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Synthesis and Properties of 6′-Fluoro-tricyclo-DNA

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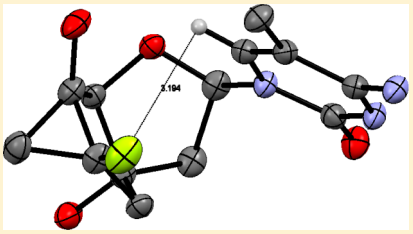
Supporting Information

ABSTRACT: The synthesis of the two fluorinated tricyclic nucleosides 6′-F-tc-T and 6′-F-tc-5^{Me}C, as well as the corresponding building blocks for oligonucleotide assembly, was accomplished. An X-ray analysis of N^4-benzoylated 6′-F-tc-^{5Me}C reavealed a 2′-exo (north) conformation of the furanose ring, characterizing it as an RNA mimic. In contrast to observations in the bicyclo-DNA series, no short contact between the fluorine atom and the H6 of the base, reminiscent of a nonclassical F···H hydrogen bond, could be observed. T_m measurements of modified oligodeoxynucleotides with complementary RNA showed slightly sequence-dependent duplex stabilization profiles with maximum ΔT_m/mod values of +4.5 °C for 6′-F-tc-^{5Me}C and +1 °C for 6′-F-tc-T. In comparison with parent tc-modified oligonucleotides, no relevant changes in T_m were detected, attributing the fluorine substituent a neutral role in RNA affinity. A structural analysis of duplexes with DNA and RNA by CD-spectroscopy revealed a shift from B- to A-type conformation induced by the 6′-F-tc-nucleosides. This is not a specific "fluorine effect", as the same is also observed for the parent tc-modifications. The two fluorinated tc-nucleosides were also incorporated into a pure tricyclo-DNA backbone and showed no discrimination in T_m with complementary RNA, demonstrating that 6′-F substitution is also compatible within fully modified tc-oligonucleotides.



INTRODUCTION

Fluorine is widely appreciated in small molecule medicinal chemistry due its metabolic stability-enhancing properties and its unique protein-binding characteristics.[1−3] For similar reasons, fluorinated DNA analogues are of interest in oligonucleotide therapeutic approaches. Among the first fluorinated oligonucleotides investigated were the 2′-deoxy-2′-fluoro RNA (F-RNA) and the 2′deoxy-2′-fluoro-arabino nucleic acids (F-ANA, Figure 1). While both analogues have been known for quite some time, their structural and biophysical features have only recently been characterized in detail. Compared to their 2′-hydroxy variants RNA and ANA, both the F-RNA and F-ANA analogues bind with higher affinity (ΔT_m = 1−2 °C/mod) to complementary RNA. The origin of the higher duplex stability in the case of F-RNA was attributed to improved hydrogen-bonding and base-stacking as a consequence of the polar C2′−F bond.[4,5] In the case of F-ANA, internucleoside F−H8 pseudo hydrogen bonds, that are particularly strong at purine/pyrimidine sequence steps, have been invoked as a stability-enhancing feature.[6,7] F-RNA and F-ANA have been shown to improve the performance of therapeutic siRNAs.[8,9] Due to its unique RNaseH activating properties, F-ANA was also investigated in classical antisense applications.[10]

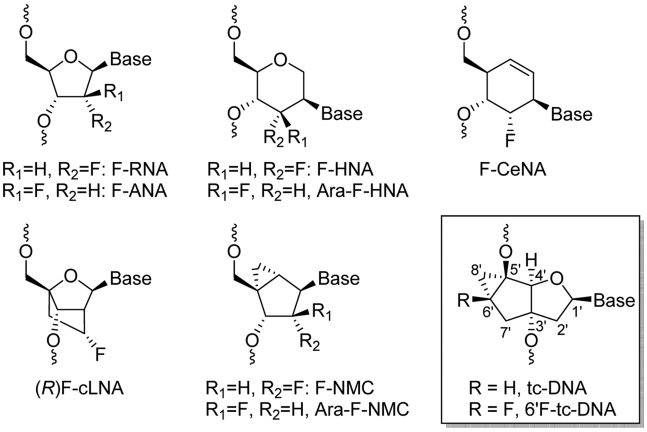


Figure 1. Chemical structures of selected fluorinated nucleic acid analogues.

Recently there has been a growing interest in investigating the effect of fluorine substitution in more complex, carbohydrate-modified oligonucleotide analogues such as F-HNA and its 2′-epimer Ara-F-HNA.[11,12] While F-HNA shows increased thermal stability (ΔT_m = +2 °C/mod) in complex with complementary RNA, the F-Ara-HNA analogue exhibits the opposite effect (ΔT_m = −3 °C/mod). The destabilization of Ara-F-HNA was attributed to repulsive steric effects of the fluoro substituent onto the 5′-adjacent nucleotide unit.[11] Also fluorinated versions of CeNA[13] and cLNA[14] were investigated. In these cases fluorine substitution does not contribute significantly to duplex stability. The most recent additions to the palette of fluorinated oligonucleotide analogues were F-NMC and Ara-F-NMC,[15] both derived from the northern methanocarbacyclic nucleoside (NMC) analogues.[16,17] Here again, F-NMC stabilized duplexes by +2.2 °C/mod on average while Ara-F-NMC was destabilizing by −2.8 °C/mod. The intrinsic contribution of the fluorine atom to thermal stability in

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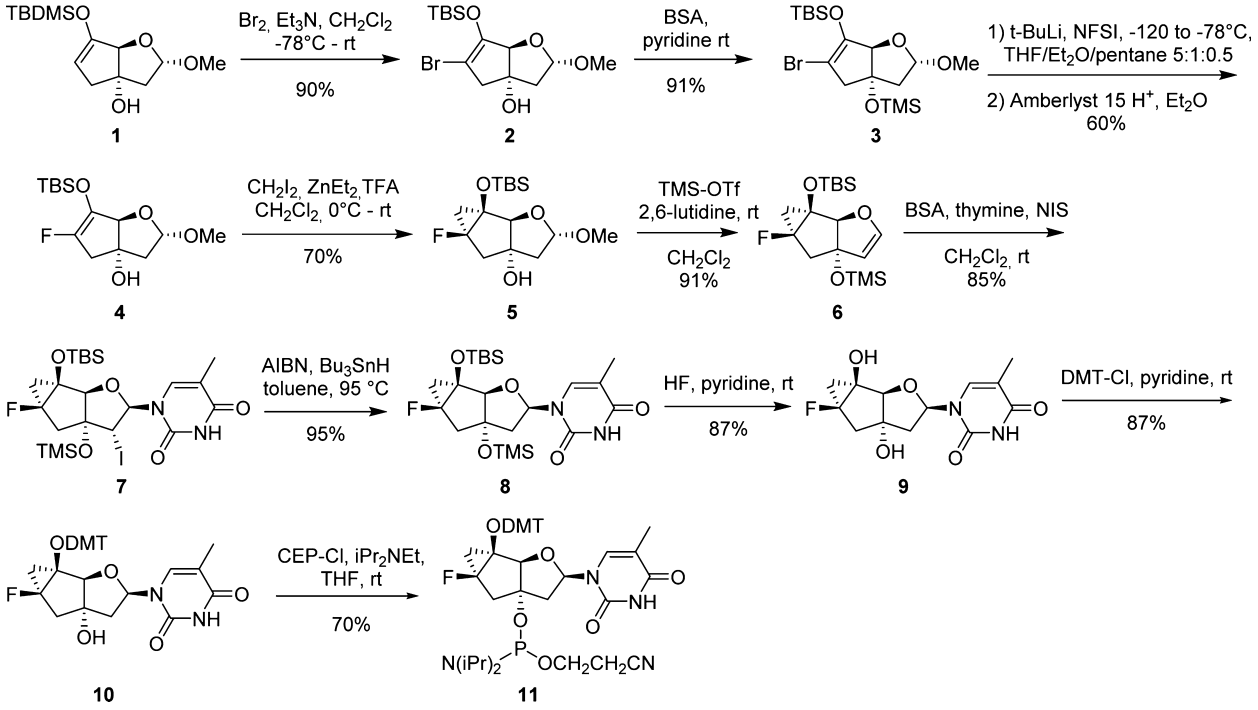
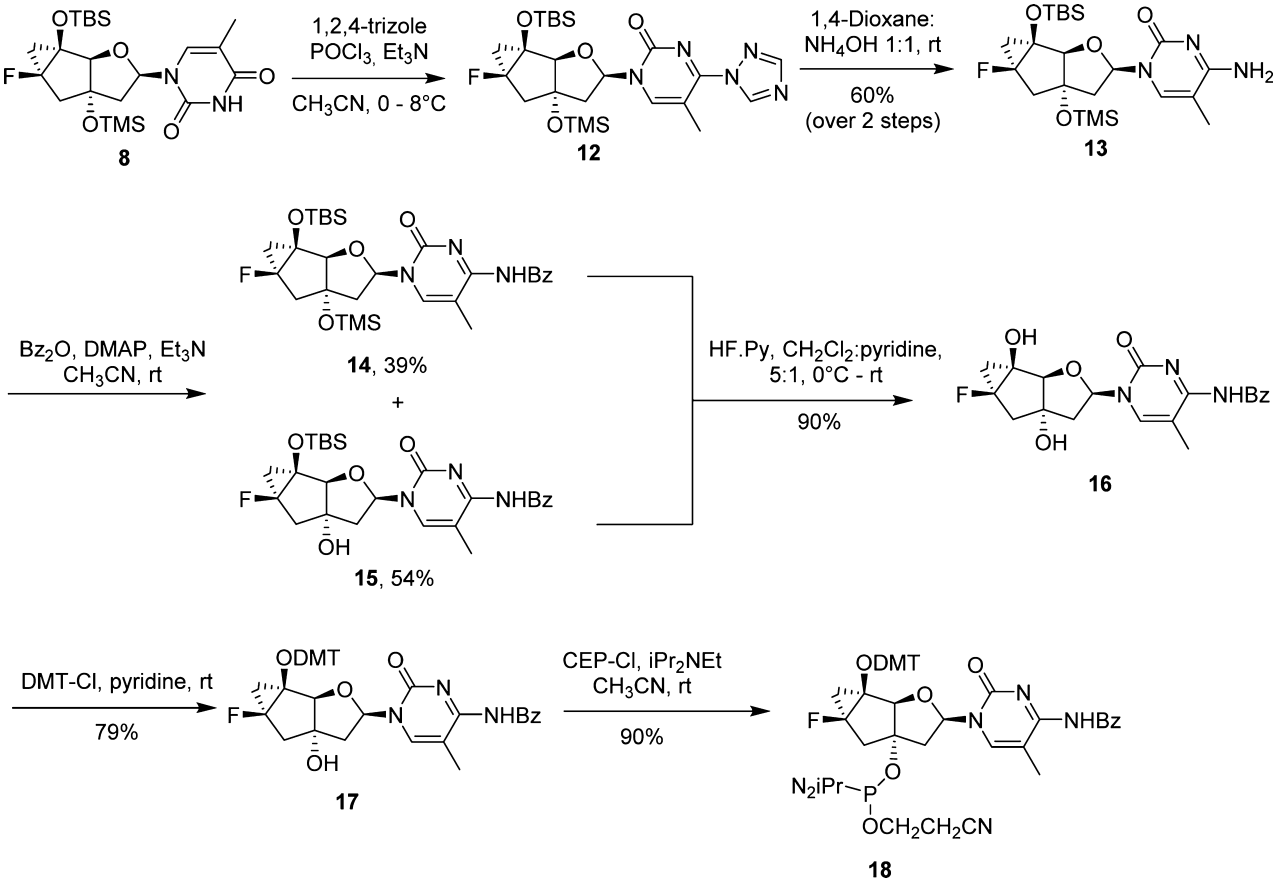
Scheme 1. Synthesis of Phosphoramidite 11



