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Unusual C7- versus Normal 5′-O-Dimethoxytritylation of 6-Arylpyrrolocytidine Analogs

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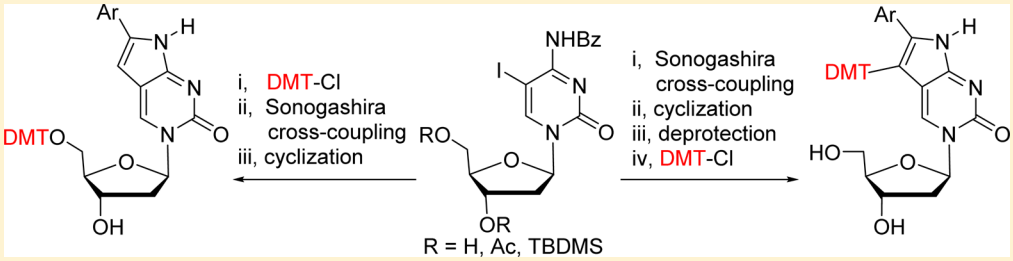
Supporting Information



ABSTRACT: Fluorescent deoxynucleosides possessing the modified bases 6-(2-benzo[*b*]furyl)- and 6-(2-furyl)pyrrolocytosine (BFpC and FpC) have been synthesized along with the quencher nucleosides possessing 6-{4-[(4-dimethylamino)azo]phenyl}-pyrrolocytosine (DABCYLpC) and 6-(*p*-nitrophenyl)pyrrolocytosine (*p*-NO_2-PhpC) nucleobase analogs. Standard treatment of BFpC, FpC, DABCYLpC, and *p*-NO_2-PhpC with dimethoxytrityl chloride (DMT-Cl) led to the unusual substitution on the C7 of the pyrrolocytosine skeleton. The desired 5′-*O*-DMT-protected nucleoside analogs were synthesized from suitably protected 5′-*O*-DMT cytidines. Subsequent phosphitylation smoothly afforded BFpC-, FpC-, DABCYLpC-, and *p*-NO_2-PhpC-derived monomers suitable for standard oligonucleotide synthesis.

INTRODUCTION

Within the framework of our continuing interest in the development of intrinsically fluorescent nucleobases[1] we have prepared and studied 6-phenylpyrrolocytidine (PhpC, **1**, Figure 1). PhpC obeys the Watson−Crick base-pairing rules with guanine (Figure 1), is generally stabilizing to hybridization when incorporated into DNA, RNA, or PNA, and possesses useful probe properties.[2] With the aim to tune the fluorescence properties associated with PhpC, we have, among other structural modifications, also prepared 2-benzo[*b*]furyl-substituted (BFpC,[3] **3**, Figure 1) and 2-furyl-substituted (FpC, **4**, Figure 1) analogs for enhanced fluorescence properties, along with the nucleobase possessing 4-[(4-dimethylaminophenyl)-azo]phenyl (DABCYLpC, **5**, Figure 1)[4] and 6-(*p*-nitrophenyl) (*p*-NO_2-PhpC, **2**, Figure 1)[2a] moieties to serve as fluorescence quenchers.[5]

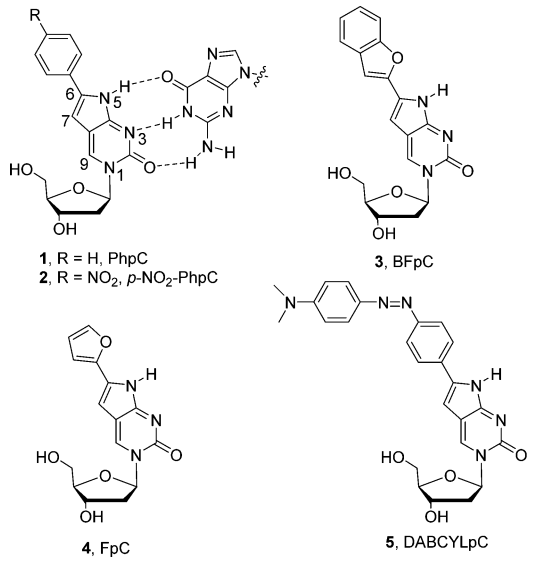


Figure 1. Chemical structures of PhpC (**1**), *p*-NO_2-PhpC (**2**), BFpC (**3**), FpC (**4**), and DABCYLpC (**5**).

Our intention to use the nucleoside analogs **2**−**5** for automated DNA oligomerization required further synthetic transformations. The "gold standard" in automated DNA synthesis requires the 5′-OH group to be protected with the acid-labile dimethoxytrityl (DMT) group.[6] The introduction of the DMT group into nucleosides has been well-documented;[7] the reagent used for this purpose, dimethoxytrityl chloride (DMT-Cl), is usually reacted with the unprotected nucleosides under basic, aprotic conditions (pyridine as solvent) for nucleobases that lack reactive amino groups.

As described below, direct treatment of nucleoside analogs **2**−**5** with DMT-Cl resulted in the formation of unusual *C*7-DMT-substituted arylpyrrolocytosine analogs. Detailed spectral characterization (1D and 2D NMR) of *C*7-DMT-substituted

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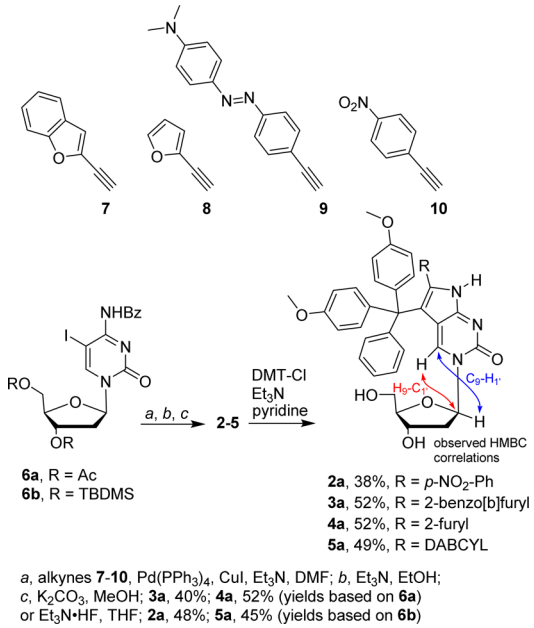
arylpyrrolocytosine analogs was carried out, and the results were compared to those acquired for genuine 5′-*O*-DMT-substituted analogs. The structural assignment of *C*7-DMT-substituted arylpyrrolocytosine analogs was also supported by quantum chemical calculations performed on the aglycon possessing *N*1-isopropyl substitution as a model for the deoxyribose.

The desired 5′-*O*-DMT-substituted phosphoramidites derived from **2**–**5** suitable for automated DNA oligomerization have been prepared by an alternate synthetic route. The phosphoramidite derived from FpC (**4**) (Figure 1) was successfully incorporated into DNA oligomers by means of automated solid-phase DNA synthesis. Hybridization studies with FpC-modified DNA oligomers revealed moderate duplex stabilization upon hybridization to the complementary strand as well as fluorescence responsiveness to the perfectly matched complement wherein diminution fluorescence signal was observed (G:pC base pair, Figure 1).

RESULTS AND DISCUSSION

Synthesis of Nucleoside Analogs 2–5. Nucleoside analogs **2**–**5** possessing substituted pyrrolocytoside nucleobases have been obtained in one-pot fashion via a reaction cascade involving a Sonogashira cross-coupling with an appropriate alkyne and subsequent 5-endo-dig cyclization.[2a] The synthesis of BFpC (**3**, Scheme 1) from protected cytosine **6a** and 2-ethynylbenzo[*b*]furan (**7**, Scheme 1) has been described elsewhere.[3]

Scheme 1. Preparation of *C*7-DMT Substituted Nucleobase Modified Nucleosides 2a–5a

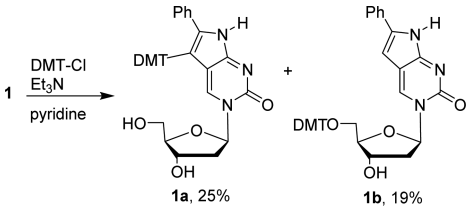


p-NO_2-PhpC (**2**, Scheme 1), FpC (**4**, Scheme 1), and DABCYLpC (**5**, Scheme 1) have been synthesized in the same manner by reacting suitably protected cytosines **6a** or **6b**[3] with commercially available *p*-NO_2-phenylacetylene (**10**), 2-ethynylfuran (**8**, prepared in two steps from fural using the modified literature protocol),[8] or DABCYL-modified alkyne **9** (Scheme 1) developed recently by our laboratory.[9] Subsequent deprotection (Scheme 1) led to preparation of nucleobase analogs **2**–**5** (Scheme 1) in reasonable overall yield (40–52%), see the Experimental Section for details. A thorough characterization of the fluorescence properties associated with BFpC (**3**) can be found elsewhere.[3] Characterization of the fluorescence properties associated with FpC (**4**) will be discussed below.

Treatment of Nucleoside Analogs 2–5 with DMT-Cl. Nucleoside analogs **2**–**5** were treated with DMT-Cl (Scheme 1) in pyridine at room temperature in the presence of Et_3N. The reaction was found to be unusually sluggish (requiring 24–72 h), compared to the same reaction with natural nucleosides, and in all four instances resulted in incomplete conversion of starting nucleosides. The reaction of **2**–**5** with DMT-Cl produced the DMT-protected products **2a**–**5a** in moderate (38–52%, Scheme 1) yield after flash column chromatography (FCC). Although 5′-*O*-DMT-protected nucleobases are known to be very sensitive to acidic environments, the isolated products **2a**–**5a** failed to display this property. When analyzed by thin-layer chromatography (TLC), the spots associated with **2a**–**5a** did not turn orange upon staining with 1 M HCl (expected due to DMT cation formation). Moreover, compounds **2a**–**5a** remained stable; i.e., no DMT was removed as judged by TLC and high-resolution mass spectrometry (HR-MS) analysis, even after prolonged exposure to strong acid (neat TFA, 12 h).

When PhpC (**1**)[3] was treated with the DMT-Cl under similar conditions for a prolonged period of time (up to 10 days was required), two products were obtained in low yields (**1a**, 25%; **1b**, 19%, Scheme 2), and their structures were assigned on the basis of the analogy with the NMR spectra acquired for **2a**–**5a**, as discussed below.

Scheme 2. Reaction of PhpC (1) with DMT-Cl



Spectroscopic Characterization of 2a–5a. Detailed spectroscopic characterization of **2a**–**5a** was carried out, and the spectra were compared to those associated with nucleoside analogs **2b**–**5b**, prepared as described later. HR-MS spectra of **2a**–**5a** were consistent with the presence of the DMT group; this was further supported by the ^1H and ^{13}C NMR spectra associated with **2a**–**5a**. As an illustrative example, the ^{1}NMR spectra for the benzo[*b*]furyl derivatives **3a,b** are shown in Figure 2. For comparison, selected ^1H NMR chemical shifts associated with compounds **2a**–**5a** are listed in Table 1.

