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5′ Modification of Duplex DNA with a Ruthenium Electron Donor–Acceptor Pair Using Solid-Phase DNA Synthesis

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Incorporation of metalated nucleosides into DNA through covalent modification is crucial to measurement of thermal electron-transfer rates and the dependence of these rates with structure, distance, and position. Here, we report the first synthesis of an electron donor–acceptor pair of 5′ metallonucleosides and their subsequent incorporation into oligonucleotides using solid-phase DNA synthesis techniques. Large-scale syntheses of metal-containing oligonucleotides are achieved using 5′ modified phosporamidites containing $[Ru(acac)_2(IMPy)]^{2+}$ (acac is acetylacetonato; IMPy is 2′-iminomethylpyridyl-2′-deoxyuridine) (**3**) and $[Ru(bpy)_2(IMPy)]^{2+}$ (bpy is 2,2′-bipyridine; IMPy is 2′-iminomethylpyridyl-2′-deoxyuridine) (**4**). Duplexes formed with the metal-containing oligonucleotides exhibit thermal stability comparable to the corresponding unmetalated duplexes (T_m of modified duplex = 49 °C vs T_m of unmodified duplex = 47 °C). Electrochemical (**3**, $E_{1/2}$ = –0.04 V vs NHE; **4**, $E_{1/2}$ = 1.12 V vs NHE), absorption (**3**, λ_{max} = 568, 369 nm; **4**, λ_{max} = 480 nm), and emission (**4**, λ_{max} = 720 nm, τ = 55 ns, $\Phi = 1.2 \times 10^{-4}$) data for the ruthenium-modified nucleosides and oligonucleotides indicate that incorporation into an oligonucleotide does not perturb the electronic properties of the ruthenium complex or the DNA significantly. In addition, the absence of any change in the emission properties upon metalated duplex formation suggests that the $[Ru(bpy)_2(IMPy)]^{2+}$[Ru-$(acac)_2(IMPy)]^{2+}$ pair will provide a valuable probe for DNA-mediated electron-transfer studies.

Introduction

We are engaged in research aimed at elucidation of distance and driving force dependencies of the rates of thermal electron transfer in structurally unperturbed DNA duplexes.[1] Recent studies support the idea of guanine oxidation as a crucial step in the long-range charge transfer observed in some duplex systems where superexchange may operate within intervening polyAT segments.[2] Support for such a mechanism is expected from the investigation of donor−acceptor labeled duplexes, where driving forces and intramolecular donor−acceptor distances can be systematically varied. The concept of site-specific and covalent attachment of donor−acceptor transition metal complexes in DNA[3] has been extended to the first demonstration of solid-phase incorporation of a donor−acceptor transition metal pair (high and low potential) into duplex DNA. Here, we report our results of the synthesis and characterization of this type of system.

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Oligonucleotides modified with transition metal complexes have been used in the development of sequence-specific DNA sensors,[4] hybridization assays,[5] and as structural probes for charge and energy-transfer processes through DNA.[3,6] A recent approach to the synthesis of nucleoside−metal complex conjugates involves direct incorporation of metalated nucleoside phosphoramidites into oligonucleotides via solid-phase DNA synthesis.[7,8] Such an approach offers rapid, simple, and efficient metal incorporation over bimolecular metalation techniques. Incorporation of metal complex conjugates into automated DNA synthesis techniques has been successfully demonstrated for chemically stable bis-(2,2′-bipyridine) ruthenium(II) complexes tethered to base residues.[8b] Strategies for the incorporation of chemically sensitive transition metal donor complexes into DNA have not been effectively demonstrated.[9] This approach would allow insertion of both donor and acceptor metal complexes into DNA with tunable electronic properties that are located at positions efficiently coupled to the DNA backbone. Herein, we report the synthesis of 5′-metalated thymidine derivatives in which both electron-donor and electron-acceptor ruthenium complexes can be incorporated into the 5′ end of oligonucleotides with little structural or electronic perturbation to either the metal complex or duplex structure of DNA.

Experimental Section

Materials and Methods. Anhydrous solvents were obtained from Fluka. Preparative chromatography was performed using aluminum oxide (activated, neutral) Brockman I, standard grade from Aldrich Chemical Co. (Milwaukee, WI), or silica, 60 Å, EM Science. Ruthenium derivatives *cis*-dichloro-bis(2,2′-bipyridine)-ruthenium(II) dihydrate and ruthenium(III) chloride were purchased from Strem Chemicals (Newburyport, MA). Oligonucleotide synthesis was carried out on an ABI 394 DNA synthesizer. DNA synthesis reagents were purchased from Glen Research. Digestion enzymes were purchased from Pharmacia. Unless otherwise specified, materials obtained from commercial suppliers were of the highest purity available and used without further purification.

