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Duplex DNA and DNA–RNA Hybrids with Parallel Strand Orientation: 2′-Deoxy-2′-fluoroisocytidine, 2′-Deoxy-2′-fluoroisoguanosine, and Canonical Nucleosides with 2′-Fluoro Substituents Cause Unexpected Changes on the Double Helix Stability

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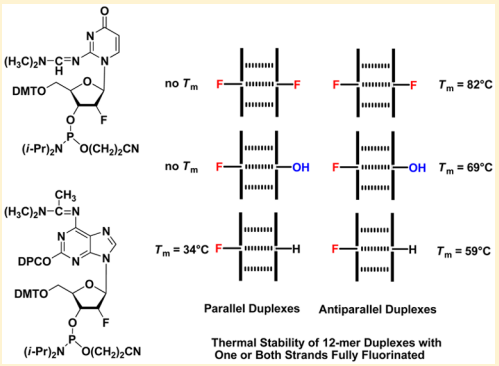
Supporting Information

ABSTRACT: Oligonucleotides with parallel or antiparallel strand orientation incorporating 2′-fluorinated 2′-deoxyribonucleosides with canonical nucleobases or 2′-deoxy-2′-fluoroisocytidine (^{F}iC$_d$, **1c**) and 2′-deoxy-2′-fluoroisoguanosine (^{F}iG$_d$, **3c**) were synthesized. To this end, the nucleosides **1c** and **3c** as well as the phosphoramidite building blocks **19** and **23** were prepared and employed in solid-phase oligonucleotide synthesis. Unexpectedly, ^{F}iC$_d$ is not stable during oligonucleotide deprotection (55 °C, aq NH$_3$) and was converted to a cyclonucleoside (**14**). Side product formation was circumvented when oligonucleotides were deprotected under mild conditions (aq ammonia–EtOH, rt). Oligonucleotides containing 2′-fluoro substituents (^{F}iC$_d$, ^{F}iG$_d$ and fluorinated canonical 2′-deoxyribonucleosides) stabilize double-stranded DNA, RNA, and DNA–RNA hybrids with antiparallel strand orientation. Unexpected strong stability changes are observed for oligonucleotide duplexes with parallel chains. While fluorinated oligonucleotides form moderately stable parallel stranded duplexes with complementary DNA, they do not form stable hybrids with RNA. Furthermore, oligoribonucleotide duplexes with parallel strand orientation are extremely unstable. It is anticipated that nucleic acids with parallel chains might be too rigid to accept sugar residues in the N-conformation as observed for ribonucleosides or 2′-deoxy-2′-fluororibonucleosides. These observations might explain why nature has evolved the principle of antiparallel chain orientation and has not used the parallel chain alignment.



INTRODUCTION

DNA with parallel strand (ps) orientation can be constructed from any single-stranded DNA of natural or artificial origin when the complementary strand consists of the four nucleosides 2′-deoxyisocytidine (iC$_d$, **1a**) or its 5-methyl derivative (^{Me}iC$_d$, **2a**), 2′-deoxyisoguanosine (iG$_d$, **3a**), dA, and dT (Figure 1).[1,2] Parallel-stranded DNA is remarkably stable but shows a lower stability than DNA with antiparallel strand (aps) orientation.[3] From that, it was concluded that the lower stability of ps DNA results from the low strength of the reverse Watson–Crick dA–dT pair (Donohue pair). Incorporation of iG$_d$–dC or iC$_d$–dG pairs in ps DNA can compensate the loss of stability.[1] Furthermore, nucleoside shape mimics with a pyrrolo[2,3-*d*]pyrimidine or a pyrazolo[3,4-*d*]pyrimidine skeleton replacing the nucleobases of dA or dT and iG$_d$ or iC$_d$ have been shown to be well accommodated in ps DNA and add additional stability to the parallel helix structure.[4]

Previously, the beneficial influence of fluorine substituents to antiviral nucleosides and to antisense oligonucleotides with antiparallel strand orientation was demonstrated.[5,6] The high fluorine–carbon bond stability (109 kcal/mol)[7] makes fluorine substituents rather resistant to metabolic transformations. Furthermore, the fluorine atom introduces lipophilicity into the molecule.[8] The target site of the fluoro modification in antisense oligonucleotides is usually the 2′-position of the sugar moiety with the fluorine substituent in the "ribo" (down) configuration as shown for **1b–3b** and **1c–3c** (Figure 1). The hydrophobic, but still polar, fluorine substituent has approximately the same atomic radius as hydrogen and is well accommodated in DNA and DNA–RNA hybrids.[9] Nevertheless, the van der Waals radius of fluorine is larger (1.47 Å) than that of hydrogen (1.20 Å) but similar to that of oxygen (1.52 Å).[10] Apart from that, fluorine has the highest electronegativity of a substituent and has therefore a great impact on the sugar conformation. The replacement of the 2′-OH group of the ribose moiety by a fluorine atom leads to a preferred 3′-*endo* (N) conformation of the sugar residue, which

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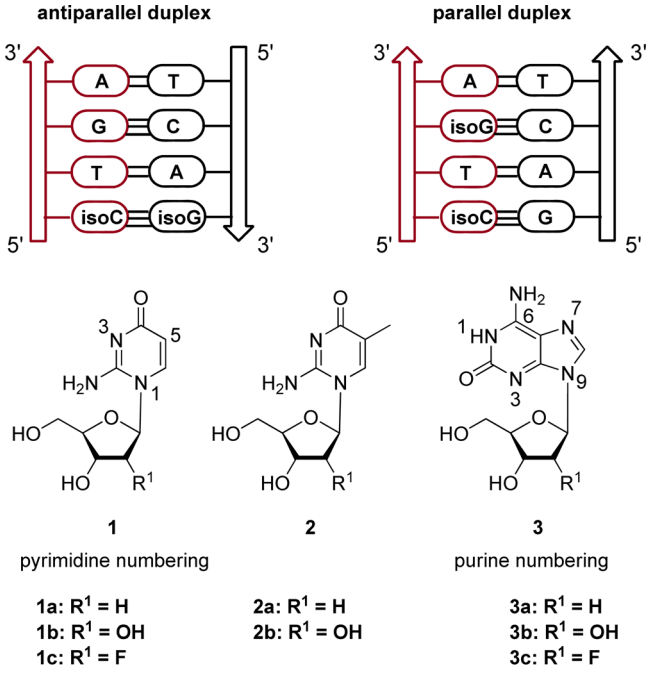


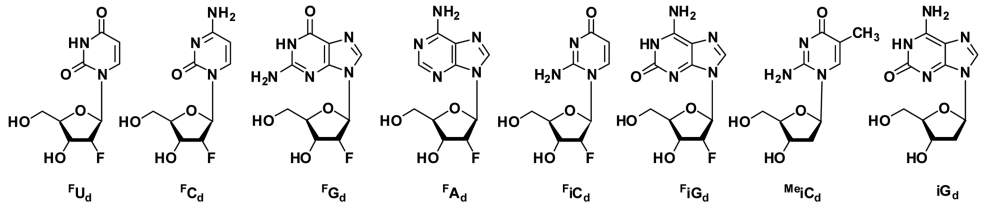
Figure 1. Motifs of strand orientation and structures of isocytosine and isoguanine nucleosides.

is the conformation of RNA duplexes.[11] In the antisense approach, entirely 2′-deoxy-2′-fluoro-modified oligonucleotides, which adopt an A conformation, show a high binding affinity to their RNA target.[9a] It was assumed that the increased pairing affinity of 2′-F-RNA compared to RNA results from a positive enthalpic effect going back to strengthening of H-bonding, stacking interactions, and a higher rigidity of the duplex backbone induced by the electron-withdrawing effect of fluorine in the 2′-position.[6b] Another interesting property of fluorine is the ease of detection by ^{19}F NMR spectroscopy and the use of the radiolabeled ^{18}F isotope as tracer in in PET imaging.[12]

In this study, the concept of parallel strand orientation which was already realized by using isocytosine nucleosides (**1a**,**b**, **2a**,**b**)[13] and isoguanine nucleosides (**3a**,**b**)[14] (Figure 1) is now combined with the unique properties of 2′-fluoro substitution, such as enhanced binding affinity and nuclease resistance. To construct parallel-stranded DNA with 2′-fluoro substituents, building blocks (phosphoramidites) of 2′-deoxy-2′-fluoroisocytidine (^{F}iC$_d$, **1c**) and 2′-deoxy-2′-fluoroisoguanosine (^{F}iG$_d$, **3c**) were prepared and applied to automatized solid-phase oligonucleotide synthesis. To this end, the precursor nucleosides 2′-deoxy-2′-fluoroisocytidine and 2′-deoxy-2′-fluoroisoguanosine were synthesized. The base-pairing properties of ^{F}iC$_d$ and ^{F}iG$_d$ in DNA duplexes with parallel chain orientation are studied and compared with those in DNA duplexes with antiparallel chains. Hybridization experiments are performed

Table 1. Synthesized Oligonucleotides and Their Molecular Masses Measured by MALDI-TOF Mass Spectra

Oligonucleotides	M.W. calcd.[a] M.W. found[b]	Oligonucleotides	M.W. calcd.[a] M.W. found[b]
5'- (AGUAUUGACCUA) (**26**)	3781.5 3781.6	5'-d(A^{F}**iG**TATT^{F}**iG**ACCTA) (**36**)	3681.4 3680.9
5'-d(TA^{F}**G**^{F}**G**TCAATACT) (**27**)	3681.4 3682.3	5'-d(TA**iGiG**T^{Me}**iC**AATA^{Me}**iC**T) (**37**)	3673.5 3673.6
5'-d(^{F}U^{F}A^{F}G^{F}G^{F}U^{F}C^{F}A^{F}A^{F}U^{F}A^{F}C^{F}U) (**28**)	3805.3 3805.0	5'-d(A^{F}**iG**TATT^{F}**iG**A^{F}**iC**^{F}**iC**TA) (**38**)	3717.4 3716.1
5'-d(^{F}A^{F}G^{F}U^{F}A^{F}U^{F}U^{F}G^{F}A^{F}C^{F}C^{F}U^{F}A) (**29**)	3805.3 3807.5	5'-d(^{F}U^{F}A^{F}iG^{F}iG^{F}U^{F}iC^{F}A^{F}A^{F}U^{F}A^{F}iC^{F}U) (**39**)	3805.3 3804.9
5'- (UAGGUCAAUACU) (**30**)	3781.5 3781.3	5'-d(A**iG**TATT**iG**A^{Me}**iC**^{Me}**iC**TA) (**40**)	3673.5 3674.0
5'-d(TA**iGiG**TCAATACT) (**31**)	3645.4 3644.9	5'-d(AT^{Me}**iC**^{Me}**iC**A**iG**TTAT**iG**A) (**41**)	3673.5 3673.3
5'-d(AGTATTGA^{Me}**iC**^{Me}**iC**TA) (**32**)	3673.5 3674.4	5'-d(ATCCAGTTATGA) (**42**)	3645.5 3645.9
5'-d(TA^{F}**iG**^{F}**iG**TCAATACT) (**33**)	3681.4 3682.3	5'- (UCAUAACUGGAU) (**43**)	3781.5 3780.6
5'-d(AGTATTGA^{F}**iC**^{F}**iC**TA) (**34**)	3681.4 3680.6	5'- (AUCCAGUUAUGA) (**44**)	3781.5 3781.1
5'-d(TAGGT^{Me}**iC**AATA^{Me}**iC**T) (**35**)	3673.5 3671.9	5'-d(^{F}A^{F}U^{F}C^{F}C^{F}A^{F}G^{F}U^{F}U^{F}A^{F}U^{F}G^{F}A) (**45**)	3805.3 3807.5



[a]Calculated on the basis of molecular weight as $[M + H]^+$. [b]Determined by MALDI-TOF mass spectrometry as $[M + H]^+$ in the linear positive mode.

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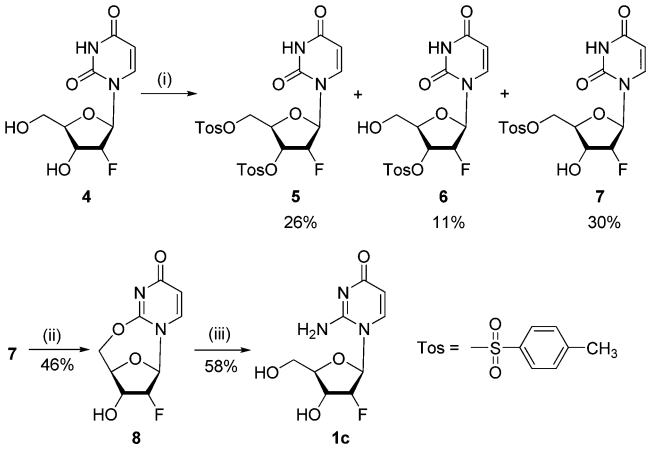
with complementary strands of DNA, RNA, and 2′-F-RNA, and the effect of the 2′-fluorine substituent is related to non-fluorinated oligonucleotides.

