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Original article

Synthesis and antiviral evaluation of *bis*(POM) prodrugs of (*E*)-[4′-phosphono-but-2′-en-1′-yl]purine nucleosides

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ABSTRACT

Seventeen *hitherto unknown bis*(POM) prodrugs of novel (*E*)-[4′-phosphono-but-2′-en-1′-yl]purine nucleosides were prepared in a straight approach and at good yields. Those compounds were synthesized by the reaction of purine nucleobases directly with the phosphonate synthon **3** bearing POM biolabile groups under Mitsunobu conditions. All obtained compounds were evaluated for their antiviral activities against a large number of DNA and RNA viruses including herpes simplex viruses 1 and 2, varicella zoster virus, Feline herpes virus, human cytomegalovirus, HIV-1 and HIV-2. Among these molecules, some of them exhibit anti-VZV and anti-HIV activity at submicromolar concentrations. This class of compound will be of further interest for lead optimization as anti-infectious agents.

1. Introduction

Acyclonucleoside phosphonates (ANPs) [1] represent a key class of antiviral drugs which has attracted considerable attention through the large number of modified nucleobase and/or acyclic chain moieties. Among ANPs, 9-[2-(phosphonomethoxy)ethyl] adenine (PMEA) [2] or (*R*)-[2-(phosphonomethoxy)propyl]adenine (PMPA) [3] exhibited strong antiviral activities against of HIV and/or HBV infections (Fig. 1). However, their antiviral efficiency is mainly hampered by their low cell penetration (<5%) but also by their ability to be converted by human/viral kinases to their triphosphate active forms at a high concentration at the right location [4]. To circumvent the poor bioavailability of ANP which are negatively charged at physiological pH, several groups [5–8] have successfully developed biolabile promoieties in which the charged phosphonic acid group is transformed into a neutral phosphonate diester(s) with decreased polarity. Based on that, PMEA and PMPA have been commercialized as their prodrug *bis*(-POM)PMEA (Adefovir, HEPSERA™) and *bis*(POC)PMPA (Tenofovir, VIREAD™), respectively.

Part of our program to discover antiviral compounds, we have recently reported a new class of acyclic nucleoside phosphonates bearing the (*E*)-4′-phosphono-but-2′-en-1′-yl side chain moiety, which can mimic the conformation of the C1–O4–C4–C5 atoms from the 2-deoxyribose in dTMP [9,10]. Having in hand an acyclic phosphonate side chain, it was rational to connect this synthon to purine base analogues which exhibit the greatest activities as antiviral agents. In this work, we aim to identify a novel series of purine ANP analogues in their prodrug form, combining our biolabile phosphonate side-chain and purine analogues selected in the literature as the lead nucleobases which possesses antiviral properties (e.g., guanine [11] and its tricyclic analogues [12,13], N^6-substituted-purines [14,15]). Herein we report the biological evaluation and the efficient one step synthesis *via* Mitsunobu reaction of (*E*)-*bis*-(POM)-[4-phosphono-but-2′-en-1′-yl]purine analogues.

2. Chemistry

We have previously reported the use of cross metathesis reaction between *bis*(POM)-allyl phosphonate **2** and crotylated

Abbreviations: VZV, varicella zoster virus; VV, vaccinia virus; HSV, herpes simplex virus; VSV, vesicular stomatitis virus; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; CC_{50}, compound concentration affording 50% inhibition of cell growth; EC_{50}, compound concentration affording 50% inhibition of the viral cytopathicity; MCC, minimum cytotoxic concentration required to afford a microscopically detectable alteration of cell morphology; MDCK, Madin–Darby canine kidney.

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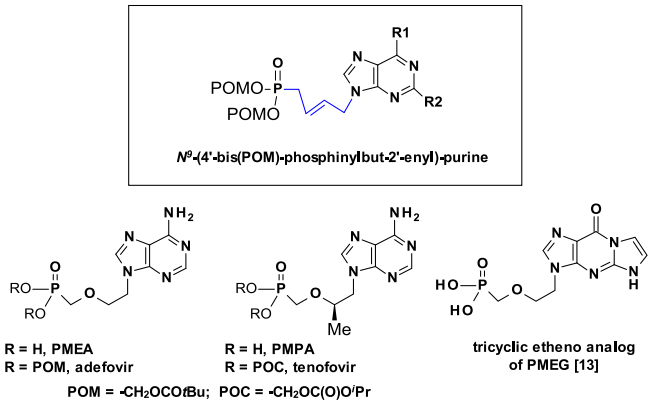


Fig. 1. Some nucleoside phosphonates and target compounds.

5-substituted uracil as an one-step efficient tool to obtain in good yield a large library of (*E*)-4-phosphono-but-2′-en-1′-yl pyrimidine nucleosides, which some of them exhibit submicromolar activities against VZV [9,16]. However, it is well-known that nitrogen containing heterocycles can decrease the catalytic metals through a poisoning mechanism. Usually, metathesis reaction involving purine nucleosides requires exocyclic amine protection to avoid catalyst poisoning and often gives very low yields. Thus, to circumvent this problem, we aimed to determine the most suitable conditions for an efficient synthesis of (*E*)-4-phosphono-but-2′-en-1′-yl purines in their prodrug form. We thought that selected purines could be directly introduced through a *N*-alkylation with the corresponding activated (*E*)-4-phosphono-but-2′-en-1′-yl counterpart either under SN2 or under Mitsunobu conditions.

Thus, starting with *bis*(POM)-allyl phosphonate (**2**), the (*E*)-*bis*(POM)-4-bromo-but-2-en-1-yl phosphonate (**3′**) was obtained in 73% yields by cross metathesis of *bis*-(POM)-allyl phosphonate (**2**) with (*E*)-1,4-dibromobut-2-ene. Unfortunately, the *N*-alkylation reaction of adenine in the presence of Cs_2CO_3 [17] and synthon **3′** in anhydrous DMF failed, because of major formation of *bis*(POM)-but-1,3-dienyl phosphonate **4** resulting from undesired bromine elimination (Scheme 1).

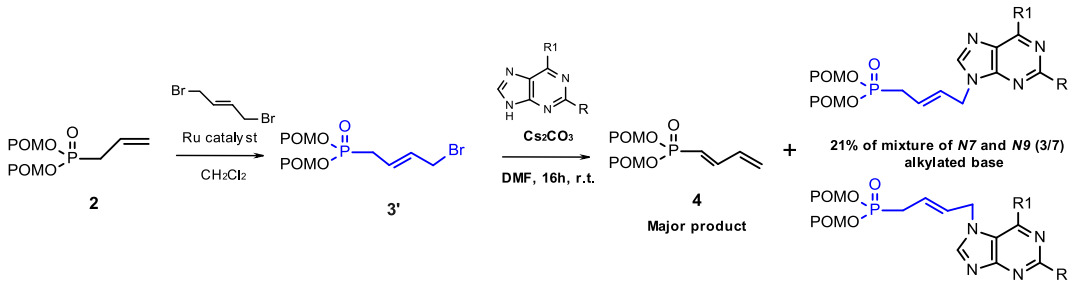
We then turned our attention to the introduction of the nucleobase under Mitsunobu conditions [18] (Scheme 2). Firstly, the *hitherto* unknown (*E*)-*bis*(POM)-4-hydroxy-but-2-en-1-yl phosphonate **3** bearing hydroxyl was prepared by cross metathesis reaction with *bis*(POM) allylphosphonate (**2**) and 2-buten-1,4-diol in 74% yields.

