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Organometallic Ruthenium Anticancer Complexes: Design, Synthesis, and Nucleic Acid Recognition

A Thesis Submitted for the Degree of

Doctor of Philosophy

by

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Abstract

Organometallic ruthenium(II) complexes $[(\eta^6\text{-arene})\text{RuCl}(\text{X})(\text{Y})]$ (X, Y are monodentate or chelating ligands) are cytotoxic to cancer cells, and complexes of the type of $[(\eta^6\text{-arene})\text{RuCl}(\text{en})]$ (en = ethylenediamine) are especially effective inhibitors of the growth of cisplatin-resistant cell lines. Introduction of the tricyclic anthracene ligands THA (1,4,9,10-tetrahydroanthracene) and DHA (9,10-dihydroanthracene) into Ru(II) complexes are shown to enhance their anticancer activities. Two novel dinuclear bis Ru(arene) complexes (arene = biphenyl, THA) were also prepared by use of the bifunctional ligand of N, N'-bis-(2'-aminoethyl)-1,6-diaminohexane and have been investigated for their cytotoxicity. They exhibit a distinct profile of antitumour activity, compared with their corresponding mononuclear complexes, which is likely to be associated with interstrand cross-linking of DNA. The distribution of the populations of the diastereomers in the dinuclear complex $[(\eta^6\text{-biphenyl})\text{RuCl}]_2(\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_6\text{NH}(\text{CH}_2)_2\text{NH}_2\text{-N,N',N'',N'''})^{2+}$ was determined in solution using NMR methods and compared with the mononuclear complex $[(\eta^6\text{-biphenyl})\text{RuCl}(\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2\text{CH}_3))]^+$.

The structures of monofunctional adducts of $\{(\eta^6\text{-arene})\text{Ru}(\text{II})(\text{en})\}^{2+}$ (arene = biphenyl, THA and DHA) with guanine derivatives have been studied in the solid state by X-ray crystallography, and in solution using 2D ^1H , ^1H NOESY and ^1H , ^{15}N HSQC NMR methods. These guanine adducts show interesting features, including Ru(II) binding to guanine N7, stereospecific hydrogen-bonding of en NH with G O6, and strong arene-nucleobase stacking. These studies suggest a potential DNA-binding mode involving simultaneous intercalation and coordination. The diamine NH group and hydrophobic arene group as well as the Cl leaving group may therefore all play important roles in anticancer activity.

Thermodynamic and kinetics studies were made of the binding of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\text{X}\}^{n+}$ (arene = THA, DHA, biphenyl, *p*-cymene, or benzene; X = Cl⁻ or H₂O) to nucleotides and nucleosides (G, I, A, C, T) using ¹H, ³¹P and ¹⁵N (¹⁵N-en) NMR spectroscopy, together with pH titrations. $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ binds only to N7 of G, to N7 and N1 of I, and to N3 of T. Binding to N3 of C was very weak, and there was almost no binding to A. The reactivity of the various binding sites of nucleobases towards Ru at neutral pH decreased in the order G(N7) > I(N7) > I(N1) > T(N3) > C(N3) > A(N7), A(N1). Such site-selectivity appears to be controlled by the en NH₂ groups, which H-bond to exocyclic oxygens but are non-bonding and repulsive towards exocyclic amino groups of nucleobases. Additionally, significant amounts of the 5'-phosphate-bound species (40-60%) were present at equilibrium in reactions with the nucleotides 5'-TMP, 5'-CMP and 5'-AMP. In contrast, no binding to the phosphodiester groups of 3',5'-cyclic-GMP (cGMP) or cAMP was detected. The rates of reaction of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\text{X}\}^{n+}$ towards cGMP decreased in the order: THA > biphenyl > DHA > *p*-cymene > benzene, suggesting that N7-binding is promoted by favourable arene-purine hydrophobic interactions. Such arene-purine hydrophobic interactions may influence the kinetics of DNA binding. These findings will aid the design of more effective anticancer complexes, as well as new site-specific DNA reagents.

Dedicated to my Parents

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Declaration

I hereby declare that except where specific reference is made to other sources, the work contained in this thesis is the original work of my own research since registration of the PhD degree in October 1999, and any collaboration has been indicated clearly. This thesis has been composed by the candidate and not been submitted, in whole or in part, for any of other degree, diploma, or other qualification.

Haimei Chen

November 2002

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Abbreviations:

Cisplatin	<i>cis</i> -[PtCl ₂ (NH ₃) ₂], <i>cis</i> -DDP
NAMI-A	[<i>trans</i> -Ru(III)Cl ₄ (DMSO)Im][ImH]
Im	imidazole
Ind	indazole
cdta	1, 2-cyclohexanediaminotetraacetate
AO	acridine orange
Eth	ethidium bromide
Tf	transferrin
HMG	high-mobility-group
LMCT	ligand-to-metal charge transfer
NMR	nuclear magnetic resonance
COSY	2D correlation spectroscopy
HSQC	heteronuclear single quantum coherence
DQF	double-quantum filtered
NOESY	2D nuclear Overhauser effect spectroscopy
NOE	nuclear Overhauser effect
TOESY	total correlation spectroscopy
HPLC	high performance liquid chromatography
UV	ultraviolet spectroscopy
WATERGATE	water suppression by gradient-tailored excitation
en	ethylenediamine
AD	adriamycin
IC ₅₀	50% inhibitory concentration
Bip	biphenyl
THA	1, 4, 9, 10 – tetrahydroanthracene
DHA	9, 10 – dihydroanthracene
Cym	<i>p</i> -cymene
Ben	benzene

5'-GMP	guanosine 5'-monophosphate
9EtG	9-ethylguanine
Guo	guanosine
cGMP	3', 5'-cyclic-GMP
5'-IMP	inosine 5'-monophosphate
Ino	inosine
5'-AMP	adenosine 5'-monophosphate
Ado	adenosine
cAMP	3', 5'-cyclic-AMP
5'-CMP	cytidine 5'-monophosphate
5'-TMP	thymidine 5'-monophosphate
TSP	sodium trimethylsilyl- <i>d</i> ₄ -propionate
Cp	η^5 -C ₅ H ₅
DMSO	dimethyl sulfoxide
DMF	<i>N, N</i> -dimethylformamide
THF	tetrahydrofuran
ca.	circa (about)

Chapter 1 Introduction

1.1 Introduction

Interest in the biological activity of ruthenium complexes, as well as the possibility of discovering ruthenium-based drugs active in the treatment of human malignancies, is mainly due to the antineoplastic activity of cisplatin.¹ Approaches to the design of new active ruthenium complexes have rapidly evolved in recent years. Currently, ruthenium is probably second only to platinum in the number of complexes that exhibit anticancer activity.² A potential advantage over platinum is that it is generally less toxic. The fate of Ru compounds in the body appears to be different from that of many other transition metals, probably attributable to its own unique structural features and chemical behaviour.³ Finding new ruthenium-based anticancer drugs will be aided by understanding existing metal agents with a view toward developing new modes of attack.

1.2 Ruthenium Chemistry

Ru (atomic number 44, atomic weight 101.07, $Kr5s^14d^7$), along with Os, Rh, Ir, Pd and Pt, forms the so-called platinum metals group. Ru has seven stable isotopes, ranging from 96 to 104, with ^{102}Ru being the most abundant at 31.6%. In medicinal inorganic chemistry, Ru compounds are often active against Pt-resistant tumours.³ Likely reasons for this are the differences in the geometric structure of the generally square-planar platinum(II) compounds and the ruthenium(II/III) species, which are nearly always six-coordinate, low spin octahedral. The most relevant oxidation states for bioinorganic chemistry are Ru II, III, IV.⁴ The three $(t_{2g})d$ orbitals are fully populated in Ru(II) complexes and have a single electronic vacancy in Ru(III) species.^{5,6} The difference of a single electron between the two oxidation states significantly affects their chemistry, so that Ru(II) usually behaves as a relatively “soft”,⁷ π -donor metal ion and Ru(III) as a borderline “hard” metal ion, which can function as a π -acceptor of ligand electron density.⁸

Ru(II) complexes with π -acceptor ligands invariably exhibit intense metal-to-ligand charge-transfer transitions in their optical spectra due to the strong back-bonding interactions, while Ru(III) complexes with π -donor ligands such as R-S⁻ and imidazole, usually show strong ligand-to-metal charge transfer transitions. The reduction potential for the Ru(III/II) couple is a strong function of the ligand environment.^{9,10,11,12} The effect of back donation of electron density is somewhat more prominent in stabilizing the lower valence state of Ru than for most other metals. The coordination of π -acceptor ligands such as carbonyl or pyridine increases the reduction potential while the addition of anionic or good electron-donor ligands causes it to decrease.^{8,13}

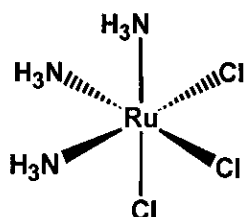
The hypothesis of “activation by reduction”, proposed by Clarke based on his systematic study of the anticancer activities of ammine-ruthenium complexes, suggests that Ru(III) complexes may serve as prodrugs, which are activated by reduction *in vivo* since Ru(II) coordinates more rapidly to biomolecules.^{14,15} *In vivo* reduction of Ru(III) to Ru(II) can occur by single-electron-transfer from proteins which exist in both the mitochondrial electron-transfer chain and in microsomal electron-transfer systems, with microsomal proteins being the more efficient.¹⁶ Oxidation of Ru(II) back to Ru(III) can occur by reaction with molecular oxygen,¹⁷ cytochrome oxidase,^{17,18} and other oxidants.

As reduction of Ru(III) to Ru(II) fills the d_{π} orbitals, the π -donor ligands are no longer able to bind sufficiently strongly to remain intact. In the case of Ru(II) ammine complexes, acido ligands are lost fairly rapidly ($k = 1\sim 10\text{ s}^{-1}$).^{19,20} Since most biological amines are protonated at neutral pH, potential biological ligands such as lysylamines or thiols on the surface of proteins at neutral pH do not readily coordinate to the Ru(II) species.⁸ Consequently the lone pairs of electrons on the nitrogens of imidazole rings of histidine (on protein) and purines (on DNA) present the readiest binding targets to Ru(II).

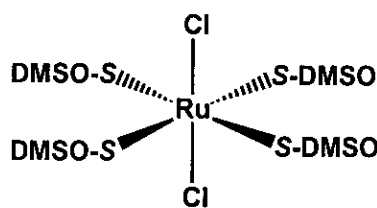
Being directly below Fe in the periodic table, and existing in both the +2, +3 oxidation states in aqueous solution as does Fe, Ru may mimic some properties of Fe. Indeed, like Fe(III), Ru(III) appears to have a high affinity for the plasma protein. HPLC studies of the anticancer complex $[\text{Ru(III)Cl}_4\text{Ind}_2]\text{HInd}$ in the blood have shown that most of the complex is bound to albumin (80%) and the remainder to transferrin.²¹ X-ray crystallographic studies of human lactoferrin have demonstrated that this indazole Ru(III) complex directly bound to the imidazole nitrogen of His253, one of the Fe^{3+} binding ligands in the N-lobe.²² Significantly, the binding of Ru(III) to transferrin is reversible. The metal complex is released at pH 4-5 in the presence of a large excess of citrate or adenosine triphosphate (ATP). This suggests that transferrin can serve as a mediator to deliver Ru(III) anticancer complexes. Transferrin uptake appears to an important mode for the specific uptake of Ru(III) by tumour cells,^{23,24} which is probably linked to the potential selective cytotoxicity. Transferrin may lower ruthenium toxicity by preventing it from binding to other biomolecules or uptake until it has been delivered to the tumour cells.²⁵ Ru-Tf complexes offer a potential new family of less toxic and more effective antitumor agents.

1.3 Ruthenium Antitumour Agents

Ru-ammine complexes were the first group of Ru complexes to undergo screening for antitumor activity.²⁶ *Fac*- $[\text{Ru(III)Cl}_3(\text{NH}_3)_3]$ (**1**) showed the best activity but its low solubility makes it unsuitable as a drug.²⁷ Clarke's hypothesis of "activation by reduction" suggests Ru(III) complexes may serve as prodrugs.



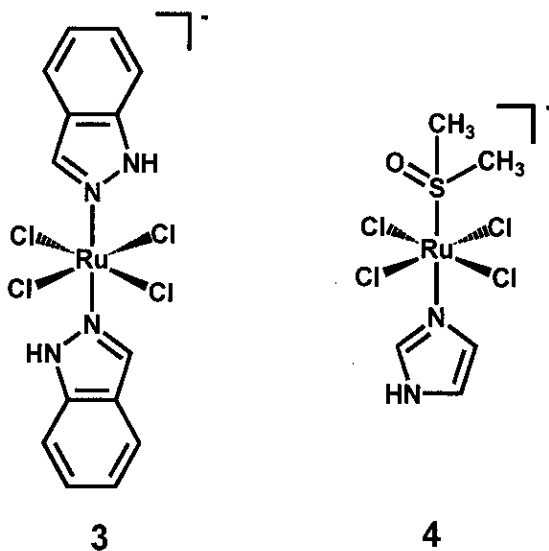
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Trans-[Ru(II)Cl₂(dms_o)₄] (2), selected for its good solubility, exhibits significant activity against solid tumour metastases, and, interestingly, much higher than the activity against the primary tumour.^{28,29}

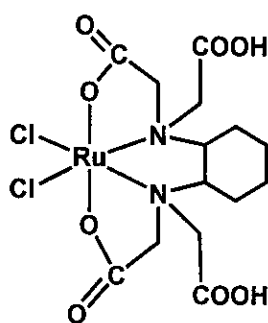
The introduction of heterocyclic ligands to Ru(III) complexes has been shown to improve their solubility as well as antitumor activity.^{30,31} The indazole complex, *trans*-[Ru(III)Cl₄Ind₂]⁻ (3) has become a candidate for clinical trial. This complex has been shown to enter cancer cells by transferrin-mediation^{32,21} and it has been cocrystallized with apolactoferrin by Baker *et al.* and analysed through X-ray crystallography.²²



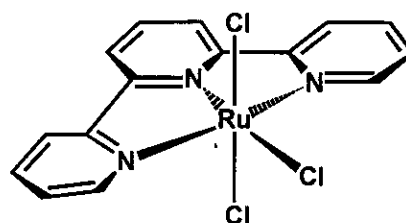
Trans-[Ru(III)Cl₄(dmso)Im]⁻ (NAMI-A) (4), which contains both dmso and a heterocyclic ligand, became the first Ru(III) antimetastatic complex to be introduced into clinical trials.³³ Sava *et al.* have shown that NAMI-A exhibits excellent antimetastatic activity³⁴ at doses genuinely devoid of toxicity through the novel mechanism of action distinguished from the traditional ones of DNA binding,^{35,36,37} which establishes a new generation of anticancer agents.

Cis-[Ru(IV)(cdta)Cl₂] (**5**) (cdta = 1,2-cyclohexanediaminetetraacetate) was reported as the first example of an active Ru(IV) antitumour complex.^{38,39} Ru polyamino-polycarboxylate complexes constitute a relatively new group of anticancer agents.

Ru-polypyridyl complexes have shown effective intercalative binding and groove-binding to DNA and have been widely used for probing DNA structure.⁴⁰ *Mer*-[Ru(terpy)Cl₃] (**6**) has been shown by Reedijk *et al* to exhibit good antitumour activity against human cervix carcinoma Hela and murine L1210 cell lines.⁴¹



5



6

1.4 Di/tri-nuclear Metal Complexes

1.4.1 Di/tri-nuclear Platinum Complexes

The development of polynuclear metal complexes represents a promising approach in the design of new anticancer agents. The platinum dimers and trimers developed by Farrell and his coworkers⁴² provide very stimulating precedents in this direction. The first representative of this new class, 1,0,1/t,t,t or BBR3464 (Figure 1.1) is now advanced to phase-II clinical trials after a highly promising Phase I trial indicated responses in colon and pancreatic cancers.⁴³ This trinuclear cationic compound shows potent cytotoxicity against both cisplatin-sensitive (L1210) and – resistant (L1210/CDDP) murine leukemia cell lines, and it is 30 times more cytotoxic

than cisplatin.⁴⁴ Also, a wider spectrum of tumours are sensitive to this agent. Within 18 human tumour xenografts studied, BBR3464 also maintains high antitumour activity in a subset of 6 tumours classified as mutant p53.

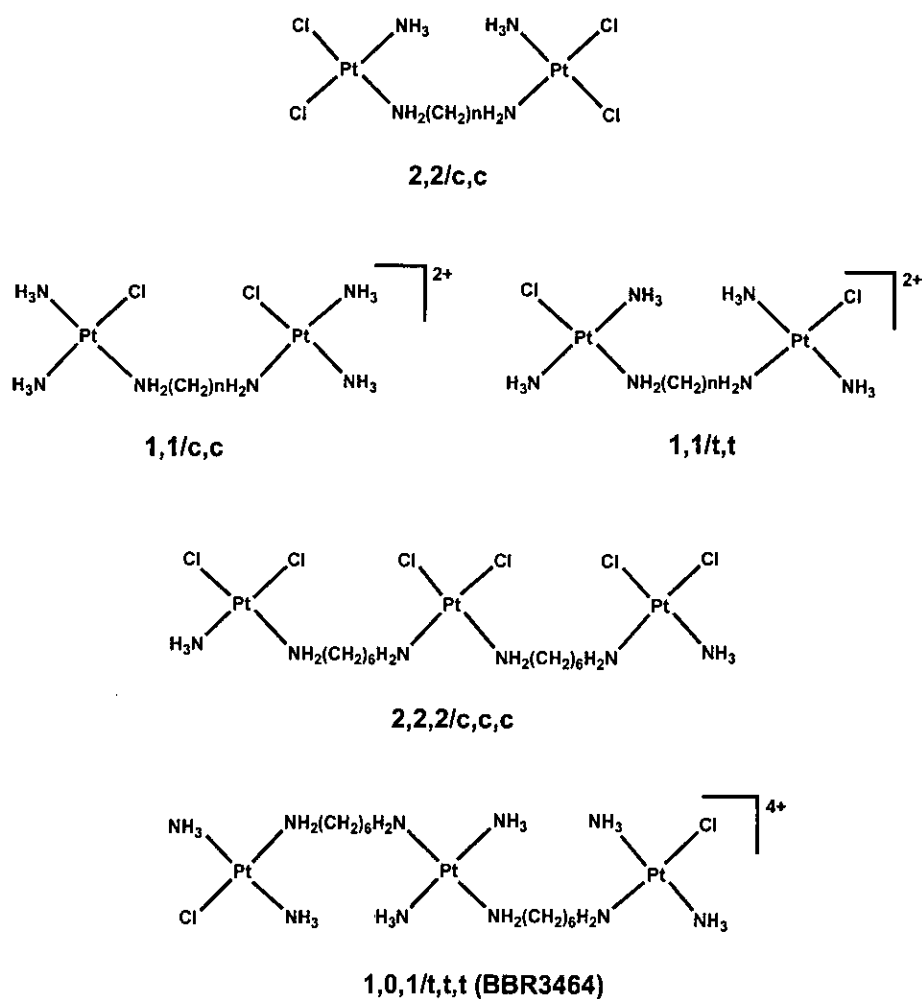


Figure 1.1 Structures of some dinuclear and trinuclear platinum anticancer compounds.⁴²

The choice of BBR3464 as a clinical candidate arose from systematic studies on the broad class of dinuclear and trinuclear Pt compounds. The structures in Figure 1.1 represent a formal array of potential DNA-binding modes from bifunctional to hexafunctional. The 2,2/c,c compound was first reported in 1988 and consists of two

cis-{PtCl₂(NH₃)₂} units linked by a flexible diamine chain.⁴⁵ The use of cisplatin as a synthon was further demonstrated by extension to trinuclear systems 2,2,2/c,c,c (Figure 1.1).⁴⁶ Most recently, extensive investigation has focused on the distinct biological activity of the pair of dinuclear isomers 1,1/c,c and 1,1/t,t (Figure 1.1) derived from their mononuclear counterparts, cisplatin and transplatin respectively.⁴⁷

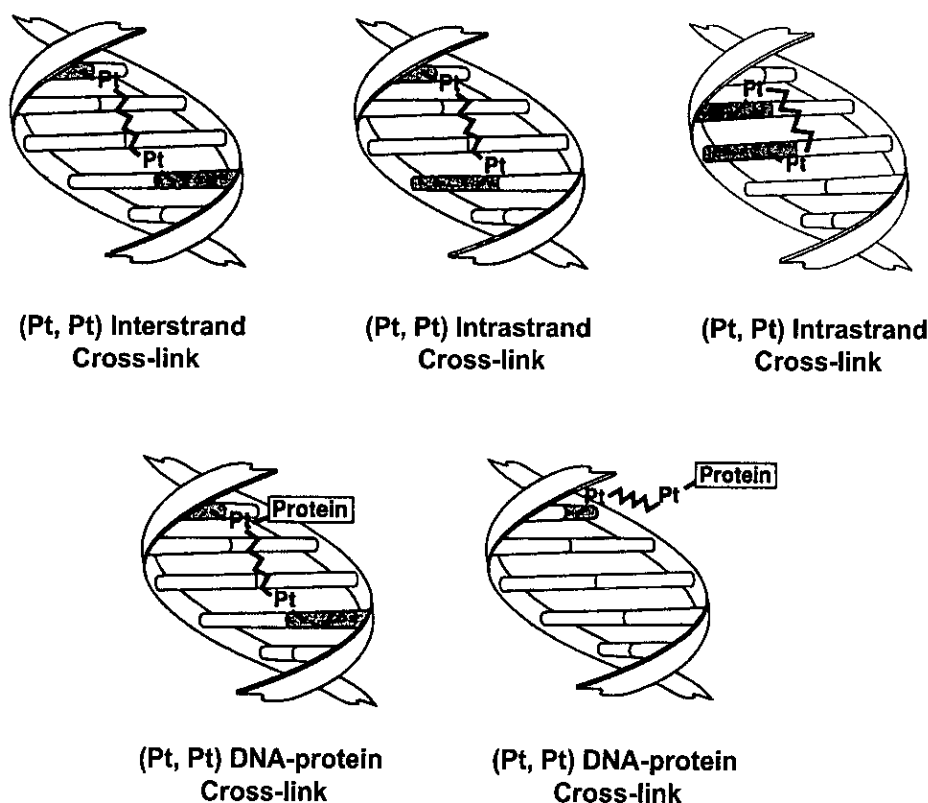


Figure 1.2 Schematic limiting modes for possible DNA-DNA and DNA-protein cross-linking induced by dinuclear platinum complexes.⁴⁷

Unlike the mononuclear case, where one isomer (cisplatin) is antitumour-active and the other (transplatin) is not, it has been found that both dinuclear isomers of 1,1/c,c and 1,1/t,t are as active as cisplatin against conventional tumour lines and the 1,1/t,t derivatives are markedly active against tumour lines resistant to cisplatin.⁴⁸ Sequencing studies^{49,50,51} on template double-stranded DNA binding of 1,1/c,c or

1,1/t,t have indicated that both isomers are very efficient with formation of interstrand cross-links (Figure 1.2), and, additionally, the 1,1/t,t derivatives are capable of forming (Pt, Pt) intrastrand cross-links to the adjacent guanines of a d(GpG) sequence.⁴⁹ The excision repair UVrABC^{52,53} complex also recognizes bis(Pt)-DNA adducts formed either by 2,2/c,c or 1,1/t,t complexes. The polyfunctional nature of the bis-Pt complexes suggested that the availability of multiple coordination sites (> 2) can be used to induce covalent cross-linking of protein to platinated DNA (Figure 1.2). These results suggest that improved antitumour activity of bis-Pt complexes could be related to the formation of DNA adducts structurally unique from those formed by *cis* and *trans*-DDP and the more efficient protein-DNA cross-linking than *cis* and *trans*-DDP. The DNA adducts will be affected by geometry and nature of the coordination sphere as well as chain length and steric effects within the linker group.⁴⁷

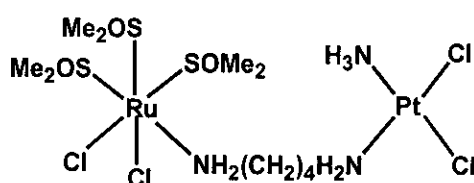
Further studies based on the 1,1/t,t series have shown that, in general, in vivo antitumour activity as well as the enhanced activity in cisplatin-resistant cancer cells are seen with longer chain lengths ($n > 4$) and are greater with the straight-chain-diamine ($n = 6$).^{47,54} Steric effects within the diamine linker were examined by replacing butane-1,4-diamine with the sterically hindered *cis*-cyclohexane-1,4-diamine, which produced very disappointing cytotoxicity.⁵⁰ It is likely that the sterically hindered compound binds more slowly to DNA, especially in the second bifunctional step.

Straight-chain flexible diamines linking two *trans*- $\{\text{PtCl}(\text{NH}_3)\}^+$ moieties gave consistently better antitumour activity in cisplatin-resistant cells and the 1,1/t,t ($n = 6$) complex was the most potent compound which can readily span a four-base pair segment of DNA.⁴⁷ 1,0,1/t,t,t (BBR3464) was thus developed by linking a precursor central molecule, a non-covalent tetra-amine $[\text{Pt}(\text{NH}_3)_2\{\text{H}_2\text{N}(\text{CH}_2)_6\text{NH}_2\}_2]^{2+}$ to two equivalents of a target terminal platinum

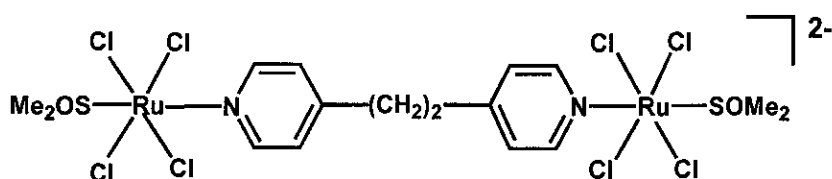
coordination sphere, $\text{trans-}\{\text{PtCl}(\text{NH}_3)_2\}^+$,⁵¹ in which the central Pt unit is capable of hydrogen-bonding interactions with DNA. The overall charge of +4 greatly enhances DNA affinity, characterised by long-range interstrand cross-linking up to six base pairs apart with significant unwinding and efficient, irreversible conversion of B- to Z-DNA.^{55,56,57} The adducts terminate DNA synthesis in vitro. Therefore, BBR3464 became the first genuinely new platinum anticancer agent not based on the “classical” cisplatin structure to enter clinical trials and its success is encouraging the development of other polynuclear metal anticancer agents.

1.4.2 Di/tri-nuclear Ruthenium Complexes

The development of heterodinuclear (Ru, Pt) metal compounds provides an alternative approach to the design of a new type of anticancer agents. This is driven by the hypothesis that alteration of the antitumour activity of drugs will be achieved by modification of the mode of DNA-DNA or DNA-protein binding in comparison to cisplatin and this mode will vary with alterations in the coordination sphere.⁵⁸



7



8

The Ru-Pt dinuclear complex $[\{cis, fac-(RuCl_2(Me_2SO)_3)\mu-NH_2(CH_2)_4NH_2-\{cis-Pt(NH_3)Cl_2\}]$ (7) has been designed^{58,53} to incorporate two inequivalent coordinations, the octahedral sphere of Ru(II) and square-planar Pt(II). This combination is capable of selective reaction at the Ru centre and effective cross-linking of DNA repair protein to DNA. Unfortunately, the hydrolytic activity, photosensitivity, and dissociation of Ru from this complex result in nonspecific biopolymer binding.⁵⁸ Analogous complexes of the type $[(bpy)_2M(dpb)PtCl_2]Cl_2$ (where M = Ru(II) or Os(II) and bpy = 2,2'-bipyridine, dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) have been synthesised to couple a chromophoric metal (Ru or Os) site to the *cis*-dichloride platinum site which provides a system with two potential modes of binding to DNA, intercalative and covalent.⁵⁹

With the success of NAMI-A as the first Ru(III) antineoplastic complex to enter into clinical trials, currently, dinuclear Ru complexes based on NAMI-A are being developed.⁶⁰ The dinuclears $Na_2[\{trans-RuCl_4(Me_2SO-S)\}_2(\mu-L)]$ and $[\{mer, cis-RuCl_3(Me_2SO-S)(Me_2SO-O)\}_2(\mu-L)]$, where L = pyrazine, pyrimidine, 4,4'-bipyridine, 1,2-bis(4-pyridyl)-ethane (etbipy) or 1,3-bis(4-pyridyl)-propane, have been synthesised with the aim of accessing their neoplastic activity. They have been found to maintain their dimeric structures in solution and undergo aquation reactions similar to those of the monomeric analogues. This indicates that they might behave as bi- or even tetra-functional agents and might maintain the antineoplastic activity of the closely related mononuclear species but with some different, and possibly enhanced, selectivity or specificity. The preliminary *in vitro* test data⁶¹ have shown that these dimers effectively reduce the number and the weight of lung metastases; in particular, the complex $Na_2[\{trans-RuCl_4(Me_2SO-S)\}_2(\mu-etbipy)]$ (8) was as effective as NAMI-A in inducing spontaneous metastases at a dosage which is about 3.5 times lower compared to that normally used for NAMI-A.

Ru-red, $[(\text{NH}_3)_5\text{Ru}^{\text{III}}\text{O}\text{Ru}^{\text{II}}(\text{NH}_3)_4\text{Ru}^{\text{III}}(\text{NH}_3)_5]^{6+}$,⁶² is a representative of another type of O-bridged mixed-valent metal complexes which have been studied for their interesting biological activity. This agent has been used as a cytological stain and a tumour imaging agent with ^{103}Ru -red. Ru-red also inhibits tumour growth, which may arise from its ability to interact with calcium metabolism. The dimeric complex $[\mu\text{-O-}\{(\text{H}_2\text{O})_2(\text{bpy})_2\text{Ru}^{\text{III}}\}_2]^{4+}$, related to Ru-red, has also been studied for its biological properties and found to bind stereoselectively to calf thymus DNA. Irreversible thermal denaturation of DNA with this complex covalently bound indicated the formation of an interstrand biadduct.⁶³

1.5 DNA Binding

Substantial progress has been made over the past years in the fundamental characterization of a variety of drug-DNA interactions. Structure-based design strategies have yielded new DNA-binding agents with clinical promise.

Cross-linking/Covalent Binding

The success of cisplatin in cancer chemotherapy derives from its ability to crosslink DNA and alter the structure. Most cisplatin-DNA adducts are intrastand d(GpG) and d(ApG) crosslinks. The structural information about the principal DNA adducts of cis-DDP have been provided by NMR structural analysis, crystallographic studies, molecular mechanics and dynamics modeling. The X-ray crystal structures of cisplatin adducts with short single-stranded deoxyoligonucleotides such as *cis*- $[\text{Pt}(\text{NH}_3)_2\{\text{dpGpG}\}]$,^{64,65} and *cis*- $[\text{Pt}(\text{NH}_3)_2\{\text{dCpGpG}\}]$,⁶⁶ have shown that the adjacent two N7-platinated guanine rings are oriented in a head-to-head manner and destacked with the dihedral angle being 80-84°. The X-ray structure of a site-specifically platinated double-stranded deoxyoligonucleotide with the sequence d(CCTCTG*G*TCTCC)-d(GGAGACCAGAGG), where G*G* represents the *cis*-

$[\text{Pt}(\text{NH}_3)_2\text{-}\{\text{d}(\text{GpG})\text{-N7}(\text{G}_6), \text{N7}(\text{G}_7)\}]$ intrastrand crosslink, has revealed⁶⁷ that the duplexes are bent (bend angle $39\text{-}55^\circ$) towards the major groove with the bend locus near the site of platination. The dihedral angle between the G6 and G7 guanine planes is 26° . A projection of this DNA structure, based on the X-ray coordinates, is shown in Figure 1.3. A similar structure had been found in solution with an early NMR structural analysis of a cisplatin-modified duplex decamer, *cis*- $[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{TCTCG}^*\text{G}^*\text{TCTC})\cdot\text{d}(\text{GAGACCGAGA})\}]$.⁶⁸ Recently a 16-base-pair double-stranded deoxyoligonucleotide containing a single *cis*- $[\text{Pt}(\text{NH}_3)_2\text{-}\{\text{d}(\text{GpG})\text{-N7}(\text{G}_8), \text{N7}(\text{G}_9)\}]$ intrastrand crosslink was crystallized as a 1:1 complex with the 89-amino-acid domain A of HMG1 from rat, affording the first detailed picture of any

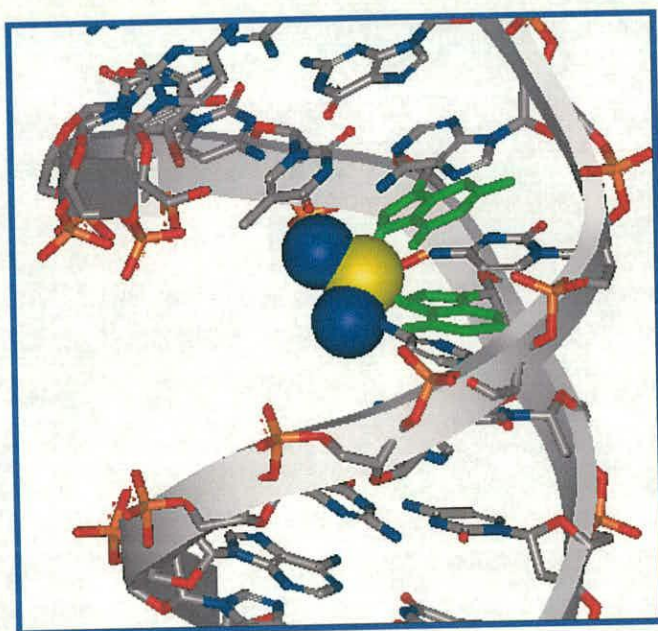


Figure 1.3 The X-ray structure of the GpG platinated DNA duplex $\text{d}(\text{CCTCTG}^*\text{G}^*\text{TCTCC})/\text{d}(\text{GGAGACCGAGG})$, where G^*G^* represents the *cis*- $[\text{Pt}(\text{NH}_3)_2\text{-}\{\text{d}(\text{GpG})\text{-N7}(\text{G}_6), \text{N7}(\text{G}_7)\}]$.⁶⁷ Colour code of *cis*- $[\text{Pt}(\text{NH}_3)_2\text{-}\{\text{d}(\text{GpG})\text{-N7}(\text{G}_6), \text{N7}(\text{G}_7)\}]$: Pt (yellow), N of NH_3 (blue), G6 and G7: (green).

complex between a structure-specific HMG domain and a DNA substrate.^{69,70} This revealed how intrastrand crosslinking unwinds and bends the duplex to facilitate the binding of proteins that contain one or more high-mobility-group (HMG) domains. Such interaction with HMG therefore blocks the cisplatin-DNA adducts from recognition by the repair apparatus, enhancing the cytotoxic properties of the drug. This provides a rational basis for the design of new chemotherapeutic strategies and of molecules with an increased affinity and specificity for cisplatin-modified DNA. Such agents could enhance the cytotoxic effect of cisplatin and related compounds by shielding damaged DNA from intracellular repair.

In contrast to the situation for cisplatin, the trans isomer, transplatin has been found to be therapeutically inactive. Transplatin cannot, for stereochemical reasons, link two adjacent guanine bases in DNA. Instead, alternative lesions, namely 1,3-intrastand (GNG) cross-links,⁴⁸ are formed which tend to rearrange into bifunctional interstrand adducts.⁷¹ Furthermore interstrand cross-links between N7 of guanine and N3 of cytosine of the same base pair (GC) have been detected *in vitro*.^{72,73} Possibly these adducts made by transplatin *in vivo* disrupt the duplex more profoundly than the intrastrand cross-link *cis*-[Pt(NH₃)₂{d(pGpG)}], and may therefore be more readily recognized and repaired by the cell.⁶⁴

Intercalation

Intercalative binding is a noncovalent stacking interaction resulting from the insertion of a planar heterocyclic aromatic ring between the base pairs of the DNA helix. The flat intercalator is held rigidly in a well-defined orientation perpendicular to the axis of the helix. This DNA binding mode was first proposed by Lerman to explain the strong affinity for DNA of certain heterocyclic aromatic dyes such as the aminoacridines.⁷⁴ Intercalators are of interest owing largely to their ability to inhibit nucleic acid synthesis *in vivo*, leading to their activity as mutagens, antibiotics,

antibacterials, trypanocides, schistosomicides and antitumour agents. Examples of intercalators^{75,76} are given in Figure 1.4.

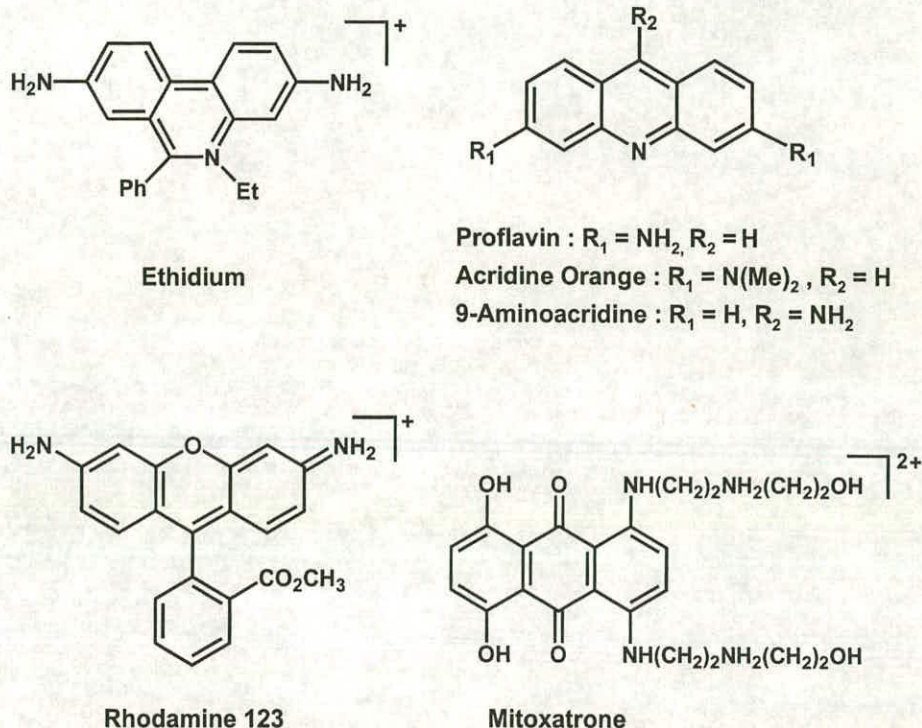
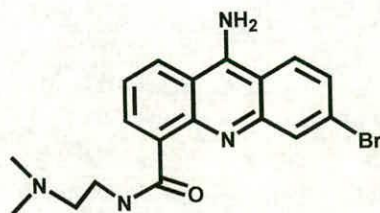
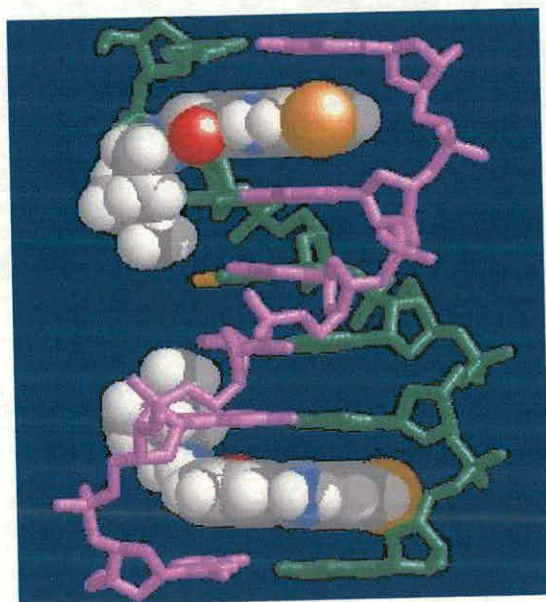


Figure 1.4 Structures of DNA intercalators and dyes.

Intercalation stabilizes, lengthens, stiffens and unwinds the DNA double helix.^{74,77,78} The binding energy for intercalation derives both from electrostatic and aromatic stacking interaction between the intercalator and DNA. Although this binding mode results in high affinities of the ligand for double-stranded DNA, the majority of the interactions are with the aromatic surfaces of the bases rather than with the distinctive functional groups in the DNA grooves. Consequently, many intercalators, such as ethidium and acridine, are relatively nonspecific DNA binding agents.⁷⁹ Sequence-specific DNA binding can be improved when the aromatic ring is substituted with special ancillary groups. One example is the acridine-4-carboxamides which have a *N*-(2-dimethylamino)ethyl-4-carboxamide side chain

(Figure 1.5). This type of agent inhibits both topoisomerases I and II.^{80,81,82} The X-ray structure of one derivative of 9-amino-6-bromo-DACA (DACA = *N*-(2-(dimethylamino)ethyl)acridine-4-carboxamide) complexed to the d(CG(5-BrU)ACG)₂ duplex shows that two molecules intercalate between the d(CG)₂ base pairs and the terminal NMe₂H⁺ group of the side chain H-bonded to N7 of one of the two guanines at the intercalation site and the neutralized N site of acridine H-bonded to the carbonyl group of the carboxamide (Figure 1.5).⁸³ Such specific major groove interactions together with the “DNA-induced” side-chain orientation which therefore blocks the groove are the major features for this type of DNA intercalator, and are likely to be important contributors to topoisomerase inhibition.



9-amino-6-bromo-DACA

DACA = *N*-[2-(dimethylamino)ethyl]-acridine-4-carboxamide

Figure 1.5 X-ray structure of 9-amino-6-bromo-DACA intercalated into the DNA duplex d(C|G(5-BrU)AC|G), where | indicates insertion.⁸³ Colour code of intercalator: O (red), N (blue), brown (Br), C (grey), H (white).

Another class of noncovalent DNA-binding agents is the groove binders, which fit into the DNA minor groove causing little perturbation of the DNA structure. One example of this class is the hairpin polyamides, designed as drugs that can target specific sequences in the DNA minor groove.⁸⁴ The NMR structure of the duplex oligonucleotide d(CGCTAACAGGC)•d(GCCTGTAGCG) bound by a six-ring hairpin polyamide ImPyPy- γ -PyPyPy- β -Dp (where Im = imidazole; Py = pyrrole; Dp = dimethylamino-propylamide) has shown that the hairpin polyamide molecule fits in the minor groove and covers its five base pair recognition site (5'-TGTTA-3'), with an binding affinity of 10^7 M^{-1} .⁸⁵

Metallointercalator

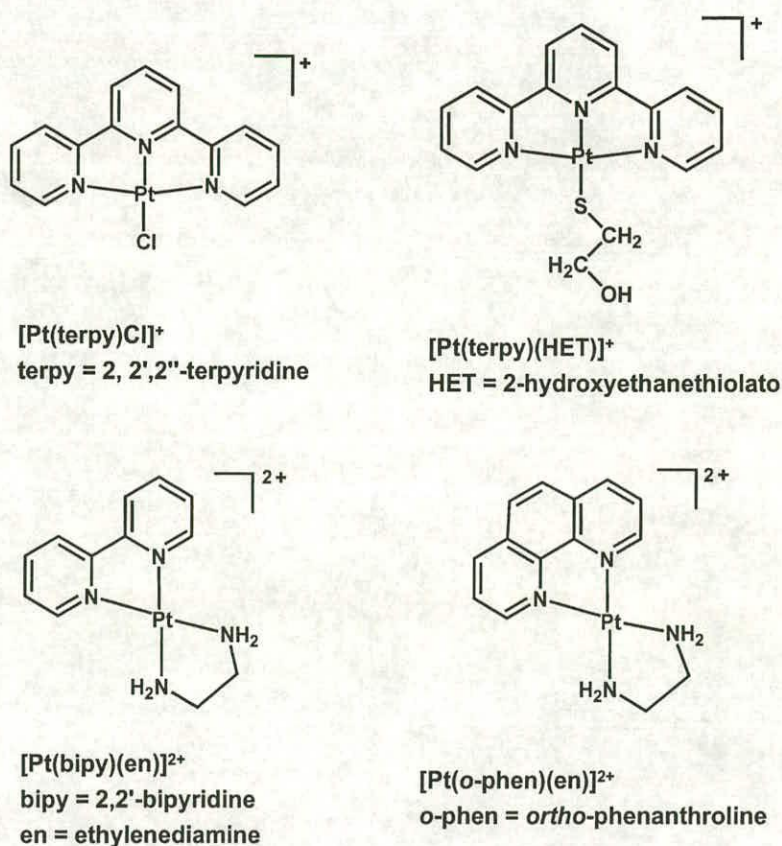
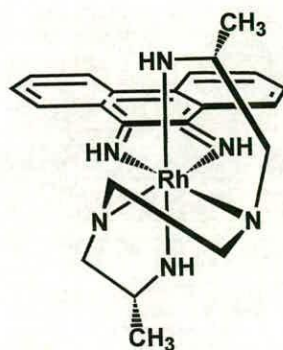
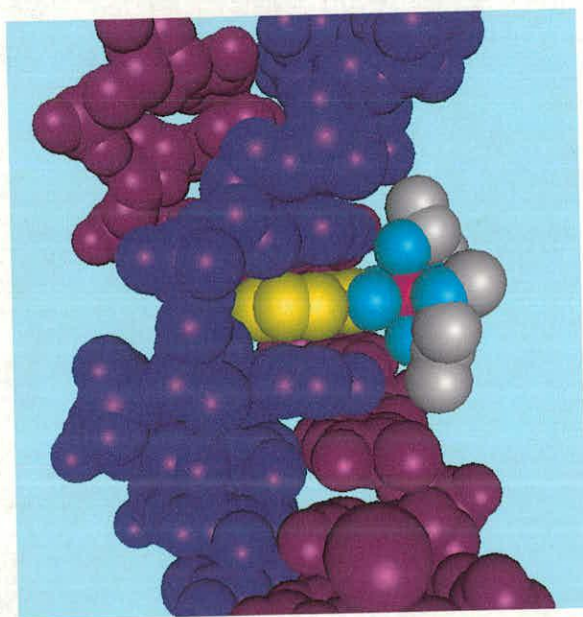


Figure 1.6 Structures and abbreviations for platinum intercalators.

Metal complexes also may bind to DNA by intercalation. It has been shown that square planar platinum(II) complexes containing a heterocyclic aromatic ligand (Figure 1.6), such as terpyridine (terpy), phenanthroline (phen), or bipyridine (bipy), bind to the helix noncovalently, inserting between the base pairs of the polynucleotide.^{86,87,88,89,90} The crystal structure of $[\text{Pt}(\text{terpy})(\text{HET})]^+$ bound to $(\text{dCpG})_2$ revealed that the flat metal cation intercalates symmetrically between base pairs with the hydroxyethanethiolato chain projecting into the major groove of this miniature DNA helix.⁹¹ These Pt metallointercalators have been utilized as electron-dense probes in X-ray diffraction experiments on nucleic acids.^{92,93,94}



$\Delta\text{-}\alpha\text{-}[\text{Rh}(\text{R}, \text{R-Me}_2\text{trien})\text{Phi}]^{3+}$

Figure 1.7 X-ray structure of the rhodium complex $\Delta\text{-}\alpha\text{-}[\text{Rh}(\text{R}, \text{R-Me}_2\text{trien})\text{Phi}]^{3+}$ bound to the duplex 5'-G(dIU)TTG|CAAC-3' oligonucleotide, where | indicates insertion.⁹⁸ Colour code of rhodium complex : Rh (purple), Phi (yellow), N (light blue), C of Me_2trien (grey).

Metallointercalation has been extended to three dimensions by Barton using octahedral Co, Zn, Ru, Os or Rh complexes containing multi heterocyclic aromatic ligands such as phenanthroline (phen), 9,10-phenanthrenequinone diimine (phi) or [3,2-*a*:2',3'-*c*]phenazine (dppz).^{95,96,97} The application of octahedral metallointercalators has permitted the targeting of specific DNA sites by matching the shape, symmetry, and functionalities of the metal complex to that of the DNA target. The crystal structure of $\Delta\text{-}\alpha\text{-}[\text{Rh}(\text{R}, \text{R-Me}_2\text{trien})\text{Phi}]^{3+}$ ((*R*, *R*-Me₂trien = 2*R*, 9*R*-diamino-4,7-diazadecane) bound to 5'-GTTGCAAC-3' duplex DNA revealed that the phi ligand is deeply inserted into the DNA base pair stack from the major groove at the site of 5'-GC-3' and the axial amine is located in a specific position to H-bond to the exocyclic oxygen of the G base with a close contact of N...O 2.9 Å. The methyl group of the ligand is a comfortable 3.5-4.1 Å distance from the thymine methyl carbon (Figure 1.7). Those interesting binding features may contribute to the DNA site recognition ability of this Rh intercalator.⁹⁸ Moreover, these metallointercalators possess interesting photophysical, photochemical, and redox properties and therefore are being developed as powerful agents for probing and targeting nucleic acids.⁹⁷

Simultaneous Covalent and Intercalative Binding

A recent development has been the design and synthesis of complexes in which an intercalator and a chemically reactive platinum functionality are covalently linked. The interactions of these and other multifunctional molecules with nucleic acids are of interest since they introduce potentially new modes of binding and can yield information on the mutual interactions of functional groups held in close proximity.

Planar aromatic ligands such as doxorubicin (dx),^{99,100} acridine orange (AO),¹⁰¹ 9-aminoacridine (9-AA),¹⁰² and ethidium bromide (Eth)¹⁰³ have been incorporated into Pt complexes as potential intercalators (Figure 1.8). For

$[\text{Pt}\{\text{AO}(\text{CH}_2)_6\text{en}\}\text{Cl}_2]^+$ complex, there is a hexamethylene chain linking the reactive platinum moiety $\{\text{Pt}(\text{en})\text{Cl}_2\}$ to the acridine orange;¹⁰¹ for $\text{cis}-[\text{Pt}(\text{NH}_3)_2(\text{N9-9-AA})\text{Cl}]^+$, the platinum moiety is bound directly to an intercalator.¹⁰² The DNA binding and modelling studies have shown that these Pt-intercalator complexes can react with DNA covalently and intercalatively, and the DNA platination can be sensitive to sequence-dependent local DNA structure modulations such as those

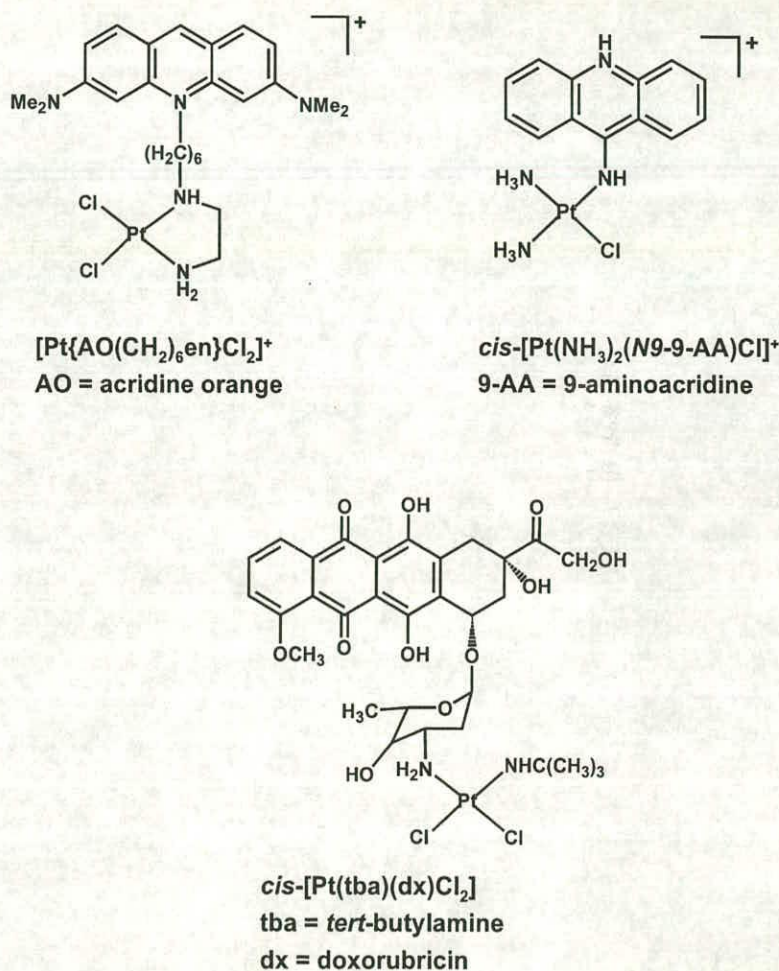


Figure 1.8 Structures and abbreviations of platinum complexes covalently linked to DNA intercalators.

accompanying intercalator binding.^{102,103,104} Although the toxicity of many Pt-intercalator complexes precludes their use as drugs, antitumor activity has been reported for $[\text{Pt}\{\text{AO}(\text{CH}_2)_6\text{en}\}\text{Cl}_2]^+$,¹⁰⁵ *cis*- $[\text{Pt}(\text{tba})(\text{N3-dx})\text{Cl}_2]$ (tba = *tert*-butylamine),⁹⁹ and for platinum complexes of mitoxantrone.¹⁰⁶

Planar aromatic ligands have also been introduced into transplatin, *trans*- $[\text{PtCl}_2(\text{NH}_3)_2]$, for development of a new class of anticancer drug (Figure 1.9). When the ammine is changed to a planar aromatic N donor ligand such as pyridine, thiazole or quinoline as in *trans*- $[\text{PtCl}_2(\text{NH}_3)\text{L}]$ (L = planar aromatic N donor), the cytotoxicity of the *trans* complex is dramatically enhanced.^{107,48} This has been attributed to the interaction of the planar aromatic ligand with the DNA duplex, perhaps partially by intercalation.^{108,109} The transplatin derivative *trans*- $[\text{PtCl}(\text{PyAc-}N,O)(\text{NH}_3)]$ (PyAc = pyridin-2-yl acetate) also exhibits enhanced cytotoxicity and appears to produce its biological activity via a “pseudobifunctional” mechanism (covalent/intercalative binding).¹¹⁰

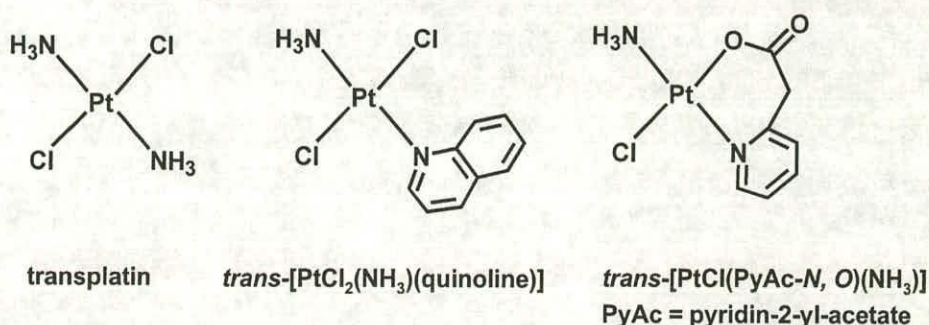


Figure 1.9 Structures and abbreviations for type *trans*- $[\text{PtCl}_2(\text{NH}_3)\text{L}]$ complexes.

1.6 Project Aims

Ruthenium compounds provide a particularly promising and versatile path toward the development of new antitumour drugs. Finding Ru compounds more

potent than cisplatin with a broad spectrum of tumor sensitivity but low toxicity is a general aim of the research. The Ru(III)-chemotherapeutic agents designed on the basis of the “activation by reduction” hypothesis have often yielded good results.¹¹¹ The determination of the modes of tumour uptake and DNA binding by Ru complexes in actual biological organisms still forms a challenge for future research.² On the other hand, the success of NAMI-A against metastasis¹¹² will stimulate more innovative studies in testing metal compounds in cell and animal systems.

Our laboratory has been investigating Ru(II) arene complexes as a potential new class of chemotherapeutic agents. The recent research results have been encouraging. The goal is to find compounds that may be active against a broad spectrum of tumours that do not respond, or acquire resistance, to cisplatin. To achieve this goal intensive research efforts are required. The aims of my project were as follows.

- (1) The design and synthesis of a series of novel (η^6 -polycyclic arene)Ru(II) complexes as potential anticancer agents as well as investigation of their anticancer activities.
- (2) Design and synthesis of di-nuclear Ru(II)(arene) complexes as potential bifunctional DNA cross-linking agents. The increased DNA binding is expected to lead to a distinct and unique profile of anticancer activity.
- (3) Investigation of the structure-activity relationships among these mono- and di-nuclear Ru(II) arene anticancer agents, including studies of their binding to nucleobases, nucleotides and polynucleotides.

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Chapter 2

Experimental Methods

2.1 Instruments

2.1.1 NMR Spectroscopy

NMR spectra were recorded on either Bruker DMX 500 (^1H , 500.13 MHz; ^{13}C , 125.72 MHz; ^{15}N , 50.66 MHz; ^{31}P , 202 MHz) or Bruker DMX 360 (^1H , 360.13 MHz) Varian ^{Unity}*INOVA* (^1H , 599.82 MHz; ^{15}N , 60.80 MHz) NMR spectrometers using TBI [^1H , ^{13}C , X] or triple resonance [^1H , ^{13}C , ^{15}N] probeheads, respectively, and equipped for z-field gradients. All spectra were acquired in 5 mm NMR tubes using standard sequences. All data processing was carried out using XWIN-NMR version 2.0 (Bruker U.K. Ltd.). ^1H NMR chemical shifts were internally referenced to 1, 4-dioxane (3.77 ppm), or to the methyl singlet of sodium trimethylsilylpropionate (TSP, 0 ppm) in aqueous solutions, or to $\text{CD}_2\text{HCOCD}_3$ (2.06 ppm) in acetone- d_6 solution, or to $(\text{CD}_2\text{H})_2\text{SO}$ (2.50 ppm) in $\text{dmsO-}d_6$ solution. ^{15}N resonances were referenced to 1 M $^{15}\text{NH}_4\text{Cl}$ in 1.5 M HCl (external) at 0 ppm and ^{31}P resonances to 85% H_3PO_4 (external) at 0 ppm. NMR work was carried out with the aid of Dr John A. Parkinson.

2.1.2 X-ray Crystallography

X-ray crystallography studies were carried out on a Bruker SMART-APEX CCD or a Stoe Stadi-4 serial diffractometer equipped with Oxford Cryosystems low-temperature devices by Dr Simon Parsons, Dr Robert O. Gould, Dr Robert A. Coxall, Iain Oswald and James Davidson. XP 5.1 was used for drawings of crystal structures.

2.1.3 CHN Analysis

CHN analysis were performed by the CHN Service at the University of Edinburgh.

2.1.4 High Performance Liquid Chromatography

The following equipment was used: Hewlett-Packard Series 1100 Chemstation, HP 1100 series quaternary pumps, HP 1100 series vacuum degasser, HP variable wavelength D₂ lamp UV/Vis detector, HP enhanced integrator, Rheodyne sample injector with 25 - 100 µl loops, Hamilton microlitre syringes. All water for HPLC was purified using a Millipore Elix 5 system, followed by an Elgastat UHQ II deioniser, and filtered using PK50, 0.45, 47 mm nylon 66 membranes from Anachem. The data were analysed using HP chemstation for Windows '95. The column and mobile phase used was dependent on the system being studied. The HPLC analysis was carried out with the aid of Dr Fuyi Wang.

2.1.5 Ultraviolet and Visible Spectroscopy

Ultraviolet and visible absorption spectra were obtained on a Perkin-Elmer λ 16 UV-Vis recording spectrophotometer using 1 cm pathlength quartz cuvettes. The temperature was controlled using a PTP1 Peltier Temperature Programmer. Spectra were normally referenced to solvent alone. Spectra were processed with UVWinlab for Windows '95.

2.2 Nuclear Magnetic Resonance Spectroscopy

The basic theory and concepts of NMR are given in detail in textbooks such as Mason¹ and Evans². The nuclei used in this study and their magnetic properties are given in Table 2.1.

Table 2.1 NMR properties of nuclei studied in this thesis.

Nucleus	Spin (I)	Natural Abundance (%)	Magnetogyric Ratio $\gamma / 10^7 \text{ rad T}^{-1} \text{ s}^{-1}$	NMR Frequency at 11.74 T (MHz)
^1H	1/2	99.98	26.75	500.13
^{31}P	1/2	100	10.80	202.4
^{15}N	1/2	0.37	-2.71	50.66

2.2.1 Exchange Effects in NMR Spectroscopy

The term “chemical exchange” in NMR refers to any process in which a nucleus exchanges between two or more different environments in which its NMR parameters (e.g. chemical shift, scalar coupling, or relaxation) differ.³ The process may be intra- or intermolecular, involving one or several species, respectively. Typical examples are isomerization or ligand exchange processes. The effect of the exchange process on the NMR spectrum depends upon its rate, relative to the magnitude of the accompanying change in NMR parameters. For example, if an ionization process is accompanied by a change in chemical shift of 250 Hz, an exchange rate of $> 1000 \text{ sec}^{-1}$ would be regarded as fast, one of $\approx 250 \text{ sec}^{-1}$ as intermediate, and one of $< 100 \text{ sec}^{-1}$ as slow on the “NMR time scale”. Each of these regions of exchange leads to characteristic effects on the NMR spectrum (Figure 2.1). In slow exchange, separate resonances are seen for the nucleus of interest in each of the two states A and B. By contrast, in fast exchange a single averaged resonance is observed. In the intermediate exchange region, there is a complex series of changes in appearance of the spectrum as the separate signals coalesce into one.

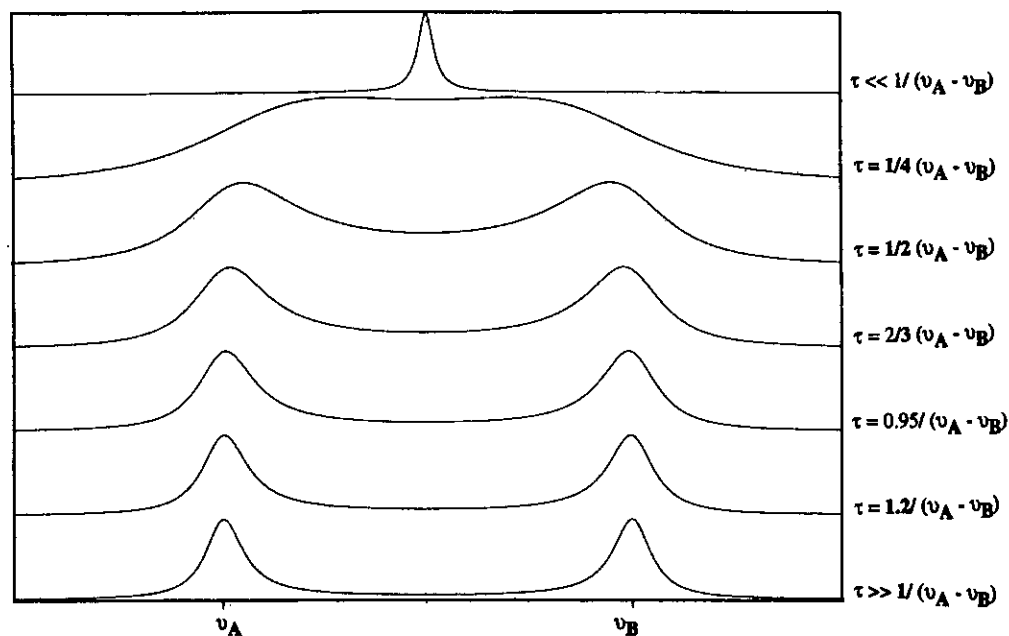


Figure 2.1 Change in chemical shifts and linewidths in the presence of chemical exchange between two equally populated environments. Bottom to top: increasing exchange rates as labelled (from ref 3). τ : exchange time; ν : resonance frequency.

For ^1H NMR experiments detecting interconversion between two isomers, isomers A and B appear at two resonance frequencies ν_A and ν_B .



If it is assumed that this exchange process obeys the first-order kinetics and depends on temperature, their exchange rate constants and activation parameters can be obtained from the temperature dependence of the resonance lines.⁴ If the coalescence temperature T_c at which the two signals just coalesce is detected, the rate constant k_c is given by:

$$k_c = 2.22 \Delta\nu$$

Here $\Delta\nu$ is the separation in Hz between the two signals in the absence of exchange. It is determined experimentally from spectra recorded at temperatures which are as

far below the coalescence temperature as possible. This equation applies only if the exchange process is first-order kinetically, if the two singlets have equal intensity, and if the exchanging nuclei are not coupled to each other. An important fact of k_c is that it is determined by the magnitude of $\Delta\nu$, which is in turn proportional to the resonance frequency. Thus T_c is not a constant, but depends on the observation frequency.