Instrumentation. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a JEOL 400 MHz and a General Electric 300 MHz spectrometer. Chemical shifts are reported relative to TMS for ^{1}H and ^{13}C NMR and to phosphoric acid for ^{31}P NMR. Electrospray mass spectrum (ESI-MS) and the MALDI-TOF were obtained with at the Mass Spectral Facility, Beckman Institute, California Institute of Technology. Absorption spectra were recorded on a HP 8452A diode array spectrometer. Emission spectra were recorded with a Shimadzu RF-5001 PC spectrometer equipped with a Hamamatsu R-928 photomultiplier tube. Relative emission quantum yields were determined by integrating the corrected emission spectra over the frequency range relative to an equally absorbing solution of ruthenium-(bipyridine)$_3^{2+}$ at 480 nm. The luminescence lifetimes were determined by using a modified Applied Photophysics laser kinetic spectrometer equipped with a Hamamatsu R-928 photomultiplier tube and a (Nd)YAG -MOPO laser as the excitation source as previously described. Kinetic analyses of the luminescence decays were performed by a nonlinear least-squares regression using Marquardt's algorithm.[12] Cyclic voltammetry, SWV, and DPV were carried out on 0.3 μM (oligonucleotides) or mM (metallonucleosides) degassed solutions with a CH Instruments 660 electrochemical workstation using a glassy carbon working electrode, Pt auxiliary electrode, and Ag/AgCl reference electrode. Runs were typically started at 0 V with a total of at least 6 segments, and scan rates of 0.03−0.05 V/s.

Lifetime Measurements. Lifetimes of the modified nucleosides were determined by laser flash photolysis in deoxygenated aqueous solution with an apparatus described previously.[13] Quantum yields were determined relative to {Ru(bpy)$_3$}$^{2+}$.[14] Values for k_r and k_{nr} were obtained using the relationships $k_r = \Phi_r/\tau o$ (25 °C) and $k = k_r + k_{nr}$, where Φ_r is the emission quantum yield at 25 °C (see Figure 1a).

Syntheses. The precursor 5′-amino-5′-deoxy-thymidine was prepared by reaction of the nucleoside with phosphonium salt and sodium azide to produce 5′-azido-5′-deoxythymidine in 78% yield.[15] Hydrogenation of this compound by Pd/C in methanol led to compound **3** in 96% yield.

1-(4-Hydroxy-5-{[(pyridin-2-ylmethylene)-amino]-methyl}-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione (2). To a mixture of 5′-amino-5′-deoxythymidine (3.13 g, 13.0 mmol) and 4 Å molecular sieves in 150 mL of anhydrous ethanol was added 2-pyridinecarboxaldehyde (1.39 g, 13.0 mmol). The solution was heated to reflux and allowed to react for 2 h. The hot solution was filtered and the solvent removed under reduced pressure to give a tan solid. Column chromatography on silica (methylene chloride: methanol) followed by recrystallization from anhydrous ethanol afforded 2.3 g of white crystals (yield: 84%). ^{1}H NMR (300 MHz, CD_3OD) δ 8.59 (d, 1 H), 8.41 (s, 1 H, H-imine), 7.99 (d, 1 H), 7.88 (m, 1 H), δ 7.42−7.59 (m, 2 H), 6.25 (t, 1 H, H-1′), 4.50 (m, 1 H, H-3′), 4.16 (m, 1 H, H-4′), 3.94 (m, 2 H, H-2′), 2.21 (m, 1 H, H-5′), 1.50 (s, 3 H, H−CH_3). ESI-MS: 331 [M + H]$^+$, 353 [M + Na]$^+$, 369 [M + K]$^+$.

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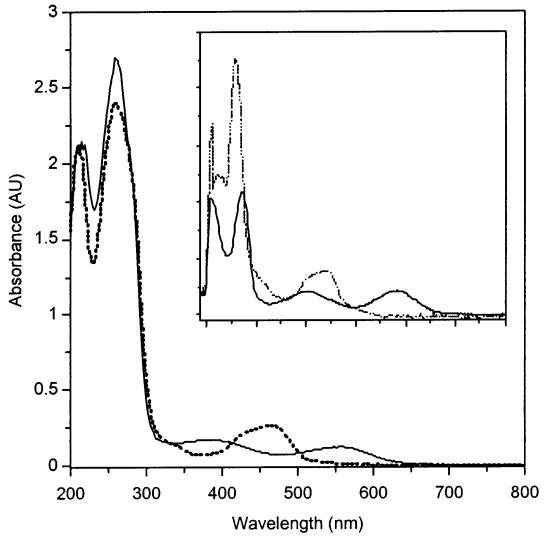

