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Synthesis of Methylenecyclopropane Analogues of Antiviral Nucleoside Phosphonates

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Abstract

Synthesis of methylenecyclopropane analogues of nucleoside phosphonates **6a**, **6b**, **7a** and **7b** is described. Cyclopropyl phosphonate **8** was transformed in four steps to methylenecyclopropane phosphonate **16**. The latter intermediate was converted in seven steps to the key *Z*- and *E*-methylenecyclopropane alcohols **23** and **24** separated by chromatography. Selenoxide eliminations (**15** → **16** and **22** → **23** + **24**) were instrumental in the synthesis. The *Z*- and *E*-isomers **23** and **24** were transformed to bromides **25a** and **25b** which were used for alkylation of adenine and 2-amino-6-chloropurine to give intermediates **26a**, **26b**, **26c** and **26d**. Acid hydrolysis provided the adenine and guanine analogues **6a**, **6b**, **7a** and **7b**. Phosphonates **6b** and **7b** are potent inhibitors of replication of Epstein-Barr virus (EBV).

Keywords

Cyclopropylphosphonates; Methylenecyclopropanes; Selenoxide eliminations; Nucleotide analogues; Antivirals

1. Introduction

Analogues of nucleoside 5'-phosphates have been a fruitful topic of research for many years. 1-3 Phosphonate derivatives which unlike nucleotides are chemically and enzymatically stable occupy a prominent place in this effort. Another important feature of these compounds is that they are capable of circumventing the first phosphorylation step in the activation of nucleoside analogues. This is frequently a limiting event in the phosphorylation sequence which ultimately leads to triphosphates. One of the first groups of such analogues which yielded biologically effective compounds are acyclic nucleoside phosphonates¹ exemplified by structures **1a** and **1b** (Chart 1). Thus, compound **1a** (adefovir) and the guanine counterpart **1b** are just two Chart 1 examples of potent antiviral agents from this class of analogues. Because acyclic chain of **1a** and **1b** has five rotatable bonds, limiting their number will improve the entropic factor and this may lead to new biologically active analogues. Indeed, an insertion of two methylene groups between carbons 2' and 3' of the guanine derivative **1b** led to furanose *cis*- and *trans*-phosphonates **2** and **3** with only three rotatable bonds. Both analogues were effective⁴⁻⁶ against human cytomegalovirus (HCMV) and the *trans*-isomer **3** is an antitumor agent.⁷⁻⁹

Our previous investigations have shown that isosteric replacement of C-O-C grouping of antiviral drugs acyclovir **4a** (B = Gua) and ganciclovir **4b** (B = Gua) with a rigid

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methylenecyclopropane moiety led to a new class of nucleoside analogues **5a** and **5b** (Chart 2), Chart 2 effective in particular against HCMV and Epstein-Barr virus (EBV).¹⁰⁻¹³ Therefore, it seemed possible that a similar replacement of the C-O-C function in acyclic phosphonates **1a** and **1b** (Chart 1) might provide new analogues with biological activity. Regardless of the results, the antiviral testing of these analogues will provide further insight into the structure-activity relationships of methylenecyclopropane analogues. For these reasons, we have synthesized phosphonates **6a**, **6b**, **7a** and **7b**.

2. Results and discussion

Diisopropyl (*E*)-2-hydroxymethyl-1-phosphonate¹⁴ (**8**) was chosen as a starting material. The reproduction of the synthetic sequence on the scale of 0.27 mol was problem-free although little details were originally provided.¹⁴ The commercially available diisopropyl methylphosphonate (**9**) and allyl bromide (**10**) were transformed to unsaturated phosphonate **11** in 70% yield (Scheme 1). Compound **11** was transformed to oxirane **12** (91%) and, finally, by an Scheme 1 intramolecular opening of the oxirane ring to cyclopropane **8** in 80% yield. Phosphonate **8** was converted to bromo derivative **13** using $\text{Ph}_3\text{P} - \text{Br}_2$ reagent but the product was inseparable from the triphenylphosphine oxide formed during the reaction and, therefore, it was used as such in the next step. Reaction with PhSeNa generated from Ph_2Se_2 and NaBH_4 gave phenylselenenyl derivative **14** still contaminated with triphenylphosphine oxide. Although it was possible to obtain pure **14** by chromatography on a small scale, this was impractical for the intended purpose. Therefore, crude **14** was oxidized with H_2O_2 to give, after chromatography, selenoxide **15** in 76% yield after three steps (**8** $\rightarrow$ **13** $\rightarrow$ **14** $\rightarrow$ **15**). β -Elimination catalyzed by *i*- Pr_2NEt gave after a prolonged reflux in toluene (50 h) methylenecyclopropane phosphonate **16** (65%). Elimination of phenylselenoxide from cyclopropanes to give methylenecyclopropanes was reported.¹⁵⁻¹⁷ Electrophilic addition of phenylselenenyl bromide¹⁸ (prepared in situ from Ph_2Se_2 and NBS) afforded intermediate **17** (88%) as a single stereoisomer as shown by NMR. It is likely that the phosphonate group of **16** directs the addition of selenium reagent to the *syn* face of double bond similar to methylenecyclopropane carboxylate function¹⁸ to give the *cis* (*Z*) isomer of **17** via phenylselenonium intermediate **18** (Scheme 2).

Scheme 2 Carbon chain extension was performed using nitrile **19** which was obtained from **17** using Me_3SiCN and Bu_4NF method¹⁹ in 45% yield. Methanolysis (HCl in MeOH) afforded ester **20** (72%) which was reduced with LiBH_4 to alcohol **21** (74%). Acetylation gave acetate **22** which was oxidized with H_2O_2 , subsequently refluxed for 24 h and then deacetylated to give the *Z*- and *E*-methylenecyclopropane phosphonates **23** and **24**. Both isomers were readily separated by chromatography to give the less polar (faster moving) *Z*-isomer **23** followed by the *E*-isomer **24** in 32 and 29% overall yield, respectively, after four steps.

Separated isomers **23** and **24** were converted to bromo derivatives **25a** and **25b** using Ph_3P and CBr_4 (67%, Scheme 3). Alkylation of adenine with **25a** or **25b** using K_2CO_3 in DMF at Scheme 3 room temperature gave intermediates **26a** or **26b** in 70 and 67% yield, respectively. Hydrolysis in refluxing 6M HCl for 20 min provided free target phosphonates **6a** (83%) and **7a** (71%). In a similar fashion, alkylation of 2-amino-6-chloropurine with bromides **25a** and **25b** afforded diisopropyl phosphonates **26c** and **26d** in 62 and 67% yield, respectively. The corresponding 7-isomers **27a** and **27b** were obtained as more polar by-products in 15% yield. Attack of the Formula 27a and 27b 7-position of a purine ring is a frequent side-reaction in alkylations of 2-amino-6-chloropurine.²⁰ The assignment of the 7-isomers followed from a general rule²¹ that the H_8 , C_8 and C_1' signals of the 7-isomers are located at a lower field than in 9-isomers whereas the opposite is true for the C_5 resonances (Table 1). Hydrolysis of phosphonate esters and hydrolytic dechlorination of **26c** and Table 1 **26d** were performed

simultaneously to give target phosphonates **6b** (85%) and **7b** (73%) after chromatography on Dowex 1.

