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Synthesis of 2′-*C*-Difluoromethylribonucleosides and Their Enzymatic Incorporation into Oligonucleotides

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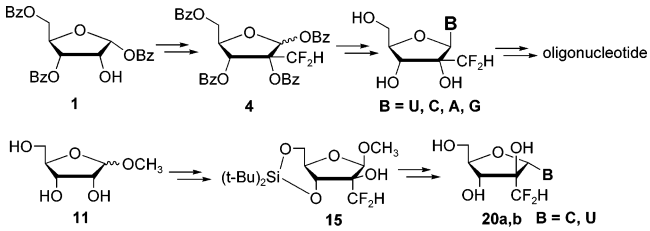

Nucleosides bearing a branched ribose have significant promise as therapeutic agents and biotechnological and biochemical tools. Here we describe synthetic entry into a new subclass of these analogues, 2′-*C*-*β*-difluoromethylribonucleosides. We constructed the glycosylating agent **4** in three steps from 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **1**. The key steps included nucleophilic addition of difluoromethyl phenyl sulfone to 2-ketoribose **2** followed by mild and efficient reductive desulfonation. Ribofuranose **4** glycosylated bis(trimethylsilyl)uracil directly, giving difluoromethyluridine **7** efficiently after deprotection. Conversion of **4** to the corresponding ribofuranosyl bromide allowed efficient access to C, A, and G analogues. A related approach starting from methyl D-ribofuranose offered synthetic entry into the diastereomeric manifold, 2′-*C*-α-difluoromethylarabino-α-pyrimidine. To incorporate 2′-*C*-*β*-difluoromethyluridine into an oligodeoxynucleotide we converted **7** to the bisphosphate and carried out successive ligation reactions using T_4 RNA ligase and T_4 DNA ligase. Analogous to natural RNA linkages, the resulting oligonucleotide undergoes hydroxide-catalyzed backbone scission at the difluoromethyluridine residue via internal transphosphorylation.

Introduction

Over the past decade 2′-*C*-branched nucleosides have garnered much attention for their potential anticancer and antiviral activities.[1−9] For example, 2′-*C*-*β*-methyladenosine and the corresponding 7-deazaadenosine analogue potently inhibit Hepatitis C Virus (HCV) RNA replication in vivo.[10,11] Within oligonucleotides, 2′-branched nucleotides impart increased nuclease resistance in biological media and have allowed artificial regulation of gene expression by antisense approaches,[12−16] self-cleaving RNA motifs,[17] or the combination thereof.[18]

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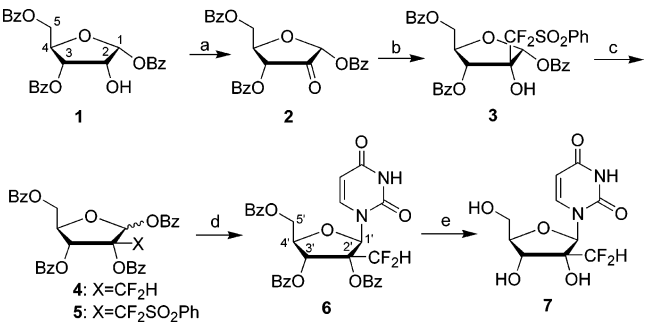
In this context, 2′-*C*-difluoromethyl ribonucleosides represent important synthetic targets as fluorination of nucleosides may enhance their therapeutic activities.[19−22]

2′-*C*-Branched nucleosides also serve as valuable probes to explore biomolecular structure and function of protein and RNA enzymes.[4,5,23,24] For example, 2′-*C*-methyluridine has been used to investigate nucleases,[23] ribonucleotide reductase,[5] and RNA polymerase.[25] 2′-*C*-Methylcytidine[26] and 2′-*C*-α-difluoromethyl-arabinouridine[27] have been used to investigate the ai5γ group II intron ribozyme and the hammerhead ribozyme, respectively. Our interest in 2′-*C*-branched ribonucleosides arises from their possible value as mechanistic agents with which to vary the pK_a of the 2′-hydroxyl group. A series of ribonucleotides bearing substituents of increasing electron-withdrawing power (CH_3, CH_2F, CHF_2, CF_3) might allow such pK_a variation, thereby enabling the application of physical organic approaches to study RNA-mediated biological processes.

Previous work has described the synthesis of several analogues in this series.[28−30] Here we aim to develop a reliable synthesis of 2′-*C*-*β*-difluoromethylribonucleosides. Two general strategies prevail for the synthesis of 2′-*C*-branched ribonucleosides: (a) a linear approach starting from natural nucleosides and (b) a convergent approach in which nucleobases are glycosylated with the appropriately modified sugar. The linear approach often suffers from poor stereoselectivity during installation of the branch. The convergent approach offers greater stereocontrol during installation of the branch by starting with a sugar bearing suitable substituents configured with the appropriate stereochemistry. The convergent approach also offers greater flexibility in that a variety of nucleobases may be glycosylated, saving synthetic steps overall. Here we develop a convergent approach for the first synthesis of 2′-*C*-*β*-difluoromethylribonucleosides and their diastereomeric counterparts, 2′-*C*-α-difluoromethyl-arabino-α-nucleosides. We exploit an enzymatic ligation procedure to incorporate the uridine analogue site-specifically into an oligodeoxynucleotide.

SCHEME 1[a]



[a] Conditions: (a) Dess−Martin periodinane, CH_2Cl_2, rt, 48 h, 98%; (b) $PhSO_2CF_2H$, LiHMDS, THF, −78 to 0 °C, 2 h, 64%; (c) (i) 5% Na−Hg, Na_2HPO_4, MeOH, THF, H_2 atmosphere, 0 °C, 45 min; (ii) BzCl, DMAP, Et_3N, CH_2Cl_2, rt, 5 h, 61% in two steps; (d) bis(trimethylsilyl)uracil, $SnCl_4$, CH_3CN, reflux, 2 days, 78%; (e) NH_3, MeOH, 4 °C, 2 days, 95%.

Results and Discussion

Synthesis of 2′-*C*-*β*-Difluoromethylribonucleosides. Diethylaminosulfur trifluoride (DAST) under mild conditions converts aldehydes, including those appended to nucleosides, to a difluoromethyl group.[22,31,32] On this basis we prepared 2′-*C*-*β*-formyluridine as a putative precursor to 2′-*C*-difluoromethyluridine via DAST-mediated fluorination. We obtained 1,3,5-tri-*O*-benzoyl-2-oxo-α-D-*erythro*-pentofuranose **2** as previously described[33] by oxidation of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose **1** with Dess−Martin periodinane. Vinylation and glycosylation generated 2′,3′,5′-tri-*O*-benzoyl-2′-vinylribouridine as described by Harry-O'kuru et al.[34] Subsequent oxidation with OsO_4/KIO_4 gave 2′-*C*-formyluridine. Treatment of this compound with DAST under various conditions gave none of the desired product, possibly due to the steric hindrance of the uracil base (Liao, X.; Piccirilli, J. A. Unpublished results).

Sabol and McCarthy prepared 2′-*C*-α-difluoromethyl-arabinocytidine in two steps from 2′-ketocytidine.[35] Addition of difluoromethyl phenyl sulfone in the presence of lithium hexamethyldisilazane in THF/HMPA gave 2′-*C*-α-(phenylsulfonyl)difluoromethyl-arabinocytidine. Subsequent reduction with SmI_2 converted the phenylsulfonyl difluoromethyl group to a difluoromethyl group. Inspired by this report, we investigated the reaction of ketoribose **2** with difluoromethyl phenyl sulfone under the same conditions. The reaction consumed starting material but yielded none of the desired product. However, in the absence of HMPA the reaction gave sulfone **3** in reasonably good yield (64%, Scheme 1). $^{19}F-^{1}H$ NOE

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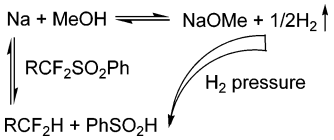

FIGURE 1. Hydrogen pressure shifts the reduction equilibrium.

experiments with **3** indicated that the phenylsulfonyl difluoromethyl group added stereoselectively to the β-face of the sugar. Irradiation of ^{19}F nuclei resulted in strong NOE signals for the 1-H (δ 7.08 ppm) and 3-H (δ 6.03 ppm) heterocyclic protons, suggesting that 1-H, 3-H, and the phenylsulfonyl difluoromethyl group reside on the same side of the ribofuranose ring.

