Chemical Synthesis and Characterization of Duplex DNA Containing a New Base Pair: A Nondisruptive, Benzofused Pyrimidine Analog

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A new base pair appropriate for incorporation into B-DNA was designed with the goal of allowing fusion of a benzene substituent across the 4 and 5 carbons of a pyrimidine analog. Such a residue may have utility in the preparation of DNA duplexes bearing precisely spatially positioned and conformationally constrained unnatural substituents such as reporter groups. The design called for the incorporation of the β-anomer of a C-linked deoxyriboside of 2-hydroxyquinoline (dQ) opposite the β-N^9 deoxyriboside of 2-aminopurine (dAP). Several duplex DNAs were synthesized containing this new base pair as well as the analog in which 2-hydroxypyridine replaces 2-hydroxyquinoline (dP). Phosphoramidites 17 and 18 were synthesized and incorporated into synthetic oligonucleotides using automated methodology. That dQ and dP had been incorporated without chemical modification was proven by enzymatic digestion of the synthetic oligonucleotides to the component nucleosides and analysis by HPLC. Native polyacrylamide gel electrophoresis revealed that admixture of complementary strands containing dP or dQ opposite dAP gave new substances with mobility comparable to a duplex DNA of the same length containing only Watson–Crick base pairs. Solution circular dichroism measurements were consistent with these substances existing in the B conformation. T_m, ΔH, and ΔS were measured for synthetic duplex DNAs containing pairings of dQ and dP with dAP, dA, dC, dG, and dT. Of these, duplexes in which dAP was the partner of dP or dQ were most thermodynamically stable (ΔG 25 °C) and highest melting, with T_m values lower by 1 to 5 °C than the corresponding dA·dT-containing duplex. Solution ^{1}H NMR measurements from δ 11–15 on an 11-mer duplex containing the dAP·dQ pair were diagnostic for the presence of 11 base pairs. The resonance for the dAP·dQ base pair was assigned on the basis of a combination of 1D NOE measurements, temperature-dependent line width, and chemical shift measurements. We conclude that dP and dQ are competent base-pairing partners for dAP in duplex DNA and are reasonable condidates for use in the design of novel base-pairing nucleoside analogs.

Introduction

The view was at one time widely held that duplex DNA came primarily in two structural classes, A and B, with the latter predominanting in aqueous solutions of sequence-random samples. This simple view has been discarded in revent years, due to the realization that the three-dimensional, ground-state structure of "B-DNA" varies considerably as a function of nucleotide sequence. Critical to this revision in dogma was the retirement of bulk DNA as the experimental system of choice and its replacement with short, synthetic DNA duplexes of defined sequence. This same substitution has revolutionized our understanding of drug–DNA[1] and protein–DNA interactions.[2] We are interested in the possibility that our understanding of the dynamics of DNA, which has largely been shaped by the study of bulk DNA, might be similarly revolutionized by the availability of synthetic DNA. The goal of these studies is the elucidation of DNA dynamics as a function of nucleotide sequence. It is at present unknown whether such differences exist. If they do, and are substantial, they will likely play a role in the kinetics of DNA–protein and DNA–drug interactions. We describe here studies enroute to a "second generation", nondisruptive nitroxide spin probe for duplex DNA dynamics. Specifically, we describe a new base pair in which one of the partners is a pyrimidine analog which possesses a benzo fusion across carbons 4 and 5. This base pair does not disrupt the duplex structure of B-DNA. Modeling indicates that in the duplex form, the fused ring projects into the major groove, providing a conformationally defined platform upon which to build further functionality. This approach is unique and may have utility beyond the application intended in our laboratory.

Design

Electron paramagnetic resonance (EPR) spectroscopy has been used for the study of DNA dynamics. The technique is highly sensitive, requiring sample sizes of only 100 pmol, and can defect motions over an 8 order of magnitude range of time scale, from motion on the subnanosecond to millisecond time scales.[3] Progress in the field has been slow, however, for several reasons. First, there is the need to install an unpaired spin at a specific site in DNA. This limitation has been overcome by the development of nitroxide-bearing monomers appropriate for standard DNA synthesis protocols. Second, the spin label must not disrupt the dynamics of duplex DNA. Assuming that, to a first approximation, spin labels which are not disruptive of structure will not likewise alter

(1) (a) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376. (b) Quigley, G. J.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J.; Rich, A. *Science* **1986**, *232*, 1255.

(2) See, for example: (a) Pavletich, N. P.; Pabo, C. O. *Science* **1991**, *252*, 809. (b) McKay, D. B.; Steitz, T. A. *Nature (London)* **1981**, *290*, 744. (c) McClarin, J. A.; Frederick, C. A.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Grable, J.; Rosenberg, J. M. *Science* **1986**, *234*, 1526.

(3) (a) Hustedt, E. EPR Studies of Polyacetylene and Spin-Labeled DNA, Ph.D. Thesis, University of Washington, 1989, and references therein. (b) Robinson, B. H.; Thomann, H.; Beth, A. H.; Fajer, P.; Dalton, L. R. In *EPR and Advanced EPR Studies of Biological Systems*; CRC Press: Boca Raton, FL, 1985, and references therein, 295.

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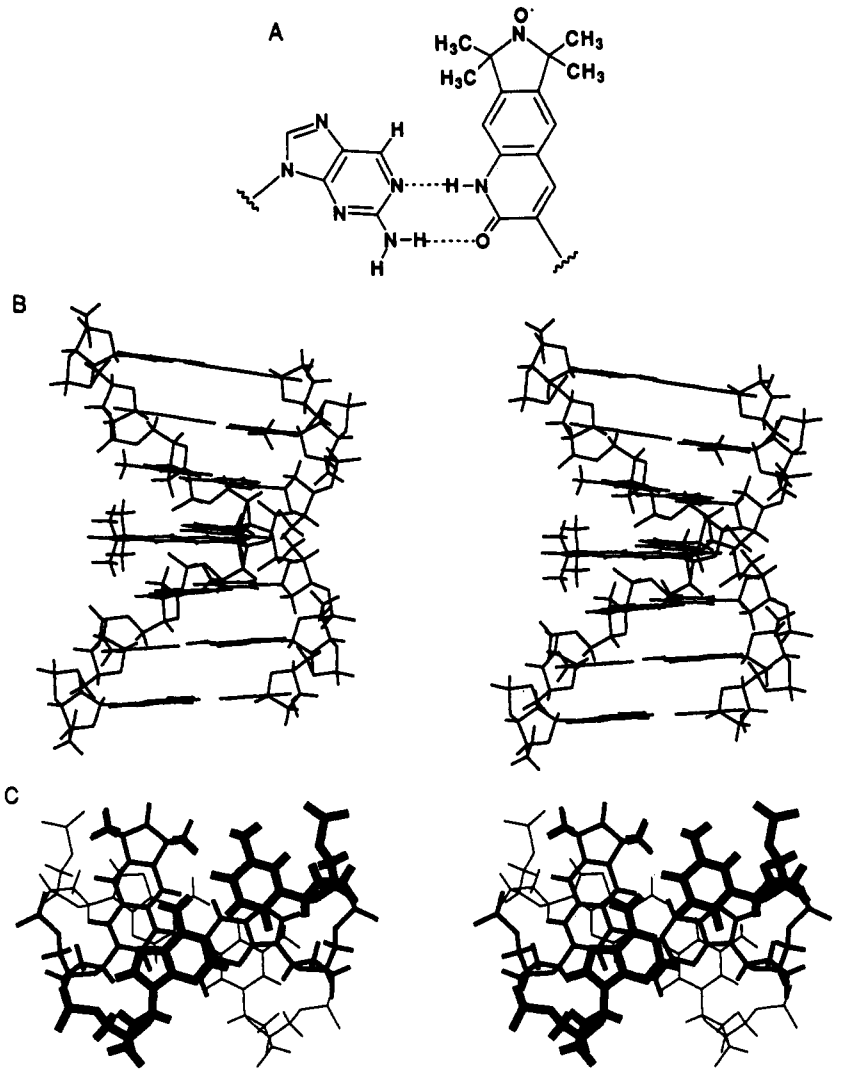
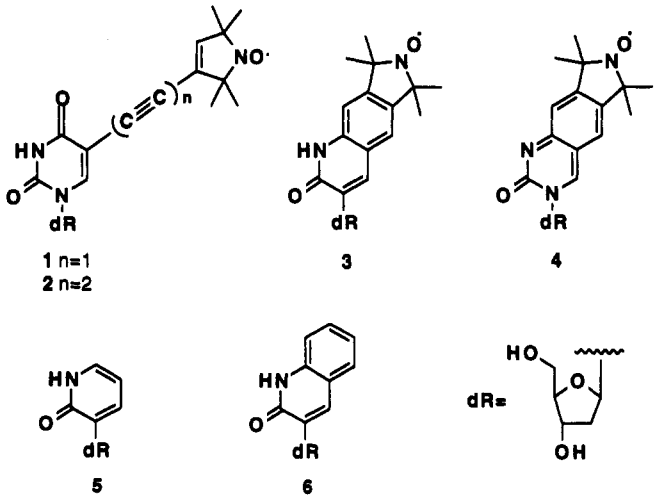


Figure 1. (A) Proposed hydrogen-bonding pattern for a rigid spin probe. Side view (B) and clipped view down helix axis (C) of computer model of base pair shown in A incorporated into a heptamer duplex in the B conformation.

dynamic properties, this requirement has been met by judicious placement of the spin label at position 5 of a pyrimidine residue, allowing the bulky spin label to project into the relatively spacious major groove (see Figure 1).[4] Third, the spin label should not possess appreciable motion independent of the DNA on the time scale of interest. As described below, no spin-labeled DNA prepared to date has satisfied this third requirement.

The synthesis of a variety of DNA structures (hairpins, single strands, duplexes) containing substructure 1 has been previously reported.[4] The EPR spectra of DNAs containing 1 exhibit line shapes characteristic of the slowest motions yet observed for spin-labeled DNA but retain features diagnostic for a relatively rapid motion about a single axis independent of the DNA. The latter has been attributed to motion of the nitroxide about the acetylene axis restricted by steric clash with the sugar phosphate backbone. Consistent with this hypothesis was the observation that a duplex DNA containing the substructure 2 exhibited an EPR spectrum indicative of unrestricted motion about a single axis.[4d] Space filling models indicate that the nitroxide moiety of 2 in duplex DNA projects well beyond the sugar phosphate backbone, allowing unrestricted rotation. Spin probe 1 was an advance over existing probes for DNA dynamics, but was not optimal.



One potential solution to the problem of independent probe motion encountered with 1 is the fusion of the nitroxide-containing ring to a heterocycle within duplex

(4) (a) Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 1299. (b) Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2303. (c) Spaltenstein, A.; Robinson, B. H.; Hopkins, P. B. *Biochemistry* **1989**, *28*, 9484. (d) Kirchner, J. J.; Hustedt, E. J.; Robinson, B. H.; Hopkins, P. B. *Tetrahedron Lett.* **1990**, *31*, 593. (e) Hustedt, E. J.; Spaltenstein, A.; Kirchner, J. J.; Hopkins, P. B.; Robinson, B. H. *Biochemistry*. In press.

DNA. Some 2 dozen analogs of the common bases have been incorporated nondisruptively into duplex DNA, including, for example, 2-aminopurine, 2,6-diaminopurine, several 7-deazapurines, 4-thiothymidine, 6-thioguanine, 2-thiothymine, 5-methyl-2-pyrimidinone, and 3-deazaadenine.[5] Base analogs that have spectroscopic labels, biological labels attached, and those containing DNA cross-linking and cleaving agents have also been synthesized and incorporated into DNA.[6] Unfortunately, none of these was directly applicable to the problem at hand.

A fused structure as required herein was without precedent. After consideration of several pyrimidine analogs, we decided upon structure **3** as a suitable target. In this structure, the bulky nitroxide-containing ring was expected to project into the spacious major groove (Figure 1). The alternative structure **4** was eliminated from consideration on the basis of literature precedent, which suggested an inappropriately high level of chemical reactivity.[7] The β-deoxyriboside of 2-aminopurine (dAP) was selected as a base-pairing partner for **3** (Figure 1), a choice encouraged by the report that thymidine forms a stable base pair with this partner.[8] A computer model of **3** base paired to dAP is shown in Figure 1.

Before embarking on a synthesis of DNA containing **3**, it seemed wise to investigate two factors critical to the success of this plan which are the subject of this paper. First, we have utilized pyrimidine analog **5** to demonstrate that the base-pairing functionality in **3** is competent to base pair to 2-aminopurine in duplex DNA. Second, we have utilized **6** to demonstrate that the major groove of B-DNA will accommodate a benzo fusion across carbons 4 and 5 of a pyrimidine analog.

