$Ru(t\text{-}Bupy)_2(DBQ)_2$ is diamagnetic and has a proton NMR spectrum consistent with the trans structure.[16] Cyclic voltammetry, in 1,2-dichloroethane (100 mV/s), shows four couples at +0.62, -0.19, -1.12, and -2.00 (vs. Fc^+/Fc), the most negative being irreversible. Assignments will be discussed elsewhere.[12]

The optical absorption spectrum consists of bands at 280 nm (13 000 M^{-1} cm^{-1}), 325 (4060), 400 (3780), and 580 (1100) in the UV and visible regions. The most interesting feature is a remarkably intense, structured band centered at 1160 nm (35 300) in the near-IR (Figure 2). It is surely significant that the species $Ru^{II}(bpy)_2(DBSq)^+$ and $Ru^{II}py_4(DBSq)^+$ show similar, structured, intense bands at comparable energies[10] and assigned as Ru(II) → DBSq metal to ligand charge-transfer (MLCT) transitions.[17]

The photoelectron spectrum[18] shows a ruthenium $3d_{3/2}$ signal at a bonding energy of 280.8 eV, being the same as that found for $[Ru^{II}(bpy)_2(DBSq)]PF_6$.[10]

There are two limiting electronic structures for the title species, $Ru^{II}(t\text{-}Bupy)_2(DBSq)_2$ and $Ru^{IV}(t\text{-}Bupy)_2(DBCat)_2$. We favor the ruthenium(II) formulation as the dominant (but not exclusive) contribution to the structure for the following reasons: (a) the near-IR absorption appears to be a MLCT feature, and its presence therefore favors Ru(II); (b) the PES bonding energy is appropriate for Ru(II), rather than Ru(III) (ca. 281.5-282 eV)[19a] or Ru(IV) (ca. 282 eV).[19b,20]

In its Ru(II) form, this would be the first structurally characterized example of a complex in which two equivalent semiquinone ligands lie in the same plane. The diamagnetism observed in this case must result from strong ligand-ligand coupling through the ruthenium center.

The species $Ru^{II}(bpy)_2(DBSq)^+$ has a structure[10,11] distorted toward the electronic isomer $Ru^{III}(bpy)_2(DBCat)^+$. We have previously proposed[10] that the existence of the low-lying, near-infrared MLCT state is responsible, through mixing into the ground state, for this distortion. The relatively long C-O bonds observed in the title compound probably reflect similar mixing and indicate that the complex has Ru(IV) character. Note that the analogous complex $Mn^{IV}(py)_2(DBCat)_2$ has C-O bond lengths (1.35 Å) significantly longer than observed here, but that this manganese(IV) complex electronically isomerized to $Mn^{II}(py)_2(DBSq)_2$ when cooled below about 250 K.[6b] The title ruthenium(II) complex does not show similar temperature dependence.

In summary, the title compound, as a consequence of intramolecular electron transfer, shows properties of being both a Ru(II) and a Ru(IV) species, but with the former dominant.

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Supplementary Material Available: Table of atomic positional and thermal parameters for $Ru(t\text{-}Bupy)_2(DBQ)_2$ (1 page); table of observed and calculated structure factors for $Ru(t\text{-}Bupy)_2(DBQ)_2$ (17 pages). Ordering information is given on any current masthead page.

(16) ^1H NMR ($CDCl_3$) δ 1.03 (s, 18 H), 1.42 (s, 18 H), 1.64 (s, 18 H), 5.85 (d, 2 H, ^4J = 5 Hz), 6.43 (2 d, 4 H, ^3J = 13, ^5J = 3 Hz), 7.07 (2 d, 4 H, ^3J = 13, ^5J = Hz), 7.73 (d, 2 H, ^4J = 5 Hz).

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(18) Photoelectron spectra were obtained courtesy of the "Surface Science Western" laboratory at the University of Western Ontario.

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(20) A bis(catecholato)ruthenium(IV) electronic isomer might have a binding energy depressed by the electron-donating catechol from the Ru(IV) into the Ru(II) binding energy region. Based on previous experience[21] it is very unlikely that such a dramatic shift would be observed; however, the possibility is being investigated.

