ACCOUNTS
of chemical research

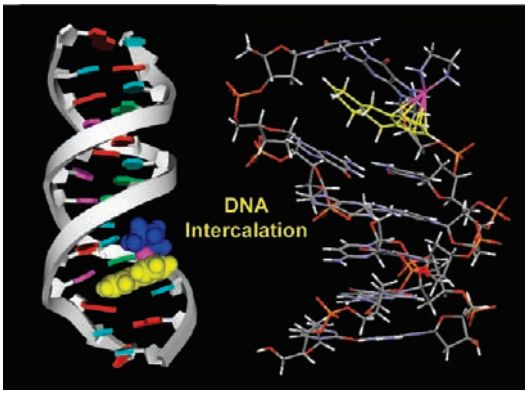
Metal Complexes as DNA Intercalators

HONG-KE LIU*,† AND PETER J. SADLER*,‡

†Jiangsu Key Laboratory of Biofunctional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210046, China, and ‡Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, U.K.

RECEIVED ON OCTOBER 15, 2010

CONSPECTUS



DNA has a strong affinity for many heterocyclic aromatic dyes, such as acridine and its derivatives. Lerman in 1961 first proposed intercalation as the source of this affinity, and this mode of DNA binding has since attracted considerable research scrutiny. Organic intercalators can inhibit nucleic acid synthesis in vivo, and they are now common anticancer drugs in clinical therapy.

The covalent attachment of organic intercalators to transition metal coordination complexes, yielding metallointercalators, can lead to novel DNA interactions that influence biological activity. Metal complexes with σ-bonded aromatic side arms can act as dual-function complexes: they bind to DNA both by metal coordination and through intercalation of the attached aromatic ligand. These aromatic side arms introduce new modes of DNA binding, involving mutual interactions of functional groups held in close proximity. The biological activity of both *cis*- and *trans*-diamine Pt^{II} complexes is dramatically enhanced by the addition of σ-bonded intercalators.

We have explored a new class of organometallic "piano-stool" Ru^{II} and Os^{II} arene anticancer complexes of the type $[(\eta^6\text{-arene})Ru/Os(XY)Cl]^+$. Here XY is, for example, ethylenediamine (en), and the arene ligand can take many forms, including tetrahydroanthracene, biphenyl, or *p*-cymene. Arene−nucleobase stacking interactions can have a significant influence on both the kinetics and thermodynamics of DNA binding. In particular, the cytotoxic activity, conformational distortions, recognition by DNA-binding proteins, and repair mechanisms are dependent on the arene. A major difficulty in developing anticancer drugs is cross-resistance, a phenomenon whereby a cell that is resistant to one drug is also resistant to another drug in the same class. These new complexes are non-cross-resistant with cisplatin towards cancer cells: they constitute a new class of anticancer agents, with a mechanism of action that differs from the anticancer drug cisplatin and its analogs. The Ru−arene complexes with dual functions are more potent towards cancer cells than their nonintercalating analogs.

In this Account, we focus on recent studies of dual-function organometallic Ru^{II}− and Os^{II}−arene complexes and the methods used to detect arene−DNA intercalation. We relate these interactions to the mechanism of anticancer activity and to structure−activity relationships. The interactions between these complexes and DNA show close similarities to those of covalent polycyclic aromatic carcinogens, especially to N7-alkylating intercalation compounds. However, Ru−arene complexes exhibit some new features. Classical intercalation and base extrusion next to the metallated base is observed for $\{(\eta^6\text{-biphenyl})Ru(ethylenediamine)\}^{2+}$ adducts of a 14-mer duplex, while penetrating arene intercalation occurs for adducts of the nonaromatic bulky intercalator $\{(\eta^6\text{-tetrahydroanthracene})Ru(ethylenediamine)\}^{2+}$ with a 6-mer duplex. The introduction of dual-function Ru−arene complexes introduces new mechanisms of antitumor activity, novel mechanisms for attack on DNA, and new concepts for developing structure− activity relationships. We hope this discussion will stimulate thoughtful and focused research on the design of anticancer chemotherapeutic agents using these unique approaches.

Published on the Web 03/29/2011 www.pubs.acs.org/accounts
10.1021/ar100140e

Introduction

Intercalative binding, as most commonly studied, is the noncovalent stacking interaction resulting from the insertion of a planar heterocyclic aromatic ring between the base pairs of the DNA double helix. This DNA binding mode was first proposed by Lerman[1] to explain the strong affinity for DNA of certain heterocyclic aromatic dyes such as the acridines. Intercalation stabilizes, lengthens, stiffens, and unwinds the DNA double helix.[1,2] The degree of unwinding varies depending on the intercalator. The ethidium cation unwinds DNA by about 26° and proflavine by about 17°.[3,4] These structural modifications can lead to functional changes, often to inhibition of transcription and replication and DNA repair processes, which make intercalators potent mutagens.

There is much interest in the ability of intercalators to inhibit nucleic acid synthesis in vivo, leading to activity as mutagens, antibiotics, antibacterials, trypanocides, schistosomicides, and antitumor agents.[5] Intercalative interactions between DNA duplexes and planar polycyclic aromatic organic intercalators,[5,6] such as ethidium bromide, acridine and its derivatives, and benzo[*a*]pyrene (BP),[7] have been thoroughly studied. Studies of bulky intercalators are rare.[8] Bis-intercalators[9,10] have also been reported, such as bis-naphthalimide, which exhibits antitumor activity.[9] A tetra-intercalator has been reported by Iverson, et. al.[11] Representative polycyclic aromatic intercalators are shown in Table 1.

There are two major modes of intercalation: classical intercalation[9,12−14] and threading intercalation.[11,15,16] Classical intercalators, such as benzo[*a*]pyrene (BP)[7] (Figure 1.1), bind to DNA duplexes with essentially all of their aromatic system inserted between GpG base pairs that form the top and bottom of the intercalation site. A threading intercalator occupies and interacts strongly with both the minor and major grooves of DNA simultaneously. For example, the threading intercalation of acridine-4-carboxamides (DACA, Figure 1.2) into the duplex 5′-d(CG(5-BrU)ACG)$_2$-3′ has been reported by Cardin.[16] Many intercalators, including ethidium and acridine, are relatively nonspecific DNA binding agents. Sequence-specific DNA binding is improved when the aromatic ring is substituted with special ancillary groups, or when bis-intercalators are involved. For example acridine-4-carboxamides have a *N*-(2-dimethylamino)ethyl)-4-carboxamide side chain (Figure 1.2).[16] This type of agent inhibits both topoisomerases I and II.[16]

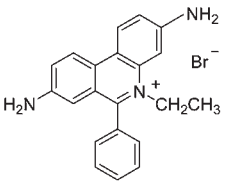
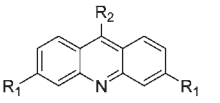
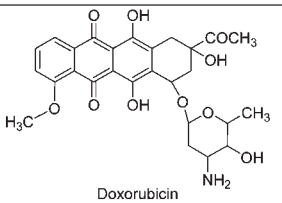
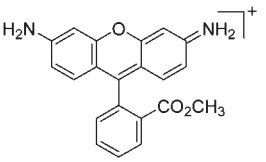
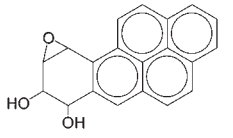
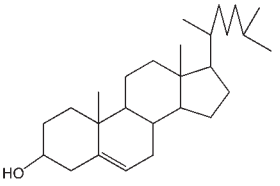
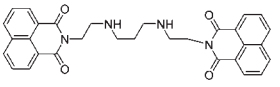
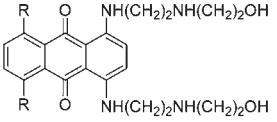
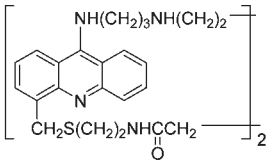
Metal complexes that can have intercalative interactions with DNA form two classes. For relatively inert coordinatively saturated square-planar Pt(II) complexes and octahedral complexes with aromatic ligands, intercalation into DNA mainly involves the aromatic ligands. Metal complexes containing σ-bonded ligands with aromatic side arms as intercalators or organometallic complexes with π-bonded arenes as intercalators can be dual-function complexes: the aromatic side arms or arene ligands can intercalate between DNA bases while the metal coordinates directly to a DNA base. Intercalative interactions between metal complexes and DNA have novel features that can influence biological activity, and this is the focus of our Account.

