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Postsynthetic Guanine Arylation of DNA by Suzuki–Miyaura Cross-Coupling

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Abstract: Direct radical addition reactions at the *C*[8]-site of 2′-deoxyguanosine (dG) can afford *C*[8]-Ar-dG adducts that are produced by carcinogenic arylhydrazines, polycyclic aromatic hydrocarbons, and certain phenolic toxins. Such modified nucleobases are also highly fluorescent for sensing applications and possess useful electron transfer properties. The site-specific synthesis of oligonucleotides containing the *C*[8]-Ar-G adduct can be problematic. These lesions are sensitive to acids and oxidants that are commonly used in solid-phase DNA synthesis and are too bulky to be accepted as substrates for enzymatic synthesis by DNA polymerases. Using the Suzuki–Miyaura cross-coupling reaction, we have synthesized a number of *C*[8]-Ar-G-modified oligonucleotides (dimers, trimers, decamers, and a 15-mer) using a range of arylboronic acids. Good to excellent yields were obtained, and the reaction is insensitive to the nature of the bases flanking the convertible 8-Br-G nucleobase, as both pyrimidines and purines are tolerated. The impact of the *C*[8]-Ar-G lesion was also characterized by electrospray ionization tandem mass spectrometry, UV melting temperature analysis, circular dichroism, and fluorescence spectroscopy. The *C*[8]-Ar-G-modified oligonucleotides are expected to be useful substrates for diagnostic applications and understanding the biological impact of the *C*[8]-Ar-G lesion.

Introduction

Modified nucleic acids are of great interest because of their applications in diagnostics, catalysis, or nanotechnology and material science.[1] Modified nucleobases with new fluorescent,[2] electrical,[3] magnetic,[4] and metal ion binding[5] properties have been used to expand the scope of applications for modified nucleic acids. They may also have detrimental consequences as their formation is highly correlated with mutagenesis and cancer.[6] In this regard, our group is interested in the properties of *C*[8]-aryl (Ar)-purine adducts that are formed by phenolic toxins.[7] Following oxidative activation to phenoxyl radicals, both *O*- and *C*-linked adducts at the *C*[8]-site of 2′-deoxyguanosine (dG) are produced as a result of the ambident (*O*- vs *C*-attack) electrophilicity of phenoxyl radicals. Pentachlorophenol (PCP) generates an *O*-linked *C*[8]-dG adduct,[8] while the natural fungal carcinogen ochratoxin A forms a *C*-linked *C*[8]-dG adduct.[9] Related *C*-linked adducts, which are the focus of the present study, are shown in Figure 1. The isomeric *para* (*p*) and *ortho* (*o*) *C*-phenolic adducts (*C*-(*p*-OHPh)-8-dG and *C*-(*o*-OHPh)-8-

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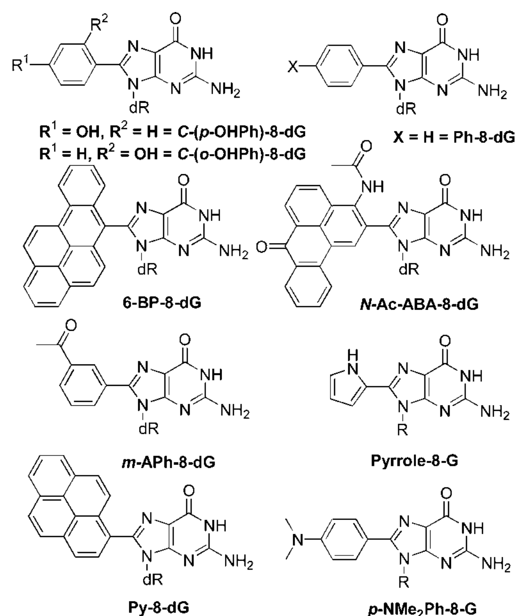

Figure 1. Structures of C^8-Ar-dG adducts derived from chemical carcinogens and structural analogues used for fluorescence sensing, G-quadruplex formation, and the study of electron transfer processes.

dG) are formed from reactions of DNA with mutagenic diazoquinones.[10] Carcinogenic arylhydrazines that produce aryl radical intermediates generate Ph-8-dG and other C^8-Ar-purine adducts bearing various *para*-substituents.[11] The polycyclic aromatic hydrocarbons (PAHs) benzo[*a*]pyrene (BP)[12] and 3-nitrobenzanthrone (NBA)[13] generate 6-BP-8-dG and *N*-Ac-ABA-8-dG, respectively. Collectively, these adducts may play a role in the biological activity of these toxins.

Some C^8-Ar-purine adducts also have desirable properties. Attachment of (*m*-acetylphenyl) to yield *m*-APh-8-dG expands the Hoogsteen edge of dG and stabilizes G-quadruplex formation that may have biomedical applications.[14] In contrast, the Sessler laboratory demonstrated that the pyrrole-8-G analogue forms a three-point Hoogsteen-type interaction with G that can disrupt G-quadruplex formation.[15] C^8-Ar-purines also act as fluorophores,[16] and phenolic adducts structurally related to *C*-(*p*-OHPh)-8-dG act as pH-sensing fluorescent probes,[17] while the isomeric *C*-(*o*-OHPh)-8-dG adduct undergoes a solvent-dependent excited-state intramolecular proton transfer (ESIPT) process that may be used to sense the local solvent environment within duplex DNA.[18] (Pyren-1-yl)-8-dG (Py-8-dG) has been touted as a duplex-sensitive optical probe and as a fluorescent donor for the investigation of charge transfer processes between DNA and peptides.[19] The C^8-dimethylanilide-G adduct *p*-NMe_2Ph-8-G has been used for the study of electron transfer in a donor−bridge−acceptor (DBA) ensemble.[20]

While C^8-Ar-purine adducts are of wide interest in the current literature, their incorporation into oligonucleotides has been problematic. Some studies have reported solid-phase DNA synthesis of oligonucleotides bearing C^8-Ar-purine adducts,[19,21−23] while others have pointed out the limitations of this strategy.[24] One of the problems may stem from their sensitivity to acid. Our studies show that C^8-Ar-dG adducts can have k_1 values for acid-catalyzed deglycosylation that are 200-fold larger than k_1 for dG.[25] Such adducts are also more prone than dG to oxidation,[26] suggesting that chemical incompatibility with synthesis conditions may present a problem for certain C^8-Ar-dG adducts.

