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Synthesis and Stability of Oligodeoxynucleotides Containing C8-Labeled 2′-Deoxyadenosine: Novel Redox Nucleobase Probes for DNA-Mediated Charge-Transfer Studies

Mark T. Tierney and Mark W. Grinstaff*

Department of Chemistry, Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina 27708

mwg@chem.duke.edu

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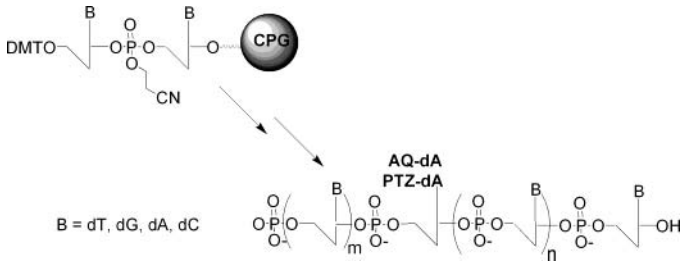
ABSTRACT



An efficient and convenient synthetic strategy to redox-labeled C8-derivatives of 2′-deoxyadenosine is described. The Pd(0) cross-coupling chemistry is amenable to *both* oxidative and reductive redox probes. The corresponding phosphoramidites of phenothiazine and anthraquinone nucleosides are amenable to automated DNA synthesis. The resulting labeled oligodeoxynucleotide strands form stable B-form duplexes with melting temperatures and CD spectra similar to those of the unlabeled analogues.

Charge-transfer reactions are pervasive throughout biology and occur in oxidative phosphorylation and respiration, nitrogen fixation, and photosynthesis. A key requirement for studying, understanding, and predicting the factors that control a charge-transfer reaction is the ability to manipulate the medium between the charge donor and acceptor to ask specific questions. Ideally, this requires a "building block" synthetic approach to the system of interest. Spectroscopic studies on site-specific labeled proteins and peptides with electron donor/acceptor probes provided key data for determining the factors that effect protein-mediated electron transfer.[1] In comparison, charge transfer in DNA is less well understood, and the majority of donors/acceptors being used are covalently attached at or intercalated near the 5′- or 3′-terminus.[2] To address this current limitation, we are synthesizing novel redox-active nucleosides and oligodeoxynucleotides[3] for DNA-mediated charge-transfer studies.[4] Herein we describe the synthesis of novel phenothiazine- and anthraquinone-2′-deoxyadenosine probes and the incorporation of these redox-active purine nucleosides in oligodeoxynucleotides.

Phenothiazine (PTZ) and anthraquinone (AQ) are ideally suited for characterizing DNA charge-transfer reactions because these probes can undergo either reductive (PTZ) or oxidative (AQ) charge-transfer reactions. The one-electron oxidized products of *N*-alkyl phenothiazine, $PTZ^{\bullet+}$ (510 nm, CH_3CN[5]), and reduced product of *N*-alkyl anthraquinonecarboxamide, $AQ^{\bullet-}$ (610 nm, DMF;[6] 600 nm, CH_3CN[5]), are spectroscopically characterized in solution. These probes are

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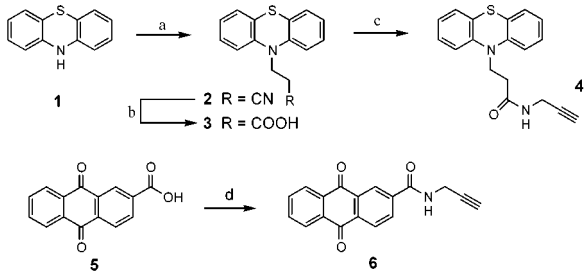
low-potential ground-state reductants or oxidants, respectively (AQ, −0.84;[7] PTZ, 0.76 V[8] vs SCE). Also, AQ labeled at the 5′-terminal phosphate or at the 2′-position of a nucleotide in DNA has been previously used for studying photoinduced oxidative damage in DNA.[2a] Previous nucleobase labeling with redox probes is limited primarily to substitution at the 5-position of deoxyuridine. A recent report, although, describes AQ attached to the *N*6-exocyclic amino group of adenine.[9] This report further substantiates the usefulness of AQ as a mechanistic charge-transfer probe. In general, reports of derivatized purines are fewer in number with only simple alkynyl (e.g., propyne), alkenyl, alkyl, and amino derivatives being described.[10]

