A ^{1}H NMR Study of the DNA Binding of Ruthenium(II) Polypyridyl Complexes

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Received September 19, 1997

^{1}H NMR spectroscopy was used to study the oligonucleotide binding of the Δ enantiomers of $[Ru(phen)_2L]^{2+}$ where the bidentate ligand L is 1,10-phenanthroline (phen), dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq) or dipyrido-[3,2-*a*:2′,3′-*c*](6,7,8,9-tetrahydro)phenazine (dpqC). The data from one- and two-dimensional NMR experiments of the oligonucleotide−metal complex binding suggest that all three ruthenium(II) polypyridyl complexes bind in the DNA minor groove. While a minimally intercalated oligonucleotide binding mode may be proposed for Δ-$[Ru(phen)_3]^{2+}$, the NMR data clearly indicate that Δ-$[Ru(phen)_2dpq]^{2+}$ binds the hexanucleotide $d(GTCGAC)_2$ by intercalation, of the dpq ligand, from the minor groove. This demonstrates that metallointercalators can intercalate from the DNA minor groove. Molecular modeling of the metal complex in the intercalation site suggests that Δ-$[Ru(phen)_2dpq]^{2+}$ binds in a "head-on" fashion with the phenanthroline rings in the minor groove and the dpq ligand inserted into the nucleotide base stack. NOESY experiments of the binding of Δ-$[Ru(phen)_2dpq]^{2+}$ with $d(GTCGAC)_2$ and $d(TCGGGATCCCGA)_2$ suggest that intercalation from the minor groove is favored at purine−purine/pyrimidine−pyrimidine sequences for this complex. The syntheses of Δ-$[Ru(phen)_2dpq]^{2+}$ and Δ-$[Ru(phen)_2dpqC]^{2+}$ are reported along with crystal structure of $[Ru(phen)_2dpq](PF_6)_2$ (monoclinic crystal system, space group $P2_1/c$, $Z = 4$, $a = 9.483(2)$ Å, $b = 33.374(6)$ Å, $c = 12.900(3)$ Å, $\beta = 110.05(2)°$, $V = 3835(2)$ Å^3).

Introduction

The DNA binding ability of inert chiral transition metal complexes has attracted considerable current interest. While recent studies have shown that metal complexes of nonintercalating ligands have significant potential as probes for sequence and structure specific DNA binding,[1−4] most attention has centered upon metal complexes that are capable of binding DNA by intercalation.[5−11] Due to their luminescent characteristics and strong DNA binding affinity the ruthenium(II) polypyridyl class of metallointercalators has received particular attention. Barton and co-workers have shown that complexes such as $[Ru(bpy)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$ (bpy = 2,2′-bipyridine, phen = 1,10 phenanthroline, and dppz = dipyridophenazine) may act as "molecular light switches" for double-helical DNA.[12,13] These complexes show no luminescence in aqueous buffer, but when bound to DNA by intercalation they luminesce brightly. More recently, $[Ru(phen)_2dppz]^{2+}$ was extensively utilized to study long-range fast electron transfer that is mediated by the stacked bases of DNA.[14−18] As the ruthenium(II) polpyridyl complexes have a wide range of applications that are dependent upon their ability to bind DNA by intercalation, it is important that a detailed description of their DNA binding specificity and intercalation geometry be established.

Initial studies of the parent ruthenium(II) polypyridyl, $[Ru(phen)_3]^{2+}$, suggested the existence of two binding modes, a minor groove surface bound interaction, and a major groove intercalated form.[19,20] More recently, Eriksson et al. proposed that both Δ- and Λ-$[Ru(phen)_3]^{2+}$ bound only in a nonintercalative fashion in the minor groove and displayed an AT binding preference.[21,22] Complexes based on the dppz ligand, such as

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S0020-1669(97)01194-4 CCC: $15.00
Published on Web 05/30/1998

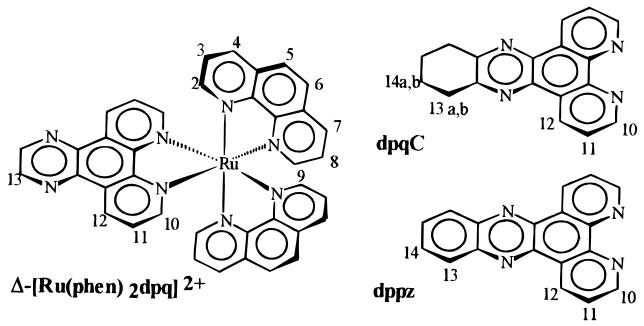

Figure 1. Structure and numbering of the Δ-$[Ru(phen)_2dpq]^{2+}$ complex and the dpqC and dppz ligands.

$[Ru(phen)_2dppz]^{2+}$, have unambiguously been shown to strongly bind ($K_a > 10^6$ M^{-1}) DNA by intercalation; however, their DNA binding geometry is still not firmly established. On the basis of similarities of the DNA binding geometry of $[Ru(phen)_2dppz]^{2+}$ with that of actinomycin D, and the retention of binding to T4 DNA in which all 5-hydroxymethylcytosine residues are glycosylated in the major groove, Lincoln et al. have proposed that the metal complex intercalates from the minor groove.[9] Alternatively, on the basis of NMR evidence Dupureur and Barton have proposed that $[Ru(phen)_2dppz]^{2+}$ intercalates from the major groove.[10,11]

Recently, we reported evidence that suggests that Δ-$[Ru(phen)_2dpq]^{2+}$ (see Figure 1), a complex similar to $[Ru(phen)_2dppz]^{2+}$, binds DNA by intercalation from the minor groove.[23] In this paper we extend our previous preliminary study and present a ^{1}H NMR study of the binding of Δ-$[Ru(phen)_2dpq]^{2+}$ with the self-complementary oligonucleotides d(TCGGGATCCCGA) and d(GTCGAC). In addition, the results of an NMR study of the binding of Δ-$[Ru(phen)_3]^{2+}$ with $d(TCGGGATCCCGA)_2$ and Δ-$[Ru(phen)_2dpqC]^{2+}$ (see Figure 1) with $d(GTCGAC)_2$ are also presented and compared to the Δ-$[Ru(phen)_2dpq]^{2+}$−oligonucleotide binding results. The results described here are discussed in terms of general DNA−ruthenium(II) polypyridyl binding.

Experimental Section

Materials. The oligonucleotides $d(GTCGAC)_2$ and $d(TCGGGATCCCGA)_2$ were obtained from Bresatec Ltd. (South Australia). D_2O (99.96%), $RuCl_3$, and 1,10-phenanthroline monohydrate were obtained from Aldrich Chemical Co., while CM-Sephadex was obtained from Pharmacia.

Synthesis and Resolution of Metal Complexes. Δ-$[Ru(phen)_3]^{2+}$ was synthesized and resolved by the method of Dwyer and Gyarfas.[24]

$[Ru(phen)_2dpq]^{2+}$. The dpq ligand (dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline) was prepared by stirring a mixture of 1,10-phenanthroline-5,6-dione (1.0 g, 4.8 mmol) and ethylenediamine (0.4 g, 6.7 mmol) in ethanol (350 mL) for 2 h at 40 °C and then at room-temperature overnight. The resulting solution was reduced in volume by rotary evaporation at 50 °C to yield a cream product. The crude product was left to stand for 8 h, methanol/water (10/90) was then added, and the product was filtered and recrystallized from methanol to give a cream solid. Yield: 1.0 g, 91% (mp 331 °C). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 9.43 (d, $J = 8.2$ Hz, 2H), 9.22 (d, $J = 4.4$ Hz, 2H), 9.13 (s, 2H), 7.92 (dd, $J = 8.2$, 4.4 Hz, 2H).

