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A 6′-Fluoro-Substituent in Bicyclo-DNA Increases Affinity to Complementary RNA Presumably by CF−HC Pseudohydrogen Bonds

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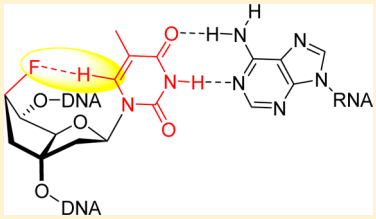
Supporting Information

ABSTRACT: The synthesis of a novel bicyclic thymidine analogue carrying a β-fluoro substituent at C6′ (6′F-bcT) has been achieved. Key steps of the synthesis were an electrophilic fluorination/stereospecific hydrogenation sequence of a bicyclo sugar intermediate, followed by an *N*-iodo-succinimide-induced stereoselective nucleosidation. A corresponding phosphoramidite building block was then prepared and used for oligonucleotide synthesis. T_m measurements of oligonucleotides with single and double incorporations showed a remarkable stabilization of duplex formation particularly with RNA as complement without compromising pairing selectivity. Increases in T_m were in the range of +1−2 °C compared to thymidine and +1−3 °C compared to a standard bc-T residue. Structural investigations of the 6′F-bcT nucleoside by X-ray crystallography showed an in-line arrangement of the fluorine substituent with H6 of thymine, however, with a distance that is relatively long for a nonclassical CF−HC hydrogen bond. In contrast, structural investigations in solution by ^{1}H and ^{13}C NMR clearly showed scalar coupling of fluorine with H6 and C6 of the nucleobase, indicating the existence of at least weak electrostatic interactions. On the basis of these results, we put forward the hypothesis that these weak CF−HC6 electrostatic interactions increase duplex stability by orienting and partially freezing torsion angle χ of the 6′F-bcT nucleoside.



INTRODUCTION

The central element of oligonucleotide-based therapeutic approaches is the selective Watson−Crick recognition of RNA by antisense oligonucleotides.[1] Depending on the nature of the RNA target and the type of chemical modification of the antisense oligonucleotide, modulation of gene expression can be tuned by exploiting different biological mechanisms. Besides the classical antisense strategy in which a mRNA is silenced by a steric block mechanism or by RNaseH-induced degradation of the message, the modulation of the splicing process has recently become an important target.[2,3] Other mechanisms include gene silencing by harnessing the siRNA machinery.[4] Moreover, the recent finding that more than 80% of all DNA is transcribed into RNA and the majority of it does not code for proteins[5] made clear that targeting noncoding RNAs such as micro-RNAs that are involved in complex genetic and epigenetic gene regulation networks by single stranded steric block oligonucleotides will add another dimension to RNA therapeutics.[6] It has been shown in the past that chemically modified oligonucleotide analogues show superior properties over natural oligonucleotides mainly due to improved RNA affinity and to higher biological stability. The class of conformationally constrained oligonucleotide analogues is of particular interest as it provides some of the strongest known RNA binders.[7,8]

Fluorine on the other hand holds a privileged role in medicinal chemistry, which is evidenced by an increasing fraction of drugs that contain organic fluorine.[9] Fluorine is known not only to enhance metabolic stability of drugs but also to change and improve significantly its protein binding properties due to its polar hydrophobic nature.[10,11] In this context also fluorine containing antisense oligonucleotides have recently been investigated (Figure 1). The structurally most simple and obvious fluorinated oligonucleotide analogues are the 2′-deoxy-2′-fluoro RNA (F-RNA) and the 2′deoxy-2′-fluoro-arabino nucleic acids (F-ANA). Although these analogues have been long known, their structural and biophysical properties, explaining to some extent the impact of the fluorine substituent on the

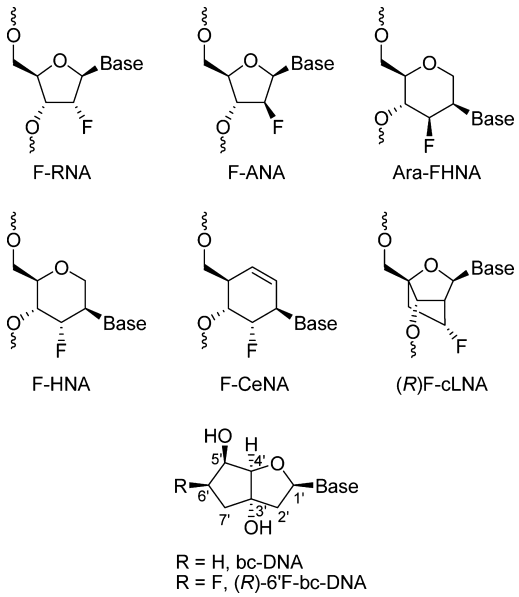


Figure 1. Chemical structures of selected fluoronucleosides as constituents of oligonucleotides.

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overall properties, has only been unraveled recently. Both analogues show improved binding affinity to complementary RNA by 1−2 °C per modification compared to their 2′-OH congeners. The origin of higher duplex stability was determined to be due to stronger hydrogen bonding and stacking of base-pairs imparted by the polar C2′−F bond in the case of F-RNA,[12,13] and to the formation of intraresidual F−H8 pseudohydrogen bonds in the case of F-ANA that are strongest at purine/pyrimidine sequence steps.[14,15] Both F-RNA and F-ANA have successfully been used in siRNA applications,[16,17] while F-ANA, because of its RNaseH activating properties, has also been shown to be a valid candidate for classical antisense applications.[18]

These results have raised interest to investigate the effect of fluorine substitution also in the context of different carbohydrate modified oligonucleotide analogues. F-HNA and its isomer Ara-F-HNA as well as 6′-methylated variants thereof were reported recently.[19,20] F-HNA showed increases in thermal stability by +2 °C/mod, whereas Ara-FHNA decreases by −3 °C/mod. The destabilization of Ara-FHNA was structurally rationalized by repulsive steric effects of the fluoro substituent.[19] Also CeNA[21] and most recently cLNA[22] have been fluorinated. While F-CeNA stabilizes duplexes with RNA slightly less compared to F-HNA (+1 °C/mod), (*R*)F-cLNA behaves roughly neutral compared to cLNA.

Increasing thermal stability is, however, not the only interest in fluorinated oligonucleotides, given the fact that serum transport, cellular uptake, and tissue distribution are the important yet unsolved problems on the way to oligonucleotide drugs. It has been shown previously in a 2−10−2 gapmer format that F-HNA showed a more potent down regulation of gene expression in liver tissue compared to LNA, despite lower affinity to its target as expressed by T_m.[19] While the origin of this behavior is yet unclear it is likely due to either improved metabolic stability or differential binding to plasma proteins facilitating trafficking. In more recent work targeting mutant huntingtin with gapmer oligonucleotides containing F-RNA or F-ANA units, differential antisense effects were observed as a matter of the nature of the fluorinated units.[23] Perhaps the most impressive case of differential binding to proteins has been shown with F-RNA that was reported to recruit the interleukin enhancer-binding factor complex (ILF2/3) when in complex with RNA, while other 2′-chemical modifications such as 2′-MOE, 2′-OMe and cEtLNA did not.[24]

On the basis of this precedence we became interested in investigating fluorinated versions of our bicyclo-DNA (bc-DNA)[25] that together with tricyclo-DNA (tc-DNA)[26,27] represents an interesting molecular platform for antisense oligonucleotides. Besides modifying the obvious 2′-position, also the 6′-β-position was for us of interest due to its spacial proximity to the edge of the nucleobase. Here we report on the synthesis of the corresponding thymine containing nucleoside, its structural properties as determined by X-ray and NMR, its incorporation into DNA, and its thermal melting properties as determined from UV-melting curves.

RESULTS

Synthesis of the Fluorinated Sugar Derivative. It was clear from the onset that a stereoselective access to a fluorine containing sugar intermediate, suitable for nucleosidation according to standard methods, had to be achieved. We envisaged the readily available bicyclic sugar **1** as starting material as it can easily be manipulated to allow for either electrophilic or nucleophilic fluorination at C5 (Figure 2).[28]

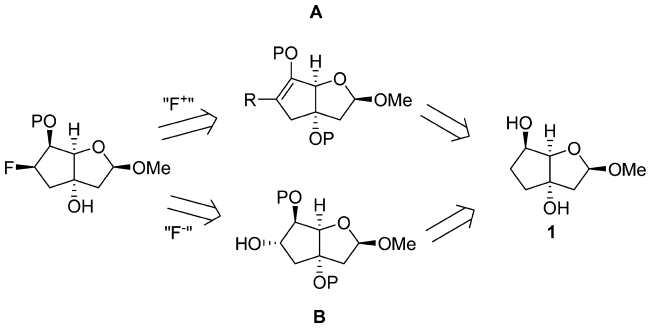
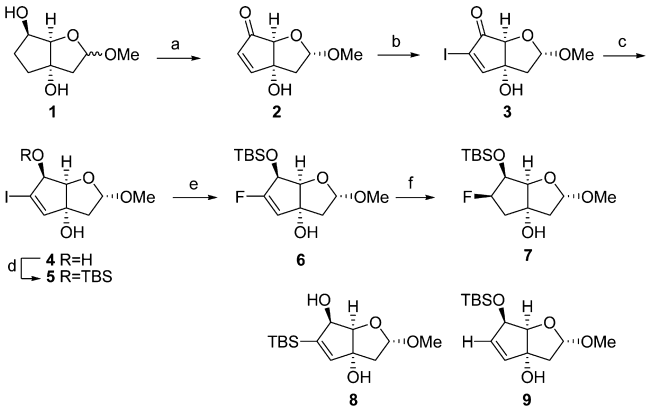


Figure 2. Retrosynthetic scheme for the introduction of fluorine either by elecrophilic or nucleophilic fluorination reagents (P: protecting groups).

