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Design, synthesis, and anti-HIV activity of 4′-modified carbocyclic nucleoside phosphonate reverse transcriptase inhibitors

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ABSTRACT

A diphosphate of a novel cyclopentyl based nucleoside phosphonate with potent inhibition of HIV reverse transcriptase (RT) (**20**, IC_{50} = 0.13 μM) has been discovered. In cell culture the parent phosphonate diacid **9** demonstrated antiviral activity EC_{50} = 16 μM, within two-fold of GS-9148, a prodrug of which is currently under clinical investigation, and within 5-fold of tenofovir (PMPA). In vitro cellular metabolism studies using **9** confirmed that the active diphosphate metabolite is produced albeit at a lower efficiency relative to GS-9148.

1. Introduction

A hallmark of the current standard of care for anti-HIV therapy is the combination of three or more drugs from multiple classes in the anti-retroviral arsenal. Most commonly, treatment regimens are built on a backbone of two drugs from the class of nucleoside (or nucleotide) reverse transcriptase inhibitors (N(t)RTIs), with additional agents added from the non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) classes.[1] As with all anti-infective therapies, resistance to clinical agents inevitably arises. One strategy for overcoming resistance is to undertake medicinal optimization of existing inhibitor classes, for example, N(t)RTIs. It was the goal of our research program to identify a novel RT inhibitor with potent activity toward the majority of N(t)RTI resistant mutations.

PMPA, **1**, is a nucleoside phosphonate, in which the phosphonate group is designed to mimic the first metabolite (monophosphate) of conventional nucleosides, thereby bypassing the often rate-limiting first phosphorylation step in activation to the triphosphate (Fig. 1). The active metabolite of **1**, diphosphophosphonate **2**, is retained inside cells for prolonged periods of time (median half life 150 h in peripheral blood mononuclear cells from patients) and consequently **1** is well suited for once-daily dosing regimens.[2] Furthermore, tenofovir phosphonamidate prodrugs have been demonstrated to effectively mask the charged nature of the phosphonate diacid and efficiently and selectively deliver high levels of active metabolites to lymphoid tissues.[3] Based on these observations, we have recently reported on a new nucleoside phosphonate RT inhibitor, GS-9148, **3**, that has a favorable resistance profile.[4] A phosphonoamidate prodrug of GS-9148, GS-9131, is able to efficiently load lymphoid cells after oral administration and is currently under clinical investigation.[5] GS-9148 retained the favorable resistance profile and potency that was demonstrated by the non-fluorinated 2′,3′-didehydro-2′,3′-dideoxy (d4) analog, **4** (Table 1). Encouraged by these results we have continued our efforts in the design and synthesis of new d4 nucleoside phosphonates. By modifying the ribose core and appending additional groups that might provide novel and favorable contacts with RT, we hoped to enhance the RT potency and antiviral activity while preserving the promising pharmacological and resistance profile of **4**.

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Table 1
Inhibition of reverse transcriptase, HIV antiviral activity, cytotoxicty, and resistance profile

Compd. No.	Code	RT HIV[a] IC_{50} (μM)	WT HIV[b] EC_{50} (μM)	MT-2[c] CC_{50} (μM)	K65R[d] fold change	M184V[d] fold change
	AZT[e]	0.05 (0.03)	0.16 (0.12)	>200	1.2 (0.7)	0.7 (0.2)
1	PMPA	0.38 (0.20)	3.6 (1.5)	>1000	4.3 (1.5)	0.7 (0.2)
3	GS-9148	1.9 (0.8)	12 (3.4)	>1000	1.1 (0.2)	0.8 (0.3)
4	d4AP	0.60 (0.16)	2.1 (1.0)	>1000	2.9 (1.0)	2.9 (1.1)
5	ABC	0.13 (0.07)	0.32 (0.15)	190 (45)	3.8 (1.8)	7.1 (4.4)
6	4′-Ethynyl-d4T	—	0.81 (0.40)	—	0.40 (0.2)	4.8 (0.1)
7	cpAP	—	52 (13.4)	—	—	—
8	4′-Me-cpAP	—	244	—	—	—
9	4′-Ethynyl-cpAP	0.13 (0.10)	16 (9.6)	1450	2.2 (0.5)	>30
21	4′-Vinyl-cpAP	0.62 (0.30)	—	—	—	—

[a] Diphosphophosphonates or nucleoside triphosphates were used in this assay.
[b] Antiviral activity in MT-2 cells using HIV-IIIa. Values are results of at least two experiments; standard deviation is given in parentheses.
[c] Cytotoxicity measured in MT-2 cells following a 5-day incubation with tested compounds.
[d] Fold resistance was determined in MT-2 cell line infected in parallel with a given mutant and a wild-type virus; standard deviation is given in parantheses.
[e] 3′-Deoxy-3-azidothymidine (zidovudine/Retrovir®).

2. Strategy

To achieve our goal of designing a new d4 nucleoside phosphonate, we combined two approaches that had shown promise in the discovery of nucleoside based RT inhibitors; (i) utilization of the carbocyclic d4 ribose replacement and (ii) 4′-substitutions onto the ribose core. During the discovery of GS-9148 we compared the RT inhibition of d4 ribose nucleosides with the corresponding d4 ribose nucleoside phosphonates and found that the IC_{50} differed by <10-fold. Thus, the d4 *ribose* phosphonates are preferred bioisosteres of the d4 *ribose* nucleoside monophosphates, in contrast to 2′,3′-dideoxy analogs, which tended to be poorer bioisosteres.[6] Triphosphate metabolites of carbocyclic d4 nucleosides also have good RT potency (e.g., triphosphate of abacavir, **5**, Fig. 1, Table 1) so it was expected that the corresponding carbocyclic d4 phosphonates would be potent RT inhibitors, at least within 10-fold of the nucleoside. Indeed, several reports on carbocyclic d4 nucleoside phosphonates support potent RT inhibition but only weak antiviral activity (e.g., **7**).[7] The introduction of 4-substitutions, for example 4-ethynyl, onto 2-deoxynucleosides has provided potent anti-HIV compounds often with hypersensitivity demonstrated by the K65R RT mutant virus.[8] More recently, the 4′-ethynyl-substituted d4T analog **6** was reported to have >10-fold improved antiviral activity relative to d4T, and in our assay, an improved K65R resistance profile (Table 1).[9] Our modeling studies (Fig. 2, Panel A) demonstrated the presence of a narrow, relatively hydrophobic 4′-pocket that can accommodate these substitutions, contributing to the observed enhancement in potency. Thus, we proposed that (i) the active metabolite of carbocyclic d4 nucleoside phosphonates would retain potent RT inhibition and (ii) 4′-substitutions should be able to take advantage of the narrow binding pocket, significantly improving both RT potency and antiviral activity and (iii) provide an optimal resistance profile toward K65R compared to PMPA (Table 1). Modeling in the RT active site suggested that the 4′-ethynyl derivative **9** would have the optimal fit (Fig. 2, panel D), but the 4′-methyl **8** would likely be poorer due to its larger van der Waals radius. Although this strategy is predicted to result in potent RT inhibitors, the permeability and metabolism to the respective diphosphophosphonates is essential for antiviral activity in cell culture. Earlier results from our nucleoside phosphonate studies indicated that phosphonamidate prodrugs can lead to significant improvements in antiviral activity.[4a] Therefore, we reasoned that the weak antiviral activity noted for analog **7**[7] was largely due to poor permeability and that a prodrug strategy, similar to GS-9131, could be applied to these analogs to improve antiviral activity. Here we report our initial studies on the synthesis and anti-HIV activity of several new 4′-modified carbocyclic nucleoside phosphonate analogs.

