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Utility of 5′-*O*-2,7-Dimethylpixyl for Solid-Phase Synthesis of Oligonucleotides Containing Acid-Sensitive 8-Aryl-Guanine Adducts

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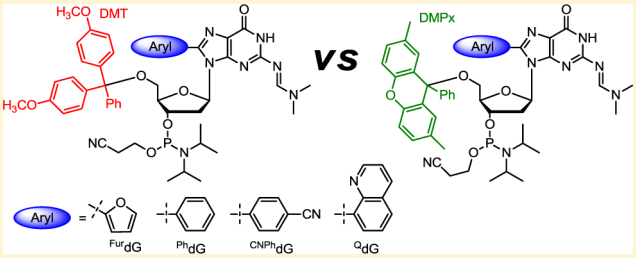
Supporting Information

ABSTRACT: To study the structural and biological impact of 8-aryl-2′-deoxyguanosine adducts, an efficient protocol is required to incorporate them site-specifically into oligonucleotide substrates. Traditional phosphoramidite chemistry using 5′-*O*-DMT protection can be limiting because 8-aryl-dG adducts suffer from greater rates of acid-catalyzed depurination than dG and are sensitive to the acidic deblock conditions required to remove the DMT group. Herein we show that the 5′-*O*-2,7-dimethylpixyl (DMPx) protecting group can be used to limit acid exposure and improve DNA synthesis efficiency for DNA substrates containing 8-aryl-dG adducts. Our studies focus on 8-aryl-dG adducts with 8-substituents consisting of furyl (^{Fur}dG), phenyl (^{Ph}dG), 4-cyanophenyl (^{CNPh}dG), and quinolyl (^{Q}dG). These adducts differ in ring size and sensitivity to acid-promoted deglycosylation. A kinetic study for adduct hydrolysis in 0.1 M aqueous HCl determined that ^{Fur}dG was the most acid-sensitive (55.2-fold > dG), while ^{Q}dG was the most resistant (5.6-fold > dG). The most acid-sensitive ^{Fur}dG was chosen for optimization of solid-phase DNA synthesis. Our studies show that the 5′-*O*-DMPx group can provide a 4-fold increase in yield compared to 5′-*O*-DMT for incorporation of ^{Fur}dG into DNA substrates critical for determining adduct impact on DNA synthesis and repair.



INTRODUCTION

Aryl radical species derived from the metabolism of polyaromatic hydrocarbons (PAHs),[1] arylhydrazines,[2] estrogens,[3] and phenolic toxins[4] can react at the 8-position of 2′-deoxyguanosine (dG) to afford carbon-linked 8-aryl-dG adducts. If left unrepaired, these lesions may initiate carcinogenesis. The 8-aryl-dG adducts are also highly emissive and are useful fluorescent nucleobase probes for detecting G-quadruplex folding[5] and for monitoring adduct conformation within duplex DNA.[6]

Attachment of the aryl substituent to the 8-position of dG can also increase nucleobase sensitivity to acid-catalyzed deglycosylation.[7] The acid-catalyzed hydrolysis of dG proceeds by a stepwise mechanism.[8] The first step involves protonation at N^7; the pK_a for N^7H$^+$-dG is 2.34.[9] The second step is rate-limiting and involves unimolecular cleavage of the glycosidic bond to release protonated guanine as a good leaving group. Direct attachment of the aryl ring to afford the C-linked 8-aryl-dG adduct has little impact on protonation at N^7.[7] However, removal of the sugar moiety in the second step releases steric strain provided by the bulky aryl substituent to increase the rate of depurination. Electron-withdrawing substituents attached to the 8-aryl moiety also increase the rate of hydrolysis due to stabilization of the developing negative charge at N^9 during glycosidic bond cleavage.[7]

To study the structural, photophysical and biological properties of 8-aryl-dG adducts in oligonucleotide substrates, our initial goal is to incorporate them into the G_3-site of the 12-mer sequence 5′-CTCG$_1$G$_2$CG$_3$CCATC (G_3 = 8-aryl-dG), which contains the recognition sequence of the *Nar*I Type II restriction endonuclease.[6] This sequence represents a "hotspot" for mutagenicity mediated by nitrogen-linked 8-aryl-dG adducts produced by arylamine carcinogens.[10] The 12-mer sequence permits analysis of adduct impact on duplex DNA structure. For N-linked arylamine adducts such duplex structures have been used to predict mutagenic outcome[10] and propensity for DNA repair.[11] A second goal is to incorporate 8-aryl-dG adducts into longer DNA substrates that can be used for primer-extension assays using DNA polymerases to assess the biological impact of these lesions. In an effort to achieve these goals and generate adducted oligonucleotide substrates containing 8-aryl-dG lesions, we developed a postsynthetic strategy using Suzuki cross-coupling,[12] which avoids synthesis of the modified phosphoramidite and exposure of the 8-aryl-dG lesion to acidic deblock (3% dichloroacetic acid (DCA) for removal of the 5′-*O*-DMT protecting group). This approach can be used to incorporate a single adduct into relatively short substrates (3−15mers).[6,12,13] However, yields can be low,[6] and the strategy becomes problematic when either incorporating multiple adducts into strands or generating longer adducted DNAs required to assess the biological impact of the lesion using DNA polymerases and DNA repair enzymes.

The limitations of the Suzuki cross-coupling approach prompted our laboratory to seek a solid-phase assisted strategy for incorporating 8-aryl-dG adducts into oligonucleotide

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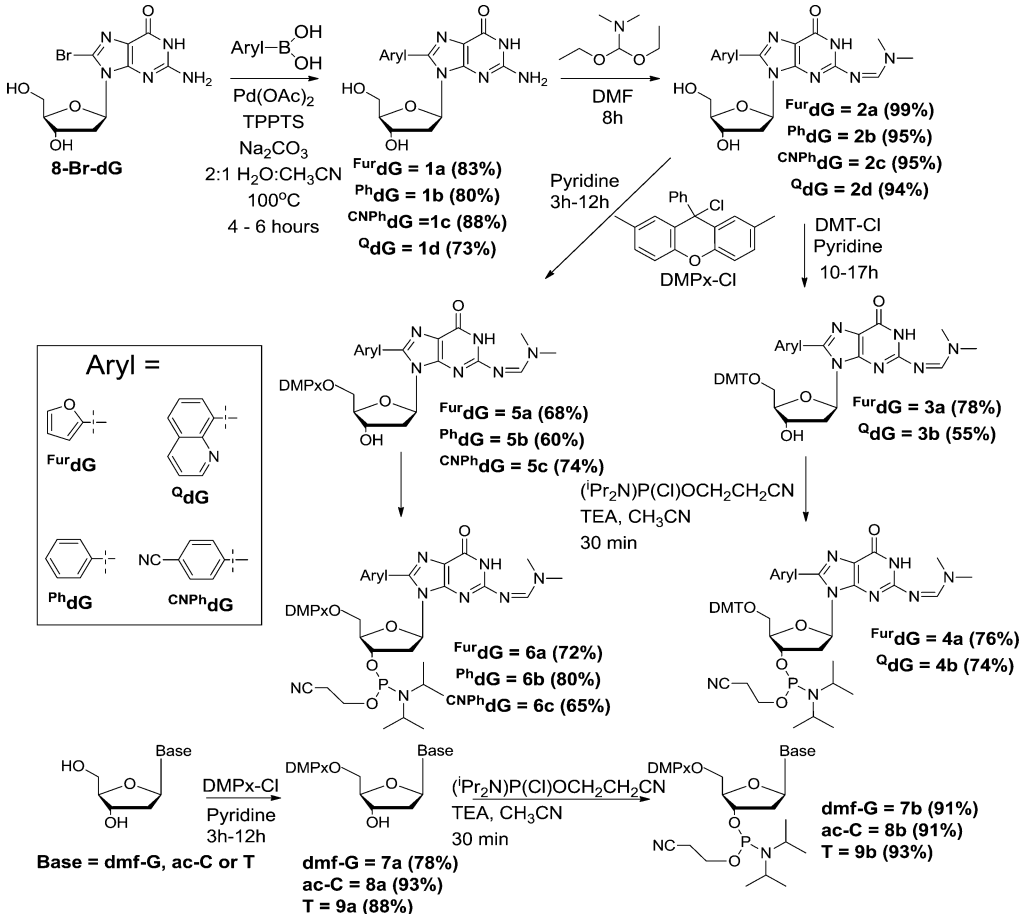
Scheme 1. Synthesis of Phosphoramidites



substrates. Inspired by recent efforts of the Yan laboratory,[14] the 2,7-dimethylpixyl (DMPx) protecting group was deemed an attractive alternative to the widely used 5′-*O*-DMT. The DMPx group is more acid-labile than DMT[15] (because of the release of an aromatic carbocation versus a benzylic carbocation) and can be efficiently removed using 0.5% DCA in dry dichloromethane (DCM).[14,15] This suggested that the DMPx group may offer a general protocol for efficient solid-phase synthesis of oligonucleotides containing acid-sensitive 8-aryl-dG adducts. In this paper we present our efforts to optimize solid-phase synthesis of *Nar*I substrates containing 8-aryl-dG adducts and demonstrate the utility of the 5′-*O*-DMPx protecting group for efficient synthesis of oligonucleotide substrates containing acid-sensitive 8-aryl-dG adducts.