Figure 1. Absorption spectra of oligonucleotides 11H (- - -) and 11L (—) at room temperature in buffer (0.8 M NaCl, 50 mM sodium phosphate, pH 7.0). 11L: $\lambda_{max} = 394$ nm ($\epsilon = 4800$ M^{-1} cm^{-1}), 568 nm ($\epsilon = 4800$ M^{-1} cm^{-1}). 11H: $\lambda_{max} = 463$ nm ($\epsilon = 8350$ M^{-1} cm^{-1}). Inset: Absorption spectra of metalated nucleosides **3** (—) and **4** (- - -) at room temperature in methanol. Complex **3**: $\lambda_{max} = 402$ nm ($\epsilon = 4600$ M^{-1} cm^{-1}), 568 nm ($\epsilon = 4600$ M^{-1} cm^{-1}). **4**: $\lambda_{max} = 468$ nm ($\epsilon = 7500$ M^{-1} cm^{-1}).

Bis(acetylacetonate)ruthenium(II) (1-(4-Hydroxy-5-{[(pyridin-2-ylmethylene)-amino]-methyl}-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione) (3). To a degassed solution of compound **2** (206 mg, 0.62 mmol) in 30 mL of anhydrous ethanol was added bis(acetylacetonate)ruthenium(II) bis(acetonitrile).[16] The reaction mixture was brought to 90 °C and allowed to reflux for 2.5 h after which a dark green solution was obtained. The reaction mixture was cooled and the solvent removed under reduced pressure. Column chromatography on silica with 2:1 tetrahydrofuran/hexane as eluent led to the isolation of two diastereomers (F1, $R_f = 0.16$; F2, $R_f = 0.25$) in 26% and 32% yield, respectively. NMR was complicated by broadening due to slight paramagnetic impurity. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83 (m), 8.65 (m), 8.0 (s), 7.68 (m, 1 H), 7.42−7.59 (m), 7.25 (m), 7.0 (s), 6.4 (d), 5.38 (m), 5.0 (s), 4.5 (m), 4.38 (m), 4.15 (m), 4.05 (m), 3,75 (t), 3.64 (d), 3.39 (s), 2.27 (m), 2.13 (m), 1.95 (s), 1.73−1.85 (m), 1.6 (m), 1.42 (s), 1.24(s), 1.22(s), 1.19 (s), 0.93 (s). Mass spectral analysis, ESI-MS: 630.2 $[M]^+$. UV ((ethanol), λ_{max} nm, (ϵ)): 208 (26 000), 274 (25 000), 402 (4600), 586 (4600). The reduction potential as measured by cyclic voltammetry in 0.1 M NH_4PF_6/ethanol at a scan rate of 0.05 V/s was found to be 0.039 V versus Ag/AgCl (0.236 V vs NHE) with a $\Delta E_p = 0.076$ V.

Bis(2,2′-bipyridine)ruthenium(II) (1-(4-Hydroxy-5-{[(pyridin-2-ylmethylene)-amino]-methyl}-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione) (4). To a degassed solution of compound **2** (500 mg, 1.5 mmol) in 50 mL anhydrous ethanol was added *cis*-dichloro-bis(2,2′-bipyridine)ruthenium(II) dihydrate (1.090 g, 1.5 mmol). The reaction mixture was brought to 100 °C and allowed to reflux for 3 h, after which a dark red solution was obtained. The reaction mixture was cooled and the solvent removed under reduced pressure. Silica gel column chromatography using 20/4/1 acetonitrile/water/saturated KNO_3 solution as eluent led to the isolation of product salts as a mixture of diastereomers ($R_f = 0.17$). The solid was taken up in a minimum of water, anion exchange was performed by the addition of solid KPF_6, and the resulting solution was extracted with methylene chloride. The solvent was removed under reduced pressure to give orange crystals (400 mg, 26% yield). NMR was complicated by existence of diastereomers and therefore not assigned. ^{1}H NMR (300 MHz, CD_3CN) δ 8.62−9.18 (m), 8.42−8.58 (m), 8.4 (d), 8.25 (d), 7.84−8.24 (m), 7.66−7.76 (m), 7.61−7.47 (m), 7.43−7.56 (m), 7.33−7.24 (m), 7.02 (dd), 5.97 (dd), 5.73 (t), 3.93 (d), 3.76 (m), 3.56 (m), 3.26 (d), 3.14 (d), 3.04 (q), 2.17 (s), 1.96 (m), 1.88 (s), 1.86 (s). Mass spectral analysis, ESI-MS: 889.2 $[M + PF_6]^+$, 372 $[M]^{+2}$. UV ((methanol), λ_{max} nm (ϵ)): 210 (42 000), 246 (25 000), 288 (49 000), 468 (7500). Emission (methanol) λ_{EM} 720 nm (λ_{EX} 480 nm). Lifetime measurements of a 23 μm solution in 800 mM NaCl, 50mM phosphate buffer, pH = 7.0, were performed: $\tau = 45$ ns (λ_{EX} 480 nm). The reduction potential as measured by cyclic voltammetry in 0.1 M TBAH/CH_3CN, scan rate of 0.05 V/s, was found to be 1.37 V versus Ag/AgCl (1.57 V vs NHE) with a $\Delta E_p = 0.091$ V.

