Automated Solid-Phase Synthesis and Photophysical Properties of Oligodeoxynucleotides Labeled at 5′-Aminothymidine with $Ru(bpy)_2(4\text{-m-}4'\text{-cam-bpy})^{2+}$

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A facile and automated procedure for the incorporation of a derivatized $Ru(bpy)_3^{2+}$ in an oligodeoxynucleotide is reported. The $Ru(bpy)_3^{2+}$−thymidine phosphoramidite is synthesized, and then incorporated in DNA using a standard protocol on an automated DNA solid-phase synthesizer. The structure of the DNA duplex is not altered after labeling with $Ru(bpy)_3^{2+}$. Photophysical studies of the novel ruthenium trisdiimine thymidine complex as well as the corresponding labeled oligodeoxynucleotides demonstrate that the favorable properties associated with the ruthenium complex are retained after covalent attachment to the nucleoside and oligodeoxynucleotide.

Introduction

Redox-active and photoactive probes covalently linked to biological macromolecules are of widespread interest for basic studies such as characterizing biologically relevant energy- and electron-transfer reactions, and for clinical applications such as detecting proteins and deoxyribonucleic acid (DNA).[1−13] Probes such as metal complexes possess a number of favorable properties for these uses including tunable electronic structures, reversible electrochemical behavior, and emissive spectroscopic states. The site-specific labeling of proteins has advanced significantly in the last 15 years, and the techniques are well-established.[1,2] However, the labeling of deoxyribonucleic acid is an area of active current research. Synthetic procedures to precisely control and vary the location of a metal complex in an oligodeoxynucleotide while capitalizing on the use of solid-phase reactions and automation are highly desirable.

The two general synthetic approaches toward labeling oligodeoxynucleotides with metal complexes are postmodification of an oligodeoxynucleotide and metal-complex-derivatized phosphoramidites for automated DNA synthesis. The former procedure typically involves reacting an oligodeoxynucleotide previously modified to contain a primary amine with a metal complex in solution.[14] This method is extensively used and is amenable to a large number of metal complexes.[12,13,15−26] However, this solution-phase reaction has drawbacks which, depending on the system, may include side reactions, poor coupling yields, and multiple chromatographic purifications.

The second approach requires the use of an automated DNA solid-phase synthesizer and a metal-complex-derivatized phosphoramidite, and possesses several advantages over the post-modification procedure. This method allows control over the location of the metal complex in the oligodeoxynucleotide while being fully automated and user-friendly. Several laboratories are using this procedure to synthesize platinated oligodeoxynucleotides,[27,28] europium texaporphyrin-labeled oligodeoxy-

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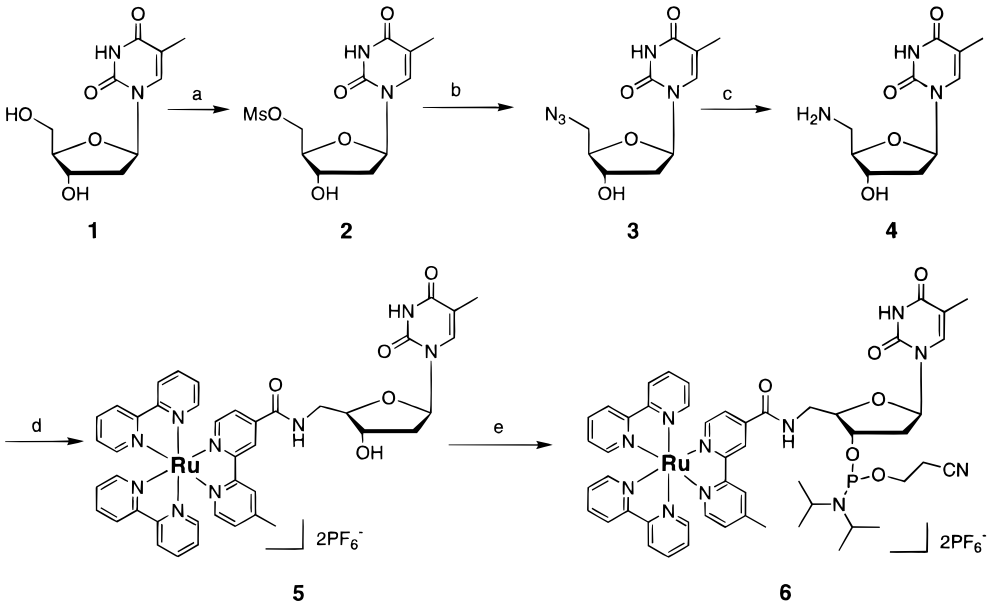
Scheme 1. Phosphoramidite Synthesis[a]