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Chiral Probe for A-Form Helices of DNA and RNA: Tris(tetramethylphenanthroline)ruthenium(II)

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There has been considerable interest in exploring local variations in the structure of DNA along the strand and in developing small molecular probes which, like DNA-binding proteins, may be targeted to particular sites or sequences.[1] We have focused on the use of chiral metal complexes in designing spectroscopic probes and photoactivated DNA cleaving agents for DNA.[2] In particular, a chiral tris(diphenylphenanthroline)cobalt(III) complex has been useful in targetting specific sites in a left-handed conformation in supercoiled plasmids and viral DNA.[3] We report here the design of a probe that is specific for the A conformation.[4] Tris(3,4,7,8-tetramethylphenanthroline)ruthenium(II), $Ru(TMP)_3^{2+}$, binds preferentially to A-form helices, displays enantiomeric discrimination in its binding, and upon irradiation with visible light cleaves A-form helices preferentially.

Figure 1 shows the result of an equilibrium dialysis experiment[5] using racemic $Ru(TMP)_3^{2+}$ and dialysis of poly(rI)·poly(rC) and poly(rG)·poly(dC), two A-form polymers, or poly[d(GC)] and calf thymus DNA, ostensibly in the B form, against the ruthenium complex.[6,7] As is evident in the plot of the ratio of bound metal per nucleotide (r_b) vs. the formal added ratio (r_f) of metal per nucleotide, the highest degree of binding is found with the double-stranded RNA. DNA-RNA hybrids show also cooperative binding by the ruthenium complex. In comparison, no binding to poly[d(GC)] is detectable and for a native, heterogeneous calf thymus DNA, at most a small level of binding is observed. Hence $Ru(TMP)_3^{2+}$ is seen to bind cooperatively to the A-form polymer under conditions where little binding to B DNA is detected.

Besides electrostatic considerations, the binding of the complex to the polynucleotide may involve hydrophobic interactions of ligands bound against the shallow groove of the A helix. We earlier found that $Ru(phen)_3^{2+}$ binds to B DNA via two modes, one intercalative, one surface bound.[7] Intercalative binding through the major groove was characterized by an increase in luminescence lifetime of the complex and the preferential binding to the right-handed helix of the Δ-isomer. The hydrophobic surface-bound mode showed no enhancement in luminescence and a small preference in binding of the Λ-isomer. It occurred to us

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(5) Dialyses in 5 mM Tris, pH 7.3, and 50 mM NaCl were performed at 25 °C for at least 48 h to achieve equilibration. Each sample consisted of 3.2 mL of dialysate containing *rac*-$Ru(TMP)_3^{2+}$ (42-250 μM) and, inside, 1 mL of 1 mM nucleotides. Free ruthenium concentrations were determined spectrophotometrically ($\epsilon_{438} = 2.45 \times 10^4$ M cm^{-1}).[6] Bound concentrations in the presence of nucleic acid were determined spectrophotometrically after dilution into buffer or ethanol ($\epsilon_{438} = 2.60 \times 10^4$ M^{-1} cm^{-1} in 75% ethanol).

(6) $[Ru(TMP)_3]Cl_2$ was synthesized as described by: Lin, C. T.; Bottcher, W.; Creutz, C.; Sutin, N. J. *J. Am. Chem. Soc.* **1976**, *98*, 6536.

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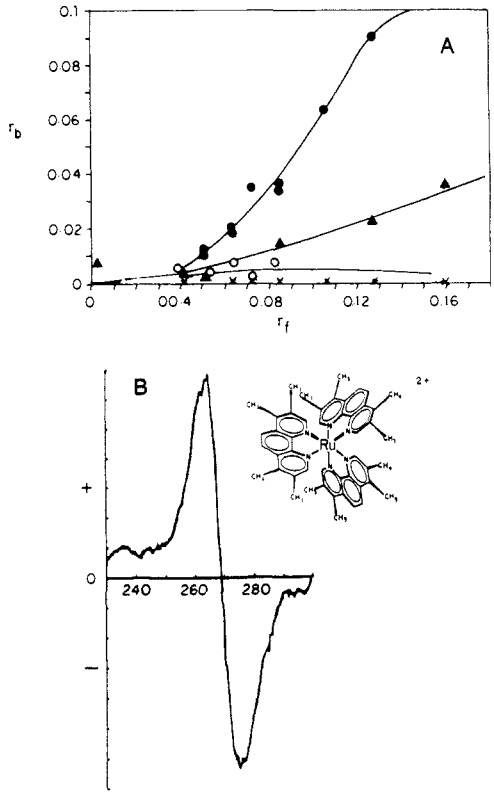

