Synthesis and Characterization of Fluorenone-, Anthraquinone-, and Phenothiazine-Labeled Oligodeoxynucleotides: 5′-Probes for DNA Redox Chemistry

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A facile and automated procedure for the synthesis of oligodeoxynucleotides possessing derivatives of 9-fluoreneone, 9,10-anthraquinone, and phenothiazine is described. The phosphoramidite approach is used to attach these redox and spectroscopic probes to the 5′-terminus of oligodeoxynucleotides in high yield (>95%). Thermal denaturation studies of labeled oligodeoxynucleotides show a slight enhancement in duplex stability relative to the unlabeled control, and circular dichroism spectra confirm B-form helical DNA structure in solution.

Introduction

Oligodeoxynucleotides possessing probes with unique spectroscopic and electrochemical properties are of interest for biophysical studies (electron transfer and radical cation migration)[1−10] and analytical applications (sequencing and DNA diagnostics).[11−17] In the analytical arena, the use of fluorescently labeled oligodeoxynucleotides in conjugation with newly developed surface modification techniques affords sensitive devices (e.g., DNA arrays) for analyzing specific DNA sequences and gene expression. Although widely used, these methods require labeling of the target DNA. Electrochemical detection of DNA, via an electron-transfer reaction, offers the benefits of detecting DNA without prior labeling of the target oligodeoxynucleotides, obtaining high-density arrays, and multiplex electrochemical sensing.[18] Successful reports using daunomycin and ferrocene labeled oligodeoxynucleotides, as well as other transition metal and organic mediators, for sensing DNA are recently described.[19−32] In addition, redox and spectroscopic probes (e.g., anthraquinone and Ru(dppz)(bpy)$_2^{2+}$) are currently used to study DNA-mediated radical cation migration in order to gain mechanistic insight into oxidative DNA damage.[1,2] These basic studies and analytical applications require an efficient, high yielding, and reproducible procedure for synthesizing oligodeoxynucleotides containing well-characterized reductive and oxidative probes. Herein we describe

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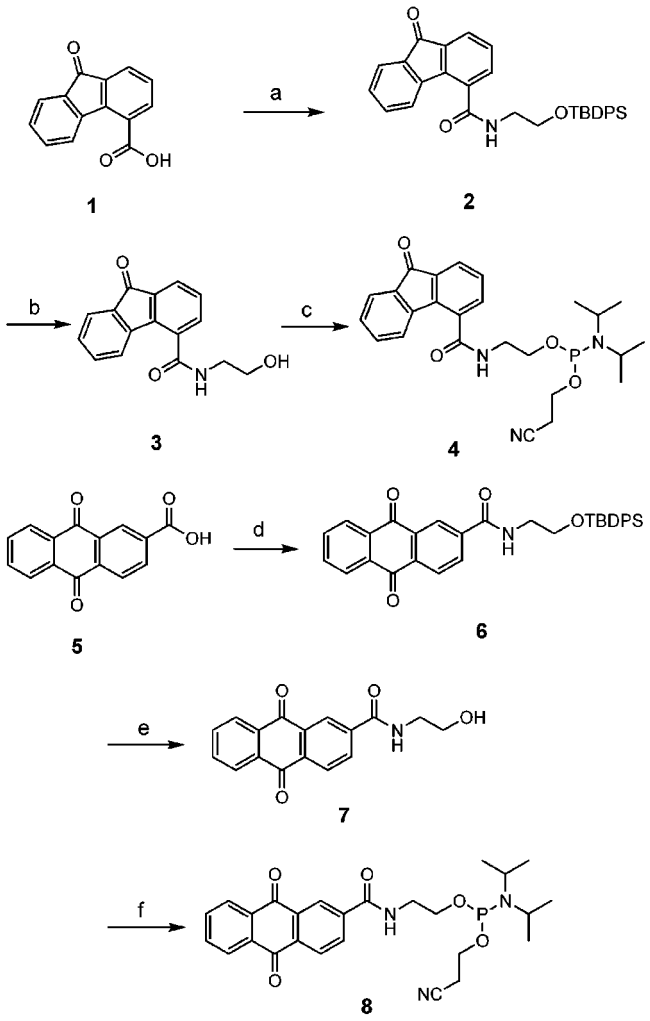
the synthesis of fluorenone, anthraquinone, and phenothiazine-derivatized phosphoramidites, and the incorporation of these three redox-active and spectroscopic probes at the 5′-terminus of oligodeoxynucleotides.

