Synthesis of 5′-Deoxy-5′-difluoromethyl Phosphonate Nucleotide Analogs

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A synthetic route to nucleoside 5′-deoxy-5′-difluoromethyl phosphonates from ribofuranosyl 5-deoxy-5-difluoromethyl phosphonate precursors is described. Methyl 5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (**7**) was converted, under mild conditions, to the suitable glycosylating agent 1-*O*-acetyl-2,3-di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (**10**). 1,2-Di-*O*-acetyl-3-*O*-benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (**16**) was also prepared as a versatile building block for nucleotide synthesis. Condensation of **10** with silylated nucleobases, followed by complete deprotection, afforded 5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-6′,6′-difluoro nucleoside analogs **22a–c**. In the case of the glycosylation of adenine, a considerable quantity of N-7 regioisomer **19** was formed. 5′,6′-Dideoxy-6′-(dihydroxyphosphinyl)-6′,6′-difluoro adenosine analog **22c** was converted into the triphosphate analog **23** using 1,1′-carbonyldiimidazole activation followed by condensation with pyrophosphate. The adenosine 3′,5′-cyclic monophosphate analog **24** was obtained through the DCC promoted intramolecular cyclization of **22c**. Dinucleoside phosphate analog **27** was prepared by DCC-catalyzed coupling of 1-[2,3-di-*O*-benzoyl-5,6-dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil (**21a**) with 2′,5′-bis(*O*-*tert*-butyldimethylsilyl)-N^4-acetylcytidine (**25**), followed by deprotection.

Introduction

Phosphonic acids often exhibit important biological properties because of their similarity to phosphates.[1] The carbon–phosphorus bond in phosphonates, unlike phosphates, is not susceptible to the hydrolytic action of phosphatases, thereby imparting greater stability under physiological conditions. In particular, alkyl phosphonate esters of nucleosides are generally more stable to nucleases and have greater cell permeability.[2]

Initial work in the synthesis of phosphonic acid analogs of nucleotides involved nonisosteric systems, that is compounds differing from the natural molecules simply by noninclusion of the normal ester oxygen. They were synthesized using previously developed reaction systems with readily available materials.[3] Later, the synthesis of isosteric analogs of nucleotides such as 5′,6′-dideoxy-6′-(dihydroxyphosphinyl) nucleosides, where the 5′-oxygen was replaced with CH_2, was reported using the Wittig reaction of a nucleoside 5′-aldehyde with diphenyl [(triphenylphosphoranylidene)methyl]phosphonate, followed by reduction.[4] This reaction is generally used for the synthesis of isosteric 5′-phosphonates. Recently, a general route to phosphonate analogs of 5′-nucleotides has been elaborated using the Arbuzov reaction.[5] The analogs of greatest interest and use thus far for biological investigations have been those of nucleoside polyphosphates. Moffatt *et al.*[4] have mentioned such analogs of nucleoside di- and triphosphates, prepared from 5′,6′-dideoxy-6′-(dihydroxyphosphinyl) nucleosides. No experimental details were presented although biochemical experiments have been performed using materials from this source[6] demonstrating that the thymidine triphosphate analog was not a substrate for DNA polymerase. In another study,[7] the 5′-deoxy-5′-methyl phosphonate analog of uridine triphosphate was incorporated into an RNA chain using *Escherichia coli* RNA polymerase. However, following incorporation, synthesis was terminated. Thus this analog acted as a pseudoterminator of RNA synthesis.

It has been suggested by Blackburn and Kent[8] that α-fluoro and α,α-difluoromethyl phosphonates should mimic phosphate esters better than the corresponding phosphonates. This assumption was based both on electronic and steric considerations. Groups such as CHF and CF_2 could be either incorporated in place of 3′- or 5′-oxygens, or be incorporated as CFH_2, CF_2H, or CF_3 in place of the hydroxyl on the phosphate. Blackburn *et al.*[9] reported the synthesis of methyl 5,6-dideoxy-6-(dihydroxyphosphinyl)-6-fluoro-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (**1**) as a mixture of diastereoisomers using the Wadsworth–Emmons condensation of protected ribofuranose 5-aldehyde with tetraisopropyl fluoromethylenebisphosphonate, followed by catalytic hydrogenation, but no α-fluoromethyl phosphonate or α,α-difluoromethyl phosphonate analogs of nucleoside 5′-phosphates were reported. The isopolar CF_2 moiety most closely resembles an oxygen group in structure and the synthesis of

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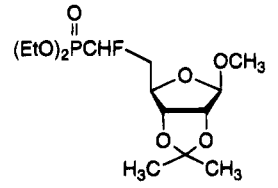
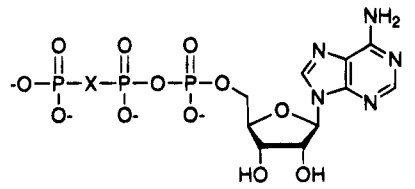
nucleoside 3′-difluoromethyl phosphonate, where a difluoromethyl group is substituted for the 3′-oxygen function, and its incorporation into a dimer was reported.[10] Recently Blackburn and Guo[11] described the synthesis of 3′- and 5′-trifluoromethyl phosphonate analogs of nucleosides. The above analogs, when incorporated into oligonucleotides, will generate a nonionic and chiral phosphonate backbone.