Reduction of **3** with SmI_2 in THF/HMPA[36] consumed the starting material but gave no desired product. Without HMPA the reaction produced a major product that lacked both the phenylsulfonyl difluoromethyl group and the 1-*C*-benzoyl group. To circumvent this apparent over-reduction, we tested alternative mild reducing agents. Among the reagents tested, including Raney Ni, Mg−MeOH−Na_2HPO_4,[37] $[(Cp_2NiAlH_2)^-Li^+]_2$,[38] and Na(Hg)−MeOH−Na_2HPO_4,[27,39] only Na(Hg)−MeOH−Na_2HPO_4 appeared suitable. Exposure of **3** to this reagent at −10 to 0 °C for 2 h followed by treatment with benzoyl chloride resulted in 15% difluoromethylribose **4** and 20% unreduced adduct **5**. Longer reaction time resulted in destruction of the sugar moiety. Using THF as solvent and stoichiometric equivalents of methanol and Na−Hg improved the yields of both **4** (25%) and **5** (30%). Strikingly, we observed that under a hydrogen atmosphere (hydrogen balloon) reduction completes in about 40 min as monitored by TLC, and the yield of **4** improves to 61% ($\beta/\alpha = 3/4$). The hydrogen pressure may shift the equilibrium for the redox reaction between sodium and methanol, minimizing depletion of sodium and thereby favoring reduction of the phenyl sulfone group (Figure 1). To the best of our knowledge, this is the first demonstration that hydrogen pressure can assist in single-electron-transfer reduction by Na−Hg−MeOH−Na_2HPO_4.

We tested difluoromethylribose **4** as a glycosylating agent for bis(trimethylsilyl)uracil. Ribose derivatives usually glycosylate persilylated nucleobases rapidly and stereoselectively to yield the β-nucleosides.[40−42] However, **4** exhibits significantly weaker glycosylating power than the corresponding ribose derivative, presumably reflecting both the strong electron-withdrawing character and the steric bulk of the difluoromethyl group, which would destabilize the putative carbocation intermediate and hinder the addition of the nucleobases at the desired β-face, respectively. Glycosylation of bis(trimethylsilyl)-uracil with **4** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at room temperature resulted in recovered starting material only. Using the stronger Lewis acid $SnCl_4$, the glycosylation reaction gave uridine **6** in about 5% yield after 2 days at room temperature. Under the same conditions at reflux in CH_3-CN (83 °C), the yield of **6** improved significantly (78%). The stereochemistry was verified by $^{19}F-^1H$ NOE experiments. Upon irradiation of the ^{19}F nuclei of the difluoromethyl group, strong NOE signals occur for CF_2H (δ 6.58 ppm), 3′-H (δ 6.08 ppm), and 6-H (δ 7.57 ppm). In NOESY spectra, 1′-H (δ 6.97 ppm) exhibits strong cross peaks with 4′-H (δ 5.01 ppm) but not with CF_2H or 3′-H. Debenzoylation of **6** with saturated ammonia in methanol at 4 °C for 2 days provided 2′-*C*-β-difluoromethyluridine **7** in 95% yield. CD spectroscopy further confirmed the β anomeric configuration: **7** exhibits a positive Cotton effect between 260 and 280 nm, as expected for a pyrimidine nucleoside in the β anomeric configuration.[43]

As observed for bis(trimethylsilyl)uracil, TMSOTf proved insufficient in the glycosylation of persilylated cytosine, adenine, and guanine (appropriately protected) with difluoromethylribose **4**, even at high temperature (120 °C). In the presence of the more activated Lewis acid $SnCl_4$, **4** decomposed, yielding no desired product, similar to a previous report.[44] Possibly $SnCl_4$ forms a "σ complex" with the stronger nucleobases, leading to undesired side reactions.[42] As a consequence of these observations, we investigated transformation of **4** to the more active difluoromethylribofuranosyl bromide **8**. At 80 °C in the presence of 30% HBr/acetic acid **4** gives the bromide **8** in 65% yield (Scheme 2). NOESY experiments supported the assigned stereochemistry, showing strong cross peaks between CF_2H (δ 6.11 ppm) and 1′-H (δ 6.99 ppm) as well as 3′-H (δ 5.43 ppm).

In the presence of HgO/$HgBr_2$,[29] bromide **8** glycosylates persilylated N^4-benzoylcytosine and N^6-benzoyladenine to give exclusively the corresponding β-nucleoside derivatives **9a** and **9b** in 65% and 50% yield, respectively. NOESY experiments revealed the stereochemistry of **9a** and **9b**: 1′-H (δ 6.09 ppm for **9a**, 6.37 ppm for **9b**) exhibits much stronger cross peaks with 4′-H (δ 4.75 ppm for **9a**, 4.78 ppm for **9b**) than with 3′-H (δ 5.63 ppm for **9a**, 6.25 ppm for **9b**). Additionally, 1′-H in **9b** lacks strong cross peaks with CF_2H (in **9a**, these two peaks are too close to determine which cross peaks occur). Debenzoylation of **9a** and **9b** in the same manner as described for **6** affords the parent nucleosides **10a** and **10b** in 96% and 93% yield, respectively.

For the synthesis of the guanosine derivative we conducted the glycosylation reaction with N^2-acetyl-O^6-diphenylcarbamoylguanine to minimize formation of the undesired *N*-7 isomer.[45,46] Persilylated N^2-acetyl-O^6-diphenylcarbamoylguanine was exposed to **8** in the presence of HgO/$HgBr_2$ to give **9c** in 31% yield. NOESY experiments verified the stereochemistry of **9c**. 1′-H (δ

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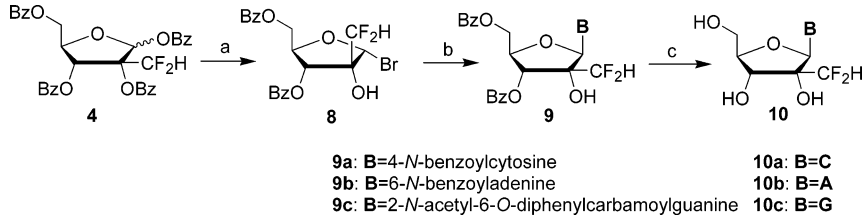
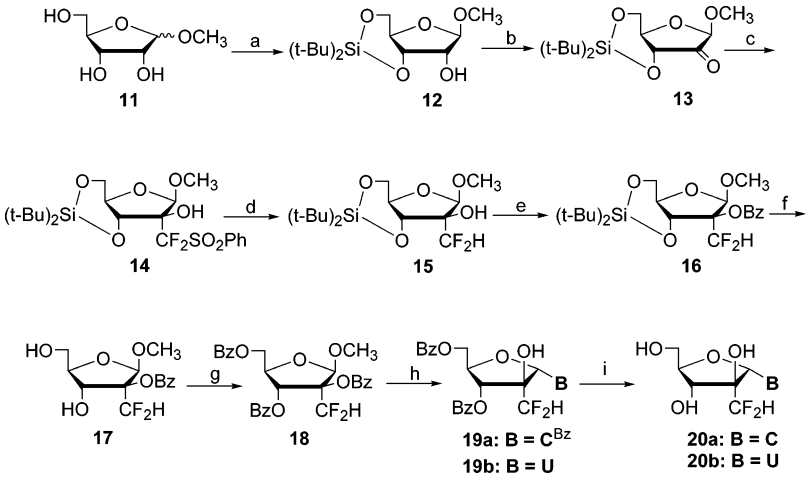
SCHEME 2[a]



[a] Conditions: (a) 30% HBr in AcOH, 80 °C, 5 h, 65%; (b) persilylated nucleobases, HgO/$HgBr_2$, toluene, 80−90 °C, 2−5 h, 31−65%; (c) for **10a** and **10b**, NH_3, MeOH, 4 °C, 2 days, 93−96%; for **10c**, NH_3/H_2O/MeOH, 60 °C, overnight, 55%.

SCHEME 3[a]

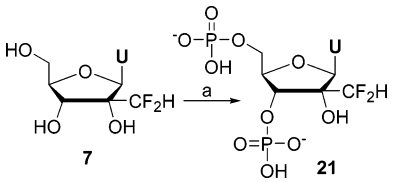


[a] Conditions: (a) (i) $(t\text{-Bu})_2Si(Tf)_2$, 0 °C to rt, 15 min; (ii) Et_3N, 5 min, 59%; (b) Dess−Martin periodinane, overnight, 91%; (c) $PhSO_2CF_2H$, LiHMDS, THF/HMPA (10/1), −78 °C, 2 h, 63%; (d) SmI_2 (4 equiv), THF/HMPA (20:1), 0 °C, 30 min, 74%; (e) BzCl, DMAP, Et_3N, 5 h, 95%; (f) pyridinium poly(hydrogen fluoride), 0 °C to rt, 15 min, 95%; (g) BzCl, DMAP, Et_3N, 5 h, 97%; (h) (i) 30% HBr in AcOH, 75 °C, 4 h; (ii) persilylated nucleobases, HgO/$HgBr_2$, 36 h, 48−55% in two steps; (i) NH_3, MeOH, 4 °C, 2 days, 84−90%.

6.23 ppm) exhibits strong cross peaks with 4′-H (δ 4.78 ppm) but not with CF_2H (δ 5.86 ppm). Incubation of **9c** at 60 °C overnight in NH_3/H_2O/MeOH followed by preparative HPLC gave the parent guanosine nucleoside **10c** in 55% yield.