Bis(acetylacetonate)ruthenium(II) (1-(4-Hydroxy-5-{[(pyridinyl-2-methylene)-amino]-methyl}-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione) Phosphoramidite (5). To a solution of ruthenium complex **3** (151 mg, 0.24 mmol) and 1-H tetrazole (16.6 mg, 0.24 mmol) in 4.8 mL anhydrous acetonitrile was added 2-cyanoethyl-tetraisopropylphosphoramidite (152 mg, 0.50 mmol, 2.1 equiv). The solution was stirred under argon at room temperature for 4.5 h. The solvents were removed under reduced pressure, and the remaining crude material was purified by column chromatography on silica with 45/45/10 dichloromethane/ethyl acetate/triethylamine to give a dark green glass (163 mg, 82% yield). $_1$H NMR spectra were complicated by mixtures of diastereomers and, therefore, are not reported. Mass spectral analysis, ESI-MS: 830.2 $[M + H]^+$. UV ((CH_2Cl_2), λ_{max} nm): 232, 274, 396, 586.

Bis(2,2′-bipyridine)ruthenium(II) (1-(4-Hydroxy-5-{[(pyridin-2-ylmethylene)-amino]-methyl}-tetrahydro-furan-2-yl)-5-methyl-1*H*-pyrimidine-2,4-dione) Phosphoramidite (6). To a solution of ruthenium complex **4** (207 mg, 0.20 mmol) and 1-H tetrazole (14 mg, 0.20 mmol) in 4 mL anhydrous acetonitrile was added 2-cyanoethyl-tetraisopropylphosphoramidite (128 mg, 0.42 mmol, 2.1 equiv). The solution was stirred under argon at room temperature for 4 h. The solvents were removed under reduced pressure, and the remaining oil was taken up in 300 mL 1% TEA in dichloromethane. The solution was washed (2 × 20 mL) with 1 M sodium bicarbonate solution and (2 × 20 mL) brine solution. The solvents were removed under reduced pressure, and the red solid was purified by column chromatography on basic alumina (Brockman I) with 97% acetonitrile, 2.5% water, and 0.5% saturated potassium hexafluorophosphate solution ($R_f = 0.55$ in 80/15/5 CH_3CN/H_2O/sat. KNO_3) to give a dark red glass (159 mg, 64% yield). H NMR spectra were complicated by mixtures of diastereomers and therefore are not reported. Mass spectral analysis, ESI-MS: 1089.2 $[M + PF_6]^+$. UV ($(CH_2Cl_2$, 5% MeOH), λ_{max} nm): 288, 466.

Oligonucleotide Synthesis and Purification. Modified oligonucleotides and controls were prepared on a 1.0 μmol scale on 500 Å CPG support. Modified nucleoside couplings were carried out directly on an ABI 394 DNA synthesizer. Acetonitrile solutions of the modified phosphoramidites (0.1−0.15 M) were introduced site specifically at the 5′ end of the oligonucleotide, trityl on, manual cleavage cycle. Coupling times were extended to 30 min with single delivery. Cleavage and deprotection steps were carried out manually by addition of 1 mL of 30% ammonium hydroxide solution followed by standing at 25 °C for 8 h. Oligonucleotides labeled with $Ru(acac)_2LL'$ metal complexes were reduced with dithionite prior to cleavage and deprotected at 25 °C for 6 h. The oligonucleotide solutions were lyophilized and purified by HPLC on a Dionex

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Nucleopac PA-100 using a mobile phase of 10−60% 1.5 M ammonium chloride, 20 mM tris, 0.5% acetonitrile gradient in 20 mM tris, 0.5% acetonitrile, and a photodiode array detector. Metalated oligonucleotides were identified by their absorbance at 260 with visible bands due to the MLCT states of the metal complexes. Following HPLC purification and lyophilization, the oligonucleotides were desalted using SepPak cartridges (Waters Corporation, MA) and submitted for mass spectral analysis. Mass spectral characterization of 11H, 8H, 11L, and 8L confirmed the desired oligonucleotides. Analyses follow. 8H Anal. Calcd: 2911. Found: 2911. UV ((H_2O), λ_{max} nm): 259, 465. 8L Anal. Calcd: 2796. Found: 2797. UV ((H_2O), λ_{max} nm): 260, 394, 563. 11H Anal. Calcd: 3856. Found: 3855. UV ((H_2O), λ_{max} nm): 258, 463. 11L Anal. Calcd: 3702. Found: 3703. UV ((H_2O), λ_{max} nm): 268, 394, 568.

