Synthesis of 2′,3′-Dideoxynucleoside 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphates and Their Inhibition of HIV-1 Reverse Transcriptase

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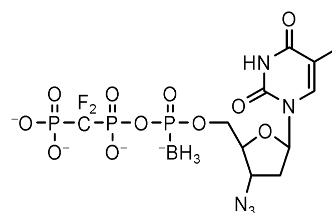
The triphosphates of antiviral 2′,3′-dideoxynucleosides (ddNs) are the active chemical species that inhibit viral DNA synthesis. The inhibition involves incorporation of ddNMP into DNA and subsequent chain termination. A conceivable strategy for antiviral drugs is to employ nucleoside 5′-triphosphate mimics that can entirely bypass cellular phosphorylation. AZT 5′-α-R_P-borano-β,γ-(difluoromethylene)triphosphate (5′-αB-$\beta\gamma CF_2$TP) has been identified as a potent inhibitor of HIV-1 reverse transcriptase (HIV-1 RT). This work was aimed at confirming that 5′-αB-$\beta\gamma CF_2$TP is a useful generic triphosphate moiety and can render antiviral ddNs with potent inhibitory effects on HIV-1 RT. Thus, 10 ddNs were converted to their 5′-αB-$\beta\gamma CF_2$-TPs via a sequence (one-pot) of reactions: formation of an activated phosphite, formation of a cyclic triphosphate, boronation, and hydrolysis. Other synthetic routes were also explored. All ddN 5′-αB-$\beta\gamma CF_2$TPs tested exhibited essentially the same level of inhibition of HIV-1 RT as the corresponding ddNTPs. A conclusion can be made that 5′-αB-$\beta\gamma CF_2$TP is a generic and promising triphosphate mimic (P3M) concerning HIV-1 RT inhibition and serum stability. It is anticipated that use of 5′-αB-$\beta\gamma CF_2$TP as P3M moiety will lead to the discovery of a new class of anti-HIV agents.

Introduction

2′,3′-Dideoxynucleoside (ddN) antiviral drugs are successively phosphorylated in cells to 2′,3′-dideoxynucleoside 5′-monophosphates (ddNMPs), 2′,3′-dideoxynucleoside 5′-diphosphates (ddNDPs), and 2′,3′-dideoxynucleoside 5′-triphosphates (ddNTPs).[1,2] The triphosphates of the antiviral nucleosides are the active chemical species that inhibit viral DNA synthesis. The inhibition of viral DNA synthesis primarily involves incorporation of ddNMP into the elongating DNA chain and subsequent chain termination.[1−4] Cellular phosphorylation of an antiviral nucleoside by kinases is a crucial process leading to an active NTP, but any alternative route that can lead to a sufficient cellular concentration of the active NTP can be very useful in the discovery of antiviral nucleosides. A conceivable strategy is to directly use a nucleotide as a drug entity that can bypass cellular phosphorylation partially or entirely. To bypass the first cellular phosphorylation of nucleosides, NMP prodrugs have been intensively explored, and several types of NMP prodrugs have shown promising in vitro activities.[5−9] However, a major obstacle to success is the inherent instability of NMPs to cellular dephosphorylating enzymes. Thus far, NMP prodrugs have not been used clinically as antiviral drugs, but NMP prodrug approach has been successfully applied to nonhydrolyzable acyclic nucleoside phosphonates, a type of NMP mimics. Currently, tenofovir disoproxil fumarate (a bis-POC prodrug of PMPA)[10] and adefovir dipivoxil (a bis-POM prodrug of PMEA)[11] are used clinically as anti-HIV and anti-HBV drugs, respectively. Since NMP mimic prodrugs were successful, it was conceivable to use stable NTP mimics (NP3Ms) as antiviral agents, which can entirely bypass cellular phosphorylation. If such an NP3M is identified, an effective cellular concentration may be achieved from a relatively low dose of a NP3M prodrug. Recently, our laboratories synthesized and evaluated a large number of NP3Ms containing relatively stable triphosphate mimics (P3Ms).[12] Among the most promising P3Ms is 5′-α-R_P-borano-β,γ-(difluoromethylene)triphosphate (5′-αB-$\beta\gamma CF_2$TP), which led to AZT 5′-R_P-αB-$\beta,\gamma CF_2$TP (**1**), a very potent inhibitor of HIV-1 reverse transcriptase (RT) with a K_i value of 9.5 nM in an assay using a poly-A as template. AZT 5′-αB-$\beta\gamma CF_2$TP also exhibited satisfactory stability in serum and cell extract.[12] A valuable P3M should be applicable to a variety of nucleosides; therefore, it is interesting to know whether the 5′-αB-$\beta\gamma CF_2$TPs of other nucleoside reverse transcriptase inhibitors (NRTIs) possess similar anti-HIV activities as NRTI triphosphates. To answer this question, this article will describe synthesis of a series of ddN 5′-αB-$\beta\gamma CF_2$TPs and their inhibitory properties on HIV-1 RT.



AZT 5’-αB-βγCF_2TP (**1**)