RESULTS AND DISCUSSION

Nucleoside Adducts and Phosphoramidite Synthesis. To test the utility of the 5′-*O*-DMPx group and draw comparison to 5′-*O*-DMT protection, four representative 8-aryl-dG adducts were synthesized using Suzuki cross-coupling procedures[16] and were converted into phosphoramidites using standard protocols (Scheme 1). The nucleoside adducts chosen for study serve different purposes. The 5-membered heteroaryl-dG analogue (^{Fur}dG) has been employed to study probe conformation in duplex DNA.[6] Furan-decorated nucleobase analogues serve as efficient fluorescent nucleobase mimics[17] and are precursors for oxidative-promoted DNA cross-link formation.[18] The 8-phenyl-dG nucleoside (^{Ph}dG) is an authentic DNA adduct produced by arylhydrazine carcinogens.[2] Attachment of the electron-withdrawing cyano group to afford ^{CNPh}dG generates a nucleoside with donor−acceptor character. Biphenyl systems with D−A character produce charge-transfer (CT) states in the emission spectra that are very sensitive to solvent polarity,[19] thereby permitting the use of fluorescence spectroscopy to help define adduct conformation within duplex DNA. The bulky 8-quinolyl-dG derivative ^{Q}dG is a model for C-linked naphthalene-dG adducts that when compared to ^{Ph}dG permits assessment of aryl ring size in promoting mutagenicity. The electron-withdrawing ring nitrogen in ^{Q}dG also provides D−A character.

Hydrolysis Kinetics. Our previous study on acid-catalyzed hydrolysis of 8-phenyl-dG adducts bearing different para and ortho substituents showed that all adducts are more prone than dG to acid-catalyzed deglycosylation.[7] 8-Phenyl-dG adducts bearing o-substituents underwent hydrolysis at a significantly slower rate than their p-substituent counterparts. These results suggested that relief of steric strain upon sugar removal played a key role in the observed rate constants. For p-adducts, a decrease in twist angle of 40° was observed upon removal of the sugar moiety to generate the planar nucleobase. However, for o-adducts, such as 8-*o*-CH_3Ph-dG, the neutral nucleobase is also highly twisted so the relief of strain is diminished upon sugar removal, and hence the rate of hydrolysis was also reduced. The most acid-sensitive adducts were those bearing electron-withdrawing p-substituents.[7]

In the present study the nucleoside adducts (^{Fur}dG, ^{Ph}dG, ^{CNPh}dG, and ^{Q}dG) also possess very different sensitivity to acid-catalyzed deglycosylation (Table 1). First order rate constants

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Table 1. Summary of First-Order Rate Constants (k_{obs}) and Half-Lives ($t_{1/2}$) for Hydrolysis of 8-Aryl-dG Adducts

adduct	k_{obs} (min^{-1}), $t_{1/2}$ (min)[a]	$k_{obs}/k_{obs(dG)}$
^{Fur}dG	2.16 ± 0.08, 0.321	55.2
^{Ph}dG	0.79 ± 0.008, 0.877	20.2
^{CNPh}dG	1.78 ± 0.02, 0.389	45.5
^{Q}dG	0.22 ± 0.002, 3.16	5.6
dG	0.0391, 17.7[b]	1

[a]Determined in 0.1 M aqueous HCl at 37 °C from the average of six kinetic runs. [b]Data for dG taken from ref 8.

(k_{obs}) were determined using UV−vis by monitoring in 0.1 M aqueous HCl at 37 °C the appearance of a peak corresponding to the deglycosylated nucleobase as a function of time, as previously described.[7,20] Under these conditions dG undergoes hydrolysis with a half-life ($t_{1/2}$) of ∼18 min.[8] Surprisingly, the ^{Fur}dG adduct was the most sensitive to acid and hydrolyzed 55 times faster than dG. Interestingly, the bulky quinolone adduct (^{Q}dG) was the least sensitive to acid with a hydrolysis rate of only 5.6-fold faster than dG. This suggested that preferential protonation of the quinoline ring N atom (pK_a 4.9[21]), as opposed to N^7 of the nucleobase, helps protect the nucleoside from acid-catalyzed hydrolysis. Since ^{Q}dG was not particularly sensitive to acid, its phosphoramidite containing 5′-*O*-DMPx was not prepared (Scheme 1).

Optimization of DNA Synthesis. For optimization of solid-phase DNA synthesis, the most acid-sensitive ^{Fur}dG was utilized. Initial experiments examined hydrolysis rates of ^{Fur}dG (**1a**), its N^2-dimethylformamidyl derivative **2a**, and the 5′-*O*-DMT analogue (**3a**) (Scheme 1) in DCA as a function of acid concentration (3, 1, and 0.5% DCA) and solvent (H_2O vs CH_3CN; hydrolysis product ^{Fur}G is not soluble in DCM, which limited the use of DCM for determination of rate data). The rate data (Table 2) demonstrated the impact of DCA

Table 2. Summary of First-Order Rate Constants (k_{obs}) and Half-Lives ($t_{1/2}$) for Hydrolysis of ^{Fur}dG and Its Derivatives

adduct	solvent	3% DCA[b]	1% DCA	0.5% DCA
1a	H_2O[a]	0.40, 1.7	0.21, 3.3	0.12, 5.9
	CH_3CN[a]	0.35, 2.0	0.15, 4.6	0.06, 11.4
2a	H_2O[a]	0.84, 0.82	0.40, 1.7	0.22, 3.15
	CH_3CN[a]	0.85, 0.81	0.114, 6.1	0.042, 16
3a	H_2O[a]	0.91, 0.76	0.40, 1.7	0.22, 3.15
	CH_3CN	0.80, 0.9	0.097, 7.1	0.041, 17

[a]Contains 0.25% DMSO. [b]k_{obs} (min^{-1}), $t_{1/2}$ (min) determined by UV−vis at 21 °C from the average of six kinetic runs; errors <5%

concentration on hydrolysis rate and the need for dry solvents as rates in H_2O were ∼5-fold faster than rates in CH_3CN. For the 5′-*O*-DMT analogue (**3a**) a $t_{1/2}$ value of 17 min was determined in CH_3CN with 0.5% DCA at 21 °C, while the corresponding value in 3% DCA was 0.9 min (19.5-fold reduction in rate of hydrolysis). The rate data also demonstrated the protecting effect of the N^2-dimethylformamidyl group in CH_3CN. In both 1 and 0.5% DCA, hydrolysis rates for **2a** were slower compared to the unprotected ^{Fur}dG (**1a**). Since 3% DCA is required to efficiently detritylate 5′-*O*-DMT, the rate data suggested that utility of the 5′-*O*-DMPx protecting group coupled with a deblock solution of 0.5% DCA in dry DCM could enable efficient incorporation of ^{Fur}dG into oligonucleotides.

