Pergamon

Tetrahedron 58 (2002) 6047–6059

TETRAHEDRON

Synthesis of phosphonate derivatives of methylenecyclopropane nucleoside analogues by alkylation–elimination method and unusual opening of cyclopropane ring

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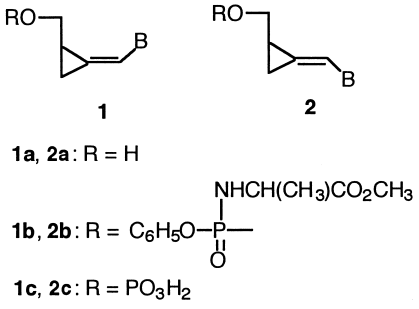
Received 14 March 2002; revised 4 June 2002; accepted 6 June 2002

Abstract—The synthesis of phosphonates of methylenecyclopropane nucleoside analogues **15a**–**18a**, **15b**–**18b** and **15c**–**18c** by alkylation–elimination approach is described. In a foreshortened series, methanesulfonate **19** was transformed by Michaelis–Becker reaction with diethyl or diisopropyl phosphite to methylenecyclopropane phosphonates **20a** or **20b**. The latter were converted to vicinal dibromides **21a** and **21b** which were then used for alkylation–elimination of nucleic acid bases (adenine) or precursors (2-amino-6-chloropurine and N^4-acetylcytosine). The intermediary *Z*+*E*-isomers **22a**+**23a** and **22b**+**23b** were dealkylated with bromo- of iodotrimethylsilane to free phosphonic acids **15a**, **16a** and phosphonate with an open cyclopropane ring **25** which were separated by ion exchange chromatography on Dowex 1. Phosphonate diesters **22c** and **23c** were separated by chromatography on silica gel, they were hydrolyzed to guanine derivatives **22d** and **23d** which were then dealkylated to give target analogues **15b**, **16b** and products of addition of hydrogen bromide or iodide across the double bond **26a** or **26b**. The *E*+*Z*-isomers **22e**+**23e** were converted to cytosine phosphonates **15c**+**16c** and cyclic phosphonate with an open cyclopropane ring **27a**. In a homologous series of phosphonates, dibromocyclopropane **34** was converted to intermediate **31** by reaction with diisopropyl methyl phosphonate and subsequent β-elimination. Compound **31** was transformed to vicinal dibromide **36**, a key component for alkylation–elimination of nucleic acid bases. The rest of the synthetic sequence followed the scheme described for the series of lower homologues to give the *Z*-isomeric phosphonates **17a**, **17b**, *E*-isomers **18a**, **18b** and *E*+*Z*-isomers **17c**+**18c** as the final products. All methylenecyclopropane phosphonates were devoid of antiviral activity with the exception of guanine derivative **15b** which inhibited the replication of varicella zoster virus (VZV) and it was non-cytotoxic.

1. Introduction

Recently, we have described a new series of nucleoside analogues where a furanose ring is replaced with methylenecyclopropane system. The purine *Z*-isomers **1a** have a broad-spectrum antiviral activity whereas pyrimidines or *E*-isomers **2a** are effective only exceptionally.[1,2] Conversion of purine analogues **1a** or **2a** to phosphoralaninate pronucleotides **1b** or **2b** improved in many cases the antiviral potency of less potent parent compounds.[3] These results have indicated that pronucleotides **1b** and **2b** are capable of delivering the free monophosphates **1c** and **2c** into the virus-infected cells. The latter metabolites are then processed to the respective di- and triphosphates in a manner similar to other nucleoside analogues.[4]



The transformation of parent analogues **1a** and **2a** to lipophilic pronucleotides **1b** and **2b** is just one strategy to introduce phosphorylated metabolites into the virus-infected cells. Alternately, it has been shown that phosphonate analogues of nucleotides which are resistant to enzymatic dephosphorylation and able to penetrate cell membrane can function as surrogates for the corresponding monophosphates. Several such phosphonates derived from furanose or acyclic nucleoside analogues exhibited antiviral activity. They can be divided into two classes: (i) 'foreshortened' phosphonates where the nucleic acid base is separated

Keywords: methylenecyclopropane nucleoside analogues; alkylation–elimination; cyclopropane ring.
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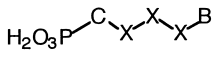
0040–4020/02/$ - see front matter
PII: S0040-4020(02)00589-6

from the phosphorus by a chain of four atoms (formula **3**), one atom shorter than in the respective nucleoside phosphates **4** and (ii) 'full-length' analogues (formula **5**) where the five-atom chain of nucleotides **4** ($C_{1'}$–$O_{1'}$–$C_{4'}$–$C_{5'}$–$O_{5'}$) is preserved. Examples of nucleoside phosphonates with antiviral activity include foreshortened analogues[5–8] **6**–**8** as well as full-length derivatives[7,9–13] **9**–**14**. Phosphonates where the chain length between a heterocyclic base and phosphonate moiety exceeds five atoms[14,15] or is shorter than four atoms[16] lack antiviral activity.



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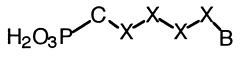
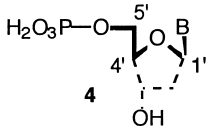


5

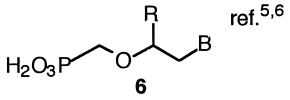
3, **5**: X = C or O



4

B = nucleic acid base

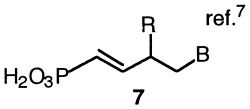
Foreshortened phosphonates:



ref.[5,6]

6

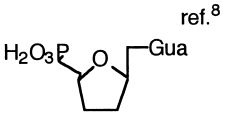
R = H, CH_2OH or CH_2F



ref.[7]

7

R = H or CH_2OH

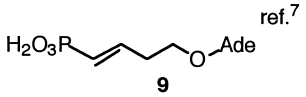


ref.[8]

8

B = nucleic acid base

'Full-length' phosphonates:



ref.[7]

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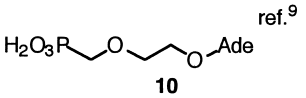

ref.[9]

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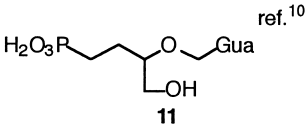

ref.[10]

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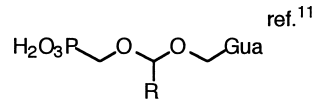
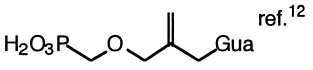


ref.[11]

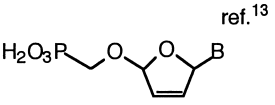
12

R = H or CH_2OH



ref.[12]

13

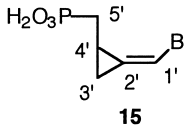


ref.[13]

14

B = nucleic acid base

Therefore, it was of interest to investigate synthetic approaches to methylenecyclopropane phosphonates **15**–**18**. For reasons mentioned above, both foreshortened (series **15** and **16**) and full-length analogues (series **17** and **18**) were of interest. Although cyclopropane phosphonates[17–20] including those comprising a nucleic acid base[21,22] are known, methylenecyclopropane phosphonates have not been described to the best of our knowledge. Alkylation–elimination approach which had been successfully exploited for synthesis of methylenecyclopropane analogues of nucleosides,[1,2] was considered as a convenient strategy. A key element in this method is the synthesis of an alkylating agent comprising a preformed phosphonate moiety that could serve for alkylation of a nucleic acid base or suitable precursor.



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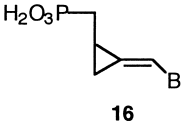

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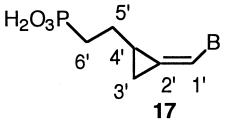

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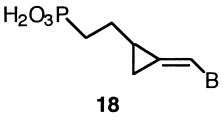
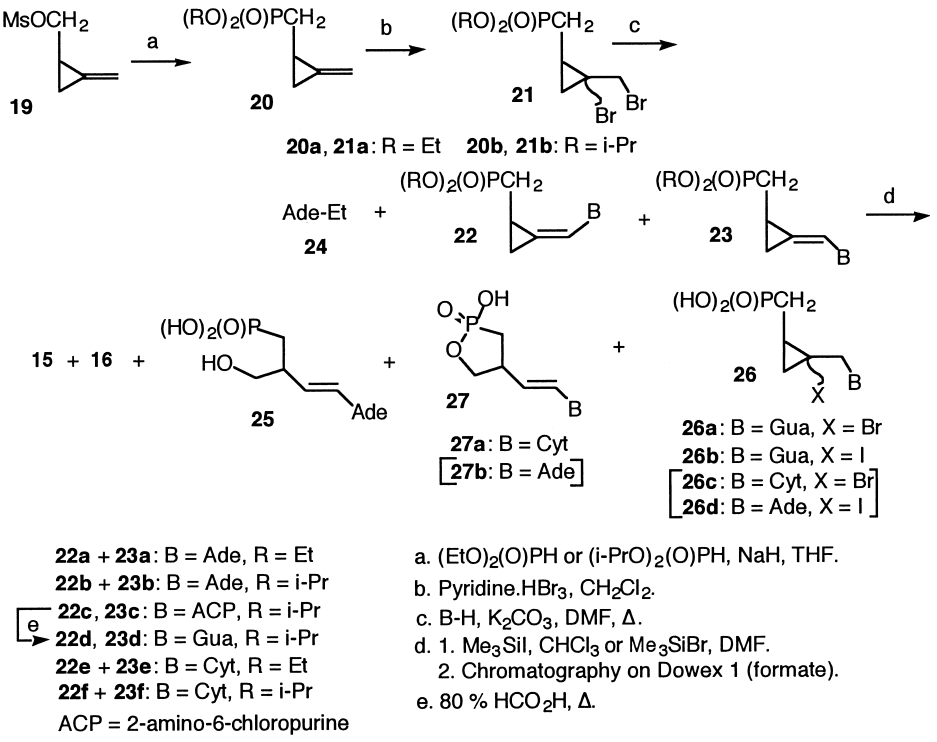


18

15a, **16a**, **17a**, **18a**: B = Ade
15b, **16b**, **17b**, **18b**: B = Gua
15c, **16c**, **17c**, **18c**: B = Cyt

2. Synthesis

A. *Foreshortened phosphonates*. For the synthesis of phosphonates **15** and **16** the known[23] (methylenecyclopropyl)-methyl methanesulfonate (**19**) served as a convenient starting material (Scheme 1). The Michaelis–Becker reaction of **19** with diethyl phosphite using NaH in THF gave diethyl (methylenecyclopropyl)methyl-phosphonate (**20a**) in 88% yield. In a similar fashion, diisopropyl phosphite afforded phosphonate **20b** (84%). Addition of bromine to **20a** via pyridinium tribromide gave *Z*,*E*-dibromo phosphonate **21a** in 96% yield. The latter intermediate was used for alkylation–elimination procedure with adenine using K_2CO_3 in DMF at 110°C to give a mixture of *Z*- and *E*-isomers **22a**+**23a** (57%) and 9-ethyladenine (**24**,



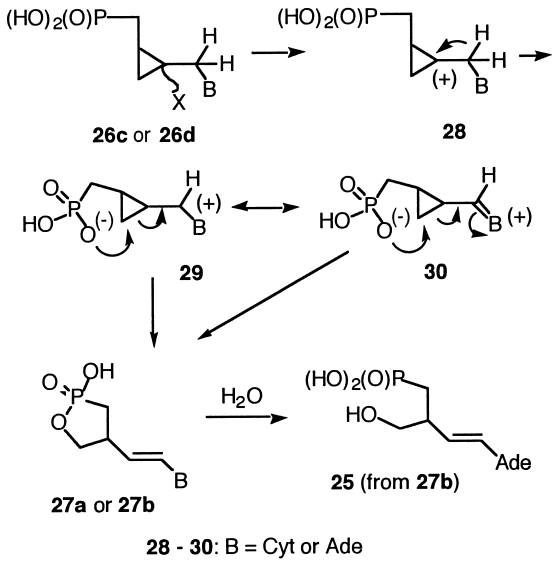
Scheme 1.

