Binding of the Δ- and Λ-Enantiomers of $[Ru(dmphen)_2dpq]^{2+}$ to the Hexanucleotide $d(GTCGAC)_2$

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^{1}H NMR spectroscopy and viscosity measurements have been used to study the oligonucleotide binding of the Δ- and Λ-enantiomers of the metal complex $[Ru(dmphen)_2dpq]^{2+}$ (dmphen = 2,9-dimethyl-1,10-phenanthroline and dpq = dipyrido[3,2-*f*:2′,3′-*h*]quinoxaline). The addition of either enantiomer to $d(GTCGAC)_2$ induced large upfield shifts and significant broadening for the hexanucleotide imino and metal complex dpq resonances. These data coupled with the observed increase in the melting transition midpoint of the hexanucleotide duplex upon addition of either enantiomer suggests that both Δ- and Λ-$[Ru(dmphen)_2dpq]^{2+}$ bind by intercalation. A significant number of metal complex to hexanucleotide NOE contacts were observed in NOESY spectra of $d(GTCGAC)_2$ with added Δ- or Λ-$[Ru(dmphen)_2dpq]^{2+}$. The observed intermolecular NOEs were consistent with both enantiomers intercalating between the G_4A_5 bases of one strand and the T_2C_3 bases of the complementary strand. Intermolecular NOEs from the dmphen protons were only observed to protons located in the hexanucleotide minor groove. Alternatively, NOE contacts from the dpq protons were observed to both major and minor groove protons. The NOE data suggest that the dpq ligand of the Δ-enantiomer intercalates deeply into the hexanucleotide base stack while the Λ-enantiomer can only partially intercalate. Viscosity measurements were consistent with the proposed intercalation binding models. The addition of the Δ-enantiomer increased the relative viscosity of the DNA solution, while a decrease in the relative viscosity of the DNA was observed upon addition of the Λ-metal complex. These results confirm our proposal that octahedral metallointercalators can intercalate from the minor groove. In addition, the results demonstrate that the left-handed enantiomer of $[Ru(dmphen)_2dpq]^{2+}$ prefers to intercalate from the narrow minor groove despite only being able to partially insert a polycyclic aromatic ligand into the DNA base stack.

Introduction

There is considerable interest in the DNA binding properties of inert transition metal complexes. Transition metal complexes have been used to further our understanding of the polymorphic nature of nucleic acid conformation,[1−3] the sequence specific interactions by small molecules and proteins,[4−6] long-range electron transfer that is mediated by the stacked bases of DNA,[7−13] and the relative flexibility of different DNA sequences.[14] As transition metal complexes have a wide range of applications that are dependent upon their ability to bind DNA, it is important that a detailed understanding of the metal complex−DNA association be obtained.

It has unambiguously been shown that metallointercalators based on the phenanthrenequinone diimine (phi) ligand intercalate from the major groove.[6,15−18] Complexes which contain nonbulky ancillary ligands, such as NH_3,[17] ethylenediamine,[18] or 2(*R*),9(*R*)-diamino-4,7-diazadecane (Me_2trien),[6] are easily accommodated in the wide major groove. The binding site selectivity of these phi-based complexes is determined by favorable van der Waals and hydrogen bond interactions with the major groove. Complexes that contain bulky ancillary ligands, such as $[Rh(phen)_2phi]^{3+}$, bind DNA by intercalation where the major groove is more open.[6,15,16]

The ruthenium(II) polypyridyl complexes are another important class of metallointercalators; however, their DNA binding is not yet well understood. Nordén and co-workers, on the basis of the similarity of the binding geometry to that of actinomycin D and photophysical studies using T4-DNA, have proposed that

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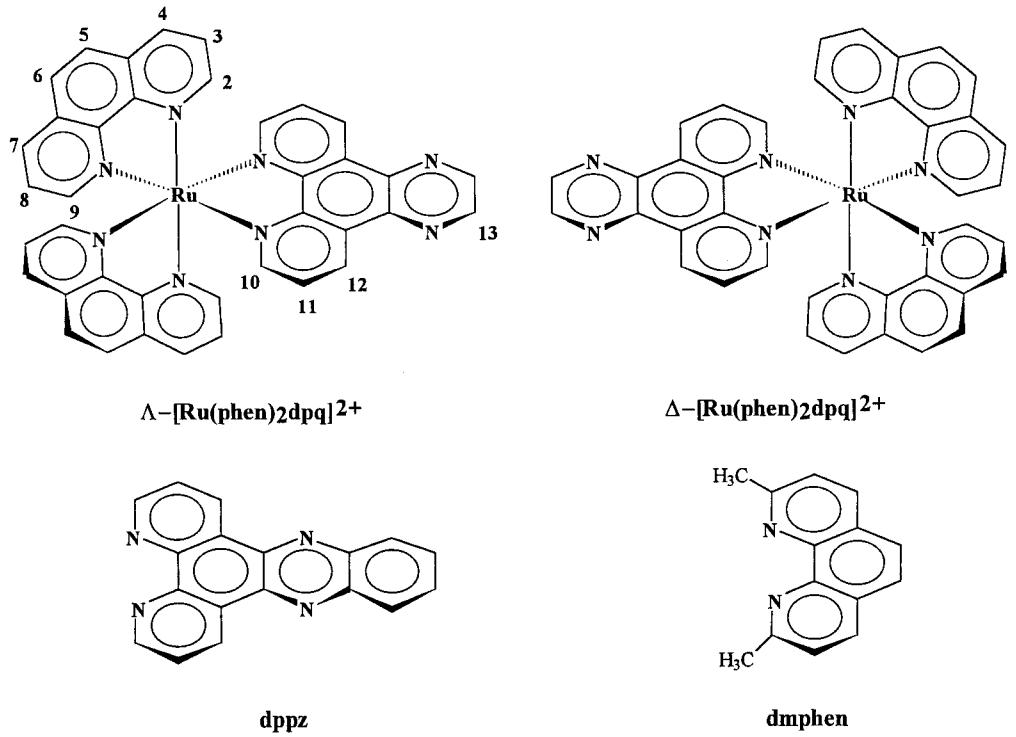

Figure 1. Structure of the Δ- and Λ-$[Ru(phen)_2dpq]^{2+}$ complexes and the dmphen and dppz ligands.

both Δ- and Λ-$[Ru(phen)_2dppz]^{2+}$ intercalate from the minor groove.[19,20] Alternatively, on the basis of both photophysical studies and NMR data, Barton and co-workers have proposed that Δ- and Λ-$[Ru(phen)_2dppz]^{2+}$ intercalate from the DNA major groove.[21−23] We have recently shown that the metal complex Δ-$[Ru(phen)_2dpq]^{2+}$ (see Figure 1), which is closely related to Δ-$[Ru(phen)_2dppz]^{2+}$, intercalates from the minor groove of a hexanucleotide.[24,25] However, as chiral metal complexes generally display enantioselectivity in their DNA binding, it cannot be assumed that Λ-$[Ru(phen)_2dpq]^{2+}$ will bind DNA in a fashion similar to that of the Δ-enantiomer.

