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Non-Platinum Chemotherapeutic Metallopharmaceuticals

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I. Introduction

Despite the resounding success of cisplatin and closely related platinum antitumor agents,[1−3] the movement of other transition-metal antitumor agents toward the clinic has been exceptionally slow. Keppler has pointed out the inherent bias in testing metal compounds in cell and animal systems, which have been proven sensitive to cisplatin, and the difficulty in formulating metal complexes, particularly those with low solubility.[4] Also, most metallopharmaceuticals have emanated from academic rather than from commercial pharmaceutical laboratories. Earlier reviews have suggested possible advantages in using transition-metal ions other than platinum, which may involve (1) additional coordination sites, (2) changes in oxidation state, (3) alterations in ligand affinity and substitution kinetics, and (4) photodynamic approaches to therapy.[5−12] While the latter may become a clinically useful method,[13] it is not addressed here as a chemotherapeutic approach.

Broadening the chemotherapeutic arsenal depends on understanding existing agents with a view toward developing new modes of attack. Indeed, few of the compounds covered here may function in a manner analogous to cisplatin, which appears to bend DNA by cross-linking adjacent guanines, thereby causing a class of DNA binding proteins to adhere to the site.[1−3] This review focuses on possible mechanistic approaches to chemotherapeutic anticancer drugs involving non-platinum metal ion complexes exclusive of metalloproteins or metal-activated antibiotics. Since DNA has often been proposed as the target of these agents, there is a particular emphasis on those that can interact with nucleic acids. Nevertheless, heavy metals are generally toxic by binding to sulfur and nitrogen sites on proteins and, thus, can interfere with a number of modes of metabolism. Several metals also exhibit action through redox activity, and gallium appears to operate through the displacement of metal ions in iron metabolism or bone.

II. Gallium: Iron Depletion, Inhibition of DNA Synthesis and Incorporation into Bone

A. Activity Against Soft Tissue Tumors

While generally only moderately effective on an experimental basis against soft-tissue tumors, gallium nitrate has exhibited clinical activity against lymphomas[14] and bladder carcinomas.[15,16] In combination with vinblastine and ifosfamide, it is effective against metastatic carcinoma of the urothelium[17] and cisplatin-resistant ovarian cancer, but patients may exhibit cardiac arrhythmias as a side effect.[18] In combination with paclitaxel (Taxol), it may be useful against cancers that are difficult to treat with existing agents.[19] A synergism may arise between Taxol's arresting cells in mitosis and gallium's inhibiting the S phase of cell replication.[20] The most commonly used gallium salt for therapeutic purposes, $Ga(NO_3)_3$, is normally administered by continuous intravenous

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Professor Michael J. Clarke earned his B.S. degree in Chemistry at the Catholic University of America in 1968. His graduate study in bioinorganic chemistry with Henry Taube at Stanford University was interspersed with teaching at San Francisco City College and a conscientious objectorship at the Washington Hospital Center. He garnered his Ph.D. degree in 1974 followed by visiting professorships at Boston University and Wheaton College. Since 1976, he has been on the faculty of Boston College, where he became Full Professor in 1985. His research on anticancer drugs, technetium chemistry, metal–coenzyme interactions, and more recently nitric oxide complexes and electron transfer through DNA has been largely directed toward demonstrating that pharmaceutical development involves more than CHNOPS. When not designing new metallopharmaceuticals, he can sometimes be found canoeing on New England's white water rivers or advocating for open space in his hometown of Newton, MA.

Dr. Fuchun Zhu received his B.S. and M.S. degrees in Chemistry from Nankai University and then worked in the Research Institute of Petroleum Processing, Beijing. He joined Professor E. Dubler and Professor H. R. Oswald's group at the University of Zürich, where he completed his Ph.D. degree in 1995 and stayed on as a postdoctoral Fellow. Following a short stay as a scientific collaborator at the University of Neuchâtel, Switzerland, he joined Professor Clarke's group in Boston College in 1998. His most recent work includes studies of the interaction of Cu with the anticancer thiopurines, molecular modeling to design ligands for metal complexes as well as to understand molecular recognition in electron transfer between chiral cobalt complexes and copper proteins, and DNA-mediated electron transfer.

infusion (200−300 mg/m^2 per day), which avoids nephrotoxicity problems.[14,21] $GaCl_3$ has been used orally and may potentiate the effect of cisplatin.[22,23]

Since Ga^{3+} is similar in size to Fe^{3+}, it mimics some of the chemistry of iron in that it binds to transferrin (Tf) and can enter cells through transferrin receptors (TfR) as well as by routes that do not require transferrin.[24,25] Because of its high charge-to-radius ratio (Pauling radius = 0.62 Å), Ga^{3+} is a relatively hard Lewis acid. The successive pK_a values for $[(H_2O)_6Ga]^{3+}$ are 2.6, 3.3, 4.4, and 6.3. Consequently,

Dominic Frasca is a native of Hartford, CT, and graduated from Providence College in 1994. His thesis work at Boston College has correlated ruthenium binding to DNA and transferrin with hypoxia and cell toxicity. His present work involves the interactions of ruthenium complexes with other biological components such as glutathione.

at neutral pH and at concentrations greater than ∼10 mM, amorphous $Ga(OH)_3$ or GaO(OH) precipitates, leaving only ∼1 μM $[Ga(OH)_4]^-$ in solution.[26] The insolubility of the phosphate salt, $GaPO_4$ (K_{sp} = 10^{-21}), is significant for the precipitation of Ga^{3+} in the kidney and its incorporation into bone.[25] The water exchange of $[(H_2O)_6Ga]^{3+}$ is fairly rapid (1.8×10^3 s^{-1}).[27] The affinities for Ga^{3+} binding to the two metal sites of transferrin are fairly high (log K_1 = 20.3 and log K_2 = 19.3) but are less than that for Fe^{3+} (log K_1 = 22.8 and log K_2 = 21.5).[28,29] Serum therapeutic levels of Ga^{3+} are thought to be 10−15 μM, and equilibrium calculations indicate that at $[Ga^{3+}]$ < 50 μM nearly all the Ga^{3+} is bound to transferrin. When large doses of Ga^{3+} are administered intravenously, the transferrin may become saturated, so that much of the Ga^{3+} may be initially present as $[(HO)_4Ga]^-$,[25,30] which may enter cells by a transferrin-independent route.[24]

The infusion of therapeutic levels of Ga^{3+} into the bloodstream results in at least 90% saturation of the serum Tf with approximately equimolar amounts of Ga^{3+} and Fe^{3+} in the transferrin.[31] This gallium loading reduces the Tf-mediated uptake of iron into cells, which is indicated by a substantial fall in hemoglobin levels and an increase in Tf receptors (TfR) on blood lymphocytes.[31] The uptake of Ga^{3+} into cells is largely dependent on TfR density on the exterior of cells,[32] and cellular iron deprivation results in enhanced sensitivity of cells to gallium.[33,34] As the enhanced requirements for nutrients arising from their generally higher metabolism leads to higher TfR densities on cancer cells, the uptake of trace (but not therapeutic)[14,21] levels of Ga^{3+} into tumors is generally higher than in other tissues. Consequently, ^{67}Ga−citrate is very useful in the radioscintigraphic imaging of tumors.[24,32] As further evidence of its ability to mimic Fe^{3+} in mammalian metabolism, Ga^{3+} is also deposited in ferritin through a phosphate-mediated pathway.[35] Elimination of $[(HO)_4Ga]^-$ occurs through the kidneys. Instances of kidney toxicity in animals may result from the formation of $Ga(OH)_3$, $GaPO_4$, or related polymers following the administration of large doses that saturate transferrin.[25] Essentially all tissues, par-

ticularly the renal cortex and bone, also utilize non-transferrin uptake routes for both Fe^{3+} and Ga^{3+},[36] and the non-transferrin route can be stimulated in cultured cells by Ga^{3+}.[33,34]

Importantly for antitumor therapy, Tf−Ga blocks DNA synthesis through inhibiting Fe^{3+} uptake. Both iron depletion and, possibly, the direct displacement[37] of Fe^{3+} by Ga^{3+} from the dinuclear iron site in the R2 subunit of ribonucleotide reductase decrease the activity of this essential enzyme,[38] which converts ribonucleotides to deoxyribonucleotides prior to their incorporation into DNA.[38−42] Displacement of Fe^{3+} from the active site with a redox-inactive metal ion prevents the enzyme from generating a tyrosine radical near the dinuclear site, which initiates the reduction of the substrate sugar by the R1 subunit of the protein.[38] The biological effects of gallium are synergistic with those of human interferon-α[43] and the ribonuclease reductase inhibitors hydroxyurea[44] and gemcitabine.[45] On the other hand, the ribonucleotide reductase inhibitors amidox, didox, or trimidox negate the effects of Ga^{3+} by complexing it.[45] A combination of $Ga(NO_3)_3$ with a TfR blocking agent, TfR antibody 42/6, exerted only a slight inhibitory effect on the growth of small cell lung cancer cell lines, which is consistent with blocking the Tf-mediated uptake pathway.[46] The combination of gallium with the iron chelator deferoxamine resulted not only in greater inhibition of cell growth, but also condensation of chromatin and, perhaps most significantly, the formation of DNA-ladder fragments that are characteristic of apoptotic cell death.[47]

Exposure of cells to Tf−Ga arrests cells in the S phase, where ribonucleotide reductase is needed to synthesize DNA.[48] After administration of Tf−Ga, the level of mRNA for transferrin receptors increases in HL60 cells but decreases in CCRF−CEM cells.[49] While HL60 cells become resistant to gallium both by increasing their number of transferrin receptors and utilizing a non-transferrin Fe-uptake pathway,[34,39] resistance in human leukemic CCRF−CEM cells results from a decreased uptake of both Fe^{3+} and Ga^{3+} coupled with increased activity of iron regulatory protein-1 (an iron-responsive element mRNA binding protein) and decreased ferritin production.[50,51] A Ga-regulated expression of TfR at the posttranscriptional level is suggested, but it is not known whether Ga^{3+} binds to the iron regulatory proteins (IRP) and what the effect of the Ga−IRP's might be on iron regulation.

Some chelates of Ga^{3+} have been investigated in an effort to increase its solubility and absorption in the body. Citrate is used to suppress the hydrolysis of Ga^{3+} in ^{57}Ga preparations used in radiodiagnostic imaging.[24,52] The compound, $[(quin)_3Ga]$, where quin = 8-hydroxyquinoline, exhibits enhanced bioavailability and toxicity after oral administration compared to $GaCl_3$.[53] Chelates with 3-hydroxy-4-pyrones and iminophenolates are reported to increase bioavailability through oral administration, particularly against bone cancers.[54,55] In combination with pyridoxal isonicotinoyl hydrazone (PIH), an effective iron-sequestering cytotoxic agent,[56] gallium seems to depress the effect of the chelate alone but the PIH appears to enhance the effect of the gallium. This is probably due to the formation of (PIH)Ga, whose uptake into cultured cells is independent of transferrin receptors.[57,58] An antitumor effect of ultrasound focused on an implanted colon carcinoma, in which a Ga−porphyrin complex had concentrated, has recently been reported.[59]

B. Bone Cancer and Hypercalcemia

The most widespread use of gallium is in combating elevated Ca^{2+} in the blood (hypercalcemia), which often results from bone cancer. Gallium nitrate is the drug of choice for this and is also useful in treating Paget's disease.[60−63] Relatively low therapeutic levels of $Ga(NO_3)_3$ (~200 mg/m^2 per day) block osteolysis and bone resorption by decreasing energy-dependent proton transport in osteoclasts[21,63,64] without altering DNA or protein synthesis.[63] In low doses, $Ga(NO_3)_3$ attenuates the pain and rate of bone loss in multiple myeloma and bone metastases.[63,65−67] Phase III studies are underway to determine its efficacy in limiting bone metastases from breast carcinoma and bone involvement in multiple myeloma.[63] It has also been suggested as a treatment for osteoporosis.[68]

Ga^{3+} incorporates into growing bone tissue at trace (ppm) levels, which increases Type I collagen, calcium, and phosphate levels in the bone, thereby increasing bone strength and density.[64,68] Most uptake of gallium into bone tissue does not occur through transferrin receptors.[69] While the mechanism is unknown, it may simply involve chemisorption of Ga^{3+} to the surface of newly formed hydroxyapatite.[25]

Gallium nitrate has an effect on intracellular signaling pathways through inhibition of a protein tyrosine phosphatase.[70] It also exhibits immunosuppressive properties, probably through Tf−Ga inhibiting T-cell activation by reducing the number of interleukin-2 receptors on T-cell surfaces. Consequently, Ga^{3+} has also been suggested for treating autoimmune diseases.[26]

III. Ruthenium: DNA and Non-DNA Modes of Activity

A. Amine and Imine Complexes

A direct correlation between cytotoxicity and DNA binding has been observed for the representative ruthenium am(m)ine anticancer compounds, *cis*-$[Cl_2(NH_3)_4Ru^{III}]Cl_2$ and (HIm)[*trans*-$[(Im)_2Cl_4Ru^{III}]$ (ICR, Figure 1a) in cell cultures.[71] Binding of $[(H_2O)(NH_3)_5Ru]^{2+}$ to both single- and double-stranded DNA occurs preferentially at $G^{\kappa 7}$,[72,73] but also occurs on A and C residues; however, glutathione alters this. Modifying the ruthenium center to *trans*-$[(H_2O)py(NH_3)_4Ru^{II}]$ causes the metal ion to bind specifically at $G^{\kappa 7}$.[74] Also consistent with DNA binding in vivo, a number of ammine, amine, and heterocyclic complexes of ruthenium exhibit inhibition of DNA replication,[75] mutagenic activity and induction of the SOS repair mechanism,[76] binding to nuclear DNA,[71,77] and reduction of RNA synthesis.[78] More recently EDTA-type complexes of Ru^{III} and even Ru^{IV} have

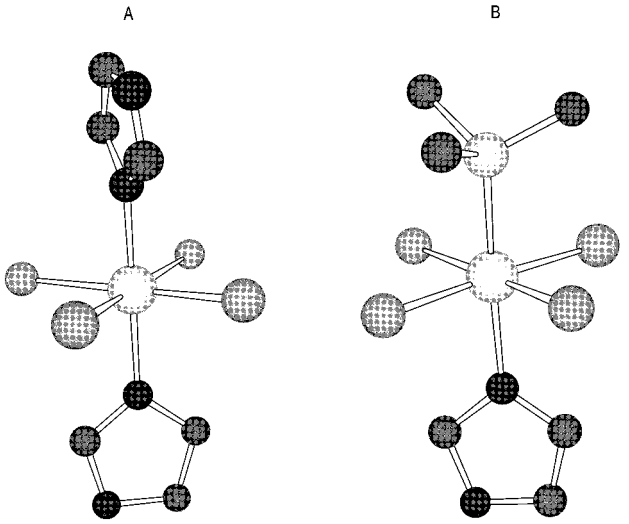

Figure 1. Structures of (a) *trans*-$[(Im)_2Cl_4Ru]^-$ [83] and (b) *trans*-$[(Me_2SO)(Im)Cl_4Ru]^-$ (NAMI).[399]

shown anticancer activity, apparently through DNA binding.[79,80]