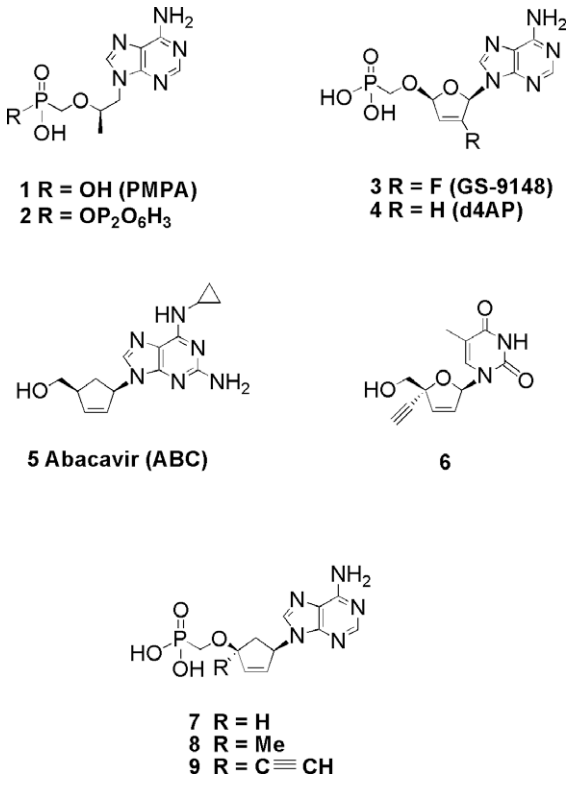


Figure 1. Previously reported nucleoside and nucleoside phosphonate RT inhibitors.

3. Synthesis

The synthetic scheme (Scheme 1) to prepare analogs **7–9** involved several departures from previously reported methods for the synthesis of **7**.[10] In particular, intermediate **13** served as an electrophile to which nucleophilic addition of the appropriate 4′-alkyl groups would ultimately yield analogs **8** and **9**. Commercially available desymmetrized cyclopentenediol **10** was reacted with 6-chloropurine under Mitsunobu conditions to provide **11** in 52% yield. Intermediate **11** was deacetylated with TEA/MeOH/H_2O (1:8:1) and then the resultant free alcohol **12** oxidized with Dess–Martin periodinane to enone **13** in 90% yield. Enone **13**

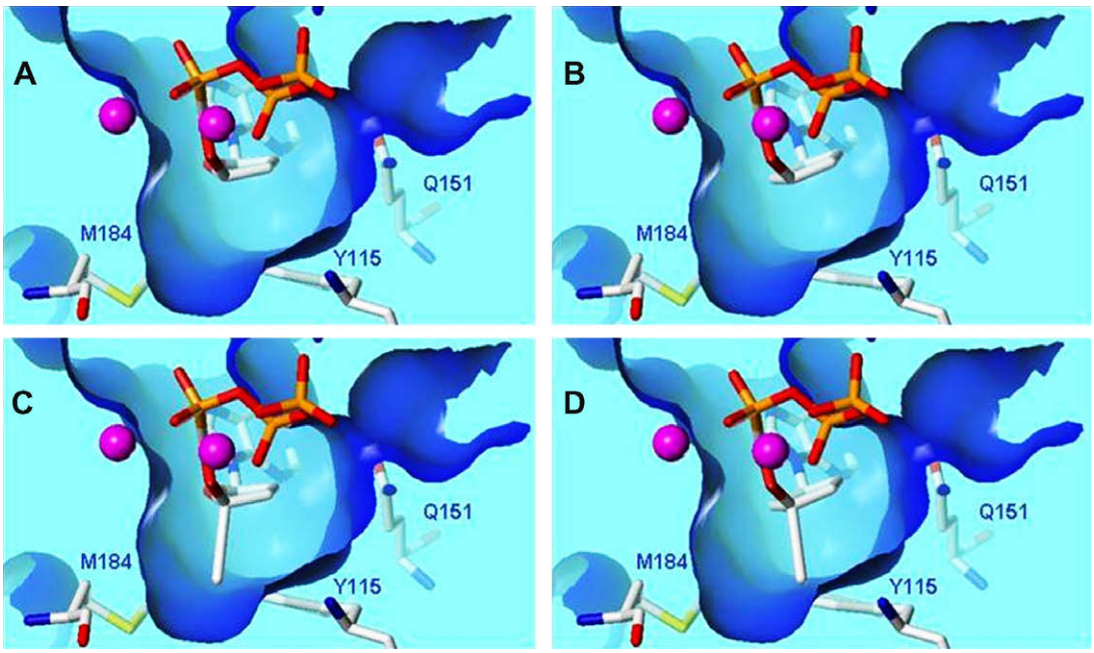
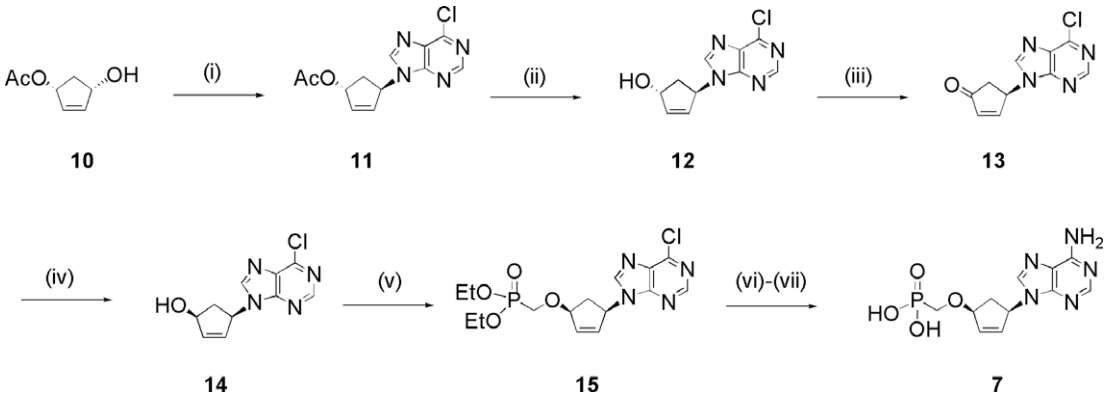


