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Synthesis of C-Nucleosides Designed To Participate in Triplex Formation with Native DNA: Specific Recognition of an A:T Base Pair in DNA

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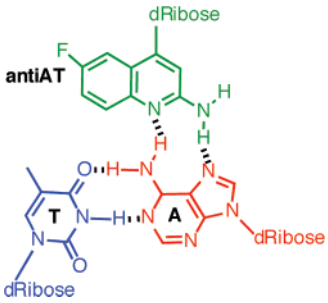

We have previously described a system of 2-aminoquinoline- and 2-aminoquinazoline-based C-deoxynucleosides (TRIPsides) that are designed to be incorporated into oligomers that can specifically bind in the major groove via Hoogsteen base pairing to any sequence of native DNA. The four TRIPsides are termed antiGC, antiCG, antiTA, and antiAT with respect to the Watson–Crick base pair targets that they bind. The first three TRIPsides have been prepared, characterized, and shown to form stable and sequence-specific triplexes. In the present study, we describe the preparation of two molecules, 2-amino-4-(2′-deoxy-β-D-ribofuranosyl)quinazoline (**7**) and 2-amino-6-fluoro-4-(2′-deoxy-β-D-ribofuranosyl)quinoline (**14**), that can serve as the remaining antiAT TRIPside. The phosphoramidites of **7** and **14** were prepared, but only the latter was successfully incorporated into DNA oligomers. It is demonstrated using UV–visible melting experiments that **14** forms sequence-specific intramolecular triplets with A:T base pairs at physiological pH.

Introduction

There is intense interest in the design of molecules that can bind sequence specifically via a triple helix motif to mixed purine/pyrimidine sequences in native Watson–Crick DNA.[1−6] To achieve this goal, we proposed triplex-forming oligomers composed of a set of four C-glycoside bases,[7] 2-amino-4-(2′-deoxy-β-D-ribofuranosyl)quinoline (**antiGC**), 2-amino-5-(2′-deoxy-β-D-ribofuranosyl)quinoline (**antiCG**), 2-amino-4-(2′-deoxy-β-D-ribofuranosyl)-quinazoline (**antiAT**), and 2-amino-5-(2′-deoxy-β-D-ribofuranosyl)quinazoline (**antiTA**), which differentiate between the four base-pairing schemes in the major groove (i.e., G:C, C:G, A:T, T:A, respectively; Scheme 1). The orientation of the triplex-forming oligomer relative to the duplex is arbitrarily defined as running antiparallel to the left strand in the major groove that runs 5′ to 3′ top to bottom. Of the four C-glycoside bases, antiTA,[7] antiGC,[8] and antiCG[9] have been synthesized by the coupling of a protected ribofuranoid glycal with a halo-

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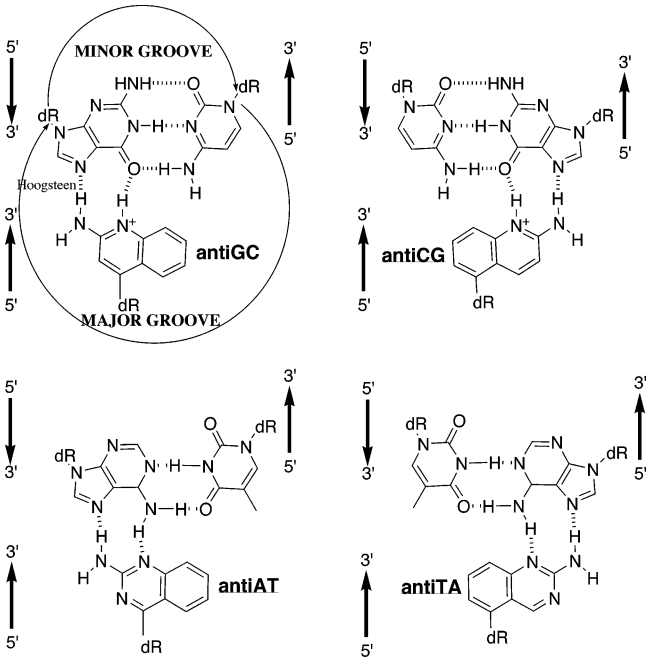
SCHEME 1. Structures of the Four TRIPsides Associated with Their Four Potential Watson–Crick Base Pair Partners



genated heterocycle using a Pd-mediated Heck-type reaction.[10–13] However, antiAT could not be synthesized by this method, presumably due to the electron-rich aminoquinazoline ring system. A literature survey indicated that antiTA was the first example of quinazoline-based C-glycoside.[7] Therefore, it was necessary to develop a new method to prepare quinazoline C-glycosides. Herein, we report the synthesis of the last of the four originally proposed C-glycosides, antiAT (**7**). Although **7** was prepared, it proved unsuitable for solid-phase synthesis using standard phosphoramidite chemistry. To mimic the H-bonding scheme of the antiAT·A:T triplet (Scheme 1), a 2-amino-6-fluoroquinoline-based C-glycoside (antiAT-F, **14**) was synthesized and efficiently incorporated in oligomers employing standard conditions. Characterization of antiAT-F shows that it forms stable intramolecular triplexes at physiological pH and with excellent specificity; that is, mismatches are highly destabilizing.

Results and Discussion

Synthesis of 7 (antiAT). The known starting materials, O-protected 2′-deoxyribonic-1-β-cyanide **1a**[14] and **1b**,[15,16] were hydrolyzed to the corresponding acids **2a** and **2b** in a refluxing mixture of HOAc and HCl (Scheme 2). **2a** and **2b** were then converted into their corresponding acid chlorides (**3a** and **3b**, respectively) by treatment with an excess of $SOCl_2$ in DMF and Et_2O at refluxing temperature. **3a** and **3b** underwent a Stille coupling with *N*-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)aniline[17] to afford **4a** and **4b**, respectively. Removal of the protecting Boc group from **4a** and **4b** gave **5a** and **5b**, respectively, in high yield. The key step in the synthesis was the elaboration of the 2-aminoquinazoline heterocycle through a low-temperature (50 °C) fusion reaction[18] of the HCl salts of **5a** and **5b** with an excess of cyanamide to yield the O-protected C-glycosides **6a** and **6b**, respectively. Deprotection of **6a** and **6b** using NaOMe in MeOH at room temperature (RT) afforded the desired **7** in 70% yield.

NOESY-NMR experiments on **7** in 10 mM Na–phosphate buffer (pH 7.0) containing 50 mM NaCl in D_2O confirmed that it adopts the expected anti conformation (data not shown). This conformational preference is critical for **7** to differentiate A:T from T:A base pairs.[7,8] The UV–vis spectrum of **7** shows absorption peaks at 230 and 352 nm. The absorption peak at 352 nm is typical for 2-aminoquinazoline heterocycles.[7] As expected, **7** showed a characteristic fluorescence emission peak at 430 nm when excited at 350 nm.[7,8]

Attempts To Incorporate antiAT (7) into Oligomers. The amino group in **7** was selectively protected by benzoylation to give **8** using standard procedures.[7] It is worth noting that attempts to protect this amino group with isobutyric anhydride or Fmoc-Cl resulted in the decomposition of the starting material and a complex mixture. Using standard methods, we sequentially converted compound **8** into the 5′-DMTr-protected **9** and the 3′-phosphoramidite derivative **10** (Scheme 2).