RESULTS AND DISCUSSION

1. Synthesis of 2′-Deoxy-2′-fluororibonucleosides. 2′-Deoxy-2′-fluororibonucleosides with canonical nucleobases are known for several decades ($^{F}A_{d}$,[15] $^{F}U_{d}$,[16] $^{F}G_{d}$[17] and $^{F}C_{d}$;[18] for structures see Table 1). Corresponding phosphoramidites were employed in oligonucleotide synthesis (for structures see Figure S1, Supporting Information).[9a,19] Several reviews on this matter appeared in the literature.[20] To the best of our knowledge, synthetic routes to access 2′-deoxy-2′-fluoroisocytidine (**1c**) and 2′-deoxy-2′-fluoroisoguanosine (**3c**) have not been reported before.

2′-Deoxy-2′-fluoroisocytidine (1c). The synthesis of 2′-deoxy-2′-fluoroisocytidine (**1c**) uses 2′-deoxy-2′-fluorouridine (**4**) as starting material (Scheme 1). This route employs similar

Scheme 1. Synthesis of 2′-Deoxy-2′-fluoroisocytidine (1c) via Tosylation of 2′-Deoxy-2′-fluorouridine (4)[a]

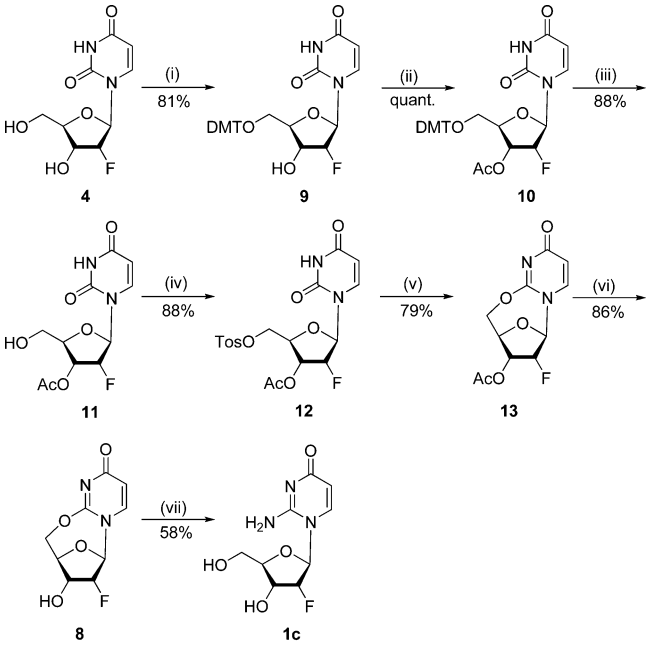


[a]Reagents and conditions: (i) *p*-toluenesulfonyl chloride, pyridine, 16 h, rt; (ii) DBU, CH_3CN, reflux, 30 min; (iii) saturated NH_3/MeOH, rt, overnight.

reaction steps as described for 2′-deoxyisocytidine.[13d,21] To this end, nucleoside **4** was tosylated with *p*-toluenesulfonyl chloride in pyridine. Different to the reaction of 2′-deoxyuridine which yields the 5′-*O*-tosylated compound as major product,[21] tosylation of 2′-deoxy-2′-fluorouridine (**4**) furnished a mixture of the 5′-*O*-tosylated compound **7** (30%) together with the 3′-*O*-derivative **6** (11%) and the 3′,5′-bis-*O*-tosylated nucleoside **5** (26%). The reaction products were separated by flash column chromatography and characterized by NMR spectra and ESI-TOF mass spectra. Modified reaction conditions did not lead to an improved yield of nucleoside **7**. Next, the 5′-*O*-tosylated **7** was treated with DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) in dry CH_3CN under reflux conditions for 30 min (TLC monitoring) affording **8** in 46% yield. Finally, treatment of the 2,5′-anhydro nucleoside **8** with saturated NH_3/MeOH at room temperature overnight furnished 2′-deoxy-2′-fluoroisocytidine (**1c**) in 58% yield (Scheme 1; for details, see the Experimental Section).

As the yield for the 5′-*O*-tosylated nucleoside **7** was unsatisfactory, an alternative route was developed (Scheme 2). To this end, the 3′- and 5′-sugar hydroxyl groups of nucleoside **4** were transiently protected.[22] A DMT residue was

Scheme 2. Synthesis of 2′-Deoxy-2′-fluoroisocytidine (1c) via the Sugar Protection and Deprotection Method[a]



[a]Reagents and conditions: (i) DMT-Cl, pyridine, rt; (ii) acetic anhydride, pyridine, rt; (iii) AcOH/H_2O (4:1), rt; (iv) *p*-toluenesulfonyl chloride, pyridine, rt; (v) DBU, CH_3CN, reflux, 30 min; (vi) K_2CO_3, MeOH, rt; (vii) saturated NH_3/MeOH, rt, overnight.

introduced in the 5′-position (→ **9**), and an acetyl group was used for 3′-protection to acquire compound **10**.[9a,22b,c] Then, the 5′-*O*-DMT group was removed and the resulting compound **11**[22b,c] was 5′-*O*-tosylated using *p*-toluenesulfonyl chloride to give **12** (88% yield). Treatment of **12** with DBU in refluxing CH_3CN furnished the 3′-*O*-acetylated 2,5′-anhydro nucleoside **13** (79%), which after treatment with K_2CO_3 in MeOH gave **8** (86%) (Scheme 2). Finally, treatment of **8** with saturated NH_3/MeOH afforded nucleoside **1c**.

In summary, the first route gives the target nucleoside **1c** in an overall yield of 8% over three steps, while the second route leads to an improved overall yield of 25% over seven steps. Therefore, if time matters, the first route is the method of choice to access 2′-deoxy-2′-fluoroisocytidine (**1c**).

As discussed in section 4, it was found that oligonucleotides with 2′-deoxy-2′-fluoroisocytidine residues are rather labile under standard oligonucleotide deprotection conditions. To verify this finding, nucleoside **1c** was treated with concentrated aqueous NH_3 at 55 °C for 16 h (standard oligonucleotide deprotection conditions). HPLC analysis of the reaction mixture indicated the formation of two side products (**14**: major and **15**: minor), while almost no starting material was left (Figure 2a and Figure S2, Supporting Information). Hence, it was proven that side product formation could be minimized when mild oligonucleotide deprotection conditions were chosen. Indeed, treatment of **1c** in a mixture of EtOH/concentrated aqueous NH_3 (1:3) at room temperature kept the main amount of **1c** intact with only a small amount of formation of the faster migrating side product **14** (Figure 2b). As deprotection at room temperature is sufficient to remove all base protecting groups from oligonucleotides (data not shown), we were confident that pure oligonucleotides containing 2′-

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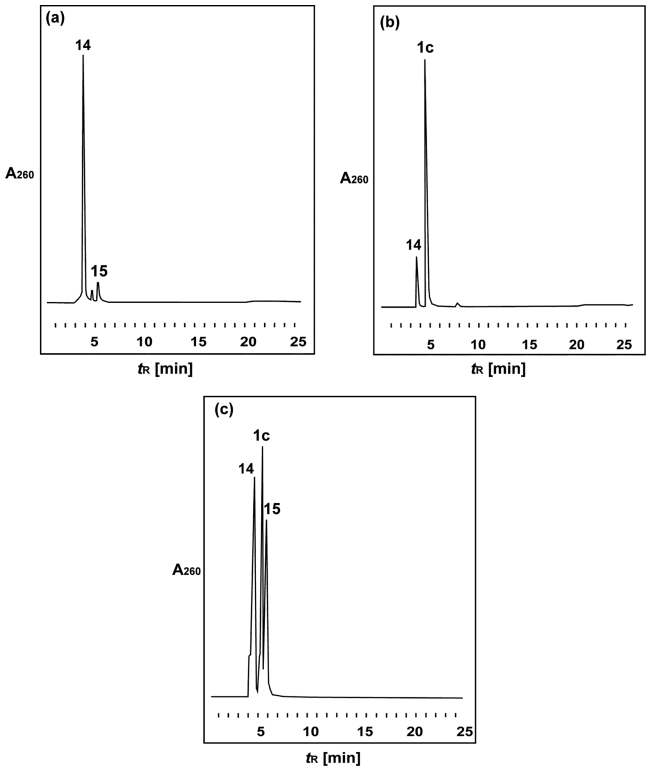
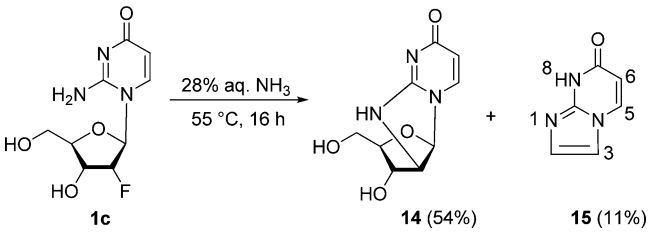


Figure 2. Reversed-phase HPLC elution profiles of the crude reaction mixtures: (a) 2′-deoxy-2′-fluoroisocytidine (**1c**) treated with concentrated aqueous ammonia (55 °C for 16 h), (b) **1c** treated with EtOH/concentrated aqueous NH_3 (1:3) (room temperature, 16 h), (c) artificial mixture of nucleoside **1c**, **14** and base **15**. The HPLC profiles were monitored at 260 nm using gradient system III.

deoxy-2′-fluoroisocytidine are accessible when mild deprotection conditions are chosen.

Then, the structure of the side products was evaluated. To this end, the 2′-fluoro nucleoside **1c** was fully converted to the side products **14** and **15** at 55 °C in concentrated aqueous ammonia (preparative scale experiment) (Scheme 3). On the

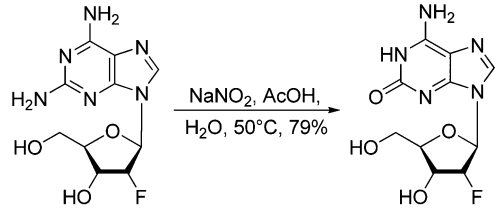
Scheme 3. Conversion of 1c to Compounds 14 and 15



basis of mass spectra and ^1H and ^{13}C NMR data, the structure of the faster migrating material was assigned to cyclonucleoside **14** and the fluorescent slower migrating compound to base **15** (Figure 2c and Figure S2, Supporting Information).[23] Cyclonucleoside formation as occurring on nucleoside **1c** is possible because of the spatial proximity of the nucleobase amino group and the 2′-fluorinated sugar carbon. This steric arrangement promotes the fluoro substituent to leave the molecule under the anchimeric assistance of the amino group. Further degradation of the molecule to compound **15** has already been reported.[23]

*2′-Deoxy-2′-fluoroisoguanosine (**3c**).* It has been verified on several examples that 2,6-diaminopurine nucleosides are suitable precursors for the synthesis of isoguanine nucleosides.[13d,14a,24] The 2-amino group can be selectively deaminated via diazotization. Consequently, 2,6-diamino-2′-deoxy-2′-fluoropurine nucleoside **16**, whose chemical and enzymatic synthesis has been reported,[25] was used as starting material for the preparation of 2′-deoxy-2′-fluoroisoguanosine (**3c**). Deamination of **16** using $NaNO_2$ in AcOH gave compound **3c** in 79% yield (Scheme 4). As the compound is now

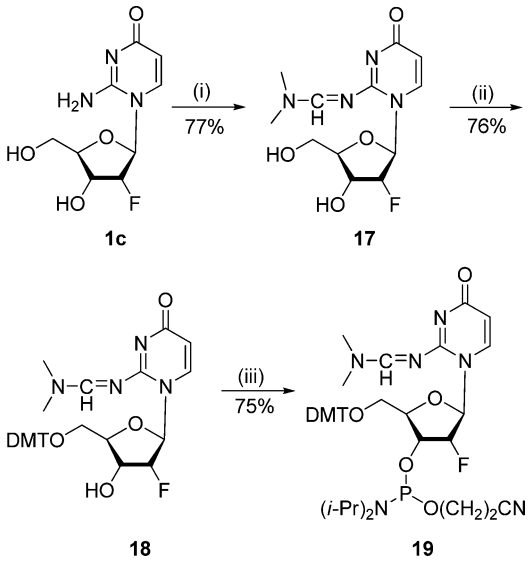
Scheme 4. Synthesis of 2′-Deoxy-2′-fluoroisoguanosine 3c



commercially available, we anticipate that similar conditions were chosen for its preparation. Nucleoside **3c** is stable under moderate alkaline or acidic conditions and shows strong gelation properties in water. The same is observed for the 2′-fluorinated 2,6-diaminopurine nucleoside **16** as well as for 2′-deoxyisoguanosine (**3a**). A detailed investigation of this matter will be described elsewhere.