Fluorenone (FL), anthraquinone (AQ), and phenothiazine (PTZ) are useful as probes for studying DNA redox chemistry since these chromophores possess several key physical and chemical properties. First, these probes can participate in either oxidative (FL and AQ) or reductive (PTZ) electron-transfer reactions and are low-potential ground-state oxidants or reductants, respectively ($FL/FL^{\bullet-}$, −1.25 (DMF);[33] $AQ/AQ^{\bullet-}$ −0.84 (DMF);[33] $PTZ^{\bullet+}/PTZ$ 0.75 (CH_3CN)[34] V vs SCE). Second, the high-energy photoexcited states (>2.0 eV) of these compounds also enable participation in photoinduced DNA charge-transfer reactions. Third, the one-electron-reduced products of anthraquinone, $AQ^{\bullet-}$ (580 nm-DMF),[33] and fluorenone, $FL^{\bullet-}$ (540 nm-DMF),[33] as well as the oxidized product of phenothiazine, $PTZ^{\bullet+}$ (510 nm-CH_3CN),[35,36] are spectroscopically and electrochemically characterized in solution. Furthermore, effects of selected substitution on the parent compound (e.g., *N*-(2-triethylammoniumethyl)anthraquinone-2-carboxamide[37] and *N*-alkylammonium phenothiazine salts[38]) can alter the aqueous redox and spectroscopic properties of the chromophores, further demonstrating their use as mechanistic probes of DNA redox chemistry.[39]

Results and Discussion

The fluorenone and anthraquinone phosphoramidites, **4** and **8,** were synthesized in three steps (Scheme 1).[40] Protection of the free primary hydroxyl of ethanolamine with a bulky *tert*-butyldiphenylsilyl group (TBDPS) greatly increased solubility and allowed for chromatographic purification of the amide products. As shown in Scheme 1, treatment of **1** with DCC/HOBt or **5** with $(COCl)_2$/DMF (cat) followed by addition of TBDPS-protected ethanol amine in the presence of DIEA afforded amides **2** or **6**, respectively, in good yields. Both products were isolated as yellow crystalline solids after silica gel purification (hexanes/ethyl acetate). Cleavage of the silyl protecting group with TBAF followed by reaction with 2-cyanoethyl-*N*,*N*′-diisopropylaminochlorophosphine, in the presence of DIEA, yielded the phosphoramidites for automated synthesis.

Scheme 1. Synthesis of Fluorenone and Anthraquinone Phosphoramidites[a]



[a] Reagents and conditions: (a) $Cl{\cdot}H_3NCH_2CH_2OTBDPS$, DCC, HOBt, DIPEA, −5 to +25 °C, 76% yield; (b) TBA^+F^-, THF, 89% yield; (c) $ClP(iPr_2N)(OCH_2CH_2CN)$, DIPEA, CH_2Cl_2, >95% yield (TLC); (d) (i) $(COCl)_2$, DMF (cat.), CH_2Cl_2, (ii) $Cl{\cdot}H_3NCH_2CH_2OTBDPS$, CH_2Cl_2, DIPEA, −5 to +25 °C, 87% yield; (e) TBA^+F^-, THF, 65% yield; (f) $ClP(iPr_2N)(OCH_2CH_2CN)$, DIPEA, CH_2Cl_2, >95% yield (TLC).

The phenothiazine phosphoramidite **12** was prepared, as shown in Scheme 2, by first reacting PTZ with *tert*-butylbromoacetate and NaOH under phase-transfer conditions[41] to afford the 2-(phenothiazinyl) ester in 99% yield. Reduction of the ester by $LiAlH_4$ was achieved in high yield, and the alcohol, **11**, was isolated in 95% yield after Kugelrohr distillation (150−175 °C; 0.1 Torr) as a pale yellow oil. Treatment of **11** with 2-cyanoethyl-diisopropylchlorophosphoramidate yielded the PTZ-phosphoramidite **12**. Typically, the phosphoramidite reactions were quenched with methanol, checked by TLC, and precipitated with mixtures of freshly distilled ether and pentane. The phosphoramidites were then diluted to a concentration of 0.2 M with anhydrous CH_3CN, and immediately used in an automated DNA synthesizer. All three phosphoramidites (**4**, **8**, and **12**) were stable under inert atmosphere.

