Synthesis and Antiviral Evaluation of Cis-Substituted Cyclohexenyl and Cyclohexanyl Nucleosides

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Received July 28, 2004

Starting from commercially available (*rac*)-3-cyclohexene-1-carboxylic acid, a series of purine and pyrimidine cis-substituted cyclohexenyl and cyclohexanyl nucleosides were synthesized through a key Mitsunobu reaction. Antiviral evaluations were performed on HIV, coxsackie B3, and herpes viruses (HSV-1, HSV-2, VZV, HCMV). Three compounds showed moderate activity against HSV-1 and coxsackie viruses. Specific computer modeling studies were performed on HSV-1 thymidine kinase in order to understand the enzyme activation of an analogue showing moderate antiviral activity.

Introduction

Nucleoside analogues play a major role in antiviral chemotherapy. Although interest in the design of these analogues have relatively decreased during the past few years, the emergence of resistance to currently FDA-approved drugs[1] has generated new interest for the search of new active nucleoside analogues. Despite the fact that the carbocyclic analogues has led to the discovery of carbovir[2] as anti-HIV agent, less efforts have been directed toward the synthesis of six-membered carbocyclic analogues.[3] However, Herdewijn et al.[4] have described D- and L-5-hydroxy-4-hydroxymethyl-2-cyclohexenylguanine (Figure 1) which selectively showed potent and selective anti-herpes virus activity (HSV-1, HSV-2, VZV, CMV).

The rationale of these findings stems mostly from the fact that the cyclohexene ring can be considered as a (bio)isostere of a saturated furanose ring.[5] Cyclohexene nucleosides analogues can also confer protection from resistance to hydrolysis, since glycosidic bond cleavage is a frequently encountered degradative pathway of nucleoside antivirals, particularly for the 2′,3′-dideoxy-nucleosides.[6] Moreover, the conformational flexibility of these cyclohexene derivatives proved to be important for antiviral activity.[7]

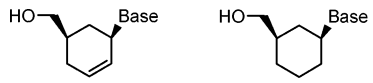
The aim of this article is to describe the synthesis and antiviral evaluation of six-membered nucleoside containing cyclohexenyl and cyclohexanyl sugars (Figure 2).

Chemistry

The known allylic alcohol derivative **7** (Scheme 1) is a requisite for the Mitsunobu coupling reaction of various nucleoside bases.[8] Iodolactonization of the commercially available precursor **1** (*rac*)-3-cyclohexene-1-carboxylic acid, followed by elimination of the iodide **2**

D-cyclohexenyl G L-cyclohexenyl G

Figure 1. D- and L-Cyclohexenylguanine nucleosides.



(±)-*cis* **I** (±)-*cis* **II**

Base: cytosin-1-yl **(I,II)**, thymin-1-yl **(I,II)** adenin-9-yl **(I,II)**, guanin-9-yl **(I** only**)**

Figure 2. General structure of cyclohexenyl and cyclohexanyl nucleosides.

using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), afforded the unsaturated lactone **3** in quantitative yield.[9] Reduction of **3** with lithium aluminum hydride, followed by the protection of the primary alcohol function of **4**, provided **5** in 58% yield from compound **3**. The preparation of the trans derivative **6** was accomplished using a Mitsunobu-type reaction on the allylic alcohol **5**. Thus, the introduction of a benzoyl protective group allowed an inversion of configuration.[10] The allylic alcohol **5** was reacted with benzoic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (PPh_3) in dry THF to give **6**. Alkaline hydrolysis of **6** afforded in 93% yield the trans allylic alcohol **7**. It should be noted that partial hydrolysis was achieved with ammonia solution. The use of sodium hydroxide was necessary to complete hydrolysis.

The corresponding *cis*-cyclohexenyl nucleosides were obtained by use of a second Mitsunobu-type reaction between the allylic alcohol **7** and pyrimidine and purine bases.[11] The synthesis of the cytosin-1-yl (**10**), thymin-1-yl (**13**), adenin-9-yl (**17**), and guanin-9-yl (**19**) derivatives are illustrated in Schemes 2 and 3.

Condensation of the common intermediate, allylic alcohol **7**, respectively with N^4-benzoylcytosine and N^3-benzoylthymine[12] in the presence of DEAD and triphenylphosphine in THF gave the cis racemic cytosine

