Synthesis, Characterization, and DNA Binding Studies of a Chromium(III) Complex Containing a Tridentate Ligand

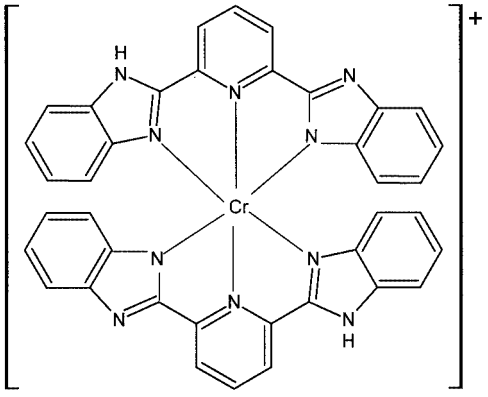
Vaidyanathan Ganesan Vaidyanathan[a] and Balachandran Unni Nair*[a]

Keywords: Chromium / Tridentate ligands / DNA cleavage / Radicals

[Cr(bzimpy)$_2$]Cl, where bzimpy is 2,6-bis(benzimidazol-2-yl)-pyridine, has been synthesized and characterized by ESI-MS, UV/Visible, and fluorescence spectra. Absorption titration and thermal denaturation experiments indicate that the complex binds to DNA with moderate strength, while viscosity measurements show that it may undergo surface binding. The fluorescence intensity of the complex increases with increasing DNA concentration, in contrast to [Cr(phen)$_3$]$^{3+}$ and [Cr(bpy)$_3$]$^{3+}$. The complex cleaves pBR322 DNA in the presence of H_2O_2.

The interaction of metal complexes with biomolecules has been extensively studied in recent decades.[1−13] Several metal complexes, which bind to DNA through different modes, have been used as probes for DNA structure in solution, as agents for mediation of strand scission of duplex DNA and as chemotherapeutic agents.[14−16] [M(LL)$_3$]$^{n+}$ metal complexes, where LL is 1,10-phenanthroline (phen) or a modified phenanthroline ligand, are particularly attractive species to recognize and cleave DNA.[17−22] Some transition metal complexes that bind covalently to DNA function as anti-tumor agents.

There has been renewed interest in biological aspects of chromium, and its extensive use in the tanning and electroplating industries has aroused much environmental concern over effluents bearing Cr^{VI} and Cr^{III}. Epidemiological and animal studies have firmly established hexavalent chromium compounds as potent carcinogens.[23−25] Chromate can form several DNA lesions, including DNA, DNA−protein, and DNA−amino acid crosslinks, strand breaks and alkali labile sites, which are responsible for its mutagenic and carcinogenic effects.[26,27] The carcinogenic activity of Cr^{VI} is due to its reduction by intracellular reductants to generate Cr^{V} and Cr^{IV} intermediates and finally Cr^{III}. The chromium(V/IV) intermediates are proposed to cause DNA damage that leads ultimately to tumor development.[28−32] Chromium(III) is believed to be non-toxic and is an essential trace element for glucose and lipid metabolism.[33] While chromium(III) picolinate complex is a therapeutic in the treatment of adult-onset diabetes,[34−35] Wetterhahn and coworkers have demonstrated that Cr(pic)$_3$ causes chromosomal damage in Chinese hamster ovary cells (CHO).[36] Chromium(III) not only affects DNA replication but also inhibits enzymatic activity in the biosynthesis of nucleotide pools, which in turn is responsible for mutations in cells.[37−38] We have demonstrated that a chromium(III) complex of a Schiff base having a donor−acceptor group exhibits nuclease activity, while [Cr(salen)(H$_2$O)$_2$]$^+$ can cleave DNA only in the presence of peroxide.[39−40] Furthermore, Sudgen et al. showed that Cr^{III} aromatic bidentate amines that can enter cells are mutagenic agents in the Ames test, as such complexes can generate a Cr^{III}/Cr^{II} active redox center.[41] These observations indicate that modification of the ligand environment would alter the binding affinities and DNA cleaving properties of the metal complex. Our present study focuses on the interaction of DNA with chromium(III) complex **1** which has a pyridine-type tridentate ligand, bzimpy.