Intercalation from the minor groove by Δ-$[Ru(phen)_2dpq]^{2+}$ was unexpected, given the steric bulk of the nonintercalating phenanthroline ligands and the narrowness of the DNA minor groove. For the Λ-enantiomer to intercalate the phenanthroline ligands must run across the groove (rather than along the groove for the Δ-enantiomer), thereby creating even greater steric clashes with the right-handed groove. In an attempt to determine the binding geometry of the Λ-enantiomer, we examined the binding of Λ-$[Ru(phen)_2dpq]^{2+}$ to a self-complementary hexanucleotide by NMR spectroscopy. However, in the two-dimensional NOE experiments that provide the most detailed information, only a few NOE cross-peaks between the hexanucleotide and the metal complex were observed. As the NMR data were not sufficient to determine the binding mode, we sought to study the DNA binding of the closely related metal complex Λ-$[Ru(dmphen)_2dpq]^{2+}$ (dmphen = 2,9-dimethyl-1,10-phenanthroline; see Figure 1).

The addition of methyl groups at the 2 and 9 positions on the phenanthroline rings of Λ-$[Ru(phen)_2dpq]^{2+}$ resulted in the observation of many intermolecular NOE cross-peaks in NOESY spectra of the hexanucleotide with added metal complex. Furthermore, the methyl groups place additional steric constraints on the intercalation process. The methyl groups are brought into close proximity to the base pairs at the intercalation site if the bases are stacked perpendicularly to the helix axis (canonical form DNA). In this paper we present a ^1H NMR study of the binding of Δ- and Λ-$[Ru(dmphen)_2dpq]^{2+}$ with the self-complementary oligonucleotide d(GTCGAC). The results indicate that both enantiomers bind by intercalation and from the minor groove.

Experimental Section

Materials. The oligonucleotide $d(GTCGAC)_2$ was obtained from Bresatec Ltd., South Australia. Ruthenium(III) chloride hydrate, 2,9-dimethyl-1,10-phenanthroline hydrate (dmphen), ethidium bromide, Hoechst 33258, potassium hexafluorophosphate, aluminum oxide (activated, neutral, Brockmann I), Amberlite IRA-400(Cl) ion-exchange resin, D_2O (99.96%), and dibenzoyl-L-tartaric acid were obtained from Aldrich Chemical Co. CM- and SP-Sephadex ion-exchange resins were obtained from Pharmacia.

Synthesis of Metal Complexes. $[Ru(dmphen)_2Cl_2]$. $RuCl_3$ (2.0 g, 9.64 mmol), lithium chloride (0.5 g), and dmphen (4.0 g, 19.3 mmol) were dissolved in *N*,*N*-dimethylformamide (50 mL) and refluxed for 10 h. The solution was cooled to room temperature, poured into acetone (250 mL), and stored at − 20 °C overnight. The crude reaction mixture was filtered and washed with acetone (100 mL), water/acetone (1:1) (20 mL), and then diethyl ether (50 mL). The black solid was dried in air. Yield: 3.69 g (65%).

$[Ru(dmphen)_2dpq](PF_6)_2$. The ligand dpq[25] (0.5 g, 2.2 mmol) was added to a stirred solution of $[Ru(dmphen)_2Cl_2]$ (1.0 g, 1.7 mmol) in ethanol/water (350/100 mL) and refluxed for 8 h. The reaction mixture was reduced to 50 mL and cooled and excess potassium hexafluorophosphate added to induce precipitation. The resulting orange solid was filtered and washed with water (100 mL) and diethyl ether (50 mL). This crude product was purified by column chromatography on aluminum oxide (activated, neutral, Brockmann I (5 cm × 30 cm))

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using acetonitrile. Ethanol (20 mL) was added to the major orange band, affording an orange product which was recrystallized from acetone/water (200 mL, 50:50). Yield: 1.43 g, 81%. ^{1}H NMR (400 MHz, d_6-acetone): δ 9.47 (d, 1H), 9.24 (s, 1H), 8.94 (d, 1H), 8.48 (d, 1H), 8.45 (d, 1H), 8.30 (d, 1H), 8.04 (d, 1H), 7.89 (d, 1H), 7.72 (dd, 1H), 7.42 (d, 1H), 2.10 (s, 3H), 2.02 (s, 3H). MS (ESMS, CH_3CN, MW = 1039.8): m/z = 895.3 (M − $PF6^-$).

Enantiomer Resolution. Resolution was achieved using a procedure similar to that described by Rutherford et al.[26] Δ- and Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ were resolved on a Sephadex SP C-25 column (100 × 1.6 cm) using sodium dibenzoyl-L-tartrate as the eluent (approximately 800 mL). The enantiomer purity of the chloride salt was assayed by CD spectroscopy. Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ (band 1) CD [λ, nm ($\Delta\epsilon$, water)] 253 (435); 273 (−559); 377 (36); 467 (−7.5); 502 (8.7); 545 (−3.1). Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ (band 2) CD [λ, nm ($\Delta\epsilon$, water)] 253 (−437); 273 (569); 377 (−40); 467 (8.1); 502 (−8.1); 545 (4.1).

Sample Preparation for NMR Analysis. The hexanucleotide d(GTCGAC)$_2$ was converted into the Na^+ form using a small CM-Sephadex column. The hexanucleotide was dissolved in 0.65 mL of phosphate buffer (10 mM, pH 7) containing 20 mM NaCl and 0.1 mM EDTA, and a trace of DSS was added as an internal chemical shift reference. For experiments carried out in D_2O the sample was repeatedly freeze-dried from D_2O and finally made up in 99.96% D_2O. The hexanucleotide concentration was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide.[27]

NMR Spectroscopy. ^{1}H NMR spectra (400 MHz) were recorded on a Varian Unityplus-400 spectrometer. Two-dimensional phase-sensitive NOESY spectra were acquired by the method of States et al.,[28] using 2048 data points in t_2 for 256 t_1 values with a pulse repetition delay of 1.7 s. DQFCOSY experiments were accumulated using 2048 data points in t_2 for 256 t_1 values using a pulse repetition delay of 1.7 s. Spectra recorded in 90% H_2O/10% D_2O were recorded using the WATERGATE solvent suppression technique of Piotto et al.[29] NOESY spectra recorded in 90% H_2O/10% D_2O were obtained using the standard NOESY pulse sequence with the WATERGATE sequence incorporated as a read pulse.