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(39) The anion radical of anthraquinone *N*-(2-triethylammoniumethyl)anthraquinone-2-carboxamide is centered at 605 nm and displays a small bathochromic shift relative to AQ, see ref 37. *N*-methylene-substituted phenothiazine salts display a small range (0.12 V) of redox potentials in aqueous solution, see ref 38.

(40) The phosphoramidite approach is conveniently used for labeling DNA, see refs 44 and 45. Recent examples of novel approaches to this technique can be found in the following. Khal, J. D.; Greenburg, M. M. *J. Am. Chem. Soc.* **1999**, *121*, 1597−1604. Kwiatkowski, M.; Samiotaki, M.; Lamminmaki, U.; Mukkala, V. M.; Andegren U. *Nucleic Acids Res.* **1994**, *22*, 2604−2611. Beilstein, A. E.; Tierney, M. T.; Grinstaff, M. W. *Comments Inorg. Chem.* **2000**, in press.

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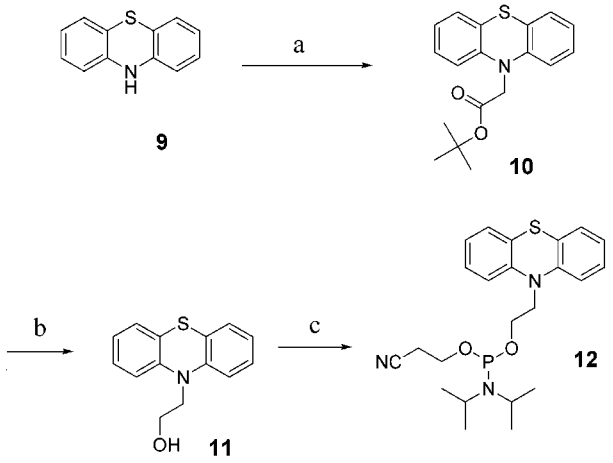
Scheme 2. Synthesis of Phenothiazine Phosphoramidites[a]



[a] Reagents and conditions: (a) *tert*-butylbromoacetate, NaOH, $NBu_4^+HSO_4^+$, methyl ethyl ketone/CH_2Cl_2 (1:1) 99%; (b) $LiAlH_4$, THF, reflux, 95% yield; (c) $ClP(iPr_2N)(OCH_2CH_2CN)$, DIEA, CH_2Cl_2, 25 °C, >95% yield (TLC).