1

Results and Discussion

Compound **1** was synthesized in good yield by reaction of the ligand bzimpy with [Cr(dmso)$_6$]$^{3+}$ generated in situ.

[a] Chemical Laboratory, Central Leather Research Institute, Adyar, Chennai 600 020, India
Fax: (internat.) +91-44-24911589
E-mail: bcunchem@rediffmail.com

The ESI mass spectrum shows the base peak at m/z = 672 due to $[Cr(bzimpy)]^+$. The ESI spectrum thus confirms the authenticity of the complex. It is noteworthy that the ligand behaves like a monoanion (through the loss of a proton from a −NH group). Similar behaviour has been observed in Mn^{II} complex of this ligand.[42] However, with $[Ru(bzimpy)_2]^{2+}$ the ligand coordinates as a neutral tridentate ligand without such a loss of a proton.[43] Despite our best efforts we could not grow diffraction quality crystals. However, the Mn^{II} complex of the same ligand has been crystallographically characterized and found to have a regular octahedral geometry.[42] The Cr^{III} complex reported here is expected to have a similar geometry. The cyclic voltammogram of the complex shows a reduction of Cr^{III} to Cr^{II} at a cathodic peak potential, E_{pc}, of −1.05 V versus SCE. Reoxidation of the Cr^{II} species occurred at −0.81 V upon reversal of the scan (Figure 1). The separation of anodic and cathodic peak potentials, ΔE_p = 240 mV for $[Cr(bzimpy)_2]^+$, indicates a quasi-reversible redox process. Such behavior is typical of many Cr^{III}/Cr^{II} couples because of the Jahn−Teller distortion expected for the Cr^{II} ion.

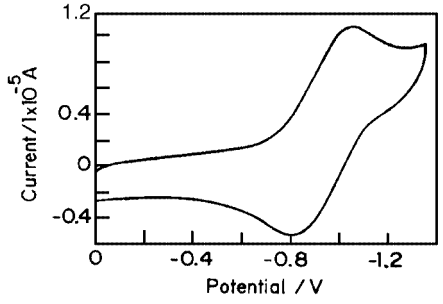


Figure 1. Cyclic voltammogram of $[Cr(bzimpy)_2]^+$

Absorption Titration

Absorption titration can monitor the interaction of a metal complex and DNA. In general, hypochromism and redshift are associated with the intercalative binding of the complex to the helix, due to strong stacking interactions between the aromatic chromophore of the complex and the base pairs of DNA. The chromium complex **1** shows an absorbance at 317 nm due to the intraligand (π−π*) transition of the ligand bzimpy. Binding of chromium(III) with DNA is expected to change markedly its electronic spectrum. Upon addition of CT DNA, the chromium(III) complex absorbance increases, and is accompanied by a small shift of 3 nm in λ_{max}, from 317 to 320 nm (Figure 2), that is consistent with groove binding, leading to small perturbations. Such a small increase in λ_{max} and hyperchromicity occurs with some porphyrin and copper complexes on their interaction with DNA.[44] This hyperchromism can be attributed to external contact (surface binding) with the duplex. The absorption data were analyzed to evaluate the intrinsic binding constant K_b, which can be determined from Equation (1), where ε_A corresponds to A_{obsd}/[Cr] and ε_F, and ε_B are the molar absorption coefficients for the free chromium complex and the chromium complex in the fully bound form, respectively.

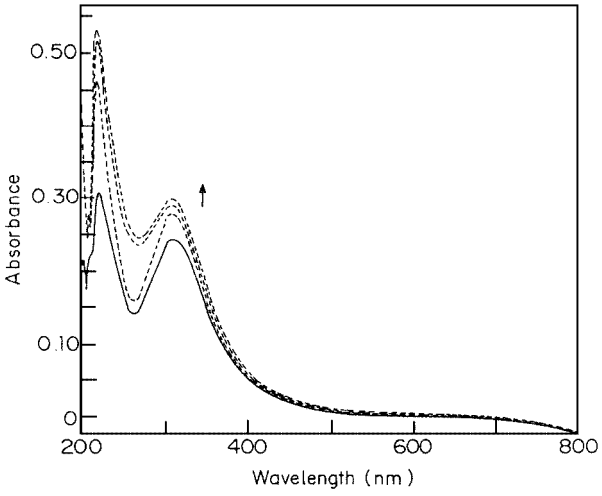


Figure 2. Electronic spectra of $[Cr(bzimpy)_2]^+$ in Hepes buffer upon addition of calf thymus DNA, [Cr] = 10 μM (—), [DNA] = 20−150 μM (- - -)

