Synthesis and DNA Binding of Novel Water-Soluble Cationic Methylcobalt Porphyrins

Jenna S. Trommel and Luigi G. Marzilli*

Department of Chemistry, Emory University, Atlanta, Georgia 30322

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Organocobalt derivatives of tetracationic water-soluble porphyrins are difficult to prepare via the typical reductive alkylation of the Co(II)(por) (porH_2 = porphyrin ligand). None have been reported. The problem may arise because the porphyrin core is made relatively electron poor by the positively charged peripheral groups. We have circumvented this problem by using the $[Co(III)(NH_3)_5CH_3]^{2+}$ reagent, which inserts the Co(III)−CH_3 moiety directly into porH_2 in water under basic conditions. The method afforded two new $[CH_3Co(por)]^{4+}$ derivatives, $[CH_3CoTMpyP(4)]^{4+}$ and $[CH_3CoTMAP]^{4+}$, where $[TMpyP(4)]^{4+}$ and $[TMAP]^{4+}$ are the coordinated, NH-deprotonated forms of *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin and *meso*-tetrakis(*N*,*N*,*N*-trimethylaniliniumyl)porphyrin, respectively. The binding of the two new $[CH_3Co(por)]^{4+}$ cations to DNA and to the synthetic DNA polymers $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ was studied. Using published criteria by which changes in DNA viscosity and in the visible and CD spectra in the Soret region can be used to assess DNA binding, we conclude that both are outside binders. A large hypochromicity of the Soret bands of the $[CH_3Co(por)]^{4+}$ cations observed upon outside binding to DNA may indicate a high degree of self-stacking. The visible absorption and CD spectra of the $[CH_3Co(por)]^{4+}$ cations in the presence of 1:1 mixtures of $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ are nearly identical to those with $[poly(dA-dT)]_2$ alone and are very different from those of $[poly(dG-dC)]_2$ alone. Thus, both cations show a high preference for outside binding at AT-rich over GC-rich DNA sites. Upon binding of each of the $[CH_3Co(por)]^{4+}$ cations to all of the DNA polymers, the Soret bands exhibit blue shifts, whereas the Soret bands of the corresponding $[(H_2O)_2Co(por)]^{5+}$ cations exhibit red shifts. The blue shifts strongly suggest that the $[CH_3Co(por)]^{4+}$ cations, particularly $[CH_3CoTMAP]^{4+}$, become five-coordinate forms to some extent on DNA binding; this result is the first good evidence for the presence at equilibrium of five-coordinate $CH_3Co(III)(N_4)$ forms in water.

Introduction

Cationic water-soluble metalloporphyrin derivatives exhibit diverse binding modes to nucleic acids. This binding mode depends on the size and charge of the porphyrin *meso* substituents (Figure 1), on the identity of the metal, and on the nature of the axial ligation.[1−5] Binding studies are motivated in part by the potential medical applications of cationic porphyrin complexes. One of the porphyrins used in this study, *meso*-tetrakis(*N*-methyl-4-pyridiniumyl)porphyrin ($[TMpyP(4)]^{4+}$, Figure 1), and its derivatives exhibit activity against human immunodeficiency virus, type 1 (HIV-1), the virus responsible for AIDS.[6,7] $[TMpyP(4)]^{4+}$ has also been studied recently as an inhibitor of telomerase, a potential target for new antitumor drugs.[8,9] In addition, numerous porphyrins, including both $[TMpyP(4)]^{4+}$ and $[TMAP]^{4+}$ ($[TMAP]^{4+}$ = *meso*-tetrakis(*N*,*N*,*N*-trimethylaniliniumyl)porphyrin, Figure 1), inhibit the formation of protease-resistant prion protein, thereby acting as potential therapeutic agents for transmissible spongiform encephalopathies.[10,11]

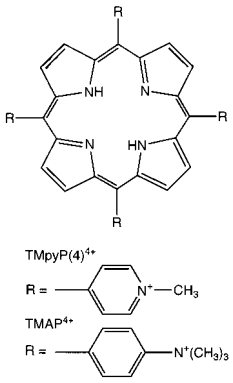

Figure 1. Structures of porphyrin ligands referred to in this study.

One complicating factor in understanding the binding of metalloporphyrins to biological targets is the relatively facile axial ligand exchange that occurs in most cases. Thus, we decided to prepare water-soluble metalloporphyrins with a tightly held covalent axial ligand. We selected Co(III) porphyrins

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which contain an axial Co−CH_3 bond (CH_3Co(por)) (porH_2 = parent porphyrin). Another advantage of CH_3Co(por) species is light sensitivity. Homolytic cleavage of the photosensitive Co−CH_3 bond to produce Co(II) and methyl radical in non-water-soluble CH_3Co(por) complexes and a variety of other CH_3-Co(III) complexes has been studied extensively.[12] DNA cleavage can be effected by photochemically activated porphyrins.[6,13,14] In addition, methyl radicals are known to initiate DNA cleavage, and a few recent examples exist of DNA cleavage by methyl radicals generated via homolysis of a metal−alkyl bond.[15−17] In the future, our goal is to study the products of irradiation of water-soluble $[CH_3Co(por)]^{4+}$ complexes bound to DNA; however, the goal of the present study was to develop synthetic methods for these porphyrins and to characterize their binding to DNA.

The most widely used synthetic route to organocobalt complexes is reductive alkylation, in which Co(II) or Co(III) complexes are reduced to Co(I) complexes and then treated with electrophilic alkylating agents.[18] This procedure has been applied to water-soluble B_{12} derivatives such as the cobalamins[19] and to non-water-soluble B_{12} models.[12] This route has also been successful in the preparation of uncharged CH_3Co(por) compounds which contain electron-rich porphyrins and which are soluble in organic solvents; examples include methylCo(III)-tetraphenylporphyrin (CH_3CoTPP)[20] and methylCo(III)octaethylporphyrin (CH_3CoOEP).[21] CH_3Co(por) complexes have been studied as models for the B_{12} coenzyme methylcobalamin.[22−24] Organocobalt porphyrins have also been prepared by using Grignard or alkyl(aryl)lithium reagents[25,26] or by transfer of the alkyl group from an N-alkylated cobalt porphyrin to the cobalt center under reducing conditions.[27] However, most of these routes utilized nonaqueous solvents.

Organocobalt(III) derivatives of cationic water-soluble porphyrins such as $[TMAP]^{4+}$ and $[TMpyP(4)]^{4+}$ (Figure 1) are unknown. We have been able to prepare salts of $[CH_3$-$CoTMAP]^{4+}$ from $[(H_2O)_2CoTMAP]^{5+}$ via a new approach combining reagents employed in previously reported reductive alkylations. However, our new combination was unsuccessful in attempts to prepare $[CH_3CoTMpyP(4)]^{4+}$ from $[(H_2O)_2$-$CoTMpyP(4)]^{5+}$. Thus, reductive alkylation of $[(H_2O)_2Co$-$(por)]^{5+}$ cations which contain more electron-poor porphyrin ligands does not appear to be a generally useful method.

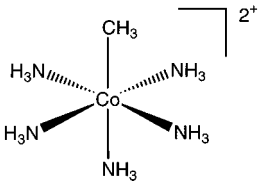

Figure 2. Pentaamminemethylcobalt(III) cation, $[Co(NH_3)_5CH_3]^{2+}$.

The $[Co(III)(NH_3)_5CH_3]^{2+}$ cation[28] (Figure 2) is the simplest organocobalt(III) compound with one coordinated carbon atom and five coordinated nitrogen atoms. The trans-labilizing methyl group promotes facile ammine ligand exchange. Substitution with simple ligands such as H_2O, NO_2^-, CN^-, and 1,2-ethylenediamine of the ammine ligands in $[Co(III)(NH_3)_5CH_3]^{2+}$ has been reported.[29,30] We have found that this substitution procedure can be extended to the tetracationic porphyrin ligands shown in Figure 1, and we have used $[Co(III)(NH_3)_5CH_3]^{2+}$ to synthesize salts of both the $[CH_3CoTMAP]^{4+}$ and the $[CH_3$-$CoTMpyP(4)]^{4+}$ cations. The binding interactions of these new $[CH_3Co(por)]^{4+}$ cations with DNA and synthetic DNA polymers were then studied as the first step toward the eventual assessment of the photochemistry of the $[CH_3Co(por)]^{4+}$ cations bound to DNA and of potential medical applications of the porphyrins. Unexpectedly, the study has generated good evidence that five-coordinate $[CH_3Co(por)]^{4+}$ complexes may exist in water.

Experimental Section

Materials. The chloride salts of $[TMAP]^{4+}$ and $[TMpyP(4)]^{4+}$ (Figure 1) were obtained from MidCentury. $[(H_2O)_2CoTMpyP(4)]^{5+}$ was prepared and isolated as the Cl^-, Br^-, and BF_4^- salts, as previously described.[31] $[(H_2O)_2CoTMAP]^{5+}$ was prepared following the same procedure used for $[(H_2O)_2CoTMpyP(4)]^{5+}$,[31] except that reflux conditions were used to decrease the reaction time. $[Co(NH_3)_5CH_3](NO_3)_2$ was prepared as reported.[28] TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy, free radical) was obtained from Aldrich. Deuterated solvents were obtained from Isotec. Stock solutions of calf thymus (CT) DNA (Worthington), $[poly(dA-dT)]_2$ (Pharmacia), and $[poly(dG-dC)]_2$ (Pharmacia) were prepared in 10 mM NaCl. Concentrations in base pairs were determined by UV spectroscopy using $\epsilon_{260} = 1.32 \times 10^4$ M^{-1} cm^{-1} (CT DNA),[32] $\epsilon_{262} = 1.32 \times 10^4$ M^{-1} cm^{-1} ($[poly(dA-dT)]_2$),[33] and $\epsilon_{254} = 1.68 \times 10^4$ M^{-1} cm^{-1} ($[poly(dG-dC)]_2$).[34] All DNA solutions were kept at −20 °C and were thawed at room temperature prior to use. PIPES10 buffer was prepared with 10 mM PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid], Sigma) at pH 7.0 in deionized water containing 100 mM NaCl.