[a] Conditions: (a) MsCl/pyridine, 0 °C, 12 h, 77% yield; (b) LiN_3/DMF, 90 °C, 3 h, 73% yield; (c) PPh_3/dioxane, NH_4OH, 25 °C, 12 h, 72% yield; (d) CDI/DMF, LH-20/THF, $Ru(bpy)_2$(4-m-4′-ca-bpy), 25 °C, 12 h, 80% yield; (e) 2-cyanoethylchloro-*N*,*N*-diisopropylphosphoramidite, DIPEA, CH_3CN, 25 °C, 2 h (yield >95%, TLC).

nucleotides,[29] and $Ru(diimine)_3^{2+}$-labeled oligodeoxynucleotides.[30−36] We are currently exploring this synthetic approach for labeling oligodeoxynucleotides with $M(diimine)_3^{2+}$ complexes at the nucleobase[33−35] and 5′-terminal phosphate.[36]

A third site for attaching transition-metal probes to an oligodeoxynucleotide is the carbohydrate backbone. Labeling the 5′-, 3′-, or 2′-position of ribose is relatively unexplored. A report in 1995 describes a reaction between a 2′-amino-modified oligodeoxynucleotide and a ruthenium complex.[24] This solution-phase procedure requires first synthesizing a 2′-amino-modified oligodeoxynucleotide, and then hybridizing it with its complement before metal complexation. Next, the duplex is denatured, and the ruthenium-labeled oligodeoxynucleotide single strand is isolated. Herein, we report an automated solid-phase procedure for labeling DNA at the 5′-aminoribose of an oligodeoxynucleotide with a substitutionally inert transition-metal complex. A $Ru(diimine)_3^{2+}$−thymidine derivative, 5′-[$Ru(bpy)_2$(4-m-4′-cam-bpy)$^{2+}$]−thymidine, is first synthesized, and then this active metallonucleoside phosphoramidite is incorporated in an oligodeoxynucleotide using an automated DNA synthesizer.

Results and Discussion

The $Ru(diimine)_3^{2+}$-derivatized 5′-deoxythymidine phosphoramidite, **6**, for automated DNA synthesis was synthesized in five steps starting from thymidine. As shown in Scheme 1, the 5′-position of thymidine was converted from a hydroxyl to an amine, by first treating thymidine dissolved in pyridine with methanesulfonyl chloride to form **2**.[37] Next, the mesyl group was substituted with an azide, and subsequently reduced with PPh_3 to yield the 5′-amino-5′-deoxythymidine, **4**. The monocarboxylic acid-derivatized $Ru(diimine)_3^{2+}$ complex, $Ru(bpy)_3$-(4-m-4′-ca-bpy)$^{2+}$, was then coupled to 5′-amino-5′-deoxythymidine, **4**, in DMF using CDI. Other amide-forming strategies using DCC or the NHS-activated ester were explored, but yielded less product. Finally, **5** was reacted with 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite in dry CH_3CN to afford the metallophosphoramidite, **6**. The ruthenium−thymidine phosphoramidite was now ready for use in an oligodeoxynucleotide synthesizer. Normal solid-phase oligodeoxynucleotide synthesis was performed,[38,39] and in the last coupling step the ruthenium-modified thymidine phosphoramidite was introduced as shown in Scheme 2. All syntheses were performed at the 1.0 μmol scale using the standard coupling protocol except that the final step proceeded for 15 min to ensure sufficient time for the Ru-$(diimine)_3^{2+}$−thymidine phosphoramidite to react with the 5′-terminal alcohol of the oligodeoxynucleotide. Once synthesized, the ruthenium-labeled oligodeoxynucleotide was cleaved from the column. Finally, the nitrogenous bases and phosphate groups were deprotected in 30% ammonium hydroxide at 55 °C for 16 h, and the oligodeoxynucleotide was purified by RP-HPLC (see Table 1).