It was determined that treatment of **8** in concentrated NH_4OH at 55 °C for 8 h, a standard method for the deprotection of oligomeric deoxynucleotides, resulted in partial decomposition of the desired product, **7**. However, it was found that **8** could be quantitatively converted into **7** in concentrated NH_4OH at 23 °C for 24 h, which provided an acceptable post-DNA synthesis deprotection procedure to remove the N^2-benzoyl group. Preliminary tests indicated that the dimethoxytrityl group in 5′-DMTr-protected **9** could not be removed by 2% DCA in CH_2Cl_2 without affecting the 2-aminoquinazoline heterocycle. This was confirmed in an oligomer synthesis, and therefore standard solid-phase synthesis, which employs 2% DCA in CH_2Cl_2 as the detritylation reagent to generate the free 5′-hydroxy group on the growing oligomer, is not suitable for preparing oligomers containing **7**. Since Lewis acids can catalyze detritylation, we successfully used a saturated $ZnBr_2$ solution in CH_3NO_2[20] to remove the dimethoxytrityl group in 5′-DMTr-protected **9** without affecting the 2-aminoquinazoline heterocycle. However, attempts to incorporate a saturated $ZnBr_2$ solution of CH_3NO_2 into the solid-phase synthesis of oligomers composed of **7** proved unsatisfactory because

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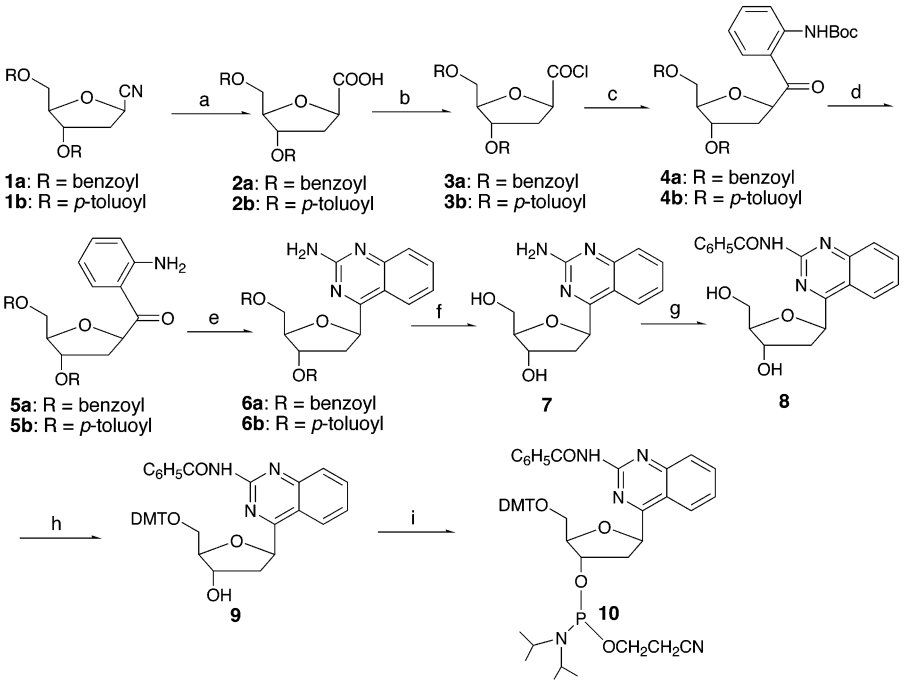
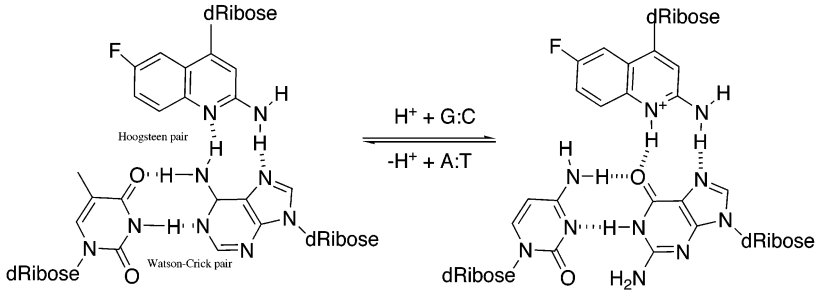
SCHEME 2. Synthesis of antiAT[a]



[a] (a) HCl/HOAc, reflux, 15 min, **2a** (75% yield), **2b** (78% yield); (b) $SOCl_2$, DMF, Et_2O, reflux, 1.5 h; (c) *N*-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)aniline, $Pd_2(dba)_3$, toluene, 85 °C, 4 h, **4a** (68% yield), **4b** (70% yield); (d) TFA, CH_2Cl_2, RT, 1 h, **5a** (92% yield), **5b** (90% yield); (e) HCl, Et_2O, RT, 5 min/NH_2CN, 50 °C, 1.5 h, **6a** (72% yield), **6b** (75% yield); (f) NaOMe, MeOH, RT, 1 h, 70% yield; (g) Me_3SiCl, pyridine, 0 °C, 30 min/PhCOCl, pyridine, RT, 2 h/NH_4OH, 0 °C, 30 min, 78% yield; (h) DMTrCl, pyridine, Et_3N, DMAP, RT, 72 h, 55% yield; (i) 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, CH_2Cl_2, RT, 1 h, 80% yield.

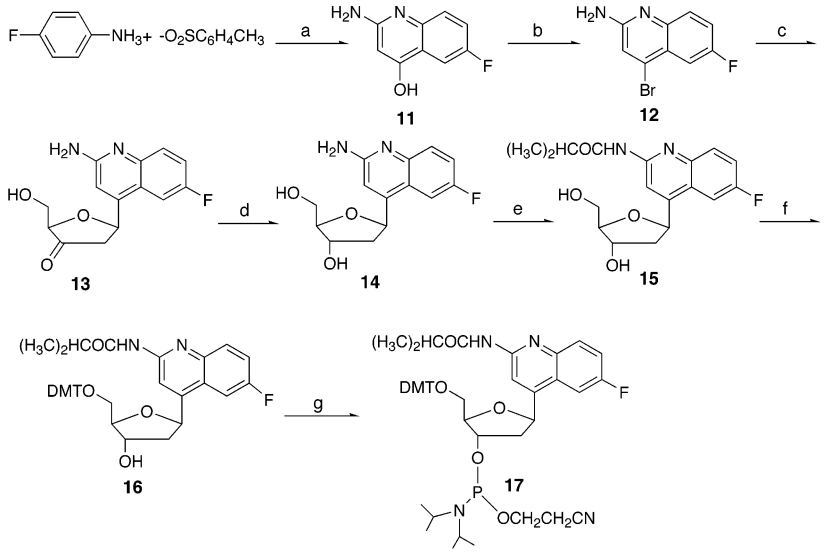
SCHEME 3. Effect of pH on Hoogsteen Binding of antiAT-F to A:T or antiAT-F$^+$ to G:C Watson−Crick Base Pair



of limited solubility of $ZnBr_2$ and the required prolonged deprotection times.