Synthesis of 2′-*C*-α-Difluoromethyl-arabino-α-pyrimidines. During the synthesis of 2′-*C*-β-difluoromethylribonucleosides an efficient synthesis of 2′-*C*-α-difluoromethyl-arabino-α-pyrimidines emerged, which we briefly describe here (Scheme 3). The strategy parallels that used above but starts from ribose and proceeds through ketone **13**. Treatment of D-ribose with sulfuric acid in methanol gave the methyl D-ribose **11** as an anomeric mixture.[47] Simultaneous protection of the 3- and 5-hydroxyl groups with di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (DTBS)[48] afforded the bis(silyl)ether **12** after careful silica gel chromatography. Subsequent oxidation with Dess−Martin periodinane afforded ketone **13**. Nucleophilic addition of difluoromethyl phenyl sulfone in the presence of lithium hexamethyldisilazane converted **13** exclusively to sulfone **14** in 61% yield. In the presence of HMPA, reduction of the sulfone **14** with SmI_2 generated the difluoromethylribose derivative **15**. The stereochemistry of compound **15** was established by $^{19}F-^{1}H$ NOE experiments. When the ^{19}F nuclei of the difluoromethyl group were irradiated, strong NOE signals occur for

SCHEME 4[a]



[a] Conditions: (a) diphosphoryl chloride, 0 °C to rt, 4 h, 50%.

CF_2H (δ 6.00 ppm) and 1-H (δ 5.05 ppm) but not for 3-H. These observations indicate that the difluoromethyl phenyl sulfone adds to the ketone **13** on the α face.

To install the stereodirecting acyl group for subsequent glycosylation, we converted **15** to **16** with benzoyl chloride in the presence of DMAP and Et_3N. Removal of the DTBS protecting group with pyridinium poly(hydrogen fluoride) (PPHF)[48] followed by benzoylation afforded the tribenzoyl difluoromethyl sugar **18**. Direct coupling of **18** with persilylated bases in the presence of $SnCl_4$ in acetonitrile gave none of the expected products, even after refluxing for 1 week. We therefore converted **18** to the more reactive 1-bromide derivative by incubating **18** in 30% HBr in acetic acid at 75 °C for 4 h. The bromide derivative readily glycosylated persilylated 4-*N*-benzoylcytosine and uracil in the presence of HgO/$HgBr_2$ to give nucleoside derivatives **19a** and **19b** in 48% and 55% yield, respectively. Debenzoylation with ammonia in methanol at 0 °C for 2 days afforded the arabinopyrimidines **20a**,**b** in

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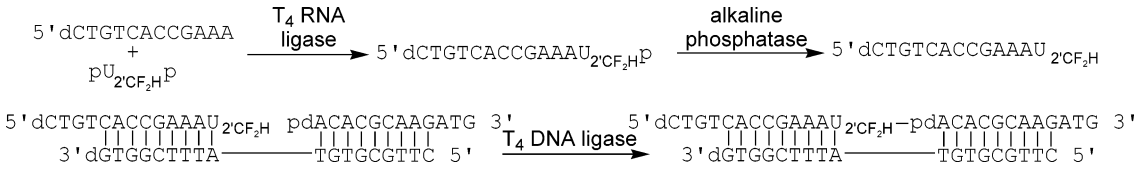
SCHEME 5



80−90% yields. We made no attempt to prepare the corresponding purine analogues. The stereochemistry of arabinonucleosides was revealed by NOESY and $^{19}F-^{1}H$ NOE experiments. For **19a**, 1′-H (δ 7.09 ppm) exhibited strong NOE cross peaks with 3′-H (δ 6.43 ppm) but not with 4′-H (δ 4.94 ppm) nor with CF_2H (δ 6.19 ppm). Lack of NOE signals for 1′-H and 3′-H in the $^{19}F-^{1}H$ NOE experiments of compounds **19a,b** and **20a,b** is also consistent with the structure assignment. Furthermore, CD spectra of **20a,b** show a negative Cotton effect in 260−280 nm, confirming the α anomeric configuration.

Enzymatic Incorporation of 2′-*C*-β-Difluoromethyluridine into Oligonucleotides. Use of 2′-β-modified nucleosides for biochemical investigations of nucleic acid structure and function requires incorporation of these analogues into oligonucleotides. Although we could prepare oligonucleotides containing the methyl analogue using standard phosphoramidite chemistry, the phosphoramidite of the trifluoromethyl analogue proved exceedingly difficult to access (Dai, Q.; Piccirilli, J. A. Unpublished results). To develop a unified and reliable approach to incorporate these analogues we exploited an enzymatic route using 3′,5′-bisphosphorylated derivatives and T_4 RNA ligase.[49,50] We chose uridine analogues to develop a representative incorporation strategy. Difluoromethyluridine 3′,5′-bisphosphate **21** (Scheme 4) was synthesized from uridine **7** using a slightly modified version of the method described by Barrio et al.[50] Reactions were conducted at room temperature instead of −10 to −15 °C to compensate for the lower reactivity of uridine analogues toward phosphorylation.[51] The reaction mixture was purified by ion-exchange chromatography on a DEAE−cellulose column, eluting with a linear gradient of 0.15−0.5 M triethylammonium bicarbonate (TEAB, pH 8.0). NMR showed that only one product, 3′,5′-bisphosphate **21** (confirmed by its action as a nucleotide donor for RNA ligation catalyzed by T_4 RNA ligase) was isolated. We observed no phosphorylation of the 2′-hydroxyl group, presumably because of the electron-withdrawing and steric effects of the difluoromethyl group.

Bisphosphate **21** (donor) was first ligated to the 5′-oligodeoxynucleotide 12mer dCTGTCACCGAAA ($(dN)_{12}$, acceptor) using T_4 RNA ligase in conjunction with an ATP regeneration system (Scheme 5).[52] Following this initial ligation the 3′-terminal phosphate group of the ligation product was removed with calf intestine alkaline phosphatase (CIAP). The resulting dephosphorylated 13mer $(dN)_{12}rU_{2'CF_2H}$ was analyzed by ^{32}P labeling of an aliquot of the reaction mixture followed by electrophoresis through an analytical 20% denaturing polyacrylamide gel (DPAGE), which revealed a conversion yield of 90% (based on the $(dN)_{12}$ acceptor). To verify the presence of the modified nucleoside we synthesized a shorter oligonucleotide ($rCCGAAAU_{2'CF_2H}$) using a similar strategy and confirmed the molecular weight by MALDI mass spectroscopy (calcd, 2237; found, 2235−2238). To synthesize the full-length chimeric RNA we incubated the 13mer $(dN)_{12}rU_{2'CF_2H}$, the donor oligonucleotide pd(ACACGCAAGATG), and a complementary DNA template in the presence of T_4 DNA ligase overnight at 16 °C (Scheme 5). The resulting 25mer ligation product $(dN)_{12}rU_{2'CF_2H}(dN)_{12}$ was purified by 20% DPAGE. We 5′-^{32}P labeled the chimeric RNA 25mer and a chemically synthesized 25mer containing natural nucleotides, $*p(dN)_{12}rU(dN)_{12}$, with γ-^{32}P-ATP and T_4 polynucleotide kinase and purified them by 20% DPAGE. The two oligonucleotides migrate with the same electrophoretic mobility and give essentially the same P1 nuclease digestion patterns, except at the position bearing the modification (see Supporting Information).

In base-catalyzed cleavage of RNA the 2′-hydroxyl group acts as a nucleophile, attacking the adjacent phosphorus center to yield 2′,3′-cyclic phosphate and 5′-hydroxyl termini. We tested whether the tertiary hydroxyl group of 2′-*C*-β-difluoromethyluridine could facilitate RNA cleavage under alkaline conditions. Cleavage of the ^{32}P-labeled 25mers was initiated with the addition of 0.1 N KOH. Aliquots were neutralized with aqueous HCl and separated by 20% DPAGE. At pH 13, both oligonucleotides react at a similar rate to give a single, faster-migrating species (Figure 2). These results indicate that the tertiary hydroxyl group in 2′-*C*-β-difluoromethyluridine participates in base-catalyzed internal transphosphorylation.

Conclusions

In summary, we established synthetic entry into a new class of branched ribonucleosides bearing a 2′-*C*-β-difluoromethyl group. We accessed the analogues of U, C,

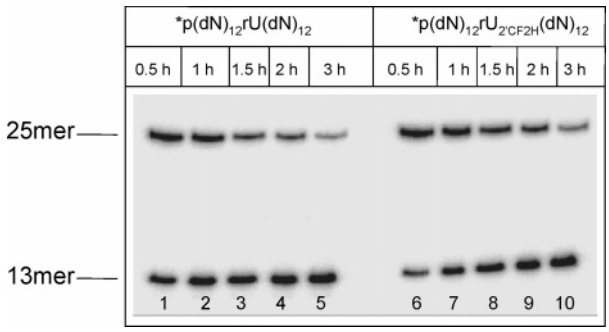


FIGURE 2. Cleavage of 5′-^{32}P-labeled (*) oligonucleotides with 0.1 N potassium hydroxide at 23 °C.