2. Intercalation by Inert Square-Planar and Octahedral Metal Complexes

Square-planar complexes containing aromatic fragments can bind to DNA by intercalation without direct metal coordination to DNA bases.[17] Lippard et al. have shown that square-planar platinum(II) complexes containing heterocyclic aromatic ligands, such as terpy, quaterpy, phen, bipy, and phi, bind to DNA duplexes noncovalently, intercalating between the base pairs (Figure 2.1).[17] The crystal structure of [Pt(terpy)(HET)]$^+$ (HET = 2-hydroxyethanethiolato-2,2′,2″-terpyridine) bound to (dCpG)$_2$ (Figure 2.2) reveals that the flat metal cation intercalates symmetrically between two GC base pairs.[18]

Metallointercalation has been extended into three dimensions by Barton and others using octahedral Rh, Ru, or Os complexes containing multi-heterocyclic aromatic ligands such as phen, phi or dppz, chrysi, phzi, dpq, dppn, and eilatin (Figures 2.3 and 2.5).[19−21] Octahedral metallointercalators permit the targeting of specific DNA sites by matching the shape, symmetry, and functionalities of the metal complex to that of the DNA target. There are two kinds of noncovalent interactions between metallointercalators and DNA: intercalation and insertion.[19] Metallointercalators unwind the DNA and insert their planar ligand between two intact base pairs, while metalloinsertors eject the bases of a single base pair, and their planar ligand acts as a π-stacking replacement in the DNA base stack.[19] Complexes with ligands phen, phi, or dppz have classic intercalation into duplex DNA, but complexes with ligands chrysi, phzi, and eilatin usually insert into duplex DNA.[19,21] Two-dimensional NMR studies[22] of Δ-α-[Rh(R, R-Me$_2$trien)phi]$^{3+}$ (R, R-Me$_2$trien = 2*R*,9*R*-diamino-4,7-diazadecane) bound to 5′-d(GAGTGCACTC)$_2$-3 duplex DNA reveal that the phi ligand intercalates classically into the DNA base-pair stack from the major groove at the G^{5}pC^6 site. The crystal structure of the adduct of [Δ-Rh(bpy)$_2$(chrysi)]$^{3+}$ with an oligonucleotide duplex

TABLE 1. Some Polycyclic Aromatic and Bulky Intercalators

Intercalator	structure	comments	reference
Ethidium bromide		Fluorescent tag, nucleic acid stain for agarose gel electrophoresis.	6
Proflavine Acridine Orange Acridine derivatives	 Proflavin: $R_1 = NH_2$, $R_2 = H$ Acridine Orange: $R_1 = N(Me)_2$, $R_2 = H$ 9-Aminoacridine: $R_1 = H$, $R_2 = NH_2$	Antitumor drugs. Can be attached to abasic sites on synthetic oligonucleotides or as side-arms on Pt and Ru anticancer complexes.	5-6
4′-Epiadriamycin Adriamycin Daunomycin Hedamycin Doxorubicin	 Doxorubicin	Cancer chemotherapy, threading and classical DNA binding modes.	5
Rhodamine	 Rhodamine 123	Dye for fluorescence microscopy, flow cytometry, fluorescence correlation spectroscopy and ELISA.	6
Benzo[a]pyrene (BP)		Can be attached to abasic sites on synthetic oligonucleotides.	7
Cholesterol		Example of bulky intercalator.	8
Bis-naphthalimide		Antitumor activity Elinafide (LU 79553) on clinical trial.	9
Miltoxantrone (R = OH) Ametantrone (R = H) Macrocyclic-bis-9-aminoacridine	 	Mono or bis(inter-duplex) intercalators.	10

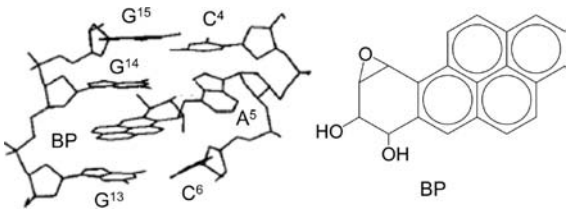


FIGURE 1.1. NMR solution structure[7] of the d($C^4A^5C^6$)–d($G^{13}G^{14}G^{15}$) segment of the 10*S*-[BP]dA·dG 9-mer duplex (5′-$G^1G^2T^3C^4$-[BP]$A^5C^6G^7A^8G^9$-3′)/(5′-$C^{10}T^{11}C^{12}G^{13}G^{14}G^{15}A^{16}C^{17}C^{18}$-3′), where the BP group attached to the A^5 base intercalates classically between the $G^{13}G^{14}$ base step of the opposite strand.

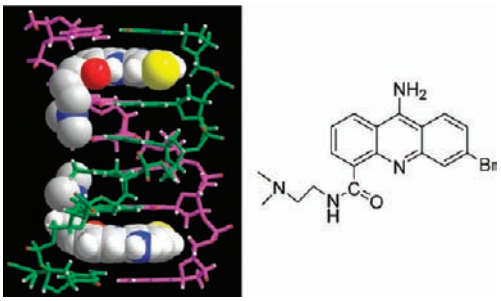


FIGURE 1.2. X-ray structure showing threading intercalation of 9-amino-6-bromo-DACA[16] (space-filling model) into the DNA duplex 5′-d(C|G(5-BrU)AC|G)$_2$-3′ (wireframe), where | indicates intercalation sites.

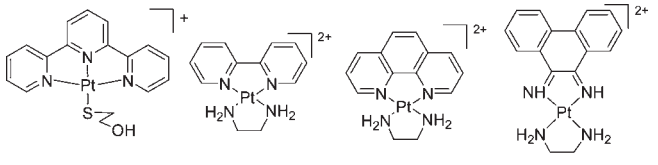


FIGURE 2.1. Platinum metallointercalators: terpy = terpyridine; phen = phenanthroline; bipy = bipyridine; phi = phenanthrenequinone diimine.

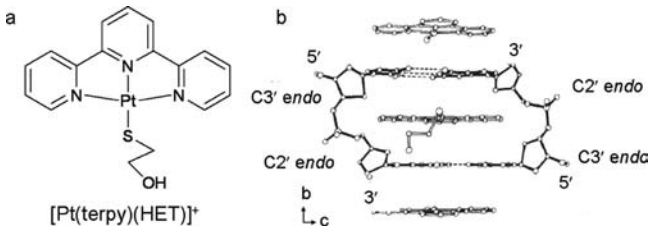


FIGURE 2.2. (a) Molecular structure of $[Pt(terpy)(HET)]^+$. (b) Crystal structure of $[Pt(terpy)(HET)]^+ \cdot d(CpG)_2$ showing HET intercalated between two GC base pairs.[18]

containing two AC mismatches 5′-d(CGGAAATTCCCG)$_2$-3′ (Figure 2.4) reveals two binding modes for $[Rh(bpy)_2(chrysi)]^{3+}$:[23] intercalation and insertion. Some bis-metallointercalators (Figure 2.5) can undergo threading, which has been extensively studied.[15,19,24]

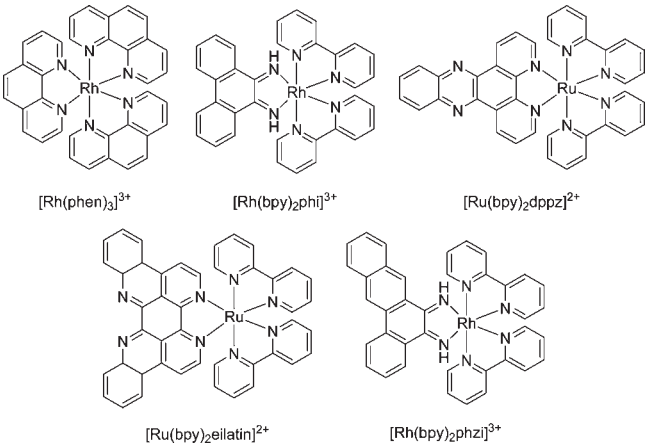


FIGURE 2.3. Metallointercalators containing phen, phi, dppz, phzi, and eilatin ligands:[19,21] dppz = dipyrido[3,2-*a*:2,3-*c*]plenazine, phzi = benzo[*a*]phenazin-5,6-quinone diimine.

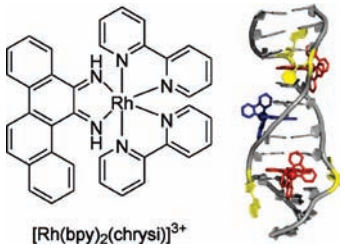


FIGURE 2.4. X-ray crystal structure of $[\Delta\text{-}Rh(bpy)_2(chrysi)]^{3+}$ bound to 5′-d(CGGAAATTCCCG)$_2$-3′ duplex:[23] chrysi = 5,6-chrysene quinine diimine. Reproduced with permission from ref 19. Copyright 2007 Royal Society of Chemistry.