Physical Measurements. Visible spectra were recorded at 25 °C with a Varian Cary 3 spectrophotometer. Circular dichroism (CD) spectra were recorded at 25 °C on a JASCO J-600 spectropolarimeter using a 1 nm bandwidth, 1 s time constant, 0.2 nm/data step resolution, and 50 nm/min scan speed. The molar ellipticity values [Θ] are reported in the units deg cm^2/dmol of porphyrin; these units will not be repeated in the text. Ten spectra were recorded in succession and averaged to improve signal-to-noise ratios. Solutions for spectroscopy were prepared by diluting an aqueous porphyrin stock solution in 10 mM NaCl (pH 7) in a 1 cm cuvette. Porphyrin concentrations were determined using the molar absorptivity (ϵ_{Soret}) at the wavelength of maximum absorbance of the Soret band, λ_{Soret} (Table 1). Aliquots of DNA or synthetic polymer

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Table 1. Visible Spectroscopic Data (H_2O) for the Porphyrins in This Study

porphyrin	λ_{Soret} (nm)	ϵ_{Soret} (M^{-1} cm^{-1}) ($\times 10^{-5}$)	pH
$[TMpyP(4)]^{4+}$	422	2.63[a]	6
$[(H_2O)_2CoTMpyP(4)]^{5+}$	437	1.68[b,c]	8
$[CH_3CoTMpyP(4)]^{4+}$	431	1.42[d]	8
$[(NH_3)_2CoTMpyP(4)]^{5+}$	438[e]	2.00[d]	11.5
$[TMAP]^{4+}$	412	4.85[a]	7
$[(H_2O)_2CoTMAP]^{5+}$	425	2.20[b]	7
$[CH_3CoTMAP]^{4+}$	417	1.80[d]	7
$[(NH_3)_2CoTMAP]^{5+}$	428[e]	2.65[d]	11.5

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were added to prepare solutions with specific *R* values, where *R* is the [porphyrin]/[DNA base pair] ratio.

NMR Spectroscopy. ^1H NMR measurements were carried out using a Varian INOVA 400 or Mercury 300 spectrometer typically with 5 mM porphyrin, unless otherwise noted. A sweep width of ∼7000 Hz was used for the $[CH_3Co(por)]^{4+}$ complexes. Signals were referenced internally to tetramethylsilane (TMS) in DMSO-d_6 and to the HOD signal at 4.8 ppm in D_2O because the (3-trimethylsilyl)propionic-2,2,3,3-d_4 acid (TSP) shift was influenced by the porphyrin complex. Most manipulations with the porphyrin complexes, particularly the $[CH_3Co(por)]^{4+}$ derivatives, were performed in dim light, and solutions for both the visible and NMR studies were covered with a dark cloth or aluminum foil, except during the experiments to determine ϵ_{Soret} values for the new porphyrins (below). Titrations of both $[(H_2O)_2CoTMAP]^{5+}$ and $[(H_2O)_2CoTMpyP(4)]^{5+}$ with NH_3 in DMSO-d_6 were performed in order to identify $[(NH_3)_2Co(por)]^{5+}$ and $[(NH_3,DMSO)Co(por)]^{5+}$ ^1H NMR signals (Table 3), which were often observed in spectra (DMSO-d_6) of initial products of the $[CH_3Co(por)]^{4+}$ syntheses. In DMSO-d_6, the monoammine derivative is presumed to have an axially bound DMSO ligand trans to NH_3. Assignments of the Co−NH_3 (3 H) and $Co(NH_3)_2$ (6 H) signals were based on the integration of each signal vs the corresponding downfield porphyrin signals.

Determination of Molar Absorptivity Values. Photosensitivity of the Co−CH_3 bond in the new $[CH_3Co(por)]^{4+}$ complexes proved to be a useful tool in determining ϵ_{Soret} in water. This procedure involved photolysis of $[CH_3Co(por)]^{4+}$ to $[Co(II)(por)]^{4+}$ followed by oxidation to $[(H_2O)_2Co(por)]^{5+}$, for which ϵ_{Soret} values have already been determined (Table 1). In general, samples of $[CH_3Co(por)]^{4+}$ (∼3−5 μM) were irradiated for ∼25 min with a 75 W lamp placed 8−10 in. from the sample to minimize heating. Details of this procedure are given in the Supporting Information, but we note here an important consideration. In this procedure, to avoid the formation of methylperoxoCo(III)porphyrins ($[CH_3OOCo(por)]^{5+}$) upon irradiation, samples were irradiated in the presence of 10 mM TEMPO and in the absence of ambient oxygen. TEMPO traps the methyl radical, thereby preventing it from being trapped by O_2 to form the $[CH_3OOCo(por)]^{5+}$ derivative. All Co porphyrins had approximately the same absorbance in H_2O as in 10 mM TEMPO in H_2O at the same pH and concentration of porphyrin. Multiple photolyses of separate samples of each $[CH_3Co(por)]^{4+}$ were performed, giving values of $\epsilon_{Soret} = 1.80 \times 10^5$ M^{-1} cm^{-1} for $[CH_3CoTMAP]^{4+}$ and $\epsilon_{Soret} = 1.42 \times 10^5$ M^{-1} cm^{-1} for $[CH_3CoTMpyP(4)]^{4+}$ (Table 1).

Viscosity Studies. Viscometric titrations of $[CH_3Co(por)]^{4+}$ (Supporting Information) were performed using Cannon-Ubbelohde semimicrodilution capillary viscometers in a circulating water bath maintained at 30.4 °C. PIPES10 buffer (1.0 mL) was added to the viscometer, and a reading was obtained. CT DNA stock solution was added to make the final DNA concentration 70 μM. The viscosity of the DNA solution was then measured. Aliquots of porphyrin stock solution were then added for the titration. Time readings were obtained with a timer accurate to ±0.01 s, and viscosity measurements were taken until three consecutive readings differed by less than ±0.1 s. The solution reduced viscosity (SRV) was calculated as the ratio of the viscosity of the porphyrin/DNA solution to that of the DNA solution alone. Readings were obtained in the dark to prevent photolysis of the Co−CH_3 bond, with just enough residual light to see the viscometer markings.

Table 2. ^1H NMR Shifts (ppm)[a] for Porphyrin Signals, 5 mM in D_2O, at pH = 7 and 25 °C

porphyrin	pyrrole	H_α	H_β	NCH_3	Co(III)−CH_3
$[TMpyP(4)]^{4+}$	*b*	8.96	9.31	*c*	
$[(H_2O)_2CoTMpyP(4)]^{5+}$	9.31	8.99	9.32	*c*	
$[CH_3CoTMpyP(4)]^{4+}$	8.96	8.82	9.21	4.73	−4.83

porphyrin	pyrrole	H_α	H_β	$N(CH_3)_3$	Co(III)−CH_3
$[TMAP]^{4+}$	*b*	8.20	8.29	3.93	
$[(H_2O)_2CoTMAP]^{5+}$	9.24	8.30	8.54	3.97	
$[CH_3CoTMAP]^{4+}$	8.82	8.20	8.33	3.90	−4.97

[a] Versus HOD at 4.80 ppm. [b] Signal too broad to assign at this concentration and temperature.[55,56] [c] This signal obscured by presaturation of the HOD signal.

Synthetic Methods. Preparation of $[CH_3CoTMAP]Cl_4$ or $[CH_3CoTMAP](BF_4)_4$ via Reductive Methylation of $[(H_2O)_2CoTMAP]Cl_5$ or $[(H_2O)_2CoTMAP](BF_4)_5$. $[(H_2O)_2CoTMAP]Cl_5$ or $[(H_2O)_2CoTMAP](BF_4)_5$ (50 mg) was dissolved in 10 mL of 1% NH_4Cl (w/w), and the solution was purged with N_2 for 30 min. In the dark, a 10-fold molar excess of CH_3I was added, followed by a 10-fold molar excess of $NaBH_4$. Extensive bubbling and the appearance of an orange-colored froth occurred. The solution was allowed to stir under N_2 for ∼30 min, when a red crystalline precipitate floating in solution appeared. Acetone (35 μL) was added to quench the excess of borohydride, and $[CH_3CoTMAP]Cl_4$ or $[CH_3CoTMAP](BF_4)_4$ was isolated by filtration. The isolated product gave a λ_{Soret} at 417 nm (Table 1) and had one upfield ^1H NMR signal (3 H) at −4.97 ppm in D_2O and at −5.02 ppm in DMSO-d_6. Assignments of the remaining ^1H NMR signals are listed in Tables 2 and 3. Occasionally, residual NH_4Cl or NH_4BF_4 (detected by a broad ^1H NMR signal around 7.1 ppm in DMSO-d_6) was removed by washing the product with water. $[CH_3CoTMAP]Cl_4$ or $[CH_3CoTMAP](BF_4)_4$ was isolated in 60−70% yield (∼30 mg, depending on counterion). MS (+ve FAB, mNBA): 916.2 (calcd 917.0 for $[CH_3CoTMAP]^{4+}$).