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_A - \varepsilon_F) \quad (1)$$

A plot of $[DNA]/(\varepsilon_A - \varepsilon_F)$ versus [DNA] revealed a moderate intrinsic binding constant K_b of $1.013 \pm 0.03 \times 10^4$ M^{-1}, which is similar to those observed for many other chromium and ruthenium complexes ($1-4 \times 10^4$ M^{-1}),[40,45] and is more in keeping with groove binding than intercalative binding. Further, since the complex is octahedral, and upon coordination the planarity of the ligand is lost, intercalative binding to DNA is highly unlikely.

Thermal Denaturation Studies

DNA melting is observed when double-stranded DNA molecules are heated and separate into two single strands; it occurs due to disruption in intermolecular forces such as π stacking and hydrogen bonding interactions between DNA base pairs. Here, a DNA melting experiment revealed that T_m of calf thymus DNA was 65±0.2 °C and 68±0.2 °C in the absence and presence of the complex, respectively. However, for the ligand alone, there is no change in DNA melting temperature. The ΔT_m of 3 °C indicates that the binding cannot be intercalative. Such a small ΔT_m is generally associated with surface binding of the metal complex. Generally one would expect a much larger increase in T_m for classical intercalators.[46−49] A small ΔT_m also indicates weak binding of the complex to DNA. This is also reflected in the binding constant.

Viscosity Measurement

Optical photophysical probes generally provide support for a binding model. Hydrodynamic measurements that are sensitive to length change are, in the absence of crystallographic data, regarded as the most critical tests of a binding model in solution. Intercalating agents are expected to destack the base pairs to accommodate the ligands, causing

elongation of the double helix and an increase in the viscosity of the DNA. However, some complexes, which bind through non-intercalative modes, decrease the viscosity initially and then increase it at higher concentrations. With chromium complex **1**, the viscosity of DNA showed a complicated dependence on complex concentration. The change in viscosity with the addition of the complex to DNA is shown in Figure 3. The viscometric data rules out classical intercalation. Similar behaviour has been observed on the addition of $[Cu(bcp)_2]^+$ to ST DNA.[50] The viscometric results could be explained in terms of a bridged structure to promote the extension of duplexes. Here the binding may involve inner-sphere complex formation via phosphate oxygen, indicating that the binding of Cr^{III} complex with DNA could be surface binding or by forming bridged adducts. Adducts formed by bridging duplexes are stabilized by hydrophobic interaction or coulombic interaction, as with $[Cu(bcp)_2]^+$ and $[Cu(dpsmp)_2(H_2O)]$.[51–53]

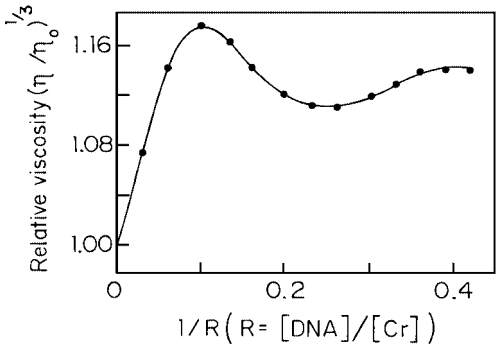


Figure 3. Effect of increasing amounts of $[Cr(bzimpy)_2]^+$ (–•–) on the relative viscosity of DNA

