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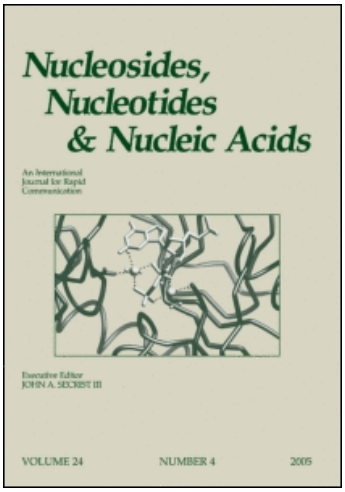

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Synthesis of 5'-Triphosphate Mimics (P3Ms) of 3'-Azido-3',5'-Dideoxythymidine and 3',5'-Dideoxy-5'-Difluoromethylenethymidine as HIV-1 Reverse Transcriptase Inhibitors

Vivek K. Rajwanshi[a]; Marija Prhavc[a]; Patrick Fagan[a]; Jennifer L. Brooks[a]; Tiffany Hurd[a]; P. Dan Cook[a]; Guangyi Wang[ab]
[a] Research Laboratories, Biota, Inc., Carlsbad, California, USA [b] Research Laboratories, Biota, Inc., Carlsbad, CA, USA

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SYNTHESIS OF 5′-TRIPHOSPHATE MIMICS (P3Ms) OF 3′-AZIDO-3′,5′-DIDEOXYTHYMIDINE AND 3′,5′-DIDEOXY-5′-DIFLUOROMETHYLENETHYMIDINE AS HIV-1 REVERSE TRANSCRIPTASE INHIBITORS

Vivek K. Rajwanshi, Marija Prhavc, Patrick Fagan, Jennifer L. Brooks, Tiffany Hurd, P. Dan Cook, and Guangyi Wang ▫ *Research Laboratories, Biota, Inc., Carlsbad, California, USA*

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▫ *3′-Azido-3′,5′-dideoxythymidine 5′-phosphonate and 3′,5′-dideoxy-5′-difluoromethylenethymidine 5′-phosphonate were prepared by multistep syntheses. The nucleoside 5′-phosphonates were converted to their triphosphates and triphosphate mimics (P3Ms) containing β,γ-difluoromethylene, β,γ-dichloromethylene, or β,γ-imodo by condensation with pyrophosphate or pyrophosphate mimics, respectively. Inhibition of HIV-1 reverse transcriptase by the nucleoside P3Ms is briefly discussed.*

Keywords 5′-Deoxynucleotides, Synthesis, Triphosphate Mimics, HIV-1 RT

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) are successively phosphorylated in cells to their 5′-triphosphates.[1,2] NRTI triphosphates are the active chemical entities that inhibit HIV viral DNA synthesis. The inhibition is effected primarily by incorporation of NRTI 5′-monophosphates into the viral DNA and subsequent chain termination.[1–4] Thus, a prerequisite for a nucleoside antiviral drug is cellular activation to its triphosphate. In order to bypass the first cellular phosphorylation of nucleosides, nucleoside 5′-monophosphte prodrugs have been intensively explored as an alternative form of nucleoside antiviral drugs.[5] So far, acyclic nucleoside phosphonate prodrugs, tenofovir disoproxil fumarate[6] and adefovir dipivoxil,[7] have been successfully developed as anti-HIV and anti-HBV drugs, respectively. It is conceivable that a stable NTP mimic (NP3M) containing a modified triphosphate moiety may be a substrate or an inhibitor of viral polymerases and potentially can be useful. Thus, use of active, stable NP3Ms or

Received 27 September 2004, accepted 31 January 2005.
Address correspondence to Guangyi Wang, Research Laboratories, Biota, Inc., 2232 Rutherford Road, Carlsbad, CA 92008, USA; E-mail: g.wang@biota-inc.com

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their prodrugs as antiviral drugs can entirely bypass cellular phosphorylation. In addition, use of NP3Ms may minimize drug resistance resulting from polymerase mutations, especially in the case of AZT-resistant HIV mutant polymerase.[8] In our search for useful NP3Ms, we identified several very promising P3Ms containing two modifications.[9] Particularly, 5′-α-R_p-borano-β,γ-(difluoromethylene)triphosphate (αB-β,γCF_2TP) rendered AZT 5′-αB-β,γCF_2TP (**1**) with a potent inhibition of HIV-1 RT (K_i 9.5 nM). AZT 5′-β,γ-(difluoromethylene)triphosphate (**2**) also exhibited a potent inhibition (K_i 41 nM).[9] In order to reveal the effects of modifications at the O5′ position of nucleosides, we have synthesized a number of 5′-deoxy-AZT P3Ms and 3′,5′-dideoxy-5′-difluoromethylenethymidine P3Ms. In this article, we describe the synthesis of the nucleoside P3Ms (**3a–d, 4a,b,d**) as shown in Figure 1 and briefly discuss their inhibition of HIV-1 RT.