Design and Synthesis of an Alternative to Quinazoline-Based antiAT. The key difference between the antiAT aminoquinazoline (**7**) and the antiGC aminoquinoline (Scheme 1) is that the former is less basic (pK_a, 4.8 versus 7.2, respectively).[7,8] Therefore, antiAT is not protonated at physiological pH and can form the two required H-bonds with an A:T base pair via its H-bond acceptor and H-donator atoms (Scheme 1). In contrast, antiGC is protonated at neutral pH and binds to G:C using its two H-bond donors.[8] We proposed that a fluoro substituent would reduce the pK_a of the 2-aminoquinoline system and thus mimic quinazoline **7** (Scheme 3). The fluoro group, based upon DNA models, was not anticipated to present any steric barriers to triplex formation. The synthesis of antiAT-F is shown in Scheme 4. The formation of the 2-amino-6-fluoroquinoline (**11**) ring system was accomplished, albeit in low yield, by the fusion of 4-fluoroaniline salt with ethyl cyanoacetate. The hydroxy group of **11** was converted into the 4-bromo compound **12** that was needed in the subsequent Heck coupling. The approach to couple **12** with the ribofuranoid glycal has been used previously by others as a route to C-glycosides[10−13] and by us to prepare the antiTA, antiGC, and antiCG TRIPsides.[7−9] The yield for the conversion of **12** to **13** is relatively poor (33%) because of the formation of significant amounts of the quinoline−quinoline coupling dimer, and efforts are underway to improve the procedure. Stereoselective reduction of ketone **13** gives the desired deoxynucleoside **14**. The pK_a of antiAT-F was calculated as 6.7 using either UV−vis or fluorescence versus pH experiments (data not shown). The conformational analysis using NOE ^{1}H NMR confirmed that **14** adopted the anti conformation with respect to the glycosidic bond (data not shown). Incorporation of the antiAT-F phosphoramidite (**17**) into oligomers followed standard procedures and gave >98% coupling

SCHEME 4. Synthesis of antiAT-F (14)[a]



[a] (a) $NCCH_2CO_2Et$, 210−260 °C, 1.25 h (15% yield); (b) $POBr_3/PBr_3$, 140−160 °C (57% yield); (c) $Pd_2(dba)_3$, 1,4-anhydro-2-deoxy-D-*erthro*-pent-1-enitol, dioxane, reflux/TBAF, AcOH, 0 °C, 5 min (33% yield); (d) $NaBH(OAc)_3$, −22 °C, 1 h (70% yield); (e) $(i\text{-}PrCO)_2O$, pyridine, RT, 2 h (47% yield); (f) DMTrCl, pyridine, Et_3N, DMAP, RT, 72 h, 58% yield; (g) 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine, CH_2Cl_2, RT, 1 h, 84% yield.

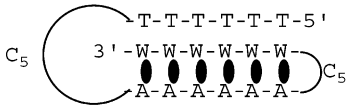
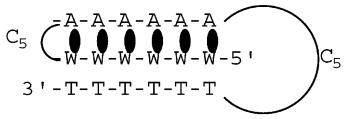
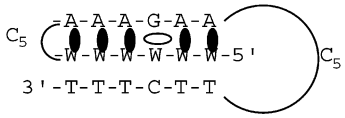
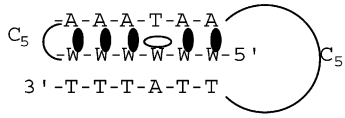
OL	potential triplex structure	pH	T_M 260 nm	T_M 325 nm
OL-1		6	31.0	n.d.
		7	33.8	32.3
		8	31.2	33.3
OL-2		6	34.0	n.d.
		7	35.7	34.9
		8	34.0	47.8
OL-3		6	38.1	n.d.
		7	37.9	n.d.
		8	41.8	n.d.
OL-4		6	38.1	n.d.
		7	37.9	n.d.
		8	41.8	n.d.

FIGURE 1. Sequences of oligomers (OL-1−4), potential triplex structures (solid vertical bonds indicate two Hoogsteen H-bonds can be formed; horizontal open bonds indicate a mismatch), and T_M's in 10 mM sodium phosphate buffer containing 200 mM NaCl as a function of pH; n.d. = not detected. W = antiAT-F (**14**).

yields. After deprotection, HPLC purification, and desalting, the purity and structure of the oligomers were confirmed by analytical HPLC and MALDI-TOF MS.

Intramolecular Triplex Formation with antiAT-F. The ability of antiAT-F (**14**) to participate in a triplex structure with A:T base pairs was evaluated by determining the stability of a series of putative intramolecular triplexes (see Figure 1 for potential structures) using UV−visible absorbance versus temperature profiles. The antiAT-F deoxynucleoside has a λ_{max} at 325 nm, which makes it convenient to monitor absorbance increases commonly observed for aromatic systems when they unstack from a triplex or duplex into a random coil. In addition, by monitoring the absorbance at 260 nm it is possible to observe the natural nucleobases as the Watson−Crick duplex segment melts. In OL-1 and OL-2, antiAT-F is correctly matched with A:T base pairs, although the directionality for triplex formation is reversed (Figure 1). The melting profile at 325 nm for OL-1 at pH 7.0 indicates a triplex structure with a T_M near 32 °C (Figure 2b). At pH 8.0, the triplex to random coil transition is broadened but the T_M remains unchanged (Figure 2b). At both pH 7.0 and 8.0 the duplex to random coil transition has a T_M of 31−34 °C (Figure 2a).