A preliminary *Z*- and *E*-isomeric assignment was based on a chromatographic mobility of compounds **23** and **24** on silica gel which conformed with the general observation in the series of methylenecyclopropane analogues that the *Z*-isomers are less polar (faster moving) than *E*-isomers.^{3,10,12,13} The NOE experiments with phosphonates **6a** and **7a** confirmed these assignments (Table 2). As expected, NOE enhancements were found between the *cis*-orientated Table 2 hydrogens, H_{2'} and H_{4'} in the *Z*-isomer **6a** and H_{2'} and H_{5'} in the *E*-isomer **7a**.

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. Guanine analogues **6b** and **7b** were potent inhibitors of replication of EBV in Daudi cells with EC₅₀/CC₅₀ (μM) 1.1 and <0.03/>300, respectively.²² Acyclovir (**4a**, B = Gua) used as a control had EC₅₀/CC₅₀ 0.33/>100. No activity was found against other tested viruses.

3. Conclusions

Synthesis of methylenecyclopropane phosphonates **6a**, **6b**, **7a** and **7b** is described. Selenoxide eliminations (Scheme 1) were key steps in the synthesis. The described methodology may find application for synthesis of cyclopropylphosphonates beyond the area of nucleotide analogues. The *Z*- and *E*-guanine analogues **6b** and **7b** were potent inhibitors of replication of Epstein-Barr virus (EBV).

4. Experimental

4.1. General Methods

The NMR spectra were determined at 300 or 400 MHz (¹H), 75 or 100 MHz (¹³C) and 162 MHz (³¹P). Mass spectra were measured in electron-impact (EI-MS) or electrospray ionization (ESI-MS, methanol - NaCl) mode. For synthesis of **11**, **12** and **8** a general procedure¹⁴ was followed.

4.1.1. Diisopropyl 3-Butenyl-1-phosphonate (11). BuLi (330 mL, 0.66 mol, 2.0 M in hexanes) was slowly added to a stirred solution of diisopropyl methylphosphonate (**9**, 78 mL, 0.40 mol) in THF (200 mL) at -78 °C under N₂. The stirring was continued for 30 min, the solution of the Li reagent was allowed to warm to rt and then it was carefully transferred to a 1-L addition funnel. The reagent was added to a solution of allyl bromide (**10**, 75 mL, 0.86 mol) in THF (200 mL) at -78 °C within 30 min under N₂. The stirring was continued for 30 min whereupon the reaction was carefully quenched by adding saturated aqueous NH₄Cl solution (300 mL). The layers were separated and the aqueous phase was extracted with EtOAc (2 × 150 mL). The combined organic phases were washed with brine (300 mL) and dried (Na₂SO₄). Evaporation of solvents gave a crude product which was chromatographed on a silica gel column in hexanes : EtOAc = 4 : 1 to 2 : 1 to afford phosphonate **11** as an oil (61.6 g, 70%). ¹H NMR (CDCl₃) δ 5.78 (ddt, 1H, J = 17.2, 10.4, 6.4 Hz, CH=), 4.98 (ddt, 1H, J = 17.2, 1.6 Hz), 4.92 (dd, 1H, J = 10.0, 1.6 Hz, CH₂=), 4.62 (m, 2H, CHO of *i*-PrO), 2.28 (m, 2H, CH₂), 1.71 (m, 2H, CH₂P), 1.25 (2d, J = 5.6 Hz, 12H, CH₃). ¹³C NMR 137.6 (d, J = 18.6 Hz, CH=), 115.1 (CH₂=), 70.1 (d, J = 6.7 Hz, CHO of *i*-PrO), 26.9 (d, J = 4.4 Hz, CH₂), 26.4 (d, J = 141.8 Hz, CH₂P), 24.3 (J = 4.4 Hz, CH₃). ³¹P NMR 30.44.

4.1.2. Diisopropyl (*E*)-2-(Oxiranyl)ethylphosphonate (12). MCPBA (126 g, 0.56 mol, 77%) was added in small portions to a solution of compound **11** (60.0 g, 0.27 mol) in CH₂Cl₂ (1 L) with stirring at 0 °C. The stirring was continued at rt for 11 h. The solution was then cooled to 0 °C again and the reaction was quenched with saturated aqueous solutions of NaHCO₃ (500

mL) and 250 Na₂S₂O₃ (250 mL). The organic layer was separated and aqueous phase was extracted with CH₂Cl₂ (3 × 250 mL). The combined organic phase was washed with saturated aqueous NaHCO₃ (3 × 200 mL) and brine (200 mL). After drying (Na₂SO₄) the solvents were evaporated to give a crude product which was chromatographed on silica gel in hexanes : EtOAc = 3 : 1 to 0 : 100 and then CH₂Cl₂ : MeOH = 10 : 1 leaving oxirane **12** (58.5 g, 91%) as an oil. ¹H NMR (CDCl₃) δ 4.63 (m, 2H, CHO of *i*-Pr), 2.93 (t, J = 5.2 Hz), 2.70 (m), 2.45 (dd, 3H, J = 5.6, 2.4 Hz, oxirane), 1.87 - 1.67 /m, 4H, (CH₂)₂/, 1.24 (m, 12H, CH₃). ¹³C NMR 70.3 (d, J = 6.7 Hz, CHO of *i*-PrO), 52.1 (d, J = 19.3 Hz) and 47.3 (oxirane), 26.1 (d, J = 5.2 Hz, CH₂), 24.2 (m, CH₃), 23.3 (d, J = 144.7 Hz, CH₂P). ³¹P NMR 29.91. EI-MS 237 (M + H, 2.8), 135 (100.0).

4.1.3. Diisopropyl (*E*)-2-Hydroxymethyl-1-cyclopropylphosphonate (8). BuLi (120 mL, 0.30 mol, 2.5 M in hexanes) was added slowly to a solution of compound **12** (58.0 g, 0.24 mol) in THF (400 mL) under N₂ at -78 °C within 30 min. The stirring was continued for 30 min whereupon the reaction was quenched with saturated aqueous NH₄Cl (100 mL) and the mixture was allowed to warm to rt. After addition of CH₂Cl₂ (600 mL), the layers were separated and the organic phase was washed with saturated aqueous NH₄Cl solution (400 mL). The combined aqueous phase was saturated with solid NaCl and it was extracted with CH₂Cl₂ (3 × 300 mL). The organic phase was washed with brine (500 mL) and it was dried (Na₂SO₄). Evaporation of the solvent afforded the crude product which was chromatographed on silica gel in CH₂Cl₂ : MeOH = 100 : 1 to 50 : 1 to 30 : 1 providing compound **8** (48.0 g, 83%). ¹H NMR (CDCl₃) δ 4.64 (m, 2H, CHO of *i*-PrO), 3.56 (ddd, J = 11.4, J = 6.4 Hz and 1.6 Hz) and 3.50 (dd, 2H, J = 11.4, 6.4 Hz, CH₂O), 2.61 (m, 1H, OH), 1.64 - 1.53 (m), 1.09 - 1.00 (m), 0.83 - 0.72 (m, 4H, cyclopropane), 1.30 (m, 12H, CH₃). ¹³C NMR 70.6, 70.5 (2d, J = 8.3 Hz, CHO of *i*-PrO), 64.9 (d, J = 4.4 Hz, CH₂O), 24.6 (d, J = 3.8 Hz, CH₃), 19.7 (d, J = 3.7 Hz), 10.2 (d, J = 196.3 Hz), 8.4 (d, J = 5.2 Hz, cyclopropane). ³¹P NMR 28.71. EI-MS 237 (M + H, 4.0), 153 (100.0). HRMS calcd for C₁₀H₂₂O₄P: 237.1256; found 237.1254.