Fluorescence Spectroscopic Study

The emission spectra of $[Cr(bzimpy)_2]^+$ in the absence and the presence of CT DNA is shown in Figure 4. The luminescence spectrum of complex **1** shows an emission band at 717 nm when excited at 320 nm. The band at 717 nm is attributable to the $^2E_g \rightarrow {}^4A_{2g}$ transition of $[Cr(bzimpy)_2]^+$. Addition of DNA causes a gradual increase in the fluorescence intensity and the emission maximum also shifts by 2 nm to a longer wavelength. This is because, in the presence of DNA, the metal complex is bound in a relatively non-polar environment compared to water. Such an increase in fluorescence intensity has been reported for certain intercalators upon their binding to DNA. The increase observed here, however, is less than that observed for classical intercalators. In contrast, $[Cr(bpy)_3]^{2+}$ and $[Cr(phen)_3]^{2+}$ show a decrease in luminescence intensity in the presence of DNA, indicating that the excited state of these complexes are strong oxidizing agents that could oxidize guanine base.[54–55] The excited state potential for complex **1** was calculated as 0.92 V from Equation (2), where $E_{0-0}(Cr^{3+}/{}^*Cr^{3+})$ is the one-electron potential corresponding to the zero-zero spectroscopic energy of the excited state.

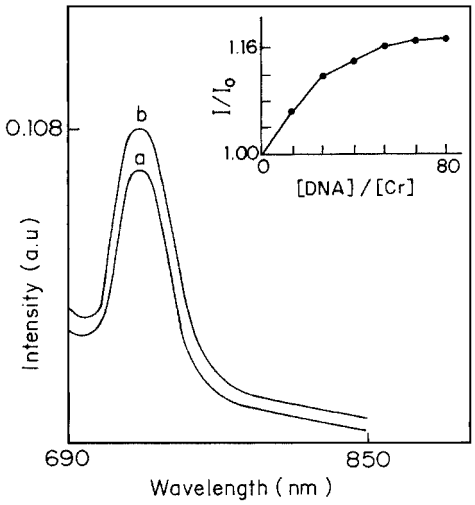


Figure 4. Emission spectra of $[Cr(bzimpy)_2]^+$ in aqueous solution in a) absence and b) presence of DNA. [Cr] = 10 mM, [DNA] = 200–800 mM. Inset: Relative emission intensity of $[Cr(bzimpy)_2]^+$ versus [DNA]/[Cr]

$$E^0({}^*Cr^{3+}/Cr^{2+}) = E^0(Cr^{3+}/Cr^{2+}) - E_{0-0}(Cr^{3+}/{}^*Cr^{3+}) \quad (2)$$

The reported excited state potentials of $[Cr(bpy)_3]^{3+}$ and $[Cr(phen)_3]^{3+}$ are 1.44 and 1.42 V, which are much greater than the 0.92 V in the present case. Hence, compared to $^*[Cr(bpy)_3]^{3+}$ and $^*[Cr(phen)_3]^{3+}$, the excited state of $[Cr(bzimpy)_2]^+$ is not a good oxidizing agent to oxidize the guanine base; as the excited state potential is low compared to that of guanine base.

DNA Cleavage by $Cr(bzimpy)_2^+$

The ability of $[Cr(bzimpy)_2]^+$ to serve as a metallonuclease has been examined. The reaction of $[Cr(bzimpy)_2]^+$ with pBR322 DNA was monitored by observing the conversion of supercoiled plasmid DNA (fastest migrating species) into the circular, nicked form (slowest migrating species). All reactions were evaluated by comparing the amount of circular plasmid to the amount present in plasmid controls. Neither relaxation nor nicking is observed in control reactions of DNA alone or with DNA and either $[Cr(bzimpy)_2]^+$ or hydrogen peroxide (Figure 5. lanes 1–3). However, with hy-

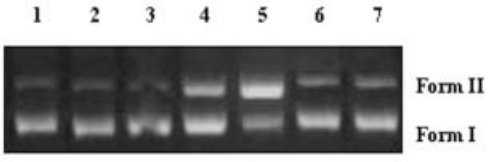


Figure 5. Nicking of pBR322 DNA by $[Cr(bzimpy)_2]^+$ over time. Lane 1, control DNA alone; lane 2, DNA + complex **1**; lane 3, DNA + H_2O_2; lanes 4 and 5, DNA + complex **1** + H_2O_2 incubated for 30 and 60 min, respectively. Lane 6, DNA + complex **1** + H_2O_2 + EtOH (1 M); lane 7, DNA + complex **1** + D-mannitol (500 mM)