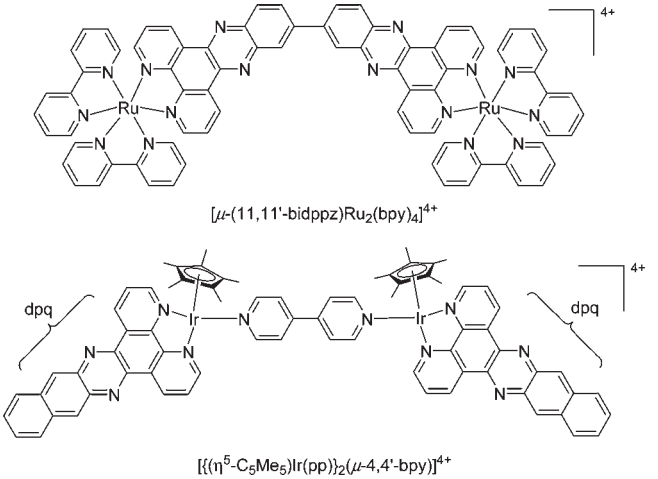


FIGURE 2.5. Bis-Ru^{II} and -Ir^{III}-metallointercalators:[15,24] pp = dipyrido[3,2-*d*: 2′,3′-*f*]quinoxaline (dpq), dppz, or benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppn).

The bleomycins (BLMs) are natural products. Cobalt bleomycin probably has the most pharmaceutical relevance of any of its metal adducts studied to date.[25] Two-dimensional NMR methods and molecular modeling have shown that

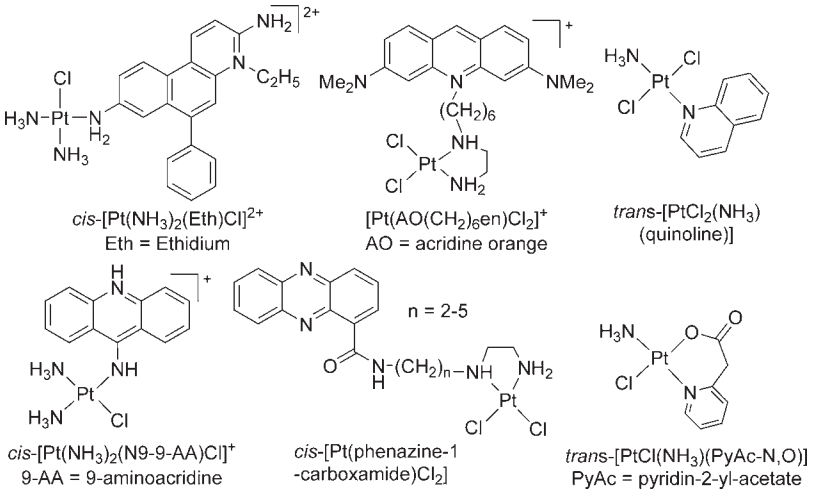


FIGURE 3.1. Platinum complexes covalently linked to organic intercalators, and quinoline and pyridine complexes.

HOO-CoPLM binds to 5′-d(CCAGGCCTGG)$_2$-3′ in a partial intercalative mode providing a basis for the sequence specificity of binding and chemical specificity of cleavage.[26]

3. Intercalation by Dual-Function Metal Complexes with σ-Bonded Side Arm Intercalators

In the late 1980s, platinum complexes having a σ-bonded side arm as an intercalator were designed.[17,27] Planar aromatic ligands such as acridine orange (AO), 9-aminoacridine (9-AA), and ethidium bromide[4,28] have been incorporated into Pt complexes as potential intercalators (Figure 3.1). These Pt−intercalator complexes can react with DNA by both coordination and intercalation, and the DNA platination can be sensitive to sequence-dependent local DNA structural modulations such as those accompanying intercalator binding. The reaction between cisplatin and ethidium (Eth) is promoted by DNA; ethidium binds to platinum via its N8 or N3.[28] Modeling shows that the ethidium is intercalated between the C^3/G^{10}:G^4/C^9 base pairs of 5′-d(CGCGCG)$_2$-3′, while the *cis*-{Pt(NH$_3$)$_2$}$^{2+}$ fragment coordinates to N7 of G^4. These complexes introduced new modes of DNA binding involving mutual interactions of functional groups held in close proximity.

Bierbach et al.[27] have described a new class of hybrid Pt compounds containing acridinylthiourea as intercalating groups (Figure 3.2). Platinum-acridinylthiourea (PT-ACRAMTU) and its analogs exhibit promising activity toward several tumor cell lines, and show only partial cross-resistance with cisplatin.[27,29] The 2D NMR solution structure[30] of PT-ACRAMTU with (5′-CCTCGTCC-3′)/(3′-GGAGCAGG-5′) shows that Pt is bound to N7 of G^5 in the major groove and

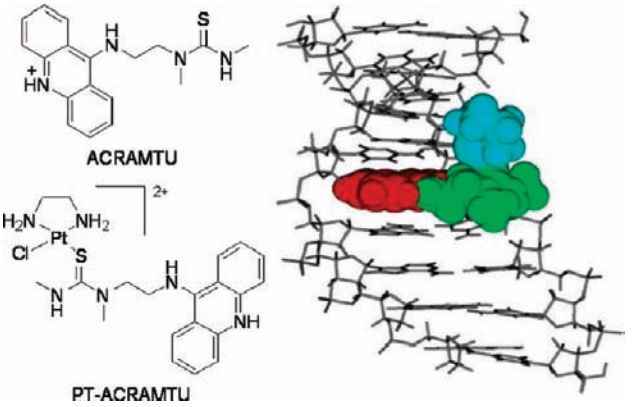


FIGURE 3.2. Structures of ACRAMTU (upper left) and Pt-ACRAMTU (bottom left) and model of a DNA duplex containing the Pt-ACRAMTU adduct (right, the platinum fragment, thiourea linker, and acridine chromophore are depicted in blue, green, and red, respectively).[30].

ACRAMTU intercalates threadingly into the central 5′-C^{4}G^5/C^{12}G^{13} base-pair step on the 5′-face of the platinated nucleobase (Figure 3.2). Intercalation of ACRAMTU does not cause helical bending but significantly augments duplex thermal stability, indicating that the PT-ACRAMTU lesion is different from that formed by cisplatin cross-linking.

Planar aromatic ligands have also been introduced into transplatin, *trans*-[PtCl$_2$(NH$_3$)$_2$], for development of a new class of anticancer drugs (Figure 3.1).[17] When one ammine is changed to a planar aromatic N-donor ligand such as L = pyridine or quinoline in *trans*-[PtCl$_2$(NH$_3$)L], the cytotoxicity of the *trans* complex is dramatically enhanced.[31] This has been attributed to interaction of the planar aromatic ligand with the DNA duplex, perhaps partially by intercalation.[32]

Other dual-function coordination complexes have also been studied (Figure 3.3).[14,33] Two-dimensional NMR shows

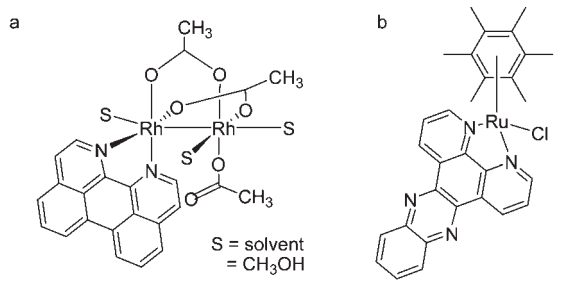


FIGURE 3.3. *cis*-$[Rh_2(dap)(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(CH_3OH)]^+$ and $[(\eta^6\text{-}C_6Me_6)RuCl(dppz)]^+$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) dual-function complexes.

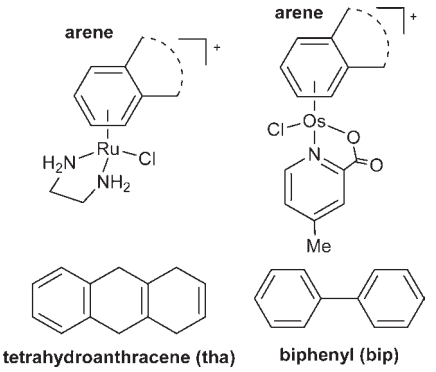


FIGURE 4.1. Structures of Ru^{II} and Os^{II} arene "piano-stool" complexes.

combined coordinative and intercalative interactions between the dirhodium(II) complex *cis*-$[Rh_2(dap)(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(CH_3OH)]$ (O_2CCH_3)[14] (dap = 1,12-diazaperylene) and the duplex (5′-GGAAGTTGAGAG-3′)/(5′-CTCTCAACTTCC-3′). One rhodium atom binds coordinatively to N7 of the A^6 base on the second strand, while the dap ligand intercalates classically into the central A^6pA^7 base step.

