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The DNA binding of the ΔΔ-, ΔΛ- and ΛΛ-stereoisomers of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$

Fiona M. Foley,[a] F. Richard Keene *[a] and J. Grant Collins *[b]

[a] *School of Pharmacy & Molecular Sciences, James Cook University, Townsville, Queensland 4811, Australia. E-mail: Richard.Keene@jcu.edu.au; Fax: +61-7-47814600*
[b] *School of Chemistry, University College, University of New South Wales, Australian Defence Force Academy, Canberra 2600, Australia*

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^{1}H NMR spectroscopy has been used to study the dodecanucleotide binding of the *rac* (ΔΔ- and ΛΛ-) and *meso* (ΔΛ stereoisomers of the dinuclear species $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine; bpm = 2,2′-bipyrimidine). The addition of the ΔΔ- and ΛΛ-enantiomers to the dodecanucleotide $d(CAATCCGGATTG)_2$ induced different shifts for the resonances from each enantiomer. On binding, the *meso* diastereoisomer exhibited twice the number of resonances observed for the free metal complex, identified as two sets of resonances with shifts similar to those induced by the ΔΔ- and ΛΛ-enantiomers of the *rac* form. In NOESY spectra of $d(CAATCCGGATTG)_2$ bound with each stereoisomer, NOE's from the metal complex were observed to the dodecanucleotide H1′ and H4′ protons, indicating that $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ bound in the dodecanucleotide minor groove. The strongest NOE's from the metal complex to $d(CAATCCGGATTG)_2$ were observed for the ΛΛ-isomer. The observed pattern of intermolecular NOE's indicated that the ΛΛ-isomer bound at the central CC/GG site as well as at the terminal CA/GT sequence of the dodecanucleotide. By contrast, NOE data of the ΛΛ-isomer associated with a second dodecanucleotide $d(CAATCGCGATTG)_2$ indicated that the ΛΛ-isomer bound at the AAT/ATT sequence as well as at the terminal base pairs in this case. It is proposed that $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ will only bind in the DNA minor groove: however, as the metal complex is not easily accommodated in the groove because of its size, it will only bind at sites where the minor groove is more open.

Introduction

There has been considerable recent interest in the DNA binding properties of inert transition metal complexes.[1–3] In particular, ruthenium(II) complexes with polypyridyl ligands have attracted considerable attention.[4–17] Complexes such as $[Ru(L)_2(dppz)]^{2+}$ (where L = 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen) and dppz = dipyridophenazine) can act as a "molecular light switch", as they show no luminescence in aqueous solution but luminesce intensely in the presence of DNA.[4] This has led to dppz complexes being used as luminescent probes for nucleic acid sequence and conformation, as well as for the study of electron transfer within the double helix.[9–15] Complexes containing dppz bind DNA strongly ($k_{ass} \approx 10^6$ M^{-1}) by intercalation of the dppz ligand between the stacked base-pairs.[4–8]

More recently, non-intercalating dinuclear ruthenium(II) complexes have been synthesised as probes for DNA structure and electron transfer reactions within DNA.[18–21] Positively-charged metal complexes can associate within the grooves of polyanionic DNA, with the binding being further stabilised by a variety of intermolecular forces such as van der Waals and hydrophobic interactions and hydrogen bonding. Dinuclear ruthenium complexes have some advantages over mononuclear complexes as photoprobes and stereochemical probes of nucleic acids, such as increased variations in shape and size. For example, DNA binding by the dinuclear complex $[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ (HAT = 1,4,5,8,9,12-hexaazatriphenylene) resulted in only a very weak luminescence enhancement, whereas a large luminescence enhancement was observed upon addition of partially denatured DNA.[20,21] It was proposed that the dinuclear ruthenium complex does not interact well due to the relative size of the metal complex relative to the dimensions of the DNA grooves. Consequently, this complex could be an excellent probe for denatured or deformed segments along double-stranded DNA.[20] Additionally, if each ruthenium centre is chiral, then a greater number of stereoisomers (and hence subtle changes in molecular shape) are available for use as stereochemical probes of nucleic acids.

Our laboratory has developed strategies for the synthesis of di- (and oligo-) nuclear complexes where the chirality and coordination environment of each polypyridyl ruthenium centre can be controlled.[22,23] However, the nature of the non-intercalative DNA binding mode (for example the groove and sequence/structure preference) of this class of metal complex has yet to be determined. In this paper we present an NMR study of the dodecanucleotide binding of the dinuclear ruthenium(II) complex $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ (shown in Fig. 1), where Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine and bpm = 2,2′-bipyrimidine. A symmetric bipyrimidine bridge was selected for the initial studies; in the terminal ligands, methyl groups were substituted on the 4- and 4′-positions of the bipyridyl rings because they significantly simplify the aromatic region in the ^{1}H NMR spectra of the three possible stereoisomers of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ (see Fig. 1), thereby facilitating the assignment of the spectra of the metal complex bound-dodecanucleotides.

Experimental

Measurements

Electronic spectra were measured on a Varian CARY 5E UV-VIS-NIR spectrophotometer, and circular dichroism (CD) using a JASCO J-715 spectropolarimeter.

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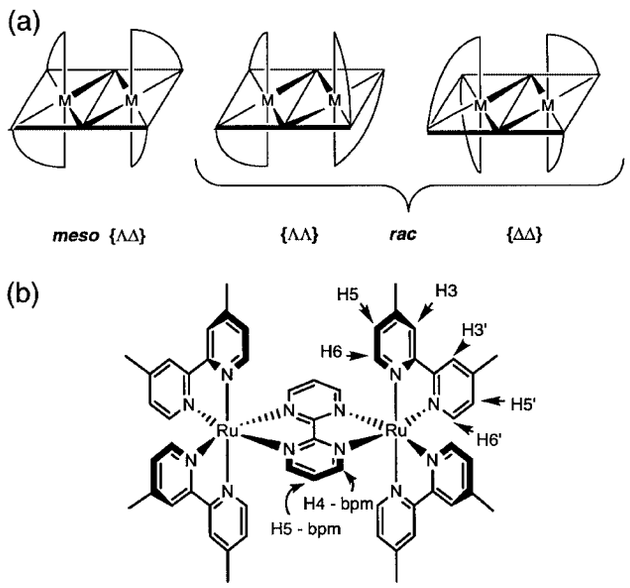

Fig. 1 Schematic representation of the three diastereoisomers of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ (a), and a structural representation of *meso*-(ΛΔ)-$[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ showing the numbering scheme used in the discussion (b).