Results and Discussion

Synthesis of Phosphoramidites. Syntheses of the C-nucleosides **5** and **6** in optically active form have been previously described[9] and are summarized in Scheme I. The synthesis commenced with the acetonide of *R*-glyceraldehyde **7**[10] which was chain extended by the method of Kishi[11] to afford in 95% yield a 9:1 mixture of diastereoisomers **8** favoring that isomer useful in deoxyriboside synthesis. The minor component was carried through the synthesis for two further routine steps, at which point it was removed chromatographically during purification of aldehyde **10**. Addition of the appropriate Gilman reagent to aldehyde **10** afforded a ca. 1:1 mixture of **11S/11R** or **12S/12R**.[12] These isomers were separated chromatographically. Based upon the observation that isomers **11S** and **12S** were converted to the α-anomers of **13** and **14**, respectively, and the assumption that Walden inversion accompanied the tetrahydrofuran-forming displacement reaction, we assigned the stereostructures shown to **11** and **12**. The Mitsunobu[13] reaction was used to convert the *R* isomers of **11** and **12** into the useful *S* isomer. In both the pyridine and quinoline sequences, the furanose ring was now closed, and the fluorinated substituent was converted to a pyridone (**5**) or quinolone (**6**).[14]

To prepare the pyridone **5** and quinolone **6** for incorporation into synthetic DNA, it remained to protect the carbonyl- and 5′-oxygen substituents and to activate the 3′-hydroxyl group. These alterations were achieved by the routine transformations shown in Scheme II.

DNA Synthesis. Except as specified, automated DNA synthesis of DNAs I, II, and III followed literature protocols.[15] To conserve the pyridone- and quinolone-containing phosphoramidites, these were coupled to the growing chain with a modified protocol using a 5-fold excess of phosphoramidite, a 10-fold excess being normal, and with a prolonged coupling time of 2 min relative to the standard 30 s. Coupling yields for the pyridone- and quinolone-containing phosphoramidites, as assayed by trityl cation release, ranged from 79 to 89 and 83 to 94%, respectively. The resulting DNAs were purified by denaturing PAGE.[16]

$^{5'}HO$CCGATXTAGCC$^{OH3'}$
$_{3'HO}$GGCTAYATCGG$_{OH5'}$ **I (X, Y)**

$^{5'}HO$CGAGGXCCAGC$^{OH3'}$
$_{3'HO}$GCTCCYGGTCG$_{OH5'}$ **II (X, Y)**

$^{5'}HO$TGCCATXTACCGT$^{OH3'}$
$_{3'HO}$ACGGTAYATGGCA$_{OH5'}$ **III (X, Y)**

To verify that the synthetic pyrimidine analogs had been incorporated into DNA in chemically unaltered form and had been completely deprotected, the dP- and dQ-containing strands of DNA I were digested to the corresponding deoxyribonucleosides using snake venom phos-

(5) (a) Strazewski, P.; Tamm, C. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 36. (b) McLaughlin, L. W.; Benseler, F.; Graeser, E.; Piel, N.; Scholtissek, S. *Biochemistry* **1987**, *26*, 7238. (c) Seela, F.; Driller, H. *Nucleic Acids Res.* **1986**, *14*, 2319. (d) Seela, F.; Kehne, A. *Biochemistry* **1987**, *26*, 2232. (e) Cosstick, R.; Li, X.; Tuli, D. K.; Williams, D. M.; Connolly, B. A.; Newman, P. C. *Nucleic Acids Res.* **1990**, *18*, 4771. (f) Chollet, A.; Kawashima, E. *Nucleic Acids Res.* **1988**, *16*, 305. (g) Jiricny, J.; Wood, S. G.; Martin, D.; Ubasawa, A. *Nucleic Acids Res.* **1986**, *14*, 6579. (h) Eritja, R.; Horowitz, D. M.; Walker, P. A.; Ziehler-Martin, J. P.; Boosalis, M. S.; Goodman, M. F.; Itakura, K.; Kaplan, B. E. *Nucleic Acids Res.* **1986**, *14*, 8135. (i) Anand, N. N.; Brown, D. M.; Salisbury, S. A. *Nucleic Acids Res.* **1987**, *20*, 8167. (j) Lin, P. K. T.; Brown, D. M. *Nucleic Acids Res.* **1989**, *17*, 10373. (k) Brown, D. M.; Lin, P. K. T. *Carbohydr. Res.* **1991**, *216*, 129. (l) Nishio, H.; Ono, A.; Matsuda, A.; Ueda, T. *Nucleic Acids Res.* **1992**, *20*, 777. (m) Piccirilli, J. A.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1991**, *30*, 10350. (n) Switzer, C.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 8322. (o) Piccirilli, J. A.; Karuch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33. (p) Piccirilli, J. A.; Krauch, T.; MacPherson, L. J.; Benner, S. A. *Helv. Chim. Acta* **1991**, *74*, 397.

(6) (a) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci.* **1985**, *82*, 968. (b) Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000. (c) Meyer, R. B., Jr.; Tabone, J. C.; Hurst, G. D.; Smith, T. M.; Gamper, H. *J. Am. Chem. Soc.* **1989**, *111*, 8517. (d) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 6966.

(7) (a) Jones, D. W. *J. Chem. Soc. C* **1969**, 1729. (b) Mruk, N. J.; Tieckelmann, H. *Tetrahedron Lett.* **1970**, *14*, 1209.

(8) (a) McLaughlin, L. W.; Leong, T.; Benseler, F.; Piel, N. *Nucleic Acids Res.* **1988**, *16*, 5631. (b) Eritja, R.; Kaplan, B. E.; Mhaskar, D.; Sowers, L. C.; Petruska, J.; Goodman, M. F. *Nucleic Acids Res.* **1986**, *14*, 5869.

(9) Solomon, M. S.; Hopkins, P. B. *Tetrahedron Lett.* **1991**, *32*, 3297.

(10) (a) Hertel, L. W.; Grossman, C. S.; Kroin, J. S. *Synth. Commun.* **1991**, *21*, 151. (b) Baer, E. *J. Am. Chem. Soc.* **1945**, *67*, 338.

(11) (a) Nagaoka, H.; Kishi, Y. *Tetrahedron* **1981**, *37*, 3873. (b) Mulzer, J.; Angermann, A. *Tetrahedron Lett.* **1983**, *24*, 2843.

(12) (a) Gribble, G. W.; Saulnier, M. G. *Tetrahedron Lett.* **1980**, *21*, 4137. (b) Marsais, F.; Bouley, E.; Queguiner, G. *J. Organomet. Chem.* **1979**, *171*, 273.

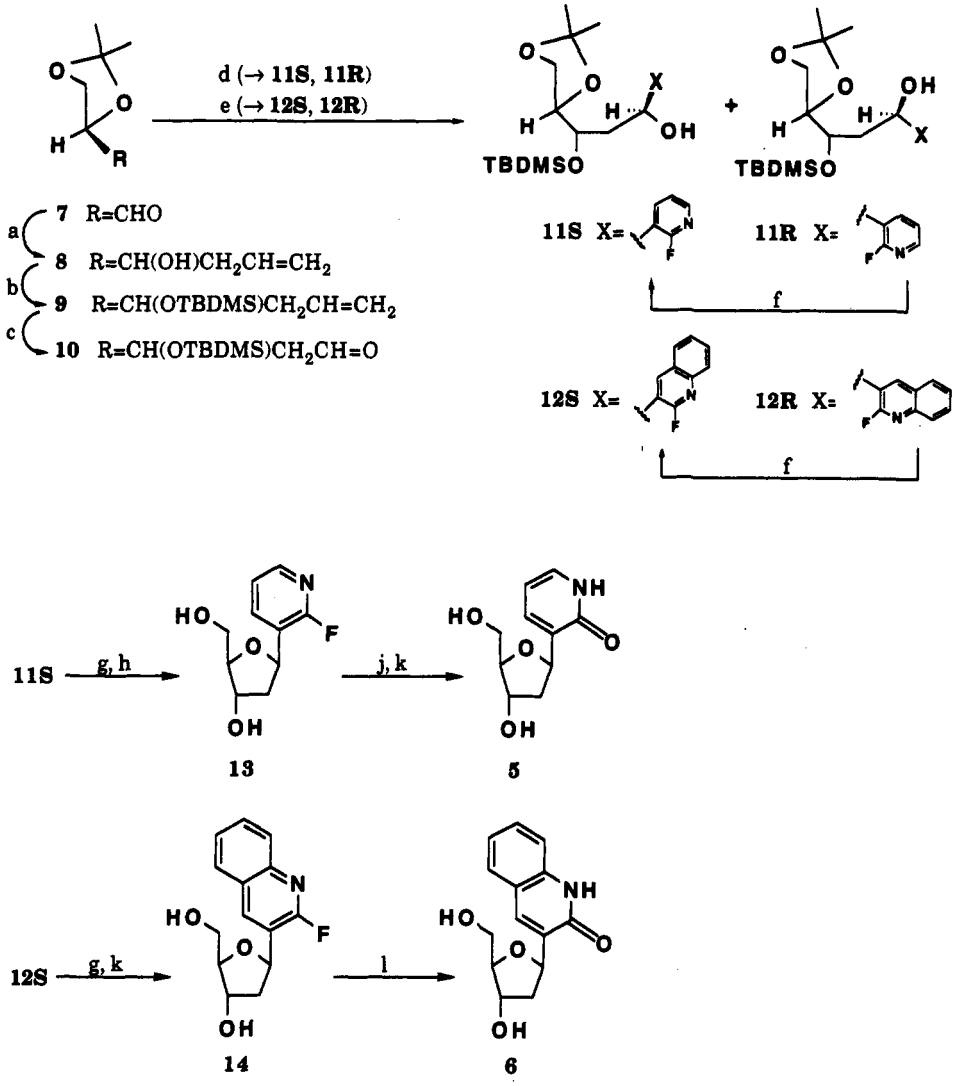
(13) Mitsunobu, O. *Synthesis* **1981**, 1.

(14) The stereochemical assignments at C-1 of **5** and **6** rest primarily on qualitative 1D NOE measurements. Reciprocal NOE's between protons 1′ and 4′ were observed in both **5** and **6**; a control experiment under these spectrometer conditions gave comparable NOE's for 2′-deoxycytidine. In experiments not described in detail in this paper, compounds **5** and **6** were tested for their stability to the conditions required in all steps of DNA synthesis and were recovered unchanged (TLC and ^{1}H NMR analysis).

(15) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655. (b) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859.

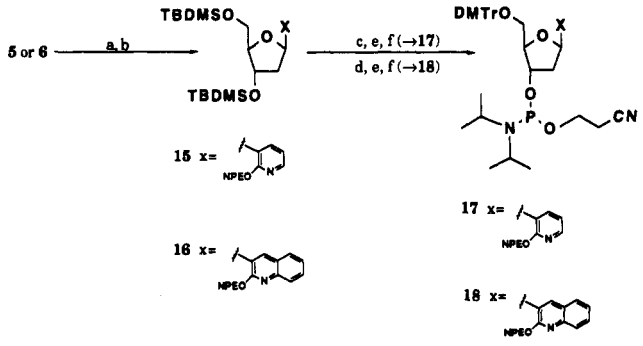
(16) Maniatas, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold spring Harbor, NY, 1982.

Scheme I. Syntheses of Nucleoside Analogs 5 and 6[a]



[a] (a) $(CH_2{=}CHCH_2)_2Zn$, THF; (b) TBDMSOTf, CH_2Cl_2; (c) O_3, MeOH; Ph_3P; (d) 2-fluoro-3-lithiopyridine, THF; H_2O; (e) 2-fluoro-3-lithioquinoline, THF; H_2O; (f) DEAD, Ph_3P, $PhCO_2H$, THF; NH_3, MeOH; (g) MsCl, Et_3N, CH_2Cl_2; (h) 4:1 TFA/$CHCl_3$; (i) $C_6H_5CH_2OH$, KOH; (j) TMSI, CH_2Cl_2; (k) 4:1 TFA/MeOH; (l) NaOH, H_2O.

Scheme II. Syntheses of Phosphoramidites Required for the Incorporation of 5 and 6 into Duplex DNA[a]



[a] (a) TBDMSCl, DMF; (b) NPEOH, $(C_6H_5)_3P$, DEAD, THF; (c) (2:1) TFA/$CHCl_3$; (d) PPTs, EtOH; (e) DMTrCl, pyridine; (f) $(((CH_3)_2CH)_2N)_2POCH_2CH_2CN$, CH_2Cl_2.

