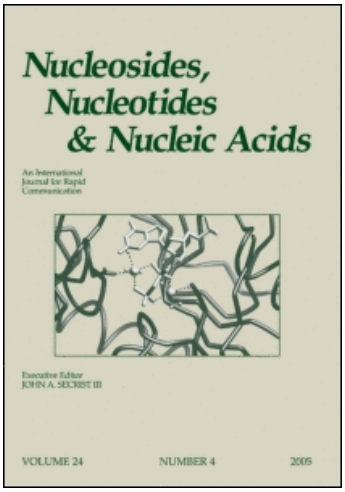


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

2',3'-Dideoxynucleoside 5'-β,γ-(Difluoromethylene) Triphosphates With α-*P*-Thio or α-*P*-Seleno Modifications: Synthesis and Their Inhibition of HIV-1 Reverse Transcriptase

Nicholas A. Boyle[a]; Patrick Fagan[a]; Jennifer L. Brooks[a]; Marija Prhavc[a]; John Lambert[a]; P. Dan Cook[a]
[a] Research Laboratories, Biota, Inc., Carlsbad, California, USA

To cite this Article Boyle, Nicholas A. , Fagan, Patrick , Brooks, Jennifer L. , Prhavc, Marija , Lambert, John and Cook, P. Dan(2005) '2',3'-Dideoxynucleoside 5'-β,γ-(Difluoromethylene) Triphosphates With α-*P*-Thio or α-*P*-Seleno Modifications: Synthesis and Their Inhibition of HIV-1 Reverse Transcriptase', Nucleosides, Nucleotides and Nucleic Acids, 24: 10, 1651 — 1664
To link to this Article: DOI: 10.1080/15257770500267055
URL: http://dx.doi.org/10.1080/15257770500267055

Nucleosides, Nucleotides, and Nucleic Acids, 24:1651–1664, 2005

ISSN: 1525-7770 print/1532-2335 online
DOI: 10.1080/15257770500267055

Taylor & Francis
Taylor & Francis Group

2′,3′-DIDEOXYNUCLEOSIDE 5′-β,γ-(DIFLUOROMETHYLENE) TRIPHOSPHATES WITH α-*P*-THIO OR α-*P*-SELENO MODIFICATIONS: Synthesis and Their Inhibition of HIV-1 Reverse Transcriptase

Nicholas A. Boyle, Patrick Fagan, Jennifer L. Brooks, Marija Prhavc, John Lambert, and P. Dan Cook □ *Research Laboratories, Biota, Inc., Carlsbad, California, USA*

□ *Nucleoside reverse transcriptase inhibitors (NRTIs) are prodrugs which require three intracellular phosphorylation steps to yield their corresponding, biologically active, nucleoside triphosphate. In order to circumvent this often inefficient phosphorylation cascade, a plausible approach is to provide the active species directly in the form of a stabilized nucleoside triphosphate mimic. We have previously shown that such a mimic, namely 5′-α-R_p-borano-β,γ-(difluoromethylene)triphosphate (5′-αBCF$_2$TP) is a generic triphosphate mimic that is biologically stable and can render antiviral ddNs with potent inhibitory activity against HIV-1 RT.*[1,2] *Herein we report the synthesis and activity against HIV-1 RT of several ddN 5′-α-modified-β,γ-(difluoromethylene)triphosphate mimics with either a non-bridging α-P-thio (5′-αSCF$_2$TP) or α-P-seleno (5′-αSeCF$_2$TP) modification. One compound, namely, AZT-5′-α-P-seleno-β,γ-(difluoromethylene)triphosphate (diastereomer I), was identified as a potent inhibitor of HIV-1 RT (K_i = 64 nM) and represents the first report of HIV-1 RT inhibition data for a nucleotide bearing an α-P-seleno modification. These triphosphate mimics may be useful in the investigation of enzyme mechanism and may have interesting properties with respect to drug resistance and polymerase selectivity.*

Keywords Triphosphate mimic; HIV-1; Reverse transcriptase; Thio triphosphate; Seleno triphosphate; AZT; Drug resistance; Polymerase selectivity

INTRODUCTION

The triphosphates of antiviral 2′,3′-dideoxynucleosides (ddNs) are the active chemical entities that inhibit viral DNA synthesis via the incorporation of ddN 5′-monophosphate (ddNMP) into DNA, resulting in chain

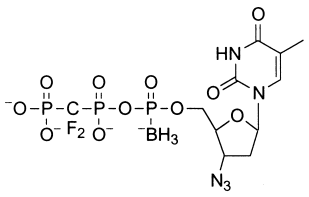
The authors dedicate this manuscript to the memory of Professor John A. Montgomery.

Received 30 January 2005; accepted 28 February 2005.

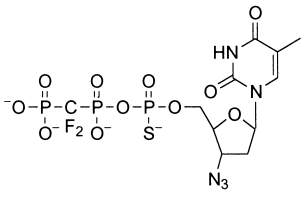
The authors thank Peter J. Nichols at the NMR facility, Department of Chemistry, Monash University.

Address correspondence to Nicholas Boyle, Ph.D., 1715 Greentree Road, Encinitas, CA 92024. E-mail: naboyle@hotmail.com

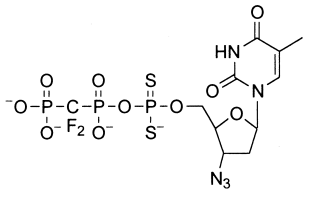
termination.[3–6] Given that ddN antiviral drugs are inefficiently phosphorylated in cells, a major advantage would be to deliver the active triphosphate species. Encouraged by the clinical successes of the monophosphate mimics tenofovir disoproxil fumarate (a *bis*-POC prodrug of PMPA)[7] and adefovir dipivoxil (a *bis*-POM prodrug of PMEA)[8] we sought to generate stable triphosphate mimics (termed P3Ms) of ddNs (ddN-P3Ms) as antiviral agents that can bypass entirely the three cellular phosphorylation steps. Recently, we reported a series of AZT-5′-triphosphate mimics (AZT-P3Ms) that possessed a combination of serum and cellular stability and potent inhibition of HIV-1 RT.[1] Most notably, an AZT-P3M (**1a**) compound bearing an 5′-α-R_P-borano-β,γ-(difluoromethylene)triphosphate moiety (αBCF$_2$TP) was shown to have a half-life in human serum of over 48 h and was a potent inhibitor of HIV-1 RT with a K_i value of 9.5 nM in an assay using a poly-A template. We subsequently showed that the αBCF$_2$TP moiety was able to render other antiviral ddNs with potent inhibitory activity against HIV-1 RT.[2] Another potent AZT-P3M from this work was 5′-α-thio-β,γ-(difluoromethylene)triphosphate (αSCF$_2$TP) (**1b**) which gave an AZT-P3M with a K_i value of 90 nM against HIV-1 RT.[9] The corresponding α,α-dithio P3M (**1c**), gave 19% inhibition at a concentration of 0.5 μM.