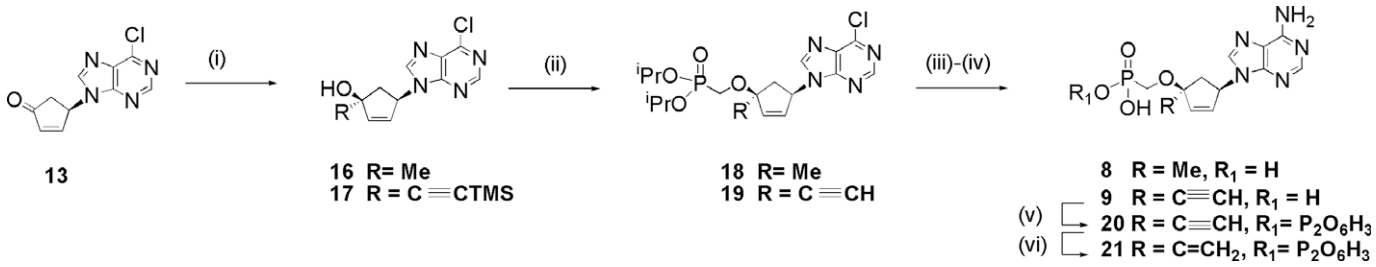
Figure 2. Model of HIV RT active site. Side view of selected nucleoside and nucleoside phosphonate inhibitors bound to the active site and location of the cavity below 4′ that is formed by residues M184 and Y115. Panel A. d4AP **4** diphosphate positioned above the 4′-cavity and coordinating to the two Mg^{2+} ions (magenta); Panel B. cd4AP **7** diphosphate. Note the tilt of the carbocyclic ring is more pronounced compared to d4AP; Panel C. 4′-Ethynyl d4AP diphosphate. The ethynyl group projects down into the narrow cavity. Panel D. 4′-Ethynyl cd4AP (**9**) diphosphate **20**. Note the projection of the ethynyl group is positioned differently to that of 4′-ethynyl d4AP (Panel C) and is situated closer to residue Y115.



Scheme 1. Reagents and conditions: (i) PPh_3, DIAD, 6-chloropurine, dioxane; (ii) Et_3N/MeOH/H_2O 1:8:1; (iii) Dess–Martin reagent; (iv) $CeCl_3$, H_2O, MeOH, $NaBH_4$; (v) trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester, LiO–*t*-Bu, THF, 50 °C; (vi) NH_3, MeOH, 50 °C; (vii) TMSBr, 2,6-lutidine, DMF, 50 °C, then MeOH and NH_4OH.

could also be prepared using a π-allyl palladium-based strategy, which introduces 6-chloropurine onto the same desymmetrized cyclopentenediol.[10] The π-allyl palladium method was equivalent in efficiency and operational difficulty and so both methods were used interchangeably for the generation of **13**. For the synthesis of **7**, the desired 4′-H group was introduced by Luche reduction of **13**, to provide **14** in 64% yield.[11] Compound **14** formed in a 4:1 ratio (based on recovered material) with **12**, which was recycled through the same synthetic steps. The diastereoselective outcome of this reduction provided good evidence that similar or better diastereoselectivity could be obtained with the addition of alternative nucleophiles in the route to **8** and **9**. The alcohol of **14** was alkylated in 79% yield with trifluoromethanesulfonic acid diethoxy phosphorylmethyl ester in the presence of LiO*t*Bu in THF to provide **15**. The chloropurine was converted into adenine by treatment with NH_3 in MeOH under pressure, and finally the phosphonate esters cleaved with TMSBr, to provide compound **7** (isolated as the ammonium salt), in 71% yield over 2 steps.

The synthesis of the 4′-alkyl substituted derivatives **8** and **9** proceeded smoothly (Scheme 2). Direct treatment of enone **13** with simple organometallic reagents resulted in some 6-chloropurine byproduct, presumably, arising via beta elimination of the nucleobase. Direct addition of organolithium reagents has been noted for similar carbocyclic enone system when poorer leaving groups relative to chloropurine are in the beta position.[11] To avoid the byproduct formation, the organolithium reagents were first transmetallated onto cerium in order to reduce their basicity. The 1,2-addition of the cerium reagents onto cyclopentenone **13** provided the intermediates **16** and **17** with virtually complete diastereoselectivity and in moderate to excellent (49–91%) yields. Reactions that were performed at higher temperatures than −78 °C, resulted in eroded stereoselectivity. Analogs **8** and **9** were then prepared following the same steps as described for analog **7**. The diphosphophosphonate **20** was prepared from **9** according to standard methods.[12] Treatment of 4′-ethynyl diphosphate **20** under Lindlar reduction conditions also afforded the 4′-vinyl diphosphophospho-



Scheme 2. Reagents and conditions: (i) 'RCeCl$_2$', THF, −78 °C; (ii) diisopropyl bromomethylphosphonate ester, LiO–*t*-Bu, THF, 50 °C; (iii) NH_3, MeOH 50 °C; (iv) TMSBr, 2,6-lutidine, DMF, 50 °C, then MeOH and NH_4OH; (v) literature conditions;[12] (vi) Lindlar catalyst, quinoline, H_2, H_2O.

nate **21**. The parent diacid of **21** could not be isolated from reduction of **9** due to poor stability of the final product.

