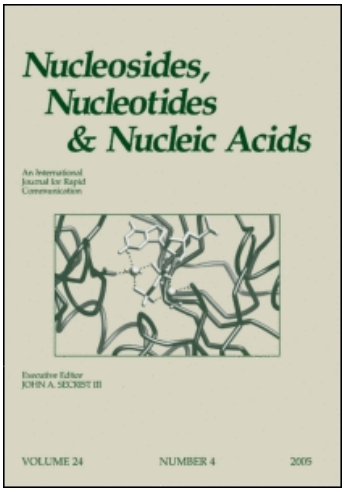

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SYNTHESIS OF "REVERSED" METHYLENECYCLOPROPANE ANALOGUES OF ANTIVIRAL PHOSPHONATES

Chengwei Li and Jiri Zemlicka □ *Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan, USA*

□ *Synthesis of "reversed" methylenecyclopropane analogues of nucleoside phosphonates* ***6a, 7a, 6b,*** *and* ***7b*** *is described. 1-Bromo-1-bromomethylcyclopropane* ***8*** *was converted to the bromocyclopropyl phosphonate* ***9*** *by Michaelis-Arbuzov reaction with triisopropyl phosphite. Base-catalyzed β-elimination and deacetylation gave the key* Z- *and* E-*hydroxymethylcyclopropyl phosphonates* ***10*** *and* ***11*** *separated by chromatography. The Mitsunobu type of alkylation of* ***10*** *or* ***11*** *with adenine or 2-amino-6-chloropurine afforded phosphonates* ***12a, 12b, 13a,*** *and* ***13b****. Acid hydrolysis furnished the adenine and guanine analogues* ***6a, 7a, 6b,*** *and* ***7b****. The* E *and* Z *configuration was assigned on the basis of NOE experiments with phosphonates* ***6b*** *and* ***7b****. All* Z- *and* E-*isomers were also distinguished by different chemical shifts of* CH_2O *or* CH_2N (H_4 *or* H_4*). Significant differences of the chemical shifts of the cyclopropane* $C_{3(3')}$ *carbons and coupling constants* $^3J_{P,C2(2')}$ *or* $^3J_{P,C3(3')}$ *selective for the* Z- *or* E-*isomers were also noted. Phosphonates* ***6a, 7a, 6b,*** *and* ***7b*** *are devoid of significant antiviral activity.*

Keywords Methylenecyclopropane phosphonates; Nucleotide analogues; michaelis-arbuzov reaction; Mitsunobu alkylation; Antivirals

INTRODUCTION

Synthesis and structure-activity relationships of antiviral methylenecyclopropane analogues of nucleosides are under active investigation in our laboratory.[1,2] Our attention has turned also to phosphonate mimics[3] of phosphorylated metabolites **1** and **2** (n = 1 or 2). The guanine analogue **1b** (n = 1) inhibited replication of varicella zoster virus (VZV) at noncytotoxic concentrations. More recently, we have described[4] a new

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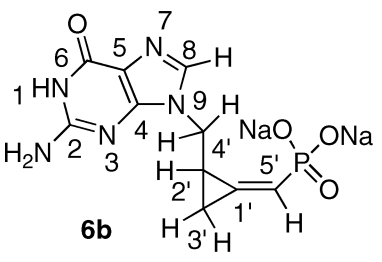
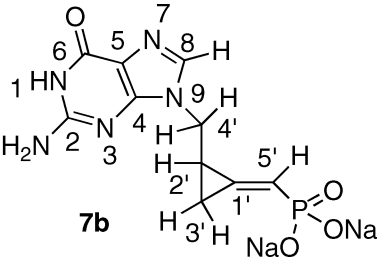
CHART 1 The NOE enhancements of relevant ^{1}H NMR signals of the *Z*- and *E*-phosphonates **6b** and **7b** in D_2O



6b



7b

6b			**7b**		
δH_{irr}	δH_{obs}	NOE (%)	δH_{irr}	δH_{obs}	NOE (%)
2.06 ($H_{2'}$)	6.14 ($H_{5'}$)	0.03	1.76 ($H_{2'}$)	6.01 ($H_{5'}$)	2.15
6.14 ($H_{5'}$)	2.06 ($H_{2'}$)	0.27	6.01 ($H_{5'}$)	1.76 ($H_{2'}$)	2.11
1.21 ($H_{3'}$)	6.14 ($H_{5'}$)	1.21	1.43 ($H_{3'}$)	6.01 ($H_{5'}$)	0
0.88 ($H_{3'}$)	6.14 ($H_{5'}$)	1.48	1.12 ($H_{3'}$)	6.01 ($H_{5'}$)	0
6.14 ($H_{5'}$)	1.21 ($H_{3'}$)	0.65	6.01 ($H_{5'}$)	1.43 ($H_{3'}$)	0
6.14 ($H_{5'}$)	0.88 ($H_{3'}$)	0.75	6.01 ($H_{5'}$)	1.12 ($H_{3'}$)	0
6.14 ($H_{5'}$)	7.72 (H_8)	0	6.01 ($H_{5'}$)	7.57 (H_8)	0.62
7.72 (H_8)	6.14 ($H_{5'}$)	0	7.57 (H_8)	6.01 ($H_{5'}$)	2.31