Analogs of triphosphates **2** where the bridging oxygen atoms were replaced by a difluoromethylene group have been successfully employed as substrates in enzymatic processes.[12] Compound **3**, 9-(5,5-difluoro-5-phosphonopentyl)guanine, has been utilized as a multisubstrate analog inhibitor of purine nucleoside phosphorylase.[13]

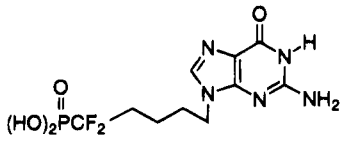


1



2

X = O, CH_2, CHF, CF_2, CCl_2

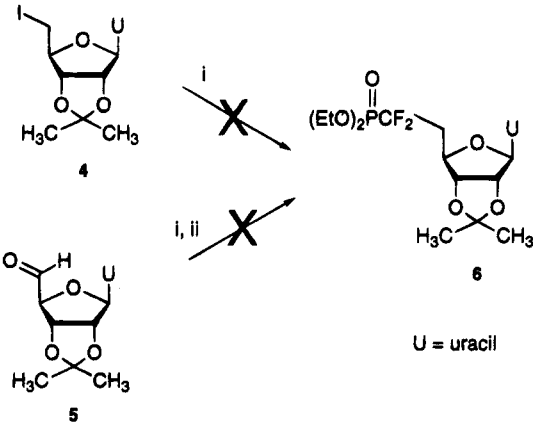


3

Phosphate-modified oligonucleotide analogs have been extensively investigated for the sequence specific inhibition of mRNA translation[14] and as biochemical probes of nucleic acid–protein interactions.[15] Normal phosphodiester linked oligoribonucleotides have relatively short half-lives in serum due to degradation by nucleases. The major types of phosphorus modifications aimed at increasing nuclease resistance include phosphorothioates, phosphorodithioates, phosphoramidates, phosphotriesters, and alkyl phosphonates.[14] The preparation of adenylate oligomers containing methylene groups in place of their 5′-oxygens was reported by Gilham *et al.*[16] by polymerization of the 5′-deoxy-5′-methyl phosphonate analog of adenosine diphosphate (ADP) with the enzyme polynucleotide phosphorylase. These modified oligonucleotides were resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5′-oxygen, but can still form stable complexes with complementary sequences. Heinemann *et al.*[17] reported the chemical synthesis of a self-complementary DNA octamer that contained a single 3′-methyl phosphonate linkage by incorporating the dimer block bearing the internal 3′-methyl phosphonate linkage using a triester approach in solution. The comparison of the crystal structure of the modified duplex with the unmodified control revealed only minor conformational differences between the two. Recently, Caruthers *et al.*[18] reported the synthesis of a 3′-phosphoramidite of a 5′-deoxy-5′-methyl phosphonate thymidine dimer and its incorporation into oligomers by the solid phase phosphoramidite method.

On the basis of the above encouraging results we undertook the synthesis of isosteric and isopolar 5′-deoxy-5′-difluoromethyl phosphonate analogs of nucleoside 5′-phosphates and polyphosphates, as well as dinucleoside phosphates that can potentially be incorporated into oligonucleotides. This modification preserves the ionic, stereochemical, and polar character of natural oligonucleotides and is expected to enhance the resistance of such modified oligonucleotides to degradation by nucleases.

Scheme 1[a]



[a] (i) $LiCF_2PO(OEt)_2$, THF, HMPA; (ii) a. $LiCF_2PO(OEt)_2$, THF, b. PhOC(S)Cl, c. *n*-Bu_3SnH, AIBN, toluene.

Results and Discussion

One common synthetic approach to α,α-difluoroalkyl phosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate.[19] However, our attempts to synthesize 5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-6′,6′-difluoro nucleosides from 5′-deoxy-5′-iodo nucleosides, *i.e.* **4**, using the above reagent were unsuccessful (Scheme 1). Starting compounds were quantitatively recovered. The reaction of uridine 5′-aldehyde **5** with diethyl (lithiodifluoromethyl)phosphonate, according to the procedure of Martin *et al.*,[20] led to a complex mixture of products. Recently, the synthesis of sugar α,α-difluoromethyl phosphonates from primary sugar triflates using the above reagent was described.[21] Unfor-

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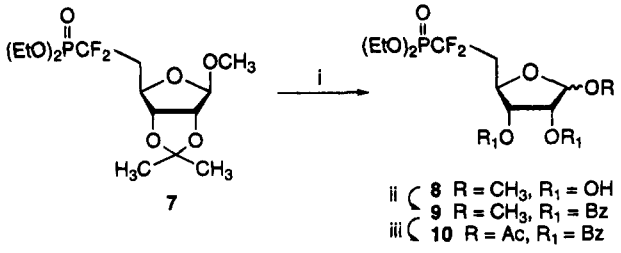
Scheme 2[a]



[a] (i) I_2-MeOH or Dowex 50 (H^+)-MeOH; (ii) BzCl, Pyr; (iii) Ac_2O, AcOH, H_2SO_4, EtOAc.