4.1.4. Diisopropyl (*E*)-2-(Phenylselenenylmethyl)-1-cyclopropylphosphonate (14). A solution of Br₂ (22 mL, 0.42 mol) in CH₂Cl₂ (80 mL) was added with stirring to PPh₃ (107 g, 0.41 mol) in CH₂Cl₂ (300 mL) at -35 to -25 °C within 15 min under N₂. The stirring was continued for 15 min, a solution of compound **8** (48.0 g, 0.20 mol) in CH₂Cl₂ (200 mL) was added, the resulting mixture was agitated for 15 min and then at rt for another 15 min. Saturated NaHCO₃ solution (200 mL) followed by saturated Na₂S₂O₃ solution (100 mL) were slowly added at 0 °C. The organic phase was separated and the aqueous layer was extracted with CH₂Cl₂ (2 × 200 mL). Combined organic phase was washed with water (500 mL) and brine (500 mL) whereupon it was dried (Na₂SO₄). The solvent was evaporated to give crude product **13** (170 g) containing Ph₃PO (ratio 1 : 2.7, estimated yield of **13** was 88%) which was employed in the next step. ¹H NMR (CDCl₃) δ 7.61 - 7.56 (m, 16H), 7.46 - 7.42 (m, 8H) and 7.38 - 7.34 (m, 16H, Ph₃PO), 4.66 - 4.54 (m, 2H, CHO of *i*-PrO), 3.31 - 3.20 (m, 2H, CH₂Br), 1.80 - 1.68 (m, 1H), 1.20 - 1.15 (m, 1H), 0.82 - 0.78 (m, 2H, cyclopropane), 1.25 (2d), 1.24 (2d, J = 5.6 Hz, 12H, CH₃). ¹³C NMR 133.3, 132.2, 132.1, 128.8 and 128.6 (Ph), 70.7 and 70.5 (2d, J = 6.3 Hz, CHO of *i*-PrO), 36.3 (d, J = 3.8 Hz), 24.4 - 24.3 (4 peaks, CH₃), 20.0 (d, J = 3.7 Hz), 15.1 (d, J = 194.8 Hz), 12.7 (d, J = 4.5 Hz, cyclopropane). ³¹P NMR 30.12 (Ph₃PO), 26.63.

A solution of (PhSe)₂ (34.9 g, 0.112 mol) in EtOH (350 mL) was refluxed till all selenide was dissolved (30 min). After cooling to rt, 4M NaOH (56 mL, 0.225 mol) was added followed by NaBH₄ (7.67 g, 0.225 mol). The mixture was refluxed for 30 min and then it was cooled to rt. The crude product **13** from the previous experiment (170 g) in EtOH (300 mL) was added and the mixture was stirred at rt for 5 h. The solvent was evaporated, saturated aqueous solution of NaHCO₃ (200 mL) was added and the mixture was extracted with EtOAc (2 × 400 mL). The organic phase was washed with brine (200 mL) and it was dried over Na₂SO₄. The solvent was evaporated and the residue was chromatographed on a silica gel column in hexanes : EtOAc

= 100 : 0 to 4 : 1 and then EtOAc : MeOH = 10 : 1 giving crude product **14** (185 g) containing Ph_3PO . A 200-mg sample of this product was rechromatographed in hexanes : EtOAc = 1 : 1 to CH_2Cl_2 : MeOH = 100 : 1) to afford compound **14** (70 mg) as an oil. ^1H NMR (CDCl_3) δ 7.51 - 7.48 (m, 2H), 7.26 - 7.21 (m, 3H, Ph), 4.67 - 4.56 (m, 2H, CHO of *i*-PrO), 2.98 (dd, 1H, J = 12.4, 7.0 Hz), 2.81 (ddd, 1H, J = 12.4, 7.0, 1.6 Hz, CH_2Se), 1.69 - 1.57 (m, 1H), 1.15 - 1.06 (m, 1H), 0.77 - 0.66 (m, 2H, cyclopropane), 1.29 - 1.25 (4d, 12H, J = 5.6 Hz, 12H, CH_3). ^{13}C NMR 133.3, 130.2, 129.30, 127.34 (Ph), 70.5, 70.4 (2d, J = 6.0 Hz, CHO of *i*-PrO), 32.0 (d, J = 4.4 Hz, CH_2Se), 24.4 - 24.3 (2 overlapped d, CH_3), 18.1 (d, 1H, J = 3.7 Hz), 14.2 (d, 1H, J = 193.9 Hz), 12.2 (d, 2H, J = 5.2 Hz, cyclopropane). ^{31}P NMR 27.80. EI-MS 376, 374 (M, 3.6, 2.1), 135 (100.0). HRMS calcd for $\text{C}_{16}\text{H}_{25}\text{O}_3\text{P}^{80}\text{Se}$: 376.0707, found 376.0709. Anal. Calcd for $\text{C}_{16}\text{H}_{25}\text{O}_3\text{PSe}$: C, 51.20; H, 6.71. Found: C, 51.37; H, 6.80.

4.1.5. Diisopropyl (*E*)-2-(Phenylselenenylmethyl)-1-cyclopropylphosphonate Se-Oxide (15). Hydrogen peroxide (30%, 220 mL) was added slowly to a solution of the crude product **14** from the previous experiment (185 g) in THF (800 mL) at 0 °C with stirring which was continued for 1 h. The mixture was then stirred at rt for 16 h. Saturated aqueous NaHCO_3 (200 mL) was slowly added and the mixture was extracted with EtOAc (6×200 mL). Combined organic phase was dried (Na_2SO_4), the solvent was evaporated and the residue was chromatographed on a silica gel column in hexanes : EtOAc : MeOH = 30 : 60 : 1 to EtOAc : MeOH = 5 : 1 leaving compound **15** (60 g, 76% in three steps) as an oil. ^1H NMR (CDCl_3) δ 7.71 - 7.65 (m, 2H), 7.44 - 7.43 (m, 3H, Ph), 4.78 - 4.61 (m, 2H, CHO of *i*-PrO), 3.03 (dd, J = 12.4, 5.8 Hz), 2.86 (dd, J = 12.4, 7.4 Hz, 2H), 2.70 (ddd, J = 12.0, 8.0, 1.6 Hz), (dd, 2H, J = 12.0, 9.6 Hz, CH_2Se), 1.52 - 1.46 (m), 1.39 - 1.31 (m), 1.19 - 1.01 (m), 0.84 - 0.77 (m), 0.76 - 0.63 (m, 4H, cyclopropane), 1.23 - 1.16 (4 overlapped d, 12H, J = 6.4 Hz, CH_3). ^{13}C NMR 140.1, 131.69, 131.67, 129.8, 126.3, 126.1 (Ph), 70.8 - 70.7 (2 overlapped d, CHO of *i*-PrO), 58.1 (d, J = 3.0 Hz), 57.9 (d, J = 3.7 Hz, CH_2Se), 24.3, 24.2 (2 overlapped d peaks, J = 5.2, 4.5 Hz, CH_3), 13.48 (d, J = 194.6 Hz), 13.45 (d, J = 195.6 Hz), 12.0 (d, J = 3.7 Hz), 11.9 (d, J = 3.7 Hz), 11.8 (d, J = 5.2 Hz), 11.2 (d, J = 5.1 Hz, cyclopropane). ^{31}P NMR 26.23, 25.99. ESI-MS 415, 413 (M + Na, 41.1, 20.2), 393, 391 (M + H, 49.7, 100.0). Anal. Calcd for $\text{C}_{16}\text{H}_{25}\text{O}_4\text{PSe}$: C, 49.11; H, 6.44. Found: C, 49.41; H, 6.42.