2. Building Block Synthesis. Having both 2′-fluoro-modified nucleosides in hand, building blocks for solid-phase oligonucleotide synthesis were prepared. To this end, the 2-amino group of 2′-deoxy-2′-fluoroisocytidine **1c** was protected at the nucleobase with a (dimethylamino)methylidene residue to give **17** in 77% yield. 4,4′-Dimethoxytritylation of the 5′-OH group afforded **18** (76%) and phosphitylation furnished **19** (75%) (Scheme 5).

Scheme 5. Synthesis of the Phosphoramidite Building Block 19[a]



[a]Reagents and conditions: (i) *N,N*-dimethylformamide dimethyl acetal, MeOH, 1 h, rt; (ii) DMT-Cl, pyridine, rt; (iii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, DIPEA, DCM, 2 h, rt.

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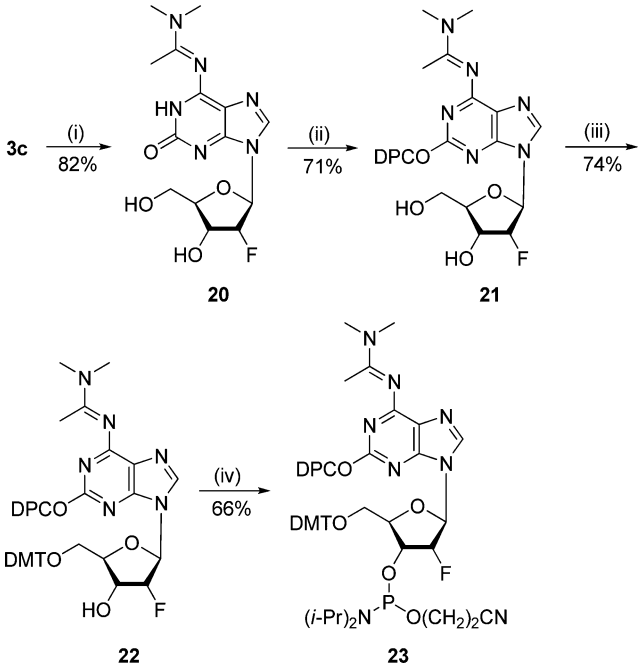
Next, 2′-deoxy-2′-fluoroisoguanosine **3c** was converted to the phosphoramidite **23**. Different to 2′-deoxyguanosine but similar to 2′-deoxyisoguanosine both, the amino group and the oxo group of the nucleobase have to be protected. All protecting groups should be removed under mild deprotection conditions. Consequently, the amino group of 2′-deoxy-2′-fluoroisoguanosine (**3c**) was protected with the (dimethylamino)ethylidene residue (→ **20**, 82%) and a *N,N*-diphenylcarbamoyl (DPC) residue was used to protect the 2-oxo group (→ **21**, 71%). 4,4′-Dimethoxytritylation of the 5′-OH group afforded **22** (74% yield) and phosphitylation gave **23** (66% yield; Scheme 6).

Scheme 6. Synthesis of the Phosphoramidite Building Block 23[a]



[a]Reagents and conditions: (i) *N,N*-dimethylacetamide dimethyl acetal, MeOH, 2 h, rt; (iii) *N,N*-diphenylcarbamoyl (DPC) chloride, pyridine, 60 min, rt; (iv) DMT-Cl, pyridine, 90 min, rt; (v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, DIPEA, DCM, 2 h, rt.

All synthesized compounds were characterized by ^{1}H, ^{13}C, and ^{19}F NMR spectra as well as ESI-TOF spectra. DEPT-135 and $^{1}H-^{13}C$ gated-decoupled NMR spectra were used to assign the ^{13}C NMR signals (Table S1 and Table S2, Supporting Information). For details see the Experimental Section (for spectra, see the Supporting Information).

3. Synthesis and Characterization of Oligonucleotides. It has been reported that the synthesis of oligonucleotides (ODNs) containing 2′-fluoro substituents is encountered with difficulties.[26] The problem results mainly from the instability of 2′-deoxy-2′-fluoropyrimidine nucleosides. In our study, it was necessary to apply conditions for oligonucleotide synthesis, deprotection, and workup under which all six 2′-deoxy-2′-fluoronucleoside residues are stable.

Oligonucleotides were prepared on the solid phase using the phosphoramidites **19** and **23** together with the phosphoramidites of nucleosides **2a** and **3a** (Figure S1, Supporting Information) as well as the standard phosphoramidites and applying the standard protocol for solid-phase oligonucleotide chemistry. Oligonucleotides containing 2′-deoxy-2′-fluoro nucleosides were cleaved from the solid support using concentrated aqueous NH_3/EtOH (3:1, v/v) and were deprotected in this solution for 48 h at room temperature (for details, see the Experimental Section). All other oligonucleotides were cleaved from CPG and finally deprotected in concentrated aqueous NH_3 at 55 °C for 16 h. The coupling yields of the modified building blocks were always higher than 95%. All synthesized oligonucleotides were purified by reversed-phase HPLC (RP-18), detritylated with 2.5% dichloroacetic acid in dichloromethane and again purified by HPLC. The contents of single peaks were isolated in all cases (Figure S3, Supporting Information). Subsequently, the molecular masses were determined by MALDI-TOF mass spectra. Table 1 displays all modified oligonucleotides used in this study together with the mass data.

The purity of the 2′-fluoro-modified oligonucleotides were further confirmed by enzymatic tandem hydrolysis with snake venom phosphodiesterase and alkaline phosphatase in 0.1 M Tris-HCl buffer (pH 8.5) at 37 °C (for details, see the Experimental Section).

For the cleavage profile of ODN **34** and ODN **38** incorporating **1c** or **3c** (2′-fluoro), see Figure 3. The mixtures

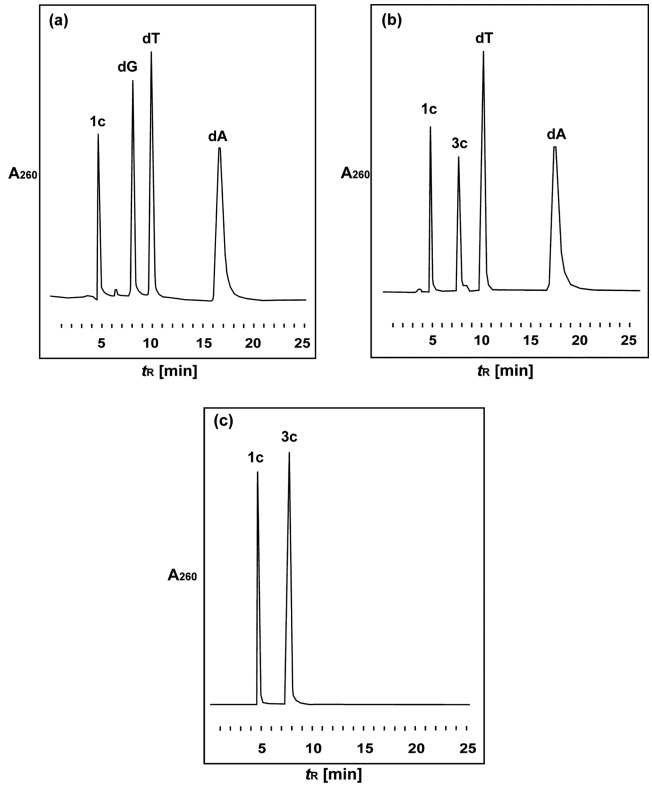


Figure 3. HPLC profiles of the enzymatic hydrolysis products of (a) ODN **34** and (b) ODN **38**. (c) Artificial mixture of purified nucleosides 2′-deoxy-2′-fluoroisocytidine (**1c**) and 2′-deoxy-2′-fluoroisoguanosine (**3c**), monitored at 260 nm using gradient system III.

obtained from the digest were analyzed by reversed-phase HPLC (RP-18, at 260 nm), showing the peaks for the canonical nucleosides and the 2′-fluoro nucleosides **1c** and **3c** (Figure 3, Figures S4 and S5, Supporting Information). In contrast to the findings made on the monomeric 2′-deoxy-2′-fluoroisocytidine nucleoside, a conversion to the side products **14** and **15** was not substantial or not observed for oligonucleotides containing

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Table 2. T_m Values of Fluorinated and Nonfluorinated 12-mer Duplexes with Antiparallel Chain Orientation[a]

DNA DUPLEXES	T_m [°C][b]	ΔT_m [°C][c]	RNA DUPLEXES or HYBRIDS	T_m [°C][b]	ΔT_m [°C][c]
5'-d(TAGGTCAATACT) (**24**) 3'-d(ATCCAGTTATGA) (**25**)	47		5'-d(TAGGTCAATACT) (**24**) 3'- (AUCCAGUUAUGA) (**26**)	46	-1
5'-d(TA^{F}G^{F}GTCAATACT) (**27**) 3'-d(AT C C AGTTATGA) (**25**)	49	+2	5'-d(TA^{F}G^{F}G TCAATACT) (**27**) 3'- (AU C C AGUUAUGA) (**26**)	51	+4
5'-d(^{F}U^{F}A^{F}G^{F}G^{F}U^{F}C^{F}A^{F}A^{F}U^{F}A^{F}C^{F}U) (**28**) 3'-d(A T C C A G T T A T G A) (**25**)	59	+12	5'-d(^{F}U^{F}A^{F}G^{F}G^{F}U^{F}C^{F}A^{F}A^{F}U^{F}A^{F}C^{F}U) (**28**) 3'- (A U C C A G U U A U G A) (**26**)	69	+22
5'-d(^{F}U^{F}A^{F}G^{F}G^{F}U^{F}C^{F}A^{F}A^{F}U^{F}A^{F}C^{F}U) (**28**) 3'-d(^{F}A^{F}U^{F}C^{F}C^{F}A^{F}G^{F}U^{F}U^{F}A^{F}U^{F}G^{F}A) (**29**)	82	+35	5'- (UAGGUCAAUACU) (**30**) 3'- (AUCCAGUUAUGA) (**26**)	61	+14
5'-d(TA **iG** **iG**TCAATACT) (**31**) 3'-d(AT^{Me}**iC**^{Me}**iC**AGTTATGA) (**32**)	53	+6	5'-d(TA ^{F}**iG** ^{F}**iG** TCAATACT) (**33**) 3'-d(AT ^{Me}**iC** ^{Me}**iC** AGTTATGA) (**32**)	54	+7
5'-d(T A^{F}**iG**^{F}**iG**TCAATACT) (**33**) 3'-d(AT ^{F}**iC**^{F}**iC**AGTTATGA) (**34**)	54	+7	5'-d(TA **iG** **iG** TCAATACT) (**31**) 3'-d(AT^{F}**iC**^{F}**iC**AGTTATGA) (**34**)	52	+5
5'-d(TAGGT^{Me}**iC** AATA^{Me}**iC**T) (**35**) 3'-d(ATCCA ^{F}**iG** TTAT ^{F}**iG**A) (**36**)	52	+5	5'-d(TA **iG** **iG** T ^{Me}**iC**AATA^{Me}**iC** T) (**37**) 3'-d(AT^{F}**iC**^{F}**iC** A ^{F}**iG** T TAT ^{F}**iG** A) (**38**)	54	+7
5'-d(^{F}U^{F}A^{F} iG^{F} iG^{F}U^{F}iC^{F}A^{F}A^{F}U^{F}A^{F}iC^{F}U) (**39**) 3'-d(A T ^{Me}**iC**^{Me}**iC** A **iG** T T A T **iG** A) (**40**)	65	+18			

[a]Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single strand concentration. [b]T_m values were determined from the melting curves by using the software MELTWIN 3.0. [c]ΔT_m was calculated as $T_m^{\text{modified duplex}} - T_m^{\text{unmodified duplex (24·25)}}$. For nucleoside structures see Table 1.