Viscosity Measurements. Viscosity experiments were carried out using a Cannon-Manning semi-micro viscometer maintained at a constant temperature of 24.9 °C in a circulating water bath. Calf thymus DNA, sonicated to approximately 200 base pair (bp) lengths, was prepared as described by Haq et al.[30] BPES buffer (8 mM sodium phosphate, 1 mM EDTA, and 25 mM NaCl), which gave a flow time of 465(±1) s, was used. The concentration of DNA was 100 mM bp, and samples were prepared to give total ligand/bp ratios of 0.15 and 0.3. Each sample was measured five times with an average flow time being calculated. Data are presented as $(\eta/\eta^\circ)^{1/3}$ versus binding ratio (r) where η is the viscosity of the DNA in the presence of the metal complex and η° is the viscosity of the DNA alone. Viscosity values were calculated from the observed flow times of the DNA-containing solutions (t) corrected for the flow time of buffer alone (t°), $\eta = t - t^\circ$.

Circular Dichroism and UV/Vis Spectra. CD were recorded at ambient temperature on a Jasco 500C spectropolarimeter, while UV/vis spectra were recorded at 260 nm on a Cary 1 UV/vis spectrometer.

Molecular Modeling. The coordinates for the Δ-and Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ were taken from the unpublished crystal structure for the closely related metal complex *rac*-[Ru(dmphen)$_2$dpqC]$^{2+}$, where dpqC = dipyrido[3,2-*a*:2′,3′-*c*](6,7,8,9-tetrahydro)phenazine. The geometry relative to the ruthenium atom is distorted from octahedral as a consequence of the small bite angles of the dpqC and dmphen ligands (78.9°, 78.9°, and 79.6°). Both dmphen ligands make an angle of approximately 80.5° with the dpqC, but with each other this angle is 103°, a deviation from octahedral geometry due to steric congestion. The ruthenium−dpqC and the ruthenium−dmphen bonds are within the ranges of 2.054(9)−2.075(9) and 2.079(9)−2.12(1) Å, respectively. The hexanucleotide binding site was constructed using MSI molecular modeling software. The docking of the metal complexes was done manually using the program 3Dmol (R. S. Vagg, Macquarie University, Australia).

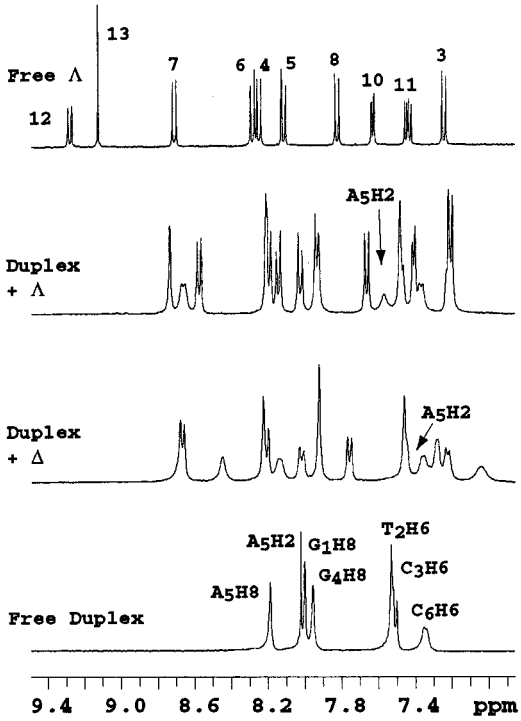

Figure 2. ^{1}H NMR spectrum of the free d(GTCGAC)$_2$ (1.3 mM) and with added Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ (duplex + Δ) and Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ (duplex + Λ), at a metal complex-to-duplex ratio (R) of 0.9. The spectrum of the free Λ-metal complex (1.0 mM) in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C is also shown (free Λ).

Results

Assignment of the Proton Resonances of d(GTCGAC)$_2$. The ^{1}H NMR resonances of the free hexanucleotide were assigned by established methods.[31−33] The observation of two resonances in the imino region (12−14 ppm) of the NMR spectrum of the hexanucleotide dissolved in 90% H_2O/10% D_2O indicated that only the terminal residue did not form a stable base pair. The solution conformation of the hexanucleotide was determined by analysis of DQFCOSY and short mixing time NOESY spectra.[31,33] In NOESY spectra of d(GTCGAC)$_2$ an NOE is observed from each base H8/H6 to its own H1′/H2′/H2″ protons as well as to the H1′/H2′/H2″ protons of the flanking 5′-sugar, consistent with a right-handed duplex. Furthermore, as the NOE cross-peak from each H8/H6 proton to its own H2′ proton is significantly larger than to the H2′ proton on the flanking 5′-sugar, it is concluded that the hexanucleotide adopts a B-type conformation in aqueous solution. However, it must be noted that the hexanucleotide in low ionic strength solution only represents a model for DNA.

One-Dimensional NMR Experiments. Figure 2 shows the ^{1}H NMR spectrum of d(GTCGAC)$_2$ upon addition of either Δ-

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Table 1. Chemical Shift Changes for the Resonances from the Δ- and Λ-Enantiomers of $[Ru(dmphen)_2dpq]^{2+}$ upon Hexanucleotide Binding, at 25 °C

ligand proton	Λ-enantiomer	Δ-enantiomer	ligand proton	Λ-enantiomer	Δ-enantiomer
		dpq			
H13	−0.39	−0.66	H11	−0.23	−0.38
H12	−0.61	−0.58	H10	−0.22	−0.35
		dmphen			
9-Me	−0.18	−0.12	H5	−0.09	−0.10
H8	−0.16	−0.07	H4	−0.05	−0.11
H7	−0.14	−0.05	H3	−0.04	−0.02
H6	−0.14	−0.08	2-Me	−0.09	−0.10

Table 2. Change in Chemical Shift for the $d(GTCGAC)_2$ Resonances upon Addition of Δ- and Λ-$[Ru(dmphen)_2dpq]^{2+}$, at a Metal Complex-to-Duplex Ratio of 0.9, in 10 mM Phosphate (pH 7) Containing 20 mM NaCl at 25 °C

	hexanucleotide proton									
	H8/H6		H1′		H2′		H2″		H3′	
	Λ	Δ	Λ	Δ	Λ	Δ	Λ	Δ	Λ	Δ
G_1	−0.06	−0.07	−0.10	−0.12	−0.09	−0.10	−0.08	−0.03	−0.03	0.00
T_2	−0.03	−0.05	−0.20	−0.27	−0.02	−0.09	−0.07	−0.25	0.00	−0.03
C_3	−0.02	−0.05	−0.17	−0.21	−0.12	−0.13	−0.11	−0.16	−0.04	−0.05
G_4	−0.01	−0.02	−0.10	−0.10	−0.06	−0.04	−0.16	−0.03	−0.01	−0.01
A_5	0.03	0.05	−0.23	−0.25	−0.01	−0.03	−0.05	−0.13	0.00	−0.01
C_6	0.02	0.02	−0.07	−0.02	−0.08	−0.04	−0.08	−0.04	−0.02	−0.01