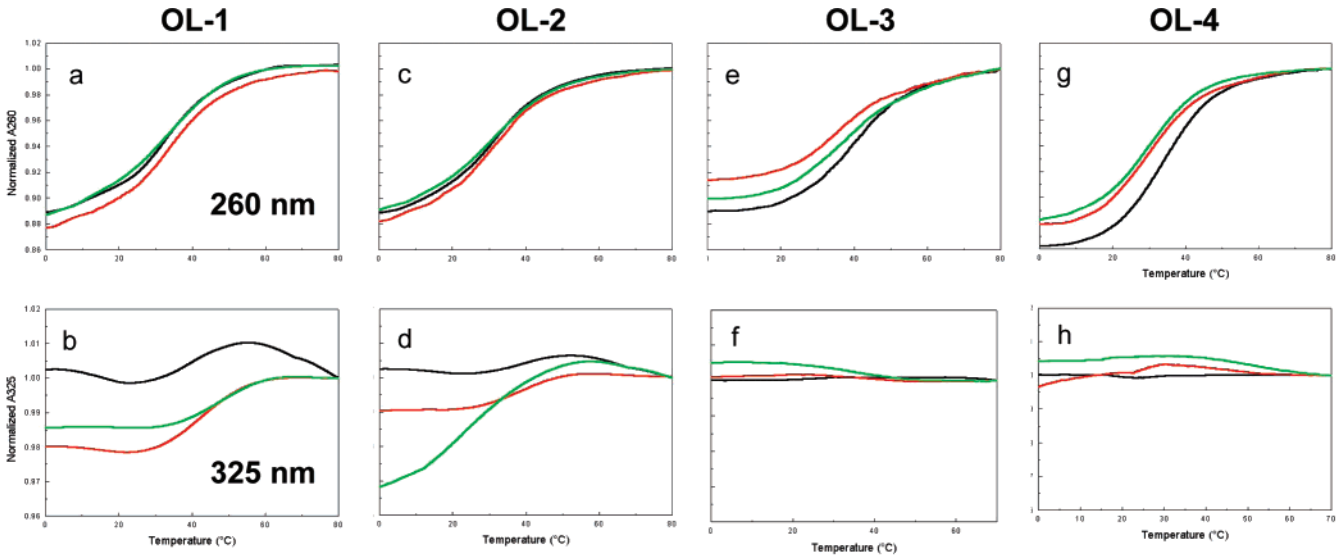


FIGURE 2. T_M curves at 260 and 325 nm in 10 mM sodium phosphate buffer containing 200 mM NaCl at pH 6.0 (black), 7.0 (red), and 8.0 (green) for: (a) OL-1 at 260 nm, (b) OL-1 at 325 nm, (c) OL-2 at 260 nm, (d) OL-2 at 325 nm, (e) OL-3 (with a G:C mismatch) at 260 nm, (f) OL-3 at 325 nm, (g) OL-4 (with a T:A mismatch) at 260 nm, and (h) OL-4 at 325 nm.

In contrast, OL-2 melts with a T_M of 34.9 and 47.8 at pH 7.0 and 8.0, respectively, and with similar hyperchromicities (Figure 2d). The T_M for the duplex stem of OL-2 is near 34−35 °C at all pH values (Figure 2c). Significantly, at pH 6.0 there remains only residual evidence for a triplex in the melting curves for either OL-1 or OL-2 (Figure 2). This is consistent with increased protonation of antiAT-F at pH 6.0, which would convert antiAT-F to antiAT-F$^+$ and from an A:T to a G:C binder (Scheme 3). The hyperchromicity and T_M at 325 nm for the melting of the third strand decreases at lower salt concentration for both OL-1 and OL-2 (data not shown).

We have previously reported that an intramolecular triplex using antiTA TRIPsides, 5′-d(antiTA$_6$-C$_5$-T$_6$-C$_5$-A$_6$), has a T_M of 25 °C at pH 7.0.[7] This is substantially lower than the >30 °C transition temperature we report here for the antiAT-F-based OL-2, which has the same folding orientation and same number of triplets. For comparison, a natural pyrimidine-motif intramolecular triplex, 5′-T$_6$-C$_5$-T$_6$-C$_5$-A$_6$, under the same conditions has a T_M of 22.7 °C.[7]

Another approach to determine the binding specificity of antiAT-F is to replace a single A:T base pair in the duplex segment of OL-2 with either a G:C (OL-3) or T:A (OL-4) base pair. These changes in the stem should introduce mismatches between the antiAT-F segment and the duplex causing a reduction in triplex stability. The results corroborate this expectation (Figure 2f,h): both OL-3 and OL-4 show no evidence of temperature-dependent hyperchromicity when monitored at 325 nm. This suggests that, at neutral pH, antiAT-F only binds stably to A:T.

We explored the possibility that antiAT-F could form an antiparallel Hoogsteen duplex with dA by synthesizing 5′-(antiAT-F)$_6$-C$_5$-(A)$_6$. Modeling shows that antiAT-F and dA can also associate via a parallel pseudo-Watson−Crick duplex. Because of the hairpin arrangement, only the former is possible in 5′-(antiAT-F)$_6$-C$_5$-(A)$_6$. The UV−visible melt for 5′-(antiAT-F)$_6$-C$_5$-(A)$_6$ showed no evidence for duplex formation at 250 or 325 nm (data not shown). Therefore, the presence of a Watson−Crick-stabilized major groove is required for the binding of the third strand. Given the opportunity to form an antiparallel Watson−Crick or Hoogsteen structure, oligomers composed of complementary sequences will invariably associate as the former in the absence of DNA distorting ligands or mismatches. A notable exception is 5′-d(ATATAT), which, in the crystal structure, does associate via Hoogsteen base pairing.[22] However, even this sequence in solution NMR studies adopts a Watson−Crick duplex.[23]

In summary, we have successfully developed a synthetic route to a C-nucleoside, antiAT-F, that specifically binds to A:T base pairs via a triplex structure. The antiAT-F glycoside provides us with the last of the four necessary monomers needed to form a stable triplex at any sequence of native DNA. In addition, we have reported a synthetic pathway for access to aminoquinazoline C-glycosides that cannot be prepared by existing methods.

Experimental Section

All reagents were of the highest grade commercially available and used without any purification. NMR studies were performed on a 500 MHz instrument.

Synthesis of 3,5-Di-*O*-benzoyl-2-deoxy-*β*-D-*threo*-pentofuranosyl Chloride (3a) and 3,5-Di-*O*-toluoyl-2-deoxy-*β*-D-*threo*-pentofuranosyl Chloride (3b). *O*-Benzoyl-protected 2′-*β*-deoxyribocyanide **1a** (12.0 g, 34.2 mmol) was refluxed in concentrated HCl (30 mL) and HOAc (120 mL) for 15 min. Volatiles were removed under vacuum, and the residue was partitioned in 100 mL of EtOAc and 200 mL of brine. The organic portion was separated, dried over $MgSO_4$, and filtered. Removal of the solvent gave 10 g of the *O*-benzoyl-protected 2′-*β*-deoxyribonic acid **2a** as a viscous syrup, which was ca.

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95% pure (based upon NMR) and used without further purification. The *O*-tolulyl-protected 2′-*β*-deoxyribonic acid **2b** was prepared using the same procedure. **2a** (10.0 g, 23.5 mmol) was mixed with $SOCl_2$ (100 mL), DMF (0.3 mL), and anhydrous Et_2O (200 mL) and refluxed for 1 h. The volatiles were removed under reduced pressure. Toluene (30 mL) was added to the residue, and the volatiles were removed under reduced pressure. The acid chloride (**3a**) was ready to use for the next step without any further purification. The *O*-tolulyl-protected 2′-*β*-deoxyribonic acid **3b** was prepared using the same procedure and also used without any further purification.