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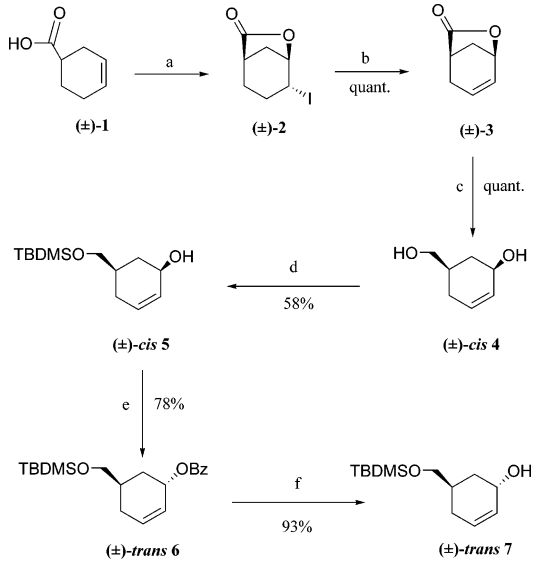
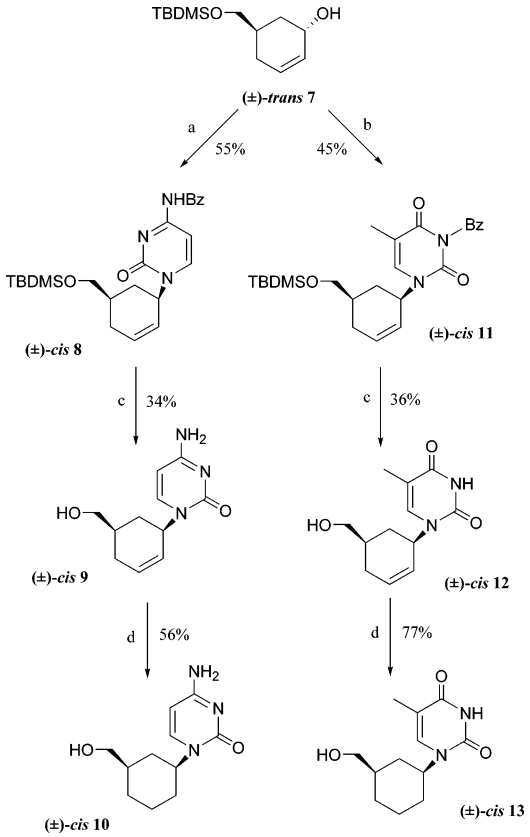
10.1021/jm0493966 CCC: $30.25
Published on Web 12/23/2004

Scheme 1[a]



[a] Reagents and conditions: (a) aq $NaHCO_3$. then aq KI/I_2; (b) DBU, toluene reflux 10 h; (c) $AlLiH_4$, THF, rt, 2 h ; (d) TBDMSCl, imidazole, DMF, rt, 5 h; (e) DEAD, PPh_3, BzOH, THF rt, 5 h ; (f) NH_3/MeOH, NaOH 2 N, rt, 3 h.

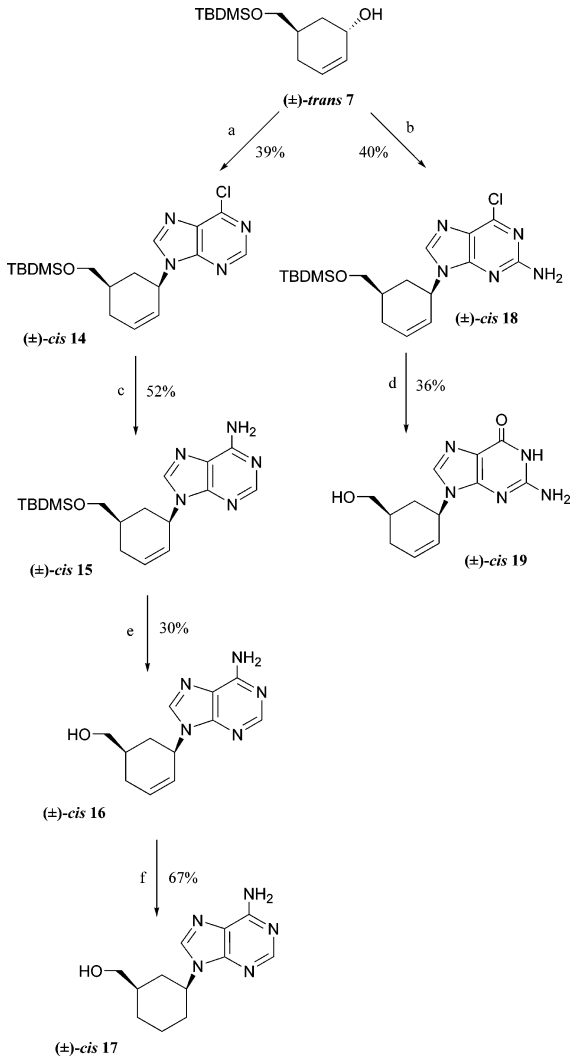
Scheme 2[a]



[a] Reagents and conditions: (a) N^4-benzoylcytosine, DEAD, PPh_3, THF, rt, 18 h; (b) N^3-benzoylthymine, DEAD, PPh_3, THF, rt, 18 h; (c) TBAF, THF, 3 h then sat. NH_3/MeOH, 20 h; (d) 10% Pd/C, H_2, EtOAc, 24 h.

and thymine derivatives **8** and **11** in 55% and 45% yield (Scheme 2). Compounds **8** and **11** were converted to compounds **9** and **12** by treatment with TBAF in THF followed by a saturated ammonia solution in MeOH. The overall yields, starting from **8** and **11**, were 34% and 36% respectively. Hydrogenation[13] of compounds **9** and

Scheme 3[a]



[a] Reagents and conditions: (a) 6-chloropurine, DEAD, PPh_3, THF, rt, 18 h; (b) 2-amino-6-chloropurine, DEAD, PPh_3, dioxane, rt, 48 h; (c) NH_3/MeOH, 80 °C, 24 h; (d) TFA/H_2O (3/1), rt, 72 h; (e) TBAF, THF, 4 h; (f) 10% Pd/C, H_2, EtOH/EtOAc, rt, 24 h.

