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# Synthesis and biological activity of tricyclic analogues of 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine

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**Abstract**—The base moiety of the potent antiherpetic agent 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine **3** was transformed into that of the tricyclic 3,9-dihydro-9-oxo-6-*R*-5*H*-imidazo[1,2-*a*]purine system. The tricyclic analogues **5a–d** were evaluated for their activity against herpes viruses as well as for cytostatic activity against HSV-1 thymidine kinase (TK) gene-transduced human osteosarcoma tumor cells. Marked activity was found against VZV. The 6-phenyl-substituted fluorescent analogues **5c** and **d** were comparable to that of parent **3** in activity against the VZV strain YS and were 3-fold less active against the VZV strain OKA. The compounds **5a–d** also showed marked activity against HSV-1 (KOS) and HSV-2 (G)—against the former generally approximately comparable to that of acyclovir **1a** and one order of magnitude lower than **3**; against the latter comparable to that of **1a** and approximately 6- to 30-fold lower than that of **3**. The most pronounced cytostatic activity (5-fold lower than that of **3**) was exhibited by compounds **5c** and **d**. Tricyclic analogues with pseudosugar moieties are intrinsically bio-active.


## 1. Introduction

The potent antiherpetic activity of acyclovir (ACV, **1a**),[1] ganciclovir (GCV, **1b**),[2,3] carba-acyclovir (HBG, **1c**)[4], and carba-ganciclovir (penciclovir, PCV, 3HM-HBG, **1d**)[5–8] results to a large extent from the flexibility of their acyclic chains.[9,10] This feature allows the pseudosugars to adopt a conformation favorable for the interaction with enzymes catalyzing phosphorylation steps and incorporation into viral DNA. However, it has been previously demonstrated that rigid side chains do not necessarily annihilate antiviral activity. The fixed conformation of some of them has been found to be suitable for enzyme requirements. An analogue of **1c**, modified by a three-membered ring fused between its C2′ and C3′ positions, the *cis*-isomer of the racemic 9-{[2′-(hydroxymethyl)cycloprop-1′-yl]methyl}guanine (**2a**) has been reported to be less active than **1a**, but comparable in activity to **1c** against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2).[10] In contrast, the *trans*-isomer of **2a**,[10] the 1′*S*,2′*R* enantiomer of **2a**[11] as well as 9-{[2′,2′-bis(hydroxymethyl)-cycloprop-1′-yl]methyl}guanine (**2b**)[10] have been found to be devoid of antiviral activity.

The racemic 9-{[*cis*-1′,2′-bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine (**3**) and especially its 1′*S*,2′*R* enantiomer (A-5021, **3a**) has appeared to be the most prominent in terms of antiviral activity among the cyclopropyl guanine analogues possessing a methylene spacer between the base and the carbocyclic ring.[12] Preliminary tests indicated that **3a** displays 40-fold, and the racemic **3** nearly 20-fold, more potent activity against HSV-1 in vitro than ACV. The 1′*R*,2′*S* enantiomer (**3b**) as well as *trans*-stereoisomer of **3** were considerably less active. According to subsequent determinations, A-5021 was 17- to 150-fold more active than ACV and ca. 60-fold more active than PCV against HSV-1, 2- to 8-fold more active than ACV and 10- to 12-fold more active than PCV against HSV-2, and 5- to 7-fold more active than ACV and 2- to 18-fold more active than PCV against VZV, depending on the viral strain and type of cells used.[13,14] The selectivity index of A-5021 is superior to those of ACV and PCV against HSV-1 and similar against HSV-2 and VZV. Antiviral effectiveness of **3a** depends on the intracellular phosphorylation by the virus-encoded thymidine kinase (TK) and is associated with a considerable intracellular stability of its

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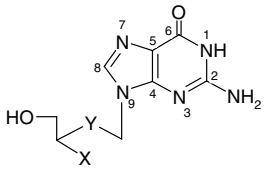
triphosphate.[15] As for **1a** and **d**, little activity was noted with **3a** against human cytomegalovirus (HCMV).[13,16] Compound **3a** has proved equipotent to ACV as an inhibitor of Epstein–Barr virus (EBV) and more potent than ACV against human herpes virus 6 (HHV-6).[17] The data reported in the literature point to the efficacy of A-5021 in HSV-infected mice.[17,18] Moreover, among all the tested acyclic and carbocyclic guanine nucleosides, A-5021 has been identified as the most potent and selective cytostatic agent against HSV-1 TK gene-transfected human osteosarcoma cells.[16] A-5021 has also demonstrated cytostatic activity, equal to that of GCV (**1b**), in HSV TK gene-transduced human lung cancer cells.[19] As compared with GCV, A-5021 is less toxic to the bone marrow cells which makes it possible to use A-5021 as an alternate to GCV in the combined gene therapy/chemotherapy of cancer.

We have previously modified the molecules of ACV and GCV by introduction of a 1,$N^2$-(ethene-1,2-diyl) bridge to form their tricyclic analogues as derivatives of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine. The substituents in the appended ring of 1,$N^2$-ethenoacyclovir (TACV) and 1,$N^2$-ethenoganciclovir (TGCV) enabled us to modify the biological and physical properties of the new compounds.[20–25] Out of a series of tricyclic analogues which have been evaluated for activity against HSV-1 and HSV-2, and subjected to a structure–antiviral activity relationship study, 6-phenyl-TACV (**4a**), 6-(4-methoxyphenyl)-TACV (**4b**), and their TGCV congeners **4c** and **d** have turned out to exhibit marked activity, similar to that of parent **1a** and **b** (Fig. 1).[22,23]

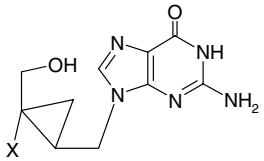
In this work, we present the synthesis and the results of biological evaluations of a series of tricyclic derivatives of **3**. The compounds **5a–d** were designed so as to combine the advantages of the pseudosugar of **3** with better aqueous solubility,[24] lipophilicity,[24,25] and fluorescence properties[22,23] of 6-substituted tricyclic analogues of ACV and GCV.