28%) arising from a competitive ethylation with diethyl phosphonate **21a**. Similar side reaction was described in alkylation of adenine with other dimethyl or diethyl phosphonate derivatives[24,25] but it was avoided by using diisopropyl esters.[25] Therefore, diisopropyl phosphonate **20b** was transformed to dibromide **21b** which was used in situ for alkylation–elimination with adenine to give *Z*+*E* isomeric mixture **22b**+**23b** in 69% yield. As expected, no alkylated adenine was detected. Isomeric mixtures **22a**+**23a** or **22b**+**23b** were not separable on a silica gel column. Therefore, the *Z*+*E*-isomers **22a**+**23a** were dealkylated[24] with Me_3SiI in $CHCl_3$ at −40°C to afford free phosphonates which were separated by chromatography on Dowex 1 (formate) using formic acid as an eluent. The *Z*-isomer **15a** (17%) was eluted first followed by *E* isomer **16a** (24%) and an unusual product of cyclopropane ring opening, phosphonate **25** (5%). Elution order of both isomers is related to the distance between the phosphonate group and nucleic acid base. Thus, the *Z* (*cis*) isomer is eluted faster than *E* (*trans*) isomer. A similar elution pattern was observed[26] for mixtures of ribonucleotides (*cisoid* 5′>*transoid* 2′ or 3′). Dealkylation of **22b**+**23b** followed by chromatography on Dowex 1 afforded the *Z*-isomer **15a** (20%), *E*-isomer **16a** (22%) and the alkene derivative **25** (4%).

Alkylation–elimination of 2-amino-6-chloropurine with diisopropyl phosphonate **21b** was perfomed with K_2CO_3 in DMF at 110°C to give the *Z* and *E*- isomers **22c**+**23c** (56%). In this case, chromatographic separation on a silica gel column was successful to afford first the *Z*-isomer **22c** (24%) followed by *E*-isomer **23c** (31%). This elution pattern followed the trend observed previously for the *Z*- and *E*-isomers of methylenecyclopropane analogues[1] **1** and **2** (B=2-amino-6-chloropurine). Hydrolysis of **22c** and **23c** with 80% formic acid furnished guanine phosphonates **22d** and **23d** in virtually quantitative yields. Dealkylation of the *Z*- and *E*-isomers **22d** and **23d** was performed using Me_3SiBr in DMF and the crude products were purified by chromatography on Dowex 1 (formate). Diisopropyl *Z*-phosphonate **22d** furnished the *E*,*Z*-bromo phosphonate **26a** (10%) and *Z*-isomer **15b** (58%). The side-product **26a** resulted from an addition of elements of HBr (generated during dealkylation) across the double bond of methylenecyclopropane phosphonate **15b**. An unsaturated derivative with an open cyclopropane ring observed in case of adenine phosphonates **15a** and **16a** (see compound **25**) was not isolated. In a similar fashion, the *E*-phosphonate **23d** afforded compound **26a** (13%) and the *E*-isomer **16b** (50%). Dealkylation of isomeric mixture **22d**+**23d** with Me_3SiI in $CHCl_3$ gave, after chromatography on Dowex 1 (formate), the *E*,*Z*-iodophosphonate **26b** (32%) followed by the target phosphonates **15b**+**16b** (25%). It is clear that an increased acidity (HI>HBr) and nucleophilicity/$I(^-)$>$Br(^-)$/facilitates the formation of side-product **26b** over that of **26a** as observed for the reactivity of alkenes toward hydrogen halides.[27]

The cytosine phosphonates **15c**+**16c** were obtained as follows. The reaction of N^4-acetylcytosine with diethyl phosphonate **21a** (K_2CO_3 in DMF at 100°C, work-up with methanol) afforded a 1.2:1 mixture of *Z*- and *E*-isomers **22e**+**23e** (70%) that were inseparable by chromatography. A similar reaction with diisopropyl phosphonate furnished a 1:1 isomeric mixture **22f**+**23f** (60%). Dealkylation of **22e**+**23e** with Me_3SiBr and subsequent chromatography on Dowex 1 afforded cyclic phosphonate **27a** (10%) followed by a mixture of the *Z*/*E*-isomers **15c**+**16c** (40%) in the ratio of 2.4:1.

The structure of **27a** isomeric with **16c** was confirmed by

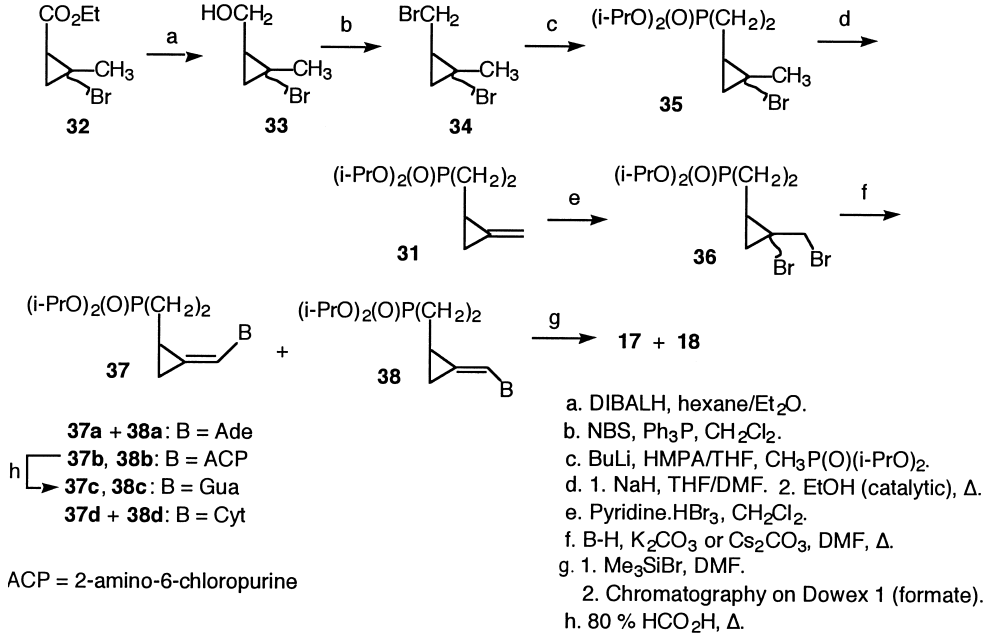


Scheme 2.

UV, NMR and mass spectra. A large downfield shift of the ^{31}P resonance (44.7 ppm) relative to acyclic phosphonates **15c** and **16c** (24 ppm) is characteristic for five-membered ring cyclic phosphonates[28] (48 ppm). It is likely that phosphonate **25** is a hydrolysis product of a similar intermediate **27b** which was not isolated. It is also possible that compounds **27a** and **27b** are generated from halogeno derivatives **26c** and **26d** (Scheme 2) although both intermediates have not been observed in contrast to guanine counterparts **26a** and **26b**. Ionization of **26c** or **26d** leads to carbocation **28** which then undergoes a 1,2-hydride shift[29] to give a resonance-stabilized ion **29** ↔ **30**. This stabilization can be expected stronger with more basic nucleosides (cytosine, adenine) than less basic guanine. An intramolecular attack of the cyclopropane ring by a phosphonate anion then gives the cyclic phosphonate **27a** or **27b**. Compound **25** is then a hydrolysis product of **27b**. It is also possible that a preferential formation of cyclic phosphonate **27a** from the *E*-isomer **16c** via **26c** can explain a shift in the *Z*/*E* isomeric ratio in favor of the *Z*-isomer **15c** (see **15c**+**16c**).

B. *Full-length phosphonates*. Compounds **17** and **18** are homologues of foreshortened phosphonates **15** and **16**. Initially, the displacement of mesyloxy group of methanesulfonate **19** (Scheme 1) with a lithium salt of diisopropyl methylphosphonate was considered as the shortest route to a key intermediate **31**. However, attempts at such an alkylation were fruitless. Therefore, a more circuitous route was adopted (Scheme 3). The sequence started with the ethyl 2-bromo-2-methylcyclopropane carboxylate[23] (**32**) which was reduced to 2-bromo-2-methylcyclopropanemethanol (**33**) with DIBALH in hexane–ether mixture in 88% yield. The latter was converted to dibromo derivative **34** using Ph_3P-NBS reagent[30] in CH_2Cl_2 (95%). Reaction with a lithium salt of diisopropyl methylphosphonate was smooth to give the bromo phosphonate intermediate **35** (86%). Elimination of the elements of HBr using NaH and a catalytic amount of ethanol[31] furnished methylenecyclopropane phosphonate **31** (64%). Addition of bromine effected by pyridinium tribromide gave the *E*,*Z*-dibromo phosphonate **36** in 91% yield. Alkylation–elimination procedure with adenine using K_2CO_3 in DMF at 110°C gave the *Z*,*E*-isomeric mixture of **37a**+**38a** (67%) which was not separable by chromatography. Dealkylation with Me_3SiBr in DMF furnished target phosphonates **17a** and **18a** which were separated in a manner similar to the lower homologues **15a** and **16a** by ion exchange chromatography on Dowex 1 (formate) in 41 and 36% yield, respectively. As in the case of foreshortened analogues **15a** and **16a**, the *Z*-isomer **17a** was eluted prior to the *E*-isomer **18a**.

Alkylation–elimination of 2-amino-6-chloropurine with dibromide **36** was performed using Cs_2CO_3 in DMF[32]



Scheme 3.

Table 1. Comparison of the relevant ^{13}C NMR chemical shifts of isomeric phosphonates and parent analogues

Isomer	$C_{3'}$ (ppm)	$C_{4'}$ (ppm)	Δppm $C_{3',4'}$
Z-**1a** (B=Ade)[a]	6.7	19.7	13
Z-**15a**	9.9	13.4	3.5
Z-**17a**	8.1	18.5	10.4
E-**2a** (B=Ade)[a]	9.7	18.1	8.4
E-**16a**	11.5	11.5	0
E-**18a**	10.3	16.8	6.5
Z-**1a** (B=Gua)[a]	6.6	19.6	13
Z-**15b**	9.6	12.8	3.2
Z-**17b**	8.1	18.3	10.2
E-**1a** (B=Gua)[a]	9.6	17.9	8.3
E-**16b**	11.2	11.4	0.2
E-**18b**	10.6	16.2	5.6

Sodium salt, D_2O.
[a] DMSO-d_6. The $C_{3'}$ and $C_{4'}$ values are from Ref. 1.

which made possible to lower the temperature and shorten the reaction time (70°C for 5 h). Chromatography on a silica gel column afforded individual isomers **37b** and **38b** in 24 and 25% yield, respectively. As in case of foreshortened analogues **22c** and **23c**, the *Z*-isomer **37b** was eluted before the *E*-isomer **38b**. Compounds **37b** and **38b** were hydrolyzed with formic acid to give guanine phosphonates **37c** and **38c** in quantitative yields. Dealkylation of the *Z*-isomer **37c** with Me_3SiBr in DMF furnished the target phosphonate **17b** (65%). In a similar fashion, the *E*-isomer **38c** afforded phosphonate **18b** (67%).