or Λ-$[Ru(dmphen)_2dpq]^{2+}$, as well as the spectra of the free hexanucleotide and Λ-metal complex. The resonances of the bound metal complexes were assigned by a combination of two-dimensional NMR experiments after the spectrum of $d(GTCGAC)_2$ with added Δ- or Λ-$[Ru(dmphen)_2dpq]^{2+}$ was recorded as a function of temperature. From the spectra of the hexanucleotide-bound metal complex recorded from 25 to 80 °C, the dpq H12 and H13 resonances are easily assigned, as the chemical shifts of these resonances approach their respective values for the free metal complex with increasing temperature. The remaining dpq protons are then assigned from the DQFCOSY experiments. The 2,9-methyls on the dmphen ligands are then specifically assigned from the NOESY spectra. An NOE cross-peak is observed from the dpq H11 proton to the 2-methyl but not to the 9-methyl, consistent with the H11 to 2-methyl and H11 to 9-methyl distances determined from a molecular model. Once the 2- and 9-methyl resonances have been assigned, the remaining dmphen resonances can be assigned from NOESY and DQFCOSY spectra.

In the NMR spectrum of $d(GTCGAC)_2$ with added Δ- or Λ-$[Ru(dmphen)_2dpq]^{2+}$, only one set of hexanucleotide and metal complex resonances are observed, indicating that neither enantiomer binds with slow exchange kinetics (on the NMR time scale). However, addition of either the Δ- or Λ-metal complexes did induce significant broadening of some resonances (see Figure 2), particularly the Δ-enantiomer, indicating that both enantiomers bind with intermediate exchange kinetics. Addition of Δ-$[Ru(dmphen)_2dpq]^{2+}$ to $d(GTCGAC)_2$ induced significant upfield shifts for the dpq resonances, with the dmphen resonances showing only small shifts (see Table 1). Addition of Λ-$[Ru(dmphen)_2dpq]^{2+}$ to the hexanucleotide induced a similar upfield shift for the H12 resonance, but smaller upfield shifts for the other dpq resonances. The large upfield shifts of the dpq resonances induced by the addition of either enantiomer and the observed intermediate exchange binding kinetics are consistent with both enantiomers binding the hexanucleotide by intercalation.

As shown in Table 2, addition of Δ- and Λ-$[Ru(dmphen)_2dpq]^{2+}$ to $d(GTCGAC)_2$ induced a similar pattern of changes in the chemical shifts of the resonances from the hexanucleotide. The A_5H2 (see Figure 2) and the sugar H1′ protons show the largest change in chemical shift, suggesting that both metal complexes bind in the hexanucleotide minor groove. Addition of the Δ- or Λ-complexes to the hexanucleotide also induced significant broadening and upfield shifts of the T_2 and G_4 imino resonances. Both enantiomers caused similar upfield shifts for the T_2 (∼0.5 ppm) and G_4 (∼0.3) imino resonances.

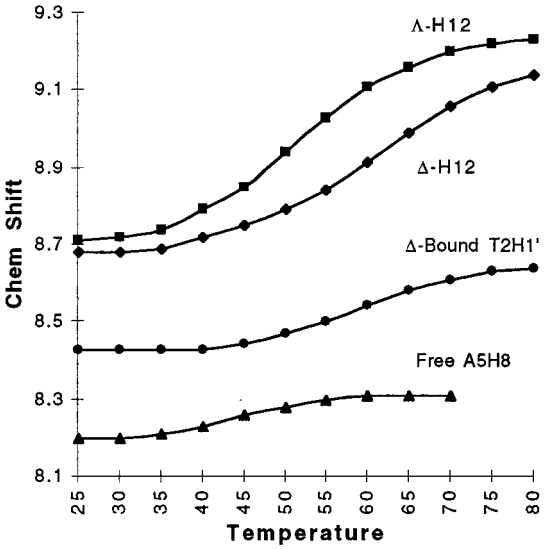

Figure 3. Melting curves of the free $d(GTCGAC)_2$ duplex and the Δ- and Λ-$[Ru(dmphen)_2dpq]^{2+}$-bound hexanucleotide duplexes. The figure shows the chemical shift of the A_5H8 resonance of the free hexanucleotide, the T_2H1' resonance of the Δ-bound hexanucleotide (chemical shift + 2.5 ppm), and the H12 resonance of the Δ- and Λ-enantiomers of the hexanucleotide-bound complexes as a function of temperature.

While it was not possible to determine a hexanucleotide binding constant for the two enantiomers, it is possible to set a minimum binding constant. The resonances from the H12 and H13 protons of both metal complexes exhibit large upfield shifts upon hexanucleotide binding. As the chemical shifts for these resonances are the same at metal complex-to-hexanucleotide ratios of 0.1 and 1.0, it can be concluded that both enantiomers bind essentially stoichiometrically at a ratio of 1.0. Given this assumption and the concentration of the metal complex−oligonucleotide solution, a minimum binding constant of 10^5 M^{-1} can be calculated.

Hexanucleotide−Duplex Melting Experiments. DNA binding by intercalation is generally characterized by an increase in the transition midpoint of the temperature dependence curve of the resonances from the oligonucleotide.[18,22] The chemical shift changes reflect the conversion from a duplex state to the totally base destacked single state, often (but inappropriately) referred to as the duplex melting temperature. Figure 3 shows the chemical shift of the A_5H8 resonance of the free hexanucleotide, the T_2H1' resonance of the Δ-bound hexanucleotide and the H12 resonance of the Δ- and Λ-enantiomers of the hexanucleotide-bound metal complexes as a function of temperature. The transition midpoint of the temperature dependence curve of the free hexanucleotide was determined to be 45 °C. Addition of either enantiomer increased the midpoint of the transition of the hexanucleotide, with the Δ-enantiomer inducing a larger increase (13 °C) than the Λ-enantiomer (8 °C). As a comparison, the change in the melting profile of the hexanucleotide duplex upon addition of either enantiomer was also determined by UV/vis spectroscopy. At the millimolar concentrations used for the