Initially, upon isolation of **3a** by column chromatography, and expecting the normal 5′-*O*-dimethoxytrityl ether, we were pleased with the correspondence of the calculated formula mass and the observed HR-MS. However, we were perplexed by aspects of the ^{1}NMR spectrum. For example, the presence of two D_2O-exchangeable signals in the spectrum for **3a** at δ 5.26 (d, J = 4.0 Hz, 1H) and 4.71 (t, J = 5.5 Hz, 1H) implied that neither 5′- nor 3′-OH present in **3** [δ 5.30 (d, J = 5.5 Hz, 1H) and 5.15 (t, J = 5.5 Hz, 1H), DMSO-d_6][3] were converted to an ether (Figure 3, panels A and B and Table 1). Another D_2O-exchangeable signal (N_5-H) associated with **3a** was present at δ 11.74 (s, 1H), discounting possible alkylation of the pyrrolo-

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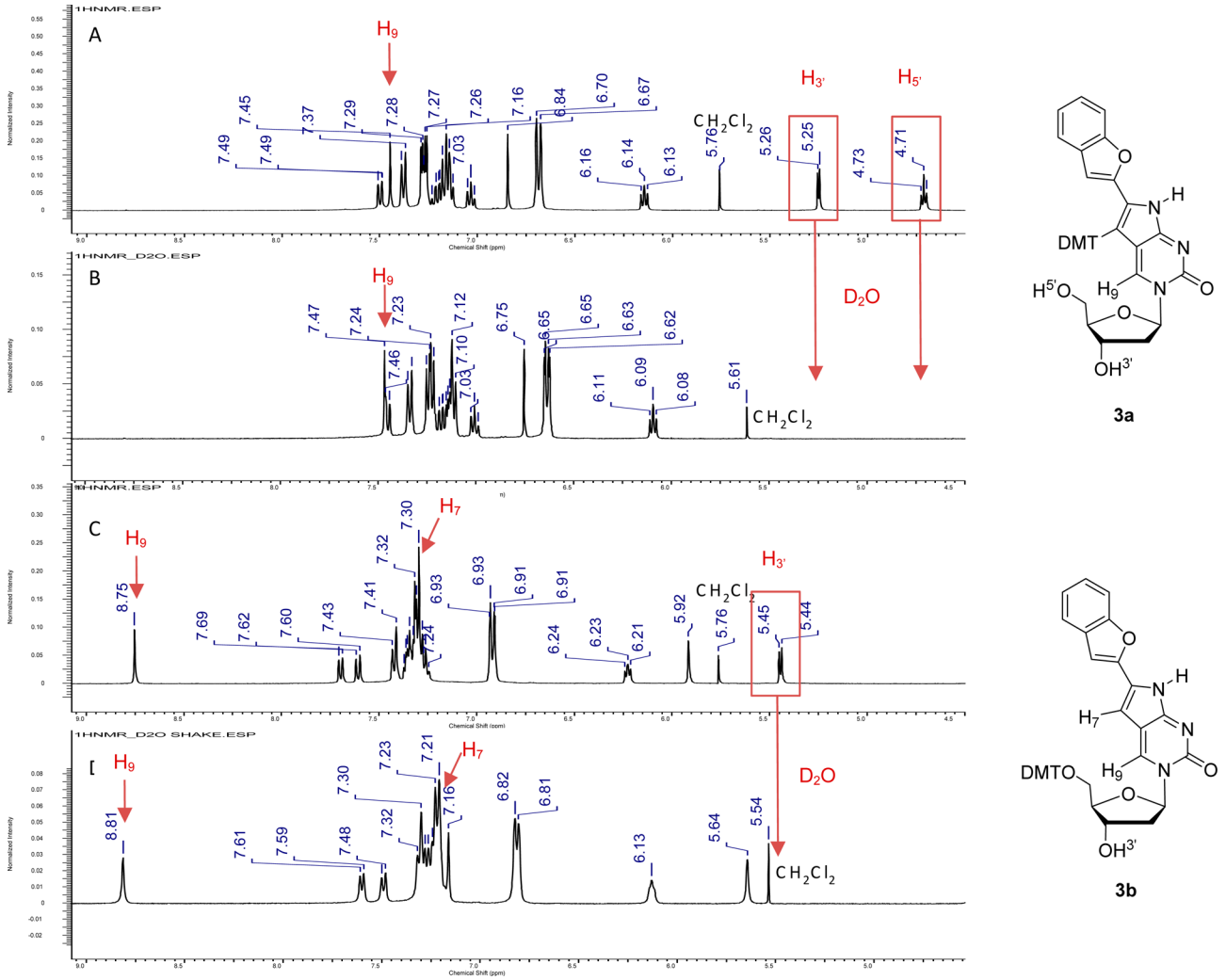

Figure 2. Comparison of the low-field region of ^{1}H NMR spectra associated with **3a** [panels A (DMSO−D6) and B (DMSO-d_6 + D_2O)] with that associated with **3b** [panels C (DMSO-d_6) and D (DMSO-d_6 + D_2O)]. Note that the signals at δ 5.26 (d, J = 4.0 Hz, 1H) and 4.71 (t, J = 5.5 Hz, 1H) associated with **3a** (panel A) disappear upon shaking with D_2O (3′- and 5′-OH groups, panel B). The signal at δ 5.45 (d, J = 5.0 Hz, 1H) associated with **3b** (panel C) disappears upon shaking with D_2O (3′-OH group, panel D), as highlighted by the red boxes and arrows. Resonances due to H_9 and H_7 protons are indicated by red labels.

nitrogen (see the Supporting Information). The presence of three D_2O-exchangeable signals in ^{1}H NMR spectra of **3a** (and similarly for **2a**−**5a**, Table 1) and the lack of reactivity toward acid treatment was consistent with C−C connectivity between the nucleobase and the DMT group. Also notable in the spectrum was the absence of the singlet typically attributable to the proton labeled H_9 of the phenylpyrrolocytosine moiety. This proton characteristically possesses a chemical shift in the approximate range δ 8.50−8.80.[10] Since there are no such signals present in the ^{1}H NMR spectra associated with **3a** (or any of **2a**−**5a**), our initial suspicion was that the DMT group was attached to C_9,[5b] which was counterintuitive to the presumed sites of the heterocycle susceptible to electrophilic substitution. Clearly, further structural analysis was needed to resolve the structural assignment. Our attempts to grow crystals of **2a**−**5a** suitable for an X-ray analysis were unsuccessful and we turned to 2D NMR spectroscopy (Figure 3). Nucleosides **3a** and **5a** were subjected to further scrutiny, while the structures of the remaining nucleosides (**2a**, **4a**) were assigned by analogy.

Below is shown the assignment for the benzo[*b*]furyl derivative **3a**, while the spectra for **5a** is presented in the Supporting Information. The notion of a C−C linkage of the DMT group was supported by the presence of a quaternary carbon in the range δ 57.9−58.8 (see the Experimental Section and in the Supporting Information) in the ^{13}C NMR spectra of **3a** (and all of **2a**−**5a**) as well as by the stability of analogs **2a**−**5a** under acidic conditions (no DMT group removal). All characteristic signals due to the presence of a deoxyribose subunit were present in the ^{1}H NMR spectrum associated with **2a**−**5a** (see the Supporting Information), thus ruling out the unlikely derivatization of the carbohydrate moiety with the DMT group

The chemical shift of the anomeric carbon present in **3a** (δ 87.2) and **5a** (δ 86.6) was determined by HSQC NMR as depicted in Figure 3 (**3a**, panel A, red arrows) or in the Supporting Information (**5a**). The HSQC NMR spectrum of **3a** indicated that the proton exhibiting δ 7.45 is correlated with a carbon with δ 139.8. Similarly, a proton with δ 7.20 (**5a**) was found to be correlated with a carbon with δ 138.3; see the Supporting Information.

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Table 1. Selected ^{1}H NMR Chemical Shifts Associated with Compounds 1a−5a and 1b−5b

compd	3′-, 5′-OH[a,b]	anomeric H[a]	H_7, H_9[a,c]
1a	5.24 (s, 1H)	6.10 (t, 1H)	7.19 (s, 1H)
	4.66 (s, 1H)		
2a	5.22 (d, 1H)	6.07 (t, 1H)	7.24 (s, 1H)
	4.62 (t, 1H)		
3a	5.26 (d, 1H)	6.14 (t, 1H)	7.45 (s, 1H)
	4.72 (t, 1H)		
4a	5.24 (d, 1H)	6.15 (t, 1H)	7.32 (s, 1H)
	4.71 (t, 1H)		
5a	5.25 (d, 1H)	6.10 (t, 1H)	7.20 (s, 1H)
	4.66 (t, 1H)		
1b	5.44 (d, 1H)	6.24 (t, 1H)	5.81 (s, 1H)
			8.66 (s, 1H)
2b	5.45 (d, 1H)	6.21 (t, 1H)	6.10 (s, 1H)
			8.77 (s, 1H)
3b	5.45 (d, 1H)	6.23 (t, 1H)	5.92 (s, 1H)
			8.75 (s, 1H)
4b	5.41 (d, 1H)	6.22 (t, 1H)	5.67 (s, 1H)
			8.59 (s, 1H)
5b	5.45 (d, 1H)	6.24 (t, 1H)	5.89 (s, 1H)
			8.71 (s, 1H)

[a]Chemical shifts are given in ppm (DMSO-d_6), and only multiplicities of the peaks are provided; for J constants see the Experimental Section. [b]Compounds **1b−5b** contain only 3′-OH group. [c]Compounds **1a−5a** contain only H_9 proton.

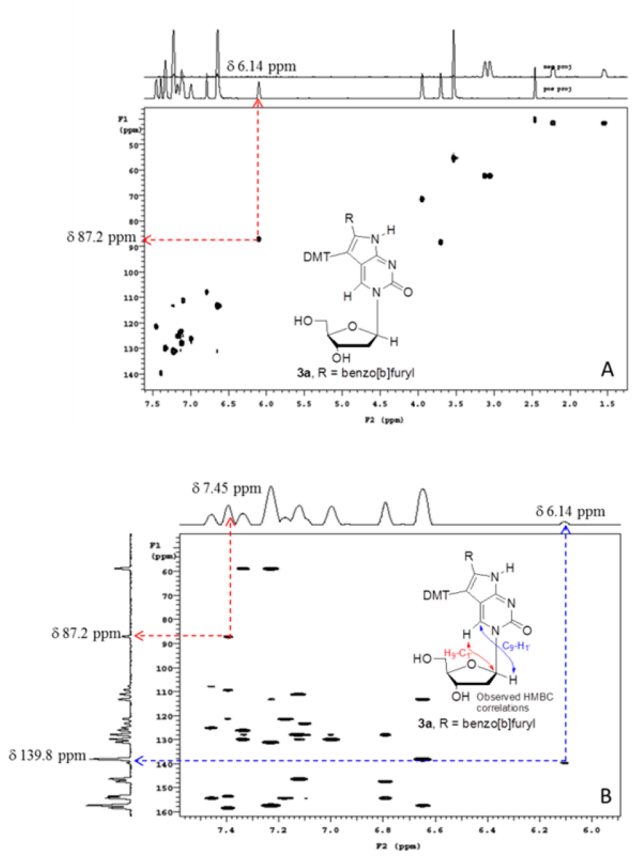

Figure 3. HSQC correlations associated with **3a** (panel A), the key correlation is indicated by red arrows. HMBC correlations associated with **3a** (panel B), where the key correlations are indicated by red and blue arrows.

