



Synthesis and DNA Binding Studies of Cobalt (III) Mixed-Polypyridyl Complex

Lan Jin and Pin Yang

LJ. State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, People's Republic of China.—PY. Institute of Molecular Science, Shanxi University, Taiyuan, People's Republic of China

Abstract

The complex of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ (where phen is *o*-phenanthroline, and dppz is dipyrrodo [3, 2-*a*: 2', 3'-*c*]phenazine) has been synthesized. This complex was characterized by elemental analysis, molar conductivity, IR and ^1H NMR spectroscopy. The interaction of the complex with calf thymus DNA has been studied using absorption and emission spectroscopy, DNA melting techniques, cyclic voltammetric, viscosity, and electrophoresis measurements. The compound shows absorption hypochromicity, fluorescence enhancement, DNA melting temperature, and the specific viscosity increased when binding to calf thymus DNA. CV measurement shows that the shifts in oxidation-reduction potential and change in peak current with addition of DNA. The complex is also shown to be more efficient photosensitizers for single strand breaks in plasmid DNA. *Journal of Inorganic Biochemistry* 68, 79–83 (1997) © 1997 Elsevier Science Inc.

Introduction

The interaction of transition metal polypyridyl coordination compounds with DNA have been extensively studied in the past few years. Due to the unusual binding properties and general photoactivity, these coordination compounds are suitable candidates as DNA secondary structure probe, photocleavers and antitumor drugs [1].

Most metal polypyridyl coordination compounds are positively charged and may thus bind electrostatically to single or double stranded DNA at low ionic strength. In the case of double stranded DNA, some coordination compounds may also bind in the major groove with one ligand inserting between two base pairs of DNA. The effect of size, shape, hydrophobicity, and the charge on the binding of the complex to DNA has been studied by changing the type of heteroaromatic ligand or metal center [2, 3]. In order to make mixed-ligand coordination compounds intercalate in DNA, the intercalated ligand needs flat, large surface area and has a special geometry that permits overlapping between aromatic ring of intercalated ligand and the base pairs of DNA.

Recently, a ruthenium (II) polypyridyl coordination complex, $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ was shown to be a remarkable luminescence light switch for DNA [4]. In aqueous

solutions, this compound lacks luminescence but it shows intense luminescence in the presence of DNA. Studies of the interaction of this complex with DNA have been concerned largely with characterization of its luminescence properties in the presence and absence of DNA. In contrast, the investigation about the complex as DNA photocleavage reagent is relatively less. In this paper, we describe the synthesis of the mixed-ligand coordination complex of cobalt (III) containing dipyrrodo [3, 2-*a*: 2', 3'-*c*]phenazine (Fig. 1) and phen, then extend our studies to the complex-DNA binding system by using a variety of physical methods. The ability of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ to induce DNA cleavage upon photoexcitation has also been researched. To our knowledge, this type of cobalt (III) mixed-ligand complex photocleaving DNA is still unknown. The results should be valuable in understanding the mode of the complex binding to DNA, as well as laying the foundation for the rational design of DNA structure probes and antitumor drugs.

Experimental

Materials and Methods

$[\text{Co}(\text{phen})_2\text{Cl}_2]\text{Cl}$ was prepared and purified according to the literature [5]. Calf thymus DNA was obtained from Sigma Chemical Company. The solution of DNA was prepared by dissolving DNA in aqueous solution and dialyzing several times against buffer until the UV absorbance ratio A_{260}/A_{280} is greater than 1.90. The concentration of the prepared DNA stock solution was expressed as DNA (ρ), and was determined according to its absorbance at 260 nm using $\epsilon_{260} = 6.6 \text{ mM}^{-1} \cdot \text{cm}^{-1}$. Ethidium bromide (EthBr) was obtained from Fluka (Buchs, Switzerland), plasmid pBR322 DNA, agarose DNA grade (high gel strength), and Tris were purchased from Beijing Sino-American Biotechnology Company. All other reagents and solvents were analytical grade reagents and were used as received.