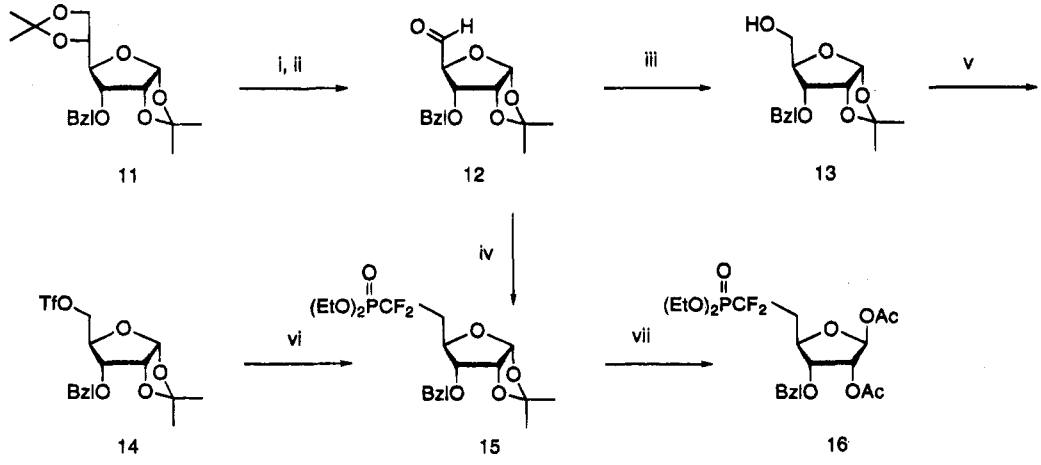
tunately,our experience is that nucleoside 5′-triflates are too unstable to be used in these syntheses. Therefore we took advantage of the successful sugar 5-difluoromethyl phosphonate syntheses and used them for the preparation of suitable glycosylating agents for the preparation of 5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-6′,6′-difluoro nucleosides.[22]

The methyl 5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (**7**) was synthesized from the 5-aldehyde according to the procedure of Martin *et al.*[20] The 2,3-*O*-isopropylidene group of **7** was cleaved under mild conditions (I_2 in methanol[23] or Dowex 50 (H^+) in methanol, Scheme 2) affording **8** as an anomeric mixture in moderate yields. All attempts to use more aggressive acidic conditions to improve the deprotection yield led to destruction of the sugar. Reprotection of the hydroxyls with benzoyl groups yielded the methyl 2,3-di-*O*-benzoyl-D-*ribo*-hexofuranoside **9**. At this point we wanted to displace the relatively unreactive anomeric *O*-methyl group with a more reactive *O*-acetyl, phenylsulfenyl, or halogen group. It was desirable to accomplish these conversions under mild conditions to preserve the integrity of the acid-sensitive difluoromethyl phosphonate riboside. When the conditions used in the classical acetolysis of methyl glycosides[24] were employed, the desired 1-*O*-acetyl sugar was obtained in less than 20% yield. Reaction of **9** with thiophenol in the presence of BF_3·Et_2O, according to the procedure of Beau and Chanteloup[25] did not proceed and the prolonged treatment caused decomposition of the sugar. Similarly, the attempted preparation of glycosyl iodide from **9** and *in situ* condensation with silylated uracil according to Earl *et al.*[26] failed to give the desired nucleosidic product. Finally, the preparation of a suitable glycosylating agent was achieved by acetolysis of **9** under mild acidic conditions (Ac_2O, AcOH, H_2SO_4, EtOAc, 0 °C)[27] to afford an anomeric mixture of 1-*O*-acetyl-2,3-di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (**10**) in high yield.[28]

As a result of our interest in sugar 5-difluoromethyl phosphonates that have a different type of protection at the 2- and 3-hydroxyls, suitable for further functionalization, we synthesized **16** in six steps from the known **11**[29] (Scheme 3). Selective cleavage of the 5,6-*O*-isopropylidene group of **11** and the oxidation of the intermediate diol with sodium metaperiodate furnished aldehyde **12** as a dihydrate. Attempts to dehydrate this compound using various drying techniques had limited success, reflected in a low yield in its conversion to the desired 5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro sugar **15**[21] using the method of Martin *et al.*[20] Reduction of **12** with sodium borohydride afforded 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentofuranose (**13**) which was converted to **15** using triflylation followed by a triflate displacement according to the procedure of Berkowitz *et al.*[21] It is worth noting that when methyl 2,3-*O*-isopropylidene-5-*O*-[(trifluoromethyl)sulfonyl]-β-D-*ribo*-pentofuranoside[30] was treated with diethyl (lithiodifluoromethyl)phosphonate no displacement product could be detected in the reaction mixture confirming that this sugar is not suitable for the displacement reaction due to the reported intramolecular displacement of the triflyloxy group by the methoxy group.[30] Compound **15** was smoothly converted into 1,2-di-*O*-acetyl derivative **16** under the conditions used for the preparation of the analogous 2,3-di-*O*-benzoyl derivative **10**. This acetolysis proceeded in a stereoselective manner yielding exclusively the β-anomer.

