.84 (s, 1H), 9.83 (s, 1H), 9.78 (s, 1H), 9.57 (s, 1H), 8.28 (t, 1H, J = 5.8 Hz), 7.93 (t, 1H, J = 5.4 Hz), 7.81 (t, 1H, J = 5.4 Hz), 7.61 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.21 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.15 (d, 1H, J = 1.4 Hz), 7.07 (s, 1H), 7.03 (d, 1H, J = 1.4 Hz), 6.87 (d, 1H, J = 1.6 Hz), 6.83 (d, 1H, J = 1.6 Hz), 5.19 (bm, 2H), 4.03 (s, 3H), 4.98 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.39 (bt, 2H), 3.37 (q, 2H, J = 7.3 Hz), 3.02 (q, 2H, J = 7.1 Hz), 2.47 (t, 2H, J = 7.1 Hz), 2.29 (m, 4H, J = 7.4 Hz), 1.82 (m, 2H, J = 7.1 Hz). 195Pt NMR 85 MHz (d7-DMF): -2420 (bs). ESI-MS calc’d for C46H66ClN20O8Pt [M – Cl]+ 1257.6 m/z;
found 1257.4 m/z. Anal. Calc. for $C_{46}H_{66}Cl_{2}N_{20}O_{8}$Pt.6$H_2$O: C, 40.57; H, 6.08; N, 19.90%; found: C, 40.84; H, 5.76; N, 19.66%.

4.2.5.2 Method 2

4.2.5.2.1 $\text{NHBoc-}L_{6}$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm (Step 1)

To $\beta$-Ala-PyPyPy-L$_4$-ImImIm (0.15 g, 0.17 mmol) in DMF (10 mL) was added HBTU (0.06 g, 0.16 mmol) and DIEA (0.06 mL, 0.34 mmol). The solution was stirred for 5 min before tert-butyl-6-aminohexylcarbamate (0.04 g, 0.19 mmol) in DMF (4 mL) was added. The solution was stirred overnight under N$_2$(g) before the solvent was removed under reduced pressure and the crude product purified using column chromatography (silica gel, 10% water/ACN, $R_f$ 0.31) to yield $\text{NHBoc-}L_{6}$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm (0.13 g, 72%) as a pale yellow powder. $^1$H NMR 300 MHz (d$_6$-DMSO): $\delta$ 10.00 (s, 1H), 9.84 (s, 1H), 9.83 (s, 1H), 9.78 (s, 1H), 9.57 (s, 1H), 8.30 (t, 1H, $J = 5.8$ Hz), 7.96 (t, 1H, $J = 5.4$ Hz), 7.79 (t, 1H, $J = 5.4$ Hz), 7.63 (s, 1H), 7.51 (s, 1H), 7.43 (s, 1H), 7.21 (d, 1H, $J = 1.4$ Hz), 7.17 (d, 1H, $J = 1.4$ Hz), 7.15 (d, 1H, $J = 1.4$ Hz), 7.07 (s, 1H), 7.03 (d, 1H, $J = 1.4$ Hz), 6.88 (d, 1H, $J = 1.6$ Hz), 6.68 (d, 1H, $J = 1.6$ Hz), 6.68 (t, 1H, $J = 5.7$ Hz), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.39 (t, 2H, $J = 7.0$ Hz), 3.37 (q, 2H, $J = 7.3$ Hz), 3.02 (q, 2H, $J = 7.2$ Hz), 2.88 (q, 2H, $J = 7.4$ Hz), 2.47 (t, 2H, $J = 7.1$ Hz), 2.31 (t, 2H, $J = 7.4$ Hz), 2.29 (t, 2H, $J = 7.4$ Hz), 1.82 (bm, 2H), 1.35 (s, 9H).
4.2.5.2.2 HSP-6 (Step 2)

The conversion of NHBoc-L₆-β-Ala-PyPyPy-L₄-ImImIm to HSP-6 was attempted according to the general procedures described in Chapter 4.2.1 and Chapter 4.2.2 with two variations. In the first step, which involved the deprotection of the amine, ethyl acetate was used instead of methanol as the solvent of choice. In the second step, the concentration of amine to metal complex was varied as transplatin (0.5 eq.) was coordinated to NH₂-L₆-β-Ala-PyPyPy-L₄-ImImIm (1 eq.). HSP-6 could not be successfully isolated, as determined by the ¹H and ¹⁹⁵Pt NMR spectra of the crude product.

4.2.6 Synthesis of Pt-L₆-β-Ala-PyPyPy-L₄'(Pt)-ImImIm

4.2.6.1 NHBoc-L₆-β-Ala-PyPyPy-L₄'(NHBoc)-ImImIm (Step 1)

To β-Ala-PyPyPy-L₄'-ImImIm (0.17 g, 0.17 mmol) in DMF (10 mL) was added HBTU (0.06 g, 0.16 mmol) and DIEA (58 µL, 0.34 mmol) and the solution stirred for 5 min. tert-Butyl-6-aminohexylcarbamate (0.04 g, 0.19 mmol) in DMF (4 mL) was added and the mixture stirred overnight under N₂(g). DMF was removed under reduced pressure to obtain a brown oil, which was purified twice by column chromatography (silica gel, 10% water/ACN, Rf 0.42) to yield the product as a yellow solid (0.08 g, 43%). ¹H NMR 300 MHz (d₆-DMSO): δ 9.96 (bs, 1H), 9.87 (s, 1H), 9.85 (s, 1H), 9.81 (s, 1H), 9.52 (s, 1H), 8.20 (t, 1H, J = 5.7 Hz), 7.92 (t, 1H, J = 5.4 Hz), 7.78 (t, 1H, J = 5.5 Hz), 7.62 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.20 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.15 (d, 1H, J = 1.4 Hz), 7.05 (s, 1H), 7.02 (d, 1H, J = 1.4 Hz), 6.95 (bd, 1H), 6.91 (d, 1H, J = 1.6 Hz), 6.81 (d, 1H, J = 1.6 Hz), 6.66 (t, 1H, J = 5.8 Hz), 4.03 (s, 3H), 3.99 (s, 3H), 3.94 (s, 3H), 3.83 (s, 6H), 3.79 (s, 3H), 3.36 (q, 2H, J = 7.3 Hz),
3.33 (t, 2H, J = 7.0 Hz), 3.02 (q, 2H, J = 7.2 Hz), 2.88 (q, 2H, J = 7.4 Hz), 2.31 (t, 2H, J = 7.4 Hz), 1.90 (bm, 2H), 1.82 (bm, 2H), 1.38 (s, 9H), 1.35 (s, 9H).

4.2.6.2 $\text{NH}_2\text{-L}_6\text{-β-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm}$ (Step 2)

The conversion of $\text{NHBoc-L}_6\text{-β-Ala-PyPyPy-L}_4'(\text{NHBoc})\text{-ImImIm}$ to $\text{NH}_2\text{-L}_6\text{-β-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm}$ was carried out according to the general procedure described in Chapter 4.2.1. The product was obtained as a white solid (0.05 g, 61%).

$^1$H NMR 300 MHz (d$_6$-DMSO): δ 11.25 (bs, 1H), 10.70 (s, 1H), 9.93 (s, 1H), 9.82 (s, 1H), 9.38 (s, 1H), 8.61 (t, 1H, J = 5.5 Hz), 8.43 (bt, 1H), 7.92 (t, 1H, J = 5.8 Hz), 7.73 (d, 1H, J = 1.4 Hz), 7.71 (s, 1H), 7.56 (d, 1H, J = 1.3 Hz), 7.53 (s, 1H), 7.22 (d, 1H, J = 1.4 Hz), 7.20 (d, 1H, J = 1.4 Hz), 7.18 (d, 1H, J = 1.4 Hz), 7.03 (d, 1H, J = 1.4 Hz), 6.96 (d, 1H, J = 1.5 Hz), 6.82 (d, 1H, J = 1.6 Hz), 6.81 (d, 1H, J = 1.6 Hz), 4.07 (s, 3H), 4.03 (s, 3H), 3.95 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.79 (s, 3H), 3.36 (q, 2H, J = 7.5 Hz), 3.04 (q, 2H, J = 7.2 Hz), 2.72 (bm, 2H), 2.31 (t, 2H, J = 7.4 Hz), 2.02 (bm, 2H), 1.82 (bm, 2H), 1.51 (bm, 2H), 1.38 (bm, 2H).