HMBC NMR spectra of **3a** and **5a** revealed the presence of the correlation between the anomeric carbon and the proton at δ 7.45 (s, 1H, **3a**, Figure 3, panel B, red arrows) and δ 7.20 (s, 1H, **5a**, Supporting Information). We have also observed the HMBC correlations between the carbon with δ 139.8 (**3a**) and the anomeric proton δ 6.14 (t, J = 6.5 Hz, 1H, **3a**) (Figure 3, panel B, blue arrows) or the analogous correlation for **5a** between ^{13}C δ 138.3 and ^{1}H δ 6.10 (t, J = 6.5 Hz, 1H) (see Supporting Information). Overall, the results of 2D NMR studies indicate that the proton at the C_9 was not substituted. In each of **2a−5a**, the pyrrolo-type proton (H_7) has been replaced and the H_9 experiences significant upfield shift due the anisotropic shielding due to the DMT group.

The possibility of the attachment of the DMT group to other sites, such as the pendant aryl group present at C_6 (p-NO_2-Ph, 2-furyl, 2-benzo[b]furyl, and DABCYL), is not consistent with the observed spectra. For instance, two doublets at δ 7.85 (d, J = 8.5 Hz, 2H) and 7.18 (d, J = 8.5 Hz, 2H) are present in the ^{1}H NMR spectrum of **2a** (p-substituted benzene ring), while four doublets (two p-substituted benzene rings) at δ 7.78 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), and 6.83 (d, J = 9.0 Hz, 2H) are present in the ^{1}H NMR spectrum of **5a** (see the Experimental Section and the Supporting Information) indicating that both the p-NO_2-Ph moiety present in **2a** and the DABCYL moiety present in **5a** are intact. In totality, these results indicate that the DMT group is attached to the C_7 of the pyrrolocytosine moiety. Although competitive electrophilic addition to the heterocycle with ether formation is somewhat surprising, a similar observation has been made previously, wherein the reaction of indole with trityl chloride in pyridine leads to the formation of C-substituted 3-tritylindole as a sole product in 75% yield.[11]

Quantum Chemical Calculations. The unusual electrophilic attack on the nucleobase, which was competitive with O-dimethoxytritylation as observed for PhpC (**1**, Scheme 2), was surprising, since it was observed to occur on the pyrrole ring irrespective of the nature of the 6-substituent. The isolated yields of the electrophilic substitution products **2a−5a** were somewhat affected by the electron-rich character of the aromatic group, yet even the p-nitrophenyl-substituted pyrrolcytosine gave the substitution product over ether formation. In order to gain some insight on the site of reactivity in the heterocyclic skeletons present in **1−5** toward electrophilic aromatic substitution, quantum chemical calculations were performed.

The calculations were performed on the aglycon possessing an isopropyl group attached to N1 rather than deoxyribose to reduce the computational burden. The structures were geometry optimized at the Hartree−Fock 6-311+G** level using their lowest-energy conformer (PM6) as a starting point. Surfaces were constructed in Spartan '14 as ionization potential maps on electron density using the H−F output (see the Supporting Information). The calculations indicate that C_7, the observed site of substitution, possesses the lowest ionization potential in almost every case. For example, PhpC (**1**, modeled with an $N1$-isopropyl, Figure 4) supports this hypothesis as well as the above-described structural assignment, indicating that reactivity occurs at C_7 (pyrrole ring) of the nucleobase analog.

Once the DMT group installs at the C_7, additional derivitization of the 5′-OH is not observed, and vice versa, most likely due to steric hindrance. This reactivity of nucleobase-modified nucleosides analogs is rather novel; to the best of our knowledge, it has only been observed once

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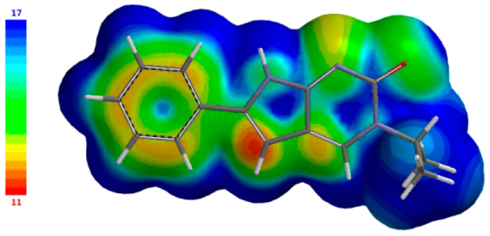


Figure 4. Ionization potential map (eV) superimposed to the 6-phenylpyrrolcytosine-derived model compound.

previously for a tricyclic analog of acyclovir with the stronger electrophile trityl chloride.[12]

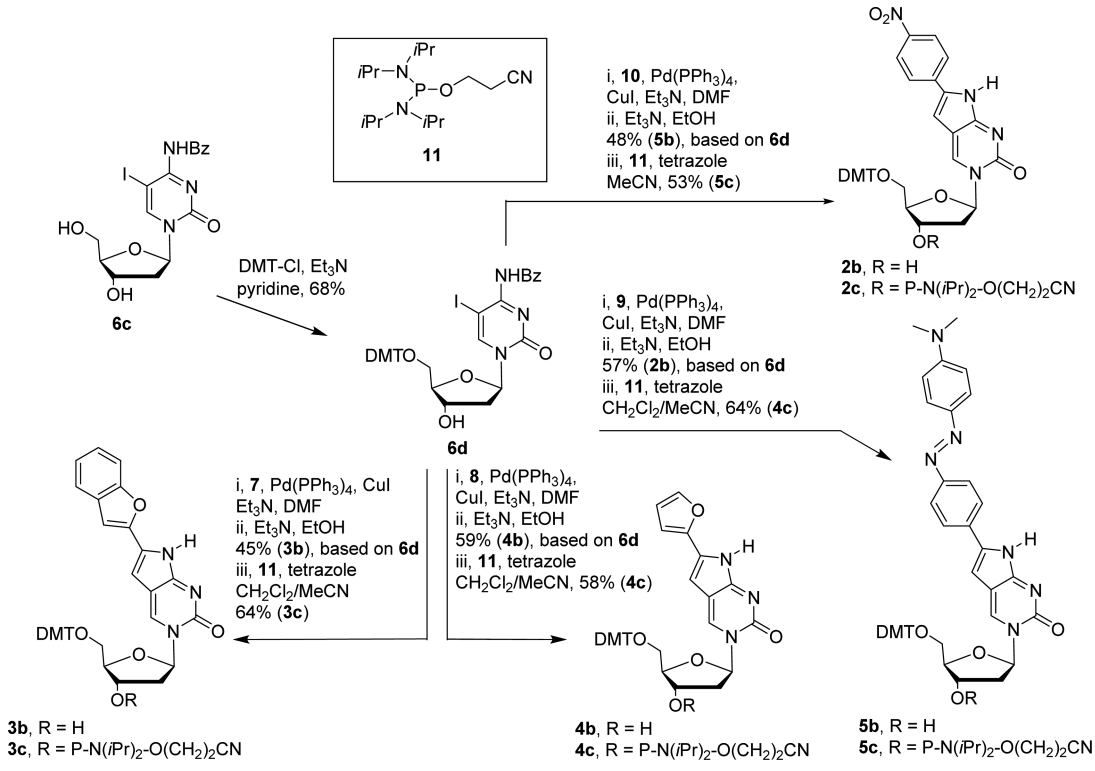
Synthesis of Phosphoramidites 2c−5c. To obtain monomers **2c−5c** suitable for automated DNA oligomerization, a different synthetic strategy was utilized. *N*4-Benzoyl-5-iodo-deoxycytosine (**6c**), prepared by iodination of deoxycytosine[13] followed by global benzoylation and subsequent 3′-, 5′-*O*-debenzoylation,[14] was reacted with DMT-Cl (Scheme 3). The reaction proceeded without difficulty and 5′-*O*-DMT-*N*4-benzoyl-5-iododeoxycytosine (**6d**) was obtained in 68% yield after FCC. The spectra associated with **6d** were fully consistent with its structure. A key step in the synthesis,[15] a reaction cascade involving a Sonogashira cross-coupling between **6d** and alkynes **7−10**, followed by subsequent 5-endo-dig cyclization, was found to proceed smoothly, furnishing the 5′-*O*-DMT protected pyrrolocytosine analogs **2b−5b** in reasonable yields (45−59%, Scheme 3) after FCC purification (see the Supporting Information for experimental details). ^{1}H and ^{13}C NMR and HR-MS spectra were fully consistent with the structure of **2b−5b** (see Table 1 for selected chemical shifts and the Supporting Information for the full spectra).

Treatment of **2b−5b** with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (**11**, Scheme 3) in the presence of tetrazole[16] proceeded smoothly, affording the phosphoramidites **2c−5c** in reasonable yield (53−64%) after the purification. We found the purification of **3c−5c** by preparative TLC (PTLC) to be more effective, as opposed to FCC, at removal of *H*-phosphonate impurities (δ ca. 5−15) as judged by ^{31}P NMR spectroscopy. The PTLC purification failed for the *p*-NO_2-PhpC-derived phosphoramidite **2c**; however, it was purified by precipitation from CH_2Cl_2/hexanes mixture as described in the Experimental Section. A complete spectral characterization of phosphoramidites **2c−5c** can be found in the Supporting Information. By utilizing the above-described synthetic methodology, the phosphoramidites **2c−5c** have been synthesized that are suitable for automated DNA oligomerization.[6]

Fluorescence Associated with FpC (4). Characterization of the fluorescence properties associated with FpC (**4**, Supporting Information) revealed that this structural modification leads to a fluorophore (Φ 0.58, EtOH, Stoke's shift 73 nm) possessing properties comparable to those of PhpC (**1**, Φ 0.61, EtOH, Stoke's shift 78 nm) developed in our laboratory previously.[2a,3] Solvatochromaticity associated with FpC (**4**, Supporting Information) also compared well with that associated with PhpC (**1**).[3]

DNA Oligomerization of Phosphoramidite 4c and Hybridization Studies. FpC-derived phosphoramidite **4c** was successfully incorporated into DNA oligomers. The DNA sequences have been assembled by automated solid-phase DNA synthesis.[6] Two sequences have been prepared as follows: GTAG-ATX-ACT (5′-O → 3′-O, sequence 1, X = FpC) and GTAG-ATC-XCT (5′-O → 3′-O, sequence 2, X = FpC). The sequences were purified by high-performance liquid chroma-

Scheme 3. Preparation of 5′-*O*-DMT-Nucleobase Analogs 2b−5b and Corresponding Phosphoramidites 4−5c



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tography (HPLC) and were characterized by MS (see the Supporting Information for details).

Hybridization studies with sequences 1 and 2 were performed at 10^{-6} M in a phosphate buffer (pH 7) containing 10 mM $MgCl_2$. Replacement of natural C with FpC resulted in moderate duplex stabilization (sequence 1, +4.0 °C, sequence 2, +5.0 °C) as indicated in Table 2, which are similar to the results obtained for PhpC (**1**).[15]

Table 2. DNA Hybridization Studies

sequence[a] (5′ → 3′)	X	T_m (°C)	ΔT_m (°C)	MM[b] DNA T_m (°C)
GTAG-ATX-ACT	C	35		<20, all MMs
GTAG-ATX-ACT	FpC	40	+5.0	<20, all MMs
GTAG-ATC-XCT	C	39		20 (MMA)
				22 (MMC)
				24 (MMT)
GTAG-ATC-XCT	FpC	43	+4.0	23 (MMA)
				28 (MMC)
				35 (MMT)

[a]Measurements were carried out as described in the Experimental Section. Each strand was present at 1 μM in a buffer containing 100 mM NaCl, 10 mM, $MgCl_2$, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. [b]MM = mismatch.