Scheme 1 shows synthesis of the 5′-deoxy-AZT P3Ms **3a, 3b, 3c, 3d** in which the 5′-oxygen is eliminated. 1-(2,5-Dideoxy-5-iodo-β-D-xylofuranosyl)thymine (**5**)[10] was converted to **6** by acetylation. Condensation of **6** with triethyl phosphite yielded the 5′-phosphonate ester **7**. After removal of the 3′-acetyl, the resulting **8** was converted to the mesylate **9**. Our first attempt to remove the acetyl group of **7** with methanolic ammonia failed owing to a partial hydrolysis of the phosphonate ester. Alcoholysis of **6** using anhydrous ethanol in the presence of sodium ethoxide yielded **8** in good yield. As expected, treatment of **9** with sodium azide at elevated temperature gave the 3′-azido derivative **10**, resulting from an S_N2 nucleophilic substitution. Synthesis of **10** was also attempted by treatment of 3′-azido-3′,5′-dideoxy-5′-iodothymidine[11] with triethyl phosphite under reflux, but did not yield

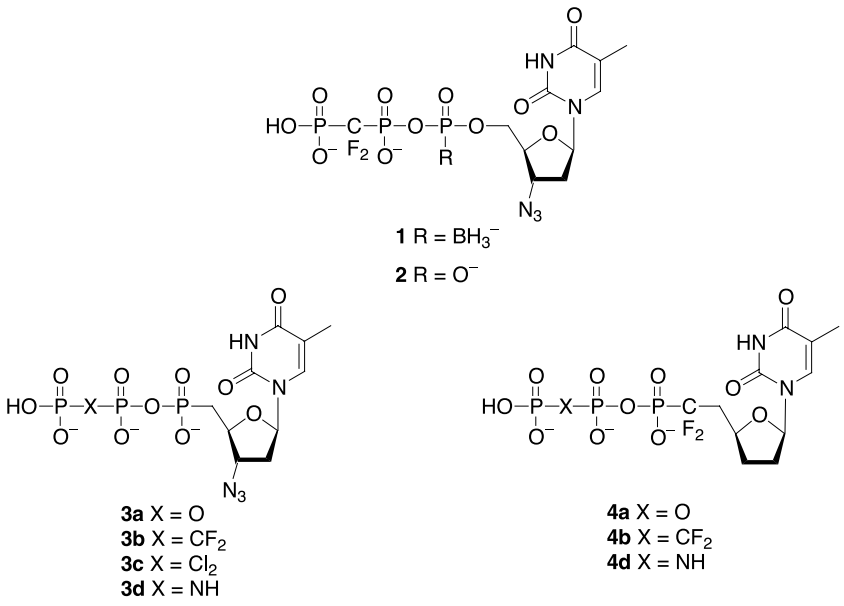

FIGURE 1 Nucleoside triphosphate mimics.

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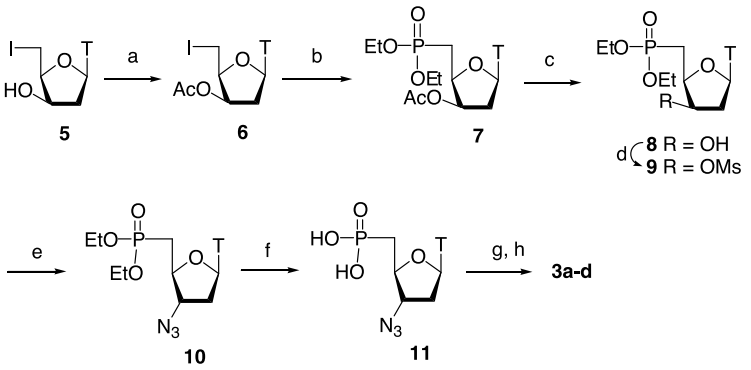

SCHEME 1 Conditions: (a) Ac_2O, pyridine, rt; (b) $P(OEt)_3$, reflux; (c) NaOEt, EtOH, 0°C; (d) MsCl, DMAP, pyridine, rt; (e) NaN_3, DMF, 80°C; (f) TMSBr, CH_3CN, 40°C; (g) 1,1′-carbonyldiimidazole, HMPA; (h) Et_3NH^+salt of pyrophosphate or a mimic thereof, HMPA.

the desired **10**. After removal of the ethyl groups with TMSBr, the resulting **11** was converted to its triethylammonium salt. Synthesis of **11** through a different route has been previously reported.[12] The triethylammonium salt of **11** was treated with 1,1′-carbonyldiimidazole in HMPA,[13] and then condensed with bis(tributylammonium) difluoromethylenediphosphonate[9] to afford, after HPLC purification, the desired **3b**. Similarly, condensations of the triethylammonium salt of **11** with the tributylammonium salts of pyrophosphate, dichloromethylenediphosphonate, and imidodiphosphate yielded **3a**, **3c** and **3d**, respectively, in low to moderate yields.

Scheme 2 shows the synthesis of 3′,5′-dideoxy-5′-(difluoromethylene)thymidine P3Ms (**4a**, **4b**, and **4d**). 2,3-Dideoxy-1-*O*-methylribofuranose (**12**, a mixture of α and β epimers)[14] was converted to the 5-*O*-triflate **13** by treatment with trifluoromethanesulfonic anhydride. Purification of **13** was a challenge because of its sensitivity to hydrolysis. Finally, **13** was obtained in a moderate yield by rapidly passing through a silica gel column and used immediately in the next reaction. Diethyl difluoromethylphosphonate was treated with LDA at −78°C and then reacted with **13** to give the 5-deoxy-5-difluoromethylene derivative **14**. Condensation of **14** with a silylated thymine in the presence of tin (IV) chloride gave the 5′-phosphonate ester **15**, consisting of two anomers (3:2). Attempts to separate the two anomers of **15** were not successful, even on HPLC. Thus, the mixture of two anomers of **15** was used for further reactions. After removal of the ethyl groups with TMSBr, the resulting **16** was converted to its triethylammonium salt, treated with 1,1′-carbonyldiimidazole[13] and then condensed with the tributylammonium salts of pyrophosphate, difluoromethylenediphosphonate and imidodiphosphate, respectively, to give the NP3Ms **4a, 4b** and **4d** as a mixture of α/β anomers. The major anomers of **15**, **16**, **4a**, **4b**, and **4d** was tentatively assigned as the α-anomers using proton NMRs and by comparison with known proton NMR data of a series of 2′,3′-unsubstituted 2′,3′-dideoxynucleotides.