drogen peroxide, $[Cr(bzimpy)_2]^+$ relaxes supercoiled pBR322 DNA in a time-dependent manner (lanes 4,5). At longer times, the supercoiled form is completely converted into the open circular form (form II) in the presence of Cr^{III} complex. The time dependence of the reaction suggests that $[Cr(bzimpy)_2]^+$ acts as a catalyst. In the presence of Cr^{III} complex, H_2O_2 produces radical species, which cause the DNA cleavage. The addition of radical traps (1 M EtOH or D-mannitol) to reaction mixtures containing complex and H_2O_2 inhibited the cleavage of DNA (lanes 6 and 7 of Figure 6). Gel electrophoresis of similar DNA-cleaving experiments with CT DNA showed that complex **1** cleaved the CT DNA in a non-specific manner, in the presence of H_2O_2. The presence of hydroxyl radicals has been shown to lead to hydrolysis of *p*-nitrophenyl phosphate ester.[56] Treatment of p-nitrophenyl phosphate with complex **1** and H_2O_2 generates *p*-nitrophenol, which has an absorption at 404 nm (Figure 6). This confirms the formation of hydroxyl radicals from hydrogen peroxide in the presence of complex **1**.

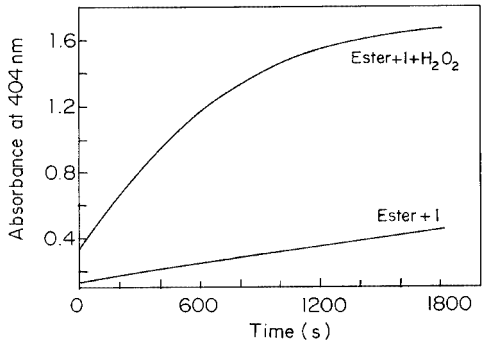


Figure 6. Kinetics of phosphate ester hydrolysis of *p*-nitrophenylphosphate by complex **1** in the presence of hydrogen peroxide

Conclusions

Chromium complexes having a tridentate ligand can bind DNA with moderate strength. Spectroscopic and viscosity measurements show that the Cr^{III} complex interacts with the DNA surface. A fluorescence study shows that the emission intensity of the complex increases on addition of DNA as the environment around the complex is more hydrophobic in the presence of DNA. The emission of both $[Cr(phen)_3]^{3+}$ and $[Cr(bpy)_3]^{3+}$ is quenched on binding to DNA. The emission of $[Cr(bzimpy)]^{2+}$, however, is not quenched by DNA as it has a lower excited state potential than that for guanine base. Gel electrophoresis shows that $[Cr(bzimpy)_2]^+$ nicks DNA in the presence of H_2O_2. The amount of nicked DNA formed increases with increasing incubation time of the Cr^{III} complex and H_2O_2 with DNA. The nicking of DNA is inhibited by radical scavengers such as ethanol and D-mannitol.

Experimental Section

Materials: *o*-Phenylenediamine, 2,6-pyridinedicarboxylic acid, and calf thymus DNA were obtained from Sigma chemicals, USA. Tris, Hepes, agarose, and pBR322 DNA were obtained from Bangalore Genei, Bangalore. All solvents were obtained from Ranbaxy, India. Milli Q water was used for preparing buffers.

Synthesis of $[Cr(bzimpy)_2]Cl$ (1): 2,6-Bis(benzimidazo-2-yl)pyridine was synthesized according to the published procedure.[57] An excess of DMSO was added to chromium(III) chloride salt (1 mmol, 0.267 g) and boiled under reflux for 1 h. The ligand bzimpy (2 mmol, 0.622 g) was then added to the resulting mixture, and refluxing was continued for a further hour. The volume of solvent was then minimised by rotary evaporation and a reddish orange precipitate was obtained on addition of diethyl ether. The precipitate was filtered off and dried in vacuo. The compound was then recrystallised from acetonitrile (yield 0.66 g, 75%); found C 64.33, H 3.34, N 19.80, Cr 7.32% calcd. for $C_{38}H_{24}ClCrN_{10}$ (M.wt. 707.5): C 64.43, H 3.41, N 19.78, Cr 7.34%. IR (KBr pellet): 3401 (N−H), 3057 (C−H), and 1615 (C=N) cm^{-1}.