As shown in Figure 1, the thermal denaturation curve for the unlabeled (**11·15**) and $Ru(diimine)_3^{2+}$-labeled (**7·15**) 13-mer oligodeoxynucleotide duplexes are similar. The decrease in the melting temperature from 42 to 39 °C is small, and this magnitude of change is also observed with the larger duplex (Table 2). These relatively small changes indicate that labeling the 5′-terminal nucleotide of the oligodeoxynucleotide does not dramatically alter the duplex structure of DNA. These results are further supported by circular dichroism (CD) spectroscopy

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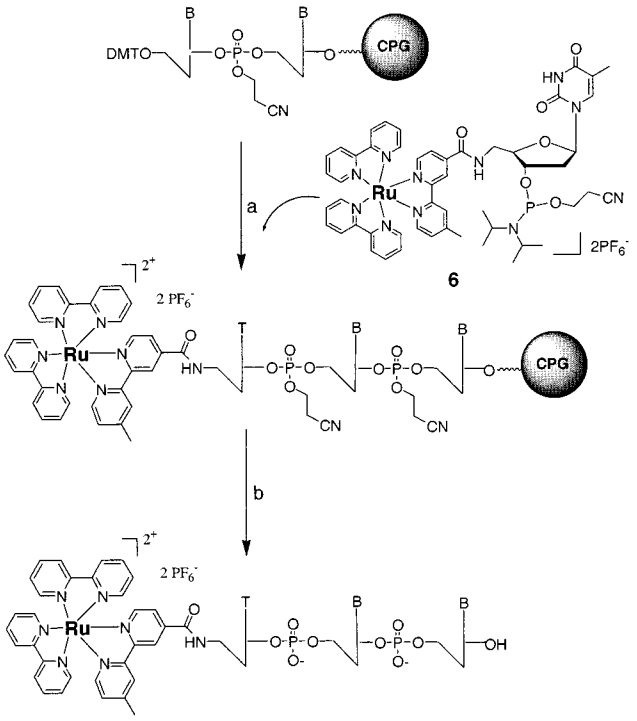
Table 1. Ruthenium-Labeled Oligodeoxynucleotides Synthesized

7	5′-^{Ru}TTCAACAGTTTGT-3′
8	5′-^{Ru}TCAACAGTTTGTAGCA-3′
9	5′-^{Ru}TGCTACCCTCTGTTGA-3′
10	5′-^{Ru}TTCAACAGTTTGTAGCA-3′
11	5′-TTCAACAGTTTGT-3′
12	5′-TCAACAGTTTGTAGCA-3′
13	5′-TGCTACCCTCTGTTGA-3′
14	5′-TTCAACAGTTTGTAGCA-3′
15	5′-ACAAACTGTTGAA-3′
16	5′-TGCTACAAACTGTTGA-3′
17	5′-TCAACAGAGGGTAGCA-3′
18	5′-TGCTACAAACTGTTGAA-3′

Scheme 2. Oligonucleotide Synthesis[a]



[a] Conditions: (a) normal synthesis; (b) 30% NH_3, 55 °C, 16 h. B = A, C, G, or T.

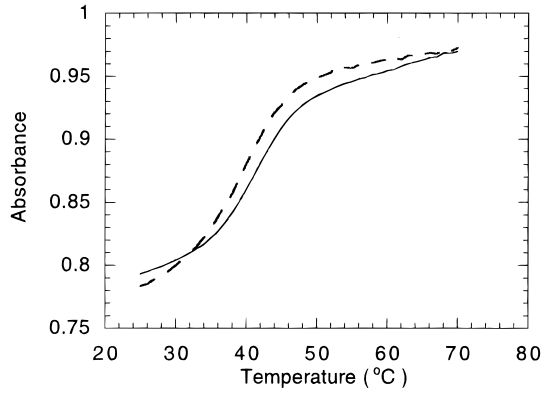


Figure 1. Melting curve profiles for Ru(diimine)$_3^{2+}$-labeled (**7·15**; dashed line) and unlabeled oligodeoxynucleotides (**11·15**; solid line).