4. Intercalation by Organometallic Ru− and Os−Arene Complexes

Organometallic Ru^{II}−arene complexes of the half-sandwich "piano-stool" type $[(\eta^6\text{-arene})Ru(XY)Cl]^+$ (arene = tetrahydroanthracene (tha), biphenyl (bip), dihydroanthracene (dha), *p*-cymene (cym), benzene (ben), for example; XY = ethylenediamine (en), for example) exhibit promising anticancer activity both in vitro and in vivo (Figure 4.1).[34−40] One of the potential targets of these complexes is DNA. The strong binding to N7 of G on DNA is enhanced by H-bonding between the en NH_2 groups and the exocyclic C6O carbonyl oxygen of G. Repulsive interactions with exocyclic amino groups of A and C nucleobases lead to high specificity for G binding (Figure 4.2).[41] The rates of reaction of $[(\eta^6\text{-arene})Ru(en)(H_2O)]^{2+}$ complexes with cGMP depend on the nature of

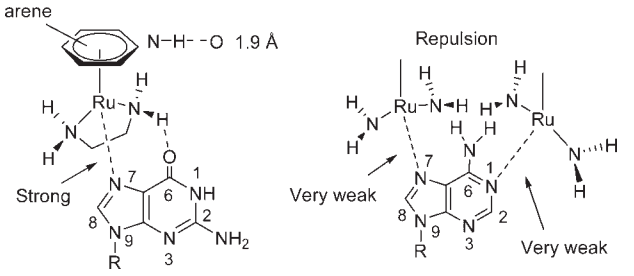


FIGURE 4.2. H-bonding and steric interactions that give rise to strong binding of $\{(\eta^6\text{-arene})Ru(en)\}^{2+}$ to guanine but very weak binding to adenine.[41]

the arene, decreasing by over an order of magnitude in the series ($\times 10^{-2}\ M^{-1}\ s^{-1}$): tha (14.72) > bip (8.06) > dha (7.08) > cym (2.49) > ben (1.13) (for abbreviations, see above).[42] Also the cytotoxic activity of these complexes shows a strong dependence on the arene:[35,36] benzene (Ru-ben, $IC_{50} = 17\ \mu M$) < *p*-cymene (Ru-cym, 10 μM), < biphenyl (Ru-bip, 5 μM), < dihydroanthracene (Ru-dha, 2 μM), < tetrahydroanthracene (Ru-tha, 0.5 μM), the latter complex being as cytotoxic as the clinical platinum drug cisplatin. We have also investigated the chemical and biological activity of half-sandwich osmium−arene complexes.[35,43−45] Their cytotoxicity (toward A2780 cells) also appears to depend on the size of the coordinated arene: benzene (Os-ben, $IC_{50} = 32.7\ \mu M$) < *p*-cymene (Os-cym, 7.6 μM), < tetrahydroanthracene (Os-tha, 4.5 μM), < biphenyl (Os-bip, 3.2 μM), the latter two complexes being as cytotoxic as carboplatin.[35,45,46]

Studies of DNA duplexes singly modified by Ru-cym (nonintercalating) and Ru-tha (intercalating) reveal that conformational distortions, recognition by DNA-binding proteins, and repair mechanisms are also dependent on the arene.[47,48] Distortions induced by Ru-cym and Ru-tha are not recognized by the DNA-binding protein HMGB1, indicating that the mechanism of antitumor activity of Ru^{II}−arene complexes does not involve recognition of their DNA adducts by HMG proteins as a crucial step, in contrast to cisplatin and its analogs.[38,39,46,47,49−52] Adducts of $\{(\eta^6\text{-}p\text{-cymene})Ru(en)\}^{2+}$ (Ru-cym) are removed from DNA more efficiently than those of Ru-tha, and both adducts are removed from DNA preferentially by mechanisms other than nucleotide excision repair (a major mechanism contributing to cisplatin resistance).[36,53] DNA binding studies on a series of complexes of the type $[(\eta^6\text{-arene})Os(XY)Cl]^{n+}$, where arene = biphenyl, for example, and XY = ethylenediamine, for example, show that these complexes all bind and distort polymeric DNA with a rate of binding comparable to that of cisplatin.[35,54] They coordinate mainly to guanine with additional noncoordinative interactions. DNA

osmiated with $\{(\eta^6\text{-arene})Os(XY)\}^{n+}$ (arene = biphenyl or *p*-cymene, XY = ethylenediamine, picolinate, or oxinate) inhibits RNA synthesis like cisplatin and the ruthenium analog Ru-bip.[54] In contrast to cisplatin, no DNA bending occurs. The unwinding angle induced in supercoiled plasmid DNA by Os−arene complexes is larger (21−27°) than that of ruthenium analogs (7−14°) or cisplatin (6° for monofunctional and 13° for bifunctional adducts).[35,36] The noncovalent interactions are greater for $[(\eta^6\text{-bip})Os(en)Cl]^+$ than those of its Ru analog.[35] Remarkably, these Ru^{II} and Os^{II} complexes are non-cross-resistant with cisplatin toward cancer cells, implying promise for tackling the common problem of developed (and intrinsic) drug resistance in chemotherapy.[35] These complexes form a new class of novel anticancer agents with a different mechanism of action compared with the anticancer drug cisplatin and its analogs.[54,55]

Detection of Arene Intercalation. DNA intercalation by ligands can be detected by several methods. (a) Circular dichroism (CD). The presence of the tricyclic tetrahydroanthracene ligand on Ru^{II} (Ru-tha) results in the appearance of a CD band centered around 370−380 nm on interaction with ct-DNA, while binding of the monocyclic Ru-cym complex to DNA does not give such a band in this region of the spectrum.[50] (b) Linear dichroism (LD). The complexes Ru-tha, Ru-dha, and Ru-bip cause a significant red shift (ca., 10 nm) of the main DNA band near 260 nm in LD spectra,[50] whereas complexes Ru-ben and Ru-cym cause no such shift. (c) Fluorescence measurements.[50] Modification of DNA by Ru-bip, Ru-dha, and Ru-tha results in a marked decrease in the fluorescence of EthBr bound to DNA, while the decrease caused by binding to Ru-cym is very small. These data indicate intercalation by Ru−arene complexes with extended arene ligands. (d) Isothermal titration calorimetry (ITC). The intercalation of porphyrins is evident from the enthalpic terms measured by ITC, which are similar to those reported for ethidium bromide with a G4 tetraplex.[56] (e) Viscosity. The binding of $[Pt(en)(phen)]^{2+}$ to duplex DNA gives rise to an increase in the relative viscosity, indicative of intercalative binding of the phen ligand.[57] (f) Melting temperature (T_m). The T_m of duplex DNA modified by intercalators Ru-bip, Ru-dha, or Ru-tha increases, while T_m decreases for DNA modified by nonintercalators Ru-ben and Ru-cym.[50] (g) NMR. Intercalation between DNA base pairs can often be recognized by distinctive NMR features,[11−14] including upfield ^1H NMR shifts of the intercalator, NOE cross-peaks between intercalator and DNA bases at sites of intercalation, and the interruption or weakening of NOE connectivities between sequential DNA nucleotides.

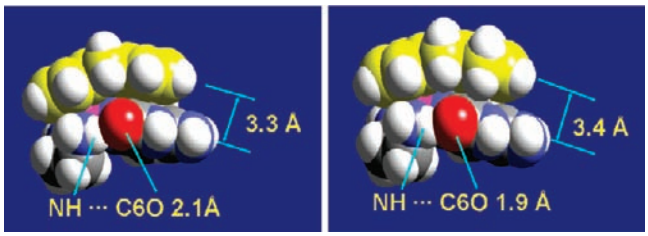


FIGURE 4.3. Crystal structures of $[(\eta^6\text{-dha})Ru(en)(9EtG)]^{2+}$ (left) and $[(\eta^6\text{-tha})Ru(en)(9EtG)]^{2+}$ (right), showing the arene−purine $\pi-\pi$ stacking and hydrogen bonding between en NH and G C6O.[41] Reproduced with permission from ref 36. Copyright 2005 Royal Society of Chemistry.