Carbon, hydrogen, and nitrogen contents were determined by a Perkin-Elmer 240c instrument, IR spectra was recorded on a Perkin-Elmer-1700 spectrophotometer with KBr as disks. ^1H NMR spectra was recorded on a Bruker AM-500MHz NMR spectrometer. Absorbance spectra was recorded on a Shimadzu UV-365 spectrophotometer. Fluorescence measurement was made with Hitachi Model-850 fluorescence spectrophotometer, excitation and emission slits were 5 nm.

The absorption titration was performed by keeping the concentration of the complex constant while varying the

Address correspondence to: Pin Yang, Institute of Molecular Science, Shanxi University, Taiyuan 030006, People's Republic of China.

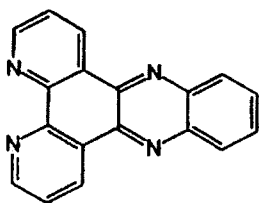


Figure 1. dppz.

nucleic acid concentration. The absorption at 374 nm was recorded after each addition of DNA. The intrinsic binding constant K was determined according to Eqn. (1) [6], where $[DNA]$ and $[Co]$ are the total concentrations of base pairs and metal complex, respectively. ϵ_A , ϵ_F , and ϵ_B correspond to the absorbance/ $[Co]$, the extinction coefficient for the free cobalt complex, and the extinction coefficient for the bound cobalt complex, respectively. K was obtained from the ratio of the slope to the Y -intercept

$$[DNA]/(\epsilon_A - \epsilon_F) = [DNA]/(\epsilon_B - \epsilon_F) + 1/K(\epsilon_B - \epsilon_F) \quad (1)$$

The DNA melting experiments were done by controlling the temperature of the sample cell with a Shimadzu circulating bath while monitoring the absorbance at 260 nm. Viscosity measurements were performed in an Ostwaldtype viscometer, immersed in a thermostated water bath maintained at 23 ± 0.1 °C. Cyclic voltammetry (CV) was performed with a BAS-100A electrochemical analytical instrument (U.S.A.) with saturated Ag/AgCl electrode as reference electrode. A saturated calomel electrode (SCE) was used in all experiments. DNA samples were electrophoresed through 1% agarose gel containing 50 mM Tris acetate, 20 mM sodium acetate and 18 mM sodium chloride, then stained with ethidium bromide. The photographs were taken under UV light.

All the measurements were conducted using solutions of the complex in Tris-HCl buffer (pH 7.0) containing 5 mmol/L Tris and 50 mmol/L NaCl.

Synthesis of $[Co(phen)_2dppz](ClO_4)_3$

$[Co(phen)_2Cl_2]Cl$ (0.526g) was dissolved in water-methanol (20 mL, 1:2 V/V), then, dppz (0.449g), prepared by the literature method [7], was added. The solution was stirred under reflux. After 3 h, the reaction mixture was cooled to room temperature and filtered. To the filtrate was added 30% $NaClO_4$ solution; a yellowish precipitate formed. The precipitate was washed with ice water and methanol, then dried under vacuum (65%). Found: C 50.17, H 2.48, N 10.79, Calc. for $C_{42}H_{26}N_8CoCl_3O_{12}$: C 50.45, H 2.62, N 11.21%. IR data (cm^{-1}): 1522 m (ν ring), 1506 m ($\delta c = c$), 1429 s (δ CCH), 1091 vs (ν ClO_4^-), 847 s (δ phen), 723 s (δ phen), 625 s (δ ClO_4^-). 1H NMR (ppm, CF_3COOD): δ 10.32 (d, 1H), 10.27 (s, 2H), 9.60 (d, 1H), 9.08 (d, 3H), 8.93 (m, 1H), 8.86 (s, 3H), 8.60 (d, 2H), 8.54 (m, 5H), 8.34 (s, 2H), 8.08 (s, 3H), 7.96 (s, 2H), 7.81 (s, 1H).