## 2. Chemistry

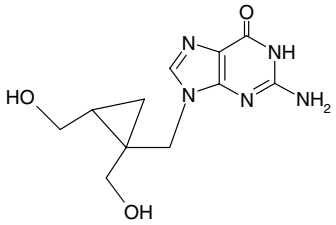
Originally, the compound **3** has been synthesized in an eleven-step procedure, starting from 1,4-dibromo-2-butene and diethyl malonate, in 3% overall yield.[12] Its active enantiomer 1′*S*,2′*R* (**3a**) has been prepared by the other eleven-step route, starting from *R*(−)-epichlorohydrin and diethyl malonate, in 4% total yield. A practical method employing the same substrates has been elaborated afterwards to obtain **3a** within six steps, in 14–19% yield.[26] For the synthesis of **3** we chose the latter procedure. We generally elaborated some details and



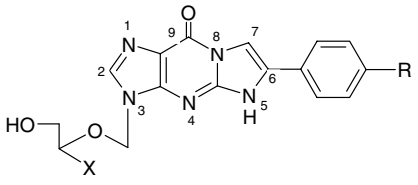
**1a** ACV X = H, Y = O
**1b** GCV X = $CH_2OH$, Y = O
**1c** HBG X = H, Y = $CH_2$
**1d** PCV X = $CH_2OH$, Y = $CH_2$



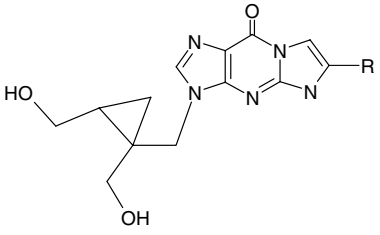
**2a** X = H
**2b** X = $CH_2OH$



**3** racemic
**3a** 1'S,2'R (A-5021)
**3b** 1'R,2'S



**4a** X = H, R = H
**4b** X = H, R = $OCH_3$
**4c** X = $CH_2OH$, R = H
**4d** X = $CH_2OH$, R = $OCH_3$



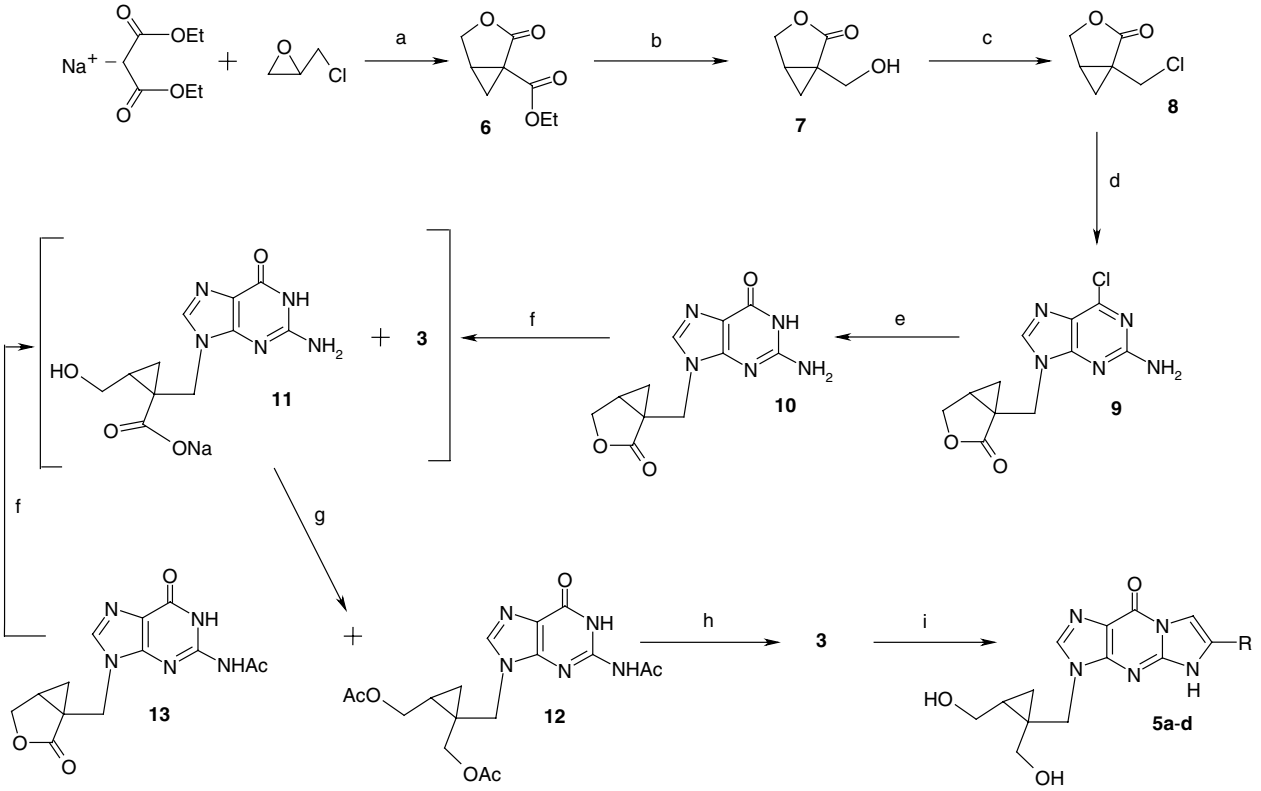
**5a** R = $CH_3$
**5b** R = $CH_2CH_3$
**5c** R = Ph
**5d** R = 4- $CH_3OPh$

