# The Cyclohexene Ring System as a Furanose Mimic: Synthesis and Antiviral Activity of Both Enantiomers of Cyclohexenylguanine

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Both enantiomers of cyclohexenylguanine were synthesized in a stereospecific way starting from the same starting material: *R*-(−)-carvone. Both compounds showed potent and selective anti-herpesvirus activity (HSV-1, HSV-2, VZV, CMV). The binding of both cyclohexene nucleosides in the active site of HSV-1 thymidine kinase was investigated, and a model for the binding of both enantiomers is proposed. The amino acids involved in binding of the optical antipodes are the same, but the interaction energy of both enantiomers is slightly different. This may be attributed to the interaction of the secondary hydroxyl function of the nucleoside analogues with Glu-225. Structural analysis has demonstrated the flexibility of the cyclohexenyl system, and this may be considered as an important conformational characteristic explaining the potent antiviral activity.

## Introduction

Anti-herpesvirus activity has been found for a whole series of carbohydrate-modified nucleoside analogues, the most commercial successful example being acyclovir.[1] Acyclovir (**1**) is a very flexible molecule, and it does not provide us with much information about structural requirements of a nucleoside analogue for antiviral activity.[2] During the last years, we and others[3,4] have synthesized conformationally restricted analogues of natural nucleosides with the aim of establishing the conformational preferences of the nucleoside for the different enzymes (kinases, polymerases) involved in its metabolic activation and action. These enzymes play a crucial role in the antiviral activity of the nucleosides.

A conclusion of these studies is that nucleoside analogues that mimic a natural furanose nucleoside in its 3′-endo conformation (northern type nucleosides) have the highest potential of showing anti-herpesvirus activity. The reason for this has not been clearly demonstrated. However, on the basis of further studies an initial hypothesis can be formulated. A cocrystallization experiment[3f] of an antiviral anhydrohexitol nucleoside (**2**) with herpes simplex virus (HSV) type 1 thymidine kinase showed a chair inversion when the nucleoside analogue is bound in the active site (and thus a conformation which is clearly different from the typical northern type). This is due to the extra stabilization of the anhydrohexitol moiety in the C1 conformation (equatorial base) via hydrogen bonding of the secondary hydroxyl group with Tyr-101 of the enzyme.[5] When the same nucleoside was used to construct an oligonucleotide, hexitol nucleic acids were obtained. These oligonucleotides hybridize strongly with RNA and DNA.[6] In both the monomeric and oligomeric structures, the base moiety is oriented axially with respect to the six-membered hexitol ring (C1 conformation, N-type mimic).[6] On the contrary, when the oligonucleotides consist of modified six-membered nucleotides with an equatorially oriented base moiety, no hybridization with DNA or RNA is possible.[7] It seems therefore possible that the conformational requirements for a six-membered nucleoside to function as a substrate for thymidine kinase and DNA polymerase (as triphosphate) may be different. This may lead us to infer that a certain degree of conformational flexibility is important for the antiviral activity of a nucleoside.

To prove this concept we synthesized the cyclohexene nucleoside analogues **3**−**6** (Figure 1). Several cyclohexane[8−16] and cyclohexene[14−19] nucleosides and unsaturated hexitol nucleosides [20,21] have been described in the literature. None of the cyclohexane nucleosides showed antiviral activity. Also, no or low antiviral activity has been described for the cyclohexene and unsaturated hexitol congeners. The cyclohexene nucleosides and unsaturated hexitol nucleosides described in the literature[14−21] are analogues of the natural dideoxy nucleosides, and the antiviral activity has mainly been determined against human immunodeficiency virus (HIV). Poor anabolism (phosphorylation) inside the cells, or for the phosphonate congeners poor penetration into the cells,[15,20] could account for this lack of activity. However, the introduction of a secondary alcohol function in cyclohexene nucleosides could increase binding to viral (and cellular) kinases[5] and facilitate phosphorylation of the nucleoside analogue.

A second concept that has become increasingly important in the field of antiviral nucleosides is the difference in biological behavior of enantiomers. It has been recognized that D- and L-nucleosides might have a different activity and toxicity spectrum.[22,2] This is most clearly demonstrated in the case of the anti-HIV nucleoside 3TC (lamivudine). The L-(−)- and D-(+)-enantiomers are equipotent, but 3TC, which corresponds

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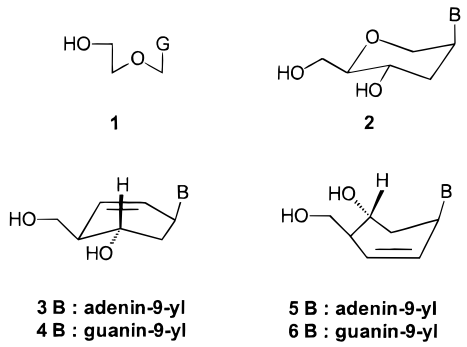



**Figure 1.** Structures of acyclovir (**1**), anhydrohexitol nucleoside (**2**), D-cyclohexenyl nucleosides (**3, 4**), and L-cyclohexenyl nucleosides (**5, 6**).

to the L-(−)-enantiomer, is less toxic than the D-enantiomer. These results stimulated us to synthesize and investigate the properties of both enantiomeric forms of the cyclohexene guanine and adenine nucleosides (**3, 4** and **5, 6**), although the former compounds have only two chiral centers while the cyclohexene nucleosides have three chiral centers.

## Synthesis

Recently we have developed an enantioselective approach toward the synthesis of D-cyclohexene nucleoside **3** (Scheme 1) using *R*-(−)-carvone (**7**) as an inexpensive starting material.[23] Here the endocyclic double bond is treated as analogous to the furanosyl oxygenation in natural nucleosides, hence resulting in the D- and L-designations for the cyclohexenyl compounds. A sequence of chemical transformations led to intermediate **8**, possessing four chiral centers and, disregarding the protecting groups $R_1$−$R_4$, a plane of symmetry. Depending on the nature of $R_1$−$R_4$, intermediate **8** allows for the synthesis of both the D- and L-cyclohexene nucleosides **3**, **4** and **5**, **6**, respectively.

As described, the synthesis of D-adenine cyclohexene nucleoside **3** was successfully accomplished by using a Mitsunobu type reaction on the allylic alcohol **9** to introduce the adenine base moiety on the cyclohexenyl ring.[23] The corresponding guanine derivative **4** has now been synthesized in a similar way. The allylic alcohol **9** was reacted with 2-amino-6-chloropurine in the presence of DEAD and triphenylphosphine in dioxane to give **10** (Scheme 2), which was converted to **4** by treatment with TFA−$H_2O$ (3:1). Under these reaction conditions the two TBDMS protecting groups were simultaneously removed. The overall yield starting from **9** was 46%. Analytically pure **4** is obtained by reversed-phase HPLC purification.

The synthesis of the L-cyclohexene nucleosides **5** and **6** was carried out by protection of the C4-$CH_2OH$ and C5-OH groups of **8b**, followed by conversion of the $OR_2$ and $OR_4$ groups into allylic alcohol **11**. This compound was then used for the introduction of the base moiety according to the same strategy as used for the D-series. Previously described intermediate **8b** ($R_1 = R_3 = H$; $R_2 = R_4$ = TBDMS) was converted into dibenzoate **12** (Scheme 3) using standard reaction conditions ($Bz_2O$, DMAP, $CH_2Cl_2$, 98%). The equatorial C3-OTBDMS protecting group was selectively removed in the presence of the axial C1-OTBDMS to give **13** by using 1 equiv of TBAF in THF at room temperature (74%). The selectivity of the desilylation reaction has been observed before.[13c] The C3 alcohol **13** was converted to the corresponding mesylate **14**, and the C1-OTBDMS group was removed using TBAF to give the alcohol **15**, which was oxidized using PDC in $CH_2Cl_2$. This oxidation was accompanied by simultaneous elimination of the C3-OMs group to afford directly the desired enone **17** (68%). The latter proved rather unstable on silica gel and was used as such following flash chromatography. Stereoselective reduction of enone **17** using $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ in MeOH gave **11** (75%). The stereochemistry at C1 of **11** was established in a similar way as for **9**.[23]

