Synthesis and Properties of New DNA Cleavage Agents Based on Oxoruthenium(IV)

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Received June 22, 1992

New aquaruthenium(II) reagents that are capable of being oxidized to hydroxoruthenium(III) and oxoruthenium(IV) have been prepared. Complexes based on $Ru(tpy)(L)OH_2^{2+}$ (L = η^2-tpt, phen, dppz, tmen; tpy = 2,2′:6′,2″-terpyridine, tpt = 2,4,6-tripyridyltriazine, phen = 1,10-phenanthroline, dppz = dipyridophenazine, and tmen = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine) have been prepared and can all be reversibly oxidized to their $Ru^{IV}O$ forms, which are component DNA cleavage agents, as is $Ru(phen)_2(py)O^{2+}$. In addition to $Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$, the η^3 complex of tpt, $Ru(tpy)(\eta^3\text{-tpt})^{2+}$, can also be prepared under similar conditions. In the presence of Ag^+ ion, a novel Ru_2Ag complex can be isolated and has been crystallographically characterized. The complex $[Ru(tpy)(\eta^3\text{-tpt})](ClO_4)_2 \cdot 0.5AgClO_4 \cdot 0.5H_2O$ crystallizes in the monoclinic space group $A2/A$ with a = 14.723 (5) Å, b = 26.061 (6) Å, c = 22.148 (6) Å, β = 106.33 (3)°, V = 8155 (5) Å^3, Z = 4, R = 0.0807, and R_w = 0.1156 for 2923 reflections with $I \geq 2\sigma(I)$. The $Ru(tpy)OH_2^{2+}$ unit can also be attached to the tmen-AO$^+$ ligand, where a *N*,*N*′,*N*′-trimethylethylenediamine function is appended via a $(CH_2)_6$ linker to the acridine orange intercalator. The $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ complex is an effective cleavage agent, but only when oxidation is performed on the complex prebound to DNA. In homogeneous solution, electrochemically reversible access of only the $Ru^{III}OH$ form is possible, probably because of oxidation of the polymethylene linker.

The development of metal complexes that cleave DNA has been pursued with the goals of obtaining new pharmaceutical agents,[1] preparing artificial restriction enzymes,[2] determining the binding loci of proteins to DNA,[3] and probing DNA structure.[4] We have recently begun a program aimed at developing new DNA cleavage agents based on oxoruthenium(IV) complexes.[5-8] One of the principal advantages of these systems is the ease with which the mechanistic aspects of the cleavage reaction can be studied from the point of view of the metal complex. In particular, the kinetics of the cleavage reaction can be studied in real time by electrochemistry and optical spectroscopy.[5] The active Ru(IV) forms can be generated electrochemically by oxidation of the $Ru(II)OH_2^{2+}$ forms according to eqs 1 and 2 (tpy = 2,2′:6′,2″-terpyridine; bpy = 2,2′-bipyridine).[9] The $Ru^{II}OH_2^{2+}$, $Ru^{III}OH^{2+}$, and $Ru^{IV}O^{2+}$ forms all have unique absorption spectra, which have been used to characterize in detail the oxidation of small molecules by $Ru^{III}OH^{2+}$ and $Ru^{IV}O^{2+}$.[10]

$$Ru(tpy)(bpy)OH_2^{2+} \rightarrow Ru(tpy)(bpy)OH^{2+} + H^+ + e^- \quad (1)$$

$$Ru(tpy)(bpy)OH^{2+} \rightarrow Ru(tpy)(bpy)O^{2+} + H^+ + e^- \quad (2)$$

We have begun a program of synthesis of derivatives of complexes based on $Ru(tpy)(bpy)OH_2^{2+}$ or $Ru(bpy)_2(py)OH_2^{2+}$ designed to have unusual DNA-binding or cleavage properties. We report here the synthesis and electronic properties of an extensive family of aquaruthenium(II) complexes containing tpy, bpy, phen, tpt, dppz, and tmen ligands (tpt = 2,4,6-tripyridyltriazine, phen = 1,10-phenanthroline, dppz = dipyridophenazine, and tmen = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Figure 1). All of these complexes can be oxidized to the oxoruthenium(IV) form and cleave DNA. Complexes containing primary amine ligands, such as en and pda, do not support the oxoruthenium(IV) functionality (en = ethylenediamine, pda = *o*-phenylenediamine).

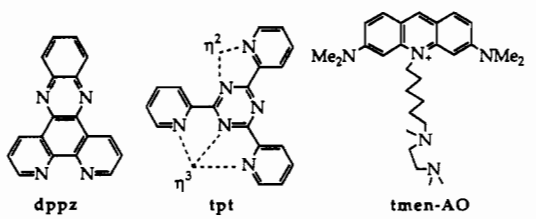

Figure 1. dppz, tpt, and tmen-AO ligands.

We have successfully tethered the $Ru^{II}OH_2^{2+}$ functionality from an acridine intercalator via a $(CH_2)_6$ linker.[11] This complex cleaves DNA, but only when activated by oxidation while bound. We have also observed an unusual coordination chemistry for the tpt ligand,[12] which reacts with $Ru(tpy)Cl_3$ to form either $Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$ or $Ru(tpy)(\eta^3\text{-tpt})^{2+}$, depending on the conditions. We have obtained the X-ray crystal structure of this latter complex, which crystallizes with an unusual four-coordinate Ag^+ ion bridging two tpt ligands.

Experimental Section

Materials. All chemicals were obtained from Aldrich and used without further purification unless noted. $Ru(tpy)Cl_3$,[13] $[Ru(tpy)(tmen)OH_2](ClO_4)_2$,[7] $[Ru(tpy)(dppz)OH_2](ClO_4)_2$,[8] $[Ru(tpy)(phen)OH_2](ClO_4)_2$,[7] and (en-AO)I[11] were prepared by literature procedures.

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(1) (a) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211. (b) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 83.
(2) (a) Dervan, P. B. *Science (Washington, D.C.)* **1986**, *232*, 484. (b) Barton, J. K. *Science (Washington, D.C.)* **1986**, *233*, 727.
(3) Uchida, K.; Pyle, A. M.; Morii, T.; Barton, J. K. *Nucleic Acids Res.* **1989**, *17*, 10259. Sigman, D. S.; Chen, C. B. *Annu. Rev. Biochem.* **1990**, *59*, 207.
(4) (a) Burkhoff, A. M.; Tullius, T. D. *Nature (London)* **1988**, *331*, 455. (b) Tullius, T. D.; Dombroski, B. A. *Science (Washington, D.C.)* **1985**, *230*, 679.
(5) Grover, N.; Thorp. H. H. *J. Am. Chem. Soc.* **1991**, *113*, 7030.
(6) Grover, N.; Gupta, N.; Thorp, H. H. *J. Am. Chem. Soc.* **1992**, *114*, 3390.
(7) Grover, N.; Gupta, N.; Singh, P.; Thorp, H. H. *Inorg. Chem.* **1992**, *31*, 2014.
(8) Gupta, N.; Grover, N.; Neyhart, G. A.; Liang, W.; Singh, P.; Thorp, H. H. *Angew. Chem.* **1992**, *104*, 1058.
(9) Meyer, T. J. *J. Electrochem. Soc.* **1984**, *131*, 221C.
(10) (a) Thompson, M. S.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 4106. (b) Thompson, M. S.; Meyer, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 5070.
(11) Bowler, B. E.; Ahmed, K. J.; Sundquist, W. I.; Hollis, L. S.; Whang, E. E.; Lippard, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 1299.
(12) Chirayil, S.; Hegde, V.; Jahng, Y.; Thummel, R. P. *Inorg. Chem.* **1991**, *30*, 2821.
(13) Leising, R. A.; Kubow, S. A.; Churchill, M. R.; Buttrey, L. A.; Ziller, J. W.; Takeuchi, K. J. *Inorg. Chem.* **1990**, *29*, 1306.

