Multinuclear Platinum(II) Complexes Containing Carboranes for Potential Use in Boron Neutron Capture Therapy

by

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B.Sc. (Hons)

A Thesis Submitted Towards the Degree of

Doctor of Philosophy

THE UNIVERSITY OF ADELAIDE
AUSTRALIA

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January 2004

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Acknowledgements

I would like to thank my supervisor, Dr Louis Rendina for giving me the opportunity to work with him on this project. I have thoroughly enjoyed my time under his supervision. His continuous support, enthusiasm, understanding and ingenuity throughout my Ph.D. have made this project enjoyable, challenging and achievable.

I would also like to thank my fellow lab members from Lab 10, for their advice and friendship. Especially Dr Daniela Caiazza and Dr Jean Todd for our organic chemistry discussions and triumphs. I have also thoroughly appreciated the company of Mike Crisp during those long days at the bench. Thanks must go to Jimmy Sykiotis for his preliminary work with the electrophoresis assay.

I would like to thank Dr George Gream for his help and friendly advice during my time at the University of Adelaide. Thanks also to Dr Simon Pyke, Dr Bruce May, Phil Clements and Graham Bull for their varying expertise, which were received gratefully.

I would also like to thank the past and present members of the MIB group for their assistance, friendships and including me in their social events.

Thanks to my friends and family, in particular to my Mum and Dad, who each in their own way have provided me with the support, desire and means to achieve all that I have.

And Ben, 'Thank you' (on any scale) will never be enough.

Contents

Abstract

Boron Neutron Capture Therapy (BNCT) is a binary method of cancer treatment that is currently undergoing clinical trials in several countries. It has several advantages over the more common, traditional treatment methods of cancer therapy. In particular, it is not essential to know the exact location of the tumour cells in order to achieve comprehensive destruction. This factor alone ensures no residual cancerous cells remain to act as foci for new tumour growth. The ability of the Neutron Capture process to induce cell death has been demonstrated to be optimal when the ^{10}B nuclei are adjacent to nuclear DNA.

Platinum anti-cancer drugs, such as cisplatin, are well known for their ability to target and bind to DNA. In particular, multinuclear platinum(II) complexes containing linear linking diamines have displayed a high affinity for DNA and an ability to overcome the biological mechanisms of DNA repair. The use of metal complexes as potential BNCT agents is unprecedented. Within this Thesis, multinuclear platinum(II) complexes containing icosahedral dicarba-*closo*-dodecaboranes (or carboranes) have been prepared for their potential application in BNCT. These compounds constitute a totally new type of boron-containing DNA-binding agents.

Chapter One presents a review of the current status of BNCT drug design and the unique biochemistry and DNA-targeting abilities of mono- and multinuclear platinum(II)-amine complexes.

The preparation and structural characterisation of bis(aminopropyl)carboranes and their corresponding multinuclear platinum(II) complexes is presented in Chapter Two. A number of dinuclear platinum(II) complexes containing both the 1,7- and 1,12-bis(aminopropyl)carboranes were prepared and characterised. Additionally, a novel method was utilised to allow the preparation of asymmetric dinuclear platinum(II) complexes containing 1,7-carborane in which each of the two platinum(II) centres possessed a unique ligand environment. The utilisation of a similar methodology permitted the preparation of novel trinuclear platinum(II) complexes, each containing two bridging bis(aminopropyl)carborane ligands.

Chapter Three describes a semi-quantitative restriction endonuclease DNA-cleavage assay, developed in our laboratories, which allows quantification of the ability of selected dinuclear platinum(II) complexes described in Chapter Two to bind to linearised III pBR322 plasmid. It was determined that the inclusion of the carborane cage did not greatly hinder the ability of the platinum(II) centres to covalently bind to DNA. Furthermore, those complexes incorporating cationic *trans*-PtN_3Cl centres were found to have a higher DNA affinity than cisplatin.

In Chapter Four, an overview of DNA-intercalators is presented, particularly focusing on the well-known 2,2':6',2''-terpyridine-platinum(II) DNA metallointercalators. The preparation of three bis(thiopropyl)carboranes containing 1,2-, 1,7- and 1,12-carboranes is described, along with the preparation and NMR spectroscopic characterisation of the corresponding dinuclear platinum(II)-2,2':6',2''-terpyridine complexes.

In summary, this Thesis presents the first examples of multinuclear platinum(II)-amine and platinum(II)-2,2':6',2''-terpyridine complexes containing carborane ligands. These complexes represent two new classes of potential BNCT agents with avid DNA-binding and *in vitro* biological activity in the absence of neutrons.

Abbreviations

δ	chemical shift
ε	extinction coefficient
μM	micromolar
A	Adenine
BBB	Blood Brain Barrier
BNCT	Boron Neutron Capture Therapy
BOC	*tert*-butyloxlcarbonyl
br	broad
bp	base pairs
n-BuLi	*n*-butylithium
^{t}Bu	*tert*-butyl
C	Cytosine
°C	degree(s) Celsius
calcd	calculated
carborane	icosahedral dicarba-*closo*-dodecaborane(12), $C_2B_{10}H_{12}$
cm	centimetre
cod	1,5-cyclooctadiene
COSY	^{1}H-^{1}H Correlated Spectroscopy
ctDNA	calf thymus DNA
d	doublet
D	deuterium
2D	two-dimensional
dmf	*N,N'*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dt	doublet of triplets
edta	ethylenediamine tetraacetate
EGF	epidermal growth factor
ethidium	3,8-diamino-5-ethyl-6-phenylphenanthridium
EtOH	ethanol
g	grams

G	guanine
h	hour(s)
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	hertz
K_d	dimerisation constant
K_s	stability constant
L(')	ligand
LDL	low density lipoproteins
J	coupling constant
$^nJ_{ij}$	n bound coupling constant between nuclei i and j
LET	linear energy transfer
m	multiplet
M^+	molecular ion
mg	milligrams
min	minutes
mL	millilitres
mmol	millimoles
m/z	mass per unit charge
NC	Neutron Capture
NCT	Neutron Capture Therapy
NER	nucleotide excision repair
nm	nanometre
NMR	nuclear magnetic resonance
oligos	oligonucleotides
OTf	triflate, trifluoromethansulfonate, $CF_3SO_3^-$
P	protecting group
PDT	photodynamic therapy
PEG	polyethylene-glycol
Ph	phenyl
qu	quintet
R_f	retention factor

s	singlet
t	triplet
tert-	tertiary
THF	tetrahydrofuran
t.l.c.	thin layer chromatography
TMS	trimethylsilyl, $SiMe_3$
trpy	2,2':6',2''-terpyridine
UV	Ultra Violet
vis	visible
X	leaving group, halogen

CHAPTER ONE

Introduction

Introduction

1.1. The Treatment of Cancer

In the treatment of cancer, a therapeutic method that selectively destroys malignant cells in the presence of normal cells is a highly valued goal. Treatments currently available for many solid tumours remain inadequate in their therapeutic management and control. These inadequacies have become most apparent in the treatment of malignant brain tumours.[1] Unlike most malignant tumours, those of the brain do not tend to metastasise elsewhere in the body, however they are highly infiltrative of the brain itself. Like most solid tumours, surgical removal of those of the brain is the method of choice. Unfortunately, there is an uncertainty with such surgery as to whether all the cancerous cells have been removed as residual cells may act as the foci for recurrences of the cancer. To ensure complete eradication of cancerous cells, surgery is usually used in combination with radiotherapy or chemotherapy.

In the case of radiotherapy, the magnitude of the dose required to kill cancer cells is such that nearby normal cells are severely compromised.[1] As a result, the ability to use radiotherapy is limited by normal tissue tolerance and the unknown location of all cancer cells. Similarly, in chemotherapy the drug effect is not confined to the tumour, thus adversely affecting normal cells and the dose of drug is once again limited by normal tissue tolerance.

The toxic effects of radiation and chemotherapy cannot be reversed,[1] and highly malignant tumours demonstrate increased resistance to radiation and chemotherapy with continued exposure to these agents. These limitations have stimulated the search for new cancer therapies.

1.1.1. Binary Systems

The concept of a binary method for the treatment of cancer involves two components, each relatively harmless to the cell. Upon combination, ideally restricted within tumour cells, a lethal cytocidal effect is generated. The tumour cells are eradicated

without compromising nearby normal tissue from which the tumour has arisen, making it a selective form of treatment for tumours, even those that are resistant to conventional therapies.

Each component of a binary system can be manipulated independently. Only when levels are optimised to maximise the cytotoxic affect toward malignant cells and produce the maximum tolerated dose to adjacent normal cells are the two components combined. Practically, at least one of the two components would be confined to tumour cells while the second component is exposed to all cells in the vicinity of the tumour. As a result, knowing the exact location of the tumour cells is not essential to achieve comprehensive destruction and no residual cancerous cells remain to act as foci for new tumour growth. Examples of some therapeutic binary systems in developmental stages include radiation sensitisers, photon activation therapy, photodynamic therapy and Neutron Capture Therapy (NCT).[2]

1.1.2. Neutron Capture Therapy

The term Neutron Capture Therapy (NCT) refers to the radiation generated from the capture reaction of thermal neutrons by certain nuclides. NCT is used as a means of selectively destroying tissue, especially malignant tumours. Thermal neutrons have an energy of 0.025 eV, well below the threshold required to ionise tissue components. However, some nuclei absorb thermal neutrons and undergo prompt fission, generating lethal energetic particles.[1] If this outburst of energy could be confined to only malignant cells, they could be destroyed selectively without affecting adjacent normal cells.

Experiments have shown that the ability of an atomic nucleus to capture a thermal neutron does not depend on the mass of the nucleus, but rather the structure of the nucleus. The likelihood of neutron capture is expressed in terms of a characteristic cross-sectional area of the nucleus, in units of 10^{-24} cm^2 or barns.[2] The suitability of a nucleus to undergo neutron capture depends on its nuclear cross-section. The nuclear cross-sections of some elements, including some commonly found in tissue, are shown in Table 1.1.[1]

In NCT the products of fission must also be considered. The products may have a considerable mass, such as α-particles from ^{10}B, or they may not, such as γ-rays from ^{157}Gd. The energy released by the neutron capture reaction must also be considered, as the objective of NCT is to retain the fission product in the tumour cell where they originate, thus preventing destruction of adjacent normal cells.

Element	Nuclear Cross-Section (barns)
^{10}B	3838
^{157}Gd	255000
^{235}U	580
^{1}H	0.332
^{6}Li	942
^{12}C	0.0034
^{14}N	1.82
^{16}O	1.8×10^{-4}
^{22}Na	32000
^{23}Na	0.43

Table 1.1 Thermal neutron capture cross-section of some nuclides, (1 barn = 10^{-24} cm^2).[1,2]

1.1.2.1. Boron Neutron Capture Therapy

After fleeting interests in the neutron capture by ^{235}U and ^{6}Li, the latest emphasis in NCT has been in ^{10}B-containing compounds. The other naturally-occurring isotope of boron, ^{11}B, is incapable of undergoing neutron capture, whereas ^{10}B has a large nuclear cross-section (3838 barns). Many different boron-containing compounds can be synthesised as boron is capable of forming stable bonds with carbon, oxygen and nitrogen. With a natural abundance of 19.8%, the non-radioactive ^{10}B nucleus is an ideal candidate for the safe treatment of cancer using the neutron capture process.[2] The major pathway (94%)[2] for thermal neutron capture by a ^{10}B nucleus is shown in Equation 1.1.

$$^{10}_{5}B + {}^{1}_{0}n \rightarrow {}^{4}_{2}He^{2+} + {}^{7}_{3}Li^{+} + 2.31\,MeV \qquad \textbf{Equation 1.1.}$$

The radiation produced by ^{10}B on neutron capture is comprised of high linear energy transfer (LET) particles. Such entities are highly energetic, but due to their relatively large size, they travel only short mean paths. Their ionisation tracks are consequently approximately 0.01μm long (approximately the diameter of a typical cell), and therefore an immense amount of energy is dissipated in a small volume. Consequently, upon neutron capture by ^{10}B in a cell, the radiant energy produced would be confined within the cell from which the fission products arose and would be of lethal magnitude, readily damaging biological materials by ionisation processes, thus achieving the primary objective of NCT.[2]

Recent research has focussed on the utilisation of ^{157}Gd nuclei for NCT.[1] ^{157}Gd has an cross-section for thermal neutrons that is 66 times that of the ^{10}B nuclei.[1] However, its use is limited by the products of the neutron capture reaction (γ-rays and Auger (low energy) electrons).[1,2] Unlike the α-particles produced from the neutron capture reaction of ^{10}B nuclei, the lack of a considerable mass of the γ-rays means the transfer of their kinetic energy takes place over a much longer path length. Consequently, the resultant ionisation paths are not confined to the cell in which the γ-rays arise and would damage any adjacent normal cells. In contrast, the low energy of the generated Auger electrons is rapidly transferred to the surrounding media, leaving considerably short ionisation tracks. The ^{157}Gd nuclei would have to be located very close to important cellular components, such as DNA, in order to successfully damage them. This makes the development of a clinically effective ^{157}Gd agent even more challenging as it must be highly selective for critical macromolecules such as DNA.

The potential application of BNCT becomes complicated when considering the tissue surrounding the tumour. The normal elemental components of tissue have considerably low nuclear cross-sections (Table 1.1) in comparison with ^{10}B. However, in the case of ^{1}H and ^{14}N nuclei, their high concentrations in tissue can potentially result in a significant level of neutron capture side-reactions, thus contributing to the

total radiation dose (derived from the tissue exposed to the beam). The neutron capture reactions of ^{1}H and ^{14}N are shown in Equations 1.2. and 1.3.

$$^{1}_{1}H + {}^{1}_{0}n \rightarrow [{}^{2}_{1}H] \rightarrow {}^{2}_{1}H + \gamma\ 2.23\,MeV \qquad \textbf{Equation 1.2.}$$

$$^{14}_{7}N + {}^{1}_{0}n \rightarrow [{}^{15}_{7}N] \rightarrow {}^{14}_{2}C + {}^{1}_{1}p\ 0.63\,MeV \qquad \textbf{Equation 1.3.}$$

To minimise the radiation contribution from ^{1}H and ^{14}N neutron capture, the BNCT agent must be somewhat tumour specific. The concentration of ^{10}B must be high in tumour cells (*ca.* 10^{9} nuclei per cell),[3,4] and high enough to offer a radiation dose that is well above the amount delivered from neutron capture by ^{1}H and ^{14}N. Ideally 85% of the radiation should be due to neutron capture by ^{10}B alone.[2]

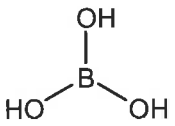
As is common in the search for a drug with a specific biological action, the challenges in the search for a suitable BNCT agent are numerous. The concentration of the agent in the tumour must be such that a therapeutic affect is achieved, however there must be tumour specificity to minimise radiation dose to surrounding tissue. Ideally, the tumour concentration should be greater than the normal tissue concentration by a factor of 3-5.[1] The BNCT agent must possess a sufficiently low toxicity so the appropriate dose can be well-tolerated by patients. The agent must not only target the tumour, but it must also persist in the cancer cell for long enough so the radiation delivered will be selective for malignant cells only.

1.2. Approaches to BNCT Drug Design

As described earlier, many different classes of boron-containing compounds can be prepared as boron is capable of forming stable bonds with carbon, oxygen and nitrogen. There is a broad spectrum of synthetic boron chemistry, from simple boronic acid analogues and single B-atom boranes to families of polyhedral boranes stabilised by three-dimensional electron delocalisation.

Boric acid (**1**) is often incorporated into compounds by replacement of a single hydroxyl group with an organic moiety, such as in the ^{10}B-containing amino acid

analogues, **2**,[5] and BPA (**3**),[6,7] one of only two compounds currently being evaluated in BNCT clinical trials.



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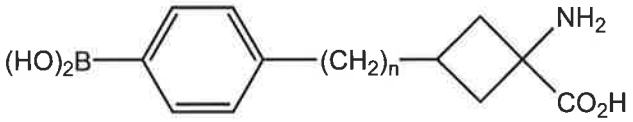

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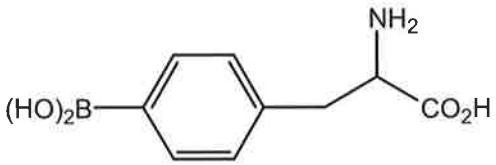

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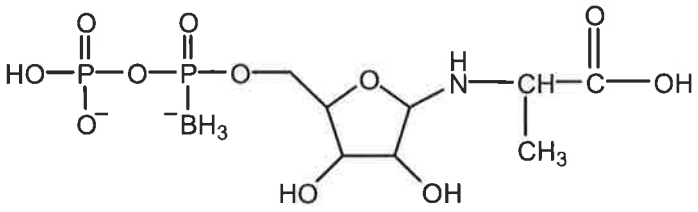
Boron can also be incorporated as a typically unstable borane,[8,9] through stabilisation of the tetra-substituted anionic centre by electron donating functional groups, such as in the boranophosphate containing nucleoside **4**.[9]



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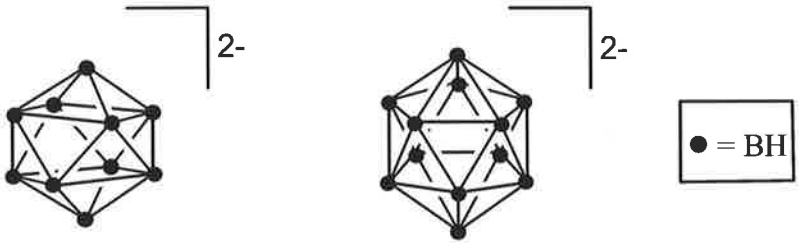
Each of the three boron-containing compounds described above contain only one boron atom. As concentration of ^{10}B nuclei within tumour cells is directly proportional to the level of neutron capture products and the consequential cell death, compounds containing multiple boron atoms offer more desirable BNCT characteristics. A compound containing multiple boron atoms could be administered in lower concentrations than those containing a single boron atom and achieve similar or higher concentrations of ^{10}B nuclei within the tumour.

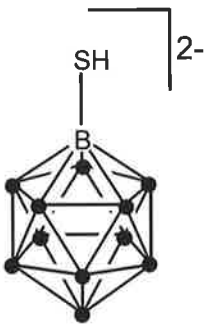
A great deal of effort has gone into developing BNCT agents containing multiple boron atoms. The most suitable sources of multiple boron atoms have, over the years,

proved to be the polyhedral borane anions and carboranes. The polyhedral borane anions $B_{10}H_{10}^{2-}$ (**5**) and $B_{12}H_{12}^{2-}$ (**6**) are often incorporated into potential BNCT agents.[10-13] Their cage-like structures are inherently stable and easily functionalised with organic moieties. The charge of the cage facilitates hydrophilicity and effective systematic biodistribution, however it can also significantly affect the biodistribution and transport of the compound into tumour cells, as it can hinder the penetration of the lipophilic cellular membrane.[1]



5 **6**

Along with **3**, the other ^{10}B-compound currently being evaluated in clinical trials is sodium borocaptate (Na_2[*closo*-$B_{12}H_{11}SH$] or BSH) (**7**).[14] **7** was first introduced in clinical trials in the 1960s and it has since demonstrated variable results. While clinical trials continue today, it is generally accepted that BNCT with **7** is comparable to contemporary photodynamic therapy in prolonging of the patient life.[15-17]



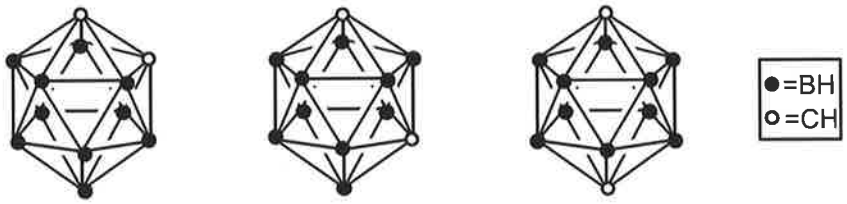
7

1.2.1. Carboranes and BNCT

Boron cluster compounds in which one or more carbon atoms form part of the boron framework are commonly called 'carboranes'. The most widely used carboranes are

the icosahedral dicarba-*closo*-dodecaboranes(12) or $C_2B_{10}H_{12}$.[18] The $C_2B_{10}H_{12}$ carboranes have become a popular choice in the development of BNCT agents. Not only do they offer relatively simple chemistry and high ^{10}B content, but also excellent kinetic stabilities and they are particularly robust *in vivo*.[2] Methods for incorporation of carboranes into various organic and biological substrates are well understood.[1]

Three isomers of $C_2B_{10}H_{12}$ carboranes are known, differing only in the arrangement of the two carbon atoms, and are referred to as 1,2- (*ortho*, **8**), 1,7- (*meta*, **9**) and 1,12- (*para*, **10**) carboranes.



8 **9** **10**

Thermal rearrangement of the least stable 1,2-carborane (**8**) at 400-500°C in an inert atmosphere gives the 1,7-isomer (**9**).[19] Further heating above 600°C allows rearrangement to the most thermodynamically stable 1,12-carborane (**10**).[20]

The high concentration of ^{10}B nuclei in the $C_2B_{10}H_{12}$ carboranes makes them ideal sources of ^{10}B nuclei in BNCT. Many analogues of naturally-occurring biological entities have been modified to include $C_2B_{10}H_{12}$ carboranes in an attempt to achieve the high intracellular concentrations of ^{10}B nuclei needed for sufficient levels of neutron capture. However, the highly lipophilic character of the cage can cause such derivatives to interact mainly with the lipophilic parts of cells, such as the cell membrane. This can greatly alter the biodistribution of the BNCT agents such that the carboranyl-compounds become trapped in the cellular membranes, unable to reach cellular DNA.[21]

1.2.2. Targeting the Cancer

The early development of BNCT was not particularly successful owing to a complete lack of tumour selectivity and impure neutron beams. Treatment was solely directed towards brain tumours and selective delivery was based on the increased permeability of the Blood Brain Barrier (BBB) in the tumour compared with normal brain.[1] The immediate limitation of this treatment was any isolated clusters of tumours that were protected by the normal BBB were consequently not targeted by any of the drug. Arterial administration rather than intravenous injection showed an elevation of drug concentration, but the BBB still restricted the entrance of some compounds into the brain, especially those of high molecular weight.[1] It was soon realised that the specific delivery of BNCT agents to tumour sites was not going to be a facile task. BNCT agents must be transported through blood vessels, across cell membranes and incorporated into cells at sufficiently high concentrations.

More recently the design of potential BNCT agents has become increasingly sophisticated. The biochemistry of tumours is a rapidly evolving subject of research. Tumours are known to possess unique membrane processes including their receptors and ion channels, allowing for the development of BNCT agents based on biochemical or physiological principles. Potential BNCT agents have been synthesised after great consideration of the biological and metabolic differences between tumour and normal cells, allowing low toxicity, selective and high concentrations of drug and persistence in tumours.

1.2.3. Optimising Neutron Capture Results

The required concentrations of ^{10}B nuclei are generally accepted to lie between 10-30 μg ^{10}B per gram of tumour or 10^9 nuclei per cell, with the lowest concentrations required if the ^{10}B is localised within the nucleus of the cell.[2,22] It is generally accepted that the target of the high LET products from the neutron capture reaction is DNA within the tumour cell nucleus. DNA is a highly sensitive cellular component and the ionisation tracks left by LET particles damage the structure of DNA and are sufficient to disrupt the delicate balance of cellular processes within the cell, causing cell death. The dose of LET particles experienced by the nucleus is strongly

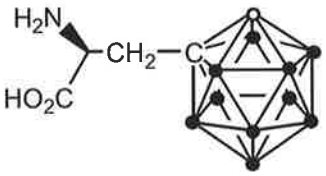
dependent on the location of the ^{10}B nuclei relative to the radiation sensitive nucleus. ^{10}B nuclei accumulating in the cell nucleus are up to five times more efficient in cell killing than the same amount of ^{10}B nuclei distributed uniformly throughout the cell.[23,24]

There have been several different approaches explored in an attempt to deliver ^{10}B nuclei as close as possible to the cell nucleus. As mentioned previously, biodelivery systems are now considered to be the most promising for targeted delivery, with a biochemical and/or physiological basis for achieving selectivity. The small atomic size of boron allows it to replace carbon in organic structures, allowing entry into such biodelivery pathways. However, it is essential that the boron substitution does not adversely affect the solubility, toxicity or tumour targeting ability of the parent compound.

1.3. Biological Pathways of BNCT Drug Delivery

1.3.1. DNA Building Blocks

Precursors to DNA synthesis are especially desirable to achieve incorporation of ^{10}B nuclei inside the cell nucleus, the primary target of neutron capture reaction products.[9] Boron-containing analogues of cellular building blocks such as nuclei acids (**4**),[9,25-27] amino acids (**11**)[5,28-33] and carbohydrates (**12**)[34] have been designed in an effort to sufficiently emulate naturally-occurring precursors needed for cellular replication. The basis for their development is that in contrast to normal cells, tumour cells have a faster rate of division and thus have a higher requirement for biochemical substrates essential for proliferation. As a result, the tumour cells would acquire a high ^{10}B-content in comparison to normal cells. However, normal cells with a rapid rate of proliferation, such as hair and gastrointestinal epithelial cells, would also acquire high ^{10}B concentrations. However, problems associated with host toxicity and adverse effects on cellular processes have been encountered.



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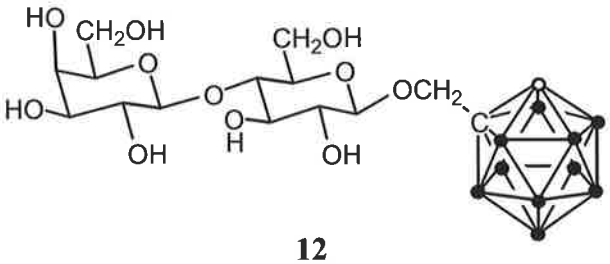
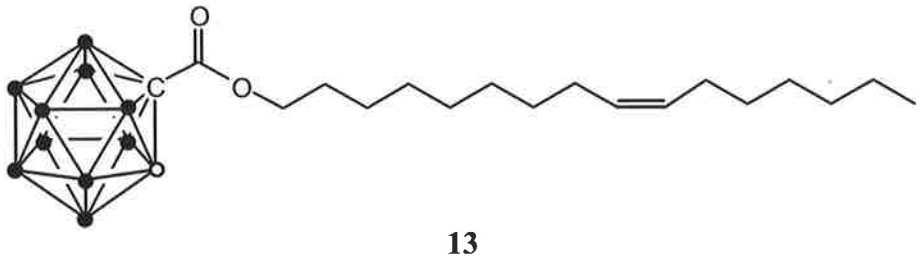


12

1.3.2. Lipoproteins

Low-density lipoproteins (LDLs) contain around 1500 molecules of cholesterol per LDL particle and normally function as the main carrier of cholesterol in blood circulation, providing 90% of cholesterol to tissues.[35,36] Tumour cells have a high uptake of LDLs compared to normal cells, as the rapidly proliferating tumour cells require high levels of cholesterol for new membrane formation.[1,36] This higher requirement for LDLs is facilitated by an over-expression of LDL receptors on the surface of the tumour cell membrane.[35]

A range of esters composed of carborane carboxylic acids and various long chain alcohols (such as **13**) have been developed to mimic cholesterol.[1,2,36,37] Cholesterol was removed from LDLs and replaced with synthetic esters allowing ^{10}B nuclei to be delivered intracellularly. *In vitro* studies have demonstrated high cellular uptake,[1,2] in some cases intracellular ^{10}B levels were up to ten times the desired concentration.[36]



13

1.3.3. Liposomes

Related to the LDLs is a class of vesicles referred to as synthetic small unilamellar liposomes. Liposomes are synthetic, composed of a phospholipid bilayer forming a spherical shell surrounding an aqueous core (Figure 1.1). Liposomes have attracted substantial interest in their use as drug delivery tools in the therapy of cancer and

infectious diseases.[38-40] Such liposomes have been labelled 'supertankers', potentially capable of delivering large quantities of ^{10}B to tumour cells.

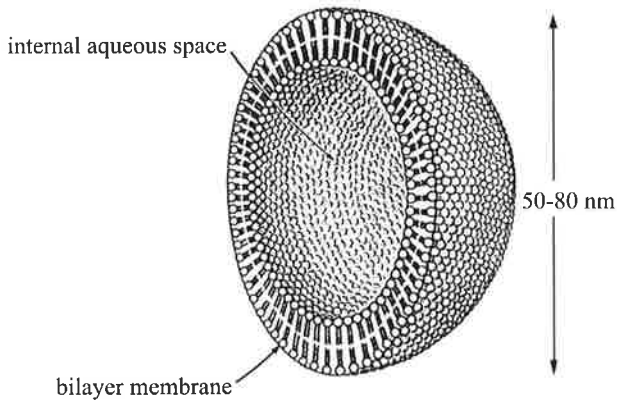
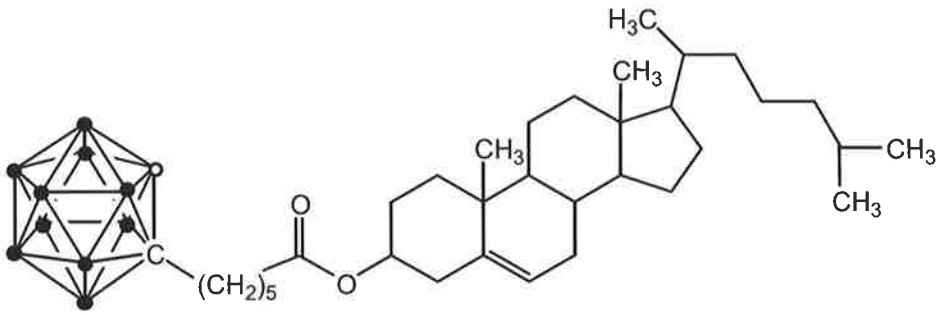


Figure 1.1 Cross-section of 'supertanker'.[2]

Liposomes are not internalised by normal cells, however they have been shown to selectively penetrate the tumour cell membrane and localise intracellularly.[2] It is believed that the liposomes are the right size to penetrate the network of immature and, consequently, abnormally porous blood vessels and capillaries that rapidly-growing cancers induce to form around them in a process known as angiogenesis.[2] As this leakage occurs around the tumour, the cancer cells are able to absorb the liposomes. The advantages of liposomes include the ability to encapsulate large amounts of water-soluble drugs in the aqueous core or lipophilic drugs in the phospholipid bilayer, allowing delivery to specific tumour sites, sparing healthy tissue from toxic effects and increasing the circulation time of the drug.[41]

Long-chain carborane ester derivatives or cholesterol esters, such as **14**, have been incorporated into the liposome phospholipid bilayer and an aqueous solution of sodium salts of anionic isocyanate polyhedral borane derivatives have been placed in the central core.[41-44]

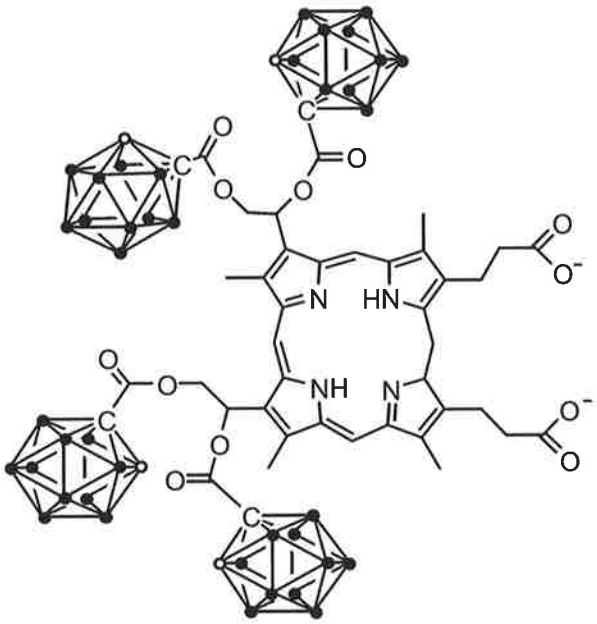


14

An advantage of liposomes is that unlike other biodelivery systems, the ^{10}B nuclei may be incorporated such that they do not alter the tumour-targeting ability of the liposome, ensuring the selective delivery of ^{10}B nuclei. Liposomes are not naturally-occurring and as a result they are readily removed from the circulatory system, leading to the development of sterically-stabilised liposomes.[39] The most frequently-used method of stabilisation involves the attachment of polyethylene-glycol (PEG) chains to the surface of the liposome, prolonging circulation time.[41-43,45-47] Attachment of a conjugate of epidermal growth factor (EGF) to the exterior of a PEG-stabilised liposome can further assist the tumour-seeking ability of the liposome as EGF-receptors are over-expressed in many tumour cells.[46,47] Liposomes loaded with 10^4-10^5 water-soluble boronated acridine molecules have been shown to be internalised following specific binding to the EGF receptor, and the ^{10}B-containing contents are evenly distributed within the cytoplasm.[46,47]

1.3.4. Porphyrins

While the precise mechanism is unclear, porphyrin macrocycles are capable of highly selective incorporation within tumour cells and persist there in the long term.[48,49] This is utilised in another binary treatment of cancer, photodynamic therapy (PDT), a photosensitising method where the macrocycle generates singlet oxygen upon irradiation with light of a specific wavelength. *In vivo* distribution studies of carborane-containing porphyrin BOPP (**15**) appear promising, showing high tumour selectivity and concentrations.[50-52] Promising research into other ^{10}B-containing porphyrins is currently underway.[53-56]



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1.3.5. Antigens and Immunoglobulins

Tumour walls have a variety of antigens for the recognition and binding of specific immunoglobulins. Some antigens are specific to, or over-expressed by, tumour cells in comparison with normal cells. The incorporation of ^{10}B-containing compounds into proteins within tumour-targeted antibodies has been successful, without causing denaturation or affecting antibody activity and specificity, allowing delivery to antigen sites in tumour cell membranes.[11,57-61] A limitation is that unlike the liposome 'supertankers' described above, the boron must be covalently bound to the antibodies to prevent loss in the vascular system or in tissues, hence results to date have achieved only low intracellular ^{10}B concentrations.

1.3.6. DNA Binders

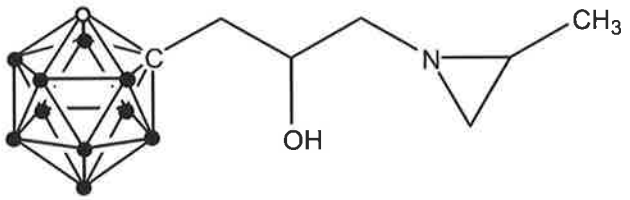
As mentioned earlier, the cytocidal effect of the neutron capture reaction involving ^{10}B nuclei is greatly enhanced when the nuclei are localised close to the nuclear DNA, directing recent BNCT developments into new DNA building blocks. DNA also contains a large number of binding sites, hence an alternative would be the development of ^{10}B-containing compounds that bind directly to the macromolecule.

Several types of DNA-binding drugs have been investigated for their potential application as ^{10}B-delivery agents.

1.3.6.1. Alkylating Agents

Electrophilic alkylating agents, such as nitrogen mustards, readily react with nucleophilic sites in DNA, resulting in its substitution (alkylation). Such lesions make DNA unstable and are thought to be responsible for cytotoxicity of nitrogen mustards such as chlorambucil, melphalan and HN2, through consequential interference with cellular processes and induction of apoptosis.[62]

Recently, icosahedral-carboranyl aziridines such as MACB (**16**) have been reported.[63-65] MACB alkylates DNA at the phosphate and purine bases *via* the aziridine group, thus irreversibly incorporating ^{10}B into the DNA molecule. Most importantly, there is evidence for selective incorporation of MACB into B16 melanoma cells and treatment of certain cell lines with MACB results in significant growth inhibition.[64]



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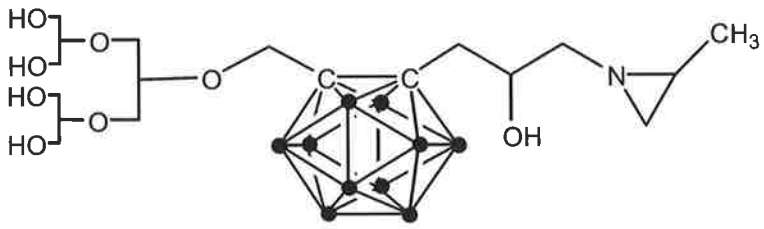


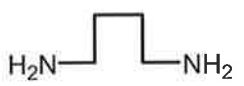
17

The development of a synthetic pathway to produce cascade polyols attached to the carborane cage has successfully increased the water solubility of a number of carborane-containing compounds.[66,67] The attachment of cascade polyols to modified aziridines (such as **17**) has also led to a substantial increase in water solubility in

comparison with MACB, consequently lowering cytotoxicity and increasing ^{10}B uptake by cancer cells.[65]

1.3.6.2. Polyamines

Low molecular weight polyamines such as putrescine (**18**), spermidine (**19**) and spermine (**20**) are ubiquitous, naturally-occurring and essential biological molecules involved in cell growth and replication.[68] Under physiological conditions, polyamines are positively charged and bind electrostatically to DNA in a tight, yet non-specific manner.[69-71] Cells are equipped with a specific transport system, allowing uptake of polyamines from the surrounding environment. The specificity of this transport system is not stringent and a wide variety of synthetic polyamine analogues are capable of exploiting this system.[72]



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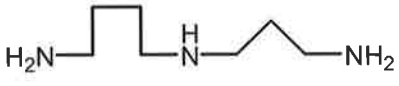

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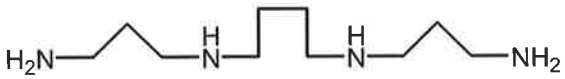

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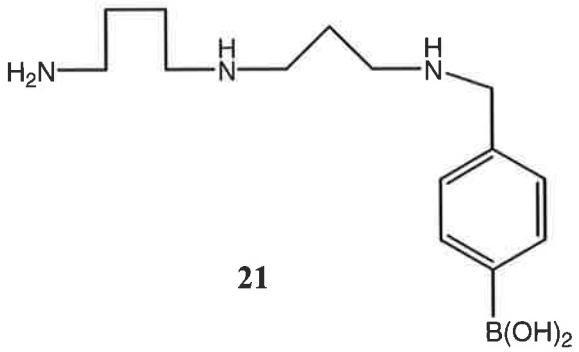
Elevated levels of polyamines are found in the nucleus of rapidly proliferating tumour cells, facilitated by the increased polyamine transport system of tumour cells.[70,71] Consequently, many tumour cells have demonstrated an uptake of synthetic polyamines, structurally related to endogenous ones.[73] This affinity of synthetic polyamines for tumour cells and DNA has led to the development of ^{10}B-containing putrescine, spermidine and spermine analogues (such as **21**) as potential ^{10}B-delivery agents for BNCT.[70-75]



21

The ^{10}B-containing spermidine, **21**, demonstrated a higher affinity for DNA than parent spermidine (**19**) and it is less toxic than the clinically-used **7** and previously reported carborane-containing polyamines.[70-72,75] Intracellular levels of ^{10}B were higher than that achieved by **3** and **7**, despite each molecule only containing one boron atom. However, it failed to achieve the ideal ^{10}B-concentration of greater than 10 μg per gram of cancer tissue.[72] Some tumour selectivity was observed, but the ratios were not sufficient for clinical use.[72] Such derivatives remain promising BNCT agents and are still being developed with modifications including increasing the number of ^{10}B atoms within the molecule.[72]

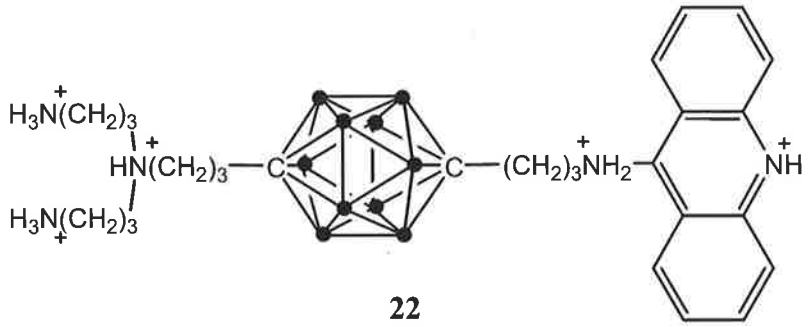
1.3.6.3. DNA Intercalators

DNA intercalators are small planar aromatic heterocyclic molecules that can insert or stack between the base pairs of double-helical DNA.[76] Many intercalators display high DNA-binding affinities and anti-cancer activity.[77]

Early research into carborane-containing acridine dyes failed to achieve selective delivery to tumour cells.[78] This is not unexpected when considering the hydrophobic nature of the icosahedral carborane cage. As seen in other biodelivery models, decreasing the hydrophobicity of such carborane-containing compounds by insertion of hydrophilic functional groups adjacent to the carborane cage has improved their biodistribution.[65]

1,12-Carborane has been functionalised to form **22**, containing both a spermidine residue and an acridine system. While the acridine functionality serves as a DNA-

intercalating fragment, the spermidine functions as both a hydrophilic water-solubilising and DNA-binding unit.[79]



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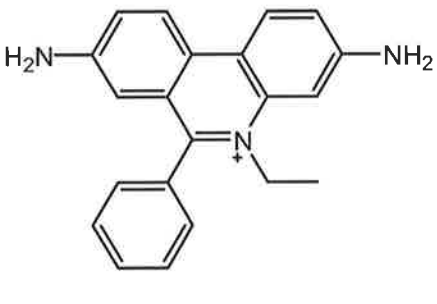
The widely studied intercalator ethidium (**23**) has also been modified to include *nido*- or 1,12-carboranylpropyl groups at the N5 (**24**) or C6 (**25**) position. While these derivatives have retained the strong DNA-binding capacity, they not only accumulate in cell nuclei, but to a greater extent in other cell constituents.[80,81] In order to increase hydrophilicity and decrease non-specific binding, an ethidium derivative containing an aminoalkyl substituted 1,12-carborane (**26**) was prepared.[81]



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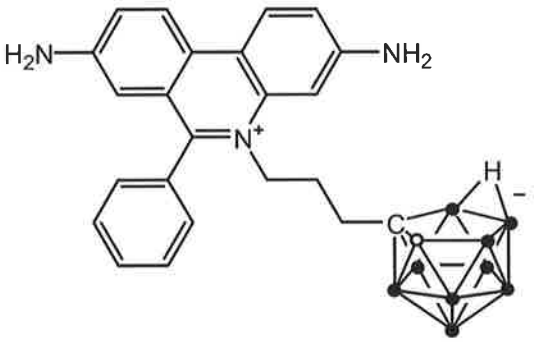

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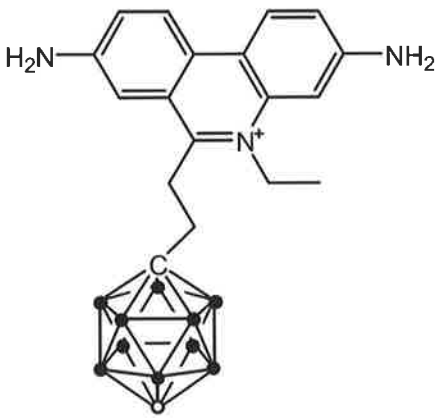

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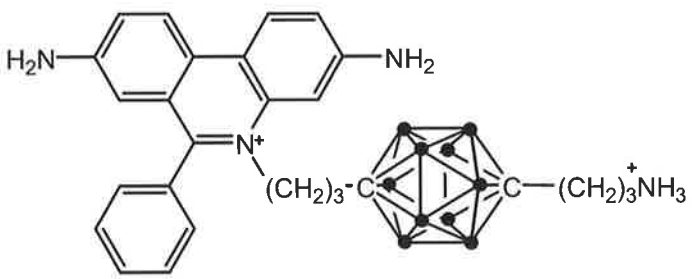


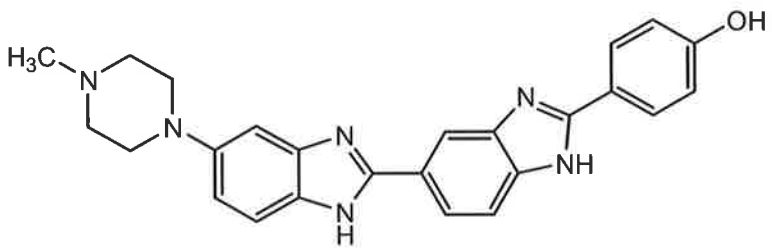
26

While such derivatives are able to intercalate DNA, they are too toxic to be considered suitable for BNCT. The lipophilicity of the carborane cage dominates their biodistribution, and the derivatives remaining trapped in the cellular membrane, thereby decreasing the ability of the ^{10}B nuclei to reach the nuclear DNA.[21,82]

1.3.6.4. DNA Groove Binders

Many DNA-binding molecules bind to the minor and major grooves of DNA. Most bind to the major groove, while some small molecules bind to the minor groove.[83] Many groove binders have anti-cancer, anti-parasitic, anti-bacterial or anti-viral activity.[84]

Hoechst 33258 (**27**) is a crescent-shaped bisbenzimidazole that is used as a fluorescent stain for *in vitro* DNA studies.[85] Hoechst 33258 exhibits a distinct selectivity for binding within AT-rich DNA sequences in the minor groove.[86] The compound was initially found to have anti-cancer activity, however clinical trials did not pass the Phase II stage.[83,87]



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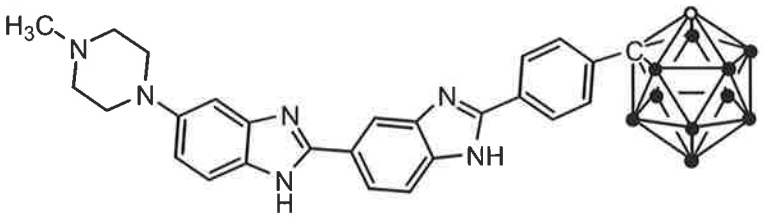
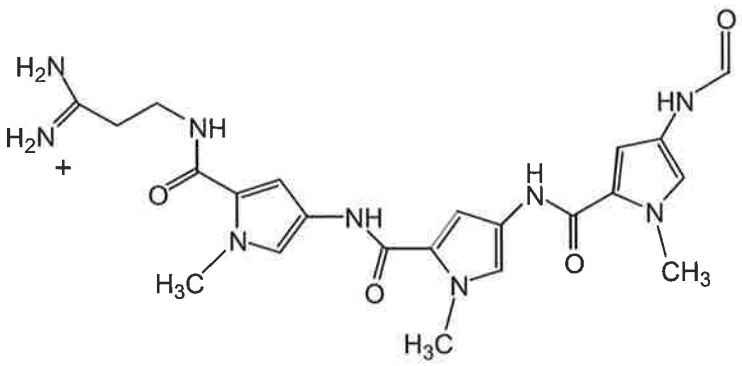
Extensive NMR studies and X-ray structure analyses of Hoechst 33258-DNA interactions have established the NH of the two benzimidazole rings form hydrogen bonds with the N3 atom of adenine and O2 atom of thymidine.[83] Based on this knowledge of the mode of DNA-binding, researchers have attempted to functionalise Hoechst 33258 to include ^{10}B in such a way that modifications do not interfere with DNA-binding and affinity. This approach allows the delivery of ^{10}B nuclei to the nucleus of the cell, optimising the efficiency of the neutron capture reaction.[22,85,88,89]

A Hoechst 33258 analogue has been prepared containing an icosahedral 1,2-carborane cage (**28**), however it demonstrates a reduced DNA-binding capacity.[22] This is most likely due to steric hindrance between the minor groove of DNA (diameter = 0.57 nm) and the bulky carborane cage (diameter = 0.86 nm).[88] An OCH_2 linker has been placed between the carborane cage and the bisbenzimidazole,[85,88] however the DNA-binding characteristics of these derivatives remains relatively weak[88] or unpublished. Further studies are currently directed towards analogues containing longer linker chains, increasing the distance between the carborane cage and the bisbenzimidazole moiety. Any minimisation of steric hindrance is expected to result in DNA-binding that is comparable to that of the parent Hoechst 33258.[88]



28

The groove binder distamycin (**29**) is a crescent shaped tripeptide that binds to the minor groove of DNA at sites of four or five successive AT base pairs.[90]



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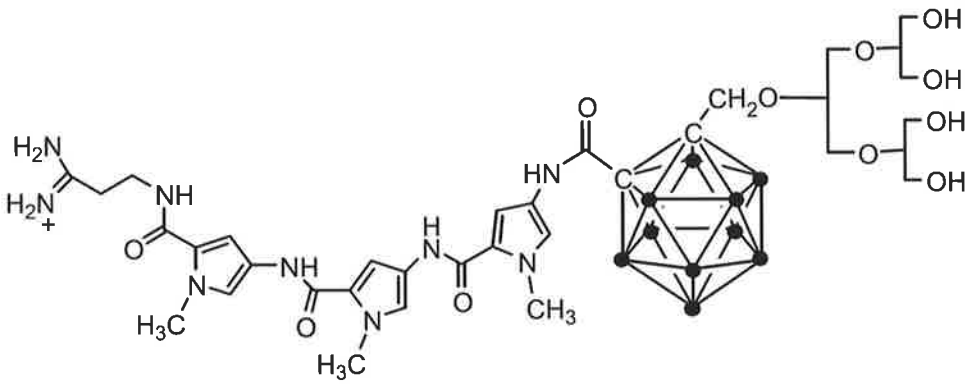
Similarly to Hoechst 33258, the binding of distamycin to DNA is dominated by hydrogen bonding interactions between the distamycin amide NH groups and N3 atom of adenine and O2 atom of thymidine.[83] Distamycin has been modified to include a carborane cage with water solubilising cascade polyols (**30**).[91] **30** was found

to bind to the same sites as parent distamycin with the four hydroxyl groups increasing the overall water solubility. Biodistribution and toxicity of **30** both *in vivo* and *in vitro* are yet to be reported.