Table 1. Crystallographic Data and Details of Data Collection of $[Ru(phen)_2dpq](PF_6)_2$

crystal system	monoclinic
space group	$P2_1/c$
a, Å	9.483(2)
b, Å	33.374(6)
c, Å	12.900(3)
β, deg	110.05(2)
V, Å^3	3835(2)
fw	983.8
D_{calcd}, g cm^{-3}	1.414
empirical formula	$C_{38}H_{24}F_{12}N_8P_2Ru$
Z	4
absorp coeff, cm^{-1}	5.93
transm coeffs	0. 945−0.930
temp, °C	21
λ, Å	0.710 69
R (F_o)[a]	0.051
R_w[b]	0.047

[a] $R = \sum(||F_o| - |F_c||)/\sum|F_o|$. [b] $R_w = (\sum w(|F_o| - |F_c|)^2/\sum wF_o^2)^{1/2}$.

$[Ru(phen)_2dpq]^{2+}$ was prepared by refluxing 1.0 g of $[Ru(phen)_2Cl_2]$ and 0.5 g (1.1 equiv) of dpq in 450 mL of 75% ethanol for 5 h. The volume was then reduced (50 mL), the solution was cooled, and excess KPF_6 was added. The resultant orange precipitate was filtered and washed with water (100 mL) and then ether (50 mL). The solid was dissolved in acetonitrile (20 mL) and applied to the head of a column (5 × 30 cm) of activated aluminum oxide (neutral Brockmann 1). The orange band was eluted with acetonitrile, and to this fraction was added water (20 mL) to yield fine orange needles (1.5 g, 80%). ^{1}H NMR (d_6-acetone, 400 MHz): δ (ppm) 9.6 (d, $J = 8.4$ Hz, 2H), 9.3 (s, 2H), 8.8 (d, $J = 8.1$ Hz, 4H), 8.5 (d, $J = 5.4$ Hz, 4H), 8.4 (s, 4H), 8.4 (d, $J = 5.1$ Hz, 2H), 7.9 (dd, $J = 5.1$, 8.1 Hz, 2H), 7.8 (dd, $J = 5.4$, 8.4 Hz, 4H). MS (ESMS, CH_3CN, MW = 983.8); m/z 838.9 (M − PF_6^-), 693.9 [M − 2(PF_6^-)].

$[Ru(phen)_2dpqC]^{2+}$. The dpqC ligand (dipyrido[3,2-*a*:2′,3′-*c*]-(6,7,8,9-tetrahydro)phenazine) was prepared by refluxing a mixture of 1,10-phenanthroline-5,6-dione (1.0 g, 4.8 mmol) and 1,2-diaminocyclohexane (cis and trans, 0.7 g, 6.3 mmol) in ethanol (120 mL) for 2 h. The resulting yellow solution was reduced in volume by rotary evaporation at 50 °C until a pale yellow solid was observed. The product was filtered and recrystallized from methanol to give pale yellow needles. Yield: 1.3 g, 95% (mp 308−310 °C). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 9.35 (d, $J = 8.0$ Hz, 2H), 9.17 (dd, $J = 8.0$, 2.0 Hz, 2H), 7.87 (dd, $J = 8.0$, 8.0 Hz, 2H), 3.20 (m, H8a,b, 4H), 2.10, (m, H9a,b, 4H).

$[Ru(phen)_2dpqC]^{2+}$ was prepared by refluxing 1.0 g of $[Ru(phen)_2Cl_2]$ and 0.6 g (1.1 equiv) of dpqC in 450 mL of 90% ethanol for 6 h. The reaction mixture was reduced in volume (50 mL) and cooled, and excess KPF_6 was added. The resultant precipitate was filtered and washed with water (100 mL) and ether (50 mL). The solid was dissolved in acetone (20 mL) and applied to the head of a column (5 × 30 cm) of activated aluminum oxide (neutral Brockmann 1). The orange band was eluted with acetone, and to this fraction was added water (20 mL) to yield fine orange needles (1.4 g, 70%). ^{1}H NMR (d_6-acetone, 400 MHz): δ (ppm) 9.4 (d, $J = 8.1$ Hz, 2H), 8.8 (d, $J = 8.1$ Hz, 4H), 8.5 (d, $J = 5.1$ Hz, 2H), 8.4 (d, $J = 5.1$ Hz, 2H), 8.4 (s, 4H), 8.4 (d, $J = 5.1$ Hz, 2H), 7.9 (dd, $J = 5.1$, 8.1 Hz, 2H), 7.8 (dd, $J = 5.1$, 8.1 Hz, 4H), 3.3 (m, 4H), 2.1 (m, 4H). MS (ESMS, CH_3CN, MW = 1037.8): m/z 893.4 (M − PF_6^-).

Enantiomer Resolution. The enantiomers of both $[Ru(phen)_2dpq]^{2+}$ and $[Ru(phen)_2dpqC]^{2+}$ (typically 50 mg) were resolved on a Sephadex CM C-25 column (40 × 2.5 cm) using 0.1 M potassium antimonyl tartrate as the eluent. The enantiomeric purity of the chloride salt was assayed by CD spectroscopy, with the Δ-enantiomers displaying negative circular dichroism at 310 and 464 nm.

Crystallography. Cell constants were determined by a least-squares fit to the setting parameters of 25 independent reflections, measured and refined on an Enraf-Nonius CAD4-F diffractometer. The crystallographic data are summarized in Table 1. Data reduction and application of Lorentz, polarization, and analytical absorption correc-

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tions were carried out using teXsan.[25] The structure was solved by direct methods using SHELXS-86[26] and refined using full-matrix least-squares methods with teXsan.[25] Hydrogen atoms were included at calculated sites with thermal parameters derived from the parent atoms. Non-hydrogen atoms were refined anisotropically. Scattering factors and anomalous dispersion terms for Ru (neutral Ru) were taken from *International Tables*.[27] Anomalous dispersion effects were included in F_c;[28] the values for *Df′* and *Df″* were those of Creagh and McAuley.[29] The values for the mass attenuation coefficients are those of Creagh and Hubbell.[30] All other calculations were performed using the teXsan[25] crystallographic software package of Molecular Structure Corporation.

Sample Preparation for NMR Analysis. Oligonucleotides were converted into the Na^+ form using a small CM-Sephadex column. The oligonucleotide was dissolved in 0.7 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. Oligonucleotide concentration was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide.[31]

Instrumental Methods. 400 MHz ^{1}H NMR spectra were recorded on a Varian Unity*plus*-400 spectrometer. Spectra recorded in 90% H_2O/10% D_2O were collected using the WATERGATE solvent suppression technique of Piotto et al.[32] Two-dimensional phase sensitive NOESY spectra were recorded by the method of States et al.,[33] 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 256−310 t_1 values with a pulse repetition delay of 1.7 s. Circular dichroism spectra (CD) were recorded at ambient temperature on a Jasco 500C spectropolarimeter. The electrospray mass spectra were obtained with a Fisons/VG Biotech Quattro (Altrincham, UK) mass spectrometer.

Molecular Modeling. The coordinates for the Δ-$[Ru(phen)_2dpq]^{2+}$ complex were taken from the crystal structure while the hexanucleotide intercalation site was constructed using Biosym molecular modeling software (Biosym Technologies, Inc). The intercalative docking of the metal complex was done manually using the molecular modeling program 3Dmol (R. S. Vagg, Macquarie University, Australia).