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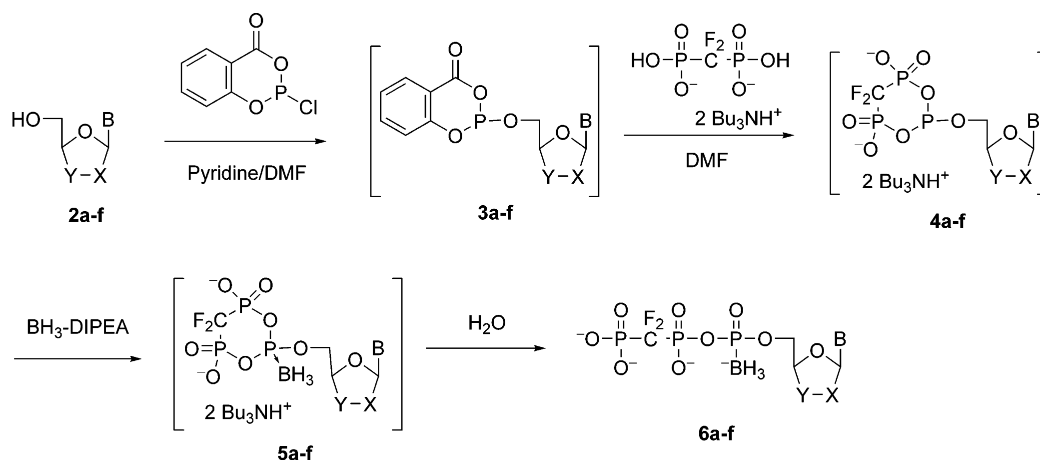
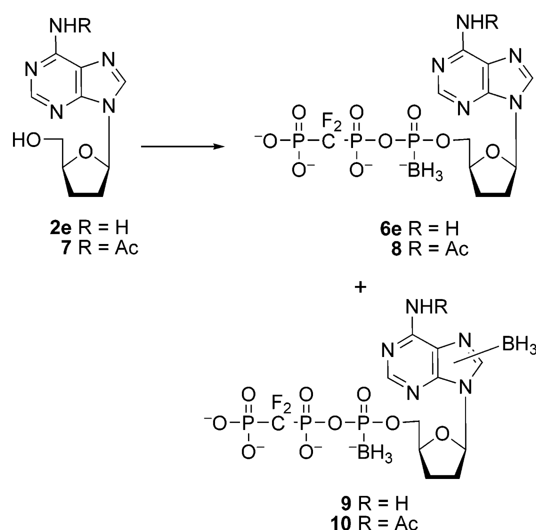
Scheme 1

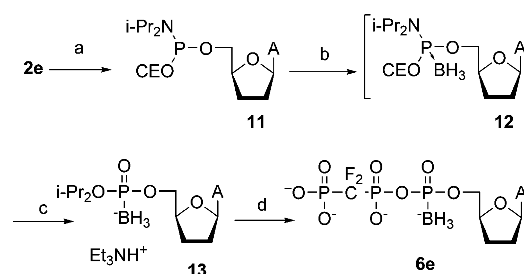


a B = thymine, X = Y = CH_2; **b** B = thymine, X, Y = CH=CH; **c** B = 5-F-cytosine, X = CH_2, Y = S (L-ribose), **d** B = uracil, X = Y = CH_2; **e** B = adenine, X = Y = CH_2; **f** B = 7-deazaguanine, X = Y = CH_2

Scheme 2



Scheme 3[a]



[a] Reaction conditions: (a) (CEO)(i-$Pr_2N)_2$PCl, DIPEA, CH_3CN, room temp, 12 h; (b) DIPEA−BH_3, room temp, 24 h; (c) (1) NH_3, MeOH, water, room temp, 24 h, (2) TEA; (d) CF_2DP−TBA, DMF, 50 °C, 3.5 h.

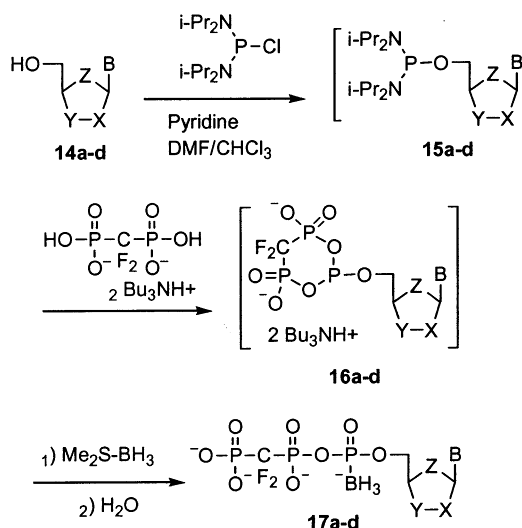
Chemistry

Compounds **6a−f** (Scheme 1) were synthesized according to a similar procedure[12] for preparation of AZT 5′-αB-$\beta\gamma$$CF_2$TPs (**1**)[12] and nucleoside 5′-α-P-boranotriphosphates.[13] Treatment of **2a−f** with 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one yielded the activated phosphites **3a−f**, which were condensed with bis(tributylammonium) difluoromethylenediphosphonate[12] to form the cyclic triphosphates **4a−f**. Treatment of **4a−f** with diisopropylethylamine−borane complex yielded, after hydrolysis, the ddN 5′-αB-$\beta\gamma$$CF_2$TPs **6a−f** in moderate yields.

In the preparation of **6e**, the adenine base of ddA (**2e**) was also boronated and the initial major product **9** contained two BH_3 as evidenced by LC−MS (Scheme 2). Surprisingly, treatment with aqueous ammonia could not remove the borane complexed with the adenine base. The boronation on the adenine base further complicated the reaction, and a mixture of products including ring-reduction products (not shown) was formed as revealed by LC−MS. After storage of the crude product at −20 °C for 2 months, a significant amount of **9** changed to **6e** which could be separated by HPLC. However, compound **9** could not be isolated in a pure form because of its lability during HPLC purification. In a previous publication,[14] N^7-cyanoborane-2′-deoxyguanosine 5′-triphosphate was prepared through phosphorylation of N^7-cyanoborane-2′-deoxyguanosine, which indicated that N^7-cyanoborane purine nucleosides was superior to N^7-borane purine nucleosides concerning chemical stability. In an attempt to minimize the formation of the diboronated product, ddA was acetylated at the N6 position and the resulting **7** was subjected to the sequential reactions as shown in Scheme 1. The rationale of using N^6-acetyl ddA (**7**) was to reduce electron density on the heterocycle, which was expected to decrease the amount of the bis-BH_3 product. Unfortunately, the acetyl at the N6 position did not change the course of the reactions and led to a complicated mixture. Actually, the formation of **8** and **10** was not observed.