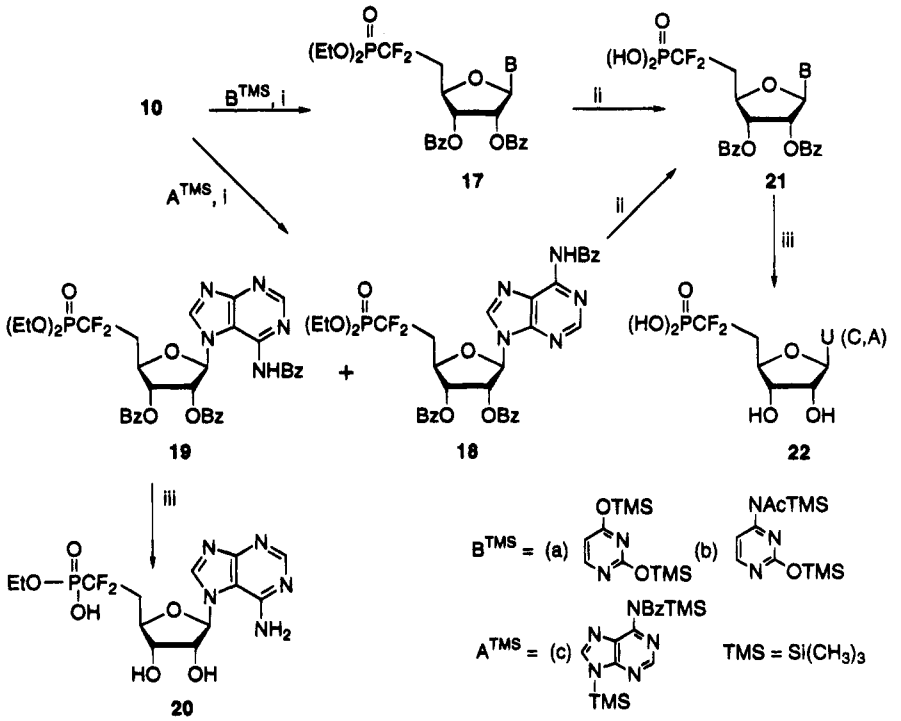
Pyrimidine and purine 5′,6′-dideoxy-6′-(diethoxyphosphinyl)-6′,6′-difluoro nucleosides were synthesized from **10** and silylated bases under Vorbrüggen conditions[31]

Scheme 3[a]



[a] (i) aq. AcOH; (ii) $NaIO_4$, H_2O; (iii) $NaBH_4$, 50% aq. EtOH; (iv) a. $LiCF_2PO(OEt)_2$, THF, b. PhOC(S)Cl, c. *n*-Bu_3SnH, AIBN, toluene; (v) Tf_2O, 2,6-di-*t*-butyl-4-methylpyridine, CH_2Cl_2; (vi) $LiCF_2PO(OEt)_2$, HMPA, THF; (vii) Ac_2O, AcOH, H_2SO_4, EtOAc.

Scheme 4[a]



[a] (i) $SnCl_4$, CH_3CN; (ii) $(CH_3)_3SiBr$, CH_3CN; (iii) conc. NH_4OH:MeOH /3:1.

($SnCl_4$ as a catalyst, refluxing acetonitrile, Scheme 4). The use of $CF_3SO_2OSi(CH_3)_3$ as a glycosylation catalyst was precluded because it was expected to lead to undesired 1-ethyluracil or 9-ethyladenine byproducts.[5] Selective N-1 glycosylation took place in the pyrimidine derivative syntheses yielding **17a** (B = uracil) in 62% yield and **17b** (B = cytosine) in 75% yield. Interestingly, the attempted condensation of methyl 2,3-di-*O*-benzoyl derivative **9** with silylated uracil under Vorbrüggen conditions failed to yield nucleoside phosphonate **17a**. The major product, isolated in low yield, showed an OCH_3 signal and uracil protons in the ^1H NMR suggesting that condensation occurred but was accompanied by the opening of the sugar ring. Since a mixture of diastereoisomers results from such an opening, the ^1H NMR spectrum was rather complex and no further attempt was made to elucidate the structure of these products. Glycosylation of silylated N^6-benzoyladenine yielded a mixture of N-9 isomer **18** and N-7 isomer **19** in 34% and 15% yield, respectively. The N-7 isomer was the slower moving product on TLC (10% methanol in dichloromethane). To clearly determine the site of sugar attachment using UV absorbance measurements, **19** was debenzoylated with methanolic ammonia, yielding the monoester **20**. A UV maximum at 271 nm compared to 260 nm of adenosine confirmed the N-7 structure[32] for **20**. The deprotection of **17a**, **17b**, and **18** was carried out by a one-pot, two-step procedure, deesterification with bromotrimethylsilane in acetonitrile followed by debenzoylation with ammonia–methanol. The first step required long reaction times at room temperature (typically three days) and a great excess of reagent. It has been reported[33] that deprotecting ethyl phosphonates usually requires prolonged reaction times or elevated temperatures. The reaction time for deprotecting **17a**, **17b**, and **18** was considerably shortened (to 30–60 min) by increasing the reaction temperature to 65 °C. Free difluoromethylphosphonic acids of peracylated nucleosides **21a–c** were obtained as chromatographically pure foams after removal of the volatiles and hydrolysis. These were treated further with concentrated NH_4OH/MeOH 2:1 mixtures to yield, after chromatography on DEAE Sephadex, fully deprotected 5′,6′-dideoxy-6′-(dihydroxyphosphinyl)-6′,6′-difluoro nucleoside analogs (82% **22a**; 87% **22b**; 82% **22c**). ^1H, ^{31}P, and ^{19}F NMR, as well as electrospray ionization mass spectrometry (ESI-MS) of these compounds confirmed their structures.

Our interest in the biological properties of these phosphate isosteres prompted us to synthesize the analogs of biologically important molecules adenosine 5′-triphosphate (ATP) and adenosine 3′,5′-cyclic monophosphate (cAMP). It was our intent that the 5′-deoxy-5′-difluoromethylene analog of ATP will substitute for ATP in the synthesis of polynucleotides by RNA polymerases.

