The work described in this thesis is the candidate's own, except where otherwise noted.

Mark James Lynch

Department of Chemistry  
Faculty of Science  
The Australian National University  
Canberra, A. C. T.
ACKNOWLEDGEMENTS

I would like to take this opportunity to give my most sincere thanks to my supervisor, Dr. J. A. Broomhead, whose general guidance, ideas and encouragement throughout this work has been greatly appreciated. I also thank him for providing me with a very fulfilling project and for always being there when I needed assistance.

I also greatly indebted to Dr. M. Sterns, for not only her supervision and assistance during the crystal structure determination, but for her support, encouragement and valuable advice throughout my degree.

My thanks are extended to Prof. J. Elix, Dr. G. Salem and the rest of the Department of Chemistry for their support, good humour and for providing an ideal environment for study.

Many thanks to Dr. G. Robertson and Dr. A. Willis for allowing me access to their diffractometer, structure solution programs and for the helpful advice that they were always willing to give.

The help with the electrochemistry that Dr. G. Heath and Dr. S. Boyd have given me is greatly appreciated. For the help with the DNA work and for the generous gift of DNA I am extremely grateful to Dr. N. Dixon.

Special thanks to Dr. L. Rendina whose learned advice I have relied upon not only during this degree but right through my undergraduate degree. Good on you Lou.

For their friendship and help within the department, and for our fortnightly visits to the Workers Club for lunch I thank Wayne Jealous and Geoff Deeble. Maybe our luck will change one day.

My final and biggest thank you is extended to my Mother, Father, sister Carmel and her family for all the love, encouragement and support they have shown me for all of my life.
TABLE OF CONTENTS

CONTENTS.......................................................................................... i

ABSTRACT............................................................................................ v

ABBREVIATIONS.................................................................................... vii

Chapter 1:
APPROACHES TO THE TREATMENT OF CANCER BASED ON
COORDINATION CHEMISTRY ................................................................. 1

1.1. INTRODUCTION.................................................................................. 2

1.2. THE TREATMENT OF CANCER........................................................ 2

1.3. THE CHEMOTHERAPY OF CANCER.................................................. 3

1.4. PLATINUM ANTICANCER DRUGS.................................................. 5
   1.4.1. Clinical aspects of cisplatin....................................................... 7
   1.4.2. Disadvantages of cisplatin....................................................... 8
   1.4.3. Second generation platinum anticancer drugs....................... 9
   1.4.4. Deoxyribonucleic acid as the target of platinum drugs........... 11
   1.4.5. Dinuclear platinum complexes for use as anticancer drugs...... 15
   1.4.6. Other transition metal complexes as potential anticancer drugs 18

1.5. THE TREATMENT OF CANCER BY BORON NEUTRON CAPTURE THERAPY ................................................. 21
   1.5.1. Introduction............................................................................... 21
   1.5.2. Early clinical trials of BNCT..................................................... 22
   1.5.3. The use of the borocaptate anion in BNCT......................... 22
   1.5.4. Other compounds used in BNCT.......................................... 24
   1.5.5. Other nuclei for use in neutron capture therapy................... 27
   1.5.6. Boronated transition metal complexes as potential agents
       for BNCT.................................................................................. 29

1.6. SUMMARY.......................................................................................... 29
Chapter 2:
THE PREPARATION AND CHARACTERIZATION OF DINUCLEAR PLATINUM COMPLEXES BRIDGED BY THE 4,4'-DIPYRAZOLYMETHANE LIGAND

2.1. INTRODUCTION

2.2. RESULTS AND DISCUSSION

2.2.1. The synthesis and characterization of cis-\{PtCl_2(NH_3)_2(\mu-dpzm)\}

2.2.2. The synthesis and characterization of trans-\{PtCl_2(Me_2SO)_2(\mu-dpzm)\}

2.2.3. The synthesis and characterization of cis-\{PtCl_2(Me_2SO)_2(\mu-dpzm)\}

2.2.4. Attempted synthesis of \{Pt(mal)(Me_2SO)_2(\mu-dpzm)\}

2.2.4. The synthesis of 4,4'-dipyrazolymethane, dpzm.

2.3. FURTHER INVESTIGATIONS

Chapter 3:
THE PREPARATION AND CHARACTERIZATION OF COMPLEXES CONTAINING THE BOROCAPTATE LIGAND

3.1. INTRODUCTION

3.2. RESULTS AND DISCUSSION

3.2.1. The synthesis and characterization of [Ru(SB_{12}H_{11})(NH_3)_5] \cdot 2H_2O

3.2.2. The synthesis and characterization of [Ru(SB_{12}H_{11})(en)_2(OH_2)]

3.2.3. The synthesis and characterization of [Ru(SB_{12}H_{11})(terpy)(OH_2)_2]

3.3. CONCLUSIONS

3.4. FURTHER INVESTIGATIONS
Chapter 4:

X-RAY STRUCTURE DETERMINATION OF PENTAAAMMINE
(1-THIOLATO-closo-UNDECAHYDRODODECABORANE)
RUTHENIUM(III) DIHYDRATE, [Ru(SB_12H_11)(NH_3)_5].2H_2O

4.1. BACKGROUND

4.2. X-RAY STRUCTURE DETERMINATION OF

4.2.1 Structure solution and refinement

4.3. RESULTS AND DISCUSSION

Chapter 5:

DNA BINDING STUDIES

5.1. INTRODUCTION

5.2. RESULTS AND DISCUSSION

5.2.1. The binding to DNA of cisplatin

5.2.2. The binding to DNA of cis-\{(PtCl_2(NH_3))_2(μ-dpzm)\}

5.2.3. The binding to DNA of cis- and trans-

5.2.4. The reaction of DNA with borocaptate complexes

5.3. DNA BINDING AND IN VITRO CYTOTOXICITY STUDIES

5.4. FURTHER STUDIES

Chapter 6:

EXPERIMENTAL DETAILS

6.1. GENERAL

6.2. SYNTHETIC PROCEDURES

6.2.1. Materials and Methods

6.2.2. Preparation of [PPh_4][PtCl_3(NH_3)]

6.2.3. Preparation of 4,4'-dipyrazolylmethane, dpzm

6.2.4. Preparation of cis-\{(PtCl_2(NH_3))_2(μ-dpzm)\}

6.2.5. Preparation of trans-\{(PtCl_2(Me_2SO))_2(μ-dpzm)\}

6.2.6. Préparation of cis-\{(PtCl_2(Me_2SO))_2(μ-dpzm)\}
6.2.7. Attempted preparation of \([\text{Pt(mal)(Me}_2\text{SO)}]_2(\mu\text{-dpzm})\) ...........................................108
6.2.8. Preparation of \([\text{RuCl(NH}_3)_5]\text{Cl}_2\) ........................................................................108
6.2.9. Preparation of \([\text{Ru(SB}_{12}\text{H}_{11})(\text{NH}_3)_5]\cdot2\text{H}_2\text{O}\) ................................109
6.2.10. Preparation of \([\text{Ru(O}_3\text{SCF}_3\text{)}_3(\text{terpy})]\) .....................................................................109
6.2.11. Preparation of \([\text{Ru(SB}_{12}\text{H}_{11})(\text{terpy})(\text{OH}_2)_2]\) ........................................110
6.2.12. Preparation of trans-\([\text{Ru(SB}_{12}\text{H}_{11})(\text{en})_2(\text{OH}_2)]\) .........................................111
6.2.13. Preparation of \([\text{N(Bu)}_4]_2[\text{B}_{12}\text{H}_{11}\text{SH}]\) ......................................................111
6.2.14. The reaction of \([\text{NiCl}_2(\text{PET}_3)_2]\) with \([\text{NBu}_4][\text{B}_{12}\text{H}_{11}\text{SH}]\) .................................................................112
6.2.15. The reaction of \([\text{PtCl(terpy)}]\text{Cl} \text{with Cs}_2\text{B}_{11}\text{H}_{12}\text{SH} \) ........................................112
6.2.16. The reaction of cis-\([\text{RuCl}_2(\text{Me}_2\text{SO})_4]\) with \(\text{Cs}_2\text{B}_{12}\text{H}_{11}\text{SH}\) ..............................................................113

6.3. PLASMID DNA-BINDING EXPERIMENTS ..........................................................113

6.3.1. Materials and Methods ........................................................................113
6.3.2. Preparation of saline-phosphate buffer ..................................................114
6.3.3. Plasmid DNA-binding experiments ......................................................114

Appendix A1: IN VITRO ANTICANCER STUDIES ....................................................115
A1.1. RESULTS OF IN VITRO ANTICANCER STUDIES ........................................116
A1.2. DISCUSSION .........................................................................................116

Appendix A2: OBSERVED AND CALCULATED STRUCTURE FACTOR AMPLITUDES
FOR \([\text{Ru(SB}_{12}\text{H}_{11})(\text{NH}_3)_5]\cdot2\text{H}_2\text{O}\) .........................................................................................118

REFERENCES ........................................................................................................132
The use of metal complexes as anticancer agents is at the present time a very active area of research. This thesis describes the synthesis, characterization and DNA binding properties of two different classes of metal complexes that have potential in cancer treatment regimes. Dinuclear platinum complexes bridged by the dpzm ligand are one type discussed, while the other class involves the use of the borocaptate anion as a ligand to produce metal complexes with a high boron content for use in boron neutron capture therapy.

Chapter One presents an overview of cancer chemotherapy with particular attention paid to transition metal anticancer drugs. Topics discussed include the discovery, clinical aspects and mode of action of cisplatin as an anticancer agent. Second generation platinum drugs, which appear likely to replace cisplatin in the clinic, are included. Also discussed are novel approaches to new classes of metal chemotherapeutic agents, which may display improved properties over cisplatin and its analogues, such as dinuclear platinum complexes, and complexes with non-platinum metals. The discussion is extended to boron neutron capture therapy and the potential use transition metal complexes may have in this form of cancer treatment.

Chapter Two discusses the synthesis and characterization of the dinuclear monobridged platinum complexes, cis-[[PtCl2(NH3)]2(μ-dpzm)], trans-[[PtCl2(Me2SO)]2(μ-dpzm)] and cis-[[PtCl2(Me2SO)]2(μ-dpzm)]. An attempt to produce [[Pt(mal)(Me2SO)]2(μ-dpzm)] is also discussed but due to its lack of solubility only a very limited characterization of this complex could be made. An improved method for the synthesis of the dpzm ligand is also described.

A discussion of the known chemistry of the borocaptate anion, in relation to its potential as a ligand in transition metal complexes, is presented in Chapter Three. This chapter describes the synthesis and characterization of the first complexes to feature coordinated borocaptate; [Ru(SB12H11)(NH3)5], [Ru(SB12H11)(terpy)(OH2)2] and trans-[Ru(SB12H11)(en)2(OH2)]. The chemical properties of the borocaptate ligand, particularly the strong electron donating effects that are manifested in these complexes, is
also discussed.

The details of the X-ray crystal structure determination of \([\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{NH}_3)_5]\cdot2\text{H}_2\text{O}\) is presented in Chapter Four. The crystals of the complex are monoclinic, space group \(P2_1/c\), with unit cell parameters \(a = 8.056(1)\), \(b = 14.240(2)\), \(c = 15.172(2)\), \(\beta = 98.48^\circ\) and \(Z = 4\). The structure was solved by the Patterson heavy atom method and was refined to \(R = 0.041\), based upon 2196 reflections. The structure features discrete molecules of the complex separated from the waters of crystallization by normal Van der Waals contacts. The geometry of the complex can be described as a distorted octahedron. The distortions probably arise from the strong electron donating properties and large steric bulk of the borocaptate ligand.

Chapter Five presents the results of time-dependent DNA binding experiments conducted with the complexes described. The complex \(\text{cis-}[\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) shows a significantly faster rate of unwinding of supercoiled circular pUC9 DNA, compared to cisplatin at equivalent concentrations, with the unwinding of Form I to Form \(I_0\) DNA occurring about 2.5 times more rapidly. The complex \(\text{trans-}[\{\text{PtCl}_2(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})]\) shows a rate of unwinding of DNA that is comparable to that of cisplatin, but after the convergence of the Form I and Form II bands little separation due to rewinding is observed, indicating that this complex has a time limited effect on DNA. Only very small effects on DNA are observed with \(\text{cis-}[\{\text{PtCl}_2(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})]\) over the course of the experiment. This suggests that either competitive reactions are involved, or that possible steric constraints prevent its binding to DNA. The interaction of the borocaptate anion and of the complexes \([\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{NH}_3)_5]\) and \([\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{en})_2(\text{OH}_2)]\) with DNA is also discussed.

Although no binding occurred, these compounds did act as DNA cutting agents. The rate of this cutting, as monitored by the appearance of linear Form III DNA, was significantly slower in the complexes than for the free ligand. The relevance of these results when compared to the data obtained from \textit{in vitro} anticancer screens presented in Appendix One is also discussed.

Chapter Six gives full experimental details for the work described in this thesis.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anal. Calc.</td>
<td>analysis calculated</td>
</tr>
<tr>
<td>ATPase</td>
<td>adenosine 5'-triphosphatase</td>
</tr>
<tr>
<td>BNCT</td>
<td>boron neutron capture therapy</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl group</td>
</tr>
<tr>
<td>ca.</td>
<td>circa (about)</td>
</tr>
<tr>
<td>D</td>
<td>deuterium</td>
</tr>
<tr>
<td>dach</td>
<td>1,2-diaminocyclohexane</td>
</tr>
<tr>
<td>dien</td>
<td>N-(2-aminoethyl)-1,2-ethanediamine</td>
</tr>
<tr>
<td>dmf</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid: A, adenine; C, cytosine; G, guanine; T, thymine</td>
</tr>
<tr>
<td>dpzm</td>
<td>4,4'-dipyrazolylmethane</td>
</tr>
<tr>
<td>dtpa</td>
<td>[(carboxymethyl)imino]-bis(ethanediylNitrilo)tetracetate</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>e.s.r.</td>
<td>electron spin resonance</td>
</tr>
<tr>
<td>en</td>
<td>1,2-ethanediamine</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl group</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii (and others)</td>
</tr>
<tr>
<td>f.a.b.-m.s.</td>
<td>fast atom bombardment-mass spectrometry</td>
</tr>
<tr>
<td>g.c.-m.s.</td>
<td>gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>i.e.</td>
<td>id est (that is to say)</td>
</tr>
<tr>
<td>int.</td>
<td>intensity</td>
</tr>
<tr>
<td>IR</td>
<td>infrared: s, strong; m, medium; w, weak; br, broad; sh, shoulder</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>mal</td>
<td>malonate</td>
</tr>
<tr>
<td>Me</td>
<td>methyl group</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>min.</td>
<td>minutes</td>
</tr>
<tr>
<td>n.a.</td>
<td>not applicable</td>
</tr>
<tr>
<td>n.m.r.</td>
<td>nuclear magnetic resonance: s, singlet; d, doublet; br, broad.</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>R</td>
<td>alkyl group</td>
</tr>
<tr>
<td>r.p.m.</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>rel.</td>
<td>relative</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>solv.</td>
<td>solvent</td>
</tr>
<tr>
<td>terpy</td>
<td>2,2',5',2''-tripyridine</td>
</tr>
<tr>
<td>tmdpz</td>
<td>3,3',5,5'-tetramethyl-4,4'-dipyrazole</td>
</tr>
<tr>
<td>tmdpzm</td>
<td>3,3',5,5'-tetramethyl-4,4'-dipyrazolylmethane</td>
</tr>
<tr>
<td>tms</td>
<td>tetramethylsilane</td>
</tr>
<tr>
<td>triflic</td>
<td>trifluoromethanesulfonic</td>
</tr>
<tr>
<td>vs.</td>
<td>versus</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
</tr>
<tr>
<td>X</td>
<td>anionic group</td>
</tr>
<tr>
<td>x.r.d.</td>
<td>X-ray powder diffraction</td>
</tr>
</tbody>
</table>
Chapter One

APPROACHES TO THE TREATMENT OF CANCER BASED ON COORDINATION CHEMISTRY
Chapter One

1.1. INTRODUCTION

This chapter presents an overview of the treatment of cancer, with particular attention paid to platinum based chemotherapeutic agents and to the use of boron compounds in boron neutron capture therapy. It includes the discovery, clinical aspects and mode of action of platinum anticancer drugs, as well as the potential dinuclear platinum complexes have as anticancer treatments. As an extension, other metal complexes that have shown promise as anticancer drugs are included. The use of boron neutron capture therapy in the treatment of cancer is also discussed. This includes the rationale behind the technique, the success it has had in the treatment of brain tumours and an overview of the boron compounds that have been investigated for use in the therapy.

This thesis describes the synthesis and characterization of transition metal complexes that have a potential use in the treatment of cancer. Two distinct approaches have been investigated. The first involved mono-bridged dinuclear platinum complexes, where the bridging ligand is 4,4'-dipyrazolylmethane. The other approach was to investigate the possible use of mercaptoundeca hydro-c/oso-dodecaborate as a ligand to produce complexes with a high boron content that could be of use in boron neutron capture therapy. The DNA binding properties of these complexes, along with results from some preliminary in vitro anticancer screens are also presented.

1.2. THE TREATMENT OF CANCER

Cancer is defined as “a cellular tumour the natural course of which is fatal”\(^1\). Cancer cells exhibit the properties of invasion and metastasis, and are characterized by reversed development. More than 270 types of human cancer have been recognised and defined histologically, but the degrees of variation within a single tumour type can be infinite. The clinical spectrum of these various diseases can be likewise infinite\(^2\).

Cancer can be described as cured only when all cancerous cells have been eradicated. There are four principal forms of treatment which may accomplish this –
surgery, radiotherapy, chemotherapy and immunotherapy. Surgery and radiotherapy are particularly useful when the tumour is solid and localised. Often, at the time of diagnosis the cancer is disseminated throughout the body as microscopic foci, which are very difficult to detect and treat by these methods. Chemotherapy has the advantage, in theory at least, that it can selectively attack these foci. The problem is in obtaining the selectivity, as both healthy cells and disease cells are fundamentally the same. Fortunately there are some differences which can be exploited to gain the necessary selectivity. Cancer cells are characterized by their more rapid rates of replication, and thus they utilize biosynthetic precursors, such as amino acids, purines and pyrimidines, at an enhanced rate. This intensified uptake means that certain anticancer drugs can be accumulated preferentially in tumour cells.

Another difference, which can be used to gain selectivity, is that a tumour grows as a cellular mass without any real form of vasculature, thus it is generally undersupplied with oxygen, making it hypoxic. This lack of oxidizing potential makes the tumour resistant to the effects of radiation, but this may be exploited by certain chemotherapeutic agents which are activated under reducing conditions to give toxic effects.

Immunotherapy is still an experimental form of treatment, relying upon the stimulation of natural mechanisms to fight the disease. It is not uncommon for cancer patients to experience what is known as a spontaneous cure, whereby their own immune system fights and destroys the cancer without any external intervention. What triggers the immune system to recognise and attack cancer cells is still unknown, and much work is still necessary in order to produce an effective treatment.

All these forms of treatment are commonly used in combination to combat and cure cancer. One particular combination treatment is boron neutron capture therapy (BNCT), which will be discussed in detail later.

1.3. **THE CHEMOTHERAPY OF CANCER**

Chemotherapy involves the use of chemicals that will destroy disease without seriously harming the patient. One of the first examples of chemotherapy successfully
treating a disease dates to the Incas of Peru, who treated malaria with a tea made from cinchona bark which is a rich source of the drug quinine. Many centuries later Paul Ehrlich laid the foundations of modern chemotherapy when he discovered that the dye trypan red could inhibit the growth of the trypanosoma parasite, which causes sleeping sickness. He later introduced Salvarsan, which was successful in the treatment of syphilis. In 1935 Gerhard Domagk reported that Prontosil, the first of the sulfonamide drugs, could cure streptococcal infections. Shortly after, the development of penicillin was achieved by Chain, Florey and Gardner, thereby establishing chemotherapy as an effective means of disease management.

The use of chemicals to treat cancer was originally based on mustard gas, 1,1'-thiobis[2-chloroethane]. It was observed that individuals heavily gassed with this during World War I suffered damage to bone marrow and lymphoid tissue. Animal studies performed with the nitrogen mustards (N-methyl-2,2'-dichlorodiethylamine and similar molecules) showed that these compounds selectively destroyed lymphoid cells and trials were undertaken for the treatment of cancers of this tissue, such as lymphosarcoma and Hodgkin's disease. Initially these drugs were highly effective in treating tumours of this type, but because of their severe toxicity to bone marrow it was impossible to continue the treatment and completely cure the patient. Many derivatives have been subsequently synthesized, the most successful being cyclophosphamide, which is still used frequently in the treatment of lymphosarcoma and Hodgkin's disease, as well as breast, ovarian and lung cancers.

Drugs of this type are known as alkylating agents as they covalently bind organic groups to DNA, RNA and certain important enzymes that are necessary for cellular function. Another class of anticancer drugs are the antimetabolites. An antimetabolite is a drug that is structurally quite similar to a compound essential to the organism. These agents act by inhibiting key enzymes in metabolic pathways vital to the cell. As cancer cells have generally a higher uptake of metabolites, these drugs are selectively accumulated, to the detriment of the cell. The antimetabolites of folic acid, such as aminopterin and methotrexate, were the first compounds of this type to be used for cancer
treatment\textsuperscript{13}. Other antimetabolites are employed in many drug regimes and are selective for a variety of cancer cells.

Certain antibiotics have been found to also have antineoplastic activity. Some of the more successful antibiotic-anticancer drugs are actinomycin D\textsuperscript{14}, daunorubicin\textsuperscript{15}, doxorubicin (adriamycin)\textsuperscript{16} and mitomycin C\textsuperscript{17}. Other natural sources have provided many active agents. For example, vinblastine and vincristine, alkaloids derived from the periwinkle plant \textit{(Vinca rosea)}, are particularly useful against leukemias and lymphomas\textsuperscript{18}, while semisynthetic derivatives of podophyllotoxin derived from the May apple \textit{(Podophyllum peltatin)} have been used against solid tumors\textsuperscript{19}.

The discovery of the antineoplastic properties of certain platinum compounds by Rosenberg \textit{et al.}\textsuperscript{20} in 1969 introduced yet another class of anticancer drugs – those based on coordination chemistry.

\textbf{1.4. PLATINUM ANTICANCER DRUGS}

Rosenberg, while conducting experiments on the effects electric fields have on cell division, noted that when such a field was applied to a culture of the bacterium \textit{Escherichia coli} (\textit{E. coli}), unusual growth characteristics were observed. The cells grew up to 300 times their normal length, but failed to divide\textsuperscript{21}. Further experiments showed that the growth was not in fact due to the electric field, but could be attributed to the presence of platinum-ammine complexes, formed in an electrochemical reaction between the platinum electrodes and the growth medium used in the experiment\textsuperscript{21-23}.

The major species identified was the hexachloroplatinate(IV) anion, [PtCl\textsubscript{6}]\textsuperscript{2-}. Sequential replacement of the chloro ligands by ammonia, over the period of the experiment, gave complexes of the form \textit{cis}-[PtCl\textsubscript{6-x}(NH\textsubscript{3})\textsubscript{x}]\textsuperscript{(2-x)-}, where \(x \leq 2\). Subsequent studies into the effects these complexes had on the growth of \textit{E. coli} demonstrated that [PtCl\textsubscript{5}]\textsuperscript{2-} was bacteriostatic, the amminepentachloroplatinum(IV) anion [PtCl\textsubscript{5}(NH\textsubscript{3})]\textsuperscript{-} had little effect on cell growth or division, but the uncharged \textit{cis}-diamminetetrachloroplatinum(IV) \textit{cis}-[PtCl\textsubscript{4}(NH\textsubscript{3})\textsubscript{2}] was found to be a potent inhibitor of the processes of cell division (Figure 1.1). Notably, the corresponding \textit{trans} isomer,
trans-[PtCl₄(NH₃)₂], was shown to be ineffective in inhibiting cell division, and thus it was concluded that cis-[PtCl₄(NH₃)₂] was the species responsible for the unusual growth observed in the original experiments²³.

The cytotoxic activity of the platinum(II) analogues cis-[PtCl₂(NH₃)₂] and trans-[PtCl₂(NH₃)₂] was examined in vitro. Again, the cis isomer was found to be active, whereas the trans isomer was essentially inactive. Rosenberg next took the intuitive step of examining the anticancer properties of platinum complexes on the premise that the inhibition of cell division would be an advantage in halting the progress of cancer cells²⁰. It was found that the complexes cis-[PtCl₄(NH₃)₂], cis-[PtCl₂(NH₃)₂], [PtCl₄(en)] and [PtCl₂(en)] (en = 1,2-ethanediamine, Figure 1.1) were highly active against Sarcoma 180 and L1210 leukemia in mice with cis-[PtCl₂(NH₃)₂] displaying the best activity. The corresponding trans–diammine isomers were once again found to be inactive.

Further animal studies conducted with cis-[PtCl₂(NH₃)₂], or cisplatin as it is now commonly known, demonstrated that it has a broad spectrum of activity against slowly or rapidly growing solid, disseminated or ascitic tumours²⁴. When compared with established and clinically available organic drugs in the National Cancer Institute (NCI) murine tumour panel, cisplatin was found to have comparable or better activity (Table 1.1)²⁵.
Table 1.1. Comparison of cisplatin\textsuperscript{25} with other drugs in the NCI murine tumour panel.\textsuperscript{†}

<table>
<thead>
<tr>
<th>Drug</th>
<th>Substantial Activity</th>
<th>Minimal Activity</th>
<th>No Activity</th>
<th>Erratic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>L1210 B16 CD8F\textsubscript{1}</td>
<td>LL Colon 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>L1210 CD8F\textsubscript{1} Colon 38</td>
<td>B16</td>
<td>LL</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>L1210 B16 CD8F\textsubscript{1}</td>
<td>Colon 38</td>
<td>LL</td>
<td></td>
</tr>
<tr>
<td>Bis(chloroethylnitrosourea)</td>
<td>L1210 CD8F\textsubscript{1}</td>
<td>B16 LL Colon 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methotrexate</td>
<td>L1210</td>
<td></td>
<td>B16 CD8F\textsubscript{1}</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LL Colon 38</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{†} L1210 = L1210 lymphoid leukemia, B16 = B16 melanotic melanoma, CD8F\textsubscript{1} = CD8F\textsubscript{1} mammary carcinoma, LL = Lewis lung carcinoma and Colon 38 = Colon 38 carcinoma.

1.4.1. Clinical aspects of cisplatin

With the completion of successful animal trials, the next step was to investigate the applicability of cisplatin to human patients. Clinical trials were started in 1971 under sponsorship from the National Cancer Institute\textsuperscript{26}. During these early stages the complex showed little or no activity against common tumours and its use was associated with severe toxicity to the patient. Cisplatin was virtually abandoned as a drug but incidental testing in patients afflicted with testicular cancer produced favourable results. Subsequently, the drug was advanced as a treatment for this cancer in combination with other drugs, with the results obtained demonstrating its excellent activity\textsuperscript{27}. Clinical trials of cisplatin were completed in 1978. It was soon approved for use in a number of countries, including Australia, where it is now a mainstay for the curative treatment of
advanced testicular cancer when used in combination with other drugs, such as vinblastine and doxorubicin. Its effectiveness against this form of cancer can be seen in the 70% of patients who are apparently cured and the 90% who are in long term remission after this form of treatment. The drug is also used to treat other cancers of the genitourinary region, notably bladder and ovarian, as well as those of the head and neck.

1.4.2. **Disadvantages of cisplatin**

Despite its success, cisplatin has a number of drawbacks. Testicular cancer accounts for less than 1% of all malignancies encountered, thus its actual spectrum of activity is limited. Dose limiting toxic side effects are also a problem associated with the use of cisplatin. It is nephrotoxic and neurotoxic, although its nephrotoxicity can be largely overcome by the administration of large amounts of intravenous fluids and by the introduction of diuretics such as D-mannitol and furosemide. The neurotoxic effects are manifested as demyelination and axonal degeneration of peripheral nerves when high or prolonged doses of the complex are administered. High frequency hearing loss (ototoxicity) can also occur, due to damage to the hair-cells of the organ of Corti. This occurs especially in young children and in some cases has resulted in total hearing loss. Other side-effects that have been reported include Raynauds phenomenon (a disorder causing chilblain like symptoms), impairment of sex hormone production, psycho-sexual difficulties, elevated blood pressure and elevated levels of blood cholesterol. Cisplatin also displays the toxic effects, which are common to other anticancer drugs, such as severe nausea and vomiting. Mild haematological toxicity is observed as well, but this is not as severe as is observed with other anticancer drugs or with radiation therapy.

Yet another major obstacle to the clinical utility of cisplatin is that cancer cells may develop resistance to its cytotoxic effects. Because of these limitations many thousands of platinum derivatives have been synthesized in the hope that better pharmacological properties could be obtained.
1.4.3. **Second generation platinum anticancer drugs**

Of all the platinum complexes screened over the past two decades or so, only very few have actually progressed to the stage of clinical trials. In order to justify the time and expense required for such a trial, the compound must show at least comparable activity to cisplatin and should display some improvement in either the spectrum of activity or the minimization of toxic side effects. One particular drug, carboplatin\(^{37}\) (diammine(1,1-cyclobutanedicarboxylato-\(O,O'\))platinum(II)), appears to be the successor to cisplatin for clinical use\(^{38,39}\) (Figure 1.2(a)). Although its activity is much the same as that of cisplatin, it can be given safely at a much higher dose\(^{40}\). Its nephrotoxicity is considerably lower and as a result the hydration regime used with cisplatin can be eliminated. Furthermore, it is now possible to give platinum chemotherapy in the course of a short visit to the outpatient's department. Unfortunately, cancer cells which are resistant to cisplatin appear to be similarly resistant to carboplatin, so this problem still remains unsolved. It would appear that both cisplatin and carboplatin act upon the same intracellular target\(^{30}\).