Materials

The two self-complementary dodecanucleotides d(CAATCCGGATTG) and d(CAATCGCGATTG) were obtained from GeneWorks South Australia. The ligand 2,2′-bipyrimidine (bpm) was obtained from Lancaster, while 4,4′-dimethyl-2,2′-bipyridine (Me_2bpy) and D_2O were obtained from Aldrich, and SP- and CM-Sephadex were obtained from Amersham-Pharmacia-Biotech. $[Ru(Me_2bpy)_2Cl_2]$ was prepared as previously described.[24]

Synthesis of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]Br_4$, and separation of stereoisomers of the cation

Bpm (30 mg) and $[Ru(Me_2bpy)_2Cl_2]$ (300 mg) were suspended in a 10% aqueous ethylene glycol solution (20 mL) and the mixture refluxed at approximately 120 °C for 5 hours. The product was purified using cation exchange chromatography (SP Sephadex C-25; eluent 0.15 M sodium 4-toluenesulfonate). The major band was collected and the green material extracted with dichloromethane after the addition of a saturated solution of KPF_6. The complex was converted to the chloride salt by stirring a suspension with an anion exchange resin (Dowex; 1 × 8) and separated into diastereoisomers using cation exchange chromatography (SP Sephadex C-25; eluent 0.15 M sodium 4-toluenesulfonate). Bands 1 and 2 were determined to be the *meso*- and racemic (*rac*-) diastereoisomers, respectively. Both eluent bands were extracted with dichloromethane after the addition of a saturated solution of KPF_6. Band 2 was converted to the chloride salt as described above and resolved using cation exchange chromatography (SP Sephadex C-25; eluent 0.075 M disodium (−)-*O,O*-dibenzoyl tartrate). Band 1 was determined to be the ΔΔ-enantiomer, and band 2 the ΛΛ-enantiomer from comparison of CD spectra with those of related species.[25] The enantiomer bands were extracted with dichloromethane after the addition of a saturated solution of KPF_6. The hexafluorophosphate salts were metathesised to the corresponding bromide salts by dissolution of the former in a minimum volume of acetone (distilled from $CaCO_3$) and adding $[(n\text{-}C_4H_9)_4N]Br$ until complete precipitation had occurred. The products were filtered and washed with cold acetone. *Meso*—^{1}H NMR (D_2O); δ 8.38 (s, 8H), 8.23 (d, 4H, J = 4.2 Hz), 7.95 (d, 4H, J = 4.2 Hz), 7.60 (d, 4H, J = 4.2 Hz), 7.49–7.46 (m, 6H), 7.3 (d, 4H, J = 4.2 Hz), 2.58 (s, 12H), 2.54 (s, 12H). *Rac*—^{1}H NMR (D_2O); δ 8.45 (s, 4H), 8.41 (s, 4H), 8.24 (d, 4H, J = 4.5 Hz), 7.63 (d, 4H, J = 4.5 Hz), 7.55 (d, 4H, J = 4.5 Hz), 7.47 (t, 2H, J = 4.2 Hz), 7.31 (d, 4H, J = 3.6 Hz), 7.27 (d, 4H, J = 3.9 Hz), 2.63 (s, 12H), 2.54 (s, 12H). CD (ΔΔ in CH_3CN): λ(nm), ($\Delta\varepsilon$(M^{-1} cm^{-1})): 387 (24), 317 (−40), 275 (−126), 255 (63), 213 (−89). UV/Vis (H_2O): λ(nm), (ε(M^{-1})): 627 (4460), 570 (3220), 414 (10080), 290 (45660). Anal. calc. for $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]Cl_4\cdot 17H_2O$: C, 43.5%; H, 5.70%, N, 10.9%. Found C, 43.6%; H, 5.65%; N, 10.7%.

Sample preparation for NMR analysis

Both dodecanucleotides were converted to the sodium salts using a CM-Sephadex column that had been equilibrated with 1 M NaCl. After elution from the CM-Sephadex column, each dodecanucleotide was freeze-dried and then dissolved in 700 μL of phosphate buffer (pH 7.0) containing 20 mM NaCl and 0.1 mM EDTA and freeze-dried again. The dodecanucleotides were then repeatedly freeze-dried from D_2O and finally dissolved in 700 μL of 99.96% D_2O. The dodecanucleotide concentration (1.2 to 1.8 mM) was determined from the A_{260} absorbance using an extinction coefficient of 6600 M^{-1} cm^{-1} per nucleotide.[26] Stock solutions of the various metal complexes (approx. 13 mM) were prepared. The solution was freeze-dried and then re-dissolved in 700 μL of D_2O. Additions of the metal complex stock solutions were made directly to the dodecanucleotide solution.

NMR spectroscopy

^{1}H, NOESY and DQFCOSY NMR experiments were recorded on a Varian Unityplus-400 NMR spectrometer. One dimensional spectra recorded in 90% H_2O–10% D_2O solution were collected using the WATERGATE solvent suppression technique of Piotto *et al.*[27] Two-dimensional (2D) phase-sensitive NOESY spectra were acquired by the method of States *et al.*,[28] recorded using 2048 or 4096 data points in t_2 for 256–360 t_1 values with a pulse repetition delay of 1.7 s. NOESY spectra were recorded on the 1 : 1 oligonucleotide : metal complex solutions for mixing times ranging from 50 to 350 ms, and at temperatures from 5 to 35 °C. DQFCOSY spectra were recorded using 2048 data points in t_2 for 256 t_1 values and a pulse repetition delay of 1.7 s. Two-dimensional NMR data sets were zero-filled to 1024 points in the t_1 dimension and apodized with either a Gaussian or a shifted sinebell function.