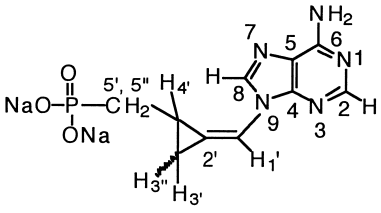
The alkylation–elimination of N^4-acetylcytosine with dibromide **36** (K_2CO_3 in DMF at 100°C) gave an isomeric mixture of phosphonates **37d**+**38d** in 68% yield inseparable by chromatography. Work-up with methanol led to a removal of the N^4-acetyl group. Dealkylation with Me_3SiBr in DMF furnished phosphonates **16c**+**17c** (79%) which were also not resolved by chromatography on Dowex 1 (formate). In contrast to foreshortened analogues (see compounds **25**, **26a**, **26b** and **27a** in Scheme 1), no side-products were isolated.

Comparison of the UV spectra of all target phosphonates with the corresponding parent analogues[1,2] established that adenine and guanine phosphonates are 9-substituted purines whereas the cytosine derivatives are 1-substituted cytosines. It is also noteworthy that electrophoretic mobility of full-length analogues **17a**, **17b** was significantly slower than those of foreshortened phosphonates **15a**, **16a**.

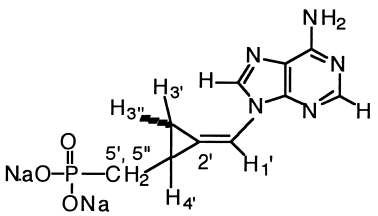
NMR spectra. The isomeric structures of target phosphonates were confirmed by NMR spectroscopy. Although spectra of phosphonates and parent analogues were determined in different solvents (D_2O vs DMSO-d_6) some comparisons relevant for isomeric assignment are possible. In a foreshortened series **15a**, **15b**, **16a** and **16b** the $H_{5'}$ protons are non-equivalent. The $\Delta\delta_{5',5''}$ of the *Z*-isomers **15a** and **15b** were 1.13 and 1.03, respectively. In contrast, the *E*-isomers **16a** and **16b** exhibited significantly lower values, 0.46 and 0.25. A similar trend was observed for the $H_{5'}$ resonances of *Z*- and *E*-isomers of parent analogues **1a** and **2a** (B=adenine or guanine).[1] In the ^{13}C NMR spectra, the $C_{3'}$ resonances of the *Z*-isomers are located at a higher field from those of the *E*-isomers (Table 1) in both series of analogues. The corresponding chemical shifts of the full-lengths analogues **17a**, **17b**, **18a** and **18b** resemble more closely the respective parent analogues than the foreshortened series **15a**, **15b**, **16a** and **16b**. This trend is also reflected in the Δppm values of $C_{3',4'}$.

The NOE experiments with adenine phosphonates **15a** and **16a** were in line with these conclusions (Table 2). It was not possible to get reliable data for both isomers at 25°C, probably due to molecular association of the *E*-isomer **16a** in solution but no difficulty was observed at 50°C. In the *Z*-isomer **15a**, NOE enhancements were observed between

Table 2. NOE data of sodium salts of (*Z*)- and (*E*)-phosphonates **15a** and **16a** at 50°C



15a (OH = ONa)



16a (OH = ONa)

$H_8\cdots H_{1'}$	1.2	$H_8\cdots H_{1'}$	0.6
$H_8\cdots H_{4'}+H_{5'}$	5.9	$H_8\cdots H_{3''}$	1.0
$H_{1'}\cdots H_8$	0.5	$H_8\cdots H_{3'}$	1.0
$H_{1'}\cdots H_{3'}$	0.5	$H_{1'}\cdots H_8$	1.2
$H_{1'}\cdots H_{3''}$	0.6	$H_{1'}\cdots H_{4'}$	0.4
$H_{3'}\cdots H_{1'}$	1.3	$H_{4'}\cdots H_{1'}$	1.0
$H_{3'}\cdots H_{4'}$	8.2	$H_{4'}\cdots H_{3'}$	2.5
$H_{3''}\cdots H_{1'}$	1.6	$H_{3''}\cdots H_8$	3.3
$H_{3''}\cdots H_{5''}$	2.8	$H_{3'}\cdots H_8$	3.4
$H_{4'}\cdots H_8$	4.1	$H_{5'}\cdots H_{1'}$	1.0
$H_{4'}+H_{5'}\cdots H_8$	4.1		
$H_{5'}\cdots H_{3''}$	8.3		
$H_{5''}\cdots H_{3'}$	3.4		

$H_{5'}$ and $H_{5''}$ were assigned arbitrarily.

the purine H_8 and proximate $H_{4'}+H_{5'}$ (4.1–5.9%) but none with the more distant $H_{3'}$ or $H_{3''}$. In contrast, the strongest interaction of H_8 of the *E*-isomer **16a** was with $H_{3'}$ and $H_{3''}$ (NOE enhancements 1.0–3.4%). A similar pattern of NOE was seen in the *Z*- and *E*-isomers of parent analogues **1** and **2** (B=adenine).[1]

It is also of interest to note that anisochronism of diastereotopic methylene, methine and methyl groups was observed in the ^{13}C NMR spectra of some diethyl and diisopropyl phosphonates but the ^{31}P resonances were in all cases isochronous. Thus, methylenecyclopropane phosphonates **20a** and **20b** exhibited signals of non-equivalent CH_2 (ethyl) and CH (isopropyl) groups (two overlapped C,P doublets at 61.9, 61.8 and 70.2, 70.1 ppm, respectively). The signals of methyl groups were isochronous. This apparently reflects the diastereotopicity of alkoxy groups in **20a** and **20b** attached to a prochiral phosphorus (the $C_{4'}$ is chiral). By contrast, diisopropyl phosphonate **31** (homologue of **20b**) exhibited only a single doublet for CH of isopropyl at 70.0 ppm. Apparently, when an extra methylene group is interposed between the chiral ($C_{4'}$) and prochiral (phosphorus) center, the diastereotopic effect is no longer observable. Non-equivalency of ethoxy groups in diethyl phosphorothioate was established by ^1H and ^{31}P NMR spectra.[33] Anisochronous ^{13}C NMR signals, were observed in compounds with geminal prochiral groups such as diisopropyl alkylmalonates[34] and 2-isopropylmalondialdehyde tetraethyl acetal.[35] Extra ^{13}C resonances were also found in diisopropyl phosphonates **37b** and **38b**. Thus, the CH signal of iPr in *Z*-isomer **37b** appeared as two overlapped doublets at 70.4 ppm and similar splitting was also observed for CH_3 groups at 24.3 ppm. By contrast, only the CH_3 resonances of the *E*-isomer **38b** were anisochronous and no such splittings were seen in the lower homologues **22c** and **23c**.

3. Biological activity

All target phosphonates were found inactive against herpesviruses (HSV-1, HSV-2, HCMV, VZV and EBV) as well as against hepatitis B virus (HBV) and HIV-1. Guanine phosphonate **15b** which inhibited VZV in HFF culture with EC_{50}/CC_{50} (μM) 2.3/>317 (cytopathic effect inhibition assay) and 24/>317 (plaque reduction assay) was an exception.

4. Experimental

4.1. General methods

See Ref. 1 The UV spectra were determined in ethanol or 0.01 M Na_2HPO_4 (pH 7). The ^1H, ^{13}C and ^{31}P NMR spectra were determined with the 300, 400 or 500 MHz instruments. For less volatile compounds the FAB-MS (thioglycerol matrix unless stated otherwise) and electrospray ionization spectra (ESI-MS, direct injection, H_2O/MeOH and NaCl or KCl) were used unless stated otherwise. Anion exchange chromatography was performed on Dowex 1X2-200 columns, 25×2.5 cm in a formate form using linear gradients of formic acid at concentrations specified in Section 4. Fractions (25 mL) were collected using a LKB Ultrorac 7000 Fraction Collector using a UV detector (254 nm) LKB Uvicord II (LKB Produkter, Bromma 1, Sweden) and Recorder Model 201 (Ross Recorders, Sparks, Nevada). The flow-rate was 5 mL/min. Maintaining a high flow-rate is essential to prevent precipitation of phosphonates in the column. Paper electrophoresis was run on a flatbed instrument (Savant Instruments, Inc., Hicksville, New York) and Whatman No. 1 paper for 2 h at 10°C and 40 V/cm in 0.02 M Na_2HPO_4 (pH 7.0). The relative mobilities refer to that of AMP=1.00.

4.1.1. Diethyl (methylenecyclopropyl)methylphosphonate (20a). Diethyl phosphite (4.1 mL, 32 mmol) was added dropwise to a stirred suspension of NaH (50% in mineral oil, 1.54 g, 32 mmol) in THF (50 mL) at 0°C. The mixture was then stirred at room temperature for 2 h whereupon it was cooled to −40°C. A solution of (methylenecyclopropyl)methyl methanesulfonate[23] **19** (2.5 g, 15.4 mmol) in THF (10 mL) was added dropwise with stirring which was continued at room temperature for 24 h. The reaction was quenched with water (10 mL) at 0°C followed by saturated aqueous $NaHCO_3$ (100 mL) and the mixture was extracted with EtOAc (3×50 mL). The combined organic phase was washed successively with saturated aqueous $NaHCO_3$, NH_4Cl, brine, and it was dried (Na_2SO_4). The solvent was removed in vacuo and the residue was chromatographed on a silica gel column in hexane/EtOAc=3:1→2:1) to give product **20a** (2.76 g, 88%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 5.52 (1H, s) and 5.41 (1H, s), 4.12 (4H, m), 1.81 (2H, m), 1.61 (1H, m), 1.33 (7H, m), 0.90 (1H, m). ^{13}C NMR 134.5 (d), 104.6, 61.9 and 61.8 (2 overlapped d, *J*=7.0 Hz), 29.6 (d, *J*=141.0 Hz), 16.7 (d, *J*=5.0 Hz), 10.2 (d, *J*=11.0 Hz), 9.0 (d, *J*=5.6 Hz). ^{31}P NMR 27.3. EI-MS 204 (M, 2.9), 148 (100.0). HRMS calcd for $C_9H_{17}O_3P$ 204.0915; found 204.0916.

4.1.2. Diisopropyl (methylenecyclopropyl)methylphosphonate (20b). The procedure described above was performed with diisopropyl phosphite (5.4 mL, 32 mmol) and the reaction mixture was stirred at 45°C for 24 h to afford product **20b** (3.1 g, 84%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 5.51 (1H, s) and 5.38 (1H, s), 4.70 (2H, m), 1.75 (2H, m), 1.60 (1H, m), 1.25 (12H, 2d+m), 0.89 (1H, m). ^{13}C NMR 134.8 (*J*=10.0 Hz), 104.4, 70.2 and 70.1 (2 overlapped d, *J*=8.0 and 7.1 Hz), 30.8 (d, *J*=142.0 Hz), 24.3 (d, *J*=5.0 Hz), 10.2 (2 overlapped d, *J*=10.0 Hz), 9.2 (*J*=5.1 Hz). ^{31}P NMR 27.0. EI-MS 233 (M+H, 0.8), 148 (100.0). HRMS calcd for $C_{11}H_{22}O_3P$ (M+H) 233.1306; found 233.1312.