Modified nucleic acids can also be prepared enzymatically by polymerase incorporations of functionalized nucleoside triphosphates (dNTPs).[27] This approach has been used for incorporation of 8-NH_2-dGTP[28] and the bulkier 8-[(2-imidazol-4-ylethyl)amino]-dGTP[29] for construction of novel DNAzymes. However, recent efforts from the Hocek laboratory[30] show that 8-substituted purine derivatives bearing groups larger than amino, bromo, or methyl are generally poor substrates for DNA polymerases and that 8-Ph-dATP is too bulky for the polymerase to accept as a substrate.[30b,c]

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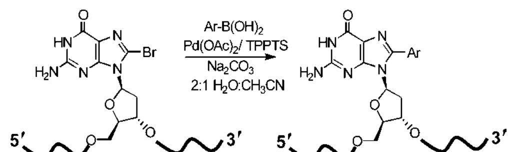
Scheme 1



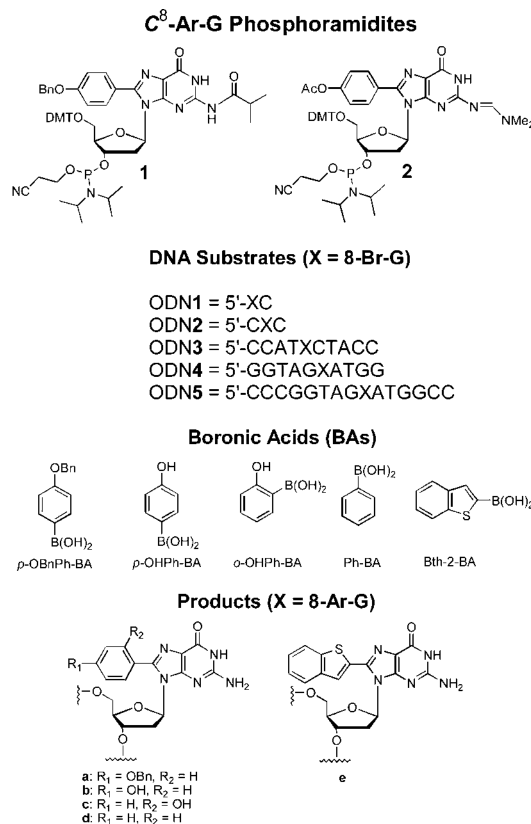
Many C^8-Ar-purine nucleoside adducts are prepared using palladium-catalyzed (Suzuki−Miyaura) cross-coupling between 8-Br-purine and the arylboronic acid.[31] This suggested that a postsynthetic strategy[32] with 8-Br-purine being the convertible nucleoside may be a general approach for the synthesis of C^8-Ar-purine-modified oligonucleotides. The Pd-catalyzed cross-coupling reaction is noted for its wide tolerance toward a variety of functionalities and applicability to extremely labile systems such as nucleoside monophosphates and triphosphates.[30] Thus, an extension of this application to oligonucleotide substrates containing 8-Br-G, as shown in Scheme 1, seemed feasible and would provide access to oligonucleotides bearing bulky acid-labile C^8-Ar-purine nucleobases. This postsynthetic strategy differs from previous Pd-catalyzed cross-coupling reactions for synthesis of uracil−alkynyl oligonucleotides,[33,34] in which the modified base is not sensitive to the solid-phase DNA synthesis reagents. In these examples the modified oligonucleotide is prepared using a semiautomated strategy that avoids lengthy phosphoramidite synthesis, but exposes the uracil−alkynyl base to acid and oxidant.[33,34]

Herein we demonstrate, for the first time, the feasibility of Suzuki−Miyaura cross-coupling for the synthesis of C^8-Ar-G-modified oligonucleotides. The ease and versatility of this protocol should allow synthesis of a wide range of C^8-Ar-purine-modified oligonucleotides that are difficult or impossible to prepare using automated solid-phase synthesis with highly functionalized C^8-Ar-purine phosphoramidites. The impact of the C^8-Ar-dG lesion on duplex stability and structure is also described and found to be a useful marker of C^8-Ar-G incorporation. The C^8-Ar-G-modified oligonucleotides are expected to act as substrates for diagnostic applications and understanding the biological impact of the C^8-Ar-dG adduct.

Results and Discussion

Building Blocks for Oligonucleotide Synthesis. The building blocks for synthesis of C^8-Ar-G-modified oligonucleotides are shown in Chart 1. The modified phosphoramidites **1** and **2** were synthesized using literature procedures (Supporting Information).[21−23] These phosphoramidites were prepared in an effort to incorporate 8-(*p*-OHPh)-dG (Figure 1) into DNA. Our synthetic efforts demonstrated the need for protection of the phenolic OH that competes with the sugar 3′-OH for reaction with the phosphitylating reagent. Thus, the *O*-benzyl (Bn)-protected derivative **1** was synthesized first and served to optimize solid-phase DNA synthesis. However, strategies for removal of the *O*-Bn protecting group (hydrogenolysis or acid[35]) proved to be incompatible with DNA, so phosphoramidite **2** containing the acetyl protecting group was prepared, in which the acetyl is removed by treatment with aqueous ammonium hydroxide after solid-phase DNA synthesis.

Chart 1. Building Blocks for the Synthesis of C^8-Ar-G Oligonucleotides



Postsynthetic arylation reactions were initially investigated on the dinucleotide ODN**1** and trinucleotide ODN**2**. This work was then extended to include the pyrimidine-rich decanucleotide ODN**3** and the purine-rich decanucleotide ODN**4**. In ODN**4** the convertible nucleoside 8-Br-G is flanked by purine bases, and it was important to establish specificity for 8-Br-G in such a sequence, as adenine bases are known to undergo direct metal-mediated arylation (although Cu(I) plays a key role in this reaction).[36] The 15-mer substrate ODN**5** served as a further test for the utility of the Pd-catalyzed postsynthetic arylation reaction. This 15-mer is an extension of the purine-rich decanucleotide ODN**4** and has greater relevance in biochemical studies, as short oligonucleotides are poor substrates for DNA polymerases and can be difficult to extend in ligation reactions due to low thermal duplex stability coupled with the effects of the adducts in reducing base pairing.[37]

Initial work also focused on the use of *p*-OBnPh-BA and *p*-OHPh-BA for reaction with the 8-Br-G-modified DNA

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substrates. This provided a direct comparison between the postsynthetic arylation reaction with automated DNA synthesis using the modified phosphoramidites **1** and **2**. The arylation reactions were then extended to include reactions of *o*-OHPh-BA, Ph-BA, and benzothiophene (Bth)-2-BA. These boronic acids (BAs) were chosen for study because the products **c** and **d** (Chart 1) are formed by chemical carcinogens[10,11] and their insertion into oligonucleotides generates substrates that can be tested for biological activity, while the nucleoside adduct of product **e** (Chart 1) has recently been synthesized[38] and shows promise as a fluorescent probe.