Determination of binding constant

For the metal complex binding to the dodecanucleotide the binding constant can be expressed as:

$$K_{ass} = \frac{[M - DNA]}{[M][DNA]} \quad (1)$$

where [M − DNA] is the concentration of metal complex bound dodecanucleotide and [DNA] and [M] are the concentrations of free dodecanucleotide and metal complex respectively. The concentrations of [M − DNA], [M] and [DNA] in the above equation can be estimated from the following equation and the initial concentrations of the metal complex and dodecanucleotide.[8]

$$\delta_{obs} = \chi_f\delta_f + \chi_b\delta_b$$

δ_{obs} is the observed chemical shift of the metal complex resonances, χ_f and χ_b are the mole fractions of free and bound metal complex and δ_f and δ_b are the chemical shifts of the resonances of the free and bound metal complex. The chemical shift of the resonances of the fully bound metal complex can be estimated from their shift at the lowest ratio of metal complex to dodecanucleotide that the resonance can be assigned. The value for δ_f is taken from the spectrum of the free metal complex in similar buffer.

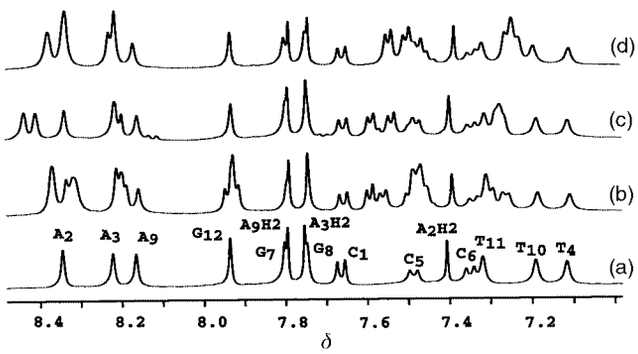

Fig. 2 ^{1}H NMR spectrum of the free d(CAATCCGGATTG)$_2$ (a) and with added [{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$, ΔΛ- (b), ΔΔ- (c) and ΛΛ- (d) at a metal complex to duplex ratio (*R*) of 1.0 at 25 °C.

Results

Assignment of the proton resonances of the free dodecanucleotides and [{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$

The ^{1}H NMR resonances of the free dodecanucleotides were assigned by standard techniques.[29–31] In 90% H_2O–10% D_2O solution, five imino resonances were observed for each dodecanucleotide at 10 °C. This indicates that both dodecanucleotides form stable double helices in the aqueous buffer, with only the terminal residue not forming a stable base-pair. In agreement with many other NMR studies of oligonucleotides,[16,30] analysis of short mixing-time NOESY spectra and DQFCOSY spectra indicated that both dodecanucleotides adopted a B-type conformation in the aqueous buffer.

The ^{1}H spectral characteristics (including COSY assignments) for the diastereoisomers of [{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ have been reported previously.[32]

Titration of d(CAATCCGGATTG)$_2$ with the ΔΔ-, ΔΛ- and ΛΛ-stereoisomers of [{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$

Fig. 2 shows the aromatic region of the ^{1}H NMR spectrum of free d(CAATCCGGATTG)$_2$, and the dodecanucleotide with added ΔΔ-, ΔΛ- and ΛΛ-stereoisomers of [{Ru(Me$_2$bpy)$_2$}$_2$-(μ-bpm)]$^{4+}$. Only one set of metal complex and dodecanucleotide resonances were observed throughout the titration of each stereoisomer. This indicates that the binding of all three stereoisomers is in the fast-exchange regime (on the NMR time scale). The addition of the ΔΔ-, ΔΛ- and ΛΛ-stereoisomers did not induce any significant changes to the chemical shift of the resonances from the dodecanucleotide aromatic protons (>0.03 ppm). However, some significant shifts were observed for the dodecanucleotide sugar resonances, particularly the H4′ protons (see Tables 1 and 2). A slightly different pattern of chemical shift changes is observed for the binding of each of the three stereoisomers.

The addition of the two enantiomers (ΔΔ- and ΛΛ-) of the *rac* diastereoisomer to the dodecanucleotide induced different shifts for the metal complex resonances (Table 3). While the induced shifts for the ΔΔ-isomer of the complex were very small, by contrast the dodecanucleotide binding of the ΛΛ-isomer induced significant upfield shifts for the H6′, H5′, H3′ and H3 resonances of the metal complex. The *meso* (ΔΛ-) diastereoisomer exhibited twice the number of resonances, compared to that observed for the free metal complex (in the phosphate buffer), upon binding to the dodecanucleotide. Two sets of resonances could be clearly identified in DQFCOSY and NOESY spectra. Interestingly, one set of resonances exhibited only very small shifts, similar to those observed for the ΔΔ-isomer, while the other ΔΛ-set of resonances exhibited a similar pattern of shifts to that observed for the ΛΛ-isomer.

As the chemical shifts of the H3′ and H3 resonances from the metal complex shifted systematically throughout the titration

Table 1 Chemical shifts (ppm) of the non-exchangeable protons of d(CAATCCGGATTG)$_2$ in 10 mM phosphate buffer containing 20 mM NaCl at 25 °C

Base	H8/H6	AH2	H1′	H2′	H2″	H3′	H4′
C$_1$	7.67		5.65	1.80	2.34	4.68	4.05
A$_2$	8.35	7.41	5.94	2.83	2.94	5.07	4.35
A$_3$	8.22	7.75	6.24	2.62	2.94	5.05	4.50
T$_4$	7.12		5.88	2.03	2.47	4.85	4.18
C$_5$	7.49		5.96	2.07	2.45	4.83	4.15
C$_6$	7.36		5.51	1.88	2.27	4.81	4.06
G$_7$	7.80		5.51	2.61	2.68	4.97	4.30
G$_8$	7.75		5.68	2.62	2.77	5.00	4.39
A$_9$	8.17	7.79	6.23	2.62	2.93	5.02	4.45
T$_{10}$	7.19		5.97	1.96	2.52	4.85	4.18
T$_{11}$	7.32		5.94	1.99	2.41	4.89	4.14
G$_{12}$	7.94		6.17	2.62	2.38	4.70	4.18

Table 2 Resonances from d(CAATCCGGATTG)$_2$ that exhibited a significant change in chemical shift upon addition of ΔΔ-, ΛΛ- and ΔΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ at a metal complex : duplex ratio of 1.0 at 25 °C