Preparation of $[CH_3CoTMAP]Br_4$ from $[Co(NH_3)_5CH_3](NO_3)_2$ and $[TMAP]Cl_4$. $[Co(NH_3)_5CH_3](NO_3)_2$ (10−11 mg, 0.90−0.95 equiv) was added to an 8.1 mM aqueous solution of $[TMAP]Cl_4$ (40 mg in 5 mL), giving a solution with a pH of ∼9.5−10. The pH was then raised to 12−12.5 by addition of 10 μL of 50% NaOH, and the solution was allowed to stir at room temperature for 1.5−2 h. Our studies suggested that $[CH_3CoTMAP]^{4+}$ formed at this stage. However, in the presence of a high concentration of NH_3/NH_4^+ in solution, $[CH_3CoTMAP]^{4+}$ decomposed, as evidenced by ^1H NMR signals for the $[(NH_3)_2CoTMAP]^{5+}$ and $[(NH_3,DMSO)CoTMAP]^{5+}$ cations in DMSO-d_6 solutions of the isolated material (Table 3). Details of the problems encountered with decomposition are given in the Supporting Information. One equivalent of $[Co(NH_3)_5CH_3]^{2+}$ yields 5 equiv of NH_3/NH_4^+ in aqueous solution once the Co−CH_3 moiety inserts into the porphyrin core; thus, less than 1 equiv of $[Co(NH_3)_5CH_3]^{2+}$ was used.

When the $[CH_3CoTMAP]^{4+}$ product was isolated as the Br^- salt after the 1.5−2 h reaction time mentioned above, the product gave ^1H NMR signals of $[TMAP]^{4+}$. The remaining $[TMAP]^{4+}$ was converted to $[Co(II)TMAP]^{4+}$ after 1.5−2 h by addition of $Co(NO_3)_2 \cdot 6H_2O$ (12 mg, 8.2 mM, 10−20 times the molar amount of $[TMAP]^{4+}$ estimated to be remaining (0.4−0.8 mM, 5−10%) after formation of $[CH_3CoTMAP]^{4+}$). Because the pH of the solution had dropped to ∼10, it was raised back to ∼12 by addition of a small amount of 50% NaOH, and the solution was stirred for ∼15 min. The pH of the reaction mixture was then lowered to ∼2 by addition of a small amount of HNO_3 (concentrated), and air was bubbled through the solution to oxidize the $[Co(II)TMAP]^{4+}$ to $[(H_2O)_2Co(III)TMAP]^{5+}$. The product isolated after ∼2−2.5 h total reaction time by addition of a saturated aqueous solution of NaBr (∼1 mL) displayed the same λ_{Soret} (417 nm) and the same ^1H NMR signals (Tables 2 and 3) as the product obtained via reductive methylation (above). If any ^1H NMR signals of $[(NH_3,DMSO)CoTMAP]^{5+}$ or $[(NH_3)_2CoTMAP]^{5+}$ were observed in a DMSO-d_6 solution of the isolated product (Table 3), the product was

Table 3. ^{1}H NMR Shifts (ppm)[a] for Porphyrin Signals, 5 mM in DMSO-d_6, at 25 °C

porphyrin	pyrrole	H_α	H_β	NCH_3	−NH	Co−CH_3	Co−NH_3
$[TMpyP(4)]^{4+}$	9.19	9.00	9.54	4.74	−3.11		
$[(DMSO)_2CoTMpyP(4)]^{5+}$	9.49	9.01	9.51	4.76			
$[CH_3CoTMpyP(4)]^{4+}$	8.94	8.82	9.42	4.70		−4.96	
$[(NH_3,DMSO)CoTMpyP(4)]^{5+}$	9.33	8.88	9.47	4.74			−4.82
$[(NH_3)_2CoTMpyP(4)]^{5+}$	9.23	8.75	9.44	4.72			−4.55

porphyrin	pyrrole	H_α	H_β	$N(CH_3)_3$	−NH	Co−CH_3	Co−NH_3
$[TMAP]^{4+}$	8.87	8.46	8.50	3.94	−2.97		
$[(DMSO)_2CoTMAP]^{5+}$	9.20	8.45[b]	8.45[b]	3.93			
$[CH_3CoTMAP]^{4+}$	8.67	8.29	8.38	3.90		−5.02	
$[(NH_3,DMSO)CoTMAP]^{5+}$	9.05	8.42[b]	8.42[b]	3.92			−4.96
$[(NH_3)_2CoTMAP]^{5+}$	8.94	8.40[b]	8.40[b]	3.91			−4.71

[a] Versus TMS. [b] These signals coalesce at this concentration.

redissolved in water and placed in the refrigerator overnight to allow slow precipitation of only $[CH_3CoTMAP]Br_4$ in 20−30% yield (10−15 mg).

Preparation of $[CH_3CoTMpyP(4)]Br_4$ from $[Co(NH_3)_5CH_3](NO_3)_2$ and $[TMpyP(4)]Cl_4$ Using an Excess of $[TMpyP(4)]Cl_4$. $[Co(NH_3)_5CH_3](NO_3)_2$ (23 mg, 0.9 equiv) was added to an 18 mM aqueous solution of $[TMpyP(4)]Cl_4$ (75 mg in 5 mL), giving a solution with a pH of ∼9.5−10. The pH was raised to 12−12.5 by addition of 10 μL of 50% NaOH, and the solution was stirred for 15−25 min. Because the isolated product from initial preparations had ^{1}H NMR signals of $[TMpyP(4)]^{4+}$, the remaining $[TMpyP(4)]^{4+}$ was converted to $[Co(II)TMpyP(4)]^{4+}$ at this point by addition of $Co(NO_3)_2\cdot 6H_2O$ (26 mg, 17.9 mM, ∼10 times the molar amount of $[TMpyP(4)]^{4+}$ estimated to be remaining (1.8 mM, 10%) after formation of $[CH_3CoTMpyP(4)]^{4+}$). Because the pH of the solution had dropped to ∼10, it was raised back to ∼12 by addition of a small amount of 50% NaOH, and the solution was stirred for ∼15 min. The pH of the reaction mixture was then lowered to ∼2 by addition of a small amount of HNO_3 (concentrated), and air was bubbled through the solution to oxidize the $[Co(II)TMpyP(4)]^{4+}$ to $[(H_2O)_2Co(III)TMpyP(4)]^{5+}$. With the solution on ice, a saturated aqueous solution of NaBr (∼1 mL) was added and a red-colored precipitate formed after 5−10 min. The isolated product, $[CH_3CoTMpyP(4)]Br_4$, obtained in ∼45% yield (∼40 mg), had one upfield ^{1}H NMR signal (3 H) at −4.83 ppm in D_2O and at −4.96 ppm in DMSO-d_6. Assignments of the remaining ^{1}H NMR signals are given in Tables 2 and 3. MS (+ve FAB, mNBA): 750.1 (calcd 750.0 for $[CH_3CoTMpyP(4)]^{4+}$).

Preparation of $[CH_3CoTMpyP(4)](BF_4)_4$ from $[Co(NH_3)_5CH_3](NO_3)_2$ and $[TMpyP(4)]Cl_4$ Using an Excess of $[Co(NH_3)_5CH_3](NO_3)_2$. $[Co(NH_3)_5CH_3](NO_3)_2$ (38 mg, 1.5 equiv) was added to an 18 mM aqueous solution of $[TMpyP(4)]Cl_4$ (75 mg in 5 mL), giving a solution with a pH of ∼10. The pH was raised to ∼12.2 by addition of 15 μL of 50% NaOH, and the solution was stirred for 5 min. The solution was then put on ice and 1 mL of 2 M $NaBF_4$ added to give a red precipitate. $[CH_3CoTMpyP(4)](BF_4)_4$ was obtained in 82% yield (81 mg) by this route, and no ^{1}H NMR signals (DMSO-d_6) for either $[TMpyP(4)]^{4+}$ or residual NH_4^+ were observed.

In both of the above preparations of $[CH_3CoTMpyP(4)]Br_4$ or $[CH_3CoTMpyP(4)](BF_4)_4$, if any ^{1}H NMR signals for $[(NH_3,DMSO)CoTMpyP(4)]^{5+}$ or $[(NH_3)_2CoTMpyP(4)]^{5+}$ were detected in DMSO-d_6 solutions of the isolated products (Table 3), the product was redissolved in water, and the solution was placed in the refrigerator overnight to allow slow precipitation of only $[CH_3CoTMpyP(4)]Br_4$ or $[CH_3CoTMpyP(4)](BF_4)_4$. Details of the problems encountered with decomposition due to the presence of a high concentration of NH_3/NH_4^+ in solution are given in the Supporting Information.

