Bioorganic & Medicinal Chemistry 17 (2009) 3892–3899

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Phosphonate analogues of cyclopropavir phosphates and their *E*-isomers. Synthesis and antiviral activity

Santosh B. Mhaske [a], Bashar Ksebati [b], Mark N. Prichard [c], John C. Drach [d], Jiri Zemlicka [a,*]

[a] Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201-1379, USA
[b] Central Instrumentation Facility, Department of Chemistry, Wayne State University, Detroit, MI 48202, USA
[c] Department of Pediatrics, The University of Alabama School of Medicine, Birmingham, AL 35233, USA
[d] Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48018-3218, USA

ARTICLE INFO

Article history:
Received 2 March 2009
Revised 10 April 2009
Accepted 12 April 2009
Available online 17 April 2009

Keywords:
Methylenecyclopropanes
Phosphonates
Alkylation–elimination
Antiviral agents
Cyclopropavir
UL97 phosphotransferase

ABSTRACT

Z- and *E*-Phosphonate analogues **12** and **13** derived from cyclopropavir and the corresponding cyclic phosphonates **14** and **15** were synthesized and their antiviral activity was investigated. The 2,2-bis(hydroxymethylmethylenecyclopropane acetate (**17**) was transformed to tetrahydropyranyl acetate **18**. Deacetylation gave intermediate **19** which was converted to bromide **20**. Alkylation with diisopropyl methylphosphonate afforded after protecting group exchange (**21** to **22**) acetylated phosphonate intermediate **22**. Addition of bromine gave the dibromo derivative **16** which was used in the alkylation–elimination procedure with 2-amino-6-chloropurine to give *Z*- and *E*-isomers **23** and **24**. Hydrolytic dechlorination coupled with removal of all protecting groups gave the guanine phosphonates **12** and **13**. Cyclization afforded the cyclic phosphonates **14** and **15**. *Z*-Phosphonate **12** was a potent and non-cytotoxic inhibitor of human and murine cytomegalovirus (HCMV and MCMV) with EC_{50} 2.2–2.7 and 0.13 μM, respectively. It was also an effective agent against Epstein-Barr virus (EBV, EC_{50} 3.1 μM). The cyclic phosphonate **14** inhibited HCMV (EC_{50} 2.4–11.5 μM) and MCMV (EC_{50} 0.4 μM) but it was ineffective against EBV. Both phosphonates **12** and **14** were as active against two HCMV Towne strains with mutations in UL97 as they were against wild-type HCMV thereby circumventing resistance due to such mutations. *Z*-Phosphonate **12** was a moderate inhibitor of replication of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) but it was a potent agent against varicella zoster virus (VZV, EC_{50} 2.9 μM). The cyclic phosphonate **14** lacked significant potency against these viruses. *E*-isomers **13** and **15** were devoid of antiviral activity.

1. Introduction

Methylenecyclopropane analogues of nucleosides are established antiviral agents, particularly effective against herpes viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpes virus 6 and 8 (HHV-6 and HHV-8).[1] The most potent are the *Z*-isomers of purine nucleosides **1** and **2** although the individual *E*-isomers **3** and **4** (Chart 1) are active against EBV, especially in the series of fluorinated analogues.[2,3] The *Z*-guanine analogue **2b**, cyclopropavir, is under preclinical development as a potential drug against human cytomegalovirus (HCMV) infections.[4–6] It is accepted that methylenecyclopropane analogues follow the intracellular activation process (monophosphate–diphosphate–triphosphate) generally established for nucleosides and their analogues. In several cases, metabolically stable mimics of nucleoside phosphates, phosphonates, have yielded antiviral agents.[7,8] These include phosphonate derivatives[9–12] of antiherpetic drugs acyclovir (Zovirax), ganciclovir (Cytovene) **5a**, **5b** and the cyclic phosphonate **6**. By contrast, phosphonates of methylenecyclopropanes **7** and **8** did not exhibit antiviral potency[13] with a single exception of compound **7b** ($n = 1$) which was a moderate inhibitor of replication of varicella zoster virus (VZV).

Whereas the cyclopropavir phosphate (**9**) is an effective prodrug of the parent compound **2b**, the pattern of antiviral activity of the cyclic phosphate **10** is different.[14] Although it had limited potency against HCMV in Towne strain of the virus, it was effective against AD169 strain with efficacy comparable to the cyclic phosphate of ganciclovir **11**. Compound **10** also exhibited potent activity against hepatitis B virus (HBV). It was therefore of interest to synthesize phosphonate analogues **12–15** and investigate their antiviral activity.

2. Results and discussion

2.1. Synthesis

Alkylation–elimination method which had been successfully exploited[13] for synthesis of phosphonates **7** and **8** formed also the basis of our approach to analogues **12–15**. The suitably

* Corresponding author. Tel.: +1 313 578 4288; fax: +1 313 832 7294.
E-mail address: zemlicka@karmanos.org (J. Zemlicka).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.04.020

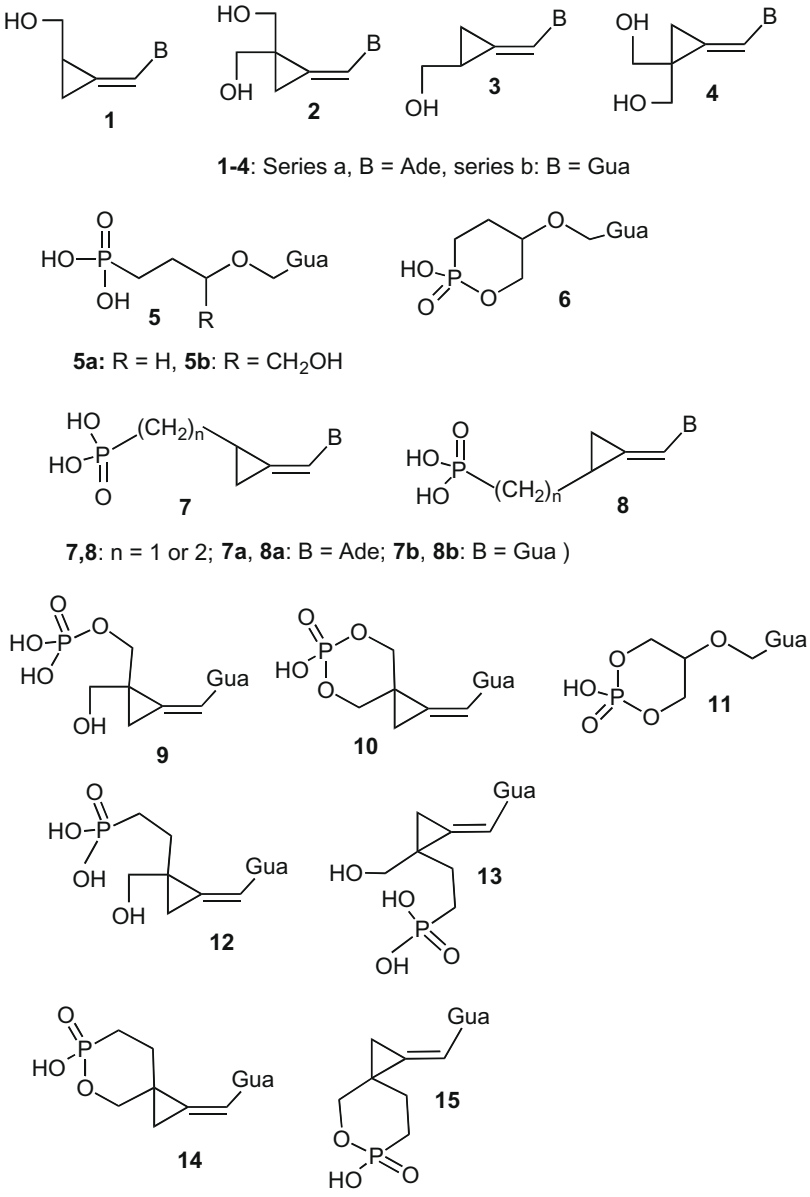
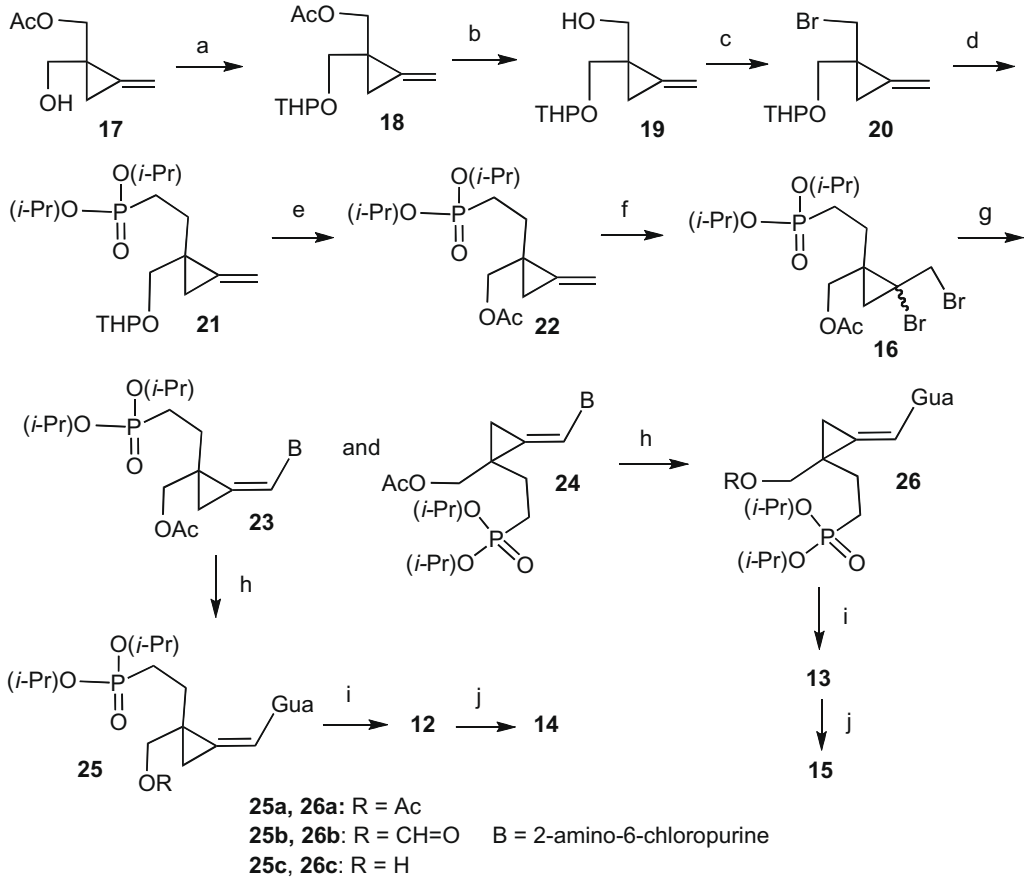