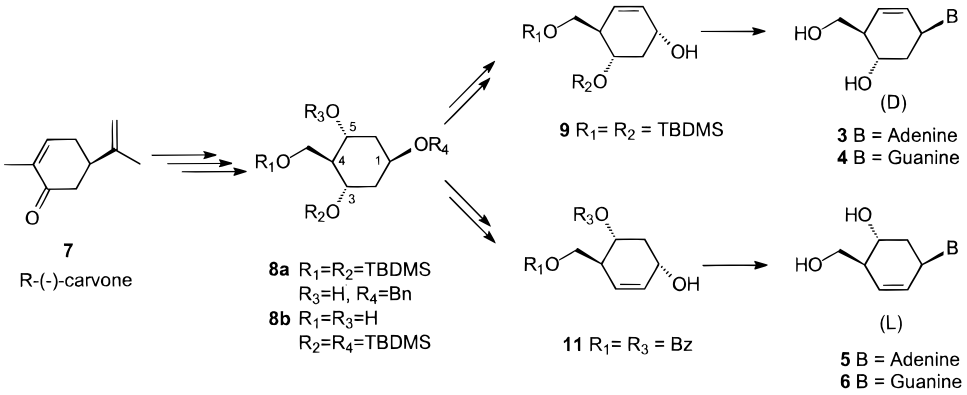
Mitsunobu reaction was then applied for introduction of the base moiety (Scheme 4). Upon reaction of **11** with adenine in the presence of DEAD and $PPh_3$ in dioxane, the desired adenine derivative **18a** was isolated in a moderate 40% yield, together with 15% of the N7-isomer **18b** (Scheme 4). Finally, removal of the benzoyl protecting groups using $K_2CO_3$ in MeOH gave the L-adenine cyclohexene nucleoside **5** in 77% yield. The corresponding L-guanine nucleoside **6** was synthesized in an analogous way. The allylic alcohol **11** was treated with 2-amino-6-chloropurine under the same reaction conditions as described above for **10**, and the 6-chloropurine **19** thus obtained was converted to the guanine derivative **20** using TFA−$H_2O$ (3:1) (58% yield from **11**). Final deprotection was carried out by heating **20** in a saturated solution of $NH_3$ in MeOH in a sealed vessel for 2 days, and reversed-phase HPLC purification gave pure L-guanine cyclohexene nucleoside **6** in 73% yield.

## Results and Discussion

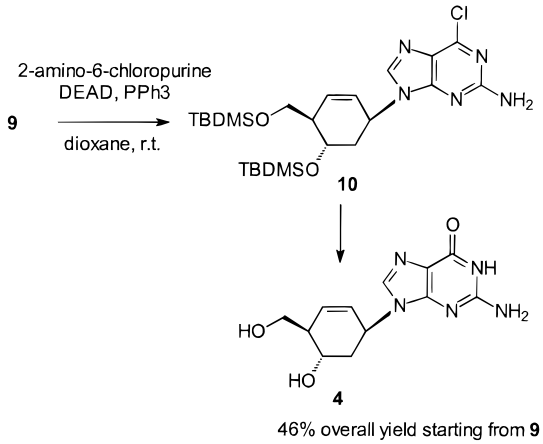
**Antiviral Activity.** The anti-herpesvirus activity of D-cyclohexenyl-G (**4**) and L-cyclohexenyl-G (**6**) and the corresponding adenine analogues was determined in human embryonic skin-muscle fibroblast ($E_6$SM: HSV-1, HSV-2) and human embryonic lung (HEL) cells [varicella-zoster virus (VZV), cytomegalovirus (CMV)] (Table 1). The source of the viruses and the methodology used to monitor antiviral activity have been described previously.[24,25] The antiviral activity was compared with the activity of known and approved antiviral drugs of which two possessed purine bases (acyclovir, ganciclovir) and two possesed pyrimidine bases (brivudine, cidofovir). The adenine nucleoside analogues only showed very low antiviral activity and, therefore, will not be further discussed.

D-Cyclohexenyl-G as well as L-cyclohexenyl-G did not show toxicity in four different cell lines (HeLa, Vero, $E_6$SM, HEL) (Table 2), pointing to their selective antiviral mode of action, as reflected by the high selectivity index of the compounds (Table 1). A salient feature is that the activity spectrum of both enantiomers is remarkably similar. Both compounds displayed the same activity against HSV-1 and HSV-2. Against VZV and CMV the potency of L-cyclohexenyl-G was about 2-fold lower than that of D-cyclohexenyl-G. Against HSV-1, the cyclohexenyl-G nucleosides were as active as acyclovir and brivudin. Against HSV-2, their activity was very similar to that of acyclovir. The cyclohexenyl-G nucleosides retained activity against the $TK^-$ strains of HSV-1 and VZV, although the activity was reduced as compared to the activity against the wild type. The

**Scheme 1**




**Scheme 2**



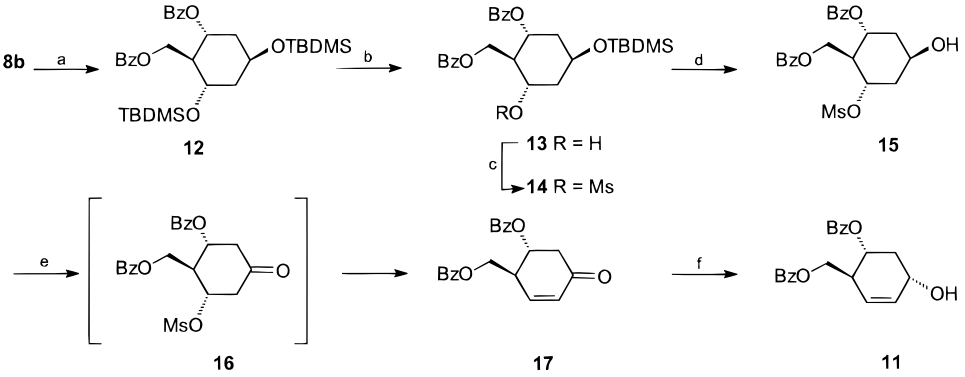

activity of D-cyclohexenyl-G against $TK^+$ and $TK^-$ VZV strains was higher than the respective activities of acyclovir and brivudin against these viruses. Finally, D-cyclohexenyl-G showed the same potency against CMV as ganciclovir. In conclusion, the activity spectrum of the newly synthesized cyclohexenyl nucleosides is very similar to that of the known antiviral compounds possessing a guanine base moiety (acyclovir, ganciclovir). Both the D- and L-enantiomers of cyclohexenyl-G are antivirally active. The high selectivity indexes observed for D- and L-cyclohexenyl-G warrant further in vivo studies with these compounds against herpesvirus infections.

**Conformational Analysis of Cyclohexene Nucleosides.** The conformational globe of a puckered cyclohexene is more complex than the pseudo-rotation cycle of five-membered rings (Figure 2).[26] The energetically most favorable puckered ring forms are located on the surface of the globe. The chair and inverted chair forms of the cyclohexene ring are located at the two poles of the globe. The half-chair forms are located at $\theta = 52.7°$, $\phi_2 = 210°$ and at $\theta = 127.3°$, $\phi_2 = 30°$. The half-chair−half-chair interconversion occurs via the boat forms. Cyclohexene rings are often locked in energy valleys along the ellipsis formed by the different boat and half-chair forms (Figure 3). For simplicity and to make comparison with furanose nucleosides possible, we designated the $^2H_3$ conformation of the cyclohexene nucleoside as S′-type and the $^3H_2$ conformation as N′-type (Figure 3). The $^3H_2$ conformation may be considered as a mimic of a furanose nucleoside in its 3′-endo form, while the $^2H_3$ conformation might be considered as a 2′-endo mimic of a furanose nucleoside. The energy of interconversion between $^3H_2$ and $^2H_3$ of cyclohexene (via the boat conformation) is about 23.4 kJ/mol.[27] The energy barrier for interconversion of the most stable conformers of a cyclohexene nucleoside (Figure 3) is not known. The energy difference between both low-energy conformations of cyclohexenyl-G (1.6 kJ/mol)[23] is similar to the energy difference between the 3′-endo and 2′-endo forms of a deoxyribose nucleoside (2.1 kJ/mol).[28] The proposed cyclohexene nucleosides are flexible molecules,[23] and structurally, they may be considered as the best six-membered mimics of natural furanoses. The reason for this is that the $\pi-\sigma^*$ interaction in cyclohexenyl nucleosides takes over the role of the anomeric effect in furanose nucleosides.[23,29] The anomeric effect in furanose nucleosides drives the S ↔ N equilibrium in the northern direction. Unfavorable 1,3-steric pseudo-diaxial interactions (the base moiety is generally more sterically demanding than the $CH_2OH$ group) together with the gauche effect (3′-OH and ring oxygen atom) favor the southern type conformation. In cyclohexene nucleosides the $\pi-\sigma^*$ interaction favors the $^3H_2$ conformation while steric effects favor the $^2H_3$ conformation (Figure 3). [23,29]

**Molecular Modeling.** D- And L-cyclohexenyl-G are the first examples of two enantiomeric nucleosides showing similar activity against a whole range of herpesviruses (HSV-1, HSV-2, VZV, CMV). When evaluated against $TK^-$ strains of HSV-1, both nucleoside analogues showed reduced antiviral activity. It is therefore reasonable to hypothesize that intracellular phosphorylation in the virus-infected cells is an important step in the enzymatic activation of cyclohexenyl-G nucleosides. In an effort to understand how enantiomeric nucleosides can be phosphorylated by the same kinases (we assume that the viral kinase is responsible for the first phosphorylation step because the activity of both enantiomeric nucleosides was reduced against $TK^-$ strains of HSV-1), we carried out a molecular modeling study using D- and L-cyclohexenyl-G and HSV-1 thymidine kinase as players.