To minimize the number of chemical transformations while recognizing the sensitivity of these redox-active chromophores to reducing and oxidizing conditions, a Pd(0)-catalyzed coupling strategy was employed. Several inorganic[3d−f,11] and organic[12] 5-labeled derivatives of uridine have been recently prepared using the Sonogashira reaction.[13] We have extended this approach to the coupling of redox-sensitive organic chromophores to the purine nucleoside 2′-deoxyadenosine. The alkynyl PTZ and AQ derivatives were prepared as shown in Scheme 1. A Michael addition of PTZ to acrylonitrile in the presence of tetrabutylammonium hydroxide produced nitrile **2**, and this procedure was a modification of an earlier method.[14] Alkaline hydrolysis yielded the carboxylic acid **3**, and subsequent coupling with propargylamine using DCC/HOBt afforded the PTZ alkyne **4**. The AQ derivative was prepared by first reacting 9,10-anthraquinone carboxylic acid with $(COCl)_2$ and DMF (cat.) to produce the acid chloride intermediate. Next, the addition of propargylamine and DIEA gave alkyne **6** in good yield.

Scheme 1. Synthesis of PTZ and AQ Alkynes



Reagents: (a) acrylonitrile, $NBu_4^+OH^-$, 87% yield; (b) NaOH, aqueous MeOH, 56% yield; (c) DCC, HOBt, DIEA, 75% yield; (d) 1) $(COCl)_2$, DMF (Cat.), CH_2Cl_2, 2) CH_2Cl_2, DIEA, propargyl amine·HCl, 92% yield.

The syntheses of the labeled 2′-deoxyadenosines are shown in Scheme 2. The purine nucleoside 8-bromo-2′-deoxyadenosine,[15] **7**, was first protected at the 5′-position with dimethoxytrityl chloride (DMT-Cl) in pyridine to give the DMT-protected nucleoside **8**. Transient protection of 3′-hydroxyl with excess TMS-Cl in pyridine followed by addition of benzoyl chloride afforded the *N*7-benzolyated intermediate. The TMS group was selectively removed in cold methanolic ammonia, and following flash chromatography, *O*5-(4,4′-dimethoxytrityl)-*N*-benzoyl-8-bromo-2′-deoxyadenosine, **9**, was obtained in high yield. The Pd(0) cross-couplings of **9** with either redox probe **4** or **6** proceeded smoothly in good yield (Scheme 2). A number of different catalysts, bases, and reaction conditions were employed to optimize these reactions.

The use of catalysts other than $Pd(P(C_6H_5)_3)_4$ (e.g., $Pd(P(C_6H_5)_3)_2Cl_2$ or $Pd(P(C_6H_5)_3)_2(OAc)_2$) in the presence of CuI yielded only insoluble solids or dark reaction mixtures with no significant product obtained. The temperature of the reaction was also critical. For the PTZ derivative **4**, coupling at 45 °C using $Pd(P(C_6H_5)_3)_4$/CuI in the presence of excess TEA occurred smoothly, with **10** obtained in 85% yield after column chromatography. However, for the anthraquinone nucleoside **14**, use of similar conditions (excess TEA)

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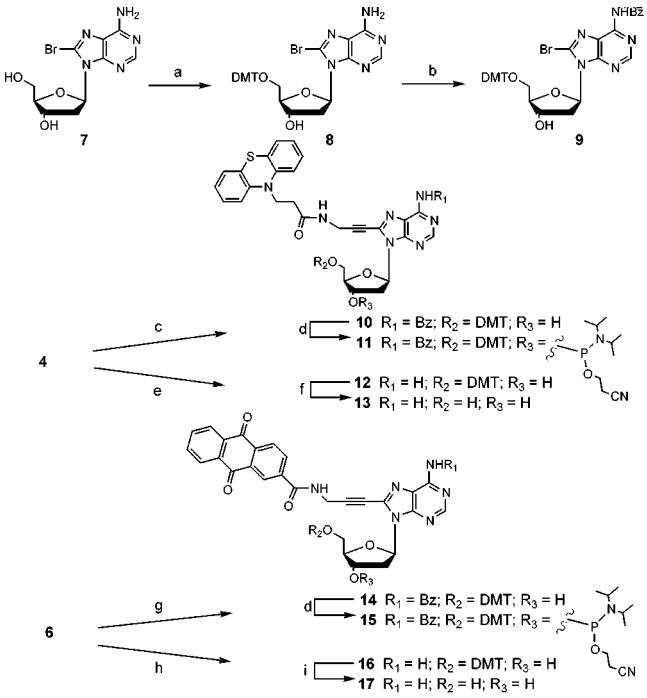
Scheme 2. Synthesis of PTZ and AQ Alkynyl-Modified Deoxyadenosines