Surprisingly this fluorination turned out to be rather tricky. In preliminary experiments with enol ethers of type A carrying a variety of classical O-protecting groups using electrophilic fluorination reagent such as Selectfluor, (*N*-fluorotriethylene-diammonium bis(tetrafluoroborate)),[29,30] or *N*-fluorodibenze-nesulfonimide (NFSI)[31] in different solvents and conditions, we found complex product mixtures containing the expected 6′-fluorinated products only in traces, along with stereoisomers and other nonidentified fluorinated products. The same was also found for intermediates of type B when treated with nucleophilic fluorination reagents. The S_N2 displacement of the OH group with diethylaminosulfur trifluoride (DAST)[32] failed, as did the substitution of a O5 mesylate with fluoride ions. Somewhat sobered by these results we turned our attention to halogen exchange reactions via a transition metal intermediate of type A (R = I).[33] Indeed this strategy finally looked promising.

The successful synthesis followed the pathway depicted in Scheme 1. The known diol **1**[28] was oxidized in a two-step

Scheme 1[a]



[a](a) IBX, 4-methoxypyridine *N*-oxide (MPO), DMSO, rt, 3 d, 2 steps, 40%; (b) I_2, pyridine/CH_2Cl_2, 0 °C to rt, 2.5 h, 67%; (c) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, 0 °C, 50 min, 89%; (d) TBDMSCl, imidazole, CH_2Cl_2, rt, 24 h, 87%; (e) *N*-fluorobenzenesulfonimide (NFSI), *n*-BuLi, THF, −78 °C, 1 h, 36%; (f) H_2, Pd−C, MeOH, rt, 2 h, 80%.

procedure with IBX[34] to enone **2** that could easily be converted to **3** by iodination with I_2 in pyridine in good yields. Compound **3** was then subjected to 1,2-reduction, leading readily and stereospecifically to **4** (reduction from the exoface of the bicyclic system), which after standard TBS-protection (→**5**) was considered to be adequate for the halogen exchange reaction.

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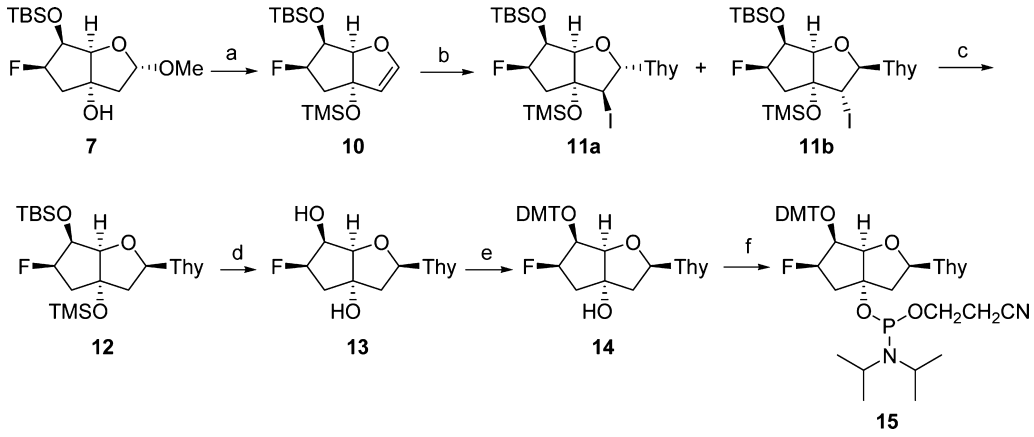
Scheme 2[a]



[a](a) TMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 1.5 h; (b) thymine, BSA, NIS, CH_2Cl_2, 0 to 4 °C, 16 h, 22% (**11a**), 43% (**11b**); (c) Bu_3SnH, AIBN, toluene, reflux, 1.75 h, 90%; (d) $Et_3N{\cdot}3HF$, THF, 5 d; (e) DMTr-OTf, pyridine, 75 °C, 11 h, 80%; (e) CEP-Cl, DIPEA, THF, rt, 2 h, 99%.

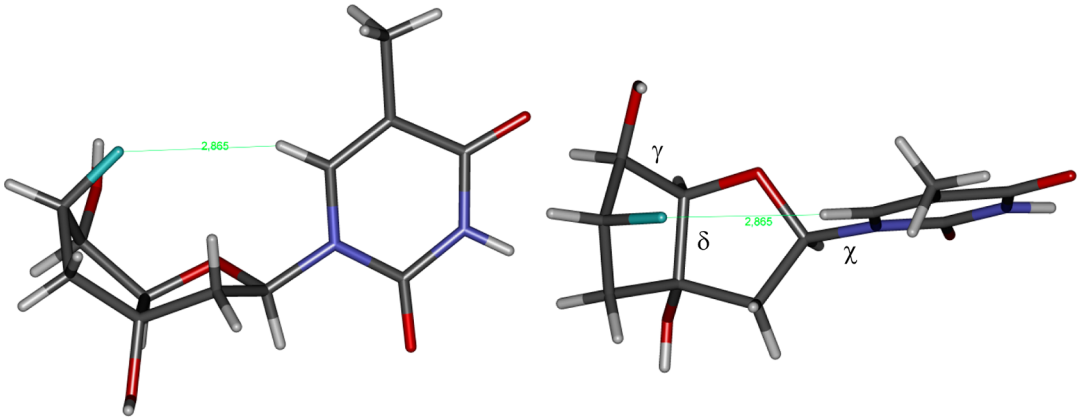


Figure 3. X-ray structure of 6′-fluorobicyclothymidine **13**; left: side view, right: top view. Torsion angles γ, δ, and χ are indicated in the top view picture for clarity.

Metal iodine exchange in **5** at −78 °C with an excess of BuLi, followed by treatment with NFSI indeed led to the fluoro-derivative **6** in 36% yield. Two side products could be identified, namely, compound **8** arising from TBS migration[35] and the C5-reduced compound **9**. Increasing the amount of NFSI did not dramatically change the ratio of **6**/**9**, and quenching of the dianion of **6** with D_2O in the absence of NFSI only led to C5-deuterated **9**, implying that metalation goes to completion under the applied conditions. Therefore the origin of the hydrogen is unclear, indicating that the mechanism of fluorine transfer may involve electron transfer processes. Catalytic hydrogenation of **6** went smoothly and, as expected, only occurred from the convex side of the bicyclic core system, giving **7** as the exclusive stereoisomer in 80% yield.

The synthesis of the building block **15** continued with the NIS-induced nucleosidation of **7** (Scheme 2). This variant was preferred over the classical Vorbrüggen nucleosidation protocol[36] based on precedence in bicyclonucleoside synthesis from which a more favorable β:α anomeric ratio was expected.[37,38] Treatment of **7** with persilylated thymine in the presence of NIS indeed led to the corresponding nucleosides in a ratio of **11b**:**11a** = 2:1. Radical deiodination of **11b** with Bu_3SnH gave nucleoside **12** that was deprotected with $Et_3N{\cdot}3HF$ to give the free 6′-fluoronucleoside **13**. To conclude the synthesis of phosphoramidite **15**, only two steps remained to be performed, namely, dimethoxytritylation followed by phosphitylation. While the latter proceeded without problems, the synthesis of the tritylated nucleoside **14** proved difficult again. No reaction was observed with the standard reagent DMTr-Cl. Only the use of an excess of the more reactive DMTr-OTf[39] at elevated temperatures provided **14** in good yields.

Structure of 13. To obtain final proof for the stereochemistry at C1′ and C6′ and to gain information on the conformational preferences of the fluorine substituent, crystals of **13** were grown and subjected to X-ray analysis (Figure 3). Selected backbone torsion angles and sugar pucker data are summarized in Table 1 and compared to that of the unmodified bcT.

Table 1. Selected Backbone Torsion Angles and Sugar Pucker Data for the bcT and 6′F-bcT-Nucleosides from X-ray Structures

	γ	δ	χ	P	ν_{max}
6′-F-bcT	149.2°	122.3°	−115.6°	123.1°	42.3
bcT[a]	149.3°	126.5°	−112.7°	128.4°	42.4

[a]Ref 40.

The furanose ring in **13** exists in an *S*-type (2′-*exo*) conformation, giving rise to a pseudorotation phase angle of 123°. The base is in the antiarrangement and torsion angle γ is in

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the antiperiplanar range, clearly positioning O5′ in a pseudoequatorial and the 6′F substituent in a pseudoaxial position. The C6′F−HC6 bonds lies in one plane with an F−H distance of 2.865 Å. This distance is larger than the 2.5 Å that generally characterize a CF−HC hydrogen bond.[41] In general, the solid state structures of the parent bcT and the 6′F-bcT are very similar.

The rather long distance between F and H6, observed in the solid state, contrasts with NMR data of the nucleoside in solution. In the case of the β-nucleoside **12b**, fluorine coupling to H6 with a J^{FH} of ∼1 Hz is observed in the ^{1}H NMR spectrum, transforming the expected quadruplet into a more complex signal. In addition, fluorine coupling to C6 with a J^{FC} of 6 Hz occurs, giving rise to a doublet in the proton decoupled ^{13}C NMR spectrum (Figure 4). In contrast, in the corresponding α-nucleoside

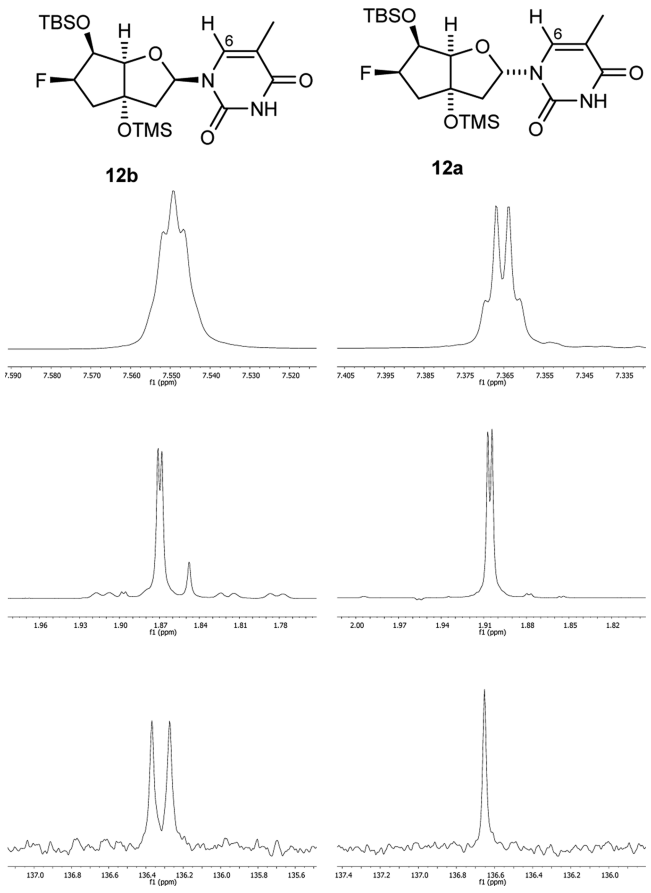

Figure 4. Section showing the H6 signal (top) and the CH_3−C5 signal (center) in the 400 MHz ^{1}H NMR spectrum as well as the C6 resonance (bottom) in the proton decoupled ^{13}C NMR spectrum at 161 MHz in $CDCl_3$ for **12b** (left column) and **12a** (right column).