Synthesis of (3′,5′-Di-*O*-benzoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)-[2-*N*-(*tert*-butoxycarbonyl)]phenyl Ketone (4a) and (3′,5′-Di-*O*-toluoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)-[2-(*N*-*tert*-butoxycarbonylamino)]phenyl Ketone (4b). 3a (10.0 g, 24 mmol) and *N*-(*tert*-butoxycarbonyl)-2-(trimethylstannyl)aniline (8.55 g, 24 mmol) were dissolved in toluene (300 mL). To this solution was added $Pd_2(dba)_3$ (200 mg, 0.5 mmol), and the mixture was heated to 85 °C for 4 h. The reaction was cooled, the volatiles were removed under reduced pressure, and the residue was purified by silica gel column chromatography (hexane to 75% hexane−Et_2O). The product **4a** was obtained as a syrup in 68% yield: ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 9 H), 2.51−2.55 (m, 1 H), 2.79−2.85 (m, 1 H), 4.51 (dd, 1 H, J = 11.5, 5.0 Hz,), 4.61 (dd, 1 H, J = 11.5, 5.0 Hz), 4.65 4.66 (m, 1 H), 5.60 (dd, 1 H, J = 9.5, 6.5 Hz), 5.63−5.65 (m, 1 H), 7.05 (t, 1 H, J = 6.5 Hz), 7.43 7.59 (m, 6 H), 7.63 (t, 1 H, J = 7.5 Hz), 8.03−8.07 (m, 4 H), 8.11 (d, 1 H, J = 9.0 Hz), 8.53 (d, 1 H, J = 9.0 Hz), 10.82 (s, 1 H); HRMS (M + Na^+) calcd for 568.1948, found 568.1931. **4b** was synthesized by the same procedure and obtained as a white solid (70% yield): ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 9 H), 2.42 (s, 3 H), 2.46 (s, 3 H), 2.49−2.54 (m, 1 H), 2.76−2.82 (m, 1 H), 4.48 (dd, 1 H, J = 12.0, 5.0 Hz), 4.61 (dd, 1 H, J = 12.0, 5.0 Hz), 4.62−4.65 (m, 1 H), 5.57−5.61 (m, 2 H), 7.05 (t, 1 H, J = 7.5 Hz), 7.25 (d, 2 H, J = 8.0 Hz), 7.30 (d, 2 H, J = 8.0 Hz), 7.56 (t, 1 H, J = 7.5 Hz), 7.95 (d, 2 H, J = 8.0 Hz), 7.99 (d, 2 H, J = 8.0 Hz), 8.02 (d, 1 H, J = 8.0 Hz), 8.53 (d, 1 H, J = 8.0 Hz), 10.83 (s, 1 H); HRMS (M + Na^+) calcd for 596.2261, found 596.2243.

Synthesis of (3′,5′-Di-*O*-benzoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)(2-amino-phenyl) Ketone (5a) and (3′,5′-Di-*O*-toluoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)(2-amino-phenyl) Ketone (5b). 4a (9.5 g) was dissolved in CH_2Cl_2 (150 mL) and treated with CF_3CO_2H (10 mL) at RT for 1 h. Then the solution was basified with 1 M NaOH (200 mL), the organic portion was separated and dried over $MgSO_4$, and the volatiles were removed. The residue was purified by silica gel column chromatography (hexanes−Et_2O from 3:1 to 1:1). The product **5a** was obtained as a syrup in 92% yield: ^{1}H NMR ($CDCl_3$) δ 2.49−2.54 (m, 1 H, H2″), 2.77−2.83 (m, 1 H, H2′), 4.50−4.53 (m, 1 H), 4.60−4.66 (m, 2 H), 5.59 (dd, 1 H, J = 9.0, 6.0 Hz, H1′), 5.63−5.64 (m, 1 H), 6.30 (bs, 2 H), 6.67−6.69 (m, 2 H), 7.32 (m, 1 H), 7.42−7.64 (m, 6 H), 7.88 (d, 1 H, J = 7.0 Hz), 8.06−8.11 (m, 4 H); HRMS (M + Na^+) calcd for 468.1423, found 468.1410. **5b** was synthesized by the same procedure and obtained as a white solid (90% yield): ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.45 (s, 3 H), 2.48−2.52 (m, 1 H), 2.75−2.81 (m, 1 H), 4.48 (dd, 1 H, J = 12.0, 4.5 Hz), 4.61 (dd, 1 H, J = 12.0, 4.5 Hz), 4.62−4.64 (m, 1 H), 5.57 (dd, 1 H, J = 6.5, 9.5 Hz), 5.61 (d, 1 H, J = 6.5 Hz,), 6.32 (bs, 2 H), 6.65−6.70 (m, 2 H), 7.24 (d, 2 H, J = 8.0 Hz), 7.28−7.31 (m, 3 H), 7.86 (d, 1 H, J = 8.5 Hz), 7.96 (d, 2 H, J = 8.0 Hz), 7.99 (d, 2 H, J = 8.0 Hz); HRMS (M + Na^+) calcd for 496.1736, found 496.1721.

Synthesis of 2-Amino-4-(3′,5′-di-*O*-benzoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)quinazoline (6a) and 2-Amino-4-(3′,5′-di-*O*-toluoyl-2′-deoxy-*β*-D-*threo*-pentofuranosyl)-quinazoline (6b). To **5a** (7.0 g) in Et_2O (100 mL) was added 2 M HCl in Et_2O (20 mL) at RT. The mixture was stirred for 5 min, the volatiles were removed, NH_2CN (3.8 g) in Et_2O (30 mL) was added, and the reaction was stirred for another 5 min. The Et_2O was removed in vacuo, and the mixture was stirred at 50 °C for 1.5 h. $CHCl_3$ (200 mL) was added followed by 1 M NaOH (200 mL) solution. The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (CH_2Cl_2 to 80% CH_2Cl_2−Et_2O). The product, **6a**, was obtained as a solid in 72% yield: ^{1}H NMR ($CDCl_3$) δ 2.61−2.65 (m, 1 H), 3.07−3.13 (m, 1 H), 4.62−4.71 (m, 3 H), 5.22 (s, 2 H), 5.77 (d, 1 H, J = 6.5 Hz), 5.90 (dd, 1 H, J = 6.0, 10.0 Hz), 7.22 (t, 1 H, J = 6.5 Hz), 7.42−7.70 (m, 8 H), 8.04 (d, 2 H, J = 7.0 Hz), 8.15 (d, 2 H, J = 6.5 Hz), 8.22 (d, 1 H, J = 9.0 Hz); HRMS (M + Na^+) calcd for 470.1717, found 470.1732. **6b** was synthesized by the same procedure and obtained as a white solid (75% yield): ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.47 (s, 3 H), 2.60−2.64 (m, 1 H), 3.03−3.09 (m, 1 H), 4.56−4.69 (m, 3 H), 5.17 (s, 2 H), 5.74 (d, 1 H, J = 6.5 Hz), 5.90 (dd, 1 H, J = 6.0, 10.0 Hz), 7.21−7.5 (m, 3 H), 7.32 (d, 1 H, J = 8.5 Hz), 7.62 (d, 1 H, J = 8.5 Hz), 7.69 (t, 1 H, J = 8.0 Hz), 7.95 (d, 2 H, J = 8.5 Hz), 8.03 (d, 2 H, J = 8.5 Hz), 8.22 (d, 1 H, J = 8.5 Hz); HRMS (M + Na^+) calcd for 498.2030, found 498.2018.