(49) England, T. E.; Uhlenbeck, O. C. *Biochemistry* **1978**, *17*, 2069−76.
(50) Barrio, J. R.; Barrio, M. C.; Leonard, N. J.; England, T. E.; Uhlenbeck, O. C. *Biochemistry* **1978**, *17*, 2077−81.
(51) Simoncsits, A.; Tomasz, J. *Biochim. Biophys. Acta* **1975**, *395*, 74−9.
(52) Brennan, C. A.; Manthey, A. E.; Gumport, R. I. *Methods Enzymol.* **1983**, *100*, 38−52.

A, and G convergently from a common intermediate, difluoromethylribofuranose **4**, prepared by difluoromethyl phenyl sulfone addition to ketoribose **2** followed by reduction to generate the difluoromethyl group. To accomplish the latter step we described a mild and efficient reduction procedure involving sodium amalgam under a hydrogen atmosphere. This finding could broaden the effectiveness and applicability of sodium amalgam as a reducing agent and of the sulfone addition/reduction sequence as a strategy to access synthetic targets containing a difluoromethyl group. Standard Hilbert−Johnson glycosylation conditions activated **4** sufficiently to generate the uridine nucleoside derivative, while synthesis of the C, A, and G analogues required conversion of **4** to the more reactive ribofuranosyl bromide. An analogous reaction sequence starting from methyl-D-ribofuranose resulted in the synthesis of 2′-*C*-α-difluoromethyl-arabino-α-pyrimidines, diastereomeric analogues configured with the opposite stereochemistry at both C2′ and C1′. To incorporate the 2′-*C*-*β*-difluoromethyluridine into oligonucleotides, we exploited an enzymatic ligation procedure which shall also allow incorporation of the corresponding methyl, fluoromethyl, and trifluoromethyl analogues. A 2′-*C*-*β*-difluoromethyl-nucleoside within an oligonucleotide mediates backbone scission using its 2′-hydroxyl group in the same manner as natural nucleotides.

Experimental Section

1,3,5-Tri-*O*-benzoyl-2-*C*-*β*-(benzenesulfonyl)difluoromethyl-α-D-ribofuranose (3). Under an argon atmosphere lithium hexamethyldisilazane (LiHMDS, 1.0 M in THF, 52 mL, 52 mmol) was added dropwise to a stirred solution of ketoribose **2** (10 g, 22 mmol) and difluoromethyl phenyl sulfone (3.8 mL, 5.0 g, 26 mmol) in anhydrous THF at −78 °C. After stirring at −78 °C for 2 h, the reaction was stirred at 0 °C for an additional 30 min followed by quenching with 200 mL of saturated aqueous ammonium chloride. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 200 mL). The combined organic phases were washed with brine, dried over magnesium sulfate, concentrated, and purified by silica gel chromatography, eluting with 25% ethyl acetate in hexanes, to give **3** (9.0 g, 64% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.0 Hz, 2H), 8.10 (d, J = 8.0 Hz, 2H), 7.99 (dd, J = 8.5, 9.5 Hz, 4H), 7.45−7.78 (m, 10H), 7.35 (t, J = 7.8 Hz, 2H), 7.10 (s, 1H), 6.05 (d, J = 7.0 Hz, 1H), 4.79 (m, 1H), 4.76 (dd, J = 13.5, 3.5 Hz, 1H), 4.56 (dd, J = 5.0, 12.0 Hz, 1H), 3.91 (br, 1H); ^{13}C NMR ($CDCl_3$) δ 166.0, 164.5, 164.0, 135.7, 133.8, 133.0, 132.6, 130.6, 130.4, 130.0, 129.9, 129.6, 129.3, 129.1, 128.8, 128.6, 128.5, 128.4, 128.2, 119.4 (t, J = 297.4 Hz), 94.5, 81.5 (t, J = 22.6 Hz), 79.3, 70.3, 62.6; ^{19}F NMR ($CDCl_3$) δ −106.3 (d, J = 248.5 Hz), −108.0 (d, J = 248.5 Hz); HRMS calcd for $C_{33}H_{26}O_{10}SNaF_2$ $[M + Na]^+$ 675.1112, found 675.1111.

1,2,3,5-Tetra-*O*-benzoyl-2-*C*-*β*-difluoromethyl-D-ribofuranose (4). To a stirred mixture of sulfone **3** (5.0 g, 7.7 mmol) and sodium hydrogen phosphate (6.6 g, 46 mmol) in anhydrous THF at 0 °C under hydrogen atmosphere was added methanol (1.9 mL, 46 mmol) followed by 5% sodium−mercury amalgam (21 g, 46 mmol). After stirring at 0 °C for 1 h, the solution was carefully decanted into chilled saturated aqueous ammonium chloride (200 mL) and extracted with ethyl acetate (2 × 200 mL). The combined organic phases were washed with brine, dried over magnesium sulfate, and concentrated. The residue was dried under high vacuum and dissolved in dry dichloromethane (125 mL). To the resulting solution at 0 °C were added *N*,*N*-(dimethylamino)pyridine (0.9 g, 7.4 mmol), triethylamine (11 mL), and benzoyl chloride (4.0 mL, 35 mmol) successively. After stirring for 5 h at ambient temperature, the reaction mixture was poured into 200 mL of ethyl ether and washed with saturated aqueous ammonium chloride and brine. The organic layer was dried over magnesium sulfate, concentrated under reduced pressure, and purified by silica gel chromatography, eluting with 12% ethyl acetate in hexanes, to give anomeric mixture **4** (2.9 g, 61% overall yield from **3**, β/α = 3/4) as a white foam. ^{1}H NMR ($CDCl_3$) δ 7.12−8.15 (m, 36H, α, β isomer), 7.06 (s, 0.75H, α isomer), 6.87 (dd, J = 53.7, 56.8 Hz, 0.75H, α isomer), 6.8 (t, J = 55.7 Hz, 1H, β isomer), 6.62 (d, J = 8.0 Hz, 0.75H, α isomer), 6.12 (d, J = 4.8 Hz, 1H, β isomer), 4.79−4.88 (m, 2.75H, α, β isomer), 4.77 (dd, J = 12.1, 3.8 Hz, 0.75H, α isomer), 4.69 (dd, J = 5.9, 11.7 Hz, 1H, β isomer), 4.56 (dd, J = 4.4, 12.2 Hz, 0.75H, α isomer); ^{13}C NMR ($CDCl_3$) δ 166.14, 165.99, 164.92, 164.89, 164.69, 164.56, 164.21, 163.97, 133.81, 133.21, 130.12, 130.08, 129.98, 129.92, 129.87, 129.83, 129.76, 129.59, 128.77, 128.67, 128.60, 128.50, 128.36, 128.33, 128.18, 111.79 (t, J = 251.1 Hz), 110.75 (t, J = 249.5 Hz), 96.20, 96.16, 94.79, 85.58 (t, J = 23.1 Hz), 83.04 (t, J = 23.4 Hz), 81.75, 79.14, 69.68, 69.34, 69.27, 63.19, 63.01; ^{19}F NMR ($CDCl_3$) δ −123.6 (d, J = 297.4 Hz), −128.8 (d, J = 301.2 Hz), −129.7 (d, J = 301.2 Hz), −131.7 (d, J = 297.4 Hz); HRMS calcd for $C_{34}H_{26}O_9NaF_2$ $[M + Na]^+$ 639.1443, found 639.1444.

2′,3′,5′-Tri-*O*-benzoyl-2′-*C*-*β*-difluoromethyluridine (6). A stirred suspension of uracil (1.1 g, 9.7 mmol) and ammonium sulfate (40 mg) in 1,1,1,3,3,3-hexamethyldisilazane (40 mL) was heated at reflux under argon until a clear solution formed (about 1 h). The clear solution was evaporated under vacuum to remove the excess 1,1,1,3,3,3-hexamethyldisilazane and further dried under high vacuum (<0.1 mmHg) for 1 h. The crude bis(trimethylsilyl)uracil obtained was dissolved in dry acetonitrile (40 mL) and transferred via cannula to a two-necked round-bottom flask (equipped with a water condenser) containing difluoromethylribofuranose **4** (2 g, 3.24 mmol). Under an argon atmosphere $SnCl_4$ (1.14 mL, 9.72 mmol) was added in one portion with vigorous stirring and exclusion of moisture. The resulting homogeneous light yellow solution was stirred under reflux for 2 days. When TLC indicated that **4** was completely consumed, the reaction was quenched carefully by the addition of 50 mL of saturated aqueous sodium bicarbonate and stirred for an additional 15 min. The mixture was extracted with methylene chloride, and the organic phase was washed with brine and dried over magnesium sulfate. After evaporation of the solvent the residue was purified by silica gel chromatography, eluting with 30% ethyl acetate in hexanes, to give **7** (1.5 g, 78% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 9.69 (br, 1H), 8.06 (d, J = 8.5 Hz, 2H), 8.02 (d, J = 8.5 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.3 Hz, 1H), 7.18−7.56 (m, 7H), 7.23 (t, J = 8.0 Hz, 2H), 6.97 (s, 1H), 6.58 (t, J = 54.8 Hz, 1H), 6.11 (d, J = 7.6 Hz, 1H), 5.62 (d, J = 8.3 Hz, 1H), 5.01 (m, 1H), 4.92 (dd, J = 2.8, 12.6 Hz, 1H), 4.65 (dd, J = 4.2, 12.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 165.9, 165.0, 164.7, 163.2, 150.2, 139.8, 134.1, 133.8, 133.5, 130.1, 129.8, 129.5, 129.2, 128.6, 128.4, 128.3, 127.8, 113.0 (t, J = 249.3 Hz), 102.1, 86.8, 84.4 (t, J = 20.6 Hz), 79.6, 68.2, 62.3; ^{19}F NMR ($CDCl_3$) δ −128.1 (d, J = 301.2 Hz), −130.5 (d, J = 301.2 Hz); HRMS calcd for $C_{31}H_{25}O_9N_2F_2$ $[M + H]^+$ 607.1528, found 607.1529.