Results

Synthesis. Reductive Alkylation Method To Prepare $[CH_3CoTMAP]^{4+}$. Initial attempts to prepare $[CH_3CoTMAP]^{4+}$ by the typical reductive alkylation procedures using $NaBH_4$ and CH_3I in water or methanol resulted in a mixture of methylated products. A less commonly utilized reductive alkylation procedure employs 10% (w/w) NH_4Cl solution conditions and Zn as the reducing agent; the aqueous NH_4Cl may provide a source of extra protons to assist in the reduction.[19] We found that using $NaBH_4$ as the reducing agent in aqueous 10% NH_4Cl (a combination of reducing agent and salt which has not been reported before) successfully permitted reductive methylation of $[(H_2O)_2CoTMAP]^{5+}$ to $[CH_3CoTMAP]^{4+}$. This method does not suffer from the use of insoluble Zn. The soluble $NaBH_4$ reducing agent can be easily quenched by addition of a small amount of acetone following reductive methylation. Because of evidence for decomposition of $[CH_3CoTMAP]^{4+}$ in solution in the presence of a high concentration of NH_3/NH_4^+ (Supporting Information), the amount of NH_4Cl was eventually decreased to only 1% (w/w). The specific mechanism of this decomposition is uncertain, but alkylcobalt(III) complexes are known to undergo base-catalyzed decomposition.[12]

The product was identified as $[CH_3CoTMAP]^{4+}$ by a ^{1}H NMR signal for the Co−CH_3 group at −5.02 ppm in DMSO-d_6 and −4.97 ppm in D_2O (Tables 2 and 3). These upfield shifts are consistent with those reported for CH_3CoTPP (−4.75 ppm, $CDCl_3$)[20,25] and CH_3CoOEP (−5.20 ppm, $CDCl_3$[21] and C_6D_6[35]). Additional support for the presence of a photosensitive Co−CH_3 bond in the product arises from the decomposition observed upon exposure to light of a solution of $[CH_3CoTMAP]^{4+}$ in D_2O, DMSO-d_6, CD_3CN, or CD_3OD. We have also prepared $[CH_3CH_2CoTMAP]^{4+}$ by this reductive alkylation technique (using ethyl iodide). The upfield CH_3CH_2 moiety had ^{1}H NMR signals at −4.89 ppm (CH_3) and −4.04 ppm (CH_2), while the downfield pyrrole signal of $[CH_3CH_2CoTMAP]^{4+}$ was at 8.66 ppm. This pyrrole signal is at the same position as that of $[CH_3CoTMAP]^{4+}$ (Table 3), consistent with the conclusion that both products are organocobalt(III) porphyrins.

The ^{1}H NMR spectrum of the product from the typical $NaBH_4/CH_3I$ method in water or methanol showed three separate upfield methyl signals, each integrating to 3 protons against their corresponding downfield signals in DMSO-d_6. These products were not separated or characterized. One of these upfield signals (−5.02 ppm) indicated that the mixture did contain a small amount of $[CH_3CoTMAP]^{4+}$, but the remaining two signals did not correlate with other species such as $[(NH_3,DMSO)CoTMAP]^{5+}$ (Table 3). These two upfield signals fall between 0 and −4 ppm, a range common for N-methylated porphyrin species.[27,36] Also, a doublet and a triplet observed in the $-N(CH_3)_3$ (trimethylanilinium) region of the ^{1}H NMR spectra of these samples implied a disruption in the C_4 symmetry of

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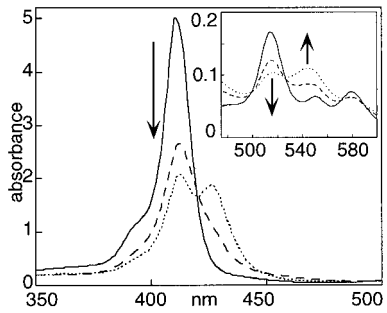

Figure 3. Visible spectra followed during the synthesis of $[CH_3CoTMAP]Br_4$ from $[TMAP]^{4+}$ (—) and 0.9 equiv of $[Co(NH_3)_5CH_3](NO_3)_2$ at room temperature, pH ~12. The Soret band decreases ~50% after ~90 min (– – –); the spectrum resulting from irradiation of an aliquot of the reaction mixture (- - -) is also shown. The inset (no irradiation depicted) shows the transition of the Q-bands with the appearance of a band at 543 nm indicating formation of $[CH_3CoTMAP]^{4+}$.

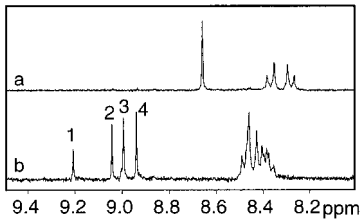

Figure 4. ^1H NMR (DMSO-d_6) aromatic region of the products isolated from reaction between $TMAPCl_4$ and 0.95 equiv of $[Co(NH_3)_5CH_3](NO_3)_2$ upon addition of Br^-: (a) fraction 1, corresponding to $[CH_3CoTMAP]Br_4$ only; (b) fraction 2, in which the pyrrole signals correspond to (1) $[(DMSO)_2CoTMAP]Br_5$, (2) $[(NH_3,DMSO)CoTMAP]Br_5$, (3) $[(Br,DMSO)CoTMAP]Br_5$, and (4) $[(NH_3)_2CoTMAP]Br_5$.

the porphyrin, a defining characteristic of N-methylated porphyrins.[27]

$[Co(NH_3)_5CH_3](NO_3)_2$ Method To Prepare $[CH_3CoTMAP]^{4+}$. $[Co(NH_3)_5CH_3]^{2+}$ (Figure 2) was also found to be a useful reagent for preparing $[CH_3CoTMAP]^{4+}$ by insertion of the $Co-CH_3$ moiety into $[TMAP]^{4+}$. This method avoids the need for reducing conditions and the exclusion of ambient oxygen required by the reductive alkylation method. During synthesis over a period of 1.5−2 h, the intensity of the $[TMAP]^{4+}$ Soret band at ~412 nm decreased by ~50% (Figure 3). This decrease is consistent with cobalt insertion on the basis of the reported molar absorptivity values of $[TMAP]^{4+}$ vs $[(H_2O)_2CoTMAP]^{5+}$ (Table 1). Other changes observed include a decrease in intensity of the longer wavelength Q-band at 514 nm and the appearance of a new band at 543 nm, which became slightly more intense than the 514 nm band (Figure 3, inset). Both $[(H_2O)_2CoTMAP]^{5+}$ and $[CH_3CoTMAP]^{4+}$ (prepared via the reductive methylation method) have a Q-band at 541 nm. The presence of $[CH_3CoTMAP]^{4+}$ in the reaction mixture was verified by the appearance of a band for $[(H_2O)_2CoTMAP]^{5+}$ (at 425 nm) after irradiation of an aliquot of the reaction mixture in the presence of ambient oxygen (Figure 3), indicating photolysis of the $Co-CH_3$ bond.

Various conditions employed for preparing $[CH_3CoTMAP]^{4+}$ by this method led to contamination by the $[TMAP]^{4+}$ salt. Because these species have the same charge, we converted any remaining $[TMAP]^{4+}$ to $[(H_2O)_2CoTMAP]^{5+}$, which is more water soluble. To improve selectivity in isolation, we employed the small Br^- counterion. The selective isolation of $[CH_3CoTMAP]Br_4$ is demonstrated by the ^1H NMR spectra in Figure 4. Refluxing conditions are normally used to increase the rate of insertion of metals into $[TMAP]^{4+}$, but *no* heat was applied to the reaction because an early synthetic attempt in which heat was used led to a large amount of $[(H_2O)_2CoTMAP]^{5+}$ in the isolated product, most likely as a result of thermolysis of the $Co-CH_3$ bond of the $[CH_3CoTMAP]^{4+}$ formed initially. Also, to minimize the risk of decomposition to $[(NH_3)_2CoTMAP]^{5+}$ caused by free NH_3/NH_4^+ in solution (Supporting Information), an excess of $[Co(NH_3)_5CH_3]^{2+}$ was not used. In fact, the use of a slight excess of $[TMAP]^{4+}$, followed by addition of $Co(NO_3)_2 \cdot 6H_2O$ to metalate the remaining $[TMAP]^{4+}$, was found to be the best way to obtain $[CH_3CoTMAP]Br_4$ by this method. The product exhibited the same λ_{Soret} in water (417 nm) and the same ^1H NMR signals (Tables 2 and 3) as $[CH_3CoTMAP]^{4+}$ obtained via the reductive methylation method. The synthesis of $[CH_3CoTMAP]^{4+}$ by two routes further confirms the identity of this product.

$[Co(NH_3)_5CH_3](NO_3)_2$ Method To Prepare $[CH_3CoTMpyP(4)]^{4+}$. Numerous variations of the modified reductive alkylation method used successfully to prepare $[CH_3CoTMAP]^{4+}$ did not afford $[CH_3CoTMpyP(4)]^{4+}$. Isolated products contained either no methylated product or a mixture of products with multiple ^1H NMR signals upfield of TMS in DMSO-d_6. We therefore focused on the $[Co(NH_3)_5CH_3]^{2+}$ reagent. The products obtained using this reagent were dependent on the amount of $[Co(NH_3)_5CH_3]^{2+}$ added and the reaction time (Supporting Information).

