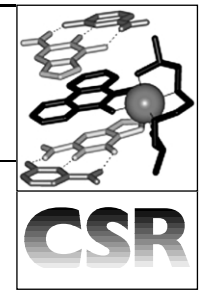
Kinetically inert transition metal complexes that reversibly bind to DNA



Clive Metcalfe[a] and Jim A. Thomas*[b]

[a] *Department of Chemistry, University of Leicester, Leicester, UK*
[b] *Department of Chemistry, University of Sheffield, Sheffield, UK.*
E-mail: james.thomas@sheffield.ac.uk; Fax: +44 (0)114 273 8673; Tel: +44 (0)114 222 9325

Received 20th December 2002
First published as an Advance Article on the web 2nd May 2003

Transition metal complexes that reversibly bind to DNA have been studied for almost 30 years. In the last few years a variety of new systems have been developed, employing a range of metal ions and ligand architectures. In many cases, high affinity binding and specific selectivities have been observed. These complexes display properties that make them attractive as probes of DNA structure and function, suggesting that they may find a rôle as prototypical tools for a spectrum of applications, from basic molecular biology to medicine. This review presents an overview of some of the structures and properties of such complexes.

1 Introduction

The central dogma of molecular biology holds that the genetic information coded within DNA can be replicated, transcribed or processed as RNA, and translated into proteins. All of these processes are initiated, regulated and terminated by molecules that bind to nucleic acids in a site-specific ways. Consequently, synthetic molecules that interact with nucleic acids find a variety of uses as biophysical and therapeutic agents. Furthermore, it is clear that in the post-genomic age such systems will become increasingly important.[1]

To understand binding of molecular substrates to DNA requires some understanding of the structure of the biopolymer. The most common form of DNA is the right-handed antiparallel double helix known as B-DNA—Figure 1. This helix defines a wide major groove and a narrower minor groove.[1a]

Small molecule binding to DNA can be reversible or irreversible, with the latter case usually involving covalent bond formation. Examples of irreversible binding agents include potent carcinogens such as aflatoxin B_1 and benzo[α]pyrene, and antitumour drugs such as cisplatin. Reversible recognition of nucleic acids characteristically involves non-covalent interactions and is usually defined by electrostatic, intercalative or major or minor groove binding motifs.[1b] Within this context, this review centres on one particular area of research; transition metal complexes that function as reversible DNA binding agents.

Transition metals centres are particularly attractive moieties for such research for not only do they exhibit well-defined coordination geometries but they also often possess distinctive electrochemical or photophysical properties, thus enhancing the functionality of the binding agent.[2] In particular, kinetically inert complexes based on d^8 square planar and d^6 octahedral metal ions possess well-developed substitution chemistry—for this reason, they are commonly employed as the central scaffold for the construction of metal-based DNA binding agents. Complexes synthesised by these methodologies have found a plethora of applications ranging from foot-printing agents to probes of electron transfer processes within DNA. It is impossible to present a single exhaustive review of such a diverse subject. Consequently, what follows is an overview of the typical structures used in this research, highlighting particularly noteworthy results. Furthermore, in many cases, recognition of DNA has been accomplished by attaching a simple metal complex to a known, well-characterised binding agent. This review will only be concerned with systems in

Clive Metcalfe graduated from the University of Sheffield with a masters degree in chemistry in 1998. He stayed on at Sheffield to study for a PhD under the supervision of Dr Jim Thomas where he studied novel metallo-intercalators for DNA, and obtained his doctorate in 2002. He then took up a post-doctoral position with Dr Emma Raven at the University of Leicester, where he is currently carrying out research into the covalent modification of the active sites of peroxidases.

Jim Thomas obtained his BSc from the University of Reading and a PhD (1993) at the University of Birmingham under the supervision of Dr Chris Jones and Professor Jon McCleverty. Following a Royal Society European Exchange Fellowship in Strasbourg with Professor J.-M. Lehn, he first arrived in Sheffield to work with Professor Chris Hunter. In 1995 he was awarded a Royal Society University Research Fellowship. His research involves the use of coordination chemistry to mediate electron transfer, self-assembly and recognition processes.

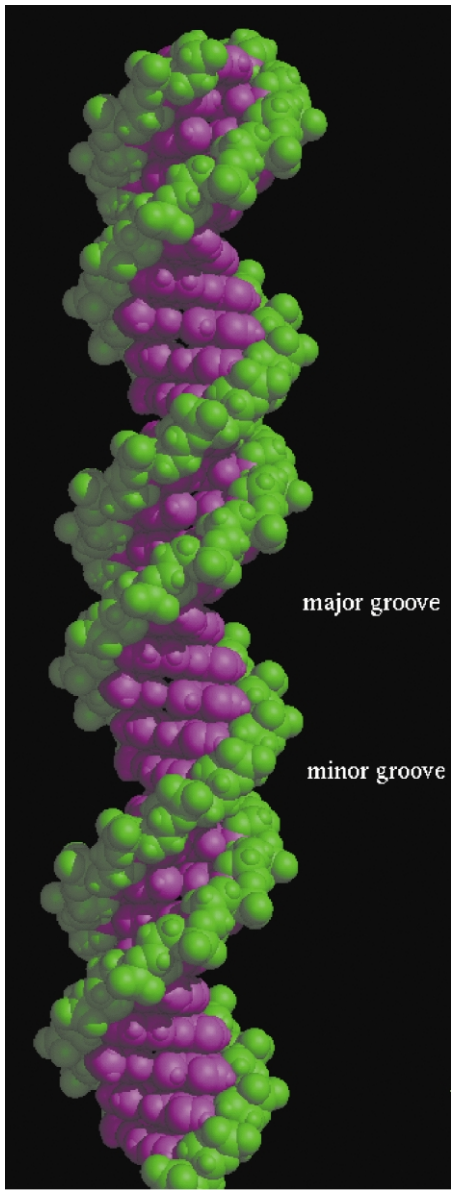


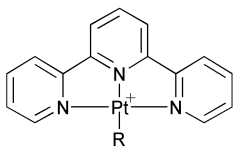
Fig. 1 Structure of B-DNA.

which DNA binding properties arise due to structural features inherent within the complex itself.

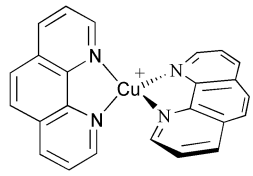
2 Monometallic complexes

2.1 Early work

Almost thirty years ago, Lippard and co-workers first established that monocationic square planar Pt^{II} complexes containing the 2,2′:6′,2″ terpyridyl (tpy) ligand, such as **1**, could bind to duplex DNA through intercalation. Later work revealed that the binding constant, K_b, for this interaction is around 10^5 M^{-1}.[3]

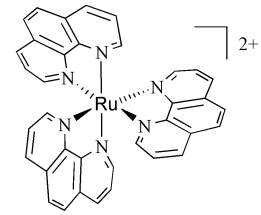


1 (R = $S(CH_2)_2OH$)

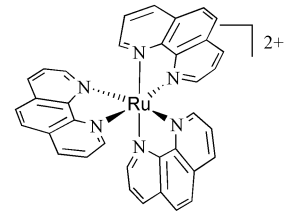


2

At around the same time, work from the Sigman group demonstrated that $[Cu(phen)_2]^+$, **2**, phen = 1,10-phenanthroline, binds reversibly in the minor groove and functioned as a synthetic DNA nuclease.[4] By the early 1980s, investigations into the interaction of octahedral metal centres with DNA were underway. While initial studies involved several phen complexes, this work swiftly focused on the $[Ru(phen)_3]^{2+}$ cation **3**.