The fluorescence response associated with DNA duplexes featuring sequences 1 and 2 was found to be somewhat sensitive to the presence of mismatch (ca. 2-fold fluorescence increase compared to complementary strand), although no fluorescence-based mismatch discrimination was observed with FpC-modified DNA oligomers. The results of these studies are depicted in Figure 5. Although not studied comprehensively herein, there appears to be some sequence context dependence on the fluorescence response. Sequence 2, which has deoxycytidine nearest neighbors to the fluorescent nucleotide, shows ca. 65% reduction in fluorescence comparing the duplex to the single strand, while sequence 1, with a deoxyadenosine neighbor, shows ca. 45% reduction in fluorescence.

CONCLUSIONS

In summary, we have described an unusual aromatic electrophilic substitution of pyrrolocytosine analogs **1**−**5** with dimethoxytrityl chloride (DMT-Cl) leading to the formation of C7-DMT-substituted nucleoside analogs **1a**−**5a**. These observations prompt us to make a cautionary note for other chemists involved in development of nucleobase analogs, as we believe that the unusual reactivity between **1**−**5** and DMT-Cl observed by us may complicate the routine DMT protection of other electron-rich aromatic and heteroaromatic nucleobase analogs. This reactivity also suggests a route for the preparation of otherwise difficult to access C7-substituted pyrrolocytosine analogs via, for example, electrophilic halogenation and further derivatization.

A simple reordering of the steps in the synthetic scheme permitted the preparation of the desired 5′-*O*-DMT-protected nucleoside analogs **2b**−**5b**; subsequently, a sufficient amount of phosphoramidites **2c**−**5c** for automated DNA oligomerization was obtained.

FpC (**4**) was found to be a relatively bright blue fluorophore possessing the properties comparable to those observed for the PhpC (**1**) described previously.[2a,3] FpC-derived phosphoramidite **4a** was successfully incorporated into DNA oligomers by means of automated solid-phase DNA synthesis. The

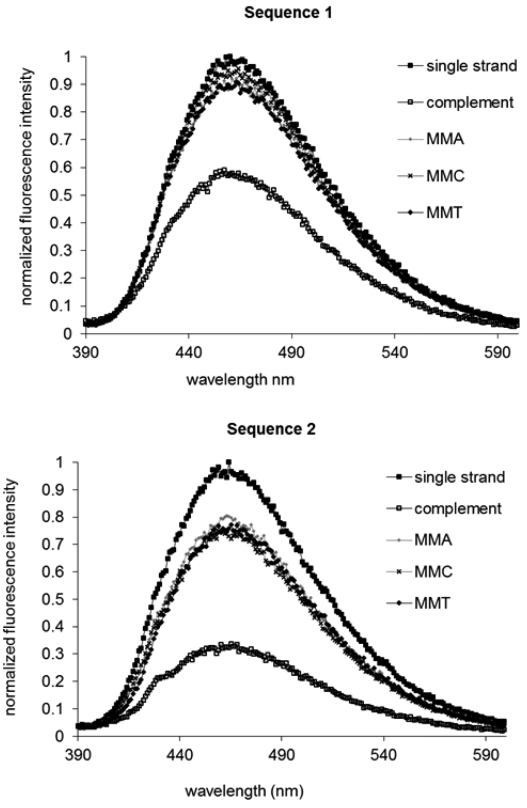

Figure 5. Fluorescence hybridization studies with FpC-modified DNA sequences 1 (top) and 2 (bottom); MM = mismatch.

incorporation of the FpC-modified nucleobase was found to have a moderate stabilizing effect (ca. +5 °C) for an internal site of substitution upon hybridization to the complementary strands of DNA and was able to discriminate perfectly matched sequences for mismatched sequences by selective quenching of the fluorescence signal, in the case of the former.

EXPERIMENTAL SECTION

General Experimental Protocols. Reagents were commercially available unless otherwise stated, and all solvents were reagent grade unless otherwise stated. Dry solvents (CH_2Cl_2, dioxane, DMF, Et_2O, THF) for chemical synthesis were obtained by drying on activated Al_2O_3 columns in a solvent purification system or by drying over 3 Å molecular sieves (Et_3N, MeCN, pyridine). Spectroscopic-grade EtOH has been used to perform spectroscopic studies. Solvents were removed under reduced pressure in a rotary evaporator, and organic extracts were dried over Na_2SO_4. Reaction mixtures involving air-sensitive reagents [$Pd(PPh_3)_4$, CuI] were degassed by repeated freeze−pump−thaw cycles using dry N_2. FCC was carried out using silica gel (SiO_2; mesh size 230−400 Å). TLC was carried out on an Al-backed silica gel plate with compounds visualized by 1 M HCl, phosphomolybdic acid stain, and UV light. PTLC was carried out on glass-backed silica gel plates (20 × 20 cm), with layer thickness 1000 μm; compounds were visualized by UV light. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million and are referenced as follows: CD_2Cl_2 (5.32 ppm) and DMSO-d_6 (2.49 ppm) for ^1H NMR and CD_2Cl_2 (54.0 ppm) and DMSO-d_6 (39.5 ppm) for ^{13}C NMR (100 MHz). Ultra-performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7 μm; 1.0 i.d. ×100 mm) and HR-ESI-MS and diode array UV detectors. The mobile phase for method A was 100% H_2O to 100% MeCN (both solvents containing 0.1% HCOOH) over 5 min, by linear gradient, and then 100% MeCN over 2 min, at flow rate 0.1 mL/min. The mobile phase for method B was 100% H_2O to 100% MeOH over 5 min, by

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linear gradient, and then 100% MeOH over 2 min, at flow rate 0.1 mL/min. HPLC purification of the DNA oligomers was carried out on a Microsorb-MW C_{18} 100 Å column (4.6 id ×250 mm), using 0.05 M ammonium acetate buffer (AAB, pH 6.5). Mobile phases for the various methods are as follows: method C (DMT-protected sequence 1), from 99% AAB−1% MeCN to 60% AAB−40% MeCN over 30 min, by linear gradient, and then 60% AAB−40% MeCN over 2 min, at flow rate 1 mL/min; method D (DMT-protected sequence 2), from 99% AAB−1% MeCN to 46% AAB−54% MeCN over 27 min, by linear gradient, at flow rate 1 mL/min; method E (sequence 1), from 99% AAB−1% MeCN to 50% AAB−50% MeCN over 25 min, by linear gradient, at flow rate 1 mL/min; method F (sequence 2), from 99% AAB−1% MeCN to 48% AAB−52% MeCN over 16 min, by linear gradient, at flow rate 1.2 mL/min. Mass spectra (MS) were obtained on a mass spectrometer using electrospray ionization (ESI) and time-of-flight (TOF) analyzer. UV−vis spectra were acquired using an UV−vis spectrophotometer equipped with temperature-controlled cell holder. Steady-state fluorescence spectra were acquired using a PTI quantmaster fluorimeter. All fluorescence measurements were performed using a 1-cm-wide four-sided quartz cuvette. UV−vis measurements were performed using a 1-cm-wide two-sided quartz glass cuvette.

Reaction of PhpC (1) with DMT-Cl. A flask containing PhpC[3] (**1**, 52 mg, 0.16 mmol) and DMT-Cl (70 mg, 0.21 mmol) was flushed with N_2, followed by the addition of dry pyridine (1 mL) and dry Et_3N (160 μL, 1.14 mmol). The reaction mixture was stirred at rt (room temperature) under N_2 atmosphere for 240 h (10 days). The reaction was quenched by the addition of MeOH (100 μL) and was coevaporated with toluene (3 × 60 mL). The residue was subjected to PTLC, eluted with CH_2Cl_2/MeOH/NH_4OH (360:39:1). The bands containing the products were carved off the plates and extracted with CH_2Cl_2/MeOH/NH_4OH (380:19:1), the SiO_2 was filtered off, and the filtrates were concentrated, yielding two fractions, the more polar 7-*C*-DMT-PhpC (**1a**, 25 mg, 25%) and the less polar 5′-*O*-DMT-PhpC (**1b**, 19 mg, 19%).

7-*C*-DMT-PhpC (**1a**): yellow solid; UPLC (method A) t_R = 5.06 min; ^{1}H NMR (DMSO-d_6) δ 11.29 (s, D_2O exch, 1H), 7.19 (s, 1H), 7.02 (m, 14H), 6.63 (m, 4H), 6.10 (t, *J* = 6.5 Hz, 1H), 5.24 (br s, D_2O exch, 1H), 4.66 (br s, D_2O exch, 1H), 4.45 (m, 1H), 3.92 (m, 1H), 3.69 (m, 1H), 3.68 (s, 6H), 3.03 (m, 1H), 2.21 (m, 1H), 1.45 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 157.6, 157.2, 157.1, 153.4, 145.5, 138.0, 137.6, 137.3, 137.2, 132.8, 131.4, 131.3, 130.2, 129.1, 127.2, 127.0, 125.9, 116.4, 112.5, 109.8, 87.6, 86.3, 70.7, 61.9, 58.0, 54.9 (2 × C), 40.8; HRMS (ESI) *m*/*z* found 630.2626 $[M + H]^+$ (calcd 630.2604 for $C_{38}H_{36}N_3O_6$).

5′-*O*-DMT-PhpC (**1b**): yellow solid; UPLC (method A) t_R = 5.59 min; ^{1}H NMR (DMSO-d_6) δ 11.80 (s, D_2O exch, 1H), 8.66 (s, 1H), 7.69 (m, 2H), 7.44 (m, 4H), 7.30 (m, 8H), 6.92 (m, 4H), 6.24 (t, *J* = 6.0 Hz, 1H), 5.81 (s, 1H), 5.44 (d, D_2O exch, *J* = 5.0 Hz, 1H), 4.45 (m, 1H), 3.98 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.41 (m, 1H), 3.32 (m, 1H), 2.43 (m, 1H), 2.20 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.9, 158.2, 158.1, 153.7, 144.3, 139.2, 135.9, 135.6, 135.2, 130.4, 129.8, 129.7, 128.8, 128.3, 128.0, 127.9, 126.8, 113.3, 96.4, 86.6, 86.1, 85.6, 68.9, 62.5, 55.0 (2 × C), 54.8, 41.5; HRMS (ESI) *m*/*z* found 630.2578 $[M + H]^+$ (calcd 630.2604 for $C_{38}H_{36}N_3O_6$).

