Synthesis, Biological Evaluation, and Structure Analysis of a Series of New 1,5-Anhydrohexitol Nucleosides

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In view of the selective anti-HSV activity of 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol,[1] a series of novel 1,5-anhydrohexitol nucleosides were synthesized and evaluated for their inhibitory activity against several viruses. The 5-iodouracil **3** and the 5-ethyluracil **4** derivatives are highly selective TK-dependent inhibitors of HSV-1 and HSV-2. Broad anti-herpes virus activity was noticed for 5-fluorocytosine **6** and 2,6-diaminopurine **10** analogues. From a transport study of **3**, using the thymidine influx competition method, one can conclude that intracellular uptake of this compound most probably occurs by passive diffusion. X-ray analysis of compounds **3** and **9** showed that the heterocyclic base of 1,5-anhydrohexitol pyrimidine and purine is placed in the axial position and that the sugar ring adopts a slightly distorted chair conformation.

Introduction

The synthesis and biological evaluation of sugar-modified nucleoside analogues have been a very active research area for a number of years. This is mainly due to their potential antiviral and antitumor activities.

During our work on the study of the antiviral activity of a new class of sugar-modified nucleoside analogues, 1,5-anhydrohexitol[1] nucleosides, we discovered that 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol[1] exhibited a marked and selective activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) at a concentration of 0.07 μg/mL. This activity probably is dependent on a specific phosphorylation by the virus-encoded thymidine kinase (TK), since this compound was inactive against TK-deficient mutants of HSV-1. Also the cytosine and guanine analogues[1] showed activity against HSV-1, HSV-2, and other herpesviruses [varicella-zoster virus (VZV) and cytomegalovirus (CMV)] at relatively low concentrations well below the cytotoxicity threshold (2 and 20 μg/mL, respectively). However, at these concentrations, the compounds proved also inhibitory to the growth of human T-cells.

These results prompted us to synthesize other 1,5-anhydrohexitol nucleosides **1**–**11** (Figure 1) in order to study the effects of structural modifications on antiviral activity and toxicity. Replacement of the 5-methyl group of natural thymidine by various substituents has led to a multitude of compounds with either cytostatic or antiviral properties,[2] i.e., 5-fluoro-2′-deoxyuridine, 5-ethyl-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. This inspired us to synthesize their 1,5-anhydrohexitol nucleoside counterparts **1**–**5** (Figure 1).

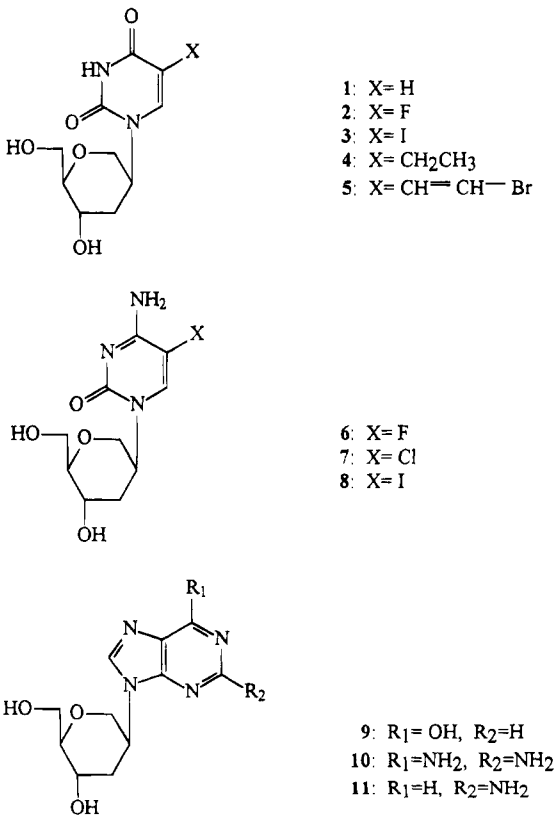

Figure 1. Structural formulas of some 1,5-anhydrohexitol nucleosides (**1**–**11**).

Since it has been noted that the 5-substituted 2′-deoxycytidines show a greater selectivity index than the corresponding 2′-deoxyuridines,[2] several 5-halogenated cytosine analogues **6**–**8** (Figure 1) were also synthesized. Because of the interesting anti-CMV activity of the guanine derivative,[1] some 1,5-anhydrohexitol nucleosides with a modified purine base moiety (**9**–**11**, Figure 1) were synthesized as well. Here, the guanine moiety is replaced by 6-hydroxypurine, 2,6-diaminopurine, and 2-aminopurine base.