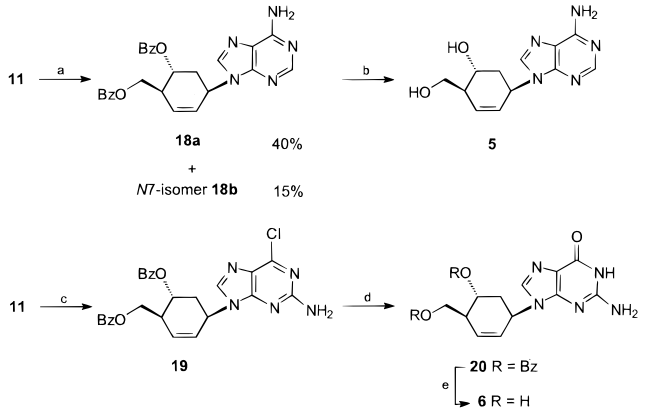
When D-cyclohexenyl-G was analyzed as a single molecule, it was observed that the conformation with the pseudo-axial base (Figure 3, N′-conformation) and an anti-glycoside torsion angle was (calculations were carried out in a vacuum) more stable than the conformation with a pseudo-equatorial base moiety (Figure 3, S′-conformation). These data are in agreement with

**Scheme 3**[a]




[a] a. $Bz_2O$, DMAP, $CH_2Cl_2$, 0 °C, 98%; b. TBAF (1 equiv), THF, rt, 74%; c. MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 98%; d. TBAF, THF, rt, 86%; e. PDC, $CH_2Cl_2$, rt, 68%; f. $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, rt, 75%.

**Scheme 4**[a]




[a] a. Adenine, DEAD, $PPh_3$, dioxane, rt; b. $K_2CO_3$, MeOH, rt, 77%; c. 2-amino-6-chloropurine, DEAD, $PPh_3$, dioxane, rt; d. TFA/$H_2O$ (3:1), rt, 2 days, 58% from **11**; e. $NH_3$/MeOH, 80 °C, 2 days, 73%.

the conformational analysis in solution using NMR spectrometry.[23]

The crystal structure of HSV-1 thymidine kinase complexed with penciclovir, as described by the group of Sanderson,[30] was used as a starting model in the docking experiment of the cyclohexenyl-G nucleosides (after removal of water molecules together with penciclovir itself and adding hydrogen atoms). D- And L-cyclohexenyl-G were docked in the active site of the enzyme with the guanine base moiety anchored to Gln-125 by double hydrogen bonds and the hydroxymethyl group anchored to Arg-163 via hydrogen bonding. The compounds having a conformation with a pseudo-axial base moiety (Figure 3, N′-conformation) could not be accommodated in the active side due to the difficulty of obtaining close interactions between the hydroxymethyl group of the nucleoside and Arg-163. On the contrary, the cyclohexenyl nucleosides nicely fit in the active site when the conformation is flipped to that with a pseudo-equatorially oriented base moiety (Figure 3, S′-conformation, and Figure 4). Both enantiomers adopt the syn conformation ($\chi$ of 18.8° for **4** and −88.5° for **6**). These results are consistent with our previous observations that hexitol nucleosides undergo chair inversions when cocrystallized with HSV-1 thymidine kinase.[3f]

The guanine bases of **4**, **6,** and penciclovir are bound in the same position and orientation. These guanine bases are stacked against Tyr-172. The guanine base of D-cyclohexenyl-G (**4**) is bound via four hydrogen bounds (covering the Watson−Crick base-pairing functionality of guanine) to Gln-125 and via three hydrogen bonds (involving the O6 and N7 of guanine) to Arg-176 (Figure 4). In the case of L-cyclohexenyl-G (**6**), only two hydrogen bonds are involved in the interaction with Arg-176. The sugar moieties of D- and L-cyclohexenyl-G have one hydrogen bond interaction between the primary hydroxyl group and Arg-163 and one hydrogen bond interaction between the secondary hydroxyl group and Glu-225. The hydrogen bond with Glu-225 is 1.3 Å longer in the L-cyclohexenyl complex than in the D-cyclohexenyl complex. A more detailed study of the interaction energy between ligands and neighboring residues reveals a slightly different binding energy for both enantiomers. The interaction energies are given in Table 3. The difference in binding is mainly due to a different contribution of the sugar moiety. The interaction between the secondary hydroxyl group of the nucleosides and Glu-225 (and, to a lesser extent, between the primary hydroxyl group and Arg-163) explains the lower stability of the L-cyclohexenyl-G/thymidine kinase complex versus the D-cyclohexenyl-G/thymidine kinase complex. Table 3 also shows the important contribution of Tyr-172 to the interaction energy.

## Conclusion

The D- and L-cyclohexenyl-G nucleosides were synthesized in a stereospecific manner starting from *R*-(−)-carvone. D-Cyclohexenyl-G as well as L-cyclohexenyl-G exhibited potent and selective anti-herpesvirus (HSV-1, HSV-2, VZV, CMV) activity. Their activity spectrum is comparable to that of the known antiviral drugs acyclovir and ganciclovir. D- And L-cyclohexenyl-G represent the most potent antiviral nucleosides containing a six-membered carbohydrate mimic that have ever been reported. They are as well the first example of two enantiomeric nucleosides, both showing similar activity against the whole series of classical herpesviruses. This finding is rather unexpected for nucleosides having three chiral centers.

The cyclohexenyl nucleosides have a flexible conformation. In solution, they appear as an equilibrium between the $^2H_3$ and $^3H_2$ conformations, and the energy difference between the conformations is low. It is expected that cyclohexenyl nucleosides can adapt their conformation easily to different enzymatic require-

**Table 1.** Antiviral Activity of D- and L-Cyclohexenyl-G in Comparison with Approved Antiviral Drugs: 50% Inhibitory Concentration ($IC_{50}$, $\mu$g/mL)

| | D-cyclohexenyl-G (**4**) | | L-cyclohexenyl-G (**6**) | | | | | |
|---|---|---|---|---|---|---|---|---|
| virus | activity | selectivity index | activity | selectivity index | brivudin | acyclovir | ganciclovir | cidofovir |
| HSV-1 (KOS)[a] | 0.002[b] | $>2 \times 10^5$ | 0.003[b] | $>5 \times 10^3$ | 0.001[b] | 0.01[b] | 0.001[b] | ND[e] |
| HSV-1 (F)[a] | 0.002[b] | $>2 \times 10^5$ | 0.003[b] | $>5 \times 10^3$ | 0.001[b] | 0.003[b] | 0.001[b] | ND |
| HSV-1 (McIntyre)[a] | 0.004[b] | $>1 \times 10^5$ | 0.004[b] | $>4 \times 10^3$ | 0.001[b] | 0.005[b] | 0.001[b] | ND |
| HSV-2 (G)[a] | 0.05[b] | $>8 \times 10^3$ | 0.07[b] | $>2.2 \times 10^2$ | >80[b] | 0.02[b] | 0.002[b] | ND |
| HSV-2 (196)[a] | 0.07[b] | $>5 \times 10^3$ | 0.1[b] | $>1.6 \times 10^2$ | >80[b] | 0.02[b] | 0.001[b] | ND |
| HSV-2 (Lyons)[a] | 0.07[b] | $>5 \times 10^3$ | 0.07[b] | $>2.2 \times 10^2$ | >80[b] | 0.02[b] | 0.001[b] | ND |
| HSV-1 ($TK^-$ KOS $ACV^r$)[a] | 0.38[b] | $>1 \times 10^3$ | 1.28[b] | >12 | >80[b] | 9.6[b] | 0.48[b] | ND |
| HSV-1 ($TK^-$/$TK^+$ VMW1837)[a] | 0.01[b] | $>4 \times 10^4$ | 0.01[b] | $>1.6 \times 10^3$ | >80[b] | 0.07[b] | 0.01[b] | ND |
| VZV (YS)[c] | 0.49[d] | >40 | 1.2[d] | >16 | 0.03[d] | 1.1[d] | ND | ND |
| VZV (OKA)[c] | 0.64[d] | >30 | 1.9[d] | >10 | 0.003[d] | 0.8[d] | ND | ND |
| VZV ($TK^-$ 07/1)[c] | 2.1[d] | >10 | 5.8[d] | >3 | >20[d] | 13[d] | ND | ND |
| VZV ($TK^-$ YS/R)[c] | 2.8[d] | >7 | 6.8[d] | >3 | >50[d] | 28[d] | ND | ND |
| CMV (AD 169)[c] | 0.6[d] | >30 | 1.5[d] | >13 | ND | ND | 0.6[d] | 0.08[d] |
| CMV (Davis)[c] | 0.8[d] | >25 | 1.7[d] | >12 | ND | ND | 0.8[d] | 0.2[d] |

[a] Activity determined in $E_6$SM cell cultures. [b] Minimum inhibitory concentration ($\mu$g/mL) required to reduce virus-induced cytopathogenicity by 50%. Virus input was 100 50% cell culture infective doses ($CCID_{50}$). [c] Activity determined in HEL cells. [d] Minimum inhibitory concentration ($\mu$g/mL) required to reduce virus plaque formation by 50%. Virus input was 20 plaque-forming units (PFU). [e] ND: not determined. The values are means of two independent determinations.