Table 2. Melting Temperatures for Ru(diimine)$_3^{2+}$-Labeled and Unlabeled Oligodeoxynucleotides

duplex	T_m (°C)	duplex	T_m (°C)
11·15	42	**10·18**	53
7·15	39	**14·18**	51

experiments.[40] CD spectra of the unlabeled (**11·15**) and Ru(diimine)$_3^{2+}$-labeled oligodeoxynucleotides (**7·15**) are similar, and the characteristic spectral features for B-DNA are present (Figure 2). Similar to when labeling other sites in an oligodeoxynucleotide,[33,35] attaching a Ru(diimine)$_3^{2+}$ to the 5′-terminal ribose of an oligodeoxynucleotide does not substantially alter the melting temperature nor the CD spectrum.

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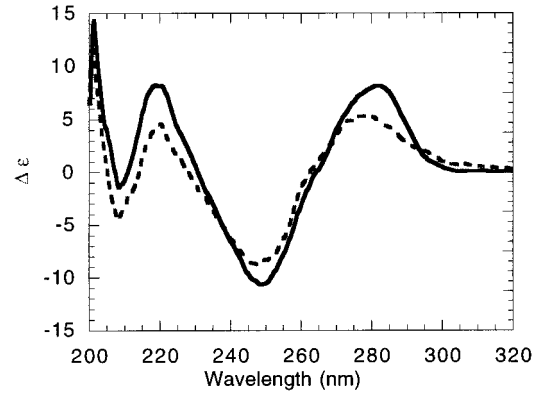

Figure 2. CD spectra of unlabeled (**11·15**; solid line) and Ru(diimine)$_3^{2+}$-labeled oligodeoxynucleotide (**7·15**; dashed line) duplexes.

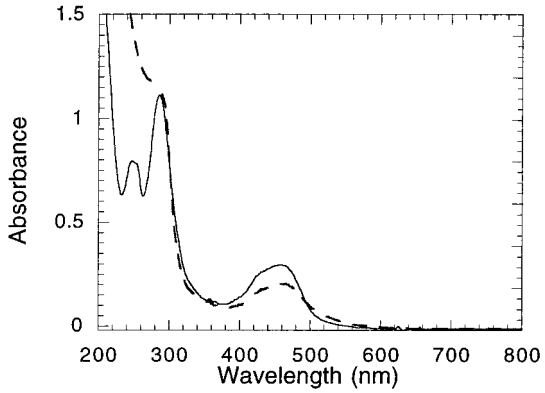

Figure 3. Electronic absorption of Ru(diimine)$_3^{2+}$−thymidine, **5** (solid line), and Ru(diimine)$_3^{2+}$-labeled single oligodeoxynucleotide strand **8** (dashed line) in aqueous solution.

The Ru(diimine)$_3^{2+}$-derivatized thymidine complex, **5**, exhibits the characteristic metal-to-ligand charge-transfer band (^{1}MLCT-^{1}A$_1$), centered at 450 nm in the absorption spectrum, analogous to that of Ru(bpy)$_3^{2+}$ (Figure 3). At higher energy the $\pi-\pi^*$ transitions of thymidine and bipyridine are present at 260 and 280 nm, respectively. Excitation of the MLCT band at 450 nm produces an emission centered at 666 nm in phosphate buffer (see Figure 5), slightly red-shifted from Ru(bpy)$_3^{2+}$ (625 nm). The emission lifetime of **5** is 430 ns under the same conditions. These absorption and emission results are also consistent with similar Ru(diimine)$_3^{2+}$ complexes designed for labeling the 5-terminal phosphate or the nucleobase of an oligodeoxynucleotide.[35,36]

To gain further insight into the excited-state electronic structure of **5**, time-resolved step-scan FTIR spectroscopy (S^2-FTIR TRS) with 10 ns time resolution probed the excited state of **5** in CD_3CN at room temperature.[41,42] The ground- and excited-state infrared $\bar{\nu}$(CO) band energies for **5** are 1679 and 1647 cm^{-1}, respectively, as shown in Figure 4. This substantial negative shift in $\bar{\nu}$ of −31 cm^{-1} is similar in magnitude to other monoamide-derivatized trisbipyridine complexes such as those