4.1.6. Diisopropyl 2-Methylene-1-cyclopropylphosphonate (16). A mixture of compound **15** (60 g, 0.153 mol) and $i\text{Pr}_2\text{NEt}$ (50 mL, 0.30 mol) in toluene (500 mL) was refluxed for 50 h. The solvent was evaporated in vacuo at rt, the residue was dissolved in THF (400 mL) and H_2O_2 (30%, 120 mL) was added to oxidize compound **14** formed as a by-product. The reaction was run at 0 °C for 1 h and at rt for 24 h. It was worked up as described in the previous experiment. Column chromatography afforded product **16** (14 g) as an oil and the starting material **15** (31 g, 79.5 mmol). The latter was subjected to another round of elimination as described above to give additional compound **16** (7.8 g), total yield 21.8 g (65%). ^1H NMR (CDCl_3) δ 5.47 (m, 2H, $\text{CH}_2=$), 4.60 (m, 2H, CHO of *i*-PrO), 1.60 - 1.45 (m, 3H, cyclopropane), 1.26 - 1.21 (4 overlapped d, 12H, J = 6.4 Hz, CH_3). ^{13}C NMR 127.0 (d, J = 9.0 Hz, $\text{C}=\text{C}$), 105.2 (d, J = 5.2 Hz, $\text{CH}_2=$), 70.7, 70.5 (2d, J = 6.0, 6.7 Hz, CHO of *i*-PrO), 24.3 - 24.0 (3d, J = 5.2 Hz, CH_3), 10.9 (d, J = 188.8 Hz, C_1), 8.2 (d, J = 5.2 Hz, C_3). ^{31}P NMR 24.23. EI-MS 219 (M + H, 0.8), 134 (100.0). HRMS calcd for $\text{C}_{10}\text{H}_{20}\text{O}_3\text{P}$ (M + H) 219.1150, found 219.1155. Anal. Calcd for $\text{C}_{10}\text{H}_{19}\text{O}_3\text{P}$: C, 55.03; H, 8.77. Found: C, 54.80; H, 8.53.

4.1.7. Diisopropyl (*Z*)-2-Bromomethyl-2-phenylselenenyl-1-cyclopropylphosphonate (17). NBS (13.5 g, 75.8 mmol) was added in portions to a stirred solution of compound **16** (14.0 g, 64.2 mmol) and $(\text{PhSe})_2$ (24.0 g, 77.0 mmol) in CH_2Cl_2 (500 mL) under N_2 at 0 °C. The stirring was continued for 1 h whereupon water (100 mL) was added followed by saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (100 mL). The layers were separated and the organic phase was washed with 2M NaOH (2×200 mL) and brine (200 mL). It was dried (Na_2SO_4), the solvent was evaporated and the residue was chromatographed on silica gel in hexanes : EtOAc = 10 : 1 to 1.5 : 1 to

give product **17** (26.5 g, 91%) as an oil. ^1H NMR (CDCl_3) δ 7.63 - 7.61 (m, 2H), 7.27 - 7.26 (m, 3H, Ph), 4.74 (m, 2H, CHO of *i*-PrO), 3.78 (dd, J = 11.2, 1.6 Hz), 3.47 (d, 2H, J = 11.2 Hz, CH_2Br), 1.72 - 1.64, 1.61 - 1.55, 1.48 - 1.44 (3m, 3H, cyclopropane), 1.34 - 1.30 (cluster of d, 12H, J = 7.0 Hz, CH_3). ^{13}C NMR 134.7, 129.4, 128.7, 128.4 (Ph), 71.1, 71.0 (2d, J = 6.6, 5.9 Hz, CHO of *i*-PrO), 42.1 (d, J = 3.7 Hz, CH_2Br), 27.1 (d, J = 4.4 Hz, C_2), 21.0 (d, J = 196.3 Hz, C_1), 21.1 (d, J = 6.0 Hz, C_3), 24.4 - 24.3 (clusters of d, CH_3). ^{31}P NMR 23.46. EI-MS 452, 454, 456 (M, 3.7, 7.7, 5.8), 213 (100.0). HRMS calcd for $\text{C}_{16}\text{H}_{24}^{79}\text{BrO}_3\text{P}^{80}\text{Se}$: 453.9812, found 453.9815. Anal. Calcd for $\text{C}_{16}\text{H}_{24}\text{BrO}_3\text{PSe}$: C, 42.31; H, 5.32. Found: C, 42.28; H, 5.12.

4.1.8. Diisopropyl (Z)-2-Cyanomethyl-2-phenylselenenyl-1-cyclopropylphosphonate (19). A mixture of compound **18** (26.0 g, 57.2 mmol), Me_3SiCN (16.0 mL, 120 mmol) and Bu_4NF (1.0 M in THF, 120 mL, 120 mmol) in MeCN (500 mL) was stirred at rt for 60 h. The volatile components were evaporated in vacuo at rt and the residue was chromatographed on silica gel in hexanes : EtOAc = 1 : 1 to 1 : 2 to give product **19** (10.3 g, 45%) as an oil. ^1H NMR (CDCl_3) δ 7.67 - 7.64 (m, 2H), 7.31 - 7.28 (m, 3H, Ph), 4.75 (m, 2H, CHO of *i*-PrO), 2.86, 2.61 (2dd, 2H, J = 17.2 Hz, J = 1.6 Hz, CH_2CN), 1.71 - 1.63 (m), 1.53 - 1.47 (m, 1H), 1.36 - 1.32 (m, 15H, cyclopropane and CH_3). ^{13}C NMR 135.1, 129.6, 128.8, 128.0 (Ph), 116.9 (CN), 71.4, 71.3 (2d, CHO of *i*-PrO), 30.2 (d, J = 3.0 Hz, CH_2CN), 21.0 (d, J = 4.5 Hz, C_2), 20.8 (d, J = 197.0 Hz, C_1), 20.4 (d, J = 5.2 Hz, C_3), 24.4 - 24.2 (cluster of d, CH_3). ^{31}P NMR 22.47. EI-MS 399, 401 (M, 3.5, 7.0). HRMS calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_3\text{P}^{80}\text{Se}$: 401.0651, found 401.0654. Anal. Calcd for $\text{C}_{17}\text{H}_{24}\text{NO}_3\text{PSe}$: C, 51.00; H, 6.04; N, 3.49. Found: C, 51.04; H, 6.07; N, 3.59.

