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Supplementary Material Available: Experimental details for preparation of the FK506 and the rapamycin matrix, isolation of the FK506 and rapamycin binding proteins, and ^{32}P labeling of Jurkat cells and autoradiography (3 pages). Ordering information is given on any current masthead page.

Enantiomeric Resolution of $Ru(phen)_3^{2+}$ and $Ru(bpy)_2ppz^{2+}$ on a DNA-Hydroxylapatite Column

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It has been demonstrated[1,2] that various six-coordinate metal complexes in which the ligands are bidentate diimines with fused aromatic ring systems are capable of enantiomerically selective interaction with double-stranded DNAs. The basis of this enantioselectivity is reported[2] to be the more favorable steric fit of the Δ isomer within the major groove of DNA induced by an intercalative interaction of one of the metal-bonded aromatic ligands. At the same time, a lesser enantioselectivity has been observed for the Λ isomer via interaction with the minor groove. Such complexes have been demonstrated[2-4] to be useful as selective reagents for cleaving both DNA and RNA. We report here a novel application of these interactions in separating enantiomers of such complexes by immobilizing double-stranded DNA on a column of hydroxylapatite and passing a racemic solution of a complex through the column. In one case described here, the first resolution of a complex is achieved by using such a column. A racemic mixture of the complex separated into two bands, which are demonstrated to contain mainly (~95% or higher purity) the separated Λ and Δ isomers.

We have been interested[5a] in the photophysical and photochemical properties of mixed-ligand diimine complexes of ruthenium(II) and have synthesized and characterized mononuclear and dinuclear complexes containing the ligands 4′,7′-phenanthrolino-5′,6′:5,6-pyrazine (ppz) (**1**)[5b] and 2,3-di-2-pyridylpyrazine (dpp) (**2**). The complexes $Ru(bpy)_2 \cdot \mathbf{1}^{2+}$ and $Ru(bpy)_2 \cdot \mathbf{2}^{2+}$ (bpy = 2,2′-bipyridine) were tested for evidence of intercalative interaction with DNA. The visible-region metal to ligand charge transfer (MLCT) band of $Ru(bpy)_2 \cdot \mathbf{1}^{2+}$, which is associated with the ppz ligand (Figure 1), shows hypochromicity in the presence of calf thymus DNA, while the MLCT bands of $Ru(bpy)_2 \cdot \mathbf{2}^{2+}$ show no hypochromicity under the same conditions. While this is not definitive proof of an intercalative interaction, it is strongly suggestive. At the least, it provides evidence of strong association of $Ru(bpy)_2 \cdot \mathbf{1}^{2+}$ with DNA. During the course of our investigations we attempted to resolve each into Δ and Λ isomers by fractional crystallization with potassium antimony tartarate without success. Because of limited amounts of complex, we have devised a novel means of separation for $Ru(bpy)_2 \cdot \mathbf{1}^{2+}$.

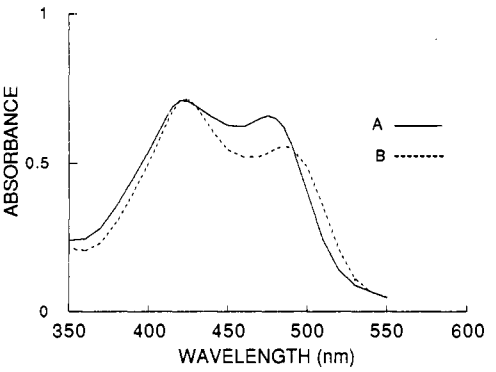
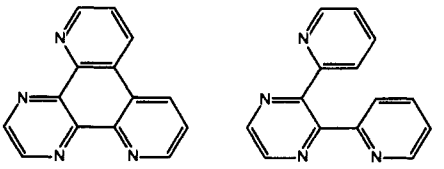


Figure 1. Absorption spectra of $Ru(bpy)_2 \cdot \mathbf{1}^{2+}$ alone (A) and in the presence of calf thymus DNA, mole ratio of DNA phosphate/Ru of 20 (B). Complex is 65 μM in both spectra.



1 **2**

A column of hydroxylapatite (BIO-GEL HTP, Biorad Laboratories), 1.6 cm × 21 cm, was first poured and washed with several column volumes of buffer (0.01 M sodium phosphate, pH 6.8; 100 mM NaCl). Calf thymus DNA (Sigma) was dissolved at a concentration of 1 mg/mL in the same buffer, and 40 mL of this solution was washed onto the column, followed by several hundred milliliters of buffer. Double-stranded DNA (and some single-stranded, if present) adsorbs[6,7] to the column under these conditions. The absorbance of the column wash at 260 nm was checked, and no DNA was detected.