Λ-**3**



Δ-**3**

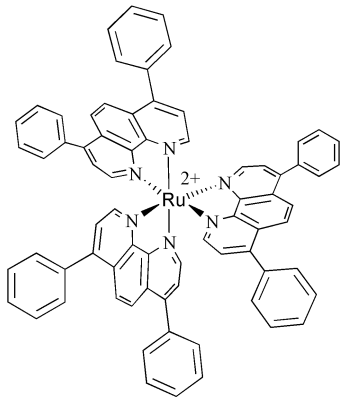
2.2 Binding studies on 3

Through NMR and photophysical studies Barton and colleagues suggested that **3** interacted with B-form DNA *via* hydrophobic contacts in the major groove, and that in one of two binding modes involved in this interaction a phenanthroline ligand intercalated into the DNA base stack. These early studies also suggested that **3** preferentially bound to GC rich sequences and that there was a small but significant preference for binding by the right-handed Δ isomer of **3**.[5] However, the binding modes and exact orientations of this relatively simple complex became open to much debate with later work suggesting that **3** did not intercalate, but bound to DNA through electrostatic and groove binding interactions within the minor groove.

The issue of intercalation was disproved once-and-for-all when viscosity data clearly indicated that **3** bound to DNA *via* a non-intercalative mode.[6] More recently, a detailed spectroscopic and modelling account by Rodger and co-workers showed that there are three possible binding mode for **3**, dependent upon the degree of saturation of the DNA by the drug complex. At all mixing ratios, Λ-**3** binds into the major groove with a single phen ligand approximately parallel to the base pair planes. However, binding of Δ-**3** is more complex. At low binding ratios, Δ-**3** binds with two phen ligands inserted into the minor groove. While at higher complex loads, binding *via* insertion of a single phen ligand into both minor and major grooves becomes more favourable.[7]

2.3 Complexes of dpphen

It was reasoned that increasing ligand surface area in complexes related to **3** would lead to a corresponding enhancement in binding affinities, thus complexes containing 4,7-diphenyl-1,10-phenanthroline, dpphen, were investigated. Luminescence quenching experiments on Λ-$[Ru(dpphen)_3]^{2+}$, Λ-**4**, indicated binding to B-form DNA exclusively through an electrostatic interaction, whereas Δ-**4** interacts with B-form DNA by intercalation of one of the dpphen ligands and subsequent threading of a pendant phenyl from the major groove to the minor groove.[8] Later circular dichroism, CD, and linear dichroism, LD, studies indicated that both enantiomers interact with DNA in the same, non-intercalative, mode.[9] One possible reason for these apparently contradictory results is the poor solubility and general hydrophobic nature of **4**. Initial studies, performed on chloride and perchlorate salts, required 1:9 DMSO/aqueous buffers solution mixtures. Whereas, the later studies on the more water-soluble acetate salt, were carried out in purely aqueous buffers. It has been suggested that the presence of DMSO could possibly alter the conformational properties of B-form DNA and falsely induce chiral discrimination.[9]

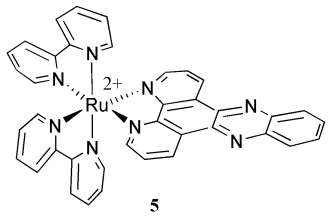


4

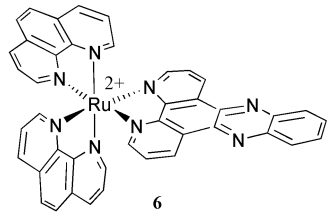
2.4 Complexes of the dppz ligand

2.4.1 $[Ru(bpy)_2(dppz)]^{2+}$. In 1990, it was reported that $[Ru(bpy)_2(dppz)]^{2+}$, **5**, (bpy = 2,2′-bipyridine, dppz = dipyrido[3,2-a:2′,3′-c]phenazine), displayed striking photophysical properties.

Electrochemical and photophysical measurements in both the ground and excited states showed that the charge transfer is directed from the metal centre to the phenazine of the dppz ligand, and that the major non-radiative deactivation pathway of this excited state was protonation of the phenazine nitrogen atoms. Thus, the excited state of **5** is extremely dependent upon its microenvironment. So, while **5** shows strong metal-to-ligand



5



6

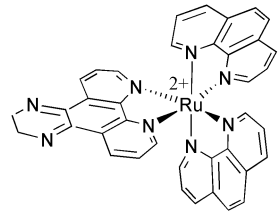
charge-transfer (MLCT) centred luminescence in hydrophobic solvents such as acetonitrile or dichloromethane, in aqueous or protic solvents the MLCT state is completely quenched. These properties lead to what has been termed the "light switch" effect: addition of DNA into aqueous solutions of **5** results in intense luminescence, indicating that the ring nitrogens of the dppz ligands are being shielded from the solvent *via* intercalation into the DNA base pair stack.[10] Consequent research on **5** and **6** has incontrovertibly established that they are high affinity intercalators with $K_b = 10^6$–10^7 M^{-1} and, unlike many classical intercalators, this recognition process is entropically driven. Furthermore, although Λ-**6** and Δ-**6** display no significant difference in binding affinity,[11] intercalative geometries, luminescent quantum yields and lifetime do show significant differences. However, despite the large number of reported studies, a more detailed model for the DNA binding mechanism of these metallo-intercalators is yet to be agreed.

Photophysical studies show that, when bound to DNA, the luminescence lifetime of both Δ-**6** and Λ-**6** display bi-exponential decay, indicating the presence of two emitting species, one with a considerably longer lifetime than the other.[11] One interpretation of this data is that **6** possesses two binding modes. In one mode, the dppz ligand is intercalated in a "side on" manner, leaving one of the ring nitrogens still accessible for excited state quenching by water. In the perpendicular mode both of the phenazine ring nitrogens are fully intercalated into the base pair stack, rendering them inaccessible by water, resulting in a longer lived excited state.[12] An alternative proposal is that the bi-exponential decay is due to a loading effect, with relatively isolated complexes bound to the DNA lattice being more accessible to solvent than closely packed ligands at the saturation point.

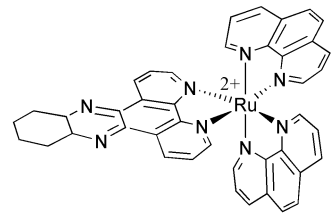
In 1997, building on initial LD, studies, Tuite and colleagues extended this latter theory by suggesting that intercalation takes place from the minor groove. They reported that binding of both Δ-**6** and Λ-**6** to T-4 DNA is not hindered in any way. T-4 DNA is 100% glycosylated at the cytosine 5-CH_2-OH position in the major groove, providing a significant steric obstacle to any binding into this groove. Additionally, there is no significant difference between the lifetimes observed with T-4 DNA and calf thymus DNA (CT-DNA). From this latter result, it was inferred that preferential binding to the AT regions of T-4 DNA, which are free from glycosylation, could be ruled out. It was concluded that if the metal complex is confined in the minor groove, then there would be no room for intercalation of **6** *via* two distinct orientations.[13]

A later luminescence study by Holmlin, *et al.*, investigated the interaction of Δ-**6** with a 1:1 mixture of poly d(AT) and poly d(GC). This indicated that 85% of complexes bound to poly d(AT). From this data, it was concluded that **6** *is* preferentially bound to AT regions in *both* T-4 DNA and CT-DNA.[14] Furthermore, this same report outlines results obtained from competition binding experiments with known major and minor groove binding agents, that provided support for major groove binding by **6**.

