Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ shows minor groove AT selectivity with oligonucleotides

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NMR studies show that the ternary octahedral Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ cation binds with AT selectivity in the minor groove of [d(CGCGATCGCG)$_2$] and [d(ATATCGATAT)$_2$] duplexes through a non-intercalative interaction.

The design of metal complexes for sequence-specific DNA-binding has attracted much attention recently,[1,2] and elucidation of the structural aspects of such binding is clearly a crucial step in the design process. Some measure of controversy exists over the general mode of interaction with DNA of complexes based on the ligand phen (1,10-phenanthroline). For example, on the basis of ^{1}H NMR data, one study concluded that Δ-[Ru(phen)$_3$]$^{2+}$ binds intercalatively in the major groove of the [(GTGCAC)$_2$] duplex, while its Λ-isomer surface binds in the minor groove.[3] In contrast, a like study indicated minor groove non-intercalative binding for both Δ and Λ forms.[4] Furthermore, other studies employing a variety of experimental techniques and nucleotides arrive at different and often contradictory conclusions regarding the mode of binding of this complex.[5–7] Recent publications[4,8,9] have indicated that a similar debate exists over the binding site preferences of Δ- and Λ-[Ru(phen)$_2$(dppz)]$^{2+}$.

The interactions of potential intercalators with oligonucleotides may be probed using ternary complex compounds based on the [Ru(*R***R**-picchxnMe$_2$)(bidentate)]$^{2+}$ cation template.[2] In such complexes the tetradentate ligand *R***R**-picchxnMe$_2$ [(1*R**,2*R**)-*N*,*N*′-dimethyl-*N*,*N*′-di(2-picolyl)-1,2-diaminocyclohexane][10] determines the chirality of the cation and its overall shape, with the bidentate ligand serving as the putative intercalator.[2] In contrast to [Ru(bidentate)$_3$]$^{2+}$, ternary complexes of this type avoid complications due to averaging between the three possible bound states. Here we report the synthesis and preliminary NMR studies on the binding of the chiral Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ complex cation† (Fig. 1) to the self-complementary duplexes [d(CGCGATCGCG)$_2$] and [d(ATATCGATAT)$_2$]. The study is the first to allow a rigorous evaluation of sequence and groove selectivities for metalloprobes based on a single phen ligand.

Titration of the metal complex into a solution of duplex [d(CGCGATCGCG)$_2$], monitored by one-dimensional ^{1}H NMR spectroscopy at 313 K (Fig. 2), shows considerable upfield shifts of all phen resonances ($-1.23 \geqslant \Delta\delta \geqslant -0.29$ ppm), consistent with significant shielding by the duplex. In contrast, the pyridyl and aliphatic protons of the picchxnMe$_2$ ligand are only slightly affected ($< \pm 0.2$ ppm). This titration also provides evidence for preferential binding at the central AT sequence of the oligonucleotide. At a 1 : 1 complex–duplex ratio, the A5H2 peak is observed to shift 0.29 ppm upfield, while each of the other DNA base protons move marginally downfield ($\leqslant 0.09$ ppm). The A5H2 upfield shift is consistent with the findings of a recent NMR study using the same oligonucleotide sequence and the Δ- and Λ-[Ru(phen)$_3$]$^{2+}$ complex enantiomers.[4]

While chemical shift changes alone are insufficient to deduce the nature of the binding interaction (for example, the AH2 proton has been noted for its sensitivity to the binding of small molecules in both the major and minor grooves[3]), our one-dimensional titration data are indicative of minor groove binding to the AT site. 2D NOESY spectra, recorded at both 303 and 273 K (1 : 1 complex–duplex ratio), provide evidence to support this conclusion. First, three NOE cross peaks are observed between A5H2 (which points into the minor groove) and the metal complex protons H3, H7 and H8 (Fig. 3). Secondly, both T6CH$_3$ and A5H8, which point directly towards the major groove and show a number of intramolecular NOEs, display no cross peaks with the protons of the cation. Thirdly, there is no measureable disruption in the intensities of the sequential H1′–H6/H8 NOE signals in the complex–duplex adduct, indicating that the regular B-form DNA helical structure is retained.[4]