4.1.3. Diethyl (*E*,*Z*)-(2-bromomethyl-2-bromocyclopropyl)methylphosphonate (21a). Pyridinium hydrobromide perbromide (5.1 g, 16 mmol) was added in portions to a solution of compound **20a** (2.76 g, 13.5 mmol) in CH_2Cl_2 (100 mL) at 0°C. The mixture was stirred at room temperature for 3 h whereupon an additional portion of CH_2Cl_2 (100 mL) was added. The organic phase was washed successively with aqueous $NaHSO_3$, 1 M HCl, $NaHCO_3$ and brine and it was dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product was chromatographed on a silica gel column using hexane–EtOAc=4:1→3:1→2:1) to give compound **21a** (4.7 g, 96%)

as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.10 (4H, m), 3.82 (dd), 3.68 (d) and 3.57 (dd, J_{AB}=12.7 Hz, total 2H), 2.30 (1H, m), 1.82 (1H, m), 1.61 (1H, m), 1.50 (1H, m), 1.35 (6H, m), 0.90 (1H, m). ^{13}C NMR 62.2 (2 poorly resolved d, J=4.5 Hz), 44.5, 41.3, 35.4 (J=15.7 Hz), 25.3 (d, J=141.8 Hz), 25.2 (d, J=5.1 Hz), 25.1 (d, J=4.5 Hz), 16.7 (J=5.2 Hz). EI-MS 285, 283 (M−Br, 61.7, 63.8), 147 (100.0). HRMS calcd for $C_9H_{17}{}^{79}BrO_3P$ (M−Br) 283.0099; found 283.0095. Anal. Calcd for $C_9H_{17}Br_2O_3P$: C, 29.70; H, 4.71; P, 8.51; Br, 43.91. Found: C, 29.65; H, 4.89; P, 8.66; Br, 43.79.

4.1.4. Diisopropyl (*E*,*Z*)-(2-bromomethyl-2-bromocyclopropyl)methyl phosphonate (21b). The procedure described above for compound **21a** was used with phosphonate **20b** (3.1 g, 13.3 mmol). Product **21b** was obtained as a colorless oil (5.1 g, 100%) in >90% purity (^{1}H NMR). ^{1}H NMR ($CDCl_3$) δ 4.62 (m, 2H), 3.72 (d), 3.62 (s) and 3.52 (d, J_{AB}=12 Hz, 2H), 2.13 (m, 1H), 1.80 (m, 1H), 1.50 (m, 1H), 1.37 (m, 1H), 1.12 and 1.10 (2s, 12H, CH_3), 0.86 (t, J=7.1 Hz, 1H). ^{13}C NMR 70.72 and 70.65 (2 d, J=3.0 and 4.0 Hz), 41.4, 35.6 (d, J=16.1 Hz), 26.4 (d, J=144.1 Hz), 25.4 (d, J=6.0 Hz), 25.2 (d, J=5.1 Hz), 24.2 (J=3.0 Hz). ^{31}P NMR 26.5, 28.0. EI-MS 395, 393, 391 (M, 7.8, 15.7, 8.2), 313, 311 (M−Br, 16.2, 18.5), 147 (100.0). HRMS calcd for $C_{11}H_{22}O_3P^{79}Br_2$ 390.9673; found 390.9677.

4.1.5. (*E*,*Z*)-9-{[2-(Diethylphosphonomethyl)cyclopropylidene]methyl}adenine (22a+23a). A mixture of compound **21a** (1.46 g, 4 mmol), adenine (0.825 g, 5.2 mmol) and flame-dried K_2CO_3 (2.76 g, 20 mmol) in DMF (25 mL) was stirred at 110°C for 18 h. The solids were filtered off and washed with DMF (2×8 mL). The solvent was evaporated and the crude product was chromatographed on a silica gel column (CH_2Cl_2/MeOH=30:1→20:1) to give a mixture of the *E*- and *Z*-isomers **22a**+**23a** (0.77 g, 57%) as a white gum and 9-ethyladenine (**24**, 0.186 g, 28%) which was identical with an authentic sample.[24] UV max (EtOH) 262 nm (ε 14,500), 227 (ε 31,000). ^{1}H NMR (DMSO-d_6) δ 8.47 and 8.41 (1H, 2s, 1.5:1), 8.18 and 8.17 (1H, 2s), 7.54 and 7.39 (1H, 2s, 1.5:1), 7.34 (2H, s), 4.03 and 3.95 (4H, m), 2.37 (0.4H, dt, ^3J=16.2 Hz and ^2J=4.8 Hz), 2.07 (1H, m), 1.84 (1.6H, m), 1.71 and 1.62 (1H, m), 1.44 and 1.31 (1H, m, 1.5:1), 1.28 (m) and 1.16 (6H, m, 1.5:1). ^{13}C NMR δ 156.7, 153.8, 153.7, 149.0, 138.8, 137.8, 119.1, 116.8, 112.0, 111.6, 61.9 (d, J=5.9 Hz), 28.8 (d, J=137.3 Hz), 27.8 (d, J=138.8 Hz), 17.0 (d, J=3.0 Hz), 16.9 (d, J=5.9 Hz), 12.0 (d, J=12.7 Hz), 11.5, 9.6, 9.3. ^{31}P NMR 30.2, 30.1. EI-MS 337 (M, 3.2), 200 (100.0). HRMS calcd for $C_{14}H_{20}N_5O_3P$ 337.1304; found 337.1303. Anal. Calcd for $C_{14}H_{20}N_5O_3P$: C, 49.85; H, 5.98; N, 20.76; P, 9.18. Found: C, 50.08; H, 5.94; N, 20.85; P, 9.31.

4.1.6. (*E*,*Z*)-9-{[2-(Diisopropylphosphonomethyl)cyclopropylidene]methyl}adenine (22b+23b). A mixture of compound **21b** (1.96 g, 5 mmol), adenine (0.88 g, 6.5 mmol) and K_2CO_3 (3.45 g, 25 mmol) in DMF (30 mL) was treated as described above for isomers **22a**+**23a** to give product **22b**+**23b** as a foam (1.26 g, 69%). UV max (EtOH) 262 nm (ε 14,300), 227 (ε 29,800). ^{1}H NMR (DMSO-d_6) 8.46 and 8.40 (1H, 2s, 1:1), 8.20 and 8.18 (2H, 2s), 7.57 and 7.37 (1H, 2s, 1:1), 7.45 and 7.44 (2H, 2s), 4.59 (2H, m), 2.34 (0.5H, dt, J=16.2 and 4.4 Hz), 2.02 (1H, m), 1.80 and 1.74 (1.5H, m), 1.57 (1H, m), 1.42 (0.5H, m), 1.15–1.14 (12.5H, m). ^{13}C NMR δ 156.7, 153.8, 153.7, 148.9, 148.8, 138.7, 137.7, 119.1, 116.56, 116.5, 112.0, 111.4, 70.2, 70.0, (2d, J=6.7 Hz), 30.1, 29.0 (2d, J=140.3 Hz), 24.5 (d, J=4.4 Hz), 24.4 (d, J=3.0 Hz), 24.3 (d, J=6.7 Hz), 11.9 (d, J=12.7), 11.7 (d, J=4.4 Hz), 9.6 (d, J=6.0 Hz), 9.5 (d, J=5.2 Hz). ^{31}P NMR 28.2, 28.1. EI-MS 365 (M, 4.0), 200 (100.0). HRMS calcd for $C_{16}H_{24}N_5O_3P$ 365.1617; found 365.1616.

4.1.7. (*Z*)-, (*E*)-9-{[2-(Phosphonomethyl)cyclopropylidene]methyl}adenine (15a, 16a) and (*E*)-9-(4-phosphono-3-hydroxymethyl-1-buten-1-yl)adenine (25). Me_3SiI (3.58 mL, 25.1 mmol) was added dropwise with stirring to a solution of isomers **22a**+**23a** (2.12 g, 6.3 mmol) in $CHCl_3$ (50 mL) at −40°C under N_2. The mixture was then allowed to warm to room temperature and the stirring was continued for 6 h. The solvent was evaporated, the resultant yellow syrup was dried at 5 torr and room temperature overnight. It was dissolved in water and the solution was lyophilized. The crude product was redissolved in water and the pH was adjusted to 8 with NH_4OH. The solution was put on the top of a Dowex-1 column (see Section 4.1), the column was washed with water till the disappearance of UV absorption and the products were eluted with a linear gradient of formic acid (0.06→0.08 and 0.08→0.12 M, 1 L each). The *Z*-isomer **15a** was eluted first followed by *E*-isomer **16a** and compound **25**. Fractions containing the products were pooled and evaporated to approximately 1/10 of the original volume. The precipitated white solids were filtered off to give the *Z*-isomer **15a** (297 mg, 17%), *E*-isomer **15a** (420 mg, 24%) and compound **25** (88 mg, 5%).

Z-Isomer **15a**: mp 237–243°C. UV max (pH 7) 261 nm (ε 13,800), 228 (ε 29,300). Electrophoretic mobility 0.80 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 8.11 (1H, s, H_8), 7.94 (1H, s, H_2), 6.94 (1H, s, $H_{1'}$), 2.08 (1H, td, J=16 Hz, $H_{5'}$) partly overlapped with 2.00 (1H, m, $H_{4'}$), 1.67 (1H, t, J=9.2 Hz, $H_{3'}$), 1.32 (1H, t, J=6.4 Hz, $H_{3''}$), 0.96 (1H, dd, J=15.6 and 11 Hz, $H_{5''}$). ^{13}C NMR 155.0 (C_6), 152.3 (C_2), 146.6 (C_4), 138.9 (C_8), 121.3 (d, ^3J=15.2 Hz, $C_{2'}$), 117.5 (C_5), 108.6 ($C_{1'}$), 31.3 (d, ^1J=129.0 Hz, $C_{5'}$), 13.4 ($C_{4'}$), 9.9 ($C_{3'}$). ^{31}P NMR 20.7. FAB-MS 282 (M+H, 29.6), 91 (100.0). Anal. Calcd for $C_{10}H_{12}N_5O_3P$: C, 42.71; H, 4.30; N, 24.90; P, 11.01. Found: C, 42.74; H, 4.53; N, 24.60; P, 10.87.

E-isomer **16a**: mp 276–287°C. UV max (pH 7) 261 nm (ε 14,600), 223 (ε 28,100). Electrophoretic mobility 0.79 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 8.00 (1H, s, H_8), 7.76 (1H, s, H_2), 6.96 (1H, s, $H_{1'}$), 1.86 (1H, m, $H_{4'}$), 1.73 (1H, td, J=5.6 and J=15 Hz, $H_{5'}$), 1.61 (1H, t, J=8.8 Hz, $H_{3'}$), 1.27 (1H, dt, J=8.0 and 15.2 Hz, $H_{5''}$) partly overlapped with 1.21 (1H, m, $H_{3''}$). ^{13}C NMR 154.6 (C_6), 152.0 (C_2), 146.2 (C_4), 137.9 (C_8), 121.1 (d, ^3J=12.1 Hz, $C_{2'}$), 117.1 (C_5), 108.9 ($C_{1'}$), 32.4 (d, ^1J=128.9 Hz, $C_{5'}$), 11.5 ($C_{3'}$, $C_{4'}$). ^{31}P NMR 21.0. FAB-MS 282 (M+H, 10.0), 91 (100.0). Anal. Calcd for $C_{10}H_{12}N_5O_3P$: C, 42.71; H, 4.30; N, 24.90; P, 11.01. Found: C, 42.60; H, 4.46; N, 24.80; P, 10.87.

Compound **25**: mp 235–238°C. UV max (pH 7) 260 nm (ε

14,800), 223 (ε 27,800). ^{1}H NMR (sodium salt, D_2O) δ 8.12 (1H, s), 7.98 (1H, s), 6.85 (1H, d, J=14.8 Hz), 6.17 (1H, dd, J=8.8 and 14.0 Hz), 3.76 (1H, dd, J=5.6 and 11.2 Hz), 3.57 (1H, d, J=6.4 and 11.2 Hz), 2.75 (1H, m), 1.56 (2H, dd, J=6.4 and 16.8 Hz). ^{13}C NMR 155.3, 152.6, 147.6, 139.9, 127.4 (d, ^3J=12.1 Hz), 120.0, 118.2, 66.0 (d, ^3J=8.0 Hz), 39.1, 31.9 (d, ^1J=128.0 Hz). ^{31}P NMR 20.5. ESI-MS (MeOH+NH_4OH) 300 (M+H, 100.0). Anal. Calcd for $C_{10}H_{14}N_5O_4P{\cdot}H_2O$: C, 37.86; H, 5.08; N, 22.08; P, 9.76. Found: C, 38.26; H, 5.29; N, 21.59; P, 10.09.