4.1.9. Diisopropyl (Z)-2-(Methoxycarbonylmethyl)-2-phenylselenenyl-1-cyclopropylphosphonate (20). A solution of compound **19** (9.8 g, 24.5 mmol) in MeOH (200 mL, saturated with gaseous HCl) was stirred at 0 °C for 1 h. The stirring was continued at rt for 20 h. The reaction was monitored by ^{31}P NMR spectra. Volatile components were evaporated at room temperature and the crude product was dissolved in MeOH - 2M HCl (1 : 1, 200 mL) and the solution was kept at rt for 22 h. Water (200 mL) was added and the mixture was extracted with EtOAc (2 $\times$ 200 mL). The organic phase was washed with saturated NaHCO_3 solution (100 mL), brine (100 mL) and it was dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed on silica gel in hexanes : EtOAc = 1 : 2 to afford compound **20** (7.6 g, 72%) as an oil. ^1H NMR (CDCl_3) δ 7.64 - 7.61 (m, 2H), 7.26 - 7.24 (m, 3H, Ph), 4.82 - 4.67 (m, 2H, CHO of *i*-PrO), 3.61 (s, 3H, CH_3O), 2.80 (dd, J = 15.8 Hz, J = 4.2 Hz), 2.32 (d, 2H, J = 15.8 Hz, $\text{CH}_2\text{CO}_2\text{Me}$), 1.63 - 1.56 (m, 1H), 1.39 - 1.28 (m, 14H, cyclopropane overlapped with CH_3). ^{13}C NMR 170.9 (C=O), 135.0, 129.2, 128.1 (Ph), 71.0, 70.7 (2d, J = 6.9, 6.0 Hz, CHO of *i*-PrO), 51.9 (CH_3O), 46.1 (d, J = 3.0 Hz, $\text{CH}_2\text{CO}_2\text{CH}_3$), 24.4, 24.3 (2d, J = 5.3, 6.6 Hz, CH_3), 23.0 (d, J = 4.9 Hz, C_2), 22.1 (d, J = 197.4 Hz, C_1), 21.0 (d, J = 4.9 Hz, C_3). ^{31}P NMR 23.58. EI-MS 432, 434 (M, 2.6, 4.8), 193 (100.0). HRMS calcd for $\text{C}_{18}\text{H}_{27}\text{O}_5\text{P}^{80}\text{Se}$: 434.0761; found 434.0759. Anal. Calcd for $\text{C}_{18}\text{H}_{27}\text{O}_5\text{PSe}$: C, 49.89; H, 6.27. Found: C, 49.78; H, 6.29.

4.1.10. Diisopropyl (Z)-2-(2-Hydroxyethyl)-2-phenylselenenyl-1-cyclopropylphosphonate (21). LiBH_4 (2M in THF, 35 mL, 70 mmol) was added via syringe to a solution of compound **20** (7.0 g, 16.1 mmol) in THF (100 mL) under N_2 at 0 °C and the resulting mixture was stirred for 1 h. The stirring was continued at room temperature for 23 h. The solution was re-cooled to 0 °C, and MeOH (50 mL) was added to quench the reaction. The saturated NH_4Cl solution (120 mL) was then added slowly (**CAUTION, gas evolution!**) at 0 °C. The mixture was extracted with EtOAc (2 $\times$ 150 mL), the organic phase was washed with saturated aqueous NH_4Cl solution and brine. It was dried (Na_2SO_4), the solvent was evaporated and the residue was chromatographed in hexanes : EtOAc = 1 : 1 to 1 : 2 and then EtOAc : MeOH = 15 : 1 leaving product **21** (4.8 g, 74%) as an oil. ^1H NMR (CDCl_3) δ 7.61 - 7.58 (m, 2H), 7.26 - 7.25 (m, 3H, Ph), 4.78 - 4.71 (m, 2H, CHO of *i*-PrO), 4.02 - 3.96, 3.83 - 3.78 (2m,

2H, CH₂O), 2.64 (bs, 1H, OH), 2.05 - 1.99 (m, 1H), 1.62 (ddd, J = 18.4, 7.2, 4.8 Hz, 1H), 1.38 - 1.28 (m, 16H, cyclopropane, CH₂CH₂O, CH₃). ¹³C NMR 133.8, 129.8, 129.2, 127.6 (Ph), 71.0 (d, J = 5.9 Hz, CHO of *i*-PrO), 61.5 (CH₂O), 42.7 (d, J = 3.0 Hz, CH₂CH₂O), 25.4 (d, J = 5.2 Hz, C₂), 21.5 (d, J = 5.2 Hz, C₃), 21.4 (d, J = 197.0 Hz, C₁), 24.5 - 24.2 (4d, J = 5.2 Hz, CH₃). ³¹P NMR 25.14. EI-MS 404, 406 (M, 5.4, 10.5), 147 (100.0). HRMS calcd for C₁₇H₂₇O₄P⁸⁰Se: 406.0812, found 406.0819. Anal. Calcd for C₁₇H₂₇O₄PSe: C, 50.38; H, 6.71. Found: C, 49.93; H, 6.31.

4.1.11. Diisopropyl (Z)- and (E)-(2-Hydroxyethylidene)-1-cyclopropylphosphonate (23) and (24). A mixture of compound **21** (4.5 g, 11.1 mmol) and Ac₂O (20 mL) in pyridine (40 mL) was stirred at rt for 6 h. The volatile components were evaporated in vacuo at rt and the residue was dissolved in EtOAc (200 mL). The organic phase was washed with 1M HCl (2 × 80 mL), brine (100 mL) and it was dried (Na₂SO₄). The solvent was evaporated and the crude acetate **22** was dissolved in THF (120 mL). Hydrogen peroxide (30%, 40 mL) was then added with stirring at 0 °C which was continued for 1 h and then at rt for 13 h, whereupon the solution was refluxed for 24 h. After cooling, water (200 mL) was added and the mixture was extracted with EtOAc (2 × 150 mL). Aqueous layer was saturated with NaCl and it was extracted again with EtOAc (2 × 100 mL). The combined organic phase was washed with saturated NaHCO₃ (2 × 100 mL), brine (100 mL) and it was dried (Na₂SO₄). The solvent was evaporated and the residue was dissolved in MeOH (50 mL) followed by addition of K₂CO₃ (1.22 g, 8.9 mmol in water (10 mL). The mixture was stirred at rt for 10 min. Water (100 mL) was added and the resulting mixture was extracted with EtOAc (2 × 150 mL). The organic phase was washed with brine (100 mL) and it was dried (Na₂SO₄). The solvent was evaporated and the crude product was chromatographed on a silica gel column in hexanes : EtOAc = 1 : 1 to 1 : 2 followed by EtOAc (100%) and, finally, EtOAc : MeOH = 25 : 1 to furnish the faster moving *Z*-isomer **23** (890 mg, 32%) followed by *E*-isomer **24** (810 mg, 29%) as oils.²⁴

Z-Isomer **23**: ¹H NMR (CDCl₃) δ 6.13 (m, 1H, CH=), 4.71, 4.61 (2m, 2H, CH₂O), 4.40 - 4.27 (m, 2H, CHO of *i*-PrO), 2.89 (bs, 1H, OH), 1.79 (m, 1H), 1.55 - 1.49 (m, 2H, cyclopropane), 1.36 - 1.23 (4d, 12H, J = 5.6 Hz, CH₃). ¹³C NMR 120.7 (d, J = 5.2 Hz, CH=), 117.7 (d, J = 9.7 Hz, C=), 71.7, 71.3 (2d, J = 6.6, 6.7 Hz, CHO of *i*-PrO), 62.3 (CH₂O), 24.3, 24.2, 24.1, 23.8 (4 overlapped d, J = 3.7, 5.1, 3.7, 5.2 Hz, CH₃), 10.5 (d, J = 190.3 Hz, C₁), 7.0 (d, J = 5.2 Hz, C₃). ³¹P NMR 25.42.

E-Isomer **24**: ¹H NMR (CDCl₃) δ 6.04 (m, 1H, CH=), 4.63 - 4.56 (2m, 2H, CHO of *i*-PrO), 4.19 (m, 2H, CH₂O), 3.54 (bs, 1H, OH), 1.61 - 1.54 (m, 3H, cyclopropane), 1.29 - 1.24 (m, 12H, CH₃). ¹³C NMR 120.1, 120.2 (2 overlapped d, C=, CH=), 71.0, 70.8 (2d, J = 6.0, 6.7 Hz, CHO of *i*-PrO), 62.5 (CH₂O), 24.3 - 24.1 (3 overlapped d, CH₃), 9.7 (d, J = 188.7 Hz, C₁), 7.3 (d, J = 4.5 Hz, C₃). ³¹P NMR 24.98.