12a, where no close contact between fluorine and H6/C6 is possible, no fluorine coupling is visible neither at H6 nor at C6, giving rise to a quadruplet for H6 in the proton and a singlet for C6 in the carbon NMR. The same behavior as for **12b** is also observed in nucleosides **13** and **14** and is thus not dependent on the protecting groups at O5′ and O3′. This fact clearly reflects a tight spatial relationship between fluorine and H6/C6 and is consistent with at least a weak pseudohydrogen bond.[14]

Synthesis of Oligonucleotides. Standard phosphoramidite solid phase synthesis was used for the preparation of oligonucleotides **ON1**−**3** containing either one or two 6′F-bcT modifications. During initial experiments we realized that the protocol had to be changed in two steps of the cycle. First, the 6′F-bcT nucleosides turned out to be notoriously difficult to detritylate. Under standard conditions (3% dichloroacetic acid (DCA) in dichloroethane (DCE), 60 s), only one-third of the trityl groups were removed. A 5-fold increase of DCA concentration was necessary to obtain full deprotection in the same time span. Although we used increased acid concentrations for detritylation of 6′F-bcT residues, we were not aware of an unusual extent of depurination as judged by HPLC during purification. The coupling step also had to be prolonged to 10 min in order to obtain coupling yields of >95%. After assembly, the oligonucleotides were deprotected in 33% NH_3 for 16 h at 55 °C and purified by HPLC, and their constitution was analyzed by ESI$^-$-MS spectrometry (Table 2).

The affinity of **ON1**−**3** to complementary DNA and RNA was assessed by UV-melting curves. The corresponding T_m data are summarized in Table 2. Inspection of the data clearly shows that isolated 6′F-bcT residues (**ON1**, **ON2**) slightly destabilize duplexes with complementary DNA but increase stability with complementary RNA by +0.4 to +1.3 °C/mod relative to DNA. Two consecutive modifications led to an increase in T_m by +1 °C/mod against DNA and +2.2 °C/mod against RNA. It clearly emerges that 6′F-bcT residues stabilize duplexes with RNA more efficiently compared to natural dT residues.

We also investigated whether pairing of 6′F-bcT was Watson−Crick selective. For this we measured T_m data from duplexes of **ON1** with natural DNA complements carrying the three noncanonical bases in opposite position (Table 3). For all mismatches the T_m values drop significantly with ΔT_m values of −9.5 °C for the T−G wobble base-pair and −12 to −15 °C for the two other mismatches. To determine whether there is a difference between unmodified and modified mismatched duplexes we determined the $\Delta\Delta T_m$ values (Table 3). In all cases the modified mismatched duplexes showed lower T_m values than the unmodified mismatched duplexes ($\Delta\Delta T_m$ = −1 to −2.9 °C), pointing to a generally higher propensity for mismatch discrimination of 6′F-bcT over dT.

To determine the influence of the 6′-fluoro-substituent within the bc-DNA backbone on DNA and RNA affinity we compared ΔT_m/mod data with that of duplexes carrying either a parent bcT residue or a bcT residue with polar amino-substituents in the same position (Table 4). Only the fluorinated bcT-residue (mod a) is consistently able to stabilize RNA duplexes while the other modifications are slightly (mod b) to strongly (mods c, d, e) destabilizing. Thus the contribution to stability is fluorine specific and not simply due to the presence of a polar substituent. We note, however, that modifications c−e are at least partially positively charged at pH 7, which may, in addition, exert a component of charge compensation within the phosphodiester backbone to affinity.

■ DISCUSSION

The findings reported here clearly show that the presence of fluorine in the 6′-position of bc-DNA significantly adds to duplex stability with RNA and to a lesser degree with DNA. It seems even that contiguous substitutions reinforce this effect. It is striking to see that a simple amino function in the same position (mod c) can destabilize duplexes with RNA by −5.4 °C/mod while the fluorine substituent stabilizes the same duplex by 2.2 °C/mod.

The structural similarity between bcT and 6′F-bcT as revealed by the X-ray structure suggests that the differences in affinity are not due to differential conformations of the nucleosides. In fact, the F6′ as the more electronegative substituent compared to the

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Table 2. Analytical Data of 6′F-bcT Containing Oligonucleotides ON1−3 and T_m Data with Complementary DNA and RNA (c(duplex) = 1.2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0)

	sequence[a]	*m/z* calcd	*m/z* found	T_m vs DNA [°C][b]	T_m vs RNA [°C][b]
ON1	d(GGATGTTCtCGA)	3720.4	3720	46.7 (−1.2)	49.6 (+1.3)
ON2	d(GGAtGTTCtCGA)	3764.5	3764	47.3 (−0.3)	49.0 (+0.4)
ON3	d(GGATGttCTCGA)	3764.5	3764	49.9 (+1.0)	52.6 (+2.2)

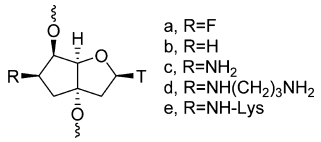
[a] T_m of unmodified duplex: 47.9 °C (vs DNA); 48.3 °C (vs RNA). [b] ΔT_m per modification in parentheses.

Table 3. Mismatch Discrimination of ON1 with Complementary DNA Containing Mismatched Bases

mismatch	T_m [°C]	ΔT_m[a] [°C]	$\Delta\Delta T_m$[b] [°C]
T−T	32.0	−15.0	−2.9
T−G	37.5	−9.5	−3.1
T−C	35.0	−12.0	−1.0

[a] ΔT_m = T_m of mismatched minus T_m of matched modified duplex (base selectivity). [b] $\Delta\Delta T_m$ = T_m of modified mismatched duplex minus T_m of unmodified mismatched duplex (backbone selectivity).

Table 4. ΔT_m-Values for Different (β)6′-Modified bcT Containing Duplexes



modification	sequence	ΔT_m/mod vs DNA [°C]	ΔT_m/mod vs RNA [°C]
a	d(GGATGTTCtCGA)	−1.2	+1.3
b[a]	d(GGATGTTCtCGA)	+1.5	−0.5
c[a]	d(GGATGTTCtCGA)	+0.8	−3.0
d[a]	d(GGATGTTCtCGA)	−0.2	−3.8
e[a]	d(GGATGTTCtCGA)	+1.5	−3.5
a	d(GGAtGTTCtCGA)	−0.3	+0.4
b[b]	d(GGAtGTTCtCGA)	+0.2	−0.7
d[a]	d(GGAtGTTCtCGA)	−0.7	−3.1
e[a]	d(GGAtGTTCtCGA)	−0.1	−1.7
a	d(GGATGttCTCGA)	+1.0	+2.2
b[a]	d(GGATGttCTCGA)	+0.6	−0.6
c[a]	d(GGATGttCTCGA)	−1.0	−5.4
d[a]	d(GGATGttCTCGA)	−1.2	−5.6
e[a]	d(GGATGttCTCGA)	−1.0	−5.5

[a] Taken from ref 42. [b] Taken from ref 43.

OH5′ is expected to adopt preferentially a pseudoaxial position due to stronger stabilization of this particular conformation by σCH−σ*CF hyperconjugation of the antiperiplanar aligned, α-configured hydrogens at C5′ and C7′ (Figure 3) rather than the alternative gauche conformation of the carbocyclic ring in which the OH5′ is in a pseudoaxial and the F6′ in a pseudoequatorial orientation.

In F-ANA/RNA duplexes that exhibit increased stability compared to ANA/RNA duplexes it has recently been shown by structural, biophysical, and computational methods that the increased stability may among other effects be due to attractive CHF pseudohydrogen bonds between 2′F and purine H8 in F-ANA purine residues which contrast with unfavorable steric interactions of the 2′-OH in ANA.[14,15] The distance between fluorine and H6 of thymine of 2.865 Å as observed in the crystal structure of **13** would at first glance preclude the existence of a strong CHF hydrogen bond that could conformationally constrain the glycosidic torsion angle into the *anti*-conformational range as a measure to increase RNA affinity. However, NMR data in solution of the 6′F-bcT nucleoside **12**−**14** clearly show scalar J^{FH} and J^{FC} 6′F−H/C6 couplings of ∼1 and 6 Hz, respectively, clearly indicating close distance electrostatic interactions of these nuclei. It thus seems likely that 6′F-bcT residues are capable of forming such pseudohydrogen bonds in duplexes under aqueous conditions. We hypothesize therefore that these are mainly responsible for the increase in RNA affinity, much in the same way as observed for F-ANA. Clearly, more structural work on the oligonucleotide level has to be done to confirm this hypothesis. Along the same lines it seems likely that steric and electrostatic repulsion with H6 of the base may be the origin of the decreased affinity observed for all β-6′*N*-substituted bcT duplexes (mods c−e, Table 4).