Results and Discussion

Characterization of the Co(III) Complex

The conductivity of the soluble complex was recorded in 10^{-3} M DMF solution on a conductivity meter. The molar conductance value of the complex in DMF is in the range of 200–400 suggesting that it is a 1:3 electrolyte [8]. The absorption bands δ (C—H) (852 cm^{-1} , 737 cm^{-1}) of phen and of the phen ring at 1558 cm^{-1} are redshifted to 847, 723, and 1506 cm^{-1} (phen ring) after coordination, which proves that phen coordinates the Co ion through N. Proton magnetic resonance indicates the presence of coordinated ligands in complex as shown by their corresponding proton resonance using the tetramethylsilane as internal standard in CF_3COOD solution.

Absorption Studies

The absorption of $Co(phen)_2dppz^{3+}$ in the visible wavelength region arises from the intraligand (IL) transition of the dppz chromophore ($\lambda = 374, 358\text{ nm}$). Figure 2 shown the behavior of the absorption spectra of the complex upon interaction with DNA from free complex to $R_t = [DNA]/[Co] = 2$. In the presence of DNA, the electronic absorption of $Co(phen)_2dppz^{3+}$ showed strong decreases in the peak intensities. Hypochromism was suggested to be due to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases [9]. In addition to the decrease in intensity, a small redshift and an isosbestic point at 388 nm were also observed in the spectra. These various spectral changes are consistent with the intercalation of $Co(phen)_2dppz^{3+}$ into the DNA base stack. The binding constant K was obtained as $7.6 \times 10^6\text{ M}^{-1}$. These features are equivalent to those observed with $Ru(phen)_2dppz^{2+}$ and suggest that cobalt complex binds by intercalation in a manner that parallels $Ru(phen)_2dppz^{2+}$ [10].

Fluorescence Studies

Binding of the complex to DNA was found to increase the fluorescence intensity. For the $Co(phen)_2dppz^{3+}$, no detectable emission is observed in aqueous solution due to quenching by hydrogen bonding between water and the phenazine nitrogens of the dppz ligand [11]. Binding to DNA, however, protects the phenazine nitrogens from water through preferential intercalation of the dppz ligand and leads to intense photoluminescence. Figure 3 shows the steady-state of $Co(phen)_2dppz^{3+}$ in buffered solution both in the presence and absence of double-stranded DNA.

To establish that the quenching of the cobalt excited state is indeed the result of interaction with water, we examined the quenching of the excited state of the complex by water in a nonaqueous solvent [12], and found that although the complex of $Co(phen)_2dppz^{3+}$ luminesces in acetonitrile, emission excited state is quenched by increasing concentrations of H_2O owing to interac-

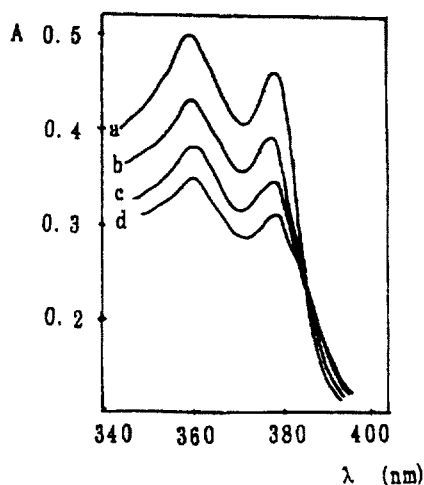


Figure 2. Absorption spectra of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ ($10 \mu\text{M}$) in the absence and presence of DNA. $R_t = [\text{DNA}]/[\text{Co}] = 0, 0.5, 1, 2$, for *a-d*, respectively.

tion between the solvent hydrogens and the phenazine nitrogens of the dppz ligand. These results are consistent with the proposed mechanism for light switch effect.