12 in EtOAc over 10% palladium on carbon gave the saturated cytosine derivative **10** and the saturated thymine derivative **13** in 56% and 77% yield, respectively. It should be noted that hydrogenation in methanol or ethanol rapidly led to a rapid $C_{1'}$–N cleavage.

Using the same conditions, the Mitsunobu-type reaction on the allylic alcohol **7** with 6-chloropurine and 2-amino-6-chloropurine gave the protected 6-chloropurine derivatives **14** and **18** in 39% and 40% yield, respectively (Scheme 3).[14]

Construction of the adenine ring from **14** was accomplished by treatment with methanolic ammonia in a sealed reaction vessel for 1 day to give **15** in 52% yield. The material was converted to pure adenine-cyclohexene nucleoside **16** by treatment with TBAF in THF. The 2-amino-6-chloropurine derivative **18** was converted to the guanine cyclohexene nucleoside **19** by treatment with TFA-H_2O (3:1). Under these conditions, the TBDMS protecting group was simultaneously removed with an overall yield of 36%. Hydrogenation of compound **16** over palladium on activated carbon gave the saturated adenine derivative **17** in 67% yield. However, hydrogenation of compound **19** either in methanol or

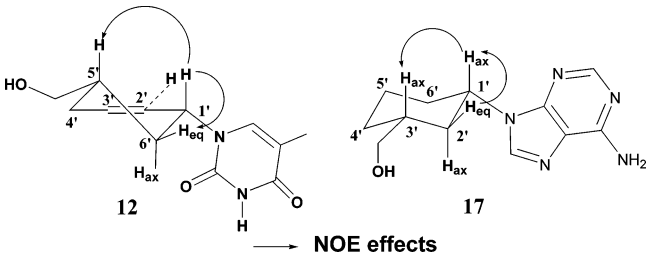


Figure 3. NOE effects on cyclohexenyl and cyclohexanyl derivatives.

ethanol led to decomposition whereas the use of neat EtOAc was unsuitable since **19** was insoluble in this solvent. Attempts using a mixture of EtOH/EtOAc were ineffective too.

Conformational Study

The configuration of the synthesized cis-substituted cyclohexenyl and cyclohexanyl nucleosides **9**, **10**, **12**, **13**, **16**, **17**, and **19** were confirmed by 1D and 2D ^{1}H NMR spectroscopy. Data are given for each compound in the Experimental Section. A detailed ^{1}H NMR conformational study was carried out on compounds **12** and **17** in DMSO-d_6 at room temperature.

For compound **12** the H-2′ resonance at $\delta = 5.49$ ppm appears as a doublet with one large coupling constant $J_{2'-3'} = 9.94$ Hz.

The absence of coupling between H-1′ and H-2′ indicates, according to the Karplus equation, that the dihedral angle between C-1′−H-1′ and C-2′−H-2′ is close to 90° (involving a quasi-null constant of coupling). That is possible only if proton H-1′ assumes a pseudoaxial position with the thymine base in a pseudoequatorial orientation. Moreover, the proton H-6′$_{ax}$ at $\delta = 1.27$ ppm represents a ddd which appears as a broad quadruplet because the coupling constant $J_{6'ax-6'eq}$ is equivalent to the constant $J_{6'ax-1'} \cong J_{6'ax-5'} = 11.6$ Hz. These data suggest a pseudoaxial position of H-1′ and H-5′. Therefore, it can be concluded that compound **12** exists predominantly in a pseudo-chair conformation with the thymine base and the hydroxymethyl group in pseudoequatorial positions. These conclusions are further supported by examination of 2D ^{1}H−^{1}H NOESY spectra. Strong NOE interactions were observed between H-1′, H-5′, and H-6′$_{eq}$ suggesting the cis configuration of the nucleosides **12** (Figure 3).

For compound **17**, the H-1′ resonance at $\delta = 3.69$ ppm appears as a triplet of triplets with coupling constants: $J_{1'-2'ax} \cong J_{1'-6'ax} = 12.15$ Hz and $J_{1'-2'eq} \cong J_{1'-6'eq} = 3.6$ Hz. Altogether, these coupling constants confirm that H-1′ occupies an axial position in a chair conformation. As previously mentioned, H-2′$_{ax}$ at $\delta = 0.81$ represents a ddd which appears as broad quadruplet resulting from three approximately equal coupling constants $J_{2'ax-2'eq} \cong J_{2'ax-1'} \cong J_{2'ax-3'} = 12.1$ Hz. These data suggest a chair conformation with the heterocyclic moiety and the hydroxymethyl group in equatorial orientations. Strong NOE interactions were observed between H-1′, H-2′$_{eq}$. and H-3′, and no correlation appears between H-1′ and H-2′$_{ax}$ (Figure 3). These findings definitively exclude any boatlike conformation.

In summary, the values of vicinal H,H coupling constants and NOE measurements lead to the conclusion that both **12** and **17** are in a chair conformation with the bases and the hydroxymethyl group orientated in equatorial position (cis configuration). Similar results were obtained for compounds **9**, **10**, **13**, **16**, and **19**.