Enzymatic Digestions of Metalated Oligonucleotides. An 8 nmol pellet of DNA was taken up in 24 μL of Nanopure water. To this solution was added 3.5 μL of a 0.5 M Tris solution, pH = 7.5, 0.8 μL of 1.0 M $MgCl_2$, 4.0 μL alkaline phosphatase (calf intestinal mucosa supplied as a solution in 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$ and 50% glycerol, Pharmacia, and diluted 10-fold with 10X-One-Phor-All-Buffer PLUS), and 2.4 μL phosphodiesterase (stock solution of 100 units at 31 units/mg) dissolved in 110 mM Tris HCl, 110 mM NaCl, 15 mM $MgCl_2$, and 50% glycerol. Each sample was centrifuged and incubated at 37 °C for 15 h. The samples were analyzed by reverse-phase HPLC (Prism RP 250 mm × 4.6 mm, 5 μm particle size) using a photodiode array detector. HPLC mobile phase: 2−75% acetonitrile gradient in 100 mM TEAC buffer over 33 min. Nucleoside retention times of modified and unmodified nucleosides were determined by independent injections. Nucleoside ratios were determined by integration of peak areas at 260 nm.

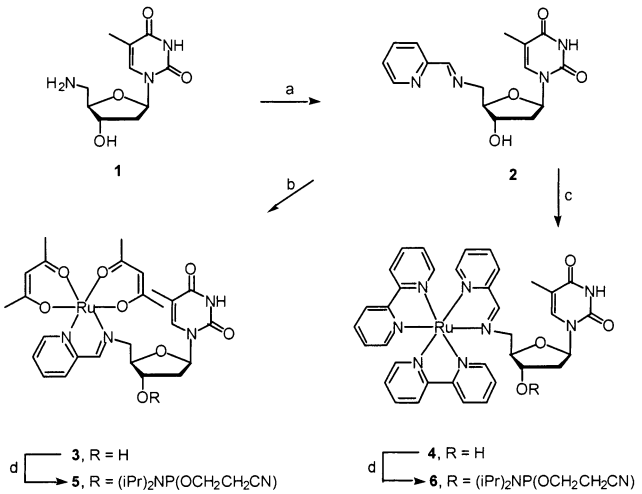
Thermal Denaturation Studies. All hybridizations and melting experiments were carried out in 800 mM NaCl (to ensure complete hybridization), 50 mM sodium phosphate buffer at pH 7.0, using a Hewlett-Packard 8452A spectrometer with a Peltier heating accessory. Degassed solutions of 1−2 μM (double stranded) were heated at 75 °C for 5 min and then allowed to cool to room temperature over the course of 8 h. Heating runs were performed on samples placed in stoppered cuvettes with a 1.0 cm path length, between 20 °C and 70 °C at a scan rate of 0.5° min^{-1} with optical monitoring at 260 nm. The melting temperature (T_m) values derived from UV melting profiles were verified over 3−4 independent runs.

Discussion

Previous results in our lab suggested that bis(acetylacetonato)ruthenium(II) imine complexes may serve as useful electron donor−acceptor complexes because of their high chemical stability and low reduction potential.[10] Our synthetic strategy required design of a ligand strongly coupled to the ribose moiety, yet distal from the 3′ phosphoramidite coupling site. Modification of the 5′ position of thymidine by covalent attachment of iminopyridine (IMPy) allowed access to both a high potential metal complex $\{Ru(bipy)_2(IMPy)\}^{2+}$ and the corresponding low potential complex $\{Ru(acac)_2(IMPy)\}^{2+}$. The synthesis of 5′-imine-5′-deoxythymidine derivative (**2**) was therefore targeted for initial investigation.