Physical Measurements: UV/Visible spectra of the complex and DNA binding studies were recorded on a Perkin−Elmer Lambda 35 spectrophotometer at 25 °C. Elemental analysis was performed using a Heraeus-CHN-Rapid Analyzer at RSIC, IIT, Madras. The emission spectra were recorded on a Hitachi 650−40 spectrofluorimeter. The electrospray ionization (ESI) mass spectrum of the complex was recorded with a Hewlett−Packard 1100 mass spectrometer equipped with an electron spray source. The infrared spectrum of the complex was recorded on a Perkin-Elmer FT-IR spectrometer. Cyclic voltammetry was performed on an EG and G PAR 173 potentiostat/Galvanostat analyzer. Tetrabutylammonium perchlorate (TBAP) was used as supporting electrolyte. The sample in dried DMSO was purged with nitrogen prior to measurement. A standard three-electrode system consisted of a glassy carbon working electrode, platinum auxiliary electrode and a saturated calomel reference electrode (SCE).

DNA Binding Experiments: All experiments involving the interaction of the complex with DNA were carried out in Hepes buffer (10 mM, pH 7.5). A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8−1.9:1, indicating that the DNA was sufficiently free from protein.[58] The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 $M^{-1}cm^{-1}$) at 260 nm.[59]

The electronic spectra of the Cr^{III} complex were monitored in both the presence and absence of DNA. The binding constant for the interaction of Cr^{III} complex **1** with DNA was obtained from absorption titration data. A fixed concentration of complex **1** (10 μM) was titrated with increasing amounts of DNA over the range 20−150 μM.

During measurement of the absorption spectra, an equal amount of DNA solution was added in both complex solution and reference solution to eliminate the absorbance of DNA itself.

Thermal denaturation studies were conducted in a Perkin−Elmer Lambda 35 spectrophotometer supplied with thermostatted cell holder. The temperature was controlled by a Peltier system (±0.1 °C). The absorbance at 260 nm was monitored for DNA (100 μM) in the absence and presence of the complex (10 μM).

Viscosity measurement was carried out on an Ostwald viscometer, immersed in a thermostatted water bath maintained at 28±0.1 °C.

The DNA concentration was kept constant (150 μM) and that of the metal complex was varied from 5 to 75 μM. The flow time was measured with a stop-watch operated manually, and each sample was measured five times and an average flow time calculated. Data are presented as $(\eta/\eta_o)^{1/3}$ versus [Cr]/[DNA], where η is the viscosity of DNA in the presence of complex, and η_o is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions corrected for the flow time of buffer alone (t_o), $\eta = t - t_o$.[60]

In steady state fluorescence experiments, the complex concentration (10 μM) was fixed while that of DNA was varied (200−800 μM). The chromium(III) complex in the presence of DNA was excited at 320 nm and the fluorescence spectra were recorded between 650 and 850 nm.

Phosphate ester hydrolysis was monitored on a Perkin−Elmer Lambda 35 spectrophotometer at 25 °C by measuring the production of p-nitrophenol at 404 nm. The background reaction was carried out with the peroxide and ester alone. A reaction mixture containing Cr^{III} complex **1** (0.025 mM), peroxide (0.1 mM, freshly prepared) and *p*-nitrophenylphosphate (1 mM) in Hepes (10 mM, pH 7.0) was used to determine the rate of *p*-nitrophenol formation at 404 nm.

For gel electrophoresis experiments, supercoiled pBR322 DNA (800 ng) was treated with Cr^{III} complex (100 μM) in Tris-EDTA buffer (10 mM, pH 8.0) and the solution was incubated for 16 h at room temperature. H_2O_2 (500 μM) was then added and a timed assay was carried out. The reactions were quenched at various times by adding loading buffer. The samples were analyzed by electrophoresis for 4 h at 50 V on a 0.8% agarose gel in Tris−boric acid−EDTA buffer. The gel was stained with 0.5 μg/mL ethidium bromide and photographed under UV light.