AZT 5′-αBCF$_2$TP (**1a**)



AZT 5′-αSCF$_2$TP (**1b**)

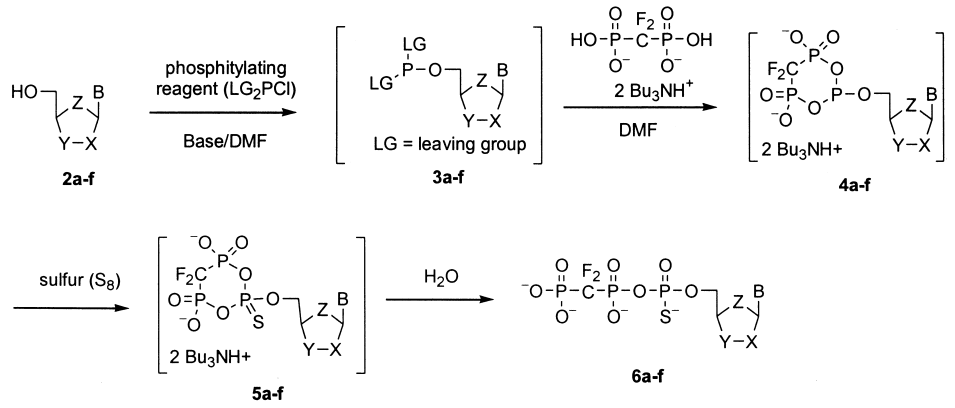


AZT 5′-α,α-SCF$_2$TP (**1c**)

In our search to further identify useful combinations of triphosphate modifications, and to explore if other ddN-αSCF$_2$TPs had similar activities against HIV-1 RT, we prepared a number of ddN β,γ-(difluoromethylene) triphosphates with either a non-bridging α-*P*-thio (5′-αSCF$_2$TP) or α-*P*-seleno (5′-αSeCF$_2$TP) modification. In the context of anti HIV-1 RT activity, there have been numerous publications regarding oligomeric phosphorodithioates,[10] but relatively few reports of ddN nucleoside triphosphates with an α-*P*-thio substitution.[1,11,12] Furthermore, described herein is the first report of HIV-1 RT inhibition data for a nucleotide bearing an α-*P*-seleno modification.

CHEMISTRY

Compounds **6a-f** (Scheme 1) were synthesized using two different phosphitylating reagents. Compounds **6a** and **6b** were prepared using 2-chloro-



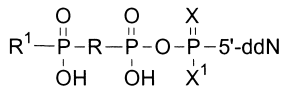
a B = thymine, X = Y = CH_2, Z = O, (dT); **b** B = thymine, X, Y = CH=CH, Z = O, (D4T); **c** B = thymine, X = CH_2, Y = CHF (F on α face), Z = O, (3'-FT); **d** B = cytosine, X = CH2, Y = S, Z = O, (L-ribose), (3TC); **e** B = guanine, X–Y = CH=CH, Z = CH_2 (carbovir); **f** B = 6-Cl-2-NH_2-purine, X, Y = CH=CH, Z = CH_2.

SCHEME 1

4*H*-1,3,2-benzodioxaphosphorin-4-one, according to a similar procedure for the preparation of AZT 5′-$\alpha$$SCF_2$TPs (**1b**)[1] and nucleoside 5′-α-*P*-thiotriphosphates.[13] Compounds **6c–6f** were prepared via *bis*(diisopropylamino)chlorophosphine, using a procedure modified from the preparation of ddN 5′-$\alpha$$BCF_2$TPs and nucleoside 5′-$\alpha$-*P*-boranotriphosphates.[2,14] In a report by Nahum et al., *bis*(diisopropylamino)chlorophosphine was found to yield adenine 5′-α-*P*-boranotriphosphates in better yields compared to other phosphitylating reagents such as 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one.[14] This is likely a result of reduced side reaction of the phosphitylating reagent with the adenine N^6. Similarly, when we used *bis*(diisopropylamino)chlorophosphine, rather than 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, as the phosphitylating reagent for the nucleosides bearing an exocyclic amino group reported herein, we obtained improved yields of nucleoside 5′-α-*P*-thiotriphosphates. In some cases, the use of *bis*(diisopropylamino)chlorophosphine with nucleoside analogs that do not bear an exocyclic amino group (for example compound **2c**) also resulted in improved yields of 5′-α-*P*-thiotriphosphates. The exact reasons for this observation were not investigated.

Treatment of **2a-f** with either 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one or *bis*(diisopropylamino)chlorophosphine yielded the activated phosphites **3a-f**, which were condensed with *bis*(tributylammonium) difluoromethylenediphosphonate[1] to form the cyclic triphosphates **4a-f**. Treatment of **4a-f** with powdered sulfur yielded, after hydrolysis, the ddN 5′-$\alpha$$SCF_2$TPs **6a-f** in moderate to good yields. Compound **6f** and the corresponding TP (abbreviated as 6-chloro carbovir-TP in Table 1) were prepared from the

TABLE 1 Inhibition of HIV-1 RT by ddN 5′-P3Ms[a]



Compound	X	X^1	R	R^1	K_i (μM)
AZT-TP	O	OH	O	O	0.0084
1a-I	O	$^{-}BH_3$	CF_2	O	0.0096
1b-I/II	O	SH	CF_2	O	0.090
1c	S	SH	CF_2	O	19%[b]
11-I	O	Se	CF_2	O	0.064
11-II	O	Se	CF_2	O	1.37
ddT-TP	O	OH	O	O	0.0052
6a-I/II	O	SH	CF_2	O	20.7%[b]
6h-I/II	O	SH	CF_2	S	29.1%[b]
D4T-TP	O	OH	O	O	0.0032
6b-I	O	SH	CF_2	O	10.0%[b]
6b-II	O	SH	CF_2	O	17.0%[c]
3′-FT-TP	O	OH	O	O	0.0093
6c-I	O	SH	CF_2	O	0.735
6c-II	O	SH	CF_2	O	0.4%[c]
3TC-TP	O	OH	O	O	0.188
6d-I/II	O	SH	CF_2	O	0.0%[c]
6g	O	OH	CF_2	O	37.1%[b]
carbovir-TP	O	OH	O	O	0.037
6e-I/II	O	SH	CF_2	O	0.0%[c]
6-chloro carbovir-TP	O	OH	O	O	12.3%[c]
6f	O	SH	CF_2	O	3.9%[c]
3′-NH_2-ddT-TP	O	OH	O	O	0.042[d]
10-I/II	O	SH	CF_2	O	13.2%[b]

Shaded boxes are for comparison and inhibition values (except those for ddT-TP, D4T-TP, 3′-FT-TP and 6-chloro carbovir-TP) have been reported previously.[1,2]

[a]Diastereomer I assigned to the ddN-P3M having shorter HPLC retention time, Diastereomer II assigned to the ddN-P3M having longer HPLC retention time, I/II refers to a mixture.