Synthesis of *p*-NO_2-PhpC (2), BFpC (3), FpC (4), and DABCYLpC (5). Nucleobase analog BFpC (**3**) was prepared as described elsewhere.[3] Round bottom flasks containing *N*4-benzoyl-5-iodo-2′,3′-di-OAc-deoxycytidine (**6a**;[3] 577 mg, 1.07 mmol) and 2-ethynylfuran (**8**; 402 mg, 4.37 mmol) or *N*4-benzoyl-5-iodo-2′,3′-di-*O*-TBDMS-deoxycytidine (**6b**;[3] 700 mg, 1.02 mmol) and DABCYL-modified alkyne (**9**; 381 mg, 1.52 mmol) or *N*4-benzoyl-5-iodo-2′,3′-di-*O*-TBDMS-deoxycytidine (**6b**;[3] 643 mg, 0.94 mmol) and *p*-NO_2-phenylacetylene (**10**; 207 mg, 1.4 mmol) were charged with N_2, and dry DMF (4 mL, **6a** + **8**; 2.5 mL, **6b** + **10**) or dry THF (6 mL, **6b** + **9**) was added. The mixtures were degassed (see General Experimental Protocols), followed by the addition of $Pd(PPh_3)_4$ (**6a** + **8**, 123 mg, 0.11 mmol; **6b** + **9**, 116 mg, 0.1 mmol; **6b** + **10**, 108 mg, 0.094 mmol) and CuI (**6a** + **8**, 41 mg, 0.21 mmol; **6b** + **9**, 19 mg, 0.1 mmol; **6b** + **10**, 36 mg, 0.19 mmol). The mixtures were degassed again, Et_3N (**6a** + **8**, 1.49 mL, 10.66 mmol; **6b** + **9**, 3 mL, 21.52 mmol; **6b** + **10**, 1.3 mL, 9.38 mmol) was added, and the mixtures were stirred (in the dark, under N_2 atmosphere) for 5 h at 50 °C (**6a** + **8**; **6b** + **10**) or 48 h at 55 °C (**6b** + **9**). EtOH and Et_3N were added (2 mL each), and the stirring was continued for 5 h at 80 °C (**6a** + **8**), 18 h at 80 °C (**6b** + **9**), or 18 h at 70 °C (**6b** + **10**). The mixtures were cooled to rt, diluted with 4% EDTA solution (50 mL) or brine (**6b** + **10**, 100 mL), and extracted with EtOAc (2 × 30 + 20 mL). The combined organic extracts were washed with brine (3 × 50 mL), dried, and concentrated. The residues were subjected to FCC on 30 g of SiO_2, eluted with Et_2O/acetone (2:1) later replaced with Et_2O/acetone (1:1, **6a** + **8**); on 40 g of SiO_2, eluted with CH_2Cl_2 later replaced with CH_2Cl_2/MeOH (95:5, **6b** + **9**); or on 80 g of SiO_2, eluted with CH_2Cl_2/MeOH (95:5, **6b** + **10**). Evaporation of the eluates afforded the desired intermediates 2,3-di-*O*-TBDMS-*p*-NO_2-PhpC (orange solid, 350 mg, 62%), 2,3-di-*O*Ac-FpC (yellow solid, 257 mg, 60%), and 2,3-di-*O*-TBDMS-DABCYLpC (red solid, 423 mg, 59%).

2,3-Di-*O*-Ac-FpC (257 mg, 0.64 mmol) was suspended in MeOH (8 mL) and the mixture was cooled to 0 °C. K_2CO_3 (221 mg, 1.6 mmol) was added and the mixture was stirred for 1 h at 0 °C. The solvent was evaporated and the residue was subjected to FCC on 25 g of SiO_2, eluted with EtOAc/MeOH (95:5) later replaced with EtOAc/MeOH (9:1). Evaporation of the eluate afforded FpC [**4**; 176 mg, 87% (52%, based on **6a**)]: yellow solid; UPLC (method A) t_R = 3.75 min; ^{1}H NMR (DMSO-d_6) δ 11.81 (s, D_2O exch, 1H), 8.67 (s, 1H), 7.80 (d, *J* = 2.0 Hz, 1H), 6.93 (d, *J* = 3.5 Hz, 1H), 6.63 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.44 (s, 1H), 6.24 (t, *J* = 6.0 Hz, 1H), 5.28 (d, D_2O exch, *J* = 4.5 Hz, 1H), 5.12 (t, D_2O exch, *J* = 5.0 Hz, 1H), 4.24 (m, 1H), 3.89 (m, 1H), 3.66 (m, 2H), 2.36 (m, 1H), 2.03 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.6, 153.8, 146.0, 143.7, 136.3, 130.7, 112.0, 108.7, 107.8, 95.3, 87.9, 87.0, 69.9, 61.0, 41.4; HRMS (ESI) *m*/*z* found 318.1079 $[M + H]^+$ (calcd 318.1090 for $C_{15}H_{16}N_3O_5$).

Separate solutions of 2,3-di-*O*-TBDMS-*p*-NO_2-PhpC (171 mg, 0.28 mmol) and di-*O*Ac-DABCYLpC (423 mg, 0.6 mmol) in THF (3 mL, the former intermediate; 7 mL, the later intermediate) were cooled to 0 °C, followed by a slow addition of Et_3N·HF (160 μL, 1 mmol, the former intermediate; 295 μL, 1.8 mmol, the later intermediate). The cooling baths were removed, and the stirring was continued for 18 h at rt. The solvents were evaporated and the residues were subjected to FCC on 25 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (360:39:1), or 20 g of SiO_2, eluted with CH_2Cl_2/MeOH (9:1). Evaporation of the eluates afforded *p*-NO_2-PhpC [**2**, 82 mg, 77% (48%, based on **6b**)] or DABCYLpC [**5**, 230 mg, 81% (48%, based on **6b**)].

p-NO_2-PhpC (**2**): orange solid; UPLC (method A) t_R = 4.00 min; ^{1}H NMR (DMSO-d_6) δ 12.04 (s, D_2O exch, 1H), 8.87 (s, 1H), 8.28 (d, *J* = 9.0 Hz, 2H), 8.08 (d, *J* = 9.0 Hz, 2H), 7.08 (s, 1H), 6.24 (t, *J* = 6.0 Hz, 1H), 5.30 (d, D_2O exch, *J* = 4.5 Hz, 1H), 5.18 (t, D_2O exch, *J* = 5.0 Hz, 1H), 4.25 (m, 1H), 3.93 (m, 1H), 3.69 (m, 2H), 2.40 (m, 1H), 2.07 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.8, 153.7, 146.3, 138.4, 136.9, 136.8, 125.7, 124.1, 108.8, 101.5, 88.1, 87.4, 69.8, 60.9, 41.5; HRMS (ESI) *m*/*z* found 373.1158 $[M + H]^+$ (calcd 373.1148 for $C_{17}H_{17}N_4O_6$).

DABCYLpC (**5**): red solid; UPLC (method A) t_R = 4.67 min; ^{1}H NMR (DMSO-d_6) δ 11.89 (s, D_2O exch, 1H), 8.76 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 2H), 7.82 (m, 4H), 6.85 (m, 3H), 6.26 (t, *J* = 6.0 Hz, 1H), 5.31 (d, D_2O exch, *J* = 4.0 Hz, 1H), 5.19 (t, D_2O exch, *J* = 5.0 Hz, 1H), 4.26 (m, 1H), 3.92 (m, 1H), 3.68 (m, 2H), 3.07 (s, 6H), 2.38 (m, 1H), 2.06 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 160.0, 153.8, 152.6, 151.8, 142.7, 138.6, 136.7, 131.4, 125.8, 124.9, 122.4, 111.6, 109.2, 98.2, 87.9, 87.1, 69.9, 60.9, 45.7, 41.5; HRMS (ESI) *m*/*z* found 475.2079 $[M + H]^+$ (calcd 475.2094 for $C_{25}H_{27}N_6O_4$).

Synthesis of *C*7-DMT-*p*-NO_2-PhpC (2a), *C*7-DMT-BFpC (3a), *C*7-DMT-FpC (4a), and *C*7-DMT-DABCYLpC (5a). Separate flasks containing *p*-NO_2-PhpC (**2**; 75 mg, 0.2 mmol), BFpC (**3**; 134 mg, 0.37 mmol), FpC (**4**; 256 mg, 0.81 mmol), or DABCYLpC (**5**; 199 mg, 0.42 mmol) and DMT-Cl (**2**, 89 mg, 0.26 mmol; **3**, 161 mg, 0.47 mmol; **4**, 328 mg, 0.97 mmol; **5**, 185 mg, 0.5 mmol) were flushed with N_2, followed by the addition of dry pyridine (**2**, 1.2 mL; **3**, 3 mL; **4**, 5 mL; **5**, 15 mL) and dry Et_3N (**2**, 200 μL, 1.45 mmol; **3**, 350 μL, 2.54

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mmol; **4**, 790 μL, 5.65 mmol; **5**, 2.5 mL, 17.94 mmol). The reaction mixtures were stirred at rt (under N_2 atmosphere) for 24 h (**2**, **3**, and **4**) or 72 h. The reactions were quenched by the addition of MeOH (200 μL), the mixtures were coevaporated with toluene (3 × 100 mL). The residues were subjected to FCC as follows: **2a**, 40 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (360:39:1); **3a**, 25 g of SiO_2, eluted with acetone/EtOAc/Et_3N (55:40:5); **4a**, 70 g of SiO_2, eluted with CH_2Cl_2/MeOH/Et_3N (90:7:3); **5a**, 25 g of SiO_2, eluted with CH_2Cl_2/MeOH/Et_3N (90:7:3). Evaporation of the eluates afforded *C*7-*p*-NO_2-PhpC (**2a**, 52 mg, 38%), *C*7-DMT-BFpC (**3a**, 127 mg, 52%), *C*7-DMT-FpC (**4a**, 249 mg, 50%), and *C*7-DMT-DABCYLpC (**5a**, 151 mg, 49%).

*C*7-*p*-NO_2-PhpC (**2a**): yellow solid; UPLC (method A) t_R = 5.07 min; ^{1}H NMR (DMSO-d_6) δ 11.49 (s, D_2O exch, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.24 (s, 1H), 7.18 (d, *J* = 8.5 Hz, 1H), 7.14 (m, 5H), 6.95 (m, 4H), 6.63 (m, 4H), 6.07 (t, *J* = 6.5 Hz, 1H), 5.22 (d, D_2O exch, *J* = 4.0 Hz, 1H), 4.62 (t, D_2O exch, *J* = 5.5 Hz, 1H), 3.90 (m, 1H), 3.39 (m, 1H), 3.63 (s, 6H), 3.00 (m, 2H), 2.23 (m, 1H), 1.45 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 157.7, 157.4, 153.3, 145.9, 145.2, 139.9, 138.7, 137.0, 136.9, 135.3, 131.5, 130.6, 130.4, 127.4, 126.1, 122.0, 118.5, 112.6, 109.6, 87.7, 86.5, 70.7, 61.9, 57.9, 54.9 (2 × C), 40.8; HRMS (ESI) *m*/*z* found 675.2449 [M + H]$^+$ (calcd 675.2455 for $C_{38}H_{35}N_4O_8$).

*C*7-DMT-BFpC (**3a**): yellow solid; UPLC (method A) t_R = 5.20 min; ^{1}H NMR (DMSO-d_6) δ 11.74 (s, D_2O exch, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.45 (s, 1H), 7.38 (m, 2H), 7.28 (m, 4H), 7.20 (m, 1H), 7.16 (m, 4H), 7.03 (m, 1H), 6.84 (s, 1H), 6.69 (m, 4H), 6.14 (t, *J* = 6.5 Hz, 1H), 5.26 (d, D_2O exch, *J* = 4.0 Hz, 1H), 4.72 (t, D_2O exch, *J* = 5.5 Hz, 1H), 3.99 (m, 1H), 3.74 (m, 1H), 3.57 (s, 6H), 3.17 (m, 1H), 3.09 (m, 1H), 2.27 (m, 1H), 1.60 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 158.5, 157.5, 154.4, 153.7, 147.5, 146.4, 139.8, 138.3 (2 × C), 131.1, 129.9, 128.0, 127.9, 126.5, 126.2, 125.1, 123.3, 121.4 (2 × C), 113.3, 111.2, 109.4, 107.9, 88.3, 87.2, 71.4, 62.3, 58.8, 55.3 (2 × C), 41.5; HRMS (ESI) *m*/*z* found 670.2581 [M + H]$^+$ (calcd 670.2553 for $C_{40}H_{36}N_3O_7$).