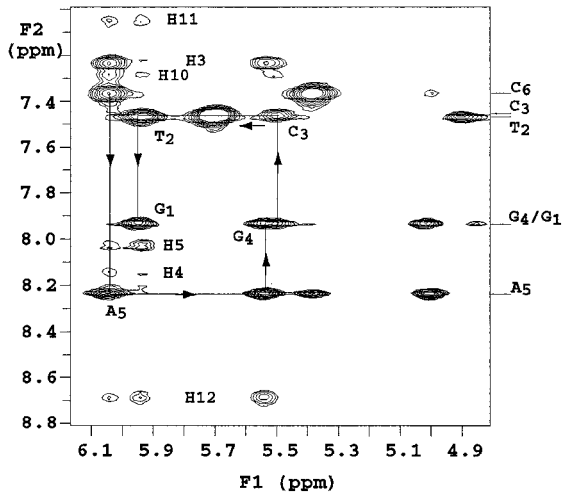

Figure 4. Expansion of the NOESY spectrum (250 ms mixing time) of Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex-to-duplex ratio of 0.9 at 25 °C. The expansion shows the NOE connectivities from the hexanucleotide base and metal complex aromatic protons (7.0−8.8 ppm) to the hexanucleotide sugar H1′ and H3′ protons (4.8−6.1 ppm). All two-dimensional spectra were recorded with samples dissolved in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl. The sequential NOE connectivities, starting from C$_6$H6−A$_5$H1′, are shown by the arrowheads. No NOEs from the metal complex to the major groove H3′ protons are observed, consistent with minor groove binding.

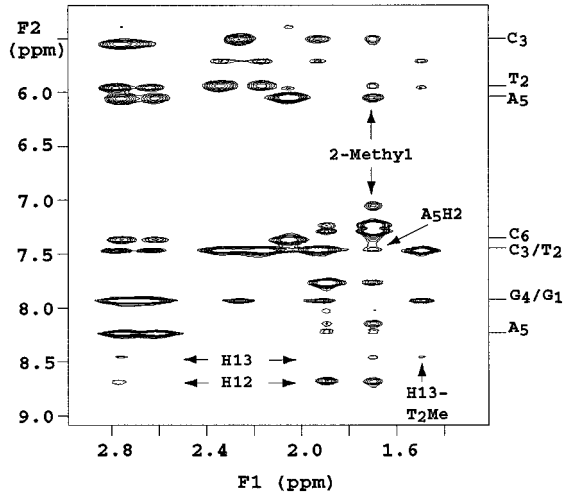

Figure 5. Expansion of the NOESY spectrum (250 ms mixing time) of Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex-to-duplex ratio of 0.9 at 25 °C. The expansion shows the hexanucleotide base and metal complex aromatic (7.4−8.8 ppm) and sugar H1′ (5.5−6.2 ppm) protons to hexanucleotide H2′/H2″, T$_2$methyl, and metal complex methyl protons (1.4−2.8 ppm) region.

UV/vis experiments, the melting temperature of the free hexanucleotide duplex (in the same buffer as used in the NMR experiments) was found to be 23 °C. Addition of either enantiomer induced a broader melting transition with an increased melting temperature, with the Δ-enantiomer inducing a slightly larger increase (14 °C) than the Λ-enantiomer (12 °C).

Two-Dimensional NMR Experiments. Two-dimensional NOE spectra of the hexanucleotide with added metal complex were recorded, at mixing times from 100 to 300 ms, to obtain a more detailed picture of the binding. Figures 4 and 5 show two expansions of a 250 ms mixing time NOESY spectrum of d(GTCGAC)$_2$ with added Δ-[Ru(dmphen)$_2$dpq]$^{2+}$. A considerable number of intermolecular NOE cross-peaks between the dmphen protons and the hexanucleotide H1′ protons are observed (see Table 3). The stronger dmphen to sugar H1′ NOE cross-peaks, such as the H3 to the A$_5$H1′ and G$_4$H1′ and the 2-methyl to the A$_5$H1′, C$_3$H1′, and T$_2$H1′, are also clearly observed in 100 ms mixing time experiments. As the sugar H1′ protons are located in the hexanucleotide minor groove, the NOESY data indicate that the dmphen ligands are bound in the minor groove. This conclusion is further supported by the observation of NOE cross-peaks between the dmphen protons and the hexanucleotide H4′/H5′/H5″ protons (located in the minor groove) and the absence of NOEs to hexanucleotide major groove protons (H8/H6, H2′, and H3′). Intermolecular NOE cross-peaks from the dpq ligand of the Δ-enantiomer to both major and minor groove protons are observed (see Table 3). Intermolecular NOEs from the dpq H10 and H11 are only observed to hexanucleotide minor groove protons, while NOEs from the dpq H13 are only observed to major groove protons (T$_2$methyl, G$_4$H8, A$_5$H8, and G$_4$H2′/H2″). By contrast, intermolecular NOEs from the dpq H12 are observed to both major and minor groove protons. The NOE data indicate that the Δ-enantiomer binds the hexanucleotide by intercalation with the dpq ligand selectively inserted between the stacked bases.

Table 3. NOE Cross-Peaks Observed between the Bound Metal Complex and d(GTCGAC)$_2$, at a Metal Complex-to-Duplex Ratio of 0.9, in 10 mM Phosphate (pH 7) Containing 20 mM NaCl at 25 °C

metal complex proton	hexanucleotide protons	
	Λ-enantiomer	Δ-enantiomer
H13	T$_2$Me, G$_4$ imino	T$_2$Me, G$_4$H8, A$_5$H8, G$_4$H2′/H2″
H12	T$_2$H1′, G$_4$H1′, T$_2$H2″, G$_4$H2′/H2″	A$_5$H1′, T$_2$H1′, G$_4$H1′, C$_3$H4′, G$_4$H2′/H2″, G$_4$H5′/H5″
H11	T$_2$H1′, C$_3$H4′, G$_4$H2′/H2″	A$_5$H1′, T$_2$H1′
H10	T$_2$H1′	A$_5$H1′, C$_3$H1′, C$_3$H4′
9-methyl	T$_2$H1′, A$_5$H2	
H8		C$_3$H4′
H6	C$_3$H4′	
H5	T$_2$H1′, T$_2$H4′, C$_3$H4′	T$_2$H1′, A$_5$H1′
H4	T$_2$H1′	A$_5$H1′
H3	T$_2$H1′, A$_5$H1′, G$_4$H1′, A$_5$H2	A$_5$H1′, G$_4$H1′
2-methyl	G$_4$H1′, T$_2$H1′, A$_5$H1′, A$_5$H2	A$_5$H1′, C$_3$H1′, T$_2$H1′, A$_5$H2, C$_3$H4′, G$_4$H5′/H5″