**Figure 1.**

introduced major modifications at the stage of preparation of compound **8** and final reduction leading to **3** (Scheme 1). We condensed diethyl malonate with racemic epichlorohydrin to obtain 1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**6**) in 67% yield (lit.: 70%[12]). The lactone **6** was hydrolyzed by the action of 1 equiv of NaOH. The resulting hydroxycarboxylic acid ester was reduced by means of 6.5 equiv of $NaBH_4$ added in several portions at intervals of 1–2 h and the reaction was carried out in 80% aq EtOH at room temperature (lit.: 5 equiv in one portion, EtOH used as a solvent, reaction under reflux[26]). Treatment with 2 N HCl gave 1-hydroxymethyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**7**) in 68% yield based on **6** (lit.: 55%[26]). In a described procedure,[26] the alcohol **7** has been reacted with 1.2 equiv of mesyl chloride and 1.5 equiv of $Et_3N$ at 0 °C to provide 1-mesyloxymethyl-2-oxo-3-oxabicyclo[3.1.0]hexane (67%), whereas its 1-chloromethyl analogue **8** has been formed in the reaction of **7** with thionyl chloride (82%). When we used 2.75 equiv of MsCl, 3 equiv of $Et_3N$ and DMAP as a catalyst at room temperature, we obtained compound **8** in 73% yield. Coupling **8** with 2-amino-6-chloropurine proceeded smoothly at 50 °C, to result in 9- and 7-substituted isomers (69% and 9%, respectively). 1-(2-Amino-6-chloropurin-9-yl)-methyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**9**) was then converted into its guanine congener **10** by treatment with 85% formic acid at 100 °C. Compound **10** was subjected to reduction with $NaBH_4$ in 90% aq EtOH at 70 °C, which led to two products observed by TLC, *i*PrOH–$NH_4OH$–$H_2O$ (7:1:2), namely the desired product **3** and, most probably, sodium 1-(guanin-9-yl)methyl-2-hydroxymethyl-1-*cyclo*-propanecarboxylate (**11**) (lit.: EtOH was used as a solvent and only compound **3** was isolated in 79% yield[26]). The mixture was not separated but submitted to acetylation under standard conditions to give 9-{[*cis*-1′,2′-bis(acetoxymethyl)cycloprop-1′-yl]methyl}-$N^2$-acetylguanine (**12**, 36% yield based on **9**) and 1-($N^2$-acetylguanin-9-yl)methyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**13**, 52%). The latter was once again reduced with $NaBH_4$ and the reaction mixture was acetylated to afford next portion of **12** (overall yield 64%). Compound **12** was deprotected with aqueous ammonia to obtain compound **3**, pure as shown by $^1H$ NMR and $^{13}C$ NMR. It was employed as a substrate for alkylation–condensation reactions with suitable bromoketones resulting in a series of novel tricyclic derivatives of **3**, bearing various substituents in the 6 position. Compounds **5a–d** were characterized by $^1H$ and $^{13}C$ NMR, and by elemental analysis.

## 3. Biological results

The newly synthesized compounds **5a–d** as well as compound **3** and other pertinent standards were examined for their inhibitory effect on the replication of HSV-1 (strain KOS), HSV-2 (strain G), HSV-1 $TK^-$ $ACV^r$ (TK-deficient, acyclovir–resistant HSV-1 KOS) strain, VZV (strains YS and OKA), VZV $TK^-$ (strain 07/1), cytomegalovirus (CMV, strains AD-169 and Davis), vaccinia virus (VV), and vesicular stomatitis virus (VSV) in human embryonic lung (HEL) cell cultures,




**Scheme 1.** Reagents and conditions: (a) anhyd EtOH, reflux; (b) 1 equiv NaOH, EtOH, rt, then $NaBH_4$, 80% aq EtOH, rt, then 2 N HCl, rt; (c) MsCl, $Et_3N$, DMAP, anhyd $CH_2Cl_2$, rt; (d) 2-amino-6-chloropurine, $K_2CO_3$, DMF, 50 °C; (e) 85% HCOOH, 100 °C; (f) $NaBH_4$, 90% aq EtOH, 70 °C; (g) $Ac_2O$, anhyd py, 90 °C; (h) $NH_4OH$, rt; (i) NaH, $BrCH_2COR$, anhyd DMF, rt; then $NH_4OH$, rt.

Coxsackie B4 virus and respiratory syncytial virus (RSV) in human HeLa cell cultures, and parainfluenza-3 virus, reovirus-1, Sindbis virus, and Punta Toro virus in Vero cell cultures. They were also evaluated for their cytostatic activity against human osteosarcoma cells [TK-deficient (OST TK$^-$)] and OST TK$^-$ cells transduced with the HSV-1 TK gene and efficiently expressing the HSV-1 TK (OST TK$^-$/HSV-1 TK$^+$).

The results of the antiviral tests (Table 1) confirmed the literature data[12] with regard to the 20-fold more potent activity of **3** (the racemic counterpart of A-5021) against HSV-1 as compared with ACV. We also found that **3** was 17-fold more potent than ACV against HSV-2 and 5- to 9-fold more potent than ACV against VZV. Compound **3** was also 3- to 4-fold more potent than GCV against HSV-1, HSV-2, and VZV. In general, introduction of the third ring into the base part of **3** reduced its antiviral activity, but to a varying extent depending on the virus type and on the type of substituent in the 6 position. The most pronounced decrease (ca. 30-fold) was observed in the case of the 6-ethyl compound (**5b**) against HSV-2 and of 6-methyl derivative (**5a**) against VZV (YS). Virtually no reduction of activity (1- to 3-fold) was noted for the 6-phenyl and 6-(4-methoxyphenyl) analogues (**5c** and **d**, respectively) against VZV (YS and OKA). These data are consistent with the trends observed previously for the tricyclic derivatives of ACV and GCV.[22,23] Thus most significant loss of activity among the tricyclic compounds bearing the above-mentioned 6-substituents and against the same viral strains as above, in comparison with parent ACV and GCV, was detected for their 6-alkyl derivatives. On the other hand, the most promising activities against HSV-1, HSV-2, and VZV were noted for the 6-phenyl and 6-(4-methoxyphenyl) derivatives **4a**–**d**.[22,23] The change of activity, however, as a result of conversion of the bicyclic parent compounds into tricyclic analogues, was not the same for **1a**,**b** and **3**. For example, 6-(4-methoxyphenyl)-TACV (**4b**) was 2-fold more active than ACV, 6-(4-methoxyphenyl)-TGCV (**4d**) was 2-fold less active than GCV,[23] and **5d** was ca. 7-fold less active than **3** against HSV-2 (G). This allows us to assume that tricyclic analogues do not decompose in cells into their respective bi-cycles, but act as intrinsically bio-active agents, or, alternatively, decompose at different rates to their corresponding bi-cycles.

Although the compounds **5a**–**d** exhibited lower antiviral activities than that of **3**, against HSV-1, HSV-2, and VZV, they were nevertheless in many cases more active than the current drug of choice ACV. Especially, the activities of **5c** and **d**, the 6-phenyl and 6-(4-methoxyphenyl) congeners of **3**, were substantial and ranged from 2-fold higher to 8-fold higher than those of ACV against tested viral strains. It is noteworthy that **5c** and **d** are intrinsically fluorescent and much more lipophilic than **1a** or **3** which may be advantageous to cross the blood–brain barrier and to detect plasma levels of the drug. The compounds **3** and **5a**–**d** were shown to be inactive or weakly active against HSV-1 TK$^-$ ACV$^r$ and VZV TK$^-$ strains. This points to the marked dependence of their aniherpetic activity on phosphorylation by the virus-encoded TK (Table 2). The evaluated compounds were found inactive against all other types of viruses tested.