30

1.3.6.5. Di- and Oligonucleotides - Antisense Agents

The term 'antisense' oligonucleotides (oligos) refers to synthetic oligonucleotides that bind specifically by Watson-Crick base pairing to complementary nucleic acids such as single-stranded DNA and RNA. Various cellular processes can be affected depending on where the oligonucleotide binds. Such binding of oligonucleotides is regularly used in Nature to inhibit and activate gene expression.[92] The preparation of oligonucleotides has been studied for their potential use in genetic engineering, to inhibit or control growth of viruses, and to specifically control the expression of oncogene or genes associated with other genetic disorders.[8]

From the view of drug design, antisense oligos enable the genetic targeting of new drugs. Once the nucleotide sequence within the target nuclei acid is known, a drug containing the appropriately sequenced oligonucleotide fragment can potentially bind to specific genes.

Antisense oligos made for BNCT have included ^{10}B in : (i) the phosphodiester bond[1,8,93]; or (ii) the nucleoside moiety.[1,94] In the case of carboranyl oligos, the thermostability of duplexes formed by the synthetic oligos with DNA was affected by the location of the carborane within the nucleic acid chain.[1] While preliminary biological studies appeared promising,[94] molecular modelling studies have

demonstrated an interference by the bulky icosahedral carborane cage on the binding of the ^{10}B-containing antisense oligos with the target nucleic acids.[1]

1.4. DNA-Targeting of Drugs by Metal Complexes

As described previously, The products of neutron capture are known to be most harmful to a cell if the neutron capture process occurs as close to the DNA as possible. Despite the immense variety of compounds developed as potential BNCT agents, until recently, the use of the unique targeting abilities of metal complexes within biological systems for specific DNA-targeting of compounds containing ^{10}B nuclei was unprecedented for BNCT.

1.4.1. Roles of Metal Complexes in Biological Systems

The participation of metallic elements is critical at all levels of biology. Naturally-occurring metals, such as K, Na, Mg, Ca, Zn, Fe and Cu, are well-known for an extensive number of both structural and chemical roles in biological systems. These roles occur mainly in proteins.

Some enzymes have a metal ion (or ions) at their active site that are critical to the function of the enzyme. Such enzymes are termed 'metalloenzymes' and several hundred have been described. The role of the metal ion in biological catalysis varies extensively within biological systems. For example, the zinc(II) ion at the active site of the well-known 'zinc finger' proteins is essential in the nuclei acid binding ability of the enzyme.[95]

There is also a rich chemistry between nucleic acids and metal complexes. While the role of metal ions in the stabilisation of chromosomal DNA is largely unexplored, Na^+ and Mg^{2+} ions are known to stabilise the RNA and DNA tertiary structure by binding to anionic phosphate groups of the phosphodiester backbone.[96-98] In addition, most of the major classes of pharmaceutical agents currently in clinical use contain metal centres.[99-101] The area of medicinal inorganic chemistry is becoming an increasingly active area in the development of new generations of therapeutics.

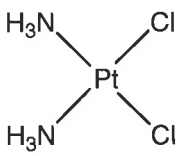
Drugs containing chelating ligands can specifically target metal ions that are naturally present in biological systems. Some metal complexes are capable of acting as biological probes, giving the biochemist information such as nucleic acid conformation[102-108] or the location of protein-binding sites on DNA.[109-111] Alternatively, some drugs contain metals centres that play key roles in their mechanism of action. Platinum(II) anti-cancer drugs are an example of such drugs, whereby the covalent interactions between the platinum(II) centres and DNA are the essential first steps of drug action, ultimately triggering cell death.

1.4.1.1. Platinum Anti-Cancer Drugs

Platinum(II)-amine complexes have become the most widely used drugs in the treatment of cancer, consequently the biochemistry of platinum(II) complexes has been extensively studied and is well understood. Various aspects of the biochemistry of platinum(II) centres, especially the DNA-binding characteristics are detailed below and indicate the suitability of platinum(II) centres to be used to achieve DNA-targeting of BNCT agents. To our knowledge the use of metal complexes as biodelivery pathways for ^{10}B-containing agents in BNCT has yet to be utilised.

1.5. DNA - The Biological Target of Platinum(II)-Amine Complexes

The clinical success of platinum(II) anti-cancer drugs, such as cisplatin (**31**), has lead to a more comprehensive understanding of the interactions between metal complexes and DNA.



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Cisplatin is widely used in the treatment of many forms of cancer. It was the first platinum drug to enter clinical trials after it successfully displayed a wide spectrum of anti-cancer activity in animal tumour models.

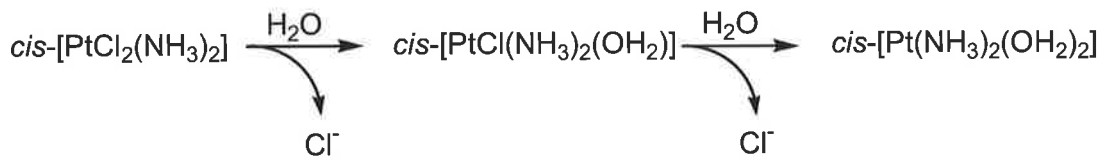
Cisplatin is highly effective against ovarian, testicular, bladder, head and neck, osteogenic and uterine carcinomas. However, it displays limited activity against other cancers such as breast and colon cancers. As a result of the clinical success of cisplatin, a great deal of research has gone into studying its mechanism of action.

A major focus has been directed towards the interactions between platinum(II) and DNA, the cellular target.[112] Cisplatin covalently binds to DNA at the N7 position of guanine residues and to a lesser extent, adenine, to form bifunctional adducts.[113] Current thinking is that the formation of such platinum(II)-DNA adducts are an essential first step in the activity of the drug, however the mechanism by which the cell is eventually killed remains elusive.

The platination of DNA by cisplatin involves several steps, including: hydrolysis of the complex; pre-association with DNA; monofunctional adduct formation and closure to bifunctional adduct.[114] The bound platinum(II) centre induces a distortion of the DNA duplex, which is recognised by the cell and can alter cellular processes, eventually leading to cell death.

1.5.1. Cisplatin Inside the Cell

Aquation of cisplatin by hydrolysis almost certainly precedes binding of cisplatin to DNA. Many details of the cellular pathway taken by cisplatin after intravenous injection into the bloodstream are well understood.[112,115] Within the plasma, where chloride ion concentration is high (103 mM), the cisplatin exists predominantly as the neutral dichloro complex. Within the cell, chloride ion concentration is much lower (4 mM), facilitating hydrolysis (Scheme 1.1).[112]



Scheme 1.1

The aqua ligand is more labile than the chloro ligand, consequently the mono-aqua complex, *cis*-$[PtCl(NH_3)_2(OH_2)]^+$, and di-aqua complex, *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$, are

considerably reactive, especially with nucleophilic cellular components such as RNA, proteins, DNA, membrane phospholipids, cytoskeletal microfilaments and thiol-containing molecules.[116] Approximately 1% of intracellular cisplatin reacts with DNA,[117] and it is generally accepted that DNA is the primary target.[118] However, there is some evidence to suggest that the other biological targets such as DNA polymerases may play an important role in the cytotoxic effect of cisplatin.[119]

1.5.2. DNA Adducts Formed by Cisplatin

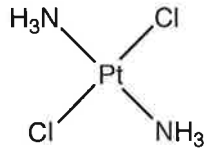
There are several ways in which cisplatin can bind to DNA in a bifunctional manner. There have been countless enzymatic digestions of cisplatin-treated DNA reported in the literature, each with a slightly different result, however all agree that cisplatin binds preferentially to adjacent guanine residues, forming 1,2-intrastrand cross-links (Figure 1.2). The 1,2-intrastrand cross-links account for 60-65% of total cisplatin-DNA adducts, followed by the 1,2-ApG-intrastrand cross-links (20-25%).[120] The 1,2-interstrand cross-links (Figure 1.2) occur at GpC sequences between the two G residues on opposite strands and account for less than 1% of total cisplatin-DNA adducts.[112,121] The 1,3-intrastrand adducts, where the bound purines are separated by one base pair, and monofunctional adducts account for the remainder of the adducts.[122]

1,2-Interstrand Cross-link 1,2-Intrastrand Cross-link

Figure 1.2

Considerable evidence suggests that the anti-cancer effects of cisplatin are the result of the formation of the 1,2-intrastrand DNA cross-links.[123] This is supported by the

finding that the clinically-ineffective *trans* isomer of cisplatin, transplatin (**32**), is unable to form the 1,2-intrastrand cross-link between neighbouring base residues due to its stereochemistry. Instead transplatin forms mainly mono-functional adducts, interstrand cross-links and intrastrand cross-links between non-adjacent base residues. These adducts are recognised by cellular proteins to a lesser extent than cisplatin-modified DNA.[118,123]



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The DNA cross-links formed by cisplatin have been extensively studied by NMR spectroscopy and X-ray methods.[118] These techniques have demonstrated the different distortions of the DNA double helix that result from the various cisplatin-DNA adducts. The 1,2-intrastrand cross-link induces a bend of 30-35° in DNA towards the major groove and a localised unwinding of the double helix by 13°. These values are highly reproducible and are independent of the sequence of base pairs flanking the platinated site.[124] The 1,3-intrastrand adduct causes a similar bend, unwinding by 23° and a denaturation of the double helix. The 1,2-interstrand cross-link causes a bend of 20-45° towards the minor groove and unwinding by 80-90°.[122]

The ability of cisplatin to bind to DNA and modify the 3-D structure has been shown to interfere with several normal cellular processes. The different structural modifications that result from the various cisplatin-DNA adducts each alter the cellular processes of cisplatin-modified DNA in various ways. The biological effects of the various cisplatin-DNA adducts (including the 1,2-intrastrand cross-link) and their and proposed roles in the anti-cancer properties of cisplatin are discussed below.

1.6. Processing and Consequences of Cisplatin-DNA Adducts

1.6.1. Cisplatin Effects on DNA Replication

Upon the discovery of cisplatin, the initial observation of impairment of cell division without simultaneous inhibition of cellular growth suggested inhibition of DNA synthesis.[125] At therapeutic doses, cisplatin can inhibit DNA replication and transcription.[112,118] Replication of DNA requires an unwinding of duplex DNA, separation of the two strands and the movement of DNA polymerases along the double helix. A common hypothesis is that the distinctive modification of the DNA duplex by cisplatin causes the DNA to be an unsuitable template for the DNA sliding clamp (a key component of the DNA polymerase holoenzyme), thus inhibiting DNA replication and contributing to cell death.[126] However, it has been discovered that the interstrand cross-link and mono-functional adducts formed by clinically-ineffective transplatin also block replication and transcription, hence the therapeutic effect of cisplatin cannot be solely attributed to its interaction with DNA and the consequent blocking of replication and transcription.[118] Instead, the anti-cancer activity of cisplatin is likely to be the result of the subsequent processing of DNA adducts involving damage recognition proteins.[127]

1.6.2. Processing of Cisplatin-Modified DNA by Proteins

Within one day of intravenous cisplatin administration, 65-98% of the drug is bound to plasma proteins.[128] This is not surprising when one considers the high concentration of such proteins and the resulting number of donor sites. Despite active research over the past few years, the exact role of cisplatin-protein adducts in the mechanism of action of cisplatin is yet to be fully elucidated.

A number of proteins preferentially bind to cisplatin-modified DNA. This recognition is presumed to be an important step in the mechanism of action, mediating the responsiveness of tumour cells to cisplatin.[129-136] Such proteins have been classified into two groups: repair proteins and architectural proteins. Repair proteins are those involved in damage recognition and initiation of repair pathways. Such proteins are involved in nucleotide excision repair (NER) that recognise other types of DNA lesions in addition to cisplatin-modified DNA.[113,130,137,138] Architectural proteins are

generally abundant proteins that normally perform an architectural role in the formation of DNA/protein or protein/protein complexes. The HMGB domain architectural proteins can bind to cisplatin-modified DNA simply because of a structural similarity to the natural binding site. This finding was completely unexpected and consequently has attracted much research into HMGB domain proteins.

1.6.2.1. The Role of HMGB Proteins in the Activity of Cisplatin

Most architectural proteins share a DNA-binding domain called a 'high mobility group box' or HMGB. This entity was first found in the proteins HMGB1 and HMGB2 and has since been found to exist in a myriad of functionally unrelated proteins.[133] HMGB1 and HMGB2 are the most abundant of the HMGB-containing proteins found in all eukaryotic cells.[130,132,134,135]

1.6.2.1.1. Normal Function of HMGB Proteins

The primary role of HMGB domain proteins has recently become known.[134] Within a cell nucleus the HMGB1 protein acts as an architectural factor, bending DNA and promoting protein assembly on DNA. Outside the cell, the proteins play important roles in the mediation of inflammation and tumour metastasis.[139,140]

The main DNA-binding role of the HMGB proteins occurs during the formation of higher order protein-DNA or protein-protein complexes and is proposed to be the stabilisation of DNA bending.[136] The loss of rigidity in bent DNA is energetically unfavourable, but necessary in the formation of complex nucleoprotein assemblies.[134] Other than their normal architectural roles, HMGB1 and HMGB2 proteins also show a preferential interaction with cisplatin-modified DNA.[135]

1.6.2.1.2. Cisplatin Modified-DNA and HMGB Proteins

It has been found that full-length HMGB1 proteins recognise and preferentially bind to the rigid directional bend in DNA induced by the 1,2-GpG and 1,2-ApG intrastrand cross-links of cisplatin, but not the more flexible non-directional bend in 1,3-

intrastrand and 1,2-interstrand cross-links, monofunctional adducts and transplatin-modified DNA.[130,136,141] Considerable evidence implicates the involvement of the stable cisplatin-DNA-HMGB protein complexes in the modulation of the cellular response to cisplatin modification of DNA.[142] Once bound, the HMGB domain proteins inhibit repair of cisplatin-DNA adducts *in vivo* and *in vitro*, by shielding the 1,2 intrastrand cross-links from removal by repair-related enzymes (such as NER), thus enabling the cisplatin adducts to persist for longer periods.[127,131,134,138] This is called 'repair shielding' and is supported by the important finding that cell sensitivity to cisplatin is increased by an overexpression of HMGB1.[133] In addition, it is feasible that in the presence of cisplatin-modified DNA, the HMGB domain proteins are hijacked from their normal functions thereby disrupting normal gene regulation and contributing to cell death.[118,133]

Testicular cancers are successfully treated by cisplatin, whereas the drug produces a response in only a few other types of cancers. A HMGB domain protein that is specifically found in testes (tsHMG) has been found to modulate the cytotoxicity consequences of cisplatin treatment.[143] This is considered evidence that a HMGB domain protein can affect cellular responses to cisplatin and may be relevant to the observation that cancer cells in specific tissue are particularly sensitive to cisplatin.

1.6.2.2. TBP Proteins and their Role in the Activity of Cisplatin

The TATA-box binding protein (TBP) is a DNA-binding protein that normally functions in transcription initiation.[144] Despite a considerably slow binding rate,[145] TBP has a 175-fold higher affinity for cisplatin-modified DNA over unmodified DNA.[146,147] Upon binding, TBP specifically shields the cisplatin 1,2-intrastrand cross-link from NER. The high binding affinity is similar to, but not as dramatic as the HMBG1 protein, and has implications for the action of cisplatin, possibly through repair shielding or due to hijack of TBP away from RNA synthesis.[146] The possible contribution of TBP to the cytotoxicity of cisplatin is further supported by the finding that TBP is 5-10 fold more highly expressed in testis compared with normal tissues.[148]

It is not possible that these enzymes have evolved to recognise specifically cisplatin-modified DNA. Their tight binding to cisplatin-DNA adducts is viewed as fortuitous

especially when one considers the major importance this interaction seems to play in the activity of cisplatin. While the full mechanism of binding of proteins to cisplatin-modified DNA remains unresolved, further studies should provide a deeper understanding into the mechanism of action of cisplatin and any mediation by proteins.

1.6.3. Cisplatin-Modified DNA Can Trigger Apoptosis

There is considerable evidence that cisplatin can kill cells through the induction of apoptosis.[116,117] Apoptosis is a ubiquitous genetically-regulated mechanism of destroying cells. It usually occurs during embryonic development, metamorphosis and general cell turnover. Numerous stimuli, including anti-cancer drugs, can induce apoptosis, however the events leading to apoptotic cell death after cisplatin treatment are not well understood.

It has been proposed that HMGB1 proteins bound to cisplatin-modified DNA, shielding from repair enzymes, could alter cell cycle events and trigger apoptosis. However, cisplatin also reacts with other cellular components that could possibly play a role in the induction of apoptosis.

It is well established that the p53 gene plays a major role in the activation of apoptosis in response to DNA-binding by cisplatin.[149-152] The p53 gene normally functions to protect the cell, controlling cellular responses to DNA damage, such as irradiation or anti-cancer drugs.[118,153] The p53 gene remains mostly latent in the absence of stress but is activated upon DNA damage, triggering apoptosis, DNA repair or cell cycle arrest, allowing genomic integrity to be maintained.[153]

1.6.4. Toxicity of Cisplatin

A major obstacle to the further widespread use of cisplatin in the treatment of cancer is the persistence of severe toxic side effects. The major dose-limiting factor is the toxic effects toward the renal system (nephrotoxicity), ranging from abnormal urinalyses to renal failure.[154] To help alleviate such symptoms, intravenous hydration and diuretics have been administered, unfortunately making treatment inconvenient

on an outpatient basis.[154] Recent developments with BGP-15, an poly(ADP-ribose) polymerase inhibitor, have been promising, decreasing certain effects of cisplatin and preventing degeneration of the kidney.[155]

As cisplatin inhibits DNA replication, normal tissues with a high rate of proliferation are adversely affected, including bone marrow, gastrointestinal epithelial cells and the skin. The nausea and vomiting caused by cisplatin are severe and deter patients from continuing treatment. Young children receiving treatment are susceptible to high frequency hearing loss (ototoxicity), while high or prolonged doses of cisplatin may cause nerve damage (neurotoxicity).[154]

1.6.5. Resistance to Cisplatin

Another major obstacle to the use of cisplatin in the clinic is resistance to chemotherapy. Some tumour lines are intrinsically resistant to chemotherapy, whereas others, initially responsive to the drugs, develop a resistance to cisplatin after continued treatment.[156] Cisplatin-resistant cells can be obtained by repeated exposure to the drug. Comparisons between the resistant cells and parent cells have allowed an understanding of the molecular basis for resistance.[118] Multiple mechanisms have been identified, however the results are often contradictory.

1.6.5.1. Altered Accumulation of Cisplatin

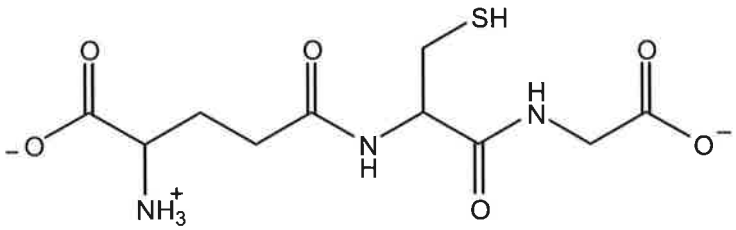
While the mechanism of cisplatin accumulation is unclear, a decrease in intracellular drug levels is observed in most resistant cells.[117,157] This is considered to be the first line response in defence against cisplatin, although such changes do not generally correlate with the magnitude of resistance observed, thus additional mechanisms most likely contribute to resistance.[117] Other experiments show no change in drug accumulation, but a higher rate of efflux is found.[118]

1.6.5.2. Intracellular Thiols Can Inactivate Cisplatin

After cisplatin passes from the blood into cells, the drug undergoes aquation to afford the reactive dicationic *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ species, which readily covalently binds

to DNA. However, the reactive aquated drug readily reacts with other biomolecules, such as proteins, before reaching the cell nucleus, hence only 1% reaches nuclear DNA.[117]

The HSAB theory predicts a very strong and rapid interaction of platinum(II) ions with S-donor ligands.[158] Thiol groups (RSH) such as those found on glutathione (GSH, **33**) and metallothionein (MT), preferentially bind to aquated cisplatin in the cytosol, sequestering the platinum(II) centre prior to reaching DNA. The formation of a strong platinum(II)-S bond deactivates the platinum(II) centre towards reaction with DNA, inhibiting the formation of cytotoxic cross-links on DNA.[159,160]



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GSH is one of the most abundant molecules in cells, and it is therefore surprising that any aquated cisplatin reaches nuclear DNA at all. Indeed there exists a subtle kinetic and thermodynamic balance between platinum(II)-S and platinum(II)-N binding in biological systems.[161] The intracellular thiol L-methionine (Met) has been demonstrated to increase the reaction rate for cisplatin with monomeric nucleotides, thus it has been suggested that this thiol mediates reaction of platinum(II) centres with DNA.[162-164] However, Met decreases the rate of reaction of cisplatin with base residues in high molecular weight DNA.[165]

It is easy to overwhelm the thiol protective mechanism in patients receiving cisplatin treatment for the first time, however prolonged exposure results in an increase in intracellular GSH levels within the cell, leading to cisplatin-resistance through increased deactivation of the platinum(II) centre.[117,157,166] Another explanation of the inhibition of cisplatin cytotoxicity by GSH is the antioxidant role of GSH, which is capable of inhibiting a cisplatin-induced apoptotic signal preventing cell death rather than affecting cisplatin-DNA adduct formation.[167]

1.6.5.3. Enhanced Repair of Cisplatin-DNA Adducts

The effects of cisplatin-modified DNA may also be diminished by the removal of platinum(II)-DNA lesions. Enhanced DNA repair has been observed in cisplatin-resistant cell lines, allowing platinum(II)-DNA lesions to be removed more rapidly than in parent cell lines.[117,157] Some tumour cell lines with deficiencies in repair pathways have been found to be highly sensitive to cisplatin.[168] In addition, the loss of DNA-mismatch repair has been shown to contribute to resistance of cisplatin. Normally mismatch repair proteins recognise but do not remove cisplatin-DNA adducts. Loss of mismatch repair can prevent attempts to insert a 'correct' base into the non-damaged strand opposite the platinum(II)-DNA adduct, a futile cycle of DNA repair that can trigger apoptosis.[117,169]

More recently an enhanced capacity to tolerate cisplatin-induced DNA damage has been demonstrated to contribute to cisplatin-resistance.[117] Resistant cells are able to replicate DNA, despite the presence of platinum(II)-DNA lesions.

1.6.5.4. Loss of p53 Function Can Contribute to Cisplatin Resistance

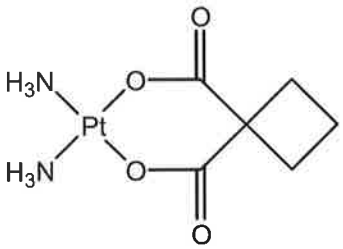
The loss of p53 function through mutation occurs in 50% of human cancers. In some cases, the loss of p53 function has shown to contribute to tumour resistance to platinum(II)-drugs, such as cisplatin, by protecting the cell from p53-triggered drug induced apoptosis.[150,153]

Multiple mechanisms of resistance to cisplatin may be operating and their relative levels are variable among different tumour lines. This makes the design of new drugs that are active in cisplatin-resistant cells difficult as the cause of the resistance cannot be identified directly. Making research in this area even more challenging is the revelation that some mechanisms of acquired resistance only occur *in vivo*.[118]

1.7. A Second Generation of Platinum(II) Anti-Cancer Drugs

After the success demonstrated by cisplatin in the treatment of cancer, the search for platinum(II) anti-cancer drugs that are effective in cell lines where cisplatin is not, was directed and dominated by the idea that it was essential for a complex to possess a *cis*-$[PtX_2(amine)_2]$ geometry, where X is a leaving group, in order to achieve anti-cancer activity.[170,171] This structure-activity relationship dominated platinum(II) drug design for over twenty years and, as a result, thousands of simple analogues of cisplatin have been synthesised and many of these have been evaluated as potential anti-cancer drugs. However, by 2002, only 30 compounds had reached clinical trials and more than half of them had been rejected.[172]

Today, along with cisplatin, three platinum(II) complexes are used clinically, Carboplatin (diammine[1,1-cyclobutane-dicarboxylato(2-)]-*O,O'*-platinum(II) **34**) is the only cisplatin analogue that has achieved worldwide approval and it is routinely used in the clinic. Oxaliplatin (*trans*-L-diaminocyclohexane oxalatoplatinum(II) or L-OHP, **35**) has been approved in France and other European countries, and Nedaplatin (*cis*-diammine-glycoloato-*O,O'*-platinum(II) or 254-S, **36**), is available only in Japan. These drugs are not more active, but they exhibit less toxic side effects than cisplatin.



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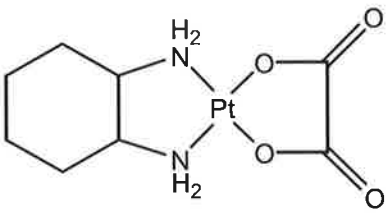

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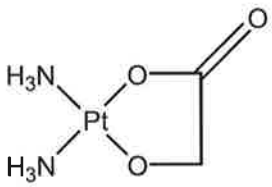

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The new platinum(II) drugs have the *cis*-$[PtX_2(amine)_2]$ geometry with carboxylate-type ligands as leaving groups. Carboplatin is less toxic than cisplatin and can be

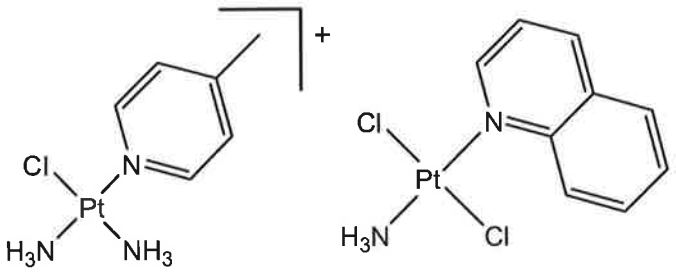
administered to the patient in larger doses. Unfortunately, the drug is only active in the same range of tumours as cisplatin.[171,172] Oxaliplatin also has a lower toxicity than cisplatin and exhibits activity against a wide range of cisplatin- and Carboplatin-resistant cell lines.[173,174] Oxaliplatin was recently approved for the treatment of metastatic colorectal cancer with 5-fluorouracil in Europe, Asia and Latin America.[175] Interesting results have also been seen in other tumour types, however the cellular and molecular aspects of the mechanism of action and limitations of this drug are still being investigated.[172,176]

Nedaplatin has been approved for use in Japan as it produced better results in pre-clinical trials than cisplatin. It is cross-resistant with cisplatin and shows no advantage in the overall survival rates, but it is less toxic. It is used in the treatment of head and neck, testicular, lung, oesophageal, ovarian and cervical cancers.[172]

The lack of a greatly altered spectrum of clinical efficacy of these cisplatin analogues in comparison with cisplatin is not surprising. The similarity of the aquation products of the *cis*-$[PtX_2(amine)_2]$ functionality to aquated cisplatin means the mechanism of action of these new platinum(II) drugs is similar to cisplatin, and would produce a very similar array of DNA adducts to those produced by cisplatin.[172] As a result, the biological consequences of these adducts would be similar to those of cisplatin. Consequently, mechanisms of resistance for cisplatin are most likely common to Carboplatin, Oxaliplatin, and Nedaplatin.

1.7.1. New Platinum(II) Drugs that Violate the Classic Structure-Activity Relationship

More recent research has been directed by the hypothesis that an alteration in the mode of DNA-binding by platinum(II) complexes compared with cisplatin would result in an altered spectrum of anti-cancer activity and possibly enhanced activity in cisplatin-resistant cells. The different adducts could result in a higher cytotoxicity or perhaps be more difficult to repair. In 1989 two exceptions to the structure-activity relationship were reported. Both complexes **37**[177,178] and **38**[179] were found to possess remarkable anti-cancer activity.



37 **38**

Complexes **37** and its analogues form monofunctional DNA lesions and block DNA replication polymerases,[178] and **38** was the first *trans*-$[PtX_2(amine)_2]$ complex with significant cytotoxicity. These findings indicated that a neutral charge and the *cis*-orientation of inert amine ligands of platinum(II) compounds are not prerequisites for anti-cancer activity.

Trans-platinum(II) complexes,[179-186] sterically hindered platinum(II) complexes,[187-192] cisplatin analogues with DNA-binding ligands[193-200] and multinuclear platinum(II) complexes[122,123,160,201-242] are unconventional complexes that violate the old structure-activity relationships and, in fact, appear to have biological properties that are quite distinct from those of cisplatin. Some compounds belonging to these families are active against cisplatin-resistant cell lines and act on DNA in a different manner to cisplatin. This is strong evidence that the cisplatin-type lesions are not the unique arbiters of cytotoxicity.

1.7.1.1. Transplatin Analogues

A wide range of *trans*-dichloro platinum(II) complexes that possess favourable therapeutic properties have been developed.[179-186] The new 'non-classical' *trans*-platinum(II) anti-cancer compounds tend to be classified according to their non-ammine N-donor ligands. *Trans*-dichloro platinum(II) complexes have been prepared with planar amines,[179-181,243] iminoether ligands,[181,244] aliphatic amines[181] or cyclic hexylamine ligands.[182,183]

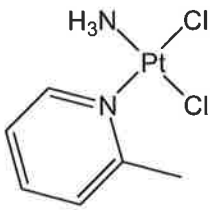
The *trans*-platinum(II) complexes often exhibit higher cytotoxicities than *cis*-analogues in cisplatin-resistant cell lines.[181,186] Some complexes within this new class have demonstrated distinctly different DNA-binding modes in comparison with the

cis-isomer, transplatin and cisplatin, such as high levels of interstrand DNA cross-link formation.[186] Their unique activity is most likely due to their ability to form novel adducts with DNA. While anti-cancer activity displayed *in vivo* is poor in comparison to cisplatin analogues,[185] results to date have been promising and there are indications that certain *trans*-platinum(II) complexes should be further developed into potential platinum(II) drugs with activity complementary to that of cisplatin.[181]

1.7.1.2. Sterically-Hindered Cisplatin Analogues

The development of cisplatin analogues with bulky planar ligands was based on the observation that increased steric hindrance decreases the rate of substitution reactions in square planar complexes.[171] Such cisplatin-analogues have been rationally designed to possess a reduced reactivity with intracellular thiols, such as GSH and Met.[180,186-192]

AMD473 (**39**) is a cisplatin analogue with a planar N-donor ligand that has recently entered Phase I clinical trials.[191] The bulky 2-methylpyridine ring provides steric hindrance and directs reactions of the platinum(II) centre.[192] It was successfully designed to be less reactive than cisplatin towards thiols and is able to overcome acquired cisplatin-resistance by detoxification mechanism of intracellular thiols. The reaction of AMD473 with DNA is slower in comparison with cisplatin, eventually platinating DNA to the same level.



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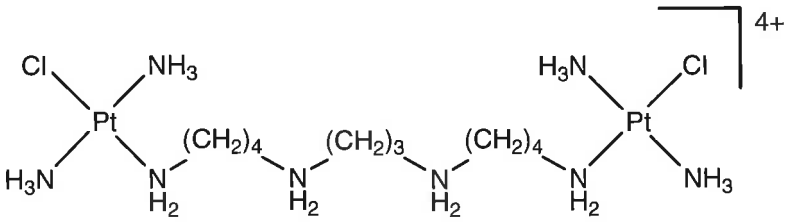
1.7.1.3. DNA-Targeted Platinum(II) Complexes

Like all areas of modern drug design, recent developments in the comprehensive understanding of cellular processes has led to the development of platinum(II) complexes with biologically-active ligands. While cisplatin has no intrinsic affinity for DNA, attachment of a DNA-affinic group could place the platinum(II) centre

closer to the primary biological target of platinum(II) drugs. As a consequence, the number of side reactions with biomolecules, such as proteins or intracellular thiols, could decrease, increasing the proportion of administered complex that reaches the DNA. Other possible consequences include altered kinetics of binding, reaction mechanism, sequence specificity and the formation of novel lesions that may escape the DNA repair processes. Additionally, incorporation of a tumour-targeting ligand may increase the concentration of platinum(II) in tumour cells over normal cells.

The elevated requirement of tumour cells for polyamines and the ability of the polyamine uptake mechanism to tolerate modified polyamines have led to the development of synthetic polyamines as drug delivery pathways (Section 1.3.6.2). DNA-binding polyamines putrescine (**18**), spermidine (**19**), and spermine (**20**) have been used as DNA-affinic ligands in platinum(II) complexes.[195-197,213] Complexes **40** and **41** demonstrate high cytotoxic activity toward cisplatin-resistant cells and induce DNA conformational changes at lower doses than the parent platinum(II) complex.[195]



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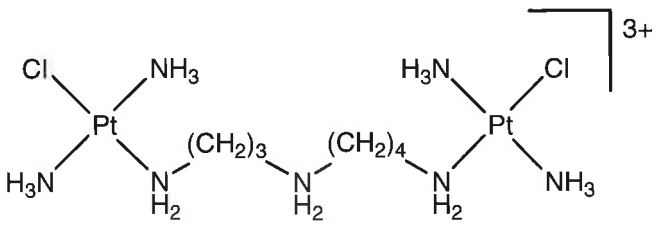

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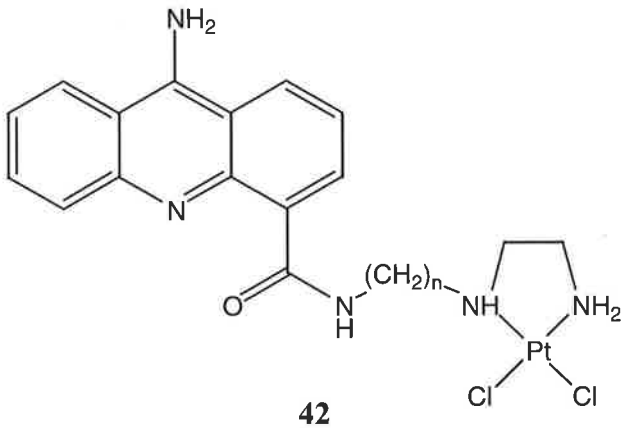
It is likely that the polyamine carrier contributes to this enhanced activity by incorporation of a high cationic charge and consequent increased electrostatic affinity for DNA.

The incorporation of an intercalating moiety into platinum(II) complexes (**42**, **43** and **44**) has resulted in an increased rate of reaction with DNA compared with parent cisplatin.[198-200] This is probably due to a different mechanism of reaction with DNA. Pre-association of the platinum(II) complex with DNA *via* intercalation places the platinum(II) centre closer to the N-7 of guanine residues, allowing direct displacement of the chloro ligand by the N-7 atom, avoiding rate-limiting hydrolytic generation of the aqua complex prior to reaction with DNA.[198]



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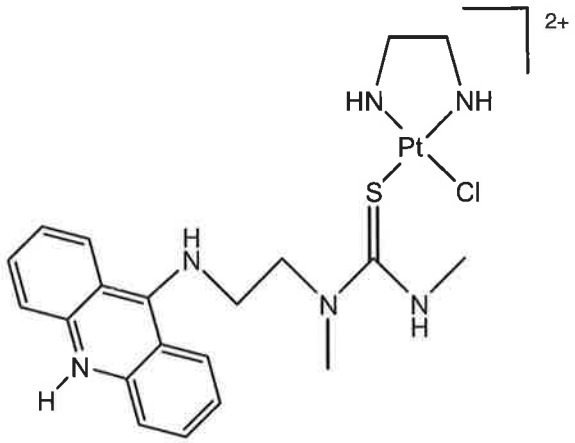
The acridine-modified thioureas platinum(II) complexes **43** and **44** are monofunctional, hence unable to cross-link DNA.[199] The high cytotoxicity of **43** is attributed to monofunctional platination of the nucleobase N-7 atom and efficient intercalation of acridine moiety. In contrast, the acridine moiety of the ineffective **44** does not intercalate DNA, most likely due to the shorter linkage between the platinum(II) centre and acridine increasing the rigidity of the molecule. The monofunctional covalent platination by **44** of DNA means the **44**-DNA adducts have little effect on DNA conformation and are readily removed by repair machinery. These findings confirm that the incorporation of a DNA-binder carrier ligand can enhance the cytotoxicity of a platinum(II) complex.



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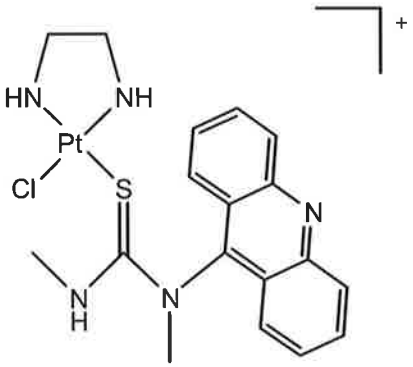


44

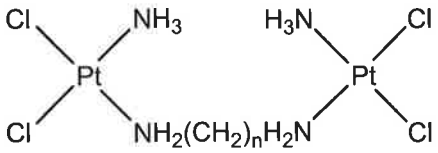
1.7.1.4. Dinuclear Platinum(II) Complexes

Farrell and co-workers have developed an extensive series of dinuclear platinum(II) complexes of the general formula *trans*- and *cis*-[{$PtCl_m(NH_3)_{3-m}$}-μ-H_2N-R-NH_2-{$PtCl_n(NH_3)_{3-n}$}]$^{\{(2-m)+(2-n)\}}$ (m or n = 0-3; R = linear or substituted aliphatic linker, typically *n*-butane, pentane or hexane) with a greater affinity for DNA in comparison to cisplatin.[122,123,160,201,202,204,205,207-228,245] This class of 'non-classical' platinum(II) complexes has been comprehensively extended and studied to include variations in the number of platinum(II) centres, linker lengths, platinum(II)-centre functionality and geometry.

1.8. Multinuclear Platinum(II) Complexes

1.8.1. Tetra-Functional Dinuclear Platinum(II) Complexes

The first example of Farrell's dinuclear chloroplatinum(II) complexes were those within the series **45**, utilising bridging diaminoalkane ligands of varying chain length (n = 4-6).[201,207,228] The DNA-binding of **45** was studied by means of a restriction endonuclease DNA cleavage assay.[207] These complexes inhibited exonuclease activity to a significantly greater extent than cisplatin and displayed high activity toward cisplatin-sensitive tumour cell lines *in vitro* and *in vivo*.[201,208]



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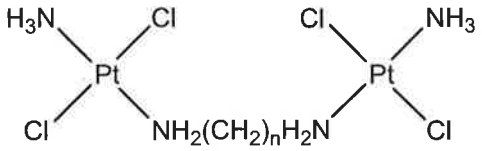
These complexes are often referred to as 'tetra-functional' as they have four labile chloro ligands and therefore four sites that are capable of forming covalent bonds with the DNA base pairs. The cytotoxicity of the tetra-functional *trans*-isomers, (**46** series, where n = 4-6) was found not to be significantly enhanced in comparison to the parent transplatin.[201,222]



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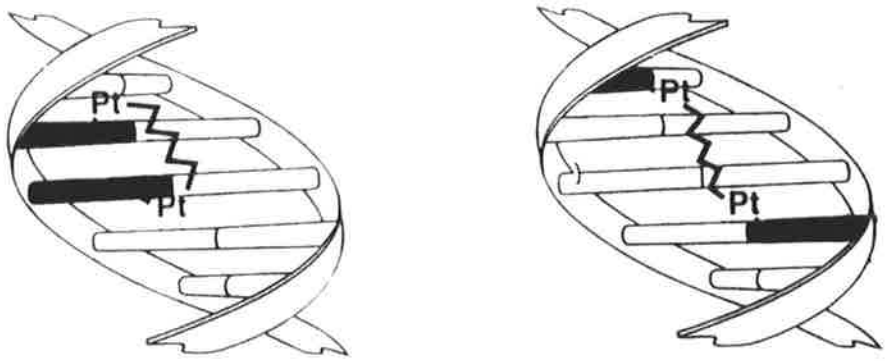
1.8.1.1. Interactions of Tetra-Functional Dinuclear Platinum(II) Complexes with DNA

The presence of two reactive platinum(II) centres, the high functionality of the platinum(II) centres, and the flexibility of the bridging bidentate ligand enables dinuclear platinum(II) complexes to form an array of unique adducts with DNA. Around 10% of the total adducts formed by **45** are novel long-range interstrand cross-links that occur at 250 times the level of interstrand cross-links formed by cisplatin and its analogues.[209] Interstrand cross-links are formed by binding of one platinum(II) atom to each strand of DNA (Figure 1.3).



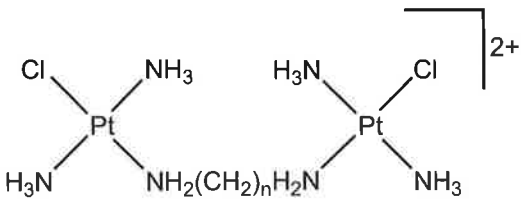
Intrastrand cross-link Interstrand cross-link

Figure 1.3 Modes of dinuclear platinum(II) complexes DNA-binding.[170]

Such long-range interstrand cross-links have attracted special interest as they cannot be formed by cisplatin and its mononuclear analogues. It was anticipated that the new long-range interstrand cross-links were responsible for the new spectrum of activity displayed by the dinuclear platinum(II) complexes and their ability to overcome cisplatin-resistance. Interstrand cross-links are significant as they involve both strands of duplex DNA, and could pose an unusual challenge to repair enzymes. Hence, dinuclear platinum(II) complexes capable of forming long-range interstrand cross-links with DNA could potentially not only prove to be non-cross resistant with cisplatin but also display a broader spectrum of activity.

1.8.2. Bi-Functional Dinuclear Platinum(II) Complexes

The extensive series of dinuclear platinum(II) complexes was expanded as the minimal structural features required for cytotoxicity were investigated. To determine the biological effects of the interstrand cross-links, the functionality of the dinuclear platinum(II) complexes was reduced from tetra- to bi-functional, decreasing the likelihood of formation of cisplatin-like intrastrand cross-links. The bifunctional dinuclear platinum(II) complexes, **47** and **48** (where n = 4-6), containing monochloro coordination spheres were prepared.[210,245] Geometric isomers can exist in this class of complex because the chloro ligand can coordinate in a *cis*- or *trans*- manner to the linking diamine.



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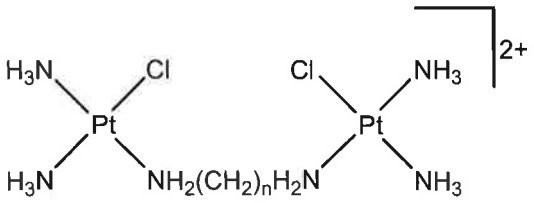


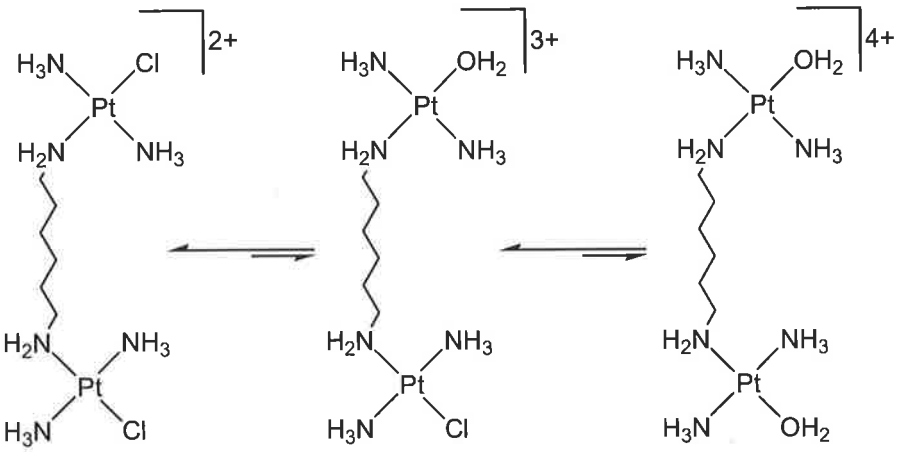
48

Both **47** and **48** type complexes show anti-cancer activity *in vivo* and *in vitro* in cisplatin-sensitive cell lines at levels that are comparable to cisplatin.[202,210] Unlike the more cytotoxic **45** series, the activity of **47** and **48** is retained in cisplatin-resistant cell lines, although **48** are less efficient at overcoming cisplatin-resistance than **47**.[210] These results demonstrate that a dinuclear platinum(II) complex with monodentate coordination spheres is sufficient to exhibit some degree of cytotoxicity, especially in cisplatin-resistant cells, further evidence that the 'classical' *cis*-$[PtX_2(amine)_2]$ unit is not an essential criterion in achieving anti-cancer activity.