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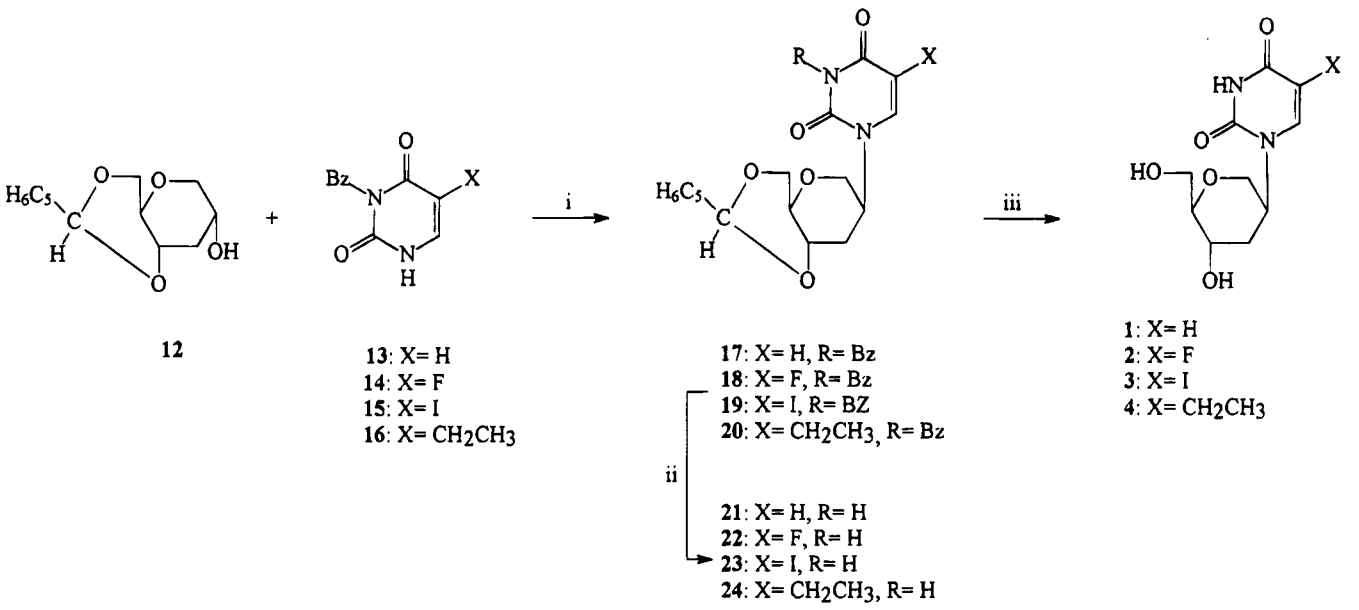
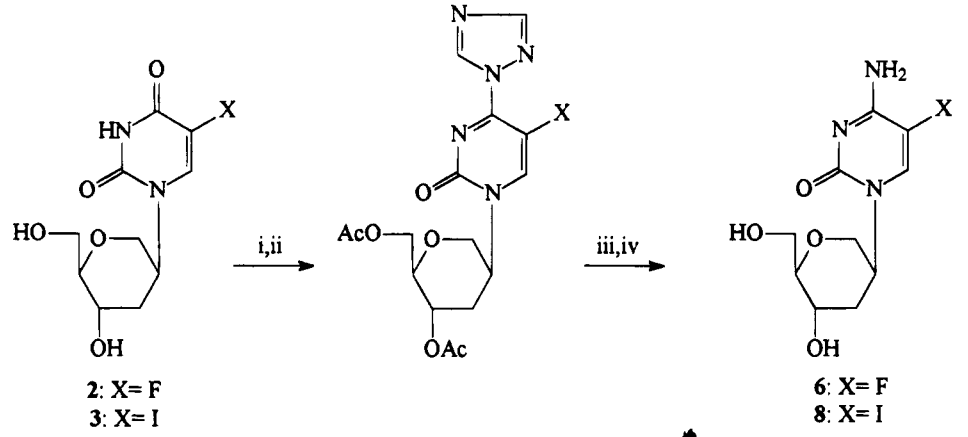
Scheme 1[a]



[a] (i) Ph_3P, DEAD, THF; (ii) NH_3/MeOH; (iii) 80% HOAc.

Scheme 2[a]



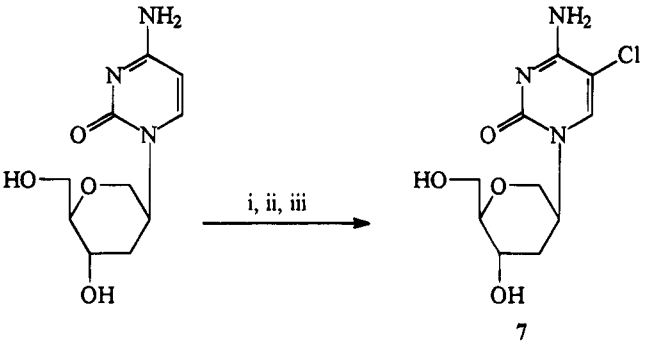
[a] (i) Ac_2O, pyridine; (ii) 1,2,4-1*H*-triazole; (iii) NH_4OH, dioxane; (iv) NH_3/MeOH.

Chemistry

Compounds **1**–**4** were synthesized starting from the alcohol **12**[1] using Mitsunobu reaction conditions[3] as described previously[1] (Scheme 1). The uracil derivative **1** was prepared in 53% yield from **12**, making use of 1.5 equiv of N^3-benzoyluracil **13**, 2 equiv of triphenylphosphine (Ph_3P), and 2 equiv of diethyl azodicarboxylate (DEAD) in THF. The benzoyl group of **17** was removed by methanol saturated with NH_3, before isolation and purification. The benzylidene moiety was hydrolyzed by 80% acetic acid at 70 °C. Likewise, N^3-benzoyl-5-fluorouracil (**14**), N^3-benzoyl-5-iodouracil (**15**), and N^3-benzoyl-5-ethyluracil (**16**) were reacted with **12** to afford **2**, **3**, and **4** in 62%, 50%, and 44% overall yield, respectively.

The 5-fluoro- and 5-iodocytosine analogues **6** and **8** were synthesized from their uracil counterparts (**2** and **3**, respectively) *via* the 4-triazolylpyrimidinone intermediates[5] (Scheme 2). After acetylation of **2** and **3** in pyridine with acetic anhydride, the acetylated compounds were treated with phosphorus oxychloride and triazole, followed by treatment with a NH_4OH–1,4-dioxane (1:3) solution. After chromatographic purification, the acetyl groups were removed by saturated methanolic ammonia to yield the 5-fluorocytosine derivative **6** and the 5-iodocytosine derivative **8** in good overall yields (62% and 66%, respectively).