4.1.12. Diisopropyl (Z)-(2-Bromoethylidene)-1-cyclopropylphosphonate (25a). A mixture of compound **23** (820 mg, 3.29 mmol), PPh₃ (2.62 g, 10.0 mmol) and CBr₄ (3.31 g, 10.0 mmol) in CH₂Cl₂ (60 mL) was stirred at rt for 4 h. The solvent was evaporated in vacuo at room temperature and the residue was chromatographed on silica gel in hexanes : EtOAc = 3 : 1 to give product **25a** (690 mg, 67%) as an oil.²⁵ ¹H NMR (CDCl₃) δ 6.13 (m, 1H, CH=), 4.66 (m, J = 6.4 Hz, 2H, CHO of *i*-PrO), 4.21 - 4.10 (m, 2H, CH₂Br), 1.71 - 1.60 (m, 3H, cyclopropane), 1.31 - 1.26 (poorly resolved 3d, 12H). ¹³C NMR 124.0 (d, J = 10.4 Hz, C=), 117.2 (d, J = 5.9 Hz, CH=), 71.2 and 70.8 (2d, J = 6.0 and 6.7 Hz, CHO of *i*-PrO), 31.3 (CH₂Br), 24.4 - 24.3 (2 overlapped d), 24.2, 24.1 (2d, J = 3.7 and 4.4 Hz, CH₃), 10.8 (d, J = 188.8 Hz, C₁), 8.4 (d, J = 4.4 Hz, C₃). ³¹P NMR 23.45. EI-MS 311, 313 (M + H, traces), 147 (100.0). HRMS calcd for C₁₁H₂₁O₃P⁷⁹Br (M + H) 311.0412, found 311.0412. Anal. Calcd for C₁₁H₂₀BrO₃P.0.5 H₂O: C, 41.27; H, 6.61. Found: C, 41.50, H, 6.36.

4.1.13. Diisopropyl (*E*)-(2-Bromoethylidene)-1-cyclopropylphosphonate (25b). The procedure described above for the *Z*-isomer **25a** was performed with *E*-isomer **24** (740 mg, 2.97 mmol) to give *E*-isomer **25b** (620 mg, 67%) as an oil. ²⁵ ¹H NMR (CDCl₃) δ 6.14 (m, 1H, CH=), 4.63 (m, 2H, CHO of *i*-PrO), 4.06 (d, *J* = 7.2 Hz, 2H, CH₂Br), 1.71 - 1.54 (2m, 3H, H₁, H₃), 1.29 - 1.25 (4 overlapped d, *J* = 6.2 Hz, 12H, CH₃). ¹³C NMR 124.5 (d, *J* = 8.9 Hz, C=), 116.8 (d, *J* = 5.2 Hz, CH=), 71.1 and 70.8 (2d, *J* = 5.9 and 6.6 Hz, CHO of *i*-PrO), 31.3 (CH₂Br), 24.3 - 24.1 (3 overlapped d, CH₃), 11.2 (d, *J* = 188.8 Hz, C₁), 7.3 (d, *J* = 5.2 Hz, C₃). ³¹P NMR 23.09. EI-MS 313, 311 (M, traces), 147 (100.0). HRMS calcd for C₁₁H₂₁O₃P⁷⁹Br (M + H) 311.0412, found 311.0404. Anal. Calcd for C₁₁H₂₀BrO₃P: C, 42.46; H, 6.47. Found: C, 42.25; H, 6.51.

4.1.14. Diisopropyl (*Z*)-2-[2-(Adenin-9-yl)ethylidene]-1-cyclopropylphosphonate (26a). A mixture of compound **25a** (350 mg, 1.12 mmol), adenine (302 mg, 2.25 mmol) and K₂CO₃ (470 mg, 3.36 mmol) in DMF (50 mL) was sonicated for 5 min and then it was stirred at room temperature for 10 h. Solvent was evaporated in vacuo and the residue was chromatographed on silica gel in CH₂Cl₂ : MeOH = 10 : 1 to afford compound **26a** (281 mg, 70%), mp 126 - 128 °C. UV max (EtOH) 262 nm (ε 13,800). ¹H NMR (CD₃SOCD₃) δ 8.13 and 8.09 (2s, 2H, H₈ and H₂), 7.22 (s, 2H, NH₂), 6.14 (m, 1H, H_{2'}), 4.94 and 4.78 (AB, *J* = 12.8 - 14.8 Hz), 4.93 and 4.80 (A'B', *J* = 14.0 - 14.4 Hz, 2H, H_{1'}), 4.62 - 4.50 (m, 2H, CHO of *i*-PrO), 1.99 (dt, 1H, H_{5'}), 1.71 (dd, *J* = 18.0 and 8.8 Hz, 1H) and 1.54 (m, 1H, H_{4'}), 1.24 - 1.18 (4 overlapped d, *J* = 6.0 Hz, 12H, CH₃). ¹³C NMR 156.6, 153.2, 149.9, 140.9, 119.3 (adenine), 125.4 (d, *J* = 8.9 Hz, C_{3'}), 114.6 (d, *J* = 5.9 Hz, C_{2'}), 70.9 and 70.6 (2d, *J* = 6.0 and 6.7 Hz, CHO of *i*-PrO), 44.4 (C_{1'}), 24.5, 24.4, 24.2 (3d, *J* = 3.6 - 4.5 Hz, CH₃), 10.6 (d, *J* = 187.2 Hz, C_{5'}), 8.3 (d, *J* = 5.1 Hz, C_{4'}). ³¹P NMR 24.04. EI-MS 365 (M, 5.2), 200 (100.0). HRMS calcd for C₁₆H₂₄N₅O₃P 365.1617, found 365.1612. Anal. Calcd for C₁₆H₂₄N₅O₃P: C, 52.59; H, 6.62; N, 19.16. Found: C, 52.80; H, 6.51; N, 19.20.

4.1.15. Diisopropyl (*E*)-2-[2-(Adenin-9-yl)ethylidene]-1-cyclopropylphosphonate (26b). The procedure described above for the *Z*-isomer **26a** was performed with *E*-isomer **25b** (190 mg, 0.61 mmol) to give *E*-isomer **26b** (148 mg, 67%), mp 165 - 167 °C. UV max (EtOH) 262 nm (ε 14,300). ¹H NMR (CD₃SOCD₃) δ 8.11 and 8.07 (2s, 2H, H₈ and H₂), 7.21 (s, 2H, NH₂), 6.11 (m, 1H, H_{2'}), 4.90 (s, 2H, H_{1'}), 4.46 (m, 2H, CHO of *i*-PrO), 1.75 (dt, 1H) and 1.34 - 1.08 (m, 14H, H_{5'}, H_{4'} and CH₃). ¹³C NMR 156.6, 153.2, 150.2, 141.4, 119.2 (adenine), 123.1 (d, *J* = 8.9 Hz, C_{3'}), 115.0 (d, *J* = 5.2 Hz, C_{2'}), 70.6 and 70.4 (2d, *J* = 5.9 and 6.0 Hz, CHO of *i*-PrO), 44.1 (C_{1'}), 24.4 (2 overlapped d) and 24.2 (d, *J* = 4.7 Hz), 9.7 (d, *J* = 185.0 Hz, C_{5'}), 7.2 (d, *J* = 5.2 Hz, C_{4'}). ³¹P NMR 23.60. EI-MS 365 (M, 3.9), 200 (100.0). HRMS calcd for C₁₆H₂₄N₅O₃P 365.1617, found 365.1617. Anal. Calcd for C₁₆H₂₄N₅O₃P: C, 52.59; H, 6.62; N, 19.16. Found: C, 52.71; H, 6.50; N, 18.92.