In total, 25 intermolecular NOE signals have been unambiguously assigned at 303 K, all supporting a single binding site model for the ruthenium complex at the central AT sequence, and involving a non-intercalative minor groove binding mode. The NOESY spectrum recorded at 273 K reveals a number of weak intermolecular NOE signals involving cation protons and

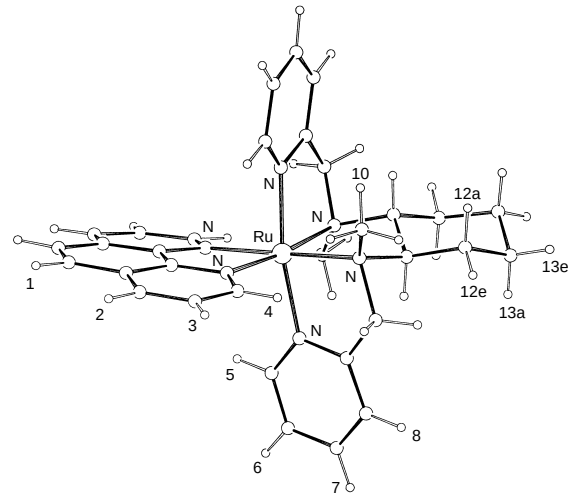

Fig. 1 The structure of the Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ cation. The proton numbering system used in the text is indicated. The Δ absolute configuration of the cation was confirmed by NMR spectroscopy of the complex. In a compensated ROESY spectrum[12] (τ_m = 50 ms, 4 kHz spinlock), using the H13a–H13e cross peak intensity as a calibration (1.78 Å), the average distances for H10–H12a (2.64 Å) and H10–H12e (3.24 Å) were measured according to the formula $r_{ij}/r_{kl} = [\sigma_{kl}/\sigma_{ij}]^{1/6}$. (Due to differences in τ_c for individual protons, an error of 15% can be expected (ref. 13). These distances correspond well to those of the computed model of the Δ-isomer shown [H10$_{(av)}$–H12a = 2.75 Å; H10$_{(av)}$–H12e = 3.35 Å], but not to those of the Λ-isomer [H10$_{(av)}$–H12a = 3.89 Å; H10$_{(av)}$–H12e = 2.94 Å).

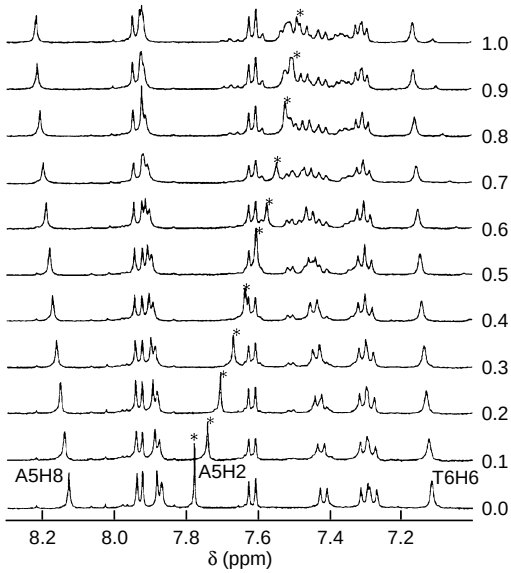

Fig. 2 One-dimensional ^{1}H NMR spectra of the nucleobase protons of [d(CGCGATCGCG)$_2$] at varying cation–duplex ratios (0.0–1.0) (313 K). Note that the resonances of A5H2 and A5H8 shift considerably with complex addition while the others are little affected.