For a pH titration experiment,



the process of protonation/deprotonation is usually very fast with respect to the difference in chemical shift between the two species A and AH^+ . Thus a single resonance is observed, whose chemical shift is the weighted average of the chemical shifts of the deprotonated species A and protonated species AH^+ ,³

$\delta_{\text{obsd}} = \delta_A (X_A) + \delta_{AH} (X_{AH})$, where X_A and X_{AH} are the fractional populations of species A and AH, respectively, with $X_A + X_{AH} = 1$.

From the chemical shift data derived from pH titrations, pK_a values of deprotonation can be calculated according to the following formula.⁵

$$\delta_{\text{obsd}} = \frac{\delta_A + \delta_{AH} \times 10^{(pK_a - pH)}}{1 + 10^{(pK_a - pH)}}$$

2.2.2 Ruthenium NMR

Ruthenium has a very broad coordination chemistry with its oxidation states varying from -II up to + VII. The most relevant oxidation states for bioinorganic chemistry are Ru II, III, and IV. Among these, the +III state cannot be readily detected by conventional high-resolution NMR methods because of its paramagnetism. The paramagnetic nature of low-spin, d^5 Ru(III) strongly affects the NMR spectra of its coordinated ligands.⁶ For example, the resonances of protons are

generally severely shifted and broadened by Ru(III). The paramagnetic effects generated by the metal centre can be used to assist in assigning the geometry of the complex.⁷ The Ru complexes studied in this thesis contain diamagnetic Ru(II) and they have all been readily studied by using ^1H , ^{31}P , and ^{15}N NMR.

Table 2.2 NMR parameters of ^{99}Ru and ^{101}Ru .

Nucleus	Spin (I)	Natural Abundance (%)	Receptivity relative to ^{13}C	Quadrupole moment, $10^{-28} Q$, $\text{m}^2 \cdot \text{A}$
^{99}Ru	5/2	12.72	0.83	0.076
^{101}Ru	5/2	17.07	1.56	0.44

Although not a technique utilised in this thesis, it is interesting to consider the potential of using ruthenium NMR. Ruthenium has two magnetically active isotopes (Table 2.2): ^{99}Ru (natural abundance = 12.7 %) and ^{101}Ru (natural abundance = 17.1 %). Both isotopes have I values of 5/2, but ^{99}Ru has a much lower quadrupole moment than ^{101}Ru and hence gives narrower spectral lines.⁸ This makes ^{99}Ru more preferable than ^{101}Ru despite the higher receptivity of the latter. ^{99}Ru and ^{101}Ru NMR in solution was first reported for RuO_4 in 1981.^{8,9} These nuclei are also useful for solid state NMR,¹⁰ for example, for investigating magnetic properties of ruthenium oxides.¹¹ ^{99}Ru has a large range of the chemical shift, for example, from -1270 ppm for RuCp_2 (in CH_2Cl_2 solution) to +7821 ppm for $[\text{Ru}(\text{NH}_3)_6]^{2+}$ (in aqueous solution)¹² and is sensitive to the environment around the metal centre which allows study of subtle electronic changes at the Ru moiety.¹³ ^{99}Ru NMR provides a powerful tool for structural analysis and for distinction of isomers in enantiomeric mixtures.^{14,15} For examples, *mer*- and *fac*- isomers of $[\text{Ru}(2,3\text{-dpp})_3][\text{PF}_6]_2$ (2,3-dpp

= 2,3-bis(2-pyridyl)pyrazine) in acetonitrile solution were distinguished by ^{99}Ru NMR at 4510 and 4499 ppm, respectively.¹⁴ Generally, ^{99}Ru NMR is sensitive to the species and requires high symmetry and high concentration (50-500 mM). Therefore it was not investigated in this thesis since the Ru(arene) complexes have low symmetry.

2.2.3 ^{31}P NMR

^{31}P is spin $\frac{1}{2}$, 100% abundant and has a receptivity relative to the proton of 0.0663. In consequence, this is an easy nucleus to observe. ^{31}P NMR has a large chemical shift, ranging up to 1362 ppm in $\text{Bu}^t\text{P}\{\text{Cr}(\text{CO})_5\}_2$, but a range from -224 ppm (P_4S_3) to +326 ppm (P_4O_{10}) is more typical.¹⁶ The chemical shift is sensitive to the coordination environment. ^{31}P NMR is a general method to investigate the reaction of phosphate groups with metal anticancer complexes.^{17,18,19} The pH effect on the chemical shift is obvious when deprotonation/protonation process happens to the phosphate group. Thus, the curve of ^{31}P NMR chemical shift as a function of pH can provide the $\text{p}K_a$ values of phosphate groups. In this thesis, ^{31}P NMR has been used to investigate the direct coordination of Ru(II) to phosphate groups of DNA nucleotides or phosphate buffer (Chapter 6) and to detect the possible H-bonding interactions between the amino NH and phosphate O group (Chapter 5). In particular, the case of direct binding leads to a large ^{31}P NMR downfield shift (up to 5 ppm), whilst H-bonding only leads to a small ^{31}P NMR downfield shift (less than 1 ppm).

2.2.4 ^{15}N NMR

^{14}N NMR spectroscopy can be useful for ammine and amine complexes,^{20,21} but ^{14}N is a quadrupolar nucleus ($I = 1$, 99.6% natural abundance), and quadrupolar relaxation is dominant when the environment of ^{14}N has a low symmetry.²² This can lead to very broad lines and a consequent reduction in sensitivity

^{15}N has spin 1/2 and is recently the isotope of choice for the observation of nitrogen, despite its low natural abundance (0.37%) and low NMR sensitivity. Its receptivity as compared to ^1H is only 3.85×10^{-6} . The sensitivity of detection can be improved by the use of multipulse techniques and inverse detection technology.^{23,24,25} For example, the sensitivity of ^{15}N can be greatly improved by the use of inverse detection methods (^1H -detected ^{15}N), by a theoretical maximum of $306 \{(|\gamma_{\text{H}}|/|\gamma_{\text{N}}|)^{5/2}\}$ with respect to directly detected ^{15}N , such that signals can be detected in aqueous solutions at concentrations of physiological relevance (5 μM).²¹ ^1H -detected inverse methods are applicable to any system which contains a ^{15}N atom with a measurable spin-spin coupling to ^1H (i.e. $^1J(^{15}\text{N}, ^1\text{H})$) in ammine, primary and secondary amines, but not tertiary amines although a longer range coupling can sometimes be utilised. In practice the best applications are for those systems with large one-bond couplings (e.g. *ca.* 73 Hz for $^{15}\text{NH}_3$).

The protons bonded to ^{15}N can be selectively detected by these methods with natural abundance ^{15}N , but it takes several hours of instrument time and requires high concentrations of complexes (> *ca.* 30 mM in a 5-mm tube). By enriching complexes with >95% ^{15}N , it is possible to work at low millimolar concentrations, and even approach micromolar concentrations of physiological relevance. Much work has recently been done to apply ^{15}N -edited ^1H NMR and 2D [^1H , ^{15}N] heteronuclear single/multiple quantum coherence (HSQC/HMQC) NMR spectroscopy to the direct detection of ammine and amine N-H resonances of ^{15}N -labeled platinum complexes in aqueous solution.^{26,27,28} [^1H , ^{15}N] HSQC (see section 2.3) NMR has proven to be a powerful method for studying DNA platination reactions by cisplatin and other platinum complexes. The technique requires the use of ^{15}N -labeled compounds and allows observation of all platinated species at low (micromolar) concentrations. The work in this thesis has involved ^{15}N NMR studies of ^{15}N in natural abundance for characterization of mono Ru(arene) diamine and dinuclear bis-Ru(arene) diamine

complexes (Chapter 4) and ^{15}N -labeled Ru compounds for reactions with DNA nucleobase derivatives (Chapters 5 and 6).

2.2.5 Water Suppression

NMR spectra for biomolecules are normally obtained from aqueous media, such as D_2O (99.9%) or $\text{H}_2\text{O}/\text{D}_2\text{O}$ (90% : 10%, v/v). For metal anticancer complexes, such as Pt am(m)ine complexes, the Pt-NH ^1H NMR resonances cannot be readily detected in D_2O solutions because the half-life for H-D exchange is only a few minutes at ambient temperatures.^{29,30} In H_2O solutions, the intense H_2O peak causes severe dynamic range problems. For the Ru(arene) diamine complexes studied in this thesis, similarly, the Ru-NH ^1H NMR resonances cannot be detected within minutes. Therefore all the studies in aqueous media were carried out in $\text{H}_2\text{O}/\text{D}_2\text{O}$ (90% : 10%, v/v). Special techniques are needed to suppress the large HOD (or H_2O) signal. “Presaturation” and “WATERGATE (WATER suppression by GrAdient Tailored Excitation)” were used in this study.³¹ The former method is applied by irradiation at frequency of the HOD (H_2O) resonance during the relaxation delay between pulses, before the observe pulse in an experiment. The disadvantage is that exchangeable NH protons can also be saturated resulting in loss of intensity. The WATERGATE method uses selective 180° pulse field gradients to select different coherence pathways, and yields a superior water suppression.

2.2.6 Two Dimensional NMR

Heteronuclear Single-Quantum Correlation (HSQC)

The heteronuclear experiments allow identification of the chemical shifts of protons and X-nuclei that are directly coupled. One obvious use of this correlation information is to allow assignments already made for one nuclear species to be transferred to the other. A more important but less obvious use is in overcoming

problems caused by overlap in the proton spectrum: in the heteronuclear 2D spectrum the proton resonances are spread out according to the shifts of the heteronuclei to which they are coupled.

The heteronuclear single quantum coherence (HSQC) technique belongs to the family of indirect (or inverse) detection techniques. An important feature of the experiment is that the proton magnetisation which is detected during t_2 originated as proton magnetisation at the start of the sequence. The main advantage of this technique is the enhanced sensitivity, a theoretical maximum of $[\gamma_H/\gamma_X]^{5/2}$ (306 for ^{15}N) with respect to direct detection, and relatively short repetition time according to the T_1 of the protons and not of the X-nuclei.³² Therefore it is possible to detect species at concentrations of physiological relevance (mM or even μM) in D_2O or aqueous solution (90% H_2O /10% D_2O) where labile NH protons can be observed. By means of selective labelling ^{15}N , this technique can also simplify a complicated spectrum in which only those protons directly attached to ^{15}N atoms are detected. The basic HSQC pulse sequences is shown in Figure 2.2.

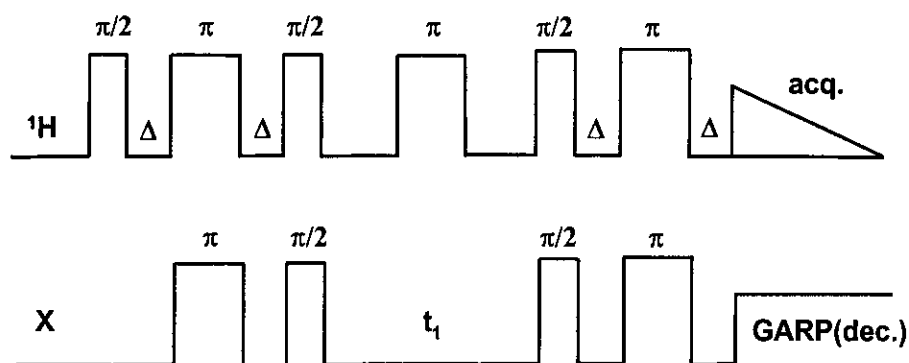


Figure 2.2 Basic HSQC pulse sequences. The delays Δ and τ are $\frac{1}{4}J$ and $\frac{1}{2}J$ respectively, where J is the one-bond HX coupling constant.

The Pt- ^{15}NH protons can be detected selectively by the use of [^1H , ^{15}N] single quantum coherence (HSQC) pulse sequences. A 1D ^1H spectrum containing only

resonances from Pt- ^{15}NH species is obtained by acquiring only the first increment in a two-dimensional experiment; resonances for CH and OH (including water) are eliminated. This is particularly useful in studies of body fluids or cell culture media, where only the signals from platinum complexes are detected and thousands of other overlapping ^1H resonances are filtered out. If ^{15}N decoupling is employed during acquisition (e.g. the GARP method), then each type of Pt-NH resonance appears as a singlet. In practice the water resonance is so intense that it is usually necessary to use additional solvent suppression techniques (e.g. presaturation). The addition of an H_2O T_2 relaxation agent (e.g. 0.5 M $(\text{NH}_4)_2\text{SO}_4$) can also be helpful to detect NH peaks very close to the H_2O peak. A large improvement in water suppression is achieved by the use of pulsed field gradients for coherence selection, for example, by use of the HSQC sequences of Stonehouse *et al.*³³

CORrelation SpectroscopY (COSY)

COSY is a two-dimensional homonuclear (H, H)-correlated NMR experiment which is used to correlate the chemical shifts of ^1H nuclei which are J-coupled to one another. It is the standard 2D experiment that can be used to identify pairs of protons that have a scalar spin-spin coupling connectivities. Two types of COSY sequences were used in this thesis: magnitude COSY^{34,35} and double-quantum filtered (DQF-) COSY³⁶. Their basic pulse sequences are shown in Figure 2.3. The 2D spectrum acquired in these magnitude COSY experiments has line-shapes in both F1 and F2 that have both absorption and dispersion contributions. This means that it is not possible to phase the spectrum so that the peaks are purely absorptive, and the spectrum must be displayed in magnitude mode. A typical resolution is 3 Hz/pt. This is sufficient for resolving large couplings, but not for resolving small ones. In order to resolve small J-couplings, it is necessary to use fine

digital resolution and this requires a significant increase in the experiment time. In general, if higher resolution is desired, it is desirable to use DQF-COSY.

More favourable lineshapes can be obtained by using the double-quantum filtered COSY sequence (DQF-COSY) (Figure 2.3b). This experiment is phase-sensitive, i.e. the cross peaks of the DQF-COSY spectrum can be displayed with pure absorption line-shapes in both F1 and F2. A phase-sensitive spectrum has a higher resolution than an otherwise equivalent magnitude spectrum because the magnitude lineshape is broader than the pure absorption lineshape. The DQF-COSY sequence has two significant advantages. First, partial cancellation of the diagonal peaks in a DQF-COSY spectrum means that the diagonal ridge is much less pronounced in a DQF-COSY spectrum than it is in a normal COSY spectrum. This makes it easier to observe cross peaks between signals which are close together in chemical shift. The double quantum filter eliminates the strong signals from, e.g., solvent ^1H 's that do not experience homonuclear J-coupling. These signals would otherwise dominate the spectrum and possibly be a source of troublesome t_1 noise.

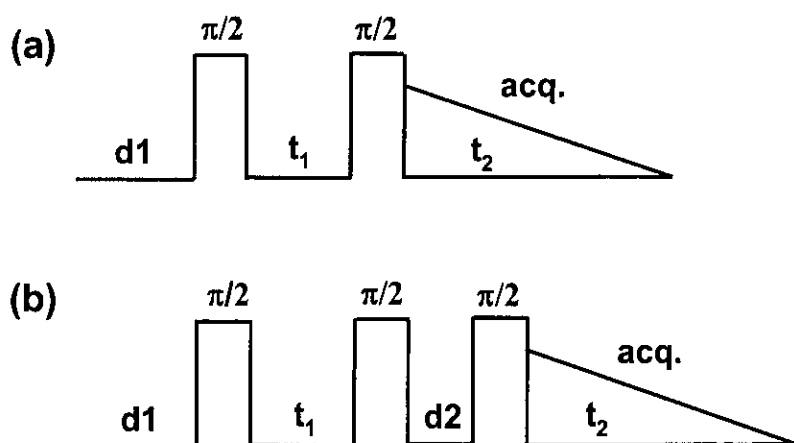


Figure 2.3 Pulse sequences for two-dimensional (a) COSY and (b) DQF-COSY.

Nuclear Overhauser Effect Spectroscopy (NOESY)

The NOESY experiment^{37,38} is often used to identify pairs of protons that are undergoing cross-relaxation, i.e. protons that would show an NOE in 1D experiments. Most commonly, NOESY is used as a homonuclear ^1H technique. In NOESY, direct dipolar couplings provide the primary means of cross-relaxation, and so spins undergoing cross-relaxation are those which are close to one another in space. Thus, the cross peaks of a NOESY spectrum indicate which ^1H 's are close to which other ^1H 's in space.

The characteristic feature of the NOESY pulse sequence (Figure 2.4) is the mixing time τ (the time during which cross relaxation occurs). The cross-peaks are generated by magnetisation transfer that takes place during the mixing time so the length of this delay must be chosen according to the rate of the transfer process. In contrast to the COSY experiment, both cross-peaks and diagonal peaks in NOESY spectra can be obtained with absorption-mode lineshapes. The experiments are therefore eminently suitable for studying macromolecules. If presaturation is used in a NOESY experiment it is usually also necessary to irradiate the solvent during the mixing time. A complicated factor is that the NOESY pulse sequence cannot distinguish between magnetisation transfer caused by cross-relaxation and magnetisation transfer by chemical exchange. In macromolecules, both give cross-peaks of the same sign as the diagonal.

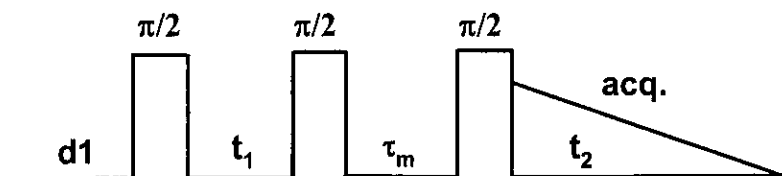


Figure 2.4 2D NOESY pulse sequence. τ_m is the mixing time.

Total Correlation Spectroscopy (TOCSY)

TOCSY provides a different mechanism of coherence transfer than COSY for 2D correlation spectroscopy.^{39,40} In TOCSY, cross peaks are generated between all members of a coupled spin network. An advantage is that the “net” coherence transfer produced can be arranged to create pure absorption mode spectra with positive intensity peaks (rather than “differential” coherence transfer which causes spectra with equal positive and negative intensities). In traditional COSY, cross-peaks have zero integrated intensity and coherence transfer is restricted to directly spin-coupled nuclei. In TOCSY, oscillatory exchange is established which proceeds through the entire coupling network so that there can be net magnetization transfer from one spin to another even without direct coupling. The isotropic mixing which occurs during the spin-lock period of the TOCSY sequence exchanges all in-phase as well as anti-phase coherences. The basic pulse sequence is shown in Figure 2.5.

The coherence transfer period of the TOCSY sequence occurs during a multiple-pulse spin-lock period. The multiple-pulse spin-lock sequence most commonly used is MLEV-17. The length of the spin-lock period determines how “far” the spin coupling network will be probed. A general rule of thumb is that $1/(10 J_{HH})$ should be allowed for each transfer step, and five transfer steps are typically desired for the TOCSY spectrum. The TOCSY spectrum can be phased so that all cross peaks and diagonal peaks have positive intensity.

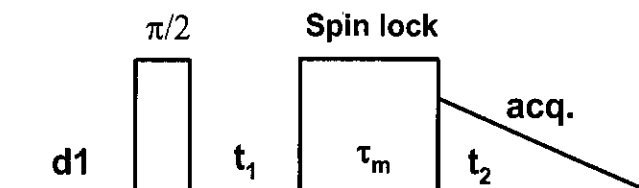


Figure 2.5 2D TOCSY pulse sequence

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Chapter 3

(η^6 -polycyclic arene)Ru(II) Complexes

3.1 Introduction

Cisplatin is an effective clinical anti-tumour drug, however, it also has many side-effects, such as nausea, neuropathy and loss of high frequency hearing.¹ Our laboratory has been developing organometallic Ru(II)(arene) complexes as novel anticancer agents. A series of nine Ru(arene) complexes with different substituted aromatic rings, has been synthesised in this laboratory² and assessed against the human ovarian cancer cell line, A2780, and drug resistant sub-clones by the Oncology Unit, Western General Hospital. The IC₅₀ values for the Ru(II) compounds in A2780 cells ranged from 6-200 μ M, compared to 0.6 μ M for cisplatin and 6 μ M for carboplatin. The two most active compounds, [$(\eta^6$ -biphenyl)Ru(en)Cl][PF₆] (7, IC₅₀ 6 μ M, where en = ethylenediamine) and [$(\eta^6$ -*p*-cymene)Ru(en)Cl][PF₆] (IC₅₀ 8 μ M) were evaluated in cisplatin-resistant A2780cis cells and multi-drug resistant 2780^{AD} (AD = adriamycin) cells. Both compounds were non-cross resistant in A2780cis, whereas cross-resistance was observed in A2780^{AD}.³

These preliminary cytotoxicity tests indicated that more potent activity was associated with chemical stability and increased hydrophobicity of the compounds.⁴ The aromatic ligand is likely to exert a larger effect on the anticancer activity, and thus, introducing variety into the arene ring allows investigation of structure-activity relationships for the Ru(arene) complexes.

In this Chapter, polycyclic ring systems are introduced into Ru(II) complexes to allow investigation of their anticancer activity. A range of novel tetrahydroanthracene (THA, C₁₄H₁₄) chloro (η^6 -C₁₄H₁₄)Ru(II) complexes (1, 2 and 3) and dihydroanthracene (DHA, C₁₄H₁₂) chloro (η^6 -C₁₄H₁₂)Ru(II) complexes (4, 5 and 6) were synthesised (Scheme 3.1) and characterised by ¹H NMR, X-ray crystallography and CHN analysis. They show interesting structural properties which may lead to exciting biological activities. The details of the synthesis and

characterisation for the ethylenediamine complexes $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**1**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**4**) will be reported in Chapter 5, but their structures are described here for comparison with other Ru(arene) complexes. In addition, the three aqua Ru(arene) complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2$ (arene = Bip (biphenyl), **8**; THA, **9**; DHA, **10**) were also prepared and characterised by ^1H NMR and X-ray crystallography. Their structural features are compared with their parent chloro complexes **7** ($[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$), **1** and **4**.

3.2 Experimental Section

3.2.1 Materials and Methods

The complex $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**7**) was prepared as previously described.² Ethylenediamine was freshly distilled over Na, and ethanol and methanol were dried over P_2O_5 . Tetrahydrofuran (THF) was dried by distillation from Na-benzophenone.

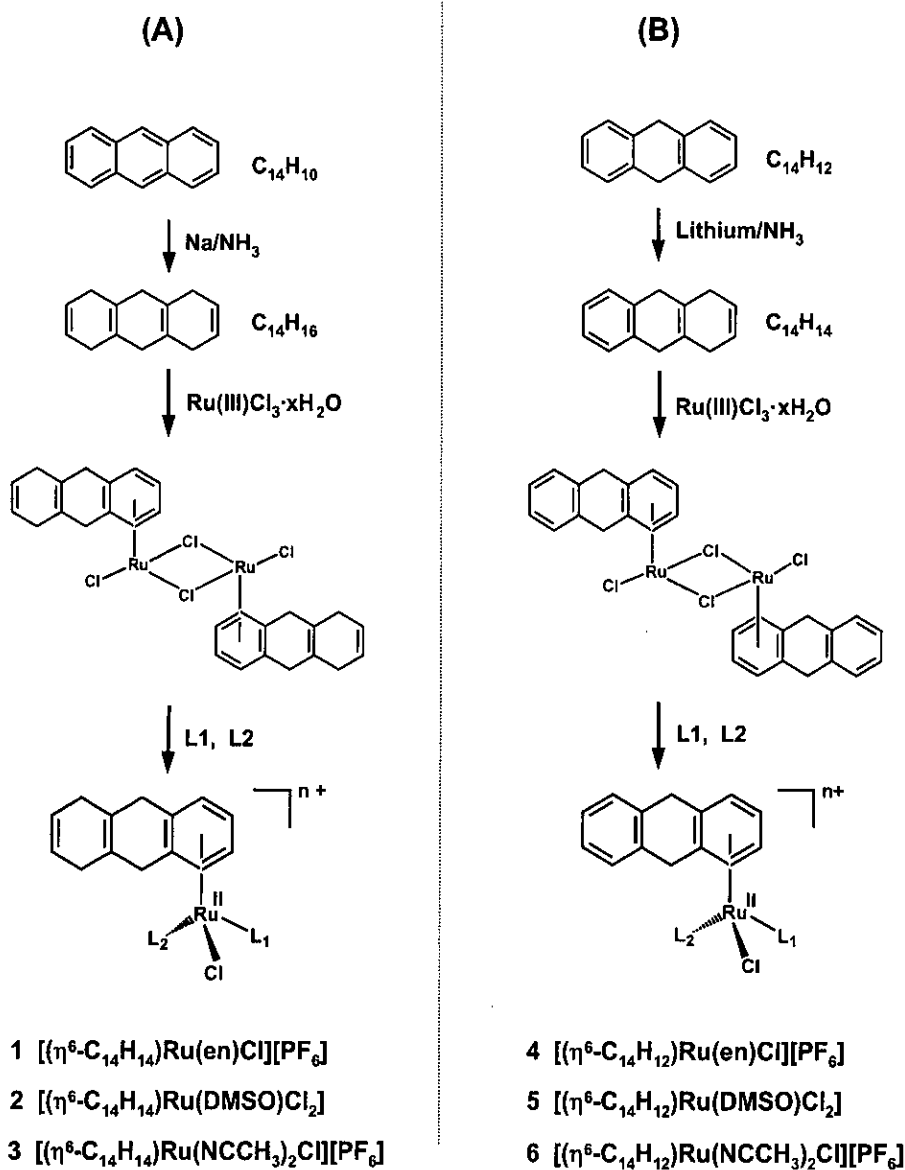
3.2.2 Preparation of Chloro Ru(arene) Complexes 1-6

1,4,5,8,9,10-Hexahydroanthracene ($\text{C}_{14}\text{H}_{16}$)⁵

Anthracene ($\text{C}_{14}\text{H}_{10}$, 4.0 g, 22.4 mmol) was dissolved in freshly dried THF (200 mL) and ethanol (40 mL). This mixture was added to liquid NH_3 (500 mL) which had been condensed under argon into a 1 litre flask equipped with a Dewar condenser, cooling bath (dry-ice/acetone) and mechanical stirrer. Sodium (10.40 g, 0.45 mol) was added in small pieces over a period of 20 min. After a further 50 min stirring at -60°C , the cooling bath was removed and the ammonia was allowed to evaporate under an argon flow with stirring. To the residue was added 50 mL water slowly to decompose the excess of sodium and then a further 150 mL. This was extracted with diethyl ether (4×250 mL) and the combined ether layers washed



Scheme 3.1 Preparation of (A) $\{(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}\}^{2+}$ complexes **1**, **2** and **3**, and (B) $\{(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}\}^{2+}$ complexes **4**, **5** and **6**.



with saturated NaCl solution (2×250 mL) and dried over MgSO_4 . Removal of diethyl ether on a rotary evaporator afforded a white solid, which was recrystallised $2 \times$ first from benzene/chloroform (1:1), and then from benzene to yield a white needle product, 98% pure by ^1H NMR. This was used for preparation of dimer $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$. Yield: 1.54 g, 8.36 mmol, 37.3 %. ^1H NMR (CDCl_3) δ : 5.68 (s, 4H), 2.51 (s, 8H), 2.37 (s, 4H).

1,4,9,10-Tetrahydroanthracene ($\text{C}_{14}\text{H}_{14}$)⁶

9,10-Dihydroanthracene ($\text{C}_{14}\text{H}_{12}$, 6.5 g, 36.1 mmol) dissolved in 540 mL THF was added to refluxing ammonia (1100 mL) which had been condensed under argon into a 2 litre flask equipped with a Dewar condenser, cooling bath (dry-ice/acetone) and mechanical stirrer. Li wire (0.66 g, 95.1 mmol) was added in small pieces over a period of 20 min. After refluxing for 2.7 h with stirring, 60 mL ethanol was added to the reaction mixture and then 130 mL water, and the ammonia allowed to evaporate overnight. This was extracted with diethyl ether (3×250 mL) and the combined ether layers washed with saturated NaCl solution (1×500 mL) and dried over MgSO_4 . Removal of ether on the rotary evaporator afforded a light yellow solid which was recrystallized $5 \times$ from benzene to remove of the hexahydroanthracene ($\text{C}_{14}\text{H}_{16}$) as white needles. The residue (3.3 g) contains the starting material $\text{C}_{14}\text{H}_{12}$ (18.6%) and $\text{C}_{14}\text{H}_{14}$ (81.4%). This mixture was directly used for preparation of dimer $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2]_2$. ^1H NMR (CDCl_3) of $\text{C}_{14}\text{H}_{14}$ δ : 7.11 (m, 4H), 5.78 (s, 2H), 3.25 (s, 4H), 2.70 (s, 4H).

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2]_2$.

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru(en)Cl}][\text{PF}_6]$ (1) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru(en)Cl}][\text{PF}_6]$ (4)

For details of preparations, see Chapter 5.

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{dmsO})\text{Cl}_2]$ (2) ⁷

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ (0.05 g, 0.07 mmol) was dissolved in dimethyl sulfoxide (2 mL) and filtered to yield a deep red solution. Slow diffusion of diethyl ether into this solution resulted in the formation of brilliant red crystals suitable for X-ray diffraction. The crystals were collected and washed thoroughly with diethyl ether (4 $\times$ 10 mL).

Yield: 0.03 g, 0.07 mmol, 49.5%. Anal. Calcd for $\text{C}_{16}\text{H}_{20}\text{Cl}_2\text{ORuS}$ (432.37): C, 44.45; H, 4.66. Found : C, 44.41; H, 4.51. ^1H NMR (CDCl_3) δ : 5.74 (s, 2H), 5.68 (m, 2H), 5.50 (m, 2H), 3.50 (m, 2H), 3.00 (m, 2H), 2.65 (m, 4H), 3.15 (s, 6H).

 $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{NCCH}_3)_2\text{Cl}][\text{PF}_6]$ (3)

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ (0.10 g, 0.144 mmol) was suspended in 10 mL acetonitrile. NH_4PF_6 (47.1 mg, 0.288 mmol) in 2 mL acetonitrile was added in one portion. The reaction was stirred at 60°C without special precautions to exclude air. After 48 h the pale brown precipitate was filtered off and orange filtrate evaporated to yield an orange solid. This was recrystallized from acetonitrile/ether to yield orange crystals.

Yield: 0.13 g, 0.238 mmol, 82.8%. Anal. Calcd for $\text{C}_{18}\text{H}_{20}\text{ClF}_6\text{N}_2\text{PRu}$ (545.86): C, 39.60; H, 3.69; N, 5.13. Found: C, 39.17; H, 3.48; N, 5.47. ^1H NMR (Acetone- d_6) δ : 5.95 (s, 2H), 5.80 (m, 4H), 3.28 (s, 4H), 2.87 (d, 4H), 2.71 (m, 6H)

 $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{dmsO})\text{Cl}_2]$ (5)

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2]_2$ (0.05 g, 0.07 mmol) was dissolved into dimethyl sulfoxide (1 mL) and filtered to yield a rose red solution. Slow diffusion of diethyl ether into this solution resulted in the formation of brilliant red crystals suitable for

X-ray diffraction. The crystals were collected and washed thoroughly with diethyl ether (3×10 mL).

Yield: 0.025 g, 0.058 mmol, 41.4%. Anal. Calcd for $C_{16}H_{18}Cl_2ORuS$ (430.35): C, 44.65; H, 4.21. Found: C, 44.08; H, 4.18. ^1H NMR ($CDCl_3$) δ : 7.25 (m, 4H), 5.75 (m, 4H), 4.25 (d, 2H), 3.80 (d, 2H), 2.97 (s, 6H).

$[(\eta^6-C_{14}H_{12})Ru(NCCH_3)_2Cl][PF_6]$ (6)

$[(\eta^6-C_{14}H_{12})RuCl_2]_2$ (0.10 g, 0.142 mmol) was suspended in 10 mL acetonitrile. NH_4PF_6 (48.6 mg, 0.298 mmol) in 2 mL acetonitrile was added in one portion. The reaction was stirred at 60°C without special precautions to exclude air. After 25 h the pale brown precipitate was filtered off and orange filtrate evaporated to yield an orange solid. This was recrystallized from acetonitrile/ether to yield orange crystals.

Yield: 0.125 g, 0.23 mmol, 81%. Anal. Calcd for $C_{18}H_{18}ClF_6N_2PRu$ (543.85): C, 39.75; H, 3.34; N, 5.15. Found: C, 39.42; H, 3.33; N, 5.14. ^1H NMR (Acetone- d_6) δ : 7.39 (m, 4H), 6.15 (m, 4H), 4.05 (m, 4H), 2.85 (s, 3H), 2.45 (s, 3H).

3.2.3 Preparation of Aqua Ru(arene) Complexes 8-10.

A solution of $AgPF_6$ (1.3 mol equiv) in H_2O was added to a solution of $[(\eta^6\text{-arene})Ru(en)Cl][PF_6]$, complex 7 (100 mg, 0.20 mmol), complex 1 (49.4 mg, 0.09 mmol) or complex 4 (61 mg, 0.12 mmol), in 20 mL H_2O , giving a yellow-orange solution and an immediate precipitate of $AgCl$ (Scheme 3.2). This mixture was stirred over night at 40°C (protected from light) and was then filtered to remove $AgCl$. Evaporation of the solution to dryness gave a dark-yellow product. This was recrystallized from water to afford a crop of fine yellow crystals. Further recrystallization from water at 4°C afforded yellow needles which were suitable for X-ray structure determination.

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2$ (**8**), yield: 31.0 mg, 24.6%. ^1H NMR (10% D_2O , pH 7.6): δ 7.80 (m, 2H), 7.618 (m, 3H), 6.116 (d, 2H), 5.991 (t, 2H), δ 5.844 (t, 1H), 5.967 (broad, 2H), 3.768 (broad, 2H), 2.455 (m, 2H), 2.390 (m, 2H).

$[(\eta^6\text{-THA})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2$ (**9**), yield: 49.0 mg, 83.6%. ^1H NMR (10% D_2O , pH 4.5): δ 6.057 (broad, 2H), 5.863 (s, 2H), 5.808 (m, 2H), 5.729 (m, 2H), 4.045 (broad, 2H), 3.357 (q, 2H), 3.015 (q, 2H), 2.760 (s, 4H), 2.448 (m, 2H), 2.368 (m, 2H).

$[(\eta^6\text{-DHA})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2$ (**10**), yield: 30.0 mg, 39.5%. ^1H NMR (10% D_2O , pH 6.7): 7.473 (m, 2H), 7.407 (m, 2H), 5.954 (m, 2H), 5.857 (m, 2H), 5.737 (broad, 2H), 3.98 (q, 2H), 3.864 (q, 2H), 3.654 (broad, 2H), 2.10 (m, 2H), 2.011 (m, 2H).

3.3 Results and Discussion

$(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{II})$ Complexes 1-3

The THA ($\text{C}_{14}\text{H}_{14}$) complexes, $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**1**), $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{DMSO})\text{Cl}_2]$ (**2**) and $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{NCCH}_3)_2\text{Cl}][\text{PF}_6]$ (**3**), were synthesised by reaction of the dimer $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ with three different ligands: ethylenediamine, DMSO and acetonitrile (Scheme 3.1). The preparation of the dimer $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ began with anthracene ($\text{C}_{14}\text{H}_{10}$) which was first reduced using sodium metal in liquid ammonia to give 1,4,5,8,9,10-hexahydroanthracene ($\text{C}_{14}\text{H}_{16}$).⁵ Then, reaction between $\text{C}_{14}\text{H}_{16}$ and hydrated RuCl_3 in refluxing ethanol afforded the yellow-brown dimer $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$,⁷ a member of the well-known chloro-bridged polyruthenium family, where the $\text{C}_{14}\text{H}_{16}$ skeleton is aromatized in one outer ring by dehydrogenation and binding to Ru as a π -bonding arene.

Table 3.1 Crystallographic data for $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{CNCH}_3)_2\text{Cl}][\text{PF}_6]$ (**3**), $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{DMSO})\text{Cl}_2]$ (**5**), $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{CNCH}_3)_2\text{Cl}][\text{PF}_6]$ (**6**), $[(\eta^6\text{-Bip})\text{Ru}(\text{en})(\text{OH}_2)][(\eta^6\text{-Bip})\text{Ru}(\text{en})(\text{OH})][\text{PF}_6]_3$ (**8**), $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2 \cdot \text{H}_2\text{O}$ (**9**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(\text{OH}_2)][\text{PF}_6]_2 \cdot 2\text{H}_2\text{O}$ (**10**).

	3	5	6	8	9	10
Empirical formula	$\text{C}_{20}\text{H}_{23}\text{ClF}_6\text{N}_3\text{PRu}$	$\text{C}_{16}\text{H}_{18}\text{Cl}_2\text{ORuS}$	$\text{C}_{20}\text{H}_{21}\text{ClF}_6\text{N}_3\text{PRu}$	$\text{C}_{28}\text{H}_{39}\text{F}_{18}\text{N}_4\text{O}_2\text{P}_3\text{Ru}_2$	$\text{C}_{16}\text{H}_{26}\text{F}_{12}\text{N}_2\text{O}_3\text{P}_2\text{Ru}$	$\text{C}_{16}\text{H}_{20}\text{F}_{12}\text{N}_2\text{O}_3\text{P}_2\text{Ru}$
Molar mass	586.90	430.33	584.89	1100.68	669.40	685.40
Crystal system	triclinic	monoclinic	triclinic	monoclinic	triclinic	triclinic
Crystal size /mm	0.84 x 0.66 x 0.08	0.80 x 0.20 x 0.14	0.74 x 0.43 x 0.18	0.43 x 0.21 x 0.09	0.28 x 0.24 x 0.18	0.30 x 0.14 x 0.12
Space group	<i>P</i> -1	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> -1	<i>C</i> 2/ <i>c</i>	<i>P</i> -1	<i>P</i> -1
Crystal habit	yellow lath	orange block	orange lath	orange plate	yellow block	orange block
<i>a</i> / Å	6.8697(18)	10.947(5)	6.921(3)	14.400(4)	9.3030(17)	11.586(3)
<i>b</i> / Å	12.706(3)	12.405(5)	12.813(5)	16.011(5)	9.8849(18)	13.319(4)
<i>c</i> / Å	14.722(4)	12.543(5)	14.613(5)	17.049(5)	13.086(2)	16.241(4)
α / deg	115.235(15)	90	64.514(18)	90	85.585(3)	98.663(4)
β / deg	96.606(16)	108.36(3)	78.041(18)	106.881(4)	88.262(3)	94.970(4)
γ / deg	93.916(15)	90	84.800(18)	90	85.679(3)	91.094(4)
<i>T</i> / K	150 (2)	220 (2)	220 (2)	150 (2)	150 (2)	150
<i>Z</i>	2	4	2	4	2	4
$R [F > 4\sigma(F)]^a$	0.0305	0.0343	0.0305	0.0524	0.0562	0.0936 ^d
R_w^b	0.0768	0.0799	0.0762	0.1254	0.1409	0.0772 ^d
GOF ^c	1.020	1.054	1.057	1.055	1.034	1.117
$\Delta\rho$ max and min, / eÅ ⁻³	+ 0.65, -0.87	+ 0.58, -0.83	+ 0.69, -0.83	+ 1.64, -1.12	+1.31, -1.02	+ 2.28, -1.21

^a $R = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^b $R_w = [\sum w(F_o^2 - F_c^2)^2 / \sum wF_o^2]^{1/2}$. ^c $\text{GOF} = [\sum w(F_o^2 - F_c^2)^2 / (n-p)]^{1/2}$, where *n* = number of reflections and *p* = number of parameters. ^d

Refinement based on *F* with 7242 data with $F > 6\sigma(F)$.

Table 3.2 Selected bond lengths (Å) and angles (deg) for complexes **3**, **5**, **6**, **8**, **9** and **10**.

Bond(s)	3	5	6	8	9	10^c
Ru1-C1	2.176(3)	2.255(5)	2.179(3)	2.207(6)	2.224(5)	2.235(7)
Ru1-C2	2.185(3)	2.217(5)	2.180(3)	2.188(6)	2.180(5)	2.176(7)
Ru1-C3	2.173(3)	2.171(5)	2.174(3)	2.164(7)	2.175(5)	2.174(7)
Ru1-C4	2.166(3)	2.181(5)	2.176(3)	2.162(7)	2.156(5)	2.164(7)
Ru1-C5	2.198(3)	2.178(5)	2.196(3)	2.173(7)	2.146(5)	2.210(7)
Ru1-C6	2.208(3) (Ru1-C14)	2.210(5)	2.202(3) (Ru1-C14)	2.201(6)	2.216(5) (-C6A)	2.207(7)
Ru1-N1	2.078(3) (Ru1-N1A)		2.073(3)	2.113(5)	2.111(4)	2.138(7)
Ru1-N2	2.078(3) (Ru1-N1B)		2.076(3)	2.130(5)	2.115(5)	2.135(6)
Ru1-Cl1	2.4072(9)	2.3935(15)	2.4041(11)			
Ru1-Cl2		2.4087(14)				
Ru1-O1				2.090(4)	2.163	2.161(5)
Ru1-S1		2.3279(14)				
N1-Ru1-N2	85.96(10) (N1A-Ru1-N1B)		85.54(10)	79.4(2)	79.44(18)	78.6(3)
N1-Ru1-Cl1	87.03(7) (N1A-Ru1-Cl1)		86.99(7)	79.4(2) (N1B-Ru1-O1W)	77.4(2) (N1B-Ru1-O1) ^b	81.5(2) (N12-Ru1-O1)
N2-Ru1-Cl1	87.35(7) (N1B-Ru1-Cl1)		87.77(7)	82.9(2) (N2B-Ru1-O1W)	87.6(2) (N2B-Ru1-O1) ^b	81.9(2) (N22-Ru1-O1)
Cl1-Ru1-Cl2		89.28(5)				
Cl1-Ru1-S1		85.00(5)				
Cl2-Ru1-S1		86.86(5)				

^a. For atom labels see Figures 3.1, 3.2 and 3.3. ^b. O1 has an occupancy factor of 0.7.^c. Refers to molecule 1 (Ru1). The data for molecule 2 (Ru2) are not listed.

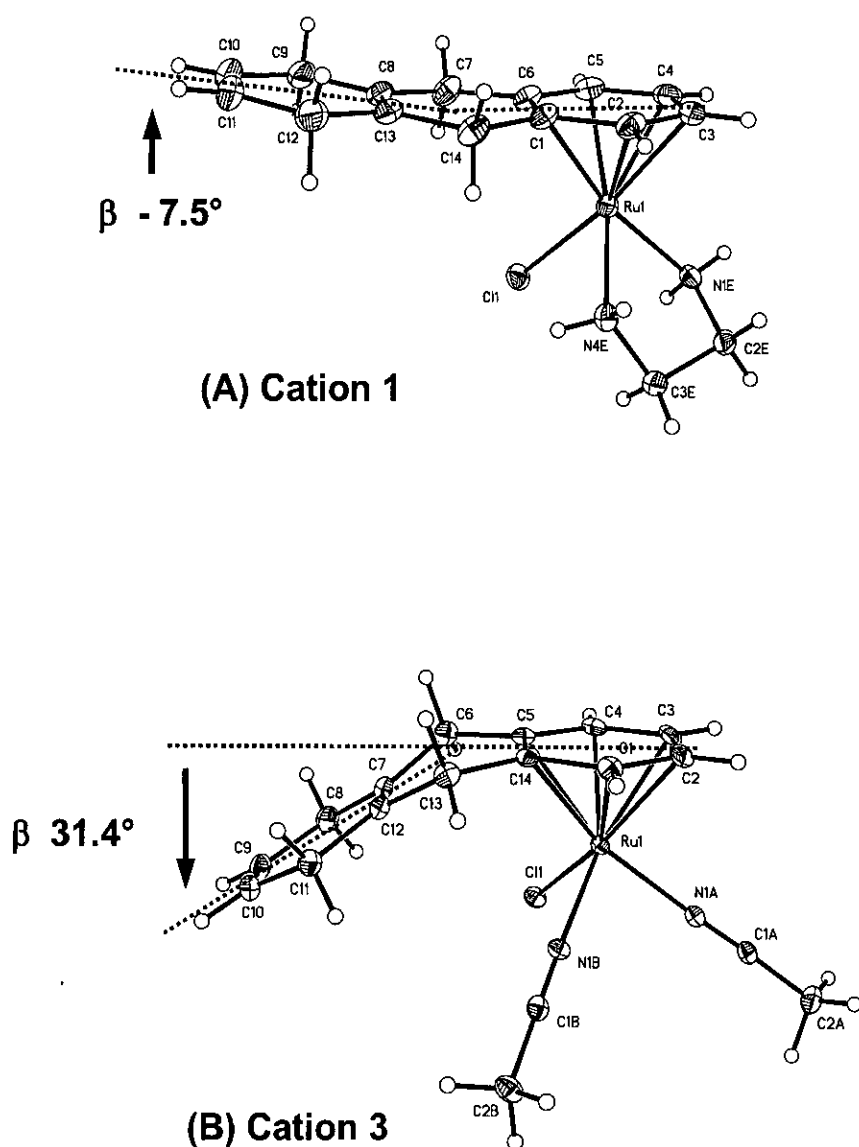


Figure 3.1 X-ray structures and atom numbering schemes for $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{CNCH}_3)_2\text{Cl}]^+$ in complex **3** (B), with the $\text{C}_{14}\text{H}_{14}$ ring conformation compared to that for $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}]^+$ in complex **1** (A, from Chapter 5), at 30% probability thermal ellipsoids. The PF_6^- counter-ion has been omitted for clarity. The hinge bending angle β is defined as in Table 3.3.

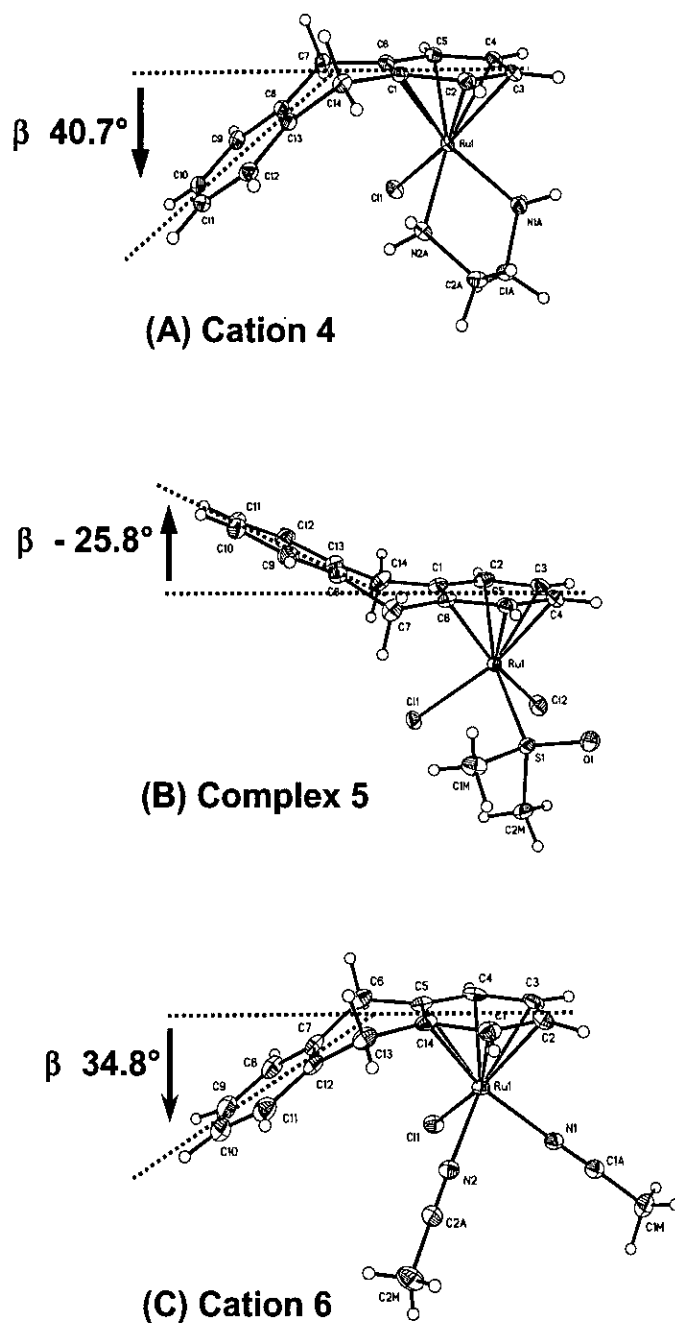


Figure 3.2 X-ray structures and atom numbering schemes for $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{DMSO})\text{Cl}_2]$ (**5**, B) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{NCCH}_3)_2\text{Cl}]^+$ in complex **6** (C), with the $\text{C}_{14}\text{H}_{12}$ ring conformation compared to that for $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}]^+$ in complex **4** (A, from Chapter 5), at 30% probability thermal ellipsoids. The PF_6^- counter-ion has been omitted for clarity. The hinge bending angle β is defined as in Table 3.3.

Crystals of **1** (Chapter 5) and **3** suitable for X-ray analysis were obtained through ether diffusion into an ethanol solution of **1** and an acetonitrile solution of **3**. The structures of cations **1** (from chapter 5) and **3** are displayed in Figure 3.1. The crystallographic data of **3** are shown in Table 3.1, and selected bond lengths and angles listed in Table 3.2. Figure 3.1 shows interesting structural features of complexes **1** and **3**. In **1**, the coordinated tricyclic-ring system is nearly flat and the outer ring is slightly tilted up from the Ru centre about C7-C14 by 7.2° . The Ru-centroid distance of 1.675 Å is normal; the two ring-junction carbons C1 and C6 are slightly further away from the metal centre than C3 and C4. In **3**, the planarity of the coordinated tricyclic-ring system is lost, and the outer ring is twisted down from the Ru centre about C5 and C14 by 31.4° , but the two ring-junction carbons C5 and C14 are still slightly further away from the metal centre than C2 and C3. The Ru-centroid distance of 1.614 Å is 0.061 Å shorter than that of **1**; the Ru-Cl distance of 2.4072(9) Å is slightly longer than 2.4033(16) Å distance found in **1**. The Ru-N $\equiv$ CCH₃ linkage is linear and the Ru-N distances are actually equal, 2.078(3) Å. The crystal structure of complex **2** has been reported,⁷ and the planarity of the coordinated tricyclic-ring system is lost too. The outer ring is significantly tilted up from the Ru centre by 23° and the other structural features are similar to those of **1** and **3**.

(η^6 -C₁₄H₁₂)Ru Complexes 4-6

An analogous polycyclic series of DHA (C₁₄H₁₂) complexes [(η^6 -C₁₄H₁₂)Ru(en)Cl][PF₆] (**4**, Chapter 5), [(η^6 -C₁₄H₁₂)Ru(DMSO)Cl₂] (**5**) and [(η^6 -C₁₄H₁₂)Ru(NCCH₃)₂Cl][PF₆] (**6**) was prepared via a similar method to that for the synthesis of (η^6 -C₁₄H₁₄)Ru complexes **1-3**. The dimer [(η^6 -C₁₄H₁₂)RuCl₂]₂ was obtained by reaction between 1,4,9,10-tetrahydroanthracene (C₁₄H₁₄) and hydrated

RuCl₃ during which the outer diene ring was dehydrogenated and aromatized (Scheme 3.1).

Their structures were all confirmed by X-ray diffraction studies and are displayed in Figure 3.2. Crystallographic data of **5** and **6** are shown in Table 3.1 and selected bond lengths and angles are listed in Table 3.2. These three molecular structures exhibit the familiar piano-stool geometry similar to those of complexes **1**, **2** (ref. 7) and **3** (Figure 3.1). An interesting feature is that the coordinated C₁₄H₁₂ ring adopts three different conformations in these three complexes in the solid state. It is of interest to examine the nature of the C₁₄H₁₂ ring in different environments.

As previously reported,^{8,9,10} there are two preferred conformations for the C₁₄H₁₂ molecular skeleton, either folded with a dihedral angle (defined as the angle between the planes of the two benzene rings) close to 146.2° (sample standard deviation 9.2°), or planar (or very nearly so); the individual values depend on the nature of the substituent. For the structures of these three (η^6 -C₁₄H₁₂)Ru complexes, the coordinated C₁₄H₁₂ rings are all folded through the central rings, exhibiting a boat conformation (Figure 3.2). In complex **4**, the ring system is twisted down towards the Ru-centre and the central ring forms a dihedral angle of 139.3°; in complex **5**, the ring system is tilted up from the Ru-centre and has a dihedral angle of 154.2°; and in complex **6**, the ring system is twisted down again with a dihedral angle of 145.2°. These can be compared with free C₁₄H₁₂ in the solid state, which has a folded shape with a dihedral angle of 144.7°, while C₁₄H₁₂·2(TNB) was found to be virtually planar.¹¹ It seems that the marked conformational change in our cases is due to the effect of metal coordination on the C₁₄H₁₂ ring but is still within the normal range.

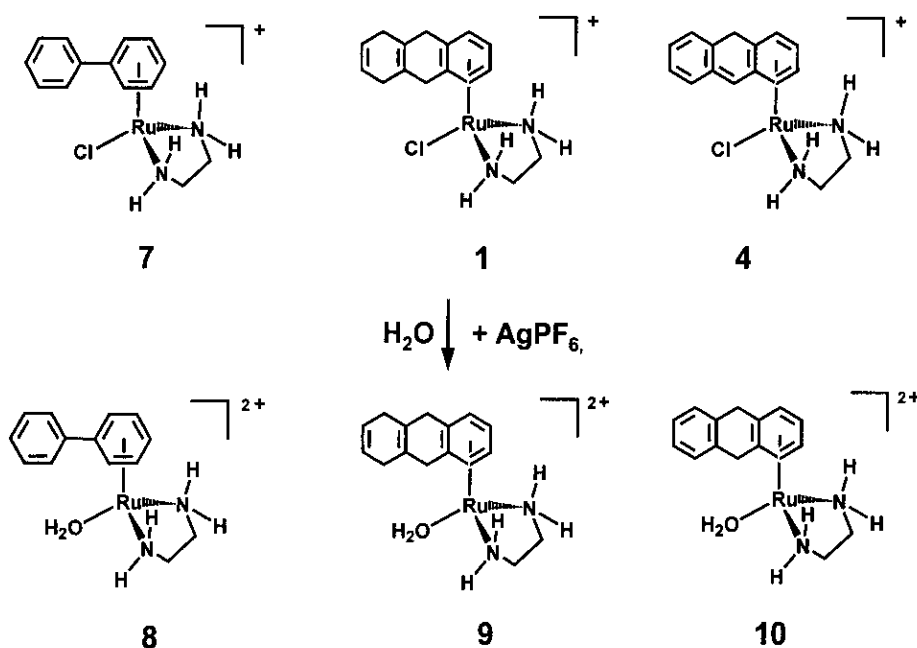
On the basis of the intriguing structures of these 6 complexes, particularly the conformational mobility of the polycyclic ring, which in the case of complex **1** can be planar, there could be several possible modes of DNA binding: (1) covalent

binding by the loss of Cl, (2) partial intercalation with DNA due to the flatness of polycyclic ring,¹² and (3) groove binding by the polycyclic arene ring which is rich in electrons and flexible in structure.^{13,14}

Aqua Ruthenium Arene Complexes

Since the ethylenediamine complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ show the promising anticancer activities¹⁵ and their aqua species are potential reactive intermediates inside cells,¹⁶ it is of interest to study the chemistry of $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{OH}_2)]^{2+}$ complexes. The coordinated chloride ligands can be effectively removed from the chloro Ru arene complexes **7**, **1** and **4** by addition of a soluble Ag⁺ salt, eg. AgNO₃, AgPF₆, accompanied by precipitation of AgCl (Scheme 3.2). The

Scheme 3.2 Preparation of aqua Ru(arene) complexes as PF₆⁻ salts.

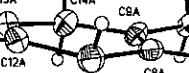


resultant aqueous solutions were found to be acidic with pH values of 3-6. For preparation of crystalline aqua Ru arene complexes **8-10**, AgPF₆ was used instead of AgNO₃, to avoid the possibility of the coordination of the nitrate ligand to Ru, as reported in preparation of crystalline structures of α -[Ru(azpy)₂(NO₃)₂]¹⁷ and *cis*-[Pt(NH₃)₂(NO₃)₂]¹⁸.

Crystallographic data for aqua ruthenium arene complexes **8**, **9** and **10** are listed in Table 3.1, and selected bond lengths and angles in Table 3.2. Comparisons of their conformations with those of their parent chloro-complexes **7**, **1** and **4**, respectively, are listed in Table 3.3. The structures of cations **8**, **9** and **10a** are shown in Figure 3.3, and cation **10b** shown in Figure 3.4.

For crystal **8**, the ratio of Ru to PF₆⁻ in the unit cell is 8 : 12, thus its formulation is determined as [(η^6 -Bip)Ru(en)(OH₂)][(η^6 -Bip)Ru(en)(OH)][PF₆]₃ (or [(η^6 -Bip)Ru(en)(OH₂)_{0.5}(OH)_{0.5}][PF₆]_{1.5}) This suggests that the O atom attached to Ru can be assigned to a 1:1 mixture of OH₂ and OH, probably due to the partial deprotonation of the coordinated OH₂ molecule. Similar situation was found in a single crystal of complex **9**, where the O atom attached to Ru again can be assigned to a 1:1 mixture of OH₂ and OH (data not shown). Another single crystal from the same batch of **9** was selected for data collection and it was found that its formulation is [(η^6 -THA)Ru(en)(OH₂)] [PF₆]₂•H₂O. There is no deprotonation of the coordinated OH₂ molecule for this crystal, but this OH₂ molecule was disordered over two positions O1 and O1' with occupancy factors of 0.7 and 0.3 respectively. For complex **10**, the crystallographic data showed that its formulation is [(η^6 -DHA)Ru(en)(OH₂)] [PF₆]₂•2(H₂O) and without deprotonation of the coordinated OH₂ molecule.

(A) cation 8



(B) cation 9

(C) cation 10a

Figure 3.3 X-ray structures and atom numbering schemes for (A) $\{[(\eta^6\text{-Bip})\text{Ru}(\text{en})(\text{OH}_2)_{0.5}(\text{OH})_{0.5}]^{1.5+}$ in complex **8**, (B) $[(\eta^6\text{-THA})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ in complex **9**, and (C) $[(\eta^6\text{-DHA})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ (**10a**) in complex **10**, at 30% probability thermal ellipsoids. Cation **10b** is shown in Figure 3.4.

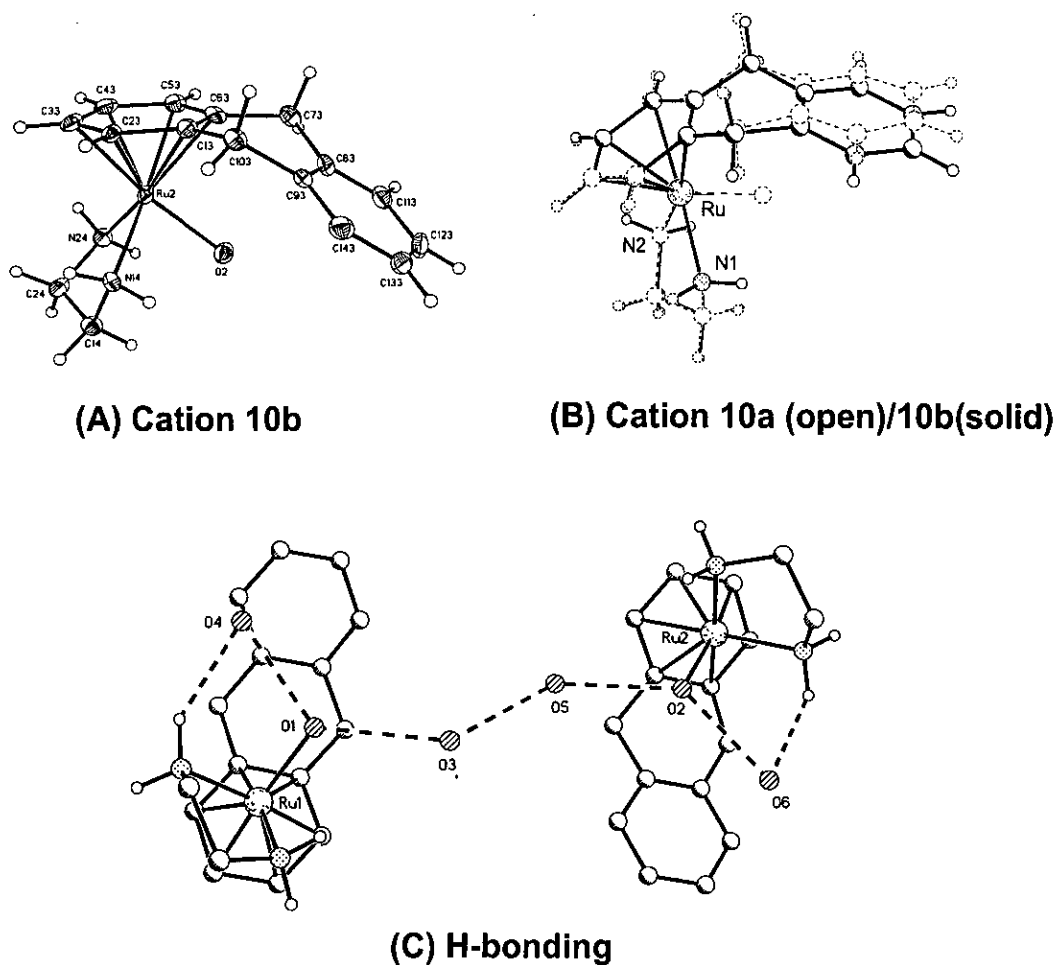
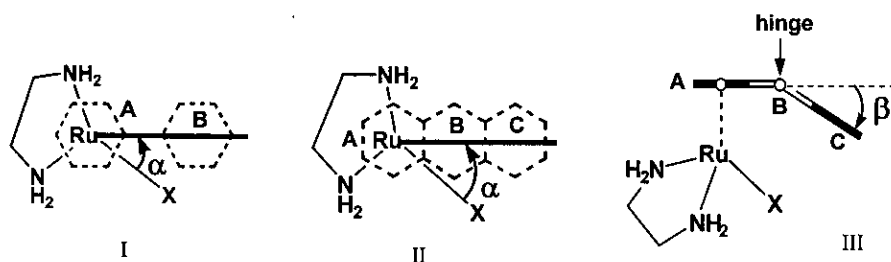


Figure 3.4 (A) X-ray structure and atom numbering scheme for $[(\eta^6\text{-DHA})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ (cation **10b**) in complex **10**. (B) Comparison of the conformations of cation **10a** (open) and **10b** (solid). Alignments are based on a superposition of the labelled atoms (Ru, N1 and N2 of en). (C) Intermolecular hydrogen bonding involving en NH and O(w) in the crystal of complex **10**.

Table 3.3 Comparison of the Conformations of Aqua Ru Arene Complexes **8-10** (Figures 3.3) and Their Parent Chloro-Complexes^a **7**, **1** and **4**. X = H₂O or Cl.**(A) Biphenyl Complex 4 (H₂O) and 1 (Cl).**

	8 (H ₂ O)	7 (Cl) ^a
Bip orientation, α (I), [$\Delta\alpha$] ^c (deg)	23.3(3) [−3.5]	26.8(2)
Bip propeller twist, χ , ^b [$\Delta\chi$] ^c (deg)	45.3(3) [+19.1]	26.2(4)
Ru-coordinated ring (centroid), Å	1.663 (3)	1.663 (3)

(B) THA Complexes 9 (H₂O) and 1 (Cl) and DHA Complexes 10 (H₂O) and 4 (Cl).

	9 (H ₂ O)	1 (Cl) ^a	10 (H ₂ O)	4 (Cl) ^a
THA/DHA orientation, α (II), [$\Delta\alpha$] ^c (deg)	63.7(2) [+18.58]	45.12(6)	32.5(3) ^f [−31.59] 35.5(3) ^g [−28.6]	64.09(8)
Hinge bending angle, β (III) ^d , [$\Delta\chi$] ^c (deg)	−1.8(3) [+5.73]	−7.53(12)	+ 31.8(4) ^f [−8.85] + 38.4 (4) ^g [−2.25]	+ 40.65(16)
Ru-coordinated ring (centroid), Å	1.664 (2)	1.676(3)	1.671(3) ^d 1.678(3) ^e	1.667(3)

^a See Chapter 5. ^b χ : twist of pendent phenyl ring (B) with respect to coordinated phenyl (A) of Bip. ^c The change of torsion angles for **8** compared to **7**. ^d The hinge is defined by C7A – C10A, see Figure 1. A + ve sign for β indicates movement towards Ru-X. ^e The change of torsion angles for **5** and **6** compared to **2** and **3**, respectively. ^f cation **10a**. ^g cation **10b**.