Figures 6 and 7 show two expansions of a 300 ms mixing time NOESY spectrum of d(GTCGAC)$_2$ with added Λ-[Ru(dmphen)$_2$dpq]$^{2+}$. Similarly to the NOESY spectra of the hexanucleotide with added Δ-enantiomer, a considerable number of intermolecular NOE cross-peaks between the dmphen protons of the Λ-complex and the hexanucleotide H1′ protons are observed. Again, the stronger intermolecular NOE cross-peaks, such as the H3 to the T$_2$H1′ and the 2-methyl to the T$_2$H1′ and G$_4$H1′, are clearly observed in 100 ms mixing time NOESY spectra. Interestingly, however, the pattern of intermolecular NOE contacts to the sugar H1′ protons from the 2-methyl protons for the bound Λ-enantiomer is significantly different from that observed for the Δ-complex. For the Δ-enantiomer the strongest NOE cross-peaks between the 2-methyl and sugar H1′ protons are to the A$_5$H1′ and C$_3$H1′, while for the Λ-enantiomer the stronger NOEs are to the T$_2$H1′ and G$_4$H1′. Intermolecular NOE cross-peaks are also observed between the dpq protons of the Λ-enantiomer and the hexanucleotide. NOE contacts between the dpq H10, H11, and H12 protons and the hexanucleotide minor groove protons are observed, while NOE cross-peaks to the hexanucleotide major groove protons are detected for the dpq H11, H12, and H13 protons (see Table 3). Again, the NOE data indicate that the Λ-enantiomer binds the hexanucleotide by intercalation with the dpq ligand selectively inserted between the stacked bases.

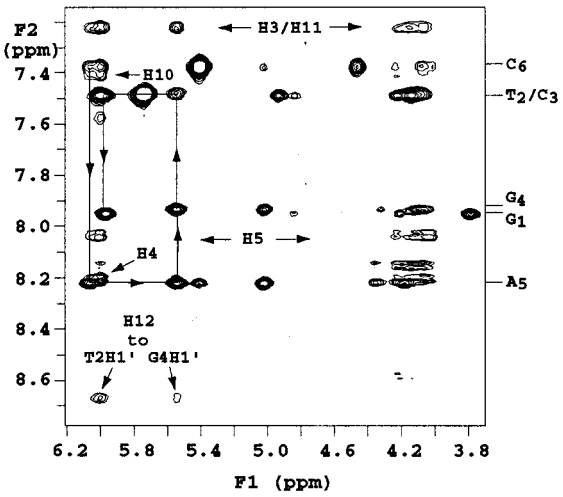

Figure 6. Expansion of the NOESY spectrum (300 ms mixing time) of Λ-$[Ru(dmphen)_2dpq]^{2+}$ and $d(GTCGAC)_2$, at a metal complex-to-duplex ratio of 0.9 at 25 °C. The expansion shows the NOE connectivities from the hexanucleotide base and metal complex aromatic protons (7.2−8.7 ppm) to the hexanucleotide sugar H1′, H3′, and H4′/H5′/H5″ protons (3.8−6.2 ppm). In NOESY spectra run at 10 °C the H3 and H11 protons are clearly resolved, and it can be established that it is the H3 that predominantly gives rise to the NOE contacts with the hexanucleotide H1′ protons.

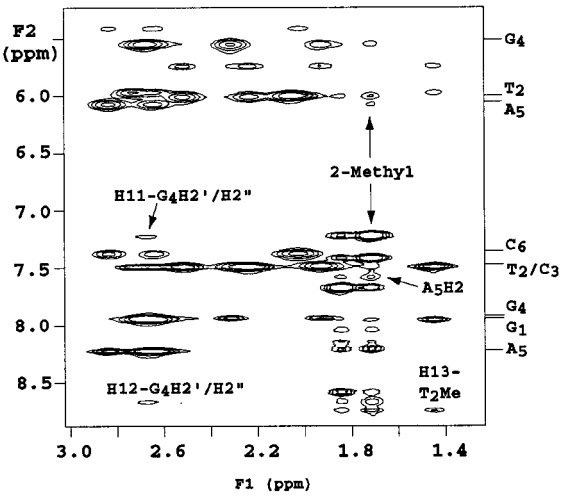

Figure 7. Expansion of the NOESY spectrum (300 ms mixing time) of Λ-$[Ru(dmphen)_2dpq]^{2+}$ and $d(GTCGAC)_2$, at a metal complex-to-duplex ratio of 0.9 at 25 °C. The expansion shows the hexanucleotide base and metal complex aromatic (7.0−8.7 ppm) and sugar H1′ (5.5−6.2 ppm) protons to hexanucleotide H2′/H2″, T_2methyl, and metal complex methyl protons (1.4−2.8 ppm) region.

In NOESY spectra of $d(GTCGAC)_2$ with added Δ-$[Ru(dmphen)_2dpq]^{2+}$ run in 90% H_2O/10% D_2O, no additional intermolecular NOEs were observed, due to the broadness of the dpq resonances (particularly at 10 °C) and the imino resonances. In NOESY spectra of the hexanucleotide with added Λ-$[Ru(dmphen)_2dpq]^{2+}$, in 90% H_2O/10% D_2O at 10 °C, the imino and dpq resonances were again very broad; however, an NOE between the dpq H13 and the G_4 imino proton was observed.

It was not possible to obtain a detailed picture of the solution conformation of the Δ- or Λ-enantiomer-bound hexanucleotide. However, analysis of 100 ms mixing time NOESY spectra indicated that the hexanucleotide maintained the basic B-type conformation upon binding of either enantiomer.

Δ-$[Ru(dmphen)_2dpq]^{2+}$−$d(GTCGAC)_2$ Binding Model. As only broad exchange-averaged resonances from both the metal complex and the hexanucleotide are observed, it was not possible to determine a quantitative picture of the metal complex−hexanucleotide binding. However, a qualitative binding model could be constructed that is in general agreement with the intermolecular NOE data. Figure 8 shows the Δ-$[Ru(dmphen)_2dpq]^{2+}$ intercalated between the G_4A_5 bases of one strand and the C_3T_2 bases on the complementary strand, with the dmphen ligands residing in the minor groove. Although the dpq ligand does not extend into the major groove, the model suggests that the metal complex is deeply intercalated. The metal complex is rotated slightly (15°) toward the G_4A_5 strand; however, as the detailed structure of the hexanucleotide binding site could not be determined, other binding orientations may be consistent with the observed NOE data.