Because of the low yield and complexity encountered in the preparation of **6e** as shown above, an alternative synthetic route[15] was explored. Thus, ddA was converted to the phosphoramidite **11**, which was treated with diisopropylethylamine−borane complex to form the 5′-P-boranophosphite **12** (Scheme 3). Removal of the 2-cyanoethyl (CE) with aqueous ammonia gave **13**, which was converted to its triethylammonium salt and then treated with bis(tetrabutylammonium) difluoromethylenediphosphonate to yield **6e**. Similarly, the treatment of **11** with diisopropylethylamine−borane complex also yielded a bis-BH_3 derivative (not shown) but to a lesser extent. In the case of 7-deazaguanosine (**2f**) that cannot be boronated on the base moiety under

Scheme 4



a B = cytosine, X = Y = CH_2, Z = O; **b** B = cytosine, X = CH_2, Y = S (L-ribose), Z = O; **c** B = thymine, X = CH_2, Y = CHF (F on α-face), Z = O; **d** B = guanine, X = Y = CH, Z = CH_2

the reaction conditions, **6f** was prepared in better yields than in the case of ddA by both synthetic routes.

It appeared that the synthetic method in Scheme 1 did not apply well to all the nucleosides, especially the nucleosides having an exocyclic amino group, therefore, an alternative synthetic procedure[16] was employed (Scheme 4). Thus, **14a**–**d** were treated with bis(diisopropylamino)chlorophosphine instead of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one. The course of the reactions[16] was similar to that in Scheme 1 except that the bis(diisopropylamino)phosphines **15a**–**d** were the active phosphite intermediates, equivalent to the intermediates **3a**–**f** in Scheme 1. Treatment of **15a**–**d** with bis(tributylammonium) difluoromethylenediphosphonate presumably yielded the cyclic triphosphates **16a**–**d**, which were subsequently subjected to boronation and hydrolysis to give **17a**–**d**.

Compounds **6a**–**f** and **17a**–**d** all consist of two diastereomers in approximately 1:1 ratio, which were separated on a reverse-phase HPLC column except for **6a**, **6f**, **17b**, and **17d**, which were not separable under the conditions used. The separated diastereomers are designated as diastereomers I and diastereomers II, according to whether they were eluted earlier (I) or later (II) from the C18 reverse-phase HPLC. Determination of the stereochemistry of the diastereomers was not conducted. Since the diastereomer I of AZT 5′-α-*P*-boranotriphosphate was previously assigned as the α-R_P-diastereomer[17,12] and was found to be a much more potent inhibitor of HIV-1 RT than the diastereomer II (S_P), we might expect the diastereomers I (shorter retention time on HPLC) of **6a**–**f** and **17a**–**d** to be more potent inhibitors of HIV-1 RT than the corresponding diastereomers II (longer retention time on HPLC).

Serum Stability

Serum stability of selected ddN 5′-αB-$\beta\gamma CF_2$TPs (Table 1) was assessed in fetal calf serum at 37 °C following a published procedure.[18] Like AZT 5′-αB-$\beta\gamma CF_2$TP, all the ddN 5′-αB-$\beta\gamma CF_2$TPs tested demonstrated satisfactory serum stability, having a half-life of more than 48 h relative to a half-life of 0.8–3 h for NTPs under the same assay conditions. The data in Table 1 also suggested a generic property of 5′-αB-$\beta\gamma CF_2$TP concerning serum stability.

Table 1. Serum Stability of ddNTPs and ddN 5′-αB-$\beta\gamma CF_2$TPs

compd	serum half-life (h)
AZT 5′-αB-$\beta\gamma CF_2$TP (**1**)	>48
ddU 5′-αB-$\beta\gamma CF_2$TP (**6d**-I)	>48
ddC 5′-αB-$\beta\gamma CF_2$TP (**17a**-I)	>48
3TC 5′-αB-$\beta\gamma CF_2$TP (**17b**-I/II)	>48
AZTTP	2
ddUTP	1
ddCTP	0.8
3TCTP	3

Inhibition of HIV-1 RT

The effectiveness of compounds as inhibitors of HIV-1 RT was determined according to a published procedure using a heteropolymeric RNA as template.[3] ddN 5′-αB-$\beta\gamma CF_2$TPs, **1**, **6b**-I, **6d**-I, **17a**-I, **17b**-I/II, **17c**-I, and **17d**-I/II exhibited similar or slightly better activities than their corresponding ddNTPs, while **6a**-I/II, **6c**-I, **6e**-I, and **6f**-I/II are slightly (2- to 4-fold) less active than their corresponding ddNTPs (Table 2). Since **6a**-I/II and **6f**-I/II each contain two diastereomers and the diastereomers II are not expected to be active,[12,17] the potency of pure **6a**-I and **6f**-I could be higher. It is not difficult to draw a conclusion from these data that 5′-αB-$\beta\gamma CF_2$TP is a promising, generic P3M concerning HIV-1 inhibition. As mentioned above, when 5′-αB-$\beta\gamma CF_2$TP was attached to the antiviral nucleosides, all the resulting ddN 5′-αB-$\beta\gamma CF_2$TPs (diastereomers I or diastereomers I/II) demonstrated essentially the same level of inhibition of HIV-1 RT as the corresponding ddNTPs. Given their enhanced biological stability (Table 1)[12] as well as their potent inhibitory effects on HIV-1 RT, these ddN 5′-αB-$\beta\gamma CF_2$TP compounds represent a new class of potential antiviral agents. In addition, αB-$\beta\gamma CF_2$TP may be attached to such nucleosides that cannot be phosphorylated by cellular enzymes, but their triphosphates are the potent inhibitors of HIV-1 RT. One example in this work is ddU 5′-αB-$\beta\gamma CF_2$TP. ddU is not phosphorylated to ddUTP in cells,[19] but ddUTP does inhibit HIV-1 RT (K_i = 0.55 μM). The corresponding ddU 5′-αB-$\beta\gamma CF_2$TP exhibited essentially the same level (K_i = 0.44 μM) of inhibition. It is expected that inhibitors more potent than ddU 5′-αB-$\beta\gamma CF_2$TP will be identified when αB-$\beta\gamma CF_2$TP is applied to more prospective nucleosides that cannot be phosphorylated in cells. By employment of useful P3Ms such as 5′-αB-$\beta\gamma CF_2$TP, the pool of potentially useful nucleosides is also enlarged. Recently, AZT and d4T 5′-α-*P*-boranotriphosphates were found to be potent inhibitors against mutant HIV-1 RT.[17] Since 5′-αB-$\beta\gamma CF_2$TP has a close structural similarity to 5′-α-*P*-boranotriphosphate, it is anticipated that ddN 5′-αB-$\beta\gamma CF_2$TPs may offer similar advantages over ddNs concerning drug resistance and selectivity. Clearly, further studies on ddN 5′-αB-$\beta\gamma CF_2$TP are justified.