4.2.6.3 Pt-L$_6$-β-Ala-PyPyPy-L$_4'$(Pt)-ImImIm (Step 3)
The synthesis of \( \text{Pt-L}_6\beta\text{-Ala-PyPyPy-L}_4'(\text{Pt})\text{-ImImIm} \) was attempted according to the general procedure described in Chapter 4.2.2, by subjecting \( \text{NH}_2\text{-L}_6\beta\text{-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm} \) (1 eq.) to activated transplatin (2, 5 or 10 eq.) in the presence of TEA (3, 6 or 11 eq.). \( \text{Pt-L}_6\beta\text{-Ala-PyPyPy-L}_4'(\text{Pt})\text{-ImImIm} \) could not be successfully isolated, as determined by the \( ^1\text{H} \) and \( ^{195}\text{Pt} \) NMR spectra of the crude product.

### 4.2.7 Synthesis of \( \beta\text{-Ala-PyPyPy-L}_4'(\text{Pt})\text{-ImImIm} \)

#### 4.2.7.1 \( \beta\text{-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm} \) (Step 1)

The conversion of \( \beta\text{-Ala-PyPyPy-L}_4'\text{-ImImIm} \) to \( \beta\text{-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm} \) was carried out according to the general procedure described in Chapter 4.2.1. The only variation was the use of ethyl acetate as the solvent of choice instead of methanol. \( \beta\text{-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm} \) (0.06 g, 83%) was obtained as a yellow solid. \( ^1\text{H} \) NMR 300 MHz (\( d_6\text{-DMSO} \)): \( \delta \) 10.59 (s, 1H), 10.38 (s, 1H), 9.93 (s, 1H), 9.87 (s, 1H), 9.43 (s, 1H), 8.52 (t, 1H, \( J = 5.6 \) Hz), 8.39 (bt, 1H), 7.65 (s, 1H), 7.52 (s, 2H), 7.21 (s, 3H), 7.17 (s, 1H), 7.03 (s, 1H), 6.93 (s, 1H), 6.82 (s, 1H), 4.02 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.85 (s, 3H), 3.81 (s, 6H), 3.38 (bm, 5H), 2.52 (bq, 2H), 2.43 (bm).
4.2.7.2 β-Ala-PyPyPy-L₄'(Pt)-ImImIm (Step 2)

The synthesis of β-Ala-PyPyPy-L₄'(Pt)-ImImIm was attempted according to the general procedure described in Chapter 4.2.2 by subjecting β-Ala-PyPyPy-L₄'(NH₂)-ImImIm (1 eq.) to activated transplatin (0.5, 1 or 2 eq.) in the presence of TEA (1.5, 2 or 3 eq.). β-Ala-PyPyPy-L₄'(Pt)-ImImIm could not be successfully isolated, as determined by the ¹H and ¹⁹⁵Pt NMR spectra of the crude product.

4.2.8 Synthesis of Pt-L₆-β-Ala-PyPyPy-L₄-ImImIm-L₆'-Pt-(Pt)(TNHLSP-6)

DNHLSP-6 (48.0 mg, 0.03 mmol) was activated by stirring in DMF (3 mL) with HBTU (11.3 mg, 0.03 mmol) and DIEA (16 µL, 0.09 mmol) for 10 min. Pt-L₆-NH₂ (12.9 mg, 0.03 mmol) was added and the solution stirred overnight under N₂(g). The solid was removed by filtration and the solvent evaporated under reduced pressure to obtain a brown/yellow oil. Acetone (4 mL) was added and the mixture cooled to 4°C
overnight. The resulting precipitate was collected and obtained as a yellow solid (0.06 g, 89%). TNHLS\textsubscript{P}-6 could not be successfully isolated, as determined by the $^1$H and $^{195}$Pt NMR spectra of the crude product.

4.3 Discussion

4.3.1 Synthesis of Pt-L$_6$-NH$_2$

The synthesis of Pt-L$_6$-NH$_2$ was carried out using 1,6-diaminohexane (Figure 4.1). The diaminohexane linker was mono-protected using di-tert-butyl-dicarbonate as described in Chapter 2.2.4. Transplatin (1 eq.) was activated using silver nitrate (0.9 eq.) by stirring overnight in DMF under dark conditions. Silver nitrate was used as the limiting reagent to avoid the double activation of transplatin. The reaction was carried out in the dark as the silver chloride by-product is not stable in the presence of light and may convert back to silver nitrate. Under dark conditions, the silver chloride was filtered using a 0.45 µm cartridge filter to obtain the activated transplatin in DMF. The protected linker (1.1 eq.) was then mixed with activated transplatin (1 eq.) and stirred overnight. Pt-L$_6$-NHBoc was precipitated, upon the addition of acetone, as a yellow/white powder (76%). The protected linker was used in excess to ensure that all the transplatin was reacted. The rationale behind this was that unreacted transplatin would be harder to separate than the organic linker from the desired platinum(II)- linker complex. The Boc-protected amine terminal of the platinum(II)-linker complex was then converted to the amine salt, NH$_2$.HCl, by stirring with HCl for 48 h (monitored by TLC: silica gel, 3% KNO$_3$ in 10% water/ACN). The complex was then stirred with Dowex-OH anion exchange resin to obtain Pt-L$_6$-NH$_2$ as a white solid (87%).
In the $^1$H NMR spectrum of Pt-L$_6$-NH$_2$ (Figure 4. 2) the amide proton was observed as a broad triplet at 6.68 ppm. The broad triplet at 5.08 ppm was assigned as the NH$_2$ directly coordinated to the platinum(II) centre, while the two platinum(II)-bound NH$_3$ groups were observed as a broad singlet at 3.96 ppm. The six aliphatic CH$_2$ groups were observed as quartets and broad multiplets between 1.24 and 2.91 ppm, while the Boc-protecting group was observed as a large singlet at 1.39 ppm.

In the $^{195}$Pt NMR spectrum of Pt-L$_6$-NH$_2$ a single platinum(II) resonance was observed at -2417 ppm, which is consistent with similar metal complexes with PtN$_3$Cl coordination spheres.$^{160,166}$

The $^1$H NMR spectrum of Pt-L$_6$-NH$_2$ (Figure 4. 3) was similar to that of Pt-L$_6$-NHBoc, except for the absence of the Boc singlet at 1.39 ppm. After the removal of the Boc-protecting group, the peak for the free amine was expected to shift upfield to $\sim$ 5 ppm; instead the peak shifted further downfield to 7.85 ppm. This indicates that the terminal amine was obtained as the amine salt (NH$_2$.HCl). The amine salt was found to couple to carboxylic acid groups in the presence of base (DIEA), therefore the isolation of the free amine was not required.

In the $^{195}$Pt NMR spectrum of Pt-L$_6$-NH$_2$ a single platinum(II) resonance was observed at -2415 ppm, which is consistent with similar metal complexes with PtN$_3$Cl coordination spheres.}\textsuperscript{160,166}
The synthesis of $\text{Pt-L}_6$-$\text{NH}_2$ provides a platinum(II) complex with a terminal amine that is capable of coupling to the carboxylic acid group of polyamides using solution phase chemistry. The carboxylic acid group on the polyamides was obtained upon cleavage from chlorotrityl resin using acetic acid and TFE.

### 4.3.2 Synthesis of Pt-L$_6$-$\beta$- Ala-Py-L$_4$-Im (HSP-2) and Pt-L$_6$-$\beta$- Ala-PyPyPy-L$_4$-ImImIm (HSP-6)

The synthesis of HSP-2 (Figure 4. 4) and HSP-6 (Figure 4. 5) was achieved by a combination of solid and solution phase chemistry techniques. For both complexes, the polyamide was made using solid phase chemistry and the carboxylic acid group was obtained when the molecule was cleaved from the resin. The carboxylic acid group allows coupling via amide bond formation with $\text{Pt-L}_6$-$\text{NH}_2$. $\text{Pt-L}_6$-$\text{NH}_2$, used as the limiting reagent, was coupled to the polyamides $\beta$-Ala-Py-L$_4$-Im and $\beta$-Ala-PyPyPy-L$_4$-ImImIm, to obtain HSP-2 and HSP-6 respectively. The carboxylic acid group was activated using HBTU prior to the addition of the amine. For HSP-2, the polyamide (67%) was obtained using solid phase chemistry (Chapter 3.2.4), before it was platinated using solution phase chemistry to obtain the complex (72%) with an overall yield of 50%. For HSP-6, the polyamide (80%) was obtained using solid phase chemistry (Chapter 3.2.6), before it was platinated using solution phase chemistry to obtain the complex (86%) with an overall yield of 69%.