![Carboplatin](a)

![Platinum compound with the dach ligand](b)

![Platinum compound with the dach ligand](c)

![Platinum compound with the dach ligand](d)

*Figure 1.2. Second generation platinum anticancer drugs. (a) Carboplatin. (b), (c) and (d) Platinum compounds with the dach ligand, which are currently undergoing clinical trials\(^{30}\).*
Another group of analogues which has received a great deal of attention involves the replacement of the ammine groups of cisplatin with 1,2-diaminocyclohexane (dach), on the basis that platinum complexes containing this ligand are known to be effective agents against the L1210 leukemia cell line resistant to cisplatin\textsuperscript{41}. However, considerable doubt now exists as to the utility of the murine leukemias as a model of cisplatin-refractory disease, since dach compounds are frequently cross-resistant with cisplatin in other platinum resistance models; for example human ovarian carcinoma xenografts\textsuperscript{42}. A total of eleven different dach complexes have entered clinical trials in the past two decades, but most have failed early in development because of either formulation difficulties or unacceptable clinical toxicity. Clinical evidence for the activity of platinum-dach complexes against resistant cells has yet to be found. However, three dach compounds remain in clinical trials\textsuperscript{30} (Figure 1.2).

Both cisplatin and carboplatin are administered intravenously. The compliance and quality of life of patients receiving platinum chemotherapy could be enhanced if the treatment could be administered orally. The Institute of Cancer Research, along with Johnson Matthey and Bristol-Myers Squibb Oncology are currently developing a cisplatin analogue which can be absorbed by the gastrointestinal tract. One of the more promising of these agents is the platinum(IV) complex \textit{cis,trans,cis}–\{PtCl\textsubscript{2}(O\textsubscript{2}CPr\textsubscript{2})(NH\textsubscript{2}C\textsubscript{6}H\textsubscript{11})\}–(NH\textsubscript{3})\}, or as it is generically known JM 216 (Figure 1.3), which has recently entered clinical trials\textsuperscript{30}. Animal studies, involving a panel of four human ovarian carcinoma xenografts in rodents, have shown that after oral administration JM 216 has an activity comparable to cisplatin and carboplatin. The toxic side effects that have been observed are similar to those observed for carboplatin\textsuperscript{30}.

\textbf{Figure 1.3.} JM 216, an orally administered platinum anticancer drug currently undergoing clinical trial.
1.4.4. **Deoxyribonucleic acid as the target of platinum drugs**

Deoxyribonucleic acid (DNA) is the primary genetic material of all forms of life, with the possible exception of a few viruses. Clearly DNA is vitally important for the survival of the cell. Aberrations in the function of DNA are thought to be responsible for the changes that occur when a healthy cell becomes cancerous.

It was realized by Rosenberg that the filamentous growth of bacterial cells under the influence of cisplatin was due to the inhibition of DNA synthesis. Other fundamental functions of the cell, such as RNA and protein syntheses were unaffected. Further experiments with cultured human cells and Ehrlich ascites cells showed that at therapeutically relevant doses cisplatin caused the inhibition of DNA synthesis. It was found that this inhibition was due to a reaction with the DNA template and not to the inhibition of the important replication enzyme DNA polymerase. Measurement of the amount of platinum bound to various macromolecular fractions of cultured HeLa cells demonstrated that a far greater amount of platinum would bind to DNA than to either RNA or protein, when the molecular weights of macromolecules were considered. The amount of cisplatin that was bound to either the RNA or protein fractions could not be lethal to the cell. Additional experiments showed that mutant cell lines, deficient in DNA-excision repair mechanisms, are far more susceptible to the action of cisplatin. The complex is mutagenic toward both eukaryotic and prokaryotic cells and produces chromosomal abnormalities in eukaryotic cells. All this evidence strongly implicates DNA as the intracellular target of cisplatin.

A two hour lag time exists between intravenous injection of cisplatin into animals and the onset of its cytotoxic action. A similar lag time is observed on incubation of cisplatin with bacterial cells. This suggests that cisplatin requires metabolic activation for it to be effective, thus making it a pro-drug. Under the high chloride concentration found in blood (ca. 103 mM) the complex remains undissociated and electrically neutral. On diffusion into the cytoplasm of the cell, the complex encounters a greatly
decreased chloride concentration (ca. 4 mM). Under these conditions a number of hydrolysis products are formed by the replacement of the labile chloro ligands. The rate limiting step for the binding of cisplatin to DNA is believed to be this hydrolysis.

DNA offers a number of potential sites where a metal could coordinate. Covalent binding can occur to the phosphodiester backbone, to the sugar residues or to the purine or pyrimidine bases. A non-covalent mode that can occur is the intercalation of planar molecules between the base pairs of DNA, which is promoted by Van der Waals forces and in many cases by electrostatic interactions. It is expected that the sp²-hybridised nitrogen atoms of the purine and pyrimidine bases would be the most favourable sites for the binding of the metal. It has been found that the N-7 atom of guanine is the most preferred site for platinum(II) coordination, but the N-7 atom of adenine is also favoured. These results have been confirmed by numerous studies.

The binding of cisplatin to DNA (Figure 1.3) can occur in either a mono- or bifunctional manner. The monofunctional manner involves the coordination of one donor atom from DNA to the platinum centre. Evidence suggests that this mode is not responsible for the anticancer activity observed. The complexes \( \text{trans-[PtCl}_2(\text{NH}_3)_2] \) and \([\text{PtCl(dien)]}^+ \) (dien = N-(2-aminoethyl)-1,2-ethanediamine), for example, bind to DNA in this manner, yet are both inactive. Bifunctional binding involves the coordination of two donor atoms to adjacent sites in the square-plane of platinum(II) and it is this mode which is most likely responsible for the anticancer properties of cisplatin. Bifunctional binding can occur in a number of possible ways. These include chelation to a single base, DNA-protein crosslinking, interstrand crosslinking and intrastrand crosslinking.

The chelation of cisplatin to a single base, for example via the N-7 and O-6 atoms of guanine has been predicted by molecular modelling studies and was one of the first proposed modes. However, no experimental evidence is available to support the actual existence of this mode.
Both cisplatin and trans-[PtCl$_2$(NH$_3$)$_2$] are capable of forming DNA-protein crosslinks in vivo. It has been estimated, however, that this mode accounts for only 0.15% of the total cisplatin-DNA adducts formed in mammalian cell lines$^{55}$. As trans-[PtCl$_2$(NH$_3$)$_2$] is more capable of forming these crosslinks, it is highly unlikely that the activity of cisplatin is due to this mode of binding$^{77,78}$.

Interstrand crosslinks are formed when there is covalent binding of cisplatin to guanine N-7 donor atoms which are on opposite strands of the DNA duplex. Estimates suggest that this accounts for less than 1% of the total adducts formed in mammalian cells$^{55}$. The role, if any, interstrand crosslinking plays in the anticancer activity of cisplatin has yet to be shown$^{60}$.

The majority of adducts formed by the binding of cisplatin to DNA involve intrastrand crosslinks. These occur when cisplatin binds to adjacent adenine and guanine bases d(ApG), to two adjacent guanine bases d(GpG), or to two guanine bases separated by another nucleotide d(GpNpG) where N may be adenine, cytosine or thymine$^{60}$. 

Figure 1.3. Schematic representation of the binding modes of cisplatin to DNA. 1. Monofunctional binding. 2. DNA-protein crosslinking. 3. Interstrand crosslinking. 4. Intrastrand crosslinking d(GpG) 5. Intrastrand crosslinking d(ApG) 6. Intrastrand crosslinking d(GpNpG) where N is adenine, cytosine or thymine$^{66}$. 
Interestingly, the attachment of cisplatin to d(GpA) sequences has not been observed. Molecular mechanics modelling suggests that this is due to a highly unfavourable interaction between the ammine ligand and the O-6 atom of the 3' guanine. The predominant adduct of bifunctional cisplatin binding has been identified as cis-\([\text{Pt(NH}_3)_2(d(GpG))]\). In vitro studies suggest that this adduct accounts for about 65% of all platinum-DNA adducts found, whereas in vivo studies indicate that this figure is about 50%. It is believed that adducts involving intrastrand crosslinks are primarily responsible for the biological effects of cisplatin. Experiments have shown that only a very low level of cisplatin is required to inhibit the replication of a platinated SV40 viral genome; 50% inhibition occurs when only about four platinum atoms are bound per genome. It is highly unlikely that the low frequency adducts would be present in such systems. Another experiment has been conducted where one cis-Pt(NH$_3$)$_2$d(GpG) intrastrand crosslink is incorporated into a E. coli bacteriophage M13 genome. The viability of the genome was only 10% of normal, demonstrating that just one platinum intrastrand crosslink can be lethal to the bacteriophage.

Although cisplatin inhibits DNA replication, thereby blocking cell division, the exact mechanism by which it does this and so kills the cell remains obscure. The biochemical basis by which cisplatin is selectively toxic toward only certain cancer cells is also uncertain. It is thought that the binding of cisplatin induces changes in the tertiary structure of the DNA duplex, such as unwinding or kinking of the molecule. This can be caused by the hydrogen-bonding of the ammine ligands with either the phosphate backbone or with adjacent nucleotide bases. These structural changes may prevent the machinery of DNA synthesis from functioning. The actual stage of the cell cycle when inhibition occurs may also be important in placing the cancer cell in a non-viable state.

Cells which are deficient in DNA repair mechanisms are far more susceptible to the actions of cisplatin than are normal cells. Conversely, in cells which are resistant to cisplatin, the platinum-DNA adducts that are formed have been found to be repaired at an enhanced rate. These observations may partly explain the selectivity cisplatin has for cancer cells. If it is assumed that cancer cells are in some manner deficient in the repair
mechanisms present within normal cells, then they would indeed be more susceptible to
the effects of cisplatin.

Recently, proteins that bind to DNA structurally modified by cisplatin have been
isolated from yeast and human cells. It has been found that a strain of yeast, which is
deficient in the gene that encodes for this protein, is two to three times more resistant to
cisplatin than a normal yeast cell.

While these results have provided clues, the actual biochemical mechanisms for the
effects cisplatin has on DNA replication are still largely unknown. It should, however, be
noted that the mechanisms of replication in eukaryotic cells are not fully understood
either. Until such time as the full process of replication has been elucidated, it is likely
that the mechanism of cisplatin's action on cancer cells will remain obscure.

1.4.5. Dinuclear platinum complexes for use as anticancer
drugs

A class of platinum complexes has been recently described which appear to be
quite effective against cisplatin resistant cell lines. These complexes involve the linking of
two cisplatin-like centres with a bridging ligand that replaces one or both of the ammine
groups. The types of bridging ligands that have given complexes, which have shown
promising anticancer results, involve diaminoalkanes, bis(dimethylaminomethyl)–
ferrocene and 4,4'-dipyrazolylmethane.

A series of diaminoalkane bridged diplatinum complexes (Figure 1.3) have been
described and their solution chemistry, nucleotide binding, DNA binding, antitumour
activity and cytotoxicity has been explored (Figure 1.4). The cis/cis isomers have shown in vitro activity against L1210 leukemia cell lines that is comparable
to cisplatin, with the 1,4-diaminobutane (n = 4) bridged complex showing the greatest
activity. In vivo results with L1210 and P388 murine leukemia screens suggest that this
complex is also the most toxic. The 1,5-diaminopentane (n = 5) bridged complex gives
activity in these screens that is much the same as cisplatin, but with a toxicity much lower
than that of the 1,4-diaminobutane bridged analogue. As expected for this type of complex, the \textit{trans/trans} analogue does not give any improved activity over \textit{trans-}[PtCl₂(NH₃)₂], which has been noted previously as being inactive. However, the cationic \textit{trans} isomer that has just one chloro group per platinum centre does show an \textit{in vitro} toxicity that is much improved in the cisplatin resistant cell line. This is the first example of a platinum species which is at least equally as active in cell lines sensitive and resistant to cisplatin. The mixed \textit{cis/trans} analogue does show reasonable activity \textit{in vitro}, which suggests that the presence of one \textit{cis} moiety is sufficient to impart anticancer properties to dinuclear platinum complexes.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram1}
\caption{Diaminoalkane bridged dinuclear platinum complexes studied as potential anticancer drugs. The alkane linkers used have been for \(n = 4, 5\) or 6 and the analogues indicated are (a) \textit{cis/cis}, (b) \textit{trans/trans}, (c) \textit{bis(chloroplatinum) trans/trans} and (d) \textit{mixed cis/trans}.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram2}
\caption{\([\eta^5\text{-PtCl(Me}_2\text{SO)}\text{Me}_2\text{NCH}_2\text{C}_5\text{H}_3]_2\text{Fe}\). A cyclometallated diplatinum species which has shown anticancer properties}
\end{figure}
A novel diplatinum complex with a ferrocene derivative as the bridging ligand has been reported recently. This compound combines features of anticancer platinum complexes with those of metallocene derivatives that have also shown anticancer properties. The water-soluble complex that has been prepared (Figure 1.5.) exhibits low toxicity and has significant activity against P388 tumours in mice.

Recent work has produced a series of doubly-bridged diplatinum complexes, where the bridging ligand was 4,4'-dipyrazolylmethane (dpzm) (Figure 1.6). It has been found that the Pt(II)—Pt(II) analogue possesses significant in vivo activity against the P388 leukemia cell line, when the complex was administered in Me₂SO. Mass-spectral evidence suggests that the major species was a Me₂SO adduct. The other complexes prepared were not found to be active. The major limitation of these complexes was their poor solubility in water.

Figure 1.6. Doubly-bridged diplatinum complexes, where the bridging ligand is 4,4'-dipyrazolylmethane, which have been investigated as potential anticancer drugs. (a) Pt(II)—Pt(II) cis-[PtCl₂₂(μ—dpzm)₂]. (b) Pt(IV)—Pt(IV) cis-[PtCl₄₂(μ—dpzm)₂]. (c) The mixed Pt(II)—Pt(IV) cis-[Cl₂Pt(μ—dpzm)₂PtCl₄]. (d) The N-substituted Pt(II)—Pt(II) cis-[PtCl₂₂(μ—1,1′-Me₂dpzm)₂].
The potential of these dinuclear-platinum anticancer drugs is quite encouraging. Dinuclear platinum complexes which are bridged by just one dpzm ligand have not been examined to date. One of the aims of this research has been to investigate such complexes as possible anticancer drugs. It was hoped that complexes of this type would have better solubilities, greater degrees of freedom with respect to their possible binding modes with DNA, and thus improved anticancer activity.

1.4.6. **Other transition metal complexes as potential anticancer drugs**

Numerous complexes with metals other than platinum, have been investigated as potential anticancer agents. Octahedral dinuclear rhodium(II) carboxylates\textsuperscript{103-105} (Figure 1.6(a)) have been found to be potent inhibitors of tumour growth, with the butyrate (R = CH\_2CH\_2CH\_3, L = H\_2O) and the propionate (R = CH\_2CH\_3, L = H\_2O) being the most effective\textsuperscript{106}. In the series of rhodium(III) compounds which have been examined, antitumour activity has been found in species of the octahedral type \textit{mer}-[RhX\_3L\_3] (L = NH\_3 or another monodentate amine, X = anionic ligand)\textsuperscript{107}.

Numerous ruthenium(II) and (III) complexes are characterized by antitumour activity\textsuperscript{103}. The main representatives are the ruthenium(III)–amine complexes, [RuCl\_3(NH\_3)\_3] (which is a mixture of \textit{fac} and \textit{mer} isomers), \textit{cis}-[RuCl\_2(NH\_3)\_4]Cl, [RuCl(NH\_3)\_5]Cl\_2, [Ru(OOCCH\_2CH\_3)(NH\_3)\_5](ClO\_4)\_2 and the salt [Ru(O\_4C\_2)(en)\_2][Ru(O\_4C\_2)\_2(en)]\textsuperscript{108-111}. The neutral ruthenium(II) complexes \textit{cis-} and \textit{trans-}[RuCl\_2(Me\_2SO)\_4] are also active, with the \textit{trans} isomer displaying the higher activity\textsuperscript{112-115}. Experimental evidence indicates that the mode of action of these complexes is the inhibition of DNA synthesis and that DNA itself is the target site, as has been found for the platinum anticancer drugs\textsuperscript{116}. Another ruthenium complex, the so-called “ruthenium red”, containing a trinuclear cation (Figure 1.6(b)) also exhibits good antitumour activity
against various experimental tumour systems\textsuperscript{117,118}. Its mode of action is fundamentally different to that of other cytostatic ruthenium complexes. Ruthenium red inhibits specifically the cell membrane Ca\textsuperscript{2+}-ATPase and impairs Ca\textsuperscript{2+} transport at the mitochondrial and cellular membrane\textsuperscript{119,120}. It is also selectively accumulated in tumour cells\textsuperscript{103,117}.

As mentioned previously metallocene complexes (Figure 1.7(a)) show antiproliferative activity against various experimental tumours, including Ehrlich ascites tumour, sarcoma 180, B16 melanoma, colon 38 carcinoma, Lewis Lung carcinoma and xenografted human carcinomas\textsuperscript{121-124}. Titanocene dichloride seems to be the most promising, although clinical trials have not yet commenced\textsuperscript{125}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.6}
\caption{(a) General formula of the dinuclear rhodium carboxylates investigated as antitumour agents (R = CH\textsubscript{3}, CH\textsubscript{2}CH\textsubscript{3}, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}, CH\textsubscript{2}OCH\textsubscript{3}; L = H\textsubscript{2}O) (b) The structure of the ruthenium red cation.}
\end{figure}

Other antitumour titanium complexes are the six coordinate bis(benzoylacetonato)-titanium(IV) dihalides and bis(alkoxides) (Figure 1.7(a))\textsuperscript{126-128}. The bis(ethoxide) derivative, known commonly as “budotitane” (Figure 1.7(b)), has progressed as far as clinical trials in Germany\textsuperscript{129}. 
Figure 1.7. (a) General formula for metallocene complexes which have displayed anticancer properties. \((M = Ti, V, Nb, Mo; \text{e.g. } X = Cl)\) (b) General formula of bis(benzoylacetonato)titanium complexes which have been advanced as cancer treatments.

Novel anticancer agents are described on almost a daily basis but few progress further than initial screening. There is however a great deal of scope left for the development of other transition metal based antineoplastic agents.
1.5. **THE TREATMENT OF CANCER BY BORON NEUTRON CAPTURE THERAPY**

1.5.1. **Introduction**

Boron neutron capture therapy (BNCT) is a type of cancer treatment that combines elements of both radiotherapy and chemotherapy for the destruction of tumours\(^{130-133}\). The basic strategy of this therapy is to introduce selectively a chemical species rich in \(^{10}\text{B}\) into tumour cells that are then irradiated with a stream of thermal energy neutrons. The nuclear reaction that takes place when the \(^{10}\text{B}\) nucleus captures a thermal energy neutron yields primary fission fragments that have a short range, high linear energy transfer (Scheme 1.1), and will destroy biological function of any macromolecule within their flight distance of about 10 \(\mu\text{m}\). Because of the relatively inert nature of neutron radiation to biological material, healthy cells that have not been dosed with \(^{10}\text{B}\) are unaffected, unlike standard methods of radiation treatment that invariably cause damage to healthy neighbouring tissue.

\[
^{10}\text{B} + \gamma \rightarrow \begin{cases}^{7}\text{Li} + ^{2}\text{He} + 2.79 \text{MeV} (6\%) \\ ^{7}\text{Li}^* + ^{4}\text{He} + 2.31 \text{MeV} (94\%) \\ ^{7}\text{Li} + \gamma + 0.48 \text{MeV} \end{cases}
\]

*Scheme 1.1. \(^{10}\text{B}\) neutron capture processes\(^{130}\).*

The advantages of using \(^{10}\text{B}\), rather than some other nuclide, are that it is non-radioactive, it comprises about 20% of naturally occurring boron, the pathlength of the fission products is such that they are confined to a radius approximately the same as that of a single cell, and the chemistry of boron suggests that it may be incorporated into a multitude of different chemical structures\(^{133}\).

For BNCT to be effective it has been calculated that if just 17 boron-neutron...
capture reactions occur within a single tumour cell, the cell will be destroyed. In order to attain this goal, a minimum of $2.5 \times 10^{12}$ neutrons per gram of tumour have to be delivered when the average concentration of $^{10}\text{B}$ is 25 \(\mu\text{g}\) per gram of tumour\(^{132}\). This figure would be of the order of two to five times lower if the $^{10}\text{B}$ were to be localized in the nucleus of the cell, where the most harm could be inflicted\(^{134}\).

### 1.5.2. Early clinical trials of BNCT

The basic strategy for BNCT was probably conceived in 1936, just four years after the discovery of the neutron\(^{131}\). The first clinical trials of this treatment were conducted from 1951 to 1961 at the Brookhaven National Laboratory and at the Massachusetts Institute of Technology. However, these early studies were less than encouraging, as they failed to achieve any regression in the tumours treated\(^{132}\). This was due mainly to the poor uptake by tumour cells of the $^{10}\text{B}$ compounds used in the study. This led to severe radiation induced side-effects on the central nervous and vascular systems that were due to high blood concentrations of boron compounds\(^{130}\).

Subsequent work by Hatanaka in the late 1960's established BNCT as a viable means of cancer control. His work involved the treatment of brain tumours and in particular glioblastoma multiforme (gliomas of Grades III-IV). This disease accounts for 45\% of all brain cancers and prior to the work of Hatanaka it was invariably fatal with one- and five-year survival rates of 4.6 and 0 \% respectively. Using BNCT, Hatanaka has dramatically increased these survival rates to 58 and 29 \%, as has been determined from a group of 107 patients treated between 1968 and 1990. Recent results, where the techniques involved have become more refined, suggest that the survival rate after five years could be as high as 60\%\(^{135}\).

### 1.5.3. The use of the borocaptate anion in BNCT

The boron-10 carrier used by Hatanaka was the mercaptoundecahydro-\textit{closo}-dodecaborate anion $[\text{B}_{12}\text{H}_{11}\text{SH}]^{2-}$, more commonly known as borocaptate (Figure 1.8).
This compound was first described in the early 1960's, in work detailing the derivative chemistry of the anions dodecahydro-\textit{closos}\textdash dodecaborate \([\text{Bi}_2\text{Hi}_2]^2-\) and decahydro-\textit{closos}\textdash decaborate \([\text{Bi}\text{OHi}_2]^2-\). Together with the anion \([\text{Bi}_0\text{Cl}_8(\text{SH})_2]^2-\), borocaptate was shown to be capable of achieving a significant concentration differential between gliomas and normal brain-blood in rodents. These results were the stimulus for the work conducted subsequently by Hatanaka. The actual biochemical and physiological processes by which these compounds localize in neoplasms remains unclear. It is apparent that the thiol group has a significant role in the observed activity, as there are major differences between the physiological effects of \(\text{B}_2\text{H}_{11}^2-\), which is essentially inert having a LD\(_{50}\) of 7 g/kg\(^2\), and its mercapto derivative.

Studies show that borocaptate will form disulfide linkages with various plasma proteins, but it is unclear whether this is the basis for its accumulation within malignant cells. It has been suggested that such boron containing proteins become endocytosed, possibly in a manner analogous to the incorporation of antibodies into malignant cells. Recent work has demonstrated a significantly greater accretion and diminished degradation of these proteins by cancer cells, when compared with the corresponding normal cells, although further experiments confirming this hypothesis have yet to be conducted.

It was discovered that the preparations of cesium borocaptate used in the initial studies were significantly contaminated with the oxidation products \([\text{B}_1\text{H}_1\text{SSB}_1\text{H}_1]^4-\) and \([\text{B}_1\text{H}_1\text{SS(O)}\text{B}_1\text{H}_1]^4-\). These compounds have been shown to achieve higher concentrations in tumour cells than does borocaptate. Unfortunately, these agents display an increased toxicity and so do not have any clinical value. However, knowledge of the mechanisms by which sulfur-containing polyhedral boranes achieve selective accumulation in tumour cells would be of interest for the design of other boron compounds that could have even better selectivity.
1.5.4. Other compounds used in BNCT

Melanoma has a high uptake of aromatic amino acids, which are converted into the pigment melanin. This has provided the motivation for the investigation of p-boronophenylalanine (Figure 1.8) as a boron carrier for BNCT. This compound has been used in studies involving animals\textsuperscript{141-143} and humans\textsuperscript{144,145} where it has been found to be a useful compound for the delivery of $^{10}$B to melanotic tissue. Clinically, this compound has been found to be effective in treating secondary lymph-node metastasis of melanoma by BNCT\textsuperscript{145}. It is of interest to note that only the L-form of this optically active compound shows any biological activity, which is in keeping with the premise that it is used as a biosynthetic precursor. The investigation of other boron analogues of amino acids\textsuperscript{146} has been conducted based on the observation that cancer cells, which grow more vigorously, require greater amounts of these biochemical building blocks that will be thus selectively incorporated into such cells.

Another approach for the delivery of boron compounds to melanomas is based on the fact that thiouracil is uniquely and selectively incorporated into such cancers during melanin formation. This has led to the investigation of a boronated thiouracil\textsuperscript{147,148} (Figure 1.9). Similarly, chlorpromazine has exhibited a high degree of localization in tissue that contains preformed melanin and efforts have been directed toward the production of boronated promazine derivatives\textsuperscript{149}; such as that shown in Figure 1.9.

Porphyrens and phthalocyanines have shown a significant ability to localize in a
variety of tumour types. *In vivo* studies involving a boronated porphyrin derivative, Mn(III)BOPP (Figure 1.10), have shown that it can produce boron concentrations of 65 μg / g of tumour in murine gliomas, with no toxic effects. In addition, the compound may be used as a contrast agent for magnetic resonance imaging, enabling the direct monitoring of the tumour. Little, if any, of the agent is seen in normal brain tissue, so it would appear to be an ideal candidate for further study\textsuperscript{150}.

*Figure 1.9. Agents advanced for use in BNCT. (a) 5-Dihydroxyboryl-2-thiouracil. (b) A boronated promazine derivative.*

In an attempt to produce a boron compound that can be incorporated directly into the DNA of rapidly dividing cells, the boronated nucleoside 5-dihydroxyboryl-2'-deoxyuridine has been prepared\textsuperscript{151} (Figure 1.10). *In vitro* studies done on cells grown in the presence of this compound suggest that it replaces thymidine to the extent of 5-13%, with a boron concentration reaching about 6 μg B / g cell, which is approaching the level required for a useful therapeutic action\textsuperscript{133}. A number of other nucleosides and nucleotides have been also investigated\textsuperscript{134,152-155}.

*In vitro* studies done on a carboranylaziridine derivative, MACB(Figure 1.11), have shown relatively high growth inhibition toward B16 melanoma and Hep G 2 liver cancer cells, when compared to results obtained from TIG-1-20 normal human foetal lung cells. This indicates that MACB possesses a selective toxicity toward certain cancer cells in its own right. Such selective toxicity has not been observed in any of the boron carriers reported previously. Apart from being a useful agent for BNCT there is the possibility
that this agent may serve as an anticancer drug, independent of any other form of treatment\textsuperscript{156}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{bnct_agents.png}
\caption{Agents advanced for use in BNCT. (a) Boronated porphyrin–manganese(III) complex MnBOPP. (b) 5–Dihydroxyboryl–2′–deoxyuridine.}
\end{figure}

Boronated derivatives of 2-nitroimidazole have been prepared for use as hypoxia-selective agents\textsuperscript{157,158}. One of these derivatives 1-(3′-ortho[nido]carboranyl-2′-hydroxy)propyl-2-nitroimidazole, CNI-1 (Figure 1.11), has been found to produce \textit{in vitro} toxicity toward KHTn rodent tumour cells only under anaerobic conditions\textsuperscript{157}. The magnitude of this toxicity is similar to that of misonidazole, which is used as a hypoxia-selective radiosensitizing drug. As a number of tumours are characterized by poor vasculature, and hence an oxygen deficiency, it would appear that compounds of this sort have plenty of potential.
Chapter One

Figure 1.11. Compounds advanced as agents for BNCT. (a) 1-Carboranyl-3-methylaziridino-2-propanol, MACB. (b) 1-(3'-Ortho[nido]carboranyl-2'-hydroxy)propyl-2-nitroimidazole, CNI-1.

Rather than use small molecular weight compounds, another approach attempted has been to boronate polyclonal or monoclonal antibodies that are targeted at specific cell receptors\textsuperscript{159,160}. The problem with this approach lies in achieving adequate boron levels in the antibody while still maintaining immunoreactivity. Also, the precipitation of water insoluble conjugates has been found to occur on boronation and certain toxic side-effects have been observed\textsuperscript{130,159,161}. Work currently in progress suggests that these problems may be overcome in the near future\textsuperscript{161}.

The use of liposomes and lipoproteins has been investigated as a means of delivering boron to cancer cells. Low density lipoprotein (LDL) is the major cholesterol transport protein within the human body and it is recognised by high affinity receptors on the surface membranes of cells\textsuperscript{162}. By modifying LDL to carry small molecular weight lipophilic boron molecules, it seems possible to achieve an uptake of boron that is about 100 times that of the borocaptate anion\textsuperscript{163,164}. The levels of $^{10}$B, which can be incorporated into cancer cells, necessary for BNCT can be easily achieved with this approach.