According to previous reports, the compound **3a** was slightly more cytostatic against OST TK$^-$/HSV-1 TK$^+$ cells than GCV, with a selectivity index of 5-fold higher than that of GCV.[16] The racemic **3** was now found to display an inhibitory effect on the proliferation of these cells that was 3-fold lower than GCV and to be at least 5-fold less toxic than GCV against OST TK$^-$ cells (Table 2). Although the tricyclic derivatives of **3** were less cytostatic than **3** against OST TK$^-$/HSV-1 TK$^+$ cells, their decrease in antiproliferative activity varied from only 2.6-fold (compound **5d**) to ca. 30-fold (compounds **5a** and **b**).

**Table 2.** Inhibitory effects of compounds **3** and **5a**–**d** on the proliferation of human osteosarcoma cells TK-deficient (OST TK$^-$) and OST TK$^-$ cells transduced with the HSV-1 TK gene (OST TK$^-$/HSV-1 TK$^+$)

| Compound | $IC_{50}$[a] (μM) | |
|---|---|---|
| | OST TK$^-$ | OST TK$^-$/HSV-1 TK$^+$ |
| **3** | >100 | 0.0070 ± 0.0001 |
| **5a** | >100 | 0.20 ± 0.02 |
| **5b** | >100 | 0.22 ± 0.02 |
| **5c** | >100 | 0.044 ± 0.009 |
| **5d** | 71 ± 10 | 0.018 ± 0.007 |
| **1a** | 30 ± 8 | 0.19 ± 0.01 |
| **1b** | 21 ± 1 | 0.0021 ± 0.0001 |

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

**Table 1.** Activity against human herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and varicella zoster virus (VZV) and cytotoxicity of compounds **3** and **5a**–**d** in human embryonic lung (HEL) cells

| Compound | MIC[a] (μM) | | | $EC_{50}$[b] (μM) | | | $CC_{50}$[c] (μM) |
|---|---|---|---|---|---|---|---|
| | HSV-1 (KOS) | HSV-2 (G) | HSV-1 (KOS) TK$^-$ACV$^r$ | VZV (YS) | VZV (OKA) | VZV TK$^-$(07/1) | |
| **3** | 0.13 | 0.96 | 16 | 0.37 | 0.31 | 47 | >50 |
| **5a** | 1.3 | 6.4 | 160 | 5.5 | 2.1 | >100 | >50 |
| **5b** | 0.96 | 32 | 120 | 3.0 | 1.5 | >100 | >50 |
| **5c** | 0.96 | 6.4 | 80 | 0.71 | 1.0 | >100 | >50 |
| **5d** | 1.3 | 6.4 | 32 | 0.4 | 0.95 | >20 | 13.5 |
| **1a** | 2.4 | 16 | 300 | 3.2 | 1.4 | 60 | 537 |
| **1b** | 0.48 | 3.2 | 7.2 | — | 1.2 | 4.7 | 53 |

[a] Minimum inhibitory concentration or compound concentration required to reduce virus-induced cytopathogenicity by 50%.
[b] Effective concentration required to reduce virus plaque formation by 50%.
[c] Cytotoxic concentration or compound concentration required to reduce cell growth by 50%.

## 4. Conclusions

The fluorescent compounds **5c** and **d**, which are specifically active against $TK^+$ HSV-1 and $TK^+$ VZV, are worth further pursuing for their anti-HSV-1 and anti-VZV activity.

The observed differential antiviral potency of the tricyclic analogues composed of the same tricyclic moiety and different pseudosugar moieties may contribute to a better understanding of their molecular mode of action.

## 5. Experimental

### 5.1. General methods

Melting points were determined on a MEL-TEMP II capillary melting point apparatus and are uncorrected. Elemental analyses were performed at Microanalytical Laboratories of the Institute of Organic Chemistry, Polish Academy of Sciences in Warsaw. Fluorescence spectra were measured on a Perkin Elmer MPF-3 fluorescence spectrophotometer (excitation at 305 nm); quantum yields were calculated relative to quinine sulfate in 0.1 N $H_2SO_4$ as a standard ($\phi_F = 0.52$). $^1H$ and $^{13}C$ NMR spectra were recorded on a Unity 300 Varian spectrometer operating at 299.95 and 75.43 MHz; tetramethylsilane was used as the internal standard; the chemical shifts are reported in parts per million ($\delta$ scale). Thin-layer chromatography (TLC) was performed on Merck precoated 60 $F_{254}$ gel plates. Column chromatography was carried out on Merck silica gel 60H (40–63 μm). Anhydrous solvents were prepared as follows: EtOH was distilled with benzene, then with $Mg(OC_2H_5)_2$; $CH_2Cl_2$ was distilled with $CaH_2$; pyridine and DMF were distilled; all the above solvents were finally dried over molecular sieves 4 Å.

### 5.2. 1-Hydroxymethyl-2-oxo-3-oxabicyclo[3.1.0]hexane (7)

To a solution of 1-ethoxycarbonyl-2-oxo-3-oxabicyclo[3.1.0]hexane[5] (**6**, 5.369 g, 31.55 mmol) in EtOH (80 ml) was added NaOH (1.262 g, 31.55 mmol) and the resulting mixture was stirred at room temperature overnight. Next day it was diluted with $H_2O$ (18 ml), while the suspension turned into clear solution, to which $NaBH_4$ (2.387 g, 63.1 mmol) was added. Subsequent portions of $NaBH_4$ (2.387, 1.492, and 1.492 g) were added after 1, 3, and 5 h, respectively (overall amount of $NaBH_4$ used 205 mmol). The reaction was allowed to proceed with effervescence at room temperature overnight. The solution was evaporated, the residue was treated with 2 N HCl (ca. 230 ml) and kept at room temperature for three days. Then it was evaporated and the solid obtained was purified by chromatography, $CH_2Cl_2$–MeOH (99:1–98:2) as an eluent to afford colorless oil (2.752 g, 68% yield). $^1H$ NMR as in lit.[26]