Results

Assignment of the Proton Resonances of d(GTCGAC)$_2$ and d(TCGGGATCCCGA)$_2$. The ^{1}H NMR resonances of the free oligonucleotides were assigned from a combination of NOESY and DQFCOSY experiments, according to well-established methods.[34−36] The solution conformation of the oligonucleotides were determined by analysis of the NOESY spectra. For a right-handed duplex each aromatic H8 and H6 proton should exhibit an NOE to its own H1′, H2′, and H2″ sugar protons as well as to the H1′, H2′, and H2″ protons on the flanking 5′-sugar (the sugar of the nucleotide in the 5′-direction).[35,36] For a B-type DNA conformation the distance between the base H8/H6 and its own H2′ is approximately 2 Å and approximately 4 Å to the H2′ proton on the flanking 5′-sugar,[36] whereas for an A-type duplex the relative distances are reversed, with the shorter distance being to the H2′ proton on the 5′-sugar.[36] In the NOESY spectra of both oligonucleotides 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. Additionally, 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 both oligonucleotides adopt a B-type conformation.

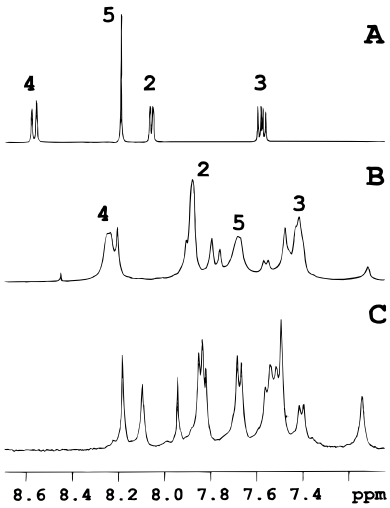

Figure 2. ^{1}H NMR spectra of the aromatic proton region of (A) Δ-$[Ru(phen)_3]^{2+}$; (B) the dodecanucleotide with added Δ-$[Ru(phen)_3]^{2+}$, at a metal complex-to-dodecanucleotide ratio of 1, in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C; and (C) the free dodecanucleotide d(TCGGGATCCCGA)$_2$.

The imino resonances in the NMR spectra of the oligonucleotides dissolved in 90% H_2O/10% D_2O were examined to determine the extent of the nucleotide base pairing. The spectra indicated that for each oligonucleotide only the terminal residue did not form a stable base-pair, with five imino resonances being observed in the spectrum of the dodecanucleotide and two imino resonances in the spectrum of the hexanucleotide at 25 °C.

Δ-$[Ru(phen)_3]^{2+}$−d(TCGGGATCCCGA)$_2$ Binding Studies. The NMR spectrum of d(TCGGGATCCCGA)$_2$ with added Δ-$[Ru(phen)_3]^{2+}$ is shown in Figure 2 together with the spectra of the free metal complex and dodecanucleotide. In agreement with other $[Ru(phen)_3]^{2+}$−oligonucleotide binding studies,[19−22] only one set of dodecanucleotide and metal complex resonances are observed, with the H2, H3, H4, and H5 phenanthroline protons equivalent to the H9, H8, H7, and H6 protons. The dodecanucleotide resonances do not significantly broaden upon addition of Δ-$[Ru(phen)_3]^{2+}$. In contrast to previous studies,[19−22] however, the resonances from the metal complex do exhibit some line broadening upon dodecanucleotide binding, particularly the H5 resonance. This indicates that Δ-$[Ru(phen)_3]^{2+}$ exhibits intermediate-to-fast exchange kinetics (on the NMR time scale) in its binding to the dodecanucleotide. The addition of the metal complex also induces selective chemical shift changes of the resonances from the dodecanucleotide (see Table 2). The largest chemical shift changes are observed for the resonances from the G_4, G_5, A_6, T_7, C_8, and C_9 residues. In particular, large shifts are observed for the H1′ and H2″ resonances of T_7, C_8, and C_9. These selective chemical shift

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Table 2. ^{1}H NMR Chemical Shifts (in ppm) of d(TCGGGATCCCGA)$_2$ and the Chemical Shift Differences Induced by the Addition of Δ-Ru(phen)$_3^{2+}$ (Numbers in Parentheses), at a Metal Complex-to-Dodecanucleotide Ratio of 0.9, in 10 mM Phosphate Buffer (pH 7) Containing 20 mM NaCl at 25 °C; Positive Numbers Indicate a Downfield Shift

	oligonucleotide proton				
	H8/H6	AH2	H1′	H2′	H2″
T_1	7.51 (−0.02)		6.08 (−0.08)	2.12 (−0.09)	2.47 (−0.11)
C_2	7.56 (−0.02)		5.58 (−0.02)	2.11 (−0.07)	2.37 (−0.05)
G_3	7.85 (0.05)		5.48 (0.00)	2.62 (0.00)	2.67 (−0.05)
G_4	7.69 (0.12)		5.70 (−0.10)	2.58 (0.07)	2.74 (−0.09)
G_5	7.68 (0.11)		5.65 (−0.09)	2.59 (−0.01)	2.73 (−0.08)
A_6	8.10 (0.10)	7.82 (−0.16)	6.22 (−0.06)	2.56 (0.02)	2.92 (−0.07)
T_7	7.14 (−0.02)		5.92 (−0.18)	2.04 (−0.10)	2.49 (−0.27)
C_8	7.54 (−0.08)		5.97 (−0.19)	2.18 (−0.17)	2.45 (−0.23)
C_9	7.52 (−0.06)		5.91 (−0.24)	2.08 (−0.10)	2.40 (−0.29)
C_{10}	7.41 (0.02)		5.48 (−0.02)	1.81 (0.01)	2.19 (−0.03)
G_{11}	7.84 (0.05)		5.69 (−0.04)	2.55 (0.05)	2.64 (−0.04)
A_{12}	8.18 (0.02)	7.94 (−0.04)	6.33 (−0.07)	2.63 (−0.02)	2.46 (−0.07)

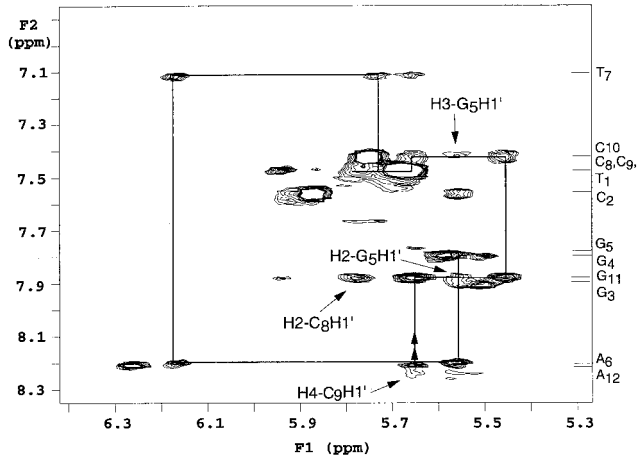

Figure 3. Expansion of the NOESY spectrum (300 ms mixing time) of d(TCGGGATCCCGA)$_2$ (1.3 mM) with added Δ-[Ru(phen)$_3$]$^{2+}$, at a metal complex-to-dodecanucleotide ratio of 0.9, in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 30 °C. The expansion shows the NOE connectivities between the metal complex and dodecanucleotide aromatic protons (7.1−8.3 ppm) and the sugar H1′ protons (5.3−6.3 ppm). The intermolecular NOE cross-peaks between Δ-[Ru(phen)$_3$]$^{2+}$ and the dodecanucleotide are indicated. The sequential NOE connectivities, starting from A_{12}H8−G_{11}H1′ (double arrowheads) and going through to G_5H8−G_5H1′, are also shown.

changes suggest that the Δ-[Ru(phen)$_3$]$^{2+}$ binds the dodecanucleotide at the T_7−C_9 region. However, changes in chemical shifts alone are insufficient evidence to establish the binding position of the metal complex on the dodecanucleotide. The resonances from the metal complex also exhibit significant upfield shifts upon binding to the dodecanucleotide. The H5 displays the largest shift (0.56 ppm) with the H4 (0.33 ppm), H3 (0.16 ppm), and H2 (0.20 ppm) displaying smaller chemical shift changes.