Much effort has gone into further elucidating the underlying mechanisms that cause the various activities of di- and mononuclear platinum(II) complexes and the differences between **47** and **48**.[122,123,160,202,205,210-212,214-220,224,225,227,246] The results of these studies are summarised below.

1.8.3. Cellular Processes involving Dinuclear Platinum(II) Complexes

The aquation of cationic PtN_3Cl complexes have been shown to be rapid relative to that of cisplatin.[247] Examination of the kinetics of hydrolysis of **47** (n = 6) has indicated the aquation rate constant is similar to other complexes containing the PtN_3Cl core and equilibrium is achieved quicker than cisplatin.[220] However the position of the equilibrium (Scheme 1.2) favours the dichloro complex. Despite the low concentration of the more reactive aquated complex, DNA-binding is rapid,[210,224] indicating that formation of the aquated complex may not be necessary prior to DNA adduct formation.[123,220]



Scheme 1.2

The rapid DNA-adduct formation by **47** and **48**, in part, may be attributed to their positive charges which experience an electrostatic attraction to the polyanionic DNA molecule, facilitating covalent reaction of platinum(II) with DNA bases.[216]

1.8.3.1. Platinum(II)-DNA Adducts Formed by Dinuclear Platinum(II) Complexes

As found for cisplatin, the inhibition of DNA replication and transcription by **47** and **48** indicates that the modification of DNA plays an important role in the mechanism of cytotoxicity.[123,214] Overall cellular accumulation and DNA platination of **47** and **48** are not significantly different to cisplatin[214,216] and overall alterations in DNA conformation upon binding of **47** and **48** are equivalent to those induced by cisplatin and its mononuclear analogues.[202,217] Therefore, the differential biological effects of the **47** and **48** complexes over cisplatin are most likely caused by other factors, such as the types of lesions formed, which on the molecular scale are significantly different to cisplatin and subtly different from each other.

The extent of interstrand cross-links formation by **47** and **48** is higher than by **45**.[123,202,210] Approximately 90% of all adducts for **48** are interstrand cross-links, compared with 75% for **47**.[123,202,214,215] While the length of the linking diamine alters the distance and spatial configuration between the reactive platinum(II) centres, thereby affecting the types of adducts formed, alterations to the length of the linking

diamine ligand give essentially identical levels of interstrand cross-link formation.[123,214,215]

The geometry of the platinum(II) coordination sphere in dinuclear platinum(II) complexes tends to have a large affect on the DNA cross-links formed.[214] The major DNA-adducts formed by **47** are 1,3- and 1,4-interstrand cross-links between guanine residues.[218] A minor amount of 1,2-interstrand cross-links and cisplatin-like 1,2-intrastrand cross-links between adjacent guanine residues are also formed.[202,215,218,224] **48** forms 1,2-, 1,3- and 1,4-interstrand cross-links with similar preference, however they do not form the cisplatin-like 1,2-intrastrand cross-link.[202,214] This is most likely due to the steric bulk of the linking diamine located *cis*- to the chloro leaving group inhibiting the formation of the crowded 1,2-intrastrand cross-link.[122,202]

1.8.3.2. Recognition of Dinuclear Platinum(II)-DNA Adducts by Cellular Proteins

The stable, directional bend of the helical axis of DNA induced by the 1,2-intrastrand cross-link of cisplatin is the structural motif recognised by HMGB proteins, playing an instrumental role in the cytotoxic activity of cisplatin.[248] The HMGB proteins also recognise DNA modified by **47** and **48**.[202,215] However, the recognition is not as efficient as for cisplatin-modified DNA, not surprising considering the bend in the DNA helical axis induced by the binding of **47** is more flexible and non-directional in comparison with the rigid directed bend induced by cisplatin.[215,219] HMGB protein binding affinities of DNA-adducts formed by cisplatin analogues have been found to be sensitive to the nature of non-leaving ligands.[249] This could also account for the poor of recognition of DNA-adducts formed by **47** and **48** by HMGB proteins.

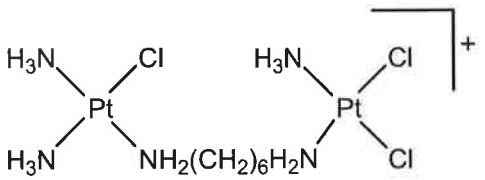
47 and **48** are the first examples of cytotoxic platinum(II) complexes that are not efficiently recognised by HMGB proteins suggesting that, in contrast to the proposals for cisplatin and its direct analogues, the mechanisms of action does not rely on the recognition by HMGB proteins as a crucial step.

1.8.3.3. Dinuclear Platinum(II) Complexes and Thiols

A study into the cytotoxicity of **47** in cells with high and low GSH levels showed them to be susceptible to deactivation by GSH, however not as much as cisplatin.[160] This is most likely due to the rapid binding of the dicationic dinuclear complexes to DNA, in comparison with cisplatin. The less time spent in the cytosol, the less opportunity exists for a platinum(II) centre to be deactivated by reaction with a S-donor ligand.[160]

1.8.4. Asymmetric Dinuclear Platinum(II) Complexes

The development of dinuclear platinum(II) complexes has led to more detailed investigations into the large variety of potential DNA-binding modes.[201,221,223,226] Complexes **49** and **50** have two platinum(II) centres containing inequivalent ligand environments and show similar potency to the **47** and **48** series and, furthermore, retain their activity in cisplatin-resistant cell lines.[226] As intermediates between the bi- and tetra-functional dinuclear platinum(II) complexes, the trifunctional complexes **49-51** are useful in studying the DNA-binding modes by each platinum(II) centre within a dinuclear platinum(II) complex. Each platinum(II) centre has a unique reaction profile with DNA, potentially allowing platination to be directed towards certain sequences based on functionality (i.e. mono- or bi-) or the type of leaving group (eg. halogen, malonate).[221]



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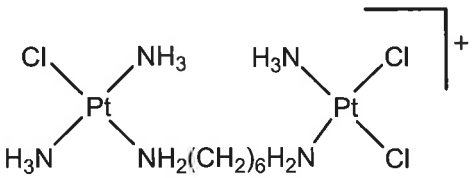
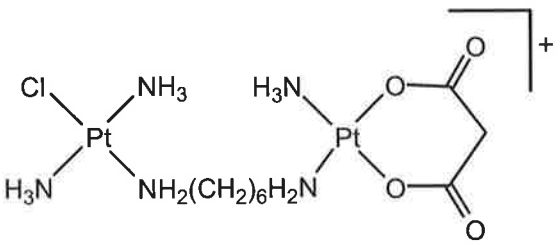


50

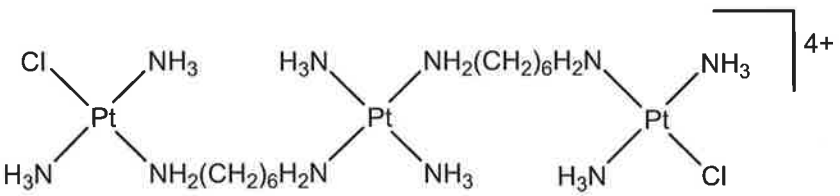


51

A comparison of the substitution kinetics displayed by the reaction of **49-51** with the nucleotide GMP gave an indication of the possibility of directive reactions with DNA.[221,226] The reaction of **50** and **51** with GMP should give rise to the same final adduct. The malonato ligand in **51** is not as labile as a chloro ligand, hence the monofunctional platinum(II) centre is the first to react with DNA. Similarly the reaction of **50** with DNA occurs predominately at the *trans*-chloro platinum(II) centre. However, for **49** both platinum(II) centres are equally reactive and compete simultaneously for DNA-binding sites.[221] These studies have provided an indication of the high reactivity of a *trans*-PtN_3Cl centre with DNA, its ability to overcome cisplatin-resistance and the selectivity that can be achieved when designing a DNA cross-linking agent.

1.8.5. Trinuclear Platinum(II) Complexes

The success of the dinuclear platinum(II) class of anti-cancer drugs has led to the development of a trinuclear platinum(II)-complex, BBR3464 (**52**).[197,203,204,211,213,216,217,229-242] **52** is the first representative of the 'non-classical' class of multinuclear platinum(II) anti-cancer drugs to enter clinical trials,[203] recently advancing into Phase II trials.[205]



52

The structure of **52** is best described as two *trans*-$[PtCl(NH_3)_2]^+$ units linked by *trans*-$[Pt(NH_3)_2(H_2N(CH_2)_6NH_2)_2]^{2+}$. Similarly to cisplatin, **47** and **48**, only two chloro ligands are present in the molecule, making **52** potentially bifunctional. However, **52** has a large separation between the two platinum(II)-centres (27.4 Å from X-ray data) and a high cationic charge.[229]

1.8.5.1. The Clinical Success of 52

52 was chosen for clinical trials due to its remarkable potency, cytotoxic at a 10- to 20-fold lower concentration than cisplatin, and its outstanding activity in cisplatin-resistant cell lines.[204,230,239,241,242] **52** also shows rapid intracellular accumulation and DNA-binding.[203,204,216,231,241] **52** is also active in mutant p53 gene cases.[203,204,230,234,235] All these observations support the hypothesis of **52** having a distinctly unique mechanism of action.

The cationic charge of **52** increases the DNA-binding affinity in comparison with cisplatin and the structurally-related **47**.[203,204,213,216] The central platinum(II)-tetraamine unit does not contribute to covalent DNA-binding, instead incorporating a dicationic charge into the linker allowing strong hydrogen bonding with the phosphodiester backbone of DNA. The strong electrostatic association increases the interaction of **52** with DNA, facilitating rapid covalent platination.[213,232] The dicationic charges of **47** and **48** also increases the rate of DNA-adduct formation compared with cisplatin, but to a lesser extent.[216]

The rapid binding of highly-charged **52** results in high levels of DNA platination.[204,232] The DNA adducts persist over time, suggesting poor DNA-adduct repair, causing a more persistent perturbation of the cell cycle than cisplatin.[242]

1.8.5.2. Cellular Processes of 52

The aquation of **52** is similar to that of **47** (n = 6).[233] While the equilibrium is established more rapidly than for cisplatin, the position of the equilibrium favours the dichloro species to a greater extent than the aquation of cisplatin.[233] Despite the low concentration of highly reactive aquated-platinum(II) centres, DNA platination is rapid, suggesting rate-limiting hydrolysis is not essential prior to DNA platination.

The high charge of **52** creates a unique charge/lipophilicity balance. Such charged complexes, including **47** and **48**, are unlikely to accumulate by passive diffusion (such as that proposed for neutral cisplatin), however cellular accumulation of multinuclear complexes, **47**, **48** and **52**, has been shown to be quite similar to that of cisplatin.[216]

Hence, the observed differential activities are unlikely to result from differences in cellular accumulation.

1.8.5.3. Interactions of 52 with DNA

While **52** achieves high levels of DNA platination, the distinct pattern of platinum(II)-DNA adducts and the cellular responses, rather than the level of platination, seem to be the major determinant of activity.[230,232,234]

1.8.5.3.1. Interstrand Cross-Link Formation by 52

Extensive studies have demonstrated the structure of DNA-adducts formed by multinuclear platinum(II) complexes differ significantly to those reported for mononuclear platinum(II) compounds such as cisplatin.[123,202,204,217]

The covalent DNA-binding of **52** is bifunctional and the overall profile is similar to that described for structurally-similar **47**.[204] The long linker of **52** was expected to increase the formation of long-range cross-links, where platinated nucleosides are separated by several intervening base pairs and the consequences of which are different to cisplatin. However, while **52** preferentially forms the longer 1,4 interstrand cross-links over other interstrand cross-links,[237] only 20% of **52**-DNA adducts are interstrand cross-links, compared with 75% of **47**-DNA adducts and 90% of **48**-DNA adducts.[123,202,204,214,215] These levels of interstrand cross-links correlate poorly with observed cytotoxic effects in cisplatin-resistant cell lines, suggesting long range interstrand cross-links are not critical factors in the unique spectrum of activity of multinuclear platinum(II) complexes, or alternatively not all interstrand cross-links are equal in their cytotoxic effect or other non-interstrand cross-links are also significant in drug mechanism.[216]

1.8.5.3.2. Cellular Processing of DNA Damage

52 is able to overcome many mechanisms associated with cisplatin-resistance, e.g. decreased drug accumulation, enhanced DNA repair or GSH content.[241] Alterations in the DNA repair systems, such as NER, do not affect the sensitivity to **52** in some cell

lines,[239] indicating **52**-DNA adducts can escape such DNA damage recognition and repair mechanisms of cisplatin-resistance.[231,234,241]

In contrast to the intrastrand cross-links of cisplatin, the intrastrand cross-links formed by **52** on duplex DNA are not recognised by the HMGB domain proteins, suggesting the processing of intrastrand cross-links of **52** by the HMGB1 protein is not relevant to the anti-cancer activity of **52**.[236] While the major interstrand cross-link (1,4-) formed by **52** does induce directional bending and local unwinding of the DNA helix, it is also not recognised by HMGB domain proteins.[237] The lack of recognition by HMGB domain proteins is not surprising when one considers the fact that, similarly to **47**,[215] the long range DNA cross-links of **52** induce a flexible, non-directional bend in DNA[237] rather than a rigid directional bend, the structural motif necessary for recognition of platinum(II)-modified DNA by the HMGB domain proteins.[129,136] The 1,4-interstrand cross-link is also not recognised or repaired by NER proteins (unlike the intrastrand cross-link of **52**),[236] therefore it is likely the 1,4-interstrand cross-links formed by **52** persist for a longer time than cisplatin-DNA adducts.[237]

52 shows superior activity against tumours carrying mutant p53 gene.[203,204,230,234,235] This result was surprising as loss of p53 was expected to lead to intrinsic resistance to platinum(II) drugs as a consequence of reduced susceptibility to apoptosis.[150-153] This indicates **52** is able to induce p53-independent apoptosis. This has important implications in the unique range of activity displayed by **52**, as the p53 gene is defective in several human tumours.

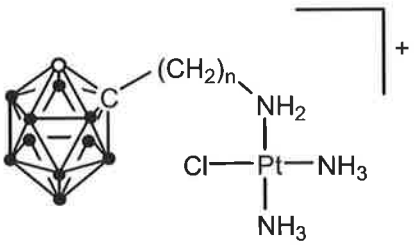
The above findings are further evidence that the high activity of **52** over cisplatin is due to the structurally unique DNA-binding modes of **52**. These significant DNA adducts mediate their biological consequences through different pathways to cisplatin, resulting in a unique mechanism of action of **52**.[232,234] Consequently, the modifications of DNA induced by the binding of **52** and cisplatin can result in different cellular responses in the same tumour model.[234] Hence **52** is new type of DNA-binding drug, rather than simply another cisplatin analogue.

1.9. Multinuclear Platinum(II) Complexes Containing (Aminopropyl)carborane Ligands for BNCT

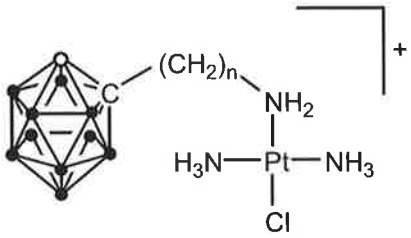
1.9.1. Recent Work

As detailed earlier, one of the most active areas of research in bio-inorganic chemistry is the study of platinum(II)-amine complexes, such as cisplatin, as avid DNA-binding agents and anti-cancer drugs. There are a variety of examples of transition metal complexes containing polyhedral boranes, however, none had been investigated for their potential application as novel DNA-targeting BNCT agents.[250-266]

Recent work within the Rendina group has led to the synthesis and isolation of platinum(II)-amine complexes **53-58**, containing 1,2-carborane functionalised at one of the carbon atoms with aminoalkyl tethers of varying lengths that were coordinated to various platinum(II) centres.[267]



53 n = 1
54 n = 2
55 n = 3



56 n = 1
57 n = 2
58 n = 3

Molecular modelling studies have demonstrated that the carborane framework comfortably fits into the major groove of duplex DNA and it is not expected to greatly decrease the ability of the platinum(II) centre to bind covalently to DNA bases. Indeed, some enhancement in DNA binding even may be observed.

1.9.1.1. Degradation of Carboranes

Unfortunately, the DNA-binding characteristics of complexes **53-58** were not determined as the complexes were hydrolytically unstable. Under aqueous conditions extensive degradation of the boron cluster was observed along with concomitant

reduction of the platinum(II) centre.[267,268] The decomposition process appeared to follow a two step pathway, initial hydrolytic degradation of the unstable *closo*-1,2-carborane cage generated the *nido*-7,8-carborane, which was then oxidised by the platinum(II) centre to afford colloidal platinum and a number of degraded cage species that gave rise to very complicated $^{11}B\{^{1}H\}$ NMR spectra.[268] The deboronation of *closo*-1,2-carboarane ligands in polar solvents (such as ethanol) has been observed in the preparation of a number of metal complexes, including palladium(II), silver (I), copper(I) and gold(I).[269,270]

The propylene-linked complexes (**55** and **58**) were of greater stability than the shorter linked analogues, indicating the relative proximity of the platinum(II)-amine centre to the carborane moiety appears to be a key factor in the stability of the complexes. Furthermore, the *cis*-dichloroplatinum(II) analogues were less stable and could not be isolated, even in the absence of water.[267,268]

1.9.2. The Advantages of Multinuclear Platinum(II) as Potential BNCT Agents

Of particular interest to the Rendina group were the DNA-binding abilities of the multinuclear platinum(II) complexes of the type described in Section 1.8, containing two or more platinum(II) centres linked by bridging bidentate diamine ligands. Multinuclear platinum(II) complexes have many advantages as DNA-binding agents over their mononuclear analogues. The presence of two or more reactive platinum(II) centres allows such complexes to bind avidly to DNA and achieve high levels of DNA platination. Multinuclear platinum(II) complexes also give rise to a unique array of DNA adducts, structurally distinct to those formed by cisplatin and its analogues.

The unique DNA adducts, such as those formed by **52**, have been shown to not be recognised by cellular DNA repair pathways as efficiently as those DNA-adducts formed by cisplatin and its mononuclear analogues.[242] Consequently, once a ^{10}B-containing multinuclear platinum(II) complex forms covalent bonds with the purine nucleotides of DNA, poor recognition of platinum(II)-DNA adducts by cellular repair pathways would allow the ^{10}B nuclei to persist near DNA for longer periods of time

than a ^{10}B-containing mononuclear complex. Such persistence of ^{10}B nuclei close to cellular DNA is a highly desired goal in BNCT.

1.9.3. Project Aims

One of the aims of this project is to prepare and fully characterise novel carborane-containing, multinuclear platinum(II)-amine complexes with varying platinum(II)-ligand environments as potential DNA-binding BNCT agents. The incorporation of the more thermodynamically stable 1,7- and 1,12-carboranes is anticipated to increase the hydrolytic stability of the platinum(II) complexes in comparison with **53-58**. We also plan to determine if the novel complexes retain the ability of the platinum(II) centres to covalently bind to DNA by comparison with the well-known DNA-cross-linking agent, cisplatin.

Due to the well known interactions of intercalators, such as platinum(II)-trpy complexes, another aim of this project is to prepare and fully characterise novel carborane-containing, dinuclear platinum(II)-trpy complexes and to evaluate their DNA-binding properties against other well known metallointercalators, by established methods.

The advantage, in each case, of using multinuclear platinum(II) complexes to target the ^{10}B nuclei to cellular DNA includes the decrease in dissociation constants (including natural removal mechanisms, such as NER) that tends accompanies bi-functional binding. Finally, due to the recognised anti-cancer activities of platinum(II)-amine and platinum(II)-trpy complexes, it is reasonable to expect cell destruction in the absence of thermal neutrons.

Furthermore, it is feasible that one may dramatically enhance tumour cell destruction by coupling the thermal neutron reactions of the ^{10}B nucleus with the DNA-binding properties of the platinum(II) centre. Indeed, an additive or perhaps synergistic therapy of certain tumours is also feasible here, particularly when one considers the intrinsic anti-cancer properties of the platinum(II) centre. Such an approach may greatly expand the clinical efficacy of platinum(II) anti-cancer drugs.

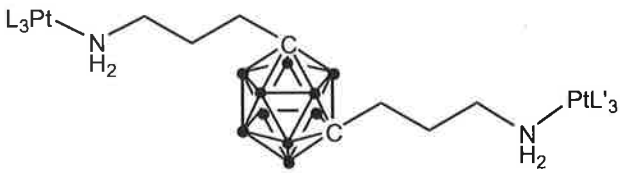
CHAPTER TWO

Multinuclear Platinum(II)-Amine Complexes Containing (Aminopropyl)carborane Ligands

Multinuclear Platinum(II)-Amine Complexes Containing (Aminopropyl)carborane Ligands

2.1. Introduction

In this Chapter, we report the preparation and characterisation of di- and trinuclear platinum(II) complexes containing bridging bidentate diamine ligands that incorporate 1,2-, 1,7- or 1,12-carborane, such as **59** and **60**. It was anticipated that incorporation of the more thermodynamically stable 1,7- and 1,12-carboranes would increase the hydrolytic stability of platinum(II) complexes in comparison with the 1,2-carborane containing complexes, **53-58**.



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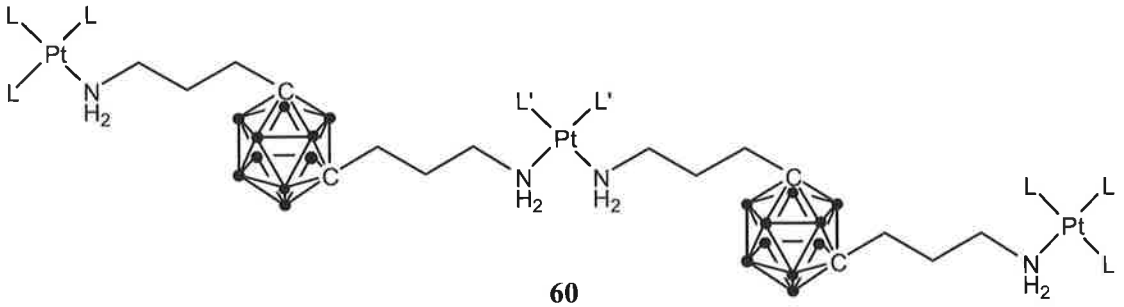

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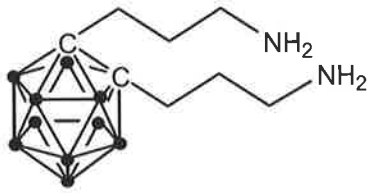
The number of ^{10}B-containing dinuclear platinum(II) complexes that can be prepared from diamines, **61-63** are almost limitless. For example, the platinum(II) coordination spheres may be equivalent or inequivalent, varying in overall charge or the number of leaving groups on each platinum(II) centre. Such variations may subtly alter the biological properties of the complex, with each variation potentially leading to a distinct interaction with DNA and, most importantly, alterations in the affinity for DNA and the covalent platinum(II)-DNA adducts that can be formed.



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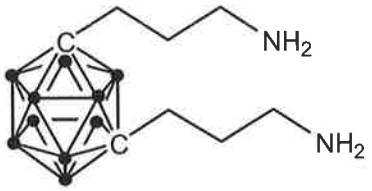

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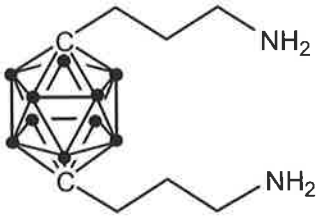

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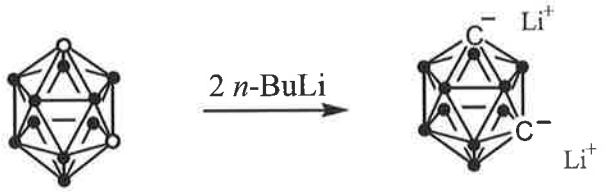
The simplest methodology for the preparation of multinuclear platinum(II) complexes such as **59** and **60** involved the initial synthesis of carborane-containing amine ligands prior to coordination to a platinum(II) centre. Due to the tendency of carboranes to degrade in the presence of platinum(II) under mild to severe reaction conditions,[250,271-273] the manipulation and handling of such complexes was best kept to a minimum.

2.2. Bis(Aminopropyl)carborane Ligands

The novel ligands described in this section are the carborane containing diamines **61-63**, which incorporate ten boron atoms in the form of di-substituted 1,2-, 1,7- and 1,12-carboranes, respectively. Both of the amine functionalities in **61-63** are available for coordination to platinum(II).

The $C_2B_{10}H_{12}$ carboranes (**8-10**) can be readily functionalised at both carbon atoms. Deprotonation of the CH groups by treatment with two equivalents of *n*-BuLi produces dilithio-carboranes with anionic charges localised on the carbon atoms (Scheme 2.1).[274] Reaction of the dilithio-carboranes enables functionalisation of the cage at the carbon atoms by nucleophilic ring opening or substitution reactions.[275-278]



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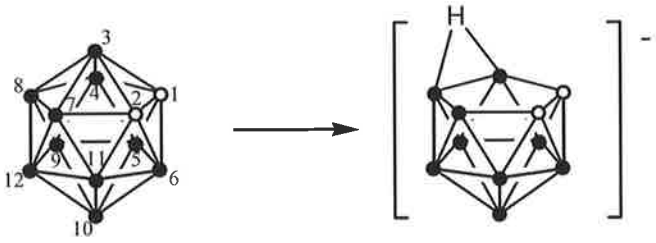
Scheme 2.1

Despite their high stability towards heat and oxidising agents, carboranes are susceptible to degradation of the icosahedral cage by nucleophiles, such as hydroxides and amines.[250,271-273] The degradation of the cage involves the selective removal of the B-3 (or B-6) atom to afford the anionic *nido*-$C_2B_9H_{12}^-$ carborane, **64** (Scheme 2.2).



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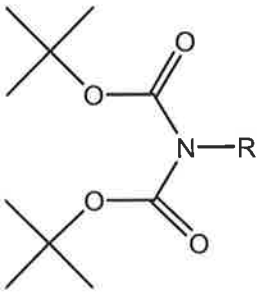
Scheme 2.2

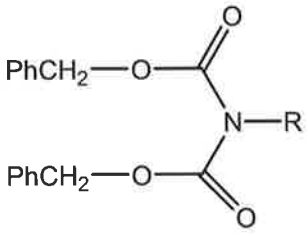
The relatively electronegative carbon atoms can activate the adjacent boron atoms toward nucleophilic attack.[273] As a result, the 1,2-carborane is the most susceptible to base degradation, as the 3- and 6-boron atoms are adjacent to two electron-withdrawing carbon atoms and hence are the most electrophilic boron atoms in the icosahedral framework.[279] Under similar conditions the degradation of the 1,7-carborane also proceeds, but not as quickly as for the 1,2-carborane.[280] The 1,12-carborane is only degraded under extremely harsh conditions, most likely as no single boron atom has two adjacent carbon atoms.[280,281]

Due to the tendency of carborane cages to be degraded, during the synthesis of diamines **61-63** it is essential to minimise the exposure of the *closo*-carborane cage to strong nucleophiles such as amines. Consequently, the amine functionality in **61-63** is

introduced as a less reactive protected entity such as the amide form by means of iminodicarboxylates (such as $N(BOC)_2$ and $N(CBZ)_2$) or phthalimides.



$N(BOC)_2$



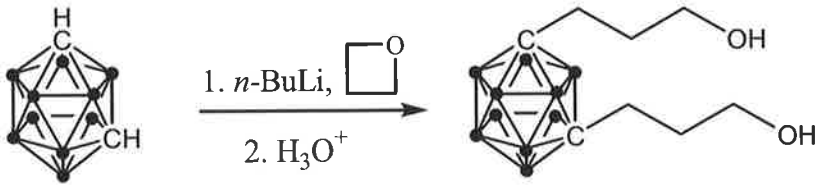
$N(CBZ)_2$

N,N-Iminodicarboxylate and phthalimide protected amines are readily prepared by *N*-alkylation using alkyl halides. A large number of mild methods are known for the preparation of precursor alkyl halides from a variety of starting materials, such as alcohols and alkenes.[282]

2.2.1. Preparation and Characterisation of Bis(aminopropyl)carborane Ligands

The five-step synthetic methodology used in the preparation of 1,7-bis(aminopropyl)-1,7-carborane (**62**) is presented below. Some aspects of this protocol were based upon reported literature procedures.[79,81,277,283-285]

In the first step of the synthesis of the bis(aminopropyl)carboranes **61-63**, the parent carborane was treated under anhydrous conditions with two equivalents of *n*-BuLi to generate the *closo*-dicarbanion (Scheme 2.3).[274] The propyl tether was then introduced by the nucleophilic ring opening of trimethylene oxide (oxetane). Any excess base was neutralised upon addition of aqueous acid, which also functioned to protonate the alkoxide anion to generate 1,7-bis(3-hydroxypropyl)-1,7-carborane (**65**).



65

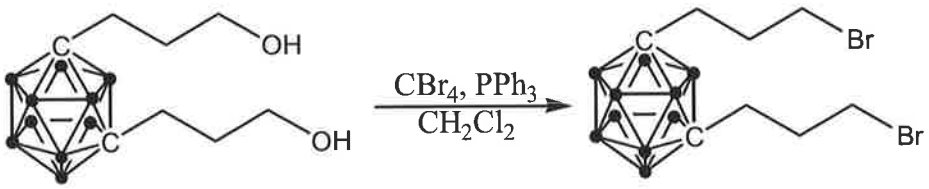
Scheme 2.3

Purification by column chromatography gave the diol in a reasonable yield (62%). The reaction conditions were successfully applied to the synthesis of both the corresponding 1,2- (**66**, 76%) and 1,12-isomers (**67**, 51%). Optimum yields were gained when using fresh *n*-BuLi. If the reaction mixture was too concentrated a violent unidentified reaction occurred which significantly reduced the overall yield of product.

One can characterise compounds such as **62**, **61**, **63** and **65** by means of $^{11}B\{^1H\}$ NMR spectroscopy.[286] Upon functionalisation of 1,2- and 1,7- and 1,12-carboranes the ^{11}B NMR signals are not significantly shifted, however the number of signals decreases as the modifications in the electronic properties of the *closo*-cage cause the boron atoms to become increasingly equivalent.

A number of methods exist for the conversion of alcohols to alkyl halides. Treatment with halogen acids (HX) and inorganic acid halides ($SOCl_2$, PCl_3 etc.) allows generation of primary halides, with the risk of elimination to give alkenes or alkynes.[282] Alternatively, treatment of alcohols with halogen gas and phosphines in dmf solution allows the preparation of alkyl halides without rearrangements.[287] Such methods generally involve highly corrosive reagents.

Instead, the alcohol functionality in **65** was treated with carbon tetrabromide and triphenylphosphine in dichloromethane to afford **68** in 86% yield (Scheme 2.4, **69** (1,2-) 73%, **70** (1,12-) 95%). These reaction conditions are mild in comparison to those mentioned above and are known to proceed within a few hours in high yields.[288] Any unreacted triphenylphosphine and by-products (bromoform and triphenylphosphine oxide) were easily removed by flash chromatography.

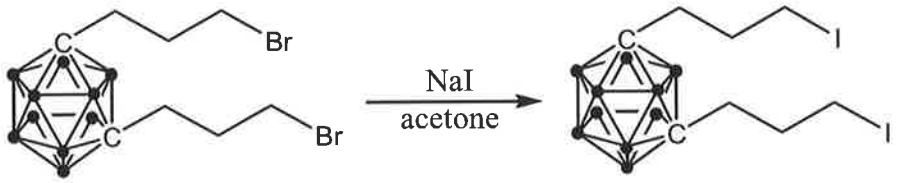


65 **68**

Scheme 2.4

The functionality at the end of the alkyl arm is significantly removed from the carborane cage and consequently does not cause notable changes in the $^{11}B\{^1H\}$ NMR spectra of these compounds. During such transformations, $^{11}B\{^1H\}$ NMR spectroscopy was most useful in determining whether degradation of the *closo*-cage to the *nido*-cage had occurred, a process that is typically accompanied by a characteristic upfield shift in $^{11}B\{^1H\}$ NMR resonances of around 30-40 ppm.[286]

The diiodo derivative (**71**) was obtained from **68** by Finkelstein reaction employing sodium iodide in acetone, in 92% yield (Scheme 2.5, **72** (1,2-) 73%, **73** (1,12-) 91%).[282] The introduction of alkyl iodide functionality was designed to facilitate the subsequent *N*-alkylation reaction, favoured by the iodide over bromide as the former is a better leaving group.

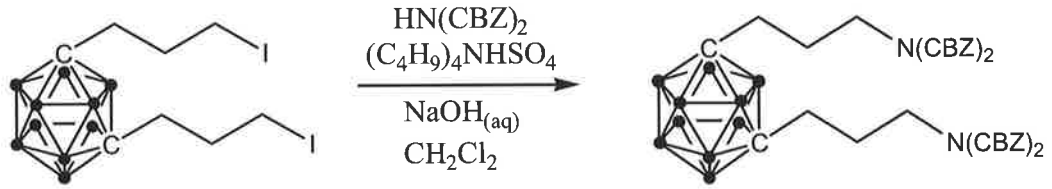


68 **71**

Scheme 2.5

The target amine derivative must be prepared from a protected-amine, due to the tendency of carboranes (particularly the 1,2-isomer) to degrade in the presence of free amines.[280] There are a high number of amino-protecting groups available.[289-294] Symmetrical iminodicarboxylates are a versatile protecting group, due to ease of introduction and removal and stability of the protected amine for long periods of time.[293] Protection also causes considerable reduction in the nucleophilicity of the amine.

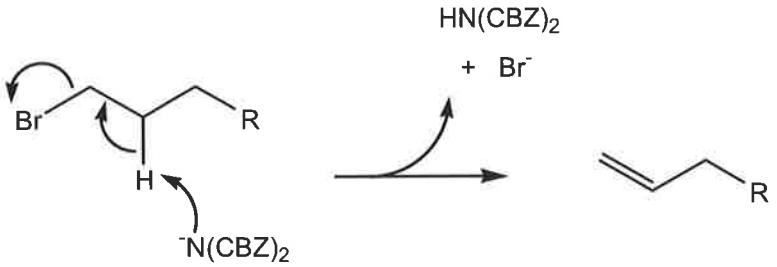
The amine functionality was introduced by the alkylation of *N,N*-dibenzyl iminodicarboxylate ($HN(CBZ)_2$) with the alkyl iodide (**71**) to generate the bis-CBZ-protected diamine (**74**, Scheme 2.6) under refluxing phase-transfer conditions.[295] Sodium hydroxide is required to deprotonate $HN(CBZ)_2$ to generate the nucleophilic iminodicarboxylate anion, $^-N(CBZ)_2$; and phase transfer conditions function to provide a lipophilic cation ($(C_4H_9)_4N^+$) to aid the solubility of the reactive anion $^-N(CBZ)_2$ in the dichloromethane layer. Unfortunately, a mixture of products was formed and **74** could not be isolated.



71 **74**

Scheme 2.6

Previous studies have found the alkylation of $HN(CBZ)_2$ by dihalides to be difficult.[81] This is most likely due to the tendency of one CBZ group to dissociate during extended periods under phase transfer conditions to afford RNH(CBZ) derivatives.[81] In some cases, rather than alkylation through nucleophilic substitution, the reactive iminodicarboxylate anion facilitates elimination of hydrogen iodide from the carborane propyl arm, generating vinylic derivatives (Scheme 2.7).[285]



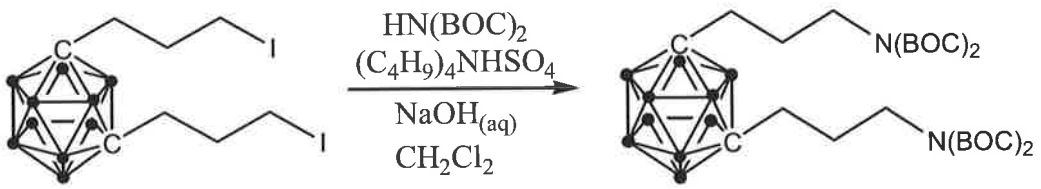
Scheme 2.7

Other methods of introducing iminodicarboxylate protecting groups have utilised Mitsunobu or Gabriel methods.[285,289,290,293,294] Adopting Mitsunobu conditions for an introduction of the CBZ protecting groups would involve the treatment of diol **65** with $HN(CBZ)_2$ in the presence of triphenylphosphine and DEAD (diethyl

azodicarboxylate).[296] However, studies within our laboratories have demonstrated that Mitsunobu conditions do not yield alkylation products in the presence of carboranes.[297]This is most likely due to the known competing base-catalysed elimination of HX to yield vinylic derivatives[285] (*cf.* Scheme 2.7) which would be facilitated by the electron-withdrawing nature of the highly aromatic carborane.

Generation of **74** under Gabriel conditions would involve the nucleophilic displacement of the halides in **68** or **71** by the potassium or sodium salt of iminodicarboxylate anion under anhydrous conditions.[293,294] Such conditions were not chosen for the synthesis of **74** as strongly basic conditions could degrade the carborane cage.[280]

Another symmetrical iminodicarboxylate, *N,N*-di-*tert*-butyl iminodicarboxylate or $HN(BOC)_2$ is commonly used in the preparation of protected amines.[289-293] The BOC protecting group was found to be more robust under the phase transfer conditions and alkylation of $HN(BOC)_2$ by **71** successfully gave the di-BOC-protected diamine (**75**) in 58% yield (Scheme 2.8, **76** (1,2-) 89%, **77** (1,12-) 48%).



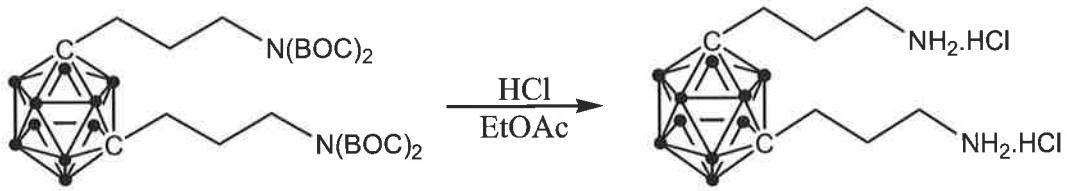
71 **75**

Scheme 2.8

Any unreacted **71** or intermediate mixed species was removed by flash chromatography, but it was difficult to separate **75** from any unreacted $HN(BOC)_2$. Several preparative silica gel columns and successive recrystallisations from *n*-hexane were necessary in order to obtain micro-analytically pure samples. However, the presence of $HN(BOC)_2$ was found not to interfere with the subsequent synthetic steps, and so in all cases impure di-BOC protected diamines were used in subsequent steps.

The BOC-protecting groups in **75** were easily removed under acidic conditions to give carbon dioxide, isobutene and the hydrochloride salt of the amine, **62**.2HCl in 69%

yield (Scheme 2.9, **61** 69%, **63** 85%). Under such conditions, any $HN(BOC)_2$ was converted to ammonium chloride and was readily removed by recrystallisation.



75 **62**.2HCl

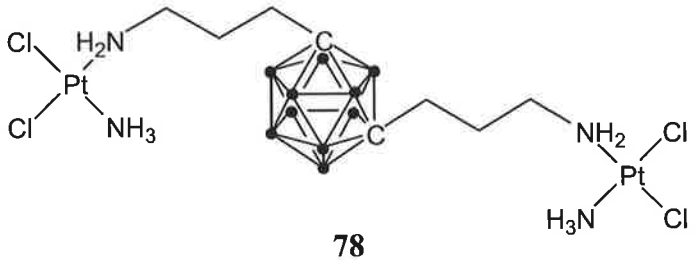
Scheme 2.9

The hydrochloride salts of amines **61-63** were isolated as stable, white powders that can be stored for extended periods of time, allowing complete characterisation. However, the free amines (**61-63**) were freshly prepared prior to complexation by platinum(II) by the addition of an aqueous solution of the corresponding hydrochloride salt to an excess of potassium carbonate in the presence of dichloromethane.

2.3. Dinuclear Platinum(II) Complexes Containing 1,7-Bis(3-aminopropyl)-1,7-carborane and 1,12-Bis(3-aminopropyl)-1,12-carborane

2.3.1. Synthesis of Complexes with *cis*-[$PtCl_2(NH_3)$(amine)] Geometry

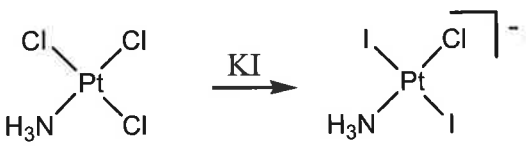
Potassium amminetrichloroplatinate(II) ($K[PtCl_3(NH_3)]$) is often used as a precursor in the synthesis of platinum(II) complexes of the type *cis*-[$PtCl_2(NH_3)$(amine)].[228,298-300] The reaction of **62** with two equivalents of $K[PtCl_3(NH_3)]$ afforded the corresponding dinuclear complex (**78**) with a *cis*-dichloro geometry, as indicated by the characteristic singlet resonance in the $^{195}Pt\{^1H\}$ NMR spectrum at δ -2156 consistent with a $PtCl_2N_2$ core.[301]



78

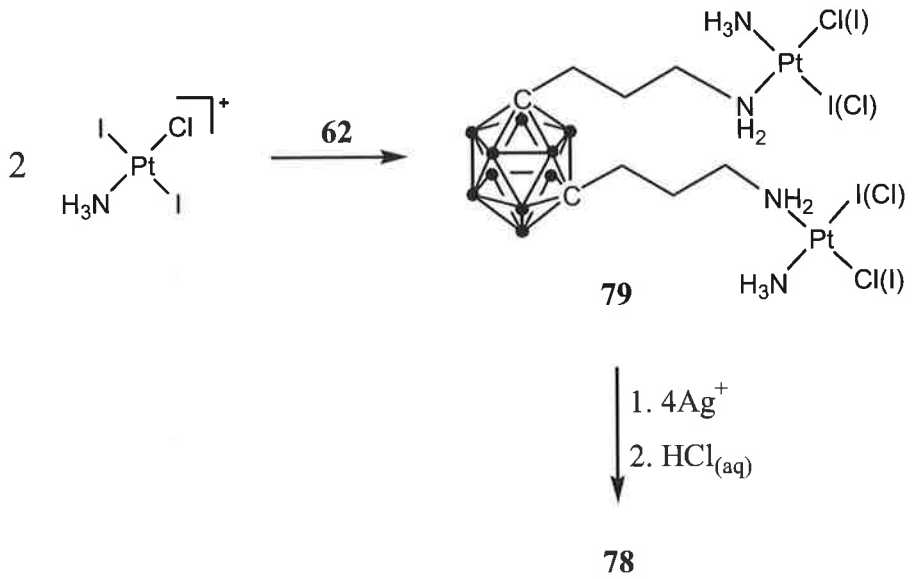
The relative *trans*-effects of the chloro and ammine ligands in the precursor $[PtCl_3(NH_3)]^-$ ensure the formation of the *cis*-geometry in the product, however an unidentified impurity was usually observed at δ -2462 in the $^{195}Pt\{^1H\}$ NMR spectrum. While this impurity remains unidentified, it is most likely a PtN_4 complex.

It is extremely difficult to purify **78** and similar complexes (such as **45**) by recrystallisation methods.[302] In order to increase the purity of **45**, $K[PtCl_3(NH_3)]$ was treated with an excess of potassium iodide to form *trans*-$[PtI_2Cl(NH_3)]^-$ (Scheme 2.10).[303] Amine substitution reactions on *trans*-$[PtI_2Cl(NH_3)]^-$ are faster than on $[PtCl_3(NH_3)]^-$ centres, favoured by the stronger *trans* effect of the iodo ligand over chloride. The presence of an iodo ligand also strongly favours formation of product with *cis*-geometry thereby decreasing yields of impurities.[303]



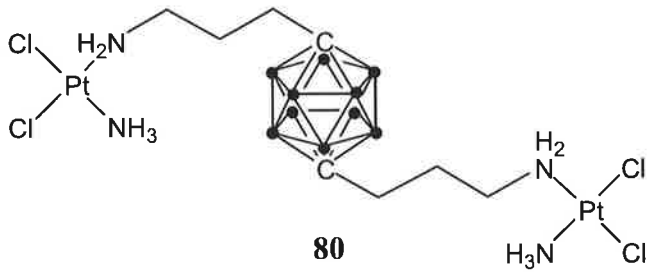
Scheme 2.10

Consequently, $K[PtCl_3(NH_3)]$ was converted to *trans*-$[PtI_2Cl(NH_3)]^-$ prior to reaction with **62** (Scheme 2.11) and **63**. The ligand amine groups displace either one of the iodo ligands in $[PtI_2Cl(NH_3)]^-$ to give the neutral mixed chloro/iodo complex (**79**), which precipitates from a methanol/water solution. Treatment with four equivalents of silver nitrate, followed by addition of aqueous hydrochloric acid precipitated the desired **78** ($^{195}Pt\{^1H\}$ NMR δ -2156).