From detailed structural and biophysical studies of F-RNA/RNA duplexes[12,13] which also show increased thermal duplex stabilities by roughly 1.8 °C/mod compared to all-RNA duplexes, it emerged that they are structurally very similar. Interestingly, F-RNA/RNA duplexes are enthalpically stabilized, and it was put forward that this enthalpic stabilization has its origin in reinforced hydrogen bonds and stacking interactions as a consequence of electronic coupling of the antiparallel aligned C−F bond with the glycosidic bond. In the case of 6′F-bcT, the distance of the CF bond to the glycosidic bond, however, excludes through-bond electronic coupling of fluorine with the base.

F-ANA and F-RNA duplexes are typically less hydrated in their grooves as compared to their 2′-OH congeners. This can be explained by the aversion of fluorine to act as a bridge for connecting water molecules in the minor groove. This has been found in high resolution X-ray structures and has been verified by osmotic stress experiments.[13] Although there clearly exist differences in hydration it appears that the overall entropy change between fluorinated and nonfluorinated duplexes is similar due to 2′-F binding less water in a more ordered way and 2′-OH binding more water with less order. It thus is inferred that the differential hydration shows less impact on duplex stability. With the limited data available for 6′F-bcDNA at this point it is difficult to assess the importance of hydration on duplex stability. Given the fact that only single or double incorporations have been investigated, the effect is expected to be very small, most likely invisible. A thorough assessment of the role of hydration in this system would require fully modified 6′F-bcDNA oligonucleotides, which are currently not at hand.

CONCLUSIONS

We have succeeded in the synthesis of 6′-fluoro bicyclothymidine and its incorporation into oligonucleotides. From T_m measurements it appeared that the 6′-fluorine substituent increases RNA affinity by a ΔT_m of 1−3 °C per modification, relative to a standard bc-T unit. Structural investigations in the solid state by X-ray crystallography revealed an in-line arrangement of fluorine and H−C6 of the base with roughly van der Waals distance. NMR experiments in solution, however, show scalar coupling in the ^1H and ^{13}C NMR to the signals of H6 and

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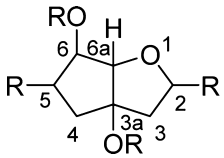
C6 of the thymine base, respectively. These couplings clearly indicate close contacts between the respective nuclei and favor the hypothesis of conformational stabilization by virtue of aligning and partially fixing the nucleosidic torsion angle χ. To the best of our knowledge this is the first time that fluorine/base electrostatic interactions have been found over distances of 7 or 8 bonds, respectively, in nucleoside chemistry. These first results clearly stimulate the study of the biophysical and structural properties of fully 6′F-bc-modified oligonucleotides with all natural nucleobases, work that is currently in progress in our laboratory.

EXPERIMENTAL SECTION

General Methods. All reactions were performed under an atmosphere of argon in oven-dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated alumina or by storage over molecular sieves (4 Å). Column chromatography (CC) was performed on silica gel 60 (230−400 mesh, neutralized with 0.1% of w/Ca). All solvents for CC were of technical grade and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel plates. Visualization was achieved either under UV light or by staining in dip solutions [Cer^{IV}-sulfate (10.5 g), phosphormolybdenic acid (21 g), concentrated H_2SO_4 (60 mL), H_2O (900 mL) or $KMnO_4$ (6 g), K_2CO_3 (40 g), 15% NaOH (3 mL) in H_2O (800 mL)] followed by heating with a heat gun. NMR spectra were recorded at 300 or 400 MHz (^{1}H), at 75 MHz (^{13}C), at 376 MHz (^{19}F) and at 162 MHz (^{31}P). Chemical shifts (δ) are reported relative to the undeuterated residual solvent peak [$CHCl_3$: 7.24 ppm (^{1}H) and 77.2 ppm (^{13}C); DMSO-d_6: 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C)]. Signal assignments are based on DEPT or APT experiments, and on ^{1}H,^{1}H and ^{1}H,^{13}C correlation experiments (COSY, HSQC). ^{1}H NMR difference-NOESY experiments were recorded at 400 MHz. Chemical shifts for ^{31}P and ^{19}F NMR are reported relative to 85% H_3PO_4 and $CFCl_3$ as external standards, respectively. Electrospray ionization in the positive mode (ion trap, ESI^+) was used for high resolution mass detection. The numbering scheme for nucleosides is outlined in Figure 1. For sugar derivatives, the extended Hantzsch−Widman system has been applied with the following numbering scheme:



(2*S*,3a*R*,6a*S*)-3a-Hydroxy-2-methoxy-3,3a-dihydro-2*H*-cyclopenta[*b*]furan-6(6a*H*)-one (2). A solution of diol **1** (4.6 g, 26.4 mmol, 3:1 mixture of α- and β-anomers) in DMSO (30 mL) was added into a stirred suspension of IBX (13.3 g, 47.5 mmol) in DMSO (10 mL) at rt. After stirring the mixture for 21 h it was diluted with CH_2Cl_2 (40 mL), stirred for another 1 h, filtered through Celite that was washed with CH_2Cl_2 (250 mL), followed by evaporation of the filtrate. The residue was then added into a stirred suspension of IBX (14.8 g, 52.8 mmol) and 4-methoxypyridine *N*-oxide (6.6 g, 52.8 mmol) in DMSO (10 mL). After stirring for 30 min at rt a clear solution formed, which turned into a dense suspension overnight. After additional stirring for 3 days, the mixture was diluted with CH_2Cl_2 (50 mL), left for 30 min and filtered over Celite. The filtrate was poured into saturated aq $NaHCO_3$ and extracted with CH_2Cl_2 (5×). The combined organic phases were dried over $MgSO_4$. After evaporation of CH_2Cl_2 the residual DMSO was removed on HV and the mixture was purified by CC (3.5% MeOH in CH_2Cl_2) to afford 1.8 g (40%) of **2** as a white solid, together with 0.44 g of the corresponding β-anomer (10%) as a yellow oil that was not further used.

Data for **2**. R_f = 0.19 (4% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52 (d, J = 5.9 Hz, 1H, H−C(4)), 6.02 (dd, J = 5.9, 0.4 Hz, 1H, H−C(5)), 5.22 (d, J = 4.3 Hz, 1H, H−C(2)), 4.36 (s, 1H, H−C(6a)), 3.40 (s, 3H, OCH_3), 3.36 (s, 1H, OH), 2.23 (d, J = 13.8 Hz, 1H, H−C(3)), 1.99 (dd, J = 13.8, 4.3 Hz, 1H, H−C(3)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.7 (s, C(6)), 161.0 (d, C(4)), 132.0 (d, C(5)), 110.0 (d, C(2)), 88.7 (d, C(6a)), 86.0 (s, C(3a)), 55.4 (q, OCH_3), 45.5 (t, C(3)); ESI^+-HRMS m/z calcd for $C_8H_{10}NaO_4$ $[M + Na]^+$ 193.0471, found 193.0475.

(2*S*,3a*R*,6a*S*)-3a-Hydroxy-5-iodo-2-methoxy-3,3a-dihydro-2*H*-cyclopenta[*b*]furan-6(6a*H*)-one (3). A solution of I_2 (4.56 g, 18.0 mmol) in dry CH_2Cl_2/pyridine (1:1, 55 mL) was added dropwise into a stirred solution of ketone **2** (1.8 g, 10.6 mmol) in dry CH_2Cl_2/pyridine (1:1, 55 mL) at 0 °C. The mixture was stirred under Ar for 30 min, and then the cooling bath was removed, and stirring continued for 2 h at rt. The solution was then diluted with 1 M HCl and extracted with CH_2Cl_2. The organic extracts were washed with 1 M aq $Na_2S_2O_3$, and the aqueous phase was again extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was purified by CC (3.5% MeOH in CH_2Cl_2) to provide 2.09 g (67%) of the title compound **3** as viscous yellow liquid.

Data of **3**. R_f = 0.57 (3.5% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.91 (d, J = 0.4 Hz, 1H, H−C(4)), 5.24 (d, J = 4.2 Hz, 1H, H−C(2)), 4.40 (s, 1H, H−C(6a)), 3.41 (s, 3H, OCH_3), 3.38 (s, 1H OH), 2.26 (d, J = 13.9 Hz, 1H, H−C(3)), 2.03 (dd, J = 13.9, 4.2 Hz, 1H, H−C(3)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 197.3 (s, C(6)), 166.3 (d, C(4)), 109.8 (d, C(2)), 102.1 (s, C(5)), 87.3 (s, C(3a)), 86.0 (d, C(6a)), 55.6 (q, OCH_3), 45.3 (t, C(3)); ESI^+-HRMS m/z calcd for $C_8H_9INaO_4$ $[M + Na]^+$ 318.9438, found 318.9435.

(2*S*,3a*R*,6*R*,6a*R*)-5-Iodo-2-methoxy-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-3a,6-diol (4). To a stirred solution of ketone **3** (2.09 g, 7.06 mmol) in MeOH (70 mL) was added $CeCl_3{\cdot}7H_2O$ (2.63 g, 7.06 mmol) at rt. After 30 min the solution was cooled to 0 °C, and $NaBH_4$ (0.267 g, 7.06 mmol) was added in three equal portions over 20 min. After 30 min ca. 1 g of silica gel was added, and the mixture evaporated. The residue was purified by CC (3.5% MeOH in CH_2Cl_2) to yield 1.87 g (89%) of the title compound **4** as a colorless oil.

Data of **4**. R_f = 0.33 (3.5% MeOH in CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.31−6.28 (m, 1H, H−C(4)), 5.20 (dd, J = 3.7, 2.2 Hz, 1H, H−C(2)), 4.65 (ddd, J = 7.4, 6.2, 1.1 Hz, 1H, H−C(6)), 4.34−4.31 (m, 1H, H−C(6a)), 3.40 (s, 3H OCH_3), 2.76 (m, 2H, OH), 2.21−2.10 (m, 2H, H−C(3)); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.9 (d, C(4)), 108.0 (d, C(2)), 105.3 (s, C(5)), 91.6 (s, C(3a)), 85.1 (d, C(6a)), 79.0 (d, C(6)), 55.4 (q, OCH_3), 47.0 (t, C(3)); ESI^+-HRMS m/z calcd for $C_8H_{11}INaO_4$ $[M + Na]^+$ 320.9594, found 320.9602.

