5-Substituted Pyrimidines with a 1,5-Anhydro-2,3-dideoxy-D-*arabino*-hexitol Moiety at N-1: Synthesis, Antiviral Activity, Conformational Analysis, and Interaction with Viral Thymidine Kinase

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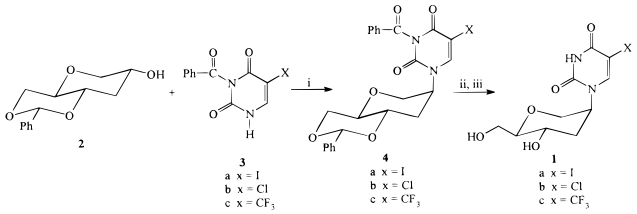
A new series of anhydrohexitol nucleosides are described. These compounds have a pyrimidine base moiety substituted in the 5-position with a chloro (**1b**), trifluoromethyl (**1c**), vinyl (**1d**), 2-thienyl (**1e**), ethynyl (**1f**) or propynyl (**1g**) substituent. The vinyl, propynyl, and, in particular, the 5-trifluoromethyl analogue showed potent activity against herpes simplex virus (HSV), **1c** with a selectivity index of >16000 against HSV-1 and >1000 against HSV-2. Conformational analysis of anhydrohexitol nucleosides using computational methods indicates that these nucleosides occur in an equilibrium between the C1 and 1C form with a ΔE of 5.9 kJ/mol. When the anhydrohexitol nucleoside is cocrystallized with the HSV-1 thymidine kinase it adopts a 1C conformation, which is opposite to the conformation found for the small molecule alone. The enzyme, apparently, induces a conformational change, and conformational flexibility of an anhydrohexitol nucleoside may be advantageous for recognition by viral enzymes.

Introduction

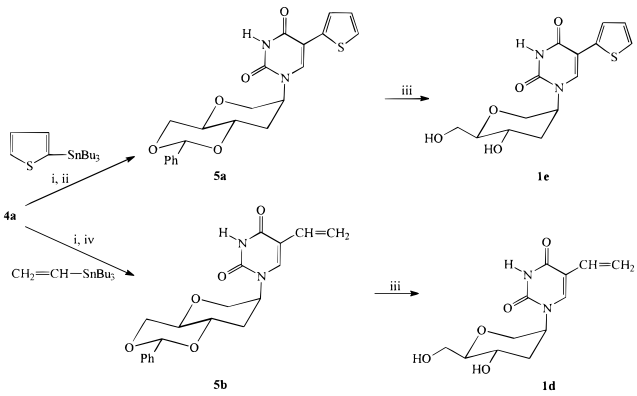
1,5-Anhydro-2,3-dideoxy-D-*arabino*-hexitol nucleosides are nucleoside analogues with a six-membered carbohydrate mimic and the base part substituted in the 2-position (Schemes 1−3, compounds **1a**−**g**). Because of the 1,3-relationship between base moiety and ring oxygen atom, the heterocycle substituent is oriented axially. This structural characteristic distinguishes this class of nucleosides from the hexose nucleosides with an anomeric positioned base moiety[1] and from their carbocyclic congeners,[2] both having equatorially oriented base moieties. The anhydrohexitol nucleosides are the sole nucleoside analogues with a six-membered carbohydrate moiety showing potent antiviral activity.[3,4] The 5-iodouracil[3] and 5-ethyluracil[4] congeners are highly selective thymidine kinase (TK)-dependent inhibitors of herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2). Broad antiherpes virus activity was noted for the 5-fluorocytosine and diaminopurine analogues.[4] From previous structure−activity relationship studies,[5] it appeared that the presence of 5-unsaturated substituents and the presence of small heterocycles in the 5-position of uracil[6] are compatible with antiviral activity. To test the hypothesis that these structural changes may also result in an increased antiviral activity in the anhydrohexitol series, we prepared compounds **1d**−**g** together with compound **1b** (as 5-chlorouracil may be a good mimic of the thymine base[7]) and **1c** (as a congener of 5-trifluoromethyl-2′-deoxyuridine[8]) and determined their antiviral activity. In the meantime we started studying the hypothesis of the conformational preference of hexitol nucleosides for antiviral activity. The 1C ⇌ C1 equilibrium of three representative examples of modified nucleosides with a six-membered carbohydrate moiety was studied using computational methods. The anhydrohexitol nucleoside with a 5-iodouracil base moiety was cocrystallized with HSV-1 thymidine kinase. From these results it can be

Scheme 1[a]



[a] (i) Ph_3P, DEAD, THF; (ii) NH_3, MeOH; (iii) 80% AcOH, 65 °C.

Scheme 2[a]



[a] (i) NH_3, MeOH; (ii) $(PH_3P)_2PdCl_2$, THF. (iii) 80% AcOH, 65 °C; (iv) $(Ph_3P)_2PdCl_2$, CH_3CN.

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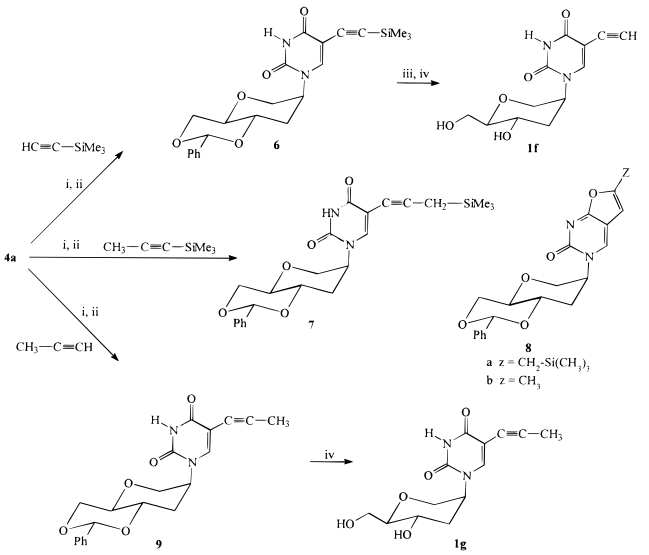
Scheme 3[a]



[a] (i) NH_3, MeOH: (ii) Et_3N, $(Ph_3P)_2PdCl_2$, CuI; (iii) KF, Et_4NBr, CH_3CN; (iv) 80% AcOH, 65 °C.

concluded that the viral thymidine kinase may induce conformational changes in an anhydrohexitol nucleoside.

Chemistry

1′,5′-Anhydro-2′,3′-dideoxy-2′-(5-iodouracil-1-yl)-D-*arabino*-hexitol (**1a**) was prepared using a Mitsunobu type condensation reaction between the alcohol **2** and N^3-benzoyl-5-iodouracil (**3a**).[9] The synthesis of **2** was performed according to a previously described eight-step procedure.[10] The Mitsunobu reaction was carried out making use of 1 equiv of **2**, 2 equiv of **3a**, 2.5 equiv of triphenylphosphine, and 2.5 equiv of diethyl azodicarboxylate in THF.[4] The product was isolated by chromatography as its *N*-benzoyl derivative as previously described for the thymine congener[11] to afford 71% of **4a**. Debenzoylation in MeOH saturated with ammonia and hydrolysis of benzylidene moiety with 80% aq AcOH at 65 °C gave **1a** in 64% yield (based on **4a**). Identical procedures were used for the synthesis of 5-chlorouracil and 5-(trifluoromethyl)uracil compounds **1b** and **1c**. The yields of condensation were 58% and 64%; the yields of two-step deprotection were 56% and 77%, respectively.

The 5-vinyluracil analogue **1d** was synthesized by the coupling reaction of debenzoylated **4a** with tributyl(vinyl)tin in the presence of bis(triphenylphosphine)palladium(II) chloride in acetonitrile at 55 °C in 85% yield (room temperature[3] was not sufficient for this transformation) giving **5b**. Deprotection of **5b** furnished **1d** in 68% yield. Replacement of iodine with 2-thienyl substituent was achieved using 2-(tributylstannyl)thiophene and a catalytic amount of bis(triphenylphosphine)palladium(II) chloride in refluxing THF overnight[12] to give 70% of **5a**. The desired **1e** was obtained by removing the benzylidene group in 80% aq AcOH at 65 °C in 69% yield.

Treatment of **4a** with methanolic ammonia in order to hydrolyze the benzoyl group and subsequent reaction with trimethylsilylacetylene, bis(triphenylphosphine)palladium(II) chloride, and copper(I) iodide[13] in triethylamine at 55 °C for 5 h resulted in the formation of the 5-(trimethylsilyl)ethynyl derivative (**6**) in 78% yield. Removal of the trimethylsilyl group was carried out by means of potassium fluoride/tetraethylammonium bromide. The reaction in refluxing acetonitrile was slower than originally reported.[14] After heating overnight, some byproducts were observed. Workup, deprotection of the sugar moiety, and chromatographic separation gave the 5-ethynyluracil compound **1f** in 42% yield based on **6**.