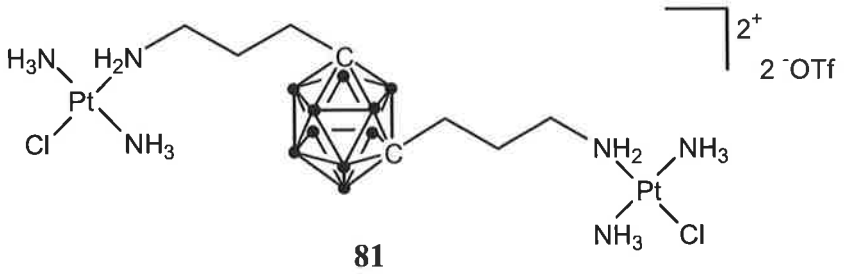
Scheme 2.11

Under identical conditions, **63** was converted into the corresponding dinuclear complex with *cis*-dichloro geometry (**80**). Once again, the formation of this complex was confirmed by a characteristic resonance in the $^{195}Pt\{^{1}H\}$ NMR spectrum at δ -2155.[301]

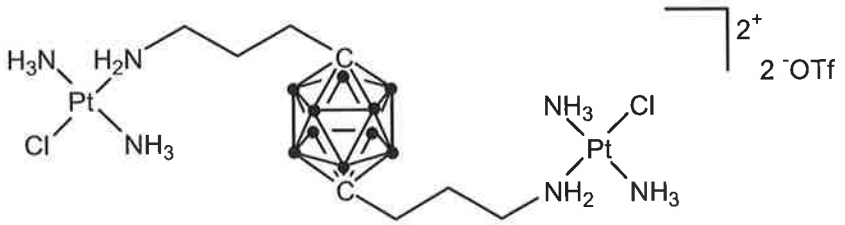


2.3.2. Synthesis of Complexes with *trans*-[PtCl(NH$_3$)$_2$(amine)] Geometry

The removal of one chloro ligand by the reaction of transplatin (**32**) with one equivalent of silver triflate in dmf solution generates the labile species *trans*-[PtCl(dmf)(NH$_3$)$_2$]OTf. Reaction of a diamine ligand (**62** or **63**) with two equivalents of *trans*-[PtCl(dmf)(NH$_3$)$_2$]$^+$ afforded the corresponding dinuclear complexes (**81** and **82**) with characteristic resonances in the $^{195}Pt\{^{1}H\}$ NMR spectrum at δ -2410 and δ -2409, respectively. The chemical shifts of these resonances are consistent with a complex possessing a PtN$_3$Cl core.[301]



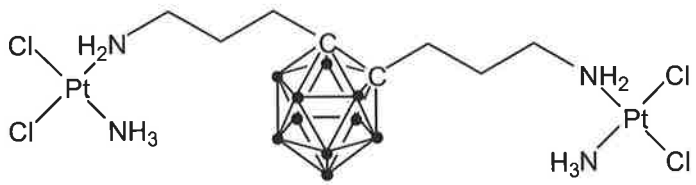
81



82

2.4. Platinum(II) Complexes Containing 1,2-Bis(3-aminopropyl)-1,2-carborane

The synthesis of the 1,2-carborane-containing dinuclear platinum(II) complex **83** was attempted by the reaction of **61** with $K[PtCl_3(NH_3)]$ in dmf/water and methanol/water solvent mixtures. However, the isolated solid was quite unstable, discolouring upon purification and on standing, signifying extensive degradation of the *closo*-carborane cage to the *nido*-cage, with concomitant reduction of the platinum(II) to platinum metal.



83

The decomposition of **83** was slower than that observed for the mononuclear complexes **53-58** and their *cis*-dichloro platinum(II) analogues (Section 1.9.1.1.),[267,268] evidence that the bi-functionalisation of 1,2-carborane somewhat increases the stability of the platinum(II) derivatives. The incorporation of the propylene tether in **83** increased the distance between the carborane and the platinum(II) centre, which

has been demonstrated to increase the overall stability of complexes of this type.[268] It was found that the degradation process could be retarded upon acidification below pH 2.[304] However, under such acidic conditions the amine becomes protonated and the complexation by the platinum(II) centre does not proceed to completion.

In the absence of water, the synthesis of **83** was achieved in high yield upon treatment of **61** with $PPh_4^+[PtCl_3(NH_3)]^-$ in dichloromethane. Unfortunately, the purification by recrystallisation was unsuccessful due to the poor solubility of the by-product (PPh_4Cl) and unreacted $PPh_4[PtCl_3(NH_3)]$. Despite the increased stability of **83**, the purity and stability of any isolated solid was consistently insufficient to allow spectral characterisation or the determination of DNA-binding affinity. Considering the poor stability of **83** in aqueous solutions, it is likely that even if **83** was successfully synthesised and purified, it would not be stable under biological conditions and consequently could not be studied as a potential BNCT agent. Consequently, further studies into platinum(II) complexes containing **61** were abandoned.

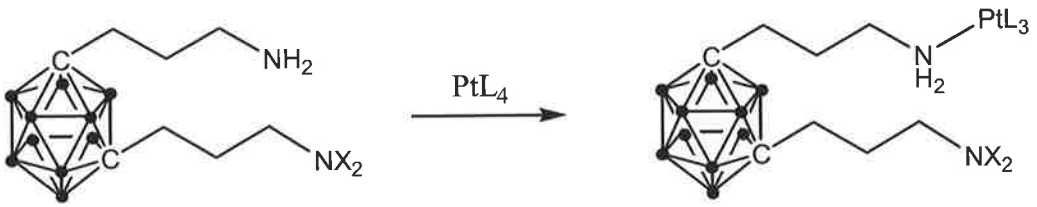
2.5. Asymmetric Dinuclear Platinum(II) Complexes Containing 1,7-Bis(3-aminopropyl)-1,7-carborane

The synthesis of dinuclear platinum(II) complexes with inequivalent coordination spheres has attracted the interest of researchers due to the potential of some complexes selectively reacting at only one of the platinum(II) centres.[170,221,226]

Many reported syntheses have used a 'dangling' diamine (such as **84**) with one of the amine groups protected as an iminodicarboxylate or a hydrochloride salt.[226,305,306] Diamine **84** is commercially available and it can be synthesised by treatment of 1,6-diaminohexane with di-*tert*-butyl dicarbonate ($O(BOC)_2$) in dioxane solution, followed by purification by extraction into water.[305]

84

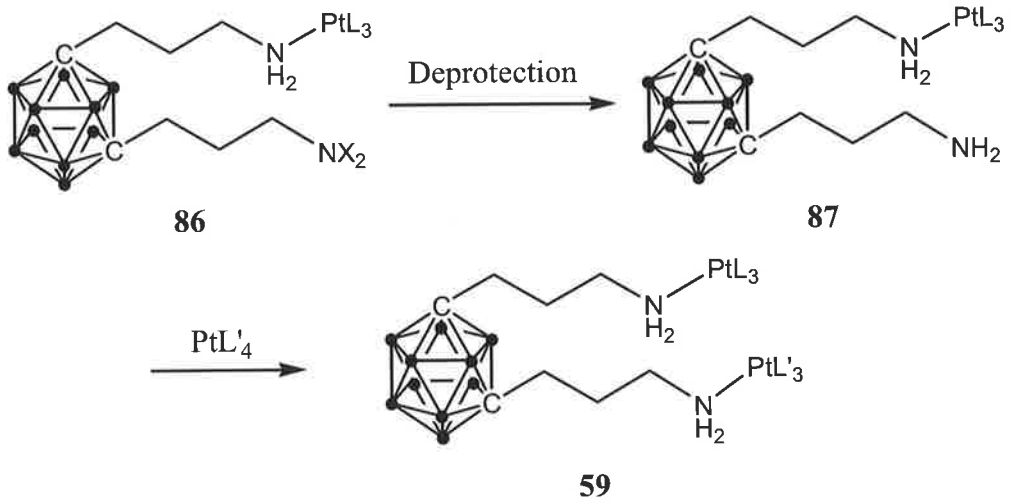
The synthesis of the analogous 'dangling' amine-carborane ligand (**85**) would allow for selective reaction with a platinum(II) centre to generate a mononuclear platinum(II) complex, **86** (Scheme 2.12). The protection of the second amine functionality ensures that it does not coordinate to platinum(II) or degrade the carborane cage.



85 **86**

Scheme 2.12 X = protecting group; L = ligand.

The amine-protecting group in **86** may then be removed to liberate mononuclear platinum(II) complex **87**, containing the second free amine, which can then complex to a second (non-identical) platinum(II) centre to generate the dinuclear complex, **59** containing two platinum(II) centres with inequivalent coordination spheres (Scheme 2.13).



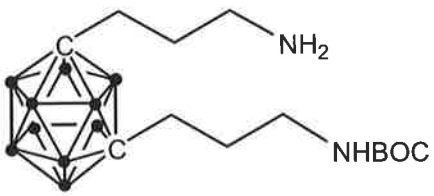
86 **87**

59

Scheme 2.13

A carborane-containing a 'dangling' amine, such as **88**, was not synthesised for a number of reasons. The synthesis of the required precursor diamine (**62**) involves several steps and it is considered to be very valuable. Furthermore, due to the

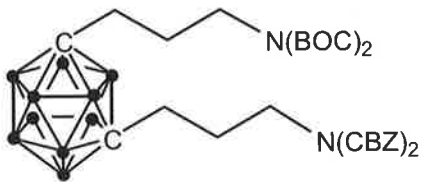
relatively limited solubility of carboranes and their derivatives (such as **88**), meant that it is unlikely that **88** could be purified easily by recrystallisation, instead requiring purification by means of column chromatography. Unfortunately, it was found in this project that amines such as **88** tended to streak on silica during chromatographic separation, resulting in low yields upon purification.



88

2.5.1. Asymmetric Protection of 1,7-Bis(3-aminopropyl)-1,7-carborane

In a novel approach, the amine groups in **62** were protected with two different protecting groups. **89** incorporates both the $N(BOC)_2$ and $N(CBZ)_2$ iminodicarboxylate protecting groups.



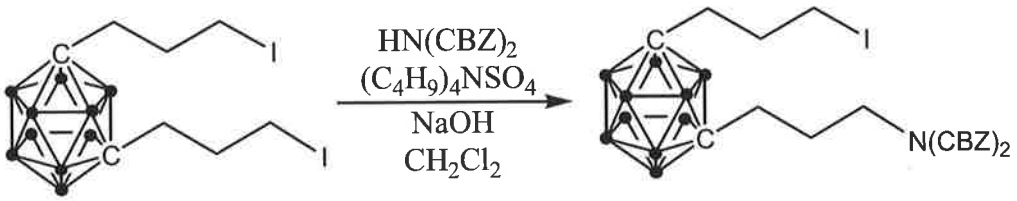
89

The CBZ protecting group may be removed selectively under hydrogenation conditions and it is stable under acidic conditions. The BOC protecting group may be removed under acidic conditions and it is stable under non-acidic hydrogenation conditions. Both deprotections occur cleanly and in high yields in the presence of carborane.[79,81,284,285,307]

2.5.1.1. Synthesis of Asymmetrically Protected 1,7-Bis(3-aminopropyl)-1,7-carborane

The reaction of **71** with one equivalent of dibenzyl iminodicarboxylate ($HN(CBZ)_2$) under phase transfer conditions gave the CBZ-protected monoamine **90** in 44% yield

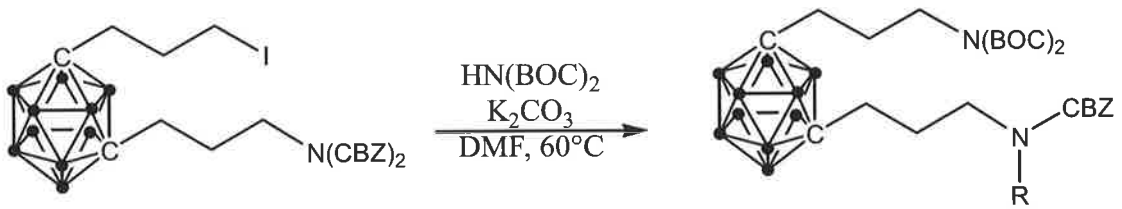
(Scheme 2.14). The reaction time was limited to 5 hours, as longer reaction times led to dissociation of one of the CBZ groups.[81]



71 **90**

Scheme 2.14

Due to the tendency of the CBZ protecting groups, such as those found in **90**, to dissociate during extended periods under phase transfer conditions, the BOC-protected amine in **89** could not be introduced under the phase transfer conditions used in the preparation of **75**. Instead, warming a solution of **90**, potassium carbonate and one equivalent of di-*tert*-butyl iminodicarboxylate in dmf solution at 60°C for 24 hours allowed the preparation of the mixed protected diamine **89** in moderate yield (Scheme 2.15).



90

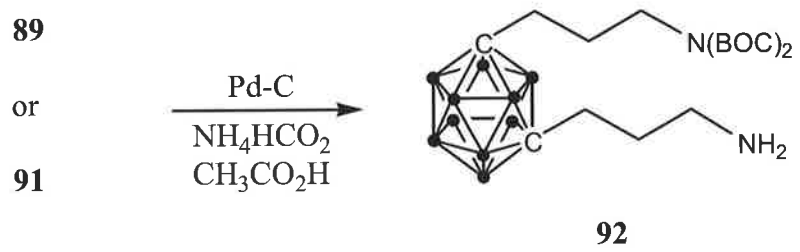
89 R = CBZ
91 R = H

Scheme 2.15

In Scheme 2.15, the potassium carbonate acts a mild base to generate the reactive anion, $^{-}N(BOC)_2$, without causing degradation of the *closo*-carborane cage and the dmf acts as a solvent able to dissolve all of the reaction substrates. If the reaction temperature exceeded 60°C or the reaction was allowed to proceed for longer periods (48 hours), one of the CBZ groups was lost to give **91**. However, **89** and **91** can be separated by flash chromatography and both may successfully undergo the following deprotection steps.

2.5.2. Selective Removal of the CBZ Protecting Groups

The CBZ protecting group(s) were removed from **89** and **91** prior to the BOC groups. This order of deprotection was chosen in order to eliminate any potential complication that may occur if a platinum(II) complex of the type **86** were subjected to conditions of hydrogenation, such as potential reduction of platinum(II) to platinum(0). Typically, a CBZ group is removed in an acidified polar solvent, such as ethanol, in the presence of a palladium catalyst under a high-pressure dihydrogen atmosphere,[284,285] however such acidic conditions would also remove the BOC protecting groups in **89** and **91**. Using related conditions to those described by Ghaneolhosseini,[79] the hydrogenation was achieved to give **92** in quantitative yields by means of palladium on carbon catalyst in a buffered ammonium formate aqueous acetic acid solution (Scheme 2.16). Due to the tendency of the free amine to degrade the *closo*-carborane cage **92** was freshly prepared prior to reaction with the platinum(II) centres and a suitable micro-analysis of **92** could not be obtained.

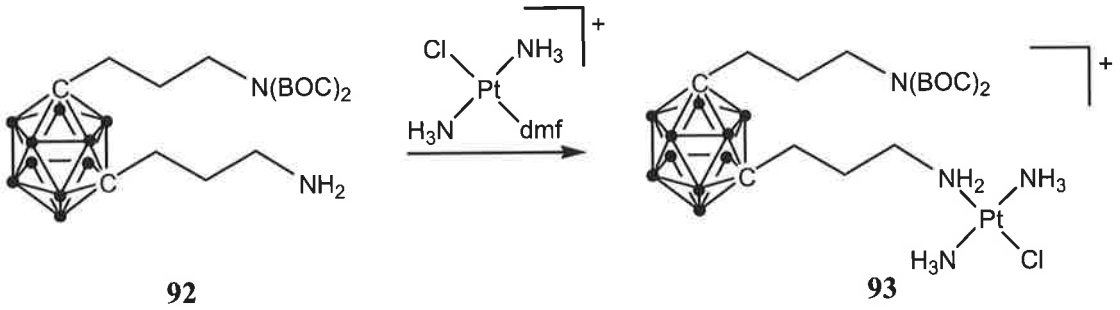


92

Scheme 2.16

2.5.3. Synthesis of Mononuclear Platinum(II)-Amine Complexes Containing 92

Treatment of transplatin with one equivalent of silver triflate generated the labile *trans*-$[PtCl(NH_3)_2(dmf)]^+$ species which readily coordinated the primary amine group in **92** to give the mononuclear platinum(II) complex **93** (Scheme 2.17). **93** was unexpectedly soluble in solvents such as methanol and ethyl acetate, allowing facile removal of any unreacted transplatin.

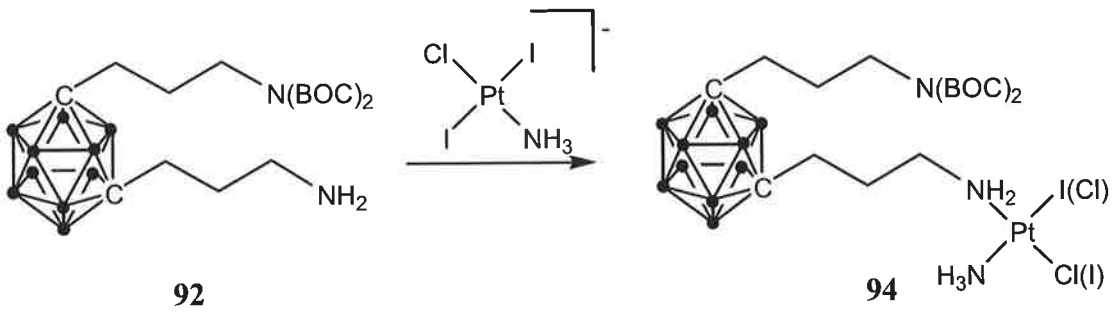


92 **93**

Scheme 2.17

The coordination of the primary amine in **92** to the platinum(II) centre was confirmed by a characteristic resonance in the $^{195}Pt\{^{1}H\}$ NMR spectrum at δ -2409, consistent with a PtN_3Cl core. There was also a change in the $^{13}C\{^{1}H\}$ NMR spectrum with the CH_2NH_2 signal shifting from δ 40.4 to δ 47.4 upon coordination by the platinum(II) centre.

Alternatively, the free primary amine in **92** was reacted with *trans*-$[PtClI_2(NH_3)]^-$ by stirring in a methanol/water solvent mixture to precipitate the mononuclear platinum(II) complex **94** (Scheme 2.18). The formation of a $PtClIN_2$ species was verified by a characteristic resonance in the $^{195}Pt\{^{1}H\}$ NMR spectrum at δ -2651.[303]



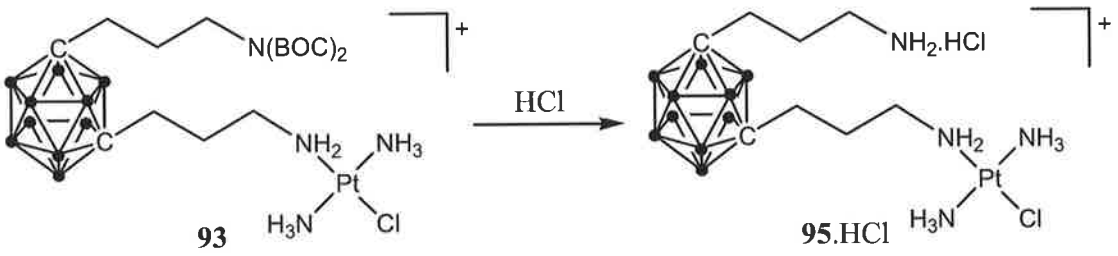
92 **94**

Scheme 2.18

2.5.4. Selective Removal of BOC Protecting Groups

The BOC protecting groups in **93**were removed by the addition of a 3 M hydrochloric acid solution liberating **95**.HCl containing the second amine functionality as its hydrochloride salt (Scheme 2.19). The deprotection was confirmed by $^{13}C\{^{1}H\}$ NMR spectroscopy with the signals corresponding to the carbonyl and *tert*-butyl groups of

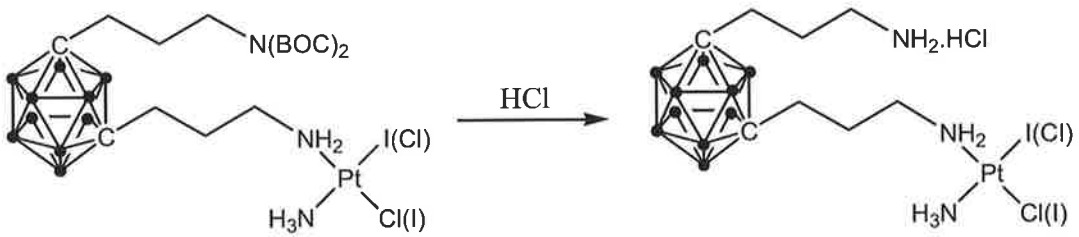
the BOC group disappearing and a shift in the non-coordinated CH_2N signal from δ 46.1 to 39.1.



93 **95**.HCl

Scheme 2.19

It is essential that all dmf was removed prior to the deprotection under the strongly acidic aqueous conditions to prevent formation of an unidentified polymer that was difficult to remove. Similarly, deprotection of the BOC protecting groups in **94** was achieved by stirring a 3 M hydrochloric acid solution to liberate the amine (**96**) as the hydrochloride salt (Scheme 2.20).



94 **96**.HCl

Scheme 2.20

During the generation of the hydrochloride salts in **95** and **96** there was no significant change in the $^{195}Pt\{^1H\}$ NMR spectra of the complexes.

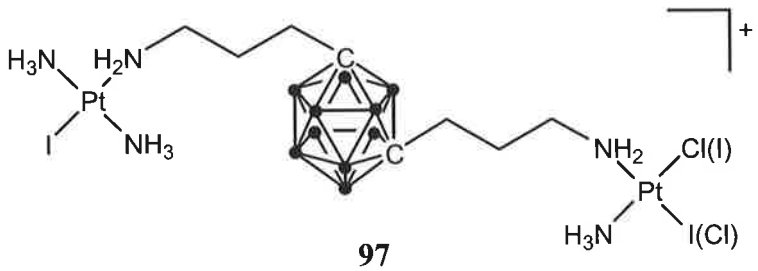
2.5.5. Synthesis of Asymmetric Dinuclear Platinum(II)-Amine Complexes Containing 1,7-Bis(3-aminopropyl)-1,7-carborane

Due to the limited solubility of both **95**.HCl and **96**.HCl the free amines were liberated *in situ* by the addition of a mild base such as potassium carbonate. The complexation of $K[PtCl_3(NH_3)]$ by the primary amine in **95** was attempted in a mixed dmf/water solvent system, essential for the solubility of all reactants. However, the

complexation proceeded very slowly, even in the presence of potassium carbonate. This was possibly due to a poorer solvation of the leaving chloro ligand by dmf in comparison to the methanol/water solvent system used in the synthesis of **78** and **80**. Furthermore, it is possible that the precipitation of **78** and **80** from the methanol/water solutions drove the complexation reactions to completion. In contrast, the reaction between $K[PtCl_3(NH_3)]$ and **95** was conducted in dmf solution, in which the platinum(II) product has a high solubility and thereby eliminating the driving force of the complexation reaction as precipitation of the product from solution does not occur.

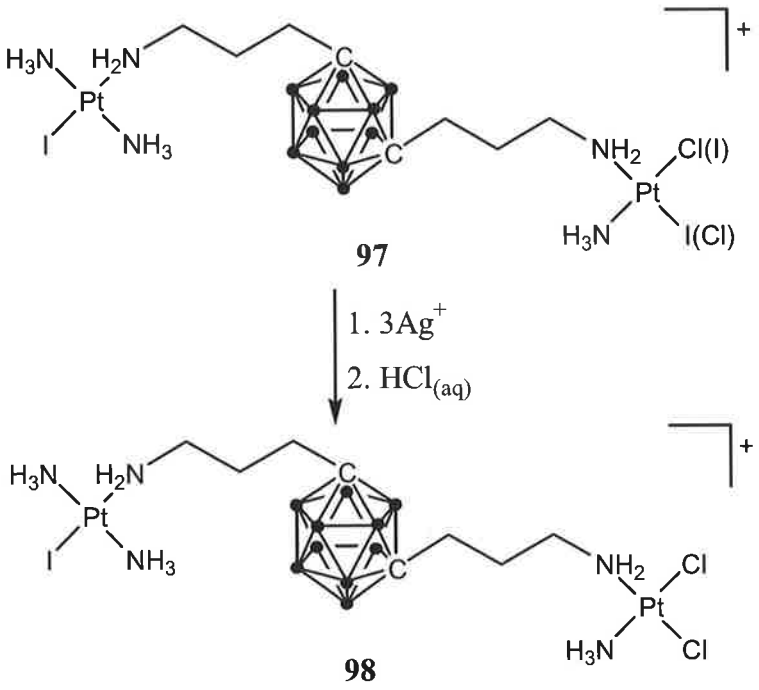
As mentioned previously, the slow rate of ligand substitution in $[PtCl_3(NH_3)]^-$, particularly in dmf solution, often necessitates the use of iodide to accelerate the reaction.[228,303] The stronger *trans* effect of iodide over chloride facilitates rapid exchange at the platinum(II) centre by primary amines, such as **95**.

The rate of reaction of **95** was much higher with *trans*-$[PtClI_2(NH_3)]^-$ in the presence of potassium carbonate, to afford the dinuclear platinum(II) complex **97** containing a mixed halogen ligand set. The presence of excess potassium iodide resulted in the exchange of the chloro ligand in the $PtClN_3$ centre to form the corresponding $PtIN_3$ centre. **97** was characterised by $^{13}C\{^1H\}$ and $^{195}Pt\{^1H\}$ NMR spectroscopy, displaying resonances at δ -2652 and δ -2851 in a 1:1 ratio which correspond to the $PtClIN_2$ and $PtIN_3$ cores, respectively.



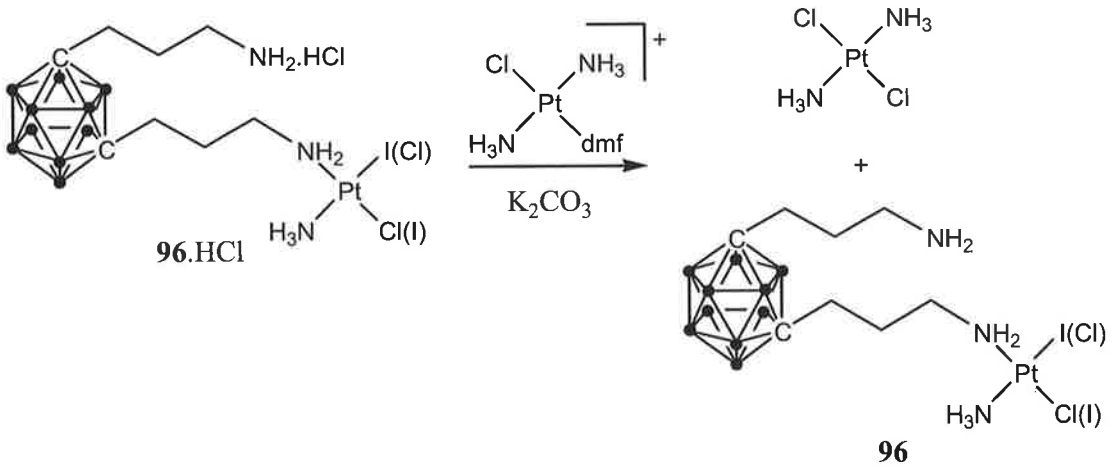
97

Upon treatment of **97** with three equivalents of silver triflate, the iodo and chloro ligands were displaced to afford the labile dmf complex. The addition of aqueous hydrochloric acid to the dmf solution formed the desired **98** ($^{195}Pt\{^1H\}$ NMR δ -2156 and -2410) which precipitated out of solution as a bright yellow solid (Scheme 2.21).



Scheme 2.21

The addition of labile *trans*-$[PtCl(dmf)(NH_3)_2]^+$ to a dmf solution of **96**.HCl in the presence of potassium carbonate did not result in coordination of the free amine in **96** by the platinum(II) centre to give **97**. Instead, the chloride anion (HCl) rapidly reacted with $[PtCl(dmf)(NH_3)_2]^+$ to generate transplatin and free **96** (Scheme 2.22).



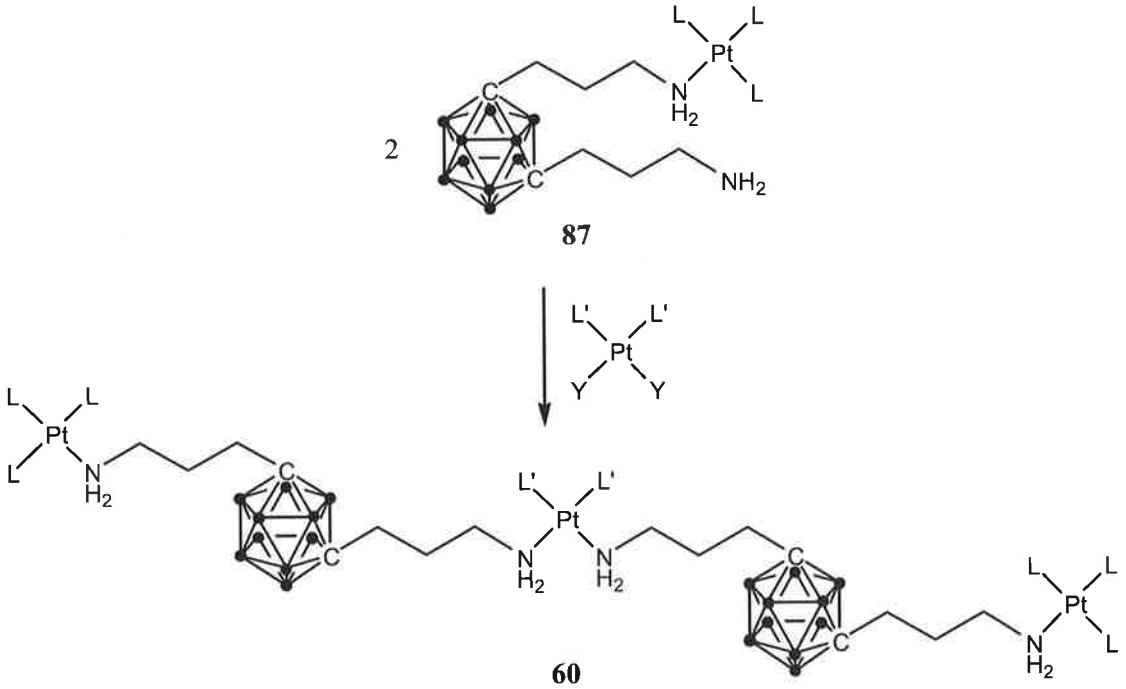
Scheme 2.22

It is possible that **96**.HCl can be neutralised in a dmf/methanol solvent mixture, resulting in the precipitation of free **96**. The free amine can then be quickly collected by filtration and reacted with *trans*-$[PtCl(dmf)(NH_3)_2]^+$ in dmf solution. The absence

of free chloride ions would then prevent formation of transplatin and allow the formation of **97**. However, due to time constraints this methodology was not investigated.

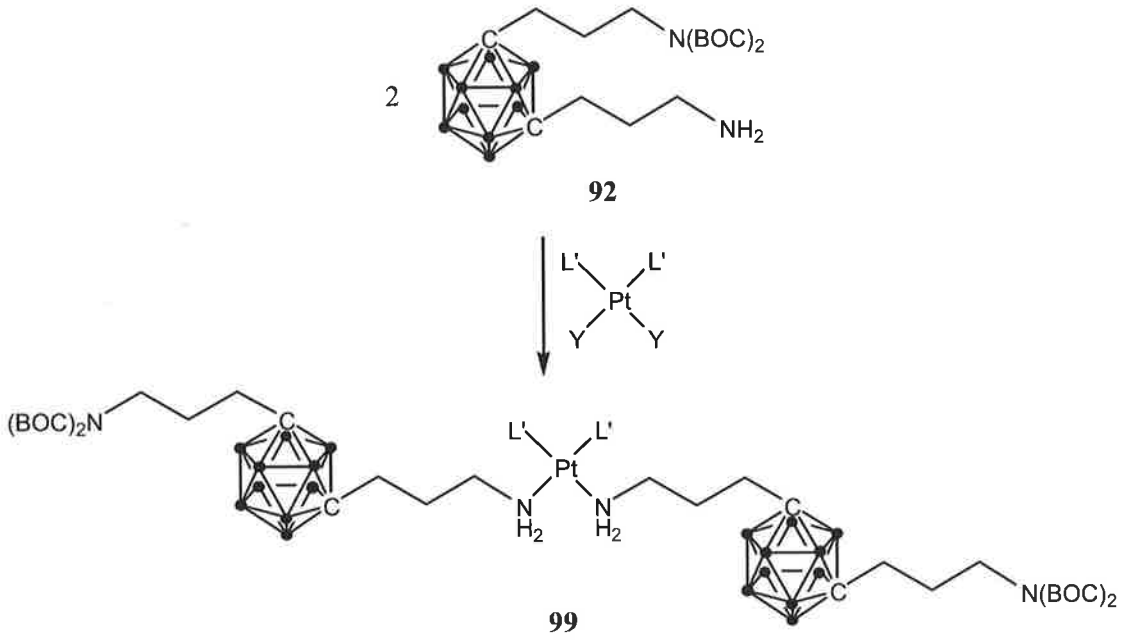
2.6. Trinuclear Platinum(II) Complexes Containing 1,7-Bis(3-aminopropyl)-1,7-carborane

Once the synthesis of the carborane-containing 'dangling' amine (**92**) was achieved, the synthesis of a novel trinuclear platinum(II) complex could be obtained by a number of synthetic pathways. For example, a mononuclear platinum(II) complex containing a second free amine, such as **87**, could be reacted with one-half equivalent of a second, inequivalent platinum(II) centre (eg. *cis*-PtL'_2Y_2) to generate the corresponding *cis*-isomer of the trinuclear platinum(II) complex, **60** (Scheme 2.23).



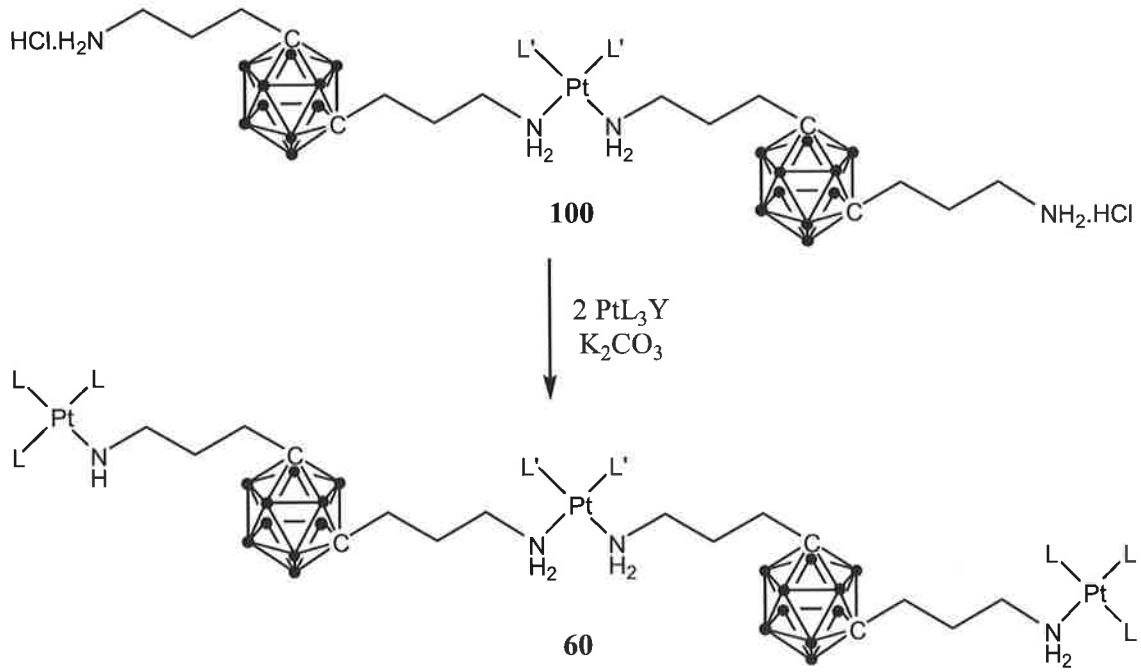
Scheme 2.23

Alternatively, two equivalents of **92** may be reacted with the same *cis*-$[PtL'_2Y_2]$ centre, containing two coordination sites that are occupied by labile monodentate ligands, to generate the mononuclear platinum complex **99** containing two carborane ligands (Scheme 2.24).



Scheme 2.24

The BOC protecting groups may be removed under standard acidic deprotection conditions to liberate **100**, containing two primary amine groups as their hydrochloride salts. The 'capping' platinum(II) centres may then be introduced to form **60** in the presence of a mild base, such as potassium carbonate (Scheme 2.25).

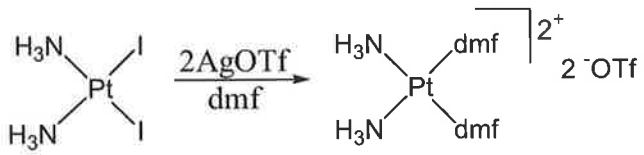


Scheme 2.25

The latter synthetic pathway has been utilised in the synthesis of non-boron containing analogues of the trinuclear platinum(II) complex **52**.[238] Stirring an aqueous solution of K_2PtCl_4 in water with two equivalents of 'dangling' amine $H_2N(CH_2)_4NHBOC$ precipitates *cis*-$[PtCl_2(H_2N(CH_2)_4NHBOC)_2]$.

An analogous reaction was attempted using $K_2[PtCl_4]$ and the carborane-containing 'dangling' amine, **92**, however no reaction occurred. $K_2[PtCl_4]$ is very water soluble, however it was not sufficiently soluble in a methanol/water solvent mixture required to solubilise **92**. Despite all efforts to establish water/methanol solvent systems to maximise the solubility of both reactants, the only precipitate that was ever isolated was the starting platinum(II) precursor complex. The reaction was not attempted in dmf solution, as the substitution of chloro ligands in a $PtCl_3N$ core by amines in this solvent, as previously discussed, does not occur at a reasonable rate and it was anticipated that the process would not go to completion.

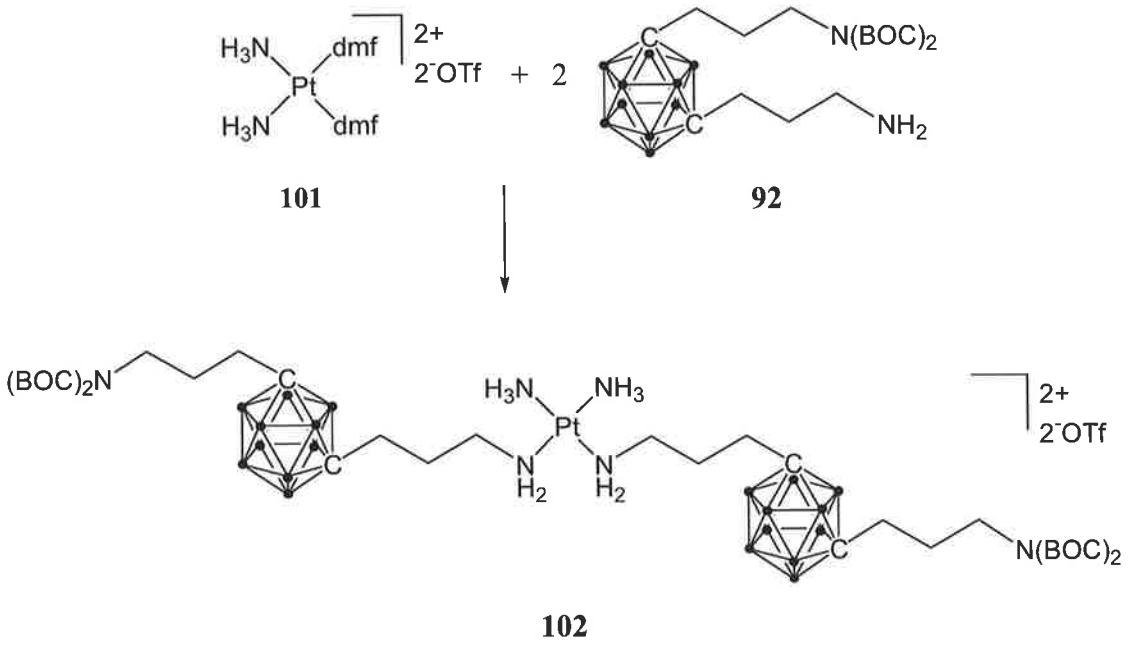
Instead, a platinum(II) centre with two labile coordination sites (**101**) was generated by reaction of *cis*-$[PtI_2(NH_3)_2]$ with two equivalents of silver triflate in dmf solution. The iodo ligands were readily removed (precipitated with Ag^+ as AgI) and replaced by the labile, *O*-donor dmf solvent molecules (Scheme 2.26). The triflate salt was chosen in order to increase the solubility of the cationic product in organic solvents.



101

Scheme 2.26

The two vacant coordination sites in **101** were readily occupied by the primary amine in **92** to afford **102** (Scheme 2.27). The coordination of the amine in **92** was supported by a shift in the $^{13}C\{^1H\}$ NMR signal corresponding to the CH_2NH_2 group from δ 40.4 to δ 46.8 and a signal at δ -2663 in the $^{195}Pt\{^1H\}$ NMR spectrum, characteristic of a PtN_4 coordination sphere.[303]

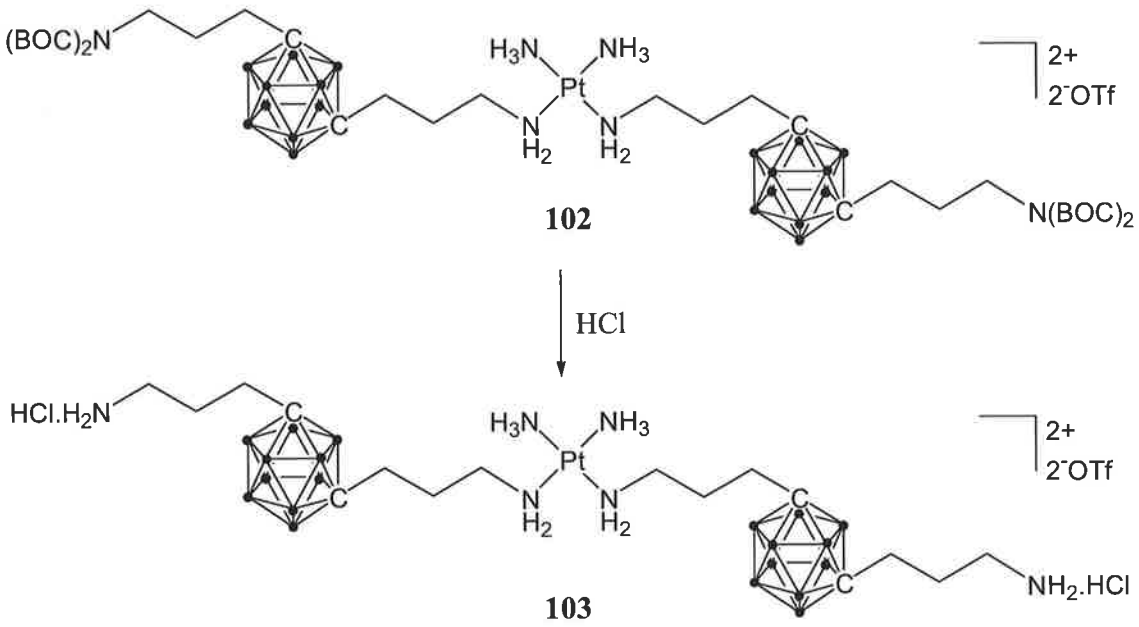


101 **92**

102

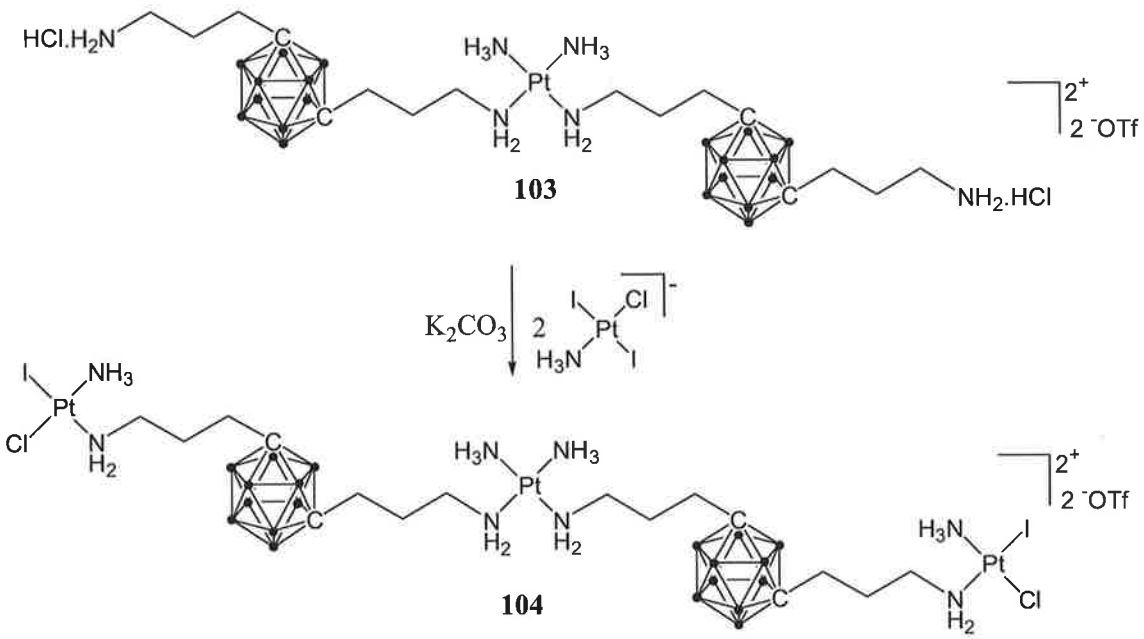
Scheme 2.27

The mononuclear platinum(II) complex, **102**, was sufficiently soluble in ethyl acetate to allow BOC-deprotection using the standard ethyl acetate/hydrochloric acid$_{(aq)}$ conditions (Scheme 2.28). As **103** formed, it separated out of solution as an oil and was isolated by decantation. Removal of the BOC protecting groups was accompanied by a characteristic shift in the $^{13}C\{^1H\}$ NMR signal from δ 46.8 to δ 39.6, corresponding to the conversion of $CH_2N(BOC)_2$ to $CH_2NH_2.HCl$. As expected, no change was observed in the $^{195}Pt\{^1H\}$ NMR spectrum of the complex.



Scheme 2.28

The terminal amines of **103** were treated with *trans*-[$PtI_2Cl(NH_3)$] in the presence of two equivalents of potassium carbonate (Scheme 2.29). The coordination of the terminal amine centres by the platinum(II) centre was confirmed by a characteristic shift in the $^{13}C\{^1H\}$ NMR signal from δ 39.6 to δ 46.0, corresponding to the conversion of $CH_2NH_2.HCl$ to $CH_2NH_2PtClI(NH_3)$ of **104**. The formation of **104** could not be confirmed by $^{195}Pt\{^1H\}$ NMR spectroscopy because, coincidently, both the PtN_4 and $PtIClN_2$ centres characteristically display shifts in the $^{195}Pt\{^1H\}$ NMR spectrum around δ -2650. Consequently, only one peak was observed in the $^{195}Pt\{^1H\}$ spectrum of **104** (δ -2646) and no signals corresponding to the precursor complex or other platinum(II) centres were observed, consistent with the formation of **104**.