DNA Melting Experiments

Other strong evidence for the intercalation of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ into helix was obtained from the DNA melting studies. Intercalation of small molecules into the double helix is known to increase the helix melting temperature, the temperature at which the double helix denatures into single-stranded DNA [13]. The extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than in the single-stranded form, hence, melting of the helix leads to an increase in the absorption at this wavelength. Thus, the helix to coil transition temperature can be determined by monitoring the absorbance of the DNA bases at 260 nm as a function of temperature. The DNA melting curves in the absence

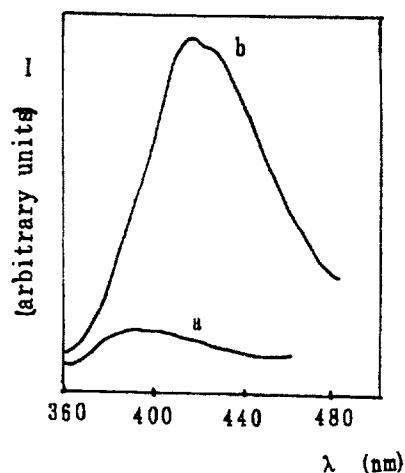


Figure 3. Fluorescence spectra of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ ($50 \mu\text{M}$) in the absence (a) and presence (b) of DNA, $R_t = [\text{DNA}]/[\text{Co}] = 44$. Sample excitation was at 330 nm.

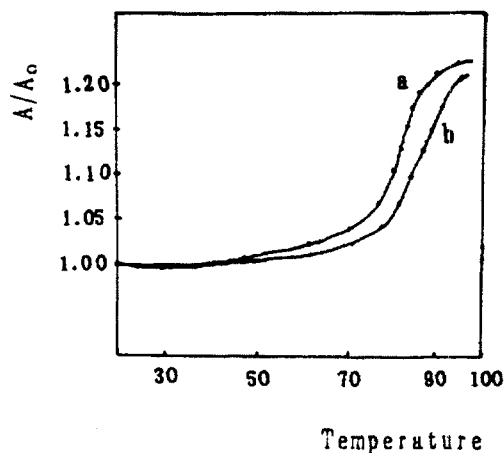


Figure 4. Plots of A/A_0 vs temperature of CT DNA ($90 \mu\text{M}$) (a) and CT DNA in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ (b) with a 9:1 ratio of DNA to $\text{Co}(\text{phen})_2\text{dppz}^{3+}$.

and in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ are presented in Figure 4. The melting temperature (T_m) of CT DNA was increased from 78°C to 86°C . The increase in the melting temperature is comparable to the values observed with the classical intercalator ethidium and lend strong support for the intercalation of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ into the helix [14].

Viscosity Measurements

The viscosity studies provide a strong argument for intercalation [15]. The viscosity measurement is based on the flow rate of a DNA solution through a capillary viscometer. The specific viscosity contribution (η) due to the DNA in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ was obtained. The results are summarized in Table 1. The results indicate that the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ has an effect on the viscosity of the DNA. The specific viscosity of the DNA sample increases obviously with the addition of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$. The viscosity increase of DNA is ascribed to the intercalative binding mode of the drug because this could cause the effective length of the DNA to increase [16]. So we think the viscosity increase of the DNA caused by the addition of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ can provide further support for the intercalative mode of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$.

CV Study

Native DNA is not reducible at the mercury electrode because the stability of the intact double helix makes the reducible bases inaccessible to the electrode. In Tris-HCl buffer, pH 7.0, the addition of DNA causes considerable

Table 1. Effect of the Drug of the Specific Viscosity of DNA

C_{drug} (mmol/L)	0.00	0.02	0.04	0.06	0.08
η	1.98	2.13	2.23	2.31	2.40

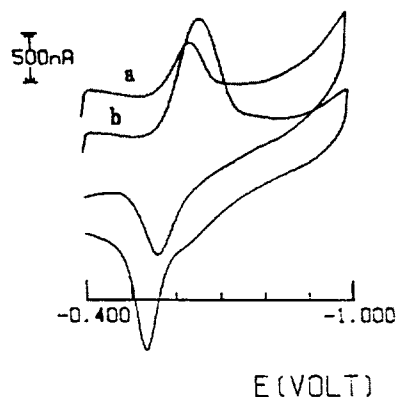


Figure 5. Cyclic voltammograms of 45 μM $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ in the absence (A) and presence (B) of 230 μM DNA. Sweep rate, 200 mv/s.