Stereoisomer	Dodecanucleotide resonances/ppm
ΛΛ	C$_1$H1′ (0.06), C$_1$H4′ (0.04), C$_6$H4′ (0.04), G$_7$H4′ (0.06), G$_{12}$H1′ (0.05), G$_{12}$H4′ (0.07)
ΔΔ	C$_1$H1′ (0.03), A$_2$H4′ (0.04), C$_5$H4′ (0.06), T$_{10}$H4′ (0.04), T$_{11}$H4′ (0.05), G$_{12}$H4′ (0.07)
ΔΛ	C$_1$H1′ (0.03), C$_1$H2′ (0.07), C$_1$H2″ (0.08), A$_3$H4′ (0.08), T$_4$H2′ (0.04), C$_6$H6 (0.04), G$_{12}$H1′ (0.05), G$_{12}$H2′ (0.08), G$_{12}$H2″ (0.05)

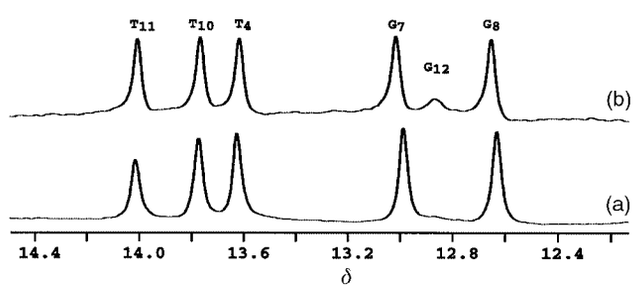

Fig. 3 ^{1}H NMR spectrum of the imino protons of the free dodecanucleotide d(CAATCCGGATTG)$_2$ (a) and with added ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ (b) in 90% H_2O–10% D_2O at 10 °C.

of the dodecanucleotide with the ΛΛ- and ΔΛ-isomers, they can be used to estimate the metal complex–dodecanucleotide binding constants. Binding constants of *ca.* 3×10^3 and 2×10^3 M^{-1} were calculated for the ΛΛ- and ΔΛ-isomers respectively.

For each of the three stereoisomers, five imino resonances were observed in NMR spectra of the metal complex-bound dodecanucleotide dissolved in 90% H_2O–10% D_2O (see Fig. 3), confirming that the double helical structure of the dodecanucleotide is maintained upon the binding of each stereoisomer. The denaturation temperature of the ΛΛ-isomer bound dodecanucleotide, determined from the transition midpoint of the temperature dependence curve of the resonances from the dodecanucleotide,[16] was found to be 63 °C.

NOESY spectra of ΔΔ-, ΔΛ- and ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$-(μ-bpm)]$^{4+}$ bound d(CAATCCGGATTG)$_2$

In order to gain further information on the dodecanucleotide binding of the metal complexes, NOESY spectra of the stereoisomers bound to d(CAATCCGGATTG)$_2$ were acquired. A similar pattern of NOE's from the metal complex to the dodecanucleotide was observed for each stereoisomer (see Table 4). However, the NOE's observed between the ΛΛ-isomer and the dodecanucleotide were stronger than the corresponding intermolecular NOE's from the ΔΔ- and ΔΛ-isomers at all

Table 3 Chemical shift changes for the resonances from ΔΔ-, ΛΛ- and ΔΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ upon dodecanucleotide binding, at a metal complex : duplex ratio of 1.0 at 25 °C

	Metal complex resonances/ppm					
	rac			*meso*		
Metal complex proton	Free	ΛΛ/oligo	ΔΔ/oligo	Free	α/oligo	β/oligo
H3′	8.45	−0.07	−0.01	8.38	−0.01	−0.06
H3	8.41	−0.07	0.00	8.38	−0.01	−0.06
H4-bpm	8.24	−0.01	−0.02	8.23	−0.01	−0.04
H6′	7.63	−0.08	−0.03	7.95	−0.01	−0.03
H6	7.55	−0.04	0.00	7.60	0.00	−0.03
H5-bpm	7.47	+0.04	+0.03	7.49	−0.01	+0.01
H5′	7.31	−0.05	−0.03	7.48	0.00	−0.02
H5	7.27	−0.02	+0.01	7.30	+0.01	−0.03

Table 4 NOE cross-peaks observed between the ΔΔ-, ΛΛ- and ΔΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ and d(CAATCCGGATTG)$_2$, at a metal complex to duplex ratio of 1.0 at 25 and 35 °C. nd = Not determined due to the overlap of the resonances at both 25 and 35 °C

	Dodecanucleotide protons			
	ΛΛ	ΔΔ	ΔΛ	
Metal complex protons			α	β
H3′/H3	C_1H4′, C_5H4′, C_6H4′	C_1H4′, C_5H4′, C_6H4′	C_1H4′, C_5H1′, G_{12}H4′	C_1H4′, G_{12}H4′
H4-bpm	C_1H1′, C_1H4′, A_2H1′, A_2H4′, C_5H4′, C_6H1′, C_6H4′	C_6H1′, G_{12}H1′	C_1H1′, C_1H4′, G_{12}H4′	C_1H1′, C_1H4′, C_6H1′, G_{12}H4′
H6′	C_1H4′, C_6H4′	C_1H4′, C_5H1′, C_6H4′	C_6H4′	C_6H4′
H6	G_{12}H1′	C_5H1′, C_6H4′, G_{12}H1′, G_{12}H4′	C_1H4′, G_{12}H4′	C_1H4′
H5′	C_1H4′, C_5H4′, C_6H4′	C_1H4′, C_6H4′	nd	nd
H5	C_1H4′, A_2H1′, C_5H1′, C_5H4′, C_6H1′, C_6H4′, G_{12}H1′	C_1H4′, C_5H4′, C_6H4′	C_1H1′	C_5H1′, C_5H4′

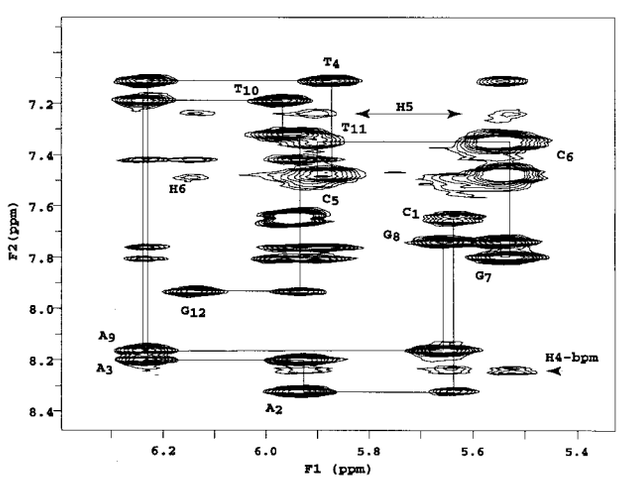

Fig. 4 Expansion of the NOESY spectrum (300 ms mixing time) of ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ and d(CAATCCGGATTG)$_2$, at a metal complex to duplex ratio of 1.0 at 35 °C. The expansion shows the NOE connectivities from the hexanucleotide base and metal complex aromatic protons (6.9 to 8.4 ppm) to the hexanucleotide sugar H1′ protons (5.4 to 6.3 ppm). The sequential NOE's connecting each base H8/H6 proton to its own H1′ proton and to the H1′ proton of the flanking 5′-sugar, and the intermolecular NOE's between the metal complex and dodecanucleotide are indicated.