4. Results and discussion

The antiviral activity of the carbocyclic nucleoside phosphonate analogs **7**–**9** is reported in Table 1. The 4′-H analog **7** was weakly active, displaying more than 20-fold reduced activity compared to the equivalent ribose analog d4AP **4**. Clearly replacement of the ribose oxygen with methylene affords weaker antiviral activity in support of the earlier reports.[7] The 4′-methyl analog **8** exhibited decreased antiviral activity with EC_{50} > 200 μM as expected from our model, but the 4′-ethynyl analog **9** had a 3-fold improved antiviral activity relative to 4′-H analog **7**. Furthermore, **9** also exhibited low cytotoxicity in MT-2 cells resulting in a selectivity of almost 100-fold. To confirm that the improved activity is at least in part due to potent RT inhibition as postulated, the diphosphophosphonate **20** was prepared and found to inhibit RT with an IC_{50} = 0.13 μM. This is comparable to the RT potency displayed by abacavir triphosphate and more potent than the diphosphates of the earlier phosphonate inhibitors **1**, **3** and **4**, respectively. The 4′-vinyl analog **21** also displayed potent RT inhibition but was weaker than the diphosphophosphonate **20**. Thus the proposed 4′-pocket in the active site of RT is sensitive to changes in the 4′-substituent, especially when this involves increasing the van der Waals radius or possibly changes in the projection angle of the 4′-substituent into the pocket.

Despite the potent RT inhibition displayed by the diphosphate **20** of 4′-ethynyl analog, **9**, the antiviral activity was still weaker than the other adenine phosphonates (**1**, **3** and **4**). This suggests that **9** may be poorly permeable, a property that can be repaired with the phosphonamidate prodrugs, or deficient in its metabolism to the diphosphophosphonate. Therefore, the metabolism of **9** in relevant T-cells (CEM-CCRF) was evaluated and compared to PMPA **1** (Fig. 3). The intracellular levels of **1** and its metabolite **2** were comparable to what was observed previously in the same cell line (Fig. 3A).[13] While slightly higher levels of the parent nucleotide **9** accumulated intracellularly relative to **1**, levels of **20** (diphosphate metabolite) were approximately 10-fold weaker than those of **2** (Fig. 3B). Therefore, the carbocyclic scaffold **9** is less efficiently metabolized and this explains the weaker antiviral activity in cell culture.

The next phase of evaluation included resistance profiling of **9** toward HIV strains with K65R and M184V mutations in RT. K65R mutant virus showed only 2.2-fold reduced susceptibility relative to WT, a profile that is superior to PMPA **1** and d4AP **4**. Given the weak activity of the 4′-H analog **7**, it was not possible to establish whether the additional 4′-ethynyl substituent had afforded the improved resistance profile as it did for the 4′-ethynyl nucleoside **6**. The weakest aspect of the resistance profile was a substantial decrease (>30-fold) in the antiviral activity against the M184V RT mutant virus compared to **6** (4.8-fold). The resistance profile of

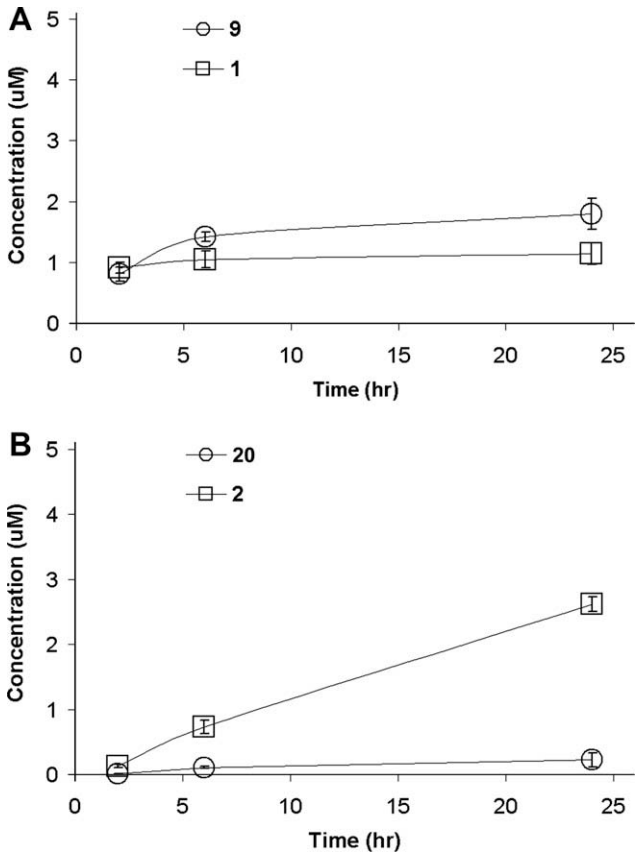


Figure 3. Intracellular accumulation of respective parent nucleoside phosphonate (Panel A) and diphosphophosphonate metabolite (Panel B) during 10 μM incubations of **9** (circles) or **1** (squares) with CEM–CCRF cells.

abacavir **5** toward M184V, shown in Table 1, indicates a 7.1-fold reduced susceptibility, likely due to the carbocyclic 4′-oxygen-to-4′-methylene replacement, since the M184V virus did not show significant resistance toward d4 ribose analogs (e.g., **3** and **4**). Therefore the >30-fold reduced susceptibility toward M184V for **9** suggests that an unfavorable synergy exists when the 4′-group and carbocyclic modifications are combined in the same molecule.

Modeling of **20** (diphosphate of **9**), and the corresponding analog with the 4′-oxygen group (i.e., 4′-ethynyl d4AP) shows a slight difference in the positions of the 4′-ethynyl group within the 4′-cavity of HIV polymerase (Fig. 2, Panel C and D). The positional difference of the 4′-ethynyl group is due to the differences in the tilt of planes for the different ring systems. In compound **20**, the 4′-methylene group is in close contact with the C8 position of the purine base and the C1 anomeric center no longer exists. The combination of these structural differences leads to the predicted difference in the ring tilt of the cyclopentene ring compared to the d4 ring.

The lateral surface of the wild-type cavity, surrounding the 4′-substituent, is mainly due to residues M184 and Y115. Our modeling shows that the 4′-ethynyl group for the ribose d4 analogs lies reasonably centered between the M184 and Y115. In contrast, the wild-type model of carbocyclic analog **20** shows that the 4′-ethynyl group lies adjacent to the surface of Y115. In the corresponding computer models of the M184V mutant, the branched valine sidechain further increases the tilt of the carbocyclic scaffold towards Y115 and thus, causes unfavorable steric contacts between the 4′-ethynyl group and Y115.

Overall, the promising K65R profile of the 4′-modified carbocyclic analogs is tempered by the poor activity against the M184V mutant virus, which is a common mutation in treatment-experienced patients.