Scheme 2. Synthesis of Phosphoramidite 18



the case of F-NMC was determined to be +0.6 °C/mod on average.[18]

In terms of biological activity it has been shown previously that F-HNA gapmers can down-regulate gene expression in vivo in liver tissue more potently than LNA despite lower target affinity.[11] Thus, the higher potency of F-HNA seems to be the consequence of either improved biostability or more efficient plasma transport or both. Other recent observations, attributing

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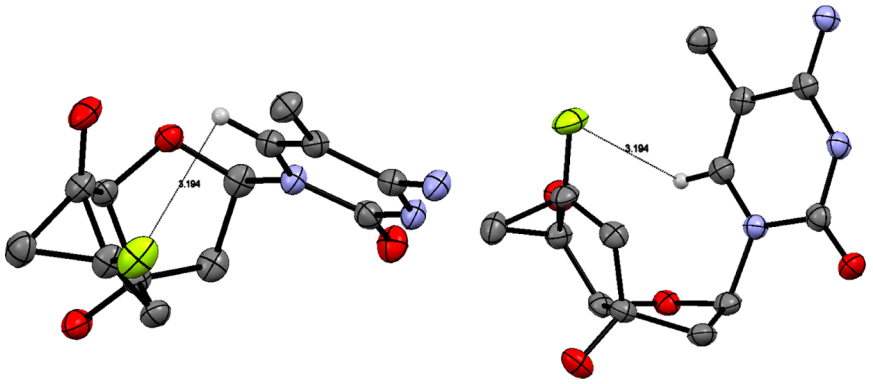

Figure 2. Ortep plot (50% probability ellipsoids) of the X-ray structure of nucleoside **16**: top-view (left) and side-view (right). Nonrelevant hydrogen atoms as well as the N^4-benzoyl residue in **16** are omitted for clarity.

a special but yet elusive role to fluorine in antisense efficacy were reported for gapmer oligonucleotides with F-RNA or F-ANA units targeting mutant huntingtin,[19] and for F-RNA antisense oligonucleotides recruiting the interleukin enhancer-binding factor complex (ILF2/3).[20]

Given these intriguing properties of fluorinated oligonucleotides, and building on earlier work on 6′-F-bicyclo-DNA,[21] we decided to investigate 6′-F-tc-DNA (Figure 1). In the following we present the synthesis and structural properties of the corresponding 6′-F-tc-nucleosides containing the bases thymine and 5-methylcytosine, as well as the influence on duplex stability and conformation of these modifications if complexed with complementary RNA and DNA.

RESULTS AND DISCUSSION

Synthesis of the Phosphoramidite Building Blocks 11 and 18. Drawing on earlier experiences during the synthesis of 6′-fluorinated bicyclo-DNA,[21] we envisaged to introduce the fluorine atom in an early step of the synthesis via electrophilic fluorination of a metalated bicyclic sugar intermediate. Consequently, we started our synthetic journey with the known bicyclic silyl enol ether **1** (Scheme 1).[22] Bromination of **1** with Br_2 at −78 °C gave the expected bromide **2** in 90% yield. To exclude side reactions during the following metal−halogen exchange, the free OH group in **2** was protected as a TMS ether (→ **3**). Electrophilic fluorination of **3** worked best if *t*-BuLi was used as lithiation reagent and if NFSI was added in one portion at a temperature of −120 °C. Temperatures above −78 °C, or addition of NFSI in multiple portions, led to substantial decomposition and thus reduced yield. The treatment with an acidic ion-exchange resin after quenching of the reaction quantitatively removed the TMS group, resulting in a 60% yield of fluoro silyl enol ether **4**. The rationale of removing the TMS group at this stage was based on the hypothesis that the corresponding hydroxyl group could help in directing the subsequent cyclopropanation reaction to the convex side of the bicyclic ring system. Indeed, cyclopropanation of **4** with a Zn−carbene complex in homogeneous solution yielded exclusively the exotricyclic sugar **5** in good yield but only if TFA was added as accelerator.[23] To prepare for β-selective nucleosidation,[24,25] compound **5** was converted to enol ether **6** with TMSOTf, which was then reacted with in situ persilylated thymine and NIS, yielding iodo-nucleoside **7** in 85% yield in a stereospecific manner. Removal of the iodine via radical reduction with Bu_3SnH finally gave the expected O-protected 6′-fluoro tricyclothymidine **8** in excellent yields. From here the synthesis of the phosphoramidite building block was completed by standard removal of the silyl protecting groups (→ **9**) followed by dimethoxytritylation (→ **10**) and phosphitylation with 2-cyanoethyl diisopropylamino chlorophosphine (CEP-Cl) to give **11** in a respectable overall yield of 13.4% starting from **1**.

Given the availability of the thymine nucleoside **8** and known procedures to interconvert pyrimidine bases on the level of nucleosides and oligonucleotides,[26,27] we next envisioned the synthesis of the building block **18** containing the base 5-methylcytosine. To this end, compound **8** was transformed into the triazolide **12** with 1,2,4-triazole and $POCl_3$ (Scheme 2). Treatment of **12** with ammonia then afforded the 5-methlytricyclocytidine **13** in 60% yield over two steps. N^4-Benzoylation of **13** with Bz_2O yielded the two nucleosides **14** and **15** that differed only by the presence or absence of the 3′-O-TMS group. This was of no harm, as the silyl groups in both **14** and **15** were removed in the next step, yielding **16** in high yield. The synthesis of the phosphoramidite building block **18** was then completed via standard dimethoxytritylation (→ **17**) followed by phosphitylation as described above.

X-ray Structure of 16. To confirm the relative configuration and to determine the effect of the fluorine substituent on the conformation of the tricyclic ring system, crystals of nucleoside **16** were grown and subjected to X-ray analysis. The molecular structure is depicted in Figure 2.

The furanose unit in **16** adopts a 2′-exo conformation with a pseudorotation phase angle *P* of 336° and a maximum torsion angle ν_{max} of 31°. It thus belongs clearly to the N-type conformation, typically adopted by RNA nucleosides.[28] The base is oriented in the central anti range (χ = −176.2°). Comparison of **16** with the structure of 6′-fluoro-bicyclo-T[21] reveals two major differences: First, the distance F−H6 in **16** (3.194 Å) is too long for a nonclassical F−H hydrogen bond while the same distance in 6′-fluoro-bc-T (2.865 Å) is indicative for such a weak interaction. Furthermore, there is no linear arrangement of F−H6−C6 in **16**, whereas this is clearly the case in 6′-fluoro-bc-T. Support for the absence of such an interaction in **16** comes also from the fact that there are no F−H6 or F−C6 couplings observable in the ^1H and ^{13}C NMR spectra of **16**, which contrasts the findings in the case of 6′-fluoro-bc-T. Thus, compared to 6′-fluoro-bc-T, the base-orienting H−F interaction of the fluorine is lost in **16**. The second structural change resides within the furanose conformation, which is 1′-exo (S-type) in the case of 6′-fluorobicyclo-T and 2′-exo (N-type) in the case of **16**. With respect to the nonfluorinated tricyclo-T nucleoside which

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Table 1. Analytical Data of Oligodeoxynucleotides ON1−ON7, Containing 6′-Fluoro-tc-T (t) or 6′-Fluoro-tc-^{5Me}C (c) Units, as well as T_m Data of Duplexes with Complementary DNA and RNA

	sequence	ESI-MS m/z calcd	ESI-MS m/z found	T_m (°C) vs DNA[a,b] (ΔT_m/mod)	T_m (°C) vs RNA[a,c] (ΔT_m/mod)
ON1	d(AACTG**t**CACG)	3067.6	3067.5	45.5 (+2.0)	44.4 (0.0)
ON2	d(AAC**t**GTCACG)	3067.6	3067.5	45.1 (+1.6)	45.4 (+1.0)
ON3	d(AAC**t**G**t**CACG)	3123.6	3123.5	43.5 (0.0)	44.3 (0.0)
ON4	d(GCA**ttttt**ACCG)	3890.7	3890.6	43.1 (−0.6)[d]	45.6 (+0.5)[e]
ON5	d(AACTGT**c**ACG)	3081.6	3081.5	44.6 (+1.1)	46.3 (+1.9)
ON6	d(AA**c**TGTCACG)	3081.6	3081.5	44.9 (+1.4)	48.9(+4.5)
ON7	d(AA**c**TGT**c**ACG)	3151.6	3151.6	48.2 (+2.4)	51.0 (+3.3)

[a]Total duplex concn: 2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0. Estimated error in T_m = ±0.5 °C. [b]T_m of unmodified duplex: 43.5 °C. [c]T_m of unmodified duplex: 44.4 °C. [d]T_m of unmodified duplex: 46.3 °C. [e]T_m of unmodified duplex: 42.9 °C

Table 2. Structure−Affinity Relationship: ΔT_m/mod Data for Oligodeoxynucleotides Containing Parent or Substituted tc-Nucleosides in Complex with Complementary RNA[a]

	tc-T	6′-F-tc-T		tc-C	tc-^{5Me}C	6′-F-tc-^{5Me}C
d(AACTG**X**CACG)	−0.1	0.0	d(AACTGT**X**ACG)	+2.0	+2.2	+1.9
d(AAC**X**GTCACG)	+1.4	+1.0	d(AA**X**TGTCACG)	+3.0	+4.2	+4.5
d(AAC**X**G**X**CACG)	+0.4	0.0	d(AA**X**TGT**X**ACG)	+2.5	+3.6	+3.3

[a]Experimental conditions as in Table 1.