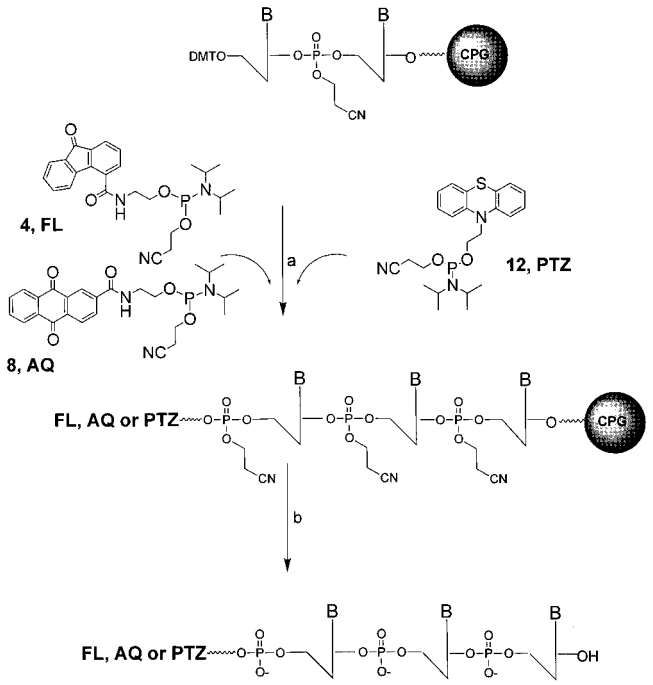
Table 1. Sequences Synthesized

compound	sequence
13	5′-**FL**-TACA-3′
14	5′-**FL**-TGCTACAAACTGTTGA-3′
15	5′-**AQ**-TCAGT-3′
16	5′-**AQ**-TGCTACAAACTGTTGA-3′
17	5′-**PTZ**-TGCCACAAACTGTTGA-3′
18	5′-**PTZ**-TGCTACAAACTGTTGA-3′
19	5′-TGCTACAAACTGTTGA-3′
20	5′-ACGATGTTTGACAACT-3′

The procedures described for synthesizing AQ, FL, and PTZ phosphoramidites are efficient and likely to be applicable for other sensitive redox probes. The AQ, FL, and PTZ precursors can be rigorously purified by column chromatography or crystallization, and the subsequent phosphoramidites reactions are clean without formation of impurities (via TLC). With regard to AQ, the method for synthesizing AQ 5′-labeled oligodeoxynucleotides via cyanoethylphosphoramidites described here is an improved procedure over the published method using methoxyphosphoramidites.[42] Silica gel chromatography was attempted with these phorphoramidites, but this led to significant decomposition. Silica gel pretreatment with TEA was also not used due to the likely formation of AQ-amine adducts.[43] All three phosphoramidites were immediately used and not stored for future use. For example, phosphoramidite **12** was found to oxidize (^{31}P NMR) when stored at −15 °C.

Solid-phase oligodeoxynucleotide syntheses were performed on an ABI 392 synthesizer,[44,45] and the redox probes were introduced at the 5′-terminus as shown in Scheme 3. All syntheses were performed at the 1.0 or 0.2 μmol scales using the standard protocol except the final coupling step proceeded for 5 min to ensure sufficient time for the phosphoramidite to react with the 5′-terminal alcohol of the oligodeoxynucleotide. Collection

Scheme 3. Solid-Phase Oligodeoxynucleotide Synthesis[a]



[a] Reagents and conditions: (a) normal synthesis; (b) FL, 0.1 M NaOH, 80% aqueous MeOH, 55 °C, 24 h; AQ, 30% NH_3, 55 °C, 16 h; PTZ, 30% NH_3, 55 °C, 16 h. B = A, C, G, or T.

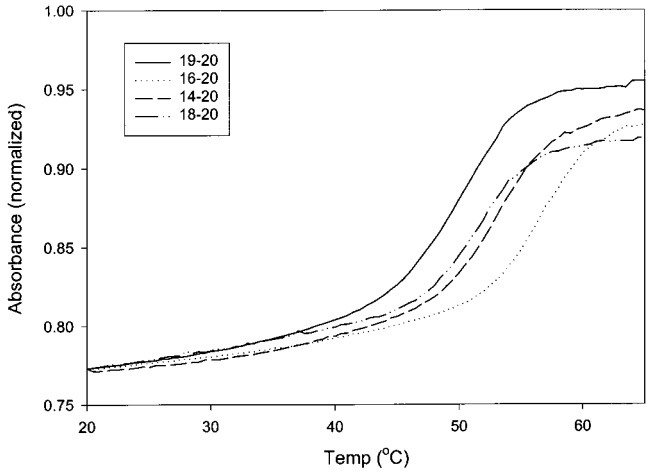


Figure 1. Melting-curve profiles for labeled and unlabeled oligodeoxynucleotides.

of the dimethoxytrityl fractions showed coupling efficiencies for the four nucleoside phosphoramidites were high (>98%), and the redox probes incorporated in >95% as determined by HPLC. Finally, cleavage from the solid support and deprotection of the nitrogenous bases and phosphate groups occurred in 0.4 M NaOH in 80% aqueous MeOH at ambient temperature for 24 h (FL) or 30% ammonium hydroxide at 55 °C for 16 h (AQ and PTZ), and purified by RP HPLC (0.1 M TEAA (aq)/CH_3CN). The labeled oligodeoxynucleotides eluted several minutes longer than the corresponding unlabeled strands. Using these protocols, a series of fluorenone-, anthraquinone-, and phenothiazine-labeled oligodeoxynucleotides were synthesized (Table 1).

