A Thymidine Phosphorylase-Stable Analogue of BVDU with Significant Antiviral Activity

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Abstract: (*E*)-5-(2-Bromovinyl)isodideoxyuridine (BVisoDDU), synthesized on the basis of molecular modeling, is selectively active against HSV-1 (three different strains) but inactive against HSV-2. Unlike BVDU, BVisoDDU is completely resistant to cleavage by thymidine phosphorylase. BVisoDDU is also the first nucleoside analogue lacking OH groups at both the 2′- and 3′-position that shows pronounced activity against HSV-1 replication.

Introduction. A variety of antiviral agents has been developed for the treatment of herpes simplex virus (HSV) infections.[1,2] Nucleoside analogues, which target the viral DNA polymerase, represent an important class of anti-HSV drugs. These nucleoside inhibitors require phosphorylation by HSV thymidine kinase (TK) to the respective deoxynucleoside triphosphate (DNTP) to express their activity. The active form of these compounds can then decrease viral replication by inhibiting the viral DNA polymerase.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVDU, Figure 1) is a potently active antiviral agent against HSV-1 and varicella zoster virus (VZV).[3] However, BVDU is a good substrate for the human pyrimidine nucleoside phosphorylases (PyNP), such as thymidine phosphorylase (TPase).[4,5,6] It is known that human blood platelets contain TPase, which induces rapid intracellular degradation of certain 5-substituted-2′-deoxy-uridines, including BVDU. The possible degradation of BVDU by PyNP before its conversion to the triphosphate may be a significant limitation of its antiviral potential.

One way to overcome the cellular instability of BVDU is to alter the nature of the glycosidic bond to the nucleobase. We have designed and synthesized an isomeric analogue of BVDU (BVisoDDU, Figure 1) in which the nucleobase was translocated from the natural 1′-position to the isomeric 2′-position. This apparently small structural modification has major enzymological ramifications. For example, isonucleosides are not expected to be substrates for nucleoside phosphorylases.[7]

Computer Modeling. Several preliminary computer modeling experiments were carried out to verify whether BVisoDDU is a suitable candidate as an anti-HSV agent. As cellular phosphorylation of the nucleoside is a requirement for antiviral activity, docking experiments with the critical phosphorylating enzyme, TK, were performed in order to obtain an indication of the probability of anti-HSV activity by BVisoDDU. The viral enzyme, HSV-1 TK, is present in infected target cells and it is significantly less selective than human kinases. If the computational experiments were to suggest that BVisoDDU binds appropriately to HSV-1 TK, then the likelihood of phosphorylation of this compound would be high. No correlation, however, can be made between the ease of phosphorylation(s) and the potential for anti-HSV activity because the target enzyme of BVisoDDUTP is the viral DNA polymerase. However, if the molecular modeling experiments gave evidence that BVisoDDU was not likely to be a substrate of HSV-1 TK, then it would most likely not be active against HSV-1. Other modeling experiments included qualitative comparisons of the electrostatic potential surface (EPS) maps of BVDU and BVisoDDU to determine similarities and differences in polarity and steric volume.

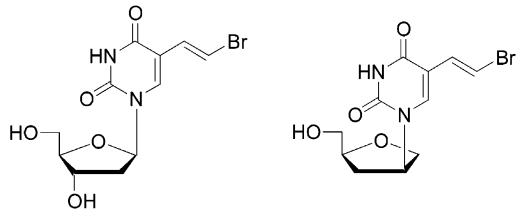

Figure 1. Structures of BVDU and BVisoDDU.

The docking experiments were executed with AutoDock 3.0 which allows automated docking of flexible ligands to an enzyme receptor employing rapid energy evaluations with the use of prior generated grid-based atomic affinity potentials. Appropriate binding positions and conformations of the ligands were determined utilizing the Lamarckian genetic algorithm (LGA). The calculations started with a population of 100 randomly positioned individuals and a maximum number of 27000 generations for the global optimization. Local search was performed using the Pseudo-Solis and Wets method for a maximum number of 3000 steps. Ten independent experiments, each with 100 runs, were carried out for every ligand.

Models of BVDU and BVisoDDU were generated in SYBYL.[8] Minimum energy conformations were calculated using TRIPOS force field and Gasteiger-Marsili charges. The parameter file for AutoGrid 3.0 (distributed with the AutoDock software bundle) was created after assigning Kollman charges to the ligands and merging nonpolar hydrogens and lone pairs. All eligible bonds were defined rotatable (five for BVDU, four for BVisoDDU).