Table 3. Analytical Data of tc-Oligonucleotides ON8−ON10, Containing 6′-Fluoro-tc-T (t), and T_m Data of Duplexes with Complementary DNA and RNA

	sequence[a]	ESI-MS m/z calcd	ESI-MS m/z found	T_m (°C) vs DNA[b,c] (ΔT_m/mod)	T_m (°C) vs RNA[b,d] (ΔT_m/mod)
ON8	d(p*AACTG***t***CACG*)	3490.5	3489.6	55.0 (0.0)	68.0 (+1.6)
ON9	d(p*AAC***t***GTCACG*)	3490.5	3489.6	53.4 (−1.6)	66.7 (+0.3)
ON10	d(p*AAC***t***G***t***CACG*)	3508.5	3507.6	53.2 (−1.8)	66.3 (−0.1)

[a]Characters in italic denote regular tc-DNA residues, and p denotes a 5′-phosphate group. [b]Total duplex concn: 2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0. Estimated error in T_m = ±0.5 °C. [c]T_m of unmodified duplex: 55.0 °C. [d]T_m of unmodified duplex: 66.4 °C

coexists in a 2′-endo (S-type) and a 4′-endo (E-type) conformation in the crystal,[29] it could well be that the fluoro atom helps to drive the furanose conformation of the tricyclic scaffold into a N-type conformation. We cannot exclude, however, that the higher propensity of N-conformation in **16** is also simply an effect of the base 5-methylcytosine. Unfortunately, there are currently no X-ray structures for the nonfluorinated tc-C or tc-^{5Me}C nucleosides available.

Synthesis of Oligonucleotides and T_m Data. Oligodeoxynucleotides **ON1−10**, containing the 6′-fluoro-tc-nucleosides (Table 1), were synthesized on a 1.3 μmol scale by standard phosphoramidite chemistry, utilizing the building blocks **11** and **18** (for details, see Experimental Section). Crude oligonucleotides were deprotected and detached from the solid support by standard ammonia treatment (33% NH_4OH, 55 °C, overnight) and purified by ion exchange HPLC. The composition of all oligonucleotides was verified by ESI-mass spectrometry (Tables 1 and 3).

Oligodeoxynucleotides containing single 6′-fluoro tc-T mutations (**ON1−3**) lead to duplexes with complementary DNA and RNA with neutral to slightly enhanced stability in a slightly sequence-dependent context, compared to unmodified duplexes. Interestingly, the stabilization (ΔT_m/mod) is a bit stronger in duplexes with complementary DNA as compared to RNA. Double substitutions in a noncontiguous order (**ON3**) tendentially lead to poorer duplex stabilization with RNA compared to multiple substitutions in a consecutive manner (**ON4**). This is in agreement with earlier observations in the series of tc-DNA[30] and has been ascribed to an incremental energetic penalty arising from increasing numbers of structural heterobackbone junctions. Replacing natural dC with 6′-fluoro-tc-^{5Me}C units (**ON5−7**) leads to a somewhat different picture. Again, in a slightly sequence-dependent context, duplex stabilization is significantly higher (up to +4.5 °C/mod) as compared to dC and more pronounced with RNA as a complement. In addition, there seems to be almost no energetic penalty as a function of the number of noncontiguous substitutions (**ON5** and **ON6** vs **ON7**). While it is known that the base thymine in the tc-DNA context is least stabilizing compared to the other three bases,[30,31] the origin of this effect is yet unknown and awaits further structural investigations.

To elucidate the role of the fluorine atom and the methyl group in 5-methylcytosine on RNA duplex stability, we compared ΔT_m/mod data with that of oligonucleotides containing tc-T, tc-C, and tc-^{5Me}C residues, respectively (Table 2). From this set of data it becomes evident that in both the T- and C-series, the 6′-fluorine atom behaves as neutral and does not significantly add to duplex stability. This is in agreement with the absence of any F−H5 pseudo hydrogen bond, as found in the X-ray structure of **16**, and supports our earlier hypothesis that this interaction is responsible for the increase in stability in the bc-DNA series.[21] At the same time, it is in agreement with the properties of other 6′-modified tc-DNA derivatives for which it was shown before that this position can be chemically modified without compromising RNA affinity.[24] In the C-series, the 5-methyl group of cytosine brings about 0.2−1.2 °C/mod of additional thermal stability also in the context of the tricyclic nucleoside structure. As for the case of 5-methyldeoxycytidine in DNA duplexes, this is most likely the consequence of improved stacking interactions

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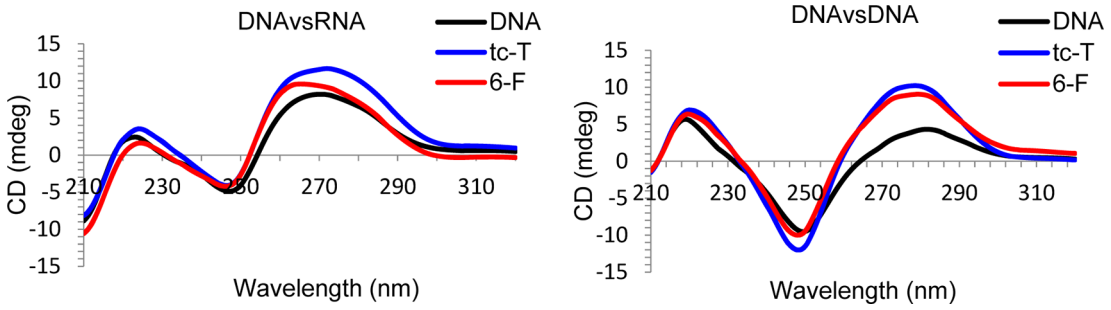

Figure 3. CD-spectra on left: DNA/RNA duplexes. CD-spectra on right: DNA/DNA duplexes. Black lines: unmodified duplexes, red lines: duplexes with **ON4**, blue lines: duplexes with **ON4** in which 6′-fluoro-tc-T was replaced by tc-T. Experimental conditions as indicated in Table 1

and/or improved hydrogen bonding induced by the molecular polarizability of the size-extended base.[32]

In the context of future applications as steric block or splice switching oligonucleotides, we also investigated the fully modified tc-oligonucleotides **ON8−10** containing 6′-fluoro-tc-T units. These oligonucleotides all carry a 5′-phosphate unit in order to confer chemical stability to the 5′-terminal nucleoside unit during oligonucleotide deprotection.[33] As can be seen from Table 3, duplexes with complementary DNA (non 5′-phosphorylated) are somewhat destabilized in the presence of the fluorine atom, while a slight stabilization in a sequence-dependent manner occurs with RNA (non 5′-phosphorylated) as complement. Thus, 6′-fluorination is fully compatible with the tc-DNA backbone and does not lead to loss of RNA affinity.

To determine the effect of 6′-F-tc-T units on duplex conformation, we measured CD-spectra of duplexes of **ON4** with complementary DNA and RNA and compared them with the corresponding unmodified duplexes and with duplexes containing tc-T instead of 6′-F-tc-T units (Figure 3). The largest structural deviation occurs in the DNA/DNA duplex series where both the 6′-F-tc-T and the tc-T units drive the duplex conformation from B to A-like. There are no significant differences between duplexes with tc-T or 6′-fluoro-tc-T, indicating that both adopt an N-type nucleoside conformation. The tendency to adopt a more A-like conformation in duplexes with tc-T or 6′-F-tc-T units is also present in the DNA/RNA duplex series. Again, there are no large differences between the tc-T- and 6′-F-tc-T-containing duplexes, perhaps with the exception that the maximum positive ellipticity around 270 nm is blue-shifted by ca. 10 nm in the case of the latter duplex, with a yet unknown implication on the helix structure.

CONCLUSION

We have accomplished the synthesis of the two 6′-fluorinated tc-nucleoside building blocks **11** and **18** and have incorporated them into oligodeoxynucleotides and tc-oligonucleotides. We analyzed complementary DNA and RNA affinity by T_m measurements and determined structural effects of fluorine substitution on duplex conformation by CD-spectroscopy. On the basis of the X-ray structure of **16** as well as on ^{1}H and ^{13}C NMR coupling data on the nucleosides and derivatives, we could not find any indications for short fluorine-base F−H6 contacts. This is in surprising contrast to findings in the 6′-fluoro-bc-DNA series, where such short contacts were observed. Compared to the nonfluorinated tc-nucleosides, we find that the fluorine substituent does not significantly alter the thermal melting properties of the corresponding duplexes, irrespective of the nature of the base (thymine vs 5-methylcytosine). This is in agreement with the absence of the glycosidic bond-constraining nature of the F−H6 interaction that adds up to +2 °C/mod in T_m in the case of the bicyclo-DNA series. The 6′-fluoro modification is also compatible with the tc-DNA backbone, as no change or even a slight increase in T_m with complementary RNA was observed. The fluorine atom also does not significantly alter the duplex conformation compared to nonfluorinated tc-DNA as can be seen from the corresponding CD-spectra. On the basis of these encouraging biophysical data, we are now planning to investigate functional efficacy, cellular uptake, and in vivo tissue distribution of antisense tc-oligonucleotides containing these 6′-F-tc-nucleosides.