### 5.3. 1-Chloromethyl-2-oxo-3-oxabicyclo[3.1.0]hexane (8)

A solution of **7** (3.297 g, 25.73 mmol) in anhyd $CH_2Cl_2$ (50 ml) was treated with anhyd $Et_3N$ (5.206 g, 51.45 mmol) and cooled to −20 °C. Then a solution of mesyl chloride (5.158 g, 45.03 mmol) in anhyd $CH_2Cl_2$ (70 ml) and DMAP (314 mg, 2.57 mmol) were added. The mixture was stirred for 30 min at temperature increasing from −20 to +5 °C, then at room temperature overnight. Additional MsCl (2.947 g, 25.73 mmol) in $CH_2Cl_2$ (20 ml) and $Et_3N$ (2.603 g, 25.73 mmol) were added and stirring was continued for six days. After this time, the mixture was adsorbed on silica gel (63–200 μm) by evaporation. The dried gel was subjected to chromatography, hexane–EtOAc (3:1–2:1) as an eluting system, to obtain **8** as a yellow oil (2.743 g, 73% yield). $^1H$ NMR as in lit.[26]

### 5.4. 1-(2-Amino-6-chloropurin-9-yl)methyl-2-oxo-3-oxabicyclo[3.1.0]hexane (9)

To a solution of 2-amino-6-chloropurine (3.158 g, 18.62 mmol) in DMF (120 ml) was added $K_2CO_3$ (3.86 g, 27.93 mmol). After being stirred with exclusion of moisture for 1 h, a solution of **8** (2.73 g, 18.62 mmol) in DMF (40 ml) was added. The resulting mixture was stirred at room temperature for 30 min, then at 50 °C overnight. It was evaporated and the residual oil was separated by chromatography, $CH_2Cl_2$–MeOH (97:3–95:5). Evaporation of appropriate fractions afforded **9** (3.594 g, 69%) and 1-(2-amino-6-chloropurin-7-yl)methyl-2-oxo-3-oxabicyclo-[3.1.0]hexane (465 mg, 9%) as white solids. $^1H$ NMR as in lit.[26]

### 5.5. 9-{[*cis*-1′,2′-Bis(acetoxymethyl)cycloprop-1′-yl]methyl}-$N^2$-acetylguanine (12)

Compound **9** (600 mg, 2.15 mmol) was heated in 85% aq formic acid (30 ml) at 100 °C for 3 h. Next, the mixture was evaporated and co-evaporated several times with anhyd toluene. 1-(Guanin-9-yl)methyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**10**) without further purification was suspended in 90% aq EtOH (30 ml) and treated with $NaBH_4$ (243 mg, 6.44 mmol). The resulting mixture was stirred at 70 °C for 3 h. TLC in $CH_2Cl_2$–MeOH (4:1) showed the disappearance of **10**, whereas TLC in *i*PrOH–$NH_4OH$–$H_2O$ (7:1:2) indicated the formation of two products (in other experiment, performed without $H_2O$, the reaction progress was slower, but also two products were detected). The volatiles were removed by evaporation and the residue was dried by co-evaporation with anhyd pyridine (3× 30 ml). The oily residue was dissolved in anhyd pyridine (25 ml) and treated with acetic anhydride (1.97 g, 19.31 mmol). The solution was stirred at 90 °C overnight and then was evaporated and co-evaporated with toluene. The products were separated by chromatography using $CH_2Cl_2$–MeOH (95:5) as an eluent. Evaporation of appropriate fractions and drying under vacuum afforded 305 mg (36% yield) of **12**. Further elution with $CH_2Cl_2$–MeOH (9:1) gave 1-($N^2$-acetylguanin-9-yl)methyl-2-oxo-3-oxabicyclo[3.1.0]hexane (**13**, 341 mg, 52% yield), which was dissolved in 90% aq EtOH (15 ml) and treated with $NaBH_4$ (128 mg,

3.37 mmol). After stirring at 70 °C for 3 h, it was evaporated and co-evaporated with anhyd pyridine. The obtained oil was dissolved in anhyd pyridine (15 ml) and treated with acetic anhydride (1.033 g, 10.12 mmol). The reaction was completed after stirring at 90 °C overnight, then the mixture was evaporated, co-evaporated with toluene, and chromatographed with $CH_2Cl_2$–MeOH (95:5) to result in 233 mg of **12**. The overall amount of isolated oily **12** was finally 538 mg (64% yield). $^1$H NMR ($CDCl_3$) $\delta$ 11.98 (s, 1H, N*H*), 9.54 (s, 1H, N*H*), 7.71 (s, 1H, C8-*H*), 4.30 (d, 1H, N-C*H*H-C1′, $J$ = 14.4 Hz), 4.29 (dd, 1H, C2′-C*H*H-O, $J$ = 11.7, 7.2 Hz), 4.17 (d, 1H, C1′-C*H*H-O, $J$ = 12.6 Hz), 3.95 (dd, 1H, C2′-CH*H*-O, $J$ = 12.0, 8.1 Hz), 3.85 (d, 1H, C1′-CH*H*-O, $J$ = 12.3 Hz), 3.66 (d, 1H, N-CH*H*-C1′, $J$ = 14.4 Hz), 2.32 (s, 3H, N-Ac), 2.04 (s, 3H, O-Ac), 1.95 (s, 3H, O-Ac), 1.84–1.96 (m, 1H, C2′-*H*), 1.07 (dd, 1H, C3′-*H*H, $J$ = 8.7, 6.0 Hz), 0.76 (t, 1H, C3′-H*H*, $J$ = 6.0 Hz). Compound **13**: $^1$H NMR (DMSO-$d_6$) $\delta$ 12.02 (s, 1H, N*H*), 11.66 (s, 1H, N*H*), 7.98 (s, 1H, C8-*H*), 4.57 (d, 1H, N-C*H*H-C1′, $J$ = 15.0 Hz), 4.27 (dd, 1H, C4′-*H*H, $J$ = 9.0, 4.8 Hz), 4.27 (d, 1H, N-CH*H*-C1′, $J$ = 14.4 Hz), 4.13 (d, 1H, C4′-H*H*, $J$ = 9.3), 2.67 (m, 1H, C5′-*H*), 2.19 (s, 3H, N-Ac), 1.47 (dd, 1H, C6′-*H*H, $J$ = 7.8, 5.1 Hz), 1.09 (t, 1H, C6′-H*H*, $J$ = 4.8 Hz).