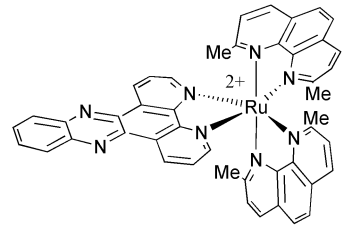
However, a series of 1-D and 2-D ^{1}H NMR studies involving the binding of the hexanucleotide $d(GTCGAC)_2$ by structures closely related to **6**, such as **7–9**, produced contrasting results. In



7



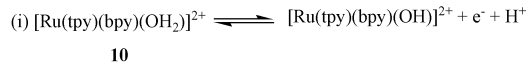
8



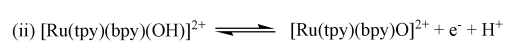
9

all cases, strong NOEs were observed between protons on auxiliary ligands of the complexes and sugar protons in the minor groove. For the extended ligands, protons close to the Ru^{II} metal centre showed NOEs to minor groove protons, while those ligand protons that are further away from the metal showed NOEs to protons in both grooves, or only the major groove. All these observations are consistent with intercalative binding involving the extended ligand and further indicate that, even for complexes such as **9**, which contain more sterically demanding 2, 9-dimethylated phen ancillary ligands, binding takes place from the minor groove side.[15]

2.4.2 [Ru(tpy)(dppz)O]$^{2+}$. Thorp and colleagues have investigated the use of Ru^{IV} complexes as DNA cleavage agents. Originally this work was carried out using the readily available **10**, which is used to electrochemically generate



10

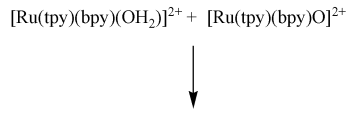


11

Scheme 1

11—Scheme 1.

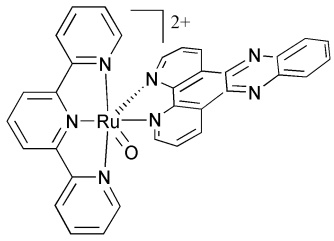
Complex **11** reacts *via* hydride transfer followed by protonation, thus regenerating **10** which in turn, reacts with **11** in a comproportionation reaction to produce the Ru^{III} species, **12**—Scheme 2. Both **11** and **12** are capable of oxidising DNA by oxidation of guanine and also at the 1′ position of sugar residues.



12

Scheme 2

However, the binding affinity of **10** is low ($K_b < 10^3\ M^{-1}$) and essentially electrostatic in nature. In an attempt to enhance this affinity, and hence the efficiency of the cleavage reaction, the system was modified by the addition of dppz. A variety of techniques confirm that K_b for the resultant complex, [Ru(tpy)(dppz)(OH$_2$)]$^{2+}$ **13**, is over three orders of magnitude larger, and that it can be used to electrochemically generates **14**. It has been found that, like **5** and **6**, **13** seems to show a binding preference for AT rich regions.[16]

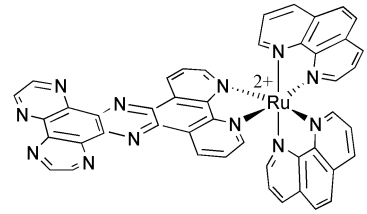


14

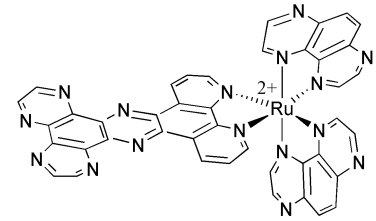
Kinetic studies on **11** and **14** reveals that oxidation of DNA occurs in two phases. The first phase is rapid and involves oxidation by bound $[Ru^{IV}O]^{2+}$ species. The second phase of oxidation takes place over a much longer time period as, following the initial oxidation, the rate-determining step becomes the dissociation of $[Ru^{II}OH_2]^{2+}$ species. This means that, for the first phase, the amount of metal complex bound to DNA controls the efficiency of the oxidation and, therefore, the complex with the higher affinity, **14**, is the most efficient cleavage agent. However, in the second phase, the more strongly bound **14** is actually a *less* efficient oxidant than the low affinity **11**.[17]

Using high-resolution electrophoresis the binding specificity of **14** has been investigated. Oxidative cleavage of; duplex DNA, HIV-1 TAR DNA and RNA, and $tRNA^{Phe}$ was carried out using **11** and **14**. Cleavage sites common to both complexes were assigned to non-intercalative binding properties, whereas cleavage sites uniquely observed for **14** were strongly implicated as sites of intercalative recognition. Additionally, to distinguish between high-affinity sites and sites made more accessible *via* intercalation of **14** at another remote site, cleavage patterns generated by **11** in the presence and absence of **1** were compared. The results confirm that intercalative recognition by the dppz ligand is very similar to classical intercalators with duplex binding enhanced over single stranded DNA and specific features such as bulge-loops being targeted. Furthermore, oxidative cleavage by **14** results in sugar lesions that are consistent with the dppz ligand being bound to the minor groove. However, since only 10% of the oxidant is consumed by sugar oxidation, these results do not exclude the possibility that the major fraction of **14** is bound in the major groove.[18]

2.4.3 Other Ru^{II} complexes with dppz-type ligands. A large number of complex based on **5** and **6**, but incorporating a structural variant of the dppz ligand have been synthesised. Many, but not all, display the light switch effect. Of particular note are complexes based on 1,10-phenanthroline[5,6-b]-1,4,5,8,9,12-hexaazatriphenylene (phehat), **15** and **16**.



15



16

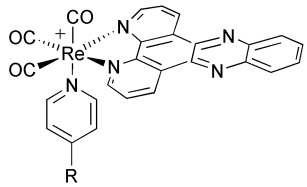
Using ligands such as 1,4,5,8,9,12-hexaazatriphenylene (HAT), Kirsch-De Mesmaeker and co-workers have developed Ru^{II} complexes with strongly oxidizing MLCT states, capable of photocleaving or forming photoadducts with nucleic acids The primary process that initiates DNA cleavage corresponds to a photo-induced electron transfer, generally from a guanine base to the excited state complex.[19] However, the relatively weak binding affinities of these complexes limit their use as very efficient DNA photoreagents. The hybrid phehat ligand incorporates the redox properties of a HAT moiety with the intercalative properties of dppz. Binding studies on **15** reveals that it displays the light switch effect of the dppz complexes and binding affinities are comparable to those of **5** and **6**. However, photo-initiated electron transfer processes from guanosine-5′-monophosphate, GMP, show low efficiency and are not observed with DNA. Contrastingly, the luminescence of more oxidizing, **16** is quenched by GMP and on binding to DNA—strongly suggesting that, in this case, photoinduced electron transfer is efficient.[20]

2.5 Complexes of dppz with metals other than ruthenium

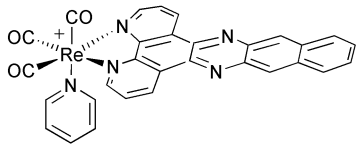
Several complexes, which are isostructural with **5** and **6**, but incorporating metals such as Os^{II}, Co^{III}, and Ni^{II} have been reported. While all the complexes show binding characteristics that are comparable to **5** and **6**, only the Os^{II} system displays a

light bulb effect, although in this latter case emission is appreciably red-shifted.[21]

2.5.1 Re^{I} complexes. In 1995, both the Schanze and Yam groups reported intercalative Re^{I} complexes of dppz, **17** and **18**.[22] Emission and transient absorption studies on **17b** in organic solvents indicate that the lowest lying excited state of the complex is not the expected MLCT, but a weakly phosphorescent dppz-based intraligand (IL) triplet state. However, while both **17a** and **17b** show a DNA light switch effect,



17a R = H, **17b** R = CH_3



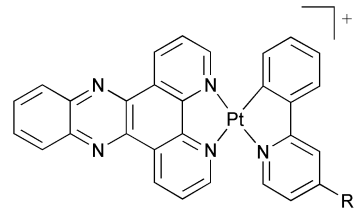
18

luminescent enhancements for **18** are much lower, with a significant drop in intensity at low [DNA]:[**18**] ratios. Subsequent work revealed that **17a** and **18** had similar binding affinities with CT-DNA—$K_b = 4 \times 10^4$–5×10^4 M^{-1}. These figures are around two orders of magnitude lower than that obtained for dicationic octahedral complexes of dppz, such as **5** and **6**, indicating that electrostatic contributions are important in DNA–metallo-intercalators interactions. The binding behaviour of **17a** and **18** with synthetic oligonucleotides was strikingly different.