Chart 1.

protected reagent **16** for alkylation–elimination was prepared as follows (Scheme 1). The previously described[15] monoacetate **17** was converted to tetrahydropyranyl (THP) derivative **18** in 82% yield using 3,4-dihydro-2*H*-pyran in CH_2Cl_2 under acid catalysis. Ammonolysis afforded intermediate **19** (98%). The latter was converted to bromide **20** in 76% yield by CBr_3–triphenylphosphine reagent. This reagent is not compatible[16] with acid-labile groups like THP but inclusion of triethylamine in the reaction mixture successfully removed this obstacle. This modification may significantly expand use of the reagent. Reaction of **20** with lithium salt of diisopropyl methylphosphonate in THF gave the phosphonate intermediate **21** (81%). The presence of THP group was considered a potential liability for the bromination step and, therefore, the THP was replaced with acetyl in a single step[17] using acetyl chloride in CH_2Cl_2 to give acetate **22** in 92% yield. Addition of bromine using pyridinium perbromide in CH_2Cl_2 was uneventful to provide dibromo derivative **16** as a mixture of *cis,trans* isomers (92%).

Alkylation–elimination of 2-amino-6-chloropurine with **16** (Cs_2CO_3, DMF, 75 °C, 20 h) furnished *Z*- and *E*-isomers **23** and **24** which were separated by column chromatography on silica gel in 31% and 30% yield, respectively. Hydrolytic dechlorination of the *Z*-isomer **23** by 80% formic acid afforded after chromatographic separation a mixture of acetyl and formyl esters **25a** + **25b** in the ratio of 4:1 and 89% yield. A smaller amount (4%) of deacylated phosphonate **25c** was also obtained. In this case, conversion to guanine moiety was accompanied by a partial deacetylation followed by formylation. Formylation of hydroxy groups in the course of this procedure was observed before.[18] Dealkylation of **25a** + **25b** with trimethylsilyl bromide in DMF followed by ammonolysis, chromatography on DEAE Sephadex in NH_4HCO_3 buffer and then Dowex 1 ($HCO_2^{(-)}$) in formic acid gave phosphonate **12** in 81% yield as a free acid. Cyclization was performed using a protocol previously employed for the corresponding cyclic phosphate[14] **10** with *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine and *N*,*N*′-dicyclohexylcarbodiimide (DCC) in pyridine. Cyclic phosphonate **14** was obtained as a free acid with the aid of Dowex 50 ($H^{(+)}$) in 85% yield. In a similar fashion, hydrolytic dechlorination of the *E*-isomer **24** furnished a mixture of acetyl and formyl esters **26a** + **26b** in the



Scheme 1. Reagents and conditions: (a) 3,4-dihydro-2*H*-pyran, $MeSO_3H$, CH_2Cl_2; (b) NH_3, MeOH, Δ; (c) CBr_4, Ph_3P, NEt_3, CH_2Cl_2; (d) $CH_3P(O)(i\text{-}PrO)_2$, BuLi, THF; (e) AcCl, CH_2Cl_2; (f) pyridine·HBr_3, CH_2Cl_2; (g) (1) B–H, Cs_2CO_3, DMF, Δ; (2) chromatography; (h) 80% HCO_2H, Δ; (i) (1) Me_3SiBr, DMF; (2) NH_4OH; (3) chromatography; (j) *N*,*N*′-dicyclohexyl-4-mopholinecarboxamidine, DCC, pyridine; (2) NH_4OH; (3) Dowex 50 ($H^{(+)}$).

ratio of 4:1 and 88% yield as well as deacetylated product **26c** (5%). Dealkylation, ammonolysis and work-up employed for the *Z*-isomer **12** gave the *E*-phosphonate **13** (80%). Cyclization as described above then afforded the cyclic *E*-phosphonate **15** in 82% yield.

2.2. The *Z*- and *E*-isomeric assignment

The UV spectra of intermediates **23**, **24** (λ_{max} 311 nm) and final products **12**, **13** (λ_{max} 267–268 nm) have indicated that the phosphonylated side chain is attached in the 9 position of the purine base as found also for phosphonates[13] **7** and **8**. As far as the *Z*/*E* isomerism is concerned, a general trend[13] that *Z*-isomers are less polar (moving faster on silica gel) than *E*-isomers was also observed with compounds **23** and **24**. In addition, the $C_{3'}$ chemical shifts of the *Z*-isomers are more shielded than those of *E*-isomers (Table 1). This was also found in analogues **7** and **8** and their derivatives.[13] However, the reversed pattern of the $C_{4'}$ chemical shifts found in analogues **7** and **8** was not followed possibly because of a quarternary character of $C_{4'}$. Final confirmation of the *Z*- and *E*-assignment came from the NOE experiments with compounds **23** and **24** (Table 2). In the *Z*-isomer **23**, the NOE enhancements were observed between the *cis*-related atoms such as the H_8 of the heterocyclic base in an *anti*-like conformation and $H_{4''}$ and $H_{5'}$ and between the $H_{1'}$ and $H_{3'}$. As expected, the strongest interactions in the *E*-isomer **22** were observed between the H_8 and $H_{3'}$ as well as $H_{1'}$ and $H_{4''}$, $H_{6'}$, $H_{5'}$. A weaker NOE enhancements were found between the $H_{1'}$ and CH_3 of the acetyl and isopropyl groups.