*C*7-DMT-FpC (**4a**): yellow solid; UPLC (method A) t_R = 4.89 min; ^{1}H NMR (DMSO-d_6) δ 11.53 (s, D_2O exch, 1H), 7.32 (s, 1H), 7.31 (m, 2H), 7.25 (m, 5H), 7.16 (m, 2H), 7.08 (m, 1H), 6.74 (m, 4H), 6.32 (m, 2H), 6.15 (t, *J* = 6.0 Hz, 1H), 5.24 (d, D_2O exch, *J* = 4.0 Hz, 1H), 4.71 (t, D_2O exch, *J* = 5.5 Hz, 1H), 3.98 (m, 1H), 3.72 (m, 1H), 3.68 (s, 6H), 3.17 (m, 1H), 3.09 (m, 1H), 2.24 (m, 1H), 1.58 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 158.0, 157.0, 153.3, 146.1, 144.9, 143.4, 138.5, 137.8, 130.6, 129.3, 127.5, 126.7, 125.6, 118.1, 112.9, 111.4, 111.1, 109.2, 87.8, 86.5, 71.0, 61.8, 58.2, 54.9 (2 × C), 41.0; HRMS (ESI) *m*/*z* found 620.2413 [M + H]$^+$ (calcd 620.2397 for $C_{36}H_{34}N_3O_7$).

*C*7-DMT-DABCYLpC (**5a**): red solid; UPLC (method A) t_R = 5.63 min; ^{1}H NMR (DMSO-d_6) δ 11.39 (s, D_2O exch, 1H), 7.78 (d, *J* = 9.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.20 (s, 1H), 7.16 (m, 4H), 7.10 (m, 1H), 7.04 (d, *J* = 8.0 Hz, 2H), 6.98 (m, 4H), 6.83 (d, *J* = 9.0 Hz, 2H), 6.63 (m, 4H), 6.10 (t, *J* = 6.5 Hz, 1H), 5.25 (d, D_2O exch, *J* = 4.0 Hz, 1H), 4.66 (t, D_2O exch, *J* = 5.5 Hz, 1H), 3.91 (m, 1H), 3.69 (m, 1H), 3.59 (s, 3H), 3.58 (s, 3H), 3.06 (m, 7H), 3.01 (m, 1H), 2.22 (m, 1H), 1.47 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 158.0, 157.5, 153.6, 152.7, 151.1, 145.7, 142.8, 137.5, 137.2, 134.2, 131.6, 130.5, 130.1, 127.5, 124.9, 120.8, 117.3, 112.8, 111.8, 110.0, 87.8, 86.6, 70.9, 62.1, 58.2, 55.0 (2 × C), 40.8, 40.1; HRMS (ESI) *m*/*z* found 777.3431 [M + H]$^+$ (calcd 777.3401 for $C_{46}H_{45}N_6O_6$).

Synthesis of 5′-*O*-DMT-*p*-NO_2-PhpC (2b), 5′-*O*-DMT-BFpC (3b), 5′-*O*-DMT-FpC (4b), and 5′-*O*-DMT-DABCYLpC (5b). Separate round-bottom flasks containing *N*4-benzoyl-5-iodo-5′-*O*-DMT-deoxycytidine (**6d**; 250 mg, 0.33 mmol) and 2-ethynylbenzo[*b*]furan (**7**, 70 mg, 0.49 mmol), 2-ethynylfuran (**8**; 110 mg, 1.2 mmol), DABCYL-modified alkyne (**9**; 103 mg, 0.41 mmol), or *p*-NO_2-phenylacetylene (**10**; 80 mg, 0.54 mmol) were charged with N_2, and dry DMF (2 mL) was added. The mixtures were degassed (see General Experimental Protocols), followed by the addition of $Pd(PPh_3)_4$ (38 mg, 0.03 mmol) and CuI (13 mg, 0.07 mmol). The mixtures were degassed again, Et_3N (460 μL, 3.3 mmol) was added, and the mixtures were stirred (in the dark, under N_2 atmosphere) for 4 h at 50 °C. EtOH and Et_3N were added (1 mL each), and the stirring was continued for 18 h at 70 °C. The mixtures were cooled to rt, diluted with brine (50 mL), and extracted with EtOAc (3 × 20 mL). Combined organic extracts were washed with brine (2 × 50 mL), dried, and concentrated. The residues were subjected to FCC on 30 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (380:19:1), later replaced with CH_2Cl_2/MeOH/NH_4OH (360:39:1, **6d** + **7**); 30 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (380:19:1, **6d** + **8** and **6d** + **10**); or 50 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (380:19:1), later replaced with CH_2Cl_2/MeOH/NH_4OH (360:39:1, **6d** + **9**). Evaporation of the eluates afforded 5′-*O*-DMT-*p*-NO_2-PhpC (**2b**; 106 mg, 48%, based on **6d**), 5′-*O*-DMT-BFpC (**3b**; 100 mg, 45%, based on **6d**), 5′-*O*-DMT-FpC (**4b**; 120 mg, 59%, based on **6d**), and 5′-*O*-DMT-DABCYLpC (**5b**; 146 mg, 57%, based on **6d**).

5′-*O*-DMT-*p*-NO_2-PhpC (**2b**): orange solid; UPLC (method A) t_R = 5.62 min; ^{1}H NMR (DMSO-d_6) δ 12.04 (s, D_2O exch, 1H), 8.77 (s, 1H), 8.29 (d, *J* = 9.0 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.42 (m, 2H), 7.31 (m, 7H), 6.92 (m, 4H), 6.21 (t, *J* = 4.5 Hz, 1H), 6.10 (s, 1H), 5.45 (d, D_2O exch, *J* = 4.5 Hz, 1H), 4.43 (m, 1H), 4.01 (m, 1H), 3.71 (s, 3H), 3.70 (s, 3H), 3.36 (m, 2H), 2.45 (m, 1H), 2.21 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.9, 158.2 (2 × C), 153.6, 146.4, 144.5, 137.9, 136.7, 136.6, 135.5, 135.2, 129.9, 129.8, 128.0, 127.9, 126.9, 125.5, 124.2, 113.3, 108.6, 87.0, 86.1, 85.8, 68.9, 62.5, 55.0 (2 × C), 54.9, 41.4; HRMS (ESI) *m*/*z* found 675.2468 [M + H]$^+$ (calcd 675.2455 for $C_{38}H_{35}N_4O_8$).

5′-*O*-DMT-BFpC (**3b**): yellow solid; UPLC (method A) t_R = 5.84 min; ^{1}H NMR (DMSO-d_6) δ 12.08 (s, D_2O exch, 1H), 8.75 (s, 1H), 7.70 (d, *J* = 7.5 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.42 (m, 2H), 7.31 (m, 10H), 6.92 (m, 4H), 6.23 (t, *J* = 6.5 Hz, 1H), 5.92 (s, 1H), 5.45 (d, D_2O exch, *J* = 5.0 Hz, 1H), 4.44 (m, 1H), 3.99 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.36 (m, 2H), 2.45 (m, 1H), 2.22 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.7, 158.2, 154.3, 153.7, 147.9, 144.3, 137.0, 135.6, 135.3, 129.9, 129.8, 129.7, 128.2, 128.0, 127.9, 126.9, 125.2, 123.5, 121.5, 113.3, 110.9, 103.4, 98.2, 86.9, 86.1, 85.7, 68.9, 62.5, 55.0 (2 × C), 41.5; HRMS (ESI) *m*/*z* found 670.2535 [M + H]$^+$ (calcd 670.2553 for $C_{40}H_{36}N_3O_7$).

5′-*O*-DMT-FpC (**4b**): yellow solid; UPLC (method A) t_R = 5.41 min; ^{1}H NMR (DMSO-d_6) δ 11.80 (s, D_2O exch, 1H), 8.59 (s, 1H), 7.77 (m, 1H), 7.40 (m, 2H), 7.27 (m, 7H), 6.89 (m, 5H), 6.60 (m, 1H), 6.22 (t, *J* = 5.5 Hz, 1H), 5.67 (s, 1H), 5.41 (d, D_2O exch, *J* = 4.5 Hz, 1H), 4.40 (m, 1H), 3.97 (m, 1H), 3.70 (2 × s, 6H), 3.33 (m, 2H), 2.40 (m, 1H), 2.18 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 159.6, 158.2, 153.8, 146.0, 144.4, 143.8, 135.9, 135.6, 135.3, 130.7, 129.8, 129.7, 128.0, 127.8, 126.9, 113.3, 112.0, 108.7, 107.8, 94.9, 86.7, 86.1 (2 × C), 85.7, 69.1, 62.7, 55.0 (2 × C), 41.5; HRMS (ESI) *m*/*z* found 620.2374 [M + H]$^+$ (calcd 620.2397 for $C_{36}H_{34}N_3O_7$).

5′-*O*-DMT-DABCYLpC (**5b**): red solid; UPLC (method A) t_R = 6.30 min; ^{1}H NMR (DMSO-d_6) δ 11.89 (s, D_2O exch, 1H), 8.71 (s, 1H), 7.82 (m, 6H), 7.43 (m, 2H), 7.31 (m, 7H), 6.93 (m, 4H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.24 (t, *J* = 5.0 Hz, 1H), 5.89 (s, 1H), 5.45 (d, D_2O exch, *J* = 4.5 Hz, 1H), 4.46 (m, 1H), 3.99 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.37 (m, 2H), 3.07 (s, 6H), 2.44 (m, 1H), 2.22 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 160.0, 158.2 (2 × C), 153.7, 152.5, 151.9, 144.4, 142.7, 138.5, 136.3, 135.6, 135.2, 131.2, 129.9, 129.8, 128.0, 127.9, 126.9, 122.4, 113.3, 111.5, 109.0, 97.5, 86.7, 86.1, 85.6, 68.9, 62.4, 55.0 (2 × C), 41.5, 39.8; HRMS (ESI) *m*/*z* found 777.3400 [M + H]$^+$ (calcd 777.3401 for $C_{46}H_{45}N_6O_6$).

Reaction of 5′-*O*-DMT-*p*-NO_2-PhpC (2b), 5′-*O*-DMT-BFpC (3b), 5′-*O*-DMT-FpC (4b), and 5′-*O*-DMT-DABCYLpC (5b) with 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosporodiamidite (11). Separate round-bottom flasks containing 5′-*O*-DMT-*p*-NO_2-PhpC (**2b**; 102 mg, 0.15 mmol), 5′-*O*-DMT-BFpC (**3b**; 100 mg, 0.15 mmol), 5′-*O*-DMT-FpC (**4b**; 120 mg, 0.19 mmol), or 5′-*O*-DMT-DABCYLpC (**5b**; 143 mg, 0.18 mmol) and tetrazole (13 mg, 0.18 mmol, to react with **2b** and **3b**; 16 mg, 0.23 mmol, to react with **4b**; 15 mg, 0.22 mmol, to react with **5b**) were flushed with N_2, followed by the addition of dry CH_2Cl_2 (1.5 mL) and MeCN (1 mL). In the case of **2b**, only dry MeCN (1.7 mL) was used. A solution of 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosporodiamidite (**11**) (54 mg, 0.18 mmol, to react with **2b** and **35**; 70 mg, 0.23 mmol, to react with **3b**; 66 mg,

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0.22 mmol, to react with **4b**) in dry MeCN (500 μL) was added, and the mixtures were stirred for 2 h (4 h for **2b**) at rt (N_2 atmosphere). The mixtures were diluted with CH_2Cl_2 (20 mL) and were washed with saturated $NaHCO_3$ solution (10 mL). The aqueous phase was extracted with CH_2Cl_2 (10 mL), and the combined organic extracts were dried and were concentrated. The residues were subjected to PTLC (see General Experimental Protocols for details), eluting the plates with CH_2Cl_2/MeOH/NH_4OH (380:19:1). The bands containing the product were carved off the plates and extracted with CH_2Cl_2/MeOH/NH_4OH (380:19:1), the SiO_2 was filtered off, and the filtrates were concentrated to afford 3′-(2-cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-BFpC (**3c**; 83 mg, 64%), 3′-(2-cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-FpC (**4c**; 92 mg, 58%), and 3′-(2-cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-DABCYLpC (**5c**; 115 mg, 64%). In the case of 5′-*O*-DMT-*p*-NO_2-PhpC (**2b**), the residue obtained after the extractive workup was subjected to FCC on 40 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (380:19:1). Evaporation of the eluate afforded the desired product containing a large amount of *H*-phosphonate contaminant. This material was dissolved in CH_2Cl_2, hexanes were added until the turbidity in the solution persisted, and the mixture was set aside for 2 h at −20 °C. The separated solid was isolated by filtration, washed with hexanes, and dried to afford 3′-(2-cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-*p*-NO_2-PhpC (**2c**, 70 mg, 53%).