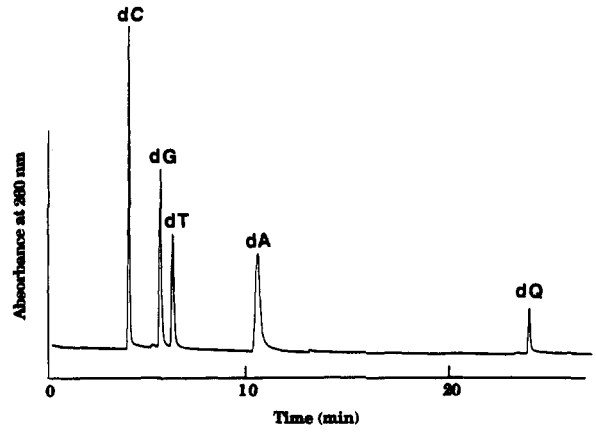


Figure 2. HPLC analysis of the enzymatic hydrolysate of 5′-d(CCGATQTAGCC). Detection was at 260 nm; gradient B.

phodiesterase and calf intestinal alkaline phosphatase. HPLC analysis of the resulting digests revealed peaks eluting at the appropriate times for diols 5 or 6. For DNA I, X = P, the residues were relased in the ratio 4.21(dC):1.1(dP):2.0(dG):2.19(dT):2.18(dA), which compares favorably with the predicted 4:1:2:2:2. Similar results were seen for DNA I, X = Q, where the released nucleoside ratio was 4.0(dC):1.90(dG):1.92(dT):2.03(dA):1.06(dQ) and the calculated ratio is 4:2:2:2:1 (Figure 2). For DNA I, X = Q, the quinolone moiety could be detected in the intact single strand by its ultraviolet absorbance at wavelengths greater than 300 nm where the standard residues of DNA do not absorb. The HPLC peak from the enzymatic digest

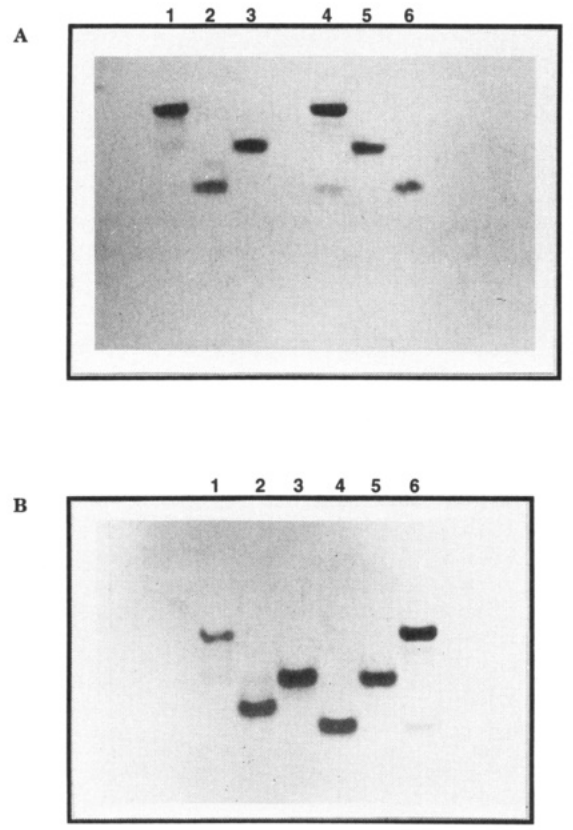


Figure 3. Native PAGE of several DNA I duplexes and component single strands demonstrating purity and similar electrophoretic mobility of analogous species. (A) Lane 1, DNA I (P,AP) duplex; lane 2, DNA I (P,AP) upper strand; lane 3, DNA I (P,AP) lower strand. Lane 4, DNA I (T,A) duplex; lane 5, DNA I (T,A) lower strand only; lane 6, DNA I (T,A) upper strand only. (B) Lane 1, DNA I (Q,AP) duplex; lane 2 DNA I (Q,AP), upper strand only; lane 3, DNA I (Q,AP) lower strand only. Lane 4, DNA I (T,A) upper strand only; lane 5, DNA I (T,A) lower strand only; lane 6, DNA I (T,A) duplex.

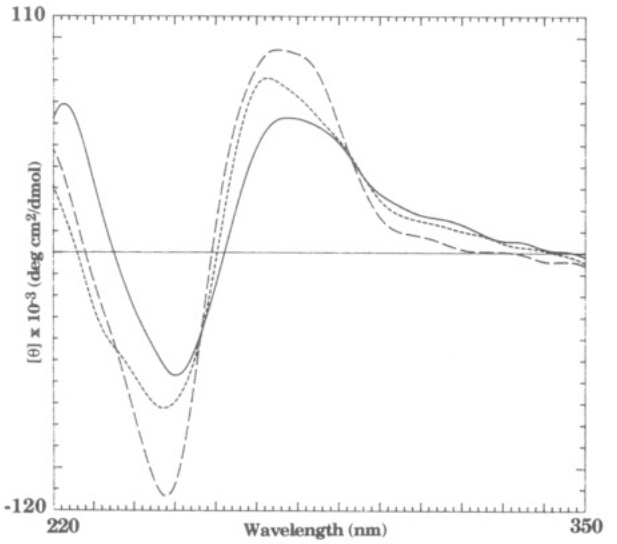


Figure 4. Circular dichroic spectra of (a) DNA I (T,A) (– –), (b) DNA I (P,AP) (- - -), and (c) DNA I (Q,AP) (—) (ca. 16 μM in single strands, 100 mM Na^+ (NaCl), 10 mM phosphate buffer, pH 7.0, 0.1 mM EDTA, 25 °C).

of DNA I, X = Q, eluting at the time expected for diol **6** had the characteristic UV spectrum of diol **6**, further confirming the incorporation of **6** as desired. These data confirmed that residues with structures **5** and **6** had been successfully incorporated into synthetic DNA.

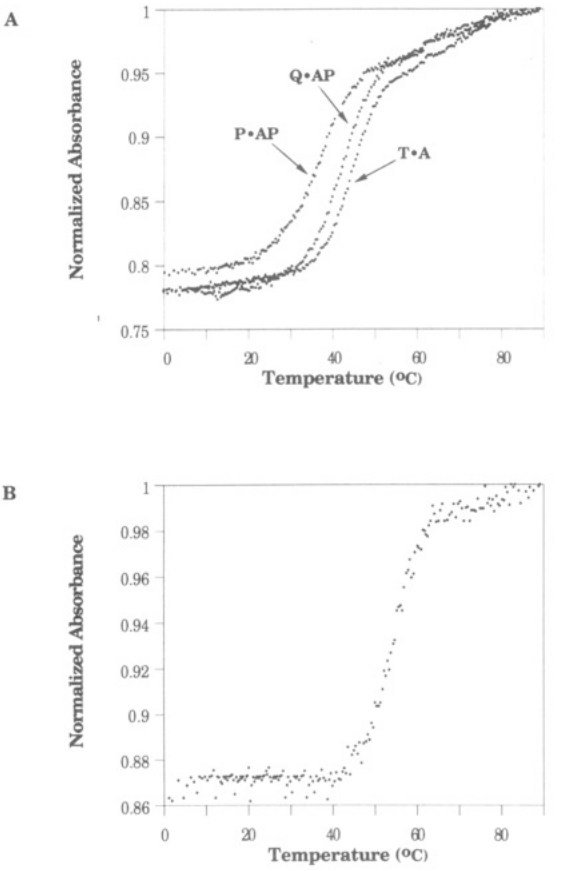


Figure 5. UV-monitored thermal denaturation of DNA duplexes. (A) DNA I monitored at 260 nm. (B) DNA III (Q,AP) monitored at 325 nm.

Characterization of Duplex DNAs Containing Pyridone and Quinolone Residues. Native PAGE[16] provided clear evidence for complexation of pyridone- and quinolone-containing DNAs with a complementary strand bearing the 2-aminopurine (AP) residue. Admixture in equimolar quantities of the complementary strands of DNA I (P,AP) and I (Q,AP) afforded a substance with PAGE mobility distinct (lower) from that of either single strand along (Figure 3). The circular dichroic spectra of these same two duplexes possessed the minima and maxima at ca. 250 and 280 nm, respectively, commonly observed for B-DNA[17] (Figure 4).

UV-monitored thermal denaturation was used to compare the P·AP and Q·AP base pairs to the corresponding A·T pair (Figure 5A). For DNAs I, II, and III, the P·AP pair-containing duplex was seen to melt some 4–5 °C lower than an A·T pair (Table I). The Q·AP pair better approximated an A·T pair, with melting temperatures lowered by 1–3 °C (Table I). To place these values in context, it should be noted that in DNAs of this size, a dysfunctional base pair lowers the melting temperature on the order of 20 °C.[18]

The fact that native DNA does not have a chromophore that absorbs past 300 nm was also used to study the AP·Q base pair. Both AP and Q absorb at 325 nm and by monitoring a thermal denaturation at this wavelength only the modified base pair was studied. A UV-monitored

(17) Ivanov, V. I.; Minchenkova, L. E.; Schyolkina, A. K.; Poletayev, A. I. *Biopolymers* **1973**, *12*, 89.

(18) (a) Aboul-ela, F.; Koh, D.; Tinoco, I. *Nucleic Acids Res.* **1985**, *13*, 4811. (b) Gaffney, B. L.; Jones, R. A. *Biochemistry* **1989**, *28*, 5881. (c) Bhattacharyya, A.; Lilley, D. M. J. *J. Mol. Biol.* **1989**, *209*, 583 and references therein.

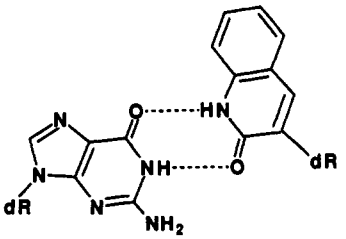
Table I. Physical Parameters Derived[a] from UV-Monitored, Thermal Denaturation Studies

DNA	X	Y	T_m[b] (°C)	ΔH (kcal/mol)	ΔS (cal/mol·K)	ΔG^{298} (kcal/mol)	hyperchromicity (260 nm, %)
I	T	A	43	90.4	259	13.2	18
I	Q	AP	42	89.6	258	12.7	23
I	P	AP	38	83.8	243	11.4	23
I	P	G	35	78.6	227	10.9	24
I	P	A	29	75.3	221	9.4	24
I	P	T	28	82.4	247	8.8	25
I	P	C	26	81.0	244	8.3	21
I	Q	G	38	77.2	221	11.4	25
I	Q	A	30	64.0	184	9.2	22
I	Q	T	34	76.1	220	10.5	23
I	Q	C	26	75.9	226	8.5	22
II	T	A	51				21
II	P	AP	47				16
II	Q	AP	48				17
III	T	A	51				28
III	P	AP	46				25
III	Q	AP	50				28

[a] See Experimental Section for details. [b] 4.5 μM in single stranded DNA, 100 mM Na^+ (NaCl), 10 mM phosphate buffer, pH 7.0, 0.1 mM EDTA.

thermal denaturation profile of the DNA III (Q,AP) at 325 nm is shown in Figure 5B.[19] The high cooperativity of the transition, T_m 55 °C, provides further evidence that the Q·AP is paired in the duplex state and melts in concert with the normal, surrounding base pairs.

Further evidence in favor of the base pairing pattern illustrated in Figure 1 for the P·AP and Q·AP base pairs was obtained by varying the residue opposite P or Q. Among the DNAs I in Table I are found all possible pairing of dA, dC, dG, and dT with dP and dQ. All combinations were lower melting than the comparable P·AP or Q·AP pair; with the exception of the P·G and Q·G pairs, the melting temperatures were some 9–12 °C lower for dP and 8–16 °C lower for dQ. The exceptional pairings P·G and Q·G melted only 3 and 4 °C lower, respectively, than the P·AP and Q·AP pairs, suggesting the possibility of wobble pairing as illustrated in **19**. The analogous wobble pairing has been observed crystallographically in a G·T pair.[20]



19

The enthalpies, entropies, and free energies of melting were determined for DNAs I (Table I) using van't Hoff analysis.[21,22] It has been shown that enthalpies obtained by the method are in good agreement with those measured calorimetrically.[21a,b] This analysis reveals that the Q·AP- and A·T-containing duplexes are closely related, differing by less than one part per hundred in both enthalpy and entropy of melting (see Table I). Interestingly, relative to the A·T-containing duplex, the duplex containing the P·AP pair had a some 7 kcal/mol lower enthalpy of melting, which was only partially offset by an entropy of melting lowered by some 16 cal/mol·K.