class of methylenecyclopropane analogues **3a**, **3b**, **4a**, and **4b** derived from antiviral phosphonates[5] **5a** and **5b** by replacing the acyclic CH_2-O-CH_2 moiety with a methylenecyclopropane unit (Chart 1). Compounds **3b** and **4b** are potent inhibitors of Epstein-Barr virus (EBV) in culture. In contrast to the symmetric CH_2-O-CH_2 moiety of analogues **5**, the "insertion" of the methylenecyclopropane unit between the base and phosphonate grouping can be achieved in two different ways. In phosphonates **3** and **4** the nucleic acid base is attached to the alkene portion via a methylene group whereas the phosphonate is connected with the cyclopropane ring. Alternately, a second group of analogues, phosphonates **6** and **7**, can be visualized, with a reversed arrangement of the base and phosphonate moieties. To the best of our knowledge, methylenecyclopropanes with phosphonate attached to the double bond have not been reported. This communication describes the synthesis of phosphonates **6a**, **6b**, **7a**, and **7b**.

RESULTS AND DISCUSSION

Synthesis

The synthesis of **6a**, **6b**, **7a**, and **7b** commenced with the Michaelis-Arbuzov reaction[6] of the previously described[7] 1-bromo-1-bromomethylcyclopropane **8** employing triisopropyl phosphite at 120–125°C for 48 h (Scheme 1). The (*Z,E*)-bromocyclopropyl phosphonate **9**

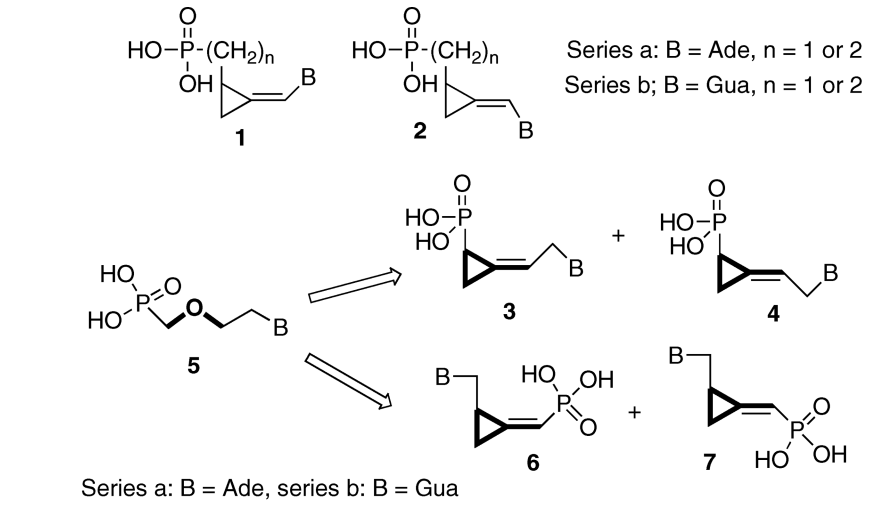
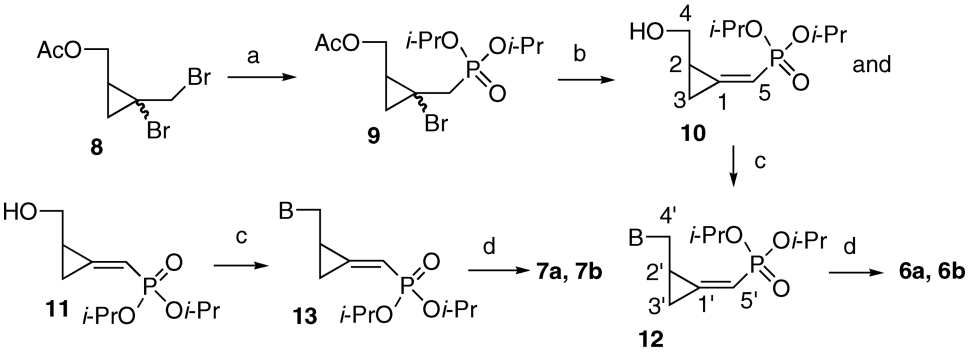


Chart 1



12a, **13a**: B = Ade, **12b**, **13b**: B = 2-amino-6-chloropurine

a. $P(Oi\text{-}Pr)_3$, 120-130 °C.
b. 2% NaOH, Et_2O.
c. B-H, Ph_3P, DEAD, THF.
d, 6M HCl, Δ.