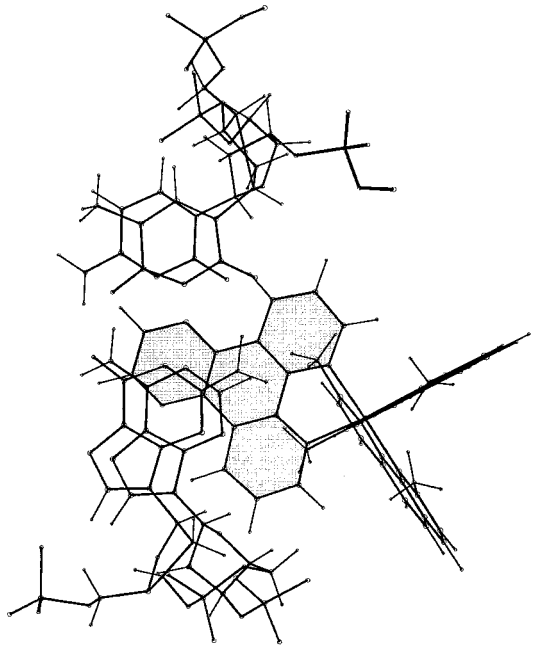

Figure 8. A model showing the intercalative binding of the Δ-$[Ru(dmphen)_2dpq]^{2+}$ complex between the G_4A_5 residues of one strand and the T_2C_3 residues of the other strand of the hexanucleotide $d(GTCGAC)_2$. The dmphen rings are located in the minor groove with the dpq ligand (shaded gray) inserted into the nucleotide base stack.

In this Δ-$[Ru(dmphen)_2dpq]^{2+}$−hexanucleotide binding model there are no van der Waals clashes between the metal complex and the hexanucleotide. Furthermore, the binding model is in good agreement with the observed intermolecular NOEs. For all observed NOEs between dpq and hexanucleotide protons the corresponding interproton distances in the binding model are ≤5.5 Å. The agreement is also reasonable for the dmphen protons, with only the distances from the H8 and H5 protons to the hexanucleotide protons being slightly greater than the maximum distance for which a direct NOE could be expected (5.5 Å). The model is also consistent with the observed changes in the chemical shift of the dpq resonances upon hexanucleotide binding. The H13 and H12 protons are positioned near the purine rings consistent with the observed ∼0.6 ppm upfield shifts; a proton located directly between the purine bases would be expected to shift upfield by ≥1 ppm.[34] The H11 and H10 protons are positioned at greater distances from the shielding ring current effects of the two purines, consistent with their smaller upfield shifts.

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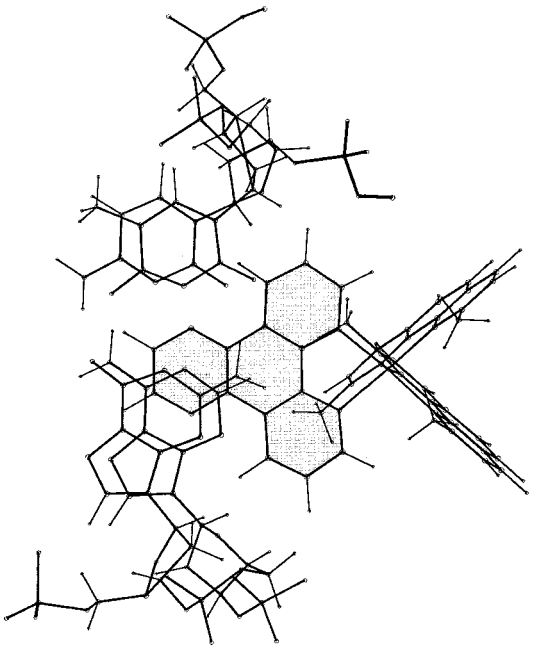

Figure 9. A model showing the intercalative binding of the Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ complex between the G_4A_5 residues of one strand and the T_2C_3 residues of the other strand of the hexanucleotide d(GTCGAC)$_2$. The dmphen rings are located in the minor groove with the dpq ligand (shaded gray) inserted into the nucleotide base stack.

Λ-[Ru(dmphen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ Binding Model. Again, it was only possible to construct a qualitative picture (using the same intercalation site as for the Δ-binding) of the metal complex−hexanucleotide binding. In the Λ-[Ru(dmphen)$_2$dpq]$^{2+}$−hexanucleotide binding model (Figure 9) there are no van der Waals clashes between the metal complex and the hexanucleotide, and the binding model is in good agreement with the observed intermolecular NOEs. Similarly to the Δ-enantiomer binding model, for all observed NOEs between dpq and hexanucleotide protons the corresponding interproton distances in the binding model are ≤5.5 Å. The agreement with the dmphen protons was again also reasonable, with only the H4 and H5 distances to the hexanucleotide protons, to which NOEs were observed, being greater than 5.5 Å.

Figure 9 shows the Λ-[Ru(dmphen)$_2$dpq]$^{2+}$ intercalated between the G_4A_5 bases of one strand and the C_3T_2 bases on the complementary strand. The dmphen ligands reside in the minor groove similarly to the Δ-enantiomer binding; however, there are two significant differences. First, the metal complex is not rotated slightly toward the G_4A_5 strand but is located in the middle of the binding site. This is evidenced, for example, by the strong intermolecular NOEs between the H3 of the metal complex to both the T_2H1′ and G_4H1′ protons (which are on opposite strands) of the hexanucleotide. In Figure 6 the intermolecular NOEs observed to the coincident H3/H11 resonances are predominantly to the H3 proton. This was established in a NOESY spectrum run at 10 °C where the H3 and H11 protons were clearly resolved. The second difference is that the Λ-enantiomer is inserted into the base stack to a lesser degree than is the Δ-enantiomer; that is, the Λ-enantiomer is only partially intercalated. This is evidenced, for example, by the relatively weaker NOEs from the metal complex H10 proton to the hexanucleotide H1′ protons in NOESY spectra of the hexanucleotide with added Λ-complex compared to those with added Δ-enantiomer.

Viscosity Measurements. As the viscosity of a DNA solution is sensitive to the addition of organic drugs and metal complexes which bind by intercalation, we examined the effect on the specific relative viscosity of DNA upon addition of Δ- or Λ-[Ru(dmphen)$_2$dpq]$^{2+}$. Figure 10 shows the change in viscosity upon addition of both enantiomers of the metal complex as well as the known groove-binding molecule Hoechst 33258,[35] and the known intercalating agent ethidium bromide.[30] Consistent with its groove-binding mode of interaction with DNA,[35] Hoechst does not alter the relative viscosity of the DNA. The addition of Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ increased the relative viscosity of the DNA solution (but to a smaller extent than ethidium bromide), consistent with intercalation.[30,36,37] Addition of the Λ-enantiomer, however, decreased the viscosity of the DNA. Such a decrease in the DNA viscosity, due to shortening of the effective length of the helix, is consistent with the Λ-enantiomer binding by partial intercalation.[36,37]

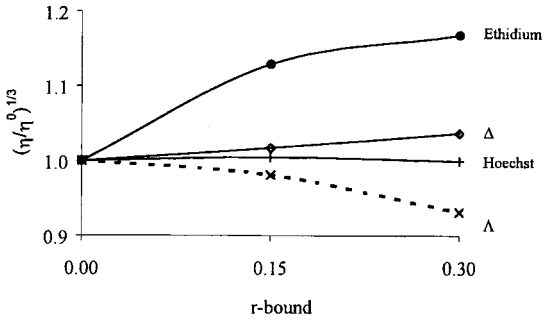

Figure 10. Change in the relative viscosity of a sonicated calf thymus DNA solution upon addition of Δ- or Λ-[Ru(dmphen)$_2$dpq]$^{2+}$, the known groove-binding molecule Hoechst 33258, and the known intercalating agent ethidium bromide. Data are presented as $(\eta/\eta^\circ)^{1/3}$ versus binding ratio (r) where η is the viscosity of the DNA in the presence of the metal complex, Hoechst, or ethidium bromide and η° is the viscosity of the DNA alone.