Table 1. Antiviral Activity of Cyclohexenyl and Cyclohexanyl Nucleosides in Comparison with Standard and Approved Antiviral Drugs: Effective Concentration or Concentration Required To Inhibit 50% of Virus-Induced Cytopathicity (EC_{50}, μM)[a]

compd	Coxsackie B3 (Vero cells)	HSV-1 Kos (Hel cells)
(±)-cis **9**	>200	>200
(±)-cis **10**	>200	62
(±)-cis **12**	>200	63
(±)-cis **13**	168	>200
(±)-cis **16**	>200	>200
(±)-cis **17**	>200	>200
(±)-cis **19**	>200	>200
DPN	5.84	n.d.
acyclovir	n.d.	0.32
gancyclovir	n.d.	0.06

[a] n.d. = Not determined. DPN: 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile.

Results and Discussion

Antiviral Activity. Evaluation of antiviral activity was done as previously described.[15] The target compounds were evaluated against HIV-1 and HIV-2, herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), vaccinia virus, coxsackie B3 virus in Vero cells, and human cytomegalovirus in Hel cells. No significant cytotoxicities were reported for any of the compounds. None of these compounds were active against any of the tested viruses except for coxsackie and herpes simplex virus type 1. Compound **13** displayed a moderate activity against coxsackie whereas **10** and **12** displayed moderate activity against wild-type HSV-1. The weak but consistent activity of these compounds against HSV-1 is encouraging and may suggest that these new nucleosides are metabolized to their 5′-triphosphates or as such recognized by the viral DNA polymerases. As intracellular phosphorylation in the virus-infected cells is postulated to be an important step in the enzymatic activation of these nucleosides, we carried out molecular modeling studies using compound **12** and HSV-1 thymidine kinase.

Molecular Modeling. Molecular modeling was performed on thymidine cyclohexenyl derivative **12** in the HSV-1 thymidine kinase (TK) active site, using "GenMol" (www.3dgenoscience.com) software with its all atoms force field, from the X-ray crystal structure of wild-type binary complex TK_{HSV-1}/5-iodouridine anhydrohexitol (AHIU) derivative (PDB ID: 1KI6).[16] HSV-1 thymidine kinase (TK_{HSV-1}) activates antiviral nucleosides such as acyclovir to the 5′-monophosphate. Further phosphorylation by cellular kinases yields the 5′-triphosphate that is a selective inhibitor of the viral polymerase. Phosphorylation of compound **12** by TK_{HSV-1} may be a prerequisite for antiviral activity. We therefore carried out a molecular modeling study.

The crystal structure of HSV-1 thymidine kinase complexed with a iodouridine anhydrohexitol derivative and dT was used as a model for this molecular modeling study (Figure 4). Indeed, since compound **12** is a six membered ring, it seemed reasonable to use a TK_{HSV-1} crystal structure with a six membered ring. The TK_{HSV-1} active site was then adapted to this type of ribose

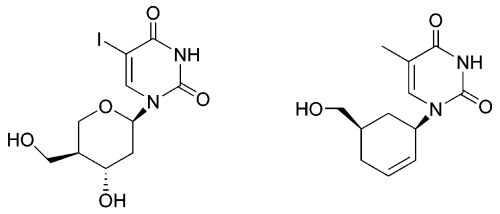


Figure 4. Structures of AHIU and compound 12 used for the molecular modeling study.

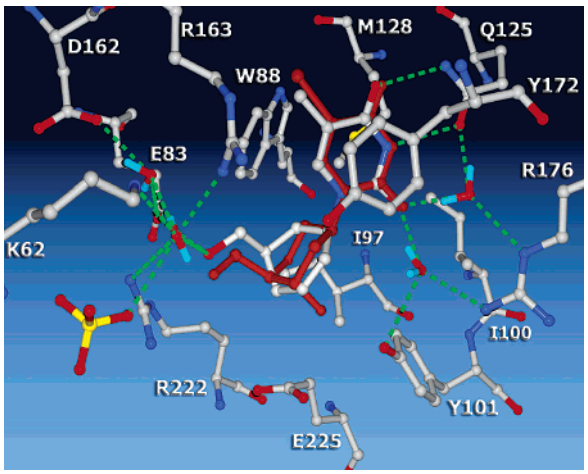


Figure 5. Molecular modeling of compound **12** and AHIU in the TK_{HSV-1} active site.

mimicking ring. Compound **12** binds in the deep pocket of the TK active site and is stabilized by hydrophobic van der Waals interactions and by direct or indirect hydrogen bonds through structural water molecules.

The active conformation of compound **12** in the TK_{HSV-1} shows a thymidine base with a pseudoequatorial orientation which is consistent with the iodouridine conformation of AHIU in TK_{HSV-1} structure (Figure 5). Moreover, the hydroxymethyl moiety of compound **12** is in an axial conformation allowing close interactions in the 5′OH binding site of TK_{HSV-1}: Glu-83, Arg-222, Arg-163 (Figure 5).

The molecular model of compound **12** is superimposed with the minimized AHIU-TK_{HSV-1} crystal structure (PDB ID: 1KI6).[16] Nucleoside analogue AHIU is displayed in brown and the model of **12** in the TK_{HSV-1} active site in atom type. Water molecules are shown with its hydrogen (oxygen in red and hydrogen in cyan). For clarity, only the hydrogen bonds between **12** and the TK_{HSV-1} active site are shown (green dotted line). This picture was generated with Swiss-PdbViewer and rendered with PovRay 3.5.