The two most useful approaches for preparing $[CH_3CoTMpyP(4)]^{4+}$ salts from $[TMpyP(4)]^{4+}$ follow: (i) addition of 0.9 equiv of $[Co(NH_3)_5CH_3]^{2+}$ over 15−25 min, followed by addition of $Co(NO_3)_2 \cdot 6H_2O$ to metalate the remaining $[TMpyP(4)]^{4+}$, and then isolation of $[CH_3CoTMpyP(4)]Br_4$; (ii) addition of 1.5 equiv of $[Co(NH_3)_5CH_3]^{2+}$ over 5 min and then isolation of $[CH_3CoTMpyP(4)](BF_4)_4$. In the first approach, an ~50% decrease in intensity of the $[TMpyP(4)]^{4+}$ Soret band along with a shift in the maximum from 422 to 431 nm (λ_{Soret} of $[CH_3CoTMpyP(4)]^{4+}$) occurred within 15−25 min. In the second approach, similar visible spectral changes were observed within only 5 min. The products isolated from both approaches were identified as $[CH_3CoTMpyP(4)]^{4+}$ by similar criteria [upfield $Co-CH_3$ shift (Tables 2 and 3) and light-sensitivity] used to identify $[CH_3CoTMAP]^{4+}$ (above). It was concluded that both approaches have their own advantages: Addition of the smaller Br^- counterion in the first approach allowed selective precipitation of only $[CH_3CoTMpyP(4)]Br_4$ over any other [Co(III)-TMpyP(4)]$^{5+}$ species. The $[CH_3CoTMpyP(4)]Br_4$ product was more water soluble than $[CH_3CoTMpyP(4)](BF_4)_4$ isolated via the second approach. Unlike the synthesis of $[CH_3CoTMAP]^{4+}$ by this method, use of an *excess* of $[Co(NH_3)_5CH_3]^{2+}$ in the second approach was found to speed the synthesis of $[CH_3CoTMpyP(4)]^{4+}$ and prevent the need for added $Co(NO_3)_2 \cdot 6H_2O$ since no $[TMpyP(4)]^{4+}$ was observed in the isolated product. In the second approach, BF_4^- was used to isolate $[CH_3CoTMpyP(4)]^{4+}$ because the product isolated by addition of Br^- showed broadened $[CH_3CoTMpyP(4)]^{4+}$ ^1H NMR signals, a finding that is unexplained at this time.

The $[CH_3CoTMpyP(4)]^{4+}$ product obtained from either approach did not decompose in either D_2O or DMSO-d_6 over an extended period of time (1−2 weeks), even though ^1H NMR signals for a small amount of residual NH_4Br or NH_4BF_4 were occasionally observed for the product in DMSO-d_6. In contrast, $[CH_3CoTMAP]^{4+}$ decomposed (in the absence of light) over a few hours (in D_2O) to a few days (in DMSO-d_6) in the presence of residual NH_3/NH_4^+ (Supporting Information). This difference in reactivity may be a result of the difference in electron density between the two porphyrins; $[TMAP]^{4+}$ has a higher pK_a than $[TMpyP(4)]^{4+}$ (pK_3 = 4.1 vs 1.4, respectively; $K_3 = H_3(P)^+ \leftrightarrow$

Table 4. Visible Spectroscopic Data for $[CH_3CoTMAP]^{4+}$ and $[CH_3CoTMpyP(4)]^{4+}$ in the Presence of CT DNA and Two Synthetic Polymers, 10 mM NaCl, at pH = 7

	CT DNA		$[poly(dA\text{-}dT)]_2$		$[poly(dG\text{-}dC)]_2$	
R[b]	λ (nm)	% H[c]	λ (nm)	% H[c]	λ (nm)	% H[c]
			$[CH_3CoTMAP]^{4+}$ [a]			
0.50	411	26	412	22	408	31
0.25	412	25	413	14	410	33
0.15	412	21	414	−2	412	31
0.05	413	14	414	−5	412	32
			$[CH_3CoTMpyP(4)]^{4+}$ [d]			
0.50	430	15	430	19	430	19
0.25	429	26	427	29	428	29
0.15	430	25	427	28	428	30
0.05	431	25	427	27	428	31

[a] $[CH_3CoTMAP]^{4+}$ alone, $\lambda = 417$ nm. [b] R = [porphyrin]/[polymer base pair]. [c] % H = % hypochromicity, defined by $[(abs_i - abs_f)/abs_i] \times 100\%$, where abs_i is the initial absorbance of the Soret band in the absence of DNA and abs_f is the final absorbance of the new Soret band after addition of DNA (a negative % H indicates *hyper*chromicity). [d] $[CH_3CoTMpyP(4)]^{4+}$ alone, $\lambda = 431$ nm.

$H_2(P) + H^+$, where P = porphyrin).[37,38] This difference in electron density most likely also plays a role in the synthesis, as the room-temperature synthesis of $[CH_3CoTMAP]^{4+}$ using $[Co(NH_3)_5CH_3](NO_3)_2$ required ~2−2.5 h, while that of $[CH_3CoTMpyP(4)]^{4+}$ using an excess of $[Co(NH_3)_5CH_3](NO_3)_2$ required only ~5 min.

DNA-Binding Studies. Viscosity. Viscosity measurements with 70 μM CT DNA in PIPES10 (Supporting Information) resulted in a slight increase in the SRV upon addition of $[CH_3CoTMAP]^{4+}$ when $R \leq 0.1$. Upon further addition of $[CH_3CoTMAP]^{4+}$, the SRV of the solution decreased as the porphyrin/CT DNA ratio increased. Likewise, a slight increase in SRV occurred upon addition of $[CH_3CoTMpyP(4)]^{4+}$ when $R \leq 0.1$, but as more $[CH_3CoTMpyP(4)]^{4+}$ was added, the SRV decreased. The lack of an increase in SRV at all R values studied confirms that both of the $[CH_3Co(por)]^{4+}$ cations are outside-binding porphyrins.

Visible and CD Spectroscopy. Binding of both $[CH_3Co(por)]^{4+}$ cations to CT DNA and the synthetic DNA polymers $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ was studied more extensively by visible and CD spectroscopies. All spectra were obtained in the presence of an excess of nucleic acid ($R \leq 0.5$). The nucleic acids have no CD bands in the visible region, so only the induced CD spectra of the bound porphyrins are observed because the free porphyrins have no CD signal.

$[CH_3CoTMAP]^{4+}$. Addition of CT DNA or synthetic DNA polymer to $[CH_3CoTMAP]^{4+}$ (~10 μM) caused a 3−9 nm *blue* shift of the Soret band, depending on the type of DNA added and on the R value (Table 4, Figures 5−7). The largest blue shift (9 nm) was observed in the presence of $R = 0.5$ $[poly(dG-dC)]_2$ (Table 4). At $R = 0.05$, the blue shifts were 5, 4, and 3 nm for $[poly(dG-dC)]_2$, CT DNA, and $[poly(dA-dT)]_2$, respectively (Table 4). Significant hypochromicity (~30%) of the Soret band was observed upon $[CH_3CoTMAP]^{4+}$ binding to $[poly(dG-dC)]_2$ at all R values (Table 4, Figure 7), while a smaller hypochromicity at high R and a hyperchromicity at low R were observed upon $[CH_3CoTMAP]^{4+}$ binding to $[poly(dA-dT)]_2$ (Table 4, Figure 6). The hypochromicity observed upon $[CH_3CoTMAP]^{4+}$ binding to CT DNA at $R \geq 0.25$ was between

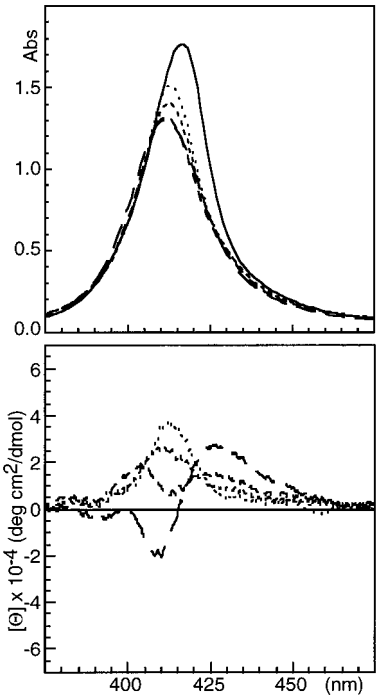

Figure 5. Visible and induced CD spectra of 10 μM $[CH_3CoTMAP]^{4+}$ alone (—) and in the presence of CT DNA at $R = 0.50$ (— - —), $R = 0.25$ (— — -), $R = 0.15$ (- - -), and $R = 0.05$ (• • •), 10 mM NaCl, pH = 7.

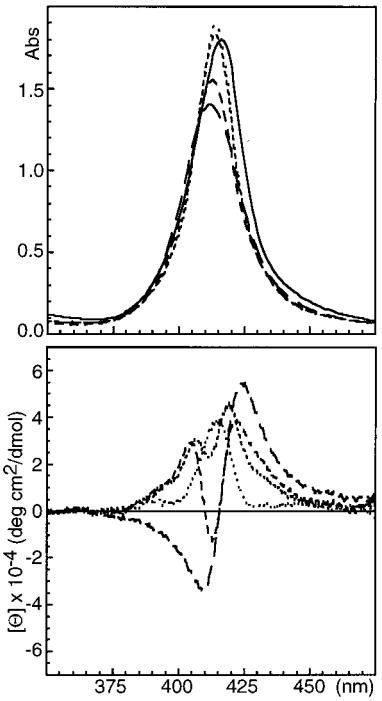

Figure 6. Visible and induced CD spectra of 10 μM $[CH_3CoTMAP]^{4+}$ alone (—) and in the presence of $[poly(dA-dT)]_2$ at $R = 0.50$ (— - —), $R = 0.25$ (— — -), $R = 0.15$ (- - -), and $R = 0.05$ (• • •), 10 mM NaCl, pH = 7.

that observed with the two synthetic DNA polymers, and no hyperchromicity was observed at $R \leq 0.25$ (Table 4, Figure 5).