Solid-Phase versus Postsynthetic DNA Synthesis. To optimize the Pd-catalyzed postsynthetic reaction, the short DNA substrates ODN**1** and ODN**2** (0.1 μmol) were utilized first and reacted with 1.2 equiv of *p*-OBnPh-BA in the presence of $Pd(OAc)_2$, $P(C_6H_4\text{-}3\text{-}SO_3Na)_3$ (TPPTS), and Na_2CO_3 in a mixture of water and CH_3CN (typical conditions for C^8-Ar-purine nucleoside synthesis[31]) and were heated at 70 °C for 24 h. The expected products ODN**1_a** and ODN**2_a** from the arylation reactions were also obtained from solid-phase synthesis using phosphoramidite **1**. These reactions were carried out on a 1 μmol scale with an extended coupling time of 4 min (standard 1 min) using standard deblock (3% $CHCl_2COOH$ in CH_2Cl_2 for 40 s) and oxidant (I_2/THF/pyridine/H_2O for 1 min). Reversed-phase HPLC results for reaction with ODN**1** are shown in Figure S1 (Supporting Information). Solid-phase synthesis produced 28 OD of the dinucleotide ODN**1_a** that eluted at ~9.5 min and showed fluorescence (λ_{ex} = 280 nm, λ_{em} = 390 nm), as expected for attachment of the C^8-Ar-G base.[16] ODN**1_a** (29 OD) was also produced from Pd-catalyzed coupling of ODN**1** (48 OD) with *p*-OBnPh-BA. This afforded an isolated yield of ~60%.[39−41] Both strategies were also successful in the synthesis of the trinucleotide ODN**2_a**. The solid-phase synthesis afforded 16 OD of ODN**2_a**, while 22 OD (~41%) was isolated from Pd coupling of *p*-OBnPh-BA with 53 OD of ODN**2**.

Having established that the Pd-catalyzed arylation chemistry is compatible with DNA, efforts were made to prepare longer strands, and the initial focus was placed on the pyrimidine-rich decanucleotide substrate ODN**3**. Figure 2 shows reversed-phase HPLC results from attempted solid-phase synthesis of ODN**3_a** and Suzuki−Miyaura coupling of *p*-OBnPh-BA with ODN**3**. In this case, solid-phase synthesis using phosphoramidite **1** (Figure 2A) generated four peaks. The major products eluting at ~2 and 5.5 min lacked fluorescence for incorporation of the C^8-Ar-G base and gave singly charged ions at *m/z* 517 and 1427 that suggested formation of truncated strands. We speculate that repetitive treatment of the modified base with acid and/or oxidant (five deblock and five oxidant steps) led to the failed sequences suggested by Figure 2A. In an effort to generate the decanucleotide using solid-phase synthesis, the deblock time was reduced to 20 s, the normal oxidant was replaced with 10-camphorsulfonyloxaziridine, and in one instance 0.5 M 2-mercaptoethanol was added to NH_4OH during deprotection. However, no full-length decanucleotide ODN**3_a** was produced. We were then eager to determine the number of bases that can be placed after 8-(*p*-OBnPh)-G. These studies led to successful solid-phase synthesis of the octanucleotide 5′-CCG-8-(*p*-OBnPh)-CTACC (17 OD, Supporting Information) containing two bases after the modified base.

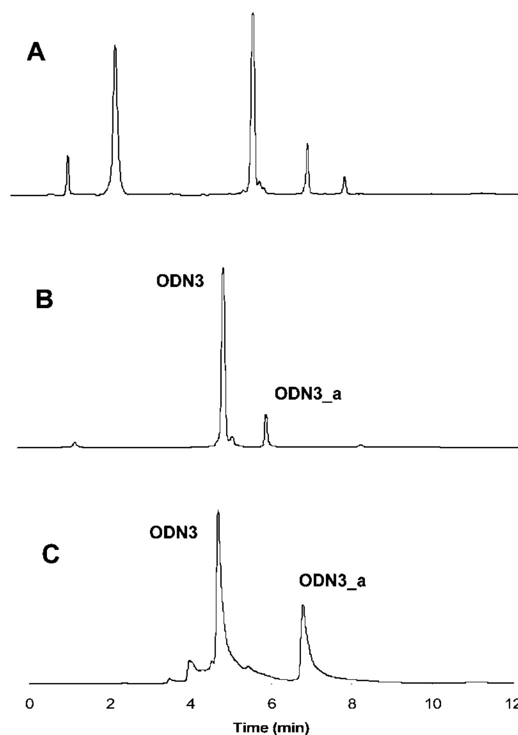

Figure 2. Reversed-phase HPLC traces of the (A) product mixture from attempted solid-phase DNA synthesis using **1**, (B) product mixture following Pd-catalyzed coupling of ODN**3** with 1.2 equiv of *p*-OBnPh-BA, and (C) product mixture following Pd-catalyzed coupling of ODN**3** with 10 equiv of *p*-OBnPh-BA using an alternative HPLC gradient protocol for slower product elution.

In contrast to the solid-phase synthesis results, the Pd-catalyzed coupling reaction between ODN**3** and *p*-OBnPh-BA (1.2 equiv) generated a single product peak at ~6 min for ODN**3_a**, as evidenced by the HPLC trace in Figure 2B that exhibited the expected fluorescence for incorporation of the C^8-Ar-G base.[16] Integration of the HPLC trace was used to establish a yield of 15% ODN**3_a**.[39] The HPLC trace shown in Figure 2C was obtained following the Pd-catalyzed reaction of ODN**3** with 10 equiv of *p*-OBnPh-BA using a slightly altered HPLC gradient protocol for slower product elution. The yield from the 10 equiv reaction was 45%, and all further Pd-catalyzed postsynthetic arylation reactions were carried out using 10 equiv of Ar-BA.

The spectral properties of the isolated product ODN**3_a** are shown in Figure 3. Its UV spectrum (Figure 3A) showed an absorbance at ~310 nm for the modified base. We have shown that C^8-Ar-dG nucleotides adopt a twisted structure with $\lambda_{max} \approx 290$ nm, while the nucleobase lacking the deoxyribose moiety is planar and absorbs at ~310 nm,[18b,25] suggesting a planar 8-(*p*-OBnPh)-G within the decanucleotide. Its ESI^- (ESI = electrospray ionization) spectrum (Figure 3B) showed the expected cluster of multiply charged peaks for the full-length decanucleotide with molecular formula $C_{108}H_{133}N_{34}O_{59}P_9$ and the mass of the most abundant isotope peak at 3129.6. An expansion of the isotopic peaks representing $[M - 2H]^{2-}$ ions is shown in

(38) Hobley, G.; Gubala, V.; Rivera-Sánchez, M. D. C.; Rivera, J. M. *Synlett* **2008**, *1510*, 1514.
(39) Yields of C^8-Ar-G-modified oligonucleotides were estimated by quantification of the isolated product by UV using ε_{260} for the unmodified oligonucleotides or from integration of the HPLC trace assuming similar absorption of the product and 8-Br-G-modified substrate. These estimations are reasonable given that log ε values of dG (4.14)[40] and 8-Br-dG (4.18)[41] are similar to that of 8-Ar-dG (~4.26).
(40) Onidas, D.; Markovitsi, D.; Marguet, S.; Sharonov, A.; Gustavsson, T. *J. Phys. Chem. B* **2002**, *106*, 11367–11374.
(41) Holmes, R. E.; Robins, R. K. *J. Am. Chem. Soc.* **1964**, *86*, 1242–1245.