Starting from dimethylallylphosphonate, compound **2** was obtained according our previous procedure, using chloromethylpivalate in the presence of sodium iodine during 48 h in 84% yield. (*E*)-4-Hydroxy-*bis*(POM)-but-2-enylphosphonate **3**, the key compound of our strategy, was prepared through cross metathesis reaction between *cis*-but-2-en-1,4-diol and **2** in the presence of 5 mol% of Ru catalyst during 16 h in CH_2Cl_2 at reflux. The resulted mixture of two isomers (*E* and *Z*) was separable by column chromatography to afford the desired isomer *trans* **3** in 74% yield. A previous attempted cross metathesis reaction with crotylalcohol afforded also (*E*/*Z*) mixture in a disappointing 32% yield, which can be mainly ascribed to the formation of the unreactive crotyl phosphonate.

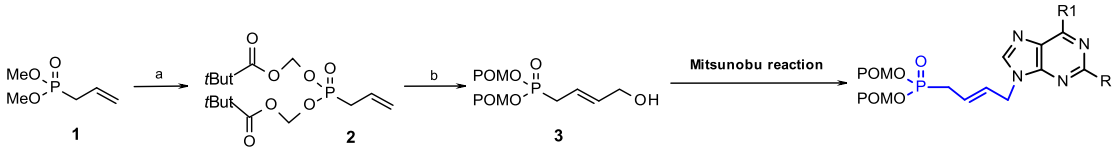
The synthesis of purine ANP analogues **9**–**13** was performed as shown in Scheme 3). The key intermediates **7** and **8** were obtained in 71% and 50% yield, respectively, through Mitsunobu reaction between appropriate 6-chloropurines (**5** or **6**) and synthon **3** in the presence of PPh_3 and DIAD in dioxane at room temperature. The Mitsunobu reaction condition gave a mixture of *N*-7 and *N*-9 alkylated bases, which were isolated by delicate column chromatography. Both isomers were determined by NMR spectroscopic data analysis including HMBC correlation between protons H2 and H2′, and carbons C4 and C5. *Bis*(POM)-ANP chloropurine derivatives **7** and **8** were first converted to hypoxanthine **9** and guanine **10** derivatives in 86% and 85% yields, respectively, using diluted HCOOH at 40 °C for 20 h. Compounds **7** and **8** underwent also nucleophilic substitution on the C6-position with cyclopropylamine to afford **11** and **12** in good yield. From compound **8**, a tricyclic derivative **13** was obtained in 48% yield by treatment with 2-chloroacetaldehyde (50 wt. % in H_2O) in a dioxane/water mixture at 70 °C for 6 h [13]. During our investigations, we did not observe the degradation of the (POM) biolabile group proving the efficiency of our strategy in terms of number of steps and overall yield. However, in order to facilitate the isolation of the *N*-9 isomers, substitutions at C6-position were alternatively proceeded before the Mitsunobu reaction. The desired C6-substituted-purine bases **14b**–**e** and **15c**–**e** were obtained from **5** or **6** by nucleophilic substitution with substituted primary amines and Et_3N in *n*BuOH with yields ranging from 80 to 92%. Compounds **14a**–**e** and **15a**,**c**,**d**,**e**,**f** can be readily converted in *bis*(POM) ANP derivatives **16a**–**e** and **17a**,**c**,**d**,**e**,**f** by coupling reaction with synthon **3** as described previously, in 38–52% overall yield. All the structures were confirmed by ^1H, ^{13}C, ^{31}P NMR and HRMS analysis.

The title *bis*(POM) (*E*)-4-phosphono-but-2′-en-1′-yl acyclic nucleosides **7**–**13** and **16**, **17** were subjected to an *in vitro* antiviral screening using a wide spectrum of DNA viruses, in HEL cell cultures, for vaccinia virus (VV), herpes simplex virus-1(KOS) (HSV-1), herpes simplex virus-2(G) (HSV-2), varicella-zoster virus (VZV TK^+ and TK^-) as summarized in Table 1.

Among the synthesized compounds some of them show potent inhibitory activities against several DNA viruses, in particular compounds **10**, **12** and **17f**, exhibiting submicromolar activities. Even if the active compounds against DNA virus replication do not cause a microscopically detectable alteration of cell morphology (MCC) at concentration up to 100 μM, they were found to be rather cytostatic. Therefore, it is currently unclear whether the activity observed for these compounds is due to a specific antiviral effect or to an antiproliferative activity. It is interesting to notice that the



Scheme 1. Purine base alkylation through the use of halogenated synthon **3′**.



Scheme 2. Synthesis of (*E*)-4-hydroxy-*bis*(POM)-but-2-enylphosphonate.Reagents and conditions (a) NaI, chloromethylpivalate, CH_3CN, reflux, 48 h, 84% (b) (*Z*)-2-buten-1,4-diol, [Ru] = catalyst (5 mol%), CH_2Cl_2, reflux, 12 h, 74%.

bis(POM)-ANP derivatives bearing 2-amino 6-substituted purines (**8**, **10**, **12** and **17a**,**f**) displayed a (sub)micromolar activity against VZV in contrast with the inactive hypoxanthine analogues (**7**, **9**, **11**). It is tempting to assume that the 2-aminopurine derivatives owe their antiviral activities upon conversion to the corresponding guanine derivatives. This assumption is in line with the rather pronounced cytostatic activity of these compounds, since guanine-bearing compounds such as PMEG are known to be quite inhibitory to cell proliferation.

Among the tested final molecules against Feline herpes virus in CRFK cell cultures (data not shown) only compound **10** showed activity against Feline herpes virus at an EC_{50} of 11 μM with no observed cytotoxicity up to 100 μM (Table 2).

All new compounds were also evaluated against HIV-1(III_B) or HIV-2(ROD) in CEM cell cultures. Whereas most of them are devoid of anti-HIV-1 and -2 activity (data not shown), we report in Table 3 the micromolar and submicromolar activities of compounds **17a** and **17f**. While compound **17a** exhibits micromolar anti-HIV-1 and anti-HIV-2 activities with an EC_{50} of 6.6 μM and 6.3 μM respectively, **17f** displayed a potent submicromolar anti-HIV-2 activity with an EC_{50} of 0.28 μM. Its selectivity index (ratio CC_{50}(CEM)/EC_{50}) was 28.5 (Table 3).