To test the efficiency of solid-phase DNA synthesis, the two phosphoramidites **6a** and **4a** (Scheme 1) were used to initially incorporate ^{Fur}dG into a thymidine decamer (5′-T_7XTT, X = ^{Fur}dG). This model decamer was employed because thymidine is the most difficult nucleoside to detritylate.[22] The reverse-phase (RP) HPLC profiles of the synthesis (1 μmol-scale) are shown in Figure 1. For synthesis using **4a** (Figure 1a) and a

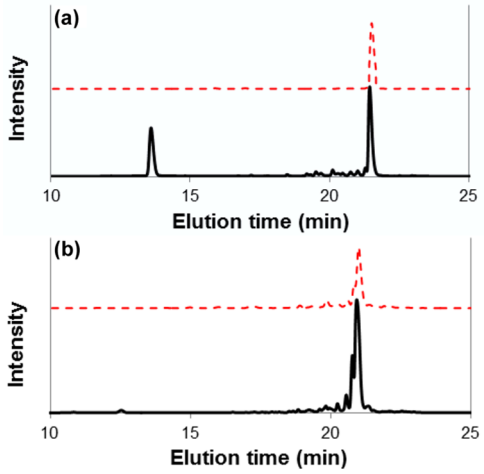

Figure 1. RP-HPLC traces of T_7XTT (X = ^{Fur}dG) synthesis using phosphoramidites **4a** (3% DCA deblock) (a) or **6a** (0.5% DCA deblock) (b), solid black trace, UV detection at 258 nm, dashed red trace, FLD (λ_{ex} = 310 nm, λ_{em} = 380 nm).

deblock solution of 3% DCA in DCM (50 s detritylation time, continuous delivery to the column) the RP-HPLC trace suggested that full length decamer was prepared and eluted at ∼22 min. This peak contained the characteristic fluorescent properties of the modified nucleobase ^{Fur}dG (dashed red trace is with fluorescence detection (FLD)). However, a dominant peak was also present at 13.6 min that lacked fluorescence. This peak either resulted from incomplete coupling of **4a** or acid-promoted depurination of ^{Fur}dG followed by hydrolysis of the abasic site during ammonolysis.[14] Synthesis of the truncated strand 5′-DMT-TXTT (Supporting Information, Figure S1) showed that coupling efficiency was not the problem. For the synthesis of T_7XTT using **6a**, normal deblock (3% DCA) and commercially available 5′-*O*-DMT thymidine phosphoramidites were employed prior to coupling with **6a**. At this point the synthesis was halted, and the deblock solution was changed to 0.5% DCA in DCM. The synthesis was then allowed to proceed to completion using **6a** and the corresponding 5′-*O*-DMPx phosphoramidite of T (**9b**, Scheme 1), which was also prepared, as this amidite is not commercially available. HPLC analysis (Figure 1b) showed the fluorescent peak for full length product. Importantly, the use of 0.5% DCA deblock eliminated formation of the decomposition peak at 13.6 min.

Synthesis of *Nar*I Substrates. To incorporate ^{Fur}dG into the 12-mer *Nar*I recognition sequence 5′-CTCGGCXCCATC (*Nar*I(12)) using the 5′-*O*-DMPx phosphoramidite **6a**, 5′-*O*-DMPx phosphoramidites of dG (**7b**) and dC (**8b**) were also synthesized (Scheme 1). Figure 2 shows HPLC traces for the synthesis of *Nar*I(12) using **4a** and commercially available 5′-*O*-DMT phosphoramidites (Figure 2a) versus the synthesis using **6a**, **7b**, and **8b** (with 0.5% DCA in DCM after the coupling of **6a**). The synthesis of *Nar*I(12) using **4a** produced full length product[6] but contained hydrolysis peaks (Figure 2a)

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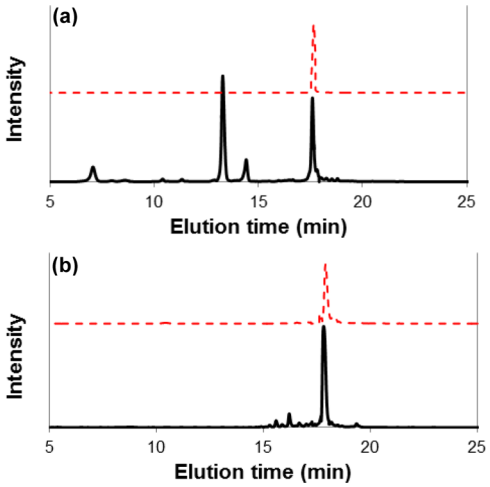

Figure 2. RP-HPLC traces of 5′-CTCGGCXCCATC (X = ^{Fur}dG) synthesis using phosphoramidites **4a** (3% DCA deblock) (a) or **6a** (0.5% DCA deblock) (b), solid black trace, UV detection at 258 nm, dashed red trace, FLD (λ_{ex} = 310 nm, λ_{em} = 380 nm).

and afforded an isolated yield of 49 nmol for *Nar*I(12). Using **6a**, **7b**, and **8b**, the hydrolysis peaks were eliminated (Figure 2b), and an isolated yield of 190 nmol was obtained, for a 4-fold increase in yield of *Nar*I(12). The 5′-*O*-DMPx phosphoramidites **6b** and **6c** (Scheme 1) were then employed to incorporate ^{Ph}dG and ^{CNPh}dG into the G_3-site of *Nar*I(12). A longer 22-mer substrate 5′-CTCGGCXCCATCCCTTACGAGC (*Nar*I(22)) suitable for primer-extension assays using DNA polymerases was also prepared. For these *Nar*I sequences ESI-MS spectra are available in the Supporting Information, and the results of the ESI-MS analysis and isolated yields of oligonucleotide product are summarized in Table 3.

Table 3. Summary of Yields and ESI-MS Data for 8-Aryl-dG-Modified *Nar*I Oligonucleotides

ODN	X	yield[a] (nmol)	calc mass	exptl *m*/*z* (ESI$^-$)[b]	exptl mass
*Nar*I(12)	^{Fur}dG[c]	49	3647.6	M^{6-} = 607.0	3648.0
	^{Fur}dG	190	3647.6	M^{7-} = 520.2	3648.4
	^{Ph}dG	216	3657.6	M^{9-} = 405.4	3657.6
	^{CNPh}dG	170	3682.6	M^{7-} = 525.1	3682.7
	^{Q}dG[c]	147	3708.6	M^{7-} = 529.0	3710.0
*Nar*I(22)	^{Fur}dG	42	6695.2	M^{11-} = 607.7	6695.7
	^{Ph}dG	53	6705.2	M^{11-} = 608.6	6705.6
	^{Q}dG[c]	26	6756.2	M^{9-} = 749.7	6756.3

[a]Isolated yield from 1 μmol-scale synthesis after oligonucleotides were purified via RP-HPLC and quantified using ε^{260} for the corresponding unmodified oligonucleotide (*Nar*I(12) = 102,100; *Nar*I(22) = 185,700 M^{-1} cm^{-1}). [b]Measured *m*/*z* from mass spectrum. [c]Oligonucleotide synthesized with 5′-*O*-DMT protection, while all others were synthesized using 5′-*O*-DMPx protection.

Applications for the 5′-*O*-DMPx Protecting Group. The results of our studies demonstrate the utility of the 5′-*O*-DMPx protecting group for solid-phase DNA synthesis of oligonucleotide substrates containing acid-sensitive 8-aryl-dG adducts. For the *Nar*I substrates reported in Table 3 it was critical to remove the final 5′-OH protecting group on-column (using acidic deblock in dry DCM) prior to cleavage of the oligonucleotide from the solid support using aqueous ammonium hydroxide. Any attempts to purify the modified oligonucleotide with the final 5′-*O*-DMPx group attached (to serve as a hydrophobic handle) resulted in degradation of the oligonucleotide through exposure of the 8-aryl-dG adduct to both acid and water using commercially available solid-phase extraction cartridges for DNA purification. A number of examples in the literature have reported use of solid-phase DNA synthesis for incorporation of 8-aryl-dG adducts into oligonucleotides using standard 5′-*O*-DMT phosphoramidites. For probe development, these include insertion of 8-pyridyl-dG,[5] where preferential protonation of the pyridyl group (pK_a 5.2[21]) would be expected to protect the base from depurination. In other examples the aryl ring is separated from the dG nucleobase by a vinyl[5b,23] or alkynyl group.[24] This reduces steric strain between the aryl ring and sugar moiety, thereby diminishing the sensitivity of the nucleoside to acid-promoted hydrolysis.[7] For DNA adduction by chemical mutagens, nucleophilic aryl radicals also attach to the 8-position of 2′-deoxyadenosine (dA).[2] In terms of sensitivity to acid-catalyzed deglycosylation, dA is 2.2 times more reactive than dG at 30 °C.[25] No information is available on the hydrolytic stability of 8-aryl-dA adducts, and very little has been reported on incorporation of 8-aryl-dA adducts into oligonucleotide substrates. A notable exception for probe development is the click-chemistry-based 8-triazole-dA,[26] which again contains ring nitrogen atoms in the 8-aryl-substituent. Chemical mutagens such as PAHs lack ring nitrogen atoms and attach directly to the 8-position of purine nucleosides, making them acid-sensitive. PAHs are ubiquitous environmental pollutants and are present in cigarette smoke and in vehicle exhaust condensate.[27] Currently, very little is known about the biological implications of C-linked 8-purine adducts produced by PAHs. We plan to use the solid-phase assisted synthesis strategies reported herein to prepare a variety of adducted *Nar*I(22) substrates to assess the differing propensities of 8-aryl-dG adducts to induce mutagenesis when the adducted DNA is replicated.