1.5.5. Other nuclei for use in neutron capture therapy

Nuclei other than $^{10}$B have been also considered for neutron capture therapy. In order to be considered, the nuclei must have a high cross section for capture of thermal neutrons (Table 1.2). Originally all potential nuclei, other than $^{10}$B, were discounted, as they were either radioactive or did not yield particles that have a high linear energy transfer. However, recent work with $^{157}$Gd, which has a capture cross section more than
50 times greater than $^{10}$B, has produced some very promising results. The process involved with neutron capture by $^{157}$Gd is associated with electron internal conversion that produces Auger electron cascade that as a consequence can cause lethal damage to tumour cells. Early experiments involved the use of GdCl$_3$ in a low ionic strength buffer that allowed ionic association of Gd$^{3+}$ with the phosphate groups of DNA. Subsequent neutron bombardment initiated a capture reaction that led to double strand cleavage of the DNA. A more effective agent for the delivery of $^{157}$Gd to DNA has been recently developed. This compound was produced by linking a bibenzimidazole, which is known to intercalate into DNA, to the chelating agent diethylenetriaminepentaacetic acid (dtpa), which acts as a carrier for gadolinium (Figure 1.12). Preliminary results show that this compound does indeed increase the rate of DNA strand breakage on irradiation with neutrons. The investigation of compounds of this type is still at a very early stage$^{165}$.

\begin{table}[H]
\centering
\caption{Thermal neutron capture cross section values of potential nuclides for neutron capture therapy.}
\begin{tabular}{lll}
\hline
Nuclide & Cross Section ($\sigma$) (barns) & Nuclide & Cross Section ($\sigma$) (barns) \\
\hline
$^3$He & 5500 & $^{155}$Gd & 58000 \\
$^6$Li & 953 & $^{157}$Gd & 240000 \\
$^{10}$B & 3837 & $^{174}$Hf & 400 \\
$^{113}$Cd & 20000 & $^{199}$Hg & 2000 \\
$^{135}$Xe$^*$ & 2720000 & $^{235}$U$^*$ & 678 \\
$^{149}$Sm & 41500 & $^{241}$Pu$^*$ & 1375 \\
$^{151}$Eu & 5900 & $^{242}$Am$^*$ & 8000 \\
\hline
\end{tabular}
\end{table}

$^*$Nuclides are radioactive
1.5.6. **Boronated transition metal complexes as potential agents for BNCT**

With the known selectivity for cancer cells displayed by a variety of transition metal complexes, it is somewhat surprising that no investigation into the use of such a compound as a tumour selective boron delivery system for use in BNCT, has been forthcoming. The complexes that could be envisaged would feature a non-labile ligand that had a high boron content and possibly a number of labile ligands that could be substituted for any of the possible sites on DNA. The borocaptate anion appears to be an ideal candidate for use as boron rich ligand. The thiol group is well known in inorganic chemistry as a good donor function, and coordination should be analogous to that of an aromatic thiol. One of the aims of the present work was to investigate the possible use of the borocaptate anion in a transition metal complex that may have an affinity for the DNA of cancer cells.

1.6. **SUMMARY**

The major aspects of the chemotherapy of cancer using coordination complexes have been discussed. These include:
Chapter One

(a) The role of chemotherapy in the treatment of cancer.

(b) The discovery of the antineoplastic activity of certain platinum complexes, notably cisplatin (cis-\([\text{PtCl}_2(\text{NH}_3)_2])\).

(c) The clinical use of cisplatin against genitourinary, head and neck cancers.

(d) The toxic side-effects associated with cisplatin use and the formation of cisplatin resistant cancers.

(e) Second generation platinum anticancer drugs, notably carboplatin, which have been found to be more effective as forms of treatment than cisplatin.

(f) Evidence that the intracellular target for platinum anticancer drugs is DNA and the possible mechanisms by which these drugs interfere with replication.

(g) Novel dinuclear platinum complexes that have displayed anticancer activity.

(h) Other transition metal complexes that have shown anticancer activity.

The treatment of cancer by boron neutron capture therapy, has also been discussed. Emphasis has been placed on:

(a) The basic strategy of this therapy.

(b) Early clinical trials of the technique.

(c) The use of the borocaptate anion in BNCT.

(d) Other compounds that have been advanced as boron delivery agents.

(e) Nuclei other than boron-10 that have been investigated for use in neutron capture therapy.

(f) The potential of using transition metal complexes as boron delivery agents.

This discussion has provided an overview of how coordination chemistry can be used to produce agents that can be employed in anticancer treatments.
Chapter Two

THE PREPARATION AND CHARACTERIZATION OF DINUCLEAR PLATINUM COMPLEXES BRIDGED BY THE 4,4'-DIPYRAZOLYL METHANE LIGAND
2.1. **INTRODUCTION**

As a ligand in metal complexes 4,4'-dipyrazolylmethane (dpzm) has received little attention, unlike the isomeric 1,1'-dipyrazolylmethane. The dpzm ligand has, however, recently attracted interest due to the anticancer properties exhibited by some of its platinum complexes\textsuperscript{101}.

Cuadro et al.\textsuperscript{166} have described the synthesis and characterization of polymeric nickel(II) and cobalt(II) complexes with dpzm and with the structurally related 3,3',5,5'-tetramethyl-4,4'-dipyrazolylmethane (tmdpz) and 3,3',5,5'-tetramethyl-4,4'-dipyrazole (tmdpz) ligands (Figure 2.1). The crystal structure of a dimeric complex of cobalt(II) with tmdpz has been reported\textsuperscript{167} and organometallic complexes of rhodium(I) with tmdpz and tmdpz have been described\textsuperscript{168}. Recently, Broomhead et al.\textsuperscript{100} have reported the synthesis and characterization of a number of dimeric and monomeric complexes of dpzm with platinum (II) and (IV). This work has been extended to dpzm complexes of copper(II), iron(II), manganese(II), chromium(II) and zinc(II), all of which appear to be polymeric in nature\textsuperscript{169}.

The dpzm ligand can bind potentially in a bidentate manner, but because of the large separation between the coordination sites and its relatively rigid nature, chelation to
one metal centre is not possible. In its complexes dpzm has been found to be exclusively bridging, except when further coordination is blocked by the formation of a hydrochloride salt in which case the coordination is monodentate\cite{100}. The bridging-bidentate mode of coordination associated with dpzm allows polymeric species to form readily, as has been observed with a number of complexes containing this ligand.

The work of Broomhead et al.\cite{100} focused on dimeric-dibridged platinum-dpzm complexes. Recent work by Farrell et al.\cite{92-94,97,98} on dimeric-monobridged platinum complexes, where the bridging ligand was an alkyldiamine, has demonstrated that this class of compounds shows substantial cytotoxicity toward cancer cell lines, particularly those that are cisplatin resistant. As an adjunct to both of these studies, this chapter presents the preparation and characterization of dimeric-monobridged platinum-dpzm complexes.

The synthesis of dpzm was first described by Trofimenko\cite{170}, who heated pyrazole and methylenebromide at 200°C in an autoclave for 3 hours. Despite the fact that only a 31% yield is reported for this preparation, no other method for synthesis has been proposed. This chapter describes a preparation of dpzm that is more applicable as a laboratory scale synthesis and does not involve the use of high-pressure apparatus.

2.2. RESULTS AND DISCUSSION

The reactions used to prepare the platinum(II)-dpzm complexes described in this chapter are outlined in Scheme 2.1. The assignment of major infrared bands and \textsuperscript{1}H n.m.r. resonances are shown in Tables 2.1 and 2.2 respectively.
Scheme 2.1. Preparative routes for the synthesis of dinuclear-monobridged platinum(II)-dpzm complexes.
**Chapter Two**

**Table 2.1. Major infrared band assignments (cm$^{-1}$) in the region 4000 -250 cm$^{-1}$ for dinuclear-monobridged platinum(II)-dpzm complexes.**

<table>
<thead>
<tr>
<th>Assignment</th>
<th>cis-[[PtCl$_2$(NH$_3$)$_2$]-($\mu$-dpzm)]</th>
<th>trans-[[PtCl$_2$(Me$_2$SO)$_2$]-($\mu$-dpzm)]</th>
<th>cis-[[PtCl$_2$(Me$_2$SO)$_2$]-($\mu$-dpzm)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>v(N-H)</td>
<td>3267, 3180, 3125</td>
<td>3225</td>
<td>3298</td>
</tr>
<tr>
<td>Aromatic v(C-H)</td>
<td>3117</td>
<td>3127</td>
<td>3123</td>
</tr>
<tr>
<td>Methylene v$_\text{as}$(C-H)</td>
<td>2974</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Methylene v$_\text{s}$(C-H)</td>
<td>2860</td>
<td>2875</td>
<td>†</td>
</tr>
<tr>
<td>Methyl v$_\text{as}$(C-H)</td>
<td>n.a.</td>
<td>3001</td>
<td>3004</td>
</tr>
<tr>
<td>Methyl v$_\text{s}$(C-H)</td>
<td>n.a.</td>
<td>2912</td>
<td>2915</td>
</tr>
<tr>
<td>v ring</td>
<td>1477, 1409, 1367</td>
<td>1476, 1367</td>
<td>1477</td>
</tr>
<tr>
<td>$\delta$(CH$_2$)</td>
<td>1437</td>
<td>1440</td>
<td>1437</td>
</tr>
<tr>
<td>Aromatic $\beta$(N-H)</td>
<td>1140</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Aromatic $\beta$(C-H)</td>
<td>1080, 1010, 1000</td>
<td>1080, 1006, 997</td>
<td>1083,1007</td>
</tr>
<tr>
<td>Ring breathing</td>
<td>975</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>$\gamma$(C-H)</td>
<td>859, 827</td>
<td>868, 833</td>
<td>869, 842</td>
</tr>
<tr>
<td>Aromatic $\gamma$(N-H)</td>
<td>747</td>
<td>749</td>
<td>749</td>
</tr>
<tr>
<td>$\gamma$ Ring</td>
<td>586</td>
<td>585</td>
<td>594</td>
</tr>
<tr>
<td>v(Pt-Cl)</td>
<td>333, 322</td>
<td>340</td>
<td>.337</td>
</tr>
<tr>
<td>$\delta$(NH$_3$)</td>
<td>1570</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>$\delta$(NH$_3$)</td>
<td>1308</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>v(Pt=N)</td>
<td>526</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>v(S=O)</td>
<td>n.a.</td>
<td>1120</td>
<td>1132</td>
</tr>
<tr>
<td>pr(CH$_3$)</td>
<td>n.a.</td>
<td>975, 933, 921</td>
<td>976, 932, 920</td>
</tr>
<tr>
<td>$\delta$$_\text{as}$(CH$_3$)</td>
<td>n.a.</td>
<td>1409</td>
<td>1405</td>
</tr>
<tr>
<td>$\delta$$_\text{s}$(CH$_3$)</td>
<td>n.a.</td>
<td>1316, 1297</td>
<td>1317, 1298</td>
</tr>
<tr>
<td>v$_\text{as}$(C-S)</td>
<td>n.a.</td>
<td>719</td>
<td>727</td>
</tr>
<tr>
<td>v$_\text{s}$(C-S)</td>
<td>n.a.</td>
<td>698</td>
<td>694</td>
</tr>
<tr>
<td>$\delta$$_\text{as}$(CSO)</td>
<td>n.a.</td>
<td>438</td>
<td>438</td>
</tr>
<tr>
<td>$\delta$$_\text{s}$(CSO)</td>
<td>n.a.</td>
<td>374</td>
<td>374</td>
</tr>
</tbody>
</table>

† These bands are obscured by those associated with the DMSO ligand.

n.a. Not applicable
Table 2.2. $^1$H n.m.r. spectral data ($\delta$, ppm) for dpzm and dpzm-platinum complexes. $^{195}$Pt coupling constants, where observed, are shown in brackets.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>dpzm</th>
<th>cis-[${\text{PtCl}_2(\text{NH}_3)}_2(\mu$-dpzm)]</th>
<th>trans-[${\text{PtCl}_2(\text{Me}_2\text{SO})}_2(\mu$-dpzm)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyrazolyl N−H</td>
<td>12.66</td>
<td>13.62</td>
<td>13.7</td>
</tr>
<tr>
<td>pyrazolyl H5</td>
<td>7.49</td>
<td>7.91</td>
<td>8.07</td>
</tr>
<tr>
<td>pyrazolyl H3</td>
<td>7.49</td>
<td>7.84</td>
<td>7.95</td>
</tr>
<tr>
<td>methylene −CH2−</td>
<td>3.71</td>
<td>3.77</td>
<td>3.86</td>
</tr>
<tr>
<td>Pt−NH$_3$</td>
<td>n.a.</td>
<td>4.43</td>
<td>n.a.</td>
</tr>
<tr>
<td>OS(CH$_3$)$_2$</td>
<td>n.a.</td>
<td>n.a.</td>
<td>3.49 (22 Hz)</td>
</tr>
</tbody>
</table>

n.a. Not applicable.

2.2.1. The synthesis and characterization of cis-[$\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu$-dpzm)]

The original approach used in the synthesis of cis-[$\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu$-dpzm)] was to react K[PtCl$_3$(NH$_3$)] with dpzm in an aqueous solution. Unfortunately this gave a product which was virtually insoluble in all solvents, including hot Me$_2$SO and hot dmf. The infrared spectrum of this product gave peaks that were very broad and poorly resolved. On the basis of these observations it was concluded that this product was polymeric in nature, and thus, of little use in the current study. It appears likely that coordinated water plays an important role in the formation of these polymers.

A more effective approach was to use the salt [PPh$_4$][PtCl$_3$(NH$_3$)] in the reaction with dpzm. This salt offered a number of synthetic advantages. It was easier to purify, especially as the preparations of the [PtCl$_3$(NH$_3$)]$^-$ anion described in the literature$^{171,172}$ were invariably contaminated with the [PtCl$_4$]$^{2−}$ anion. Fractional recrystallization of the crude [PPh$_4$][PtCl$_3$(NH$_3$)] in dmf/chloroform gave a product of high purity, free of any [PPh$_4$]$_2$[PtCl$_4$] formed as a by-product. The presence of the tetraphenylphosphonium counter-ion imparts good solubility in organic solvents, particularly methylene chloride which was found to be useful in this preparation. Due to the lack of solubility dpzm has in methylene chloride it was, however, necessary to conduct the reaction in a mixture of
methylene chloride and ethanol. The use of this solvent system prevents the formation of polymeric products, as cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) is insoluble and thus further reactions cannot occur. This solvent also prevents the formation of undesirable solvolysis products as its components are only weakly coordinating.

A cis geometry is expected for the product due to the increased trans directing influence of the chloro groups over the ammine group of the \([\text{PtCl}_3(\text{NH}_3)]^-\) anion\(^{173,174}\). The mechanism by which trans directed substitution occurs in square-planar platinum(II) complexes has been extensively discussed\(^ {175}\). It is possible to assign the geometry of the complex on the basis of the frequencies observed for the v(Pt–Cl) bands\(^ {176}\). For trans-\([\text{PtCl}_2(\text{NH}_3)_2]\) this band is seen as a single absorption at 365 cm\(^{-1}\), while for cis-\([\text{PtCl}_2(\text{NH}_3)_2]\), two absorptions are observed at 323 and 330 cm\(^{-1}\). For cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) two absorptions are present at 320 and 330 cm\(^{-1}\), which is as expected for a cis isomer. Bands associated with the dpzm, ammine and chloro ligands can also be seen in the infrared spectrum. The assignment of these bands is given in Table 2.1.

The \(^1\text{H}\) n.m.r. spectrum of cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) in D\(_7\)-dmf supports the proposed structure (Table 2.2). The resonance associated with the pyrazolyl N–H protons is seen at 13.62 ppm, which is significantly downfield from that observed for the free ligand (12.66 ppm). This shift can be attributed to a deshielding effect associated with the coordination of platinum to the neighbouring nitrogen atom. In both instances the peak is quite broad, which would be due to the electrical quadrupole moment of the \(^{14}\text{N}\) nucleus to which the proton is partially coupled. The addition of deuterium oxide to the n.m.r. solution results in the loss of this peak, indicating that this proton exchanges freely. The rate of exchange is fast enough to prevent coupling to the adjacent pyrazolyl H5 proton. The assignment of the pyrazolyl H5 and H3 protons to the resonances at 7.91 and 7.84 ppm respectively is based upon previous assignments of these protons, where the H5 proton was invariably seen further downfield than the H3 proton. The differences in the magnitudes of the \(^{195}\text{Pt}-\(^1\text{H}\) coupling constants have previously been used in the assignment of these protons\(^ {100}\). Unfortunately, in the present case it was not possible to
observe these couplings because of the close proximity of the two resonances.

The broad resonance at 4.43 ppm can be assigned to the NH$_3$ ligand, confirming the mixed ligand nature of the complex. This resonance is in the same general area as had been observed by Muir et al. in a series of complexes of the form [PtCl$_2$(NH$_3$L)], where L is a thiazole or imidazole ligand$^{177}$. The methylene resonance is observed at much the same position as it is in the free dpzm ligand.

When the $^1$H n.m.r. spectrum of the complex dissolved in D$_6$-Me$_2$SO is recorded a number of peaks are observed, which is indicative of a rapid solvolysis reaction. A $^1$H n.m.r. study performed with cisplatin in this solvent system has demonstrated that at least five different species are formed as a result of ammonia exchange reactions that are promoted by the large kinetic *trans* effect of the sulfur-bound Me$_2$SO ligand$^{173}$. For a dinuclear complex, such as *cis*-[$\{PtCl$_2$(NH$_3$)$\}_2(\mu$–dpzm)]$, the number of possible isomers that may be formed must be at least 25. Clearly, Me$_2$SO is not a suitable solvent for the characterization of complexes of this type.

### 2.2.2. The synthesis and characterization of *trans*-[$\{PtCl$_2$(Me$_2$SO)$\}_2(\mu$–dpzm)]

This complex can be conveniently synthesized from the salt K[PtCl$_3$(Me$_2$SO)] and the dpzm ligand. The reaction was performed in aqueous ethanol (1:1 ethanol/water) so as to promote the precipitation of the product before any polymeric species could form. The starting material, K[PtCl$_3$(Me$_2$SO)], is conveniently prepared from K$_2$[PtCl$_4$] and a stoichiometric amount of Me$_2$SO$^{178}$.

As expected, the substitution reaction between dpzm and K[PtCl$_3$(Me$_2$SO)] proceeded with the formation of the *trans* product. This is due to the strong *trans* directing effect exhibited by the sulfur-bound Me$_2$SO ligand, which is comparable to that of dimethyl sulfide and ethylene$^{179}$.

The infrared spectrum of *trans*-[$\{PtCl$_2$(Me$_2$SO)$\}_2(\mu$–dpzm)] is dominated by bands produced by the Me$_2$SO ligand. The S–O stretching frequency is dependent on whether Me$_2$SO is sulfur or oxygen bonded. When it is oxygen bonded, this band occurs
at a lower frequency than in the free ligand, but when it is sulfur bonded it is observed at a higher frequency\textsuperscript{180}. In the present example the S–O stretching frequency is 1120 cm\textsuperscript{-1} compared to 1055 cm\textsuperscript{-1} for uncomplexed Me\textsubscript{2}SO\textsuperscript{180}, which indicates the sulfur bonded nature of the ligand that is typical of platinum–Me\textsubscript{2}SO complexes. Unfortunately, a number of peaks due to Me\textsubscript{2}SO obscure the $\nu$(Pt–Cl) bands, which makes assignment of geometry based upon these frequencies difficult. However, chemical evidence strongly suggests that the complex would have a \textit{trans} configuration. The bright yellow colour of the complex is also indicative of a \textit{trans} arrangement\textsuperscript{178}.

The $^1$H n.m.r. spectrum of $\textit{trans}$-\{[PtCl\textsubscript{2}(Me\textsubscript{2}SO)]\textsubscript{2}(\mu–dpzm)\} dissolved in D\textsubscript{6}-Me\textsubscript{2}SO again displays a large number of peaks and is thus indicative of a rapid solvolysis reaction. When a freshly prepared sample dissolved in D\textsubscript{7}-dmf is run, the spectrum is much simpler and is similar to that obtained for $\textit{cis}$-\{[PtCl\textsubscript{2}(NH\textsubscript{3})]\textsubscript{2}(\mu–dpzm)\}. The broad peak at 13.55 ppm can be attributed to the pyrazolyl N–H resonance. The H3 and H5 protons are only separated by a shift of 0.1 ppm and this is further complicated by the formyl proton of dmf at 8.02 ppm which lies between the two peaks. As a result, the magnitudes of the $^{195}$Pt–H couplings could not be determined. The assignment of the H3 and H5 protons is again based upon the observation that the H5 resonance generally lies further downfield than the H3 resonance\textsuperscript{100}. Thus, it is proposed that the H5 resonance occurs at 8.07 ppm with the H3 at 7.95 ppm. These shifts are downfield from that observed for $\textit{cis}$-\{[PtCl\textsubscript{2}(NH\textsubscript{3})]\textsubscript{2}(\mu–dpzm)\} and probably arise from the inductive effect of the \textit{trans} Me\textsubscript{2}SO ligand.

The methylene bridge protons are seen as a sharp singlet at 3.86 ppm, which is much the same as that observed for $\textit{cis}$-\{[PtCl\textsubscript{2}(NH\textsubscript{3})]\textsubscript{2}(\mu–dpzm)\} and free dpzm. The methyl groups of the Me\textsubscript{2}SO ligand are also seen as a sharp singlet at 3.44 ppm. This indicates that free rotation about the Pt–S bond occurs with the Me\textsubscript{2}SO ligand having no preferred conformation. At 300 MHz the $^{195}$Pt–S–C–$^1$H coupling is only barely observable, but at 80 MHz this coupling is clearly visible and has been found to be 22 Hz.

When the spectrum was run 3 days later, it was clear that extensive solvolysis had
occurred. The peaks in the n.m.r. spectrum that had been assigned to the H3 and H5 protons were barely visible and peaks corresponding to free dpzm (3.71 ppm) and free Me₂SO (2.60 ppm) could be discerned.

2.2.3. The synthesis and characterization of cis-[[PtCl₂(Me₂SO)]₂(μ–dpzm)]

The preparation of cis-[[PtCl₂(Me₂SO)]₂(μ–dpzm)] has been achieved by two different methods. It has been observed previously that platinum–Me₂SO complexes of the form trans-[[PtCl₂A(Me₂SO)]], where A is an amine ligand, can be converted to the more thermodynamically stable cis isomer by heating to about 150°C¹⁸¹,¹⁸² or by dissolving the complex in Me₂SO for a number of days¹⁸³. Attempts to isomerize trans-[[PtCl₂(Me₂SO)]₂(μ–dpzm)] in the solid state by the use of elevated temperatures (~150°C) resulted in decomposition of the complex. In Me₂SO solution the isomerization was successfully completed after a period of about 3 days.

A more direct method for the synthesis of cis-[[PtCl₂(Me₂SO)]₂(μ–dpzm)] involved the substitution of one of the Me₂SO ligands of cis-[[PtCl₂(Me₂SO)]₂] with dpzm. It has long been known that one of the two Me₂SO ligands of cis-[[PtCl₂(Me₂SO)]₂] can be displaced by amine-like ligands, whereas a single Me₂SO can be replaced only with difficulty. The reaction is promoted by a mutual cis-labilizing effect, presumably due to steric rather electronic repulsions of the Me₂SO ligands, and proceeds with retention of configuration¹⁸⁴. The actual reaction of dpzm with cis-[[PtCl₂(Me₂SO)]₂] produced an off-white product that was identical to that obtained by the isomerization of trans-[[PtCl₂(Me₂SO)]₂(μ–dpzm)].

The infrared spectrum of cis-[[PtCl₂(Me₂SO)]₂(μ–dpzm)] shows a spectrum that is quite similar to that obtained for the trans isomer. The frequency due to S–O stretching mode v(S=O) in this case is observed at 1132 cm⁻¹, which is slightly higher than is observed for the trans isomer. A similar situation has been observed with the cis- and trans- isomers of [PtCl₂(py)(Me₂SO)] (py = pyridine), where the v(S=O) bands were
observed at 1142 and 1134 cm\(^{-1}\) respectively\(^{178}\). The assignment of other bands is given in Table 2.1.

The \(^1\)H n.m.r. spectrum of cis-\([\{\text{PtCl}_2(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})]\) could not be obtained in either D\(_6\)-Me\(_2\)SO or D\(_7\)-dmf because of rapid solvolysis reactions that produced a number of different species in the solutions. These solvolysis reactions would be promoted by the large \textit{trans} labilizing effect the Me\(_2\)SO ligand has on the chloro ligand. These results indicate that when dissolved in either of these solvents, this complex does not maintain structural integrity. Because of the lack of solubility \(\text{cis-}\[\{\text{PtCl}_2(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})]\) has in other solvents further analyses were not possible.

2.2.4. \textit{Attempted synthesis of }\[\{\text{Pt}(\text{mal})(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})\]

It is known that the substitution of the chloro ligands by a dicarboxylate group in a platinum drug can give a product with desirable biological features\(^{38,39}\). It was hoped that the incorporation of malonate into a dinuclear complex would give a complex with enhanced solubility in aqueous media.

\[
\text{Scheme 2.2. Preparation of } [[\text{Pt}\text{mal}(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})] \text{ from } [\text{Pt}(\text{mal})(\text{Me}_2\text{SO})_2] \text{ and dpzm.}
\]
Chapter Two

The reaction of \([\text{Pt(mal)(Me}_2\text{SO)}]_2\) with dpzm, conducted according to the general procedure described by Bitha et al.\(^{185}\), did give a product that may well have been the desired \([\{\text{Pt(mal)(Me}_2\text{SO)}\}_2(\mu-\text{dpzm})]\). Its infrared spectrum suggested the presence of the malonato, \(\text{Me}_2\text{SO}\) and dpzm ligands. The microanalytical values obtained for carbon, hydrogen and nitrogen were similar to the values expected. However, the product was found to be totally insoluble in all common solvents, including hot water, hot dmf and hot \(\text{Me}_2\text{SO}\). Even in hot aqua regia no solubility was observed! This total lack of solubility meant that any further analysis or purification was not possible.

2.2.4. The synthesis of 4,4'-dipyrazolylmethane, dpzm.

The synthesis of this compound was originally described by Trofimenko\(^{170}\) who obtained it in only 30% yield from the reaction of pyrazole and methylenebromide at 200°C under pressure. Because of the low yield and the use of pressure to achieve the reaction a more convenient method was sought.

It was found that the hydrobromide salt of 1,1'-dipyrazolylmethane, which can be prepared in high yield by a method that uses phase transfer catalysis\(^{186}\), could be converted to dpzm by heating it at 200°C for just one hour. It was necessary to use the hydrobromide salt so as to limit the volatility of the 1,1'-dipyrazolylmethane, which would otherwise sublime. Also, the presence of bromide ions probably assists in stabilizing the carbonium ion intermediate that is believed to form during the reaction\(^{102}\). The only other product from the reaction that was detected in any appreciable quantity was 4-bromopyrazole, which was identified by gas-chromatography mass-spectrometry. This by-product is probably formed by nucleophilic attack of bromide on the pyrazole groups. The overall yield of this reaction approached 60%, which included sublimation of the dpzm product to give a sample of high purity.
2.3. **FURTHER INVESTIGATIONS**

Despite some very promising biological results, the lack of solubility in water represents a major obstacle to the advancement of dpzm-bridged dinuclear platinum complexes as anticancer agents. One of the main priorities of any future research on this class of compounds would be to address this problem. Better solubility may be gained by replacement of the chloro leaving groups with ligands that have a net ionic charge, such as the phosphonocarboxylates, for example. Another approach may be to derivatize the dpzm ligand with solubilizing functions, such as hydroxy groups on the methylene bridge, in the hope that better biological properties in the resulting complexes can be obtained.
THE PREPARATION AND CHARACTERIZATION OF COMPLEXES CONTAINING THE BOROCAPTATE LIGAND
3.1. INTRODUCTION

The borocaptate anion was first described in 1964\textsuperscript{136}, but despite its usefulness as an agent in boron neutron capture therapy (BNCT) its chemistry has not been extensively explored. The original synthetic procedure for the preparation of borocaptate involved the reaction of the \textit{closo}-dodecahydrododecaborate anion ($B_{12}H_{12}^{2-}$) with hydrogen sulfide at 100°C for 4 hours in a pressure vessel. The crude product was neutralized with cesium hydroxide and this precipitated the salt $Cs_{2}B_{12}H_{11}SH$. The yield from this preparation was moderate (28%) and the product was reported to be contaminated with other boranes\textsuperscript{139}. Other synthetic procedures have been subsequently described. Tolpin \textit{et al.}\textsuperscript{139} used a synthetic route based on the reaction of $N$-methylbenzothiazole-2-thione with \textit{closo}-$B_{12}H_{11}^{2-}$. A similar method has been used by Nakagawa and Nagai\textsuperscript{187}, who used $N$-methylthiopyrrolidone as the thiolating agent (Scheme 3.1).

![Scheme 3.1. Preparative routes to the borocaptate anion. The \textit{closo}-dodecahydrododecaborane anion has been thiolated with $N$-methylbenzothiazole-2-thione\textsuperscript{139}, $N$-methylthiopyrrolidone\textsuperscript{187} or hydrogen sulfide\textsuperscript{136} to produce the borocaptate anion. (Labelling of the $B$-$H$ groups has been omitted for clarity in this and subsequent schemes).](image)

The crystal structure of cesium borocaptate has been described\textsuperscript{188}. It is composed of a regular dodecahedron with boron atoms located at the vertices. Hydrogen atoms are projected directly out from the centre of the dodecahedron, as is the thiol substituent. No
other structural studies involving the borocaptate anion have been reported.

The known chemistry of the borocaptate anion centres about the thiol group. This group is oxidised by atmospheric oxygen in aqueous solution to yield a disulfide. Subsequent oxidation in turn yields a thiosulfinate and a sulfinylsulfone\(^\text{189}\) (Scheme 3.2).

The mechanism of these reactions has been studied by electron spin resonance spectroscopy and are believed to involve the formation of superoxide adducts\(^\text{190}\). Apart from oxygen other agents have been used to oxidize the borocaptate anion and these produce similar results\(^\text{139,191}\). All the oxidation products can be converted back into the parent thiol by reduction with dithiothreitol under basic conditions\(^\text{139}\). The disulfide when dissolved in acidified organic solvents is converted to an intensely coloured free radical, with the electron apparently distributed about the two sulfur atoms. The stability of the radical is undoubtedly due to the high charge (4-) on the anion and the aromatic nature of the boron cage\(^\text{191}\).