None of the compounds displayed a specific antiviral effect against a wide panel of other viruses including vesicular-stomatitis virus, influenza virus type A (H1N1 and H3N2) and B in MDCK cell cultures, para-influenza-3 virus, reovirus-1, Sindbis virus and Punta Toro virus in Vero cell cultures and Vesicular stomatitis, Coxsackie B4 and respiratory syncytial virus in HeLa cell cultures (data not shown).

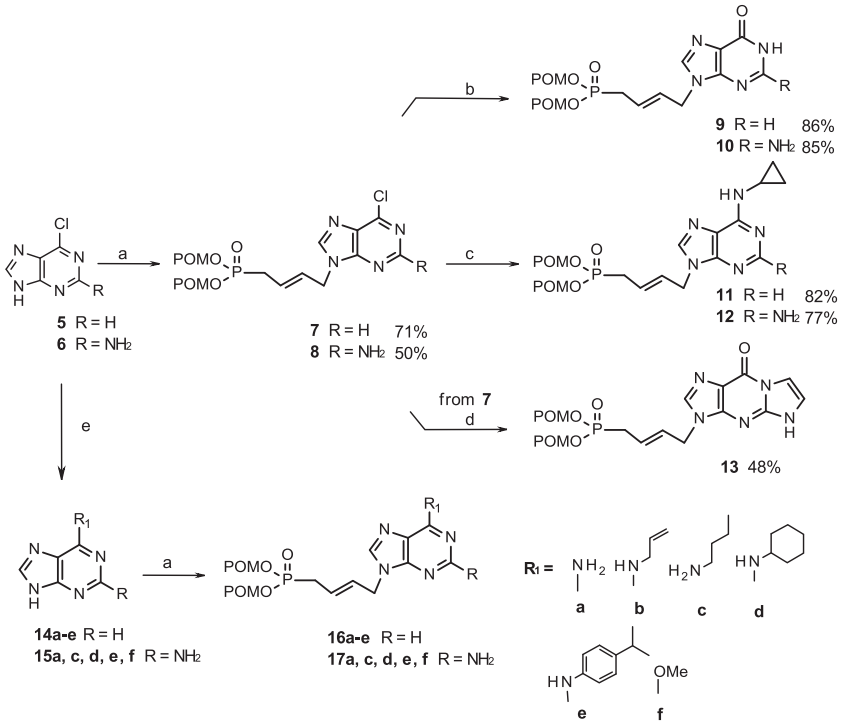
3. Conclusion

In summary, a novel series of 4-phosphono-but-2-en-1-yl purine nucleosides *bis*(POM) prodrugs, were successfully synthesized by an efficient one step strategy under Mitsunobu conditions between purines and *bis*-(POM) (*E*)-4-phosphono-but-2-enol synthon **3**. All synthesized molecules have been evaluated against several DNA and RNA viruses. Submicromolar activities against HIV and VZV were displayed for some of these compounds, which reinforce the choice of our unsaturated side chain for the development of antiviral agents. This encouraging result allows us to prepare new ANPs in their prodrug form bearing a modified heterocycle moiety in our future drug discovery program.

4. Experimental section

4.1. Chemistry

Commercially available chemicals were of reagent grade and used as received and solvents were dried following standard procedure. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel $60F_{254}$, E.



Scheme 3. Reagents and conditions (a) (*E*)-4-hydroxy-*bis*(POM)-but-2-enylphosphonate **3**, PPh_3, DIAD, dioxane, r.t., 12 h; (b) H_2O/formic acid (1/1), 40 °C, 20 h; (c) cyclopropylamine/CH_2Cl_2 (1/10), 40 °C; 20 h (d) 1,4-dioxane/H_2O (1/1), 2-chloroacetaldehyde (50 wt. % in H_2O), 70 °C, 6 h, 48%; (e) appropriate amine derivatives, Et_3N, *n*BuOH, reflux, 16h.

Table 1
Antiviral activity and cytotoxicity of ANP prodrugs against VV, HSV and VZV in HEL cell cultures.

Compounds	EC_{50}[a] μM						Cytotoxicity μM	
	VV (Lederle)	HSV-1		HSV-2 (G)	VZV		MCC[b] (μM)	CC_{50}[c] (μM)
		(KOS)	(TK^- KOS ACV)		TK^+ (OKA)	TK^- (07/1)		
7	>20	>20	>20	>20	>20	>20	100	10.5
8	>20	>20	>20	>20	**4.4**	**2.2**	>100	5.4
9	>100	>100	>100	>100	>100	>20	>100	51.9
10	100	**3.9**	**5.8**	**7.8**	**1.05 ± 0.2**	**0.8 ± 0.4**	>**100**	**4.1 ± 0.1**
11	>100	>100	>100	>100	55.7	47.2	>100	39.6
12	**20**	**16.3**	**16.3**	**7.2**	**0.8 ± 0**	**0.8 ± 0**	>**100**	**2.3 ± 0.1**
13	>100	>100	>100	>100	17.3	40.7	>100	nd
16a	>100	>100	20	45	12.56	13.53	>100	11.6
17a	>20	20	20	20	**6.8 ± 1.2**	**12.0 ± 0**	>100	**36 ± 1**
16b	>100	>100	>100	>100	>100	>100	>100	>100
16c	>100	>100	>100	>100	100	>100	>100	37.2
17c	>100	>100	>100	>100	32.4	43.6	>100	60
16d	>100	>100	>100	>100	20	>20	100	11.4
17d	>100	>100	>100	>100	6.34	20	100	9
16e	>100	>100	>100	>100	>20	>20	100	32.3
17e	>100	>100	>100	>100	>20	>100	>100	46
17f	**16 ± 4**	**6 ± 3**	**7.5 ± 4.5**	**6.5 ± 2.5**	**0.1 ± 0.4**	**0.5 ± 0.3**	>**100**	**1.6 ± 0.4**
Acyclovir	>250	0.4	250	0.4	2.7	15.0 ± 1.5	>100	134 ± 10
Brivudine	5	0.08	250	50	0.01	117	>100	339
Cidofovir	10	1.2	1	1	nd	nd	nd	nd

Bold numbers represents the compounds which possess an antiviral activity <10 μM.
[a] 50% Effective concentration or compound concentration required to reduce virus-induced cytopathicity by 50%.
[b] Minimum compound concentration that causes a microscopically detectable alteration of cell morphology.
[c] 50% Cytostatic concentration or compound concentration required to reduce cell growth by 50%.