Conclusion

Synthesis of a series of ddN 5′-α-*P*-borano-β,γ-(difluoromethylene)triphosphates by three different approaches has been described. All ddN 5′-αB-$\beta\gamma CF_2$TPs

Table 2. Inhibition of HIV-1 RT by ddN 5′-αB-$\beta\gamma$CF$_2$TPs[a]

ddNTP	K_i (μM)	ddN 5′-αBCF$_2$TP (compd no.)	K_i (μM)
AZTTP	0.091 ± 0.030	AZTαBCF$_2$TP (**1**)	0.094 ± 0.026
dTTP	0.061 ± 0.009	dTαBCF$_2$TP (**6a**-I/II)	0.236 ± 0.032
d4TTP	0.056 ± 0.0134	d4TαBCF$_2$TP (**6b**-I)	0.045 ± 0.127
FTCTP	0.501 ± 0.083	FTCαBCF$_2$TP (**6c**-I)	1.77 ± 0.245
ddUTP	0.545 ± 0.097	ddUαBCF$_2$TP (**6d**-I)	0.438 ± 0.135
ddATP	0.02 ± 0.004	ddAαBCF$_2$TP (**6e**-I)	0.047 ± 0.011
7-deaza-ddGTP	0.025 ± 0.004	7-deaza-ddGαBCF$_2$TP (**6f**-I/II)	0.071 ± 0.025
ddC	0.051 ± 0.011	ddCαBCF$_2$TP (**17a**-I)	0.023 ± 0.008
3TCTP	0.188 ± 0.039	3TCαBCF$_2$TP (**17b**-I/II)	0.314 ± 0.122
3′-F-dTTP	0.071 ± 0.019	3′-F-dTαBCF$_2$TP (**17c**-I)	0.046 ± 0.016
Carbovir TP	0.037 ± 0.011	Carbovir-αBCF$_2$TP (**17d**-I/II)	0.034 ± 0.010

[a] Diastereomer I assigned to the ddN 5′-αB-$\beta\gamma$CF$_2$TPs having shorter HPLC retention time. Diastereomer II assigned to the ddN 5′-αB-$\beta\gamma$CF$_2$TPs having longer HPLC retention time. I/II refers to a mixture.

tested exhibited essentially the same level of inhibition of HIV-1 RT as the corresponding ddNTPs. A conclusion can be made that αB-$\beta\gamma$CF$_2$TP is a promising, generic P3M concerning HIV-1 inhibition and serum stability. It is anticipated that use of 5′-αB-$\beta\gamma$CF$_2$TP as the P3M moiety of nucleoside 5′-triphosphate mimics will lead to more potent anti-HIV agents. Progress in synthesis and in vitro evaluation of ddN 5′-αB-$\beta\gamma$CF$_2$TP prodrugs have been made and will be reported in due time.

Experimental Section

^{1}H NMR spectra were recorded on a Varian Mercury 300 NMR spectrometer. Tetramethylsilane was used as internal reference for ^{1}H NMR, 85% phosphoric acid as external reference for ^{31}P NMR, and CFCl$_3$ as external reference for ^{19}F NMR. Bis(tributylammonium) difluoromethylenediphosphonate was prepared according to an established procedure.[12] Anhydrous solvents purchased from Aldrich were used directly in the reactions without further treatment unless otherwise indicated. AZTTP was purchased from Amersham, and ddCTP and ddUTP were purchased from Trilink Biotechnologies. ddATP and 7-deaza-ddGTP were prepared, respectively by reactions with phosphorus oxychloride and subsequent condensations with pyrophosphate according to a widely used procedure.[20] 3TCTP, 3′-F-dTTP, and Carbovir-TP were prepared according to a published procedure.[21] FTCTP, dTTP, and d4TTP were prepared by the procedure in ref 21 except that 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one was used as the phosphytilation reagent. The purity of 3′-F-dTTP was 94.6% and of FTCTP was 93.8%. All the other NTPs had a purity of more than 98% as determined by reverse-phase HPLC.

Purification of ddN 5′-αB-$\beta\gamma$CF$_2$TPs. ddN 5′-αB-$\beta\gamma$CF$_2$TPs were purified by anion exchange (AX) chromatography using a 10 mm × 160 mm Mono Q column (Pharmacia). Initial conditions were typically 0−35 mM NaCl. A linear elution gradient was typically initiated at 0−35 mM NaCl and terminated at 350 mM to 1 M NaCl in two to three column volumes at 6.5 mL/min. A constant concentration of 50 mM Tris, pH 8, was maintained throughout the purification. Fractions containing the target compounds were collected and desalted by reverse-phase HPLC (RP-HPLC) using a Luna C18 250 mm × 21 mm column (Phenomenex) with a flow rate of 10 mL/min. Elution gradients were generally from 0% to 20% to 95% methanol in 20−60 min at a constant concentration of triethylammonium acetate (50 mM). ddN 5′-αB-$\beta\gamma$CF$_2$TPs of this work, unless otherwise specified, were purified using both the anion-exchange HPLC and then RP-HPLC. Fractions containing desired ddN 5′-αB-$\beta\gamma$CF$_2$TPs were collected and lyophilized. Compounds that did not require anion exchange HPLC purification were purified by RP-HPLC only, using the same conditions as described above. Yields of all the ddN 5′-αB-$\beta\gamma$CF$_2$TPs of this work were calculated on the basis of their UV absorbance and are reported in milimoles.