The X-ray crystal structure of TK complexed with BVDU (PDB code: 1ki8) was used as the enzyme template.[9] The protein was set up by removing all water molecules, the BVDU structure, and nonpolar hydrogens. Finally, lone pairs were merged, followed by the addition of Kollman united atom partial charges and solvent parameters. A grid box of 40 Å × 40 Å × 40 Å

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Table 1. Energy Values of the Lowest Total Docked Energy of Each Experiment with the Corresponding Estimated Binding Free Energy Obtained from the BVDU Docking

experiment no.	ΔG_{total}[a] (kcal/mol)	ΔG_{bind}[b] (kcal/mol)
1	−9.48	−9.25
2	−9.11	−8.38
3	−8.58	−7.94
4	−9.26	−8.78
5	−8.49	−8.57
6	−9.46	−8.85
7	−9.26	−9.04
8	−9.01	−8.63
9	−9.46	−9.42
10	−9.47	−9.09

[a] Lowest total docked energy of 100 runs. [b] Estimated free energy of binding (from same run as ΔG_{total}).

Figure 2. Crystal structure of BVDU (yellow) in the active site of TK (enzyme not displayed) superimposed with the structures from the top four docking experiments.

with grid spacing of 0.375 Å was centered around the original location of the bound BVDU. At first, a series of 10 docking experiments was carried out with the BVDU model to determine the value of the subsequent calculations with BVisoDDU. The lowest total docked energy of each experiment is shown in Table 1. The correlating structure of every best fit and the original molecule from the X-ray crystal structure were then superimposed for visual comparison. Figure 2 illustrates how the models of BVDU obtained with AutoDock align very well with the crystal structure with respect to position, orientation, and conformation of the ligands.

Another 10 docking experiments were then executed with BVisoDDU. The results of the run with the overall lowest total docked energy were then used for data evaluation. We found that BVisoDDU aligns similarly to BVDU within the active site of TK. The positions of the base moieties are almost identical. Hence, the sugar moiety of the isomeric nucleoside appears rotated relative to the 2′-deoxyribosyl group of BVDU so that the 2′-C of BVisoDDU assumes a position close to the 1′-C of the natural nucleoside (Figure 3).

Finally, EPS maps of both compounds were rendered after assigning Gasteiger−Hückel charges to the binding BVDU, isolated from the crystal structure complex, and to the lowest total docked energy structure of BVisoDDU. The two molecules show matching regions

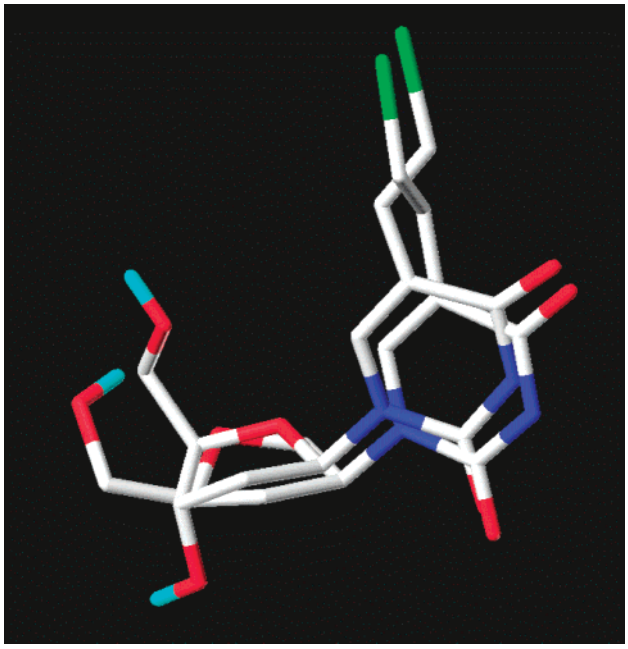


Figure 3. Orientation and conformation of BVisoDDU (overall lowest total docked energy model) and BVDU (X-ray crystal structure) in their bound state within the same coordinate system.