Acknowledgments

V. G. V thanks the CSIR for a research fellowship.

[1] S. M. Hecht, *Acc. Chem. Res.* **1986**, *19*, 383−391.
[2] T. D. Tullius, B. A. Dombroski, *Science* **1985**, *235*, 679−681.
[3] A. M. Burkoff, T. D. Tullius, *Nature* **1988**, *331*, 455−457.
[4] A. M. Burkoff, T. D. Tullius, *Cell* **1987**, *48*, 935−943.
[5] W. K. Pogozelski, T. K. Tullius, *Chem. Rev.* **1998**, *98*, 1089−1108.
[6] C. J. Burrows, J. G. Muller, *Chem. Rev.* **1998**, *98*, 1109−1152.
[7] A. E. Friedman, J. K. Barton, *J. Am. Chem. Soc.* **1990**, *112*, 4960−4962.
[8] R. M. Hartshorn, J. K. Barton, *J. Am. Chem. Soc.* **1992**, *114*, 5919−5925.
[9] C. J. Murphy, J. K. Barton, *Methods Enzymol.* **1993**, *226*, 576−594.
[10] Y. Jenkins, A. E. Friedman, N. J. Turro, J. K. Barton, *Biochemistry* **1992**, *31*, 10809−10816.
[11] C. Turro, S. H. Bossmann, Y. Jenkins, J. K. Barton, N. J. Turro, *J. Am. Chem. Soc.* **1995**, *117*, 9026−9032.
[12] N. Gupta, N. Grover, G. A. Neyhart, W. Liang, P. Singh, H. H. Thorpe, *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1048−1050.
[13] X.-H. Zou, B.-H. Ye, H. Li, Q.-L. Zhang, H. Chao, J.-G. Liu, L.-N. Ji, *J. Biol. Inorg. Chem.* **2001**, *6*, 143−150.
[14] C. J. Burrows, S. E. Rokita, *Acc. Chem. Res.* **1994**, *27*, 295−301.
[15] T. D. Tullius, Metal-DNA chemistry, ACS Symposium Series 402, American Chemical Society, Washington DC, **1989**, pp. 1−23.
[16] A. R. Banerjee, J. A. Jaeger, D. H. Turner, *Biochemistry* **1993**, *32*, 153−163.
[17] D. S. Sigman, *Acc. Chem. Res.* **1986**, *19*, 180−186.
[18] A. M. Pyle, J. K. Barton, *Prog. Inorg. Chem.* **1990**, *38*, 413−475.
[19] S. Sigman, A. Mazumdar, D. M. Perrin, *Chem. Rev.* **1993**, *93*, 2295−2316.
[20] K. Naing, M. Takashani, M. Taniguchi, A. Yamagishi, *Inorg. Chem.* **1995**, *34*, 350−356.
[21] F. L. Liu, K. A. Meadows, D. R. Millin, *J. Am. Chem. Soc.* **1993**, *115*, 6699−6704.
[22] Y. Xiong, X.-F. He, X.-H. Zou, J.-Z. Wu, X.-M. Chen, L.-N. Ji, R.-H. Li, J.-Y. Zhou, K.-B. Yu, *J. Chem. Soc., Dalton Trans.* **1999**, 19−24.
[23] *IARC*, **1990**, *49*, 49−256.
[24] U. Glaser, D. Hochrainer, H. Kloppel, H. Oldiges, *Toxicology* **1986**, *42*, 219−232.
[25] A. Leonard, R. R. Lauwreys, *Mutat. Res.* **1980**, *76*, 227−239.
[26] S. De Flora, M. Bagnasco, D. Serra, P. Zanacchi, *Mutat. Res.* **1990**, *238*, 99−172.
[27] J.-L. Yang, Y.-C. Hsieh, C.-W. Wu, T.-C. Lee, *Carcinogenesis* **1992**, *12*, 2053−2057.
[28] S. Kawanishi, S. Inoue, S. Sano, *J. Biol. Chem.* **1986**, *261*, 5952−5958.
[29] X. Shi, N. S. Dalal, *Environ. Health. Perspect.* **1994**, *102*, 231−236.
[30] A. Kortenkamp, G. Oetken, D. Beyersmann, *Mutat. Res.* **1990**, *232*, 155−161.
[31] A. Mikalsen, J. Alexander, H. Wallin, M. I. Sundberg, R. A. Andersen, *Carcinogenesis* **1991**, *12*, 825−831.