Thus, compound **12** adopts a half chair conformation and allows this compound to mimic the active South 3′exo conformation for the natural nucleoside substrates of TK_{HSV-1} such as dT (PDB ID: 1KIM,[16] Figure 6). The 5′OH hydroxymethyl of compound **12** is hydrogen bonded to Glu-83 and via a water molecule to Lys 62, Arg 163, Arg 222, and the sulfate anion that is located in a phosphate binding loop, residues 59 g 63 (GMGKT) corresponding to the phosphate binding site of the ATP cofactor. A second water molecule allows to extend the hydrogen bond network to Asp-162 (Figure 5). Compound **12** is a dideoxynucleoside analogue (ddN) and

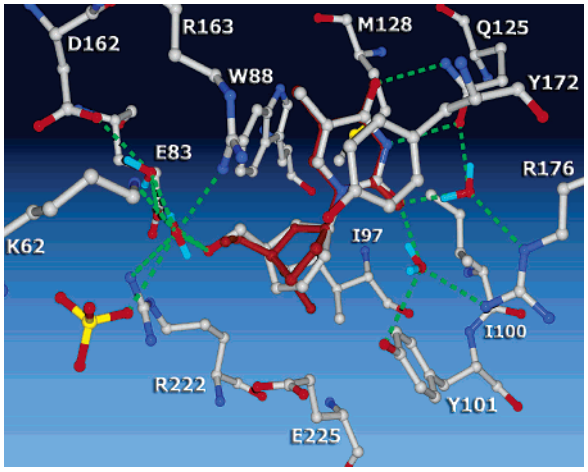


Figure 6. Molecular modeling of compound **12** and dT in the TK_{HSV-1} active site.

lacks the hydrogen bond network with the 3′OH TK_{HSV-1} binding pocket.

The molecular model of compound **12** is superimposed with the minimized 2′-deoxythymidine-TK_{HSV-1} crystal structure (PDB ID: 1KIM).[16] Natural nucleoside substrate dT of TK_{HSV-1} is displayed in brown and the model of **12** in the TK_{HSV-1} active site in atom type. Water molecules are shown with its hydrogen (oxygen in red and hydrogen in cyan). For clarity, only the hydrogen bonds between **12** and TK_{HSV-1} active site are shown (green dotted line).

This picture were generated with Swiss-PdbViewer and rendered with PovRay 3.5.

In the case of AHIU (Figure 5), its 3′OH makes hydrogen bonds with Tyr-101 and Glu-225. These specific interactions increase the binding affinity as well as the stability of the complex with TK_{HSV-1}. However, the cyclohexenyl of TK_{HSV-1} ring has fine geometrical complementarities with the ribose ring binding pocket. The cyclohexenyl ring provides favorable hydrophobic van der Waals interactions with the hydrophobic pocket of TK_{HSV-1} shaped by the side chain of hydrophobic residue Trp-88, Met-85, Ile-97 and alkyl side chain of Arg-222, as previously described (Figures 5 and 6).[17] The thymine moiety of compound **12** undergoes π-stacking interactions with Tyr-172 and van der Waals contacts with Met-128 as well as Ile-100. Structurally, thymine, which is sandwiched between Tyr-172 and Met-128 (Figure 5) allows a fine orientation of the nucleobase for the Watson−Crick-like hydrogen bonds to Gln-125. Thymine is strongly hydrogen bonded to N3 and O4 with Oϵ1 and Nϵ2 of Gln-125 respectively (Figure 4). O2 thymine nucleobase as well provides three additional water-mediated hydrogen bonds with Arg-176, Oζ Tyr-101, and Oϵ1 of Gln-125. This strong hydrogen bond network on thymine affords an excellent anchorage point for compound **12** as well as a fine orientation in regard with Gln-125 for the Watson−Crick-like interactions. The existence of these specific interactions allows compound **12** to have favorable interaction with the nucleoside binding site of TK_{HSV-1} and a correct orientation of its 5′OH for the phosphorylation with ATP cofactor. The relatively weak activity of compound **12** could be explained by the loss of 3′OH interactions with residue Glu-225 and Tyr-101 of TK_{HSV-1} 3′OH binding pocket (Figures 5 and 6). Moreover, the 1,3 substitution

of cyclohexenyl ring requires a half chair conformation to mimic the south 3′OH exo active conformation of natural nucleoside substrates for TK_{HSV-1}. Therefore, this energy consuming half chair conformation decreases the stability of the active complex **12**/TK_{HSV-1} in regard with the phosphorylation.

In summary, the syntheses of cyclohexenyl and cyclohexyl nucleoside derivatives were successfully achieved. Conformational studies, as well as molecular modeling performed on thymidine cyclohexenyl derivative **12** in the HSV-1 thymidine kinase (TK) active site, allowed us to understand the observed moderate antiviral activity. These results will be taken into account in order to optimize the encouraging activity profile of compound **12**.

Experimental Section

Molecular Modeling. The molecular modeling was performed with GenMol (www.3dgenoscience.com) with its all atoms force field, from the X-ray crystal structure of wild-type binary complex TK_{HSV-1}/AHIU derivative (PDB ID: 1KI6). The first entity (A) of the TK_{HSV-1}/AHIU crystal structure in the asymmetric crystal unit was used for the molecular modeling study. All crystallographic water molecules corresponding to the first entity (A) were kept. All hydrogen atoms were added to the enzyme and water from the Biopolymer module of GenMol. Hydrogen positions were then optimized. Energy refinements were done in a vacuum with a sigmoid distance-dependent dielectric model.[18] The construction of the nucleoside analogue **12** was based on X-ray conformation of AHIU in the crystal structure (1KI6).[16] All atom charges were computed with GenMol for TK, nucleoside, water molecules, and phosphate. Finally, compound **12** was docked into TK_{HSV-1} active site from the positioning of AHIU in the X-ray structure binary complex 1KI6. The resulting binary complex was then optimized using GenMol all atom force field with a gradient of energy up to 0.01 $kcal.mol^{-1}Å^{-1}$.