2′-*C*-*β*-Difluoromethyluridine (7). Ammonia was bubbled into a solution of tribenzoyluridine **6** (500 mg, 0.83 mmol) in methanol (25 mL) at 0 °C for 0.5 h, and the resulting solution was stirred at 4 °C for 2 days. After TLC indicated the reaction was complete, the reaction solution was concentrated under vacuum, and the residue was purified by silica gel chromatography, eluting with 10% methanol in chloroform, to give **7** (231 mg, 95% yield) as a white powder. ^{1}H NMR (CD_3OD) δ 8.06 (d, J = 8.1 Hz, 1H), 6.04 (s, 1H), 5.86 (t, J = 54.7 Hz, 1H), 5.69 (d, J = 8.1 Hz, 1H), 4.49 (d, J = 9.1 Hz, 1H), 3.99 (dd, J = 1.9, 12.5 Hz, 1H), 3.94 (m, 1H), 3.79 (dd, J = 2.6, 12.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 166.1, 152.2, 143.3, 116.3 (t, J = 246.0 Hz), 102.3, 91.2, 83.5, 81.0 (t, J = 20.5 Hz), 68.3,

60.2; ^{19}F NMR (CD_3OD) δ −132.1 (d, J = 298.4 Hz), −133.2 (d, J = 298.4 Hz); HRMS calcd for $C_{10}H_{13}O_6N_2F_2$ $[M + H]^+$ 295.0742, found 295.0742.

3,5-Di-*O*-benzoyl-2-*C*-*β*-difluoromethyl-α-D-1-ribofuranosyl Bromide (8). A stirred mixture of difluoromethylribofuranose **4** (2 g, 3.2 mmol) and 30% HBr in acetic acid (15 mL) was stirred and heated to 80 °C for 5 h in a dry, sealed heavy-wall pressure tube. After it was cooled the reaction mixture was diluted with methylene chloride (100 mL) and transferred to a separation funnel containing ice. The organic layer was washed subsequently with ice water (45 mL), ice-cooled saturated aqueous sodium bicarbonate (2 × 45 mL), and ice water (45 mL). The organic layer was dried over magnesium sulfate, concentrated, and purified by silica gel chromatography, eluting with 10% ethyl acetate in hexanes, to give **8** (1 g, 65% yield) as a yellowish foam. ^{1}H NMR ($CDCl_3$) δ 8.08 (d, J = 7.2 Hz, 2H), 8.01 (d, J = 7.2 Hz, 2H), 7.62 (t, J = 7.4 Hz, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.48 (t, J = 8.0 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 6.98 (s, 1H), 6.11 (dd, J = 54.4, 55.8 Hz, 1H), 5.43 (d, J = 6.0 Hz, 1H), 4.83 (m, 1H), 4.77 (dd, J = 3.7, 12.2 Hz, 1H), 4.63 (dd, J = 5.2, 12.3 Hz, 1H).

3′,5′-Di-*O*-benzoyl-4-*N*-benzoyl-2′-*C*-*β*-difluoromethylcytidine (9a). A stirred suspension of 4-*N*-benzoylcytosine (1.0 g, 4.8 mmol) and ammonium sulfate (18 mg) in 1,1,1,3,3,3-hexamethyldisilazane (25 mL) was heated at reflux under argon until a clear solution formed in about 1 h. The clear solution was evaporated under vacuum to remove the excess 1,1,1,3,3,3-hexamethyldisilazane and further dried under high vacuum (<0.1 mmHg) for 1 h. The crude bis(trimethylsilyl) 4-*N*-benzoylcytosine obtained was dissolved in dry toluene (25 mL) and transferred via cannula to a two-necked round-bottom flask (equipped with a water condenser) containing bromide **8** (0.57 g, 1.2 mmol). To this solution was added HgO (0.5 g) and $HgBr_2$ (0.5 g). The reaction mixture was heated at 90 °C under an argon atmosphere for 5 h. When TLC indicated that **8** was completely consumed, the reaction was cooled to room temperature and quenched by addition of methanol (10 mL) and water (5 mL). The resulting mixture was stirred for an additional 15 min and extracted with methylene chloride. The organic phase was separated, washed with brine, and dried over magnesium sulfate. After evaporation of the solvent, the residue was purified by silica gel chromatography, eluting with 1% methanol in chloroform, to give **9a** (0.47 g, 65% yield) as a pale yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.01 (br, 1H), 8.07 (m, 5H), 7.90 (d, J = 7.4 Hz, 2H), 7.68 (d, J = 7.0 Hz, 1H), 7.41−7.64 (m, 9H), 6.09 (s, 1H), 6.08 (t, J = 54.6 Hz, 1H), 5.63 (s, 1H), 4.88 (dd, J = 2.9, 12.2 Hz, 1H), 4.75 (m, 1H), 4.69 (dd, J = 5.0, 12.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 166.1, 165.4, 163.0, 156.5, 144.5, 133.7, 133.5, 133.3, 132.7, 129.9, 129.6, 129.2, 129.0, 128.5, 127.6, 115.4 (t, J = 249.9 Hz), 97.2, 92.1, 78.9, 78.1 (t, J = 21.6 Hz), 70.9, 62.4; ^{19}F NMR ($CDCl_3$) δ −129.4 (d, J = 297.4 Hz), −132.3 (d, J = 297.4 Hz); HRMS calcd for $C_{31}H_{26}O_8N_3F_2$ $[M + H]^+$ 606.1688, found 606.1665.

3′,5′-Di-*O*-benzoyl-6-*N*-benzoyl-2′-*C*-*β*-difluoromethyladenosine (9b). A stirred suspension of 6-*N*-benzoyladenine (0.8 g, 3.4 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (10 mL) and dry pyridine (5 mL) was heated at reflux under argon until a clear solution formed (∼1 h). The clear solution was evaporated under vacuum to remove the excess 1,1,1,3,3,3-hexamethyldisilazane and pyridine. The residue was further dried under high vacuum (<0.1 mmHg) for 1 h. The crude bis-(trimethylsilyl) 6-*N*-benzoyladenine obtained was dissolved in dry toluene (15 mL), which was transferred via cannula to a double-necked round-bottom flask (equipped with a water condenser) containing bromide **8** (0.53 g, 1.1 mmol). To this solution was added HgO (440 mg) and $HgBr_2$ (440 mg). The resulting reaction mixture was heated at 90 °C under an argon atmosphere for 5 h. When TLC indicated that **8** was completely consumed, the reaction was cooled to room temperature and quenched by addition of methanol (10 mL) and water (5 mL). The resulting mixture was stirred for an additional 15 min and extracted with methylene chloride. The organic phase was separated, washed with brine, and dried over magnesium sulfate. After evaporation of the solvent, the residue was purified by silica gel chromatography, eluting with 40% ethyl acetate in hexanes, to give **9b** (352 mg, 50% yield) as pale yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.07 (s, 1H), 8.83 (s, 1H), 8.15 (s, 1H), 8.08 (d, J = 8.3 Hz, 2H), 8.03 (t, J = 7.1 Hz, 4H), 7.62 (q, J = 7.4 Hz, 2H), 7.52−7.57 (m, 3H), 7.49 (t, J = 7.85 Hz, 2H), 7.41 (t, J = 7.85 Hz, 2H), 6.36 (s, 1H), 6.25 (d, J = 8.3 Hz, 1H), 5.91 (t, J = 53.7 Hz, 1H), 4.90 (dd, J = 2.4, 11.7 Hz, 1H), 4.72−4.79 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.2, 165.4, 164.5, 153.0, 151.4, 149.7, 141.9, 133.9, 133.6, 133.4, 132.8, 129.9, 129.7, 129.3, 128.9, 128.6, 128.5, 127.8, 123.0, 113.7 (t, J = 251.5 Hz), 90.8, 82.8 (t, J = 19.8 Hz), 78.2, 70.9, 62.9; ^{19}F NMR ($CDCl_3$) δ −127.6 (d, J = 297.4 Hz), −130.4 (d, J = 297.4 Hz).