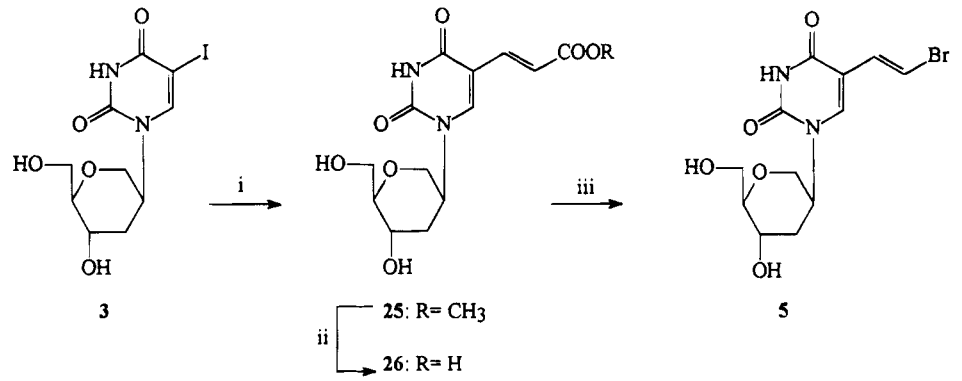
Scheme 3[a]



[a] (i) Ac_2O, pyridine; (ii) NCS, pyridine; (iii) NH_3/MeOH.

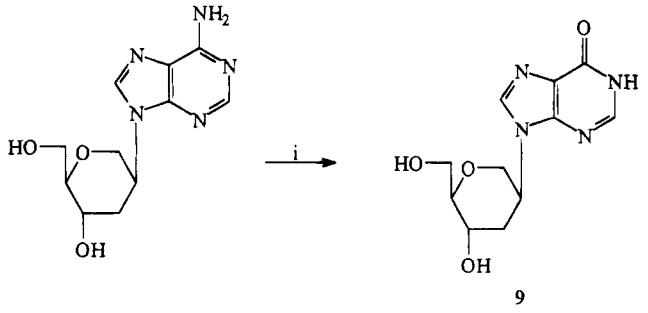
The chlorination of the C-5 position of the cytosine ring of 1,5-anhydro-2,3-dideoxy-2-(cytosin-1-yl)-D-*arabino*-hexitol[1] was carried out with *N*-chlorosuccinimide (NCS) as described for the synthesis of chlorinated uridines[6] (Scheme 3). After acetylation with acetic anhydride in pyridine, the protected cytosine analogue

Scheme 4[a]



[a] (i) CH_2=$CHCOOCH_3$, $Pd(OAc)_2$, Ph_3P, dioxane; (ii) NaOH, HCl; (iii) NBS, DMF.

Scheme 5[a]



[a] (i) Adenosine deaminase, phosphate buffer (pH 7.5).

was treated with 1.3 equiv of NCS for 30 min at 100 °C. Chromatographic purification, followed by treatment with methanol saturated with ammonia, yielded 46% of the 5-chlorocytosine analogue **7**.

The procedure for the synthesis of the (*E*)-5-(2-bromovinyl)uracil analogue **5** is based on the Heck reaction[7] starting from the 5-iodouracil derivative **3** via the methyl ester **25** (Scheme 4). The latter can be obtained by reaction between **3**, methyl acrylate, and a catalytic amount of palladium acetate.[8] The ester is hydrolyzed to give the free acid **26**, which on reaction with *N*-bromosuccinimide (NBS)[9] gives the desired product **5** in an overall yield of 33%. J^3 coupling constant proved the *E* configuration of the bromovinyl function.

1,5-Anhydro-2,3-dideoxy-2-(6-hydroxypurin-9-yl)-D-*arabino*-hexitol **9** was synthesized from its adenosine counterpart[1] by the action of adenosine deaminase (Scheme 5). Adenosine deaminase is known to effect hydrolytic deamination at the C-6 of related purine nucleosides.[10] Treatment of the adenosine analogue with this enzyme resulted in a clean conversion of **9** in 86% yield.

The other purine derivatives **10** and **11** were synthesized starting from 1,5-anhydro-4,6-*O*-benzylidene-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol, **28**, which was obtained by alkylation of 2-amino-6-chloropurine with the tosylate **27**[1] (Scheme 6). Treatment of **28** with methanol saturated with ammonia in a Parr pressure reactor at 100 °C[11] gave, after removal of the benzylidene moiety, the desired 2,6-diaminopurine derivative in 69% yield (starting from **28**). For the synthesis of the 6-deoxyguanine derivative, compound **28** was subjected to hydrogenation on a Pd/C catalyst[12] to give, after treatment with 80% HOAc, **11** in 62% yield.