Binding of $[CH_3CoTMAP]^{4+}$ to all three DNA polymers at high R induced a conservative CD spectrum with a negative band at short wavelength followed by a positive band at longer wavelength, crossing zero at the Soret maximum (Figures 5−7). At lower R values, the induced CD spectrum was no longer

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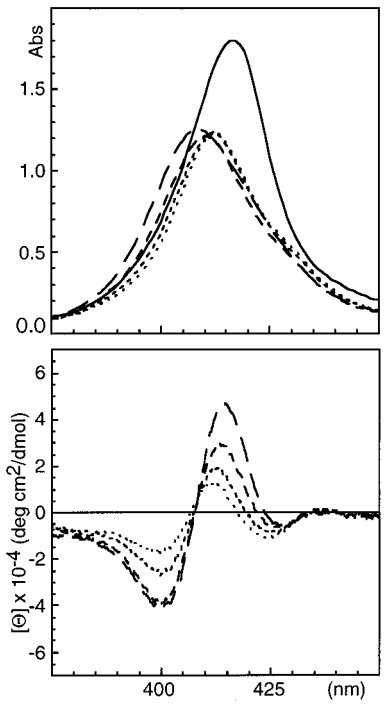

Figure 7. Visible and induced CD spectra of 10 μM $[CH_3CoTMAP]^{4+}$ alone (—) and in the presence of $[poly(dG-dC)]_2$ at $R = 0.50$ (— · —), $R = 0.25$ (— — —), $R = 0.15$ (- - -), and $R = 0.05$ (· · ·), 10 mM NaCl, pH = 7.

conservative and had one positive band at 413 nm with $\Theta \approx 4.0 \times 10^4$ at $R = 0.05$ with both CT DNA (Figure 5) and $[poly(dA-dT)]_2$ (Figure 6). However, with $[poly(dG-dC)]_2$, the induced CD spectrum of $[CH_3CoTMAP]^{4+}$ remained conservative in appearance at all R values studied (Figure 7). Overall, the visible and the induced CD spectra of $[CH_3CoTMAP]^{4+}$ bound to CT DNA were more similar to the respective spectra obtained for binding to $[poly(dA-dT)]_2$ than those obtained for binding to $[poly(dG-dC)]_2$.

The relative $[CH_3CoTMAP]^{4+}$ binding preference for $[poly(dA-dT)]_2$ vs $[poly(dG-dC)]_2$ was determined by competitive binding experiments (not shown). A solution of $[CH_3CoTMAP]^{4+}$ (10 μM)/$[poly(dA-dT)]_2$ at $R = 0.05$ gave a positive induced CD signal ($\Theta \approx 4.0 \times 10^4$ at 413 nm). Upon addition of an equal amount of $[poly(dG-dC)]_2$, the CD spectrum was essentially unchanged, exhibiting only a very slight decrease in the intensity over 2 h. Also, the visible spectrum did not change upon addition of $[poly(dG-dC)]_2$, with the Soret band at 414 nm retaining approximately the same hyperchromicity observed in the presence of $[poly(dA-dT)]_2$ alone (~2%, Table 4). In a similar experiment in which $[poly(dG-dC)]_2$ was first added to 10 μM $[CH_3CoTMAP]^{4+}$ ($R = 0.05$), a blue shift of the Soret band to 412 nm with ~31% hypochromicity was observed. Upon addition of an equivalent amount of $[poly(dA-dT)]_2$, the Soret band shifted instantly to 414 nm and increased in intensity to only 2% hypochromicity (with respect to the original $[CH_3CoTMAP]^{4+}$ spectrum; not shown). After 2 h, the 414 nm band increased to give a 1% hyperchromicity for the $[CH_3CoTMAP]^{4+}$ band, a value close to that observed in the presence of $[poly(dA-dT)]_2$ alone (Table 4). The low-intensity conservative CD signal observed in the presence of $[poly(dG-dC)]_2$ alone also changed instantly upon addition of $[poly(dA-dT)]_2$ to give a positive induced signal ($\Theta \approx 4.0 \times 10^4$ at 413 nm). *These results indicate that $[CH_3CoTMAP]^{4+}$ has a high preference for binding to $[poly(dA-dT)]_2$ over $[poly(dG-dC)]_2$.*

$[CH_3CoTMpyP(4)]^{4+}$. The DNA binding of $[CH_3CoTMpyP(4)]^{4+}$ was also studied (Supporting Information). Addition of CT DNA or a synthetic polymer to $[CH_3CoTMpyP(4)]^{4+}$ (~10 μM) caused a blue shift of its Soret band; the blue shifts [1−4 nm, depending on the DNA added and on the R value (Table 4)] were smaller than those observed for $[CH_3CoTMAP]^{4+}$. Also, in contrast to the results obtained with $[CH_3CoTMAP]^{4+}$, approximately the same percent hypochromicity was observed for the Soret band of $[CH_3CoTMpyP(4)]^{4+}$ regardless of which DNA was added (Table 4). The induced CD spectrum observed when $[CH_3CoTMpyP(4)]^{4+}$ was bound to CT DNA showed one broad positive band at low R (0.05), $\Theta \approx 4 \times 10^4$ at 440 nm. Addition of $[poly(dA-dT)]_2$ gave similar results as with CT DNA (not shown). On binding of $[CH_3CoTMpyP(4)]^{4+}$ to $[poly(dG-dC)]_2$ at $R \geq 0.25$, a *negative* induced CD signal of very low intensity was observed at 432 nm (Θ of ca. -2.6×10^4) and at $R \leq 0.15$, two small negative signals were observed at 415 and 437 nm (Θ of ca. -1.8×10^4 each) (Supporting Information). This result is in contrast to the low-intensity *positive* signals observed in CD experiments with $[(H_2O)_2CoTMpyP(4)]^{5+}$ and $[poly(dG-dC)]_2$.[5]

Competitive binding experiments were performed with $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ to determine the $[CH_3CoTMpyP(4)]^{4+}$ binding preference (not shown). A sample of $[CH_3CoTMpyP(4)]^{4+}$ (4.5 μM)/$[poly(dA-dT)]_2$ at $R = 0.05$ gave a positive induced CD signal at 440 nm with $\Theta = 4.1 \times 10^4$. Addition of an equal amount of $[poly(dG-dC)]_2$ did not change the CD spectrum when checked after 2 h. Similarly, the Soret band of $[CH_3CoTMpyP(4)]^{4+}$ did not change in position or intensity once $[poly(dG-dC)]_2$ was added (however, $[CH_3CoTMpyP(4)]^{4+}$ has roughly the same λ_{Soret} and hypochromicity in the presence of both $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$, individually (Table 4)). *These results demonstrate that $[CH_3CoTMpyP(4)]^{4+}$ prefers AT-binding sites over GC-binding sites, as found for $[CH_3CoTMAP]^{4+}$.*

Discussion

Synthesis. Organocobalt derivatives of tetracationic water-soluble porphyrins are difficult to prepare via typical alkylation of the Co(I)(por) obtained by reduction of the Co(II) or Co(III) precursor. The problem may arise because the porphyrin core is made relatively electron poor by the positively charged peripheral groups. We have overcome this difficulty and successfully prepared $[CH_3CoTMAP]^{4+}$ from $[(H_2O)_2CoTMAP]^{5+}$ by using a new reductive alkylation approach combining $NaBH_4$ as the reducing agent with 1% (w/w) aqueous NH_4Cl.

$[Co(NH_3)_5CH_3](NO_3)_2$ proved to be a useful alternative reagent for preparing $[CH_3CoTMAP]^{4+}$ and the only reagent we found for preparing $[CH_3CoTMpyP(4)]^{4+}$. We could not prepare the latter via reductive methylation, most likely because $[CH_3CoTMpyP(4)]^{4+}$ is unstable in the presence of $NaBH_4$ (experiments not shown). Substitution of the ammine ligands of $[Co(NH_3)_5CH_3]^{2+}$ with the bidentate 1,2-ethylenediamine ligand and other simple ligands has been documented,[29,30] but we provide the first example of this substitution procedure with quadridentate porphyrin ligands, effectively inserting the Co−CH_3 moiety into the porphyrin core.