The syntheses of the ligand 5′-deoxy-5′-iminomethyl-(2-pyridyl) thymidine and the corresponding metal complexes are shown in Scheme 1. Thymidine is initially functionalized to 5′-amino-5′-deoxy-thymidine (**1**), followed by Schiff base

Scheme 1 [a]



[a] Reactants: (a) 2-pyridinecarboxaldehyde, 4 Å molecular sieves, ethanol, 80 °C, 2 h, 84%; (b) bis(acetoacetonate)ruthenium(II) bis(acetonitrile), ethanol, 80 °C, 1 h, 71%; (c) ruthenium(II) bis(bipyridine) dichloride hydrate, ethanol, 80 °C, 2 h, 26%; (d) 2-cyanoethyltetraisopropyl-phosphoramidite, 1H-tetrazole, acetonitrile, 25 °C, 4 h, 82%.

condensation with 2-pyridine carboxaldehyde to give 5′-deoxy-5′-iminomethyl-(2-pyridyl) thymidine (**2**) in 84% yield from starting thymidine. Reaction of nucleoside ligand **2** with bis(acetylacetonate)ruthenium(II) bis(acetonitrile) in refluxing ethanol leads to a mixture of diastereomers of complex **3**, $\{Ru(acac)_2(T\text{-}IMPy)\}^{2+}$, in 71% yield. Chromatographic separation of a diastereomeric mixture of **3** on silica leads to 21% and 50% of two diastereomers. Reaction of ligand **2** with *cis*-dichloro-bis(2,2′-bipyridine)ruthenium(II) dihydrate under similar conditions leads to complex **4**, $\{Ru(bipy)_2(T\text{-}IMPy)\}^{2+}$, in 26% yield. Chromatographic resolution on silica of the corresponding diastereomers of **4** was not easily achieved. Therefore, diastereomeric mixtures of **4** were used for the following chemical transformations.

Phosphitylation of metal complexes **3** and **4** allows direct incorporation into oligonucleotides by standard solid-phase DNA synthesis techniques in high yield. Metalated nucleosides **3** and **4** phosphitylation were incorporated into the 5′ end of oligonucleotides of varying length (8−14 bases) by introduction of a 0.1 M acetonitrile solution of the phosphoramidite. Phosphoramidite coupling was carried out on an ABI DNA synthesis machine followed by standard capping/oxidation/deprotection/cleavage steps. HPLC ion exchange purification gave the metalated oligonucleotides in 30−46% isolated yield for the described series (oligonucleotides 5′ labeled with the low potential complex $\{Ru(acac)_2(T\text{-}IMPy)\}^{2+}$ are 8L, 9L, 10L, 11L, 14L, and those 5′ labeled with the high potential complex $\{Ru(bpy)_2(T\text{-}IMPy)\}^{2+}$ are 8H, 9H, 10H, 11H, 14H). Single strand complements of each series (8−14 LC for 8−14L, and 8−14 HC for 8−14H) were synthesized using standard DNA synthesis and HPLC purification techniques. The MALDI-TOF mass spectral analyses of metalated oligonucleotides confirm the expected mass for 11H (Calcd: 3855 amu. Found: 3855.44 amu.) and 11L (Calcd: 3702 amu. Found: 3702.34 amu.). In addition, enzyme digestions of 8H, 8L, 11H, and 11L were carried out with alkaline phosphatase

(calf intestinal mucosa) and phosphodiesterase I (*Crotalus adamanteus* venom) in 0.5 M Tris, 1.0 M $MgCl_2$, and were analyzed by HPLC using independent injections of ruthenium-labeled and nonlabeled nucleosides. The results confirm the expected base composition of ruthenium modified oligonucleotides and suggest that the metal complexes can be successfully incorporated into oligonucleotides using standard solid-phase techniques without chemical modification of the complexes.

Thermal denaturation studies suggest little perturbation of the stability of DNA duplexes upon metal complex incorporation. Denaturation studies were carried out on degassed solutions of duplex DNA in 800 mM NaCl, 50 mM sodium phosphate buffer at pH 7.0. The unmetalated 11-mer duplex 11LC/11HC exhibits a melting temperature of 47 °C, whereas the corresponding bismetalated duplex 11L/11H exhibits essentially the same melting temperature of 48 °C. Contributions to thermal denaturation from nucleotide base pairing, as well as electrostatic interactions, preclude detailed analysis of the thermodynamic contributions. Qualitatively, however, the cooperative behavior expected for thermal denaturation of duplex DNA is maintained, and little perturbation of the stability of the duplex is suggested.

Spectroscopic characterization of the metalated nucleosides **3** and **4** reveals little perturbation of the electronic structure of isolated metal complexes upon ligation to thymidine (Figure 1). The low potential complex **3** shows characteristic MLCT bands in ethanol at $\lambda_{max} = 402$ nm ($\epsilon = 4600$ L mol^{-1} cm^{-1}), assigned to a ruthenium(II)−acac d(π)−π* transition, and $\lambda_{max} = 586$ nm ($\epsilon = 4600$ L mol^{-1} cm^{-1}), assigned to a ruthenium(II)−(iminomethyl-pyridine) d(π)−π* transition.[9a] A moderate bathochromic shift of 10 nm in the long wavelength transition is evident upon ligation as compared to that of the model complex $\{Ru(acac)_2(IMPy)\}^{2+}$, with $\lambda_{max} = 402$ nm ($\epsilon = 4600$ L mol^{-1} cm^{-1}) and $\lambda_{max} = 576$ nm ($\epsilon = 4600$ L mol^{-1} cm^{-1}). The high potential complex **4** reveals a typical broad MLCT absorption band in methanol at $\lambda_{max} = 468$ nm ($\epsilon = 7500$ L mol^{-1} cm^{-1}) due to a ruthenium(II)−(bipyridine) d(π)−π* transition, closely resembling that of the corresponding model system $\{Ru(bpy)_2(T\text{-}IMPy)\}^{2+}$.[11]