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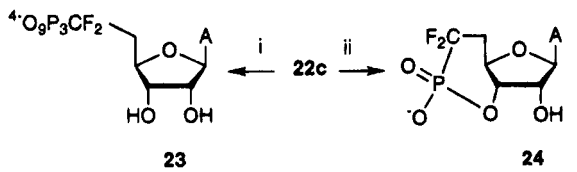
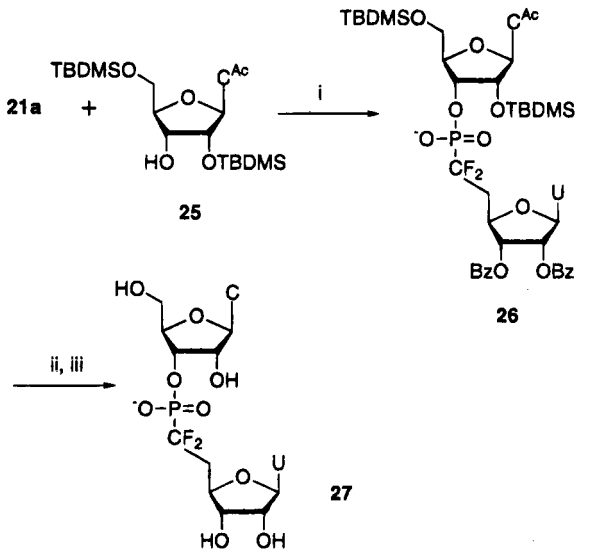
Scheme 5[a]



23 24

[a] (i) a. 1,1'-carbonyldiimidazole, DMF, b. $P_2O_7^{4-}$; (ii) N,N'-dicyclohexyl-4-morpholinecarboxamidine, DCC, Pyr.

Scheme 6[a]



[a] (i) DCC, Dowex 50 ($PyrH^+$), Pyr; (ii) Conc. NH_4OH:MeOH/2:1; (iii) TBAF, THF.

Such synthetic polynucleotides would possess many interesting features, in particular nonhydrolyzable P–C bonds. By using the standard procedure[34] for the preparation of nucleoside triphosphates from nucleoside monophosphates, *i.e.* activation of the phosphate (phosphonate) group with 1,1-carbonyldiimidazole followed by condensation of the intermediate phosphorimidazolidate with tributylammonium pyrophosphate, 5',6'-dideoxy-6'-(dihydroxyphosphinyl)-6',6'-difluoro adenosine analog **22c** (Scheme 5) was converted to the 5'-triphosphate analog **23** in 80% yield. The 3'-cyclic monophosphate analog **24** was prepared using DCC-promoted intramolecular cyclization of **22c** according to the procedure of Smith *et al.*[35]

Finally, the synthesis of a dimer incorporating the 5'-difluoromethylene functionality was undertaken (Scheme 6). DCC-promoted condensation of 1-[2,3-di-*O*-benzoyl-5,6-dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil (**21a**) with a suitably protected cytidine monomer **25** under the conditions introduced by Khorana *et al.*[36] yielded U(5'-3')C dimer **26** in 64% yield. The two-step, one-pot deprotection of **26** using concentrated NH_4OH/MeOH 2:1, followed by TBAF in THF yielded the deprotected dimer **27**. The dimer was purified by DEAE Sephadex column chromatography followed by RP-HPLC. The pure dimer **27** was obtained from **26** in 84% yield.

The incorporation of the difluoromethyl phosphonate dimers into oligonucleotides as well as the results on the substrate and/or inhibitor properties of the nucleoside 5'-triphosphate analogs in the enzymatic reactions will be reported elsewhere.

Experimental Section

General Methods. All reactions were carried out under a positive pressure of argon in anhydrous solvents. Starting materials were purchased from Aldrich (except *N*-benzoyladenine from Lancaster Synthesis Inc., Windham, NH). Commercially available anhydrous solvents were employed without purification. The organic extracts of crude products were dried over anhydrous Na_2SO_4. Analytical thin-layer chromatography (TLC) was performed with Merck Art.5554 Kieselgel 60 F_{254} plates and flash column chromatography using Merck 0.040–0.063 mm silica gel 60. Solvents used for TLC of charged phosphonates were (A) *i*-PrOH/concd NH_4OH/H_2O 7:1:2, and (B) *i*-PrOH/concd NH_4OH/H_2O 6:3:1. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. NMR spectra were recorded at 400.075 MHz for ^1H, 161.947 MHz for ^{31}P, and 376.414 MHz for ^{19}F. $CDCl_3$ was used as a solvent unless indicated otherwise. Chemical shifts in ppm refer to TMS, H_3PO_4, and $CFCl_3$, respectively. RP-HPLC was performed utilizing a Hamilton PRP-1 5 μ (250 × 4.1 mm) column. General conditions employed a binary mixture of eluents A and B: A = 50 mM triethylammonium acetate (TEAA) pH 8, B = CH_3CN at a flow rate of 2 mL/min. Positive ion and negative ion electrospray ionization mass spectra (ESI-MS) are reported as *m/z* (relative intensity).

Methyl 5,6-Dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (8). A. Compound **7**[20] (7.2 g, 19.2 mmol) was dissolved in a 1% solution of I_2 in MeOH (90 mL). The solution was refluxed overnight and evaporated to a syrup that was partitioned between $CHCl_3$ and 5% aqueous $Na_2S_2O_3$. The aqueous layer was washed two times with $CHCl_3$, the organic layers were combined, dried, and evaporated to dryness. Chromatography using a 2–10% gradient of MeOH in CH_2Cl_2 afforded the product (2.6 g, 40%, or 72% based on isolated starting material) as a syrup, α/β ratio = 20/80. Anal. Calcd for $C_{11}H_{21}F_2O_7P$: C, 39.53; H, 6.33. Found: C, 39.66; H, 6.41.