Two-dimensional NMR studies of the interactions of Ru-bip with 5′-d$(CGGCCG)_2$-3′ reveal that the arene ligand intercalates between G^3/C^4 or C^5/G^6 base pairs.[13] (h) X-ray crystallography. Wang et al. have reported a 1.8 Å resolution crystal structure of a macrocyclic bis-9-aminoacridine complex bound to 5′-$(CGTACG)_2$-3′ by intercalation.[10]

Adducts of Ru−Arene Complexes with Nucleobases and DNA. We have determined the structures of monofunctional adducts of the Ru−arene complexes[41,42] with guanine derivatives by X-ray crystallography and 2D NMR methods. Strong $\pi-\pi$ arene−nucleobase stacking (center-to-center distances of 3.45 Å for Ru-tha and 3.31 Å for Ru-dha) is present in the crystal structures of $[(\eta^6\text{-tha})Ru(en)(9EtG\text{-}N7)]^{2+}$ and $[(\eta^6\text{-dha})Ru(en)(9EtG\text{-}N7)]^{2+}$ (Figure 4.3). Significant reorientations and conformational changes of the arene ligands in $[(\eta^6\text{-arene})Ru(en)(G\text{-}N7)]^{2+}$ complexes are observed in the solid state, with respect to those of the parent chlorido-complexes $[(\eta^6\text{-arene})Ru(en)Cl]^+$,[41] which maximize intra- or intermolecular stacking of extended arene rings with the purine ring. This flexibility makes simultaneous arene−base stacking and N7-covalent binding compatible.

The tetrahydroanthracene complex Ru-tha is 10× more toxic to cancer cells than the biphenyl complex Ru-bip.[36] The extended nonaromatic rings B and C of Ru-tha are more bulky than bip in Ru-bip (Figure 4.1). Two-dimensional NMR experiments,[58]show the presence of a novel penetrating intercalation of rings B and C of Ru-tha into a DNA hexamer, selectively between two base pairs, G^3/C^{10}:C^4/G^9 or G^6/C^7:C^5/G^8 (Figure 4.4). Large low-field shifts for protons on rings B and C of Ru-tha are observed for the monoruthenated DNA adduct. Deshielding of intercalator NMR resonances is rare.

The dinuclear Ru−arene complex $[\{(\eta^6\text{-bip})RuCl(en)\}_2(CH_2)_6]^{2+}$ (Figure 4.5)[59] can undergo double intercalation into DNA via induced-fit recognition involving epimerization at the dynamic stereogenic centers. DNA cross-linking by $\{((\eta^6\text{-bip})RuCl(en))_2(CH_2)_6\}^{2+}$ is observed, and the complex generates interstrand cross-links with similar efficiency to

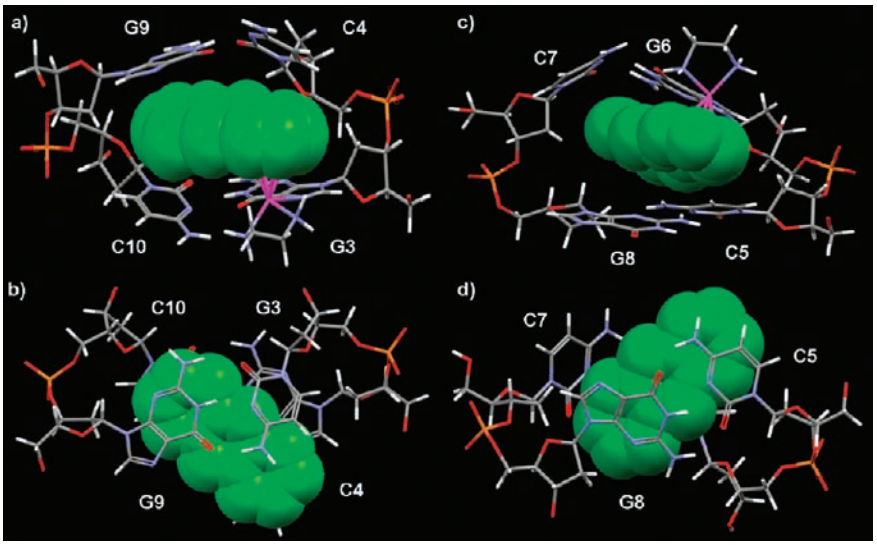


FIGURE 4.4. Model of duplex DNA ruthenated at N7 of G3 (a,b) or N7 of G6 (c,d) with $\{(\eta^6\text{-tha})\text{Ru(en)}\}^{2+}$,[58] showing a novel penetrative intercalation. The tetrahydroanthracene ligand (tha) is in green space filling form: (a, c) side views; (b, d) top views.

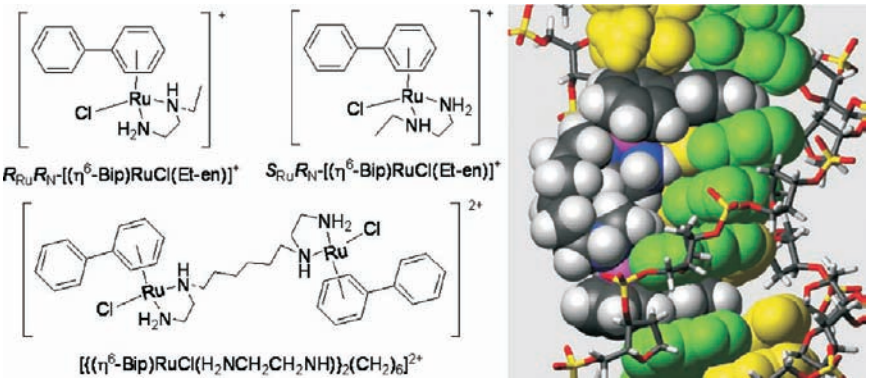


FIGURE 4.5. (left) Mono- and dinuclear-Ru(II) complexes.[59] (right) Model of a 1,3-interstrand cross-link formed by a dinuclear Ru(II) complex on the DNA duplex (5′-AATGTCTAA-3′)/(3′-TTACAGATT-5′), showing bis-intercalation. DNA bases, one strand green, second strand yellow; backbone sugars and phosphate groups, sticks; BisRu(bip), Ru purple, N blue, C black, H gray. Reproduced with permission from ref 36. Copyright 2005 Royal Society of Chemistry.

cisplatin; 1,3-GG interstrand and 1,2-GG and 1,3-GTG intrastrand cross-links are observed for 20-mer DNA duplexes.[59] This dinuclear Ru−arene complex blocks intercalation of ethidium considerably more than mononuclear analogs, and both of the free phenyl rings can contribute to the DNA intercalation (Figure 4.5).

Further work[12] has provided evidence that coordinative and intercalative interactions of Ru-bip can affect the base-pairing of duplex DNA. The fragment $\{(\eta^6\text{-bip})\text{Ru(en)}\}^{2+}$ from Ru-bip is highly specific for G N7 in the 14-mer DNA duplex d(5′-ATACATGGTACATA-3′)/(3′-TATGTACCATGTAT-5′) but relatively labile; it can migrate to other G residues at high temperature. Ruthenium−arene intercalation is dynamic in nature: equilibria can exist between intercalated and nonintercalated conformers, Figure 4.6. In one conformer, the base T17 is partially extruded from its Watson−Crick base pair in the helix and the pendent phenyl ring of biphenyl (bip) is involved in $\pi-\pi$ stacking with DNA bases either via classical intercalation between the bases or with the partially extruded T base.[12,60] Cross-linking of bases by cisplatin can also give rise to extruded bases,[60] and such distortions are thought to affect histone protein and nucleosome recognition. The displacement, or flip-out, of bases near the modified sites is common for DNA modified covalently by aromatic or bulky intercalators and may act as a trigger for nucleotide excision repair (NER).[8]

5. Comparison with Dual-Mode Organic Intercalators

Polycyclic aromatic hydrocarbons,[61] heterocyclic amines,[62] their nitro derivatives, and food mutagens[61,62] are well-known classes of environmental pollutants that cause the

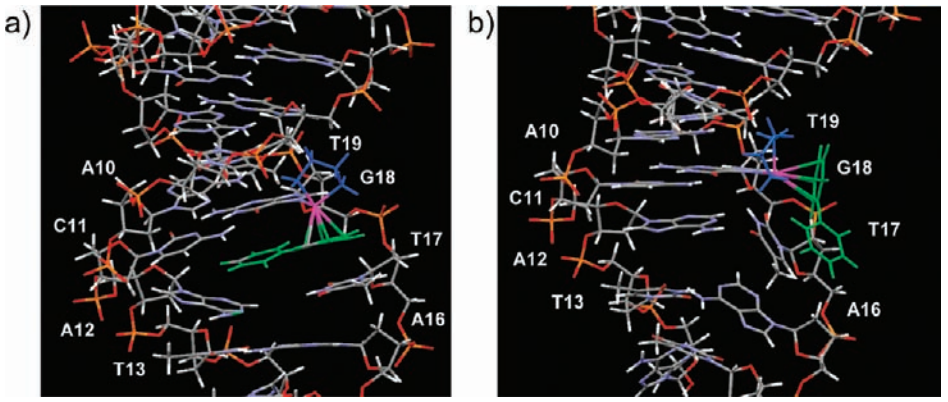


FIGURE 4.6. Molecular models of two conformers of duplex (5′-ATACATGGTACATA-3′)/(3′-TATGTACCATGTAT-5′) ruthenated at N7 of G18 with $\{(\eta^6\text{-bip})\text{Ru(en)}\}^{2+}$: (a) the biphenyl arene is intercalated; (b) it is stacked on T17 as a flipped-out base. Reproduced from ref. 12 with permission. Copyright 2006 Wiley-VCH.[12]

formation of bulky DNA lesions in vivo. They are dual-function organic intercalators, which can modify DNA bases covalently and exhibit intercalative interactions. Oligonucleotides containing these groups have been studied extensively.[8,61,63] The modification of DNA bases by polycyclic aromatic carcinogens induces various structural distortions, including double-helix bending, diminished base-stacking, classical or threading intercalation, and impaired Watson−Crick pairing that can lead to flip-out of the nucleotide opposite the modified site. The enhanced lesion dynamics may play an important role in facilitating NER.