temperatures that NOESY spectra were recorded. Fig. 4 shows the aromatic to the sugar H1′ region of a NOESY spectrum of the dodecanucleotide with added ΛΛ-isomer, at a metal complex to dodecanucleotide duplex ratio (*R*) of 1.0. The sequential intraduplex NOE cross-peaks between the dodecanucleotide base and sugar H1′ protons expected for B-type DNA were observed in this expansion.

Intermolecular NOE cross-peaks between the ΛΛ-isomer and the dodecanucleotide were also observed—in particular, between the protons from the ΛΛ-isomer and the dodecanucleotide sugar H1′ and H4′ protons of C_1, A_2, C_5, C_6 and G_{12}. As the H1′ and H4′ protons are located in the dodecanucleotide minor groove, it is concluded that all three stereoisomers bind the dodecanucleotide in the minor groove. The absence of any NOE's from the ΛΛ-isomer to any dodecanucleotide protons that are located in the major groove (base H8/H6 and sugar H2′/H2″ and H3′) supports this conclusion. The observed pattern of intermolecular NOE's also indicates that the metal complex binds at the central CC/GG site as well as at the terminal CA/GT sequence. Most minor groove binding agents, both organic and transition metal complexes, are selective for A/T rich sequences.[33] Interestingly, no NOE's from the metal complex were observed to the A_3T_4/A_9T_{10} protons at 25 or 35 °C, indicating that the metal complex did not bind at the symmetric A/T rich sites on the dodecanucleotide. However, it was noted that very weak NOE's were observed between metal complex and A_3T_4/A_9T_{10} minor groove protons at 5 °C.

ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$–d(CAATCGCGATTG)$_2$ binding

The ΛΛ-isomer (as well as the ΔΔ- and ΔΛ-isomers) bound d(CAATCCGGATTG)$_2$ at the central CCGG site, although no intermolecular NOE's were observed to the G_7 and G_8 protons. This suggests that the metal complex binds in a fashion that reduces any steric clashes with the guanine 2-amino protons which protrude into the minor groove. To further examine this proposal, the binding of the ΛΛ-isomer to the dodecanucleotide d(CAATCGCGATTG)$_2$ was studied. The only difference in base sequence in this second dodecanucleotide is that the central CCGG sequence has been changed to CGCG, thereby changing the positions of the guanine amino groups within the minor groove. As previously observed for the CCGG dodecanucleotide, the ΛΛ-isomer bound the CGCG dodecanucleotide in the fast-exchange regime. The metal complex H6′ and H3′

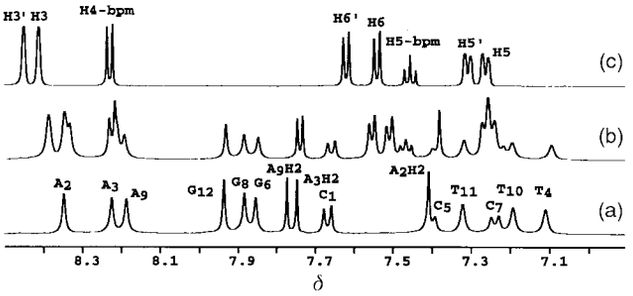

Fig. 5 ^{1}H NMR spectrum of the free d(CAATCGCGATTG)$_2$ (a) and with added ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ (b), at a metal complex to duplex ratio (*R*) of 1.0 at 25 °C. The spectrum of the free ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ is also shown (c).

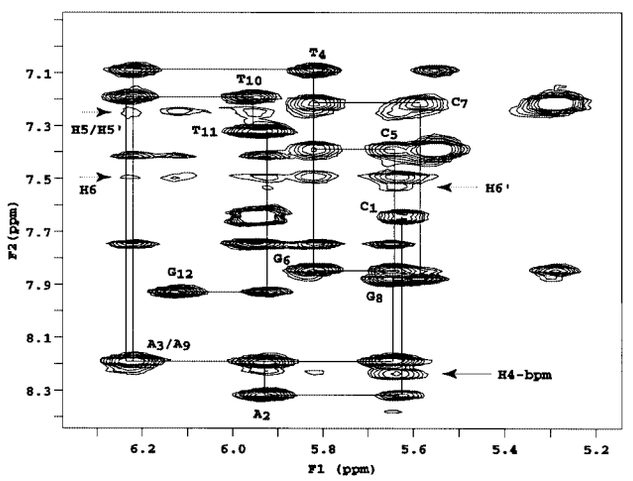

Fig. 6 Expansion of the NOESY spectrum (300 ms mixing time) of (ΛΛ)-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ and d(CAATCGCGATTG)$_2$, at a metal complex to duplex ratio of 1.0 at 35 °C. The expansion shows the NOE connectivities from the hexanucleotide base and metal complex aromatic protons (6.9 to 8.4 ppm) to the hexanucleotide sugar H1′ protons (5.2 to 6.3 ppm). The sequential NOE's, connecting each base H8/H6 proton to its own H1′ proton and to the H1′ proton of the flanking 5′-sugar, and the intermolecular NOE's between the metal complex and dodecanucleotide are indicated. At 35 °C the H1′ protons of T_4 and G_6 are coincident. However, at 25 °C the two resonances are resolved and hence allow the assignment of the intermolecular NOE from the metal complex H6 to the T_4H1′ and not the G_6H1′.

resonances exhibited a 0.07 ppm shift upon binding (see Fig. 5), and a binding constant of *ca.* 1×10^4 M^{-1} was calculated from these shifts. The denaturation temperature of the ΛΛ-isomer bound CGCG dodecanucleotide was found to be 67 °C.