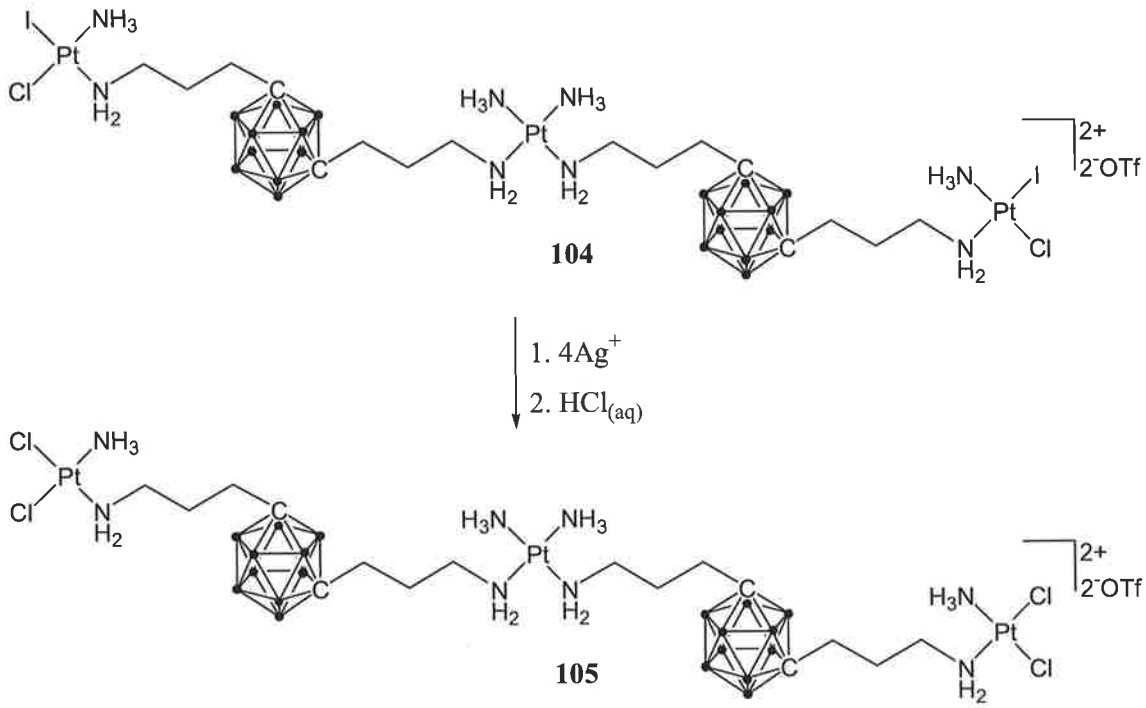


103

104

Scheme 2.29

In order to confirm that the signal observed at δ -2646 in the $^{195}Pt\{^{1}H\}$ NMR spectrum of **104** was due to two different platinum(II) centres, **104** was treated with four equivalents of silver triflate, followed by an excess of hydrochloric acid to liberate two new *cis*-$PtCl_2N_2$ centres in **105** (Scheme 2.30). This conversion was confirmed by the observation in the $^{195}Pt\{^{1}H\}$ NMR spectrum of **105** of two signals at δ -2157 and δ -2651 in a 2:1 ratio, corresponding to the *cis*-$PtCl_2N_2$ and PtN_4 centres, respectively.



Scheme 2.30

2.7. Conclusions

The first examples of multinuclear platinum(II)-amine complexes containing 1,2-, 1,7- and 1,12-dicarba-*closo*-dodecaboranes were prepared in this work. Three bis(aminopropyl)carboranes containing 1,2- (**61**), 1,7- (**62**) and 1,12-dicarba-*closo*-dodecaboranes (**63**) were prepared and their abilities to act as ligands in multinuclear platinum(II) complexes were investigated. The platinum(II) complexes containing the less thermodynamically stable 1,2-dicarba-*closo*-dodecaborane could not be isolated due to the relatively rapid degradation of the *closo*-carborane cage with concomitant reduction of the platinum(II) to platinum metal.

Symmetrical dinuclear platinum(II) complexes containing 1,7 and 1,12-dicarba-*closo*-dodecaboranes containing both the *trans*-[$PtCl(NH_3)_2$(amine)] (**81** and **82**) and *cis*-[$PtCl_2(NH_3)$(amine)] (**78** and **80**) centres were successfully isolated. A novel method of preparing asymmetric dinuclear platinum(II) complexes containing 1,7-dicarba-*closo*-dodecaboranes was also developed. The new methodology also achieved the preparation of novel trinuclear platinum(II) complexes, **104** and **105**, each containing two bridging bis(aminopropyl)carborane ligands.

In summary, this chapter reports the development of a new class of DNA-binding BNCT agents. Their ability to retain the DNA-targeting ability of platinum(II) complexes, by means of a semi-quantitative restriction endonuclease DNA-cleavage assay is reported in Chapter Three.

CHAPTER THREE

DNA-Binding and Anti-Cancer Studies of Platinum(II)-Amine Complexes

DNA-Binding and Anti-Cancer Studies of Platinum(II)-Amine Complexes

3.1. Circular DNA Plasmids

In addition to chromosomal DNA, bacteria may also possess plasmid DNA which exists in the cytoplasm of the cell. Plasmid DNA is circular and much smaller than the chromosome, consisting of only a few thousand base pairs and replicates independently of chromosomal DNA. Plasmid DNA possesses specific functions, such as involvement in defence mechanisms or sexual reproduction.[308]

Many naturally-occurring duplex DNA molecules, such as bacterial plasmids, exist as covalently closed circles with no free 5' or 3' ends.[308] Such circular DNA molecules exhibit a very interesting topological property known as *supercoiling*.

Supercoiling results from the fact that the number of times the two chains of the DNA double helix can twist around each other cannot be changed. When DNA has the normal number of base pairs per helical turn, it is in the relaxed state. Circular DNA is usually over- or under-twisted, so the molecule relieves the helical stress by twisting around itself. As a result virtually all circular DNA exists in the supercoiled state known as Form I (Figure 3.1).[309-311]

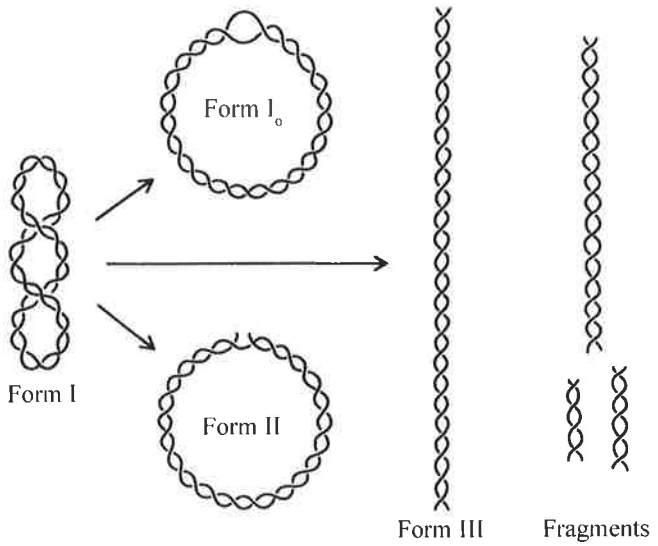


Figure 3.1

The cleavage of one strand of Form I DNA relieves the constraints of circular DNA and, through twisting of the helix strands, supercoiling is removed. The DNA now exists as a relaxed open circular form known as Form II. Double-stranded cleavage gives linear Form III DNA, which can then be cut to give smaller fragments.

The decrease in density of circular DNA resulting from strand cleavage of supercoiled DNA results in decreased DNA migration through an agarose medium when subjected to gel electrophoresis. Consequently, Forms I, II and III DNA can be separated easily using this technique.

3.1.1. The pBR322 Plasmid

The DNA used in the DNA-binding studies in this Chapter is the well-known circular plasmid DNA, pBR322.[312] The pBR322 plasmid is a 4363 base pair DNA molecule that normally exists in the superhelical form (Form I).

One advantage of using the pBR322 plasmid in DNA-binding experiments is its small size. Any slight structural or density changes, such as those induced upon covalent binding of cisplatin and other platinum(II) complexes, are easily detected by techniques such as electrophoresis. The pBR322 plasmid also contains a number of unique restriction endonuclease recognition sequences that can be used to examine the binding of metal complexes (Figure 3.2).

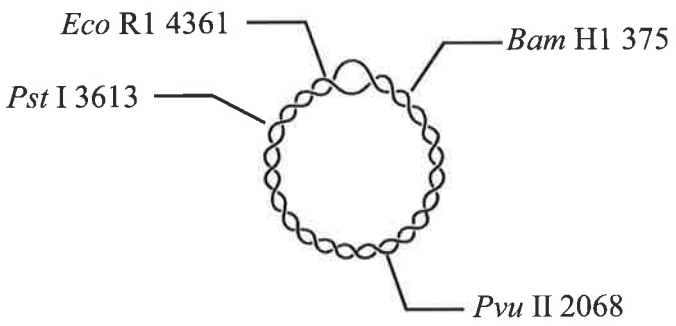


Figure 3.2 Restriction sites within the pBR322 plasmid. Numbering begins at the first T residue of the unique *Eco* RI site.[312]

3.2. Restriction Endonucleases

Endonucleases are enzymes that specifically cleave polynucleotides such as DNA by recognition of a specific nucleotide sequence. Such enzymes are very useful in the study of interactions of small molecules with DNA. The binding of small molecules, such as cisplatin and other platinum(II) complexes, at or near the restriction site in pBR322 plasmid can inhibit the restriction enzyme activity, preventing the double-stranded cleavage of Forms I and II DNA to linear Form III.[313] The degree of enzyme inhibition can be a useful indication as to the site and extent of interactions of small molecules with DNA.

The restriction sequences of some commonly used endonucleases (Table 3.1) are known and unique in the pBR322 plasmid.

Enzyme	Restriction sequence
Bam H1	5'-GGATCC-3'
Eco RI	5'-GAATTC-3'
Pst I	5'-CTGCAG-3'
Pvu II	5'-CAGCTG-3'

Table 3.1 Restriction sequences of some endonucleases.[154]

Many restriction endonuclease DNA-cleavage assays have been developed in the literature enabling semi-quantitative determination of the DNA-binding of metal complexes.[207,313-315] We have developed a semi-quantitative assay that allows a comparison in binding of our new platinum(II)-amine complexes to the pBR322 plasmid with that of cisplatin.

3.2.1. The Principles of Restriction Endonuclease DNA Cleavage Studies.

A titration assay (inhibition of enzyme cleavage measured as a function of platinum(II) complex concentration) is a simple and rapid method to determine the binding affinity for pBR322 of platinum(II) complexes prepared in this work and

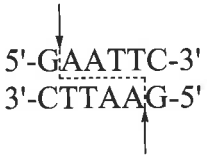
allows comparison with the classical DNA cross-linking agent, cisplatin and other well-known DNA-binding compounds.

Incubation of pBR322 DNA with various concentrations of platinum(II) complex results in formation of platinum(II)-DNA adducts. The incubation is carried out at 37°C in an aqueous saline-phosphate buffer to mimic the conditions inside a typical cell. Incubation for 2 hours allows adequate time for hydrolysis of the chloro ligands, forming a reactive, aquated platinum(II) centre which can readily react with the plasmid.

The incubation mixture usually required a small amount (*ca* 0.5% (*v/v*)) of dmf, necessary for the dissolution of complexes owing to their poor aqueous solubility. Alterations in the pH and ionic strength or the presence of solvents, such as glycerol, DMSO and dmf have been found to decrease the specificity of *Eco* RI, consequently increasing the number of cleavage sites in pBR322.[316,317] However, the variation in recognition specificity of *Eco* RI in the assays discussed in this Chapter has been shown not to be altered in the presence of up to 30% (*v/v*) dmf.

The reaction of the platinum(II) complex with DNA is quenched by addition of NaCl, converting any unbound platinum(II)-aqua species back to the less reactive chloro-platinum(II) centres. The increased ionic strength also disrupts any electrostatic attraction between the cationic platinum(II) centres and DNA, leaving only platinum(II) that is covalently bound to DNA. To ensure the restriction enzyme is not affected by any free platinum(II) complex, the DNA samples were subjected to spin dialysis to remove any unbound platinum(II) complex prior to reaction with the restriction enzyme. While there is no evidence that cisplatin does bind to restriction enzymes in these assays,[313] the binding properties of dinuclear platinum(II) complexes are not known. It is important to remove any unbound platinum(II) to ensure any observed inhibition of enzyme activity is due to platinum(II)-modification of DNA rather than modification of the enzyme.

The *Eco* RI restriction endonuclease cleaves the sequence at the phosphodiester bonds indicated by arrows, converting Forms I and II pBR322 to Form III.



The platinum(II)-modified pBR322 was treated with restriction endonuclease *Eco* RI, and after 2 hours the enzyme reaction was quenched by addition of EDTA. Forms I, II and III were separated by electrophoresis.

In electrophoresis an electric field is applied to a gel medium immersed in a buffered solution and charged molecules within the gel migrate towards one electrode or another. Within this assay, the negatively charged DNA molecules are loaded at the cathode end and migrate towards the positive anode. The velocity of this migration depends on two factors, size and charge. The driving motion is the charge of the molecule and the strength of the electric field (which is constant). Resisting this motion is the frictional force exerted on the particle as it moves through the gel, which varies with the size and shape of the molecule. Since the Forms I, II and III of DNA differ in their molecular dimensions, their mobilities vary enough to be separated using the electric field.

The gels were illuminated from below with an UV-light (302 nm) and photographed. All photographs in this Chapter depict the movement of DNA from the negative electrode (top) to positive electrode (bottom). The camera detects the DNA from the visible light emitted by the fluorescing intercalated ethidium bromide dye.[318] Densitometric scanning allows analysis of digital photographs. Inhibition is assessed from the relative proportion of density of the bands (cleaved *vs.* uncleaved), indicating the degree of enzyme activity and therefore the extent of DNA binding by a platinum(II) complex.

3.3. Results and Discussion

3.3.1. *Eco* RI DNA-Cleavage Studies Using Form I and II pBR322

The considerable distance between the two platinum(II) centres within the dinuclear platinum(II) complexes prepared in this work allows the formation of a wide variety of long range cross-links on DNA. Unfortunately, during preliminary experiments some of the pBR322 DNA treated with platinum(II) complexes **45** (n = 6) and **78**, failed to enter the agarose gel during electrophoresis (Figure 3.3, lanes 3-6). This phenomenon has previously been observed with circular-DNA modified by other dinuclear platinum(II) and palladium(II) complexes, including **47**.[209,319-321]

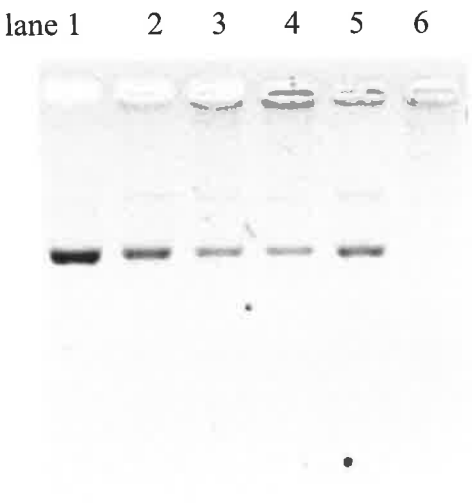


Figure 3.3 Gel electrophoresis of highly **78**-modified pBR322 plasmid followed by digestion with *Eco* RI. Lanes corresponding to DNA samples incubated with (left to right) 0 (control), 30, 51, 81, 106 and 132 μM **78**.

While the exact nature of platinum(II)-adducts formed on DNA by the novel dinuclear platinum(II) complexes presented herein have not been elucidated, the lack of migration of pBR322 plasmid highly modified by **78** and **45** (n = 6) upon application of the electric field is most likely a consequence of the formation of interhelical cross-links by the flexible dinuclear platinum(II) complexes. This would result in a DNA matrix of high molecular weight and low electrophoretic mobility, thus preventing the platinum(II)-modified plasmid from entering the gel.

In order to retard the formation of interhelical cross-links, pBR322 was incubated with *Pst* I restriction enzyme to convert Forms I and II to linear Form III. Incubation of Form III pBR322 with high levels of **78** and **45** (n = 6), followed by a 2 hour incubation with *Eco* RI enzyme, generated DNA fragments that migrated readily through the agarose gel.

3.3.2. *Eco* RI DNA-Cleavage Studies Using Form III pBR322

The inhibition of *Eco* RI action by **78**, **80**, **81** and **82** was successfully studied with pBR322 DNA that was previously linearised with *Pst* I. The distance between the *Eco* RI and *Pst* I restriction sites is almost 750 bp. Consequently, cleavage of Form III pBR322 by *Eco* RI results in two smaller fragments, Fragment A (3615 bp) and Fragment B (748 bp). The difference in size between Form III, Fragment A and Fragment B results in significant differences in electrophoretic mobility of the three polynucleotides and allows them to be readily separated by electrophoresis.

Inhibition of *Eco* RI activity in the presence of cisplatin, **78**, **80**, **81** and **82** were assessed from the relative proportion of density of the bands, cleaved (Fragment A + Fragment B) versus uncleaved (Form III). The gel illustrated in Figure 3.4 shows Form III pBR322 after a 2 hour incubation with various concentrations of cisplatin, followed by a 2 hour incubation with *Eco* RI enzyme.

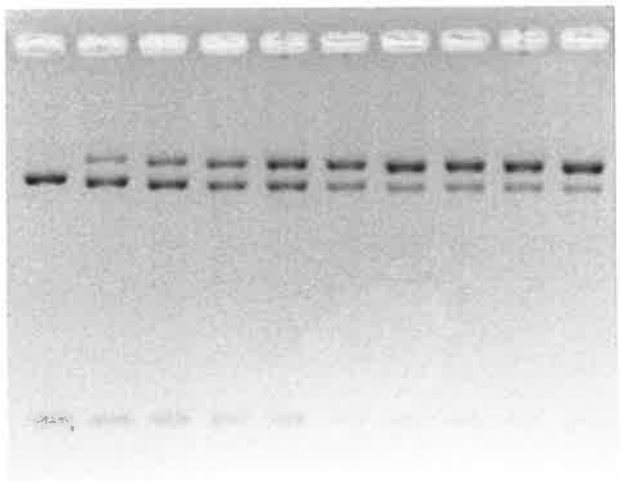


Figure 3.4 Gel electrophoresis of Form III pBR322 plasmid followed by digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 12, 22, 33, 45, 55, 67, 78, 88 and 100 μM cisplatin.

From the control lane ([Pt] = 0 μM) it can be seen that the enzyme has completely cleaved all Form III pBR322 to give Fragments A and B. As the concentration of cisplatin increases there are increasing amounts of inhibition of DNA cleavage by *Eco* RI, the intensity of the bands corresponding to Fragments A and B decrease and those corresponding to Form III pBR322 increase. This is consistent with increased inhibition of DNA cleavage by *Eco* RI restriction enzyme, resulting from an increasing amount of platinum(II) binding at or near the *Eco* RI restriction site.

The concentration of cisplatin required to reach 20% inhibition of *Eco* RI activity was noted to be 14 μM. A comparison of this value with the concentration of novel multinuclear complexes prepared in this work required to achieve the same level of enzyme inhibition (20%) allows a determination of the relative abilities of the platinum(II) complexes to inhibit *Eco* RI activity which is a direct result of their relative abilities to bind covalently to Form III pBR322.

3.3.2.1. *Eco* RI Inhibition by Complexes with *cis*-[$PtCl_2(NH_3)$(amine)] Geometry

It can be seen from the gel depicted in Figure 3.5 that **78** inhibits *Eco* RI poorly in comparison with cisplatin.

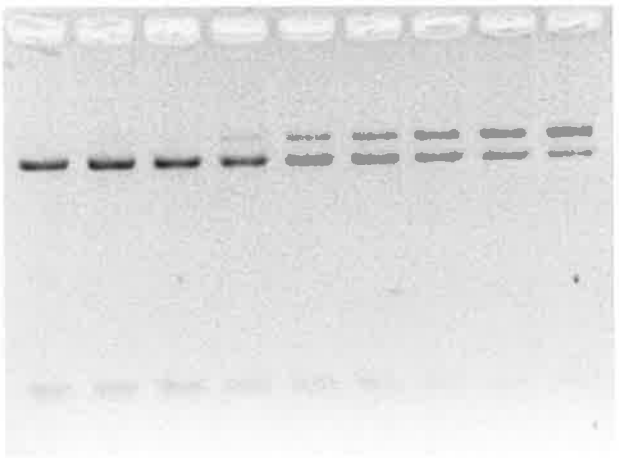


Figure 3.5 Gel electrophoresis of Form III pBR322 plasmid following digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 12, 23, 35, 47, 57, 81, 92 and 104 μM of **78**.

The concentration of **78** required to achieve 20% inhibition of *Eco* RI activity is 49 μM. As a result of the relatively poor DNA-binding affinity of **78**, any inhibition of *Eco* RI activity by **78** (up to 23 μM) is undetectable by this method.

Similarly, **80** inhibits *Eco* RI restriction enzyme poorly in comparison with cisplatin, as displayed from the gel depicted in Figure 3.6. The concentration of **80** required to achieve 20% inhibition of *Eco* RI activity is 20 μM. At concentrations of **80** higher than 33 μM, the migration of platinum(II)-modified DNA begins to decrease and DNA remains in the wells of the agarose gel.

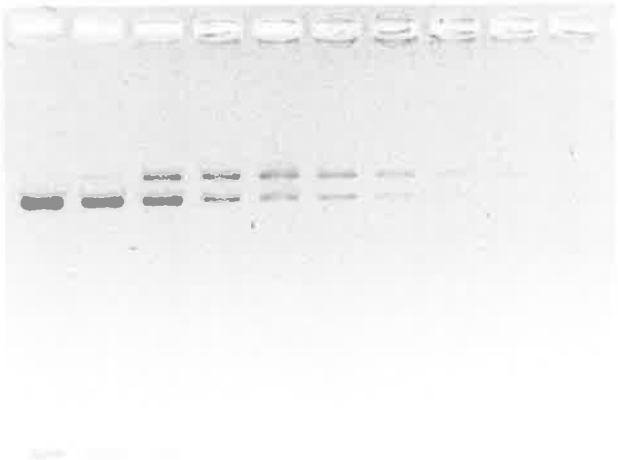


Figure 3.6 Gel electrophoresis of Form III pBR322 plasmid following digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 11, 21, 33, 44, 54, 66, 77, 87 and 99 μM **80**.

3.3.2.2. *Eco* RI Inhibition by Complexes with *trans*-[PtCl(NH$_3$)$_2$(amine)] Geometry

It is evident from Figure 3.7 that **81** inhibits *Eco* RI restriction enzyme to a greater extent than cisplatin. The concentration required to achieve 20% inhibition of *Eco* RI activity is 3 μM.

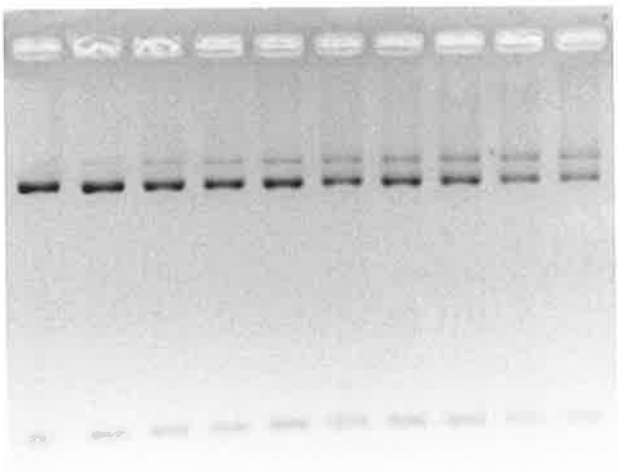


Figure 3.7 Gel electrophoresis of Form III pBR322 plasmid following digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 0.4, 0.6, 0.9, 1.3, 1.6, 1.9, 2.2, 2.5 and 2.9 μM **81**.

Similarly to **81**, it can be seen from the gel depicted in Figure 3.8 that **82** inhibits *Eco* RI activity considerably better than cisplatin and the complexes with *cis*-$[PtCl_2(NH_3)(amine)]$ geometry, **78** and **80**. The **82** concentration required to achieve 20% inhibition of *Eco* RI activity is 2.1 μM.

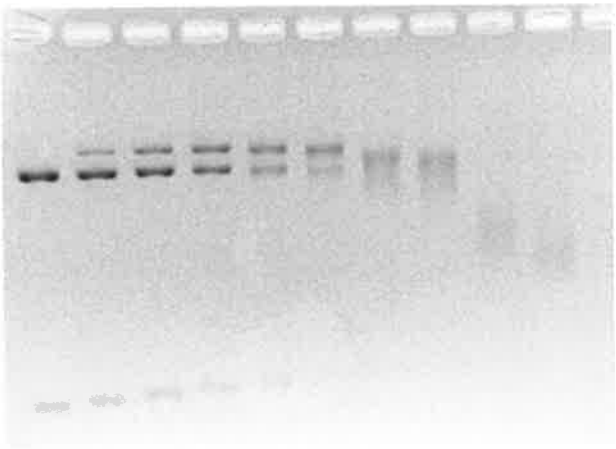


Figure 3.8 Gel electrophoresis of Form III pBR322 plasmid following digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 1.1, 2.1, 3.2, 4.3, 5.3, 6.4, 7.5, 8.5 and 9.6 μM **82**.

3.3.3. Comments on the Restriction Endonuclease DNA-Cleavage Studies

By means of *Eco* RI DNA-cleavage experiments, the concentrations of the platinum(II) complexes required to reach 20% inhibition of enzyme activity are summarised in Table 3.2.

Compound	Concentration required for 20% inhibition of *Eco* RI activity (μM)
cisplatin	14
78	49
80	20
81	3
82	2

Table 3.2

The presence of two reactive platinum(II) centres in complexes **78**, **80**, **81** and **82** was expected to increase the extent of DNA platination in comparison with cisplatin. It was also anticipated that the inclusion of the carboranes would not significantly hinder DNA binding. The main factor that appears to affect the DNA-binding affinity of **78**, **80**, **81** and **82** was the platinum(II) ligand environment and charge of the platinum(II) centres, as discussed below.

3.3.3.1. *Eco* RI Inhibition by Complexes with a *trans*-[PtCl(NH$_3$)$_2$(amine)] Geometry

The most potent of the dinuclear platinum(II) complexes investigated using this DNA-binding assay were the dicationic complexes **81** and **82**. These complexes require less than one quarter the concentration of cisplatin to achieve an equivalent amount of enzyme inhibition. Electrostatic interactions alone are unlikely to account for the strong binding of the charged dinuclear platinum(II) complexes to DNA. These would be reversed upon the addition of NaCl used to quench reaction of the platinum(II)-complex with DNA, and under the conditions of the electrophoresis experiment. It is

assumed that the extent of inhibition of *Eco* RI activity is indicative of the ability of the complex to covalently bind to DNA. This is evidence that the inclusion of the carborane moiety in **81** and **82** does not significantly decrease the ability of the platinum(II) centres to form covalent bonds with DNA.

The ability of **81** and **82** to inhibit *Eco* RI activity at a concentration that is considerably lower than **78**, **80** and cisplatin would be facilitated by the dicationic charges in **81** and **82**. The electrostatic attraction to the negatively charged DNA phosphodiester backbone could directly enhance the kinetics of DNA-binding by **81** and **82** over neutral mononuclear cisplatin. The dicationic charges of **81** and **82** could also enhance the aqueous solubility of the complexes, which incorporate a considerably lipophilic carborane ligand. This would decrease the likelihood of precipitation of platinum(II) complexes during periods of incubation in the DNA-binding assay.

In addition, it has been well-established that the DNA-binding affinity of complexes containing PtN_3Cl centres, such as **47** and **52**, is high. The aquation of PtN_3Cl complexes (such as $[Pt(NH_3)_3Cl]^+$, **47** (n = 6) and **52**) occur much more rapidly than those containing PtN_2Cl_2 coordination spheres, such as cisplatin.[220,247] Despite the hydrolysis equilibrium favouring the dichloro complex and the consequent low concentration of the more reactive aquated platinum(II) centres, DNA-binding by complexes containing PtN_3Cl centres is rapid, indicating formation of the aquated platinum(II) centre may not be necessary prior to DNA adduct formation and the N7 of DNA purine bases directly displace the chloro-platinum(II) ligand.[123,220] It is possible that the kinetics of aquation of **81** and **82** are similar to the well-known PtN_3Cl complexes and contribute to their displayed higher affinity for DNA over cisplatin, **78** and **80** accordingly.

3.3.3.1.1. Streaking of Bands at High Concentrations of Complexes with a *trans*-$[PtCl(NH_3)_2(amine)]$ Geometry

At concentrations higher than 3.3 μM and 4.3 μM for complexes **81** and **82**, respectively, considerable streaking of DNA through the agarose gel was observed (Figures 3.8 and 3.9). Fortunately, the streaking occurs after the point of 20%

inhibition of enzyme activity, allowing comparison of the *Eco* RI inhibition effect of **81** and **82** to be compared with those of cisplatin, **78** and **80**.

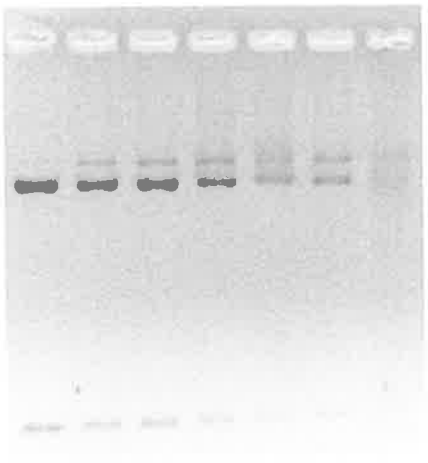


Figure 3.9 Gel electrophoresis of Form III pBR322 plasmid following digestion with *Eco* RI. Lanes corresponding to DNA samples previously incubated with (left to right) 0 (control), 1.2, 1.6, 2.3, 3.3, 4.0 and 4.9 μM **81**.

Streaking is often caused by rapid and effective covalent binding by dicationic platinum(II) complexes to the polyanionic DNA molecule, resulting in extensive charge neutralisation. Such neutralisation could retard the migration of the DNA through the gel, however in the gels depicted in Figures 3.8 and 3.9 an increase in DNA mobility is seen at high platinum(II) concentrations. These significant increases in electrophoretic mobility are most likely a result of 'DNA collapsing'.

The intramolecular repulsive forces that exist between the polyanionic charges of DNA are essential to maintain the DNA tertiary structure. Neutralisation, such as the binding of inorganic cations, often causes spontaneous collapse of the DNA tertiary structure.[322-327] Therefore, it is most likely that DNA molecules highly-modified by **81** or **82** tend to collapse. While the collapsed DNA remains overall negatively charged, the macromolecules are markedly condensed, resulting in an increased electrophoretic mobility and the observed rapidly moving and diffuse bands seen in Figures 3.8 and 3.9 above threshold concentrations.[322,328]

3.3.3.2. *Eco* RI Inhibition by Complexes with a *cis*-[$PtCl_2(NH_3)$(amine)] Geometry

As determined by the restriction endonuclease assay, the neutral complexes **78** and **80** were considerably less efficient at DNA-binding than **81**, **82** and cisplatin. To achieve 20% inhibition of *Eco* RI activity, the concentrations of **78** and **80** needed to be approximately 3.5 and 2.5 times the concentration of cisplatin, respectively. It is assumed that the poor inhibition of *Eco* RI activity by **78** and **80** may be indicative of poor DNA-binding.

It is important to note that, despite the comparatively poor DNA-binding affinities of **78** and **80**, the ability of the two platinum(II) centres to modify DNA is not completely lost. There are a number of factors that could result in the poor DNA-binding of **78** and **80** in comparison with **81**, **82** and cisplatin. **78** and **80** are neutral, hence unlike **81** and **82** there is no electrostatic attraction to the negatively charged DNA prior to hydrolysis. This would result in a considerably slower reaction between DNA and the platinum(II) centres, thus decreasing the levels of platinum(II)-DNA adducts formed within the set incubation period. It is interesting to note that the non-boron-containing analogues (**45**) have been determined by similar assay methods to inhibit *Eco* RI restriction endonuclease activity significantly better than cisplatin.[207] Consequently, the presence of the bulky carborane moiety in **78** and **80** must be reducing the capacity of the two metal centres to bind to DNA. However, the DNA-binding capability of the charged carborane-containing complexes, **81** and **82**, does not appear to be adversely affected by the steric bulk of the carborane. Thus it is most likely that the presence of the lipophilic carborane would, together with the absence of charge, decrease the water solubility of **78** and **80** in comparison with cisplatin and dicationic **81** and **82**. The low water solubility of **78** and **80** could increase the likelihood of precipitation during the conditions of the assay. Any precipitation of complex will decrease the concentration of platinum(II) complexes in solution, a factor that would decrease the levels of platinum(II)-DNA adducts formed within the set incubation periods and strongly influence the results of the DNA-binding assay.

The observation in Figure 3.6 of DNA-streaking after incubation with high levels of **80** is consistent with interhelical cross-linking observed upon incubation of the

circular pBR322 plasmid with the dinuclear complexes (Section 3.3.1.). However, such streaking is not observed in studies with **78** (Figure 3.6). It is unlikely that substitution of the 1,7-carborane moiety in **78** with the 1,12-carborane of **80** would perhaps increase the formation of interhelical cross-links. It is more likely that the notable difference between the two complexes is due to low solubility of the highly platinated DNA or charge neutralisation of the DNA macromolecule.

3.4. Anti-Cancer Screening

The two novel complexes **78** and **81** were screened against a range of tumour cell lines *in vitro* at the Andrew Durant Drug Testing Facility at the Peter MacCallum Cancer Institute in Melbourne, Australia. In addition, the well-known anti-cancer agent, cisplatin, was used as a control. The cell lines that were tested included L1210 murine leukaemia cells and its cisplatin-resistant variant (L1210/DDP), along with the cisplatin sensitive (2008) and resistant (C13) human ovarian carcinoma cell lines.

It is reasonable to expect cell destruction in the absence of neutrons through DNA-modification by the novel complexes prepared in this project, and the concentrations (μM) required to achieve 50% inhibition of cell growth in each cell line (IC_{50}) are shown in Table 3.3.

Cell Line	78	81	Cisplatin
L1210	2.0	1.1	0.5
L1210/DDP	2.5	1.4	6.9
2008	13	5.4	0.6
C13	13	5.6	10

Table 3.3 IC_{50} (μM) values for various platinum(II) complexes. **78** and **81** were dissolved in DMSO. (n = 2). L1210 and L1210/DDP cell lines were assayed using a CC assay. 2008 and C13 cell lines were assayed using SRB assay.

The results of the anti-cancer screening for **78** and **81** are encouraging. Most importantly, the values are within the same order of magnitude as the values for

cisplatin. This indicates that the compounds are able to enter the cells without difficulty. This is extremely promising, as achieving uptake of boron drugs by cells is a highly valued goal in BNCT.

The IC_{50} values also indicate the compounds are cytotoxic in the absence of neutrons. The mechanism of inducing cell death is not known. Further research must be conducted in order to establish the mode of action and whether the complexes are reaching nuclear DNA. The cytotoxic effects of **78** and **81** in the absence of neutrons are not as essential to their proposed application as BNCT agents as their ability to bind to DNA. A small cytotoxic effect of **78** and **81** is not undesirable and could, in some cases, enhance the anti-cancer effects of the compounds perhaps *via* some synergistic effect. However, high anti-cancer activity thorough action of platinum(II) on DNA is not desirable as this would cause cell death at low platinum(II) concentrations, prior to the accumulation of sufficient levels of ^{10}B nuclei.

Complex **81** is consistently more active than **78** in all cell lines that were tested. This is likely to be due to the dicationic nature of **81** (compared to the neutral **78**), which would increase the aqueous solubility of the complex and also enhance its DNA-binding affinity through an electrostatic attraction to the polyanionic DNA. Additionally the positive charge could potentially assist the internalisation of the dinuclear platinum(II) complex by the cells, balancing out the lipophilicity of the carborane cage.

In both cisplatin sensitive cell lines (L1210 and 2008) complexes **78** and **81** are not as effective as cisplatin at inhibiting cell growth. This could be due to poor solubility or perhaps a less efficient or slower mechanism of cellular internalisation of **78** and **81** compared with cisplatin. This is not unexpected considering the incorporation of the sterically large, lipophilic carborane.

The anti-cancer activities of **78** and **81** in cisplatin-resistant cell lines (L1210/DDP and C13) are not considerably different from those observed in the parent cell lines, indicating the mechanism of cisplatin-resistance does not adversely affect the action of **78** and **81**. This is consistent with the observation that DNA-adducts formed by

dinuclear platinum(II) complexes appear to be able to overcome many mechanisms associated with cisplatin-resistance.[210,226]

3.4.1. Correlations with DNA-binding studies

The high cytotoxic effect of **81** over **78** could mistakenly be attributed to the greater ability of **81** to bind to DNA, as demonstrated from *Eco* RI inhibition studies in Section 3.3.2.2. However, the mechanism of inducing cell death during the anti-cancer screens are not known and further tests must be conducted to establish if **78** and **81** are reaching and binding to DNA prior to cell death, in which case the affinity for DNA would be an critical factor in the remarkable anti-cancer activity of **81**.

3.5. Conclusion

A semi-quantitative restriction endonuclease DNA-cleavage assay has allowed determination of the ability of **78**, **80**, **81** and **82** to bind to linear pBR322 plasmid in comparison with cisplatin.

In general, it was found that the presence of the carborane cage did not significantly reduce the DNA-targeting ability of the platinum(II) centres. In fact, the dicationic complexes **81** and **82** achieved higher degrees of DNA-binding than cisplatin. These results support the potential application of **78**, **80**, **81** and **82** as DNA-targeting BNCT agents as they indicate the platinum(II) centres are capable of avidly binding to DNA.

78 and **80** were screened against L1210 and 2008 cell lines and their cisplatin variants. The IC_{50} values were close to, and in some cases lower than, the values displayed by cisplatin. The DNA-binding and *in vitro* anti-cancer results are most encouraging and support the further development of **78**, **80**, **81** and **82** as BNCT agents as they indicate the compounds are readily taken up by cancer cells, a highly valued goal in BNCT.

CHAPTER FOUR

Multinuclear Platinum(II)-Trpy Complexes Containing (Thioalkyl)carborane Ligands.

Multinuclear Platinum(II)-Trpy Complexes Containing (Thioalkyl)carborane Ligands.

4.1 Intercalators and Intercalation

Classical intercalation involves the insertion of a planar molecule between two neighbouring base pairs of DNA (Figure 4.1).[76] The binding energy for intercalation arises from Van der Waals forces and aromatic stacking interactions between the planar, heterocyclic intercalator and DNA. If the intercalator is positively charged, there is additional stabilisation of the DNA complex by electrostatic attraction to the negatively charged DNA phosphate backbone. The binding of intercalators to duplex DNA causes many changes in the physical properties of both DNA and the intercalator.[329-331] The unwinding of base pairs and helical backbone to accommodate an intercalator causes lengthening, stiffening and unwinding of the DNA helix. The planar intercalator is rigidly held between the aromatic heterocyclic base pairs and it is stabilised by π-π stacking and dipole-dipole interactions.[329,331]

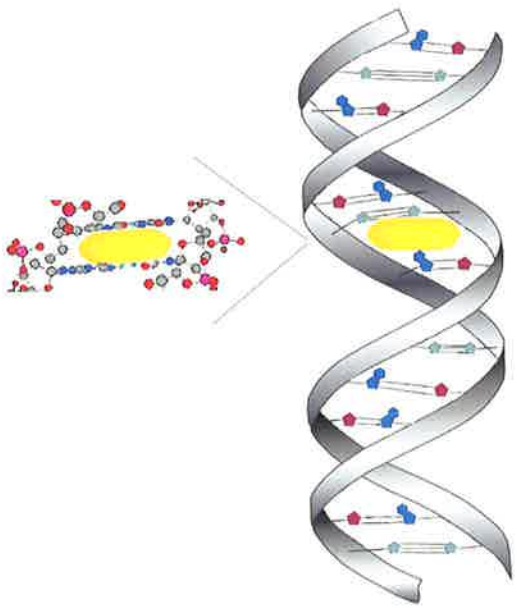


Figure 4.1

Intercalation is relatively rapid in comparison with the covalent reaction of metal complexes, such as 'cisplatin-type' platinum(II) complexes, with DNA as prior

hydrolysis is not required.[332,333] Although intercalation can be thermodynamically favourable ($\Delta G°$ = -20-40 kJ mol^{-1}),[334-336] the interaction with DNA is kinetically labile.[154]

Some metal complexes, such as those in Figure 4.2, are capable of intercalating DNA and these are known as 'metallointercalators'.[332,333,337-349] In all cases, at least one of the aromatic ligands has the capacity to reversibly bind to DNA.

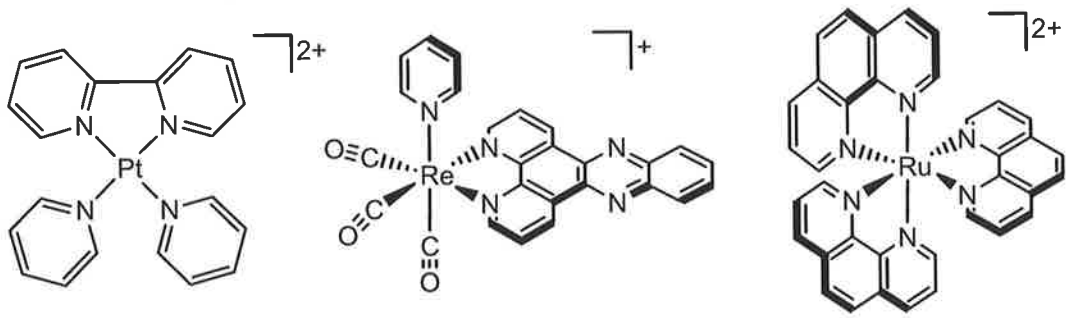
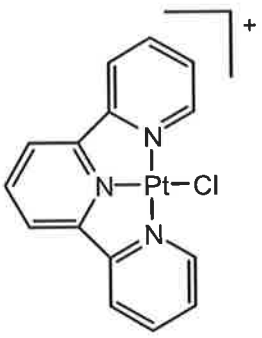


Figure 4.2

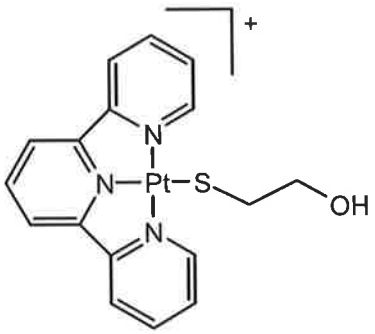
Metallointercalators are well-known for their DNA-binding affinities and have attracted considerable attention for their role as antibiotic,[350] anti-protozoal[351] and, in particular, anti-cancer agents.[77,200,352-356] In some cases, DNA-intercalation is considered to be critical to the mode of anti-cancer activity of intercalators, while others adversely alter DNA topoisomerases.[357-360]

4.1.1 Platinum(II)-Trpy Metallointercalators

The interaction of square planar platinum(II) complexes containing the 2,2':6',2''-terpyridine (trpy) ligand with DNA is of particular interest to researchers ever since the discovery that $[Pt(trpy)Cl]^+$ (**106**) and $[Pt(HET)(trpy)]^+$ (**107**) are capable of binding strongly to DNA by intercalation, competitively inhibiting the binding of the well-known intercalator, ethidium bromide (**23**), to ctDNA.[337] Intercalation of platinum(II)-trpy complexes produces an unwinding angle in DNA similar to that induced upon intercalation of ethidium bromide.[361]



106



107

While **106** both intercalates and, through the loss of the chloro ligand, covalently binds to DNA, **107** interacts predominantly *via* intercalation, as covalent binding is slowed dramatically due to the inert character of the Pt-S bond.[337,362]

In recent times some platinum(II)-trpy complexes such as **107** have attracted extensive interest due to their DNA-sequence specificity,[339] anti-protozoal[351] and anti-cancer activity.[342,352,363]

4.1.2 'Non-Intercalative' Modes of DNA-Binding

Most intercalators self-associate in aqueous solution through π-π stacking, forming dimers.[329] Metallointercalators are also capable of forming aggregated dimers, with the well-known stacking behaviour of planar d^8-metals such as platinum(II) and palladium(II), further enhanced by the presence of aromatic ligands.[329] In some cases, larger high molecular weight aggregates composed of multiple intercalators form in solution and on the surface of the DNA helix, particularly at high metallointercalator concentrations.[344]

At low concentrations, platinum(II)-trpy complexes intercalate DNA, however at higher concentrations, the complexes also form aggregated dimers and bind to DNA in a non-intercalated, external mode.[331,339] The exact details of this external DNA-binding mode are not fully elucidated, however there are indications of high molecular weight species containing extended aggregates of platinum(II) complexes on the surface of the nucleic acid.[344]

4.2 Consequences of Intercalation on DNA Structure and the Intercalator

The changes that occur upon DNA intercalation allow the interaction to be monitored by a variety of physical methods.[329-331] Measurements of DNA viscosity,[364] electrophoretic mobility,[361] and $^{31}P\{^{1}H\}$ NMR spectroscopy of the phosphodiester backbone[365] assess changes in the helical nature of DNA. Circular dichroism[348,366] and luminescence polarisation[367] demonstrate changes in molecular rigidity and orientations. ^{1}H NMR spectroscopy can also be used to detect the effects on the intercalator of base-pair ring currents.[348,365] Monitoring changes in the UV-visible absorption of the aromatic intercalating moiety is the most convenient method to detect intercalation of a chromophore into DNA.[331]

4.2.1 Detection of Intercalation Using UV-Visible Absorption Spectroscopy

The absorption and fluorescence spectra of the intercalator is usually perturbed by the electronic interactions with DNA and such spectroscopic methods are often used to quantify the interaction.[331,344,364,366,368] Upon intercalation, absorbance maxima shift to longer wavelengths (lower energy of transitions - termed a 'bathochromic shift') and the molar extinction coefficient is reduced ('hypochromism').[331] The fluorescence of the well-known intercalator ethidium bromide (**23**) is enhanced 20-fold upon DNA intercalation.[318]

4.2.2. Effects of 'Non-Intercalative' DNA-Binding on UV-Visible Spectra

While a shift in the UV-visible absorption spectra of the intercalating-chromophore is consistent with intercalation, it does not necessarily indicate intercalation. A non-intercalating interaction, such as dimerisation, external or groove binding, may produce changes in UV-visible spectra identical to that induced by intercalation, giving false indications of intercalation.[331] The non-intercalative modes of DNA binding also complicate deconvolution of UV-visible spectra and determination of stability constants of the intercalation process. In such cases, the presence of an isosbestic point is not sufficient proof that only two species are present and further

details of the binding mechanism must be investigated before intercalation can be proven.