The above strategy was also used to synthesize the 5-(1-propynyl) analogue **1g**. Starting from debenzoylated **4a** and using propargyltrimethylsilane in triethylamine (the same catalysts and the same procedure as for preparing **6**), the 5-(3-trimethylsilyl-1-propynyl) derivative (**7**) was obtained in 37% yield as well as the fluorescent cyclized product **8a**. Attempted desilylation of **7** resulted in the formation of 1′,5′-anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-{6-methylfurano[2,3-*d*]pyrimidin-2-one-1-yl}-D-arabino-hexitol (**8b**) as the sole compound. Therefore we decided to use an alternative approach using gaseous propyne, which was generated by dehydrohalogenation of 1,2-dibromopropane and directly introduced into the reaction mixture. A preliminary attempt using tetrakis(triphenylphosphine)-palladium(0), copper(I) iodide, dimethylformamide, and 2 equiv of triethylamine[15] failed probably because of an inappropriate ratio of both catalysts. Another experiment, performed with Pd(II) catalyst in Et_3N at 55 °C according to the described method,[16] was also not successful, and resulted in formation of homogeneous **8b**. It was found that conducting the reaction at room temperature is absolutely necessary and that a reaction time of 3.5 h gives the optimal amount of the expected product in the mixture. Thus treating of **4a** with methanolic ammonia and then coupling with propyne catalyzed by bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide in triethylamine at room temperature gave only a trace of **8b** and 65% of **9**. The obtained yield is comparable with that reported previously for the synthesis of 5-(1-propynyl)-2′-deoxyuridine.[17] Removal of the benzylidene group proceeded without difficulties to afford **1g** in 70% yield.

The structures of the newly synthesized compounds were confirmed by proton and carbon nuclear magnetic resonance spectra (and compared with literature data on the corresponding 5-substituted 2′-deoxyuridines: iodo,[4] chloro,[7] trifluoromethyl,[18] 2-thienyl,[12,19] vinyl,[13,20] ethynyl[16] and 1-propynyl[16]), ultraviolet spectra, and mass spectrometry. In addition, the compounds (**1b**−**g**), which were tested for biological activity, were analyzed by elemental analysis.

Antiviral Activity

All compounds (**1a**−**g**) were evaluated for their antiviral activity against HSV-1 and HSV-2 as described previously[21] (Table 1). The antiviral data against HSV-1 were identical for the different strains tested (KOS, F, McIntyre). Likewise, the antiviral activity against HSV-2 was the same for all strains tested (G, 196, Lyons). The 5-chloro substituted compound **1b** was far less active against HSV-1, HSV-2, and varicella-zoster virus (VZV) than the 5-iodinated compound **1a**. In contrast to what might have been expected from the furanose series, the 5-thienyl compound (**1e**) and the

Table 1. Cytotoxicity and Antiherpes Activity of **1b**−**g** As Determined in E_6SM Cell Cultures

compd	min cytotoxic concn[a] (μg/mL)	minimum inhibitory concentration[b] (μg/mL) HSV-1[c]	HSV-2[d]	HSV-1 (TK⁻ B2006)	VZV[e] (YS)	CMV[e,f] (AD-169)
1a	>400	0.025	0.50	> 400	>100	>40
1b	>400	>400	>400	>400	>50	>50
1c	>400	0.025	0.38	9.8	49	>50
1d	>400	0.075	9.6	240	40	>50
1e	>400	240	240	240	>50	>50
1f	>400	48	>400	>400	>50	>50
1g	>400	0.38	16	48	24	>50
BVDU[g]	≥200	0.015	>80	3.2	0.0003	ND
ACV[g]	≥200	0.075	0.38	1.92	0.10	ND

[a] Required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenicity by 50%. [c] Reported values are the mean value of the antiviral activities determined against KOS, F, and McIntyre strain. [d] Reported values are means of G, 196, and Lyons strains. [e] Activity against varicella-zoster virus and cytomegalovirus was determined in HEL cells. [f] For anti-CMV activity, HPMPC (IC_{50}: 0.42 μg/mL) was used as reference. [g] BVDU: 5-bromovinyl-2′-deoxyuridine; ACV: acyclovir. ND: not determined.

5-ethynyl compound (**1f**) did not show significant antiviral activity. Elongation of the ethynyl substituent with one methyl group (giving the 5-propynyl substituted compound **1g**) increased the anti-HSV-1 activity (relative to **1f**) with a factor of 100 and the anti-HSV-2 activity with a factor of >25. This structure−function effect is the opposite of what might be expected from the data available from the study of 5-substituted 2′-deoxyuridine derivatives.[16] The 5-vinyl compound (**1d**) had similar activity as acyclovir against HSV-1 but was clearly less active against HSV-2. The 5-trifluoromethyl analogue (**1c**) emerged as the most interesting compound of this new series of hexitol nucleosides. Its activity profile against HSV-1 and HSV-2 was similar to that of acyclovir. The selectivity index of **1c** was >16 000 against HSV-1 and >1000 against HSV-2. When evaluated against TK⁻ strains of HSV-1, the antiviral activity of **1c** decreased by a factor of 400. Also, these data are similar to the data found for acyclovir. This indicates that phosphorylation of the primary hydroxyl group of **1c** is a crucial metabolic step leading to its antiviral activity. The change of the sugar moiety of 5-trifluoromethyl-2′-deoxyuridine from the deoxyribosyl[21] to the anhydrohexitol moiety thus results in maintenance of antiviral activity but loss of cellular toxicity. This might be due to the lack of interference of the 5′-monophosphate of **1c** with the host cell thymidylate synthase. The high specificity of the compound may also be demonstrated by its lack of anti-VZV and anti-CMV (CMV, cytomegalovirus) activity (Table 1). These in vitro data warrant further exploration of the in vivo efficacy of **1c** in the treatment of HSV infections, and, in particular, as a substitute for TFT in the treatment of ocular HSV infections.

Conformational Analysis of Pyranose and Hexitol Monomers Using Computational Methods

In an effort to understand the relationship between the structure of pyranose and hexitol nucleosides and their antiviral activity we first analyzed the conformational preference of three nucleoside analogues with a six-membered sugar moiety (Figure 1). Compound A is an example of an anhydrohexitol nucleoside as described in this and former publications.[3,4] Compound B has the same organization of functional groups as A, with the exception of the position of the ring-oxygen atom. This nucleoside analogue (B) has an anomeric center which might influence its conformational preference. Compound C is a pyranose nucleoside, from which the conformational analysis was described before.[22] While compound A demonstrates antiviral activity, compound C does not. The synthesis of compound B is in progress in our laboratory. The lowest energy conformations (kJ/mol) of compounds A, B, and C with the base moiety situated in an equatorial position or in an axial orientation were calculated (Experimental Section) together with the energy difference between both conformations (Table 2, Figure 2). For compound A, the energy difference between both conformations (axial and equatorial oriented base moieties) is small, indicating a possible interconversion at room temperature. Using computational calculations, the 1C conformation (with an equatorial base moiety) seems to be the lowest energy form. In solution phase (NMR) and in solid phase (X-ray) the opposite (C1) conformation is preferred,[3,4] indicating again the difficulties in predicting conformations of molecules by computational methods, especially when energy differences are low. A comparison of the energies at the 6-31G** level of theory

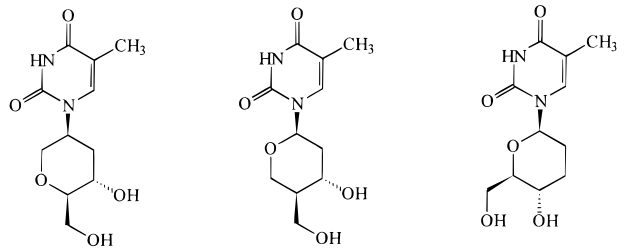


Figure 1. Structure of three different nucleosides analogues with a six-membered carbohydrate (mimic) used for conformational analysis by computational methods. (A) 1,5-anhydro-2,3-dideoxy-2-(thymin-1-yl)-D-*arabino*-hexitol, (B) 1-(2,4-dideoxy-4-*C*-hydroxymethyl-α-L-lyxopyranosyl)-thymine, (C) 1-(2,3-dideoxy-β-D-*erythro*-hexopyranosyl)thymine.