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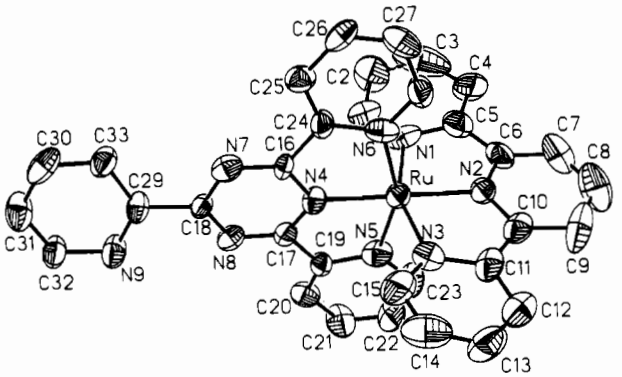


Figure 2. ORTEP diagram of the $Ru(tpy)(\eta^3\text{-tpt})^{2+}$ cation (50% probability level; hydrogen atoms omitted for clarity).

Table I. Crystal Data for $[Ru(tpy)(\eta^2\text{-tpt})](ClO_4)_2\cdot 0.5AgClO_4\cdot 0.5H_2O$

empirical formula: $C_{33}H_{25}Ag_{0.5}N_9O_{10.5}Cl_{2.5}Ru$	$F_{000} = 3808$
fw = 957.82	μ(Mo Kα) = 8.28 cm^{-1}
cryst dimens: 0.52 × 0.27 × 0.015 mm	no. of reflcns measd tot.: 10230 unique: 4695 (R_{int} = 0.013)
cryst syst: monoclinic	function minimized: $\sum\omega(\|F_o\| - \|F_c\|)^2$
lattice params	least-squares weights: $1/[\sigma^2(F) + 0.001F^2]$
a = 14.723 (5) Å	no. of observns, $I \geq 2\sigma(I)$: 2923
b = 26.061 (6) Å	$R = \sum\|\|F_o\| - \|F_c\|\|/\|F_o\| = 0.0807$
c = 22.148 (6) Å	$R_2 = [\sum w(\|F_o\| - \|F_c\|)^2/\sum wF_o^2]^{1/2}$ = 0.1156
β = 106.33 (3)°	goodness of fit indicator: 1.013
V = 8155 (5) Å^3	
space group: $A2/A$	
Z = 4	
D_{calcd} = 1.55 g/cm^3	

Complexes. [Ru(tpy)(en)Cl]Cl. $Ru(tpy)Cl_3$ (0.5 g, 1.14 mmol) was refluxed in 100 mL of 3:1 $EtOH/H_2O$ with en (0.068 g, 1.14 mmol), LiCl (0.05 g), and NEt_3 (0.25 mL). The solution was filtered hot and the volume of the filtrate was reduced by half by rotary evaporation. The solution was chilled for 24 h, and the resulting black solid was collected, washed with water, and air-dried. Yield: 59%.

[Ru(tpy)(en)OH$_2$](ClO$_4$)$_2$. [Ru(tpy)(en)Cl]Cl was treated with 2 equiv of $AgClO_4$ in 1:1 acetone/water. The solution was refluxed for 1.5 h and cooled to room temperature. The solid AgCl was filtered off, and the filtrate was allowed to evaporate slowly in air, precipitating a black, microcrystalline solid. Anal. Calcd: C, 33.38; H, 3.44; N, 11.46. Found: C, 37.08; H, 3.12; N, 12.49. Yield: 63%.

[Ru(tpy)(pda)OH$_2$](ClO$_4$)$_2$. This complex was prepared by the same method as the en complex. Anal. Calcd: C, 38.24; H, 3.19; N, 10.62. Found: C, 38.89; H, 3.23; N, 10.52. Overall yield based on $Ru(tpy)Cl_3$: 66%.

[Ru(tpy)(η^2-tpt)Cl]Cl. $Ru(tpy)Cl_3$ (0.110 g, 0.25 mmol) and tpt (0.080 mg, 0.25 mmol) were refluxed in 20 mL of 1:1 ethanol/water for 24 h. The reaction mixture was cooled to room temperature, and an aqueous solution of $NaClO_4\cdot H_2O$ (0.037 g, 0.26 mmol) was added. The volume was reduced by half by rotary evaporation, the solution was chilled for 12 h, and a crude solid was collected and dried. The crude solid was chromatographed on alumina in 1:1 acetonitrile/toluene. The second fraction was collected and rotary evaporation yielded a black microcrystalline solid. Yield: 77%.

[Ru(tpy)(η^2-tpt)OH$_2$](ClO$_4$)$_2\cdot$0.5AgClO$_4\cdot$2H$_2$O. This complex was prepared from the chloro complex by the same method as the other $Ru(tpy)(L)OH_2^{2+}$ complexes. Anal. Calcd: C, 39.49; H, 2.89; N, 12.56. Found: C, 39.54; H, 2.88; N, 12.24. Yield: 84%.

(tmen-AO)(PF$_6$). A 1.0-g sample of (en-AO)I (1.92 mmol), 24 mL of formic acid (88%), 20 mL of formaldehyde (37%), and 2.5 mL of water were brought to reflux. After 24 h, 25 mL of water was added, and the solution was cooled to 5 °C. The pH was adjusted to >12 by adding a concentrated NaOH solution while maintaining the temperature of the reaction mixture at ≤25 °C. The solution was extracted with five 100-mL portions of chloroform, which were combined and evaporated to dryness. The orange solid was dissolved in a minimum amount of water and treated with 1 mL of a saturated aqueous solution of NH_4PF_6. The resulting orange solid was filtered, washed with water, and air-dried. MS (EI): (M – PF_6 + H), 451, 24%; (M – PF_6 – CH_3), 436, 28%; (M – PF_6 – $(CH_3)_2NCH_2$), 393, 100%. The proton NMR spectrum is identical to that reported for (en-AO)Cl except that a new peak that can be readily assigned to the three added methyl groups is present at δ (ppm) 2.25 (9H). Yield: 81%.

[Ru(tpy)(tmen-AO)Cl](PF$_6$)$_2$. $Ru(tpy)Cl_3$ (0.1 g, 0.227 mmol) and (tmen-AO)(PF_6) (0.135 g, 0.227 mmol) were refluxed for 4.5 h in 3:1 $EtOH/H_2O$ containing LiCl (0.01 g) and NEt_3 (0.05 mL). The solution was filtered hot, and the volume of the filtrate was reduced by half by rotary evaporation. A saturated, aqueous solution of NH_4PF_6 (2 mL) was added. The resulting orange-brown solid was collected, washed with water, and air-dried. The FAB mass spectrum (nitrobenzyl alcohol matrix) showed a series of peaks centered at m/z = 965.2 which gave the appropriate theoretical ion distribution for M – PF_6. Yield: 83%.

[Ru(tpy)(tmen-AO)OH$_2$](PF$_6$)$_3$. A 0.1-g sample of [Ru(tpy)(tmen-AO)Cl](PF_6)$_2$ was refluxed in 20 mL of acetone/H_2O for 4 h. The reaction was cooled, and 1 mL of an aqueous, saturated solution of NH_4PF_6 was added. The resulting solution was allowed to evaporate slowly in air, and an orange-brown solid precipitated. The FAB mass spectrum (nitrobenzyl alcohol matrix) showed a series of peaks centered at m/z = 948.3 which gave the appropriate theoretical ion distribution for M – $(PF_6)_2$. The molecular ion spectrum and simulation are given in the supplementary material. No signal attributable to the chloro complex was observed. Anal. Calcd: C, 41.7; H, 4.61; N, 9.05. Found: C, 41.9; H, 5.10; N, 8.41.