Table 3. T_m Values of Fluorinated and Nonfluorinated 12-mer Duplexes with Parallel Chain Orientation[a]

Duplexes Without 2'-Fluoro Substitution	T_m [°C][b]	ΔT_m [°C][c]	Duplexes With 2'-Fluoro Substitution[d]	T_m [°C][b]	ΔT_m [°C][c]
5'-d(TA G G T C AATACT) (**24**) 5'-d(AT^{Me}**iC**^{Me}**iC**A**iG**TTAT**iG**A) (**41**)	36	-	5'-d(^{F}U^{F}A ^{F}G ^{F}G^{F}U^{F}C^{F} A^{F}A^{F}U^{F}A^{F}C^{F}U) (**28**) 5'-d(A T^{Me}**iC**^{Me}**iC** A **iG** T T A T **iG** A) (**41**)	39	+3
5'- (UA G G UCAAUACU) (**30**) 5'-d(AT^{Me}**iC**^{Me}**iC**A**iG**TTAT**iG**A) (**41**)	34	-2	5'-d(^{F}U^{F}A^{F}iG^{F}iG^{F}U^{F}iC^{F}A^{F}A^{F}U^{F}A^{F}iC^{F}U) (**39**) 5'-d(A T C C A G T T A T G A) (**42**)	34	-2
5'- (U CAUAACU G G AU) (**43**) 5'-d(A**iG**TATT**iG**A^{Me}**iC**^{Me}**iC**TA) (**40**)	31	-5	5'- (U C AUAA C U G G AU) (**43**) 5'-d(A^{F}**iG** TA TT^{F}**iG**A^{F}**iC**^{F}**iC**TA) (**38**)	<10	-
5'-d(TA**iG** **iG**T ^{Me}**iC**AATA^{Me}**iC**T) (**37**) 5'- (AU C C A GU UAU GA) (**44**)	33	-3	5'-d(^{F}U^{F}A^{F}iG^{F}iG^{F}U^{F}iC^{F}A^{F}A^{F}U^{F}A^{F}iC^{F}U) (**39**) 5'- (A U C C A G U U A U G A) (**44**)	<10	-
			5'-d(^{F}U^{F}A^{F}iG^{F}iG^{F}U^{F}iC^{F}A^{F}A^{F}U^{F}A^{F}iC^{F}U) (**39**) 5'-d(^{F}A^{F}U ^{F}C ^{F}C^{F} A ^{F}G^{F}U^{F}U^{F}A^{F}U ^{F}G^{F}A) (**45**)	<10	-

[a]Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, 10 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration. [b]T_m values were determined from the melting curves by using the software MELTWIN 3.0. [c]ΔT_m was calculated as $T_m^{\text{modified duplex}} - T_m^{\text{unmodified duplex (24·41)}}$. [d]For nucleoside modifications see Table 1.

1c when mild deprotection conditions (EtOH/concentrated aqueous NH_3, 1:3, 48 h) were used (for heating condition see Figure S6, Supporting Information). This finding was confirmed by MALDI-TOF mass spectra and HPLC profiles from the enzymatic digestion of ODNs **34** and **38** showing the intact isonucleosides **1c** and **3c** together with the canonical nucleosides without side product formation (Figure 3a,b). An HPLC profile of the artificial mixture of **1c** and **3c** is shown for comparison (Figure 3c).

4. Effect of 2′-Fluoro Substitution on the Stability of Duplexes with Parallel and Antiparallel Strand Orientation. Functionalization of regular oligonucleotides with 2′-fluoro substituents increases the binding affinity to complementary DNA and even more to RNA when both duplex

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strands are in antiparallel orientation.[6b,9c] As nothing is known on the fluoro effect on ps DNA, a series of 12-mer oligonucleotides containing 2′-deoxy-2′-fluoroisocytidine (**1c**) and 2′-deoxy-2′-fluoroisoguanosine (**3c**) as well as their nonfluorinated counterparts **2a** and **3a** as a comparison were studied. For duplexes with parallel strands, the duplex 5′-d(TAGGTCAATACT) (**24**)·5′-d(AT^{Me}iC^{Me}iCAiGTTATiGA) (**41**) was selected as reference duplex and for comparison aps duplex 5′-d(TAGGTCAATACT) (**24**)·3′-d-(ATCCAGTTATGA) (**25**) was chosen (Table 2 and 3). These duplexes were partially modified with **1c**, **3c**, **2a**, or **3a** or were fully fluorinated using the fluorinated canonical nucleosides $^{F}U_d$, $^{F}G_d$, $^{F}C_d$, and $^{F}A_d$ (for structures, see Table 1). Hybridization experiments were performed with single-stranded DNA, RNA, and 2′-F-RNA.

Due to the low hydrolytic stability of iC_d, the oligonucleotide **41** and related oligomers used for the construction of ps DNA contain always $^{Me}iC_d$ (Table 3).[1c,e] For the fluorinated iC_d (**1c**) a structural stabilization by the 5-methyl group was not necessary as the fluorinated compound is more stable than the nonfluorinated counterpart. Moreover, it is known that pyrimidine 5-methyl groups stabilize the DNA duplex structure depending on the content and positioning in duplex DNA. This matter has already been investigated on parallel and antiparallel DNA.[1c,e,27,28] To demonstrate this effect for the 12-mer duplexes used in this study, duplex 5′-d(UAGGUCAAUACU) (**24-dU**)·3′-d(AUCCAGUUAUGA) (**25-dU**) was prepared (replacing dT by dU),[1c] T_m values were determined and compared to those obtained for duplex **24·25** containing dT. The difference in the T_m values between duplex **24·25** (containing dT) and **24-dU·25-dU** (containing dU) amounts to only 2 °C (see Table S3, Supporting Information). The effect is even smaller when one strand was fluorinated (ΔT_m = +1 °C; **28·25** vs **28·25-dU**).

Duplexes with Antiparallel Chain Orientation. In a first series of experiments, duplex stability was studied on helices with antiparallel chain orientation containing canonical nucleobases (Table 2; for thermodynamic data, see Table S4, Supporting Information). In the context of antisense therapeutics, the upper strands can be considered as antisense oligonucleotides and the lower ones as RNA or DNA target. The data displayed in Table 2 show that the most stable duplex is the fully 2′-fluorinated duplex **28·29** with aps orientation (T_m = 82 °C) (for melting curves see Figure S7, Supporting Information). Its T_m is significantly higher than that of the corresponding RNA duplex **30·26** (T_m = 61 °C). 2′-Fluorination of one strand of duplex DNA (**24·25** vs **28·25**) causes a T_m increase of 12 °C, while for the DNA−RNA hybrid **28·26** a T_m increase of 22 °C was observed. The changes observed for these 12-mer duplexes are in line with previous reports made on other fluorinated oligonucleotides.[6b,9a] Hydrophobic properties of the fluorine residue which affect hydration by changing the water spine in the helix groove also have to be considered.[29]

It is accepted that the T_m increase induced by the fluorine substituents results from conformational changes of the DNA B-form to the A-form. Both forms show significantly different CD spectra. Exemplarily, CD spectra were determined for selected fluorinated and nonfluorinated 12-mer duplex DNA, duplex RNA, and DNA−RNA hybrids (see Table 2 and Figure 4, Figure S8 Supporting Information). According to Figure 4, RNA duplex **30·26**, fluorinated DNA-RNA hybrid **28·26** as well as duplex DNA **28·29** with both strands fluorinated are in the A

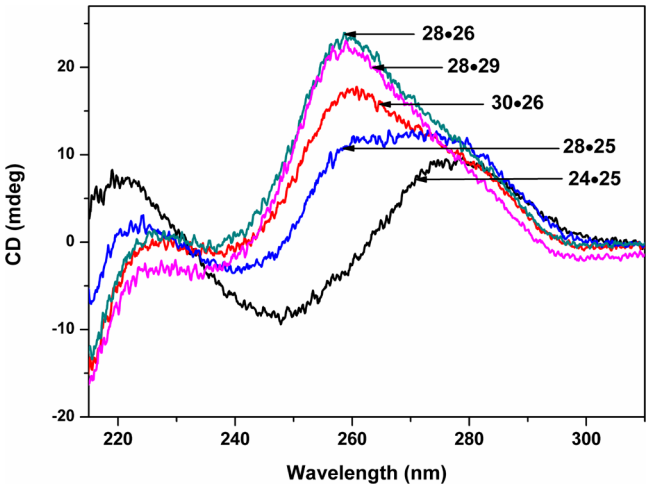

Figure 4. CD spectra of fluorinated and nonfluorinated oligonucleotide duplexes measured in 0.1 M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate (pH 7.0) with a single-strand concentration of 5 μM + 5 μM.

form. The DNA−DNA duplex **28·25** with one strand fluorinated adopts an intermediate conformation while the reference DNA−DNA duplex **24·25** is in the B form.

Comparison of the stability of duplex **24·25** containing entirely canonical base pairs (dA−dT and dG−dC) with that of duplex **31·32** comprising iG_d−$^{Me}iC_d$ pairs shows an additional stabilization by the "iso-pairs" (ΔT_m = 6 °C) as this base pair is more stable than the dG−dC pair (Table 2, lower part). DNA−DNA duplexes or DNA−RNA hybrids show almost the same duplex stability in this sequence motif. The new base pair **1c·3c** can be considered to be as stable as the iG_d−$^{Me}iC_d$ pair (**31·32** vs **33·34**, Table 2, lower part, Figure 5). However, a further significant T_m increase by the 2′-fluoro modification was not observed in this sequence context.

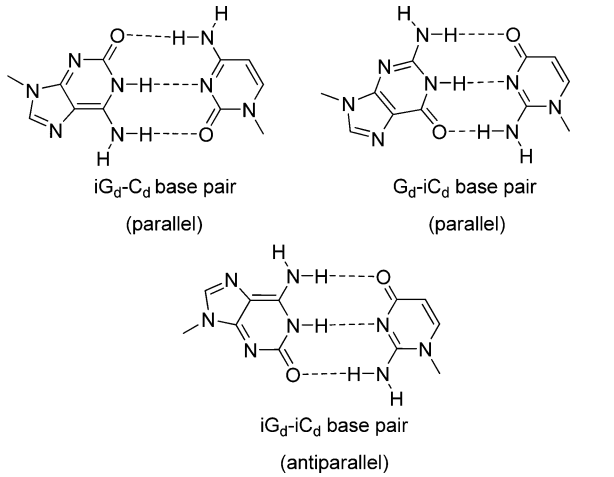

Figure 5. Base-pair motifs of DNA with parallel and antiparallel chain orientation.

Duplexes with Parallel Chain Orientation. Ps DNA represents an autonomous nucleic acid structure and was realized by changing the Watson−Crick motif to the reverse mode (dA-dT base pair) and/or by replacing dG by 2′-deoxyisoguanosine (iG_d) resulting in a iG_d−dC base pair or by

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substituting dC by 2′-deoxy-5-methylisocytidine ($^{Me}iC_d$) in the dC−dG pair (Figure 5).[3,13,14,30]

As mentioned above, nucleoside shape mimics with a pyrrolo[2,3-*d*]pyrimidine or a pyrazolo[3,4-*d*]pyrimidine skeleton replacing the nucleobases of dA or dT and iG_d or iC_d have been shown to be well accommodated in ps DNA and add additional stability to the parallel helix structure.[4] However, it remains to be shown if modifications in the sugar moiety are accepted by ps DNA. In this context, only the influence of a 2′-methoxy group on ps DNA stability was reported.[31] As discussed above, the 2′-fluoro (down) substituent has a positive influence on the stability of 12-mer duplexes with antiparallel chain orientation, and a similar behavior was anticipated for ps DNA. Consequently, a series of duplexes with parallel chain orientation was constructed, and the stability was studied by thermal denaturation experiments.

To this end, the influence of the 2′-fluoro substituent on the sugar puckering (*N* vs *S* conformation, Figure 6) of the monomeric "isonucleosides" 2′-deoxy-2′-fluoroisoguanosine (**3c**) and 2′-deoxy-2′-fluoroisocytidine (**1c**) was investigated. As the stability data of 2′-fluorinated duplexes will be compared with those of oligoribonucleotide and oligodeoxyribonucleotide duplexes, the conformational analysis was extended to a number of fluorinated and nonfluorinated nucleosides with canonical bases.[32]

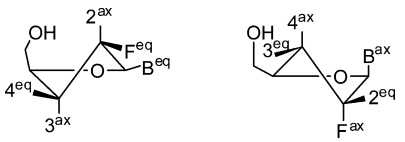

Figure 6. *N* and *S* conformation for fluoronucleosides. B corresponds to nucleobase. ax: axial; eq: equatorial.