EXPERIMENTAL SECTION

General Methods. All reactions were performed under argon in oven-dried glassware. Solvents were dried by filtration over 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. Compounds were visualized either under UV light or by staining in dip solution A: Cer^{IV}-sulfate (10.5 g), phosphormolybdenic acid (21 g), concd H_2SO_4 (60 mL), H_2O (900 mL); or B: $KMnO_4$ (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 or 100 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). ^{13}C signal multiplicities include ^{1}H- and ^{19}F-couplings. ^{1}H NMR difference-NOESY experiments were recorded at 400 MHz. Chemical shifts for ^{31}P and ^{19}F NMR (fully proton coupled) 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 tc-nucleosides is outlined in Figure 1. For non-nucleoside derivatives, von Baeyer nomenclature has been applied.

*(1S,3R,5S)-7-Bromo-8-tert-butyldimethylsilyloxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-en-5-ol (**2**).* To a stirred solution of silyl enol ether **1** (10.6 g, 37.0 mmol) in dry CH_2Cl_2 (100 mL) was added dropwise a solution of bromine (2.1 mL, 40.7 mmol) in CH_2Cl_2 (200 mL) over a period of 30 min at −78 °C, followed by Et_3N (7.7 mL, 55.48 mmol). The cooling bath was subsequently removed, and the temperature was allowed to rise to rt. Stirring was continued for another 2 h. Then the reaction mixture was poured into H_2O and extracted with Et_2O. The combined organic layers were washed with water, dried over $MgSO_4$, and evaporated. The residual dark oil was purified by CC (hexane/EtOAc 4:1) to give the title compound **2** (12.1 g, 90%) as a yellow oil.

Data for **2**. R_f = 0.33 (hexane/EtOAc 7:3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.04 (d, J = 4.1 Hz, 1H, H-C(3)), 4.57 (m, 1H, H-C(1)), 3.37

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(s, 3H, OMe), 3.15 (s, 1H, OH), 2.76 (d, J = 15.3 Hz, 1H, H_b-C(6)), 2.67 (dd, J = 2.2, 15.3 Hz, 1H, H_a-C(6)), 2.25 (d, J = 13.5 Hz, 1H, H_b-C(4)), 2.02 (dd, J = 4.1, 13.5 Hz, 1H, H_a-C(4)), 0.97 (s, 9H, *t*-Bu), 0.24, 0.20 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.4 (s, C(8)), 105.3 (d, C(3)), 96.7 (s, C(7)), 90.4 (d, C(1)), 83.4 (s, C(5)), 54.7 (q, OMe), 47.2 (t, C(6)), 44.8 (t, C(4)), 25.8 (q, *t*-Bu), 18.4 (s, *t*-Bu), −3.9, −4.2 (2 × s, 2 × CH_3); ESI$^+$-HRMS m/z calcd for $C_{14}H_{25}BrNaO_4Si$ $[M + Na]^+$ 387.0603, 389.0583, found 387.0598, 389.0577.

*(1S,3R,5S)-7-Bromo-8-tert-butyldimethylsilyloxy-3-methoxy-5-trimethylsilyloxy-2-oxabicyclo[3.3.0]oct-7-ene (**3**).* To a stirred solution of bromo silyl enol ether **2** (12.0 g, 32.74 mmol) in dry pyridine (170 mL) was added BSA (12 mL, 49.11 mmol) at rt, and the mixture was left overnight. The reaction mixture was diluted with sat. aq $NaHCO_3$ and extracted with Et_2O. The combined organic layers were dried over $MgSO_4$ and evaporated, and the residual oil was purified by CC (hexane/EtOAc 95:5, with 0.5% of Et_3N) to yield the title compound **3** (13.0 g, 91%) as a yellow oil.

Data for **3**. R_f = 0.68 (hexane/EtOAc 9:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.99 (dd, J = 1.3, 5.3 Hz, 1H, H-C(3)), 4.64 (t, J = 1.4 Hz, 1H, H-C(1)), 3.35 (s, 3H, OMe), 2.73 (d, J = 1.5 Hz, 2H, H-C(6)), 2.33 (dd, J = 1.3, 13.7 Hz, 1H, H_b-C(4)), 2.06 (dd, J = 5.3, 13.7 Hz, 1H, H_a-C(4)), 0.97 (s, 9H, *t*-Bu), 0.24, 0.21 (2s, 2 × 3H, 2 × CH_3), 0.15 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.6 (s, C(8)), 105.7 (d, C(3)), 96.4 (s, C(7)), 90.3 (d, C(1)), 84.8 (s, C(5)), 55.2 (q, OMe), 49.5 (t, C(6)), 48.4 (t, C(4)), 25.8 (q, *t*-Bu), 18.4 (s, *t*-Bu), 1.9 (q, TMS), −3.9, −4.2 (2q, 2 × CH_3); ESI$^+$-HRMS m/z calcd for $C_{17}H_{33}BrNaO_4Si_2$ $[M + Na]^+$ 459.0998, 461.0978, found 459.1002, 461.0981.

*(1S,3R,5S)-7-Fluoro-8-tert-butyldimethylsilyloxy-3-methoxy-2-oxabicyclo[3.3.0]oct-7-en-5-ol (**4**).* To a stirred solution of bromo silyl enol ether **3** (6.14 g, 14.04 mmol) in dry THF (211 mL) and ether (42 mL) was added dropwise a solution of *t*-BuLi (1.7 M in pentane, 16.5 mL, 28.08 mmol) at −78 °C. After being stirred for 20 min, the reaction mixture was further cooled to −120 °C and NFSI (8.85 g, 28.08 mmol) was added at once, followed by another portion of *t*-BuLi (24.8 mL, 42.12 mmol). The reaction mixture was stirred for 2 h and then allowed to warm to −80 °C. After being quenched with water (210 mL), the mixture was warmed to rt and extracted with EtOAc. The combined organic phases were dried over $MgSO_4$ and evaporated. The residue was then dissolved in dry ether (200 mL) and treated with amberlyst 15 (6.1 g), and the mixture was stirred for 2 h at rt. The amberlyst was then filtered off, and SiO_2 was added to the filtrate prior to evaporation. Purification by CC (CH_2Cl_2/hexane 7:3 → CH_2Cl_2, + 1% Et_2O) gave the title comound **4** (2.56 g, 60%) in form of a yellowish solid.

Data for **4**. R_f = 0.53 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.05 (d, J = 4.1 Hz, 1H, H-C(3)), 4.58 (dt, J = 1.8, 8.0 Hz, 1H, H-C(1)), 3.38 (s, 3H, OMe), 3.11 (d, J = 2.3 Hz, 1H, OH), 2.62 (m, 2H, H-C(6)), 2.28 (d, J = 13.4 Hz, 1H, H_b-C(4)), 1.97 (dd, J = 4.1, 13.4 Hz, 1H, H_a-C(4)), 0.95 (s, 9H, *t*-Bu), 0.19, 0.17 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5 (d, J(C,F) = 272.2 Hz, C(7)), 128.6 (d, J(C,F) = 4.0 Hz, C(8)), 104.8 (d, C(3)), 89.9 (dd, J(C,F) = 5.3 Hz, C(1)), 79.7 (d, J(C,F) = 11.6 Hz, C(5)), 54.7 (q, OMe), 47.5 (t, C(4)), 37.4 (td, J(C,F) = 18.6 Hz, C(6)), 25.7 (q, *t*-Bu), 18.3 (s, *t*-Bu), −4.3 (qd, J = 1.7 Hz), CH_3), −4.56 (qd, J = 2.1 Hz, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −138.5 (s, br); ESI$^+$-HRMS m/z calcd for $C_{14}H_{25}FNaO_4Si$ $[M + Na]^+$ 327.1404, found 327.1412.

*(1S,2S,4S,6R,8S)-2-tert-Butyldimethylsilyloxy-4-fluoro-8-methoxy-9-oxatricyclo[4.3.0^{1,6}.0^{2,4}]nonan-6-ol (**5**).* To dry CH_2Cl_2 (97 mL) was added a solution of Et_2Zn (1 M in hexane, 48.2 mL 48.20 mmol). The mixture was cooled to 0 °C, and a solution of TFA (3.69 mL, 48.21 mmol) in CH_2Cl_2 (48 mL) was slowly added. After the mixture was stirred for 20 min, a solution of CH_2I_2 (7.76 mL, 96.42 mmol) in CH_2Cl_2 (48 mL) was added. After another 20 min of stirring, a solution of fluoro silyl enol ether **4** (2.45 g, 8.04 mmol) in CH_2Cl_2 (48 mL) was added, and the ice bath was removed. After 5 h of stirring, the reaction mixture was quenched with sat. aq NH_4Cl and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with sat. $NaHCO_3$, dried over $MgSO_4$, concentrated, and purified by CC (hexane/EtOAc, 9:1) to yield the title compound **5** (1.79 g, 70%) as a colorless oil.

Data for **5**. R_f = 0.33 (hexane/EtOAc 3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.10 (dd, J = 1.6, 5.2 Hz, 1H, H-C(8)), 3.89 (d, J = 6.0 Hz, 1H, H-C(1)), 3.37 (s, 3H, OMe), 2.49 (m, 1H, H_b-C(5)), 2.43 (dd, J = 5.3, 14.0 Hz, 1H, H_b-C(7)), 2.29 (dd, J = 1.1 Hz, J = 13.4 Hz, 1H, H_a-C(5)), 2.10 (s, br, 1H, OH), 2.06 (dd, J = 1.6, 14.0 Hz, 1H, H_a-C(7)), 1.33 (ddd, J = 2.5, 7.5, 21.5 Hz, 1H, H_b-C(3)), 1.19 (dd, J = 7.5, 8.5 Hz, 1H, H_a-(3)), 0.91 (s, 9H, *t*-Bu), 0.17 (s, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 105.7 (d, C(8)), 88.7 (dd, J(C,F) = 3.8 Hz, C(1)), 83.2 (d, J(C,F) = 268.2 Hz, C(4)), 82.1 (d, J(C,F) = 5.5 Hz, C(6)), 63.7 (d, J(C,F) = 8.3 Hz, C(2)), 54.9 (q, OMe), 49.9 (t, C(7)), 44.4 (td, J(C,F) = 16.5 Hz, C(5)), 25.9 (q, *t*-Bu), 21.7 (td, J(C,F) = 10.3 Hz, C(3)), 18.3 (s, *t*-Bu), −3.9 (q, CH_3), −4.0 (qd, J(C,F) = 1.8 Hz, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −194.9 (m); ESI$^+$-HRMS m/z calcd for $C_{15}H_{27}FNaO_4Si$ $[M + Na]^+$ 341.1560, found 341.1561.