The effectiveness of compounds **3a**–**d** and **4a**,**b**,**d** as inhibitors of HIV-1 reverse transcriptase was determined using a fluorometric assay[9,15] and poly(A)

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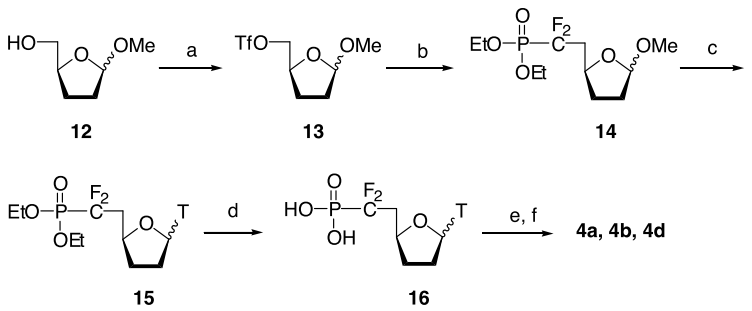

SCHEME 2 Conditions: (a) $(CF_3SO_2)_2O$, 2,6-di-*t*-Bu-4-Me-pyridine, CH_2Cl_2, −15 to −5°C; (b) i-Pr_2NH, BuLi, $CHF_2P(O)(OEt)_2$, HMPA, THF, −78°C; (c) bis(TMS)thymine, $SnCl_4$, CH_3CN, 75°C; (d) TMSBr, rt; (e, f) same as (g, h) in Scheme 1.

homopolymer RNA as template. AZTTP, AZT 5′-αB-β,γCF_2TP (**1**) and AZT 5′-β,γ-CF_2TP (**2**) are very potent inhibitors of HIV-1 RT with K_i values of 8.4, 9.5, 41 nM, respectively.[9] Compared to **2**, its 5′-deoxy derivatives **3b** showed only moderate activity, with 25% inhibition at 10 μM concentration. Compounds **3a**, **3c**, and **3d** have a similar level (30–44%) of inhibition. Compounds **4a, 4b** and **4d** in which 5′-oxygen is replaced with a difluoromethylene showed slightly stronger (41–58%) inhibition at 10 μM concentration. It appears that modifications at the O5′ position have a predominant impact on the activity, overshadowing the effects of β,γ-bridge modifications. Although compounds **3** and **4** were designed as incorporable substrate inhibitor of HIV-1 RT, conformational changes induced by the O5′-modifications probably impaired their incorporation, as revealed by the poor K_i values.

In summary, synthesis of several P3Ms of 3′-azido-3′,5′-dideoxythymidines **3a**–**d** and 3′,5′-dideoxy-5′-difluoromethylenethymidines **4a**,**b**,**d** has been described. The synthetic strategy is expected to apply to other nucleosides. Modifications at the O5′ position of nucleotides are not well tolerated by HIV-1 RT and decrease the inhibitory effect of the NP3Ms. However, successful synthesis of compounds **3** and **4** increased the pool of novel NP3Ms, and applications of these NP3Ms in areas beyond HIV-1 RT inhibition are being actively explored in these laboratories.

EXPERIMENTAL

^{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. Tributylammonium pyrophosphate was purchased from Sigma and used without further treatment. Dichloromethylenediphosphonic acid disodium salt, and imidodiphosphate sodium salt were purchased from Sigma and converted to their tributylammonium salts,[9] respectively. Difluoromethylenediphosphonic acid was prepared from tetraisopropyl methylenediphosphonate by reacting with

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N-fluorobenzenesulfonimide in the presence of sodium bis(trimethylsilyl)amide and subsequent treatment with TMSBr and then converted to its bis(tributylammonium) salt according to a procedure developed in this laboratory.[9] Anhydrous solvents purchased from Aldrich were used directly without further treatment unless as indicated. Triethylammonium bicarbonate (TEAB, 1.0 M, pH 8.5) was purchased from Fluka.

Purification of NP3Ms

The NP3Ms were purified by anion exchange (AX) chromatography using a 10 × 160 mm Mono Q column (Pharmacia). A linear elution gradient of NaCl from 0 to 35 mM to 350–1000 mM and a constant concentration of 50 mM Tris, pH 8 were used. Fractions containing the target compounds were collected and desalted by reversed phase HPLC (RP-HPLC) using a Luna C18 250 × 21 mm column (Phenomenex) with an elution gradient of methanol from 0 to 20% to 95% and a constant concentration of triethylammonium acetate (50 mM). Fractions containing desired NP3Ms were collected and lyophilized. The final NP3M products were triethylammonium salts. The yields of all NTP mimics of this work were calculated on the basis of UV absorbance.

LCMS and HPLC Analysis of NTP Mimics

Mass spectra and purity of the NP3Ms were obtained using on-line HPLC mass spectrometry on a ThermoFinnigan (San Jose, CA) Deca XP plus. A Phenomenex Luna C18(2) or C5), 75 × 2 mm, 3-μm particle size was used for RP-HPLC. A 0 to 50% linear gradient of acetonitrile in 10 mM N,N′-dimethyl-n-hexylammonium acetate, pH 7 was performed in series with MS detection in the negative ionization mode. Nitrogen gas and a pneumatic nebulizer were used to generate the electrospray. All NP3Ms were subjected to the LCMS analysis.