Synthesis of 2-Amino-4-(2′-deoxy-*β*-D-*threo*-pentofuranosyl)quinazoline (7). 6a or **6b** (5.5 g) was dissolved in $CHCl_3$ (10 mL) and MeOH (100 mL). To this solution was added NaOMe (1.8 g), and the mixture was stirred at RT for 1 h. The volatiles were removed under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2 to 90% CH_2Cl_2−Et_2O). The product was recrystallized from Et_2O−EtOH as white crystals (2.02 g, 70% yield): mp 95−97 °C; ^{1}H NMR (DMSO-d_6) δ 2.09−2.13 (m, 1 H), 2.48−2.53 (m, 1 H), 3.43−3.46 (m, 2 H), 3.87−3.90 (m, 1 H), 4.25−4.30 (m, 1 H), 4.78 (t, 1 H, J = 6.0 Hz), 5.15 (d, 1 H, J = 4.5 Hz), 5.61 (dd, 1 H, J = 6.5, 9.5 Hz), 6.74 (s, 2 H), 7.20 (t, 1 H, J = 8.5 Hz), 7.44 (d, 1 H, J = 8.0 Hz), 7.66 (d, 1 H, J = 8.5 Hz), 8.17 (d, 1 H, J = 8.0 Hz); HRMS (M + H^+) calcd for 262.1192, found 262.1179.

Synthesis of N^2-Benzoyl-4-(2′-deoxy-*β*-D-*threo*-pentofuranosyl)quinazoline (8). 7 (1.2 g) was dissolved in dry pyridine (100 mL) was cooled in an ice bath and then $(CH_3)_3SiCl$ (10 mL) was added. The reaction was stirred for 0.5 h before the ice bath was removed. Then benzoyl chloride (8.6 mL) was added, the reaction was stirred at RT for 2.5 h, and then H_2O (20 mL) was added at 0 °C. After 20 min, NH_4OH (20 mL) was added at 0 °C, and the reaction was stirred for 0.5 h. The volatiles were removed under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2 to 95% CH_2Cl_2−MeOH). The product was recrystallized from CH_2Cl_2−EtOH as an off-white solid (1.31 g, 78% yield): mp 88−90 °C; ^{1}H NMR (DMSO-d_6) δ 2.16−2.21 (m, 1 H), 2.55−2.64 (m, 1 H), 3.40−3.50 (m, 2 H), 3.91−3.94 (m, 1 H), 4.30−4.33 (m, 1 H), 4.72 (t, 1 H, J = 5.5 Hz), 5.17 (d, 1 H, J = 4.0 Hz), 5.82 (dd, 1 H, J = 6.5, 9.0 Hz), 7.5−7.54 (m, 2 H), 7.60−7.65 (m, 2 H), 7.88 (d, 1 H, J = 8.0 Hz), 7.92−7.98 (m, 1 H), 8.00 (d, 2 H, J = 7.5 Hz), 8.48 (d, 1 H, J = 8.0 Hz), 11.12 (s, 1 H); HRMS (M + H^+) calcd for 388.1273, found 388.1283.

Synthesis of N^2-Benzoyl-4-[2′-deoxy-*β*-D-*threo*-pentofuranosyl-5′-*O*-(4,4′-dimethoxytrityl)]quinazoline (9). 8 (1.3 g) was dissolved in pyridine (60 mL), and 4,4′-dimethoxytrityl chloride (1.4 g), Et_3N (5 mL), and a catalytic amount of DMAP were added. The reaction was stirred at RT for 72 h, after which time the volatiles were removed under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 to 97% CH_2Cl_2−MeOH). The product was obtained as a light yellow solid (1.3 g, 55% yield): ^{1}H NMR ($CDCl_3$) δ 2.41−2.45 (m, 1 H), 2.87−2.93 (m, 1 H), 3.20 (dd, 1 H, J = 8.5, 10.0 Hz,), 3.46 (dd, 1 H, J = 8.5, 10.0 Hz), 3.68 (s, 3 H), 3.69 (s, 3 H), 4.21−4.24 (m, 1 H), 4.67 (m, 1 H), 6.05 (dd, 1 H, J = 3.0, 9.0 Hz), 6.72 (d, 2 H, J = 1.0 Hz), 6.74 (d, 2 H, J = 1.0 Hz), 7.11 (t, 1 H, J = 7.5 Hz), 7.20 (t, 2 H, J = 8.0 Hz), 7.26 (t, 2 H, J = 7.5 Hz), 7.40−7.55 (m, 10 H), 7.89 (m, 1 H), 8.10 (d, 1 H, J = 8.5 Hz), 8.37 (d, 1 H, J = 8.5 Hz), 8.55 (s, 1 H). HRMS (M + H^+) calcd for 668.2761, found 668.2758.

Synthesis of N^2-Benzoyl-4-[2′-deoxy-β-D-*threo*-pentofuranosyl3′-*O*-(2-cyanoethoxy)(diisopropylamino)-phosphino-5′-*O*-(4,4′-dimethoxytrityl)]quinazoline (10). **9** (0.5 g) was dissolved in dry CH_2Cl_2 (30 mL) and cooled in ice, and Hunig's base (400 μL) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphoramidite (280 μL) were added. The reaction was stirred at RT for 1 h, the solvent was removed, and the residue was purified by silica gel column chromatography (CH_2Cl_2−hexanes−Et_2O−Et_3N, 33:66:100:1). The final product was obtained as a light yellow solid (0.53 g, 80% yield): ^{1}H NMR ($CDCl_3$) δ 1.09−1.22 (m, 14 H), 2.49−2.67 (m, 2 H), 2.90−2.96 (m, 1 H), 3.08−3.12 (m, 1 H), 3.46−3.55 (m, 1 H), 3.59−3.64 (m, 1 H), 3.67 (s, 3 H), 3.68 (s, 3 H), 3.70−3.82 (m, 1 H), 3.88−3.91 (m, 1 H), 4.33−4.39 (m, 1 H), 4.75 (m, 1 H), 6.03 (m, 1 H), 6.71 (m, 4 H), 7.12 (m, 1 H), 7.16−7.28 (m, 4 H), 7.41−7.56 (m, 10 H), 7.91 (m, 1 H), 8.13 (dd, 1 H, J = 2.5, 8.5 Hz), 8.41 (t, 1 H, J = 8.0 Hz), 8.54 (s, 0.5 H), 8.63 (s, 0.5 H); HRMS-FAB ($M + H^+$) calcd for 867.3761, found 867.3772.