SCHEME 1 Synthesis of methylenecyclopropane phosphonates.

was obtained in 92% yield. Elimination of the elements of HBr was effected using 2% NaOH in ether to give the key *Z*- and *E*-methylenecyclopropane phosphonates **10** and **11** under simultaneous deacetylation. The isomers were separated by chromatography on silica gel in 46 and 28% yield, respectively. The heterocyclic bases were introduced by Mitsunobu type of alkylation[8–10] using phosphonates **10** or **11**, triphenylphosphine, diethyl azodicarboxylate (DEAD) and adenine or 2-amino-6-chloropurine in tetrahydrofuran. Reaction of adenine with the *Z*-isomer **10** gave phosphonate **12a** in 47% yield whereas a similar transformation of the *E*-isomer **11** afforded phosphonate **13a** (50%). Alkylation of

2-amino-6-chloropurine with **10** gave the respective *Z*-phosphonate **12b** in 50% yield and the *E*-isomer **11** furnished compound **13b** (87%). In the last step, the isopropyl groups were removed by hydrolysis in 6M HCl. Thus, adenine diisopropyl phosphonates **12a** and **13a** furnished target phosphonates **6a** and **7a** after chromatography on Dowex 1 (formate) in 80 and 96% yield, respectively. In case of 2-amino-6-chloropurine phosphonates **12b** and **13b**, the hydrolytic dechlorination to guanine also occurred giving phosphonates **6b** and **7b** in 85–86% yield.

Determination of *E,Z* Configuration and NMR Spectra

In the series of methylenecyclopropane analogues, the *Z*-isomers move faster on silica gel than the *E*-isomers.[2] Faster chromatographic mobility of the *Z*-isomer **10** relative to *E*-isomer **11** was in accord with this trend. This assignment was confirmed by NOE experiments with the *Z*- and *E*-isomeric guanine phosphonates **6b** and **7b** (Table 1). Strong NOE enhancements observed in compound **6b** between the *cis* oriented $H_{3'}$ and $H_{5'}$ (0.65–1.48) were indicative of a *Z* configuration. Interactions between the *trans* disposed $H_{2'}$ and $H_{5'}$ were significantly weaker (0.03–0.27). As expected, no NOE effect was found between the purine H_8 and $H_{5'}$. By contrast, strong NOE enhancements were observed between the *cis* oriented $H_{2'}$ and $H_{5'}$ of the *E*-isomer **7b** (2.11 and 2.15) whereas no interaction between the $H_{3'}$ and $H_{5'}$ was detected. The *E* configuration of **7b** was further confirmed by a strong NOE effect between the H_8 and $H_{5'}$ (0.62–2.31). In an *anti*-like conformation (the $H_{3'}$ facing phosphonate moiety of **7b**), the H_8 can come much closer to $H_{5'}$ in the *E*-isomer **7b** than *Z*-isomer **6b**.

There are simpler ways to distinguish between the *Z*- and *E*-isomers of the methylenecyclopropane phosphonates applicable for compounds comprising a nucleic acid base (**12a**, **12b**, **13a**, **13b**, **6a**, **6b**, **7a**, and **7b**) and those lacking it (**10**, **11**) using some typical signal patterns or coupling constants of the ^1H and ^{13}C NMR spectra. The $H_4(H_{4'})$ of all the *Z*-isomers are non-equivalent with $\Delta\delta$ values between 0.85 and 1.20 (Table 2). By contrast, the $H_4(H_{4'})$ protons of the *E*-isomers are either equivalent ($\Delta\delta = 0$) or their differences of chemical shifts are very small ($\Delta\delta = 0.02$–0.11). In the ^{13}C NMR spectra (Table 3), the coupling constants $^3J_{P,C}$ of the carbons located *trans* to the phosphorus /$C_2(C_{2'})$ of the *E*-isomers and $C_3(C_{3'})$ of *Z*-isomers/ are larger (14.9–18.7 Hz) than those positioned *cis* /$C_3(C_{3'})$ of the *E*-isomers and $C_2(C_{2'})$ of *Z*-isomers/ (5.1–7.6 Hz). It should also be noted that the $C_3(C_{3'})$ signals of the *Z*-isomers appear at a higher field than those of the *E*-isomers. This is in line with other methylenecyclopropane analogues[2] including phosphonates[3] **12** and **13**.

Phosphonates **6a**, **6b**, **7a**, and **7b** were tested against the following viruses in culture: HSV-1, HSV-2, HCMV, EBV, VZV, HIV-1 and HBV. No significant antiviral activity or cytotoxicity was found.

TABLE 2 The $H_4(H_{4'})$ chemical shifts of the *Z*- and *E*-methylenecyclopropane phosphonates

Isomer[a]	H_4 or $H_{4'}$ (δ)	$\Delta\delta$	Isomer[a]	H_4 or $H_{4'}$ (δ)	$\Delta\delta$
Z-**10**	4.14, 3.00	1.14	*E*-**13b**	4.07	0
E-**11**	3.54, 3.43[b]	0.11	*Z*-**6a**	4.64, 3.53[c]	1.11
	3.52, 3.45[b]	0.07			
Z-**12a**	4.89, 4.04	0.85	*E*-**7a**	3.92[c]	0
E-**13a**	4.22, 4.16[b]	0.06	*Z*-**6b**	4.61, 3.54[c]	1.07
	4.20, 4.18[b]	0.02			
Z-**12b**	4.84, 3.64	1.20	*E*-**7b**	3.79[c]	0

[a]$CDCl_3$.
[b]AB system.
[c]D_2O, sodium salt.