**Table 2.** Cytotoxicity of D- and L-Cyclohexenyl-G in Four Different Cell Lines (concn in $\mu$g/mL)

| cell line | D-cyclohexenyl-G | L-cyclohexenyl-G | brivudin | acyclovir | ganciclovir | cidofovir |
|---|---|---|---|---|---|---|
| HeLa[a] | 400 | 400 | ≥400 | ND[c] | ND | ND |
| Vero[a] | 400 | 400 | ≥400 | ND | ND | ND |
| $E_6$SM[a] | >400 | >16 | ≥400 | ≥400 | >100 | ND |
| HEL[b] | >20 | >20 | >50 | >50 | >50 | >50 |
| HEL[a] | 11 | >20 | >200 | >200 | >50 | >50 |

[a] Minimum cytotoxic concentration causing a microscopically detectable alteration of cell morphology. [b] Cytotoxic concentration required to reduce cell growth by 50%. [c] ND: not determined.

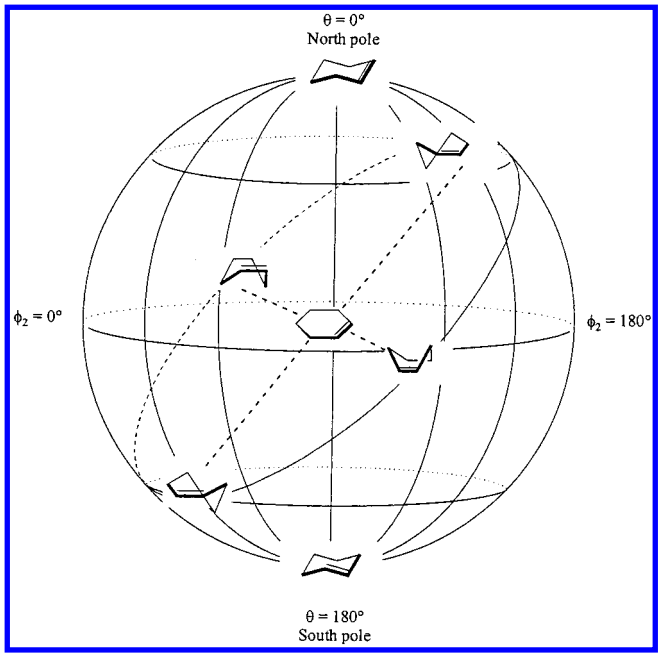



**Figure 2.** Conformational globe of puckered cyclohexene showing the different boat and half-chair forms.

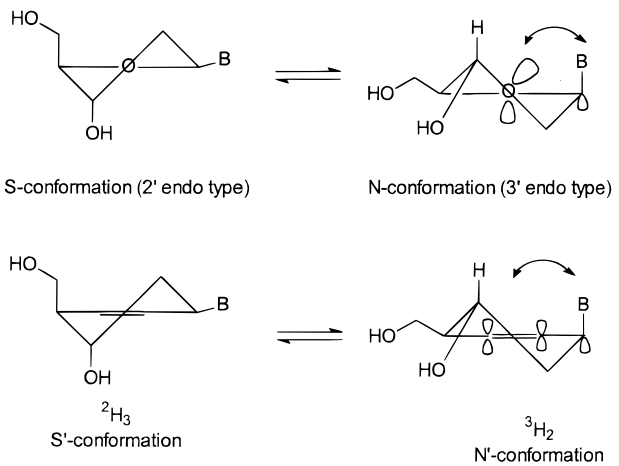



**Figure 3.** Comparison of the northern ⇌ southern conformational equilibrium between a furanose nucleoside and a cyclohexene nucleoside.

ments. This flexibility may be an important structural determinant in their potent antiviral activity. The activity of D- and L-cyclohexenyl-G is lower against $TK^-$ strains of HSV-1 and VZV than against $TK^+$ strains. This indicates that the viral TK plays an important role in their biological activity, at least against those viruses for which these data are available. Therefore we investigated the binding of D- and L-cyclohexenyl-G in the active site of HSV-1 thymidine kinase using molecular modeling. This study revealed that both enantiomeric cyclohexene nucleosides are bound in the active site in a high-energy conformation (pseudo-equatorial orientation of the base moiety in the syn conformation). These data confirm previous observations that HSV-1 thymidine kinase may induce a conformational change in nucleoside upon binding.[3f] In the X-ray structure of the complex, the anhydrohexitol nucleoside is, likewise, bound in a high-energy conformation.[3f] The present study illustrates for the first time how enantiomeric guanine-derived nucleosides can be bound in the active site of HSV-1 thymidine kinase. Since the observations of Fischer at the turn of the 20th century, it has been believed that enzymes do show discrimination in the way of binding of optical antipodes. However, this study indicates that in special situations the binding mode of

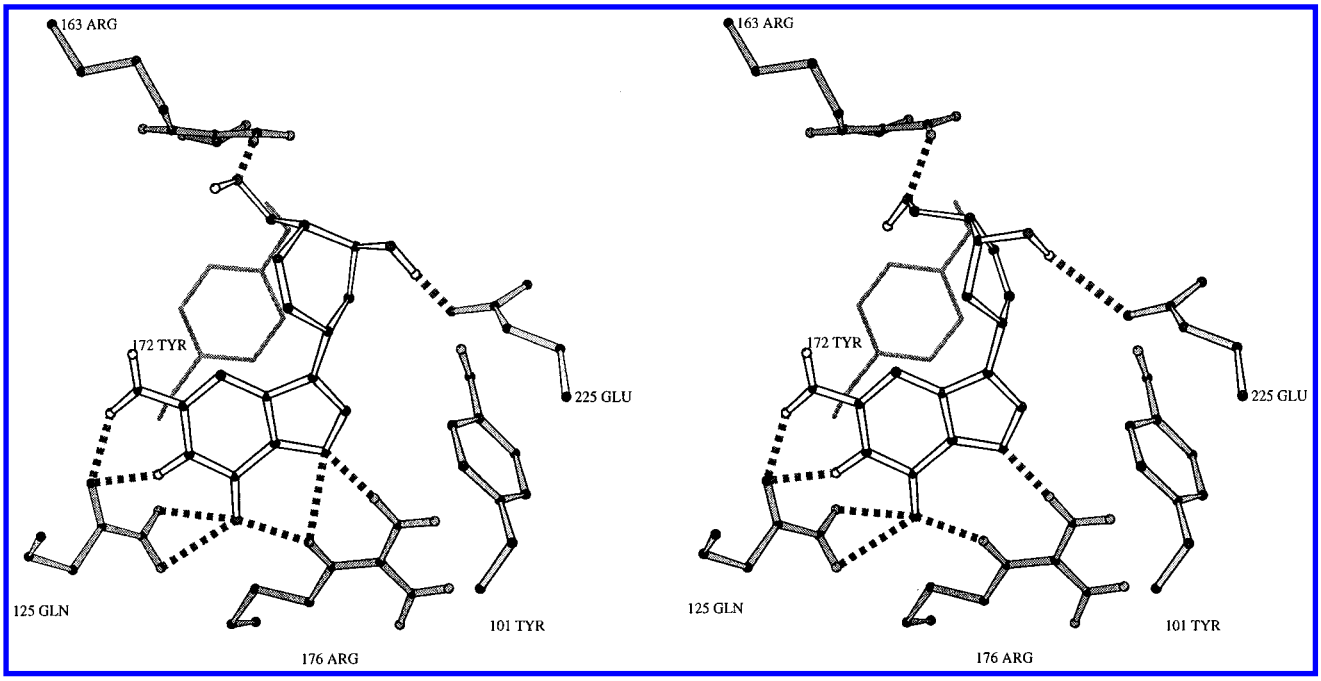



**Figure 4.** Plot of the minimized structure of HSV-1 thymidine kinase in complex with **4** (left) and **6** (right). Hydrogen bonds are plotted if the distance donor-hydrogen to acceptor is less than 2.7 Å and are marked by dotted lines. In the complex with **4**, the double bond in the sugar points to the front, while in the complex at the right, this double bond points to the back.