[Ru(tpy)(η^3-tpt)](ClO$_4$)$_2\cdot$0.5AgClO$_4\cdot$2.5H$_2$O. [Ru(tpy)(η^3-tpt)](ClO_4)$_2$ was prepared according to published procedures.[12] This complex (0.045 g, 0.053 mmol) was refluxed with 0.015 g of $AgClO_4$ in 10 mL of 3:1 acetone/water for 2 h. The solution was filtered, and the filtrate was allowed to evaporate slowly. Orange, diffraction-quality crystals eventually precipitated. Anal. Calcd: C, 39.85; H, 2.82; N, 12.68. Found: C, 39.51; H, 3.08; N, 12.10. Yield: 82%.

[Ru(phen)$_2$(py)OH$_2$](PF$_6$)$_2\cdot$2H$_2$O. This complex was prepared via $[Ru(phen)_2(NO)(NO_2)](PF_6)_2$, which was prepared by published procedures.[14] This complex was converted to $[Ru(phen)_2(py)(NO)](PF_6)_3$ by the same procedure used for the analogous bpy complex (yield 35%).[15] The nitrosyl complex was then converted to $[Ru(phen)_2(py)OH_2](PF_6)_2$ using the published procedure for the bpy complex.[17] We found that this procedure was easier with the phen derivative than a shorter, alternative route for the bpy derivative.[17] Anal. Calcd: C, 39.43; H, 3.04; N, 7.91. Found: C, 39.44; H, 3.10; N, 8.09.

[Ru(tpy)(tmen)O](ClO$_4$)$_2$. This complex was prepared from $Ru(tpy)(tmen)OH_2^{2+}$ by oxidation with Cl_2 according to published procedures.[5] Anal. Calcd: C, 37.89; H, 4.06; N, 10.50. Found: C, 37.79; H, 4.05; N, 10.06.

Measurements. Cyclic voltammetry was performed as described[7,16] using a PAR 273A potentiostat with either tin-doped indium oxide or edge-oriented pyrolytic graphite working electrodes. Pourbaix diagrams were measured using phosphate buffer from pH 2 to pH 10 or 0.1 M $HClO_4$ for pH 1. Values for pK_a's were determined by spectrophotometric titration. Optical spectra were obtained using an HP8452 dioide-array spectrophotometer.

Controlled-potential electrolysis was performed in the same cell used for cyclic voltammetry. Solutions were diluted with bromphenol blue loading buffer and loaded onto 1% agarose gels containing ethidium bromide and electrophoresed for approximately 1 h at 44 V. The gels were photographed under UV light. Plasmid $\phi X174$ DNA (rf I) was purchased from Pharmacia and used as received.

X-ray Crystallography. The structure of $Ru(tpy)(\eta^3\text{-tpt})^{2+}$ was solved by the Patterson method. Block-diagonal least-squares refinement yielded R = 0.0807 and R_w = 0.1156 for 2923 reflections with $I > 2\sigma(I)$ measured on a Nicolet P3/F diffractometer up to 2θ = 43° at 25 °C (Mo Kα radiation, λ = 0.710 73 Å). Crystal data for $C_{33}H_{25}N_9O_{10.5}Ag_{0.5}Cl_{2.5}Ru$: monoclinic, $A2/A$, Z = 4, a = 14.723 (5) Å, b = 26.061 (6) Å, c = 22.148 (6) Å, β = 106.33 (3)°, V = 8155 (5) Å^3, D_{calc} = 1.55 g/cm^3, μ(Mo Kα) = 8.28 cm^{-1}.

(14) Godwin, J. B.; Meyer, T. J. *Inorg. Chem.* **1971**, *10*, 471.
(15) Callahan, R. W.; Meyer, T. J. *Inorg. Chem.* **1977**, *16*, 575.
(16) Thorp, H. H. *J. Chem. Educ.* **1992**, *69*, 250.
(17) Moyer, B. A.; Meyer, T. J. *Inorg. Chem.* **1981**, *20*, 436. Moyer, B. A.; Meyer, T. J. *J. Am. Chem. Soc.* **1978**, *100*, 3601.

Table II. Atomic Coordinates (×10⁴) and Isotropic Thermal Parameters

	x	y	z	U,[a] Å²		x	y	z	U,[a] Å²
Ru	−2208 (1)	1154 (1)	2535 (1)	38 (1)	C21	−106 (11)	1280 (7)	4655 (7)	67 (6)
N1	−2532 (7)	409 (4)	2683 (5)	52 (5)	C22	256 (10)	1090 (6)	4221 (8)	66 (7)
N2	−1449 (7)	763 (4)	2064 (5)	41 (4)	C23	−340 (9)	1042 (6)	3575 (7)	55 (6)
N3	−1599 (8)	1745 (4)	2166 (5)	44 (4)	C24	−4202 (9)	1473 (5)	2098 (6)	40 (5)
N4	−2930 (7)	1515 (4)	3028 (5)	36 (4)	C25	−5118 (9)	1538 (5)	1745 (6)	43 (5)
N5	−1253 (7)	1203 (4)	3407 (5)	48 (4)	C26	−5381 (9)	1322 (6)	1167 (7)	50 (6)
N6	−3541 (7)	1226 (4)	1872 (5)	45 (4)	C27	−4752 (11)	1081 (6)	940 (7)	58 (6)
C1	−3086 (11)	244 (7)	3013 (8)	74 (7)	C28	−3814 (10)	1034 (6)	1309 (6)	52 (6)
C(2)	−3318 (14)	−285 (6)	3086 (10)	86 (9)	C29	−4494 (9)	2266 (5)	4020 (6)	42 (5)
C3	−2982 (18)	−597 (8)	2729 (11)	125 (12)	N9	−4032 (8)	2476 (5)	4573 (5)	53 (5)
C4	−2281 (12)	−471 (6)	2380 (9)	76 (8)	C30	−5982 (10)	2531 (6)	4134 (8)	67 (7)
C5	−2124 (10)	42 (6)	2379 (7)	60 (6)	C31	−5524 (11)	2747 (6)	4709 (7)	62 (7)
C6	−1505 (10)	264 (5)	2054 (7)	52 (6)	C32	−4592 (10)	2722 (6)	4895 (7)	53 (6)
C7	−928 (14)	−21 (7)	1744 (7)	82 (8)	C33	−5486 (9)	2272 (6)	3797 (7)	54 (6)
C8	−370 (16)	234 (7)	1471 (9)	94 (9)	Cl1	2182 (3)	1935 (2)	1536 (2)	67 (2)
C9	−287 (13)	765 (8)	1496 (8)	86 (9)	O1	1557 (8)	2356 (4)	1430 (6)	77 (5)
C10	−894 (9)	1041 (6)	1797 (6)	51 (6)	O2	2988 (11)	2053 (9)	2077 (8)	173 (10)
C11	−981 (9)	1584 (6)	1842 (6)	52 (5)	O3	2589 (11)	1913 (6)	1023 (7)	120 (8)
C12	−503 (11)	1960 (7)	1571 (7)	60 (7)	O4	1663 (12)	1507 (6)	1608 (13)	198 (15)
C13	−661 (12)	2460 (7)	1618 (9)	72 (8)	Cl2	4876 (3)	683 (2)	3622 (2)	83 (2)
C14	−1319 (14)	2608 (6)	1881 (9)	82 (8)	O5	4748 (17)	180 (7)	3710 (12)	213 (15)
C15	−1748 (11)	2239 (7)	2188 (8)	69 (7)	O6	4146 (13)	974 (8)	3747 (11)	171 (11)
C16	−3836 (8)	1651 (5)	2764 (6)	41 (5)	O7	5777 (9)	873 (6)	3992 (6)	108 (7)
N7	−4353 (7)	1904 (4)	3048 (5)	44 (4)	O8	4801 (10)	775 (7)	2962 (7)	129 (8)
C17	−2559 (8)	1616 (5)	3636 (6)	40 (5)	Ag	−2500	2381 (1)	5000	56 (1)
N8	−3033 (7)	1857 (4)	3969 (5)	40 (4)	ow	921 (17)	−37 (8)	269 (10)	75 (10)
C18	−3907 (8)	1993 (5)	3680 (5)	33 (5)	Cl3	7503	1257 (3)	0	105 (4)
C19	−1584 (8)	1431 (5)	3881 (6)	38 (5)	O9	7567 (14)	1582 (10)	548 (8)	178 (11)
C20	−1050 (10)	1442 (5)	4475 (7)	47 (5)	O10	8492 (21)	1093 (9)	152 (10)	211 (15)