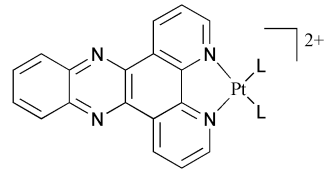
While the interaction of **17a** with poly(dA).poly(dT) results in a 13-fold enhancement in luminescence, no enhancement is observed in the interaction of **17a** with poly(dG).poly(dC). It was concluded that **17a**, like **6**, has a preference for AT sites. Surprisingly, it was found that the interaction of **18** with poly(dA).poly(dT) results in a considerable enhancement in luminescence intensity, while for the analogous experiment with poly(dG).poly(dC) luminescence is quenched. It was concluded that the combined effects of luminescent quenching at GC sites and emission enhancement at AT sites explains the data obtained for the interaction of **18** with CT-DNA[22c]

2.5.2 Pt^{II} complexes. Recently, Che and colleagues have investigated the properties of monocationic and dicationic Pt^{II} complexes of dppz.[23] Although acetonitrile solutions of **19** and **20** display room temperature luminescence, the excited state responsible for emission is not the same. **19** displays a relatively high-energy—477 nm—structured luminescence that is consistent with emission from an IL of the cyclometalated phenyl pyridine. Luminescence from concentrated, frozen acetonitrile solutions is observed at 675 nm and this has been assigned to excimer emission due to coupling between the ground and excited state of [pt(dppz)] moieties. **20** displays a low energy, less structured emission at 558 nm, that was assigned to a [5d(Pt)→π*(dppz)] ^{3}MLCT, with no solid-state emission being observed.

Binding to DNA was studied using absorption and emission titrations. Absorption titrations on **19** and **20** reveal that, despite differences in charge, $K_b \approx 1 \times 10^4$ M^{-1} for both complexes. Emission studies were more complex. Aqueous solutions of **19** and **20** are non-emissive. However, while **20** shows no



19 R = 4-*tert*-butyl



20a L = 1-methylimidazole
20b L = 4-aminopyridine

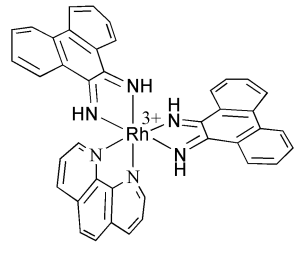
luminescence in the presence of CT-DNA, the analogous experiment with **19** results in intense photoluminescence at 650 nm. Since only one **19** ion can intercalate between two base pairs it seems unlikely that this emission is from the excimer state. Consequently, the emission was ascribed to a exciplex between **19** and the DNA base pairs. It was also found that **19** is up to 40 times more cytotoxic towards certain human epithermal carcinoma cell lines than cisplatin. Despite **19** being a intercalator the possibility that the cytotoxicity is due to inhibition of cellular functions other than DNA replication was not be ruled out.

2.6 Complexes of phi and related ligand

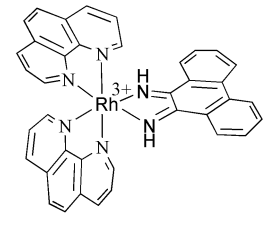
2.6.1 Rhodium complexes of phi. In contrast to the dppz ligand, complexes of 9,10-phenanthrenequinone, phi, intercalate with the long axis of the phi ligand parallel to the long axis of the base pair.

The phi ligand projects far from the metal centre due to the use of imines as the coordinating chelator. The first report on DNA binding by complexes of phi concerned Ru^{II} systems, but subsequent work has largely concerned Rh^{III} complexes.

Barton and colleagues reported that the complexes **21** and **22**. bind to DNA with $K_b \geq 10^7$ $M^{-1}$and, on photo-excitation at 310–356 nm, both compounds efficiently cleave duplex DNA. However, while **21** cleaves DNA in a sequence-neutral fashion, **22** cleaves selectively at 5′-pyr-pyr-pur-3′ steps (particular at 5′-C*C*AG-3′), and homopyrimidine sites, whereas cleavage is particularly suppressed at 5′-pur-pur-pyr-3′ sites.



21

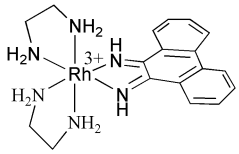


22

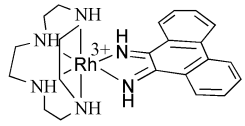
These observations were interpreted as being a consequence of shape selectivity in the binding process. In B-DNA, propeller twisting of 5′-pyr-pyr-pur-3′ steps leads to steric clashes that result in an opening of the major groove and placing of pyrimidines in less sterically demanding positions. It was reasoned that **22** binds *via* the major groove and, due to possible steric clashes between pyrimidines and the H2 and H9 hydrogens of coordinated phen ligands, the complexes shows a

binding preference for these more opened major groove sites. In contrast, the major groove sites at 5′-pur-pur-pyr-3′ sites are not only narrower, but the pyrimidines are unfavourably aligned relative to the ancillary phen ligands of **22**.[24] Later work showed that more structurally complex analogues of **22** displayed enhancements in sequence selectivity and that these effects could be explained by a consideration of steric and van der Waals interactions between ancillary ligands and residues in the major groove. Furthermore, because of this preference for relatively open helical sites, it was found that, while **22** does not bind to the narrower groove of double helical RNA or single stranded regions of RNA, the complex targets sites of tertiary interaction where the groove is more open. This fact has been used to probe the nature of specific peptide–RNA interactions involved in the replication of immuno-deficiency viruses.[25]

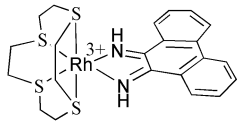
The influence of ancillary ligands with hydrogen bonding sites on DNA binding specificities of the [Rh^{III}(phi)] unit was also investigated. It was found that complexes that incorporate hydrogen donor groups, for example **23** and **24**, showed specific binding preference for 5′-GC-3′ sites, whereas the structurally analogous **25**, containing hydrogen bond acceptor sites shows