Table 1
Comparison of the $C_{3'}$ ^{13}C NMR chemical shifts of isomeric methylenecyclopropane phosphonates

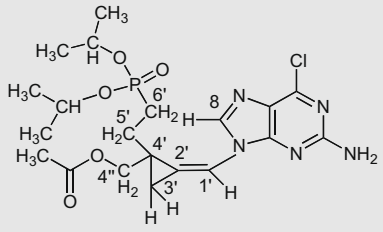
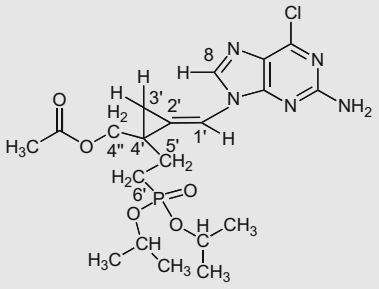
Compound[a]	Isomer	$C_{3'}$ (ppm)	Compound[a,b]	Isomer	$C_{3'}$ (ppm)
23	*Z*	12.3	**7**[c,d,e]	Z	8.8
24	*E*	17.0	**8**[c,d,e]	*E*	11.2
25c	*Z*	11.9	**7b**[e]	*Z*	9.1
26c	*E*	15.9	**8b**[e]	*E*	11.4
12	*Z*	11.8	**7b**	*Z*	8.1[f]
13	*E*	16.0	**8b**	*E*	10.6[f]

[a] CD_3SOCD_3 as solvent unless stated otherwise. For numbering of atoms see Table 2.
[b] The values are from Ref. 13.
[c] $CDCl_3$.
[d] B = 2-Amino-6-chloropurine.
[e] Diisopropyl ester, *n* = 2.
[f] Sodium salt, D_2O, *n* = 2.

2.3. Antiviral activity

All phosphonates were tested against the following viruses: Human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), hepatitis B virus (HBV) and hepatitis C virus (HCV). As mentioned at the outset, the first series of phosphonates of methylenecyclopropanes **7** and **8** designed as analogues of acyclovir phosphonates **5a** lacked any significant antiviral activity.[13] It is therefore surprising (and

Table 2
NOE data of the *Z*- and *E*-isomers **23** and **24** (500 MHz, $CDCl_3$).

Compound	H_{irr}	δ	H_{obs}	δ	NOE
23 	H_8	8.10	CH_3 of Ac	1.97	1.50, 2.66[a]
	H_8	8.10	$H_{5'}$	2.20	1.81
	H_8	8.10	$H_{4''}$	3.83	0.84
	$H_{5'}$	2.20	H_8	8.10	3.85, 4.73[a]
	$H_{4''}$	3.83	H_8	8.10	0.85, 0.95[a]
	$H_{3'}$	1.46	$H_{1'}$	7.26	2.43, 4.24[a]
	$H_{3'}$	1.39	$H_{1'}$	7.26	1.47, 2.89[a]
	$H_{1'}$	7.26	$H_{3'}$	1.46	0.55
	$H_{1'}$	7.26	$H_{3'}$	1.39	0.51
	$H_{1'}$	7.26	$H_{3'}$	1.39 + 1.46	0.64
24 	H_8	8.17	$H_{3'}$	1.66	1.54
	H_8	8.17	$H_{3'}$	1.58	1.37
	$H_{3'}$	1.66	H_8	8.17	2.16, 3.82[a]
	$H_{3'}$	1.58	H_8	8.17	2.28, 3.16[a]
	$H_{1'}$	7.46	$H_{4''}$	4.16	0.04
	$H_{1'}$	7.46	$H_{4''}$	4.03	0.28
	$H_{1'}$	7.46	$H_{6'}$, $H_{5'}$	1.84	1.10
	$H_{1'}$	7.46	CH_3 of Ac	2.09	0.17
	$H_{1'}$	7.46	CH_3 of *i*-Pr	1.30	0.21
	CH_3 of Ac	2.09	$H_{1'}$	7.46	0.13

[a] Two different scans.

rewarding) that among analogues **12**–**15** new potent antivirals were found. Thus, the *Z*-isomeric phosphonate **12** and the corresponding cyclic derivative **14** which can be regarded as mimics of ganciclovir phosphonate **5b** and cyclic phosphonate **6**, are potent and non-cytotoxic inhibitors of replication of HCMV comparable to ganciclovir (Table 3). Analogues **12** and **14** were virtually equipotent against the Towne strain of HCMV with EC_{50}'s 2.2 and 2.4 μM, respectively. Against the AD169 strain, phosphonate **12** was somewhat more effective (EC_{50} 2.7 μM) than the cyclic derivative **14** (EC_{50} 11.6 μM). In comparison with the cyclic phosphate[14] **10**, cyclic phosphonate **14** was significantly more effective in Towne strain and somewhat less potent in AD169 strain. Both phosphonates **12** and **14** inhibited murine cytomegalovirus (MCMV) with EC_{50} 0.13 and 0.4 μM, respectively, surpassing the cyclic phosphate[14] **10**. Comparison with cyclopropavir[4] (**2b**) reveals reduced potency of the phosphonates **12** and **14** against HCMV in vitro but against MCMV the efficacy was about the same (Table 3). A distinct advantage of phosphonates **12** and **14** is circumventing the first step of activation (phosphorylation) of cyclopropavir (**2b**) most likely catalyzed by HCMV UL97 phosphotransferase.[5] Therefore, analogues **12** and **14** may overcome resistance of cyclopropavir (**2b**) caused by mutations in this enzyme.[19,20] To test this hypothesis, cyclopropavir (**2b**) and its phosphonates **12** and **14** were assayed in two strains of Towne HCMV with mutations in UL97 that are known to produce resistance to cyclopropavir.[19,20] Strain E8 has two point mutations[19] in UL97 whereas strain 2696r has most of UL97 sequence deleted.[20] In both strains, cyclopropavir (**2b**) was 13–47 times less active than in wild-type Towne strain (Table 4). In contrast, phosphonates **12** and **14** were as active against both mutant strains as they were against wild-type Towne strain of HCMV. We conclude, therefore, that **12** and **14** do circumvent the necessity of UL97 to phosphorylate cyclopropavir (**2b**) to produce their activity.

Table 3
Inhibition of human and murine cytomegalovirus (HCMV and MCMV) and Epstein-Barr virus (EBV) replication by methylenecyclopropane phosphonates

Compound	EC_{50}/CC_{50} (μM)			
	HCMV/HFF			
	Towne[a,b]	AD169[c,d]	MCMV/MEF[a]	EBV/Akata[e]
2b	0.46/>100[f]	0.49/>380[a,f]	0.27/>380[f]	0.22/46[g]
10	20/>100[h]	6.0/>301[a,h]	7.2/>301[h]	0.96/150[h,i]
12	2.2/>100	2.7/>300[a]	0.13/100	3.1/>100
13	>100/>100	>300/>300	—	>100/>100
14	2.4/>100	11.6/>300[a]	0.4/>100	>100/>100
15	>100/>100	>300/>300	—	>100/>100
Control	2.9/>100[j]	0.09/>100[a,j]	2.6/>100[j]	8.4/>100[k]

[a] Plaque reduction assay in HFF cultures.
[b] Visual cytotoxicity of HFF's in cells unaffected by virus.
[c] Cytopathic effect (CPE) assay.
[d] Cytotoxicity by neutral red uptake.
[e] DNA hybridization assay.
[f] Ref. 4.
[g] Ref. 21.
[h] Ref. 14.
[i] Daudi cells, viral capsid antigen (VCA) assay. The EC_{50}/CC_{50} in H-1 cells (DNA hybridization assay) was >20/>100 μM, cytotoxicity was determined in CEM cells.
[j] Ganciclovir.
[k] Acyclovir.

The cyclic phosphonate **14** may be either a prodrug of **12** or have an entirely different mechanism of action. It is interesting that against EBV in Akata cells, only the *Z*-isomeric phosphonate **12** was effective (EC_{50} 3.1 μM) whereas the corresponding cyclic phosphonate **14** was inactive. This precludes that the latter analogue can function as a prodrug of phosphonate **12** under the conditions of this assay. Interestingly, the cyclic phosphate **10** was active against EBV although the assays used were different.[14]

Table 4
Activity of phosphonates **12** and **14** against drug resistant HCMV

Compound	EC_{50}[a] (μM) Virus strain		
	Towne[b]	2696r[c]	E8[d]
12	3.5	3	3.2
14	4	3	4
Cyclopropavir (**2b**)	0.6	28	8

[a] Data from a plaque reduction assay with four drug concentrations in duplicate.
[b] Wild-type virus from which isolates 2696r and E8 were obtained.
[c] Virus isolated for resistance to cyclopropavir (**3b**) that has a truncated UL97 gene.[20]
[d] Virus with two point mutations[19] introduced into gene UL97.