Table 1 summarizes the anticancer activity of a representative selection of ruthenium complexes against animal tumor models. While the activity of $[CH_3CH_2CO_2(NH_3)_5Ru](ClO_4)_2$ suggests that monoacido complexes can be active, multichloro compounds such as *cis*-$[Cl_2(NH_3)_4Ru]Cl$, *fac*-$[Cl_3(NH_3)_3Ru]$,[81,82] and (HIm)*trans*-$[Cl_4(Im)_2Ru]$[83,84] exhibit the best activity against primary tumors. While *fac*-$[Cl_3(NH_3)_3Ru]$ showed good antitumor activity in several tumor screens, its low solubility makes it unsuitable as a drug.[85,86] *mer*-$[Cl_3(tpy)Ru]$ (tpy = 2,2′:6′,2′-terpyridine) exhibits antitumor activity midway between those of cisplatin and carboplatin in the L1210 cell line[87] and is cytotoxic against human cervix carcinoma HeLa and murine L1210 tumor cell lines. It also exhibits in vivo activity against the murine lymphosarcoma LS/BL.[88] Several related complexes of the type Cl_3LRu, where L = 2-(2′-pyridyl)-1,10-phenanthroline, 2-(2′-quinolyl)-1,10-phenanthroline, and 2-(2′-benzo[*g*]quinolyl)-1,10-phenanthroline, have also been tested.[89] In *mer*-$[Cl_3(H_2O)(dmtp)_2Ru]$, where dmtp = 5,7-dimethyl[1,2,4]triazolo[1,5-*a*]pyrimidine-N3, the dmtp ligands are in trans positions and the aqua ligand is readily substituted.[90]

Solubility can be enhanced by increasing the number of chlorides, and *trans*-complexes of the type $(LH)[Cl_4L_2Ru]$ (where L = imidazole or indazole) have shown good results against P388 lymphocytic leukemia, Walker 256 carcinosarcoma, Stockholm ascitic tumor, subcutaneously transplanted B16 melanoma, intramuscularly growing sarcoma 180, Ehrlich ascites, and MAC 15A colon tumor and are particularly effective against colorectal tumors.[83,91−96] Activity was observed against nonsmall cell lung, breast, and renal cancers and clonogenic cells from freshly explanted human tumors.[97−99]

These and other multiacido ruthenium(III) complexes, particularly di- through tetrachloro complexes, appear to be transported in the blood by transferrin and albumin (HSA), with the major portion (80%) binding to the latter.[100−104] Albumin can bind up to five (hydrolyzed) $[Cl_4L_2Ru]^-$,[105] which leads to a loss of structure in its α-helical domains. Quenching of the HSA Trp 214 fluorescence is consistent with Ru binding to the nearby His 242 so as to alter the local structure and expose Trp 214 to water.[106] Similarly, a substantial reduction of heme and bilirubin binding is attributed to Ru−histidine coordination at or near the HSA−heme binding site.

Both *trans*-$[Cl(SO_2)(NH_3)_4Ru]^+$[107] and *mer*-$[Cl_3(terpy)Ru]$ (see Table 1) form interstrand cross-links in DNA, and the latter binds two guanine derivatives in a trans configuration.[108] Interstrand cross-linking has also been suggested for *cis*-diaqua polypyridyl complexes.[109]

Solubility can be increased by utilizing dialkylsulfoxide (R_2SO) analogues, such as *trans*-$[Cl_2(Me_2SO)_4Ru]$, $[Cl_3(Me_2SO)_2BRu]$ (B = imidazole or indazole), and Na-*trans*-$[Cl_4(R_2SO)_2BRu]$, where R = methyl and tetramethylene.[110−112] Interestingly, some of these do not show significant activity against P388 lymphocytic and L1210 murine leukemia (Table 1) but are effective against tumor metastases.[113−115] *mer*-$[Cl_3(Me_2SO)_3Ru]$, *trans*-$[Cl_4(Me_2SO)_2Ru]^-$, and *mer,cis*-$[Cl_3(H_2O)_2(Me_2SO)Ru]$ all produce DNA interstrand cross-links.[110] Overall, the broad class of

Table 1. Antitumor Activity of Representative Ruthenium Complexes[a]

compound	dose (mg/kg)	*T/C* (%)	*E*° (V)	ref
$[CH_3CH_2COO(NH_3)_5Ru^{III}]ClO_4$	12.5	163	−0.05	400
$[(C_4O_4)(NH_3)_5Ru^{III}](F_3CSO_3)$[b]	21.2	140	−0.09	401
cis-$[Cl_2(NH_3)_4Ru^{III}]Cl$	12.5	157	−0.10	400
fac-$[Cl_3(NH_3)_3Ru^{III}]$	50	189		400
$[Cl_3(1,5\text{-dimethyltetrazole})_3Ru^{III}]$	80	179		93
mer-$[Cl_3(terpy)Ru^{III}]$			∼0	108,400
$(ImH)[Cl_4Im_2Ru^{III}]$ (Im = imidazole)	209.3	156	−0.29	402
$(pdta\text{-}H_3)Ru^{IV}$ 120	152		130	
H[*cis*-$Cl_2(pdta)Ru^{III}$]	60	210	∼−0.01	79,80
mer-$[Cl_3(Me_2SO)_2BRu^{III}]$ B = NH_3, Im		110−143		110,111
Na[*trans*-$(Im)(Me_2SO)Cl_4Ru^{III}$]	40	170	0.235	110,111
cis-$[I(NO)(NH_3)_4Ru^{III}]I_2$	25	144	∼0.1	249
$(IndH)[Cl_4(Ind)_2Ru^{III}]$	91.1	133		93
μ-$(CH_3CO_2)_4Ru_2Cl$	32	133		93
cis-$[Cl_2(Me_2SO)_4Ru^{II}]$	565	125		113

[a] *T/C* values are expressed as the 100 times the ratio of the lifetime of animals treated with the ruthenium drug to that for the untreated animals. Values listed are for the most common initial screens, i.e., P388 or L1210. In some cases, *T/C* values on other screens were considerably higher or lower. [b] C_4O_4 = squarate anion.

multichloro ruthenium(III) antitumor agents appears to differ from cisplatin by favoring interstrand rather than intrastrand cross-links.

As with cisplatin, adjacent intrastrand $G^{\kappa 7}-G^{\kappa 7}$ cross-links with *cis*-ruthenium ions are possible but are sterically more crowded by the octahedral geometry.[116−119] For example, *trans*-$[Cl_4(Me_2SO)_2Ru]^-$ reacts with d(GpG) to yield a macrocyclic chelate with the likely formulation, *cis*-$[d(G^{\kappa 7}pG^{\kappa 7})Cl(H_2O)(Me_2SO)_2Ru^{II}]$, in which the sugars are in anti configurations and the guanines are destacked in a head-to-head arrangement.[120] A way around the steric constraints that also extends the cross-linking possibilities is to tether two metal centers together. The Ru−Pt dinuclear complex, $[\{cis,fac\text{-}(RuCl_2(Me_2SO)_3)\}\mu\text{-}NH_2(CH_2)_4NH_2\text{-}\{cis\text{-}(Pt(NH_3)Cl_2)\}]$, rapidly loses Me_2SO and chloride from the ruthenium center and cross-links DNA repair proteins to DNA.[121,122] The DNA lesion responsible for efficient DNA−protein cross-linking is most probably a DNA−DNA interstrand cross link by the platinum end of the molecule. Unfortunately, the hydrolytic activity, photosensitivity, and dissociation of Ru from this complex result in nonspecific biopolymer binding.[122] Analogous complexes of the type $[(bpy)_2M(dpb)PtCl_2]Cl_2$ (where M = Ru^{II} or Os^{II} and dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) also form both intrastrand DNA cross-links, due to the *cis*-Cl_2Pt^{II} moiety, and interstrand cross-links, which are probably made through the second metal center.[123]

Some nitrosylruthenium(II) species may be active by releasing toxic nitric oxide upon reduction in vivo.[124−126] Ford has recently reviewed the advantages of photodynamic approaches to releasing NO from ruthenium complexes.[127] A unique "photodynamic" approach is to use the Mössbauer absorption of γ-rays by ruthenium complexes bound to DNA to induce Auger electrons to damage the nucleic acid.[128]

B. Polyaminopolycarboxylate Complexes

Ruthenium complexes with polyaminopolycarboxylic chelating ligands constitute a relatively new group of anticancer compounds.[129,130] These complexes are six-coordinate, octahedral, and highly water soluble. In $[Cl_2(cdta)Ru^{IV}]$, where cdta = 1,2-cyclohexanediaminotetraacetate, the chlorides are cis to one another and the carboxylates appear to be labile. The $Ru^{IV,III}$ reduction potential occurs at 0.78 V, while that for the $Ru^{III,II}$ couple is at −0.01 V,[130] so that Ru^{III} or even Ru^{II} species are present in vivo. This suggests that these complexes may actually belong to the class of multi-acido ruthenium(III) complexes, whose activity involves transport by transferrin.

A labile Ru^{III} complex, *cis*-$[Cl_2(pdta)Ru^{III}]$, where pdta = 1,2-propylendiaminetetraacetate, also shows good antitumor activity, possibly by cross-linking guanines on DNA; and a model complex, $[(Gua)_2(pdta)Ru^{III}]$, has been isolated. The chlorides dissociate to produce a number of reactive Ru^{III} species; however, the metal ion maintains its oxidation state as well as the pdta ligand.[131] The complex rapidly binds to albumin, apotransferrin, or diferric transferrin to produce relatively stable adducts in which $(pdta)Ru^{III}$ is probably bound at the protein surface. Electrophoretic assays show that *cis*-$[Cl_2(pdta)Ru^{III}]$ damages nuclear DNA and significantly alters the conformation of plasmid pHV14 DNA.[132] Moreover, this compound inhibits DNA recognition and DNA lysis by restriction enzymes.[133]

Interestingly, *cis*-$[Cl_2(pdta)Ru^{III}]$ also stimulates NADPH oxidase and a respiratory burst in phagocytic neutrophils. Consequently, it may induce the generation of superoxide, which may be partly responsible for its cytotoxicity, and serve as a catalyst in its production. Finally, it elicits phosphorylation of tyrosine residues, possibly through the involvement of protein kinase.[79,80] A minor disadvantage of the Ru^{III} polyaminopolycarboxylates is that they are generally anionic, which increases the work function for binding to DNA. While $[(H_2O)(edta)Ru^{II}]^{2-}$ coordinates to both the N7 (30%) and N3 (70%) sites on 5′-GMP, the Ru^{III} form yields only the N7 isomer in abundance at a second-order rate constant of 30 M^{-1} s^{-1} (27 °C). $[(5'\text{-GMP})(edta)Ru^{III}]^{n-}$ has a reduction potential of 0.01 V (22 °C) but ionizes a proton from N1 at a pK_a of 7.2, which should cause its reduction potential to decreases at higher pH.[134]

Shepherd has illustrated the potential of Ru^{II} polyaminopolycarboxylates as anticancer agents in binding to the C5−C6 olefinic bonds of pyrimidines, particularly as binuclear agents to span the major groove of DNA.[135] For example, $[(hedta)Ru^{II}]^-$ binds to the usual N3 position of pyrimidines but can also bind in a η^2-fashion to C5−C6.[136] A distribution between η^1 binding at both N1 and N3 of pyrimidine, which can be either stereochemically rigid or fluxional, as well as η^2-binding is observed.[137] $[Ru_2^{II}(ttha)(DMU)_2]^{2-}$ (ttha = triethylenetetraminehexaacetate; DMU = 1,3-dimethyluracil) models a potential interstrand cross link between uracils.[135] Since $[(edta)Ru^{II}]^-$ and related complexes are rapidly air-oxidized, their activity as η^2-agents would depend on the Ru^{III} form being activated by reduction in hypoxic tumors (see section III.G). Because η^2-coordination across C5−C6 increases $E°$ for $Ru^{III/II}$ to ∼0.5 V, the η^2-Ru^{II}−DNA adducts may be stable in vivo. While Ru^{II} can be stabilized with a variety of π-acceptor ligands,[138] e.g., $E°$ for $[py(edta)Ru^{II}]^-$ is 0.1 V and that for $[bpy(edta)Ru^{II}]^-$ is 0.57 V, π-bonding sufficient to stabilize against autoxidation would likely eliminate formation of the η^2-pyrimidine bond.

C. Dimethyl Sulfoxide Complexes

Dimethyl sulfoxide complexes of both Ru^{II} and Ru^{III} exhibit antitumor activity and are relatively nontoxic with LD_{50}'s up to ∼1 g/kg, but correspondingly high doses are necessary to obtain a therapeutic effect in animals.[6] Both *cis*- and *trans*-$[Cl_2(Me_2SO)_4Ru]$ show only marginal activity against the primary tumor of the Lewis lung carcinoma. The cis isomer is similarly effective against MCa mammary carcinoma, an intramuscular implanted solid tumor of CBA mice that produces lung metastases.[139] The cis isomer induces filamentous growth and λ-prophage activity as well as inhibiting the growth of bacteria with defective DNA repair systems,[140,141] while the trans isomer markedly inhibits both primary tumor growth and metastases of B16 melanoma in mice.