Synthesis of 2-Amino-4-hydroxy-6-fluoroquinoline (11). 4-Fluoroanilinium toluene-*p*-sulfonate (76 g) was heated at 260 °C for 5 min under N_2, and ethyl cyanoacetate (30 g) was added dropwise through an air condenser within 5 min. Then the temperature of the reaction was allowed to drop to 220−250 °C. After 90 min of heating, the orange slurry was cooled, $CHCl_3$ (100 mL) was added, and the resulting mixture was refluxed overnight to dissolve the residue. This solution was vigorously mixed with H_2O (100 mL), EtOH (40 mL), and saturated Na_2CO_3 solution (100 mL). The resulting slurry was filtered, and the product, a pale-yellow solid, was washed with H_2O and dried in vacuo over P_2O_5 (7.4 g, 15% yield): ^{1}H NMR (DMSO-d_6) δ 5.26 (bs, 1 H), 6.22 (s, 2 H), 7.32 (m, 2 H), 7.55 (d, 1 H, J = 8.5), 10.79 (bs, 1 H, OH); HRMS-FAB (M^+) calcd for 178.0542, found 178.0545.

Synthesis of 2-Amino-4-bromo-6-fluoroquinoline (12). **11** (7.2 g) was heated with $POBr_3$ (22.0 g) in PBr_3 (20 mL) at 140−160 °C under N_2 for 19 h. The reaction was cooled, carefully basified with 2 M NaOH (250 mL), and thrice extracted with $CHCl_3$ (30 mL). The organic layer was concentrated, and the residue was purified by silica gel chromatography using $CHCl_3$−acetone−Et_3N (75:25:1) to afford the product, which was crystallized from benzene−CH_2Cl_2−hexane to furnish off-white crystals (5.5 g, 57% yield): ^{1}H NMR ($CDCl_3$) δ 4.70 (bs, 2 H), 7.10 (s, 1 H), 7.37 (m, 1 H), 7.66 (m, 2 H); HRMS-FAB (M^+) calcd for 239.9698, found 239.9707.

Synthesis of 4-[β-D-glyceropentofuran-3′-ulos-1′-yl]-2-amino-6-fluoroquinoline (13). 1,4-Anhydro-3-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-erythro-pent-1-enitol[13] (2.13 g) and **12** (1.21 g) were dissolved in dioxane (100 mL) under N_2. Then $Pd_2(dba)_3$ (0.81 g) and (t-Bu)$_3$P (0.71 mL) were added to the reaction. After the reaction had been purged with N_2 for 15 min, dicyclohexylmethylamine (1.35 mL) was added and the mixture was refluxed under N_2 for 20 h. The reaction was cooled and filtered, and the filtrate was concentrated in vacuo. The residue was subjected to silica gel chromatography with CH_2Cl_2 to CH_2Cl_2−MeOH (gradient from 100 to 95%) to give the desired nucleoside and the quinoline−quinoline dimer side product in a ratio 2:1 (total 1.95 g). This mixture was dissolved in THF (40 mL) containing HOAc (0.4 mL) and Bu_4NF (5 mL, 1 M in THF) and stirred at 0 °C for 50 min. Then NH_4OH (2 mL) was added, and the solution was concentrated in vacuo. The residue was subjected to silica gel column chromatography with CH_2Cl_2 to 91% CH_2Cl_2−MeOH to give the desired desilylated product (0.46 g, 33% overall yield): ^{1}H NMR ($CDCl_3$) δ 2.23 (dd, 1 H, J = 13.0, 11.0 Hz), 3.08 (dd, 1 H, J = 13.0, 11.0 Hz), 4.01−4.17 (m, 2 H), 4.20 (m, 1 H), 5.63 (dd, 1 H, J = 7.5, 5.5 Hz), 6.15 (bs, 2 H), 7.25 (s, 1 H), 7.28 (m, 2 H), 7.67 (m, 1 H); HRMS-FAB (M^+) calcd for 276.0910, found 276.0914.

Synthesis of 4-[2′-Deoxy-β-D-*threo*-pentofuranosyl]-2-amino-6-fluoroquinoline (antiAT-F) (14). **13** (1.1 g) was dissolved in AcOH and CH_3CN (100 mL, 1:1) and stirred under N_2 at −23 °C. $NaHB(OAc)_3$ (1.4 g) was added to the cooled solution, and the mixture was stirred at −23 °C for 30 min. The reaction was concentrated, and the residue was chromatographed on silica gel using CH_2Cl_2−MeOH (gradient from 10:1 to 2:1). The crude product was obtained as a pale yellow powder, which was crystallized from Et_2O−EtOH to yield an off-white powder (0.77 g, 70% yield): mp 69−70 °C; ^{1}H NMR (DMSO-d_6) δ 1.73−1.78 (m, 1 H, C2′−H), 2.33−2.37 (m, 1 H, C2″−H), 3.45 (dd, 1 H, J = 11.5, 6.0, C5′−H), 3.56 (dd, 1 H, J = 11.5, 6.0, C5″−H), 3.85−3.88 (m, 1 H, C4′−H), 4.18 (m, 1 H, C3′−H), 4.77 (bs, 1 H, OH3′), 5.15 (bs, 1 H, OH5′), 5.45 (dd, 1 H, J = 10.0, 5.5, C1′−H), 6.37 (s, 2 H, NH2), 6.96 (s, 1 H), 7.32−7.36 (m, 1 H), 7.41−7.49 (m, 2 H); ^{19}F NMR (DMSO-d_6) δ −90.26 (one single peak with multiple splits, referenced by TFA in H_2O); HRMS-FAB ($M + H^+$) calcd for 278.1067, found 278.1070.

Synthesis of N^2-Isobutyryl-4-[2′-deoxy-β-D-*threo*-pentofuranosyl]-2-amino-6-fluoroquinoline (15). **14** (0.88 g) in dry pyridine (50 mL) was cooled in an ice bath and treated with TMS−Cl (4.8 mL) under N_2. The reaction was stirred at 0 °C for 30 min, then isobutyric anhydride (2.62 mL) was added, and the reaction was stirred for an additional 2 h at RT under N_2. The reaction was then cooled in an ice bath, and cold H_2O (6.25 mL) was added. After 15 min, concentrated NH_4OH (6.25 mL) was added to give a solution approximately 2 M in NH_3. This mixture was stirred for another 30 min and then concentrated under reduced pressure to afford an oil that was purified on silica gel with CH_2Cl_2−MeOH (10:1) to furnish the desired product as a pale yellow solid (0.53 g, 47% yield): ^{1}H NMR ($CDCl_3$) δ 1.31 (dd, J = 6.0, 0.5, 6 H), 2.13−2.18 (m, 1 H, C2′−H), 2.57−2.64 (m, 2 H, including C2″−H), 3.86 (dd, 1 H, J = 17.5, 4.5, C5′−H), 4.01 (dd, 1 H, J = 17.5, 4.5, C5″−H), 4.21 (dd, 1 H, J = 7.5, 3.5, C4′−H), 4.57 (m, 1 H, C3′−H), 5.77 (dd, 1 H, J = 9.0, 8.5, C1′−H), 7.43 (m, 1 H), 7.53 (m, 1 H), 7.84 (m, 1 H), 8.13 (s, 1 H), 8.68 (s, 1 H); HRMS-FAB (M^+) calcd for 348.1485, found 348.1492.