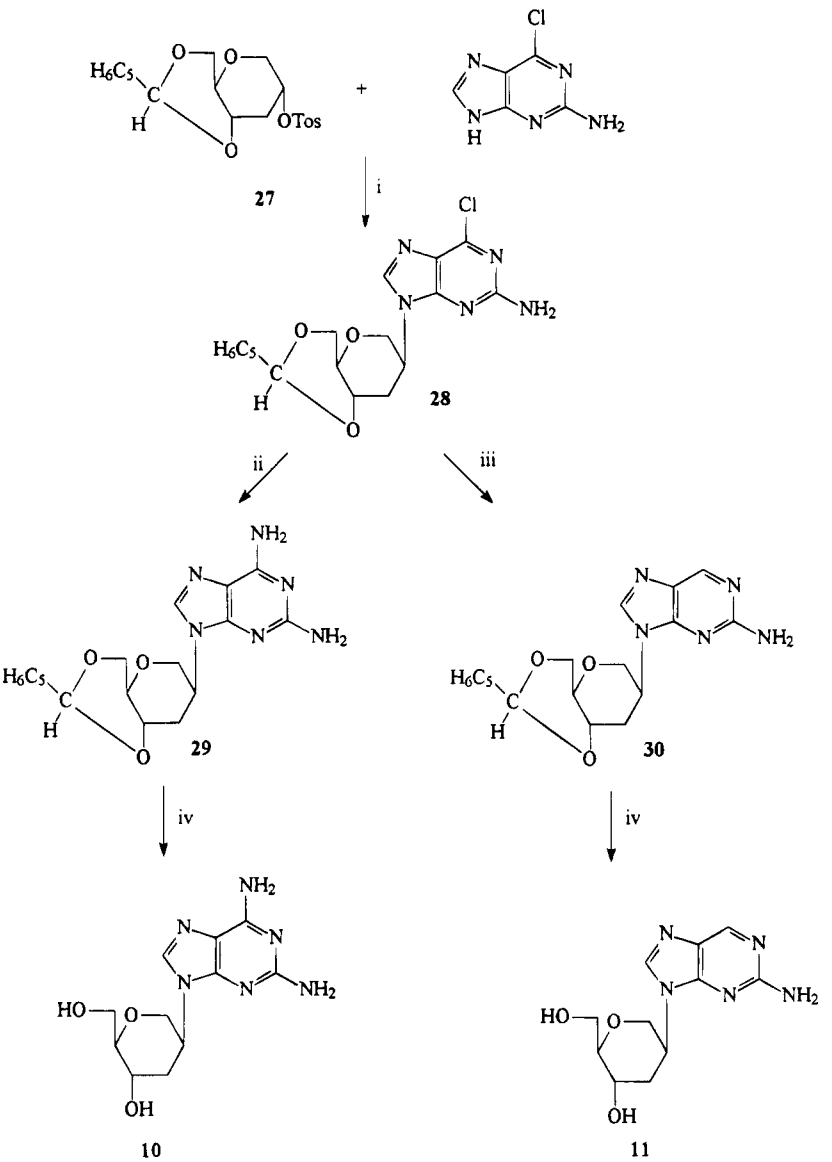
X-ray Crystallographic Study

The three-dimensional structure of compound 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol, **3**, the most potent anti-herpes agent among the 1,5-anhydrohexitol nucleosides, was analyzed by single-crystal X-ray crystallography. A plot of this compound with the atomic numbering scheme is shown in Figure 2.

The orientation of the heterocyclic base with respect to the sugar moiety is given by the torsion angle χ [C(5′)−C(1′)−N(1)−C(2)], which has a value of −150.6° in the anti position. Using the method of Cremer and Pople[13] the phase angles Φ = 168.06 and θ = 9.68 were calculated with a total puckering amplitude Q = 0.575 for the sequence C(1′)−C(2′)−C(3′)−C(4′)−O(4′)−C(5′). These parameters describe a slightly distorted chair conformation with a total puckering amplitude Q only slightly lower than that for an ideal cyclohexane chair (Q = 0.63 Å).[12] Of the three sugar ring substituents, only the heterocyclic base is placed in an axial position, while both the primary and secondary hydroxyl were found to be in an equatorial position. This is in contrast with nucleoside analogues with a normal pyranose moiety, where the base is placed in an equatorial position.[14] The X-ray results are similar to the conformation deduced from previously studied NMR spectra.[1] Figure 3 shows the best molecular fit of **3** and 5-iodo-2′-deoxyuridine (IDU)[15] using the 5-iodouracil fragment.

As the position of the O(5′) in IDU and O(41′) in **3** oxygen atoms is strongly influenced by intermolecular hydrogen bonds in the crystal lattice, it is difficult to compare O(5′)···O(3′) distances in nucleosides with the analogue distances in oligonucleotides. It is indeed expected that these modified nucleosides have to be phosphorylated and incorporated into DNA to exert their antiviral activity. However, the C(5′)···O(3′) distance of 3.73 Å in IDU is clearly in the range observed in B-DNA oligomers (3.21−3.75 Å for the Dickerson−Drew dodecamer[16]), which is not the case for the comparable C(41′)···O(3′) distance of 2.97 Å in **3**. As a consequence, the incorporation of 1,5-anhydrohexitol nucleosides in DNA will strongly influence its three-dimensional structure. In order to evaluate if the same conformational preference is found in the purine series, a single-crystal X-ray analysis was undertaken with the inosine analogue **9**. The asymmetric unit contains two molecules (A and B), which are depicted in Figure 4, and two water molecules. The axial, anti orientation

Scheme 6[a]



[a] (i) K_2CO_3, 18-crown-6, DMF; (ii) NH_3/MeOH; (iii) H_2, Pd/C (10%); (iv) 80% HOAc.

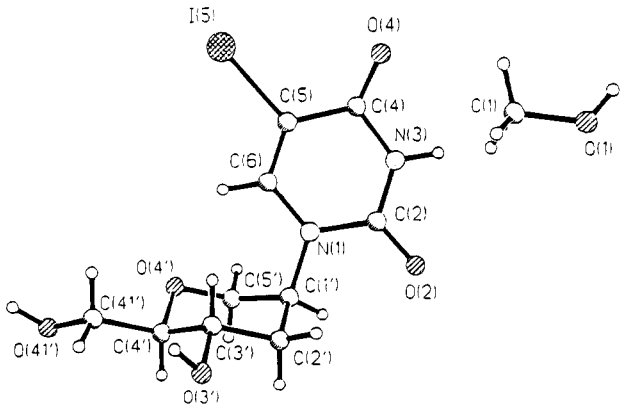


Figure 2. Crystal structure of 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol.

of the base moiety is confirmed in the purine series, as well as the chair conformation of the 1,5-anhydrohexitol ring.

Nucleoside Transport Study

The design of nucleoside drugs which may have therapeutic value is greatly aided by an understanding of the mechanism by which existing compounds exert their effect *in vivo*. Since the activity of many of these compounds depends upon their entry into intracellular metabolic pathways, the ability to cross cell membranes is a prerequisite for their effectiveness. Therefore it is useful to examine the effects of structural modification on membrane transportability.