![Scheme 3.2. The oxidation of the borocaptate anion. Oxidizing agents, [O], that have been investigated include hydrogen peroxide\(^\text{189}\), 2-chloroperbenzoic acid\(^\text{139}\), 2-iodosobenzoate\(^\text{191}\) and oxygen\(^\text{189}\). The actual product that can be isolated is dependent upon the conditions and the time allowed for the reaction.](image)

Electrophilic substitution reactions occur readily on the thiol group. The borocaptate anion reacts with trimethylsulfonium iodide to produce (B\(_{12}\)H\(_{11}\)S(CH\(_3\))\(_2\))\(^-\).
(Scheme 3.3) which is the more stable sulfonium ion\textsuperscript{136}. Disubstituted sulfonium salts are also produced when borocaptate is reacted with alkyl halides\textsuperscript{192}. These are formed via the monosubstituted thioether (Scheme 3.4). Only when a bulky alkyl halide, such as 2-iodopropane, is used can the reaction be halted at the monosubstituted thioether. The sulfonium salts produced are quite stable when compared to the corresponding alkyl sulfonium salts. They will only alkylate amines with great difficulty. Removal of an alkyl group from the sulfonium salt could only be achieved with the use of a strong base, such as tetramethylammonium hydroxide in acetonitrile, and then only with the bis(cyanoethyl) and bis(cyanomethyl) derivatives. A synthetic route for the production of thioether derivatives of borocaptate based on this reaction has been devised\textsuperscript{192}.

\[
\begin{align*}
\text{Scheme 3.3. The reaction of the borocaptate anion with the trimethylsulfonium cation.}
\end{align*}
\]

\[
\begin{align*}
\text{Scheme 3.4. The synthesis of borocaptate derived sulfonium salts via a thioether intermediate. The reaction only halts at the thioether when a sterically demanding R group is used.}
\end{align*}
\]

Thioesters of borocaptate with carboxylic acids are also remarkably stable against hydrolysis, with pseudo-first-order reaction rate constants similar to or smaller than those of carboxylic acid esters. The thioesters described\textsuperscript{192} have been prepared by the reaction of the corresponding acid chloride with the borocaptate anion(Scheme 3.5).
The apparent stability of these organo-sulfur derivatives must be attributed to the strong electron donating properties of the closo-dodecaborane cage structure. This is also borne out in the unusually high pKₐ of the thiol group that has been determined to be 13.4. Although Gabel et al.¹⁹² have attributed this to the “the strong electron-withdrawing character of the borate cage”, this cannot be the case as electron withdrawal would decrease the strength of the S–H bond and so produce a lower pKₐ. Electron withdrawal would also serve to destabilize sulfonium ions and thioesters. A species which has formally a charge of 2⁻ cannot be viewed as electron deficient, and it would appear that a significant amount of this charge resides on the sulfur atom.

Scheme 3.5. The preparation of thioester derivatives of the borocaptate anion with acid chlorides (R = CH₃, C₆H₅).

The coordination chemistry of thio ligands is extensive. There is nothing obvious to suggest that the sulfur of the borocaptate anion could not also act as a donor in metal complexes. In view of the known affinity a number of metal complexes have for DNA and the apparent need to develop boron compounds that display just this property, it is somewhat surprising that attempts to prepare borocaptate-metal complexes have not been reported previously. Additionally, there are no reports of any studies aimed at elucidating possible interactions the borocaptate anion may have with metal ions in patients receiving this drug, and thus, evidence for complex formation would be of interest.
3.2. RESULTS AND DISCUSSION

Initial attempts to produce borocaptate complexes focused on platinum(II) and nickel(II) metal centres. The starting materials with which complexation was attempted were [PtCl(terpy)]Cl and [NiCl₂(PEt₃)₂]. When mild reaction conditions were used only starting materials were returned. With more vigorous conditions, mixtures of solid materials, which could only be described as decomposition products, were obtained. When the borocaptate anion was reacted with the ruthenium(II) complex, cis-[RuCl₂(Me₂SO)₄], a number of different water soluble species, which could not be satisfactorily purified, were formed. The use of ruthenium(III) species was more successful.

3.2.1. The synthesis and characterization of [Ru(SB₁₂H₁₁)(NH₃)₅]·2H₂O

The synthesis of [Ru(SB₁₂H₁₁)(NH₃)₅] has been achieved by the reaction of [RuCl(NH₃)₅]Cl₂ with cesium borocaptate in a warm aqueous solution (Scheme 3.6). The reaction mixture quickly turned deep-blue and on standing, crystals of the product formed. The reaction probably proceeds via either the aquated species [Ru(NH₃)₅(OH₂)]³⁺ or the hydroxo species [Ru(NH₃)₅(OH)]²⁺, both of which are known to be present in a neutral aqueous solution¹⁹³. Substitution with the sulfur of borocaptate occurs with the loss of the thiol proton to give a neutral species. Because of the large pK_a of the thiol group (13.4) it is expected that the loss of the proton occurs after substitution¹⁹².

The infrared spectrum of the complex is dominated by the very intense peak due to the v(B–H) band at 2475 cm⁻¹. This peak is slightly lower in frequency than it is in the free borocaptate (2486 cm⁻¹), which may be due to a lowering of the electron density of the boron cage with subsequent weakening of the B–H bonds. Other peaks and their assignments are given in Table 3.1. The location of most of these peaks is very similar in the complex to their positions found in the spectra of the parent moieties. The exception is
the band assigned to the NH₃ rocking vibration (760 cm⁻¹) which is significantly reduced in energy from that observed for [RuCl(NH₃)₅]Cl₂ (801 cm⁻¹). This band has been used as a measure of the strength of the M–N bond. The reduction in frequency observed here suggests that there has been a weakening of the Ru–N bonds, which is probably due to the stronger electron donating effects of the borocaptate ligand over the chloro ligand.

\[
\left[ \begin{array}{c}
\text{H}_3\text{N} \\
\text{H}_3\text{N} \\
\text{Ru} \\
\text{H}_3\text{N} \\
\text{Cl} \\
\end{array} \right]^{2+} + \left[ \begin{array}{c}
\text{SH} \\
\text{B} \\
\text{H}_3\text{N} \\
\text{H}_3\text{N} \\
\end{array} \right]^{2-} \rightarrow \text{Ru}^{2+} \left[ \begin{array}{c}
\text{H}_3\text{N} \\
\text{H}_3\text{N} \\
\text{NH}_3 \\
\text{H}_3\text{N} \\
\text{S} \text{B} \\
\end{array} \right]^{-2} \\
\text{HCl} \\
\]

Scheme 3.6. The preparation of [Ru(SB₁₂H₁₁)(NH₃)₅] in aqueous solution.

The changes in the ultraviolet and visible spectrum on complex formation are quite dramatic. From a pale-orange solution of [RuCl(NH₃)₅]Cl₂ and a colourless solution of Cs₂B₁₂H₁₁SH is produced the intensely blue product, [Ru(SB₁₂H₁₁)(NH₃)₅]·2H₂O. The ultraviolet and visible spectrum of this complex was measured in Me₂SO solution and this displayed two reasonably broad bands at 588 and 440 nm. The \( \varepsilon_{\text{max}} \) values for these absorptions are 1320 and 1190 M⁻¹cm⁻¹ respectively. These relatively high values suggest that the bands are due to charge transfer. With formally a 3+ charge on the ruthenium centre and a 3- charge on the borocaptate ligand, the presence of charge transfer bands is hardly surprising and the transfer undoubtedly occurs from the ligand to the metal. With a large electron density thought to manifested on the sulfur atom, borocaptate must be viewed as a π-base ligand. Ruthenium(III) with its low-spin d⁵ arrangement of electrons has a t₂g orbital available for overlap with just such a ligand and this promotes the formation of a stable complex. The solution spectrum of [Ru(SB₁₂H₁₁)(NH₃)₅]·2H₂O in Me₂SO remains unchanged after several days, which is an indication of the high stability of the complex.
Table 3.1. Infrared bands of the complex [Ru(SB_{12}H_{11})(NH_{3})_{5}]-2H_{2}O, with comparisons to the parent compounds from which it is derived.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>[Ru(SB_{12}H_{11})(NH_{3})<em>{5}]-2H</em>{2}O</th>
<th>[RuCl(NH_{3})<em>{5}]Cl</em>{2}</th>
<th>Cs_{2}B_{12}H_{11}SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>\nu(N-H_{3})</td>
<td>3321</td>
<td>3300</td>
<td>n.a.</td>
</tr>
<tr>
<td>\nu(N-H_{3})</td>
<td>3252</td>
<td>3200</td>
<td>n.a.</td>
</tr>
<tr>
<td>\nu(B-H)</td>
<td>2475</td>
<td>n.a.</td>
<td>2486</td>
</tr>
<tr>
<td>\delta(H-N-H)</td>
<td>1609</td>
<td>1617</td>
<td>n.a.</td>
</tr>
<tr>
<td>\delta(H-N-H)</td>
<td>1294</td>
<td>1297</td>
<td>n.a.</td>
</tr>
<tr>
<td>\rho(NH_{3})</td>
<td>760</td>
<td>801</td>
<td>n.a.</td>
</tr>
<tr>
<td>[B_{12}H_{11}S—]^{2-}</td>
<td>1055</td>
<td>n.a.</td>
<td>1053</td>
</tr>
<tr>
<td>[B_{12}H_{11}S—]^{2-}</td>
<td>963</td>
<td>n.a.</td>
<td>976</td>
</tr>
<tr>
<td>[B_{12}H_{11}S—]^{2-}</td>
<td>840</td>
<td>n.a.</td>
<td>843</td>
</tr>
<tr>
<td>[B_{12}H_{11}S—]^{2-}</td>
<td>722</td>
<td>n.a.</td>
<td>718</td>
</tr>
</tbody>
</table>

n.a. = not applicable.

The cyclic voltammogram of [Ru(SB_{12}H_{11})(NH_{3})_{5}]-2H_{2}O in dmf at a platinum electrode is shown in Figure 3.1. This shows a number of electrochemical processes. A tentative reaction scheme which may explain the nature of these couples is shown in Scheme 3.7.

Figure 3.1. Cyclic voltammogram of [Ru(SB_{12}H_{11})(NH_{3})_{5}] in dmf, 0.5 M NBu_{4}BF_{4} and about 10^{-3} M in sample, run at 100 mV s\(^{-1}\) in the range -1.0–1.0 V vs Ag/AgCl at -20 °C.
Scheme 3.7. The oxidation and reduction chemistry of \([\text{Ru(SBi}_{12}\text{H}_{11})(\text{NH}_3)_5]\cdot 2\text{H}_2\text{O}\) as observed by cyclic voltammetry in dmf solution. Redox couples are referenced against the NHE according to the method of Duff and Heath\textsuperscript{196}. Arrows marked with a \(X\) indicate that the couple was not reversible in the direction indicated.

A cathodic process for \([\text{Ru(SBi}_{12}\text{H}_{11})(\text{NH}_3)_5]\) is seen to occur at -0.30 V, relative to the normal hydrogen electrode (NHE). The relatively small intensity of this process suggests that it is an one electron reduction. Being a borohydride, the borocaptate anion is in a very reduced state. It is probable, therefore, that further reduction of this group would not occur at such a moderate potential. Hence, it is assumed that this reduction is associated with the metal centre and thus would correspond to the reduction of ruthenium(III) to ruthenium(II). Ruthenium(II), which is d\(^6\), does not have an available \(t_2g\) orbital that can accept \(\pi\) electrons from the sulfur atom of the borocaptate ligand. Thus, the ruthenium(II)-borocaptate complex generated electrochemically, is unstable and dissociates irreversibly. The solvated ruthenium(II) species so formed can be oxidised back to a ruthenium(III) species but only at a more positive potential.

Recent work by Lever\textsuperscript{197} has suggested that the redox potential, \(E_{1/2}\), of a couple \(M(n)/M(n - 1)\) for a complex species can be estimated according to the formula:

\[
E_{1/2} = S_M[\Sigma E_L(L)] + I_M
\]
where $S_M$ and $I_M$ are values determined experimentally for the particular couple involved and $E_L(L)$ is what is known as the ligand electrochemical parameter. While the $S_M$ and $I_M$ values for the ruthenium(III)/ruthenium(II) couple are known (and are 0.97 and -0.04 V respectively\(^{197}\)) the $E_L(L)$ value for the borocaptate ligand has not been determined. The value $E_L(SB_{12}H_{11})$ can be calculated from the experimental data, and by incorporating the known value of $E_L(NH_3)$ that has been quoted as 0.07 V\(^{197}\). That is:

$$-0.30 = 1.01(5 \times 0.07 + E_L(SB_{12}H_{11})) - 0.063$$

$$\therefore E_L(SB_{12}H_{11}) = -0.6 \text{ V}$$

This value is quite similar to that obtained for the related thiophenolato ligand that has a stated $E_L(SC_6H_5) = -0.53 \text{ V}$. This value is amongst the most negative of any determined thus far. This large negative value of $E_L(SB_{12}H_{11})$ would make reductions of borocaptate complexes difficult. Conversely, high oxidation state complexes should be stabilized by the presence of the borocaptate ligand.

From the estimated value of $E_L(SB_{12}H_{11})$ it is possible to estimate the $E_{1/2}(Ru^{IV/III})$, which is given by Duff and Heath\(^{196}\) as:

$$E_{1/2}(Ru^{IV/III}) = 1.01SE_L + 1.523$$

Thus, $E_{1/2}(Ru^{IV/III})$ for $[Ru(SB_{12}H_{11})(NH_3)_5]$ is approximately 1.3 V. This was not observed in this experiment because at these potentials decomposition of the solvent dmf occurs. The unidentified anodic processes that are observed are assumed to be associated with the oxidation of the borocaptate ligand itself.

The magnetic moment, $\mu$, of the complex was determined by the Gouy method\(^{198}\). In order for the determination to be made, the molar magnetic susceptibility, $\chi_m$, for the borocaptate anion had to be measured. This was found to be $-204 \times 10^{-6}$ cgs units. The magnetic moment of the complex was thus calculated to be 1.68 BM. This corresponds to a value due entirely to a spin-only low spin $d^5$ system. That is, the paramagnetism observed is due to the one unpaired electron on the metal centre. It is not unusual for $d^5$ complexes of ruthenium to have a contribution to their magnetic moment attributable to
spin-orbit coupling\textsuperscript{199}, but this is not found in this borocaptate complex.

The \textsuperscript{1}H n.m.r. spectrum was of little value in the determination of the structure of the complex. It featured a broad peak at 8.00 ppm that is due to the NH\textsubscript{3} ligands and another broad peak at 3.64 ppm due to the B–H groups. The B–H protons of the borocaptate thioether, sulfonium salt and thioester derivatives, are seen as broad resonances between 0 and 2 ppm\textsuperscript{192}. An effect due to metal coordination of the borocaptate anion is reflected in the downfield shift observed for the proton resonances of the boron cage in this complex.

Definitive proof as to the actual nature of the complex was obtained by a single crystal X-ray structure analysis (see Chapter Four).

3.2.2. \textit{The synthesis and characterization of trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})]}\textsuperscript{198}

The reaction of trans-[RuCl\textsubscript{2}(en)\textsubscript{2}]Cl with Cs\textsubscript{2}B\textsubscript{12}H\textsubscript{11}SH in water produced a pale yellow precipitate which was identified as the salt trans-[RuCl\textsubscript{2}(en)\textsubscript{2}][B\textsubscript{12}H\textsubscript{11}SH]. Because of the ready formation of this salt an alternate procedure was required. The method used for this synthesis involved the preparation of the trifluoromethanesulfonato (triflato) intermediate\textsuperscript{200-202}. The addition of trifluoromethanesulfonic (triflic) acid to trans-[RuCl\textsubscript{2}(en)\textsubscript{2}]Cl proceeded with the liberation of hydrogen chloride gas and produced a dark-orange oil. The excess acid was removed and the triflato complex was dissolved in water. This was treated with Cs\textsubscript{2}B\textsubscript{12}H\textsubscript{11}SH to produce the desired complex, which was obtained as an amorphous green-brown powder (Scheme 3.8).
A number of significant shifts in the infrared bands associated with the ethylenediamine ligand between the parent dichloro complex and the borocaptate complex are observed (Table 3.2). Molecular modelling studies indicate that there exists a great degree of steric crowding about the ruthenium centre in this borocaptate complex. This would have a direct influence on the ethylenediamine groups which is reflected in the higher frequencies obtained for a number of the bands. The $\nu$(B–H) frequency is again somewhat lower than what is found in the free borocaptate anion, and is comparable to that found for [Ru(SB$_{12}$H$_{11}$)(NH$_3$)$_5$]. The presence of water can be seen in the broad $\nu$(O–H$_2$) frequency at 3435 cm$^{-1}$. 

*Scheme 3.8. Preparative route to trans-[Ru(SB$_{12}$H$_{11}$)(en)$_2$(OH$_2$)] via the bis(triflato) intermediate.*
Table 3.2. Infrared bands of the complex trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})], with comparison to the parent compounds from which it is derived.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})]</th>
<th>trans-[RuCl\textsubscript{2}(en)\textsubscript{2}] Cl</th>
<th>Cs\textsubscript{2}B\textsubscript{12}H\textsubscript{11}SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ν(O–H\textsubscript{2})</td>
<td>3435</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{a}(N–H\textsubscript{2})</td>
<td>3297</td>
<td>3247</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{g}(N–H\textsubscript{2})</td>
<td>3254</td>
<td>3178</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{a}(C–H\textsubscript{2})</td>
<td>2942</td>
<td>2981</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{g}(C–H\textsubscript{2})</td>
<td>2884</td>
<td>2946</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν(B–H)</td>
<td>2475</td>
<td>n.a.</td>
<td>2486</td>
</tr>
<tr>
<td>δ(NH\textsubscript{2})</td>
<td>1580</td>
<td>1595</td>
<td>n.a.</td>
</tr>
<tr>
<td>δ(CH\textsubscript{2})</td>
<td>1454</td>
<td>1445</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{a}(C–N)</td>
<td>1163</td>
<td>1123</td>
<td>n.a.</td>
</tr>
<tr>
<td>ν\textsubscript{g}(C–N)</td>
<td>1108</td>
<td>1101</td>
<td>n.a.</td>
</tr>
<tr>
<td>[B\textsubscript{12}H\textsubscript{11}S−]\textsubscript{2}\textsuperscript{−}</td>
<td>1045</td>
<td>n.a.</td>
<td>1053</td>
</tr>
<tr>
<td>[B\textsubscript{12}H\textsubscript{11}S−]\textsubscript{2}\textsuperscript{−}</td>
<td>976</td>
<td>n.a.</td>
<td>976</td>
</tr>
<tr>
<td>[B\textsubscript{12}H\textsubscript{11}S−]\textsubscript{2}\textsuperscript{−}</td>
<td>837</td>
<td>n.a.</td>
<td>843</td>
</tr>
<tr>
<td>[B\textsubscript{12}H\textsubscript{11}S−]\textsubscript{2}\textsuperscript{−}</td>
<td>718</td>
<td>n.a.</td>
<td>718</td>
</tr>
</tbody>
</table>

n.a. = not applicable.

The $^1$H n.m.r. spectrum of trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})] in D\textsubscript{7}–dmf is poorly resolved. This is probably due to the paramagnetism of the d\textsuperscript{5} ruthenium metal centre, but it could also be due to a solvolysis reaction. In Me\textsubscript{2}SO a blue solution is formed from the green-brown trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})]. Undoubtedly this is produced by a solvolysis reaction. The $^1$H n.m.r. spectrum obtained in this solvent is again poorly resolved.

The magnetic moment, $\mu$, of trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(en)\textsubscript{2}(OH\textsubscript{2})] was 1.08 BM. This value is much lower than the theoretical value expected for one unpaired electron in a low-spin d\textsuperscript{5} system of 1.73 BM\textsuperscript{199}. The complex trans-[Ru(SB\textsubscript{12}H\textsubscript{11})(NH\textsubscript{3})\textsubscript{5}] also has a relatively low value for its magnetic moment, which suggests that this may be a feature of borocaptate coordination to ruthenium(III). The poor solubility characteristics of the product have prevented further analysis.
3.2.3. **The synthesis and characterization of \([\text{Ru}(\text{SB}_{12}H_{11})(\text{terpy})(\text{OH}_2)_2]\).**

The direct treatment of \([\text{RuCl}_3(\text{terpy})]\) with \(\text{Cs}_2\text{B}_{12}H_{11}\text{SH}\) failed to give any reaction. Thus, it was necessary to convert \([\text{RuCl}_3(\text{terpy})]\) to a more reactive intermediate. The procedure that was used to prepare \([\text{Ru}(\text{SB}_{12}H_{11})(\text{terpy})(\text{OH}_2)_2]\) was via the tris(trifluoromethanesulfonato) complex (Scheme 3.9), in similar manner to that used to produce the ethylenediamine analogue\(^{203,204}\). In this case it was possible to actually isolate the triflato complex, \([\text{Ru}(\text{O}_3\text{SCF}_3)_3(\text{terpy})]\), as a dark-green crystalline solid. This compound was very air sensitive, but its infrared spectrum indicated the presence of terpyridine and triflate, and the absence of any peaks that were due to chloro ligands.

The triflato intermediate was dissolved in water and then reacted with the borocaptate anion which rapidly gave the product as a brown amorphous powder. Molecular modelling studies suggest that the aquo ligands would be in a *cis* geometry, with respect to each other, with the borocaptate ligand perpendicular to the plane formed by the terpyridine ligand. This would be necessary because of the extreme steric crowding that would result if the borocaptate was in the same plane as the terpyridine ligand; the borocaptate is far too bulky to occupy this position.

The infrared spectrum is a mixture of bands derived from both the terpyridine and the borocaptate ligands (Table 3.3). The band associated with the B–H stretching mode is at a higher frequency than is observed in the free borocaptate anion, rather than at a lower frequency as observed in \([\text{Ru}(\text{SB}_{12}H_{11})(\text{NH}_3)_5]\) and \([\text{Ru}(\text{SB}_{12}H_{11})(\text{en})_2(\text{OH}_2)]\). This is most likely due to an effect associated with the presence of the aromatic terpyridine ligand. Bands associated with the terpyridine ligand are at essentially the same frequencies as they are in the parent complex \([\text{RuCl}_3(\text{terpy})]\), with the exception of the band assigned to the out of plane wagging mode, \(\omega(\text{C–H})\), which is found at a somewhat lower energy. If the borocaptate was having a direct steric effect on the terpyridine ligand then it is expected that this mode would be at a higher energy, being less favourable. As this is not the case it can be tentatively concluded that the borocaptate lies out of the plane...
Chapter Three

of the terpyridine ligand, as is depicted in Scheme 3.9.

Scheme 3.9. The preparative route to \([\text{Ru(SB}_{12}\text{H}_{11})(\text{terpy})(\text{OH}_2)_2]\), via the tris(triflato) derivativ.

Table 3.3. Infrared bands of the complex \([\text{Ru(SB}_{12}\text{H}_{11})(\text{terpy})(\text{OH}_2)_2]\), with comparisons to the parent compounds from which it is derived.

<table>
<thead>
<tr>
<th>Assignment</th>
<th>([\text{Ru(SB}<em>{12}\text{H}</em>{11})(\text{terpy})(\text{OH}_2)_2])</th>
<th>([\text{RuCl}_3(\text{terpy})])</th>
<th>Cs(<em>2\text{B}</em>{12}\text{H}_{11}\text{S}^\text{-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\nu(\text{O-H}_2))</td>
<td>3445</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>(\nu(\text{C-H}))</td>
<td>3078</td>
<td>3060</td>
<td>n.a.</td>
</tr>
<tr>
<td>(\nu(\text{B-H}))</td>
<td>2500</td>
<td>n.a.</td>
<td>2486</td>
</tr>
<tr>
<td>(\nu(\text{C-C}))</td>
<td>1601, 1468, 1449, 1388</td>
<td>1595, 1468, 1445, 1387</td>
<td>n.a.</td>
</tr>
<tr>
<td>(\delta(\text{C-H}))</td>
<td>1285, 1244, 1162</td>
<td>1280, 1233, 1156</td>
<td>n.a.</td>
</tr>
<tr>
<td>(\omega(\text{C-H}))</td>
<td>767</td>
<td>783</td>
<td>n.a.</td>
</tr>
<tr>
<td>([\text{B}<em>{12}\text{H}</em>{11}\text{S}^-]^\text{-2})</td>
<td>1051</td>
<td>n.a.</td>
<td>1053</td>
</tr>
<tr>
<td>([\text{B}<em>{12}\text{H}</em>{11}\text{S}^-]^\text{-2})</td>
<td>963</td>
<td>n.a.</td>
<td>976</td>
</tr>
<tr>
<td>([\text{B}<em>{12}\text{H}</em>{11}\text{S}^-]^\text{-2})</td>
<td>837</td>
<td>n.a.</td>
<td>843</td>
</tr>
<tr>
<td>([\text{B}<em>{12}\text{H}</em>{11}\text{S}^-]^\text{-2})</td>
<td>733</td>
<td>n.a.</td>
<td>718</td>
</tr>
</tbody>
</table>

n.a. = not applicable.
The complex \([\text{Ru(SB}_{12}\text{H}_{11})(\text{terpy})(\text{OH}_{2})_{2}]\) is only soluble in \(\text{Me}_2\text{SO}\). Its \(^1\text{H}\) n.m.r. spectrum indicates that this occurs with solvolysis as a number of species can be detected. The paramagnetism again makes the spectrum poorly resolved.

The magnetic moment, \(\mu\), of the solid was determined to be 1.31 BM. This value is again lower than that expected for a low spin \(d^5\) system, which is 1.73 BM\(^{199}\). This low value is, however, in keeping with what has been observed for the other complexes of borocaptate described. With poor solubility further analyses were not possible.

3.3. CONCLUSIONS

It is possible to prepare metal complexes featuring the borocaptate anion as a ligand, albeit with some difficulty. Being a dianion, borocaptate has a strong tendency to form insoluble salts with a number of cationic metal complexes. With anionic metal complexes, there is a considerable electrostatic repulsion to be overcome before any kind of association can proceed. The sterically demanding size of the borocaptate ligand also makes coordination unfavourable.

While it is difficult to produce complexes with borocaptate, it is not impossible as the preceding examples have demonstrated. Complexation has been found to be achievable with ruthenium(III) species. Other metals and oxidation states, which were tried but failed to give a reaction or a characterizable product, were platinum(II), nickel(II) and ruthenium(II).

Borocaptate has been found to coordinate in a monodentate fashion with formally, at least, a 3− charge and is clearly a very electron rich ligand. This would favour charge transfer, which would occur from the ligand to the metal centre, in its complexes. This opens up the possibility that the borocaptate ligand may be effective in stabilizing unusually high oxidation states.

The possible coordination of borocaptate to a metal centre should not be ignored in any discussion of the \textit{in vivo} action of the borocaptate anion, especially considering the
variety of metal ions to be found in biological systems.

3.4. **FURTHER INVESTIGATIONS**

The poor aqueous solubilities observed for the borocaptate complexes represents a serious impediment to their further development as boron delivery agents for BNCT. However, the idea of using a metal complex to deliver boron specifically to the DNA of cancer cells is not without merit, if a suitable ligand with a high boron content is used. The general investigation of the borocaptate anion as a ligand may be warranted. Unusually high oxidation states of metal complexes are likely to be accessible when borocaptate is present.

Investigations into the possible *in vivo* coordination of the borocaptate anion may also produce some interesting results. Coordination studies which focus on metals commonly found in biological systems, such as iron(III), would be of interest in determining the possible fate of borocaptate within an organism, and whether that fate is linked to a metal centre.
X-RAY STRUCTURE DETERMINATION OF PENTAAMMINE(1-THIOLATO-c/oso-UNDECA-
HYDRODODECABORANE)RUTHENIUM(III) DIHYDRATE, [Ru(SB_{12}H_{11})(NH_{3})_{5}].2H_{2}O
4.1. BACKGROUND

The scattering of X-rays by a crystal is produced by the interaction of the X-rays with the electrons of the atoms present. The scattering pattern formed discloses the atomic arrays associated with the crystals' structure. The scattering strength of an atom is proportional to the number of electrons it possesses, which is given by its atomic number. Because the distances between the electrons of an atom are about the same as the wavelength of the incident X-rays, the scattered waves interfere with one another, so the preceding statement is only strictly applicable for scattering in the forward direction. The total scattering amplitude in other directions decreases with increasing scattering angle because of the partially destructive interference produced by electrons scattering at different points within the atom. The ratio between the amplitude of the radiation scattered by an atom, $E_a$, and that of an isolated electron, $E_e$, (under identical conditions) is known as the atomic scattering factor $f$:

$$f = \frac{E_a}{E_e}$$

The sum of the scattering factors, with respect to the position of the $J$ atoms (represented by fractional coordinates $x_j, y_j, z_j$) within the crystal unit cell gives the complex quantity $F_{hkl}$ which is the structure factor:

$$F_{hkl} = f_1 e^{i\phi_1} + f_2 e^{i\phi_2} + f_3 e^{i\phi_3} + \ldots + f_J e^{i\phi_J}$$

where

$$\phi_j = 2\pi(hx_j + ky_j + lz_j)$$

This characterizes the diffraction maxima, or reflections, with indices $hkl$. Thus for a particular arrangement of atoms in a cell, there is a specific set of diffraction maxima $F_{hkl}$.

A crystal structure solution is achieved by comparing the observed diffraction pattern with that calculated from a model of the structure. The degree of agreement between the observed and calculated is indicative of the accuracy of the model. The observed intensity of a reflection $I_{hkl}$ is related to the structure factor of that reflection $F_{hkl}$ as:
For the purposes of computing structure factors it is more convenient to express them in terms of their real and imaginary components, that is:

\[
F_{hkl} = |F_{hkl}| \cos \phi + i|F_{hkl}| \sin \phi \\
= (\sum_i f_j \cos \phi_j) + i(\sum_i f_j \sin \phi_j)
\]

As crystals are periodic structures, the electron density \( \rho \) at a point \( x, y, z \) in a unit cell of volume \( V \) can be represented by a three dimensional Fourier series:

\[
\rho_{xyz} = \frac{1}{V} \sum_h \sum_k \sum_l |F_{hkl}| \cos \left[ 2\pi(hx + ky + lz) - \alpha \right]
\]

where \( \alpha \) is the phase angle, defined as:

\[
\alpha = \tan^{-1}\frac{\sum_i f_j \sin \phi_j}{\sum_i f_j \cos \phi_j}
\]

If the structure factor amplitude and phase angles for each reflection were known, then the electron density could be calculated for all values of \( x, y, z \) to give an electron density map with atomic nuclei located in regions of high electron concentration. Unfortunately, only the structure factor amplitudes can be determined from experimentally observed intensities. This is known as the phase problem.

If one or more atoms within a structure have an atomic number substantially greater than the majority of the atoms present, then these heavy atoms will dominate the scattering and the phase angle for the whole structure can by approximated to that of the heavy atoms alone. The positions of the heavy atoms can be located by using the Patterson function, which is a vectorial representation of the atomic composition of the unit cell and is expressed thus:

\[
P_{uvw} = \frac{1}{V} \sum_h \sum_k \sum_l |F_{hkl}|^2 \cos \left[ 2\pi(hu + kv + lw) \right]
\]
The intensity of each peak in this three-dimensional contour representation is proportional to the product of the electron density of the two atoms which define the interatomic vector \( u, v, w \).