NOESY spectra of d(TCGGGATCCCGA)$_2$ with added Δ-[Ru(phen)$_3$]$^{2+}$ were recorded at metal complex-to-dodecanucleotide ratios (R) of 0.9 and 1.8 over a range of temperatures. Relatively long mixing time experiments were required to observe metal complex−dodecanucleotide NOE cross-peaks, in agreement with Eriksson et al.[21,22] Figure 3 shows the base H8/H6 to sugar H1′ region of a NOESY spectrum of the dodecanucleotide with added Δ-[Ru(phen)$_3$]$^{2+}$ (R = 0.9). In addition to the expected dodecanucleotide sequential NOEs, NOE cross-peaks between the metal complex and the H1′ protons of G_5, C_8, and C_9 are observed. The C_8H1′ resonance cannot be directly assigned from the base H8/H6 to sugar H1′ region shown in Figure 3. The C_8H1′ resonance was assigned by examination of the H1′ to H2′/H2″ region in the same NOESY spectrum, after the C_8H2′/H2″ resonances had been assigned from the base H8/H6 to sugar H2′/H2″ region of the spectrum. Consistent with the assignment of the C_8H1′ is the observation of NOE cross-peaks from the A_6H2 (7.67 ppm) to both the C_8H1′ (5.78 ppm) and T_7H1′ (5.74 ppm) resonances in both the free and Δ-[Ru(phen)$_3$]$^{2+}$ bound dodecanucleotide. An NOE between an AH2 resonance and the closest cross-strand H1′ resonance (and the H1′ resonance of the same strand 3′-nucleotide) is generally observed in NOESY spectra of oligonucleotides, and has been used as an indicator of minor groove width.[37,38] In agreement with the assignment of the T_7H1′ (5.74 ppm), is the observation of three NOE cross-peaks from the T_7H6 in Figure 3. For a B-type DNA duplex, NOEs from the T_7H6 to the A_6H1′ (6.16 ppm), to the T_7H1′ (5.74 ppm) and to the C_8H5 (5.67 ppm) protons are expected and observed.

As the H1′ protons are located in the dodecanucleotide minor groove, the NOE cross-peaks observed between the Δ-[Ru(phen)$_3$]$^{2+}$ and the H1′ protons of G_5, C_8, and C_9 suggest that the metal complex binds in the minor groove at the C_8C_9 (and equivalent G_4G_5) region. This conclusion is supported by the observation of NOE cross-peaks between protons from the metal complex to the minor groove H4′/H5′/H5″ protons of C_8 and C_9 (data not shown).

The metal complex binding does not induce any significant structural changes to the conformation of the dodecanucleotide. As the observed intermolecular NOE cross-peaks represent an average for the H2, H3, H4, and H5 protons from all three phenanthroline rings it is not possible to determine an accurate picture of the metal complex−dodecanucleotide binding.

Δ-[Ru(phen)$_2$dpq]$^{2+}$ Binding Studies. [Ru(phen)$_2$dpq]$^{2+}$ Crystal Structure. The structure consists of the dipositive Ru complex and two PF_6^- anions. One of the anions is rotationally disordered over two sites. An ORTEP[39] diagram of the structure of [Ru(phen)$_2$dpq]$^{2+}$ with the atomic numbering scheme is shown in Figure 4. The geometry about the Ru atom is distorted from octahedral as a consequence of the small bite angles of the bidentate ligands (78.4−80.0°). The phen ligands each make an angle of close to 90° with the dpq ligand but make an angle of 79° with each other, revealing a further but unexpected deviation from octahedral geometry. This deviation may be a consequence of the crystal packing. The dpq ligands are stacked with respect to each other and make T-shaped contacts with the face of one of the phen ligands. The dpq ligand and one of

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(38) Chuprina, V. P.; Lipanov, A. A.; Fedoroff, O. Y.; Kim, S.-G.; Kintanar, A.; Reid, B. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9087.
(39) Johnson, C. K. *ORTEP, A Thermal Ellipsoid Plotting Program*; Oak Ridge National Laboratories, Oak Ridge, TN, 1965.

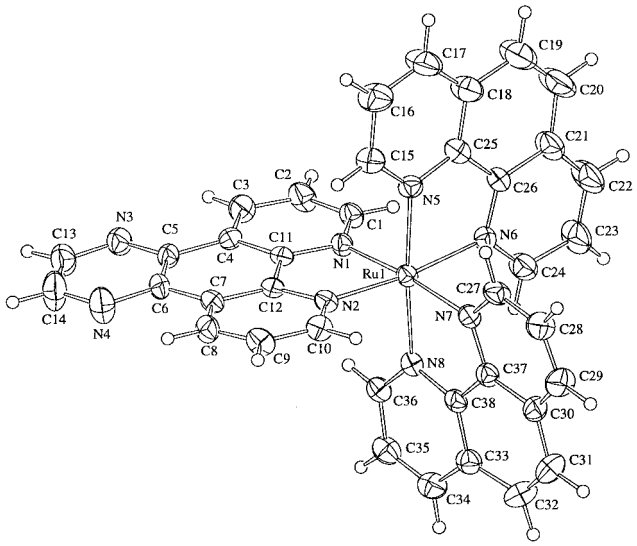

Figure 4. ORTEP plot of the $[Ru(phen)_2dpq]^{2+}$ cation giving the crystallographic atom numbering. The 30% probability ellipsoids are shown.

Table 3. Selected Distances (Å) and Angles (deg) in $[Ru(phen)_2dpq]^{2+}$

	Distances		
Ru(1)−N(1)	2.043(5)	Ru(1)−N(2)	2.063(5)
Ru(1)−N(5)	2.071(6)	Ru(1)−N(6)	2.073(6)
Ru(1)−N(7)	2.068(5)	Ru(1)−N(8)	2.065(6)
	Bond Angles		
N(1)−Ru(1)−N(2)	79.6(2)	N(1)−Ru(1)−N(5)	88.2(2)
N(1)−Ru(1)−N(6)	96.9(2)	N(1)−Ru(1)−N(7)	174.0(2)
N(1)−Ru(1)−N(8)	94.7(2)	N(2)−Ru(1)−N(5)	95.4(2)
N(2)−Ru(1)−N(6)	173.0(2)	N(2)−Ru(1)−N(7)	97.4(2)
N(2)−Ru(1)−N(8)	89.1(2)	N(5)−Ru(1)−N(6)	78.4(2)
N(5)−Ru(1)−N(7)	97.2(2)	N(5)−Ru(1)−N(8)	175.0(2)
N(6)−Ru(1)−N(7)	86.7(2)	N(6)−Ru(1)−N(8)	97.3(2)
N(7)−Ru(1)−N(8)	80.0(2)		

the phen ligands are planar to within 0.08 Å, but the other is slightly folded about its central axis, resulting in deviation of up to 0.12 Å. Ru−N bond lengths cover a narrow range [2.043(5)−2.073(6) Å], and the geometries of the phen and dpq ligands are similar. Selected bond lengths and angles are listed in Table 3. Listings of atom coordinates, complete tables of bond lengths and angles, anisotropic thermal parameters, and details of least-squares planes calculations are included in the Supporting Information.

Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ Binding. The resonances of the free and d(GTCGAC)$_2$-bound Δ-$[Ru(phen)_2dpq]^{2+}$ were assigned by a combination of NMR experiments. For the free metal complex, the NMR resonances from the phenanthroline protons are easily distinguished from the dpq protons by integration of the resonances. Then, within each ring system the resonances were assigned to particular protons from a COSY spectrum and by comparison to previously reported assignments for Δ-$[Ru(phen)_3]^{2+}$ and Δ-$[Ru(phen)_2dppz]^{2+}$.[11,22] The resonances of the bound Δ-$[Ru(phen)_2dpq]^{2+}$ were assigned through a combination of COSY and NOESY experiments, and by recording the one-dimensional spectrum as a function of temperature. The H13 and H12 were assigned by following their resonances over the temperature range 25−65 °C, with the H11 and H10 resonances then being assigned from COSY experiments. For the phenanthroline resonances, two separate spin systems are observed: the (H2, H3, and H4) and the (H9, H8, and H7). The H2 resonance (8.19 ppm) was assigned through the observation of a weak NOE between the resonance at 8.19 ppm and the H11 (dpq) proton, as the H2−H11 distance is 1 Å shorter than the H9−H11 distance.

