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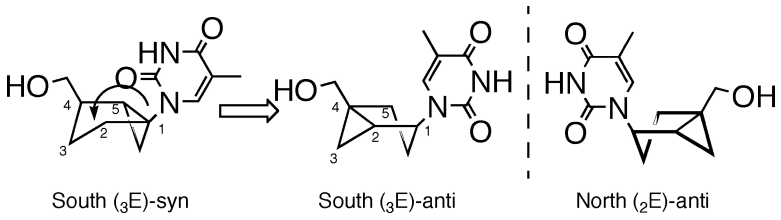

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Understanding How the Herpes Thymidine Kinase Orchestrates Optimal Sugar and Nucleobase Conformations To Accommodate Its Substrate at the Active Site: A Chemical Approach

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Abstract: The herpes virus thymidine kinase (HSV-tk) is a critical enzyme for the activation of anti-HSV nucleosides. However, a successful therapeutic outcome depends not only on the activity of this enzyme but also on the ability of the compound(s) to interact effectively with cellular kinases and with the target viral or cellular DNA polymerases. Herein, we describe the synthesis and study of two nucleoside analogues built on a conformationally locked bicyclo[3.1.0]hexane template designed to investigate the conformational preferences of HSV-tk for the 2′-deoxyribose ring. Intimately associated with the conformation of the 2′-deoxyribose ring is the value of the C−N torsion angle χ, which positions the nucleobase into two different domains (syn or anti). The often-conflicting sugar and nucleobase conformational parameters were studied using North and South methanocarbadeoxythymidine analogues (**6** and **7**), which forced HSV-tk to make a clear choice in the conformation of the substrate. The results provide new insights into the mechanism of action of this enzyme, which cannot be gleaned from a static X-ray crystal structure.

Introduction

Human herpes viruses HSV-1 and HSV-2 infections are widespread and can be life-threatening to immunocompromised patients.[1] In the United States, HSV infections are a significant public health problem affecting nearly 150 million Americans, of which 40−60 million are infected with HSV-2 and an additional 1 million infections occur each year.[2] Genital herpes, caused by either HSV-1 or HSV-2, has become the leading cause of genital ulcers.[3] Genital ulcers increase the risk of HIV infection augmenting its morbidity and mortality.[4] If the herpes virus is not totally eradicated after the initial infection, it can persist latently in host neurons, posing a risk of periodical reactivation over a lifetime.[5] The current treatments for HSV infections are principally nucleoside analogues. Despite their safety and efficacy, many nucleoside analogues have limited oral bioavailability, must be administered early in the disease process for maximal benefit, and can become ineffective due to the development of drug resistance, particularly in immunocompromised patients.[2,6] The need to overcome these problems requires a commitment to develop novel drugs, including new nucleoside analogues. The development of new nucleosides analogues will require a better understanding of their mechanism of action.

Successful therapy using nucleoside analogues depends on the ability of the analogues to interact effectively with a triad of anabolic kinases and with the target viral or cellular DNA polymerases. In the specific case of HSV-infected cells, the viral kinase (HSV-tk) accepts a broader range of substrates (nucleoside analogues) for the first phosphorylation step than the more stringent cellular thymidine kinase (tk1), which phosphorylates thymidine almost exclusively.[7,8] Because only infected cells contain HSV-tk, a favorable therapeutic index depends on the differential specificity of HSV-tk and tk1 and the ability of subsequent phosphorylation steps to produce a 5′-triphosphate

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|| Department of Clinical Pharmacology, Ben-Gurion University of the Negev.

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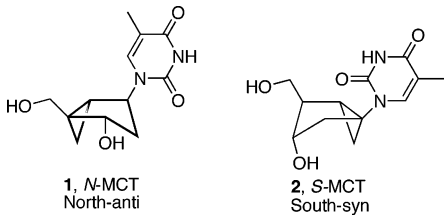
and on host or viral polymerases incorporating the resulting triphosphate. Although the second and third phosphorylation steps are normally carried out by cellular kinases, HSV-tk can also function as a thymidylate kinase, performing the second phosphorylation step.[9,10] In the end, it is the fine balance between the ability of a nucleoside to function as substrate both for kinases and for polymerases that determines its usefulness as a drug.

We have demonstrated, in previous studies, that the conformation of the sugar ring plays a critical role in determining the affinity of kinases and polymerases for nucleosides.[11] Using nucleoside analogues built on a rigid template, the bicyclo[3.1.0]-hexane scaffold, we produced two isomers of methanocarba (MC) thymidine (T), one with the pseudosugar moiety locked in the North (*N*) hemisphere of the pseudorotational cycle (**1**, *N*-MCT) and the other with it locked in the antipodean South (*S*) conformation (**2**, *S*-MCT). These two isomers display opposite affinities for HSV-tk and DNA polymerases.[11] We found that for the second and third phosphorylation steps *S*-MCT was the preferred substrate, but that despite the high levels of *S*-MCT-5′-triphosphate formed, only negligible amounts were incorporated into DNA. On the other hand, because *N*-MCT was a poorer substrate for these kinases, lower levels of *N*-MCT-5′-triphosphate were produced. Nevertheless, *N*-MCT-5′-triphosphate was efficiently incorporated into DNA by the host polymerase; as a result, *N*-MCT (**1**) is a potent anti-HSV drug (EC_{50} = 0.02 μM) while *S*-MCT (**2**) was completely ineffective.[10,12]