![Figure 4. 4: The synthesis of HSP-2 using solution phase chemistry.](image-url)
In the $^1$H NMR spectrum of HSP-2 (Figure 4. 6) one amide singlet was observed at 9.73 ppm and three amide triplets were observed at 8.40, 7.92 and 7.83 ppm. The four singlets at 7.31, 7.07, 6.96 and 6.61 ppm were assigned as the four aromatic hydrogens. The NH$_2$ directly coordinated to the platinum(II) centre was observed as a broad peak at 5.41 ppm, while the two platinum(II)-bound NH$_3$ groups were observed as a broad singlet at 4.00 ppm. The two singlets at 3.92 and 3.75 ppm were assigned as the two N-CH$_3$ groups. The eleven CH$_2$ groups were observed in the aliphatic region between 1.77 and 3.34 ppm.

Figure 4. 6: The $^1$H NMR spectrum of HSP-2 (Figure 4.4) in d$_6$-DMSO at 35°C.
In the $^1$H NMR spectrum of **HSP-6** (Figure 4.7) five amide singlets were observed between 9.57 and 9.98 ppm and three amide triplets were observed at 8.28, 7.93 and 7.81 ppm. The ten singlets/doublets between 6.83 and 7.61 ppm were assigned as the ten aromatic hydrogens. The NH$_2$ group directly coordinated to the platinum(II) centre was observed as a broad peak at 5.19 ppm, while the six singlets between 4.03 and 3.78 ppm were assigned as the six N-CH$_3$ groups. The two platinum(II)-bound NH$_3$ groups resonate at the same frequency as the N-CH$_3$ groups and therefore could not be independently assigned. The eleven CH$_2$ groups were observed in the aliphatic region between 1.82 and 3.39 ppm.

![Figure 4.7: The $^1$H NMR spectrum of HSP-6 (Figure 4.5) in d$_6$-DMSO at 35°C.](image)

In the $^{195}$Pt NMR spectra of **HSP-2** and **HSP-6** single platinum(II) resonances were observed at -2425 and -2420 ppm respectively (**Appendix IV, Section A4.2.6 and A4.2.7**). As mentioned earlier, the chemical shifts are consistent with similar metal complexes with PtN$_3$Cl coordination spheres.$^{160,166}$

**HSP-2** and **HSP-6** displayed poor solubility in aqueous solutions; as a result both complexes were dissolved in 1% DMSO in 50% water/ACN for the purposes of mass
spectrometry. In the ESI mass spectrum of HSP-2 (Appendix IV, Section A4.5.6) one prominent ion peak occurs at \( m/z \) 767.3 and corresponds to the parent metal complex with a 1+ charge. Fragmentation ions were also observed. The peak at \( m/z \) 503.3 was assigned as the parent ion minus the \( \text{trans} \)-chlorodiamine platinum(II) centre. The peak at \( m/z \) 696.2 was assigned as the parent ion with a 1+ charge minus the coordinated chloride and two amine groups. In the ESI mass spectrum of HSP-6 (Appendix IV, Section A4.5.7) one prominent ion peak occurs at \( m/z \) 1257.4 and corresponds to the parent metal complex with the 1+ charge. Fragmentation ions were also observed. The peak at \( m/z \) 993.4 was assigned as the parent ion minus the \( \text{trans} \)-chlorodiamine platinum(II) centre. Elemental analysis shows that HSP-2 contains one water of crystallisation, while HSP-6 contains six waters of crystallisation and 2.5 molecules of DMF. The DMF can be removed by further drying under vacuum at 40-50 ºC.

The successful synthesis of HSP-2 and HSP-6 paves the way for the preparation of more complex molecules, such as single hairpins with three platinum(II) groups or multiple linked hairpins with one or more platinum(II) groups.

The preparation of HSP-6 was also attempted using a different method, where \( L_6 \) was coupled to the polyamide prior to the coordination of transplatin. The polyamide component was synthesised using solid phase chemistry and then cleaved off the resin to obtain \( \beta\)-Ala-PyPyPy-L\(_4\)-ImImIm. \( \beta\)-Ala-PyPyPy-L\(_4\)-ImImIm was then activated using HBTU and DIEA before it was coupled to \( \text{tert} \)-butyl-6-aminohexylcarbamate to obtain \( \text{NHBoc-} L_6\beta\)-Ala-PyPyPy-L\(_4\)-ImImIm (72.3%) (Figure 4. 8).

In the \(^1\)H NMR spectrum of \( \text{NHBoc-} L_6\beta\)-Ala-PyPyPy-L\(_4\)-ImImIm (Figure 4. 9) five amide protons (two broad peaks and three singlets) were observed between 9.57 and 10.00 ppm. The remaining four amide protons were observed as triplets at 8.30, 7.96, 7.79 and 6.68 ppm. The ten aromatic hydrogens were observed as 4 singlets and 6 doublets between 6.85 and 7.63 ppm. The six singlets between 3.80 and 4.03 ppm were assigned as the six \( N\)-CH\(_3\) groups. The peaks between 1.82 and 3.39 ppm correspond to the eleven CH\(_2\) groups and were observed as triplets and quartets, while the Boc group was observed as a large singlet at 1.35 ppm.
The removal of the Boc-protecting group from \( \text{NHBoc-L}_6\text{-}\beta\text{-Ala-PyPyPy-L}_4\text{-ImImIm} \) (Figure 4. 10) was achieved by stirring the polyamide with HCl for 30 min. The solvent was removed and the residue stirred with Dowex-OH anion exchange resin to obtain the free amine. The \(^1\)H NMR spectrum of \( \text{NH}_2\text{-L}_6\text{-}\beta\text{-Ala-PyPyPy-L}_4\text{-ImImIm} \) (Figure 4. 11) was similar to that of \( \text{NHBoc-L}_6\text{-}\beta\text{-Ala-PyPyPy-L}_4\text{-ImImIm} \). The major exception was the absence of the Boc singlet (1.35 ppm) and one
amide peak (6.68 ppm). The resulting free amine group was thought to resonate at the same frequency as the $N$-CH$_3$ groups and therefore could not be observed.

The last step involved the coordination of activated transplatin to the free amine on the polyamide (Figure 4.10). Transplatin was activated overnight with silver nitrate before it was added, as the limiting reagent, to the polyamide and stirred in the dark overnight. However, the reaction was unsuccessful as determined by the $^1$H and $^{195}$Pt NMR spectra. This may be due to the lack of a base, such as TEA, which would deprotonate the free amine, thereby allowing it to attack the carboxylic acid. As seen in Chapter 3, TEA was used for all reactions in which a platinum(II) centre was coordinated to a free amine on the end of a polyamide.

Figure 4.10: An alternate scheme for the preparation of HSP-6.
Figure 4.11: The $^1$H NMR spectrum of NH$_2$-L$_6$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm (Figure 4.10 in blue) in d$_6$-DMSO at 35°C. The impurities, which include methanol, acetone and ethyl acetate, are denoted with a red arrow.

4.3.3 Synthesis of Pt-L$_6$-$\beta$-Ala-PyPyPy-L$_4$'(Pt)-ImImIm

The synthesis of Pt-L$_6$-$\beta$-Ala-PyPyPy-L$_4$'(Pt)-ImImIm (Figure 4.12) was carried out using a similar procedure to that of Pt-L$_6$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm. The Boc-mono-substituted hexane linker was coupled using HBTU and DIEA to $\beta$-Ala-PyPyPy-L$_4$'(NHBoc)-ImImIm to produce NHBoc-L$_6$-$\beta$-Ala-PyPyPy-L$_4$'(NHBoc)-ImImIm. The polyamide consists of two Boc-protected amines that were deprotected using HCl and Dowex-OH anion exchange resin to produce NH$_2$-$\beta$-Ala-PyPyPy-L$_4$'(NH$_2$)-ImImIm and provide two possible sites for the coordination of transplatin. The polyamide was then reacted with different concentrations of activated transplatin (2, 5 or 10 eq.) in the presence of TEA. The metal complex was used in excess to ensure the platination of both amine sites. Despite using TEA to deprotonate the free amine, the product could not be isolated as indicated by the $^1$H and $^{195}$Pt NMR spectra.
In the $^1$H NMR spectrum of $\text{NHBoc-}\beta\text{-Ala-PyPyPy-L}_4'\text{(NHBoc)-ImImIm}$ (Figure 4. 13) five amide protons (two broad peaks and three singlets) were observed between 9.52 and 9.96 ppm. Four amide protons were observed as triplets at 8.20, 7.92, 7.78 and 6.66 ppm and one broad amide doublet was observed at 6.95 ppm. The ten aromatic hydrogens were observed as four singlets and six doublets between 6.81 and 7.96 ppm.
7.62 ppm. The six singlets between 3.79 and 4.03 ppm were assigned as the six $N$-CH$_3$ groups. The peaks between 1.82 and 3.36 ppm correspond to the ten CH$_2$ groups and one CH group and were observed as triplets and quartets. The two Boc groups were observed as two large singlets at 1.38 and 1.35 ppm.