**Table 3.** Interaction Energy (kcal/mol) between D- and L-Cyclohexenyl-G and the Active Site Residues[a]

| | D-cyclohexenyl-G | | | L-cyclohexenyl-G | | |
|---|---|---|---|---|---|---|
| | vdw | electr | total | vdw | electr | total |
| all 5 Å residues | −22.6 | −76.5 | −99.1 | −29.1 | −49.9 | −79.0 |
| base | −16.1 | −35.3 | −51.4 | −15.4 | −36.6 | −52.0 |
| sugar | −6.5 | −41.2 | −47.8 | −13.7 | −13.3 | −27.0 |
| Glu-225 | 6.2 | −34.3 | −28.1 | −1.7 | −8.5 | −10.2 |
| Arg-176 | 0.3 | −19.3 | −19.0 | 0.4 | −20.3 | −19.9 |
| Gln-125 | 1.0 | −15.8 | −14.5 | 1.1 | −15.9 | −14.8 |
| Arg-163 | 1.1 | −10.5 | −9.4 | 0.9 | −6.9 | −5.9 |
| Tyr-172 | −8.1 | −0.3 | −7.8 | −7.1 | −0.6 | −7.8 |
| Arg-222 | −2.1 | −1.6 | −3.7 | −1.6 | −1.9 | −3.5 |
| Ile-100 | −2.6 | −0.3 | −2.9 | −2.2 | −0.4 | −2.6 |
| Ile-97 | −2.9 | 0.2 | −2.7 | −2.3 | 0.2 | −2.1 |
| Met-128 | −2.9 | 0.6 | −2.3 | −3.9 | 0.5 | −3.3 |
| Trp-88 | −1.7 | 0.0 | −1.7 | −2.0 | 0.0 | −2.0 |
| Met-231 | −1.4 | −0.3 | −1.7 | −1.3 | −0.3 | −1.6 |
| His-58 | −1.5 | 0.3 | −1.2 | −2.1 | 0.5 | −1.7 |
| Lys-62 | −0.3 | −1.1 | −1.4 | −0.3 | −0.6 | −0.9 |
| Gln-221 | −1.1 | 0.3 | −0.9 | −0.6 | 0.4 | −0.2 |
| Ala-168 | −0.8 | 0.1 | −0.7 | −0.8 | 0.0 | −0.8 |
| Ala-167 | −0.4 | −0.1 | −0.5 | −0.5 | 0.0 | −0.6 |
| Glu-83 | −0.9 | 0.4 | −0.5 | −1.0 | 0.6 | −0.4 |
| Ala-124 | −0.3 | −0.2 | −0.5 | −0.2 | −0.2 | −0.4 |
| Pro-173 | −0.5 | 0.3 | −0.2 | −0.5 | 0.3 | −0.2 |
| Met-182 | −0.2 | −0.1 | −0.3 | −0.2 | −0.1 | −0.3 |
| Tyr-239 | −0.2 | 0.0 | −0.2 | −0.2 | 0.2 | −0.1 |
| Met-121 | −0.7 | 0.8 | 0.1 | −0.7 | 0.8 | 0.1 |
| Tyr-132 | −0.4 | 0.9 | 0.4 | −0.5 | 0.8 | 0.3 |
| Tyr-101 | −2.2 | 2.7 | 0.5 | −1.6 | 1.4 | −0.2 |

[a] Residues having atoms at a distance less than 5 Å from the ligand were included in the calculation; vdw = van der Waals energy contribution, electr = electrostatic energy contribution.

enantiomers to a specific enzyme might be similar. The amino acids in thymidine kinase involved in binding of D- and L-cyclohexenyl-G are the same. Given the fact that the guanine base moiety is bound to the enzyme via a web of hydrogen bonds superimposed on a stacking interaction, it is not unexpected that chemical modifications of the purine base of guanine nucleosides have mostly led to a decrease of anti-herpes activity. The difference in binding energy of both enantiomers is primarily due to a difference in hydrogen-bonding interaction between the secondary hydroxyl groups of the nucleoside and Glu-225. Whether this difference has any biological relevance remains the subject of further studies. Because an antiviral guanine nucleoside is bound in the HSV-1 thymidine kinase in a slightly different way than an antiviral pyrimidine nucleoside,[5,30] it is to be expected that this study cannot be extrapolated, per se, to all anti-herpesvirus nucleosides.

In summary, D-cyclohexenyl-G (**4**) and L-cyclohexenyl-G (**6**) are broad-spectrum anti-herpesvirus agents with very similar antiviral activity. The flexibility of the cyclohexene ring may be an important structural determinant leading to the observed biological activity.

## Experimental Section

**General Methods.** Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8740 UV/vis spectrophotometer. $^1$H and $^{13}$C NMR were determined with a 200-MHz Varian Gemini apparatus or with a Varian Unity 500 spectrometer with tetramethylsilane as internal standard for the $^1$H NMR spectra and DMSO-$d_6$ (39.6 ppm) or $CDCl_3$ (76.9 ppm) for the $^{13}$C NMR spectra (s = singlet, d = doublet, dd = double doublet, t = triplet, br s = broad singlet, br d = broad doublet, m = multiplet). Liquid secondary ion mass spectra (LSIMS) with $Cs^+$ as primary ion beam were recorded on a Kratos Concept IH (Kratos, Manchester, U.K.) mass spectrometer equipped with a MASPEC II[32] data system (Mass Spectrometry Services Ltd., Manchester, U.K.). Samples were dissolved in glycerol (GLY)/thioglycerol (THGLY)/*m*-nitrobenzyl alcohol (NBA) and the secondary ions were accelerated at 7 kV. Scans were performed at 10 s/decade from *m*/*z* 1000 down to *m*/*z* 50. Precoated Machery-Nagel Alugram SIL G/$UV_{254}$ plates were used for TLC; the spots were examined with UV light and sulfuric acid/anisaldehyde spray. Elemental analyses were done at the University of Konstanz, Germany.

**9-[(1*S*,4*R*,5*S*)-5-(*tert*-Butyldimethylsilyloxy)-4-(*tert*-butyldimethylsilyloxymethyl)-2-cyclohexenyl]-2-amino-6-**

**chloropurine (10).** To a mixture of **9** (130 mg, 0.35 mmol), 2-amino-6-chloropurine (119 mg, 0.70 mmol), and $PPh_3$ (184 mg, 0.70 mmol) in dry dioxane (7 mL) under $N_2$ at room temperature was added a solution of DEAD (110 $\mu$L, 0.70 mmol) in dry dioxane (3 mL) over a period of 1.5 h. The reaction mixture was stirred at room temperature for 2 days and concentrated. The residue was chromatographed on silica gel ($CH_2Cl_2$−MeOH 50:1 then 20:1) to yield crude **10** (170 mg) as a yellow foam: $^1$H NMR ($CDCl_3$) $\delta$ −0.10 (s, 3H), −0.04 (s, 3H), 0.07 (s, 6H), 0.82 (s, 9H), 0.90 (s, 9H), 2.04 (t, 2H, $J$ = 5.6 Hz), 2.27 (m, 1H), 3.66 (dd, 1H, $J$ = 9.9, 5.1 Hz), 3.77 (dd, 1H, $J$ = 9.9, 4.4 Hz), 3.98 (m, 1H), 5.21 (m, 1H), 5.43 (s, 2H, $NH_2$), 5.79 (dm, 1H, $J$ = 9.9 Hz), 6.00 (dm, 1H, $J$ = 9.9 Hz), 7.79 (s, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ −5.6 (q), −5.5 (q), −5.1 (q), −4.8 (q), 17.8 (s), 18.2 (s), 25.6 (q), 25.8 (q), 36.0 (t), 46.9 (d), 49.2 (d), 62.9 (t), 64.6 (d), 124.4 (d), 125.4 (s), 134.4 (d), 141.3 (d), 151.1 (s), 153.3 (s), 159.1 (s).

**9-[(1*S*,4*R*,5*S*)-5-Hydroxy-4-hydroxymethyl-2-cyclohexenyl]guanine (4).** Crude **10** (170 mg) was treated with TFA−$H_2O$ (3:1, 10 mL) at room temperature for 2 days. The reaction mixture was concentrated and coevaporated with toluene (2×). The residue was chromatographed on silica gel ($CH_2Cl_2$−MeOH 10:1 then 1:1) to afford **4** (45 mg, 46% overall yield starting from **9**): mp >230 °C; UV $\lambda_{max}$ (MeOH) 253 nm; $^1$H NMR ($CD_3OD$) $\delta$ 1.94−2.27 (m, 3H), 3.77 (d, 2H, $J$ = 4.7 Hz), 3.85 (m, 1H), 5.17 (m, 1H), 5.88 (dm, 1H, $J$ = 10.2 Hz), 6.09 (dm, 1H, $J$ = 10.2 Hz), 7.73 (s, 1H); $^{13}$C NMR ($CD_3OD$) $\delta$ 37.1 (t), 47.7 (d), 50.6 (d), 63.1 (t), 64.8 (d), 125.8 (d), 135.4 (d), 138.5 (d); LSIMS (THGLY/NBA) 278 (M + H)$^+$; HRMS calcd for $C_{12}H_{16}N_5O_3$ (M + H)$^+$ 278.1253, found 278.1270. Anal. Calcd for $C_{12}H_{15}N_5O_3 \cdot 0.77H_2O$: C 49.49, H 5.73, N 24.05. Found: C 49.45, H 5.55, N 24.22.