Merck). Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck). The ^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Varian $Inova_{Unity}$ 400 spectrometer (400 MHz) in (d_4) methanol, $CDCl_3$, shift values in parts per million relative to $SiMe_4$ as internal reference. High Resolution Mass spectra were performed by the Mass Spectrometry Center of Blaise Pascal University (Program Masslynx 4.0) (Aubière, France).

4.1.1. (E)-Bis(POM)-4-hydroxy-but-2-en-1-yl phosphonate (**3**)

To a dry CH_2Cl_2 solution (30 mL) of *bis*(POM) allylphosphonate (500 mg, 1.41 mmol), and 2-buten-1,4-diol (604 mg, 2.82 mmol) under argon was added $RuCl_2(PPh_3)$IMesBenzylidene Nolan's catalyst (60 mg, 0.071 mmol, 5%). After 48 h stirring at 40 °C, volatiles were evaporated and residue was purified by silica gel column chromatography (AcOEt/Petroleum ether 1:1) to give pure (*E*)-*bis*(POM)-4-hydroxy-but-2-en-1-yl phosphonate **3** (402 mg, 1.06 mmol, 74%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.88–5.79 (m, 1H, $H_{P-CH2-CH=CH}$), 5.73–5.55 (m, 5H, $H_{P-CH2-CH=CH}$, $H_{O-CH2-O}$), 4.11 (t, J = 7.4 Hz, 2H, H_{CH2-OH}), 2.68 (dd, J = 22.4, 7.3 Hz, 2H, H_{P-CH2}), 2.03 (s, 1H, H_{OH}), 1.22 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 135.9, 135.7, 119.0, 118.9, 81.6, 81.5, 62.9, 38.7, 31.4, 30.1, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.54. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{16}H_{29}NaO_8P$: 403.1498; found 403.1518.

Table 2
Anti-Feline Herpes Virus activity and cytotoxicity of compound **10** in Crandell-Rees Feline Kidney cells.

Compound	EC_{50}[a] (μM) Feline Herpes Virus	CC_{50}[b] (μM)
10	11 ± 0.3	>100
Ganciclovir	1.2 ± 0.3	>100

[a] 50% Effective concentration or compound concentration required to inhibit virus-induced cytopathicity by 50%.
[b] 50% Cytotoxic concentration or compound concentration required to reduce the viability of CRFK cells by 50%.

4.1.2. (E)-Bis(POM)-4-bromo-but-2-en-1-yl phosphonate (**3′**)

To a dry CH_2Cl_2 solution (40 mL) of *bis*-(POM)-allylphosphonate **2** (1 g, 2.82 mmol) and *trans*-1,4-dibromo-but-2-en (1.21 g, 5.64 mmol) under argon was added $RuCl_2(PPh_3)$IMesBenzylidene Nolan's catalyst (120 mg, 0.14 mmol, 5%). After 12 h stirring at 40 °C, volatiles were evaporated and the residue was purified by silica gel column chromatography (AcOEt/Petroleum ether 1:1) to give pure (*E*)-*bis*-(POM)-4-bromo-but-en-1-yl phosphonate **3′** (904 mg, 2.04 mmol, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.93–5.83 (m, 1H, $H_{P-CH2-CH=CH}$), 5.74–5.62 (m, 5H, $H_{P-CH2-CH=CH}$ and $H_{O-CH2-O}$), 3.92 (dd, J = 7.5, 3.5 Hz, 2H, $_{CH2-Br}$), 2.70 (dd, J = 22.7, 7.3 Hz, 2H, H_{P-CH2}), 1.23 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 132.1, 131.9, 122.7, 122.6, 81.2, 38.3, 31.2, 30.9, 29.5, 26.5. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.77. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{16}H_{28}O_7NaBrP$: 465.0654, found: 465.0671.

4.1.3. (E)-Bis(POM)-but-1,3-dien-1-yl phosphonate (**4**)

To a dry DMF solution (1 mL) of adenine (29 mg, 0.215 mmol) and cesium carbonate (74 mg, 0.0225 mmol) was added (*E*)-*bis*-(POM)-4-bromo-but-en-1-yl phosphonate **3′** (100 mg, 0.225 mmol) in DMF (0.5 mL). After 24 h stirring at room temperature, all

Table 3
Anti-HIV-1 and HIV-2 activity in CEM cells and cytostatic activity of compounds **17a** and **17f**.

Compounds	EC_{50}[a] (μM)		IC_{50}[b] (μM)			SI[c]
	HIV-1	HIV-2	L1210	CEM	HeLa	
17a	6.6 ± 3.0	6.3 ± 3.2	128 ± 25	46 ± 27	128 ± 25	7.3
17f	>0.4	**0.28 ± 0.014**	37 ± 6	8.0 ± 2	37 ± 6	28.5

Bold numbers represents the compounds which possess an antiviral activity <10 μM.
[a] 50% Effective concentration or compound concentration required to protect CEM cells against the cytopathicity (giant cell formation) of HIV by 50%.
[b] 50% Cytostatic concentration or compound concentration required to reduce L1210, CEM and HeLa cell proliferation by 50%.
[c] Selectivity index; ratio CC_{50}/EC_{50} in CEM cell culture.

volatiles were evaporated and the residue was purified by silica gel column chromatography (AcOEt/Petroleum ether 1:3) to give by-product **4** as major compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (ddd, J = 22.2, 16.9, 10.6 Hz, 1H, H_{CH}), 6.38 (tdd, J = 16.9, 10.4, 1.8 Hz, 1H, H_{CH}), 5.81–5.50 (m, 7H, H_{CH2}, H_{CH}, $H_{O-CH2-O}$), 1.20 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 149.7, 149.6, 135.4, 135.1, 126.2, 117.9, 116.0, 81.5, 81.5, 38.7, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 18.86.

4.1.4. N^9-(4′-Bis(POM)-phosphinyl-but-2′-enyl)-6-chloropurine (**7**)

To a dioxane solution (3 mL) of **3** (100 mg, 0.261 mmol), 6-chloropurine **5** (49 mg, 0.313 mmol), and triphenylphosphine (82 mg, 0.313 mmol) under argon at 10 °C was added dropwise di-*iso*propylazodicarboxylate (63 mg, 0.313 mmol). After 12 h stirring at room temperature, volatiles were evaporated, and residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 1:99) to give compound **7** (97 mg, 0.187 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H, H_2), 8.14 (s, 1H, H_8), 5.94–5.85 (m, 1H, $H_{2'}$), 5.80–5.71 (m, 1H, $H_{3'}$), 5.71–5.67 (m, 4H, $H_{O-CH2-O}$), 4.88 (t, J = 5.2 Hz, 2H, $H_{1'}$), 2.71 (dd, J = 22.8, 7.1 Hz, 2H, $H_{4'}$), 1.21 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 152.0, 151.6, 151.1, 144.7, 131.6, 128.9, 128.7, 124.6, 124.5, 81.6, 81.5, 45.5, 45.4, 38.7, 31.4, 30.0, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.05; HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_30N_4NaO_7PCl$: 539.1438; found 539.1449.