1, *N*-MCT
North-anti

2, *S*-MCT
South-syn

The contrasting conformational preference of kinases and polymerases demonstrates the importance of the inherent flexibility of the ribose (or 2′-deoxyribose) ring, which allows it to adapt to the conformational demands of the two types of enzymes. Conventional nucleosides in solution normally undergo rapid equilibration between the *N* and *S* puckering domains, as described in the pseudorotational cycle (0° → 360°, Figure 1).[13,14] The *N* conformations encompass a range between 342° and 18° ($_2$E → ^{3}T$_2$ → ^{3}E), where E and T correspond to envelope (E) and twisted (T) conformations of the tetrahydrofuran ring, and the super- or subscripted numbers denote the atoms on the five-member ring that project above or below the plane of the sugar ring. At the other extreme, the antipodean *S* conformations oscillate between 162° and 198° (^{2}E → ^{2}T$_3$ → $_3$E). The ideal conformations of ^{3}T$_2$ and ^{2}T$_3$ correspond to 0° and 180° in the pseudorotational cycle. The bicyclo[3.1.0]hexane

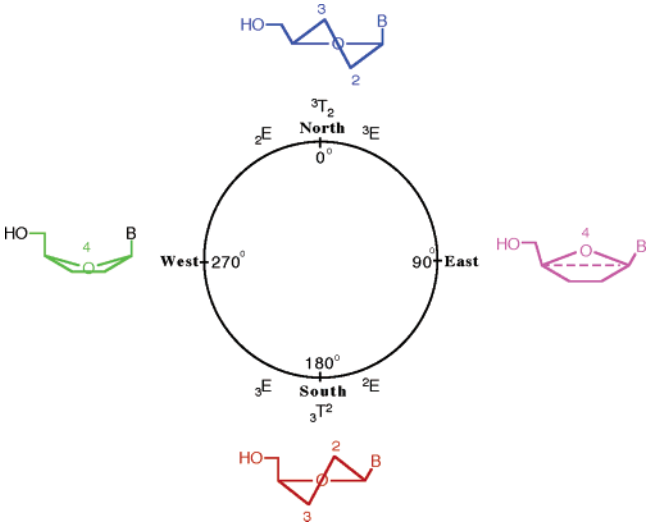


Figure 1. Pseudorotational cycle of the furanose ring in nucleosides. The populated ranges for *N* ($_2$E → ^{3}T$_2$ → ^{3}E) and *S* (^{2}E → ^{2}T$_3$ → $_3$E) conformations are indicated.

nucleosides, because of their rigid pseudoboat conformations, lock the positions of *N*-MCT and *S* -MCT at $_2$E (342°) and $_3$E (198°), respectively (Figure 1).

The strong preference of *S*-MCT for the second and third phosphorylation steps was demonstrated by the *S*/*N* ratios of phosphorylated metabolites of 4.6 and 2.5, respectively.[11] Surprisingly, the first phosphorylation step performed by HSV-tk did not show the same penchant, as the observed *S*/*N* ratio of 0.9 even appeared to suggest an opposite preference.[11] A proposal to explain this apparent deviation from the behavior of the other kinases is that the nucleobase in *S*-MCT is in an orientation not easily recognized by the enzyme. Both X-ray structures of *N*-MCT[15] and *S*-MCT,[16] and computational analysis,[11] showed that the preferred conformation for the nucleobase in *N*-MCT was anti, while the preferred conformation for the nucleobase in *S*-MCT was syn. The anti or syn conformation describes the orientation of the nucleobase relative to the sugar ring (see structures **1** and **2**), and it is determined by the value of the torsion angle χ of the C−N bond that connects the base to the sugar.[14] The crystallographic data and the ab initio calculations for *N*-MCT and *S*-MCT mentioned above are in perfect agreement with the trend observed with conventional nucleosides where χ tends to be anti when the sugar pucker is *N* and syn when the sugar pucker is *S*.[17] This propensity is much stronger for the locked bicyclo[3.1.0]hexane nucleosides because the energy barriers between anti and syn are much larger for *N*-MCT and *S*-MCT than for conventional nucleosides.[11] Remarkably, the crystal structures of HSV-tk in complexes with *N*-MCT or *S*-MCT show that the nucleobase in both compounds is in the anti conformation with the fused cyclopropane ring neatly tucked under the cyclopentane ring and away from neighboring amino acids.[18,19] Unfortunately, X-ray structures are static models of stable end-points that do not shed useful

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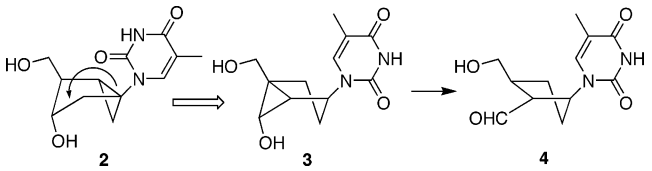
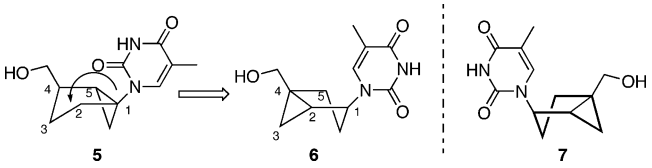
Scheme 1



Scheme 2



light on the energy required for the ligand to reach the bound conformation. These X-ray structures also confirmed that *S*-MCT was the better substrate mimic because the locked cyclopentane ring of the bicyclo[3.1.0]hexane pseudosugar perfectly followed the contour of the sugar ring of the natural substrate, thymidine.[11] Moreover, the critical 3′-OH group of *S*-MCT is in an axial disposition and formed a well-defined bifurcated H-bond with the OH of Tyr101 and the carboxylate group of Glu225, identical to what was observed with thymidine.[19] In contrast, the equatorial 3′-OH in *N*-MCT was displaced 3.7 Å away from Glu225 and 4.1 Å away from Tyr101, showing that the *N* sugar pucker prevented the formation of the H-bonds.[18] The puzzling question was why the first phosphorylation step of HSV-tk was not so efficient when *S*-MCT appeared to be such a good mimic? Was the higher energy barrier required to switch the nucleobase from syn to anti the critical factor negatively affecting the first phosphorylation step? If this is the problem, could we shift the position of the nucleobase in *S*-MCT from syn to anti and generate an analogue that would be efficiently phosphorylated?