(2*S*,3a*R*,6*R*,6a*R*)-6-(*tert*-Butyldimethylsilyloxy)-5-iodo-2-methoxy-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-3a-ol (5). To a stirred solution of diol **4** (1.87 g, 6.27 mmol) in dry CH_2Cl_2 (65 mL) were added imidazole (0.94 g, 13.8 mmol) and TBDMSCl (1.04 g, 6.90 mmol). After stirring for 24 h at rt, the mixture was diluted with 1 M HCl and extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, evaporated, and purified by CC (EtOAc/hexane 3:7) to yield the title compound **5** (2.26 g, 87%) as colorless oil.

Data of **5**. R_f = 0.46 (EtOAc/hexane 3:7); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.29 (s, 1H, H−C(4)), 5.16 (dd, J = 4.5, 1.0 Hz, 1H, H−C(2)), 4.63 (dd, J = 5.8, 0.7 Hz, 1H, H−C(6)), 4.20 (d, J = 5.8 Hz, 1H, H−C(6a)), 3.37 (s, 3H, OCH_3), 2.85 (s, 1H, OH), 2.16 (dd, J = 13.6, 4.5 Hz, 1H, H−C(3)), 2.07 (dd, J = 13.6, 1.0 Hz, 1H, H−C(3)), 0.92 (s, 9H; H−$C(CH_3)_3$), 0.16, 0.12 (2 × s, 2 × 3H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.0 (d, C(4)), 108.5 (d, C(2)), 104.8 (s, C(5)), 91.3 (s, C(3a)), 87.2 (d, C(6a)), 80.5 (d, C(6)), 55.0 (q, OCH_3), 46.5 (t, C(3)), 26.1 (q, $C(CH_3)_3$), 18.8 (s, $C(CH_3)_3$), −4.3, −4.6 (2 × q, $Si(CH_3)_2$); ESI^+-HRMS m/z calcd for $C_{14}H_{25}INaO_4Si$ $[M + Na]^+$ 435.0459, found 435.0459.

(2*S*,3a*R*,6*S*,6a*R*)-6-(*tert*-Butyldimethylsilyloxy)-5-fluoro-2-methoxy-3,3a,6,6a-tetrahydro-2*H*-cyclopenta[*b*]furan-3a-ol (6). To a stirred solution of **5** (1.75 g, 4.24 mmol) and *N*-fluorobenzenesulfonimide (NFSI, 1.61 g, 5.09 mmol) in dry THF (40 mL) under Ar was added *n*-BuLi (1 M in hexanes, 10.6 mmol, 17.0 mmol) at −78 °C within 15 min. The reaction mixture was stirred for 1 h and then quenched at −78 °C with H_2O. After warming up to rt, saturated aq NH_4Cl was added, and the mixture was extracted with Et_2O. The combined organic phases were dried over $MgSO_4$ and evaporated. The residue was purified by CC (EtOAc/hexane 3:7) to give pure compound **8** (0.222 g, 19%) as yellow oil and 1.01 g of a mixture of compounds **6** and **9**. Subsequent chromatography (Et_2O/CH_2Cl_2 5:95 → 25:75) of

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this mixture yielded the title compound **6** (0.468 g, 36%) as a white solid and compound **8** (0.379 g, 33%) as colorless oil.

Data for ***6****.* R_f = 0.49 (Et_2O/CH_2Cl_2 1:9); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (s, 1H, H−C(4)), 5.17 (td, J = 4.2, 0.9 Hz, 1H, H−C(2)), 4.61 (dd, J = 6.2, 0.5 Hz, 1H, H−C(6)), 4.19 (dd, J = 6.2, 1.9 Hz, 1H, H−C(6a)), 3.38 (s, 3H, OCH_3), 2.96 (d, J = 2.0 Hz, 1H, OH), 2.14 (dd, J = 13.6, 1.0 Hz, 1H, H−C(3)), 2.08 (dd, J = 13.6, 4.2 Hz, 1H, H−C(3)), 0.88 (s, 9H, $C(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.7 (d, $^1J(C,F)$ = 291 Hz, C(5)), 109.0 (dd, $^2J(C,F)$ = 8 Hz, C(4)), 108.8 (d, C(2)), 86.5 (dd, $^3J(C,F)$ = 7 Hz, C(6a)), 86.3 (d, $^3J(C,F)$ = 11 Hz, C(3a)), 71.3 (d, $^2J(C,F)$ = 20 Hz, C(6)), 55.1 (q, OCH_3), 47.3 (td, $^4J(C,F)$ = 3 Hz, C(3)), 25.9 (q, $C(CH_3)_3$), 18.7 (s, $C(CH_3)_3$), −4.6, −5.1 (2 × q, $Si(CH_3)_2$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −123.52; ESI$^+$-HRMS m/z calcd for $C_{14}H_{25}FNaO_4Si$ $[M + Na]^+$ 327.1398, found 327.1409.

Data for ***8****.* R_f = 0.23 (Et_2O/CH_2Cl_2 1:9); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.05 (d, J = 0.6 Hz, 1H, H−C(4)), 5.19 (dt, J = 6.4, 3.2 Hz, 1H, H−C(2)), 4.77 (dt, J = 6.1, 0.6 Hz, 1H, H−C(6)), 4.39 (d, J = 6.0 Hz, 1H, H−C(6a)), 3.40 (s, 3H, OCH_3), 2.67 (sbr, 1H, OH), 2.53 (d, J = 6.1 Hz, 1H, OH), 2.17−2.10 (m, 2H, H−C(3)), 0.89 (s, 9H, $C(CH_3)_3$), 0.09, 0.08 (2 × s, 2 × 3H, H−$Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.9 (s, C(5)), 145.5 (d, C(4)), 108.9 (d, C(2)), 92.4 (d, C(6a)), 87.8 (s, C(3a)), 77.6 (d, C(6)), 55.3 (q, OCH_3), 47.5 (t, C(3)), 27.0 (q, $C(CH_3)_3$), 16.9 (s, $C(CH_3)_3$), −5.2, −5.9 (2 × q, $Si(CH_3)_2$); ESI$^+$-HRMS m/z calcd for $C_{14}H_{26}NaO_4Si$ $[M + Na]^+$ 309.1493, found 309.1496.

Data for ***9****.* R_f = 0.37 (Et_2O/CH_2Cl_2 1:9); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.88 (dd, J = 5.8, 0.8 Hz, 1H, H−C(4,5)), 5.71 (dd, J = 5.8, 2.2 Hz, 1H, H−C(4,5)), 5.16 (dd, J = 4.7, 0.9 Hz, 1H, H−C(2)), 4.73 (ddd, J = 5.7, 2.1, 0.9 Hz, 1H, H−C(6)), 4.24 (d, J = 5.7 Hz, 1H, H−C(6a)), 3.38 (s, 3H, OCH_3), 2.82 (s, 1H, OH), 2.18 (dd, J = 13.5, 4.7 Hz, 1H, H−C(3)), 2.07 (dd, J = 13.5, 0.9 Hz, 1H, H−C(3)), 0.87 (s, 9H, $C(CH_3)_3$), 0.08 (s, 6H, H−$Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 135.6, 134.8 (2 × d, C(4,5)), 109.0 (d, C(2)), 91.1 (s, C(3a)), 88.2 (d, C(6a)), 74.7 (d, C(6)), 54.9 (q, OCH_3), 46.9 (t, C(3)), 26.1 (q, $C(CH_3)_3$), 18.6 (s, $C(CH_3)_3$), −4.4, −4.8 (2 × q, $Si(CH_3)_2$); ESI$^+$-HRMS m/z calcd for $C_{14}H_{26}NaO_4Si$ $[M + Na]^+$ 309.1493, found 309.1504.

(2*S*,3a*R*,5*R*,6*S*,6a*R*)-6-(*tert*-Butyldimethylsilyloxy)-5-fluoro-2-methoxyhexahydro-2*H*-cyclopenta[*b*]furan-3a-ol (7). Through a solution of compound **6** (0.440 g, 1.44 mmol) in dry MeOH (40 mL) was bubbled Ar, and then 10% Pd−C (88 mg) was added. The Ar atmosphere was replaced by H_2 and the mixture was vigorously stirred for 2 h at rt. The resulting suspension was filtered through Celite, which was thoroughly washed with MeOH. After evaporation of the filtrate the residue was purified by CC (Et_2O/CH_2Cl_2 5:95) to give the title compound 7 (0.355 g, 80%) as a white solid.

Data for ***7****.* R_f = 0.41 (Et_2O/CH_2Cl_2 1:9); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.12 (d, J = 4.3 Hz, 1H, H−C(2)), 4.97 (ddddd, J = 51.6, 7.0, 6.4, 3.4, 0.9 Hz, 1H, H−C(5)), 4.17 (dddd, J = 10.8, 4.8, 3.3, 0.9 Hz, 1H, H−C(6)), 4.11 (d, J = 4.9 Hz, 1H, H−C(6a)), 3.34 (s, 3H, OCH_3), 2.88 (s, 1H, OH), 2.25 (ddd, J = 16.9, 13.9, 7.1 Hz 1H, H−C(4)), 2.21 (dd, J = 13.3, 4.3 Hz, 1H, H−C(3)), 2.12 (d, J = 13.3 Hz, 1H, H−C(3)), 2.08 (dt, J = 14.2, 6.5 Hz 1H, H−C(4)), 0.89 (s, 9H, $C(CH_3)_3$), 0.09, 0.08 (2 × s, 2 × 3H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 107.6 (d, C(2)), 93.9 (dd, $J(C,F)$ = 192 Hz, C(5)), 89.0 (dd, $^3J(C,F)$ = 4 Hz, C(6a)), 85.0 (d, $^3J(C,F)$ = 6 Hz, C(3a)), 73.6 (d, $^2J(C,F)$ = 15 Hz, C(6)), 54.7 (q, OCH_3), 48.6 (t, C(3)), 40.3 (dd, $^2J(C,F)$ = 19 Hz, C(4)), 26.0 (q, $C(CH_3)_3$), 18.6 (s, $C(CH_3)_3$), −4.6, −4.8 (2 × q, $Si(CH_3)_2$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.84 (dtd, $J(H,F)$ = 51.6, 15.7, 11.1 Hz); ESI$^+$-HRMS m/z calcd for $C_{14}H_{27}FNaO_4Si$ $[M + Na]^+$ 329.1555, found 329.1548.