Table 2. Lowest Energy and Energy Differences (kJ/mol) between the 1C and C1 Conformation of Compounds A, B, and C

	compound A		compound B		compound C	
equatorial base	−294.8	[1C]	−251.6	[C1]	−310.1	[C1]
axial base	−288.9	[C1]	−234.3	[1C]	−284.6	[1C]
Δ*E*	5.9		16.9		25.5	

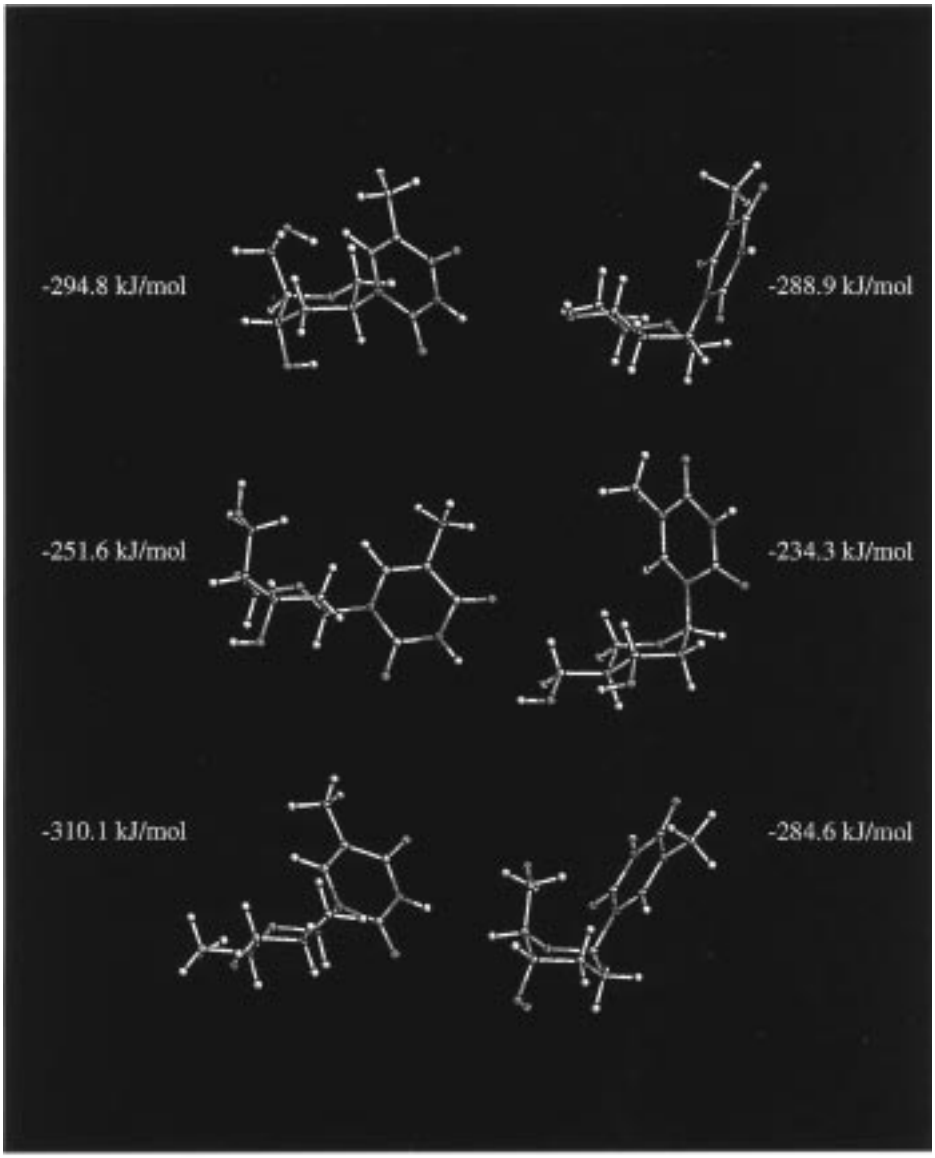


Figure 2. Lowest energies of compounds A, B, and C adopting conformations with an equatorial (left site) or axial (right site) base moiety.

confirms the above-mentioned observation (similar energies and differences). In contrast, larger energy differences are observed with compounds B and C. These compounds predominantly occur in the C1 conformation with an equatorial oriented base moiety. The easiest way to explain these results is by the preference of the largest substituents (hydroxymethyl group and especially the nucleobase) to obtain a nonhindered position (equatorial position or axial position with, as far as possible, absence of 1,3-diaxial interactions due to the position of the oxygen atom in the 3-position with respect to the bulky substituent). Stereoelectronic effects such as anomeric effect, gauche effects, and hydrogen bonding apparently play a minor role in this game. In compound A where the forces dictating the preferential conformation do not work in the same direction, the energy difference between the C1 and 1C form becomes low (5.9 kJ/mol). In conclusion, (a) the eventual occurrence of an equilibrium between both conformations of A in a biological medium should not be overlooked when trying to correlate structure with antiviral activity, and (b) compounds B and C are frozen in C1 conformation (ΔE = 16.9 and 25.5 kJ/mol, respectively) and this preference might play a role in explaining their biological (in)activity.

X-ray Crystallography Study of the Binding Hexitol Nucleoside to HSV-1 Thymidine Kinase

From Table 1, it may be concluded that compounds **1a** and **1c** demonstrate the highest activity against HSV-1. Because of its easier availability we determined the structure of compound **1a** in complex with the viral thymidine kinase.

The two molecules of TK in the asymmetric unit of the crystal have similar folds, with a root mean square difference of C-α positions of only 0.25 Å after superposition.

A first difference map of the complex, in which **1a** was not included in refinement, shows the ligand in a binding mode in which the 5-substituted iodine occupies the same region of the active site as do the iodo group of 5-iodo-2′-deoxyuridine (IDU) and the bromovinyl and bromothienyl moieties of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and 5-(5-bromothien-2-yl)-2′-deoxyuridine,[23]

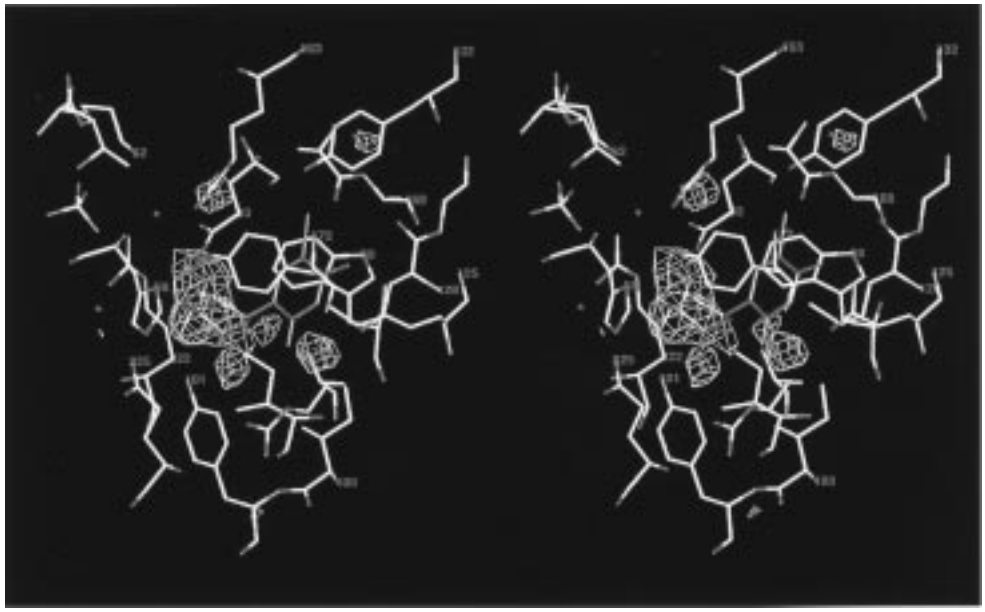


Figure 3. Relaxed stereoview of the 3σ difference electron density map in the TK (molecule I) active site calculated on the basis of refinement of structure into the active site of which only the 5-iodouracil moiety has been fitted. Density gives the shape and position of the anhydrohexitol moiety as well as the positions of nearby water molecules.

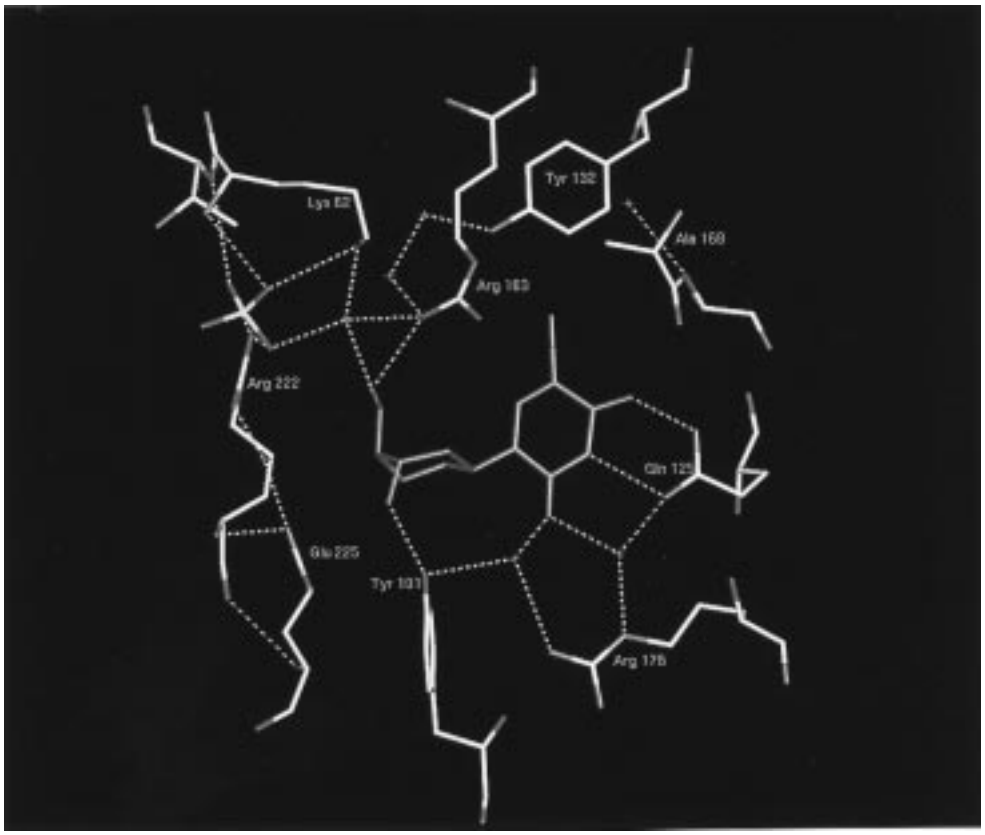


Figure 4. TK (molecule I) active site residues, **1a**, and waters, indicating pattern of hydrogen bonding. For clarity, groups above and below the approximate plane of the diagram, providing important van der Waals interactions (see text), are not shown.