To evaluate whether incorporation into a DNA duplex leads to significant perturbations in the electronic structure of the complexes, the absorption and emission spectroscopy of single strand 11H, 11L and duplexes 11L/11LC, 11H/11HC, and 11L/11H were investigated. The absorption spectra of the metalated oligonucleotides 11L and 11H exhibit intense MLCT transitions of their respective nucleoside analogues, along with an intense π−π* transition due to the oligonucleotide bases. The electronic absorption spectrum of the ruthenium modified oligonucleotides is the sum of the spectra of the ruthenium complex and the unmodified oligonucleotides suggesting little electronic perturbation of the electronic structure of either the metal complexes or DNA upon functionalization (Figure 1). Thus, while there is a slight increase in extinction coefficient observed, the λ_{max} absorption values shift by only 3−5 nm upon metal complex incorporation into oligonucleotides, suggesting only slight electronic perturbations.

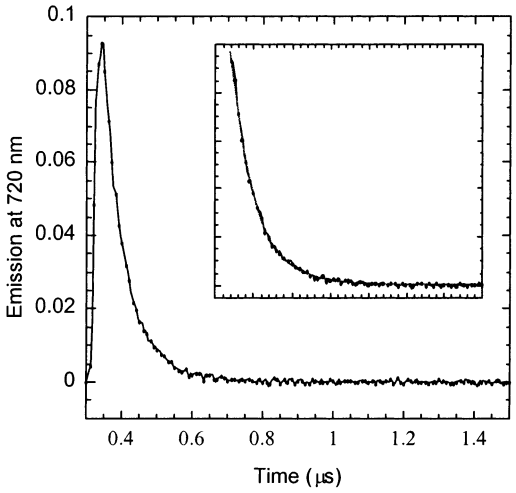


Figure 2. Emission spectra of oligonucleotide 11H at room temperature in buffer (0.8 M NaCl, 50 mM sodium phosphate, pH 7.0). Inset: monoexponential fit to emission decay at 720 nm with lifetime of $\tau = 65$ ns.

Excited-state lifetimes were measured by laser flash photolysis in degassed aqueous solution. The emission spectra of complex **4** consists of a band at $\lambda_{max} = 720$ nm that decays monoexponentially with a lifetime of $\tau = 54$ ns ($\Phi = 3.5 \times 10^{-3}$) in degassed aqueous buffer (800 mM NaCl, 50 mM NaP_i, pH = 7.0). Once incorporated into the DNA single strand, the lifetime of the excited state of **4** in aqueous buffer increases with a lifetime of $\tau = 65$ ns (Figure 2). Incorporation into duplex DNA (11H−11HC) leads to no significant change in lifetime or quantum yield ($\tau = 66$ ns). The lifetime is independent of duplex length as evidenced by no significant change in the lifetime for the series of duplexes (8−14 base pairs: $\tau = 65$−66 ns). Finally, incorporation of the acceptor complex **3** into duplex DNA (8L−8H) leads to a monoexponential decay of the excited state with a 7% decrease in lifetime ($\tau = 63$ ns), suggesting little contribution of energy transfer in the donor−acceptor bismetalated duplex.

The half wave reduction potentials of complexes **3** and **4** were measured by cyclic voltammetry and square wave and differential scanning polarimetry. Cyclic voltammetry of the dication of **3** exhibits a half-wave potential of 236 mV versus NHE in 0.1 M NH_4PF_6/EtOH which is nicely reversible with a ΔIP of 76 mV (Figure 3). The Ru(II/III) half wave potential of **4** was measured using DPV and CV in both aqueous 800 mM NaCl and acetonitrile solution. In aqueous solution, the first reduction is partially reversible and occurs at −1.197 V versus NHE while it is fully reversible in 0.1 M TBAH/CH_3CN with a half potential of −1.567 V and a ΔIP of 91 mV. Further reduction waves are observed at −1.29, −1.45, and −1.63 V vs NHE corresponding to the II/I, I/0, and 0/−1 reductions of $Ru(bpy)_3^{2+}$. While the first reduction is 200 mV lower than that of $Ru(bpy)_3^{2+}$, the second, third, and fourth are comparable, suggesting that the LUMO of the metal complex is lowered relative to that of $Ru(bpy)_3^{2+}$. DFT calculations (B3LYP/LACVP**) performed on the metal complexes $Ru(bpy)_3^{2+}$ and $Ru(bpy)_2(IMPy)^{2+}$ suggest HOMO/LUMO splittings of 3.589 and 3.456 eV, respectively. The decrease in HOMO/LUMO gap of 0.13 eV is accompanied