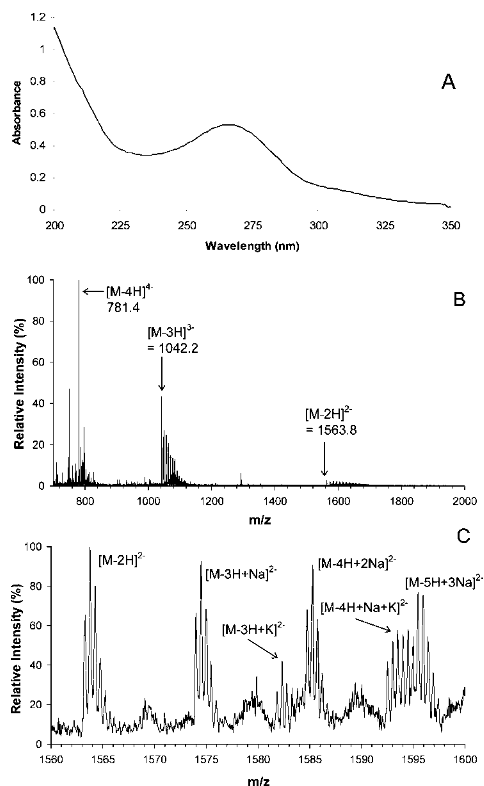

Figure 3. Spectral properties of the decanucleotide ODN **3_a** generated by Pd coupling of ODN**3** with *p*-OBnPh-BA: (A) UV spectrum (λ_{max} = 270 and 310 nm (shoulder), (B) ESI$^-$ spectrum, (C) expansion of the cluster of peaks containing $[M - 2H]^{2-} = 1563.8$.

Figure 3C. The most abundant isotopic peak contains a single ^{13}C and 107 ^{12}C atoms.

With phosphoramidite **2** (Chart 1), only the dinucleotide ODN**1_b** could be prepared using solid-phase DNA synthesis, as attempted syntheses of the trinucleotide ODN**2_b** and decanucleotide ODN**3_b** generated failed sequences (data not shown). The failed trinucleotide synthesis suggested that the labile acetylphenolic protecting group in **2** may have presented a problem and that further optimization would be required for successful synthesis. However, both ODN**2_b** and ODN**3_b** were prepared in yields of 60% and 79%, respectively, using the postsynthetic arylation route with 10 equiv of *p*-OHPh-BA (see the Supporting Information for spectral properties of modified DNAs). These results further highlighted the difficulty of the automated solid-phase DNA synthetic procedure for synthesis of C^8-Ar-G-modified oligonucleotides[24] and demonstrated the ease and applicability of the Suzuki–Miyaura cross-coupling procedure. This suggested that the postsynthetic strategy may be employed for insertion of a range of C^8-Ar-G adducts into DNA.

Scope of Pd-Catalyzed Arylation of Oligonucleotides. To test the general applicability of the Pd-catalyzed postsynthetic arylation procedure, the boronic acids shown in Chart 1 were reacted with DNA substrates ODN**2**, ODN**3**, and ODN**4**. Representative HPLC chromatograms for reactions of the purine-rich decanucleotide ODN**4** with *p*-OBnPh-BA, *o*-OHPh-BA, Ph-BA, and Bth-2-BA are shown in Figure 4. In each case the product peaks labeled in the chromatograms showed the expected fluorescence for insertion of C^8-Ar-G[16] and gave the expected *m/z* values for full-length decanucleotide (Supporting Information), confirming specificity for arylation at 8-Br-G. In Figure 4A for reaction of ODN**4** with *p*-OBnPh-BA, integration of the HPLC peaks for unreacted ODN**4** and the product ODN**4_a** afforded a yield of 71%. A similar yield (77%) was obtained for ODN**4_b** from reaction with *p*-OHPh-BA. The results for the isomeric *o*-OHPh-BA shown in Figure 4B were very pleasing given our interest in adducts derived from phenolic toxins. Virtually no peak for starting material was observed, and a yield of 87% for ODN**4_c** was determined. A remarkably clean chromatogram was obtained from reaction of ODN**4** with Ph-BA (Figure 4C) with almost complete conversion into the ODN**4_d** product (97%). In contrast, reaction of Bth-2-BA with ODN**4** (Figure 4D) showed a significant amount of unreacted ODN**4** and gave the lowest yield of 39% for ODN**4_e**. The variability in yield was not surprising given that arylation of halonucleosides with TPPTS/$Pd(OAc)_2$ range from 60% to 99%.[31] The results with ODN**4** demonstrated the specificity of the Ar-BAs for the convertible nucleoside 8-Br-G when flanked by purine bases.

As a further test of the Pd-catalyzed arylation reaction, the longer 15-mer substrate ODN**5** was reacted with the parent Ph-BA. Figure S2 (Supporting Information) shows HPLC chromatograms of the product mixture following Suzuki–Miyaura cross-coupling of ODN**5** with 10 equiv of Ph-BA. In Figure S2A, the bottom trace shows UV detection at 258 nm, while

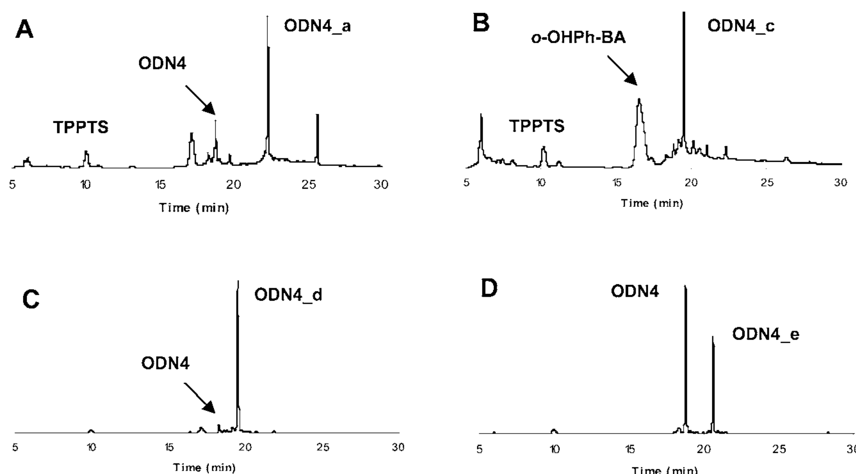

Figure 4. Reversed-phase HPLC traces of product mixtures following Pd-catalyzed coupling of ODN**4** with 10 equiv of Ar-BA: (A) ODN**4** + *p*-OBnPh-BA, (B) ODN**4** + *o*-OHPh-BA, (C) ODN**4** + Ph-BA, (D) ODN**4** + Bth-2-BA.