For the Bip complex **8**, the propeller twist of the pendant phenyl with respect to the coordinated phenyl of the Bip ligand is 45.3° , which is 19.1° larger than that for the chloro complex **7** (Table 3.3). The long axis of Bip is rotated away from the Ru-O vector by 23.3° , which is 3.5° smaller than that for complex **7**. The distance from Ru to the centroid of coordinated phenyl (A) is $1.66 \text{ \AA}$, similar to that in complex **7**.

For the THA complex **9**, the tricyclic ring system (THA) is nearly flat. The outer ring (C) is slightly bent up away from the O atom (hinge bending) by ca. 1.8° , similar to that (7.5°) in complex **7**. The long axis through the THA ligand is rotated away from the Ru-O vector by 66.62° , which is 21.5° larger than that in complex **7**. The distance from Ru atom to the centroid of coordinated ring (A) is $1.66 \text{ \AA}$, similar to that in complex **7**.

For the DHA complex **10**, there are two kinds of independent cations (two **10a** cations and two **10b** cations, Figures 3.3 and 3.4) in the asymmetric unit. The structures of these two cations **10a** and **10b** differ significantly only in the orientation and hinge bending of the DHA ring (Figure 3.4). The outer ring (C) is bent down towards the O atom by 31.8° in cation **10a**, and by 38.4° in cation **10b**, comparable to that (40.65°) in complex **4**. The long axis through the DHA ligand is rotated away from the Ru-O vector by 32.5° in cation **10a** and by 35.5° in cation **10b**, which are ca. 30° smaller than that in complex **4**. The distance from the Ru to the centroid of coordinated ring (A) is $1.67 \text{ \AA}$ in both **10a** and **10b**, similar to that in complex **4**. There are interesting chains of intermolecular hydrogen-bonds: $\text{O4}\cdots\text{O1}\cdots\text{O3}\cdots\text{O5}\cdots\text{O2}\cdots\text{O6}$ and $\text{en NH}\cdots\text{O4/O6}$, involving both cations **10a** and **10b** in the crystal packing (with the distances of $\text{O4}\cdots\text{O1}$ $2.650 \text{ \AA}$, $\text{O1}\cdots\text{O3}$ $2.805 \text{ \AA}$, $\text{O3}\cdots\text{O5}$ $2.952 \text{ \AA}$, $\text{O5}\cdots\text{O2}$ $2.726 \text{ \AA}$, $\text{O2}\cdots\text{O6}$ $2.702 \text{ \AA}$, $\text{O4}\cdots\text{N12}$ $3.272 \text{ \AA}$ and $\text{O6}\cdots\text{N14}$ $3.096 \text{ \AA}$, Figure 3.4).

The X-ray structures of **8-10** revealed that an Ru-O bond length for coordinated water of 2.1-2.2 Å, within the range reported for other aqua Ru arene compounds.¹⁹ The coordinated OH₂ molecule showed the acidity through protonation/deprotonation as found in the solid state of complexes **8** and **9**. In each case, the coordinated OH₂ molecule is deprotonated in about 50% of the unit cell to provide charge neutrality. There is an interesting arene conformation for each aqua compound as shown in Figure 3.3 and Table 3.3. Noticeably, the propeller twist of the biphenyl in **8** is 19.1° larger than that in chloro complex **7**. For aqua THA complex **9**, the small hinge bending of 1.8° away from the O atom shows that tricyclic ring system is still rather flat as in chloro THA complex **1**. For the aqua DHA complex **10**, the outer ring (C) is significantly bent down toward the O atom by 31.8° (38.4°), comparable with the hinge bending of 40.65° in the chloro-complex **4**. Such large hinge bending toward the O or Cl atom in the DHA complexes **10** and **4** may impose a steric constraint to hydrolysis of Ru-Cl and may also influence the rate of G N7 coordination in reactions with DNA.

3.4 Conclusion

A series of novel chloro (η^6 -C₁₄H₁₄)Ru(II) complexes (**1**, **2** and **3**) and (η^6 -C₁₂H₁₂)Ru(II) complexes (**4**, **5** and **6**) was synthesised with the aim of investigating their anticancer activity. The preliminary cytotoxicity data of these six complexes against A2780 human ovarian cancer cells show that, the two ethylenediamine complexes, [(η^6 -THA)Ru(en)Cl][PF₆] (**1**) and [(η^6 -DHA)Ru(en)Cl][PF₆] (**4**), obtained the increased cytotoxicity with IC₅₀ values 0.5 µM for **1** and 2 µM for **4**, compared with 6 µM for [(η^6 -Bip)Ru(en)Cl][PF₆] (**7**) (see Appendix Table). The dimethyl sulfoxide and acetonitrile complexes, **2**, **3**, **5** and **6**, however, recorded IC₅₀ values greater than 100 µM and were defined essentially as inactive.²⁰ Noticeably, these four compounds alone contain more reactive monodentate ligands, dimethyl

sulfoxide and acetonitrile, in comparison to the more stable chelating en ligand in **1** and **4**. Those preliminary data suggest that stable bidentate ligands, a more hydrophobic arene ring and a single leaving group (Cl) are associated with the increased cytotoxicity.¹⁵

The aqua complexes $[(\eta^6\text{-Bip})\text{Ru}(\text{en})(\text{OH}_2)]^{2+}$ (**8**) $[(\eta^6\text{-THA})\text{Ru}(\text{en})(\text{OH}_2)]^{2+}$ (**9**) and $[(\eta^6\text{-DHA})\text{Ru}(\text{en})(\text{OH}_2)]^{2+}$ (**10**), potentially reactive intermediates inside cells, were also prepared from the chloro complexes **7**, **1** and **4**, respectively, by removal of the Cl ligand with Ag^+ . Their molecular structures were determined by X-ray crystallography. These showed interesting features, particularly in the conformation of the polycyclic rings, as compared with their parent chloro complexes. This maybe relevant to the biological activities for these novel chloro and aqua Ru(arene) complexes and will be discussed further in Chapters 5 and 6.

3.5 References

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Chapter 4

Synthesis and Characterisation of Dinuclear Bis Ru(arene) Complexes

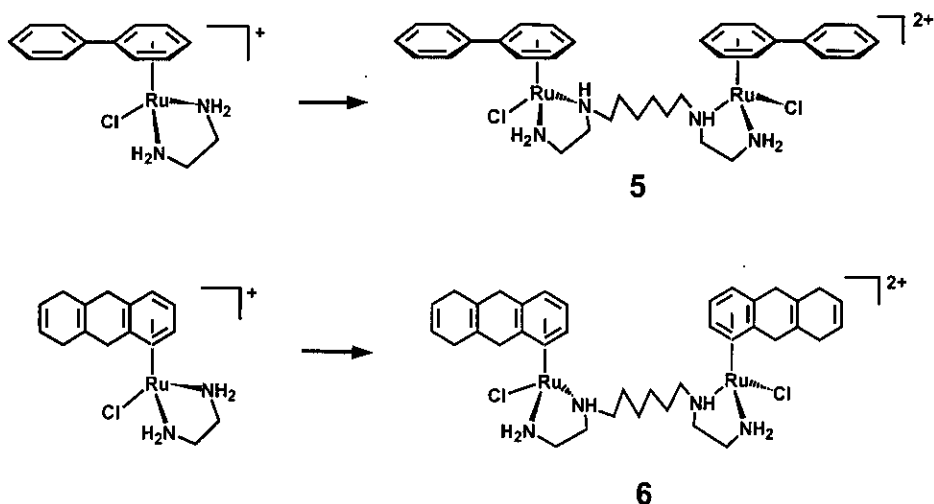
4.1 Introduction

Organometallic ruthenium(II) complexes of the type $[(\eta^6\text{-arene})\text{Ru(II)(en)Cl}][\text{PF}_6]$ (en = ethylenediamine) have high and reproducible *in vitro* or *in vivo* anticancer activity. The activity increases with increase in size of the $\eta^6\text{-arene}$. For example, complexes which contain $\eta^6\text{-tetra-}$ or $\text{di-hydroanthracene}$ rings (THA, DHA) or biphenyl (Bip) have much higher activities than compounds containing a single ring such as $\eta^6\text{-benzene}$ (Ben) or $\eta^6\text{-cymene}$ (Cym).^{1,2} A potential target for these organometallic Ru(II) complexes is DNA and the preferred binding site appears to be guanine.² The structures of guanine adducts of THA, DHA and Bip complexes show stereospecific hydrogen-binding of en NH with G O6, and strong arene-nucleobase stacking, both in the solid state by X-ray crystallography, and in solution by NMR methods (Chapter 5). These studies have revealed several DNA recognition features and suggested a potential DNA-binding mode, involving simultaneous intercalation and covalent coordination with DNA for organometallic Ru(II) complexes. The diamine NH group and hydrophobic arene group as well as the Cl leaving group may therefore all play important roles in anticancer activity. These studies have stimulated the design of multi-functional arene-Ru(II) antitumour agents. One approach involves the formation of dinuclear bis Ru(arene) agents by linking two Ru(arene) units with a tetraamine ligand, where each Ru centre would behave similarly to the active mononuclear species. This type of dimeric species would be a potential bifunctional agent and facilitate the formation of DNA interstrand crosslinks or DNA-protein crosslinks leading to a distinct and unique profile of antitumour activity.

There are precedents which have exploited a similar concept. The platinum dimers and trimers developed by Farrell, et al,³ one of which, the trinuclear compound BBR3464,^{4,5,6} is currently in phase-II clinical trial, represent a very stimulating precedent in this sense. Dimers such as

$[(\text{PtCl}(\text{NH}_3)_2)_2(\text{NH}_2(\text{CH}_2)_4\text{NH}_2)]^{2+}$,⁷ derived from *cis*- $[\text{PtCl}_2(\text{NH}_3)_2]$ upon replacement of one ammonia with a bridging aliphatic diamine, maintain structure motifs and coordination environments very similar to those of the monomeric parent compound (cisplatin). They are as active as cisplatin against conventional tumour lines, but are remarkably active also against tumour lines resistant to cisplatin, apparently because the co-ordination-induced modifications of DNA are different from those induced by the mononuclear complex.^{8,9} Currently, due to the success of NAMI-I in clinical trials against solid tumour metastasis, the antineoplastic activity of the dimers $\text{Na}_2[\{trans\text{-RuCl}_4(\text{Me}_2\text{SO}-S)_2(\mu\text{-L})\}]$ and $[\{mer,cis\text{-RuCl}_3(\text{Me}_2\text{SO}-S)(\text{Me}_2\text{SO}-O)\}_2(\mu\text{-L})]$ where L is pyrazine, pyrimidine, 4,4'-bipyridine, 1,2-bis(4-pyridyl)-ethane or 1,3-bis(4-pyridyl)-propane, based on NAMI-A, are being investigated.^{10,11}

Chart 4.1 The structures of dinuclear bis Ru(arene) complexes compared with their mononuclear Ru(arene) analogues.



In this chapter, the design and synthesis of the bifunctional ligand of N, N'-bis-(2'-aminoethyl)-1,6-diaminohexane (**4**, $\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_6\text{NH}(\text{CH}_2)_2\text{NH}_2$, Scheme 4.1), and its reactions with the dimers $[(\eta^6\text{-biphenyl})\text{RuCl}_2]_2$ and $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$, respectively, to form two dinuclear complexes $[(\eta^6\text{-$

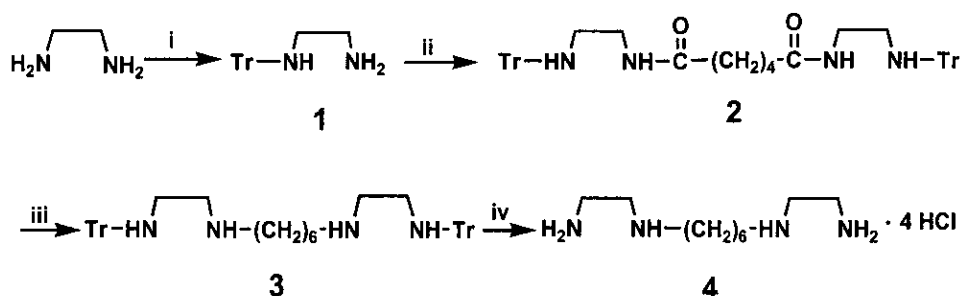
biphenyl)RuCl)₂(H₂N(CH₂)₂NH(CH₂)₆NH(CH₂)₂NH₂-*N,N',N'',N'''*)][PF₆]₂ (**5**), and [(η⁶-C₁₄H₁₄)RuCl)₂(H₂N(CH₂)₂NH(CH₂)₆NH(CH₂)₂NH₂-*N,N',N'',N'''*)][PF₆]₂ (**6**) are described. Their structures have been studied by 2D [¹H, ¹H] TOCSY, COSY, NOESY, and [¹H, ¹⁵N] HSQC NMR methods. In addition, the mononuclear complex [(η⁶-biphenyl)Ru(Et-en)Cl][PF₆] (**7**, where Et-en = N-ethylethylenediamine, H₂N(CH₂)₂NH(CH₂CH₃)) was also synthesized for comparison, due to the similarity of its structure to each unit of the dinuclear complex **5**. This mono Ru compound was characterised by X-ray crystallography and 2D [¹H, ¹H] COSY and NOESY and [¹H, ¹⁵N] HSQC NMR methods. It exhibits interesting diastereomerism as compared with complex **5**.

4.2 Experimental Section

4.2.1 Materials and Methods

The starting materials [(η⁶-biphenyl)RuCl₂]₂ and [(η⁶-C₁₄H₁₄)RuCl₂]₂ were prepared as previously described (Ref 2 and Chapter 5). Ethylenediamine, triethylamine and N-ethylethylenediamine were freshly distilled over Na. Tetrahydrofuran (THF) was dried by distillation from Na-benzophenone. triphenylmethyl chloride (99%) and adipoyl chloride (98%, ClCO(CH₂)₄COCl) was purchased from the Arcos Chemical Co. All other chemicals were AR grade and were used as received.

Scheme 4.1 i) TrCl, CH₂Cl₂, r.m., 12 h; ii) Adipoyl chloride, triethylamine, CHCl₃, reflux, 2 h; iii) LiAlH₄-THF, reflux, 25 h; iv) 6.0 M HCl, reflux, 3 h.



4.2.2 Preparation of N,N'-bis-(2'-aminoethyl)-1,6-diaminohexane

(a) N-tritylethylenediamine (1)

A solution of trityl chloride (5.57 g, 20 mmol) in dichloromethane (25 mL) was slowly added to a solution of ethylenediamine (8 mL, 120 mmol) in dichloromethane (75 mL) with stirring at room temperature. The addition was accomplished within 1 h and the reaction stirred overnight. The white salt was filtered off and the filtrate washed with water and dried over anhydrous sodium sulphate. Dichloromethane was removed by rotary evaporation and the residue dissolved in methanol (30 mL). A white precipitate begun to form after shaking for a while and the mixture kept in the fridge for 5 h and then filtered off. The methanol filtrate was reduced in volume to 10 mL and kept in the fridge overnight. A white solid precipitated. This was collected as the desired product, and washed with diethyl ether and dried in *vacuo*.

Yield: 4.5 g, 14.88 mmol, 74.4%, mp 96 °C. ^1H NMR (CDCl_3) δ : 7.10-7.49 (m, 15H, aromatic H), 2.80 (t, 2H, CH_2), 2.20 (t, CH_2).

(b) N,N'-bis(2'-tritylaminoethyl)-1,6-diamidohexane (2)

N-Tritylethylenediamine (1.5 g, 4.96 mmol) and triethylamine (1.0 g, 7.29 mmol) were dissolved in chloroform (35 mL) and cooled in an ice bath. To this solution was added adipoyl chloride (0.36 mL, 2.48 mmol) in chloroform (10 mL) slowly with stirring. After addition, the mixture was refluxed for 2 h and cooled to room temperature. This was filtered to give a clear chloroform filtrate (see below). The filtered precipitate was dissolved in dichloromethane (100 mL). This was washed with water and then saturated NaCl solution and dried over anhydrous sodium sulphate. Removal of the solvent by rotary evaporation gave a white product. The chloroform filtrate was also washed with water and saturated NaCl solution, and

dried over anhydrous sodium sulphate. After removal of chloroform, a further crop of product formed.

Yield: 1.40 g, 1.91 mmol, 77% mp 200-202 °C

Anal. Calcd for $C_{48}H_{50}O_2N_4 \cdot H_2O$ (732.96) : C, 78.66; H, 7.15; N, 7.64. Found: C, 78.81; H, 6.73; N, 7.55. ^1H NMR ($CDCl_3$) δ : 7.15-7.50 (m, 15H, aromatic H), 3.23 (q, 4H, 2CH₂), 2.22 (m, 8H, 4CH₂), 1.63 (m, 4H, 2CH₂).

(c) N,N'-bis(2'-tritylaminoethyl)-1,6-diaminohexane (3)

Into a solution of N,N'-bis(2'-tritylaminoethyl)-1,6-diamidohexane (1.3g, 1.82 mmol) in dry THF was added a suspension of $LiAlH_4$ (0.69 g, 18.18 mmol) in dry THF (20 mL) under argon with vigorous stirring. After the addition, the reaction was maintained under gentle reflux with stirring for 25 h. This was cooled to 4°C. The reaction product and excess of hydride were decomposed by the dropwise addition of H_2O (0.69 mL), followed by 15%(w/v) NaOH solution (0.69 mL) and H_2O (2.07 mL) in succession. After vigorous stirring for 30 min, the mixture was filtered by suction and the resulting cake was washed thoroughly with dichloromethane. The combined filtrate was concentrated to dryness on a rotary evaporator and the resulting light-yellow residue dissolved in dichloromethane (50 mL). This was washed with water and then saturated NaCl solution, and dried over anhydrous sodium sulphate. Removal of dichloromethane on a rotary evaporator afforded a colorless, waxy solid.

Yield: 1.20 g, 1.75 mmol, 96%. ^1H NMR ($CDCl_3$) δ : 7.10-7.49 (m, 15H, aromatic H), 2.71 (t, 4H, 2CH₂), 2.50 (t, 4H, 2CH₂), 2.28 (t, 4H, 2CH₂), 1.49 (m, 4H, 2CH₂), 1.32 (m, 4H, 2CH₂).

(d) N,N'-bis(2-aminoethyl)-1,6-diaminohexane tetrahydrochloride (4)

A mixture of N,N'-bis(2'-tritylaminoethyl)-1,6-diaminohexane (1.0 g, 1.45 mmol) and 6 M HCl (30 mL) was refluxed for 3 h. Then the mixture was filtered and the filtrate was concentrated to about 3 mL in *vacuo*. Addition of methanol into the concentrated solution afforded a white salt.

Yield: 0.46 g, 1.32 mmol, 92%. m.p. 244°C (decomp.). Anal. Calcd for C₁₀H₂₆N₄•4HCl (348.09): C, 34.48; H, 8.68; N, 16.09. Found: C, 34.26; H, 8.77; N, 16.24.

¹H NMR (D₂O) δ: 3.35 (m, 8H, 4CH₂), 3.10 (t, 4H, 2CH₂), 1.69 (m, 4H, 2CH₂), 1.40 (m, 4H, 2CH₂). ¹³C NMR (D₂O) δ: 25.9, 35.0, 44.5, 48.2.

4.2.3 Preparation of Dinuclear Bis Ru(arene) Complexes

[((η⁶-biphenyl)RuCl₂)₂(H₂N(CH₂)₂NH(CH₂)₆NH(CH₂)₂NH₂-N,N',N'',N''')][PF₆]₂ (5)

[(η⁶-biphenyl)RuCl₂]₂ (0.106 g, 0.162 mmol) in 10 mL water was refluxed for 2.5 h and then cooled to 50°C. Into this suspension was added a methanol solution of N,N'-bis(2-aminoethyl)-1,6-diaminehexane (0.162 mmol) which was obtained by the treatment of N,N'-bis(2'-aminoethyl)-1,6-diaminohexane tetrahydrochloride (4, 56.39 g, 0.162 mmol) with 1.294 ml 0.501 M KOH-MeOH. The mixture was heated to reflux for 1.5 h. This was filtered while hot. NH₄PF₆ (0.175 g, 1.07 mmol) was added into the bright yellow filtrate to yield a bright yellow precipitate, which was recrystallized from methanol/ether.

Yield: 0.10 g, 0.093 mmol, 57.5%

Anal. Calcd for C₃₄H₄₆Cl₂F₁₂N₄P₂Ru₂ (1073.74): C, 38.03; H, 4.32; N, 5.22. Found: C, 37.86; H, 4.25; N, 5.20. ¹H NMR (DMSO-d₆): δ 7.80 (m, 4H), 7.50 (m, 6H), 6.95, 6.7 (broad, NH), 6.31, 6.25, 6.20 (m, 4H), 6.00, 5.93, 5.87, 5.78 (m, 6H), 4.60, 6.65, 3.80 (broad, NH), 3.3-1.0 (broad, CH₂) MS (FAB) (M-H)⁺ : m/z 783.11630 (Calc. m/z 783.11988, Dev.4.50 ppm)



$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ (0.15 g, 0.213 mmol) in 10 mL methanol was stirred under argon at 60°C. Into this suspension was added a methanol solution of N,N'-bis(2-aminoethyl)-1,6-diaminohexane (0.213 mmol), which was obtained by treatment of N,N'-bis(2-aminoethyl)-1,6-diaminohexane tetrahydrochloride (**4**, 73.97 g, 0.213 mmol) with 1.697 mL 0.501 M KOH-MeOH solution. The mixture was adjusted to pH 6.5 and stirred at 60°C for a further 1.5 h. This was filtered while hot and concentrated to 6 mL. Addition of NH_4PF_6 (0.25 g, 1.53 mmol) into the concentrated solution afforded a yellow precipitate. This was recrystallized from methanol/ether.

Yield: 0.09 g, 0.0796 mmol, 37.4%. Anal. Calcd for $\text{C}_{38}\text{H}_{54}\text{Cl}_2\text{F}_{12}\text{N}_4\text{P}_2\text{Ru}_2$ (1129.85): C, 40.39; H, 4.81; N, 4.95. Found: C, 40.30; H, 4.49; N, 4.21. ^1H NMR (DMSO-d_6): δ 6.82, 6.35 (broad, NH), 5.72 (s, 4H), 5.6, 5.45 (m, 8H), 4.52, 3.80, 3.72 (broad, NH), 3.15 (m, 8H), 2.65 (m, 8H), 2.87, 2.70 (broad, CH_2), 2.5-1.2 (broad, CH_2). MS (FAB) (M-H^+): m/z 839.18129 (Calc. m/z 839.18248, Dev. -1.42 ppm)

4.2.4 Preparation of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}][\text{PF}_6]$ (**7**)

$[(\eta^6\text{-biphenyl})\text{RuCl}_2]_2$ (0.10 g, 0.158 mmol) was refluxed in water (10 mL) for 3 h and then cooled down to 80°C. To this suspension was added N-ethylethylenediamine (37 mg, 0.42 mmol). The brown suspension immediately became dark green. This was then slowly heated to reflux again for a further 1.5 h and filtered while hot. NH_4PF_6 (0.2 g, 1.23 mmol) was added to the yellowish filtrate and the flask briefly shaken. A yellow precipitate began to form almost immediately. After standing at 4°C overnight, the precipitate was collected, washed with a little methanol followed by diethyl ether and dried in *vacuo*. This was recrystallised from methanol/ether.

Yield: 0.08 g, 0.153 mmol, 48.3%. Anal. Calcd for $C_{16}H_{22}ClF_6N_2PRu$ (523.85): C, 36.68; H, 4.23; N, 5.35. Found: C, 36.00; H, 4.37; N, 5.24.

4.2.5 X-ray Crystallography

Diffraction data were collected for isomers **7A** and **7B** with Mo K_α radiation, $\lambda = 0.71073$ Å. For isomer **7A**, a Stoë Stadi-4 serial diffractometer was used at 220 K, and absorption corrections were made using ψ -scans. For isomer **7B**, a Bruker Smart Apex ccd diffractometer was used at 150 K, and absorption correction made using the program SADABS.¹² Both structures were solved by combined Patterson and direct methods (DIRDIF¹³). All hydrogen atoms were placed in calculated positions and allowed to ride on the nitrogen or carbon atoms to which they are bonded. All non-hydrogen atoms were refined with anisotropic displacement parameters. In both isomers, the refined displacement parameters suggest a slight disordering of the PF_6^- ions.

4.2.6 NMR Spectroscopy.

^1H NMR spectra were acquired on a Bruker DMX 500 ($^1H = 500$ MHz) NMR spectrometer using TBI [^1H , ^{13}C , X] or triple resonance [^1H , ^{13}C , ^{15}N] probeheads, respectively, and equipped with z-field gradients.

1D ^1H NMR spectra were typically acquired with 32 transients into 32 K data points over a spectral width of 6.0 kHz using a double pulsed-field gradient spin-echo pulse sequence. Typically, 2D [^1H , ^1H] NOESY NMR spectra were acquired with 8 transients for each of 512 t_1 increments (TPPI). The mixing time was 1 s, and the relaxation delay was 2 s. 2D [^1H , ^1H] COSY and DQFCOSY spectra were acquired with 32 transients for each of 512 t_1 increments (TPPI). 2D [^1H , ^1H] TOCSY spectra were acquired with 16 transients into 4096 complex data points

(acquisition time 0.51 s) for each t_1 increments. A spin-lock time of 60 ms was employed.

2D [^1H , ^{15}N] HSQC NMR spectra (optimised for $^1J_{\text{NH}} = 73$ Hz) were acquired using the sequence of Stonehouse *et. al.*¹⁴ A GARP decoupling sequence was used for ^{15}N decoupling during acquisition. 2D [^1H , ^{15}N] HSQC NMR data were acquired with 140 transients into 2048 data points over a ^1H (F2) spectral width of 5 kHz (10 ppm centred at 5 ppm) for each of 512 t_1 increments (TPPI), and over a ^{15}N (F1) spectral width of 3.04 kHz (60 ppm, centre at -10 ppm) using a relaxation delay of 2 s between transients. The NH proton resonances from sample 5 (90 mM) and 7 (40 mM) in DMSO- d_6 solutions were observable within a few hours with ^{15}N at *natural abundance*. Typically, 2D [^1H , ^{15}N] NMR HSQC spectra were acquired over a 15 h period.

All data processing was carried out using XWIN-NMR version 2.0 (Bruker U.K. Ltd.). ^1H NMR chemical shifts were internally referenced to $(\text{CD}_2\text{H})_2\text{SO}$ (2.5 ppm) in DMSO- d_6 solution. ^{15}N resonances were referenced to 1 M $^{15}\text{NH}_4\text{Cl}$ in 1.5 M HCl (external) at 0 ppm.

4.3 Results and Discussion

4.3.1 [$(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}$][PF₆] (7)

The reaction of [$(\eta^6\text{-biphenyl})\text{RuCl}_2$]₂ with N-ethylethylenediamine yields complex 7 as a mixture of diastereomers which exhibit chirality both at the metal centre and at the substituted N atom of the N-ethylethylenediamine ligand (Figure 4.1).

X-ray Structure of Complex 7

The X-ray structures of diastereomers 7A and 7B for complex 7 are shown in Figure 4.1. These two diastereomers crystallised in the same batch but in two

Table 4.1 Crystallographic data for the two diastereomers **7A** and **7B** of complex $[(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}][\text{PF}_6]$.

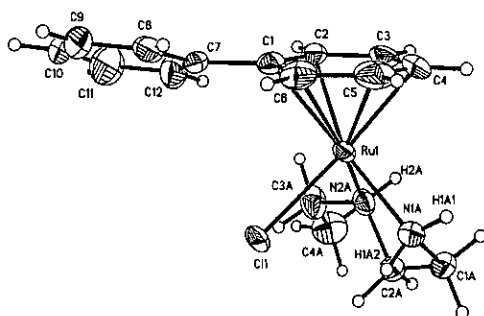
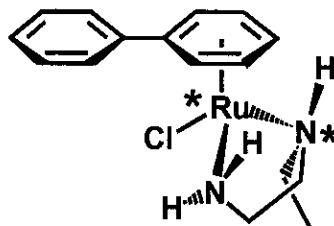
	7A	7B
Formula	$\text{C}_{16}\text{H}_{22}\text{ClF}_6\text{N}_2\text{PRu}$	$\text{C}_{16}\text{H}_{22}\text{ClF}_6\text{N}_2\text{PRu}$
Molar mass	523.85	523.85
Crystal system	Monoclinic	Monoclinic
Space group	$\text{P2}_1/\text{n}$	$\text{P2}_1/\text{n}$
Colour	Orange lump	Yellow flake
Crystal size / mm	0.31 x 0.31 x 0.20	0.18 x 0.05 x 0.04
$a / \text{\AA}$	10.701(3)	14.076(2)
$b / \text{\AA}$	12.284(4)	8.2819(13)
$c / \text{\AA}$	15.237(5)	16.115(3)
α / deg	90	90
β / deg	93.82(3)	90.554(3)
γ / deg	90	90
T / K	220(2)	150(2)
Z	4	4
$R [F > 4\sigma(F)]^a$	0.0682	0.0733
R_w^b	0.1960	0.1487
GOF ^c	1.022	1.383
$\Delta\rho$ max and min, / $\text{e}\text{\AA}^{-3}$	+1.988 , -1.329	+1.182, -1.135

^a $R = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^b $R_w = [\sum w(F_o^2 - F_c^2)^2 / \sum wF_o^2]^{1/2}$. ^c $\text{GOF} = [\sum w(F_o^2 - F_c^2)^2 / (n-p)]^{1/2}$, where n = number of reflections and p = number of parameters.

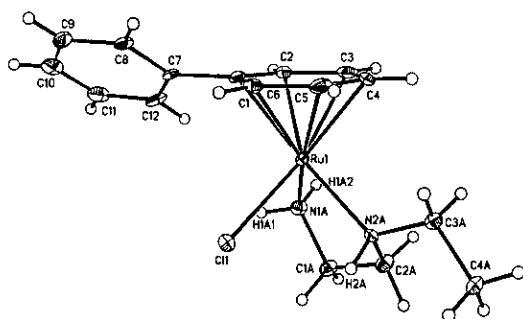
Table 4.2 Selected Bond Lengths (Å) and Angles (deg) for the two diastereomers **7A** and **7B** of complex $[(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}][\text{PF}_6]$.

atoms	7A	7B
Ru1-N1A	2.112(6)	2.109(7)
Ru1-N2A	2.142(6)	2.148(6)
Ru1-Cl1	2.392(2)	2.408(2)
Ru1-C1	2.220(7)	2.223(7)
Ru1-C2	2.193(8)	2.175(8)
Ru1-C3	2.147(7)	2.186(8)
Ru1-C4	2.159(8)	2.192(8)
Ru1-C5	2.178(9)	2.181(9)
Ru1-C6	2.181(7)	2.175(8)
Ru-(centroid) coord-ring	1.667	1.669(4)
N1A-Ru1-N2A	79.4 (3)	79.8(3)
N1A-Ru1-Cl1	83.29 (19)	83.0(2)
N2A-Ru1-Cl1	87.22 (18)	83.26(18)
Bip propeller twist	19.5 (5)	35.6(4)
Rotation of bip from Ru1-Cl1	29.44	46.1(3)

(A)

**7A** ($R_{Ru}R_N$)

(B)



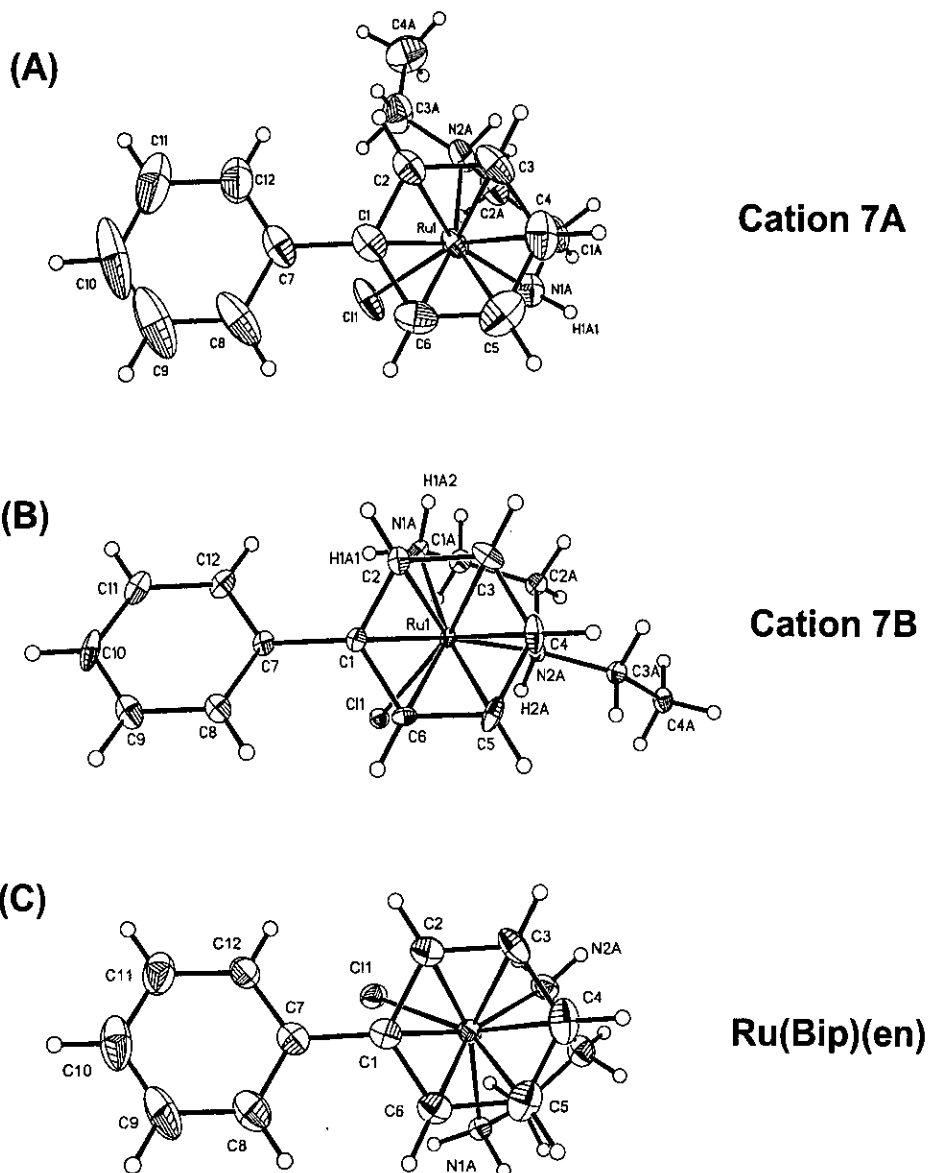


Figure 4.2 Projections of the diastereomers **7A** and **7B** of $[(\eta^6\text{-Bip})\text{Ru}(\text{Et-en})\text{Cl}]^+$ along the Ru-centroid axis of the arene group, showing the relative dispositions of the biphenyl rings and Cl1, N1A and N2A bearing the ethyl substituent, which are compared with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (ref 2).

independent single crystals. These two single crystals showed different appearances: orange lump for **7A** and yellow flake for **7B**, and thus both were selected for X-ray data collection. The absolute configurations of **7A** and **7B** can be assigned¹⁵ as $R_{Ru}R_N$ and $S_{Ru}R_N$. Their corresponding enantiomers **7A'** ($S_{Ru}S_N$) and **7B'** ($R_{Ru}S_N$) were also present in their respective unit cells (not shown). Crystallographic data for both isomers **7A** and **7B** are listed in Table 4.1, and selected bond lengths and angles in Table 4.2. In **7A**, the pendant biphenyl ring is twisted out of the plane of the coordinated ring by about 19.5° , and in **7B**, by 35.6° (Table 4.2), compared with 26.8° in $[(\eta^6\text{-biphenyl})Ru(en)Cl]^+$ (Chapter 5). The distance from Ru atom to the centroid of the coordinated phenyl is 1.667 Å for **7A**, very similar to that for **7B** (1.669 Å). The Ru-Cl distance for **7A** is 2.392 Å, a little shorter than that for **7B** (2.408 Å), and the distances of Ru1-N1A (2.112 Å) and Ru1-N2A (2.142 Å) are close to those of **7B** (2.109, 2.148 Å).

The pyramidal conformation of Ru1-Cl1, -N1A and -N2A for both isomers is an interesting feature (Figure 4.2, Table 4.2). The angles of N1A-Ru1-Cl1 (83.29°) and N2A-Ru1-N1A (79.4°) angles in **7A** are comparable with values of 83.0° and 79.8° respectively, found in **7B**, while the angle of N2A-Ru1-Cl1 (87.22°) in **7A** is larger than that of **7B** (83.0°). Noticeably, the long axis of the biphenyl ligand is rotated away from the Ru-Cl1 vector by 29.4° for **7A** while 46.1° for **7B**, compared with 26.2° for the structure of $[(\eta^6\text{-biphenyl})Ru(en)Cl]^+$ (Chapter 5). This suggests that the three legs of Ru1-Cl1, Ru1-N1A and Ru1-N2A are much more staggered with the Ru-C bonds for **7A**, similar to the case for $[(\eta^6\text{-biphenyl})Ru(en)Cl]^+$, but are much more eclipsed for **7B** (Figure 4.2). In addition, the ethyl substituent on the N2A atom (chiral) points down towards the Cl atom for **7A**, while it is tilted up away from the Cl atom for **7B**. As a result, the NH proton on N2A atom points up toward the coordinated phenyl ring in **7A** while it points down away from the

coordinated phenyl ring in **7B**. This represents the most distinguishing feature for these two diastereomers. The conformation of the pendant-down ethyl group as well as the staggered legs as found in isomer **7A** may be energetically favourable, compared with the conformation in **7B** with the tilt-up ethyl group as well as the eclipsed legs, and therefore accounts for the high stereoselectivity for formation of isomer **7A** in DMSO- d_6 (0.737 : 0.263 equilibrium ratio for **7A** : **7B**, based on the intensity of NH cross-peaks in 2D [^1H , ^{15}N] HSQC NMR spectrum of complex **7**, Figure 4.5A).

NMR Structure of Complex 7

The ^1H NMR spectrum of **7** was acquired immediately after dissolving the sample in DMSO- d_6 , and did not change over a period of more than 10 days at ambient temperature. It contains two sets of signals, indicating the presence of two diastereomers in solution as found in crystals. 2D [^1H , ^{15}N] HSQC, 2D [^1H , ^1H] COSY and NOESY spectra were also acquired and thus allowed signal assignment for both diastereomers.

The 2D [^1H , ^{15}N] HSQC spectrum contains two sets of cross-peaks for the Et-en NH resonances, accounting for two diastereomers (Figure 4.5A). There are three cross-peaks at δ 6.625/-24.183, δ 3.804/-24.183 and δ 6.940/-7.130 for NH_{u1} , NH_{d1} and NH_{u2} of isomer **7A** and another three cross-peaks at δ 6.623/-28.171, δ 4.522/-28.171 and δ 3.864/10.746 for $\text{NH}_{\text{u1}'}$, $\text{NH}_{\text{d1}'}$ and $\text{NH}_{\text{d2}'}$ of isomer **7B**. These two sets of cross-peaks have the intensity ratio of 0.737 : 0.263. According to previous NMR studies for structures of the type $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$,¹⁶ the high frequency signal is for NH_{u} proton which points up toward the phenyl ring, whereas the low frequency signal is for NH_{d} proton which points down away from the phenyl ring. In the 2D COSY spectra (Figure 4.3), the Et-en region (A) shows the correlations of $\text{H}_{\text{d1}}\text{-H}_{1,2}$, $\text{H}_{1,2}\text{-H}_{3,4}$, and $\text{H}_7\text{-H}_{5,6}$ for **7A** and of $\text{H}_{\text{d2}'}\text{-H}_{5',6'}$, $\text{H}_{\text{d2}'}\text{-H}_{3',4'}$ and $\text{H}_7'\text{-H}_{5',6'}$ for **7B**,

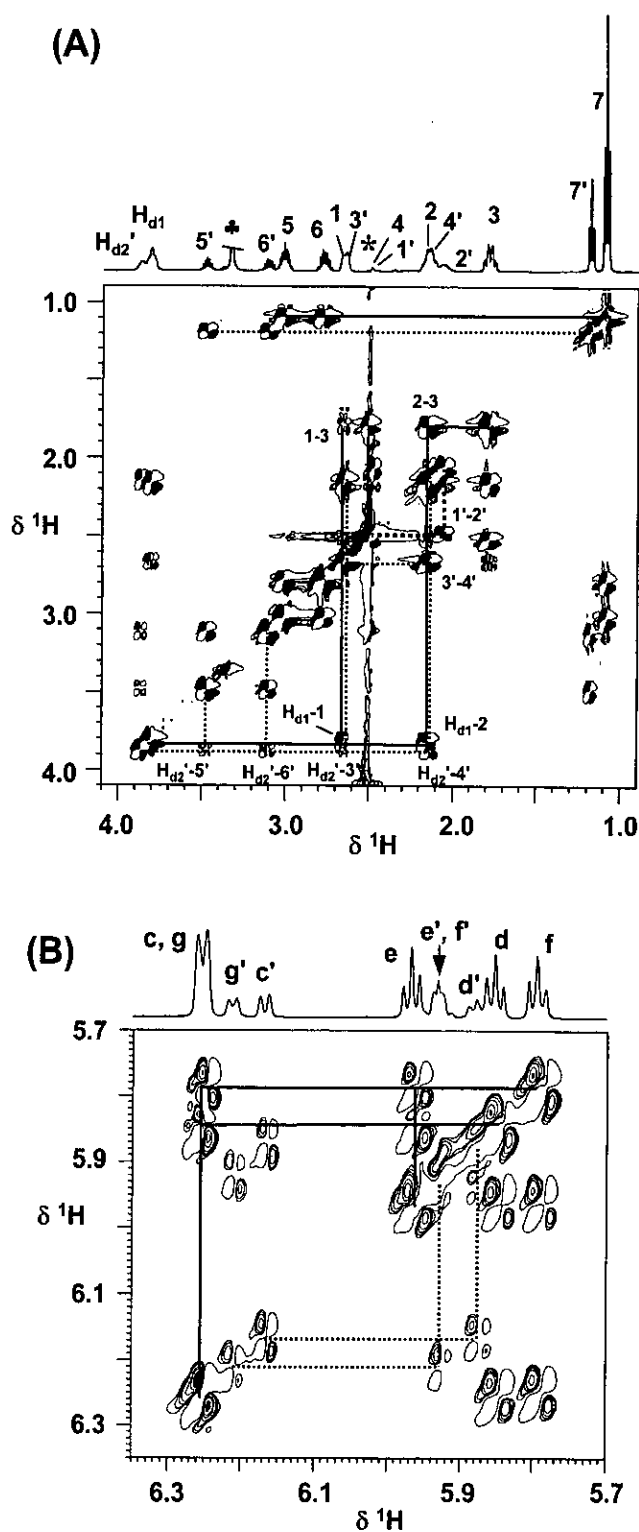


Figure 4.3 2D DQFCOSY ^1H NMR spectrum of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}]^+$ (**7**) in $\text{DMSO-}d_6$, showing assignment of (A) Et-en and (B) biphenyl resonances for the two diastereomers **7A** (solid lines) and **7B** (dashed lines). In (A), the DOH signal is

at δ 3.35 ($\clubsuit$); and H4 and H1' are overlapped with the $(\text{CD}_2\text{H})_2\text{SO}$ signal at δ 2.5 ($\ast$) and are suppressed by irradiation in the 1D ^1H NMR spectrum. For structures, see Figure 4.4A.

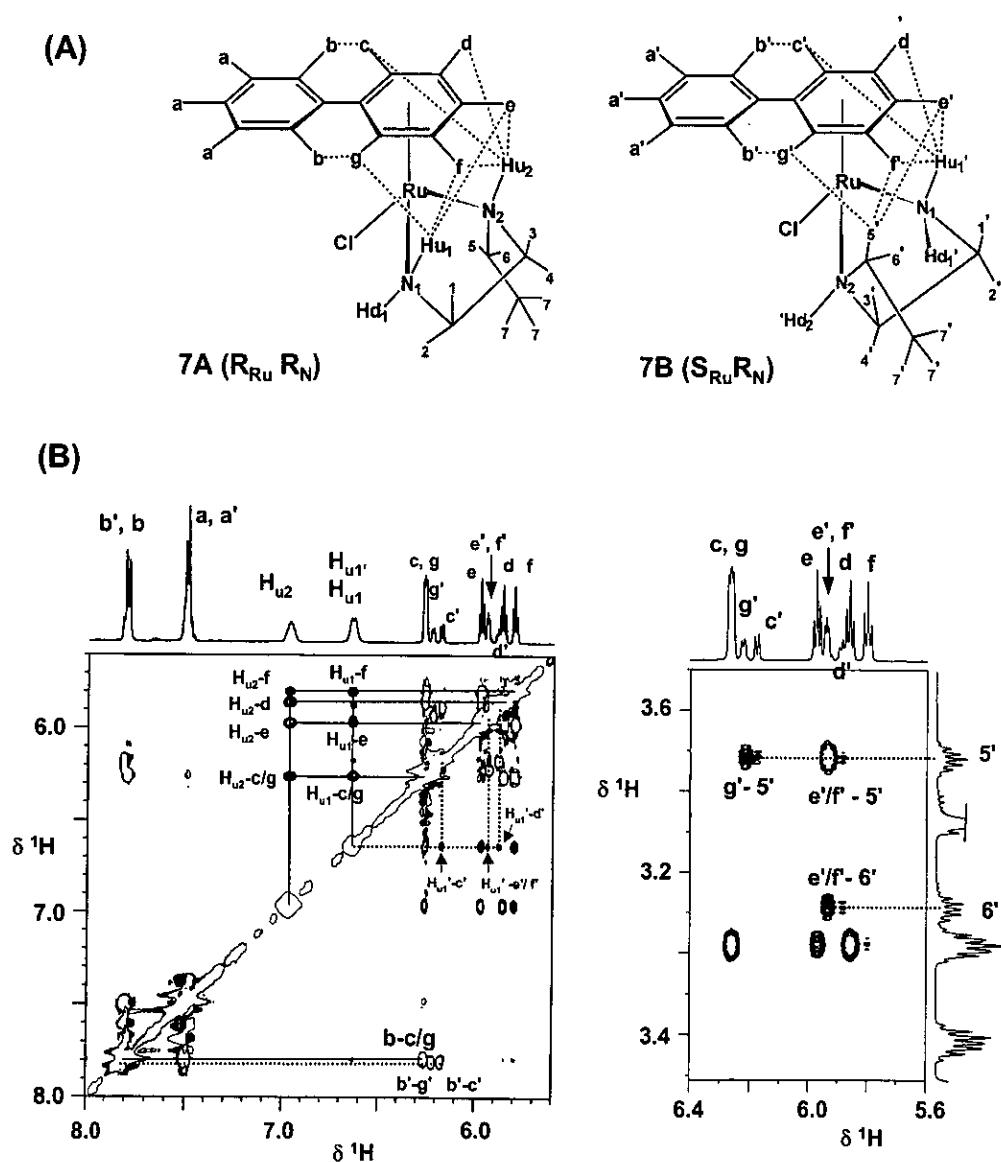


Figure 4.4 NOE data for $[(\eta^6\text{-biphenyl})\text{Ru}(\text{Et-en})\text{Cl}]^+$ (**7**). (A) Structure with NOEs for the two diastereomers **7A** and **7B** indicated by dotted lines. (B) Regions of the 2D NOESY ^1H NMR spectrum in $\text{DMSO}-d_6$ at 298 K.

and the aromatic region (B) shows the correlations of H_c-H_d , H_g-H_f , H_e-H_d and H_e-H_f for **7A** and of $H_g'-H_f'$, $H_c'-H_d'$, $H_e'-H_d'$ for **7B**. 2D NOESY spectra (Figure 4.4B) show that, for isomer **7A** there are strong NOEs between the protons NH_{u1} and NH_{u2} of the Et-en ring and the protons c, d, e, f and g of the coordinated phenyl ring, while the NOEs between NH_{d1} and the coordinated phenyl ring are very weak; for isomer **7B** there are strong NOEs between Et-en NH_{u1}' and the phenyl protons c', d', e' and f', and between the ethyl protons 5' and the phenyl protons g', f' and e', and but no NOEs between Et-en NH_{d1}' , NH_{d2}' and the phenyl protons, indicating that the proton $H5'$ together with proton NH_{u1}' points up towards the phenyl ring and the protons NH_{d1}' and NH_{d2}' point down away from the phenyl ring. These NMR studies reveal that the solution structure of two isomers **7A** and **7B** (Figure 4.4A) are largely consistent with the conformations found in the solid state (Figure 4.1). Noticeably in both solid and solution state, the chiral N atom which bears the ethyl substituent has one NH proton which points up toward the coordinated phenyl ring and appears at high frequency for isomer **7A**, while the proton points down away from the coordinated phenyl ring and appears at low frequency for isomer **7B**.

4.3.2 Dinuclear Bis Ru(arene) Complexes 5 and 6

Synthesis of Linear Tetraamine Ligand

The route used for the preparation of the linear tetraamine ligand, N, N'-bis(2-aminoethyl)-1,6-diaminohexane, was similar to that previously reported for the synthesis of hexaamine ligands.¹⁷ The general aim was to assemble a ligand in a convergent fashion by C-N bond formation via nucleophilic displacement at a terminal N of amino group.¹⁸ The route shown in the Scheme 4.1 was proved to be mild, efficient and easy to handle, and may be generally useful for the preparation of this type of tetraamine ligand with a variety of chain lengths. In addition, this method will be applicable to the preparation of ¹⁵N-labeled bis(2-aminoethyl)-1,6-

diaminohexane based on the availability of $^{15}\text{N}(\text{en})$ obtained via the use of ^{15}N -glycine in our laboratory. It is well known that ^{15}N -labelled amine ligands are useful in the study of the interaction between metal-amine complexes and DNA via [^1H , ^{15}N] 2D NMR methods.¹⁹ Therefore, this new synthetic method has potential applications related to biological research.

Preparation of Dinuclear Complexes 5 and 6

The two dinuclear bis Ru(arene) complexes **5** and **6** were prepared via reaction of $[(\eta^6\text{-biphenyl})\text{RuCl}_2]_2$ or $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2]_2$ with a stoichiometrically equivalent amount of N,N'-bis(2'-aminoethyl)-1,6-diaminohexane, obtained by treating its tetrahydrochloride salt **4** with four molar equivalents of KOH in MeOH solution. FAB mass spectra were obtained for each of the complexes **5** and **6** and confirmed the composition of the molecular ions, $[(\eta^6\text{-biphenyl})\text{RuCl}_2(\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_6\text{NH}(\text{CH}_2)_2\text{NH}_2\text{-N,N',N'',N'''})]^{2+}$ (**5**), and $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2(\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_6\text{NH}(\text{CH}_2)_2\text{NH}_2\text{-N,N',N'',N'''})]^{2+}$ (**6**). For cation **5**, the peak at 783.116 is assignable to $[5\text{-H}]^+$ (calcd m/z 783.119). For cation **6**, the peak at 839.181 is assignable to $[6\text{-H}]^+$ (calcd m/z 839.182).

NMR Studies of Complexes 5 and 6

^1H NMR spectra of **5** and **6** were acquired immediately after dissolution in DMSO- d_6 , and did not change over a period of more than 10 days at ambient temperature. In each case, the spectrum is highly overlapped both in the aromatic region (δ 5.6-6.4) and in the linker CH_2 region (δ 0.9-3.4). This is complicated by the presence of more than one species in solution. Diastereomer formation is

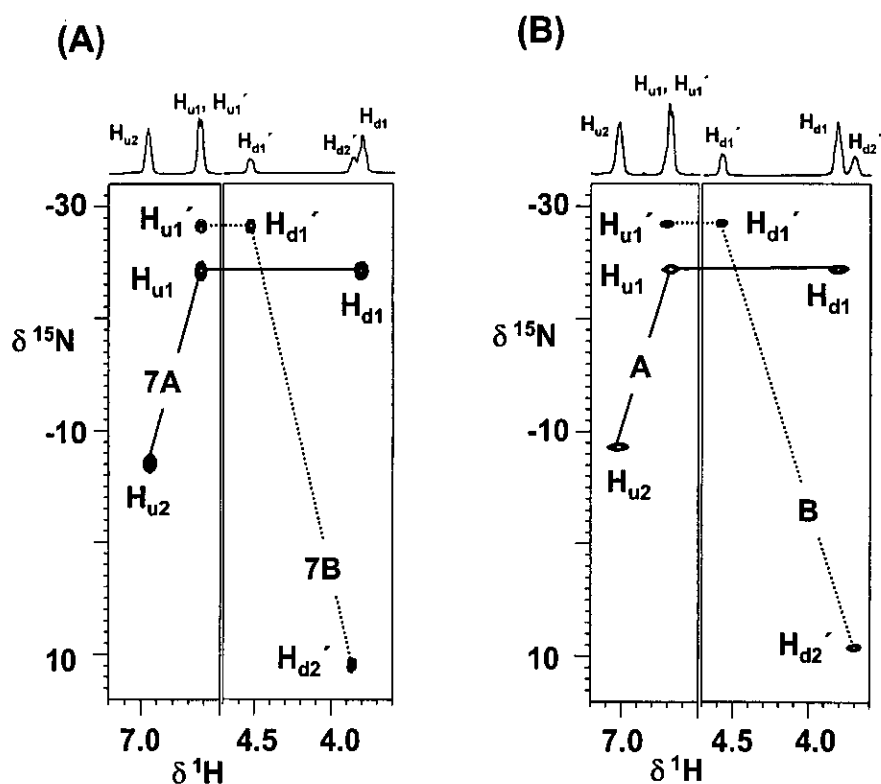
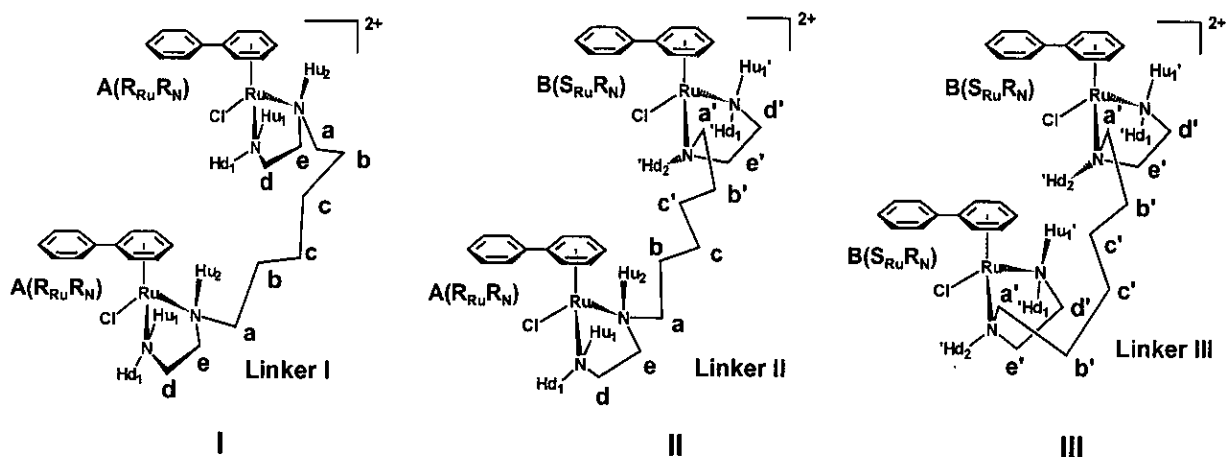


Figure 4.5 Comparison of $[^1\text{H}, ^{15}\text{N}]$ HSQC spectra of (A) $[(\eta^6\text{-Bip})\text{Ru}(\text{Et-en})\text{Cl}]^+$ (**7**), and (B) $[\{(\eta^6\text{-Bip})\text{RuCl}\}_2(\text{H}_2\text{N}(\text{CH}_2)_2\text{NH}(\text{CH}_2)_6\text{NH}(\text{CH}_2)_2\text{NH}_2\text{-}N,N',N'',N''')\}]^{2+}$ (**5**) in $\text{DMSO-}d_6$ solution. In each case, two sets of NH peaks can be identified due to their connectivities in COSY, NOESY and TOCSY spectra, and by integration, corresponding to two conformations of A and B (based on the conformations of **7A** and **7B**, Figure 4.4A). A and B cross-peaks for complex **5** have an intensity ratio of 0.729 : 0.271, consistent with that for **7A** and **7B** of complex **7** (with a ratio of 0.737 : 0.263).

unavoidable for this type of dinuclear complex. The linear tetraamine ligands themselves exhibit no chiral centres (Figure 4.8). However they contain two prochiral nitrogen atoms (N_1 , N_2). These two amine nitrogens become chiral when bound to two metal atoms, and correspondingly the two metal centres also become chiral too. Thus, there are four chiral atoms ($Ru_1N_1N_2Ru_2$) in bis(Ru-arene)-tetraamines complexes, resulting in a total of 16 possible configurations (Figure 4.8). In order to understand such interesting diastereomerization, NMR analysis for the dinuclear complexes was carried out in detail. First complex **5** was studied.

The solid and solution studies of mononuclear complex **7** provide a basis to understand the stereochemistry of dinuclear bis Ru(Bip) complex **5**. Specifically there is one NH proton on the chiral N atom (bearing the ethyl substituent) which has a characteristic chemical shift: to high frequency when in isomer **7A** where NH (H_{u2}) points up toward the coordinated phenyl; to low frequency when in isomer **7B** where NH (H_{d2}) points down away from the coordinated phenyl ring. This provides a fingerprint for analysis of the configurations of the Ru(Bip) units in complex **5**. The dinuclear complex **5** can be treated as an assemble: two $[(\eta^6\text{-Bip})Ru(\text{Et-en})Cl]^+$ units analogous to **7** linked by a CH_2CH_2 chain through the ethyl side chain (Figure 4.4A). Since complex **7** is a mixture of two diastereomers: **7A**($R_{Ru}R_N/S_{Ru}S_N$) and **7B**($S_{Ru}R_N/R_{Ru}S_N$), both Ru(Bip) units of **5** can be treated as three combinations of AA (**I**), AB (**II**) or BB (**III**), see Figure 4.9. The A and B units can be treated to have the similar conformation as that for diastereomers **7A** and **7B** of complex **7**, respectively (Figure 4.4A). The AA combination (**I**) consists of four configurations (based on the absolute configuration of Ru-N-N-Ru): RRRR, SSRR/RRSS and SSSS. The AB combination (**II**) consists of eight configurations: SRRR/RRRS, RSRR/RRSR,

(A)



(B)

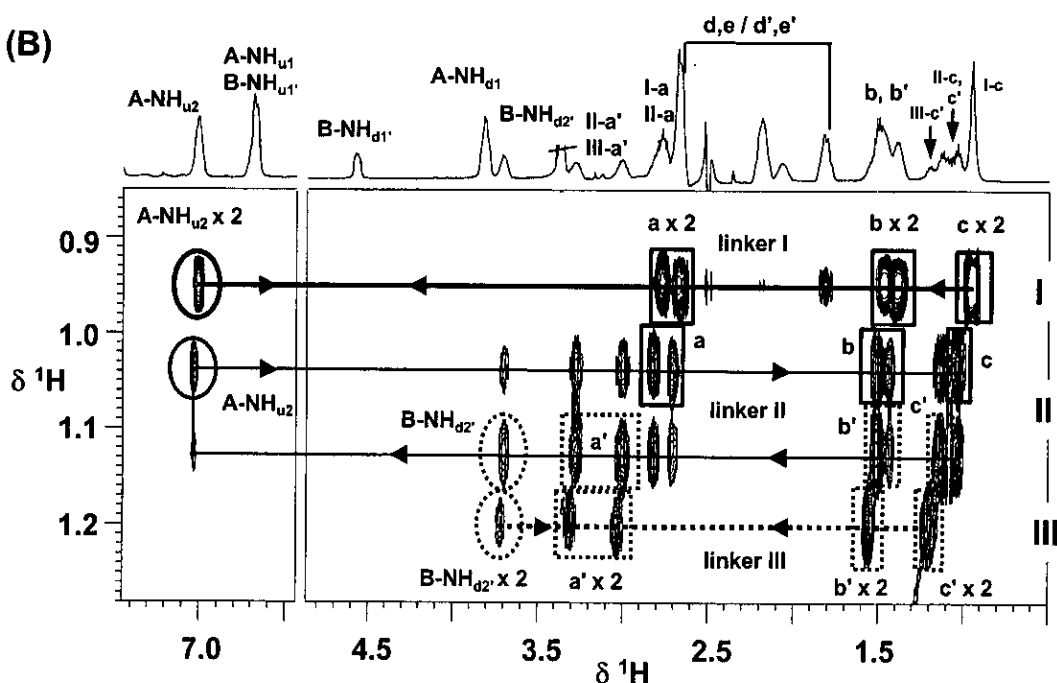


Figure 4.6 (A) The structures of three types of configurations I, II and III for dinuclear complex 5. (B) 2D TOCSY NMR spectrum of NH and linker CH₂ region of Bis-Ru(Bip) in dmso-*d*₆ solution (90 mM) suggested the presence of three spin-systems for the linker. Cross-peak connectivities of NH_{u2}→a→b→c→c'→b'→a→NH_{u2} for I (thick solid line), NH_{u2}→a→b→c→c'→b'→a'→NH_{d2'} for II (thin solid line); and NH_{d2'}→a'→b'→c'→c'→b'→a'→NH_{d2'} for III (dotted line). Note that each of the labels a, b, c, d, e, a', b', c', d' and e' represent a

pair of protons on the same C. Each pair of protons are inequivalent and one appears at high frequency and the other at low frequency, but specific assignments were not made for both protons.