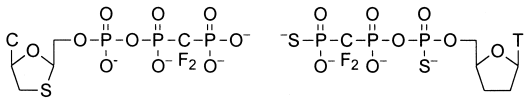
[b]Percentage inhibition at 0.5 μM of compound.

[c]Percentage inhibition at 10 μM of compound.

[d]K_i value reported by Kedar et al.[21] A heteropolymeric RNA template was used for compounds **6d-g** and their respective ddN-TPs. A homopolymeric RNA template (poly-A) was used for compounds **6a-c**, **6h**, **10**, and **11** and their respective ddN-TPs.

6-chloro precursor of carbovir and although this precursor nucleoside does not exhibit anti-HIV activity, it was prepared for SAR and NMR purposes.

The versatile cyclic intermediate **4** can be treated with a variety of oxidants and nucleophiles to generate a diverse set of modified triphosphates.[1] Treatment of intermediate **4d** with iodine[15] followed by hydrolysis gave **6g** and treatment of **5a** with lithium sulfide gave α,γ-*P*-dithio **6h** (ddT 5′-α,γ-SCF_2TP) as the major *P*-dithio-substituted product.[16] Minor products with a mass corresponding to other *P*-dithio-substituted products were observed but were not isolated.



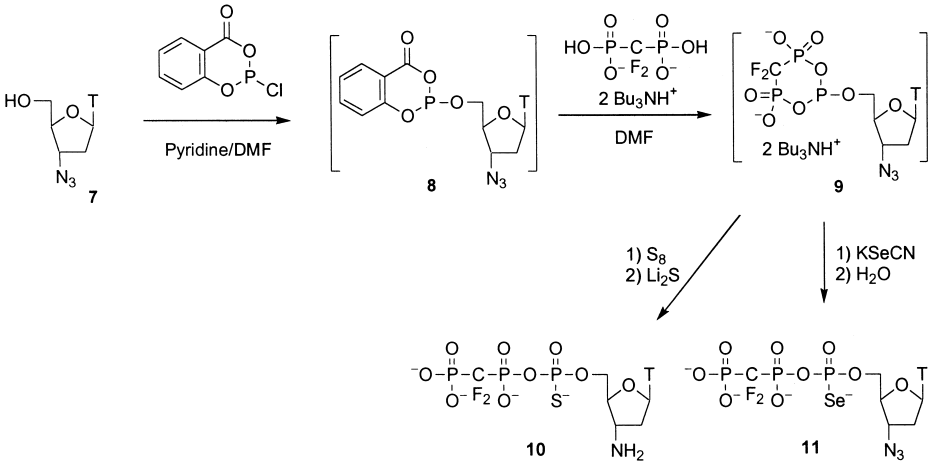
3TC 5′-αOCF$_2$TP (**6g**) ddT 5′-α,γ-SCF$_2$TP (**6h**)

In a similar fashion, AZT, **7**, was phosphitylated to give intermediate **8**, which was converted to cyclic triphosphate **9** as described previously.[1] Treatment of **9** with sulfur followed by lithium sulfide gave a mixture of compounds including 3′-amino α-*P*-thio compound **10**, presumably via sulfide-mediated reduction (Scheme 2).[17] Furthermore, treatment of **9** with potassium selenocyanate as an oxidant gave, after hydrolysis, α-*P*-seleno compound **11** (Scheme 2).[18]

Analysis of ddN 5′-α-*P*-substituted-β,γ-difluoromethylenetriphosphate ^{31}P NMR spectra of this work and previously published data[1,2] reveals similar chemical shifts and splitting patterns for both β-P and γ-P. A representative example of a ^{31}P NMR spectrum for these compounds is shown in Figure 1 for a single diastereoisomer of compound **6c**, termed **6c-I**.

However, comparison of the α-P chemical shifts for ddN 5′-β-*P*-substituted-β,γ-difluoromethylenetriphosphates reveals distinct differences. Within a series of AZT 5′-α-*P*-substituted-β,γ-difluoromethylenetriphosphates, the similarity in the chemical shifts for β-P and γ-P and the substituent effect on α-P are evident (Table 2).

As observed by their respective ^{31}P NMR spectra, compounds **6a-6f**, **6h**, and **10-11** all consist of two diastereomers in approximately 1:1 ratio. The respective diastereomers of **6b**, **6c**, and **11** were separated by reverse-



SCHEME 2

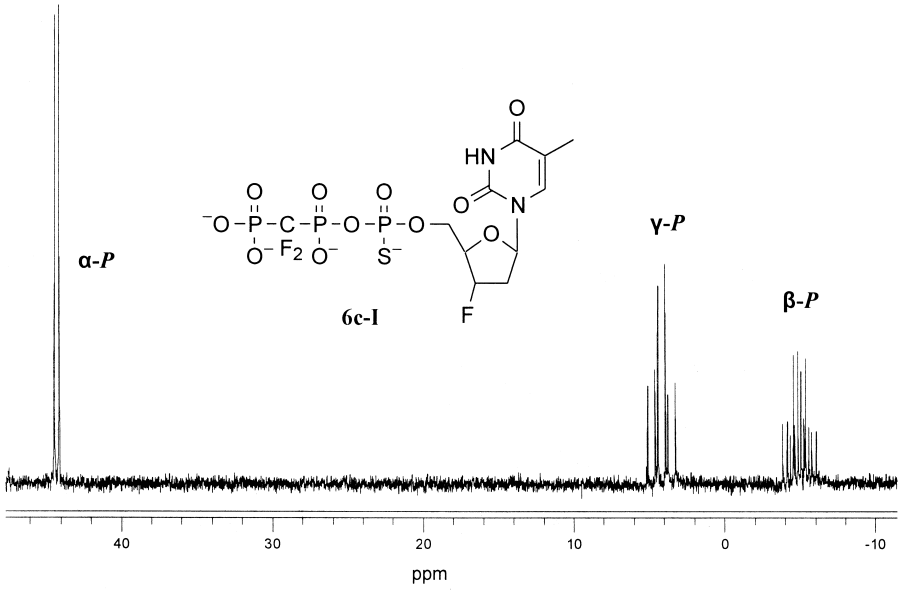
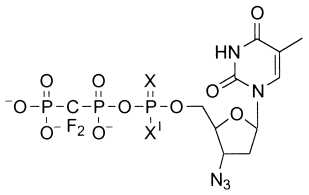


FIGURE 1 ^{31}P NMR proton-decoupled spectrum of compound **6c-I** in D_2O. Sample recorded on a Bruker DPX300 NMR spectrometer.