Chemistry. General Methods. Melting points were determined in capillary tubes with a 9100 Electrothermal (Fisher Scientific) apparatus and are uncorrected. The ^1H NMR and ^{13}C NMR spectra were determined with a BRUKER AMX 200 MHz and referenced to the solvent. Chemical shifts are expressed in ppm and coupling constants (*J*) are in hertz (*s* = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, dt = double triplet, tt = triple triplet, m = multiplet, dm = double multiplet, triple multiplet). FAB^+ mass spectra (MS) were obtained on a JEOL DX-100 mass spectrometer (Laboratoire de Mesures Physiques RMN, Dr. Astier, USTL, Montpellier, France) using a cesium ion source and a glycerol−thioglycerol (GT) matrix. Elemental microanalyses were determined by Service Central d'Analyse CNRS Vernaison-Lyon France and gave combustion values for C, H, N within 0.4% of the theoretical values. Preparative flash column chromatographies were performed using silica gel (Merck) G60 230−240 mesh. Analytical thin layer chromatographies were performed on silica gel 60F 254 aluminum plates (Merck) of 0.2 mm thickness. The spots were examined with UV light and Ceric Dip spray. Preparative TLC was done on glass plates EM Science silica gel 60F 254 (1.0 mm or 2.0 mm layer).

(±)-4-Iodo-6-oxabicyclo[3.2.1]octan-7-one (2). 3-Cyclohexene-1-carboxylic acid **1** (5 g, 39.7 mmol) was added to a solution of $NaHCO_3$ (9.95 g, 119 mmol) in water (96 mL), and the resulting mixture was stirred until it became homogeneous. The flask was then protected from light, and the mixture was treated in one portion with a solution of KI (39.50 g, 238 mmol) and iodine (10.57 g, 41.7 mmol) in water (96 mL). The reaction mixture was stirred at room temperature for 16 h and then extracted with $CHCl_3$. The organic extracts were combined, washed with 10% aqueous $Na_2S_2O_3$, 10% aqueous $NaHCO_3$, and water, and then dried ($MgSO_4$). Removal of the solvent in vacuo yielded **2** (9.60 g, 97%) as a yellow solid: mp 133 °C; MS (GT, FAB^+): 125 $(M - I)^+$, 253 $(M + 1H)^+$.

(±)-6-Oxabicyclo[3.2.1]oct-3-en-7-one (3). Iodolactone **2** (13.6 g, 54 mmol) was dissolved in dry toluene (120 mL) containing 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (12.48 g, 81 mmol), the mixture was heated at reflux for 6 h, cooled, and filtered, and the filtrate was concentrated under reduced pressure to give an oil. This crude oil was directly used in the next step.

(±)-*cis*-5-(Hydroxymethyl)-2-cyclohexen-1-ol (4). The crude lactone **3** (6.7 g, 54 mmol) was dissolved in dry THF (200 mL) and added dropwise to $LiAlH_4$ (3.07 g, 81 mmol) dissolved in dry THF (200 mL) at 0 °C. A white precipitate formed. After 1 h, water (6.7 mL), 15% aqueous NaOH (6.7 mL), and water (20 mL) were added sequentially, and the mixture was allowed to warm to room temperature with stirring over several hours. The salts were removed by filtration, and the solution was dried over $MgSO_4$ and concentrated at room temperature. Recrystallization from cyclohexane/acetone gave **4** (5.4 g, quantitative) as white solid: mp 82 °C.

(±)-*cis*-5-[(*tert*-Butyldimethylsilyloxy)methyl]-2-cyclohexen-1-ol (5). Diol **4** (2 g, 15.6 mmol) was dissolved in dry DMF (30 mL). Imidazole (2.12 g, 31 mmol) was added and dissolved. The solution was cooled to 0 °C, *tert*-butyldimethylsilyl chloride (2.35 g, 15.6 mmol, in 5 mL DMF) was added, and the reaction mixture was allowed to warm to room temperature and stirred for 10 h. Brine was added and the solution extracted with ether and washed with water. $MgSO_4$ was added, and the solution was filtered and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (dichloromethane) to give **5** (2.2 g, 58%).

(±)-*trans*-1-Benzoyloxy-5-[(*tert*-butyldimethylsilyl-oxy)-methyl]-2-cyclohexene (6). A solution of diethyl azodicarboxylate (DEAD) (2.86 mL, 18.14 mmol) in THF (10 mL) was added dropwise to a stirred solution of the alcohol **5** (2.2 g, 9.07 mmol), triphenylphosphine (4.76 g, 18.14 mmol), and benzoic acid (2.21 g, 18.14 mmol) in THF (40 mL) at room temperature. After 14 h the mixture was partitioned between chloroform (200 mL) and saturated aqueous sodium hydrogen carbonate (200 mL). The aqueous layer was further extracted with chloroform, and the combined organic extracts were dried, filtered, and evaporated to give a white residue. This latter was purified by flash chromatography (dichloromethane) to give **6** (2.6 g, 78%). MS (GT, FAB^+): 225 $(M - OBz)^+$; 347 $(M + 1H)^+$.