4.1.5. N^9-(4′-Bis(POM)-phosphinyl-but-2′-enyl)-2 amino-6-chloropurine (**8**)

To a dioxane solution (4.5 mL) of **3** (160 mg, 0.417 mmol), 2-amino-6-chloropurine **6** (107 mg, 0.627 mmol), and triphenylphosphine (164 mg, 0.627 mmol) under argon at 10 °C was added dropwise di-*iso*propylazodicarboxylate (127 mg, 0.627 mmol). After 12 h stirring at room temperature, volatiles were evaporated, and residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 2:98) to give compound **8** (112 mg, 0.209 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 1H, H_8), 5.88–5.79 (m, 1H, $H_{2'}$), 5.72–5.59 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 5.28 (s, 2H, H_{NH2}), 4.65 (t, J = 5.1 Hz, 2H, $H_{1'}$), 2.70 (dd, J = 22.7, 7.2 Hz, 2H, $H_{4'}$), 1.20 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 159.1, 153.6, 151.3, 141.8, 129.4, 129.3, 125.1, 123.6, 123.5, 81.6, 81.5, 44.9, 38.7, 31.4, 30.0, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.35. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{31}N_5O_7NaPCl$: 554.1547, found: 554.1566.

4.1.6. N^9-(4′-Bis(POM)-phosphinyl-but-2′-enyl)hypoxanthine (**9**)

A solution of **7** (24 mg, 0.046 mmol) in a (1:1) mixture of water (0.75 mL) and formic acid (0.75 mL) was stirred for 20 h at 40 °C. After evaporation of all volatiles, the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 5:95) to give compound **9** (20 mg, 0.040 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.94 (s, 1H, H_{NH}), 8.17 (s, 1H, H_2), 7.81 (s, 1H, H_8), 5.94–5.83 (m, 1H, $H_{2'}$), 5.78–5.60 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 4.78 (t, J = 5.2 Hz, 2H, $H_{1'}$), 2.72 (dd, J = 22.7, 7.2 Hz, 2H, $H_{4'}$), 1.21 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 159.1, 148.9, 145.0, 139.7, 129.7, 129.5, 124.5, 123.8, 123.7, 81.6, 45.3, 38.7, 31.4, 30.0, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.40. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{24}H_{26}N_4O_8Na$: 521.1648; found 521.1625.

4.1.7. N^9-(4′-Bis(POM)-phosphinyl-but-2′-enyl)guanine (**10**)

A solution of compound **8** (22 mg, 0.041 mmol) in a (1:1) mixture of water (0.75 mL) and formic acid (0.75 mL) was stirred for 20 h at 40 °C. After evaporation of all volatiles, the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 10:90) to give compound **10** (18.0 mg, 0.035 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.11 (s, 1H, H_{NH}), 7.60 (s, 1H, H_8), 6.65 (s, 2H, H_{NH2}), 5.95–5.84 (m, 1H, $H_{2'}$), 5.73–5.62 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 4.60 (t, J = 4.7 Hz, 2H, $H_{1'}$), 2.72 (dd, J = 22.6, 7.3 Hz, 2H, $H_{4'}$), 1.20 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 159.0, 153.9, 151.4, 137.2, 130.6, 130.4, 122.7, 122.6, 116.8, 81.7, 81.6, 44.8, 38.7, 31.4, 30.0, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.04. HRMS (ESI): m/z $[M + Na]^+$ calcd for $C_{21}H_{32}N_5O_8NaP$: 536.1886; found 536.1890.

4.1.8. N^9-(4′-Bis(POM)-phosphinyl-2′-butenyl)-6-cyclopropylaminopurine (**11**)

A solution of compound **7** (24 mg, 0.046 mmol) in (1:10) mixture of cyclopropylamine (0.2 mL) and dichloromethane (2 mL) was stirred for 20 h at 40 °C. After evaporation of all volatiles, the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 4:96) to give compound **11** (20.4 mg, 0.038 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H, H_2), 7.75 (s, 1H, H_8), 5.95 (s, 1H, H_{NH}), 5.93–5.84 (m, 1H, $H_{2'}$), 5.69–5.61 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 4.78 (t, J = 5.1 Hz, 2H, $H_{1'}$), 3.04 (d, J = 3.0 Hz, 1H, H_{NHCH}), 2.70 (dd, J = 22.6, 7.3 Hz, 2H, $H_{4'}$), 1.21 (s, 18H, $H_{C(CH3)3}$), 0.94 (td, J = 8.4, 6.9 Hz, 2H, $H_{NHCHCH2}$), 0.68–0.64 (m, 2H, $H_{NHCHCH2}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 155.8, 153.3, 149.0, 139.5, 130.1, 129.9, 123.2, 123.0, 119.8, 81.6, 81.5, 44.8, 38.7, 31.5, 30.1, 26.8, 23.7, 7.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.57. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{37}N_5O_7P$: 538.2431; found 538.2430.

4.1.9. N^9-(4′-Bis(POM)-phosphinyl-2′-butenyl)-2-amino-6-cyclopropylaminopurine (**12**)

A solution of compound **8** (20 mg, 0.037 mmol) in (1:10) mixture of cyclopropylamine (0.2 mL) and dichloromethane (2 mL) was stirred for 20 h at 40 °C. After evaporation of all volatiles, the residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2 3:97) to give compound **12** (16.0 mg, 0.029 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H, H_2), 5.89–5.81 (m, 1H, $H_{2'}$), 5.76 (s, 1H, H_{NH}), 5.67–5.57 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 4.79 (s, 2H, H_{NH2}), 4.61 (t, J = 5.1 Hz, 2H, $H_{1'}$), 3.05–2.95 (m, 1H, H_{NHCH}), 2.69 (dd, J = 23.0, 7.0 Hz, 2H, $H_{4'}$), 1.22 (s, 18H, $H_{C(CH3)3}$), 0.85 (td, J = 6.9, 5.4 Hz, 2H, $H_{NHCHCH2}$), 0.63–0.58 (m, 2H, $H_{NHCHCH2}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 160.1, 156.3, 151.0, 136.9, 130.6, 130.4, 122.4, 122.3, 114.6, 81.6, 44.4, 44.3, 38.7, 31.4, 30.0, 26.8, 23.7, 7.4. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.77; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{38}N_6O_7P$: 553.2531; found 553.2540.