Table 1. Yields and ESI⁻ MS Analysis of Pd-Catalyzed Postsynthetic DNA Synthesis of C^8-Ar-G-Modified Oligonucleotides[a]

DNA	Ar-BA (amt, equiv)	product formula	yield (%)	calcd mass[d]	exptl m/z (ESI⁻)[e]	exptl mass[d]
ODN**1**	*p*-OBnPh- (1.2)	$C_{32}H_{35}N_8O_{11}P$	60[b]	738.2	$[M - H]^- = 737.2$	738.2
ODN**2**	*p*-OBnPh- (1.2)	$C_{41}H_{47}N_{11}O_{17}P_2$	41[b]	1027.3	$[M - H]^- = 1026.1$	1027.1
ODN**3**	*p*-OBnPh- (1.2)	$C_{108}H_{133}N_{34}O_{59}P_9$	15[c]	3129.6	$[M - 2H]^{2-} = 1563.8$	3129.6
ODN**3**	*p*-OBnPh- (10)	$C_{108}H_{133}N_{34}O_{59}P_9$	45[c]	3129.6	$[M - 2H]^{2-} = 1563.3$	3129.6
ODN**4**	*p*-OBnPh- (10)	$C_{113}H_{133}N_{44}O_{59}P_9$	71[c]	3329.6	$[M - 5H]^{5-} = 664.9$	3329.5
ODN**2**	*p*-OHPh- (10)	$C_{34}H_{41}N_{11}O_{17}P_2$	60[b]	937.2	$[M - H]^- = 936.2$	937.2
ODN**3**	*p*-OHPh- (10)	$C_{101}H_{127}N_{34}O_{59}P_9$	79[b]	3039.6	$[M - 3H]^{3-} = 1012.2$	3039.6
ODN**4**	*p*-OHPh- (10)	$C_{106}H_{127}N_{44}O_{59}P_9$	77[c]	3239.6	$[M - 4H]^{4-} = 808.9$	3239.6
ODN**2**	*o*-OHPh- (10)	$C_{34}H_{41}N_{11}O_{17}P_2$	39[b]	937.2	$[M - H]^- = 936.3$	937.3
ODN**3**	*o*-OHPh- (10)	$C_{101}H_{127}N_{34}O_{59}P_9$	83[b]	3039.6	$[M - 2H]^{2-} = 1012.2$	3039.6
ODN**4**	*o*-OHPh- (10)	$C_{106}H_{127}N_{44}O_{59}P_9$	87[b]	3239.6	$[M - 4H]^{4-} = 808.9$	3239.6
ODN**2**	Ph- (10)	$C_{34}H_{41}N_{11}O_{16}P_2$	49[b]	921.2	$[M - H]^- = 920.3$	921.3
ODN**3**	Ph- (10)	$C_{101}H_{127}N_{34}O_{58}P_9$	55[b]	3023.6	$[M - 3H]^{3-} = 1006.9$	3023.7
ODN**4**	Ph- (10)	$C_{106}H_{127}N_{44}O_{58}P_9$	97[c]	3223.6	$[M - 4H]^{4-} = 804.9$	3223.6
ODN**5**	Ph- (10)	$C_{151}H_{187}N_{59}O_{88}P_{14}$	83[b]	4669.8	$[M - 5H]^{5-} = 932.95$	4669.8
ODN**2**	Bth-2- (10)	$C_{36}H_{41}N_{11}O_{16}P_2S$	47[b]	977.2	$[M - H]^- = 976.1$	977.1
ODN**3**	Bth-2- (10)	$C_{103}H_{127}N_{34}O_{58}P_9S$	78[b]	3079.5	$[M - 2H]^{2-} = 1538.7$	3079.4
ODN**4**	Bth-2- (10)	$C_{108}H_{127}N_{44}O_{58}P_9S$	39[c]	3279.6	$[M - 4H]^{4-} = 818.9$	3279.6

[a] Conditions: molar ratios Br-DNA:Ar-B(OH)$_2$ = 0.8 or 0.1, Br-DNA:TPPTS = 15, TPPTS:Pd(OAc)$_2$ = 25, and Na_2CO_3:Br-DNA = 2 for a total volume of 350 μL of 2:1 water/acetonitrile, heated under argon at 70 °C for 24 h. [b] Isolated yield derived using ε_{260} for the unmodified oligonucleotides. [c] Yield derived from integration of the HPLC trace. [d] Monoisotopic mass of most abundant isotopologue. [e] Measured *m/z* (spectra presented in the Supporting Information).

the top trace shows fluorescence detection ($\lambda_{ex} = 290$ nm, $\lambda_{em} = 390$ nm). The product ODN**5_d** eluted as a sharp peak at 19.3 min, as readily apparent in the fluorescence trace. From the UV trace, no discernible peak for unreacted ODN**5** was observed. Spiking the sample with ODN**5** gave a new sharp peak eluting at 18.7 min that did not overlap with the product peak (Figure S2B). The excess Ph-BA was noted as a broad peak at 17.2 min in both the UV and fluorescence traces. In the UV trace the broad peak eluting just before Ph-BA at 16.7 min was not identified. The fluorescence trace also showed peaks at 28−30 min that were not identified; these peaks showed no appreciable UV absorbance at 258 nm. To determine the yield of ODN**5_d**, the product peak was isolated and quantified by UV spectroscopy.[39] This provided an excellent yield of 83% for ODN**5_d**. ESI⁻ MS analysis provided an ion at $[M - 5H]^{5-} = 932.95$ corresponding to the full-length 15-mer with a monoisotopic mass of 4669.8 for the most abundant isotopologue.

The results of these studies are summarized in Table 1. Using 10 equiv of Ar-BA, the yields of the Pd-catalyzed reactions ranged from 39% to 97%. Surprisingly, some of the lowest yields were obtained using the trinucleotide substrate ODN**2** (39−60%). Variation in the nature of the bases flanking the convertible 8-Br-G nucleoside had little effect on the reaction yield. ESI⁻ MS spectra of the starting 8-Br-G substrates ODN**1**−**5** along with UV−vis and ESI⁻ MS spectra of the modified oligonucleotides are given in the Supporting Information. The MS data confirmed the identity of the product oligonucleotides and provided a check for purity, as did HPLC analysis of the final isolated product.