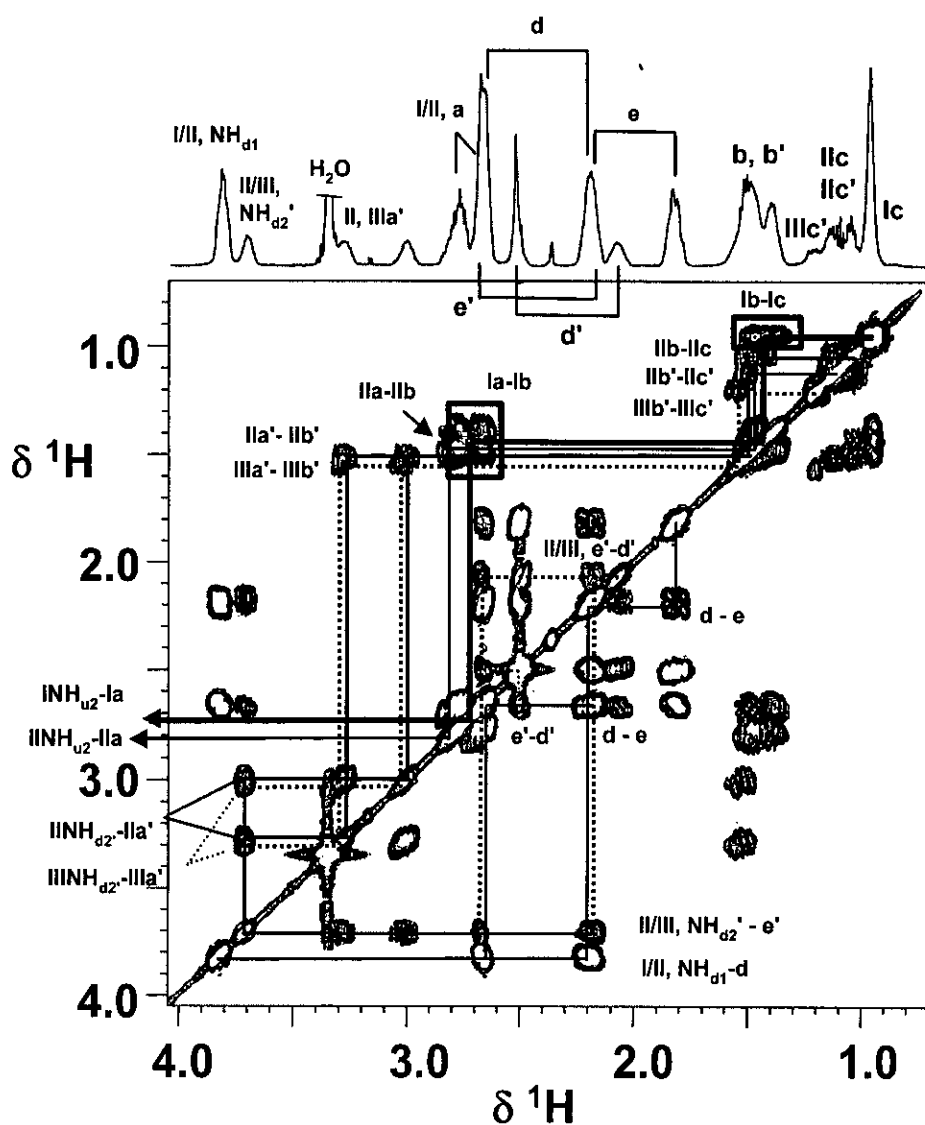
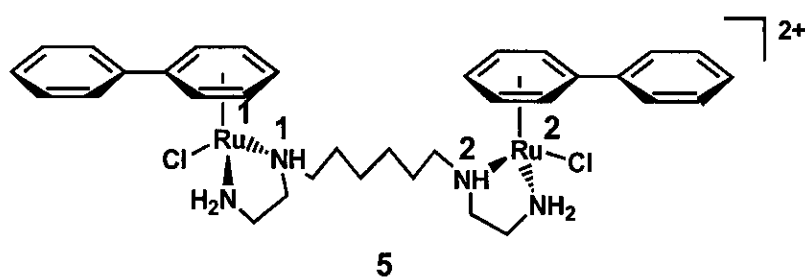


Figure 4.7 2D COSY ^1H NMR spectrum of dinuclear complex **5**, showing assignment of NH and linker CH_2 resonances for the three types of configurations **I**, **II** and **III**. The cross-peak connectivities: $\text{NH}_{\text{u}2} \rightarrow \text{a}$, $\text{a} \rightarrow \text{b}$, $\text{b} \rightarrow \text{c}$, $\text{c} \rightarrow \text{c}$, $\text{c} \rightarrow \text{b}$, $\text{b} \rightarrow \text{a}$, $\text{a} \rightarrow \text{NH}_{\text{u}2}$ (**I**, thick solid line), $\text{NH}_{\text{u}2} \rightarrow \text{a}$, $\text{a} \rightarrow \text{b}$, $\text{b} \rightarrow \text{c}$, $\text{c} \rightarrow \text{c}'$, $\text{c}' \rightarrow \text{b}'$, $\text{b}' \rightarrow \text{a}'$, $\text{a}' \rightarrow \text{NH}_{\text{d}2}'$ (**II**, thin solid line); $\text{NH}_{\text{d}2}' \rightarrow \text{a}'$, $\text{a}' \rightarrow \text{b}'$, $\text{b}' \rightarrow \text{c}'$, $\text{c}' \rightarrow \text{c}'$, $\text{c}' \rightarrow \text{b}'$, $\text{b}' \rightarrow \text{a}'$, $\text{a}' \rightarrow \text{NH}_{\text{d}2}'$ (**III**, dotted line). For structures, see Figure 4.6.



Four chiral atoms: Ru1, N1, N2, Ru2

16 possible configurations:

Ru1	N1	N2	Ru2
R	R	R	R
R	R	R	S
R	R	S	R
R	S	R	R
S	R	R	R
S	S	R	R
R	S	S	R
R	R	S	S
R	S	S	S
S	S	S	R
S	S	S	S
R	S	R	S
S	R	S	R
S	R	R	S
S	S	R	S
S	R	S	S

Figure 4.8 Possible configurations for dinuclear complex 5.

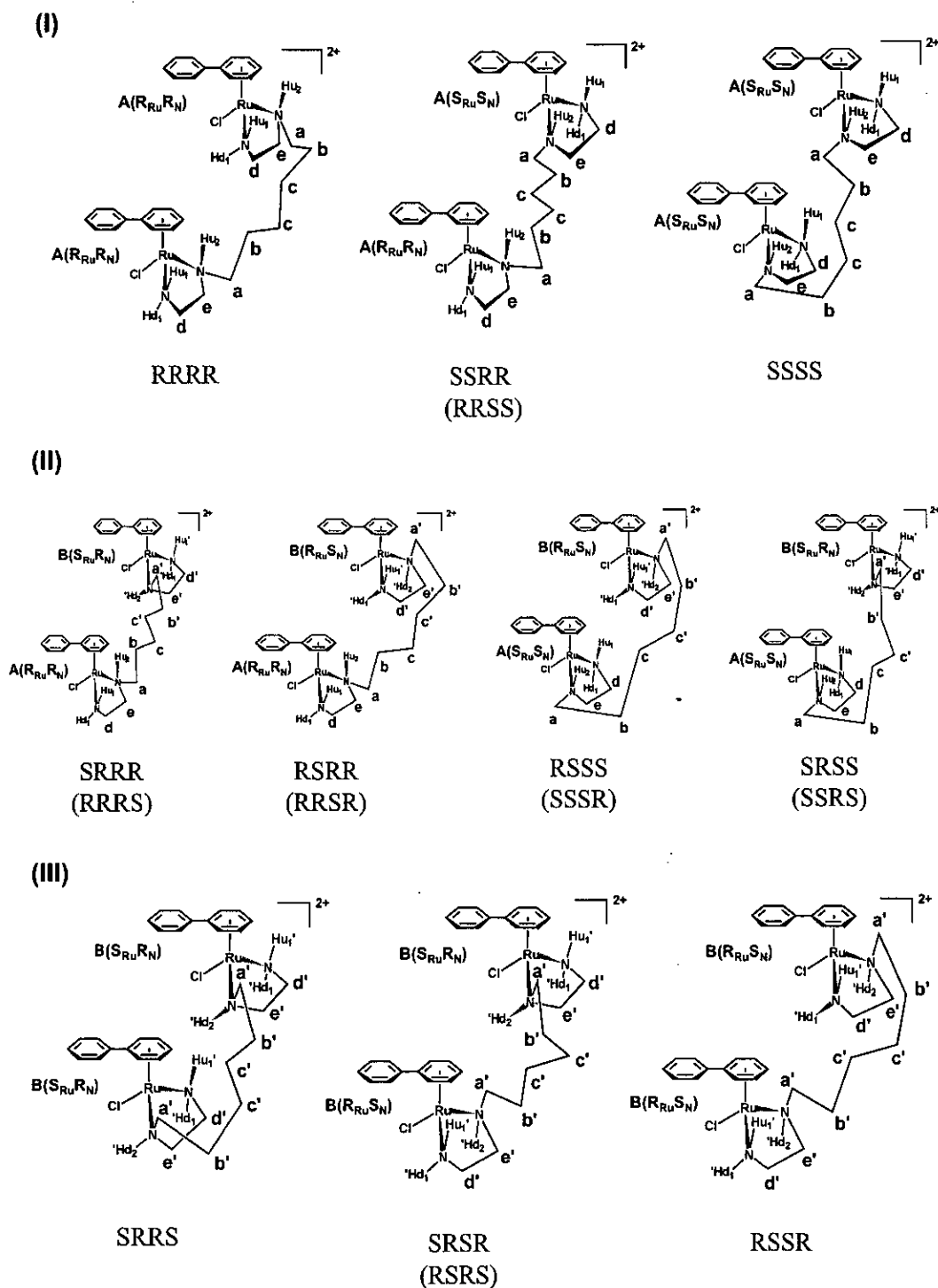


Figure 4.9 The structures of the 16 possible configurations of dinuclear complex 5. Each structure can be a combination of AA, AB, or BB-type configurations. The four configurations RRRR, SSRR, RRSS and SSSS would show the identical NMR signals as type I since RRRR and SSSS, SSRR and RRSS are mirror isomers and RR

and SS on each end of molecule are mirror images. Each end has little influence on the other. Similarly, the eight configurations SRRR/RRRS, RSRR/RRSR, RSSS/SSSR and SRSS/SSRS (reversed in direction) would show identical NMR signals as type **II**, and the six configurations SRRS, SRSR/RSRS and RSSR would show the identical NMR signals as type **III** (Figure 4.6).

RSSS/SSSR and SRSS/SSRS; and the BB combination (**III**) consists of four configurations : SRRS, SRSR/RSRS and RSSR.

The 2D [^1H , ^{15}N] HSQC spectrum of complex **5** (90 mM in DMSO- d_6) contains two major sets of cross-peaks, almost identical to that for complex **7** (Figure 4.5B). This suggests that complex **5** contains both A and B units either within the same molecule or in different molecules, and one Ru unit has little influence on the other Ru unit in solution. There are three cross-peaks at δ 6.677/-24.372, δ 3.809/-24.372 and δ 7.011/-8.646 (average positions) for the NH_{u1} , NH_{d1} and NH_{u2} of unit A, and another three cross-peaks at δ 6.703/-28.396, δ 4.574/-28.396 and δ 3.70/9.155 (average position) for the $\text{NH}_{u1'}$, $\text{NH}_{d1'}$ and $\text{NH}_{d2'}$ of unit B. This analysis is aided by studies of the NH signal on the chiral N atom: NH_{u2} signal at high frequency as the marker for a conformation of **7A**-type and the $\text{NH}_{d2'}$ signal at low frequency as the marker for a conformation of **7B**-type. These two sets of cross-peaks have an intensity ratio of 0.729 : 0.271, again consistent with that for complex **7** (with a ratio of 0.737 : 0.263).

The 2D [^1H , ^1H] TOCSY spectrum shows that there are three spin-systems for the linkers, indicating that there are three major conformations (Figure 4.6): **I** (AA), **II** (AB) and **III** (BB) for complex **5**. The cross-peak connectivities are $\text{NH}_{u2} \rightarrow a \rightarrow b \rightarrow c \rightarrow c \rightarrow b \rightarrow a \rightarrow \text{NH}_{u2}$ for **I**, $\text{NH}_{u2} \rightarrow a \rightarrow b \rightarrow c \rightarrow c' \rightarrow b' \rightarrow a' \rightarrow \text{NH}_{d2'}$ for **II**; and $\text{NH}_{d2'} \rightarrow a' \rightarrow b' \rightarrow c' \rightarrow c' \rightarrow b' \rightarrow a' \rightarrow \text{NH}_{d2'}$ for **III**. 2D [^1H , ^1H] COSY data (Figure 4.7) show the coupling pathways for these three spin systems: (**I**) $\text{NH}_{u2} \rightarrow a$, $a \rightarrow b$, $b \rightarrow c$, $c \rightarrow c$, $c \rightarrow b$, $b \rightarrow a$, $a \rightarrow \text{NH}_{u2}$; (**II**) $\text{NH}_{u2} \rightarrow a$, $a \rightarrow b$, $b \rightarrow c$, $c \rightarrow c'$, $c' \rightarrow b'$, $b' \rightarrow a'$, $a' \rightarrow \text{NH}_{d2'}$; (**III**) $\text{NH}_{d2'} \rightarrow a'$, $a' \rightarrow b'$, $b' \rightarrow c'$, $c' \rightarrow c'$, $c' \rightarrow b'$, $b' \rightarrow a'$, $a' \rightarrow \text{NH}_{d2'}$. Therefore,

analysis of 2D ^1H TOCSY and COSY cross-peak connectivities allowed “walks” from one Ru(Bip) unit through the alkylene linker to the second Ru(Bip) unit, and thus to the assignment of major conformations for dinuclear complex **5**. The TOCSY NMR study for complex **6** gave very similar results as for complex **5**.

These NMR analyses reveal the diastereotopic nature for this type of dinuclear Bis Ru(arene) complex. The formation of 16 configurations is unavoidable during the preparations of **5** and **6** since no specific methods to control the nature of chiral centers was used. Since two Ru units on each molecule have little influence on each other, the four configurations of RRRR, SSRR, RRSS and SSSS would show the identical NMR signals as type **I**, the eight configurations of SRRR/RRRS, RSRR/RRSR, RSSS/SSSR and SRSS/SSRS would show the identical NMR signals as type **II**, and the six configurations of SRRS, SRSR/RSRS and RSSR would show the identical NMR signals as type **III** (Figure 4.9).

For complex **7**, there are two diastereomers present in DMSO- d_6 solution with **7A** 73.7% and **7B** 26.3% at equilibrium. Based on this, the *statistical ratio* for the combinations of AA, AB and BB should be 67.32%, 24.07% and 8.60%. The observed ratios of conformations I, II and III are 67.7% : 24.0% : 8.3% in DMSO- d_6 solution, based on the cross-peak intensities of I, II, and III in the TOCSY spectrum. This result is consistent with the *statistical ratios* of I(AA), II(AB) and III(BB), suggesting that it is energetically unfavourable for the Ru(Bip) unit to adopt the B-type configuration in which the NH proton on the chiral N points down away from the coordinated phenyl ring while the ethyl substituent points up towards it.

4.4 Concluding Remarks

A new method has been used for synthesis of the bifunctional ligand N,N'-bis(2'-aminoethyl)-1,6-diaminohexane. Two novel dinuclear bis (Ru-arene) complexes **5** and **6** were prepared with this tetraamine ligand, as potential DNA

cross-linking agents. They are presently undergoing cytotoxicity tests. The cytotoxicity data against A2780 cell lines are promising. They are at present under study as potential DNA interstrand cross-linking agents.

NMR studies of the dinuclear complex **5** revealed diastereotopic behaviour. This can be understood with the aid of studies of complex **7**, the mono chiral Ru(arene) complex. Each end of the dinuclear complex **5** was found to have a similar stereo-chemistry as the two diastereomers **7A** and **7B**, and would therefore have a similar chemical behaviour to complex **7**. Thus, further investigations of DNA binding for the two diastereomers **7A** and **7B** will be interest. It will be interest to investigate the influence on DNA binding of the steric hindrance from the ethylenediamine aliphatic substituent in isomers **7A** and **7B**, in comparison with the analogue species $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}]^+$.

In view of the importance of steric-selectivity in biochemistry, eg. chiral recognition of DNA by drugs,²⁰ the resolution of diastereomers of the dinuclear complexes **5** and **6** is also of interest. For the resolution of the isomeric mixture, an efficient HPLC method or fractional crystallization could be used.²¹ Selective crystallization of one diastereomer²² of the dinuclear complexes could also be attempted.

4.5 References

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Chapter 5

Arene-Nucleobase Stacking and Stereospecific Hydrogen-bonding in Guanine Adducts

5.1 Introduction

Ruthenium complexes offer potential for the design of anticancer drugs.¹ The ammine complex *fac*-[Ru(III)Cl₃(NH₃)₃], has long been known to be active, but its poor aqueous solubility has prevented its clinical use.² Recently other Ru complexes such as *trans*-[Ru(II)Cl₂(DMSO)₄], *trans*-[HIn][Ru(III)Cl₄(Ind)₂] (Ind = indazole), *mer*-[Ru(III)(terpy)Cl₃] (terpy = 2, 2' : 6', 2''-terpyridine), α -[Ru(azpy)₂]Cl₂ (azpy = 2-(phenylazo)pyridine) and [Ru(IV)(cdta)Cl₂] (cdta = 1, 2-cyclohexanediaminetetraacetate) have also been reported to be highly active.³ *Trans*-[HIm][Ru(III)Cl₄(DMSO)(Im)] (Im = imidazole) (NAMI-A) recently became the first Ru complex to enter clinical trials. NAMI-A is relatively non-toxic, but active against tumour metastases.⁴ Ru(III) antitumor complexes may serve as prodrugs which are reduced to active Ru(II) species *in vivo*.⁵ We have found that organometallic Ru(II) complexes of the type $[(\eta^6\text{-arene})\text{Ru(II)(en)Cl}][\text{PF}_6]$ (en = ethylenediamine) exhibit anticancer activity both *in vitro*,^{6,7} and *in vivo*,⁸ including activity against cisplatin-resistant cancer cells.

A potential target for these organometallic Ru(II) complexes is DNA and in initial studies we observed strong selective binding to N7 of guanine (G) bases on DNA oligomers.⁷ It is now important to elucidate potential modes of interaction of these Ru arene complexes with DNA and, in particular, to investigate whether monofunctional binding induces significant structural changes in DNA. Such understanding will be aided by investigations of interactions with mononucleobase derivatives. However, to date only one X-ray crystal structure of a Ru arene adduct with a G derivative, that of $[(\eta^6\text{-C}_6\text{H}_6)\text{Ru(II)(9EtG-N7)}_2(\text{H}_2\text{O})][\text{CF}_3\text{SO}_3]_2 \cdot 2\text{H}_2\text{O}$, appears to have been reported.⁹ Also there are very few reported X-ray crystallographic studies of other Ru(II) and Ru(III) -nucleobase, -nucleoside, or -nucleotide complexes.

Hydrophobic interactions between the arene ligand in $[(\eta^6\text{-arene})\text{Ru(II)(en)Cl}]^+$ and DNA could produce a driving force for DNA binding.¹⁰ Such interactions have already been shown to be of importance in the biological activity of some platinum anticancer agents. Planar aromatic ligands such as doxorubicin,¹¹ acridine orange,¹² 9-aminoacridine,¹³ and ethidium bromide¹⁴ have been incorporated into Pt complexes as potential intercalators. Also earlier studies of the binding of $[\text{Pt(terpy)Cl}]^+$ to calf thymus DNA revealed intercalation as well as covalent interactions.¹⁵ The high antitumor activity of *trans*- $[\text{PtCl}_2(\text{NH}_3)\text{L}]$ (L = planar aromatic N donor such as pyridine, thiazole or quinoline) in comparison with transplatin, *trans*- $[\text{PtCl}_2(\text{NH}_3)_2]$,^{16,17} has been attributed to the interaction of the quinoline ligand with DNA duplex, perhaps partially by intercalation,^{18,19} and the cytotoxic transplatin derivative *trans*- $[\text{PtCl}(\text{PyAc-}N,O)(\text{NH}_3)]$ (PyAc = pyridin-2-yl-acetate) also appears to exhibit “pseudobifunctional” behavior (covalent/intercalative binding).²⁰ Hydrophobic interactions with nucleobases play a key role in determining the recognition of cisplatin-modified DNA by the high-mobility group protein (HMG1),²¹ and also contribute to formation of stereoselective GG intrastand crosslinks on DNA by the anticancer drug *cis*- $[\text{PtCl}_2(\text{NH}_3)(2\text{-picoline})]$.²² The presence of intercalator ligands in coordinatively-saturated octahedral Ru(II), Os(II), and Rh(III) complexes increases DNA affinity and gives rise to highly specific recognition of DNA base sequences via shape selection.²³ Also the introduction of aliphatic amine ancillary ligands into Rh(III)(phi) complexes (phi = 9, 10-phenanthrenequinone diimine) has optimised site-recognition through specific H-bonding and van der Waals contacts between the ancillary ligands and DNA bases.²⁴ Hydrogen-bonding plays an important role in modulating the DNA sequence recognition of platinum am(m)ine complexes and stabilization of platinated adducts.²⁵ Pt-am(m)ine NH protons are often involved in H-bonding with an oxygen

of the 5'-phosphodiester linkage or with G O6.²⁶ The diamine ligand in $[(\eta^6\text{-arene})\text{Ru}(\text{II})(\text{en})\text{Cl}]^+$ complexes has potential for H-bonding interactions.

The aim of this work is to gain insight into the factors which influence the interaction of $[(\eta^6\text{-arene})\text{Ru}(\text{II})(\text{en})]^{2+}$ with guanine derivatives, including covalent Ru binding to N7, hydrophobic interactions of the arene ligand, and en NH hydrogen bonding. The molecular structures of the parent chloro anticancer complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$, where arene = biphenyl (Bip, complex 1), 1, 4, 9, 10-tetrahydroanthracene (THA, 2), and 9, 10-dihydroanthracene

Chart 5.1 Ligands and complexes studied in this work and NMR numbering scheme (X = Cl or guanine derivatives, see Table 5.1)

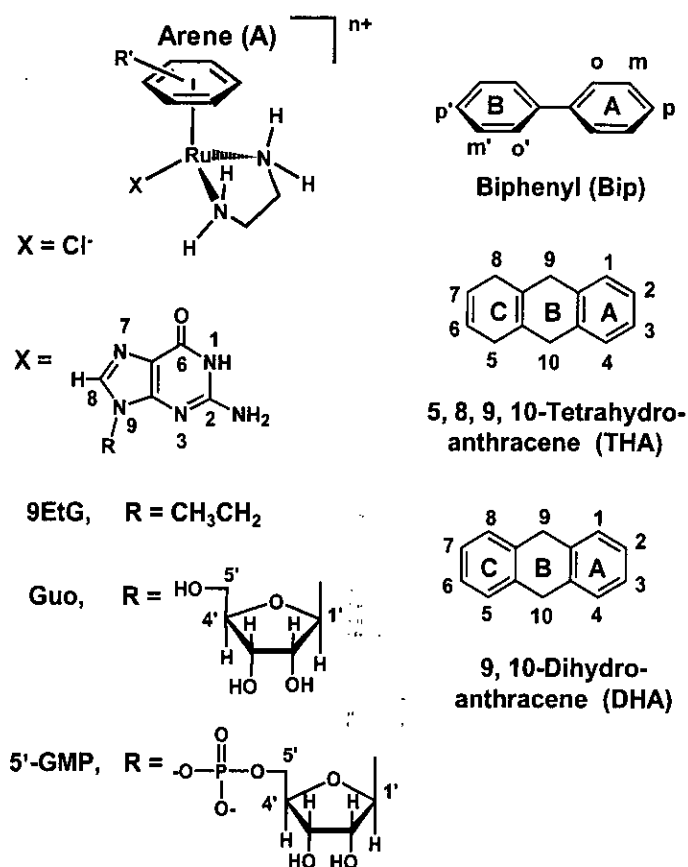


Table 5.1 Structural studies of $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{X})]^{n+}$ complexes reported in this work. For structures and abbreviations, see Chart 5.1.

Arene	X	Complex No.	Structure ^a	
Bip	Cl	1	X-ray	NOESY (DMF- <i>d</i> ₆) ^b
	9EtG	4	X-ray	NOESY (acetone- <i>d</i> ₆) ^b
	Guo	5	X-ray	NOESY (10% D ₂ O in H ₂ O) ^b
	5'-GMP	8^c	—	NOESY (10% D ₂ O in H ₂ O) ^b
THA	Cl	2	X-ray	NOESY (DMSO- <i>d</i> ₆) ^b
	9EtG	6	X-ray	— ^c
	5'-GMP	9^c	—	— ^c
DHA	Cl	3	X-ray	—
	9EtG	7	X-ray	— ^c
	5'-GMP	10^c	—	— ^c

^a All crystals studied as PF₆⁻ salts. ^b Solvent for NMR. ^c Arene resonances too broad for NOESY studies. ^c Since the charge on 5'-GMP is dependent on pH, no overall charge is assigned to the 5'-GMP adducts discussed in this chapter.

(DHA, **3**), and those of their 9-ethylguanine (9EtG), guanosine (Guo) and guanosine 5'-monophosphate (5'-GMP) adducts (Chart 5.1), have been studied, where possible both in the solid state by X-ray crystallography and in solution using NMR methods (Table 5.1). These studies will aid optimisation of the design of this new class of anticancer agents.

5.2 Experimental Section

5.2.1 Materials

9-Ethylguanine (Sigma), guanosine hydrate (9-[β -D-ribofuranosyl]guanine) (Aldrich) and Na₂-5'-GMP (Aldrich) were used without further purification. ¹⁵NH₂(CH₂)₂¹⁵NH₂ (¹⁵N-en),²⁷ 1, 4, 5, 8, 9, 10-Hexahydroanthracene (C₁₄H₁₆),²⁸ 1, 4, 9, 10-Tetrahydroanthracene (C₁₄H₁₄),²⁹ [(η^6 -biphenyl)Ru(en)Cl]PF₆ (**1**) and [(η^6 -biphenyl)Ru(¹⁵N-en)Cl]PF₆ (¹⁵N-**1**)⁷ were prepared according to the published procedures. Other complexes were prepared as described below.

5.2.2 Preparation of Complexes

[(η^6 -C₁₄H₁₄)RuCl₂]₂.³⁰ 1, 4, 5, 8, 9, 10-Hexahydroanthracene (1.0 g, 5.43 mmol) was added to a solution of RuCl₃·3H₂O (0.84 g, 3.18 mmol) in dry ethanol (60 mL) and refluxed under Ar for 48 h. Filtration of the hot reaction mixture gave a yellow-brown solid which was washed with fresh ethanol (2 mL) followed by diethyl ether (4×10 mL) and dried under vacuum. Yield: 0.96 g (85.5 %). ¹H NMR (DMSO-*d*₆): δ 5.83 (m, 2H), 5.71 (s, 2H), 5.61 (m, 2H), 3.21 (m, 2H), 3.05 (m, 2H), 2.5 (m, 4H).

[(η^6 -C₁₄H₁₄)RuCl(en)][PF₆] (**2**). [(η^6 -C₁₄H₁₄)RuCl₂]₂ (0.205 g, 0.289 mmol) was stirred in dry methanol (25 mL) under Ar at 333 K. Ethylenediamine (48 μ L, 0.75 mmol) was added in one portion. The reaction was stirred for 3 h and filtered, and NH₄PF₆ (0.4 g, 2.45 mmol) added. The volume was reduced to ca. 6 mL on a rotary evaporator. After standing at 277 K overnight, a yellow microcrystalline precipitate formed, which was collected by filtration, washed with fresh methanol (0.5 mL), followed by diethyl ether (5 mL) and dried in *vacuo*. Yellow crystals suitable for X-ray diffraction were obtained by recrystallisation from ethanol/ether. Yield: 0.1 g (32.9%). Anal. Calcd for C₁₆H₂₂ClF₆N₂PRu (523.85): C, 36.68; H, 4.23; N, 5.35%. Found: C, 36.20; H, 4.17; N, 5.34%. ¹H NMR (DMSO-*d*₆): δ 6.29 (broad,

2H), 5.75 (s, 2H), 5.61 (m, 2H), 5.50 (m, 2H), 4.10 (broad, 2H), 3.18 (m, 4H), 2.62 (s, 4H), 2.32 (m, 2H), 2.23 (m, 2H).

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2]_2$. 1, 4, 9, 10-Tetrahydroanthracene (0.45 g, 2.49 mmol) was added to a solution of $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (0.48 g, 1.83 mmol) in dry ethanol (45 mL). The reaction was heated to reflux under Ar for 48 h. Filtration of the hot reaction mixture gave a brown solid which was washed with fresh ethanol (1 mL) followed by diethyl ether (4×10 mL) and dried under vacuum. Yield: 0.57 g (88.5%). ^1H NMR ($\text{DMSO-}d_6$): δ 7.27 (m, 4H), 5.89 (m, 4H), 3.92 (m, 4H).

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}(\text{en})][\text{PF}_6]$ (3). $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2]_2$ (0.10 g, 0.142 mmol) was stirred in dry methanol (10 mL) under Ar at 333 K. Ethylenediamine (24 μL , 0.359 mmol) was added in one portion. The reaction was maintained at 333 K with stirring for 5 h and filtered. The volume was reduced to ca. 4 mL with a rotary evaporator and to this solution was then added a solution of NH_4PF_6 (0.20 g, 1.227 mmol) in methanol (2 mL). On shaking, a yellow solid precipitated. This mixture was allowed to stand overnight at 277 K. The precipitate was then collected by filtration, washed with fresh methanol (0.5 mL), followed by diethyl ether (5 mL), and dried under vacuum. Recrystallisation from benzyl alcohol / ether gave yellow crystals suitable for X-ray diffraction studies. Yield: 0.1 g (67.5%). Anal. Calcd for $\text{C}_{16}\text{H}_{20}\text{ClF}_6\text{N}_2\text{PRu}$ (521.83) : C, 36.82; H, 3.86; N, 5.37%. Found: C, 36.50; H, 3.85; N, 5.38%. ^1H NMR ($\text{DMSO-}d_6$): δ 7.20 (m, 4H), 6.19 (broad, 2H), 5.70 (m, 4H), 3.95 (m, 4H), 3.86 (broad, 2H), 2.22 (m, 2H), 2.05 (m, 2H).

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})][\text{PF}_6]_2 \cdot (\text{MeOH})$ (4). $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (1) (0.10 g, 0.20 mmol) was dissolved in water (30 mL) and 9EtG (37.63 mg, 0.21 mmol) was added. The mixture was stirred for 2 d at 313 K.

After filtration, the volume was reduced to 8 mL and then NH_4PF_6 (0.2 g, 1.2 mmol) was added. A precipitate was obtained overnight at 277 K. Recrystallization from methanol gave yellow crystals suitable for X-ray diffraction studies. Yield: 0.12 g (73.5 %). Anal. Calcd for $\text{C}_{22}\text{H}_{31}\text{F}_{12}\text{N}_7\text{O}_2\text{P}_2\text{Ru}$ (816.55): C, 32.36; H, 3.83; N, 12.01%. Found: C, 32.19; H, 3.80; N, 12.34%.

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})][\text{PF}_6]_2 \cdot 3.75(\text{H}_2\text{O})$ (5) was prepared by reacting $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (1) (89.7 mg, 0.18 mmol) with Guo (53.8 mg, 0.19 mmol) in water (20 mL) at 313 K for 4 d. The solution was filtered and then NH_4PF_6 (0.16 g, 1.0 mmol) was added. After shaking, the reaction mixture was then kept at 277 K overnight. The precipitate was recrystallised from water. Slow evaporation of the aqueous solution gave yellow-green crystals suitable for X-ray diffraction studies. Yield: 80.0 mg (48.1%). Anal. Calcd for $\text{C}_{24}\text{H}_{38.5}\text{F}_{12}\text{N}_7\text{O}_{8.75}\text{P}_2\text{Ru}$ (956.11): C, 30.15; H, 4.05; N, 10.60%. Found: C, 30.17; H, 3.86; N, 10.42%.

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N7})][\text{PF}_6]_2 \cdot (\text{MeOH})$ (6). $[(\eta^6\text{C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (2) (70.68 mg, 0.14 mmol) was reacted with 9EtG (26.34 mg, 0.147 mmol) in water (30 mL) at 313 K for 2 d under Ar. The mixture was then filtered. The volume of the filtrate was reduced to 10 mL and NH_4PF_6 (0.16 g, 1 mmol) was added. Yellow crystals suitable for X-ray diffraction studies were obtained on recrystallization of the precipitate from MeOH. Yield: 62.0 mg (52.4 %). Anal. Calcd for $\text{C}_{24}\text{H}_{35}\text{F}_{12}\text{N}_7\text{O}_2\text{P}_2\text{Ru}$ (844.60): C, 34.13; H, 4.18; N, 11.61%. Found: C, 34.28; H, 4.62; N, 11.96 %.

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-N7})][\text{PF}_6]_2 \cdot 2(\text{MeOH})$ (7). $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (3) (34.8 mg, 0.067 mmol) was reacted with 9EtG (12.0 mg, 0.067 mmol) in water (15 mL) at 313 K for 2 d under Ar. The mixture was then filtered.

The volume of the filtrate was reduced to 4 mL and NH_4PF_6 (0.16 g, 1 mmol) was added. Recrystallisation of the precipitate from MeOH gave the yellow crystals suitable for X-ray diffraction studies. Yield: 32.5 mg (55.5 %). Anal. Calcd for $\text{C}_{25}\text{H}_{37}\text{F}_{12}\text{N}_7\text{O}_3\text{P}_2\text{Ru}$ (874.63): C, 34.33; H, 4.26; N, 11.21 %. Found: C, 34.35; H, 4.55; N, 11.69 %.

5.2.3 Preparation of NMR Samples

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (8). A solution containing this complex was prepared by mixing $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (1) (2.43 mg, 4.9 μmol) with an aliquot (122.3 μL) of a stock solution of $\text{Na}_25'\text{-GMP}$ (40 mM) in 10% D_2O / 90% H_2O (total volume 700 μL). This solution was kept at 310 K for 16 h. The final Ru concentration was 7.0 mM and $\text{pH} = 7.3$. Then sodium trimethylsilyl[2,2,3,3- d_4]propionate (TSP, 2 μL of a 50 mM solution in D_2O) was added and the solution was filtered into a 5 mm NMR tube.

$[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N-en})(9\text{EtG-N7})]^{2+}$ ($^{15}\text{N-4}$), $[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N-en})(\text{Guo-N7})]^{2+}$ ($^{15}\text{N-5}$) and $[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N-en})(5'\text{-GMP-N7})]$ ($^{15}\text{N-8}$) were prepared by the following procedure. Typically, $[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N-en})\text{Cl}][\text{PF}_6]$ ($^{15}\text{N-1}$) (1.77 mg, 3.55 mmol) and 9EtG (0.636 mg, 3.55 mmol) or Guo (1.0 mg, 3.55 mmol) or $\text{Na}_25'\text{-GMP}$ (88.75 μL of a 40.0 mM solution) were reacted in 10% D_2O / 90% H_2O (total volume 700 μL) at 310 K for 24 h. The reaction was then complete and the mixture was filtered directly into NMR tubes. For the spectra in acetone- d_6 or DMSO- d_6 , the filtrate was lyophilized and then redissolved in acetone- d_6 or DMSO- d_6 (700 μL). The final Ru concentration was 5 mM.

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (6 and $^{15}\text{N-6}$) and $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (9 and $^{15}\text{N-9}$) were prepared by *in situ* reactions of

stoichiometric amounts of $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**) (0.91 mg, 1.73 μmol) and 9EtG (0.31 mg, 1.73 μmol) or $\text{Na}_25'\text{-GMP}$ (43.25 μL of a 40.0 mM solution)) in 10% D_2O / 90% H_2O (total volume 680 μL). The mixture was degassed by bubbling Ar for 10 min, then sealed and kept at 310 K for 10 h. TSP (2 μL of a 50 mM solution in D_2O) was added and the resulting mixture filtered into NMR tubes under Ar. To prepare ^{15}N -**6** and ^{15}N -**9**, ^{15}N -**2** was used. The final Ru concentration was 2.5 mM. The pH of solution was 6.3 for 9EtG adducts, and 7.4 for 5'-GMP adducts.

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-N7})]$ (**7**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (**10**) were prepared by *in situ* reactions of $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**3**) (0.98 mg, 1.88 μmol) and 9EtG (0.34 mg, 1.88 μmol) or $\text{Na}_25'\text{-GMP}$ (47.0 μL of a 40.0 mM solution) in 10% D_2O /90% H_2O (total volume 740 μL) at 310 K for 10 h; then TSP (2 μL of a 50 mM solution in D_2O) was added. The mixtures were filtered into NMR tubes under Ar. The final Ru concentration was 2.5 mM. The pH of solution was 6.2 for complex **7**, and 7.1 for complex **10**.

5.2.4 Methods and Instrumentation

X-ray Crystallography. All diffraction data were collected using a Bruker (Siemens) Smart Apex ccd diffractometer equipped with an Oxford Cryosystems low-temperature device operating at 150 K. Absorption corrections for all data sets were performed with the multi-scan procedure SADABS;³¹ structures were solved using either Patterson or direct methods (SHELXTL³² or DIRDIF³³); all except complex **5** were refined against F^2 using SHELXTL. For complex **2**, all H-atoms were located in a difference map and refined freely. In other structures these atoms were placed in calculated positions, except those attached to nitrogen, which were refined freely. The difference in the strategy for H-atom treatment adopted for **2** was as a result of the rather unexpected non-planarity of the hydrocarbon ligand: the H-

atom positions were refined without difficulty, which gave confidence in the quality of the data set and the correctness of the model presented. We confirmed these structural features by repeating the structure determination of this compound using a different sample. All non-H atoms in all structures were modelled with anisotropic displacement parameters. In **4**, one of the crystallographically-independent PF_6^- anions is disordered in an approximately 50:50 ratio about a common FPF axis. Similarity restraints were applied to the geometry and the displacement parameters for this ion.

The crystal structure of **5** was complicated by both twinning and pseudosymmetry effects. Several crystals were investigated, and all showed significant multiplicity. The diffraction patterns were indexed using the program GEMINI,³⁴ and a sphere of data was collected. An absorption correction was applied (assuming point group 1) by the multi-scan procedure SADABS. The structure solved easily by direct methods, yielding the positions of a pair of centrosymmetrically-related $[\text{Ru}(\text{C}_{12}\text{H}_{10})(\text{en})(\text{Guo})]$ fragments and four PF_6^- anions, again in two centrosymmetrically-related pairs. A rather noisy region of electron density was observed next to the N9 atoms of the guanine rings. The ribose sugar was used in a chirally-pure form in the synthesis of this material, and so the space group P-1 is physically impossible. The true space group must be P1, but the difficulty in resolving the ribose fragments can be ascribed to the dominant effect on the phases of the centrosymmetric part of the structure. Two ribose fragments were eventually picked out of difference maps by ensuring that only peaks which corresponded to the known enantiomer of the sugar were selected. Further modelling revealed that while one pair of PF_6^- anions (based on P1 and P3) conform closely to inversion symmetry, the other pair differ in orientation. At this stage, further difference peaks were assigned to water of crystallisation, to give a conventional R-factor of 10%. It was clear at this stage that the centrosymmetric sub-structure could

be constrained by applying the inversion relationship as part of the model. The advantage of this procedure is that it effectively cures ill-conditioning of the least squares and minimises the number of parameters which need be refined against the rather weak data set obtained.³⁵ A refinement of this type was set up using the program CRYSTALS³⁶ for both fractional coordinates and displacement parameters. The ribose sugars, the PF₆⁻ anions based on P2 and P4, and the water molecules were all refined independently. Analysis of poorly-fitting data at this stage revealed that most for which $F_o > F_c$ had $h = 0$; the program ROTAX³⁷ was used to identify the likely cause as twinning via a 2-fold rotation about the direct a -axis, which can be expressed by the matrix

$$\begin{pmatrix} 1 & 0 & 0 \\ -0.045 & -1 & 0 \\ -0.200 & 0 & -1 \end{pmatrix}.$$

This was confirmed by re-analysis of the diffraction pattern using GEMINI. This is clearly not a symmetry operation of the lattice, and it accounts for the difficulty encountered in indexing the diffraction pattern prior to data collection. A recently introduced feature of CRYSTALS allows this kind of twin matrix to be input directly without the need to split overlapping data manually. H-atoms attached to N and C were placed by conventional methods and reidealised at the end of each refinement cycle. Coordinates for H-atoms attached to oxygen in the OH groups and water molecules were calculated using the program CALC-OH,³⁸ and thereafter allowed to ride on their parent atoms. Refinement was performed against F using 6014 data with $F > 4\sigma(F)$ and Chebychev polynomial weights.³⁹ The final R-factor was 6.65% ($R_w = 6.95\%$) for 665 parameters. The final difference map showed no feature outwith $\pm 1.8 \text{ e}\text{\AA}^{-3}$. The twin scale factors refined to 0.66 and 0.44; the Flack parameter was not refined, but set at zero to avoid the need to model both non-

merohedral and racemic twinning; the known chirality of the sugar justifies this course of action.

In the Tables of H-bonding data, esd's are given in cases where parameters have been refined.

NMR Spectroscopy. ^1H NMR spectra were acquired on either Bruker DMX 500 (^1H = 500 MHz) or Varian Unity INOVA (^1H = 600 MHz) NMR spectrometers using TBI [^1H , ^{13}C , X] or triple resonance [^1H , ^{13}C , ^{15}N] probe-heads, respectively, and equipped with z-field gradients.

1D ^1H NMR spectra were typically acquired with 64 transients into 32 K data points over a spectral width of 6.0 kHz using a double pulsed-field gradient spin-echo pulse sequence. Typically, 2D [^1H , ^1H] NOESY NMR spectra were acquired with 32 transients for each of 1024 t_1 increments (TPPI). The mixing time was 1 s, and the relaxation delay was 2 s. 2D [^1H , ^1H] COSY and DQFCOSY spectra were acquired with 32 transients for each of 512 t_1 increments (States or TPPI). Water suppression was achieved by presaturation or by using a WATERGATE pulsed-field-gradient sequence.⁴⁰

Both 1D ^{15}N -edited ^1H NMR spectra and 2D [^1H , ^{15}N] HSQC NMR spectra (optimised for $^1J_{\text{NH}} = 73$ Hz) were acquired using the sequence of Stonehouse *et al.*⁴¹ A GARP decoupling sequence was used for ^{15}N decoupling during acquisition. Typically, 1D ^1H - $\{^{15}\text{N}\}$ data were acquired with 16 transients into 1024 data points over a spectral width of 2.5 kHz (5.0 ppm) with a relaxation delay of 2.0 s. 2D [^1H , ^{15}N] HSQC NMR data were acquired with 4 transients into 1024 data points over a ^1H (F2) spectral width of 2.5 kHz (5 ppm, centred at the water resonance) for each of 256 t_1 increments (TPPI), and over a ^{15}N (F1) spectral width of 0.76 kHz (15 ppm, centre at -28 ppm) using a relaxation delay of 1.4 s between transients. Typically, 1D

$^1\text{H}\{-^{15}\text{N}\}$ spectra were acquired over a 10 min period and 2D [^1H , ^{15}N] NMR HSQC spectra were acquired over a 30 min period.

^{31}P NMR spectra were obtained on either Bruker DMX 500 (^{31}P = 202 MHz) or Bruker DMX 360 (^{31}P = 146 MHz) NMR spectrometers at 298 K, using inverse-gated ^1H decoupling. Typical 1D $^{31}\text{P}\{-^1\text{H}\}$ NMR spectra at 202 MHz were acquired with 512 transients into 42 K data points over a spectral width of 40.7 kHz (200.8 ppm) using a relaxation delay of 1.3 s. Typical 1D $^{31}\text{P}\{-^1\text{H}\}$ NMR spectra at 146 MHz were acquired with 256 transients into 32 K data points over a spectral width of 14.6 kHz (100.28 ppm) using a relaxation delay of 0.5 s.

All data processing was carried out using XWIN-NMR version 2.0 (Bruker U.K. Ltd.). ^1H NMR chemical shifts were internally referenced to 1, 4-dioxane (3.77 ppm) or to the methyl singlet of TSP (0 ppm) in aqueous solutions, or to $\text{CD}_2\text{HCOCD}_3$ (2.06 ppm) in acetone- d_6 solution. ^{15}N resonances were referenced to 1 M $^{15}\text{NH}_4\text{Cl}$ in 1.5 M HCl (external) at 0 ppm, and ^{31}P resonances to 85% H_3PO_4 (external) at 0 ppm.

pH Measurement. The pH values of NMR samples in 10% D_2O / 90% H_2O were measured at ca. 298 K directly in the NMR tube, before and after recording NMR spectra, using a Corning 240 pH meter equipped with an Aldrich micro combination electrode calibrated with Aldrich buffer solutions at pH 4, 7, and 10. The pH values of samples were adjusted with dilute solutions of HCl and NaOH. No correction has been applied for the effect of deuterium on the glass electrode.

Calculations of pK_a values. The pH titration curves were fitted to the Henderson-Hasselbalch equation using the program KALEIDAGRAPH,⁴² with the assumption that the observed chemical shifts are weighted averages according to the populations of the protonated and deprotonated species.

5.3 Results

5.3.1 General

The molecular structures of the parent chloro anticancer complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$, where arene = biphenyl (Bip, complex **1**), **5**, **8**, **9**, **10**-tetrahydroanthracene (THA, **2**) or **9**, **10**-dihydroanthracene (DHA, **3**) (Chart 5.1) and those of their 9EtG, Guo and 5'-GMP adducts were been studied, where possible both in the solid state and in solution (Table 5.1). The X-ray structure of complex **1** has been reported previously⁷ and those of **2** and **3** are reported here. Yellow 9EtG and Guo adducts of $\{(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\}^{2+}$ were successfully crystallized from MeOH and H₂O, respectively. The structures of the chloro complex **1** and its 9EtG, Guo and 5'-GMP adducts (**4**, **5** and **8**) in solution were studied by NMR methods, including 2D [¹H, ¹⁵N] HSQC NMR using ¹⁵N-en complexes, NOE measurements, pH and temperature dependences. For the anthracene derivatives, the 9EtG adducts **6** and **7** were successfully crystallized, but NMR work in solution was limited to 1D ¹H and 2D [¹H, ¹⁵N] NMR studies since broad resonances and slow chemical exchange processes complicated the interpretation of NOESY data.

5.3.2 X-ray Crystal Structures of Biphenyl Complexes

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})][\text{PF}_6]_2 \cdot (\text{MeOH})$ (**4**). In complex **4**, Ru(II) adopts the familiar “three-leg piano-stool” geometry. It is π -bonded to the hexadentate biphenyl ligand and σ -bonded to the N atoms of ethylenediamine and N7 of 9EtG (Figure 5.1). Crystallographic data are listed in Table 5.2, selected bond lengths and angles in Tables 5.3A and 5.3C, and selected H-bonding interactions in Table 5.4A. The propeller twist of the pendent phenyl with respect to the coordinated phenyl of the biphenyl ligand is 13.7°. The long axis of biphenyl is rotated away from the Ru-N7 vector by 136.0° (Table 5.5A) and therefore adopts the *anti* orientation with respect to the purine base. Atom O6 of 9EtG is strongly H-bonded

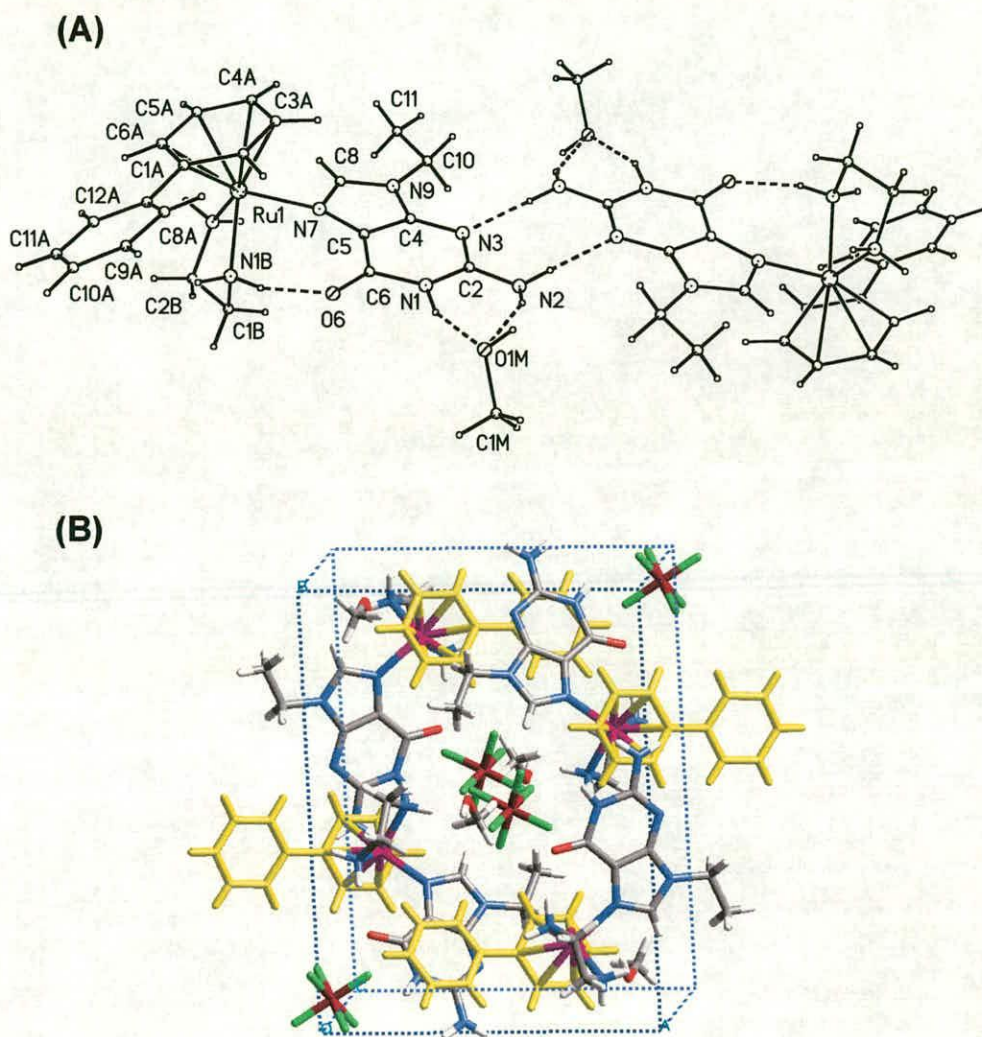


Figure 5.1 X-ray crystal structure of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})][\text{PF}_6]_2 \cdot (\text{MeOH})$ (**4**). (A) The H-bond network of the dimer. The atom numbering scheme is shown for one cation. PF_6^- ions are omitted for clarity. (B) Tetrameric aggregate in the unit cell (as viewed along the c axis). The core of this cyclic tetramer is filled by two PF_6^- ions and two MeOH molecules. The disordered PF_6^- ions are omitted for clarity. Two pendent phenyl rings inside the unit cell stack onto the purine bases of adjacent cations. Color code: Ru purple, biphenyl yellow, N blue, O red, C of G and MeOH gray, H of G and MeOH white, F of PF_6^- green and P of PF_6^- dark red.

Table 5.2 Crystallographic data for $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**), $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**3**), $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot (\text{MeOH})$ (**4**), $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-}N7)][\text{PF}_6]_2 \cdot 3.75(\text{H}_2\text{O})$ (**5**), $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot (\text{MeOH})$ (**6**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot 2(\text{MeOH})$ (**7**)

	2	3	4	5	6	7
Formula	$\text{C}_{16}\text{H}_{22}\text{ClF}_6\text{N}_2\text{PRu}$	$\text{C}_{16}\text{H}_{20}\text{ClF}_6\text{N}_2\text{PRu}$	$\text{C}_{22}\text{H}_{31}\text{F}_{12}\text{N}_7\text{O}_2\text{P}_2\text{Ru}$	$\text{C}_{24}\text{H}_{38.5}\text{F}_{12}\text{N}_7\text{O}_{8.75}\text{P}_2\text{Ru}$	$\text{C}_{24}\text{H}_{35}\text{F}_{12}\text{N}_7\text{O}_2\text{P}_2\text{Ru}$	$\text{C}_{25}\text{H}_{37}\text{F}_{12}\text{N}_7\text{O}_3\text{P}_2\text{Ru}$
Molar mass	523.85	521.83	816.55	956.11	844.60	874.63
Crystal system	Monoclinic	orthorhombic	Monoclinic	Triclinic	Monoclinic	Triclinic
Space group	$\text{P}2_1/\text{c}$	Pbca	$\text{P}2_1/\text{n}$	$\text{P}1$	$\text{P}2_1/\text{n}$	$\text{P}-1$
Colour	Orange	Yellow	Yellow	Yellow	Pale Yellow	Yellow
$a / \text{\AA}$	12.171(3)	11.1220(18)	13.2927(14)	8.850(3)	16.6279(11)	10.6486(17)
$b / \text{\AA}$	8.4733(18)	12.576(2)	15.9583(17)	11.543(4)	9.0528(6)	12.317(2)
$c / \text{\AA}$	18.946(4)	25.593(4)	15.0878(16)	17.556(5)	21.5446(15)	13.006(2)
α / deg	90	90	90	103.254(4)	90	73.957(2)
β / deg	103.854(3)	90	108.613(2)	92.885(4)	99.677(1)	79.483(2)
γ / deg	90	90	90	90.983(4)	90	84.977(2)
T / K	150(2)	150(2)	150(2)	150(2)	150(2)	150(2)
Z	4	8	4	2	4	2
$R [F > 4\sigma(F)]^a$	0.0230	0.0444	0.0895	0.0665	0.0593	0.0291
R_w^b	0.0625	0.1055	0.220	0.0695	0.1391	0.0768
GOF ^c	1.062	1.038	1.222	0.981	1.132	1.035
$\Delta\rho$ max and min / $\text{e}\text{\AA}^{-3}$	+0.722, -0.371	2.328, -2.190	2.00, -1.21	1.76, -1.84	1.12, -0.80	0.56, -0.45

^a $R = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^b $R_w = [\sum w(F_o^2 - F_c^2)^2 / \sum wF_o^2]^{1/2}$. ^c $\text{GOF} = [\sum w(F_o^2 - F_c^2)^2 / (n-p)]^{1/2}$, where n = number of reflections and p = number of parameters.

Table 5.3 Selected Bond Lengths (Å) and Angles (deg) for Complexes 1-7.(A) Bond lengths (Å) for biphenyl complexes **1**, **4** and **5**.

atoms	1 ^a	4	5
Ru1-N1B	2.110(5)	2.104(6)	2.099(6)
Ru1-N2B	2.121(5)	2.126(5)	2.133(6)
Ru1-N7/C11	2.408(15)	2.128(5)	2.120(5)
Ru1-C1A	2.244(6)	2.221(6)	2.240(6)
Ru1-C2A	2.185(6)	2.185(7)	2.181(6)
Ru1-C3A	2.160(6)	2.177(7)	2.158(7)
Ru1-C4A	2.152(5)	2.190(7)	2.194(7)
Ru1-C5A	2.138(5)	2.168(7)	2.171(6)
Ru1-C6A	2.190(5)	2.205(7)	2.158(6)
Ru-(centroid) coord-ring	1.663(3)	1.676(3)	1.667(3)

^a From ref. 7.(B) Bond lengths (Å) for THA complexes **2** and **6** and DHA complexes **3** and **7**.

Atoms	2	6	3	7
Ru1-N1B	2.1243(17)	2.119(3)	2.139(3)	2.1169(17)
Ru1-N2B	2.1307(18)	2.134(3)	2.134(3)	2.1189(16)
Ru1-N7/C11	2.405 (6)	2.128(3)	2.407(9)	2.1173(15)
Ru1-C1A	2.2248(19)	2.221(4)	2.203(3)	2.2344(18)
Ru1-C2A	2.151(2)	2.173(4)	2.166(3)	2.1820(19)
Ru1-C3A	2.169(2)	2.184(4)	2.154(3)	2.1709(19)
Ru1-C4A	2.180(2)	2.169(4)	2.184(3)	2.1408(19)
Ru1-C5A	2.197(2)	2.202(4)	2.181(3)	2.1965(19)
Ru1-C6A	2.2350(19)	2.226(4)	2.192(3)	2.2300(18)
Ru-(centroid) coord. ring	1.6769(10)	1.6831(17)	1.6596(14)	1.6783(9)

(C) Bond angles (deg) for biphenyl complexes **1**, **4** and **5**

	1 ^a	4	5
N1B-Ru1-N2B	79.20 (18)	79.5(2)	79.5(2)
N1B-Ru1-N7/Cl1	85.60 (12)	87.2(2)	86.0(2)
N2B-Ru1-N7/Cl1	82.88 (11)	86.3(2)	86.8(2)

^a Ref. 7.(D) Bond angles (deg) for THA complexes **2** and **6** and DHA complexes **3** and **7**

	2	6	3	7
N1B-Ru1-N2B	78.66(7)	79.22(13)	79.24 (10)	79.36(7)
N1B-Ru1-N7/Cl1	84.59(5)	87.07(12)	85.66 (8)	87.93(6)
N2B-Ru1-N7/Cl1	83.18(6)	84.41(12)	84.48 (8)	84.44(6)

to an en NH proton (H1B1) with a N1B...O6 distance 2.761 Å and N1B-H1B1...O6 angle of 162.9° (Table 5.4A). Guanine-guanine pairing via two H-bonds between the G N2H and the G N3 (N2...N3 3.015 Å, N2-H2X...N3 172.0°, Figure 5.1) is present. In addition, for each molecule, the second G N2 proton (H2Y) together with the G N1 proton (H1X)) forms two H-bonds to the O of MeOH (O1M) (N2...O1M 2.983 Å, N1...O1M 2.844 Å, Figure 5.1). One PF₆⁻ counter anion is beneath the guanine plane and H-bonded to the en NH proton (H2B2) (N2B...F4 2.952 Å); the second PF₆⁻ ion (disordered) is above the guanine plane and its presence prevents intramolecular arene-purine stacking.

In one unit cell, four molecules form a cyclic aggregate when viewed along the *c* axis (see Figure 5.1B). The core of this cyclic tetramer is filled by two PF₆⁻ ions and two solvent CH₃OH molecules. The pendent phenyl ring stacks onto the purine base of an adjacent cation with a centroid-centroid separation of 4.0 Å and a dihedral angle of 4.5° (see space-filling model in Figure 5.2A). Tetramers are stacked on top

Table 5.4 Selected H-bonding Interactions for Complexes **4**, **5**, **6** and **7**. O1M is MeOH oxygen.**(A) Complex 4** $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot (\text{MeOH})$

D-H...A	H...A (Å)	D...A (Å)	$\angle\text{D-H...A}$ (deg)
N1B-H1B1...O6 (intra)	1.868	2.761(8)	162.91
N2-H2X...N3 (inter)	2.123	3.015(9)	171.96
N2-H2Y...O1M	2.168	2.983(12)	151.25
N1-H1X...O1M	2.004	2.844(11)	155.19

(B) Complex 5 $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-}N7)][\text{PF}_6]_2 \cdot 3.75(\text{H}_2\text{O})$ (Cations **I** and **II**)

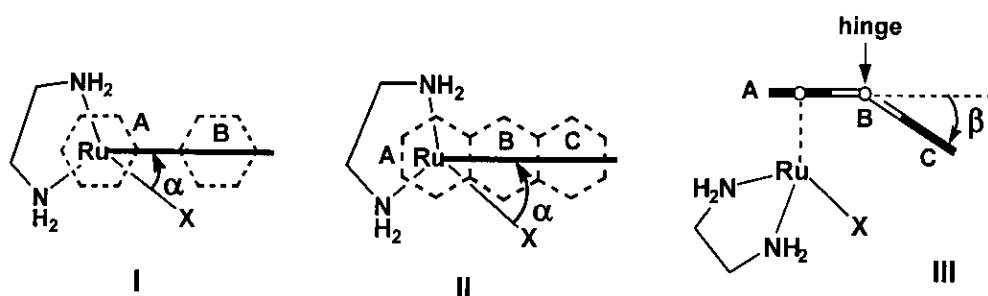
Cation I				Cation II			
D-H...A	H...A (Å)	D...A (Å)	$\angle\text{D-H...A}$ (deg)	D-H...A	H...A (Å)	D...A (Å)	$\angle\text{D-H...A}$ (deg)
N14-H141...O61 (intra)	1.8899	2.799(8)	158.97	N18-H181...O65 (intra)	1.8899	2.799(8)	158.97
N14-H142...O65(II) (inter)	2.5454	3.212(7)	127.28	N18-H182...O61(I) (inter)	2.5454	3.212(7)	127.28
N24-H241...O52	2.7750	3.714(7)	169.28	N28-H281...O69(W)	2.1963	3.083(13)	154.72
N11-H11...O29(W)	1.9772	2.841(16)	150.65	N15-H15...O19(W)	2.0708	2.949(16)	153.63
N21-H212...O29(W)	2.0335	2.861(17)	144.66	N25-H252...O19(W)	2.2352	3.050(16)	143.47
N21-H211...O99(W)	2.2411	3.136(17)	156.44	N25-H251...O32(I) (inter)	1.7916	2.715(11)	162.79

(C) Complex 6 $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot (\text{MeOH})$

D-H...A	H...A (Å)	D...A (Å)	$\angle\text{D-H...A}$ (deg)
N1B-H1B1...O6 (intra)	1.919	2.812(4)	163.30
N2-H2X...N3 (inter)	2.349	3.105(4)	168.07
N2-H2Y...O1M	2.403	3.085(5)	142.04
N1-H1X...O1M	2.161	2.829(6)	160.84

(D) Complex 7 $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot 2(\text{MeOH})$

D-H...A	H...A (Å)	D...A (Å)	$\angle\text{D-H...A}$ (deg)
N1B-H1NB...O6 (intra)	2.081	2.840(2)	163.09
N1B-H1NA...O6 (inter)	2.237	3.031(2)	146.19
N2-H2X...N3 (inter)	2.299	3.085(3)	162.19
N2B-H2NA...O1M	2.112	2.895(3)	162.50

Table 5.5 Comparison of the Conformations of Guanine Adducts and their Parent Chloro-Complexes (see Figures 5.4 and 5.24). X = Cl or N7 of G.**(A) Biphenyl Complexes 1, 4 and 5**

	1 (Cl)	4 (9EtG)	5 (Guo)
Bip orientation, α (I), $[\Delta\alpha]^b$, (deg)	26.8(2)	136.0(3) [+109.2]	13.2(3) [-13.6]
Bip propeller twist, χ^a , $[\Delta\chi]^b$ (deg)	26.2(4)	13.7(5) [-12.5]	17.9(4) [-8.3]
Ring B- G distance (Å) /dihedral angle (deg)	—	4.022(5) / 4.48(5) (Inter) ^c	3.801(4) / 23.8(4) (Intra) ^c 4.199(4) / 23.0(6) (Inter) ^c

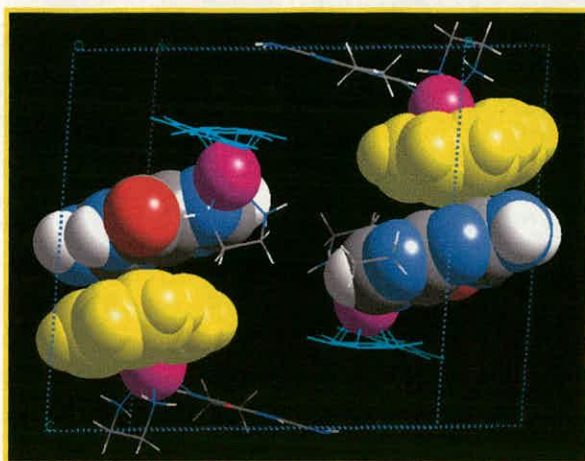
^a χ : twist of pendent phenyl ring (B) with respect to coordinated phenyl (A) of Bip. ^b The change of torsion angles for 4 and 5 compared to 1. ^c Ring B and six-membered ring of G of an adjacent cation. ^d Ring B and five-membered ring of G of the same cation. ^e Ring B with six-membered ring of G of an adjacent cation.

(B) THA Complexes 2 and 6 and DHA Complexes 3 and 7.

	2 (Cl)	6 (9EtG)	3 (Cl)	7 (9EtG)
THA/DHA orientation, α (II), $[\Delta\alpha]^b$, (deg)	45.12(6)	25.62(14) [-19.5]	64.09(8)	20.88(7) [-43.2]
Hinge bending angle, β (III) ^a , $[\Delta\beta]^b$ (deg)	-7.53(12)	+ 27.8(2) [+ 35.33]	+ 40.65(16)	+ 31.95(9) [-8.7]
Ring C - G distance (Å) /dihedral angle (deg)	—	3.447(9) / 3.30(17) (Intra) ^c	—	3.310(12) / 3.07(8) (Intra) ^c

^a The hinge is defined by C7A – C10A, see Figures 5.5 and 5.6. A + ve sign for β indicates movement towards Ru-X. ^b The change of torsion angles for 6 and 7 compared to 2 and 3, respectively. ^c Ring C and purine ring of G of the same cation.

(A) Complex 4



(B) Complex 5

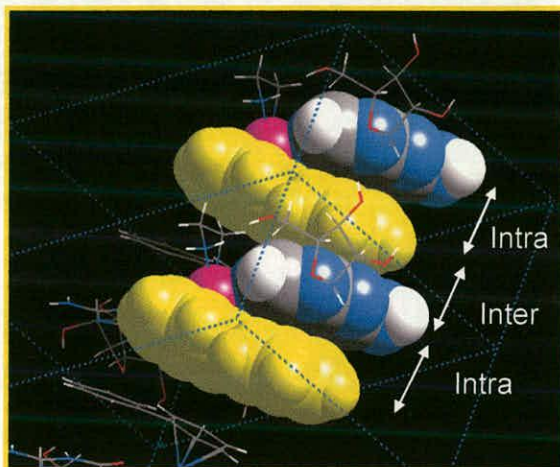


Figure 5.2 Space-filling models of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (**4**) and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (**5**). Colour code: phenyl ring yellow, Ru purple, O red, N blue, H white, C of G gray. Other parts of molecules not involved in this stacking are shown as stick models. (A) Stacking in complex **4** along the *c* axis: intermolecular stacking between pendant phenyl ring (yellow) and purine six-membered ring (centroid-centroid distance 4.0 Å, dihedral angle 4.5°). (B) Stacking in complex **5** along the *a* axis: intramolecular stacking between pendant phenyl ring and purine five-membered ring (centroid-centroid distance 3.8 Å, dihedral angle 23.8°), and intermolecular stacking between six-membered purine ring and pendant phenyl ring of an adjacent cation (centroid-centroid distance 4.2 Å, dihedral angle 23.0°).

of one another along the *c* axis. In the same layer, tetramers are connected by four sets of base pairs via H-bonds N2-H2X...N3 along *a* and *b* axes. Figure 5.1A shows base-pairing along the *b* axis.