**(1*S*,2*S*,3*R*,5*R*)-3-Benzoyl-2-benzoylmethyl-1,5-di(*tert*-butyldimethylsilyloxy)cyclohexane (12).** To a solution of **8b** (2.2 g, 5.64 mmol) in dry dichloromethane (20 mL) at 0 °C under $N_2$ were added DMAP (3.44 g, 28.2 mmol, 5 equiv) and $Bz_2O$ (3.83 g, 16.92 mmol, 3 equiv) sequentially and in portions. After stirring at 0 °C for 2 h, the reaction was quenched with ice. The reaction mixture was poured into $CH_2Cl_2$ (250 mL), washed with water and brine, dried over $Na_2SO_4$, and concentrated. The crude product was chromatographed on silica gel (*n*-hexanes−EtOAc 10:1) to yield **12** (3.3 g, 98%) as a light yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 0.03 (s, 3H), 0.06 (s, 3H), 0.13 (s, 3H), 0.17 (s, 3H), 0.89 (s, 9H), 1.01 (s, 9H), 1.58 (m, 2H), 2.09 (m, 2H), 2.37 (m, 1H), 4.29 (br s, 1H), 4.40 (td, 1H, $J$ = 10.3, 4.0 Hz), 4.49 (dd, 1H, $J$ = 11.4, 2.0 Hz), 4.61 (dd, 1H, $J$ = 11.4, 2.2 Hz), 5.66 (td, 1H, $J$ = 11.0, 4.6 Hz), 7.37−7.44 (m, 4H), 7.49−7.59 (m, 2H), 8.02 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ −5.3 (q), −5.2 (q), −5.1 (q), −4.5 (q), 17.8 (s), 25.6 (q), 25.7 (q), 38.2 (t), 42.4 (t), 49.5 (d), 60.0 (t), 65.2 (d), 66.4 (d), 68.5 (d), 128.3 (d), 129.6 (d), 130.3 (s), 130.4 (s), 132.8 (d), 165.6 (s), 166.5 (s).

**(1*S*,2*S*,3*R*,5*S*)-3-Benzoyl-2-benzoylmethyl-5-(*tert*-butyldimethylsilyloxy)cyclohexanol (13).** A solution of 1 M TBAF in THF (5.38 mL, 5.38 mmol) was added slowly to a solution of **12** (3.23 g, 5.38 mmol) in THF (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and further at room temperature for 3 h. Ice was added and the reaction mixture was poured into EtOAc (300 mL) which was washed with $NH_4Cl$ solution, water, and brine, dried over $Na_2SO_4$, and concentrated. The crude product was purified on silica gel (*n*-hexanes−EtOAc 5:1 then 1:1) to yield **13** (1.93 g, 74%) as a white foam: $^1$H NMR ($CDCl_3$) $\delta$ 0.02 (s, 3H), 0.09 (s, 3H), 0.81 (s, 9H), 1.50−1.63 (m, 2H), 1.96 (m, 1H), 2.13 (m, 1H), 2.32 (m, 1H), 3.24 (d, 1H, $J$ = 4.8 Hz, −OH), 4.04 (m, 1H), 4.25 (m, 1H), 4.33 (dd, 1H, $J$ = 11.4, 2.2 Hz), 5.04 (dd, 1H, $J$ = 11.4, 2.2 Hz), 5.60 (td, 1H, $J$ = 11.0, 4.5 Hz), 7.36−7.60 (m, 6H), 8.06 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ −5.2 (q), 17.6 (s), 25.4 (q), 38.2 (t), 40.8 (t), 50.4 (d), 59.8 (t), 64.1 (d), 66.1 (d), 68.4 (d), 128.4 (d), 129.6 (d), 129.8 (d), 130.4 (s), 133.0 (d), 133.2 (d), 165.6 (s), 167.6 (s); LSIMS (THGLY/TFA) 485 (M + H)$^+$; HRMS calcd for $C_{27}H_{37}O_6Si$ (M + H)$^+$ 485.2359, found 485.2376.

**(1*S*,2*S*,3*R*,5*S*)-3-Benzoyl-2-benzoylmethyl-5-(*tert*-butyldimethylsilyloxy)-1-(methanesulfonyloxy)cyclohexane (14).** To a solution of **13** (1.90 g, 3.92 mmol) in dry dichloromethane (20 mL) at 0 °C under $N_2$ were added slowly triethylamine (2.71 mL, 19.6 mmol, 5 equiv) and MsCl (456 $\mu$L, 5.89 mmol, 1.5 equiv) sequentially. After stirring at 0 °C for 1 h, the reaction was quenched with ice. The reaction mixture was poured into $CH_2Cl_2$ (250 mL), washed with a saturated $NH_4Cl$ solution, water, and brine, dried over $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel (*n*-hexanes−EtOAc 1:1) to afford **14** (2.17 g, 98%) as a white foam: $^1$H NMR ($CDCl_3$) $\delta$ 0.13 (s, 3H), 0.15 (s, 3H), 0.96 (s, 9H), 1.62 (td, 1H, $J$ = 12.0, 2.0 Hz), 1.85 (td, 1H, $J$ = 12.0, 2.0 Hz), 2.26−2.58 (m, 3H), 2.98 (s, 3H), 4.34 (m, 1H), 4.50 (dd, 1H, $J$ = 11.4, 2.0 Hz), 4.60 (dd, 1H, $J$ = 11.4, 2.0 Hz), 5.28 (td, 1H, $J$ = 11.1, 4.8 Hz), 5.69 (td, 1H, $J$ = 11.0, 4.8 Hz), 7.42 (m, 4H), 7.57 (m, 2H), 8.03 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ −5.3 (q), −5.2 (q), 17.8 (s), 25.5 (q), 37.8 (t), 38.1 (q), 39.9 (t), 46.7 (d), 58.9 (t), 65.9 (d), 67.8 (d), 75.9 (d), 128.5 (d), 129.6 (d), 129.9 (s), 133.1 (d), 165.4 (s), 166.3 (s); LSIMS (THGLY/GLY) 563 (M + H)$^+$; HRMS calcd for $C_{28}H_{39}O_8SSi$ (M + H)$^+$ 563.2135, found 563.2188.

**(1*R*,3*S*,4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-3-(methanesulfonyloxy)cyclohexanol (15).** To a solution of **14** (2.15 g, 3.82 mmol) in THF (50 mL) at room temperature was added slowly a 1 M solution of TBAF (7.64 mL, 7.64 mmol, 2 equiv) in THF. The reaction was stirred at room temperature for 2.5 h and quenched with ice. After standard workup and purification on silica gel (*n*-hexanes−EtOAc 1:1), **15** (1.55 g, 86%) was obtained as a white foam: $^1$H NMR ($CDCl_3$) $\delta$ 1.71 (td, 1H, $J$ = 12.1, 2.2 Hz), 1.90 (td, 1H, $J$ = 12.2, 2.3 Hz), 2.29−2.65 (m, 4H), 3.00 (s, 3H), 4.41 (m, 1H), 4.52 (dd, 1H, $J$ = 11.7, 2.8 Hz), 4.61 (dd, 1H, $J$ = 11.7, 2.8 Hz), 5.27 (td, 1H, $J$ = 10.6, 4.8 Hz), 5.65 (td, 1H, $J$ = 10.6, 4.7 Hz), 7.42 (m, 4H), 7.55 (m, 4H), 8.02 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ 37.3 (t), 38.2 (q), 39.0 (t), 46.8 (d), 59.3 (t), 65.1 (d), 67.9 (d), 75.8 (d), 128.5 (d), 129.7 (d), 133.2 (d), 133.3 (d), 165.6 (s), 166.4 (s); LSIMS (THGLY/TFA) 449 (M + H)$^+$; HRMS calcd for $C_{22}H_{25}O_8S$ (M + H)$^+$ 449.1270, found 449.1244.

**(4*S*,5*R*)-5-Benzoyl-4-benzoylmethylcyclohex-2-en-1-one (17).** A mixture of **15** (500 mg, 1.12 mmol) and PDC (2.1 g, 5.60 mmol, 5 equiv) in dry $CH_2Cl_2$ (30 mL) was stirred vigorously at room temperature for 24 h. The reaction mixture was filtered through Celite and washed with $CH_2Cl_2$. The filtrate was concentrated and the residue was chromatographed on silica gel (*n*-hexanes−EtOAc 2:1 then 1:2) to yield starting material **15** (100 mg, 20%) and enone **17** (267 mg, 68%) as a light yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 2.73 (dd, 1H, $J$ = 16.5, 8.8 Hz), 3.10 (dd, 1H, $J$ = 16.5, 4.4 Hz), 3.27 (m, 1H), 4.50 (dd, 1H, $J$ = 11.3, 4.7 Hz), 4.66 (dd, 1H, $J$ = 11.3, 5.5 Hz), 5.66 (ddd, 1H, $J$ = 8.8, 7.3, 4.4 Hz), 6.26 (dd, 1H, $J$ = 10.2, 2.2 Hz), 6.96 (dd, 1H, $J$ = 10.2, 3.3 Hz), 7.40−7.63 (m, 6H), 8.01 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ 41.3 (d), 42.1 (t), 63.2 (t), 70.0 (d), 128.6 (d), 129.5 (2s), 129.7 (d), 129.8 (d), 131.4 (d), 133.5 (d), 146.5 (d), 165.5 (s), 166.4 (s), 195.8 (s).