Having established that DNAs containing one Q·AP pair form duplexes comparable in stability to one with an A·T pair, we sought direct spectroscopic confirmation of the Q·AP base pair. Nuclear magnetic resonance measurements are especially valuable in this regard, revealing the individual base pairs in short DNA duplexes through their "imino" hydrogen resonances.[23] The ^1H NMR spectrum of DNA I (Q,AP) between 11.5 and 13.5 ppm was strongly temperature dependent, with nine distinct, sharp resonances at 15 °C (Figure 6). At lower temperature (5 °C), evidence of two additional, broad resonances was found, which we presumed to be based pairs 1 and 11; at higher temperature, two of the original nine resonances broadened, and these were assigned as base pairs 2 and 10. Through a series of 1D NOE measurements in which resonances marked as 4 through 9 in Figure 6 were irradiated and thus revealed the identity of their immediate neighbors, an order in space of eight (3–10) of the protons represented by the nine sharp resonances could be assigned (see Figure 6). Together with the temperature-dependent broadening observations, this allowed the resonance assignments shown in Figure 6. Because of the pseudosymmetry of this sequence, the polarity of the contiguous protons could not be assigned (5′ → 3′ vs 3′ → 5′); however, because the Q·AP pair is centrally located, it can unequivocally be assigned to the resonance at 12.29 ppm. The temperature-dependent behavior of this resonance is qualitatively comparable to the other centrally located G·C and A·T pairs, showing no signs of aberrant line broadening, supporting the conclusion that the Q·AP pair is comparable in stability to an A·T pair.

Conclusions

It is thus demonstrated that both the pyridone **5** (dP) and the quinolone **6** (dQ) form stable base pairs opposite the deoxyriboside of 2-aminopurine (dAP). Circular dichroism spectra of these duplexes possess maxima and minima corresponding to those of B-DNA. This pairing of P or Q to AP is preferred over pairing to A, C, G, or T. The thermodynamic stability of P·AP is somewhat less than the corresponding A·T pair; Q·AP is comparable in stability to A·T. Proton NMR measurements revealed a low field resonance assigned to the imino hydrogen of the Q·AP pair, the line shape of which was roughly equivalent to a typical A·T or G·C pair.

Experimental Section

General Procedures. Air- or water-sensitive reactions were conducted under a positive argon atmosphere. Commercial

(19) The UV-monitored thermal denaturation was run the same buffer as in previous experiments, but because of the reduced signal afforded by the single base pair being monitored, the concentration (56 μM) of DNA was ten times that of experiments monitored at 260 nm. This concentration difference qualitatively accounts for the higher observed melting temperature.

(20) Hunter, W. N.; Brown, T.; Kneale, G.; Anand, N. N.; Rabinovich, D.; Kennard, O. *J. Biol. Chem.* **1987**, *262*, 9962.

(21) (a) Review: Breslauer, K. J. in *Thermodynamic Data for Biochemistry and Biotechnology*; Hinz, H., Ed., Academic Press: New York, 1986; pp 402–427. (b) Albergo, D. D.; Marky, L. A.; Breslauer, K. J.; Turner, D. H. *Biochemistry* **1981**, *20*, 1409. (c) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601. (d) LeBlanc, D. A.; Morden, K. M. *Biochemistry* **1991**, *30*, 4042.

(22) Benight, A. S.; Schurr, J. M.; Flynn, P. F.; Reid, B. R.; Wemmer, D. E. *J. Mol. Biol.* **1988**, *200*, 377.

(23) (a) Patel, D. J.; Kozlowski, S. A.; Ikuta, S.; Itakura, K.; Bhatt, R.; Hare, D. R. *Cold Spring Harbor Symp. Quant. Biol.* **1983**, *47*, 197. (b) For a review on sequence specific assignments and their use in NMR studies of DNA structure, see: Reid, B. R. *Q. Rev. Biophys.* **1987**, *20*, 1. (c) Kearns, D. R.; Patel, D. J.; Schulman, R. G. *Nature* **1971**, *229*, 338. (d) Chou, S.-H.; Wemmer, D. E.; Hare, D. R.; Reid, B. R. *Biochemistry* **1984**, *23*, 2257.

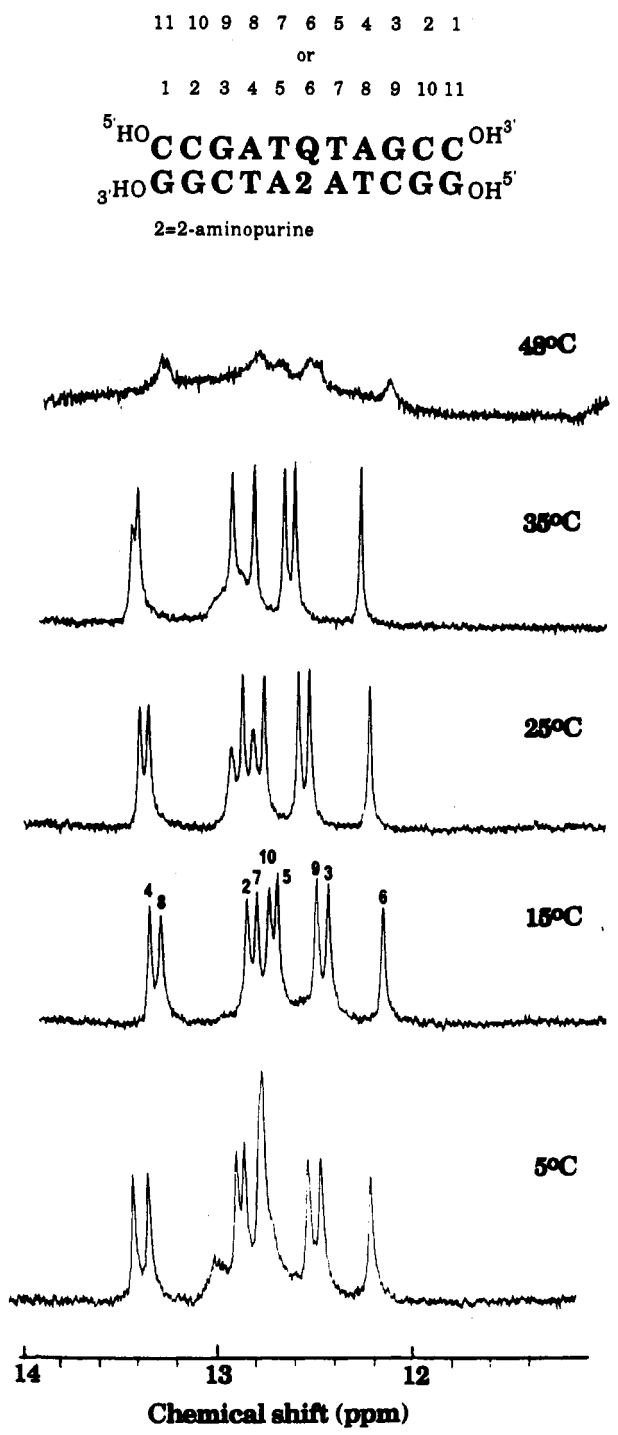


Figure 6. ^{1}H NMR spectra of the "imino region" of DNA I (Q,AP) (ca. 0.7 mM of single strands, 100 mM Na^+ (NaCl), 10 mM phosphate buffer, pH 7.0, 0.1 mM EDTA, 10% D_2O) as a function of temperature. The two possible resonance assignments were derived from difference NOE measurements.[23]

reagents were used as received, except for the following: acetonitrile, dichloromethane, fluoropyridine, pyridine, and triethylamine were distilled under argon from calcium hydride; tetrazole was sublimed at reduced pressure and 140 °C and tetrahydrofuran was distilled under argon from benzophenone ketyl. The 2-deoxyriboside of 2-aminopurine was synthesized starting from triisobutyryl-2-deoxyguanosine[24] using the method of McLaughlin.[8a] DNA synthesis reagents were from Applied Biosystems. Alkaline phosphatase (calf intestinal) was from Amersham. Phosphodiesterase I (Crotalus adamanteus venom) was from Pharmacia. Buffer A for UV melts, CD, native PAGE, and NMR was 10 mM phosphate (pH 7.0), 100 mM Na^+ ion (NaCl), and 0.1 mM EDTA. TE buffer was 10 mM Tris (pH 8.0), 1 mM EDTA. Loading buffer was 90% aqueous deionized formamide containing 10 mM Tris (pH 7.5), 0.1% xylene cyanol, and 0.1 mM sodium EDTA for denaturing gels and 16% glycerol/water for nondenaturing gels. Column chromatography was performed under slight positive pressure on Merck silica gel 60 (230–400 mesh); thin-layer chromatography was performed on precoated silica gel 60 plates (0.25 mm). Ozone was generated from a Welsbach T408 ozonator. Infrared spectra (IR) were recorded on a Perkin-Elmer Model 257 grating infrared spectrophotometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were determined on a Bruker AC200 (200 MHz), a Bruker AF300 (300 MHz), or a Bruker AM500 (500 MHz) spectrometer and, unless otherwise noted, are reported in parts per million downfield from internal tetramethylsilane (0.00 ppm). Coupling constants (J) are given in hertz. ^{31}P NMR spectra were determined on a Bruker AC200 (200 MHz) spectrometer and are reported in parts per million downfield from external phosphoric acid (0.00 ppm). Imino spectra and NOE experiments were carried out on a Bruker AM500 (500 MHz) spectrometer and are reported in parts per million downfield from an external DSS (0.00 ppm) standard. Low resolution mass spectra (LRMS) were measured on a Hewlett-Packard 5985 mass spectrometer; high resolution mass spectra (HRMS) as well as LRMS (FABS) were measured on a VG 7070H double-focusing mass spectrometer. Ultraviolet (UV) spectra were measured on a Perkin-Elmer Lambda 3A or a Hewlett-Packard Model 8450A UV/vis spectrometer and are reported as wavelength in nanometers. UV-monitored thermal denaturations were done using a Hitachi 100-80 UV spectrophotometer connected to a Haake KT2 water bath. Water was purified on a Millipore Milli-Q deionizer. Aqueous samples were concentrated on a Savant Speed Vac concentrator. CD measurements were carried out on a Jasco J700 in a 0.5-cm cell. HPLC analytical and preparative separations were performed on an Alltech, 5 μm, C18, 250-mm × 4.6-mm column, using SSI 200B pumps controlled by an SSI controller and sequential SSI 500 UV/vis and Waters Lambda-Max Model 481LC detectors outputting to both an HP 3390A electronic integrator and a Linear Model 255/MM recorder and a Linear Model 156 recorder, respectively. Solvent gradients were run at 1 mL/min as follows: gradient A, solvent A = 100% 0.1 M ammonium formate; solvent B = 50% methanol/0.1 M ammonium formate; isocratic 99% A for 10 min, 10-min linear gradient to 95% A, isocratic 5 min, 20-min linear gradient to 0% A, isocratic 5 min, 10-min linear gradient to initial conditions; gradient B, solvent A = 0.1 M ammonium acetate; solvent B = 100% CH_3CN; isocratic 92% A for 7 min, 13-min linear gradient to 70% A, 10-min linear gradient to 60% A, 10-min linear gradient to 30% A, 15-min linear gradient to initial conditions; gradient C, solvent A = 100% 0.1 M ammonium formate; solvent B = 50% methanol/0.1 M ammonium formate; isocratic 99% A for 10 min, 10-min linear gradient to 97% A, isocratic 10 min, 20-min linear gradient to 30% A, isocratic 5 min, 5-min linear gradient to 0% A, isocratic 5 min, 15-min linear gradient to initial conditions. Unmodified DNAs were synthesized and purified as described by Kirchner et al.[25]

Allylic Alcohol 8. A 3-neck, 1-L flask was charged with 300 mL of tetrahydrofuran and cooled to 0 °C, and 37 mL (37 mmol, 1 M in ether) of allylmagnesium bromide was added. Zinc bromide,[26] 50 mL (50 mmol, 1 M in THF), was then added dropwise over a 0.25-h period. The solution became yellowish and then gradually turned a milky grey. After 10 min, the mixture was warmed to 25 °C and then cooled to −78 °C. (*R*)-2,3-*O*-Isopropylidene-D-glyceraldehyde (7),[10] 1.3 g (10 mmol), was added in 50 mL of tetrahydrofuran over 20 min. After 0.25 h the reaction was quenched with 15 mL of water and allowed to warm to 25 °C over 1 h. The solution was decanted from the solids, concentrated in vacuo, and then dissolved in ether. The ether solution was washed with saturated aqueous ammonium chloride, dried (Na_2SO_4), filtered, and concentrated to a pale yellow oil. The crude product was used without purification in the next step. A small portion was purified by column chromatography on silica gel (5% Et_2O:CH_2Cl_2) to yield 1.71 g (95%) of 11 as a

(24) Gaffney, B. L.; Marky, L. A.; Jones, R. A. *Tetrahedron* 1984, *40*, 3.