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})][\text{PF}_6]_2 \cdot 3.75\text{H}_2\text{O}$ (5) crystallizes with two independent $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ cations (**I** and **II**, Figure 5.3) in the asymmetric unit, which also contains four PF_6^- counterions and nine water molecules (three with half-occupancy). The structures of these two cations differ significantly only in the orientation of the ribose ring. Selected bond lengths and angles are listed

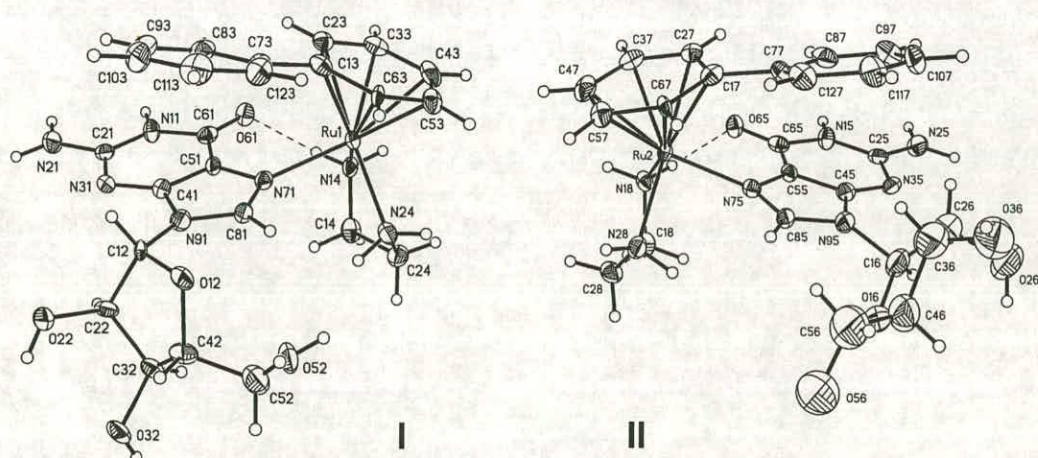


Figure 5.3 Two independent cations (**I** and **II**) of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ in the asymmetric unit of the X-ray structure of complex **5**, showing the atom numbering scheme and 30% probability thermal ellipsoids. H-bonding interactions of en NH with G O6 are indicated by dotted lines.

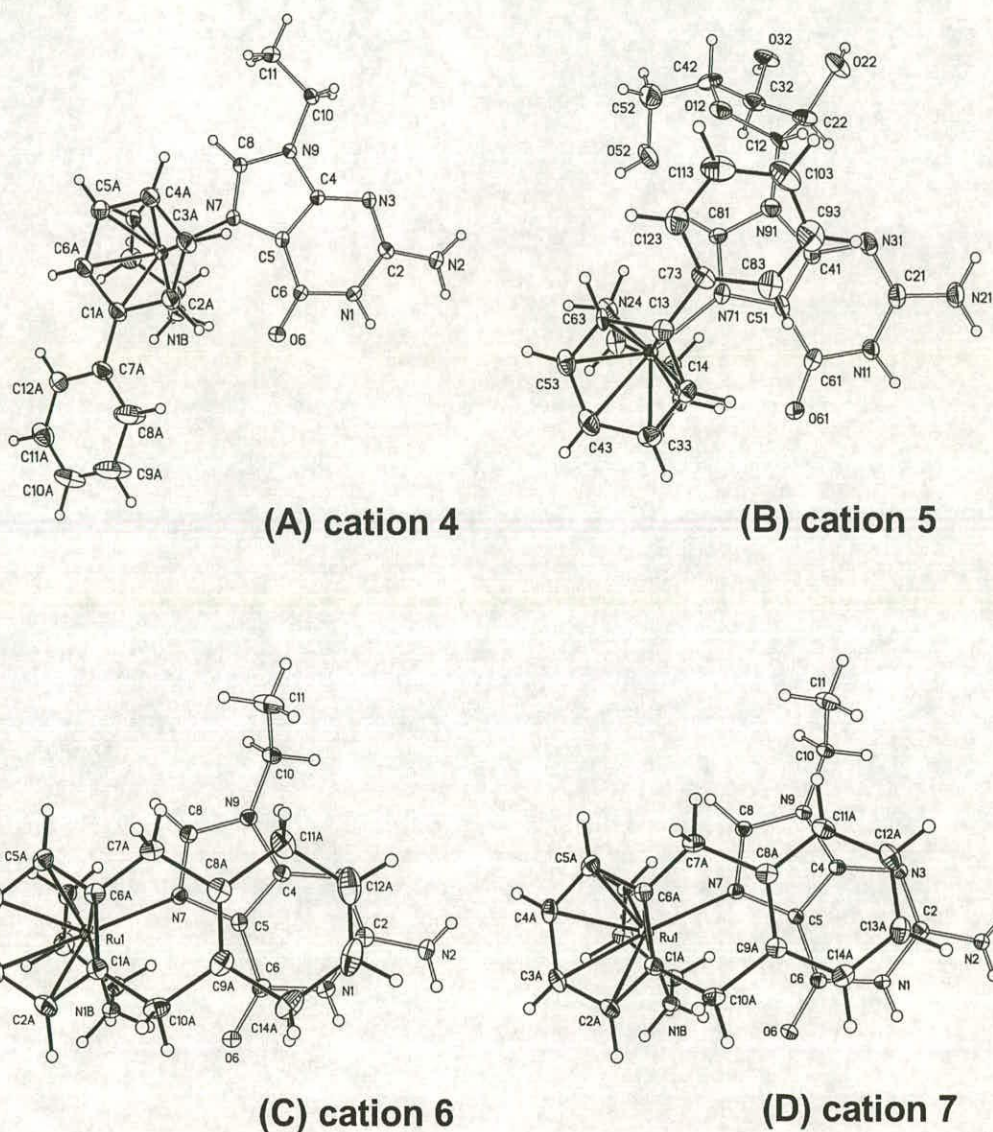


Figure 5.4 Projections of the structures of cations. (A) $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (**4**), (B) $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (**5**), (C) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (**6**), and (D) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (**7**) (30% probability thermal ellipsoids), showing arene (heavy line)-guanine base (light line) stacking in (B), (C) and (D) with the arene rings *syn* to G bases, whereas in (A), the arene ring is *anti* to the G base.

Table 5.6 Characterisation of the Ribose Ring Conformations (**I** and **II**) in Crystalline $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})][\text{PF}_6]_2 \cdot 3.75(\text{H}_2\text{O})$ (**5**).

Parameter	Torsion angle (deg)	I (Ru1)	II (Ru2)
χ_{CN}	O1'-C1'-N9-C8	6.09 (9)	-11.03 (11)
	O1'-C1'-N9-C4	174.48 (6)	147.82 (6)
Φ_{OO}	O1'-C4'-C5'-O5'	-70.67 (7)	51.53 (7)
Φ_{OC}	C3'-C4'-C5'-O5'	48.79 (7)	169.33 (5)
ν_0	C4'-O1'-C1'-C2'	12.20 (7)	11.68 (7)
ν_1	O1'-C1'-C2'-C3'	-28.18 (6)	-30.54 (7)
ν_2	C1'-C2'-C3'-C4'	33.59 (6)	35.91 (6)
ν_3	C2'-C3'-C4'-O1'	-26.72 (7)	-29.50 (7)
ν_4	C3'-C4'-O1'-C1'	-9.62 (7)	-11.11 (7)
P		-12.69	-12.17
Φ_m		-12.505	-11.948

^aTorsion angles $\nu_0 - \nu_4$ and the pseudorotational parameters P and Φ_m are defined according to the conventions of Ref. 43. ^bTheoretical: C2'-endo (S type) $P = 144 \sim 190^\circ$, C3'-endo (N-type) $P = 0 \sim 36^\circ$, C2'-exo/C3'-endo $P = -36^\circ \sim 0$.

in Tables 5.3A and 5.3C. The torsion angles $\nu_0 - \nu_4$ and the pseudorotational parameters P and ϕ_m for ribose ring characterize the ring conformation⁴³ and are listed in Table 5.6. In both these cations, the sugar puckers take the N- type (C2'-exo/C3'-endo) conformation ($P = -12.69^\circ$ for cation **I** and -12.17° for cation **II**).

The long axis of the biphenyl ligand is rotated away from the Ru-N7 vector by 13.2° . The propeller twist of biphenyl is 17.9° (Table 5.5A). When viewed normal to the plane of the purine base (Figure 5.4B), there is overlap of the pendent phenyl ring with the purine five-membered ring (distance 3.8 Å, dihedral angle 23.8° , Table 5.5A). The six-membered ring of the same purine stacks with a pendent phenyl ring of an adjacent cation (distance 4.2 Å, dihedral angle 23.0° , Table 5.5A). Thus the

pendent phenyl ring is stacked alternately with the purine five-membered and six-membered rings to form a columnar-type structure (as viewed down *c*, see Figure 5.2B).

Table 5.7 Possible Hydrogen Bonding Interactions^a Involving Water^c or Sugar Oxygens of [(η⁶-biphenyl)Ru(en)(Guo-*N*7)][PF₆]₂•3.75(H₂O) (**5**).

interaction	distance (Å)	interaction	distance (Å)
O22(I) ^b ...N35(II)	2.84(1)	O26(II)···O16(II)	3.01(1)
O32(I)···O69(W)	3.08(2)	O36(II)···O59(W)	2.70(2)
O52(I)···F19	3.08(2)	O56(II)···O16(II)	2.72(2)
O29(W)···F15	2.87(2)	O19(W)···F3	2.85(1)
O29(W)···N21(I)	2.86(1)	O19(W)···F11	3.15(2)
O29(W)···F20	3.22(2)	O19(W)···F16	3.09(2)
O39(W)···F24	3.21(1)	O49(W)···F8	3.25(1)
O39(W)···O89(W)	3.17(4)	O49(W)···F9	2.83(2)
O39(W)···O52(I)	2.89(1)	O59(W)···O99(W)	2.77(2)
O79(W)···F10	3.14(2)	O59(W)···O22(I)	2.88(2)
O79(W)···F12	3.26(2)	O69(W)···O56(II)	2.88(2)
O79(W)···O32(I)	2.55(2)	O69(W)···O49(W)	2.77(1)
O89(W)···O12(I)	2.81(3)	O69(W)···F7	3.20(1)
O99(W)···O16(II)	2.79(2)	O69(W)···F8	3.28(1)
O99(W)···O52(I)	2.76(2)		

^a A generous maximum donor···acceptor distance of 3.30 Å is used as the cut-off.

^b Parentheses after atom labels designate oxygen from cation I or II or from water(W). ^c Three water molecules (O39, O79 and O89) are at half-occupancy.

Selected H-bonds for **5** are listed in Table 5.4B. For cations **I** and **II**, there is strong intramolecular H-bonding between an en NH and G O6, N14···O61(I) (N18···O65(II)) distance of 2.799 Å, and N14-H141···O61(I) (N18-H181···O65(II))

angle of 159.0° . Intermolecular base-pairing between guanine bases of **5** is absent. Instead, N2H, N3 and N1H of Guo are H-bonded to PF_6^- and H_2O . Most of the water molecules and PF_6^- ions are within H-bonding distances to the NH groups of en or O(5'), O(3'), O(2') and O(1') atoms of the ribose ring, and thus form a complicated H-bonding network (possible H-bonding interactions involving water molecules and ribose are listed in Table 5.7).

5.3.3 X-ray Crystal Structures of THA and DHA Complexes

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**3**).

The structures of the cations of **2** and **3** are shown in Figure 5.5. The crystallographic data are listed in Table 5.2, and selected bond lengths and angles in Tables 5.3B and 5.3D. In the THA ($\text{C}_{14}\text{H}_{14}$) complex **2**, the tricyclic ring system is nearly flat. The outer ring (C) is slightly bent up away from the Cl ligand about the C7A-C10A hinge by 7.5° . The long axis through the THA ligand is rotated away from the Ru-Cl

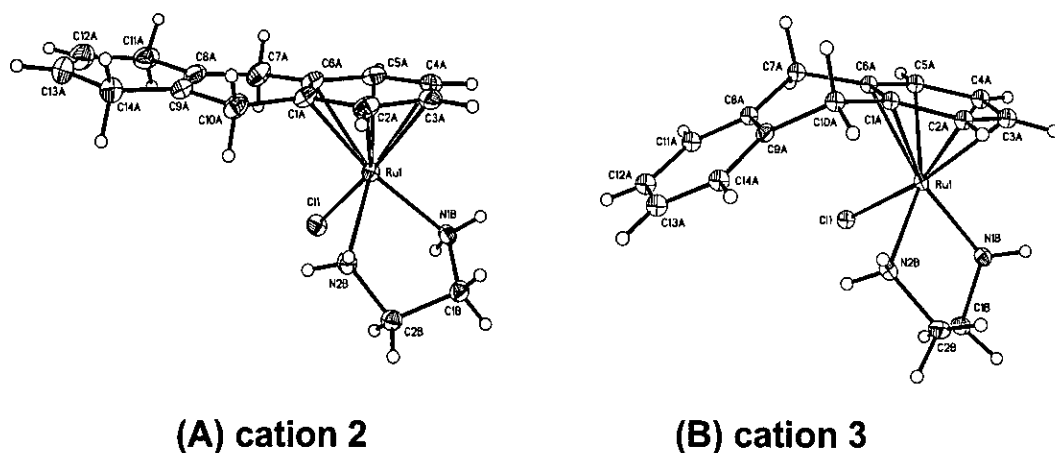


Figure 5.5 X-ray structures and atom numbering schemes for (A) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}]^+$ in complex **2**, and (B) $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}]^+$ in complex **3**, at 30% probability thermal ellipsoids.

vector by 45.1° (Table 5.5B). In the DHA ($C_{14}H_{12}$) complex **3**, the tricyclic ring system deviates significantly from planarity. The outer ring (C) is bent down toward the Cl ligand about the C7A-C10A hinge by 40.6° . The long axis of DHA is rotated away from the Ru-Cl vector by 64.1° (Table 5.5B). In both complexes, there are H-bonding interactions between NH protons of en and F atoms of PF_6^- ions. No intermolecular arene ring stacking is observed for either complex.

$[(\eta^6-C_{14}H_{14})Ru(en)(9EtG-N7)][PF_6]_2 \cdot (MeOH)$ (6) and $[(\eta^6-C_{14}H_{12})Ru(en)(9EtG-N7)][PF_6]_2 \cdot 2(MeOH)$ (7). Both of these crystal structures show the presence of strong intramolecular π - π arene-nucleobase stacking. As can be seen in Figure 5.6, the outer ring (C) of THA / DHA lies directly over the purine base (see also Figures 5.4C and 5.4D). The centroid-centroid separation between ring C and the purine ring is 3.45 Å for **6** and 3.31 Å for **7**, with dihedral angles of 3.3° and 3.1° , respectively (Table 5.5B). Ring C is bent down towards the purine about the C7A-C10A hinge by 27.8° in **6**, and by 31.9° in **7**. The long axes of THA (**6**) and DHA (**7**) are rotated away from the Ru-N7 vectors by 25.6° and 20.9° , respectively. There is intramolecular H-bonding between en NH and G O6 (N1B $\cdots$ O6 2.812 Å and N1B-H1B1 $\cdots$ O6 163.3° for **6**, N1B $\cdots$ O6 2.840 Å and N1B-H1NB $\cdots$ O6 163.1° for **7**, Tables 5.4C and 5.4D). Additionally for **7**, the second H atom (H1NA) on the same en N1B is H-bonded to O6 of a neighbouring G base (Figure 5.8) with N1B $\cdots$ O6 distance of 3.031 Å. For both structures, dimers are formed via a pair of H-bonds between G N2H and G N3 (N2 $\cdots$ N3 3.105 Å for **6**, 3.085 Å for **7**). For **6**, the second G N2H proton (H2Y) together with G N1H proton (H1X) form two H-bonds to O of MeOH (N2 $\cdots$ O1M 3.085 Å, N1 $\cdots$ O1M 2.829 Å, Figure 5.7). No intermolecular arene-base ring stacking is observed for complexes **6** or **7**.

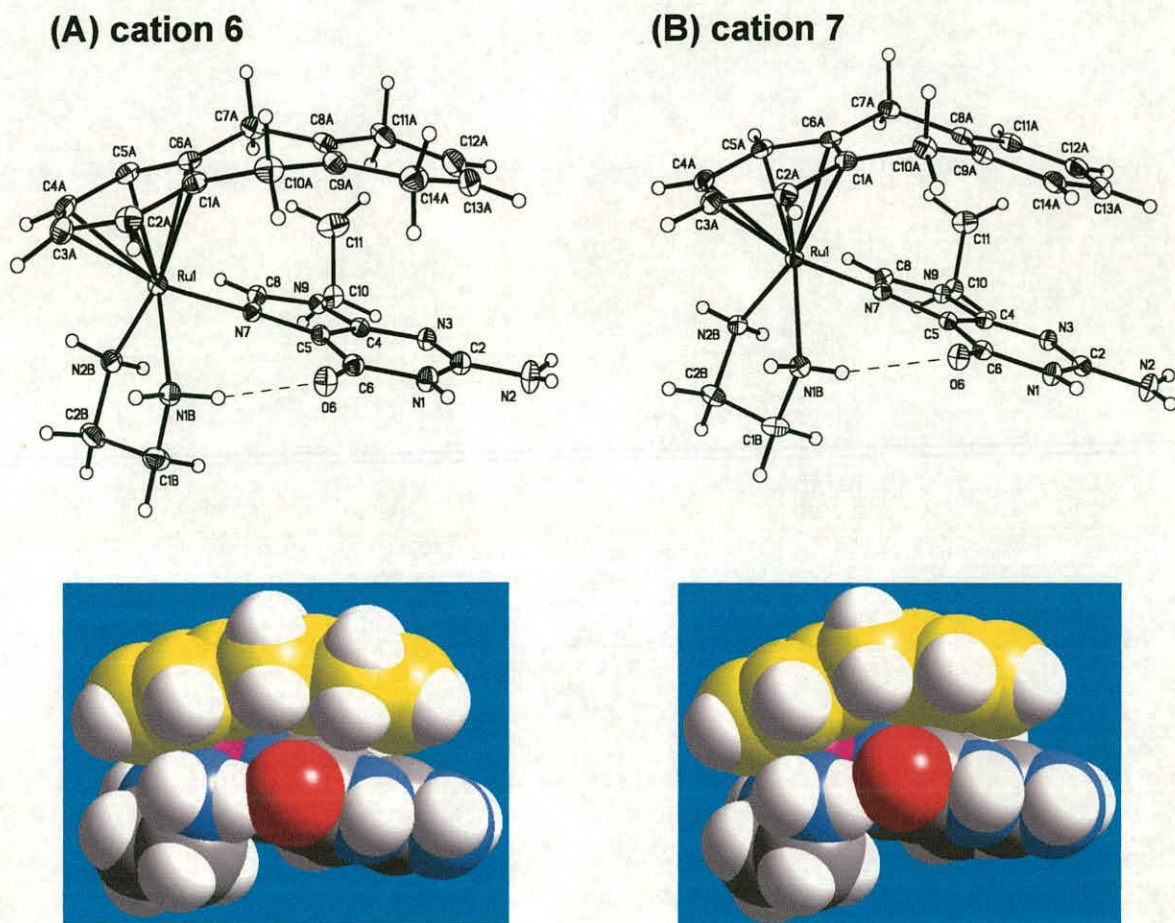


Figure 5.6 X-ray structures and atom numbering schemes for (A) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N}7)]^{2+}$ in complex **6**, and (B) $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-N}7)]^{2+}$ in complex **7**, at 30% probability thermal ellipsoids. The space-filling models clearly show the intramolecular arene-guanine base stacking and H-bonding interactions between en NH and G O6. Color code: C of $\text{C}_{14}\text{H}_{14}$ and $\text{C}_{14}\text{H}_{12}$ yellow, C of 9EtG and en gray, Ru purple, O red, N blue, and H white.

5.3.4 NMR Structures of Biphenyl Complexes in Solution

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (1) in $\text{DMF-}d_6$. The ^1H NMR spectrum of complex **1** at 298 K was assigned using 2D COSY (Figure 5.9) and NOESY ^1H NMR spectroscopy (Figure 5.10). The aromatic resonances are divided into two groups: the high-field resonances (δ 6.28, 6.08 and 5.98) corresponding to the protons H(o), H(p) and H(m) of the coordinated phenyl ring (A), and the low-field resonances (δ 7.86 and 7.52) corresponding to the protons H(o'), H(m') and H(p') of the pendent phenyl ring (B) (H(m') and H(p') overlap at δ 7.52). The strong NOE

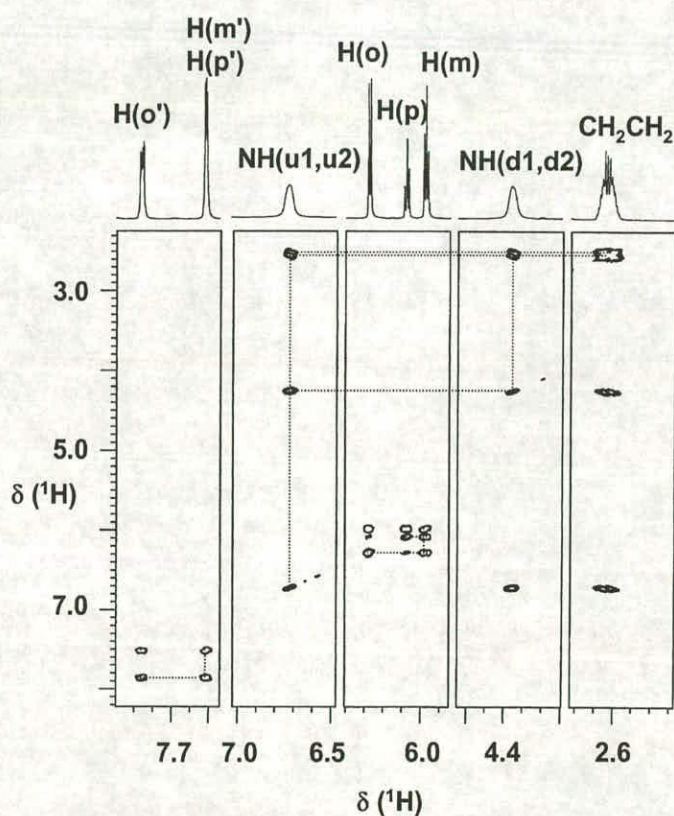


Figure 5.9 2D COSY ^1H NMR spectrum of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**1**) in $\text{DMF-}d_6$ at 298 K showing assignment of arene and en resonances. For structure, see Figure 5.10.

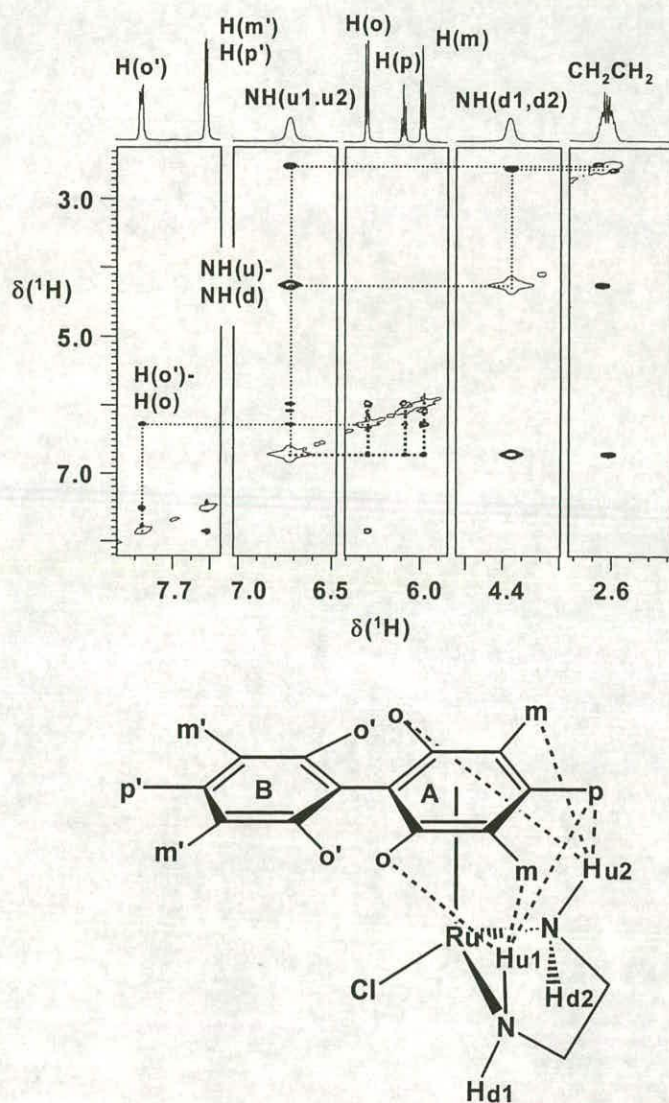


Figure 5.10 2D NOESY ^1H NMR spectrum of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}]^+$ (1) in $\text{DMF-}d_6$ at 298 K. Bottom: structure with inter-ligand NOEs indicated by dotted lines.

between H(o') and H(o) is in accordance with the distance of 2.2 Å determined for the X-ray crystal structure of **1**.⁷ Only one resonance is observed for the ortho protons and one for the meta protons.

The four en NH protons give rise to two broad resonances at δ 6.72 and 4.26. These are assigned to the two types of protons on the same N: NH(u1, u2) (δ 6.72 which point up towards ring A, and NH(d1, d2) (δ 4.26) which point down away from ring A.⁴⁴ NH(u1, u2) show NOEs to H(o), H(m), and H(p) of ring A with medium intensity compared with the NOE of NH(u)-NH(d), whilst the NOEs between NH(d1, d2) and the ring A protons are very weak. These NOE intensities ($\propto 1/r^6$) are in accordance with the crystal structure of **1**⁷ in which the biphenyl ligand is in the *syn* orientation with respect to the Cl ligand ($\alpha = 26.8^\circ$, see Table 5.5A and Figure 5.24). The average distances of NH(u1, u2) protons to H(o), H(m) and H(p) are 3.65 Å, 3.54 Å and 2.71 Å, respectively, whilst the average distances of NH(d1, d2) protons to H(o), H(m) and H(p) are longer, 3.84 Å, 3.71 Å and 4.41 Å, respectively.

[(η^6 -biphenyl)Ru(en)(9EtG-N7)][PF₆]₂•(MeOH) (4**) in Acetone-*d*₆**

Crystalline complex **4** is poorly soluble in water and therefore [¹H, ¹H] 2D NMR spectra were acquired for acetone-*d*₆ solutions. ¹H NMR signal assignments were obtained from 2D DQFCOSY (Figure 5.11) and NOESY (Figure 5.12) ¹H NMR data. Compared to the parent chloro-complex **1** in DMF-*d*₆, the signal for the NH(d1, d2) protons of **4** was surprisingly broad and low-field shifted to δ 5.34 whereas the signal for NH(u1, u2) protons at δ 6.49 was relatively sharp. These assignments for en-NH protons were confirmed by the 2D [¹H, ¹⁵N] HSQC NMR spectrum of ¹⁵N-**4** in acetone-*d*₆ solution. Correlations between NH(u1, u2) and en CH₂CH₂ were observed in the DQFCOSY NMR spectrum (Figure 5.11), but cross-peaks for

NH(d1, d2) correlations with en CH₂CH₂ were too broad to detect. The NOE cross-peak between NH(d1, d2) and NH(u1, u2) was broad, but observable. NOEs were observed between the ethyl protons of 9EtG and H(m'), H(o') and H(p') of ring B (Figure 5.12B). These, together with NOEs between H8 and H(o')/H(o), and between

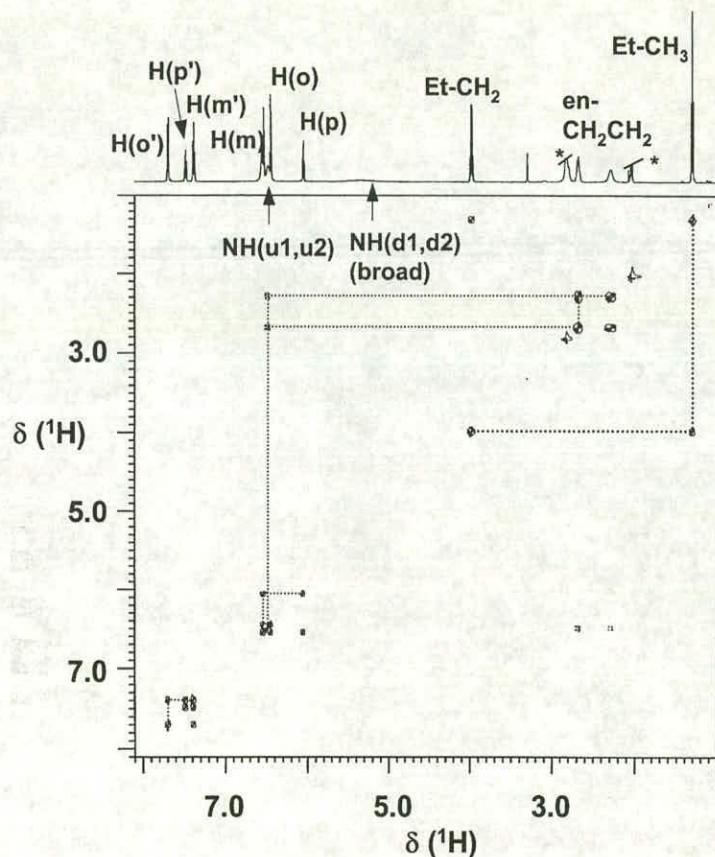
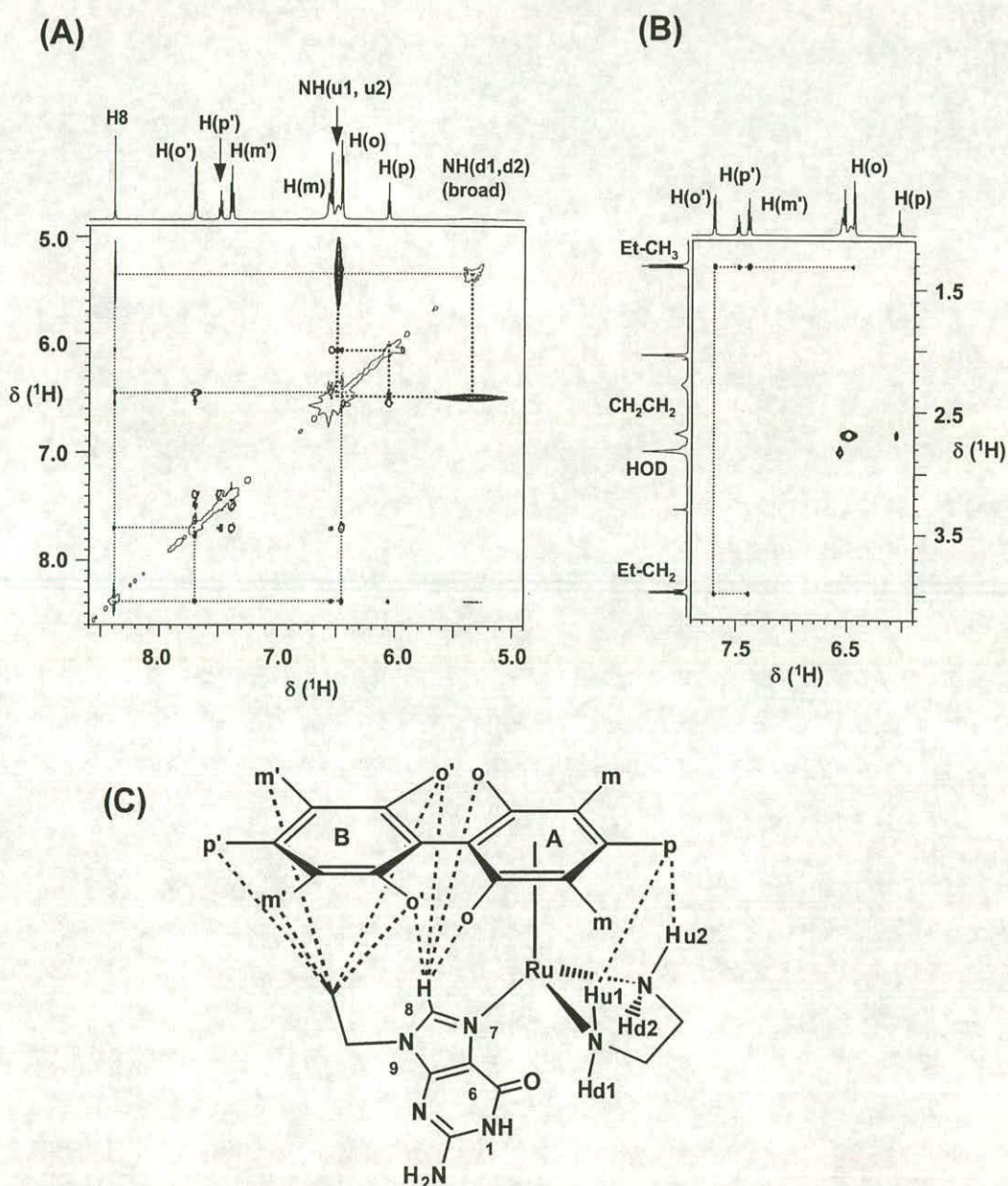


Figure 5.11 2D DQFCOSY ¹H NMR spectrum of [(η⁶-biphenyl)Ru(en)(9EtG-N7)]²⁺ (**4**) in acetone-*d*₆ at 298 K, showing assignment of arene and en resonances. The resonance for NH(d) is very broad but can be detected in the 2D [¹H, ¹⁵N] HSQC NMR spectrum of ¹⁵N-**4**. (* = solvent). For structure, see Figure 5.12.



NH(u1, u2) and H(p) (Figure 5.12A), indicate that a *syn* conformation predominates for the pendent phenyl ring (B) with respect to the G base. Also the presence of NOEs between H8 and NH(d1, d2) (broad) and the absence of NOE cross-peaks between H8 and NH(u1, u2) suggests that H8 of 9EtG points towards NH(d1, d2). The cross-peaks NH(d1, d2) in the 2D [^1H , ^{15}N] HSQC NMR spectrum of ^{15}N -4 in DMSO- d_6 solution sharpened as the temperature was raised from 295 to 310 K, Table 5.10.

$[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (5) in Aqueous Solution. 2D

NOESY and DQFCOSY ^1H NMR spectra of complex 5 in 10% D_2O / 90% H_2O at

Table 5.8 Selected Proton Chemical Shifts (ppm) for Cations $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})\text{Cl}]^+$ (1), $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ (1a) and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (4) and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (5) and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (8).

Com- plex	δ (^1H)								
	H(o')	H(m')	H(p')	H(o)	H(m)	H(p)	NH (u)	NH(d)	H8
1 ^a	7.86	7.52	7.52	6.28	5.98	6.09	6.72	4.26	—
1 ^b	7.79	7.59	7.59	6.17	5.98	5.90	6.02	3.98	—
1a ^b	7.82	7.63	7.63	6.21	6.09	5.93	6.08	4.14	—
4 ^c	7.70	7.39	7.48	6.45	6.54	6.06		5.34	
($\Delta\delta$) ^e	(-0.12)	(-0.24)	(-0.15)	(0.24)	(0.45)	(0.13)	6.49	(broad)	8.38
4 ^b	7.46	7.30	7.46	6.30	6.40	5.72			
($\Delta\delta$) ^e	(- 0.36)	(- 0.33)	(- 0.17)	(0.09)	(0.31)	(- 0.21)	6.53	broad ^f	8.10
5 ^b	7.49	7.34	7.48	6.30	6.41	5.74	6.52 (u1)		
($\Delta\delta$) ^e	(- 0.33)	(- 0.29)	(- 0.15)	(0.09)	(0.32)	(- 0.19)	6.56 (u2)	broad ^f	8.37
8 ^d	7.48	7.31	7.45	6.36	6.46	5.68	6.32 (u1)	5.26 (d1)	
($\Delta\delta$) ^e	(- 0.34)	(- 0.32)	(- 0.18)	(0.15)	(0.37)	(- 0.25)	6.36 (u2)	5.11 (d2)	8.71

^a DMF- d_6 , 298 K; ^b 10% D_2O / 90% H_2O , 298 K; ^c acetone- d_6 , 298 K; ^d 10% D_2O / 90% H_2O , 310 K, ^e $\Delta\delta = \delta(\text{G}) - \delta(1\text{a})$, change in shift upon species 1a binding to G base. ^f too broad to observe.

Table 5.9 ^1H NMR Chemical Shifts of the Non-exchangeable Protons of Guanosine, $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-}N7)][\text{PF}_6]_2$ (**5**), 5'-GMP and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-}N7)]$ (**8**) in 10% D_2O / 90% H_2O .

proton	δ (^1H)			
	Guanosine ^a	Complex 5	5'-GMP ^b	Complex 8
H8	7.97	8.36	8.20	8.71
H1'	5.88	5.77	5.92	5.84
	(6.05) ^c	(4.49) ^c	(6.10) ^c	(5.78) ^c
H2'	4.70	4.38	4.70	4.64
H3'	4.40	4.24	4.50	4.41
H4'	4.20	4.21	4.30	4.30
H5'/H5''	3.89/3.82	3.96/3.84	4.0/3.96	4.1/4.0

^aBased on the excess guanosine in reaction mixture for **5** at 298 K. ^bBased on the excess 5'-GMP in reaction mixture for **8** at 310 K. ^c $^3J(\text{H1'}/\text{H2'})$ in Hz.

298 K were used to assign the proton resonances (Tables 5.8 and 5.9). In contrast to the parent chloro-complex **1** in $\text{DMF-}d_6$, the en NH(d1, d2) signals for **5** were too broad to detect at ambient temperature, but the NH(u) signals were reasonably sharp (as for **4**). The 2D [^1H , ^{15}N] HSQC NMR spectrum for $^{15}\text{N-5}$ at 298 K contained two cross-peaks for NH(u1, u2) at δ 6.51/-29.38 and δ 6.55/-28.87, but no cross-peaks for NH(d1, d2) (see Table 5.10). When the temperature was raised to 328 K, broad peaks for NH(d1, d2) appeared. At this temperature, the four cross-peaks at δ 6.45/-29.1, 4.98/-29.1, 6.48/-28.62, and 4.65/-28.62, can be assigned to NH(u1), NH(d1), NH(u2) and NH(d2), respectively (Figure 5.13A and Table 5.10). In contrast to complexes **1** and **4**, the two ^{15}N atoms of en in **5** are magnetically non-equivalent, as expected in view of the chirality of the sugar. But as for **1** and **4**, only one resonance is observed for the ortho protons and one for the meta protons. This is probably due

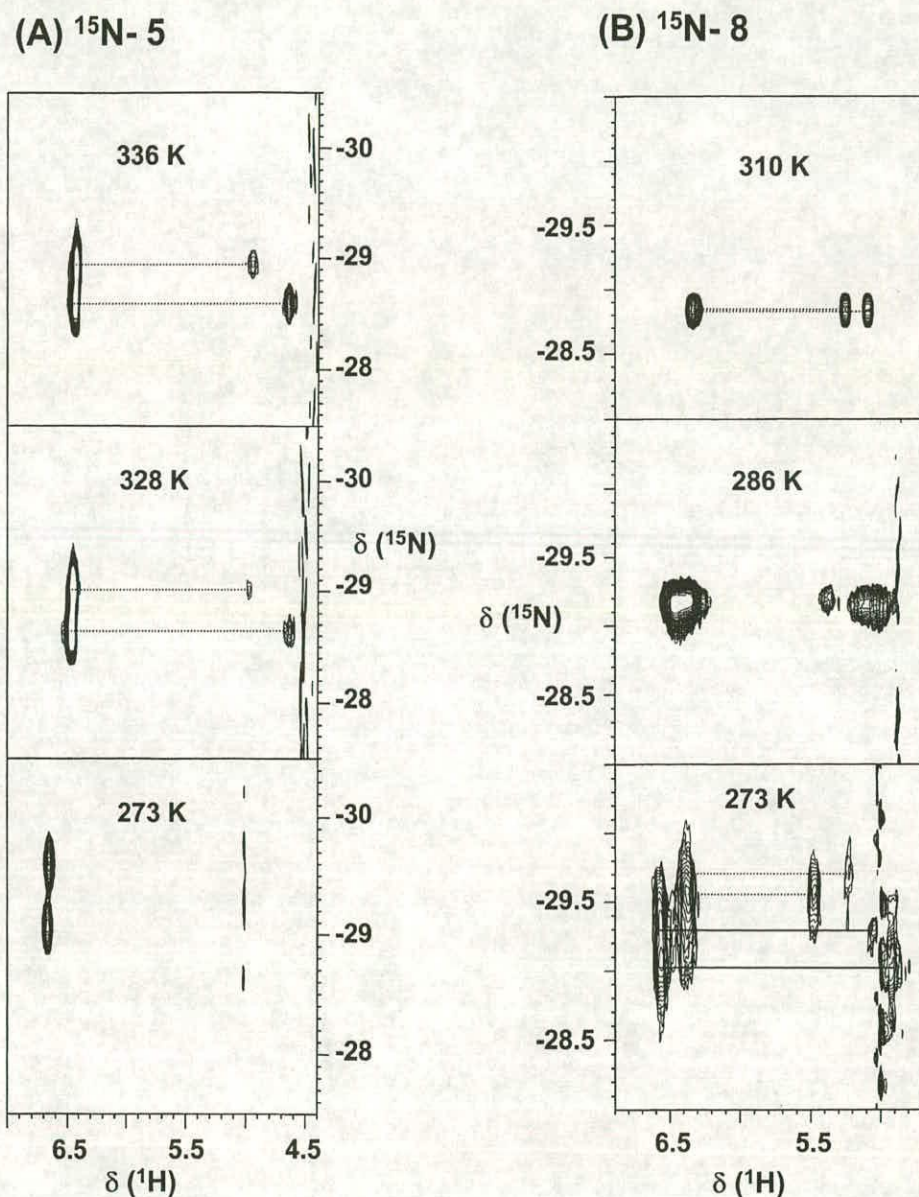


Figure 5.13 2D [^1H , ^{15}N] HSQC NMR spectra of (A) $[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N}\text{-en})(\text{Guo-}N7)]$ (^{15}N -5) and (B) $[(\eta^6\text{-biphenyl})\text{Ru}(^{15}\text{N}\text{-en})(5'\text{-GMP-}N7)]$ (^{15}N -8) in 10% D_2O / 90% H_2O at various temperatures (T). For ^{15}N -5, the en NH(d1, d2) resonances are too broad to observe at $T < 328$ K. For ^{15}N -8, two sets of en NH cross-peaks are present at 274 K, attributable to two isomers.

Table 5.10 ^1H and ^{15}N NMR data for $[(\eta^6\text{-Bip})\text{Ru}(^{15}\text{N}_2\text{-en})(9\text{EtG-N7})]^{2+}$ ($^{15}\text{N-4}$), $[(\eta^6\text{-Bip})\text{Ru}(^{15}\text{N}_2\text{-en})(\text{Gua-N7})]^{2+}$ ($^{15}\text{N-5}$), $[(\eta^6\text{-THA})\text{Ru}(^{15}\text{N}_2\text{-en})(9\text{EtG-N7})]^{2+}$ ($^{15}\text{N-6}$) and $[(\eta^6\text{-THA})\text{Ru}(^{15}\text{N}_2\text{-en})(5'\text{-GMP-N7})]^{2+}$ ($^{15}\text{N-9}$) at Various Temperatures.

Complex (NMR solvent)	T/K	en $\delta(^1\text{Hu})$, $\delta(^1\text{Hd})$ ($\delta^{15}\text{N}$)	
		$\delta(^1\text{Hu1})$, $\delta(^1\text{Hd1})$ ($\delta^{15}\text{N}$)	$\delta(^1\text{Hu2})$, $\delta(^1\text{Hd2})$ ($\delta^{15}\text{N}$)
$^{15}\text{N-4}$ (DMSO- d_6)	328	6.87, 5.12 ^a (-26.90)	6.87, 5.12 ^a (-26.90)
	310	6.90, 5.08 ^a (-26.85)	6.90, 5.08 ^a (-26.85)
	295	6.94, broad ^b (-26.88)	6.94, broad ^b (-26.88)
$^{15}\text{N-5}$ (DMSO- d_6)	328	6.84, 5.15 ^a (-26.75)	6.87, 5.04 ^a (-26.50)
	310	6.89, 5.14 ^a (-26.78)	6.92, 5.02 ^a (-26.50)
	291	6.94, broad ^a (-26.76)	6.97, broad ^a (-26.46)
$^{15}\text{N-5}$ (10% D ₂ O/90% H ₂ O)	336	6.42, 4.90 ^a (-28.95)	6.44, 4.69 ^a (-28.69)
	328	6.45, 4.98 ^a (-29.10)	6.48, 4.65 ^a (-28.62)
	298	6.51, broad ^b (-29.38)	6.55, broad ^b (-28.87)
	273	6.65, broad ^b (-29.60)	6.68, broad ^b (-29.50)
$^{15}\text{N-6}^c$ (10% D ₂ O/90% H ₂ O)	310	6.40, broad ^b (-29.38)	6.40, broad ^b (-29.38)
	283	6.50, 3.85 ^a (-29.35)	6.50, 3.85 ^a (-29.35)
		6.43, 6.0 ^a (-29.74)	6.43, 6.0 ^a (-29.74)
	274	6.55, 3.89 (29.40)	6.55, 3.89 (29.40)
		6.45, 6.05 (-29.92)	6.45, 6.05 (-29.92)
$^{15}\text{N-9}^c$ (10% D ₂ O/90% H ₂ O)	328	6.19, broad ^b (-28.50)	6.19, broad ^b (-28.50)
	308	6.19, broad ^b (-27.92)	6.25, broad ^b (-27.89)
		6.27, broad ^b (-29.28)	6.27, broad ^b (-29.28)
	278	6.19, 4.95 (-28.90)	6.25, 4.65 (-28.80)
		6.41, 5.90 (-30.32)	6.44, 5.84 (-30.10)

^a signal is broad and weak ; ^b too broad to observe; ^c For $^{15}\text{N-6}$ and $^{15}\text{N-9}$ at low temperature there are two sets of NH cross-peaks attributable to the presence of two isomers, probably due to slow rotation of THA rings about arene-Ru π -bonds.

to fast rotation of the biphenyl ligand about the arene-Ru π -bond together with fast propeller twisting on the NMR time scale. A series of 2D [^1H , ^{15}N] HSQC NMR spectra of ^{15}N -**5** in DMSO- d_6 were also acquired at various temperatures (Table 5.10). The NH(d1, d2) resonances was also too broad to detect at 298 K in this solvent but appeared when the temperature was raised to 310 K. The similarity of the results for DMSO- d_6 and aqueous solutions suggests that overlap with the water resonance is not the reason why the NH(d1, d2) resonances of **5** are unobservable in aqueous solution at low temperature.

In the 2D NOESY ^1H NMR spectrum of **5** (Figure 5.14), NOEs were observed for H8-H(o'), H8-H(o), NH(u1, u2)-H(m) and NH(u1, u2)-H(p), indicating that the *syn* conformation predominates for ring B with respect to the G base. A similar orientation for ring B occurs in the crystal structure, and the observed NOEs are in agreement with the average crystallographic distances of NH(u1, u2)-H(m) 2.70 Å, NH(u1, u2)-H(p) 3.37 Å, NH(u1, u2)-H(o) 4.73 Å, H8-H(o) 3.35 Å, H8-H(o') 3.82 Å, H8-H(m) 4.68 Å, H8-H(m') 4.81 Å, H8-H(p) 5.85 Å and H8-H(p') 5.40 Å. The NOE cross-peaks for H8-H1', H8-H2', H8-H3' and H8-H4' have intensity ratios of ca. 5 : 1 : 1 : 1, respectively (Figure 5.15A). The strong NOE for H8-H1' observed in this spectrum, suggests that a *syn* orientation predominates for the G base with respect to the ribose ring. This conformational feature is different from the solid state where an *anti* orientation was found for **5**. The value of $^3J(\text{H1}'/\text{H2}') = 4.49$ Hz is lower than that for free Guo (6.05 Hz), suggesting that the sugar conformation changes when Guo binds to Ru.

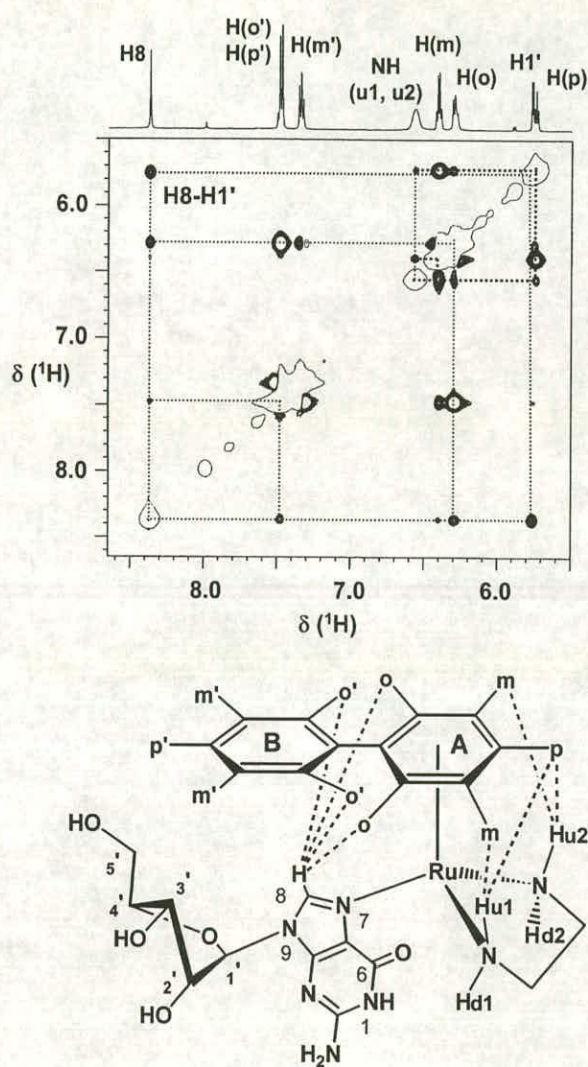


Figure 5.14 NOE data for $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (**5**). Top: 2D NOESY ^1H NMR spectrum for **5** in 10% D_2O / 90% H_2O at 298 K. Bottom: structure with NOEs indicated by dotted lines.

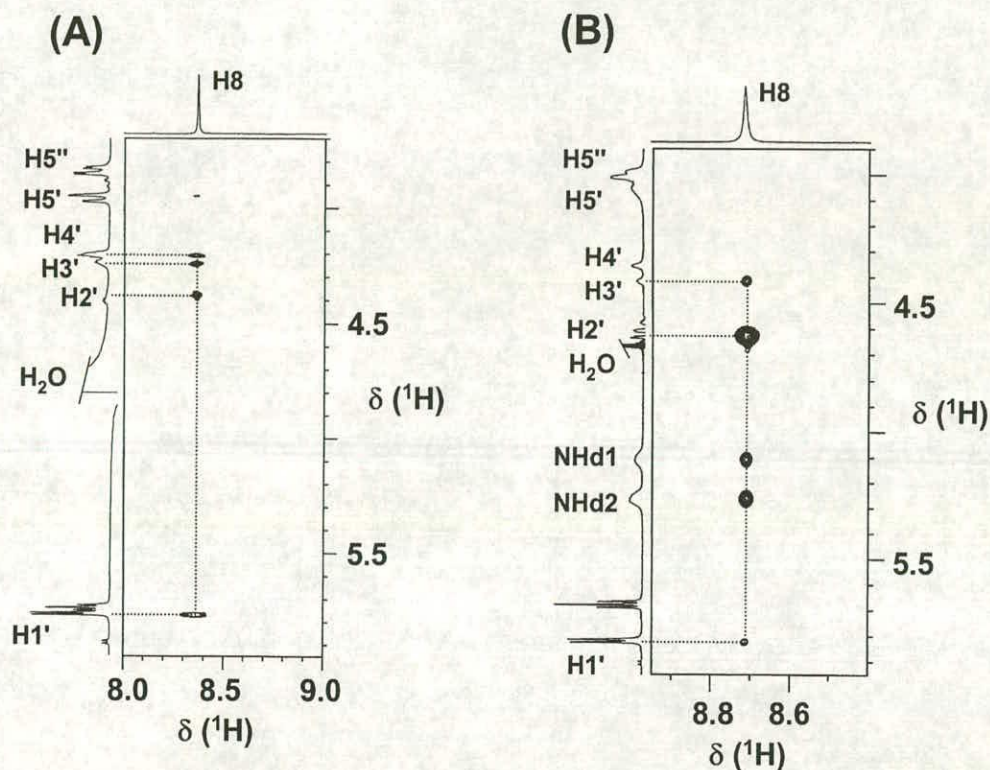


Figure 5.15 2D ^1H NOESY NMR spectra of (A) $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (5), and (B) $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (8), in 10% D_2O / 90% H_2O , showing the guanine H8-sugar ring connectivities. In (A) the intensity ratio of NOE cross-peaks for H8-H1' : H8-H2' : H8-H3' : H8-H4' is ca. 5 : 1 : 1 : 1. The stronger NOE for H8-H1' suggests that a *syn* conformation predominates. $^3J(\text{H1'}/\text{H2'}) = 4.49$ Hz (6.05 Hz for free Guo). In (B) the intensity ratio of NOE cross-peaks of H8-H1' : H8-H2' : H8-H3' is ca. 1 : 10 : 1.5. There is no H8-H4' NOE cross-peak. The strong NOE between H8 and H2' indicates C2'-endo (S) and *anti* conformations for the sugar and base, which fits well with their conformations in canonical B-DNA. $^3J(\text{H1'}/\text{H2'}) = 5.78$ Hz (6.10 Hz for free 5'-GMP).

[(η^6 -biphenyl)Ru(en)(5'-GMP-N7)] (8) in Aqueous Solution. 2D NOESY and DQFCOSY ^1H NMR spectra of **8** in 10% D_2O / 90% H_2O at 310 K were used to assign the protons NMR resonances (Tables 5.8 and 5.9). The four en NH resonances at δ 6.36, 5.27, 6.39 and 5.09 can be assigned to NH(u1), NH(d1), NH(u2) and NH(d2), respectively. All are of reasonable intensity at this temperature. Their assignments were confirmed by reference to the 2D [^1H , ^{15}N] HSQC ^1H NMR spectrum of ^{15}N -**8** (Figure 5.13B). The inequivalence of en NH_2 can be accounted for by the chirality of the sugar ring of 5'-GMP. Only one resonance is observed for the ortho protons and one for the meta protons of biphenyl, as for **5**. In the 2D NOESY NMR spectrum (Figure 5.16), the NOE cross-peaks for H8-H(o'), H8-H(o) and NH(u1, u2)-H(p) indicate that the *syn* conformation predominates for the biphenyl ring with respect to the G base. There are NOEs between H8 and NH(d1, d2), but not between H8 and NH(u1, u2), suggesting that H8 of G points toward NH(d1, d2). The ratio of the intensities of the NOE cross-peaks H8-H1', H8-H2' and H8-H3' is ca. 1 : 10 : 1.5, respectively (Figure 5.15B). There is no H8-H4' NOE cross-peak. The strong NOE for H8-H2', and the absence of an NOE between H8 and H4', suggest a C2'-endo (S) *anti* conformation for the sugar ring. The value of $^3J(\text{H1}'/\text{H2}') = 5.78$ Hz for **8** is similar to that for the free 5'-GMP (6.10 Hz). These data suggest that the C2'-endo anti-glycosidic sugar conformation of 5'-GMP is not altered by N7-binding to Ru.

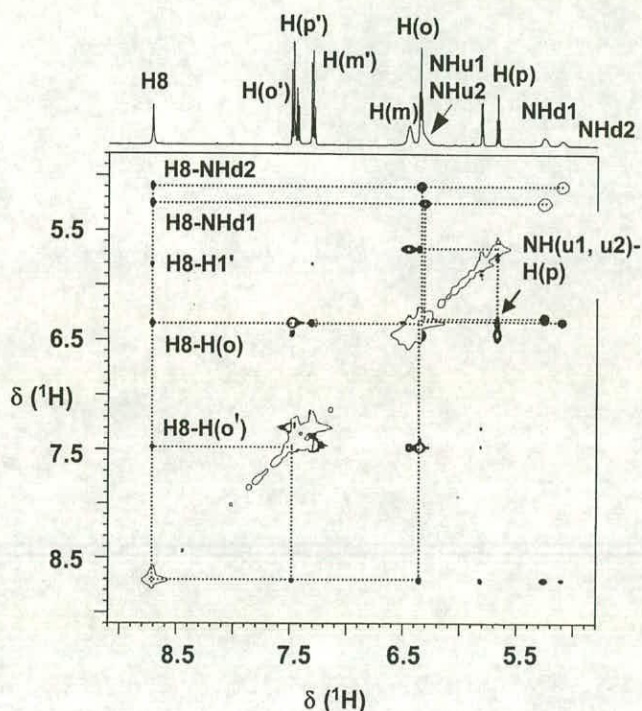


Figure 5.16 2D NOESY ^1H NMR spectrum of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (**8**) in 10% D_2O / 90% H_2O at 310 K, showing NOEs for H8-H(o'), H8-H(o), and H(p)-NH(u1,u2). The labelling scheme for **8** is similar to that for **5** in Figure 5.14.

5.3.5 NMR Structures of THA and DHA Complexes in Solution

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}]^+$ (**2**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}]^+$ (**3**) in $\text{DMSO-}d_6$. The 2D NOESY ^1H NMR spectrum of **2** in $\text{DMSO-}d_6$ provided a basis for the assignment of ^1H NMR resonances of **2** (Figure 5.17). There are strong NOEs between NH(u1, u2) and NH(d1, d2), between NH(u1, u2) and H(1, 4) / H(2, 3), between H(1, 4) and H(8, 9), and between H(8, 9) and H(5, 8). No NOEs were observed between NH(d1, d2) and aromatic protons. For **3**, the resonances were assignable directly from the 1D ^1H NMR spectrum (Figure 5.20). In each case, one set of sharp signals was observed for the THA/DHA ring.

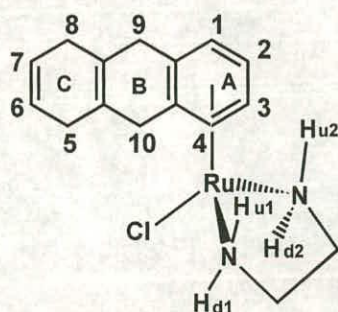
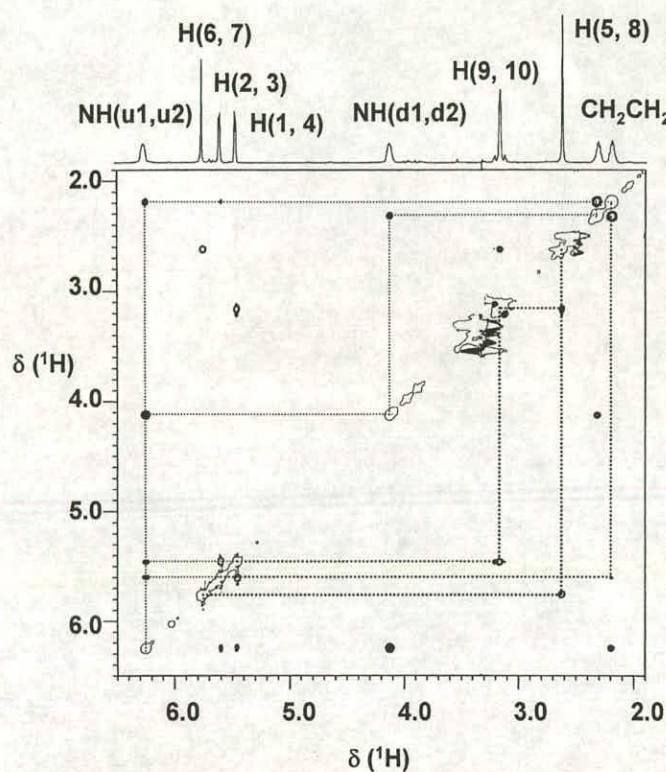


Figure 5.17 2D NOESY ^1H NMR spectrum of $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**) in $\text{DMSO-}d_6$ at 298 K, showing assignments of the arene and en resonances. NOEs of $\text{NH}(\text{u}1, \text{u}2)\text{-NH}(\text{d}1, \text{d}2)$, $\text{NH}(\text{u}1, \text{u}2)\text{-H}(1, 4)$, $\text{NH}(\text{u}1, \text{u}2)\text{-H}(2, 3)$, $\text{H}(1, 4)\text{-H}(8, 9)$, and $\text{H}(8, 9)\text{-H}(5, 6)$ are indicated by dotted lines.

$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (6 and $^{15}\text{N-6}$) and $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (9 and $^{15}\text{N-9}$) in Aqueous Solution. In contrast to the ^1H NMR resonances for the biphenyl complexes **4**, **5** and **8**, the resonances of the tetrahydroanthracene rings of complexes **6** and **9** in the aqueous solution were very broad, as were their G base and en ligand ^1H NMR resonances (Figures 5.18 and 5.19). When solutions of **6** and **9** were heated to 339 K and 318 K, respectively, the peaks became sharper, but the resolution was not sufficient for NOESY studies. On cooling to 278 K, more than two sets of signals were detected in both cases, but chemical exchange processes complicated the interpretation of the NOESY data. 2D [^1H , ^{15}N] HSQC NMR spectra of complexes $^{15}\text{N-6}$ and $^{15}\text{N-9}$ in aqueous solutions were acquired to confirm the assignment of the en NH signals, and the low temperature spectra suggested the existence of two isomers (Table 5.10).

$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (7) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$ (10) in Aqueous Solution. The ^1H NMR resonances of the dihydroanthracene complexes **7** and **10** were very broad at ambient temperature. For complex **10**, two H8 signals were observed at 298 K (Figures 5.20 and 5.21). When the solutions of complexes **7** and **10** were heated to 339 K and 318 K, respectively, the signals became sharper but the resolution was not sufficient for 2D NOESY studies. At 278 K, more than two sets of signals were detected in both cases, but NOESY experiments at low temperatures were not attempted.

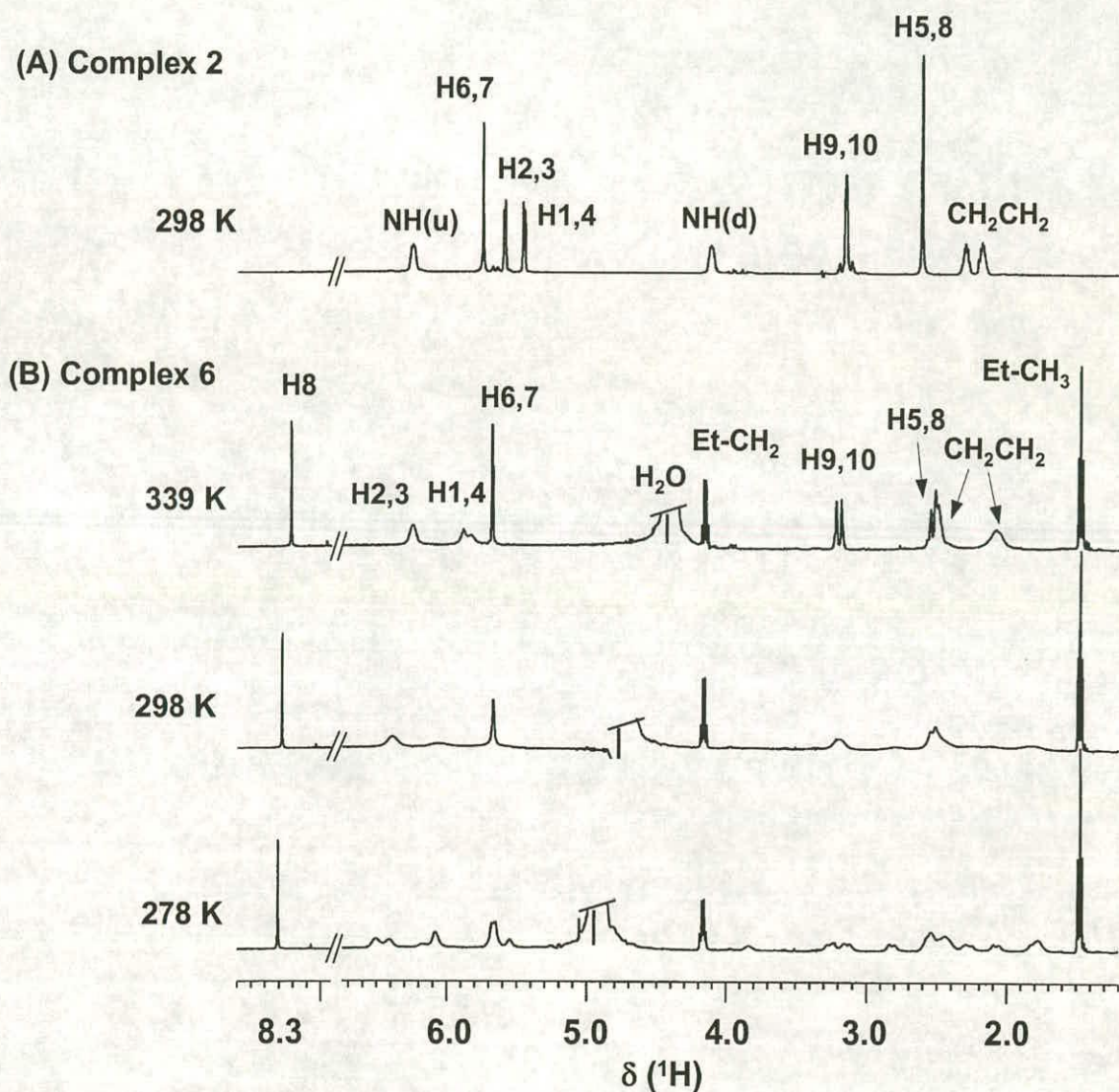


Figure 5.18 ^1H NMR spectra of (A) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**) in $\text{DMSO-}d_6$ at 298 K, (B) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(9\text{EtG-}N7)]$ (**6**) in 10% D_2O / 90% H_2O at various temperatures showing that the resonances of the tetrahydroanthracene rings are broad at 298 K but separate into two sets at low temperature (278 K).