The $^1$H NMR spectrum of NH$_2$-β-Ala-PyPyPy-L$_4$(NH)$_2$-ImImIm (Figure 4. 14) was similar to that of NHBoc-β-Ala-PyPyPy-L$_4$(NHBoc)-ImImIm. The major exception was the absence of the two Boc singlets from 1.38 and 1.35 ppm and two amide peaks from 6.95 and 6.68 ppm. The resulting two free amine groups are thought to resonate at the same frequency as the N-CH$_3$ groups and therefore could not be observed.
4.3.4 Synthesis of $\beta$-Ala-PyPyPy-L$_4'(Pt)$-ImImIm

The synthesis of $\beta$-Ala-PyPyPy-L$_4'(Pt)$-ImImIm (Figure 4. 15) was carried out in two steps. In the first step, $\beta$-Ala-PyPyPy-L$_4'(NHBoc)$-ImImIm was stirred with HCl followed by Dowex-OH anion exchange resin to produce $\beta$-Ala-PyPyPy-L$_4'(NH_2)$-ImImIm and thereby provide a possible site for the coordination of transplatin. In the second step, the polyamide was reacted with different concentrations of activated transplatin (0.5, 1 or 2 eq.) in the presence of TEA. In all three experiments, the product could not be isolated as indicated by the $^1$H and $^{195}$Pt NMR spectra. This could be due to the fact that the free amine of one polyamide molecule is forming an amide bond with the free acid group of another, leaving no free amines for the activated transplatin to coordinate to.
The $^1$H NMR spectrum of $\beta$-Ala-PyPyPy-L$_4$'($N\text{H}_2$)-ImImIm (Figure 4. 16) was similar to that of $\beta$-Ala-PyPyPy-L$_4$'(NHBoc)-ImImIm. The only exception was the absence of the Boc singlet from 1.39 ppm and the amide peak from 6.89 ppm. The free amine is thought to resonate at a similar frequency as the $N$-CH$_3$ groups and therefore could not be observed. Solvent impurities (methanol, acetone and ethyl acetate) were also observed. These impurities can be removed by further evaporation under reduced pressure.
Figure 4. 16: The $^1$H NMR spectrum of $\beta$-Ala-PyPyPy-L$_4$(NH$_2$)-ImImIm (Figure 4.15 in blue) in d$_6$-DMSO at 35 °C. The impurities, which include methanol, acetone and ethyl acetate, are denoted with a red arrow.

4.3.5 Synthesis of $\text{Pt-L}_6$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6'$-Pt-(Pt) (TNHLSP-6)

The synthesis of TNHLSP-6 (Figure 4. 17) was carried out by coupling DNHLSP-6 and Pt-L$_6$-NH$_2$. The dinuclear polyamide complex consists of a free carboxylic acid group, which can form an amide bond with the free amine on the platinum(II)-linker complex. The reaction was carried out by activating the acid group of DNHLSP-6 using HBTU and DIEA, followed by the addition of Pt-L$_6$-NH$_2$ and stirring overnight under N$_2$(g). However, the product could not be isolated as determined by the $^1$H and $^{195}$Pt NMR spectra.
4.3.6 Synthesis of Pt-β-Ala-COOH

The synthesis of Pt-β-Ala-COOH was attempted in order to obtain a platinum(II) complex with a carboxylic acid group, which could be coupled to the free amine on a polyamide molecule (Figure 4. 18). The coupling would be carried out using the previously stated coupling conditions \textit{i.e.} stirring in DMF with HBTU as the activating agent and DIEA under N\textsubscript{2}(g) overnight.

Figure 4. 18: The coupling of Pt-β-Ala-COOH to a polyamide.
The synthesis of Pt-β-Ala-COOH was carried out by stirring activated transplatin in DMF with an excess eq. of β-alanine for 18 h(Figure 4. 19). The reaction proved to be unsuccessful and it is believed that the free amine of one β-alanine molecule attacks the carboxylic acid group of another to form an amide bond (the mechanism for amide bond formation is represented in Scheme 2 of Chapter 3.3). This was supported by the $^1$H NMR spectrum, which revealed amide triplets resonating around 8 ppm.

![Figure 4. 19: The synthetic scheme for the preparation of Pt-β-Ala-COOH.](image)

4.4 Conclusion

The preparation of Pt-L$_6$-NH$_2$ provides a route, via solution phase chemistry, for the coupling of an extra platinum(II) centre to the sequence selective polyamides. The addition of Pt-L$_6$-NH$_2$ to the organic polyamides, β-Ala-Py-L$_4$-Im and β-Ala-PyPyPy-Py-L$_4$-ImImIm was carried out successfully to obtain the mononuclear platinum(II) sequence selective complexes, HSP-2 and HSP-6. Pt-L$_6$-NH$_2$ was used as the limiting reagent, while any excess polyamide was removed by precipitating the metal complexes from acetone. However, when Pt-L$_6$-NH$_2$ was coupled to the dinuclear complex, DNHLSP-6, the desired product could not be isolated from a mixture of mono-, di- and trisubstituted complexes.

Attempts to coordinate activated transplatin to the polyamides via solution phase chemistry were not successful, despite using a similar method to that implemented in solid phase chemistry.
CHAPTER 5: BIOLOGICAL STUDIES

5.1 Introduction

The anticancer properties of cisplatin and subsequent platinum(II) based drugs (such as BBR3464) have been linked to the formation of cytotoxic DNA adducts. The majority of these adducts are formed through the N7 of guanine and adenine bases. Examining the formation of coordinate covalent bonds to guanosine has previously been used to investigate the ability of platinum(II) complexes to form adducts with DNA. Wheate et al. have investigated the coordinate covalent binding between guanosine and trans-[{Pt(NH3)2Cl}2µ-dpzm]2+ (dpzm is 4,4'-dipyrazolylmethane) (Figure 5.1) by monitoring the rate of adduct formation using 1H NMR spectroscopy. As the metal complex binds the N7 of guanosine, the intensity of the H8 and H1' of unbound guanosine decreases gradually until they have completely disappeared. Two new signals corresponding to H8 and H1' of bound guanosine gradually appear further downfield. The rate of adduct formation can be determined by measuring the increase or decrease in the intensity of either peak on an hourly basis.

The platinum(II) sequence selective polyamides have displayed poor solubility in aqueous solutions. As a result, growth inhibition tests such as IC50 could not be accurately determined. As guanosine binding experiments are typically carried out in D2O, attempts to enhance their solubility were investigated.
5.2 Experimental

The chemicals, reagents and instruments used to carry out the experiments described in this chapter are listed in Appendix I, Section A1.1 and Section A1.2.

5.2.1 Solubilising the platinum(II) polyamides

5.2.1.1 Using α-, β- and γ-cyclodextrin

α-, β- or γ-Cyclodextrin (65.2 µmol, 10 × 1 eq.) was added over a period of 2 h to HLSP-6 or DNHLSP-6 (6.52 µmol, 1 eq.) in water (50 mL) at 50 ºC and the mixture stirred for 3 h.

5.2.1.2 Using silver nitrate

HLSP-6 (12.9 µmol, 1 eq.) was stirred with silver nitrate (12.1 µmol, 0.95 eq.) in water (50 mL) for 18 h.

5.2.1.3 Using HBTU

HLSP-6 (12.9 µmol, 1 eq.) was stirred with HBTU (12.1 µmol, 0.95 eq.) and DIEA (36.3 µmol) in water (50 mL) for 15 min.

5.2.2 Guanosine binding

5.2.2.1 Binding of guanosine to HLSP-6

The guanosine binding of HLSP-6 was conducted using 1:1 guanosine:HLSP-6 at 2 mM concentrations in 50% d$_7$-DMF/D$_2$O at 37 ºC. Scans were recorded at zero time and then on an hourly basis over 50 h.