B. Compound **7** (3.0 g, 8.01 mmol) was dissolved in MeOH (30 mL) and Dowex 50WX8 (H^+) (10 g) was added. The mixture was stirred 5 days at rt and filtered, and the filtrate was evaporated to a syrup and chromatographed as in A to yield 0.88 g, 33% of the deprotected product (82% yield based on the isolated starting material).

Methyl 2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (9). Compound **8** (4.4 g , 13.2 mmol) was dissolved in pyridine (23 mL) and the solution was cooled to 0 °C. Benzoyl chloride (5 mL, 43 mmol) was added dropwise to the stirred solution under Ar, and the reaction mixture was stirred at 0 °C for 4 h and then at rt overnight. The reaction was then poured into ice–water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with 5% aqueous $NaHCO_3$, dried, and evaporated to dryness. Chromatography using a 15–50% gradient of EtOAc in hexane yielded the product as a syrup (5.9 g, 82%), α/β ratio = 20/80. Anal. Calcd for $C_{25}H_{29}F_2O_9P$: C, 55.35; H, 5.39. Found: C, 55.19; H, 5.36.

1-*O*-Acetyl-2,3-di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranoside (10). Dry **9** (0.65 g, 1.2 mmol) was dissolved in EtOAc (10 mL). The solution was cooled to −15 °C and mixed with a cooled (−15 °C) solution of EtOAc (19.5 mL), acetic anhydride (11.0 mL), acetic acid (8.3 mL) and concd H_2SO_4 (0.05 mL). The solution was kept at 0 °C for 16 h. The reaction was diluted with $CHCl_3$ (75 mL) and poured into cold 5% aqueous $NaHCO_3$ (100 mL). The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (3 × 25 mL). The combined organic layers were washed with brine, dried, and evaporated to dryness to give chromatographically and spectrally pure

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material as a syrup (0.62 g, 91%), α/β ratio = 40/60. Anal. Calcd for $C_{26}H_{29}F_2O_{10}P$: C, 54.74; H, 5.12. Found: C, 54.74; H, 5.22.

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentodialdo-1,4-furanoside (12). 3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**11**)[29] (18 g, 51.37 mmol) was dissolved in 75% aqueous acetic acid (130 mL) and the solution kept at rt for 24 h. 1-Butanol (100 mL) was added and the solution evaporated to a syrup and coevaporated with 1-butanol (3 × 50 mL). The residue was dissolved in water (100 mL) and $NaIO_4$ (12.64 g, 59 mmol) was added portionwise under vigorous stirring. The stirring was continued at rt for 1 h and then EtOH (500 mL) was added. Salts were filtered off and washed with water, and the filtrate was evaporated to a syrup *in vacuo* and dried by several coevaporations with toluene. The resulting residue was purified using a 10–50% EtOAc gradient in hexane. Fractions containing the desired product were pooled and concentrated *in vacuo*. The purified residue was dissolved in CH_2Cl_2 (500 mL), activated molecular sieves 4Å (100 g) were added, and the mixture was set aside for 48 h. The molecular sieves were filtered off and the filtrate was evaporated to dryness affording **12** as a colorless syrup (11.8 g, 82%).

3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (13). To a cold, well-stirred solution of **12** (11.5 g, 41 mmol) in 50% aqueous EtOH (300 mL) was added portionwise $NaBH_4$ (3.1 g, 82 mmol). The solution was stirred at rt for 2 h and then neutralized with 10% aqueous acetic acid. EtOH was removed under reduced pressure at 40 °C and the aqueous layer extracted with $CHCl_3$ (2 × 250 mL). Extracts were combined, dried, and evaporated to dryness at 40 °C. The residue was purified using a 30–60% EtOAc gradient in hexane. Fractions containing the desired product were combined and evaporated to a syrup (10.2 g, 88%) which had identical spectral properties to a compound prepared by Berkowitz *et al.*[21]

3-*O*-Benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-1,2-*O*-isopropylidene-β-D-*ribo*-hexofuranose (15). A. By using the two-step procedure of Martin *et al.*[20] 5-aldehyde **12** (1.08 g, 3.77 mmol) was converted to **15** (0.39 g, 22%). **B.** By using the procedure of Berkowitz *et al.*[21] **14** (1 g, 2.3 mmol) was converted to **15** (1 g, 96%).

1,2-Di-*O*-acetyl-3-*O*-benzyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranose (16). Compound **15** (0.39g, 0.88 mmol) was converted to **16** (0.27 g, 63%) in the same manner as described for the synthesis of **10**. Anal. Calcd for $C_{20}H_{29}F_2O_9P$: C, 49.80; H, 6.06. Found: C, 50.06; H, 6.09.

1-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil (17a). Compound **10** (570 mg, 1 mmol) was dissolved in acetonitrile (17 mL) and added, under argon, to silylated uracil base. The latter was prepared by refluxing uracil (224 mg, 2 mmol) with 1,1,1,3,3,3-hexamethyldisilazane/pyridine 1:1 (4 mL) until complete dissolution occurred followed by the removal of volatiles under reduced pressure and coevaporation with toluene (2 x 10 mL). Tin(IV) chloride was added (129 μL, 1.1 mmol) and the mixture was heated under reflux for 2 h. After cooling to rt, the mixture was diluted with CH_2Cl_2 (100 mL) and extracted with 5% aqueous $NaHCO_3$ (50 mL) and H_2O (30 mL). The organic layer was dried and concentrated to a syrup. The product was purified using a 1–5% methanol in CH_2Cl_2 gradient to yield the product (388 mg, 62%) as a white foam. Anal. Calcd for $C_{28}H_{29}F_2N_2O_{10}P$: C, 54.02; H, 4.70; N, 4.50. Found: C, 53.93; H, 4.90; N, 4.46.