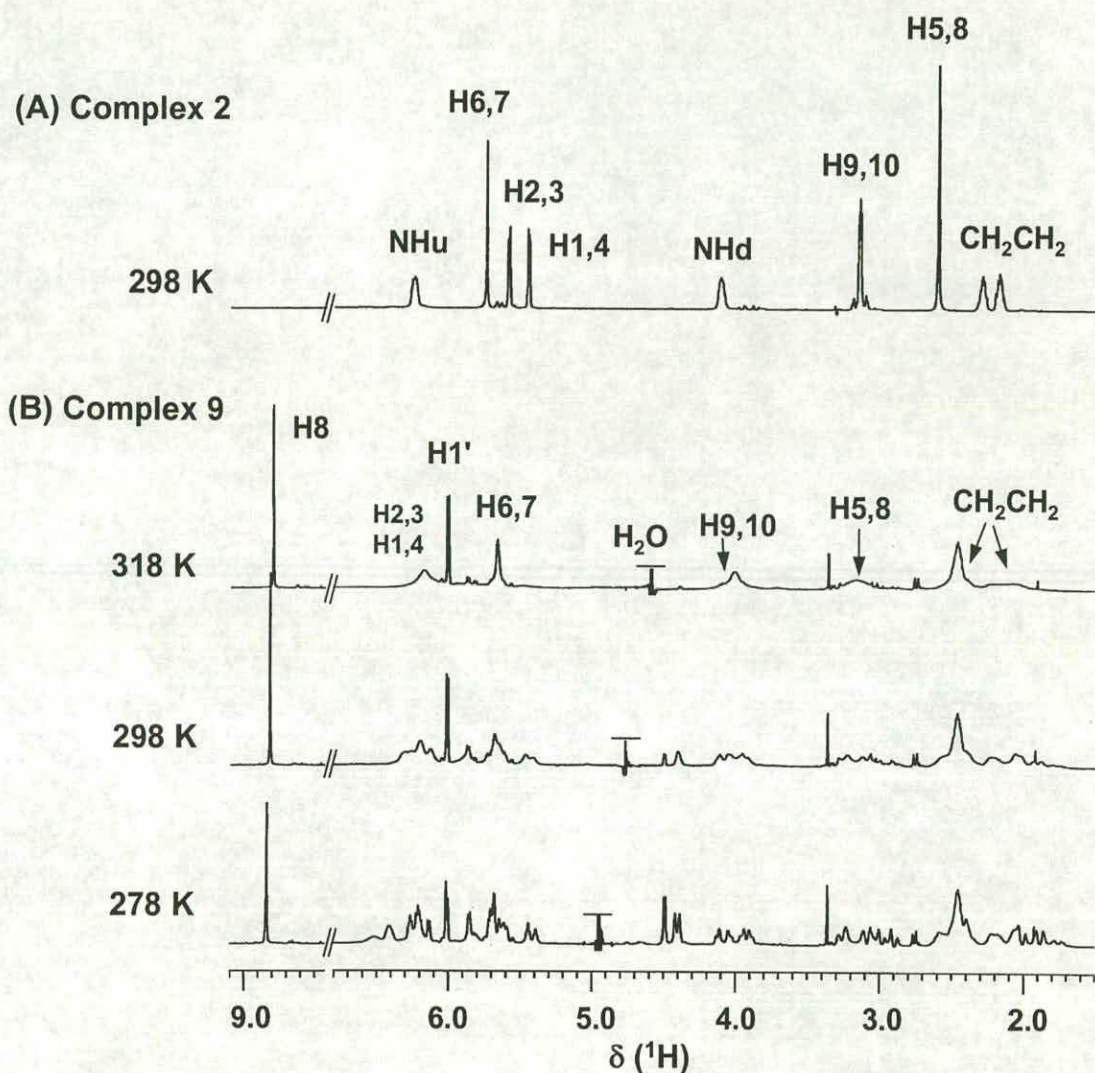


Figure 5.19 ^1H NMR spectra of (A) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**2**) in $\text{DMSO}-d_6$ at 298 K, and (B) $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})(5'\text{-GMP}-N7)]$ (**9**) in 10% D_2O / 90% H_2O at various temperatures showing that the resonances of the tetrahydroanthracene rings are broad at 298 K but separate into two sets at low temperature (278 K).

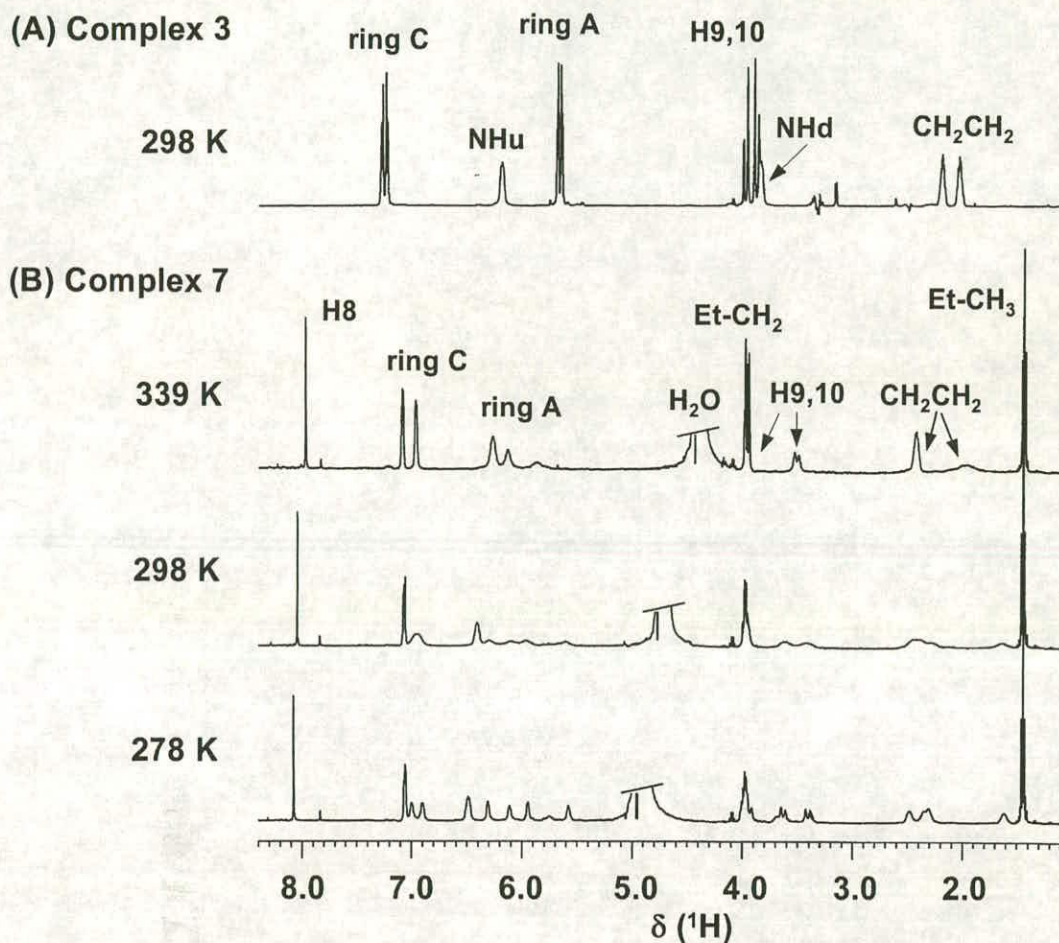


Figure 5.20 ¹H NMR spectra of (A) [(η^6 -C₁₄H₁₂)Ru(en)Cl][PF₆] (**3**) in DMSO-*d*₆ at 298 K, and (B) [(η^6 -C₁₄H₁₂)Ru(en)(9EtG-*N*7)] (**7**) in 10% D₂O/ 90% H₂O at various temperatures showing that the resonances of the dihydroanthracene rings are broad at 298 K but separate into two sets at low temperature (278 K).

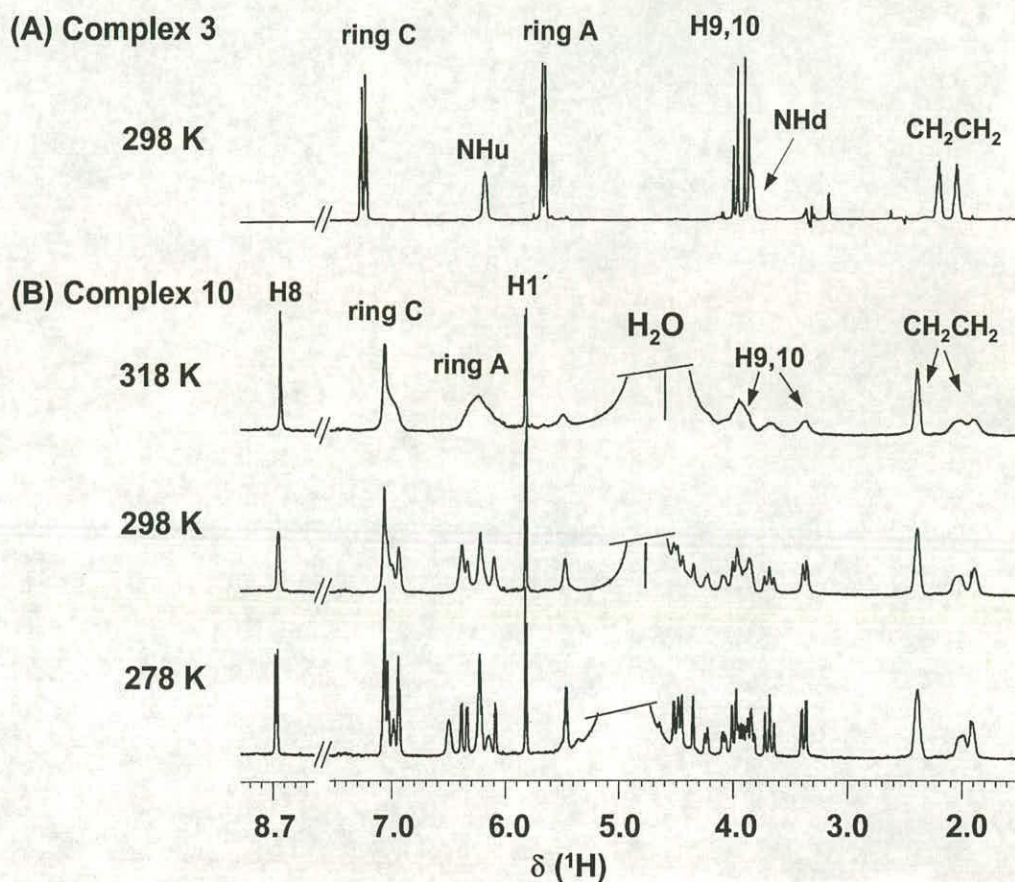


Figure 5.21 ^1H NMR spectra of (A) $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (**3**) in $\text{DMSO-}d_6$ at 298 K, and (B) $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})(5'\text{-GMP-}N7)]$ (**10**) in 10% D_2O / 90% H_2O at various temperatures showing that the resonances of the dihydroanthracene rings are broad at 298 K but separate into two sets at low temperature (278 K).

5.3.6 pH Dependence of NH Resonances of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-}N7)]$ (**8**).

We used ^{15}N -edited $^1\text{H}\{^{15}\text{N}\}$ NMR and pH titration methods to investigate H-bonding interactions between en NH and the 5'-phosphate or O6 groups of 5'-GMP in ^{15}N -**8**. The 2D $[^1\text{H}, ^{15}\text{N}]$ HSQC NMR spectrum of ^{15}N -**8** at

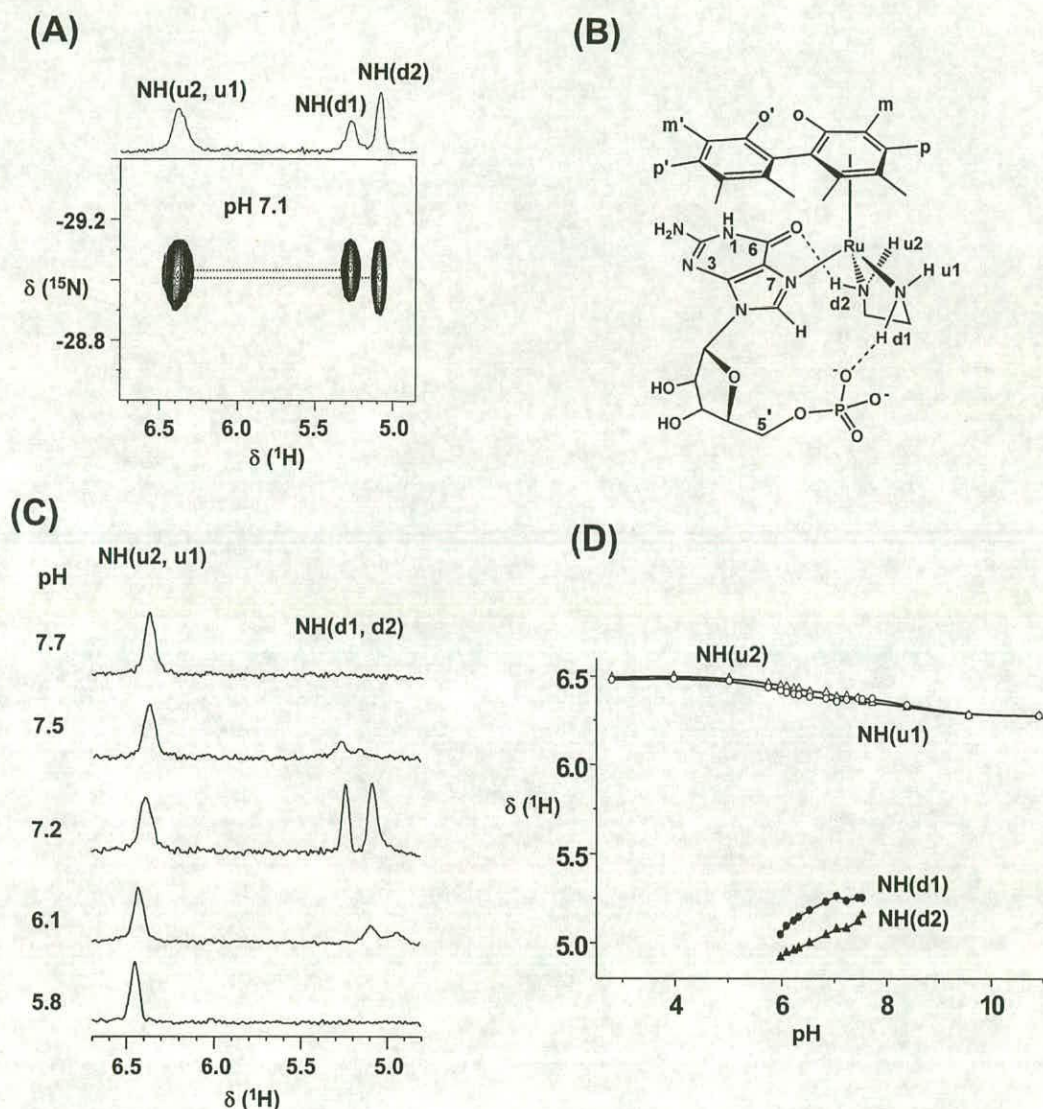


Figure 5.22 (A) 2D ^1H , ^{15}N HSQC NMR spectrum of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{N}_2\text{-en})(5'\text{-GMP-N7})]$ (^{15}N -8) at pH 7.1 (Ru 5 mM, 10% D_2O / 90% H_2O , 298 K). (B) Structure with proposed H-bonds indicated by dotted lines. (C) ^{15}N -edited $^1\text{H}\{^{15}\text{N}\}$ spectra at various pH values. (D) Plot of the chemical shifts of en $^{15}\text{NH}_2$ ^1H NMR resonances of ^{15}N -8 versus pH. The large variation in shift of the NH(d1, d2) protons can be accounted for by H-bonding interactions with the 5'-phosphate and G O6 groups, promoted by deprotonation of $-\text{OPO}_3\text{H}$ and N1H , respectively. The NH(d) resonances were too broad to observe both at high pH and at the low pH.

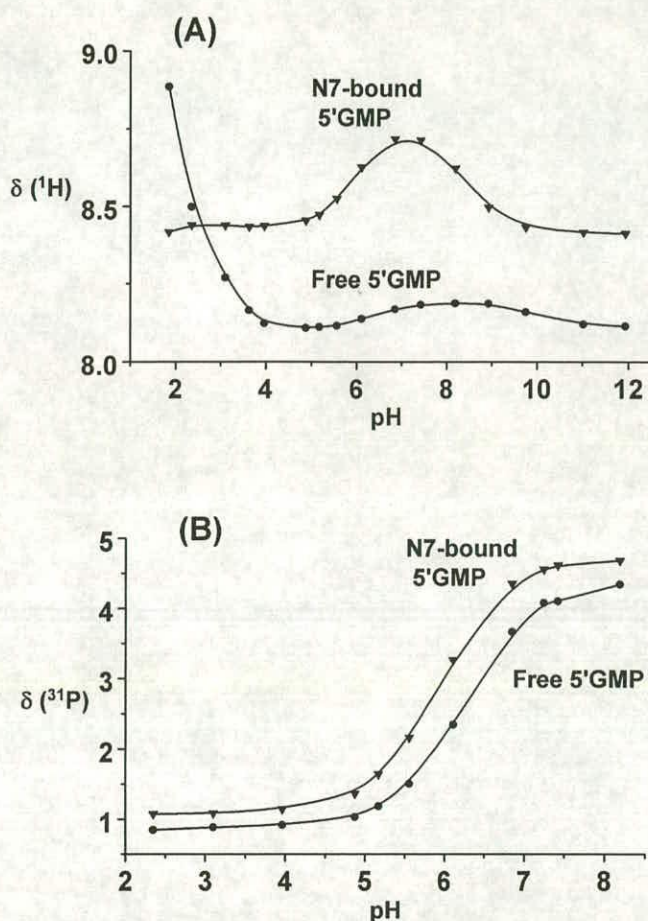


Figure 5.23 pH dependences of the 5'-GMP H8 ^1H (A) and phosphate ^{31}P (B) NMR resonances for $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-N7})]$, **8** ($\blacktriangledown$) and free 5'-GMP ($\bullet$) at 298 K, showing the lack of protonation of N7 for **8** at low pH and deprotonation of N1 and phosphate at higher pH. The corresponding pK_a (1) values were determined to be 5.96 (^1H), 5.93 (^{31}P) for **8** and 6.30 (^1H), 6.24 (^{31}P) for free 5'-GMP (protonation of the phosphate group), and second pK_a (2) 8.45 (^1H) for **8** and 9.85 (^1H) for free 5'-GMP (deprotonation of N1 of G).

298 K showed four cross-peaks at δ 6.36/-29.04, 5.27/-29.04, 6.39/-29.01, and 5.09/-29.01, assignable to NH(u1), NH(d1), NH(u2) and NH(d2) (Figure 5.22A). 2D [^1H , ^{15}N] HSQC NMR spectra of ^{15}N -**8** were recorded over the pH range 2.8-10.9 and enabled the two NH(u) resonances to be distinguished on the basis of small differences in their ^{15}N chemical shifts. A plot of chemical shift of each en NH signal

versus pH is shown in Figure 5.22D. The two NH(u1, u2) protons have the same pH dependence and shift slightly to high-field as the pH is increased; the two NH(d1, d2) protons also show similar pH behaviours and shift greatly to low-field over the range from pH 5.98 to 7.53. However, at pH < 5.98 or pH > 7.53 these two NH(d) peaks became too broad to observe.

^1H NMR and ^{31}P NMR pH titrations at 298 K were also carried out for a solution of unlabelled $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(5'\text{-GMP-}N7)]$ (**8**), containing a 10% excess of 5'-GMP. At pH 7.1, ^1H NMR peaks at δ 8.71 and δ 8.20 were observed for H8 protons of **8** and free 5'-GMP, respectively; ^{31}P NMR peaks at δ 4.61 and δ 3.96 were observed for the 5'-phosphate of **8** and free 5'-GMP, respectively. Plots of δ (^1H) and δ (^{31}P) against pH are shown in Figure 5.23. The curves were fitted to the Henderson-Hasselbalch equation, giving $\text{p}K_a$ values of 5.96 for **8** and 6.30 for free 5'-GMP (^1H curves). These values are attributed to deprotonation of the 5'-phosphate groups. The $\text{p}K_a$ values determined from the ^{31}P curves (Figure 5.23B, 5.93 and 6.24) are in good agreement with the values from ^1H curves. Also $\text{p}K_a$ values of 8.45 for complex **8** and 9.85 for free 5'-GMP were determined from the ^1H curves. These values are attributed to deprotonation of G N1H.

5.4 Discussion

5.4.1 General

There are many potential applications of organometallic chemistry in medicine. Examples include the anticancer activity of cyclopentadienyl complexes such as titanocene dichloride,⁴⁵ which has been on clinical trial, and receptor-targeted anti-oestrogen organometallic complexes.^{46,47} The work in this chapter is concerned with organometallic Ru(II) complexes of the type $[(\eta^6\text{-arene})\text{Ru}(\text{II})(\text{en})\text{Cl}][\text{PF}_6]$ which exhibit promising anticancer activity. Initial *in vitro* and *in vivo* data suggest that the most active complexes contain the most hydrophobic η^6 -arene ligands, examples being complexes **2** and **3** which contain the

tricyclic η^6 -anthracene derivatives THA and DHA.⁶⁻⁸ In general, $(\eta^6\text{-arene})\text{Ru(II)}$ bonds are relatively stable in water,⁴⁸ and are more resistant to hydrolysis than the Cp ligands in titanocene dichloride, $[\text{Cp}_2\text{TiCl}_2]$.⁴⁹ The target for Ru(II) arene complexes may be DNA and we found that $\{(\eta^6\text{-arene})\text{Ru(II)(en)}\}^{2+}$ exhibits a high specificity for binding to guanine (G).⁷ The aim of the present work is to elucidate the nature of the interactions which give rise to such specificity. Therefore the structures and dynamics of adducts of $\{(\eta^6\text{-arene})\text{Ru(II)(en)}\}^{2+}$ with G derivatives have been studied both in the solid state and in solution.

Very few X-ray structures of Ru-nucleobase adducts have been reported previously.⁵⁰ The structure for $[(\eta^6\text{-biphenyl})\text{Ru(II)(en)(Guo-}N7)][\text{PF}_6]_2 \cdot 3.75\text{H}_2\text{O}$ determined in the present work appears to be the first reported X-ray structure for a ruthenium nucleoside complex. Also no X-ray structures of η^6 -coordinated DHA complexes appear to have been reported previously. Hydrophobic interactions of the arene ligand and H-bonds involving en NH protons appear to exert a strong influence on the conformations of these guanine adducts.

5.4.2 Arene-guanine Base Stacking

Strong arene-nucleobase stacking is present in the crystal structures of $[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru(en)(9EtG-}N7)]^{2+}$ (**6**) and $[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru(en)(9EtG-}N7)]^{2+}$ (**7**). Figures 5.6A and 5.6B show that π - π stacking between the outer ring (C) of THA or DHA and the guanine base is almost ideal⁵¹: ring C is parallel to and fully overlaps the purine ring (see also Figures 5.4C and 5.4D). The THA ligand in complex **6** has a diene ring C, but it adopts a very similar conformation to the DHA ligand in complex **7** which contains an aromatic ring C. The interplanar (centroid-centroid) distance is 3.45 Å for complex **6** and 3.31 Å for **7**, with dihedral angles of 3.3° and 3.1°, respectively. The π -stacking of aromatic rings and unsaturated groups, or even between unsaturated groups themselves, has been widely investigated in studies of

crystal engineering, and energy transfer. Diene stacking has been employed in the design and control of polymer-chain structures.⁵² For the biphenyl adduct **4**, $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-}N7)][\text{PF}_6]_2 \cdot \text{MeOH}$, intramolecular arene-nucleobase stacking is precluded by the *anti* orientation of 9EtG with respect to biphenyl. Here, there is intermolecular stacking between the pendent phenyl ring and the purine six-membered ring (Figures 5.1B and 5.2A) with a centroid-centroid distance of 4.0 Å and dihedral angle of 4.5°. Such intermolecular arene-nucleobase stacking may stabilize the cyclic tetramer structure in the unit cell (Figure 5.1B). For the adduct **5**, $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-}N7)][\text{PF}_6]_2 \cdot 3.75(\text{H}_2\text{O})$, there are two types of stacking: intramolecular stacking of the pendent phenyl ring with the purine five-membered ring (3.8 Å, 23.8°), and intermolecular stacking of the purine six-membered ring with an adjacent pendent phenyl ring (4.2 Å, 23.0°). These occur alternately giving a columnar-type structure (Figure 5.2B). For aromatic systems, π - π interactions are usually accompanied by interplanar separations of 3.3-3.8 Å.⁵¹ However, longer distances for π - π stacking have been also reported (up to 4.7 Å).^{52a,52c,53} The arene-nucleobase stacking in the biphenyl adducts **4** and **5** are accompanied by longer centroid-centroid distances (average 4.0 Å), compared with that of THA and DHA adducts **6** and **7** (average 3.4 Å). This can be accounted for by the higher degree of freedom^{53c} for movement of the biphenyl ring (free propeller twisting of phenyl ring in Bip, but relatively rigid tricyclic frames of THA and DHA).

The extensive arene-nucleobase stacking but not arene-arene or base-base stacking in $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{G-}N7)]^{2+}$ adducts represents a new structural feature for adducts of metal anticancer complexes with nucleobases. Intra- or inter-molecular base-base stacking has been reported in the X-ray structures of some Pt complexes of nucleobases, nucleosides and nucleotides. For example, *cis*- $[(\text{tn})\text{Pt}(\text{Me-5'-GMP})_2]$, *cis*- $[(\text{tn})\text{Pt}(5'\text{-IMP})_2]$ (tn = trimethylenediamine) and *cis*- $[(\text{NH}_3)_2\text{Pt}(5'\text{-IMP})_2]$ all have intracomplex base-base stacking with dihedral angles of 39.6°, 38.2° and 40.7°,

respectively.⁵⁴ Such features are relevant to the intrastrand cross-linking by *cis*-platinum complexes on DNA.⁵⁵ In the adducts of *cis*-[Pt(NH₃)(Guo)₂]Cl_{1.5}(ClO₄)_{0.5}·7H₂O and *cis*-[Pt(en)(Guo)₂]Cl_{1.5}I_{0.5}·2H₂O, one guanine base is in nearly parallel contact with a guanine base on a neighboring cation at distances of 3.34 Å and 3.31 Å, respectively.⁵⁶ Stacking of acridine orange (AO) rings at a distance of 3.5 Å was found in the complex [Pt{AO(CH₂)₆(en)}(ox)](NO₃)·7H₂O and the phi ligands of [Rh(NH₃)₄(phi)]³⁺ (phi = 9, 10-phenanthrenequinone diimine) cations stack in dimer pairs at a distance of 3.4 Å. This kind of self-stacking of aromatic rings has been interpreted as an indication of an ability to intercalate.^{12,57} The arene-base stacking in [(η⁶-arene)Ru(en)(G-N7)]²⁺ adducts indicates potential for intercalation or hydrophobic interactions of the arene ring with duplex DNA. Hydrophobic interactions between the arene ligand and purine base could provide a driving force for DNA binding. A recent analysis of free energy contributions to DNA binding, suggests that intercalation of ethidium, propidium, daunorubicin and adriamycin is driven by hydrophobic effects and van der Waals contacts within the intercalation site, minimising the solvent-accessible surfaces of the drug.¹⁰ Recognition of specific sequences by DNA minor groove binding proteins is often dominated by hydrophobic protein-DNA interactions.⁵⁸ The X-ray crystal structure of HMG1 domain A bound to a cisplatin-modified 16-base-pair deoxyoligonucleotide shows that the cisplatin intrastrand crosslink destacks the G₈-G₉ purine rings and leads to exposure of their hydrophobic surfaces in the minor groove. Phe37 intercalates at the site of the intrastrand cross-link *cis*-[Pt(NH₃)₂-{d(GpG)-N7(G₈),-N7(G₉)}], stacking onto the G₉ base at a distance of 3.5 Å.⁵⁹ Favorable intercalation directs protein binding and orientation.²¹

5.4.3 Arene Conformation

The conformations of the parent chloro-complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ and guanine adducts $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{G-N7})]^{2+}$ are compared in Figure 5.24. In each case, there is a significant re-orientation and conformational change of the arene ring with respect to the parent chloro-complex, as a result of arene-nucleobase interaction. For the biphenyl-guanine adducts **4** and **5**, the biphenyl ligand is rotated away from the Ru-N7 vector (around the arene-Ru π bond) by 109.2° for **4**, and rotated back by 13.6° for **5**, compared to the parent chloro-complex **1**, and is involved in inter- or intra-molecular stacking with the purine ring. A decrease of ca. 10° in propeller twist of biphenyl for **4** and **5** (compared to **1**) may also be driven by the phenyl-purine stacking force since there is no stacking interaction in **1**.

Arene-nucleobase stacking forces in **6** and **7** appear to cause large changes in the conformations of the THA and DHA ligands compared with their parent chloro-complexes **2** and **3**, respectively (Figures 5.24C and 5.24D). When the Cl ligand in complex **2** is substituted by N7-G (complex **6**), the THA ligand rotates back around the arene-Ru π -bond by 19.5° (Table 5.5B) and re-locates to a position where ring C is completely overlapped with the G base. Moreover, the planarity of this tricyclic frame is lost and ring C bends down toward the purine about the C7A-C10A hinge by 27.8° (Table 5.5B). Similarly, the DHA ligand is rotated away from the Ru-Cl vector by 64.1° in **3**, and rotated back around the arene-Ru π -bond by 43.2° in **7** compared to **3**, whilst ring C is bent down toward the Cl ligand about the C7A-C10A hinge by 40.6° in **3** and toward the purine by 31.9° in **7**. The increase of 35.3° in hinge bending angle for **6** and decrease of 8.7° for **7** compared with their parent complexes **2** and **3**, respectively, allow ring C to make parallel contact with the purine in either case. Thus π -stacking interactions between the arene ring and nucleobase lead to arene ring re-orientation and conformational adjustment.

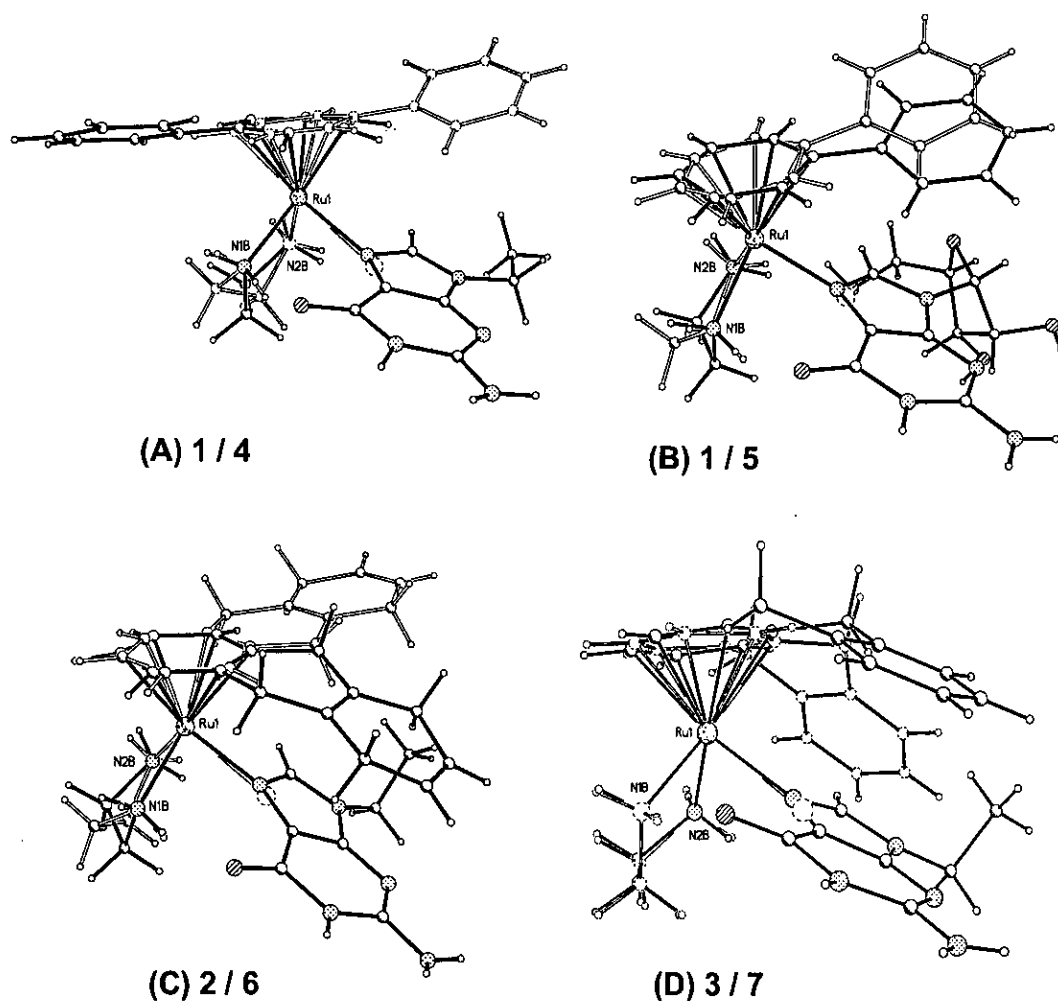


Figure 5.24 Comparison of the conformations of the parent chloro-complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ (light line) and the guanine adducts $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{G-N7})]^{2+}$ (heavy line). Arene = Bip in (A) and (B), THA in (C), and DHA in (D); Guanine derivative = 9EtG in (A), (C) and (D), Guo in (B). Alignments are based on a superposition of the labelled atoms (Ru1, N1B and N2B of en). In each case there is a significant re-orientation of the arene with respect to the parent chloro-complex, resulting in arene-nucleobase interaction.

The arene ligands process flexibility through rotation around the arene-Ru π -bonds, propeller twisting for Bip, and hinge-bending for THA and DHA. These allow optimisation of the geometry for simultaneous arene-base stacking with N7-covalent binding. When the Ru(arene) complex approaches DNA, the arene ligand could therefore adjust its conformation to accommodate interactions with the guanine base. This property may reduce the steric demands of the Ru drug and enhance DNA affinity. The steric requirement of the metal complex itself directly influences the rate of DNA binding and hence affects anticancer activity. The coordination of octahedral ruthenium complexes to DNA is more sterically demanding than for square-planar four-coordinated platinum complexes.^{60,57} The antitumor complex *mer*-[Ru(III)(terpy)Cl₃] (terpy = 2, 2' : 6', 2'' - terpyridine) can bind two guanine bases and rate of binding to calf thymus DNA is faster than the inactive complex *cis*-[Ru(II)(bpy)₂Cl₂] (bpy = 2, 2' - bipyridine) which has one more rigid heterocyclic ring and binds to only one guanine base.^{3c,50a,61} The binding of DNA bases to α -[Ru(II)(azpy)₂Cl₂] (azpy = 2-(phenylazo)pyridine) is sterically less-hindered than for *cis*-[Ru(II)(bpy)₂Cl₂]. The azpy ligand has flexibility since one ring is free from coordination to Ru, compared with the rigid coordinated bpy ligand. α -[Ru(II)(azpy)₂Cl₂] has a very high cytotoxicity.^{3d,62} The higher DNA reactivity of Na[*trans*-Ru(III)(Im)(DMSO)Cl₄] compared to Na[*trans*-Ru(III)(Im)₂Cl₄] (Im = imidazole) may also be related to the presence of two bulky heterocyclic axial ligands in the latter.⁶³

Comparison between the crystal structures of complexes **4** and **5** suggests that the biphenyl ligand can adopt *anti* or *syn* conformations with respect to the G base (Figures 5.4A and 5.4B). In the crystal structures of **6** and **7**, the THA and DHA ligands both adopt a *syn* orientation (Figures 5.4C and 5.4D). In general, the orientation of arene ligand in G adducts may be different in the solid state compared

to solution since both crystal packing and solvent effects can play a role. The solution structures of guanine adducts were studied using 2D NOESY NMR experiments in water or in acetone (dependent on solubility). The NOEs for its biphenyl adducts **4**, **5** and **8** reveal that a *syn* orientation of the biphenyl ligand with respect to the G base predominates in solution in each case (Figures 5.12, 5.14 and 5.16). Accordingly, downfield shifts for resonances of the pendent phenyl ring protons ($H(p')$, $H(o')$ and $H(m')$) in these three G adducts relative to those of the aqua biphenyl complex **1a** are observed. This effect is most pronounced for $H(o')$: $\Delta\delta = -0.36$ (**4**), -0.33 (**5**), -0.34 (**8**) ppm (Table 5.8). Taken together, the *syn* orientation appears to be favoured for $[(\eta^6\text{-biphenyl})Ru(en)(G-N7)]$ adducts both in solid state and in solution. This can be attributed to hydrophobic interactions between the phenyl ring and purine base.

5.4.4 Fluxional Behavior of Arene Adducts

Structural studies on THA and DHA adducts in solution were also attempted. Compared with the parent chloro complexes **2** and **3**, the ^1H NMR resonances of the THA and DHA rings in the guanine adducts **6**, **7**, **9**, **10** are broadened (Figures 5.18-5.21). This may be due to the restricted rotation of the THA and DHA ligands. 2D $[^1H, ^{15}N]$ HSQC NMR spectra of $^{15}N\text{-6}$ (274 K) and $^{15}N\text{-9}$ (278 K) in water both show two sets of NH cross-peaks attributable to the presence of two isomers A and B (probably corresponding to *anti* and *syn* conformations) (Table 5.10). The 2D NMR EXSY-NOESY spectra (278 K) of $^{15}N\text{-9}$ (not shown) indicated exchange among two possible conformations at low temperature. The fluxionality of organometallic complexes has been previously investigated.⁶⁴ Very small rotational barriers have been calculated for $(\eta^6\text{-benzene})Ru(II)$ complexes and restricted rotation about the Ru-arene π bond has been observed only on the presence of particular electronic and steric factors.⁶⁵ For biphenyl adducts, there is no obvious broadening of the biphenyl

ring resonances for complexes **4**, **5** and **8** at various temperatures (273 - 318 K), indicating free rotation of the biphenyl ligand. However, the resonances for Guo and 5'-GMP in **5** and **8** do broaden at low temperature. In the case of ^{15}N -**8**, two sets of en NH [^1H , ^{15}N] cross-peaks were present at 274 K (Figure 5.13B) and the H8 ^1H signal separated into two. This can be attributed to slow rotation of G at low temperature. Thus it appears that G rotation is more restricted than biphenyl rotation. This is not surprising since the G base forms one leg of the “piano-stool” and its movement is restricted by both the arene and the en ligands. Synchronous movements of the pendent phenyl ring and the G base may be possible with propeller twisting of the pendent phenyl ring. Such movement would not be possible for THA and DHA adducts which have relatively rigid tricyclic rings and explains why free rotation of the biphenyl rings is little affected by the steric factors from the G base in the biphenyl adducts in contrast to the THA and DHA adducts.

5.4.5 H-bonding Interactions

The crystal structures of the guanine adducts **4**, **5**, **6** and **7** show that en NH protons are involved in stereospecific H-bonding. The average $\text{N}\cdots\text{O}$ distance of 2.8 Å and $\text{N-H}\cdots\text{O}$ angle of 163° are indicative of the strong $\text{N-H}\cdots\text{O}$ hydrogen-bonding between one en NH(d) proton and the G O6 oxygen (Table 5.4). H-bonding interactions between the other en NH(d) proton and ribose O(5') in the Guo adduct **5** are not present since the $\text{N}\cdots\text{O}$ distances, 3.7 Å in cation **I** and 5.3 Å in cation **II**, are long as in the reported Pt-Guo adducts.⁶⁶ The two NH(u) protons of en are not involved in the intramolecular H-bonding with G O6 but in relatively weak H-bonding with the counterion PF_6^- or with G O6 of a neighbouring molecule. Such stereo-specific H-bonding suggests that the en ligand may locate in a specific position to H-bond to the neighboring exocyclic oxygen atoms of the bases (or a phosphate group) and thus influence the site recognition of $\{(\eta^6\text{-arene})\text{Ru(II)(en)}\}^{2+}$

when covalently bound to DNA. The site-recognition ability of octahedral metallointercalators has been improved by the introduction of aliphatic ancillary ligands. For example, $[\text{Rh}(\text{NH}_3)_4\text{phi}]^{3+}$, $[\text{Rh}(\text{en})_2\text{phi}]^{3+}$ and $[\text{Rh}[12]\text{aneN}_4\text{phi}]^{3+}$ (phi = 9, 10-phenanthrenequinone diimine) bind to 5'-GC-3' sites. This sequence specificity is related to H-bonding between the axial amines of these metal complexes and the O6 of G.²³ The crystal structure of $\Delta\text{-}\alpha\text{-}[\text{Rh}[(\text{R},\text{R})\text{Me}_2\text{trien}]\text{phi}]^{3+}$ bound to 5'-GTTGCAAC-3' clearly shows sequence-specific interactions between the thymine methyl group and DNA, including H-bonding between axial amines and G O6 with an average N...O distance of 2.9 Å and H-N...O angle of 165°. ²⁴ Platinum am(m)ine complexes also show H-bonding involving am(m)ine ligands in their nucleobase adducts in the solid state. For example, there is intermolecular H-bonding between the G O6 and the en NH of a neighboring molecule in $[\text{Pt}(\text{en})(\text{Guo})_2]\text{Cl}_{1.5}\text{I}_{0.5}\cdot 2\text{H}_2\text{O}$,^{56b} weak intramolecular H-bonding between an ammine NH and a G O6 in *cis*- $[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{CpGpG})\text{-N7(2),N7(3)}\}]^{2+}$,⁶⁷ and weak intermolecular ammine-O6 H-bonding but stronger intramolecular H-bonding with the terminal 5'-phosphate in *cis*- $[\text{Pt}(\text{NH}_3)_2\{\text{d}(\text{pGpG})\text{-N7(1),N7(2)}\}]^{2+}$.⁶⁰ Such H-bonding interactions of NH ligands are thought to play a major role in determining the nature of platinated DNA adducts,^{26a} and this may be an important factor for the antitumour activity.⁶⁸ Similarly, en NH H-bonding may be important for the activity of Ru(II) arene complexes. Moreover, Ru-N bonds form the legs of the piano stool and impose much tighter steric constraints in comparison with planar Pt am(m)ine complexes. The NH(d) but not NH(u) protons are correctly oriented for intramolecular H-bonding with G O6. This strong intramolecular NH...O6 H-bonding may partly account for the high selectivity of $\{(\eta^6\text{-arene})\text{Ru(II)(en)}\}^{2+}$ binding to guanine with little binding to adenine, cytosine, or thymine. Although many reported Pt and Ru complexes also bind covalently to adenine derivatives,^{26a,1} this does not appear to be the case for $[(\eta^6\text{-arene})\text{Ru(en)Cl}]^+.$ ⁷

Guanine is involved in intermolecular N2H...N3 base pairing in the three 9EtG adducts **4** (Figure 5.1A), **6** (Figure 5.7) and **7** (Figure 5.8). Such dimerisation has been observed in a number of Pt(II) crystal structures containing purine derivatives.⁶⁹ In the case of complex **5**, base pairing is not observed; instead, the NH2 of G participates in H-bonding with O(32) of the sugar from a neighbouring cation, and with O(19)W of water (Table 5.4B). The overall structure of **5** contains a complicated H-bonding network involving the en group, purine, PF₆⁻ ion and water molecules (9 per unit cell). Water is an important participant in intercalation complexes, and the specific binding of water to DNA complexes can make a significant contribution to the free energy of drug binding.⁷⁰ The presence of numerous water molecules together with PF₆⁻ ions contribute to the stabilisation of the columnar-type stacking in [(η⁶-biphenyl)Ru(en)(Guo-*N7*)](PF₆)₂·3.75H₂O (**5**). The 9EtG adducts **4**, **6** and **7** also form interesting polymeric structures in the solid state. Figure 5.8 shows the right-handed helical chain-type structure of **7**, which displays G-G base pairing, intramolecular N-H...O6 H-bonds, intermolecular N-H...O6 H-bonds and intramolecular stacking of ring C with the purine of 9EtG.

Attempts to prepare X-ray-quality single crystals of [(η⁶-biphenyl)Ru(en)(5'-GMP-*N7*)] (**8**) were not successful, but NMR studies allowed investigation of NH H-bonding interactions in aqueous solution. ³¹P and ¹⁵N-edited ¹H NMR and 2D [¹H, ¹⁵N] NMR methods have been used previously to detect NH H-bonding to 5'-phosphate and G O6 groups in Pt-am(m)ine adducts of G derivatives.^{26d,71,72,73} Similarly, ¹⁵N-edited ¹H{¹⁵N} NMR and ³¹P NMR spectroscopy together with pH titration methods were used to investigate the role of en NH protons in ¹⁵N-**8**.

With increase in pH from 2.8 to 10.9, the two NH(u1, u2) protons of ¹⁵N-**8** exhibit a similar pattern of chemical shift changes, becoming slightly deshielded, whilst the two NH(d1, d2) protons also exhibit the same behaviour but become greatly shielded (Figure 5.22). Similar data have been reported for [Pt(¹⁵N-en)(5'-

AMP)₂]²⁺, for which one en NH is capable of H-bonding with 5'-phosphate and becomes greatly shielded, but the other proton on the same en N is incapable of H-bonding and becomes slightly deshielded as the pH increases.^{26d} The NH(d1, d2) protons of **8** point towards 5'-GMP as shown in the solution structure of **8** (Figure 5.16), and are capable of H-bonding with the 5'-phosphate⁷⁴ and G O6. This H-bonding is promoted by deprotonation of the 5'-phosphate and N1H groups.^{71b} The pK_a of the 5'-phosphate group of **8** is 5.93, 0.31 units lower than that of free 5'-GMP (6.24, Figure 5.23B), attributable to the increased stabilization of deprotonated phosphate via H-bonding. This can be compared to a lowering of the 5'-phosphate pK_a value for [Pt(en)(5'-GMP)₂]²⁺ by 0.38 units,^{26d} and for [Pt(en)(5'-AMP)₂] by 0.4 units.⁷⁵ The pK_a of G N1H of **8** is 8.45, 1.28 units lower than that of free 5'-GMP (9.73⁷⁶). For comparison, the pK_a of G N1H is lowered by ca 0.9-1.3 when guanine derivatives are coordinated to Pt(II) via N7,⁷⁷ e.g. 1.1 units for [Pt(dien)(5'-GMP)].^{71b} Lowerings of 0.8 for [Ru(II)(NH₃)₅(HGuo)]²⁺ and of 2.2 for [Ru(III)(NH₃)₅(HGuo)]³⁺ have been reported. The acidifying effect of Ru(II) is therefore lower than that of Ru(III), attributable not only to the lower charge of Ru(II), but also to a smaller degree of back donation of electron density.^{2,78} The larger lowering of pK_a (1.28) in complex **8** may be associated partly with N7 coordination to Ru(II) and partly with strong (N1⁻)-C=O (N1=C-O⁻) H-bonding with en NH.²

At pH > 7.53, NH(d1) and NH(d2) resonances of ¹⁵N-**8** both became too broad to observe. This may be due to more restricted rotation of G about Ru-N7 due to the formation of stronger H-bonding at high pH or due to base-catalysed proton exchange.⁷⁹ The effect of restricted rotation of G on the broadening of en NH(d1, d2) resonances was also observed for [(η⁶-biphenyl)Ru(II)(¹⁵N-en)(Guo-N7)]²⁺ (¹⁵N-**5**). The two NH(d) resonances disappeared when temperature was below 328 K, but sharpened above 328 K due to faster G rotation (see Figure 5.13A and Table 5.10).

The NH(d1, d2) signals of ^{15}N -**8** also disappeared at $\text{pH} < 5.96$ (Figure 5.22), perhaps due to overlap with the water resonance. The en NH resonances of $[\text{Pt}(^{15}\text{N}\text{-en})(5'\text{-GMP})_2]$ and $[\text{Pt}(^{15}\text{N}_2\text{-dien})(5'\text{-GMP})]$ have been reported to disappear, but outside a much wider pH range (3-9.5).^{26d,71b} The pH dependence of the chemical shift of NH(u1, u2) of ^{15}N -**8** show little evidence of strong H-bonding with the 5'-phosphate or G O6 groups. This result is in accordance with the X-ray crystal structures of $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(9\text{EtG-N7})]^{2+}$ (**4**) and $[(\eta^6\text{-biphenyl})\text{Ru}(\text{en})(\text{Guo-N7})]^{2+}$ (**5**): NH(u1, u2) are on the opposite side to NH(d1, d2) and point away from 5'-GMP. The high stereo-specificity leads to the formation of strong H-bonds.

5.5 Conclusions

Organometallic Ru(II) complexes have been designed which incorporate several DNA recognition features. The Ru(II) center has a reactive coordination site and can form monofunctional adducts with DNA. The π -bonded arene ligand has potential to form hydrophobic and van der Waals contacts with nucleobases, and the diamine ligand has NH groups available for H-bonding.

The structures of monofunctional adducts of $[(\eta^6\text{-arene})\text{Ru}(\text{II})(\text{en})\text{Cl}][\text{PF}_6]$ (arene = Bip, **1**; THA, **2**; DHA, **3**) with guanine derivatives have been determined in the solid state by X-ray crystallography, and in solution using 2D [^1H , ^1H] NOESY and [^1H , ^{15}N] HSQC NMR methods. These guanine adducts show interesting features, including Ru(II) binding to guanine N7, stereospecific hydrogen-bonding of en NH with G O6 (with average N...O distance 2.8 Å), and strong arene-nucleobase stacking (at distances of 3.3 - 4.2 Å). The presence of arene-nucleobase interactions suggests that the η^6 -arene ligand could intercalate into DNA. The arene ligands especially process conformational flexibility through rotation around the arene-Ru π -bonds, propeller twisting for biphenyl, and hinge bending for the anthracene derivatives THA and DHA. These make simultaneous arene-nucleobase stacking and

N7-covalent binding compatible. The conformational flexibility can also reduce the steric requirements of the Ru drug and enhance DNA affinity. Stereospecific H-bonding to G O6 involves only en NH(d) protons (oriented away from the arene) and not NH(u) protons (oriented toward the arene) and partly accounts for the high preference of $\{(\eta^6\text{-arene})\text{Ru(en)}\}^{2+}$ for binding to G versus A.

These model compounds suggest a new potential DNA-binding mode for ruthenium anticancer drugs, involving simultaneous intercalation and covalent coordination with DNA. Such features may lead to highly specific recognition of bases in DNA duplexes, and may be useful for optimising the anticancer activity of organometallic Ru(II) arene anticancer complexes. Initial studies using CD, LD, and melting experiments⁸⁰ suggest that monofunctional binding of Ru(II) arene can cause significant perturbation to the structure of calf thymus DNA, accompanied by intercalation in some cases.

5.6 Reference

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Chapter 6

Highly Selective Binding of Organometallic Ruthenium Ethylenediamine Complexes to Nucleic Acids: Novel Recognition Mechanisms

6.1 Introduction

Studies of the molecular recognition of DNA and RNA are of fundamental importance in post-genomic chemistry. The rational design of new DNA-binding agents that recognise specific sequences or structures, and can modify specific DNA functions such as replication and transcription, provides an effective approach for development of novel chemotherapeutic anticancer drugs.¹ Noncovalent interactions play key roles in biological molecular recognition.² Metal coordination, combined with, for example, hydrogen bonding, hydrophobic and electrostatic interactions, can effectively enhance site- and base-selective recognition of nucleic acids. Selective coordination to nucleobases is influenced by structural and electronic factors arising from the nucleobase and the complex itself.³ Both hydrogen-bonding and non-bonding repulsive interactions between the exocyclic groups on the bases and the chelate ligands in metal complexes can lead to selective reactions. Hydrophobic interactions can increase DNA affinity and give rise to highly specific recognition of DNA base sequences.⁴ Zn(II) macrocyclic tetraamine (1, 4, 7, 10-tetraazacyclododecane, cyclen) complexes with quinolyl or naphthyl pendants selectively bind to AT-rich regions of DNA mainly via Zn(II)-N(3)(thymine) coordination,⁵ whereas incorporation of anthraquinone pendants into the cyclen ring allows recognition of G bases via a dominant π - π stacking interaction.⁶ There is precedent for incorporating potential DNA intercalators into metal anticancer complexes. Examples include $[\text{Pt}(\text{AO})(\text{CH}_2)_6(\text{en})\text{Cl}_2]^+$, *cis*- $[\text{Pt}(\text{NH}_3)_2(\text{N9-AA})]$ (AO = acridine orange; N9-AA = 9-aminoacridine),^{7,8} and *trans*- $[\text{PtCl}_2(\text{NH}_3)\text{L}]$ (L = planar aromatic N donor such as pyridine, thiazole, or quinoline),⁹ where the additional intercalation or hydrophobic interaction after covalent binding of the Pt to nucleobases can lead to enhanced activity.

Organometallic ruthenium(II) complexes of the type $[(\eta^6\text{-arene})\text{RuCl}(\text{X})(\text{Y})]$ (X, Y are monodentate or chelating ligands) are cytotoxic to cancer cells, including

cisplatin-resistant cell lines. Preliminary structure-activity data have shown that compounds are active when X, Y belong to a chelating diamine such as ethylenediamine (en) or its derivatives containing NH groups in the amine. The complex $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]\text{PF}_6$ is active *in vivo* against the A2780 xenograft model of human ovarian cancer, and is also active against A2780cis, the cisplatin-resistant xenograft.¹⁰ The cytotoxicity increases with increase in size of the η^6 -arene. For example, complexes which contain η^6 -tetra- or di-hydroanthracene rings (THA, DHA) or biphenyl (Bip) have much higher activities than compounds containing a single ring such as η^6 -benzene (Ben) or η^6 -cymene (Cym).^{10,11} A potential target for these organometallic Ru(II) complexes is DNA, and the preferred binding site appears to be guanine.¹¹ The structures of guanine adducts of THA, DHA and Bip complexes and show stereospecific hydrogen-binding of en NH with G O6, and strong arene-nucleobase stacking, both in the solid state by X-ray crystallography, and in solution by NMR methods.^{12,13} These studies have revealed several DNA recognition features and suggested a potential DNA-binding mode, involving simultaneous intercalation and covalent coordination. The diamine NH group and hydrophobic arene group as well as the Cl leaving group may therefore all play important roles in anticancer activity.

It is now important to understand how stereospecific hydrogen-bonding can lead to specific DNA site-recognition and whether arene-purine hydrophobic interactions influence the kinetics of DNA binding. Therefore, both the thermodynamics and kinetics of the binding of $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{X}]^{n+}$ complexes (arene = tetra-, di-hydroanthracene (THA, DHA), biphenyl (Bip), *p*-cymene (Cym) and benzene (Ben); X = Cl or H₂O, Chart 6.2) to mononucleotides and mononucleosides (G, I, A, C, and T, Chart 6.1) have been studied by ¹H, ³¹P, ¹⁵N (¹⁵N-en) NMR spectroscopy, together with pH titrations. These studies show that these novel organometallic ruthenium ethylenediamine anticancer complexes are

highly selective in their recognition of nucleic acid bases and that the nature of the coordinated arene has a dramatic effect on the kinetics of binding. Such insights can direct new synthetic work for optimization of drug activity.

Chart 6.1 Structures and atom numbering for nucleobases and their derivatives.

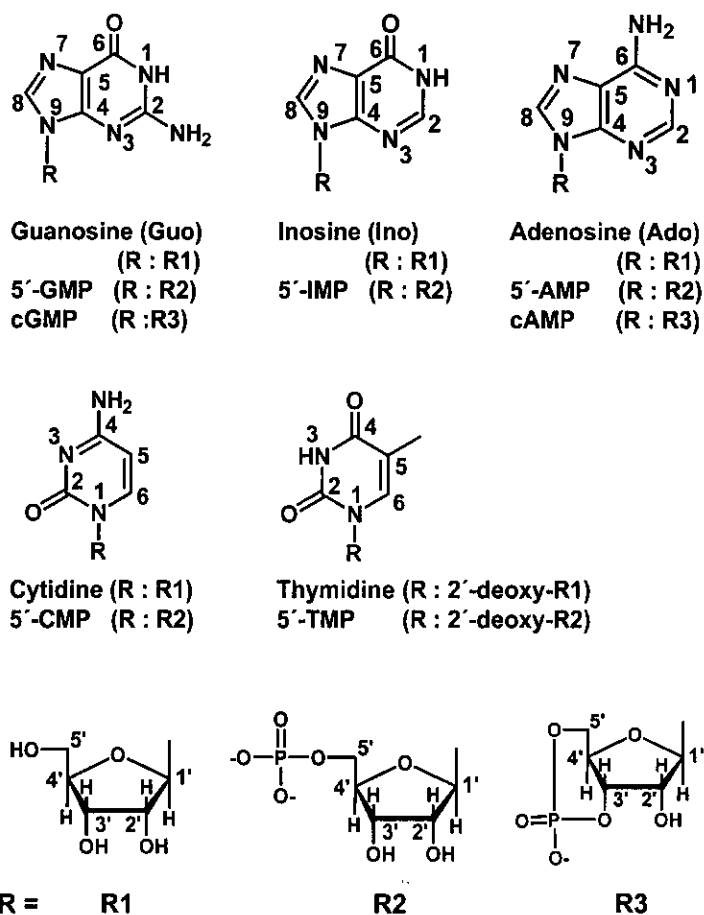
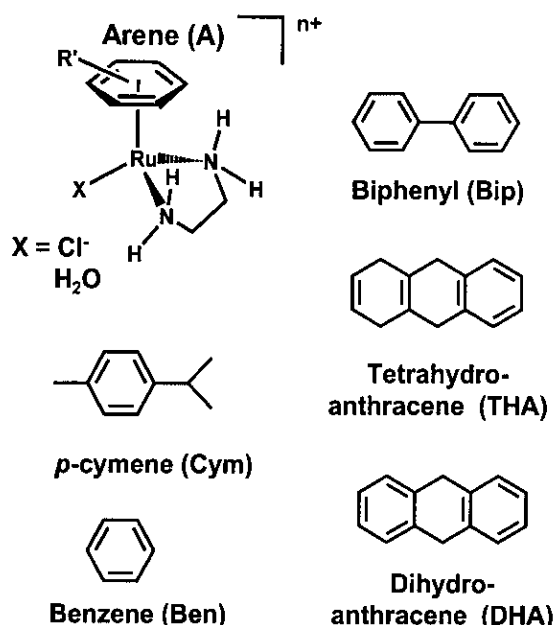


Chart 6.2 Arene complexes studied in this work

6.2 Experimental Section

6.2.1 Materials

Chloro complexes $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$ (arene = Bip, THA, DHA, Cym, and Ben, Chart 6.2) were synthesized as described previously.^{11,12} The mononucleotides 5'-GMP, 3'-GMP, 3', 5'-cyclic GMP, 5'-IMP, 5'-AMP, 3', 5'-cyclic AMP, 5'-CMP and 5'-TMP (all as sodium salts), and the nucleosides guanosine, inosine, adenosine, cytidine and thymidine were purchased from Sigma or Aldrich. The purity was checked by ^1H and ^{31}P NMR spectroscopy. D_2O (99.98%) was obtained from Aldrich. All NMR experiments were performed in 10% D_2O /90% H_2O .

6.2.2 Preparation of Aqua Ru(arene) Complexes

An aliquot of a 100 mM aqueous solution of AgNO_3 (0.98 mol equiv) was added to a solution of $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$, giving a yellow-orange solution and an immediate precipitate of AgCl . This mixture was kept at ambient temperature overnight (protected from light) and was then filtered to remove AgCl . The resulting yellow solutions¹⁴ were used freshly and directly for reactions with 5'-GMP and cGMP.

6.2.3 pH Titrations

For the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (12 mM) with 5'-GMP (12 mM), ^1H and ^{31}P NMR pH titrations were performed at 298 K within the first six hours, in order to record ^1H and ^{31}P NMR resonances for the intermediates $\text{Ru-O}(\text{PO}_3)\text{GMP}$ and $\text{Ru-O}(\text{PO}_3)\text{GMPN7-Ru}$ (Scheme 6.1).

For the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (8 mM) with cGMP (9 mM), the mixture was incubated at 298 K for 24 h. This sample was then used directly for recording ^1H and ^{31}P NMR spectra at various pH values.

For the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (12 mM) with 5'-IMP (14 mM), the mixture was incubated at 298 K for 24 h, followed by addition of a 14% molar excess of Ru. This mixture was used for a pH titration, which was completed within a further four hours. The resultant ^1H and ^{31}P NMR spectra were recorded. The H8 and H2 chemical shifts of all six IMP species were subsequently plotted as a function of pH.

For the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (5 mM) with inosine (6 mM), the mixture was incubated at 298 K for 12 h. ^1H NMR spectra were then recorded at various pH values.

For reactions of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (10 mM) with 5'-CMP, 5'-TMP or thymidine (10 mM), the mixtures were incubated at 310 K for 48 h, and ^1H and ^{31}P NMR spectra were then recorded at various pH values.

For reactions of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-AMP, ten parallel experiments were performed in ten NMR tubes under the similar conditions with various pH values over the range of 3-10. For each sample, the mixture of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ and 5'-AMP (1:1, 5 mM) with an adjusted pH was incubated at 310 K for 24 h, after which the pH value was determined again and used as the equilibrium pH value. Subsequently, ^1H and ^{31}P NMR spectra were recorded. The amount of phosphate-bound adduct at each of the ten equilibrium pH values was determined by integration of either ^1H or ^{31}P NMR peaks.

6.2.4 Competitive Reactions of 5'-GMP, 5'-AMP, 5'-CMP, and 5'-TMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$

5'-GMP was mixed with one of three other nucleotides in a 1:1 mol ratio (5 mM, GMP + AMP, GMP + CMP, or GMP + TMP). Each of the combinations was then added to an aqueous solution of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (final concentration 5 mM). The reaction mixtures were monitored immediately by ^1H and ^{31}P NMR spectroscopy at 298 K or 310 K. The amounts of free and Ru-bound nucleotides were determined by peak integration.