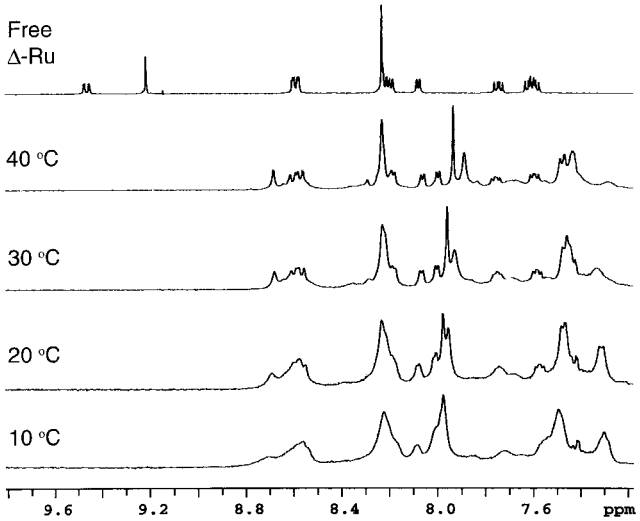

Figure 5. ^{1}H NMR spectra of d(GTCGAC)$_2$ (1 mM) with added Δ-$[Ru(phen)_2dpq]^{2+}$, at a metal complex-to-hexanucleotide ratio (R) of 0.8, as function of temperature. The spectrum of the free metal complex (1 mM), in 10 mM phosphate buffer (pH 7) containing 20 mM NaCl at 25 °C is also shown (Free Δ-Ru). The assignments of the resonances from the free metal complex and the hexanucleotide with added metal complex (R = 1) are given in Tables 4 and 5.

Table 4. ^{1}H NMR Chemical Shifts of the Free Δ-$[Ru(phen)_2dpq]^{2+}$ and the Hexanucleotide-Bound Δ-$[Ru(phen)_2dpq]^{2+}$, in 10 mM Phosphate Buffer (pH 7) Containing 20 mM NaCl at 25 °C

ligand proton	free $[Ru(phen)_2dpq]^{2+}$	d(GTCGAC)$_2$ bound $[Ru(phen)_2dpq]^{2+}$	change in shift upon binding
dpq			
H13	9.22	8.69	−0.53
H12	9.45	8.58	−0.87
H11	7.74	7.30	−0.44
H10	8.22	8.08	−0.14
phen			
H9	8.08	8.00	−0.08
H8	7.60	7.57	−0.03
H7	8.59	8.57	−0.02
H5/H6	8.23	8.23	0.00
H4	8.59	8.59	0.00
H3	7.60	7.73	0.13
H2	8.19	8.19	0.00

Addition of Δ-$[Ru(phen)_2dpq]^{2+}$ to d(GTCGAC)$_2$ induced large upfield chemical shift changes for the dpq resonances (see Table 4). These large upfield shifts of the dpq resonances are consistent with the metal complex binding the hexanucleotide by intercalation.[10,11] By contrast, resonances from the phenanthroline rings exhibited only small chemical shift changes upon addition of Δ-$[Ru(phen)_2dpq]^{2+}$ to d(GTCGAC)$_2$, suggesting that the dpq ring system selectively intercalates. Addition of the metal complex to the hexanucleotide also induced significant broadening of the resonances (due to intermediate exchange on the NMR time scale) from both the Δ-$[Ru(phen)_2dpq]^{2+}$ and d(GTCGAC)$_2$ (see Figure 5), again consistent with intercalation.[10,11,21] At temperatures below 25 °C the metal complex and hexanucleotide resonances were even broader, indicating that the intermediate exchange kinetics could not be changed to a slow exchange regime.

Significant chemical shift changes were also observed for various hexanucleotide resonances upon addition of Δ-$[Ru(phen)_2dpq]^{2+}$ (see Table 5). In particular, the resonances from the H1′ protons, which are located in the DNA minor groove,

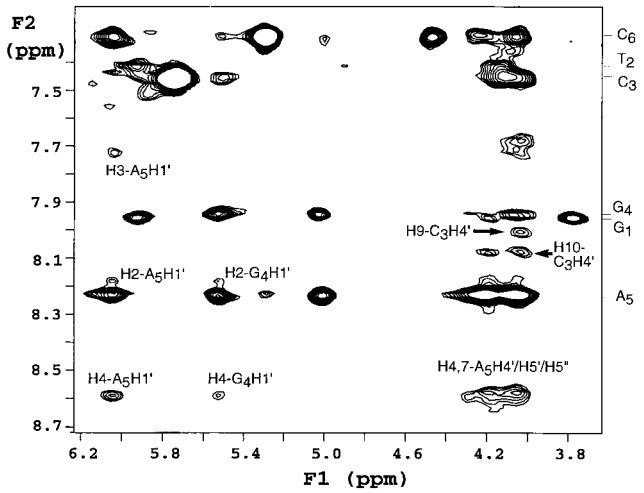

Figure 6. Expansion of the NOESY spectrum (350 ms mixing time) of Δ-[Ru(phen)$_2$dpq]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex-to-duplex ratio of 1, showing the NOE connectivities from the hexanucleotide base and metal complex protons (7.3−8.8 ppm) to the hexanucleotide sugar H1′, H3′, and H4′ protons (3.8−6.2 ppm). NOEs between the metal protons and hexanucleotide sugar H1′ and H4′ protons are shown. The H4−A$_5$H1′ and H4,7−A$_5$H4′/H5′/H5″ cross-peaks were also observed in 150 and 250 ms mixing time NOESY experiments, while the H9 and H10 to C$_3$H4′ NOE cross-peaks were also observed in 250 ms mixing time experiments. No NOEs from the metal complex to the major groove G$_4$H3′ and A$_5$H3′ protons are observed, consistent with minor groove binding.

Table 5. ^{1}H NMR Chemical Shifts (in ppm) of d(GTCGAC)$_2$ and the Chemical Shift Differences Induced by the Addition of Δ-[Ru(phen)$_2$dpq]$^{2+}$ at a Metal Complex-to-Duplex Ratio of 1 (Numbers in Parentheses), in 10 mM Phosphate (pH 7) Containing 20 mM NaCl at 25 °C

	oligonucleotide proton				
	H8/H6	H1′	H2′	H2″	H3′
G$_1$	8.01 (−0.05)	6.07 (−0.14)	2.73 (−0.12)	2.81 (−0.09)	4.85 (−0.02)
T$_2$	7.52 (−0.10)	6.21 (−0.15)	2.25 (−0.05)	2.59 (−0.14)	4.93 (−0.03)
C$_3$	7.51 (−0.05)	5.72 (−0.20)	2.05 (−0.14)	2.42 (−0.14)	4.87 (−0.07)
G$_4$	7.96 (−0.02)	5.64 (−0.11)	2.74 (−0.02)	2.79 (−0.07)	5.03 (0.00)
A$_5$	8.19 (0.04)	6.29 (−0.25)	2.65 (0.01)	2.89 (−0.06)	5.02 (−0.01)
C$_6$	7.35 (−0.04)	6.07 (−0.02)	2.09 (0.00)	2.09 (0.00)	4.48 (−0.01)

of T$_2$, C$_3$, and A$_5$ exhibited large upfield shifts. Alternatively, the resonances from protons located in the major groove (H8/H6, H2′, and H3′) showed significantly smaller shifts upon addition of the metal complex. In spectra of the hexanucleotide recorded in 90% H$_2$O/10% D$_2$O at a range of temperatures, the addition of Δ-[Ru(phen)$_2$dpq]$^{2+}$ induced upfield shifts and extensive broadening of the imino resonances (which precluded the observation of metal complex−imino cross-peaks in NOESY experiments).