DNA Sequence. In addition to confirmation of oligonucleotide structure by ESI⁻ MS, it was found that ESI MS/MS was particularly useful for confirming the site of the C^8-Ar-G lesion within the oligonucleotide. Due to the lability of the C^8-Ar-G nucleobase, product ion spectra show loss of the modified base with subsequent fragmentation of the resulting abasic site to allow the site of the C^8-Ar-G adduct to be determined. This is highlighted in Figure 5, which shows the product ion spectrum of $[M - 2H]^{2-}$ from ODN**3_a** (5′-CCATG-8-(*p*-OBnPh)-CTACC). The spectrum is considerably simpler than that of the corresponding unmodified decanucleotide (not shown) and is dominated by loss of the G-8-(*p*-OBnPh) nucleobase as a neutral molecule to give the $[M - 2H - X]^{2-} = 1397.2$ ion. This base loss then promotes breakage of the 3′-C−O bond at the sugar moiety to give the singly charged fragment ions at *m/z* 1501.2 (a w_5 fragment according to the nomenclature of McLuckey et al.[42]) and *m/z* 1292.2 (an a_5-B fragment[42]). The ion at *m/z* 1292.2 still contains the sugar moiety from the modified nucleobase, but it is now present as a furan ring.[42] Other ions at *m/z* 1390.1, 1212.2, and 1083.2 are fragment ions of *m/z* 1501.2. These results are very similar to those observed previously by Zhang and Gross[43] for oligonucleotides containing abasic sites, in which complementary fragment ions (a_n and w_n) are produced at the abasic site to allow its location to be determined. In this case, the labile G-8-Ar nucleobase is lost first to initiate fragmentation of the oligonucleotide at the modified base position. This type of positional mapping, depurination followed by cleavage of the phosphodiester

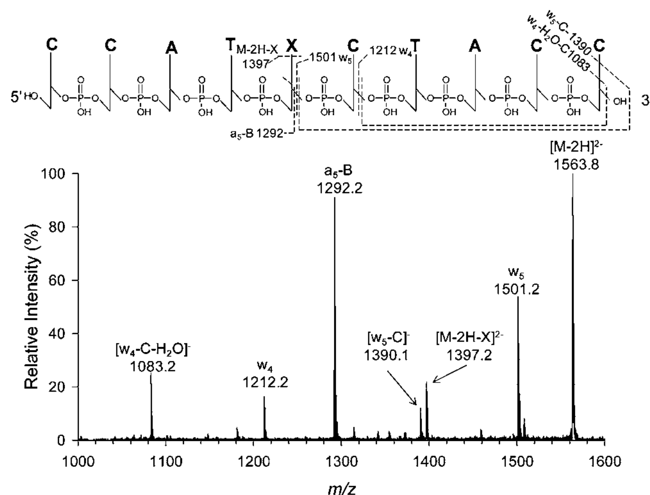

Figure 5. Product ion spectrum of ESI-produced $[M - 2H]^{2-}$ of ODN**3_a** (5′-CCATG-8-(*p*-OBnPh)-CTACC), where X = G-8-(*p*-OBnPh).

(42) (a) McLuckey, S. A.; Habibi-Goudarzi, S. *J. Am. Soc. Chem. Soc.* **1993**, *115*, 12085–12095. (b) Wu, J.; McLuckey, S. A. *Int. J. Mass Spectrom.* **2004**, *237*, 197–241.
(43) Zhang, L.-K.; Gross, M. L. *J. Am. Soc. Mass Spectrom.* **2002**, *13*, 1418–1426.

Table 2. Melting Temperatures (T_m) of Decanucleotides[a]

decanucleotide	T_m (°C)	ΔT_m (°C)
5′-CCATGCTACC	44	
CCATG-8-Br-CTACC (ODN**3**)	39	−5
CCATG-8-(*p*-OBnPhG)-CTACC (ODN**3_a**)	27	−17
CCATG-8-(*p*-OHPh)-CTACC (ODN**3_b**)	27	−17
CCATG-8-(*o*-OHPh)-CTACC (ODN**3_c**)	31	−13
CCATG-8-Ph-CTACC (ODN**3_d**)	31	−13
CCATG-8-(2-Bth)-CTACC (ODN**3_e**)	33	−11

[a] Conditions: 200 mM NaCl, 50 mM triethylamine acetate, pH 7.2, 1 mM EDTA, 0.5 A_{260}/mL of each oligonucleotide (modified and complementary strand 5′-GGTAGCATGG).

backbone, is established for carcinogens that undergo metabolic activation to form DNA adducts at the N^7 position of G and can be used to determine the chemical stability of DNA adducts.[44] That C^8-Ar-G-modified oligonucleotides behave in a similar fashion highlights the lability of the C^8-Ar-G lesion.

Duplex Stability and Structure. The modified pyrimidine-rich ODN**3** decanucleotides were hybridized to the complementary strand 5′-GGTAGCATGG, and the effect of the C^8-Ar-G adduct on the thermal stability of the DNA duplex was measured using UV melting temperature analysis (T_m) by monitoring the absorbance at 260 nm versus temperature. These data are summarized in Table 2 and demonstrate that the C^8-Ar-G adduct destabilizes the duplex considerably (11−17 °C), while the 8-Br-G adduct shows a more modest 5 °C destabilization for the decanucleotide. These findings are in accord with previous results showing bulky C^8-Ar-G lesions to significantly decrease duplex stability.[19,45]

The melting temperature analysis showed 8-(*p*-OHPh)-G to have a dramatic impact on helix stability ($\Delta T_m = -17$ °C), while ODN**3_e** bearing 8-(2-Bth)-G generated the most stable helix of the C^8-Ar-G-modified duplexes ($\Delta T_m = -11$ °C). To determine the impact of these C^8-Ar-G lesions on the duplex structure, circular dichroism (CD) measurements of decanucleotides ODN**3_b** and ODN**3_e** were carried out. Figure 6 shows CD spectra for the modified and unmodified decanucleotide duplexes. The unmodified duplex shows roughly equal positive (275 nm) and negative (244 nm) bands, with a crossover at 260 nm that is characteristic of normal B-form DNA.[46] The duplex of ODN**3_b** (Figure 6A) shows a broad positive band at 271 nm and a negative band at 224 nm that is decreased in intensity compared to the negative band at 244 nm for the unmodified duplex. These changes in CD features suggest more A-like character for the right-handed duplex of ODN**3_b**.[47] In contrast, the duplex of ODN**3_e** shows characteristics that closely resemble the normal B-form DNA of the unmodified duplex (Figure 6B). The positive induced CD signal at ~325 nm in Figure 6B (open circles) is assigned to the benzothiophene group and results from the chromophore residing in the chiral environment of the DNA duplex. These CD measurements show that the 8-(*p*-OHPh)-G lesion has a greater impact on the duplex structure than the 8-(2-Bth)-G adduct, which correlates with the impact of these lesions on the duplex stability.