**(1S,4*S*,5*R*)-5-Benzoyl-4-benzoylmethylcyclohex-2-en-1-ol (11).** To a solution of **17** (267 mg, 0.76 mmol) in MeOH (10 mL) at room temperature under $N_2$ was added $CeCl_3 \cdot 7H_2O$ (426 mg, 1.14 mmol, 1.5 equiv). The mixture was stirred for 0.5 h and a clear solution was obtained. $NaBH_4$ (35 mg, 0.91 mmol, 1.2 equiv) was added in portions and $H_2$ evolved. The reaction mixture was stirred for 1 h and quenched with ice. The mixture was stirred for 15 min and concentrated. The residue was taken up into EtOAc, washed with $H_2O$ and brine, dried over $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel (*n*-hexanes−EtOAc 10:1) to give **11** (200 mg, 75%) as a light yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 1.77 (d, 1H, $J$ = 7.2 Hz), 1.93 (ddd, 1H, $J$ = 12.1, 10.2, 8.0 Hz), 2.54 (ddd, 1H, $J$ = 12.1, 5.8, 3.3 Hz), 3.00 (m, 1H), 4.32 (dd, 1H, $J$ = 11.4, 5.5 Hz), 4.44 (dd, 1H, $J$ = 11.4, 5.5 Hz), 4.50 (m, 1H), 5.35 (ddd, 1H, $J$ = 10.2, 7.3, 2.9 Hz), 5.78 (dt, 1H, $J$ = 10.2, 1.8 Hz), 5.97 (dt, 1H, $J$ = 10.2, 2.5 Hz), 7.34−7.60 (m, 6H), 8.00 (m, 4H); $^{13}$C NMR ($CDCl_3$) $\delta$ 36.6 (t), 40.9 (d), 46.6 (t), 65.8 (d), 69.9 (d), 126.6 (d), 128.4 (d), 128.5 (d), 129.7 (d), 129.8 (s), 130.9 (s), 132.7 (d), 133.1 (d), 133.2 (d), 166.0 (s), 166.5 (s); LSIMS (THGLY/TFA) 353 (M + H)$^+$; HRMS calcd for $C_{21}H_{21}O_5$ (M + H)$^+$ 353.1389, found 353.1440.

**9-[(1*R*,4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-2-cyclohexenyl]adenine (18a).** To a mixture of **11** (65 mg, 0.18 mmol), adenine (48 mg, 0.36 mmol, 2 equiv), and $PPh_3$ (94 mg, 0.36 mmol, 2 equiv) in dry dioxane (4 mL) under $N_2$ at room temperature was added a solution of DEAD (56 $\mu$L, 0.36 mmol, 2 equiv) in dry dioxane (3 mL) over a period of 1 h. The reaction mixture was stirred at room temperature overnight and concentrated. The residue was chromatographed on silica gel ($CH_2Cl_2$−MeOH 50:1, 20:1, 10:1) to yield **18a** (33 mg, 40%) as a white solid: UV $\lambda_{max}$ (MeOH) 231 and 263 nm; $^1$H NMR ($CDCl_3$) $\delta$ 2.48 (ddd, 1H, $J$ = 13.6, 8.3, 5.8 Hz), 2.57 (ddd, 1H, $J$ = 13.6, 6.0, 3.2 Hz), 4.50 (dd, 1H, $J$ = 10.4, 5.0 Hz), 4.63 (dd, 1H, $J$ = 10.4, 6.1 Hz), 5.53 (m, 2H), 5.92 (s, 2H), 6.09 (dm, 1H, $J$ = 10.0 Hz), 6.17 (dm, 1H, $J$ = 10.0 Hz), 7.41 (m, 4H), 7.57 (m, 2H), 7.86 (s, 1H), 8.04 (m, 4H), 8.35 (s, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ 32.4 (t), 40.6 (d), 48.7 (d), 64.3 (t), 68.2 (d), 120.1 (s), 126.8 (d), 128.5 (d), 128.6 (d), 129.6 (d), 129.7 (d), 131.0 (d), 133.4 (d), 138.8 (d), 149.8 (s), 153.1 (d), 155.8 (s), 165.8 (s), 166.5 (s); LSIMS (THGLY/NBA) 470 $(M + H)^+$; HRMS calcd for $C_{26}H_{24}N_5O_4$ $(M + H)^+$ 470.1828, found 470.1845.

**9-[(1*R*,4*S*,5*R*)-5-Hydroxy-4-hydroxymethyl-2-cyclohexenyl]adenine (5).** Compound **18a** (33 mg, 0.07 mmol) was treated with anhydrous $K_2CO_3$ (100 mg) in MeOH (3 mL) at room temperature for 3 h. A small portion of silica gel was added to the reaction mixture and the solvent was evaporated. The residue was chromatographed on silica gel ($CH_2Cl_2$−MeOH 10:1, 1:1) to give **5** (14 mg, 77%): $^1$H NMR ($CD_3OD$) $\delta$ 2.02−2.32 (m, 3H), 3.79−3.90 (m, 3H), 5.35 (m, 1H), 5.93 (dm, 1H, $J$ = 9.9 Hz), 6.15 (dm, 1H, $J$ = 9.9 Hz), 8.09 (s, 1H), 8.21 (s, 1H); $^{13}$C NMR ($CD_3OD$) $\delta$ 37.2 (t), 47.7 (d), 51.1 (d), 63.0 (t), 64.7 (d), 120.6 (s), 125.4 (d), 136.0 (d), 141.6 (d), 150.3 (s), 153.8 (d), 157.5 (s); LSIMS (THGLY/TFA) 262 $(M + H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_2$ $(M + H)^+$ 262.1304, found 262.1323.

**9-[(1*R*,4*S*,5*R*)-5-Benzoyl-4-benzoylmethyl-2-cyclohexenyl]guanine (20).** Compound **11** (160 mg, 0.45 mmol) was treated with 2-amino-6-chloropurine (153 mg, 0.90 mmol, 2 equiv) in the presence of $PPh_3$ (235 mg, 0.90 mmol, 2 equiv) and DEAD (140 $\mu$L, 0.90 mmol, 2 equiv) in dry dioxane (12 mL) at room temperature overnight. After concentration and purification on silica gel ($CH_2Cl_2$−EtOAc 1:1), crude **19** (500 mg) was obtained, which was treated with $CF_3COOH/H_2O$ (3:1, 12 mL) at room temperature for 2 days. The reaction mixture was concentrated and coevaporated with toluene. The residue was purified on silica gel ($CH_2Cl_2$−MeOH 20:1) to yield **20** (126 mg, 58% over two steps) as a white solid: UV $\lambda_{max}$ (MeOH) 251 and 256 nm; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.30 (ddd, 1H, $J$ = 13.6, 8.3, 5.9 Hz), 2.42 (ddd, 1H, $J$ = 13.6, 6.4, 3.2 Hz), 3.00 (m, 1H), 4.52 (m, 2H), 5.17 (m, 1H), 5.37 (m, 1H), 6.03 (dm, 1H, $J$ = 10.2 Hz), 6.11 (dm, 1H, $J$ = 10.2 Hz), 6.45 (s, 2H), 7.51 (m, 4H), 7.66 (m, 2H), 7.69 (s, 1H), 7.95 (m, 4H), 10.61 (s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 31.4 (t), 40.0 (d, overlapped with DMSO-$d_6$ peak), 47.9 (d), 64.4 (t), 68.5 (d), 116.9 (s), 127.0 (d), 128.9 (d), 129.3 (d), 129.4 (d), 130.2 (d), 133.6 (d), 135.7 (d), 150.9 (s), 153.8 (s), 156.9 (s), 165.3 (s), 165.8 (s); LSIMS (THGLY/GLY) 486 $(M + H)^+$; HRMS calcd for $C_{26}H_{24}N_5O_5$ $(M + H)^+$ 486.1777, found 486.1816.