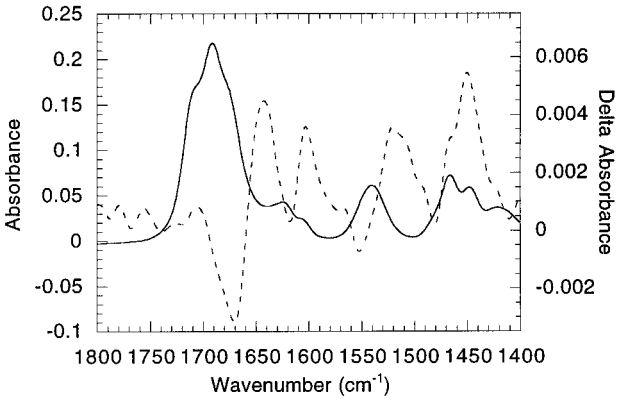

Figure 4. FT-IR ground-state (solid line) and laser-induced (dashed line) ΔA spectra in CD_3CN of **5**.

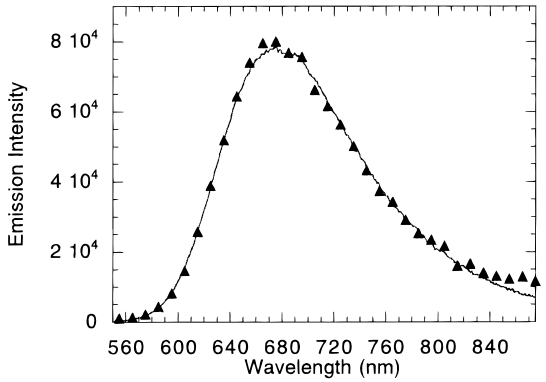

Figure 5. Emission spectra of **5** (solid triangles) and ruthenium-labeled duplex **7·15** (solid line) in phosphate buffer (10^{-6} M chromophore and DNA concentrations for the emission experiments). Corrected emission spectra recorded at 298 K (excitation at 450 nm).

containing a propargylamide and ethanolamide.[35,36] The frequency shift to *lower* energy for $\bar{\nu}$(CO) indicates that the MLCT excited-state electron resides on the asymmetrically substituted bipyridine and that significant C═O character is present in the lowest π^* excited state. The relatively large shift in $\bar{\nu}$(CO) suggests considerable metal−ligand polarization of the MLCT excited state, with the receiving orbital localized primarily on the amide-substituted pyridine ring. The metal dπ−ligand π^* orbital overlap is also substantial in this complex. Finally, these data are in agreement with previous work on related asymmetric and symmetric amide- and ester-substituted analogues of $Ru(bpy)_3^{2+}$, indicating that on the nanosecond time scale the excited-state electron is localized on the modified bipyridine rather than delocalized over all three ligands or exchanging between the ligands.[41−44]

Once **5** is incorporated in the oligodeoxynucleotide, **7**, the emission maximum shifts to higher energy from 666 to 677 nm. The emission maximum is essentially unchanged after hybridization, and the labeled duplex, **7·15**, emission is centered at 674 nm (Figure 5). The emission lifetime of this ruthenium-labeled oligodeoxynucleotide, **7**, is 572 ns, and that of the modified oligodeoxynucleotide duplex, **7·15**, is 586 ns at 25 °C in phosphate buffer.[45] Other $Ru(diimine)_3^{2+}$-modified DNA duplexes also only show small changes in the lifetime between the single strand and duplex.[35] The $Ru(diimine)_3^{2+}$ excited state is not quenched in the presence of DNA, and this observation is consistent with the redox potentials of A, C, G, and T.[46] The lack of a significant change in the emission maximum or lifetime upon hybridization indicates that the local environment or the electronic structure of the $Ru(diimine)_3^{2+}$ complex is not altered.

Conclusion

In summary, a facile and automated solid-phase procedure for site-specific labeling of oligodeoxynucleotides at the carbohydrate backbone with a substitutionally inert, coordinatively saturated metal complex is reported. The synthesis and spectroscopic properties of this novel $Ru(bpy)_3^{2+}$-derivatized thymidine are described. Time-resolved S^2FTIR experiments support the assignment of the excited state on the bipyridine covalently linked to the 5′-amino-5′-deoxythymidine. Incorporation of this ruthenium−thymidine in an oligodeoxynucleotide does not disrupt the B-form duplex. The favorable photophysical properties of the $Ru(bpy)_3^{2+}$ complex are retained when the complex is covalently attached to DNA. These results further demonstrate the generality of the metallophosphoramidite approach for labeling oligodeoxynucleotides, and the ease with which metal complex probes can be covalently attached to various sites (nucleobase, phosphate, or ribose) in DNA.