Design of an anti-South Analogue

Ab initio calculations suggested that the fused cyclopropane ring adjacent to the C−N bond was the source of the high-energy barrier between the syn and anti conformations of *S*-MCT.[11] The integrity of the bicyclo[3.1.0]hexane, which maintains a rigid pseudoboat conformation, suggested that we could transpose one bond of the fused cyclopropane ring in *S*-MCT (**2**) to the opposite side of the molecule, generating the isomeric bicyclo[3.1.0]hexane nucleoside **3** shown in Scheme 1. This rearrangement would maintain the desired *S*-like conformation; however, it produced a structure that, based on literature precedent, would be unstable and prone to ring-opening to give **4**, even at room temperature (Scheme 1).[20] We then considered removing the OH function, as in compound **5**, which after an identical bond transposition would produce a stable dideoxy analogue, *S*-MCdT (**6**, Scheme 2). As shown is Scheme 2, the new compound **6** corresponds to the optical antipode of the dideoxy analogue of *N*-MCdT (compound **7**). These two compounds, the L- and D-isomers of *N*-MCdT (Figure 2), are the two compounds used to test our hypothesis. The L-isomer **6**

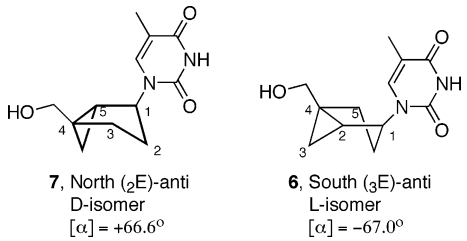


Figure 2. Structures of the D- (**7**) and L-isomer (**6**) of *N*-MCdT. The optical antipode (compound **6**) has a bicylo[3.1.0]hexane ring that mimics the *S* conformation. In both compounds, the disposition of the nucleobase is anti.

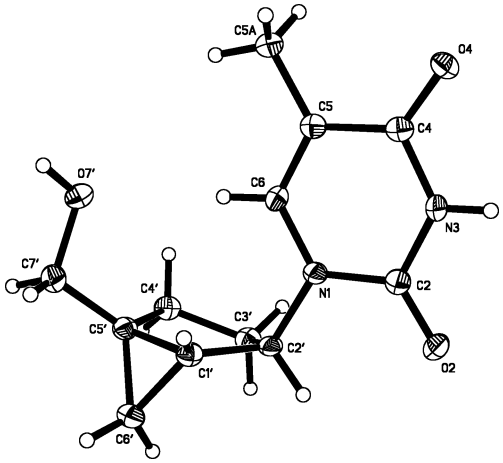
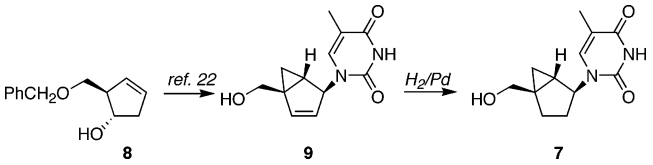


Figure 3. Crystal structure of *S*-MCdT (**6**). Displacement ellipsoid plot drawn at the 50% probability level. The numbering system used here corresponds to the IUPAC name.

Scheme 3



has the desired shape to be considered a South mimic, which, according to the numbering shown (nucleoside numbering), is equivalent to an $_3$E conformation identical to that of *S*-MCT (**2**).[21] As expected, the X-ray structure of **6** (Figure 3) confirmed the anti disposition of the base. Because both compounds (**6** and **7**) now have the C−N bond in the anti conformation, the enzyme's choice between **6** and **7** would be governed exclusively by its preference for a specific sugar conformation, *N* or *S*.

Synthesis

Synthesis of the D-isomer (*N*-MCdT, **7**) was performed by catalytic hydrogenation of compound **9**[22] (Scheme 3). Starting with the same chiral cyclopentenol (**8**)[23,24] used for the synthesis

(19) Schelling, P.; Claus, M. T.; Johner, R.; Marquez, V. E.; Schulz, G. E.; Scapozza, L. Biochemical and structural characterization of (South)-methanocarbathymidine that specifically inhibits growth of herpes simplex virus type 1 thymidine kinase-transduced osteosarcoma cells. *J. Biol. Chem.* **2004**, *279*, 32832−32838.

(20) Creary, X. *J. Org. Chem.* **1975**, *40*, 3326−3331.

(21) Because there are six carbons in the bicyclo[3.1.0]hexans system, but only five are required to define the shape of the embedded five-member ring in the pseudorotational cycle, one carbon has to be omitted. Normally, as in compound **7**, the omitted carbon is the one at the tip of the fused cyclopropane ring. However, in the case of compound **6**, the numbering system corresponds to that of the progenitor compound **5**; thus the carbon formerly at the tip of the cyclopropane ring was omitted. The new five member ring envisioned in **6** assumes a virtual bond between carbons 1 and 5.

(22) Choi, Y.; George, C.; Comin, M. J.; Barchi, J. J., Jr.; Kim, H. S.; Jacobson, K. A.; Balzarini, J.; Mitsuya, H.; Boyer, P. L.; Hughes, S. H.; Marquez, V. E. *J. Med. Chem.* **2003**, *46*, 3292−3299.