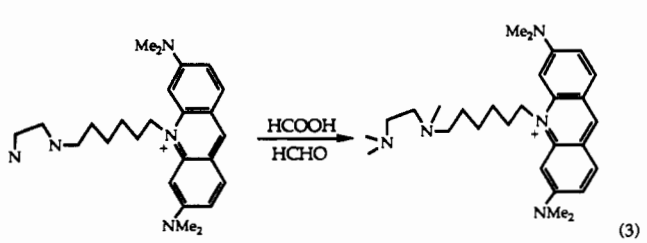
[a] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

Results

Synthesis. The syntheses of all of the $Ru(tpy)(L)OH_2^{2+}$ complexes are based on the general procedure of Meyer et al.[18] In this procedure, the neutral $Ru(tpy)Cl_3$ starting material is refluxed in ethanol/water with 1 equiv of the bidentate ligand in the presence of NEt_3 as a reductant. This reaction yields the $[Ru(tpy)(L)Cl]Cl$ precursor, which is treated with 2 equiv of $Ag(ClO_4)$. After the precipitated AgCl is filtered off, the desired $[Ru(tpy)(L)OH_2](ClO_4)_2$ species is obtained.

The preparation of the $Ru(L)_2(py)OH_2^{2+}$ complexes is less straightforward.[17] In the case of $Ru(phen)_2(py)OH_2^{2+}$, we find that even though more steps are involved, it is more desirable to go through the $[Ru(phen)_2(py)(NO)](PF_6)_3$ species rather than $[Ru(phen)_2(py)Cl]Cl$. With this procedure, we find that the desired complex can be obtained in acceptable yield and purity.

The tethered acridine–ethylenediamine ligand was prepared by the method of Bowler et al.[11] As we will discuss below, reversible formation of $Ru^{III}OH^{2+}$ or $Ru^{IV}O^{2+}$ according to eqs 1 and 2 does not occur if an NH functionality is coordinated to the $Ru^{II}OH_2$ center. Thus, we needed to methylate the nitrogen donors of the ethylenediamine portion of the en-AO⁺ ligand. We find that treatment with an excess of refluxing formic acid/formaldehyde leads to methylation of the ethylenediamine function without disruption of the acridine portion of the molecule (eq 3). Reaction of $(tmen\text{-}AO)PF_6$ with $Ru(tpy)Cl_3$ proceeds in



(3)

an analogous fashion to the reactions of the other bidentate ligands,

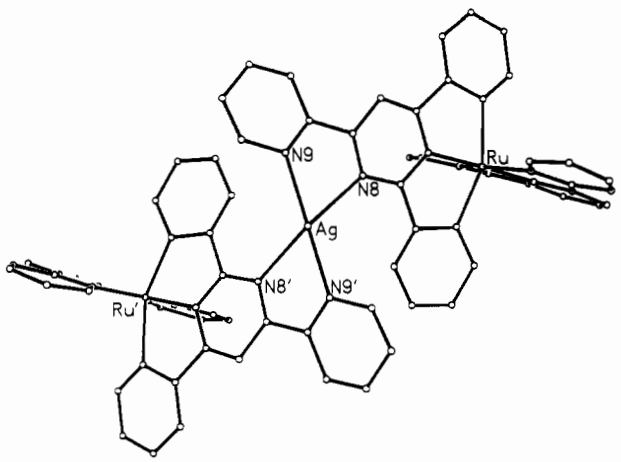


Figure 3. Structure of the entire Ru_2Ag unit. Hydrogen atoms were omitted for clarity.

affording $Ru(tpy)(tmen\text{-}AO)Cl^{2+}$ and then $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ after heating in acetone/water.

The $Ru(tpy)(\eta^2\text{-}tpt)OH_2^{2+}$ complex was prepared by the usual synthetic route, and the isolated complex shows all of the characteristic properties of the $Ru^{II}OH_2$ functionality. Slight alteration of the reaction conditions, however, led instead to $Ru(tpy)(\eta^3\text{-}tpt)^{2+}$. Following treatment with $AgClO_4$, diffraction-quality crystals of $[Ru(tpy)(\eta^3\text{-}tpt)](ClO_4)_2{\cdot}0.5AgClO_4{\cdot}2.5H_2O$ precipitated. Thus, the tpt ligand can bind in two hapticities (Figure 1), one of which (η^2) allows the formation of the $Ru^{II}OH_2$ moiety, and the other of which (η^3) blocks any available coordination site.

The X-ray crystal structure of the $[Ru(tpy)(\eta^3\text{-}tpt)]^{2+}$ cation is shown in Figure 2; the crystal data are given in Table I, the fractional coordinates in Table II, and selected bond lengths and angles in Table III. The coordination about the ruthenium center is typical for a bis(tridentate) polypyridyl coordination, with an average Ru–N bond length of 2.039 Å.[19] The N1–Ru–N3 and

(18) Takeuchi, K. J.; Thompson, M. S.; Pipes, D. W.; Meyer, T. J. *Inorg. Chem.* **1984**, *23*, 1845.

(19) Thummel, R. P.; Jang, Y. *Inorg. Chem.* **1986**, *25*, 2527.

Table III. Selected Bond Lengths (Å) and Angles (deg)

Bond Lengths			
Ru–N1	2.047 (12)	Ru–N2	2.006 (12)
Ru–N3	2.062 (11)	Ru–N4	1.966 (11)
Ru–N5	2.048 (10)	Ru–N6	2.104 (9)
N8–Ag	2.586 (10)	N9–Ag	2.201 (11)
Ag–N9′	2.201 (11)	Ag–N8′	2.586 (10)
Bond Angles			
N1–Ru–N2	77.9 (5)	N1–Ru–N3	156.7 (5)
N2–Ru–N3	78.9 (5)	N1–Ru–N4	100.2 (5)
N2–Ru–N4	177.4 (4)	N3–Ru–N4	103.1 (4)
N1–Ru–N5	92.1 (4)	N2–Ru–N5	100.8 (4)
N3–Ru–N5	93.8 (4)	N4–Ru–N5	77.4 (4)
N1–Ru–N6	89.0 (4)	N2–Ru–N6	103.3 (4)
N3–Ru–N6	94.6 (4)	N4–Ru–N6	78.5 (4)
N5–Ru–N6	155.6 (5)	N8–Ag–N9	70.1 (4)
N8–Ag–N8′	116.2 (5)	N9–Ag–N8′	117.3 (4)
N8–Ag–N9′	117.2 (4)	N9–Ag–N9′	167.2 (7)
N8′–Ag–N9′	70.1 (4)		

Table IV. Electronic Absorption of Chlororuthenium(II) Complexes in Aqueous Solution

complex	λ (nm) (ε (M^{-1} cm^{-1}))[a]
Ru(tpy)(en)Cl^+	464 (10 000), 314 (25 000), 272 (21 000), 226 sh (22 000)
Ru(tpy)(pda)Cl^+	506 (16 000), 316 (22 000), 280 sh (20 000), 274 (21 000), 232 (28 000)
Ru(tpy)(tmen)Cl^+	520 (3900), 476 (3700), 364 sh (2600), 320 (28 000), 278 (18 000), 234 (18 000)
Ru(tpy)(dppz)Cl^+	488 (12 000), 372 (14 000), 360 (14 000), 314 (37 000), 274 (65 000), 232 sh (33 000)
Ru(tpy)(phen)Cl^+	474 (8800), 410 sh (5700), 314 (29 000), 264 (52 000), 226 (43 000)
Ru(tpy)(tmen-AO)Cl^{2+}	496 (52 000), 472 sh (31 000), 318 (16 000), 296 sh (26 000), 272 (57 000), 234 (27 000)

[a] All data taken at pH 7.