1-(3-*O*-Acetyl-2,5-dideoxy-5-iodo-β-D-*threo*-pentofuranosyl)thymine (6). Acetic anhydride (7.0 mL, 75.4 mmol) was added to a stirred solution of 5.1 g (14.5 mmol) of **5**[10] in anhyd. pyridine (50 ml) at 0°C under argon. The mixture was stirred at rt for 17 h, cooled in an ice bath, quenched with water (15 ml), stirred for 30 min, concentrated to dryness, and co-evaporated with toluene three times. Chromatography on silica gel with 1–3% MeOH in EtOAc gave 4.4 g (77%) of **6** as an off-white foam; ^{1}H NMR (DMSO-d_6) δ 1.81 (d, J = 0.9 Hz, CH_3, 3H), 2.04–3.00 (m, $COCH_3$, H-2′, 4H), 2.73–2.83 (m, H-2′, 1H), 3.41 (d, J = 6.9 Hz, H-5′, 2H), 4.23–4.29 (m, H-4′, 1H), 5.27–5.29 (m, H-3′, 1H), 6.15 (dd, J = 2.7, 8.1 Hz, H-1′, 1H), 7.42 (d, J = 1.2 Hz, H-6, 1H), 11.29 (s, NH, 1H).

1-[3-*O*-Acetyl-2,5-dideoxy-5-(di-*O*-ethylphosphono)-β-D-*threo*-pentofuranosyl]thymine (7). A stirred solution of **6** (4.4 g, 12.5 mmol) in freshly distilled triethyl phosphite (50 ml) under argon was heated at 180°C for 30 h.

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The mixture was concentrated to dryness and the residue was chromatographed on silica gel with 2–4% EtOH in CH_2Cl_2 to give 2.4 g (48%) of **7** as a light-yellow foam; ^{1}H NMR (DMSO-d_6) δ 1.17–1.23 (m, 2 × CH_3, 6H), 1.98 (dd, J = 2.7, 15.2 Hz, H-2′, 1H), 2.04 (s, $COCH_3$, 3H), 2.23–2.32 (m, H-5′, 2H), 2.75–2.81 (m, H-2′, 1H), 3.92–4.02 (m, 2 × CH_2, 4H), 4.18–4.26 (m, H-4′, 1H), 5.14 (t, J = 4.2 Hz, H-3′, 1H), 6.06 (dd, J = 2.7, 8.4 Hz, H-1′, 1H), 7.39 (d, J = 1.2 Hz, H-6, 1H), 11.28 (s, NH, 1H); ^{31}P NMR (DMSO-d_6) δ 28.15 (s).

1-[2,5-Dideoxy-5-(di-*O*-ethylphosphono)-β-D-*threo*-pentofuranosyl]thymine (8). NaOEt (0.28 g, 4.03 mmol) was added to a stirred solution of **7** (1.08 g, 2.68 mmol) in anhyd. EtOH (75 ml) at 0°C under argon. The mixture was stirred at 0°C for 2 h, and then more NaOEt (47.5 mg, 0.67 mmol) was added. The mixture was stirred for another hour at 0°C and then neutralized with DOWEX 50WX8-100 ion exchange resin. The resin was filtered and washed with EtOH (60 ml). The filtrate was concentrated and the residue was chromatographed on silica gel with 3–3.5% EtOH in CH_2Cl_2 to give 0.62 g (64%) of **8** as a glassy solid; ^{1}H NMR (DMSO-d_6) δ 1.17–1.24 (m, 2 × CH_3, 6H), 1.75 (s, CH_3 3H), 1.83–2.62 (m, H-2′, H-5′, 4H), 3.92–4.12 (m, H-3′, H-4′, 2 × CH_2, 6H), 5.4 (d, J = 3.3 Hz, C-3-OH, D_2O exchangeable), 5.16 (m, H-3′, 1H), 6.03 (dd, J = 3.3, 8.4 Hz, H-1′, 1H), 7.71 (d, J = 1.2 Hz, H-6, 1H), 11.23 (s, NH, 1H).

1-[2,5-Dideoxy-5-(di-*O*-ethylphosphono)-3-*O*-mesyl-β-D-*threo*-pentofuranosyl]thymine (9). To a stirred solution of **8** (1.3 g, 3.69 mmol) in anhyd. pyridine (30 ml) at 0°C under argon were added successively DMAP (0.225 g, 1.85 mmol) and methanesulfonyl chloride (0.42 mL, 5.54 mmol). The mixture was stirred at rt for 13 h, cooled in an ice bath, quenched with water (15 ml), stirred for 20 min, concentrated, and co-evaporated with toluene three times. Chromatography on silica gel with 2–3% EtOH in CH_2Cl_2 gave 1.5 g (92%) of **9** as an off-white foam; ^{1}H NMR (DMSO-d_6) δ 1.18–1.26 (m, 2 × CH_3, 6H), 1.78 (s, CH_3 3H), 2.25–2.33 (m, H-2′, H-5′, 3H), 2.85–2.94 (m, H-2′, 1H), 3.30 (s, CH_3SO_3, 3H), 3.95–4.04 (m, 2 × CH_2, 4H), 4.26–4.30 (m, H-4′, 1H), 5.15 (m, H-3′, 1H), 6.11 (dd, J = 3.3, 8.4 Hz, H-1′, 1H), 7.38 (s, H-6, 1H), 11.32 (s, NH, 1H); ^{31}P NMR (DMSO-d_6) δ 28.16 (s).