LC−MS and HPLC Analysis of ddN 5′-αB-$\beta\gamma$CF$_2$TPs. Mass spectra and purity of the ddN 5′-αB-$\beta\gamma$CF$_2$TPs were obtained using on-line HPLC mass spectrometry on a ThermoFinnigan (San Jose, CA) Deca XP plus instrument. A Phenomenex Luna C18(2) or C5), 75 mm × 2 mm, 3 μm particle size was used for RP-HPLC. A 0−50% linear gradient (15 min) of acetonitrile in 10 mM *N,N*′-dimethyl-*n*-hexylammonium acetate, pH 7, was performed in series with mass spectra detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. The mass range of 150−1500 was typically sampled.

(−)-2′,3′-Dideoxy-3′-thia-5-fluorocytidine (FTC) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6c). A freshly prepared solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (24.6 mg, 0.121 mmol) in anhydrous DMF (0.5 mL) was added via syringe to a solution of FTC (25 mg, 0.101 mmol) and anhydrous pyridine (50 μL) in 0.5 mL of anhydrous DMF at 0 °C under argon. After the mixture was stirred at room temperature for 1 h, tributylamine (0.144 mL, 0.607 mmol) was added, followed by a solution of bis(tributylammonium) difluoromethylenediphosphonate (88.3 mg, 0.15 mmol) in anhydrous DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h and borane−diisopropylethylamine complex (174 μL, 1.0 mmol) was added at 0 °C. After being stirred at room temperature for 16 h, the mixture was cooled in an ice bath, quenched with water (5 mL), and stirred at room temperature for 30 min. HPLC purification as described above yielded 0.012 mmol of **6c**-I and 0.012 mmol of **6c**-II as the triethylammonium salt. Data for **6c**-I are as follows. ^{1}H NMR (D$_2$O): δ 2.81−2.96 (m, H-2′, 2H), 4.11−4.31 (m, H-5′, 2H), 5.30 (m, H-4′, 1H), 6.10 (m, H-1′, 1H), 8.05 (d, $^3J_{H-F}$ = 6 Hz, H-6, 1H). ^{31}P NMR (D$_2$O): δ −2.76 (m, P$_\beta$), 4.85 (dt, $^2J_{P-P}$ = 57.5 Hz, $^2J_{P-F}$ = 75.9 Hz, P$_\gamma$), 84.94 (br, P$_\alpha$). ^{19}F NMR (D$_2$O) δ −164.79 (d, $^3J_{F-H}$ = 6 Hz, 5-F), −118.48 (t, $^2J_{F-P}$ = 83.2 Hz, CF$_2$). MS *m/z*: 518.50 (M − H)$^-$. HPLC analysis: 99.4% purity.

3′-Deoxythymidine (dT) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6a). Following the same procedure as described for **6c**, the reaction starting with 3′-deoxythymidine (66.3 mg, 0.150 mmol) yielded, after HPLC purification, 0.0096 mmol of **6a** (two diastereomers) as the triethylammonium salt. ^{1}H NMR (D$_2$O): δ 1.94 (s, 5-CH$_3$, 3H), 2.14−2.32 (m, H-2′, H-3′, 4H), 3.88−4.09 (m, H-5′, 2H), 4.17 (m, H-4′, 1H), 5.92 (m, H-1′, 1H), 7.52, 7.55 (2s, two isomers, H-6, 1H). ^{31}P NMR (D$_2$O): δ −4.20 (m, P$_\beta$), 4.48 (dt, $^2J_{P-P}$ = 58.8 Hz, $^2J_{P-F}$ = 80.4 Hz, P$_\gamma$), 85.16 (br, P$_\alpha$). ^{19}F NMR (D$_2$O) δ −120.24, (m, CF$_2$). MS *m/z*: 497.10 (M − H)$^-$. HPLC analysis: 98.4% purity.

2′,3′-Didehydro-2′,3′-dideoxythymidine (d4T) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6b). Following the same procedure as described for **6c**, the reaction starting with 3′-deoxy-2′,3′-didehydrothymidine (25 mg, 0.111 mmol) yielded, after HPLC purification, 0.0044 mmol of **6b**-I and 0.0038 mmol of **6b**-II as the triethylammonium salt. Data for **6b**-I are as follows. ^{1}H NMR (D$_2$O): δ 1.76 (s, 5-CH$_3$, 3H), 4.02 (m, H-5′, 2H), 4.93 (m, H-4′, 1H), 5.75 (m, H-2′, 1H), 6.38 (m, H-3′, 1H), 6.75 (m, H-1′, 1H), 7.39 (s, H-6, 1H). ^{31}P NMR (D$_2$O): δ −4.07 (m, P$_\beta$), 4.58 (dt, $^2J_{P-P}$ = 58.3 Hz, $^2J_{P-F}$ = 80.0 Hz, P$_\gamma$), 85.42 (br, P$_\alpha$). ^{19}F NMR (D$_2$O) δ −119.48, (m, CF$_2$). MS *m/z* 495.20 (M − H)$^-$. HPLC analysis: 94.9% purity.