Having identified the relative positions of the heavy atoms, a Fourier synthesis may be produced using the phase angles calculated from these positions and the observed structure factor amplitudes. New atom positions found by this synthesis are then added to the model structure and another Fourier synthesis is calculated. This procedure is repeated until all the atomic positions are located. An alternative approach is to use a difference Fourier synthesis, calculated as the difference between the observed electron density \( \rho_{\text{obs}} \) and the calculated density \( \rho_{\text{calc}} \). A difference Fourier can reveal where insufficient or excessive electron density is included in the model structure and thus can be used to detect the positions of light atoms.

4.2. \textit{X-Ray Structure Determination of} 
\[ \text{[Ru(SB}_{12}H_{11})(NH}_{3}\text{)]}_{5}\cdot2H_{2}O \]

The crystals used in this determination were obtained directly from the reaction of \[ \text{[RuCl(NH}_{3}\text{)]}_{5}\text{Cl}_{2} \] with \( \text{Cs}_{2}\text{B}_{12}H_{11}\text{SH} \) in aqueous solution. The density of these crystals was determined by flotation in a mixture of chloroform and dibromomethane.

A needle-like crystal of approximate dimensions \( 0.20 \times 0.05 \times 0.01 \) mm was mounted lengthwise on a quartz glass fibre with Araldite adhesive. Preliminary X-ray photographic data was obtained on a Weissenberg camera using CuK\( \alpha \) radiation. Results from a zero level Weissenberg photograph suggested a monoclinic crystal mounted on the unique axis. Systematic absences were noted on the \( h0l \) net when \( l \) was odd and on \( 0k0 \) axis when \( k \) was odd. This defines the space group P2\(_1\)/c (Number 14). A first level Weissenberg photograph was taken in order that all the unit cell dimensions could be approximated using a geometric method\(^{207}\).

More accurate unit cell dimensions were obtained by a least squares calculation of the settings of 23 reflections \( (10^\circ < 2\theta < 45^\circ) \) centred on a Philips PW1100/20
diffractometer with graphite monochromated MoK$_\alpha$ radiation. An intensity data set of 2617 reflections, in the form $+h, +k, \pm l$ was collected in the ranges $0 \leq h \leq 8, 0 \leq k \leq 10$ and $-10 \leq l \leq 10$. Three standard reflections, $0 2 \overline{1}, 2 \overline{2} 0$ and $1 \overline{2} 1$, were measured every two hours in order to monitor variations in intensity. The intensities of these standard reflections dropped by 4.5, 2.6 and 4.9% respectively during the period of data acquisition.

A superior data set was collected on a Rigaku AFC6-R instrument using graphite monochromated CuK$_\alpha$ radiation from a 12 kW rotating anode generator. Unit cell parameters were obtained from 25 high angle reflections ($60^\circ < \theta < 80^\circ$), while the data set consisted of a total 2884 reflections (2176 observed, 688 weak and 20 symmetry related). This data set was of the form $+h, +k, \pm l$ and was collected in the ranges $0 \leq h \leq 9, 0 \leq k \leq 16, -16 \leq l \leq 16$. The standard reflections, $\overline{1} 2 0, 0 2 \overline{2}$ and $1 \overline{2} \overline{1}$ were measured after every 75 reflections. Over the 29 hours required for the data to be acquired the standards dropped in intensity 2.2, 3.5 and 4.1% respectively.

4.2.1. Structure solution and refinement

The structure solution and refinement were performed on the RSC4 node of the Research School of Chemistry's Minivax computer. The following computer programs from either the ANUCRYS$^{208}$, SHELX-86, XTAL3.0 or XTAL3.2 program libraries were employed in the solution.

- **ABSORB - XTAL3.2:** For the application of Gaussian, analytical and spherical absorption corrections, by G. Davenport, N. Spadaccini and J. Stewart.
- **ADDREF - XTAL3.0/3.2:** For the placement and reduction of reflection data onto a binary file, by G. Davenport and S. Hall.
- **BONDAT - XTAL3.0/3.2:** For generating atom coordinates from molecular geometry, by R. Doherty, J. Stewart and H. Flack.

CRYLSQ - XTAL3.0/3.2: For full-matrix least squares structure factor refinement, by R. Olthof-Hazekamp.

CRYSTAL STRUCTURE SOLUTION - SHELX-86: For the Patterson heavy atom solution of atomic coordinates, by G. M. Sheldrick.


FOURR - XTAL3.0/3.2: For three-dimensional Fourier syntheses, by J. Stewart, J. Holden, R. Doherty and S. Hall.

GENEV - XTAL3.2: For the normalization of structure factors, by S. Hall and V. Subramanian.

HYDGEN83 - ANUCRYS: For the calculation of hydrogen atom positions.

ORTEP - XTAL3.0/3.2: For calculating thermal ellipsoid data for plotting, by G. Davenport, S. Hall and W. Dreissig.

PEAKPIK - XTAL3.0/3.2: For the location of electron density peaks in a Fourier map, by S. Hall and R. Doherty.

PWDECO - ANUCRYS: For the analysis of standard reflections.

PWREDU - ANUCRYS: For data reduction, application of Lorentz-polarization and decay corrections.


SORTRF - XTAL3.2: For the sorting and merging of reflection data, by S. Hall, N. Spadaccini and J. Stewart.

The data obtained from the Philips instrument was corrected first for crystal degradation and Lorentz-polarization effects using the PWDECO and PWREDU programs. The integrated intensities, $I$, were corrected as follows:
\[ I = [c_p - (t_p / t_b)(B_1 + B_2)] \]

where \( c_p \) = scan count, \( t_p \) = scan time (s), \( t_b \) = background count time (s), and \( B_1 \) and \( B_2 \) are the measured background counts either side of the peak. The correction for Lorentz-polarization (\( L_p \)), which was also applied, is given by:

\[ L_p = \frac{\cos^2 \theta + \cos^2 \theta_m}{\sin \theta (1 + \cos^2 \theta_m)} \]

where \( \theta \) is the reflection angle and \( \theta_m \) is the monochromator angle. The final observed structure factor amplitudes, \( |F_0| \), are thus given by:

\[ |F_0| = \sqrt{\frac{I}{L_p}} \]

The reflections were then sorted and the mean of equivalent reflections calculated by the SHELX-86 CRYSTAL STRUCTURE SOLUTION program. This gave a set 965 reflections with \( |F_0| \geq 4\sigma(|F_0|) \) that were used in the refinement. The structure was solved by the Patterson heavy atom method. This located both the ruthenium and sulfur atoms at 0.120, 0.198, 0.011 and 0.173, 0.067, -0.75 respectively.

The data was converted to XTAL3.0 format and, with the positions of the ruthenium and sulfur atoms obtained from the Patterson solution, was used in a least-squares refinement. The selection criterion for observed reflections was set to \( |F_0| \geq 2\sigma(|F_0|) \), and this gave a set of 1382 reflections that were used for further refinement. The isotropic thermal parameters were arbitrarily set at 3.5 Å\(^2\) for ruthenium and 5.0 Å\(^2\) for sulfur. A difference Fourier was calculated and further atom positions were thus located. The function \( K \) was minimised in the least squares refinements:

\[ K = \sum w(|F_o| - |F_c|)^2 \]

where \( |F_c| \) = the calculated structure-factor amplitude and \( w \) = assigned weight, initially set to one.

The refinement process was repeated until all non-hydrogen atoms were found, including the oxygens due to the two waters of crystallization. The R-factor, which is a
measure of the data quality and the correctness of the structure, was at this stage 0.169. This index is defined as:

\[ R = \frac{\sum | | F_o | - | F_c | |}{\sum | F_o |} \]

Because the intensity of this data set was very weak, and thus a relatively small number of reflections were observed, further refinement could not be achieved. It was at this stage that the more intense data, obtained from the Rigaku instrument using CuK\(\alpha\) radiation was introduced and used for further refinement.

The DIFDAT program was applied to the data in order to scale it according to the standard reflections and to present it in a form usable by XTAL3.2. A correction for crystal absorption effects was applied, using the analytical method described by Alcock\(^209\), available within the ABSORB program. The data was then sorted, using SORTRF, and equivalent reflections were merged. A correction for Lorentz-polarization was applied by the program ADDREF. The Lorentz-polarization was in this case given by:

\[ L_p = \frac{\sin 2\theta}{(T1 + T2)} \]

where \( T1 = (1 - C)(B + (1 - B)\cos^2 2\theta \cos^2 \theta_m) / (B + (1 - B)\cos^2 2\theta_m) \)

\( T2 = C(B + (1 - B)\cos^2 2\theta \cos^2 \theta_m) / (B + (1 - B)\cos^2 2\theta_m) \)

and B is the fraction of intensity with the electric field parallel to the plane of the monochromator and was set at 0.5, while C is the monochromator perfection factor and this was also 0.5. The structure factors were calculated as described previously. This gave a set of 2196 reflections with \( |F_o| \geq 2\sigma(|F_o|) \). A least-squares refinement of this data set, using the atom positions already located with their corresponding isotropic thermal parameters, gave a R-factor of 0.080.

The program REGWT was used to produce a weighting scheme for least-squares refinement. The assigned weight, \( w \), was given by:

\[ w = \frac{1}{\sigma^2 |F_o| + p |F_o|^2} \]
where \( p \) is the instrumental uncertainty. This scheme reduces the effect of very strong maxima on the residual R-factor and the minimisation function \( K \).

Refinement was continued with anisotropic thermal parameters for all atoms except hydrogen. These parameters were satisfactory except for the oxygen of the second water molecule. Its thermal parameters were very large and this was thought to be due to partial occupancy of its site. Refinement of its population parameter gave a value of 0.90, which effectively indicates full occupancy. This refinement did, however, improve the R-factor and, therefore, the population parameter was not reset to 1.0. The large thermal parameters of this oxygen indicate that it is either statistically or thermally disordered.

The hydrogen atoms on each boron atom and on one of the solvent water molecules were located from difference Fourier synthesis. These hydrogens were included in the refinement with isotropic thermal parameters assigned arbitrarily as 0.06 \( \AA^2 \) for boron hydrogens and 0.10 \( \AA^2 \) for water hydrogens. This resulted in a reduction of the R-factor to 0.053. The possible positions of the hydrogen atoms about the nitrogen atoms were calculated with HYDGEN83. Comparison of these values with difference Fourier contour maps gave approximately the correct positions for these atoms, which were included in the refinement with thermal parameters assigned as 0.10 \( \AA^2 \).

Least-squares refinement with all atoms accounted for, except for the protons of the second water molecule, produced a R-factor of 0.041. The final difference Fourier synthesis revealed no residual electron density greater than 0.58 electrons / \( \AA^3 \). The weighted residual factor \( R_w \) was calculated to be 0.053, where \( R_w \) is given by:

\[
R_w = \sqrt{\frac{\sum w(|F_o| - |F_c|)^2}{\sum w|F_o|^2}}
\]

The standard deviation of an observation of unit weight or goodness of fit, \( S \), is defined as:

\[
S = \sqrt{\frac{\sum w(|F_o| - |F_c|)^2}{m - n}}
\]

where \( m \) = the number of unique reflections and \( n \) = the number of variables. This value
should be very close to unity if the weighting scheme has been chosen correctly. The final $S$ value in this case was found to be 1.294.

Figure 4.1. The molecular structure of $[\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{NH}_{3})_{5}]$, without hydrogen atoms. Thermal ellipsoids are represented at 30% probability.
Figure 4.2. The molecular structure of $[\text{Ru(SB}_{12}\text{H}_{11})(\text{NH}_3)_5]$ with hydrogen atoms present. Thermal ellipsoids are represented at 30% probability.
Figure 4.3. Stereoscopic view of the unit cell contents of $[\text{Ru}(\text{SB}_{12} \text{H}_{11})(\text{NH}_3)_5] \cdot 2\text{H}_2\text{O}$.
Hydrogen atoms have been omitted for clarity.
Table 4.1. Crystal data for [Ru(SB$_{12}$H$_{11}$)(NH$_3$)$_5$]-2H$_2$O

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>RuB$<em>{12}$H$</em>{26}$N$_5$S·2H$_2$O</td>
</tr>
<tr>
<td>Formula weight</td>
<td>395.14</td>
</tr>
<tr>
<td>Crystal dimensions</td>
<td>0.20 x 0.05 x 0.01 mm</td>
</tr>
<tr>
<td>Systematic absences</td>
<td>$h0l$: $l$ odd</td>
</tr>
<tr>
<td></td>
<td>$0k0$: $k$ odd</td>
</tr>
<tr>
<td>Space group</td>
<td>P2$_1$/c</td>
</tr>
<tr>
<td>Unit cell dimensions (Å)</td>
<td></td>
</tr>
<tr>
<td>Photograph</td>
<td>$a = 7.97$, $b = 14.3$, $c = 15.0$, $\beta = 99.6^\circ$</td>
</tr>
<tr>
<td>Philips Diffractometer</td>
<td>$a = 8.069(1)$, $b = 14.254(1)$,</td>
</tr>
<tr>
<td></td>
<td>$c = 15.185(4)$, $\beta = 98.371^\circ$</td>
</tr>
<tr>
<td>Rigaku Diffractometer</td>
<td>$a = 8.056(1)$, $b = 14.240(2)$,</td>
</tr>
<tr>
<td></td>
<td>$c = 15.172(2)$ $\beta = 98.48^\circ$</td>
</tr>
<tr>
<td>Volume</td>
<td>1721.3 Å$^3$</td>
</tr>
<tr>
<td>Measured density, $D_m$</td>
<td>1.50 g cm$^{-3}$</td>
</tr>
<tr>
<td>Calculated density, $D_c$</td>
<td>1.52 g cm$^{-3}$</td>
</tr>
<tr>
<td>Boundary faces of crystal, $hkl$</td>
<td></td>
</tr>
<tr>
<td>(distance to origin, mm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\overline{1} \ 4 \ 9$ (0.021) 1 3 6 (0.024)</td>
</tr>
<tr>
<td></td>
<td>$\overline{0} \ 1 \ 0$ (0.034) 0 $\overline{1}$ 0 (0.027)</td>
</tr>
<tr>
<td></td>
<td>$\overline{1} \ 4 \ 7$ (0.024) 1 $\overline{4}$ 6 (0.027)</td>
</tr>
<tr>
<td></td>
<td>$3 \ \overline{1} \ 3$ (0.097) $\overline{3}$ 1 3 (0.097)</td>
</tr>
<tr>
<td>$F_{000}$</td>
<td>804</td>
</tr>
<tr>
<td>Formula units per cell, $Z$</td>
<td>4</td>
</tr>
<tr>
<td>Linear absorption coefficient, $\mu$</td>
<td>10.03 cm$^{-1}$ (Mo $K_{\alpha}$)</td>
</tr>
<tr>
<td>(radiation used)</td>
<td>86.82 cm$^{-1}$ (Cu $K_{\alpha1}$)</td>
</tr>
</tbody>
</table>
Table 4.2. Summary of data collection for [Ru(SB\textsubscript{12}H\textsubscript{11})(NH\textsubscript{3})\textsubscript{5}]\textsubscript{2}H\textsubscript{2}O

<table>
<thead>
<tr>
<th>Description</th>
<th>Philips PW1100/20</th>
<th>Rigaku AFC6-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffractometer used for data collection</td>
<td>Philips PW1100/20 (graphite monochromator)</td>
<td>Rigaku AFC6-R (graphite monochromator)</td>
</tr>
<tr>
<td>Radiation used for data collection</td>
<td>MoK\textsubscript{α} ((\lambda = 0.7107 \text{ Å}))</td>
<td>CuK\textsubscript{α1} ((\lambda = 1.54051 \text{ Å}))</td>
</tr>
<tr>
<td>Radiation Source</td>
<td>Fine focus sealed tube</td>
<td>12 kW rotating anode generator</td>
</tr>
<tr>
<td>Number of reflections used to refine cell dimensions</td>
<td>23 in the range 10° ≤ 2θ ≤ 30°</td>
<td>25 in the range 80° ≤ 2θ ≤ 100°</td>
</tr>
<tr>
<td>Range of 2θ in data collection</td>
<td>4° – 45°</td>
<td>3° – 120°</td>
</tr>
<tr>
<td>Form of data collected</td>
<td>+(h), +k, ±l</td>
<td>+(h), +k, ±l</td>
</tr>
<tr>
<td>Transmission factors: Max. Min.</td>
<td>0.7160</td>
<td>0.5142</td>
</tr>
<tr>
<td>Monochromator 2θ</td>
<td>12.2°</td>
<td>26.50°</td>
</tr>
<tr>
<td>Dispersion factor ((\Delta))</td>
<td>0.34 tan θ</td>
<td>0.300 tan θ</td>
</tr>
<tr>
<td>Scan mode</td>
<td>2θ – ω scan</td>
<td>2θ – ω scan</td>
</tr>
<tr>
<td>Scan speed</td>
<td>1° / minute</td>
<td>8° / minute</td>
</tr>
<tr>
<td>Scan width</td>
<td>0.90 + 0.34 tan θ</td>
<td>1.260 + 0.300 tan θ</td>
</tr>
<tr>
<td>Total background count time</td>
<td>20 s</td>
<td>Half the scan time</td>
</tr>
<tr>
<td>Standard reflections (% drop in intensity)</td>
<td>0 2 (\bar{1}) (4.5), 2 (\bar{2}) 0 (2.6), 1 2 0 (4.9)</td>
<td>(\bar{1}) 2 0 (2.2), 0 2 2 (3.5), (\bar{1}) 2 (\bar{1}) (4.1)</td>
</tr>
<tr>
<td>Frequency standards were measured</td>
<td>Every 2 hours</td>
<td>Every 75 reflections</td>
</tr>
<tr>
<td>Total number of reflections measured</td>
<td>2617 (2269 unique)</td>
<td>2889 (2551 unique)</td>
</tr>
<tr>
<td>Number considered observed (</td>
<td>F_o</td>
<td>≥ 2\sigma</td>
</tr>
<tr>
<td>Instrumental uncertainty value ((\rho))</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Method used to solve structure</td>
<td>Patterson heavy atom</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Least-squares refinement method</td>
<td>Full-matrix</td>
<td></td>
</tr>
<tr>
<td>Function minimised</td>
<td>$K = \sum w(</td>
<td>F_o</td>
</tr>
<tr>
<td>Initial weights</td>
<td>$w = 1.0$</td>
<td></td>
</tr>
<tr>
<td>Final weights</td>
<td>$\frac{1}{\sigma^2</td>
<td>F_o</td>
</tr>
<tr>
<td>Absorption correction method</td>
<td>Analytical method of Alcock$^{209}$</td>
<td></td>
</tr>
<tr>
<td>Anomalous dispersion included for</td>
<td>Ru, S, N, O, B</td>
<td></td>
</tr>
<tr>
<td>Final $R$-factor</td>
<td>0.041</td>
<td></td>
</tr>
<tr>
<td>Final $R_w$-factor</td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>Final $S$ (goodness of fit)</td>
<td>1.294</td>
<td></td>
</tr>
<tr>
<td>Observations ($m$): variables ($n$)</td>
<td>2196 : 191</td>
<td></td>
</tr>
<tr>
<td>Ratio of maximum parameter shift to error in final cycle</td>
<td>0.07022</td>
<td></td>
</tr>
<tr>
<td>Final difference map: highest peak</td>
<td>$0.58 \text{ e/Å}^3$</td>
<td></td>
</tr>
<tr>
<td>Deepest trough</td>
<td>$-0.29 \text{ e/Å}^3$</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.4. Atomic coordinates and site occupancy in the asymmetric unit of [Ru(SB\(_{12}H_{11})(NH_3)\(_5\)]\(\cdot\)2H\(_2\)O*  

<table>
<thead>
<tr>
<th>Atom</th>
<th>x/a</th>
<th>y/b</th>
<th>z/c</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru</td>
<td>0.8785(5)</td>
<td>0.30197(2)</td>
<td>0.51011(2)</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.8309(2)</td>
<td>0.43187(9)</td>
<td>0.4268(1)</td>
<td></td>
</tr>
<tr>
<td>N(1)</td>
<td>1.1364(6)</td>
<td>0.3110(4)</td>
<td>0.4921(3)</td>
<td></td>
</tr>
<tr>
<td>N(2)</td>
<td>0.6265(6)</td>
<td>0.2927(3)</td>
<td>0.5348(3)</td>
<td></td>
</tr>
<tr>
<td>N(3)</td>
<td>0.8213(6)</td>
<td>0.2112(3)</td>
<td>0.4011(3)</td>
<td></td>
</tr>
<tr>
<td>N(4)</td>
<td>0.9442(6)</td>
<td>0.1757(3)</td>
<td>0.5930(3)</td>
<td></td>
</tr>
<tr>
<td>N(5)</td>
<td>0.9401(6)</td>
<td>0.3870(3)</td>
<td>0.6237(3)</td>
<td></td>
</tr>
<tr>
<td>B(1)</td>
<td>0.6544(8)</td>
<td>0.4375(4)</td>
<td>0.3311(4)</td>
<td></td>
</tr>
<tr>
<td>B(2)</td>
<td>0.7015(9)</td>
<td>0.4834(4)</td>
<td>0.2272(4)</td>
<td></td>
</tr>
<tr>
<td>B(3)</td>
<td>0.5905(9)</td>
<td>0.5535(4)</td>
<td>0.2980(4)</td>
<td></td>
</tr>
<tr>
<td>B(4)</td>
<td>0.4531(8)</td>
<td>0.4801(4)</td>
<td>0.3467(4)</td>
<td></td>
</tr>
<tr>
<td>B(5)</td>
<td>0.4744(8)</td>
<td>0.3641(4)</td>
<td>0.3084(4)</td>
<td></td>
</tr>
<tr>
<td>B(6)</td>
<td>0.6273(9)</td>
<td>0.3657(4)</td>
<td>0.2327(4)</td>
<td></td>
</tr>
<tr>
<td>B(7)</td>
<td>0.5491(9)</td>
<td>0.4367(4)</td>
<td>0.1401(4)</td>
<td></td>
</tr>
<tr>
<td>B(8)</td>
<td>0.5270(9)</td>
<td>0.5536(4)</td>
<td>0.1799(4)</td>
<td></td>
</tr>
<tr>
<td>B(9)</td>
<td>0.3776(9)</td>
<td>0.5515(5)</td>
<td>0.2547(4)</td>
<td></td>
</tr>
<tr>
<td>B(10)</td>
<td>0.3023(9)</td>
<td>0.4347(5)</td>
<td>0.2603(5)</td>
<td></td>
</tr>
<tr>
<td>B(11)</td>
<td>0.4101(9)</td>
<td>0.3631(4)</td>
<td>0.1898(4)</td>
<td></td>
</tr>
<tr>
<td>B(12)</td>
<td>0.3504(9)</td>
<td>0.4810(5)</td>
<td>0.1571(4)</td>
<td></td>
</tr>
<tr>
<td>O(1)</td>
<td>1.1238(7)</td>
<td>0.2201(4)</td>
<td>0.2873(3)</td>
<td></td>
</tr>
<tr>
<td>O(2)</td>
<td>0.842(1)</td>
<td>0.5952(7)</td>
<td>0.0449(5)</td>
<td>0.90(2)</td>
</tr>
</tbody>
</table>

* Estimated standard deviations shown in parentheses
Table 4.5 Atomic coordinates and isotropic thermal parameters* of the hydrogen atoms of [Ru(SB$_{12}$H$_{11}$)(NH$_3$)$_5$]-2H$_2$O

<table>
<thead>
<tr>
<th>Atom</th>
<th>x/a</th>
<th>y/b</th>
<th>z/c</th>
<th>U(Å$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(2)</td>
<td>0.8409</td>
<td>0.4925</td>
<td>0.2138</td>
<td>0.060</td>
</tr>
<tr>
<td>H(3)</td>
<td>0.6422</td>
<td>0.6180</td>
<td>0.3308</td>
<td>0.060</td>
</tr>
<tr>
<td>H(4)</td>
<td>0.4160</td>
<td>0.4954</td>
<td>0.4138</td>
<td>0.060</td>
</tr>
<tr>
<td>H(5)</td>
<td>0.4542</td>
<td>0.3011</td>
<td>0.3449</td>
<td>0.060</td>
</tr>
<tr>
<td>H(6)</td>
<td>0.7218</td>
<td>0.3052</td>
<td>0.2351</td>
<td>0.060</td>
</tr>
<tr>
<td>H(7)</td>
<td>0.5889</td>
<td>0.4226</td>
<td>0.0731</td>
<td>0.060</td>
</tr>
<tr>
<td>H(8)</td>
<td>0.5687</td>
<td>0.6142</td>
<td>0.1366</td>
<td>0.060</td>
</tr>
<tr>
<td>H(9)</td>
<td>0.2799</td>
<td>0.6045</td>
<td>0.2584</td>
<td>0.060</td>
</tr>
<tr>
<td>H(10)</td>
<td>0.1779</td>
<td>0.4242</td>
<td>0.2767</td>
<td>0.060</td>
</tr>
<tr>
<td>H(11)</td>
<td>0.3534</td>
<td>0.2962</td>
<td>0.1557</td>
<td>0.060</td>
</tr>
<tr>
<td>H(12)</td>
<td>0.2483</td>
<td>0.4853</td>
<td>0.0985</td>
<td>0.060</td>
</tr>
<tr>
<td>H(1N1)</td>
<td>1.1459</td>
<td>0.2971</td>
<td>0.4324</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2N1)</td>
<td>1.1752</td>
<td>0.3732</td>
<td>0.5063</td>
<td>0.100</td>
</tr>
<tr>
<td>H(3N1)</td>
<td>1.1996</td>
<td>0.2678</td>
<td>0.5309</td>
<td>0.100</td>
</tr>
<tr>
<td>H(1N2)</td>
<td>0.6090</td>
<td>0.3351</td>
<td>0.5811</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2N2)</td>
<td>0.5518</td>
<td>0.3080</td>
<td>0.4822</td>
<td>0.100</td>
</tr>
<tr>
<td>H(3N2)</td>
<td>0.6036</td>
<td>0.2303</td>
<td>0.5526</td>
<td>0.100</td>
</tr>
<tr>
<td>H(1N3)</td>
<td>0.7600</td>
<td>0.1589</td>
<td>0.4177</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2N3)</td>
<td>0.7572</td>
<td>0.2436</td>
<td>0.3531</td>
<td>0.100</td>
</tr>
<tr>
<td>H(3N3)</td>
<td>0.9233</td>
<td>0.1895</td>
<td>0.3828</td>
<td>0.100</td>
</tr>
<tr>
<td>H(1N4)</td>
<td>0.8616</td>
<td>0.1288</td>
<td>0.5776</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2N4)</td>
<td>1.0506</td>
<td>0.1529</td>
<td>0.5828</td>
<td>0.100</td>
</tr>
<tr>
<td>H(3N4)</td>
<td>0.9490</td>
<td>0.1918</td>
<td>0.6541</td>
<td>0.100</td>
</tr>
<tr>
<td>H(1N5)</td>
<td>1.0048</td>
<td>0.3510</td>
<td>0.6698</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2N5)</td>
<td>1.0043</td>
<td>0.4391</td>
<td>0.6097</td>
<td>0.100</td>
</tr>
<tr>
<td>H(3N5)</td>
<td>0.8407</td>
<td>0.4079</td>
<td>0.6438</td>
<td>0.100</td>
</tr>
<tr>
<td>H(1O1)</td>
<td>1.1240</td>
<td>0.1905</td>
<td>0.3438</td>
<td>0.100</td>
</tr>
<tr>
<td>H(2O1)</td>
<td>1.1824</td>
<td>0.2877</td>
<td>0.2969</td>
<td>0.100</td>
</tr>
</tbody>
</table>

* The isotropic thermal parameter is defined as:

$$T_{iso} = -8\pi^2 U \left[ \frac{\sin^2 \theta}{\lambda^2} \right]$$

where $U = \text{mean-square amplitude of the atomic vibration}^{210}$.  

---

Chapter Four
**Table 4.6. Intramolecular bond lengths and angles for the asymmetric unit of [Ru(SB12Hn)(NH3)5]·2H2O**

<table>
<thead>
<tr>
<th></th>
<th>Bond Lengths (Å)</th>
<th>Bond Angles (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The ruthenium coordination sphere:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru-S</td>
<td>2.241(1)</td>
<td>S-Ru-N(1) 88.1(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-Ru-N(2) 93.6(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-Ru-N(3) 93.7(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-Ru-N(4) 176.1(1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S-Ru-N(5) 89.2(1)</td>
</tr>
<tr>
<td>Ru-N(1)</td>
<td>2.139(5)</td>
<td>N(1)-Ru-N(2) 177.2(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(1)-Ru-N(3) 92.3(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(1)-Ru-N(4) 88.1(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(1)-Ru-N(5) 87.4(2)</td>
</tr>
<tr>
<td>Ru-N(2)</td>
<td>2.122(5)</td>
<td>N(2)-Ru-N(3) 89.8(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(2)-Ru-N(4) 90.2(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(2)-Ru-N(5) 90.4(2)</td>
</tr>
<tr>
<td>Ru-N(3)</td>
<td>2.095(4)</td>
<td>N(3)-Ru-N(4) 87.4(2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N(3)-Ru-N(5) 177.0(2)</td>
</tr>
<tr>
<td>Ru-N(4)</td>
<td>2.214(5)</td>
<td>N(4)-Ru-N(5) 89.7(2)</td>
</tr>
<tr>
<td>Ru-N(5)</td>
<td>2.104(4)</td>
<td></td>
</tr>
<tr>
<td><strong>The sulfur atom:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-B(1)</td>
<td>1.879(6)</td>
<td>Ru-S-B(1) 121.2(2)</td>
</tr>
<tr>
<td><strong>The boron cage environment:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average B-B</td>
<td>1.78(2)</td>
<td>Mean acute B-B-B 60.0(5)</td>
</tr>
<tr>
<td>Average B-H</td>
<td>1.12(3)</td>
<td>Mean obtuse B-B-B 108.0(5)</td>
</tr>
<tr>
<td><strong>The water of crystallization:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O(1)-H(1O1)</td>
<td>0.955(5)</td>
<td>H(1O1)-O(1)-H(2O1) 109.3(5)</td>
</tr>
<tr>
<td>O(1)-H(2O1)</td>
<td>1.073(5)</td>
<td></td>
</tr>
</tbody>
</table>

* Estimated standard deviations shown in parentheses.
Table 4.7. Selected dihedral angles for the asymmetric unit of [Ru(SB_{12}H_{11})(NH_{3})_{5}]·2H_{2}O*

<table>
<thead>
<tr>
<th>Dihedral Angle</th>
<th>Value (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(1)-S-Ru-N(1)</td>
<td>130.4(3)</td>
</tr>
<tr>
<td>B(1)-S-Ru-N(2)</td>
<td>-51.8(3)</td>
</tr>
<tr>
<td>B(5)-B(1)-S-Ru</td>
<td>18.0(6)</td>
</tr>
<tr>
<td>B(3)-B(1)-S-Ru</td>
<td>159.4(4)</td>
</tr>
<tr>
<td>B(6)-B(1)-S-Ru</td>
<td>-61.6(6)</td>
</tr>
<tr>
<td>B(1)-S-Ru-N(5)</td>
<td>-142.2(3)</td>
</tr>
<tr>
<td>B(1)-S-Ru-N(3)</td>
<td>38.2(3)</td>
</tr>
<tr>
<td>B(2)-B(1)-S-Ru</td>
<td>-132.7(3)</td>
</tr>
<tr>
<td>B(4)-B(1)-S-Ru</td>
<td>91.9(4)</td>
</tr>
</tbody>
</table>

Table 4.8. Intramolecular bond distances of the boron cage of [Ru(SB_{12}H_{11})(NH_{3})_{5}]·2H_{2}O*.