4.1.10. (E)-N^3-(4′-Bis(POM)-phosphinylbut-2′-enyl)-3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine (**13**)

To a 1,4-dioxane/H_2O (2 mL, 1/1, v/v) solution of N^9-(4′-*bis*(-POM)-phosphinylbut-2′-enyl)-2-amino-6-chloropurine **8** (116 mg, 0.218 mmol) was added 2-chloroacetaldehyde (2.5 mL, 50 wt. % in H_2O). The resulting mixture was stirred 6 h at 70 °C and was extracted with ethyl acetate. Combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel in CH_2Cl_2/MeOH 98:2 afforded 56 mg (48%) of desired product **13** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.63 (s, 1H, NH), 7.66 (d, J = 2.8 Hz, 1H, H_7), 7.64 (s, 1H, H_2), 7.25 (d, J = 7.9 Hz, 1H, H_7), 5.85 (dq, J = 16.0, 5.6 Hz, 1H, $H_{2'}$), 5.70–5.60 (m, 5H, $H_{O-CH2-O}$, $H_{3'}$), 4.65 (t, J = 4.8 Hz, 2H, $H_{1'}$), 2.72 (dd, J = 22.4, 7.2 Hz, 2H, $H_{4'}$), 1.18 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.92, 152.28, 150.32, 146.12, 138.07, 130.32 (d, J = 15 Hz), 122.52 (d, J = 11.6 Hz), 115.91 (d, J = 18.2 Hz), 107.35, 81.67 (d, J = 6.2 Hz), 38.66, (d, J = 2.1 Hz), 31.28, 29.89, 26.76. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.81; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{23}H_{33}N_5O_8P$: 538.20613, found: 538.20578.

4.1.11. General procedure 1: for modification at C-6 position of 6-chloropurine (5) or 2-amino-6-chloropurine (**6**)

To a solution of 2-amino-6-chloropurine or 6-chloropurine (1 eq.) in *n*-butanol was added substituted amine (3 eq.) and triethylamine (3 eq.). The mixture is stirred during 16 h at reflux then the solvent is evaporated under reduced pressure. The crude is

purified on a silica gel column chromatography with dichloromethane and methanol (96/4) as eluant to give pure desired product **14b**–**e**, **15c**–**e**.

*4.1.11.1. 6-Allylaminopurine (**14b**).* From 6-chloropurine, using general procedure 1 and allylamine, compound **14b** was obtained as a yellow solid (84%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1H, H_2), 8.08 (s, 1H, H_8), 6.03 (ddt, J = 21.1, 10.4, 5.3 Hz, 1H, H_{C2}), 5.29 (dd, J = 17.2, 1.5 Hz, 1H, H_{C3b}), 5.16 (dd, J = 10.3, 1.4 Hz, 1H, H_{C3a}), 4.24 (d, J = 12.4 Hz, 2H, H_{C1}). ^{13}C NMR (100 MHz, CD_3OD) δ 152.28, 151.90, 150.65, 144.22, 136.02, 126.18, 114.80, 41.99. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_8H_{10}N_5$: 176.0936, found 176.0927.

*4.1.11.2. 6-n-Butylaminopurine (**14c**).* From 6-chloropurine, using general procedure 1 and n-butylamine, compound **14c** was obtained as a white solid (91%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.51 (s, 1H, H_2), 7.99 (s, 1H, H_8), 3.38 (dt, J = 8.6, 5.2 Hz, 2H, H_{C1}), 1.55 (tt, J = 7.6, 5.3 Hz, 2H, H_{C2}), 1.44–1.33 (m, 2H, H_{C3}), 0.98 (t, J = 6.6 Hz, 3H, H_{C4}). ^{13}C NMR (100 MHz, CD_3OD) δ 152.54, 151.90, 150.71, 144.22, 124.57, 43.71, 30.87, 20.23, 14.02. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_{14}N_5$: 192.1249, found 192.1240.

*4.1.11.3. 2-Amino-6-n-butylaminopurine (**15c**).* From 2-amino-6-chloropurine, using general procedure 1 and *n*-butylamine, compound **15c** was obtained as a white solid (85%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.48 (s, 1H, H_8), 3.36 (dt, J = 19.4, 7.5 Hz, 2H, H_{C1}), 1.55 (qt, J = 7.7 Hz, 2H, H_{C2}), 1.45–1.30 (m, 2H, H_{C3}), 0.99 (t, J = 6.5 Hz, 3H, H_{C4}). ^{13}C NMR (100 MHz, CD_3OD) δ 158.84, 154.28, 153.25, 144.22, 117.38, 43.71, 30.87, 20.23, 14.02. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_9H_{15}N_6$: 207.1358; found 207.1356.

*4.1.11.4. 6-Cyclohexylaminopurine (**14d**).* From 6-chloropurine, using general procedure 1 and cyclohexylamine, compound **14d** was obtained as a white solid (92%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.50 (s, 1H, H_2), 8.00 (s, 1H, H_8), 3.28 (qt, J = 7.5 Hz, 1H, $H_{CHcyclohexyl}$), 1.95 (dt, J = 7.3, 5.7 Hz, 2H, $H_{CH2cyclohexyl}$), 1.72–1.65 (m, 3H, $H_{CH2cyclohexyl}$), 1.43 (dt, J = 7.3, 5.8 Hz, 2H, $H_{CH2cyclohexyl}$), 1.40–1.32 (m, 3H, $H_{CH2cyclohexyl}$). ^{13}C NMR (100 MHz, CD_3OD) δ 153.82, 152.42, 150.90, 144.22, 124.22, 52.78, 33.42, 33.11, 25.92, 24.92, 24.52. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{11}H_{16}N_5$: 218.1406; found 218.1413.

*4.1.11.5. 2-Amino-6-cyclohexylaminopurine (**15d**).* From 2-amino-6-chloropurine, using general procedure 1 and cyclohexylamine, compound **15d** was obtained as a white solid (92%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 1H, H_8), 3.50 (qt, J = 7.0 Hz, 1H, $H_{CHcyclohexyl}$), 1.88–1.80 (m, 2H, $H_{CH2cyclohexyl}$), 1.70–1.62 (m, 3H, $H_{CH2cyclohexyl}$), 1.47–1.40 (m, 2H, $H_{CH2cyclohexyl}$), 1.38–1.30 (m, 3H, $H_{CH2cyclohexyl}$). ^{13}C NMR (100 MHz, CD_3OD) δ 158.56, 154.58, 144.22, 115.76, 52.78, 33.42, 33.11, 25.92, 24.92, 24.52. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{11}H_{17}N_6$: 233.1515, found 233.1517.