TABLE 2 ^{31}P NMR Data of AZT 5′-*α*-*P*-Substituted-*β*,*γ*-difluoromethylenetriphosphates in D_2O



Compound	X	X^1	Chemical shifts,[a] ppm		
			P_α	P_β	P_γ
AZT 5′-*β*,*γ*-CF_2-TP[b]	O	O	−10.77 (d)	−3.95 (m)	4.16 (dt)
1a-I[b]	O	BH_3	84.02 (br)	−4.24 (m)	4.37 (ddt)
1b-I/II[b]	O	S	46.82 (d), 46.65 (d)[c]	−1.83 (m)	6.87 (dt)
1c[b]	S	S	100.61 (d)	−4.75 (m)	4.25 (dt)
11-I	O	Se	33.92 (d)	−3.42 (m)	4.66 (dt)
11-II	O	Se	33.86 (d)	−4.30 (m)	4.38 (dt)

[a] ^{31}P NMR at 121 MHz.
[b] Data reported in Wang et al.[1].
[c] Chemical shift value updates the value reported by Wang et al.[1]

phase HPLC and compounds **6a**, **6d**, **6e**, **6f**, **6h**, and **10** were not separable under the conditions used. The separated diastereomers are designated as diastereomers I and diastereomers II, according to whether they were eluted first (I) or second (II) from the C18 reverse-phase HPLC. Determination of the stereochemistry of the diastereomers was not conducted. Diastereomer I of AZT 5′-α-*P*-boranotriphosphate was previously assigned as the α-R_P-diastereomer and was found to be a much more potent inhibitor of HIV-1 RT than the diastereomer II (S_P).[1,19] Similar observations were made for diastereomer I (S_P) of nucleoside triphosphates with an α-*P*-thio modification.[1,11,12] We therefore may expect that diastereomer I (shorter retention time on HPLC) of the ddN-αSCF$_2$TP and ddN-αSeCF$_2$TP compounds reported herein to be more potent inhibitors of HIV-1 RT than the corresponding diastereomers II (longer retention time on HPLC) and tentatively assign diastereomer I for these compounds as having α–S_p stereochemistry, which corresponds to the α–R_p conformation of AZT 5′-α-*P*-boranotriphosphate.

Inhibition of HIV-1 RT

The effectiveness of compounds as inhibitors of HIV-1 RT was determined according to published procedures.[1,5,25] AZT-5′-αSCF$_2$TP was previously shown by us to be a potent inhibitor of HIV-1 RT (Table 1).[1] We therefore synthesized a number of ddNs bearing this P3M to investigate if similar inhibition properties would be observed. As expected, diastereomer II of the ddN-P3Ms tested were generally less active or completely inactive against HIV-1 RT. With respect to inhibition against HIV-1 RT, the 5′-αSCF$_2$TP modification did not render the ddNs reported here with activity comparable with their corresponding ddN triphosphates (Table 1). The most potent ddN 5′-αSCF$_2$TPs tested in this work was 3′-FT 5′-αSCF$_2$TP, **6c-I**, which exhibited approximately 20-fold lower activity relative to 3′-FT-TP with a K_i of 0.735 μM. Among the triphosphates of clinically used NRTIs, the triphosphate of 3TC is a relatively weak inhibitor of HIV-1 RT ($K_i = 0.188$ μM). Replacement of the β,γ-*P*-bridging oxygen with CF$_2$ gave compound **6g**, 3TC 5′-$\beta\gamma$-CF$_2$TP, which exhibited weak (37.1%) inhibition at 0.5 μM. This loss in activity was reduced further upon introduction of an α-*P*-thio substituent (compound **6d**, 3TC 5′-αSCF$_2$TP), which essentially had zero inhibition.

Although there have been a few reports regarding nucleoside 5′-α-*P*-seleno-triphosphates (αSeTPs),[15,20] the anti HIV-1 RT activity for this class of compounds has, hitherto, remained unreported in the literature. Interestingly, diastereomer **I** of AZT-5′-αSeCF2TP, compound **11-I**, was a potent inhibitor of HIV-1 RT with a K_i of 64.0 nM and the slower isomer, **11-II**, was weakly active with a K_i of 1.37 μM.

EXPERIMENTAL SECTION

^{1}H, ^{19}F, and ^{31}P NMR spectra were recorded on a Bruker DPX300 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.[1] Anhydrous solvents purchased from Aldrich were used directly in the reactions without further treatment unless indicated.

Purification of ddN-P3Ms

ddN-P3Ms were purified by anion exchange (AX) chromatography using a 10 × 160 mm Mono Q column (Pharmacia). Initial conditions were typically 0 to 35 mM NaCl. A linear elution gradient was typically initiated at 0 to 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 × 21 mm, 10-μm particle size column (Phenomenex), with a flow rate of 10 mL/min. Elution gradients were generally from 0–20% to 95% methanol in 20–60 min at a constant concentration of triethylammonium acetate (50 mM). Fractions containing desired ddN 5′-αSCF_2TPs or ddN 5′-αSeCF_2TPs were collected and lyophilized. Yields of all the ddN-P3Ms of this work were calculated based on their UV absorbance and are reported in milligrams.

LCMS and HPLC Analysis of ddN-P3Ms

Mass spectra and purity of the ddN-P3Ms were obtained using on-line HPLC mass spectrometry on a ThermoFinnigan (San Jose, California) Deca XP plus. A Phenomenex Luna (C18 or C5), 75 × 2 mm, 3-μm particle size was used for RP-HPLC. A 0 to 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.

HIV-1 Reverse Transcriptase Inhibition Assays

HIV-1 reverse transcriptase inhibition assays were performed as previously described.[1,5,25] A heteropolymeric RNA template was used for compounds **6d-g** and a homopolymeric RNA template (poly-A) was used for compounds **6a-c**, **6h**, **10**, and **11**.