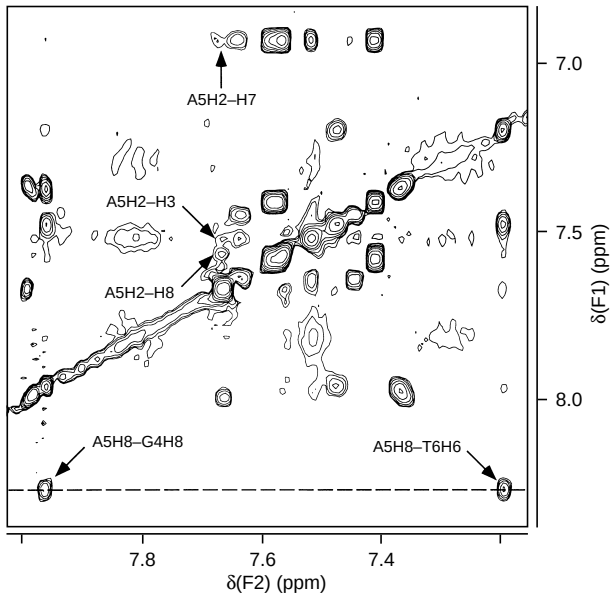

Fig. 3 A ^{1}H NOESY spectrum of the Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$–[d(CGCGATCGCG)$_2$] adduct (1 : 1 ratio, 273 K) showing NOE cross peaks involving the complex protons H3, H7 and H8 and the A5H2 proton in the minor groove. The broken line indicates the positions at which any A5H8–complex cross peaks would be observed if present. These studies were carried out using Bruker AMX600/DMX600 and Varian XL400 NMR spectrometers ([oligonucleotide] = 1.25 mM in 100% D_2O containing 10 mM NaH_2PO_4, 10 mM NaCl, 0.01% NaN_3, uncorrected pD 7.00). Proton assignments were made using DQF-COSY, TOCSY (τ_m = 80 ms) and NOESY (τ_m = 400 ms) spectra. Assignments for the free oligonucleotide were consistent with published data (ref. 14).

those on C1 and G10, suggesting that a secondary weaker binding mode exists at lower temperatures. This may involve aromatic stacking, which could occur at the termini of the duplex. None of these NOE signals are observed at 303 K, and there are no significant chemical shift changes for the C1H6 and G10H8 protons ($\Delta\delta \leqslant 0.01$ ppm), indicating that this interaction is weak.

A similar titration of the cation with a [d(ATATCGATAT)$_2$] duplex[11] was carried out at 298 K. On addition of the complex an immediate broadening of the A7H2, A7H8 and T4CH$_3$ resonances occurs, and at a 1 : 1 ratio significant broadening extends throughout the spectrum. 2D TOCSY and NOESY spectra at 313 K (1 : 1 complex–duplex ratio, τ_m = 400 ms) allowed the assignment of all resonances for the cation and nucleotide base, H1′, H2′, and H2″ protons (the A3-, A7- and A9-H2 resonances were not assignable due to severe line broadening even at higher temperatures). Ten weak intermolecular NOE cross peaks were observed, predominantly between the pyridyl protons of the cation and H1′ sugar protons of the T4, C5, A7, T8, A9 and T10 nucleobases. No NOE cross peaks were observed involving the major groove AH8 or TCH$_3$ protons. These results are consistent with binding occurring in the minor groove at the several AT sites available in this duplex. Thus it is clear that the preferential AT binding observed with the [d(CGCGATCGCG)$_2$] sequence is not due simply to the higher stability of the central part of that oligonucleotide, a potential problem with short DNA duplex sequences.

In conclusion, we have shown that Δ-*cis*-α-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ binds preferentially to AT sequences in duplex oligonucleotides, the predominant molecular binding component being the phen ligand. A range of chemical shift, line broadening and NOE evidence has been used to demonstrate that binding occurs non-intercalatively in the minor groove, thereby contributing significantly to the debate regarding the role of the phen ligand as a DNA-binding epitope. The present work provides a firm basis from which to evaluate the DNA binding properties of other ternary complexes incorporating known intercalating bidentate ligands and based on the [Ru(*R***R**-picchxnMe$_2$)(bidentate)]$^{2+}$ template.[2]

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Footnotes and References

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† Isomeric mixtures containing both Δ-*cis*-α- and Δ-*cis*-β-[Ru(*RR*-picchxnMe$_2$)(phen)]$^{2+}$ are obtained by heating solutions containing stoichiometric quantities of *RR*-picchxnMe$_2$ (ref. 10) and $RuCl_3 \cdot nH_2O$ in propane-1,2-diol under reflux for *ca.* 1 h, followed by addition of a slight molar excess of phen. The isomeric forms of the product may be separated by cation exchange chromatography. In the present work, the complex was used in the chloride form.

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