Experimental Section

Abbreviations: bpy = 2,2′-bipyridine; 4-m-4′-ca-bpy = 4-methyl-2,2′-bipyridine-4′-carboxylic acid; 4-m-4′-cam-bpy = 4-methyl-2,2′-bipyridine-4′-carboxyamide; DIPEA = *N*,*N*-diisopropylethylamine; CDI = carbonyldiimidozole; DMT = dimethoxytrityl; MLCT = metal-to-ligand charge transfer.

All solvents were dried and distilled prior to use. Absorption spectra were measured on a Hewlett-Packard 8452 diode array spectrometer. Emission spectra were recorded on a Spex Fluorolog-2 emission spectrometer. CD spectra were recorded on a JASCO J-710 spectropolarimeter. Reversed-phase (RP) HPLC was performed on a Rainin HPLC instrument with a C18 column monitoring at 254 and/or 450 nm. NMR spectra were recorded on a Varian Invoa 400 MHz spectrometer.

Syntheses. 5′-*O*-Methanesulfonylthymidine, 2.[37] Thymidine **1** (2.90 g, 12.0 mmol) was dissolved in 10 mL of dry pyridine and cooled to −10 °C. Next, mesyl chloride (14 mmol) was added dropwise over a period of 20 min. The reaction mixture was maintained at 0° C for 12 h. The next day, 10 mL of methanol was added to quench the reaction, and the solvents were evaporated via high vacuum. The resulting crude product was checked by TLC and purified by column chromatagraphy. A white powdered solid, **2**, was obtained (2.95 g, 77% yield). ^{1}H NMR (DMSO): δ 1.78 (s, 3H, 5-methyl), 2.08−2.22 (m, 2H, C2′), 3.22 (s, 3H, mesyl), 3.98 (q, 1H, C4′), 4.28 (m, 1H, C3′), 4.40 (m, 2H, C5′), 5.50 (s, 1H, 3′-OH), 6.22 (t, 1H, C1′), 7.48 (s, 1H, C6), 11.25 (s, 1H, N3). MS (FAB): calcd MW 320, found M + H 321.

5′-Azido-5′-deoxythymidine, 3. A solution of **2** (1.84 g, 5.75 mmol) in 15 mL of DMF containing lithium azide (1.60 g, 32.6 mmol) was stirred at 90 °C under nitrogen. After 3 h, the reaction was stopped, and the mixture was cooled to room temperature, and then poured into 600 mL of ice−water. The resulting white precipitate was obtained via filtration. Column chromatagraphy yielded **3** (1.12 g, 73% yield). ^{1}H NMR (DMSO): δ 1.78 (s, 3H, 5-methyl), 2.08−2.22 (m, 2H, C2′), 3.57 (d, 2H, C5′), 3.85 (q, 1H, C4′), 4.20 (m, 1H, C3′), 5.50 (s, 1H, 3′-OH), 6.22 (t, 1H, C1′), 7.48 (s, 1H, C6), 11.25 (s, 1H, N3). MS (FAB): calcd MW 267, found M + H 268.

5′-Amino-5′-deoxythymidine, 4. 5′-Azido-2′-deoxythymidine, **3** (1.12 g, 4.2 mmol), and triphenylphosphine (1.74 g, 6.7 mmol) were

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dissolved in 30 mL of dioxane and stirred for 3 h. A concentrated ammonia solution (15 mL) was then added to the reaction mixture. The reaction was determined to be complete by TLC 12 h later. The solvents were removed, and the resulting residue was partially dissolved in ether/petrolum ether (1:1). A solid was obtained via filtration, and was further purified by column chromatagraphy to yield **4** (0.73 g, 72% yield). ^{1}H NMR (DMSO): δ 1.78 (s, 3H, 5-methyl), 2.08−2.18 (m, 2H, C2′), 2.75 (s, 2H, amine), 3.37 (s, 2H, C5′), 3.75 (q, 1H, C4′), 4.20 (m, 1H, C3′), 5.20 (s, 1H, 3′-OH), 6.20 (t, 1H, C1′), 7.68 (s, 1H, C6). MS (FAB): calcd MW 241, found M + H 242.