*(1S,2S,4S,6R)-2-tert-Butyldimethylsilyloxy-4-fluoro-6-trimethylsilyloxy-9-oxatricyclo[4.3.0^{1,6}.0^{2,4}]non-7-ene (**6**).* To a solution of compound **5** (1.51 g, 4.38 mmol) and 2,6-lutidine (2.80 mL, 24.20 mmol) in dry CH_2Cl_2 (10 mL) was added TMSOTf (2.14 mL, 11.84 mmol) dropwise at 0 °C. After being stirred for 2.5 h at rt, the reaction mixture was diluted with AcOEt and washed with saturated $NaHCO_3$, and the aqueous phase was extracted with AcOEt. The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was purified by CC (hexane/Et_2O 95:5) to give the title compound **6** (1.43 g, 91%) as light brownish oil.

Data for **6**. R_f = 0.83 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.33 (d, J = 2.7 Hz, 1H, H-C(8)), 5.15 (d, J = 2.7 Hz, 1H, H-C(7)), 4.24 (d, J = 6.0 Hz, 1H, H-C(1)), 2.52 (ddd, J = 2.8, 11.6, 12.9 Hz, 1H, H_b-C(5)), 2.37 (dd, J = 1.1, 12.9 Hz, 1H, H_a-C(5)), 1.45 (ddd, J = 2.8, 7.4, 10.1 Hz, 1H, H_b-C(3)), 1.02 (dd, J = 7.4, 8.4 Hz, 1H, H_a-C(3)), 0.90 (s, 9H, *t*-Bu), 0.15, 0.11 (2s, 2 × 3H, 2 × CH_3), 0.09 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.7 (d, C(8)), 108.4 (d, C(7)), 94.1 (dd, J(C,F) = 4.1 Hz, C(1)), 87.1 (d, J(C,F) = 14.2 Hz, C(6)), 84.4 (d, J(C,F) = 250.2 Hz, C-(4)), 64.8 (d, J(C,F) = 8.4 Hz, C(2)), 48.7 (td, J(C,F) = 14.4 Hz, C(5)), 26.0 (q, *t*-Bu), 22.9 (td, J(C,F) = 10.6 Hz, C(3)), 18.4 (s, *t*-Bu), 2.0 (q, TMS), −3.8 (q, CH_3), −4.3 (qd, J(C,F) = 2.8 Hz, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −192.6 (m); ESI$^+$-HRMS m/z calcd for $C_{17}H_{32}FO_3Si_2$ $[M + H]^+$ 359.1874, found 359.1873.

*(5′-O-(tert-Butyldimethylsilyl)- 3′-O-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-2′-iodo-5′,6′-methano-β-D-ribofuranosyl)-thymine (**7**).* To a suspension of thymine (1.49 g, 11.80 mmol) and compound **6** (1.41 g, 3.93 mmol) in CH_2Cl_2 (20 mL) was added BSA (2.88 mL, 11.80 mmol), and the mixture was stirred at rt for 2 h to become a clear solution. Then *N*-iodsuccinimide (1.32 g, 5.90 mmol) was added and the mixture stirred overnight. The reaction was quenched with sat. $NaHCO_3$ (30 mL) and a 10% aq solution of $Na_2S_2O_3$ (10 mL). The aqueous phase was extracted with EtOAc, and the combined organic phases were dried over $MgSO_4$ and evaporated. CC (hexane/EtOAc 9:1) afforded nucleosides **7** (2.05 g, 85%) as a yellowish solid.

Data for **7**. R_f = 0.39 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.10 (s, 1H, NH), 7.76 (d, J = 0.9 Hz, 1H, H-C(6)), 6.38 (d, J = 2.6 Hz, 1H, H-C(1′)), 4.56 (d, J = 2.6 Hz, 1H, H-C(2′)), 4.19 (d, J = 5.7 Hz, 1H, H-C(4′)), 2.45 (d, J = 14.2 Hz, 1H, H_b-C(7′)), 2.34 (m, 1H, H_a-C(7′)), 1.91 (d, J = 0.9 Hz, 3H, CH_3), 1.50 (ddd, J = 2.2, 7.8, 20.9 Hz, 1H, H_b-C(8′)), 1.19 (t, J = 8.4 Hz, 1H, H_a-C(8′)), 0.94 (s, 9H, *t*-Bu), 0.23 (s, 9H, TMS), 0.22, 0.20 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.3 (s, CO), 150.4 (s, CO), 135.4 (d, C(6)), 110.9 (s, C(5)), 96.8 (d, C(1′)), 91.2 (dd, J(C,F) = 3.7 Hz, C(4′)), 83.9 (d, J(C,F) = 11.4 Hz, C(3′)), 81.4 (d, J(C,F) = 250.9 Hz, C(6′)), 62.7 (d, J(C,F) = 8.2 Hz, C(5′)), 41.5 (d, C(2′)), 41.3 (td, J(C,F) = 16.3 Hz, C(7′)), 25.7 (q, *t*-Bu), 21.7 (td, J(C,F) = 10.2 Hz, C-(8′)), 18.0 (s, *t*-Bu), 12.4 (q, CH_3), 2.1 (q, TMS), −3.6 (q, CH_3), −4.0 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.2 (m); ESI$^+$-HRMS m/z calcd for $C_{22}H_{37}FIN_2O_5Si_2$ $[M + H]^+$ 611.1270, found 611.1265.

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*(5′-O-(tert-Butyldimethylsilyl)- 3′-O-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)thymine (**8**).* To a solution of iodonucleoside **7** (2.05 g, 3.35 mmol) in toluene (40 mL) were added Bu_3SnH (1.1 mL, 4.02 mmol) and azoisobutyronitrile (AIBN, 165 mg, 1.00 mmol) at rt. After heating to reflux for 1 h, the solvent was evaporated and the residue purified by CC (hexane/EtOAc 8:2) to give nucleoside **8** (1,54 mg, 95%) as a colorless solid.

Data for **8**. R_f = 0.52 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (s, 1H, NH), 7.82 (d, J = 1.0 Hz, 1H, H-C(6)), 6.03 (dd, J = 1.4, 6.4 Hz, 1H, H-C(1′)), 4.07 (d, J = 5.6 Hz, 1H, H-C(4′)), 2.62 (dd, J = 6.4, 13.7 Hz, 1H, H_b-C(2′)), 2.53 (dd, J = 1.4, 13.7 Hz, 1H, H_a-C(2′)), 2.37 (dd, J = 1.8, 13.7 Hz, 1H, H_b-C(7′)), 2.13 (m, 1H, H_a-C(7′)), 1.91 (d, J = 1.0 Hz, 3H, CH_3), 1.40 (ddd, J = 2.4, 7.7, 21.0 Hz, 1H, H_b-C(8′)), 1.18 (dd, J = 7.7, 9.1 Hz, 1H, H_a-C(8′)), 0.94 (s, 9H, *t*-Bu), 0.23, 0.19 (2s, 2 × 3H, 2 × CH_3), 0.13 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.2 (s, CO), 150.2 (s, CO), 136.0 (d, C(6)), 110.2 (s, C(5)), 93.0 (dd, J(C,F) = 4.0 Hz, C(4′)), 89.6 (d, C(1′)), 82.4 (d, J(C,F) = 12.3 Hz, C(3′)), 81.43 (d, J(C,F) = 249.7 Hz, C(6′)), 63.2 (d, J(C,F) = 8.1 Hz, C(5′)), 47.3 (t, C(2′)), 44.7 (td, J(C,F) = 14.9 Hz, C(7′)), 25.7 (q, *t*-Bu), 20.4 (td, J(C,F) = 10.0 Hz, C(8′)), 18.0 (s, *t*-Bu), 12.3 (q, CH_3), 2.0 (q, TMS), −3.7 (q, CH_3), −3.8 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.9 (m); ESI$^+$-HRMS m/z calcd for $C_{22}H_{38}FN_2O_5Si_2$ $[M + H]^+$ 485.2303, found 485.2295.

*(2′-Deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)thymine (**9**).* To a solution of compound **8** (1.48 g, 3.05 mmol) and pyridine (6 mL) in CH_2Cl_2 (30 mL) was added HF−pyridine (1.5 mL, 60.6 mmol) at 0 °C. After the mixture was stirred overnight at rt, SiO_2 (7 g) was added, and the mixture stirred for another 15 min. After evaporation, the adsorbed product was purified by CC (hexane/EtOAc/EtOH 5:5:1) to yield the title compound **9** (797 mg, 87%) as a white foam.

Data for **9**. R_f = 0.22 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.81 (d, J = 0.9 Hz, 1H, H-C(6)), 6.13 (dd, J = 4.0, 6.9 Hz, 1H, H-C(1′)), 3.99 (d, J = 5.7 Hz, 1H, H-C(4′)), 2.56 (dd, J = 6.9, 13.9 Hz, 1H, H_b-C(2′)), 2.45 (dd, J = 4.0, 13.9 Hz, 1H, H_a-C(2′)), 2.38 (m, 2H, H-C(7′)), 1.93 (d, J = 0.9 Hz, 3H, CH_3), 1.43 (ddd, J = 2.4, 7.4, 20.9 Hz, 1H, H_b-(8′)), 1.28 (m, 1H, H_a-C(8′)); ^{13}C NMR (CD_3OD, 100 MHz) δ 166.6 (s, CO), 152.1 (s, CO), 137.7 (d, C(6)), 111.0 (s, C(5)), 91.5 (dd, J(C,F) = 3.7 Hz, C(4′)), 89.0 (d, C(1′)), 84.4 (d, J(C,F) = 248.0 Hz, C(6′)), 80.9 (d, J(C,F) = 12.1 Hz, C(3′)), 63.4 (d, J(C,F) = 8.4 Hz, C(5′)), 49.0 (t, C(2′)), 45.1 (td, J(C,F) = 15.6 Hz, C(7′)), 21.1 (td, J(C,F) = 10.4 Hz, C(8′)), 12.4 (q, CH_3); ^{19}F NMR (CD_3OD, 376 MHz) δ −200.4 (m); ESI$^+$-HRMS m/z calcd for $C_{13}H_{16}FN_2O_5$ $[M + H]^+$ 299.1038, found 299.1037.