As shown in Figure 1, the thermal denaturation curves for the unlabeled (**19•20**) and fluorenone- (**14•20**),

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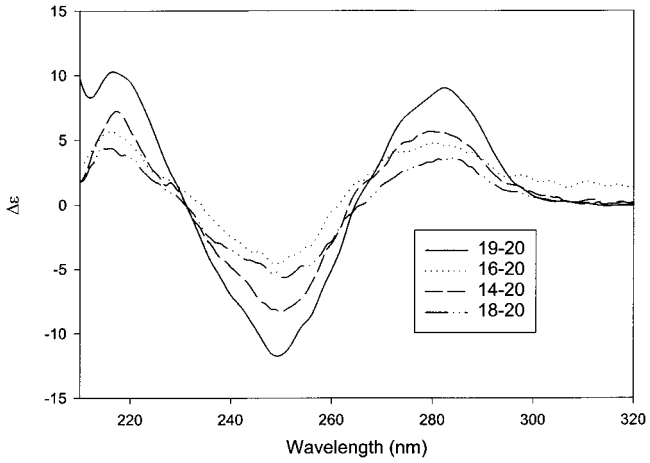

Figure 2. Circular dichroism spectra for unlabeled and labeled oligodeoxynucleotides.

anthraquinone- (**16•20**), and phenothiazine-labeled (**18•20**) 16-mer oligodeoxynucleotide duplexes are similar. An increase is observed in the melting temperature from 49 °C (**19•20**) to 56 °C (**16•20**) for the AQ-labeled strand. The relatively small increase (7 °C) in thermal stability is consistent with previously synthesized AQ-labeled oligodeoxynucleotides and likely reflects the hydrophobic redox probe "end capping" the duplex.[42] The FL DNA displays a smaller increase in T_m (5 °C) than the AQ analogue. The duplex labeled with PTZ, an electron rich compound, shows the smallest increase (3 °C) in T_m. The variance in T_m with similar-sized structures is attributed to both hydrophobic and $\pi-\pi$ electronic interactions. Such a conformation would not, however, be expected to dramatically alter DNA duplex structure. Circular dichroism (CD) spectroscopy experiments further support the conclusion that the duplex is not significantly altered. CD spectra of the unlabeled (**19•20**), FL- (**14•20**), AQ- (**16•20**), and PTZ-labeled duplexes (**18•20**) are shown in Figure 2. Their spectral features are similar, and consistent with B-form DNA.[46] This relatively small change in T_m and the similarity in the CD spectra between the unlabeled and labeled duplexes indicate that labeling the 5′-terminal of the oligodeoxynucleotide with a reductive or oxidative probe does not dramatically alter the duplex structure of DNA.

In summary, a reproducible, facile, and automated solid-phase procedure for site-specific labeling of oligodeoxynucleotides at the 5′-terminus with 9-fluorenone, 9,10-anthraquinone, and phenothiazine is reported. These redox probes require the specific handling and purification procedures as detailed herein to ensure synthesis and isolation of labeled oligodeoxynucleotides in high yield. Importantly, these results demonstrate that FL-, AQ-, or PTZ-labeled oligodeoxynucleotides form stable B-form duplexes at room temperature, possess enhanced stability relative to unlabeled duplexes, and are amenable to further studies. Besides their potential applications for electrochemical sensing of nucleic acids, these oligodeoxynucleotides containing well-defined redox and spectroscopic probes are of use for fundamental studies of DNA-mediated charge-transfer reactions.[47]

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Experimental Section

Reagents were purchased from Aldrich or Acros as highest-purity grades and used without further purification. All solvents were freshly distilled under inert atmosphere prior to use unless otherwise noted. Dichloromethane (CH_2Cl_2) and acetonitrile (CH_3CN) were distilled from CaH_2. Ethyl ether and THF were distilled from sodium/benzophenone ketyl. Anhydrous DMF was obtained from Aldrich. All reactions were performed under inert atmosphere unless otherwise noted. Absorption spectra and melting curves were measured on a diode array spectrometer. CD spectra were recorded on a Spectropolarimeter. NMR spectra were recorded on a spectrometer operating at 400 or 300 MHz. Chemical ionization mass spectra were obtained on an HP 5988A spectrometer using NH_3. Fast atom bombardment mass spectra (FABMS) were obtained using a 3-nitrobenzyl alcohol matrix. Abbreviations used in this section include DIEA (diisopropylethylamine), DCC (dicyclohexylcarbodiimide), and HOBt (hydroxybenzotriazole).