In a similar fashion, the *E*+*Z*-isomers **22b**+**23b** (1.2 g, 3.39 mmol) and Me_3SiI (1.88 mL, 13.1 mmol) in $CHCl_3$ gave **15a** (184 mg, 20%), **16a** (203 mg, 22%) and **25** (55 mg, 4%).

4.1.8. (*Z*)- and (*E*)-2-Amino-6-chloro-9-{[2-(diisopropylphosphonomethyl)cyclopropylidene]methyl}purine (22c) and (23c). A mixture of compound **21b** (3.92 g, 10 mmol), 2-amino-6-chloropurine (2.03 g, 12 mmol) and K_2CO_3 (6.9 g, 50 mmol) in DMF (80 mL) was stirred at 110°C for 18 h. The solids were filtered off and washed with DMF (2×20 mL). The solvent was evaporated and the crude product was chromatographed on a silica gel column (CH_2Cl_2/MeOH=30:1) to give a mixture of isomers **22c**+**23c** (2.25 g, 56%) which were resolved by repeated (3 times) chromatography. Solvent EtOAc/MeOH=40:1→30:1 eluted the faster moving *Z*-isomer **22c** (0.96 g, 24%) and CH_2Cl_2/MeOH=30:1 afforded the slower *E*-isomer **23c** (1.24 g, 31%).

Z-isomer **22c**: mp 172–174°C. UV max (EtOH) 311 nm (ε 7500), 233 (ε 27,900). ^{1}H NMR ($CDCl_3$) δ 7.99 (1H, s), 7.17 (1H, s), 5.78 (2H, brs), 4.67 (2H, m), 2.23 (1H, ddd, J=4.0, 15.2 and 18.6 Hz), 2.06 (1H, m), 1.62 (1H, td, ^3J=9.0 Hz, ^2J=1.6 Hz), 1.49 (1H, ddd, J=9.4, 15.4 and 18.2 Hz), 1.23 (13H, m). ^{13}C NMR 159.9, 152.5, 151.5, 139.9, 125.0, 117.0 (d, ^3J=14.9 Hz), 111.2, 70.8 (d, ^2J=6.7 Hz), 30.1 (d, ^1J=142.5 Hz), 24.3 (d, ^3J=3.7 Hz), 11.9 (d, ^2J=4.5 Hz), 10.4 (d, ^3J=4.5 Hz). ^{31}P NMR 27.5. EI-MS 399, 401 (M, 8.3, 2.8), 234 (100.0). HRMS calcd for $C_{16}H_{23}ClN_5O_3P$ 399.1227; found 399.1224. Anal. Calcd for $C_{16}H_{23}{}^{35}ClN_5O_3P$: C, 48.07; H, 5.80; N, 17.52, P, 7.75. Found: C, 48.28; H, 5.81; N, 17.31; P, 8.14.

E-isomer **23c** (isomeric purity 85%): mp 157–160°C. UV max (EtOH) 310 nm (ε 7300), 225 (ε 25,400). ^{1}H NMR ($CDCl_3$) δ 8.06 (1H, s), 7.46 (1H, s), 6.45 (2H, brs), 4.75 (2H, m), 2.04 (1H, m), 1.87 (1, m), 1.74 (1H, dt, J=9.0 Hz), 1.56 (1H, ddd, J=8.4, 14.6 and 18 Hz), 1.31 (13H, m). ^{13}C NMR 160.2, 152.5, 151.4, 138.5, 124.6, 115.8 (d, 3J= 8.1 Hz), 112.2, 70.7, 30.58 (d, ^1J=144.1 Hz), 24.3 (d, ^3J=4.0 Hz), 11.7 (d), 9.3 (d, 3J=6.7 Hz). ^{31}P NMR 27.6. EI-MS 399, 401 (M, 7.6, 3.5), 234 (100.0). HRMS calcd for $C_{16}H_{23}{}^{35}ClN_5O_3P$ 399.1227; found 399.1220. Anal. Calcd for $C_{16}H_{23}ClN_5O_3P$: C, 48.07; H, 5.80; N, 17.52, P, 7.75. Found: C, 48.21; H, 5.72; N, 17.54; P, 8.07.

4.1.9. (*Z*)- and (*E*)-9-{[2-(Diisopropylphosphonomethyl)-cyclopropylidene]methyl}guanine (22d) and (23d). A solution of compound **22c** (0.92 g, 2.3 mmol) in formic acid (80%, 20 mL) was heated at 90°C for 6 h whereupon it was evaporated. An aqueous solution of the residue was lyophilized to give the *Z*-isomer **22d** (0.86 g, 98%) as a white solid. In a similar fashion, *E*-isomer **23d** (1.14 g, 100%) was obtained from compound **23c** (1.21 g, 3.0 mmol).

Z-isomer **22d**: mp 215–220°C. UV max (EtOH) 270 nm (ε 11,300), 227 (ε 28,400). ^{1}H NMR (DMSO-d_6) δ 11.85 (1H, s), 8.70 (1H, s), 7.11 (1H, s), 6.56 (2H, brs), 4.75 (2H, m), 2.17 (1H, m), 2.11 (1H, m), 1.67 (1H, m), 1.60 (1H, m), 1.24 (13H, m). ^{13}C NMR 156.1, 154.4, 150.5, 134.7, 121.7, 111.4, 110.7, 70.8, 30.7 (d, ^1J=143.5 Hz), 24.6, 12.01, 10.5. ^{31}P NMR 28.0. ESI-MS (3-nitrobenzyl alcohol+NaCl/KCl) 801 (2M+K, 10.1), 785 (2M+Na, 9.2), 763 (2M+H, 74.6), 420 (M+K, 7.7), 404 (8.9), 382 (M+H, 100.0).

E-isomer **23d**: mp 185–188°C. UV max (EtOH) 269 nm (ε 12,000), 229 (ε 24,800). ^{1}H NMR (DMSO-d_6) δ 11.68 (1H, s), 8.82 (1H, s), 7.34 (1H, s), 6.56 (2H, brs), 4.77 (2H, m), 2.02 (2H, m), 1.77 (1H, m), 1.64 (1H, m), 1.31 (13H, m). ^{13}C NMR 155.9, 154.4, 150.3, 134.3, 122.8, 111.9, 110.6, 70.0, 30.56 (d, ^1J=143.0 Hz), 24.4, 11.8, 9.46. ^{31}P NMR 29.7. FAB-MS (3-nitrobenzyl alcohol+KCl) 420 (M+K, 88.3), 382 (M+H, 100.0).

4.1.10. (*Z*)-, (*E*)-9-{[2-(Phosphonomethyl)cyclopropylidene]methyl}guanine (15b), (16b) and 9-{[1-Bromo-2-(phosphonomethyl)cyclopropyl]methyl}guanine (26a). Me_3SiBr (1.5 mL, 11.0 mmol) was added dropwise with stirring to a solution of compound **22d** (840 mg, 2.2 mmol) in DMF (50 mL) at −40°C. The mixture was then allowed to warm to room temperature and stirred for 10 h. The work-up and chromatography followed the procedure described for compounds **15a**, **16a** and **25** using a linear gradient of formic acid (0.08→0.18 M and 0.18→0.28 M, 1 L each). Compound **26a** (85 mg, 10%) was eluted first followed by the *Z*-isomer **15b** (380 mg, 58%). Compound **26a** (122 mg, 13%) and the *E*-isomer **16b** (369 mg, 50%) were obtained from intermediate **23d** (952 mg, 2.5 mmol) following a similar procedure.

Z-isomer **15b**: mp>350°C (decomp.). UV max (pH 7) 265 nm (ε 13,400), 229 (ε 32,900). Electrophoretic mobility 1.11 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 7.75 (1H, s), 6.71 (1H, s), 2.05 (1H, t, J=15.4 Hz), 1.86 (1H, m), 1.53 (1H, t, J=9 Hz), 1.18 (1H, m), 0.97 (1H, td, J=15.6 and 11.2 Hz). ^{13}C NMR 158.5, 153.5, 149.4, 136.6, 115.3, 120.2 (d, ^3J=15.1 Hz), 109.0, 30.8 (d, ^1J=130.0 Hz), 12.8, 9.6. ^{31}P NMR 22.0. ESI-MS (H_2O/MeOH) 595 (2M+H, 27.8), 298 (M+H, 100.0). Anal. Calcd for $C_{10}H_{12}N_5O_4P{\cdot}H_2O$: C, 38.10; H, 4.48; N, 22.22; P, 9.83. Found: C, 37.86; H, 4.59; N, 22.64; P, 9.70.

E-isomer **16b**: mp>350°C (decomp.). UV max (pH 7) 266 nm (ε 11,800), 223 (ε 30,600). Electrophoretic mobility 1.08 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 7.86 (1H, s), 7.05 (1H, m, 1.86 (1H, m), 1.62 (2H, m), 1.37 (1H, td, J=7.2 and 15.2 Hz), 1.20 (1H, m). ^{13}C NMR 168.3, 161.3, 150.2, 135.4, 117.1, 120.8 (d, ^3J=11.1 Hz), 110.0, 32.6 (d, ^1J=129.0 Hz), 11.4 (d, ^2J=4.0 Hz), 11.2 (d, ^3J=8.1 Hz). ^{31}P NMR 21.1. ESI-MS (MeOH+NaCl) 298 (M+H, 100.0), 320 (M+Na, 37.7), 595 (2M+Na, 5.7), 617 (2M+Na, 20.7). Anal. Calcd for $C_{10}H_{12}N_5O_4P{\cdot}0.8H_2O$: C, 38.54; H, 4.40; N, 22.47; P, 9.94. Found: C, 38.66; H, 4.67; N, 22.43; P, 10.12.

Compound **26a** (*E*,*Z*-isomers): mp 280–290°C. UV max (pH 7) 253 nm (ε 14,100), 200 (ε 21,400). ^{1}H NMR (sodium salt, D_2O) δ 7.90 and 7.85 (1H, 2s), 4.47 and 4.20 (2H, J_{AB}=15.2 Hz), 2.04 (1H, td, J=2.4 and 16.8 Hz), 1.85 (1H, m), 1.43 (1H, m), 1.20 (1H, m). ^{13}C NMR 159.4, 154.0, 152.1, 140.2, 115.8, 49.8, 36.5, 28.8 (d, ^1J=127.9 Hz), 24.9, 22.7. ^{31}P NMR 20.9, 19.9. ESI-MS (H_2O/MeOH+NaCl/KCl) 418 and 416 (M+K, 36.0 and 31.1), 402 and 400 (M+Na, 25.6 and 26.2), 380 and 378 (M+H, 99.4 and 100.0).

4.1.11. (*Z*)-, (*E*)-9-{[2-(Phosphonomethyl)cyclopropylidene]methyl}guanine (15b), (16b) and (*E*,*Z*)-9-{[1-iodo-2-(phosphonomethyl)cyclopropyl]methyl}guanine (26b). The procedure described above was followed using Me_3SiI (0.33 mL, 2.5 mmol) and isomeric mixture **22d**+**23d** (190 mg, 0.5 mmol) in $CHCl_3$ (10 mL) at −40°C. The crude product was purified by chromatography on Dowex 1 column using formic acid (0.10→0.30 M, 1 L each) to give compound **26b** (68 mg, 32%) and a mixture of *Z*- and *E*-isomers **15b**+**16b** (38 mg, 25%).