Table 5
Inhibition of herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV) replication by methylenecyclopropane phosphonates

Compound	EC_{50}/CC_{50} (μM)			
	HSV-1/BSC-1[a]	HSV-1/HFF[b,c]	HSV-2/HFF[b,d]	VZV/HFF[b,d]
2b	>100/>100[e]	>380/>380[e]	>380[e]	>380[e]
10	20/>100[f]	>301/>301[f]	242[f]	>301[f]
12	15/>100	59.4/>300	76.5	2.9[g]
13	100/>100	>300/>300	>300	191
14	40/>100	>300/>300	>300	>300
15	100/>100	>300/>300	>300	>300
Acyclovir	0.3	1.3/>300	1.2	4.4[g]

[a] ELISA in BSC-1 cells was used for compounds **2b** and **12**; other compounds were assayed by plaque reduction in BSC-1 cells. Cytotoxicity was determined in replicating KB cells.
[b] Cytopathic effect (CPE) assay.
[c] Cytotoxicity by neutral red uptake.
[d] For cytotoxicity see HSV-1.
[e] Ref. 4.
[f] Ref. 14.
[g] Plaque reduction assay.

Against α-herpes viruses, the most potent activity was found for analogue **12** in varicella zoster virus (VZV), EC_{50} 2.9 μM whereas cyclic phosphonate **14** and cyclopropavir[4] (**2b**) were ineffective (Table 5). Interestingly, phosphonate **7b** ($n = 1$) was also effective against VZV but to a much lesser extent[13] (EC_{50} 24 μM) than **12**. Similar to other methylenecyclopropane analogues,[1] only moderate activity was found against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2). Phosphonate **12** was the most effective against HSV-1 in a plaque assay in BSC-1 cells (EC_{50} 15 μM) but it was less potent against HSV-1 and HSV-2 in a cytopathic effect (CPE) inhibition assay in HFF cultures. Regardless, we hypothesize that the activity of **12** against the α-herpes viruses is the result of delivering this monophosphate analogue into virus-infected cells thereby circumventing the necessity of an initial phosphorylation step by a viral-specified kinase. This would explain the activity of **12** against the α-herpes viruses compared to the inactivity of **2b**.

All analogues including the cyclic phosphonate **14** were inactive against HBV and HCV. By contrast, the cyclic phosphate **10** is an effective anti-HBV agent.[14] The *E*-isomers **13** and **15** were devoid of potency against all tested viruses.

3. Conclusion

Phosphonates **12**, **13**, **14** and **15** were synthesized and they were evaluated for antiviral activity. The *Z*-phosphonates **12** and **14** were effective inhibitors of replication of HCMV and MCMV in HFF and MEF culture. Compounds **12** and **14** also inhibited two Towne strains of HCMV with mutations in UL97. Phosphonate **12** was effective against EBV in Akata cells and VZV in HFF culture whereas cyclic phosphonate **14** was inactive. Analogue **12** was a moderate inhibitor of HSV-1 and HSV-2. The *E*-isomers **13** and **15** were devoid of antiviral activity.

4. Experimental

4.1. General methods

The UV spectra were measured in ethanol and NMR spectra were determined on Varian instruments at 300, 400 or 500 MHz (^1H), 75 or 100 MHz (^{13}C) and 121 or 161 MHz (^{31}P) in $CDCl_3$ unless stated otherwise. Mass spectra were determined in electrospray ionization (ESI-MS) mode using methanol–sodium acetate or by negative ESI-MS.

4.2. 2-Acetoxymethyl-2-(tetrahydropyranyloxy)methyl-1-methylenecyclopropane (18)

To a solution of monoacetate[15] **17** (5.0 g, 32.04 mmol) and 3,4-dihydro-2*H*-pyran (7.31 mL, 80.1 mmol) in CH_2Cl_2 (50 mL) was added methanesulfonic acid (0.03 mL, 0.46 mmol) in CH_2Cl_2 (2 mL) dropwise with stirring at 0 °C. The stirring was continued for 6 h. The reaction was quenched with triethylamine (0.07 mL, 0.50 mmol), the solvent was evaporated, the residue was dissolved in ethyl acetate (60 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (3×20 mL) and brine (3×20 mL) whereupon it was dried ($MgSO_4$) and the solvent was evaporated. The crude product was chromatographed on a silica gel column using ethyl acetate–hexane (0.5:10) to furnish compound **18** (6.3 g, 82%) as a sirup. ^1H NMR (400 MHz) δ 5.52, 5.48 (2t, $J = 2$–3 Hz, 1H), 5.41 (s, 1H, CH_2=), 4.63, 4.61 (2t, $J = 3$–4 Hz, 1H, CHO of THP), 4.17–4.05 (m, 2H, CH_2OAc), 3.81 (m, 1H), 3.68 (t, $J = 9.6$ Hz, 1H, CH_2OTHP), 3.46 (m, 1H), 3.39 (dd, $J = 10.0$, 3.4 Hz, 1H, CH_2O of THP), 2.05 (s, 3H, CH_3), 1.8–1.5 (cluster of m, 6H, $3 \times CH_2$ of THP), 1.29–1.26 (m, 2H, H_3). ^{13}C NMR (100 MHz) 171.3 (C=O), 135.5, 135.1 (C=), 105.11, 105.05 (CH_2=), 98.6, 98.2 (CHO of THP), 69.2, 68.9, 66.51, 66.48, 62.3, 61.9 (CH_2O), 30.8, 30.7, 25.68, 25.65, 19.6, 19.3 ($3 \times CH_2$ of THP), 23.9, 23.8 (C_2), 21.2 (CH_3), 14.1, 13.9 (C_3). ESI-MS 241 (7.4, M+H), 263 (100.0, M+Na). Anal. Calcd for $C_{13}H_{20}O_4$: C, 64.98; H, 8.39. Found: C, 65.08; H, 8.35.

4.3. 2-Hydroxymethyl-2-(tetrahydropyranyloxy)methyl-1-methylenecyclopropane (19)

A solution of compound **18** (5.7 g, 23.74 mmol) in NH_3/MeOH (30%, 100 mL) was stirred at 0 °C for 30 min and at room temperature for 16 h. The volatile components were evaporated and the product **19** obtained as a sirup (4.6 g, 98%) was used directly in the next step. For analysis, a sample of **19** was chromatographed on a silica gel column using ethyl acetate–hexane (1:5). ^1H NMR (400 MHz) δ 5.50, 5.48 (2t, $J = 2.4$ Hz, 1H), 5.40 (poorly resolved t, 1H, CH_2=), 4.63 (2 overlapped t, 1H, CHO of THP), 3.94–3.36 (cluster of m, 6H, CH_2O), 2.69, 2.61 (2t, $J = 6$ Hz, 1H, OH), 1.84–1.52 (cluster of m, 6H, $3 \times CH_2$ of THP), 1.31–1.20 (m, 2H, H_3). ^{13}C NMR (100 MHz) 135.9, 135.5 (C=), 104.5, 104.4 (CH_2=), 99.22, 99.15 (CHO of THP), 72.2, 71.9, 67.29, 67.27, 62.81, 62.7 (CH_2O), 30.83, 30.78, 25.5, 19.9, 19.8 ($3 \times CH_2$ of THP), 26.6, 26.5 (C_2), 14.1, 14.0 (C_3). ESI-MS 199 (5.0, M+H), 221 (100.0, M+Na). Anal. Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.34; H, 9.38.

4.4. 2-Bromomethyl-2-(tetrahydropyranyloxy)methyl-1-methylenecyclopropane (20)

Triphenylphosphine (25.15 g, 95.9 mmol) in CH_2Cl_2 (25 mL) was added to a solution of CBr_4 (31.8 g, 95.9 mmol) in CH_2Cl_2 (75 mL) at −5 °C with stirring which continued for another

10 min. Triethylamine (16.04 mL, 0.12 mol) was added, followed by a dropwise addition of compound[3] **19** (3.8 g, 19.18 mmol) in CH_2Cl_2 (15 mL) over a period of 10 min. Reaction was complete in 30 min. The reaction mixture was diluted with hexane (130 mL), the insoluble portion was filtered and it was washed with hexane (50 mL). The filtrate was concentrated and the crude product was chromatographed on a silica gel column using ethyl acetate–hexane (0.2:10) to furnish compound **20** (3.79 g, 76%) as a sirup. ^{1}H NMR (400 MHz) δ 5.56, 5.54 (2t, J = 2.8–3.0 Hz, 1H), 5.37 (poorly resolved d, 1H, CH_2=), 4.66, 4.64 (partially overlapped 2t, J = 3.6 Hz, 1H, CHO of THP), 3.89–3.47 (cluster of m, 6H, CH_2Br, CH_2O), 1.89–1.49 (m, 6H, 3 × CH_2 of THP), 1.43–1.42 (m, 1H), 1.31–1.28 (2 poorly resolved t, 1H, H_3). ^{13}C NMR (100 MHz) 137.6, 137.5 (C=), 104.83, 104.79 (CH_2=), 98.9, 98.5 (CHO of THP), 69.1, 68.9, 62.5, 62.1 (CH_2O), 39.1 (CH_2Br), 30.8, 30.7, 25.67, 25.65, 19.7, 19.4 (3 × CH_2 of THP), 26.1, 26.0 (C_2), 17.2, 17.1 (C_3). ESI-MS 283, 285 (98.8, 100.0, M+Na). Anal. Calcd for $C_{11}H_{17}BrO_2{\cdot}0.05H_2SiO_3$: C, 49.84; H, 6.50. Found: C, 49.90; H, 6.47.