SYNTHESIS

2′,3′-Dideoxythymidine (ddT) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6a). A freshly prepared solution of 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (45.1 mg, 0.22 mmol) in anhydrous DMF (0.5 mL) was added via syringe to a solution of 2′,3′-dideoxythymidine (42.0 mg, 0.186 mmol) and anhydrous pyridine (100 μL) in 0.5 mL of anhydrous DMF at 0°C under argon. After stirring at room temperature for 1 h, tributylamine (300 μL) was added, followed by a solution of *bis*(tributylammonium) difluoromethylenediphosphonate (140.8 mg, 0.24 mmol) in anhydrous DMF (1 mL). The reaction mixture was stirred at room temperature for 1 h then sulfur (11.9 mg, 0.372 mmol) was added at 0°C. After stirring at room temperature for 1 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 29.0 mg, 25.6% of **6a** (two diastereoisomers) as a triethylammonium salt. ^{1}H NMR (D_2O): δ 1.88 (s, 5-CH_3, 3H), 1.95–2.40 (m, H-2′, H-3′, 4H), 4.05–4.37 (m, H-4′, H-5′, 3H), 6.05 (m, H-1′, 1H), 7.71 and 7.72 (2s, 2 isomers, H-6, 1H); ^{31}P NMR (D_2O): δ −2.65 (m, P_β), 5.01 (dt, $^2J_{P\text{-}P}$ = 58.1 Hz, $^2J_{P\text{-}F}$ = 74.4 Hz, P_γ), 44.12 (d, J = 38.9 Hz, P_α) and 44.40 (d, J = 38.8 Hz, P_α); ^{19}F NMR (D_2O): δ −118.71 (m, CF_2); MS *m/z* 515.3 $(M\text{-}H)^-$; HPLC analysis, 99.1% purity.

2′,3′-Didehydro-2′,3′-dideoxythymidine (d4T) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6b). Following the same procedure as described for **6a**, the reaction starting with 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) (25 mg, 0.111 mmol) yielded, after HPLC purification, 5.9 mg of **6b-I** and 3.0 mg of **6b-II** as triethylammonium salts, total yield = 15.6%. **6b-I**: ^{1}H NMR (D_2O): δ 1.85 (s, 5-CH_3, 3H), 4.17 (m, H-5′, 2H), 5.06 (m, H-4′, 1H), 5.85 (m, H-2′, 1H), 6.47 (m, H-3′, 1H), 6.88 (m, H-1′, 1H), 7.57 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −3.93 (m, P_β), 4.25 (dt, $^2J_{P\text{-}P}$ = 58.9 Hz, $^2J_{P\text{-}F}$ = 76.9 Hz, P_γ), 43.27 (d, $^2J_{P\text{-}P}$ = 38.8 Hz, P_α); ^{19}F NMR (D_2O): δ −117.51 (apparent t, $^2J_{F\text{-}P}$ = 79.7 Hz, CF_2); MS *m/z* 513.3 $(M\text{-}H)^-$; HPLC analysis, 99.4% purity. **6b-II**: ^{1}H NMR (D_2O): δ 1.85 (s, 5-CH_3, 3H), 4.16 (m, H-5′, 2H), 5.07 (m, H-4′, 1H), 5.87 (m, H-2′, 1H), 6.49 (m, H-3′, 1H), 6.88 (m, H-1′, 1H), 7.54 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −3.19 (m, P_β), 4.75 (dt, $^2J_{P\text{-}P}$ = 58.8 Hz, $^2J_{P\text{-}F}$ = 76.2 Hz, P_γ), 44.36 (d, $^2J_{P\text{-}P}$ = 38.7 Hz, P_α); ^{19}F NMR (D_2O): δ −118.44 (apparent t, $^2J_{F\text{-}P}$ = 84.6 Hz, CF_2); MS *m/z* 513.3 $(M\text{-}H)^-$; HPLC analysis, 90.6% purity.

2′,3′-Dideoxy-3′-fluorothymidine (3′-F-ddT) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6c). A freshly prepared solution of *bis*(diisopropylamino)chlorophosphine (64 mg, 0.24 mmol) in 0.5 mL of anhydrous chloroform was added via syringe to a stirred solution of 3′-F-ddT (53 mg, 0.218 mmol) and diisopropylethylamine (49.3 μL, 0.28 mmol) in anhydrous

DMF (2 mL) at 0°C under argon. The resulting solution was stirred at 5°C for 2 h, then *bis*(tributylammonium) difluoromethylenediphosphonate (165.0 mg, 0.283 mmol) in DMF (1 mL) was added. The reaction mixture was stirred at room temperature for 4 h then sulfur (14.0 mg, 0.436 mmol) was added at 0°C. The resulting mixture was stirred at room temperature for 1 h, cooled with ice, and quenched by addition of water (5 mL). The mixture was stirred at room temperature for 0.5 h, and purified by HPLC purification to give 29.8 mg of isomer **6c-I** and 32.7 mg of isomer **6c-II** as triethylammonium salts, total yield = 48.8%. **6c-I**: ^{1}H NMR (D_2O): δ 1.87 (s, 5-CH_3, 3H), 2.26–2.61 (m, H-2′, 2H), 4.12 and 4.26 (2m, H-5′, 2H), 4.47 (dd, $^3J_{H\text{-}H} = 4.6$ Hz, $^3J_{H\text{-}F} = 27.6$ Hz, H-4′, 1H), 5.48 (dd, $^3J_{H\text{-}H} = 4.6$ Hz, $^3J_{H\text{-}F} = 52.7$ Hz, H-3′, 1H), 6.35 (dd, $J = 5.4, 9.3$ Hz, H-1′, 1H), 7.77 (d, $J =$ 1.3 Hz, H-6, 1H); ^{31}P NMR (D_2O): δ −4.96 (m, P_β), 4.18 (dt, $^2J_{P\text{-}P} = 60.2$ Hz, $^2J_{P\text{-}F} = 82.2$ Hz, P_γ), 44.15 (d, $^2J_{P\text{-}P} = 37.1$ Hz, P_α); ^{19}F NMR (D_2O): δ −174.75 (m, 3′-F), −120.45 (m, CF_2); MS *m/z* 533.4 $(M\text{-}H)^-$; HPLC analysis, 99.8% purity. **6c-II**: ^{1}H NMR (D_2O): δ 1.85 (s, 5-CH_3, 3H), 2.25–2.61 (m, H-2′, 2H), 4.15–4.30 (m, H-5′, 2H), 4.49 (dd, $^3J_{H\text{-}H} = 2.5$ Hz, $^3J_{H\text{-}F} =$ 27.7 Hz, H-4′, 1H), 5.48 (dd, $^3J_{H\text{-}H} = 4.3$ Hz, $^3J_{H\text{-}F} = 52.5$ Hz, H-3′, 1H), 6.36 (dd, $J = 5.4, 9.4$ Hz, H-1′, 1H), 7.77 (d, $J = 1.2$ Hz, H-6, 1H); ^{31}P NMR (D_2O): δ −4.90 (m, P_β), 4.20 (dt, $^2J_{P\text{-}P} = 60.4$ Hz, $^2J_{P\text{-}F} = 82.1$ Hz, P_γ), 43.96 (d, $^2J_{P\text{-}P} = 38.0$ Hz, P_α); ^{19}F NMR (D_2O): δ −174.35 (m, 3′-F), −120.45 (dd, $^2J_{F\text{-}P} = 86.7$ Hz, $^2J_{F\text{-}P} = 82.2$ Hz, CF_2); MS *m/z* 533.4 $(M\text{-}H)^-$; HPLC analysis, 96.7% purity.