Chloride loss from *cis*-$[Cl_2(Me_2SO)_4Ru]$ is suppressed at serum concentrations of chloride, while the O-bound Me_2SO rapidly dissociates.[142] As with cisplatin, maintenance of the neutral complex in blood probably facilitates its crossing lipid membranes to enter the cell, whereas the lower level of intracellular chloride favors chloride loss and DNA binding. In *trans*-$[Cl_2(Me_2SO)_4Ru]$, all the Me_2SO's are S-bound and two are rapidly lost in water to yield the *cis*-diaqua species, which then undergoes reversible chloride dissociation to give *fac*-$[(H_2O)_3Cl(Me_2SO)_2Ru]^+$.[142] While individual guanosines bind reversibly to *trans*-$[Cl_2(Me_2SO)_4Ru]$[143] and 5′-dGMP forms an N7−PO_4 chelate rather than a bis-5′-dGMP complex,[116] NMR evidence indicates a fairly stable macrocyclic chelate with d(GpG).[120] This has the likely formulation, *cis*-$[d(G^{\kappa 7}pG^{\kappa 7})Cl(H_2O)(Me_2SO)_2Ru^{II}]$, in which the sugars are in anti configurations and the guanines are destacked in a head-to-head arrangement similar to that of cisplatin.[120] Coupled with the similar chloride substitution rates of these complexes to those of cisplatin, an analogous mechanism might be expected. On the other hand, their activity against cisplatin-resistant strains suggests a different overall mechanism of action.[144] Since a number of Ru^{III} and Ru^{II} dimethyl sulfoxide complexes show similar activities and have redox potentials that are biologically accessible, it is likely that both oxidation states are available in vivo and coordinate to biopolymers such as nucleic acids and transferrin. Both *trans*-$[Cl_4(Me_2SO)_2Ru]^-$ and *mer*-$[Cl_3(Me_2SO)_3Ru]$ undergo rapid loss of a dimethyl sulfoxide ligand in aqueous solution.[145] In the case of the former, the resulting *trans*-$[Cl_4(H_2O)(Me_2SO)Ru]^-$ then undergoes a slower loss of chloride. In the case of the latter, the resulting *cis*-$[Cl_3(H_2O)(Me_2SO)_2Ru]$ loses an additional dimethyl sulfoxide.[146]

Coordination of *cis*-$[Cl_2(Me_2SO)_4Ru]$ to DNA does not significantly affect the conformation of B-DNA and increases its thermal stability. *trans*-$[Cl_2(Me_2SO)_4Ru]$ binds much more rapidly to DNA with greater changes in its CD spectra, which is attributed to disruption of the DNA structure due to cross-linking.[113] Both the cis and trans isomers induce the B to Z transition in poly(dGdC), with the trans complex being much more effective. DNA extracted from cells, which were separately treated with each isomer, showed a 5-fold higher content of Ru in the trans case.[115,142,147]

Topoisomerase II (DNA gyrase), an important enzyme in the nuclei of rapidly dividing cells, may be inhibited by some ruthenium complexes. By altering the topological properties of DNA, this enzyme helps maintain the structural organization of the mitotic chromosomal scaffold in the replication, transcription, recombination, and segregation of chromosomal pairs during cell division.[148,149] As these roles are particularly important in proliferating cancers, selectively targeting topoisomerase II could inhibit neoplastic cells division and possibly induce apoptosis by fragmenting DNA. Topoisomerase II activity is inhibited by what is reported to be $[Cl_2(Me_2SO)(C_6H_6)Ru^{II}]$ but not by $[(saldox)_2Ru^{II}]$ (*sic*), where sal = salicylaldoximate.[148] Unfortunately, the hydrolysis of the former was not considered and the latter was thought to be a square planar complex. The species introduced into solution were probably *cis*-$[Cl_2(Me_2SO)(C_6H_6)Ru^{II}]$ and conceivably *trans*-$[Cl_2(saldox)_2Ru^{III}]^+$; however, characterization was inadequate for both complexes. The former can hydrolyze to *fac*-$[(H_2O)_3(C_6H_6)Ru^{II}]^{2+}$ and was likely present in equilibria between aqua and chloro ions.[150] Whatever the species in solution, both complexes bound to DNA and similarly inhibited DNA replication and cell proliferation. Since the benzene complex interfered with the DNA-stimulated ATPase activity of topoisomerase II by allowing DNA cleavage but inhibiting re-ligation, the formation of an enzyme−Ru−DNA cleavage complex is suspected.[148]

D. NAMI: Antimetastatic Activity via Possible Impairment of a Matrix Proteinase

Since metastatic cancer is particularly difficult to treat, the antimetastatic activity of the ruthenium dimethyl sulfoxide complexes, particularly Na-*trans*-$[Cl_4(Me_2SO)(Im)Ru]$ (NAMI, Figure 1b), represents an important development. Such complexes could be of particular use in minimizing the growth of undetected micrometastases following surgery or radiotherapy.[151,152] While structurally similar to (ImH)-*trans*-$[Cl_4(Im)_2Ru]$ (ICR, $E° = -0.24$ V), NAMI has a significantly higher $Ru^{III/II}$ reduction potential (0.235 V)[83,96,146] owing to the π-acceptor effect of the S-bound DMSO, which also exerts a kinetic trans effect. Relatively high concentrations (>100 μM) are needed to produce a cytotoxic effect, which depends on the lipophilicity of the complex and the presence of serum and plasma proteins.[153] Of those tested, the most lipophilic complex, Na-*trans*-$[RuCl_4(TMSO)Iq]$ (TMSO = tetramethylensulfoxide; Iq = isoquinoline), causes DNA fragmentation similar to *cisplatin* while the most promising, NAMI, is virtually devoid of any effect on DNA. NAMI has good water solubility and is active against a broad range of tumors including Lewis lung carcinoma, B16 melanoma, and MCa mammary carcinoma.[154] In animals, doses of 22−66 mg/kg/day had a significant antimetastatic effect and NAMI can be administered orally.[111,153]

Of particular note is that (1) only a very low fraction of the NAMI reaches the tumor target, (2) its activity appears to be independent of its concentration in tumor cells, and (3) its mechanism of action does not involve DNA binding.[155] NAMI may increase resistance to the formation of metastases,[153,156] but this is not through an enhanced antigenicity or an immunological response.[151,157] At levels that cause a dramatic reduction in lung metastases, NAMI greatly alters the ratio between the mRNAs of MMP-2 (a metalloproteinase capable of degrading the extracellular matrix) and TIMP-2, the specific tissue inhibitor of this enzyme.[158,159] This corresponds with a pronounced increase of extracellular matrix components in the tumor parenchyma and around tumor blood vessels, which probably hinders metastasis formation and blood flow to the tumor.[160] Overall, NAMI appears to down regulate type-IV collagenolytic activity and the metastatic potential of MCa mammary

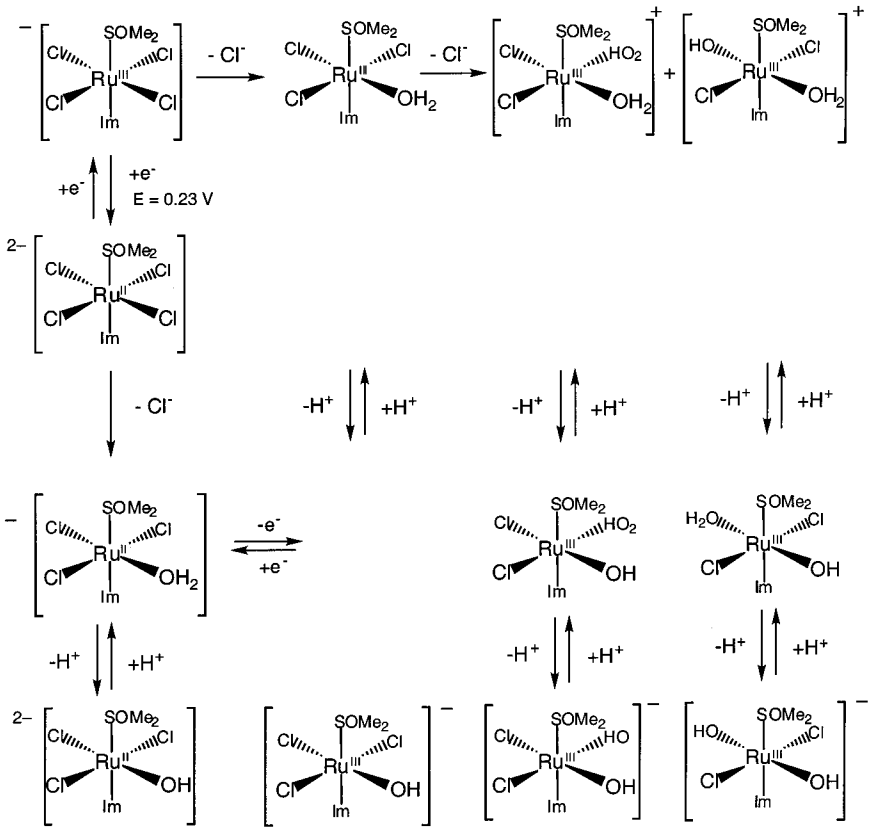

Figure 2. Hydrolysis products of Na[*trans*-(Me_2SO)(Im)Cl_4Ru] (NAMI) in aqueous solution.

carcinoma.[157] Combining NAMI with 5-fluoruracil achieved better results in mice against the solid metastasizing MCa mammary carcinoma and lymphocytic leukemia P388.[161]

Figure 2 illustrates the various equilibria of NAMI under physiological conditions. Further loss of DMSO and imidazole following chloride dissociation results in polyoxo complexes of ruthenium. At 25 °C, hydrolysis of the first chloride occurs within an hour while the second takes more than twice as long. At physiological pH, *trans*-[Cl_4(Im)(Me_2SO)Ru]$^-$ is more labile to substitution than *trans*-[$Cl_4(Im)_2Ru]^-$ ($t_{1/2}$ = 19.7 h at 25 °C).[96] Chloride loss for the former is catalyzed by reduction to Ru^{II}, which is expected to occur under physiological conditions and is enhanced in vitro by traces of biological reductants, such as ascorbic acid or cysteine.[112,162] Consequently, a redox−catalytic (activation by reduction) mechanism is suspected. The pK_a values of the aqua species have not been determined, but no shift in ^{1}H NMR resonances were noted for *mer*-[Cl_3(Me_2SO)(Im)-(H_2O)Ru^{II}]$^-$ between pH 3 and 9.

While the antimetastatic action of NAMI does not appear to involve DNA binding, 80−90% of the complex in solution binds to calf thymus DNA within 24 h at 37 °C. Such binding stabilizes DNA in low salt media (0.01 M $NaClO_4$), but alterations in the CD spectrum suggest unwinding of the DNA. Binding also inhibits the B to Z transition in poly(dG−dC). DNA interstrand cross-linking efficiency by NAMI is only ∼1%, but it significantly inhibits DNA and RNA polymerases, with termination occurring preferentially at guanine residues.[151]

NAMI-A, (ImH)-*trans*-[Cl_4(Me_2SO)(Im)Ru], has improved pharmacological properties over NAMI in that it is a more stable and reproducible solid.[163] Analogous complexes of the type (LH)-*trans*-[Cl_4(Me_2SO)-(L)Ru], where L = NH_3, 1-methyl imidazole, pyridine, and substituted pyridines, have also been prepared.[164] In mice, NAMI-A exhibits similar pharmacological properties to NAMI in that it causes a dose-dependent reduction in MCa mammary carcinoma metastases to the lung. It selectively interferes with the growth of Lewis lung, MCa mammary carcinoma, and TS/A adenocarcinoma metastases already settled in the lungs in a manner that is independent of the stage of the metatatic growth[165] and not simply related to a larger concentration in the lungs than in other tissues.[166] When administered intraperitoneally, NAMI-A appears to be rapidly cleared from the blood by the kidneys.[166] It is rapidly distributed to the body with only 10% of the original dose remaining in the blood after 5 min. The ruthenium concentration in the kidney peaks 10 min after the injection and is markedly higher than in any other tissue analyzed, but some is also retained in the liver and then bowel.[151] Total retention 2 h after intravenous injection is about 85% of the administered dose. NAMI-A significantly increases the percentage of $CD8^+$ cells at three dose levels, while $CD4^+$ cells increased only at the lowest dose and remain unchanged at medium and high doses. Like NAMI, NAMI-A significantly increases the thickness of the connective tissue of the tumor capsule and around tumor blood vessels and impairs MMP-2, possibly at the level of its gene and/or its inhibitor TIMP-2.[151] NAMI-A appears to be less toxic than cisplatin, does

not modify cell growth, and causes a transient cell cycle arrest of tumor cells in the premitotic G2/M phase; whereas cisplatin causes a dose-dependent disruption of cell cycle phases and reduces of cell proliferation.[165]

E. DNA Binding

There is little interaction between "$RuCl_3$" and calf-thymus DNA at low [Ru], but at $[Ru]/[P]_{DNA} = 2$ and elevated temperatures, the metal binds with some indication of cross-linking.[167] Since cationic metal complexes have an electrostatic attraction to polyanionic nucleic acids, the rate of RNA binding by $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ proceeds fairly rapidly and is strongly ionic-strength dependent. For tRNA's, the rate for binding to guanine N7 sites ($G^{\kappa 7}$) is rate $= k[Ru][P_{RNA}]$, where $k = 5.96\ M^{-1}\ s^{-1}$ at 25 °C and $\mu = 0.045$.[73] In DNA, the initial reaction also involves $G^{\kappa 7}$ sites, which are relatively exposed in the major groove of B-DNA, while a second reactive phase probably has to do with coordination of interior sites exposed upon separation of the nucleic acid strands.[72] Covalent Ru−DNA binding constants are 5100 and 7800 M^{-1} for helical and single-stranded CT-DNA, respectively, but are somewhat lower for RNA, probably because of the additional sugar oxygen, which has a modest effect on the basicity of the purine N.[7]

Migration of Ru between DNA sites is possible. While the initial binding of adenosine by $[(H_2O)(NH_3)_5Ru^{II}]$ appears to be largely at N1, N7 coordination occurs with 1MeAdo.[168,169] Electrochemical measurements suggest the pK_a(N6) of $[(Ado^{\kappa 1})(NH_3)_5Ru^{III}]$ to be 8.2. Consequently, at neutral pH the fraction ionized at N6 presents an excellent binding site for Ru^{III}, to which this metal ion rapidly linkage isomerizes.[86] Once coordinated at the exocyclic amine, hydrogen bonding occurs between two ammine ligands and the anionic N1 of adenine (or N3 of cytosine), but this is negated upon protonation of the pyrimidine ring, which causes the metal to rotate about the N_{exo}−C bond. The pK_a values for the N1 and N3 sites for the isocytosine (ICyt) complex are 2.9 and 10.0, respectively, and the corresponding values for 6Me-ICyt are 3.1 and 10.2. The $\Delta H^{\ddagger}$ for rotation about the C−N bond is 13 kcal lower than in the free ligand, because appreciable π-bonding between the Ru^{III} and the amide decreases the π-interactions between the amide and the pyrimidine ring.[170] With adenosine, a second motion of the metal occurs upon reduction to Ru^{II}, which rapidly linkage isomerizes from N6 to the adjacent π-acceptor ring nitrogen, N1.[86] Square wave voltammetry of $[(NH_3)_5Ru^{III}]_n$−DNA shows an increase in the current peak for pyrimidine ring coordination at $A^{\kappa 1}$, while those for exocyclic $A^{\kappa 6}$ and $C^{\kappa 4}$ coordination and possibly $G^{\kappa 7}$ decrease with increasing reductive electrolysis time.