N2–Ru–N4 angles of 156.7 and 155.6° are typical for coordination of tpy to Ru(II).[7,8,13,20]

The complex crystallizes with 0.5 $AgClO_4$ in the asymmetric unit. The silver ion lies on a 2-fold axis, bridging two of the free nitrogens on the tpt ligand. This results in an effectively trinuclear Ru_2Ag complex, as illustrated in Figure 3. The environment of the silver ion is four-coordinate, but severely distorted, because of the constraints of the bis(bipyridyl)-type coordination. The Ag–N9 (and N9′) bond length is much shorter at 2.201 Å than the Ag–N8 bond length of 2.586 Å. The bite angles of the bipyridyl functions (N8–Ag–N9 and N8′–Ag–N9′) are 70.1°. The remaining angles about silver are N9–Ag–N8′ = 117.3°, N9–Ag–N9′ = 167.2°, N8–Ag–N8′ = 116.2°, and N8–Ag–N9′ = 117.3°. Thus, the coordination is probably best described as a severely distorted tetrahedron. To our knowledge, this is only the second example of a crystal structure of a four-coordinate Ag(I) complex. The crystal structure of $Ag(py)_4^+$ has been determined,[21] and this complex exhibits a similar average Ag–N bond length of 2.322 Å but a geometry much closer to tetrahedral with two distinct bond angles of 112.3 and 108.1°.

Electronic Properties. The chloro complexes all exhibit characteristic Ru → π*(py) MLCT transitions in the 450–520-nm range of the visible spectrum and polypyridyl ligand transitions in the UV (Table IV). The visible spectrum of the tmen-AO^+ complex is dominated by the intense 496-nm band due to the acridine functionality. The extinction coefficient of this band is approximately 1×10^4 M^{-1} cm^{-1} higher in Ru(tpy)(tmen-AO)Cl^{2+} than in the free tmen-AO^+ ligand, suggesting that the Ru → tpy MLCT transition overlaps with the acridine band. The cyclic voltammograms of the chloro complexes show one-electron oxidations in acetonitrile that can be readily assigned to Ru(III/II) couples.[17,18] The potentials span the range of 0.53–1.15 V (Table V).

Table V. Electrochemical Data in Acetonitrile Solution

complex	E(III/II) (V[a])	ΔE_p (mV)
Ru(tpy)(en)Cl^+	0.53	60
Ru(tpy)(pda)Cl^+	0.96	70
Ru(tpy)(tmen)Cl^+	0.76	76
Ru(tpy)(dppz)Cl^+	0.87	60
Ru(tpy)(phen)Cl^+	0.80	80
Ru(tpy)(tmen-AO)Cl^{2+}	1.15	60
Ru(tpy)(en)$(CH_3CN)^{2+}$	0.92	70
Ru(tpy)(pda)$(CH_3CN)^{2+}$	1.42	70
Ru(tpy)(η^3-tpt)$^{2+}$	0.98	

[a] V vs Ag/AgCl. All measurements were made in a 0.1 M tetrabutylammonium hexafluorophosphate in acetonitrile solution.

The electronic spectra of the aqua complexes also show MLCT and ligand manifolds (Table VI), analogous to those of the chloro derivatives. The MLCT bands shift to lower energy by 15–30 nm upon deprotonation of the aqua ligand. The shift to lower energy can be understood from the greater electron-donating character of OH^- relative to OH_2, which makes the Ru center easier to oxidize and thereby shifts the MLCT manifold to lower energy. The shifts in the absorption spectra with pH can be used to determine the pK_a's of the coordinated aqua ligands, which are given in Table VII.

The cyclic voltammetry of $L_5RuOH_2^{2+}$ complexes is particularly characteristic, showing two closely spaced oxidation waves corresponding to eqs 1 and 2. This voltammetric behavior is a useful screen for complexes that can be converted to the oxidatively competent $Ru^{III}OH$ and $Ru^{IV}O$ forms that are DNA cleavage agents. This behavior has been reported for Ru(tpy)(tmen)OH_2^{2+},[7] Ru(tpy)(bpy)OH_2^{2+},[18] Ru(tpy)(phen)OH_2^{2+},[7] and $Ru(bpy)_2(py)OH_2^{2+}$.[17] The complexes $Ru(phen)_2(py)OH_2^{2+}$, Ru(tpy)(dppz)OH_2^{2+}, and Ru(tpy)(η^2-tpt)OH_2^{2+} all exhibit this characteristic pattern, as shown in Figure 4. The dppz complex is very poorly resolved, possibly due to adsorption of the planar dppz ligand to the electrode surface. However, coulometry confirms that the wave corresponds to a two-electron oxidation (see below). The Ru(tpy)(η^2-tpt)OH_2^{2+} complex also exhibits a pH-dependent, multielectron oxidation at higher potentials, which we ascribe to ligand oxidation. Multielectron oxidation in an aqueous solution of (η^3-tpt)Cu^{II} to [bis(2-pyridylcarbonyl)aminato]copper(II) has been demonstrated.[22] This reaction involves oxidation and hydrolysis, which accounts for the observed pH dependence observed for Ru(tpy)(η^2-tpt)OH_2^{2+} in Figure 5.

The pH dependence of the voltammetry shown in Figure 4 can be used to construct the Pourbaix diagrams for $Ru(phen)_2(py)OH_2^{2+}$ and Ru(tpy)(η^2-tpt)OH_2^{2+} that are shown in Figure 5. The $Ru(phen)_2(py)OH_2^{2+}$ complex gives a Pourbaix diagram very similar to that for $Ru(bpy)_2(py)OH_2^{2+}$.[17] Analysis of the diagram for Ru(tpy)(η^2-tpt)OH_2^{2+} is complicated by the fact that the couples are unresolved at pH = 7–9. Nevertheless, the pH dependence of both couples is within experimental error of the 59 mV/pH unit required for one-electron/one-proton couples (eqs 4 and 5). Ceric and electrochemical oxidation are consistent

$$Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+} \rightarrow Ru(tpy)(\eta^2\text{-tpt})OH^{2+} + H^+ + e^- \quad (4)$$

$$Ru(tpy)(\eta^2\text{-tpt})OH^{2+} \rightarrow Ru(tpy)(\eta^2\text{-tpt})O^{2+} + H^+ + e^- \quad (5)$$

with these assignments (see below). Because the dppz complex does not exhibit resolved (IV/III) and (III/II) couples at any pH, a Pourbaix diagram was not constructed. However, the single wave does exhibit a pH dependence of 60 mV/pH unit, appropriate for a two-electron/two-proton (or one-electron/one-proton) redox

(20) Seok, W. K.; Ph.D. Thesis, University of North Carolina at Chapel Hill, 1988.

(21) Nilsson, E.; Oskarsson, Å. *Acta Chem. Scand.* **1982**, *36*, 605.