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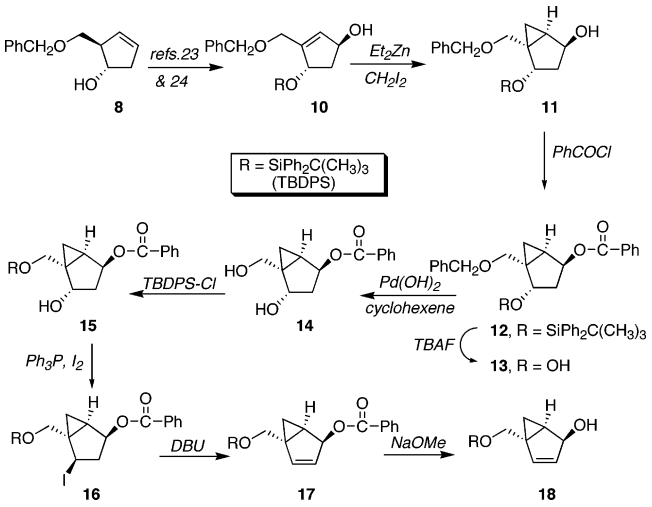
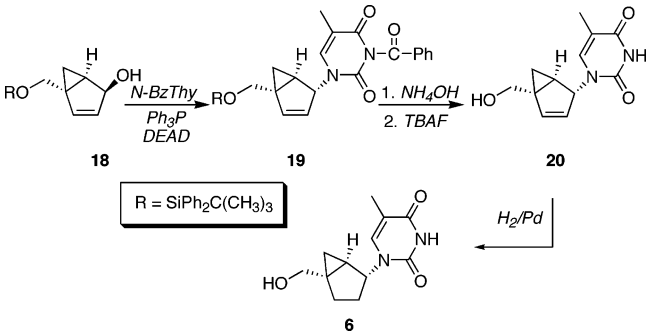
Scheme 4



Scheme 5



of **7**, the intermediate **10** was generated according to our published methodology.[24] Following the hydroxyl-directed cyclopropanation of **10**, the key bicyclo[3.1.0]hexane carbocyclic precursor **11** was obtained (Scheme 4). From that point onward, the synthesis mirrored the approach used for the synthesis of **9** to give the optical antipode **20** (Schemes 4 and 5). Catalytic hydrogenation of **20** afforded the L-isomer (*S*-MCdT, **6**).

Phosphorylation by HSV-tk and Discussion

The unfavorable ratio of *S*/*N* metabolites that was originally determined for *S*-MCT (**2**, 72 ± 5.4 pmol/10^6 cells)[11] and *N*-MCT (**1**, 76.0 ± 0.9 pmol/10^6 cells)[11] for the first phosphorylation step in HSV-infected Vero cells after 6 h was 0.9.[11] The poor selectivity of the *S* conformation was proposed to be the result of conflicting conformational preferences of HSV-tk for the sugar pucker and the nucleobase disposition. Under the same experimental conditions as those used for *S*-MCT and *N*-MCT, there was only a slight drop in monophosphate production (60.7 pmol/10^6 cells, Table 1) when *N*-MCdT, which lacks the 3′-OH, was the substrate. Levels of all phosphate metabolites in uninfected cells were very low and varied between 0.1 and 0.4 pmol/10^6 cells.

In contrast to the low and invariant levels of monophosphate metabolites generated from *S*-MCT, *N*-MCT, and *N*-MCdT, the yield of the *S*-MCdT monophoshate increased to 137 pmol/10^6 cells in HSV-tk infected cells, which translated to a favorable *S*/*N* ratio of 2.3 after 6 and 24 h (Table 1). The increase in the *S*/*N* ratio from 0.9 to 2.3 for the first phosphorylation step clearly indicates that if the nucleobase is not constrained in the syn conformation, HSV-tk has a clear preference for the substrates in the *S* conformation.

Table 1. Levels of *N*-MCdT (**7**) and *S*-MCdT (**6**) Phosphates (pmol/10^6 cells) in HSV-1-Infected Vero Cells[a]

	N-MCdT		*S*-MCdT	
	6 h	24 h	6 h	24 h
MP	60.7 ± 3.21	706 ± 21	137 ± 8.65	1609 ± 18
DP	1.214 ± 0.21	45.6 ± 6.30	1.221 ± 0.16	37.9 ± 2.68
TP	0.721 ± 0.16	9.40 ± 1.40	0.215 ± 0.08	5.06 ± 0.80

[a] MP = monophosphate; DP = diphosphate; TP = triphosphate. Cells were treated with 10 μM, 10 μCi/mL of drug. Data are mean ± SD (n = 3).

The relatively low levels of di- and triphosphate metabolites of *N*-MCdT and *S*-MCdT produced in the ensuing step, and the surprising lack of discrimination between *N* and *S* beyond the first step, contrasts with what was seen with *N*-MCT and *S*-MCT, suggesting that the 3′-OH group plays a critical role in substrate recognition by HSV-tk for the second phosphorylation step. We know that HSV-tk is a multifunctional enzyme that exhibits thymidylate kinase activity, but that, in this role, it has a narrower range of substrates.[9,25] Consistent with this more stringent substrate recognition, the 3′-OH appears to play a critical role for the second phosphorylation step. On the other hand, for the first phosphorylation step HSV-tk is shown here to be exquisitely sensitive to the conformation of the sugar and the syn/anti disposition of the nucleobase as we predicted. The low overall metabolism of *N*-MCdT and *S*-MCdT, which leads to very low levels of 5′-triphosphates in HSV-infected Vero cells, explains why these compounds poorly inhibited the growth (IC_{50} > 1000 μM) of murine tumor cells (MC38) transfected with HSV-tk (data not shown).

Evidently, modified nucleosides such as *N*-MCdT (**7**) and *S*-MCdT (**6**) might partially behave as substrates and partially as inhibitors of the enzyme. Although future kinetic experiments will address this issue, preliminary IC_{50} values measuring the ability of these compounds to inhibit thymidine phosphorylation by HSV-tk (9.91 and 2.65 μM for **7** and **6**, respectively)[26] clearly show that the better inhibitor with the lower IC_{50} value is also the better substrate as demonstrated by direct phosphorylation with the radiolabed substrates (Table 1).