4.1.16. (*Z*)-2-[2-(Adenin-9-yl)ethylidene]-1-cyclopropylphosphonate (6a). Compound **26a** (185 mg, 0.50 mmol) was refluxed in 6M HCl (12 mL) for 20 min. The volatile components were evaporated in vacuo at 40 °C. Water (3 mL) was added to the residue, the solid was filtered off, washed with water (1 mL) and it was dried in vacuo to give product **6a** (128 mg, 83%), mp 288 - 290 °C. UV max (H₂O) 262 nm (ε 13,500). ¹H NMR (D₂O, sodium salt) δ 7.99 and 7.89 (2s, 2H, H₈ and H₂), 5.83 (bs, 1H, H_{2'}), 4.77 (s, H_{1'} overlapped with HDO), 1.63 (t, 1H, H_{5'}), 1.40 - 1.31 (m, 2H, H_{4'}). ¹³C NMR 155.0, 151.9, 148.1, 142.3, 118.1 (adenine), 130.1 (d, *J* = 8.3 Hz, C_{3'}), 111.0 (d, *J* = 5.3 Hz, C_{2'}), 45.4 (C_{1'}), 13.5 (d, *J* = 166.4 Hz, C_{5'}), 7.3 (d, C_{4'}). ³¹P NMR 17.84. ESI-MS 282 (M, 100.0). Negative ESI-MS 282 (M, 1.5), 281 (M - H, 13.2), 280 (M - 2H, 100.0). Anal. Calcd for C₁₀H₁₂N₅O₃P·0.8H₂O: C, 40.63; H, 4.64; N, 23.69. Found: C, 40.87; H, 4.88; N, 23.57.

4.1.17. (*E*)-2-[2-(Adenin-9-yl)ethylidene]-1-cyclopropylphosphonate (7a). The procedure for the *Z*-isomer **6a** was followed with *E*-isomer **26b** (92 mg, 0.25 mmol) to afford *E*-isomer

7a (50 mg, 71%), mp 297 - 300 °C. UV max (H₂O) 261 nm (ϵ 13,800). ¹H NMR (D₂O, sodium salt) δ 8.00 and 7.98 (2s, 2H, H₈ and H₂), 5.96 (bs, 1H, H_{2'}), 4.78 (s, C_{1'} overlapped with HDO), 1.29 (bs, 1H, H_{5'}), 0.71 (m, 2H, H_{4'}). ¹³C NMR 155.3, 152.3, 148.9, 143.0, 118.1 (adenine), 127.7 (d, J = 8.1 Hz, C_{3'}), 110.4 (d, J = 4.2 Hz, C_{2'}), 44.9 (C_{1'}), 12.7 (d, J = 164.2 Hz, C_{5'}), 5.7 (d, J = 3.6 Hz, C_{4'}). ³¹P NMR 17.40. Negative ESI-MS 282 (M, 4.2), 281 (M - H, 8.7), 280 (M - 2H, 59.3), 113 (100.0). Anal. Calcd for C₁₀H₁₂N₅O₃P·0.8 H₂O: C, 40.63; H, 4.64; N, 23.69. Found: C, 40.57; H, 4.19; N, 23.38.

4.1.18. Diisopropyl (Z)-2-[2-(2-Amino-6-chloropurin-9-yl)ethylidene]-1-cyclopropylphosphonate (26c) and 7-Isomer 27a. A mixture of compound **25a** (450 mg, 1.46 mmol), 2-amino-6-chloropurine (510 mg, 3.07 mmol) and K₂CO₃ (621 mg, 4.53 mmol) in DMF (50 mL) was sonicated for 2 min at rt and then it was stirred for 24 h. The solvent was evaporated in vacuo at rt and the crude product was chromatographed on a silica gel column in EtOAc : MeOH = 30 : 1 to 10 : 1 followed by CH₂Cl₂ - MeOH = 10 : 1 to give (Z,9)-isomer **26c** (340 mg, 62%) as a followed by (Z,7)-isomer **27a** (84 mg, 15%) as sirups.

(Z,9)-Isomer **26c**: UV max (EtOH) 224 nm (ϵ 27,200), 249 (ϵ 5,900), 311 (ϵ 7,700). ¹H NMR (CDCl₃) δ 7.96 (s, 1H, H₈), 6.08 (m, 1H, H_{2'}), 5.27 (bs, 2H, NH₂), 4.93 (poorly resolved half of 2AB's), 4.80 and 4.79 (J_{AB} = J_{A'B'} = 14.4 Hz, 2H, H_{1'}), 4.75 - 4.63 (m, 2H, CHO of *i*-PrO), 1.77 - 1.63 (m, 3H, H_{5'} and H_{4'}), 1.32 - 1.24 (3d, J = 6.0 - 6.4 Hz, 12H, CH₃). ¹³C NMR 159.3, 153.7, 151.2, 142.9, 125.3 (purine), 125.7 (d, J = 9.6 Hz, C_{3'}), 113.8 (d, J = 5.2 Hz, C_{2'}), 71.4, 71.1 (2d, J = 6.6 Hz, CHO of *i*-PrO), 44.6 (C_{1'}), 24.3 - 24.1 (cluster of d, CH₃), 11.1 (d, J = 191.1 Hz, C_{5'}), 8.4 (d, J = 5.2 Hz, C_{4'}). ³¹P NMR 23.19. EI-MS 399, 401 (24.3, 8.7), 234 (100.0). HRMS calcd for C₁₆H₂₃N₅O₃P³⁵Cl 399.1227, found 399.1226. Anal. Calcd for C₁₆H₂₃ClN₅O₃P: C, 48.06; H, 5.79; N, 17.51. Found: C, 47.93; H, 5.86; N, 17.36.

(Z,7)-Isomer **27a**: ¹H NMR (CDCl₃) δ 8.23 (s, 1H, H₈), 6.17 (m, 1H, H_{2'}), 5.46 (bs, 2H, NH₂), 5.19 (poorly resolved half of 2AB's), 5.07 and 5.05 (J_{AB} = 14.8 Hz, J_{A'B'} = 14.4 Hz, 2H, H_{1'}), 4.73 - 4.60 (m, 2H, CHO of *i*-PrO), 1.74 - 1.60 (m, 3H, H_{5'} and H_{4'}), 1.31 - 1.19 (4 partly overlapped d, J = 6.4 Hz, 12H, CH₃). ¹³C NMR 164.1, 159.6, 148.9, 143.7, 116.3 (purine), 126.0 (d, J = 8.9 Hz, C_{3'}), 114.4 (d, J = 5.2 Hz, C_{2'}), 71.4 and 71.2 (2d, J = 6.7 Hz, CHO of *i*-PrO), 47.9 (C_{1'}), 24.4 - 24.2 (3 partly overlapped d) and 24.1 (d, J = 5.2 Hz, CH₃), 11.1 (d, J = 190.3 Hz, C_{5'}), 8.5 (d, J = 5.2 Hz, C_{4'}). ³¹P NMR 22.90.