### 5.6. 9-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}guanine (3)

A solution of **12** (2.286 g, 5.84 mmol) in 25% aqueous ammonia (55 ml) was stirred at room temperature for two days. Next, it was concentrated and the resulting suspension was co-evaporated several times with MeOH. Acetamide observed in the crude material ($^1$H NMR) was fully removed by sublimation under vacuum at 40–50 °C, to obtain pure **3** as a white solid: mp 297–299 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 10.54 (s, 1H, N*H*, $D_2O$ exchangeable), 7.72 (s, 1H, C8-*H*), 6.43 (s, 2H, N$H_2$, $D_2O$ exchangeable), 4.68 (t, 1H, O*H*, $D_2O$ exchangeable), 4.54 (t, 1H, O*H*, $D_2O$ exchangeable), 3.99 (d, 1H, N-C*H*H-C1′, $J$ = 14.4 Hz), 3.80 (d, 1H, N-CH*H*-C1′, $J$ = 14.4 Hz), 3.58 (m, 1H, C2′-C*H*H-OH; on $D_2O$ addition dd, $J$ = 12.0, 6.6 Hz), 3.40 (dd, 1H, C2′-CH*H*-OH, $J$ = 12.0, 6.0 Hz), 3.23–3.36 (m, 2H, C1′-C*HH*-OH; on $D_2O$ addition d, $J$ = 12.6 Hz and d, $J$ = 12.0 Hz), 1.23 (m, 1H, C2′-*H*), 0.88 (dd, 1H, C3′-*H*H, $J$ = 9.0, 4.8 Hz), 0.39 (t, 1H, C3′-H*H*, $J$ = 5.4 Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 157.05 (*C*-6), 153.46 (*C*-2), 151.60 (*C*-4), 137.89 (*C*-8), 116.30 (*C*-5), 60.68 (C1′-*C*$H_2$-OH and C2′-*C*$H_2$-OH), 47.63 (C1′-*C*$H_2$-N), 26.90 (*C*-1′), 24.51 (*C*-2′), 14.04 (*C*-3′). Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.73; H, 5.88; N, 26.20.

### 5.7. General procedure for the alkylation–condensation reactions

To a suspension of **3** in anhyd DMF (20 ml/1 mmol of **3**) was added sodium hydride as 60% suspension in oil (1.3 equiv). After being stirred with exclusion of moisture for 1–2 h at room temperature, the resulting solution was treated with bromoketone (1.2 equiv). The reaction mixture was stirred at room temperature for next 1.5–4 h, made alkaline by addition of 25% aqueous ammonia, and left overnight. The volatiles were evaporated and the residual oil was chromatographed on silica gel column using $CH_2Cl_2$–MeOH (6:1) or (9:1–7:1) for **5a**,**b** and **5c**,**d**, respectively. Fractions containing the main product were evaporated and the residual solid was subjected to further work-up.

### 5.8. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-6-methyl-9-oxo-5*H*-imidazo[1,2-*a*]purine (5a)

Crude material after chromatography was dissolved in MeOH–$H_2O$ (5:1), and then the solution was kept at +5 °C for 4 h. The precipitate formed (69% yield) was recrystallized from MeOH at +5 °C, to afford colorless spangles: mp 268–270 °C (dec). $^1$H NMR (DMSO-$d_6$) $\delta$ 12.34 (br s, 1H, N*H*), 7.93 (s, 1H, C2-*H*), 7.33 (d, 1H, C7-*H*, $J$ = 1.2 Hz), 4.69 (t, 1H, O*H*), 4.57 (t, 1H, O*H*), 4.11 (d, 1H, N-C*H*H-C1′, $J$ = 14.1 Hz), 3.96 (d, 1H, N-CH*H*-C1′, $J$ = 14.1 Hz), 3.60 (m, 1H, C2′-C*H*H-OH), 3.43 (dd, 1H, C2′-CH*H*-OH, $J$ = 12.0, 6.0 Hz), 3.26–3.35 (m, 2H, C1′-C*HH*-OH), 2.24 (d, 3H, 6-C$H_3$, $J$ = 1.2 Hz), 1.30 (m, 1H, C2′-*H*), 0.92 (dd, 1H, C3′-*H*H, $J$ = 9.0, 5.1 Hz), 0.41 (t, 1H, C3′-H*H*, $J$ = 5.4 Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 151.15 (*C*-9), 150.25 (*C*-3a), 145.64 (*C*-4a), 138.96 (*C*-2), 125.83 (*C*-6), 115.07 (*C*-9a), 103.12 (*C*-7), 60.76, 60.64 (C1′-*C*$H_2$-OH, C2′-*C*$H_2$-OH), 47.69 (C1′-*C*$H_2$-N), 26.76 (*C*-1′), 24.43 (*C*-2′), 13.95 (*C*-3′), 10.47 (6-*C*$H_3$). Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.30; H, 5.78; N, 23.00.

### 5.9. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-6-ethyl-9-oxo-5*H*-imidazo[1,2-*a*]purine (5b)

Crude material after preliminary separation was next rechromatographed twice using EtOAc–MeOH (5:1) as an eluent to yield 44% of solid **5b**. Analytical sample was prepared by crystallization from MeOH at +5 °C to give white needles: mp 241–244 °C. $^1$H NMR (DMSO-$d_6$) $\delta$ 12.40 (s, 1H, N*H*), 7.93 (s, 1H, C2-*H*), 7.34 (t, 1H, C7-*H*, $J$ = 1.2 Hz), 4.68 (t, 1H, O*H*), 4.57 (t, 1H, O*H*), 4.12 (d, 1H, N-C*H*H-C1′, $J$ = 14.4 Hz), 3.96 (d, 1H, N-CH*H*-C1′, $J$ = 14.1 Hz), 3.60 (m, 1H, C2′-C*H*H-OH), 3.44 (dd, 1H, C2′-CH*H*-OH, $J$ = 12.0, 6.0 Hz), 3.27–3.37 (m, 2H, C1′-C*HH*-OH), 2.61 (qd, 2H, 6-C$H_2$, $J$ = 7.5, 1.2 Hz), 1.30 (m, 1H, C2′-*H*), 1.24 (t, 3H, 6-$CH_2CH_3$, $J$ = 7.5 Hz), 0.92 (dd, 1H, C3′-*H*H, $J$ = 8.7, 4.8 Hz), 0.42 (t, 1H, C3′-H*H*, $J$ = 5.1 Hz). $^{13}$C NMR (DMSO-$d_6$) $\delta$ 151.19 (*C*-9), 150.28 (*C*-3a), 145.73 (*C*-4a), 138.92 (*C*-2), 131.73 (*C*-6), 115.02 (*C*-9a), 102.02 (*C*-7), 60.76, 60.60 (C1′-*C*$H_2$-OH, C2′-*C*$H_2$-OH), 47.63 (C1′-*C*$H_2$-N), 26.78 (*C*-1′), 24.39 (*C*-2′), 18.32 (6-*C*$H_2$CH$_3$), 13.92 (*C*-3′), 12.19 (6-CH$_2$*C*H$_3$). Anal. Calcd for $C_{15}H_{19}N_5O_3$: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.62; H, 6.16; N, 21.92.