Nucleoside transport across mammalian cell membranes occurs by a facilitated diffusion mechanism[17] via a "carrier" in the cell membrane which accepts wide variety of pyrimidine and purine nucleosides as substrates. It is also known that this mechanism is more sensitive to changes in the sugar moiety than that of the heterocyclic base.[18]

In the present study, the 5-iodo-2′-deoxyuridine (IDU) analogue **3** was tested as an inhibitor of thymidine influx under zero-*trans* conditions.[19] (Zero-*trans* influx refers to transfer of nucleoside from the outside (*cis*-side) of the membrane to the inside (*trans*-side), where the concentration of nucleoside is assumed to be zero.)

Competitive inhibition of nucleoside influx is indicative of an interaction between inhibitory substances and a permeant binding site of the transport mechanism,

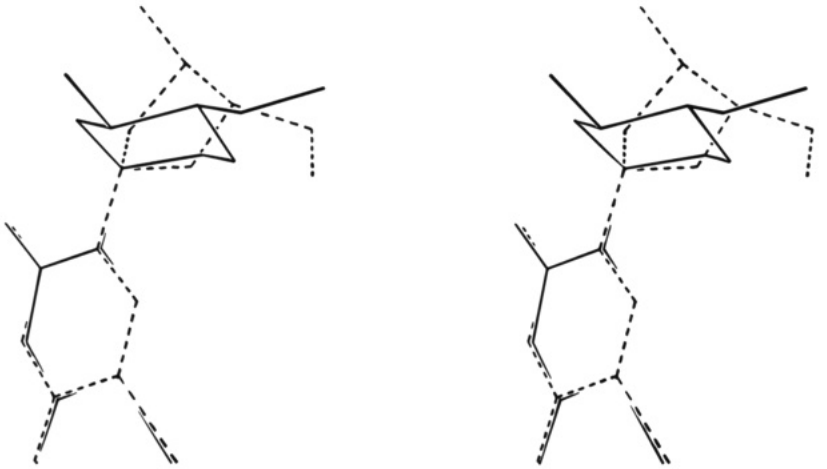


Figure 3. Stereospecific view of the best molecular fit of **3** (—) and 5-iodo-2′-deoxyuridine (– – –) using the 5-iodouracil fragment.

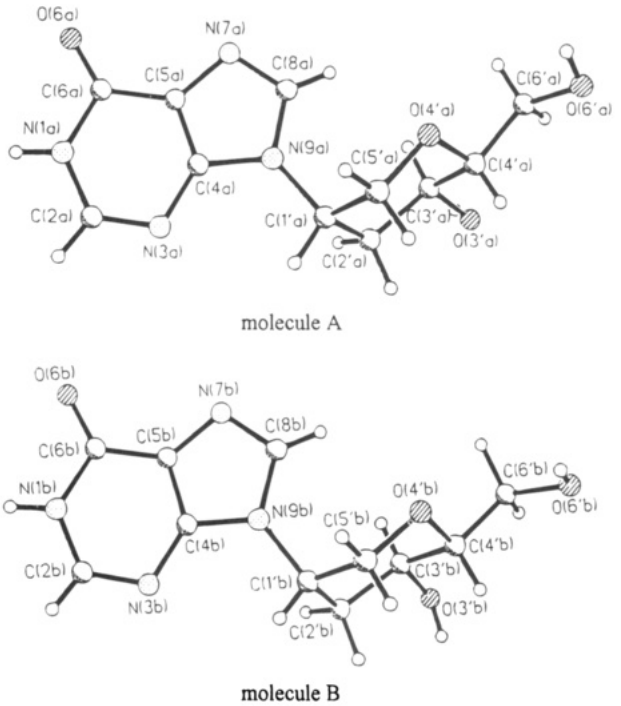


Figure 4. The crystal structure of 1,5-anhydro-2,3-dideoxy-2-(6-hydroxypurin-9-yl)-D-*arabino*-hexitol.

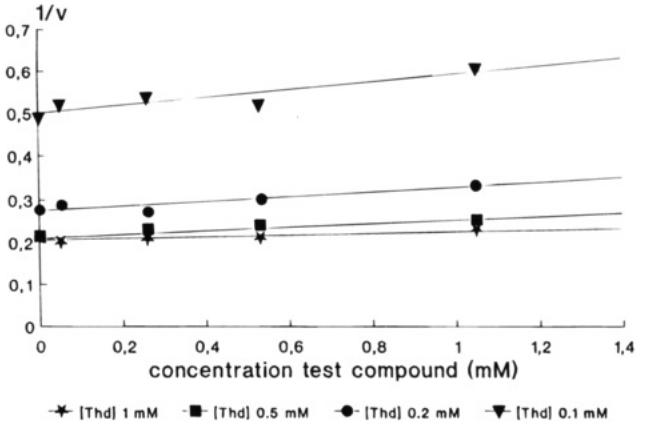


Figure 5. Estimation of the inhibitor constant according to the method of Dixon [the ordinate has units of (nmol of thymidine/10^{10} cells/s)$^{-1}$]. Each line is labeled with the thymidine concentration used (1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol (**3**)).

although it does not demonstrate that the inhibiting substance utilizes the transport mechanism.

A transport study of the IDU analogue **3** was done using the thymidine influx competition method. Membrane transport was assessed by competition studies which measured the effects of this novel nucleoside analogue on the nitrobenzyl thioinosine (NBMPR) sensitive zero-*trans* influx of 6-[^{3}H]thymidine. These studies were undertaken with fresh mouse erythrocytes using the methodology reported in detail elsewhere.[19,20] Data were plotted according to Dixon[21] as shown in Figure 5 to obtain inhibition constants (K_i) as intersects of the abcis. The K_i's (mM) for the reference compounds 2′-deoxyuridine and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine are 0.11 ± 0.02 and 0.023 ± 0.001 mM, respectively. Inhibition constants are assumed to reflect the affinities for the transport site. Compared to the reference compounds, the test compound **3** showed a very large K_i (≫1 mM). Therefore, we can conclude that this compound is not a good substrate for the binding site of the transporter. Intracellular uptake of this modified nucleoside, most probably, occurs by passive diffusion.