23



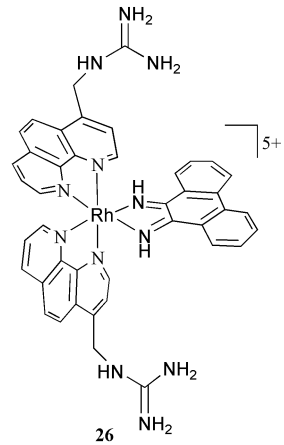
24



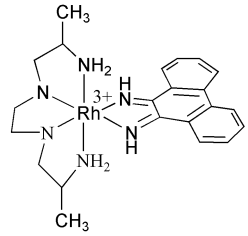
25

no such preference but targets sites such as 5′-ACT-3′. It was concluded that systems containing axial ammine auxiliary ligands are capable of interacting with guanine O6 atoms in the major groove, thus resulting in the observed specificities for complexes such as **23** and **24**, while the binding preferences of **25** was attributed to a shape selection process. Consequent studies involving the binding of Δ-**23** and Λ-**23** to specific sequences revealed that only the former showed a preference for GC sites and modelling and detailed NMR experiments offered more evidence for intercalative binding via the major groove.[26]

The use of non-covalent binding motifs in such systems was extended by employing 4-(guanidylmethyl)-1,10-phenanthroline, MGP, as an auxiliary ligand. This ligand contains a guanadinium group—a moiety known to hydrogen bond with G N7 and O6 atoms. It was postulated that systems such as **26**



26



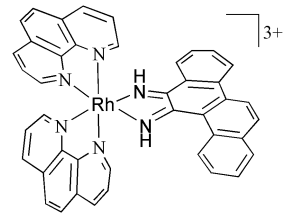
27

would target more extended sequences, with flanking G sites. Surprisingly, it was found that Δ-**26** showed a significant preference for a 6 base pair sequence; 5′-CATCTG-3′, while Λ-**26** recognised 5′-CATATG-3′. Although these sequences both have centrosymmetric 3′-guanine bases, they appeared to be too large to be spanned by a single **26** cation. NMR studies indicated that the target site for Λ-**26** is substantially unwound, thus facilitating symmetrical binding of this relatively large sequence. The situation with Δ-**26** is considerably more complex with evidence pointing to two binding conformations at the same site.[27] Consequent studies have shown that Λ-**26** binds with affinities that are comparable to transcription factors and can even selectively inhibit transcription factor binding when the target contains the 5′-CATATG-3′ sequence.[28]

The hypothesis that [Rh^{III}(phi)] complexes bind into the major groove, was recently confirmed by an X-ray crystallography study. Using information obtained from the previous studies outlined above, the Δ-α-isomer of **27** was designed to bind preferentially to the sequence 5′-TG|CA-3′ (where | indicates phi insertion). Co-crystallisation of Δ-α-**27** with an eight base-pair oligonucleotide, 5′-G-dIU-TGCAAC-3′ (dIU, 5-iodo-deoxy-uridine) resulted in a 1.2 Å resolution structure—Figure 2. At this resolution it was possible to delineate water molecules mediating interactions between the intercalator and bases. At the intercalation site, an ordered water molecule binds to guanine *via* a hydrogen bond donation to the guanine-N7, while simultaneously functioning as a hydrogen bond acceptor to axial amines of the metal complex.[29]

Very recently, the [Rh^{III}(phi)] unit has been used as an intercalative platform for the design of artificial nucleases, where a metal-binding peptide has been covalently attached to an ancillary ligand. When the peptide is coordinated to Zn^{II}, the complex hydrolytically cleaves DNA, while related systems, with coordinated Cu^{II}, cleave oxidatively.[30]

2.6.2 Rh^{III} complexes of chrysi. The complex **28**, containing the 5,6-chrysinequinone diimine (chrysi) ligand, was designed



28

to be too large to easily intercalate into standard base-pair steps, so that base mismatch sites would be targeted for recognition. Initial experiments showed that this complex had well over an order of magnitude binding preference for CC mismatches relative to B-form DNA and photo-cleavage occurs at the 3′ side of the mismatch.

Subsequent work revealed that, while **28** can recognize and cleave a single mismatch within a 2725 base pair sequence of plasmid DNA, affinities for mismatches vary, with helix destabilization being a factor in determining the binding affinity of the metal complex for specific mismatched sites.[31]

2.6.3 A square planar metal complex of phi. Although crystallographic data demonstrated that **1** intercalates into a dinucleotide *via* the major groove,[32] it is unclear whether square planar metallo-intercalators bind to longer sequences in such a manner. In an attempt to explore this issue further, the interaction of [Pt(en)(phi)]$^{2+}$, (en = ethylenediamine) **29**, with the hexanucleotide d(GTCGAC)$_2$ was explored. NMR studies indicate that **29** intercalates from the minor groove at the GA/TC site with the leading edge of the phi rings extending into the major groove. It was suggested that these two apparently contradictory results imply that the formation of structured grooves is a factor in the determination of groove access.[33]

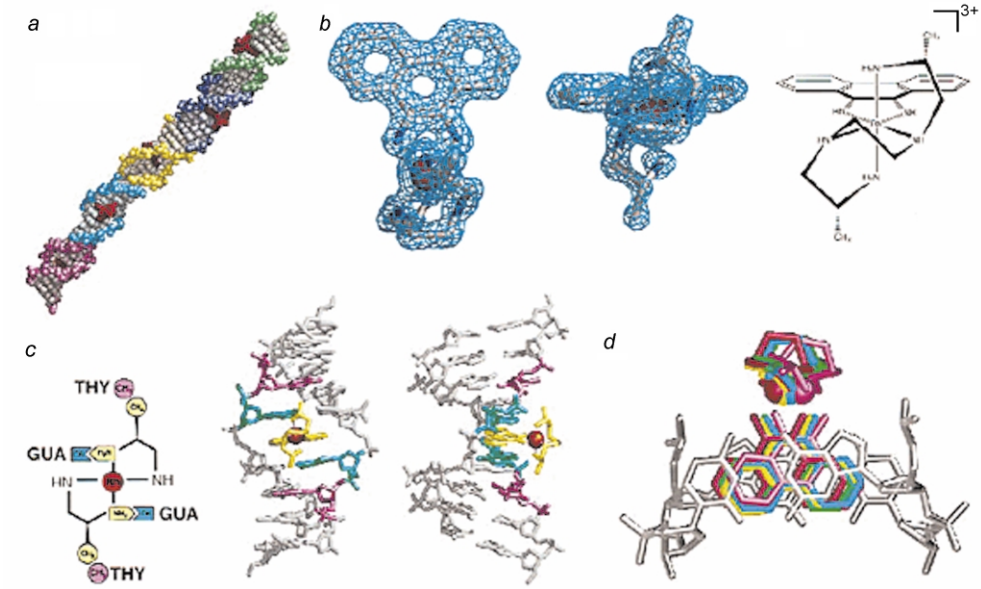
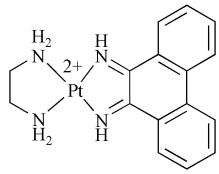


Fig. 2 Overall structure of the **27** bound to the DNA oligonucleotide 5′-G(dIU)TGCAAC-3′.*a*, The five complexes in the asymmetric unit of the crystal. *b*, Representative omit |Fo| − |Fc| electron density map for one of the intercalators, contoured at 4.0 , viewed from two perpendicular orientations with the chemical structure indicated on the right. *c*, Two views of one of the Rh–DNA complexes, one into the major groove, the other rotated about the helix axis. Thymine is purple and guanine is green. A schematic DNA binding model of the predicted contacts is shown to the left. *d*, View down the helix axis of the intercalation sites. The five independent complexes were superimposed using only the DNA atoms. The intercalator of each complex is shown, in different colors, relative to one of the binding sites. Reprinted with permission from C. L. Kielkopf, *et al.*, *Nat. Struct. Biol.*, 2000, **7**,117.