(25) Kirchner, J. J.; Sigurdsson, S. Th.; Hopkins, P. B. *J. Am. Chem. Soc.* 1992, *114*, 4021.

(26) Brown, D. S.; Ley, S. V. *Tetrahedron Lett.* 1988, *29*, 4869.

yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (3 H, s, CH_3), 1.43 (3 H, s, CH_3), 1.99 (1 H, bs, OH), 2.18–2.34 (2 H, m, -CH_2CHC), 3.76–4.05 (3 H, m, -CH_2CHOH), 5.12–5.22 (2 H, m, -CHCH_2), 5.78–5.91 (1 H, m, -CHCH$_2$); MS (EI) *m/e* 157 (M^+ – 15), 131, 115, 101, 73; IR ($CHCl_3$) 3540, 2995, 1595, 1370, 1206, 1150, 1060 cm^{-1}.

Silyl Ether 9. To the alcohol **8**, 1.50 g (8.72 mmol), were added sequentially 20 mL of dichloromethane, 2.33 g (2.53 mmol) of 2,6-lutidine, and 3.45 g (13.2 mmol) of *tert*-butyldimethylsilyl triflate at 0 °C. After 20 min, the reaction was quenched with saturated aqueous sodium bicarbonate. The dichloromethane layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to a clear, colorless oil. The crude product was purified by column chromatography on silica gel (10% EtOAc:hexane) to yield 1.99 g (80%) of **9** as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (6 H, s, 2 × CH_3), 0.83 (9 H, s, C(CH_3)$_3$), 1.32 (3 H, s, CH_3), 1.38 (3 H, s, CH_3), 2.29 (2 H, dd, -CH_2-, *J* = 6.5 and 6.5), 3.67–4.05 (4 H, m, -CH_2CHOSi, -CHO), 4.94–5.16 (2 H, m, -CHCH_2), 5.60–5.94 (1 H, m, -CHCH$_2$); MS (EI) *m/e* 270 (M^+ – 15), 244, 228, 185, 115, 101.

Aldehyde 10. The alkene **9**, 1.48 g (5.17 mmol), in 200 mL of methanol was cooled to –78 °C. Ozone was bubbled through the solution until a blue color persisted. The reaction was complete as shown by TLC analysis. The excess ozone was removed by sparging with argon for 1 h. After adding a solution of triphenylphosphine, 2.13 g (8.16 mmol), in 100 mL of dichloromethane over the course of 1 h, the solution was allowed to warm to 25 °C. The solvents were removed in vacuo, and the residue was loaded directly onto a silica gel column. Elution with 10% EtOAc:hexane afforded the aldehydes separately in 80% ((2*R*,3*S*)-**10**) and 4% ((2*R*,3*R*)-**10**) yields as clear, colorless oils. (**2*R*,3*S***)-**10**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (3 H, s, CH_3), 0.09 (3 H, s, CH_3), 0.82 (9 H, s, C(CH_3)$_3$), 1.29 (3 H, s, CH_3), 1.36 (3 H, s, CH_3), 2.27–2.52 (2 H, m, -CH_2CHO), 3.72–4.23 (4 H, m, -CH_2O-, CH-O, CHOSi), 9.78 (1 H, t, -CHO, *J* = 3.5); MS (EI) *m/e* 273 (M^+ – 15), 244, 231, 187, 173, 129, 101; IR ($CHCl_3$) 2848, 2725, 1720, 1455, 1370, 1258, 1205, 1070, 840 cm^{-1}.

Alcohols 11S/11R. A solution of 0.33 g (3.3 mmol) of diisopropylamine in 10 mL of tetrahydrofuran was cooled to 0 °C and treated with 2.5 mL of *n*-butyllithium (3.3 mmol, 1.3 M in hexane). After 0.25 h the solution was cooled to –78 °C and a solution of 2-fluoropyridine, 0.32 g (3.3 mmol), in 5 mL of tetrahydrofuran was added over 4 min. The solution was stirred for 30 min, at which time a solution of the aldehyde **10**, 1.0 g (3.47 mmol), was added in 5 mL of tetrahydrofuran. After 20 min, 1 mL of saturated sodium bicarbonate was added to the yellow solution, and the reaction mixture was allowed to warm to 25 °C over the course of 1.5 h. After concentration in vacuo, the residue was partitioned between water and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo to yield 1.57 g of a yellow, viscous oil. The crude material was purified by column chromatography on silica gel (5% Et_2O:CH_2Cl_2) to afford 1.07 g (84%) of the alcohols **11S** and **11R** as pale yellow glass. The diastereomers were separated by column chromatography on silica gel (i.e., 0.71 g of the mixture was chromatographed (20% EtOAc:hexane) to afford 50% **11S**, 30% **11R**, and 16% of an **11S**/**11R** mixture). **Diastereomer 11S**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (6 H, s, 2 × CH_3), 0.86 (9 H, s, C(CH_3)$_3$), 1.35 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 2.02 (2 H, dd, CH_2, *J* = 5 and 5), 3.06 (1 H, d, OH, *J* = 4), 3.70–4.22 (4 H, m, -CHCH$_2$O, -CHOSi), 5.21 (1 H, m, -CHOH), 7.19 (1 H, ddd, H5, *J* = 2, 5, and 7), 7.93–8.09 (2 H, m, H4, H6); LRMS (EI) *m/e* 284 (M^+ – 101), 2.70, 252, 198, 178, 126, 101; HRMS (FABS, 3NBA) *m/e* calcd 386.2172, found 386.2159; IR ($CHCl_3$) 3440, 2940, 2860, 1650, 1478, 1240, 1075, 842 cm^{-1}.

Diastereomer 11R: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.11 (3 H, s, CH_3), 0.12 (3 H, s, CH_3), 0.87 (9 H, s, C(CH_3)$_3$), 1.35 (3 H, s, CH_3), 1.42 (3 H, s, CH_3), 1.86 (1 H, ddd, CH_2, *J* = 6, 10, and 16), 2.08 (1 H, ddd, CH_2, *J* = 3, 5, and 15), 3.80–4.21 (5 H, m, -CHCH$_2$O, -CHOSi, OH), 5.16 (1 H, bd, -CHOH, *J* ≈ 10), 7.18 (1 H, ddd, H5, *J* = 2, 5, and 7), 7.94–8.10 (2 H, m, H4, H6); LRMS (EI) *m/e* 284 (M^+ – 101) 270, 252, 198, 178, 126, 101; HRMS (FABS, 3NBA) *m/e* calcd 386.2172, found 386.2159.

Methanesulfonate Ester of 11S. To the alcohol **11S**, 101 mg (0.26 mmol), were added sequentially 6 mL of dichloromethane, 52 mg (0.52 mmol) of triethylamine, and 41 mg (0.36 mmol) of methanesulfonyl chloride. The solution was stirred for 0.25 h at 0 °C and then quenched with saturated aqueous sodium bicarbonate. The dichloromethane layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to yield 120 mg (87%) of the methanesulfonate ester of **11S** as a pale yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) 0.05 (3 H, s, CH_3), 0.12 (3 H, s, CH_3), 0.87 (9 H, s, C(CH_3)$_3$), 1.25 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 2.06–2.47 (2 H, m, -CH_2), 2.89 (3 H, s, -SO_2CH_3), 3.63–4.30 (4 H, m, -CH_2CHO, CHOSi-), 5.99 (1 H, m, H5), 8.21 (1 H, m, H6).

Benzoate Ester of 11R. To the alcohol **11R**, 594 mg (1.54 mmol), were added sequentially 807 mg (3.08 mmol) of triphenylphosphine, 20 mL of THF, and 376 mg (3.08 mmol) of benzoic acid. Once the solution was homogeneous, 0.48 mL (30.8 mmol) of diethyl azodicarboxylate was added, and the yellow solution stirred at 25 °C for 1 h. After concentration in vacuo, the residue was partitioned between saturated aqueous sodium bicarbonate and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo to a yellow oil. The oil was purified by column chromatography on silica gel (100% CH_2Cl_2) to afford 613 mg (80%) of the benzoate of **11R** as a clear, colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$): 0.01 (3 H, s, CH_3), 0.02 (3 H, s, CH_3), 0.88 (9 H, s, C(CH_3)$_3$), 1.28 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 1.98 (1 , ddd, -CH_2, *J* = 3.8, 7.5 and 10.8), 2.38 (1 H, ddd, -CH_2, *J* = 3.4, 9.5, and 13.9), 3.7–4.03 (4 H, m, -CHOSi, -CHCH$_2$), 6.20 (1 H, dd, -CHO-, *J* = 3.8 and 9.9), 7.14 (1 H, ddd, H4, *J* = 4.8 and 7.0), 7.39–7.60 (3 H, m, Ar), 7.78 (1 H, ddd, H5, *J* = 1.8, 7.4, and 9.3), 8.05 (2 H, m, 2 × Ar), 8.14 (1 H, m, H6); IR ($CHCl_3$) 3095, 2930, 2870, 1726, 1606, 1581, 1443, 1370, 1269, 1071 cm^{-1}.

Alcohol 11S from Mitsunobu Inversion. To the benzoate of **11R**, 610 mg (1.24 mmol), was added 30 mL of a saturated solution of ammonia in methanol which was heated to 45 °C for 17 h. The reaction mixture was concentrated in vacuo to 15 mL, and 20 mL of fresh reagent was added. After 12 h, the reaction was complete by TLC analysis and was concentrated to dryness in vacuo. The residue was partitioned between water and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude material was purified by column chromatography on silica gel (CH_2Cl_2 then 3% Et_2O:CH_2Cl_2) to afford 432 mg (92%) of **11S** as a white, waxy solid. The spectra data of this compound were identical to compound **11S** generated directly by cyclization.

Fluorpyridine C-Nucleoside 13. To the methanesulfonate ester of **11S**, 199 mg (0.42 mmol), were added 2.5 mL of a 4:1 mixture of trifluoroacetic acid and chloroform. The mixture turned yellow and then green over the course of 1.5 h, when the reaction was complete by TLC analysis. The mixture was concentrated in vacuo and the residue loaded directly onto a silica gel column. Elution with 5% methanol:CH_2Cl_2 provided 78 mg (87%) of the diol **13** as a white solid: ^{1}H NMR (200 MHz, CD_3OD) δ 1.81 (1 H, ddd, H2′ or 2″, *J* = 6, 10, and 13), 2.25 (1 H, m, H2′ or 2″), 3.59 (2 H, d, H5′, H5″, *J* = 5), 3.89 (1 H, m, H4′), 4.25 (1 H, m, H3′), 5.22 (1 H, dd, H1′, *J* = 6 and 10), 7.22 (1 H, ddd, H5, *J* = 2, 5, and 9), 8.00–8.12 (2 H, m, H4, H6); LRMS (FABS, 3NBA), *m/e* 214 (M^+ + 1), 194, 118.

Pyridone Nucleoside 5. The fluoropyridine nucleoside **13**, 414 mg (1.94 mmol), in 26.0 mL of a 0.075 M solution of potassium hydroxide in benzyl alcohol was heated to 100 °C for 7 h. The benzyl alcohol was then removed by distillation at 90 °C/0.5 mm and the resulting residue purified by column chromatography on silica gel. Elution with 10% methanol:CH_2Cl_2 afforded 583 mg (90%) of the benzyl ether as a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.53 (1 H, bs, OH), 1.80–1.99 (2 H, m, H2′ or 2″, OH), 2.25–2.35 (1 H, ddd, H2′ or 2″, *J* = 2.3, 5.9, and 8.2), 3.71 (2 H, m, H5′ and 5″), 3.72 (1 H, m, H4′), 4.30 (1 H, m, H3′), 5.27 (1 H, dd, H1′, *J* = 6.0 and 9.7), 5.36 (2 H, s, -CH_2), 6.83 (1 H, dd, H4, *J* = 5.0 and 7.2), 7.18–7.38 (5 H, m, Ar), 7.66 (1 H, m, H5), 8.01 (1 H, dd, H6, *J* = 1.9 and 5.0); MS (FABS, 3-NBA) *m/e* 301 (M^+ + 1), 212, 91; IR ($CHCl_3$) 3600, 3020, 1605, 1445, 1365, 1250, 1080 cm^{-1}.