Unlike $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$,[72] covalent binding of *trans*-$[(H_2O)(py)(NH_3)_4Ru^{II}]^{2+}$ to DNA is $G^{\kappa 7}$-specific,[74] with $K_G = 1 \times 10^4\ M^{-1}$. Polypyridyl complexes of Ru^{II} are also generally considered to be G-specific.[109,171,172] Extensive ^1H NMR and EPR studies, including paramagnetic contact shift determinations, have been done on $[L(NH_3)_5Ru^{III}]^{3+}$ and *trans*-$[L(py)(NH_3)_4Ru^{III}]^{3+}$, where L = purines, pyrimidines, their nucleosides, nucleotides, and other heterocyclic ligands bound at various positions, which facilitate the determination of binding site.[173−177]

Pyridine ligands (Pyr) slow DNA binding by *trans*-$[(H_2O)(Pyr)(NH_3)_4Ru^{II}]^{2+}$ relative to $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ and favor of $Ru^{III/II}$ reduction by about 150 mV. At $\mu = 0.05$, DNA binding by these complexes follows the rate law $d[Ru^{III}-DNA]/dt = k[Ru^{III}][P_{DNA}]$, where $k = 0.17-0.21\ M^{-1}\ s^{-1}$ for various pyridine ligands.[74] A strong dependence on ionic strength indicates that ion-pairing with DNA occurs prior to binding. The air oxidation of $[(py)(NH_3)_4Ru^{II}]_n$−DNA to $[(py)(NH_3)_4Ru^{III}]_n$−DNA at pH 6 occurs with a pseudo-first-order rate constant of $5.6 \times 10^{-4}\ s^{-1}$ at $\mu = 0.1$ and 25 °C.[74]

Stabilization of Ru^{II} by pyridine ligands also promotes the disproportionation of Ru^{III} to the corresponding complexes of Ru^{II} and, presumably, Ru^{IV}, which leads to other products for both monomers (discussed here) and DNA (discussed below). For *trans*-$[(L)(py)(NH_3)_4Ru^{III}]^{3+}$, disproportionation follows the rate law $d[Ru^{II}]/dt = k_o[Ru^{III}] + k_1[OH^-][Ru^{III}]$. For L = Ino, $k_o = 2.7 \times 10^{-4}\ s^{-1}$ and $k_1 = 70\ M^{-1}\ s^{-1}$; for L = Guo, $k_o = 2.9 \times 10^{-4}\ s^{-1}$ and $k_1 = 6.4\ M^{-1}\ s^{-1}$.[175] Surprisingly, the rate-limiting step in the dominant, hydroxide-dependent pathway is not electron transfer between Ru^{III}'s but probably deprotonation of an ammine. The electron-donating amine at C2 or ionization of the purine at N1 or N9 slows the disproportionation by suppressing ammine ionization, so that the ordering of k_1's for various ligands is Ino > 1MeGuo > Guo ≈ dGuo > 9MeGua ≫ Gua. Activation parameters for k_1 (pH = 11.50) with L = Guo are $\Delta H^{\ddagger} = 17.4$ kcal/mol and $\Delta S^{\ddagger} = 2.4$ cal/mol·K. Following the disproportionation of *trans*-$[Guo^{\kappa 7}(py)(NH_3)_4Ru^{III}]$, the appearance of *trans*-$[Gua^{\kappa 7}(py)(NH_3)_4Ru^{III}]$ and free ribose is consistent with general acid hydrolysis of the glycosidic bond induced by Ru^{IV}, which is subsequently reduced. The rate of appearance of *trans*-$[Gua^{\kappa 7}(py)(NH_3)_4Ru^{III}]$ (pH 9.2−11.9) is complicated by purine loss, anation, and possibly redox reactions, so that a net hydroxide dependence of approximately $[OH^-]^{1/2}$ was observed. Activation parameters for the N-glycolysis reaction (pH 11.90) with L = $Guo^{\kappa 7}$ are $\Delta H^{\ddagger} = 24.6$ kcal/mol and $\Delta S^{\ddagger} = 8.9$ cal/mol·K. In the presence of oxygen, *trans*-$[8OGuo(py)(NH_3)_4Ru^{III}]$ was detected as a minor product.[175]

In the anticancer complex, *trans*-$[Cl_4(Im)_2Ru]^-$, aquation occurs stepwise by sequential loss of two chlorides at an initial rate of $9.6 \times 10^{-6}\ s^{-1}$ at 25 °C and $5.26 \times 10^{-5}\ s^{-1}$ at 37 °C. Aquation is accompanied by a drop in pH, which may be due to proton loss from the corresponding aqua complexes.[96] The formation of blue-green ($\lambda_{max} = 585$ nm) precipitates in serum and physiological buffer suggest that hydrolyzed forms anate to form carbonato or carboxylato species.[178] Reaction of *trans*-$[Cl_4(Im)_2Ru]^-$ with histidine at pH 4−5 leads to $[Cl_2(His)_4Ru]Cl$ with no histidine adducts occurring at pH 3 and a mixture of products obtained above pH 6.[179] Presumably, this complex cross links $G^{\kappa 7}$ sites on DNA, but this has not been shown. Aged (but not fresh) solutions of *trans*-$[Cl_4(Im)_2Ru]^-$ inhibit DNA polymerization, suggesting that hydrolysis occurs before

binding. The surprisingly high reduction potential of this complex (−0.24 V) may allow in vivo reduction, which would cause the chlorides to dissociate more rapidly, and an activation by reduction mechanism has been postulated.[95,179] Sadler has plausibly suggested that a hydrido intermediate may be responsible for the high $E°$,[96] which may also account for the rapid reaction between *trans*-$[Cl_4(Im)_2Ru]^-$ and glutathione (see section III.E).[179]

The L-enantiomer of *cis*-$[Cl_2(phen)_2Ru^{II}]$ selectively associates with B-DNA through electrostatic and hydrophobic interactions before coordinating, presumably at $G^{\kappa 7}$.[171] Indeed, a number of mono- and diaqua polypyridyl complexes of Ru^{II} covalently bind to DNA but at a relatively low level ($[Ru]_{DNA}/[P]_{DNA} = 0.01-0.02$).[172] Since bulky aromatic ligands present considerable steric hindrance to cis coordination, *cis*-$[Cl_2(bpy)_2Ru^{II}]$ coordinates only one 9MeHyp or 9EtGua with the bipyridyls imposing a significant barrier for rotation about the Ru−N7 bond.[180] In contrast, coordination of 9MeHyp and 9EtGua to *mer*-$[Cl_3(tpy)Ru]$ allows for two N7-coordinated purines in trans positions.[87] Covalent binding of the tpy complex to DNA occurs with about 2% interstrand cross-linking, presumably through *trans*-$(G^{\kappa 7})_2$ coordination, which may be responsible for its antitumor activity. While binding of monoaqua complexes with polyaromatic ligands generally produces small increases in the DNA melting temperature, ΔT_m, larger increases and irreversible melting curves are seen for diaqua complexes such as $[(H_2O)_2(phen)_2Ru]^{2+}$, which suggest interstrand cross-links, possibly between guanine residues in a head-to-tail arrangement.[109,120]

Tethering $[(H_2O)_2(bpy)_2Ru]^{2+}$ to the oligomers 5′-GCAC*TCAG-3′ and 5′-GCACT*CAG-3′, where C* and T* are bases modified with a linker arm terminating in a primary tethering amine, allow duplex formation with their complementary strands. DNA sequences labeled with metal ions may be used to identify and cleave specific sites through base-pairing and photochemical or redox attack.[181,182]

F. Modulation of DNA Binding by Glutathione

Glutathione (γ-glutamate−cysteine−glycine) is the most common cellular nonprotein thiol.[183] In cells, it exists predominately in the reduced form (GSH) at concentrations of 0.1−10 mM but is readily oxidized to the disulfide (GSSG, $E°' = -0.246$ V vs NHE).[184] Glutathione helps protect cells from reactive oxygen intermediates, UV radiation, and heavy metal toxicity.[185] In the latter case, GSH scavenges and sequesters heavy metal ions by coordinating them through its sulfhydryl, thereby inhibiting their binding to proteins and nucleic acids.[183,186−189] In some cases, GSH reduces metal ions, such as CrO_4^{2-} and Pt^{IV} anticancer drugs,[190] to species that coordinate or otherwise react with DNA.[189,191−194] On the other hand, GSH binding to Pt^{II} appears to contribute to cisplatin resistance in tumor cells.[195,196] GSH (0.1 M, pH 6, apparently in air) rapidly reduces *trans*-$[Cl_4(Im)_2Ru]^-$ ($E° = -0.24$ V), which then dissociates its imidazole ligands within 1 h. GSH coordination of Ru^{III} followed by electron transfer has been assumed.[179]

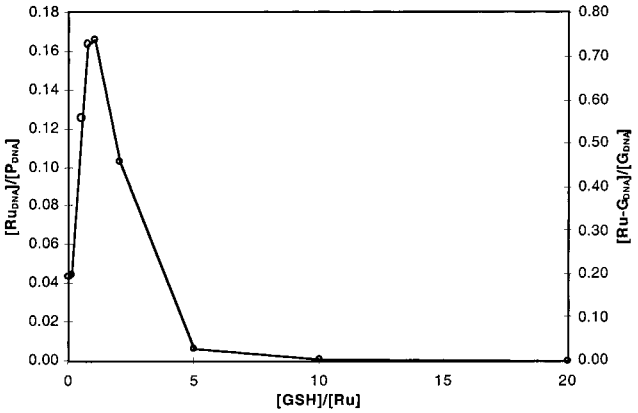

Figure 3. DNA binding of $[Cl(NH_3)_5Ru]^{+2}$ as a function of [GSH] when allowed to react for 1.5 h under argon.

The aerobic reaction of GSH with $[Cl(NH_3)_5Ru^{III}]^{2+}$ is first order in [GSH] and yields only $[OH(NH_3)_5Ru^{III}]^{2+}$ and GSSG. Since GSH only slowly reduces $[Cl(NH_3)_5Ru]^{2+}$ under physiological conditions ($t_{1/2} = \sim 10$ min) and the Ru^{II} product is readily oxidized by air, this mode of activating Ru to bind to biopolymers by reduction may not be important in tissues under normal oxygen tensions but may be in the hypoxic environment of tumors (see section III.G).[71,197] Since oxygen also effectively prevents GSH coordination, this could circumvent some thiol-based resistance to ruthenium ammine anticancer agents.

The anaerobic reaction of GSH with $[Cl(NH_3)_5Ru^{III}]^{2+}$ yields first $[OH(NH_3)_5Ru^{III}]^{2+}$ and then $[GS(NH_3)_5Ru^{III}]^{+}$ at neutral pH, both through redox catalysis. The reaction appears to proceed through reduction of Ru^{III} by GSH to give $[H_2O(NH_3)_5Ru^{II}]^{2+}$, followed by coordination to produce $[GSH(NH_3)_5Ru^{II}]^{2+}$ and then oxidation of the latter ion by $[OH(NH_3)_5Ru^{III}]^{2+}$ or GSSG to yield $[GS(NH_3)_5Ru^{III}]$.[198]

The reduction of $[OH(NH_3)_5Ru^{III}]^{2+}$ by GSH proceeds by a preequilibrium mechanism according to the rate law $d[Ru^{II}]/dt = k[Ru^{III}][GSH]/(K_{ip} + [GSH])$, where $K_{ip} = 1.98 \times 10^{-3}$ M^{-1} and $k = 2.34 \times 10^{-3}$ s^{-1}. The reduction potential of $[(GS)(NH_3)_5Ru^{III}]$ is pH dependent with $E = E° - 0.59 \log\{K_a/([H^+] + K_a)\}$, where $E° = -0.44$ V and $pK_a = 7.1$. While $[GS(NH_3)_5Ru^{III}]$ is stable for extended periods under inert atmosphere, it dissociates in air, yielding $[HO(NH_3)_5Ru^{III}]$ at high pH with k_{obs} (s^{-1}) $= (k_1K_a + k_2[H^+])/([H^+] + K_a)$, where $k_1 = 9.1 \times 10^{-6}$ s^{-1}, $k_2 = 1.2 \times 10^{-4}$ s^{-1} M^{-1}, and $pK_a = 12$.[198]

As shown in Figure 3, at $[GSH]/[Ru^{III}] \leq 1$, the coordination of $[Cl(NH_3)_5Ru^{III}]^{2+}$ to DNA is facilitated by GSH reduction to the more substitution-labile $[H_2O(NH_3)_5Ru^{II}]^{2+}$. However, at $[GSH]/[Ru^{III}] \geq 1$, DNA binding is inhibited by GSH, which coordinates the Ru^{II} and facilitates oxidation back to Ru^{III} because of the low $E°$ of $[GS(NH_3)_5Ru^{III}]^{+}$. Consistent with this is the increased toxicity of $[Cl(NH_3)_5Ru^{III}]^{2+}$ to Jurkat T-cells, when GSH levels are suppressed.[198] Inhibition of DNA binding by GSH is most evident at $G^{\kappa 7}$, and GSH removes most of the metal ion from $G^{\kappa 7}$ sites on DNA. It is less effective in preventing binding or removing the metal from $A^{\kappa 6}$ and $C^{\kappa 4}$ sites owing to the lower $Ru^{III,II}$ reduction potential,[168] when the metal ion is attached to the exocyclic ammine of these

ligands. The ability of adenine to provide strong π-binding sites for both Ru^{II} (N1) and Ru^{III} (ionized N6) may account for its maintaining ruthenium binding even at high [GSH]. Such altering of DNA binding at physiological concentrations of GSH may have a significant effect on the mechanism of ruthenium antitumor compounds by favoring A and C binding over G,[198] but speciation of nuclear DNA with respect to Ru-binding has not yet been determined.

Simple ruthenium complexes of ammine and heterocyclic nitrogen ligands, such as $[(4\text{-picoline})(NH_3)_5Ru]Cl_2$, also possess a remarkable immunosuppressant activity, which greatly exceeds that of the clinically used cyclosporin. Remarkably, this activity is exhibited within an electrochemical window of 100−400 mV.[174,199,200] Consequently, biological reductants may also be involved in the immunosuppressant behavior of this new class of ruthenium pharmaceuticals. The reaction of GSH with $[(4\text{-picoline})(NH_3)_5Ru]^{3+}$ and $[(NH_3)_6Ru]^{3+}$ also produces some $[GS(NH_3)_5Ru^{III}]^{2+}$, which is indicative of a reduced intermediate that eliminates nitrogen ligands, and suggests that GSH is conceivably involved in the ruthenium immunosuppressant activity.

G. DNA Damage Generated by Covalently Bound Ru

There are a number of ways by which ruthenium can generate strand breaks in DNA. Ruthenium(III) functions as a general acid in promoting the hydrolysis of the N−glycosidic bond in $[(dG^{\kappa 7})(NH_3)_5Ru^{III}]$ ($t_{1/2}$ = 1.5 days, 56 °C, pH 7).[201] Consequently, apurinic sites followed by hydrolysis of DNA might be induced by Ru^{III} at guanine sites in DNA, but this has not been observed.[72] Ru^{III} N7-coordinated to nucleosides facilitates their base-catalyzed air oxidation to 8-oxonucleosides.[176,202] This reaction probably proceeds by hydroxyl attack at C8 induced by Ru^{III} followed by sequential single-electron transfers via the Ru to oxygen. Following autoxidation, the glycosidic bond undergoes base-catalyzed cleavage; however, this also has not been observed to cleave DNA.[72] In air, the rate of autoxidation to 8-O-purines is first order in $[L(NH_3)_5Ru^{III}]$ and $[OH^-]$ with $k/(10^{-2}\ M^{-1}\ s^{-1})$ for L = dGuo, Guo, 1MeGuo, and Ino of 3.5, 6.6, 20, and 77, respectively. Autoxidation is hindered by the electron-donating amine at C2 and proton ionization N1.[11,176]

Ruthenium(IV) at $G^{\kappa 7}$ on DNA is a stronger general acid catalyst, which also better facilitates guanine autoxidation. A convenient route to Ru^{IV} is through the disproportionation of $[py(NH_3)_5Ru^{III}]$ to Ru^{II} and Ru^{IV} species.[175,203] Disproportionation of $[py(NH_3)_4Ru^{III}]_n$−DNA occurs according to the rate law $d[Ru^{II}-G_{DNA}]/dt = k_o[Ru^{III}-G_{DNA}] + k_1[Ru^{III}-G_{DNA}][OH^-]$, where $k_o = 5.4 \times 10^{-4}\ s^{-1}$ and $k_1 = 8.8\ M^{-1}\ s^{-1}$ at 25 °C, $\mu = 0.1$. The slower appearance of $[(Gua^{\kappa 7})(py)(NH_3)_4Ru^{III}]$ following disproportionation under argon, which occurs according to the rate law $d[Ru^{III}-G]/dt = k_o[Ru^{III}-G_{DNA}] + k_1[OH^-][Ru^{III}-G_{DNA}]$ ($k_o = 5.74 \times 10^{-5}\ s^{-1}$, $k_1 = 1.93 \times 10^{-2}\ M^{-1}\ s^{-1}$, T = 25 °C, $\mu = 0.1$), is consistent with lysis of the N−glycosidic bond by Ru^{IV}-induced general acid hydrolysis. In air, a Ru-induced autoxidation of guanine also occurs. The ratio of [Ru-8OG]/[Ru-G] and their net rates of appearance are 1.7 at pH 11, 25 °C. Small amounts of phosphate glycolate indicate a minor oxidative pathway involving C4′ of the sugar.