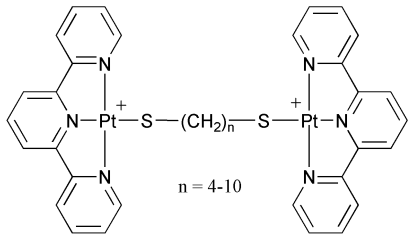
29

3 Oligometallic complexes

It is well established that polyfunctional intercalating agents can enhance the affinity and selectivity of DNA binding. Indeed, several naturally occurring antibiotics are bis-intercalating.[34] In this context, the design of oligo-metallic systems designed to interact with DNA has been investigated.

3.1 Square planar complexes

In the mid-1980s McFadyen and co-workers reported the synthesis of a series of binuclear [Pt^{II}(tpy)] complexes, **30**,

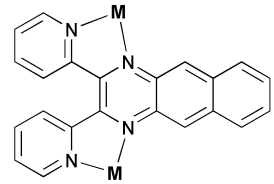


30

based on the same binding motif as **1**. Depending on the length of the linker, a variety of binding modes were displayed, including mono- and bis-intercalation. Although binding affinities were enhanced these effects were modest and there was a concomitant loss in the sequence specificity.[35]

3.2 Bimetallic Ru^{II} systems

In 1993 Carson, *et al.*, reported bimetallic complexes, **31** and **32**, containing a single potentially intercalative site that is held at 90° to the intermetallic axis.

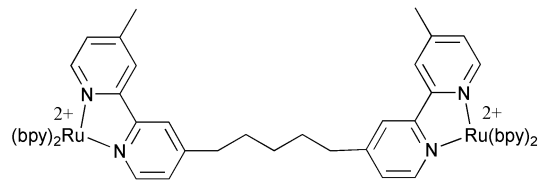


31, **M** = $[Ru(NH_3)_4]^{2+}$

32, **M** = $[Ru(bpy)_2]^{2+}$

It was found that **31** binds to CT-DNA with an affinity that is comparable to **6**, although the binding site size is appreciably larger. Contrastingly, **32** binds to DNA weakly, if at all. These results were explained by considering the steric demand of the auxiliary ligands. While the ammonia ligands are sufficiently small to fit into the groove during intercalation of the bridging ligand, the bpy spectator ligands are large enough to prevent significant interaction of the benzo[*g*]-quinoxaline moiety.[36]

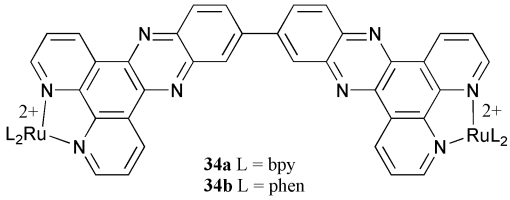
In 1995, Kelly and co-workers described the synthesis of linked $[Ru(bpy)_3]^{2+}$ units, such as **33**. Whereas the monomer only binds to DNA weakly at low ionic strengths, **33** shows a



33

two orders of magnitude enhancement in binding and is less sensitive to ionic strength. Later work revealed that, as would be expected, the interaction is electrostatically dominated and that there are several binding modes.[37]

Lincoln and Nordén reported a novel dimeric system, based on covalently linked dppz units, **34**.[38] Both ΔΔ and ΛΛ homochiral forms were isolated. Optical spectroscopy and competitive binding studies indicated that, while this system binds to DNA avidly ($K_b \approx 10^{12}$ M^{-1}), the interaction is non-intercalative, and that the binding geometries of the ΔΔ and ΛΛ isomers of **34a** are similar. However, the ΔΔ and ΛΛ isomers of **34b** show strikingly different behaviour. These data



34a L = bpy
34b L = phen

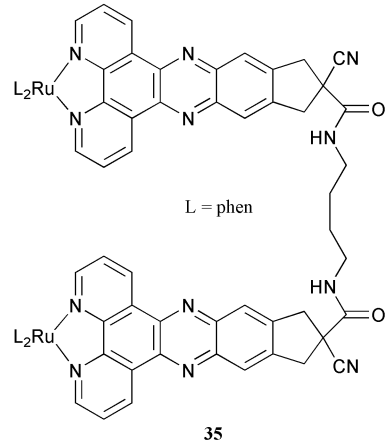
were interpreted as indicating that the bi(dppz) ligand binds to DNA with its concave side facing the duplex, and that, for **34a** and ΛΛ-**34b**, one $[RuL_2]^{2+}$ moiety is placed in each groove. In contrast, the data for the ΔΔ-**34b** suggests that the metal units may be in the same groove.[38a] Very recent work on this system has yielded surprising results.

Following a serendipitous observation on a sample of ΔΔ-**34b** and CT-DNA left at room temperature for two weeks, it was found that a major change in binding geometry had occurred. Even at elevated temperatures and high salt concentrations equilibration to this second binding mode takes almost one day. Furthermore, although the original study on this system had reported that **34** was non-luminescent, even in the presence of DNA, the alternative binding mode for ΔΔ-**34b** results in luminescent enhancements that are comparable with those observed for **5** and **6**. It was proposed that the complex switches from groove-binding to intercalation, reached by threading one of the $[Ru(phen)_2]^{2+}$ moieties through the DNA duplex, thereby intercalating one of the bridging dppz ligands between the DNA base pairs and leaving one metal centre in each groove.[38b]

The phenomenon of DNA threading is well established in other systems such as naturally occurring antibiotics and a related bimetallic complex, also reported by Nordén and co-workers, displays similar behaviour. Using units related to dppz tethered together with a longer, more flexible, linker the three stereoisomers of **35** were synthesised.

Luminescence, LD, and other studies are consistent with intercalation of both planar ligands. Due to the connectivities within this system, this mode of intercalation infers that the complex must thread into DNA by opening up base pairs. This results in the intercalating ligands being separated by two base pairs, and the $Ru(phen)_2]^{2+}$ units being held in the opposite groove—Figure 3.

Estimates of the DNA binding constant for **35**, are almost four orders of magnitude higher than **6**. Although this figure is the same for all three stereoisomers, the kinetics of the binding process differ. ΛΛ-**35** dissociates about an order of magnitude faster than the ΔΔ form and also displays a larger salt dependence for dissociation.[39] It was suggested that this is due



L = phen

35

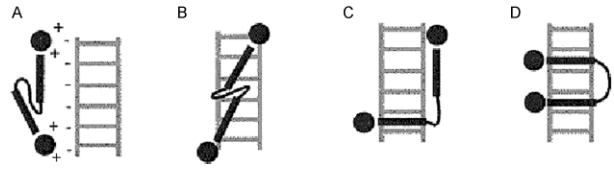
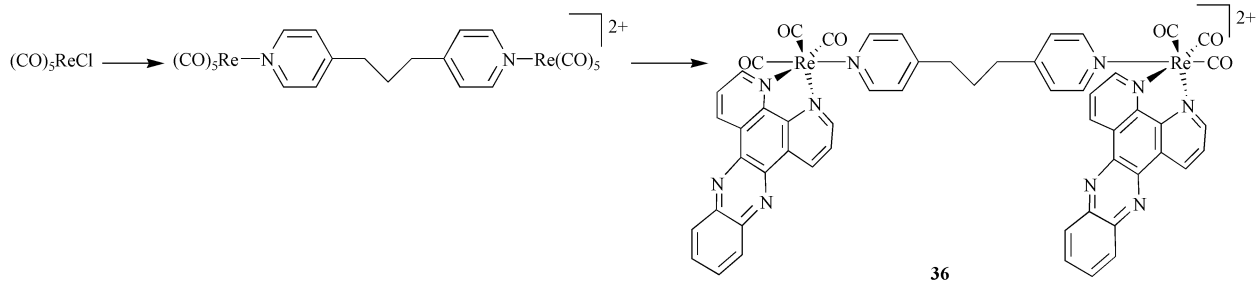


Fig. 3 Schematic DNA-interaction modes of **35.**(A)*Electrostatic external binding.*(B) *Groove binding.*The subunits positioned in either minor or major groove of DNA. (C) *Monointercalation.*One subunit intercalated the other one either in a groove or freely dangling (D) *Bis-intercalation.*Both subunits intercalated, either in the minor or the major groove, the bridging chain residing in the opposite groove. Reprinted with permission from B, Önfelt, *et al*., *J. Am. Chem. Soc*., 2001, **123**, 3630–3637.

to a smaller conformational change in DNA during the unthreading of ΛΛ-**35** compared to the ΔΔ form. Further observations also indicated that intercalation of all three forms of **35** is faster with $[poly(dA\text{-}dT)]_2$ than with $[poly(dG\text{-}dC)]_2$. This is consistent with a threading mechanism, as GC base pairs are more stable than AT steps.