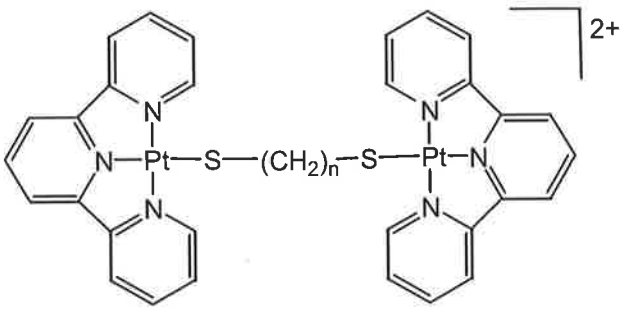
Alternatively, the intercalated, dimerised and aggregated forms of the metallointercalator can each give unique UV-visible spectra. In this case, UV-visible absorption detection of intercalation would be limited due to significant deviations from Beers law, preventing Scatchard determinations of the stability constants for intercalation.[369]

Such deviations from Beers Law can be adjusted using the Schwartz dimerisation model.[339] If the equilibrium constant of the dimerisation process (K_d) is known, a dimerisation model can account for deviations from linear behaviour, arising from dimerisation. If there is not significant levels of aggregation occurring over the concentration range of interest, modifications to the formula allow appropriate adjustments and a complete Scatchard analysis of the affinity of a metallointercalator for DNA.[339,343]

4.3. Dinuclear Platinum(II)-Trpy Complexes

Connecting two intercalating moieties to form a 'bis-intercalator' frequently increases the DNA-binding affinity due to a decrease in the dissociation rate in comparison with the mono-intercalator.[350,354,355,359,370-372] Some bis-intercalators have also exhibited anti-cancer activities.[200,354,357,373,374]

The platinum(II)-trpy complexes **108-114** (n = 4-10) contain two platinum(II) centres linked by an simple dithiol and have been shown to bifunctionally intercalate DNA with higher affinities for DNA over their mononuclear analogues.[342,343] As found for other platinum(II)-trpy complexes, these dinuclear complexes require a GC base pair at the intercalation site and display growth inhibition of L1210 cells.[342]

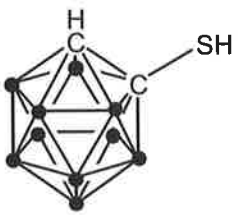


108-114

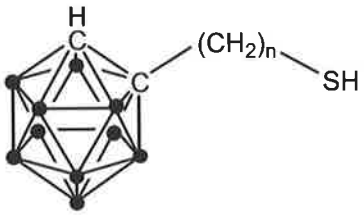
In many cases, a variation in the rigidity or length of the linker between the two intercalating moieties within a bis-intercalator can alter the mode of binding and the ability of the compound to bis-intercalate.[354,370,371,375-377] In the case of **108-114**, those complexes with short linkages display mono-intercalation or bis-intercalation in adjacent sites, with one base pair separating the two intercalating chromophores. Intermediate-linked centres intercalate bifunctionally across a short range, such as two base pairs. The longest linked centres tend to show mixed mono- and bis-intercalation. During mono-intercalation, the non-intercalated chromophore may bind externally to the DNA or aggregate with a chromophore from an adjacent molecule.[342]

4.3.1. Dinuclear Platinum(II)-Trpy Complexes Containing (Thioalkyl)carborane Ligands

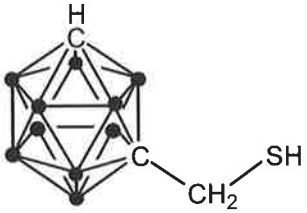
Several examples of transition metal complexes containing thiol-carborane ligands have been reported in the literature.[257-262,266] These complexes incorporate gold(I), gold(III), silver(I), palladium(II), iridium(III), ruthenium(II) and platinum(II) metal centres and most utilise the monodentate ligand, **115**. The application of these published examples include polymer synthesis and catalysis. However, none have been investigated for their potential DNA-targeting applications in BNCT.



115

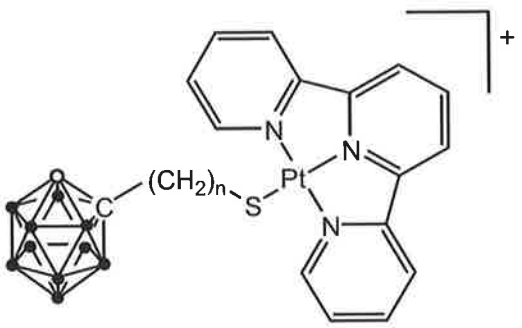


116 n=0
117 n=1
118 n=2
119 n=3

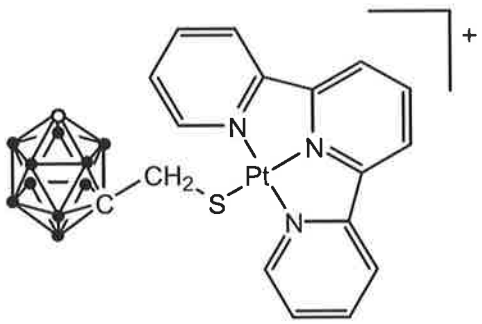


120

Recent work within the Rendina group has led to the development of a series thioalkyl carboranes (**116-120**) and their corresponding cationic platinum(II)-trpy complexes (**121-125**), for potential application in BNCT. These complexes have demonstrated a propensity to intercalate ctDNA.[267,378]

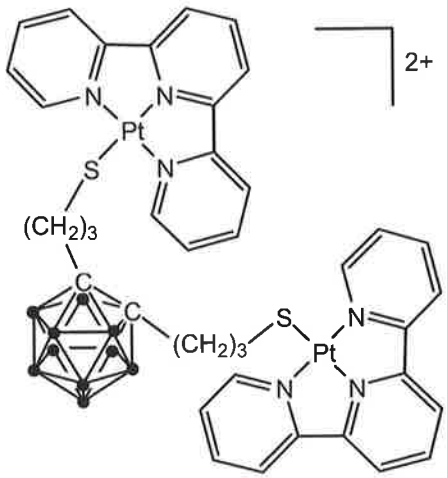


121 n=0
122 n=1
123 n=2
124 n=3

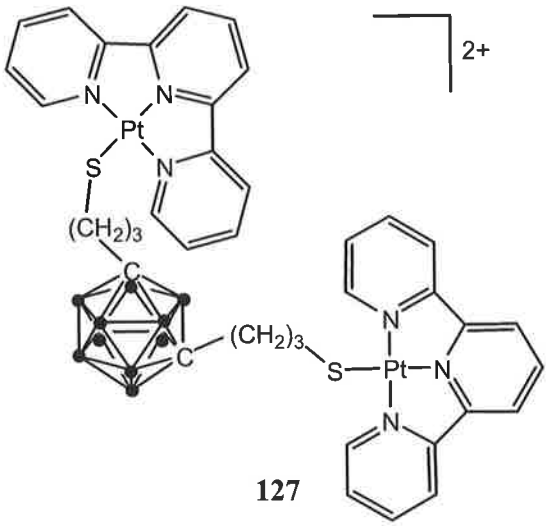


125

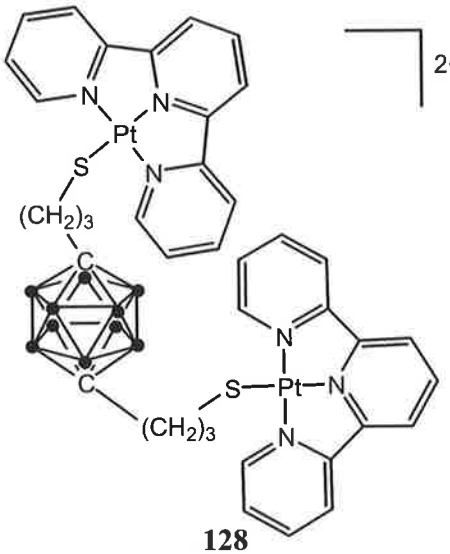
It was the purpose of the research detailed in this Chapter to extend this series to dinuclear platinum(II)-trpy complexes incorporating bis(thiopropyl) 1,2-, 1,7- and 1,12-carboranes ligands (**126**, **127** and **128** respectively).



126



127



128

It was anticipated that substitution of the carboranes with two platinum(II)-trpy chromophores would decrease the dissociation of **126**, **127** and **128** from DNA in comparison with the mono-intercalators **121-125** (as observed for bis-intercalators **108-114**),[342,343] allowing the compound (hence, the ^{10}B nuclei) to persist near the DNA for longer periods.

Intercalation is a strong but kinetically labile interaction,[154] consequently once **126**, **127** and **128** are bifunctionally intercalated and if one of the platinum(II)-trpy moieties dissociates from the DNA, the compound still remains bound to the macromolecule. The free non-intercalated platinum(II)-trpy moiety remains close to the DNA and may bind externally to the DNA or aggregate with an adjacent molecule. It is most likely that the free platinum(II)-trpy portion may intercalate DNA

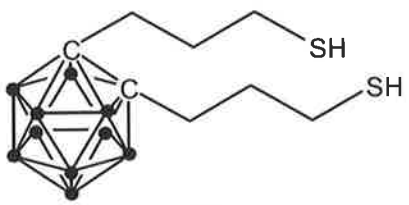
once again. It is important in the potential application of **126**, **127** and **128** in BNCT that ^{10}B nuclei are localised near the DNA for an extended period of time, maximising cellular damage by the products of neutron capture.

Preliminary modelling studies have indicated the carboranes comfortably fit into the major groove of duplex DNA.[379] These results, along with the excellent DNA-binding abilities of **121-125**, indicate the inclusion of the bulky carboranes in **126**, **127** and **128** are unlikely to hinder the ability of the platinum(II)-trpy moiety to intercalate DNA.

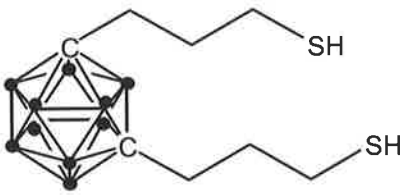
4.4. Platinum(II)-Trpy Complexes Containing Bis(thiopropyl) Carborane Ligands

4.4.1. Preparation and Characterisation of Bis(thiopropyl) Carborane Ligands

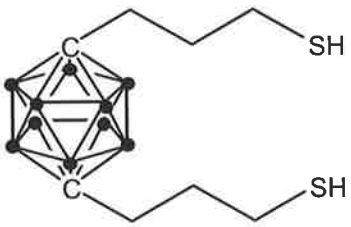
There exists no precedence for the synthesis of the three desired bridging bidentate ligands **129-131** required in the synthesis of **126**, **127** and **128**. The synthetic procedure was initially studied using the 1,7-carborane isomer (**130**) due to its moderate stability and reasonable price.



129

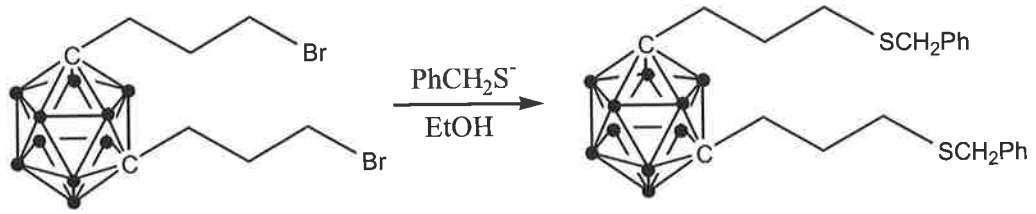


130



131

Reaction of the nucleophilic thiolate, $PhCH_2S^-$, in ethanol with 1,7-bis(3-bromopropyl)-1,7-carborane (**68**) was found to give the S-benzyl protected carborane (**132**) in 95% yield (Scheme 4.1).

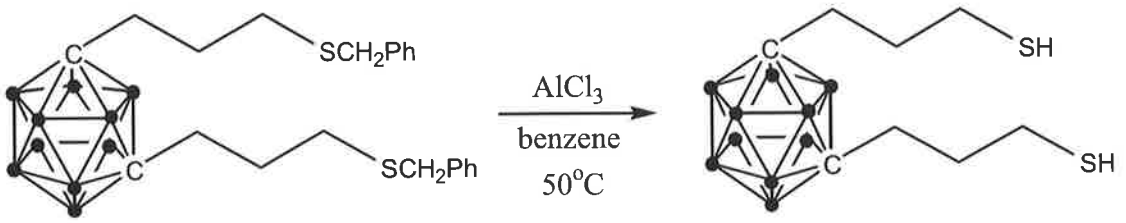


68 **132**

Scheme 4.1

This synthetic pathway was successfully applied to the synthesis of the 1,2- (**133**, 90%) and 1,12-isomers (**134**, 96%), however some care must be taken. The $PhCH_2S^-$ anion was generated *in situ* by deprotonation of benzyl mercaptan using sodium ethoxide. However, the exposure of carborane cages to strongly basic conditions (such as ethoxide) causes partial degradation of the *closo*-polyhedral framework.[250,271-273,279,280] Therefore, during the generation of the $PhCH_2S^-$ anion, an excess of benzyl mercaptan was added to the ethoxide solution in order to ensure all of the ethoxide was consumed and the only notable nucleophilic species present in solution was the less basic $PhCH_2S^-$ anion.

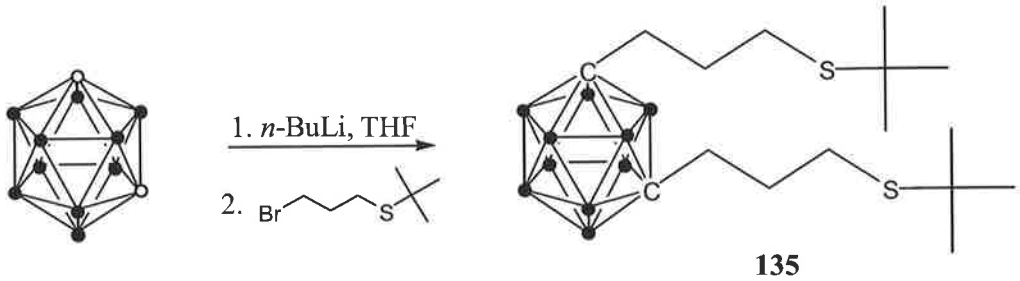
The benzyl thioether groups of **132-134** were successfully removed upon treatment with freshly sublimed aluminium chloride in benzene at 50°C, to give the di-thiopropyls, **129**, **130** and **131** as low melting point solids (Scheme 4.2). Small quantities of unidentified impurities were detected by ^1H NMR spectroscopy but the product could not be successfully purified by recrystallisation, distillation, sublimation or chromatography methods. Characterisation of **129**, **130** and **131** was confirmed by means of high resolution mass spectrometry, with molecular ion peaks detected at *m/z* 292.



132 **130**

Scheme 4.2

In an alternative approach, the *tert*-butyl protected di-thiol, **135**, was generated in reasonable yield from the parent carborane using established methods for **116-120** (Scheme 4.3).[267]



135

Scheme 4.3

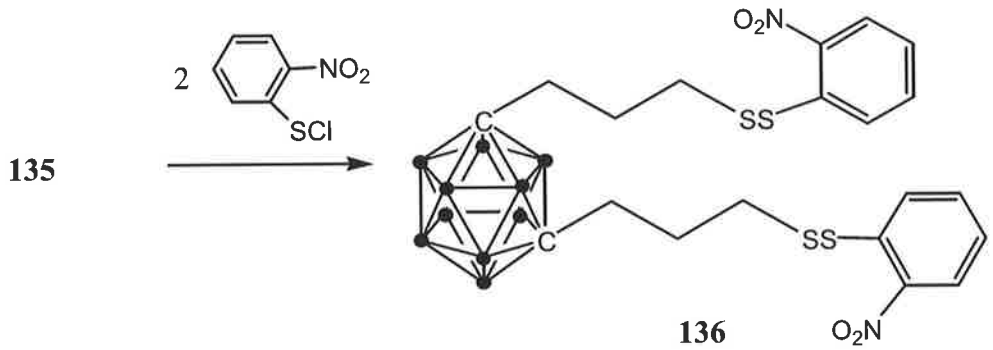
The *tert*-butyl thioether protecting group can usually be removed in the presence of a carborane upon treatment with aluminium chloride in dry benzene[380] and such conditions have allowed the synthesis of **116-120** in 28-56% yield.[267] However, despite an increased temperature and reaction time, the deprotection of **135** under such conditions was unsuccessful.

In some cases, mercurium salts such as mercury acetate allow removal of *tert*-butyl thioether protecting groups.[381] This procedure has been successfully applied to carborane-containing *tert*-butyl thioethers.[382] However, cleavage of the thioether of **135** with mercury acetate in trifluroracetic acid was also unsuccessful.

The *tert*-butyl thioether functionality is very stable, requiring relatively harsh conditions to achieve its removal. In some cases, electrophilic agents such as perchloric and hydrochloric acids are unable to remove it.[383] This is exemplified by the poor yield of **116-120** (28-56%) using aluminium chloride in benzene, indicating that these reaction conditions were not sufficiently effective at cleavage of the *tert*-

butyl thioether. Hence it is not surprising that the same reaction conditions were unable to cleave both *tert*-butyl thioethers of **135**.

Another efficient and convenient removal of the *tert*-butyl thioether protecting group involves the addition of 2-nitrobenzenesulfenyl chloride in glacial acetic acid to form the *S*-nitrophenylsulfenyl disulfide derivative (**136**, Scheme 4.4) in quantitative yield.[384]

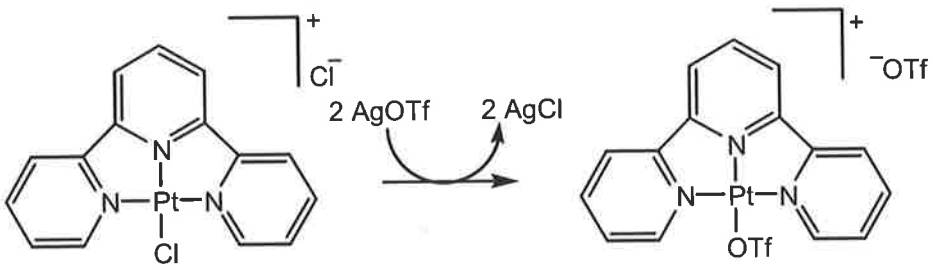


Scheme 4.4

Unfortunately, under standard reduction conditions (sodium borohydride)[384-386] only a small amount of deprotection was detectable and longer reaction times did not increase yield of product. Other methods of reduction, such as zinc dust in refluxing glacial acetic acid, also failed to cleave the disulfide.

4.4.2. Preparation of Dinuclear Platinum(II)-Trpy Complexes Containing Bis(thiopropyl) Carborane Ligands

There are many methods that have been used in the preparation of thiolato-platinum(II)-trpy complexes in the literature.[339,342,362,378,387,388] The complexes (**121-125**) made previously within the Rendina group were generated by the reaction of the thiol-carborane ligands (**116-120**) with [Pt(trpy)OTf]OTf, generated by the reaction of **106** with two equivalents of silver triflate (Scheme 4.5).[267,378]



106

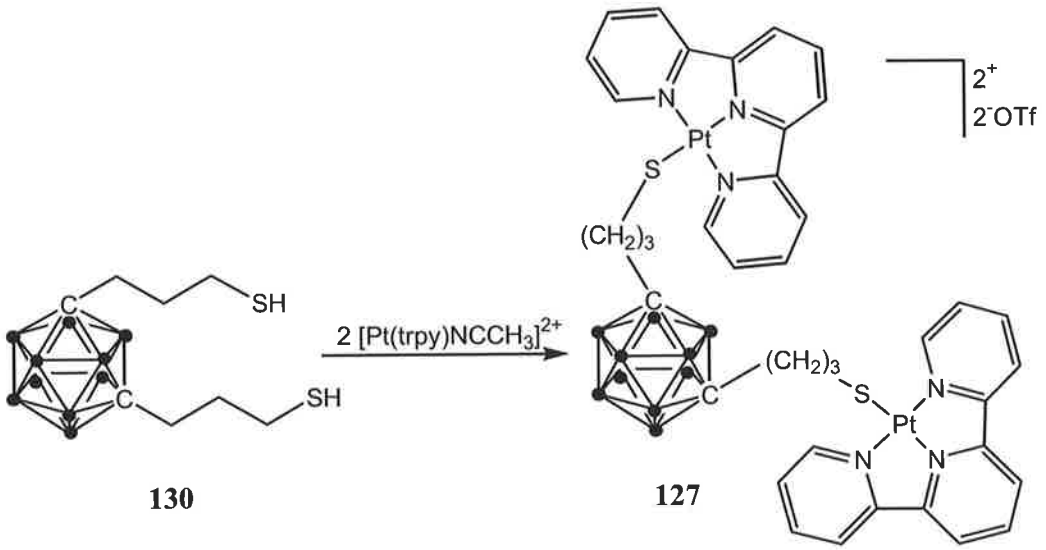
Scheme 4.5

The use of silver to generate the labile species $[Pt(trpy)OTf]^+$ has been successfully applied in the synthesis of a variety of platinum(II)-trpy complexes.[389-391] However, the removal of the chloro ligand from the inner coordination sphere of **106** was very slow, largely due to the attractive force between the negatively charged chloro ligand and the cationic complex. As a result, the reaction did not go to completion, as indicated by $^{195}Pt\{^1H\}$ NMR spectroscopy. Another method for the generation of [Pt(trpy)OTf]OTf involves treatment of **106** in refluxing triflic acid for 24 hours.[392] However, $^{195}Pt\{^1H\}$ NMR spectroscopy indicated this reaction did not go to completion.

The labile nature of the CH_3CN ligand has been utilised in the synthesis of platinum(II)-trpy complexes by means of the reactive precursor, $[Pt(trpy)CH_3CN]^{2+}$.[332,351,363,393-402] $[Pt(trpy)CH_3CN](OTf)_2$ and $[Pt(trpy)CH_3CN](NO_3)_2$ were prepared as yellow crystalline solids in high yield by a modified literature procedure using $[Pt(cod)I_2]$[403] as the precursor. The highly labile acetonitrile ligand in $[Pt(trpy)CH_3CN]^{2+}$ can easily be displaced, consequently the yellow crystals are very reactive and must be freshly prepared and stored under a N_2 atmosphere.

Addition of $[Pt(trpy)CH_3CN](OTf)_2$ to a colourless dmf solution of **130** resulted in an immediate colour change from pale yellow to deep purple, indicating substitution of the S-donor thiol for the N-donor acetonitrile ligand (Scheme 4.6). Addition of diethyl ether precipitated the desired micro-analytically pure **127** as the bis-triflate salt in 87% yield. Coordination of the thiol by the platinum(II) centre was also confirmed by NMR spectroscopy, with a shift in the $^{13}C\{^1H\}$ spectrum from δ 23.9 (CH_2SH) to δ

30.9 (CH_2SPt) and a characteristic peak in the $^{195}Pt\{^1H\}$ spectrum at δ -3205 (PtN_3S core). Similar reaction conditions were successfully applied to the synthesis of **126** (84%) and **128** (61%).



130 **127**

Scheme 4.6.

In an attempt to increase the water solubility of **128**, the bis-nitrate salt of **128** was prepared by the reaction of two equivalents of $[Pt(trpy)CH_3CN](NO_3)_2$ with **131** in dmf solution. The solubility of **128**.$2NO_3$ in dmf solution was notably poor in comparison with **128**.2OTf.

4.4.3. NMR Spectroscopic Characterisation of 126, 127 and 128

The identity of **126**, **127** and **128** were confirmed by micro-analysis and multinuclear (^1H, ^{13}C, ^{11}B and ^{195}Pt) NMR spectroscopy, as detailed in the Experimental Section. 2D NMR (COSY, HMBC and HMQC) experiments were performed to allow complete assignment of all ^1H and ^{13}C NMR signals for **126**, **127** and **128**, and the data are presented in Tables 4.1 and 4.2.

Using 200 and 300 MHz magnets, the ^1H NMR signals were considerably broadened, making complete assignment of all signals difficult. It is not uncommon for molecules of molecular weight over 1000 such as **126**, **127** and **128** to give broad NMR signals due to slow tumbling. Increasing the magnetic field to 600 MHz sufficiently

sharpened the ^{1}H NMR signals for **126** and **128** allowing clarification of all signals and their coupling constants, whereas the signals in the ^{1}H NMR spectrum of **127** still remained broad at 600 MHz.

^{1}H NMR spectroscopy is highly sensitive and coordination of a ligand to a platinum centre can often be detected *via* spin-spin coupling between the ^{195}Pt nuclei and ligand ^{1}H nuclei up to four bonds away.[404-406] The ^{195}Pt nucleus has a spin of ½, the same as ^{1}H nuclei. Consequently, ^{195}Pt-^{1}H coupling obeys the same multiplicity rules as ^{1}H-^{1}H coupling. The coupling of ^{1}H nuclei with the (33.8% abundant) ^{195}Pt nuclei splits a corresponding amount of the ^{1}H NMR signal into a doublet, generating 1:4:1 ^{1}H resonances, a spectral feature considered diagnostic of Pt-ligand bond formation. Such couplings can also give information regarding stereochemical information.[407] Such splitting is often referred to as ^{195}Pt 'satellites'.

In Pt-N-C-H fragments, such as in **126**, **127** and **128**, $^{3}J_{PtH}$ can vary from 10 to 60 Hz.[408] The values of $^{3}J_{PtH}$ depend on a number of factors, including the oxidation state of the ^{195}Pt centre and the nature of the ligands.[409] The value of $^{3}J_{Pt\text{-}N\text{-}C\text{-}H}$ also shows a Karplus-type[410] dependence on the dihedral angle between the plane of Pt-N-C and N-C-H.[408,409,411] Such satellites are observed in the 200 MHz NMR spectra of **126, 127** and **128** for the H2 atom of the trpy ligands. The satellites are considerably broad and have a coupling constant ($^{3}J_{PtH}$) of 40.0-40.6 Hz. The satellites are not observed at higher fields of 300 or 600 MHz. The broadening of the ^{195}Pt satellites of ^{1}H, ^{13}C and ^{15}N resonances with increasing field strength is characteristic of Pt-H couplings.[407,412] This is due to the relaxation of the heavy ^{195}Pt nuclei *via* the chemical shift anisotropy (CSA) mechanism.[412] CSA contribution tends to increase with the molecular weight and with the square of the applied field.[412,413]

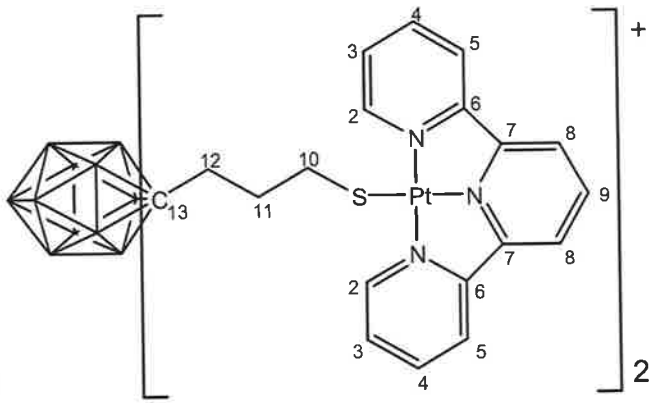


Table 4.1 ^{1}H NMR spectroscopic data for **126**, **127** and **128**[a]

Complex	H2	H3	H4	H5	H8	H9	H10	H11	H12
126	9.39 (dd, $^{4}J_{HH}$ = 1.8, $^{3}J_{HH}$ = 5.4) [$^{3}J_{PtH}$ = 40.6]	8.04 (dt, $^{4}J_{HH}$ = 1.8, $^{3}J_{HH}$ = 6.0)	8.52 (dt, $^{4}J_{HH}$ = 1.8, $^{3}J_{HH}$ = 7.8)	8.68 (d, $^{3}J_{HH}$ = 8.4)	8.72 (d, $^{3}J_{HH}$ = 8.4)	8.58 (t, $^{3}J_{HH}$ = 8.4)	2.63 (m)	1.95 (m)	*b*
127	9.51 (br s) [$^{3}J_{PtH}$ = 40.6]	8.10 (br s)	8.62 (t, $^{3}J_{HH}$ = 7.2)	8.78 (br s)	8.84 (br s)	8.74 (br s)	2.59 (br s)	1.75 (br s)	2.20(br s)
128	9.46 (d, $^{3}J_{HH}$ = 5.4) [$^{3}J_{PtH}$ = 40.0]	8.10 (dt, $^{4}J_{HH}$ = 1.2, $^{3}J_{HH}$ = 5.4)	8.61 (dt, $^{4}J_{HH}$ = 1.2, $^{3}J_{HH}$ = 7.8)	8.78 (d, $^{3}J_{HH}$ = 7.8)	8.84 (d, $^{3}J_{HH}$ = 8.4)	8.73(t, $^{3}J_{HH}$ = 8.4)	2.48 (t, $^{3}J_{HH}$ = 6.6)	1.55 (m)	1.89 (m)

[a]Measured in d_7-dimethylformamide at 600 MHz; coupling constants in Hertz. [b]Equivalent shift to H10.

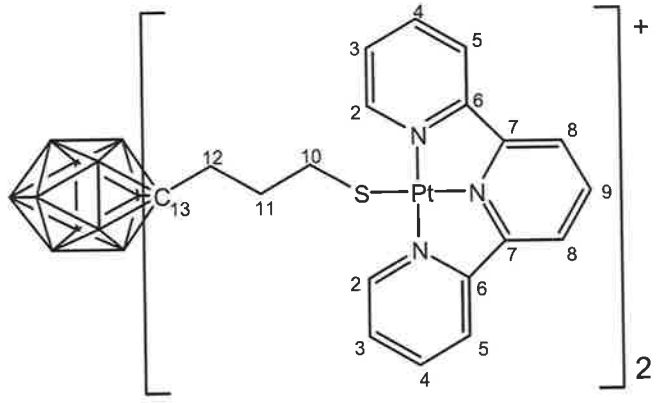


Table 4.2 ^{13}C and ^{195}Pt NMR assignments for **126**, **127** and **128**[c]

Complex	δ(^{13}C)												δ(^{195}Pt)
	C2	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C13**	
126	152.9	129.9	143.0	126.6	159.8	154.2	125.2	142.8	*e*	*e*	34.8	81.7	-3196
127	153.1	129.9	143.2	126.7	160.0	154.3	125.2	*d*	30.9	36.0	36.3	77.2	-3205
128	153.1	129.9	143.0	126.6	160.1	154.3	125.2	142.9	30.2	35.8	37.2	80.1	-3206

[c]Measured in d_7-dimethylformamide at 600 MHz; coupling constants in Hertz. [d] Equivalent to C4. [e] Resonance masked by solvent peaks.

4.5. UV-Visible Absorption Titration Studies of DNA-Binding by 126, 127, 128 and 107

Preliminary DNA-binding studies of **121-125** with ctDNA were conducted by monitoring the changes in the absorbance of UV-visible (280-400 nm) light upon addition of ctDNA into a solution of the respective complex.[267,378] The buffered solutions for UV-visible absorption measurements of **121-125** contained 20% methanol and 80% water (*v/v*) due to the low aqueous solubility of the complexes. As ctDNA concentrations were increased a progressive bathochromic shift was observed in absorbance maxima, accompanied by hypochromism, features consistent with DNA-intercalation of platinum(II)-trpy metallointercalators.[329-331,337,344]

Due to the poor solubility of platinum(II) complexes **121-125** in 1 mM phosphate buffer in 20% methanol in water (*v/v*), K_d could not be measured, preventing determination of the stability constant (K_s) for the intercalation of **121-125** by ctDNA using an adjusted Scatchard analysis.[267]

4.5.1. Solvent Systems

It is important to maximise the level of water within the solvent system to ensure biological conditions are mimicked and prevent the precipitation of DNA. The presence of up to 20% methanol (or 25% ethanol) in a buffered solution has been determined to prevent precipitation of calf thymus DNA.[414] However, the binding affinities of groove binder Hoechst 33258 and its analogues to calf thymus DNA were found to decrease by two orders of magnitude in the presence of 20% methanol (or 25% ethanol) in aqueous buffered solutions, in comparison with pure water.[88,415] The presence of non-aqueous solvents also caused shifts in λ_{max} of the UV-visible absorbance bands and a 10-fold enhancement of fluorescence quantum yield.[414]

When DNA-titrations were conducted in the presence of small amounts of methanol or dmf, the affinity of **107** for DNA was found to be lower than in pure water as the lower dielectric constant of the solvent system increases the stabilisation of free unbound non-polar metallointercalator, resulting in a shift in the position of equilibrium toward the unbound species. This lowers the overall stability constant for

the intercalation process. Due to the low solubility in methanol/water mixture, 2% dmf was required to solubilise **126**, **127** and **128**. It was anticipated that once the variations in UV-visible spectra of **126**, **127** and **128** upon addition of ctDNA were measured in a dmf/methanol/water (2:18:80) system, a comparison with the variations observed for **107** in the same solvent system would give an indication of the relative DNA-binding affinity (K_s) of **126, 127** and **128** in pure water, as K_s for **107** for ctDNA in pure water has been determined to be approximately 10^5 M^{-1}.[329]

4.5.2. UV-Visible Absorption Titration Studies of 107 with ctDNA

To allow a comparison with well-known metallointercalators and to determine the suitability of the chosen solvent systems in detecting intercalation of ctDNA by a platinum(II)-trpy complex, the changes in UV-visible spectrum of the well-known metallointercalator, **107**.2NO_3, were monitored upon titration with of ctDNA. The variations observed in the UV-visible spectrum of a 12.5 μM solution of **107** in buffered dmf/methanol/water (2:18:80) in the presence of 0-118.1 μM ctDNA are shown in Figure 4.3.

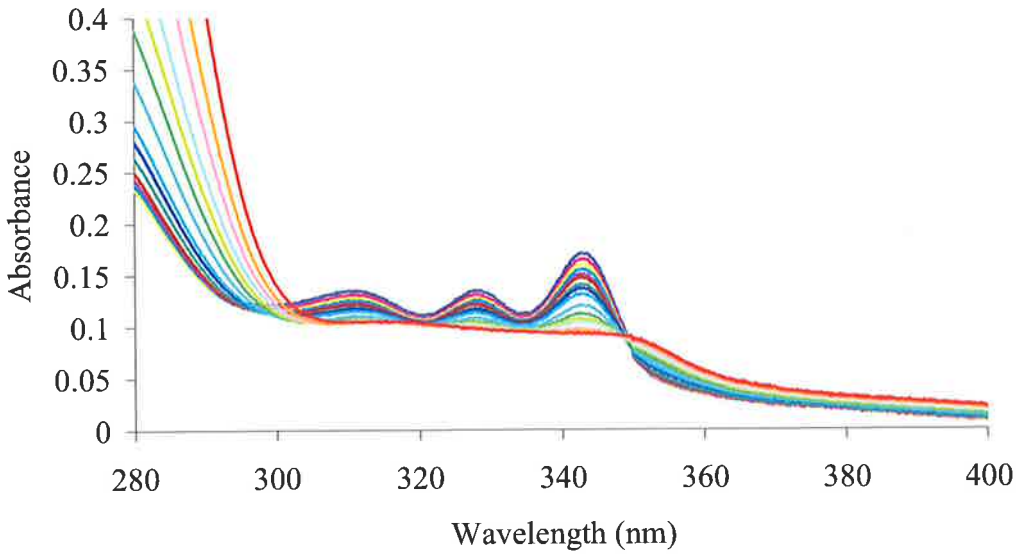


Figure 4.3 UV-vis spectra (280-400 nm) of **107** (12.5 μM) in dmf/methanol/water (2:18:80) (*v/v*) in the presence of 0.0, 0.8, 1.9, 3.8, 5.6, 7.5, 11.2, 14.9, 18.5, 27.6, 36.5, 45.2, 53.8, 70.6, 86.9 and 118.1 μM ctDNA.

107 appears to act as a classic metallointercalator under our experimental conditions. The addition of ctDNA resulted in hypochromism of the UV-visible spectra and a well-defined isosbestic point was obtained at 349 nm. These results indicate a clean reaction between **107** and ctDNA and it is most likely that only two forms of the chromophoric metallointercalator exist in solution, i.e. free and intercalated. Dimerisation and aggregation of **107** does not appear to be complicating the UV-visible spectra in this concentration range. However, dimerised and aggregated forms of **107** may display chromophore absorption spectra identical to the unbound or intercalated forms, in which case they cannot be detected by this method.

4.5.3. UV-Visible Absorption Titration Studies of 128 with ctDNA

The UV-visible spectrum of a 12.5 μM solution of **128**.2OTf in dmf/methanol/water (2:18:80) (*v/v*) was monitored upon the addition of various volumes of an aqueous solution of ctDNA (1.13 mM) and are displayed in Figure 4.4. Uniform decreases in absorbance measurements were observed between 360-300 nm, i.e. hypochromism, consistent with an intercalation of the platinum(II)-trpy moiety.

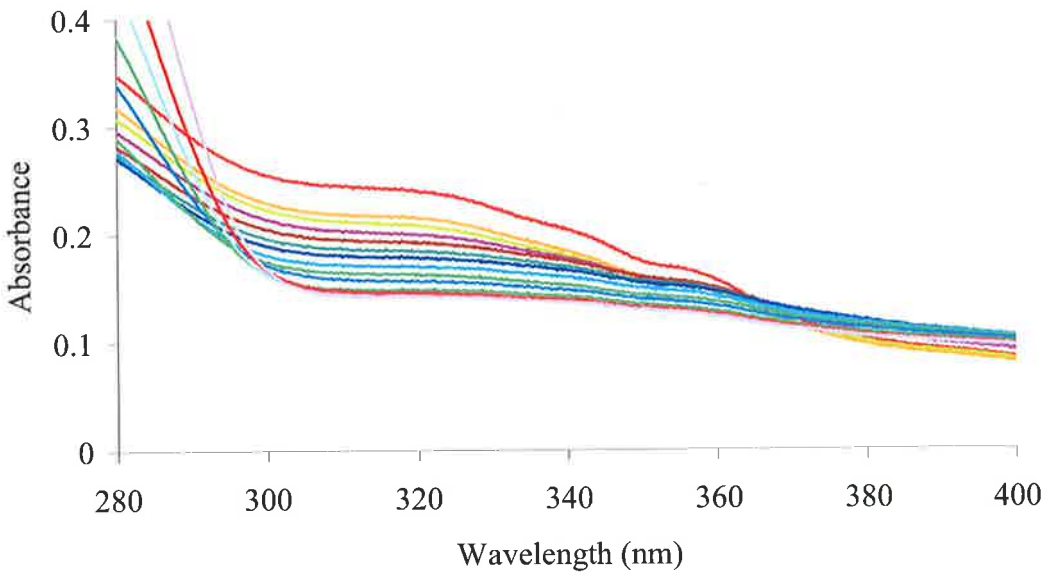


Figure 4.4 UV-visible spectra (280-400 nm) of **128** (12.5 μM) in dmf/methanol/water (2:18:80) (*v/v*) in the presence of 0, 1.9, 3.7, 5.6, 7.5, 9.3, 11.2, 14.9, 18.5, 27.6, 36.5, 45.2, 53.8 and 70.6 μM ctDNA.

Due to the poor aqueous solubility of **128**, the UV-visible spectra in dmf/methanol/water (2:18:80) (*v/v*) were significantly broadened in comparison with methanol/dmf (98:2) (*v/v*) (Figure 4.5). This broadening was fully reversible. When a solution of **128** in dmf/methanol/water (2:18:80) (*v/v*), displaying broad UV-visible absorbance spectra (blue line) was reduced to dryness and re-dissolved in methanol/dmf (98:2) (*v/v*), the UV-visible spectra gained more features (pink line) in (Figure 4.5). Such broadening was also observed for the bis-triflate salts of **126** and **127**.

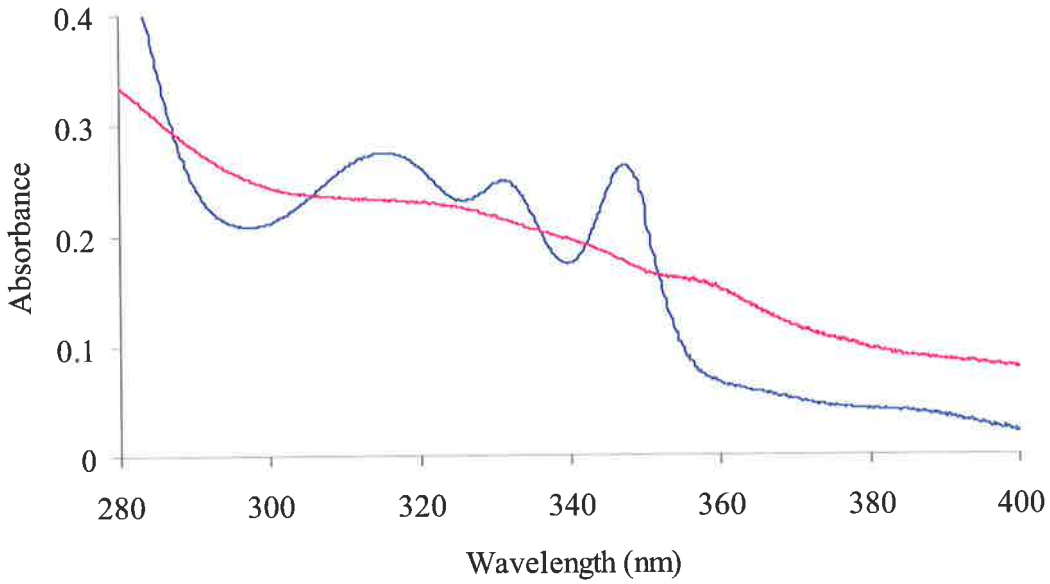


Figure 4.5 UV-visible spectra (280-400 nm) of **128** (12.5 μM) in (i) methanol/dmf (98:2) (*v/v*) (blue line) and (ii) dmf/methanol/water (2:18:80) (*v/v*) (pink line).

The broadening in the presence of high levels of water is considered to be a result of the complex precipitating as a fine suspension. It does not appear that the precipitate settles over time, as no change in the UV-visible spectra of **128** in the absence of ctDNA was observed over long periods of time. Such precipitation would decrease the levels of platinum(II)-trpy centres in solution, hence it would be difficult to detect any small amounts of intercalation by this method as the changes in the UV-visible absorption spectra appear to be dominated by precipitation and the consequent increased turbidity of the solution.

The decreases in absorbance of **128** seen in Figure 4.4 were initially attributed to hypochromism. However, the lack of any observable isosbestic points or bathochromic shifts together with the poor reproducibility of the result undermine the credibility of these DNA-titration experiments and intercalation does not appear to be occurring.

4.5.3.1. UV-Visible Absorption Spectra of 128.2NO_3

In an attempt to increase the water solubility of **128**, the nitrate salt was prepared by the reaction of two equivalents of $[Pt(trpy)CH_3CN](NO_3)_2$ with **131** in dmf solution. The nitrate salt of **128** was poorly soluble in dmf in comparison with the triflate salts, however its water solubility was insufficient to alter the results of UV-visible titration experiments and variations in UV-visible spectra upon the addition of ctDNA were identical to those observed for the triflate salts (Figure 4.4).

4.5.4. UV-Visible Absorption Titration Studies of 126 and 127 with ctDNA

Upon the addition of small quantities of calf thymus DNA to solutions of **126**.2OTf or **127**.2OTf, an unexpected increase in UV-visible absorbance was observed from 310-360 nm. This change is not characteristic of intercalation and indicates other modes of binding, such as dimerisation or aggregation on the surface of DNA are occurring, in preference to intercalation. This change in absorbance upon addition of DNA reaches a limit at DNA concentrations of 2 μM.

4.5.5. Implications of UV-Visible Absorption Titration Results

Not only does the poor solubility of **126**, **127** and **128** in aqueous solvents prevent detection and quantification of their ability to intercalate DNA, but it also indicates the complexes may not be well suited in their proposed application as BNCT agents. It is a requirement of all pharmaceuticals that they be stable and soluble under physiological conditions, such as high aqueous solvent conditions.

The limited aqueous solubility of **126**, **127** and **128** could be overcome by introducing water-solubilising functionalities, such as polar side chains,[65-67] or converting the carborane cage from the *closo*- to anionic *nido*-form.[28,80]

4.6. Anti-Cancer Screening

The dinuclear complexes, **126**, **127** and **128**, were screened along with the mononuclear platinum(II)-trpy complex, **122** (previously synthesised in the Rendina laboratories) against two tumour cell lines *in vitro* at the Andrew Durant Drug Testing Facility at the Peter MacCallum Cancer Institute in Melbourne, Australian. In addition, the well-known anti-cancer agent, cisplatin, was also tested as a control. The cell lines tested were the L1210 murine leukaemia cells and the cisplatin-resistant variation L1210/DDP.

It is reasonable to expect treatment with **122**, **126**, **127** and **128** causes cell destruction in the absence of neutrons. Some DNA intercalators and bis-intercalators, including platinum(II)-trpy complexes, are known to possess anti-cancer activity.[200,342,360,363,396] The mode of this activity is thought to be through intercalation of the platinum(II)-trpy moiety and consequent interruption to cellular processes, action on topoisomerases[357-360] or interruption of the cell membrane causing cell lysis.[342]

The concentrations (μM) of cisplatin, **122**, **126**, **127** and **128** required to achieve 50% inhibition of cell growth (IC_{50}) are presented in Table 4.3.

Cell Line	cisplatin	122	126	127	128
L1210	0.5	1.6	0.9	7.4	5.3
L1210/DDP	6.9	0.9	0.8	10	7.0

Table 4.3 IC_{50} (μM) values (n = 2) for various platinum(II) complexes. **126**, **127** and **128** were dissolved in dmf prior to addition to the cell lines. Determined using a CC assay.

The results of the anti-cancer screening for **122**, **126**, **127** and **128** are encouraging. For all platinum(II)-trpy complexes, the IC_{50} values are not significantly higher than

the values obtained for cisplatin. This appears to indicate that the compounds are able to enter the cells without difficulty. This is extremely promising, as achieving an uptake of boron into cancer cells is a highly valued goal in BNCT.

The potencies of **122**, **126**, **127** and **128** appear to be comparable in both the cisplatin-sensitive L1210 and cisplatin-resistant L1210/DDP cell lines. This indicates the mechanism of inducing cell death by **122**, **126**, **127** and **128** must not be affected by the cisplatin-resistance mechanisms operating in the L1210/DDP cell line.

In comparison with cisplatin, **122** and **126** are considerably potent in the two cell lines tested, especially in the cisplatin-resistant L1210/DDP cell line. In contrast, **127** and **128** are not as active. It appears that the inclusion of 1,7- and 1,12-carborane moieties in **127** and **128** adversely affects the ability of these complexes to exert a cytotoxic effect. The poor results in the anti-cancer screening may result from the poor aqueous solubility of carborane-containing complexes, **126**, **127** and **128**, despite their dicationic charges. It is possible that the exceptional activity of **126** over **127** and **128** results from a slightly higher solubility of **126** in aqueous solutions. While **122**, **126**, **127** and **128** are soluble in dmf, **122** is also soluble in methanol solution and unlike **126**, **127** and **128**, it does not precipitate out of solution upon dilution with water. Any precipitation of platinum(II) complexes during the long incubation periods with cell lines would prevent the full cytotoxic effect from being exerted, resulting in the observation of weak anti-cancer potency. The causes of various activities cannot be identified with certainty until the mechanism of cytotoxicity for the complexes is determined.