Conclusions

The relocation of the cyclopropane ring from the fused bicyclo[3.1.0]hexane system in compound **5** created *S*-MCdT (**6**, Scheme 2). This bond transposition was used to free the nucleobase from the preferred syn conformation. The resulting conformational change allowed HSV-tk to recognize *S*-MCdT as the preferred substrate, relative to the antipodal *N*-MCdT (**7**), based on the shape of the pseudosugar ring. If one subtracts 5 kcal/mol for the intramolecular hydrogen bond energy between the 5′-OH and the 2-oxopyrimidine carbonyl, which is favored in calculations performed in a vacuum, from the total calculated energy barrier of 15 kcal/mol needed to flip the pyrimidine ring in *S*-MCT from syn into anti,[11] 10 kcal/mol would be required

(24) Marquez, V. E.; Russ, P.; Alonso, R.; Siddiqui, M. A.; Hernandez, S.; George, C.; Nicklaus, M. C.; Dai, F.; Ford, H., Jr. *Helv. Chim. Acta* **1999**, *82*, 2119−2129.
(25) Chen, M. S.; Summers, W. P.; Walker, J.; Summers, W. C.; Prusoff, W. H. *J. Virol.* **1979**, *30*, 942−945.
(26) Balzarini, J. Katholieke Universiteit Leuven, Rega Institute, Leuven, Belgium. Personal communication.

to flip the base. Removing this energy barrier allowed HSV-tk to select its substrate based exclusively on sugar pucker; the difference in the levels of monophoshates formed from two of the substrates with opposite locked pseudosugar conformations clearly supports our hypothesis that the *S* conformation is preferred by HSV-tk.

These results, in conjunction with data from our previously published work on *N*-MCT and *S*-MCT,[11] provide insights into the multifaceted role of the sugar ring in nucleosides and demonstrates the ability of kinases and polymerases to discriminate among their substrates on the basis of sugar and nucleobase conformations. Furthermore, our study highlights how the substrate preferences for each step can be affected, positively or negatively, by minor structural modifications. On the basis of the results presented here, we can conclude that for the first step HSV-tk activity is quite insensitive to the presence or absence of a 3′-OH, but very responsive to both sugar and nucleobase conformation preferring the sugar in the *S* conformation and the base in the anti disposition. For the second step, however, HSV-tk becomes extremely sensitive to the 3′-OH and still maintains its preference for substrates with the *S* conformation. The sensitivity of both kinases and polymerases to subtle structural changes may help explain why ostensibly similar nucleoside analogues often produce widely different biological effects ranging from inactive to extremely toxic. It is hoped that the results presented here will contribute to the design of more specific and selective antiviral and antitumor agents such as *N*-MCT. This compound is a very effective and selective anti-herpes agent, both in vitro and in vivo, which, despite its lower affinity for HSV-tk and cellular kinases, has excellent properties as a substrate for DNA polymerases.[10] The potential use of *N*-MCT as a clinical agent is being actively pursued.

Experimental Methods

Preparation of Cell Extract for Metabolite Analysis. Vero cell cultures (4×10^6/25 mL flask) were infected with 1 PFU/cell of HSV-1. After a 2 h incubation period, the cells were incubated with radioactive [Me-^{3}H]-*N*-MCdT or [Me-^{3}H]-*S*-MCdT, 10 μM, 5 μCi/mL. These compounds were purchased from Moravek Biochemicals, Brea, CA [specific activities of 1100 and 1250 dpm/pmol for [Me-^{3}H]-*N*-MCdT and [Me-^{3}H]-*S*-MCdT, respectively]. As a control, uninfected cells were treated with the radioactive compounds. At the end of 6 and 24 h incubation periods, the cells were washed three times with PBS, trypsinized, and recovered by centrifugation. The dry pellets were suspended in 250 μL of 60% methanol (HPLC grade) and heated at 95 °C for 3 min. After centrifugation at 12 000*g* for 10 min, the clear supernatant fractions were evaporated under nitrogen and redissolved in 250 μL of water. Aliquots of this solution were analyzed by anion-exchanged (SAX-10) HPLC.

HPLC Separation of Metabolites. The separation of [^{3}H]-*N*-MCdT, [^{3}H]-*S*-MCdT, and their phosphorylated metabolites was carried out using a Hewlett-Packard 1100 HPLC with a diode-array UV absorption detector. A Partisil-10 SAX column (250 × 4.6 mm) was used with the following elution program: 0−5 min, 100% buffer A (0.01 M ammonium phosphate, native pH); 5−20 min, linear gradient to 25% buffer B (0.7 M ammonium phosphate with 10% methanol); 20−30 min, linear gradient to 100% buffer B; 30−40 min 100% buffer B; 40−55 min, linear gradient to 100% buffer A and equilibration. The flow rate was 2 mL/min. One-minute fractions were collected, and radioactivity was measured by scintillation spectrometry. The retention times were as follows: *N*- and *S*-MCdT (4 min), *N*- and *S*-MCdT-monophosphate (11 min), *N*- and *S*-MCdT-diphosphate (22 min), and *N*- and *S*-MCdT-triphosphate (31 min). Fractions containing radiolabeled compounds were quantitated on the basis of the known specific activity of the parent tritiated compounds.