(22) Lerner, E. I.; Lippard, S. J. *Inorg. Chem.* **1977**, *16*, 1546.

Table VI. Electronic Absorption of Aquaruthenium(II) Complexes in Aqueous Solution

complex	λ (nm) (ε (M^{-1} cm^{-1}))[a]
$Ru(tpy)(en)OH_2^{2+}$	466 (12 000), 328 sh (16 000), 312 (25 000), 278 sh (20 000), 272 (22 000), 226 sh (25 000)
$Ru(tpy)(pda)OH_2^{2+}$	496 (24 000), 314 (21 000), 280 (22 000), 272 (22 000), 234 (29 000)
$Ru(tpy)(tmen)OH_2^{2+}$	524 (5000), 470 sh (4100), 372 sh (2600), 320 (37 000), 276 (22 000), 234 (20 000)
$Ru(tpy)(dppz)OH_2^{2+}$	482 (12 000), 372 (14 000), 358 (14 000), 334 sh (16 000), 312 (39 000), 274 (60 000)
$Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$	478 (9000), 330 (12 000), 300 (23 000), 280 (25 000), 274 (24 000), 240 (14 000)
$Ru(tpy)(phen)OH_2^{2+}$	474 (9600), 406 sh (6600), 314 (29 000), 264 (52 000), 226 (46 000)
$Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$	496 (55 000), 474 sh (37 000), 316 sh (12 000), 292 (27 000), 272 (48 000), 234 (24 000)
$Ru(phen)_2(py)OH_2^{2+}$ [b]	466 (10 000), 422 sh (11 000), 318 sh (6800), 266 (85 000), 224 (64 000)

[a] All data taken at pH 7 unless otherwise noted. [b] 1 M $HClO_4$.

Table VII. pK_a Values of Aquaruthenium(II) Complexes

complex	pK_a	complex	pK_a
$Ru(tpy)(en)OH_2^{2+}$	9.1	$Ru(tpy)(phen)OH_2^{2+}$	9.6
$Ru(tpy)(pda)OH_2^{2+}$	9.2	$Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$	8.7
$Ru(tpy)(tmen)OH_2^{2+}$	10.1	$Ru(phen)_2(py)OH_2^{2+}$	10.3
$Ru(tpy)(dppz)OH_2^{2+}$	8.6	$Ru(tpy)(bpy)OH_2^{2+}$	9.7[a]
$Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$	8.7	$Ru(bpy)_2(py)OH_2^{2+}$	10.8[b]

[a] Reference 18. [b] Reference 16.

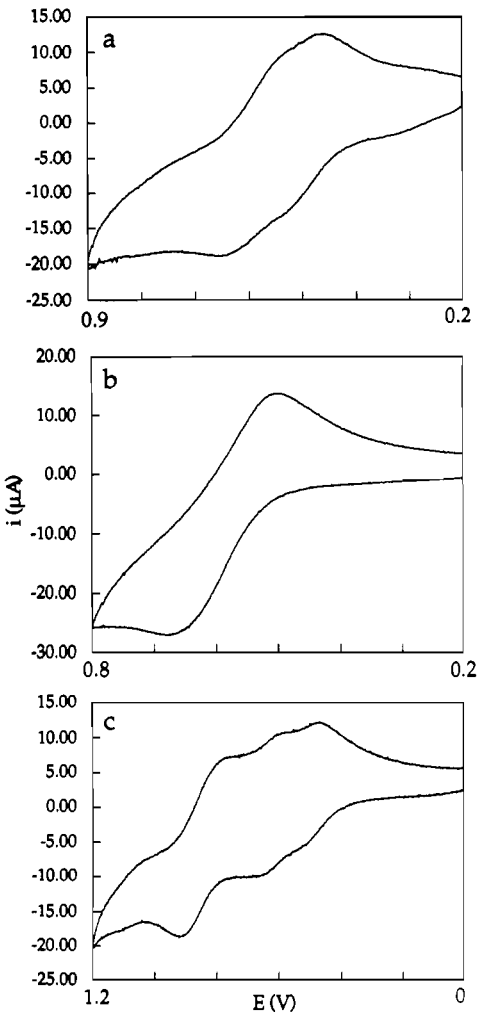


Figure 4. Cyclic voltammograms of (a) $Ru(phen)_2(py)OH_2^{2+}$, pH 7; (b) $Ru(tpy)(dppz)OH_2^{2+}$, pH 7; and (c) $Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$, pH 6. Conditions: indium oxide working electrode, Pt-wire auxillary, Ag/AgCl reference, 100 mV/s scan rate, 0.1 M phosphate buffer.

couple. Coulometric oxidation at 0.8 V does demonstrate that the complex is oxidized by 2.0 electrons, consistent with the net reaction shown in eq 6.

$$Ru(tpy)(dppz)OH_2^{2+} \rightarrow Ru(tpy)(dppz)O^{2+} + 2H^+ + 2e^- \quad (6)$$

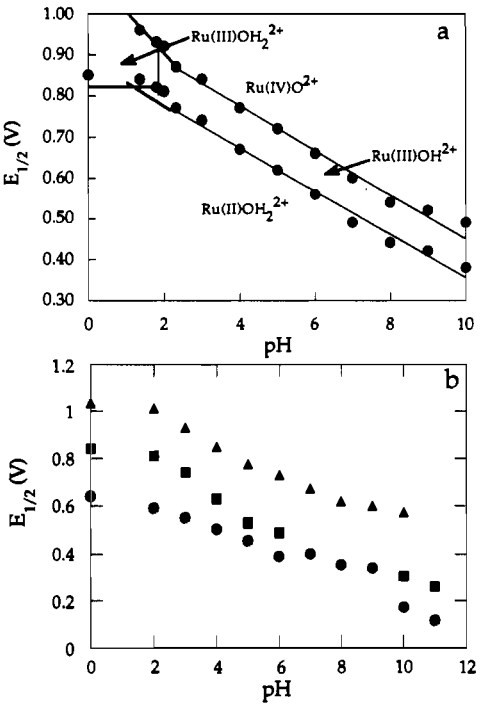


Figure 5. (a) Pourbaix diagram for $Ru(phen)_2(py)OH_2^{2+}$. (b) Pourbaix diagram for $Ru(tpy)(\eta^2\text{-tpt})OH_2^{2+}$, showing Ru(III/II) (●), Ru(IV/III) (■), and ligand-based (▲) couples.

The cyclic voltammograms of the complexes $Ru(tpy)(en)OH_2^{2+}$ and $Ru(tpy)(pda)OH_2^{2+}$ do not exhibit the characteristic two-wave pattern of the other $RuOH_2$ complexes. These complexes exhibit irreversible oxidations at approximately the same potentials as the other derivatives (Figure 6). Since $Ru(tpy)(tmen)OH_2^{2+}$ does show $Ru^{III}OH$ and $Ru^{IV}O$ formation, we conclude that in order to access the $Ru^{IV}O$ oxidation state, the coordinated nitrogen cannot have an N–H bond, as in en or pda. In this case, the N–H bond must be rapidly oxidized once the Ru center is oxidized. This effect has been studied recently in en complexes of Ru(II) and hexafluoroacetylacetonate ($hfac^-$).[23] Oxidation of the ruthenium center in these $hfac^-$ complexes results in the oxidative dehydrogenation of the en ligand. It was for this reason that we performed the methylation of the $en\text{-}AO^+$ ligand (eq 3).

The cyclic voltammogram of the $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ complex only exhibits one pH-dependent oxidation wave at 0.49 V at pH 7. Thus, only oxidation to $Ru^{III}OH$ is possible in this complex. While the reasons for this remain unclear, we have succeeded in electrocatalytically cleaving DNA with $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ at only 0.5 V, demonstrating that this initial oxidation wave is responsible for formation of a catalytically active species, probably $Ru^{III}OH$.