(3a*R*,5*R*,6*S*,6a*R*)-6-(*tert*-Butyldimethylsilyloxy)-5-fluoro-3a-trimethylsilyloxy-tetraydrocyclopenta[*b*]furan (10). To a solution of 7 (58 mg, 0.19 mmol) and 2,6-lutidine (11 μL, 0.99 mmol) in CH_2Cl_2 (3.5 mL) was added TMSOTf (0.1 mL, 0.55 mmol) dropwise at 0 °C under Ar. After 15 min, the cooling bath was removed, and stirring was continued for 75 min at rt. The mixture was then diluted with saturated aq $NaHCO_3$ and extracted with EtOAc. The organic extract was washed with saturated aq $NaHCO_3$, and the aqueous phase extracted again with EtOAc. After drying over $MgSO_4$ and evaporation of all volatiles the crude **10** (pale brown oil) was used for the next reaction step without further purification.

Data for ***10****.* R_f = 0.51 (EtOAc/hexane 5:95); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.38 (d, J = 2.7 Hz, 1H, H−C(2)), 5.09 (d, J = 2.7 Hz, 1H, H−C(3)), 4.90 (dtdd, J = 52.5, 5.2, 3.4, 0.8 Hz, 1H, H−C(5)), 4.37 (d, J = 5.8 Hz, 1H, H−C(6a)), 4.27 (dddd, J = 16.6, 5.7, 3.4, 0.9 Hz, 1H, H−C(6)), 2.37 (ddd, J = 16.8, 13.8, 5.4 Hz, 1H, H−C(4)), 2.11 (ddd, J = 22.0, 13.8, 5.1 Hz, 1H, H−C(4)), 0.89 (s, 9H, $C(CH_3)_3$), 0.10, 0.09 (2 × s, 15H; $Si(CH_3)_2$, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.9 (dd, $J(C,F)$ = 1 Hz, C(2)), 107.8 (d, C(3)), 93.7 (dd, $J(C,F)$ = 191 Hz, C(5)), 90.1 (dd, dd, $^3J(C,F)$ = 3 Hz, C(6a)), 89.5 (d, $^3J(C,F)$ = 4 Hz, C(3a)), 74.4 (dd, $^2J(C,F)$ = 16 Hz, C(6)), 44.3 (td, $^2J(C,F)$ = 18 Hz, C(4)), 26.0 (q, $C(CH_3)_3$), 18.6 (s, $C(CH_3)_3$), 2.1 (q, $Si(CH_3)_3$), −4.6, −4.7 (2 × q, $Si(CH_3)_2$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −201.8; ESI$^+$-HRMS m/z calcd for $C_{16}H_{31}FNaO_3Si_2$ $[M + Na]^+$ 369.1688, found 369.1699.

(5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-2′-iodo-3′-*O*-trimethylsilyl-α-D-ribofuranosyl)thymine (11a) and (5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-2′-iodo-3′-*O*-trimethylsilyl-β-D-ribofuranosyl)thymine (11b). To a suspension of thymine (95 mg, 0.76 mmol) in dry CH_2Cl_2 (3 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (230 μL, 0.95 mmol) and the resulting mixture was stirred for 3 h at rt. A solution of glycal **10** (72 mg, 0.19 mmol) in CH_2Cl_2 (2 mL) was then added, and the suspension was cooled to 0 °C. Solid *N*-iodosuccinimide (64 mg, 0.28 mmol) was added, and the mixture was stirred at 0 °C for 4 h and left for 12 h without stirring at 4 °C. The mixture was diluted with saturated aq Na_2CO_3 and extracted with EtOAc; the organic phase was successively washed with saturated aq Na_2CO_3 (2 ×), saturated aq $NaHCO_3$, and 1 M $Na_2S_2O_3$. The aqueous phases were extracted with EtOAc (3×). The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated, and the residue was purified by CC (EtOAc/hexane 25:75) to afford **11a,b** (76 mg, 67%) as a mixture of isomers, which were separated by a second chromatography (Et_2O/hexane 40:60) to give 25 mg (22%) of **11a** and 49 mg (43%) of **11b**.

Data for ***11b****.* R_f = 0.33 (EtOAc/hexane 25:75); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.93 (s, 1H, NH), 7.24 (sbr, 1H, H−C(6)), 6.35 (dd, J = 9.7, 1.6 Hz, 1H, H−C(1′)), 5.12−4.85 (m, 1H, H−C(6′)), 4.25−4.04 (m, 3H, H−C(2′,4′,5′)), 2.26 (td, J = 15.7, 1.8 Hz, 1H, H−C(7′)), 1.88 (d, J = 1.2 Hz, 3H, CH_3−C(5)), 1.82 (ddd, J = 34.7, 15.1, 4.3 Hz, 1H, H−C(7′)), 0.88 (s, 9H, $C(CH_3)_3$), 0.21 (s, 9H, $Si(CH_3)_3$), 0.10, 0.07 (2 × s, 2 × 3H, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.8 (s, C(4)), 150.8 (s, C(2)), 134.9 (dd, $J(C,F)$ = 5 Hz, C(6)), 112.1 (s, C(5)), 95.2 (dd, $J(C,F)$ = 187 Hz, C(6′)), 89.0 (d, C(1′)), 86.4 (s, C(3′)), 85.6 (d, C(4′)), 73.1 (dd, $^2J(C,F)$ = 16 Hz, C(5′)), 38.8 (td, $^2J(C,F)$ = 18 Hz, C(7′)), 38.1 (dd, $J(C,F)$ = 6 Hz, C(2′)), 25.9 (q, $C(CH_3)_3$), 18.5 (s, $C(CH_3)_3$), 12.7 (q, CH_3−C(5)), 2.2 (q, $Si(CH_3)_3$), −4.5, −4.8 (2 × q, $Si(CH_3)_2$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −194.1; ESI$^+$-HRMS m/z calcd for $C_{21}H_{36}FIN_2NaO_5Si_2$ $[M + Na]^+$ 621.1084, found 621.1088.

Data for ***11a****.* R_f = 0.33 (EtOAc/hexane 25:75); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (s, 1H, NH), 7.03 (q, J = 1.2 Hz, 1H, H−C(6)), 6.01 (d, J = 9.4 Hz, 1H, H−C(1′)), 4.91 (dtd, J = 53.0, 6.1, 3.9 Hz, 1H, H−C(6′)), 4.56 (d, J = 9.4 Hz, 1H, H−C(2′)), 4.34 (d, J = 4.5 Hz, 1H, H−C(4′)), 4.28 (dt, J = 12.1, 4.3 Hz, 1H, H−C(5′)), 2.55 (ddd, J = 19.7, 14.7, 6.8 Hz, 1H, H−C(7′)), 2.17 (td, J = 14.9, 5.7 Hz, 1H, H−C(7′)), 1.93 (d, J = 1.2 Hz, 3H, CH_3−C(5)), 0.90 (s, 9H, $C(CH_3)_3$), 0.22 (s, 9H, $Si(CH_3)_3$), 0.10, 0.08 (2 × s, 2 × 3H, H−$Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.5 (s, C(4)), 149.9 (s, C(2)), 136.2 (d, C(6)), 111.9 (s, C(5)), 93.1 (d, C(1′)), 91.6 (dd, $J(C,F)$ = 192 Hz, C(6′)), 87.0 (s, C(3′)), 86.8 (d, C(4′)), 74.3 (dd, $^2J(C,F)$ = 15 Hz, C(5′)), 45.2 (td, $^2J(C,F)$ = 20 Hz, C(7′)), 35.1 (d, C(2′)), 26.0 (q, $C(CH_3)_3$), 18.7 (s, $C(CH_3)_3$), 12.8 (q, CH_3−C(5)), 2.5 (q, $Si(CH_3)_3$), −4.6, −4.8 (2 × q, $Si(CH_3)_2$) ppm; ^{19}F NMR ($CDCl_3$, 376 MHz) δ −198.1; ESI$^+$-HRMS m/z calcd for $C_{21}H_{36}FIN_2NaO_5Si_2$ $[M + Na]^+$ 621.1084, found 621.1082.

(5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-3′-*O*-trimethylsilyl-β-D-ribofuranosyl)thymine (12). To a solution of nucleoside **11*β*** (49 mg, 0.08 mmol) and Bu_3SnH (33 μL, 0.12 mmol) in dry toluene (2 mL) was added azaisobutyronitrile (AIBN, 9 mg, 0.06 mmol), and the mixture was heated to reflux. After 1.75 h the

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solvent was removed in vacuo, and the residue was purified by CC (EtOAc/hexane 3:7) to give the title compound **12** (35 mg, 90%) as a white solid.