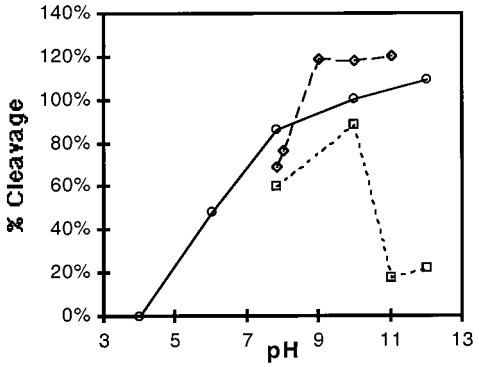

Figure 4. Plot of the estimated percent cutting in pBR322 DNA versus reactant $[Ru]/[P_{DNA}]$ for *trans*-$[(H_2O)(py)(NH_3)_4Ru^{II}]$ coordinated to DNA as a function of pH under three different reaction conditions. No [DTT] or $[H_2O_2]$ is present. (2) (□) long-dashed line; Ru^{II} incubated with DNA for 1.5 h before pH adjusted to that indicated with the reaction subsequently proceeding for 10 min; (2) (○) solid line, Ru^{II} incubated with DNA for 1.5 h, DNA precipitated, and then pH adjusted to that indicated with the reaction subsequently proceeding for 10 min. $[P_{DNA}] = 42.4\ \mu M$, $[Ru]/[P_{DNA}] = 0.1$.

In air, a dynamic steady-state arises in which reduction of Ru^{IV} produces additional $Ru^{II}-G_{DNA}$, which is air-oxidized to $Ru^{III}-G_{DNA}$ followed by disproportionation back to $Ru^{II}-G_{DNA}$ and $Ru^{IV}-G_{DNA}$. The $Ru^{IV}-G_{DNA}$ can hydrolyze to give Ru−G or undergo autoxidation to yield Ru−8OG products. If 8OG production proceeds by single electron transfer to oxygen, then $Ru^{III}-8OG$ should result and is observed. Since sugar oxidation by Ru^{IV} is also evident, this represents another route back to $Ru^{II}-G_{DNA}$. This dynamic system slowly, but catalytically, damages DNA.[74] Figure 4 represents the increase in cleavage of pBR322 plasmid $[py(NH_3)_4Ru^{III}]_n$−DNA in air as a function of pH relative to controls, which were handled identically but without Ru. Since some cleavage occurs at neutral pH, the disproportionation routes to DNA damage may be active in cells. Pointing out differences in chemistry between bulk solution and the DNA environment, the complex $[HO(DAMP)(bpy)Ru^{III}]^{2+}$, where DAMP = 2,6-bis-((dimethylamino)methyl)pyridine, is stable in solution but disproportionates following a zero-order rate law upon electrostatically binding to calf thymus DNA.[204]

Strand cleavage of plasmid DNA can also occur by Fenton, Haber−Weiss, or oxo−metal ion chemistry for a number of ruthenium(III) ammines. However, the covalently bound metal in $[(NH_3)_5Ru^{III}]_n$−DNA is ineffective at generating oxygen radicals.[72] It may be that oxo−ruthenium(IV) ions, such as $[O(NH_3)_5Ru^{IV}]^{2+}$, are more effective at cleaving DNA and are prevented from forming when the metal ion is bound to DNA so that it has six firmly coordinated nitrogen ligands. Surprisingly, base-catalyzed cleavage by covalently bound $[Cl(py)(NH_3)_4Ru^{III}]$ is more efficient than O_2 activation, even at neutral pH.[74]

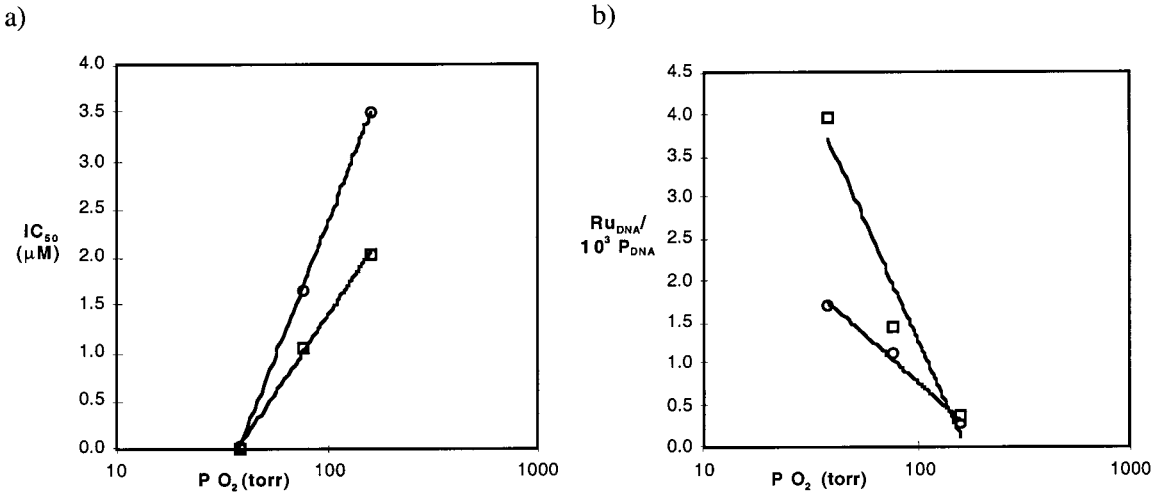

Figure 5. Toxicity and DNA binding for ruthenium anticancer complexes as a function of hypoxia. (a) Correlation between toxicity (IC_{50} values) and log[P_{O_2}]. (b) Correlation between amount of Ru bound to DNA at [Ru] = 10 μM and log[P_{O_2}]. (○) *cis*-[$Cl_2(NH_3)_4Ru$]Cl (CCR); (□) [ImH]*trans*-[$(Im)_2Cl_4Ru$] (ICR).[71]

DNA oxidation by aqua polypyridyl complexes of Ru^{III} probably involves a slow disproportionation to give oxoruthenium(IV) species,[205,206] which can oxidize both sugar and guanine residues to cleave DNA. Electrochemically generated $[(bpy)_3Ru]^{3+}$ preferentially and catalytically oxidizes mismatched guanines in DNA.[207] Oxo−Ru^{IV}−polypyridyl complexes, such as $[O(tpy)(bpy)Ru]^{2+}$ ($E°$ = 0.80 V) cleave DNA directly through C1′-sugar or C8-guanine oxidation.[205,208,209] Oxidation of dG by O=Ru^{IV} at C8, which occurs by oxygen-atom transfer that may involve a C8−O−Ru^{III} link,[210] is about 7 times that of its sugar oxidation at C1′. Since the Ru^{II} produced by reduction of oxo−Ru^{IV} comproportionates with the higher oxidation state to form Ru^{III}, only 50% of the original Ru^{IV} reacts with substrate. Of this Ru^{IV}, about 50% is consumed by the sugar or guanine oxidation pathways.[211−213] Oxidation appears to proceed past the two-electron 8OG product, so that the guanine may collapse to an oxazolone derivative, which may be the true base-labile component.[214] Oxidation of the mRNA from the iron recognition element by $[O(tpy)(bpy)Ru]^{2+}$ produces a single scission at G_8, whereas an analogous DNA fragment is cleaved at nearly every site.[215] DNA binding by $[O(tpy)(bpy)Ru]^{2+}$ is largely electrostatic (K(est) ≈ 660 m^{-1}),[206,216,217] whereas the inclusion of a strongly intercalating ligand in $[O(tpy)(dppz)Ru]^{2+}$ results in a high-affinity cleaving agent (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine).[209,218,219]

H. Activation by Reduction

More than two decades ago, in what has become known as the "activation by reduction" hypothesis, it was suggested that Ru^{III} complexes may serve as prodrugs, which are activated by reduction in vivo to coordinate more rapidly to biomolecules.[75,85] Indeed, glutathione and a number of redox proteins are capable of reducing Ru^{III} complexes in vivo.[197] The low O_2 content and lower pH in tumor cells should favor Ru^{II}, which is generally more actively binding than Ru^{III}, and thus provide for selective toxicity. In 50 mm diameter solid tumors, the relative electrochemical potential is ∼100 mV lower than in the surrounding normal tissue and this difference is greatest in the center of the tumor.[220]

In vivo reduction to Ru^{II} can occur by single-electron-transfer proteins, which exist in both the mitochondrial electron-transfer chain and in microsomal electron-transfer systems, with microsomal proteins being the more efficient.[197] Ammineruthenium complexes can also be reduced by transmembrane electron-transport systems, so that it is not necessary for the complexes to enter cells in order to be reduced.[221] Oxidation of Ru^{II} back to Ru^{III} can occur by molecular oxygen,[222] cytochrome oxidase,[222−224] and other oxidants.

As reduction of Ru^{III} to Ru^{II} fills the d_π orbitals, those ligands that π-donate are no longer able to do so and bind less strongly. In the case of Ru^{II} am(m)ine complexes, acido ligands are lost fairly rapidly (k = 1−10 s^{-1}).[225,226] Since most biological amines are protonated at neutral pH, potential ligands such as lysylamines on the surfaces of proteins do not readily coordinate. Thiolato complexes are also possible, but these are often kinetically unstable,[227] particularly in air (see section III.E). Consequently, the lone pairs of imidazole rings on histidine and purines present the readiest targets. Owing to the higher metabolism of tumor cells, oxygen is rapidly utilized. In rapidly growing tumors, the growth of new blood vessels (termed neovascularization or angiogenesis) often fails to keep pace so that the tissue becomes hypoxic or even anoxic.[228−232] Glycolysis then becomes the primary energy source, and the production of lactic acid tends to lower tumor pH,[233] which may facilitate the release of Ru on histidine or tyrosinate sites on transferrin (see section III.H) and also favors the reduction of complexes with a pH-dependent reduction potential.[96,103,104,234]

Figure 5a illustrates the effect of hypoxia in increasing the toxicity of the anticancer agents *trans*-$[Cl_4(Im)_2Ru]^-$ and *cis*-$[Cl_2(NH_3)_4Ru]^+$ against HeLa cells in tissue culture as reflected by their lower IC_{50}'s with decreasing P_{O_2}. Figure 5b shows that DNA binding at the same extracellular ruthenium concen-

tration (10 μM) also increases with increasing hypoxia.[71]

I. Transferrin Transport

The elevated requirements of tumor cells for nutrients coupled with their higher membrane permeability and angiogenesis with associated increased blood flow result in both specific and nonspecific uptake of metallopharmaceuticals. Specific intake for several metal ions appears to be mediated by transferrin.[29,235,236] Tracer studies with $^{103}RuCl_3$ demonstrate substantial transferrin (Tf) binding,[100−102] and injection of $^{103}Ru^{III}$−Tf results in high tumor uptake of the metal.[101,102,237] Consistent with Tf-transport into cells, inhibition of HeLa cell growth in tissue culture increased with added transferrin for both *cis*-$[Cl_2(NH_3)_4Ru]Cl$ (CCR) and (ImH)*trans*-$[(Im)_2Cl_4Ru]$ (ICR).[71] Nonspecific uptake is facilitated by the increased permeability of tumor cells, and some cationic complexes may also enter through endocytosis following binding to anionic sites on the cell surface, while neutral ones may diffuse through the cell membrane. Since small ions are excreted fairly readily by the kidneys, it is likely that the nonspecific uptake of ruthenium ions into tumors occurs within a few hours while the transferrin-mediated uptake might extend over a somewhat longer time scale.[101] The diaqua intermediates of NAMI and its indazole analogue bind to apotransferrin with a 2:1 stoichiometry with the heterocycles remaining attached. The metal ion can be reversibly removed with citrate.[238]

While most of the antitumor agent, *trans*-$[Cl_4(Ind)_2Ru]^-$, in the blood is bound by albumin,[178] transferrin uptake appears to be the more important mode of transport to the tumor. Binding of *trans*-$[Cl_4(Im)_2Ru]^+$ to apotransferrin takes several hours, while *trans*-$[Cl_4(Ind)_2Ru]^-$ takes only a few minutes.[105] Hydrolytic intermediates are formed in the buffer, and the carbonato complex may be taken into the protein.[178] Since these complexes do not bind to Al^{III}_2−Tf, binding is suggested to occur around both iron binding sites. Crystallographic data shows that *trans*-$[Cl_4(Im)_2Ru]^-$ binds to a histidine at both iron binding sites.[103,104,239] Nevertheless, as transferrin has 17 histidine residues, it binds multiple molecules of $[(H_2O)(NH_3)_5Ru]^{2+}$, which are converted to Ru^{III}−histidine adducts following oxidation,[240] and also appears to bind *trans*-$[Cl_4(Im)_2Ru]^-$ and *cis*-$[Cl_2(pdta)Ru^{III}]$ at surface sites.[104,132,239] The *trans*-$[Im-Ru^{III}-Im]$ core ions can be removed from Tf by acidification in the presence of citrate, so that the heterocyclic ligands remain coordinated even within the protein. Transferrin uptake may lower ruthenium toxicity by preventing it from other binding or uptake until it has been delivered to the cells. Consequently, Ru−Tf complexes may provide a new family of less toxic and more effective antitumor agents. Indeed, Tf labeled with *trans*-$[Cl_4L_2Ru]^-$ (L = heterocyclic base) exhibited equal or better antiproliferative activity against human colon cancer cells than the parent complexes.[241] Unlike $Tf-Fe^{III/II}$,[242] the $Tf-Ru^{III/II}$ reduction potential should be biologically accessible. Such reduction should facilitate release of Ru from histidine sites on transferrin, particularly in the lower pH of tumor tissue or the transferrin endosome (pH 5.6).[243]

Overall, it is likely that many ruthenium anticancer agents are transported to the tumor via transferrin and are then activated by reduction within the cell to bind to DNA.[71] By analogy to Ga^{3+}, it is also conceivable that some of the anticancer activity of Ru involves depleting Fe from cells and proteins.