4.5. 2-[(2-Diisopropylphosphono)ethyl]-2-(tetrahydropyranyloxymethyl-1-methylenecyclopropane (21)

1-Butyllithium (1.6 M in hexanes, 12.1 mL, 19.29 mmol) was added to a solution of diisopropyl methylphosphonate (3.35 mL, 18.15 mmol) in THF (25 mL) at −78 °C with stirring which was continued for 30 min. Compound **20** (2.95 g, 11.34 mmol) in THF (15 mL) was then slowly added over a period of 10 min and the reaction mixture was stirred for 2 h at −78 °C. The reaction mixture was then allowed to warm to room temperature and, after 30 min, it was quenched with saturated aqueous NH_4Cl (15 mL). Ethyl acetate (80 mL) was added, the organic phase was washed with brine (3 × 30 mL), saturated aqueous $NaHCO_3$ (3 × 30 mL) and brine (3 × 30 mL). After drying ($MgSO_4$), the solvent was evaporated and the crude product was chromatographed on a silica gel column using ethyl acetate–hexane (2:1) to furnish phosphonate **21** (3.3 g, 81%) as a sirup. ^{1}H NMR δ 5.44, 5.41 (poorly resolved 2t, 1H), 5.35 (s, 1H, CH_2=), 4.70–4.57 (m, 3H, CH of *i*-PrO, CHO of THP), 3.82 (m, 1H), 3.47 (m, 1H, CH_2O of THP), 3.66, 3.22 and 3.59, 3.32 (2AB's, J = 10.4 and 10.6 Hz, 2H, CH_2OTHP), 1.93–1.49 (cluster of m, 10H, CH_2 of THP, H_4 and H_5), 1.30–1.28 (2 poorly resolved d, 12H, CH_3), 1.17–1.04 (m, 2H, H_3). ^{13}C NMR 138.3, 137.7 (C=), 104.0, 103.8 (CH_2=), 98.6, 98.1 (CHO of THP), 71.3, 70.7 (CH_2O of THP), 70.0 (d, J = 6.7 Hz, CH of *i*-PrO), 62.4, 62.0 (CH_2OTHP), 30.8, 30.7, 25.68, 25.65, 19.7, 19.4 (CH_2 of THP), 26.84, 26.80 (2 overlapped d, J = 4.5 Hz, C_4), 24.58, 24.54 (2d, J = 140.2 Hz, C_5), 24.57, 24.36 (C_2), 24.3 (d, J = 3.8 Hz, CH_3), 15.1, 14.7 (C_3). ^{31}P NMR (161 MHz) 31.24. ESI-MS 361 (6.3, M+H), 283 (100.0, M+Na). Anal. Calcd for $C_{18}H_{33}O_5P$: C, 59.98; H, 9.23. Found: C, 60.24; H, 9.44.

4.6. 2-(Acetoxymethyl)-2-[2-(diisopropylphosphono)ethyl]-1-methylenecyclopropane (22)

Acetyl chloride (8.9 mL, 0.13 mol) was added to a solution of phosphonate **21** (3.0 g, 8.33 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at room temperature for 6 h, it was concentrated to a half of its original volume and the reaction was quenched with saturated aqueous $NaHCO_3$ (30 mL). Ethyl acetate (50 mL) was then added and the organic layer was washed with saturated aqueous $NaHCO_3$ (3 × 25 mL) and brine (3 × 25 mL). After drying ($MgSO_4$), the solvents were evaporated and the crude product was chromatographed on a silica gel column using ethyl acetate–hexane (1:1) to give acetate **22** (2.44 g, 92%) as a sirup.

^{1}H NMR (400 MHz) δ 5.47, 5.40 (2 poorly resolved t, 2H, CH_2=), 4.67 (m, 2H, CH of *i*-PrO), 3.97 (s, 2H, CH_2OAc), 2.06 (s, 3H, CH_3 of Ac), 1.92–1.62 (cluster of m, 4H, H_4 and H_5), 1.30 (d, J = 2.4 Hz), 1.29 (d, 1.6 Hz, 12H, CH_3 of *i*-PrO), 1.20, 1.11 (poorly resolved t of AB, J_{AB} = 10 and 9.2 Hz, 2H, H_3). ^{13}C NMR (100 MHz) 171.2 (C=O), 136.7 (C=), 104.8 (CH_2=), 70.1 (d, J = 6.7 Hz, CHO of *i*-PrO), 68.0 (CH_2OAc), 26.6 (d, J = 4.5 Hz, H_4), 24.4 (d, J = 140.9 Hz, H_5), 24.2 (d, J = 4.4 Hz, CH_3 of *i*-PrO), 23.5 (d, J = 21.5 Hz, C_2), 21.1 (CH_3 of Ac), 14.9 (C_3). ^{31}P NMR (161 MHz) 30.57. ESI-MS 319 (13.3, M+H), 341 (100.0, M+Na). Anal. Calcd for $C_{15}H_{27}O_5P$: C, 56.59; H, 8.55. Found: C, 56.56; H, 8.64.

4.7. (*cis,trans*)-2-(Acetoxymethyl)-2-[2-(diisopropylphosphono)ethyl]-1-bromo-1-bromomethylcyclopropane (16)

Pyridinium hydrobromide perbromide (3.32 g, 10.37 mmol) was added in three portions to a solution of acetate **22** (2.2 g, 6.92 mmol) in CH_2Cl_2 (50 mL) at-20 °C. Reaction mixture was stirred for 1 h whereupon it was diluted with diethyl ether (50 mL). The precipitate was filtered off and it was washed with diethyl ether (25 mL). The organic phase was washed with saturated aqueous $Na_2S_2O_3$ (3 × 25 mL), water (3 × 25 mL) and brine (3 × 25 mL). The solvents were evaporated to give dibromophosphonate **16** (3.03 g, 92%) as a sirup. ^{1}H NMR (400 MHz) δ 4.69 (m, 2H, CH of *i*-PrO), 4.43–3.68 (4 overlapped AB's, 4H, CH_2Br, CH_2OAc), 2.10, 2.08 (2s, 3H, CH_3 of Ac), 2.27–2.19, 2.04–1.67 (cluster of m, 4H, H_4 and H_5), 1.24–1.36 (cluster of d, CH_3 of *i*-PrO), 1.12 (d, J = 7.6 Hz, part of H_3 obscured by CH_3, total 14H). ^{31}P NMR (161 MHz) 29.55, 29.44. ESI-MS 477, 479, 481 (M+H, 9.6, 16.7, 7.4), 499, 501, 503 (M+Na, 47.5, 100.0, 49.4), Anal. Calcd for $C_{15}H_{27}Br_2O_5P$: C, 37.68; H, 5.69. Found: C, 37.72; H, 5.73.

4.8. (*Z*)- and (*E*)-2-Amino-6-chloro-9-{[2-(2-diisopropylphosphonoethyl)-2-(acetoxymethyl)cyclopropylidene]methyl}purine (23 and 24)

A mixture of dibromophosphonate **16** (2.7 g, 5.67 mmol), 2-amino-6-choropurine (0.96 g, 5.67 mmol) and Cs_2CO_3 (9.24 g, 28.36 mmol) in DMF (30 mL) was stirred at room temperature for 5 h and at 75 °C for 20 h. After cooling, the insoluble portion was filtered off and it was washed with DMF (10 mL). The solvent was evaporated in vacuo and the crude product was chromatographed on a silica gel, using methanol–CH_2Cl_2 (0.3:10) to obtain the *Z*-isomer **23** (850 mg, 31%) followed by *E*-isomer **24** (820 mg, 30%).