NOESY spectra of d(GTCGAC)$_2$ with added Δ-[Ru(phen)$_2$dpq]$^{2+}$ ($R = 1$) were recorded at 25, 35, and 45 °C for mixing times ranging from 150 to 400 ms. In addition to the expected intraduplex sequential NOE cross-peaks, a number of intermolecular NOE cross-peaks between the Δ-[Ru(phen)$_2$dpq]$^{2+}$ and d(GTCGAC)$_2$ were observed. Of note are the NOE cross-peaks from the metal complex H2, H3, and H4 to the minor groove G$_4$H1′ and A$_5$H1′ protons (see Figure 6). These data strongly suggest that the Δ-[Ru(phen)$_2$dpq]$^{2+}$ binds in the hexanucleotide minor groove. Consistent with this proposal is the observation of NOEs between the metal complex and hexanucleotide H4′ (located in the minor groove) and H5′/H5″ (most accessible from the minor groove) protons (see Figure 6). The combined one- and two-dimensional NMR results indicate that the Δ-[Ru(phen)$_2$dpq]$^{2+}$ binds the hexanucleotide

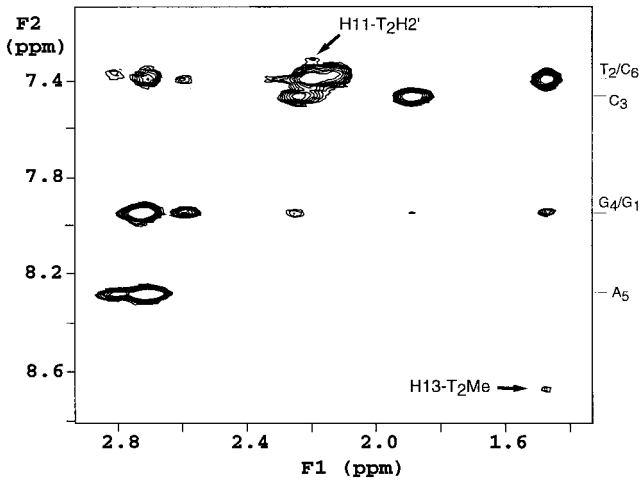

Figure 7. Expansion of the NOESY spectrum (250 ms mixing time) of Δ-[Ru(phen)$_2$dpq]$^{2+}$ and d(GTCGAC)$_2$, at a metal complex to duplex (2 mM) ratio of 1, in 10 mM phosphate (pH 7) containing 20 mM NaCl at 35 °C, showing the hexanucleotide base and metal complex aromatic (7.4−8.8 ppm) to hexanucleotide H2′/H2″ and T methyl (1.4 to 2.8 ppm) region. NOEs between the metal complex H13 and H11 and the hexanucleotide major groove T$_2$Me and T$_2$H2′ protons are shown.

by intercalation at the G$_4$A$_5$/T$_2$C$_3$ sequence. Consistent with this intercalation model is the observation of an NOE between the H13 (dpq ring) and the major groove T$_2$ methyl protons (see Figure 7). This indicates that the dpq ring system spans the stacked base pairs (intercalation) and extends into the major groove. As the metal complex binds by intercalation, a selective loss of intensity of the sequential H8/H6−H1′/H2′/H2″ NOEs might be expected because the distance between the stacked bases at the intercalation site will significantly increase. No clear selective loss in the intensity of the intrastrand sequential NOE cross-peaks was detected. However, as the metal complex can intercalate between either T$_2$ and C$_3$ or the symmetrically related G$_4$ and A$_5$, only a partial loss of intensity of the sequential NOEs would be expected. Additionally, the different degrees of broadening of the various hexanucleotide resonances, induced by the addition of Δ-[Ru(phen)$_2$dpq]$^{2+}$, hinder the observation of a selective partial loss of the sequential NOEs.

The basic B-type conformation of the hexanucleotide is maintained upon the Δ-[Ru(phen)$_2$dpq]$^{2+}$, binding. The NOE cross-peak intensity from each base H8/H6 to its own sugar protons was found to decrease in the order H2′ > H1′ > H3′ consistent with B-type DNA.

In the one-dimensional NMR spectrum of d(GTCGAC)$_2$ with added Δ-[Ru(phen)$_2$dpq]$^{2+}$ a second set of resonances from a bound metal complex was observed. The NMR spectrum of the free Δ-[Ru(phen)$_2$dpq]$^{2+}$ indicates that the second binding form is not due to a metal complex impurity. CD measurements and analysis of the ^{1}H NMR spectrum of the metal complex with added potassium antimonyl tartrate (compared to spectra of various enantiomeric mixtures with added potassium antimonyl tartrate) indicate that the Δ-[Ru(phen)$_2$dpq]$^{2+}$ is >95% enantiomerically pure. The second set of resonances was therefore assigned to a minor (approximately 15% of the metal complex) Δ-[Ru(phen)$_2$dpq]$^{2+}$ binding form. As a separate set of broad resonances are observed for this minor hexanucleotide binding form, it is concluded that the minor form exhibits intermediate exchange in its hexanucleotide binding but is in slow exchange with the major hexanucleotide binding form. This is further evidenced by the observation of two distinct sets of metal complex cross-peaks in COSY experiments, even at 45

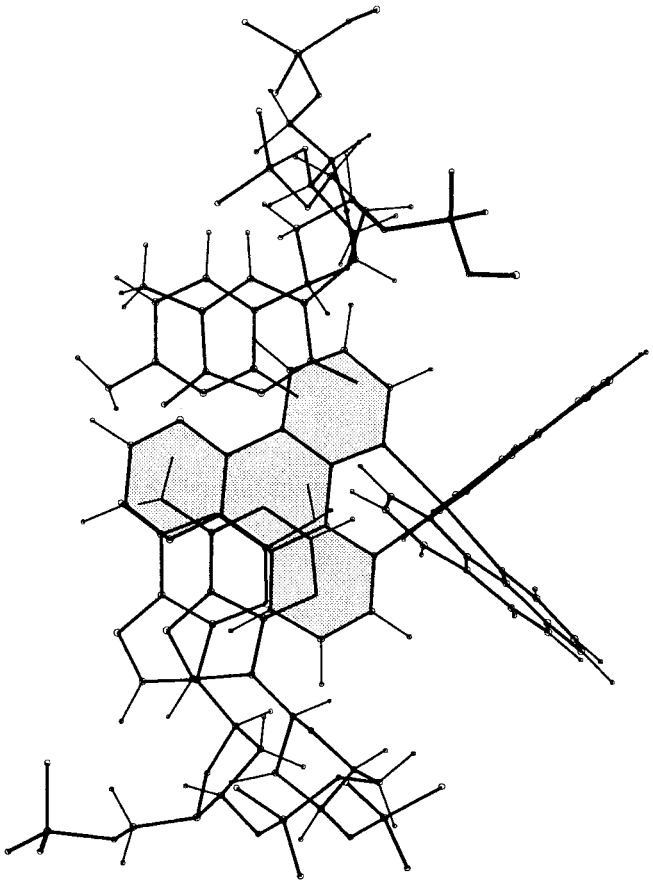

Figure 8. Model showing the intercalative binding of the Δ-[Ru(phen)$_2$dpq]$^{2+}$ complex between the G_4A_5 residues of one strand and the T_2C_3 residues of the other strand of the hexanucleotide d(GTCGAC)$_2$. The phenanthroline rings are located in the minor groove with the dpq ring (gray shaded) inserted into the nucleotide base stack. The metal complex is shown binding in a "head-on fashion" (5° rotation toward the T_2C_3 strand). In this model there are several metal complex-to-hexanucleotide H−H distances that are not consistent with van der Waals requirements. These van der Waals clashes could be removed by inserting the metal complex less deeply into the intercalation site. However, as canonical form B-type DNA was used to model the intercalation site, the van der Waals clashes could also be removed by slight conformational changes of the hexanucleotide at the intercalation site.