Synthesis of N^2-Isobutyryl-4-[2′-deoxy-β-D-*threo*-pentofuranosyl-5′-*O*-(4,4′-dimethoxytrityl)]-2-amino-6-fluoroquinoline (16). 15 (0.4 g) was dissolved in dry pyridine (10 mL) under a N_2 atmosphere, and 4,4′-dimethoxytrityl chloride (0.5 g) and DMAP (10 mg) were added. Another aliquot of trityl chloride was added after 3 h, and the reaction was stirred under N_2 at RT for a total of 6 h. After concentration, the residue was dissolved in $CHCl_3$ (30 mL) and washed thrice with saturated $NaHCO_3$ (30 mL) and thrice with H_2O (30 mL). The solution was concentrated, and the residue was chromatographed on silica gel with CH_2Cl_2−MeOH−Et_3N (gradient from 100:0:1 to 100:1:1). The final product was a white solid (0.43 g, 58% yield): ^{1}H NMR ($CDCl_3$) δ 1.28 (dd, J = 7.5, 6.5, 6 H), 2.20−2.25 (m, 1 H, C2′−H), 2.46−2.51 (m, 1 H, C2″−H), 2.55−2.61 (m, 1 H), 3.36 (dd, 1 H, J = 9.5, 5.5, C5′−H), 3.51 (dd, 1 H, J = 9.5, 5.5, C5″−H), 3.79 (s, 6 H), 4.09−4.12 (m, C4′−H), 4.43−4.45 (m,1 H, C3′−H), 5.65 (dd, 1 H, J = 10.0, 6.0, C1′−H), 6.80−6.82 (m, 4 H), 7.18−7.48 (m, 10 H), 7.68 (dd, 1 H, J = 9.5, 2.5), 7.83 (dd, 1 H, J = 9.5, 5.5), 8.00 (bs, 1 H), 8.61 (s, 1 H); HRMS-FAB ($M + H^+$) calcd for 651.2871, found 651.2862.

Synthesis of N^2-Isobutyryl-4-[2′-deoxy-β-D-*threo*-pentofuranosyl-3′-*O*-(2-cyanoethoxy)(diisopropylamino)-phosphino-5′-*O*-(4,4′-dimethoxytrityl)]-2-amino-6-fluoroquinoline (17). 16 (0.1 g) dissolved in CH_2Cl_2 (10 mL) and cooled in an ice bath under N_2 atmosphere was treated with Hunig's base (200 μL) followed by 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (90 μL). The reaction was stirred at 0 °C for 10 min and then at RT for 45 min. After concentration in vacuo, the residue was purified by column chromatography on silica gel with CH_2Cl_2−hexanes−Et_2O−Et_3N (50:100:150:1). The final product was a white powder (0.11 g, 84% yield): ^{1}H NMR ($CDCl_3$) δ 1.12−1.30 (m, 20 H), 2.18−2.28 (m, 1 H, C2′−H), 2.47−2.67 (m, 3 H, including C2″−H), 3.38−3.42 (m, 2 H, C5′−H, C5″−H), 3.31−3.57 (m, 1 H), 3.60−3.70 (m, 1 H), 3.78 (s, 6 H), 4.31 (bs, 1 H, C4′−H), 4.55 (m,1 H, C3′−H), 5.65 (dd, 1 H, J = 10.5, 5.0, C1′−H), 6.77−6.81 (m, 4 H), 7.16−

7.45 (m, 10 H), 7.74 (dd, 1 H, J = 10.5, 3.0), 7.83 (dd, 1 H, J = 9.5, 5.5), 7.96 (s, 1 H), 8.64 (s, 1 H); HRMS-FAB ($M + H^+$) calcd for 851.3950, found 851.3967.

pK_a of antiAT-F (14). To **14** (1.62 mg) dissolved in H_2O was added NaOAc buffer to give solutions (1.24 μM final concentration) with measured pH values of 4.6, 5.5, 5.9, 6.2, 6.6, 6.8, 7.2, 7.5, and 8.3. Two methods were used to measure the pK_a. First, the UV spectra of these solutions were recorded from 200 to 400 nm. The UV spectra at the different pH values were analyzed by plotting absorbance at 235 nm as a function of pH to calculate the pK_a. Second, the fluorescence emission spectra of these solutions were recorded from 300 to 600 nm with excitation at 330 nm. The fluorescence spectra at different pH values were analyzed by plotting I (391 nm), I (396 nm), I (402 nm), or the shifts of maximum wavelength, respectively, as a function of pH to calculate the pK_a. Both methods gave a calculated pK_a of 6.7.

NMR Conformational Studies. The conformational analysis of the antiAT (**7**) and antiAT-F (**14**) TRIPsides was determined by ^{1}H NMR on a 500-MHz spectrometer using NOESY with presaturation field strength γB_1 of 50 Hz and a mixing time of 0.4 s at 25 °C in 10 mM sodium phosphate buffer (pH 7.0) in D_2O containing 50 mM NaCl. For antiAT (**7**), the sample concentration was 15.6 mM, and the relaxation delay for the NOESY experiment was 2.0 s. For antiAT-F (**14**), the sample concentration was 14.8 mM, and the relaxation delay was 2.1 s.

Synthesis and Purification of Oligomers. The oligomers were synthesized on a 200 nmol scale using standard phosphoramidite solid-phase methods with coupling yields greater than 98%. After the synthesis, the resin was collected and treated with NH_4OH at 55 °C for 8 h to cleave the oligomer off the column and remove the acyl protecting groups. The crude oligomers (as the 5′-dimethoxytrityl derivatives) were purified by reverse-phase HPLC, the trityl group was removed with 80% HOAc, and the oligomers were repurified and desalted on a Sephadex G20 column. The incorporation of the modified nucleotides was determined by UV−visible spectroscopy and MALDI-TOF MS.

UV−Visible Melting Studies. Absorbance versus temperature profiles were measured simultaneously at 260 and 325 nm with a thermoelectrically controlled UV−vis spectrophotometer. The absorbance was monitored in the temperature range of 0−80 °C, by increasing the temperature at a rate of ~0.4 °C/min. In these experiments, the 325-nm wavelength was used to monitor the absorbance changes of the antiAT-F C-glycosides, while the 260 nm follows the changes of the nucleobases. First derivative and shape analysis of the resulting melting curves affords transition temperatures, T_M.

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Supporting Information Available: NMR spectra and HRMS for compounds **4a**−**17**, effect of salt concentration (50 versus 200 mM NaCl) on UV−visible melting curves of OL-1−4, plots of the effect of pH on UV−visible and fluorescence spectra of antiAT-F (**14**). This material is available free of charge via the Internet at http://pubs.acs.org.

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