5. Conclusions

A novel carbocyclic nucleoside phosphonate **9** bearing a 4′-ethynyl group has been synthesized and found to demonstrate in vitro anti-HIV activity only 2–5-fold weaker than nucleoside phosphonates that have been explored in the clinic. The diphosphate of **9** is a potent inhibitor of HIV RT and can be produced in T-cells albeit with reduced efficiency compared to PMPA. A favorable resistance profile toward K65R was achieved but was offset by a decreased susceptibility by the prevalent M184V mutant. Further development of **9** towards optimal phosphonamidate prodrugs will likely allow efficient delivery the phosphonate to the lymphatic system and provide a novel nucleotide RT inhibitor for the treatment of HIV.

6. Experimental

6.1. General

All reactions were performed with reagent-grade materials under an atmosphere of nitrogen. Solvents were reagent-grade or better. Silica gel chromatography was performed using EMD Chemicals Silica Gel 60. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian 300- or 500-MHz NMR. NMR shifts are reported relative to solvent residual. Low-resolution LC mass spectra (LCMS) were recorded on a Thermo Finnigan LCQ Advantage, electrospray ionization (ESI) mass spectrometers. The LCMS was equipped with a Phenomenex Synergi 4 μm Polar-RP C18 column (30 × 4.6 mm), eluting with a gradient of 5–100% solvent B in Solvent A over 2.5 min at a flow rate of 2 mL/min (Solvent A = 0.1% AcOH in water, Solvent B = 0.1% AcOH in acetonitrile). HPLC analysis was carried out on Agilent 1100 series HPLC equipped with a phenomex luna C18 5 μm column (4.6 mm × 250 mm) eluting with a 2–70% gradient of solvent B in solvent A over 30 min at 1 mL/min flow rate (Solvent A = 0.1% TFA in water, Solvent B = 0.1% TFA in acetonitrile). High Resolution Mass Spectroscopy (HRMS) was determined by UC Berkeley Mass Spectrometry Facility using ESI ionization. Ion exchange HPLC (HPLCie) analysis for diphosphophosphonates was performed on a Agilent 1100 series analytical HPLC, equipped with a DIONEX DNAPac PA-100 column (250 × 4 mm) eluting with a gradient of 0–50% solvent B in Solvent A over 12 min followed by 100% B for 5 min (Solvent A = water, Solvent B = 0.5 mM triethylammonium bicarbonate buffer pH 8.5). Preparative reverse-phase chromatography of phosphonic diacids (e.g., **9**) was performed on a Gilson Liquid Handler, C-18 column, eluant neat H_2O for 3 min, then ramp to 40% MeCN over 15 min. Preparative ion-exchange was carried out on a Varian Star HPLC system equipped with an Amersham Biosciences, HiTrap DEAE FF, 5 mL disposable column. Elution was a gradient of 0–80% solvent B over 18 min at a flow rate of 5.0 mL/min (Solvent A = water, Solvent B = 0.5 mM triethylammonium bicarbonate buffer pH 8.5). Melting points are uncorrected. Optical rotation $[\alpha]_D$ was measured on a Autopol V analytical instrument at 25 °C in methanol.

6.2. Chemistry

6.2.1. (1*R*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-enyl acetate (11)

(*1S*,*4R*)-*cis*-4-Acetoxy-2-cyclopenten-1-ol **10** (0.308 g, 2.17 mmol) was dissolved in dioxane (22 mL). To this solution was added 6-chloropurine (0.671 g, 4.34 mmol), forming a yellow suspension. Triphenylphosphine (1.14 g, 4.34 mmol) was added and the mixture stirred under N_2 at room temperature. Diisopropylazo dicarboxylate (DIAD, 0.877 g, 0.840 mL, 4.34 mmol) was added dropwise by syringe over 5 min. (*Note*: on larger scale, significant exotherm was observed, and the DIAD needed to be added over longer periods of time with initial cooling to 0 °C). The orange color of the DIAD was slowly lost and the suspension became green. The mixture was stirred for 12 h, with protection from light, after which time it was filtered through a plug of Celite, which was washed generously with dioxane. The liquids were concentrated in vacuo and the resulting syrup was chromatographed on silica gel, eluting with 25–75% EtOAc/hexanes, to provide **11** in 52% yield (0.312 g, 1.12 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 2.06 (s, 3H), 2.56–2.61 (m, 2H), 5.97–6.03 (m, 1H), 6.04–6.07 (m, 1H), 6.25–6.34 (m, 1H), 6.33–6.36 (m, 1H), 8.51 (s, 1H), 8.72 (s, 1H). ^{13}C NMR (75.3 MHz, $CDCl_3$) δ 21.0, 38.5, 59.6, 78.2, 131.9, 133.7, 136.8, 142.9, 151.2, 151.4, 151.9, 170.7. A small amount of material was recrystallized to high purity from hexanes/EtOAc; prisms (mp = 109.0–109.5 °C). Anal. Calcd for $C_{12}H_{11}ClN_4O_2$: C, 51.72; H, 3.98; N, 20.10. Found: C, 51.59; H, 4.07; N, 19.84.

6.2.2. (1*R*, 4*R*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-enol (12)

Compound **11** (0.168 g, 0.60 mmol) was dissolved in Et_3N/MeOH/H_2O (v/v/v) 1:8:1 (20 mL total) and stirred for 18 h. The mixture was concentrated in vacuo and purified by silica gel chromatography, eluting with 20:1 CH_2Cl_2/CH_3OH, to afford **12** in 66% yield (0.094 g, 0.40 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 2.36–2.52 (m, 2H), 5.19–5.23 (m, 1H), 5.95–6.04 (m, 1H), 6.12 (ddd, 1H, *J* = 5.4, 2.1, 0.9 Hz), 6.32 (d app t, 1H, *J* = 5.7, 2.1 Hz), 8.45 (s, 1H), 8.64 (s, 1H). ^{13}C NMR (75.3 MHz, CD_3OD) δ 41.9, 61.9, 76.7, 131.8, 132.6, 141.9, 146.5, 151.1, 152.7, 152.9. A small amount of material was recrystallized to high purity from hexanes/EtOAc; white needles (mp = 122.0–122.5 °C,[14] 123–125 °C). Anal. Calcd for $C_{10}H_9ClN_4O$: C, 50.75; H, 3.83; N, 23.68. Found: C, 50.98; H, 3.91; N, 23.71.