2-(*tert*-Butyldiphenylsiloxy)ethylamine. 2-(*tert*-Butyldiphenylsiloxy)ethylamine was prepared according to the literature procedure and isolated as the hydrochloride salt.[48]

***N*-(2-(*tert*-Butyldiphenylsiloxy)ethyl)-9-fluoreneone-4-carboxamide, 2.** To a solution of 9-fluorenone-4-carboxylic acid (2.216 g; 9.883 mmol) in DMF (100 mL) were added 2-(*tert*-butyldiphenylsiloxy)ethylamine hydrochloride (3.653 g; 10.89 mmol) and HOBt (1.345 g; 9.953 mmol). The mixture was cooled to −5 °C, and DIEA (2.07 mL; 11.88 mmol) was added followed by DCC (2.449 g; 11.87 mmol). The mixture was stirred for 48 h and then quenched by the addition of a small amount of 5% aqueous acetic acid. The mixture was stirred for an additional 0.5 h and filtered. The solvent was removed under vacuum at 45 °C to give a yellow residue. The residue was dissolved in ethyl acetate (100 mL) and washed with several equal portions of 5% aqueous acetic acid, saturated $NaHCO_3$, water, and brine. Column chromatography (silica gel, 1:3 ethyl acetate/hexanes) gave **2** as a yellow oil that spontaneously solidified upon standing (76%). ^{1}H NMR ($CDCl_3$): 7.65 (m, 6H), 7.35 (m, 9H), 6.30 (br t, 1H), 3.90 (q, 2H), 1.03 (s, 9H). FAB−MS *m*/*z*: 506 $(M+H)^+$.

***N*-(2-Hydroxyethyl)-9-fluorenone-4-carboxamide, 3.** *N*-(2-(*tert*-butyldiphenylsiloxy)ethyl)-9-fluorenone-2-carboxamide (0.2115 g; 0.419 mmol), **2**, was dissolved in THF (10 mL) and tetrabutylammonium fluoride·xH_2O was added in small portions slowly until TLC showed complete consumption of the starting material. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with equal portions of 5% aqueous citric acid, Na_2HCO_3, water, and brine. Following removal of the solvent, column chromatography (silica gel, 5% MeOH in CH_2Cl_2) gave alcohol **3** as a yellow solid (89%). ^{1}H NMR (DMSO-d_6/$CDCl_3$ 1:1): 8.6 (bt, 1H), 7.6 (d, 1H), 7.7−7.2 (m, 6H), 4.5 (br, 1H), 3.68 (t, 2H), 3.5 (t, 2H). FAB−HRMS *m*/*z*: $(M+H)^+$calcd for $C_{16}H_{14}O_3N$, 268.0974; found, 268.0974.

***N*-(2-(*tert*-Butyldiphenylsiloxy)ethyl)-9,10-anthraquinone-2-carboxamide, 6.** A 0.777 g portion of 9,10-anthraquinone-2-carboxylic acid (3.079 mmol) was suspended in CH_2Cl_2 (20 mL). Oxallyl chloride (1.34 mL; 0.154 mmol) was added followed by DMF (1 drop). Immediate formation of gas was observed and the reaction was stirred until a yellow homogeneous solution was observed. The mixture was stirred an additional 1 h. Next, the volatiles were removed under high vacuum. The yellow residue was dissolved in CH_2Cl_2 (25 mL) and cooled to −5 °C. 2-(*tert*-Butyldiphenylsiloxy)ethylamine hydrochloride (1.137 g; 3.386 mmol) was added followed by DIEA (1.81 mL; 10.14 mmol). Next, the mixture was diluted with CH_2Cl_2 (50 mL) and washed with equal portions of water, 10% aqueous $NaCO_3$, water, and brine. Removal of the solvents followed by column chromatography (silica gel, 1% EtOH in $CHCl_3$) gave **6** as a yellow solid (87%). ^{1}H NMR ($CDCl_3$): 8.56

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(d, 1H), 8.35 (m, 3H), 8.15 (dd, 1H), 7.82 (m, 2H), 7.70 (m, 1H), 7.63 (m, 4H), 7.40 (m, 6H), 6.68 (bt, 1H), 3.89 (t, 2H), 3.64 (q, 2H), 1.08 (s, 9H). FAB−MS *m*/*z*: $(M)^-$ 533.1.