(±)-*trans*-5-[(*tert*-Butyldimethylsilyl)oxy]methyl]-2-cyclohexen-1-ol (7). Compound **6** (2.2 g, 9.07 mmol) was dissolved in THF (8 mL). A saturated ammonia solution in MeOH (10 mL) and a solution of NaOH 2N (15 mL) were added at room temperature. The mixture was stirred for 3 h until no starting material appears on TLC. It was then evaporated, and the residue was partitioned between chloroform and water. The aqueous layer was further extracted with chloroform, and the combined organic extracts were dried over $MgSO_4$. The residue was purified by flash chromatography (dichloromethane/MeOH: 9.5/0.5) to afford **7** (1.35 g, 93%). MS (GT, FAB^+): 109 $(M - TBDMSiO)^+$; 154 $(M - tBu)^+$; 225 $(M - OH)^+$; 243 $(M + 1H)^+$.

***cis*-(±)-1-{5′-[(*tert* Butyldimethylsilyloxy)methyl]-2′-cyclohexenyl}*N*⁴-benzoylcytosine (8).** DEAD (2.16 mL, 13.77 mmol) dissolved in freshly distilled THF (15 mL) was added dropwise over 5 min to a 0 °C stirred suspension of **7** (1.67 g, 6.88 mmol), N^4-benzoylcytosine (2.96 g, 13.77 mmol), and triphenylphosphine (3.61 g, 13.77 mmol) in anhydrous THF (50 mL) under argon. The mixture was stirred for 3 h at 0 °C and then at room temperature for 20 h. Evaporation of the volatiles and purification by flash chromatography (10−15% EtOAc/cyclohexane) yielded **8** (1.89 g, 60%). MS (GT, FAB^+): 216 $(BBz + 1H)^+$; 382 $(M - tBu)^+$; 440 $(M + 1H)^+$.

***cis*-(±)-1-[5′-(Hydroxymethyl)-2′-cyclohexenyl]cytosine (9).** Compound **8** (595 mg, 1.05 mmol) dissolved in dry THF (7 mL) was stirred with a 1 M solution of TBAF (1.58 mL, 1.58 mmoL) for 4 h at room temperature. The solution

was then evaporated and the residue treated with a saturated ammonia solution in MeOH (10 mL) for 24 h. Evaporation of the volatiles and purification by flash chromatography (EtOAc) yielded **9** (80 mg, 34%): mp 167−168 °C. MS (GT, FAB$^+$): 112 (B + 1H)$^+$; 222 (M + 1H)$^+$. Anal. ($C_{11}H_{15}N_3O_2$) C, H, N.

***cis*-(±)-1-[3′-(Hydroxymethyl)cyclohexanyl]cytosine (10).** A suspension of compound **9** (18 mg, 0.08 mmol) and 10% palladium on carbon (8 mg) in EtOAc (5 mL) was hydrogenated under atmospheric pressure for 24 h. The reaction mixture was filtered through Celite and concentrated to afford compound **10** (10 mg, 56%): mp 166 °C. MS (GT, FAB+): 112 (B + 1H)$^+$; 224 (M + 1H)$^+$. Anal. ($C_{11}H_{17}N_3O_2$) C, H, N.

***cis*-(±)-1-{5′-[(*tert*-Butyldimethylsilyloxy)methyl]-2′-cyclohexenyl}-*N*^{3}-benzoylthymine (11).** DEAD (571 μL, 3.63 mmol) dissolved in anhydrous dioxane (5 mL) was added dropwise over 15 min to a 0 °C stirred suspension of **7** (440 mg, 1.81 mmol), *N*^{3}-benzoylthymine (836 mg, 3.63 mmol), and triphenylphosphine (952 mg, 3.63 mmol) in anhydrous dioxane (20 mL) under argon. The mixture was stirred for 3 h at 0 °C and then at room temperature for 30 h. Evaporation of the volatiles and purification by flash chromatography (10−20% EtOAc/cyclohexane) yielded **11** (355 mg, 45%).

***cis*-(±)-1-[5′-(Hydroxymethyl)-2′-cyclohexenyl]thymine (12).** Compound **11** (350 mg, 0.77 mmol) dissolved in dry THF (10 mL) was stirred with a 1 M solution of TBAF (1.15 mL, 1.15 mmoL) for 4 h at room temperature. The solution was then evaporated and the residue treated with a saturated ammonia solution in MeOH (10 mL) for 48 h. Evaporation of the volatiles and purification by flash chromatography (dichloromethane/methanol: 98/2) yielded **12** (80 mg, 44%) as a white solid: mp 171−172 °C. MS (GT, FAB$^+$): 127 (B + 1H)$^+$, 237 (M + 1H)$^+$, 473 (2M)$^+$. Anal. ($C_{12}H_{16}N_2O_3$) C, N, H.