3.3 Bimetallic Re^{I} systems

In an attempt to develop a more facile route to bis-dppz systems, Metcalfe, *et al*. investigated derivatives of **17**. It was found that bimetallic structures could be formed by linking $[fac\text{-}(CO)_3Re(dppz)]^+$ units with commercially available tether ligands, thus creating achiral structures such as **36**—Scheme 3.[40] The DNA binding characteristics of **36** were compared with monomers such as **17** using absorption titrations. While the interaction of the monomers with CT-DNA produce classical saturation binding curves and binding parameters comparable with those reported by Yam and colleagues, the titration curve for **36** was more complex, appearing to approach saturation at [DNA-]:[complex] ratios of around 10:1. Binding parameters for this initial event closely resemble those of the monometallic systems. However, on the addition of more DNA further hypochromicity in absorption bands is observed, resulting in a



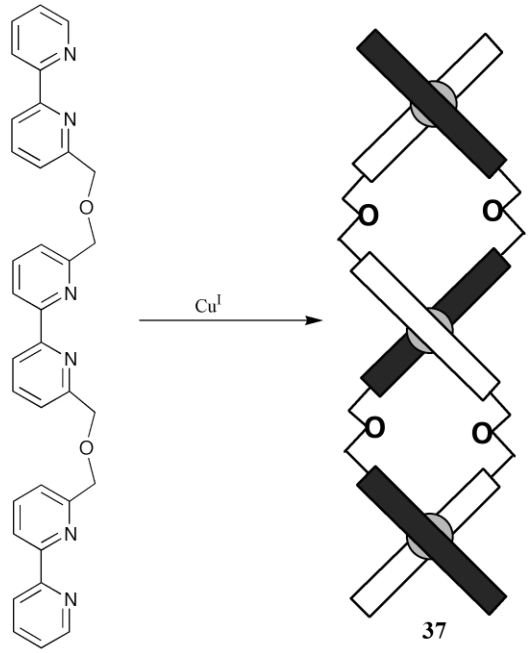
36

Scheme 3 Synthesis of achiral bimetallic Re^{I} complexes containing two dppz ligands.

shallower binding curve which does not reach saturation, even at higher [DNA]:[complex] ratios. Interestingly, apparent saturation in the first binding event for **36** occurs when the percentage hypochromicity is half that observed for the monomeric systems. These observations indicate that the propane tether is insufficiently long for both rhenium centres to intercalate into the same duplex and that the second event is due to a cross-linking interaction of the second rhenium centre with another DNA duplex.

3.4 Helicate complexes and DNA

3.4.1 double helicates. In 1995, Schoentjes and Lehn reported on the DNA binding properties of a series of previously synthesised double-helical polynuclear copper (I) complexes such as **37**—Scheme 4.



Scheme 4 Example of double helicates studied by Schoentjes and Lehn. Ligands shown as black and white strands; shaded circle represent Cu^{I}.

It was reasoned that the size of complexes such as **37** (17 Å by 6 Å) and their hydrophobic surfaces would make them compatible with major groove of B-DNA.[41] Absorption and emission titrations indicated that the helicates did interact with DNA *via* an external binding mode. Physical studies such as DNA-melting experiments demonstrated that binding affinities increase as a function of the helicate length and that GC sequences are apparently preferentially bound. It was also found that the helicates were capable of inhibiting the cleavage of DNA by restriction enzymes and that they were in themselves DNA photocleavage agents. It was concluded that these helicates were major groove binders, although the possibility of multiple binding sites was not ruled out.

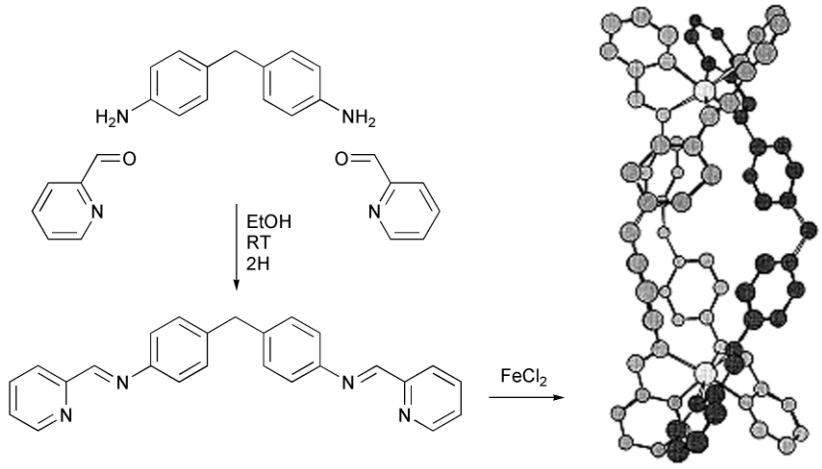
3.4.1 Triple helicates. More recently, Hannon, *et al*. have investigated the interaction of a Fe^{II} triple helical cylinder **38**, with DNA. Modelling suggested that this cylinder was too large to fit into the minor groove of B-DNA, but is the correct size and shape to fit into the major groove. CD and LD studies indicate that the cylinder interacts with DNA, resulting in bending of the duplex. Atomic force microscopy revealed that, even at moderate cylinder loading, DNA undergoes intramolecular coiling. The cylinder binds with high affinity, with $K_b \geq 10^7$ M^{-1}, and further detailed studies on the resolved enantiomers of **38** demonstrated significant differences in binding modes.

While the left handed helical, M-enantiomer preferentially binds in the major groove, the binding site of the right-handed, P-enantiomer was less easily assigned. Since steric considerations appear to preclude extensive insertion of this cylinder into this minor groove, it was suggested that this P-enantiomer lies along the surface of the minor groove perhaps interacting with two phosphate units of the DNA backbones. These observed preferences were explained by analogy with Roger and colleagues previous studies on **3**, *vide ultra*. Thus, like Λ-**3**, the M-enantiomer preferentially adopts a major groove mode with partial insertion of one of its chelates between DNA bases, while both P-**38** and Δ-**3** exhibit more complex binding motifs.[42]

4 Conclusion and outlook

The design of metal complexes that display high affinity binding to duplex DNA in both the minor and major groove is now well established. In some cases, systems have been developed that compete effectively with naturally occurring DNA-binding systems. Sequence and/or structurally specific binding has also been observed. The inclusion of metal ions within these binding agents offers increased functionality, as the examples of optical probes and cleavage agents outlined in this review illustrate.