Biological Activity

Compounds **1**—**11** were evaluated for their inhibitory effect on the cytopathogenicity of herpes simplex virus type 1 (HSV-1) (strains Kos, F and McIntyre), thymidine kinase deficient (TK$^-$) HSV-1 (strain B2006), herpes simplex virus type 2 (HSV-2) (strains G, Lyons and 196), the clinical TK$^-$/TK$^+$ HSV-1 isolate VMW-1837, and vaccinia virus (VV) in human embryonic skin muscle (E_6SM) fibroblast cell cultures.[22,23] As reference compounds (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 5-iodo-2′-deoxyuridine (IDU), and (*S*)-9-(3-hydroxy-2-(phosphonylmethoxy)propyl)adenine (HPMPA) (Table 1) were included. Compounds **3**, **4**, **5**, **6**, **8**, and **10** showed good to excellent activity against HSV-1 and HSV-2. Compounds **3** and **4** effected a 50% reduction of the cytopathogenicity induced by HSV-1 at a concentration of 0.02 μg/mL. They showed activity against HSV-2 at a concentration of 0.4 μg/mL. Compounds **3** and **4** are much less active against TK$^-$ HSV-1, which indicates that their anti-HSV activity must rely on the virus-encoded TK. Among the tested compounds the 5-fluorocytosine **6** and the 2,6-diaminopurine **10** analogues

Table 1. Activity against Herpesviruses HSV-1 and HSV-2 and Cytotoxicity of Compounds **1**–**11** in E_6SM Cell Cultures

		MIC[b] (μg/mL)					
		HSV-1			HSV-2		
compd	MCC[a] (μg/mL)	KOS	F	McIntyre	G	196	Lyons
1	>400	>400	>400	>400	>400	>400	>400
2	>400	>400	>400	>400	>400	>400	>400
3	>400	0.02	0.01	0.04	0.4	0.7	0.4
4	>400	0.02	ND	ND	0.4	ND	ND
5	>400	0.4	2	0.4	150	70	70
6	>400	0.2	ND	ND	2	ND	ND
7	>400	300	150	300	300	300	300
8	>400	2	0.7	2	7	20	20
9	>400	>400	300	>400	>400	300	300
10	>400	0.2	ND	ND	0.7	ND	ND
11	>400	20	ND	ND	20	ND	ND
BVDU	>400	0.004	0.004	0.2	>200	300	70
IDU	>400	0.2	0.2	1	0.2	0.2	0.2
HPMPA	>400	0.2	0.2	2	0.02	0.07	0.1

[a] MCC = minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology. [b] MIC = minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%. ND, not determined.

Table 2. Activity against Herpesviruses TK-HSV-1, VV, and VSV and Cytotoxicity of Compounds **1**–**11** in E_6SM Cell Cultures

		MIC[b] (μg/mL)			
compd	MCC[a] (μg/mL)	TK^- HSV-1 (B2006)	TK^-/TK^+ HSV VMW-1837	VV	VSV
1	>400	>400	>400	>400	>400
2	>400	>400	300	>400	>400
3	>400	>400	4	200	>400
4	>400	≥400	0.4	200	>400
5	>400	>100	70	>100	>100
6	>400	0.07	2	0.2	>200
7	>400	≥400	300	>400	>400
8	>400	300	70	150	>400
9	>400	≥400	300	>400	>400
10	>400	7	0.7	7	>200
11	>400	150	70	>200	>200
BVDU	>400	40	70	2	>200
IDU	>400	40	10	0.7	>400
HPMPA	>400	1	1	0.7	>400

[a] MCC = minimum cytotoxic concentration, or concentration required to cause a microscropically detectable alteration of normal cell morphology. [b] MIC = minimum inhibitory concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%.

were the only ones showing activity against TK^- HSV-1 and VV.

None of the compounds proved cytotoxic at a concentration up to 400 μg/mL, as monitored by a microscopically detectable alteration of normal cell (E_6SM) morphology (Table 1).

The 1,5-anhydrohexitol nucleosides were also evaluated for their inhibitory effect on the cytopathogenicity of varicella-zoster virus (VZV, strains OKA and YS), thymidine kinase deficient (TK^-) VZV (strains 07/1 and YS/R), and cytomegalovirus (CMV) strains AD169 and Davis in human embryonic lung (HEL) cells (Tables 2 and 3). In this case acyclovir (ACV) and ganciclovir (DHPG) were also included as reference compounds. Only the (*E*)-5-(2-bromovinyl)uracil analogue **5** exhibited activity against VZV at a concentration of about 1.4 μg/mL. It was 20–30-fold less active against TK^- VZV, indicating that the anti-VZV activity of **5** depends on phosphorylation by the virus-encoded thymidine kinase. Although compound **3** and **4** were very active against HSV-1 and HSV-2, they were inactive against VZV and CMV, which underlines the specificity of these compounds. None of the compounds could inhibit CMV plaque formations. Compound **6** proved active against VZV and CMV at a concentration of about 3 and 0.5–1 μg/mL, respectively, while affecting host cell growth at a concentration of 2 μg/mL. Compound **10**, however, proved inhibitory to VZV (both TK^+ and TK^-) and CMV at a concentration of about 1–5 μg/mL, which was about 10-fold lower than the concentration required to inhibit host cell growth. In fact, from the present series of compounds, compound **10** emerged as the only one showing selective activity against CMV (selectivity index >10).