4′-Methyl-2,2′-bipyridine-4-carboxylic acid was synthesized following the procedure described by Meyer and Erickson[47] (yield 77%). MS (FAB): calcd for $C_{12}H_{10}N_2O_2$ 214, found [M + H] 215. ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3); 7.15−9 (m, 6H, py).

[Bis(2,2′-bipyridine)(4′-methyl-2,2′-bipyridine-4′-carboxylic acid)ruthenium(II) Bis(hexafluorophosphate). A solution of (*cis*-dichlorobisbipyridine)ruthenium(II) (0.54 g, 1.0 mmol) in 50 mL of ethanol was stirred under N_2 for 10 min. Next, 4-methyl-2,2′-bipyridine-4′-carboxylic acid (0.26 g, 1/2 mmol) was added to the solution, and the reaction was refluxed for 5 h. The reaction was stopped, and the mixture was cooled to 25 °C before a saturated aqueous solution of $NH_4PF_6^-$ was added. The red precipitate was collected by filtration (0.87 g, 91% yield).

***N*-(5′-(5′-Deoxythymidine)-4′-methyl-2,2′-bipyridine-4′-carboxamide)bis(2,2′-bipyridine)ruthenium(II) Bis(hexafluorophosphate), 5.** A solution of Ru(bpy)$_2$(4-m-4′-ca-bpy) (0.20 g, 0.21 mmol) and CDI (0.06 g, 0.38 mmol) in 2 mL of dry DMF was stirred under nitrogen for 1 h at 25 °C. The mixture was then diluted with 3.5 mL of dry THF followed by addition of the LH-20 resin (0.09 g) to quench the excess CDI. An hour later, LH-20 was removed, and **4** (0.05 g, 0.22 mmol) was added to the reaction mixture. After being stirred for 12 h, the reaction was stopped, and the solvents were removed. Column chromatagraphy yielded a red solid, **5** (0.19 g, 80% yield). MS (HR-FAB): calcd for $C_{42}H_{39}O_5N_9F_6PRu$ 996.1759, found 996.1791. ^{1}H NMR (DMSO): δ 1.60 (d, 3H, 5-methyl), 2.00−2.22 (m, 2H, C2′), 2.5 (s, 3H, CH_3-bpy), 3.57 (m, 2H, C5′), 3.80 (q, 1H, C4′), 4.20 (m, 1H, C3′), 5.30 (d, 1H, 3′OH), 6.10 (t, 1H, C1′), 7.32−9.08 (m, 24H, bpy, C6, amide H), 11.25 (s, 1H, N_3).

***N*-(5′-(3′-Cyanoethoxydiisopropylaminophosphine-5′-deoxythymidine)-4′-methyl-2,2′-bipyridine-4′-carboxamide)bis(2,2′-bipyridine)ruthenium(II) Bis(hexafluorophosphate), 6.** 2-Cyanoethyldiisopropylchlorophosphoramidite (50 μL, 0.22 mmol) was added to a solution of **5** (0.193 g, 0.17 mmol) in dry CH_3CN (8.5 mL) containing diisopropylethylamine (0.1 mL). The reaction mixture was stirred under nitrogen for 2 h. Solvents were then removed, and the solid was rinsed with hexane. The red solid was then check by ^{31}P NMR ($CDCl_3$): δ 148.3 and 148.4 ppm observed for the diastereomers (PF_6^- multiplet at −150 ppm). TLC: >95% yield.

Oligodeoxynucleotide Syntheses. Oligodeoxynucleotide syntheses were performed on a commercial ABI 395 DNA synthesizer from the 3′- to 5′-end using standard automated DNA synthesis protocols as shown in Scheme 2 (1.0 μmol scale). A 0.1 M solution of **6** in dry acetonitrile was prepared and installed on the DNA synthesizer in a standard reagent bottle. Normal solid-phase oligodeoxynucleotide synthesis was performed. In the last step, the ruthenium-modified thymidine phosphoramidite was introduced and allowed to react with the oligodeoxynucleotide for 15 min. The Ru(bpy)$_3^{2+}$-labeled oligodeoxynucleotide was cleaved from the column and deprotected in 30% NH_4OH at 55 °C for 16 h. The ruthenium-modified oligodeoxynucleotide exhibited one peak in an HPLC trace, with a retention time greater than that of the corresponding unmodified oligodeoxynucleotide. Electrospray mass spectrometry of the metallooligodeoxynucleotide confirmed formation [e.g., **7**, electrospray (calcd 4545; found +2 (2275.33) and +3 (1516.79) ion states); **10**, calcd (5789 found +2 (2896.77) and +3 (1931.34) ion states)].