It seems likely that future systems will show further enhancements in both affinity and selectivity. The synthesis of such systems, coupled with an understanding of their binding



Scheme 5 The molecular structure of the ligand and the tetracationic triple helical supramolecular cylinder, **38**. Reprinted with permission from M. J. Hannon, *et al*., *Angew. Chem., Int. Ed.*., 2001, **40**, 880.

characteristics at a molecular level, offers the promise of new technologies for medical and molecular science. A spectrum of potential applications is apparent, from diagnostics, through therapeutics to the possibly of modulating gene expression.

5 Acknowledgements

For our work at Sheffield we gratefully acknowledge the support of The Royal Society (J. A. T.), the EPSRC (C. M. and J. A. T.), and all our research colleagues. We are indebted to the referees of this review for their suggestions and corrections.

6 References

1 (*a*) S. Neidle, *Nucleic Acid Structure and Recognition*, OUP, Oxford, UK, 2002; (*b*) D. S. Johnson and D. L. Boger, in *Comprehensive Supramolecular Chemistry*, **Vol. 4**, (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, and F. Vögtle), Pergamon, Oxford, 1996.
2 S. J. Lippard, *Acc. Chem. Res.*, 1979, **11**, 211.
3 See: S. J. Lippard and M. Howe-Grant, *Biochemistry*, 1979, **18**, 5762and the references therein.
4 D. S. Sigman, T. W. Bruice, A. Mazumder and C. L. Sutton, *Acc. Chem. Res.*, 1993, **26**, 98.
5 J. K. Barton, A. T. Danishefsky and J. M. Goldberg, *J. Am. Chem. Soc*, 1984, **106**, 2172.
6 S. Satyanarayana, J. C. Dabrowiak and J. B. Chaires, *Biochemistry*, 1992, **31**, 9319.
7 D. Z. M. Coggan, I. S. Haworth, P. J. Bates, A. Robinson and A. Rodger, *Inorg. Chem.*, 1999, **38**, 4486.
8 A. E. Friedman, C. V. Kumar, N. J. Turro and J. K. Barton, *Nucleic Acids Res.*, 1991, **19**, 2595.
9 H.-K. Kim, P. Lincoln, B. Nordén and E. Tuite, *Chem. Commun*, 1997, 2375.
10 A. E. Friedman, J.-C. Chambron, J.-P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960.
11 C. Hiort, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1993, **115**, 3448.
12 Y. Jenkins, A. E. Friedman, N. J. Turro and J. K. Barton, *Biochemistry*, 1992, **31**, 10809.
13 E. Tuite, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1997, **119**, 239.
14 R. E. Holmlin, E. D. A. Stemp and J. K. Barton, *Inorg. Chem.*, 1998, **37**, 29.
15 See: A. Greguric, I. D. Greguric, T. W. Hambley, J. R. Aldrich-Wright and J. G. Collins, *J. Chem. Soc., Dalton Trans.*, 2002, 849 and references therein.
16 H. H. Thorp, *Adv. Inorg. Chem.*, 1995, **43**, 127.
17 G. A. Neyhart, N. Grover, S. R. Smith, W. A. Kalsbeck, T. A. Fairley, M. Cory and H. H. Thorp, *J. Am. Chem. Soc.*, 1993, **115**, 4423.
18 P. J. Carter, C.-C. Cheung and H. H. Thorp, *J. Am. Chem. Soc*, 1998, **120**, 632.
19 I. Ortmans, C. Moucheron and A. Kirsch-De Mesmaeker, *Coord. Chem. Rev*, 1998, **168**, 233.
20 C. Moucheron, A. Kirsch-De Mesmaeker and S. Choua, *Inorg. Chem.*, 1997, **36**, 584.
21 R. E. Holmlin, J. A. Yao and J. K. Barton, *Inorg. Chem.*, 1999, **38**, 174.
22 (*a*) H. D. Soeffler, N. B. Thornton, S. L. Temkin and K. S. Schanze, *J. Am. Chem. Soc.*, 1995, **117**, 7119; (*b*) V. W. W. Yam, K. K. W Lo, K. K. Cheung and R. Y. C. Kong, *J. Chem. Soc., Chem. Commun.*, 1995, 1191; (*c*) V. W. W. Yam, K. K. W. Lo, K. K. Cheung and R. Y. C. Kong, *J. Chem. Soc., Dalton Trans.*, 1997, 2067.
23 C.-M. Che, M. Yang, K. H. Wong, H.-L. Chan and W. Lam, *Chem. Eur. J.*, 1999, **5**, 3350.
24 A. Sitlani, E. C. Long, A. M. Pyle and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 2303.
25 See : H. R. Neenhold and T. M. Rana, *Biochemistry*, 1995, **34**, 6303 and references therein.
26 T. P. Shields and J. K. Barton, *Biochemistry*, 1995, **34**, 15049.
27 S. J. Franklin and J. K. Barton, *Biochemistry*, 1998, **37**, 16093.
28 D. T. Odorn, C. S. Palmer and J. K. Barton, *Biochemistry*, 1999, **38**, 5155.
29 C. L. Kielkopf, K. E. Erkila, B. P. Hudson, J. K. Barton and D. C. Rees, *Nat. Struct. Biol.*, 2000, **7**, 117.
30 K. D. Copeland, M. P. Fitzsimmons, R. P. Houser and J. K. Barton, *Biochemistry*, 2002, **41**, 343.
31 See: B. A. Jackson, V. Y. Alekseyev and J. K. Barton, *Biochemistry*, 1999, **38**, 4655 and references therein.
32 A. H.-J Wang, J. Nathans, G. van der Marel, J. H. van Boom and A. Rich, *Nature*, 1978, **276**, 471.
33 J. G. Collins, R. M. Rixon and J. R. Aldrich-Wright, *Inorg. Chem.*, 2000, **39**, 4377.
34 See for example: B. H. Geierstanger and D. E. Wemmer, *Annu. Rev. Biophys. Biomol. Struct.*, 1995, **24**, 463.
35 W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos and B. L. Hillcoat, *Biochem. J.*, 1986, **238**, 757; W. D. McFadyen, L. P. G. Wakelin, I. A. G. Roos and B. L. Hillcoat, *Biochem. J.*, 1987, **242**, 177.
36 D. L. Carson, D. H. Huchital, E. J. Mantilla, R. D. Sheardy and W. R. Murphy, *J. Am. Chem. Soc.*, 1993, **115**, 6424.
37 F. O' Reilly J. Kelly and A. Kirsch-De Mesmaeker, *Chem. Commun.*, 1995, 1013; F. O' Reilly J. Kelly, *New J. Chem.*, 1998, 215; F. O' Reilly J. Kelly, *J. Phys. Chem B*, 2000, **104**, 7206.
38 (*a*) P. Lincoln and B. Nordén, *Chem. Commun.*, 1995, 2145; (*b*) L. M. Wilhelmsson, F. Westerland, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 2002, **124**, 12092.
39 B. Önfelt, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1999, **121**, 10846; B. Önfelt, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 2001, **123**, 3630.
40 C. Metcalfe, M. Webb and J. A. Thomas, *Chem. Commun.*, 2002, 2026.
41 B. Schoentjes and J.-M. Lehn, *Helv. Chim. Acta*, 1995, **78**, 1.
42 See : I. Meistermann, V. Moreno, M. J. Prieto, E. Moldrheim, E. Sletten, S. Khalid, P. M. Rodger, J. C. Peburdy, C. J. Isaacs, A. Rodger and M. Hannon, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 5069 and references therein.