respectively (although **1a** and IDU penetrate it less deeply than the last two). A subsequent difference map, following a refinement in which the atoms of the base were included while those of the cyclic group remained excluded, is shown in Figure 3 (for molecule I). The difference electron density is consistent with a 1C chair conformation. Apart from accommodation of the extra ring carbon, the binding mode of the anhydrohexitol of **1a** is very similar to the binding of the ribose moiety of deoxythymidine (dT) and of the other uracil analogues, as observed previously.[23] Its secondary hydroxyl group (Figure 4) makes a hydrogen bond with Tyr-101 and via a water to Arg-176. His-58 is close to this 3′-hydroxyl but not quite in hydrogen bond range. There are minor differences only between the active sites of TK molecules I and II, involving slightly altered side chain conformations for Glu-38, Tyr-132, Arg-163, Arg-222, and Glu-225. With molecule II of the asymmetric unit, but not

with molecule I, Glu-225 is also in hydrogen-bonding range. The hydroxyl of the hydroxymethyl group hydrogen binds to guanidinium of Arg-163, and via a water molecule to Glu-83, and through a second water to Tyr-132. Molecule II has the Arg-163 side chain further away (as with the dT-bound structure[23]), and there is no direct hydrogen bond. This hydroxyl is also hydrogen-bonded through an intermediate water to the sulfate ion and the side chain of Lys-62. In contrast to the crystal structure determined for the small molecule alone,[4] the base and the hydroxymethyl group substitute respectively equatorially and axially. Except as indicated, this pattern of contacts is similar to that seen in the dT-bound (native) complex. In addition to the hydrogen bond interactions, van der Waals contacts occur between the substituted base and Ile-100, Met-128, Tyr-132, Ala-168, and Tyr-172 (see Figure 3) and between the six-carbon ring and His-58, Ile-97, and Arg-222. These results demonstrate that the viral thymidine kinase is able to induce conformational change(s) in the anhydrohexitol nucleoside **1a** (the so-called induced-fit principle).

Discussion

The structure−activity relationship (SAR) study of anhydrohexitol nucleosides reveals that, although some similarities exist with the SAR of 5-substituted 2′-deoxyribopyrimidine nucleosides, there are enough dissimilarities to consider the anhydrohexitol nucleosides as a separate class of antivirals. Indeed, some congeners (i.e., **1e** and **1f**) that are active against HSV in the deoxyuridine series are not active in the anhydrohexitol series. The anhydrohexitol nucleosides are generally more selective against HSV than 5-substituted 2′-deoxyuridine nucleosides. The latter compounds mostly show activity against HSV as well as VZV, which is not the case for the anhydrohexitol analogues. The higher discriminative properties of anhydrohexitol nucleosides may result in less toxic compounds as demonstrated here with **1c**. The trifluoromethyl analogue (**1c**) has an activity profile against HSV-1 and HSV-2 similar to acyclovir but lacks the toxicity of its 5-trifluoromethyl-2′-deoxyuridine congener. A possible explanation for that may be the lack of interference of the 5′-monophosphate of **1c** with host cell thymidylate (dTMP) synthase. For the 5-substituted 2′-deoxyuridines a direct correlation has been found between their cytotoxic (cytostatic) activity and their inhibitory effect on dTMP synthase,[24] and 5-trifluoromethyl-2′-deoxyuridine (TFT) appeared to be one of the most potent compounds in this regard. The comparative effects of the 5′-monophosphates of **1c** and TFT on dTMP synthase are now under further investigation.

There is no doubt that conformational preference of nucleosides may play a role in their antiviral activity.[25] This may be studied by using model nucleosides with a more rigid conformation than natural 2′-deoxynucleosides. As an example, two 2′-deoxy-methanocarba-nucleosides were synthesized as mimics of 2′-deoxynucleosides fixed in the northern or southern conformation.[26] Antiherpes activity was demonstrated for the former series, but not for the latter. These studies are very useful but not easy to interpret because (a) besides conformational restriction also new sterical effects are introduced and (b) antiviral activity is a result of interaction with several metabolic viral/human enzymes, and it is difficult to guess which enzyme is responsible for the discrimination between both modified nucleosides. Nucleosides have to be phosphorylated to their 5′-mono-, 5′-di-, and 5′-triphosphate before interfering with DNA synthesis. The structural requirements for the different phosphorylation steps and for interaction with DNA polymerases may be different. For the study of this conformation−function relationship, it would be advantageous to have at our disposition an antiviral nucleoside which may occur in several model conformations which are interconvertible at room temperature. Cocrystallization experiments of the nucleoside itself or one of its phosphorylated forms with the different enzymes involved in the metabolism of the antiviral nucleoside might give us a better insight in the critical role played by the conformational preferences for antiviral activity.

Although anhydrohexitol nucleosides occur both in solid phase and solution in a C1 conformation,[3,4] computational calculations indicate the possibility of the existence of a C1 ⇌ 1C equilibrium with a ΔE of 5.9 kJ/mol. This energy barrier may be easily overcome in a biological medium, which means that both conformations may exist in vivo. In the C1 form, the anhydrohexitol nucleoside may be considered as a mimic of a furanose nucleoside in the C3′-*endo* conformation[2], while in the 1C form the resemblance with one of the two most common puckering conformations of a furanose nucleoside (C2′-*endo* and C3′-*endo*) is less clear. Cocrystallization experiments between 1′,5′-anhydro-2′,3′-dideoxy-2′-(5-iodouracil-1-yl)-D-*arabino*-hexitol **1a** and the thymidine kinase of HSV-1 indicate that the nucleoside adopts the uncommon 1C conformation. This means that (a) the enzyme is able to induce a conformational change in the carbohydrate moiety of the nucleosides (the *anti*-position of the base moiety where the amide functions of the base is hydrogen bonded with Gln-125 is not changed) and (b) adopting a C3′-*endo* conformation of a furanose nucleoside does not seem to be a strict requirement for binding to the viral TK. In the cocrystallized conformation, the secondary hydroxyl group of the hexitol nucleosides is hydrogen binding with Tyr-101 (and Glu-225), and the primary hydroxyl group of the modified nucleoside is connected to Arg-163 and via a water molecule to Gln-83. This situation is very similar to the binding mode of thymidine itself.[6,23] As far as cocrystallization data may be used as a model for studying the binding of substrates to enzymes, it can be concluded that the antiviral activity of anhydrohexitol nucleosides and of (N)methanocarba-T[26] cannot be due to the requirement of the viral thymidine kinase to accept only N-type nucleosides. However, these data should be interpreted very carefully as it is not a dynamic study and conformations may be changed during phosphorylation reactions. The cocrystallization of the 6′-monophosphate of anhydrohexitol nucleosides with the HSV-1 TK is underway, as the binding mode of the phosphorylated form may be different from the binding of the unphosphorylated form.

Although at this moment still speculative, the antiviral activity of anhydrohexitol nucleosides seems to be better explained by the preference of the nucleoside

analogue to take a C1 conformation at the level of the DNA polymerase. When anhydrohexitol nucleotides are oligomerized, hexitol nucleic acids (HNA) are formed. Structural analysis demonstrates that HNA adopt an A-form conformation[27−29] and are mimics of dsRNA where nucleotides occur in C3′-*endo* conformations. The axial position of the base moiety is important to obtain an helical oligonucleotide structure. With equatorial oriented base moieties (1C conformation of hexitol nucleoside) no regular nucleic acid helix can be obtained.[27,29] It should not be forgotten that the first four nucleotides at the 3′-end (important for minor-groove recognition by DNA polymerases) of the DNA polymerase-primer complex[30,31] adopt a C3′-*endo* conformation (except for the first nucleotide which occupies a C2′-*endo* conformation)[31] characteristic of A-form DNA and that the DNA outside the minor-groove recognition region adopts the regular B-form conformation (C2′-*endo* sugar pucker).[30,31] In conclusion, the thymidine kinase of HSV-1 remains an important selectivity filter for antiviral activity but the observed relationship between antiviral activity and the preference for N-type puckered nucleosides[3,4,25,26] is likely due to a selection at the level of the DNA polymerases.

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 NMR and ^{13}C NMR were determined with a 200 MHz Varian Gemini apparatus 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 MASPEC2 data system (Mass Spectrometry Services Ltd., Manchester, U.K.). Samples were directly dissolved in glycerol (gly)/thioglycerol (thgly)/*m*-nitrobenzyl alcohol (nba) and the secondary ions 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 (in solvent systems: A CH_2Cl_2−MeOH 98:2, B CH_2Cl_2−MeOH 9:1, C CH_2Cl_2−EtOAc 4:1); the spots were examined with UV light and sulfuric acid/anisaldehyde spray. Anhydrous THF was stored on sodium/benzophenone, refluxed, and distilled. Elemental analyses were done at the University of Konstanz, Germany.