Relatively strong metal complex–dodecanucleotide NOE cross-peaks were observed in NOESY spectra, at 25 and 35 °C, of the CGCG dodecanucleotide with added ΛΛ-isomer (see Fig. 6 and Table 5). Again, all the intermolecular NOE's were to the dodecanucleotide minor groove protons. In a similar manner to the binding to the CCGG dodecanucleotide, intermolecular NOE's from the ΛΛ-isomer were detected to the terminal C_1, A_2 and G_{12} sugar protons. However, unlike the case of binding to the CCGG dodecanucleotide, intermolecular NOE's were observed to protons in the A/T rich regions (H1′ and H4′ protons of A_3T_4/A_9T_{10}) of the CGCG dodecanucleotide. This data indicates that for the CGCG dodecanucleotide the ΛΛ-isomer binds at the AAT/ATT sequence as well as at the terminal base pairs, consistent with the observation of NOE's from the ΛΛ-isomer to the H2 protons of A_2, A_3 and A_9 (see Table 5).

It was anticipated that the ΛΛ-isomer would not bind at the CGCG site of the CGCG dodecanucleotide due to unfavourable interactions with the guanine amino groups. However, it is also possible that the AAT/TTA sequence of the CGCG dodecanucleotide may be a more favourable binding site than

Table 5 NOE cross-peaks observed between the ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$-(μ-bpm)]$^{4+}$ and d(CAATCGCGATTG)$_2$, at a metal complex to duplex ratio of 1.0 at 25 and 35 °C. nd = Not determined due to the overlap of the resonances at both 25 and 35 °C

Metal complex protons	Dodecanucleotide protons
H3′	C_1H1′, A_2H4′, A_3H4′, T_4H4′, C_5H1′, T_{11}H4′
H3	nd
H4-bpm	C_1H1′, A_2H1′, A_2H2, A_2H4′, A_3H4′, T_4H1′, T_4H4′, C_5H1′, T_{11}H4′
H6′	C_1H1′, C_5H1′, T_{11}H1′
H6	C_1H1′, A_2H4′, A_3/A_9H1′, A_3H4′, A_9H2, T_4H1′, C_5H1′, T_{11}H1′, T_{11}H4′, G_{12}H1′
H5-bpm	C_1H1′, T_4H1′, C_5H1′, T_{11}H4′
H5′/H5	C_1H1′, A_3/A_9H1′, A_9H2, T_4H4′, C_5H1′, T_{10}H1′, T_{11}H4′, G_{12}H1′
Me′/Me	A_2H2, A_3H2

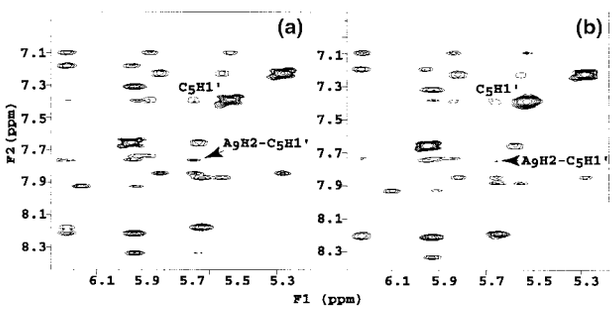

Fig. 7 Expansions of the NOESY spectra (75 ms mixing time) of the free dodecanucleotide d(CAATCGCGATTG)$_2$ at 25 °C (a) and the dodecanucleotide with added (ΛΛ-[{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ at a metal complex to duplex ratio of 1.0 at 25 °C (b).

the corresponding site in the CCGG dodecanucleotide. The interstrand distance between an AH2 and the closest cross-strand H1′ has been shown to be a good indicator of the minor groove width.[34–36] In canonical B-type DNA this distance is approximately 5 Å,[36] which is close to the maximum inter-proton distance for which an NOE would be observed in a short mixing-time NOESY spectrum. In short mixing-time NOESY spectra of the free CGCG dodecanucleotide at 25 °C, NOE cross-peaks of medium intensity were observed between the AH2 protons and H1′ protons on the complementary DNA strand, in particular between the A_9H2 and the C_5H1′ protons and the A_3H2 and T_{11}H1′ protons. These interstrand NOE's are consistent with the well-documented narrowing of the minor groove at A/T sequences.[33] However, the interstrand AH2-H1′ NOE's from the CGCG dodecanucleotide were significantly weaker than the corresponding interstrand NOE's from the CCGG dodecanucleotide (based on the cross-peak volumes compared to that of the C_5H6–C_5H5 NOE cross-peak). This indicates that the minor groove at the A/T sequences of the CGCG dodecanucleotide may be wider than the minor groove at the corresponding A/T sequences of the CCGG dodecanucleotide. Furthermore, upon addition of the ΛΛ-isomer to the CGCG dodecanucleotide, the interstrand NOE's (at 25 °C) between the AH2 protons and the closest cross-strand H1′ protons showed a further decrease in intensity, particularly the A_9H2 to C_5H1′ NOE (see Fig. 7). This suggests that for the CGCG dodecanucleotide the binding of the ΛΛ-isomer further widens the minor groove at the A/T sequences to accommodate the relatively bulky metal complex.

Discussion

The dodecanucleotide binding of the three stereoisomers of the dinuclear ruthenium(II) complex [{Ru(Me$_2$bpy)$_2$}$_2$(μ-bpm)]$^{4+}$ has been studied by NMR spectroscopy. Given that [Ru-(bpy)$_3$]$^{2+}$ does not bind DNA by intercalation,[37] it was expected

that neither would $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$, but would associate in either the major or minor groove of the dodecanucleotide. The NMR data are consistent with the metal complex binding within the dodecanucleotide grooves. Intercalative binding by metal complexes is characterised by large upfield shifts (0.5 to 1.0 ppm) and significant broadening of the resonances from the protons on the intercalating ligand (due to intermediate exchange kinetics).[16] The binding of the various stereoisomers of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ induced no significant line broadening or large upfield shifts for the resonances from the metal complex.