Interactions between dual-function transition metal complexes and DNA show some similarities to those of covalent polycyclic aromatic carcinogen−DNA adducts,[61] especially to the N7-alkylating-intercalation compounds. However, Ru−arene complexes exhibit some new features. For Ru-bip, classical intercalation causes a large distortion of the DNA helix and can lead to base extrusion next to the modification site. Such structural distortions and lower stability have also been observed for the formamidopyrimidine AFB-α-FAPY DNA adduct.[64] For the DNA adduct with the nonaromatic bulky tetrahydroanthracene intercalator Ru-tha, a novel penetrating intercalation is observed in which Ru-tha coordinates to N7 of guanine. C−H···X (X = O or N) hydrogen bonds between protons of ring C of tha and O or N atoms of the base opposite the ruthenated nucleotides are observed but no displacement of the base opposite or next to the modified nucleotide. However, the local structure of the ruthenated double helix is highly distorted, which is similar to that observed for polycyclic aromatic carcinogen-modified DNA adducts. Such a highly distorted double helix is not observed for the DNA adducts of platinum complexes containing ethidium, acridine, or acridinylthiourea derivatives ACRAMTU as side arm intercalators, where threading intercalation occurs but little helical bending. Furthermore, sequence-dependent modification is observed for the Ru−arene complexes and polycyclic aromatic carcinogen-modified DNA adducts but not for platinum complexes with different side arm intercalators. This similarity between the dual-function Ru−arene lesions and N7-alkylation intercalation compounds might provide insight into structure−activity relationships for these complexes. These findings help to explain why ruthenium−arene complexes have a different mechanism of antitumor activity compared with cisplatin and are recognized differently by nucleotide repair enzymes.

6. Conclusion

This Account has focused on new dual-function metal complexes that can interact with DNA by both coordination and intercalation modes of binding. Such dual mode binding produces unusual DNA distortions and novel mechanisms of biological activity. Ru−arene complexes with such dual functions (e.g., Ru-bip or Ru-tha) are more potent toward cancer cells than their nonintercalating analogs (e.g., Ru-*p*-cym or Ru-ben)[36] or less intercalating isomers (*p*-terphenyl arene complexes are more potent than *o*- or *m*-terphenyl complexes). This design concept can be applied to structure−activity relationships for other organometallic anticancer complexes, including cyclopentadienyl complexes,[65] and introduces novel mechanisms of action. The unusual nature of such DNA distortions affects recognition by DNA binding proteins and repair enzymes and hence the downstream processes related to apoptosis and cancer cell death.

We thank NSF (Grants 20871069, 20833006), JSSF (Grant BK2008428), the Priority Academic Program Development of

Jiangsu Higher Education Institutions (PAPD), ERC (Grant 247450), EPSRC, and Science City (ERDF/AWM) for their support for our work, our collaborators for their contributions, and members of EC COST Action D39 for stimulating discussions.

BIOGRAPHICAL INFORMATION

Hongke Liu is a Professor in the College of Chemistry and Materials Science at Nanjing Normal University. He obtained his BA and MA at Northwestern University, China, and gained his Ph.D. in Chemistry from Nanjing University. From 2003 to 2005, he spent two years as a Wellcome Trust Travelling Research Fellow in Peter Sadler's laboratory at the University of Edinburgh. His current research focuses on bioinorganic chemistry and biofunctional materials.

Peter Sadler holds a Chair of Chemistry at the University of Warwick and is also a European Research Council Advanced Investigator. He obtained his BA, MA, and DPhil at the University of Oxford and spent two years as a Medical Research Council Research Fellow at the University of Cambridge and National Institute for Medical Research before being appointed as a Lecturer at Birkbeck College, University of London, where he subsequently became Reader in Biological Inorganic Chemistry and Professor of Chemistry. From 1996 to 2007, he was Crum Brown Chair of Chemistry at the University of Edinburgh. His research interests are centered on the chemistry of metals in medicine.

FOOTNOTES

*To whom correspondence should be addressed. Fax 0086-25-83599188, e-mail liuhongke@njnu.edu.cn; or fax 00-44-24 765 23819, e-mail P.J.Sadler@warwick.ac.uk.

REFERENCES

1 Lerman, L. S. Structural Considerations in Interaction of DNA and Acridines. *J. Mol. Biol.* **1961**, *3*, 18–30.

2 Bauer, W.; Vinograd, J. The Interaction of Closed Circular DNA with Intercalative Dyes III. Dependence of the Buoyant Density upon Superhelix Density and Base Composition. *J. Mol. Biol.* **1970**, *54*, 281–298.

3 Neidle, S.; Abraham, Z. Structural and Sequence-Dependent Aspects of Drug Intercalation into Nucleic Acids. *CRC Crit. Rev. Biochem.* **1984**, *171*, 73–121.

4 Keck, M. V.; Lippard, S. J. Unwinding of Supercoiled DNA by Platinum-ethidium and Related Complexes. *J. Am. Chem. Soc.* **1992**, *114*, 3386–3390.

5 Martinez, R.; Chacon-Garcia, L. The Search of DNA-Intercalators as Antitumoral Drugs: What It Worked and What Did Not Work. *Curr. Med. Chem.* **2005**, *12*, 127–151.

6 Neidle, S., Warring, M. J., Eds.; *Molecular Aspects of Anti-Cancer Drug Action*; MacMillian: London, 1983.

7 Yeh, H. J. C.; Sayer, J. M.; Liu, X.; Altieri, A. S.; Byrd, R. A.; Lakshman, M. K.; Yagi, H.; Schurter, E. J.; Gorenstein, D. G.; Jerina, D. M. NMR Solution Structure of a Nonanucleotide Duplex with a dG Mismatch Opposite a 10S Adduct Derived from Trans Addition of a Deoxyadenosine N^6-Amino Group to (+)-(7R,8S,9S,10R)-7,8-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene: An Unusual syn Glycosidic Torsion Angle at the Modified dA. *Biochemistry* **1995**, *34*, 13570–13581.

8 Gomez-Pinto, I.; Cubero, E.; Kalko, S. G.; Monaco, V.; van der Marel, G.; van Boom, J. H.; Orozco, M.; Gonzalez, C. Effect of Bulky Lesions on DNA - Solution Structure of A DNA Duplex Containing A Cholesterol Adduct. *J. Biol. Chem.* **2004**, *279*, 24552–24560.

9 Gonzalez-Bulnes, L.; Gallego, J. Indirect Effects Modulating the Interaction between DNA and a Cytotoxic Bisnaphthalimide Reveal a Two-Step Binding Process. *J. Am. Chem. Soc.* **2009**, *131*, 7781–7791.

10 Yang, X. L.; Robinson, H.; Gao, Y. G.; Wang, A. H. J. Binding of a Macrocyclic Bisacridine and Ametantrone to CGTACG Involves Similar Unusual Intercalation Platforms. *Biochemistry* **2000**, *39*, 10950–10957.

11 Lee, J.; Guelev, V.; Sorey, S.; Hoffman, D. W.; Iverson, B. L. NMR Structural Analysis of a Modular Threading Tetra Intercalator Bound to DNA. *J. Am. Chem. Soc.* **2004**, *126*, 14036–14042.

12 Liu, H. K.; Berners-Price, S. J.; Wang, F. Y.; Parkinson, J. A.; Xu, J. J.; Bella, J.; Sadler, P. J. Diversity in Guanine-Selective DNA Binding Modes for an Organometallic Ruthenium Arene Complex. *Angew. Chem., Int. Ed.* **2006**, *45*, 8153–8156.