<table>
<thead>
<tr>
<th>Bond Distance</th>
<th>Value (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(1)-B(2)</td>
<td>1.798(9)</td>
</tr>
<tr>
<td>B(1)-B(4)</td>
<td>1.78(1)</td>
</tr>
<tr>
<td>B(1)-B(5)</td>
<td>1.778(9)</td>
</tr>
<tr>
<td>B(2)-B(3)</td>
<td>1.80(1)</td>
</tr>
<tr>
<td>B(3)-B(4)</td>
<td>1.76(1)</td>
</tr>
<tr>
<td>B(3)-B(8)</td>
<td>1.789(9)</td>
</tr>
<tr>
<td>B(4)-B(5)</td>
<td>1.767(9)</td>
</tr>
<tr>
<td>B(4)-B(9)</td>
<td>1.762(9)</td>
</tr>
<tr>
<td>B(5)-B(10)</td>
<td>1.779(9)</td>
</tr>
<tr>
<td>B(5)-B(11)</td>
<td>1.799(9)</td>
</tr>
<tr>
<td>B(6)-B(11)</td>
<td>1.77(1)</td>
</tr>
<tr>
<td>B(7)-B(12)</td>
<td>1.78(1)</td>
</tr>
<tr>
<td>B(9)-B(12)</td>
<td>1.776(9)</td>
</tr>
<tr>
<td>B(11)-B(12)</td>
<td>1.796(9)</td>
</tr>
<tr>
<td>B(2)-B(6)</td>
<td>1.795(8)</td>
</tr>
<tr>
<td>B(2)-B(8)</td>
<td>1.785(9)</td>
</tr>
<tr>
<td>B(3)-B(9)</td>
<td>1.74(1)</td>
</tr>
<tr>
<td>B(4)-B(10)</td>
<td>1.773(9)</td>
</tr>
<tr>
<td>B(5)-B(7)</td>
<td>1.769(8)</td>
</tr>
<tr>
<td>B(6)-B(11)</td>
<td>1.78(1)</td>
</tr>
<tr>
<td>B(7)-B(12)</td>
<td>1.75(1)</td>
</tr>
<tr>
<td>B(8)-B(12)</td>
<td>1.79(1)</td>
</tr>
<tr>
<td>B(10)-B(11)</td>
<td>1.79(1)</td>
</tr>
</tbody>
</table>

Table 4.9. Selected bond distances (Å) and angles (°)* for pentaammineruthenium(III)-sulfur donor complexes^{211-213}.

<table>
<thead>
<tr>
<th>Atom</th>
<th>-SB_{12}H_{11}</th>
<th>-SRR'</th>
<th>-SO(CH_{3})_{2}</th>
<th>-SSRu(NH_{3})_{5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distances (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru–S</td>
<td>2.241(1)</td>
<td>2.374(9)</td>
<td>2.188(3)</td>
<td>2.193(3)</td>
</tr>
<tr>
<td>Ru–N_{trans}</td>
<td>2.214(5)</td>
<td>2.12(2)</td>
<td>2.203(8)</td>
<td>2.183(6)</td>
</tr>
<tr>
<td>mean Ru–N_{axial}</td>
<td>2.12(2)</td>
<td>2.108(5)</td>
<td>2.155(2)</td>
<td>2.125(7)</td>
</tr>
<tr>
<td>Angles (°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru–S–N_{trans}</td>
<td>176.1(1)</td>
<td>176.2(3)</td>
<td>175.4(2)</td>
<td>180</td>
</tr>
<tr>
<td>Ru–S–X†</td>
<td>121.2(2)</td>
<td>112(3)</td>
<td>116(2)</td>
<td>111.1(5)</td>
</tr>
</tbody>
</table>

† X is the substituent atom on sulfur

* Estimated standard deviations shown in parentheses.
### Table 4.10. Bond angles in the boron cage of [Ru(SB\textsubscript{12}H\textsubscript{11})(NH\textsubscript{3})\textsubscript{5}].2H\textsubscript{2}O\textsuperscript{*}.

<table>
<thead>
<tr>
<th>Bond Angle</th>
<th>Value (°) ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-B(1)-B(2)</td>
<td>117.4(4)</td>
</tr>
<tr>
<td>S-B(1)-B(5)</td>
<td>128.8(4)</td>
</tr>
<tr>
<td>B(2)-B(1)-B(4)</td>
<td>107.9(4)</td>
</tr>
<tr>
<td>B(3)-B(1)-B(4)</td>
<td>59.3(4)</td>
</tr>
<tr>
<td>B(4)-B(1)-B(5)</td>
<td>59.6(4)</td>
</tr>
<tr>
<td>B(1)-B(2)-B(3)</td>
<td>59.3(3)</td>
</tr>
<tr>
<td>B(1)-B(2)-B(8)</td>
<td>107.4(5)</td>
</tr>
<tr>
<td>B(3)-B(2)-B(8)</td>
<td>59.9(4)</td>
</tr>
<tr>
<td>B(7)-B(2)-B(8)</td>
<td>60.0(4)</td>
</tr>
<tr>
<td>B(1)-B(3)-B(8)</td>
<td>108.1(4)</td>
</tr>
<tr>
<td>B(2)-B(3)-B(8)</td>
<td>59.7(4)</td>
</tr>
<tr>
<td>B(4)-B(3)-B(9)</td>
<td>60.4(4)</td>
</tr>
<tr>
<td>B(1)-B(4)-B(9)</td>
<td>108.7(5)</td>
</tr>
<tr>
<td>B(3)-B(4)-B(9)</td>
<td>59.3(4)</td>
</tr>
<tr>
<td>B(5)-B(4)-B(10)</td>
<td>60.3(4)</td>
</tr>
<tr>
<td>B(1)-B(5)-B(10)</td>
<td>108.4(5)</td>
</tr>
<tr>
<td>B(4)-B(5)-B(10)</td>
<td>60.0(4)</td>
</tr>
<tr>
<td>B(1)-B(6)-B(2)</td>
<td>60.3(3)</td>
</tr>
<tr>
<td>B(2)-B(6)-B(7)</td>
<td>108.6(4)</td>
</tr>
<tr>
<td>B(2)-B(7)-B(6)</td>
<td>60.2(3)</td>
</tr>
<tr>
<td>B(2)-B(7)-B(12)</td>
<td>107.2(5)</td>
</tr>
<tr>
<td>B(6)-B(7)-B(12)</td>
<td>108.1(5)</td>
</tr>
<tr>
<td>B(11)-B(7)-B(12)</td>
<td>60.7(4)</td>
</tr>
<tr>
<td>B(2)-B(8)-B(9)</td>
<td>107.6(4)</td>
</tr>
<tr>
<td>B(3)-B(8)-B(9)</td>
<td>58.6(4)</td>
</tr>
<tr>
<td>B(7)-B(8)-B(12)</td>
<td>60.2(4)</td>
</tr>
<tr>
<td>B(3)-B(9)-B(8)</td>
<td>61.2(4)</td>
</tr>
<tr>
<td>B(4)-B(9)-B(8)</td>
<td>109.2(5)</td>
</tr>
<tr>
<td>B(8)-B(9)-B(10)</td>
<td>108.3(5)</td>
</tr>
<tr>
<td>B(4)-B(10)-B(5)</td>
<td>59.7(4)</td>
</tr>
<tr>
<td>B(4)-B(10)-B(12)</td>
<td>107.4(5)</td>
</tr>
<tr>
<td>B(5)-B(10)-B(12)</td>
<td>108.2(5)</td>
</tr>
<tr>
<td>B(11)-B(10)-B(12)</td>
<td>106.1(4)</td>
</tr>
<tr>
<td>B(5)-B(11)-B(10)</td>
<td>59.4(4)</td>
</tr>
<tr>
<td>B(6)-B(11)-B(10)</td>
<td>107.9(4)</td>
</tr>
<tr>
<td>B(7)-B(11)-B(12)</td>
<td>59.6(4)</td>
</tr>
<tr>
<td>B(7)-B(12)-B(9)</td>
<td>108.5(5)</td>
</tr>
<tr>
<td>B(8)-B(12)-B(9)</td>
<td>60.2(4)</td>
</tr>
<tr>
<td>B(9)-B(12)-B(10)</td>
<td>59.8(4)</td>
</tr>
<tr>
<td>Estimated standard deviations shown in parentheses.</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.11. Anisotropic thermal-parameters* of the non-hydrogen atoms in the asymmetric unit of [Ru(SB\textsubscript{12}H\textsubscript{11})(NH\textsubscript{3})\textsubscript{5}]*\textsubscript{2}H\textsubscript{2}O\textsuperscript{‡}

<table>
<thead>
<tr>
<th>Atom</th>
<th>$U_{11}$</th>
<th>$U_{22}$</th>
<th>$U_{33}$</th>
<th>$U_{12}$</th>
<th>$U_{13}$</th>
<th>$U_{23}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru(1)</td>
<td>.0406(3)</td>
<td>.0366(3)</td>
<td>.0329(3)</td>
<td>-.0032(2)</td>
<td>.0048(2)</td>
<td>.0012(1)</td>
</tr>
<tr>
<td>S(1)</td>
<td>.064(1)</td>
<td>.0443(7)</td>
<td>.0463(7)</td>
<td>-.0154(7)</td>
<td>-.0097(7)</td>
<td>.0100(6)</td>
</tr>
<tr>
<td>N(1)</td>
<td>.042(3)</td>
<td>.074(4)</td>
<td>.054(3)</td>
<td>-.003(2)</td>
<td>.009(2)</td>
<td>-.004(2)</td>
</tr>
<tr>
<td>N(2)</td>
<td>.044(3)</td>
<td>.049(3)</td>
<td>.051(3)</td>
<td>-.002(2)</td>
<td>.013(2)</td>
<td>.007(2)</td>
</tr>
<tr>
<td>N(3)</td>
<td>.057(3)</td>
<td>.036(2)</td>
<td>.050(3)</td>
<td>-.005(2)</td>
<td>.003(2)</td>
<td>-.009(2)</td>
</tr>
<tr>
<td>N(4)</td>
<td>.048(3)</td>
<td>.055(3)</td>
<td>.049(3)</td>
<td>.002(2)</td>
<td>.007(2)</td>
<td>.011(2)</td>
</tr>
<tr>
<td>N(5)</td>
<td>.054(3)</td>
<td>.051(2)</td>
<td>.036(2)</td>
<td>-.006(2)</td>
<td>.010(2)</td>
<td>-.003(2)</td>
</tr>
<tr>
<td>B(1)</td>
<td>.048(4)</td>
<td>.036(3)</td>
<td>.037(3)</td>
<td>-.000(3)</td>
<td>.003(3)</td>
<td>.003(2)</td>
</tr>
<tr>
<td>B(2)</td>
<td>.053(4)</td>
<td>.043(3)</td>
<td>.038(3)</td>
<td>.001(3)</td>
<td>.008(3)</td>
<td>.007(3)</td>
</tr>
<tr>
<td>B(3)</td>
<td>.067(5)</td>
<td>.035(3)</td>
<td>.037(3)</td>
<td>-.003(3)</td>
<td>.003(3)</td>
<td>-.001(2)</td>
</tr>
<tr>
<td>B(4)</td>
<td>.049(4)</td>
<td>.045(3)</td>
<td>.040(3)</td>
<td>.007(3)</td>
<td>.011(3)</td>
<td>-.001(3)</td>
</tr>
<tr>
<td>B(5)</td>
<td>.041(4)</td>
<td>.039(3)</td>
<td>.042(3)</td>
<td>.003(3)</td>
<td>.004(3)</td>
<td>.002(2)</td>
</tr>
<tr>
<td>B(6)</td>
<td>.053(4)</td>
<td>.039(3)</td>
<td>.036(3)</td>
<td>.006(3)</td>
<td>.003(3)</td>
<td>.001(2)</td>
</tr>
<tr>
<td>B(7)</td>
<td>.064(5)</td>
<td>.046(3)</td>
<td>.033(3)</td>
<td>.006(3)</td>
<td>.000(3)</td>
<td>.000(3)</td>
</tr>
<tr>
<td>B(8)</td>
<td>.058(4)</td>
<td>.046(3)</td>
<td>.037(3)</td>
<td>.009(3)</td>
<td>.007(3)</td>
<td>.002(3)</td>
</tr>
<tr>
<td>B(9)</td>
<td>.069(5)</td>
<td>.047(4)</td>
<td>.043(4)</td>
<td>.017(3)</td>
<td>.014(3)</td>
<td>.005(3)</td>
</tr>
<tr>
<td>B(10)</td>
<td>.049(4)</td>
<td>.053(4)</td>
<td>.049(4)</td>
<td>.001(3)</td>
<td>.009(3)</td>
<td>.005(3)</td>
</tr>
<tr>
<td>B(11)</td>
<td>.055(4)</td>
<td>.039(3)</td>
<td>.046(4)</td>
<td>.006(3)</td>
<td>-.002(3)</td>
<td>.001(3)</td>
</tr>
<tr>
<td>B(12)</td>
<td>.057(4)</td>
<td>.052(3)</td>
<td>.040(3)</td>
<td>.014(3)</td>
<td>-.004(3)</td>
<td>.006(3)</td>
</tr>
<tr>
<td>O(1)</td>
<td>.087(4)</td>
<td>.094(3)</td>
<td>.076(3)</td>
<td>-.018(3)</td>
<td>.013(3)</td>
<td>-.014(3)</td>
</tr>
<tr>
<td>O(2)</td>
<td>.23(1)</td>
<td>.21(1)</td>
<td>.088(5)</td>
<td>-.114(8)</td>
<td>.062(6)</td>
<td>-.024(6)</td>
</tr>
</tbody>
</table>

* The anisotropic thermal parameter is defined as:

$$T_{\text{aniso}} = -2\pi^2(U_{11}\mu^2 + U_{22}\nu^2 + U_{33}\rho^2 + 2U_{12}\mu\nu\cos\gamma + 2U_{13}\mu\rho\cos\alpha + 2U_{23}\nu\rho\cos\alpha)$$

where $U_{ij}$ expresses the mean square amplitudes of vibration axes and $U_{ij}$ ($i \neq j$) represents the ellipsoid orientation\textsuperscript{210}.

‡ Estimated standard deviations shown in parentheses.
4.3. RESULTS AND DISCUSSION

The crystal structure of the title complex consists of discrete molecules [Ru(SB_{12}H_{11})(NH_3)_5] separated by normal Van der Waals contacts. Also present are two molecules of water per molecule of complex, which are also separated by normal Van der Waals contacts. Figure 4.1 shows an ORTEP view of the non-hydrogen atoms of the complex with atom labelling scheme. Another ORTEP view of the complex with all atoms present is shown in Figure 4.2. A stereoscopic view of the unit cell contents is presented in Figure 4.3. Summaries of crystal data, data collection, and structure solution and refinement are given in Tables 4.1, 4.2 and 4.3. The atomic coordinates of the non-hydrogen atoms of [Ru(SB_{12}H_{11})(NH_3)_5]-2H_2O are given in Table 4.4, while the coordinates of the hydrogen atoms are given in Table 4.5. Tables 4.6, 4.7, 4.8 and 4.10 present bond distances and bond angles and Table 4.11 gives the anisotropic thermal parameters of the non-hydrogen atoms. A comparison of selected bond distances and angles found for pentaammineruthenium(III)–sulfur donor ligand type complexes, which have been determined by X-ray crystallography, is shown in Table 4.9.

The structure of [Ru(SB_{12}H_{11})(NH_3)_5] is composed of the borocaptate ligand linked to ruthenium by the sulfur atom with five nitrogen atoms completing a distorted octahedron. The borocaptate ligand consists of a regular icosahedron with boron atoms occupying the vertices in a cage-like structure. Each boron has a hydrogen atom, which projects directly away from the centre of the cage, except for B(1) which is bonded to sulfur. Boron to boron distances within the cage structure (average 1.78 Å) do not vary significantly from one another (σ = 0.02) and are in good agreement with those reported for the free ligand (1.77 Å)\textsuperscript{188}. Bond angles (Table 4.9) are also close in value to each other and do not vary significantly from the ideal values of 60° and 108°. This uniformity in bond distances and angles probably reflects the three-dimensional delocalization of the \textit{closo}-dodecaborate structure\textsuperscript{214}. The boron to hydrogen distances found in this study are essentially the same as those reported\textsuperscript{188} for the free ligand (1.12 Å compared with 1.2 Å), as is the boron to sulfur distance (1.879 Å compared to 1.90 Å).
The boron icosahedron is positioned between the N(2) and N(3) atoms, with B(5) situated between these two atoms at distances of about 3.7 and 3.6 Å respectively (Figure 4.4). The dihedral angle Ru-S-B(1)-B(5) is 18° and this puts B(6) 3.6 Å away from N(3). The sulfur atom in turn is moved away from N(2) and N(3), toward N(1) and N(5), so as to reduce the trans N(4)-Ru-S angle to 176°. The angle about the sulfur atom, Ru-S-B(1), of 121° is significantly greater than the tetrahedral angle of 109.5° expected. This is probably a result of steric interactions, but possible electronic factors can not be wholly discounted. The B(1)-S bond does not sit at 122° with respect to the B(2), B(3), B(4), B(5) and B(6) atoms as expected but is distorted away from B(5) and B(6) and towards B(3) by about 7°. All of these effects combine to minimise steric interactions between the boron cage and the ammonia ligands.

\[ \text{Figure 4.4. Non-bonding distances (Å) between the boron cage and ammonia ligands} \]

The ammonia ligands are also perturbed from an ideal octahedral arrangement. It was expected that the N(2)-Ru-N(3) angle would be greater than 90° due to the effect of the interposing B(5), but this is not the case. It is in fact the N(1)-Ru-N(3) angle that is opened up to 92.3°, with the N(1)-Ru-N(5) angle reduced to 87.4°. No obvious steric effects are available to account for this observation.

The complex [Ru(SB_{12}H_{11})(NH_{3})_{5}] is not only the first structurally characterized compound featuring borocaptate coordinated to a metal centre but it also appears to be the first pentaammineruthenium(III)-thiolate to be so characterized. Similar compounds such as pentaammineruthenium(III)-thioethers\textsuperscript{111}, pentaammineruthenium(III)-Me_{2}SO\textsuperscript{212} and
bis(pentaammineruthenium(III))–disulfide have been characterized structurally (see Table 4.9.). The bond distances for ruthenium to sulfur atoms give an indication as to the degree of \( \pi \)-bonding between the metal and the ligand. The thioether complexes appear to have little or no \( \pi \) orbital interaction, while those of the disulfide and \( \text{Me}_2\text{SO} \) display considerably shorter bond distances and hence strong interactions. The borocaptate complex also displays a relatively short ruthenium to sulfur distance when compared to the thioether complexes but it is somewhat longer than that of the other two. This probably reflects the bulkiness of the borocaptate ligand rather than any diminished ability at \( \pi \) interaction. Strong \( \pi \) interactions also produce an obvious trans effect which is reflected in the distance between ruthenium and the nitrogen atom in the position directly opposite the sulfur. For the thioether complexes this distance is essentially the same as the ruthenium to nitrogen distances for the ammine ligands in the axial positions, however for the \( \text{Me}_2\text{SO} \) and disulfide ligands it is much longer. For the borocaptate ligand this length is at its longest (2.214 Å), but the distance for the \( \text{Me}_2\text{SO} \) ligand is only marginally shorter (2.203 Å).

There also appears to be a somewhat less noticeable cis effect. The average bond lengths of the ruthenium to the axial nitrogen atoms in the borocaptate and disulfide complexes are marginally longer than those in the thioether complexes, while in the \( \text{Me}_2\text{SO} \) complex the corresponding distances are much longer. This may indicate that the nature of the \( \pi \) interaction between these ligands and the ruthenium centre is different.

A general decrease in the angle between sulfur, ruthenium and the trans nitrogen atoms to 176° most probably reflects steric interactions. In the case of the unhindered disulfide this angle is 180°, which is as expected. The ruthenium-sulfur-substituent atom angles also increase depending on the bulkiness or on the number of substituents.
Chapter Five

DNA BINDING STUDIES
5.1. **INTRODUCTION**

It is generally believed that the *in vivo* target of cisplatin and a number of other anticancer agents, is the DNA of the cancerous cells (see Chapter One). Thus, study of the interactions between potential agents and DNA can provide insights into the usefulness of such compounds as anticancer treatments.\(^{215}\)

Bacterial cells may contain accessory chromosomes, called plasmids, that replicate independently of the host chromosome. Plasmids generally are a covalently closed circle of duplex DNA.\(^{217}\) They are relatively small in size consisting of only a few thousand base-pairs and often confer antibiotic resistance to their host cell.\(^{216}\) A feature of all circular DNA molecules is that the absolute number of times the two strands of the double-helix twist about each other cannot be altered. As a result, the formation of an appropriate number of *supercoils* in the opposite direction is produced in the DNA molecule when the average number of base pairs per helical turn is altered. Supercoiling is then the further twisting of the duplex DNA molecule into a more condensed tertiary structure.\(^{218-220}\) The condensed supercoiled DNA is known as Form I DNA. Supercoiling may be present in either the positive (right-handed) or negative (left-handed) direction and it is due to the overwinding (positive) or underwinding (negative) of the DNA duplex. If a duplex molecule is overwound the topological constraints involved in such a structure result in the number of positive supercoils exactly equalling the number of subtracted twists of the double helix caused by a decreased rotation angle. Similarly, if the duplex is underwound the number of negative supercoils exactly equals the number of added twists brought about by an increased rotation angle. The cutting, or *nickling*, of a single strand of the duplex molecule relieves these constraints, removing the supercoiling. The resulting relaxed circular DNA is known as Form II DNA. The cutting of both of the DNA strands results in the formation of linear or Form III DNA (see Figure 5.1). Form I DNA is much more compact or more dense than Form II DNA. Form III DNA is not as constrained as Form II, but it is likewise not as compact as Form I and so its density is intermediate. These density changes are translated directly into differences in the physical properties of the various forms.
Chapter Five

Figure 5.1. A schematic representation of the various forms of DNA (not drawn to the same scale)\textsuperscript{218}.

The constraints can also be removed by the interaction of the DNA with certain chemical agents. The dye ethidium bromide (Figure 5.2), which intercalates between the base pairs of DNA, can cause local untwisting of the double helix. A sufficiently high concentration will completely untwist the molecule leaving it as the fully relaxed Form I\textsubscript{0} DNA. The addition of more ethidium bromide will promote twisting in the opposite direction causing the duplex to writhe into an oppositely orientated supercoil. These structural changes are directly translated into density changes that can be easily observed by sedimentation by an ultracentrifuge or gel electrophoresis (see Figure 5.3.)\textsuperscript{218}. Cisplatin also promotes the untwisting of DNA and similar results have been obtained\textsuperscript{63,221-224}. However, cisplatin does not intercalate with DNA as ethidium bromide does, but rather it binds covalently to the nucleoside bases\textsuperscript{225-227}.

It has been proposed\textsuperscript{228} that on reaction with DNA, platinum complexes alter the electronic distribution within the coordinated bases. This causes a change in the orientation of the phosphodiester backbone, which produces localised denaturation of the
duplex. The torsion that results from this non-separable denaturation can unwind and subsequently rewind the DNA helix. The time that is required for Form I DNA to comigrate with Form II DNA reflects the effectiveness of the particular platinum complex as an unwinding agent.

![Ethidium Bromide Structure](image)

*Figure 5.2. The structure of ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide)*

![Sedimentation Velocity Diagram](image)

*Figure 5.3. The effect of adding ethidium bromide to supercoiled DNA, as demonstrated by the changes in sedimentation velocity with increasing ethidium bromide concentration. The changes to the structure of DNA which are shown have been confirmed by electron microscopy.*
Chapter Five

Electrophoresis involves the movement of charged species through a medium, such as agarose gel, under the influence of an electric potential. The rate at which a species moves is dependent on its overall charge, structure and density\(^\text{229}\). As the various forms of DNA have effectively the same charge, separation by electrophoresis is based mainly upon density differences that are a direct result of structural differences. With a time dependent experiment the rate at which an agent interacts with DNA can be followed to give a profile that is characteristic of the binding. As any structural change is translated into a density change, this sort of experiment provides a useful probe into the nature of the interactions the agent may have with DNA. It should be noted, however, that the fact that a complex binds to DNA does not necessarily imply that it will be an active anticancer agent. For instance trans-\([\text{PtCl}_2(\text{NH}_3)_2]\) binds readily to DNA, but it is not regarded as cytotoxic\(^\text{74}\).

In this chapter the binding or the reaction of the compounds \(\text{cis-}\left[[\text{PtCl}_2(\text{NH}_3)]_2(\mu-\text{dpzm})\right] , \text{trans-}\left[[\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})\right], \text{cis-}\left[[\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})\right], \text{Cs}_2\text{B}_{12}\text{H}_{11}\text{SH}, \left[\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{NH}_3)_5\right] \) and \(\left[\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{en})_2(\text{OH}_2)\right] \) to plasmid DNA is discussed and compared with the binding of the known anticancer agent cisplatin. The particular plasmid used in these experiments is known as pUC9, which is derived from the well known pBR322 plasmid\(^\text{230}\). The studies involved are known as time-dependent binding experiments and involve the sampling of mixtures of DNA and complex, which have been incubated for varying times, and then observing the changes that have occurred in the DNA by gel electrophoresis. The results of these experiments provide insights into the interaction of the complex with DNA and also the rate at which these interactions occur.
5.2. RESULTS AND DISCUSSION

5.2.1. The binding to DNA of cisplatin.

Time-dependent DNA binding experiments were performed with Form I pUC9 DNA which contained a small amount of Form II DNA. All the reactions conducted were maintained at a constant temperature of 36.0°C. It is pertinent to compare the DNA binding abilities of any potential anticancer complex with the well established effects of cisplatin. Thus, an experiment involving cisplatin was performed so that direct comparisons could be made.

The main feature of the binding of cisplatin to Form I DNA is the gradual unwinding of the supercoil to give the relaxed Form I DNA that comigrates with Form II DNA. The unwinding of the supercoils is accompanied by a progressive broadening of the band, which probably reflects statistical differences in the binding between individual DNA molecules. Further binding of cisplatin results in supercoiling in the opposite direction that causes the divergence of Form I and Form II DNA bands.

![Figure 5.4. Plot of the electrophoretic mobility of closed Form I (■) and nicked Form II (○) pUC9 DNA through agar gel after reaction with cis-[PtCl₂(NH₃)₂] for various times. The Rf values have been normalized against that of the Form I control and thus represent migration from bottom to top.](image-url)
Form II DNA is also affected by the binding of cisplatin, though not as dramatically. What is observed is that with increased binding its mobility also increases. This can be attributed to the shortening of the macromolecule due to localized denaturing or microloop formation induced by platinum crosslinks that may serve to disrupt the hydrogen-bonding between the base pairs of DNA. Thus, single stranded regions of DNA that are produced would collapse, and reduce the overall length of the DNA molecule. Binding is generally believed to involve the addition of Pt(NH$_3$)$_2^{2+}$ adducts. Thus, platinum binding should serve to reduce the net charge on the DNA molecule, reducing its electrophoretic mobility. Clearly, the structural effects of complex binding predominate over the electrostatic effects, as the binding of platinum adducts is seen to increase the mobility of the plasmid.

Under the conditions of the experiment, the comigration of Form I with Form II occurred after 5 hours. This is comparable to what has been determined previously. This time-dependence of the electrophoretic mobilities of the plasmid reflects the kinetics of the reaction of the complex with DNA.

5.2.2. The binding to DNA of cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)]]

A similar binding study, conducted with the complex cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)]] under identical conditions (Figure 5.5), produced a profile that was similar to that of cisplatin. That is, an untwisting of the supercoiled structure produced relaxed circular DNA Form I$_0$. Further binding produced a DNA structure that is supercoiled in the opposite direction. The major difference between the two was that cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)]] caused the comigration of Form I with Form II DNA after only two hours, compared to five hours with cisplatin binding. It should be noted that at equivalent concentrations cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)]] has twice the platinum, and thus twice the coordination sites available, than what cisplatin has. Even allowing for this cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)]] displays a much faster binding with DNA than its parent complex does. Hence, it can be regarded as a superior unwinding agent. This may be due
to either kinetic or stereochemical reasons. For instance, cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) may have better kinetic properties, such as the rate with which it binds to DNA, which increases its binding or that once bound to DNA it may exert a greater influence on the tertiary structure of DNA, so that each molecule bound produces a greater degree of unwinding. Since the amount of platinum bound to DNA has not been determined it is not possible to attribute the differences in the experiments to either of these mechanisms.