The ΔΔ-enantiomer interacted differently with the d(CAATCCGGATTG)$_2$ dodecanucleotide compared with the ΛΛ-enantiomer, as shown by the different changes in the chemical shift of the resonances from the enantiomers upon binding. Many examples of enantioselective binding by mononuclear transition metal complexes have been previously reported, for both binding by intercalation and within the major and minor grooves of DNA.[1–3,38,39] Addition of the *meso*- (ΔΛ) isomer to d(CAATCCGGATTG)$_2$ resulted in a doubling of the resonances from the metal complex: one set of resonances from the ΔΛ-isomer exhibited a similar pattern of shifts observed for the addition of the ΛΛ-isomer, while the other set of resonances exhibited shifts consistent with those observed upon addition of the ΔΔ-isomer. This suggests that the monomeric units display enantioselective binding even when part of the same molecule. Furthermore, the binding geometry of each monomeric unit appears to be dependent upon its absolute configuration and not upon the interaction of the two units as a whole. A similar observation was also made by Önfelt *et al.* for the DNA binding of dinuclear intercalating complex $[\{Ru(phen)_2\}_2\{\mu\text{-c4(cpdppz)}_2\}]^{4+}$ (c4(cpdppz)$_2$ = *N,N′*-bis(12-cyano-12,13-dihydro-11*H*-cyclopenta[*b*]dipyrido[3,2-*h*:2′,3′-*j*]-phenazine-12-carbonyl)-1,4-diaminobutane).[40]

From the pattern of intermolecular NOE's observed between the various stereoisomers and both dodecanucleotides, it is concluded that $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ binds in the minor groove, which is somewhat surprising given the relative dimensions of the metal complex and the DNA grooves. The metal complex is approximately 15.7 Å long and 8.0 Å wide, while for canonical form B-type DNA the width of the major groove and minor grooves are 11.6 Å and 6.0 Å, respectively.[33] The depth of both grooves is approximately 8.5 Å.[33] It is generally considered that minor groove binding is stabilised by van der Waals interactions, hydrogen bonding, electrostatic interactions and dehydration of the bound water molecules.[33,41] For organic binding drugs, like the benzimidazoles, it has been proposed that the van der Waals interactions are the most important.[41] It is probable that the van der Waals interactions are also important for the DNA binding of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ and, although the metal complex is relatively bulky, the major groove may still be too wide to allow close van der Waals interactions.

Most organic compounds and mononuclear polypyridyl ruthenium(II) complexes that bind DNA non-intercalatively, bind in the minor groove with a strong preference for A/T rich sequences.[33,42,43] This binding preference is considered to be due to the more favourable van der Waals and electrostatic interactions compared to G/C rich sequences.[33] In addition, hydrogen bonds can be formed to N3 atoms of adenines or O2 atoms of thymines. Binding at G/C rich sequences in the minor groove is also influenced by possible steric clashes with the guanine 2-amino group.[33,44] Interestingly, the three stereoisomers of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ bound the d(CAATCCGGATTG)$_2$ dodecanucleotide at the central CCGG sequence and at the CA/GT terminal residues, rather than at the expected A/T rich regions. A possible explanation for these observations could relate to the width of the dodecanucleotide groove relative to the size of the metal complex. The DNA minor groove is particularly narrow at A/T rich sequences,[33] and consequently

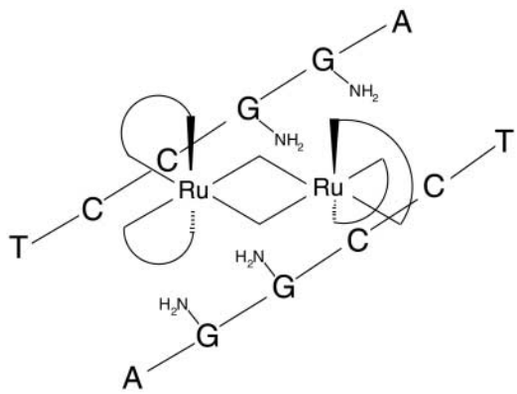

Fig. 8 A schematic showing the ΛΛ-stereoisomer of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ binding to the central CCGG region of the dodecanucleotide d(CAATCCGGATTG)$_2$. The metal complex is bound across the minor groove in a manner that positions only one bidentate ligand from each ruthenium into the groove and close to the cytosine residues and away from the guanine amino groups. If the central C and G residues on each strand were interchanged (*i.e.* C to G and G to C) the guanine amino groups would sterically hinder the metal complex binding. Due to its size, the dinuclear metal complex cannot lie deeply along the minor groove.

the relatively bulky $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ may not be easily accommodated in the minor groove at the AAT/TTA sites. Instead, the metal complex binds at the central CCGG site, which contains a more open minor groove, and at the terminal CA/GT site where the minor groove would also be considerably widened due to the fraying of the terminal base-pair.

While binding in the minor groove at the symmetric CCGG site was concluded, no NOE's from the protons on the metal complex to the two guanine residues were observed. As previously noted, this indicates that the metal complex binds at the CCGG site in such a manner that avoids steric clashes with the guanine 2-amino groups (which protrude into the groove—a schematic model is shown in Fig. 8). For the binding of the ΛΛ-isomer to the CGCG dodecanucleotide d(CAATCGCGATTG)$_2$, the binding was found to be predominantly at the AAT/TAA sites. This could be due to the positioning of the guanine amino groups within the minor groove of the CGCG sequence being such that the ΛΛ-isomer can not avoid substantial steric clashes, thus making binding at this site unfavourable.

Alternatively, it is possible that the minor groove width at the AAT/TTA site is significantly wider in the CGCG dodecanucleotide and hence a more favourable binding site. As the approximate binding constant for the ΛΛ-isomer with the CGCG dodecanucleotide was greater than with the CCGG dodecanucleotide, it is probable that minor groove is indeed wider at the AAT/TTA site for the CGCG dodecanucleotide. This proposal is also consistent with the weaker NOE's between the AH2 protons and the closest cross-strand H1′ protons observed for the CGCG dodecanucleotide.