Compound **26b** (*E*,*Z*-isomers): mp 290–300°C. UV max (pH 7) 253 nm (ε 14,300), 200 (ε 20,800). ^{1}H NMR (sodium salt, D_2O) δ 7.86 and 7.81 (1H, 2s), 4.41 and 4.10 (2H, d and m, J_{AB}=15.2 Hz), 2.02 (1H, td, J=3.4 and 19.2 Hz), 1.83 (1H, m), 1.39 (1H, dd, J=2.8 and 7.2 Hz), 1.27 (1H, ddd, J=17.4, 14.2 and 10.4 Hz), 1.16 (1H, t, J=7.4 Hz). ^{13}C NMR 160.2, 154.4, 152.4, 141.1, 116.5, 50.7, 36.9, 29.5 (d, ^1J=127.9 Hz), 25.3, 23.0. ^{31}P NMR 23.1, 21.7. FAB-MS 425 (M, 0.3), 297 (M−HI, 3.4), 93 (100.0). Anal. Calcd for $C_{10}H_{13}IN_5O_4P$: C, 28.25; H, 3.08; N, 16.47; P, 7.29. Found: C, 28.41; H, 3.25; N, 16.50; P, 7.01.

4.1.12. (*E*,*Z*)-9-{[2-(Diethylphosphonomethyl)cyclopropylidene]methyl}cytosine (22e+23e). A stirred mixture of compound **21a** (2.67 g, 7.3 mmol), N^4-acetylcytosine (1.35 g, 8.8 mmol) and K_2CO_3 (5.0 g, 36 mmol) in DMF (80 mL) was heated at 110°C for 16 h. Methanol (10 mL) was added dropwise, heating was discontinued and the mixture was stirred for 3 h. After cooling, the solution was evaporated and the crude product was chromatographed on a silica gel column (CH_2Cl_2/MeOH=15:1→10:1) to give a mixture of *Z*- and *E*-isomers **22e**+**23e** as a white gum (1.60 g, 70%). UV max (EtOH) 296 nm (ε 12,800), 231 (ε 12,300), 205 (ε 19,600). ^{1}H NMR (DMSO-d_6) δ 7.94 (d, J=8.0 Hz) and 7.80 (1H, d, J=7.2 Hz, 1:1.2), 7.47 (d), 7.45 (s) and 7.27 (d, total 3H), 5.83 (1H, 2 overlapped d), 3.94 (4H, m), 1.65 (2H, m), 1.22 (6H, m, 6H), 2.25 (dt), 1.99 (m), 1.42 (t), 1.13 (m), 0.83 (t, total 3H). ^{13}C NMR 166.1, 154.7, 154.6, 141.6, 140.8, 117.0, 116.7, 112.5, 112.4, 96.0, 95.8, 61.7, (t, ^2J=6.7 Hz), 28.7 (d, ^1J=138.0 Hz), 28.0 (d, ^1J=137.3 Hz) 16.9, 11.1 (d, ^3J=12.7 Hz), 10.6, 8.2 (d, ^3J=5.9 Hz), 7.49. ^{31}P NMR 30.4, 30.0. EI-MS 314 (M+H, 8.5), 43 (100.0). HRMS calcd for $C_{13}H_{20}N_3O_4P$: 313.1191; found 313.1188.

4.1.13. (*E*,*Z*)-9-{[2-(Diisopropylphosphonomethyl)cyclopropylidene]methyl}cytosine (22f+23f). The procedure described above for **22e**+**23e** was followed using N^4-acetylcytosine (1.42 g, 9.26 mmol), compound **21b** (2.79 g, 7.1 mmol), K_2CO_3 (4.91 g, 35.5 mmol) and DMF (80 mL). The isomeric mixture of **22f**+**23f** was obtained as a white gum (1.59 g, 60%). UV max (EtOH) 296 nm (ε 13,000), 231 (ε 12,300), 206 (ε 20,500). ^{1}H NMR ($CDCl_3$) δ 8.19 (bs) and 8.03 (1H, bs) and 7.75 (1H, bs, 1:1), 7.60 (d, J=7.2 Hz), 7.43 (d) and 7.25 (d, total 2H), 6.02 (1H, d, J=7.6 Hz), 4.49 (2H, m), 1.98 (t, J=14.6 Hz), 1.76 (m), 1.64 (m), 1.59 (m), 1.48 (m), 1.32 (m), 1.08 (m) and 0.99 (m, total 17H). ^{13}C NMR 164.5, 154.3, 141.6, 140.7, 117.6, 116.6, 112.5, 112.4, 95.9, 95.7, 70.7, 70.5, 30.4 (d, ^1J=143.1 Hz), 29.8 (d, ^1J=142.5 Hz), 24.3, 11.3, 10.9, 9.0, 8.2). ^{31}P NMR 27.9, 27.3. EI-MS 341 (M, 3.6), 43 (100.0). HRMS calcd for $C_{15}H_{24}N_3O_4P$: 341.1504; found 341.1501.

4.1.14. (*E*)-, (*Z*)-9-{[2-(Phosphonomethyl)cyclopropylidene]methyl}cytosine (15c+16c) and cyclic phosphonate 27a. Isomers **22e**+**23e** (1.56 g, 5 mmol) in DMF (50 mL) were treated with Me_3SiBr (3.4 mL, 25.0 mmol) at −40°C as described for compounds **15b** and **16b**. The crude product was purified by chromatography on Dowex 1 using formic acid (0.0→0.08 and 0.08→0.18 M, 1 L each) to give a mixture of *E*,*Z*-isomers **15c**+**16c** (512 mg, 40%) and cyclic phosphonate **27a** (135 mg, 11%).

Isomers **15c**+**16c**. Mp 230–235°C. UV max (pH 7) 291 nm (ε 12,000), 228 (ε 12,500), 201 (ε 16,600). ^{1}H NMR (sodium salt, D_2O) δ 7.78 (1H, d) and 7.66 (1H, d, J=7.5 Hz, 2.4:1), 7.10 (d, J=1.5 Hz) and 6.93 (1H, s, 2.4:1), 5.85 (d) and 5.79 (1H, d, J=7.5 Hz, 1:2.4), 1.87 (dt), 1.77 (m), 1.61 (m), 1.57 (dd), 1.50 (m), 1.38 (m), 1.06 (m), 1.05 (m), 0.91 (m, total 5H). ^{13}C NMR 166.0, 157.1, 157.0, 142.8, 142.5, 19.8, 119.7, 115.2, 114.8, 96.6, 96.2, 31.9 (d, ^1J=131.0 Hz), 31.2 (d, ^1J=130.0 Hz), 12.3, 10.6 (d, J=8.3 Hz), 9.6 (d, J=3.8 Hz), 8.8. ^{31}P NMR 24.3, 23.6. ESI-MS (H_2O/MeOH) 258 (M+H, 100.0). Anal. Calcd for $C_9H_{12}N_3O_4P$: C, 42.01; H, 4.70; N, 16.34; P, 12.05. Found: C, 42.13; H, 4.72; N, 16.16; P, 11.81.

Compound **27a**. UV max (pH 7) 291 nm (ε 14,200), 228 (ε 13,100), 201 (ε 20,400). ^{1}H NMR (sodium salt, D_2O) δ 7.63 (1H, d, J=7.2 Hz), 6.84 (1H, d, J=13.6 Hz), 5.92 (1H, dd, J=7.2 Hz), 5.71 (1H, dd, J=8.0 and 13.6 Hz), 4.12 (1H, ddd, J=7.2, 8.8 and 16.0 Hz), 3.65 (1H, td, J=9.6 and 4.8 Hz), 3.22 (1H, m), 1.93 (1H, m), 1.56 (1H, td, J=10.4 and 14.4 Hz). ^{13}C NMR 166.2, 148.3, 146.3, 125.5, 125.0, 95.5, 68.5, 38.5, 26.8 (d, ^1J=120.2 Hz). ^{31}P NMR 44.7. ESI-MS (H_2O/MeOH) 258 (M+H, 100.0).

Diisopropyl phosphonates **22f**+**23f** (716 mg, 2.1 mmol) when treated with Me_3SiBr (1.4 mL, 10.6 mmol) under similar conditions gave phosphonates **15c**+**16c** (250 mg, 50%) and cyclic phosphonate **27a** (78 mg, 16%).

4.1.15. Ethyl (*E*,*Z*)-2-bromo-2-methylcyclopropane carboxylate (32). The described procedure[23] was adapted for a large-scale preparation as follows. Ethyl diazoacetate (173.9 g, 1.65 mol) was added with the aid of a syringe pump (20 mL/h) to a mixture of 2-bromo-1-propene (260.1 g, 2.15 mol, Chemsampco, Gray Court, South Carolina, distilled before use) and $Rh_2(OAc)_4$ (221 mg, 0.5 mmol) at room temperature with stirring (Dry Ice condenser). The stirring was continued for 16 h, the unreacted crude alkene was distilled off (bath temperature

60°C, the receiver was cooled at −78°C, 135.4 g, 52%) and the residue was distilled in vacuo to give ester **32** in two fractions: bp 92–98°C/28 torr, 137.22 g (40.2%, based on ethyl diazoacetate, 65% *Z*(*trans*) isomer, bp 98–116°C/28 torr (80.82 g, 23.7, 90% *E*(*cis*)-isomer). The ^{1}H NMR data corresponded to those reported for the *cis*/*trans* isomeric mixture.[23]

4.1.16. (*Z*)-2-Bromo-2-methylcyclopropanemethanol (33). A 1 M solution of DIBALH in hexane (100 mL, 100 mmol) was added dropwise into ethyl 2-bromo-2-methylcyclopropane carboxylate (**32**, 65% *Z*-isomer, 8.28 g, 40 mmol) in Et_2O (30 mL) at 0°C with stirring under N_2 over a period of 30 min. The stirring was continued for another 2 h. The reaction was quenched with water (5 mL) and 4 M HCl (70 mL) was added to dissolve the gel formed. The mixture was extracted with Et_2O (3×30 mL). The combined organic phase was washed successively with 1 M HCl, H_2O, saturated $NaHCO_3$, H_2O, brine and it was dried (Na_2SO_4). After evaporation of the solvent, product **33** was obtained as a colorless oil. Distillation in vacuo afforded 5.48 g (83%), bp 102–108°C/28 torr, 77% *Z*-isomer. The ^{1}H and ^{13}C NMR data corresponded to those reported[23] for the 1*R*,2*S* (*Z*)-isomer of **33**.

4.1.17. (*E*,*Z*)-1-Bromo-1-methyl-2-bromomethylcyclopropane (34). NBS (9.91 g, 55.7 mmol) was added to a solution of compound **33** (77% *Z*-isomer, 4.59 g, 27.8 mmol) in CH_2Cl_2 (14 mL). A solution of PPh_3 (10.94 g, 41.7 mmol) in CH_2Cl_2 (14 mL) was then added with stirring at such a rate that the temperature did not exceed 40°C. The resulting reddish solution was stirred at room temperature for 1 h whereupon it was added dropwise with stirring into pentane (300 mL). The precipitate was filtered off and the solvent was distilled off at an atmospheric pressure to give a clear syrup which was stirred again with pentane (30 mL). The solids were removed by filtration and the filtrate was evaporated to a colorless oil. Distillation gave product **34** (79% *Z*-isomer, 6.04 g, 95%, bp 102–106°C/50 torr).

Another experiment performed with a 1:1 isomeric mixture of compound **33** gave after distillation in vacuo using a Vigreux column the *Z*-isomer of **34** (bp 85–90°C/20 torr, 3.52 g, 44%) and *E*-isomer of **34** (bp 95–98°C/20 torr, 4.0 g, 50%).