5.2.2.2 Binding of guanosine to DNHLSP-6

The guanosine binding of DNHLSP-6 was conducted using 1:1 guanosine:DNHLSP-6 at 2 mM concentrations in 30% d$_7$-DMF/D$_2$O at 37 ºC. The experiment was also conducted using 2:1 guanosine:DNHLSP-6 at 4 and 2 mM concentrations respectively in 30% d$_7$-DMF/D$_2$O at 37 ºC. Scans were recorded at zero time and then on an hourly basis over 20 h for both experiments.
5.3 Discussion

5.3.1 Solubilising the platinum(II) polyamides

In an attempt to increase the solubility of the platinum(II) complexes, molecular hosts such as $\alpha$-, $\beta$- and $\gamma$-cyclodextrins were investigated, along with the activating agents HBTU and AgNO$_3$.

$\alpha$-, $\beta$- and $\gamma$-Cyclodextrins (Figure 5.2) are polymers of sugar rings ($\alpha$ is 6, $\beta$ is 7 and $\gamma$ is 8 sugar rings) widely used to solubilise drugs. The polymers consist of hydroxyl groups that can interact with polyamides and thereby enhance their aqueous solubility. HLSP-6 and DNHLSP-6 were separately stirred in water with each of the three forms of cyclodextrins. The mixtures were stirred at 50 °C with the concentration of cyclodextrin continuously increasing from 1 to 10 eq. over a period of 2 h. Unfortunately, the complexes did not display any increase in solubility that would result upon the encapsulation of the polyamides by the sugar polymers.

Figure 5.2: The chemical structure of $\alpha$-cyclodextrin.

In other attempts, HLSP-6 was stirred with 0.95 eq. of silver nitrate in water. It was hypothesised that the platinum(II) group would be activated by the silver nitrate and thereby creates a more water soluble complex. However, this was not the case and the activated complex formed did not display any enhanced aqueous solubility. This experiment was based on the observation that when transplatin was activated with silver nitrate, the activated complex became more water soluble.
The last attempt to increase the solubility of the metal complexes was carried out by stirring HLSP-6 with 0.95 eq. of HBTU (Figure 5. 3). HBTU is an activating agent used to activate carboxylic acid groups prior to the formation of amide bonds. It was noted that when the pyrrole rings, imidazole rings and various other linkers were activated with HBTU prior to coupling with the amine resin, they instantly dissolved in DMF, the reaction solvent. Unlike the polyamide components, activated HLSP-6 did not display enhanced solubility.

![Figure 5. 3: The chemical structure of HBTU.](image)

### 5.3.2 Guanosine binding

#### 5.3.2.1 Binding of guanosine to HLSP-6

The analysis of the binding of HLSP-6 to guanosine (1:1) was conducted in 50% d$_7$-DMF as the complex displayed poor solubility in neat D$_2$O. At zero time, the H$_1'$ and H$_8$ protons of guanosine were observed as a doublet and a singlet at 5.92 and 8.16 ppm respectively. After 1 h the intensity of the H$_8$ resonance decreased by 24.5%, while two new resonances appeared at 5.98 and 8.79 ppm. As the reaction proceeded the intensities of the unbound guanosine signals continued to decrease until the 40$^{th}$ h, where the H$_1'$ and H$_8$ signals completely disappeared (Figure 5. 4). The $^{195}$Pt NMR of the guanosine-platinum(II) complex could not be carried out due to poor solubility.
Figure 5.4: The $^1$H NMR array experiment of 1:1 guanosine:HLSP-6 in 50% d$_7$DMF/D$_2$O at 37 °C.

The data obtained from the $^1$H NMR array experiment of 1:1 guanosine:HLSP-6 revealed that 50% of guanosine ($t_{1/2}$) was coordinated to the platinum(II) complex within 5.96 h. The reaction then proceeded very slowly until it reached completion by the 40$^{th}$ h. The percentage of bound guanosine was determined by monitoring the integrals of the $H_8$ proton at 8.16 ppm, the area of which was plotted as a function of time (Figure 5. 5).

![Figure 5. 5: The rate of guanosine coordination to HLSP-6 measured over 40 h and monitored by $^1$H NMR spectroscopy at 37 ºC ($t_{1/2}$ is 5.96 h).](image)

5.3.2.2 Binding of guanosine to DNHLSP-6

The analysis of the binding of DNHLSP-6 to guanosine was conducted using 1:1 and 2:1 guanosine:DNHLSP-6. d$_7$-DMF was used to enhance the solubility of the metal complex. At the initial time of mixing (for both 1:1 and 2:1 guanosine:DNHLSP-6), $H_1'$ and $H_8$ of guanosine were observed as a doublet and a singlet at 5.92 and 8.16 ppm respectively. When the experiment was conducted at a 1:1 guanosine:DNHLSP-6 ratio, the intensity of the $H_8$ resonance decreased by 27.8% after 1 h, while three new resonances appeared at 5.98, 8.78 and 8.79 ppm. As the reaction proceeded the intensity of the unbound guanosine signals continued to decrease until the 10$^{th}$ h, where the $H_1'$ and $H_8$ signals completely disappeared (Figure 5. 6). When the experiment was conducted at a 2:1 guanosine:DNHLSP-6 ratio, the unbound guanosine signals disappeared by the 13$^{th}$ h (Figure 5. 7). The $t_{1/2}$ for the rate of coordination of DNHLSP-6 to guanosine was 2.39 h when 1:1 guanosine:DNHLSP-6
was used (Figure 5. 8) and 2.61 h when 2:1 guanosine:DNHLSP-6 was used (Figure 5. 9). The $^{195}$Pt NMR of the (1:1) and (2:1) guanosine-DNHLSP-6 complexes could not be carried out as the complexes displayed poor solubility.

Figure 5. 6: The $^1$H NMR array experiment of 1:1 guanosine:DNHLSP-6 in 30% d$_7$-DMF/D$_2$O at 37 ºC.
Figure 5.7: The $^1$H NMR array experiment of 2:1 guanosine:DNHLSP-6 in 30% d$_7$-DMF/D$_2$O at 37 °C.
Figure 5.8: The rate of guanosine coordination to DNHLSP-6 (1:1) measured over 20 h and monitored by $^1$H NMR spectroscopy at 37 ºC ($t_{1/2}$ is 2.39 h).

Figure 5.9: The rate of guanosine coordination to DNHLSP-6 (2:1) measured over 20 h and monitored by $^1$H NMR spectroscopy at 37 ºC ($t_{1/2}$ is 2.61 h).

The covalent binding of DNHLSP-6 to the N$_7$ of guanosine was complete in 10 h when 1:1 guanosine:DNHLSP-6 was used and 13 h when 2:1 guanosine:DNHLSP-6 was used. The presence of two platinum(II) centres on the polyamide permits the coordination of two guanosine molecules. Thus, when the concentration of guanosine was double that of DNHLSP-6, there was enough platinum(II) centres for all the guanosine to coordinate to, although the coordination required an extra 3 h compared to when 1:1 guanosine:DNHLSP-6 was used. The appearance of two H$_8$ singlets at 8.78 and 8.79 ppm was attributed to the two possible coordination sites of the
complex, which have slightly different chemical environments. The $H_1'$ proton remained as a singlet in both cases, as a result of its proximity from both platinum(II) centres.

5.4 Conclusion

Solubilising agents, such as $\alpha$-, $\beta$- and $\gamma$-cyclodextrins, were investigated to improve the aqueous solubility of the platinum(II) complexes. However, the complexes did not display any interaction with the hydroxyl groups of the cyclodextrins and solubility remained an issue. The metal complexes were also stirred with AgNO$_3$ and HBTU, but yet again the experiment was unsuccessful as the complexes remained insoluble in aqueous mediums.

Guanosine binding experiments revealed that the mononuclear complex, HLSP-6, coordinated to guanosine within 40 h while the dinuclear complex, DNHLSP-6, coordinated to guanosine within 10 h when 1:1 guanosine:DNHLSP-6 was used and 13 h when 2:1 guanosine:DNHLSP-6 was used. These results are consistent with other platinum(II) based drugs that have been shown to coordinate with guanosine and adenosine to form different DNA adducts.$^{76,77}$
CHAPTER 6: CONCLUSION

Five platinum(II)-based sequence selective polyamides, HLSP-6, DNHLSP-6, DNHLSP-10, HSP-2 and HSP-6, have been synthesised. HLSP-6, DNHLSP-6 and DNHLSP-10 were synthesised using solid phase chemistry. HSP-2 and HSP-6 were prepared using a combination of solid and solution phase chemistry. The polyamide components of HSP-2 and HSP-6 were prepared using solid phase chemistry, after which the polyamides were cleaved from the resin and the platinum(II) centre was added using solution phase chemistry. Both techniques proved to be efficient and effective in synthesising the target compound in terms of yield, purity and time. These compounds form the basis for exploring more complex and water soluble platinum(II)-based DNA sequence selective agents that may display enhanced DNA sequence recognition and higher cytotoxicity.