EXPERIMENTAL SECTION

General Methods

The NMR spectra were determined with 400 (^1H), 100 (^{13}C), 162 (^{31}P) MHz in $CDCl_3$ and UV spectra were determined in ethanol unless stated otherwise. For atom numbering see formulas **10**, **12**, and Table 1. The NMR spectra of phosphonates **6a**, **6b**, **7a**, and **7b** were recorded after conversion to sodium salts by addition of NaOH to the free acids in D_2O. The mass spectra were measured in electrospray ionization mode (ESI-MS, methanol–NaCl) or as indicated.

Diisopropyl (*E*,*Z*)-2-(Acetoxymethyl)-1-(bromocyclopropyl)-1-methylphosphonate (9). Triisopropyl phosphite (14.58 g, 70 mmol) was heated at 120–125°C in a three-necked flask equipped with an addition funnel, short-path distillation apparatus and thermometer. *cis/trans*-1-(acetoxymethyl)-2-bromo-2-(bromomethyl)cyclopropane[7] (**8**, 18.55 g, 65 mmol) was then

TABLE 3 The $C_2(C_{2'})$ and $C_3(C_{3'})$ chemical shifts and the corresponding $^3J_{P,C}$ coupling constants of the *Z*- and *E*-methylenecyclopropane phosphonates

Isomer[a]	$C_2(C_{2'})$, ppm	$^3J_{P,C2(2')}$ (Hz)	$C_3(C_{3'})$, ppm	$^3J_{P,C3(3')}$ (Hz)
Z-**10**	19.0	7.6	7.4	18.7
E-**11**	18.3	17.9	9.8	6.7
Z-**12a**	17.4	6.6	9.3	18.6
E-**13a**	15.7	18.6	10.9	6.7
Z-**12b**	14.6	7	9.4	18.6
E-**13b**	15.2	17.9	11.0	6.0
Z-**6a**	15.9[b]	5.6	7.8	14.9
E-**7a**	13.8[b]	15.7	9.9	5.9
Z-**6b**	16.1[b]	5.8	7.6	15.6
E-**7b**	14.0[b]	16.4	9.6	5.1

[a]$CDCl_3$.
[b]D_2O, sodium salt.

added with stirring at 120–130°C within a period of 1 hour. The stirring was continued for additional 48 hours. After cooling, the crude product was chromatographed on a silica gel column with hexanes—EtOAc (3 : 1) to give compound **9** (22.04 g, 92%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.58 (m, 2H, CHO of *i*-PrO), 4.14 (dd, J = 11.6, 5.2 Hz), 4.00 (m), 3.82 (dd, 2H, J = 12.0, 8.8 Hz, H_4), 2.24, 2.03 (2 m, 2H, H_5), 1.90, 1.70 (s + m, 4H, CH_3 of Ac + H_2), 1.14, 0.88 (2 m, 14H, CH_3 of *i*-Pr + H_3). ^{13}C NMR: 170.9, 170.7 (C = O), 70.8 (2 overlapped d, CHO of *i*-PrO), 67.1, 63.3 (C_4), 40.4, 35.2 (2d, J = 144.8, 149.2 Hz, C_5), 31.5, 27.6 (2d, J = 8.0, 7.5 Hz, C_1), 27.2, 23.2 (2d, J = 9.7, 8.3 Hz, C_2), 24.2, 24.1 (2d, J = 3.7, 4.4 Hz, CH_3 of *i*-Pr), 21.26, 21.32 (2 overlapped d, J = 7.5, 6.7 Hz, C_3), 21.01, 20.95 (CH_3 of Ac). ^{31}P NMR 24.7, 23.6. ESI-MS 371, 373 (M + H, 81.4, 81.4), 393, 395 (M + Na, 100.0, 100.0). Anal. Calcd for $C_{13}H_{24}BrO_5P$: C, 42.06; H, 6.52. Found: C, 42.08; H, 6.41.

Diisopropyl (*Z*) and (*E*)-2-(Hydroxymethyl)cyclopropylidenemethylphosphonates (10) and (11). A solution of phosphonate **9** (10.08 g, 27 mmol) in Et_2O (400 mL) was shaken with 2% NaOH (400 mL, 0.2 mol) at room temperature until all starting material disappeared (40 minutes). The ether layer was separated and the aqueous phase was extracted with Et_2O (3 × 400 mL). The combined organic phase was dried (Na_2SO_4), solvent was evaporated in vacuo and the residue was chromatographed using EtOAc-hexanes-MeOH (3 : 1 : 0.2) to give the *Z*-isomer **10** (23.1 g, 46%) followed by *E*-isomer **11** (1.9 g, 28%) as colorless oils.