The compounds **1**–**11** were also evaluated against a wide variety of RNA viruses [i.e., vesicular stomatitis virus (VSV) (Table 2), poliovirus type 1, coxsackie B4 virus, reovirus type 1, parainfluenza virus type 3, Sindbis virus, and Semliki forest virus (data not shown)] and found inactive against these viruses up to the highest concentrations tested (400 μg/mL). They were also tested against human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in MT-4 cells, and again found inactive at the highest concentration tested (100 μg/mL).

Conclusion

Two compounds, the 5-iodouracil **3** and the 5-ethyl derivative **4** proved to be highly selective, viral TK-dependent inhibitors of HSV-1 and HSV-2. However, they are not active against VZV, which is in contrast to the spectrum of known TK-dependent antiviral agents such as BVDU. Conversion of the 5-iodouracil analogue into its cytosine counterpart **8** resulted in a slight decrease of activity, without change of viral specificity. The 5-fluorocytosine **6** showed the broadest anti-DNA virus activity spectrum, but was also the most inhibitory to host cell growth. The 2,6-diaminopurine derivative **10** showed activity against all herpes viruses (HSV-1, HSV-2, VZV, and CMV) at a concentration that was 10–100-fold lower than the cell growth inhibitory concentration, whereas the (*E*)-5-(2-bromovinyl) analogue **5** exhibited selective activity only against TK^+ HSV-1 and TK^+ VZV.

Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Ultraviolet spectra were recorded with a Philips PU 8700 UV/vis spec-

Table 3. Antiviral Activity against Herpesviruses TK$^+$ VZV, TK$^-$ VZV, and CMV and Cytotoxicity of Compounds **1**–**11** in HEL Cells

		IC_{50} (μg/mL)[b]					
		TK$^+$ VZV		TK$^-$ VZV		CMV	
compd	CC_{50}[a] (μg/mL)	OKA	YS	07/1	YS/R	AD-169	Davis
1	>50	>40	>40	>40	>40	>40	>40
2	>50	40	>40	>40	40	>40	>40
3	>50	>100	>100	>100	>100	>40	>40
4	>50	>50	>50	>50	>50	>50	>50
5	50	1.4	1.4	20	40	>10	23
6	2	2.9	3.5	5	7	0.9	0.5
7	>50	40	>40	>40	>40	>40	>40
8	>50	>40	>40	ND	7.6	>40	>40
9	>50	>40	25	>40	>40	>40	>40
10	25	1.2	2	2.4	5	2	0.8
11	140	60	80	90	100	>50	>50
IDU	>50	0.17	0.4	4	4	20	>50
BVDU	>50	0.0007	0.0015	8	30	>100	>100
DHPG	>200	0.02	0.072	0.1	0.9	2.9	6.2
ACV	>200	0.04	0.15	3	5	20	15

[a] 50% cytotoxic concentration, or concentration required to reduce cell growth by 50%. [b] 50% inhibitory concentration, or concentration required to reduce virus plaque formation by 50%. ND, not determined.

trophotometer. The ^{1}H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer and a Varian Gemini 200 MHz with tetramethylsilane as internal standard for the ^{1}H NMR spectra and DMSO-d_6 (39.6 ppm) or $CHCl_3$ (76.9 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, br s = broad signal, m = multiplet). Electron impact mass spectra (EIMS) and chemical ionization mass spectra (CIMS) were obtained using a KRATOS Concept 1H mass spectrometer. Precoated Macherey-Nagel Alugram Sil G/UV$_{254}$ plates were used for TLC, and the spots were examined with UV light and sulfuric acid–anisaldehyde spray. Column chromatography was performed on Janssen Chimica silica gel (0.060–0.200 nm). Preparative TLC was done on glass plates coated with Macherey-Nagel silica gel P/UV$_{254}$. Anhydrous solvents were obtained as follows: methanol and ethanol were refluxed on magnesium methoxide (I_2, Mg, MeOH, or EtOH) overnight and then distilled; dimethylformamide was dried over BaO and distilled under reduced-pressure; dichloromethane was stored on calcium hydride, refluxed, and distilled; tetrahydrofuran and dioxane were refluxed overnight on lithium aluminium hydride and distilled; pyridine was refluxed overnight on potassium hydroxide and distilled. Elemental analyses were obtained from Dr. W. Pfleiderer, Konstanz, Germany.