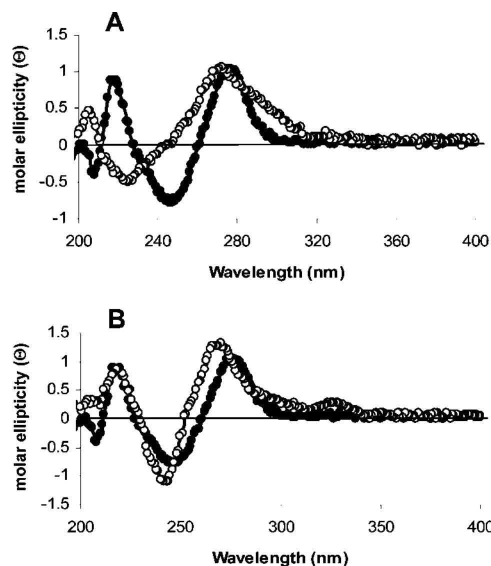

Figure 6. CD spectra of the unmodified duplex (closed circles) versus adducted duplexes (open circles) [(A) ODN**3_b** and (B) ODN**3_e**] obtained at 5 °C in 10 mM triethylamine acetate, pH 7.1, 40 mM NaCl, 0.5 A_{260}/mL of each oligonucleotide (complementary strand 5′-GGTAGCATGG).

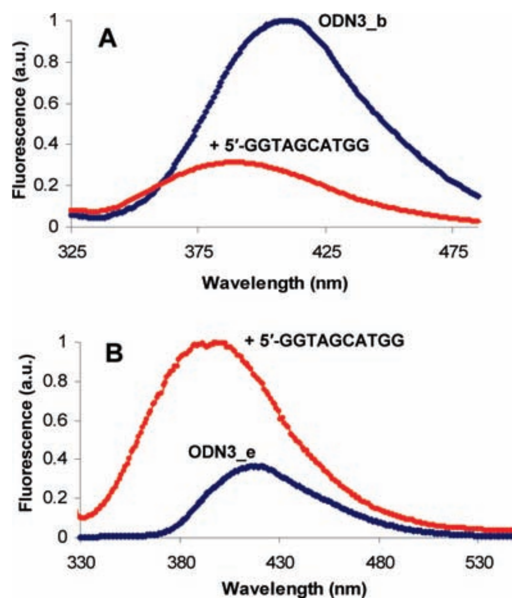

Figure 7. Normalized emission spectra of (A) ODN**3_b** (blue) + complementary strand (red) and (B) ODN**3_e** (blue) + complementary strand (red) obtained at 5 °C in 10 mM triethylamine acetate, pH 7.1, 40 mM NaCl, 0.5 A_{260}/mL of each oligonucleotide (complementary strand 5′-GGTAGCATGG).

Fluorescence emission spectroscopy was utilized to probe the environment of 8-*p*-OHPh-G and 8-(2-Bth)-G in more detail. The modified nucleosides are violet-blue fluorophores with emission maxima at ~380−420 nm.[16,17] Figure 7A shows emission spectra for ODN**3_b** in the absence and presence of the complementary strand 5′-GGTAGCATGG, while the corresponding spectra for ODN**3_e** are shown in Figure 7B. The emission of the 8-*p*-HOPh-G lesion in ODN**3_b** at 408 nm (λ_{ex} = 290 nm) is significantly quenched upon hybridization and shows a blue shift to 390 nm. When ODN**3_e** (λ_{ex} = 315 nm, λ_{em} = 420 nm) is hybridized to the complementary strand, the emission of the duplex also exhibits a blue shift (λ_{em} = 400 nm), but its intensity is enhanced 3-fold compared to the

(44) Marzilli, L. A.; Wang, D.; Kobertz, W. R.; Essigmann, J. M.; Vouros, P. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 676–682.
(45) (a) Elmquist, C. E.; Stover, J. S.; Wang, Z.; Rizzo, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 11189–11201. (b) Takamura-Enya, T.; Ishikawa, S.; Mochizuki, M.; Wakabayashi, K. *Chem. Res. Toxicol.* **2006**, *19*, 770–778.
(46) Gray, D. M.; Ratliff, R. L.; Vaughan, M. R. *Methods Enzymol.* **1992**, *211*, 389–406.
(47) (a) Gray, D. M.; Liu, J.-J.; Ratliff, R. L.; Allen, F. S. *Biopolymers* **1981**, *20*, 1337–1382. (b) Clark, C. L.; Cecil, P. K.; Singh, D.; Gray, D. M. *Nucleic Acids Res.* **1997**, *25*, 4098–4105.

emission of the single-strand ODN**3_e**. These studies show that C^8-Ar-G lesions are sensitive to the DNA microenvironment. The ability of ODN**3_e** to report hybridization with significantly increased emission, coupled with its relative ease of preparation through postsynthetic arylation, suggests wide use of the Suzuki–Miyaura methodology for generation of fluorescent DNAs with diagnostic applications.

Conclusions

We have utilized Suzuki–Miyaura cross-coupling for the site-specific synthesis of modified oligonucleotides containing the *C*-linked C^8-Ar-G lesion. The significance of this work stems from the fact that certain C^8-Ar-dG adducts are sensitive to acids and oxidants, making solid-phase DNA synthesis using highly functionalized C^8-Ar-dG phosphoramidites problematic. These adducts are also too bulky to be used as substrates for DNA polymerases, precluding an enzymatic strategy for their synthesis. Suzuki–Miyaura cross-coupling avoids the use of acids and oxidants, and the chemistry is compatible with DNA. Our studies demonstrate that good to excellent yields of C^8-Ar-G-modified decanucleotide product are obtained and that the reaction in insensitive to the nature of the bases flanking the convertible 8-Br-G nucleobase. The reaction can also be carried out with 15-mer substrates that are more relevant for molecular biology studies, including primer-extension assays using DNA polymerases. Our studies also highlight the use of ESI MS/MS to confirm the site of the C^8-Ar-G lesion within the oligonucleotides and demonstrate the potential utility of the C^8-Ar-G nucleobase for fluorescent sensing applications within duplex DNA. Our laboratory now plans to use Suzuki–Miyaura cross-coupling to generate a range of modified DNA substrates, including those resulting from postsynthetic C^8-arylation of adenine, to understand the biological and structural impact of the C^8-Ar-purine lesion.

Experimental Section

General Methods. Unless otherwise noted, commercial compounds were used as received and, in general, were purchased from Aldrich (Milwaukee, WI) with the exception of all boronic acids, which were from Frontier Scientific (Logan, UT), dG from ChemGenes (Wilmington, MA), and tris(2-sulfophenyl)phosphine trisodium salt (TPPTS) from Strem Chemical (Newburyport, MA). Pyridine was distilled from KOH and stored over 3 Å molecular sieves. Water used for buffers and spectroscopic solutions was obtained from a Milli-Q filtration system (18.2 MΩ). 8-Bromo-2′-deoxyguanosine (8-Br-dG) was prepared by treating dG with *N*-bromosuccinimide in water/acetonitrile.[31] Moisture-sensitive reactions were performed under an argon atmosphere using oven-dried (120 °C) glassware, syringes, and needles. Compounds were dried under vacuum and over P_2O_5 (12–24 h) prior to use in water-sensitive reactions. NMR spectra were recorded on a Bruker Avance 300 DPX spectrometer (^{1}H, 300.1 MHz; ^{13}C, 75.5 MHz; ^{31}P, 121.4 MHz) in DMSO-d_6 or $CDCl_3$. High-resolution mass spectra were obtained with a Micromass/Waters Global Ultima quadrupole time-of-flight (Q-TOF) instrument using electrospray ionization.