It is almost certain that the effects observed when cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) binds to DNA are due to covalent binding, similar to that seen with cisplatin binding. Ionic or hydrogen-bonding interactions that may occur would be reversed under the high salt concentration used to quench the reaction. Positively charged platinum species, which are weakly bound, would be separated from the DNA molecule during electrophoresis. A mode involving the intercalation of the complex cannot entirely be ruled out. This could occur through \(\pi\)-stacking non-covalent interactions between the base-pairs of DNA with the pyrazole rings and square-planar platinum centres of the complex. However, molecular modelling studies suggest that this would not be a favourable mode. For the pyrazole rings of dpzm to achieve the necessary coplanarity unfavourable steric interactions between the H3 or H5 and H3' or H5' protons would occur. The cutting or nicking of DNA is not observed with this complex. Redox type behaviour of the complex with DNA can be thus ruled out.

With tetrafunctional binding modes possible cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) can produce a greater number of different adducts when bound to DNA than cisplatin, which is at best only bifunctional. It appears possible that cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) could form both interstrand and intrastrand crosslinks that could bridge a number of nucleotides. DNA-protein crosslinks could also be formed. Unlike the dinuclear-dibridged platinum-dpzm complexes that have shown anticancer activity\(^{100-101}\), cis-\([\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu-\text{dpzm})]\) possesses coordinated ammonia groups, which are regarded as a definite advantage in producing anticancer properties\(^{231-236}\). It is not constrained into a 16-membered ring system as the dibridged analogues are, and thus has a greater degree of freedom to form unique adducts when bound to DNA. It still retains a pyrazole type
N–H group, which allows possible binding to d(GpA) sequences of DNA; a mode that is not accessible to cisplatin\textsuperscript{79}, but is accessible to $\beta\text{-}[\text{Cl}_2\text{Pt}(\mu\text{-dpzm})_2\text{PtCl}_2]$\textsuperscript{102}.

When compared to the dinuclear-monobridged complexes described by Farrell \textit{et al.}\textsuperscript{92,93}, cis-[$\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu\text{-dpzm})$] is more constrained. Adducts formed between DNA and cis-[$\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu\text{-dpzm})$] would be more rigidly held. This is likely to produce a greater effect on the tertiary structure of DNA, which may maximise cytotoxic effects; if it is assumed that the more affected the DNA molecule is, the less the replication mechanisms are able to function. The bridging ligand used by Farrell's group that produced the greatest cytotoxic effect was 1,4-diaminobutane\textsuperscript{98}. It is of interest to note that the maximum distances possible between the coordination sites of 1,4-diaminobutane and dpzm are about the same (~6 Å). It seems likely that the distance between platinum centres in dinuclear complexes would be important in determining the degree of activity.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5_5}
\caption{Plot of the electrophoretic mobility of closed Form I (■) and nicked Form II (●) pUC9 DNA through 1.5\% agarose gel after reaction with cis-[$\{\text{PtCl}_2(\text{NH}_3)\}_2(\mu\text{-dpzm})$] for various times. The $R_f$ values have been normalized against that of the Form I control and thus represent migration from bottom to top.}
\end{figure}
The preliminary nature of the studies presented makes the assignment of definite binding modes difficult. However, the DNA binding experiments performed do indicate that $\text{cis-}[[\text{PtCl}_2(\text{NH}_3)]_2(\mu-\text{dpzm})]$ has a large effect on plasmid DNA when compared to the known anticancer agent cisplatin. These results do not specify the actual usefulness of $\text{cis-}[[\text{PtCl}_2(\text{NH}_3)]_2(\mu-\text{dpzm})]$ as an anticancer drug, as many other factors are involved beside that of simple DNA binding. However, it is expected that this complex should show, in relatively simple in vitro systems, at least similar activity to that of cisplatin.

5.2.3. The binding to DNA of cis- and trans-$[[\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})]$.

An interesting comparison between two similar complexes has been obtained with the results of the binding studies conducted with cis- and trans-$[[\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})]$ (Figures 5.6 and 5.7). The trans isomer certainly binds to DNA with Form I comigrating with Form II after 3 to 4 hours, however, the two forms do not seem to separate with longer reaction times. It appears that either further binding of this complex with DNA does not promote further twisting of the DNA molecule or more probably a competing reaction occurs with the buffer solution, which inactivates the complex with respect to its DNA binding.

The cis isomer on the other hand shows only very marginal binding with DNA. Form I DNA does not comigrate with Form II over the period of the experiment (14 hours). $^1$H n.m.r. studies (Chapter Two) have shown that the cis isomer quickly undergoes a solvolysis reaction in dmf and this is probably the major factor that disrupts its binding to DNA. It is probable that the solvolysis products formed do not readily react with DNA and/or do not give adducts that alter the structure of DNA and produce untwisting.
Chapter Five

Figure 5.6. Plot of the electrophoretic mobility of closed Form I (■) and nicked Form II (●) pUC9 DNA through 1.5% agarose gel after reaction with trans-[(PtCl\(_2\)(Me\(_2\)SO))\(_2\)(μ-dpzm)] for various times. The \( R_f \) values have been normalized against that of the Form I control and thus represent migration from bottom to top.

Figure 5.7. Plot of the electrophoretic mobility of closed Form I (■) and nicked Form II (●) pUC9 DNA through 1.5% agarose gel after reaction with cis-[(PtCl\(_2\)(Me\(_2\)SO))\(_2\)(μ-dpzm)] for various times. The \( R_f \) values have been normalized against that of the Form I control and thus represent migration from bottom to top.
It is probable from these results that neither isomer will show significant anticancer activity. The trans isomer would not because its binding to DNA appears to be deactivated after about five hours incubation and also because trans isomers rarely show anticancer properties. The slow rate of binding does not necessarily rule out the cis isomer as an anticancer agent. Carboplatin, for example, is also known to have a very slow rate of binding to DNA when it is studied in similar experiments\(^40\). However, the loss of structural integrity that is observed in the cis isomer when it is in solution would suggest that it would not be active.

5.2.4. The reaction of DNA with borocaptate complexes.

The effects that the borocaptate anion and transition metal complexes of this anion have on plasmid DNA have been investigated.

![Figure 5.8. Plot of the electrophoretic mobility of closed Form I (■), nicked Form II (●) and linear Form III (▲) pUC9 DNA through 1.5% agarose gel after reaction with Cs\(_2\)B\(_{12}H_{11}\)SH for various times. The Rf values have been normalized against that of the Form I control and thus represent migration from bottom to top.](image)
The time-dependent reaction of the borocaptate anion with DNA produced an electrophoresis profile that showed no variation in the mobilities of the different forms of DNA over the course of the reaction (Figure 5.8). This indicates that no binding has occurred. What is observed is the sequential cutting of supercoiled Form I DNA to produce the relaxed circular Form II, which is further cut to produce the linear Form III DNA. The initially intense Form I DNA band faded over the period of the reaction, while the Form II DNA band increased in intensity to a maximum after about 8 hours after which it started to decrease again. After 5 hours a band attributable to Form III DNA was discernible and this increased in intensity during the remainder of the experiment.

![Electrophoretic mobility of DNA forms](image)

**Figure 5.9.** Plot of the electrophoretic mobility of closed Form I (■), nicked Form II (●) and linear Form III (▲) pUC9 DNA through 1.5% agarose gel after reaction with [Ru(SB12H11)(NH₃)₅] for various times. The Rf values have been normalized against that of the Form I control and thus represent migration from bottom to top.

The strand scission reaction that is involved is probably mediated by an oxygen species, most probably the superoxide radical, which has been implicated in the reaction mechanisms of other agents that give similar effects with DNA, such as bleomycin²³⁷,²³⁸ streptonigrin²³⁹ and bis(phenanthroline)copper(II)²⁴⁰,²⁴¹. An electron spin resonance
study of the auto-oxidation of the borocaptate anion has shown that superoxide radicals are generated. In order to confirm the actual involvement of superoxide in the borocaptate mediated cutting of DNA, further experiments in oxygen free systems or in the presence of the enzyme superoxide dismutase, which destroys superoxide as it is formed, would need to be conducted. These experiments were beyond the scope of the present investigation.

The ruthenium-borocaptate complexes, [Ru(SB12H11)(NH3)5] (Figure 5.9) and [Ru(SB12H11)(en)2(OH2)] (Figure 5.10), display profiles similar to the borocaptate anion, in that no binding occurs but rather the cutting of DNA is observed. The time taken for the appearance of Form III DNA is the same for both complexes and is about 9 hours. This is much slower than that found for the free ligand and probably indicates that on coordination there is a lowering of the reduction potential of the borocaptate group.

![Figure 5.10. Plot of the electrophoretic mobility of closed Form I (■), nicked Form II (○) and linear Form III (▲) pUC9 DNA through 1.5% agarose gel after reaction with [Ru(SB12H11)(en)2(OH2)] for various times. The Rf values have been normalized against that of the Form I control and thus represent migration from bottom to top.](image-url)
Chapter Five

5.3. DNA BINDING AND IN VITRO CYTOTOXICITY STUDIES

In vitro cytotoxicity studies have been conducted on the platinum complexes described (see Appendix One). These results were obtained from Me$_2$SO solutions of the various complexes with P388 murine tumour, L1210 murine leukemia and L1210 cisplatin resistant murine leukemia cell lines.

The results of the in vitro studies conducted with cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)] show that it is highly active against P388 tumour cells and is also active against both L1210 cell lines. These results agree with those obtained from the DNA binding studies, assuming that activity is directly related to DNA binding. It appears, in this case at least, that DNA binding studies can be used as a convenient means of determining the potential of DNA targeted anticancer drugs.

As predicted from its poor DNA binding cis-[[PtCl$_2$(Me$_2$SO)$_2$(μ-dpzm)] shows very little activity in all the cancer cell screens analysed. Surprisingly however, the trans isomer showed no activity whatsoever. This could have been due to the use of Me$_2$SO as the solvent in the in vitro studies. Results from $^1$H n.m.r. experiments (Chapter Two) suggest that a rapid solvolysis of this complex occurs in Me$_2$SO. This demonstrates that Me$_2$SO is inappropriate for use as solvent in biological assays involving platinum complexes, and that comparisons between different assays, which use different solvents, should not be made.

5.4. FURTHER STUDIES

The results from the time-dependent DNA binding studies and the in vitro anticancer screens suggest that cis-[[PtCl$_2$(NH$_3$)$_2$(μ-dpzm)] warrants further investigation. The next logical stage of biological testing is in vivo screening against murine cancer systems. The behaviour of this complex in animal models would be of
interest considering that it has shown at least as impressive results as any dinuclear platinum anticancer agent reported to date.

Conversely, cis–[[PtCl₂(Me₂SO)]₂(μ–dpzm)] has shown only poor DNA binding and only slight cytotoxicity toward cancer cells. Thus, further testing of this compound is not warranted.

The complex trans–[[PtCl₂(Me₂SO)]₂(μ–dpzm)] has shown some ability to bind with DNA, but has given no cytotoxic activity against the in vitro screens. This may be due to the use of Me₂SO as the solvent in the in vitro experiments. It would be of interest to know whether trans–[[PtCl₂(Me₂SO)]₂(μ–dpzm)] still gives the same results when a poorly coordinating solvent, such as dmf, is used in the experiment. Results of biological studies on platinum anticancer drugs that have been conducted in Me₂SO should be viewed with caution.
Chapter Six

EXPERIMENTAL DETAILS
6.1. **GENERAL**

Elemental analyses were measured by the Australian National University Microanalytical Service. Carbon, hydrogen and nitrogen analyses were obtained using a Carlo Erba 1106 automatic analyser. Chlorine and sulfur analyses were performed on a QIC instrument.

$^1$H n.m.r. spectra were obtained in D$_7$-dmf from either Varian *Gemini-300* spectrometer ($^1$H at 300 MHz) or a Varian *CFT-20* spectrometer ($^1$H at 80 MHz) with tms internal reference using 5mm quartz tubes, unless otherwise noted.

IR spectra were recorded on a Perkin-Elmer 683 Grating Infrared spectrometer and also on a Perkin-Elmer Series 1600 FT-IR as KBr disks, unless specified otherwise.

Gas chromatography-mass spectrometry (g.c.-m.s.) analyses were conducted on a Hewlett-Packard 5970 Series Mass Selective Detector, coupled to a 5980A Series Gas Chromatograph, using an *Ultra* 2 non-polar column and a column head pressure of 5 kpa. The temperature program used was: 0-2 min. 100°C; 2-10 min. ramp at 15°C / min. to 220°C; 10-15 min. hold at 220°C.

X-ray powder diffraction patterns were obtained using a Philips PW 1049 Counter Diffractometer (Philips PW 1010 generator) using Ni-filtered CuK$_\alpha$ ($\lambda = 1.5418$ Å) radiation.

Ultraviolet-visible spectra were recorded on a Shimadzu UV-160 spectrophotometer with the solvent as specified.

Magnetic susceptibilities were measured by the Gouy method in the solid state, at the temperature specified. The corrections for the diamagnetism of the ligands are as given in the literature.$^{242}$

Mass spectra were obtained using the fast atom bombardment (f.a.b.-m.s.) technique (Research School of Chemistry, Australian National University, Canberra, Australia). The samples were dissolved in Me$_2$SO prior to application onto the sample stage. Glycerol was used as the matrix.
Electrophoresis gels were illuminated from beneath with an ultraviolet lamp and then photographed with a Polaroid MP-4 camera using Polaroid type 55 positive-negative film and a red filter.

Cyclic voltammetric measurements made for \([\text{Ru(SB}_{12}\text{H}_{11})(\text{NH}_{3})_{5}]\cdot\text{2H}_{2}\text{O}\) were performed with E.G. & G. Model 173 potentiostat/galvanostat, Model 179 Digital Coulometer, Model 178 electrometer and Model 175 universal programmer, using a non-aqueous Ag/AgCl reference electrode\(^{196}\), a platinum working micro-electrode and a platinum wire counter electrode. Electrochemical solutions were 0.5 M NBu\(_4\)BF\(_4\) in anhydrous dmf with a sample concentration of about 10\(^{-3}\) M. All solutions were purged and maintained under a nitrogen atmosphere. Scan rate was 100 mVs\(^{-1}\). Cell temperatures were maintained by immersion in a constant temperature bath and were monitored by a digital thermometer probe within the cell solution.

6.2. SYNTHETIC PROCEDURES

6.2.1. Materials and Methods

Tetraphenylphosphonium bromide, [Ru(NH\(_3\))\(_6\)]Cl\(_3\) (Aldrich Chemical Co.) and cesium borocaptate (Boron Biologicals) were obtained commercially. Trifluoromethanesulfonic (triflic) acid was kindly supplied by Dr. L. M. Rendina (Research School of Chemistry, ANU). Other chemicals; l,l'-Dipyrazolylmethane\(^{186}\), cis-\([\text{PtCl}_2(\text{NH}_3)_2]^{243}\), K[PtCl\(_3\)(NH\(_3\))]\(^{172}\), K[PtCl\(_3\)(Me\(_2\)SO)]\(^{178}\), cis-\([\text{PtCl}_2(\text{Me}_2\text{SO})_2]^{244}\), cis-\([\text{PtCl}_2(\text{Me}_2\text{SO})_2]^{244}\), cis-\([\text{PtCl}_2(\text{Me}_2\text{SO})_2]^{244}\), trans-\([\text{RuCl}_2(\text{en})_2]\)Cl\(^{246}\), [NiCl\(_2\)(PEt\(_3\))\(_2\)]\(^{247}\), [PtCl(terpy)]Cl\(^{248}\) and cis-\([\text{RuCl}_2(\text{Me}_2\text{SO})_4]^{249}\) were all prepared according to literature methods. Milli-Q ultra-high purity water was used. All other reagents and organic solvents were of analytical grade or better and were used with no further purification, unless otherwise noted.
6.2.2. Preparation of tetraphenylphosphonium amminetrichloroplatinum(II), \([\text{PPh}_4][\text{PtCl}_3(\text{NH}_3)]\)

A solution of cis-[PtCl2(NH3)2] (0.35 g, 1.17 mmol) in concentrated hydrochloric acid (15 mL) was heated at reflux with powdered platinum metal catalyst (0.03 g) for 2 hours, whereupon an orange solution with a quantity of a yellow precipitate was formed. The precipitate, consisting of platinum metal and unreacted cis-[PtCl2(NH3)2], was filtered off and washed with cold water (60 mL). To the combined filtrates was added \([\text{PPh}_4]\)Br (0.48 g, 1.15 mmol). This mixture was stirred for two hours and then cooled to 5°C. The pale yellow precipitate that formed was collected, washed with water, ethanol and ether, and dried under vacuum. The solid was dissolved in dichloromethane and a small amount of green precipitate was removed. Ether was added to precipitate the product, which was collected and then dissolved in dmf. A small volume of chloroform was added and the brown crystals of \([\text{PPh}_4][\text{PtCl}_4]\) that formed on cooling were removed. The volume of the filtrate obtained was decreased by rotary-evaporation under reduced pressure. A small amount of ethyl acetate was added to promote the precipitation of the pale-orange product. The mixture was cooled at 5°C and the product was again collected, washed with ether and dried in a vacuum (yield 0.40 g, 52%). Anal. Calc. for PtC24H23NCl3P: C, 43.82; H, 3.52; N, 2.13. Found: C, 44.06; H, 3.76; N, 2.04. IR (cm\(^{-1}\)): 3322(w), 3252(m), 3187(sh), 3074(sh), 3053(m), 3019(w), 1585(m), 1481(m), 1440(sh), 1435(s), 1338(w), 1315(w), 1291(m), 1190(m), 1160(m), 1109(s), 1073(w), 1026(w), 994(m), 759(m), 749(sh), 724(s), 691(s), 616(w), 527(s), 457(w), 435(w), 332(sh), 320(m).

6.2.3. Preparation of 4,4'-dipyrazolylmethane, \(\text{dpzm}\)

1,1'-Dipyrazolylmethane (1.00 g, 6.75 mmol) was converted to its' hydrobromide salt by dissolving it in concentrated hydrobromic acid (5 ml). The excess acid solution was removed by rotary evaporation to leave an off white crystalline mass. Acetone (10
ml) was added and the salt was collected, washed further with a small quantity of acetone and dried at the pump.

This salt was heated in a sublimation apparatus at 200°C for 1 hour. A dark oily residue that deposited on the cooler surfaces was identified by g.c.-m.s. as being 4-bromopyrazole. Rt 2.3 min, m/z- 148(100%), 146(100%), 121(15%), 119(23%), 94(12%), 92(12%), 40(48%), 38(24%).

The solid residual mass was neutralised with excess potassium carbonate solution. The off white precipitate was collected and dried in a vacuum. By g.c.-m.s. analysis, this was identified as 4,4'-dipyrazolylmethane. Rt 8.4 min, m/z- 148(100%), 147(84%), 120(24%), 119(16%), 94(18%), 93(17%), 81(12%), 66(14%), 54(16%), 39(24%). The compound was further purified by sublimation under reduced pressure at 200°C. (Yield 0.57 g, 57%). Anal. Calc. for C₇H₈N₄ requires: C, 56.74; H, 5.44; N, 37.81. Found: C, 57.02; H, 5.52; N, 38.07. IR: 3137(br), 3054(sh), 2952(s), 2844(sh), 1570(w), 1507(m), 1443(m), 1393(s), 1349(s), 1297(w), 1267(m), 1220(w), 1184(w), 1036(s), 1059(sh), 1044(m), 999(s), 956(s), 926(m), 917(m), 870(s), 815(s), 742(s), 707(m), 655(m), 649(sh), 642(sh), 615(m), 607(m), 330(w), 317(w). ¹H n.m.r.: 12.66 ppm (br, 2 H, N-H), 7.49 ppm (s, 4 H, Pz-H), 3.71 ppm (s, 2 H, -CH₂-)

6.2.4.  **Preparation of [SP-4-2]-{(μ-4,4'-dipyrazolylmethane-N,N')bis(amminedichloroplatinum), cis-[PtCl₂(NH₃)₂(μ-dpzm)]**

The salt [PPh₄][PtCl₃(NH₃)] (0.36 g, 0.55 mmol) was dissolved in dichloromethane (30 ml). To it was added a solution of dpzm in ethanol (3 ml). The cloudy mixture was stirred overnight. The yellow product that formed was collected, washed with dichloromethane, water, ethanol and ether, and dried in a vacuum (yield 0.18 g, 0.25 mmol, 92% yield). Anal. Calc. for Pt₂C₇H₁₄N₆Cl₄ requires: C, 11.77; H, 1.98; N, 11.77. Found: C, 11.92; H, 2.16; N, 11.90. IR: 3267(br s), 3180(s), 3125(sh), 3117(s), 2974(m), 2860(sh), 1477(m), 1437(m), 1409(s), 1367(w), 1308(s),
1245(w), 1140(s), 1080(m), 1010(sh), 975(w), 859(m), 827(m), 747(m), 682(w), 586(m), 504(w), 333(m), 322(sh). \(^1\)H n.m.r.: 13.7 ppm (br, 1 H, N–H), 7.91 ppm (s, 1 H, Pz–H5), 7.84 ppm (s, 1 H, Pz–H3), 4.43 ppm (br, 3 H, Pt–NH₃), 3.77 ppm (s, 2 H, –CH₂–). X-ray powder diffraction data [\(\theta\) angle (relative intensity)]: Amorphous.

6.2.5.  \textit{Preparation of [SP-4-1]-([\mu-4,4'-dipyrazolylmethane-N,N']bis(dichloro(S–dimethylsulfoxide)platinum), trans-[[PtCl}_{2}(Me₂SO)]₂(\mu-dpzm)]}

The salt K[PtCl₃(Me₂SO)] (0.33 g, 0.79 mmol) was dissolved in water (5 ml). Ethanol (5 ml) was added and the solution was stirred magnetically. The ligand, dpzm (0.39 mmol, 0.058 g) dissolved in 1:1 ethanol-water (2 ml), was added dropwise over a 10 minute period. After 1 minute the solution became cloudy. The reaction was left to proceed overnight. The yellow solid that formed was filtered off from the colourless mother liquor and was then washed with ethanol and ether, and dried at the pump (yield 0.30 g, 92% yield). Anal. Calc. for Pt₂C₁₁H₂₀N₄Cl₄S₂O₂ requires: C, 15.80; H, 2.41; N, 6.70; S, 7.67; Cl 16.96. Found: C, 16.22; H, 2.43; N, 6.60; S, 7.19; Cl, 17.59. IR: 3548(br m), 3328(s), 3127(br s), 3071(sh), 3001(s), 2875(w), 1623(w), 1574(w), 1521(w), 1476(m), 1440(m), 1409(s), 1374(sh), 1345(sh), 1316(m), 1297(m), 1238(w), 1120(br s), 1080(s), 1024(s), 1000(sh), 975(s), 933(sh), 921(m), 878(m), 867(sh), 833(m), 749(s), 719(sh), 698(m), 613(m), 585(m), 506(w), 440(s), 375(m), 340(s), 320(sh). \(^1\)H n.m.r.: 8.07 ppm (s, 2 H, Pz–H5), 8.02 ppm (s, 1 H, Pz–H3), 7.95 ppm (s, 1 H, Pz–H3), 3.86 ppm (s, 2 H, –CH₂–), 3.50 ppm (s, 12 H, OS(CH₃)₂). X-ray powder diffraction data [\(\theta\) angle (relative intensity)]: 11.6 (35), 12.0 (100), 12.3 (77), 12.7 (42), 13.3 (88), 14.9 (99), 16.5 (44), 18.3 (59), 19.0 (72), 19.8 (61), 20.3 (31), 20.9 (37), 22.0 (87), 23.4 (42), 24.4 (58), 25.0 (25), 25.6 (28), 26.7 (38), 27.2 (35), 28.9 (49), 29.8 (57), 30.2 (35), 30.5 (32), 32.5 (26), 33.2 (21), ...
6.2.6. Preparation of \([\text{SP-4-2]}-(\mu-4,4'-\text{dipyrazolylmethane-N,N'})\text{bis(dichloro(S-dimethylsulfoxide)}\text{platinum}), \text{cis-}[\text{PtCl}_2(\text{Me}_2\text{SO})]_2-(\mu-\text{dpzm})\].

**Method A:** The complex \(\text{cis-}[\text{PtCl}_2(\text{Me}_2\text{SO})]_2\) (0.43 g, 1.02 mmol) was dissolved in warm water (15 ml). To this was added dropwise dpzm (0.075 g, 0.51 mmol) dissolved in warm water (5 ml). A pale yellow precipitate formed immediately. The mixture was left for 2 hours and the precipitate was collected. It was washed with water, ethanol and finally ether, and dried in a vacuum (yield 0.39 g, 0.47 mmol, 91% yield).

**Method B:** A small quantity of \(\text{trans-}[\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})\] (0.10 g, 0.12 mmol) was dissolved in Me2SO (3 ml). To this was added 0.1 M hydrochloric acid (1.5 ml) and the resulting mixture was left for 3 days. The precipitate that formed was collected, washed with water, ethanol and chloroform, and dried at the pump. The product was identical analytically and spectroscopically to that obtained by Method A.
6.2.7. **Attempted preparation of** \([\text{SP-}4-3]-(\mu-4,4'\text{-dipyrazolylmethane-N,N'})\text{bis(malonato(S-dimethylsulfoxide)platinum),} \) 
\([\{\text{Pt(mal)}(\text{Me}_2\text{SO})\}_2(\mu-\text{dpzm})]\). 

To a stirred solution of \([\text{Pt(mal)}(\text{Me}_2\text{SO})_2] \) (0.36 g, 0.79 mmol) in water (10 ml) was added dpzm (0.06 g, 0.40 mmol) dissolved in water (3 ml). A white precipitate was formed immediately and the mixture was stirred for a further 8 hours. The precipitate (0.33 g, 0.37 mmol) was collected washed with water, ethanol and ether, and dried at the pump. The product was totally insoluble in hot water, hot dmf, hot Me₂SO and aqua regia. *Anal.* Calc. for Pt₂C₁₇H₂₃N₄O₁₀S₂ requires: C, 22.75; H, 2.58; N, 6.24. Found: C, 19.71; H, 2.42; N, 6.90. IR: 3432(br s), 3119(sh), 3004(m), 2916(m), 1635(br s), 1589(s), 1387(br), 1355(br), 1250(m), 1132(s), 1083(s), 1024(s), 976(m), 922(m), 826(m), 746(m), 697(m), 620(w), 440(m), 375(m).

6.2.8. **Preparation of pentaamminechlororuthenium(III) chloride, \([\text{RuCl(NH}_3)_5]\text{Cl}_2\)** 

Commercial red \([\text{Ru(NH}_3)_6]\text{Cl}_3 \) (4.00 g, 12.9 mmol) was refluxed in 6 M hydrochloric acid (120 ml) for 3 hours whereupon a buff precipitate was observed. Precipitation was completed by cooling at 4°C for 16 hours. The solid was collected and then dissolved in hot 0.1 M hydrochloric acid. A dark insoluble material was removed by filtration at this stage. The filtrate was cooled at 4°C for 16 hours and the yellow-orange crystals of \([\text{RuCl(NH}_3)_5]\text{Cl}_2\) that formed were collected, washed with ethanol and ether, and dried at the pump (yield 3.2 g, 85%). IR (cm⁻¹): 3222(br), 1617(s), 1297(s), 801(s), 488(m), 466(m), 455(m), 295(s), 255(s).
6.2.9. Preparation of pentaammine(1-thiolato-closoundecahydrododecaborane)ruthenium(III) dihydrate, \([\text{Ru(SB}_{12}\text{H}_{11})(\text{NH}_{3})_{5}]\cdot\text{2H}_2\text{O}\)

A warm solution of Cs₂B₁₂H₁₁SH (0.20 g, 0.45 mmol) in water (5 ml) was filtered through a 45 μm syringe filter unit. To this was added dropwise through a 45 μm syringe filter unit \([\text{RuCl(NH}_{3})_{5}]\text{Cl}_2\) in warm (~40°C) water (5 ml). After about 5 seconds an intense deep-blue formed, and shortly after, a crystalline precipitate was evident. The mixture was left at room-temperature for 15 minutes and was then cooled at 5°C for 1 hour. The product was collected, washed with water, ethanol-water (1:1) and ethanol, and dried under vacuum. The product consisted of fine dark-blue needles (yield 0.14 g, 87% yield). Anal. Calc. for RuB₁₂H₂₆N₅S·2H₂O requires: H, 7.65; N, 17.72; S, 8.11. Found: H, 6.85; N, 16.69; S, 7.52. IR: 3611(m), 3423(m), 3321(s), 3252(s), 3181(sh), 2475(s), 1609(m), 1294(s), 1313(sh), 1055(m), 963(m), 840(m), 806(w), 760(m br), 722(m), 455(w), 402(w), 330(w). ¹H n.m.r. 8.00ppm (br, NH₃), 3.64ppm (br, B₁₂H₁₁). Ultraviolet-visible spectrum (Me₂SO, 9.9 x 10⁻⁵ M): 583nm (ε = 1300 M⁻¹cm⁻¹), 440nm (ε = 1200 M⁻¹cm⁻¹). Magnetic susceptibility (19.8°C); \(\chi_g = 2.25 \times 10^{-6}\) cgs. Cyclic voltammetry couples vs. Ag/AgCl (V): +0.29(+0.39), +0.08(+0.18), (-0.55), -0.24(-0.34). X-ray powder diffraction data (2θ angle (relative intensity)): 9.50 (23), 12.00(37), 12.90(15), 14.70(40), 15.30(59), 19.00(100), 20.00(25), 22.30(19), 23.10(12), 24.00(31), 25.80(11), 27.60(13), 29.70(14), 30.40(13), 32.70(13), 33.80(11), 35.20(14), 35.60(12), 36.30(11), 38.70(11), 41.40(12), 42.00(9).