■ CONCLUSIONS

The results of our studies demonstrate the utility of 5′-*O*-2,7-dimethylpixyl (DMPx) as a protecting group of 8-aryl-dG phosphoramidites for solid-phase assisted synthesis of oligonucleotide substrates. 8-Aryl-dG adducts are produced by a number of chemical mutagens that undergo bioactivation to afford aryl radical species that attach to the 8-position of purine nucleosides. These adducts also possess impressive fluorescent properties that make them desirable for use as fluorescent nucleobase probes. Unfortunately, 8-aryl-dG adducts can be sensitive to acid-catalyzed deglycosylation, which limits the utility of standard solid-phase synthesis using 5′-*O*-DMT protection and a deblock solution consisting of 3% dichloroacetic acid (DCA) in dry dichloromethane (DCM). The 5′-*O*-DMPx group can be efficiently removed using 0.5% DCA in DCM, which limits exposure of the modified 8-aryl-dG lesion to acid during solid-phase synthesis. Using 8-furyl-dG (^{Fur}dG) as a representative acid-sensitive 8-aryl-dG adduct (55-fold more reactive than dG in 0.1 M HCl at 37 °C), our results demonstrate that ^{Fur}dG is much more stable in 0.5% DCA in CH_3CN versus 3% DCA (19.5-fold reduction in hydrolysis rate). This increased stability provides a 4-fold increase in oligonucleotide solid-phase synthesis using 5′-*O*-DMPx phosphoramidites. Thus, the 5′-*O*-DMPx protection strategy provides a more general method for insertion of 8-aryl-dG

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adducts into oligonucleotide substrates and does not alter the reagents used for the traditional 5′-*O*-DMT solid-phase synthesis. These results demonstrate a real application for 5′-*O*-DMPx protection.

EXPERIMENTAL SECTION

Materials and Methods. Boronic acids, $Pd(OAc)_2$, 3,3′,3″-phosphinidynetris-(benzenesulfonic acid) trisodium salt (TPPTS), DCA and other commercial compounds were used as received. Pyridine, DCM, and acetonitrile were distilled over CaH_2 and stored under nitrogen. The synthesis of 8-bromo-2′-deoxyguanosine (8-Br-dG) was performed as described previously.[6] Suzuki cross-coupling reactions of boronic acids with 8-Br-dG to afford ^{Fur}dG,[6] ^{Ph}dG,[7] ^{CNPh}dG,[7] and ^{Q}dG were performed as described previously by Western and co-workers.[16] NMR spectra were recorded on 300, 400, or 600 MHz spectrometers in either DMSO-d_6, $CDCl_3$, CD_2Cl_2, or CD_3OD referenced to TMS (0 ppm) or the respective solvent. All UV−vis were recorded with baseline correction and stirring using 10 mm light path quartz glass cells. Any water used for buffers or spectroscopic solutions was obtained from a filtration system (18.2 MΩ). High-resolution mass spectra were recorded on a Q-ToF instrument, operating in nanospray ionization at 0.5 μL/min detecting positive ions.

Kinetics. The kinetic study was carried out using a UV−vis spectrophotometer equipped with a constant temperature water bath. Hydrolysis reactions were followed by monitoring formation of the deglycosylated nucleobase at its absorption maximum, as previously outlined.[7,20] For hydrolysis of the nucleoside adducts ^{Fur}dG, ^{Ph}dG, ^{CNPh}dG, and ^{Q}dG in 0.1 M HCl at 37 °C, the reactions were initiated by injection of 20 μL of a 4 mM stock solution (DMSO) of the nucleoside adduct into a Teflon-capped 10 mm UV cell containing 1980 μL of aqueous 0.1 M HCl that had been incubating at 37 °C for 15 min. For hydrolysis of ^{Fur}dG (**1a**), N^2-(dimethylformamidyl)-8-(2″-furyl)-2′-deoxyguanosine (**2a**), and 5′-*O*-(4,4′-dimethoxytrityl)-N^2-(dimethylformamidyl)-8-(2″-furyl)-2′-deoxyguanosine (**3a**) in water or dry CH_3CN containing 3, 1, or 0.5% DCA, stock solutions (4 mM) of **1a**, **2a**, and **3a** were prepared in DMSO for kinetic measurements in water. The same DMSO stock solutions of **1a** and **2a** were employed for reactions carried out in dry CH_3CN, while a separate stock solution of **3a** in CH_3CN (4 mM) was used for kinetic measurements of **3a** in CH_3CN containing various concentrations of DCA. Reactions were initiated as outlined for the kinetic runs in 0.1 M HCl. All measurements were conducted in parallel using the multicell changer, with six first-order rate constant values for hydrolysis obtained for each nucleoside in each set of conditions to allow for determination of mean ± standard deviation.

Oligonucleotide Synthesis. Oligonucleotide synthesis of T_7XTT, 5′-DMT-TXTT (X = ^{Fur}dG) and 8-aryl-dG modified *Nar*I(12) (5′-CTCGGCXCCATC) and *Nar*I(22) (5′-CTCGGCXCCATC-CCTTACGAGC) with X = ^{2Fur}dG, ^{Ph}dG, ^{CNPh}dG, or ^{Q}dG was carried out on a 1 μmol scale using an automatic DNA synthesizer. For all modified phosphoramidites with 5′-*O*-DMT protection, synthesis was carried out using standard β-cyanoethylphosphoramidite chemistry (unmodified phosphoramidites (bz-dA-CE, ac-dC-CE, dmf-dG-CE, and dT-CE), activator (0.25 M 5-(ethylthio)-1*H*-tetrazole in CH_3CN), oxidizing agent (0.02 M I_2 in THF/pyridine/H_2O, 70/20/10, v/v/v), deblock (3% DCA in dry DCM), cap A (THF/2,6-lutidine/acetic anhydride), cap B (methylimidazole in THF), and solid supports (5′-DMT-dC(Ac), 1000 Å controlled pore glass (CPG), or 5′-DMT-dT 1000 Å CPG), as outlined previously.[6] For phosphoramidites with 5′-*O*-DMPx protection, standard DNA synthesis conditions and 5′-*O*-DMT phosphoramidites were utilized prior to coupling with the modified phosphoramidite. At this point the synthesis was halted, and the normal deblock solution (3% DCA in dry DCM) was replaced with 0.5% DCA in dry DCM. The synthesis was then allowed to proceed to completion using 5′-*O*-DMPx phosphoramidites (modified (**6a**−**c**), and unmodified (**7b**, **8b**, **9b**), Scheme 1). Following synthesis, oligonucleotides were cleaved from the solid support and deprotected using 2 mL of 30% ammonium hydroxide solution at 55 °C for 12 h and purified by RP-HPLC.

Oligonucleotide Purification. The 8-aryl-G-modified *Nar*I(12) and *Nar*I(22) oligonucleotide solutions were first filtered using syringe filters (PVDF 0.20 μm) and concentrated under diminished pressure. Purification was performed using an HPLC instrument equipped with an autosampler, a diode array detector (monitored at 258 nm and λ_{abs} of the incorporated modified nucleoside), fluorescence detector (monitored at λ_{ex} and λ_{em} of the incorporated modified nucleoside), and autocollector. Separation was carried out at 50 °C using a 5 μm reversed-phase (RP) semipreparative C18 column (100 × 10 mm) with a flow rate of 3.5 mL/min, and various gradients of buffer B in buffer A (buffer A = 95:5 aqueous 50 mM TEAA, pH 7.2/acetonitrile; buffer B = 30:70 aqueous 50 mM TEAA, pH 7.2/acetonitrile). Collected DNA samples were lyophilized to dryness and redissolved in 18.2 MΩ water for quantification by UV−vis measurement using ε_{260}. Extinction coefficients were obtained from the following Web site: http://www.idtdna.com/analyzer/applications/oligoanalyzer. The 8-aryl-G modified oligonucleotides were assumed to have the same extinction coefficient as the natural *Nar*I(12) (102 100 M^{-1} cm^{-1}) and *Nar*I(22) (185 700 M^{-1} cm^{-1}) oligonucleotides.