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′-(*N*^{3}-benzoyl-5-iodouracil-1-yl)-2′,3′-dideoxy-D-*arabino*-hexitol (4a). To a solution of *N*^{3}-benzoyl-5-iodouracil[9] (**3a**, 2.053 g, 6.0 mmol), 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol[10] (**2**, 709 mg, 3.0 mmol) and triphenylphosphine (1.97 g, 7.5 mmol) in anhydrous THF (75 mL) was added a solution of diethyl azodicarboxylate (1.18 mL, 7.5 mmol) in anhydrous THF (15 mL) over a period of 4 h under N_2 at room temperature. The reaction mixture was then stirred intensively under N_2 at room temperature overnight and evaporated, and the resulting oil was dissolved in CH_2Cl_2 and purified by silica gel column chromatography (0.035−0.070 mm, 23 cm × 4.5 cm) using CH_2Cl_2. The fractions containing product **4a** were evaporated to give 1.194 g (71%) of white solid: UV (MeOH) λ_{max} 253 nm (ϵ 26440), 286 nm (ϵ 13480); LSIMS (thgly) *m*/*e* 561 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.06 (m, 1H, 3′-H), 2.48 (br d, 1H, 3′-H, J = 13.8 Hz), 3.54 (m, 1H, 5′-H), 3.80 (m, 1H, 4′-H), 3.82 (t, 1H, 6′-H, J = 10.6 Hz), 4.04 (dd, 1H, 1′-H, J = 14.2 Hz, J = 3.5 Hz), 4.28 (br d, 1H, 1′-H, J = 14.2 Hz), 4.37 (dd, 1H, 6′-H, J = 10.5 Hz, J = 4.8 Hz), 4.72 (br s, 1H, 2′-H), 5.65 (s, 1H, PhCH), 7.36−7.67 (m, 8H, aromatic H), 7.92 (d, 2H, aromatic H, J = 7.2 Hz), 8.57 (s, 1H, 6-H) ppm; ^{13}C NMR ($CDCl_3$) δ 32.78 (C-3′), 52.54 (C-2′), 67.83 (C-5), 68.73, 68.81 (C-6′, C-1′), 73.49 (C-4′), 74.40 (C-5′), 102.09 (CHPh), 126.07, 128.40, 129.27, 130.56, 130.95, 135.35, 136.95 (Ph), 146.53 (C-6), 149.50 (C-2), 158.77 (C-4), 167.67 (NC=O) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-(5-iodouracil-1-yl)-D-*arabino*-hexitol (1a). Compound **4a** (213 mg, 0.38 mmol) was dissolved in MeOH saturated with NH_3 (30 mL) and kept overnight at room temperature. Evaporation left a solid which was treated with 80% aqueous acetic acid (30 mL). The solution was heated at 65 °C for 4 h. After evaporation and coevaporation with toluene (3×) and ethanol (2×), the residue was adsorbed on silica gel (0.060−0.200 mm) by evaporation with EtOH. It was purified (26 cm × 3 cm column) using CH_2Cl_2−MeOH 93:7 as eluent. Compound **1a** [89 mg (64%)] was crystallized from MeOH. Analytical data were identical to that of an authentic sample.[4]

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′-(*N*^{3}-benzoyl-5-chlorouracil-1-yl)-2′,3′-dideoxy-D-*arabino*-hexitol (4b). *N*^{3}-Benzoyl-5-chlorouracil[9] (**3b**, 1.003 g, 4.0 mmol), the alcohol **2**[10] (472 mg, 2.0 mmol) and Ph_3P (1.312 g, 5.0 mmol) in anhydrous THF (60 mL), and DEAD (788 μL, 5.0 mmol) in anhydrous THF (13 mL) were used for the reaction that was performed as described for **4a**. The crude reaction mixture was dissolved in CH_2Cl_2 and chromatographed on a silica gel column (0.035−0.070 mm, 20 cm × 4 cm) using CH_2Cl_2 → CH_2Cl_2−MeOH 99:1 as eluent. Fractions containing product **4b** were evaporated to give 544 mg (58%). The isolated material was crystallized from CH_2Cl_2−MeOH: UV (MeOH) λ_{max} 254 nm (ϵ 19060), 285 nm (ϵ 10880); LSIMS (thgly) *m*/*e* 469 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.08 (m, 1H, 3′-H), 2.47 (br d, 1H, 3′H, J = 14.2 Hz), 3.53 (m, 1H, 5′-H), 3.78 (m, 1H, 4′-H), 3.84 (t, 1H, 6′-H, J = 10.2 Hz), 4.05 (dd, 1H, 1′-H, J = 13.8 Hz, J = 3.4 Hz), 4.29 (br d, 1H, 1′-H, J = 13.6 Hz), 4.38 (dd, 1H, 6′-H, J = 10.6 Hz, J = 5.0 Hz), 4.73 (br s, 1H, 2′-H), 5.65 (s, 1H, PhCH), 7.36−7.68 (m, 8H, aromatic H), 7.93 (d, 2H, aromatic H, J = 7.6 Hz), 8.38 (s, 1H, 6-H) ppm; ^{13}C NMR ($CDCl_3$) δ 32.93 (C-3′), 52.79 (C-2′), 68.94 (C-6′, C-1′), 73.65 (C-4′), 74.65 (C-5′), 102.29 (CHPh), 109.12 (C-5), 126.26, 128.60, 129.49, 130.78, 131.15, 135.65; 137.13 (Ph), 139.13 (C-6), 149.13 (C-2), 158.07 (C-4), 167.61 (NC=O) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-(5-chlorouracil-1-yl)-D-*arabino*-hexitol (1b). Starting from **4b** (494 mg, 1.05 mmol) and following the same method as for **1a** (80 mL of NH_3/MeOH, then 80 mL of 80% aq AcOH), the title compound **1b** was obtained in pure form after purification on silica gel (0.035−0.070 mm, 22 cm × 4 cm) using CH_2Cl_2−MeOH 93:7 as eluent [162 mg (56%)]. An analytical sample was crystallized from ethyl acetate: mp 172 °C; UV (MeOH) λ_{max} 281 nm (ϵ 9120); LSIMS (thgly) *m*/*e* 277 $(M + H)^+$, 275 $(M - H)^-$; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H, 3′-H), 2.12 (br d, 1H, 3′H, J = 13.2 Hz), 3.14 (m, 1H, 5′-H), 3.58 (m, 3H, 4′-H, 6′-H), 3.75 (dd, 1H, 1′-H, J = 13.2 Hz, J = 3.5 Hz), 4.06 (br d, 1H, 1′-H, J = 12.9 Hz), 4.50 (br s, 1H, 2′-H), 4.69 (br s, 1H, 6′-OH), 4.93 (br s, 1H, 4′-OH), 8.29 (s, 1H, 6-H), 11.81 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 35.03 (C-3′), 51.27 (C-2′), 60.39 (C-6′), 60.83 (C-4′), 66.95 (C-1′), 82.66 (C-5′), 106.37 (C-5), 140.55 (C-6), 150.31 (C-2), 159.27 (C-4) ppm. Elemental analysis $C_{10}H_{13}N_2O_5Cl \times 0.75\ H_2O$ (C, H, N).

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′-[*N*^{3}-benzoyl-5-(trifluoromethyl)uracil-1-yl]-2′,3′-dideoxy-D-*arabino*-hexitol (4c). *N*^{3}-Benzoyl-5-(trifluoromethyl)uracil[9] (**3c**, 1.705 g, 6.0 mmol), the alcohol **2**[10] (709 mg, 3.0 mmol) and Ph_3P (1.97 g, 7.5 mmol) in anhydrous THF (60 mL), and DEAD (1.18 mL, 7.5 mmol) in anhydrous THF (15 mL) were used for the reaction that was performed as described for **4a**. The crude foam was dissolved in CH_2Cl_2 and chromatographed on a silica gel column (0.035−0.070 mm, 22 cm × 4 cm, eluent CH_2Cl_2) affording 964 mg (64%) of **4c**: UV (MeOH) λ_{max} 256 nm (ϵ 29550), ∼ 280 nm (sh); LSIMS (thgly) *m*/*e* 503 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.13 (m, 1H, 3′-H), 2.48 (br d, 1H, 3′H, J = 14.2 Hz), 3.58 (m, 1H, 5′-H), 3.73 (m, 1H, 4′-H), 3.78 (t, 1H, 6′-H, J = 10.2 Hz), 4.07 (dd, 1H, 1′-H, J = 13.9 Hz, J = 3.3