Z-Isomer **10**: ^{1}H NMR δ 6.04 (d, J = 20.8 Hz, H_5), 5.30 (bs, 1H, OH), 4.65 (m, 2H, CHO of *i*-PrO), 4.14 (dd, 1H, J = 10.4, 4.0), 3.00 (t, 1H, J = 10.4, H_4), 2.09 (m, 1H, H_2), 1.47 (m, 1H), 1.02 (m, 1H, H_3), 1.35, 1.33, 1.30, 1.28 (4 partially overlapped d, 12H, J = 5.6–6.8 Hz, CH_3). ^{13}C NMR 152.5 (d, J = 7.5 Hz, C_1), 107.8 (d, J = 191.1 Hz, C_5), 71.3, 71.2 (2d, J = 5.5, 6.0 Hz, CHO of *i*-PrO), 65.8 (C_4), 24.3, 24.22, 24.18, 24.1 (4 overlapped d, J = 3.7–4.4 Hz, CH_3), 19.0 (d, J = 7.6 Hz, C_2), 7.4 (d, J = 18.7 Hz, C_3). ^{31}P NMR 16.75. ESI-MS 249 (M + H, 68.3), 271 (M + Na, 100.0), Anal. Calcd for $C_{11}H_{21}O_4P$: C, 53.20; H 8.53. Found: C, 52.99; H, 8.38.

E-Isomer **11**: ^{1}H NMR δ 6.05 (ddd, 1H, J = 22.7, 4.0, 2.4 Hz, H_5), 4.60 (m, 2H, CHO of *i*-PrO), 3.54, 3.43; 3.52, 3.45 (2AB, 2H, J = 11.2 Hz, H_4), 3.22 (bs, OH), 1.83 (m, 1H, H_2), 1.52 (m, 1H), 1.20 (poorly resolved m, 1H, H_3), 1.26, 1.23 (2d, 12H, J = 6.4 Hz, CH_3). ^{13}C NMR 149.4 (d, J = 4.5 Hz, C_1), 108.3 (d, J = 194.1 Hz, C_5), 70.6 (d, J = 4.5 Hz, CHO of *i*-PrO), 63.9 (C_4), 24.2 (2 overlapped d, J = 5.2 Hz, CH_3), 18.3 (d, J = 17.9 Hz, C_2), 9.8 (d, J = 6.7 Hz, C_3). ^{31}P NMR 15.49. ESI-MS 249 (M + H, 51.7), 271 (M + Na, 54.3), 519 (2M + Na, 100.0). Anal. Calcd for $C_{11}H_{21}O_4P$: C, 53.22; H 8.53. Found: C, 53.39; H, 8.43.

Synthesis of diisopropyl phosphonates 12a, 12b, 13a, and 13b. General Method. A mixture of *Z*- or *E*-hydroxymethylphosphonate **10** or **11** (0.75 g, 3 mmol)), Ph_3P (1.57 g, 6 mmol) and adenine or 2-amino-6-chloropurine (6 mmol) in THF (100 mL) was stirred at room temperature for 10 minutes.

The mixture was cooled to 0°C and diethyl azodicarboxylate (DEAD, 1.05 g, 6 mmol) in THF (20 mL) was added dropwise. The mixture was stirred overnight at room temperature and the solvent was evaporated. The crude product was chromatographed on a silica gel column to give phosphonates **12a**, **12b**, **13a**, or **13b**.

(*Z*)-9-[1-(Diisopropylphosphonomethylene)cyclopropylmethyl]adenine (12a). The general method was followed with adenine (0.81g, 6 mmol) and the *Z*-isomer **10**. The crude product was chromatographed in $CHCl_3$-MeOH (5 : 0.1) and EtOAc-MeOH (5 : 0.4) to give the *Z*-phosphonate **12a** (0.52g, 47%) as a yellow solid, mp 108–110°C. UV λ_{max} (EtOH) 261 nm (ε 13900), 206 (ε 24200). ^{1}H NMR δ 8.34, 8.00 (s, 2H, H_8, H_2), 6.13, 6.12 (poorly resolved ddd, 3H, J = 21.1 Hz overlapped with bs, $H_{5'}$ + NH_2), 4.89 (dd, 1H, J = 14.4, 4.0 Hz), 4.04 (dd, 1H, J = 14.4, 8.0 Hz, H_4), 4.70 (m, 2H, CHO of *i*-PrO), 2.21 (m, 1H, $H_{2'}$), 1.48 (m. 1H), 1.27 (2 m. 2H, $H_{3'}$), 1.38, 1.36, 1.35, 1.31 (4d, 12H, J = 4.0–6.5 Hz, CH_3). ^{31}C NMR 155.8, 153.1, 150.4, 141.0, 119.5 (adenine), 147.6 (d, J = 5.9 Hz, $C_{1'}$), 110.4 (d, J = 192.5 Hz, $C_{5'}$), 70.87, 70.85 (2 overlapped d, J = 5.5—6.0 Hz, CHO of *i*-PrO), 44.7 ($C_{4'}$), 24.4–24.2 (2d, CH_3), 17.4 (d, J = 6.6 Hz, $C_{2'}$), 9.3 (d, J = 18.6 Hz, $C_{3'}$). ^{31}P NMR 14.78. ESI-MS 366 (M + H, 96.1), 388 (M + Na, 31.6), 753 (2M + Na, 100.0). Anal. Calcd for $C_{16}H_{24}N_5O_3P$: C, 52.60; H 6.62; N, 19.17. Found: C, 52.52; H, 6.60; N, 19.32.