6.2.3. (*R*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-enone (13)

Compound **12** (1.26 g, 5.33 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL). To this solution was added Dess–Martin reagent (2.94 g, 6.9 mmol). The mixture was stirred for 2 h at ambient temperature. The mixture was concentrated to ¼ volume and the product purified directly by silica gel chromatography, eluting with EtOAc. A second column (also eluting with EtOAc) was necessary to remove traces of Dess–Martin reagent-related impurities to provide **13** in 90% yield (1.13 g, 4.8 mmol) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 2.84 (dd, 1H, *J* = 18.6, 3.0 Hz), 3.11 (dd, 1H, *J* = 18.6, 7.2 Hz), 6.08–6.11 (m, 1H), 6.55 (dd, 1H, *J* = 5.7, 2.1), 7.83 (dd, 1H, *J* = 5.7, 2.4 Hz), 8.58 (s, 1H), 8.71 (s, 1H). ^{13}C NMR (75.3 MHz, DMSO-d_6) δ 40.8, 54.9, 131.2, 136.6, 146.5, 149.0, 151.5, 151.7, 159.8, 205.4. LRMS $[M-H]^+$, 235.0. Recrystallized from hot EtOAc/hexanes; large prisms, mp = 135.5–136 °C). Anal. Calcd for $C_{10}H_9ClN_4O$: C, 51.19; H, 3.01; N, 23.88. Found: C, 50.91; H, 3.02; N, 23.73.

6.2.4. (1*S*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-enol (14)

Compound **13** (0.117 g, 0.5 mmol) was dissolved in MeOH (5 mL) with 5 drops of H_2O from a standard glass pipet. To this mixture was added anhydrous $CeCl_3$ (0.123 g, 0.5 mmol) and the mixture sonicated until most of the $CeCl_3$ had dissolved. With rapid stirring, $NaBH_4$ (0.019 g, 0.5 mmol) was added. The mixture was stirred for 2 h until TLC indicated completion. The mixture was worked up by neutralization with the dropwise addition of 1 N HCl. The mixture was concentrated in vacuo. Isolation of the desired compound **14** (and separation from diastereomer **12**) was carried out by silica gel chromatography (120 g, prepacked, silica gel column, ISCO Combiflash), eluting with a gradient of 0–10% CH_3OH/CH_2Cl_2 over 30 min. The title compound **14** was isolated in 64% yield (0.075 g, 0.32 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 1.89 (d app t, 1H, J = 14.4, 3.9 Hz), 3.01–3.11 (m, 1H), 4.86–4.89 (m, 1H, obscured by OH of solvent), 5.69–5.73 (m, 1H), 6.08–6.11 (m, 1H), 6.31–6.34 (m, 1H), 8.56 (s, 1H), 8.72 (s, 1H). ^{13}C NMR (75.3 MHz, CD_3OD) δ 42.0, 59.9, 75.7, 131.7, 132.6, 141.0, 146.9, 151.2, 152.8. Compound **12** (0.017 g, 0.07 mmol, 14% yield) was also recovered.

6.2.5. Diethyl ((1*S*,4*R*)-4-(6-chloro-9*H*-purin-9-yl)cyclopent-2-enyloxy)methylphosphonate (15)

Li*Ot*Bu solution (1 M in hexanes, 1.26 mL, 1.26 mmol) was added to a solution of **14** (0.10 g, 0.42 mmol) in THF (2 mL) and cooled to 0 °C. After stirring the mixture for 10 min trifluoro-methanesulfonic acid diethoxy-phosphoryl methyl ester (0.54 g, 1.68 mmol) was added and the resultant mixture stirred until LCMS indicated no starting material remained. The reaction was quenched by the addition of acetic acid (few drops) and then diluted with CH_2Cl_2 (10 mL). The organic solution was washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was subjected to ISCO Combiflash purification eluting with a gradient of 0–10% CH_3OH/EtOAc to provide **15** in 79% yield, (0.129 g, 0.33 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 1.29-1.38 (m, 6H), 2.11 (d, 1H, J = 14.7 Hz), 2.93–3.03 (m, 1H), 3.98–4.04 (m, 2H), 4.11–4.22 (m, 4H), 4.77 (m, 1H), 5.77 (m, 1H), 6.28 (m, 1H), 6.51 (m, 1H), 8.50 (s, 1H), 8.72 (s, 1H). ^{31}P NMR (121.4 MHz, CD_3OD) δ 22.3. LRMS $[M+H]^+$, 386.9.

6.2.6. ((1*S*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)cyclopent-2-enyloxy)methylphosphonic diacid (7)

Compound **15** (0.024 g, 0.058 mmol) was aminolyzed and deprotected to give **7**[10] (0.016 g, 71% yield) in a manner similar to that described for conversion of **19** to **9** below. ^{1}H NMR (300 MHz, D_2O) δ 1.78 (d app t, 1H, J = 14.7, 4.0 Hz), 2.81–2.91 (m, 1H), 3.55 (app d, 2H, J = 9.5 Hz), 5.32–5.42 (m, 1H), 6.04–6.06 (m, 1H), 6.30–6.32 (m, 1H), 8.07 (s, 1H), 8.10 (s, 1H). ^{31}P NMR (121.4 MHz, D_2O) δ 15.9. LRMS $[M-H]^+$, 311.7.

6.2.7. (1*S*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)-1-methyl-cyclopent-2-enol (16)

According to the same procedure used for the conversion of **13** to **17** below, **13** (0.239 g, 1.02 mmol) was also converted to **16**; the lithium TMS-acetylide was replaced with a commercial solution of MeMgBr in Et_2O (3 M). After work-up, automated silica-gel chromatography (ISCO Combiflash, 120 g pre-packed silica gel column) eluting with 50% EtOAc/hexanes provided the desired product **16** in 49% yield as a clear oil (single diastereomer, 0.125 g, 0.50 mmol). ^{1}H NMR (500 MHz, CD_3OD) δ 1.47 (s, 3H), 2.15 (dd, 1H, J = 14.0, 4.0 Hz), 2.76 (dd, 1H, J = 14.5, 8.0 Hz), 5.75–5.78 (m, 1H), 5.96 (dd, 1H, J = 5.5, 2.5 Hz), 6.20 (dd, 1H, J = 5.5, 1.5 Hz), 8.57 (s, 1H), 8.73 (s, 1H). LRMS $[M-H]^+$, 251.1.