MS Analysis of Oligonucleotides. MS experiments for DNA identification were conducted on a quadrupole ion trap SL spectrometer. Masses were acquired in the negative ionization mode with an electrospray ionization source. Oligonucleotide samples were prepared in 90% Milli-Q filtered water/10% methanol containing 0.1 mM ammonium acetate. Full scan MS spectra were obtained by direct infusion at a rate of 5−10 μL/min.

Phosphoramidite Synthesis. Synthesis of the phosphoramidite with 5′-*O*-DMT protection for ^{Fur}dG (**4a**)[6] has been previously published using the synthetic strategy outlined in Scheme 1. Unmodified dmf-dG, ac-dC, and T nucleosides were purchased and protected with the 5′-*O*-DMPx protocol described below before conversion to their corresponding phosphoramidite (Scheme 1).

i. N^2 Protection. 8-Aryl-2′-deoxyguanosine (3.3 mmol) was placed in a round-bottom flask (RBF) and reverse filled with argon. Dry DMF (15 mL) was added, followed by dimethylformamide-diethyl-acetal (2.7 mL, 13.5 mmol), and the mixture was allowed to stir until completion overnight at room temperature. The reaction mixture was then evaporated to dryness, and the solid washed with MeOH and dried to yield product.

ii. DMT Protection. N^2-(Dimethylformamidyl)-8-aryl-2′-deoxyguanosine (2.7 mmol) was coevaporated from dry pyridine (3 × 5 mL) in a RBF. The RBF was then fitted with a dropping funnel and reverse filled with argon, and 7 mL of dry pyridine was added to the RBF. DMT-Cl (1.28 g, 3.78 mmol) was placed in the dropping funnel and dissolved in 3 mL of dry pyridine. The 3 mL of DMT-Cl/pyridine solution was then allowed to add dropwise over 30 min. The reaction was allowed to stir at room temperature under argon and was monitored by TLC. Upon completion, the mixture was diluted with DCM (10 mL) and washed with water (2 × 10 mL). TEA (1 mL) was added, and the mixture was evaporated to yield an oil. The oil was then loaded onto a silica column and eluted with MeOH:CH_2Cl_2:TEA (5:90:5) to afford the product.

iii. DMPx Protection. N^2-(Dimethylformamidyl)-8-aryl-2′-deoxyguanosine (1.67 mmol) was placed in a RBF, coevaporated from dry pyridine (3 × 5 mL), and reverse filled with argon. The RBF was then fitted with a dropping funnel containing DMPx-Cl (0.68 g, 1.91 mmol). Dry pyridine (10 mL) was then added to the RBF as well as 4 mL of dry pyridine to the dropping funnel. The RBF was then placed in an ice bath and allowed to cool to 0 °C. The DMPx-Cl/pyridine solution was then delivered to the RBF over 30 min, and the reaction was monitored by TLC. Upon completion, the mixture was diluted with ethyl acetate (10 mL) and washed with water (2 × 10 mL). TEA (1 mL) was added, and the mixture was evaporated to dryness. The solid was then recrystallized by first dissolving in DCM (3 mL), followed by the addition of hexanes (10 mL). The resulting suspension was filtered, and if the product was sufficiently pure it was carried forward; otherwise it was loaded onto a silica column and eluted with MeOH:DCM:TEA (5:90:5) to afford the product.

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iv. Phosphoramidite Reaction. The 5′-*O*-DMT(DMPx)-N^2-(dimethylformamidyl)-8-aryl-2′-deoxyguanosine (0.706 mmol) was coevaporated from dry THF (3 × 5 mL), reverse filled with argon, and dissolved in 10 mL of dry, degassed DCM. To this was added dry, degassed TEA (0.4 mL, 2.83 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.24 mL, 1.06 mmol). The reaction was monitored via TLC and, upon completion (20−40 min), was washed successively with saturated, degassed sodium bicarbonate solution. The organic phase was separated, dried with Na_2SO_4, and either purified by recrystallization by initially dissolving in DCM and dripping into hexanes at −78 °C or loaded onto a flash chromatography column eluting with 92:5:3 DCM:MeOH:TEA. Phosphoramidites were isolated as their corresponding diastereomers, which were typically off-white foams.

v. Synthesis of DMPx-Cl. Following a published protocol,[15] tolyl ether (1 g, 0.01 mol), benzoic acid (0.75 g, 0.01 mol), zinc chloride (2.0 g, 0.02 mol), and phosphorus oxychloride (1.5 mL, 0.02 mol) were heated at 95 °C for 2 h. The mixture was cooled to room temperature and ethyl acetate (2.5 mL) was added to form a suspension. The suspension was poured into stirring water at room temperature and heated under reflux for 15 min and then allowed to cool to room temperature overnight. Mixture was filtered and washed with water. The damp cake was suspended with 30 mL of methanol and stirred to boil for 2 or 3 min. The resultant suspension was allowed to cool to room temp over a period of 3 h and was then filtered, washed with methanol, and dried to give DMPx-OMe as a white solid in 64% yield: mp 135−137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.57−7.53 (m, 2H), 7.45−7.40 (m, 2H), 7.35−7.33 (m,1H), 7.24 (m, 4H), 7.14 (s, 2H), 3.09 (s, 3H), 2.39 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ =149.9, 149.3, 132.7, 130.1, 129.9, 129.4, 129.3, 128.0, 126.6, 126.57, 122.7, 116.1, 76.3, 51.2, 21.0. DMPx-OMe (2.0 g, 6.9 mmol) was azeotroped with dry toluene (2 × 10 mL), and the residue was dissolved in dry toluene (10 mL). Acetyl chloride (5.8 mL, 81.8 mmol) was added, and the reaction was left to stir for 16 h at room temperature under argon. Upon completion the reaction was evaporated to dryness and coevaporated with dry toluene (3 × 10 mL). The product was further dried under a vacuum at 40 °C for 3 h to afford DMPx-Cl in quantitative yield as an orange/brown solid: mp 108−109 °C.^{1}H NMR (300 MHz, CD_2Cl_2) δ = 7.63−7.60 (m, 2H), 7.45−7.42 (m, 2H), 7.29−7.25 (m, 4H), 7.05 (s, 2H), 2.26 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ = 149.3, 143.5, 134.4, 133.1, 130.4, 129.2, 128.5, 125.3, 117.1, 85.7, 21.0.