2′,3′-Dideoxyuridine (ddU) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6d). Following the same pro-

cedure as described for **6c**, the reaction starting with 2′,3′-dideoxyuridine (2.4 g. 9.018 mmol) yielded, after HPLC purification, 1.28 mmol of **6d**-I and 1.42 mmol of **6d**-II as triethylammonium salts. Data for **6d**-I are as follows. ^{1}H NMR (D_2O): δ 0.34 (m, BH_3, 3H), 1.84−2.07 (m, H-2′, H-3′, 3H), 2.25−2.34 (m, H2′ or H-3′, 1H), 3.96−4.03 (m, H-5′, 2H), 4.13−4.28 (m, H-4′, 1H), 5.91 (dd, J = 3.0, 6.6 Hz, H-1′, 1H), 6.03 (d, J = 7.8 Hz, H-5, 1H), 8.05 (d, J = 7.8 Hz, H-6, 1H). ^{31}P NMR (D_2O): δ −4.65 (m, P_β), 4.71 (ddd, $^2J_{P-P}$ = 59 Hz, $^2J_{P-F}$ = 80 Hz, $^2J_{P-F}$ = 80 Hz, P_γ), 86.04 (br, P_α). ^{19}F NMR (D_2O): δ −119.31 (m, CF_2). MS *m/z*: 483.30 (M − H)$^-$. HPLC analysis: 99.7% purity. Data for **6d**-II are as follows. ^{1}H NMR (D_2O, TEA$^+$ form): δ 0.36 (m, BH_3, 3H), 1.83−2.03 (m, H-2′, H-3′, 3H), 4.02−4.25 (m, H-3′, H-4′, H-5′, 4H), 5.92 (dd, J = 3.0, 6.6 Hz, H-1′, 1H), 6.00 (d, J = 7.8 Hz, H-5, 1H), 8.07 (d, J = 7.8 Hz, H-6, 1H). ^{31}P NMR (D_2O): δ −4.47 (m, P_β), 4.47 (dt, $^2J_{P-P}$ = 59 Hz, $^2J_{P-F}$ = 82 Hz, P_γ), 83.11 (br, P_α). ^{19}F NMR (D_2O): δ −120.28 (m, CF_2). MS *m/z* 483.3 (M − H)$^-$. HPLC analysis: 96.9% purity.

2′,3′-Dideoxyadenosine (ddA) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6e). Method A. Following the same procedure as described for **6c**, the reaction starting with 2′,3′-dideoxyadenosine (71 mg, 0.30 mmol) yielded the unintended product **9** bearing an additional borane on the base, which made up ∼40% of total product (LC−MS). After treatment of the crude with aqueous ammonia, **9** was largely intact. After storage of the crude in the freezer for 2 months, a significant amount of the desired **6e** was observed on LC−MS. HPLC purification gave 0.011 mmol of **6e**, ∼0.005 mmol of a mixture of **6e** and **9** (ratio 2:3), and ∼0.013 mmol of **9** (83% purity) as triethylammonium salts.

Method B. To a solution of 2′,3′-dideoxyadenosine (235 mg, 1.0 mmol) and diisopropylethylamine (348 μL, 2.0 mmol) in anhydrous acetonitrile (10 mL) under argon was added 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (245 μL, 1.1 mmol), and the mixture was stirred at room temperature for 12 h. Diisopropylethylamine−borane (191 μL, 1.1 mmol) was added then. After the mixture was stirred for 1 day under argon, the solvent was removed *in vacuo* and the residue was treated with a mixture of methanol (2 mL) and aqueous ammonia (3 mL) at room temperature for 1 day. The mixture was concentrated *in vacuo* and purified on silica gel column using 0.5% Et_3N in CH_2Cl_2/MeOH (10:1) as eluent to yield **13** (244 mg, 70% purity, MS: *m/z* 395.5 (M − H)$^+$), part of which (90 mg, 0.15 mmol) was reacted with bis(tributhylammonium) (difluoromethylen)disphosphonate (175 mg, 0.3 mmol) in anhydrous DMF (2 mL) under argon at 50 °C for 3.5 h. After the mixture was cooled to room temperature, the reaction was quenched with TEAB buffer (1.0 M, pH 8.5, 3 mL). HPLC purification gave 0.004 mmol of **6e**-I and 0.005 mmol of **6e**-II as triethylammonium salts. Data for **6e**-I are as follows. ^{1}H NMR (D_2O) δ 0.82 to −0.24 (br, BH_3, 3H), 2.12, 2.22, 2.49, 2.58 (4m, 2H-2′, 2H-3′), 4.02 (m, 2 H-5′, 2H), 4.15 (m, H-4′, 1H), 6.31 (dd, H-1′, J = 5.9, 2.3 Hz, 1H), 8.19 (s, H2 or H-8, 1H), 8.43 (s, H-2 or H8, 1H). ^{31}P (D_2O): δ −1.26 (m, P_β), 5.90 (dt, P_γ, $^2J_{P-F}$ = 71.1 Hz, $^2J_{P-P}$ = 56.2 Hz), 85.4 (br, P_α). ^{19}F (D_2O): δ −119.02 (ddd, CF_2, $^2J_{F-P}$ = 86.2 Hz, $^2J_{F-P}$ = 72.4 Hz, $^4J_{F-P}$ = 7.4 Hz). MS *m/z*: 506.70 (M − H)$^-$. HPLC analysis: 99.1% purity. Data for **6e**-II area as follows. MS *m/z*: 506.70 (M − H)$^-$. HPLC analysis: 99.6% purity.