6.2.8. (1*S*,4*R*)-4-(6-Chloro-9*H*-purin-9-yl)-1-trimethylsilylethynylcyclopent-2-enol (17)

The following procedure was modified from literature experimental.[18] $CeCl_3$ (anhydrous beads, 1.39 g, 5.3 mmol) was suspended in THF (5 mL) and stirred under Ar for 48 h, during which time a fine suspension formed. In a second, flame-dried round-bottom flask, TMS–acetylene (746 μL, 5.3 mmol) was dissolved in anhydrous THF (5 mL). The TMS–acetylene solution, and the flask containing the $CeCl_3$ were both cooled to −78 °C. To the TMS–acetylene solution, *n*-BuLi solution (2.5 M in hexanes, 2.11 mL, 5.3 mmol) was added dropwise by syringe. Both mixtures were stirred for 20 min under Ar and then the lithium TMS–acetylide solution was transferred via canula into the rapidly-stirred $CeCl_3$ suspension, forming a faint yellow-orange color. The mixture was stirred 10 min at −78 °C, removed from the ice-bath for 5 min, to ensure complete transmetallation onto cerium, and then recooled to −78 °C and stirred for an additional 30 min. The vacuum desiccator-dried enone **13** (0.206 g, 0.88 mmol) was dissolved in anhydrous THF (5 mL), cooled to −78 °C, and transferred *via* canula into the contents of the flask containing the cerium acetylide salt. After stirring the mixture for 10 min at −78 °C, TLC and LCMS both indicated complete consumption of starting material and the formation of one product. The mixture was quenched by addition of 10 drops of HOAc, warmed to ambient temperature, and diluted into 1:1 EtOAc/H_2O (100 mL). The layers were separated and the EtOAc washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL). The EtOAc was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. Silica gel chromatography with 25–50% EtOAc/hexanes afforded the desired product **17** in 91% yield as a white powder (0.267 g, 0.80 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.16 (s, 9H), 2.30 (dd, 1H, J = 14.1, 4.8 Hz), 2.95 (dd, 1H, J = 14.4, 8.4 Hz), 5.71–5.76 (m, 1H), 6.12–6.22 (m, 2H), 8.55 (s, 1H), 8.79 (s, 1H). ^{13}C NMR (75.3 MHz, CD_3OD) δ *−0.2,* 59.9, 76.7, 89.3, 108.2, 131.5, 132.5, 142.4, 146.9, 151.2, 152.7, 152.8. LRMS $[M-H]^+$, 333.0. A small amount of this material was recrystallized from hot EtOAc/hexanes. Fine crystals separated on cooling, (mp = 179.5–180.0 °C). Anal. Calcd for $C_{15}H_{17}ClN_4OSi$: C, 54.12; H, 5.15; N, 16.83. Found: C, 53.85; H, 5.23; N, 16.65.

6.2.9. Diisopropyl ((1*S*,4*R*)-4-(6-chloro-9*H*-purin-9-yl)-1-methylcyclopent-2-enyloxy)methylphosphonate (18)

Compound **16** (0.125 g, 0.5 mmol) was converted into **18** (0.056 g, 0.13 mmol, 26% yield) in the same way that **17** was converted to **19** below. ^{1}H NMR (500 MHz, CD_3OD) δ 1.28–1.36 (m, 12H), 1.49 (s, 1H), 2.42 (dd, 1H, J = 14.5, 5.0 Hz), 2.63 (dd, 1H, J = 14.5, 8.0 Hz), 3.82–3.94 (m, 2H), 4.66–4.84 (m, 2H), 5.75–5.78 (m, 1H), 6.16 (dd, 2H, J = 5.5, 2.5 Hz), 6.30 (dd, 1H, J = 5.5, 2.0 Hz), 8.58 (s, 1H), 8.71 (s, 1H).

6.2.10. Diisopropyl ((1*S*,4*R*)-4-(6-chloro-9*H*-purin-9-yl)-1-ethynylcyclopent-2-enyloxy)methylphosphonate (19)

To compound **17** (0.275 g, 0.83 mmol) was added Li*Ot*Bu solution (1 M in THF, 3.30 mL, 3.3 mmol). *Note: no additional solvent was added.* To this was added diisopropyl bromomethylphosphonate (1.28 g, 4.9 mmol). The mixture was heated at 50 °C for 2 h under N_2. The flask was then cooled to ambient temperature, and charged with additional Li*Ot*Bu solution (1 M in THF, 3.30 mL, 3.3 mmol) and diisopropyl bromomethylphosphonate (1.28 g, 5.0 mmol). The mixture was heated to 50 °C and stirred under N_2 until LCMS indicated no starting material remained. The reaction mixture was concentrated in vacuo and ½ the resultant residue loaded directly onto silica gel column (120 g, prepacked silica column, automated purification on ISCO Combiflash), and eluted with a gradient of 0–10% CH_3OH/EtOAc over 35 min. The remaining ½ of the reaction mixture was purified in an identical manner. Com-

pound **19** (0.110 g, 2.6 mmol, 32% yield) was isolated as an impure brown oil that crystallized on standing. ^{1}H NMR (300 MHz, CD_3OD) δ 1.29–1.34 (m, 12H), 2.54 (dd, 1H, J = 14.7, 3.0 Hz), 3.07 (dd, 1H, J = 14.7, 8.1 Hz), 3.36 (s, 1H), 3.97–4.02 (m, 2H), 4.68–4.74 (m, 2H), 5.90–5.94 (m, 1H), 6.34 (dd, 1H, J = 5.7, 2.7 Hz), 6.49 (dd, 1H, J = 5.4, 1.5 Hz), 8.51 (s, 1H), 8.73 (s, 1H). ^{31}P NMR (121.4 MHz, CD_3OD) δ 19.9.

6.2.11. ((1*S*,4*R*)-4-(6-Amino-9*H*-purin-9-yl)-1-methyl-cyclopent-2-enyloxy)methylphosphonic acid (8)

Compound **18** was converted into **8** in the same way as **19** was converted into **9** below. ^{1}H NMR (300 MHz, D_2O) δ 1.33 (s, 3H), 1.97 (dd, 1H, J = 14.4, 5.1 Hz), 2.49 (dd, 1H. J = 14.4, 8.1 Hz), 3.31–3.45 (m, 2H), 5.36 (br m, 1H), 5.99 (dd, 1H, J = 5.7, 1.8 Hz), 6.17 (dd, 1H, J = 5.7, 2.1 Hz), 8.00 (s, 1H), 8.04 (s, 1H). LRMS $[M+H]^+$, 325.8, t_R = 0.51 min.