*(5′-O-((4,4′-Dimethoxytriphenyl)methyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)thymine (**10**).* To a solution of nucleoside **9** (428 mg, 1.44 mmol) in pyridine (20 mL) was added DMTrCl (1,46 g, 4,31 mmol) at rt, and the mixture was stirred for 2 days. Then reaction was diluted with sat. $NaHCO_3$ and extracted with EtOAc. The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was purified by CC (hexane/EtOAc 8:2 → EtOAc, 1% Et_3N) to give the title compound **10** (751 mg, 87%) as a yellowish foam.

Data for **10**. R_f = 0.38 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.62 (brs, 1H, NH), 8.04 (d, J = 0.8 Hz, 1H, H-C(6)), 7.47 (m, 2H, H-arom), 7.37 (dd, J = 8.9, 10.7 Hz, 4H, H-arom), 7.16 (m, 3H, H-arom), 6.75 (dd, J = 7.6, 8.9 Hz, 4H, H-arom), 5.76 (dd, J = 1.4, 6.5 Hz, 1H, H-C(1′)), 3.70 (s, 3H, OMe), 3.69 (s, 3H, OMe), 2.33 (dd, J = 6.5, 14.1 Hz, 1H, H_b-(2′)), 2.23 (m, 3H, H_a-C(2′)), H-C(4′), H_b-C(7′)), 2.09 (m, 1H, H_a-C(7′)), 1.98 (d, J = 0.8 Hz, 3H, CH_3), 1.71 (m, 1H, H_b-C(8′)), 0.84 (dd, J = 8.6, 9.7 Hz, 1H, H_a-C(8′)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.7 (s, CO), 158.95, 158.93 (2s, 2 × C-arom), 150.5 (s, CO), 145.8, 136.5, 136.4 (3s, 3 × C-arom), 136.3 (d, C-6), 131.2, 131.1, 128.8, 127.8, 127.2, 113.13, 113.08 (7d, 7 × C-arom), 110.3 (s, C(5)), 90.6 (dd, J(C,F) = 3.8 Hz, C(4′)), 88.82 (s), 88.81 (d, C(1′)), 83.3 (d, J(C,F) = 247.8 Hz, C(6′)), 80.5 (d, J(C,F) = 12.4 Hz, C(3′)), 64.9 (d, J(C,F) = 7.8 Hz, C(5′)), 55.4 (q, 2 × OMe), 47.9 (t, C(2′)), 44.0 (td, J(C,F) = 16.0 Hz, C(7′)), 20.2 (td, J(C,F) = 9.5 Hz, C(8′)), 12.5 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −193.4 (m); ESI$^+$-HRMS m/z calcd for $C_{34}H_{33}FN_2O_7Na$ $[M + Na]^+$ 623.2164, found 623.2150.

*(5′-O-(4,4′-Dimethoxytriphenyl)methyl)-3′-O-(2-cyanoethoxy)-diisopropylaminophosphanyl-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)thymine (**11**).* To a solution of compound **10** (4.24 g, 7.06 mmol) and diisopropylethylamine (4.67 mL, 28.24 mmol) in CH_3CN (142 mL) was added 2-cyanoethoxy-diisopropylaminochlorophosphine (3.94 mL, 17.65 mmol) at rt. After being stirred for 2 h at rt, the mixture was diluted with EtOAc and washed with sat. aq $NaHCO_3$. The aqueous phases were extracted with EtOAc, the combined organic phases were dried ($MgSO_4$) and evaporated, and the resulting crude product was purified by CC (hex/EtOAc 1:1, 1% NEt_3). The purified product was dissolved in CH_2Cl_2 (10 mL) and slowly added to ice-cold hexane (220 mL), and the precipitate was collected. This procedure was repeated 7× to yield the pure title compound **11** (3,96 g, 70%) as a white amorphous solid.

Data for **11**. R_f = 0.55 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.72 (brs, 1H, NH), 8.01 (dd, J = 1.0, 5.6 Hz, 1H, H-C(6)), 7.48 (d, J = 8.1 Hz, H-arom, 2H,), 7.38 (t, J = 9.0 Hz, 4H, H-arom), 7.18 (m, 3H, H-arom), 6.75 (dd, J = 6.9, 8.7 Hz, 4H, H-arom), 5.83 (dd, J = 1.9, 5.9 Hz, 1H, H-C(1′)), 3.72 (m, 6H, 2 × OMe), 3.63 (m, 1H, OCH_2), 3.50 (m, 1H, OCH_2), 3.37 (m, 2H, 2 × (Me_2CH)N), 2.76 (m, 1H, H_b-C(7′)), 2.62 (m, 1H, H_b-C2′)), 2.48 (t, J = 6.3 Hz, 2H, CH_2CN), 2.32 (m, 2H, H_a-C(2′), H-(4′)), 2.02 (m, 1H, H_a-C(7′)), 2.02 (s, 3H, CH_3), 1.72 (m, 1H, H_b-C(8′)), 1.01 (m, 12H, 2 × $(CH_3)_2$CHN), 0.84 (m, 1H, H_a-C(8′)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.29, 164.26 (s, CO), 159.0 (m, 2 × C-arom), 150.1 (s, CO), 145.8, 145.7 (s, C-arom), 136.5 (m, C-arom), 136.0 (d, C(6)), 131.2, 131.0, 128.8, 127.8, 127.3, 127.2, (6d, 6 × C-arom), 117.6, 117.5 (2s, CN), 113.14, 113.10 (2d, 2 × C-arom), 110.22, 110.19 (2s, C(5)), 91.3 (d, C(4′)), 89.5, 89.3 (2d, C(1′)), 89.0, 88.9 (2s), 83.8, 83.7 (2d, J(C,F) = 11.6 and 12.4 Hz C-(3′)), 83.3, 83.20 (2d, J(C,F) = 248.0, Hz C(6′)), 64.5 (m, C(5′)), 58.0, 57.7 (2td, J(C,P) = 19.5 Hz, OCH_2), 55.4, 55.3 (2q, 2 × OMe), 45.9, 45.4 (2td, J(C,P) = 9.7 and 12.9 Hz, C(2′)), 43.4, 43.3 (2dd, J(C,P) = 6.7 Hz, Me_2CH), 42.1 (m, C(7′)), 24.50, 24.45, 24.42, 24.37 (4q, Me_2CH), 20.5, 20.4 (2td, CH_2CN J(C,P) = 3.6 Hz, 4.1 Hz), 20.0 (m, C(8′)), 12.5 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −193.8 (m), −193.6 (m); ^{31}P NMR ($CDCl_3$, 161 MHz): 145.0, 142.9; ESI$^+$-HRMS m/z calcd for $C_{43}H_{50}FN_4O_8PNa$ $[M + Na]^+$ 823.3243, found 823.3276.

*(5′-O-(tert-Butyldimethylsilyl)- 3′-O-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-4-(1H-1,2,4-triazol-1-yl)thymine (**12**).* A suspension of 1,2,4-triazole (19.81 g, 0.29 mol) in CH_3CN was cooled to 0 °C and treated consecutively with $POCl_3$ (2.97 mL, 31.87 mmol) and Et_3N (40.9 mL, 293.25 mmol). The resulting mixture was stirred for 50 min before compound **8** (6.18 g, 12.75 mmol), dissolved in CH_3CN (105 mL), was added. After completion (TLC control, 3.5 h), the reaction was quenched with sat. aq $NaHCO_3$ (200 mL). The ice bath was removed, and the mixture was reduced to half of the volume in vacuo. Then EtOAc (200 mL) was added, and the volume was again reduced to one-third. After being poured onto H_2O/sat. aq NaCl 1:1, the resulting mixture was extracted with EtOAc. The combined organic phases were dried ($MgSO_4$) and evaporated. The crude compound **12** was used directly in the next step without further purification. For analytical data, a sample was purified by CC (hexane/EtOAc 50:50).

Data for **12**. R_f = 0.35 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.28 (s, 1H, H-C(5″)), 8.53 (s, 1H, H-C(6)), 8.11 (s, 1H, H-C(3″)), 6.18 (t, J = 3.9 Hz, 1H, H-C(1′)), 4.18 (d, J = 5.6 Hz, 1H, H-C(4′)), 2.76 (d, J = 3.9 Hz, 2H, H-C(2′)), 2.45 (s, 3H, CH_3), 2.34 (dd, J = 2.3, 13.9 Hz, 1H, H_b-C(7′)), 1.85 (m, 1H, H_a-C(7′)), 1.44 (ddd, J = 2.3, 7.7, 21.0 Hz, 1H, H_b-C(8′)), 1.20 (dd, J = 7.7, 9.1 Hz, 1H, H_a-C(8′)), 0.96 (s, 9H, *t*-Bu), 0.27, 0.22 (2s, 2 × 3H, 2 × CH_3), 0.13 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.6 (s, C(4)), 154.0 (s, CO), 153.6 (d, C(3″)), 147.6 (d, C(6)), 145.2 (d, C(5″)), 106.0 (s, C(5)), 93.5 (dd, J(C,F) = 3.9 Hz, C(4′)), 91.6 (d, C(1′)), 82.3 (d, J(C,F) = 12.1 Hz, C(3′)), 81.3 (d, J(C,F) = 249.3 Hz, C(6′)), 63.3 (d, J(C,F) = 8.1 Hz, C(5′)), 46.4 (t, C(2′)), 45.0 (td, J(C,F) = 14.7 Hz, C(7′)), 25.7 (q, *t*-Bu), 20.2 (td, J(C,F) = 10.0 Hz, C(8′)),

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18.0 (s, *t*-Bu), 16.9 (q, CH_3), 2.0 (q, TMS), −3.7 (q, CH_3), −3.8 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.7 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{24}H_{39}FN_5O_4Si_2$ $[M + H]^+$ 536.2519, found 536.2503.