6.2.10. Preparation of tris(trifluoromethanesulfanato)(2,2',5',2''-tripyridine)ruthenium(III), \([\text{Ru(O}_3\text{SCF}_3)]_3(\text{terpy})]\)

Into a three-necked round bottom flask (50 ml), flushed with nitrogen, was placed \([\text{RuCl}_3(\text{terpy})]\)·½H₂O (0.20 g, 0.45 mmol). To this was added dropwise trifluoromethanesulfonic (triflic) acid (1.2 ml). This gave a deep-green solution that was
heated for 3 hours, after which time the nitrogen effluent gave a negative AgNO₃ test for chloride. The mixture was left at room-temperature for a further 16 hours. Ether was added, producing a dark-green crystalline precipitate. This product was collected, washed thoroughly with ether (3 x 20 ml), and dried in a vacuum (Yield 0.34 g, 96% yield). The product hydrolysed rapidly in air to give a dark-purple solid. Anal. Calc. for RuC₁₈H₁₁N₃F₉O₉S₃·3H₂O (after hydrolysis) requires: C, 25.87; H, 2.05; N, 4.92. Found: C, 25.63; H, 1.67; N, 4.92. IR: 3406(br), 3093(m), 1602(m), 1476(m), 1453(m), 1354(s), 1313(sh), 1235(s), 1202(s), 1099(sh), 1055(sh), 1027(s), 956(s), 903(sh), 825(w), 776(s), 732(m), 518(m), 461(m), 348(w).

6.2.11. Preparation of (OC-6-23)-diaquo(1-thiolato-closo-undecahydrododecaborane)(2,2'5',2''-tripyridine)ruthenium(III), [Ru(SB₁₂H₁₁)(terpy)(OH₂)₂]

The complex [Ru(O₃SCF₃)₃(terpy)] (0.29 g, 0.37 mmol) was placed in water (5 ml) and heated at 60°C for 1 hour, giving a dark aqua-green solution. This was filtered through a 45 μm syringe filter unit. To this solution was added a similarly filtered solution of Cs₂B₁₂H₁₁SH (0.18 g, 0.40 mmol) in water (5 ml), which caused a rapid colour change to dark maroon. A dark precipitate became evident within 5 minutes. The mixture was left for 16 hours and the precipitate was collected, washed with water and acetone, and dried in a vacuum at 60°C for 5 hours (Yield 0.17 g, 85% yield). The complex dissolves readily in base and can be reprecipitated with acid. Anal. Calc. for RuB₁₂C₁₅H₂₆N₃O₂S requires: C, 33.16; H, 4.82; N, 7.74. Found: C, 31.68; H, 4.67; N, 7.05. IR: 3445(br), 3078(sh), 2500(s), 1691(m), 1601(m), 1468(m), 1449(s), 1388(m), 1285(m), 1244(m), 1162(m), 1093(w), 1051(m), 963(m), 837(m), 767(s), 733(sh), 669(w), 647(w), 574(w), 527(w), 494(w). Magnetic susceptibility (20.4°C); χg = 0.598 x 10⁻⁶ cgs.
6.2.12. Preparation of \((\text{OC-6-23})\text{-aquobis}(1,2\text{-ethanediamine})(1\text{-thiolato-closo-}\text{undecahydrododecaborane})\text{ruthenium(III)}, \text{trans-}[\text{Ru}(\text{SB}_{12}\text{H}_{11})(\text{en})_{2}(\text{OH}_{2})]\)

In a three-necked round bottom flask, equipped with a nitrogen bubbler, was placed \text{trans-}[\text{RuCl}_{2}(\text{en})_{2}]\text{Cl} (0.29 g, 0.89 mmol). To this was added triflic acid (2 ml), which caused some vigorous bubbling, and produced a dark orange solution. This was left for 20 minutes at room temperature and then heated at 110°C for 16 hours. After cooling back to room-temperature, ether (20 ml) was added giving a dark orange-brown oil and an ether layer. The ether layer was discarded and fresh ether was added and mixed with the oil. The ether was again discarded and the washing was repeated.

The residual oil was dissolved in water (20 ml), giving a dark tea-coloured solution. To this was added Cs\(_2\)B\(_{12}\)H\(_{11}\)SH (0.39 g, 0.89 mmol) dissolved in water (10 ml). This mixture was set aside for 2 hours. The brown precipitate that had formed was collected, washed with water and ethanol, and dried in a vacuum (yield 0.26 g, 93% yield). *Anal. Calc. for RuB\(_{12}\)C\(_4\)H\(_{29}\)N\(_4\)OS requires: C, 10.44; H, 6.35; N, 12.18. Found: C, 10.96; H, 6.58; N, 11.18. IR: 3579(br), 3425(br), 3297(s), 3254(s), 3156(sh), 2942(m), 2884(m), 2475(s), 1580(s), 1454(m), 1396(w), 1365(w), 1316(w), 1284(m), 1163(m), 1108(m), 1045(s), 1003(w), 976(m), 900(w), 837(m), 718(m), 578(w), 534(w). Magnetic susceptibility (20.6°C); \(\chi_{g} = 0.512 \times 10^{-6}\) cgs.*

6.2.13. Preparation of bis(tetrabutylammonium) 1-mercapto-closo-undecahydrododecaborate(2-), \([\text{N(Bu)}_{4}]_{2}[\text{B}_{12}\text{H}_{11}\text{SH}]\)

To a solution of Cs\(_2\)B\(_{12}\)H\(_{11}\)SH (0.20 g, 0.45 mmol) in water (2 ml) was added dropwise a solution of tetrabutylammonium bromide in water (2 ml). The white precipitate that formed was collected. It was then dissolved in methylene chloride (10 ml)
Chapter Six

and this solution was dried with anhydrous MgSO₄. The MgSO₄ was removed and the solvent was evaporated off to leave the product as a white powder (0.11 g, 0.30 mmol, 64% yield). This product was used without any further purification. IR: 2961(s), 2873(s), 2732(w), 2478(s), 1988(w), 1845(w), 1470(s), 1420(w), 1380(m), 1362(w), 1319(w), 1283(w), 1243(w), 1169(m), 1151(m), 1108(m), 1060(sh), 1043(s), 978(sh), 865(m), 925(w), 882(m), 848(w), 835(m), 803(w), 740(m), 717(m), 668(w), 618(w).

6.2.14. The reaction of [NiCl₂(PEt₃)₂] with [NBu₄][B₁₂H₁₁SH]

No reaction was observed when [NBu₄]₂[B₁₂H₁₁SH] (0.38 g, 0.57 mmol) was dissolved with [NiCl₂(PEt₃)₂] in refluxing benzene, only starting materials were returned.

6.2.15. The reaction of [PtCl(terpy)]Cl with Cs₂B₁₁H₁₂SH

To [PtCl(terpy)]Cl (0.223 g, 0.428 mmol) dissolved in water (20 ml) was added dropwise AgNO₃ (0.144 g, 0.845 mmol) in water (10 ml). This mixture was stirred in the dark, under a nitrogen atmosphere, for 16 hours. This was then heated on a hot water bath to dissolve the orange precipitate that was formed. The white AgCl precipitate that remained was removed by centrifugation at 5000 r.p.m.

To the mother liquor was added Cs₂B₁₁H₁₂SH (0.185 g, 0.422 mmol) in water (5 ml). The solution imediatedly went brown with a flocculent precipitate present. This was stirred at room-temperature overnight under a nitrogen atmosphere.

The red-brown precipitate that had formed was collected by centrifugation at 5000 r.p.m. The pellet that was isolated was dried in a vacuum overnight, to give a black flaky solid. The solid was not soluble in dmf or Me₂SO. IR (peaks generally poorly resolved): 3580(s br), 3073(m), 2457(s br), 1597(m), 1519(w), 1473(m), 1450(m), 1440(m), 1395(m), 1313(m), 1248(m), 1162(m), 1137(w), 1093(w), 1045(m), 1028(m), 955(m), 823(m), 769(s), 721(m), 658(w), 616(w), 450(m).
6.2.16. **The reaction of cis-**[**RuCl_2(Me_2SO)_4]** **with**

**Cs_2B_{12}H_{11}SH**

To a stirred solution of cis-[RuCl_2(Me_2SO)_4] (0.58 g, 1.20 mmol) in water (25 ml) was added Cs_2B_{12}H_{11}SH (0.50 g, 1.19 mmol) in water (25 ml). The resulting yellow solution was stirred at room temperature for 16 hours.

The clear brown solution that formed was evaporated under reduced pressure to a small volume (~10 ml), whereupon precipitation occurred. Absolute ethanol (50 ml) was added and the precipitate was collected. A further quantity of product was obtained by adding more ethanol and cooling the mixture at 5°C overnight (yield 0.70 g). **Anal.** Calc. for RuB_{12}CsC_4H_{29}O_5S_3 (i.e. Cs[Ru(SB_{12}H_{11})(OH)_2_(Me_2SO)_2]) requires: C, 7.78; H, 4.74; N, 0.00. Found: C, 7.71; H, 4.88; N, 0.00. **IR:** 3595(br s), 3433(br s), 3013(m), 2924(m), 2496(s), 1617(br m), 1412(m), 1295(m), 1096(s), 1016(s), 971(m), 928(m), 837(m), 720(m), 680(m), 494(w), 423(m), 384(w), 372(sh). F.a.b.-m.s.: 709(5%), 619(9%), 617(8%), 600(15%), 543(8%), 541(16%), 540(8%), 539(15%), 538(10%), 463(9%), 461(8%), 451(8%), 450(7%), 439(9%), 419(15%), 355(18%), 312(24%), 301(21%), 292(19%), 287(14%), 286(100%), 245(11%), 211(40%).

6.3. **PLASMID DNA-BINDING EXPERIMENTS**

6.3.1. **Materials and Methods**

Plasmid pUC9 DNA in sterile water was kindly supplied by Dr. N. Dixon (Research School of Chemistry, ANU). Tris(hydroxymethyl)aminomethane (Tris) (Sigma Chemical Co.), agarose (standard low-mr) (Bio-Rad), and 3,8-diamino-5-ethyl-6-phenylphenanthridium bromide (ethidium bromide) (Aldrich Chemical Co.) were obtained commercially. The metal complexes used were prepared as described in Section 6.2. All other reagents were of analytical grade or better.
6.3.2. Preparation of saline-phosphate buffer

Sodium chloride (1.75 g, 29.4 mmol) was dissolved in a mixture of KH$_2$PO$_4$ (0.1 M, 21.8 ml) and K$_2$HPO$_4$ (0.1 M, 78.2 ml). An 100-fold dilution of this gave a buffer solution with pH = 7.4 at 20°C ([Cl$^-$] = 3 mM and [PO$_4^{3-}$] = 1 mM).

6.3.3. Plasmid DNA-binding experiments

To a freshly prepared solution of cis-[PtCl$_2$(NH$_3$)$_2$] (0.067 mM, 100 µl) in NaCl (4.5 mM) and phosphate (1.5 mM) buffer was added pUC9 DNA (200 µg / ml, 50 µl) and this stock solution was incubated at 37.0°C ± 0.1°C. At various times (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 14 hours) a sample (10 µl) was removed, the reaction was quenched by the addition of NaCl (1 M, 2.5 µl), and the sample was stored at -5°C.

To the samples was added tracking dye (0.2% (w/v) bromophenol blue and 53% (w/v) glycerol, 6 µl), and gel electrophoresis was carried out in a horizontal slab gel composed of agarose (1.5% (w/v)) and TBE buffer (90 mM Tris, 90 mM boric acid and 2.2 mM Na$_2$H$_2$edta·2H$_2$O, pH = 8.3 at 22°C), loaded with 14 µl of sample per well. This was run at 30 V for 24 hours at room temperature. The gel was stained with TBE buffer containing ethidium bromide (0.5 pg / ml) for 12 hours and photographed as described previously.

The above procedure was repeated with the mole equivalent of cis-[[PtCl$_2$(NH$_3$)$_2$](µ-dpzm)], trans-[[PtCl$_2$(Me$_2$SO)$_2$](µ-dpzm)], cis-[[PtCl$_2$(Me$_2$SO)$_2$](µ-dpzm)], Cs$_2$B$_{12}$H$_{11}$SH, [Ru(SB$_{12}$H$_{11}$)(NH$_3$)$_5$]·2H$_2$O and trans-[Ru(SB$_{12}$H$_{11}$)-(en)$_2$(OH$_2$)]
Appendix One

IN VITRO ANTICANCER STUDIES
A1.1.  **RESULTS OF IN VITRO ANTICANCER STUDIES**

The platinum complexes described (Chapter Two) have been screened against a number of cancer cell lines *in vitro* by Dr. L. K. Webster at the Andrew Durant Drug Testing Facility (Experimental Chemotherapy and Pharmacology Unit), Peter MacCallum Cancer Institute, Melbourne, Australia. These results are presented in Table A1.1 along with those obtained for cisplatin, which was used as a positive reference.

*Table A1.1.*  In vitro screens of platinum complexes, expressed as $IC_{50}^*$ ($\mu$M) for a 48 hour continuous exposure of the cells to the compound dissolved in $Me_2SO$ followed by counting using a Coulter counter.

<table>
<thead>
<tr>
<th>Compound</th>
<th>P388</th>
<th>L1210</th>
<th>L1210/cisplatin resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-${[PtCl_2(NH_3)]_2(\mu-dpzm)}$</td>
<td>0.4</td>
<td>5.2</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cis-${[PtCl_2(Me_2SO)]_2(\mu-dpzm)}$</td>
<td>$&gt;20$</td>
<td>$&gt;20$</td>
<td>$&gt;20$</td>
</tr>
<tr>
<td></td>
<td>$&gt;100$</td>
<td></td>
<td>$&gt;50$</td>
</tr>
<tr>
<td>trans-${[PtCl_2(Me_2SO)]_2(\mu-dpzm)}$</td>
<td>Not determined</td>
<td>No response up to 100$\mu$g/ml</td>
<td>Not determined</td>
</tr>
<tr>
<td>cis-$[PtCl_2(NH_3)_2]$</td>
<td>0.5 (saline or $Me_2SO$)</td>
<td>0.8 (saline)</td>
<td>6.2 (saline)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.4 ($Me_2SO$)</td>
</tr>
</tbody>
</table>

$IC_{50}$ is defined as the concentration ($\mu$M) required to produce of 50% inhibition of growth in the culture.

A1.2.  **DISCUSSION**

The complex *cis-[[$PtCl_2(NH_3)_2]_2(\mu-dpzm)$]* displays activity that is comparable to cisplatin in P388 murine tumour cells. In the L1210 murine leukemia cell line this activity is somewhat reduced in comparison. In the L1210 cell line, which is resistant to the effects of cisplatin, the activity of *cis-[[$PtCl_2(NH_3)_2]_2(\mu-dpzm)$]* remains essentially the same as it is in the normal cell line. The resistance factor$^{98}$, calculated as the ratio $IC_{50}$ cisplatin resistant / $IC_{50}$ normal cell line, for this complex is 1.03. Thus, it can be seen that *cis-[[$PtCl_2(NH_3)_2]_2(\mu-dpzm)$]* is as toxic toward cisplatin resistant L1210 cells as it...
Appendix One

is to normal L1210 cells. To achieve this the complex must have a mode of action that is quite unlike that of cisplatin.

The other complexes screened, cis– and trans-\([\text{PtCl}_2(\text{Me}_2\text{SO})]_2(\mu-\text{dpzm})\) have not shown any significant activity in the cell lines tested. This may be due to the use of Me\(_2\text{SO}\) as the solvent for these complexes. It is known from \(^1\text{H}\) n.m.r. studies (Chapter Two) that rapid solvolysis reactions occur when these complexes are dissolved in this solvent, which may lead to a complete loss of structural integrity.

The use of Me\(_2\text{SO}\) as the solvent for cis-\([\text{PtCl}_2(\text{NH}_3)]_2(\mu-\text{dpzm})\) may explain the variability in the results obtained for this complex, as it too will undergo a rapid solvolysis in Me\(_2\text{SO}\). If a solvent that does not give such dramatic solvolysis effects was used, such as dmf for instance, then even better cytotoxicities might be observed. Clearly, the lack of solubility in water is an impediment to the biological effectiveness of these complexes and further efforts should be directed at overcoming this problem.

In light of the good cytotoxicity cis-\([\text{PtCl}_2(\text{NH}_3)]_2(\mu-\text{dpzm})\) has displayed, particularly against P388 tumour cells, further in vitro and in vivo testing of this compound is warranted, despite the fact that it may be necessary to use Me\(_2\text{SO}\) as the solvent in these assays.
Appendix Two

OBSERVED AND CALCULATED STRUCTURE FACTOR AMPLITUDES FOR
[Ru(SB_{12}H_{11})(NH_{3})_{5}]*2H_{2}O
### Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>FobS</th>
<th>Fcals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>131</td>
<td>Focal</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>4</td>
<td>149</td>
<td>Focal</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>6</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>8</td>
<td>78</td>
<td>75</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>10</td>
<td>68</td>
<td>67</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>12</td>
<td>67</td>
<td>66</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>14</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>16</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>3</td>
<td>67</td>
<td>62</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>4</td>
<td>42</td>
<td>41</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>5</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>6</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>7</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>8</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>9</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>10</td>
<td>13</td>
<td>62</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>11</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>12</td>
<td>43</td>
<td>44</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>13</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>14</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>15</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>16</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
<td>88</td>
<td>85</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>1</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>2</td>
<td>219</td>
<td>195</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>3</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>4</td>
<td>139</td>
<td>131</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>5</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>6</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>7</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>8</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>9</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>10</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>11</td>
<td>56</td>
<td>55</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>12</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>13</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>14</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>15</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>16</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>1</td>
<td>126</td>
<td>120</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>3</td>
<td>159</td>
<td>154</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>4</td>
<td>86</td>
<td>81</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>5</td>
<td>144</td>
<td>141</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>6</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>7</td>
<td>122</td>
<td>122</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>8</td>
<td>44</td>
<td>49</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>9</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>10</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>11</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>12</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>13</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>14</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>15</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>16</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>1</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>2</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>3</td>
<td>74</td>
<td>68</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>4</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>5</td>
<td>54</td>
<td>52</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>6</td>
<td>37</td>
<td>35</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>7</td>
<td>44</td>
<td>39</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>8</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>9</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>10</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>11</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>1</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>2</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>3</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>4</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td>34</td>
<td>33</td>
</tr>
</tbody>
</table>
## Appendix Two

<table>
<thead>
<tr>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>38</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>37</td>
<td>8</td>
</tr>
<tr>
<td>38</td>
<td>4</td>
</tr>
<tr>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
</tr>
</tbody>
</table>
### Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcalc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>7</td>
<td>2122</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>8</td>
<td>2426</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>9</td>
<td>1515</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>10</td>
<td>1617</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>11</td>
<td>109</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>12</td>
<td>1818</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>13</td>
<td>87</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>14</td>
<td>1616</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-15</td>
<td>1011</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-14</td>
<td>3434</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-13</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-12</td>
<td>3536</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-11</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-10</td>
<td>2626</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-9</td>
<td>2524</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-8</td>
<td>3636</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-7</td>
<td>4544</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-6</td>
<td>3940</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-5</td>
<td>05</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-4</td>
<td>5961</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-3</td>
<td>7176</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-2</td>
<td>5756</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>-1</td>
<td>6970</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>0</td>
<td>5755</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>1</td>
<td>5858</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>2</td>
<td>4243</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>3</td>
<td>4344</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>4</td>
<td>3436</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>5</td>
<td>5452</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>6</td>
<td>52</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>3939</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>8</td>
<td>77</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>9</td>
<td>6262</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>10</td>
<td>1111</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>11</td>
<td>5961</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>12</td>
<td>52</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>13</td>
<td>4950</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>14</td>
<td>1212</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-14</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-13</td>
<td>2424</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-12</td>
<td>42</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-11</td>
<td>2426</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-10</td>
<td>44</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-9</td>
<td>3130</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-8</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-7</td>
<td>1111</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-6</td>
<td>2422</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-5</td>
<td>4550</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-4</td>
<td>5453</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-3</td>
<td>4343</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-2</td>
<td>8784</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>-1</td>
<td>4949</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
<td>7273</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>1</td>
<td>3535</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>2</td>
<td>5960</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>3</td>
<td>2525</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>4</td>
<td>5050</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>5</td>
<td>2324</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>6</td>
<td>4443</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>7</td>
<td>1012</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
<td>5958</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>9</td>
<td>412</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>10</td>
<td>6769</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>11</td>
<td>1010</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>12</td>
<td>5659</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>13</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>14</td>
<td>3636</td>
<td>9</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>-14</td>
<td>3839</td>
<td>11</td>
</tr>
</tbody>
</table>
### Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>-1</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>1</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>3</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>5</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-16</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-14</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-10</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>- 6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-12</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>-2</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>4</td>
<td>81</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>6</td>
<td>87</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>8</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>10</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>12</td>
<td>51</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>14</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-16</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-15</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-14</td>
<td>7</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-13</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-12</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-11</td>
<td>47</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-10</td>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-9</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>- 8</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-7</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>- 6</td>
<td>66</td>
<td>67</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>- 5</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>- 4</td>
<td>67</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-3</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>- 2</td>
<td>179</td>
<td>173</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>-1</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0</td>
<td>121</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>158</td>
<td>152</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>74</td>
<td>76</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>5</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
<td>80</td>
<td>81</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>7</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>8</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>10</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>11</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>12</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>13</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>14</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>15</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-16</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-15</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-14</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-13</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-12</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>11</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>9</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>7</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6</td>
<td>67</td>
<td>64</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>5</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>53</td>
<td>58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>-16</td>
<td>49</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>53</td>
<td>58</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>-16</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>h</td>
<td>k</td>
<td>l</td>
<td>Fobs</td>
<td>Fcal</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>9</td>
<td>57</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>11</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>12</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>13</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-15</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-14</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-13</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-12</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-11</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-10</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-8</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-7</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-6</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-5</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-4</td>
<td>62</td>
<td>63</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-3</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-2</td>
<td>54</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>0</td>
<td>66</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>1</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>2</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>4</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>5</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>7</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>8</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>9</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>10</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>11</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>12</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>13</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>14</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-14</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-13</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-12</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-11</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-10</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-9</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-8</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-7</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-6</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-5</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-3</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-2</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-1</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>2</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>5</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>6</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>7</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>8</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>9</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>10</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>11</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>12</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>13</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>-13</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>-12</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>-11</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>-10</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>-9</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>h</td>
<td>k</td>
<td>1</td>
<td>Fobs</td>
<td>Fcal</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>-8</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>-6</td>
<td>80</td>
<td>79</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>-4</td>
<td>110</td>
<td>113</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>-2</td>
<td>217</td>
<td>205</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>129</td>
<td>134</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
<td>51</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>6</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>8</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>10</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>14</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-16</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-15</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-14</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-13</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-11</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-9</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-8</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-6</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-5</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-4</td>
<td>69</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-3</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-2</td>
<td>180</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-1</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>96</td>
<td>91</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4</td>
<td>58</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>6</td>
<td>45</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>7</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>8</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>10</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>11</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>12</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>13</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>14</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>15</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-16</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-15</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-14</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-13</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-12</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-11</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-10</td>
<td>77</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-9</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-8</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-7</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-6</td>
<td>103</td>
<td>104</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-5</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-4</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-3</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-2</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>-1</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>0</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>4</td>
<td>55</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>5</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>6</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>7</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>8</td>
<td>44</td>
<td>45</td>
</tr>
</tbody>
</table>
## Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7</td>
<td>-3</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>-2</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>-1</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>1</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>2</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>3</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>4</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>5</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>6</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>7</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>8</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>10</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>12</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>13</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-14</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-13</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-12</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-10</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 9</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 8</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 7</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 6</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 5</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 4</td>
<td>58</td>
<td>57</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 3</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 2</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>- 1</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>2</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>5</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>9</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>11</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>12</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-13</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-12</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-11</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-10</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-9</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-8</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-7</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-6</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-5</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-4</td>
<td>61</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-2</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>-1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0</td>
<td>63</td>
<td>69</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>1</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>2</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>3</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>4</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>5</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>6</td>
<td>55</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>7</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>8</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>9</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>10</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>h</td>
<td>k</td>
<td>1</td>
<td>Fobs</td>
<td>Fcal</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>9</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>10</td>
<td>42</td>
<td>43</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>11</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>12</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>13</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-16</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-15</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-14</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-13</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-12</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-11</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-10</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-9</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-8</td>
<td>76</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-7</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-6</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-5</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-4</td>
<td>79</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-3</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-2</td>
<td>107</td>
<td>104</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>-1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>91</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>69</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>4</td>
<td>89</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>5</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>6</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>7</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>9</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>11</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>12</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>13</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-15</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-14</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-13</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-12</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-11</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-10</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-9</td>
<td>67</td>
<td>67</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-8</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-7</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-6</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-5</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-4</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-3</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-2</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>-1</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>1</td>
<td>102</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>2</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>5</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>7</td>
<td>67</td>
<td>68</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>9</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>11</td>
<td>24</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>12</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>13</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>14</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>15</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>16</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>17</td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>
### Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>1</th>
<th>Fobs</th>
<th>1</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9</td>
<td>-6</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-5</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-4</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-3</td>
<td>24</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-2</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>-1</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>1</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>3</td>
<td>26</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>4</td>
<td>4</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>5</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>6</td>
<td>25</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>7</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>8</td>
<td>33</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>10</td>
<td>32</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-11</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-10</td>
<td>42</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-9</td>
<td>9</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-8</td>
<td>39</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-7</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-6</td>
<td>44</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-5</td>
<td>14</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-4</td>
<td>46</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-3</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-2</td>
<td>67</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>-1</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>0</td>
<td>57</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1</td>
<td>24</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>2</td>
<td>59</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>4</td>
<td>49</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>5</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>6</td>
<td>56</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>8</td>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>9</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-10</td>
<td>22</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-9</td>
<td>20</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-8</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-7</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-6</td>
<td>24</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-5</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-4</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-3</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-2</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>-1</td>
<td>24</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>0</td>
<td>23</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>1</td>
<td>27</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>2</td>
<td>22</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>3</td>
<td>22</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>4</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>5</td>
<td>22</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>8</td>
<td>27</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-9</td>
<td>19</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-8</td>
<td>31</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-7</td>
<td>17</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-6</td>
<td>27</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-5</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-4</td>
<td>37</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-3</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-2</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>-1</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>0</td>
<td>38</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>1</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>2</td>
<td>27</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4</td>
<td>1</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>2</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>3</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>4</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>5</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>6</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>7</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>11</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>12</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-14</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-13</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-12</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-11</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-9</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-8</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-7</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-6</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-5</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-3</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-2</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>-1</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>1</td>
<td>69</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3</td>
<td>83</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>56</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>6</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>7</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>9</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>11</td>
<td>43</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-13</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-12</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-11</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-10</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-9</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-8</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-7</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-6</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-5</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-3</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-2</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>-1</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>0</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>2</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>4</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>5</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>6</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>8</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-13</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-12</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-11</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-10</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-9</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-8</td>
<td>27</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>7</td>
<td>-7</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-6</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-5</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-4</td>
<td>32</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-3</td>
<td>44</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-2</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-1</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>0</td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>2</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>3</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>5</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>7</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>9</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>10</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>11</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>12</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>13</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>14</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>15</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>16</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>17</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>18</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>19</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>20</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>21</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>22</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>23</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>24</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>25</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>26</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>27</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>28</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>29</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>30</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>31</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>32</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>33</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>34</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>35</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>36</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>37</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>38</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>39</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>40</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>41</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>42</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>43</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>44</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>45</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>h</td>
<td>k</td>
<td>1</td>
<td>Fobs</td>
<td>Fcal</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-2</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>-1</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>4</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>5</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>6</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>7</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>8</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>9</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>10</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-14</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-13</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-12</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-11</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-10</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-8</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-7</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-6</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-5</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-4</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-3</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-2</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>-1</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>4</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>5</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>6</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>7</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>9</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>10</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-13</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-12</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-11</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-10</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-9</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-8</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-7</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-6</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-5</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-4</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-2</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>-1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>4</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>6</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>7</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>8</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>9</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-13</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-12</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-11</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-9</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>-7</td>
<td>46</td>
<td>49</td>
</tr>
</tbody>
</table>
## Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>11</td>
<td>0</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>2</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-12</td>
<td>32</td>
<td>35</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-10</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-8</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-6</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-4</td>
<td>67</td>
<td>71</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-2</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>2</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-12</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-9</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-8</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-7</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-6</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-5</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-4</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-3</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-2</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>-1</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>2</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>4</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>6</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>8</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>2</td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-12</td>
<td>33</td>
<td>31</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-10</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-9</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-8</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-7</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-6</td>
<td>42</td>
<td>44</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-5</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-4</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-3</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-2</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>-1</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>4</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>5</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>6</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>7</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-12</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-11</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-10</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-9</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-7</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-6</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-5</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-3</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-2</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>-1</td>
<td>23</td>
<td>23</td>
</tr>
</tbody>
</table>
## Appendix Two

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>Fobs</th>
<th>Fcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>0</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>2</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>4</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>5</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-1</td>
<td>-9</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-2</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-3</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-4</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-5</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-7</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-9</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-10</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-11</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-12</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-13</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-14</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-15</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-16</td>
<td>14</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-17</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-18</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-19</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>-20</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-1</td>
<td>-8</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-2</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-3</td>
<td>-6</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-4</td>
<td>-5</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-5</td>
<td>-4</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-6</td>
<td>-3</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-7</td>
<td>-2</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-8</td>
<td>-1</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-9</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-11</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-12</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>-13</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-1</td>
<td>-8</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-2</td>
<td>-7</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-3</td>
<td>-6</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-4</td>
<td>-5</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-5</td>
<td>-4</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-6</td>
<td>-3</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-7</td>
<td>-2</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-8</td>
<td>-1</td>
<td>48</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>-9</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-1</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-2</td>
<td>2</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-4</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-6</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>-7</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-1</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-2</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-3</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-5</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-6</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-7</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-8</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>-9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>-1</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>-2</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>-1</td>
<td>4</td>
<td>26</td>
</tr>
<tr>
<td>9</td>
<td>-2</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-3</td>
<td>10</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-4</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>
References

CITATION CONVENTION: Inorganic Chemistry


(3) Burchenal, J. H.; Burchenal, J. R. in *Cancer the Outlaw Cell*; 2nd ed.; La Fond, R. E. (Editor); American Chemical Society: Washington, 1988, p 201.

(4) Hollinshead, A. C. in *Cancer the Outlaw Cell*; 2nd ed.; La Fond, R. E. (Editor); American Chemical Society: Washington, 1988, p 237.


References


References


References


References


References


References


References


References


References


(208) McLaughlin, G. M.; Robertson, G. B.; Taylor, D.; Whimp, P. O., *The ANUCRYS Structure Determination Package*; Research School of Chemistry, The Australian National University, P.O. Box 4, Canberra A.C.T., 2601, Australia.
References


References


References