1-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]-*N*⁴-acetylcytosine (17b). Compound **10** (1 g, 1.75 mmol) was condensed with silylated N^4-acetylcytosine in the same manner as for the synthesis of **17a** to afford, after chromatographic purification, **17b** as a white foam (0.87 g, 75%). Anal. Calcd for $C_{30}H_{32}F_2N_3O_{10}P$: C, 54.30; H, 4.86; N, 6.33. Found: C, 54.07; H, 4.98; N, 6.22.

9-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]-*N*⁶-benzoyladenine (18) and 7-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(diethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]-*N*⁶-benzoyladenine (19). Compound **10** (1 g, 1.75 mmol) was condensed with silylated N^6-benzoyladenine in the same manner as for the synthesis of **17a**. Purification using a 1–5% methanol in CH_2Cl_2 gradient eluted first the N-9 isomer **18**, obtained as a white foam (450 mg, 34%). Anal. Calcd for $C_{36}H_{34}F_2N_5O_9P$: C, 57.68; H, 4.57; N, 9.34. Found: C, 57.61; H, 4.73; N, 9.42.

Fractions containing the slower moving N-7 isomer were combined and evaporated to dryness to yield 200 mg (15%) of **19** as a white foam. Anal. Calcd for $C_{36}H_{34}F_2N_5O_9P$: C, 57.68; H, 4.57; N, 9.34. Found: C, 57.49; H, 4.53; N, 9.23.

7-[5,6-Dideoxy-6-(ethoxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]adenine Sodium Salt (20). Compound **19** was dissolved in concd NH_4OH/MeOH 2:1 (15 mL) and the solution was heated at 60 °C overnight. The mixture was then evaporated to dryness and chromatographed on a DEAE Sephadex A-25 (HCO_3^-) column using 0.01–0.25 M triethylammonium bicarbonate (TEAB) buffer (pH 7.8) as eluent. Fractions containing the product were combined and concentrated *in vacuo*, and the remaining buffer was removed by several coevaporations with methanol. The residue was dissolved in water and passed through a Dowex 50 WX8 (Na^+) column. Removal of the solvent *in vacuo* yielded the sodium salt of **20** as a white powder (80 mg, 70%): UV (H_2O) λ_{max} 271 nm, λ_{min} 234 nm; MS/ESI⁺ *m/z* 410.2 $(M + H)^+$.

1-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil (21a). Compound **17a** (0.96 g, 1.54 mmol) was dissolved in acetonitrile (12 mL). Bromotrimethylsilane (4.0 mL, 30 mmol) was added dropwise to the stirred solution under argon and was then heated at 65 °C for 1 h. The volatiles were removed *in vacuo* under anhydrous conditions and the residue coevaporated several times with toluene and methanol. Aqueous methanol was added and the solution evaporated to dryness yielding chromatographically pure **21a**.

1-[5,6-Dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil Sodium Salt (22a). The above material was dissolved in the mixture of MeOH/concd ammonia (1:2, 20 mL) and heated at 60 °C overnight. Volatiles were removed *in vacuo* and the residue dissolved in 0.01 M TEAB and applied to a DEAE Sephadex A-25 (HCO_3^-) column. Elution using a 0.01–0.25 M TEAB gradient followed by removal of the buffer by evaporation and then multiple coevaporations with methanol yielded a syrup that was dissolved in H_2O and passed through a column of Dowex 50 WX8 (Na^+). Evaporation of the eluate to dryness yielded the sodium salt of **22a** as a white powder, 508 mg (82%): MS/ESI⁻ *m/z* 356.9 $(M - 2H)^-$.

1-[5,6-Dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]cytosine Sodium Salt (22b). Compound **17b** (0.85 g, 1.28 mmol) was deprotected and purified in the same way as described for the preparation of uracil derivative **22a** to give the sodium salt of **22b** as a white powder (446 mg, 87%): MS/ESI⁻ *m/z* 355.9 $(M - 2H)^-$.

9-[5,6-Dideoxy-6-(dihydroxyphosphinyl)-6,6-difluoro-β-D-*ribo*-hexofuranosyl]adenine Sodium Salt (22c). Compound **18** (390 mg, 0.52 mmol) was deprotected and purified in the same way as described for the preparation of uracil derivative **22a** to give the sodium salt of **22c** as a white powder (181 mg, 82%): MS/ESI⁻ *m/z* 380.0 $(M - 2H)^-$.

Adenosine 5′-Triphosphate Analog 23. A solution of the pyridinium salt of **22c**, obtained by passing a solution of the sodium salt (40 mg, 0.1 mmol) through a column of Dowex 50 WX8 (Pyr^+) resin, in water (10 mL) and tributylamine (30 μL), was evaporated to dryness *in vacuo*. The residue, rendered anhydrous by repeated addition and evaporation of anhydrous pyridine, was dissolved in anhydrous DMF (2 mL), and 1,1-carbonyldiimidazole (80 mg, 0.5 mmol) was added. The mixture was stirred at rt for 5 h and a solution of tributylammonium pyrophosphate (0.5 mmol) in DMF (5 mL) was added dropwise with vigorous mixing. The mixture was stirred overnight at rt and the resulting precipitate removed by filtration. The filtrate was treated with MeOH (10 mL) and the solution evaporated *in vacuo* at 40 °C. The resulting residue was dissolved in water and applied on a DEAE Sephadex A-25 (HCO_3^-) column. Elution with 0.01–0.6 M TEAB afforded **23** which was further purified by RP-HPLC

(linear gradient 0–20% B in 30 min, t_R 23.5 min): MS/ESI$^-$ m/z 539.9 $(M - H)^-$. The resulting triethylammonium salt was dissolved in EtOH and precipitated as the sodium salt with a solution of $NaClO_4$ in acetone to yield **23** as white powder (49 mg, 80%).