Oligonucleotide Synthesis. A MerMade 12 automatic synthesizer (BioAutomation Corp.) was used for modified oligonucleotide synthesis according to the manufacturer protocol for a 1 μmol scale DNA synthesis optimized for MerMade CPG columns (standard CPG loaded with 3′-C, 1000 Å pore size, loading 30 μmol/g). An extended coupling time of 4 min (standard 1 min) was employed using standard deblock (3% $CHCl_2COOH$ in CH_2Cl_2 for 40 s, which was reduced to 20 s in some instances) and oxidant (I_2/THF/pyridine/H_2O for 1 min). All normal phosphoramidites were obtained from Glen Research, while DNA synthesis reagents were from EMD. After the synthesis, the oligonucleotides were cleaved from the solid support and deprotected using 2 mL of 30% ammonium hydroxide solution at 55 °C for 12 h. The solutions were then concentrated under diminished pressure to ∼100 μL using a ThermoSavant DNA 120 SpeedVac at medium drying rate. The solutions were then purified using an Agilent 1200 series HPLC instrument equipped with an autosampler, an autocollector, a diode array detector (monitored at 258 nm), and a fluorescence detector (λ_{ex} = 290 nm; λ_{em} = 390 nm). Separation was carried out using a Phenomenox Clarity 3 μm Oligo-RP C18 column (50 × 4.60 mm, 3 μm) and various gradients of buffer B in buffer A (buffer A, aqueous 50 mM triethylammonium acetate (TEAA), pH 7.2/acetonitrile (95:5); buffer B, aqueous 50 mM TEAA, pH 7.2/acetonitrile (25:75).

Suzuki–Miyaura Coupling Reactions with 8-Br-G-Modified Oligonucleotides. Modified oligonucleotides containing 8-Br-dG of the desired sequence and length on a 1 μmol scale were custom-made by Sigma Genosys (Canada) using standard phosphoramidites and 8-Br-dG-CE phosphoramidite purchased from Glen Research. Oligonucleotides were cleaved from the solid support and deprotected in ammonium hydroxide at room temperature for 24 h (to lessen debromination). The synthesized DMT-ON oligonucleotides were desalted, purified, and deprotected on reversed-phase cartridges and rechecked for correct mass and bromine isotopic distribution by ESI MS at Guelph (see the Supporting Information for ESI MS spectra of starting DNA substrates ODN**1**–**5**). For Suzuki–Miyaura coupling of brominated oligonucleotides (Br-DNA) (0.1 μmol), the other reaction components were initially prepared as 1000× stock solutions in deoxygenated 2:1 water/acetonitrile. Through serial dilution the reagents were added via syringe to the brominated oligonucleotides in septum-capped vials at molar ratios Br-DNA:Ar-B$(OH)_2$ = 0.8 or 0.1, Br-DNA:TPPTS = 15, TPPTS:Pd$(OAc)_2$ = 25, and Na_2CO_3:Br-DNA = 2 for a total volume of 350 μL of 2:1 water/acetonitrile. The resulting solutions were heated under argon at 70 °C for 24 h and then concentrated under diminished pressure and purified by HPLC using the conditions outlined for the modified oligonucleotides. Yields were determined either by quantifying the isolated oligonucleotide by UV using ε_{260} (nearest-neighbor model) for the unmodified oligonucleotides or from integration of the HPLC trace.[39]

MS of Oligonucleotides. Synthesized oligonucleotides were dissolved in 50% water in methanol with 0.1 mM ammonium acetate for mass spectral analysis. Full-scan mass spectra were acquired by infusion through an ESI source in negative mode using either a Finnigan LCQ Deca ion trap or a Waters Q-TOF Micro quadrupole time-of-flight instrument. Oligonucleotides are particularly fragile molecules in the gas phase, and the use of gentle conditions in the ion source and ion optics regions of the instruments was essential for their detection by ESI. For the ion trap, a low flow of sheath gas (20 arbitrary units), the absence of capillary and tube lens voltages (0 V), and low multipole offset voltages (5.5 and 8.0 V for offsets 1 and 2, respectively) were found to give good detection of most of the oligonucleotides analyzed. In the Q-TOF instrument, similarly low voltages were used for the cone (14 V) and extraction cone (1 V) as well as a lower than normal collision energy in the quadrupole collision cell (4 V). Tandem mass spectra were collected using the Q-TOF instrument with the above conditions and a cone voltage of 30 V that gave slightly better transmission of the higher m/z lower charge states and a collision energy of 20 V.

Thermal Melting. UV melting studies were carried out on a Cary 300-Bio UV–vis spectrophotometer equipped with a 6 × 6 multicell block Peltier, stirrer, and temperature controller with Probe Series II. Equal amounts of the adducted decanucleotide and the complementary strand 5′-GGTAGCATGG (0.5 unit each) were dissolved in 0.5 mL of buffer (200 mM NaCl, 50 mM triethylamine acetate, pH 7.2, 1 mM EDTA). The UV absorption at 260 nm was monitored as a function of temperature. Samples were heated to 85 °C for 3 min, and then the temperature was decreased at a rate

of 0.5 °C/min from 85 to 5 °C.[48] The melting temperatures of the duplexes were calculated by determining the first derivative of the melting curve.

Circular Dichroism and Emission Measurements. CD measurements were performed on a Jasco J-815 CD spectrometer at 5 °C in 10 mM triethylamine acetate, pH 7.1, 40 mM NaCl, 0.5 A_{260}/mL of each oligonucleotide. Samples were scanned from 400 to 200 at 0.5 nm intervals averaged over 1 s in a 1 mm light path quartz cuvette. Fluorescence emission spectra were recorded on a Cary Eclipse fluorescence spectrophotometer equipped with a 1 × 4 multicell block Peltier, stirrer, and temperature controller with Probe Series II. Standard 10 mm light path quartz glass cells from Hellma GmbH & Co. were used for fluorescence measurements. All fluorescence spectra were recorded at 5 °C with baseline correction, and excitation and emission slit widths were kept constant at 5.0 nm.

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Supporting Information Available: Experimental section for phosphoramidite synthesis, NMR spectra of synthetic products, MS spectra of starting substrates ODN**1**−**5**, UV and MS spectra of *C*[8]-Ar-G-modified oligonucleotides, and Figures S1 and S2 described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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