13 Liu, H. K.; Wang, F. Y.; Parkinson, J. A.; Bella, J.; Sadler, P. J. Ruthenation of Duplex and Single-Stranded d(CGGCCG) by Organometallic Anticancer Complexes. *Chem.—Eur. J.* **2006**, *12*, 6151–6165.

14 Kang, M.; Chouai, A.; Chifotides, H. T.; Dunbar, K. R. 2D NMR Spectroscopic Evidence for Unprecedented Interactions of Cis- $[Rh_2(dap)\text{-}(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(CH_3OH)](O_2CCH_3)$ with a DNA Oligonucleotide: Combination of Intercalative and Coordinative Binding. *Angew. Chem., Int. Ed.* **2006**, *45*, 6148–6151.

15 Nazif, M. A.; Bangert, J. A.; Ott, I.; Gust, R.; Stoll, R.; Sheldrick, W. S. Dinuclear Organoiridium(III) Mono- and Bis-intercalators with Rigid Bridging Ligands: Synthesis, Cytotoxicity and DNA Binding. *J. Inorg. Biochem.* **2009**, *103*, 1405–1414.

16 Todd, A. K.; Adams, A.; Thorpe, J. H.; Denny, W. A.; Wakelin, L. P. G.; Cardin, C. J. Major Groove Binding and "DNA-Induced" Fit in the Intercalation of a Derivative of the Mixed Topoisomerase I/II Poison N-(2-(Dimethylamino)ethyl)acridine-4-carboxamide (DACA) into DNA: X-ray Structure Complexed to $d(CG(5\text{-}BrU)ACG)_2$ at 1.3 Å Resolution. *J. Med. Chem.* **1999**, *42*, 536–540.

17 Barnes, K. R.; Lippard, S. J. Cisplatin and Related Anticancer Drugs: Recent Advances and Insights. In *Metal Complexes in Tumor Diagnosis and as Anticancer Agents*; Sigel, A., Sigel, H., Eds.; Metal Ions in Biolgical Systems; Marcel Dekker: New York, 2004; Vol. 42; pp 143−177.

18 Wang, A. H.; Nathans, J.; van der Marel, G.; van Boom, J. H.; Rich, A. Molecular Structure of a Double Helical DNA Fragment Intercalator Complex between Deoxy CpG and a Terpyridine Platinum Compound. *Nature* **1978**, *276*, 471–474.

19 Zeglis, B. M.; Pierre, V. C.; Barton, J. K. Metallo-Intercalators and Metallo-Insertors. *Chem. Commun.* **2007**, 4565–4579.

20 Elias, B.; Kirsch-De Mesmaeker, A. Photo-reduction of Polyazaaromatic Ru(II) Complexes by Biomolecules and Possible Applications. *Coord. Chem. Rev.* **2006**, *250*, 1627–1641.

21 Luedtke, N. W.; Hwang, J. S.; Nava, E.; Gut, D.; Kol, M.; Tor, Y. The DNA and RNA Specificity of Eilatin Ru(II) Complexes as Compared to Eilatin and Ethidium Bromide. *Nucleic Acids Res.* **2003**, *31*, 5732–5740.

22 Hudson, B. P.; Dupureur, C. M.; Barton, J. K. ^{1}H NMR Structural Evidence for the Sequence-Specific Design of an Intercalator: Δ-α-$[Rh[(R,R)\text{-}Me_2trien]phi]^{3+}$ Bound to d-$(GAGTGCACTC)_2$. *J. Am. Chem. Soc.* **1995**, *117*, 9379–9380.

23 Pierre, V. C.; Kaiser, J. T.; Barton, J. K. Insights into Finding a Mismatch through the Structure of a Mispaired DNA Bound by a Rhodium Intercalator. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 429–434.

24 Nordell, P.; Lincoln, P. Mechanism of DNA Threading Intercalation of Binuclear Ru Complexes: Uni- or Bimolecular Pathways Depending on Ligand Structure and Binding Density. *J. Am. Chem. Soc.* **2005**, *127*, 9670–9671.

25 Chen, J; Stubbe., J. Nature and Nurture-Lessons from Chemical Carcinogenesis. *Nat. Rev. Cancer* **2005**, *5*, 102–112.

26 Wu, W.; Vanderwall, D. E.; Turner, C. J.; Hoehn, S.; Chen, J.; Kozarich, J. W.; Stubbe, J. Solution Structure of the Hydroperoxide of Co(III) Phleomycin Complexed with d-$(CCAGGCCTGG)_2$: Evidence for Binding by Partial Intercalation. *Nucleic Acids Res.* **2002**, *30*, 4881–4891.

27 Baruah, H.; Barry, C. G.; Bierbach, U. Platinum-Intercalator Conjugates: From DNA-Targeted Cisplatin Derivatives to Adenine Binding Complexes as Potential Modulators of Gene Regulation. *Curr. Top. Med. Chem.* **2004**, *4*, 1537–1549.

28 Sundquist, W. J.; Bancroft, D. P.; Chassot, L.; Lippard, S. J. DNA Promotes the Reaction of cis-Diamminedichloroplatinum(II) with the Exocyclic Amino Groups of Ethidium Bromide *J. Am. Chem. Soc.* **1988**, *110*, 8559–8560.

29 Ma, Z.; Choudhury, J. R.; Wright, M. W.; Day, C. S.; Saluta, G.; Kucera, G. L.; Bierbach, U. A Non-Cross-Linking Platinum-Acridine Agent with Potent Activity in Non-Small-Cell Lung Cancer. *J. Med. Chem.* **2008**, *51*, 7574–7580.

30 Baruah, H.; Wright, M. W.; Bierbach, U. Solution Structural Study of a DNA Duplex Containing the Guanine-N7 Adduct Formed by a Cytotoxic Platinum-Acridine Hybrid Agent. *Biochemistry* **2005**, *44*, 6059–6070.

31 Farrell, N. Current Status of Structure-Activity Selationships of Platinum Anticancer Drugs: Activation of the Trans Geometry, in Interactions of Metal Ions with Nucleotides, Nucleic Acids, and their Constituents. *Met. Ions Biol. Syst.* **1996**, *32*, 603–639.

32 Bierbach, U.; Qu, Y.; Hambley, T. W.; Peroutka, J.; Nguyen, H. L.; Doedee, M.; Farrell, N. Synthesis, Structure, Biological Activity, and DNA Binding of Platinum(II) Complexes of the Type Trans-$[PtCl_2(NH_3)L]$ (L = Planar Nitrogen Base). Effect of L and Cis/Trans Isomerism on Sequence Specificity and Unwinding Properties Observed in Globally Platinated DNA. *Inorg. Chem.* **1999**, *38*, 3535–3542.

33 Frodl, A.; Herebian, D.; Sheldrick, W. S. Coligand Tuning of the DNA Binding Properties of Bioorganometallic (η^6-arene) Ruthenium(II) Complexes of the Type [(η^6-arene)Ru(amino acid)(dppz)]$^{n+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine), n=1−3. *J. Chem. Soc., Dalton Trans.* **2002**, 3664–3673.

34 Bruijnincx, P. C. A.; Sadler, P. J. New Trends for Metal Complexes with Anticancer Activity. *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206.

35 Peacock, A. F. A.; Sadler, P. J. Medicinal Organometallic Chemistry: Designing Metal Arene Complexes as Anticancer Agents. *Chem.—Asian J.* **2008**, *3*, 1890–1899.

36 Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. Organometallic Chemistry, Biology and Medicine: Ruthenium Arene Anticancer Complexes. *Chem. Commun.* **2005**, 4764–4776.

37 Wang, F. Y.; Xu, J. J.; Habtemariam, A.; Bella, J.; Sadler, P. J. Competition between Glutathione and Guanine for a Ruthenium(II) Arene Anticancer Complex: Detection of a Sulfenato Intermediate. *J. Am. Chem. Soc.* **2005**, *127*, 17734–17743.

38 Wang, F. Y.; Habtemariam, A.; van der Geer, E. P. L.; Fernandez, R.; Melchart, M.; Deeth, R. J.; Aird, R.; Guichard, S.; Fabbiani, F. P. A.; Lozano-Casal, P.; Oswald, I. D. H.; Jodrell, D. I.; Parsons, S.; Sadler, P. J. Controlling Ligand Substitution Reactions of Organometallic Complexes: Tuning Cancer Cell Cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 18269–18274.