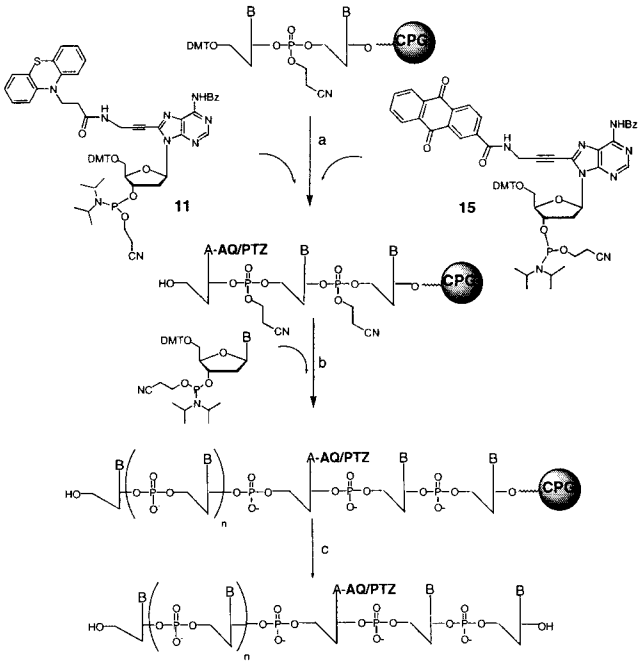


Reagents: (a) DMT-Cl, DMAP (cat), pyridine, 81% yield; (b) 1)TMS-Cl (5 eq.), benzoyl chloride (5 eq.), pyridine, 2) NH_3/MeOH, 5 °C, 81% yield; (c) **9,** $Pd(PPh_3)_4$ (0.1 eq.), CuI (0.2 eq.), TEA (excess), 45 °C, DMF, 85% yield; (d) $ClP(iPr_2N)(OCH_2CH_2CN)$ (1.1 eq.), DIEA (1.5 eq.), CH_3CN, >95% yield (TLC). (e) **8,** $Pd(PPh_3)_4$ (0.1 eq.), CuI (0.2 eq.), DIEA (1.5 eq.), DMF, 75% yield; (f) 2% Cl_3CCO_2H, CH_2Cl_2, 75% yield; (g) **9**, $Pd(PPh_3)_4$ (0.1 eq.), CuI (0.2 eq.), DIEA (1.5 eq.), 45 °C, DMF, 85% yield; (h) **8,** $Pd(PPh_3)_4$ (0.1 eq.), CuI (0.2 eq.), DIEA (1.5 eq.), DMF, 75% yield; (i) 2% Cl_3CCO_2H, CH_2Cl_2, 95% yield;

produced a dark colored reaction mixture. Although the starting material was completely consumed, **14** was obtained in low yield (<10%). Alternatively, use of 1.5 equiv of DIEA in DMF resulted in isolation of **14** in good yield. Moreover, the use of TEA-pretreated silica gel led to darkening of the pale yellow mixture, whereas no TEA pretreatment yielded significant detritylation and product decomposition. However, pretreatment of the silica gel with a 1% pyridine solution in CH_2Cl_2 allowed chromatographic purification and isolation of **14** in 85% yield with no significant detritylation observed nor discoloring of the product. Finally, the labeled *O*5-DMT-*N*7-benzoyl protected adenosines **10** and **14** were treated with 2-cyanoethylchloro-*N*,*N*′-diisopropylphosphoramidite in the presence of a slight excess of DIEA at −5 °C and slowly warmed to room temperature. Since these phosphoramidites were found to be unstable to standard laboratory conditions, precipitation under inert atmosphere was employed. Typically, the reactions were quenched with CH_3OH and precipitated using degassed $(CH_3CH_2)_2O$/petroleum ether. The resulting solids were dried extensively under high vacuum. The nucleobase-labeled phosphoramidites **11** and **15** were checked by ^{31}P NMR and then diluted to a concentration of 0.1 M with CH_3CN for use with an automated DNA synthesizer.

Automated solid-phase oligodeoxynucleotide syntheses at the 1.0 μmol scale were performed as shown in Scheme 3.[16]

Scheme 3. Oligodeoxynucleotide Synthesis



(a) extended reaction time (5 min), (b) normal synthesis, (c) 30% NH_3, 55 °C, 16 h. B = A, C, G, or T.