decrease of the peak current of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$. Additionally, the peak potentials, both E_{pc} and E_{pa} , have a small shift vs a solution without DNA shown in Figure 5. We think that $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ intercalates into the base pairs of DNA by the dppz planar. Because of the intercalation, $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ is not readily accessible to the electrode, thus causing the peak currents of the CV waves to diminish. Moreover, the obvious shift of peak potentials indicates the ion interaction mode between the drug and DNA [17]; here this mode may be the interaction between $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ and DNA via the phosphate group DNA.

Photochemistry Reaction of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ with CT DNA

Some transition metal polypyridyl coordination compounds can cleave DNA when irradiated at 254 nm light [18]. The irradiation of calf thymus DNA in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ was studied so as to determine the efficiency with which it sensitises DNA cleavage. This can be achieved by monitoring the absorption spectra of the complex-DNA system under 254 nm light. Figure 6 shows the change of absorption spectra of the complex-DNA system (the background spectra involving buffer was deducted automatically by the instrument). With no irradiation, the decrease in A_{260} of the complex-DNA system comparing to the total sum of absorbance of the complex and DNA alone suggested the reaction between the cobalt and DNA. In the presence of light, the A_{260} of the system increased obviously. Meanwhile, we try observing the absorbance change of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ or DNA with identical concentration during the same time under 254 nm light, and found that DNA did not change, while the $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ had a small enhancement in absorption. The net spectral change is a "hyperchromic effect". This spectral change process reflected the corresponding change of DNA in its conformation and structures after the drug bound to DNA. The hyperchromism effect resulted from the damage of the DNA double-helix structure. Therefore, the above process reflected the sec-

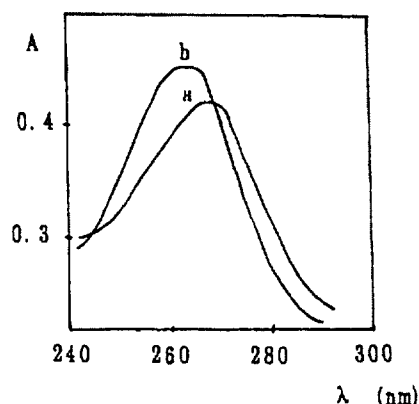


Figure 6. Absorption spectra of the $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ —DNA system ($C_{\text{DNA}} = 16 \mu\text{M}$, $R_f = [\text{DNA}]/[\text{Co}] = 2$) without light (a) and after irradiation at 254 nm for 20 min (b).

ondary structural damage to DNA. This result will be further supported by the electrophoresis experiment.

Photoactivated Cleavage of pBR322 DNA by $\text{Co}(\text{phen})_2\text{dppz}^{3+}$

The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subject to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoils will relax to generate a slower-moving open circular form (Form II) [18]. If both strands are cleaved, a linear form (Form III) will be generated that migrates between Form I and II. Figure 7 shows gel electrophoretic separation of pBR 322 DNA after incubation with cobalt complexes and irradiation for variable times. Figure 7(A) reveals the conversion of Form I to Form II after a 40 min irradiation in the presence of varying concentrations of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$. From Figure 7(B), it can be seen that with extended irradiation times, Form I of pBR 322 DNA diminishes gradually, whereas the amount of Form II increases. This is the result of single-stranded cleavage of pBR 322 DNA. Neither irradiation of the DNA at 254 nm without cobalt nor incubation with cobalt without light yielded significant strand scission. It is likely that the reduction of Co(III) is the important step leading to DNA cleavage. Here, the

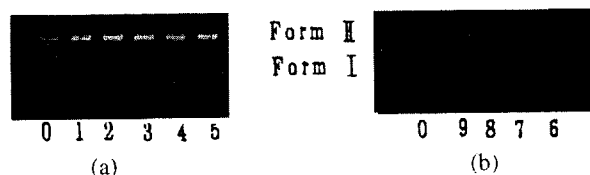


Figure 7. (A) Cleavage of pBR 322 DNA in the presence of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ after 40 min irradiation at 254 nm light. DNA alone (lane 0), the concentration of $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ was 2.5, 5, 7.5, 10, 15 μM (lanes 1–5). (B) pBR 322 DNA was incubated with $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ (10 μM) and irradiated for 5, 10, 20, 30 min (lanes 6–9) at 254 nm light.