4.7. Correlations with DNA-Binding Studies

Together, the dicationic charges and two intercalating moieties within **126**, **127** and **128** was expected to facilitate an avid and more persistent intercalation of DNA in comparison with mono-intercalators such as **107** and **122**.[342] However, despite **122** possessing only a single positive charge and one intercalating platinum(II)-trpy moiety, it appears to exert a similar cytotoxic effect to **126**. It appears that the ability of **126**, **127**, **128** or **122** to intercalate DNA may not affect their cytotoxic potency. Cellular studies with dinuclear complexes **108-114** have resulted in extensive cell

lysis,[342] suggesting that some dinuclear platinum(II)-trpy complexes exert their toxic effects on the cell membrane rather than the nuclear DNA. The cationic charge of such complexes may impede cellular internalisation and cause cell death due to membrane binding rather than DNA intercalation.

4.8. Conclusions

The first examples of dinuclear platinum(II)-trpy complexes containing 1,2-, 1,7- and 1,12-dicarba-*closo*-dodecaboranes were prepared in this work. Through the application of a novel synthetic methodology three bis(thiopropyl)carboranes containing 1,2- (**129**), 1,7- (**130**) and 1,12-dicarba-*closo*-dodecaboranes (**131**) were synthesised in moderate yields. The triflate salts of the dinuclear platinum(II)-trpy complexes **126**, **127** and **128** containing 1,2-, 1,7- and 1,12-dicarba-*closo*-dodecaboranes, respectively, were synthesised and fully characterised by multinuclear NMR spectroscopy. In order to assess their potential application as DNA-binding agents, the interaction of **126**, **127** and **128**, along with the well-known metallointercalator **107**, with ctDNA were investigated, by monitoring changes in the UV-visible absorbance spectra.

Despite the dicationic charges of **126**, **127** and **128**, the solubility of these complexes in aqueous solutions that were designed to mimic physiological conditions were found to be insufficient to allow suitable UV-visible absorbance spectra to be recorded. The preparation of the nitrate salt of **128** did not sufficiently increase the aqueous solubility in order to achieve reliable and reproducible UV-visible absorbance spectra.

The triflate salts of **126**, **127** and **128** were screened against L1210 cell line and the cisplatin-resistant variant, L1210/DDP, allowing a comparison of IC_{50} values with cisplatin and the carborane-containing mononuclear platinum(II)-trpy complex, **122**. The IC_{50} values of **122**, **126**, **127** and **128** were not considerably higher than the IC_{50} values displayed by cisplatin. However, the IC_{50} values of **126** were lower than those of cisplatin. While the mechanism of inducing cell death by the complexes remains unclear, the excellent IC_{50} values indicate **122**, **126**, **127** and **128** are capable of entering cancerous cells, a highly valued goal in BNCT.

Future Work

The high of DNA-binding affinity displayed by carborane containing dinuclear platinum(II)-amine complexes, **78**, **80**, **81** and **82** is encouraging and supports their application as DNA-binding BNCT agents. The next stage is to assess the anticancer activity of **78**, **80**, **81** and **82** in the presence of thermal neutrons. Boron Neutron Capture experiments will be conducted in collaboration with Assoc. Prof. J. Coderre at MIT (USA).

In addition, various modifications of the dinuclear platinum(II)-amine complexes, **78**, **80**, **81** and **82** can be investigated. The limited aqueous solubility of these complexes may be overcome by introducing water-solubilising functionalities, such as polar side chains, carboxylate ligands or incorporating a charge, perhaps by converting the carborane cage from the *closo*- to anionic *nido*-form or by incorporation of polyamine-type carborane linkers such as derivatives of putrescine, spermidine and spermine. In addition, assorted platinum(II) ligand environments and different alkyl arm lengths can give unique DNA-binding profiles. Variations in length and distances between binding sites on the backbone can alter binding kinetics, levels of interstrand cross-links formation and sequence preference. NMR spectroscopy studies on model dinucleotides could assist in identifying the nature and kinetics of DNA binding.

Preliminary investigations have uncovered a novel method of preparing asymmetric carborane-containing di- and trinuclear platinum(II) complexes. The experimental conditions are yet to be optimised to achieve maximum purity and yields. The DNA-binding affinities of these complexes are yet to be determined, however the methods used for the symmetrical dinuclear complexes **78**, **80**, **81** and **82** would most likely give satisfactory results. By variations in complex charge, platinum(II) ligand environment, alkyl arm length and inclusion of water-solubilising functionalities, the number of potential BNCT agents that may be prepared is numerous.

Unfortunately, the poor water solubility of the carborane-containing dinuclear platinum(II)-trpy complexes, **126**, **127** and **128**, prevents their DNA-binding affinity to be determined under the conditions of UV-Visible Absorption titration studies

attempted. Quantification of the DNA-binding affinities of such carborane-containing dinuclear platinum(II)-trpy complexes could be made by introducing water-solubilising functionalities, such as charge, polar side chains or converting the *closo*-carborane to the anionic *nido*-carborane form. Alternatively, the conditions of the UV-Visible Absorption titration studies (namely, solvent systems) may be altered to maximise the solubility of **126**, **127** and **128** without precipitation of ctDNA or NMR studies on model dinucleotides could identify the nature and kinetics of DNA binding. Pending these results, the next stage would be to determine the anticancer activity of **126**, **127** and **128** in the presence of thermal neutrons.

The results of *in vitro* anti-cancer screening of **78**, **81**, **126**, **127** and **128** indicate they are cytotoxic in the absence of thermal neutrons. The mechanism by which they are inducing cell death is not known, and may not involve DNA binding. Further research must be conducted to establish the method of inducing cell death and to determine if these compounds are able to pass through the cell membrane and reach nuclear DNA.

Achieving selective delivery to tumour cells can be complicated. However, synthetic liposomes, termed supertankers, loaded with various ^{10}B-containing compounds have been shown to selectively penetrate the tumour cell membrane and localise intracellularly.[2,39,41-47] The carborane-containing dinuclear platinum(II)-amine and platinum(II)-trpy complexes prepared in this thesis would require several modifications to be included in such supertankers, but studies into the exploitation of this tumour-targeting methodology are worthwhile.

CHAPTER FIVE

Experimental

Experimental

5.1. General

All multinuclear 1-D NMR spectra were recorded at 298 K on a Varian Gemini 2000 NMR Spectrometer equipped with an Oxford 300 MHz magnet (^{1}H at 300.10 MHz, ^{13}C at 75.48 MHz, ^{11}B at 96.30 MHz, and ^{195}Pt at 64.38 MHz), or a Varian Gemini 200 NMR Spectrometer equipped with an Oxford 200 MHz magnet (^{1}H at 199.98 MHz, ^{13}C at 50.29 MHz). ^{1}H and ^{13}C{^{1}H} NMR chemical shifts are reported in ppm relative to tetramethylsilane (TMS). ^{195}Pt{^{1}H} NMR chemical shifts were referenced to a sealed external standard of 0.1 M $Na_2[PtCl_6]$ in D_2O. ^{11}B{^{1}H} NMR chemical shifts were referenced to a sealed external standard of $BF_3.OEt_2$. Coupling constants are reported in Hz. Melting points were determined using a Kofler hot-stage apparatus under a Reichert microscope and are uncorrected. Thin layer chromatography (t.l.c.) was carried out on Kieselgel 60 F_{254} (Merck) on aluminium backed plates. Unless otherwise stated reagents were obtained from Aldrich and were used without further purification. Solvents for recrystallisation and chromatography were distilled prior to use. Tetrahydrofuran (THF) was dried by fresh distillation from sodium benzophenone ketyl. CH_2Cl_2 was freshly distilled from CaH_2. *N,N*-Dimethylformamide (dmf) was predried over $MgSO_4$, followed by distillation under reduced pressure. Ethanol was freshly distilled from Mg/I_2.

Elemental analyses were performed by Chemical and Microanalytical Services Pty Ltd, Belmont, Victoria (Australia). High Resolution Mass Spectra were run in the Department of Chemistry at the University of Adelaide.

5.2. Materials and Methods

$K_2[PtCl_4]$ (Pressure Chemicals), *trans*-$[PtCl_2(NH_3)_2]$ (Strem) and 1,2-, 1,7- and 1,12-carborane (KatChem and Dexsil) were obtained commercially and used without further purification. **107**,[362] $HN(CBZ)_2$,[292] $BrCH_2CH_2CH_2S^tBu$,[267] $K[PtCl_3NH_3]$,[298,416] $PPh_4[PtCl_3NH_3]$,[417] $Pt(COD)Cl_2$,[418] $Pt(COD)I_2$,[419] *cis*-$[PtCl_2(NH_3)_2]$[420] and *cis*-

$[PtI_2(NH_3)_2]$[420] were prepared from literature procedures. **122** was synthesised by Dr. Jean A. Todd.[267]

5.3. Synthesis

1,2-Bis(3-aminopropyl)-1,2-carborane (61)

A solution of **76** (2.352 g, 3.57 mmol) in a homogenous mixture of 10 M HCl (30 cm^3) and ethyl acetate (70 cm^3) was stirred at room temperature for 6 h. The solvent was removed *in vacuo* and recrystallisation from methanol/diethyl ether gave **61**.2HCl (645 mg, 69%) as a white solid. ^1H NMR (200 MHz, D_2O): δ 2.99 (t, 4H, $^3J_{HH}$ = 7.6 Hz, C**H**$_2$N), 2.37 (m, 4H, C**H**$_2$C$_{cage}$), 1.93 (m, 4H, C**H**$_2$CH$_2$N). $^{13}C\{^1H\}$ NMR (200 MHz, CD_3OD): δ 81.0 (**C**$_{cage}$), 40.2 (**C**H$_2$N), 33.2 (**C**H$_2$C$_{cage}$), 29.0 (**C**H$_2$CH$_2$C$_{cage}$). $^{11}B\{^1H\}$ NMR (300 MHz, $CDCl_3$): δ -3.54 (s, 2B), -9.29 (s, 8B). Calcd for $C_8H_{28}B_{10}Cl_2N_2$: C: 29.00%, H: 8.51%, N: 8.45%; Found: C: 28.86%, H: 8.61%, N: 8.50%.

To a solution of **61**.2HCl (30 mg, 9.06×10^{-5} mol) in water (1 cm^3) in the presence of dichloromethane (2 cm^3) was added saturated $K_2CO_{3(aq)}$ (1 cm^3) with vigorous stirring. The organic layer was dried over anhydrous Na_2SO_4 and reduced *in vacuo* to give **61** as a clear oil (10 mg, 43%).

1,7-Bis(3-aminopropyl)-1,7-carborane (62)

A solution of **75** (1.703 g, 2.58 mmol) in a homogenous mixture of 10 M HCl (20 cm^3) and ethyl acetate (45 cm^3) was stirred at room temperature for 5 h. The solvent was removed *in vacuo* to give a pale-yellow solid. Purification by recrystallisation from methanol/diethyl ether gave **62**.2HCl (645 mg, 69%) as a white solid. ^1H NMR (200 MHz, CD_3OD): δ 3.05 (t, 4H, $^3J_{HH}$ = 7.4 Hz, C**H**$_2$N), 2.31 (m, 4H, C**H**$_2$C$_{cage}$), 1.91 (m, 4H, C**H**$_2$CH$_2$N). $^{13}C\{^1H\}$ NMR (200 MHz, CD_3OD): δ 76.6 (**C**$_{cage}$), 40.2 (**C**H$_2$N), 34.9 (**C**H$_2$C$_{cage}$), 29.2 (**C**H$_2$CH$_2$C$_{cage}$). $^{11}B\{^1H\}$ NMR (300 MHz, CD_3OD): δ -6.05 (s, 2B), -9.77 (s, 6B),-11.78 (s, 2B). Calcd for $C_8H_{28}B_{10}Cl_2N_2$: C: 29.00%, H: 8.51%, N: 8.45%; Found: C: 29.05%, H: 8.67%, N: 8.42%.

To a solution of **62**.2HCl (59 mg, 0.178 mmol) in water (2 cm^3) in the presence of dichloromethane (2 cm^3) was added saturated $K_2CO_{3(aq)}$ (1 cm^3) with vigorous stirring. The organic layer was dried over anhydrous Na_2SO_4 and reduced *in vacuo* to give **62** as a clear oil (27 mg, 58%).

1,12-Bis(3-aminopropyl)-1,12-carborane (63)

A solution of **77** (340 mg, 5.16×10^{-4} mol) in a homogenous mixture of 10 M HCl (4.5 cm^3) and ethyl acetate (10.5 cm^3) was stirred at room temperature for 5 h. The solvent was removed *in vacuo* to give a white solid. Recrystallisation from methanol/diethyl ether gave **63**.2HCl (106 mg, 62%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 2.85 (t, 4H, $^3J_{HH}$ = 6.8 Hz, C**H**$_2$N), 1.7 (m, 4H, C**H**$_2$C$_{cage}$), 1.5 (m, 4H, C**H**$_2$CH$_2$N).

To a solution of **63**.2HCl (51 mg, 1.54×10^{-4} mol) in water (4 cm^3) in the presence of dichloromethane (5 cm^3) was added saturated $K_2CO_{3(aq)}$ (1 cm^3) with vigorous stirring. The organic layer was dried over anhydrous Na_2SO_4 and reduced *in vacuo* to give **63** as a clear oil (19 mg, 48%).

1,7-Bis(3-hydroxypropyl)-1,7-carborane (65)

A solution of 1,7-carborane (860 mg, 5.96 mmol) in dry, distilled THF (30 cm^3) was stirred under nitrogen at -78°C. A solution of *n*-BuLi (2.5 M, 6 cm^3, 15 mmol) in dry, distilled THF (25 cm^3) was added dropwise over 10 min and the mixture was stirred for a further 40 min at -78°C. The reaction was slowly warmed to room temperature. Oxetane (1.1 cm^3, 16.9 mmol) was added and the solution was heated to reflux for 4 h. The addition of $HCl_{(aq)}$ (2 M, 15 cm^3, 30 mmol) quenched the reaction. The aqueous layer was extracted with ethyl acetate (2 × 30 cm^3), and the combined organic layers were washed with water (50 cm^3) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a crude white solid. Purification by flash chromatography on silica (67% ethyl acetate in *n*-hexane, R_f = 0.31) gave **65** (963 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$ + drop d_6-acetone): δ 3.56 (t, 4H, $^3J_{HH}$ = 6.3 Hz, C**H**$_2$OH), 2.03 (m, 4H, C**H**$_2$C$_{cage}$), 1.62 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{13}C{^{1}H} NMR

(300 MHz, $CDCl_3$ + drop d_6-acetone): δ 75.5 (C_{cage}), 61.4 ($\mathbf{C}H_2OH$), 33.4 ($\mathbf{C}H_2C_{cage}$), 32.7 ($CH_2\mathbf{C}H_2C_{cage}$). $^{11}B\{^1H\}$ NMR (300 MHz, $CDCl_3$ + drop d_6-acetone): δ -7.66 (s, 2B), -11.45 (s, 6B), -13.84 (s, 2B). Calcd for $C_8H_{24}B_{10}O_2$: C: 36.90%, H: 9.29%; Found: C: 36.84%, H: 9.18%.

1,2-Bis(3-hydroxypropyl)-1,2-carborane (66)

A solution of 1,2-carborane (990 mg, 6.86 mmol) in dry, distilled THF (40 cm^3) was stirred under nitrogen at -78°C for 10 min. A solution of *n*-BuLi (2.5 M, 7.2 cm^3, 18.0 mmol) in dry, distilled THF (20 cm^3) was slowly added dropwise and the mixture stirred at -78°C for 30 min. The reaction was slowly warmed to room temperature. Oxetane (1.5 cm^3, 16.9 mmol) was added and the solution was heated to reflux for 7 h. The addition of $HCl_{(aq)}$ (2 M, 18 cm^3, 30 mmol) quenched the reaction. The aqueous layer was extracted with ethyl acetate (2 × 50 cm^3) and the combined organic layers were washed with water (50 cm^3) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a white solid. The solid was stirred with chloroform (4 cm^3) to remove the impurities and **66** (1.35 g, 76%) was isolated by vacuum filtration. Mp 125-126°C. ^1H NMR (200 MHz, $CDCl_3$ + drop d_6-acetone): δ 3.65 (t, 4H, $^3J_{HH}$ = 5.4 Hz, $\mathbf{C}H_2OH$), 2.35 (m, 4H, $\mathbf{C}H_2C_{cage}$), 1.81 (m, 4H, $CH_2\mathbf{C}H_2C_{cage}$). $^{13}C\{^1H\}$ NMR (200 MHz, $CDCl_3$ + drop d_6-acetone): δ 79.6 (C_{cage}), 61.3 ($\mathbf{C}H_2OH$), 32.4 ($\mathbf{C}H_2C_{cage}$), 31.6 ($\mathbf{C}H_2CH_2C_{cage}$). $^{11}B\{^1H\}$ NMR (300 MHz, $CDCl_3$ + drop d_6-acetone): δ -5.17 (s, 2B), -11.00 (s, 8B). Calcd for $C_8H_{24}B_{10}O_2$: C: 36.90%, H: 9.29%; Found: C: 36.87%, H: 9.35%.

1,12-Bis(3-hydroxypropyl)-1,12-carborane (67)

To a solution of 1,12-carborane (0.98 g, 6.79 mmol) in dry THF (50 cm^3) at -78°C was added dropwise a solution of *n*-BuLi (1.6 M, 12 cm^3, 19.2 mmol) in dry, distilled THF (40 cm^3) over 10 min. The mixture was slowly warmed to room temperature over 30 min. Oxetane (1.1 cm^3, 16.9 mmol) in THF (30 cm^3) was added and the solution was heated to reflux for 18 h. The addition of $HCl_{(aq)}$ (2 M, 20 cm^3, 40 mmol) quenched the reaction. The aqueous layer was extracted with ethyl acetate (2 × 50 cm^3), and the combined organic layers were washed with water (75 cm^3) and dried

over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a crude white solid. Purification by flash chromatography on silica (67% ethyl acetate in *n*-hexane, R_f = 0.26) gave **67** (907 mg, 51%). ^{1}H NMR (300 MHz, $CDCl_3$ + drop d_6-acetone): δ 3.46 (t, 4H, $^3J_{HH}$ = 6.3 Hz, C**H**$_2$OH), 1.73 (m, 4H, C**H**$_2$C$_{cage}$), 1.39 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$ + drop d_6-acetone): δ -13.22 (s, 10B).

1,7-Bis(3-bromopropyl)-1,7-carborane (68)

To a solution of **65** (947 mg, 3.64 mmol) and carbon tetrabromide (3.145 g, 9.48 mmol) in dry, distilled dichloromethane (40 cm^3) at 5°C, was added dropwise to a solution of triphenylphosphine (2.89 g, 11.0 mmol) in dry, distilled dichloromethane (30 cm^3) over 30 min under nitrogen. The reaction was warmed to room temperature and stirred for 2 h. The solvent was removed *in vacuo* to afford an orange residue. The residue was stirred with diethyl ether (20 cm^3) for 30 min, and the remaining solid was removed by filtration. The filtrate was reduced to dryness *in vacuo* to give a yellow residue which was purified by squat chromatography on silica (2% diethyl ether in *n*-hexane, R_f = 0.38) to give **68** (1.21 g, 86%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.31 (t, 4H, $^3J_{HH}$ = 6.3 Hz, C**H**$_2$Br), 2.10 (m, 4H, C**H**$_2$C$_{cage}$), 1.91 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$): δ 74.7 (**C**$_{cage}$), 35.4 (**C**H$_2$Br), 32.5 (**C**H$_2$C$_{cage}$), 31.9 (**C**H$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -7.45 (s, 2B), -11.24 (s, 6B), -13.79 (s, 2B). Calcd for $C_8H_{22}B_{10}Br_2$: C: 24.88%, H: 5.74%; Found: C: 24.83%, H: 5.81%

1,2-Bis(3-bromopropyl)-1,2-carborane (69)

A solution of triphenylphosphine (6.340 g, 24.2 mmol) in dry, distilled dichloromethane (30 cm^3) was added dropwise under nitrogen to a solution of **66** (2.033 g, 7.81 mmol) and carbon tetrabromide (6.587 g, 19.9 mmol) in dry, distilled dichloromethane (80 cm^3) at 5°C. The reaction was warmed to room temperature and stirred for 2 h. The solvent was removed *in vacuo* to afford an orange residue. The residue was stirred with dichloromethane (3 cm^3) and diethyl ether (40 cm^3) for 30 min. The remaining solid was removed by filtration and washed with diethyl ether

(20 cm^3). The filtrate and washings were reduced *in vacuo* to give an orange residue that was purified by squat chromatography on silica. Elution with 15% ethyl acetate in *n*-hexane (R_f = 0.55) gave, after removal of solvent *in vacuo*, **69** (2.20 g, 5.7 mmol, 73%). Mp 49-50°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.42 (t, 4H, $^3J_{HH}$ = 6.0 Hz, C**H**$_2$Br), 2.39 (m, 4H, C**H**$_2$C$_{cage}$), 2.10 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{13}C{^{1}H} NMR (300 MHz, $CDCl_3$): δ 78.7 (**C**$_{cage}$), 33.5 (**C**H$_2$Br), 32.1 (**C**H$_2$C$_{cage}$), 31.9 (**C**H$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -4.80 (s, 2B), -10.46 (s, 8B). Calcd for $C_8H_{22}B_{10}Br_2$: C: 24.88%, H: 5.74%; Found: C: 24.87%, H: 5.76%

1,12-Bis(3-bromopropyl)-1,12-carborane (70)

To a solution of **67** (817 mg, 3.14 mmol) and carbon tetrabromide (2.69 g, 8.11 mmol) in dry dichloromethane (60 cm^3) at 0°C was added dropwise a solution of triphenylphosphine (2.55 g, 9.71 mmol) in dichloromethane (40 cm^3). The reaction mixture was allowed to warm slowly to room temperature and stirring was continued for 2 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica (*n*-hexane, R_f = 0.40) to give **70** as a white solid (1.15 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.21 (t, 4H, $^3J_{HH}$ = 8.7 Hz, C**H**$_2$Br), 1.7 (m, 8H, C**H**$_2$C$_{cage}$, C**H**$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -13.08 (s, 10B).

1,7-Bis(3-iodopropyl)-1,7-carborane (71)

A solution of **68** (483 mg, 1.25 mmol) and sodium iodide (1.05 g, 7.01 mmol) in distilled acetone (20 cm^3) was heated to reflux for 24 h. The solvent was removed *in vacuo* from the cooled solution to afford a mixture containing both yellow and orange solids. The solids were dissolved in diethyl ether (20 cm^3) and water (20 cm^3). The aqueous layer was extracted with diethyl ether (20 cm^3) and the combined organic layers were washed with water (20 cm^3), brine (20 cm^3) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **71** as a yellow oil (551 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (t, 4H, $^3J_{HH}$ = 6.0 Hz, C**H**$_2$I), 1.98 (m, 4H, C**H**$_2$C$_{cage}$), 1.78 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$): δ 74.5 (**C**$_{cage}$), 37.6 (**C**H$_2$C$_{cage}$), 33.1 (**C**H$_2$CH$_2$C$_{cage}$), 4.12 (**C**H$_2$I). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$):

δ -7.48 (s, 2B), -11.30 (s, 6B), -13.86 (s, 2B). Calcd for $C_8H_{22}B_{10}I_2$: C: 20.01%, H: 4.62%; Found: C: 20.04%, H: 4.52%

1,2-Bis(3-iodopropyl)-1,2-carborane (72)

A solution of **69** (199 mg, 0.52 mmol) and sodium iodide (530 mg, 3.53 mmol) in distilled acetone (10 cm^3) was heated to reflux for 24 h. The solvent was removed *in vacuo* from the cooled solution to afford a mixture containing both yellow and orange solids. The solids were dissolved in diethyl ether (10 cm^3) and water (15 cm^3). The aqueous layer was extracted with diethyl ether (10 cm^3). The combined organic layers were washed with brine (10 cm^3) and dried ($MgSO_4$) and the solvent was removed *in vacuo* to give **72** (180 mg, 73%), a yellow solid. Mp 63-64°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.20 (t, 4H, $^3J_{HH}$ = 6.2 Hz, C**H**$_2$I), 2.35 (m, 4H, C**H**$_2$C$_{cage}$), 2.03 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$): δ 78.5 (**C**$_{cage}$), 35.8 (**C**H$_2$C$_{cage}$), 32.6 (**C**H$_2$CH$_2$C$_{cage}$), 4.2 (**C**H$_2$I). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -4.79 (s, 2B), -10.55 (s, 8B). Calcd for $C_8H_{22}B_{10}I_2$: C: 20.01%, H: 4.62%; Found: C: 20.02%, H: 4.66%.

1,12-Bis(3-iodopropyl)-1,12-carborane (73)

A solution of **70** (453 mg, 1.17 mmol) and sodium iodide (1.15 g, 7.67 mmol) in distilled acetone (50 cm^3) was heated to reflux for 24 h. The solvent was removed *in vacuo* from the cooled solution to afford a mixture containing both yellow and orange solids. The solids were dissolved in diethyl ether (40 cm^3) and water (50 cm^3). The aqueous layer was extracted with diethyl ether (40 cm^3). The combined organic layers were washed with water (50 cm^3), brine (30 cm^3) and dried ($MgSO_4$). The solvent was removed *in vacuo* to give **73** as a yellow solid (513 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.98 (t, 4H, $^3J_{HH}$ = 6.6 Hz, C**H**$_2$I), 1.70 (m, 8H, C**H**$_2$C$_{cage}$, C**H**$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -13.02 (s, 10B).

_N,N_-Di-*tert*-butyloxycarbonyl-1,7-bis(3-aminopropyl)-1,7-carborane (75)

To a stirred solution of tetrabutylammonium hydrogensulfate (4.38 g, 12.9 mmol) and aqueous sodium hydroxide (2 M, 13 cm^3, 26 mmol) in distilled dichloromethane (80 cm^3) was added a solution of di-*tert*-butyl iminodicarboxylate (2.81 g, 12.9 mmol) in distilled dichloromethane (25 cm^3). After stirring for 30 min, a solution of **71** (3.09 g, 6.45 mmol) in distilled dichloromethane (50 cm^3) was added dropwise to the mixture. The reaction was then heated to reflux for 20 h. The aqueous layer was extracted with dichloromethane (3 × 40 cm^3). The combined organic extracts were reduced *in vacuo* to afford a yellow residue that was stirred with diethyl ether (100 cm^3) for 20 min to precipitate tetrabutylammonium iodide, which was removed by vacuum filtration. The filtrate was dried over anhydrous $NaSO_4$ and reduced *in vacuo* to afford a yellow oil that was purified by flash chromatography on silica (elution with *n*-hexane followed by gradients up to 30% diethyl ether in *n*-hexane) and recrystallisation from *n*-hexane to give **75** (2.48 g, 58%). Mp 103°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.47 (t, 4H, $^3J_{HH}$ = 7.0 Hz, C**H**$_2$N), 1.87 (m, 4H, C**H**$_2$C$_{cage}$), 1.50 (m, 40H, C(C**H**$_3$)$_3$, C**H**$_2$CH$_2$N). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$): δ 152.5 (CO), 82.5 (**C**(CH$_3$)$_3$), 75.2 (**C**$_{cage}$), 45.5 (**C**H$_2$N), 34.2 (**C**H$_2$C$_{cage}$), 29.3 (**C**H$_2$CH$_2$C$_{cage}$), 28.1 (**C**H$_3$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -7.61 (s, 2B), -11.18 (s, 6B), -13.90 (s, 2B). Calcd for $C_{28}H_{58}B_{10}N_2O_8$: C: 51.04%, H: 8.87%, N: 4.25%; Found: C: 51.20%, H: 8.96%, N: 4.19%.

_N,N_-Di-*tert*-butyloxycarbonyl-1,2-bis(3-aminopropyl)-1,2-carborane (76)

To a stirred solution of tetrabutylammonium hydrogensulfate (3.12 g, 9.18 mmol) and aqueous sodium hydroxide (2 M, 10 cm^3, 20 mmol) in distilled dichloromethane (100 cm^3) was added a solution of di-*tert*-butyl iminodicarboxylate (1.907 g, 8.78 mmol) in distilled dichloromethane (40 cm^3). After stirring for 30 min, a solution of **72** (1.917 g, 3.99 mmol) in distilled dichloromethane (40 cm^3) was added dropwise. The reaction was then heated to reflux for 7 h. Water (50 cm^3) was added and the aqueous layer was extracted with dichloromethane (2 × 50 cm^3). The combined organic extracts were reduced *in vacuo* to afford a yellow residue, which was stirred with diethyl ether (75 cm^3) for 20 min to precipitate tetrabutylammonium iodide, removed by vacuum filtration. The filtrate was dried over anhydrous Na_2SO_4 and

reduced *in vacuo* to produce a yellow oil that was purified by flash chromatography on silica. Elution with dichloromethane followed by gradients up to 5% diethyl ether in dichloromethane gave, after removal of solvent *in vacuo* **76** (2.35 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.55 (t, 4H, $^{3}J_{HH}$ = 6.9 Hz, C**H**$_2$N), 2.13 (m, 4H, C**H**$_2$C$_{cage}$), 1.84 (m, 4H, C**H**$_2$CH$_2$N), 1.51 (s, 36H, C(C**H**$_3$)$_3$). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$): δ 152.4 (CO), 82.5 (**C**(CH$_3$)$_3$), 82.4 (**C**$_{cage}$), 45.2 (**C**H$_2$N), 32.3 (**C**H$_2$C$_{cage}$), 28.9 (**C**H$_2$CH$_2$C$_{cage}$), 28.0 (**C**H$_3$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -4.89 (s, 2B), -10.69 (s, 8B).

_N,N_-Di-_tert_-butyloxycarbonyl-1,12-bis(3-aminopropyl)-12-carborane (77)

To a vigorously stirred solution of tetrabutylammonium hydrogensulfate (769 mg, 2.26 mmol) and aqueous sodium hydroxide (2 M, 2 cm^3, 4.0 mmol) in distilled dichloromethane (55 cm^3) was added a solution of di-*tert*-butyl iminodicarboxylate (487 mg, 2.24 mmol) in distilled dichloromethane (15 cm^3). After stirring for 30 min, a solution of **73** (513 mg, 1.07 mmol) in distilled dichloromethane (30 cm^3) was added dropwise to the mixture. The reaction was heated to reflux for 16 h. The aqueous layer was extracted with dichloromethane (2 × 30 cm^3) and the combined organic extracts were reduced *in vacuo* to afford a white solid, which was stirred with diethyl ether (200 cm^3) for 40 min to precipitate tetrabutylammonium iodide, which was removed by vacuum filtration. The filtrate was dried over anhydrous $NaSO_4$ and reduced *in vacuo* to afford a colourless oil which was purified by flash chromatography on silica (10% diethyl ether in *n*-hexane, R_f = 0.14) to give **77** (340 mg, 48%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.36 (t, 4H, $^{3}J_{HH}$ = 7.2 Hz, C**H**$_2$N), 1.60 (m, 4H, C**H**$_2$C$_{cage}$), 1.50 (s, 36H, C(C**H**$_3$)$_3$), 1.40 (m, 4H, C**H**$_2$CH$_2$N). ^{13}C{^{1}H} NMR (300 MHz, $CDCl_3$): δ 152.5 (CO), 82.5 (**C**(CH$_3$)$_3$), 82.3 (**C**$_{cage}$), 45.5 (**C**H$_2$N), 35.0 (**C**H$_2$C$_{cage}$), 28.9 (**C**H$_2$CH$_2$C$_{cage}$), 28.0 (**C**H$_3$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -13.09 (s, 10B).

μ-{1,7-Bis(aminopropyl)-1,7-carborane-*N,N'*}-bis[*cis*-amminedichloro platinum(II)] (78)

A solution of potassium iodide (108 mg, 0.65 mmol) in H_2O (0.83 cm^3) was added to a solution of $K[Pt(NH_3)Cl_3]$ (75 mg, 0.21 mmol) in H_2O (1.04 cm^3), previously adjusted to pH 7 with 0.1 M NaOH. The solution was stirred for 15 min at room temperature. A solution of **62** (27 mg, 0.104 mmol) in distilled methanol (2 cm^3) was added and stirring was continued for 4 h. The resulting yellow precipitate was collected by centrifugation and washed successively with cold water, methanol, diethyl ether and dried under vacuum to afford **79** (99 mg, 0.098 mmol, 94%).

To a solution of **79** (99 mg, 0.098 mmol) in distilled dmf (2 cm^3) was added a solution of silver nitrate (62 mg, 0.366 mmol) in dmf (2 cm^3). The solution was stirred in the dark for 18 h. Insoluble AgI and AgCl was removed by filtration through a pad of Celite filter aid and washed with dmf (1 cm^3). To the filtrate and washings was added a second solution of silver nitrate (25 mg, 0.148 mmol) in dmf (1 cm^3) and the solution was stirred in the dark for 9 h. Once again, insoluble AgI and AgCl was removed by filtration through a pad of Celite. To the filtrate was added 1.0 M $HCl_{(aq)}$ (0.20 cm^3, 0.20 mmol) to precipitate unreacted Ag^+ as AgCl. The solution was left in a refrigerator overnight and AgCl was removed by filtration through a pad of Celite. To the filtrate was added 10 M $HCl_{(aq)}$ (0.10 cm^3, 1.0 mmol), followed by 0.1 M $HCl_{(aq)}$ (20 cm^3). The solution was placed in a refrigerator overnight to allow precipitation of a yellow solid. The solid was collected by centrifugation, washed with methanol, diethyl ether and air dried. Recrystallisation from dmf/0.1 M $HCl_{(aq)}$ gave **78** as a yellow solid (51 mg, 59% overall). ^1H NMR (200 MHz, d_7-dmf): δ 4.63 (br s, 4H, NH_2), 3.89 (br s, 6H, NH_3), 2.40 (m, 4H, CH_2N), 1.79 (m, 4H, CH_2C_{cage}), 1.50 (m, 4H, CH_2CH_2N). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 75.6 (C_{cage}), 45.5 (CH_2N), 33.1 (CH_2C_{cage}), 30.4 ($CH_2CH_2C_{cage}$). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -11.39 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2156. Calcd for $C_8H_{32}B_{10}Cl_4N_4Pt_2$: C: 11.65%, H: 3.91%, N: 6.80%; Found: C: 12.33%, H: 4.23%, N: 6.94%.

μ-{1,12-Bis(aminopropyl)-1,12-carborane-*N,N'*}-bis[*cis*-amminedichloro platinum(II)] (80)

A solution of potassium iodide (76 mg, 4.58×10^{-4} mol) in H_2O (1.0 cm^3) was added to a solution of $K[Pt(NH_3)Cl_3]$ (54.4 mg, 1.52×10^{-4} mol) in H_2O (1.0 cm^3), previously adjusted to pH 7 with 0.1 M NaOH. The solution was stirred for 15 min at room temperature. A solution of **63** (19.5 mg, 7.54×10^{-4} mol) in distilled methanol (4 cm^3) was added and the stirring was continued for 2 h. The yellow precipitate was collected by centrifugation, washed successively with H_2O, methanol, diethyl ether and dried under vacuum to give the mixed iodo/chloro complex, $[\{Pt(NH_3)ClI\}_2\text{-}1,7\text{-}NH_2(CH_2)_3CB_{10}H_{10}C(CH_2)_3NH_2]$ (70 mg, 6.9×10^{-4} mol, 91%).

To a solution of the mixed iodo/chloro complex (70 mg, 6.9×10^{-4} mol) in distilled dmf (2 cm^3) was added a solution of silver triflate (70 mg, 2.72×10^{-4} mol) in dmf (2 cm^3). The solution was stirred overnight in the dark. Insoluble AgI and AgCl were removed by filtration through a pad of Celite. To the filtrate was added a second portion of silver triflate (23 mg, 8.95×10^{-5} mol) in dmf (1 cm^3). The solution was stirred for 12 h in the dark. Insoluble AgI and AgCl were removed by filtration through a pad of Celite and to the filtrate was added $HCl_{(aq)}$ (1.0 M, 0.20 cm^3, 0.20 mmol) to precipitate unreacted Ag^+ as AgCl. AgCl was removed by filtration through a pad of Celite. To the filtrate and washings was added 0.1 M $HCl_{(aq)}$ (20 cm^3) and the solution was placed in fridge overnight to precipitate **80**. The precipitate was collected by centrifugation, washed with methanol, diethyl ether and dried *in vacuo* (29 mg, 46% overall). ^1H NMR (300 MHz, d_7-dmf): δ 4.87 (s, NH), 4.19 (s, NH), 2.62 (m, 4H, C**H**$_2$N), 1.76 (m, 4H, C**H**$_2$C$_{cage}$), 1.56 (m, 4H, C**H**$_2$CH$_2$N). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 80.3 (C_{cage}), 47.2 (CH_2N), 35.5 (**C**H$_2$C$_{cage}$), 31.7 (**C**H$_2$CH$_2$N). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -8.79 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2155. Calcd for $C_8H_{32}B_{10}Cl_4N_4Pt_2$: C: 11.65%, H: 3.91%, N: 6.80%; Found: C: 12.50%, H: 4.15%, N: 7.00%.

μ-{1,7-Bis(aminopropyl)-1,7-carborane-*N,N'*}-bis[*trans*-diamminechloro platinum(II)] bis(triflate) (81)

A solution of silver trifuoromethanesulfonate (48 mg, 0.187 mmol) in dmf (0.50 cm^3) was added dropwise to a suspension of transplatin (50 mg, 0.167 mmol) in dmf (0.50 cm^3). The solution was stirred in the dark for 18 h. AgCl was removed by filtration through a pad of Celite. To the filtrate was added a solution of **62** (19 mg, 0.074 mmol) in dmf (0.50 cm^3) and stirring was continued for 48 h. 0.1 M $HCl_{(aq)}$ (3 cm^3) was added and refrigerated overnight. A white precipitate was collected by centrifugation, washed with water, ethanol and diethyl ether and dried under vacuum to afford **81** (18 mg, 22%). ^{1}H NMR (300 MHz, CD_3OD + drop d_7-dmf): δ 2.74 (t, 4H, $^3J_{HH}$ = 7.5 Hz, C**H**$_2$N), 2.10 (m, 4H, C**H**$_2$C$_{cage}$), 1.78 (m, 4H, C**H**$_2$CH$_2$N). ^{13}C{^{1}H} NMR (300 MHz, d_7-dmf): δ 75.6 (**C**$_{cage}$), 46.0 (**C**H$_2$N), 33.4 (**C**H$_2$C$_{cage}$), 30.6 (**C**H$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, d_7-dmf): δ -11.00 (s, 10B). ^{195}Pt{^{1}H} NMR (300 MHz, d_7-dmf): δ -2410. Calcd for $C_{10}H_{38}B_{10}Cl_2F_6N_6O_6Pt_2S_2$: C: 11.06%, H: 3.53%, N: 7.74%; Found: C: 10.96%, H: 4.09%, N: 8.11%.

μ-1,12-Bis(aminopropyl)-1,12-carborane-*N,N'*-bis[*trans*-diamminechloro platinum(II)] bis(triflate) (82)

A solution of silver triflate (36 mg, 1.40×10^{-4} mol) in dmf (2.0 cm^3) was added dropwise to a suspension of transplatin (44 mg, 1.47×10^{-4} mol) in dmf (3 cm^3). The solution was stirred overnight in the dark. Insoluble AgCl was removed by filtration through a pad of Celite. To the filtrate was added a solution of **63** (19 mg, 7.35×10^{-5} mol) in dmf (3 cm^3) and stirring was continued overnight. The solution was reduced to dryness *in vacuo* and the residual white solid was recrystallised from dmf/0.1 M $HCl_{(aq)}$ to give **82** as a white solid (57 mg, 71%). ^{1}H NMR (300 MHz, CD_3OD + drop d_7-dmf): δ 2.64 (t, 4H, $^3J_{HH}$ = 7.5 Hz, C**H**$_2$N), 1.76 (m, 4H, C**H**$_2$C$_{cage}$), 1.56 (m, 4H, C**H**$_2$CH$_2$N). ^{13}C{^{1}H} NMR (300 MHz, d_7-dmf + CD_3OD): δ 78.3 (**C**$_{cage}$), 49.4 (**C**H$_2$N), 34.0 (**C**H$_2$C$_{cage}$), 30.7 (**C**H$_2$CH$_2$C$_{cage}$). ^{11}B{^{1}H} NMR (300 MHz, d_7-dmf): δ -9.15 (s, 10B). ^{195}Pt{^{1}H} NMR (300 MHz, d_7-dmf): δ -2409. Calcd for $C_{10}H_{38}B_{10}Cl_2F_6N_6O_6Pt_2S_2$: C: 11.06%, H: 3.53%, N: 7.74%; Found: C: 10.50%, H: 4.35%, N: 8.23%.

7-(3-Iodopropyl)-*N,N*-dibenzyloxycarbonyl-1-(3-aminopropyl)-1,7-carborane (90)

To a stirred solution of tetrabutylammonium hydrogensulfate (790 mg, 2.33 mmol) and aqueous sodium hydroxide (2 M, 2.4 cm^3, 5.00 mmol) in distilled dichloromethane (50 cm^3) was added a solution of dibenzyl iminodicarboxylate (664 mg, 2.33 mmol) in distilled dichloromethane (20 cm^3). After stirring for 30 min, a solution of **68** (1.12 g, 2.32 mmol) in distilled dichloromethane (25 cm^3) was added dropwise to the mixture. The reaction was then heated to reflux for 6 h (longer reaction times results in the elimination of one of the CBZ groups). The aqueous layer was extracted with dichloromethane (2 × 20 cm^3). The combined organic extracts were reduced *in vacuo* to afford a yellow residue, which was stirred with diethyl ether (100 cm^3) for 30 min to precipitate the tetrabutylammonium iodide, which was removed by vacuum filtration. The filtrate was dried over anhydrous $NaSO_4$ and reduced *in vacuo* to afford a yellow oil that was purified by flash chromatography on silica. Elution with 10-25% diethyl ether in *n*-hexane gave **90** (660 g, R_f = 0.35 44%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (s, 10H, Ph), 5.24 (s, 4H, C**H**$_2$Ph), 3.61 (t, 2H, $^3J_{HH}$ = 7.2 Hz, C**H**$_2$N), 3.07 (t, 2H, $^3J_{HH}$ = 6.6 Hz, C**H**$_2$I), 2.04 (m, 2H, ICH$_2$CH$_2$C**H**$_2$), 1.83 (m, 4H, (CBZ)$_2$NCH$_2$CH$_2$C**H**$_2$, ICH$_2$C**H**$_2$), 1.62 (m, 2H, NCH$_2$C**H**$_2$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -7.72 (s, 2B), -11.24 (s, 6B), -13.97 (s, 2B). ^{13}C{^{1}H} NMR (300 MHz, $CDCl_3$): δ 153.3 (CO), 135.1 (Ph), 128.7 (Ph), 128.5 (Ph), 128.2 (Ph), 75.0 (C_{cage}), 74.5 (C_{cage}), 68.8 (**C**H$_2$Ph), 45.9 (CH$_2$N), 37.6 (**C**H$_2$CH$_2$CH$_2$I), 33.9 (**C**H$_2$CH$_2$CH$_2$N), 33.1 (**C**H$_2$CH$_2$I), 29.2 (**C**H$_2$CH$_2$N), 4.2 (**C**H$_2$I). Calcd for $C_{24}H_{36}B_{10}INO_4$: C: 45.21%, H: 5.69%, N: 2.20%; Found: C: 45.19%, H: 5.73%, N: 2.20%.

_N,N_-Di-*tert*-butyloxycarbonyl-7-(3-aminopropyl)-_N,N_-dibenzyloxycarbonyl-1-(3-aminopropyl)-1,7-carborane (89)

and

_N,N_-Di-*tert*-butyloxycarbonyl-7-(3-aminopropyl)-_N_-benzyloxycarbonyl-1-(3-aminopropyl)-1,7-carborane (91)

A solution of **90** (1.40 g, 2.20 mmol), potassium carbonate (666 mg, 4.82 mmol) and di-*tert*-butyl iminodicarboxylate (528 mg, 2.43 mmol) in dry distilled dmf (20 cm^3) was warmed to 60°C for 24 h. The solution was reduced to dryness *in vacuo*. The white solid was dissolved in CH_2Cl_2 (100 cm^3) and H_2O (100 cm^3) and the aqueous solution was extracted with another portion of CH_2Cl_2 (100 cm^3). The combined organic layers were washed with H_2O (100 cm^3), brine (100 cm^3), dried over $MgSO_4$ and reduced *in vacuo* to give a yellow oil. Flash chromatography on silica (10-50% diethyl ether in *n*-hexane) gave **91** (783 mg, R_f = 0.12, 60%) and **89** (270 mg, R_f = 0.23, 16%) as colourless oils. **89** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (s, 10H, Ph), 5.23 (s, 4H, C**H**$_2$Ph), 3.60 (t, 2H, $^3J_{HH}$ = 6.9 Hz, C**H**$_2$N(CBZ)$_2$), 3.47 (t, 2H, $^3J_{HH}$ = 6.9 Hz, C**H**$_2$N(BOC)$_2$), 1.86 (m, 4H, (CBZ)$_2$NCH$_2$CH$_2$C**H**$_2$, (BOC)$_2$NCH$_2$CH$_2$C**H**$_2$), 1.62 (m, 4H, (CBZ)$_2$NCH$_2$C**H**$_2$, (BOC)$_2$NCH$_2$C**H**$_2$), 1.50 (s, 18H, C(CH$_3$)$_3$. ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -7.84 (s, 2B), -11.29 (s, 6B), -13.82 (s, 2B). ^{13}C{^{1}H} NMR (300 MHz, $CDCl_3$): δ 153.3 (CO), 152.5 (CO), 135.1 (Ph), 128.7 (Ph), 128.5 (Ph), 128.2 (Ph), 82.5 (**C**(CH$_3$)$_3$), 75.3 (C_{cage}), 75.0 (C_{cage}), 68.8 (**C**H$_2$Ph), 45.9 (**C**H$_2$N(CBZ)$_2$), 45.5 (**C**H$_2$N(BOC)$_2$), 34.2 (**C**H$_2$C$_{cage}$), 34.0 (**C**H$_2$C$_{cage}$), 29.3 (NCH$_2$**C**H$_2$), 29.2 (NCH$_2$**C**H$_2$), 28.1 (C(**C**H$_3$)$_3$). Calcd for $C_{34}H_{54}B_{10}N_2O_8$: C: 56.18%, H: 7.49%, N: 3.85%; Found: C: 56.11%, H: 7.33%, N: 3.90%. **91** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H, Ph), 5.09 (s, 2H, C**H**$_2$Ph), 4.70 (br s, 1H, NH), 3.46 (t, 2H, $^3J_{HH}$ = 7.2 Hz, C**H**$_2$N(BOC)$_2$), 3.11 (q, 2H, $^3J_{HH}$ = 6.6 Hz, C**H**$_2$NH(CBZ)), 1.86 (m, 4H, (CBZ)$_2$NCH$_2$CH$_2$C**H**$_2$, (BOC)$_2$NCH$_2$CH$_2$C**H**$_2$), 1.62 (m, 4H, (CBZ)$_2$NCH$_2$C**H**$_2$, (BOC)$_2$NCH$_2$C**H**$_2$), 1.50 (s, 18H, C(CH$_3$)$_3$). ^{11}B{^{1}H} NMR (300 MHz, $CDCl_3$): δ -7.82 (s, 2B), -11.36 (s, 6B), -13.77 (s, 2B).