6.2.12. ((1*S*,4*R*)-4-(6-Amino-9H-purin-9-yl)-1-ethynyl-cyclopent-2-enyloxy)methylphosphonic acid (9)

Compound **19** (41 mg, 0.099 mmol) was dissolved in MeOH (2 mL). The solution was transferred to a small glass pressure tube and cooled to −78 °C. A stream of anhydrous NH_3 (g) was passed through the tube, and condensed until the volume of liquid in the tube had doubled. The pressure tube was sealed and heated to 80 °C for 12 h. The solution was concentrated in vacuo and the product was isolated by silica gel chromatography, eluting with 4–20% MeOH/CH_2Cl_2. The resultant residue crystallized to a waxy solid on standing (0.037 g, 0.088 mmol, 89% yield). The material was carried on without further characterization. The aminolyzed intermediate (0.0238 g, 0.057 mmol) was dissolved in anhydrous DMF (5 mL) and the solution was heated to 50 °C. In one aliquot, trimethylsilyl bromide (112 μL, 0.85 mmol) was added and the mixture stirred at 50 °C until no starting material or mono-dealkylated intermediate remained, as determined by LCMS. The mixture was cooled to ambient temperature and 2,6-lutidine (200 μL) was added followed by 10 drops of MeOH. The mixture was concentrated in vacuo to a brown residue, and co-evaporated from conc aqueous NH_4OH (2 × 5 mL). The resultant solid was triturated with acetone (2 × 2 mL) and the residue purified by automated reverse-phase chromatography as described in the general procedures. Combination of the appropriate fractions and lyophilization provided **9** in 21% yield (6.7 mg) as a white powder (ammonium salt). ^{1}H NMR (300 MHz, D_2O) δ 2.18 (dd, 1H, J = 14.1, 3.9 Hz), 2.91 (dd, 1H, J = 14.1, 8.4 Hz), 3.00 (s, 1H), 3.50–3.60 (m, 2H), 5.42 (br m, 1H), 6.10 (dd, 1H, J = 5.4, 2.1 Hz), 6.30 (br d, 1H, J = 5.4 Hz), 7.91 (s, 1H), 7.96 (s, 1H). ^{31}P NMR (121.4 MHz, D_2O) δ 14.9. LRMS $[M-H]^+$ 334.2 t_R = 1.22 min. HPLC t_R = 10.81 min. HRMS $C_{13}H_{13}N_5O_4P$ $[M-H]^+$ req. 334.0692, found 334.0693. $[\alpha]_D$ = +139.2 (2.7 mmol, MeOH).

6.2.13. ((1*S*,4*R*)-4-(6-Amino-9*H*-purin-9-yl)-1-ethynyl-cyclopent-2-enyloxy)methylphosphonic acid diphosphate (20)

Prepared from **9** according to literature procedures.[12] ^{1}H NMR (300 MHz, D_2O) δ 2.29 (d, 1H, J = 14 Hz), 2.94–3.08 (m, 2H), 3.84–3.89 (m, 2H), 5.60 (br, s, 1H), 6.15 (br s, 1H), 6.38 (br s, 1H), 8.10 (s, 1H), 8.19 (s, 1H). ^{13}C NMR (75.3 Hz, D2O) δ 57.2, 60.5, 62.7, 76.1, 81.4, 82.6 (d, J = 67 Hz), 114.9, 118.1, 132.7, 138.0, 141.4, 148.4, 149.5, 153.8. ^{31}P NMR (121.4 MHz, D_2O) δ 8.72 (d, J = 26 Hz), −10.70 (d, J = 80 Hz), −23.16 (t, J = 74 Hz). HPLCie t_R = 8.69 min. LRMS $[M-H]^+$, 493.9, t_R = 0.26 min. HRMS $C_{13}H_{15}N_5O_{10}P_3$ $[M-H]^+$ req. 494.0032, found 494.0028.

6.2.14. ((1*S*,4*R*)-4-(6-Amino-9H-purin-9-yl)-1-vinyl-cyclopent-2-enyloxy)methylphosphonic acid diphosphate (21)

Compound **20** (20 mg, 0.04 mmol) was dissolved in H_2O (10 mL). Quinoline (32 mg, 0.2 mmol) and Lindlar-catalyst (10 mg) were added under an atmosphere of N_2. The suspension was evacuated and purged with H_2 three times and vigorously stirred under an atmosphere of H_2 for 16 h. The suspension was filtered and purified by ion exchange chromatography (C-18 column, eluant neat H_2O for 10 min, then ramp to 90% MeCN over 20 min). Combination of the appropriate fractions and lyophilization yielded **21** (15.4 mg) product as a white powder. ^{1}H NMR (300 MHz, D_2O) δ 2.10 (d, 1H, J = 13 Hz), 2.71–2.74 (m, 1H), 3.03 (br s, 1H), 3.61 (br s, 1H), 5.18–5.28 (m, 2H), 5.44 (br s, 1H), 5.94–6.00 (m, 1H), 6.11 (br s, 1H), 6.31 (br s, 1H), 8.12 (s, 1H), 8.19 (s, 1H). ^{31}P NMR (121.4 MHz, D_2O) δ 10.3, −9.9, −21.5. HPLCie t_R = 8.67 min. LRMS $[M-H]^+$ 496.02, t_R = 0.04 min. HRMS $C_{13}H_{17}N_5O_{10}P_3$ $[M-H]^+$ req. 496.0183, found 496.0184.

6.3. Biological assays

The procedures for measuring the antiviral activity toward wild-type HIV, cytotoxicity, resistance due to K65R, and M184V RT mutations, and the inhibition of HIV RT enzyme have been reported previously.[6]

6.4. Intracellular metabolism

The uptake and phosphorylation of **9** relative to that of **1** was studied in the T-cell line CEM-CCRF. Cells were cultured and experiments conducted as previously described.[13] Samples were analyzed using ion pairing liquid chromatography coupled to positive mode mass spectrometry as previously described.[15] The mass spectrometer was operated in multiple reaction monitoring mode using the parent/daughter mass transitions of 288.1/176.4, 448.3/176.4, 336.1/224.1 and 496.2/224.1 for **1**, **2**, **9** and **20**, respectively.

6.5. Molecular modeling

The structure of the ternary complex of HIV-1 RT, primer/template, and dTTP was used as the starting point for all modeling studies.[16] The X-ray model was converted into its corresponding minimized model using Sybyl (Sybyl Molecular Modeling Software, version 6.9, Tripos Inc., St. Louis MO). The X-ray of dTTP was modified to dATP, and the corresponding template base was modified into thymidine. The model of RT-dATP complex was energy minimized as previously described.[17] After minimization, modeling tools were again used to convert the dATP into the carbocyclic diphosphate analog **20**. The new model was also energy minimized and utilized in this study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.12.028.

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