*4.1.11.6. 6-(4-Isopropyl)phenylaminopurine (**14e**).* From 6-chloropurine, using general procedure 1 and 4-isopropylaniline, compound **14e** was obtained as a white solid (88%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 1H, H_2), 8.06 (s, 1H, H_8), 7.21 (d, J = 7.5 Hz, 2H, H_{arom}), 6.94–6.86 (m, 2H, H_{arom}), 3.04 (hept, J = 6.5 Hz, 1H, $H_{CHisopropyl}$), 1.33 (d, J = 6.4 Hz, 6H, $H_{CH3isopropyl}$). ^{13}C NMR (100 MHz, CD_3OD) δ 153.24, 149.26, 148.16, 144.22, 143.38, 138.38, 127.96, 127.56, 124.31, 123.63, 123.41, 34.20, 23.57, 23.17. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{14}H_{16}N_5$: 254.1406, found 254.1406.

*4.1.11.7. 2-Amino-6-(4-isopropyl)phenylaminopurine (**15e**).* From 2-amino-6-chloropurine, by using general procedure 1 and 4-isopropylaniline, compound **15e** was obtained as a white solid (83%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.79 (s, 1H, H_8), 7.69 (d, J = 8.5 Hz, 2H, H_{arom}), 7.20 (d, J = 8.5 Hz, 2H, H_{arom}), 2.88 (hept, J = 6.8 Hz, 1H, $H_{CHisopropyl}$), 1.24 (d, J = 6.9 Hz, 6H, $H_{CH3isopropyl}$). ^{13}C NMR (100 MHz, CD_3OD) δ 156.65, 153.24, 149.72, 144.22, 143.38, 138.38, 127.96, 127.56, 123.63, 123.41, 114.33, 34.20, 23.57, 23.17. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{14}H_{17}N_6$: 269.1515, found 269.1511.

*4.1.12. General procedure 2: for Mitsunobu reaction to obtain compounds **16a**–**f** and **17a**–**f***

To a dioxane solution (4.5 mL) of **3** (160 mg, 0.417 mmol), purine (0.627 mmol), and triphenylphosphine (164 mg, 0.627 mmol) under argon at 10 °C was added dropwise di-*iso*-propylazodicarboxylate (127 mg, 0.627 mmol). After 12 h stirring at room temperature, volatiles were evaporated, and residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$ 2:98) to give pure desired compound.

*4.1.12.1. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)adenine (**16a**).* From 6-aminopurine, using general procedure 2, compound **16a** was obtained as a colourless oil (45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H, H_2), 7.81 (s, 1H, H_8), 5.95–5.85 (m, 1H, $H_{2'}$), 5.74–5.60 (m, 7H, $H_{3'}$, $H_{O-CH2-O}$, H_{NH2}), 4.80 (t, J = 5.0 Hz, 2H, $H_{1'}$), 2.72 (dd, J = 22.6, 7.3 Hz, 2H, $H_{4'}$), 1.23 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 155.4, 153.1, 149.9, 140.1, 130.0, 129.8, 123.4, 123.2, 119.6, 81.6, 44.9, 38.7, 31.5, 30.1, 26.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.50. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{33}N_5O_7P$: 498.2118; found 498.2127.

*4.1.12.2. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-2,6-diaminopurine (**17a**).* From 2,6-diaminopurine, by using general procedure 2, compound **17a** was obtained as a colourless oil (41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H, H_8), 5.94–5.76 (m, 1H, $H_{2'}$), 5.71–5.58 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 5.54 (s, 2H, H_{NH2}), 4.80 (s, 2H, H_{NH2}), 4.61 (t, J = 5.2 Hz, 2H, $H_{1'}$), 2.69 (dd, J = 22.6, 7.2 Hz, 2H, $H_{4'}$), 1.21 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.26, 175.83, 164.27, 159.42, 149.75, 140.52, 133.44, 122.34, 121.87, 83.85, 82.95, 46.24, 39.86, 39.47, 32.31, 26.99, 26.78. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.42; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{21}H_{34}N_6O_7P$: 513.2227; found 513.2225.

*4.1.12.3. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-6-allylaminopurine (**16b**).* From **14b**, using general procedure 2, compound **16b** was obtained as a colourless oil (52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, H_2), 7.75 (s, 1H, H_8), 6.10–5.76 (m, 1H, $H_{2'}$), 5.75–5.54 (m, 5H, $H_{3'}$, $H_{O-CH2-O}$), 5.25 (ddd, J = 13.7, 11.6, 1.4 Hz, 2H, H_{C3}), 4.78 (t, J = 5.0 Hz, 2H, $H_{1'}$), 4.33 (d, J = 11.6 Hz, 2H, H_{C1}), 2.71 (dd, J = 22.6, 7.2 Hz, 2H, $H_{4'}$), 1.21 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.26, 175.83, 158.08, 152.42, 151.06, 141.07, 136.02, 133.44, 126.22, 121.87, 114.80, 83.85, 82.95, 46.24, 41.99, 39.86, 39.47, 32.31, 26.99, 26.78. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.57; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{24}H_{37}N_5O_7P$: 538.2431; found 538.2440 calculated.

*4.1.12.4. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-6-butylaminopurine (**16c**).* From **14c**, by using general procedure 2, compound **16c** was obtained as a colourless oil (49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H, H_2), 7.73 (s, 1H, H_8), 5.85–5.93 (m, 1H, $H_{3'}$), 5.71–5.58 (m, 5H, $H_{2'}$ $H_{O-CH2-O}$), 4.85 (d, J = 5.3 Hz, 1H, $H_{1'a}$), 4.74 (d, J = 5.4 Hz, 1H, $H_{1'b}$), 3.44 (t, J = 4.9 Hz, 1H, H_{C1a}), 3.35 (t, J = 4.9 Hz, 1H, H_{C1b}), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 2.01 (s, 1H, H_{NH}), 1.58 (ddt, J = 12.8, 7.6, 5.0 Hz, 2H, H_{C2}), 1.46–1.36 (m, 2H, H_{C3}), 1.28 (s, 18H, $H_{C(CH3)3}$), 0.99 (t, J = 6.6 Hz, 3H, H_{C4}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 157.21, 152.47,

151.00, 141.06, 133.43, 125.05, 121.87, 83.40, 46.23, 43.71, 39.66, 32.65, 31.97, 30.87, 26.88, 20.22, 14.01. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.48. HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{41}N_5O_7P$: 554.2744; found 554.2739.

*4.1.12.5. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-2-amino-6-butylaminopurine (**17c**).* From **15c**, using general procedure 2, compound **17c** was obtained as a colourless oil (49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H, H_8), 5.77–5.63 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.78 (dd, J = 9.2, 5.5 Hz, 2H, $H_{1'}$), 3.44 (t, J = 5.0 Hz, 1H, H_{C1a}), 3.35 (t, J = 4.9 Hz, 1H, H_{C1b}), 2.66 (dd, J = 11.9, 5.7 Hz, 2H, $H_{4'}$), 1.99 (s, 1H, H_{NH}), 1.58 (ddt, J = 12.8, 7.6, 5.0 Hz, 2H, H_{C2}), 1.44–1.35 (m, 2H, H_{C3}), 1.28 (s, 18H, $H_{C(CH3)3}$), 0.99 (t, J = 6.6 Hz, 3H, H_{C4}). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 161.23, 159.32, 150.89, 141.06, 133.43, 122.90, 121.87, 83.40, 46.23, 43.71, 39.66, 32.65, 31.97, 30.87, 26.88, 20.22, 14.01. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.50; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{25}H_{42}N_6O_7P$: 569.6170; found 569.6164.