***N*-(2-Hydroxyethyl)-9,10-anthraquinone-2-carboxamide, 7.** *N*-(2-(*tert*-Butyldiphenylsiloxy)ethyl)-9,10-anthraquinone-2-carboxamide (1.416 g; 2.653 mmol), **6**, was dissolved in THF (50 mL) and tetrabutylammonium fluoride·xH_2O was added in small portions slowly until TLC showed complete consumption of the starting material. The mixture was diluted with ether and a small amount of aqueous acetic acid (10 mL) was added. Yellow crystals then formed. This solid was filtered, washed with water and isopropyl alcohol, and extensively dried under vacuum to give **7** (65%). ^{1}H NMR (DMSO-d_6/$CDCl_3$ 1:1): 8.87 (bt, 1H), 8.57 (s, 1H), 8.28 (m, 1H), 8.18 (m,3H), 7.88 (m, 2H), 4.76 (t, 1H), 3.51 (q, 2H), 3.34 (q, 2H). FAB−HRMS *m*/*z*: $(M+H)^+$ calcd for $C_{17}H_{14}NO_4$, 296.0923; found, 296.0919.

***tert*-Butyl 2-(10-phenothiazinyl)acetate, 10.** Phenothiazine (2.30 g; 0.012 mol), *tert*-butylbromoacetate (0.12 mol), and tetrabutylammonium hydrogen sulfate (0.40 g; 0.0012 mol) were dissolved in CH_2Cl_2/methyl ethyl ketone (1:1, 80 mL). A 50% aqueous NaOH solution (40 mL) was added with vigorous stirring and a green color immediately developed. The mixture was stirred overnight. Next, the mixture was diluted with CH_2Cl_2 (100 mL), washed with several equal portions of water followed by brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure. Column chromatography (silica gel, 1:3−1:2 CH_2Cl_2/petroleum ether) gave **10** as a white solid (93%). ^{1}H NMR (CD_2Cl_2): 7.14 (m, 4H), 7.08 (m, 2H), 6.61 (d, 2H), 4.41 (s, 2H) 1.48 (s, 9H). CI−MS (NH_3) *m*/*z*: $(M+H)^+$ 314 .

10-(2-Hydroxyethyl)phenothiazine, 11. Ester **10** (3.30 g; 0.010 mol) was dissolved in THF (30 mL). This solution was transferred via cannula to a gently stirring solution of $LiAlH_4$ in ethylene glycol dimethyl ether (211 mL; 0.5 M soln) cooled to −78 °C. The mixture was allowed to slowly warm to room temperature and then heated to reflux for 3 h. The mixture was cooled and quenched by the careful addition of 1 M aqueous HCl (500 mL). The solution was extracted with ether (4 × 150 mL). The ether extract was then washed with brine (3 × 200 mL) and dried (Na_2SO_4). Removal of the solvent gave an oil that was distilled in a Kugelrohr apparatus (120−150 °C; 0.05 Torr) to give **11** as a pale yellow oil (96%). ^{1}H NMR (CD_2Cl_2): 7.14 (m, 4H), 7.08 (m, 2H), 6.61 (d, 2H), 1.9 (br, 1H). FAB−HRMS *m*/*z*: $(M)^+$ calcd for $C_{14}H_{13}NOS$, 243.0722; found, 243.0726.