7-Deaza-2′,3′-dideoxyguanosine (7-deaza-ddG) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (6f). Method A. Following the same procedure as described for **6e**, the reaction starting with 7-deaza-2′,3′-dideoxyguanosine (63 mg, 0.25 mmol) yielded, after HPLC purification, 0.075 mmol of **6f** (two diastereomers) as the triethylammonium salt. ^{1}H NMR (D_2O, TEA$^+$ form): δ 0.8 to −0.2 (br, BH_3, 3H), 2.35−1.95 (3m, 2H-2′, 2H-3′, 4H), 4.06−3.84 (2m, 2 H-5′, 2H), 4.23 (m, H-4′, 1H), 6.12 (dd, H-1′, J = 6.6 Hz, 4.8 Hz, 1H), 6.38 (d, H-7, J = 3.5 Hz, 1H), 7.00 (t, H-8, J = 4.0, 1H). ^{31}P (D_2O): δ −1.79 (m, P_β), 5.33 (dt, P_γ, $^2J_{P-F}$ = 72.6 Hz, $^2J_{P-P}$ = 57.0 Hz), 85 (br, P_α). ^{19}F (DMSO-d_6): δ −120.93 (m, CF_2). MS *m/z*: 521.70 (M − H)$^-$. HPLC analysis: 99.5% purity.

Method B. Procedure via 5′-Borano(*N,N*-diisopropylamino)phosphoramidate. Following the same procedure as described in Method B for **6e**, the reaction starting with 7-deaza-2′,3′-dideoxyguanosine (251 mg, 1.0 mmol) yielded, after HPLC purification, 7-deaza-2′,3′-dideoxyguanosine 5′-borano(*N,N*-diisopropyl)phosphoramidate (isomer A, 0.072 mmol, MS *m/z* 410.5; isomer B, 34 mg, MS *m/z* 410.5) as triethylammonium salts. The two isomers were separately reacted with bis(tributhylammonium) difluoromethylenebisphosphonate to give 0.013 mmol of **6f**-I/II and 0.011 mmol of **6f**-I/II.

2′,3′-Dideoxycytidine (ddC) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (17a). A solution of bis(diisopropylamino)chlorophosphine (56 mg, 0.21 mmol) in 1 mL of anhydrous chloroform was added with a syringe to a stirred solution of 2′,3′-dideoxycytidine (40 mg. 9.02 mmol) and diisopropylethylamine in anhydrous DMF (2 mL) at 0 °C under argon. The resulting solution was stirred at 0 °C for 2 h, and bis(tributylammonium) difluoromethylenediphosphonate (164 mg,0.283 mmol) in DMF (1.5 mL) was added. The reaction mixture (dark-red) was stirred at room temperature for 4 h and borane−dimethyl sulfide complex (0.95 mL) was added at 0 °C. The resulting mixture was stirred at room temperature for 14 h, cooled with ice, and quenched by slow addition of water (5 mL). The mixture was stirred at room temperature for 2 h and washed with diethyl ether three times. The aqueous layer was subjected to HPLC purification to give 0.012 mmol of **17a**-I and 0.013 mmol of **17a**-II as triethylammonium salts. Data for **17a**-I are as follows. ^{1}H NMR (D_2O): δ 0.36 (m, BH_3, 3H), 1.84−2.07 (m, H-2′, H-3′, 3H), 2.25−2.34 (m, H2′ or H-3′, 1H), 3.96−4.03 (m, H -5′, 2H), 4.13−4.28 (m, H-4′, 1H), 5.91 (dd, J = 3.0, 6.6 Hz, H-1′,1H), 6.03 (d, J = 7.8 Hz, H-5, 1H), 8.05 (d, J = 7.8 Hz, H-6,1H). ^{31}P NMR (D_2O): δ −4.65 (m, P_β), 4.71 (dt, $^2J_{P-P}$ = 61 Hz, $^2J_{P-F}$ = 83 Hz, P_γ), 86.04 (br, P_α). ^{19}F NMR (D_2O): δ −120.23 (ddt, $^2J_{F-P}$ = 85 Hz, $^2J_{F-P}$ = 85.3 Hz, $^2J_{F-P}$ = 55.3 Hz, CF_2). MS *m/z*: 482.60 (M − H)$^-$. HPLC analysis: 99.3% purity. Data for **17a**-II are as follows. ^{1}H NMR (D_2O): δ 0.36 (m, BH_3, 3H), 1.83−2.03 (m, H-2′, H-3′, 4H), 4.02−4.25 (m, H-4′, H-5′, 3H), 5.92 (dd, J = 3.0, 6.6 Hz, H-1′, 1H), 6.00 (d, J = 7.8 Hz, H-5, 1H), 8.07 (d, J = 7.8 Hz, H-6, 1H). ^{31}P NMR (D_2O): δ −4.47 (m, P_β), 4.47 (dt, $^2J_{P-P}$ = 59 Hz, $^2J_{P-F}$ = 81 Hz, P_γ), 83.11 (br, P_α). ^{19}F NMR (D_2O): δ −120.28 (ddt, $^2J_{F-P}$ = 83 Hz, $^2J_{F-P}$ = 85.2 Hz, $^2J_{F-P}$ = 63 Hz, CF_2). MS *m/z*: 482.60 (M − H)$^-$. HPLC analysis: 99.7% purity.

(−)-2′,3′-Dideoxy-3′-thiacytidine (3TC) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (17b). Following the same procedure as described for **17a**, the reaction starting with 3TC (35 mg. 9.018 mmol) yielded, after HPLC purification, 0.015 mmol of **17b** (two diastereomers) as the triethylammonium salt. ^{1}H NMR (D_2O): δ 0.36 (m, BH_3), 3.01−3.44 (m, H-2′, 2H), 4.10−4.21, 4.24−4.34 (m, H-5′, 2H), 5.31−5.34 (m, H-4′, 1H), 5.93 (m, H-5, 1H), 6.14−6.18 (m, H-1′, 1H), 7.97−8.03 (m, H-6, 1H). ^{31}P NMR (D_2O): δ −4.43 to −3.7 (m, P_β), 3.61−5.42 (m, P_α), 85.74 (br, P_γ). ^{19}F NMR (D_2O): δ −120.33 to −119.74 (m, CF_2). MS *m/z* 500.60 (M − H)$^-$. HPLC analysis: 99.2% purity.