The conformation study was performed using the program PSEUROT (version 6.3).[33] The input used the following coupling constants: ^3J(H1′, H2′), ^3J(H2′, H3′), ^3J(H3′, H4′), ^3J(H1′, F), and ^3J(H3′, F). The coupling constants were taken from well-resolved ^{1}H NMR spectra measured in D_2O or DMSO-d_6. Figure 7 summarizes not only the data of the isonucleosides but also those of the corresponding canonical DNA and RNA constituents and their 2′-fluorinated derivatives. From Figure 7 it is apparent that the pentofuranose moiety of all fluoronucleosides prefers the *N*-conformation. The degree of the *N* population is higher for the fluorinated pyrimidine nucleosides than for those with a purine skeleton. This is valid for the nucleosides with canonical nucleobases as well as for isonucleosides. On the contrary, purine and pyrimidine ribo- and 2′-deoxyribonucleosides prefer the S-type sugar pucker as long as they are not constituents of a nucleic acid. A comparison of 2′-deoxy-2′-fluoroisoguanosine (**3c**), isoguanosine (**3b**), and 2′-deoxyisoguanosine (**3a**) shows that the 2′-fluoro substituent increases the *N*-conformer population significantly, which is the conformation of the sugar moiety in RNA.[34] The same is valid for the 2′-fluorinated isocytidine **1c**. However, the conformational parameters determined for nucleosides are not necessarily the same as those found in oligonucleotide duplexes.

Next, the stability of fluorinated and nonfluorinated duplexes with parallel strand orientation was studied. At first, nonfluorinated duplexes were measured (Table 3, left row). As expected from previous observations by our laboratory, the T_m values of ps 12-mer duplexes are about 15 °C lower than those with aps orientation.[1c] The iG_d−dC and the $^{Me}iC_d$−dG base pairs both stabilize the duplex structure compared to duplexes containing only dA−dT base pairs. Surprisingly, the complete fluorination of one strand did not increase the duplex stability over that of the nonfluorinated counterpart. Only a small positive effect was found for duplex **28**·**41**, while another duplex (**39**·**42**) was slightly less stable (for melting curves see

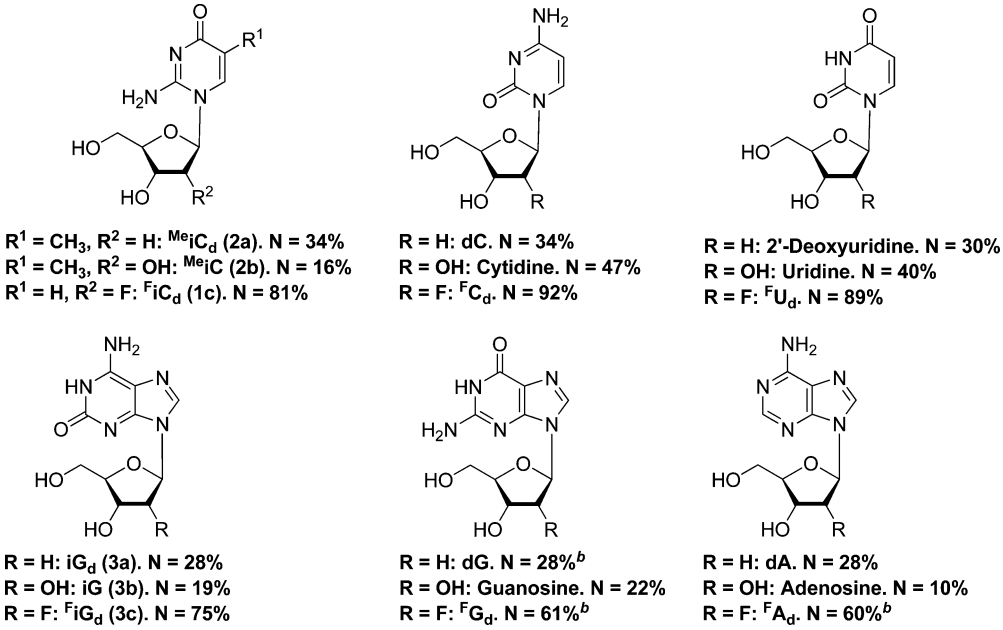

Figure 7. Conformer population of ribonucleosides, 2′-deoxyribonucleosides, and 2′-deoxyribo-2′-fluoronucleosides determined in D_2O or [b] DMSO-d_6. The conformational analysis of the sugar moiety in solution was performed using the program PSEUROT (version 6.3).[33] The input used the following coupling constants: ^3J(H1′, H2′), ^3J(H2′, H3′), ^3J(H3′, H4′), ^3J(H1′, F), ^3J(H3′, F).

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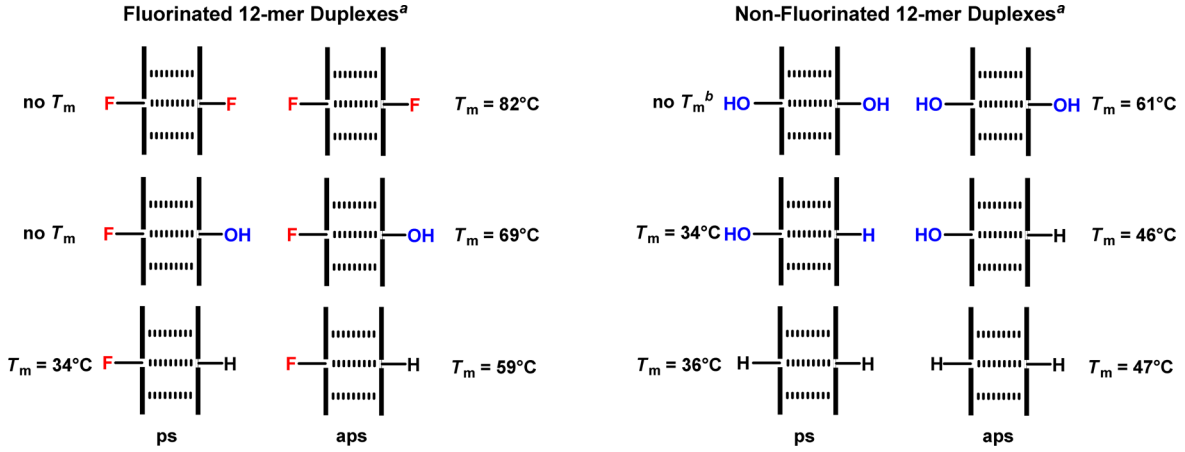

Figure 8. Stability of 12-mer duplexes with parallel and antiparallel chain orientation. (a) T_m data taken from Tables 2 and 3. (b) According to hybridization of oligonucleotides **46** and **47**.

Figure S9, Supporting Information). This result was unexpected as aps DNA shows a strong duplex stabilizing response to 2′-fluorination. More surprisingly, fluorinated oligonucleotides could not be hybridized with complementary RNA under parallel chain orientation. A T_m value was not detectable for duplexes **43**·**38**, **39**·**44**, and **39**·**45** (Table 3, right row). Taken together, fluorinated oligonucleotides are able to form ps duplexes of significant stability with complementary non-fluorinated DNA (for thermodynamic data, see Table S5, Supporting Information); ps hybrids of fluorinated DNA strands and RNA strands are unstable.

We anticipate that the unexpected behavior upon 2′-fluoro functionalization is a special feature of parallel DNA. According to NMR measurements and modeling studies, parallel DNA forms a unique structure with a number of properties similar to aps DNA, while others are different.[35] The orientation of the nucleobases in ps DNA is anti, and the sugar conformation is in the S-domain similar to aps DNA. The helix is right handed with a diameter of about 20 nm, which is almost the same for ps and aps DNA. The reduced stability results from weaker stacking interactions and the weaker hydrogen bonding of the dA−dT Donohue base pair. The helical parameters of ps and aps DNA are different, leading to two grooves with almost identical width (8−9 Å) for ps DNA compared to 6 and 12 Å width in aps DNA. The interstrand C1′−C1′ distance in ps DNA is ∼11.4 Å, which is almost 2 Å larger than in aps DNA.[35]

According to our findings, we propose that parallel DNA is less flexible than canonical DNA, which exists in various structures (A and B form). Consequently, ps DNA reacts sensitively to sugar backbone changes. 2′-Fluoro "down" nucleosides which favor the N-conformation might add too much strain to the helix and drive the nucleobases in an unfavorable pairing position.[31] Factors like steric clashes by the 2′-fluoro substituents also have to be considered. This leads to the question if RNA with the sugar residues in the N conformation is able to form stable duplexes with parallel strand alignment. Toward this end, we tried to construct a parallel-stranded 25-mer RNA−RNA duplex by hybridizing the complementary RNA single strands 5′-(AAAAAAAAAAUAA-UUUUAAAUAUUUU) (**46**) and 5′-(UUUUUUUUUUA-UUAAAAUUUAUAAAA) (**47**). Both strands do not contain iG_d and $^{Me}iC_d$ residues, and the strand orientation is solely controlled by the sequence, a methodology which was already applied to 25-mer duplex DNA.[27] In agreement with our observations made on 2′-fluoro-2′-deoxyribonucleosides, it was not possible to generate a parallel RNA duplex (no T_m), while the corresponding antiparallel duplex showed a T_m value of 52 °C.

This finding confirms that DNA with parallel strand orientation is limited to duplex DNA and DNA−RNA hybrids, while two fluorinated DNA strands do not hybridize under parallel strand alignment.

■ CONCLUSION AND OUTLOOK

A diversity of complementary oligonucleotides with fluorine substitution at the 2′-position of 2′-deoxyisoguanosine and 2′-deoxyisocytidine and at the canonical DNA residues was synthesized. To this end, syntheses for 2′-deoxy-2′-fluoroisocytidine (**1c**) and 2′-deoxy-2′-fluoroisoguanosine (**3c**) were developed. 2′-Deoxy-2′-fluoroisonucleosides **1c** and **3c** were converted to building blocks (phosphoramidites) for solid-phase oligonucleotide synthesis. Unexpectedly, 2′-deoxy-2′-fluoroisocytidine is not stable in concentrated aqueous ammonia at 55 °C (16 h treatment) and was converted to the cyclonucleoside **14**. Side product formation of nucleoside **1c** was minimized, and not observed during oligonucleotide deprotection with an aqueous ammonia−EtOH mixture (3:1, v/v) at room temperature.

As anticipated, 2′-fluorine substituents lead to a significant increased stability in antiparallel duplexes depending on the number of fluorine modifications. The fluorinated $^{F}iG_d$·$^{F}iC_d$ (**3c**·**1c**) base pair is as stable as its nonfluorinated counterpart and more stable than the dG−dC pair. The situation changes for fluorinated duplexes with parallel chain orientation. Although fluorinated DNA single strands form stable ps duplexes with nonfluorinated DNA, they are less stable than their antiparallel counterparts (Figure 8). Furthermore, the fluorine functionalization does not add additional stability to the DNA duplex structure. Apparently, fluoro nucleoside residues which prefer the N-conformation have difficulty adopting the required sugar conformation in parallel DNA, which appears to be closer to S than to N.