_N,N_-Di-*tert*-butyloxycarbonyl-7-(3-aminopropyl)-1-(3-aminopropyl)-1,7-carborane (92) (Conditions described are for **89** and are identical to **91**)

A solution of **89** (245 mg, 0.413 mmol) in distilled ethyl acetate was added to an ammonium formate solution (15 cm^3, 1.02 M in 80% aqueous acetic acid). A slurry of Pd-C (10%, 47 mg) in the ammonium formate solution (2 cm^3) was added with stirring. The solution was stirred for 15 h under a H_2 atmosphere at room temperature. The catalyst was removed by filtration through Celite. The filtrate was reduced *in vacuo* to give a colourless residue that was dissolved in ethyl acetate (100 cm^3) and H_2O (100 cm^3). The organic layer was washed with dilute $NaHCO_{3(aq)}$, dried over Na_2SO_4, and reduced *in vacuo* to give **92** as a colourless oil (150 mg, 79%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.47 (t, 2H, $^3J_{HH}$ = 6.6 Hz, C**H**$_2$N(BOC)$_2$), 2.86 (t, 2H, $^3J_{HH}$ = 7.5 Hz, C**H**$_2$NH$_2$), 2.06 (m, 2H, NH$_2$CH$_2$CH$_2$C**H**$_2$C$_{cage}$), 1.92 (m, 2H, (BOC)$_2$NCH$_2$CH$_2$C**H**$_2$C$_{cage}$), 1.75 (m, 2H, NH$_2$CH$_2$C**H**$_2$CH$_2$C$_{cage}$), 1.61 (m, 2H, (BOC)$_2$NCH$_2$C**H**$_2$CH$_2$C$_{cage}$), 1.50 (s, 18H, C(C**H**$_3$)$_3$). $^{11}B\{^1H\}$ NMR (300 MHz, $CDCl_3$): δ -7.86 (s, 2B), -11.24 (s, 6B), -13.67 (s, 2B). $^{13}C\{^1H\}$ NMR (300 MHz, $CDCl_3$): δ 152.5 (CO), 82.5 (**C**(CH$_3$)$_3$), 75.4 (C$_{cage}$), 75.0 (C$_{cage}$), 45.5 (**C**H$_2$N(BOC)$_2$), 40.4 (**C**H$_2$NH$_2$), 34.2 (**C**H$_2$CH$_2$CH$_2$N(BOC)$_2$), 34.0 (**C**H$_2$CH$_2$CH$_2$NH$_2$), 31.1 (CH$_2$**C**H$_2$CH$_2$N(BOC)$_2$), 29.3 (CH$_2$**C**H$_2$CH$_2$NH$_2$), 28.1 (C(**C**H$_3$)$_3$). Calcd for $C_{18}H_{42}B_{10}N_2O_4$: C: 47.14%, H: 9.23%, N: 6.11%; Found: C: 47.69%, H: 9.58%, N: 5.78%.

[*trans*-Diammine{1-(*N,N*-di-*tert*-butyloxycarbonyl-3-aminopropyl)-7-aminopropyl-1,7-carborane}chloroplatinum(II)] triflate (93)

To a suspension of transplatin (46.6 mg, 0.155 mmol) in dmf (1cm^3) was added a solution of silver triflate (38.4 mg, 0.149 mmol) in dmf (2 cm). The solution was stirred overnight in the absence of light. Silver chloride was removed by filtration through a pad of Celite and the filtrate was added dropwise to a solution of **92** (68.2 mg, 1.49×10^{-4} mol) in dmf (4 cm^3). After stirring for 12 h, the solvent was removed *in vacuo* to give a pale yellow residue. The residue was extracted with methanol and the supernatant was reduced *in vacuo* to give **93** as a pale yellow oil (126 mg, 97%). ^1H NMR (200 MHz, d_7-dmf): δ 5.69 (s, 2H, NH$_2$), 4.20 (s, 6H, NH$_3$), 3.52 (t, 2H, $^3J_{HH}$ = 6.9 Hz, C**H**$_2$N(BOC)$_2$), 3.22 (s, 2H, C**H**$_2$NH$_2$Pt), 2.15 (m, 2H,

CH_2C_{cage}), 2.05 (m, 2H, CH_2C_{cage}), 1.84 (s, 2H, $\mathbf{CH_2}CH_2C_{cage}$), 1.70 (m, 2H, $\mathbf{CH_2}CH_2C_{cage}$), 1.53 (s, 18H, $C(CH_3)_3$). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 153.4 (CO), 83.0 ($\mathbf{C}(CH_3)_3$), 76.9 (C_{cage}), 47.4 ($\mathbf{CH_2}NH_2Pt$), 46.1 ($\mathbf{CH_2}N(BOC)_2$), 34.7 ($C_{cage}\mathbf{CH_2}$), 34.4 ($C_{cage}\mathbf{CH_2}$), 32.2 ($C_{cage}CH_2\mathbf{CH_2}$), 30.3 ($C_{cage}CH_2\mathbf{CH_2}$), 28.3 ($C(\mathbf{CH_3})_3$). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -6.15 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2409. Calcd for $C_{19}H_{48}B_{10}ClF_3N_4O_7PtS$: C: 26.16%, H: 5.55%, N: 6.42%; Found: C: 25.77%, H: 5.03%, N: 5.66%.

cis-Ammine{1-(*N*,*N*-di-*tert*-butyloxycarbonyl-3-aminopropyl)-7-aminopropyl-1,7-carborane}chloroiodoplatinum(II) (94)

To a solution of $K[PtCl_3(NH_3)]$ (17 mg, 4.7×10^{-5} mol) in water (0.2 cm^3) was added a solution of potassium iodide (25 mg, 15 mmol) in water (0.5 cm^3). The solution was stirred for 15 minutes at room temperature and added dropwise to a solution of **92** (22 mg, 4.8×10^{-5} mol) in methanol (7 cm^3). The solution was stirred overnight at room temperature. The orange solid was collected by centrifugation, washed with methanol and diethyl ether to give **94** as a yellow solid (11 mg, 29%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 153.3 (CO), 82.9 ($\mathbf{C}(CH_3)_3$), 76.8 (C_{cage}), 47.0 ($\mathbf{CH_2}NH_2Pt$), 46.1 ($\mathbf{CH_2}N(BOC)_2$), 34.7 ($C_{cage}\mathbf{CH_2}$), 34.5 ($C_{cage}\mathbf{CH_2}$), 32.4 ($C_{cage}CH_2\mathbf{CH_2}$), 30.3 ($C_{cage}CH_2\mathbf{CH_2}$), 28.3 ($C(\mathbf{CH_3})_3$). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -7.23 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2651. Calcd for $C_{18}H_{45}B_{10}ClIN_3O_4Pt$: C: 25.95%, H: 5.44%, N: 5.04%; Found: C: 25.54%, H: 4.98%, N: 4.69%.

trans-Diammine{1,7-bis(aminopropyl)-1,7-carborane-*N*}chloroplatinum(II) hydrochloride (95)

To a solution of **93** (126 mg, 0.144 mmol) in ethyl acetate (2 cm^3) was added a homogenous mixture of HCl (10 M, 3 cm^3) and ethyl acetate (7 cm^3). The solution was stirred for 3 h at room temperature. The solvent was removed *in vacuo* to give **95** as a white solid (96 mg, 99%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 76.5 (C_{cage}), 76.1 (C_{cage}), 47.0 ($\mathbf{CH_2}NH_2Pt$), 39.1 ($\mathbf{CH_2}NH_2.HCl$), 34.0 ($C_{cage}\mathbf{CH_2}$), 33.7 ($C_{cage}\mathbf{CH_2}$), 31.5 ($\mathbf{CH_2}CH_2NH_2Pt$), 27.9 ($\mathbf{CH_2}CH_2NH_2.HCl$). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -7.28 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2408. Calcd for

$C_9H_{33}B_{10}Cl_2F_3N_4O_3PtS$: C: 15.26%, H: 4.69%, N: 7.91%; Found: C: 15.88%, H: 4.89%, N: 7.99%.

cis-Ammine{1,7-bis(aminopropyl)-1,7-carborane-_N_}chloroiodoplatinum(II) hydrochloride (96)

To a solution of **94** (11 mg, 1.34×10^{-5} mmol) in methanol (2 cm^3) was added a homogenous mixture of HCl (10 M, 1 cm^3) and ethyl acetate (3cm^3). The solution was stirred for 3 h at room temperature. The solvent was removed *in vacuo* to give **96** as a yellow solid (8 mg, 89%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 76.8 (C_{cage}), 47.0 (**C**H_2NH_2Pt), 39.1 (**C**H_2NH_2.HCl), 34.5 (C_{cage}**C**H_2), 33.7 (C_{cage}**C**H_2), 30.4 ($C_{cage}CH_2$**C**H_2), 27.9 (**C**$H_2CH_2NH_2$.HCl). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2655. Calcd for $C_8H_{30}B_{10}Cl_2IN_3Pt$: C: 14.36%, H: 4.52%, N: 6.28%; Found: C: 14.41%, H: 4.99%, N: 6.03%.

{ μ-1,7-Bis(aminopropyl)-1,7-carborane}-_N_-[_cis_-amminechloroiodo platinum(II)]-_N'_-[_trans_-diamminechloroplatinum(II)] triflate (97)

To a solution of $K[PtCl_3(NH_3)]$ (45.4 mg, 1.27×10^{-4} mol) in water (0.20 cm^3) was added a solution of potassium iodide (63 mg, 3.79×10^{-4} mol) in water (0.60 cm^3). The brown solution was stirred for 15 min at room temperature, followed by addition to a solution of **95** (89 mg, 1.25×10^{-5} mol) in dmf (5 cm^3), and potassium carbonate (17 mg, 1.23×10^{-4} mol). The mixture was stirred overnight at room temperature. The solution was reduced *in vacuo* to approx. 1 cm^3 and filtered to remove insoluble inorganic salts (KI, KCl, etc). The remaining solvent was removed *in vacuo* to give **97** as an orange solid. $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2652, -2851. Calcd for $C_9H_{35}B_{10}ClF_3I_2N_5O_3Pt_2S$: C: 9.50%, H: 3.10%, N: 6.15%; Found: C: 8.77%, H: 3.69%, N: 6.52%.

cis-Diammine-bis{1-(*N*,*N*-di-*tert*-butyloxycarbonyl-3-aminopropyl)-7-aminopropyl-1,7-carborane-*N*}platinum(II) bis(triflate) (102)

To a solution of *cis*-$[PtI_2(NH_3)_2]$ (33 mg, 6.8×10^{-5} mol) in dmf (2 cm^3) was added a solution of silver triflate (35 mg, 1.36×10^{-4} mol) in dmf (1 cm^3). The solution was stirred at room temperature for 30 min in the absence of light. Insoluble silver iodide was removed by filtration through a pad of Celite. The filtrate was added to a solution of **92** (62 mg, 1.35×10^{-4} mol) in dmf (2 cm^3). The solution was stirred overnight and the solvent was removed *in vacuo* to yield the **102** as a colourless oil (98 mg, 99%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 153.4 (CO), 82.9 (**C**$(CH_3)_3$), 76.9 (C_{cage}), 76.8 (C_{cage}), 46.8 (**C**H_2NH_2Pt), 46.1 (**C**$H_2N(BOC)_2$), 34.7 (C_{cage}**C**H_2), 34.3 (C_{cage}**C**H_2), 32.2 (**C**$H_2CH_2NH_2Pt$), 30.3 (**C**$H_2CH_2N(BOC)_2$), 28.3 (C(**C**$H_3)_3$). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2664. Calcd for $C_{38}H_{90}B_{20}F_6N_6O_{14}PtS_2$: C: 31.60%, H: 6.28%, N: 5.82%; Found: C: 31.83%, H: 6.07%, N: 5.36%.

cis-Diammine-bis{1,7-bis(aminopropyl)-1,7-carborane-*N*}platinum(II) hydrochloride bis(triflate) (103)

To solution of **102** (98 mg, 6.8×10^{-5} mol) in ethyl acetate (2 cm^3) was added a homogenous mixture of $HCl_{(aq)}$ (10 M, 0.5 cm^3) and ethyl acetate (2 cm^3). The reaction mixture was stirred for 2 h at room temperature during which time the product separated as a yellow oil. Soon after the solvent was removed by decantation, the residue solidified. The solid was dried *in vacuo* to give **103** as a pale-yellow solid (56 mg, 74%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 77.1 (C_{cage}), 76.7 (C_{cage}), 46.9 (**C**H_2NH_2Pt), 39.6 (**C**H_2NH_2.HCl), 34.5 (**C**H_2C_{cage}), 34.4 (**C**H_2C_{cage}), 32.3 (**C**$H_2CH_2NH_2Pt$), 28.6 (**C**$H_2CH_2NH_2$.HCl). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2642. Calcd for $C_{18}H_{60}B_{20}Cl_2F_6N_6O_6PtS_2$: C: 19.36%, H: 5.41%, N: 7.52%; Found: C: 19.48%, H: 5.87%, N: 7.31%.

104

A solution of potassium iodide (49 mg, 2.95×10^{-4} mol) in water (0.20 cm^3) was added to a solution of $K[Pt(NH_3)Cl_3]$ (36 mg, 1.01×10^{-4} mol) in water (0.5 cm^3). The solution was stirred for 15 min and then added to a stirred solution of **103** (56 mg,

5.01×10^{-5} mol) in dmf (5 cm^3), followed by solid K_2CO_3 (15 mg, 1.09×10^{-4} mol). After stirring for 2 h, the solvent was removed *in vacuo* to give a orange solid. The crude solid was recrystallised from dmf/0.1 M $HCl_{(aq)}$ to give **104** as an orange solid (41 mg, 46%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 77.0 (C_{cage}), 76.8 (C_{cage}), 46.8 (**C**H_2NH_2Pt), 46.0 (**C**H_2NH_2Pt), 34.6 (C_{cage}**C**H_2), 34.4 (C_{cage}**C**H_2), 32.0 (**C**$H_2CH_2NH_2$), 31.6 (**C**$H_2CH_2NH_2$). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -7.35 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2646. Calcd for $C_{18}H_{64}B_{20}Cl_2F_6I_2N_8O_6Pt_3S_2$: C: 12.06%, H: 3.60%, N: 6.25%; Found: C: 13.05%, H: 3.77%, N: 5.68%.

105

A solution of **104** (72 mg, 4.02×10^{-5} mol) in dmf (4 cm^3) was added a solution of silver triflate (41 mg, 1.60×10^{-4} mol) in dmf (1 cm^3). The solution was stirred at room temperature for 3 h in the absence of light. Insoluble AgI and AgCl were removed by filtration through a pad of Celite and the filtrate was stirred overnight with hydrochloric acid (0.3 M, 2 cm^3, 0.6 mmol). HCl (0.1 $M_{(aq)}$) was added to precipitate **105** as a yellow solid (10 mg, 15%). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 76.7 (C_{cage}), 76.5 (C_{cage}), 46.7 (**C**H_2NH_2Pt), 46.4 (**C**H_2NH_2Pt), 34.2 (**C**H_2C_{cage}), 34.1 (**C**H_2C_{cage}), 31.9 ($CH_2CH_2C_{cage}$), 31.4 ($CH_2CH_2C_{cage}$). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -2162 (s, [$PtCl_2N_2$]), -2653 (s, [PtN_4]). Calcd for $C_{18}H_{64}B_{20}Cl_4F_6N_8O_6Pt_3S_2$: C: 13.43%, H: 4.01%, N: 6.96%; Found: C: 12.96%, H: 4.36%, N: 6.25%.

μ-(1,2-bis(3-propanethiolato)-1,2-carborane)-bis(2,2':6',2"-terpyridine)platinum(II) bis(triflate) (126.2OTf)

To a solution of **129** (9.6 mg, 32.8×10^{-6} mol) in dmf (2 cm^3) was added crystalline [Pt(trpy)CH_3CN](OTf)$_2$ (50.6 mg, 65.9×10^{-6} mol). The solution immediately turned dark purple and stirring was continued for 12 h. Diethyl ether was added to precipitate **126** (40 mg, 84%) as the purple triflate salt. ^1H NMR (600 MHz, d_7-dmf): δ 9.34 (dd, 4H, $^3J_{HH}$ = 5.4 Hz, $^4J_{HH}$ = 1.8 Hz [$^3J_{PtH}$ = 40.6 Hz], H2), 8.72 (d, 4H, $^3J_{HH}$ = 8.7 Hz, H8), 8.67 (d, 4H, $^3J_{HH}$ = 8.4 Hz, H5), 8.58 (t, 2H, $^3J_{HH}$ = 7.5 Hz, H9), 8.52 (dt, 4H, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 7.8, H4), 8.04 (dt, 4H , $^4J_{HH}$ = 1.8 Hz, $^3J_{HH}$ = 6.0, H3), 2.63 (m,

8H, CH_2S + CH_2C_{cage}), 1.95 (m, 4H, CH_2CH_2S). $^{13}C\{^1H\}$ NMR (600 MHz, d_7-dmf): δ 159.8 (C6), 154.2 (C7), 152.9 (C2), 143.0 (C4), 142.8 (C9), 129.9 (C3), 126.6 (C5), 125.2 (C8), 81.7 (C_{cage}), 34.8 (C12), the two remaining signals for two carbons of the propyl chain are obscured by dmf peaks. $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -1.71 (s, 2B), -6.74 (s, 8B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -3196. Calcd for $C_{40}H_{44}B_{10}F_6N_6O_6Pt_2S_4$: C: 33.24%, H: 3.07%, N: 5.81%; Found: C: 33.30%, H: 3.10%, N: 5.78%.

μ-(1,7-bis(3-propanethiolato)-1,7-carborane)-bis(2,2':6',2"-terpyridine)platinum(II) bis(triflate) (127.2OTf)

To a solution of **130** (9.8 mg, 33.5×10^{-6} mol) in dmf (2 cm^3) was added crystalline $[Pt(trpy)CH_3CN](OTf)_2$ (51.4 mg, 67.0×10^{-6} mol). The solution immediately turned dark purple and stirring was continued for 12 h. Diethyl ether was added to precipitate **127** (44 mg, 87%) as the black triflate salt. ^1H NMR (600 MHz, d_7-dmf): δ 9.51 (s, 4H, [$^3J_{PtH}$ = 40.6], H2), 8.84 (s, 4H, H8), 8.78 (s, 4H, H5), 8.74 (s, 2H, H9), 8.62 (t, 4H, $^3J_{HH}$ = 7.2 Hz, H4), 8.10 (s, 4H, H3), 2.59 (s, 4H, CH_2S), 2.20 (s, 4H, CH_2C_{cage}), 1.77 (s, 4H, CH_2CH_2S). $^{13}C\{^1H\}$ NMR (300 MHz, d_7-dmf): δ 160.0 (C6), 154.3 (C7), 153.1 (C2), 143.2 (C4 + C9), 129.9 (C3), 126.7 (C5), 125.2 (C8), 77.2 (C_{cage}), 36.3 (C12), 36.0 (C11), 30.9 (C10). $^{11}B\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -7.25 (s, 10B). $^{195}Pt\{^1H\}$ NMR (300 MHz, d_7-dmf): δ -3205. Calcd for $C_{40}H_{44}B_{10}F_6N_6O_6Pt_2S_4$: C: 33.24%, H: 3.07%, N: 5.81%; Found: C: 33.26%, H: 2.96%, N: 5.87%.

μ-(1,12-bis(3-propanethiolato)-1,12-carborane)-bis(2,2':6',2"-terpyridine)platinum(II) bis(triflate) (128.2OTf)

To a solution of **131** (15 mg, 51.3×10^{-6} mol) in dmf (2 cm^3) was added crystalline $[Pt(trpy)CH_3CN](OTf)_2$ (79 mg, 103×10^{-6} mol). The solution immediately turned dark purple and stirring was continued for 12 h. Diethyl ether was added to precipitate a dark green solid. Further fractional recrystallisation gave **128** (45 mg, 61%) as the green triflate salt. ^1H NMR (600 MHz, d_7-dmf): δ 9.46 (d, 4H, $^3J_{HH}$ = 5.4 Hz [$^3J_{PtH}$ = 40.0 Hz], H2), 8.84 (d, 4H, $^3J_{HH}$ = 8.4 Hz, H8), 8.78 (d, 4H, $^3J_{HH}$ = 7.8 Hz, H5), 8.73 (t, 2H, $^3J_{HH}$ = 8.4, H9), 8.61 (dt, 4H, $^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 7.8 Hz, H4), 8.10 (dt, 4H,

$^4J_{HH}$ = 1.2 Hz, $^3J_{HH}$ = 5.4 Hz, H3), 2.48 (t, 4H, $^3J_{HH}$ = 6.6 Hz, C**H**$_2$S), 1.89 (m, 4H, C**H**$_2$C$_{cage}$), 1.55 (m, 4H, C**H**$_2$CH$_2$S). ^{13}C{^{1}H} NMR (300 MHz, d$_7$-dmf): δ 160.1 (C6), 154.3 (C7), 153.1 (C2), 143.0 (C4), 142.9 (C9), 129.9 (C3), 126.6 (C5), 125.2 (C8), 80.1 (C$_{cage}$), 37.2 (C12), 35.8 (C11), 30.2 (C10). ^{11}B{^{1}H} NMR (300 MHz, d$_7$-dmf): δ -9.27 (s, 10B). ^{195}Pt{^{1}H} NMR (300 MHz, d$_7$-dmf): δ -3206. Calcd for $C_{40}H_{44}B_{10}F_6N_6O_6Pt_2S_4$: C: 33.24%, H: 3.07%, N: 5.81%; Found: C: 33.17%, H: 2.98%, N: 5.77%.

128.2NO$_3$

To a solution of **131** (5.1 mg, 1.74 × 10^{-5} mol) in dmf (2 cm^3) was added [Pt(trpy)CH$_3$CN](NO$_3$)$_2$ (18.5 mg, 3.48 × 10^{-5} mol). The solution immediately turned dark purple. After 1 h the product began to precipitate out of solution. Stirring was continued for 12 h. The precipitate was collected by centrifugation and washed with methanol, diethyl ether and air dried to give **128** as the purple triflate salt (8.5 mg, 38%). ^{195}Pt{^{1}H} NMR (300 MHz, d$_7$-dmf): δ -3208.

1,2-Bis(3-sulfenylpropyl)-1,2-carborane (129)

A suspension of freshly sublimed aluminium chloride (218 mg, 1.63 mmol) in dry benzene (30 cm^3) was stirred under a N$_2$ atmosphere at 50°C for 30 min. **133** (131 mg, 0.277 mmol) was added and stirring was continued at 50°C for 24 h. The crude reaction mixture was poured through a pad of silica, followed by a portion of CH$_2$Cl$_2$ (100 cm^3). The filtrate was reduced *in vacuo* to afford a crude red oil. Purification by flash chromatography on silica (10% ethyl acetate in 2 mM HCl in *n*-hexane, R$_f$ = 0.15) gave **129** as a yellow oil (44 mg, 54%). ^{1}H NMR (CDCl$_3$): δ 2.56 (dt, 4H, $^3J_{HH}$ = 8.1, 6.6 Hz, C**H**$_2$SH), 2.31-2.36 (m, 4H, C**H**$_2$C$_{cage}$), 1.81-1.91 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$), 1.39 (t, 2H, $^3J_{HH}$ = 7.8 Hz, S**H**). ^{11}B{^{1}H} NMR (CDCl$_3$): δ -4.89 (s, 2B), -10.59 (s, 8B). ^{13}C{^{1}H} NMR (CDCl$_3$, 200 MHz): δ 75.1 (**C**$_{cage}$), 33.4 (C$_{cage}$**C**H$_2$), 33.4 (**C**H$_2$CH$_2$SH), 23.9 (**C**H$_2$SH). Mass Spec: *m/z* 292 (M$^+$), 258 (M-SH$_2^+$).

1,7-Bis(3-sulfenylpropyl)-1,7-carborane (130)

A suspension of freshly sublimed aluminium chloride (368 mg, 2.76 mmol) in dry benzene (30 cm^3) was stirred under a N_2 atmosphere at 50°C for 30 min. **132** (221 mg, 0.467 mmol) was added and stirring was continued for 24 h. The crude reaction mixture was passed through a pad of silica, followed by a portion of CH_2Cl_2 (100 cm^3). The filtrate was reduced *in vacuo* to afford crude yellow oil (203 mg). Purification by flash chromatography on silica (0-5% ethyl acetate in *n*-hexane, R_f = 0.14) gave **130** as a yellow oil (50 mg, 37%). ^{1}H NMR ($CDCl_3$): δ 2.44 (qu, 4H, $^3J_{HH}$ = 6.9 Hz, C**H**$_2$SH), 2.00-2.06 (m, 4H, C**H**$_2$C$_{cage}$), 1.61-1.71 (m, 4H, C**H**$_2$CH$_2$C$_{cage}$), 1.32 (t, 2H, $^3J_{HH}$ = 8.1 Hz, SH). ^{11}B{^{1}H} NMR ($CDCl_3$): δ -7.57 (s, 2B), -11.35 (s, 6B), -13.88 (s, 2B). ^{13}C{^{1}H} NMR ($CDCl_3$, 200 MHz): δ 75.1 (**C**$_{cage}$), 35.5 (C$_{cage}$**C**H$_2$), 33.9 (**C**H$_2$CH$_2$SH), 23.9 (**C**H$_2$SH). Mass Spec: *m/z* 292 (M^+), 258 (M-SH_2^+).

1,12-Bis(3-sulfenylpropyl)-1,12-carborane (131)

A suspension of freshly sublimed aluminium chloride (246 mg, 1.84 mmol) in dry benzene (50 cm^3) was stirred under a N_2 atmosphere at 50°C for 30 min. **134** (143 mg, 0.302 mmol) was added and stirring was continued at 50°C for 24 h. The crude reaction mixture was passed through a pad of silica, followed by a portion of CH_2Cl_2 (100 cm^3). The filtrate was reduced *in vacuo* to afford a crude colourless oil. Purification by flash chromatography on silica (2% ethyl acetate in *n*-hexane, R_f = 0.32) gave **131** as a colourless oil (44 mg, 50%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.34 (dt, 4H, $^3J_{HH}$ = 7.8, 7.0 Hz, C**H**$_2$SH), 1.68-1.74 (m, 4H, C**H**$_2$C$_{cage}$), 1.42-1.52 (m, 4H, C**H**$_2$C$_{cage}$), 1.25 (t, 2H, $^3J_{HH}$ = 7.8 Hz, S**H**). ^{11}B{^{1}H} NMR ($CDCl_3$): δ -13.09 (s, 10B). ^{13}C{^{1}H} NMR ($CDCl_3$, 300 MHz): δ 78.5 (**C**$_{cage}$), 36.3 (C$_{cage}$**C**H$_2$), 33.5 (**C**H$_2$CH$_2$SH), 23.8 (**C**H$_2$SH). Mass Spec: *m/z* 292 (M^+), 258 (M-SH_2^+).

1,7-Bis[3-(benzylsulfenyl)propyl]-1,7-carborane (132)

To a solution of sodium ethoxide (0.74 mmol) in dry ethanol (25 cm^3) was added benzyl mercaptan (116 mg, 0.93 mmol). The solution was stirred at room temperature under a N_2 atmosphere for 1 h. The solution was then added *via* canula to a solution of

68 (112 mg, 0.29 mmol) in dry ethanol (10 cm^3) and stirring was continued overnight. The reaction mixture was poured onto H_2O (50 cm^3) and CH_2Cl_2 (150 cm^3) and the aqueous solution was extracted with another portion of CH_2Cl_2 (100 cm^3). The combined organic extracts were washed with H_2O (100 cm^3), brine (50 cm^3) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a crude oil (140 mg). Purification by flash chromatography on silica (20–33% CH_2Cl_2 in *n*-hexane, R_f = 0.17-0.28) gave **132** as a colourless oil (130 mg, 95%). ^1H NMR ($CDCl_3$): δ 7.24-7.32 (m, 10H, Ph), 3.67 (s, 4H, S**C**$\mathbf{H_2}$Ph), 2.31 (t, 4H, $^3J_{HH}$ = 6.9 Hz, CH_2**C**$\mathbf{H_2}$S), 1.91-1.97 (m, 4H, **C**$\mathbf{H_2}C_{cage}$), 1.52-1.60 (m, 4H, **C**$\mathbf{H_2}CH_2C_{cage}$). $^{13}C\{^1H\}$ NMR (200 MHz, $CDCl_3$): δ 138.2 (Ph), 128.7 (Ph), 128.5 (Ph), 127.0 (Ph), 75.3 (C_{cage}), 36.3 (S**C**H_2Ph), 35.7 (C_{cage}**C**H_2), 30.5 (**C**H_2S), 29.2 (**C**$H_2CH_2C_{cage}$). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -7.75 (s, 2B), -11.44 (s, 6B), -14.01 (s, 2B). Calcd for $C_{22}H_{36}B_{10}S_2$: C: 55.89%, H: 7.67%, Found: C: 55.27%, H: 6.86%.

1,2-Bis[3-(benzylsulfenyl)propyl]-1,2-carborane (133)

To a solution of sodium ethoxide (0.914 mmol) in dry ethanol (40 cm^3) was added benzyl mercaptan (360 mg, 0.93 mmol). The solution was stirred at room temperature under N_2 atmosphere for 1 h. The solution was then added *via* canula to a solution of **69** (353 mg, 0.29 mmol) in dry ethanol (20 cm^3). Stirring was continued overnight. The reaction mixture was poured onto water (100 cm^3) and CH_2Cl_2 (100 cm^3) and the aqueous solution was extracted with another portion of CH_2Cl_2 (100 cm^3). The combined organic extracts were washed with H_2O (2 × 100 cm^3), brine (50 cm^3) and dried over anhydrous $MgSO_4$. The solution was reduced *in vacuo* to afford a crude yellow oil. Purification by flash chromatography on silica (20% CH_2Cl_2 in *n*-hexane, R_f = 0.21) gave **133** as a colourless oil (390 mg, 90%). ^1H NMR ($CDCl_3$): δ 7.22-7.34 (m, 10H, Ph), 3.68 (s, 4H, S**C**$\mathbf{H_2}$Ph), 2.40 (t, 4H, $^3J_{HH}$ = 6.9 Hz, CH_2**C**$\mathbf{H_2}$S), 2.13-2.19 (m, 4H, **C**$\mathbf{H_2}C_{cage}$), 1.69-1.77 (m, 4H, **C**$\mathbf{H_2}CH_2C_{cage}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 200 MHz): δ 138.0 (Ph), 128.8 (Ph), 128.6 (Ph), 127.2 (Ph), 79.3 (C_{cage}), 36.3 (S**C**H_2Ph), 33.7 (**C**H_2C_{cage}), 30.5 (CH_2**C**H_2S), 28.8 (**C**$H_2CH_2C_{cage}$). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -4.89 (s, 2B), -10.60 (s, 8B). Calcd for $C_{22}H_{36}B_{10}S_2$: C: 55.89%, H: 7.67%, Found: C: 55.90%, H: 7.71%.

1,12-Bis[3-(benzylsulfenyl)propyl]-1,12-carborane (134)

To a solution of sodium ethoxide (3.13 mmol) in dry ethanol (50 cm^3) was added benzyl mercaptan (481 mg, 3.87 mmol). The solution was stirred at room temperature under N_2 atmosphere for 1 h. The solution was then added *via* canula to a solution of **70** (502 mg, 1.30 mmol) in dry ethanol (50 cm^3). Stirring was continued overnight. The reaction mixture was poured onto H_2O (100 cm^3) and CH_2Cl_2 (100 cm^3) and the aqueous solution was extracted with another portion of CH_2Cl_2 (100 cm^3). The combined organic extracts were washed with H_2O (2 × 100 cm^3), brine (50 cm^3) and dried over anhydrous $MgSO_4$. The solution was reduced *in vacuo* to afford a crude yellow oil. Purification by flash chromatography on silica (20-33% CH_2Cl_2 in *n*-hexane, R_f = 0.17-0.30) gave **134** as a white solid (590 mg, 96%). Mp 65-66°C. ^1H NMR ($CDCl_3$): δ 7.23-7.33 (m, 10H, Ph), 3.63 (s, 4H, S**C**H_2Ph), 2.20 (t, 4H, $^3J_{HH}$ = 7.2 Hz, CH_2**C**H_2S), 1.58-1.67 (m, 4H, **C**H_2C_{cage}), 1.30-1.41 (m, 4H, **C**$H_2CH_2C_{cage}$). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 200 MHz): δ 138.2 (Ph), 128.8 (Ph), 128.5 (Ph), 127.0 (Ph), 78.7 (C_{cage}), 36.6 (S**C**H_2Ph), 36.2 (**C**H_2C_{cage}), 30.5 (S**C**H_2CH_2), 28.8 (**C**$H_2CH_2C_{cage}$). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -13.13 (s, 10B). Calcd for $C_{22}H_{36}B_{10}S_2$: C: 55.89%, H: 7.67%, Found: C: 55.80%, H: 7.71%.

1,7-Bis[3-(tert-butylsulfenyl)propyl]-1,7-carborane (135)

To a solution of 1,7-carborane (275 mg, 1.91 mmol) in dry THF (30 cm^3) at -78°C under N_2 atmosphere, was slowly added a solution of *n*-BuLi (3 cm^3, 2.5 M in *n*-hexane, 7.50 mmol) in THF (30 cm^3). The solution was slowly warmed to room temperature and a solution of $BrCH_2CH_2CH_2S^tBu$ (0.90 g, 4.28 mmol) in THF (20 cm^3) was added dropwise. Stirring was continued overnight at room temperature. The reaction mixture was added to $HCl_{(aq)}$ (1 M, 50 cm^3, 50 mmol) and ethyl acetate (30 cm^3). The aqueous layer was extracted with another portion of ethyl acetate (50 cm^3). The combined organic extracts were washed with H_2O (2 × 30 cm^3), brine (30 cm^3) and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to afford a yellow oily solid (900 mg). Purification by flash chromatography on silica (20% CH_2Cl_2 in *n*-hexane, R_f = 0.28) gave **135** as a white solid (428 mg, 56%). ^1H NMR ($CDCl_3$): δ 2.43 (t, 4H, $^3J_{HH}$ = 6.9 Hz, CH_2S), 2.00-2.06 (m, 4H, **C**H_2C_{cage}),

1.56-1.68 (m, 4H, $CH_2CH_2C_{cage}$), 1.30 (s, 18H, $C(CH_3)_3$). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -7.54 (s, 2B), -11.38 (s, 6B), -13.89 (s, 2B).

136

To a solution of **135** (0.143 g, 3.53×10^{-4} mol) in glacial acetic acid (10 cm^3) was added nitrobenzenesulfenyl chloride (135 mg, 7.12×10^{-4} mol). After stirring for 3 h at room temperature, the solvent was removed *in vacuo* to give a yellow residue. The residue was dissolved in CH_2Cl_2 (60 cm^3) and washed with dilute $NaHCO_{3(aq)}$ (50 cm^3), water (2 × 50 cm^3), dried over $MgSO_4$ and the solvent was removed *in vacuo* to give **136** as a yellow solid (203 mg, 97%). ^1H NMR ($CDCl_3$): δ 8.28 (dd, 2H, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.3 Hz, Ph), 8.23 (dd, 2H, $^3J_{HH}$ = 8.2 Hz, $^4J_{HH}$ = 1.3 Hz, Ph), 7.71 (dt, 2H, $^3J_{HH}$ = 1.3 Hz, $^4J_{HH}$ = 8.2 Hz, Ph), 7.38 (dt, 2H, $^4J_{HH}$ = 1.3 Hz, $^3J_{HH}$ = 8.2 Hz, Ph), 2.64 (t, 4H, $^3J_{HH}$ = 7.1 Hz, CH_2S), 2.02 (m, 4H, CH_2C_{cage}), 1.71 (m, 4H, CH_2CH_2S).

[Pt(trpy)CH_3CN]$(OTf)_2$

To a suspension or Pt(cod)I_2 (236 mg, 0.42 mmol) in acetone (3 cm^3) was added a solution of silver triflate (215 mg, 0.84 mmol) in acetone (1 cm^3). The mixture was stirred in the absence of light for 20 min at room temperature. Insoluble AgI was removed by filtration through a pad of Celite. The filtrate was added to a solution of 2,2':6',2''-terpyridine (81 mg, 0.35 mmol) in acetonitrile (1 cm^3). After stirring for 1 h at room temperature, the yellow precipitate was collected by centrifugation and recrystallised from hot acetonitrile to give [Pt(trpy)CH_3CN]$(OTf)_2$ as yellow crystals (162 mg, 61%).

[Pt(trpy)CH_3CN]$(NO_3)_2$

To a suspension or Pt(cod)I_2 (192 mg, 0.35 mmol) in acetone (2 cm^3) was added a solution of silver nitrate (116 mg, 0.68 mmol) in acetonitrile (1 cm^3). The mixture was stirred in the absence of light for 30 min at room temperature. Insoluble AgI was removed by filtration through a pad of Celite. The filtrate was added to a solution of 2,2':6',2''-terpyridine (64 mg, 0.27 mmol) in acetonitrile (2 cm^3). After stirring for

1 h at room temperature, the yellow precipitate was collected by filtration (130 mg, 71%).

5.4. DNA-Binding Electrophoresis Experiments

5.4.1. General

Gel electrophoresis was performed on a Sub Cell® GT electrophoresis cell connected to a Power Pac 300 power supply. Agarose gels were illuminated from below with an UV-light (302 nm) and photographed with a CCD camera in conjunction with the Bio-Rad Gel-Doc® 1000 system. Molecular Analyst® software (V2.1.2., Bio-Rad) was used in the densitometric scanning and analysis of digital photographs. Plasmid pBR322 DNA (250 μg/mL) was obtained from New England BioLabs and Boehringer Mannheim. *Bam* H1, *Pvu* II, *Pst* I and *Eco* R1 restriction endonucleases (10 units/μL) were obtained from Boehringer Mannheim. Spin dialysis of DNA solutions were performed in Microspin™ G-50 columns containing Sephadex G-50 (Amersham Pharmacia Biotech) using a HF 120 Capsule centrifuge (Tomy). Linear pBR322 DNA was recovered from the restriction reaction with *Pst* I using a High Pure PCR Product Purification Kit purchased from Roche Diagnostics GmbH.

Tris(hydroxy-methyl)aminomethane (Trizma base) (Sigma), agarose powder LE (Seakem, FMC) KH_2PO_4 (BDH), K_2HPO_4 (BDH), NaCl (BDH), bromophenol blue (Astral Scientific), boric acid (Sigma), Na_2H_2edta (BDH) and 3,8-diamino-5-ethyl-6-phenylphenanthridium bromide (ethidium bromide, Bio-Rad) were obtained commercially. **45** (n = 6)[201] and cisplatin[420] were synthesised according to literature procedures. The aqueous solutions were prepared using water purified by means of the Milli-Q reagent system.

5.4.2. Preparation of Form III (linearised) pBR322

A solution containing pBR322 DNA (20 μl, 1 μg/μL), water (35 μL), *Pst* I (20 μl, 10U/μl) and the corresponding supplied enzyme buffer (5× concentrate, 5 μL) was incubated at 37°C for 3 hours. The enzymatic reaction was quenched by addition of saturated EDTA solution (10 μL). Form III DNA was purified in quantitative yields

using a High Pure PCR Product Purification Kit (Roche). The concentration of the resulting DNA stock solutions were approximately 0.2 μg/μL and were stored in the freezer until required.

5.4.3. Buffer Preparation

The saline-phosphate buffer was stored as a 100× concentrate, containing NaCl (300 mM), KH_2PO_4 (21.8 mM) and K_2HPO_4 (78.2 mM). TBE (Tris borate; pH 8.3 at 22°C) buffer was stored as a 10× concentrate, containing tris(hydroxymethyl)aminomethane (0.90 M), boric acid (0.90 M) and Na_2H_2edta (22 mM).

5.4.4. Agarose Gel Preparation

A 1% (*w/v*) agarose gel was prepared by dissolving agarose powder (2 g) in TBE buffer (200 cm^3) by heating the mixture in a microwave oven for 3 min. When the solution had cooled slightly ethidium bromide solution (1 drop, 10 mg/cm^3) was added and the mixture was poured into the tray of the electrophoresis cell to give a slab of 1 cm thickness. A fine-toothed comb was placed at the cathode end of the tray. After 20 minutes, the electrophoresis tray was filled with TBE buffer (1× conc, 1.7 L), ethidium bromide (2 drops, 10 mg/cm^3) and the comb was carefully removed from the gel.

5.4.5. Concentration-Dependent Enzyme Inhibition Studies with Plasmid pBR322 DNA

Various volumes (0-6 μL) of freshly prepared platinum(II) complex solution (in 0.5% (*v/v*) dmf in H_2O) were added to solutions of Form III pBR322 DNA (4 μL) and 10× saline-phosphate buffer (2 μL). Each solution was made up to a final volume of 20 μL by addition of various amounts of H_2O and dmf (to give 0.15% (*v/v*) dmf in H_2O) and incubated at 37°C for 2 h. 5 μL of 2 M NaCl solution was then added to each solution to quench the reaction. Unbound platinum(II) complex was removed using a spin column of G50 Sephadex. The digestions were initiated by addition to the eluant of chilled H_2O (5.5 μL), appropriate enzyme buffer (2.5 μL, 10× concentrate) and *Eco*

RI restriction enzyme (1 μL, 10 units/μL). The solutions were incubated at 37°C for 2 h and the reaction was quenched by the addition of 0.5 M $Na_2H_2edta.2H_2O$ solution (6.2 μL). Following the addition of tracking dye (3 μL, 0.2% (*w/v*) bromophenol blue and 53% (*v/v*) glycerol in H_2O), 35 μL aliquilots were loaded into the agarose gel and run in TBE buffer containing ethidium bromide. The gel was run at 50 V for 6 h at room temperature and then photographed under UV light.

5.5. UV-Visible Absorption Titration Experiments

5.5.1. General

Water was purified using the Milli-Q reagent system. Bulk methanol was purified by distillation prior to use. $[Pt(HET)(trpy)]NO_3$ was synthesised according to the literature procedure.[362]

All UV-Visible absorption titration experiments were carried out in standard aqueous pH 6.8, 1× phosphate buffer. Unless otherwise specified, the solutions also contained 2% dmf and 18% methanol due to low aqueous solubility of the complex. The phosphate buffer was prepared and stored as the 10× concentrate, containing 10 mM sodium phosphate and 30 mM sodium chloride.

Calf thymus DNA (sodium salt, Type I, Sigma) was dissolved in 1× phosphate buffer (1 mg/cm^3) and purified by dialysis for 12 h. DNA concentrations were determined spectrophotometrically to be 1.13 mM using $\varepsilon^{260nm} = 1.31 \times 10^4$.[366] For the titration experiments, aliquots of DNA stock solution were dispensed using automated pipettes into a known volume (3.0 cm^3) of platinum(II) solution and reference solution (3.0 cm^3) .

5.5.2. Sample Preparation

The solutions of platinum(II)-trpy complexes for UV-Visible absorption measurements were prepared as follows: **107** (1.96 mg, 3.45 μmol) was dissolved in water (5 cm^3) to give a 691 μM solution. A volume of 1.005 cm^3 was diluted with water (10 cm^3) to give a 69.4 μM solution. 1.8 cm^3 was diluted in 10× phosphate

buffer (1cm^3), dmf (0.20 cm^3), methanol (1.8 cm^3) and water (5.2 cm^3) to generate 12.5 μM solutions in dmf/methanol/water (2:18:80) (*v/v*).

126-128 (1 mg) were dissolved in dmf (1 cm^3) to give 691.9 μM solutions. Each sample (1 cm^3) was diluted with methanol (9 cm^3) to give a 69.2 μM solution (dmf/methanol (1:9)). 1.8 cm^3 was diluted in 10× phosphate buffer (1cm^3), dmf (0.020 cm^3), methanol (0.18 cm^3) and water (7 cm^3) to generate 12.5 μM solutions in dmf/methanol/water (2:18:80) (*v/v*).

Alternatively, the **128** solution in dmf (180 μL, 691.9 μM) was diluted with dmf (20 μL) and methanol (9.8 cm^3) to give 12.5 μM solutions in dmf/methanol (2:98) (*v/v*).

5.5.3. Instrumentation

Spectrophotometric Measurements were made at 25°C with a Cary 300Bio UV-Visible spectrophotometer against an appropriate reference solution containing 1× phosphate buffer. The solutions were scanned in a quartz cell (1cm × 1cm) from 550 to 250 nm at a scan rate of 100 nm per minute.

5.6. Anti-Cancer Screening

All anti-cancer testing was performed in the Andrew Durant Drug Testing Facility at the Peter MacCallum Cancer Institute in Melbourne, Australia. **117** was synthesised by Dr. Jean A. Todd.[267,378]

5.6.1. Cell Counting (CC) Test

Cells are placed into wells of a culture plate. The drugs are dissolved and diluted to a range of concentrations. 5 μL of each drug solution is added to the wells of the plate. Six wells are used as controls: 5 μL vehicle is added to 4 wells (solvent controls) and the remaining two wells represent blank controls. The plate is then incubated at 37°C in a humidified 5% CO_2, 95% air atmosphere for 48 hr after which the cells are diluted and counted using a Sysmex particle counter.

The percent cell growth at each drug concentration is determined as the average cell number in the drug treated wells/ average cell number of the vehicle control wells×100. The results are plotted as percent cell growth against drug concentration. The IC_{50} (read from the dose response curve) is defined as the drug concentration that results in a 50% reduction in cell growth.

5.6.2. Sulforhodamine (SRB) Test

Cells are placed into the wells of two culture plates and incubated overnight at 37°C in a humidified 5% CO_2, 95% air atmosphere. One plate is then fixed with TCA (as a measure of cells present at the time of addition of drug). Drugs are dissolved to make solutions of concentrations spanning a 4-log range. 100 μL of each drug solution is then added to wells of the second plate. The plate is then incubated for a further 72 hr after which viable cells are measured using the sulforhodamine B (SRB) assay.[421,422] Cells are measured by reading the absorbance at 550 nm using an automatic plate reader. The mean absorbance for time zero growth (Tz), control growth (C) and test drug growth (Ti) is determined and the percentage growth is calculated at each drug concentration as:

$[(Ti-Tz)/(C-Tz)] \times 100$ for concentrations where $Ti \geq Tz$

$[(Ti-Tz)/Tz] \times 100$ for concentrations where $Ti < Tz$

The IC_{50} is the drug concentration that result in a 50% reduction in the net cellular protein increase in control cells following drug incubation

CHAPTER SIX

References

References

Citation convention: J Am. Chem. Soc.

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