39 Magennis, S. W.; Habtemariam, A.; Novakova, O.; Henry, J. B.; Meier, S.; Parsons, S.; Oswald, I. D. H.; Brabec, V.; Sadler, P. J. Dual Triggering of DNA Binding and Fluorescence via Photoactivation of a Dinuclear Ruthenium(II) Arene Complex. *Inorg. Chem.* **2007**, *46*, 5059–5068.

40 Bugarcic, T.; Novakova, O.; Halamikova, A.; Zerzankova, L.; Vrana, O.; Kasparkova, J.; Habtemariam, A.; Parsons, S.; Sadler, P. J.; Brabec, V. Cytotoxicity, Cellular Uptake, and DNA Interactions of New Monodentate Ruthenium(II) Complexes Containing Terphenyl Arenes. *J. Med. Chem.* **2008**, *51*, 5310–5319.

41 Chen, H.; Parkinson, J. A.; Parsons, S.; Coxall, R. A.; Gould, R. O.; Sadler, P. J. Organometallic Ruthenium(II) Diamine Anticancer Complexes: Arene-Nucleobase Stacking and Stereospecific Hydrogen-Bonding in Guanine Adducts. *J. Am. Chem. Soc.* **2002**, *124*, 3064–3082.

42 Chen, H.; Parkinson, J. A.; Morris, R. E.; Sadler, P. J. Highly Selective Binding of Organometallic Ruthenium Ethylenediamine Complexes to Nucleic Acids: Novel Recognition Mechanisms. *J. Am. Chem. Soc.* **2003**, *125*, 173–186.

43 Peacock, A. F. A.; Habtemariam, A.; Fernandez, R.; Walland, V.; Fabbiani, F. P. A.; Parsons, S.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. Tuning the Reactivity of Osmium(II) and Ruthenium(II) Arene Complexes under Physiological Conditions. *J. Am. Chem. Soc.* **2006**, *128*, 1739–1748.

44 Peacock, A. F. A.; Parsons, S.; Sadler, P. J. Tuning the Hydrolytic Aqueous Chemistry of Osmium Arene Complexes with N,O-Chelating Ligands to Achieve Cancer Cell Cytotoxicity. *J. Am. Chem. Soc.* **2007**, *129*, 3348–3357.

45 van Rijt, S. H.; Mukherjee, A.; Pizarro, A. M.; Sadler, P. J. Cytotoxicity, Hydrophobicity, Uptake, and Distribution of Osmium(II) Anticancer Complexes in Ovarian Cancer Cells. *J. Med. Chem.* **2010**, *53*, 840–849.

46 Kostrhunova, H.; Florian, J.; Novakova, O.; Peacock, A. F. A.; Sadler, P. J.; Brabec, V. DNA Interactions of Monofunctional Organometallic Osmium(II) Antitumor Complexes in Cell-Free Media. *J. Med. Chem.* **2008**, *51*, 3635–3643.

47 Novakova, O.; Kasparkova, J.; Bursova, V.; Hofr, C.; Vojtiskova, M.; Chen, H.; Sadler, P. J.; Brabec, V. Conformation of DNA Modified by Monofunctional Ru(II) Arene Complexes: Recognition by DNA Binding Proteins and Repair. Relationship to Cytotoxicity. *Chem. Biol.* **2005**, *12*, 121–129.

48 Castellano-Castillo, M.; Kostrhunova, H.; Marini, V.; Kasparkova, J.; Sadler, P. J.; Malinge, J. M.; Brabec, V. Binding of Mismatch Repair Protein MutS to Mispaired DNA Adducts of Intercalating Ruthenium(II) Arene Complexes. *J. Biol. Inorg. Chem.* **2008**, *13*, 993–999.

49 Novakova, O.; Malina, J.; Suchankova, T.; Kasparkova, J.; Bugarcic, T.; Sadler, P. J.; Brabec, V. Energetics, Conformation, and Recognition of DNA Duplexes Modified by Monodentate Ru(II) Complexes Containing Terphenyl Arenes. *Chem.—Eur. J.* **2010**, *16*, 5744–5754.

50 Novakova, O.; Chen, H.; Vrana, O.; Rodger, A.; Sadler, P. J.; Brabec, V. DNA Interactions of Monofunctional Organometallic Ruthenium(II) Antitumor Complexes in Cell-free Media. *Biochemistry* **2003**, *42*, 11544–11554.

51 Cohen, S. M.; Lippard, S. J. In *Progress in Nucleic Acid Research and Molecular Bioliogy*; Moldave, K., Ed.; Academic Press: San Diego, CA, 2001; Vol. 67, p 93.

52 Williams, J. P.; Lough, J. A.; Campuzano, I.; Richardson, K.; Sadler, P. J. Use of Ion Mobility Mass Spectrometry and a Collision Cross-Section Algorithm To Study an Organometallic Ruthenium Anticancer Complex and Its Adducts with a DNA Oligonucleotide. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3563–3569.

53 Selvakumaran, M.; Pisarcik, D. A.; Bao, R.; Yeung, A. T.; Hamilton, T. C. Enhanced Cisplatin Cytotoxicity by Disturbing the Nucleotide Excision Repair Pathway in Ovarian Cancer Cell Lines. *Cancer Res.* **2003**, *63*, 1311–1316.

54 Pizarro, A. M.; Sadler, P. J. Unusual DNA Binding Modes for Metal Anticancer Complexes. *Biochimie* **2009**, *91*, 1198–1211.

55 Pizarro, A. M.; Habtemariam, A.; Sadler, P. J. Activation Mechanisms for Organometallic Anticancer Complexes. In *Medicinal Organometallic Chemistry*; Jaouen, G., Metzler-Nolte, N., Eds.; Topics in Organometallic Chemistry 32; Springer-Verlag: Berlin, 2010; pp 21—56, ISBN 978-3-642-13184-4.

56 Haq, I.; Trent, J. O.; Chowdhry, B. Z.; Jenkins, T. C. Intercalative G-Tetraplex Stabilization of Telomeric DNA by a Cationic Porphyrin. *J. Am. Chem. Soc.* **1999**, *121*, 1768–1779.

57 Brodie, C. R.; Collins, J. G.; Aldrich-Wright, J. R. DNA Binding and Biological Activity of Some Platinum(II) Intercalating Compounds Containing Methyl-Substituted 1,10-Phenanthrolines. *Dalton Trans.* **2004**, 1145–1152.

58 Liu, H. K.; Parkinson, J. A.; Bella, J.; Wang, F. Y.; Sadler, P. J. DNA Penetrative DNA Intercalation and G-base Selectivity of an Organometallic Tetrahydroanthracene Ru^{II} Anticancer Complex. *Chem. Sci.* **2010**, *1*, 258–270.

59 Chen, H.; Parkinson, J. A.; Novakova, O.; Bella, J.; Wang, F. Y.; Dawson, A.; Gould, R.; Parsons, S.; Brabec, V.; Sadler, P. J. Induced-Fit Recognition of DNA by Organometallic Complexes with Dynamic Stereogenic Centers. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14623–14628.

60 Danford, A. J.; Wang, D.; Wang, Q.; Tullius, T. D.; Lippard, S. J. Platinum Anticancer Drug Damage Enforces A Particular Rotational Setting of DNA in Nucleosomes. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12311–12316.

61 Lukin, M.; de los Santos, C. NMR Structures of Damaged DNA. *Chem. Rev.* **2006**, *106*, 607–686.

62 Brown, K.; Hingerty, B. E.; Guenther, E. A.; Krishnan, V. V.; Broyde, S.; Turteltaub, K. W.; Cosman, M. Solution Structure of the 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine C8-Deoxyguanosine Adduct in Duplex DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8507–8512.

63 Geacintov, N. E.; Cosman, M.; Hingerty, B. E.; Amin, S.; Broyde, S.; Patel, D. J. NMR Solution Structures of Stereoisomeric Covalent Polycyclic Aromatic Carcinogen-DNA Adducts: Principles, Patterns, and Diversity. *Chem. Res. Toxicol.* **1997**, *10*, 111–146.

64 Brown, K. L.; Voehler, M. W.; Magee, S. M.; Harris, C. M.; Harris, T. M.; Stone, M. P. Structural Perturbations Induced by the Alpha-Anomer of the Aflatoxin B-1 Formamido-pyrimidine Adduct in Duplex and Single-Strand DNA. *J. Am. Chem. Soc.* **2009**, *131*, 16096–16107.

65 Liu, Z.; Habtemariam, A.; Pizarro, A. M.; Fletcher, S. A.; Kisova, A.; Vrana, O.; Salassa, L.; Bruijnincx, P. C. A.; Clarkson, G. J.; Brabec, V.; Sadler, P. J. Organometallic Half-sandwich Iridium Anticancer Complexes. *J. Med.Chem.* **2011**, in press.