DNA Binding by Porphyrins. $[TMpyP(4)]^{4+}$ vs $[TMAP]^{4+}$. The two porphyrins studied here ($[TMpyP(4)]^{4+}$ and $[TMAP]^{4+}$) and their metal derivatives contributed greatly to our early understanding of binding modes. Three different porphyrin−DNA binding modes have been invoked in previous studies: intercalation, outside binding with self-stacking, and simple outside binding without self-stacking (including groove bind-

ing).[39] $[TMpyP(4)]^{4+}$ appears to intercalate (mainly in GC sites), but $[TMAP]^{4+}$ is only an outside binder.[6,39−42] This difference led to the belief that the thickness of $[TMAP]^{4+}$ prevented intercalation.[40,41] Later, it was shown that bulky *meso* substituents still allowed intercalation, provided that the *meso* substituents were 4- or 3-pyridinium groups.[43−45] However, even with 4-pyridinium *meso* groups, metalloporphyrins with axial ligands are outside binders and do not intercalate because of steric interference.[6,46] Metal complexes of $[TMpyP(4)]^{4+}$ with either no axial ligands or only weak ones (e.g., Ni(II), Cu(II), and Au(III)) can also intercalate at GC sites and form outside-bound complexes at AT sites through electrostatic interactions or groove binding.[4,6]

Recent work suggests that intercalation may be only partial, involving mainly the pyridinium groups.[47,48] Nevertheless, the presence of axial ligands appears to prevent even this partial intercalative interaction.[46] Derivatives of $[TMpyP(4)]^{4+}$ have a relatively low tendency to stack compared to other porphyrins.[46] From recent work,[43−45] it is now clear that $[TMAP]^{4+}$ is not an intercalator since it lacks pyridinium groups. $[TMAP]^{4+}$ is electron rich and binds to the outside of DNA in a self-stacking manner.[6,40,41]

Outside binders interact preferably in AT-rich regions of DNA.[6,43] For relatively rigid porphyrins (such as $[TMpyP(4)]^{4+}$ and $[TMAP]^{4+}$ and their metal derivatives), this preference could reasonably be attributed to distortion of AT regions; this distortion may maximize electrostatic interactions.[4,45,49] However, studies with tentacle porphyrins (which have charged groups on flexible arms) still support the AT-binding preference and indicate that the DNA does not undergo a significant distortion.[43−45] The reasons for the AT-binding preference are not completely understood.

Certain experimental observations support each of the different binding modes. For the intercalative binding mode, these observations include an increase in DNA solution viscosity, an ability to unwind superhelical DNA, downfield ^{31}P NMR shifts, and upfield imino ^{1}H NMR shifts.[4,39−42,50,51] In addition, spectroscopic criteria correlate with intercalation, namely, a large red shift and large hypochromicity of the Soret band and a negative induced CD signal for this band.[5,39,41] Experimental observations used to support outside binding include the lack of an increase in DNA solution viscosity, an inability to unwind superhelical DNA, no shift of ^{31}P and ^{1}H NMR signals, small red shifts (≤8 nm) and small hypochromicity (≤30%) or hyperchromicity of the Soret bands, and the appearance of a single positive band in the visible CD spectrum.[40−42,50,51] In addition, Fiel has suggested that significant hypochromicity of the Soret band, along with a conservative induced CD, indicates outside binding with self-stacking.[40−42] Recent reports have shown that the relatively flexible and electron-rich tentacle porphyrins self-stack on the outside of DNA, with extensive self-stacking favored at high R values and at high salt concentrations and moderate self-stacking favored at intermediate R values and at low salt concentrations.[43−45]

Viscosity Studies. Because there is still inadequate understanding of the relationship between CD spectra and binding mode, we conducted viscosity measurements (Supporting Information) with the $[CH_3Co(por)]^{4+}$ cations. The lack of an increase in DNA solution viscosity observed for both cations established that they are outside binders.

Spectroscopic Studies. $[CH_3CoTMAP]^{4+}$. Visible and CD spectroscopic results with $[CH_3CoTMAP]^{4+}$ were consistent with its assignment as an outside binder from viscosity experiments. In particular, hyperchromicity of the Soret band in the presence of $[poly(dA-dT)]_2$ at $R \leq 0.15$ (Table 4) and positive induced CD bands at low R with both CT DNA (Figure 5) and $[poly(dA-dT)]_2$ (Figure 6) support outside binding with relatively little self-stacking. The 14−26% hypochromicity and low-intensity conservative CD bands observed at $R \geq 0.25$ with both CT DNA (Figure 5) and $[poly(dA-dT)]_2$ (Figure 6) suggest moderate self-stacking of $[CH_3CoTMAP]^{4+}$. Significant hypochromicity (∼32%, Table 4) and conservative induced CD signals (Figure 7) were observed at *all* R values for $[CH_3CoTMAP]^{4+}$ outside binding to $[poly(dG-dC)]_2$, indicating more extensive self-stacking of $[CH_3CoTMAP]^{4+}$ with this polymer.

Of note, a blue shift of the Soret band of $[CH_3CoTMAP]^{4+}$ occurred upon addition of any of the three polymers (Table 4), whereas red shifts were observed for $[(H_2O)_2CoTMAP]^{5+}$ (unpublished studies). Blue-shifting of a porphyrin Soret band upon DNA binding has been reported for $[VOTMpyP(4)]^{4+}$ [1] and for $[NiTMpyP(2)]^{4+}$ [2] ($[TMpyP(2)]^{4+}$ = *meso*-tetrakis(*N*-methyl-2-pyridiniumyl)porphyrin). The vanadyl porphyrin has one loosely held axial water ligand, whereas the nickel porphyrin has two such axial water ligands.[1,2] In studies with these porphyrins, Raman spectroscopy was used to identify the five-(5C) and six-coordinate (6C) forms of $[VOTMpyP(4)]^{4+}$ [1] and the four-coordinate (4C) and 6C forms of both $[NiTMpyP(2)]^{4+}$ and $[NiTMpyP(4)]^{4+}$.[2] Well-defined Raman vibrational bands clearly indicate the presence of a mixture of forms with different coordination numbers in various solvents and in the presence of several polymers.[1,2] 4C $[NiTMpyP(4)]^{4+}$ is an intercalator, while 4C $[NiTMpyP(2)]^{4+}$ is an outside binder because the 2-pyridinium groups do not allow intercalation.[2] Both 5C and 6C $[VOTMpyP(4)]^{4+}$ are outside binders due to the presence of axial ligands, verified most definitively by the lack of an increase in DNA solution viscosity.[1]

Raman data for the relative amounts of species with different coordination number were used to develop criteria for assessing coordination number by visible spectroscopy. In coordinating solvents (e.g., water, methanol, pyridine), only one Soret maximum for $[VOTMpyP(4)]^{4+}$ (for a 6C species) was observed, while in less highly coordinating solvents (e.g., acetonitrile, dimethylformamide, acetone), another band appeared (for a 5C species), blue-shifted from the original Soret band.[1] In acetone, this 5C band dominated the spectrum, while a shoulder to the red indicated that only a small amount of 6C [VOTMpyP-

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Table 5. Soret Bands of $[CH_3Co(por)]^{4+}$ Cations in Different Solvents

	$[CH_3CoTMAP]^{4+}$			$[CH_3CoTMpyP(4)]^{4+}$
solvent	λ_{Soret} 6C[a] (nm)	λ_{Soret} 5C[b] (nm)	relative abs λ_{Soret} 6C/λ_{Soret} 5C	λ_{Soret} 6C[a] (nm)
pyridine	432			445
methanol	419			433
water	417			431
acetonitrile	418	405	0.74	433
acetone	418	405	0.58	425

[a] 6C is six-coordinate $[CH_3Co(por)]^{4+}$. [b] 5C is five-coordinate $[CH_3Co(por)]^{4+}$.

$(4)]^{4+}$ remained.[1] The use of these Raman and visible spectral criteria for aqueous solutions containing DNA led to the conclusion that $[VOTMpyP(4)]^{4+}$ binds to DNA as a mixture of 5C and 6C forms. Similar Raman and visible spectroscopic methods were used to establish that the equilibrium between 6C and 4C $[NiTMpyP(4)]^{4+}$ in water was shifted entirely toward the 4C form upon binding of $[NiTMpyP(4)]^{4+}$ to DNA.[2]

Although Raman methods cannot be used to study a change in coordination number of $[CH_3CoTMAP]^{4+}$ due to its light-sensitivity, the blue shifts of the Soret band of $[CH_3CoTMAP]^{4+}$ upon DNA binding strongly suggest that some of the bound form is a 5C species. The λ_{Soret} of $[CH_3CoTMAP]^{4+}$ in several solvents is listed in Table 5 (spectra shown in Supporting Information). In water, methanol, and pyridine, only one Soret maximum was observed at 417, 419, and 432 nm, respectively; this band is assigned to a 6C species. The 15 nm red shift of the Soret band in pyridine compared to water clearly indicates coordination of the N-donor (pyridine) to form 6C $[CH_3CoTMAP(pyridine)]^{4+}$. However, in acetonitrile and acetone, the main Soret band appears at 405 nm, with only a shoulder at 418 nm. The 405 nm band, assigned to the 5C species, is larger in acetone than in acetonitrile (Table 5). This result is consistent with the finding that the largest amounts of 5C $[VOTMpyP(4)]^{4+}$ and 4C $[NiTMpyP(4)]^{4+}$ and $[NiTMpyP(2)]^{4+}$ were also found in acetone.[1,2]

In the studies of $[VOTMpyP(4)]^{4+}$ binding to DNA and to synthetic polymers, only $[poly(dG-dC)]_2$ caused a noticeable blue shift of the Soret band (3 nm) in 10 mM NaCl.[1] However, the increase in absorption in the low-wavelength region upon $[VOTMpyP(4)]^{4+}$ binding to all DNA polymers was indicative of an increased percentage of the 5C species. With $[CH_3CoTMAP]^{4+}$, all polymers cause a blue shift of the Soret band. Furthermore, this shift is largest with $[poly(dG-dC)]_2$ (Table 4), possibly indicating that the solvent environment around this polymer preferentially causes formation of more of the 5C species. These parallel effects for the $[VOTMpyP(4)]^{4+}$ and $[CH_3CoTMAP]^{4+}$ derivatives provide clear evidence that $[CH_3CoTMAP]^{4+}$ becomes 5C to an appreciable extent on DNA binding.