3′,5′-Di-*O*-benzoyl-2-*N*-acetyl-6-*O*-diphenylcarbamoyl-2′-*C*-*β*-difluoromethylguanosine (9c). 2-*N*-Acetyl-6-*O*-diphenylcarbamoylguanine (0.64 g, 1.6 mmol) was coevaporated three times with anhydrous 1,2-dichloroethane (10 mL) under vacuum. Absolute 1,2-dichloroethane (15 mL) and bis(trimethylsilyl)acetamide (0.81 mL, 3.3 mmol) were added at room temperature. The mixture was stirred at 80 °C until the base dissolved (∼15 min). Solvent was evaporated, and the residue was further dried under high vacuum (<0.1 mmHg) for 1 h. The crude bis(trimethylsilyl) 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine obtained was dissolved in anhydrous toluene (15 mL) and transferred via cannula to a double-necked round-bottom flask (equipped with a water condenser) containing bromide **8** (0.25 g, 0.53 mmol). To this solution was added HgO (120 mg) and $HgBr_2$ (120 mg). The resulting reaction mixture was heated at 80 °C under an argon atmosphere for 2 h. When TLC indicated that **8** was completely consumed, the reaction was cooled to room temperature and quenched by addition of methanol (10 mL) and water (5 mL). The resulting mixture was stirred for an additional 15 min and extracted with methylene chloride. The organic phase was separated, washed with brine, and dried over magnesium sulfate. After evaporation of the solvent the residue was purified by silica gel chromatography, eluting with 40% ethyl acetate in hexanes, to give **9c** (128 mg, 31% yield) as a pale yellow foam. ^{1}H NMR ($CDCl_3$) δ 8.23 (br, 1H), 8.10 (s, 1H), 8.07 (d, J = 8.4 Hz, 2H), 8.01 (d, J = 8.4 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.54 (t, J = 7.4 Hz, 1H), 7.24−7.45 (m, 14H), 6.27 (s, 1H), 6.12 (s, 1H), 5.86 (t, J = 54.3 Hz, 1H), 4.94 (dd, J = 3.7, 12.1 Hz, 1H), 4.77 (m, 1H), 4.69 (dd, J = 5.4, 12.1 Hz, 1H), 2.29 (s, 3H); ^{19}F NMR ($CDCl_3$) δ −131.5 (d, J = 293.6 Hz), −132.5 (d, J = 293.6 Hz); HRMS calcd for $C_{40}H_{33}O_9N_6F_2$ $[M + H]^+$ 779.2277, found 779.2274.

2′-*C*-*β*-Difluoromethylcytidine (10a). A solution of **9a** (200 mg, 0.33 mmol) in ammonia-saturated methanol (10 mL) was stirred at 4 °C for 2 days. When TLC indicated that the reaction was complete, the solvent was removed under vacuum, and the residue was purified by silica gel chromatography, eluting with 10% methanol in ethyl acetate, to give **10a** (93 mg, 96% yield) as a white powder. ^{1}H NMR (CD_3OD) δ 8.05 (d, J = 7.5 Hz, 1H), 6.03 (s, 1H), 5.89 (d, J = 7.5 Hz, 1H), 5.86 (t, J = 55.1 Hz, 1H), 4.49 (d, J = 8.9 Hz, 1H), 4.00 (dd, J = 1.9, 12.5 Hz, 1H), 3.94 (m, 1H), 3.82 (dd, J = 2.8, 12.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 167.5, 158.2, 143.4, 116.0 (t, J = 245.5 Hz), 95.8, 91.9, 83.3, 80.8 (t, J = 21.0 Hz), 68.0, 60.3; ^{19}F NMR (CD_3OD) δ −131.4 (d, J = 284.2 Hz), −133.9 (d, J = 284.2 Hz); HRMS calcd for $C_{10}H_{14}O_5N_3F_2$ $[M + H]^+$ 294.0902, found 294.0903.

2′-*C*-*β*-Difluoromethyladenosine (10b). A solution of **9b** (180 mg, 0.28 mmol) in ammonia-saturated methanol (10 mL) was stirred at 4 °C for 2 days. When TLC indicated that the reaction was complete, the solvent was removed under vacuum, and the residue was purified by silica gel chromatography, eluting with 14% methanol in chloroform, to give **10b** (83 mg, 93% yield) as a white powder. ^{1}H NMR (CD_3OD) δ 8.44 (s, 1H), 8.19 (s, 1H), 6.21 (s, 1H), 5.66 (t, J = 54.3 Hz, 1H), 4.82 (d, J = 8.9 Hz, 1H), 4.06 (m, 2H), 3.88 (dd, J = 2.7, 12.5 Hz, 1H); ^{19}F NMR (CD_3OD) δ −131.2 (d, J = 293.6 Hz), −136.5

(d, J = 293.6 Hz); HRMS calcd for $C_{11}H_{14}O_4N_5F_2$ [M + H]$^+$ 318.1014, found 318.1013.

2′-*C*-β-Difluoromethylguanosine (10c). Guanosine **9c** (50 mg, 0.064 mmol) was dissolved in methanol (3 mL) and 33% aqueous ammonia (3 mL) and heated at 60 °C overnight. When TLC indicated that the reaction was complete, the reaction mixture was concentrated, dissolved in water (5 mL), washed with chloroform (2 × 5 mL), concentrated again, and purified by reverse-phase HPLC (gradient triethylammonium acetate/acetonitrile/water) to give **10c** (12 mg, 55% yield) as a white powder. ^{1}H NMR (CD_3OD) δ 7.84 (s, 1H), 5.92 (s, 1H), 5.63 (t, J = 53.0 Hz, 1H), 4.61 (d, J = 8.5 Hz, 1H), 3.94 (m, 1H), 3.88 (dd, J = 3.0, 12.5 Hz, 1H), 3.77 (dd, J = 1.5, 12.5 Hz, 1H); ^{19}F NMR (CD_3OD) δ −130.7 (d, J = 297.4 Hz), −135.0 (d, J = 297.4 Hz); UV (H_2O) λ_{max} 209.7, 252.0 nm; HRMS calcd for $C_{11}H_{14}O_5N_5F_2$ [M + H]$^+$ 334.0963, found 334.0964.

Methyl 3,5-*O*-Di-*tert*-butylsilanediyl-β-D-ribofuranoside (12). Methyl β-D-ribofuranose **11** (5.0 g, 30.5 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry DMF at 0 °C. To this cold solution under argon was added slowly di-*tert*-butylsilyl ditriflate (13.7 g, 16.6 mL, 31.1 mmol) with vigorous stirring. After being stirred at room temperature for 15 min, the reaction mixture was treated with dried triethylamine (13 mL, 93 mmol). The reaction mixture was stirred for an additional 5 min and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography, eluting with 14% ethyl acetate in hexanes, to afford **12** as a white solid (5.5 g, 59% yield). ^{1}H NMR ($CDCl_3$) δ 4.92 (s, 1H), 4.43 (dd, J = 4.5, 8.5 Hz, 1H), 3.95−4.10 (m, 5H), 3.42 (s, 3H), 1.09 (s, 9H), 1.04 (s, 9H); LRMS (positive-ion FAB) calcd for $C_{14}H_{28}O_5Si$ [M + H]$^+$ 305, found 305.

Methyl 3,5-*O*-Di-*tert*-butylsilanediyl-2-oxo-β-D-*erythro*-ribofuranoside (13). Compound **12** (0.98 g, 3.2 mmol) in dry methylene chloride (5 mL) was added to a solution of Dess−Martin periodinane (2.29 g, 4.8 mmol) in dry methylene chloride (15 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, and the residue was triturated with diethyl ether (30 mL). After filtration through a pad of magnesium sulfate the organic solution was stirred with an equal volume of $Na_2S_2O_3{\cdot}5H_2O$ (3.7 g) in saturated aqueous sodium bicarbonate until the organic layer became clear. The organic layer was separated, washed with brine, dried, and evaporated. The residue was purified by silica gel chromatography, eluting with 11% ethyl acetate in hexanes, to afford **13** as a clear oil (0.89 g, 91% yield). ^{1}H NMR ($CDCl_3$) δ 4.74 (d, J = 10.0 Hz, 1H), 4.52 (m, 1H), 3.74−4.38 (m, 3H), 3.57 (d, J = 5.0 Hz, 3H), 1.07 (m, 18H); LRMS (positive-ion FAB) calcd for $C_{14}H_{26}O_5Si$ [M + H]$^+$ 303, found 303.