Hz), 4.31 (br d, 1H, 1′-H, J = 16.5 Hz), 4.39 (dd, 1H, 6′-H, J = 10.5 Hz, J = 4.8 Hz), 4.77 (br s, 1H, 2′-H), 5.64 (s, 1H, PhCH), 7.36−7.69 (m, 8H, aromatic H), 7.93 (d, 2H, aromatic H, J = 7.2 Hz), 8.70 (s, 1H, 6-H) ppm; ^{13}C NMR ($CDCl_3$) δ 32.54 (C-3′), 52.69 (C-2′), 68.72 (C-6′, C-1′), 73.40 (C-4′), 74.45 (C-5′), 102.15 (CHPh), 105.24 (C-5, ^2J = 33.2 Hz), 121.64 (CF_3, ^1J = 271.1 Hz), 126.04, 128.37, 129.31, 130.50, 130.91, 135.53, 136.86 (Ph), 142.80 (C-6, ^3J = 6.0 Hz), 148.95 (C-2), 157.26 (C-4), 167.21 (NC═O) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-[5-(trifluoromethyl)uracil-1-yl]-D-*arabino*-hexitol (1c). Starting with **4c** (866 mg, 1.72 mmol) and following the same method as for **1a** (100 mL of NH_3/MeOH, then 80 mL of 80% aq AcOH), the title compound was obtained, which was purified on silica gel (0.035−0.070 mm, 24 cm × 4 cm, using CH_2Cl_2−MeOH 93:7 as eluent) yielding **1c** [412 mg (77%)]. An analytical sample was crystallized from CH_2Cl_2−MeOH: mp 189 °C; UV (MeOH) λ_{max} 266 nm (ϵ 13050); LSIMS (thgly) m/e 311 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.74 (m, 1H, 3′-H), 2.15 (br d, 1H, 3′H, J = 13.6 Hz), 3.15 (m, 1H, 5′-H), 3.53 (m, 2H, 4′-H, 6′-H), 3.63 (m, 1H, 6′-H), 3.78 (dd, 1H, 1′-H, J = 13.2 Hz, J = 3.3 Hz), 4.12 (br d, 1H, 1′-H, J = 12.8 Hz), 4.54 (br s, 1H, 2′-H), 4.63 (t, 1H, 6′-OH, J = 5.2 Hz), 4.93 (d, 1H, 4′-OH, J = 5.3 Hz), 8.57 (s, 1H, 6-H), 11.86 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 35.00 (C-3′), 51.52 (C-2′), 60.63 (C-4′, C-6′), 67.12 (C-1′), 82.94 (C-5′), 102.22 (C-5, ^2J = 31.7 Hz), 122.90 (CF_3, ^1J = 269.2 Hz), 144.61 (C-6, ^3J = 6.0 Hz), 150.37 (C-2), 159.14 (C-4) ppm. Elemental analysis $C_{11}H_{13}N_2O_5F_3$ (C, H, N).

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-[5-(2-thienyl)uracil-1-yl]-D-*arabino*-hexitol (5a). Compound **4a** (840 mg, 1.5 mmol) was treated with NH_3/MeOH (80 mL) and kept overnight at room temperature and evaporated. The solid residue was dissolved in anhydrous THF (60 mL), and to this solution bis(triphenylphosphine)palladium(II) chloride (28 mg, 0.04 mmol) was added followed by 2-(tributylstannyl)-thiophene (1.903 g, 5.1 mmol). The mixture was refluxed and stirred overnight under N_2. TLC evaluation (A) showed a violet spot of the product (R_f = 0.61) and only a trace of debenzoylated starting material (R_f = 0.56). The mixture was evaporated, redissolved in CH_2Cl_2, applied onto a silica gel column (0.060−0.200 mm, 18 cm × 4 cm), and eluted with CH_2Cl_2 → CH_2Cl_2−MeOH 99:1. Fractions containing the title compound were evaporated to afford 432 mg (70%) of **5a**: UV (MeOH) λ_{max} 262 nm (ϵ 10920), 320 nm (ϵ 10540); LSIMS (thgly) m/e 411 $(M - H)^-$; ^{1}H NMR ($CDCl_3$) δ 2.14 (m, 1H, 3′-H), 2.48 (br d, 1H, 3′-H, J = 13.3 Hz), 3.59 (m, 1H, 5′-H), 3.75 (m, 1H, 4′-H), 3.81 (t, 1H, 6′-H, J = 9.1 Hz), 4.08 (dd, 1H, 1′-H, J = 13.6 Hz, J = 3.4 Hz), 4.33 (br d, 1H, 1′-H, J = 16.5 Hz), 4.41 (dd, 1H, 6′-H, J = 10.5 Hz, J = 4.6 Hz), 4.83 (br s, 1H, 2′-H), 5.59 (s, 1H, PhCH), 7.09 (dd, 1H, 4″-H, J = 5.1 Hz, J = 3.7 Hz), 7.30−7.48 (m, 7H, Ph, 3″, 5″-H), 8.54 (s, 1H, 6-H), 8.81 (br s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 32.81 (C-3′), 51.76 (C-2′), 68.90 (C-6′, C-1′), 73.62 (C-4′), 74.25 (C-5′), 102.09 (CHPh), 109.92 (C-5), 124.39, 125.34, 126.03 (C-3″, C-4″, C-5″), 127.29, 128.35, 129.21 (Ph), 133.64 (C-2″), 136.95 (Ph), 137.34 (C-6), 149.77 (C-2), 160.81 (C-4) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-[5-(2-thienyl)uracil-1-yl]-D-*arabino*-hexitol (1e). Compound **5a** (402 mg, 0.97 mmol) was dissolved in 80% aq AcOH and heated at 65 °C for 3 h. The mixture was evaporated and coevaporated with toluene (3×) and with EtOH (2×). The product (observed by TLC, B) was purified chromatographically using silica gel 0.035−0.070 mm, 22 cm × 4 cm column, and CH_2Cl_2−MeOH 93:7 as eluent to give 217 g (69%) of **1e**. An analytical sample was crystallized from 50% aq EtOH: mp 208 °C; UV (MeOH) λ_{max} 261 nm (ϵ 12090), 321 (ϵ 12220); LSIMS (gly) m/e 323 $(M - H)^-$; ^{1}H NMR (DMSO-d_6) δ 1.79 (m, 1H, 3′-H), 2.17 (br d, 1H, 3′-H, J = 12.2 Hz), 3.17 (m, 1H, 5′-H), 3.66 (m, 3H, 4′-H, 6′-H), 3.82 (dd, 1H, 1′-H, J = 12.8 Hz, J = 3.0 Hz), 4.15 (br d, 1H, 1′-H, J = 13.0 Hz), 4.61 (br s, 1H, 2′-H), 4.78 (t, 1H, 6′-OH, J = 5.4 Hz), 4.95 (d, 1H, 4′-OH, J = 5.3 Hz), 7.04 (dd, 1H, 4″-H, J = 5.1 Hz, J = 3.7 Hz), 7.39 (dd, 1H, 3″-H, J = 3.7 Hz, J = 1.2 Hz), 7.45 (dd, 1H, 5″-H, J = 5.1 Hz, J = 1.1 Hz), 8.61 (s, 1H, 6H), 11.69 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 35.42 (C-3′), 50.95 (C-2′), 60.39 (C-6′), 60.49 (C-4′), 67.36 (C-1′), 82.69 (C-5′), 107.71 (C-5), 122.70, 125.74, 126.70 (C-3″, C-4″, C-5″), 134.41 (C-2″), 138.74 (C-6), 150.13 (C-2), 161.45 (C-4) ppm. Elemental analysis $C_{14}H_{16}N_2O_5S$ (C, H, N).