Z-Isomer **23**: Mp 174–176 °C. UV λ_{max} 311 nm (ε 7,700), 231 (ε 26,700). ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 8.16 (s, 1H, H_8), 7.28 (s, 1H, $H_{1'}$), 7.05 (br s, 2H, NH_2), 4.46 (m, 2H, CH of *i*-PrO), 4.27, 4.02 (AB, J = 11.7 Hz, 2H, $H_{4''}$), 2.05–1.74 (m, 2H, $H_{6'}$, overlapped with 1.89 (s, CH_3 of Ac, 3H), 1.68–1.42 (m, $H_{5'}$ partially overlapped with split AB, J = 9.2 Hz, 4H, $H_{3'}$), 1.19–1.12 (m, 12H, CH_3 of *i*-PrO). ^{13}C NMR (75 MHz) 170.6 (C=O, Ac), 160.8, 153.2, 150.4, 140.9, 123.9 (purine), 120.6 ($C_{2'}$), 112.4 ($C_{1'}$), 70.0 (d, J = 6 Hz, CH of *i*-PrO), 68.2 ($C_{4''}$), 26.5 (d, J = 22.2 Hz, $C_{5'}$), 25.2 (d, J = 2.8 Hz, $C_{4'}$), 24.4–24.3 (2 overlapped d, J = 4.0 Hz, CH_3 of *i*-PrO), 22.8 (d, J = 140.1 Hz, $C_{6'}$), 21.0 (CH_3 of Ac), 12.3 ($C_{3'}$). ^{31}P NMR (121 MHz) 30.57. ESI-MS 486, 488 (M+H, 14.4, 3.8). 508, 510 (M+Na, 100.0, 33.7). Anal. Calcd for $C_{20}H_{29}ClN_5O_5P$: C, 49.44; H, 6.02; N, 14.41. Found: C. 49.62; H, 6.05; N, 14.33.

E-Isomer **24**: Mp 86 °C. UV λ_{max} 311 (ε 7,400), 229 nm (ε 28,600). ^{1}H NMR (400 MHz, CD_3SOCD_3) δ 8.42 (s, 1H, H_8), 7.43 (s, 1H, $H_{1'}$), 7.01 (br s, 2H, NH_2), 4.51 (m, 2H, CH of *i*-PrO), 4.11, 3.97 (AB, J = 11.2 Hz, 2H, $H_{4''}$), 2.03 (s, 3H, CH_3 of Ac), 1.82–1.59 (s + cluster of m, 6H, $H_{5'}$ and $H_{6'}$ overlapped with $H_{3'}$), 1.20 (d, J = 6 Hz, 12H, CH_3 of *i*-PrO). ^{13}C NMR (100 MHz) 171.0 (C=O), 160.8, 153.3, 150.3, 140.2, 123.8 (purine), 120.0 ($C_{2'}$), 111.8 ($C_{1'}$), 69.9 (d, J = 5.9 Hz), 67.2 ($C_{4''}$), 26.6 (d, J = 5 Hz, $C_{4'}$), 24.5, 24.4, 24.2 (2 overlapped d, $C_{5'}$ and CH_3 of *i*-PrO), 23.9 (d, J = 138.7 Hz, $C_{6'}$), 21.4 (CH_3

of Ac), 17.0 ($C_{3'}$). ^{31}P (161 MHz) 30.21. ESI-MS 486, 488 (M+H, 19.1, 5.0). 508, 510 (M+Na, 100.0, 19.8, 32.3). Anal. Calcd for $C_{20}H_{29}ClN_5O_5P$: C, 49.44; H, 6.02; N, 14.41. Found: C, 49.34; H, 5.99; N, 14.38.

4.9. (*Z*)-9-{[2-(2-Diisopropylphosphono)ethyl)-2-(acetoxy/formyloxy/methyl)cyclopropylidene]methyl}guanine (25a + 25b) and (*Z*)-9-{[2-(2-diisopropylphosphono)ethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}guanine (25c)

A solution of compound **23** (600 mg, 1.24 mmol) in formic acid (80%, 30 mL) was heated at 70 °C for 6 h. Formic acid was evaporated in vacuo, the residue was dissolved in water and the solution was lyophilized. The crude product was chromatographed on a silica gel column using methanol–CH_2Cl_2 (0.5:10) to obtain a mixture of acetate and formate **25a** + **25b** (510 mg, 89%). The ratio **25a/25b** determined from the acetyl and formyl ^{1}H NMR signals was 4:1.

Further elution of the column using methanol–CH_2Cl_2 (1:5) gave hydroxymethyl phosphonate **25c**. Rechromatography in methanol–CH_2Cl_2 (0.5:10) afforded **27c** (21 mg, 4%).

Z-Isomers **25a** + **25b**. UV λ_{max} 271, 229 nm. ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 10.70 (s, 1H, NH), 8.25 (s, 0.25H, CH═O), 7.78, 7.77 (2 overlapped s, 1H, H_8), 7.19, 7.17 (2 overlapped s, 1H, $H_{1'}$), 6.56 (s, 2H, NH_2), 4.47–4.45 (m, 2H, CH of *i*-PrO), 4.30, 3.98 (2 overlapped AB's, J = 11.4 Hz, 2H, $H_{4''}$), 2.01, 1.95 (2 overlapped s, 2.25H, CH_3 of Ac), 1.72–1.19 (cluster of m, 6H, $H_{5'}$, $H_{6'}$, $H_{3'}$), 1.19–1.13 (cluster of d, 12H, CH_3 of *i*-PrO). ^{13}C NMR (100 MHz) 170.7 (C═O of Ac), 162.7 (CH═O), 157.3, 154.7, 150.6, 134.7, 118.9, 118.3, 117.1, 112.6, 112.4 (guanine, $C_{2'}$, $C_{1'}$), 71.0, 70.0 (2 overlapped d, J = 5.2 Hz, CH of *i*-PrO), 68.1, 67.6 ($C_{4''}$), 26.1 (2 overlapped d, J = 21.6 and 22.1 Hz, $C_{5'}$), 25.3 (poorly resolved d, $C_{4'}$), 24.4, 24.36, 24.6 (2 overlapped d, CH_3 of *i*-PrO), 23.0 (d, J = 141.9 Hz), 22.9 (d, J = 140.4 Hz, $C_{6'}$), 21.1 (CH_3 of Ac), 12.4 ($C_{3'}$). ^{31}P NMR (121 MHz) 30.29, 30.19. ESI-MS 454 (M+H, **25b**, 30.0), 468 (M+H, **25a**, 100.0), 476 (M+Na, **25b**, 32.3), 490 (M+Na, **25a**, 100.0).

Z-Isomer **25c**. Mp 232–234 °C. UV λ_{max} 273 (ε 12,300), 231 nm (ε 30,100). ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 10.66 (br s, 1H, NH), 8.21 (s, 1H, H_8), 7.09 (s, 1H, $H_{1'}$), 6.57 (s, 2H, NH_2), 5.25 (poorly resolved t, 1H, OH), 4.45 (m, 2H, CH of *i*-PrO), 3.80, 3.22 (poorly resolved split AB, J = 12.0 Hz, 2H, $H_{4''}$), 2.01–1.94 and 1.69–1.32 (2 poorly resolved m, 4H, $H_{5'}$, $H_{6'}$), 1.31, 1.25 (AB, J = 8.7 Hz, 2H, $H_{3'}$), 1.17–1.12 (poorly resolved m, 12H, CH_3 of *i*-PrO). ^{13}C NMR (75 MHz) 157.3, 154.7, 150.4, 134.5, 120.1 (guanine), 117.0 ($C_{2'}$), 111.1 ($C_{1'}$), 69.9 (d, J = 4.0 Hz, CH of *i*-PrO), 65.4 ($C_{4''}$), 28.7 (d, J = 21.1 Hz, $C_{5'}$), 24.9 (d, J = 3.8 Hz, $C_{4'}$), 24.4 (d, J = 4.0 Hz, CH_3 of *i*-PrO), 23.1 (d, J = 141.0 Hz), 11.9 ($C_{3'}$). ^{31}P NMR (121 MHz) 30.57. ESI-MS 426 (M+H, 100.0), 448 (M+Na, 30.9). Anal. Calcd for $C_{18}H_{28}N_5O_5P{\cdot}0.2H_2O$: C, 50.38; H, 6.99; N, 16.33. Found: C, 50.38; H, 6.65; N, 16.12.