As a test of the resolving capabilities of the column, we passed a 0.100-mL sample of racemic $Ru(phen)_3^{2+}$ (1.2 mM) through the column, eluting with the column buffer. Peak ratios for ultraviolet absorption bands of the complex were routinely checked to exclude the presence of DNA in the eluted samples. Circular dichroism spectra of the fractions eluted showed that the fractions eluting first were enriched in Λ-$Ru(phen)_3^{2+}$ and that the tailing fractions were enriched in Δ-$Ru(phen)_3^{2+}$. This was not unexpected since Δ-$Ru(phen)_3^{2+}$ has been shown,[2] primarily via

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(5) (a) Fuchs, Y.; Lofters, S.; Dieter, T.; Shi, W.; Morgan, R.; Strekas, T. C.; Gafney, H. D.; Baker, A. D. *J. Am. Chem. Soc.* **1987**, *109*, 2691-2697. (b) The ppz ligand was prepared by the reaction of 4,7-phenanthroline-5,6-dione with ethylenediamine (ref 5). Its proton NMR spectrum is in accord with its structure [δ 7.90, dd (2 H's meta to N's of pyridine rings); δ 9.05, d (2 H's para to N's of pyridine rings); δ 9.25, s (2 H's of pyrazine ring); 9.29, d, overlapping with δ 9.25 signal (2 H's ortho to N's of pyridine rings]. The $[(bpy)_2Ru(ppz)]^{2+}$ complex was prepared by the reaction of $Ru(bpy)_2Cl_2$ with ppz (ref 5) in the standard fashion for $[(bpy)_2RuL]^{2+}$ complexes. The complex, examined as the dichloride salt, gave a satisfactory elemental analysis. Calcd for $Ru(bpy)_2ppzCl_2 \cdot 7H_2O$: C, 48.4; H, 4.53; N, 13.3. Found: C, 48.0; H, 4.25; N, 12.9. As expected, the proton and ^{13}C NMR spectra of the complex are complicated owing to the low symmetry of the complex, resulting in a much greater number of chemically distinct hydrogen atoms and carbon atoms than, e.g., $Ru(bpy)_2phen^{2+}$. The proton NMR in CD_3CN at 200 MHz consists of a number of overlapping peaks in the region δ 7-9.3. Six of the eight protons of the coordinated ppz are assignable because they appear above δ 9 (cf. data for free ligand above), whereas the bpy protons appear at δ 8.5 or lower (Constable, E. C.; Kewis, J. *Inorg. Chim. Acta* **1983**, *70*, 251). An exact analysis must await the preparation of a complex containing deuterated bipyridines so that the spectrum can be simplified. This work is in progress. The complex contains at least 19 different carbon atoms (14 on the ppz ligand and five on the bpy ligands), assuming the two bipyridine rings to be approximately equivalent, and as many as 24 if the actual inequivalence of the bipyridine ligands is reflected in the ^{13}C spectrum. The 50-MHz ^{13}C spectrum shows, in fact, 19 peaks, but again an exact assignment must await studies on simpler model complexes. The visible absorption spectrum of the complex is typical of those of other $(bpy)_2RuL^{2+}$ complexes in showing two MLCT bands. An in-depth analysis of the resonance Raman spectra of the complex obtained at wavelengths corresponding to the two MLCT excitations (ref 5) has been reported and is consistent with the proposed structure.

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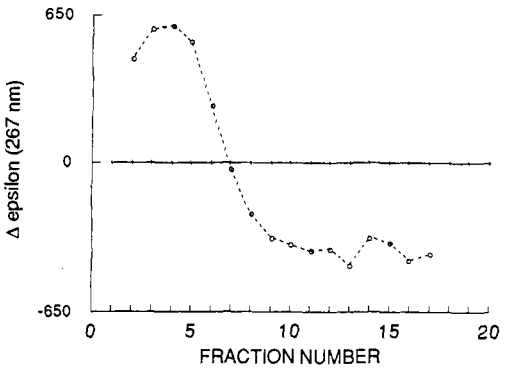

Figure 2. $\Delta\epsilon$(267nm) of fractions of $Ru(phen)_3^{2+}$ eluted from DNA-hydroxylapatite column.

equilibrium dialysis binding studies, to bind more strongly to double-stranded DNA. The column results, then, paralleled the results obtained in equilibrium dialysis experiments. A plot of the $\Delta\epsilon$(267nm) versus column fraction is presented in Figure 2. The published value[8] of $\Delta\epsilon$(267nm) is +540 for the Λ isomer. Clearly, the leading fractions are significantly enriched in the Λ isomer and the tailing fractions in the Δ isomer.