(*E*)-9-[1-(Diisopropylphosphonomethylene)cyclopropylmethyl]adenine (13a). The general method was followed with a mixture of the *E*-isomer **11** (1.0 g, 4 mmol), Ph_3P (2.1 g, 8 mmol), adenine (1.08 g, 8 mmol) and DEAD (1.37 g, 8 mmol). Chromatography in $CHCl_3$-MeOH (5 : 0.2) gave the *E*-phosphonate **13a** (0.73 g, 50%) as a pale yellow solid, mp 138–139°C. UV λ_{max} (EtOH) 261 nm (ε 14200), 207 (ε 22400). ^{1}H NMR δ 8.32, 8.00 (2s, 2H, H_8, H_2), 6.16 (bs, 2H, NH_2), 6.10 (poorly resolved dd, 1H, J = 22.4 Hz, $H_{5'}$), 4.66 (m, 2H, CHO of *i*-PrO), 4.22, 4.16 (J_{AB} = 14.4—14.8 Hz), 4.20, 4.18 (J_{AB} = 10.4–10.8, 2 partly overlapped AB, 2H, $H_{4'}$), 2.15 (m, 1H, $H_{2'}$), 1.72 (poorly resolved dt, 1H, J = 10.8 Hz), 1.48 (m, 1H, $H_{3'}$), 1.31, 1.30, 1.28, 1.26 (4d, 12H, J = 5.7–6.5 Hz, CH_3). ^{13}C NMR: 155.9, 153.2, 150.2, 140.1, 119.8 (adenine), 146.2 (d, J = 5.2 Hz, $C_{1'}$), 110.6 (d, J = 193.2 Hz, $C_{5'}$), 70.7 (d, J = 6.0 Hz, CHO of *i*-PrO), 45.8 ($C_{4'}$), 24.3 (s, CH_3), 15.7 (d, J = 18.6 Hz, $C_{2'}$), 10.9 (d, J = 6.7 Hz, $C_{3'}$). ^{31}P NMR 14.22. ESI-MS 366 (M + H, 100.0), 388 (M + Na, 26.3). Anal. Calcd for $C_{16}H_{24}N_5O_3P$: C, 52.60; H 6.62; N, 19.17. Found: C, 52.79; H, 6.46; N, 19.11.

(*Z*)-2-Amino-6-chloro-9-[1-(diisopropylphosphonomethylene)cyclopropyl]-methylpurine (12b). The general procedure was followed with 2-amino-6-chloropurine (1.01 g, 6 mmol) and the *Z*-isomer **10**. The crude product was chromatographed in CH_2Cl_2–MeOH (5 : 0 to 5:0.4), EtOAc—MeOH (5 : 0.2) and, finally, $CHCl_3$-MeOH (50 : 1) to give the *Z*-phosphonate **12b** (0.60 g, 50%) as a white solid, mp 181–183°C. UV λ_{max} (EtOH) 310 nm (ε 8050), 249 (ε 6000), 222 (ε 25500), 203 (ε 18500). ^{1}H

NMR δ 7.83 (s, 1H, H_8), 6.14 (poorly resolved ddd, 1H, 20.8, 2.0 Hz, $H_{5'}$), 4.84 (dd, 1H, J = 14.8, 4.0 Hz), 3.64 (dd, 1H, J = 14.6, 9.0 Hz, $H_{4'}$), 4.76 (m, 2H, CHO of *i*-PrO), 2.24 (m, 1H, $H_{2'}$), 1.49, 1.21 (2 m, 2H, $H_{3'}$), 1.38, 1.37, 1.33, 1.30 (4d, 12H, J = 5.7–6.6 Hz, CH_3). ^{13}C NMR 159.9, 154.1, 151.6, 142.3, 125.0 (2-amino-6-chloropurine), 146.9 (d, J = 5.9 Hz, $C_{1'}$), 110.9 (d, J = 193.2 Hz, $C_{5'}$), 71.0 (d, J = 6.0 Hz, CHO of *i*-PrO), 45.2 ($C_{4'}$), 24.4, 24.3 (2d, J = 4.5, 6.0 Hz, CH_3), 14.6 (d, J = 7 Hz, $H_{2'}$), 9.4 (d, J = 18.6 Hz, $C_{3'}$). ^{31}P NMR 14.79. ESI-MS 400, 402 (M + H, 100.0, 34.8), 422, 424 (M + Na, 17.6, 6.0). Anal. Calcd for $C_{16}H_{23}N_5O_3PCl$: C, 48.07; H 5.80; N, 17.52. Found: C, 48.12; H, 5.71; N, 17.52.