Co(III) complex is photoreduced, with perhaps concomitant hydroxide oxidation that is responsible for cleavage [18]. The mechanism of photoactivated cleave DNA with $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ may be similar to that of $\text{Co}(\text{phen})_3^{3+}$ [19].

Conclusion

We have shown $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ to bind to DNA by intercalation of the dppz ligand. Evidence for intercalation is provided by the strong hypochromism in the IL $\pi-\pi^*$ transition centered on the dppz ligand observed by visible spectroscopy. This intercalation of the dppz ligand gives rise to the molecular light switch properties of the complex since emission in aqueous solution is observed only when the dppz nitrogens are protected from solvent, as provided by intercalation. When irradiated at 254 nm, $\text{Co}(\text{phen})_2\text{dppz}^{3+}$ is capable of inducing single-strand scissions of DNA. The above results clearly indicate that it is possible to design molecular systems that can bind to DNA avidly and achieve photoactivated DNA damage. This complex should continue to be useful as a tool for probing DNA.

This work is supported by the National Science Foundation of China.

References

1. J. K. Barton, A. T. Danishefsky, and J. M. Goldberg, *J. Am. Chem. Soc.* **106**, 2171 (1984).
2. J. K. Barton, *Science* **233**, 727 (1986).
3. M. Carter, M. Rodriguez, and A. J. Bard, *J. Am. Chem. Soc.* **111**, 8901 (1989).
4. A. E. Friedman, J. C. Chambron, J. P. Sauvage, N. J. Turro, and J. K. Barton, *J. Am. Chem. Soc.* **112**, 4960 (1990).
5. G. Grassini-Strazza and S. Isola, *J. Chromatogr.* **154**, 127 (1978).
6. A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro, and J. K. Barton, *J. Am. Chem. Soc.* **111**, 3051 (1989).
7. J. E. Dickeson and L. A. Summers, *Aust. J. Chem.* **23**, 1023 (1970).
8. W. J. Geary, *Coord. Chem. Rev.* **7**, 81 (1971).
9. S. A. Tysoe, A. D. Baker, and T. C. Streckas, *J. Phys. Chem.* **97**, 1707 (1993).
10. C. Hiort, P. Lincoln, and B. Norden, *J. Am. Chem. Soc.* **115**, 3448 (1993).
11. R. E. Holmlin and J. K. Barton, *Inorg. Chem.* **34**, 7 (1995).
12. Y. Jenkins, A. E. Friedman, N. J. Turro, and J. K. Barton, *Biochem.* **31**, 10809 (1992).
13. D. J. Patel, *Acc. Chem. Res.* **12**, 118 (1979).
14. D. J. Patel and L. L. Canuel, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3343 (1976).
15. D. S. Sigma, A. Mazuder, and D. M. Perrin, *Chem. Rev.* **93**, 2297 (1993).
16. F. Liu, A. Meadows, and D. R. McMillin, *J. Am. Chem. Soc.* **115**, 6699 (1993).
17. J. Sun and D. K. Y. Solaiman, *J. Inorg. Biochem.* **40**, 271 (1990).
18. J. K. Barton and A. L. Rapheal, *J. Am. Chem. Soc.* **106**, 2466 (1984).
19. M. B. Fleisher, K. C. Waterman, N. J. Turro, and J. K. Barton, *Inorg. Chem.* **25**, 3549 (1986).

Received November 7, 1996; accepted January 29, 1997