Data for ***12****.* R_f = 0.32 (EtOAc/hexane 3:7); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.04 (s, 1H, NH), 7.55 (m, 1H, H−C(6)), 6.29 (ddd, J = 9.3, 5.4, 0.8 Hz, 1H, H−C(1′)), 5.06−4.80 (m, 1H, H−C(6′)), 4.20−4.04 (m, 2H, H−C(4′,5′)), 2.61 (ddd, J = 13.7, 5.4, 2.6 Hz, 1H, H−C(2′)), 2.39 (ddd, J = 15.7, 14.8, 1.7 Hz, 1H, H−C(7′)), 2.08 (dd, J = 13.7, 9.3 Hz, 1H, H−C(2′)), 1.87 (d, J = 1.2 Hz, 3H, CH_3−C(5)), 1.85 (ddd, J = 37.3, 14.8, 4.0 Hz, H−C(7′)), 0.89 (s, 9H; $C(CH_3)_3$), 0.15 (s, 9H, $Si(CH_3)_3$)), 0.11, 0.09 (2 × s, 2 × 3H; $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.2 (s, C(4)), 150.6 (s, C(2)), 136.3 (dd, J(C,F) = 7 Hz, C(6)), 111.0 (s, C(5)), 96.1 (dd, J(C,F) = 186 Hz, C(6′)), 88.6 (d, C(4′)), 88.5 (dd, ^3J(C,F) = 1 Hz, C(3′)), 85.7 (d, C(1′)), 73.3 (dd, ^2J(C,F) = 16 Hz, C(5′)), 48.1 (td, J(C,F) = 4 Hz, C(2′)), 41.8 (td, ^2J(C,F) = 18 Hz, C(7′)), 25.9 (q, $C(CH_3)_3$), 18.5 (s, $C(CH_3)_3$), 12.6 (q, CH_3−C(5)), 2.1 (q, $Si(CH_3)_3$), −4.5, −4.8 (2 × q, $Si(CH_3)_2$); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −194.9; ESI$^+$-HRMS m/z calcd for $C_{21}H_{37}FN_2NaO_5Si_2$ $[M + Na]^+$ 495.2117, found 495.2117.

(2′-Deoxy-3′,5′-ethano-6′-fluoro-β-D-ribofuranosyl)thymine (13). The solution of **12** (0.136 g, 0.29 mmol) in dry THF (4 mL) was treated with $Et_3N{\cdot}3HF$ (0.66 mL, 4.02 mmol) at ambient temperature for 5 days. After quenching the reaction by addition of silica gel, all volatiles were removed in vacuo, and the product was isolated by CC (EtOAc/EtOH 10:1) to afford title compound **13** (81 mg, 99%) as a white solid.

Data for ***13****.* R_f = 0.20 (EtOAc/MeOH 10:1); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.28 (s, 1H, NH), 7.66 (sbr, 1H, H−C(6)), 6.21 (dd, J = 9.6, 5.4 Hz, 1H, H−C(1′)), 5.56, 5.35 (2 × sbr, 2H, OH), 5.10−4.94 (m, 1H, H−C(6′)), 4.06 (dbr, J = 22.8 Hz, 1H, H−C(5′)), 3.94 (d, J = 5.7 Hz, 1H, H−C(4′)), 2.33−2.23 (m, 2H), 2.01 (dd, J = 13.2, 9.8 Hz, 1H), 1.89 (ddd, J = 31.4, 14.4, 4.8 Hz, 1H, H−C(2′,7′)), 1.76 (d, J = 0.8 Hz, 3H, CH_3−C(5)); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.6 (s, C(4)), 150.3 (s, C(2)), 136.0 (dd, J(C,F) = 5 Hz, C(6)), 109.4 (s, C(5)), 95.8 (dd, J(C,F) = 182 Hz, C(6′)), 87.3 (d, C(4′)), 84.2 (d, C(1′)), 83.9 (s, C(3′)), 71.7 (dd, ^2J(C,F) = 16 Hz, C(5′)), 47.2 (t, C(2′)), 41.7 (td, ^2J(C,F) = 18 Hz, C(7′)), 12.3 (q, CH_3−C(5)); ^{19}F NMR (CD_3OD, 376 MHz) δ −197.5; ESI$^+$-HRMS m/z calcd for $C_{12}H_{16}FN_2O_5$ $[M + H]^+$ 287.1038, found 287.1043.

(5′-*O*-((4,4′-Dimethoxytriphenyl)methyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-β-D-ribofuranosyl)thymine (14). To a solution of **13** (75 mg, 0.26 mmol) in dry pyridine (2 mL) stirred at 75 °C was added DMTr-OTf (0.468 g, 1.03 mmol) in portions over 10 h and the reaction was stirred for additional 1 h at the same temperature. The mixture was diluted with saturated aq $NaHCO_3$ and extracted with EtOAc, and extracts were dried over $MgSO_4$. The title compound **14** (0.125 g, 81%) was isolated by CC (CH_2Cl_2/EtOH 96:4) as a white solid.

Data for ***14****.* R_f = 0.59 (EtOAc); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.37 (s, 1H, NH), 7.62 (sbr, 1H, H−C(6)), 7.50−7.48 (m, 2H), 7.42−7.20 (m, 7H), 6.97−6.84 (m, 4H, H-DMTr), 6.23 (dd, J = 8.9, 5.9 Hz, 1H, H−C(1′)), 5.45 (s, 1H, OH), 3.96−3.67 (m, 9H, H−C(6′,5′,4′,OCH_3)), 2.34 (ddd, J = 13.6, 5.6, 2.2 Hz, 1H, H−C(2′)), 2.11 (t, J = 16.1, Hz, 1H, H−C(7′)), 2.03 (dd, J = 13.8, 9.4 Hz, 1H, H−C(2′)), 1.80 (d, J = 0.8 Hz, 3H, CH_3−C(5)), 1.49 (ddd, J = 43.7, 15.0, 2.9 Hz, 1H, H−C(7′)); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.6 (s, C(4)), 158.30 (s, DMTr), 150.2 (s, C(2)), 145.4, 136.1, 135.9 (3 × s, DMTr), 135.3 (dd, J(C,F) = 8 Hz, C(6)), 129.7, 127.9, 127.5, 126.8, 113.3, 113.2 (6 × d, DMTr), 109.6 (s, C(5)), 95.8 (dd, J(C,F) = 181 Hz, C(6′)), 87.4 (d, C(4′)), 86.6 (s, C-DMTr), 84.7 (d, ^3J(C,F) = 2 Hz, C(3′)), 84.4 (d, C(1′)), 73.7 (dd, ^2J(C,F) = 15 Hz, C(5′)), 55.0 (q, OCH_3), 47.2 (t, C(2′)), 40.6 (td, ^2J(C,F) = 17 Hz, C(7′)), 12.5 (q, CH_3−C(5)); ^{19}F NMR (DMSO-d_6, 376 MHz) δ −192.6; ESI$^+$-HRMS m/z calcd for $C_{33}H_{33}FN_2NaO_7$ $[M + Na]^+$ 611.2164, found 611.2177.

(5′-*O*-((4,4′-Dimethoxytriphenyl)methyl)-3′-*O*-(2-cyanoethoxy)-diisopropylaminophosphanyl-2′-deoxy-3′,5′-ethano-6′-fluoro-β-D-ribofuranosyl)thymine (15). To a solution of **14** (0.125 g, 0.21 mmol) and *N*-ethyldiisopropylamine (0.22 mL, 1.27 mmol) in dry THF (2.2 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.14 mL, 0.64 mmol) at rt. After the solution had been stirred for 2 h, the mixture was poured into saturated aq $NaHCO_3$ and extracted with EtOAc, and extracts were dried over $MgSO_4$. After filtration and evaporation, the residual oil was purified by CC (EtOAc/Hexane 6:4) to give the title compound **15** (0.165 g, 99%) as a white foam.

Data for ***15****.* R_f = 0.53 (CH_2Cl_2/EtOH 96:4); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.64 (sbr, 1H, NH), 7.80 (sbr, 1H, H−C(6)), 7.65−7.54 (m, 2H), 7.51−7.47 (m, 4H), 7.33−7.23 (m, 3H), 6.88−6.84 (m, 4H, H-DMTr), 6.42−6.37 (m, 1H, H−C(1′)), 4.13 (d, J = 6.9 Hz, 1H, H−C(4′)), 3.95−3.74 (m, 9H, H−C(6′,5′,OCH_2, OCH_3)), 3.68−3.54 (m, 3H, CH-^{i}Pr, OCH_2), 3.02−2.93 (m, 1H, H−C(2′)), 2.69−2.53 (m, 2H, CH_2CN), 2.40−2.31 (m, 1H, H−C(7′)), 2.24−2.11 (m, 1H, H−C(2′)), 1.99, 1.98 (2 × d, J = 1.0 Hz, 3H, CH_3−C(5)), 1.87−1.61 (m, 1H, H−C(7′)), 1.17, 1.14 (2 × d, J = 6.8 Hz, 12H, CH_3-^{i}Pr); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.0 (s, C(4)), 159.0 (s, DMTr), 150.6, 150.5 (2 × s, C(2)), 145.5, 136.6 (3 × s, DMTr), 136.5, 136.4 (2 × dd, J(C,F) = 5 Hz, C(6)), 136.3 (s, DMTr), 130.3, 128.2, 127.3, (4 × d, DMTr), 117.8 (2 × s, CN), 113.6, 113.5 (2 × d, DMTr), 111.4 (s, C(5)), 95.5, 95.4 (2 × dd, J(C,F) = 182 Hz, C(6′)), 90.2, 90.1 (2 × s, C(3′)), 87.8 (s, C-DMTr), 87.1, 87.0 (2 × d, C(4′)), 85.5 (d, C(1′)), 74.2 (dd, ^2J(C,F) = 16 Hz, C(5′)), 58.1 (2 × td, ^2J(C,P) = 18 Hz, OCH_2), 55.5 (q, OCH_3), 46.8 (m, C(2′)), 43.7, 43.6 (2 × d, CH-^{i}Pr), 40.3 (m, C(7′)), 24.8, 24.7, 24.5, 24.4 (4 × qd, ^3J(C,P) = 7 Hz, CH_3-^{i}Pr), 40.6 (td, ^2J(C,F) = 17 Hz, C(7′)), 20.5, 20.4 (2 × td, ^3J(C,P) = 8 Hz, CH_2CN), 12.9 (q, CH_3−C(5)); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −192.6, −193.0; ^{31}P NMR ($CDCl_3$, 122 MHz) δ −142.9, −142.2; ESI$^+$-HRMS m/z calcd for $C_{42}H_{50}FN_4NaO_8P$ $[M + Na]^+$ 811.3243, found 811.3225.