### 5.10. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-9-oxo-6-phenyl-5*H*-imidazo[1,2-*a*]purine (5c)

Product isolated by chromatography (73% yield) was next recrystallized from MeOH at room temperature to give yellowish crystals: mp 282–284 °C (dec at ca. 245 °C). Fluorescence emission ($H_2O$): $\lambda_{max}$ 411 nm; $\phi_F = 0.092$. $^1H$ NMR (DMSO-$d_6$) $\delta$ 13.02 (br s, 1H, N*H*), 8.21 (s, 1H, C7-*H*), 7.99 (s, 1H, C2-*H*), 7.92, 7.37–7.51 (d + m, 5H, Ph), 4.72 (br s, 1H, O*H*), 4.60 (t, 1H, O*H*), 4.16 (d, 1H, N-C*H*H-C1′, $J = 14.4$ Hz), 4.03 (d, 1H, N-CH*H*-C1′, $J = 14.1$ Hz), 3.62 (m, 1H, C2′-C*H*H-OH), 3.48 (dd, 1H, C2′-CH*H*-OH, $J = 12.0$, 4.5 Hz), 3.28–3.41 (m, 2H, C1′-C*HH*-OH), 1.32 (m, 1H, C2′-*H*), 0.94 (dd, 1H, C3′-*H*H, $J = 8.4$, 4.8 Hz), 0.45 (t, 1H, C3′-H*H*, $J = 5.4$ Hz). $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 151.26 (*C*-9), 150.58 (*C*-3a), 146.36 (*C*-4a), 139.22 (*C*-2), 128.99 (*C*-6), 128.99 (Ph: *C*-2″,6″), 128.67, 124.98 (Ph: *C*-3″,5″ and *C*-4″), 127.99, (Ph: *C*-1″), 115.23 (*C*-9a), 103.15 (*C*-7), 60.86, 60.65 (C1′-*C*$H_2$-OH, C2′-*C*$H_2$-OH), 47.70 (C1′-*C*$H_2$-N), 26.81 (*C*-1′), 24.41 (*C*-2′), 13.93 (*C*-3′). Anal. Calcd for $C_{19}H_{19}N_5O_3 \cdot 0.4H_2O$: C, 61.25; H, 5.36; N, 18.80. Found: C, 61.37; H, 4.97; N, 18.48.

### 5.11. 3-{[*cis*-1′,2′-Bis(hydroxymethyl)cycloprop-1′-yl]methyl}-3,9-dihydro-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo[1,2-*a*]purine (5d)

Chromatographically purified product (85%) was suspended in EtOAc–MeOH (4:1). The insoluble solid was isolated and dissolved in MeOH, then the solution was kept at +5 °C to appear a white crystalline material: mp 227–229 °C (dec). Fluorescence emission ($H_2O$): $\lambda_{max}$ 386 nm; $\phi_F = 0.045$. $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.91 (s, 1H, N*H*), 8.07 (s, 1H, C7-*H*), 7.98 (s, 1H, C2-*H*), 7.85, 7.04 (2× d, 4H, Ph), 4.70 (t, 1H, O*H*), 4.59 (t, 1H, O*H*), 4.15 (d, 1H, N-C*H*H-C1′, $J = 14.1$ Hz), 4.02 (d, 1H, N-CH*H*-C1′, $J = 14.1$ Hz), 3.81 (s, 3H, C$H_3$), 3.62 (m, 1H, C2′-C*H*H-OH), 3.47 (dd, 1H, C2′-CH*H*-OH, $J = 12.0$, 6.0 Hz), 3.27–3.41 (m, 2H, C1′-C *HH*-OH), 1.31 (m, 1H, C2′-*H*), 0.93 (dd, 1H, C3′-*H*H, $J = 8.4$, 4.8 Hz), 0.44 (t, 1H, C3′-H*H*, $J = 5.4$ Hz). $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 159.58 (Ph: *C*-4″), 151.26 (*C*-9), 150.44 (*C*-3a), 146.25 (*C*-4a), 139.12 (*C*-2), 129.06 (*C*-6), 126.52, 114.45 (Ph: *C*-2″,6″ and *C*-3″,5″), 120.48 (Ph: *C*-1″), 115.20 (*C*-9a), 101.67 (*C*-7), 60.84, 60.64 (C1′-*C*$H_2$-OH, C2′-*C*$H_2$-OH), 55.26 (O*C*$H_3$), 47.67 (C1′-*C*$H_2$-N), 26.82 (*C*-1′), 24.41 (*C*-2′), 13.92 (*C*-3′). Anal. Calcd for $C_{20}H_{21}N_5O_4 \cdot 1H_2O$: C, 58.10; H, 5.61; N, 16.94. Found: C, 57.75; H, 5.42; N, 16.78.

### 5.12. Antiviral assays

The antiviral assays were based on inhibition of virus-induced cytopathicity in human embryonic lung (HEL) cells [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), and HSV-1 (KOS) $TK^-$ ($ACV^r$)] or virus-induced plaque formation in HEL cells [varicella-zoster virus (VZV) (YS, OKA, and VZV $TK^-$ (07/1))].[27] Confluent cell cultures in microtiter 96-well plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (200, 40, 8, ... μM) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds.