Figure 1. (A) Plot indicating the binding of $Ru(TMP)_3^{2+}$ to nucleic acids after dialyses of A-form polymers poly(rI)·poly(rC) (●) and poly(rG)·poly(dC) (▲) and B-form calf thymus DNA (○) and poly d(GC) (×) in buffer with *rac*-$Ru(TMP)_3^{2+}$ at 25 °C, where r_b is the ratio of bound ruthenium to nucleotide concentration and r_f is the formal added ratio of metal per nucleotide. The preferential binding of $Ru(TMP)_3^{2+}$ to A-helices is evident. (B) Circular dichroism obtained after the dialysis of poly(rI)·poly(rC) against *rac*-$Ru(TMP)_3^{2+}$. Dialysis against the double-stranded RNA leads to the enrichment in the solution of the less-favored Δ-isomer. The insert shows the structure of $Ru(TMP)_3^{2+}$.

that this surface binding of phenanthroline complexes might provide the basis for the A-form probe, since a notable feature of the A-helix is the topology of its shallow minor groove, with the major groove deepened, narrowed, and largely inaccessible. The tetramethylated derivative was prepared so as to maximize interactions with an A-form helix in the minor groove and to preclude binding to B DNA either by intercalation, owing to the thickness of the ligand, or in a groove-bound fashion, because of the greater length of the complex. Consistent with this model, no increase in luminescence accompanies binding of $Ru(TMP)_3^{2+}$ to double-stranded RNAs.[8] In fact, luminescence titrations of $Ru(TMP)_3^{2+}$ with poly(rI)·poly(rC) show decreases (40%) in intensity which likely result from self-quenching upon cooperative binding to the helix.

Also consistent with this binding model is the enantiomeric preference for Λ-$Ru(TMP)_3^{2+}$. Figure 1B shows the circular dichroism obtained in the dialysate after equilibration of poly(rI)·poly(rC) against *rac*-$Ru(TMP)_3^{2+}$.[9] Optical enrichment in

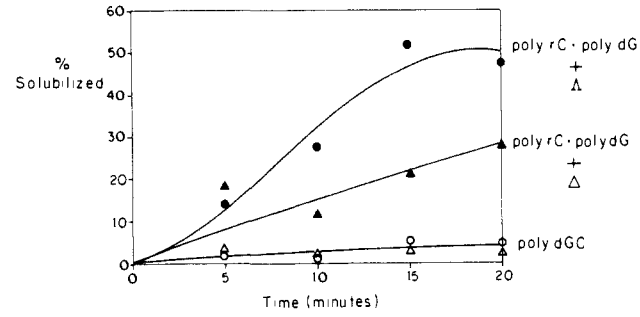

Figure 2. Photocleavage of A-form (closed) poly(rC)·poly(d[^{3}H]-G) and B-form (open) poly[d([^{3}H]-GC)] with Λ- (circles) and Δ-$Ru(TMP)_3^{2+}$ (triangles) in buffer containing 1.2 mM histidine and irradiation for increasing time at 442 nm using a 1000-W Hg/Xe lamp (Oriel) with monochromator. Cleavage was monitored by the retention on filters of acid-precipitable radioactivity and is plotted as the percentage counts solubilized as a function of irradiation time. With photoactivation, enantioselective cleavage of the A-form helix is apparent.

the Δ-isomer occurs in the dialysate, which indicates the preferential binding of the Λ-isomer to the helix.[11] At low binding ratios, the level of discrimination is 92%. In binding *against* the right-handed helix, a complementary symmetry at the metal center is found.[13]

Additionally, in the presence of irradiation with visible light, the A-conformation-specific binder becomes an A-conformation-specific DNA cleaver.[14] Phenanthroline and bipyridyl complexes of ruthenium(II), upon irradiation, serve as efficient singlet oxygen sensitizers,[15] and photoactivated cleavage of DNA mediated by singlet oxygen has been demonstrated for $Ru(bpy)_3^{2+}$ and $Ru(phen)_3^{2+}$.[14c,16] Figure 2 shows the result of photocleavage experiments using $Ru(TMP)_3^{2+}$. Tritiated polynucleotides[17] were irradiated for increasing periods of time in the presence of 20 μM $Ru(TMP)_3^{2+}$ and precipitated with trichloroacetic acid and acid-precipitable counts measured. As is evident in Figure 2, with increasing irradiation, cleavage by $Ru(TMP)_3^{2+}$ of the A-form polymers to an acid-soluble size is observed. Consistent with the binding results, $Ru(TMP)_3^{2+}$ cleaves the DNA–RNA hybrid poly(rC)·poly[d([^{3}H]-G)] where little cleavage is found for B-like poly[d([^{3}H]-GC)].[19] Furthermore, as was seen with binding,