Collection and analysis of the trityl fractions during automated synthesis showed efficient phosphoramidite coupling throughout the procedure, with both the standard pyrimidine and purine nucleosides (>95%). The coupling of **6** to the oligodeoxynucleotide occurred in good yield (50%) as determined by HPLC.

HPLC Purification of the Oligodeoxynucleotides. HPLC purification of the modified oligodeoxynucleotides was accomplished on a Rainin HPLC instrument. Reversed phase chromatography was performed on a C18 column (25 cm × 4.6 mm) with acetonitrile (ACN) and 0.1 M triethylamine acetate (TEAA) as eluting solvents. A flow rate of 3 mL/min was used, and the concentration of ACN was increased from 5% to 50% over 35 min. The retention times of the modified oligodeoxynucleotides were well separated from those of the unmodified oligodeoxynucleotide products (>2 min).

Melting Curves. The stability of the duplex formed between the two complementary oligodeoxynucleotides was determined from the melting curve profiles as a function of temperature. The T_m value was determined from the first derivative. Two complementary oligodeoxynucleotide solutions were combined, and the resulting solution was heated to 80 °C for 5 min (5 mM NaH_2PO_4, 50 mM NaCl, pH 7). The sample was then allowed to cool to room temperature over 3 h. After cooling, the thermal denaturation experiment was performed using the following parameters on a HP UV−vis instrument: (a) monitoring wavelength, 260 nm, (b) temperature range, 20−70 °C, (c) temperature step, 1.0 °C, (d) averaging time constant, 15 s, (e) rate of change for the temperature step, 1 °C/min, and (f) equilibrium time, 60 s.

S^{2}FTIR. The transient data reported here were measured on a step-scan modified Bruker IFS88 spectrometer with a standard globar source and dry air purge. The sample was dissolved in CD_3CN to give an IR absorbance between 0.125 and 0.5 in a 250 mm path length cell for the amide bond analyzed. Samples were deoxygenated by sparging with argon for 60 min and were loaded into a CaF_2-window cell by syringe under argon. Data collection and analysis was performed as previously reported.[36]

Emission Spectra. Emission spectra were recorded on a Spex Fluorolog-2 emission spectrometer equipped with a 450 W Xe lamp and cooled Hammamatsu R928 photomultiplier. The recorded emission spectra were corrected for spectrometer response. Spectra were obtained without the use of polarizers. The calibration curve was obtained using a NIST-calibrated standard lamp (Optronics Laboratories, Inc. model 220M), controlled with a precision current source at 6.5 W (Optronics Laboratories, Inc. model 65) by following the procedure recommended by the manufacturer. The error in the emission maxima was 2 nm. The spectra were obtained in buffer (5 mM NaH_2PO_4, 50 mM NaCl, pH 7) at room temperature in a 1 cm quartz cell using right angle observation of emitted light.

Lifetimes. A Laser Photonics LN1000 Nitrogen Laser-LN102 dye laser (coumarin 460 dye, Exciton) was used as the irradiation source. The emission was monitored at a right angle using a Macpherson 272 monochromator and Hammamatsu R666-10 PMT. The signal was processed by a LeCroy 7200A transient digitizer interfaced with an IBM-PC. The excitation wavelength was 460 nm, and the monitoring wavelength was 640 nm. The power at the sample was 40 μJ/pulse as measured by a Molectron J3-09 power meter. The measured instrument lifetime response was 10 ns (fwhm). The acquired emission decay curves were analyzed by locally written software based on the Marquardt algorithm. Lifetimes were obtained without polarizers as well as with both perpendicular and parallel light. The error in the lifetime measurement was 25 ns. Lifetimes were obtained in phosphate buffer (5 mM NaH_2PO_4, 50 mM NaCl, pH 7) at room temperature.

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Supporting Information Available: Lifetime data on the ruthenium-labeled oligodeoxynucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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