The results of this study also provide an explanation for the observation that the similar dinuclear complex $[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ did not interact well within the DNA grooves but did bind partially-denatured DNA strongly.[20] It was suggested that this metal complex could be an excellent probe for denatured or deformed segments of DNA.[20] The NMR data of the dodecanucleotide binding of $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ indicated that the metal complex is too bulky to be easily accommodated in the DNA minor groove. Consequently, the metal complex binds regular duplex DNA with relatively lower affinity than other cationic compounds that can associate more deeply within the minor groove at A/T rich sequences. For example, the smaller dinuclear complex $[(en)Pt(\mu\text{-dpzm})_2Pt(en)]^{4+}$ (en = ethylenediamine) was found to bind in the minor

groove at A/T rich sequences with a higher affinity (binding constant of $\approx 10^5$ M^{-1}) than that observed for $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$.[45]

Acknowledgements

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References

1 B. Nordén, P. Lincoln, B. Akerman and E. Tuite, *Met. Ions Biol. Syst.*, 1996, **33**, 177.
2 K. E. Erkkila, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777.
3 Y. Xiong and L.-N. Ji, *Coord. Chem. Rev.*, 1999, **185**, 711.
4 A. E. Friedman, J.-C. Chambron, J.-P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960.
5 C. Hiort, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1993, **115**, 3448.
6 Y. Jenkins and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 8736.
7 R. M. Hartshorn and J. K. Barton, *J. Am. Chem. Soc.*, 1992, **114**, 5919.
8 M. Eriksson, M. Leijon, C. Hiort, B. Nordén and A. Graslund, *Biochemistry*, 1994, **33**, 5031.
9 C. J. Murphy, M. R. Arkin, Y. Jenkins, N. D. Ghatlia, S. H. Bossman, N. J. Turro and J. K. Barton, *Science*, 1993, **262**, 1025.
10 L. S. Schulman, S. H. Bossmann and N. J. Turro, *J. Phys. Chem.*, 1995, **99**, 9283.
11 E. D. A. Stemp, M. R. Arkin and J. K. Barton, *J. Am. Chem. Soc.*, 1995, **117**, 2375.
12 C. Moucheron, A. Kirsch-De Mesmaeker and S. Choua, *Inorg. Chem.*, 1997, **36**, 584.
13 C. G. Coates, L. Jacquet, J. J. McGarvey, S. E. Bell, A. H. R. Al-Obaidi and J. M. Kelly, *J. Am. Chem. Soc.*, 1997, **119**, 7130.
14 P. Lincoln, E. Tuite and B. Nordén, *J. Am. Chem. Soc.*, 1997, **119**, 1454.
15 E. Tuite, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1997, **119**, 239.
16 C. M. Dupureur and J. K. Barton, *Inorg. Chem.*, 1997, **36**, 33.
17 R. B. Nair and C. J. Murphy, *J. Inorg. Biochem.*, 1998, **69**, 129.
18 P. Lincoln and B. Nordén, *Chem. Commun.*, 1996, 2145.
19 X.-H. Zou, B.-H. Ye, H. Li, J.-G. Liu, Y. Xiong and L.-N. Ji, *J. Chem. Soc., Dalton Trans.*, 1999, 1423.
20 O. Van Gijte and A. Kirsch-De Mesmaeker, *J. Chem. Soc., Dalton Trans.*, 1999, 951.
21 A. Brodkorb, A. Kirsch-De Mesmaeker, T. J. Rutherford and F. R. Keene, *Eur. J. Inorg. Chem.*, 2001, 2151.
22 F. R. Keene, *Coord. Chem. Rev.*, 1997, **166**, 121 and references cited therein.
23 F. R. Keene, *Chem. Soc. Rev.*, 1998, **27**, 185 and references cited therein.
24 P. A. Anderson, R. F. Anderson, M. Furue, F. R. Keene and B. D. Yeomans, *Inorg. Chem.*, 2000, **39**, 2721.
25 T. J. Rutherford, O. Van Gijte, A. Kirsch-De Mesmaeker and F. R. Keene, *Inorg. Chem.*, 1997, **36**, 4465.
26 T. Maniatis, E. F. Fritsch and J. Sambrook, *Molecular Cloning*, Cold Spring Harbor Laboratory, NY, USA, 1982.
27 M. Piotto, V. Saudek and V. Sklenar, *J. Biomol. NMR*, 1992, **2**, 661.
28 D. J. States, R. A. Haberkorn and D. J. Ruben, *J. Magn. Reson.*, 1982, **48**, 286.
29 R. M. Scheek, R. Boelens, N. Russo, J. H. van Boom and R. Kaptein, *Biochemistry*, 1984, **23**, 1371.
30 J. Feigon, W. Leupin, W. A. Denny and D. R. Kearns, *Biochemistry*, 1983, **22**, 5943.
31 D. J. Patel, L. Shapiro and D. Hare, *J. Biol. Chem.*, 1986, **261**, 1223.
32 N. C. Fletcher, P. C. Junk, D. A. Reitsma and F. R. Keene, *J. Chem. Soc., Dalton Trans.*, 1998, 133.
33 S. Neidle, *DNA Structure and Recognition*, IRL Press, Oxford, 1994.
34 J. G. Nadeau and D. M. Crothers, *Proc. Natl. Acad. Sci.*, 1989, **86**, 2622.
35 M. Katahira, H. Sugeta, Y. Kyogoku, R. Fujisawa and K. Tomita, *Nucleic Acid Res.*, 1988, **16**, 8619.
36 N. V. Hud and J. Feigon, *J. Am. Chem. Soc.*, 1997, **119**, 5756.
37 C. V. Kumar, J. K. Barton and N. J. Turro, *J. Am. Chem. Soc.*, 1985, **107**, 5518.
38 Q. Xu, S. R. B. Jampani, H. Deng and W. H. Braunlin, *Biochemistry*, 1995, **34**, 14059.
39 T. A. Watt, J. G. Collins and A. P. Arnold, *Inorg. Chem.*, 1994, **33**, 609.
40 B. Önfelt, P. Lincoln and B. Nordén, *J. Am. Chem. Soc.*, 1999, **121**, 10846.
41 A. Czarny, D. W. Boykin, A. A. Wood, C. M. Nunn, S. Neidle, M. Zhao and W. D. Wilson, *J. Am. Chem. Soc.*, 1995, **117**, 4716.
42 M. Eriksson, M. Leijon, C. Hiort, B. Nordén and A. Gräslund, *Biochemistry*, 1994, **33**, 5031.
43 E. M. Proudfoot, J. P. Mackay, R. S. Vagg, K. A. Vickery, P. A. Williams and P. Karuso, *Chem. Commun.*, 1997, 1623.
44 U. Sehlstedt, S. K. Kim and B. Nordén, *J. Am. Chem. Soc.*, 1993, **115**, 12258.
45 N. J. Wheate and J. G. Collins, *J. Inorg. Biochem.*, 2000, **78**, 313.