*4.1.12.6. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-6-cyclohexylaminopurine (**16d**).* From **14d**, by using general procedure 2, compound **16d** was obtained as a colourless oil (42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H, H_2), 8.14 (s, 1H, H_8), 5.73–5.59 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.97 (d, J = 5.4 Hz, 1H, $H_{1'a}$), 4.48 (d, J = 5.4 Hz, 1H, $H_{1'b}$), 3.78 (tt, J = 7.9, 3.8 Hz, 1H, $H_{CHcyclohexyl}$), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 1.92 (dqd, J = 11.4, 5.7, 2.1 Hz, 2H, $H_{CH2cyclohexyl}$), 1.78 (s, 1H, H_{NH}), 1.72 (tdd, J = 11.5, 5.7, 2.2 Hz, 2H, $H_{CH2cyclohexyl}$), 1.67–1.52 (m, 6H, $H_{CH2cyclohexyl}$), 1.28 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 157.04, 151.05, 150.21, 141.06, 133.43, 124.13, 121.87, 83.40, 52.78, 46.23, 39.66, 33.31, 32.65, 31.97, 26.88, 25.91, 24.72. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.54; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{27}H_{43}N_5O_7P$: 580.6417; found 580.6410.

*4.1.12.7. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-2-amino-6-cyclohexylaminopurine (**17d**).* From **15d**, using general procedure 2, compound **17d** was obtained as a colourless oil (50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H, H_8), 5.73–5.60 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.92 (d, J = 5.3 Hz, 1H, $H_{1'a}$), 4.51 (d, J = 5.3 Hz, 1H, $H_{1'b}$), 3.71–3.62 (m, 1H, $H_{CHcyclohexyl}$), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 2.14–2.03 (m, 3H, H_{NH} and $H_{CH2cyclohexyl}$), 1.75–1.56 (m, 8H, $H_{CH2cyclohexyl}$), 1.30 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 159.80, 159.56, 151.27, 141.06, 133.43, 122.02, 121.69, 83.40, 52.78, 46.23, 39.66, 33.31, 32.65, 31.97, 26.88, 25.91, 24.72. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.51; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{27}H_{44}N_6O_7P$: 595.6333; found 595.6322.

*4.1.12.8. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-6-(4-isopropyl) phenylaminopurine (**16e**).* From **14e**, using general procedure 2, compound **16e** was obtained as a colourless oil (39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H, H_2), 7.87 (s, 1H, H_8), 7.72 (d, J = 7.5 Hz, 2H, H_{arom}), 7.22 (d, J = 7.5 Hz, 2H, H_{arom}), 5.72–5.59 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.96 (d, J = 5.4 Hz, 1H, $H_{1'a}$), 4.48 (d, J = 5.2 Hz, 1H, $H_{1'b}$), 3.86 (s, 1H, H_{NH}), 2.99 (hept, J = 6.4 Hz, 1H, $H_{CHisopropyl}$), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 1.34 (d, J = 6.4 Hz, 6H, $H_{CH3isopropyl}$), 1.30 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 153.12, 151.64, 143.37, 141.06, 138.38, 133.43, 127.76, 123.52, 123.04, 121.87, 83.40, 46.23, 39.66, 34.19, 32.65, 31.97, 26.88, 23.37. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.48; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{43}N_5O_7P$: 616.2900; found 616.2909.

*4.1.12.9. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-2-amino-6-(4-isopropyl)phenylaminopurine (**17e**).* From **15e**, by using general procedure 2, compound **17e** was obtained as a colourless oil (45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H, H_2), 7.22 (d, J = 7.5 Hz, 2H, H_{arom}), 6.93 (d, J = 7.5 Hz, 2H, H_{arom}), 5.71–5.58 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.91 (d, J = 5.2 Hz, 1H, $H_{1'a}$), 4.49 (d, J = 5.4 Hz, 1H, $H_{1'b}$), 3.89 (s, 1H, H_{NH}), 3.04 (hept, J = 6.3 Hz, 1H, $H_{CHisopropyl}$), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 2.11 (s, 2H, H_{NH2}), 1.33 (d, J = 6.4 Hz, 6H, $H_{CH3isopropyl}$), 1.30 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 161.65, 155.21, 150.38, 143.37, 141.06, 138.38, 133.43, 127.76, 123.52, 122.63, 121.87, 83.40, 46.23, 39.66, 34.19, 32.65, 31.97, 26.88, 23.37. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.42; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{30}H_{44}N_6O_7P$: 631.6748; found 631.6754.

*4.1.12.10. N^9-(4′-Bis(POM)-phosphinylbut-2′-enyl)-2-amino-6-methoxypurine (**17f**).* From 2-amino-6-methyoxypurine, by using general procedure 2, compound **17f** was obtained as a colourless oil (51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1H, H_8), 5.73–5.61 (m, 6H, $H_{2'}$, $H_{3'}$, $H_{O-CH2-O}$), 4.76 (d, J = 5.3 Hz, 1H, $H_{1'a}$), 4.56 (d, J = 5.4 Hz, 1H, $H_{1'b}$), 3.99 (s, 3H, H_{OMe}), 3.02 (s, 2H, H_{NH2}), 2.66 (dd, J = 11.9, 5.4 Hz, 2H, $H_{4'}$), 1.26 (s, 18H, $H_{C(CH3)3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.04, 164.04, 160.79, 149.49, 137.51, 133.43, 127.28, 121.87, 83.40, 53.95, 46.23, 39.66, 32.65, 31.97, 26.88. ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.51; HRMS (ESI): m/z $[M + H]^+$ calcd for $C_{22}H_{35}N_5O_8P$: 528.2223; found 528.2228.

4.2. Antiviral activity assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity or plaque formation in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV), and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus (1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures) or with 20 plaque forming units (PFU) (for VZV) in the presence of varying concentrations (100, 20,… μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity or viral plaque (VZV) plaque formation by 50%. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. Alternatively, the cytostatic activity of the test compounds was measured based on inhibition of cell growth. HEL cells were seeded at a rate of 5×10^3 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC_{50}, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls. The methodology of the anti-HIV assays was as follows: human CEM ($\sim 3 \times 10^5$ cells/cm^3) cells were infected with 100 $CCID_{50}$ of HIV(III_B) or HIV-2(ROD)/ml and seeded in 200 μL wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.08.042.

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