1-[3-Azido-3,5-dideoxy-5-(di-*O*-ethylphosphono)-β-D-*erythro*-pentofuranosyl]thymine (10). A stirred mixture of **9** (1.5 g, 3.41 mmol) and NaN_3 (0.44 g, 6.8 mmol) in anhyd. DMF (25 mL) under argon was heated at 80°C for 14 h. Precipitate was filtered and the filtrate was concentrated. Chromatography on silica gel with 3–3.5% EtOH in CH_2Cl_2 gave 0.73 g (55%) of **10** as a white foam; ^{1}H NMR (DMSO-d_6) δ 1.18–1.25 (m, 2xCH_3, 6H), 1.79 (s, CH_3 3H), 2.23–2.50 (m, H-2′, H-5′, 4H), 3.94–4.04 (m, 2 × CH_2, H-4′, 5H), 4.37–4.40 (m, H-3′, 1H), 6.06 (t, J = 6.6 Hz, H-1′, 1H), 7.57 (d, J = 1.2 Hz, H-6, 1H), 11.33 (s, NH, 1H); ^{31}P NMR (DMSO-d_6) δ 27.73 (s).

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3′-Azido-3′,5′-dideoxythymidine 5′-(β,γ-difluoromethylene)-triphosphate (3b). TMSBr (0.5 ml, 3.65 mmol) was added to a stirred solution of **10** (0.2 g, 0.52 mmol) in anhyd. acetonitrile (5.5 ml) under argon. The mixture was stirred at 40°C for 4 h, concentrated to dryness, and co-evaporated with anhydrous acetonitrile twice. The residue was co-evaporated with MeOH three times, dissolved in water (3.0 ml), and washed with ether twice. The aqueous solution was lyophilized to afford 0.181 g (0.55 mmol) of **11** as a light-yellow foam, which was dissolved in water (2.5 ml), neutralized with 1.34 ml of 1.0 M TEAB, and lyophilized.

Part of the resulting triethylammonium salt of **11** (69.3 mg, 0.13 mmol) was dissolved in HMPA (2.5 ml) and 1,1′-carbonyldiimidazole (107 mg, 0.65 mmol) was added at rt. The mixture was stirred for 2 h, treated with MeOH (35 µl), and stirred for 45 min. A solution of bis(tributylammonium) difluoromethylenediphosphonate (397 mg, 0.68 mmol)[9] in HMPA (2.5 ml) was added, and the resulting solution was stirred at rt for 4 h, cooled in an ice bath, and quenched with water (4 ml). HPLC purification gave 31.6 µmol of **3b** as triethylammonium salt; ^{1}H NMR (D_2O): δ 1.76–2.36 (m, CH_3, H-2′, H-5′, 7H), 4.15–4.27 (m, H-3′, H-4′, 2H), 5.99 (t, J = 6.9 Hz, H-1′, 1H), 7.48 (s, H-6, 1H); ^{31}P NMR (D_2O): 13.64 (d, $^2J_{P-P}$ = 33 Hz, P_α), 4.65 (ddt, $^2J_{P-P}$ = 58 Hz, $^2J_{P-F}$ = 78 Hz, P_β), −3.72 to −2.72 (m, P_γ); ^{19}F NMR (D_2O): −119.62 (dd, $^2J_{P-F}$ = 77 Hz, $^2J_{P-F}$ = 77 Hz, CF_2); MS *m/z* 524.4 $(M-H)^-$. HPLC analysis: 100% purity.

3′-Azido-3′,5′-dideoxythymidine 5′-(β,γ-dichloromethylene)triphosphate (3c). Following the procedure described for **3b**, starting from triethylammonium salt of **11** (59.1 mg, 0.11 mmol) and bis(tributylammonium) dichloromethylenediphosphonic[13] (288 mg, 0.51 mmol), 14.9 µmol of **3c** as triethylammonium salt was obtained. MS *m/z* 556.7, 558.3 $(M-H)^-$. HPLC analysis: 86.3% purity.

3′-Azido-3′,5′-dideoxythymidine 5′-β,γ-imidotriphosphate (3d). Following the procedure described for **3b**, starting from triethylammonium salt of **11** (50.0 mg, 0.09 mmol) and tetrakis(tributylammonium) imidodiphosphate[13] (427 mg, 0.46 mmol), 62.5 µmol of **3d** as triethylammonium salt was obtained. ^{1}H NMR (D_2O): δ 1.76–2.33 (m, CH_3, H-2′, H-5′, 7H), 4.08–4.22 (m, H-3′, H-4′, 2H), 5.96 (t, J = 6.9 Hz, H-1′, 1H), 7.43 (d, J = 1.2 Hz, H-6, 1H); ^{31}P NMR (D_2O): 12.55 (d, $^2J_{P-P}$ = 26 Hz, P_α), −0.52 (P_γ), −9.91 (P_β); MS *m/z* 489.5 $(M-H)^-$. HPLC analysis: 83.3% purity.