Consequently, hybridization of single-stranded fluorinated oligonucleotides with complementary RNA strands did not result in duplex formation. This is also valid for 25-mer RNA strands which did not hybridize with parallel chain alignment. These findings might shed more light on the question why evolution has used the principle of antiparallel chain

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Table 4. ^{13}C NMR Data for Isocytosine Nucleosides[a,b]

	C(2)[b]	C(4)[b]	C(5)[b]	C(6)[b]	C(1′) ($^2J_{F,C}$)	C(2′) ($^1J_{F,C}$)	C(3′) ($^2J_{F,C}$)	C(4′)	C(5′)	CH_3 NCH_3	OCH_3 qC	C=O N=CH
1a[e]	154.2	169.7	106.7	138.1	87.6	[c]	69.8	87.4	60.7			
1c	154.3	169.5	107.2	138.2	89.1	92.3	67.5	84.1	59.5			
					(34.9)	(188)	(15.7)					
2a[d]	154.0	170.3	114.2	133.9	88.0	[c]	69.8	86.7	60.7	13.5		
4	150.3	163.2	101.6	140.4	87.2	93.6	67.4	83.2	59.3			
					(34.2)	(185)	(16.2)					
5	150.1	163.7	101.9	143.6	91.8	90.4	73.6	76.7	67.3	21.2		
					(37.4)	(187)	(15.2)			21.3		
6	150.3	163.2	102.0	141.9	89.0	90.3	74.7	80.9	59.3	21.2		
					(35.1)	(190)	(14.6)					
7	150.1	163.2	101.9	141.5	89.4	92.6	68.0	79.4	69.3	21.1		
					(36.2)	(185)	(16.6)					
8	156.8	170.4	108.8	142.8	95.9	95.1	71.2	87.4	74.2			
					(40.1)	(192)	(15.5)					
9	150.1	163.2	101.4	140.9	88.6	93.5	67.9	81.0	62.0		55.0	
					(35.9)	(184)	(16.6)				85.8	
10	150.1	163.3	101.8	142.4	90.6	91.0	69.8	79.2	62.2	20.2	55.0	169.5
					(36.3)	(186)	(15.0)				85.9	
11	150.3	163.1	102.1	141.2	88.1	90.8	69.7	81.5	60.0	20.4		169.6
					(34.5)	(189)	(14.4)					
12	150.5	163.7	102.4	143.4	91.8	91.1	69.9	78.1	69.4	20.7		169.9
					(37.2)	(187)	(15.0)			21.5		
13	156.8	170.4	109.1	143.1	95.6	93.7	73.1	84.5	74.1	20.2		169.6
					(40.0)	(195)	(13.8)					
14	156.9	136.8	106.0	171.0	91.0	65.6	75.9	89.3	61.4			
17	156.8	169.9	107.6	137.2	88.1	94.2	66.6	82.7	58.6	34.6		157.8
					(33.9)	(186)	(16.3)			40.6		
18	157.0	170.0	107.8	137.2	88.9	94.4	67.5	80.9	61.2	34.9	55.1	158.0
					(34.6)	(186)	(16.7)			40.9	85.9	

[a]Measured in DMSO-d_6 at 298 K. [b]Pyrimidine numbering. [c]Superimposed by DMSO-d_6. [d]Reference 1b. [e]Reference 1c.

Table 5. ^{13}C NMR Data for Purine-2,6-diamine and Isoguanine Nucleosides[a,b]

	C(2)[b]	C(4)[b]	C(5)[b]	C(6)[b]	C(8)[b]	C(1′) ($^2J_{F,C}$)	C(2′) ($^1J_{F,C}$)	C(3′) ($^2J_{F,C}$)	C(4′)	C(5′)	NMe_2	=CMe	C=O CH=N
3c	155.5[c]	151.7	109.0	154.5[c]	137.4	85.1	92.7	68.1	84.1	60.3			
						(32.4)	(187)	(15.7)					
3b[e]	152.2	[d]	109.8	155.9	138.4	87.8	73.0	70.9	85.6	61.7			
3a[f,g]	156.7	152.5	109.3	154.1	137.5	83.7	[d]	71.1	88.1	62.1			
16	156.3	151.2	113.3	160.3	135.5	85.0	93.3	68.4	83.9	60.5			
20	156.7[c]	153.7	113.0	156.4[c]	139.7	85.2	92.8	68.4	84.3	60.5	[d]	18.8	164.1
						(32.5)	(187)	(15.6)			38.1		
21	151.4[c]	151.3[c]	123.9	155.6	140.9	85.6	93.4	68.3	84.1	60.3	37.8	17.4	160.8,
						(33.0)	(187)	(15.9)			38.3		162.6
22	151.5[c]	151.3[c]	123.8	155.7	140.9	85.9	93.2	68.8	81.5	62.9	37.8	17.2	160.8,
						(34.0)	(185)	(16.4)			38.4		162.5

[a]Measured in DMSO-d_6 at 298 K. [b]Purine numbering. [c]Tentative. [d]Not detected. [e]Reference 14d. [f]Reference 14c. [g]Measured in DMSO-d_6/0.4 M aq NH_4OAc.

orientation. We are currently investigating this matter in more depth.

EXPERIMENTAL SECTION

General Methods and Materials. All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40−60 μM, for flash chromatography) at 0.4 bar. UV spectra: λ_{max} (ε) in nm, ε in dm^3 mol^{-1} cm^{-1}. NMR spectra were measured at 300.15 MHz for ^{1}H, 75.48 MHz for ^{13}C, 121.52 MHz for ^{31}P, and 282.4 MHz for ^{19}F. The J values are given in hertz; δ values are given in ppm relative to Me_4Si as internal standard. For NMR spectra recorded in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^{1}H NMR and 39.50 ppm for ^{13}C NMR. The ^{13}C NMR signals were assigned on the basis of DEPT-135 and ^{1}H−^{13}C gated-decoupled NMR spectra (Tables 4 and 5; for coupling constants see Tables S1and S2 in the Supporting Information). Reversed-phase HPLC was carried out on a 4 × 250 mm RP-18 (10 μM) LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller, and an integrator. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer. Molecular masses of oligonucleotides were determined by MALDI-TOF mass

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spectrometry in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix (Table 1). The melting temperature curves were measured with a UV−vis spectrophotometer equipped with a Cary thermoelectrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor with a heating rate of 1 °C min^{-1}. T_m values were determined from the melting curves using the software MELTWIN, version 3.0.[36]

Synthesis, Purification, and Characterization of Oligonucleotides. The oligonucleotides were synthesized on an automated DNA synthesizer on a 1 μmol scale employing standard phosphoramidites as well as the phosphoramidites **1c** and **3c**. After cleavage from the solid support, the oligonucleotides were deprotected in concentrated aqueous ammonia solution for 16 h at 55 °C. Oligonucleotides containing 2′-deoxy-2′-fluoro nucleosides were cleaved from the solid support by storage of the support in concentrated aqueous NH_3/EtOH (3:1, v/v) for 1 h. The supernatant was separated from the CPG and kept in concentrated aqueous NH_3/EtOH (3:1, v/v) for 48 h at room temperature. The purification of the "trityl-on" oligonucleotides was carried out on reversed-phase HPLC using the following gradient system at 260 nm: (A) MeCN; (B) 0.1 M (Et_3NH)OAc (pH 7.0):MeCN, 95:5; gradient I: 0−3 min 10−15% A in B, 3−15 min 15−50% A in B, flow rate 0.8 mL/min. The purified "trityl-on" oligonucleotides were treated with 2.5% $CHCl_2COOH$/CH_2Cl_2 for 2 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC (column 4 × 250 mm) with gradient II: 0−20 min 0−20% A in B, 20−25 min, 20% A in B, flow rate 0.8 mL/min. The oligonucleotides were desalted on RP-18 (column 4 × 125 mm) using water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2:3). The oligonucleotides were lyophilized on a Speed-Vac evaporator to yield colorless or yellow solids which were frozen at −24 °C. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode. Extinction coefficients ε_{260} (H_2O) of nucleosides are **dA**, 15 400; **dG**, 11 700; **dT**, 8800; **dC**, 7300; ^{F}**iC**$_d$ 5100, ^{F}**iG**$_d$ 4400, **iG**$_d$ 4300,[1a] ^{Me}**iC**$_d$ 6100,[1b] ^{F}**A**$_d$ 14600, ^{F}**G**$_d$ 11600, ^{F}**U**$_d$ 9400, ^{F}**C**$_d$ 6900.

Tandem Enzymatic Hydrolysis of Oligonucleotides. The enzymatic hydrolysis of **ODN-34** and **ODN-38** was performed using snake-venom phosphodiesterase (EC 3.1.15.1, *Crotallus adamanteus*) and alkaline phosphatase (EC 3.1.3.1, *Escherichia coli*) in 0.1 M Tris−HCl buffer (pH 8.5) at 37 °C. The enzymatic digestion products were analyzed by reversed-phase HPLC using the following gradient. Gradient III: 0−25 min 100% B; flow rate 0.7 mL/min. [B = 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5)].

Tosylation of 1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)uracil (4) with *p*-Toluenesulfonyl Chloride. 2′-Deoxy-2′-fluorouridine (**4**)[16] (3.2 g, 13 mmol) was dissolved in dry pyridine (20 mL). The solution was cooled to 0 °C (ice-bath), *p*-toluenesulfonyl chloride (2.8 g, 14.7 mmol) was added, and the solution was stirred overnight at room temperature. A second portion of *p*-toluenesulfonyl chloride was introduced (0.5 g, 2.62 mmol), and stirring was continued for 3 h until the starting material was consumed (TLC monitoring, CH_2Cl_2/MeOH, 95:5). The solution was diluted with CH_2Cl_2 (50 mL), and the reaction was quenched upon addition of water (20 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic phase was washed with saturated $NaHCO_3$ solution (2 × 50 mL) and H_2O (2 × 50 mL) and dried (Na_2SO_4). The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/MeOH, 95:5). Three products (**5**−**7**) were isolated.

1-[2-Deoxy-2-fluoro-3,5-di-*O*-(*p*-toluenesulfonyl)-β-D-ribofuranosyl]uracil (5). From the fastest migrating zone, compound **5** was isolated as colorless foam (1.9 g, 26%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5): R_f 0.70. λ_{max} (MeOH)/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 23900), 256 (9800). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.37, 2.42 (2s, 6H, 2 × CH_3), 3.88−4.04 (m, 2H, 2 × H-5′), 4.16−4.20 (m, 1H, H-4′), 5.10−5.20 (m, 1H, H-3′), 5.39 (dd, J = 5.1 Hz, J = 52.5 Hz, 1H, H-2′), 5.61 (d, J = 8.1 Hz, 1H, H-5), 5.68 (d, J = 24.6 Hz, 1H, H-1′), 7.37−7.39 (m, 2H, arom. H), 7.47−7.50 (m, 2H, arom. H), 7.55 (d, J = 7.8 Hz, 1H, H-6), 7.63−7.66 (m, 2H, arom. H), 7.79−7.82 (m, 2H, arom. H), 11.46 (s, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −193.851. ESI-TOF: m/z calcd for $C_{23}H_{23}FN_2O_9S_2$ [M + Na]$^+$ 577.0721, found 577.0736.

1-[2-Deoxy-2-fluoro-3-*O*-(*p*-toluenesulfonyl)-β-D-ribofuranosyl]uracil (6). From the second zone, compound **6** was isolated as colorless foam (0.590 g, 11%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5): R_f 0.50. λ_{max} (MeOH)/nm 226 (ε/dm^3 mol^{-1} cm^{-1} 14400), 257 (9900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.42 (s, 3H, CH_3), 3.33 (m, superimposed by DMSO, 1H, H-5′), 3.50−3.54 (m, 1H, H-5′), 4.04 (m, 1H, H-4′), 5.05−5.14 (m, 2H, H-3′, HO-5′), 5.31 (dd, J = 2.4 Hz, J = 52.2 Hz, 1H, H-2′), 5.64 (d, J = 8.1 Hz, 1H, H-5), 5.84 (dd, J = 1.8 Hz, J = 18.3, 1H, H-1′), 7.48−7.51 (m, 2H, arom. H), 7.76 (d, J = 8.1 Hz, 1H, H-6), 7.83−7.85 (m, 2H, arom. H), 11.44 (bs, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −198.862. ESI-TOF: m/z calcd for $C_{16}H_{17}FN_2O_7S$ [M + Na]$^+$ 423.0633, found 423.0619.

1-[2-Deoxy-2-fluoro-5-*O*-(*p*-toluenesulfonyl)-β-D-ribofuranosyl]uracil (7). From the slowest migrating zone, compound **7** was isolated as colorless foam (1.55 g, 30%). TLC (silica gel, CH_2Cl_2/MeOH, 95:5): R_f 0.45. λ_{max} (MeOH)/nm 224 (ε/dm^3 mol^{-1} cm^{-1} 14800), 260 (9900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.39 (s, 3H, CH_3), 3.92−3.96 (m, 1H, H-5′), 4.18−4.24 (m, 2H, H-4′, H-5′), 4.30−4.34 (m, 1H, H-3′), 5.10 (dd, J = 4.8 Hz, J = 53.1 Hz, 1H, H-2′), 5.57 (dd, J = 4.8 Hz, J = 8.1 Hz, 1H, H-5), 5.74−5.81 (m, 2H, H-1′, HO-3′), 7.43−7.48 (m, 3H, H-6, arom. H), 7.76−7.79 (m, 2H, arom. H), 11.42 (bs, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −199.750. ESI-TOF: m/z calcd for $C_{16}H_{17}FN_2O_7S$ [M + Na]$^+$ 423.0633, found 423.0630.