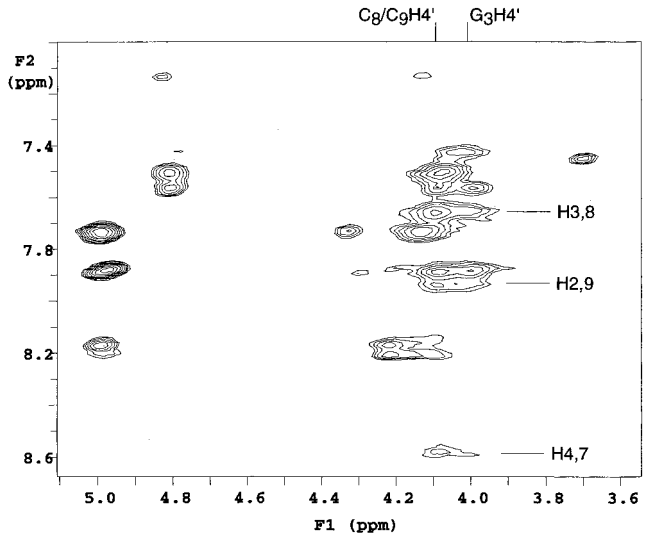

Figure 9. Expansion of the NOESY spectrum (250 ms mixing time) of Δ-[Ru(phen)$_2$dpq]$^{2+}$ and d(TCGGGATCCCGA)$_2$, at a metal complex-to-duplex (1.3 mM) ratio of 1, in 10 mM phosphate (pH 7) containing 20 mM NaCl at 35 °C, showing the dodecanucleotide base and metal complex aromatic (7.1−8.6 ppm) to hexanucleotide H3′ and H4′ (3.6−5.1 ppm) region. NOEs between the metal complex and dodecanucleotide H4′ protons are shown.

°C (data not shown). It was not possible to establish the binding position of this minor hexanucleotide binding form.

Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ Binding Model. Due to the significant selective broadening of the exchange-averaged resonances in the spectra of the Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$, it is not possible to determine a quantitative binding model on the basis of the NOE data. In addition, no one qualitative model was found to satisfy all the distance constraints imposed by the observation of intermolecular NOE connectivities. This suggests that the Δ-[Ru(phen)$_2$dpq]$^{2+}$ intercalates at the GA/TC site with several different orientations or that the metal complex intercalates at both the GA/TC and the central CG sites. However, a simple model can be proposed which is, in general, consistent with the observed NOEs between the phenanthroline protons and hexanucleotide minor groove protons as well as the NOEs between the dpq ligand and hexanucleotide major (T_2Me and T_2H2′) and minor groove protons. The model is also consistent with the observed upfield chemical shift changes of the dpq resonances upon binding. Figure 8 shows the Δ-[Ru(phen)$_2$dpq]$^{2+}$ intercalated between the G_4A_5 residues of one strand and the T_2C_3 residues of the other strand. The phenanthroline rings are located in the minor groove with the dpq ring inserted into the nucleotide base stack. The terminal ring of the dpq ligand projects out into the major groove, with the H13−T_2Me and H11−T_2H2′ distances being less than 4 Å in each case. In this model the metal complex is slightly rotated toward the pyrimidine rings. This 5° rotation places the exchange-averaged dpq H12 protons more directly into the strong shielding region of the two purine rings on one strand, consistent with the large upfield shift observed for the H12 resonance upon hexanucleotide binding. This model is also consistent with the smaller chemical shift changes observed for the dpq H10, H11, and H13 protons, as these protons are not located in the strong shielding regions of the aromatic bases. The 5° rotation also slightly reduces the distances between the phenanthroline and hexanucleotide protons that gave observable intermolecular NOEs. Larger rotation of the metal complex within the intercalation site did not produce a better overall model.

Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(TCGGGATCCCGA)$_2$ Binding. The binding of the metal complex to the dodecanucleotide d(TCGGGATCCCGA)$_2$ was studied in order to further examine the possible GA sequence selectivity observed in the Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ binding experiments. Addition of the metal complex to the dodecanucleotide at 25 °C induced extreme broadening of the resonances from the Δ-[Ru(phen)$_2$dpq]$^{2+}$. The larger degree of line broadening of the resonances from the metal complex (compared to the Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ experiments) precluded a detailed two-dimensional NMR study being carried out. However, at 35 °C the exchange-averaged phenanthroline protons could be assigned and some intermolecular NOE contacts with dodecanucleotide protons were observed in NOESY spectra. Although only a few intermolecular contacts were observed, relatively strong NOEs between the phenanthroline protons and the H4′ protons of C_8, C_9, and G_3 were detected (see Figure 9). Alternatively, no intermolecular NOEs between the metal complex and the hexanucleotide major groove protons were observed. These data suggest that the Δ-[Ru(phen)$_2$dpq]$^{2+}$ complex again binds in the minor groove but at the GGG/CCC

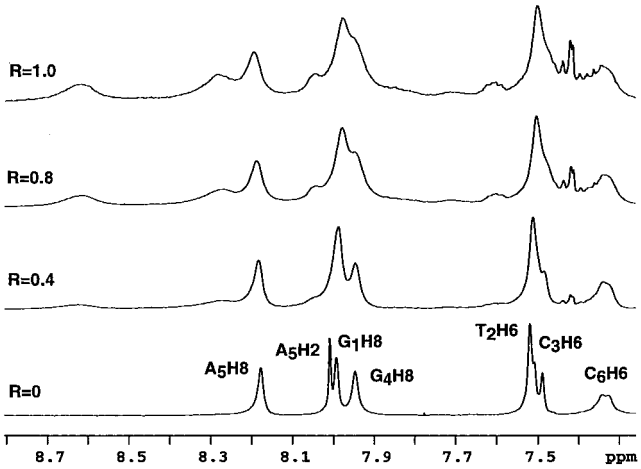

Figure 10. ^{1}H NMR spectra of d(GTCGAC)$_2$ (1.3 mM) as a function of added Δ-[Ru(phen)$_2$dpqC]$^{2+}$ in 10 mM phosphate (pH 7) containing 20 mM NaCl at 25 °C. The metal complex-to-hexanucleotide ratio (*R*) is indicated for each spectrum.

sequence rather than the GA/TC site. The extent of the line broadening of the resonances from the metal complex may indicate that the metal complex can bind at one of two (or more) possible sites at the GGG/CCC sequence.

Δ-[Ru(phen)$_2$dpqC]$^{2+}$−d(GTCGAC)$_2$ Binding Studies. The NMR spectra of d(GTCGAC)$_2$ at various ratios of added Δ-[Ru(phen)$_2$dpqC]$^{2+}$ are shown in Figure 10. The resonances of the Δ-[Ru(phen)$_2$dpqC]$^{2+}$ are significantly broader than the resonances in the equivalent Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ spectra. The increased exchange broadening of the Δ-[Ru(phen)$_2$dpqC]$^{2+}$ resonances is most likely due to the Δ-[Ru(phen)$_2$dpqC]$^{2+}$ complex having a slower rate of exchange between the free and hexanucleotide-bound form than the Δ-[Ru(phen)$_2$dpq]$^{2+}$ complex. No intermolecular NOE cross-peaks were observed in the NOESY spectra of d(GTCGAC)$_2$ with added Δ-[Ru(phen)$_2$dpqC]$^{2+}$ ($R = 1$) at either 25 or 40 °C. This is most likely due to the increased line width of the hexanucleotide and metal complex resonances. However, it was possible to determine the chemical shifts of the resonances from metal complex-bound hexanucleotide. In a fashion similar to the Δ-[Ru(phen)$_2$dpq]$^{2+}$ binding, the addition of Δ-[Ru(phen)$_2$dpqC]$^{2+}$ to the hexanucleotide induced significant upfield shifts for the minor groove H1′ resonances but only small shifts for the major groove H8/H6, H2′, and H3′ resonances. This suggests that the two complexes bind in a similar fashion.