5′-*O*-DMPx-N^2-(dimethylformamidyl)-8-(furan-2-yl)-2′-deoxyguanosine (5a). Isolated 0.76 g (1.13 mmol) as an off-white powder corresponding to a 68% yield: mp 225−227 °C; ^{1}H NMR (600 MHz, CD_2Cl_2) δ = 8.99 (bs, 1H), 8.12 (s, 1H), 7.44 (d, *J* = 1.0 Hz, 1H), 7.28−6.87 (m, 12H), 6.63 (dd, *J* = 6.0 Hz, 7.8 Hz, 1H), 6.50 (m, 1H), 4.55, (m, 1H), 4.14 (m, 1H) 3.45 (m,1H), 3.31 (m,1H), 3.15 (m,1H), 3.05 (s, 3H), 2.86 (s, 3H), 2.26 (m, 1H), 2.19 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (600 MHz, CD_2Cl_2) δ = 157.84, 157.81, 156.2, 151.1, 149.7, 149.6, 149.4, 145.3, 144.0, 140.1, 133.22, 133.17, 130.4, 130.3, 129.5, 129.3, 128.2, 126.9, 126.4, 122.9, 122.8, 121.2, 116.5, 116.1, 112.9, 112.1, 86.3, 84.7, 76.2, 73.3, 65.3, 46.5, 41.7, 39.0, 35.3, 20.9, 20.5; HRMS calcd for $C_{38}H_{37}N_6O_6^+$ [M + H$^+$] 673.2776, found 673.2775.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-N^2-(dimethyl-formamidyl)-8-(furan-2-yl)-2′-deoxyguanosine (6a). Isolated as an off-white foam in 72% yield (0.44 g, 0.51 mmol): ^{1}H NMR (300 MHz, CD_2Cl_2) δ = 9.20 (bs,1H), 8.21 (m, 1H), 7.54 (m, 1H), 7.42−6.87 (m, 12H), 6.67 (m, 1H), 6.56 (m, 1H), 4.81 (m, 1H), 4.27 (m, 1H), 3.80−3.34 (m, 6H), 3.19−3.10 (m, 4H), 2.92 (s, 3H), 2.56 (m, 2H), 2.42 (m,1H), 2.21 (m, 3H), 1.89 (m, 3H), 1.19−1.09 (m, 12H); ^{13}C NMR (300 MHz, CD_2Cl_2) δ = 158.0, 157.9, 156.3, 151.2, 149.8, 149.7, 148.6, 145.5, 144.1, 140.3, 133.4, 133.3, 130.5, 130.4, 129.6, 129.5, 127.0, 123.0 122.9, 121.3, 117.7, 116.2, 113.0, 112.2, 86.5, 84.8, 76.3, 73.4, 65.4, 57.9, 46.6, 43.3, 41.9, 39.1, 35.4, 23.4, 23.2, 21.0, 20.6, 20.2; ^{31}P NMR (300 MHz, CD_2Cl_2) δ = 149.3, 149.0; HRMS calcd for $C_{47}H_{54}N_8O_7P^+$ [M + H$^+$] 873.3848; found 873.3852.

N^2-(Dimethylformamidyl)-8-phenyl-2′-deoxyguanosine (2b).[28] Isolated 1.25 g (3.14 mmol) of a white powder corresponding to a 95% yield: mp 218−220 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.46 (s, 1H), 8.49 (s, 1H), 7.65−7.53 (m, 5H), 6.09 (t, *J* = 6 Hz, 1H), 5.21 (d, *J* = 6.0 Hz, 1H), 4.88 (t, *J* = 6.0 Hz, 1H), 4.42 (bs, 1H), 3.81 (bs, 1H), 3.67 (m, 1H), 3.61 (m, 1H) 3.33 (m, 1H), 3.22 (s, 3H), 3.04 (s, 3H) 2.08 (s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ = 158.1, 157.5, 156.8, 150.7, 148.1, 130.1, 129.5, 129.1, 128.7, 120.1, 87.7, 84.8, 71.0, 62.0, 40.8, 37.0, 34.6.

5′-*O*-DMPx-N^2-(dimethylformamidyl)-8-phenyl-2′-deoxyguanosine (5b). Isolated 0.68 g (1.0 mmol) as a white powder corresponding to a 60% yield: mp 195−198 °C; ^{1}H NMR (600 MHz, CD_2Cl_2) δ = 9.11 (bs, 1H), 8.12 (s, 1H), 7.70 (d, *J* = 7 Hz, 2H), 7.43−7.38 (m, 3H), 7.31 (d, *J* = 7.4 Hz, 2H), 7.21 (t, *J* = 7.4 Hz, 2H), 7.13−7.08 (m, 2H), 7.02 (m, 2H), 6.93 (m, 3H), 6.24 (t, *J* = 6.4 Hz, 1H), 4.43 (m, 1H), 4.14 (m, 1H), 3.52 (t, *J* = 9.2 Hz, 1H), 3.34 (dd, *J* = 2.8, 9.4 Hz, 1H), 3.11 (m, 1H), 3.07 (s, 3H), 2.85 (s, 3H), 2.20 (s, 3H), 2.11 (s, 3H) 2.10 (m,1H); ^{13}C NMR (600 MHz, CD_2Cl_2) δ = 158.2, 158.0, 156.1, 151.6, 149.9, 149.8, 149.7, 149.6, 133.4, 133.3, 130.9, 130.5, 130.4, 130.1, 129.7, 129.5, 129.0, 128.4, 127.0, 126.5, 123.0, 121.0, 116.7, 116.2, 86.7, 85.0, 76.3, 73.4, 65.5, 41.9, 38.5, 35.5, 21.1, 20.7; HRMS calcd for $C_{40}H_{39}N_6O_5^+$ [M + H$^+$] 683.2982; found 683.2985.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-N^2-(dimethylformamidyl)-8-phenyl-2′-deoxyguanosine (6b). Isolated as an off-white foam in 80% yield (0.5 g, 0.57 mmol): ^{1}H NMR (300 MHz, CD_2Cl_2) δ = 9.16 (bs, 1H), 8.19−8.11 (m, 1H), 7.61−6.86 (m, 16H), 6.13 (m, 1H), 4.52 (m, 1H), 4.11 (m, 1H), 3.90 (m, 2H), 3.80−3.51 (m, 4H), 3.46 (s, 3H), 3.43 (s, 3H), 2.68−2.50 (m, 3H), 2.15 (m, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.05−0.99 (m, 12H); ^{13}C NMR (300 MHz, CD_2Cl_2) δ = 158.4, 158.2, 156.3, 151.8, 150.1, 150.0, 149.9, 149.8, 133.54, 133.49, 131.1, 130.7, 130.6, 130.3, 129.8, 129.7, 129.2, 128.6, 127.2, 126.7, 123.2, 123.19, 121.2, 116.9, 116.4, 86.9, 85.1, 76.5, 73.6, 65.7, 58.5, 43.9, 43.85, 42.1, 38.7, 35.6, 23.1, 23.0, 22.9, 21.3, 20.9, 20.2; ^{31}P NMR δ = 148.74, 148.67; HRMS calcd for $C_{49}H_{56}N_8O_6P^+$ [M + H$^+$] 883.4055; found 883.4060.

N^2-(Dimethylformamidyl)-8-(4″-cyanophenyl)-2′-deoxyguanosine (2c). Isolated 1.33 g (3.14 mmol) of a yellow powder corresponding to a 95% yield: mp 179−182 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 8.48 (s, 1H), 8.00 (d, *J* = 8.3 Hz, 2H), 7.85 (d, *J* = 8.3 Hz, 2H), 6.11 (t, *J* = 7.7 Hz, 1H), 5.75 (bs, 1H), 5.30 (bs, 1H), 4.44 (m, 1H), 3.86 (m, 1H), 3.67 (dd, *J* = 3.5, 12 Hz, 1H), 3.55 (dd, *J* = 3.8, 11.9 Hz, 1H), 3.24 (m, 1H), 3.09 (s, 3H), 3.00 (s, 3H), 2.07 (m, 1H); ^{13}C NMR (600 MHz, DMSO-d_6) δ = 157.7, 151.6, 145.0, 142.5, 141.9, 135.0, 133.0, 132.6, 129.7, 128.1, 121.3, 118.6, 111.4, 88.3, 85.2, 71.3, 62.3, 40.5, 37.3, 34.4; HRMS calcd for $C_{20}H_{22}N_7O_4^+$ [M + H$^+$] 424.1733, found 424.1724.