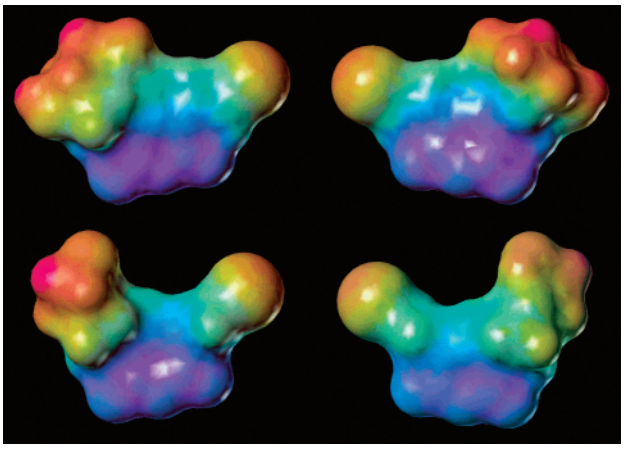


Figure 4. EPS maps of BVDU (front view, top left; back view, top right) and BVisoDDU (front view, bottom left; back view, bottom right). Regions of high electrostatic potential are shown in red and negative potentials in purple/blue.

Table 2. Comparison of the Total Docked Free Energy and Binding Free Energy of the Best Models of BVDU and BVisoDDU

ligand	ΔG_{total} (kcal/mol)	ΔG_{bind} (kcal/mol)
BVDU	−9.48	−9.25
BVisoDDU	−9.15	−8.93

of high and low electrostatic potentials due to their structural semblance (Figure 4).

The results of the computer modeling experiments indicate striking similarities in the modes and strengths of binding between BVDU and BVisoDDU to HSV-1 TK (Table 2). The use of the crystal structure based enzyme template of thymidine kinase complexed with BVDU was assumed to be adequate taking into account the similar steric volumes and EPS maps of the two compounds. As a consequence, no significant changes in the enzyme conformation were expected. Nevertheless, adverse enzyme−ligand interactions caused by the

Table 3. Inhibitory Activity of BVisoDDU and BVDU against Virus Replication in E_6SM Cell Cultures

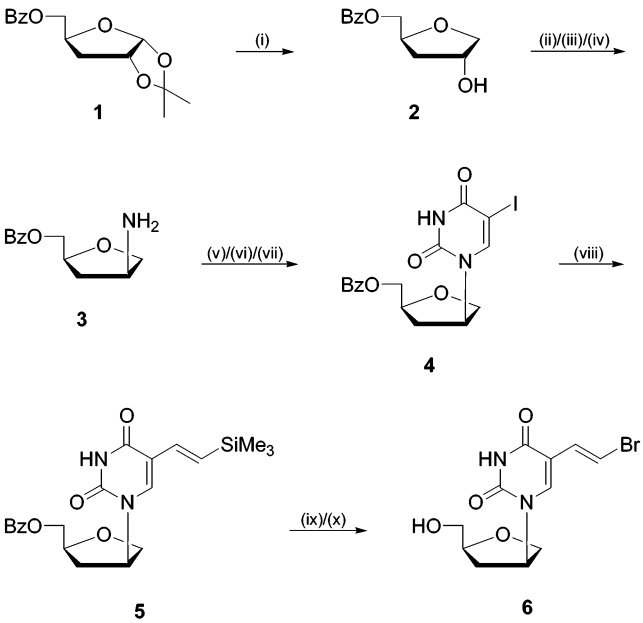
		EC_{50}[b] (μg/mL)								
		HSV-1			HSV-2					
compound	MCC[a] (μg/mL)	KOS	F	McIntyre	G	196	Lyons	VV	VSV	HSV-1 TK⁻ KOS ACV[r]
BVisoDDU	>400	2.6	1.9	9.6	240	240	240	>400	>400	>400
BVDU	>400	0.026	0.026	0.026	240	> 400	240	1.9	>400	240
ribavirin	>400	48	48	240	80	240	240	240	>400	240
acyclovir	>400	0.077	0.077	0.077	0.384	0.384	0.384	>400	>400	48
ganciclovir	>100	0.019	0.019	0.019	0.019	0.032	0.019	>100	>100	2.4

[a] Minimal cytotoxic concentration or compound concentration required to cause a microscopic detectable alteration of the host cells. [b] 50% Effective concentration of compound required to inhibit virus-induced cytopathicity by 50%.

lack of the 2′-OH or by the altered glycosidic bond (BVisoDDU is an L-related analog) would have produced unfavorable binding free energies. Our data, however, showed that even though the total docked energy values for BVisoDDU were slightly more positive than for BVDU, they were within a relatively close range. Thus, the new isomeric nucleoside was considered a potential substrate for phosphorylation by HSV-1 TK.