Synthetic Methods. (1′*S*,2′*S*,5′*R*)-1-[5-(Hydroxymethyl)bicyclo-[3.1.0]hex-2-yl]-5-methyl-1,3-dihydropyrimidine-2,4-dione (7). A stirred solution of **9**[22] (38.5 mg, 0.164 mmol) in methanol (5 mL) was reduced under a hydrogen-filled balloon atomosphere in the presence of 5% Pd/C for 1 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under vacuum. The residue was purified by silica gel flash chromatography ($CHCl_3$:MeOH, 25:1) to give **7** (38 mg, 98%) as a white solid, mp 173−175 °C; $[\alpha]^{22}_D$ +66.6° (*c* 0.35, MeOH); ^{1}H NMR (DMSO-d_6) δ 11.12 (br s, 1H, NH), 7.83 (dd, 1H, J = 1.2 Hz, H-6), 4.98 (br s, 1H, OH), 4.68 (d, 1H, J = 6.6 Hz, H-2′), 3.84 (d, 1H, J = 11.1 Hz, C*H*HOH), 3.21 (d, 1H, J = 11.1 Hz, CH*H*OH), 1.90−2.00 (irregular q, 1H, H-4′a), 1.60−1.78 (overlapping s and m, 4H, CH_3 and H-4′b), 1.40−1.58 (m, 2H, H-3), 1.16 (dd, 1H, $J \approx$ 8.8, 3.9 Hz, H-1′), 0.55 (dd, 1H, $J \approx$ 8.5, 5.2 Hz, H-6′a), 0.46 (irregular t, 1H, H-6′b); ^{13}C NMR (DMSO-d_6) δ 165.3, 151.7, 139.0, 109.7, 64.8, 57.7, 32.9, 29.9, 25.9, 25.3, 11.3, 11.2; FAB MS (*m*/*z*, relative intensity) 237 (MH^+, 100). Anal. Calcd for $C_{12}H_{16}N_2O_3 \cdot 0.30H_2O$: C, 59.58; H, 6.86; N, 11.59. Found: C, 59.32; H, 6.86; N, 11.56.

(1*R*,2*S*,4*S*,5*S*)-4-(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)-5-[(phenylmethoxy)methyl]bicyclo[3.1.0]hexan-2-ol (11). Under an argon atmosphere, a 1.0 M solution of diethyl zinc in hexanes (16 mL) was added dropwise to a solution of **10**[23,24] (6.40 g, 14 mmol) in anhydrous CH_2Cl_2 (100 mL) at 0 °C. After 15 min of stirring, a solution of diiodomethane (1.3 mL, 16 mmol) in anhydrous methylene chloride (7 mL) was rapidly added via syringe. Second equal portions of diethyl zinc (16 mL) and diiodomethane (7 mL) solutions were added sequentially after 15 min, and after an additional 15 min the reaction mixture was allowed to reach room temperature. The reaction was quenched after 4 h by the addition of a saturated solution of NH_4Cl. The aqueous layer was extracted with CH_2Cl_2 three times, and the organic phase was dried ($MgSO_4$) and concentrated under vacuum to give a yellowish oil, which was used in the next step without further purification. An analytical sample for characterization was purified by silica gel flash chromatography (hexanes:EtOAc, 8:2) to afford compound **10** as an oil; $[\alpha]^{22}_D$ −42.1° (*c* 1.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.56−7.63 (m, 4H, Ph), 7.18−7.36 (m, 11H, Ph), 4.71 (irregular sextet, 1H, H-2), 4.30−4.8 (overlapping doublets, 2H, J = 12.0 Hz and J = 4.7 Hz, OC*H*HPh and H-4), 4.31 (d, 1H, J = 12.0 Hz, OCH*H*Ph), 4.23 (dd, 1H, J = 9.5, 1.3 Hz, C*H*HOBn), 2.94 (d, 1H, J = 9.5 Hz, CH*H*OBn), 1.62 (dd, 1H, J = 14.4, 7.6 Hz, H-3α), 1.52 (irregular quintet, 1H, H-1), 1.28 (br s, 1H, OH), 1.01 (s, 9H, $C(CH_3)_3$), 0.95 (m, 1H, H-3*β*), 0.65 (irregular t, 1H, H-6a), 0.58 (irregular t, 1H, H-6b); ^{13}C NMR ($CDCl_3$) δ 138.7, 136.2, 136.0, 134.9, 133.9, 129.8, 129.7, 128.5, 127.9, 127.8, 127.7, 75.0, 73.0, 72.3, 70.6, 39.7, 34.5, 27.4, 27.2, 19.6, 10.4; FAB MS (*m*/*z*, relative intensity) 473 (MH^+, 2), 455 ($MH^+ - H_2O$, 12). Anal. Calcd for $C_{30}H_{26}O_3Si \cdot 0.25H_2O$: C, 75.49; H, 7.60. Found: C, 75.49; H, 7.73.

(1*R*,2*S*,4*S*,5*S*)-4-(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)-5-[(phenylmethoxy)methyl]bicyclo[3.1.0]hex-2-yl Benzoate (12). A stirred solution of **11** (19.6 g, 41.4 mmol) in CH_2Cl_2 (250 mL) was cooled to 0 °C and treated with pyridine (10 mL, 124 mmol) and benzoyl chloride (5.8 mL, 49.7 mmol). The mixture was allowed to reach room temperature and stirred for 3 h. After the addition of a saturated solution of $NaHCO_3$, the aqueous layer was extracted with CH_2Cl_2. The combined organic extract was washed with $NaHCO_3$, water and brine, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by silica gel flash chromatography (hexanes:EtOAc, 60:1) to give **12** (22 g, 92%) as a colorless syrup; $[\alpha]^{22}_D$ −52.1° (*c* 0.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.88−7.92 (m, 2H, Ph), 7.60−7.66 (m, 4H, Ph), 7.41−7.46 (m, 1H, Ph), 7.18−7.36 (m, 13H, Ph), 5.69 (irregular sextet, 1H, H-2), 4.52 (d, 1H, J = 4.8 Hz, H-4), 4.39 (AB q, 2H, J = 11.9 Hz, OCH_2Ph), 4.28 (dd, 1H, J = 9.7, 1.3 Hz, C*H*HOBn), 2.98 (d, 1H, J = 9.7 Hz, CH*H*OBn), 1.78−1.88 (m, 2H, H-1, H-3α),