Z-isomer: ^{1}H NMR ($CDCl_3$) δ 3.45 (1H, dd, J=7.5 and 10.8 Hz), 3.30 (1H, dd, J=9.0 and 10.5 Hz), 2.00 (1H, m), 1.81 (3H, s), 1.47 (1H, dd, J=6.9 and 9.6 Hz), 0.71 (1H, t, J=6.4 Hz). ^{13}C NMR δ 33.3, 32.4, 29.7, 25.2, 23.8. EI-MS 149 (66.9) and 147 (71.6, M−Br), 67 (100.0). HRMS calcd for $C_5H_8{}^{79}Br$ (M−Br) 146.9809; found 146.9808.

E-Isomer: ^{1}H NMR ($CDCl_3$) δ 3.64 (1H, m), 3.47 (1H, m), 1.76 (3H, s), 1.17 (2H, m), 0.96 (1H, m). ^{13}C NMR δ 38.6, 36.5, 30.3, 27.7, 25.5. A mixture of both isomers was used in a subsequent step.

4.1.18. Diisopropyl (*E*,*Z*)-2-[(2-bromo-2-methylcyclopropyl)ethyl]-1-phosphonate (35). HMPA (5.74 mL, 33 mmol) was added to a solution of BuLi (1.6 M, 20 mL, 32 mmol) in THF (50 mL). The mixture was stirred at room temperature for 10 min and then it was cooled to −78°C. Diisopropyl methylphosphonate (6.2 mL, 33 mmol) was added and the resulting mixture was stirred at −78°C for 30 min. A solution of compound **34** (mixture of *E*/*Z* isomers, 2.28 g, 10 mmol) in THF (10 mL) was then added dropwise with stirring which was continued fo 6 h at −78°C. The reaction was quenched with saturated NH_4Cl (50 mL) and the product was extracted with EtOAc (3×50 mL). The combined organic phase was washed successively with brine, aqueous $NaHCO_3$, brine, and dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed on a silica gel column in hexane/EtOAc=10:1→5:1→3:1) to give product **35** (2.8 g, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.58 (2H, m), 1.79 (2H, m), 1.67 (5H, m+s), 1.16 (m+s), 0.81 (m), 0.62 (m), 0.34 (t, total 15H). ^{31}P NMR 30.2, 29.8. EI-MS 328, 326 (M, 0.30, 0.23), 163 (100.0). HRMS calcd for $C_{12}H_{24}O_3P^{79}Br$ 326.0646; found 326.0643.

4.1.19. Diisopropyl 2-(methylenecyclopropyl)ethyl-1-phosphonate (31). NaH (1.76 g, 36.7 mmol) was added in portions to a solution of compound **35** (3.0 g, 9.17 mmol) in THF (60 mL) and DMF (20 mL). The stirred mixture was heated in an oil bath (70°C), EtOH (0.115 mL, 2 mmol) was added and the heating was continued for 6 h. After cooling to 0°C the reaction was quenched by saturated aqueous NH_4Cl (100 mL). The product was extracted with EtOAc (4×50 mL). The organic phase was washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4) and it was evaporated. Chromatography on a silica gel column in hexane/EtOAc=10:1→5:1→3:1 gave product **31** (1.45 g, 64%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 5.41 (1H, d), 5.34 (1H, bs), 4.65 (2H, m), 1.83–1.74 (2H, m), 1.63 (2H, m), 1.48 (1H, m), 1.38 (12H, 2s), 1.24 (1H, m), 0.76 (1H, m). ^{13}C NMR 136.0, 103.3, 70.0 (^2J=5.9 Hz), 26.6 (d, ^1J=117.1 Hz), 26.2 (d, ^2J=19.4 Hz), 24.3, 16.3 (d, ^3J=20.1 Hz), 9.6. ^{31}P NMR 30.7. ESI-MS (NaCl) 515 (2M+Na, 100.0), 269 (M+Na, 19.5), 247 (M+H, 83.2).

4.1.20. Diisopropyl *E*,*Z*-[2-(2-bromomethyl-2-bromocyclopropyl)ethyl]-1-phosphonate (36). Pyridinium hydrobromide perbromide (4.68 g, 14.6 mmol) was added in portions with stirring to a solution of compound **31** (3.0 g, 12.2 mmol) in CH_2Cl_2 (100 mL) at 0°C. The stirring was continued for 3 h at room temperature. The reaction was quenched with saturated aqueous $NaHSO_3$ (100 mL) and the water phase was extracted with CH_2Cl_2 (2×50 mL). The combined organic phase was washed successively with aqueous $NaHSO_3$, 1 M HCl, $NaHCO_3$, brine and then it was dried over Na_2SO_4. Solvent was evaporated and the crude product was chromatographed using hexane/EtOAc=4:1→3:1→2:1) to give product **36** (4.5 g, 91%) as an oil. The ^{31}P NMR spectrum indicated 83% purity of the *E*/*Z*-isomeric mixture of **36** which was used as such in the next step. ^{1}H NMR ($CDCl_3$) δ 4.64 (2H, m), 3.82, 3.57 (2d, J_{AB}=11.2, 12 Hz), 3.73, 3.52 (J_{AB}=11.2 Hz, total 2H), 2.0–1.6 (4H, 2m), 1.46 (1H, m), 1.30 (12H, 2s+m), 1.03 (t, J=6.8 Hz), 0.95 (m), 0.74 (t, J=7.0 Hz, total 2H). ^{31}P NMR 29.8, 29.4. EI-MS 409, 407, 405 (1.39, 2.83, 1.57, M+H), 327, 325 (6.93, 6.68, M−Br), 79 (100.0). HRMS calcd for $C_{12}H_{23}{}^{79}BrO_3P$ (M−Br) 325.0568; found 325.0568.

4.1.21. (*Z*,*E*)-9-{[(2-Diisopropylphosphonoethyl)cyclopropylidene]methyl}adenine (37a+38a). A mixture of compound **36** (2.1 g, 5.1 mmol), adenine (905 mg, 6.7 mmol) and K_2CO_3 (3.6 g, 25.7 mmol) in DMF (30 mL) was stirred at 110°C for 20 h. After cooling, the solids were filtered off and washed with DMF (2×5 mL). The solvent was evaporated in vacuo and the crude product was chromatographed on a silica gel column (CH_2Cl_2/MeOH=25:1→15:1) to give isomeric mixture **37a+38a** (1.3 g, 67%) as a white gum. UV max (EtOH) 262 nm (ε 14,200), 226 (ε 29,900). ^{1}H NMR (DMSO-d_6) δ 8.47, 8.35 (1H, 2s, 1:1), 8.17 (1H, s), 7.56, 7.36 (3H, 2s), 4.55, 4.47 (2H, 2m), 2.09 (m), 1.86 (m), 1.69 (m), 1.51 (m) and 1.35 (m, total 7H), 1.21, 1.16 (12H, 2d, J=8.4 Hz). ^{31}P NMR 30.3, 30.0. EI-MS 379 (M, 2.7), 378 (M−H, 11.0), 200 (100.0). HRMS calcd for $C_{17}H_{25}N_5O_3P$ (M−H) 378.1695; found 378.1693. Anal. Calcd for $C_{17}H_{26}N_5O_3P$: C, 53.82; H, 6.91; N, 18.46; P, 8.16. Found: C, 53.94; H, 6.83; N, 18.35; P, 8.34.

4.1.22. (*Z*)- and (*E*)-9-{[2-(Phosphonoethyl)cyclopropylidene]methyl}adenine (17a) and (18a). Me_3SiBr (2.3 mL, 17 mmol) was added dropwise with stirring to a solution of isomers **37a+38a** (1.27 g, 3.4 mmol) in DMF (40 mL) at −40°C. The mixture was then allowed to warm to room temperature and the stirring was continued for 8 h. Solvents were evaporated and the isomers **17a** and **18a** were separated by chromatography on Dowex 1 as described for phosphonates **15a** and **16a**. The *Z*-isomer **17a** was eluted first (397 mg, 41%) followed by the *E*-isomer **18a** (362 mg, 36%).

Z-Isomer **17a**: mp 261–265°C. UV max (pH 7) 260 nm (ε 12,900), 224 (ε 23,500). Electrophoretic mobility 0.60 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 8.13 and 7.97 (2H, 2s), 6.96 (1H, s), 1.94 (2H, 2 overlapped m), 1.52–1.31 (4H, m), 1.15 (1H, t, J=6.4 Hz). ^{13}C NMR 155.0, 152.3, 146.6, 138.8, 121.4, 117.5, 108.8, 28.3 (d, ^1J=130.0 Hz), 26.7, 18.5 (d, ^2J=21.1 Hz), 8.1. ^{31}P NMR 22.6. ESI-MS (MeOH) 296 (M+H, 100.0). Anal. Calcd for $C_{11}H_{14}N_5O_3P.H_2O$: C, 42.18; H, 5.15; N, 22.36; P, 9.89. Found: C, 41.93; H, 5.08; N, 22.20; P, 10.26.

E-isomer **18a**: mp 270–273°C. UV max (pH 7) 261 nm (ε 14,100), 226 (ε 30,200). Electrophoretic mobility 0.54 of AMP. ^{1}H NMR (sodium salt, D_2O) δ 8.19 and 7.95 (2H, 2s), 7.11 (1H, s), 1.84 (1H, m), 1.64–1.55 (5H, m), 1.20 (1H, m). ^{13}C NMR 154.9, 152.2, 146.6, 138.2, 120.8, 117.3, 108.7, 28.9 (d, ^1J=130.0 Hz), 27.6, 16.8 (d, ^2J=21.2 Hz), 10.3. ^{31}P NMR 22.8. ESI-MS (MeOH) 296 (M+H, 100.0). Anal. Calcd for $C_{11}H_{14}N_5O_3P{\cdot}0.8H_2O$: C, 42.67; H, 5.08; N, 22.62; P, 10.00. Found: C, 42.56; H, 4.97; N, 22.70; P, 10.37.

4.1.23. (*Z*)- and (*E*)-2-Amino-6-chloro-9-[2-(diisopropylphosphonoethyl)cyclopropylidene]ethyl]purine (37b) and (38b). A mixture of compound **36** (6.5 g, 16 mmol), 2-amino-6-chloropurine (4.06 g, 19.2 mmol) and Cs_2CO_3 (15.6 g, 48 mmol) in DMF (150 mL) was stirred at room temperature for 4 h and then at 70°C for 5 h. The solids were filtered off and washed with DMF (2×30 mL). The solvent was evaporated and the crude product was chromatographed on a silica gel column (CH_2Cl_2/MeOH=40:1) to give an isomeric mixture of **37b+38b** (3.8 g, 58%) which was separated by repeated chromatography using EtOAc/MeOH=40:1→30:1 for faster moving *Z*-isomer **37b** and then CH_2Cl_2/MeOH=30:1 for slower moving *E*-isomer **38b** to give **37b** (1.62 g, 24%, isomeric purity >95%) and **38b** (1.66 g, 25%, 90% isomeric purity).