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-(5-vinyluracil-1-yl)-D-*arabino*-hexitol (5b). Compound **4a** (807 mg, 1.44 mmol) was treated with NH_3/MeOH (80 mL) and stirred overnight at room temperature. After evaporation, the solid residue was dissolved in CH_3CN (50 mL) and to this solution bis(triphenylphosphine)palladium(II) chloride (25 mg, 0.036 mmol) was added followed by tributyl(vinyl)tin (1.554 g, 4.9 mmol). The mixture was stirred vigorously and heated at 55 °C for 5 h under N_2. TLC evaluation (C, 2× developed) demonstrated a black spot of the product (R_f = 0.56) and practically no debenzoylated substrate (R_f = 0.65). The reaction mixture was evaporated, dissolved in CH_2Cl_2−MeOH 99:1, applied onto a silica gel column (0.060−0.200 mm, 18 cm × 4 cm), and eluted with CH_2Cl_2−MeOH 99:1. Fractions containing the title compound were evaporated to afford 530 mg of material that was still contaminated with tributyl(vinyl)-tin (0.25 equiv according to ^{1}H NMR). The calculated yield of **5b** itself was 85% (435 mg): UV (MeOH) λ_{max} 235 nm (ϵ 9790), 293 nm (ϵ 8050); LSIMS (thgly) m/e 355 $(M - H)^-$; ^{1}H NMR ($CDCl_3$) δ 2.09 (m, 1H, 3′-H), 2.47 (br d, 1H, 3′H, J = 13.8 Hz), 3.55 (m, 1H, 5′-H), 3.73 (m, 1H, 4′-H), 3.76 (t, 1H, 6′-H, J = 10.3 Hz), 4.03 (dd, 1H, 1′-H, J = 13.6 Hz, J = 3.4 Hz), 4.26 (br d, 1H, 1′-H, J = 14.8 Hz), 4.37 (dd, 1H, 6′-H, J = 10.5 Hz, J = 4.8 Hz), 4.76 (br s, 1H, 2′-H), 5.29 (dd, 1H, vinylic 2″-H, J = 11.3 Hz, J = 1.4 Hz), 5.58 (s, 1H, PhCH), 6.02 (dd, 1H, vinylic 2″-H, J = 17.6 Hz, J = 1.4 Hz), 6.46 (dd, 1H, vinylic 1″-H, J = 17.6 Hz, J = 11.5 Hz), 7.34−7.49 (m, 5H, Ph), 8.12 (s, 1H, 6-H), 9.17 (br s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 32.86 (C-3′), 51.71 (C-2′), 68.85 (C-6′, C-1′), 73.66 (C-4′), 74.27 (C-5′), 102.07 (CHPh), 112.50, 116.06 (C-5, CH_2 vinylic), 126.06, 127.93, 128.37, 137.01 (Ph), 129.22 (CH vinylic), 138.93 (C-6), 150.05 (C-2), 161.83 (C-4) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-(5-vinyluracil-1-yl)-D-*arabino*-hexitol (1d). Compound **5b** (435 mg, 1.22 mmol) was converted into **1d** using the same procedure as described for **1e**. Chromatography yielded (CH_2Cl_2−MeOH 93:7 as eluent) 222 mg (68%) of the title product. An analytical sample was crystallized from CH_2Cl_2−MeOH: mp > 300 °C (dec); UV (MeOH) λ_{max} 238 nm (ϵ 11370), 292 (ϵ 9930); LSIMS (thgly) m/e 269 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.74 (m, 1H, 3′-H), 2.11 (br d, 1H, 3′-H, J = 14.8 Hz), 3.15 (m, 1H, 5′-H), 3.64 (m, 3H, 4′-H, 6′-H), 3.77 (dd, 1H, 1′-H, J = 13.1 Hz, J = 3.3 Hz), 4.06 (br d, 1H, 1′-H, J = 12.0 Hz), 4.56 (br s, 1H, 2′-H), 4.70 (t, 1H, 6′-OH, J = 6.1 Hz), 4.92 (d, 1H, 4′-OH, J = 5.3 Hz), 5.09 (dd, 1H, vinylic 2″-H, J = 11.3 Hz, J = 2.1 Hz), 5.93 (dd, 1H, vinylic 2″-H, J = 17.6 Hz, J = 2.2 Hz), 6.38 (dd, 1H, vinylic 1″-H, J = 17.6 Hz, J = 11.4 Hz), 8.20 (s, 1H, 6-H), 11.42 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 35.25 (C-3′), 50.62 (C-2′), 60.19 (C-6′), 60.45 (C-4′), 67.09 (C-1′), 82.44 (C-5′), 110.24, 113.90 (C-5, CH_2 vinylic), 129.38 (CH vinylic), 142.02 (C-6), 150.26 (C-2), 162.24 (C-4) ppm. Elemental analysis $C_{12}H_{16}N_2O_5 \times 1H_2O$ (C, H, N).

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-[5-(trimethylsilyl)ethynyluracil-1-yl]-D-*arabino*-hexitol (6). Compound **4a** (1.194 g, 2.13 mmol) was dissolved in NH_3/MeOH (100 mL) and stirred overnight at room temperature, and the reaction mixture was evaporated. The solid residue was treated with deoxygenated (by flushing with N_2) triethylamine (85 mL), trimethylsilylacetylene (628 mg, 6.39 mmol), bis(triphenyl-phosphine)palladium(II) chloride (35 mg, 0.05 mmol), and copper(I) iodide (35 mg, 0.18 mmol). The suspension was stirred at 55 °C for 5 h under N_2. The volatiles were removed in vacuo. The residue was dissolved in CH_2Cl_2 (ca. 50 mL), and washed with 5% aq disodium EDTA (2 × 50 mL) and H_2O (50 mL). The organic layer was dried with Na_2SO_4, concentrated, and applied onto a silica gel column (0.060−0.200 mm, 19 cm × 5 cm). The column was eluted with CH_2Cl_2−MeOH 99:1, giving 708 mg (78%) of **6**: UV (MeOH) λ_{max} 234 nm (ϵ 10990), 298 nm (ϵ 13250); LSIMS (thgly) m/e 427 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 0.27 (br s, 9H, 3 × CH_3), 2.09

(m, 1H, 3′-H), 2.45 (br d, 1H, 3′H, J = 13.2 Hz), 3.54 (m, 1H, 5′-H), 3.74 (m, 1H, 4′-H), 3.82 (t, 1H, 6′-H, J = 10.2 Hz), 4.02 (dd, 1H, 1′-H, J = 13.9 Hz, J = 2.6 Hz), 4.27 (br d, 1H, 1′-H, J = 13.9 Hz), 4.37 (dd, 1H, 6′-H, J = 10.3 Hz, J = 4.5 Hz), 4.74 (br s, 1H, 2′-H), 5.63 (s, 1H, PhCH), 7.35−7.50 (m, 5H, Ph), 8.28 (s, 1H, 6-H), 8.98 (br s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 32.88 (C-3′), 52.44 (C-2′), 68.81, 68.89 (C-6′, C-1′), 73.57 (C-4′), 74.53 (C-5′), 95.41, 100.06, 100.42 (ethynyl C-1″, C-2″, C-5), 102.14 (CHPh), 126.22, 128.52, 129.37, 137.18 (Ph), 145.53 (C-6), 149.85 (C-2), 161.25 (C-4) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-(5-ethynyluracil-1-yl)-D-*arabino*-hexitol (1f). To a solution of **6** (810 mg, 1.9 mmol) in CH_3CN (80 mL) potassium fluoride (221 mg, 3.8 mmol) and tetraethylammonium bromide (798 mg, 3.8 mmol) were added. The suspension was intensively stirred and heated under reflux (N_2) overnight (TLC, A: **6**: R_f = 0.37, product of desilylation: R_f = 0.32). The residual oil, after evaporation, was extracted in CH_2Cl_2 (8×). The combined organic phase was dried with Na_2SO_4 and evaporated to give 720 mg of the crude oil. This oil was heated in 80% aq AcOH (50 mL) at 65 °C for 3 h. The mixture was evaporated and coevaporated with toluene (3×) and with EtOH (2×). It was chromatographed using silica gel (0.060−0.200 mm, 28 cm × 5 cm column) and CH_2Cl_2−MeOH 93:7 as eluent giving **1f**, 211 mg (42%). The isolated product was crystallized from MeOH: mp 235−237 °C (dec); UV (MeOH) λ_{max} 228 nm (ϵ 9510), 292 (ϵ 11350); LSIMS (nba) m/e 267 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H, 3′-H), 2.12 (br d, 1H, 3′-H, J = 13.3 Hz), 3.16 (m, 1H, 5′-H), 3.59 (m, 3H, 4′-H, 6′-H), 3.75 (dd, 1H, 1′-H, J = 13.6 Hz, J = 3.0 Hz), 4.06 (br d, 1H, 1′-H, J = 9.4 Hz), 4.08 (s, 1H, ethynyl H), 4.50 (br s, 1H, 2′-H), 4.67 (m, 1H, 6′-OH), 4.91 (d, 1H, 4′-OH, J = 4.9 Hz), 8.29 (s, 1H, 6-H), 11.61 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 35.08 (C-3′), 51.33 (C-2′), 60.55 (C-6′), 61.06 (C-4′), 66.99 (C-1′), 76.86 (ethynyl CH), 82.81 (C-5′), 83.62 (ethynyl C), 97.00 (C-5), 147.18 (C-6), 150.21 (C-2), 161.94 (C-4) ppm. Elemental analysis $C_{12}H_{14}N_2O_5 \times 0.5\ H_2O$ (C, H, N).

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-[5-(3-trimethylsilyl-1-propynyl)-uracil-1-yl]-D-*arabino*-hexitol (7). Compound **4a** (969 mg, 1.73 mmol) was debenzoylated using NH_3/MeOH (70 mL) overnight at room temperature. Starting with the obtained crude residue, deoxygenated (by flushing with N_2) triethylamine (85 mL), propargyltrimethylsilane (874 mg, 7.78 mmol), bis(triphenylphosphine)palladium(II) chloride (42 mg, 0.06 mmol), and copper(I) iodide (38 mg, 0.20 mmol) and using the same procedures as for **6**, compound **7** was obtained. The reaction mixture itself was composed of unreacted substrate (TLC, A, 3× developed: R_f = 0.69), **7** (R_f = 0.74), and fluorescent product **8a** (R_f = 0.67). The mixture was separated by column chromatography (silica gel 0.035−0.070 mm, 24 cm × 5 cm, CH_2Cl_2 → CH_2Cl_2−MeOH 99.5:0.5 as eluent) to yield 281 mg (37%) of **7** [UV (MeOH) λ_{max} 231 nm (ϵ 11770), 298 nm (ϵ 11900); LSIMS (thgly) m/e 441 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 1.74 (s, 2H, CH_2−Si), 4.73 (br s, 1H, 2′-H), 5.60 (s, 1H, PhCH), 8.19 (s, 1H, 6-H), 8.39 (br s, 1H, NH) ppm] and 120 mg (including impurities) of 1′5′-anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-{6-(trimethylsilylmethyl)-furano[2,3-*d*]-pyrimidin-2-one} (**8a**) [^{1}H NMR ($CDCl_3$) δ 2.15 (s, 2H, CH_2−Si), 5.01 (br s, 1H, 2′-H), 5.56 (s, 1H, PhCH), 5.97 (s, 1H, 5-H), 8.51 (s, 1H, 4-H) ppm].