5′-*O*-DMPx-N^2-(dimethylformamidyl)-8-(4″-cyanophenyl)-2′-deoxyguanosine (5c). Isolated 0.87 g (1.23 mmol) of a yellow powder corresponding to a 74% yield: mp 170−173 °C (d); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.37 (bs, 1H), 7.99 (s, 1H), 7.95 (d, *J* = 8.2 Hz, 2H), 7.83 (d, *J* = 8.2 Hz, 2H), 7.19−7.04 (m, 7H), 6.87−6.83 (m, 2H), 6.75−6.72 (m, 2H), 6.05 (m, 1H), 5.24 (m, 1H), 4.40 (m, 1H), 4.03 (m, 1H), 3.4 (t, *J* = 9.5 Hz, 1H), 3.26 (m, 1H), 3.08 (m, 1H), 3.01 (s, 3H), 2.78 (s, 3H), 2.11 (s, 3H), 2.04 (m, 1H), 1.62 (s, 3H); ^{13}C NMR (600 MHz, DMSO-d_6) δ =157.6, 157.4, 156.4, 150.5, 149.3, 148.5, 148.4, 146.2, 134.6, 132.6, 132.2, 131.7, 130.1, 129.7, 129.5, 128.5, 128.4, 127.9, 126.4, 125.5, 122.3, 122.26, 120.7, 118.6, 116.2, 115.5, 111.7, 86.7, 84.6, 74.9, 71.3, 64.7, 40.9, 40.4, 37.1, 34.7, 20.4, 19.9; HRMS calcd for $C_{41}H_{38}N_7O_5^+$ [M + H$^+$] 708.2929; found 708.2932.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-N^2-(dimethylformamidyl)-8-(4″-cyanophenyl)-2′-deoxyguanosine (6c). Isolated as a white foam in 65% yield (0.42 g, 0.46 mmol): ^{1}H NMR (300 MHz, CD_3OD) δ = 8.21 (s, 1H), 7.95−7.84 (m, 4H), 7.29−6.78 (m, 11H), 6.15 (m, 1H), 4.95 (m, 1H), 4.37−4.20 (m, 1H), 3.84−3.47 (m, 6H), 3.30−3.17 (m,1H), 3.16 (m, 3H), 2.97 (m, 3H), 2.62 (m,2H), 2.16 (m, 3H), 2.15 (m,1H), 1.75 (m, 3H), 1.30 (d, *J* = 5.2 Hz, 6H), 1.27 (d, *J* = 5.2 Hz, 6H); ^{13}C NMR (300 MHz, CD_3OD) δ = 160.3, 159.0, 158.1, 152.5, 151.5, 150.8, 150.5, 149.0, 135.4, 133.9, 133.7, 133.4, 131.4, 131.2, 130.9, 130.6, 128.8, 127.6, 127.4, 127.3, 124.0, 123.8, 121.7, 119.5, 119.3, 117.3, 116.8, 114.5,

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87.5, 86.4, 77.2, 70.0, 65.9, 60.3, 59.7, 59.5, 46.7, 46.65, 44.7, 44.6, 44.5, 42.0, 35.6, 25.1, 25.0, 23.2, 20.8, 20.3; ^{31}P NMR δ = 149.40, 149.12; HRMS calcd for $C_{50}H_{55}N_9O_6P^+$ [M + H$^+$] 908.4007; found 908.4013.

8-(8″-Quinolyl)-2′-deoxyguanosine (^{Q}dG, 1d). Isolated 2.2 g (5.6 mmol) of a yellow solid corresponding to a 73% yield: mp 215−218 °C (d); ^{1}H NMR (600 MHz, DMSO- d$_6$) δ = 10.74 (s, 1H), 8.89 (d, J = 2.6 Hz, 1H), 8.50 (dd, J = 1.4, 8.3 Hz, 1H), 8.19 (dd, J = 1.0, 8.3 Hz, 1H), 7.92 (bs, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.61 (dd, J = 4.1, 8.3 Hz, 1H), 6.31 (bs, 2H), 5.51 (bs, 1H), 5.04 (bs, 1H), 4.90 (d, J = 4.1 Hz, 1H), 4.27 (bs, 1H), 3.55 (m, 2H), 3.41 (bs, 1H), 3.18 (bs, 1H), 2.09 (bs, 1H); ^{13}C NMR (600 MHz, DMSO-d_6) δ = 157.7, 157.2, 151.3, 150.5, 146.9, 146.3, 136.9, 132.8, 130.9, 129.7, 128.0, 126.6, 122.5, 120.8, 87.8, 85.5, 71.2, 62.4, 38.0; HRMS calcd for $C_{19}H_{19}N_6O_4^+$ [M + H$^+$] 395.1462; found 395.1468.

***N*^{2}-(Dimethylformamidyl)-8-(8″-quinolyl)-2′-deoxyguanosine (2d).** Isolated 1.39 g (3.1 mmol) as a yellow powder corresponding to a 94% yield: mp 188−192 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.45 (bs, 1H), 8.90 (m, 1H), 8.50 (m, 2H), 8.21 (d, J = 8.2 Hz, 1H), 7.95 (d, J = 6.7 Hz, 1H), 7.75 (t, J = 7.7 Hz, 1H), 7.62 (dd, J = 4.2, 8.2 Hz), 5.56 (bs, 1H), 4.97 (m, 1H), 4.89 (m, 1H), 4.30 (bs, 1H), 4.09 (m, 1H), 3.57−3.45 (m, 2H), 3.16−3.12 (m, 4H), 3.03 (s, 3H), 2.10 (bs, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ = 158.1, 157.4, 157.0, 151.1, 150.2, 146.7, 146.1, 136.6, 132.6, 130.5, 129.4, 127.7, 126.3, 122.0, 120.6, 87.5, 85.4, 70.9, 62.1, 48.6, 37.4, 34.5; HRMS calcd for $C_{22}H_{23}N_7O_4K^+$ [M + K$^+$] 488.1449; found 488.1438.

5′-*O*-DMT-*N*^{2}-(dimethylformamidyl)-8-(8″-quinolyl)-2′-deoxyguanosine (3b). Isolated 1.12 g (1.49 mmol) of a yellow powder corresponding to a 55% yield: mp 178−181 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.39 (bs, 1H), 8.88 (bs, 1H), 8.50 (d, J = 7.8 Hz, 1H), 8.19 (m, 2H), 8.02 (bs, 1H), 7.78 (bs, 1H), 7.62 (m, 1H), 7.26 (m, 2H), 7.17−7.11 (m, 7H), 6.76 (m, 4H), 5.65 (bs, 1H), 5.08 (bs, 1H), 4.42 (bs, 1H), 3.70 (s, 6H) 3.61 (m, 1H), 3.21 (bs, 1H), 3.07 (m, 1H), 3.00 (m, 4H), 3.91 (s, 3H), 1.78 (bs, 1H); ^{13}C (600 MHz, DMSO-d_6) 158.0, 157.9, 157.6, 151.2, 150.2, 145.0, 136.7, 135.7, 135.6, 132.8, 130.5, 129.7, 129.4, 127.8, 127.6, 126.5, 122.1, 113.0, 112.95, 87.0, 86.5, 85.2, 71.0, 64.5, 55.0, 54.97, 45.7, 40.7, 40.1, 37.9, 34.6, 30.7; HRMS calcd for $C_{43}H_{42}N_7O_6^+$ [M + H$^+$] 752.3197; found 752.3191.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMT-*N*^{2}-(dimethylformamidyl)-8-(8″-quinolyl)-2′-deoxyguanosine (4b). Isolated as a white foam in 74% yield (0.5 g, 0.52 mmol): ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.40 (bs, 1H), 8.92 (m, 1H), 8.53 (m, 1H), 8.26−8.19 (m, 2H), 8.00 (bs, 1H), 7.77 (m, 1H), 7.66 (dd, J = 4.2, 8.3 Hz, 1H), 7.29−6.73 (m, 13H), 5.71 (bs, 1H), 5.10 (d, J = 4.5 Hz,1H), 4.41 (bs, 1H), 3.73−3.68 (m, 7H), 3.64 (m, 1H), 3.23−3.18 (m, 2H), 3.18−3.07 (m,3H), 3.06−2.94 (s, 6H), 2.12 (bs, 1H), 0.97−0.81 (m, 12H); ^{13}C NMR (300 MHz, CDCl$_3$) δ = 154.5, 154.4, 154.1, 147.6, 146.7, 141.5, 133.2, 132.2, 132.0, 129.2, 127.0, 126.1, 125.9, 124.3, 124.1, 123.0, 118.6, 117.5, 109.5, 109.4, 82.0, 81.1, 80.1 67.1, 61.0, 58.2, 51.5, 51.45, 43.3, 43.1, 42.1, 37.2, 36.5, 36.4, 35.6, 34.4, 31.1, 27.2, 23.8, 23.6, 20.9; ^{31}P NMR δ = 149.32, 148.88; HRMS calcd for $C_{52}H_{59}N_9O_7P^+$ [M + H$^+$] 952.4275; found 952.4278.