Adenosine 3′,5′-Cyclic Phosphate Analog 24. A mixture of the triethylammonium salt of **22c** (90 mg, 0.15 mmol), *N,N′*-dicyclohexyl-4-morpholinecarboxamidine (45 mg, 0.15 mmol), pyridine (15 mL), and water (5 mL) was concentrated *in vacuo* and dried by coevaporation with pyridine (3 × 20 mL). The residue was dissolved in pyridine (15 mL) and the solution added dropwise over 30 min to a stirred refluxing solution of DCC (61 mg, 0.3 mmol) in pyridine (15 mL). The solution was heated under reflux for a further 2 h and evaporated to dryness. The residue was partitioned between water and ether and filtered, and the aqueous layer was separated and chromatographed on a DEAE Sephadex A-25 (HCO_3^-) column. Elution with 0.005–0.15 M TEAB and conversion to the sodium salt using Dowex 50 WX8 (Na^+) yielded **24** as a white powder (61 mg, 59%): MS/ESI$^+$ m/z 364.2 $(M + H)^+$.

2′,5′-Bis(*O*-*tert*-butyldimethylsilyl)-N^4-acetylcytidine (25). N^4-Acetylcytidine (4 g, 14 mmol) was suspended in THF (200 mL), and pyridine (6 mL) and silver nitrate (5.23 g, 30.8 mmol) were added. The mixture was stirred for 15 min and then *tert*-butyldimethylsilyl chloride (4.64 g, 30.8 mmol) was added. The reaction was stirred for 24 h at rt and filtered. Methanol (30 mL) was added to the filtrate which was then evaporated to a syrup. The desired faster moving product was purified using a 20–50% gradient of EtOAc in hexane, yielding **25** as a white foam (4.4 g, 61%). The structure of **25** was unequivocally determined by a ^1H double resonance experiment; D_2O exchange of the 3′-hydroxyl proton of **25** followed by irradiation of H4′ caused the collapse of the H3′ signal from ddd to d. Anal. Calcd for $C_{23}H_{43}N_3O_6Si_2$: C, 53.77; H, 8.44; N, 8.18. Found: C, 53.55; H, 8.28; N, 8.13. The slower moving 3′,5′-isomer was isolated as a white foam (0.94 g, 13%).

Protected U(5′-3′)C-Dinucleoside Phosphate Analog 26. 1-[2,3-Di-*O*-benzoyl-5,6-dideoxy-6-(dihydroxyphosphinyl-6,6-difluoro-β-D-*ribo*-hexofuranosyl]uracil (**21a**) (436 mg, 0.77 mmol) was dissolved in pyridine, evaporated to dryness, and coevaporated two times with pyridine. The residue was dissolved in pyridine, and Dowex 50 WX8 (pyr^+ form) (1.77 g, dried by repeated coevaporations with pyridine) was added followed by addition of 2′,5′-bis(*O*-*tert*-butyldimethylsilyl)-N^4-acetylcytidine (**25**) (514 mg, 1.0 mmol) and DCC (2.1g, 10.18 mmol). The mixture was stirred, protected from light, at rt for 6 days after which time water (10 mL) was added. The mixture was stirred for 2 h and then filtered, and the filtrate was extracted with $CHCl_3$ (5 × 20 mL). The combined organic layers were dried and evaporated to dryness. The residue was chromatographed using a 2–5% methanol in CH_2Cl_2 gradient containing 1% NEt_3 for elution. Evaporation of the appropriate fractions yielded **26** as a white foam (570 mg, 64%): MS/ESI$^-$ m/z 1060.0 $(M - H)^-$.

U(5′-3′)C-Dinucleoside Phosphate Analog 27. Compound **26** (350 mg, 0.33 mmol) was dissolved in a mixture of MeOH (5 mL) and concd NH_4OH (10 mL) and the solution was kept at rt for 48 h and evaporated to dryness *in vacuo*. The residual foam was dissolved in THF (10 mL) and 1 M TBAF in THF (4 mL) was added. The reaction mixture was kept at rt for 48 h, evaporated to a syrup, and partitioned between water and $CHCl_3$. The aqueous layer was extracted twice with $CHCl_3$ and then applied to a DEAE Sephadex A-25 (HCO_3^-) column. Elution with 0.005–0.15 M TEAB and RP-HPLC (linear gradient 0–40% B in 40 min, t_R 22.6 min), followed by the conversion of the product to the sodium salt using Dowex 50 WX8 (Na^+) yielded **27** as a white solid (170 mg, 84%): MS/ESI$^+$ m/z 584.3 $(M + H)^+$.

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Supplementary Material Available: Complete ^1H NMR spectral data for compounds **11**, **16**, and **25**; ^1H and ^{31}P NMR spectral data for **8–10**, **17a**, **17b**, **18–27**; ^{19}F NMR spectral data for **22a**, **23**, and **27** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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