(*E*)-2-Amino-6-chloro-9-[1-(diisopropylphosphonomethylene)cyclopropyl]-methylpurine (13b). The *E*-phosphonate **13b** was prepared by the general procedure using 2-amino-6-chloropurine and the *E*-isomer **11**. The crude product was chromatographed using $CHCl_3$-MeOH (5:0 to 5:0.4) followed by EtOAc-MeOH (5:0.2) to afford compound **13b** (0.99 g, 87%) as a colorless solid, mp 58–60°C. UV λ_{max} (EtOH) 311 nm (ε 7200), 249 (ε 5700), 222 (ε 24600), 203 (ε 18400). ^{1}H NMR δ 7.77 (s, 1H, H_8), 6.10 (poorly resolved ddd, 20.4 Hz, 1H, $H_{5'}$), 5.28 (bs, 2H, NH_2), 4.66 (m, 2H, CHO of *i*-PrO), 4.07 (2 overlapped AB, 2H, $H_{4'}$), 2.10 (m, 1H, $H_{2'}$), 1.72 (poorly resolved dt, 1H, J = 10.2 Hz), 1.49 (m, 1H, $H_{3'}$), 1.31, 1.30, 1.28, 1.26 (d, 12H, J = 5.7–7.3 Hz). ^{31}C NMR 159.4, 153.9, 151.6, 141.9, 125.3 (2-amino-6-chloropurine), 145.9 (d, 10.5 Hz, $C_{1'}$), 110.7 (d, J = 193.2 Hz, $C_{5'}$), 70.8 (d, J = 6.0 Hz), 45.8 ($C_{4'}$), 24.30, 24.26 (2 overlapped d, J = 4.4, 3.5 Hz, CH_3), 15.2 (d, J = 17.9 Hz, $C_{2'}$), 11.0 (d, J = 6.0 Hz, $C_{3'}$). ^{31}P NMR 14.16. ESI-MS 400, 402 (M + H, 90.8, 30.2), 422, 424 (M + Na, 100.0, 36.1). Anal. Calcd for $C_{16}H_{23}N_5O_3PCl$: C, 48.11; H 5.81; N, 17.52. Found: C, 47.91; H, 5.74; N, 17.47.

(*Z*)-9-[2-(Phosphonomethylene)cyclopropylmethyl]adenine (6a). A solution of diisopropyl *Z*-phosphonate **12a** (0.20 g, 0.55 mmol) in 6M HCl (12 mL) was refluxed for 30 minutes. After cooling, the volatile components were evaporated in vacuo, the residue was dissolved in water (3 mL) and the pH was adjusted to >8 with 1M NH_4OH. The solution was put on the top of Dowex 1-X2-200/HCO_2^-/column (2.2 × 10 cm) which was eluted with water and 0.1 M HCO_2H (500 mL). UV absorbing fractions were pooled and the volatile components were evaporated to give the *Z*-phosphonate **6a** (0.12 g, 80%) as a white solid, mp >300°C. UV λ_{max} (0.02M Na_2HPO_4, pH 7.0) 262 nm (ε 13900), 208 (ε 14400). ^{1}H NMR (sodium salt, D_2O) δ 7.97, 7.80 (2s, 2H, H_8, H_2), 6.13 (d, 1H, J = 16.4 Hz, $H_{5'}$), 4.64 (d, 1H, J = 14.4 Hz), 3.53 (poorly resolved dd, 1H, $H_{4'}$), 2.00 (bs, 1H, $H_{2'}$), 1.14, 0.79 (2bs, 2H, $H_{3'}$). ^{13}C NMR: 154.9, 151.9, 148.4, 142.1, 117.7 (adenine), 134.2 (J = 3.3 Hz, $C_{1'}$), 119.2 (d, J = 170.1 Hz, $C_{5'}$), 46.1 ($C_{4'}$), 15.9 (d, J = 5.6 Hz, $C_{2'}$), 7.8 (d, J = 14.9 Hz, $C_{3'}$). ^{31}P NMR 10.10. Negative ESI-MS (MeOH) 280 (M - H, 100.0). 561 (2M - H, 12.0). Anal. Calcd for $C_{10}H_{12}N_5O_3P$: C, 42.71; H 4.30; N, 24.90. Found: C, 42.60; H, 4.34; N, 24.80.