4.10. (*E*)-9-{[2-(2-Diisopropylphosphonoethyl)-2-(acetoxy/formyloxy/methyl)cyclopropylidene]methyl}guanine (26a + 26b) and (*E*)-9-{[2-(2-diisopropylphosphono)ethyl)-2-(hydroxymethyl)cyclopropylidene]methyl}guanine (26c)

The procedure described above for the *Z*-isomers **25a** + **25b** and **25c** was repeated with the *E*-isomer **24** (600 mg, 1.24 mmol) to give **26a** + **26b** (502 mg, 88%) and **26c** (26 mg, 5%). The ratio **26a**/**26b** determined as described above for **25a**/**25b** was 4:1.

E-Isomers **26a** + **26b**. UV λ_{max} 271, 229 nm. ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 10.67 (br s, 1H, NH), 8.29 (s, 0.2H, CH═O), 8.03 (s, 1H, H_8), 7.33 (poorly resolved t, 1H, $H_{1'}$), 6.53 (s, 2H, NH_2), 4.52 (m, 2H, CH of *i*-PrO), 4.20, 4.12 and 4.11, 3.97 (2AB, J = 11.6 Hz, 2H, $H_{4''}$), 2.04 (s, 2.4H, CH_3 of Ac), 1.9–1.5 (m, 6H, $H_{5'}$, $H_{6'}$, $H_{3'}$), 1.21, 1.20 (2 partly overlapped d, J = 6–6.3 Hz, CH_3 of *i*-PrO). ^{31}P NMR (121 MHz, CD_3SOCD_3) 30.21. ESI-MS 454 (M+H of **26b**, 11.8), 468 (M+H of **26a**, 39.1), 476 (M+Na of **26b**, 12.1), 490 (M+Na of **26a**, 100.0).

E-Isomer **26c**. Mp 154–156 °C. UV λ_{max} 271 nm (ε 11,100), 228 (ε 27,800). ^{1}H NMR (300 MHz, CD_3SOCD_3) δ 10.77 (br s, 1H, NH), 8.02 (s, 1H, H_8), 7.27 (s, 1H, $H_{1'}$), 6.61 (s, 2H, NH_2), 4.85 (t, J = 5.6 Hz, 1H, OH), 4.52 (m, 2H, CH of *i*-PrO), 3.37 ($H_{4''}$, overlapped with H_2O), 1.94–1.58 (m, 4H, $H_{5'}$, $H_{6'}$), 1.49 (s, 2H, $H_{3'}$), 1.22–1.19 (2 poorly resolved d, 12H, CH_3 of *i*-PrO).

^{13}C NMR (75 MHz) 157.4, 154.7, 150.6, 134.4, 120.2 (guanine), 117.0 ($C_{2'}$), 111.0 ($C_{1'}$), 69.9 (d, J = 7.0 Hz, CH of *i*-PrO), 65.0 ($C_{4''}$), 27.0 (d, J = 20.2 Hz, $H_{5'}$), 26.4 (d, J = 4.3 Hz, $H_{4'}$), 24.1 (d, J = 139.0 Hz, $H_{6'}$), 24.5 (d, J = 3 Hz, CH_3 of *i*-PrO), 15.9 ($C_{3'}$). ^{31}P NMR (121 MHz) 30.84. ESI-MS 448 (M+Na, 100.0), 426 (M+H, 90.6). Anal. Calcd for $C_{18}H_{28}N_5O_5P{\cdot}1.2H_2O$: C, 48.34; H, 6.71; N, 15.67. Found: C, 47.98; H, 6.46; N, 15.45.

4.11. (*Z*)-9-{[2-(Hydroxymethyl)l-2-(2-phosphonoethyl)-cyclopropylidene]methyl}guanine (12)

Bromotrimethylsilane (3.74 mL, 28.90 mmol) was added dropwise to a solution of phosphonate **25a** + **25b** (450 mg, 0.97 mmol) in DMF (20 mL) at room temperature with stirring which was continued for 24 h. The solvent was evaporated in vacuo and the residue was dissolved in aqueous NH_4OH (30%, 30 mL). After stirring for 3 h at room temperature, the volatile components were evaporated and the aqueous solution of the crude product was lyophilized. It was chromatographed on DEAE Sephadex (40–120 mesh, $HCO_3^{(-)}$) column, using a linear gradient of 0–0.3 M (500 mL each) NH_4HCO_3. The fractions containing the compound **12** were lyophilized. The product was loaded on Dowex-1 column (X2, 200 mesh, $HCO_2^{(-)}$) which was eluted first with water (100 mL) followed by formic acid (0.8 M, 800 mL). The fractions containing the compound were lyophilized to give phosphonate **12** (286 mg, 81%) as a white solid, mp: >300 °C. UV λ_{max} (pH 7) 268 nm (ε 12,800), 230 (ε 29,800). ^{1}H NMR (400 MHz, D_2O, sodium salt) δ 7.97 (s, 1H, H_8), 7.05 (s, 1H, $H_{1'}$), 3.80, 3.55 (AB, J = 12.2 Hz, 2H, $H_{5''}$), 2.06, 1.73 (2 m, 2H), 1.43, 1.41 (m overlapped with s, 4H, $H_{5'}$, $H_{6'}$, $H_{3'}$). ^{13}C NMR (100 MHz, CD_3SOCD_3) 157.3, 154.6, 150.3, 135.0, 120.3, 117.0, 110.8 (guanine, $C_{2'}$, $C_{1'}$), 65.4 ($C_{4''}$), 29.01 (d, J = 20.2 Hz, $C_{5'}$), 25.5 (d, J = 8.2 Hz, $C_{4'}$), 24.6 (d, J = 136.5 Hz, $C_{6'}$), 11.8 ($C_{3'}$). ^{31}P NMR (121 MHz, D_2O, sodium salt) 23.83. Negative ESI-MS 340 (M−H). Anal. Calcd for $C_{12}H_{16}N_5O_5P{\cdot}0.8H_2O$: C, 40.52; H 4.99; N, 19.69. Found: C, 40.50; H, 5.01; N, 19.59.

4.12. (*E*)-9-{[2-(Hydroxymethyl)l-2-(2-phosphonoethyl)-cyclopropylidene]methyl}guanine (13)

The *E*-isomer **13** was prepared from a mixture of acetate and formate **26a** + **26b** (450 mg, 0.98 mmol) as described above for *Z*-isomer **12** to give phosphonate **13** (279 mg, 80%) as a white solid, mp >300 °C. UV λ_{max} (pH 7) 267 nm (ε 14,500), 230 (ε 38,800). ^{1}H NMR (300 MHz, D_2O) δ 8.23 (s, 1H, H_8), 7.22 (s, 1H, $H_{1'}$), 3.56, 3.48 (AB, J = 11.7 Hz, 2H, $H_{4''}$), 1.84 (m, 1H), 1.68–1.54 (m, 3H, $H_{5'}$, $H_{6'}$), 11.47 (s, 2H, $H_{3'}$). ^{13}C NMR (100 MHz, CD_3SOCD_3) 157.4, 154.6, 150.5, 134.3, 120.7, 116.9, 110.6 (guanine, $C_{2'}$, $C_{1'}$), 65.1 ($H_{4''}$), 27.5 (d, J = 20.2 Hz, $C_{5'}$), 27.2 ($C_{4'}$), 26.2 (d, J = 135.8 Hz, $H_{6'}$), 16.0 ($C_{3'}$). ^{31}P NMR (121 MHz, D_2O) 26.91. Negative ESI-MS 340 (M−H, 100.0). Anal. Calcd for $C_{12}H_{16}N_5O_5P{\cdot}0.9H_2O$: C, 40.32; H, 5.02; N, 19.59. Found: C, 40.35; H, 5.15; N, 19.61.