Discussion

The results of this study strongly suggest that both enantiomers of [Ru(dmphen)$_2$dpq]$^{2+}$ bind the hexanucleotide d(GTCGAC)$_2$ by intercalation and from the minor groove. As intermolecular NOE cross-peaks between the dmphen ligands and the d(GTCGAC)$_2$ protons were only observed to minor groove protons upon binding of either the Δ- or Λ-enantiomers, it is concluded that both enantiomers bind in the hexanucleotide minor groove. The observed large upfield changes in chemical shift of the resonances from the hexanucleotide H1′ and A_5H2 protons support this conclusion.

Addition of Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ to d(GTCGAC)$_2$ induced significant broadening of the resonances from the metal complex, indicating intermediate exchange binding kinetics. The addition of the Δ-enantiomer caused a 13 °C increase in the midpoint of the temperature dependence curve of the resonances from the hexanucleotide. Large upfield shifts of the T_2 and G_4 imino and dpq resonances were observed upon addition of the metal complex. By contrast, only relatively small changes in chemical shift of the dmphen resonances were observed. The addition of Δ-[Ru(dmphen)$_2$dpq]$^{2+}$ increased the relative viscosity of the DNA solution. These observations taken together strongly suggest that the Δ-complex binds the hexanucleotide by intercalation. Consistent with the intercalation binding model are the observed NOEs from the metal complex H10 and H11

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(36) Kapicak, L.; Gabbay, E. J. *J. Am. Chem. Soc.* **1975**, *97*, 403.
(37) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9319.

(closest to the metal center) to the T_2, C_3, G_4, and A_5 minor groove protons, the H12 to both major and minor groove protons, and the H13 to major groove protons only.

The Λ-enantiomer shows fast to intermediate exchange kinetics in its hexanucleotide binding along with significant upfield shifts of the hexanucleotide imino and metal complex dpq resonances. Similarly again to the Δ-enantiomer, the Λ-isomer binding induces an increase in the midpoint of the temperature dependence curve of the resonances from the hexanucleotide, and the NOE data are consistent with intercalation. However, it appears that the Λ-complex does not intercalate as deeply as the Δ-enantiomer. Consistent with the proposed partially intercalated model is the observed decrease in the viscosity of the DNA solution upon addition of Λ-$[Ru(dmphen)_2dpq]^{2+}$.

The results presented here confirm our initial finding that Δ-enantiomers of polypyridyl octahedral complexes can intercalate from the DNA minor groove.[25] The NMR data additionally suggest that intercalation is still favored from the minor groove when additional steric bulk is added to the complex. However, the addition of the 2,9-methyl groups on the phenanthroline ligands does affect the degree to which the dpq ligand is inserted between the stacked base pairs of the hexanucleotide. The NMR data suggest that the Δ-$[Ru(dmphen)_2dpq]^{2+}$ complex intercalates less deeply than Δ-$[Ru(phen)_2dpq]^{2+}$. For Δ-$[Ru(dmphen)_2dpq]^{2+}$ the H13 resonance exhibits a slightly larger upfield shift than the H12 resonance does upon hexanucleotide binding. Alternatively, for Δ-$[Ru(phen)_2dpq]^{2+}$ binding the H12 resonance exhibits a significantly larger upfield shift than the H13 resonance (0.87 and 0.53 ppm, respectively).[25] This is consistent with the H13 proton of Δ-$[Ru(phen)_2dpq]^{2+}$ extending further out into the major groove and the H12 proton being located more directly between the center of the hexanucleotide purine bases. The proposed deep but not full intercalation into the DNA base stack may account for the relatively small increase in the relative viscosity of the DNA solution (compared to ethidium) observed upon addition of the Δ-$[Ru(dmphen)_2dpq]^{2+}$ complex.

More importantly, the results presented here indicate that the Λ-complex also intercalates from the minor groove even though the dpq ligand can only be partially inserted into the hexanucleotide base stack, due to steric clashes with the groove. This suggests that metallointercalators based upon the dpq ligand have a strong preference for the DNA minor groove.

For the development of metallointercalator probes for use as therapeutic agents, it is important that the factors which govern groove selectivity be well understood. Haq et al. have demonstrated that the intercalation of Δ- and Λ-$[Ru(phen)_2dppz]^{2+}$ with DNA is entirely entropically driven, through hydrophobic interactions, changes in hydration, and the release of counterions upon binding.[30] Presumably (as noted by the authors), the favorable enthalpy contribution due to the stacking of the intercalating ligand with the DNA bases is outweighed by the removal of the base−base stacking at the intercalation site and other types of molecular interactions. The square planar platinum complex (terpyridyl)(2-hydroxyethanethiolate)platinum(II) was shown to intercalate from the major groove of a dinucleotide.[38] This may suggest that the entropic factors are maximized by intercalation from the major groove. However, it is likely that the enthalpy term for the binding of the nonbulky planar platinum(II) complex is also favorable.

As Λ-$[Ru(dmphen)_2dpq]^{2+}$ could more fully intercalate from the wide major groove, the observed minor groove binding suggests that the extent of the overlap between the intercalator and DNA base pairs may not be the critical factor in groove specificity. The aromatic overlap between the intercalating ligand from the metal complex and the oligonucleotide bases can result in favorable van der Waals (negative ΔH) and hydrophobic interactions (positive ΔS). Although the Λ-complex only partially intercalates, water could still be effectively excluded from the intercalating ligand; however, partial intercalation would not maximize the van der Waals interactions. As previously noted, the DNA binding of Λ- and Δ-$[Ru(phen)_2dppz]^{2+}$ is entirely entropically driven, and over one-third of the binding free energy may arise from polyelectrolyte contributions, primarily the release of counterions.[30] From the results presented here it could be speculated that these entropic factors are maximized in the minor groove for the $[Ru(dmphen)_2dpq]^{2+}$ complex. Furthermore, the release of counterions and changes in the hydration of both the DNA groove and the metal complex may be the more important determinants of groove selectivity.

Acknowledgment. We thank A. Greguric for his help in the resolution of the Δ- and Λ-enantiomers of the $[Ru(dmphen)_2dpq]^{2+}$ complex.

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