6.2.5 Kinetic Studies

The reactions between chloro or aqua Ru(arene) complexes and nucleotides (5'-GMP, cGMP, 5'-IMP, 5'-AMP, cAMP, 5'-CMP, or 5'-TMP), or nucleosides (guanosine, inosine, adenine, cytosine or thymidine), were carried out at a concentration of 5 mM Ru and 5 mM nucleobase in 10% D_2O /90% H_2O in NMR tubes. Most solutions were unbuffered to avoid interreaction of buffer components with Ru(II). The solution pH was determined at the commencement of the reaction and after the establishment of equilibrium. The kinetic studies of various chloro or aqua Ru(arene) complexes with cGMP were carried out in 100 mM NaClO_4 at 298

K, pH 7.0. Due to the poor aqueous solubility of chloro Ru(THA) and Ru(DHA), the kinetics of reactions of various chloro Ru(arene) complexes with cGMP were performed at a concentration of 2 mM for both reactants. ^1H , ^{31}P NMR or ^{15}N NMR data were recorded at appropriate time intervals. The first spectrum was acquired 10–15 min after mixing of the reactants, to allow time for temperature equilibration of the sample in the probe. Quantitation of the reactions was achieved by integration of the peaks for the base protons H8 (G), H8 and H2 (I and A), H6/H5 (C) and C(5)H₃ (T), or by integration of ^{31}P NMR peaks, or [^1H , ^{15}N] cross-peaks.

6.2.6 Methods and Instruments

NMR Spectroscopy. ^1H NMR spectra were acquired on a Bruker DMX 500 (^1H = 500 MHz) NMR spectrometer using TBI [^1H , ^{13}C , X] or triple resonance [^1H , ^{13}C , ^{15}N] probe-heads, respectively, and equipped with z-field gradients.

1D ^1H NMR spectra were typically acquired with 64 transients into 32 K data points over a spectral width of 6.0 kHz. Water suppression was achieved by presaturation. ^{31}P NMR spectra were obtained on a Bruker DMX 500 (^{31}P = 202 MHz) NMR spectrometer at 298 K, using inverse-gated ^1H decoupling. Typically 1D ^{31}P - $\{^1\text{H}\}$ NMR spectra were acquired with 512 transients into 42 K data points over a spectral width of 40.7 kHz (200.8 ppm) using a relaxation delay of 1.3 s. Both 1D ^{15}N -edited ^1H NMR spectra and 2D [^1H , ^{15}N] HSQC NMR spectra (optimised for $^1J_{(\text{NH})} = 73$ Hz) were acquired using the sequence of Stonehouse *et. al.*¹⁵ A GARP decoupling sequence was used for ^{15}N decoupling during acquisition. Typically 1D ^1H - $\{^{15}\text{N}\}$ NMR data were acquired with 16 transients into 1024 data points over a spectral width of 2.5 kHz (5.0 ppm) with a relaxation delay of 2.0 s. 2D [^1H , ^{15}N] HSQC NMR data were acquired with 4 transients into 1024 data points over a ^1H (F2) spectral width of 2.5 kHz (5 ppm, centered at the water resonance) for each of 256 t_1 increments (TPPI), and over a ^{15}N (F1) spectral width of 0.76 kHz (15 ppm,

centered at -28 ppm) using a relaxation delay of 1.4 s between transients. Typically, 1D $^1\text{H}\{-^{15}\text{N}\}$ NMR spectra were acquired over a 10 min period and 2D [^1H , ^{15}N] HSQC NMR spectra were acquired over a 30 min period.

All data processing was carried out using XWIN-NMR version 2.0 (Bruker U.K. Ltd.). ^1H NMR chemical shifts were internally referenced to 1, 4-dioxane (3.77 ppm) or to the methyl singlet of TSP (0 ppm) in aqueous solutions. ^{31}P resonances were referenced to 85% H_3PO_4 (external) at 0 ppm, and ^{15}N resonances to 1 M $^{15}\text{NH}_4\text{Cl}$ in 1.5 M HCl (external) at 0 ppm.

pH Measurements. The pH values of NMR samples in 10% $\text{D}_2\text{O}/90\%$ H_2O were measured at 298 K directly in the NMR tube, before and after recording NMR spectra, using a Corning 240 pH meter equipped with an Aldrich micro combination electrode calibrated with Aldrich buffer solutions at pH 4 , 7 , and 10 . The pH values were adjusted with dilute HClO_4 and NaOH . No correction has been applied for the effect of deuterium on the glass electrode.

Calculation of pK_a Values. The pH titration curves were fitted to the Henderson-Hasselbalch equation using the program KALEIDAGRAPH,¹⁶ with the assumption that the observed chemical shifts are weighted averages according to the populations of the protonated and deprotonated species.

Determination of Rate Constants. The reactions of aqua $\text{Ru}(\text{arene})$ complexes with cGMP ($1:1$, 5 mM) ($\text{Ru-H}_2\text{O} + \text{cGMP} \rightarrow \text{Ru-N7cGMP}$) were analysed as second-order processes (see Figure 6.12). The second-order rate constants (k) were determined by fitting kinetic curves of concentration versus reaction time for formation Ru-N7cGMP using the appropriate equation and the program Scientist (see Figure 6.13).

6.3 Results

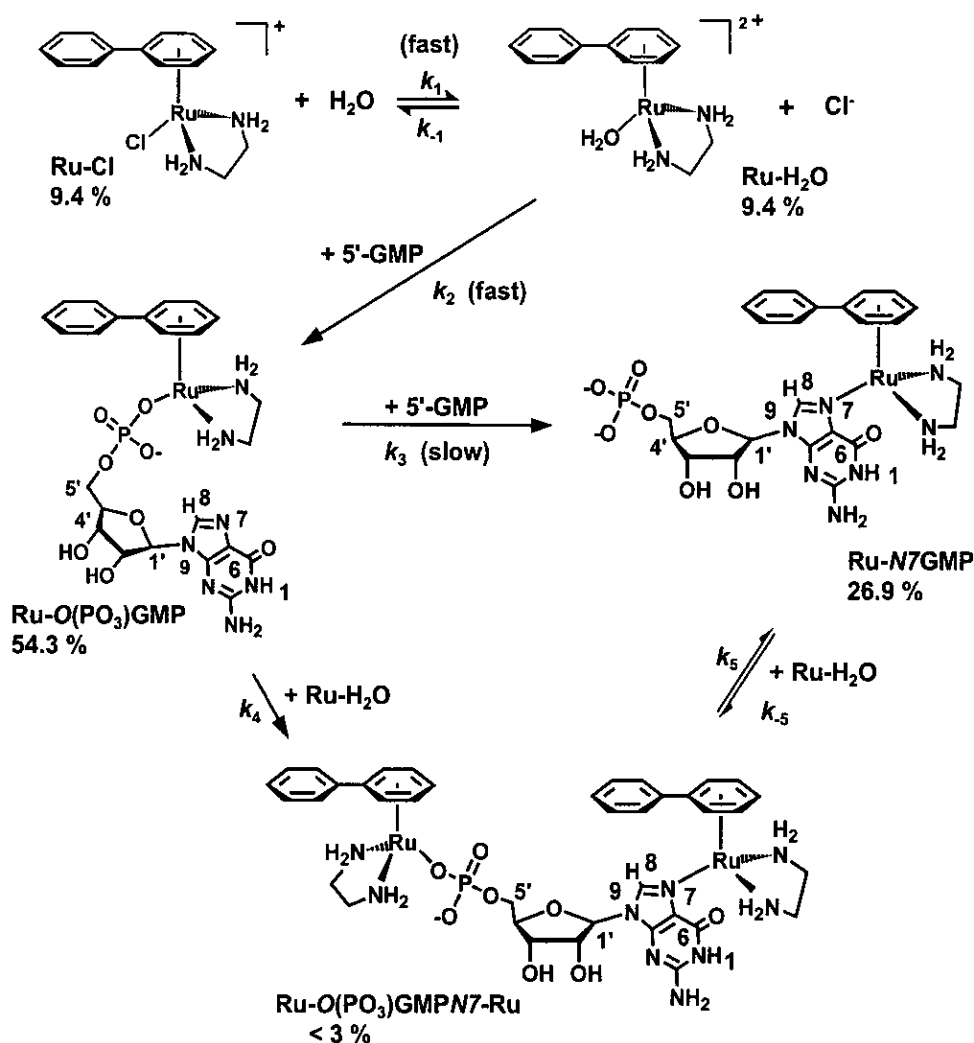
6.3.1 General

In order to investigate the specificity of the binding of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ to nucleobases, reactions of the anticancer complex $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with various nucleosides and nucleotides (see Chart 6.1) in aqueous solution were studied by ^1H , ^{15}N and ^{31}P NMR spectroscopy. The binding sites for $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ on nucleobase derivatives were identified by ^1H NMR and ^{31}P NMR pH titrations, and each adduct was characterized by pK_a values derived from the pH titration curves. Some competition experiments were also performed to study the relative binding preferences of $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ towards mononucleotides. The influence of pH, chloride and phosphate on the rate of binding of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\text{X}\}^{n+}$ (arene = Bip, THA, DHA, Cym, or Ben, and X = Cl or H_2O) complexes to 5'-GMP and cGMP was also investigated by ^1H and ^{31}P NMR. The detailed kinetics for the reactions with cGMP were analyzed.

6.3.2 Reactions of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with Nucleotides and Nucleosides

Guanine Forms only N7 Adducts. Phosphate-bound Intermediates Detected for 5'-GMP but not for cGMP. The H8 region of the NMR spectra of 5'-GMP after reaction with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.15, 298 K) for 0.25–22 h are shown in Figure 6.1A. Three new H8 signals were observed: peak b (δ 8.027) appeared rapidly (during the reaction mixing time) and was shifted to low frequency relative to free 5'-GMP (peak a, δ 8.175), but disappeared at later times; peak d at higher frequency (δ 8.453) was evident after 2 h and finally disappeared as well; peak c appeared at higher frequency (δ 8.733), increased in intensity with time, and was the only final product observed for this reaction. An identical reaction, which was followed by ^{31}P NMR (Figure 6.1B), revealed corresponding changes in intensities of ^{31}P NMR peaks (a–d) which were consistent with the ^1H NMR results. However, for ^{31}P , there were large shifts in peaks b (δ 9.017) and d (δ 9.237) to high frequency relative to the resonance for free 5'-GMP (peak a, δ 3.899), compared with the slight high frequency shift of peak c (δ

Scheme 6.1 Scheme for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-GMP (5 mM, 1:1) in aqueous solution at pH = 7.15, 298 K. Species distribution (%) is shown after 55 min reaction, and is based on $^{15}\text{NH}_2\text{-en}$ ^1H NMR intensities (except for the dinuclear species which was based on H8 intensities). The intermediate $\text{Ru-O}(\text{PO}_3)\text{GMP}$ (peak b) formed during the reaction mixing time, slowly rearranged into Ru-N7GMP (peak c), and then eventually disappeared after 22 h (see Figure 6.1A). The intermediate $\text{Ru-O}(\text{PO}_3)\text{GMPN7-Ru}$ (peak d) was evident after 2 h, and eventually disappeared after 22 h; it persisted if Ru was present in excess. When more Ru-Cl (50%) was added to the final mixture, more of species d (30%) formed after 24 h at 310 K.



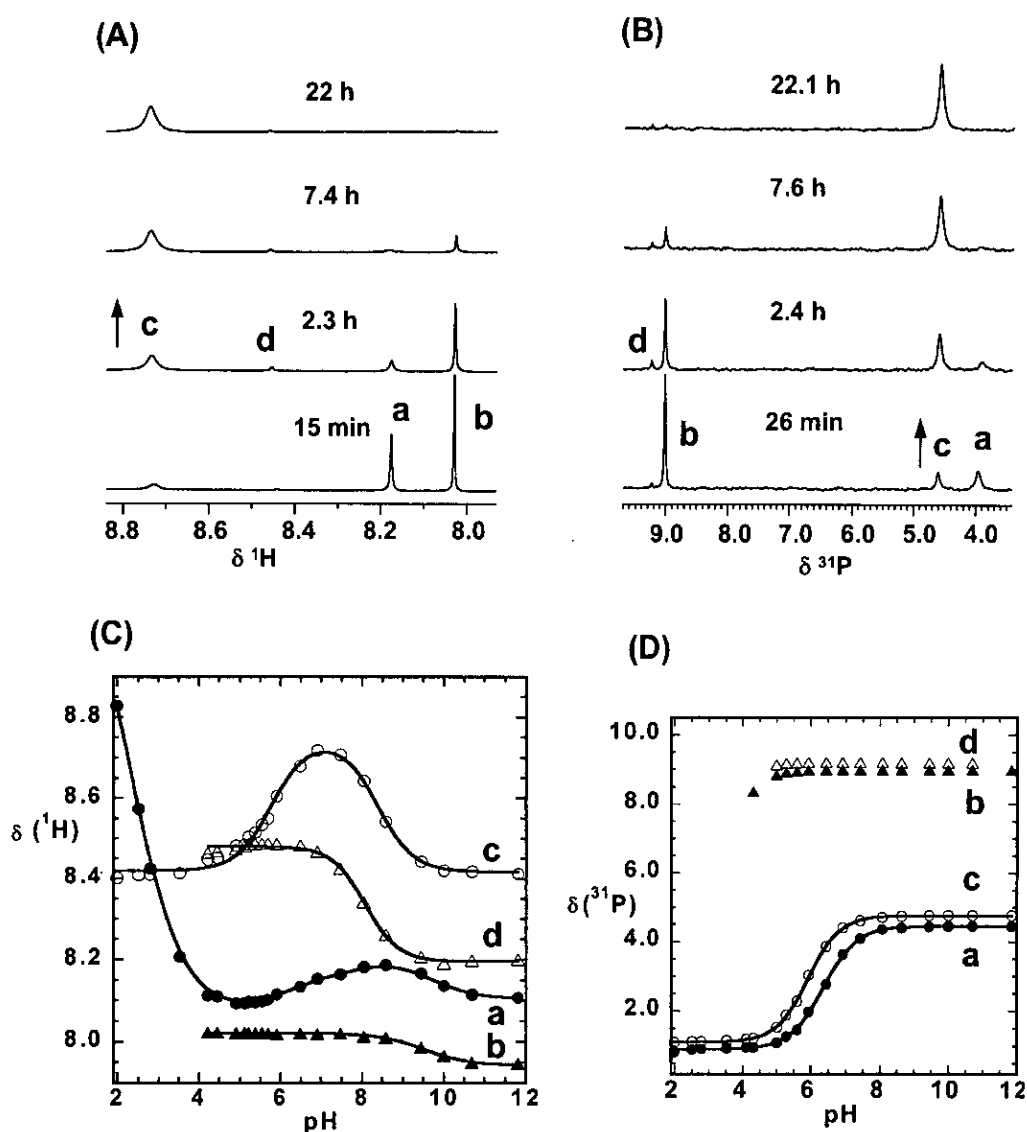


Figure 6.1 (A) ^1H , and (B) ^{31}P NMR spectra for the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-GMP (1:1, 5 mM, pH 7.15, 298 K) at various reaction times. Plots of (C) H8 ^1H NMR chemical shift, and (D) ^{31}P NMR chemical shift vs. pH for 5'-GMP species a-d. The curves are computer-fitted with the pK_a values listed in Table 6.1. The pH titration was performed for a similar reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (12 mM) with 5'-GMP (12 mM). Peak assignments: a, free 5'-GMP; b, $\text{Ru-O}(\text{PO}_3)\text{GMP}$; c, Ru-N7GMP and d, $\text{Ru-O}(\text{PO}_3)\text{GMPN7-Ru}$. See Scheme 6.1 for structures.

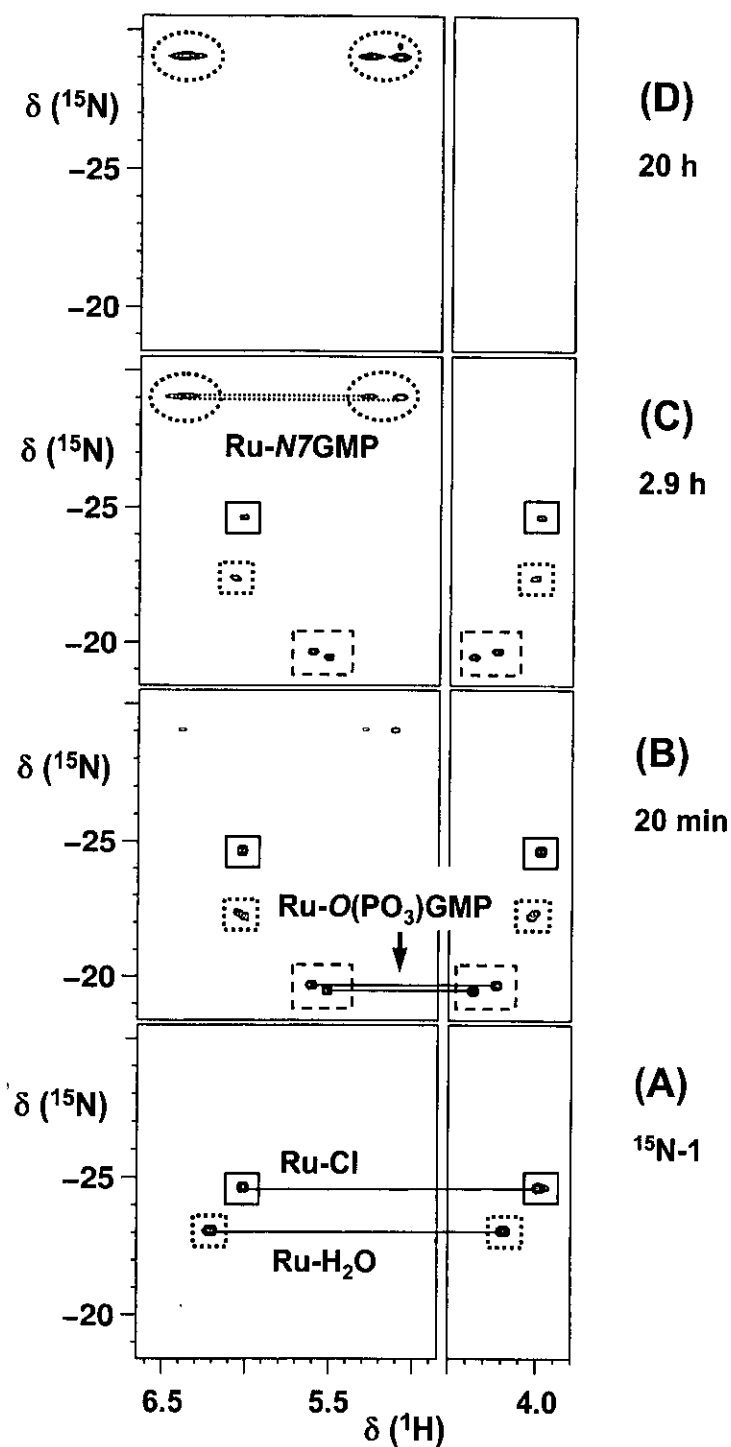


Figure 6.2 2D ^1H , ^{15}N HSQC NMR spectrum of (A) $[(\eta^6\text{-Bip})\text{Ru}(^{15}\text{N-en})\text{Cl}]^+$ (5 mM, pH 6.20) at 298 K, and (B) 20 min, (C) 2.9 h, and (D) 20 h after addition of 5'-GMP (1:1, pH 7.15). The cross-peaks for $\text{Ru-O(PO}_3\text{)GMPN7-Ru}$ are very weak and

not shown. For the chloro and aqua adducts, the two protons on each ^{15}N are non-equivalent, but the two ^{15}N atoms have the same shift, whereas in the GMP adducts, the four NH protons are all non-equivalent. Small changes in the shifts of the cross-peaks for Ru-H₂O in (B, pH 7.15) compared with those in (A, pH 6.20) are due to deprotonation (weighted average of aqua and hydroxo shifts; $\text{p}K_{\text{a}}$ of Ru-H₂O : 7.7).

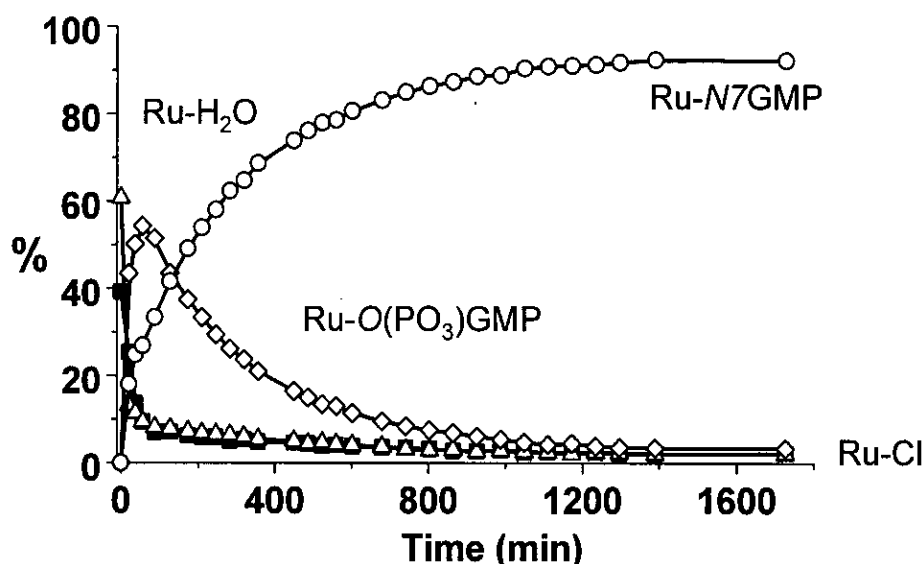


Figure 6.3 Species distribution versus time for the reaction of 5'-GMP with $[(\eta^6\text{-Bip})\text{Ru}(^{15}\text{N-en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.15) at 298 K, as determined from integration of $^{15}\text{NH}_2\text{-en } ^1\text{H}$ NMR peaks (shown in Figure 6.4). Labels: ■, Ru-Cl, Δ, Ru-H₂O, ◇, Ru-O(PO₃)GMP, ○, Ru-N⁷GMP. For structures of species, see Scheme 6.1.

4.482). The reactions were complete within ca. 22 h.

In order to identify the intermediates and products, a pH titration on a similar solution containing 12 mM Ru and 12 mM 5'-GMP was carried out during the first 6 h of reaction. Peaks b and d disappeared at pH values < 4, but the other peaks could be followed over the pH range 2-12. The ^1H and ^{31}P NMR titration curves are shown in Figures 6.1C and 6.1D, and associated pK_a values were determined by computer fits to the Henderson-Hasselbalch equation. The characteristic pK_a values associated with peaks a, b, c and d, allow the intermediates to be assigned as Ru- $\text{O}(\text{PO}_3)\text{GMP}$ (b), Ru- $\text{O}(\text{PO}_3)\text{GMPN7-Ru}$ (d), and final product as Ru- N7GMP (c) (for structures see Scheme 6.1). Their pK_a values are listed in Table 6.1. Three pK_a values were determined for free 5'-GMP: 9.81, 6.49 and 2.57, attributable to the deprotonation of N1H, PO_3H , and N7, respectively. Ru- $\text{O}(\text{PO}_3)\text{GMP}$ (peak b) undergoes deprotonation of N1H (pK_a of 9.51), but no deprotonation of PO_3H . Ru- N7GMP (peak c) undergoes deprotonation of PO_3H (pK_a of 5.77) and N1H (pK_a of 8.39), but no deprotonation of N7. Ru- $\text{O}(\text{PO}_3)\text{GMPN7-Ru}$ (peak d) undergoes deprotonation of N1H, but no deprotonation of PO_3H and N7. Notable is the lowering of the $\text{pK}_a(\text{N1})$ value by 1.42 units for Ru- N7GMP (peak c) and by 1.74 units for Ru- $\text{O}(\text{PO}_3)\text{GMPN7-Ru}$ (peak d) compared to free GMP (peak a).

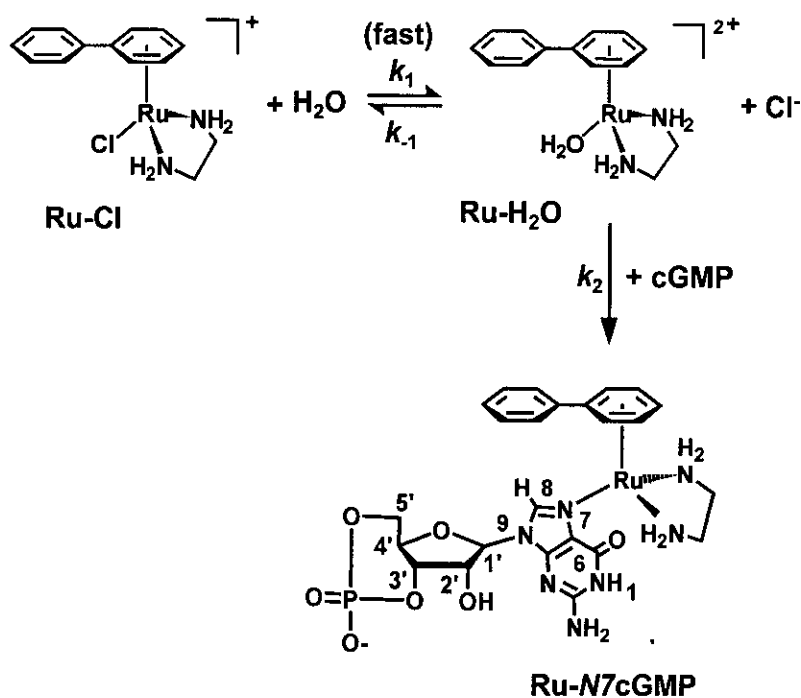
A similar reaction with 5'-GMP was also monitored by 2D [^1H , ^{15}N] HSQC NMR by use of ^{15}N -labeled $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (Figure 6.2). Four species labelled Ru-Cl, Ru- H_2O , Ru- $\text{O}(\text{PO}_3)\text{GMP}$ and Ru- N7GMP were detected during the time course of the reaction (Figures 6.2 and 6.3). This result, together with the ^1H and ^{31}P NMR time courses, allowed reaction pathways to be drawn up, as shown in Scheme 6.1. First, the chloro complex Ru-Cl undergoes hydrolysis in water to give the reactive aqua complex Ru- H_2O , followed by rapid reaction with the 5'-phosphate of 5'-GMP, and then a slow displacement of bound phosphate by N7. The species

distribution determined after 55 min of reaction is shown in Scheme 6.1.

The reaction of 3', 5'-cyclic GMP (cGMP) with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.28, 298 K) was monitored by ^1H and ^{31}P NMR for a period of ca. 12 h. After 11 min, a new H8 ^1H NMR peak (b, δ 8.221) was observed, shifted to high frequency by 0.337 ppm compared to free cGMP (peak a, δ 7.884), Figure 6.4A. This was the only new H8 signal observed, and after 12 h was the only H8 peak in the spectrum. In the ^{31}P spectrum (Figure 6.4B), only one new peak appeared (b, δ -1.022). This peak was shifted to low frequency by only 0.043 ppm relative to that for cGMP (peak a, δ -0.979). A pH titration of a similar reaction solution gave pK_a values of 9.69 and 2.08 for peak a (free cGMP), and a single pK_a value of 8.13 for the product peak b, as derived from the H8 ^1H curves (Figure 6.4C and Table 6.1). Peak b can be assigned as Ru-*N7*cGMP (see Scheme 6.2). pK_a values of 9.67 (peak a) and 8.13 (peak b) are attributable to deprotonation of N1H. The pK_a value of 2.08 (peak a) is attributable to protonation of N7. Notable is the lowering of the pK_a for N1 by 1.54 units relative to free cGMP (peak b). During the reaction, no phosphate-bound adducts were detected. The reaction course is shown in Scheme 6.2. After 10 h, > 95% of the cGMP was present as Ru-*N7*cGMP.

The reaction of guanosine (Guo) with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, 310 K, pH 7.20) was followed by ^1H NMR for 1 d. Only one new peak assignable¹² to Ru-*N7*Guo appeared at high frequency (δ 8.22) relative to free Guo (δ 7.9), and after 14 h, > 97% of Guo was present as Ru-*N7*Guo. A similar reaction was performed at pH 9.29, and only the Ru-*N7*Guo adduct was present after 14 h (Table 6.3).

Scheme 6.2 Scheme for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with cGMP in aqueous solution at pH = 7.28, 298 K.



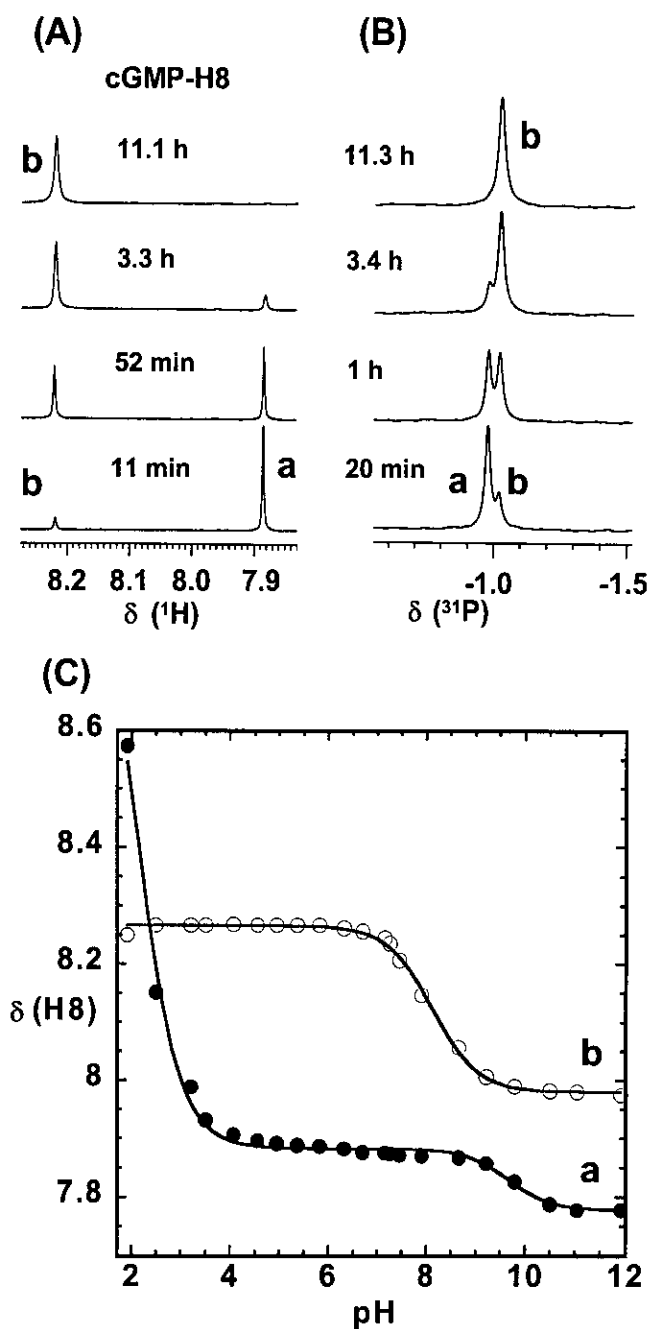


Figure 6.4 (A) ^1H , and (B) ^{31}P NMR spectra for the reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with cGMP (1:1, 5 mM, pH 7.28, 298 K) at various reaction times. Peak assignments: a, free cGMP; b, Ru-*N*7cGMP. See Scheme 6.2 for structures. (C) Plots of H8 ^1H NMR chemical shifts of cGMP species a and b vs. pH for a similar reaction with 8 mM Ru and 9 mM cGMP. The curves are computer best fits giving the pK_a values listed in Table 6.1.

Table 6.1 Selected Chemical Shifts and pK_a Values for 5'-GMP and cGMP and Their $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ Adducts at 298 K.

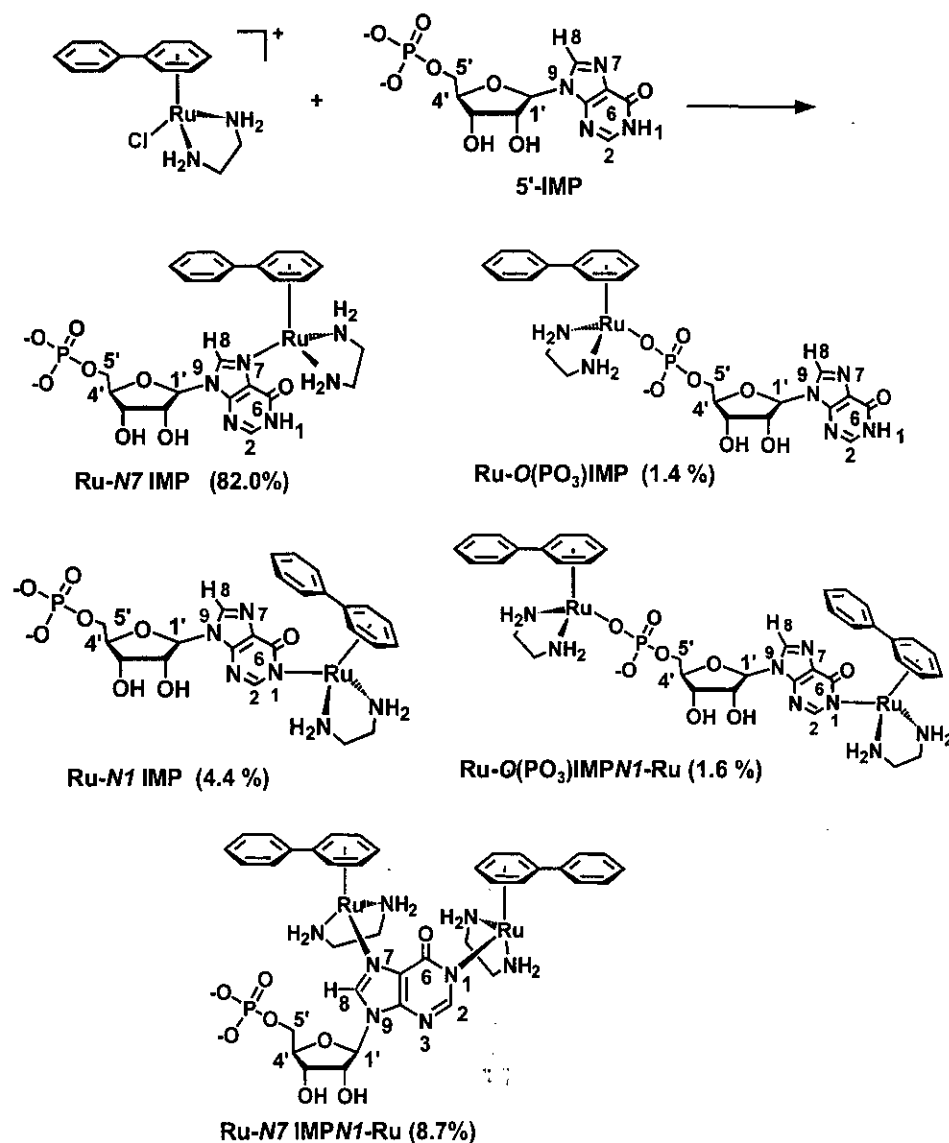
Species ^a (peak) ^b	pK_a (group)	δ (H8) ^c	δ (³¹ P) ^c
5'-GMP (a)	9.81 ± 0.10 (N1H)	8.175	3.899
	6.49 ± 0.18 (-PO ₃ H)		
	2.57 ± 0.01 (N7)		
Ru-O(PO ₃)GMP (b)	9.51 ± 0.05 (N1H)	8.027	9.017
Ru-N7GMP (c)	8.39 ± 0.06 (N1H)	8.733	4.582
	5.77 ± 0.04 (-PO ₃ H)		
Ru-O(PO ₃)GMPN7-Ru (d)	8.07 ± 0.04 (N1H)	8.453	9.237
cGMP (a)	9.67 ± 0.24 (N1H)	7.884	-0.979
	2.08 ± 0.02 (N7)		
Ru-N7cGMP (b)	8.13 ± 0.03 (N1H)	8.221	-1.022

^a For structures see Schemes 6.1 and 6.2. ^b For peak labels see Figures 6.1 and 6.4.

^c For 5'-GMP, pH 7.20 and for cGMP, pH 7.28.

Ru(II) Binds to N7 and N1 of Inosine, and Phosphate-Bound Intermediates are Formed with 5'-IMP. The reaction between 5'-IMP and $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.26) was monitored by ¹H and ³¹P NMR spectroscopy. During the reaction, the pH value dropped from 7.26 (when mixed) to 6.55 (after 3 d at 310 K). The H8 and H2 regions of the ¹H NMR spectra of 5'-IMP at different reaction times are shown in Figures 6.5A-C. ³¹P NMR peak assignments are given in Table 6.2. During the course of the reaction, 5 new sets of H8 and H2 peaks (peaks b, c, d, e and f) were seen. Of these, 3 sets (peaks c, d and e) are due to intermediates: their intensities decreased at later reaction times and almost disappeared after 3 days at 310 K. The intermediates and products were identified by a ¹H NMR pH titration on a similar solution (Figure 6.5D). It can be seen that peaks

Scheme 6.3 Scheme for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-IMP (1:1, 5 mM) in aqueous solution at pH = 7.28, 298 K. Species distribution (%) is shown after 24 h reaction at 310 K, based on H8 and H2 intensities.



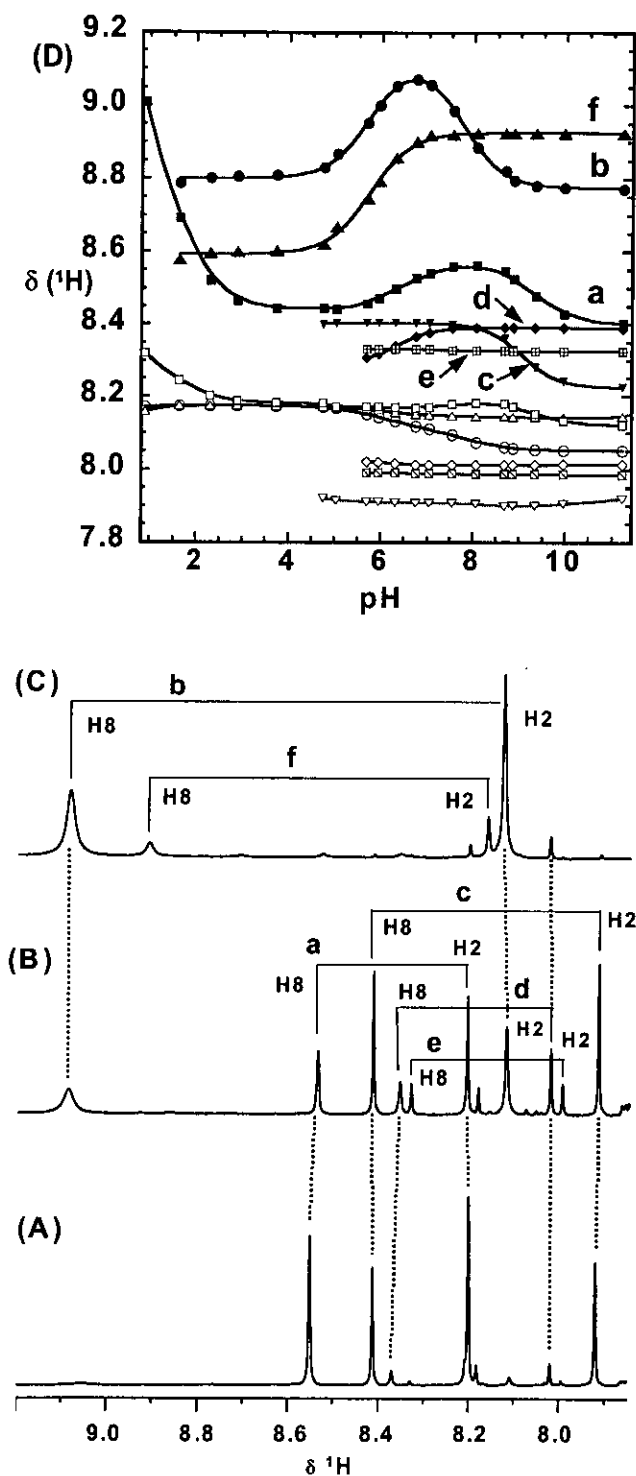
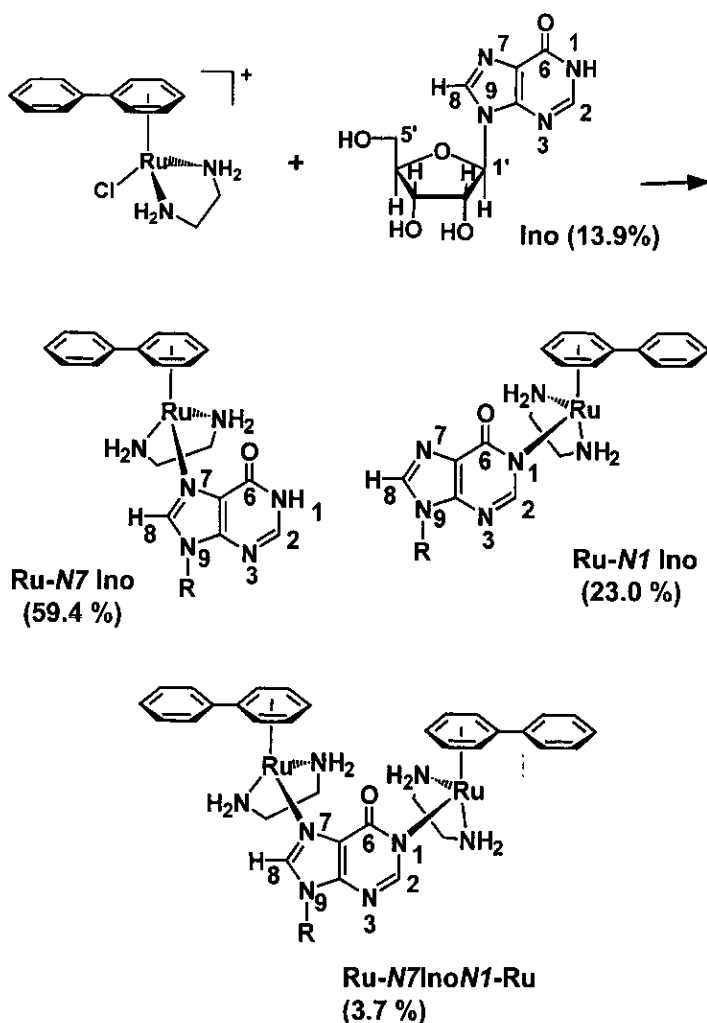


Figure 6.5 The H8 and H2 region of ^1H NMR spectra for the reaction of 5'-IMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, initial pH 7.26) at 298 K after (A) 13 min (pH 7.15), (B) 3.7 h (pH 6.74), and (C) after a further 3 days at 310 K (pH 6.55). (D) Plots showing the variation with pH of H8 (solid symbols) and H2 (open symbols)

^1H NMR chemical shifts for species a-f. pK_a values were determined from H8 shifts. Assignments: a, H8 (■) and H2 (□), free 5'-IMP; b, H8 (●) and H2 (○), Ru-N7IMP; c, H8 (▼) and H2 (▽), Ru-O(PO_3)IMP; d, H8 (◆) and H2 (◇), Ru-N1IMP; e, H8 (⊕) and H2 (⊞), Ru-O(PO_3)IMP/N1-Ru; and f, H8 (▲) and H2 (Δ), Ru-N7IMP/N1-Ru. For structures see Scheme 6.3. Note that d, e and f are N1-bound species.

Scheme 6.4 Scheme for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (5 mM) with inosine (5.5 mM) in aqueous solution at pH = 7.22. Species distribution (%) is shown after 24 h reaction at 310 K, based on H8 and H2 intensities.



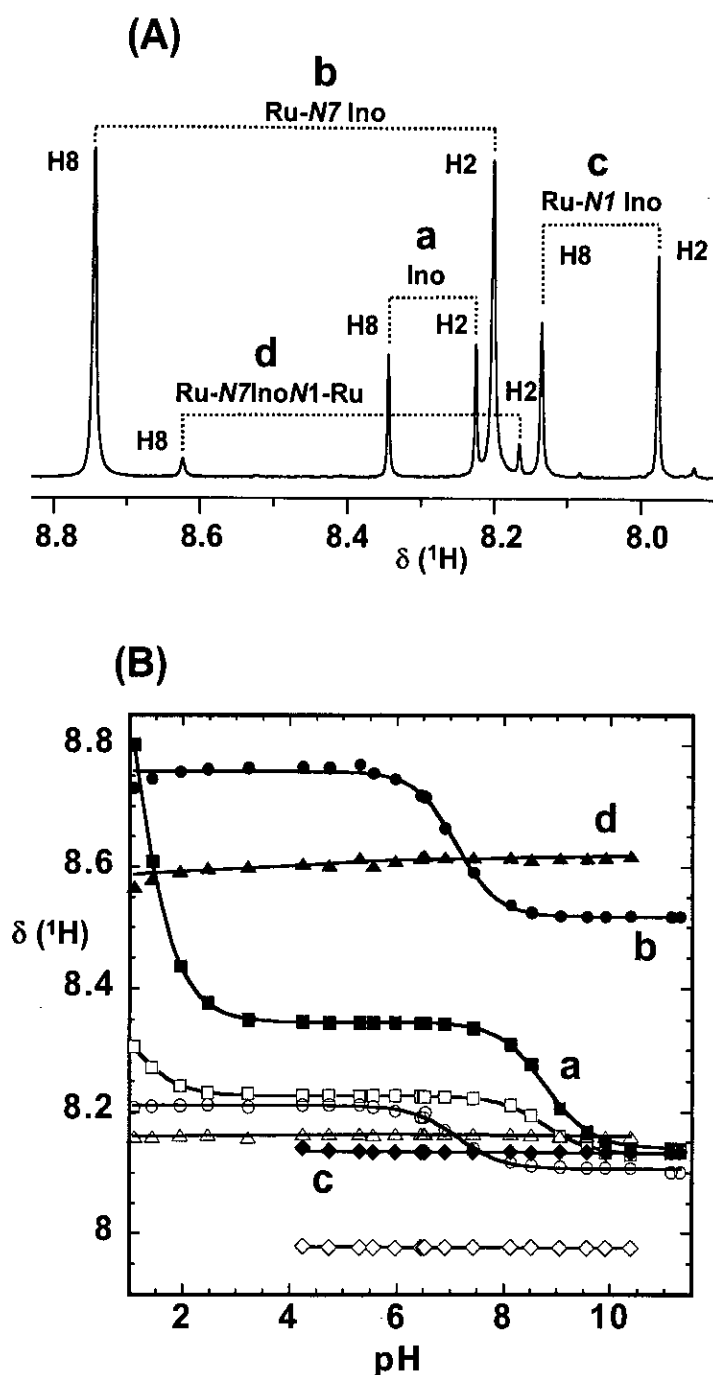


Figure 6.6 (A) The H8 and H2 region of the ^1H NMR spectrum of inosine (5.5 mM) after reaction with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (5 mM) for 24 h at 310 K with initial pH 7.22. (B) Plots of H8 and H2 chemical shifts vs. pH for the five species a-d detected during the above reaction. Peaks labels: H8, solid symbols; H2, open symbols, i.e. a, H8 (■) and H2 (□); b, H8 (●) and H2 (○); c, H8 (◆) and H2 (◇); d, H8 (▲) and H2 (△).

Table 6.2 Selected Chemical Shifts and pK_a Values for 5'-IMP and Inosine (Ino) and Their $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ Adducts at 298 K.

Species ^a (peak) ^b	pK_a (group) ^c	δ (H8) ^d	δ (H2) ^d
5'-IMP (a)	9.22 $\pm$ 0.05 (N1H) 6.36 $\pm$ 0.05 (PO ₃ H) 1.35 $\pm$ 0.01 (N7)	8.497	8.169
Ru-N7IMP (b)	7.74 $\pm$ 0.03 (N1H) 5.71 $\pm$ 0.03 (PO ₃ H)	9.053	8.131
Ru-O(PO ₃)IMP (c)	9.04 $\pm$ 0.03 (N1H)	8.404	7.909
Ru-N1IMP (d)	6.35 $\pm$ 0.05 (PO ₃ H)	8.339	8.016
Ru-O(PO ₃)IMPNI-Ru (e)	—	8.330	7.99
Ru-N7IMPNI-Ru (f)	5.73 $\pm$ 0.03 (PO ₃ H)	8.856	8.155
Ino (a)	8.79 $\pm$ 0.02 (N1H) 0.909 $\pm$ 0.003 (N7)	8.344	8.225
Ru-N7Ino (b)	7.11 $\pm$ 0.04 (N1H)	8.745	8.201
Ru-N1Ino (c)	—	8.135	7.977
Ru-N7Ino NI-Ru (e)	—	8.624	8.165

^a For structures see Schemes 6.3 and 6.4. ^b For peak labels see Figures 6.5 and 6.6. ^c Based on H8 chemical shifts. ^d pH 6.31 for 5'-IMP and pH 6.19 for Ino. For 5'-IMP, the corresponding ³¹P NMR resonances appeared at δ 3.278 for peak a, δ 8.983 for c, and δ 8.272 for e, δ 3.410, 4.393 and 1.612 for peaks b, d and f, but specific assignments were not made.

Table 6.3 Species Distribution and N(7)/N(1) Ratios for Ino and Guo Binding by $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$.

B	pH change over 24 h	Species distribution (%) ^a				N(7)/N(1) ^b
		Free B	Ru- <i>N7B</i>	Ru- <i>N1B</i>	Ru- <i>N7BN1</i> -Ru	
Ino	5.18-5.53	15.4	74.7	8.6	1.3	7.72
	7.22-6.19	13.9	59.4	23.0	3.7	2.36
	10.0-10.30	13.9	15.5	70.0	0.5	0.227
Guo	7.20-7.25	100				
	9.29-9.38	100				

^a Based on integration of ^1H NMR resonances. Conditions: Ru : **B** 5 mM : 5 mM for Guo and 5 mM : 5.5 mM for Ino, after 24 h incubation at 310 K. No added salt.

^b $\text{N}(7)/\text{N}(1) = \{[\text{Ru-}N7\text{B}] + [\text{Ru-}N7\text{BN1-Ru}]\} / \{[\text{Ru-}N1\text{B}] + [\text{Ru-}N7\text{BN1-Ru}]\}$, where **B** = Ino or Guo.

c, d and e disappeared at lower pH (< 5.8). From ^1H H8 shifts, the $\text{p}K_a$ values associated with all 6 sets of IMP peaks were determined and are listed in Table 6.2. Free 5'-IMP (a), $\text{p}K_a$ values of 9.22, 6.36 and 1.35 were determined for N1H, PO_3H and N7, respectively, whereas peak b has two associated $\text{p}K_a$ values of 7.74 and 5.71 assignable to N1H and PO_3H , but no $\text{p}K_a$ assignable to N7. Peak b can therefore be assigned as Ru-*N7IMP*. Peak c has an N1H $\text{p}K_a$ value of 9.04 and no associated phosphate $\text{p}K_a$, and is assigned to Ru-*O(PO₃)IMP*. Peak d has a PO_3H $\text{p}K_a$ of 6.35 but no N1H $\text{p}K_a$, and is assigned to Ru-*N1IMP*. Peak e has no measurable $\text{p}K_a$ values at all and is assignable to Ru-*O(PO₃)IMP*-Ru. Peak f has a PO_3H $\text{p}K_a$ of 5.73, but no N7 and N1 $\text{p}K_a$ values and is assigned to Ru-*N7IMP*-Ru. The distribution of 5'-IMP species at pH 6.46 after 24 h at 310 K was determined by H8 and H2 peak

integration and is shown in Scheme 6.3.

The reaction of inosine (Ino, 5.5 mM) with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (5 mM) was followed by ^1H NMR for 2 d at 310 K with an initial pH of 7.22. After 1 d, the pH value of the solution had decreased to 6.19. A representative ^1H NMR spectrum for the H8 and H2 region is shown in Figure 6.6A. Three products are formed corresponding to 3 new sets of H8 and H2 peaks: b, d and c. The products were identified by ^1H NMR pH titration (Figure 6.6B, note that product c disappeared at $\text{pH} < 4.0$). The pK_a values are derived from H8 chemical shifts¹⁷ and are listed in Table 6.2. Peak a has two associated pK_a values of 8.79 and 0.91, attributable to protonation of N1H and N7 of free inosine. Peak b has one pK_a value of 7.11, attributable to the protonation of N1H of Ru-N7Ino. After 24 h at 310 K, the species distribution was determined to be: Ru-N7Ino: 59.4%, Ru-N1Ino: 23.0%, Ru-N7InoN1-Ru: 3.7% (Scheme 6.4). Species distributions and N(7)/N(1) binding ratios for reactions of Ino with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ at various pH values (5.5–10.3) are listed in Table 6.3. With increase in pH, the N(7)/N(1) binding ratio decreased significantly from 7.72 (pH 5.5) to 0.22 (pH 10.3).

Little Reaction with cAMP or Adenosine, and Only 5'-Phosphate Binding for 5'-AMP. The reactions of 5'-AMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, 310 K) at various pH values were monitored for 24 h by ^1H and ^{31}P NMR spectroscopy (Figures 6.7A and 6.7B). A rapid reaction was observed (within the time of mixing) which gave rise to one new set of H2 and H8 ^1H NMR signals (peak b, H2 δ 8.006 and H8 δ 8.402, pH 6.8), shifted to low frequency relative to free 5'-AMP (peak a, H2 δ 8.240 and H8 δ 8.555) and in slow exchange on the NMR time scale. Similarly, one new signal (peak b, δ 9.125) was observed in the ^{31}P NMR spectrum (Figure 6.7B) shifted to high frequency by 5.568 ppm (pH 6.8). The variation in intensity of ^{31}P NMR peak b with pH is shown in Figure 6.7C. It can be seen that it reached its maximum intensity at pH ca. 7.2 and decreased in

intensity at either lower or higher pH; at pH 3.0, only free 5'-AMP peaks were seen, and at pH 9.6, > 90% of 5'-AMP was free. The variations of ^1H (H8 and H2) and ^{31}P

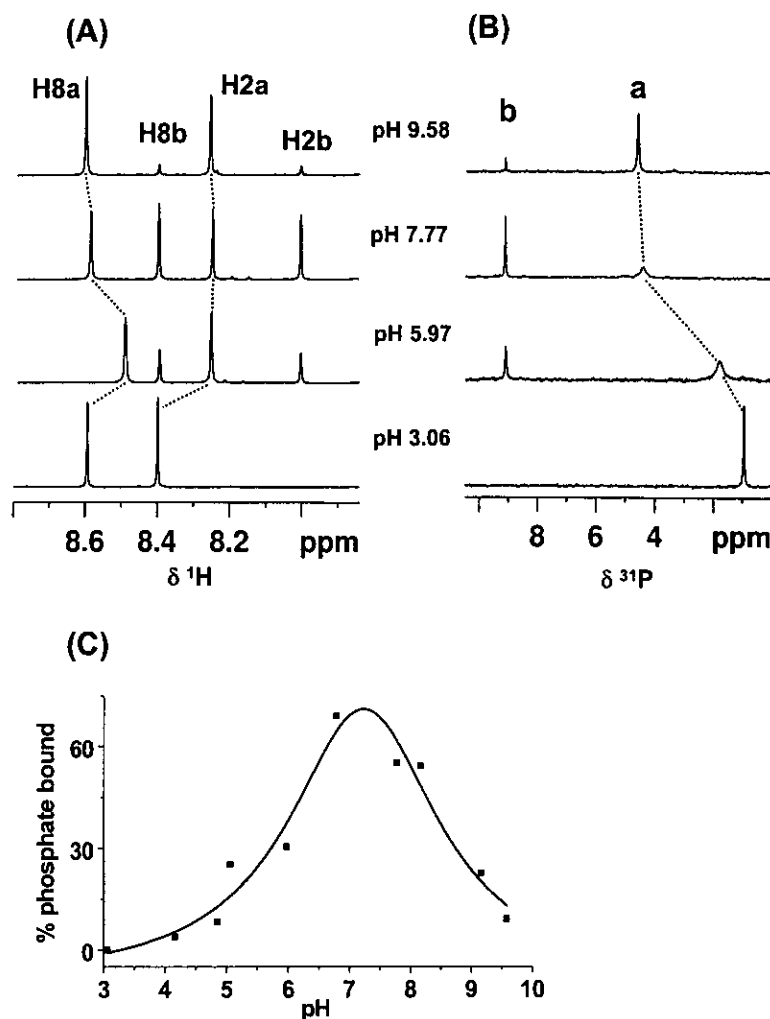


Figure 6.7 (A) ^1H , and (B) ^{31}P NMR spectra for the reaction of 5'-AMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM) after 24 h at 310 K at various pH values. Peak assignments: a, free 5'-AMP; b, $\text{Ru-O}(\text{PO}_3)\text{AMP}$. (C) Plot showing the variation with pH of species b as a percentage of the total 5'-AMP present, based on ^{31}P NMR peak intensities. There is negligible binding of Ru to the purine base of 5'-AMP over the pH range of 3.0-10.0.

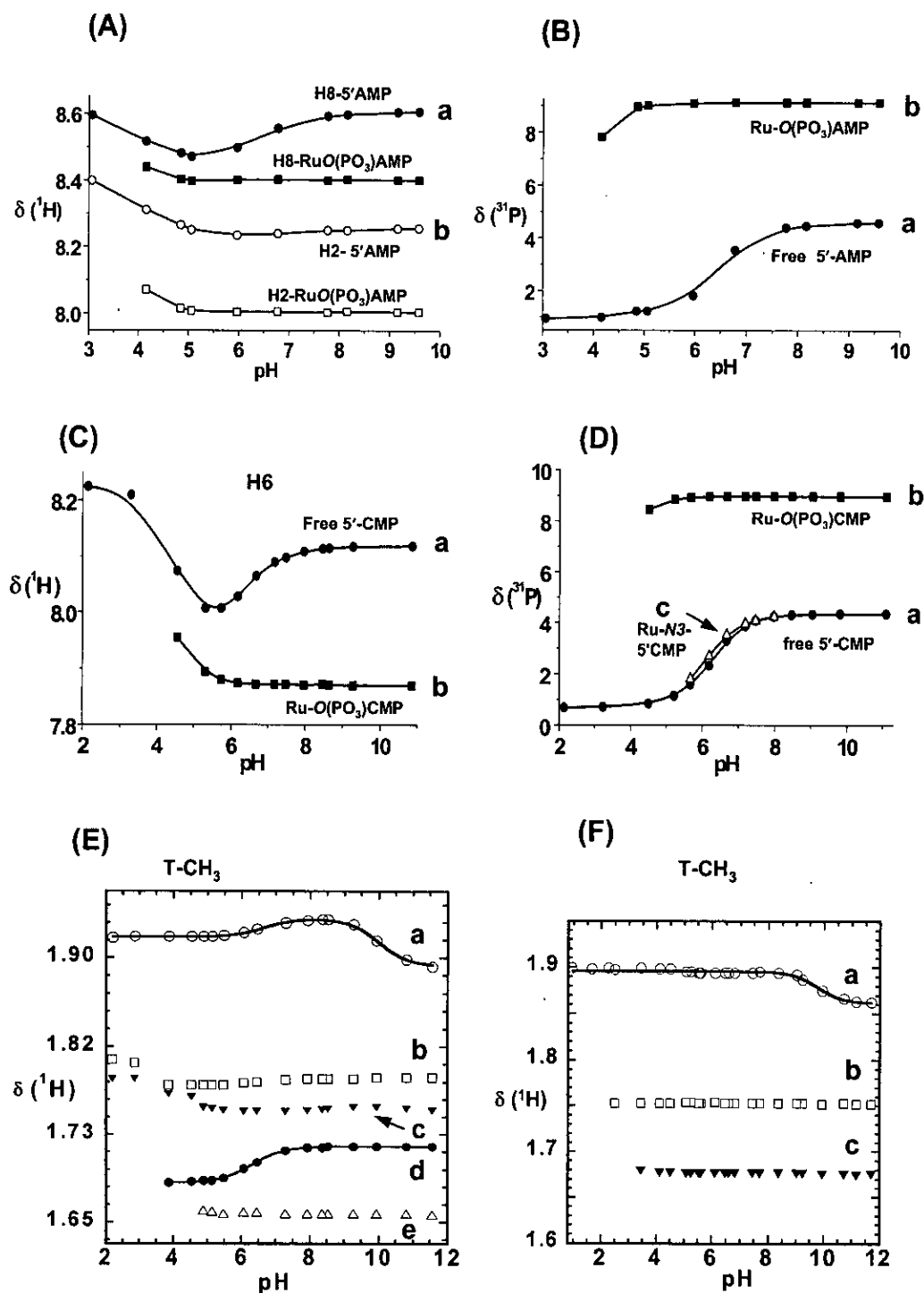


Figure 6.8 ^1H and ^{31}P NMR pH titration curves for species present during reaction of (A, B) AMP-Ru(Bip), (C, D) CMP-Ru(Bip), (E) TMP-Ru(Bip), ^1H NMR and (F) thymidine-Ru(Bip), ^1H NMR. ^{31}P plot for TMP-Ru(Bip) was not attempted because of peak overlap.

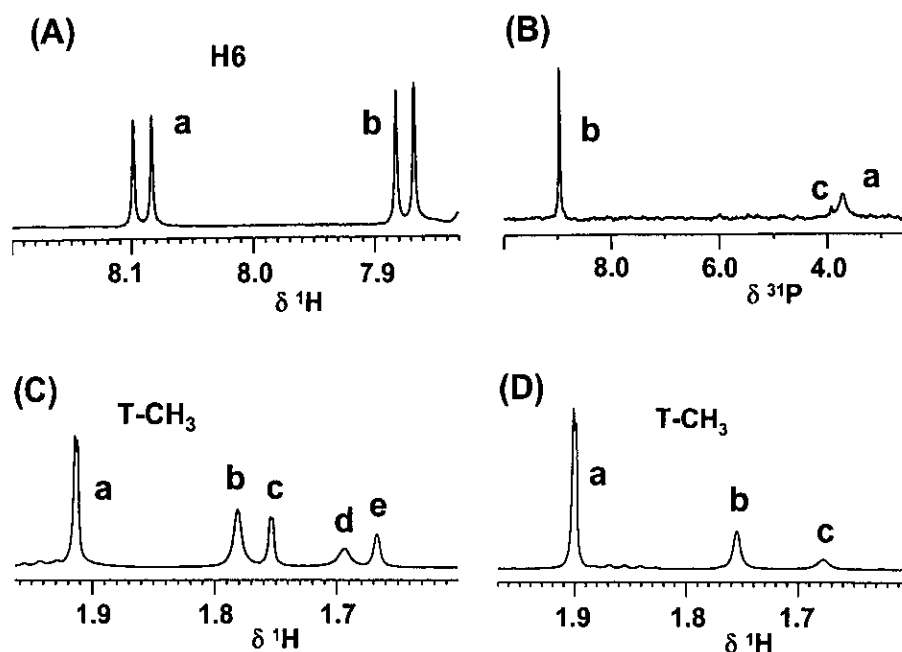


Figure 6.9 Top: (A) ^1H , and (B) ^{31}P NMR spectra for reaction of 5'-CMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.2, after 24 h at 298 K). Bottom: The T-CH₃ region of ^1H NMR spectrum for reactions of (C) 5'-TMP with Ru(Bip) (1:1, 10 mM, pH 5.9, after 2.5 d at 310 K), and (D) thymidine with Ru(Bip) (1:1, 5 mM, pH 6.55, after 3 d at 310 K).

NMR chemical shifts with pH are shown in Figures 6.8A and 6.8B, and pK_a values determined from H8 ^1H NMR shifts are listed in Table 6.4. Free 5'-AMP (peak a) has two associated pK_a values of 6.41 and 4.05, attributable to the deprotonation of PO_3H and N1H, respectively. Peak b can be assigned to the phosphate-bound adduct $\text{Ru-O}(\text{PO}_3)\text{AMP}$ and has no measurable pK_a value within the pH range 4-10.

Reactions of cAMP and adenosine (Ado) with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, 310 K) were monitored by ^1H NMR at different pH values (4.0, 7.0, and 9.0). Only small amounts (< 5%) of products were formed for both cAMP and Ado, even after 3 d at 310 K (Table 6.7). These are possibly N7/N1 adducts (based on H8 and

H2 chemical shifts). pH titrations were not attempted due to the low concentrations of products.

Table 6.4 Selected Chemical Shifts and pK_a Values for 5'-AMP and 5'-CMP and 5'-TMP and Thymidine and Their $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ Adducts at 298 K.