(8) Luminescence titrations, with excitation at 438 nm and emission measured at 610 nm, of 5 μM $Ru(TMP)_3^{2+}$ with as much as 0.3 mM poly(rA)·poly(rU), poly(rI)·poly(rC) or calf thymus DNA showed no increase in emission intensity. Measurements of emission lifetimes, using a PRA single photon counter, revealed in all cases single-exponential decays with lifetimes for the ruthenium complex (4.5 μM) in aerated solution in the absence of nucleic acid found to be 641 ns and in the presence of poly(rI)·poly(rC) and calf thymus DNA, 654 and 668 ns, respectively. Lifetimes of ~2 μs would be expected with intercalation.[2c,7]

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(11) Absolute configurations were assigned as for $Ru(phen)_3^{2+}$. See: McCaffery, A. J.; Mason, S. F.; Norman, B. J. *J. Chem. Soc. A* **1969**, 1428.

(12) Consistent with the cooperativity in binding, a loss in enantiomeric selectivity occurs with increasing binding. The selectivity (Δ bound/total bound) is reduced from 92% where r_b = 0.003 to 56% where r_b = 0.06.

(13) An analogous complementarity was evident in the coordination of bis(phenanthroline)ruthenium(II) to DNA. See: Barton, J. K.; Lolis, E. *J. Am. Chem. Soc.* **1985**, *107*, 708. This chiral selectivity may be distinguished from the like symmetry evident with intercalation.[2]

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(17) The tritiated polymers were synthesized according to the method adapted from Sigman and co-workers.[18] Polymerization was monitored through the incorporation of radioactivity into acid-precipitable form. Poly([^{3}H]-dG-dC) was synthesized with *E. coli* DNA polymerase I (40 units) and poly[d(GC)] (0.8 mM) as template and 0.4 mM d(CTP) and 0.4mM d([^{3}H]-GTP) (1700 mCi/mmol) as substrates. Immediately before use in the cleavage assay, the polymer was treated with S1 nuclease (5 units/mg of polymer), precipitated with ethanol, and the polymer was resuspended with 400 μM unlabeled poly(dG-dC). Poly(rC)·poly[d([^{3}H]-G)] was prepared by using AMV reverse transcriptase (66 units) with poly(rC)·oligo(dG_{12-18}) as template and 4 mM NaPPi, 0.4 mM d[^{3}H]-GTP) (33.9 Ci/mmol). The solution was incubated for 30 min at 45 °C and, after S1 treatment and ethanol precipitation, was resuspended and diluted with 400 μM poly(rG)·poly(dC).

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enantiomeric selectivity occurs as well with cleavage. With Λ-$Ru(TMP)_3^{2+}$, twice the cleavage efficiency of the A-form polymer is observed in comparison with Δ-$Ru(TMP)_3^{2+}$.

In summary, Λ-$Ru(TMP)_3^{2+}$ has been shown to bind selectively to A-form polynucleotides and in the presence of light to cleave A-form polymers. This chiral probe should be useful to investigate DNA conformational heterogeneity in mapping sites in the A conformation along the helical strand. The role played by DNA secondary structures in protein recognition and in the expression of genetic information may be better understood by using this and other chiral complexes which are targeted specifically to different conformations.

Acknowledgment. We are grateful to the National Institute of General Medical Science (GM33309) for their financial support. We also thank Dr. D. Sigman for methods of polynucleotide synthesis.