All platinum(II) sequence selective polyamides were characterised by $^1$H and $^{195}$Pt NMR spectroscopy. The complexes were further tested for purity using mass spectrometry and elemental analysis.

One disadvantage of the platinum(II) polyamides investigated in this study was their poor solubility in aqueous solutions. A small amount of DMSO or DMF was added to solubilise the metal complexes for the purposes of mass spectrometry and guanosine binding experiments; however, the metal complexes precipitated out of the aqueous medium used for cytotoxicity assays. As a result, the IC$_{50}$ values of the complexes could not be accurately calculated. In order to enhance aqueous solubility, it is recommended that future platinum(II) polyamides are developed with a 3+ charge, which is distributed throughout the molecule.

The results of this study establish the protocols for the synthesis of more complex molecules, such as single hairpins with three platinum(II) centres or multiple linked hairpins with one or more platinum(II) centres. One such compound is the trinuclear platinum(II) complex (TNHLSP-6, Figure 4. 17), where two platinum(II) centres can be added using solid phase chemistry and the third platinum(II) centre may be added using solution phase chemistry. It is expected that the trinuclear complex will display
increased water solubility as a result of its 3+ charge. The synthesis of TNHLSP-6 through the formation of an amide bond between the free amine of Pt-L₆-NH₂ and the carboxylic acid group of DNHLSP-6 was carried out with little success.

Another approach for the synthesis of a trinuclear complex could involve the synthesis of a transplatin-based linker that consists of an Fmoc-protected amine terminal and a carboxylic acid group terminal (Figure 6. 1). Such a molecule could be employed using solid phase chemistry as the hairpin linker which connects two polyamide chains (Figure 6. 2).

![Figure 6. 1: The chemical structure of NHFmoc-L₃-Pt-L₃-COOH.](image)

![Figure 6. 2: NHFmoc-L₃-Pt-L₃-COOH acting as a hairpin linker in solid phase chemistry.](image)

Future work will focus on the development of platinum(II) complexes with multiple hairpins. These complexes are expected to display increased DNA selectivity and binding affinity. Such studies include a trinuclear platinum(II) complex (Figure 6. 3) where the terminal platinum(II) centres are linked to the central platinum(II) centre through two separate hairpin polyamides. This complex will have a 4+ charge and is expected to bind DNA sequences up to 12 base pairs in length. Such a complex would be capable of recognising and binding tandem duplex telomere sequences, like d(TTAGGGTTAGGG)ₙ.
Figure 6.3: The chemical structure of a trinuclear platinum(II) complex with a 4+ charge.

- ● represents a pyrrole ring
- ■ represents an imidazole ring
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APPENDIX I

A1.1 Materials

A1.1.1 Chemicals and reagents
A1.1.2 NMR sample preparation

A1.2 Instrumentation

A1.2.1 Nuclear Magnetic Resonance
A1.2.2 Dynavac™ freeze drier
A1.2.3 Rotary evaporator and Eyela™ water bath SB-650
A1.2.4 Heidolph MR 3001 K magnetic stirrer and EKT 3001 temperature control
A1.2.5 Protein synthesiser
A1.2.6 Mass spectrometer
A1.2.7 Cytotoxicity assays
A1.2.8 Hydrogenation apparatus
A1.2.9 Balances

A1.1 Materials

A1.1.1 Chemicals and reagents

N-Methyl-1H-imidazole-2-carboxylic acid was purchased from Bachem. 4-(Fmoc-amino)butyric acid, (9-fluorenyl)methyl chloroformate (Fmoc-Cl), acetic anhydride and Fmoc-β-alanine were purchased from Fluka. N-Methyl-1H-imidazole, transplatin, di-tert-butyl dicarbonate (Boc₂O), triethylamine, anhydrous dimethylformamide, 1-Methyl-2-pyrrolidinone, tetrahydrofuran, trifluoroacetic acid, 2,2',2''-trifluoroethanol, piperidine, pyridine, 4-aminobutyrate hydrochloride, diisopropylethylamine, potassium nitrate, (dimethylamino)propylamine, potassium cyanide, sodium tert-butoxide, ninhydrin, 10% Pd/C, Dowex® Monosphere® 550A (OH) anion exchange resin, carbon tetrachloride and α-, β- and γ-cyclodextrins were purchased from the Sigma-Aldrich Chemical Company. 2-Chloro-chlorotrityl resin, Fmoc-β-alanine-OH-WANG resin, 2,6-Fmoc-Lysine-(Fmoc)-OH and 2-(1H-benzotriazole-1-YL)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU) were purchased from Auspep. 2-Boc-4-Fmoc-L-diaminobutyric acid was purchased from PepTech Corporation. N-Methyl-1H-pyrrole and tert-butyl alcohol were purchased from Alfa Aesar. Silver nitrate, anhydrous magnesium sulphate and anhydrous sodium sulphate were purchased from BDH. Trichloroacetic acid and thionyl chloride were purchased from Riedel-de Haën. 1,2-diaminoethane, 1,3-diaminopropane and 1,6-diaminohexane were supplied by Lancaster. All reagents and solvents, including dichloromethane, acetonitrile, hexane, diethyl ether, ethyl acetate, sulphuric acid, 70% nitric acid, hydrochloric acid, acetic acid, sodium hydroxide, ethanol, methanol, phenol, chloroform, distilled water, brine, lithium hydroxide and sodium bicarbonate, were of analytical grade or better.

A1.1.2 NMR sample preparation

The product samples (2-10 mg), used for characterisation by NMR spectroscopy, were dissolved in 0.6 mL of d₆-DMSO (Cambridge Isotope), D₂O (Cambridge Isotope), CDCl₃ (Cambridge Isotope) or d₇-DMF (Sigma-Aldrich Chemical Company).
A1.2 Instrumentation

A1.2.1 Nuclear Magnetic Resonance

$^1$H, $^{195}$Pt and NOESY NMR spectra were obtained on a 300 MHz Varian Mercury linked to a Sun workstation® or a 400 MHz Bruker Avance spectrometer. NMR experiments were run at 35 ºC for d$_6$-DMSO and 25 ºC for D$_2$O/d$_7$-DMF/CDCl$_3$, unless otherwise stated. $^1$H and NOESY NMR experiments were referenced internally to the solvent, while $^{195}$Pt NMR experiments were externally referenced to K$_2$PtCl$_4$ (-1631 ppm). For one-dimensional spectra, a spectral width of 5,000 Hz was used with 50,000 data points, a d1 of 0.5 s and an acquisition time of 3 s. Two-dimensional NOESY NMR spectra were obtained using a spectral width of 5000 Hz with 256 increments in the t$_1$ dimension, 2048 increments in the t$_2$ dimension, a mixing time of 0.5-0.8 s and a relaxation delay of 5 s.

A1.2.2 Dynavac™ freeze drier

The Dynavac™ freeze drier was used to lyophilise aqueous solutions. The samples were frozen in liquid nitrogen before they were placed under reduced pressure in order for the water to sublime.

A1.2.3 Rotary evaporator and Eyela™ water bath SB-650

The rotary evaporator was used to evaporate organic solvents under reduced pressure. The vacuum pump, an RZ20, was supplied by John Morris Scientific.

A1.2.4 Heidolph MR 3001 K magnetic stirrer and EKT 3001 temperature control

The magnetic stirrers used were Heidolph MR 3001 K connected to a temperature probe (EKT 3001). This allows samples to be stirred at up to 3000 rpm between r.t and 300-400 ºC.
A1.2.5 Protein synthesiser

Machine-assisted protocols: Machine assisted synthesis was performed on a Symphony Quartet 3.21 protein synthesiser on a 0.28 mmol scale. Each cycle of monomer addition involved a DCM wash (7 units), a DMF wash (7 units), deprotection with 20% piperidine/DMF (7 units) for 3 min, draining the reaction vessel, a DMF wash (7 units), deprotection for 17 min (7 units), a DMF wash (7 units), a DCM wash (7 units), a DMF wash (2 × 7 units), draining the reaction vessel, coupling for 3.5 h (10 h when coupling to an imidazole ring) and finally draining the reaction vessel. The activated acids (0.62 mmol) were added manually to the reaction vessel at the end of every deprotection cycle. The cycle was interrupted, reaction vessel vented, the activated acid added and the cycle was resumed. Once the polyamide was assembled, the resin was washed with DMF (2 × 7 units) and DCM (3 × 7 units) before it was dried for 1 h under N$_2$(g). In the case of platinum(II) polyamides, the resin was washed with DMF (2 × 7 units), brine (2 × 7 units), water (2 × 7 units), DMF (2 × 7 units) and DCM (3 × 7 units). Each volume unit represents 1.15 mL.