Species (Peak) ^a	pK_a ^b (group)	δ (¹ H) ^c	δ (³¹ P) ^c
5'-AMP (a)	6.41 ± 0.07 (-PO ₃ H) 4.05 ± 0.08 (N1H)	8.555	3.557
Ru-O(PO ₃)AMP (b)	—	8.402	9.125
5'-CMP (a)	6.43 ± 0.04 (-PO ₃ H) 4.36 ± 0.03 (N3H)	8.091	3.696
Ru-O(PO ₃)CMP (b)	—	7.875	8.960
Ru-N3CMP (c)	6.12 ± 0.08 (-PO ₃ H)	— ^d	3.917
5'-TMP (a)	9.99 ± 0.03 (N3H) 6.65 ± 0.07 (PO ₃ H)	1.914	1.95
Ru-N3TMP (d)	6.32 ± 0.02 (PO ₃ H)	1.694	2.05
Ru-O(PO ₃)TMPN3-Ru (b, c and e)	—	1.781, 1.754, 1.667	— ^e
Thymidine (a)	9.76 ± 0.12 (N3H)	1.900	
Ru-N3Thymidine (b and c)	—	1.755, 1.677	

^a For peak labels see Figures 6.7 and 6.9. ^b Based on H8 or H6 or T-CH₃ chemical shift. ^c H8 and pH 6.8 for 5'-AMP, H6 and pH 7.10 for 5'-CMP, CH₃ and pH 5.81 for 5'-TMP, and CH₃ and pH 5.37 for thymidine. ^d Peak c of H6 is overlapped with phenyl resonances in the ¹H NMR spectra. ^e ³¹P NMR resonances at ca. δ 9.0 for Ru-O(PO₃) adducts, but specific assignments were not made for peaks b, c and e due to overlap.

5'-CMP Forms 5'-Phosphate Adduct and a Minor N3 Adduct.

Reactions of 5'-CMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.23, 310 K) were followed for up to 3 d, and ^1H and ^{31}P NMR spectra are shown in Figures 6.9A and 6.9B. A rapid reaction was observed and gave rise to a new H6 ^1H NMR peak shifted to low frequency (b, δ 7.875), which accounted for ca. 56% of the total 5'-CMP after 1 d. There was a corresponding new ^{31}P NMR peak (b, δ 8.960) shifted to high frequency by 5.264 ppm. In the ^{31}P NMR spectrum, an additional minor peak c appeared at 3.917 ppm. The corresponding H6 signal for peak c was not detected, perhaps due to peak overlap with the phenyl resonances. The ^1H and ^{31}P NMR pH titration curves are shown in Figures 6.8C and 6.8D (note that peak b disappears at pH < 4.2). pK_a values were determined from H6 ^1H NMR shifts and are listed in Table 6.4. Free 5'-CMP (peak a) has pK_a values of 6.43 and 4.36, attributable to the deprotonation of PO_3H and N3H , respectively. Peak b can be assigned to $\text{Ru-O}(\text{PO}_3)\text{CMP}$, and has no measurable pK_a value within the pH range 4-11. Peak c has an associated pK_a value of 6.12 (based on ^{31}P NMR titration), attributable to the deprotonation of PO_3H and can be assigned to the Ru-N3CMP product.

The reaction of cytidine with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.2) was monitored by ^1H NMR. Based on new biphenyl ^1H resonances, only 13% of cytidine reacted even after 3 d at 310 K (Table 6.7), possibly via N3 binding. A pH titration was not attempted due to the low concentration of the product.

Ru(II) binds to Phosphate and N3 of 5'-TMP. Reactions of 5'-TMP with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ (1:1, 5 mM, pH 7.2, 298 K) were followed by ^1H and ^{31}P NMR spectroscopy for up to 3 d. Due to the overlap of H5 and H6 and H1' signals of TMP with those for biphenyl protons, the analysis for this reaction was based on the T- CH_3 signal. A rapid reaction was observed within 10 min and gave rise to one new T CH_3 peak c at δ 1.754 shifted to low frequency relative to that for free TMP (peak a, δ 1.914). After 2 h, peaks b (δ 1.781), d (δ 1.694) and e (δ 1.667) appeared

and increased in intensity with time, with a decrease in intensity of peak c. The pH value of the solution decreased steadily during the reaction from 7.3 (initial) to 6.20 (1 d). The reaction reached equilibrium after 1.5 d, at which time the relative intensities of 5'-TMP CH₃ peaks were a: 39%, b: 24%, c: 15%, d: 12% and e: 10%. A representative ¹H NMR spectrum of the T CH₃ region is shown in Figure 6.9C, and ¹H NMR pH titration curves are shown in Figure 6.8E. pK_a values were determined for peaks a and d (Table 6.4). Free 5'-TMP has pK_a values of 9.99 and 6.65 for N3H and PO₃H, respectively. Peak d shows one pK_a value of 6.32 for PO₃H, and the absence of deprotonation of N3H, and is assigned to Ru-N3TMP. Peaks b, c and d all have no associated deprotonation for PO₃H and N3H, and can be assigned to adducts of the type Ru-O(PO₃)TMPN3-Ru, possibly isomers.¹⁸

The reaction of [(η⁶-Bip)Ru(en)Cl]⁺ with thymidine (1:1, 5 mM, pH 7.20) was monitored by ¹H NMR at 310 K. The pH of the solution decreased from 7.20 (initial) to 5.37 after 3 d, compatible with N3 coordination to Ru with concomitant N3H deprotonation. Two new thymidine CH₃ ¹H NMR peaks (b δ 1.755 and c δ 1.677) appeared at lower frequency compared to that for free thymidine (peak a, δ 1.90; Figure 6.9D). The pH titration curves for peaks a, b and c are shown in Figure 6.8F, and pK_a values for free thymidine and its adducts are listed in Table 6.4. For free thymidine (peak a), the pK_a value of 9.76 is attributed to deprotonation of N3H. Peaks b and c both have no associated deprotonation of N3H and disappeared at pH < 3, and can therefore be assigned to two isomeric forms of Ru-N3Thymidine adducts.¹⁹ After 3 d, N3-bound adducts accounted for 34.7% of the total thymidine. In contrast to the sharp doublet signal for the T CH₃ protons of free thymidine (peak a), signals b and c were broad, perhaps due to an exchange process.

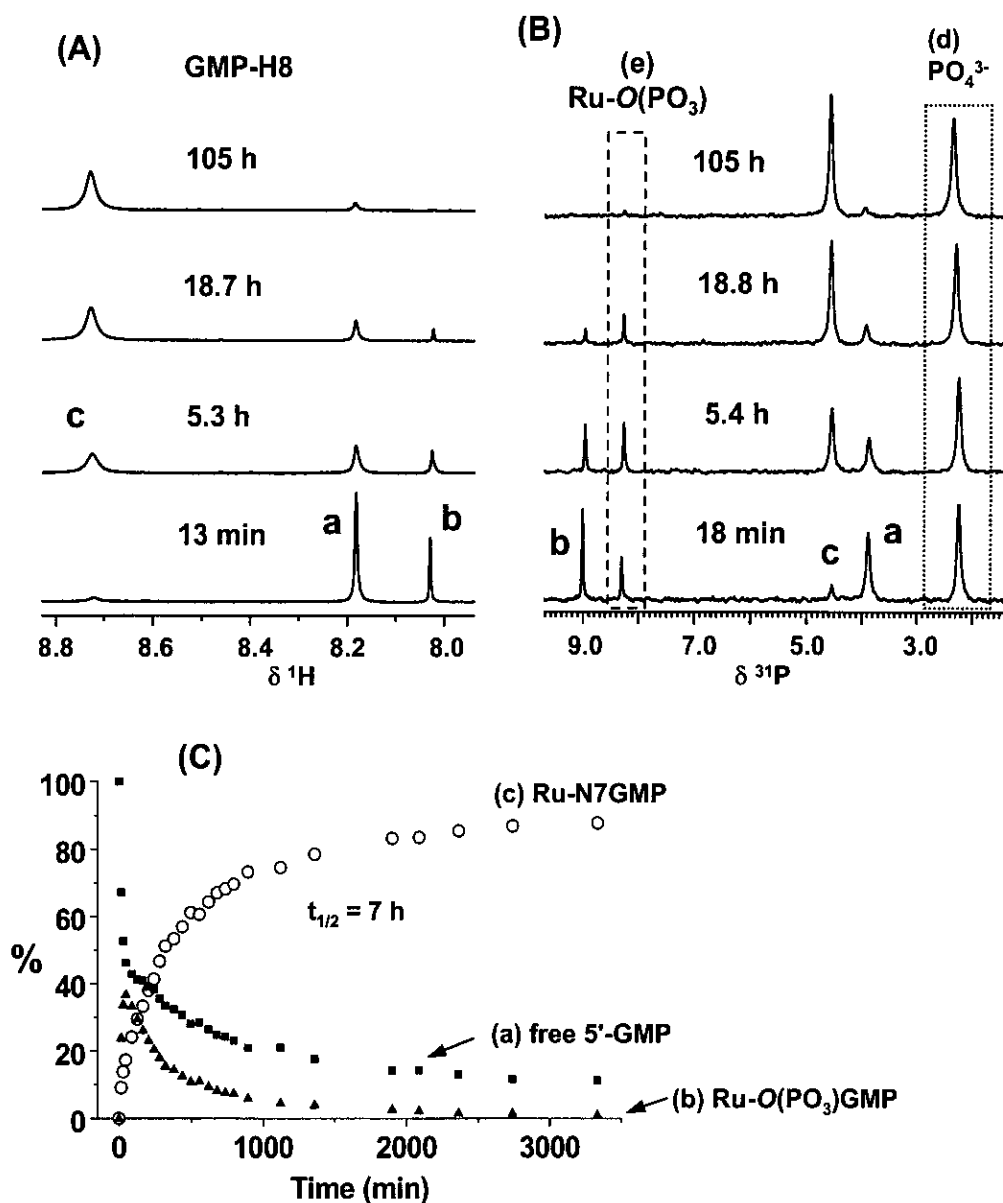


Figure 6.10 (A) ^1H , and (B) ^{31}P NMR spectra for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-GMP (1:1, 5 mM, pH 7.24) in 5 mM phosphate buffer at 298 K. Peak assignments: a, free 5'-GMP; b, Ru-O(PO_3)GMP; c, Ru-N7GMP; d, HPO_4^{2-} ; e, Ru-O(PO_3). Note that the species Ru-O(PO_3)GMP (b), and Ru-O(PO_3) (e), rapidly formed during the mixing time, and both decreased in concentration because of displacement by G N7, which was almost complete after 4 d. (C) Distribution of species versus reaction time. Labels for species as above. Phosphate-bound species b and e are intermediates. Distribution plots for species d and e are not shown.

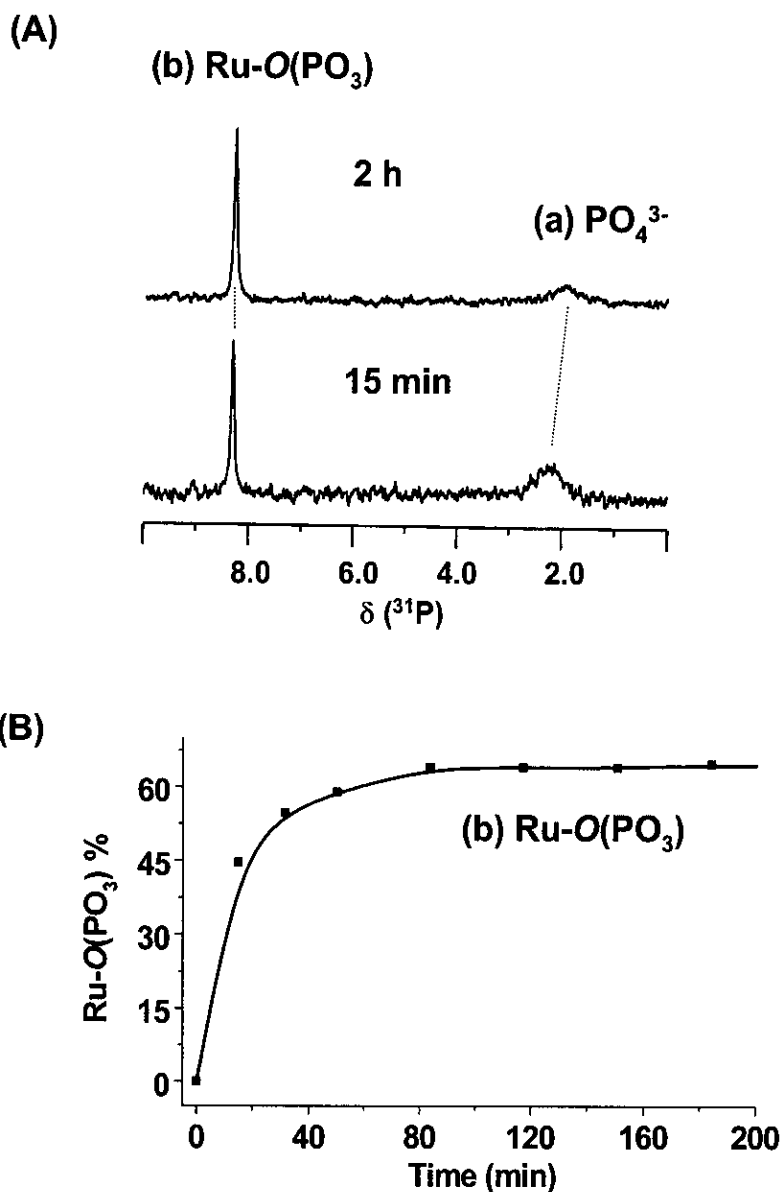


Figure 6.11 (A) ³¹P NMR spectra for reaction of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with Na_2HPO_4 (5 mM, 1:1, 298 K, initial pH 7.26) after 15 min and 2 h (pH 6.95). Note peak a (HPO_4^{2-}) at δ 2.20 and peak b ($\text{Ru-O}(\text{PO}_3)$) at 8.307. (B) Plot showing the percentage of phosphate present as $\text{Ru-O}(\text{PO}_3)$, based on ³¹P NMR peak intensities. The reaction reached equilibrium after 2 h when 64% of the phosphate was present as $\text{Ru-O}(\text{PO}_3)$. The equilibrium constant (K) for formation of $\text{Ru-O}(\text{PO}_3)$ was determined to be 3.2.

Only 5'-GMP Forms Adducts with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ in Competition with 5'-AMP, 5'-CMP, or 5'-TMP. Reactions of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with 5'-GMP in competition with either 5'-AMP, 5'-CMP or 5'-TMP in a 1:1 molar ratio (5 mM) at pH 7.2 were monitored by ^1H and ^{31}P NMR at 310 K. In each case, after 2 d, the only new peaks observed corresponded to those for Ru-N7GMP (data not shown).

6.3.3 Kinetic Studies

Phosphate, Chloride and Hydroxide Decrease the Rate of GMP N7-Binding. The kinetics of reactions of chloro and aqua Ru(Bip) with 5'-GMP and cGMP in various solutions (different NaCl and phosphate concentrations) and at various pH values were monitored by ^1H and ^{31}P NMR spectroscopy. The observed half-lives ($t_{1/2}$) for N7-binding are listed in Table 6.5. The kinetics of reaction of chloro Ru(Bip) with Na_2HPO_4 (5 mM, 1:1, 298 K, initial pH 7.26) were also monitored by ^1H and ^{31}P NMR spectroscopy (Figure 6.11). The reaction reached equilibrium after 2 h with the equilibrium constant (K) for formation of Ru-O(PO_3) was determined to be 3.2.

For the reactions of 5'-GMP with chloro Ru(Bip) in 100 mM NaCl, the $t_{1/2}$ value for formation of Ru-N7GMP was 7 h with 89% of the 5'-GMP bound as Ru-N7GMP after two weeks. In 5 mM phosphate buffer, the $t_{1/2}$ was 5 h, with 95% of GMP bound as Ru-N7GMP after 4 days (Figure 6.10). In 50 mM phosphate buffer, the $t_{1/2}$ was more than 15 h, with only 50 % Ru-N7GMP formed even after one week. Under similar conditions at pH ca.7.3 in H_2O , the reaction of 5'-GMP with aqua Ru(Bip) was 1.7 times faster than that with chloro Ru(Bip), and the reaction of Ru(Bip) with cGMP was 3 times faster than with 5'-GMP. No phosphate binding was detected for cGMP reactions, see Figure 6.4 and Scheme 6.2. Therefore chloride, dianionic phosphate ions and 5'-phosphate groups compete with N7 for binding to

Ru(II).

The reaction rate was also dependent on pH (Table 6.5). For the reactions with 5'-GMP, $t_{1/2}$ for formation of Ru-N7GMP increased from 1.1 h to 5.0 h when the pH was raised from 5.37 to 9.40. Similarly for cGMP, the reactions were much faster at low pH. The $t_{1/2}$ for formation of Ru-N7cGMP increased from 0.5 h to 4.3 h when the pH was increased from 5.60 to 9.22. Since the pK_a of H_2O in $[(\eta^6\text{-Bip})Ru(en)(H_2O)]^{2+}$ is 7.7, the hydroxo complex Ru-OH is the predominant species in solution at high pH,²⁰ and thus slows down Ru-N7 binding.

Table 6.5 Half-lives ($t_{1/2}$) for formation of Ru-N7 bound adducts of 5'-GMP and cGMP with $[(\eta^6\text{-Bip})Ru(II)(en)Cl]^+$ (Ru-Cl) and $[(\eta^6\text{-Bip})Ru(II)(en)(H_2O)]^{2+}$ (Ru-H₂O).

Ru-Cl/H ₂ O	G	Conditions ^a	pH ^b	$t_{1/2}$ (h) ^c
Ru-Cl	5'-GMP	100 mM NaCl	7.25, 7.25	7.0
		5 mM phosphate	7.24, 7.30	5.0
		50 mM phosphate	7.22, 7.18	15.0
		H ₂ O	5.37, 5.41	1.1
			7.22, 7.26	3.1
			9.40, 9.13	5.0
Ru-H ₂ O	5'-GMP	H ₂ O	7.34, 7.59	1.8
			9.11, 9.40	4.0
Ru-Cl	cGMP	H ₂ O	7.30, 7.60	1.0
Ru-H ₂ O	cGMP	H ₂ O	5.60, 5.88	0.5
			7.16, 7.69	0.8
			9.22, 9.64	4.3

^a Ru : G = 1 : 1, 5 mM, 298 K. ^b pH (start), pH (equilibrium). ^c $t_{1/2}$ from concentration versus time plots; rate constants were not determined.

Arene-purine Hydrophobic Interactions Greatly Enhance N7-binding to GMP. Reactions of five Ru(arene) (arene = Bip, THA, DHA, Ben and Cym) complexes with cGMP were investigated by ^1H and ^{31}P NMR under physiologically-relevant conditions. Fitted plots of concentration vs time for the reaction of aqua Ru(arene) with cGMP (1:1, 5 mM, pH 7.0) are shown in Figure 6.14A, and the second order rate constants (k)²¹ were determined by Scientist kinetic model (Figures 6.12 and 6.13) and are listed in Table 6.6. Reactions of cGMP with aqua Ru(THA), Ru(Bip) and Ru(DHA) complexes were more than 3 times faster ($k = 14.7, 8.0$ and $7.1 \times 10^{-2} \text{ M}^{-1}\cdot\text{s}^{-1}$, respectively) than those for aqua Ru(Cym) and Ru(Ben) complexes ($k = 2.5$ and $1.1 \times 10^{-2} \text{ M}^{-1}\cdot\text{s}^{-1}$, respectively). For the reactions of chloro Ru(arene) complexes with cGMP (1:1, 2 mM), there were two steps: Ru-Cl hydrolysis followed by Ru-N7 binding (see Scheme 6.2). The observed $t_{1/2}$ values for formation of Ru-N7 adducts are listed in Table 6.6. No attempt was made to determine the rate constants. Plots of concentration against time are shown in Figure 6.14B. The sequence of $t_{1/2}$ values for the reactions of chloro Ru(arene) is consistent with that for analogous reactions of the aqua Ru(arene) complexes: Ru(THA) < Ru(Bip) < Ru(DHA) << Ru(Cym) < Ru(Ben) (Table 6.6). For the THA, Bip and DHA complexes, 100% of the N7-bound product was observed after 2 d, whereas only ca 80% of the N7-bound product formed for Cym and Ben complexes after 4 d.

(A) // Kinetic model $A+B=P$ ($A=B$)
 IndVars : T
 DepVars : A, P
 Params : A0, P0, K2
 $A := A0 / (1 + K2 * A0 * T)$
 $P := P0 + K2 * SQR(A0) * T / (1 + K2 * A0 * T)$



$$[cGMP] = 0.005 / (1 + k_2 \times 0.005 T)$$

$$[Ru-N7cGMP] = k_2 \times (0.005)^2 T / (1 + k_2 \times 0.005 T)$$

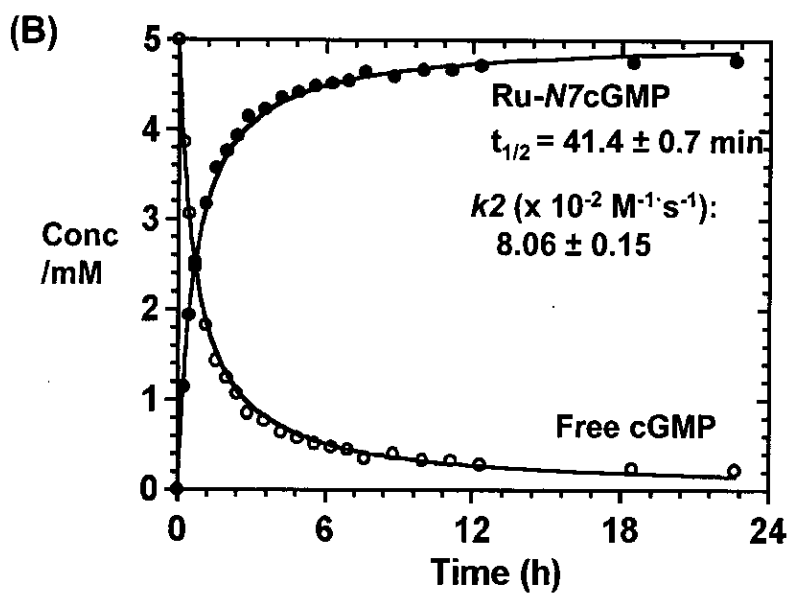


Figure 6.12 (A) Scientist kinetic model for second-order reactions studied in this work. (B) Variation in the concentration of free cGMP and the product of Ru-N7cGMP with time during reaction of cGMP with $[(\eta^6\text{-Bip})Ru(en)(H_2O)]^{2+}$ (1:1, 5 mM, pH 7.0, 100 mM $NaClO_4$, 298 K). The curves are best fits to second-order kinetics.

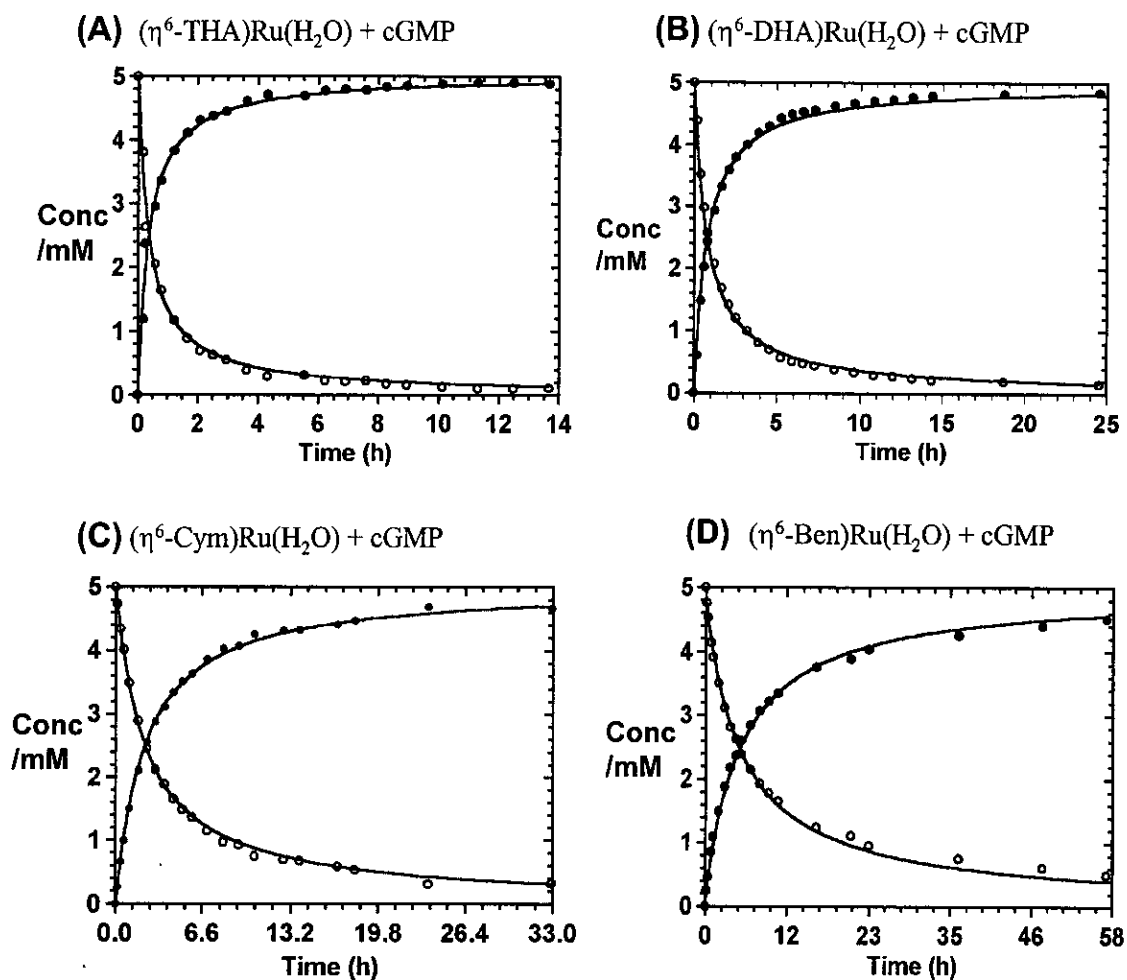


Figure 6.13 Variation of the concentration of free cGMP and Ru-*N*7cGMP with time during reactions with (A) $[(\eta^6\text{-THA})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$, (B) $[(\eta^6\text{-DHA})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$, (C) $[(\eta^6\text{-Cym})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$, (D) $[(\eta^6\text{-Ben})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ (1:1, 5 mM, pH 7.0, 100 mM NaClO_4 , 298 K). The curves are best fits to second order kinetics.

Table 6.6 Rate Constants (k) and Half-lives ($t_{1/2}$) for Reactions of Ru(arene) complexes with cGMP.^a

Aqua Ru(arene) ^b	Rate constant ($\times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$)	$t_{1/2}$ (h) ^c	Chloro Ru(arene) ^b	$t_{1/2}$ (h) ^d
Ru(THA)(H ₂ O)	14.72 ± 0.69	0.38 ± 0.02	Ru(THA)Cl	1.1
Ru(Bip)(H ₂ O)	8.06 ± 0.15	0.69 ± 0.01	Ru(Bip)Cl	2.0
Ru(DHA)(H ₂ O)	7.08 ± 0.20	0.78 ± 0.02	Ru(DTA)Cl	3.6
Ru(Cym)(H ₂ O)	2.49 ± 0.03	2.23 ± 0.03	Ru(Cym)Cl	7.1
Ru(Ben)(H ₂ O)	1.13 ± 0.02	4.94 ± 0.09	Ru(Ben)Cl	13

^a Conditions: Ru : G = 1:1, 100 mM NaClO₄, 298 K, 5 mM for aqua Ru(arene) and 2 mM for chloro Ru(arene); pH was adjusted to 7.0 at reaction commencement and found to be ca. 7.2 at equilibrium for each reaction. ^b For the THA, Bip and DHA complexes, 100% of the N7-bound products observed after 2 d, whereas only ca 80% of the N7-bound products formed for Cym and Ben complexes after 4 d. ^c Calculated from $t_{1/2} = 1/kC^0$, $C^0 = 5.0 \text{ mM}$. ^d $t_{1/2}$ from concentration versus time plots; rate constants were not determined.

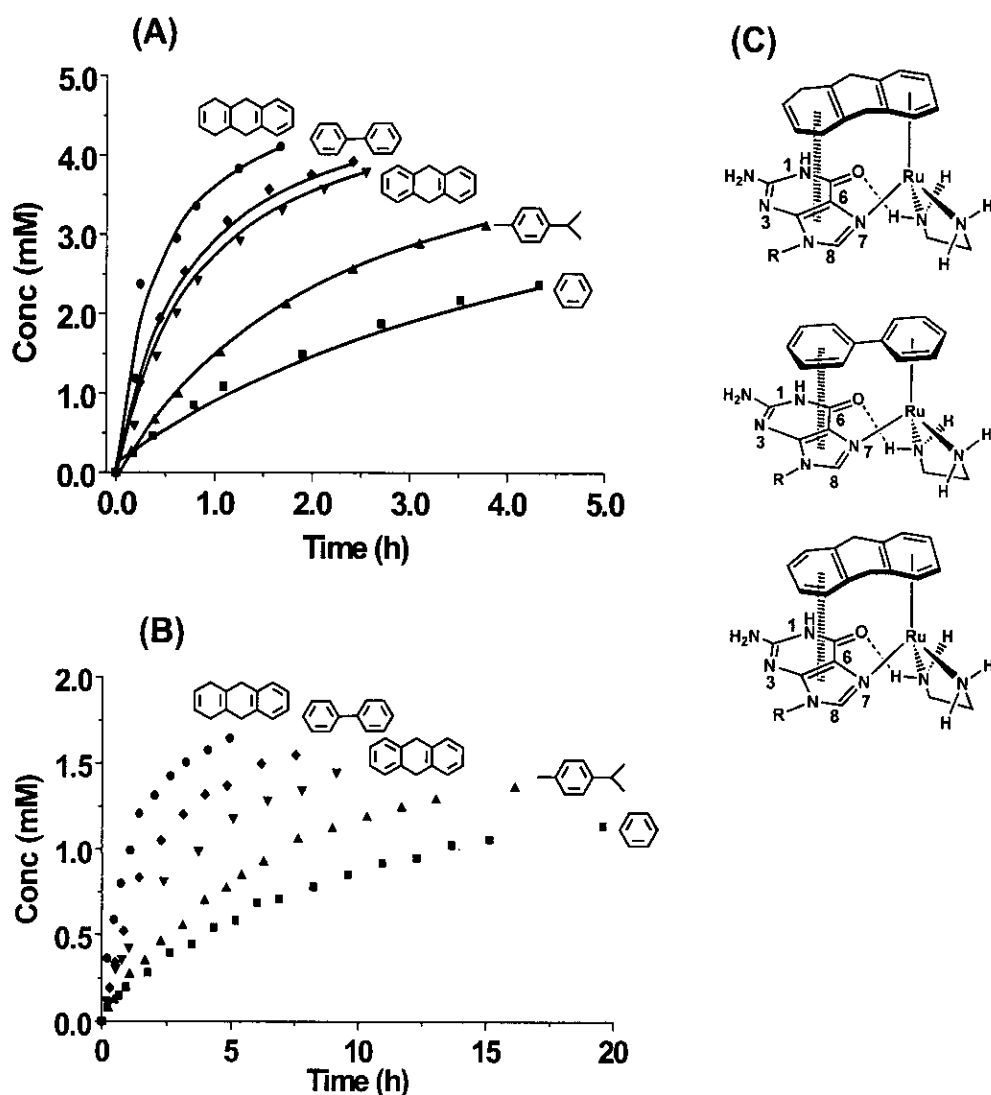


Figure 6.14 Plots showing the variation of the concentration of $(\eta^6\text{-arene})\text{Ru-N7cGMP}$ products with time during the reaction of (A) $[(\eta^6\text{-arene})\text{Ru}(\text{en})(\text{H}_2\text{O})]^{2+}$ (5 mM), (B) $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ (2 mM) with cGMP (1:1, pH 7.0), 100 mM NaClO_4 , 298 K, based on the integration of G H8 ^1H NMR peaks. The curves in (A) are computer-fits to second order kinetics giving the rate constants listed in Table 6.6. These reactions were all followed for periods of between 24 h and 4 days (see Figures 6.12 and 6.13). The reactions of the chloro complexes in (B) were followed for up to 1 week, and the half-lives for the formation of Ru-N7 adducts are shown in Table 6.6. No attempt was made to fit these data and determine the rate constants. (C) Possible π - π stacking between arene and purine in cGMP adducts with Ru(THA), Ru(Bip) and Ru(DHA).

6.4 Discussion

6.4.1 Identification of Binding Sites

Purines exhibit a dichotomy between binding at N1 and N7. The proton favors N1. In neutral solution, N7 is usually the predominant binding site for transition metal ions on N9-substituted purines such as adenosine and guanosine. However, in adenine derivatives, N1 is only slightly less favorable than N7.²² N1-substituted pyrimidines (cytidine, thymidine, uridine) offer only one favorable ring binding site, N3.²³ Reactions of purine and pyrimidine nucleosides and nucleotides with Pt(II) and Pd(II) complexes, for example, have been investigated previously, usually by ¹H and ³¹P NMR spectroscopy.^{24,25,26} Resolution of the N7-N1 dichotomy is unequivocal in the cases of Pt(II) and Pd(II) because the metal ion is both diamagnetic and, on the NMR time scale, in slow exchange between the two sites. Similarly, in this study, the diamagnetic nature of Ru(II), together with slow ligand exchange, allow the acid-base properties of the products to be individually assessed by pH titrations, and the populations of all species in solution can be determined through measurement of NMR peak areas. ³¹P NMR was also used to reveal directly the interaction between Ru(II) and the phosphate group.^{26c,27} Thus, it has been able to assess the selectivity of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ complexes towards binding sites on purine and pyrimidine nucleic acid derivatives.

Adducts of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ with nucleosides and nucleotides exhibited characteristic ¹H and ³¹P NMR signals, and acid-base properties which allow their structures to be assigned (Figures 6.1-6.9, and Tables 6.1-6.4). In the cases of 5'-GMP, cGMP, Guo, 5'-IMP and Ino, metallation at N7 gave Ru-N7 products with high frequency shifts of 0.3-0.6 ppm for H8 resonances, but slight low frequency shifts of 0.02-0.04 ppm for H2 resonances, compared with free ligands. Metallation at N1 (only found in the reactions with 5'-IMP and Ino) gave Ru-N1 products with

low frequency shifts of 0.16-0.21 ppm for H8 and 0.22-0.26 ppm for H2 resonances. A similar chemical shift behavior for H8 and H2 protons upon metallation at N7 or N1 sites of purines has been reported for other metal ions.^{25a} For all nucleotides, the ³¹P NMR resonances exhibit large downfield shifts of ca. 5 ppm when the phosphate group coordinates to the Ru center (Tables 6.1-6.4).

The pH dependences of ¹H and ³¹P NMR chemical shifts of nucleobase derivatives were studied in detail. Protonation of N7, N1, N3 or 5'-phosphate produced significant low- or high-frequency shifts of ¹H or ³¹P NMR resonances (Figures 6.1, 6.4, 6.5, 6.6, 6.7 and 6.9). Ru(II) binding to N7, N1, N3 or the 5'-phosphate group prevents the protonation of that site and therefore no change in chemical shift due to protonation is expected. Therefore the Ru(II) binding sites can be identified, as shown in Tables 6.1, 6.2 and 6.4. It is notable that small amounts of dinuclear adducts²⁸ of the type Ru-N7BNI-Ru and Ru-O(PO₃)BNI(N7)-Ru (**B** = nucleosides and nucleotides) were detected in solution. Binding site identification was supported by the following. (1) The significant pH decrease (from 7.2 to ca. 6) during reactions with I and T derivatives, is indicative of N1-coordination to Ru for 5'-IMP or inosine, and N3-coordination for 5'-TMP and thymidine, with concomitant deprotonation. This was not observed for reactions with G and A and C derivatives for which almost no N1 or N3-binding occurred (Table 6.7). (2) Ru-N7 adducts have lowered pK_a values of 1.4-1.7 units for N1, and of 0.6-0.7 units for 5'-phosphate groups, but Ru-O(PO₃) binding causes only a small lowering of pK_a values of 0.18-0.30 for N1, and Ru-N1 binding has little effect on the pK_a values of 5'-phosphate groups, relative to the free ligands (Tables 6.1, 6.2 and 6.4). These data are consistent with metallation at N7 which usually acidifies the proton at N1,^{29,30,31} and consistent with the presence of an outer-sphere interaction involving H-bonding of the 5'-phosphate group to an en NH proton in Ru-N7 adducts (but not in Ru-N1 adducts) which can contribute to the larger lowering of the pK_a value of 5'-PO₃H for Ru-N7

Table 6.7 Reactions of Nucleosides and Nucleotides with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$.

Nucleoside /Nucleotide	Species	% Reaction ^a			pH	
		1 d	3 d	0	1 d	3 d
Guanosine (Guo)	Ru- <i>N7</i> Guo	100	100	7.20	7.25	7.37
	Ru- <i>N1</i> Guo	0	0			
Inosine (Ino)	Ru- <i>N7</i> Ino	59.4	54.3	7.22	6.19	5.9
	Ru- <i>N1</i> Ino	23.0	22.8			
	Ru- <i>N7</i> Ino/ <i>N1</i> -Ru	3.7	7.7			
Thymidine	Ru- <i>N3</i> Thymidine	30.6	34.7	7.20	5.19	5.37
Cytidine	Ru- <i>N3</i> Cytidine	11.6	13.5	7.24	7.19	7.33
Adenosine (Ado)	Ru- <i>N7</i> / <i>N1</i> Ado	< 3	< 5	7.24	7.28	7.30
5'-GMP	Ru- <i>N7</i> GMP	> 95	100 ^b	7.10	7.22	7.26
	Ru- <i>O</i> (PO ₃)GMP	< 5	0			
cGMP	Ru- <i>N7</i> cGMP	100		7.28	7.26	
5'-IMP	Ru- <i>N7</i> IMP	82.0	79.7	7.28	6.46	6.55
	Ru- <i>N1</i> IMP	4.4	3.1			
	Ru- <i>N7</i> IMP/ <i>N1</i> -Ru	8.7	12.5			
	Ru- <i>O</i> (PO ₃)IMP/ <i>N1</i> -Ru	1.6	0.7			
5'-TMP	Ru- <i>O</i> (PO ₃)IMP	1.4	0.5			
	Ru- <i>N3</i> adducts (b, c, d, e)	58.0	56.8 (2 d)	7.23	6.03	6.23 (2 d)
5'-CMP	Ru- <i>O</i> (PO ₃) adducts (b, c and e)	46.8	47.2 (2 d)			
	Ru- <i>N3</i> CMP	8.6	7.4	7.23	7.10	7.10
5'-AMP	Ru- <i>O</i> (PO ₃)CMP	56.1	40.7			
	Ru- <i>N7</i> / <i>N1</i> products	3.1	3.5 (2 d)	7.04	6.78	6.90 (2 d)
cAMP	Ru- <i>O</i> (PO ₃)AMP	68.6	63.5 (2 d)			
	Ru- <i>N7</i> / <i>N1</i> products	<5	<5	7.30	7.27	7.10

^a Based on total nucleoside/nucleotide and determined by integration of ¹H or ³¹P NMR resonances. Reaction conditions: Ru : nucleobase 5 mM : 5 mM (except for inosine : 5 mM : 5.5 mM), and at 310 K (298 K for cGMP). ^b To this sample NaCl (100 mM) was added and the solution was incubated at 310 K; reversal of 5'-GMP binding was very slow and amounted to only ca. 10% after 1 week.

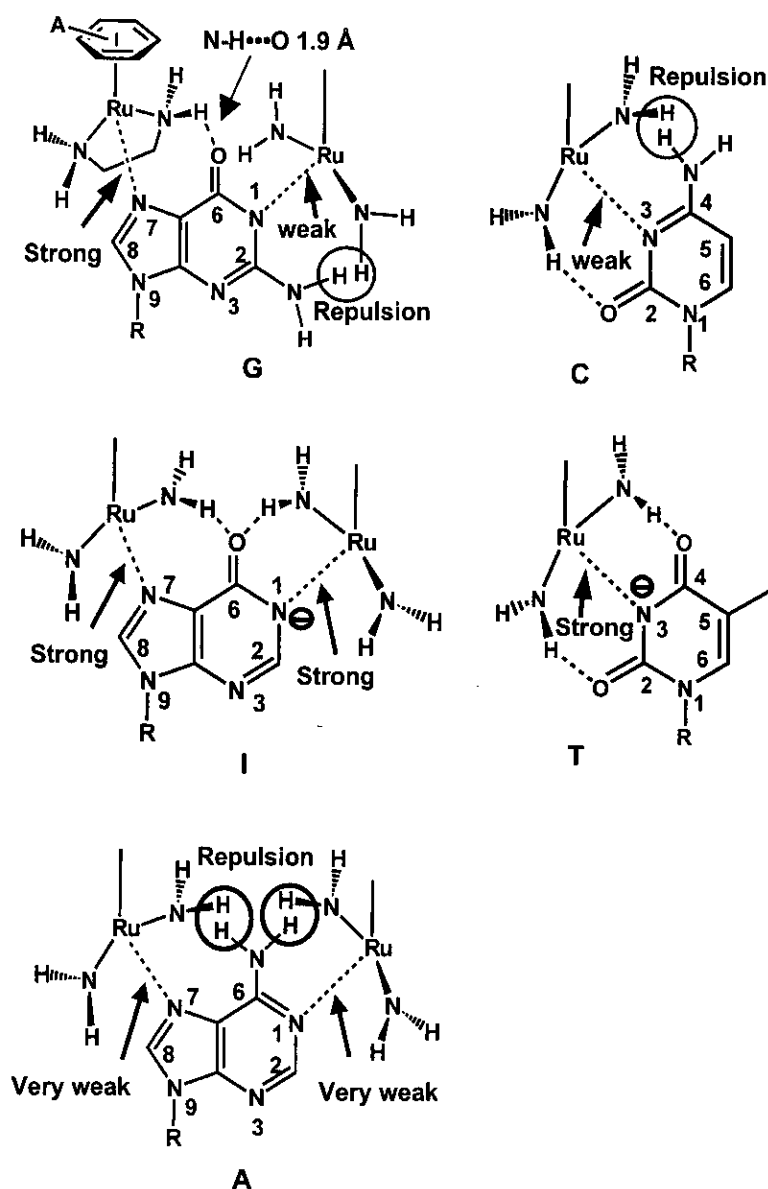


Figure 6.15 Regio- and stereo- specificities: H-bonds and nonbonding repulsions for the interaction of $\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ with nucleobases, as detected in nucleoside and nucleotide reactions. For clarity, the arene and the CH_2CH_2 of the en ring have been omitted, except for the G adduct. For the purine adducts, the diagrams indicate in a general way the reactions with N7 and N1 and are not intended to apply specifically to the dinuclear adducts.

adducts compared to Ru-N1 adducts.^{25b,32,33} (3) Ru-O(PO₃), Ru-N1 and Ru-N3 adducts disappeared at pH < 5.0 (Figures 6.1, 6.5, 6.6 and 6.7), due to protonation of N1 and the 5'-phosphate group.³⁴

6.4.2 Selective Recognition of Nucleobases

A summary of the extent of reaction of various nucleosides and nucleotides with $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ is given in Table 6.7. Nucleotides and their corresponding nucleosides have similar affinities for Ru, except for a significant amount of 5'-phosphate binding in the former case. A schematic representation of the possible interactions between the nucleobases and the en NH₂ groups is given in Figure 6.15. H-bonding and non-bonding repulsive interactions, in addition to the electronic properties of the various nucleobase coordination sites,³⁵ appear to play key roles in these site-selective reactions.^{3b} For G, N7 is the favoured site for Ru(arene) binding. Our previous studies of Ru-N7 guanine adducts have revealed a strong H-bonding interaction between one en NH (pointing towards the G base) and G C6O with N...O distance 2.8 Å and N-H...O angle 163°, which is present in the X-ray crystal structures of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})(9\text{EtG})]^{2+}$ and $[(\eta^6\text{-Bip})\text{Ru}(\text{en})(\text{Guo})]^{2+}$. Such high stereospecific H-bonding has also been detected in aqueous solution by NMR.¹² Thus, coordination at N7 is stabilized by H-bonding of en NH₂ with G C6O and coordination at N1 is unfavourable due to the repulsive interaction of en NH₂ with G2 NH₂. This is consistent with the data for reactions of Ru(Bip) with Guo, 5'-GMP and cGMP: only Ru-N7 adducts were detected (and no Ru-N1 adducts) over the pH range 2-12 (Tables 6.1 and 6.3). Inosine (I), in contrast to G, has no NH₂ group. Coordination at either N7 or N1 can be stabilized by H-bonding of en NH with C6O, and significant amounts of Ru-N7 and Ru-N1 adducts were detected for reactions of inosine and 5'-IMP with Ru(Bip) (Table 6.7). Binding at N1 is promoted at high pH

with the concomitant deprotonation of N1H (Table 6.3). Adenine (A) has a C6 NH₂ group and coordination at either N7 or N1 of A is weakened by repulsive interactions with the exocyclic amino group: no base-binding was observed for reactions of Ru(Bip) with Ado, cAMP or 5'-AMP over the range pH 3.1-9.6 (Table 6.7, Figure 6.7). For thymine (T), significant amounts of Ru-N3 adducts were formed in reactions of Ru(Bip) with both thymidine and 5'-TMP at pH ca. 6.0 (Table 6.7). Coordination at N3 may be favoured by H-bonds between en NH and the exocyclic oxygens at C2 and C4.³⁶ For C, only small amounts of Ru-N3 adducts (ca. 10%) were found for reactions of cytidine and 5'-CMP (Table 6.7). Coordination at N3 is weak due to the repulsive interaction of en NH with the C4 NH₂ group.

Based on the above nucleoside and nucleotide studies, the reactivity of the various binding sites of nucleobases³⁷ towards Ru(Bip) at neutral pH decreases in the order G(N7) > I(N7) > I(N1), T(N3) > C(N3) > A(N7), A(N1). In competitive binding experiments with 5'-GMP versus either 5'-AMP or 5'-CMP or 5'-TMP, the only final adduct was $[(\eta^6\text{-Bip})\text{Ru(II)(en)(N7-GMP)}]$. Also in enzymatic digestion experiments, it was found that the binding of $[(\eta^6\text{-}p\text{-cymene})\text{Ru(en)Cl}]^+$ to a 14-mer oligonucleotide occurred specifically at guanine sites.¹¹ Experiments on the inhibition of RNA synthesis show that the termination sites produced by $\{(\eta^6\text{-arene})\text{Ru(en)}\}^{2+}$ adducts of DNA are at guanine residues.³⁸ Such a high specificity for guanine-N7 does not appear to have been reported for other metal-based anticancer agents. Many other Pt and Ru anticancer complexes also bind covalently to adenine. The complex $[\text{Ru(II)(NH}_3)_5\text{Cl}]^+$ reacts with many nitrogen bases,^{31b} preferentially with the N7 of guanine,^{31a} N1 of adenine³⁹ and N4 of cytosine.^{31b} For *cis*- $[\text{Ru(II)(DMSO)}_4\text{Cl}_2]$, the reaction sites in double-stranded polynucleotides are thought to be N7 of both guanine and adenine.⁴⁰ The trifunctional organometallic complex $[(\eta^6\text{-Ben})\text{Ru(H}_2\text{O)}_3]^{2+}$ binds to both N7 and N1 of adenine.⁴¹ *Cis*- $\{(\text{NH}_3)_2\text{Pt(II)}\}^{2+}$ binds to adenosine at both N7 (55%) and N1 (45%),⁴² and the

monofunctional complex $\{(\text{dien})\text{Pt}(\text{II})\}^+$ also binds to adenosine at both N7 (50%) and N1 (50%).^{23a} *cis*- $\{(\text{NH}_3)_2\text{Pt}(\text{II})\}^{2+}$ forms both GG and AG intrastrand cross-links that account for about 65% and 25%, respectively, of DNA platinations.⁴³ Organometallic metallocene dichloride antitumor agents of the type $[\text{Cp}_2\text{MCl}_2]$ (M = Mo, Ti) exhibit little, if any, coordinative selectivity in nucleotide competition experiments.^{44,45} The anti-HIV agent $\text{Zn}(\text{II})$ -[12]aneN₄ shows a high selectivity for N3 of thymine and uridine derivatives amongst all of the nucleobases, with formation of cyclen NH H-bonds to the C2 and C4 carbonyl oxygens of T or U.⁴⁶

The ability of the NH proton of ethylenediamine to act as an H-bond donor towards an exocyclic oxo group but not towards an amino group appears to play an important role in controlling site recognition of the nucleobases by these Ru(arene) anticancer agents. When the en ligand is replaced by H-bond acceptor ligands (such as acetylacetonate derivatives), Ru(arene) can also recognise adenine bases.⁴⁷ Initial data suggest that there is a correlation between the anticancer activity of Ru(arene) complexes and the presence of an en NH H-bond donor group. Activity is lost when the en NH₂ hydrogens are substituted, e.g. with methyl groups.⁴⁸ The choice of the specific chelating ligand appears to be an effective way of controlling selectivity for nucleobase recognition by octahedral Ru complexes. The steric constraints for nucleobase binding to an octahedral site are much more demanding compared to those for binding at a square-planar site, e.g. Pt(II).

6.4.3 Phosphate Binding

The first stage in reactions of $[(\eta^6\text{-Bip})\text{Ru}(\text{en})\text{Cl}]^+$ with nucleotides (5'-GMP, 5'-IMP, 5'-AMP, 5'-CMP and 5'-TMP) involved rapid 5'-phosphate binding (Figures 6.1 and 6.5, Table 6.7).⁴⁹ The phosphate adducts of 5'-GMP and 5'-IMP rearranged into N7 and N1 adducts, but for adenine, cytidine, and thymidine nucleotides, significant amounts of phosphate complexes were still present at equilibrium (Table

6.7). For 5'-AMP reactions, it can be seen that Ru-O(phosphate) binding is sensitive to pH, reaching a maximum at pH 7.2 (Figure 6.7). Lower pH values promoted protonation of the 5'-phosphate group, and higher pH values promoted the competitive binding of OH⁻ and thus reduced the formation of the 5'-phosphate adducts. Similarly, inorganic dianionic phosphate ions were also found to bind rapidly to $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ to form a Ru-O(PO₃) product (Figure 6.11). Thus binding of $\{(\eta^6\text{-Bip})\text{Ru}(\text{en})\}^{2+}$ to N7 of 5'-GMP was significantly retarded by phosphate buffers at neutral pH (Figure 6.10 and Table 6.5). In contrast, no binding of Ru(II) towards the phosphodiester group of cGMP or cAMP was detected (Figure 6.4).

At neutral pH, *cis*-[Pt(NH₃)₂Cl₂] forms monodentate adducts with the inorganic ortho-, pyro- and tri-phosphate ions,⁵⁰ and an N7, PO macrochelate *cis*-[Pt(NH₃)₂(5'-GMP-N7, PO)] with the nucleotide 5'-GMP, but does not bind to the phosphate group of the methyl phosphate diester of 5'-GMP (Me-5'-GMP).⁵¹ $\{\text{Cp}_2\text{Mo}\}^{2+}$ coordinates to nucleotides (5'-dAMP, 5'-dCMP and 5'-dTMP) through Mo-N7/N3 and Mo-O(phosphate) chelation, but does not show direct phosphate coordination to the methyl-phosphate diester of 5'-dGMP (Me-5'-dGMP)), diphenyl phosphate ((C₆H₅O)₂P(O)OH), or diethyl phosphate ((CH₃CH₂O)₂P(O)OH).⁵² Zn(II) cyclen also acts as a good monotopic receptor for dianionic phosphate monoesters.⁵³ ¹H NMR studies of reactions of Zn(II)-bis(cyclen) with 5'-dTMP and 5'-dTDP indicate that the terminal phosphate dianion interacts with one of the Zn(II)-cyclens and the imido anion of dT bound to the other Zn(II)-cyclen.⁵⁴ Zinc-containing carboxylate-bridged heterodimetallic complexes can react with the phosphodiester ligand diphenyl phosphate to form bis(phosphate) complexes.⁵⁵ $\{(\text{NH}_3)_5\text{Ru}(\text{III})\}^{3+}$ does not appear to coordinate directly to 5'-phosphate groups of nucleotides,⁵⁶ but the chelation of N7 and phosphate group of 5'-(d)GMP with *cis/trans*-[Ru(II)Cl₂(DMSO)],⁵⁷ and N7,O(P)-macrochelation of adenosine and guanosine 5'-mono-, -di-, and -tri-phosphates⁴¹ with $[(\eta^6\text{-Ben})\text{Ru}(\text{II})(\text{H}_2\text{O})_3]^{2+}$ have both been

detected by ^1H and ^{31}P NMR pH titrations. The latter organometallic complex is also capable of coordinating to the phospho- diester group of 5'-ADP.⁴¹

The $\{(\eta^6\text{-arene})\text{Ru(II)(en)}\}^{2+}$ complexes studied here bind to dianionic phosphate and phosphomonoesters, but very weakly to phosphodiester. This has potential biological implications. The reversible binding to phosphate⁵⁸ as well as transient binding to membrane phospholipids may facilitate cellular uptake⁵⁹ of Ru(arene) species. Direct Ru(II) coordination with the backbone phosphodiester groups of DNA will be weak, but electrostatic interactions and H-bonding may be involved in the initial recognition of $[(\eta^6\text{-arene})\text{Ru(II)(en)(H}_2\text{O)}]^{2+}$ prior to binding to guanine N7, as proposed for some Pt complexes.⁶⁰

6.4.4 Kinetic Studies

The rate of reaction of $[(\eta^6\text{-Bip})\text{Ru(en)X}]^{n+}$ with N7 of GMP depends upon whether Cl^- , H_2O , OH^- , or phosphate occupies the available coordination position (X). Chloro Ru(arene) complexes rapidly hydrolyze in water to give more reactive aqua Ru(arene) species (Scheme 6.1, and Figures 6.2 and 6.3). Hydrolysis is suppressed in the presence of 100 mM chloride ions. The intracellular chloride concentration (ca. 4-23 mM) is significantly lower than the extracellular concentration (ca. 103 mM).⁶¹ Thus reactive Ru- H_2O species are likely to predominate inside cells under physiological conditions. The reactions of $[(\eta^6\text{-Bip})\text{Ru(en)(H}_2\text{O)}]^{2+}$ with both 5'-GMP and cGMP are more than 3 times slower at pH 9 compared to pH 5 to 7 (Table 6.5). This is attributable to the lower reactivity of Ru-OH compared to Ru-OH₂ (as is also the case for am(m)ino Pt(II) complexes), since the pK_a values for $[(\eta^6\text{-arene})\text{Ru(en)(H}_2\text{O)}]^{2+}$ complexes studied here are all within the range 7.7-8.25.^{20,62}

6.4.5 Arene-purine Hydrophobic Interactions

There appear to be few previous studies of the mechanisms of ligand substitution reactions of (η^6 -arene)Ru complexes. Reactions of aqua Ru(arene) complexes with N7 of cGMP obey second-order kinetics and therefore appear to proceed via an associative pathway involving a seven-coordinate transition state.⁶³ The rates of reaction of [$(\eta^6$ -arene)Ru(II)(en)(H₂O)]²⁺ complexes (and chloro complexes) with cGMP depends markedly on the nature of the arene, decreasing by over an order of magnitude in the series: THA > Bip > DHA >> Cym > Ben (Figure 6.14 and Table 6.6). There is no correlation between the rates of cGMP N7-binding and pK_a values of aqua Ru(arene) complexes.⁶⁴ The faster rates of reaction for [$(\eta^6$ -arene)Ru(II)(en)(H₂O)]²⁺ complexes containing the large arene ligands THA, Bip and DHA⁶⁵ imply lower activation free energies ($\Delta G^\ddagger$) for formation of seven-coordinate transition states. A significant contribution to $\Delta G^\ddagger$ may arise from π - π stacking of the arene and purine ring¹² in the transition state (negative $\Delta H^\ddagger$). Such an interaction is not possible for the *p*-cymene and benzene complexes. Arene-purine π - π stacking may also result in a positive contribution to $\Delta S^\ddagger$ through desolvation of cGMP in the reaction transition state.

Hydrophobic interactions could produce a driving force for DNA binding. A recent analysis of free energy contributions to DNA binding suggests that intercalation of ethidium, propidium, daunorubicin, and adriamycin is driven by hydrophobic effects and van der Waals contacts within the intercalation site.⁶⁶ Studies of the interaction of thymine derivatives with zinc cyclen containing a pendant acridine show that acridine-thymine ring-stacking increases the affinity of Zn(II) for dT N3.⁶⁷ For many other reported metal agents, however, the presence of a rigid aromatic rings, e.g. quinoline in *trans*-[Pt(quinoline)(NH₃)(9EtG)Cl]²⁺,⁶⁸ has been found to significantly retard the rate of N7-binding of 5'-GMP. In contrast, for the Ru(arene) complexes studied here, the flexibility of large arene ligands reduces the steric requirements and makes simultaneous arene-nucleobase stacking and N7-

covalent binding compatible. Our initial investigations of reactions of calf-thymus DNA show that chloro Ru THA, DHA and Bip complexes bind to DNA faster than the Cym complex, and CD, LD experiments suggest that the larger arene rings (THA, DHA and Bip) are oriented on DNA in a manner compatible with intercalation.³⁸ The rate of DNA binding appears to be specifically enhanced by hydrophobic interactions between the arene and the purine base.

6.5 Conclusions

It is shown here that anticancer complexes of the type $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$ are highly selective in their recognition of binding sites on nucleosides and nucleotides. This arises not only from the differences in basicity between the binding sites, but also from the demanding constraints imposed on the reactive monofunctional site in these pseudo-octahedral “piano-stool” Ru(II) arene complexes.

$\{(\eta^6\text{-arene})\text{Ru}(\text{en})\}^{2+}$ complexes exhibit a remarkably high preference for binding to guanine N7. Such binding is stabilized by H-bonding between the C6O of G and NH of the en ligand.¹² The lack of binding to adenine can be attributed to unfavorable interactions between the C6 NH₂ group and Ru en NH₂. Such a repulsive interaction also occurs for G when Ru binds to N1, but, if this NH₂ group is absent, as in inosine derivatives, then a significant amount of binding to N1 is observed. Similarly, binding of Ru to N3 of C derivatives is weak due to the repulsive interaction with the C4 NH₂ group. Binding to N3 of T is significant, but was not competitive with binding to G N7 at neutral pH. It is clear that nucleobase site-selectivity is strongly related to interactions with the en NH₂ group which is attractive toward exocyclic carbonyl oxygens but repulsive towards exocyclic amino groups of nucleobases.

Reactions of the chloro Ru(II) arene complexes with nucleotides proceed via

hydrolysis followed by rapid binding to the phosphate monoester group. In contrast, binding to the monoanionic phosphodiester group of nucleotides is weak and was not detected. Bound phosphate groups are then readily displaced by base nitrogens. Studies of the kinetics of binding of $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{X}]^{n+}$ ($\text{X} = \text{H}_2\text{O}, \text{Cl}$) to cGMP suggested that arene-purine base-stacking plays a significant role in stabilizing the transition state in the associative substitution reactions. Reactions of complexes containing arenes (THA, Bip and DHA) which can take part in π - π stacking are up to an order of magnitude faster than those containing arenes which cannot (Cym, Ben). Such kinetic effects could play a role in the biological activity of this class of complexes.

These studies provide a basis for investigating the recognition of duplex DNA by octahedral arene Ru(II) complexes. It is anticipated that it should be possible to enhance the discrimination of DNA base recognition by appropriate modification of the chelating ligand. By appropriate choice of arene it may also be possible to achieve a high degree of selectivity via kinetic effects due to π - π arene-base stacking interactions (intercalation). Dual mode G N7/intercalative binding may also induce novel structural distortions in duplex DNA and hence the recognition by DNA binding proteins and repair enzymes. Consideration of all these factors allow optimization of the design of this series of anticancer complexes.

6.6 References

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- (35) In spite of the similar acid-base properties of the nucleophilic sites of guanosine (G) and inosine (I) (see Tables 1 and 2, and ref 3b), $\{(\eta^6\text{-Bip})Ru(en)\}^{2+}$ coordinates to the N1 site of I but not of G. This suggests that interactions between the Ru en NH_2 groups and the exocyclic groups on the bases play a major role in controlling site selectivity.
- (36) The $pK_a(N3H)$ value of thymidine is 9.76 (Table 3). Protonation of N3 competes with Ru-N3 binding, and partially explains T did not fully react with Ru(Bip) at lower pH (7.2-5.8).
- (37) In Watson-Crick double-helical DNA, N3 of thymine and cytosine would not be expected to be available for binding to Ru(II), since N3 is involved in H-bonding in base-pairs. N3 binding would therefore be restricted to regions of single-stranded DNA. Competitive experiments at pH 7.2 show that N3-binding to T or C is weak compared to G N7.

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Bip),²⁰ 8.25 (Ru-Cym, unpublished data) and 7.9 (Ru-Ben; Ref. 62). Thus at pH 7.0, aqua complexes should predominate for all these arene complexes.

(65) The slower rate of reaction of DHA compared to THA complexes, may arise from the difference in hinge-bending of the arene. In both the chloro and aqua Ru(DHA) complexes, the tricyclic ring system of DHA is bent down towards the Ru-Cl(O) bond by ca. 40° (refs. 12 and 20), which may impose a steric constraint on coordination of N7. For the Ru(THA) complexes, such a steric constraint would be smaller since the ring system is bent up away from Ru by ca. 7° (refs 12 and 20).

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Appendix Table. In *Vitro* Cytotoxicity Data for Ru(arene) complexes in this thesis, and for Cisplatin, on Human Tumor Cell Lines^a

Test complexes	IC50 (μ M)		
	A2780 (ovarian)	A2780 ^{cis} fold resistant	A2780 ^{AD} fold resistant
cisplatin	0.5	12	8
$[(\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$	0.4	1	>100
$[(\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{en})\text{Cl}][\text{PF}_6]$	2	0.5	92
$[(\eta^6\text{-Bip})\text{Ru}(\text{Et-en})\text{Cl}][\text{PF}_6]$	7	0.6	27
$[\eta^6\text{-C}_{14}\text{H}_{14})\text{RuCl}_2(\text{dmsO})]$	>100		
$[\eta^6\text{-C}_{14}\text{H}_{14})\text{Ru}(\text{CNCH}_3)_2\text{Cl}][\text{PF}_6]$	>100		
$[\eta^6\text{-C}_{14}\text{H}_{12})\text{RuCl}_2(\text{dmsO})]$	>100		
$[\eta^6\text{-C}_{14}\text{H}_{12})\text{Ru}(\text{CNCH}_3)_2\text{Cl}][\text{PF}_6]$	>100		

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- (1) Postgraduate English Courses, Edinburgh University, Oct 1999-May 2000.
- (2) Course on "Metals in Medicine", Prof. Peter J. Sadler, Edinburgh University, 1999.
- (3) Weekly Inorganic Section Seminars during terms, 1999-2002.
- (4) Weekly Chemistry Department Seminars during terms, 1999-2002.
- (5) CCPN lectures, "Efficient and Rapid Structure Determination by NMR", Heriot-Watt University, Edinburgh, 6-8 April, 2001.
- (6) CCPN lectures, "NMR of non-ideal proteins: Producing and characterising samples for NMR studies", Homerton College, Cambridge, 22-24 March, 2002.
- (7) Bruker NMR Training, Chemistry Department, Edinburgh University, 18-19 March 2002.
- (8) "XP tutorials" by Dr Andy Parkin, 7 February 2001; "CSD tutorials" by Dr Andy Parkin, 17 October 2001; CRYSTALS Workshop by CCD, 21 November, 2001; "Molecular Xtals ab Initio Workshop" by Dr Carole Morrison, 7 August 2001; "Computer in Chemistry", Chemistry Department, 23 May 2001.
- (9) Postgraduate courses on Crystallography by Dr Simon Parsons, Edinburgh University, 19 Nov-10 Dec 2001.
- (10) NMR Spectroscopy Lectures by Dr Dušan Uhrín, Edinburgh University, 9-21 Oct 2002.

Publications:

(1) Highly Selective Binding of Organometallic Ruthenium Ethylenediamine Complexes to Nucleic Acids: Novel Recognition Mechanisms

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(2) Organometallic ruthenium(II) diamine anticancer complexes: arene-nucleobase stacking and stereospecific hydrogen-bonding in guanine adducts

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(5) Inhibition of cancer cell growth by ruthenium(II) arene complexes

Robert E. Morris, Rhona E. Aird, Piedad del S. Murdoch, **Haimei Chen**, Jeff Cummings, Nathan D. Hughes, Simon Parsons, Andrew Parkin, Gary Boyd, Duncan I. Jodrell and Peter J. Sadler *J. Med. Chem.* **2001**, *44*, 3616-3621.