Discussion

In agreement with the recent studies of Eriksson et al., the results presented here indicate that Δ-[Ru(phen)$_3$]$^{2+}$ binds DNA in the minor groove.[21,22] With some exceptions,[40−42] nonintercalating minor groove binding inert transition metal complexes and organic molecules show a distinct preference for AT sequences.[43−48] Consistent with this preference, Eriksson et al. reported that Δ-[Ru(phen)$_3$]$^{2+}$ bound preferentially to the central AT region of the decanucleotide d(CGCGATCGCG)$_2$.[21,22] Although the dodecanucleotide used in this study, d(TCGGGATCCCGA)$_2$, contained the same central sequence (5′-GATC), the Δ-[Ru(phen)$_3$]$^{2+}$ did not bind at the AT sequence but at the adjacent CC/GG bases. This indicates that sequence-dependent structural features do influence the minor groove binding of this metal complex.

Although it may not be strictly valid, it is interesting to compare the chemical shift changes of the phenanthroline resonances upon addition of Δ-[Ru(phen)$_3$]$^{2+}$ to the dodecanucleotide to previously reported ones. Rehmann and Barton reported upfield shifts of 0.42, 0.31, and 0.16 ppm for the phenanthroline H5, H4, and H2 protons, respectively, for Δ-[Ru(phen)$_3$]$^{2+}$−d(GTGCAC)$_2$ binding and 0.31, 0.28, 0.16, and 0.17 ppm for the H5, H4, H3, and H2 protons, respectively, for d(CGCGCG)$_2$ binding.[19] In this study the phenanthroline H4, H3, and H2 resonances exhibited similar upfield shifts upon addition of the metal complex to the dodecanucleotide; however, the H5 shifted 0.56 ppm upfield. This increased upfield shift of the H5 resonance could be induced if the H5 proton was positioned between the stacked DNA bases. Additionally, in contrast to previous Δ-[Ru(phen)$_3$]$^{2+}$−oligonucleotide binding studies, the resonances from the metal complex also exhibited significant broadening upon addition to the dodecanucleotide. The increased upfield shift of the H5 resonance coupled with the broadening of the resonances from the metal complex upon dodecanucleotide binding may suggest that Δ-[Ru(phen)$_3$]$^{2+}$ binds by partial intercalation.

While the results from the Δ-[Ru(phen)$_3$]$^{2+}$ binding may tentatively allow a minimally intercalated model to be proposed, the binding data of Δ-[Ru(phen)$_2$dpq]$^{2+}$ with d(GTCGAC)$_2$ strongly suggest that this metal complex can fully intercalate, and do so from the minor groove. Lincoln et al. have proposed that the related metal complex Δ-[Ru(phen)$_2$dppz]$^{2+}$ could intercalate from the minor groove.[9] The results of the Δ-[Ru(phen)$_2$dpq]$^{2+}$−d(GTCGAC)$_2$ binding studies provide strong NMR evidence that metal complexes can intercalate from the minor groove.

The results of the Δ-[Ru(phen)$_2$dpq]$^{2+}$−hexanucleotide binding study presented here can be directly compared to the NMR study previously reported for Δ-[Ru(phen)$_2$dppz]$^{2+}$ by Dupureur and Barton,[11] as the same hexanucleotide was used in both studies. The one-dimensional spectra of d(GTCGAC)$_2$ with added Δ-[Ru(phen)$_2$dpq]$^{2+}$ show few similarities to the corresponding spectra of the hexanucleotide with added Δ-[Ru(phen)$_2$dppz]$^{2+}$. In particular, the spectra of d(GTCGAC)$_2$ with added Δ-[Ru(phen)$_2$dppz]$^{2+}$ are characterized by large chemical shift changes for the major groove H8/H6 resonances and small shifts for the minor groove H1′ resonances. Alternatively, the hexanucleotide spectra with added Δ-[Ru(phen)$_2$dpq]$^{2+}$ are characterized by small shifts for the major groove H8/H6 resonances and large chemical shift changes for the minor groove H1′ resonances. These observations indicate that the binding of the two complexes is significantly different.

The dppz ligand differs from the dpq ligand only by the addition of another aromatic ring on the intercalating quinoxaline section of the dpq ligand. This suggests that the length of the intercalating segment may be an important parameter in the determination of the binding geometry. Dupureur and Barton

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have suggested that the Δ-$[Ru(phen)_2dppz]^{2+}$ complex may bind DNA with several intercalative geometries, including an asymmetric binding mode where the complex is canted toward one strand.[10,11] This canted structure may increase the amount of direct overlap between the intercalating ligand and the DNA bases. It is possible that the extra length of the dppz ligand allows significant aromatic−aromatic overlap to be obtained in a canted structure, whereas the shorter dpq ligand is forced to bind in a more "head-on" fashion. If this were the case, then the more canted structure may be favored in the major groove where there is more room for the complex to rotate toward one strand. Alternatively, the shorter dpq ligand is forced to bind in a "head-on" orientation which is favored from the minor groove.

Due to the extent of the exchange broadening of the resonances in Δ-$[Ru(phen)_2dpqC]^{2+}$−hexanucleotide spectra, a detailed binding study could not be carried out. However, the chemical shift changes observed for the hexanucleotide upon addition of the metal complex suggest that Δ-$[Ru(phen)_2dpqC]^{2+}$ binds in a manner similar to Δ-$[Ru(phen)_2dpq]^{2+}$ in the minor groove. This observation is consistent with the proposal that the length of the intercalating segment may govern the DNA binding geometry. The dpqC ligand is similar in length to the dppz ligand but contains a nonaromatic terminal ring, which would result in its aromatic ring−DNA base overlap potential being equivalent to that of the dpq ligand.

The NMR data indicated that Δ-$[Ru(phen)_2dpq]^{2+}$ may have some sequence selectivity in its oligonucleotide binding. With the hexanucleotide $d(GTCGAC)_2$ the metal complex bound at the GA/TC site, whereas with the dodecanucleotide $d(TCGGGATCCCGA)_2$ the metal complex bound at a GG/CC site. Although conclusions on sequence selectivity drawn from NOESY spectra of an exchange-averaged system can only be tentative, it appears that intercalation of the metal complex from the minor groove is favored at purine−purine/pyrimidine−pyrimidine sequences. Interestingly, the NOE data indicated that the parent ruthenium(II) polypyridyl, Δ-$[Ru(phen)_3]^{2+}$, also bound at a purine−purine/pyrimidine−pyrimidine sequence. As Δ-$[Ru(phen)_3]^{2+}$ could, at most, only minimally intercalate, the results suggest that the possible purine−purine/pyrimidine−pyrimidine sequence selectivity may be due to favorable minor groove dimensions at these sequences.

Acknowledgment. We thank Professor R. S. Vagg (Macquarie University, Australia) for access to the Jasco 500C spectropolarimeter.

Supporting Information Available: For the $[Ru(phen)_2dpq]^{2+}$ crystal structure the following data tables are available: positional coordinates for non-hydrogen atoms; bond lengths with estimated standard deviations; bond angles with estimated standard deviations; thermal parameters of non-hydrogen atoms; positional coordinates and thermal parameters for the hydrogen atoms; and least-squares planes (10 pages). Ordering information is available on any current masthead page.

IC971194V