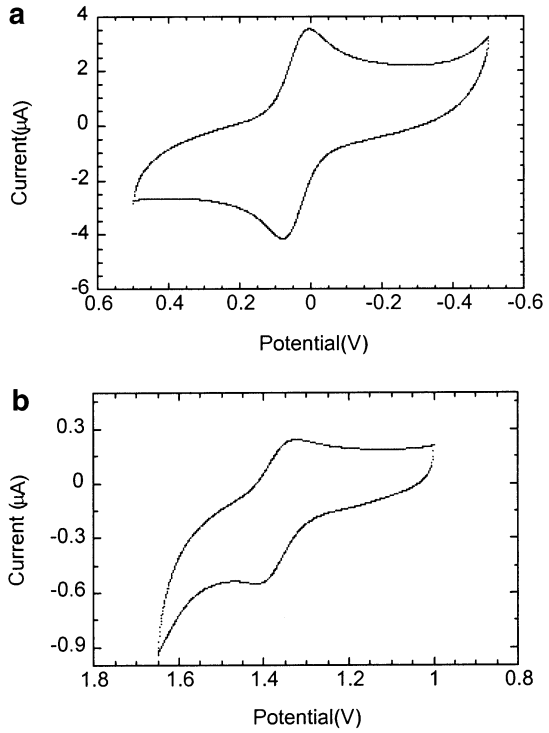


Figure 3. Room-temperature cyclic voltammograms of metalated nucleosides **3** (left) in 0.1 M NH_4PF_6/EtOH and **4** (right) in 0.1 M TBAH/acetonitrile with a scan rate of 0.05 V/s, Ag/AgCl//Pt/glassy carbon electrode. Half wave potentials for the two dicationic species are 236 mV and 1.567 V vs NHE.

by a decrease in the energies of both the HOMO and LUMO energies upon bipyridine substitution, suggesting an increased electronegativity of iminomethylpyridine relative to bipyridine, consistent with the electrochemical data.

Electrochemical measurements were performed on metalated oligonucleotides in order to evaluate the extent of electronic coupling between donor−acceptor complexes and oligonucleotide in the series of metalated oligonucleotides. Cyclic voltammetry of a representative 11-base length single stranded 11L shows a reversible wave at 0.0 V versus Ag/AgCl//Pt/glassy carbon electrode in 800 mM NaCl, 50 mM NaP_i, pH = 7.0. The half wave potential therefore decreases by only ca. 30 mV upon incorporation into DNA, suggesting only slight electronic perturbations. On the other hand, square wave voltammetry of the high potential oligonucleotide, 11H, exhibits an oxidation potential of 1.365 V versus NHE (Ag/AgCl//Pt/glassy carbon electrode in 800 mM NaCl, 50 mM NaP_i, pH = 7.0), a shift of 168 mV from the parent metallonucleoside, suggesting either significant through space interactions with the DNA backbone, or increased hydrophobicity of the metal complex environment in DNA. The latter interpretation is supported by the spectral shifts observed in the absorption spectroscopy of **4** in hydrophobic solvents.

In conclusion, we have demonstrated a generalized approach to the synthesis of 5′ metalated thymidines **3** and **4** which provide an electron donor−acceptor pair with marked spectroscopic signatures of the Ru(II) oxidation state (402 nm, 568 nm vs 468 nm), and significant driving force for electron transfer (1.4 V). We have then demonstrated successful incorporation of metallonucleosides **3** and **4** into duplex DNA through solid-phase DNA synthesis techniques. Absorption, emission, and electrochemical spectroscopy suggest little to no electronic perturbation of the metallonucleosides upon DNA incorporation. Evaluation of the stability of metalated duplex DNA though thermal denaturation studies suggests, in addition, little to no decrease in the stability of DNA upon metallonucleoside incorporation. We have thus synthesized the first DNA duplex incorporating a covalently attached transition metal donor−acceptor pair in which electronic and structural distortions are minimized. Such systems allow systematic investigation of intramolecular donor−acceptor distance and driving force dependence rate measurements of thermal electron transfer in duplex DNA and will provide valuable mechanistic insight into charge transfer through DNA.

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