J. Di- and Trinuclear Ruthenium Complexes

Mixed-valent, μ-carboxylato complexes of the type μ-$[(RCO_2)_4ClRu_2]$ (R = CH_3, CH_3CH_2)[93] are active against P388 lymphocytic leukemia,[93] possibly by binding to DNA along the lines of the structurally similar rhodium complexes (see section IV.A). Complexes with μ-*N*,*N*′-diphenylformamidinate and μ-(fluoroanilino)pyridinates have also been prepared.[244−246] The compound μ-$[(F_3CCO_2)_4(F_3CCO_2)Ru_2]$ forms *cis*-$[\mu$-$(F_3CCO_2)_4$-μ-(9EtGua)$Ru^{II}_2(CH_3OH)_2]^{2+}$ where 9EtGua = 9-ethylguanine in which the guanines bridge between the two Ru^{II} atoms in a N7−O6 head-to-tail fashion.[247]

Since preparations of the mixed-valent complex ruthenium red, $[(NH_3)_5Ru^{III}ORu^{IV}(NH_3)_4ORu^{III}(NH_3)_5]^{6+}$,[248] have been used as a cytological stain for over a century, its biological properties have been well reported,[249,250] including a remarkable immunosuppressant activity.[251] These preparations bind to polyanions such as plant pectins and the protective mucopolysaccharide coat on some tumor cells.[252] Consequently, Ru red concentrates in tumors[253] and inhibits tumor growth. Ruthenium red preparations block Ca^{2+} transport in a number of biological systems, and this may also have an effect on tumors.[254,255] However, it is a dimeric impurity in Ru red preparations, μ-O-$[X(NH_3)_4Ru]_2^{3+}$ (Ru-360, X = Cl^- or OH^-),[256] that is responsible for most of the inhibition of Ca^{2+} uptake in mitochondria.[257,258] Most notably, Ru-360 specifically blocks uptake of Ca^{2+} into the mitochondria of cardiac myocytes with an IC_{50} of 0.2 nm, but it does not exhibit antitumor activity in cell culture screens.[258]

The complex μ-O-$[(H_2O)_2(bpy)_2Ru^{III}]_2^{4+}$ coordinates to DNA at relatively low levels ($[Ru]_{DNA}/[P]_{DNA}$ = 0.02) with low stereoselectivity, which may favor the LL isomer. Coordination at this level also stabilizes the thermal melting of DNA by about 8 °C. Irreversible thermal denaturation of DNA with this complex covalently bound has been taken as an indication of interstrand cross-links.[109]

IV. Dimeric Complexes of Rhodium and Other Metal Ions: DNA and Protein Interactions. Monomeric Complexes of Rhodium

A. Dimeric μ-Acetato Dimers of Rh^{II} and Other Transition-Metal Ions

The rhodium acetate dimer, $[\mu$-$(CH_3CO_2)_4Rh_2(H_2O)_2]$, and related complexes have shown good antitumor activity[259] but toxic effects have prevented their use. Recent structural studies suggest their activity may

bear analogy to that of cisplatin by binding to adjacent guanines on DNA;[247,260,261] however, activity deriving from protein binding at sulfhydryl sites remains a possibility.[262,263] The rhodium dimer is much more inhibitory toward *E. coli* DNA polymerase I than RNA polymerase[264] and exhibits good antitumor activity against the Ehrlich ascites tumor, sarcoma 180, and P388 lymphocytic leukemia but little activity against L1210 and B16 melanoma.[265]

Activity increases in the series $[(RCO_2)_4Rh_2]$ with the lipophilicity of R and is independent of the reduction potential.[266,267] The butyrate complex inhibits DNA synthesis during S-phase development and is most toxic to cycling cells.[264] More recently, complexes of the type $[(\mu\text{-L})_2(\mu CH_3CO_2)Rh_2]^{2+}$, where L = bpy or phen, have also shown activity.[268] The analogous rhenium propanoate, $[\mu\text{-}(RCO_2H_5)_2(H_2O)_2\text{-}Br_4Re_2]$ (R = C_2H_5), shows significant activity against B16 melanoma subcutaneously transplanted in mice.[269] The rhodium complexes bind to a variety of proteins, including serum albumin, and irreversibly inhibit proteins with cysteines in the active site.[263] Up to 7 molecules of $[(CH_3CO_2)_4Rh_2]$ bind to histidyl imidazoles on human serum albumin affecting both its conformation and binding sites for other molecules.[270,271] In mammals, the butyrate complex requires a plasma concentration of 0.8 μM to be effective; however, the half-life for the drug is 1 h.[265] Maintaining the concentration at 0.8 μM for 6 h results in host toxicity.[265,272] Injected glutathione decreased the toxicity of μ-$[(RCO_2)_4Rh_2]$, where R = C_2H_5, but enhanced its antitumor activity against P388 ascites tumors.[273]

Decomposition in mammals involves displacement of the μ-acetato ligands, which enter the cell's acetate pool with a half-life of 1 h and are eventually oxidized to CO_2.[266] Dunbar has suggested that since $[(\mu\text{-}RCO_2)_2Rh_2(CH_3CN)_6]^{2+}$ and even $[(H_2O)_8Rh_2]^{4+}$ are kinetically stable,[274] it may be that the Rh−Rh core remains intact in vivo, if one or more μ-acetates are lost.[247,275] Unlike other amino acids, cysteine irreversibly coordinates to the $[(CH_3CO_2)_4Rh_2]$ through the thiolate sulfurs (and possibly acetates) in a way that sequentially displaces the μ-acetates leading to the ultimate complex, which is formulated as $[(Cys)_4\text{-}(H_2O)_2Rh_2]$.[263] Methionine forms a complex with $[(CH_3CO_2)_4Rh_2]$ in which the sulfurs of two methionines coordinate to the axial positions.[260] Glutathione appears to form a bis-chelating complex formulated as $[(CH_3CO_2)_2(GSH)_2(H_2O)_4Rh_2]$. Contrary to earlier studies,[263,276] these results suggests that the dirhodium core remains intact upon thiol binding.[260]

In water, the μ-acetate complex reacts much more readily with adenosine than with guanosine, cytidine, or uridine[266,277] while adenine tends to form an unreactive, insoluble polymer. Equilibrium binding constants for adenosine with several μ-carboxylato complexes range from 1100 to 4500 M^{-1}.[278] As shown in Figure 6a, apical coordination of purines has been observed in crystal structures of μ-$(CH_3CO_2)_4Rh_2(L)_2$, when L = Ado,[279] 1MeAdo$^{\kappa 7}$, azathioprine$^{\kappa 3}$,[280] caffeine$^{\kappa 9}$, or theophyline$^{\kappa 9}$, with the latter two showing possible steric repulsions between O6 and the μ-acetato ligands.[281] Axial cross-linking of nonadjacent

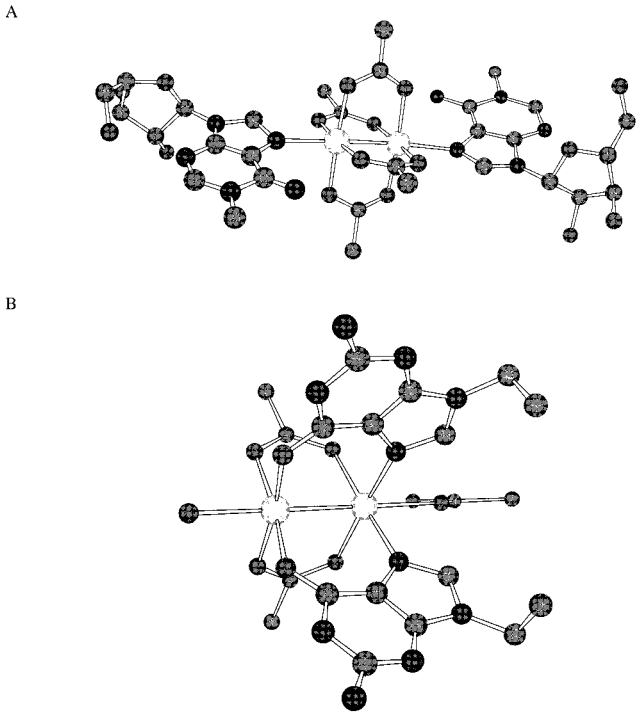

Figure 6. Structure of (a) $[(\mu\text{-}O_2CCH_3)_4Rh_2(Ado^{\kappa 7})_2]$ showing apical N7 coordination of the adenosines[279] and (b) $[(\mu\text{-}O_2CCH_3)_2(\mu\text{-}9EtGua)_2Rh_2(Me_2CO)(H_2O)]^{2+}$ illustrating the head to head arrangement of the guanines.[282] Hydrogen atoms are removed for clarity.

adenine$^{\kappa 7}$ sites was initially suggested as the mode of inhibiting DNA synthesis.

In methanol or water at 30−50 °C, 9-ethylguanine (9EtGua) reacts slowly with μ-$(CH_3CO_2)_4Rh_2$ to yield two isomeric products, in which the N1-ionized guanines bridge between the two metal atoms through their N7 and O6 sites (μ-G^{6,7}) in both head-to-head (Figure 6b) and head-to-tail fashions.[247] Neutral (N1 protonated) guanines can also bridge in this way, and reaction of μ-$(CH_3CO_2)_2[(CH_3CN)_6Rh_2]^{2+}$ or the analogous trifluoroacetato complex with 9EtGua in acetone yields the head-to-head isomer of $[\mu\text{-}(RCO_2)_2\text{-}\mu\text{-}(9EtGua)_2(Me_2CO)(H_2O)Rh_2]^{2+}$.[282] The analogous head-to-tail isomer $[\mu\text{-}(RCO_2)_2\text{-}\mu\text{-}(9EtGua)_2(CH_3CN)_2Mo_2]^{2+}$ has also been reported.[282]

Substitution of purines onto μ-$(CH_3CO_2)_4Rh_2$ may proceed by analogy to the attack of 2,2′-bipyridine, which appears to take place through initial monodentate coordination at an apical position followed by displacement of a bridging acetato to give (bpy)-$Rh(\mu\text{-}CH_3CO_2)_3Rh(O_2CCH_3)$.[283,284] The chelating ligand is then in a position to rearrange to entirely equatorial coordination by displacing a second μ-acetato, which occurs in a syn geometry, but might alternatively occur in a bridging fashion.[284]

Since the exocyclic site of adenine becomes a good ligand upon deprotonation,[168] $[(9EtAde^-)Rh\text{-}(\mu CH_3CO_2)_2Rh(bpy)(O_2CCH_3)]^+$ has also been prepared in which 9EtAde$^-$ is an equatorially coordinated, N6-ionized, 9-ethyladenine that also interacts through N1 with the apical position of a second dimer. Head-to-tail μ-A^{6,7} bridges occur in the dimolybdenum model complex *cis*-$[(\mu\text{-}CHF_2CO_2)_2(\mu\text{-}9EtAde)_2(MeCN)_2Mo_2]^{2+}$ in which the N1(H)−

N6(imino) or zwitterionic tautomer occurs.[285] The use of *N,N′-p*-tolylformamidinate (DTolF) provides extra stability to the dimer, and reaction of μ-[(DTolF)$_2$-(CH$_3$CN)$_2$Rh$_2$]$^{2+}$ with 9EtAde yielded the head-to-tail *cis*-[μ-(CHF$_2$CO$_2$)$_2$(μ-9EtAde)$_2$(MeCN)Rh$_2$]$^{2+}$.[286] The dirhenium compound, *cis*-[μ-(CH$_3$CH$_2$CO$_2$)$_2$(μ-9EtAde)$_2$Re$_2$]Cl$_2$, binds to adenine at the N1 and N6 positions, where thymine normally engages in Watson−Crick base-pairing, which could play a role in the carcinostatic activity of tetrapropionatodirhenium complexes that inhibit DNA replication.[287]

Bear has developed systematic synthesis for a number of dirhodium(II) μ-tetraamidate and μ-tetraamidinate complexes, which undergo two metal-centered one-electron oxidations; however, biological activities have not been reported.[259,288]

Early results indicated that μ-(RCO$_2$)$_4$Rh$_2$ reacts with single-stranded poly(dA) and DNA but not with poly(dG), poly(C), or double helical DNA.[289] However, Dunbar's recent synthetic studies suggest that modes of binding other than apically to A^7 (Figure 6a) may be important, particularly μ-A^{6,7} and μ-G^{6,7} as shown in Figure 6b.[247] The Rh−Rh bond distance (2.5−2.7 Å) is shorter than that between DNA base pairs, and ^{1}H NMR studies and molecular modeling of dirhodium bound to dGpG and the single-stranded oligonucleotide d(5′-CCTCTGGTCTCC-3′) suggest an intrastrand *cis*-(μ-G^{6,7}−G^{6,7}) head-to-head cross link[289] that would bend the DNA similar to cisplatin and possibly lead to HMG protein binding.[1,2,260]

B. Monomeric Rhodium Complexes

The square-planar, rhodium(I) compound, [(COD)(PMI)Rh]Cl, where COD = cyclooctadiene and PMI = 2-pyridinalmethylimine, exhibits activity against MCa mammary carcinoma metastases in the lung and Lewis lung carcinoma.[290] Organometallic compounds of the type [(CO)$_2$(dtc)Rh], where dtc = dithiocarbamate, have shown activity against several tumor cell lines.[292]

A number of rhodium(III) analogues of ruthenium(III) antitumor complexes also show antineoplastic activity; however, Rh^{III} is unlikely to be activated by reduction, which may account for its generally lower activity. Like *fac*-[Cl$_3$(NH$_3$)$_3$Ru], *mer*-[Cl$_3$(NH$_3$)$_3$Rh] is also active but insoluble.[293] While *mer,cis*-[Cl$_3$(Me$_2$SO)$_2$LRh] (L = NH$_3$ and imidazole) exhibit significant activity against some tumor cell lines, *mer,cis*-[Cl$_3$(Me$_2$SO)(Im)$_2$Rh], Na[*trans*-[Cl$_4$(Me$_2$SO)(Im)Rh], and (HIm)*trans*-[Cl$_4$(Im)$_2$Rh] were essentially inactive.[294] Na-*trans*-[Cl$_4$(Me$_2$SO)(Im)Rh] and *mer,cis*-[Cl$_3$(Me$_2$SO)$_2$(Me$_2$S*O*)Rh] modestly inhibited the growth of the primary MCa mammary tumor implanted in mice, and the latter compound may also inhibit metastases of this tumor in the lung.[294]

Some rhodium metallointercalators exhibit such remarkably specific DNA binding as to suggest new types of DNA-targeting agents. The sterically bulky DNA intercalator Δ-[(chrysi)(bpy)$_2$Rh]$^{3+}$ [295] binds specifically to destabilized regions near base pair mismatches and recognizes a single mismatch in a 2725 base pair plasmid DNA. Such sterically demanding intercalators may have application in mismatch-specific chemotherapeutic agents or in detecting mutations.[296] The metallointercalator Λ-1-[phi(mgp)$_2$Rh]$^{5+}$ binds tightly and specifically to 5′-CATATG-3′ in the major groove of double helical DNA by a combination of direct readout and geometric shape selection. When this binding site was engineered into the AP-1 recognition element of the major-groove binding bZIP transcription factor yAP-1 (the yeast analogue of mammalian AP-1), 50% competitive binding with yAP-1 occurred at a rhodium concentration of 120 nM relative to 3 μM when the binding site was not present.[297] Consequently, targeting specific transcription sites presents yet another avenue of development for transition-metal anticancer drugs.