Collection and analysis of the DMT fractions during automated synthesis showed efficient phosphoramidite coupling throughout the procedure for the standard nucleoside couplings (>98%). Extended reaction times (5 min) were employed to ensure high coupling efficiencies for both the AQ-dA and PTZ-dA phosphoramidites (>98%). Following this protocol, a series of oligodeoxynucleotides were synthesized containing a redox probe at different positions in the oligodeoxynucleotide sequence (see Table 1). The

Table 1. Oligodeoxynucleotides Synthesized

18.	5'-TGCTACAA**A***CTGTTGA-3'
19.	5'-TGCT**A***CAAACTGTTGA-3'
20.	5'-TGCTACAA**A**$^{\#}$CTGTTGA-3'
21.	5'-TGCT**A**$^{\#}$CAAACTGTTGA-3'
22.	5'-TGCTACAAACTGTTGA-3'
23.	5'-ACGATGTTTGACAACT-3'

A*= PTZdA; **A**$^{\#}$= AQdA

oligodeoxynucleotides were purified by RP-HPLC (TEAA (aq)/CH_3CN).

To characterize the electronic properties of these novel AQ and PTZ nucleoside chromophores in aqueous solution, the synthesis of the fully unprotected nucleoside was

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performed (see Scheme 2). The UV−vis spectra (3:1 CH_3CN/H_2O) of **13** and **17** contain absorptions at λ_{max} (nm) 255 and 295 for PTZ-dA and λ_{max} 258 and 330 (sh) for AQ-dA, in agreement with previous data for 2′-deoxyadenosine, AQ, and PTZ, respectively.[8,17]

Thermal denaturation profiles for the unlabeled and labeled duplexes provide important information concerning the effect of C-8 purine substitution on duplex stability. Melting temperatures (T_m) are shown in Table 2. Only a small decrease (~3 °C) in melting temperature is observed when labeling at base 5 (**19·23** and **21·23**). When the modified dA residue is incorporated at base 8, an additional decrease (~2 °C) is observed. These relatively small decreases in T_m indicate that nucleobase labeling at the C8-position of deoxyadenosine does not dramatically alter the DNA duplex structure.[18] Furthermore, the T_m values appear to be independent of the linkage and the type of label. Circular dichroism (CD) spectroscopy further supports a well-formed duplex structure. CD spectra of **18·23**, **19·23**, **20·23**, **21·23**, and **22·23** (Figure 1) are similar, and the characteristic spectral features for B-DNA[19] are present. In summary, an efficient synthetic procedure to redox-labeled C8-derivatives of 2′-deoxyadenosine is described. The Pd(0) cross-coupling chemistry is amenable to *both* oxidative and reductive redox probes as demonstrated by the successful synthesis of PTZ and AQ derivatives. The phosphoramidites of these redox-labeled nucleosides couple efficiently during automated synthesis. Stable B-form duplexes are readily formed with the labeled oligodeoxynucleotide strands. These two novel purine probes expand the current repertoire of well-defined redox and spectroscopic probes available for studying DNA-mediated charge transfer and oxidative DNA damage.

Table 2. Melting Temperature (T_m) of Duplexes

duplex	melting temperature (T_m) ± 0.5 °C
18·23	43.5
19·23	46.0
20·23	43.5
21·23	45.5
22·23	49.0

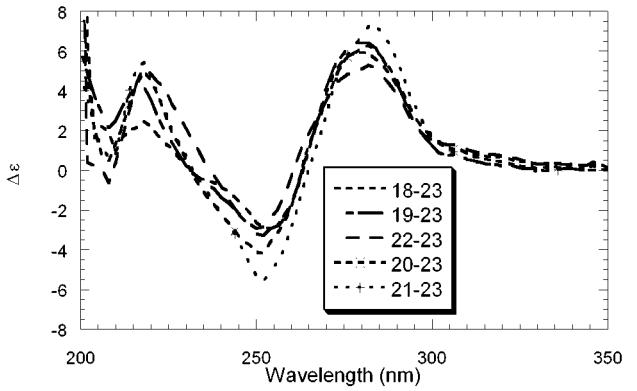

Figure 1. Circular dichroism (CD) spectra of oligodeoxynucleotide duplexes.

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Supporting Information Available: Detailed synthetic procedures. UV−vis spectra of modified nucleotides **13** and **17**. Heating and cooling profiles and first derivative traces for duplexes **18·23**, **19·23**, **20·23**, **21·23**, and **22·23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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