The blue shifts (Table 4) suggest 5C $[CH_3CoTMAP]^{4+}$ is a more preferred form when the cation binds to $[poly(dG-dC)]_2$ than when it binds to CT DNA or $[poly(dA-dT)]_2$. Conservative induced CD signals were observed for $[CH_3CoTMAP]^{4+}$ at all *R* values with $[poly(dG-dC)]_2$ (Figure 7), which, together with the significant hypochromicity observed with this polymer, suggests more extensive self-stacking than observed with the other two polymers. Thus, it is reasonable to suggest that self-stacking is more favored with the 5C form than with the 6C form, which has two axial ligands.

$[CH_3CoTMpyP(4)]^{4+}$. In addition to viscosity results, small positive induced CD signals with CT DNA (Supporting Information) and $[poly(dA-dT)]_2$, together with the lack of a substantial shift in λ_{Soret} (Table 4), all support outside binding for $[CH_3CoTMpyP(4)]^{4+}$. As found for $[CH_3CoTMAP]^{4+}$, the Soret band of $[CH_3CoTMpyP(4)]^{4+}$ exhibited a blue shift in the presence of all three types of DNA (Table 4), although the extent of this shift (only 1−4 nm) was less than that observed for $[CH_3CoTMAP]^{4+}$ (Table 4). This blue shift is in contrast to the small red shifts observed for $[(H_2O)_2CoTMpyP(4)]^{5+}$.[5,52,53]

The λ_{Soret} of $[CH_3CoTMpyP(4)]^{4+}$ in several solvents is listed in Table 5 (spectra shown in Supporting Information). In all solvents used, only one Soret maximum was observed. In water, acetonitrile, methanol, and pyridine, this band corresponds to a 6C species. The 14 nm red shift of the Soret band in pyridine compared to water clearly indicates that $[CH_3CoTMpyP(4)(pyridine)]^{4+}$ is formed. In acetone, the slight blue shift to 425 nm may indicate formation of some 5C $[CH_3CoTMpyP(4)]^{4+}$, but no resolved band for a 5C species was observed. Also, the result that no blue shift was observed in acetonitrile (in contrast to that observed for $[CH_3CoTMAP]^{4+}$) indicates a lesser propensity of $[CH_3CoTMpyP(4)]^{4+}$ to exist in its 5C form. These results are most likely a consequence of the more electron-poor nature of $[CH_3CoTMpyP(4)]^{4+}$ compared to $[CH_3CoTMAP]^{4+}$. Cations containing the $[Co(III)TMpyP(4)]^{5+}$ moiety are expected[1,2,31] to have two axial ligands because the porphyrin is electron poor; thus, formation of the 5C species is not so favored as for $[CH_3CoTMAP]^{4+}$.

The blue shift observed upon $[CH_3CoTMpyP(4)]^{4+}$ binding to DNA (Table 4), while also supporting formation of a 5C species, was less than that observed for $[CH_3CoTMAP]^{4+}$. This result indicates that upon DNA binding, $[CH_3CoTMpyP(4)]^{4+}$ has a greater preference than $[CH_3CoTMAP]^{4+}$ to remain in its 6C form rather than to adopt the 5C form. Approximately the same degree of blue shift and hypochromicity of the $[CH_3CoTMpyP(4)]^{4+}$ Soret band was observed with CT DNA, $[poly(dA-dT)]_2$, and $[poly(dG-dC)]_2$ (Table 4), suggesting that none of these polymers has a greater ability than the others to cause $[CH_3CoTMpyP(4)]^{4+}$ to become 5C. These results are in contrast to those obtained upon DNA binding of $[CH_3CoTMAP]^{4+}$.

Addition of $[poly(dG-dC)]_2$ to $[CH_3CoTMpyP(4)]^{4+}$ induced weak *negative* CD bands at all *R* values (Supporting Information), in contrast to the positive induced CD signals observed on addition of CT DNA or $[poly(dA-dT)]_2$. These negative bands are in contrast to the weak *conservative* CD bands observed for $[CH_3CoTMAP]^{4+}$ at all *R* values with $[poly(dG-dC)]_2$ (Figure 7) and to the weak *positive* CD bands observed for $[(H_2O)_2CoTMpyP(4)]^{5+}$ with $[poly(dG-dC)]_2$.[5] The negative induced CD signals observed for $[CH_3CoTMpyP(4)]^{4+}$ with this polymer demonstrate the inadequacy of CD spectroscopy alone in assigning porphyrin−DNA binding modes, because negative induced CD signals have normally been associated with intercalative binding. Also, the very weak conservative CD

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signals observed for $[CH_3CoTMpyP(4)]^{4+}$ at $R \geq 0.25$ with CT DNA and $[poly(dA-dT)]_2$ and lack of conservative signals with $[poly(dG-dC)]_2$ at any R value indicate very little stacking of this porphyrin, consistent with the known low tendency for stacking by $[TMpyP(4)]^{4+}$ derivatives.[40,41,46] It is also noteworthy that the hypochromicity and the blue shifts decrease at the high R value of 0.50. This pattern is atypical. Self-stacking is favored by a higher coverage of the DNA surface by outside binders. For example, the dependence on R for $[CH_3CoTMAP]^{4+}$ is the common type of behavior. We do not completely understand this unique behavior of $[CH_3CoTMpyP(4)]^{4+}$. However, a more complete assessment may emerge from our planned studies of the photochemical behavior of these adducts.

$[CH_3Co(por)]^{4+}$ Competitive Binding Experiments. The visible absorption and CD spectra of the cations in the presence of 1:1 mixtures of $[poly(dA-dT)]_2$ and $[poly(dG-dC)]_2$ are nearly identical to those with $[poly(dA-dT)]_2$ alone and are very different from those with $[poly(dG-dC)]_2$ alone (not shown). Thus, both $[CH_3Co(por)]^{4+}$ cations exhibit high binding selectivity to $[poly(dA-dT)]_2$ over $[poly(dG-dC)]_2$. As mentioned, the spectral data support preferential binding of the cations to AT regions of CT DNA. A similar high binding preference for AT regions was reported for $[TMAP]^{4+}$ [41,42] and for a variety of $[TMpyP(4)]^{4+}$ derivatives with metals bearing axial ligands (e.g., Co, V(O), Zn, Fe, Mn).[1,46,49,54]

Conclusions

Novel water-soluble tetracationic methylcobalt(III) porphyrins ($[CH_3Co(por)]^{4+}$) were prepared by two routes. The reductive alkylation method is preferable for the Co complex of the porphyrin with intermediate electron-richness, $[TMAP]^{4+}$, as found for the Co complex of the electron-rich TPP, and is not useful for the Co complex of the electron-poor $[TMpyP(4)]^{4+}$. In contrast, the $Co-CH_3$ direct transfer method using $[Co(NH_3)_5CH_3](NO_3)_2$, although useful as an alternative route to $[CH_3CoTMAP]^{4+}$, inserts the $Co-CH_3$ moiety more effectively into the $[TMpyP(4)]^{4+}$ ligand, which has more acidic NH groups than those of $[TMAP]^{4+}$. The $Co-CH_3$ direct transfer method has the advantage that reducing conditions and exclusion of oxygen are unnecessary. This method may prove to be useful for the preparation of other water-soluble organocobalt complexes containing electron-poor macrocyclic equatorial ligands.

From viscosity measurements and visible and CD spectroscopy, we conclude that both of these new $[CH_3Co(por)]^{4+}$ complexes are outside binders. In addition, competitive binding experiments have shown that this outside binding occurs with AT selectivity. Blue shifts of the $[CH_3Co(por)]^{4+}$ Soret bands observed upon DNA binding strongly suggest formation of some five-coordinate form. Significant hypochromicity of these Soret bands suggests a greater amount of stacking compared to that observed for the six-coordinate $[(H_2O)_2Co(por)]^{5+}$ cations, a result which may be attributed to a greater stacking tendency by the five-coordinate $[CH_3Co(por)]^{4+}$ species. The blue shift on DNA binding was more extensive for $[CH_3CoTMAP]^{4+}$ than for $[CH_3CoTMpyP(4)]^{4+}$, indicating that $[CH_3CoTMAP]^{4+}$ has a higher propensity to convert to the five-coordinate form. This result is consistent with the higher electron-richness of $[CH_3CoTMAP]^{4+}$ compared to that of $[CH_3CoTMpyP(4)]^{4+}$. These experiments provide the first evidence for the existence of five-coordinate organocobalt(III)(N_4) complexes at equilibrium in aqueous solution.

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Supporting Information Available: Procedure for determination of ϵ_{Soret} values for the $[CH_3Co(por)]^{4+}$ cations; information on the decomposition of the $[CH_3Co(por)]^{4+}$ cations at high concentrations of NH_3/NH_4^+, including a table and representative ^1H NMR spectra; SRV plots of $[CH_3Co(por)]^{4+}$; visible and induced CD spectra of $[CH_3CoTMpyP(4)]^{4+}$ in the presence of CT DNA and $[poly(dG-dC)]_2$; and visible spectra of each $[CH_3Co(por)]^{4+}$ in a variety of solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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