A1.2.6 Mass spectrometer

Positive ion ESI mass spectra were acquired using a Micromass (Wyntheshawe, UK) Quattro Micro™ spectrometer equipped with a Z-spray probe at the University of Wollongong. Solutions containing concentrations ranging between 10 and 50 µM were injected into the instruments at a flow rate of 10 µL min$^{-1}$. The source and desolvation temperatures were 150 and 120 ºC respectively. The capillary tip potential and cone voltage were 2500 and 50 V respectively. Between 10 and 50 acquisitions were summed to obtain spectra, which were calibrated against a standard CsI solution (750 mM) over the same $m/z$ range.

A1.2.7 Cytotoxicity assays

Cytotoxicity assays (IC$_{50}$) were carried out by the Peter MacCallum Cancer Institute (Melbourne), Research Division. The murine leukemia cancer cell line L1210 was grown in complete medium containing RPMI 1640 supplemented with 5% fetal bovine serum without antibiotics. Standard conditions for cell maintenance were used at 37 ºC

humidified incubator with 5% CO₂. Cells were plated in 96-well plates (100 µL per well) at a concentration of 4 × 10⁴ cells per milliliter in complete medium and were then treated with a range of metal complex concentrations in 100 µL medium (0.00013-50 µM). The cells were incubated for 48 h under standard conditions before drug cytotoxicity was determined using an RB-based growth inhibition assay. The assay was carried out by adding 50 µL MTT solutions (5 mg/mL) to the cell/drug mixtures and incubating for 2 h in standard conditions. The cells were harvested and mixed with DMSO (200 µL) before the optical density (OD₅₉₀) was read on a plate reader (MultisKan EX, LabSystems, Finland). Absorbance values at each drug concentration were plotted (y-axis) against the corresponding concentration of drug (x-axis) and the IC₅₀ values were calculated from the resulting dose-response curve. Data given are averages derived from between two and six independent experiments tested in duplicates or triplicates.

A1.2.8 Hydrogenation apparatus

The thermally protected apparatus was supplied by PARR Instrument Co, Moline, IL USA and is capable of maintaining pressures up to 100 psi. The motor was supplied by GE Motors Industrial System.

A1.2.9 Balances

Chemicals were weighed using a Mettler Toledo XS205 Dual Range with a maximum weight of 81/220 g and a minimum weight of 0.01/0.1 mg or a Mettler Toledo UMX5 with a maximum weight of 5.1 g and a minimum weight of 0.1 µg.
Appendix II

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A2.5 Determining the loading (mmol/g) of Fmoc-β-alanine-chlorotrityl resin 179
A2.1 Preparation of fuming nitric acid

Fuming nitric acid was prepared by mixing 70% nitric acid with concentrated sulphuric acid in a 1:1 ratio. The mixture was distilled at 60 °C and only the initial 1/3 of the total volume was collected. The distilled product was flushed with N₂(g) to remove any nitrous oxide. The pale yellow solution obtained was ~ 95% w/w nitric acid. The acid was sealed with a glass stopper and stored in the fridge.

A2.2 Purification using column chromatography

The packing material used for column chromatography was silica gel 60 (0.003-0.040 mm). The glass column was wet packed using the minimum volume of solvent. The packing was flushed three times with the chosen solvent before a layer of sand (1 mL) was added to stabilise the head of the column. The product was then added, eluted and small fractions were collected (< 1 mL). Each fraction was spotted on a Silica Gel F₂₅₄ TLC, using 0.25 mm thick pre-coated UV sensitive silica gel aluminium plates, and visualised under UV. Compounds were visualised with short-wave ultraviolet light at 254 nm.

A2.3 Purification of transplatin, trans-[Pt(NH₃)₂Cl₂]

Transplatin⁵ (2.00 g, 6.67 mmol) was mixed with water (300 mL, pH 5) and stirred at 100 °C until the solid dissolved. The solution was quickly filtered and the filtrate cooled to 0 °C for 1 h. The resulting precipitate was filtered to yield the product (0.80 g, 40.0%) as a yellow solid. ¹H NMR 300 MHz (d₆-DMSO): δ 4.35 (b, 6H). ¹⁹⁵Pt NMR 85 MHz (d₇-DMF): δ -2114 (s).

A2.4 Ninhydrin test

Three solutions were prepared such that Solution 1 contains 70% phenol/methanol, Solution 2 contains 5% ninhydrin/ethanol and Solution 3 contains 0.2 mM KCN/pyridine prepared from a stock solution of 0.1 M KCN/water. Once the solid phase coupling was

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⁵ Transplatin supplied by Sigma Aldrich was impure and could not be used unless purified.
started, the reaction was interrupted every 30 min where 2-5 resin beads were removed and placed in a test tube. The beads were washed with methanol/water (70%, 3 × 3 mL) before a mixture of Solutions 1 + 2 + 3 (1:1:1, 3 mL) was added and the beads heated at 110 ºC for 10 min. After which methanol/water (70%, 2-3 mL) was added to quench the solution. The solvent was decanted and the beads washed with methanol/water (70%, 3 × 3 mL) before they were observed under the microscope. Blue or red beads indicate the presence of free amines, while yellow beads indicate the presence of protected amines only. The time required for each coupling reaction was determined as the time where blue/red beads could not be observed.

**A2.5 Determining the loading (mmol/g) of Fmoc-β-alanine-chlorotrityl resin**

\[
\text{Loading}_{(2\text{-chloro-chlorotrityl resin})} = 1 \text{ mmol/g}
\]

\[
W_{(2\text{-chloro-chlorotrityl resin})} = X \text{ g (eg. } X = 0.5003 \text{ g)}
\]

\[
W_{(\text{Fmoc-β-alanine-chlorotrityl resin})} = Y \text{ g (eg. } Y = 0.5826 \text{ g)}
\]

\[
W_{(\text{increase})} = W_{(\text{Fmoc-β-alanine-chlorotrityl resin})} - W_{(2\text{-chloro-chlorotrityl resin})} = 0.0823 \text{ g}
\]

\[
\text{M.w.} = \text{M.w.}(\text{Fmoc-β-alanine-OH}) - [\text{M.w.}(1\text{H}) + \text{M.w.}(1\text{Cl})] = 275 \text{ g/mol}
\]

\[
\text{Number of moles} = \frac{W_{(\text{increase})}}{\text{M.w.}} = 0.2993 \text{ mmol}
\]

\[
\text{Loading}_{(\text{Fmoc-β-alanine-chlorotrityl resin})} = \frac{\text{Number of moles}}{W_{(\text{Fmoc-β-alanine-chlorotrityl resin})}}
\]

\[
= 0.5137 \text{ mmol/g}
\]
APPENDIX III

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A3.1 Structure abbreviations

- 9-Fluorenylmethyl chloroformate (Fmoc-Cl)

\[
\text{HO-Cl}
\]

- Di-tert-butyldicarbonate (Boc)

\[
\text{O-C(O-OC-O-C(O-O-)}
\]

- Fmoc-β-Ala-OH-WANG resin (WANG resin)

\[
\text{O-Cl}
\]

- 2-Chloro-chlorotrityl resin

\[
\text{Cl-Cl}
\]
• 2-(1H-benzotriazole-1-y1)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)

$$\begin{align*}
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\end{array}
\begin{array}{c}
\text{N} \\
\text{N} \\
\end{array}
\begin{array}{c}
\text{P} \\
\text{F} \\
\text{F} \\
\text{F} \\
\text{F} \\
\end{array}
\end{align*}$$

• tert-Butyl-2-aminoethylcarbamate (L₂)

\[ \text{H}_2\text{N}-\text{NHBOc} \]

• tert-Butyl-3-aminopropylcarbamate (L₃)

\[ \text{H}_2\text{N} \quad \text{NHBOc} \]

• (Dimethylamino)propylamine (L₃')

\[ \begin{array}{c}
\text{N} \\
\text{H}_2\text{N} \\
\end{array} \]

• 4-(Fmoc-amino)butyric acid (L₄)

\[ \text{HO} \quad \text{NHBOc} \]
- 2-Boc-4-Fmoc-L-diaminobutyric acid (L₄')

- tert-Butyl-6-aminohexylcarbamate (L₆)

- 2,6-Fmoc-Lysine-(Fmoc)-OH (L₆')
A3.2 Polyamide abbreviations

HLSP-6 is shown here as an example to how all polyamides were abbreviated. The polyamide consists of a \( \beta \)-alanine, three pyrrole rings, a \( \gamma \)-aminobutyrate linker, three imidazole rings, a \( \gamma \)-aminobutyrate linker and a transplatin group. Therefore, it is abbreviated as follows: \( \beta\text{-Ala-PyPyPy-L}_4\text{-ImImIm-L}_4\text{-Pt} \). All other polyamides were abbreviated using the same procedure.