*(5′-O-tert-Butyldimethylsilyl)-3′-O-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**13**).* To a solution of crude compound **12** from the previous step (10 g), dissolved in 1,4-dioxane (110 mL), was added concd NH_4OH (110 mL), and the mixture was stirred for 2 h at rt. The solvent was evaporated and the residue dissolved in EtOAc and extracted with H_2O and sat. NaCl. The aqueous phases were extracted with EtOAc and the combined organic layers dried ($MgSO_4$) and evaporated. The residue was purified by CC (EtOAc → EtOAc/EtOH 9:1) to yield the title compound **13** (3.70 g, 60%) as a white foam.

Data of **13**. R_f = 0.48 (EtOAc/EtOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92 (d, *J* = 0.7 Hz, 1H, H-C(6)), 6.08 (m, 1H, H-C(1′)), 4.12 (m, 1H, H-C(4′)), 2.64 (d, *J* = 3.4 Hz, 2H, H-C(2′)), 2.28 (dd, *J* = 2.0, 13.7 Hz, 1H, H_b-C(7′)), 1.99 (m, 1H, H_a-C(7′)), 1.94 (d, *J* = 0.7 Hz, 3H, CH_3), 1.37 (ddd, *J* = 2.4, 7.6, 21.0 Hz, 1H, H_b-C(8′)), 1.17 (dd, *J* = 7.6, 9.1 Hz, 1H, H_a-C(8′)), 0.94 (s, 9H, *t*-Bu), 0.24, 0.19 (2s, 2 × 3H, 2 × CH_3), 0.10 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1 (s, C(4)), 156.3 (s, CO), 139.0 (d, C(6)), 101.6 (s, C(5)), 92.9 (dd, *J*(C,F) = 3.9 Hz, C(4′)), 90.3 (d, C(1′)), 82.3 (d, *J*(C,F) = 12.3 Hz, C(3′)), 81.5 (d, *J*(C,F) = 249.0 Hz, C(6′)), 63.3 (d, *J*(C,F) = 8.0 Hz, C(5′)), 46.9 (t, C(2′)), 44.7 (td, *J*(C,F) = 14.7 Hz, C(7′)), 25.7 (q, *t*-Bu), 20.2 (td, *J*(C,F) = 9.9 Hz, C(8′)), 18.0 (s, *t*-Bu), 13.0 (q, CH_3), 2.0 (q, TMS), −3.7, −3.8 (2q, 2 × CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −198.0 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{22}H_{39}FN_3O_4Si_2$ $[M + H]^+$ 484.2458, found 484.2450.

*N^4-Benzoyl-1-(5′-O-(tert-butyldimethylsilyl)-3′-O-(trimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**14**) and N^4-Benzoyl-1-(5′-O-(tert-butyldimethylsilyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**15**).* To a solution of nucleoside **13** (492 mg, 1.01 mmol) and DMAP (25 mg, 0.20 mmol) in CH_3CN (20 mL) was added Bz_2O (253 mg, 1.12 mmol), and the mixture was stirred for 1.5 h at rt. Then Et_3N (0.28 mL, 2.03 mmol) was added and the mixture stirred overnight. After evaporation of the solvents, the residue was dissolved in EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$) and evaporated. The crude product was purified by CC (hexane/EtOAc 95:5 → Hex:EtOAc 50:50) to give compound **14** (39%) as a white foam and compound **15** (54%) as a white solid.

Data for **14**. R_f = 0.62 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.46 (s, 1H, NH), 8.32 (d, *J* = 7.3 Hz, 2H, H-arom), 8.03 (m, 1H, H-C(6)), 7.52 (m, 1H, H-arom), 7.44 (m, 2H, H-arom), 6.09 (dd, *J* = 1.1, 6.5 Hz, 1H, H-C(1′)), 4.11 (d, *J* = 5.6 Hz, 1H, H-C(4′)), 2.67 (dd, *J* = 6.5, 13.7 Hz, 1H, H_b-C(2′)), 2.60 (dd, *J* = 1.1, 13.7 Hz, 1H, H_a-C(2′)), 2.38 (dd, *J* = 1.9, 13.7 Hz, 1H, H_b-C(7′)), 2.12 (d, *J* = 0.9 Hz, 3H, CH_3), 2.11 (m, 1H, H_a-C(7′)), 1.43 (ddd, *J* = 2.4, 7.7, 21.0 Hz, 1H, H_b-C(8′)), 1.20 (dd, *J* = 7.7, 9.0 Hz, 1H, H_a-C(8′)), 0.97 (s, 9H, *t*-Bu), 0.26, 0.22 (2s, 2 × 3H, 2 × CH_3), 0.14 (s, 9H, TMS); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.8 (s, C(4)), 160.4 (s, CO), 147.9 (s, CO), 137.49 (s, C-arom), 137.45 (d, C(6)), 132.4, 130.0, 128.2 (3d, 3 × C-arom), 111.4 (s, C(5)), 93.2 (dd, *J*(C,F) = 4.0 Hz, C(4′)), 90.1 (d, C(1′)), 82.3 (d, *J*(C,F) = 12.3 Hz, C(3′)), 81.4 (d, *J*(C,F) = 249.5 Hz, C(6′)), 63.2 (d, *J*(C,F) = 8.1 Hz, C(5′)), 47.1 (t, C(2′)), 44.8 (td, *J*(C,F) = 14.9 Hz, C(7′)), 25.7 (q, *t*-Bu), 20.3 (td, *J*(C,F) = 10.1 Hz, C(8″)), 18.0 (s, *t*-Bu), 13.4 (q, CH_3), 2.0 (q, TMS), −3.7 (q, CH_3), −3.8 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.9 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{29}H_{43}FN_3O_5Si_2$ $[M + H]^+$ 588.2720, found 588.2714.

Data for **15**. R_f = 0.30 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (m, 2H, H-arom), 8.07 (d, *J* = 0.8 Hz, 1H, H-C(6)), 7.48 (m, 4H, H-arom, NH), 6.09 (dd, *J* = 2.3, 5.6 Hz, 1H, H-C(1′)), 4.13 (d, *J* = 5.5 Hz, 1H, H-C(4′)), 2.64 (m, 2H, H-C(2′)), 2.35 (dd, *J* = 1.6, 13.9 Hz, 1H, H_b-C(7′)), 2.20 (m, 1H, H_a-C(7′)), 2.12 (d, *J* = 0.8 Hz, 3H, CH_3), 1.46 (ddd, *J* = 2.4, 7.8, 21.0 Hz, 1H, H_b-C(8′)), 1.28 (m, 1H, H_a-C(8′)), 0.97 (s, 9H, *t*-Bu), 0.25, 0.21 (2s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.4 (s, C(4)), 160.5 (s, CO), 148.2 (s, CO), 137.9 (d, C(6)), 137.2 (s, C-arom), 132.6, 123.0, 128.3 (3d, 3 × C-arom), 111.5 (s, C(5)), 91.8 (dd, *J*(C,F) = 3.8 Hz, C(4′)), 90.0 (d, C(1′)), 81.5 (d, *J*(C,F) = 249.1 Hz, C(6′)), 80.7 (d, *J*(C,F) = 12.0 Hz, C(3′)), 63.5 (d, *J*(C,F) = 8.2 Hz, C(5′)), 47.9 (t, C(2′)), 44.8 (td, *J*(C,F) = 15.8 Hz, C(7′)), 25.7 (q, *t*-Bu), 20.5 (td, *J*(C,F) = 10.1 Hz, C(8′)), 18.0 (s, *t*-Bu), 13.4 (q, CH_3), −3.7 (q, CH_3), −3.8 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −197.6 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{26}H_{35}FN_3O_5Si$ $[M + H]^+$ 516.2325, found 516.2328.

*N^4-Benzoyl-1-(2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**16**).* To separate solutions of nucleosides **14** and **15** (1.85 g, 3.15 mmol and 1.24 g, 2.41 mmol, respectively) and pyridine (9.6 and 6.0 mL, respectively) in CH_2Cl_2 (40 and 30 mL, respectively) was added HF−pyridine (3.3 and 2.1 mL, respectively) at 0 °C. After the mixture was stirred for 24 h at rt, silica gel (1 g per 300 mg starting material) was added, and the mixture was stirred for another 15 min. After evaporation, the adsorbed products were purified by CC (EtOAc) to give the title compound **16** (1.13 g from **14**, 867 mg from **15**, 90% together) as white foams.

Data for **16**. R_f = 0.48 (EtOAc); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.23 (m, 2H, H-arom), 8.11 (m, 1H, H-C(6)), 7.58 (m, 1H, H-arom), 7.47 (m, 2H, H-arom), 6.15 (dd, *J* = 3.1, 7.0 Hz, 1H, H-C(1′)), 4.08 (d, *J* = 5.6 Hz, 1H, H-C(4′)), 2.65 (dd, *J* = 7.0, 14.0 Hz, 1H, H_b-(2′)), 2.53 (dd, *J* = 3.1, 14.0 Hz, 1H, H_a-C(2′)), 2.32 (m, 2H, H-C(7′)), 2.14 (d, 3H, *J* = 0.9 Hz, CH_3), 1.45 (dd, *J* = 7.5, 20.9 Hz, 1H, H_b-C(8′)), 1.31 (t, *J* = 7.5 Hz, 1H, H_a-C(8′)); ^{13}C NMR (CD_3OD, 75 MHz) δ 162.1 (s, C(4)), 148.0 (s, CO), 143.4 (s, CO), 137.7 (d, C(6)), 137.6 (s, C-arom), 133.6, 130.5, 129.3 (3d, 3 × C-arom), 112.0 (s, C(5)), 91.9 (dd, *J*(C,F) = 3.7 Hz, C(4′)), 90.2 (d, C(1′)), 84.3 (d, *J*(C,F) = 248.1 Hz, C(6′)), 80.8 (d, *J*(C,F) = 12.0 Hz, C(3′)), 63.4 (d, *J*(C,F) = 8.5 Hz, C(5′)), 48.2 (t, C(2′)), 45.2 (td, *J*(C,F) = 15.5 Hz, C(7′)), 21.1 (td, *J*(C,F) = 10.3 Hz, C(8′)), 13.8 (q, CH_3); ^{19}F NMR (CD_3OD, 376 MHz) δ −200.4 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{20}H_{21}FN_3O_5$ $[M + H]^+$ 402.1460, found 402.1461.