Z-isomer **37b**: mp 154–156°C. UV max (EtOH) 311 nm (ε 8900), 227 (ε 30,100). ^{1}H NMR ($CDCl_3$) δ 7.98 (1H, s), 7.18 (1H, s), 5.61 (2H, brs), 4.66 (2H, m), 2.19 (1H, m), 2.00 (1H, m), 1.78 (2H, m), 1.49 (2H, m), 1.23 (12H, d), 1.11 (1H, t, J=7.0 Hz). ^{13}C NMR 159.8, 152.6, 151.6, 139.8, 125.2, 118.9, 110.8, 70.4 (2 overlapped d, ^2J=5.9 Hz), 25.9 (d, ^1J=142.5 Hz), 25.4 (d, ^3J=4.4 Hz), 24.3 (2 overlapped d, ^3J=5.2 Hz), 18.3 (d, ^2J=18.6 Hz), 8.8. ^{31}P NMR 30.3 ppm. EI-MS 415, 413 (M, 5.0, 7.4), 234 (100.0). HRMS calcd for $C_{17}H_{25}{}^{35}ClN_5O_3P$ 413.1384; found 413.1385. ESI-MS (MeOH+NaCl) 849, 851 (2M+Na, 29.6, 35.9), 438, 436 (M+Na, 38.9, 100.0), 416, 414 (M+H, 10.8, 18.0). Anal. Calcd for $C_{17}H_{25}ClN_5O_3P$: C, 49.34; H, 6.09; Cl, 8.57; N, 16.92; P, 7.48. Found: C, 49.42; H, 6.17; Cl, 8.71; N, 16.82; P, 7.62.

E-Isomer **38b**: mp 130–133°C. UV max (EtOH) 310 nm (ε 9600), 230 (ε 35,700). ^{1}H NMR ($CDCl_3$) 8.16 (1H, s), 7.35 (1H, s), 5.62 (2H, brs), 4.68 (2H, m), 1.83 (2H, m), 1.72 (2H, m), 1.64 (1H, td), 1.25 (13H, m), 1.19 (1H, m). ^{13}C NMR 159.7, 152.5, 151.5, 138.9, 125.0, 117.4, 110.6, 70.3 (d, ^2J=6.6 Hz), 26.8 (d, ^1J=142.6 Hz), 26.4 (d, ^3J=4.4 Hz), 24.3 (2 overlapped d, ^2J=7.5 and 3.8 Hz), 16.1 (d, ^2J=19.4 Hz), 11.2. ^{31}P NMR 29.9. ESI-MS (MeOH+NaCl) 851, 849 (2M+Na, 35.3, 46.1), 438, 436 (M+Na, 38.9, 100.0), 416, 414 (M+H, 15.0, 44.3). Anal. Calcd for $C_{17}H_{25}ClN_5O_3P{\cdot}0.25H_2O$: C, 48.81; H, 6.14; Cl, 8.47; N, 16.74; P, 7.40. Found: C, 48.80; H, 6.15; Cl, 8.36; N, 16.84; P, 7.29.

4.1.24. (*Z*)- and (*E*)-9-{[2-(Diisopropylphosphonoethyl)-cyclopropylidene]methyl}guanine (37c) and (38c). A solution of compound **37b** (1.60 g, 3.87 mmol) in formic acid (80%, 30 mL) was heated at 90°C for 6 h whereupon it was evaporated. The residue was lyophilized from water to give the *Z*-isomer **37c** (1.53 g, 100%) as a white gum. UV max (EtOH) 271 nm (ε 11,900), 228 (ε 26,100). ^{1}H NMR (DMSO-d_6) δ 11.54 (1H, s), 8.75 (1H, s), 7.14 (3H, s), 4.44 (2H, m), 2.10 (1H, m), 1.82 (1H, m), 1.66 (2H, m), 1.52 (1H, m), 1.39 (1H, m), 1.17 (13H, m). ^{13}C NMR 155.8, 155.3, 149.8, 135.1, 122.8, 111.9, 110.7, 69.9 (d, ^2J=5.9 Hz), 25.5 (d, ^1J=139.6 Hz), 25.2, 24.4 (d, ^3J=3.7 Hz), 18.1 (d, ^2J=20.9 Hz), 9.1. ^{31}P NMR 30.22. ESI-MS (MeOH+NaCl) 813 (2M+Na, 36.8), 418 (M+Na, 100.0), 396 (M+H, 24.6).

Compound **38b** was converted to the corresponding *E*-isomer **38c** by the same procedure. UV max (EtOH) 271 nm (ε 11,700), 228 (ε 25,200). ^{1}H NMR (DMSO-d_6) δ 11.60 (1H, s), 8.92 (1H, s), 7.32 (1H, s), 7.21 (2H, brs), 4.50 (2H, m), 1.89 (1H, m), 1.80 (2H, m), 1.70 (1H, m), 1.53 (1H, m), 1.35 (1H, m), 1.19 (13H, m). ^{13}C NMR 155.9, 155.1, 149.7, 134.7, 123.0, 111.2, 110.4, 69.9 (d, ^2J=6.6 Hz), 26.3, 26.1 (d, ^1J=139.6 Hz), 24.5, 16.3 (d, ^2J=20.2 Hz), 11.4. ^{31}P NMR 30.19. ESI-MS (MeOH+NaCl) 813 (2M+Na, 10.2), 418 (M+Na, 100.0), 396 (M+H, 23.4).

4.1.25. (*Z*)- and (*E*)-9-{[2-(Phosphonoethyl)cyclopropylidene]methyl}guanine (17b) and (18b). The procedure described for compounds **15b** and **16b** was followed. Me_3SiBr (0.95 mL, 7.2 mmol) was added dropwise to a solution of compound **37c** (474 mg, 1.2 mmol) in DMF (40 mL) at −40°C. The crude product was purified by chromatography on Dowex-1 as described for compound **15b** to give the *Z*-isomer **17b** (242 mg, 65%). Mp>350°C (decomp.). UV max (pH 7) 266 nm (ε 12,900), 228 (ε 30,800). ^{1}H NMR (sodium salt, D_2O) δ 7.88 (1H, s), 6.91 (1H, s), 1.95 (1H, m), 1.86 (1H, m), 1.43 (3H, m), 1.27 (1H, m), 1.08 (1H, t, J=6.4 Hz). ^{13}C NMR 163.2, 157.3, 149.9, 136.4, 116.3, 121.1, 109.3, 28.3 (d, ^1J=130.0 Hz), 26.9, 18.3 (d, ^3J=20.2 Hz), 8.1. ^{31}P NMR 22.7. ESI-MS (MeOH+NaCl) 334 (M+Na, 27.4), 312 (M+H, 25.0), 100 (100.0). Anal. Calcd for $C_{11}H_{14}N_5O_4P{\cdot}0.3H_2O$: C, 41.73; H, 4.65; N, 22.12; P, 9.78. Found: C, 42.02; H, 4.96; N, 21.82; P, 10.04.

The *E*-isomer **18b** (475 mg, 67%) was obtained following a similar procedure from compound **38c** (900 mg, 2.28 mmol). Mp>350°C (decomp.). UV max (pH 7) 266 nm (ε 12,400), 229 (ε 32,400). ^{1}H NMR (sodium salt, D_2O) δ 7.81 (1H, s), 6.89 (1H, s), 1.75 (1H, m), 1.66 (3H, m), 1.52 (2H, m), 1.15 (1H, m). ^{13}C NMR 158.3, 153.6, 149.2, 135.6, 115.2, 119.5, 108.8, 28.1 (d, J=131.8 Hz), 27.0, 16.2 (d, ^3J=20.5 Hz), 10.6. ^{31}P NMR 25.7. ESI-MS (MeOH+NaCl) 334 (M+Na, 36.9), 311 (M+H, 33.9), 100 (100.0). Anal. Calcd for $C_{11}H_{14}N_5O_4P$: C, 42.45; H, 4.53; N, 22.50; P, 9.95. Found: C, 42.22; H, 4.72; N, 22.31; P, 10.07.

4.1.26. (*Z*+*E*)-9-{[2-(Diisopropylphosphonoethyl)cyclopropylidene]methyl}cytosine (37d+38d). A stirred mixture of compound **36** (2.03 g, 5.0 mmol), N^4-acetylcytosine (1.15 g, 7.5 mmol) and K_2CO_3 (3.45 g, 25 mmol) in DMF (70 mL) was heated under N_2 at 100°C for 16 h. Methanol (10 mL) was added dropwise and the mixture was stirred for another 3 h. The solvents were evaporated and the crude product was chromatographed on a silica gel column using CH_2Cl_2/MeOH=15:1→10:1 to give an isomeric mixture **37d**+**38d** as a white gum (1.21 g, 68%). UV max (EtOH) 296 nm (ε 13,100), 230 (ε 13,700), 204 (ε 19,600). ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J=7.2 Hz) and 7.80 (1H, d, J=7.2 Hz, 1.3:1), 7.44 (s) and 7.22 (1H, s), 7.41 (2H, brs, NH_2), 5.82 (1H, 2 overlapped d), 4.51 (2H, m), 1.97 (m), 1.78–1.32 (cluster of m's) and 0.98 (m, total 9H), 1.18 (12H, m). ^{13}C NMR δ 166.22, 166.16, 154.8, 154.7, 141.4, 141.0, 116.6, 116.0, 114.3, 114.0, 95.8, 95.7, 69.9 (d, ^2J=3.0 Hz), 69.8 (d, ^2J=5.9 Hz), 26.6 (d, ^3J=3.4 Hz), 26.3 (d, ^1J=140.2 Hz), 25.8 (d), 25.7 (d, ^1J=140.4 Hz), 24.4 (d, ^3J=3.5 Hz), 24.39 (d, ^3J=4.5 Hz), 17.0 (d, ^2J=20.2 Hz), 14.0 (d, ^2J=19.4 Hz), 10.5, 7.5. ^{31}P NMR 30.3, 30.0. ESI-MS (MeOH+NaCl) 733 (2M+Na, 49.4), 378 (M+Na, 100.0), 356 (M+H, 4.0). Anal. Calcd for $C_{16}H_{26}N_3O_4P$: C, 54.08; H, 7.37; N, 11.82; P, 8.72. Found: C, 53.82; H, 7.12; N, 11.65; P, 8.84.

4.1.27. (*Z*+*E*)-9-{[2-(Phosphonoethyl)cyclopropylidene]methyl}cytosine (17c+18c). Isomers **37d**+**38d** (1.10 g, 3.1 mmol) in DMF (40 mL) at −40°C were treated with Me_3SiBr (2.5 mL, 18.6 mmol) following a similar procedure described for compounds **15c**+**16c** to give compounds **17c**+**18c** (677 mg, 79%). Mp 229–231°C (decomp.). UV max (pH 7) 291 nm (ε 12,400), 228 (ε 13,300), 199 (ε 18,500). ^{1}H NMR (sodium salt, D_2O) δ 7.96 (d, J=7.6 Hz) and 7.80 (1H, d, J=7.6 Hz, 1.8:1), 7.21 (s) and 7.05 (1H, s, 1.8:1), 6.01 (d, J=7.2 Hz) and 5.95 (1H, d, J=7.2 Hz, 1:1.8), 1.85 (m), 1.70 (m), 1.55 (m), 1.38 (m), 1.13 (m), 1.02 (m, total 7H). ^{13}C NMR 166.3, 166.1, 157.3, 143.3, 142.8, 121.1, 120.3, 115.1, 114.6, 96.5, 96.1, 28.4 (2 overlapped d, ^1J=137.0 Hz), 27.5, 27.0, 18.0 d, ^2J=20.7 Hz), 15.4 (d, ^2J=22.2 Hz), 9.6, 7.4. ^{31}P NMR 24.2, 23.8. ESI-MS (NaCl) 316 (M+2Na−H, 294 (M+Na, 100.0), 272 (M+H, 88.9). Anal. Calcd for $C_{10}H_{14}N_3O_4P$: C, 44.29; H, 5.20; N, 15.49; P, 11.42. Found: C, 44.35; H, 5.40; N, 15.28; P, 11.61.

Acknowledgements

Our thanks are due to Dr Lew M. Hrihorczuk, Central Instrumentation Facility, Department of Chemistry, Wayne State University, for mass spectra. The work described herein was supported by US Public Health Service Grant RO1-CA32779 (J. Z.) from the National Cancer Institute and Contract NO1-AI85347 (E. R. K.) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

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