We then attempted resolution of $Ru(bpy)_2 \cdot 1^{2+}$ on the same column. A 1.0-mL sample of the complex (0.7 mM) was eluted through the column and was observed to separate into two distinct bands, separated by several centimeters just before final elution from the column. Again, peak ratios for ultraviolet absorption bands were checked to eliminate the possibility of DNA coelution. The circular dichroism spectra of the bands indicated that they were indeed enriched in the two enantiomers. The first band eluted gave $\Delta\epsilon$(289nm) = (−)120 and $\Delta\epsilon$(273nm) = (+)38 while the second band gave $\Delta\epsilon$(289nm) = (+)115 and $\Delta\epsilon$(273nm) = (−)32. A second pass of pooled and concentrated fractions from each band through the column was performed. CD spectra of these samples showed the same $\Delta\epsilon$ values within a few percent, indicating that the first pass through the column gave approximately 95% enantiomerically pure fractions. Equilibrium dialysis experiments performed in our laboratory with this complex qualitatively paralleled the column results. That is, the same isomer whose flow through the column is retarded is found to bind more strongly to DNA in solution. It seems reasonable, then, that the previously described preferred steric fit of the Δ isomer as it intercalates within the major groove of the adsorbed DNA retards its flow through the column relative to the Λ isomer, resulting in the observed separation. Because of the analogy with the $Ru(phen)_3^{2+}$ results,[9] we propose to tentatively assign the leading fractions as the Λ isomer and the tailing fractions as the Δ isomer. The maximal CD values for the Λ isomer are as follows: $\Delta\epsilon$(289nm) = (−)130; $\Delta\epsilon$(273nm) = (+)40; $\Delta\epsilon$(393nm) = (+)15.8; $\Delta\epsilon$(503nm) = (−)4.0. The Δ isomer has the same values with opposite signs.

Additional trials with several other complexes, including $Ru(bpy)_2phen^{2+}$, have indicated that this method is generally useful in accomplishing significant enantiomeric fractionation for complexes of this type, when at least one of the ligands shows evidence of potential intercalation with DNA (i.e., several fused aromatic rings). At present this is only a strong correlation and does not provide definitive evidence of the basis for the resolving power of DNA-hydroxylapatite columns. However, with the ability to separate enantiomers of such complexes, we look forward to studying, via a variety of spectroscopic methods, the effect of binding individual isomers to DNA and contributing to the resolution of this question.

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Formation of Organozincate Ions from Diorganozinc Compounds and Potassium Alkoxides

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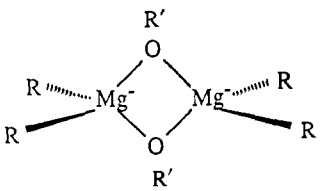
The objective of this work is to determine what species form upon mixing diorganozinc compounds and alkali-metal alkoxides. In related work, addition of alkali-metal alkoxides and some other salts to diorganomagnesium compounds was found to prepare solutions and mixtures that generally are more reactive than conventional organomagnesium compounds.[1] These preparations often are similar in reaction behavior to those obtained from diorganomagnesium compounds and crown ethers or cryptands, in which magnesiate ions (such as R_3Mg^-) are known to be significant species.[2,3] In fact, **1** was recently shown to be the probable structure for a prominent species in solutions prepared from R_2Mg and R'OK or R'ONa.[4] It seemed likely that addition of alkali-metal alkoxides to diorganozinc compounds might also form more reactive solutions that would contain species structurally similar to those formed in the Mg systems.



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Except for investigations of compositions and structures of zincates involving hydride as the added anion,[5] we could find only a few related, prior studies of R_2Zn-salt combinations. One publication[6] reported that diorganozinc compounds react with carbon monxide after, but not before, addition of *t*-BuOK. Tetraalkylammonium halides are reported[7] to somewhat increase the rate of reaction of Pr_2Zn and benzaldehyde and to significantly increase the ratio of addition to reduction product, and salts[8] of composition $Pr_4N^+RZnX_2^-$ have been isolated. There have also been a few physical studies of zincate species, prepared from R_2Zn and RLi or RK, in which only organic groups are attached to zinc.[9]

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