3′-Azido-3′,5′-dideoxythymidine 5′-triphosphate (3a). Following the procedure described for **3b**, starting from triethylammonium salt of **11** (50.0 mg, 0.09 mmol) and tributylammonium pyrophosphate (295 mg, 0.54 mmol), 26.5 µmol of **3a** as triethylammonium salt was isolated. ^{1}H NMR (D_2O): δ 1.73–2.11 (m, CH_3, H-2′, H-5′, 7H), 4.05–4.21 (m, H-3′, H-4′, 2H), 5.95 (t, J = 6.6 Hz, H-1′,

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1H), 7.43 (d, J = 1.2 Hz, H-6, 1H); ^{31}P NMR (D_2O): 12.65 (d, $^2J_{P-P}$ = 25.6 Hz, P_α), −9.14 (m, P_γ), −21.52 (m, P_β); MS *m/z* 490 $(M-H)^-$. HPLC analysis: 98.1% purity.

6-Diethylphosphono-6,6-difluoro-1-*O*-methyl-2,3,5,6-tetradeoxy-D-allofuranose (14). To a stirred, ice-cold solution of trifluoromethanesulfonic anhydride (2.43 ml, 14.4 mmol) in anhyd. CH_2Cl_2 (100 ml) under argon was added 2,6-di-*tert*-butyl-4-methylpyridine (2.96 g, 14.4 mmol). The solution was cooled to −20°C and 1.9 g (14.4 mmol) of **12** in anhyd. CH_2Cl_2 (75 mL) was added dropwise. The mixture was stirred at −15 to −5°C for 45 min, then poured into ice-cold aqueous $NaHCO_3$ (1%, 1 L) and vigorously shaken. The aqueous layer was extracted with CH_2Cl_2 twice. Combined organic layer was dried (Na_2SO_4), concentrated, and rapidly chromatographed on silica gel using hexanes and hexanes/Et_2O (3:1) as eluents. Fractions containing the triflate **13** were concentrated and used immediately for the next step; ^{1}H-NMR (CD_2Cl_2): δ 2.26–1.66 (m, 2H-2, 2H-3, 4H), 3.34 (s, CH_3, 3H), 4.62–4.35 (m, H-4, 2H-5, 3H), 5.03 (dd, H-1, J = 3.8 Hz, J = 0.9 Hz, 0.5H), 5.07 (dd, H-1, J = 4.8 Hz, J = 1.3 Hz, 0.5H).

To a solution of diisopropylamine (6.05 ml, 43.2 mmol) and HMPA (7.52 ml, 43.2 mmol) in anhyd. THF (40 ml) at −78°C under argon was added n-butyllithium (27 mL of 1.6 M in hexanes). The mixture was stirred at 0°C for 1 h and then cooled to −78°C. To this solution were added dropwise *via* transfer needles a cold (−78°C) solution of diethyl difluoromethylphosphonate (6.8 ml, 43.2 mmol) in THF (40 ml) and after 30 min a cold (−78°C) solution of the triflate **13** in THF (90 ml). The mixture was stirred at −78°C for 2 h, then poured into cold (−10°C), saturated aqueous NH_4Cl, and diluted with Et_2O. The aqueous layer was extracted with EtOAc (2 × 150 ml), and the combined organic layer dried (Na_2SO_4) and concentrated. Chromatography on silica gel with hexanes and hexanes/EtOAc (10:1) yielded **14** as a colorless oil (1.33 g; 31% for 2 steps); ^{1}H-NMR (α,β, CD_2Cl_2) δ 1.38 (t, $2CH_2CH_3$, J = 7.1 Hz, 6H), 1.48–1.62 and 1.76–2.25 (2m, 2H-2, 2H-3, 2H-5, 6H), 3.31 (s, CH_3, 3H), 4.26 (m, $2CH_2CH_3$, 4H), 4.95 (dd, H-1, J = 8.5 Hz, J = 4.1 Hz, 0.25H), 5.00 (dd, H-1, J = 5.2 Hz, J = 1.8 Hz, 0.75H); ^{31}P (CD_2Cl_2): δ 7.84 (t, $^2J_{P-F}$ = 105.9 Hz); ^{19}F (CD_2Cl_2): δ-111.47 (dm, $^2J_{F-P}$ = 106.3 Hz).

1-[6-Diethylphosphono-6,6-difluoro-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (15). Compound **14** (1.33 g, 4.4 mmol) in acetonitrile (30 ml) was added to the solution of bis(trimethylsilyl)thymine in acetonitrile. The latter was prepared by refluxing thymine (1.11 g, 8.8 mmol) with bis(trimethyl)silyl acetamide (2.3 ml, 8.8 mmol) in acetonitrile (30 ml) for 15 min. The mixture was cooled to 0°C when $SnCl_4$ (4.4 ml, 1.0 M in CH_2Cl_2) was added dropwise, and then heated at 75°C for 45 min. After cooling in an ice bath, it was poured into ice-cold aqueous $NaHCO_3$ (5%, 200 ml) and extracted with CH_2Cl_2 three times. The combined extract was dried (Na_2SO_4), concentrated, and chromatographed on silica gel with 2% MeOH in CH_2Cl_2 to yield **15** (a mixture of two diastereomers, 0.8 g, 46%; α/β 3/2) as a viscous oil; ^{1}H-NMR ($CDCl_3$) δ 1.40 (t,

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$2CH_2CH_3$, J = 7.0 Hz, 6H), 1.94 (s, C5-Me, 3H), 1.66–1.89, 1.95–2.09, and 2.19–2.64 (3m, 2H-2′, 2H-3′, 2H-5′, 6H), 4.29 (m, $2CH_2CH_3$, 4H), 4.70 and 4.40 (2m, H-4′(α) and H-4′(β), 1H), 6.01 (dd, H-1′(β, J = 7.0 and 3.8 Hz, 0.4H), 6.05 (t, H-1′(α), J = 5.9 Hz, 0.6H), 7.17 (q, H-6(β), J = 1.2 Hz, 0.4H), 7.21 (q, H-6(α), J = 1.2 Hz, 0.6H), 8.76 (br s, NH, 1H); ^{31}P ($CDCl_3$): δ 7.47 (t, P(β), $^2J_{P-F}$ = 105.1 Hz), 7.45(t, P(α), $^2J_{P-F}$ = 104.4 Hz). ^{19}F ($CDCl_3$): δ −111.42 to −110.61 [m, CF_2(α), CF_2(β)].