L-2′,3′-Dideoxy-3′-thiacytidine (3TC) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6d). Following the same procedure as described for **6c**, the reaction starting with 3TC (47 mg, 0.205 mmol) yielded, after HPLC purification, 51.6 mg, 48.6% of **6d** (two diastereoisomers) as a triethylammonium salt; ^{1}H NMR (D_2O): δ 3.37 and 3.55 (2m, H-2′, 2H), 4.31 and 4.44 (2m, H-5′, 2H), 5.48 (m, H-4′, 1H), 6.09 (d, $J = 7.7$ Hz, H-5, 1H), 6.31 (t, $J = 4.3$ Hz, H-1′, 1H), 8.05 (d, $J = 7.5$ Hz, H-6, 1H); ^{31}P NMR (D_2O): δ −1.54 (m, P_β), 5.53 (dt, $^2J_{P\text{-}P} = 71.8$ Hz, $^2J_{P\text{-}F} = 57.1$ Hz, P_γ), 44.68 (d, $J = 38.0$ Hz, P_α) and 44.78 (d, $J = 38.2$ Hz, P_α); ^{19}F NMR (D_2O): δ −119.18 (dd, $^2J_{F\text{-}P} = 88.1$ Hz, $^2J_{F\text{-}P} = 71.9$ Hz, CF_2); MS *m/z* 518.0 $(M\text{-}H)^-$; HPLC analysis, 99.9% purity.

Carbocyclic 2′,3′-Didehydro-2′,3′-dideoxyguanosine (Carbovir) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6e). Following the same procedure as described for **6c**, the reaction starting with carbovir (41.3 mg, 0.167 mmol) yielded, after HPLC purification, 16.3 mg, 18.2% of **6e** (two diastereomers) as a triethylammonium salt. ^{1}H NMR (D_2O): δ 1.59 (m, CH*H*′, 1H), 2.70 (m, C*H*H′, 1H), 3.06 (m, under triethylammonium salt signal, H-4′), 4.01 (m, H-5′, 2H), 5.37 (m, H-1′, 1H), 5.83 and 6.19 (double multiplet,

CH=CH, 2H), 7.82 (m, H-8, 1H); ^{31}P NMR (D_2O): δ −3.30 (m, P_β), 4.78 (dt, $^2J_{P\text{-}P}$ = 58.9 Hz, $^2J_{P\text{-}F}$ = 76.5 Hz, P_γ), 44.26 (d, $^2J_{P\text{-}P}$ = 39.1 Hz, P_α) and 44.34 (d, $^2J_{P\text{-}P}$ = 39.2 Hz, P_α); ^{19}F NMR (D_2O): δ −118.70 (dd, $^2J_{F\text{-}P}$ = 87.9 Hz, $^2J_{F\text{-}P}$ = 77.2 Hz, CF_2); MS *m/z* 536.9 $(M\text{-}H)^-$. HPLC analysis: 99.8% purity.

Carbocyclic 2′,3′-Didehydro-2′,3′-dideoxy-6-chloroguanosine (6-Cl-Carbovir) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (6f). Following the same procedure as described for **6c**, the reaction starting with 6-chloro carbovir (44.3 mg, 0.167 mmol) yielded, after HPLC purification, 45.6 mg, 49.2% of **6f** (two diastereomers) as a triethylammonium salt: ^{1}H NMR (D_2O): δ 1.72 (m, CH*H*′, 1H), 2.63 (m, C*H*H′, 1H), 3.06 (m, H-4′, 1H), 4.01 (m, H-5′, 2H), 5.49 (m, H-1′, 1H), 5.89 and 6.24 (double multiplet, CH=CH, 2H), 8.17 (d, J = 3.2 Hz, H-8, 1H); ^{31}P NMR (D_2O): δ −3.59 (m, P_β), 4.66 (dt, $^2J_{P\text{-}P}$ = 59.1 Hz, $^2J_{P\text{-}F}$ = 77.0 Hz, P_γ), 44.33 (m, P_α); ^{19}F NMR (D_2O): δ −118.77 (apparent t, $^2J_{F\text{-}P}$ = 83.0 Hz, CF_2); MS *m/z* 554.6 $(M\text{-}H)^-$; HPLC analysis, 98.6% purity.

L-2′,3′-Dideoxy-3′-thiacytidine (3TC) 5′-β,γ-(Difluoromethylene)triphosphate (6g). Following a similar procedure as described for **6c**, the reaction starting with 3TC (25 mg, 0.109 mmol) was modified by replacing sulfur with iodine (55.4 mg, 0.218 mmol) and yielded, after HPLC purification, 28.2 mg, 51.5% of **6g** as a triethylammonium salt; ^{1}H NMR (D_2O): δ 3.49 (dd, J = 5.3 Hz, 12.3 Hz, H-2′, 2H), 4.20 and 4.35 (2m, H-5′, 2H), 5.41 (m, H-4′, 1H), 6.07 (d, J = 7.7 Hz, H-5, 1H), 6.25 (t, J = 4.8 Hz, H-1′, 1H), 8.06 (d, J = 7.6 Hz, H-6, 1H); ^{31}P NMR (D_2O): δ −10.11 (d, J = 30.1 Hz, P_α), −4.26 (m, P_β), 4.16 (dt, $^2J_{P\text{-}P}$ = 81.9, $^2J_{P\text{-}F}$ = 59.8, P_γ); ^{19}F NMR (D_2O): δ −120.43 (dd, $^2J_{F\text{-}P}$ = 87.3 Hz, $^2J_{F\text{-}P}$ = 82.0 Hz, CF_2); MS *m/z* 502.8 $(M\text{-}H)^-$; HPLC analysis, 99.9% purity.

2′,3′-Dideoxythymidine (ddT) 5′-α,γ-*P*-Dithio-β,γ-(difluoromethylene) triphosphate (6h). Following a similar procedure as described for **6a**, the reaction starting with ddT (42.0 mg, 0.186 mmol) was modified as follows: after addition of sulfur (11.9 mg, 0.372 mmol) at 0°C, the mixture was stirred at room temperature for 1 h, cooled with ice, treated with lithium sulfide (42.7 mg, 0.93 mmol), and stirred for 1 h at room temperature. Water (5 mL) was added and the resulting mixture was extracted with diethyl ether (3 × 10 mL). The aqueous layer was purified by HPLC purification to give 10.71 mg, 10.8% of **6h** (two diastereomers) as a triethylammonium salt; ^{1}H NMR (D_2O): δ 1.88 (s, 5-CH_3, 3H), 1.99–2.45 (m, H-2′, H-3′, 4H), 4.02–4.40 (m, H-4′, H-5′, 3H), 6.06 (m, H-1′, 1H), 7.70 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −1.51 (m, P_β), 45.04 (d, J = 37.6 Hz, P_α) and 45.22 (d, J = 37.7 Hz, P_α), 47.46 (dt, $^2J_{P\text{-}P}$ = 53.7 Hz, $^2J_{P\text{-}F}$ = 77.0 Hz, P_γ); ^{19}F NMR (D_2O): δ −116.24

(dd, $^2J_{F\text{-}P}$ = 86.3 Hz, $^2J_{F\text{-}P}$ = 79.0 Hz, CF_2); MS *m/z* 531.3 $(M\text{-}H)^-$; HPLC analysis, 99.9% purity.