(*E*)-9-[2-(phosphonomethylene)cyclopropylmethyl]adenine (7a). The experiment was performed as in the case of the *Z*-isomer **6a** with the diisopropyl *E*-phosphonate **13a** (0.30 g, 0.82 mmol), reflux for 1hour. Yield 0.22 g (96%) of the *E*-isomer **5a**, mp >300°C. UV λ_{max} (0.02 M Na_2HPO_4, pH 7.0) 262 nm (ε 14500), 208 (ε 22700). ^{1}H NMR (sodium salt, D_2O) δ 7.93, 7.79 (2s, 2H, H_8, H_2), 6.10 (poorly resolved ddd, J = 16.0 Hz, 1H, $H_{5'}$), 3.92 (m, 2H, $H_{4'}$), 1.82 (bs, 1H, $H_{2'}$), 1.55, 1.24 (2m, 2H, $H_{3'}$). ^{13}C NMR 154.9, 151.9, 148.0, 141.8, 117.8 (adenine), 135.0 (d, J = 4.5 Hz, $C_{1'}$), 118.3 (d, J = 169.4 Hz, $C_{5'}$), 46.7 ($C_{4'}$), 13.8 (d, J = 15.7 Hz, $C_{2'}$), 9.9 (d, J = 5.9 Hz, $C_{3'}$). ^{31}P NMR 11.16. Negative ESI-MS (MeOH) 280 (M - H, 100.0), 561 (2M - H, 8.4). Anal. Calcd for $C_{10}H_{12}N_5O_3P$: C, 42.71; H 4.30; N, 24.90. Found: C, 42.50; H, 4.50; N, 24.69.

(*Z*)-9-[2-(phosphonomethylene)cyclopropylmethyl]guanine (6b). The procedure described for the *Z*-phosphonate **4a** was followed using the diisopropyl *Z*-phosphonate **12b** (0.40 g, 1.0 mmol). Elution of the Dowex 1 column with water was followed by a linear gradient of 0.1 M (500 mL) to 0.5 M HCO_2H (500 mL) to give after concentration of the UV absorbing fractions the Z-phosphonate **6b** (0.26 g, 86%), mp >300°C. UV λ_{max} (0.02 M Na_2HPO_4, pH 7.0) 253 nm (ε 13100), 208 (ε 22000). ^{1}H NMR (sodium salt, D_2O) δ 7.77 (s, 1H, H_8), 6.14 (dd, 1H, J = 16.4, 1.6 Hz, $H_{5'}$), 4.61 (dd, 1H, J = 14.8, 3.2 Hz,), 3.54 (dd, 1H, J = 14.6, 9.8 Hz, $H_{4'}$), 2.06 (m, 1H, $H_{2'}$), 1.21, 0.88 (2m, 2H, $H_{3'}$). ^{13}C NMR 168.5, 161.3, 151.7, 138.9, 117.6 (guanine), 134.7 (d, J = 3.0 Hz, $C_{1'}$), 119.0 (d, J = 171.6 Hz, $C_{5'}$), 45.5 ($H_{4'}$), 16.1 (d, J = 5.8 Hz, $C_{2'}$), 7.6 (d, J = 15.6 Hz, $C_{3'}$). ^{31}P NMR 10.23. Negative ESI-MS (MeOH) 296 (M - H, 100.0), 593 (2M - H, 28.1). Anal. Calcd for $C_{10}H_{12}N_5O_4P{\cdot}1.1H_2O$: C, 37.88; H 4.51; N, 22.09. Found: C, 37.93; H, 4.29; N, 21.82.

(*E*)-9-[2-(phosphonomethylene)cyclopropylmethyl]guanine (7b). Hydrolysis of the diisopropyl *E*-phosphonate **13b** (0.60 g,1.5 mmol) followed the protocol for the adenine *Z*-isomer **6a**. Elution of the Dowex 1 column with water was followed by 0.5 M HCO_2H (500 mL) to give the *E*-isomer **7b** (0.38 g, 85%), mp >300°C. UV λ_{max} (0.02 M Na_2HPO_4, pH 7.0) 253 nm (ε 12400), 209 (ε 19300). ^{1}H NMR (sodium salt, D_2O) δ 7.57 (s, 1H, H_8), 6.01 (d, 1H, J = 16.0 Hz, $H_{5'}$), 3.79 (m, 2H, $H_{4'}$), 1.76 (bs, 1H, $H_{2'}$), 1.43, 1.12 (2bs, 1H, $H_{3'}$). ^{13}C NMR 168.3, 161.1, 151.3, 138.4, 117.6 (guanine), 135.5 (d, J = 3.6 Hz, $C_{1'}$), 117.9 (d, J = 170.1 Hz, $C_{5'}$), 46.0 ($C_{4'}$), 14.0 (d, J = 16.4 Hz, $C_{2'}$), 9.6 (d, J = 5.1 Hz, $C_{3'}$). ^{31}P NMR 10.90. ESI-MS (MeOH) 298 (M + H, 100.0), 595 (2M + H, 28.1). Anal. Calcd for $C_{10}H_{12}N_5O_4P{\cdot}0.85H_2O$: C, 38.43; H 4.42; N, 22.40. Found: C, 38.68; H, 4.43; N, 22.05.

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