Preparative Oxidations. The complexes $Ru(bpy)_2(py)OH_2^{2+}$ and $Ru(tpy)(bpy)OH_2^{2+}$ can be oxidized to the $Ru^{IV}O$ forms using a variety of chemical oxidants, including Br_2, Cl_2, and

(23) Hoshino, Y.; Okuyama, F.; Nanba, A.; Shimizu, K.; Sato, G. P. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 876.

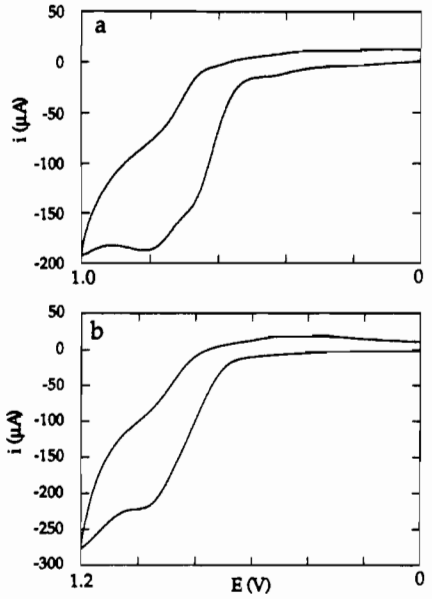


Figure 6. Cyclic voltammograms at pH 7 of (a) Ru(tpy)(en)OH_2^{2+} and (b) Ru(tpy)(pda)OH_2^{2+}. Same conditions as Figure 4.

Table VIII. Electrochemical Data in Aqueous Solution

complex	E(III/II) (V[a])	E(IV/III) (V)
Ru(tpy)(tmen)OH_2^{2+}	0.45	0.57
Ru(tpy)(dppz)OH_2^{2+}	0.59[b]	
Ru(tpy)(η^2-tpt)OH_2^{2+} [c]	0.39	0.49
Ru(tpy)(tmen-AO)OH_2^{3+}	0.49	
Ru(phen)$_2$(py)OH_2^{2+}	0.49	0.60
Ru(tpy)(phen)OH_2^{2+} [d]	0.51	0.61
Ru(bpy)$_2$(py)OH_2^{2+} [e]	0.42	0.54
Ru(tpy)(bpy)OH_2^{2+} [f]	0.49	0.62

[a] V vs SSCE. All measurements made in 50 mM phosphate buffer (pH 7), unless otherwise noted. [b] (IV/II) couple. [c] pH 6. [d] Reference 7. [e] Reference 17. [f] Reference 18.

Table IX. Electronic Absorption of Oxoruthenium(IV) and Hydroxoruthenium(III) Complexes in Aqueous Solution

complex	λ (nm) (ε (M^{-1} cm^{-1}))[a]
Ru(tpy)(tmen)O^{2+}	452 sh (1000), 502 sh (610), 354 (10 000), 340 (12 000), 290 (14 000)
Ru(tpy)(dppz)O^{2+}	384 (12 700), 360 (13 600), 316 sh (19 600)
Ru(tpy)(η^2-tpt)O^{2+}	536 sh (2300), 494 (2900), 358 sh (1800), 328 sh (4400), 284 (11 000)
Ru(tpy)(phen)O^{2+}	312 (16 500), 272 (39 000)
Ru(tpy)(tmen-AO)OH^{3+}	496 (42 000), 476 sh (33 000), 316 sh (8700), 290 (21 000), 270 (40 000)
Ru(phen)$_2$(py)O^{2+}	466 (10 000), 422 sh (11 000), 318 sh (6800), 266 (85 000), 224 (64 000)

[a] All data taken at pH 7 unless otherwise noted. [b] 1 M $HClO_4$.

Ce(IV).[9,10,17,18] Likewise, we find that Ru(tpy)(tmen)OH_2^{2+}, Ru(phen)$_2$(py)OH_2^{2+}, and Ru(tpy)(phen)OH_2^{2+} can all be oxidized to the stable $Ru^{IV}O$ forms with Cl_2 in aqueous solution (Table IX). The optical spectra of these two complexes are generally featureless, with broad absorptions in the visible region. Addition of a single drop of 2-propanol to these solutions results in the quantitative regeneration of the $Ru^{II}OH_2$ forms, as indicated by the optical spectrum. Extraction and analysis by published procedures[24] shows acetone to be the organic product, as observed for Ru(tpy)(bpy)O^{2+}.[10]

Oxidation of the other derivatives seems to require special conditions. For Ru(tpy)(η^2-tpt)OH_2^{2+}, oxidation with excess oxidant is unsuccessful because of the ligand oxidation at 0.8 V (Figure 4c). However, careful addition of 2 equiv of Ce(IV) results in formation of the $Ru^{IV}O$ form, which quantitatively returns to the $Ru^{II}OH_2$ form upon addition of 2-propanol (Figure 8).

(24) Thorp, H. H.; Van Houten, J.; Gray, H. B. *Inorg. Chem.* **1989**, *28*, 889.

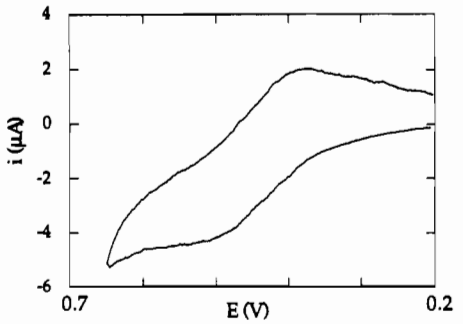


Figure 7. Cyclic voltammogram of Ru(tpy)(tmen-AO)OH_2^{3+}, pH 7. Same conditions as Figure 4.

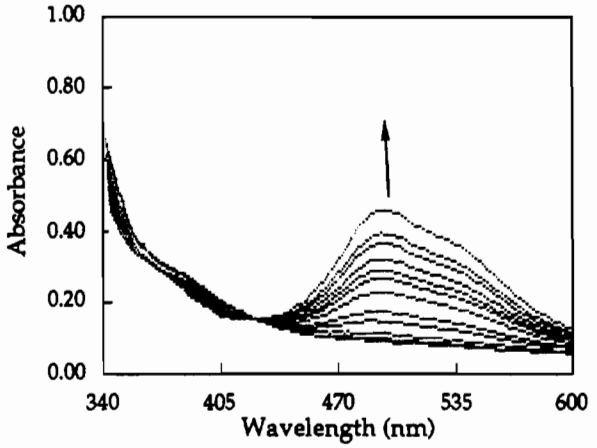


Figure 8. Electronic spectra taken every 10 min during the oxidation of 2-propanol by Ru(tpy)(η^2-tpt)O^{2+} in aqueous solution.

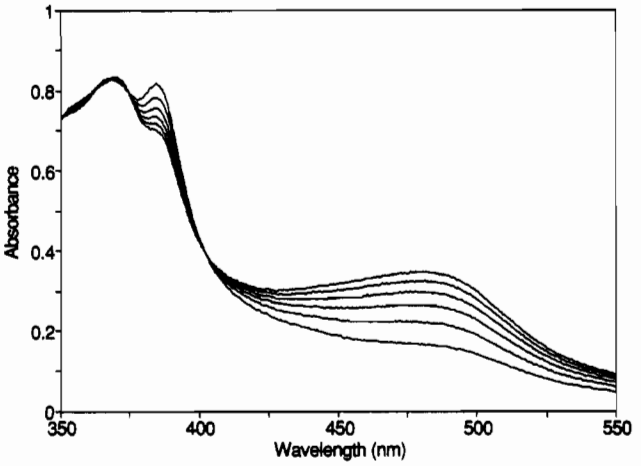


Figure 9. Electronic spectra taken every 10 min during the oxidation of 2-propanol by Ru(tpy)(dppz)O^{2+} in aqueous solution.