2′,3′-Dideoxy-3′-aminothymidine (3′-NH_2-ddT) 5′-α-*P*-Thio-β,γ-(difluoromethylene)triphosphate (10). Following a similar procedure as described for **6a**, the reaction starting with AZT (192 mg, 0.718 mmol) was modified as follows: after addition of sulfur (46.0 mg, 1.4 mmol) at 0°C, the mixture was stirred at room temperature for 1 h, cooled with ice, then treated with lithium sulfide (165 mg, 3.59 mmol) and stirred for 1 h at room temperature. Water (5 mL) was added and the resulting mixture was extracted with diethyl ether (3 × 10 mL). The aqueous layer was purified by HPLC purification to give 13.05 mg, 3.4% of **10** (two diastereoisomers) as a triethylammonium salt; ^{1}H NMR (D_2O): δ 2.03 (s, 5-CH_3, 3H), 2.54–2.61 (m, H-2′, 2H), 4.12–4.36 (m, H-3′, H-4′, H-5′, 4H), 6.21–6.27 (m, H-1′, 1H), 7.62 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −2.17 (m, P_β), 4.84 (dt, $^2J_{P\text{-}P}$ = 57.0, $^2J_{P\text{-}F}$ = 75.1, P_γ), 44.45 (d, $^2J_{P\text{-}P}$ = 37.4 Hz, P_α) and 44.98 (d, $^2J_{P\text{-}P}$ = 36.8 Hz, P_α); ^{19}F NMR (D_2O): δ −119.00 (dd, $^2J_{F\text{-}P}$ = 87.6 Hz, $^2J_{F\text{-}P}$ = 75.2 Hz, CF_2); MS *m/z* 531.0 $(M\text{-}H)^-$. HPLC analysis: 99.9% purity.

2′,3′-Dideoxy-3′-azidothymidine (AZT) 5′-α-*P*-Seleno-β,γ-(difluoromethylene)triphosphate (11). Following a similar procedure as described for **6a**, the reaction starting with AZT (100 mg, 0.374 mmol) was modified by replacing sulfur with potassium selenocyanate[18] (269 mg, 1.87 mmol) and yielded, after HPLC purification, 5.51 mg of isomer **11-I** and 5.34 mg of isomer **11-II** as triethylammonium salts, total yield = 4.8%. **11-I**: ^{1}H NMR (D_2O): δ 1.90 (s, 5-CH_3, 3H), 2.43 (m, H-2′, 2H), 4.22 (m, H-4′, H-5′, 3H), 4.55 (m, H-3′, 1H), 6.22 (t, *J* = 7.0 Hz, H-1′, 1H), 7.73 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −3.42 (m, P_β), 4.66 (dt, $^2J_{P\text{-}P}$ = 59.6 Hz, $^2J_{P\text{-}F}$ = 76.3 Hz, P_γ), 33.92 (d, $^2J_{P\text{-}P}$ = 42.2 Hz, P_α); ^{19}F NMR (D_2O): δ −119.10 (apparent t, $^2J_{F\text{-}P}$ = 80.5 Hz, CF_2); MS *m/z* 604.2 $(M\text{-}H)^-$; HPLC analysis, 92.0% purity. **11-II**: ^{1}H NMR (D_2O): δ 1.90 (s, 5-CH_3, 3H), 2.43 (m, H-2′, 2H), 4.23 (m, H-4′, H-5′, 3H), 4.57 (m, H-3′, 1H), 6.22 (t, *J* = 6.9 Hz, H-1′, 1H), 7.73 (s, H-6, 1H); ^{31}P NMR (D_2O): δ −4.30 (m, P_β), 4.38 (dt, $^2J_{P\text{-}P}$ = 60.2 Hz, $^2J_{P\text{-}F}$ = 79.3 Hz, P_γ), 33.86 (d, $^2J_{P\text{-}P}$ = 42.0 Hz, P_α); ^{19}F NMR (D_2O): δ −120.00 (apparent t, $^2J_{F\text{-}P}$ = 83.5 Hz, CF_2); MS *m/z* 604.2 $(M\text{-}H)^-$; HPLC analysis, 97.0% purity.

CONCLUSION

The synthesis of a number of ddN 5′-β,γCF_2TP bearing an α-*P*-seleno or an α-*P*-thio moiety were prepared using one of two general methods. Of all the ddN 5′-αSCF_2TPs evaluated against HIV-1 RT, one compound, **6c-I**, exhibited reasonable activity (0.73 μM), which was approximately 20-fold

lower than the corresponding triphosphate. Previously, we reported that when the 5′-αBCF$_2$TP P3M was attached to a number of antiviral nucleosides, all the resulting ddN 5′-αBCF$_2$TPs (diastereomers I or diastereomers I/II) demonstrated essentially the same level of inhibition of HIV-1 RT as the corresponding ddNTPs.[2] From the results reported here, the αSCF$_2$TP moiety does not appear to be a generic P3M concerning HIV-1 RT inhibition.

AZT 5′-αSeCF$_2$TP was separated into two diastereomers, **11-I** and **11-II**. Compound **11-I** exhibited potent inhibition of HIV-1 RT with a K_i of 64.0 nM and **11-II** was weakly active with a K_i of 1.37 μM. Given the potency compound **11-I**, it will be interesting to investigate whether the 5′-αSeCF$_2$TPs of other NRTIs possess similar HIV-1 RT activities. Furthermore, recently we have reported that the R_p diastereomer of AZT 5′-αBCF$_2$TP, **1a-I**, has potent activity against mutant HIV-1 RT and is highly selective for the intended polymerase (HIV-1 RT) over host polymerases α, β, and γ.[22] This is very significant given the implication that many of the toxic side effects of current NRTI drugs are related to host polymerase toxicity.[23,24] In an extension to the work described here, it will be interesting to investigate if ddN-P3Ms with an α-*P*-seleno modification may offer similar advantages to ddN-P3Ms with an α-*P*-borano modification with respect to polymerase selectivity and activity against mutant HIV-1 RT.