Oligonucleotide Synthesis. Syntheses of oligonucleotides were performed on the 1.3 μmol scale of a DNA synthesizer using standard solid-phase phosphoramidite chemistry. Oligomers were assembled using the manufacturer's protocols on nucleoside preloaded dA-CPG 500 (48 μmol/g). Natural phosphoramidites (dT, dC4Bz, dA6Bz,dG2dmf) were coupled as a 0.1 M solution in CH_3CN, 6′-fluorobicyclophosphoramidite **15** as 0.15 M solution in CH_3CN. The coupling step was 90 s for natural phosphoramidites. An extended coupling step of 10 min for the modified phosphoramidite was necessary to achieve >95% coupling efficiency (trityl assay). As a coupling reagent, 5-(ethylthio)-1*H*-tetrazole (0.25 M in CH_3CN) was used. Capping was performed with a solution of DMAP (0.5 M in CH_3CN, Cap A) and a solution of 25% Ac_2O and 12.5% sym-collidine in CH_3CN (Cap B). Oxidation was performed with a solution of 20 mM I_2 and 0.45 M sym-collidine in 2:1 CH_3CN/H_2O. Detritylation after coupling of natural phosphoramidites was carried out using a solution of 3% dichloroacetic acid in dichloroethane. In order to achieve complete detritylation after coupling of the modified phosphoramidite **15**, 15% dichloroacetic acid in dichloroethane was used.

Oligonucleotide Deprotection and Purification. Deprotection of the oligonucleotides and detachment from the solid support was carried out using standard conditions (concentrated aq NH_3 for 16 h at 55 °C). The solutions were centrifuged after deprotection, the supernatants were collected, and the remaining beads were washed with 2 × 0.5 mL of H_2O. The combined supernatants were then concentrated to dryness. Crude oligomers were purified by ion-exchange HPLC (Dionex DNA Pac-PA200 or DNA Pac-PA100). As mobile phases, the following buffers were prepared: (A) 25 mM Trizma (2-amino-2-hydroxymethyl-1,3-propanediol) in H_2O, pH 8.0; (B) 25 mM Trizma, 1.25 M NaCl in H_2O, pH 8.0. Linear gradients of B in A were used (typically 0−50% B in A over 30 min), with a 1 mL/min flow rate and detection at 260 nm. Purified oligonucleotides were desalted over Sep-Pak cartridges, quantified at 260 nm with a Nanodrop spectrophotometer, and analyzed by ESI−mass spectrometry. Solutions of oligonucleotides in H_2O were then stored at −18 °C.

UV-Melting Curves. UV-melting curves were recorded on a Varian Cary Bio100 UV−vis spectrophotometer. Absorbances were monitored at 260 nm, and the heating rate was set to 0.5 °C/min. A cooling−heating−cooling cycle in the temperature range 20−80 °C was applied. T_m values were obtained from the maximum of the first derivative curves and reported as the average of at least three ramps (±1 °C error). To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out in NaCl (150 mM), NaH_2PO_4 (10 mM) buffer at pH 7.0 with a duplex concentration of 1.2 μM.

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■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra of compounds **2**−**15**, analytical data of oligonucleotides and X-ray structural data (CIF) and packing diagram of compound **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Bennett, C. F.; Swayze, E. E. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 259−293.
(2) Lacerra, G.; Sierakowska, H.; Carestia, C.; Fucharoen, S.; Summerton, J.; Weller, D.; Kole, R. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*, 9591−9596.
(3) Wood, M. J. A.; Gait, M. J.; Yin, H. *Brain* **2010**, *133*, 957−972.
(4) Watts, J. K.; Deleavey, G. F.; Damha, M. J. *Drug Discovery Today* **2008**, *13*, 842−855.
(5) The ENCODE project consortium. *Nature* **2012**, *489*, 57−74.
(6) Stenvang, J.; Petri, A.; Lindow, M.; Obad, S.; Kauppinen, S. *Silence* **2012**, *3*, 1−17.
(7) Leumann, C. J. *Bioorg. Med. Chem.* **2002**, *10*, 841−854.
(8) Zhou, C. Z.; Chattopadhyaya, J. *Curr. Opin. Drug Discovery Dev.* **2009**, *12*, 876−898.
(9) Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359−4369.
(10) Biffinger, J. C.; Kim, H. W.; DiMagno, S. G. *ChemBioChem* **2004**, *5*, 622−627.
(11) Dalvit, C.; Vulpetti, A. *ChemMedChem* **2011**, *6*, 104−114.
(12) Patra, A.; Paolillo, M.; Charisse, K.; Manoharan, M.; Rozners, E.; Egli, M. *Angew. Chem., Int. Ed. Engl.* **2012**, *51*, 11863−11866.
(13) Pallan, P. S.; Greene, E. M.; Jicman, P. A.; Pandey, R. K.; Manoharan, M.; Rozners, E.; Egli, M. *Nucleic Acids Res.* **2011**, *39*, 3482−3495.
(14) Anzahaee, M. Y.; Watts, J. K.; Alla, N. R.; Nicholson, A. W.; Damha, M. J. *J. Am. Chem. Soc.* **2011**, *133*, 728−731.
(15) Watts, J. K.; Martin-Pintado, N.; Gomez-Pinto, I.; Schwartzentruber, J.; Portella, G.; Orozco, M.; Gonzalez, C.; Damha, M. J. *Nucleic Acids Res.* **2010**, *38*, 2498−2511.
(16) Allerson, C. R.; Sioufi, N.; Jarres, R.; Prakash, T. P.; Naik, N.; Berdeja, A.; Wanders, L.; Griffey, R. H.; Swayze, E. E.; Bhat, B. *J. Med. Chem.* **2005**, *48*, 901−904.
(17) Dowler, T.; Bergeron, D.; Tedeschi, A.-L.; Paquet, L.; Ferrari, N.; Damha, M. J. *Nucleic Acids Res.* **2006**, *34*, 1669−1675.
(18) Kalota, A.; Karabon, L.; Swider, C. R.; Viazovkina, E.; Elzagheid, M.; Damha, M. J.; Gewirtz, A. M. *Nucleic Acids Res.* **2006**, *34*, 451−461.
(19) Egli, M.; Pallan, P. S.; Allerson, C. R.; Prakash, T. P.; Berdeja, A.; Yu, J.; Lee, S.; Watt, A.; Gaus, H.; Bhat, B.; Swayze, E. E.; Seth, P. P. *J. Am. Chem. Soc.* **2011**, *133*, 16642−16649.
(20) Pallan, P. S.; Yu, J.; Allerson, C. R.; Swayze, E. E.; Seth, P.; Egli, M. *Biochemistry* **2011**, *51*, 7−9.
(21) Seth, P. P.; Yu, J.; Jazayeri, A.; Pallan, P. S.; Allerson, C. R.; Østergaard, M. E.; Liu, F.; Herdewijn, P.; Egli, M.; Swayze, E. E. *J. Org. Chem.* **2012**, *77*, 5074−5085.
(22) Seth, P. P.; Pallan, P. S.; Swayze, E. E.; Egli, M. *ChemBioChem* **2013**, *14*, 58−62.
(23) Østergaard, M. E.; Southwell, A. L.; Kordasiewicz, H.; Watt, A. T.; Skotte, N. H.; Doty, C. N.; Vaid, K.; Villanueva, E. B.; Swayze, E. E.; Frank Bennett, C.; Hayden, M. R.; Seth, P. P. *Nucleic Acids Res.* **2013**, *41*, 9634−9650.
(24) Rigo, F.; Hua, Y.; Chun, S. J.; Prakash, T. P.; Krainer, A. R.; Bennett, C. F. *Nat. Chem. Biol.* **2012**, *8*, 555−561.
(25) Bolli, M.; Trafelet, H. U.; Leumann, C. *Nucleic Acids Res.* **1996**, *24*, 4660−4667.
(26) Renneberg, D.; Leumann, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 5993−6002.
(27) Murray, S.; Ittig, D.; Koller, E.; Berdeja, A.; Chappell, A.; Prakash, T. P.; Norrbom, M.; Swayze, E. E.; Leumann, C. J.; Seth, P. P. *Nucleic Acids Res.* **2012**, *40*, 6135−6143.
(28) Haziri, A. I.; Silhar, P.; Renneberg, D.; Leumann, C. J. *Synthesis* **2010**, 823−827.
(29) Banks, R. E. *J. Fluorine Chem.* **1998**, *87*, 1−17.
(30) Singh, R. P.; Shreeve, J. M. *Acc. Chem. Res.* **2004**, *37*, 31−44.
(31) Differding, E.; Ofner, H. *Synlett* **1991**, 187−189.
(32) Singh, R. P.; Shreeve, J. M. *Synthesis* **2002**, 2561−2578.
(33) Jeong, L. S.; Yoo, S. J.; Lee, K. M.; Koo, M. J.; Choi, W. J.; Kim, H. O.; Moon, H. R.; Lee, M. Y.; Park, J. G.; Lee, S. K.; Chun, M. W. *J. Med. Chem.* **2002**, *46*, 201−203.
(34) Nicolaou, K. C.; Montagnon, T.; Baran, P. S. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 993−996.
(35) Lautens, M.; Delanghe, P. H. M.; Goh, J. B.; Zhang, C. H. *J. Org. Chem.* **1992**, *57*, 3270−3272.
(36) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279−1286.
(37) Silhár, P.; Leumann, C. J. *Bioorg. Med. Chem.* **2010**, *18*, 7786−7793.
(38) Luisier, S.; Leumann, C. J. *Heterocycles* **2010**, *82*, 775−790.
(39) Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716−744.
(40) Tarköy, M.; Bolli, M.; Schweizer, B.; Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481−510.
(41) Thalladi, V. R.; Weiss, H.-C.; Bläser, D.; Boese, R.; Nangia, A.; Desiraju, G. R. *J. Am. Chem. Soc.* **1998**, *120*, 8702−8710.
(42) Lietard, J.; Ittig, D.; Leumann, C. J. *Bioorg. Med. Chem.* **2011**, *19*, 5869−5875.
(43) Luisier, S.; Leumann, C. J. *ChemBioChem* **2008**, *9*, 2244−2253.