2,5′-Anhydro-2′-deoxy-2′-fluorouridine (8). *Method A (from Compound 7).* Compound 7 (0.320 g, 0.79 mmol) was dissolved in dry CH_3CN. Then DBU (1,8-diazabicyclo[5.4.0]undec-7-en, 120 μL, 0.122 g, 0.8 mmol) was added, and the reaction mixture was heated under reflux for 45 min. The solvent was evaporated, and the remaining residue was subjected to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/MeOH, 90:10). Evaporation of the main zone afforded compound **8** as colorless solid (0.083 g, 46%). TLC (silica gel, CH_2Cl_2/MeOH, 90:10): R_f 0.40. λ_{max} (MeOH)/nm 237 (ε/dm^3 mol^{-1} cm^{-1} 12000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.10 (d, J = 12.3, 1H, H-4′), 4.48−4.52 (m, 3H, H-3′, OCH_2), 5.48 (dd, J = 3.3, J = 51.3, 1H, H-2′), 5.63 (s, 1H, H-5), 5.94 (d, J = 6.6 Hz, 1H, HO-3′), 6.02 (d, J = 18.0 Hz, 1H, H-1′), 7.99 (d, J = 6.3 Hz, 1H, H-6). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −196.663. ESI-TOF: m/z calcd for $C_9H_9FN_2O_4$ [M + Na]$^+$ 251.0439, found 251.0437.

*Method B (from Compound **13**).* Compound **13** (0.390 g, 1.43 mmol) was suspended in MeOH (10 mL), K_2CO_3 (0.020 g, 0.15 mmol) was added, and the reaction mixture was stirred for 20 min. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/MeOH, 95:5). Compound **8** was obtained as colorless solid (0.281 g, 86%). Analytical data were identical to the data described for method A.

1-[3-*O*-Acetyl-2-deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl] uracil (10). Compound **9**[9a] (5.4 g, 9.84 mmol) was dissolved in dry pyridine, acetic anhydride (3.24 g, 3.0 mL, 31.7 mmol) was added, and the solution was stirred for 30 min. The solvent was evaporated, and the remaining oily residue was coevaporated with toluene (3 × 10 mL) and MeOH (3 × 10 mL) to afford **10** as a yellowish foam in quantitative yield. The compound was directly used for the next step. An analytical sample of the compound was purified by FC (silica gel, column 15 × 4 cm, CH_2Cl_2/acetone, 4:1). TLC (silica gel, CH_2Cl_2/acetone, 4:1): R_f 0.60. λ_{max} (MeOH)/nm 235 (ε/dm^3 mol^{-1} cm^{-1} 22600), 260sh (11000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.07 (s, 3H, CH_3), 3.29−3.30 (m, 2H, 2 × H-5′), 3.73 (s, 6H, OCH_3), 4.17−4.22 (m, 1H, H-4′), 5.26−5.37 (m, 1H, H-3′), 5.53 (d, J = 7.8 Hz, 1H, H-5), 5.54 (dd, J = 5.4 Hz, J = 52.8 Hz, 1H, H-2′), 5.88 (d, J = 22.2 Hz, 1H, H-1′), 6.86−6.90 (m, 4H, arom. H), 7.23−7.38 (m, 9H, arom. H), 7.79 (d, J = 8.1 Hz, 1H, H-6), 11.50 (bs, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −197.685. ESI-TOF: m/z calcd for $C_{32}H_{31}FN_2O_8$ [M + Na]$^+$ 613.1957, found 613.1952.

1-[3-*O*-Acetyl-2-deoxy-2-fluoro-β-D-ribofuranosyl]uracil (11). Crude compound **10** (5.8 g, 9.84 mmol) was dissolved in glacial acetic acid/MeOH (4:1, 50 mL, v/v), and the mixture was stirred for 12 h at

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room temperature. The solvent was evaporated, and the remaining residue was coevaporated with MeOH (3 × 10 mL) and toluene (3 × 10 mL). The resulting foam was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/acetone, 4:1) to afford compound **11** (2.5 g, 88%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 4:1): R_f 0.35. λ_{max} (MeOH)/nm 258 (ε/dm^3 mol^{-1} cm^{-1} 8700). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H, CH_3), 3.55−3.60 (m, 1H, H-5′), 3.68−3.71 (m, 1H, H-5′), 4.12−4.14 (m, 1H, H-4′), 5.16−5.27 (m, 2H, H-3′, OH-5′), 5.41 (dt, J = 3.6 Hz, J = 52.5 Hz, 1H, H-2′), 5.68 (d, J = 8.1 Hz, 1H, H-5), 5.95 (dd, J = 3.0 Hz, J = 18.9 Hz, 1H, H-1′), 7.86 (d, J = 7.8 Hz, 1H, H-6), 11.46 (bs, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −202.456. ESI-TOF: m/z calcd for $C_{11}H_{13}FN_2O_6$ [M + Na]$^+$ 311.0650, found 311.0654.

1-[3-*O*-Acetyl-2-deoxy-2-fluoro-5-*O*-(*p*-toluenesulfonyl)-β-D-ribofuranosyl]uracil (12). Compound **11** (2.9 g, 10.06 mmol) was dissolved in dry pyridine (15 mL), *p*-toluenesulfonyl chloride (6.329 g, 33.2 mmol) was added, and the resulting mixture was stirred for 12 h at room temperature. The reaction was quenched by the addition of ice-cold water (10 mL). Then CH_2Cl_2 (25 mL) was added, and the organic layer was washed saturated $NaHCO_3$ solution (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried and filtrated, and the solvent was evaporated. The remaining residue was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/acetone, 4:1) furnishing compound **12** (3.9 g, 88%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 4:1): R_f 0.70. λ_{max} (MeOH)/nm 224 (ε/dm^3 mol^{-1} cm^{-1} 14400), 260 (10400). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.06 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 4.17−4.23 (m, 2H, 2 × H-5′), 4.37−4.40 (m, 1H, H-4′), 5.12−5.22 (m, 1H, H-3′), 5.51 (dd, J = 5.4 Hz, J = 52.5 Hz, 1H, H-2′), 5.64 (d, J = 7.8 Hz, 1H, H-5), 5.77 (d, J = 22.2 Hz, 1H, H-1′), 7.41−7.44 (m, 2H, arom. H), 7.61 (d, J = 8.1 Hz, 1H, H-6), 7.74−7.77 (m, 2H, arom. H), 11.47 (s, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −197.012. ESI-TOF: m/z calcd for $C_{18}H_{19}FN_2O_8S$ [M + Na]$^+$ 465.0738, found 465.0740.

3′-*O*-Acetyl-2,5′-anhydro-2′-deoxy-2′-fluorouridine (13). Compound **12** (1.5 g, 3.4 mmol) was dissolved in dry CH_3CN (20 mL), DBU (1,8-diazabicyclo[5.4.0]undec-7-en, 540 μL, 0.550 g, 3.6 mmol) was added, and the reaction mixture was heated under reflux for 50 min. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/MeOH, 90:10). Evaporation of the main zone afforded compound **13** as colorless solid (0.730 g, 79%). TLC (silica gel, CH_2Cl_2/MeOH, 90:10): R_f 0.50. λ_{max} (MeOH)/nm 237 (ε/dm^3 mol^{-1} cm^{-1} 11900). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H, CH_3), 4.20 (d, J = 12.6 Hz, 1H, H-5′), 4.57 (d, J = 12.6 Hz, 1H, H-5′), 4.84 (s, 1H, H-4′), 5.49 (d, J = 5.1 Hz, 1H, H-3′), 5.79 (dd, J = 6.0 Hz, J = 49.5 Hz, 1H, H-2′), 5.97 (d, J = 7.5 Hz, 1H, H-5), 6.12 (d, J = 18.3 Hz, 1H, H-1′), 7.98 (d, J = 7.2 Hz, 1H, H-6). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −195.067. ESI-TOF: m/z calcd for $C_{11}H_{11}FN_2O_5$ [M + Na]$^+$ 293.0544, found 293.0542.

2-Amino-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)pyrimidin-4(3*H*)-one (1c). Compound **8** (0.280 g, 1.23 mmol) was dissolved in MeOH saturated with NH_3 at 0 °C (50 mL), and the solution was stirred for 12 h at room temperature. The solvent was evaporated, and the remaining residue was adsorbed on silica gel and applied to FC (silica gel, column 15 × 4 cm, CH_2Cl_2/MeOH, 80:20). Evaporation of the main zone afforded compound **1c** as colorless solid (0.173 g, 58%). TLC (silica gel, CH_2Cl_2/MeOH, 80:20): R_f 0.30. λ_{max} (MeOH)/nm 210 (ε/dm^3 mol^{-1} cm^{-1} 23200), 227(sh) (11700), 251(sh) (5800). λ_{max} (H_2O)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 5100). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.58−3.74 (m, 2H, H-5′), 3.90−3.92 (m, 1H, H-4′), 4.13−4.22 (m, 1H, H-3′), 5.07 (dd, J = 3.9 Hz, J = 53.1 Hz, 1H, H-2′), 5.37 (bs, 1H, HO-5′), 5.58 (d, J = 7.8 Hz, 1H, H-5), 5.74 (d, J = 5.4 Hz, 1H, HO-3′), 5.90 (dd, J = 3.3 Hz, J = 15.9 Hz, 1H, H-1′), 7.06 (bs, 2H, NH_2), 7.66 (d, J = 7.8 Hz, 1H, H-6). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −204.934. ESI-TOF: m/z calcd for $C_9H_{12}FN_3O_4$ [M + H]$^+$ 246.0885, found 246.0892.

Conversion of 2-Amino-1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-pyrimidin-4(1*H*)-one (1c) to Cyclic Byproducts 14 and 15. Compound **1c** (0.1 g, 0.33 mmol) was dissolved in aqueous concentrated NH_3, and the reaction mixture was heated at 55 °C in a steel bomb for 16 h. The solvent was evaporated, the remaining residue was subjected to FC (silica gel, CH_2Cl_2/MeOH, 70:30), and two products (**14**, **15**) were isolated.

2,2′-Imino-1-(2-deoxy-β-D-arabinofuranosyl)uracil (14). From the slower migrating zone compound **14** was isolated as colorless solid (0.050 g, 54%). TLC (silica gel, CH_2Cl_2/MeOH, 70:30) R_f 0.27. λ_{max} (H_2O)/nm 260 (ε/dm^3 mol^{-1} cm^{-1} 4000), 263 (4100). ^{1}H NMR (DMSO-d_6, 300 MHz): 3.10−3.27 (m, 2H, 2 × H-5′), 3.95−4.00 (m, 1H, H-4′), 4.11 (s, 1H, H-3′), 4.19 (d, J = 6.6 Hz, 1H, H-2′), 4.92 (bs, 1H, HO-5′), 5.49 (d, J = 7.5 Hz, 1H, H-5), 5.62 (bs, 1H, HO-3′), 6.12 (d, J = 6.3 Hz, 1H, H-1′), 7.56 (d, J = 7.5 Hz, 1H, H-6), 8.47 (s, 1H, NH). ESI-TOF: m/z calcd for $C_9H_{11}N_3O_4$ [M + Na]$^+$ 248.0642, found 248.0654.

Imidazo[1,2-*a*]pyrimidin-7-(8*H*)-one (15). From the faster migrating zone, compound **15** was isolated as a colorless solid (0.006 g, 11%). TLC (silica gel, CH_2Cl_2/MeOH, 70:30): R_f 0.47. ^{1}H NMR (DMSO-d_6, 300 MHz): 6.00 (d, J = 7.5 Hz, 1H, H-6), 7.00 (s, 1H, H-2), 7.33 (s, 1H, H-3), 8.28 (d, J = 7.8 Hz, 1H, H-5), 12.29 (br s, 1H, NH). Analytical data of **14** and **15** are in agreement in ref 23.

1-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-2-[[(dimethylamino)-methylidene]amino]-pyrimidin-4(1*H*)-one (17). Compound **1c** (0.510 g, 2.08 mmol) was dissolved in MeOH (10 mL), and *N*,*N*-dimethylformamide dimethyl acetal (0.900 g, 1.0 mL, 7.6 mmol) was added. The resulting mixture was stirred for 1 h at room temperature. The solvent was evaporated, and the remaining residue was applied to FC (silica gel, column 10 × 4 cm, CH_2Cl_2/MeOH, 85:15). Evaporation of the main zone afforded compound **17** as a colorless solid (0.481 g, 77%). TLC (silica gel, CH_2Cl_2/MeOH, 4:1): R_f 0.40. λ_{max} (MeOH)/nm 242 (ε/dm^3 mol^{-1} cm^{-1} 17500), 282 (25200). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.00 (s, 3H, NCH_3), 3.18 (s, 3H, NCH_3), 3.58−3.65 (m, 1H, H-5′), 3.78−3.83 (m, 1H, H-5′), 3.88−3.91 (m, 1H, H-4′), 4.04−4.14 (m, 1H, H-3′), 4.94 (dd, J = 3.6 Hz, J = 52.8 Hz, 1H, H-2′), 5.27 (t, J = 4.8 Hz, 1H, 5′−OH), 5.63 (d, J = 6.0 Hz, 1H, 3′−OH), 5.70 (d, J = 7.8 Hz, 1H, H-5), 6.32 (d, J = 16.8 Hz, 1H, H-1′), 7.96 (d, J = 7.8 Hz, 1H, H-6), 8.58 (s, 1H, N=CH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −202.563. ESI-TOF: m/z calcd for $C_{12}H_{17}FN_4O_4$ [M + H]$^+$ 301.1307, found 301.1310.