REFERENCES

1. Wang, G.; Boyle, N.; Chen, F.; Rajappan, V.; Fagan, P.; Brooks, J.L.; Hurd, T.; Leeds, J.M.; Bruice, T.W.; Rajwanshi, V.K.; Jin, Y.; Prhavc, M.; Cook, P.D. Synthesis of novel AZT 5′-triphosphate mimics (P3Ms) and their inhibitory effects on HIV-1 reverse transcriptase. J. Med. Chem. **2004**, 47, 6902–6913.
2. Boyle, N.A.; Rajwanshi, V.; Prhavc, M.; Wang, G.; Fagan, P.; Chen, F.; Ewing, G.; Brooks, J.L.; Hurd, T.; Bruice, T.W.; Cook P.D. Synthesis of dideoxynucleoside 5′-α-*P*-borano-β,γ-(difluoromethylene) triphosphates and their inhibition of wild type and mutant HIV reverse transcriptases. J. Med. Chem. **2005**, 48(7), 2695–2700.
3. Herdewijn, P.; Balzarini, J.; De Clercq, E. 2′,3′-Dideoxynucleoside analogues as antiviral agents. In *Advances in Antiviral Drug Design*; De Clerck, E., Ed.; JAI Press, Inc.: Greenwich, 1993; Vol. 1.
4. St. Claire, M.H.; Richards, C.A.; Spector, T.; Weinhold, K.J.; Miller W.H.; Langlois, 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.
5. 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 immunodefficiency 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.
6. 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.
7. De Clercq, E. Antiviral drugs: Current state of the art. J. Clin. Virol. **2001**, 22, 73–89.
8. Bayes, M.; Rabasseda, X.; Prous, J.R. Gateways to clinical trials. Methods Find. Exp. Clin. Pharmacol. **2003**, 25, 53–76.

9. The data reported in Wang et al.[1] corresponds to a 1:1 α-R_P/α-S_P diastereomeric mixture.
10. For a review see: Marshall, W.S.; Caruthers, M.H. Phosphorodithioate DNA as a potential therapeutic drug. Science **1993**, 259, 1564–1570.
11. Schneider, B.; Meyer, P.; Sarfati, S.; Mulard, L.; Guerreiro, C.; Boretto, J.; Janin, J.; Veron, M.; Deville-Bonne, D.; Canard, B. Activation of anti-reverse transcriptase nucleotide analogs by nucleoside diphosphate kinase: Improvement by α-boranophosphate substitution. Nucleoside, Nucleotide and Nucl. Acids **2001**, 20, 297–306.
12. Dobrikov, M.I.; Grady, K.M.; Shaw, B.R. Introduction of the α-P-borano-group into deoxynucleoside triphosphates increases their selectivity to HIV-1 reverse transcriptase relative to DNA polymerases. Nucleosides, Nucleotides and Nucl. Acids **2003**, 22, 275–282.
13. Ludwig, J.; Eckstein, F. Rapid and efficient synthesis of nucleoside 5′-*O*-(1-thiotriphosphates), 5′-triphosphates and 2′,3′-cyclophosphorothioates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one. J. Org. Chem. **1989**, 54, 631–635.
14. Nahum, V.; Zundorf, G.; Levesque, S.A.; Beaudoin, A.R.; Reiser, G.; Fischer, B. Adenosine 5′-*O*-(1-boranotriphosphate) derivative as novel $P2Y_1$ receptor agonists. J. Med. Chem. **2002**, 45, 5384–5396.
15. Wyrzykiewicz, T.K.; Cook, P.D. Process for the preparation of alpha modified nucleoside triphosphates and compounds therefrom. ISIS Pharmaceuticals, Inc. PCT WO 03/008432 A1, Jan. 30, 2003.
16. For an example of the use of this reagent for the preparation of dithiotriphosphates see: Ludwig, J.; Eckstein, F. Synthesis of nucleoside 5′-*O*-(1,3-dithiotriphosphates) and 5′-*O*-(1,1-dithiotriphosphates). J. Org. Chem. **1991**, 56, 1777–1783.
17. Kazemi, F.; Kiasat, A.; Sayyahi, S. Chemoselective reduction of azides with sodium sulfide hydrate under solvent free conditions. Phosphorus, Sulfur Silicon Relat. Elem. **2004**, 179, 1813–1817.
18. For an example of the use of this reagent see: Mori, K.; Boiziau, C.; Cazenave, C.; Matsukura, M.; Subasinghe, C.; Cohen, J.S.; Broder, S.; Toulme, J.J.; Stein, C.A. Phosphoroselenoate oligodeoxynucleotides: Synthesis, physiochemical characterization, anti-sense inhibitory properties and anti-HIV activity. Nucleic Acids Res. **1989**, 17, 8207–8219.
19. Meyer, P.; Scheider, B.; Sarfati, S.; Deville-Bonne, D.; Guerreiro, C.; Boretto, J.; Janin, J.; Veron, M.; Canard, B. Structural basis for activation of α-boranophosphate nucleotide analogues targeting drug-resistant reverse transcriptase. EMBO **2000**, 19, 3520–3529.
20. Carrasco, N.; Huang, Z. Enzymatic synthesis of phosphoroselenoate DNA using thymidine 5′-(α-P-seleno)triphosphate and DNA polymerase for x-ray crystallography via MAD. J. Am. Chem. Soc. **2004**, 126, 448–449.
21. Kedar, P.S.; Abbotts, J.; Kovács, T.; Lesiak, K.; Torrence, P.; Wilson, S.H. Mechanism of HIV reverse transcriptase: Enzyme–primer interaction as revealed through studies of a dNTP analogue, 3′-azido-dTTP. Biochemistry **1990**, 29, 3603–3611.
22. Boyle, N.A.; Cook, P.D.; Wang, G.; Bruice, T.W.; Leeds, J.; Brooks, J.; Fagen, P.; Rajwanshi, V.; Prhavc, M.; Hurd, T.; Chen, F. Nucleoside triphosphate mimics (P3Ms) and their prodrugs (P3M-PDs): A new class of agents with potent activity against resistant HIV-1. 17th International Conference on Antiviral Research (ICAR), May 2–5, 2004; International Society of Antiviral Research: Tuscon, AZ, 2004.
23. Lewis, W.; Day, B.J.; Copeland, W.C. Mitochondrial toxicity of NRTI antiviral drugs: An integrated cellular perspective. Nature **2003**, 2, 812–822.
24. Lee, H.; Hanes, J.; Johnson, K.A. Toxicity of nucleoside analogues used to treat AIDS and the selectivity of the mitochondrial DNA polymerase. Biochemistry **2003**, 42, 14711–14719.
25. Seville, M.; West, A.B.; Cull, M.G.; McHenry, C.S. Fluorometric assay for DNA polymerases and reverse transcriptase. BioTechniques **1996**, 21, 664–672.