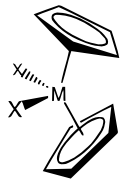

Figure 7. Structure of metallocene dihalides.

V. Metallocenes and Titanium(IV)

A. Metallocenes

The antitumor activity of metallocene dihalide complexes X$_2$Cp$_2$M (Figure 7, Cp = η^5-cyclopentadienide and X = halide) is dependent upon the metal ion with M = Ti, V, Nb, and Mo showing marked activity, Ta and W exhibiting marginal effectiveness, and Zr and Hf being inactive.[298,299] The diagonal relationship between the active metal ions (Ti−Nb and V−Mo) suggests that active complexes fall within a window of size and substitution reactivity of the metal ion. Cationic complexes of the type [X$_2$Cp$_2$M]$^+$, where M = Nb, Mo, and Re, are also active,[300,301] as are ferrocenium ions,[302] but mechanistic studies are lacking. Results of phase I clinical trials of the most successful of these agents, Cl$_2$Cp$_2$Ti, indicate a maximum tolerable dose of 315 or 140 mg/m^2 per week. The toxic dose of Cl$_2$Cp$_2$V in mice (0.28 mmol/kg, intraperitoneal) is not much higher than the therapeutic dose (0.20−0.24 mmol/kg),[303] and the metal is rapidly cleared from the blood through both the kidney and liver.[304] Dose-limiting toxic effects of Cl$_2$Cp$_2$Ti include nephrotoxicity and elevation of creatinine and bilirubin levels, which are cumulative but reversible.[305,306] While these compounds were initially tested as possible analogues of cisplatin, the effectiveness of Cl$_2$Cp$_2$Ti against platinum-resistant cell lines indicates a different mechanism of action[307] that may lead to new therapeutic options against ovarian cancer.[308,309] In a phase II clinical trial, there was no efficacy in patients with metastatic renal-cell carcinoma who received 270 mg/m^2 of Cl$_2$Cp$_2$Ti every 3 weeks for 6 weeks, but the toxicities and side effects were mild to moderate.[310]

Like NAMI, Cl$_2$Cp$_2$Ti inhibits collagenase type IV activity (IC$_{50}$ of ~0.2 mM in Walker 256 carcinosarcoma). Titanocene also prevented the growth of new blood vessels into tumors at concentrations that were

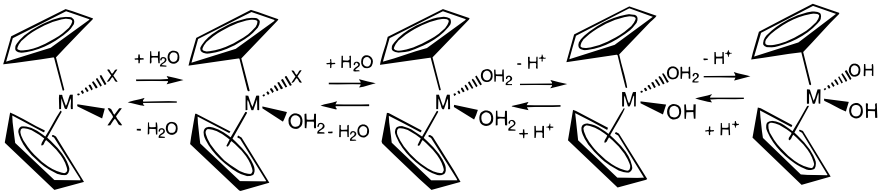

Figure 8. Hydrolysis scheme for metallocene dihalides in aqueous media.

without effect on growth or cytotoxicity of endothelial cells or Walker 256 cells in culture.[311,312] Cl_2Cp_2Ti takes 2−3 days to show selective tumor uptake, but the metal ion accumulates in cellular regions rich in nucleic acids with highest concentrations in the chromatin. The citrate complex of Ti^{IV} binds strongly to the Fe^{3+} binding sites in Tf as does Cl_2Cp_2Ti, which may provide a transport mechanism into tumors.[313] Tumor cells treated with Cl_2Cp_2Ti revealed a premitotic G_2 block combined with marked mitotic depressions and additional accumulations at the G_1/S boundary.[314] Recent work suggests that metallocene dichlorides inhibit protein kinase C (PKC) activity, which is involved in regulating cellular proliferation, and DNA topoisomerase II, which could be involved in the premitotic G_2 block.[315]

The antitumor activity of the of Cl_2Cp_2M probably depends on the hydrolysis of the metal (Figure 8), which for (M = Ti, V, Zr, and Mo) proceeds much faster than cisplatin.[315] Rapid dissociation of chloride from Cl_2Cp_2Ti results in up to five hydrolytic products in buffer solutions.[316] Loss of the first chloride is too rapid to be easily measured, and that for the second proceeds with half-lives of 45 min or less. Variations in the leaving group, X, have substantially less effect than variations between metal ions, even when X is neutral.[314] The most acidic of the aquated metallocenes discussed here is $[(H_2O)_2Cp_2Ti]^{2+}$ with $pK_{a1} = 3.51$, $pK_{a2} = 4.35$, so that it exists as the neutral $[(OH)_2Cp_2Ti]$ at neutral pH, while the least acidic $[(OH_2)_2Cp_2Mo]^{2+}$ ($pK_{a1} = 5.5$, $pK_{a2} = 8.5$) exists as $[(OH)(OH_2)Cp_2Mo]^+$ at pH 7.[317−319] This difference in aquated forms between metals may modulate their activity in that those forming neutral species under physiological conditions probably enter cells more easily. The hydrolyzed species of Cl_2Cp_2Ti appear to have a high affinity for plasma proteins.[316] The Cp ligand is also lost at different rates with that for Cl_2Cp_2Zr being the fastest ($t_{1/2} = 14.1$ h) and that for Cl_2Cp_2Ti being the slowest ($t_{1/2} = 57$ h).[317] Dissociation of the Cp occurs more rapidly in DMSO to give inactive products.[320]

Initial studies suggested a correlation between DNA binding and antitumor activity in that immediate complexation of the DNA nucleotides occurs with the antitumor active metallocenes (M = Ti and Mo), whereas the biologically inactive metallocenes (M = Hf and Zr) do not bind significantly to DNA.[314,319,321,322] Other early studies suggested that Ti and V complexes inhibit DNA synthesis more than protein synthesis.[299] However, later studies indicate that metallocene dihalides do not bind strongly to DNA at neutral pH and do not suppress DNA-processing enzymes, so that it is unlikely that their activity involves nucleic acids.[315,324] Nevertheless, their binding to DNA and its constituent bases is briefly surveyed below as an example of the DNA interactions of early transition metallocenes.

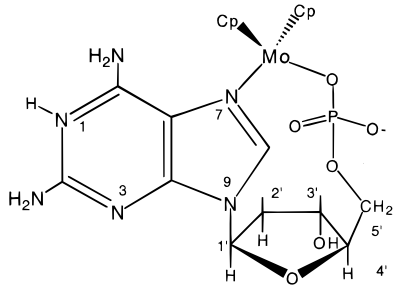

Figure 9. Complex formed between Cp_2Mo^{IV} and 5′-AMP in aqueous solution, which is representative of that between molybdocene and other nucleotides in that it shows both heterocyclic nitrogen and phosphate binding.[319,323]

Following the hydrolysis of Cl_2Cp_2V, $[(H_2O)Cp_2V]^{2+}$ binds fairly rapidly and selectively to nucleotide phosphates and hydrogen bonds strongly to anionic phosphodiesters in the solid state; consequently, it has relatively little effect on base pairing.[318] The "softer" Cl_2Cp_2Mo forms 1:1 complexes with 5′-NMP's (N = G, A, C, and T) with little selectivity and substantial exchange between the ligands. Since it bonds to both the phosphates and heterocyclic bases of nucleotides after the general manner shown in Figure 9, it generates major conformational changes with nucleotides but appears to leave Watson−Crick hydrogen bonding intact.[319,323] ^{31}P NMR studies also indicate a prevalence of binding to phosphate oxygen in sonicated calf thymus DNA,[322] but this is likely to the single-stranded ends created by sonication. Cl_2Cp_2Mo does not bind to either single- or double-stranded oligonucleotides at pH 7 and binds to the G and possibly C sites of only single-stranded oligonucleotides below pH 4 with no alterations in the ^{31}P NMR.[324] Aquated Cl_2Cp_2Mo is capable of enhancing the hydrolysis of phosphate diesters by a factor of 10^4 at pH 4 through facilitating the attack of a coordinated water or hydroxide following phosphate binding.[325]

Structural studies indicate that the Cp groups sterically prevent cross-linking DNA. In $[(1,3Me_2\text{-}Xan^-)Cp_2Ti^{III}]$, the Ti is chelated between the purine N7 and O6, suggesting a similar mode for Ti^{IV}−guanine binding. The structure of $[(5'\text{-dGMP})Cp_2Mo]_2$ reveals a macrocycle with each Mo coordinated to the N7 of one 5′-dGMP and a phosphate oxygen of the second 5′-dGMP. In solution, this species is in equilibrium with the monomeric N7/PO_4 chelate.[323] In $[(9MeAde^-)Cp_2Mo]PF_6$, the metal is chelated between N1 and an ionized N6 of 9-methyladenine, which in solution is in equilibrium with the N6/N7 chelate. Similarly, the structure of $[(1MeCyt^-)Cp_2Mo]PF_6$ exhibits chelation between N3 and the

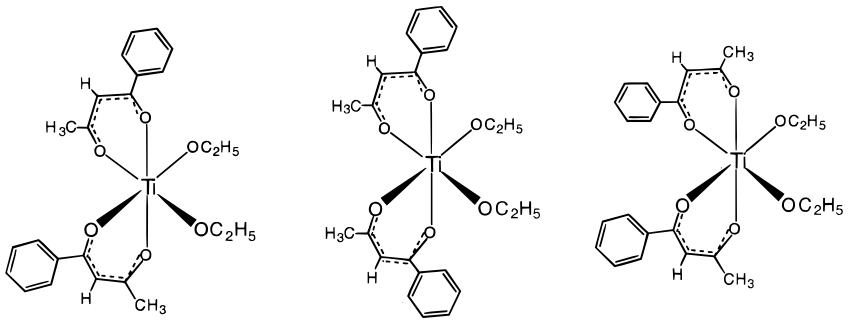

Figure 10. Structures of thermally interconvertible Budotitane geometric isomers with their relative abundances.[400]

exocyclic N4.[323] Both crystal structures are consistent with partial M−N π-bonding, with a concomitant weakening of the exocyclic-N π-bond to the pyrimidine ring. In aqueous solution, the Mo is chelated by the N7 and phosphate oxygen of 5′-dAMP (Figure 9) and the N3/PO_4 of 5′-dCMP, with the latter ligand also exhibiting monodentate phosphate binding. 5′dTMP also forms a N3/PO_4 chelate.[323]

B. Budotitane

Budotitane, *cis*-[$(CH_3CH_2O)_2(bzac)_2Ti^{IV}$], where bzac = 1-phenylbutane-1,3-diketonate, was the first non-platinum transition-metal anticancer agent to be tested in clinical trials (Figure 10).[326,327] The dimeric complexes, μ-O-*cis*-[$X_2(bzac)_2Ti^{IV}]_2$, are also active.[328] In the series *cis*-[$X_2(bzac)_2M^{IV}$], activity varied in the following order Ti ≈ Zr > Hf > Mo > Sn > Ge,[326] which is roughly inverse to the rates of X dissociating from the metal ion. As with Cl_2Cp_2Ti, the leaving group (X) is lost fairly rapidly; consequently, it exerts little influence on activity. Since the ethoxy group has the slowest rate of hydrolysis, it was chosen for formulation into an administrable drug. The slower hydrolysis of the diketonates, which results in the formation of insoluble particulates that eventually yield TiO_2, causes particular difficulties for the formulation and shelf life of budotitane and related complexes.[7] Aqueous dissolution of the drug formulated with glycerinepolyethylene−glycolericinoleate and 1,2-propylene glycol yields micelles in which the compound is protected from hydrolysis.[326] While such formulated solutions are stable for hours, the components cannot be sufficiently well characterized to advance to further clinical trials. Little is known about the mechanism of budotitane, except that it is distinct from that of cisplatin.[4]

Figure 10 shows the three cis geometric isomers of budotitane, which are in thermal equilibrium ($\Delta H^{\ddagger}$ = 10−12 kcal/mol), so that no isomerically pure complexes have been isolated.[329] The cis isomers appear to be favored on steric grounds. Hydrolysis of the ethoxy groups in water proceeds with a half-life of about 20 s, followed by condensation of the hydrolyzed species, probably by the formation of μ-oxo or μ-hydroxy bonds, to yield oligomeric compounds with the formulation $[Ti(bzac)_2O]_n$. The diketonate moieties also hydrolyze but with a half-life on the order of hours.

Budotitane is quite effective against a number of ascites tumors and induced colorectal tumors in animals.[330] Clinical trials indicate it to be fairly well tolerated by patients with the dose-limiting side effect being cardiac arrhythmia.[327] The maximum tolerable dose is 230 mg/m^2 on a biweekly schedule, with 180 mg/m^2 being suggested for any future studies. Higher doses lead to liver toxicity, kidney toxicity, and a reversible loss of taste.[330] In vitro and in vivo experiments with budotitane revealed no significant DNA damage;[331] however, the diaqua complex binds strongly to DNA.[95] While no data are available, perhaps the biological mechanism of budotitane may bear analogies to those of Cp_2TiCl_2 and NAMI.