1.25−1.34 (m, 1H, H-3β), 1.03 (s, 9H, C(CH_3)$_3$), 0.72 (irregular t, 1H, H-6a), 0.67 (irregular t, 1H, H-6b); ^{13}C NMR ($CDCl_3$) δ 166.9, 138.7, 136.2, 136.1, 134.7, 133.8, 133.0, 130.8, 129.9, 129.8, 129.7, 128.5, 127.9, 127.9, 127.8, 127.7, 76.1, 74.3, 73.1, 70.5, 36.7, 34.9, 27.3, 24.9, 19.7, 11.3; FAB MS (*m*/*z*, relative intensity) 577 (MH^+, 2), 455 (MH^+ − PhCOOH, 15), 321 (MH^+ − TBPSiOH, 26). Anal. Calcd for $C_{37}H_{40}O_3Si\cdot0.8H_2O$: C, 75.17; H, 6.96. Found: C, 75.20; H, 7.82.

(1*R*,2*S*,4*S*,5*S*)-4-Hydroxy-5-[(phenylmethoxy)methyl]bicyclo[3.1.0]-hex-2-yl Benzoate (13). A stirred solution of **12** (16.7 g, 29 mmol) in THF (150 mL) at room temperature was treated dropwise with a 1 M solution of tetra-*n*-butylammonium fluoride (TBAF, 71.6 mL) in THF. The reaction mixture was stirred overnight and concentrated under vacuum. The residue was purified by silica gel flash chromatography (hexanes:EtOAc, 4:1) to give 12.05 g (89%) of **13** as a colorless syrup; $[\alpha]^{22}_D$ −70.5° (*c* 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.94−7.98 (m, 2H, Ph), 7.46−7.50 (m, 1H, Ph), 7.22−7.38 (m, 7H, Ph), 5.78 (irregular sextet, 1H, H-2), 4.50 (AB q, 2H, *J* = 12.0 Hz, OCH_2Ph), 4.38 (d, 1H, *J* = 5.1 Hz, H-4), 3.79 (d, 1H, *J* = 10.4 Hz, C*H*OBn), 3.46 (d, 1H, *J* = 10.4 Hz, CH*H*OBn), 2.14 (dd, 1H, *J* = 14.2, 7.9 Hz, H-3α), 1.92 (irregular quintet, 1H, H-1), 1.56 (m, 1H, H-3β), 0.78 (dd, 1H, *J* = 5.7, 4.0 Hz, H-6a), 0.51 (dd, 1H, *J* = 7.8, 5.7 Hz, H-6b); ^{13}C NMR ($CDCl_3$) δ 166.8, 137.8, 133.0, 133.7, 129.8, 128.8, 128.5, 128.1, 128.0, 76.4, 74.4, 73.4, 72.8, 37.1, 33.3, 25.0, 11.4; FAB MS (*m*/*z*, relative intensity) 339.2 (MH^+, 3), 321 ($MH^+ - H_2O$, 34), 231 ($MH^+ - PhCH_2$-OH, 22). Anal. Calcd for $C_{21}H_{22}O_4\cdot0.4H_2O$: C, 73.03; H, 6.65. Found: C, 72.78; H, 6.75.

(1*R*,2*S*,4*S*,5*S*)-4-Hydroxy-5-(hydroxymethyl)bicyclo[3.1.0]hex-2-yl Benzoate (14). A solution of **13** (10.18 g, 30.1 mmol) in ethanol (200 mL) was stirred in the presence of 20% $Pd(OH)_2$ on charcoal (2.54 g) and cyclohexene (100 mL). As the reaction mixture was heated, hydrogen evolution started at ca. 80 °C, and further heating at reflux continued for 12 h. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated under vacuum to give 7.4 g (99%) of diol **14** as white solid, which was used directly in the next step. An analytical sample was obtained after silica gel flash chromatography (hexanes:EtOAc, 1:1), mp 81−82 °C; $[\alpha]^{22}_D$ −88.4° (*c* 0.63, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.93−7.98 (m, 2H, Ph), 7.44−7.50 (m, 1H, Ph), 7.33−7.39 (m, 2H, Ph), 5.79 (irregular sextet, 1H, H-2), 4.45 (d, 1H, *J* = 5.0 Hz, H-4), 3.99 (br d, 1H, *J* = 12.0 Hz, C*H*OH), 3.56 (br d, *J* = 12.0 Hz, CH*H*OH), 3.20−3.40 (broad overlapping singlets, 2H, OH), 2.15 (dd, 1H, *J* = 14.5, 7.9 Hz, H-3α), 1.96 (irregular quintet, 1H, H-1), 1.61 (m, 1H, H-3β), 0.78 (dd, 1H, *J* = 5.6, 3.8 Hz, H-6a), 0.51 (dd, 1H, *J* = 7.5, 6.0 Hz, H-6b); ^{13}C NMR ($CDCl_3$) δ 167.1, 133.15, 130.50, 129.8, 128.5, 76.5, 74.9, 65.5, 37.6, 35.3, 24.6, 11.6; FAB MS (*m*/*z*, relative intensity) 249 (MH^+, 6.7), 231 ($MH^+ - H_2O$, 63). Anal. Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.63; H, 6.49.