1-[2-Deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-2-[[(dimethylamino)methylidene]amino]pyrimidin-4(1*H*)-one (18). Compound **17** (0.240 g, 0.80 mmol was dissolved in dry pyridine (5 mL), 4,4′-dimethoxytrityl chloride (0.700 g, 2.08 mmol) was added in portions, and the reaction mixture stirred until the starting material was completely consumed (∼5 h, TLC monitoring). The solution was diluted with CH_2Cl_2 (20 mL) and extracted with 5% $NaHCO_3$ solution. The organic layer was dried, and the solvent was evaporated. The remaining residue was subjected to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH, 90:10 containing 1 pipet triethylamine per 500 mL of solvent). Evaporation of the main zone afforded **18** (0.365 g, 76%) as a colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 9:1): R_f 0.55. λ_{max} (MeOH)/nm 237 (ε/dm^3 mol^{-1} cm^{-1} 35500), 282 (33000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.00 (s, 3H, NCH_3), 3.19 (s, 3H, NCH_3), 3.31−3.40 (m, 2H, 2 × H-5′), 3.71 (s, 6H, 2 × OCH_3), 4.02−4.06 (m, 1H, H-4′), 4.27−4.39 (m, 1H, H-3′), 5.00 (dd, J = 3.9 Hz, J = 52.5 Hz, 1H, H-2′), 5.33 (d, J = 7.8 Hz, 1H, HO-3′), 5.72 (d, J = 6.9 Hz, 1H, H-5), 6.33 (d, J = 18.3 Hz, 1H, H-1′), 6.88−6.91 (m, 4H, arom. H), 7.24−7.40 (m, 9H, arom. H), 7.81 (d, J = 7.8 Hz, 1H, H-6), 8.60 (s, 1H, N=CH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −201.243. ESI-TOF: m/z calcd for $C_{33}H_{35}FN_4O_6$ [M + H]$^+$ 603.2613, found 603.2631.

1-[2-Deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-2-[[(dimethylamino)methylidene]amino]pyrimidin-4(1*H*)-one 3′-*O*-[(2-Cyanoethyl)-(*N*,*N*-diisopropyl)]-phosphoramidite (19). Compound **18** (0.360 g, 0.60 mmol) was dissolved in dry CH_2Cl_2 (10 mL), (*i*-Pr)$_2$NEt (0.138 g, 180 μL, 1.07 mmol) and 2-cyanoethyl *N*,*N*-diisopropyl phosphoramidochloridite (0.191 g, 180 μL, 0.81 mmol) were added, and the reaction mixture was stirred for 1 h at room temperature. The solution was diluted with CH_2Cl_2 (20 mL) and extracted with 5% $NaHCO_3$ solution. The organic layer was dried, and the solvent was evaporated. The remaining residue was subjected to FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH/triethylamine 94.5:5:0.5). Evaporation of the

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main zone afforded **19** (0.361 g, 75%) as a colorless foam. TLC (silica gel, CH_2Cl_2/MeOH/triethylamine, 90:9:1): R_f 0.55. ^{19}F NMR ($CDCl_3$, 282.4 MHz): δ −201.07, −201.04, −201.01, −200.98. ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ 151.23, 151.17, 150.59, 150.52. ESI-TOF: m/z calcd for $C_{42}H_{52}FN_6O_7P$ $[M + H]^+$ 803.3692, found 803.3674.

6-Amino-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-1,9-dihydro-2*H*-purin-2-one (3c). Compound **16**[25] (0.568 g, 2.00 mmol) was suspended in H_2O (18 mL) at 50 °C, and $NaNO_2$ (0.541 g, 7.73 mmol) in H_2O (4 mL) was introduced. Then, acetic acid (0.833 g, 0.79 mL, 13.87 mmol) was added dropwise to the solution at 50 °C in 2 min. The resulting clear solution was stirred for 20 min at 50 °C. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with water (8 mL) and cooled in an ice bath, and then concd aq NH_3 was added dropwise until the pH reached 8. Then the solution was evaporated, and the remaining residue was redissolved in 50 mL of water. The pH of the solution was adjusted to 5−6, and the solution was passed through a pad of RP-18 silica gel (mesh 40−63 μM, 50 g). Inorganic salts were removed with water (2 × 10 mL), and the nucleoside was eluted with methanol (2 × 50 mL). The combined methanol portions were evaporated to obtain **3c** as a pale yellow solid (0.450 g, 79%). TLC (silica gel, *i*-PrOH/H_2O/NH_3, 70:20:10) R_f 0.75. λ_{max} (H_2O)/nm 247 (ε/dm^3 mol^{-1} cm^{-1} 8300), 260 (4400), 292 (9600). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.53−3.72 (m, 2H, 2 × H-5′), 3.94 (bs, 1H, H-4′), 4.35−4.42 (m, 1H, H-3′), 5.32 (d, J = 52.8 Hz, 1H, H-2′), 5.98 (d, J = 15.3 Hz, 1H, H-1′), 7.98 (s, 1H, H-8). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −205.9. ESI-TOF: m/z calcd for $C_{10}H_{12}FN_5O_4$ $[M + Na^+]$ 308.0766, found 308.0775.

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-6-[[1-(dimethylamino)ethylidene]amino]-1,9-dihydro-2*H*-purin-2-one (20). To a suspension of compound **3c** (0.660 g, 2.31 mmol) in methanol (40 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (0.826 g, 0.91 mL, 6.20 mmol), and the resulting mixture was stirred at room temperature for 2 h (TLC monitoring). The solvent was evaporated, and the residue was coevaporated with methanol and subjected to FC (silica gel, CH_2Cl_2/MeOH, 85:15) to obtain **20** (0.670 g, 82%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH, 70:30) R_f 0.51. λ_{max} (MeOH)/nm 337 (ε/dm^3 mol^{-1} cm^{-1} 18900), 251 (9000). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.12 (s, 3H, CH_3), 3.07 (s, 3H, CH_3), 3.15 (s, 3H, CH_3), 3.51−3.58 (m, 1H, H-5′), 3.68−3.72 (m, 1H, H-5′), 3.92 (br s, 1H, H-4′), 4.33−4.42 (m, 1H, H-3′), 5.27−5.46 (m, 2H, H-2′, 5′−OH), 5.70 (d, J = 5.7 Hz, 1H, 3′−OH), 5.97 (dd, J = 3.3 Hz, J = 16.5 Hz, 1H, H-1′), 8.03 (s, 1H, H-8), 11.02 (br s, 1H, NH). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −205.8. ESI-TOF: m/z calcd for $C_{14}H_{19}FN_6O_4$ $[M + Na^+]$ 377.1344, found 377.1347.

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-6-[[1-(dimethylamino)ethylidene]amino]-1,9-dihydro-2*H*-purin-2-yl Diphenyl Carbamate (21). To a solution of compound **20** (0.610 g, 1.72 mmol) in pyridine (10 mL) were added *N*,*N*-diphenylcarbamoyl chloride (0.598 g, 2.58 mmol) and (*i*-Pr)$_2$EtN (0.334 g, 0.44 mL, 2.58 mmol). The mixture was stirred at room temperature for 60 min, poured into 5% aqueous $NaHCO_3$ (20 mL), and extracted with CH_2Cl_2 (3 × 40 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated, and the residue was applied to FC (silica gel, CH_2Cl_2/MeOH, 96:4) to yield **21** (0.670 g, 71%) as a colorless foam. TLC (silica gel, CH_2Cl_2/MeOH, 90:10) R_f 0.56. λ_{max} (MeOH)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 32300), 310 (27800). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.11 (s, 3H, CH_3), 3.12 (s, 3H, CH_3), 3.14 (s, 3H, CH_3), 3.56−3.63 (m, 1H, H-5′), 3.73−3.78 (m, 1H, H-5′), 3.98−3.99 (m, 1H, H-4′), 4.42−4.48 (m, 1H, H-3′), 5.18 (t, J = 5.1 Hz, 1H, 5′−OH), 5.39 (dt, J = 3.0 Hz, J = 52.8 Hz, 1H, H-2′), 5.75 (br s, 1H, 3′−OH), 6.21 (dd, J = 2.4 Hz, J = 16.7 Hz, 1H, H-1′), 7.28−7.33 (m, 2H, Ar-H), 7.42−7.44 (m, 8H, Ar-H), 8.43 (s, 1H, H-8). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −204.7. ESI-TOF: m/z calcd for $C_{27}H_{28}FN_7O_5$ $[M + Na^+]$ 572.2028, found 572.2013.

9-[2-Deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-6-[[1-(dimethylamino)ethylidene]amino]-1,9-dihydro-2*H*-purin-2yl-diphenylcarbamate (22). To a solution of compound **21** (0.5 g, 0.91 mmol) in pyridine (1 mL) was added 4,4′-dimethoxytrityl chloride (0.462 g, 1.36 mmol). The mixture was stirred at room temperature for 90 min. After completion of the reaction (TLC monitoring), 40 mL of CH_2Cl_2 was added and the mixture washed with 5% aq $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and coevaporated with toulene (2 × 5 mL), and the residue was applied to FC (silica gel, CH_2Cl_2/acetone, 84:16) to obtain compound **22** (0.575 g, 74%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.29. λ_{max} (MeOH)/nm 234 (ε/dm^3 mol^{-1} cm^{-1} 48000), 309 (23500). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.07 (s, 3H, CH_3), 3.11 (s, 6H, 2 × CH_3), 3.16−3.29 (m, 2H, 2 × H-5′), 3.68 (s, 6H, 2 × OCH_3), 4.09−4.10 (m, 1H, H-4′), 4.60−4.67 (m, 1H, H-3′), 5.51 (dd, J = 3.6 Hz, J = 52.7 Hz, 1H, H-2′), 5.75 (d, J = 5.1 Hz, 1H, 3′−OH), 6.26 (d, J = 18.9 Hz, 1H, H-1′), 6.75−6.80 (m, 4H, Ar-H), 7.14−7.40 (m, 19H, Ar−H), 8.30 (s, 1H, H-8). ^{19}F NMR (DMSO-d_6, 282.4 MHz): δ −202.3. ESI-TOF: m/z calcd for $C_{48}H_{46}FN_7O_7$ $[M + Na^+]$ 874.3335, found 874.3315.

9-[2-Deoxy-2-fluoro-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-6-[[1-(dimethylamino)ethylidene]amino]-1,9-dihydro-2*H*-purin-2yl Diphenyl Carbamate 3′-*O*-[(2-Cyanoethyl)-(*N*,*N*-diisopropyl)]phosphoramidite (23). To a solution of compound **22** (0.250 g, 0.29 mmol) in dry CH_2Cl_2 (3 mL) were added (*i*-Pr)$_2$NEt (0.065 g, 85 μL, 0.50 mmol) and 2-cyanoethyl diisopropylphosphoramidochloridite (0.103 g, 98 μL, 0.44 mmol), and the reaction mixture was stirred for 90 min at room temperature. After completion of the reaction (TLC monitoring), the reaction mixture was diluted with CH_2Cl_2 (30 mL), poured into 5% $NaHCO_3$ solution (30 mL), and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The residual foam was applied to FC (silica gel, eluted with CH_2Cl_2/acetone, 85:15). Evaporation of the main zone afforded **23** (0.205 g, 66%) as a colorless foam. TLC (silica gel, CH_2Cl_2/acetone, 80:20) R_f 0.53. ^{31}P NMR ($CDCl_3$, 121.5 MHz): δ 151.5, 151.4, 151.2, 151.1. ^{19}F NMR ($CDCl_3$, 282.4 MHz): δ −201.54, −201.56, −201.66, −201.69. ESI-TOF: m/z calcd for $C_{57}H_{63}FN_9O_8P$ $[M + Na^+]$ 1074.4413, found 1074.4376.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H−^{13}C coupling constants, HPLC profiles, copies of ^{1}H, ^{13}C, ^{31}P, ^{19}F NMR, DEPT-135, and ^{1}H−^{13}C gated-decoupled NMR spectra, melting profiles, T_m values, thermodynamic data, and CD spectra. 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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