4.1.19. Diisopropyl (E)-2-[2-(2-Amino-6-chloropurin-9-yl)ethylidene]-1-cyclopropylphosphonate (26d) and 7-Isomer 27b. The experiment was performed as described for the *Z*-isomer **26c** with compound **25b** (280 mg, 1.8 mmol), 2-amino-6-chloropurine (305 mg, 1.8 mmol) and K₂CO₃ (373 mg, 2.7 mmol) in DMF (50 mL). Column chromatography on silica gel in CH₂Cl₂ - MeOH (20 : 1) gave the *E*-isomer **26d** (244 mg, 67%) as a sirup and 7-isomer **27b** (56 mg, 15%).

(E,9)-Isomer **26d**: UV max (EtOH) 224 nm (ϵ 27,100), 248 (ϵ 5,900), 310 (ϵ 7,700). ¹H NMR (CDCl₃) δ 7.80 (s, 1H, H₈), 6.10 (m, 1H, H_{2'}), 5.20 (bs, 2H, NH₂), 4.83 (bs, 2H, H_{1'}), 4.67 - 4.59 (m, 2H, CHO of *i*-PrO), 1.62 (poorly resolved dt), 1.52 - 1.44 (m) and 1.38 ~ 1.23 (m, 15H, H_{5'}, H_{4'} and CH₃). ¹³C NMR 159.4, 153.9, 151.4, 142.6, 125.0 (purine), 124.4 (d, J = 8.9 Hz, C_{3'}), 113.6 (d, J = 5.2 Hz, C_{2'}), 71.08 and 70.93 (2d, J = 6.7 Hz, CHO of *i*-PrO), 44.5 (C_{1'}), 24.32 - 24.11 (cluster of d, CH₃), 10.5 (d, J = 189.5 Hz, C_{5'}) and 7.3 (d, J = 4.4 Hz, C_{4'}). ³¹P NMR 22.98. EI-MS 399, 401 (34.6, 14.3), 234 (100.00). HRMS calcd for C₁₆H₂₃N₅O₃P³⁵Cl 399.1227, found 399.1222. Anal. Calcd for C₁₆H₂₃ClN₅O₃P: C, 48.06; H, 5.79; N, 17.51. Found: C, 48.23; H, 5.97; N, 17.35.

(E,7)-Isomer **27b**: ¹H NMR (CDCl₃) δ 8.04 (s, 1H, H₈), 6.14 (bs, 1H), 5.59 (bs, 2H, NH₂), 5.09 (bs, 2H, H_{1'}), 4.61 (m, 2H, CHO of *i*-PrO), 1.60 (poorly resolved t, 1H, H_{5'}), 1.45 - 1.37

(m, 1H) and 1.27 - 1.22 (m, 13H, H_{4'} + CH₃). ¹³C NMR 164.0, 159.7, 148.8, 143.9, 116.5 (purine), 124.4 (d, J = 9.8 Hz, C_{3'}), 114.4 (d, J = 5.2 Hz, C_{2'}), 71.1 and 70.9 (2d, J = 6.7 Hz, CHO of *i*-PrO), 47.9 (C_{1'}), 24.3 - 24.1 (m, CH₃), 10.3 (d, J = 189.6 Hz, C_{5'}), 7.3 (d, J = 5.2 Hz, C_{4'}). ³¹P NMR 22.86.

4.1.20. (Z)-2-[2-(Guanin-9-yl)ethylidene]-1-cyclopropylphosphonate 6b. A solution of compound **26c** (270 mg, 0.67 mmol) in 6M HCl (9 mL) was refluxed for 20 min. The volatile components were evaporated in vacuo at 40 °C, the residue was dissolved water (5 mL) and the pH was adjusted with NH₄OH to 9.0. The solution was put on the top of Dowex 1×2 column (200 mesh, AcO⁽⁻⁾) which was eluted first with water (300 mL) and then 0.5 M AcOH (900 mL). The UV absorbing fractions were collected and they were evaporated to dryness. The residue was dried in vacuo to give product **6b** (170 mg, 85%), mp 222 - 224 °C. UV max (H₂O) 253 nm (ε 13,300), 270 (shoulder, ε 10,300). ¹H NMR (D₂O, sodium salt) δ 7.61 (s, 1H, H₈), 5.67 (s, 1H, H_{2'}), 4.63 (overlapped with HDO, 2H, CH₂N), 1.44 (m, 1H, H_{5'}) and 1.16 (m, 2H, H_{4'}). ¹³C NMR 158.5, 153.5, 150.6, 139.4, 115.6 (purine), 131.3 (d, J = 7.1 Hz, C_{3'}), 110.1 (C_{2'}), 45.2 (C_{1'}), 13.9 (d, J = 164.2 Hz, C_{5'}), 7.2 (C_{4'}). ³¹P NMR 17.66. Negative ESI-MS 296 (M - H, 100.0), 297 (M, 13.5). Anal. Calcd for C₁₀H₁₂N₅O₃P·1.4H₂O: C, 37.25; H, 4.62; N, 21.72. Found: C, 37.15; H, 4.23; N, 22.08.

4.1.21. (E)-2-[2-(Guanin-9-yl)ethylidene]-1-cyclopropylphosphonate 7b. The experiment was performed as described for the *Z*-isomer **6b**. From the *E*-isomer **26d** (100 mg, 0.25 mmol) 55 mg (73%) of **7b** was obtained, mp 254 - 256 °C. UV max (H₂O) 253 nm (ε 12,800), 270 (shoulder, ε 9,500). ¹H NMR (D₂O, sodium salt) δ 7.71 (s, 1H, H₈), 5.98 (m, 1H, H_{2'}), 4.80 (overlapped with HDO, 2H, H_{1'}), 1.30 (poorly resolved t, 1H, H_{5'}) and 0.79 (m, 2H, H_{4'}). ¹³C NMR 165.2, 158.7, 151.6, 139.7, 116.9 (purine), 126.9 (d, J = 7.1 Hz, C_{3'}), 110.8 (C_{2'}), 44.4 (C_{1'}), 12.5 (d, J = 164.2 Hz, C_{5'}), 5.6 (d, C_{4'}). ³¹P NMR 17.52. Negative ESI-MS 296 (M - H, 100.0), 297 (M, 19.8). Anal. Calcd for C₁₀H₁₂N₅O₃P·1.5 H₂O: C, 37.04; H, 4.67; N, 21.59. Found: C, 37.26; H, 4.42; N, 21.60.

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25. Compounds **25a** and **25b** are stable neat at -10 °C but they are unstable in solution

Chart 1.

Chart 2.

Scheme 1.

Scheme 2.

Scheme 3.

Formula 27a and 27b.

Graphical Abstract.

Table 1.

Selected Chemical Shifts of the ^1H (δ) and ^{13}C NMR (ppm) of the 9- and 7-isomers **26c**, **27a**, **26d** and **27b** in CDCl_3 .

Compound	H_8	C_8	C_5	$\text{C}_{1'}$
(Z,9)- 26c	7.96	142.9	125.3	44.6
(Z,7)- 27a	8.23	148.9	116.3	47.9
(E,9)- 26d	7.80	142.6	125.0	44.5
(E,7)- 27b	8.04	148.8	116.5	47.9

Table 2.
Selected NOE Data of the *Z*- and *E*-Isomers **6a** and **7a**.

Isomer	H _{irr} (δ)	H _{obs} (δ)	%NOE
<i>Z</i> - 6a	H _{2'} (5.83)	H _{4'} (1.40)	0.3
	H _{4'} (1.40)	H _{2'} (5.83)	1.35
<i>E</i> - 7a	H _{2'} (5.96)	H _{5'} (1.29)	1.11
	H _{5'} (1.29)	H _{2'} (5.96)	0.89