3′-(2-Cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-*p*-NO_2-PhpC (**2c**): orange solid; ^{1}H NMR (CD_2Cl_2) δ 12.15 (br s, D_2O exch, 1H), 9.07 (s, 0.6H), 9.04 (s, 0.4 H), 8.25 (d, *J* = 8.5 Hz, 2H), 8.04 (d, *J* = 9.0 Hz, 2H), 7.51 (m, 2H), 7.36 (m, 7H), 6.88 (m, 4H), 6.43 (m, 1H), 5.74 (s, 1H), 4.86 (m, 1H), 4.24 (m, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.62 (m, 6H), 2.83 (m, 1H), 2.63 (m, 1H), 2.53 (m, 2H), 1.19 (m, 9H), 1.11 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_2Cl_2) δ 161.0, 159.5, 159.4, 154.9, 147.6, 144.9 (2 × C), 139.0 (2 × C), 138.4, 136.9 (2 × C), 136.3, 136.2, 135.9 (2 × C), 130.9 (2 × C), 130.8 (2 × C), 129.0 (2 × C), 128.7, 127.7 (2 × C), 126.8, 124.7, 118.3, 118.2, 113.9, 110.3 (2 × C), 101.4, 101.3, 88.3 (2 × C), 87.7 (2 × C), 86.1 (2 × C), 86.0, 85.9, 71.2, 71.0, 62.2, 62.0, 59.1, 58.9 (2 × C), 58.7, 55.8 (3 × C), 44.0, 43.9, 41.9 (2 × C), 41.5 (2 × C), 25.0, 24.9 (3 × C), 24.8, 23.3, 23.2, 21.0, 20.9 (2 × C), 20.8; ^{31}P NMR (CD_2Cl_2) δ 149.3, 148.8; HRMS (ESI) *m*/*z* found 875.3534 $[M + H]^+$ (calcd 875.3533 for $C_{47}H_{52}N_6O_9P$).

3′-(2-Cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-BFpC (**3c**): yellow solid; ^{1}H NMR (CD_2Cl_2) δ 12.41 (br s, D_2O exch, 1H), 8.89 (s, 0.5H), 8.84 (s, 0.5 H), 8.02 (m, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.53 (m, 3H), 7.34 (m, 9H), 6.90 (m, 4H), 6.50 (m, 1H), 5.95 (s, 0.5H), 5.94 (s, 0.5H), 4.81 (m, 1H), 4.28 (m, 1H), 3.87 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.60 (m, 5H), 2.85 (m, 1H), 2.55 (m, 3H), 1.21 (m, 9H), 1.13 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_2Cl_2) δ 160.9, 160.8, 159.4 (2 × C), 155.6, 155.0 (2 × C), 148.4, 144.9, 144.8, 137.4, 136.3, 136.2, 136.0, 131.9 (2 × C), 130.8 (2 × C), 130.7 (2 × C), 129.8, 128.9 (2 × C), 128.6, 127.7 (2 × C), 125.4, 123.6, 122.2, 118.3, 118.2, 113.9, 111.4, 110.4 (2 × C), 105.3, 99.2, 99.1, 88.3, 87.6, 86.2 (2 × C), 86.0 (2 × C), 72.5, 72.4, 71.8, 71.6, 62.7, 62.4, 59.1, 58.9 (2 × C), 58.8, 58.4, 55.8 (2 × C), 55.7, 43.9, 43.8, 42.0 (2 × C), 41.6 (2 × C), 30.3, 25.0, 24.9, 24.8, 23.2 (2 × C), 22.0, 20.9 (2 × C), 20.8; ^{31}P NMR (CD_2Cl_2) δ 149.1, 148.7; HRMS (ESI) *m*/*z* found 870.3630 $[M + H]^+$ (calcd 870.3632 for $C_{49}H_{53}N_5O_8P$).

3′-(2-Cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-FpC (**4c**): yellow solid; ^{1}H NMR (CD_2Cl_2) δ 12.00 (br s, D_2O exch, 1H), 8.76 (s, 0.5H), 8.71 (s, 0.5 H), 7.58 (m, 1H), 7.50 (m, 3H), 7.33 (m, 7H), 6.87 (m, 4H), 6.54 (m, 1H), 6.44 (m, 1H), 5.74 (s, 0.5H), 5.71 (s, 0.5H), 4.75 (m, 1H), 4.26 (m, 1H), 3.85 (m, 1H), 3.77 (2 × s, 3H), 3.76 (2 × s, 3H), 3.59 (m, 5H), 2.82 (m, 1H), 2.63 (t, *J* = 6.5 Hz, 1H), 2.50 (t, *J* = 6.5 Hz, 1H), 2.45 (m, 1H), 1.19 (m, 9H), 1.11 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_2Cl_2) δ 160.7, 159.4 (2 × C), 155.0 (2 × C), 146.8, 144.9 (2 × C), 143.4, 136.3, 136.2 (2 × C), 136.0, 132.4, 132.3, 130.8 (2 × C), 130.7 (2 × C), 128.9, 128.8, 128.6, 127.7, 127.6, 118.3, 118.2, 113.8, 112.6, 110.6, 110.5, 109.2, 96.3, 96.2, 88.1, 87.5, 86.2, 86.1 (2 × C), 86.0, 72.9, 72.7, 72.0, 71.8, 62.9, 62.5, 59.1, 59.0 (2 × C), 58.8, 58.4, 55.8, 55.7, 43.9, 43.8, 42.0 (2 × C), 41.6 (2 × C), 25.0 (2 × C), 24.9 (2 × C), 24.8, 21.0, 20.9 (2 × C), 20.8; ^{31}P NMR (CD_2Cl_2) δ 149.0, 148.7; HRMS (ESI) *m*/*z* found 820.3485 $[M + H]^+$ (calcd 820.3475 for $C_{45}H_{51}N_5O_8P$).

3′-(2-Cyanoethyldiisopropylphosphoramidite)-5′-*O*-DMT-DABCYLpC (**5c**): red solid; ^{1}H NMR (CD_2Cl_2) δ 11.90 (br s, D_2O exch, 1H), 8.93 (s, 0.5H), 8.88 (s, 0.5 H), 7.94 (m, 2H), 7.88 (m, 3H), 7.53 (m, 2H), 7.37 (m, 8H), 6.89 (m, 4H), 6.79 (m, 2H), 6.46 (m, 1H), 5.73 (s, 0.5H), 5.71 (s, 0.5H), 4.83 (m, 1H), 4.25 (m, 1H), 3.83 (m, 1H), 3.77 (2 × s, 3H), 3.76 (2 × s, 3H), 3.60 (m, 5H), 3.09 (s, 6H), 2.84 (m, 1H), 2.59 (t, *J* = 6.0 Hz, 1H), 2.50 (m, 3H), 2.49 (t, *J* = 6.5 Hz, 1H), 1.19 (m, 9H), 1.11 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_2Cl_2) δ 160.9 (2 × C), 159.4 (2 × C), 154.9 (2 × C), 153.3, 153.2, 145.0, 144.9, 144.2, 140.2 (2 × C), 137.1, 136.3, 136.2, 136.0, 135.9, 131.7, 130.9 (2 × C), 130.8 (2 × C), 129.0, 128.9, 128.7, 127.7, 127.6, 126.7, 125.5,123.2, 118.3, 118.2, 113.9, 112.0, 110.8, 110.7, 98.3 (2 × C), 88.1 (2 × C), 87.6, 86.0 (2 × C), 86.1 (2 × C), 72.2, 72.1, 71.3, 71.2, 62.5, 62.1, 59.2, 59.0, 58.8, 55.8 (2 × C), 43.9, 43.8, 42.0 (2 × C), 41.6 (2 × C), 40.7, 25.0, 24.9 (2 × C), 24.8, 21.0, 20.9, 20.8; ^{31}P NMR (CD_2Cl_2) δ 149.2, 148.8; HRMS (ESI) *m*/*z* found 977.4470 $[M + H]^+$ (calcd 977.4479 for $C_{55}H_{62}N_8O_7P$).

Preparation of *N*4-Benzoyl-5-iodo-5′-*O*-DMT-deoxycytidine (6d). A round-bottom flask containing *N*4-benzoyl-5-iodo-2′-deoxycytidine (**6c**;[13,14] 687 mg, 1.46 mmol) and DMT-Cl (643 mg, 1.9 mmol) was flushed with N_2, followed by the addition of dry pyridine (8 mL) and dry Et_3N (1.47 mL, 10.52 mmol). The resulting solution was stirred for 18 h at rt (under N_2 atmosphere), the reaction was quenched with MeOH (200 μL), diluted with toluene (150 mL), and concentrated. The residue was subjected to FCC on 90 g of SiO_2, eluted with CH_2Cl_2/MeOH/NH_4OH (380:19:1). Evaporation of the eluate afforded *N*4-benzoyl-5-iodo-5′-*O*-DMT-deoxycytidine (**6d**; 751 mg, 68%): yellow solid; UPLC (method A) t_R = 6.31 min; ^{1}H NMR (DMSO-d_6) δ 12.92 (s, D_2O exch, 1H), 8.23 (m, D_2O exch, 3H), 7.62 (m, 1H), 7.53 (m, 2H), 7.42 (m, 2H), 7.32 (m, 7H), 7.23 (m, 1H), 6.91 (m, 4H), 6.11 (t, *J* = 6.0 Hz, 1H), 5.37 (d, D_2O exch, *J* = 4.5 Hz, 1H), 4.24 (m, 1H), 3.97 (m, 1H), 3.73 (s, 6H), 3.21 (m, 3H), 2.30 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 178.1, 158.1, 156.6, 147.2, 146.3, 144.7, 136.3, 135.5, 135.4, 132.8, 129.7, 129.5, 128.3, 128.0, 127.7, 126.7, 113.3, 86.3, 86.1, 85.9, 70.4, 69.6, 63.6, 55.0 (2 × C), 40.3; HRMS (ESI) *m*/*z* found 760.1540 $[M + H]^+$ (calcd 760.1520 for $C_{37}H_{35}IN_3O_7$).