- \( \beta\text{-Ala-Py-L}_4\text{-Im} \)

- \( \beta\text{-Ala-ImIm} \)

- $\beta$-Ala-PyPyPy-L$_4$-ImImIm

- $\beta$-Ala-PyPyPy-L$_4$'-ImImIm

- $\beta$-Ala-Py-L$_4$-Pt

- β-Ala-Im-L₄-Pt

- β-Ala-PyPyPy-L₄-ImImIm-L₆'-Pt-(Pt) (DNHLSP-6)

- β-Ala-PyPyImImIm-L₄'-PyPyPyPyPy-β-Ala-L₆'-Pt-(Pt) (DNHLSP-10)

- **Pt-L₆-β-Ala-Py-L₄-Im (HSP-2)**

- **Pt-L₆-β-Ala-PyPy-Py-L₄-ImImIm (HSP-6)**

- **Pt-L₆-β-Ala-PyPy-Py-Py-L₄'(Pt)-ImImIm**

- $\beta$-Ala-PyPyPy$_4$(Pt)-ImImIm

- Pt-L$_6$-$\beta$-Ala-PyPyPy$_4$-ImImIm-L$_6'$-Pt-(Pt) (TNHLSP-6)
APPENDIX IV

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A4.5.3  β-Ala-PyPyPy-L₄-ImImIm-L₄-Pt (HLSP-6)  242
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A4.1.2  2-Trichloroacetyl-N-methyl-1H-imidazole (Im A)
A4.1.3 4-Nitro-2-trichloroacetyl-N-methyl-1H-imidazole (Im B)
A4.1.4  *tert*-Butyl-4-nitro-N-methyl-1H-imidazole-2-carboxylate (Im C)
A4.1.5 \textit{tert}-Butyl-4-(amino)-N-methyl-1H-imidazole-2-carboxylate (ImD-amino)
A4.1.6  *tert*-Butyl-4-[(9-fluorenylethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylate (Im D)
A4.1.7 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-imidazole-2-carboxylic acid (Im E)

A4.1.8  N-Methyl-1H-pyrrole (Py)
A4.1.9  2-Trichloroacetyl-N-methyl-1H-pyrrole (Py A)
A4.1.10 4-Nitro-2-trichloroacetyl-N-methyl-1H-pyrrole (Py B)
A4.1.11  *tert*-Butyl-4-nitro-\(N\)-methyl-1H-pyrrole-2-carboxylate (Py C)
A4.1.12  *tert*-Butyl-4-[(9-fluorenlymethoxycarbonyl)amino]-N-methyl-1H-pyrrole-2-carboxylate (Py D)
A4.1.13 4-[(9-Fluorenylmethoxycarbonyl)amino]-N-methyl-1H-pyrrole-2-carboxylic acid (Py E)
A4.1.14  *tert*-Butyl-2-aminoethylcarbamate (L2)
A4.1.15  tert-Butyl-3-aminopropylcarbamate (L₃)
A4.1.16  *tert*-Butyl-6-aminohexylcarbamate (L₆)
A4.1.17  (N-Methyl-1H-imidazole-2-carboxamido)butyric acid (Im-L4-COOH)

A4.1.18  $\beta$-Ala-Py-L$_4$-Im

A4.1.19 \( \beta \)-Ala-ImIm
A4.1.20  \( \beta \)-Ala-PyPyPy-L\textsubscript{4}-ImImIm
A4.1.21  \( \beta \text{-Ala-Py-L}_4 \text{-Pt} \)
A4.1.22 $\beta$-Ala-Im-L$_4$-Pt
A4.1.23 \( \beta \text{-Ala-PyPyPy-L}_4\text{-ImImIm-L}_4\text{-Pt (HLSP-6)} \)
A4.1.24 $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6'$-Pt-(Pt) (DNHLSP-6)
A4.1.25  \( \beta \)-Ala-PyPyImImIm-L\(_4\)'-PyPyPyPyPyPy-\( \beta \)-Ala-L\(_6\)'-Pt-(Pt) (DNHLSP-10)
A4.1.26  Pt-L₆-NHBoc

\[
\begin{align*}
\text{A4.1.27} & \quad \text{Pt-L}_{4}\text{-NH}_2 \\
\end{align*}
\]
A4.1.28 \( \text{Pt-L}_6\beta\text{-Ala-Py-L}_4\text{-Im} \) (HSP-2)
A4.1.29  NHBoc-\(\delta^\beta\)-Ala-PyPyPy-L,4-ImImIm
A4.1.30 \( \text{NH}_2\text{L}_6\beta\text{-Ala-PyPyPy-L}_4\text{-ImImIm} \)
A4.1.31  Pt-L₆-β-Ala-PyPyPy-L₄-ImImIm (HSP-6)
A4.1.32  NHBoc-Lαβ-Ala-PyPyPy-L4'(NHBoc)-ImImIm
A4.1.33  \( \text{NH}_2-L_{6-\beta}\text{-Ala-PyPyPy-L}_4'(\text{NH}_2)\text{-ImImIm} \)
A4.1.34 $\beta$-Ala-PyPyPy-L$_4'(\text{NH}_2)$-ImImIm
A4.1.35 Transplatin
A4.2.1  \( \beta \)-Ala-Py-L\(_4\)-Pt

A4.2.2  \( \beta \)-Ala-Im-L4-Pt
A4.2.3  β-Ala-PyPyPy-L₄-ImImIm-L₄-Pt (HLSP-6)
A4.2.4 $\beta$-Ala-PyPyPy-$L_4$-ImImIm-$L_6$'-Pt-(Pt) (DNHLSP-6)
A4.2.5 $\beta$-Ala-PyPyImImIm-L$_4$'-PyPyPyPyPy-\(\beta\)-Ala-L$_6$'-Pt-(Pt) (DNHLSP-10)
A4.2.6  Pt-L₆-β-Ala-Py-L₄-Im (HSP-2)
Pt-L$_6$-$\beta$-Ala-PyPyPy-L$_4$-ImImIm (HSP-6)
A4.2.8 Transplatin
A4.3.1  1:1 Guanosine:HLSP-6
A4.3.2  1:1 Guanosine:DNHLSP-6
A4.3.3 2:1 Guanosine:DNHLSP-6
A4.4.1 Im E
A4.5.1  β-Ala-Py-L₄-Pt

C_{22}H_{23}ClN_{4}O_{2}Pt
Exact Mass: 561.13
Mol. Wt.: 561.9

A4.5.2 $\beta$-Ala-Im-L$_3$-Pt
A4.5.3  β-Ala-PyPyPy-L₄-ImImIm-L₄-Pt (HLSP-6)

A4.5.4  $\beta$-Ala-PyPyPy-L$_4$-ImImIm-L$_6$'-Pt-(Pt) (DNHLS-6)
A4.5.5 \( \beta \text{-Ala-PyPyImImIm-L}_4'\text{-PyPyPyPyPy \text{-Ala-L}_6'\text{-Pt-(Pt) (DNLSP-10)} } \)

C_{24}H_{44}Cl_{10}O_{4}Pt
Exact Mass: 766.29
Mol. Wt.: 767.2

A4.5.6 Pt-L_{4}\beta-Ala-Py-L_{4}\Im (HSP-2)
A4.5.7 \( \text{Pt-L}_{6}\beta\text{-Ala-PyPyPy-L}_{4}\text{-ImImIm} \) (HSP-6)