3′-Deoxy-3′-fluorothymidine (3′-F-dT) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (17c). Following the same procedure as described for **17a**, the reaction starting with 3′-deoxy-3′-fluorothymidine (coevaporated with pyridine three times, 50 mg, 0.205 mmol) and Hunigs base (46 μL, 0.266 mmol), instead of diisopropylethylamine, yielded, after HPLC purification, 0.028 mmol of **17c**-I and 0.053 mmol of **14c**-II as triethylammonium salts. data for **17c**-II are as follows. ^{1}H NMR (D_2O): δ 1.80 (s, 5-CH_3, 3H), 2.09−2.52 (m, H-2′, 2H), 3.92−4.01, 4.13−4.22 (2m, H-5′, 2H), 4.40 (d, $^3J_{H-F}$ = 27.3 Hz, H-4′, 1H), 5.42 (dd, $^3J_{H-H}$ = 4.4 Hz, $^3J_{H-F}$ = 52.5 Hz, H-3′, 1H), 6.24 (dd, J = 5.6, 5.3 Hz, H-1′, 1H), 7.63 (m, H-6, 1H). ^{31}P NMR (D_2O): δ −2.12 (m, P_β), 5.02 (dt, $^2J_{P-P}$ = 56.7 Hz, $^2J_{P-F}$ = 73.4 Hz, P_γ), 83.2 (br, P_α). ^{19}F NMR (D_2O) δ −174.0 (m, 3′-F), −117.4 (m, CF_2). MS *m/z*: 515.50 (M − H)$^-$. HPLC analysis: 99.5%.

Carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine (Carbovir) 5′-α-*P*-Borano-*β*,*γ*-(difluoromethylene)triphosphate (17d). Following the same procedure as described for **17a**, the reaction starting with Carbovir (350 mg, 1.4 mmol, coevaporated with pyridine three times) yielded, after HPLC

purification, 0.146 mmol of **17d** (two diastereomers) as the triethylammonium salt. ^{1}H NMR (D_2O): δ 1.59 (m, CH*H*′, 1H), 2.63 (m, C*H*H′, 1H), 2.90 (m, H-4′), 3.87 (m, H-5′, 2H), 5.32 (m, H-1′, 1H), 5.73 and 6.07 (double multiplet, CH=CH, 2H), 7.72 (m, H-8, 1H). ^{31}P NMR (D_2O): δ −4.20 (m, P_β), 4.65 (dt, $^2J_{P-P}$ = 58.3 Hz, $^2J_{P-F}$ = 78.4 Hz, P_γ), 85.88 (br, P_α). ^{19}F NMR (D_2O) δ −119.33, (m, CF_2). MS *m/z*: 518.70 (M − H)$^-$. HPLC analysis: 99.3% purity.

Serum Stability Assessment Using Serum. The stability of nucleotide mimics was assessed in fetal calf serum, generally following a published procedure.[21] Fetal calf serum purchased from HyClone Corporation was mixed 1:1 with each compound containing Tris-HCl buffer and $MgCl_2$. The final concentrations of the reaction components were as follows: 50 mM Tris-HCl, pH 7.4, 0.1 mM $MgCl_2$, 500 μM nucleotide mimic, 10% (v/v) fetal calf serum. The reaction mixtures were incubated at 37 °C. At appropriate times, aliquots of 25 μL were removed and added to 65 μL of ice-cold methanol. These solutions were incubated for at least 1 h at −20 °C. After incubation, samples were centrifuged for 20 min at high speed in a microcentrifuge. The supernatant was transferred to a clean tube, and the extract was dried under vacuum in a LabConco Centrivap concentrator. The dried extracts were resuspended in deionized water and filtered to remove particulates and then analyzed on reverse-phase HPLC. The reverse-phase HPLC column used for the analysis was a Phenomenex C18 Aqua column (3 mm × 150 mm). The HPLC flow rate was 0.5 mL/min with the following buffer system: 5 mM tetrabutylammonium acetate, 50 mM ammonium phosphate, and an acetonitrile gradient running from 5% to as high as 60%. The amount of remaining parent compound at each time point was used to determine the half-life of the compound. Time points were only taken through 48 h so that if greater than 50% of a compound was still intact after 48 h of incubation, the half-life was expressed as >48 h. Unmodified nucleoside triphosphates were used as positive controls.

HIV-1 Reverse Transcriptase Inhibition Assays (Heteropolymeric RNA Template). Assay buffer conditions were as follows (120 μL total/reaction): 50 mM Tris-HCl, pH 8.1; 6.5 mM $MgCl_2$; 100 mM NaCl; 10 mM DTT; 50 uM dNTPs (dATP, TTP, dGTP, dCTP); 5 μg/mL primed ribosomal RNA (*E. coli*); 10 units HIV reverse transcriptase (purified, type B, 66 kDa subunit). HIV-1 RT DNA polymerase activity was measured in a reaction buffer containing primed ribosomal RNA template and dNTPs diluted to appropriate concentrations in assay buffer and pipetted into 1.5 mL microcentrifuge tubes. The ddN 5′-αBCF_2TPs were diluted in buffer and tested at various concentrations up to 5 μM final concentration. The reaction was initiated by addition of the enzyme and allowed to proceed at 42 °C for 60−90 min. The reaction was quenched by addition of 12 μL of 0.2 M EDTA, pH 8.0. Blank reactions were prepared in parallel with the test reactions in which either enzyme or dNTP was omitted from the reactions, substituted by an appropriate volume of enzyme diluent or assay buffer, respectively. An amount of 50 μL each of reaction mixture was transferred to the well of a 96-well plate (in duplicate). An amount of 200 μL of diluted PicoGreen dsDNA quantitation reagent was added to each well of a 96-well plate and incubated at room temperature for 5 min. Plate wells were read on a microplate fluorometer. The wells were excited at 480 nm, and the fluorescence emission intensity (RFU) was measured at 520 nm. The percentage of inhibition and inhibition constants (K_i) were determined.

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