Synthesis. The 3′-deoxy sugar **1** was prepared from 1,2-*O*-isopropylidine-α-D-xylofuranose in two steps, as previously described by us.[10] Cleavage of the isopropylidine protecting group and deoxygenation of the 1′-C was achieved upon treatment with Et_3SiH and $BF_3{\cdot}Et_2O$ in CH_2Cl_2. The resulting compound **2** was then mesylated with MsCl in CH_2Cl_2/Et_3N, followed by an azide substitution at the 2′-position using NaN_3 in DMF at 70 °C. Reduction with hydrogen in the presence of palladium/carbon catalyst in EtOAc/EtOH produced amino sugar **3**. Subsequent coupling with in situ generated 3-methoxyacryloyl isocyanate (from 3-methoxyacryloyl chloride and silver cyanate) yielded an open chain intermediate. Cyclization was induced in the presence of 2 N H_2SO_4 in dioxane at 100 °C. The resulting isomeric nucleoside was then treated with ICl in CH_2Cl_2 to form the 5-iodo compound **4**. Coupling with tributyl(trimethylsilylvinyl)stannane catalyzed by $(Ph_3P)_2$-Pd(II)Cl_2 in CH_3CN at 50 °C yielded the vinyl intermediate **5**. Treatment of **5** with LiBr and XeF_2 in benzene followed by deprotection with methanolic ammonia resulted in the target molecule, BVisoDDU, **6** (Scheme 1).

Scheme 1. Synthetic Scheme for BVisoDDU[a]



[a] (i)Et_3SiH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (ii) MsCl, CH_2Cl_2, Et_3N; (iii) NaN_3, DMF, 55 °C; (iv) Pd/C, H_2, EtOAc/EtOH; (v) 3-methoxyacryloyl isocyanate, toluene; (vi) dioxane, H_2SO_4, 100 °C; (vii) ICl, CH_2Cl_2, *n*-$Bu_3SnHCCH(SiMe_3)$, $(Ph_3P)_2Pd(II)Cl_2$, CH_3CN, 50 °C; (ix) LiBr, XeF_2, benzene; (x) NH_3/MeOH.

Biological Methods. The antiviral assays were based on inhibition of virus-induced cytopathicity in human embryonic skin-muscle and human embryonic lung [E_6SM (HSV-1, HSV-2, VV, VSV) or HEL (VZV, CMV)] cell cultures. Briefly, confluent cell cultures in 96-well microtiter plates 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 (400, 200 and 100 μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were treated with the test compounds.

Results and Discussion. BVisoDDU inhibited HSV-1 replication at concentrations between 1.9 and 9.6 μg/mL (Table 3). It was not active against HSV-2 strains, vaccinia virus (VV) and vesicular stomatitis virus (VSV) in E_6SM cells, or against cytomegalovirus (CMV, strains Davis and AD-169) and VZV (strains YS and OKA) in HEL cells at concentrations up to 200 μM (data not shown). BVisoDDU was also inactive against an acyclovir-resistant thymidine kinase (TK)-deficient HSV-1 strain (Table 3). This experimental observation suggests that BVisoDDU requires activation (phosphorylation) by the HSV-1 TK before it can exert its anti-HSV-1 activity in cell culture. The initial modeling studies, therefore, correctly predicted that the new isomeric nucleoside would be a substrate of HSV-1 TK. BVisoDDU showed a somewhat similar spectrum of antiviral activity as BVDU, in that both clearly discriminated between HSV-1 and HSV-2. However, BVisoDDU was less efficient than BVDU in inhibiting HSV-1 strains, and it did not show activity against VZV. According to the docking experiments, this disparity in anti-HSV-1 activity cannot be explained exclusively by different phosphorylation efficiencies because both compounds showed relatively similar binding strengths to thymidine kinase. We suggest, therefore, that the difference in activity may be based, at least in part, on the greater inhibitory affect of BVDUTP compared to BVisoDDUTP toward HSV-1 DNA polymerase. Unlike BVDU, BVisoDDU, which lacks a normal glycosidic bond, was completely resistant toward cleavage by thymidine phosphorylase. BVisoDDU is also the first example of a 2′,3′-dideoxynucleoside that shows pronounced activity against HSV-1 replication.

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