VI. Vanadium: Peroxidase Activity and Inhibition of Nucleo-Enzymes

Vanadium is an essential trace element, which is generally present in all mammalian tissues at about 10 μM or less, but very little intracellular vanadium is free of macromolecular binding. Of that occurring in small molecules, most is probably bound in analogy to phosphate and lesser amounts to glutathione and ascorbate.[332] A number of biological reductants[333] can convert the vanadate core, $[O_2V]^+$, in complexes such as *cis*-$[(OH)_2O_2V^V]^-$, to the vanadyl core, $[OV^{IV}]^{2+}$.[334] The vanadyl ion binds proteins and other cellular components at both oxygen and nitrogen sites,[335] and transferrin appears to be involved in its transport and metabolism.[336−338] Vanadium in the drinking water of Sprague−Dawley rats elevates their glutathione levels with a concomitant elevation in glutathione S-transferase activity in the liver.[304]

Vanadate(V) and/or vanadyl ions inhibit a number of phosphatases, ATPases, nucleases, kinases, etc.[336] Vanadate esters of sugars of the form $[(RO)OV]^{2+}$ mimic phosphate esters and are potent kinase inhibitors,[339] and the monovanadate esters inhibits a number of nucleases.[340,341] In marine vanadium haloperoxidases, vanadate(V) is bound to a histidyl imidazole nitrogen at the active site.[342] Vanadate and vanadyl ions mimic the effect of insulin,[343,344] probably by inhibiting a protein phosphotyrosine phosphatase (PPTK) that is selective for a cytosolic protein tyrosine kinase.[345] This appears to result in inhibition of the insulin receptor kinase, which is responsible for inactivation of the insulin receptor. Vanadium inactivation of PPTK is presumed to occur

through vanadium peroxidase action on a critical cysteine at the PPTK tyrosine phosphate binding site.[339] Vanadyl ion in the presence of H_2O_2 also causes lipid peroxidation in isolated rat hepatocytes.[334]

Both vanadyl sulfate and sodium orthovanadate (5−10 μM) inhibit the growth of proliferating tumor cells in culture but have little effect on nonproliferating cells, and cytotoxicity is enhanced when H_2O_2 is added.[346] In vivo subcutaneous injections of orthovanadate into mice containing MDAY-D2 tumors resulted in the inhibition of tumor growth by 85−100%.[346,347] The insulin-mimetic compound [O(maltolato)$_2$V][343] has also proven effective against MDAY-D2 tumors implanted in mice when administered in a slow-release paste.[347]

Hydrogen peroxide displaces oxo groups from vanadate complexes to form side-bound peroxo vanadates.[339] Activity against the L1210 murine leukemia system has been observed for the peroxo compounds $(NH_4)_4[O(O(O_2)_2V)_2]$, $M_3[O(O_2)_2(C_2O_4)V]$ ($M = K^+$, NH_4^+), and $NH_4[O(O_2)(malato)V]$. In the presence of hydrogen peroxide, vanadyl ion and $[O(H_2O)_3(phen)V]^{2+}$, which exhibits anticancer activity in tissue culture, cleave DNA possibly by generating hydroxyl radicals.[334,348,349] Vanadyl bleomycin in the presence of H_2O_2 preferentially cleaves G−A(5′-3′) sites in DNA in a mechanism different from that of Fe^{III}−bleomycin.[350] Most interestingly, vanadyl sulfate induces the autoxidation of deoxyguanosine to 8-oxo-deoxyguanosine in very low yields in air by a mechanism that appears to involve peroxide and is capable of cleaving DNA.[351] Since vanadate and vanadyl can bind to imidazole nitrogens, it is possible that coordination of these ions to purine N7 sites on nucleic acids could poise them toward oxidatively damaging nucleic acids through either oxo-atom transfer or generation of hydroxyl radicals. A vanadium(III) complex of L-cysteine has shown significant antitumor activity in mice.[352,353] Dietary vanadyl sulfate also inhibits mammary carcinomas in rats.[354] Overall, the peroxidase activity of vanadium appears to be important in its anticancer activity, possibly through oxidative DNA damage; however, activity through inhibiting enzymes involved in DNA metabolism may also be possible.

VII. Tin

A. Toxicity and Anticancer Activity

A series of organotin(IV) compounds of the types X_2R_2Sn and $X_2R_2L_2Sn$, where X = halide or pseudohalide, R = organic group, and L = a nitrogen ligand (py) or L_2 = a bidentate nitrogen ligand (en, bpy, etc.), were initially tested as possible analogues of cisplatin, but little activity was found.[355] The toxicity of organotin complexes has been reported to follow the order $R_3Sn > R_2Sn > RSn$ and to increase with the chain length of R, with alkyl complexes often being more toxic than aryl ones.[355−357] However, more recent results on the activity of a number of organotin compounds against tumor cell lines seems to point to a necessary balance between solubility and lipophilicity in order to optimize their efficacy.[358−369] Immune suppression (see section VII.B) and neurotoxicity have been reported as the most significant side effects.[367] Triethyltin compounds attack the myelin of the central nervous system, while trimethyltin compounds may cause neuronal hyperexcitation.[357] The biological activity of organotin compounds may depend on their affinity for protein thiols.[370]

A number of organotin dipeptide complexes of the type $R_2Sn(AA)_2$, where R = methyl, ethyl, *n*-butyl, cyclohexyl, and phenyl and AA = dipeptide or mercapto amino acid, have shown modest activity against P388 lymphocytic leukemia cells, and several have been structurally characterized.[371] The dipeptide complexes are pentacoordinate with GlyGly coordinated in a nearly planar fashion through the both the amine and carboxylate termini as well as the amidate nitrogen. The dipeptide complexes hydrolyze slowly and precipitate diorganotin oxides.[371] When a number of 2,6-pyridinedicarboxylato complexes of the type $C_5H_3N(COO)_2RR'Sn$ were tested against MCF-7 and WiDr tumor cell lines, the compound with R = R′ = *n*-butyl was the most active.[358,372−374] In an attempt to improve solubility with salicylate ligands, in the series $(sal)_2R_2Sn-O-SnR_2(sal)_2$, where sal = salicylate or salicylate derivative, the most active was with 5-methoxysalicylate.[373,375−377] In complexes of the type $(n\text{-}Bu)_2(benz)_2Sn$, where benz = benzoate or benzoate derivative, the complex with 4-hydroxy-3-methoxybenzoate was the most active.[373,378−381] When benz = fluorobenzoate, the difluorobenzoates were more active than the monofluorobenzoates with 2,3-difluorobenzoate being marginally the most active.[382−384] Of the series $R_3(benz)Sn$, the complexes are more active when R = phenyl than when R = n-Bu. When $(Ph)_3SnL$, where Ph = phenyl and L = 5-sulfosalicylate, 5-aminosalicylate, 4-fluorobenzoate, $(n\text{-}Bu)_3Sn$(2,6-difluorobenzoate), and $(n\text{-}Bu)_2SnL_2$, L = 2,4-dihydroxybenzoate, 2,5-dihydroxybenzoate, and pentafluorophenylacetate, were tested against the colon 26 tumor implanted in mice, all showed good results, but their toxicity was generally considerably higher than cisplatin.[373]

Somewhat unusual tin complexes, such as the seven-coordinate $(n\text{-}Bu)_2(Cy_2HN)_2$(2,6-pyridinedicarboxylato)$_2$Sn (Cy = cyclohexyl)[385] and dimeric μ-oxo complexes with carborane moieties (1,2- and 1,7-dicarba-*closo*-dodecaborane) tethered via carboxylates to a μ-oxo-distannic core, have also shown fairly good activity against a number of cell lines.[367,386] In the solid state, a dimer of dimers structure is seen for the carborane adduct $\{[Bu_2(RCO_2)_2Sn_2]_2O\}_2$, where RCO_2 = 2-phenyl-1,2-dicarba-*closo*-dodecaborane-1-carboxylato; however, in solution, the complex exists as the dimer, $[n\text{-}Bu_2(RCO_2)_2Sn_2]_2O$, in which the carboxylates are monodentate.[386,387]

When $[\eta^5\text{-}(C_6H_5)_5Ph_5]_2Sn^{II}$, where Ph = phenyl or benzyl, were tested against Ehrlich ascites tumor in female CF1 mice, cure rates of 40−90% were obtained over fairly broad dose ranges. The toxicity of these compounds was relatively low, with the LD_{10} values between 460 and 500 mg/kg. The free cyclopentadiene ligands were also active but at a level less than that of the stannocene complexes.[388] Triethyltin(IV)lupinylsulfide hydrochloride exhibited IC_{50} values of

approximately 0.7 μM against three human ovarian cancer cell lines (SW 626, IGROV 1, and OVCAR-3).[389]

The low aqueous solubility of tin compounds is a significant problem, which can be partially addressed through the use of hydrophilic leaving groups.[367,373] Formulation may require colloidal suspensions or DMSO to enhance solubility.[367] While hydrolysis seems to be necessary for the activity of organotin compounds, the lipophilicity of the more stable carbon-bound ligands appears to control their toxicity,[4,355] with the *n*-butyl group apparently the most effective.[373] Similarly, the reported efficacy of various tin complexes is $(n\text{-}Bu)_2SnX_2$ > $(n\text{-}Bu)_3SnX$ > Ph_3SnX.[373] This may simply point out the necessity of balancing lipophilicity with solubility or a true structural requirement, perhaps analogous to that of cisplatin, for cis leaving groups. Given their hydrophobic character, a mechanism analogous to that of titanocene dichloride might be suspected. The compounds $(n\text{-}Bu)_3Sn$(5′AMP) and $(n\text{-}Bu)_3Sn$(5′GMP) appear to be monomeric with one $(n\text{-}Bu)_3Sn$ bound to the phosphate and the other to the 3′-oxygen.[390] Studies with CT-DNA, 5′-GMP, 5′-ATP, and 5′-di$(CGCGCG)_2$ indicate phosphate binding below pH 7, but that phosphate is unable to compete with hydroxide binding above this pH.[391] Coordination to the sugar is most likely to occur at pH > 9, particularly when adjacent deprotonated hydroxyls are available to chelate the Sn^{IV}.[391−393]

B. Possibly Related Immunological Effects

Since tributyl and triphenyl tin compounds are widely used as fungicides and herbicides, some immunological effects have been observed in mammals.[357,394] Triphenyltin acetate and chloride exert a selective toxic effect on the immune system in vivo by inhibiting T-cell response,[395,396] and both $(^nBu)_2SnCl_2$ and $(^nBu)_3SnCl$ caused atrophy of the thymus by selectively reducing the number of rapidly proliferating lymphoblasts.[397] Mouse thymocytes treated with triphenyltin acetate in cell culture exhibit chromatin condensation, cell membrane fragmentation, and formation of membrane-bound apoptotic bodies suggestive of apoptosis.[395] The effect of organotin on cellular immune systems may involve their hydrophobic intracellular distribution and the inhibition of intracellular phospholipid transport between organelles by impairing the structure and functions of the Golgi apparatus and the endoplasmic reticulum. Consequently, organotin complexes have been associated with the activation of nuclear endonucleases associated with apoptotic DNA cleavage.[398,399] Organotin compounds may also be cytotoxic to adenocarcinoma cells by inducing tumor necrosis factor alpha (TNF-α). In combination with macrophages, Sn^{IV}−protoporphyrin induces mitogenicity in peripheral T cells, and this effect is enhanced by low levels of interleukin-2 (IL-2).[356] However, the Sn^{IV}−protoporphyrin is not cytotoxic and inhibits IL-2 cytotoxicity. IL-2 is synergistic with Sn^{IV}−protoporphyrin in stimulating TNF-α and IFN-γ production by human PBMC (80% lymphocytes and 20% monocytes isolated from blood). While the mutagenic activity of both Sn^{IV}−protoporphyrin and hemin required the presence of macrophages, only hemin appeared to function by oxidatively inducing DNA damage.[356]

VIII. Conclusion

While several classes of complexes, such as metallocene and organotin agents, were initially tested with a vision toward behavior analogous to cisplatin, relatively few exhibit this. NAMI is a particularly good example; even though it is from a family of complexes that may bind DNA similarly to cisplatin, its antimetastatic activity is independent of DNA coordination and probably depends on the regulation of a matrix proteinase. Metallocenes may also inhibit this class of enzymes. On the other hand, the dirhodium complexes, which were initially thought to function by binding to thiols on proteins, may act by binding adjacent DNA guanines similarly to cisplatin. Gallium ions interfere with ribonucleotide reductase. The lipophilicity of tin complexes is important in both their antitumor and anti-immune effects. Many types of complexes appear to be transported by transferrin,[236] and some ruthenium complexes give evidence of being activated by reduction inside the cell.

The dirhodium class of compounds may be too toxic for clinical use, but a better understanding of their mechanism may lead to improvements elsewhere. Cisplatin is extraordinarily effective against testicular cancer, where a specific DNA binding protein appears to grip the cisplatin−DNA lesion particularly tightly. If the dimeric complexes function analogously to cisplatin, they may provide different specificities toward DNA binding proteins, thereby offering promise against other cancers, at least in determining how to subtly tune the metal−DNA lesion to DNA binding proteins.

The formulation and solution characterization of lipophilic drugs that depend on hydrolysis, such as budotitane and organotin compounds, present particular problems in developing anticancer metallopharmaceuticals. Another issue is that tissue culture methods may not be adequate for screening complexes, such as those of ruthenium, which utilize multiple biological mechanisms in transport and macromolecular binding. New approaches, such as peroxovanadium complexes, are promising if they can be suitably targeted to DNA or essential enzymes in tumor cells rather than random oxidation events. With the exception of cisplatin, there is relatively little mechanistic information on how metal anticancer drugs function, but it is clear that metal ions can work by a variety of different routes. Combining drugs with different modes of action often synergizes their effects, so probing the various metallopharmaceutical mechanisms is likely to yield a wider range of effective chemotherapeutic agents.

IX. Abbreviations

The superscript k#, as in $G^{\kappa 7}$, indicates the coordinating atom when linkage isomers are possible.

Ade, A	adenine
5′-AMP	adenosine-5′-monophosphate
bpy	2,2′-bipyridyl
bzac	1-phenylbutane-1,3-diketonate
CD	circular dichroism
cdta	1,2-cyclohexanediaminotetraacetate
chrysi	5,6-chrysenequinone diimine
Cyt, C	cytosine
5′-CMP	cytidine-5′-monophosphate
Cp	cyclopentadienide
dppz	dipyrido[3,2-*a*:2′,3′-*c*]phenazine
en	ethylenediammine
9EtGua	9-ethylguanine
5′-dGMP	deoxyguanosine-5′-monophosphate
Gua, G	guanine
dGuo	guanosine
dGuo, dG	2′-deoxyguanosine
Hyp	hypoxanthine
ICyt	isocytidine
ICR	imidazolium *trans*-tetrachlorobisimidazoleruthenium(III)
Im	imidazole
Ind	indazole
Isn	isonicotinamide
Me	methyl
Et	ethyl
mgp	methylguanidinium phenanthroline
1MeAdo	1-methyladenosine
$1MeCyt^-$	deprotonated 1-methylcytosine
7MeGua	7-methylguanine
1MeGuo	7-methylguanosine
7MeHyp	7-methylhypoxanthine
6MeICyt	6-methylisocytosine
$1,3Me_2Xan^-$	deprotonated 1,3-dimethylxanthine
MM2	molecular mechanics 2
NAMI	Na[*trans*-(Me_2SO)(Im)Cl_4Ru]
NAMI-A	ImH[*trans*-(Me_2SO)(Im)Cl_4Ru]
NHE	normal hydrogen electrode
8OG	8-oxoguanine
PIH	pyridoxal isonicotinoyl hydrazone
quin	8-hydroxyquinoline
Ox	oxalate
pdta	1,2-propylenediamminetetraacetate
phen	1,10-phenanthroline
phi	phenanthrenequinone diimine
py	pyridine
Pyr	pyridine derivative
pyrazine	pz
saldox	salicylaldoxime, 2-HO−C_6H_4CH=NOH
SSCE	standard saturated calomel electrode
5′-TMP	thymidine-5′-monophosphate
5′-dTMP	deoxythymidine-5′-monophosphate
tpy	2,2′:6′,2′-terpyridine
Tf	transferrin
TfR	transferrin receptor
trien	triethylenetetraammine

X. Acknowledgment

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XI. References

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