Thus far, we can prepare Ru(tpy)(dppz)O^{2+} only electrochemically. However, we find from coulometry that oxidation of an aqueous solution of Ru(tpy)(dppz)OH_2^{2+} at 0.8 V proceeds with the passage of 2.0 oxidizing equiv to produce an optical spectrum characteristic of the $Ru^{IV}O$ complexes. Addition of 2-propanol or DNA results in the regeneration of $Ru^{II}OH_2$ (Figure 9).

The Ru(tpy)(tmen-AO)OH_2^{3+} complex can be oxidized by only one electron, as shown in the cyclic voltammetry (Figure 7). We assign this oxidation to the one-electron/one-proton reaction

$$Ru^{II}(tpy)(tmen\text{-}AO)OH_2^{3+} \rightarrow Ru^{III}(tpy)(tmen\text{-}AO)OH^{3+} + H^+ + e^- \quad (7)$$

This electrochemical oxidation can be performed preparatively, with passage of 1.0 electron to produce a solution giving the

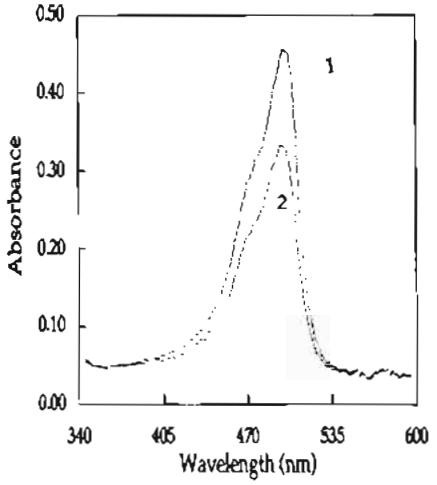


Figure 10. Electronic spectra of (1) $Ru^{II}(tpy)(tmen\text{-}AO)OH_2^{3+}$ and (2) $Ru^{III}(tpy)(tmen\text{-}AO)OH^{3+}$.

optical absorption spectrum shown in Figure 10. The extinction coefficient of the acridine band at 496 nm decreases from 5.5×10^4 M^{-1} to 4.4×10^4 M^{-1} cm^{-1} upon oxidation. The value for the oxidized form is close to that of the free tmen-AO^+ ligand. Since the $Ru^{III}OH$ forms are essentially transparent in the visible region,[17,18] this is consistent with loss of the MLCT absorption overlapping with the acridine band upon oxidation of Ru(II) to Ru(III). Identical results are obtained upon treatment with 1 equiv of Ce(IV).

While the oxidation of $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ appears quasi-reversible in the cyclic voltammogram, addition of 2-propanol to the solution of the chemically or electrochemically oxidized species does not quantitatively regenerate the $Ru^{II}OH_2$ form, as in the other cases described here. We suspect that this is because the $Ru^{III}OH$ functionality oxidizes its own methylene linker in competition with oxidation of an added substrate. Nevertheless, if the complex is bound to DNA prior to oxidation, DNA cleavage readily occurs upon electrochemical activation. Thus, the ability to activate the bound complexes permits the attachment of the ruthenium-based cleaving function to binding moieties that may be readily oxidized in homogeneous solution.

DNA Cleavage Chemistry. We have already demonstrated the DNA-cleavage ability of $Ru(tpy)(bpy)O^{2+}$,[5] $Ru(tpy)(phen)O^{2+}$,[7] $Ru(tpy)(tmen)O^{2+}$,[7] and $Ru(tpy)(dppz)O^{2+}$.[8] Shown in Figure 11 are the results of electrocatalytic cleavage of supercoiled plasmid *φX174* DNA for the other derivatives described here. The gel shows conversion of supercoiled (form I) *φX174* DNA to nicked circular (form II) DNA upon electrolysis at 0.8 V in the presence of $Ru(bpy)_2(py)OH_2^{2+}$, $Ru(phen)_2(py)OH_2^{2+}$, and $Ru(tpy)(\eta^2\text{-}tpt)OH_2^{2+}$. Also shown is the cleavage of DNA by $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ electrolyzed at 0.5 V. We observe no cleavage by the $Ru^{II}OH_2$ forms or upon electrolysis in the absence of metal complex; we have published these controls elsewhere for $Ru(tpy)(bpy)OH_2^{2+}$ and $Ru(tpy)(tmen)OH_2^{2+}$.[5,7]

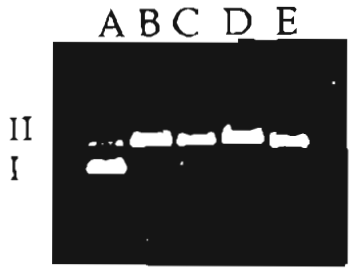


Figure 11. Photograph of a 1% agarose gel showing the results of electrophoresis of 60 μM *φX174* DNA after electrolysis at 0.8 V in the presence of 40 μM (A) $Ru(bpy)_2(py)OH_2^{2+}$, (B) $Ru(phen)_2(py)OH_2^{2+}$, and (C) $Ru(tpy)(\eta^2\text{-}tpt)OH_2^{2+}$ and at 0.5 V in the presence of 40 μM (D) $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$.

Conclusions

In summary, we have demonstrated that a family of DNA cleavage agents can be prepared using existing synthetic methodology. In general, reaction of $Ru(tpy)Cl_3$ with bidentate polypyridyl or tertiary amine ligands affords complexes from which oxoruthenium(IV) or hydroxoruthenium(III) complexes can be readily prepared. These complexes are effective oxidants and DNA cleavage agents that can be studied in the manner we have discussed.[5-8]

The observation of two hapticities for tpt is unusual. While the tpy ligand has previously been shown to bind in both η^3 and η^2 fashions,[25] this has not been demonstrated in a complex containing a relatively labile ligand such as aqua. This suggests that the complex $Ru(\eta^3\text{-}tpy)(\eta^2\text{-}tpy)OH_2^{2+}$ may also exist, and efforts to prepare this species are underway in our laboratory.

The ability to utilize the $RuOH_2$-based cleaving function tethered to oxidizable groups has been demonstrated. Although $Ru^{III}OH$ and $Ru^{IV}O$ are capable of oxidizing the tmen-AO^+ ligand, DNA is apparently oxidized preferentially when the bound $Ru^{II}OH_2$ form is activated by electrolysis. The electrochemical activation procedure may then offer a means of using binding functionalities (i.e. oligonucleotides) that would ordinarily undergo self-oxidation.

Acknowledgment. The support of this research by the North Carolina Biotechnology Center and the Donors of the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged. H.H.T. thanks the David and Lucile Packard Foundation for a Fellowship in Science and Engineering and the National Science Foundation for a Presidential Young Investigator Award.

Supplementary Material Available: Figures showing the mass spectrum and a simulation for $Ru(tpy)(tmen\text{-}AO)OH_2^{3+}$ and drawings of the cation and perchlorate counterions of $Ru(tpy)(\eta^3\text{-}tpt))(ClO_4)_2\text{-}0.5AgClO_4$ showing the complete atomic labeling and tables giving a summary of the crystal data and details of the X-ray data collection, atomic fractional coordinates, anisotropic thermal parameters, complete interatomic distances and angles, and hydrogen atom parameters (10 pages). Ordering information is given on any current masthead page.

(25) Abel, E. W.; Long, N. J.; Orrell, K. G.; Osborne, A. G.; Pain, H. M.; Sik, V. *J. Chem. Soc., Chem. Commun.* **1992**, 303.