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Tetramethyleneethane, a Ground-State Triplet

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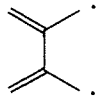
The question whether tetramethyleneethane[1] (I) is or is not a ground-state triplet is a central issue in understanding π-bonding



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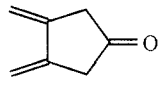

II

in conjugated hydrocarbons. Hund's first rule[2] suggests, as trimethylenemethane is,[3] that I should be a ground-state triplet. However, tetramethyleneethane (I) is an even, alternate, disjoint hydrocarbon.[4,5] In such molecules the nonbonding orbitals (or appropriate linear combination of them) are geographically isolated from each other and do not span common atoms.[4-7] Accordingly, the electron repulsion that provides the basis for Hund's rule is minimized and the singlet, according to this line of reasoning, can be the ground state.[4-7] This rationale has provided a means for understanding the contrasting multiplicities of ground-state trimethylenemethane[3]—triplet— and ground-state cyclobutadiene[7]—singlet. Tetramethyleneethane (I) is an important testing ground for these ideas.

Tetramethyleneethane (I), most probably in its singlet state, is a central intermediate in the dimerization of allene[8] as well as in the thermal rearrangement of 1,2-dimethylenecyclobutane.[9]

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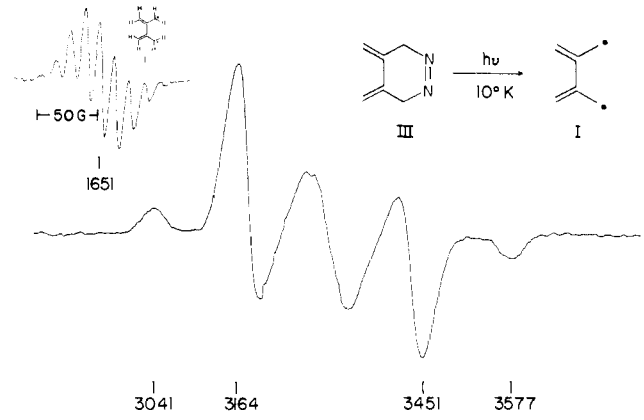
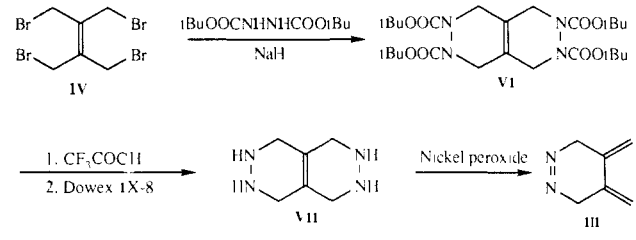


Figure 1. ESR spectrum of tetramethyleneethane (I) from irradiation of III at 265 nm and 10 K. Shown here are the $\Delta m = 1$ and (inset) $\Delta m = 2$ lines.

The triplet state of I was first observed[1] in 1970 following irradiation of the ketone II at 77 K. This experiment was not completely satisfactory because the diradical I is not stable in liquid nitrogen and continuous irradiation was required in order to observe the ESR spectrum of the triplet I. Under these circumstances, it was not possible to establish the multiplicity of the ground state of I. Following our experiments a tetramethyleneethane diradical incorporated into a six-membered ring was reported,[10] but in this experiment the nature of the ground state was also left unresolved.

We decided to explore the diradical I starting from the azo precursor III with the conviction that the structure of the diradical I would be more certain if it could be generated from two independent precursors. Temperatures below the boiling point of liquid nitrogen were used to prevent untimely decomposition of the diradical I. The synthetic strategy was important because at least one attempted synthesis of the azo compound III had not been successful.[11] This molecule contains the reactive s-cis diene system, which is highly susceptible to polymerization. Therefore, it is essential to keep the diene masked until the final step of the synthesis.

In our synthesis leading to III, the tetrabromide IV was treated with di-*tert*-butyl hydrazodicarboxylate (V) in the presence of sodium hydride yielding the double adduct VI.



Synthetic Scheme

The oxygen-sensitive dihydrazine VII was obtained upon removal of the *tert*-butyl groups and decarboxylation in trifluoroacetic acid followed by passage through a column of Dowex 1-X8 basic ion-exchange resin. Oxidation of VII with nickel peroxide in 1:2 carbon tetrachloride–chloroform-*d* at −78 °C yielded the azo diene III. The latter is very sensitive and too reactive to be isolated in the pure state; it must be kept cold and in solution. Even under these circumstances, however, good spectroscopic corroboration of its structure has been obtained. The proton NMR spectrum shows the two-proton exocyclic vinyl methylenes as singlets at δ 5.45 and 5.04 and a four-proton allylic methylene singlet at δ 4.68. The proton-coupled carbon-13 NMR spectrum

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