To the benzyl ether, 90 mg (0.29 mmol), were added sequentially 10 mL of dichloromethane and 86 mg (0.43 mmol) of trimethylsilyl iodide at 25 °C. The reaction immediately turned yellow and a gummy white solid precipitated. The mixture was stirred for 3 h at which time it was complete as indicated by TLC analysis. Methanol, 0.4 mL, was added to quench the reaction and the solution was concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2, then

2% methanol:CH_2Cl_2, and finally 5% methanol:CH_2Cl_2) to afford 56 mg (70%) of **5** as a white foam: ^{1}H NMR (200 MHz, D_2O) δ 1.88 (1 H, ddd, H2′ or 2″, J = 6, 10, and 13), 2.18 (1 H, ddd, H2′ or 2″, J = 2, 6, and 16), 3.56 (2 H, m, H5′, H5″), 3.90 (1 H, m, H4′), 4.25 (1 H, m, H3′), 5.07 (1 H, dd, H1′, J = 6 and 10), 6.43 (1 H, dd, H5, J = 7), 7.33 (1 H, dd, H6, J = 2 and 7), 7.68 (1 H, m, H4); LRMS (FABS, 3NBA) m/e 212 (M^+ + 1), 176, 122; HRMS (FABS, 3NBA) m/e calcd 212.0904, found 212.0920; ^{13}C NMR (75 MHz, CD_3OD) δ 41.6, 63.9, 73.9, 77.5, 88.7, 108.4, 133.2, 134.7, 139.1, 163.8; UV (MeOH) λ_{max} 223 (8839 M^{-1} cm^{-1}), 300 nm (6068 M^{-1} cm^{-1}).

Protected Pyridone Nucleoside 15. To the pyridone diol **5**, 176 mg (0.83 mmol), was added imidazole, 417 mg (6.86 mmol), and 2.5 mL of dimethylformamide. Once the mixture had become homogeneous, *tert*-butyldimethylsilyl chloride, 517 mg (3.43 mmol), was added. The mixture was stirred for 14 h at 25 °C and then concentrated in vacuo to a yellow residue. The residue was partitioned between water and dichloromethane. The organic layer was washed with water, dried (Na_2SO_4), filtered, and concentrated in vacuo to a residue that was purified by column chromatography on silica gel (2% Et_2O:CH_2Cl_2) to yield 150 mg (41%) of the 2′,5′-disilylated derivative of **5** and 246 mg of the corresponding O^2,$O^{2'}$,$O^{5'}$-trisilylated compound. The trisilylated compound was treated with Amberlite IRC-50H$^+$ ion exchange beads (2 g) in methanol (60 mL). After 0.5 h the beads were removed by filtration and the filtrate was concentrated in vacuo to 179 mg of the disilylated compound for a 90% combined yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (6 H, s, 2 × CH_3), 0.06 (6 H, s, 2 × CH_3), 0.88 (9 H, s, $C(CH_3)_3$), 0.89 (9 H, s, $C(CH_3)_3$), 1.78 (1 H, m, H2′ or H2″), 2.40 (1 H, dd, H2′ or 2″, J = 3, 6, and 10), 3.69 (2 H, m, H5′ and 5″), 3.88 (1 H, m, H4′), 4.33 (1 H, m, H3′), 5.25 (1 H, dd, H1′, J = 7 and 7), 6.23 (1 H, dd, H5, J = 7 and 7), 7.25 (1 H, m, H6), 7.69 (1 H, m, H4), 11.8 (1 H, bs, NH); MS (EI) m/e 439 (M^+), 424, 382, 208, 176, 122; IR ($CHCl_3$) 3140, 2925, 1645, 1610, 1520, 1468, 1380, 1250, 1105, 840 cm^{-1}.

To the disilylated derivative of compound **5**, 315 mg (0.71 mmol), were added triphenylphosphine, 280 mg (1.07 mmol), and 2-nitrophenethyl alcohol, 178 mg (1.07 mmol). The resulting mixture was dried in vacuo for 1 h. After placing the mixture under argon, 14 mL of anhydrous THF was added followed by diethyl diazodicarboxylate, 186 mg (1.07 mmol). After 1 h the reaction was concentrated in vacuo to a yellow oil and partitioned between water and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (1% Et_2O:CH_2Cl_2, then 3% Et_2O:CH_2Cl_2) to afford 294 mg (71%) of **15** as a colorless oil: NMR (200 MHz, $CDCl_3$) δ 0.04 (6 H, s, CH_3), 0.05 (6 H, s, CH_3), 0.87 (9 H, s, $C(CH_3)_3$), 0.88 (9 H, s, $C(CH_3)_3$), 1.61 (1 H, m, H2′ or 2″), 2.15 (1 H, ddd, H2′ or 2″, J = 2, 6, and 8), 3.17 (2 H, t, -CH_2Ar, J = 6), 3.66 (2 H, m, H5′ and H5″), 3.90 (1 H, m, H4′), 4.32 (1 H, m, H3′), 4.57 (2 H, m, -CH_2O), 5.21 (1 H, dd, H1′, J = 6 and 10), 6.84 (H, dd, H5, J = 5 and 7), 7.42 (2 H, m, 2 × Ar), 7.78 (1 H, m, H6), 7.97 (1 H, m, H4), 8.13 (2 H, m, 2 × Ar); IR ($CHCl_3$) 3095, 2942, 2860, 1590, 1519, 1437, 1343, 1249, 1084, 1031, 837, 773 cm^{-1}.

Phosphoramidite 17. The disilylated, NPE protected nucleoside **15**, 38 mg (0.064 mmol), was dissolved in 2.4 mL of a 2:1 mixture of trifluoroacetic acid/dichloromethane, and the solution was stirred for 28 h at 25 °C. The reaction was concentrated in vacuo and resuspended in CH_2Cl_2, a few drops of saturated aqueous sodium bicarbonate were added to neutralize the residual TFA, and the mixture was reconcentrated. The residue was purified by column chromatography on silica gel (10% methanol:CH_2Cl_2) to yield 14 mg (80%) of the NPE-protected nucleoside of **5** as a colorless glass: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50–1.92 (3 H, m, H2′ or 2″, 2 × OH), 2.14 (1 H, ddd, H2′ or 2″, J = 2, 6, and 8), 3.19 (2 H, t, -CH_2Ar, J = 6), 3.77 (2 H, m, H5′ and H5″), 3.97 (1 H, m, H4′), 4.36 (1 H, m, H3′), 4.59 (2 H, t, -CH_2Ar, J = 6), 5.17 (1 H, dd, H1′, J = 6 and 10), 6.88 (H, dd, H5, J = 5 and 7), 7.43 (2 H, m, 2 × OH), 7.67 (1 H, m, H6), 8.03 (1 H, m, H4), 8.15 (1 H, m, 2 × Ar); LRMS (FABS, 3-NBA) m/e 361 (M^+ + 1).

The NPE-protected pyridone nucleoside of **5**, 90 mg (0.25 mmol), was dissolved in 2.0 mL of dry pyridine and concentrated in vacuo to drive off residual water. 4,4-Dimethoxytrityl chloride, 101 mg (0.3 mmol), and 4-(*N*,*N*-dimethylamino)pyridine, 1.5 mg (0.012 mmol), were added, and the mixture was dried in vacuo for 1 h. Pyridine, 5.0 mL, was then added and the mixture was stirred for 4 h at 25 °C. The mixture was quenched with 0.02 mL of methanol, stirred for 15 min, and then concentrated in vacuo to an orange oil. The crude compound was purified by column chromatography on silica gel (CH_2Cl_2 and then 1–2% MeOH:CH_2Cl_2) to afford 147 mg (99% based on recovered starting material) of the dimethoxytrityl-protected nucleoside of **15** as a white foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.64–1.80 (2 H, m, H2′ or 2″, OH), 2.16 (1 H, ddd, H2′ or 2″, J = 2, 6, and 8), 3.16 (2 H, t, -CH_2Ar, J = 6), 3.28 (2 H, m, H5′ and 5″), 3.76 (6 H, s, 2 × OCH_3), 4.01 (1 H, m, H4′), 4.31 (1 H, m, H3′), 4.56 (2 H, t, -CH_2O, J = 6), 5.19 (1 H, dd, H1′, J = 6 and 10), 6.69–6.85 (5 H, m, 4 × Ar, H5), 7.15–7.44 (11 H, m, H5, 11 × Ar), 7.72 (1 H, m, H6), 7.99 (1 H, m, H4), 8.14 (2 H, m, 2 × Ar); IR ($CHCl_3$) 3560, 2970, 1585, 1495, 1430, 1360, 1230, 1075, 1005, 900, 870 cm^{-1}; LRMS (FABS, 3-NBA) m/e 663 (M^+ + 1), 303.

A mixture of 48 mg (0.072 mmol) of the dimethoxytrityl-protected nucleoside of **15** and 8.2 mg (0.048 mmol) of diisopropylammonium tetrazolide was dried in vacuo for 1 h. Dichloromethane, 1.5 mL, was added followed by 25 mg (0.085 mmol) of bis(diisopropylamino)(2-cyanoethoxy)phosphine. The solution was stirred for 2 h at which time it was partially evaporated and loaded onto a silica gel column. Elution with 5% Et_2O:CH_2Cl_2 gave a clear oil that was lyophilized from benzene to afford 55 mg (90%) of phosphoramidite **17** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.00–1.19 (12 H, m, 4 × CH_3), 1.75 (1 H, m, H2′ or H2″), 2.26–2.65 (3 H, m, H2′ or H2″, CH_2CN), 3.12–3.80 (14 H, m, H5′, H5″, -CH_2O and 2 × $CHCH_3$, 2 × OCH_3, CH_2Ar), 4.20 (1 H, m, H4′), 4.36–4.70 (3 H, m, H3′, CH_2 Ar), 5.17 (1 H, m, H1′), 6.72–6.85 (5 H, m, 4 × Ar, H5), 7.16–7.53 (11 H, m, 11 × Ar), 7.78 (1 H, m, H6), 8.04 (1 H, m, H4), 8.13 (2 H, m, 2 × Ar); ^{31}P NMR (121 MHz, C_6H_6) δ 148.59, 148.37; LRMS (FABS, 3-NBA) m/e 863 (M^+ + 1), 303, 201.

Alcohols 12S and 12R. A solution of 63 mg (0.63 mmol) of diisopropylamine in 3.5 mL of tetrahydrofuran was cooled to 0 °C and treated with 0.5 mL of *n*-butylithium (0.63 mmol, 1.26 M in hexane). After 15 min, the solution was cooled to −78 °C and a solution of 2-fluoroquinoline,[27] 92 mg (0.63 mmol), in 0.25 mL of tetrahydrofuran was added over 4 min. The solution was stirred for 2 h and turned a deep red. A solution of the aldehyde, **10**, 273 mg (0.94 mmol), was added in 0.25 mL of tetrahydrofuran. The reaction became clear yellow within 5 min. After 1 h the mixture was allowed to warm to −35 °C over 2 h and then quenched with a water/THF mixture. After warming to 25 °C over the course of 1.5 h, the mixture was concentrated in vacuo and the residue was partitioned between water and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo to 0.97 g of an orange oil. The crude material was purified by column chromatography on silica gel (CH_2Cl_2 and then 3% Et_2O:CH_2Cl_2) to afford 49 mg (29%) of **12S** as a colorless glass, 42 mg (25%) of **12R** as a colorless oil, and 73 mg (44%) of mixed fractions. **Diastereomer 12S**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.13 (3 H, s, CH_3), 0.14 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 1.39 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 1.90 (1 H, ddd, -CH_2, J = 6, 10, and 16), 2.22 (1 H, m, CH_2), 3.81–4.18 (4 H, m, -CH_2CH, -CHOSi), 4.41 (1 H, d, OH, J = 2), 5.26 (1 H, bd, $CHOH$, J = 10), 7.52 (1 H, m, H6), 7.68 (1 H, m, H7), 7.86 (2 H, m, H5, H8), 8.42 (1 H, d, H4, J = 10). **Diastereomer 12R**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (3 H, s, CH_3), 0.06 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 1.39 (3 H, s, CH_3), 1.45 (3 H, s, CH_3), 2.13 (2 H, m, -CH_2), 3.70–4.28 (5 H, m, -CH_2CH, -CHOSi, OH), 5.35 (1 H, bd, $CHOH$, J = 10), 7.52 (1 H, m, H6), 7.68 (1 H, m, H7), 7.80–7.98 (2 H, m, H5, H8), 8.52 (1 H, d, H4, J = 7); HRMS (FABS, 3NBA) m/e calcd 436.2316, found 436.2269; IR ($CHCl_3$) 3400, 3005, 1620, 1677, 1500, 1420, 1350, 1200, 1070, 930 cm^{-1}.