**9-[(1*R*,4*S*,5*R*)-5-Hydroxy-4-hydroxymethyl-2-cyclohexenyl]guanine (6).** A mixture of **20** (85 mg) in an ammonium MeOH solution (75 mL) was sealed and heated at 80 °C for 2 days. After cooling to room temperature, the mixture was concentrated and the residue was purified by reversed-phase HPLC (4% $CH_3CN$ in water) to afford **6** (36 mg, 75%) as a white powder: mp 255 °C dec; UV $\lambda_{max}$ (MeOH) 254 nm; $^1$H NMR (500 HMz, DMSO-$d_6$) $\delta$ 1.85 (m, 1H), 1.98 (m, 1H), 2.11 (m, 1H), 3.54 (dd, 1H, $J$ = 10.3, 5.5 Hz), 3.60 (dd, 1H, $J$ = 10.3, 4.8 Hz), 3.70 (m, 1H), 4.68 (br s, 1H, −OH), 4.75 (br s, 1H, −OH), 4.99 (m, 1H), 5.77 (dm, 1H, $J$ = 9.8 Hz), 5.97 (dm, 1H, $J$ = 9.8 Hz), 6.57 (s, 2H, $-NH_2$), 7.50 (s, 1H), 10.8 (br s, 1H, −NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 35.9 (t), 46.4 (d), 48.2 (d), 61.5 (t), 62.7 (d), 116.9 (s), 124.8 (d), 133.7 (d), 135.6 (d), 150.8 (s), 154.1 (s), 157.6 (s); LSIMS (THGLY/NBA) 278 $(M + H)^+$; HRMS calcd for $C_{12}H_{16}N_5O_3$ $(M + H)^+$ 278.1253, found 278.1247. Anal. Calcd for $C_{12}H_{15}N_5O_3 \cdot 1.5H_2O$: C 47.35, H 5.96, N 23.03. Found: C 47.46, H 5.64, N 22.87.

**Molecular Modeling. Force field and parameters:** The AMBER 4.1 all-hydrogen parameter database of 1994[31] was used for thymidine kinase without modification. For the substrates, the same parameter set was used but missing bond and angle parameters were taken from comparable bonds or angles from the AMBER force field. Particularly the parameters for the double bond in the sugar moiety (C5′, C6′, H5′, and H6′, respectively) were extracted from the AMBER atom types CM, and H4. Partial atomic charges for the substrates were calculated using the standard procedure called RESP,[32] of fitting the electrostatic potential at the 6-31G* level of theory using GAMESS.[33] All energy refinements and calculations using the Sander and Anal modules of AMBER were done in vacuum, with a distance-dependent dielectric constant and a cutoff of 9 Å for the nonbonded interactions.

**Small molecules:** Molecules **4** and **6** were built starting from a riboguanine monomer generated by the Nucgen program in the AMBER software. A simple text editor was used to modify the Protein Data Bank (pdb) structure files. The energy minimization was performed in Sander to relax the structures. A conformational search was performed on the ligand **4** by the QCPE software DGEOM95 (QCPE-590).[34,35] Two major low-energy conformational clusters were found, the first group showing pseudo-axial and the second cluster having all pseudo-equatorial conformations of the base moiety. The energy as a function of the torsion angle C6′−C1′−N9−C4 was calculated using the Sander module of AMBER. This calculation revealed that the most stable conformation for **4** is pseudo-axial, with the torsion angle C6′−C1′−N9−C4 equal to 200° (anti) and about 3 kcal/mol (in vacuum) more stable than the semi-equatorial form. Since structure **6** is the mirror structure of **5**, the same low-energy conformations are expected, and hence the different conformations of **8** were created by coordinate transformations from structure **4**.

**Enzyme:** The 2.37 Å resolution crystal structure of HSV-1 thymidine kinase in complex with penciclovir, pdb entry 1KI3,[30,36] was used as a starting point for all modeling experiments because compounds **4** and **6** also contain the guanine base moiety.

The first entity (A) in the asymmetric crystal unit was used. All crystallographic waters were removed. Penciclovir was also removed, but its coordinates were saved in a separate file to be used as a fitting template in the docking experiments. Residues with missing heavy atoms were removed; the final enzyme contained residues 47−71, 77−149, 152−263, and 281−374. Hydrogens were added by the Edit module of the AMBER 4.1 software, and the energy of the system was relaxed until the energy gradient dropped below 0.01 kJ/mol·Å. During this minimization the non-hydrogen atoms were kept fixed at their X-ray positions by a harmonic potential with a force constant of 5000 kcal/mol. This final structure was used as a starting model in the docking experiments.

**Docking Experiments.** During the docking experiments the following interactions between **4**, **6,** and the viral TK were respected: (1) the base moiety is anchored by double hydrogen bonds to Gln-125; (2) the 5′-OH group is hydrogen bonding to Arg-163 to keep it in the right position for phosphorylation.

In a first simulation the pseudo-equatorial and pseudo-axial conformations of molecule **4** were fit on the penciclovir guanine base coordinates using the Carnal module of AMBER. Those structures were docked in the active site of thymidine kinase. The compound with an axial base conformation could not be accommodated in the active site because (1) position of the primary hydroxyl (equivalent of O5′ in a natural nucleoside) is not close to Arg-163 and (2) major steric clashes. This steric hindrance in the axial conformation could not be removed by turning the dihedral angle C6′−C1′−N9−C4. This procedure was repeated for molecule **6**, leading to the same conclusion.

The final docking experiments were done as follows. The guanine base in the equatorial conformation of molecule **4** was fitted onto the base of the penciclovir molecule using Carnal. The resulting molecule was docked in the active site of the

prepared thymidine kinase structure. The dihedral angle C6′−C1′−N9−C4 was rotated manually to make the primary hydroxyl close to Arg-163. The complex was energy-minimized in the Sander module. The substrate and residues with atoms closer than 5 Å to the original penciclovir substrate were allowed to move free; all others were fixed to their initial positions (X-ray coordinates for heavy atoms, built coordinates for hydrogens) by a force constant of 5000 kcal/mol. Three additional distance restraints were added: two for the double hydrogen bond from the guanine base to Gln-125 (atoms O6, H1A−HE21, OE1, respectively) and one hydrogen bond from the hydroxymethyl group to Arg-163 atom HH21 (force constant = 100 kcal/mol). After the whole system was relaxed, the minimization was continued but now the distance restraints were removed. This procedure was repeated for structure **6**. The final structures are shown in Figure 4.

**Conformation.** Both ligands fit well into the active site. The molecules **4** and **6** have a pseudo-equatorial conformation when bound to the TK enzyme. Both molecules adapt the syn conformation, the torsion angle $\chi$[C6′−C1′−N9−C4] is 18.8° in **4** and −88.5° (or 271.5°) for molecule **6**. Superimposing the 1KI3 structure, having penciclovir in the active site, onto the structure with the predicted binding orientations of structures **4** and **6** reveals that the guanine base is located in the same position and orientation in the three complexes.

**Hydrogen bonds:** The guanine bases of the cyclohexenyl nucleosides are at the expected location making a double hydrogen bond from H1/N1 and O6 to Gln-125. The Gln-125 $NH_2$ group is slightly rotated so that a double hydrogen bond to O6 becomes possible. O6 is also involved in a hydrogen bond to Arg-176 HE (hydrogen of NE). A hydrogen bond exists from $H_2A$ (hydrogen of base nitrogen N2) to Gln-125 OE1. The base nitrogen N7 is making two hydrogen bonds to Arg-176 HH21 (hydrogen of $NH_2$) and HE (hydrogen of NE) in the case of **4**. In **6** only one hydrogen bond exists to HH21. The 'sugar' in **4** and **6** has similar hydrogen bonds: one hydrogen bond from the primary hydroxyl group to Arg-163 HH21 and another from 3′-OH to Glu-225 OE2. The Tyr-101 OH group is out of reach of the sugar 3′-OH. Instead, it is hydrogen bonding to the Glu-225 OE1 atom.

**Energies:** From the hydrogen bond pattern it is not very clear if there is a difference in binding strength between molecules **4** and **6**. Therefore the interaction energy between ligands and neighboring residues in the active site was studied in detail using the Anal module of AMBER. The results are given in Table 3. The interaction of the active site with the base moiety is the same for molecules **4** and **6**. The sugar in **4** is more stabilized by the binding to thymidine kinase than **6**. The main contribution to this stabilization energy is coming from the closer contact of the 3′-OH group of **4** to Glu-225 than in the complex with **6** and hence results in a better interaction. While the stacking of the guanine base with Tyr-172 is, like in the penciclovir and acyclovir complexes, 1KI3 and 2KI5 pdb entries, not as good as in the deoxythymidine complexes (1KIM entry), there is clearly a stabilizing contribution (−8 kcal/mol) from this interaction for the binding of D- and L-cyclohexenyl-G.

**Antiviral Activity Assays.** The antiviral assays were based on an inhibition of virus-induced cytopathicity in either $E_6$SM, HeLa, Vero, or HEL cell cultures, following previously established procedures.[24,25] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required 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 of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

**Cytotoxicity Assays.** The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology ($E_6$SM, HeLa, Vero) or inhibition of normal cell growth (HEL), as previously described.[37]

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