1-[6,6-Difluoro-6-phosphono-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (16). To an ice-cold solution of **15** (550 mg, 1.6 mmol) in anhyd. acetonitrile (15 ml) under argon was added dropwise TMSBr (4.2 ml, 20 equiv). The mixture was stirred at rt for 1 day. Volatiles were removed in vacuo and the residue coevaporated several times with toluene and finally partitioned between Et_2O and water. The aqueous layer was neutralized with 1.0 M TEAB, washed with Et_2O twice, and concentrated to give the triethylammonium salt of **16** as a white solid (630 mg; 76%, α/β: 3:2); ^{1}H NMR (D_2O) δ 1.71 (s, CH_3, 3H), 1.60–1.81, 1.94, 2.03–2.44 (3m, 2H-2′, 2H-3′, 2H-5′, 6H), 4.67 and 4.31 (2m, H-4(α) and H-4(β), 1H), 5.92 (dd, H-1′(β), J = 7.5 and 3.1 Hz, 0.4H), 5.99 (t, H-1′(α), J = 5.9 Hz, 0.6H), 7.40 and 7.39 (2s, H-6(α) and H-6(β), 1H); ^{31}P (D_2O): δ 5.68 (t, P(β), $^2J_{P-F}$ = 95.5 Hz), 5.72 (t, P(α), $^2J_{P-F}$ = 95.5 Hz); ^{19}F (D_2O): δ −113.85 to −110.30 (m, CF_2(α), CF_2(β)); MS m/z 339 $(M-H)^-$.

1-[6,6-Difluoro-6-(β,γ-difluoromethylenetriphosphono)-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]thymine (4b). To a solution of triethylammonium salt of **16** (94 mg, 0.17 mmol) in HMPA (2.5 ml) under argon was added 1,1′-carbonyldiimidazole (110 mg, 0.68 mmol). The mixture was stirred at rt for 4 h when bis(tributylammonium) difluoromethylenediphosphonate (400 mg, 4 equiv.) in HMPA (2 ml) was added. After 8 h, the mixture was poured into 1.0 M TEAB (10 ml), and purified by HPLC to yield 17.2 μmol of **4b** as triethylammonium salt; ^{1}H NMR (D_2O) δ 1.76 (s, CH_3, 3H), 1.88–2.03, 2.05–2.56 (2m, 2H-2′, 2H-3′, 2H-5′, 6H), 4.37 (m, H-4′, 1H), 5.93 (dd, H-1′(β), J = 7.3 and 3.5 Hz, 0.35H), 5.98 [t, H-1′(α), J = 6.0 Hz, 0.65H], 7.39 and 7.33 (2q, H-6(α) and H-6(β), J = 1.2 Hz, 1H); ^{31}P (D_2O): δ −1.93 to −4.39 (m, P_β, P_γ), 0.4.38 (m, P_α); ^{19}F (D_2O): δ −110.4 to −113.9 (m, C6′-F_2), −119.8 (dd, PCF_2P, J_{FP} = 78.3 and 82.3 Hz); MS m/z 533.4 $(M-H)^-$. HPLC analysis: 95.9%.

1-[6,6-Difluoro-6-(β,γ-imidotriphosphono)-2,3,5,6-tetradeoxy-α/β-D-allofuranosyl]-thymine (4d). Following the procedure for **4b**, starting from triethylammonium salt of **16** (94 mg, 0.17 mmol) and tetrakis (tributylammonium) imidodiphosphate (270 mg, 1.2 equiv.), 14.6 μmol of **4d** as triethylammonium salt was isolated; ^{1}H NMR (D_2O) δ 1.99 (s, CH_3, 3H), 1.88–1.98, 2.05–2.56 (2m, 2H-2′, 2H-3′, 2H-5′, 6H), 4.32 (m, H-4′, 1H), 5.89 (dd, H-1′(β), J = 6.8 and 3.5 Hz, 0.35H), 5.98 (t, H-1′(α), J = 6.0 Hz, 0.65H), 7.36 and 7.30 (2s, H-6(α) and H-6(β),1H); ^{31}P (D_2O): β −3.38 (m, P_α), −1.90 to −4.20 (m, P_γ), −21.0 to

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−22.1 (m, P_β). ^{19}F (D_2O): δ −110.3 to −114.1 (m, C6′-F_2); MS *m/z* 498.4 $(M-H)^-$. HPLC analysis: 80.4%.