General Procedure for the Preparation of Phosphoramidites 4, 8, and 12. To a suspension of alcohol **3**, **7**, and **11** (1 equiv) in CH_3CN or CH_2Cl_2 was added DIEA (5 equiv). Next, 2-cyanoethyldiisopropylchlorophosphoramidite (1.1 equiv) was added slowly, dropwise, to the gently stirring mixture. The mixture slowly became homogeneous and was allowed to stir until TLC showed complete conversion of the starting material. MeOH was added (2 drops) and most of the solvent removed under reduced pressure. Degassed ether/hexanes (1:1) was added causing the separation of the product as an oil. The mixture was decanted and the residue washed with additional degassed ether/hexanes. The residue was dried extensively under vacuum. A sample was checked by ^{31}P NMR, and the remainder dissolved to a concentration of 0.1 M (based on starting material) with acetonitrile and loaded on an automated DNA synthesizer. These operations were carried out under inert atmosphere with minimal exposure to light.

***N*-(2-(*β*-Cyanoethyl-*N*′,*N*″-diisopropylphosphino)ethyl)-9-fluorenone-4-carboxamide, 4.** Phosphoramidite **4** was obtained from *N*-(2-hydroxyethyl)-9-fluoreneone-2-carboxamide (0.124 g; 0.466 mmol) in CH_2Cl_2 (15 mL). ^{31}P NMR: 148.74.

***N*-(2-(*β*-Cyanoethyl-*N*′,*N*″-diisopropylphosphino)ethyl)-9,10-anthraquinone-2-carboxamide, 8.** Phosphoramidites **8** was obtained from *N*-(2-hydroxyethyl)-9,10-anthraquinone-2-carboxamide (0.129 g; 0.437 mmol) in CH_3CN (15 mL). ^{31}P NMR: 148.73.

10-(2-(*β*-Cyanoethyl-*N*′,*N*″-diisopropylphosphino)ethyl)phenothiazine, 12. Phosphoramidite **12** was obtained from 10-(2-hydroxyethyl)phenothiazine (0.224 g; 0.920 mmol) in CH_2Cl_2 (15 mL). ^{31}P NMR: 148.69 ppm.

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 3 (1.0 mmol scale). A 0.1 M solution of **4**, **8**, or **12** 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 phosphoramidite was introduced and allowed to react with the oligodeoxynucleotide for 5 min. Redox-labeled oligodeoxynucleotides were deprotected in 0.1 M NaOH 80%, aqueous MeOH at 55 °C for 24 h (FL) or 30% ammonium hydroxide at 55 °C for 16 h and purified by HPLC (AQ and PTZ). The FL-, AQ-, or PTZ-labeled oligodeoxynucleotides exhibited one peak in an HPLC trace, with retention times greater than the corresponding unlabeled oligodeoxynucleotide.

HPLC Purification and Characterization of the Oligodeoxynucleotides. HPLC purification of the modified oligodeoxynucleotides was accomplished on a Rainin HPLC instrument. Reverse-phase chromatography was performed on a C18 column (25 cm × 4.6 mm) with acetonitrile (ACN) and 0.1 M aqueous 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 30% over 35 min. The retention times of the labeled oligodeoxynucleotides were well separated from the unlabeled oligodeoxynucleotide products (>2 min). ESI mass spectra confirmed formation of the desired labeled oligonucleotides.

Mass Spectra Data for the Labeled Oligodeoxynucleotides. 13: ESI calcd, 1488; found, 1487. **14**: ESI calcd, 5207; found, 5209. **15**: ESI calcd, 1835; found, 1835. **16**: ESI calcd, 5239; found, 5237. **17**: ESI calcd, 5168; found, 5170. **18**: ESI calcd, 5184; found, 5185.

Melting Curves. The stability of the duplex formed between 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 of the absorbance as a function of the temperature. Two complementary oligodeoxynucleotide solutions were combined and the solution containing the duplex oligodeoxynucleotide was heated to 90 °C for 2 min (5 mM NaH_2PO_4, 50 mM NaCl, pH = 7). The solution was then allowed to slowly cool to room temperature over several hours. The thermal denaturation experiment was performed on an HP UV−Vis using the following parameters: (a) monitoring wavelength, 260 nm, (b) temperature range, 20−70 °C, (c) temperature step, 1.0 °C, (d) rate of change for the temperature step, 1 °C/min, and (e) equilibration time, 60 s.

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Supporting Information Available: ^{1}H NMR spectra of alcohols **3, 7**, and **11**. MALDI or ESI mass spectra of labeled oligodeoxynucleotides **13**−**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

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