### 5.13. Inhibition of cell proliferation

To evaluate the cytostatic activity of the nucleoside analogues against human osteosarcoma cells, OST $TK^-$ and osteosarcoma cells transduced with the TK gene of HSV-1, OST $TK^-$/HSV-1 $TK^+$ cells,[28] $10^4$ cells/well were plated in 96-well microtiter plates (Falcon) and allowed to adhere. Cells were subsequently incubated at 37 °C in a humidified $CO_2$-controlled atmosphere, in the presence of 5-fold dilutions (in normal growth medium) of the compounds. After three days, the cells were detached with trypsin solution (Gibco) and counted in a Coulter Counter (Coulter Electronics, Ltd, Harpenden Hertz, UK). The $IC_{50}$ was defined as the drug concentration required to inhibit cell proliferation by 50%.

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## References and notes

1. Elion, G. B.; Furman, P. A.; Fyfe, J. A.; de Miranda, P.; Beauchamp, L.; Schaeffer, H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5716.
2. Field, A. K.; Davies, M. E.; DeWitt, C.; Perry, H. C.; Liou, R.; Germershausen, J.; Karkas, J. D.; Ashton, W. T.; Johnston, D. B. R.; Tolman, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4139.
3. Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.
4. Larsson, A.; Alenius, S.; Johansson, N. G.; Öberg, B. *Antiviral Res.* **1983**, *3*, 77.
5. Tippie, M. A.; Martin, J. C.; Smee, D. F.; Matthews, T. R.; Verheyden, J. P. H. *Nucleosides Nucleotides* **1984**, *3*, 525.
6. Datema, R.; Johansson, N. G.; Öberg, B. *Chem. Scripta* **1986**, *26*, 49.
7. MacCoss, M.; Tolman, R. L.; Ashton, W. T.; Wagner, A. F.; Hannah, J.; Field, A. K.; Karkas, J. D.; Germershausen, J. I. *Chem. Scripta* **1986**, *26*, 113.
8. Harnden, M. R.; Jarvest, L. R.; Bacon, T. H.; Boyd, M. R. *J. Med. Chem.* **1987**, *30*, 1636.
9. Birnbaum, G. I.; Cygler, M.; Shugar, D. *Can. J. Chem.* **1984**, *62*, 2646.
10. Ashton, W. T.; Meurer, L. C.; Cantone, C. L.; Field, A. K.; Hannah, J.; Karkas, J. D.; Liou, R.; Patel, G. F.; Perry, H. C.; Wagner, A. F.; Walton, E.; Tolman, R. L. *J. Med. Chem.* **1988**, *31*, 2304.

11. Pierra, C.; Olgen, S.; Cavalcanti, S. C. H.; Cheng, Y.-C.; Schinazi, R. F.; Chu, C. K. *Nucleosides Nucleotides Nucleic Acids* **2000**, *19*, 253.
12. Sekiyama, T.; Hatsuya, S.; Tanaka, Y.; Uchiyama, M.; Ono, N.; Iwayama, S.; Oikawa, M.; Suzuki, K.; Okunishi, M.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 1284.
13. Iwayama, S.; Ono, N.; Ohmura, Y.; Suzuki, K.; Aoki, M.; Nakazawa, H.; Oikawa, M.; Kato, T.; Okunishi, M.; Nishiyama, Y.; Yamanishi, K. *Antimicrob. Agents Chemother.* **1998**, *42*, 1666.
14. Onishi, T.; Mukai, C.; Nakagawa, R.; Sekiyama, T.; Aoki, M.; Suzuki, K.; Nakazawa, H.; Ono, N.; Ohmura, Y.; Iwayama, S.; Okunishi, M.; Tsuji, T. *J. Med. Chem.* **2000**, *43*, 278.
15. Ono, N.; Iwayama, S.; Suzuki, K.; Sekiyama, T.; Nakazawa, H.; Tsuji, T.; Okunishi, M.; Daikoku, T.; Nishiyama, Y. *Antimicrob. Agents Chemother.* **1998**, *42*, 2095.
16. De Clercq, E.; Andrei, G.; Snoeck, R.; De Bolle, L.; Naesens, L.; Degreve, B.; Balzarini, J.; Zhang, Y.; Schols, D.; Leyssen, P.; Ying, C.; Neyts, J. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 271.
17. Neyts, J.; Naesens, L.; Ying, C.; De Bolle, L.; De Clercq, E. *Antiviral Res.* **2001**, *49*, 115.
18. Iwayama, S.; Ohmura, Y.; Suzuki, K.; Ono, N.; Nakazawa, H.; Aoki, M.; Tanabe, I.; Sekiyama, T.; Tsuji, T.; Okunishi, M.; Yamanishi, K.; Nishiyama, Y. *Antiviral Res.* **1999**, *42*, 139.
19. Hasegawa, Y.; Nishiyama, Y.; Imaizumi, K.; Ono, N.; Kinoshita, T.; Hatano, S.; Saito, H.; Shimokata, K. *Cancer Gene Ther.* **2000**, *7*, 557.
20. Boryski, J.; Golankiewicz, B.; De Clercq, E. *J. Med. Chem.* **1988**, *31*, 1351.
21. Boryski, J.; Golankiewicz, B.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 2380.
22. Golankiewicz, B.; Ostrowski, T.; Andrei, G.; Snoeck, R.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 3187.
23. Golankiewicz, B.; Ostrowski, T.; Goslinski, T.; Januszczyk, P.; Zeidler, J.; Baranowski, D.; De Clercq, E. *J. Med. Chem.* **2001**, *44*, 4284.
24. Balzarini, J.; Ostrowski, T.; Goslinski, T.; De Clercq, E.; Golankiewicz, B. *Gene Ther.* **2002**, *9*, 1173.
25. Zielenkiewicz, W.; Golankiewicz, B.; Perlovich, G. L.; Kozbial, M. *J. Solution Chem.* **1999**, *28*, 731.
26. Onishi, T.; Matsuzawa, T.; Nishi, S.; Tsuji, T. *Tetrahedron Lett.* **1999**, *40*, 8845.
27. McGuigan, C.; Yarnold, C. J.; Jones, G.; Velazquez, S.; Barucki, H.; Brancale, A.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *J. Med. Chem.* **1999**, *42*, 4479.
28. Degreve, B.; De Clercq, E.; Balzarini, J. *Gene Ther.* **1999**, *6*, 162.