Methyl 2-*C*-α-(Benzenesulfonyl-1′,1′-difluoro)methyl-3,5-*O*-di-*tert*-butylsilane-diyl-β-D-arabinofuranoside (14). To a stirred solution of 13 (1.5 g, 4.8 mmol) and difluoromethyl phenyl sulfone (1.1 g, 0.73 mL, 6.8 mmol) in dry THF/HMPA (10/1, 70 mL) at −78 °C under argon was added dropwise LiHMDS (1.0 M in THF, 8 mL). After stirring at −78 °C for 2 h, the reaction was quenched with saturated aqueous ammonium chloride (30 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 30 mL). The combined organic phases were washed with brine (30 mL), dried over sodium sulfate, concentrated, and purified by silica gel chromatography, eluting with 14% ethyl acetate in hexanes, to afford 14 (1.5 g, 63% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 8.02 (d, J = 7.5 Hz, 2H), 7.79 (t, J = 7.5 Hz, 1H), 7.65 (t, J = 8.0 Hz, 2H), 5.57 (s, 1H), 4.41 (dd, J = 5.0, 9.0 Hz, 1H), 4.21 (d, J = 10 Hz, 1H), 3.90−3.95 (m, 2H), 3.79−3.83 (m, 1H), 3.77 (s, 3H), 1.04 (s, 9H), 0.95 (s, 9H).

Methyl 2-C-α-Difluoromethyl-3,5-*O*-di-*tert*-butylsilane-diyl-β-D-arabinofuranose (15). To a mixture of **14** (1.5 g, 3.0 mmol) and dry HMPA (10 mL) at 0 °C under argon was added slowly commercial SmI_2 in THF (0.1 M) with vigorous stirring. The reaction mixture became dark blue after addition of ∼150 mL of the SmI_2 solution. After 10 min at room temperature the reaction mixture was poured into saturated aqueous ammonium chloride (100 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 60 mL). The combined organic phases were washed with brine, dried over magnesium sulfate, concentrated, and purified by silica gel chromatography, eluting with 14% ethyl acetate in hexanes, to afford **15** (0.81 g, 74% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 6.05 (dd, J = 53.5, 56.5, 1H), 5.08 (s, 1H), 4.40 (dd, J = 5.0, 9.0 Hz, 1H), 4.11 (dd, J = 4.6, 9.8 Hz, 1H), 3.93 (m, 1H), 3.81 (m, 1H), 3.64 (s, 3H), 3.39 (s, 1H), 1.08 (s, 9H), 1.01 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 114.4 (t, J = 246.0 Hz), 100.5, 83.5, 77.3 (t, J = 21.6 Hz), 71.8, 68.2, 57.7, 27.3, 26.9; ^{19}F NMR ($CDCl_3$) δ −130.4 (d, J = 296.4 Hz), −137.4 (d, J = 296.4 Hz); HRMS calcd for $C_{15}H_{28}F_2O_5Si$ [M + H]$^+$ 355.1752, found 355.1751.

Methyl 2-*C*-α-Difluoromethyl-2-*O*-benzoyl-3,5-*O*-di-*tert*-butylsilanediyl-β-D-arabinofuranoside (16). To a stirred solution of 4-(dimethylamino)pyridine (0.37 g, 3.0 mmol) in dry methylene chloride (20 mL) was added dry triethylamine (4.7 mL) followed by benzoyl chloride (0.69 mL, 5.9 mmol). After 5 min a solution of compound **15** (0.90 g, 2.5 mmol) in dry methylene chloride (50 mL) was added. The reaction was kept at room temperature overnight. When TLC showed no starting material remaining, the reaction mixture was diluted with methylene chloride (50 mL), washed with water (3 × 80 mL), dried over magnesium sulfate, concentrated, and purified by silica gel chromatography, eluting with 14% ethyl acetate in hexanes, to afford **16** (1.11 g, 95% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 7.48−8.11 (m, 5H), 6.73 (t, J = 52.5 Hz, 1H), 5.38 (s, 1H), 4.66 (d, J = 9.5 Hz, 1H), 4.45 (dd, J = 4.5, 9.0 Hz, 1H), 3.94−4.06 (m, 2H), 3.51 (s, 3H), 1.12 (s, 9H), 1.06 (s, 9H).

Methyl 2-*C*-α-Difluoromethyl-2-*O*-benzoyl-β-D-arabinofuranoside (17). HF−pyridine (1.6 mL, 61 mmol) was carefully mixed with pyridine (8 mL) at 0 °C. This cold mixture was added dropwise to a stirred solution of **16** (1.1 g, 2.4 mmol) in THF (10 mL). After being stirred at room temperature for 10 min, the reaction mixture was diluted with pyridine (30 mL) and then methylene chloride (100 mL). The resulting organic solution was washed with water (120 mL) and aqueous sodium bicarbonate (100 mL), dried over sodium sulfate, concentrated, and purified by silica gel chromatography, eluting with 50% ethyl acetate in hexanes, to afford **17** (0.74 g, 95%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.49−8.08 (m, 5H), 6.48 (dd, J = 53.5, 55.5 Hz, 1H), 5.26 (s, 1H), 4.85 (dd, J = 3.0, 7.0 Hz, 1 H), 4.26 (dd, J = 3.3, 7.0 Hz, 1H), 3.99 (b, 1H), 3.90 (m, 1H), 3.75 (m, 1H), 3.60 (s, 3H), 2.27 (b, 1H).

Methyl 2-*C*-α-Difluoromethyl-2,3,5-tri-*O*-benzoyl-β-D-arabinofuranoside (18). To a stirred solution of 4-(dimethylamino)pyridine (0.74 g, 6.0 mmol) in dry methylene chloride (50 mL) was added dry triethylamine (9.4 mL), followed by benzoyl chloride (1.4 mL, 12 mmol). After 5 min a solution of compound **17** (0.72 g, 2.3 mmol) in dry methylene chloride (20 mL) was added. The reaction was kept at room temperature overnight, and TLC showed that no starting material remained. The reaction mixture was diluted with methylene chloride (50 mL), washed with water (3 × 80 mL), dried over sodium sulfate, concentrated, and purified by silica gel chromatography, eluting with 20% ethyl acetate in hexanes, to afford **18** (1.2 g, 97% yield) as a white foam. ^{1}H NMR ($CDCl_3$) δ 8.11 (d, J = 8.5 Hz, 2H), 8.08 (d, J = 8.5 Hz, 2H), 8.03 (d, J = 8.1 Hz, 2H), 7.57 (t, J = 7.5 Hz, 2H), 7.42−7.49 (m, 5H), 7.32 (t, J = 7.8 Hz, 2H), 6.74 (t, J = 55.0 Hz, 1H), 6.33 (d, J = 6.0 Hz, 1H), 5.55 (s, 1H), 4.61−4.71 (m, 3H), 3.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.0, 165.3, 165.0, 133.7, 133.6, 133.0, 130.1, 129.9, 129.6, 128.5, 128.2, 112.7 (t, J = 252.5 Hz), 102.5, 84.5 (t, J = 20.1 Hz), 77.9, 65.5, 55.8; ^{19}F NMR ($CDCl_3$) δ −127.5 (d, J = 301.1 Hz), −130.2 (d, J = 301.1 Hz); HRMS calcd for $C_{28}H_{25}F_2O_8$ [M + H]$^+$ 527.1518, found 527.1517.

4-*N*-Benzoyl-1-(2-*C*-α-difluoromethyl-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl)cytosine (19a). To a pressure tube

containing **18** (0.45 g, 0.86 mmol) was added a solution of HBr in acetic acid (30%, 10 mL). The tube was sealed and heated to 75 °C for 2 h with stirring. After cooling to room temperature, the reaction mixture was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (15 mL), washed with brine, dried over sodium sulfate, concentrated under vacuum, and subsequently dissolved in dry benzene (10 mL). With exclusion of moisture, this solution was added to a solution of 4-*N*-benzoyl bis(trimethylsilyl)cytosine (5 mL, prepared in situ from 4-*N*-benzoyl cytosine (0.59 g, 0.26 mmol), 1,1,1,3,3,3-hexamethyldisilazane (10 mL), and 22 mg ammonium sulfate) in dry benzene. To this clear solution were added HgO (0.28 g) and $HgBr_2$ (0.28 g), and the reaction mixture was stirred at room temperature under argon for 36 h. The reaction was diluted with ethyl acetate (15 mL), washed with aqueous potassium iodide (3 × 10 mL) and brine (10 mL), dried over sodium sulfate, concentrated, and purified by silica gel chromatography, eluting with 50% ethyl acetate in hexanes, to afford **19a** (0.25 g, 48%) as a white foam. ^{1}H NMR ($CDCl_3$) δ 9.06 (s, 1H), 8.08 (d, J = 7.5 Hz, 2H), 8.07 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H), 7.71 (d, J = 7.3 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.34−7.56 (m, 8H), 7.09 (s, 1H), 6.43 (s, 1H), 6.19 (t, J = 53.9 Hz, 1H), 4.93 (m, 2H), 4.79 (dd, J = 4.7, 11.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 166.2, 165.2, 163.1, 156.9, 144.5, 133.9, 133.3, 133.1, 132.9, 129.8, 129.7, 129.5, 128.8, 128.6, 128.4, 128.3, 127.9, 114.9 (t, J = 249.8 Hz), 97.1, 92.5, 83.8, 81.3 (t, J = 21.8 Hz), 78.2, 64.6, 60.4; ^{19}F NMR ($CDCl_3$) δ −131.06; HRMS calcd for $C_{31}H_{26}F_2O_8N_3$ $[M + H]^+$ 606.1688, found 606.1688.