Attempted Synthesis of 1g from 7. When compound **7** was heated as described for **6** in the synthesis of **1f**, only 1′,5′-anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-{6-methylfurano-[2,3-*d*]-pyrimidin-2-one}-D-*arabino*-hexitol (**8b**) was obtained [δ 2.39 (s, 3H, CH_3), 5.01 (br s, 1H, 2′-H), 5.55 (s, 1H, PhCH), 6.20 (s, 1H, 5-H), 8.61 (s, 1H, 4-H) ppm].

1′,5′-Anhydro-4′,6′-*O*-benzylidene-2′,3′-dideoxy-2′-[5-(1-propynyl)uracil-1-yl]-D-*arabino*-hexitol (9). Compound **4a** (756 mg, 1.35 mmol) was treated overnight at room temperature with NH_3/MeOH (80 mL) and evaporated. Propyne was generated by the dropwise addition of 1,2-dibromopropane (58.1 g, 288 mmol) to a refluxing mixture of KOH (18.0 g, 320 mmol) and *n*-BuOH (200 mL) over a period of 1 h. The gaseous propyne was introduced through a discharge tube into a cooled (−70 °C) mixture of crude debenzoylated substrate, deoxygenated triethylamine (150 mL), bis(triphenylphosphine)palladium(II) chloride (22 mg, 0.031 mmol), and copper(I) iodide (22 mg, 0.116 mmol) while stirring, and the reaction mixture was further stirred intensively under N_2 for 3.5 h at room temperature. Workup as described for **6** (chromatography was performed using silica gel 0.035−0.70 mm, 24 cm × 4 cm, and CH_2Cl_2 → CH_2Cl_2−MeOH 99:1 as eluent) yielded **9** [324 mg (65%)]: UV (MeOH) λ_{max} 230 nm (ϵ 11840), 296 nm (ϵ 12210); LSIMS (thgly) m/e 369 $(M + H)^+$; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H, propynyl CH_3), 2.13 (m, 1H, 3′-H), 2.45 (br d, 1H, 3′H, J = 13.7 Hz), 3.54 (m, 1H, 5′-H), 3.74 (m, 1H, 4′-H), 3.83 (t, 1H, 6′-H, J = 10.1 Hz), 4.02 (dd, 1H, 1′-H, J = 13.8 Hz, J = 3.5 Hz), 4.25 (br d, 1H, 1′-H, J = 14.8 Hz), 4.36 (dd, 1H, 6′-H, J = 10.5 Hz, J = 4.8 Hz), 4.73 (br s, 1H, 2′-H), 5.61 (s, 1H, PhCH), 7.34−7.49 (m, 5H, Ph), 8.20 (s, 1H, 6-H), 8.75 (br s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$) δ 4.26 (propynyl CH_3), 31.91 (C-3′), 51.84 (C-2′), 68.13 (C-6′, C-1′), 73.13 (C-4′), 73.58 (C-5′), 72.55, 89.18 (propynyl C-1″, C-2″), 98.59 (C-5), 100.99 (CHPh), 126.31, 128.21, 128.97, 137.90 (Ph), 144.84 (C-6), 150.12 (C-2), 162.19 (C-4) ppm.

1′,5′-Anhydro-2′,3′-dideoxy-2′-[5-(1-propynyl)uracil-1-yl)-D-*arabino*-hexitol (1g). Compound **9** (264 mg, 0.72 mmol) was converted into **1g** using the same procedure as described for **1e**. Column chromatography yielded (CH_2Cl_2−MeOH 93:7 as eluent) 141 mg (70%) of the title product. An analytical sample was crystallized from MeOH to give white needles: mp 256−258 °C (dec); UV (MeOH) λ_{max} 230 nm (ϵ 11060), 296 nm (ϵ 12630); LSIMS (thgly) m/e 279 $(M - H)^-$; ^{1}H NMR (DMSO-d_6) δ 1.72 (m, 1H, 3′-H), 1.98 (s, 3H, propynyl CH_3), 2.12 (br d, 1H, 3′-H, J = 14.3 Hz), 3.15 (m, 1H, 5′-H), 3.55 (m, 3H, 4′-H, 6′-H), 3.66 (m, 1H, 6′-H), 3.74 (dd, 1H, 1′-H, J = 13.2 Hz, J = 3.3 Hz), 4.05 (br d, 1H, 1′-H, J = 12.6 Hz), 4.48 (br s, 1H, 2′-H), 4.66 (t, 1H, 6′-OH, J = 5.9 Hz), 4.91 (d, 1H, 4′-OH, J = 5.1 Hz), 8.14 (s, 1H, 6-H), 11.55 (br s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6) δ 4.22 (propynyl CH_3), 35.11 (C-3′), 51.15 (C-2′), 60.59 (C-6′), 61.19 (C-4′), 66.94 (C-1′), 72.40, 88.98 (propynyl C-1″, C-2″), 82.83 (C-5′), 98.36 (C-5), 145.29 (C-6), 150.17 (C-2), 162.09 (C-4) ppm. Elemental analysis $C_{13}H_{16}N_2O_5$ (C, H, N).

Computational Methods. Model building and a Monte Carlo conformational search were carried out using MacroModel 5.0.[32] The force field Amber 4.1[33] was employed, taking into account all nonbonded interactions closer than 9 Å. The water surrounding was mimicked by use of the GB/SA method,[34] and 10 000 steps of the Monte Carlo conformational search of all rotable bonds were undertaken. The energies of the resulting structures were minimized by truncated the Newton Conjugate Gradient method,[35] and similar structures were grouped together. For compound A, additional ab initio energy calculations at the 6-31G** level of theory were performed using GAMESS[36] for the lowest energy conformations with the base in equatorial and axial orientation.

Crystallization Experiments. (1) Crystallization and Data Collection of the TK/1a Complex. Thymidine kinase was expressed as residues 11 to 376 of the complete 376 amino acid peptide and crystallized using the hanging-drop technique with ammonium sulfate as precipitant as described previously.[23,37−39] Initially, crystals of the TK/deoxythymidine complex were grown which were well ordered, having dimensions typically 0.3 × 0.1 × 0.5 mm^3 and belonging to the orthorhombic space group $C222_1$ with two molecules per asymmetric unit. The exchange of deoxythymidine bound to the protein for **1a** was made by washing the crystals five times in a 3 mL solution containing **1a**, 33% saturated ammonium sulfate, and 100 mM Tris-HCl pH 6.75. Crystals were flash-frozen as described previously.[23] Unit cell dimensions were a = 113.6 Å, b = 116.4 Å, c = 108.4 Å. Crystals diffracted to 2.2 Å resolution although anisotropic diffraction limited the resolution by about 0.5 Å in the b* direction compared with the plane perpendicular to b*. Full three-dimensional data were collected on a RAXIS-II imaging plate, using an RAXIS camera fitted with Yale Mirrors (Molecular Structure Corporation), mounted on an R-AXIS 5.0 KW X-ray generator. Data

from 90 frames were processed with DENZO/SCALEPACK[40] and TRUNCATE from the CCP4 suite.[41] The *R*-symm for intensities was 0.119. 26 808 data were present. In the highest resolution frame used (2.48 Å − 2.37 Å), 46% of data had $I/\sigma I > 2.0$. [*R*-symm $= \sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the measured intensity of an individual reflection and $\langle I\rangle$ the mean of repeated measurements.]

(2) Refinement Procedure. Coordinates of the latest best TK/dT model (with ligand and active site water molecules excluded) were used as a starting point for refinement by X-PLOR[42,43] of atomic positions and temperature factors against data from the TK/**1a** complex. Rigid-body refinement placed the starting coordinates optimally in the particular unit cell for these data, after which positional and temperature factor refinement was done; finally a difference map was calculated with X-PLOR so that the ligand could be located from modeling to positive difference Fourier electron density. Modeling was done on an Evans and Sutherland Freedom workstation using the program O.[44] Following incorporation of the ligand in the model, further rounds of positional refinement and modeling were done. Once finally modeled, the complex was taken through a double round of positional (100 cycles) and temperature refinement (20 cycles). 25 870 data having $F/\sigma F$ better than 2.0 and comprising 88% of data to 2.37 Å resolution gave an *R* value for the working set of data of 0.226 (*R*-free = 0.306); RMS-bond for the atomic structure was 0.015 Å, and a mean temperature factor of 15.3 Å^2 was obtained. [The *R* value is a measure of the agreement between calculated and observed X-ray data: $R = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. *R*-free is similarly defined for a randomly chosen set of reflections excluded from driving refinement. RMS-bond (X-PLOR software) is a measure of the discrepancy between refined and standard bond lengths. The mean *B* value is for main chain atoms after two pairs of positional and *B* factor refinement rounds.] A total of 250 water molecules have been identified. It was necessary to calculate an anisotropic overall *B* value to the data set prior to refinement. Coordinates will be deposited with the Protein Data Bank at Brookhaven (entry code 1K16).

Antiviral Activity Measurement. The source of the viruses and the methodology used to monitor antiviral activity have been described previously.[21,45]

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