4.13. *Z*-Cyclic phosphonate 14

A mixture of phosphate **12** (150 mg, 0.44 mmol), DCC (727 mg, 3.52 mmol) and *N*,*N*′-dicyclohexyl-4-morpholinecarboxamidine (194 mg, 0.66 mmol) in pyridine (15 mL) was refluxed under N_2 with stirring for 12 h. Pyridine was evaporated in vacuo and water

(50 mL) was added to the residue. The aqueous layer was extracted with dichloromethane (5 × 25 mL) and it was filtered through a cotton plug. Aqueous NH_3 (30%, 10 mL) was added and the resulting reaction mixture was stirred overnight at room temperature. The volatile components were evaporated and the product was absorbed on Dowex-50 column (WX 2, 200 mesh, $H^{(+)}$) which was eluted with water (600 mL). The appropriate fractions were concentrated to give cyclic phosphonate **14** (121 mg, 85%) as a white solid, mp >300 °C. UV λ_{max} (H_2O) 268 nm (ε 12,800), 227 (ε 28,700). ^{1}H NMR (300 MHz, D_2O) δ 8.54 (s, 1H, H_8), 7.06 (s, 1H, $H_{1'}$), 4.10, 3.84 (2t, J = 10.8, 11.9 Hz, 2H, $H_{4''}$), 2.00 (m, 1H), 1.83–1.39 (m, 3H, $H_{5'}$, $H_{6'}$), 1.54, 1.45 (AB, J = 9.3 Hz, 2H, $H_{3'}$ partly overlapped with $H_{5'}$, $H_{6'}$). ^{13}C NMR (100 MHz, CD_3SOCD_3) 157.3, 154.6, 150.3, 135.8, 117.4, 117.2, 113.3 (guanine, $C_{2'}$, $C_{1'}$), 73.2 (d, J = 5.0 Hz, $H_{4''}$), 29.8 (d, J = 8.1 Hz, $C_{4'}$), 25.4 (d, J = 8.2 Hz, $C_{5'}$), 23.6 (d, J = 126.9 Hz, $C_{6'}$), 15.7 ($C_{3'}$). ^{31}P NMR (121 MHz, D_2O) 22.41. Negative ESI-MS 322 (M−H, 100.0). Anal. Calcd for $C_{12}H_{14}N_5O_4P{\cdot}H_2O$: C, 42.23; H, 4.73; N, 20.52. Found: C, 42.45; H, 4.83; N, 20.26.

4.14. *E*-Cyclic phosphonate 15

The *E*-isomer **13** (150 mg, 0.44 mmol) was subjected to the same procedure as *Z*-isomer **12** (see above) to give cyclic phosphonate **15** (116 mg, 82%) as a white solid, mp >300 °C. UV λ_{max} (H_2O) 268 nm (ε 10,400), 229 (ε 28,500). ^{1}H NMR (300 MHz, D_2O, sodium salt) δ 7.96 (s, 1H, H_8), 7.22 (s, 1H, $H_{1'}$), 3.94, 3.86 (split AB, J = 12.9 Hz, 2H, $H_{4''}$), 1.87 (dt, J = 18.9, 6.0 Hz, 2H, $H_{6'}$), 1.74–1.63 (m, 2H, $H_{5'}$), 1.57, 1.49 (split AB, 2H, $H_{3'}$). ^{13}C NMR (100 MHz, CD_3SOCD_3) 157.4, 154.7, 150.7, 134.3, 118.6, 116.9, 111.4 (guanine, $C_{2'}$, $C_{1'}$), 73.3 (d, J = 5.2 Hz), 29.8 (d, J = 7.5 Hz), 24.4 (d, J = 126.9 Hz, $C_{6'}$), 22.8 (d, J = 8.2 Hz, $C_{4'}$), 16.5 ($C_{3'}$). ^{31}P NMR (121 MHz, D_2O, sodium salt) 22.52. Negative ESI-MS 322 (M−H, 100.0). Anal. Calcd for $C_{12}H_{14}N_5O_4P{\cdot}H_2O$: C, 42.23; H, 4.73; N, 20.52. Found: C, 42.25; H, 4.85; N, 20.17.

4.15. Antiviral assays

The antiviral assays were performed as described previously.[4,15] The HCMV assays were performed using HFF cell culture with two strains of virus, Towne and AD169, in a plaque reduction or cytopathic effect (CPE) inhibition assay. MCMV was assayed in mouse embryonic fibroblasts (MEF) by plaque reduction. The EBV DNA hybridization assay was run in Akata cells. The HSV-1 assays were performed in BSC-1 cells by ELISA and, together with HSV-2, in HFF cells by CPE inhibition assays. The VZV assays were run in HHF culture using CPE or plaque reduction assays. The results are summarized in Tables 3–5.

Acknowledgements

We thank L. M. Hryhorczuk from the Central Instrumentation Facility, Department of Chemistry, Wayne State University for mass spectra. We also thank Kathy Borysko and Julie Breitenbach of the University of Michigan for performing the antiviral assays. The work described herein was supported by US Public Health Service Grant RO1-CA32779 and contract NO1-AI30049 from the National Institutes of Health, Bethesda, Maryland.

References and notes

1. Zemlicka, J. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; Elsevier: Amsterdam, 2007; pp 113–165.
2. Zhou, S.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Matsumi, S.; Mitsuya, H.; Zemlicka, J. *J. Med. Chem.* **2004**, *47*, 6964.
3. Zhou, S.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Tamiya, S.; Mitsuya, H.; Zemlicka, J. *J. Med. Chem.* **2006**, *49*, 6120.
4. Zhou, S.; Breitenbach, J. M.; Borysko, K. Z.; Drach, J. C.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Zemlicka, J. *J. Med. Chem.* **2004**, *47*, 566.
5. Kern, E. R.; Kushner, N. L.; Hartline, C. B.; Williams-Azziz, S. L.; Harden, E. A.; Zhou, S.; Zemlicka, J.; Prichard, M. N. *Antimicrob. Agents Chemother.* **2005**, *49*, 1039.
6. Kern, E. R.; Bidanset, D. J.; Hartline, C. B.; Yan, Z.; Zemlicka, J.; Quenelle, D. C. *Antimicrob. Agents Chemother.* **2004**, *48*, 4745.
7. *Nucleotide Analogues as Antiviral Agents*; Martin, J. C., Ed.ACS Symposium Series 401; American Chemical Society: Washington, DC, 1989.
8. Holy, A. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K., Ed.; Elsevier: Amsterdam, 2002; pp 167–238.
9. Prisbe, E. J.; Martin, J. C.; McGee, D. P. C.; Barker, M. F.; Smee, D. F.; Duke, A. E.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1986**, *29*, 671.
10. Reist, E. J.; Sturm, P. A.; Pong, R. Y.; Tanga, M. J.; Sidwell, R. W. In *Nucleotide Analogues as Antiviral Agents*; Martin, J. C., Ed.; ACS Symposium Series 401; American Chemical Society: Washington, DC, 1989; pp 17–34.
11. Reist, E. J.; Bradford, W. W., III; Ruhland-Frisch, B. L.; Sturm, P. A.; Zaveri, N. T.; Huffman, J.; Sidwell, R. W. *Nucleosides Nucleotides* **1994**, *13*, 539.
12. Huffman, J. H.; Sidwell, R. W.; Morrison, A. G.; Coombs, J.; Reist, E. J. *Nucleosides Nucleotides* **1994**, *13*, 607.
13. Guan, H.-P.; Qiu, Y.-L.; Ksebati, M. B.; Kern, E. R.; Zemlicka, J. *Tetrahedron* **2002**, *58*, 6047.
14. Yan, Z.; Kern, E. R.; Gullen, E.; Cheng, Y.-C.; Drach, J. C.; Zemlicka, J. *J. Med. Chem.* **2005**, *48*, 91.
15. Li, C.; Prichard, M. N.; Korba, B. E.; Drach, J. C.; Zemlicka, J. *Bioorg. Med. Chem.* **2008**, *16*, 2148.
16. Wagner, A.; Heitz, M.-P.; Mioskowski, C. *Tetrahedron Lett.* **1989**, *30*, 557.
17. Bakos, T.; Vincze, I. *Synth. Commun.* **1989**, *19*, 523.
18. Qiu, Y.-L.; Ksebati, M. B.; Ptak, R. G.; Fan, B. Y.; Breitenbach, J. M.; Lin, J.-S.; Cheng, Y.-C.; Kern, E. R.; Drach, J. C.; Zemlicka, J. *J. Med. Chem.* **1998**, *41*, 10.
19. Breitenbach, J. M.; Borysko, K. Z.; Zemlicka, J.; Drach, J. C. 19th International Conference on Antiviral Research, San Juan, Puerto Rico; May 7–11, 2006, Abstract 61, *Antiviral Res.* **2006**, *70*, A69.
20. Borysko, K. Z.; Gentry, B. G.; Breitenbach, J. M.; Zemlicka, J.; Drach, J. C. 21st International Conference on Antiviral Research, Montreal, Quebec, Canada, April 13–17, 2008, Abstract 98, *Antiviral Res.* **2008**, *78*, A54.
21. Prichard, M. N.; Daily, S. L.; Jefferson, G. M.; Perry, A. L.; Kern, E. R. *J. Virol. Methods* **2007**, *144*, 86.