1-(2-*C*-α-Difluoromethyl-3,5-di-*O*-benzoyl-α-D-arabinofuranosyl)uracil (19b). Compound **19b** was synthesized by the similar procedure used for **19a** with a yield of 55% as a white foam. ^{1}H NMR ($CDCl_3$) δ 10.20 (s, 1H), 8.12 (d, J = 7.5 Hz, 2H), 7.98 (d, J = 7.5 Hz, 2H), 7.66−7.46 (m, 8H), 6.47 (s, 1H), 6.13 (t, J = 55.0 Hz, 1H), 5.86 (d, J = 5.0 Hz, 1H), 5.78 (t, J = 10.5 Hz, 2H), 4.92 (d, J = 4.0 Hz, 1H), 4.79 (m, 2H).

1-(2-*C*-α-Difluoromethyl-α-D-arabinofuranosyl)cytosine (20a). In a pressure tube **19a** (0.25 g, 0.41 mmol) was dissolved in methanol (20 mL). This solution was cooled to 0 °C and saturated with ammonia. The pressure tube was sealed and kept at 4 °C with stirring for 2 days. When TLC showed that the reaction was complete, the mixture was evaporated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 10% methanol in chloroform, to afford **20a** (0.10 g, 84%) as a white solid. ^{1}H NMR (CD_3OD) δ 7.90 (d, J = 7.6 Hz, 1H), 6.20 (s, 1H), 5.91 (d, J = 7.7 Hz, 1H), 5.90 (t, J = 54.0 Hz, 1H), 4.37 (m, 1H), 4.26 (d, J = 3.4 Hz, 1H), 3.75 (d, J = 5.2 Hz, 2H); ^{13}C NMR (CD_3OD) δ 167.6, 158.7, 143.5, 116.2 (t, J = 243.9 Hz), 95.8, 93.5, 90.4, 83.3 (t, J = 20.6 Hz), 77.9, 63.1; ^{19}F NMR (CD_3OD) δ −136.9, −137.01; HRMS calcd for $C_{10}H_{14}F_2N_3O_5$ $[M + H]^+$ 294.0902, found 294.0901.

1-(2-*C*-α-Difluoromethyl-α-D-arabinofuranosyl)uracil (20b). Using the similar procedure as described for **20a**, compound **20b** was obtained in 90% yield as a white solid. ^{1}H NMR (CD_3OD) δ 8.00 (d, J = 8.2 Hz, 1H), 6.21 (s, 1H), 5.95 (t, J = 53.2 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 4.43 (t, J = 5.3 Hz, 1H), 4.19 (s, 1H), 3.77 (dd, J = 6.5, 11.6 Hz, 1H), 3.70 (dd, J = 5.3, 11.6 Hz, 1H); ^{19}F NMR (CD_3OD) δ −135.9 (d, J = 296.4 Hz), −138.8 (d, J = 296.4 Hz); HRMS calcd for $C_{10}H_{13}F_2N_2O_6$ $[M + H]^+$ 295.0742, found 295.0741.

2′-β-Difluoromethyluridine 3′,5′-Bisphosphate (21). Uridine **7** (50 mg, 0.17 mmol) was stirred in anhydrous diphosphoryl chloride (0.24 mL, 1.7 mmol) under argon in an ice bath. The reaction was allowed to warm to room temperature over ~4 h. After TLC (n-PrOH:30% aqueous NH_3:H_2O, 6:3:1, v:v:v) showed that the reaction was complete, ice-chilled water (5 mL) was added, and the mixture was kept at 0 °C for 10 min before addition of an ice-cooled 0.5 M triethylammonium bicarbonate (TEAB) solution (20 mL, pH 8.0). The resulting colorless solution was concentrated under vacuum, maintaining the temperature below 30 °C. The residue was coevaporated three times with 10 mL of 50% methanol in water, washed with chloroform (3 × 5 mL), dried under vacuum, and redissolved in 50 mL of water. The solution was adjusted to pH 7 with triethylamine, filtered, and chromatographed on a DEAE cellulose column (2.5 × 25 cm) using a linear gradient of 0.15−0.5 M TEAB, pH 8.0 (700 mL). The fractions (peaked around 0.35 M TEAB) containing uridine bisphosphate were pooled, evaporated to dryness, and coevaporated several times with 10 mL of 50% methanol in water to remove TEAB buffer, giving **21** (38 mg) in 50% yield. ^{1}H NMR (D_2O) δ 7.85 (d, J = 8.0 Hz, 1H), 6.04 (s, 1H), 5.93 (t, J = 53.5 Hz, 1H), 5.82 (d, J = 8.0 Hz, 1H), 4.80 (t, J = 9.3 Hz, 1H), 4.15 (m, 2H), 3.77 (t, J = 5.3 Hz, 1H); ^{31}P NMR (D_2O; peak position changes with pH) δ 4.35 (triplet in nondecoupled ^{31}P NMR), 3.47 (doublet in nondecoupled ^{31}P NMR); ^{19}F NMR (D_2O) δ −129.07 (d, J = 301.2 Hz), −134.90 (d, J = 301.2 Hz); HRMS calcd for $C_{10}H_{14}N_2O_{12}P_2F_2$ $[M + H]^+$ 455.0068, found 455.0068.

Incorporation of Uridine Bisphosphate (21) into an Oligonucleotide. In a total volume of 10 μL, 3 mM uridine bisphosphate **22**, 0.3 mM 5′ DNA fragment d(CTGTCACCGAAA), 0.4 mM ATP, 8 mM spermine, 1 mM creatine phosphate, 10 mM magnesium chloride, 10 mg/mL bovine serum albumin, 1 mM hexaaminecobalt(III) chloride, 20 mM DTT, 170 units/mL myokinase, 175 units/mL phosphocreatine kinase, and 2000 units/mL T_4 RNA ligase were incubated in 50 mM Tris-HCl, pH 8.0, at 25 °C for 3 days. After the ligase was deactivated by incubating at 100 °C for 5 min, 1 μL of 10× alkaline phosphatase buffer (500 mM Tris-HCl, pH 9.0, 10 mM $MgCl_2$) and 1 μL of 30 units/μL calf intestine phosphatase (CIP) were added to the solution and incubated at 37 °C for 30 min to remove the 3′-phosphate. The phosphatase was deactivated in the same manner as above, and the reaction mixture was purified by 20% denaturing polyacrylamide gel electrophoresis (DPAGE). The correct band was visualized by UV shadowing, excised with a clean razor blade, and eluted in TE buffer (10 mM Tris, pH 8.0; 0.1 mM EDTA) at 4 °C overnight. The eluant was extracted by phenol/chloroform/isoamyl alcohol (25/24/1), precipitated three times by *n*-butanol, dried under vacuum (speed vac), and redissolved in water. About 90% of the starting DNA fragment became ligated based on ^{32}P labeling of the mixture before gel purification. To prepare the full-length chimeric oligonucleotide, the 13mer [d(CTGTCACCGAAA)r$U_{2'CF_2H}$, final concentration 20 μM], phosphorylated 3′ fragment pd(ACACGCAAGATG) and splint DNA template (dCTTGCGTGTATTTCGGTG) were preannealed in T_4 DNA ligase buffer (1X containing 50 mM Tris-HCl, 10 mM magnesium chloride, 10 mM DTT, 1 mM ATP, 25 g/mL bovine serum albumin) by incubating at 90 °C for 1 min followed by slow cooling to 4 °C. Hexaaminecobalt(III) chloride (1 mM) and 8 pmol/μL T_4 DNA ligase were added, and the mixture was incubated at 16 °C overnight. The full-length chimeric product was purified and precipitated by the same procedure as described above for the 5′ fragment.

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Supporting Information Available: ^{1}H NMR spectra of **3**, **4**, **6**−**8**, **9a**−**c**, **10a**−**c**, **15**−**18**, **19a,b**, **20a,b,** and **21**; ^{13}C NMR spectra of **3**, **4**, **6**, **7**, **9a,b**, **10a**, **15**, **18**, **19a**, and **20a**; ^{19}F−^{1}H NOE spectra of **3** and **6**; NOESY spectra of **6**, **8**, **9a**,**b**, and **19a**; ^{31}P spectra of **21**; MALDI mass spectroscopy of r$CCGAAAU_{2'CF_2H}$; P1 nuclease digestion of the oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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