Preparation of Alkynes 7−10. 2-Ethynylbenzo[*b*]furan (**7**)[3] and DABCYL-modified alkyne **9**[9] have been prepared as described previously, while *p*-NO_2-phenylacetylene (**10**) is commercially available. A modified literature procedure[8] has been used to prepare 2-ethynylfuran (**8**) as follows. A flask containing PPh_3 (6.56 g, 25 mmol), CBr_4 (8.29 g, 25 mmol), and Zn dust (1.64 g, 25 mmol) was flushed with N_2. Dry CH_2Cl_2 (60 mL) was added and the mixture was stirred for 24 h at rt (under N_2 atmosphere). The mixture was then cooled to room temperature, fural (830 μL, 10 mmol) was added, the cooling bath was removed, and the stirring was continued for a further 18 h at rt (under N_2 atmosphere). The reaction mixture was filtered through a short Celite pad; the filter was washed with CH_2Cl_2, the filtrate was concentrated, and the residue was subjected to FCC on 50 g of SiO_2, eluted with petroleum ether. Evaporation of the eluate afforded 2-(2,2-dibromovinyl)furan (2.29 g, 91%) as a slightly yellow oil. 2-(2,2-Dibromovinyl)furan (800 mg, 3.18 mmol) was dissolved in dry Et_2O (20 mL), and the solution was charged with N_2, followed by cooling to −78 °C. A 1.5 M solution of *t*-BuLi in pentane (8 mL, 12.1 mmol) was added slowly and the mixture was stirred for 1 h (under N_2 atmosphere) while the cooling bath was allowed to gradually warm up. The reaction was the quenched with saturated NH_4Cl solution (20 mL), the organic layer was separated, and the aqueous layer was extracted with Et_2O (20 mL). The combined organic extract was dried and was concentrated (water bath kept at rt) to yield crude 2-ethynylfuran (**8**, 290 mg, 99%) as a pale orange oil used for the subsequent step immediately without further purification. ^{1}H NMR spectra of both 2-(2,2-dibromovinyl)furan and 2-ethynylfuran (**8**) were in agreement with those previously reported.

Quantum Yield (Φ) and Molar Extinction Coefficient (ε) Determination of FpC (4). The quantum yield (Φ) associated with FpC (**4**) was determined in EtOH using 9,10-diphenylanthracene (Φ

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0.90) as a standard.[17] The quantum yield of FpC (**4**) was determined to be 0.58.

The molar extinction coefficient (ε) associated with FpC (**4**) was determined in EtOH by constructing Lambert–Beer's plots [$A = \varepsilon cl$,[3] wherein A is the absorbance, ε is the molar extinction coefficient, c is the concentration, and l is the light path length (~1 cm)]. Plots of concentration versus absorbance using five different concentrations were used. The molar extinction coefficient of FpC (**4**) was determined to be 3.26×10^4 M^{-1} cm^{-1} (260 nm) and 6.65×10^3 M^{-1} cm^{-1} (379 nm, excitation wavelength).

Preparation, Purification, and Characterization of DNA Sequences 1 and 2. DNA sequences 1 and 2 were prepared by automated solid-phase DNA synthesis using standard methods as supplied by the manufacturer. Coupling times for unmodified nucleoside phosphoramidite reagents were 60 and 300 s for modified nucleosides. The individual resins obtained after each DNA oligomerization were suspended in concentrated NH_4OH solution, agitated at room temperature for 30 min, and then incubated at 55 °C in a sealed vial for 12 h. Afterward, NH_3 was allowed to evaporate, and the resin was separated by filtration by passing through Pasteur pipet with a cotton plug. The separate solutions containing crude DMT-protected sequences 1 and 2 were frozen and lyophilized. The pellets were dissolved in water (700 μL) and were subjected to HPLC purification as described in General Experimental Protocols: for DMT-protected sequence 1, t_R = 29.1 min (method C); for DMT-protected sequence 2, t_R = 24.5 min (method D).

The fractions containing pure DMT-protected DNA sequences 1 and 2 were combined, frozen, and lyophilized; the residues were dissolved in H_2O (150 μL)/AcOH (600 μL); and the solutions were set aside for 30 min at rt, diluted with water, frozen, and lyophilized. The residues were dissolved in water (800 μL each) and were subjected to HPLC purification as described in General Experimental Protocols. Sequence 1: t_R = 15.4 min (method E); MS (ESI-TOF) m/z found 3114.1 $[M - H]^+$ (calcd 3114.5). Sequence 2: t_R = 11.3 min (method F); MS (ESI-TOF) m/z found 3090.3 $[M - H]^+$ (calcd 3090.5).

Hybridization Studies with DNA Sequences 1 and 2. The hybridization properties of the modified DNA oligomers with fully matched, complementary DNA and single-mismatch-containing sequences were studied by temperature-dependent UV spectroscopy (i.e., melting experiments). Thermal denaturation experiments were carried out in a buffer containing 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na_2HPO_4, 0.1 mM EDTA at pH 7.0. T_m experiments were performed at 10^{-6} M strand concentration. Samples were heated to 95 °C and were allowed to cool to room temperature slowly (ca. 3 h). Denaturation was performed from 20 to 85 °C at a scan rate of 0.5 °C/min. ΔT_m values are the difference between the T_m of the sequences 1 and 2 containing unnatural nucleobase and control sequences containing cytosine. The T_m values are an average of three measurements and are rounded to the nearest 0.5 °C. T_m values were estimated for cooperative transitions by the first-derivative method. Temperature-dependent UV spectra that lacked upper and lower baselines or lacked sigmoidal shape were deemed not to be cooperative transitions. Samples for the fluorescence measurements associated with the DNA oligomers were prepared in the same manner as those used for the determination of T_m values, using the same buffer, pH value, and concentration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01584.

Spectral characterization of **2**, **4**, **5**, **1a–5a**, **1b–5b**, **2c–5c**, and **6d**; computed ionization potential maps for models of **1–5** and atom coordinate tables and absolute energies from the quantum calculations; and characterization of the DNA sequences (sequences 1 and 2) (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) For the recent reviews on fluorescent nucleobase analogs, see the following: (a) Dodd, D. W.; Hudson, R. H. E. *Mini-Rev. Org. Chem.* **2009**, *6*, 378–391. (b) Sinkeldam, R. W.; Greco, N. J.; Tor, Y. *Chem. Rev.* **2010**, *110*, 2579–2619. (c) Wilhelmsson, L. M. *Q. Rev. Biophys.* **2010**, *43*, 159–183.

(2) (a) Hudson, R. H. E.; Dambenieks, A. K.; Viirre, R. D. *Synlett* **2004**, 2400–2402. (b) Wojciechowski, F.; Hudson, R. H. E. *J. Am. Chem. Soc.* **2008**, *130*, 12574–12575. (c) Wojciechowski, F.; Hudson, R. H. E. *Org. Lett.* **2009**, *11*, 4878–4881. (d) Hu, J.; Dodd, D. W.; Hudson, R. H. E.; Corey, D. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6181–6184. (e) Wahba, A. S.; Azizi, F.; Deleavey, G. F.; Brown, C.; Robert, F.; Carrier, M.; Kalota, A.; Gewirtz, A. M.; Pelletier, J.; Hudson, R. H. E.; Damha, M. J. *ACS Chem. Biol.* **2011**, *6*, 912–919. (f) Torres, A. G.; Fabani, M. M.; Vigorito, E.; Williams, D.; Al-Obaidi, N.; Wojciechowski, F.; Hudson, R. H. E.; Seitz, O.; Gait, M. J. *Nucleic Acids Res.* **2012**, *40*, 2152–2167.

(3) Elmehriki, A. A. H.; Suchý, M.; Chicas, K. J.; Wojciechowski, F.; Hudson, R. H. E. *Artif. DNA* **2014**, *5*, e29174.

(4) (a) Moustafa, M. E.; Hudson, R. H. E. *Nucleosides, Nucleotides Nucleic Acids* **2011**, *30*, 740–751. (b) DABCYL has become the classic quencher since its demonstration in peptide and oligonucleotide applications: Matayoshi, E. D.; Wang, G. T.; Krafft, G. A.; Erickson, J. *Science* **1990**, *247*, 954–958. and (c) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303–308.

(5) (a) Chicas, K.; Hudson, R. H. E. Expanding the Nucleic Acid Chemists Toolbox: Fluorescent Cytidine Analogs. In *Fluorescent Analogs of Biomolecular Building Blocks Design and Applications*. Wilhelmsson, M., Tor, Y., Eds.; John Wiley & Sons: Hoboken, NJ, 2016; pp 174–203. (b) Ettles, C. Progress toward Synthesis of Molecular Beacons Incorporating DABCYL Analog Quenchers. M.Sc. thesis, The University of Western Ontario, London, Canada, 2013.

(6) (a) The DMT group for 5′-OH protection is well-established: Smith, M.; Rammler, D. H.; Goldberg, I. H.; Khorana, H. G. *J. Am. Chem. Soc.* **1962**, *84*, 430–440. (b) Caruthers, M. H. *Science* **1985**, *230*, 281–285.

(7) Reese, C. B. *Org. Biomol. Chem.* **2005**, *3*, 3851–3868 and references cited therein..

(8) Kyi, S.; Wongkattiya, N.; Warden, A. C.; O'Shea, M. S.; Deighton, M.; Macreadie, I.; Graichen, F. H. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4555–4557.

(9) Charles, M. Synthesis and Spectroscopic Studies of Substituted Pyrrolocytidines. M.Sc. thesis, The University of Western Ontario, London, Canada, 2012. For the azo coupling between 4-iodoaniline and *N*,*N*-dimethylaniline to prepare the corresponding iodinated intermediate, see the following: Siemeling, U.; Bruhn, C.; Meier, M.; Schirrmacher, C. *Z. Naturforsch.* **2008**, *63b*, 1395–1401.

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(10) Chemical shifts (DMSO-d_6) of protons adjacent to C9 and C7 of an authentic sample of 5′-*O*-DMT-4-(methoxyphenyl)pyrrolocytosine available in our laboratory were found to be at δ 8.58 (s, 1H) and 5.68 (s, 1H). The sample was prepared as described previously: Ghorbani-Choghamarni, A. Preparation of Some New Reagents and Their Applications in Organic Reactions: Synthesis of Modified Nucleobases and Their Incorporation into DNA Oligomers and Their Evaluation of Fluorescence Properties. Ph.D. thesis, Bu Ali Sina University, Hamedan, Iran, 2007.
(11) Butskus, P. F.; Raguotene, N. V. *Khim. Geterotsikl. Soedin.* **1970**, *6*, 1056−1057.
(12) Zeidler, J.; Golankiewicz, B. *Tetrahedron* **1998**, *54*, 2941−2952.
(13) Chang, P. K.; Welch, A. D. *J. Med. Chem.* **1963**, *6*, 428−430.
(14) Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.
(15) 5′-*O*-DMT-PhpC was prepared by this route: Hudson, R. H. E.; Ghorbani-Choghamarani, A. *Synlett* **2007**, *2007*, 0870−0873.
(16) (a) Nielsen, J.; Taagaard, M.; Marugg, J. E.; van Boom, J. H.; Dahl, O. *Nucleic Acids Res.* **1986**, *14*, 7391−7403. (b) Asanuma, H.; Shirasuka, K.; Takarada, T.; Kashida, H.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 2217−2223.
(17) Eaton, D. F. *Pure Appl. Chem.* **1988**, *60*, 1107−1114.

DOI: 10.1021/acs.joc.6b01584
J. Org. Chem. 2016, 81, 8415−8425