*N^4-Benzoyl-1-(5′-O-((4,4′dimethoxytriphenyl)methyl)-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**17**).* To a stirred solution of compound **16** (606 mg, 1.51 mmol) in pyridine (20 mL) was added DMTrCl (1.54 g, 4.53 mmol) at rt. After 2 days, the mixture was poured onto sat. aq $NaHCO_3$ and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and evaporated, and the crude material was purified by CC (hexane/EtOAc 8:2 → 6:4, +0,2% Et_3N) to give the title compound **17** (843 mg, 79%) as a yellowish foam.

Data for **17**. R_f = 0.44 (hexane/EtOAc 1:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.37 (brs, 1H, NH), 8.26 (m, 2H, H-arom, H-C(6)), 7.42 (m, 9H, H-arom), 7.20 (m, 4H, H-arom), 6.77 (dd, *J* = 7.6, 9.0 Hz, 4H, H-arom), 5.85 (dd, *J* = 1.5, 6.4 Hz, 1H, H-C(1′)), 3.73 (s, 3H, OMe), 3.72 (s, 3H, OMe), 2.39 (dd, *J* = 1.5, 14.1 Hz, 1H, H_b-C(2′)), 2.33 (dd, *J* = 6.4, 14.1 Hz, 1H, H_a-C(2′)), 2.22 (d, *J* = 0.7 Hz, 3H, CH_3), 2.21 (m, 2H, H-C(4′), H_b-C(7′)), 2.11 (m, 1H, H_a-C(7′)), 1.77 (ddd, *J* = 2.1, 8.3, 20.4 Hz, 1H, H_b-C(8′)), 1.56 (brs, 1H, OH), 0.85 (dd, *J* = 8.3, 9.6 Hz, 1H, H_a-C(8″)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.3 (s, C(4)), 160.4 (s, CO), 159.08, 159.06 (2s, 2 × C-arom), 147.9 (s, CO), 145.9 (s, C-arom), 137.5 (d, C(6)), 137.4, 136.44, 136.37 (3s, 3 × C-arom), 132.6, 131.2, 131.1, 130.0, 128.9, 128.3, 127.9, 127.4, 113.19, 113.15 (10d, 10 × C-arom), 111.1 (s, C(5)), 90.9 (dd, *J*(C,F) = 4.1 Hz, C(4′)), 89.4 (d, C(1′)), 89.0 (s), 83.2 (d, *J*(C,F) = 248.1 Hz, C(6′)), 80.8 (d, *J*(C,F) = 12.2 Hz, C(3′)), 64.9 (d, *J*(C,F) = 7.9 Hz, C(5′)), 55.4 (q, 2 × OMe), 47.9 (t, C(2′)), 44.2 (td, *J*(C,F) = 16.2 Hz, C(7′)), 20.2 (td, *J*(C,F) = 9.7 Hz, C(8′)), 13.6 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −193.5 (m); ESI$^+$-HRMS *m*/*z* calcd for $C_{41}H_{39}FN_3O_7$ $[M + H]^+$ 704.2767, found 704.2771.

*N^4-Benzoyl-1-(5′-O-((4,4′-dimethoxytriphenyl)methyl)- 3′O-(2-cyanoethoxy)diisopropylaminophosphanyl-2′-deoxy-3′,5′-ethano-6′-fluoro-5′,6′-methano-β-D-ribofuranosyl)-5-methylcytosine (**18**).* To a solution of compound **17** (2.37 g, 3.37 mmol) and diisopropylethylamine (2.23 mL, 13.47 mmol) in CH_3CN (50 mL) was added (2-cyanoethoxy)diisopropylaminochlorophosphine (1.88 mL, 8.42 mmol). After being stirred for 2 h at rt, the mixture was diluted with EtOAc and washed with sat. aq $NaHCO_3$. The aqueous phases were extracted with EtOAc, the combined organic phases were dried ($MgSO_4$) and evaporated, and the crude product was purified by

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CC (hexane/EtOAc 1:1, +1% NEt_3) to give the title compound **18** (2.78 g, 90%) as a yellowish foam.

Data for **18**. R_f = 0.71 (hexane/EtOAc 1:3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 13.39 (brs, 1H, NH), 8.25 (m, 3H, H-arom, H-C(6)), 7.43 (m, 9H, H-arom), 7.18 (m, 3H, H-arom), 6.76 (m, 4H, H-arom), 5.88 (d, J = 6.8 Hz, 1H, H-C(1′)), 3.72 (m, 6H, 2 × OMe), 3.62 (m, 1H, OCH_2), 3.49 (m, 1H, OCH_2), 3.36 (m, 2H, 2 × $(Me_2CH)N$), 2.76 (m, 1H, H_b-C(7′)), 2.62 (m, 2H, H-C(2′)), 2.47 (m, 2H, CH_2CN), 2.36 (m, 1H, H-C(4′)), 2.21 (s, 3H, CH_3), 2.01 (m, 1H, H_a-C(7′)), 1.74 (m, 1H, H_b-C(8′)), 1.01 (m, 12H, 2 × $(CH_3)_2CHN$), 0.85 (m, 1H, H_a-C(8′)); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.7 (s, C(4)), 160.4 (s, CO), 159.1, 159.04, 159.02, 159.01 (4s, 4 × C-arom), 147.8 (s, CO), 145.70, 145.65 (2s, C-arom), 137.4 (d, C(6)), 136.43, 136.39 136.32 (3s, 3 × C-arom), 132.4, 131.2, 131.0, 130.0, 128.8, 128.2, 127.9, 127.3, 127.2, (9d, 9 × C-arom), 117.5, 117.4 (2s, CN), 113.2, 113.1 (2d, 2 × C-arom), 111.31, 111.27 (2s, C(5)), 91.5 (md, C(4′)), 90.0, 89.9 (2d, C(1′)), 89.03, 88.98 (2s), 83.7 (m, C(3′)), 83.3, 83.2 (2d, J(C,F) = 248.6 Hz, C(6′)), 64.63, 64.55 (2d, J(C,F) = 3.6 Hz, C(5′)), 58.0, 57.7 (2td, J(C,P) = 19.4 Hz, OCH_2), 55.34, 55.30 (2q, 2 × OMe), 45.7, 45.2 (2td, J(C,P) = 9.7, 12.9 Hz, C(2′)), 43.4, 43.3 (2dd, J(C,P) = 12.6 Hz, 2 × Me_2CH), 42.2, 42.1 (2td, J (C,F) J (C,P) = 10.5, 12.3 Hz, C(7′)), 24.53, 24.47, 24.40, 24.35 (4q, 2 × Me_2CH), 20.4, 20.3 (2td J(C,P) = 2.0, 2.7 Hz, CH_2CN), 20.0, 19.9 (2td, J(C,F) = 10.2 Hz, C(8′)), 13.5 (q, CH_3); ^{19}F NMR ($CDCl_3$, 376 MHz) δ −193.7 (m), −193.5 (m); ^{31}P NMR ($CDCl_3$, 161 MHz) d 145.1, 143.0; ESI$^+$-HRMS m/z calcd for $C_{50}H_{56}FN_5O_8P$ $[M + H]^+$ 904.3845, found 904.3846.

Oligonucleotide Synthesis and Purification. Oligonucleotides **ON1**−**10** were synthesized by standard solid-phase phosphoramidite methodology on a 1.3 μmol scale on a Pharmacia LKB Gene Assembler Special DNA Synthesizer using a slightly modified DNA synthesis program. Natural phosphoramidites (dT, dC^4Bz, dA^6Bz, dG^2dmf) were coupled as a 0.1 M solution in CH_3CN, and tricyclophosphoramidites as 0.15 M solutions in CH_3CN, with the exception of 6′-F-tc-T, 6′-F-tc-$^{5Me}C^4Bz$, and tc-A that were used as 0.15 M solutions in DCE. The coupling step was 90 s for natural phosphoramidites and 12 min for tricyclo-phosphoramidites. As coupling reagent, 5-(ethylthio)-1*H*-tetrazole (0.25 M in CH_3CN) was used. Capping, oxidation, and detritylation were carried out using standard solutions as described in the manufacturer's protocol. Deprotection of the oligonucleotides after assembly and detachment from solid support was effected by standard ammonia treatment (33% aq NH_3, 16 h, 55 °C). The crude oligomers were purified by ion-exchange HPLC using a DNAPAC PA200, 4 × 250 mm analytical column (Dionex). Mobile phases A: 25 mM TRIZMA in H_2O, pH 8.0. B: 25 mM TRIZMA, 1.25 M NaCl in H_2O, pH 8.0. or A: 10 mM NaOH in H_2O, pH 12.0. B: 10 mM NaOH, 1.5 M NaCl in H_2O, pH 12.0, flow 1 mL/min detection at 260 nm. Purified oligonucleotides were desalted over Sep-Pak cartridges, quantified at 260 nm using extiction coefficients as determined previously for tricyclo-nucleosides,[31] and analyzed by ESI$^-$-mass spectrometry. Oligonucleotides were then stored at −18 °C.

UV Melting Curves. 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 derivative curves using Varian WinUV software. To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out in 150 mM NaCl, 10 mM Na-phosphate, pH 7.0, with duplex concentration of 2 μM.

CD-Spectroscopy. CD-spectra were recorded using the same buffer conditions and oligonucleotide concentrations as for UV melting curves. All CD-spectra were collected at 20 °C between 210 to 320 nm at a 50 nm/min rate and were baseline-corrected against buffer. The reported spectra correspond to the average of at least three scans.

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra of compounds **2**−**18** and X-ray structural data (CIF) of compound **16**. 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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