Benzoate Ester of 12R. To the alcohol **12R**, 56 mg (0.12 mmol), were added sequentially 62 mg (0.24 mmol) of triphenylphosphine, 3.0 mL of THF, and 29 mg (0.24 mmol) of benzoic acid. Once the solution was homogeneous, 42 mg (0.24 mmol) of diethyl azodicarboxylate was added and the solution was stirred at 25 °C. After 10 min, the bright yellow solution had become pale yellow and was concentrated in vacuo. The residue was resuspended in ether and the precipitate removed by filtration. The filtrate was dried (Na_2SO_4), filtered, and concentrated in vacuo to a yellow oil. The oil was purified by column chroma-

(27) Hamer, J.; Link, W. J.; Jurjevich, A.; Vigo, T. L. *Rec. Trav. Chim.* **1962**, *81*, 1058.

tography on silica gel (10% EtOAc:hexane) to afford 55 mg (86%) of the benzoate of **12R** as a pale yellow glass: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04 (3 H, s, CH_3), 0.05 (3 H, s, CH_3), 0.90 (9 H, s, $C(CH_3)_3$), 1.26 (3 H, s, CH_3), 1.39 (3 H, s, CH_3), 2.16 (1 H, ddd, $-CH_2$, J = 4, 7, and 11), 2.47 (1 H, m, -CH32), 3.73–4.04 (4 H, m, -CHOSi, $-CHCH_2$), 6.35 (1 H, dd, -CHO, J = 4 and 10), 7.24–7.83 (6 H, m, H5 or H8, H6, H7, 3 × Ar), 7.91 (1 H, m, H5 or H8), 8.07 (2 H, m, 2 × Ar), 8.21 (1 H, m, H4); IR ($CHCl_3$) 2920, 1735, 1610, 1580, 1500, 1420, 1250, 1070, 920, 830 cm^{-1}.

Methanesulfonate Ester of 12S. To the alcohol **12S**, 50 mg (0.11 mmol), was added 3.0 mL of dichloromethane. The mixture was cooled to 0 °C and treated sequentially with triethylamine, 22 mg (0.22 mmol), and 16 mg (0.14 mmol) of methanesulfonyl chloride. The solution was stirred for 0.5 h and then quenched with saturated aqueous sodium bicarbonate. The dichloromethane layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to yield 55 mg (88%) of the methanesulfonate ester of **12S** as a yellow oil. The mesylate was taken on without further purification to the next step: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (3 H, s, CH_3), 0.16 (3 H, s, CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 1.20 (3 H, s, CH_3), 1.32 (3 H, s, CH_3), 2.28 (1 H, m, $-CH_2$-), 2.47 (1 H, m, $-CH_2$-), 2.87 (3 H, s, SO_2CH_3), 3.63–3.99 (4 H, m, $-CH_2CHO$, -CHOSi-), 6.11 (2 H, dd, CHOMs, J = 6 and 8), 7.56 (1 H, dd, H6, J = 8 and 8), 7.71–7.96 (3 H, m, H7, H5, H8), 8.31 (1 H, d, H4, J = 10).

Fluoroquinolone Nucleoside 14. To the methanesulfonate ester of **12S**, 56 mg (0.1 mmol), was added 4.75 mL of a 4:1 mixture of trifluoroacetic acid and methanol. After 20 min, the reaction was transferred to a larger flask by rinsing with MeOH:CH_2Cl_2 and the reaction concentrated in vacuo at 25 °C. The remaining TFA was quenched with saturated aqueous sodium bicarbonate and extracted several times with dichloromethane. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to a residue that was purified by column chromatography on silica gel. Elution with 3% MeOH:CH_2Cl_2 provided 23 mg (86%) of **14** as a waxy, white solid: ^{1}H NMR (200 MHz, CD_3OD) δ 1.87 (1 H, ddd, H2′ or 2″, J = 6, 10, and 16), 2.33 (1 H, ddd, H2′ or 2″, J = 2, 6, and 16), 3.63 (2 H, m, H5′, H5″), 3.93 (1 H, m, H4′), 4.26 (1 H, m, H3′), 5.30 (1 H, dd, H1′, J = 5 and 10), 7.48 (1 H, dd, H6 or H7, J = 7), 7.64 (1 H, dd, H6 or H7, J = 2 and 7), 7.74 (1 H, d, H5 or H8, J = 8), 7.86 (1 H, d, H5 or H8, J = 8), 8.52 (1 H, d, H4, J = 10); LRMS (FABS, 3NBA) m/e (M^+ + 1) 264, 109; UV (MeOH) λ_{max} 306, 299 (sh), 293, 263, 222 nm.

Quinolone C-Nucleoside 6. To the fluoroquinoline nucleoside **14**, 80 mg (0.3 mmol), was added 2.0 mL of 0.1 N aqueous sodium hydroxide. The mixture was heated to 90 °C for 3 h. The mixture was neutralized with Amberlite IRC-50H$^+$ ion exchange beads to a pH of 7. The beads were removed by filtration and washed with methanol. The filtrate was concentrated in vacuo and the resulting residue purified by column chromatography on silica gel. Elution with 10% methanol:CH_2Cl_2 afforded 66 mg (83%) of **6** as a white solid: ^{1}H NMR (200 MHz, D_2O) δ 1.95 (1 H, ddd, H2′ or 2″, J = 6, 10, and 16), 2.39 (1 H, ddd, H2′ or 2″, J = 2, 6, and 16), 3.75 (2 H, m, H5′, H5″), 4.09 (1 H, m, H4′), 4.40 (1 H, m, H3′), 5.18 (1 H, dd, H1′, J = 6 and 10), 7.18 (2 H, m, H6, H7), 7.39 (2 H, m, H5, H8), 7.89 (1 H, m, H4); HRMS (FABS, 3NBA) m/e calcd 262.1077, found 262.1050; UV (MeOH) λ_{max} 226, 270, 325 nm; fluorescence (MeOH) λ_{ex} = 320 nm; λ_{em} = 425 nm.

Protected Quinolone Nucleoside 16. To the quinolone diol **6**, 65 mg (0.25 mmol), were added imidazole, 207 mg (3.20 mmol), and 2.0 mL of dimethylformamide. Once the mixture had become homogeneous, *tert*-butyldimethylsilyl chloride, 229 mg (1.51 mmol), was added and the mixture was stirred for 4.5 h at 25 °C. The reaction was quenched with methanol (0.05 mL) and partitioned between ether and water. The ether layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to a residue that was purified by column chromatography on silica gel (2% Et_2O:CH_2Cl_2) to yield 77 mg (63%) of the 2′,5′-disilylated derivative of **6** and 30 mg of the corresponding O^2,$O^{2'}$,$O^{5'}$-trisilylated compound. The trisilylated compound was treated with Amberlite IRC-50H$^+$ ion exchange beads (220 mg) in methanol/dichloromethane (6 mL/2 mL). After 0.5 h, the beads were removed by filtration and the filtrate was concentrated in vacuo to yield 19 mg of the disilylated derivative of **6** as a colorless oil, for a 78% combined yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.05 (12 H, m, 4 × CH_3), 0.89 (9 H, s, $C(CH_3)_3$), 0.92 (9 H, s, $C(CH_3)_3$), 1.78 (1 H, ddd, H2′ or H2″, J = 6, 10, and 13), 2.54 (1 H, ddd, H2′ or 2″, J = 3, 6, and 13), 3.74 (2 H, m, H5′ and 5″), 3.98 (1 H, m, H4′), 4.38 (1 H, m, H3′), 5.36 (1 H, dd, H1′, J = 6 and 9), 7.15 (1 H, m, H6 or H7), 7.40–7.56 (3 H, m, H5, H8, H6, or H7), 7.98 (1 H, m, H4), 10.6 (1 H, bs, NH); IR ($CHCl_3$) 3140, 2925, 1645, 1610, 1520, 1468, 1380, 1250, 1105, 840 cm^{-1}.

To disilylated **6**, 96 mg (0.19 mmol), were added triphenylphosphine, 77 mg (0.29 mmol), and 2-nitrophenethyl alcohol, 49 mg (0.29 mmol), and the resulting mixture was dried in vacuo for 2 h. After the mixture was placed under argon, 3.5 mL of anhydrous THF was added followed by diethyl diazodicarboxylate, 51 mg (0.29 mmol). After 0.75 h, the mixture was concentrated in vacuo to a yellow oil. The residue was partitioned between water and dichloromethane. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude compound was purified by column chromatography on silica gel (1% Et_2O:CH_2Cl_2 and then 3% Et_2O:CH_2Cl_2) to afford 70 mg (57%) of **16** as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (3 H, s, CH_3), 0.07 (3 H, s, CH_3), 0.09 (6 H, s, 2 × CH_3), 0.88 (9 H, s, $C(CH_3)_3$), 0.90 (9 H, s, $C(CH_3)_3$), 1.64 (1 H, ddd, H2′ or H2″, J = 6, 10, and 13), 2.22 (1 H, dd, H2′ or 2″, J = 2, 6, and 13), 3.24 (2 H, m, CH_2Ar), 3.73 (2 H, m, H5′ and 5″), 3.98 (1 H, m, H4′), 4.34 (1 H, m, H3′), 4.75 (2 H, m, $-OCH_2$), 5.31 (1 H, dd, H1′, J = 6 and 10), 7.34 (1 H, m, H6 or H7), 7.45 (2 H, m, 2 × Ar), 7.56 (1 H, m, H6 and H7), 7.68 (1 H, m, H5 or H8), 7.78 (1 H, m, H5 or H8), 8.15 (3 H, m, H4, 2 × Ar); IR ($CHCl_3$) 2927, 2854, 1624, 1521, 1426, 1344, 1256, 1092, 835, 776 cm^{-1}.

Phosphoramidite 18. To the disilylated NPE-protected quinolone nucleoside **16**, 70 mg (0.11 mmol), were added 3.0 mL of ethanol and 135 mg (0.54 mmol) of pyridinium *p*-toluenesulfonic acid. The mixture was heated at 55 °C for 62 h. The mixture was then concentrated in vacuo and partitioned between CH_2Cl_2 and water. The dichloromethane was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (3% methanol:CH_2Cl_2) to yield 34 mg (88%) of the NPE-protected derivative of **6** as a colorless glass: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.70 (1 H, bs, OH), 1.82 (1 H, dd, H2′ or 2″, J = 6, 10, and 13), 2.02 (1 H, bs, OH), 2.25 (1 H, ddd, H2′ or 2″, J = 2, 6, and 13), 3.27 (2 H, dd, $-CH_2Ar$, J = 6 and 6), 3.85 (2 H, m, H5′ and 5″), 4.06 (1 H, m, H4′), 4.40 (1 H, m, H3′), 4.78 (2 H, dd, $-CH_2O$, J = 6 and 6), 5.29 (1 H, dd, H1′, J = 6 and 10), 7.38 (1 H, m, H6 or H7), 7.48 (2 H, m, 2 × Ar), 7.60 (2 H, m, H6 or H7, H5 or H8), 7.71 (1 H, m, H5 or H8), 8.08 (1 H, s, H4), 8.18 (2 H, m, 2 × Ar); ^{13}C NMR (75 MHz, $CDCl_3$) δ 35.3, 42.4, 63.4, 65.5, 73.7, 75.6, 86.9, 123.7, 124.4, 125.8, 126.8, 127.4, 129.3, 129.9, 133.9; LRMS (FABS, 3NBA) m/e 361 (M^+ + 1); UV (MeOH) λ_{max} 319, 306, 269 nm.