(1*R*,2*S*,4*S*,5*S*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-4-hydroxybicyclo[3.1.0]hex-2-yl Benzoate (15). Imidazole (4.91 g, 72.2 mmol) was added to a stirred solution of diol **14** (8.15 g, 32.80 mmol) in CH_2Cl_2 (200 mL) at 0 °C. Immediately after, *tert*-butylchlorodiphenyl silane (9.0 mL, 34.44 mmol) dissolved in CH_2Cl_2 (30 mL) was added dropwise. The mixture was stirred at 0 °C for 20 min and quenched with brine (20 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by silica gel flash column chromatography (hexanes: EtOAc, 9:1) to give 12.34 g (77%) of **15** as a syrup: $[\alpha]^{22}_D$ −81.2° (*c* 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.93−7.98 (m, 2H, Ph), 7.58−7.64 (m, 4H, Ph), 7.44−7.49 (m, 1H, Ph), 7.31−7.41 (m, 8H, Ph), 5.80 (irregular sextet, 1H, H-2), 4.43 (d, 1H, *J* = 4.8 Hz, H-4), 4.08 (d, 1H, *J* = 11.4 Hz, C*H*OSi), 3.48 (d, 1H, *J* = 11.4 Hz, CH*H*OSi), 2.15 (dd, 1H, *J* = 14.3, 8.1 Hz, H-3α), 1.74 (irregular quintet, 1H, H-1), 1.53 (m, 1H, H-3β), 1.00 (s, 9H, C(CH_3)$_3$), 0.68 (dd, 1H, *J* = 5.6, 3.8 Hz, H-6a), 0.31 (dd, 1H, *J* = 7.8, 5.8 Hz, H-6b); ^{13}C NMR δ 166.8, 135.8, 133.0, 132.9, 132.8, 130.8, 130.3, 130.2, 129.8, 128.5, 128.2, 128.1, 76.5, 74.9, 67.2, 37.2, 34.6, 27.0, 25.2, 19.3, 11.0; FAB MS (*m*/*z*, relative intensity) 487 (MH^+, 24.5), 469 ($MH^+ - H_2O$, 86). Anal. Calcd for $C_{30}H_{34}O_4Si$: C, 74.04; H, 7.04. Found: C, 74.01; H, 6.93.

(1*R*,2*S*,4*R*,5*S*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-4-iodobicyclo[3.1.0]hex-2-yl Benzoate (16). Starting from **15** (1.51 g, 3.11 mmol), this compound was prepared in the same manner as its optical antipode[22] to give **16** (1.62 g, 88%) as a colorless syrup: $[\alpha]_D^{23}$ −27.2° (*c* 0.60 $CHCl_3$). Anal. Calcd for $C_{30}H_{33}IO_3Si$: C, 60.40; H, 5.58. Found: C, 60.73; H, 5.63.

(1*R*,2*S*,5*S*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-bicyclo[3.1.0]hex-3-en-2-yl Benzoate (17). Starting from **16** (1.57 g, 2.63 mmol), this compound was prepared in the same manner as its optical antipode[22] to give **17** (640 mg, 52%) as a colorless syrup: $[\alpha]_D^{23}$ +42.1° (*c* 0.43 $CHCl_3$). Anal. Calcd for $C_{30}H_{32}O_3Si$: C, 76.88; H, 6.88. Found: C, 76.55; H, 7.05.

(1*R*,2*S*,5*S*)-5-[(2,2-Dimethyl-1,1-diphenyl-1-silapropoxy)methyl]-bicyclo[3.1.0]hex-3-en-2-ol (18). Starting from **17** (619 mg, 1.32 mmol), this compound was prepared in the same manner as its optical antipode[22] to give **18** (475 mg, 99%) as a colorless syrup: $[\alpha]_D^{23}$ +11.2° (*c* 0.43 $CHCl_3$). Anal. Calcd for $C_{23}H_{28}O_2Si$: C, 75.78; H, 7.74. Found: C, 75.53; H, 7.79.

(1′*R*,2′*R*,5′*S*)-1-{5-[(2,2-Dimethyl-1,1-silapropoxy)methyl]bicyclo-[3.1.0]hex-3-en-2-yl}-5-methyl-3-(phenylcarbonyl)-1,3-dihydropyrimidine-2,4-dione (19). Starting from **18** (480 mg, 3.41 mmol), this compound was prepared in the same manner as its optical antipode[22] to give **19** (340 mg, 45%) as a colorless syrup: $[\alpha]_D^{23}$ −49.6° (*c* 0.16, $CHCl_3$). Anal. Calcd for $C_{35}H_{36}N_2O_4Si\cdot0.7H_2O$: C, 71.33; H, 6.39; N, 4.75. Found: C, 71.30; H, 6.28; N, 4.64.

(1′*R*,2′*R*,5′*S*)-1-[5-(Hydroxymethyl)bicyclo[3.1.0]hex-3-en-2-yl]-5-methyl-1,3-dihydropyrimidine-2,4-dione (20). Starting from **19** (310 mg, 0.53 mmol), this compound was prepared in the same manner as its optical antipode[22] to give **20** (90 mg, 75%) as a white solid: mp 182 °C; $[\alpha]_D^{23}$ −166.5° (*c* 0.37, CH_3OH). Anal. Calcd for $C_{12}H_{14}N_2O_3\cdot0.25H_2O$: C, 60.37; H, 6.12; N, 11.73. Found: C, 0.70; H, 6.37; N, 11.35.

(1′*R*,2′*R*,5′*S*)-1-[5-(Hydroxymethyl)bicyclo[3.1.0]hex-2-yl]-5-methyl-1,3-dihydropyrimidine-2,4-dione (6). Starting from **20** (38.5 mg, 0.164 mmol), this compound was prepared in the same manner as its optical antipode (compound **7**) to give **6** (38 mg, 98%) as a white solid: mp 173−175 °C; $[\alpha]_D^{23}$ −67.0° (*c* 0.17, CH_3OH).

Acknowledgment. This paper is dedicated to the memory of Muttaiya Sundaralingam.

Supporting Information Available: Complete crystallographic data on compound **6**, including crystal data and structure refinement, atomic coordinates, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, torsion angles, and hydrogen bonds (seven tables). X-ray crystallographic data, in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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