[32] M. Sugiyama, K. Tsuzuki, R. Ogura, *J. Biol. Chem.* **1991**, *266*, 3383−3386.
[33] R. A. Anderson, *Biol. Trace Element Res.* **1992**, *32*, 19−24.
[34] R. A. Anderson, N. Cheng, N. A. Bryden, M. M. Polansky, N. Cheng, J. Chi, J. Feng, *Diabetes* **1997**, *46*, 1786−1791.
[35] A. Ravina, L. Slezack, *Harefuah* **1993**, *125*, 142−145.
[36] D. M. Stearns, J. P. Wise, S. R. Patierno, K. E. Wetterhahn, *FASEB J.* **1995**, *9*, 1643−1648.
[37] S. E. Kohlami, M. Gattke, E. M. McIntosh, B. A. Kunz, *J. Mol. Biol.* **1991**, *220*, 933−946.
[38] J. Singh, E. T. Snow, *Biochemistry* **1998**, *37*, 9371−9378.
[39] R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, B. U. Nair, *Biochem. Biophys. Res. Commun.* **2000**, *271*, 731−734.
[40] R. Vijayalakshmi, M. Kanthimathi, V. Subramanian, B. U. Nair, *Biochim. Biophys. Acta* **2000**, *1475*, 157−162.
[41] K. D. Sugden, R. D. Geer, S. J. Rogers, *Biochemistry* **1992**, *31*, 11626−11631.
[42] R. Rajan, R. Rajaram, B. U. Nair, T. Ramasami, S. K. Mandal, *Dalton Trans.* **1996**, 2019−2020.
[43] V. G. Vaidyanathan, B. U. Nair, *J. Inorg. Biochem.* **2002**, *91*, 405−412.
[44] R. F. Pasternack, E. J. Gibbs, J. J. Villafranca, *Biochemistry* **1983**, *22*, 2406−2414.
[45] M. Erikkson, M. Leijon, G. Hiort, B. Norden, A. Graslund, *J. Am. Chem. Soc.* **1992**, *114*, 4933−4934.
[46] M. Cory, D. D. McKee, J. Kagan, D. W. Henry, J. A. Miller, *J. Am. Chem. Soc.* **1985**, *107*, 2528−2536.
[47] M. J. Waring, *J. Mol. Biol.* **1965**, *13*, 269−282.
[48] J. M. Kelly, A. B. Tossi, D. J. McConell, C. OhUigin, *Nucleic Acids Res.* **1985**, *13*, 6017−6034.
[49] G. A. Neyhart, N. Grover, S. R. Smith, W. A. Kalsbeck, T. A. Fairley, M. Cory, H. H. Thorp, *J. Am. Chem. Soc.* **1993**, *115*, 4423−4428.
[50] F. Liu, K. A. Meadows, D. R. McMillin, *J. Am. Chem. Soc.* **1993**, *115*, 6699−6704.
[51] P. G. Schultz, J. S. Taylor, P. B. Dervan, *J. Am. Chem. Soc.* **1982**, *104*, 6861−6863.
[52] S. Mahadevan, M. Palaniandavar, *Inorg. Chem.* **1998**, *37*, 693−700.
[53] G. Yang, F. Z. Wu, L. Wang, L. NianFi, X. Tina, *J. Inorg. Biochem.* **1997**, *66*, 141−144.

[54] M. A. Jamieson, N. Serpone, M. Z. Hoffman, *Coord. Chem. Rev.* **1981**, *39*, 121−179.
[55] R. T. Watson, N. Desai, J. Wildsmith, J. F. Wheeler, N. A. P. Kane-Maguire, *Inorg. Chem.* **1999**, *38*, 2683−2687.
[56] A. Parand, A. C. Royer, T. L. Cantrell, M. Weitzel, N. Memon, J. B. Vincent, M. W. Crowder, *Inorg. Chim. Acta* **1998**, *268*, 211−219.
[57] A. W. Addison, P. J. Burke, *J. Heterocycl. Chem.* **1981**, *18*, 803−805.
[58] J. Marmur, *J. Mol. Biol.* **1961**, *3*, 208−218.
[59] M. F. Reichmann, S. A. Rice, C. A. Thomas, P. Doty, *J. Am. Chem. Soc.* **1954**, *76*, 3047−3053.
[60] G. Cohen, H. Eisenberg, *Biopolymers* **1969**, *8*, 45−55.
Received March 29, 2003