5′-*O*-DMPx-*N*^{2}-(dimethylformamidyl)-2′-deoxyguanosine (7a). Isolated 0.79 g (1.30 mmol) of a white powder corresponding to 78% yield: mp 113−118 °C (d); ^{1}H NMR (300 MHz, CDCl$_3$) δ = 9.50 (bs, 1H), 8.43 (s, 1H), 7.65 (s, 1H), 7.31 (m, 2H), 7.22−6.95 (m, 7H), 6.90 (m, 2H), 6.33 (t, J = 6.9 Hz, 1H), 4.55 (m, 1H), 4.13 (m, 1H), 3.16 (m, 2H), 3.02 (s, 3H), 2.99 (s, 3H), 2.54 (m, 2H), 2.13 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (300 MHz, CDCl$_3$) δ = 158.1, 158.0, 156.6, 150.1, 149.3, 149.28, 148.5, 132.8, 132.7, 130.2, 130.1, 129.3, 129.1, 127.9, 126.6, 126.3, 122.2, 122.0, 120.3, 116.0, 85.9, 83.3, 76.1, 72.5, 64.0, 45.7, 41.3, 40.8, 35.1, 30.9, 20.7, 20.68; HRMS calcd for $C_{34}H_{35}N_6O_5^+$ [M + H$^+$] 607.2669; found 607.2672.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-*N*^{2}-(dimethylformamidyl)-2′-deoxyguanosine (7b). Isolated as a white foam in 91% yield (0.52g, 0.642 mmol): ^{1}H NMR (300 MHz, CD$_2$Cl$_2$) δ = 9.70 (bs, 1H), 8.45 (m, 1H), 7.60 (m, 1H), 7.26−6.86 (m, 11H), 6.21 (m, 1H), 4.58 (m, 1H), 4.16 (m, 1H), 3.74−3.37 (m, 5H), 3.12 (m, 2H), 3.01 (s, 6H), 2.43−2.39 (m, 2H), 2.37 (m,1H), 2.07 (m, 6H), 1.11−1.00 (m, 12H); ^{13}C NMR (300 MHz, CD$_2$Cl$_2$) δ = 161.1, 161.0, 159.6, 153.0, 152.31, 152.28, 151.5, 135.8, 135.7, 133.2, 133.1, 132.3, 132.1, 130.8, 129.6, 129.3, 125.2, 125.0, 123.2, 119.0, 117.7 88.9, 86.3, 79.1, 75.4, 67.0, 57.2, 44.3, 43.8, 42.8, 38.0, 33.9, 23.74, 23.68, 22.7, 19.6; ^{31}P NMR δ = 148.93, 148.72; HRMS calcd for $C_{43}H_{52}N_8O_6P^+$ [M + H$^+$] 807.3748; found 807.3752.

5′-*O*-DMPx-*N*^{4}-(acetyl)-2′-deoxycytidine (8a). Isolated 0.86 g (1.55 mmol) of a white powder corresponding to a 93% yield: mp 115−118 °C (d); ^{1}H NMR (300 MHz, CD$_2$Cl$_2$) δ = 9.03 (bs, 1H), 8.21 (d, J = 7.5 Hz, 1H), 7.36−7.11 (m, 10H), 6.92 (m, 2H), 6.22 (t, J = 6.1 Hz, 1H), 4.35 (m, 1H), 4.06 (m, 1H), 3.24 (dd, J = 3.2, 10.6 Hz, 1H), 3.10 (dd, J = 4.1, 10.6 Hz, 1H), 2.78−2.70 (m, 1H), 2.22 (m, 10H); ^{13}C NMR (300 MHz, CD$_2$Cl$_2$) δ = 170.6, 162.8, 155.6, 150.0, 149.9, 148.7, 144.6, 133.42, 133.37, 130.8, 129.5, 129.3, 128.3, 127.2, 126.8, 122.5, 122.2, 116.6, 96.3, 87.9, 87.0, 76.9, 71.9, 63.6, 42.4, 25.2, 21.0; HRMS calcd for $C_{32}H_{32}N_3O_6^+$ [M + H$^+$] 554.2294; found 554.2296.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-*N*^{4}-(acetyl)-2′-deoxycytidine (8b). Product isolated in 91% yield as a foam (0.48 g, 0.64 mmol): ^{1}H NMR (300 MHz, CDCl$_3$) δ = 10.25 (bs, 1H), 8.22−8.05 (m, 1H), 7.33−6.78 (m, 12H), 6.17 (m, 1H), 4.32 (m, 1H), 4.09 (m, 1H), 3.73−3.41 (m, 4H), 3.17 (m, 1H), 2.98 (m, 1H), 2.70 (m, 1H), 2.52 (t, J = 6.3 Hz, 2H), 2.32 (m, 1H), 2.22 (s, 3H), 2.16 (m, 6H), 0.95 (m, 12H);^{13}C NMR (300 MHz, CDCl$_3$) δ = 169.1, 168.9, 161.4, 154.1, 148.4, 147.1, 143.11, 143.09, 131.9, 129.3, 128.0, 127.8, 126.8, 125.6, 125.3, 121.0, 120.7, 117.1, 115.0, 94.81, 94.79, 86.4, 85.4, 75.4, 70.3, 62.1, 58.1, 43.4, 43.4, 40.8, 23.7, 23.6, 23.2, 23.1, 20.6, 20.5, 19.5, 19.4; ^{31}P NMR δ = 149.55, 149.23; HRMS calcd for $C_{41}H_{49}N_5O_7P^+$ [M + H$^+$] 754.3369 found 754.3373.

5′-*O*-DMPx-2′-deoxythymidine (9a). Isolated 0.81 g (1.47 mmol) of a white powder corresponding to a 88% yield: mp 139−141 °C (d); ^{1}H NMR (600 MHz, DMSO-d_6) δ = 11.33 (s, 1H), 7.53 (s, 1H), 7.27−7.24 (m, 4H), 7.16 (m, 3H), 7.11 (m, 2H), 7.04 (bs, 1H), 6.97 (m, 1H), 6.20 (t, J = 7 Hz, 1H), 5.32 (d, J = 4.4 Hz, 1H), 4.31 (m, 1H), 3.82 (m, 1H), 3.13 (dd, J = 2.8, 10.4 Hz, 1H), 3.06 (dd, J = 4.0, 10.4 Hz, 1H), 2.27 (m, 1H), 2.18 (m, 1H), 2.14 (s, 3H), 3.10 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (600 MHz, DMSO-d_6) δ = 163.6, 150.4, 149.0, 148.7, 148.4, 135.7, 132.5, 132.4, 130.4, 130.2, 128.3, 128.22, 128.18, 126.8, 125.6, 122.1, 121.9, 116.1, 109.5, 85.3, 83.6, 75.5, 70.8, 63.7, 20.3, 20.2, 11.7; HRMS calcd for $C_{31}H_{30}N_2O_6Na^+$ [M + Na$^+$] 549.2002; Found 549.1995.

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-DMPx-2′-deoxythymidine (9b). Product isolated in 93% yield as a foam (0.48 g, 0.66 mmol): ^{1}H NMR (400 MHz, CD$_2$Cl$_2$) δ = 9.22 (bs, 1H), 7.67 (s, 1H), 7.35−7.06 (m, 10H), 6.93 (m, 1H), 6.35 (m, 1H), 4.55 (m, 1H), 4.13−4.07 (m, 1H), 3.63−3.55 (m, 4H), 3.30−3.27 (m, 1H), 3.17−3.14 (m, 1H), 2.53 (m, 1H), 2.41−2.33 (m, 3H), 2.24 (s, 3H), 2.18 (s, 3H), 1.63 (s, 3H), 1.18 (d, J = 6.8 Hz, 12H); ^{13}C NMR (400 MHz, CD$_2$Cl$_2$) δ = 164.9, 164.8, 151.1, 150.1, 149.6, 149.0, 136.1, 133.33, 133.30, 130.8, 130.7, 129.3, 129.0, 128.4, 127.2, 126.6, 122.4, 122.1, 118.2, 111.4, 85.6, 85.0, 76.9, 74.8, 74.6, 63.7, 60.7, 58.8, 58.6, 43.6, 43.5, 40.4, 24.8, 24.7, 24.6, 24.5, 21.0, 20.8, 20.7, 14.3, 12.3, 1.12; ^{31}P NMR δ = 149.23, 148.33; HRMS calcd for $C_{40}H_{48}N_4O_7P^+$ [M + H$^+$] 727.3260; found 727.3264.

■ ASSOCIATED CONTENT

Supporting Information

Figure S1 described in the text, NMR spectra of synthetic samples, and ESI-MS spectra of modified *Nar*I oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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