1-[6,6-Difluoro-2,3,5,6-tetradeoxy-6-triphosphono-α/β-D-allofuranosyl]thymine (4a). Following the procedure for **4b**, starting from triethylammonium salt of **16** (102 mg, 0.19 mmol) and tributylammonium salt of pyrophosphate (246 mg), 24 μmol of **4a** as triethylammonium salt was isolated; ^1H NMR (D_2O) δ 1.90 (s, CH_3, 3H), 1.86–2.03, 2.05–2.56 (2m, 2H-2′, 2H-3′, 2H-5′, 6H), 4.23–4.6 (m, H-4′, 1H), 5.98 and 5.94 [2m, H-1′(α), H-1′(β), 1H], 7.39 and 7.32 (2s, H-6(α) and H-6(β), 1H); ^{31}P (D_2O): δ −7.46 to −8.66 (m, P_β), −3.95 (m, P_α), −21.0 to −22.1 (m, P_γ); ^{19}F (D_2O): δ −118 (m, C6′-F_2); MS *m/z* 499.5 $(M-H)^-$. HPLC analysis: 94.8%.

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REFERENCES

1. Herdewijn, P.; Balzarini, J.; De Clercq, E. 2′-3′-Dideoxynucleoside analogues as antiviral agents. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press, Inc.: Greenwich, 1993; Vol. 1.
2. St. Claire, M.H.; Richards, C.A.; Spector, T.; Weinhold, K.J.; Langlois, Miller A.J.; Furman, P.A. 3′-Azido-3′-deoxythymidine triphosphate as an inhibitor and substrate of purified human immunodeficiency virus reverse transcriptase. Antimicrob. Agents Chemother. **1987**, *31*, 1972–1977.
3. Parker, W.B.; White, E.L.; Shaddix, S.C.; Ross, L.J.; Buckheit, R.W., Jr.; Germany, J.M.; Secrist, J.A., III; Vince, R.; Shannon, W.H. Mechanism of inhibition of human immunodeficiency virus Type 1 reverse transcriptase and human DNA polymerases α, β, γ by the 5′-triphosphates of Carbovir, 3′-azido-3′-deoxythymidine, 2′,3′-dideoxyguanosine, and 3′-deoxythymidine. J. Biol. Chem. **1991**, *266*, 1754–1762.
4. Jeffrey, J.L.; Feng, J.Y.; Qi, C.C.R.; Anderson, K.S.; Furman, P.A. Dioxolane guanosine 5′-triphosphate, an alternative substrate inhibitor of wild-type and mutant HIV-1 reverse transcriptase. J. Biol. Chem. **2003**, *278*, 18971–18979.
5. Wagner, C.R.; Iyer, V.V.; McIntee, E.J. Pronucleotides: toward the in vivo delivery of antiviral and anticancer nucleotides. Med. Res. Rev. **2000**, *20*, 417–451.
6. De Clercq, E. Antiviral drugs: current state of the art. J. Clin. Virol. **2001**, *22*, 73–89.
7. Bayes, M.; Rabasseda, X.; Prous, J.R. Gateways to clinical trials. Methods Find Exp. Clin. Pharmacol. **2003**, *25*, 53–76.
8. Selmi, B.; Deval, J.; Borreto, J.; Canard, B. Nucleotide analogue binding, catalysis and primer unblocking in the mechanisms of HIV reverse transcriptase-mediated resistance to nucleoside analogues. Antivir. Ther. **2003**, *8*, 143–154.
9. Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J.L.; Hurd, T.; Leeds, J.M.; Rajwanshi, V.K.; Jin, Y.; Prhavc, M.; Bruice, T.W.; Cook, P.D. Synthesis of AZT 5′-triphosphate mimics and their inhibitory effects on HIV-1 reverse transcriptases. J. Med. Chem. **2004**, *47*, 6902–6913.
10. Horwitz, J.P.; Chua, J.; Noel, M. Nucleosides. V. The monomesylates of 1-(2′-deoxy-β-D-lyxofuranosyl)-thymine. J. Org. Chem. **1964**, *29*, 2076–2078.
11. Lin, T.-S.; Shen, Z.-Y.; August, E.M.; Brankovan, V.; Yang, H.; Ghazzouli, I.; Prusoff, W.H. Synthesis and antiviral activity of several 2,5′-anhydro analogs of 3′-azido-3′-deoxythymidine (AZT), 3′-azido-2′,3′-dideoxyuridine (AZU), 3′-azido-2′,3′-dideoxy-5-halouridines, and 3′-deoxythymidine against human

immunodeficiency virus (HIV-1) and Rauscher-Murine leukemia virus (R-MuLV). J. Med. Chem. **1989**, *32*, 1891–1895.

12. Hakimelahi, G.H.; Moosavi-Movahedi, A.A.; Sadeghi, M.M.; Tsay, S.-C.; Hwu, J.R. Design, synthesis, and structure-activity relationship of novel dinucleotide analogs as agents against herpes and human immunodeficiency viruses. J. Med. Chem. **1995**, *38*, 4648–4659.
13. Freeman, G.A.; Rideout, J.L.; Miller, W.H.; Reardon, J.E. 3′-Azido-3′,5′-dideoxythymidine-5′-methylphosphonic acid diphosphate: synthesis and HIV-1 reverse transcriptase inhibition. J. Med. Chem. **1992**, *35*, 3192–3196.
14. Krecmerova, M.; Hrebabecky, H.; Masojidkova, M.; Holy, A. 2′,3′-Dideoxy- and 3′-azido-2′,3′-dideoxynucleosides of 5-phenyl-2(1*h*)-pyrimidinone. Preparation of 2′,3′-dideoxypentofuranoses. Collect. Czechoslov. Chem. Commun. **1996**, *61*, 478–488.
15. Seville, M.; West, A.B.; Cull, M.G.; McHenry, C.S. Fluorometric assay for DNA polymerases and reverse transcriptase. BioTechniques **1996**, *21*, 664–672.

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