The NPE-protected quinoline nucleoside of **6**, 27 mg (0.065 mmol), was dissolved in 2.0 mL of dry pyridine and concentrated in vacuo to drive off residual water. 4,4-Dimethoxytrityl chloride, 26 mg (0.079 mmol), and 4-(*N*,*N*-dimethylamino)pyridine, 0.4 mg (0.003 mmol), were then added and the mixture was dried in vacuo for 16 h. Pyridine, 2.0 mL, was then added and the mixture was stirred for 21 h at 25 °C. The reaction was stopped with 0.02 mL of methanol and the solution was stirred for 15 min and then concentrated in vacuo to an orange oil. The crude compound was purified by column chromatography on silica gel (CH_2Cl_2 and then 2–15% Et_2O:CH_2Cl_2) to afford 32 mg (99% based on recovered starting material) of the dimethoxytrityl derivative of NPE-protected **6** as a white foam: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.80 (1 H, m, H2′ or 2″), 2.26 (1 H, ddd, H2′ or 2″, J = 3, 6, and 9), 3.26 (2 H, dd, CH_2Ar, J = 6 and 6), 3.37 (2 H, m, H5′ and 5″), 3.77 (6 H, s, 2 × OCH_3), 4.08 (1 H, m, H4′), 4.35 (1 H, m, H3′), 4.77 (2 H, dd, $-CH_2O$, J = 6 and 6), 5.31 (1 H, dd, H1′, J = 6 and 9), 6.81 (4 H, m, 4 × Ar), 7.18–7.31 (5 H, m, H6 or H7, 4 × Ar), 7.35 (4 H, m, 4 × Ar), 7.48 (4 H, m, 4 × Ar), 7.58 (2 H, m, H5 or H8, H6 or H7), 7.79 (1 H, d, H5 or H8, J = 8), 8.19 (3 H, m, 2 × Ar, H4); IR ($CHCl_3$) 3560, 2970, 1585, 1495, 1430, 1360, 1230, 1075, 1005, 900, 870 cm^{-1}; LRMS (FABS, 3NBA) m/e 663 (M^+ + 1), 303.

To 47 mg (0.066 mmol) of the DMTr-protected, NPE derivative of **6** was added 7.6 mg (0.044 mmol) of diisopropylammonium tetrazolide. The mixture was dried in vacuo for 1 h. Dichloromethane, 1.5 mL, was added followed by 22 mg (0.072 mmol) of bis(diisopropylamino)(2-cyanoethoxy)phosphine. The solution was stirred for 3 h at which time it was partially evaporated and loaded onto a silica gel column. Elution with 5% Et_2O:

CH_2Cl_2 gave a colorless oil that was lyophilized from benzene to afford 45 mg of phosphoramidite 18 as a white solid (75%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.04–1.24 (12 H, m, 4 × CH_3), 1.84 (1 H, m, H2′ or H2″), 2.42–2.61 (3 H, m, H2′ or H2″, CH_2CN), 3.24–3.84 (14 H, m, H5′, H5″, -CH_2O and 2 × C*H*CH_3, 2 × OCH_3, CH_2Ar), 4.25 (1 H, m, H4′), 4.40 (1 H, m, H3′), 4.78 (2 H, m, -CH_2O), 5.34 (1 H, dd, H1′, J = 6 and 9), 6.80 (4 H, m, 4 × Ar), 7.22–7.59 (14 H, m, 11 × Ar, H6, H7, H8, or H5), 7.80 (1 H, m, H5 or H8), 8.17 (2 H, m, 2 × Ar), 8.28 (1 H, m, H4); ^{31}P NMR (121 MHz, C_6H_6) δ 149.68; ^{31}P NMR (121 MHz, $CDCl_3$) δ 148.72, 148.45; LRMS (FABS, 3NBA) *m/e* 913 (M^+ + 1), 303, 201.

Synthesis and Purification of Modified Oligonucleotides. DNAs containing modified bases were synthesized using standard procedures except that the coupling time for each step was increased from 30 s to 2 min. After cleavage from the CPG resin (28% aqueous ammonia), the protecting groups were removed by two methods. DNAs containing 2-aminopurine were diluted with 1 mL of 28% aqueous ammonia and heated to 55 °C for 72 h in a screw cap test tube. The resulting solution was concentrated in a vacuum centrifuge at 25 °C. DNA strands containing the NPE-protected pyridone or quinolone were diluted with 1 mL of 28% aqueous ammonia and heated to 55 °C for 15 h in a screw cap test tube. This step removed all of the protecting groups except the NPE group. The resulting solution was concentrated in a vacuum centrifuge at 25 °C. To the residue were added 1 M DBU in pyridine (0.5 mL/30 OD) and 4 mg of cetyltrimethylammonium bromide.[28] The DNA suspension was sonicated in intervals over 5 min and heated at 55 °C for 24 h. The reactions were quenched with 1.1 equiv of acetic acid and concentrated in a vacuum centrifuge at 25 °C. The DNA was then dissolved in 1 mL of water and applied to a ca. 10-mL column containing Biorad AG-50W×X8 (Na^+ form) ion exchange resin to exchange the cetyltrimethylammonium group for sodium ion. Fractions (1 mL) were collected and those fractions that had an absorbance at 260 nm were pooled and concentrated. The DNA usually eluted in fractions 3–8. The crude DNA was analyzed by analytical PAGE and purified by preparative PAGE.

Extinction coefficients for the DNAs were calculated using the method of Tinoco.[29] Modified bases were substituted into the calculation by taking the average of the extinction coefficients (260 nm) for the nearest neighbor doublets. These extinction coefficients were then used when calculating the concentrations of these DNAs for subsequent experiments.

Enzymatic Hydrolysis and Quantitation of Nucleosides by HPLC. DNAs containing modified bases (0.2 OD) in 30 mL of 500 mM Tris buffer (10 mM $MgCl_2$, pH 8.9) were treated with calf intestinal phosphatase, 2 μL (2 units), and phosphodiesterase I, 10 μL (1 unit), at 37 °C for 3 h. HPLC analysis was carried out on 10 μL of the above reaction mixture using gradient A (pyridone containing DNAs), gradient B (quinolone containing DNAs), or gradient C (2-aminopurine containing DNAs). Peaks were identified by comparison of retention times of those of commercial samples except for compounds 5, 6, and dAP. Quantitation was based on response factors obtained from an enzymatic hydrolysate of 5′-d(CCGATTTAGCC) for dP- and dQ-containing DNAs and 5′-d(GGCTAAATCGG) for dAP-containing DNAs. These response factors were as follows: gradients A and C, dC, 1; dT, 1.16; dG, 1.91; dA, 2.0; gradient B, dC, 1; dT, 1.23; dG, 1.85; dA, 2.0. For DNA containing dP, detection was at 260 and 300 nm. For DNA containing dAP, detection was at 260 and 310 nm. DNA containing dQ was monitored at 260 nm.

Isolation and Characterization of 6 from 5′-d(CCGATQTAGCC). 5′-d(TGCCATQTACCGT) (5.6 OD) was dissolved in 9 μL of aqueous 10 mM $MgCl_2$, 500 mM Tris (pH 8.9), and 60 μL of water and treated with 2 μL (2 units) of alkaline phosphatase and 10 μL (1 unit) of phosphodiesterase I at 37 °C for 4 h. The most strongly retained substance, t_R 23 min, was collected from an analytical HPLC column using gradient A and concentrated in a vacuum centrifuge at 25 °C. A 1-mL sample of the gradient directly after the peak was collected and concentrated to dryness for use as a blank. The dried compound was dissolved in 1 mL of methanol and the UV spectrum was determined relative to the blank sample prepared in an identical manner. This spectrum was compared to a spectrum of 6 that had not been incorporated into DNA.

Nondenaturing PAGE. Samples of DNA (1.5 μmol of single strands) were dissolved in 10 mL of buffer A, heated to 90 °C for 5 min, and then cooled to 25 °C over 2.5 h. Nondenaturing loading buffer (2 μL) was added to each sample prior to 30% PAGE. Gels were prepared as follows: 16.6 mL of 30% acrylamide stock solution, 2.5 mL of 10× TBE buffer,[16] 5.3 mL of water, and 0.5 mL of 10% aqueous ammonium persulfate were mixed and polymerization initiated by addition of 50 mL of TEMED. The gel was poured (preassembled 0.75-mm gel frame) and after 1 h electrophoresed on a Hoefer SE600 gel stand for 1 h using 1× TBE[16] buffer at 4 °C. Samples and marker dyes (xylene-cyanol and bromophenol blue in loading buffer) were loaded and the gel were run at 250 V/10 mA with a Biorad Model 2000/200 power supply for 8 h until the faster moving dye had traveled to within the bottom 2 in. of the gel. The gel was then stained by exposure to a mixture of 5 mL of Stains-all solution, 60 mL of formamide, and 40 mL of water in the dark for 12 h. Bands were visualized by destaining the gel in water.

Circular Dichroism Spectra. The dried DNAs (16 μmol of single strands) were dissolved in 1 mL of buffer A. Spectra were taken in a 0.5-cm cuvette at 25 °C. The bandwidth was 1.0 nm, the sensitivity was 0.02°, and the response time was 0.5 s. The scan speed was 100 nm/min with a step resolution of 0.2 nm; 16 scans were taken for each sample and the resulting curve was subjected to noise reduction processing.

Measurement and Analysis of Melting Curves. Samples were prepared by dissolving 6.75 μmol of single strand DNA in 1.5 mL of buffer A. The samples were heated to 90 °C for 5 min and then cooled to 25 °C over 2.5 h, followed by purging with helium for 5 min. UV-monitored thermal denaturation was performed in a 1-cm cell in a locally assembled apparatus consisting of a Physitemp type IT-18 thermocouple, a Perkin-Elmer Lambda 3A UV/vis spectrophotometer, and a Techne RB-5 refrigeration bath all interfaced to an IBM-PC. The absorbance was set to either 260 or 325 nm. The melting curves were obtained by heating at 0.5 °C/min.

Absorbance versus temperature curves were converted into θ versus temperature curves (where θ is fraction of oligomers in the single strand state) by subtracting upper and lower base lines.[21,22] These upper and lower linear base lines define temperature-dependent extinction coefficients for the initial and final states. T_ms listed in Table I are at the maximum of the first derivative of the smoothed θ versus temperature curves.

Thermodynamic data were generated from van't Hoff plots (ln K versus $1/T$) of the data using the kaleidagraph graphics package. For non-self-complementary molecules the equilibrium constant, K, for formation of a duplex from single strands,[21a] assuming a two-state model, was calculated using eq 1, where α

$$K = \frac{2\alpha}{[C_T(1-\alpha)^2]} \tag{1}$$

is defined as the fraction of strands in the duplex state and C_T is the total concentration in single strands. Values for K were calculated by using θ, where $\alpha = 1 - \theta$. Plots of ln K versus $1/T$ from $T = T_m \pm 10$ °C were then used to determine values of the enthalpy, ΔH, and entropy, ΔS, from eq 2:

$$\ln K = -\frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R} \tag{2}$$

Values for ΔG were then calculated by substitution of ΔH and ΔS into eq 3:

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

^1H NMR Imino Spectra and NOE Experiments. ^1H NMR imino and NOE experiments were performed on a Bruker AM 500 NMR at 500 MHz with ca. 0.70 mM in single strands in aqueous 10 mM phosphate buffer, 100 mM sodium ion (NaCl), 0.1 mM EDTA, pH 7.0, containing 10% deuterium oxide. The dried DNA was dissolved in buffer and placed in a 5-mm tube. The spectra were obtained using a water suppression program with a 1-1 hard pulse sequence.[23c,d] NOE Experiments were run at 20 °C. On-resonance frequencies were chosen by recording an initial spectrum, and recording the frequencies of the resonances

(28) Xu, Y-Z; Swann, F. *Nucleic Acids Res.* **1990**, *18*, 4061.
(29) Borer, P. N in *Handbook of Biochemistry and Molecular Biology*, 3rd ed., Fasman, G. D., Ed.; CRC Press: Cleveland, Vol. 1, p 589.

to be irradiated from the transformed spectra. NOE experiments were accumulated by the method of Hare and Reid.[30]

Computation. Computation was performed on a Silicon Graphics 4D-25 IRIS workstation utilizing Insight II (version 1.1.0) for structure building.

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(30) Hare, D. R.; Reid, B. R. *Biochemistry* **1982**, *21*, 1835.

Supplementary Material Available: ^{1}H NMR difference NOE spectra for imino region of I(Q,AP) and ^{1}H NMR spectra of **11S**, methanesulfonate ester of **11S**, **13**, benzyl ether of **5**, **5**, bis-TBDMS ether of **5**, NPE ether of **5**, NPE, DMTr-**5**, **17**, **12S**, methanesulfonate ester of **12S**, **14**, **6**, bis-TBDMS ether of **6**, **16**, NPE ether of **6**, NPE, DMTr-**6** and **18** (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.