***cis*-(±)-1-[3′-(Hydroxymethyl)cyclohexanyl]thymine (13).** A suspension of compound **12** (30 mg, 0.13 mmol) and 10% palladium on carbon (20 mg) in EtOAc (15 mL) was hydrogenated at atmospheric pressure for 24 h. The reaction mixture was filtered through Celite and concentrated to afford compound **13** (24 mg, 77%): mp 171 °C. MS (GT, FAB$^+$): 127 (B + 1H)$^+$, 239 (M + 1H)$^+$, 477 (2M)$^+$. Anal. ($C_{12}H_{18}N_2O_3$) C, H, N.

***cis*-(±)-6-Chloro-9-{5′-[(*tert*butyldimethylsilyloxy)-methyl]-2′-cyclohexenyl}purine (14).** DEAD (904 μL, 1 g, 5.77 mmol) dissolved in freshly distilled THF (10 mL) was added dropwise over 15 min to a 0 °C stirred suspension of **7** (700 mg, 2.88 mmol), 6-chloropurine (892 mg, 5.77 mmol), and triphenylphosphine (1.51 g, 5.77 mmol) in anhydrous THF (30 mL) under argon. The mixture was stirred for 1 h at 0 °C and then at room temperature for 76 h. Evaporation of the volatiles and purification by flash chromatography (40% EtOAc/cyclohexane) yielded **14** (420 mg, 39%): mp 99 °C. MS (GT, FAB$^+$): 155 (B + 1H)$^+$, 379 (M + 1H)$^+$.

***cis*-(±)-9-{5′-[(*tert*-Butyldimethylsilyloxy)methyl]-2′-cyclohexenyl}adenine (15).** Compound **14** (400 mg, 1.055 mmol) was treated with a saturated ammonia solution in MeOH (10 mL). The mixture was heated at 80 °C for 24 h and cooled. Evaporation of the volatiles and purification by flash chromatography (10% MeOH/dichloromethane) yielded **15** (200 mg, 52%). MS (GT, FAB$^+$): 136 (B + 1H)$^+$, 360 (M + 1H)$^+$, 719 (2M)$^+$.

***cis*-(±)-9-[5′-(Hydroxymethyl)-2′-cyclohexenyl]adenine (16).** Compound **15** (200 mg, 0.55 mmol) dissolved in dry THF (5 mL) was stirred with a 1 M solution of TBAF (834 μL, 0.834 mmol) for 4 h at room temperature. The solution was then evaporated, and the residue was dissolved in EtOAc, washed with 10% aqueous citric acid, and water, and then dried ($MgSO_4$). Evaporation of the volatiles and purification by flash chromatography (10% MeOH/dichloromethane) yielded **16** (40 mg, 30%): mp 173−174 °C. MS (GT, FAB$^+$): 136 (B + 1H)$^+$, 246 (M + 1H)$^+$, 491 (2M)$^+$. Anal. ($C_{12}H_{15}N_5O$) C, H, N.

***cis*-(±)-9-[3′-(Hydroxymethyl)cyclohexanyl]adenine (17).** A suspension of compound **16** (46 mg, 0.187 mmol) and 10% palladium on carbon (20 mg) in EtOAc (6 mL) and EtOH (6 mL) was hydrogenated under atmospheric pressure for 24 h. The reaction mixture was filtered through Celite and concentrated to afford compound **17** (30 mg, 67%): mp 168 °C. MS (GT, FAB$^+$): 136 (B + 1H)$^+$, 248 (M + 1H)$^+$. Anal. ($C_{12}H_{17}N_5O$) C, H, N.

***cis*-(±)-2-Amino-6-chloro-9-{5′-[(*tert*-butyldimethylsilyl-oxy)methyl]-2′-cyclohexenyl}purine (18).** DEAD (600 μL, 618 g, 3.55 mmol) dissolved in dry dioxane (10 mL) was added dropwise over a period 2 h to a stirred suspension of **7** (430 mg, 1.775 mmol), 2-amino-6-chloropurine (602 mg, 355 mmol), and triphenylphosphine (931 mg, 3.55 mmol) in anhydrous dioxane (30 mL) under argon at room temperature. The mixture was stirred for 48 h. Evaporation of the volatiles and purification by flash chromatography (2% MeOH/dichloromethane) yielded **18** (280 mg, 40%) as a white foam: mp 135 °C. MS (GT, FAB$^+$): 170 (B + 1H)$^+$; 394 (M + 1H)$^+$; 782 (2M + 1H)$^+$.

***cis*-(±)-9-[5′-(Hydroxymethyl)-2′-cyclohexenyl]guanine (19).** Compound **18** (250 mg, 0.634 mmol) was treated with TFA/H_2O (3:1, 8 mL) for 72 h at room temperature. The reaction mixture was then concentrated and coevaporated twice with toluene. The residue was cooled and treated with MeOH/NH_4OH (10:1, 15 mL). Volatiles were removed and the residue was purified by flash chromatography (MeOH/dichloromethane 1:4) yielded **19** (60 mg, 36%): mp 174 °C. MS (GT, FAB$^+$): 262 (M + 1H)$^+$; 562 (2M + 1H)$^+$. Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

Acknowledgment. We wish to thank Dr. Robert Faure (Université Aix-Marseille III, St. Jérôme, Marseille, France) for his expertise in conducting NMR experiments and Mrs. Miette Stuyck, Mrs. Ann Absillis, Mrs. Anita Vanlierde, and Mrs. Frieda De Meyer for excellent technical assistance.

Supporting Information Available: Elemental analysis and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0493966