1,5-Anhydro-2,3-dideoxy-2-uracil-1-yl-D-*arabino*-hexitol (1). A suspension of 1.95 g (9 mmol) of N^3-benzoyluracil[4] (**13**), 1.41 g (6 mmol) of the alcohol 1,5-anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol[1] (**12**), and 3.15 g (12 mmol) of Ph_3P in 100 mL of anhydrous THF was treated with 1.9 mL (12 mmol) of DEAD in 40 mL of anhydrous THF. The solution was stirred overnight at room temperature, after which the volatiles were removed *in vacuo*. The residue **17** was dissolved in 100 mL of methanol saturated with ammonia. Evaporation and coevaporation with toluene left an oil which was purified on silica gel (CH_2Cl_2–MeOH, 99:1, 98:2). This yielded 3.5 g of crude **21** which also contained hydrazine dicarboxylate. The crude **21** was taken up in 75 mL of 80% acetic acid and heated at 80 °C for 5 h. After evaporation and coevaporation with toluene, the residue was dissolved in water and extracted with ether. The water layer was concentrated and purified on silica gel (CH_2Cl_2–MeOH, 85:15). Crystallization from MeOH–Et_2O afforded **1** as white crystals (776 mg, 3.2 mmol, 53% overall yield): mp 159 °C; UV (MeOH) λ_{max} 266 nm (ϵ 10 600); CIMS (iC_4H_{10}) *m/e* 243 [M + H]$^+$, 113 [B + 2H]$^+$; ^{1}H NMR (DMSO-d_6) δ 1.50–2.49 (m, 2H, H-3′, H-3″), 2.95–3.30 (m, 1H, H-5′), 3.32–4.15 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.50 (m, 2H, 6′-OH, H-2′), 4.90 (m, br s, 1H, 4′-OH), 5.30 (d, J = 7.9 Hz, 1H, H-5), 8.01 (d, J = 7.8 Hz, 1H, H-6), 11.25 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 35.4 (C-3′), 50.7 (C-2′), 60.6, 60.9 (C-6′, C-4′), 67.2 (C-1′), 82.7 (C-5′), 100.8 (C-5), 143.3 (C-6), 151.1 (C-2), 163.9 (C-4). Anal. ($C_{10}H_{14}N_2O_5$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-fluorouracil-1-yl)-D-*arabino*-hexitol (2). Starting with 1.48 g (6.3 mmol) of the alcohol **12** and 2.21 g (9.45 mmol) of N^3-benzoyl-5-fluorouracil[4] (**14**) and following the same method as described for **1**, 1.02 g (3.9 mmol, 62% overall yield) of **2** was obtained as white crystals: mp 239 °C; UV (MeOH) λ_{max} 273 nm (ϵ 10 000); CIMS (iC_4H_{10}) *m/e* 261 [M + H]$^+$, 131 [B + 2H]$^+$; ^{1}H NMR (DMSO-d_6) δ 1.25–1.90 (m, 1H, H-3′), 2.28 (m, 1H, H-3″), 3.20 (m, 1H, H-5′), 3.42–4.10 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.41–4.78 (m, 2H, 6′-OH, H-2′), 4.92 (m, 1H, 4′-OH), 8.32 (dd, 1H, J = 7.5 Hz, H-6), 11.98 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 34.9 (C-3′), 50.8 (C-2′), 60.2, 60.6 (C-6′, C-4′), 66.9 (C-1′), 82.5 (C-5′), 127.8 (J = 34.2 Hz, C-6), 139.4 (J = 228.3 Hz, C-5), 149.6 (C-2), 157.0 (J = 26.9 Hz, C-4). Anal. ($C_{10}H_{13}N_2O_5F$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol (3). Starting with 2.48 g (10.54 mmol) of the alcohol **12**, 5.42 g (15.81 mmol) of N^3-benzoyl-5-iodouracil[4] (**15**), 5.59 g (21.08 mmol) of Ph_3P, and 3.32 mL (21.08 mmol) of DEAD in anhydrous THF, using the same procedure as described for **1**, 1.92 g (5.22 mmol, 50% overall yield) of the 5-iodouracil derivative **3** was obtained: mp 220 °C; UV (MeOH) λ_{max} 283 nm (ϵ 13 500); CIMS (NH_3) *m/e* 369 [M + H]$^+$, 239 [B + 2H]$^+$; ^{1}H NMR (DMSO-d_6) δ 1.5–1.90 (m, 1H, H-3′), 1.95–2.25 (m, 1H, H-3″), 3.18–4.20 (m, 6H, H-1′, H-1″, H-5′, H-4′, H-6′, H-6″), 4.50 (m, H-2′), 4.67 (t, J = 5.5 Hz, 1H, 6′-OH), 4.91 (d, J = 4.7 Hz, 1H, 4′-OH), 8.41 (s, 1H, H-6), 11.64 (br s, NH); ^{13}C NMR (DMSO-d_6) δ 35.3 (C-3′), 51.4 (C-2′), 60.7, 61.1 (C-4′, C-6′), 67.2 (C-1′), 68.3 (C-5), 82.7 (C-5′), 147.9 (C-6), 150.9 (C-2), 160.9 (C-4). Anal. ($C_{10}H_{13}N_2O_5I$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-ethyluracil-1-yl)-D-*arabino*-hexitol (4). Using the same procedure as described for **1**, and starting from 470 mg (2 mmol) of the alcohol **12** and 736 mg (3 mmol) of N^3-benzoyl-5-ethyluracil[4] (**16**), the 5-ethyluracil derivative **4** was obtained in 44% overall yield (240 mg, 0.89 mmol). The final purification consisted of a preparative thin layer chromatography (CH_2Cl_2–MeOH, 85:15) followed by HPLC purification on a Rogel column (MeOH–H_2O, 50:50) and lyophilization: UV (MeOH) λ_{max} 270 nm (ϵ 10 600); EIMS *m/e* 270 [M]$^+$, 141 [B + 2H]$^+$; ^{1}H NMR (DMSO-d_6) δ 0.91–1.12 (t, J = 7.4 Hz, 3H, CH_3), 1.59–1.84 (m, 1H, H-3′ax), 1.98–2.30 (m, 3H, CH_2, H-3′eq), 3.05–3.22 (m, 1H, H-5′), 3.25–3.88 and 3.95–4.16 (m, H-4′, H-6′, H-6″, H-1′, H-1″), 4.51 (s, 1H, H-2′), 4.78 (s, 1H, 6′-OH), 4.98 (s, 1H, 4′-OH), 7.85 (s, 1H, H-6), 11.21 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 13.2 (CH_3), 19.7 (CH_2), 35.4 (C-3′), 50.2 (C-2′), 60.4, 60.7 (C-4′, C-6′), 67.1 (C-1′), 82.5 (C-5′), 114.1 (C-5), 138.4 (C-6), 150.8 (C-2), 163.8 (C-4). Anal. ($C_{12}H_{18}N_2O_5{\cdot}H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-[(*E*)-5-(2-bromovinyl)uracil-1-yl]-D-*arabino*-hexitol (5). A mixture of triethylamine (450 μL, 3.23 mmol), triphenylphosphine (45 mg, 0.17 mmol), and palladium acetate (20 mg, 0.087 mmol) in 6 mL of dry dioxane was heated to reflux on a heating mantle giving a black solution. After the solution was cooled to just below reflux temperature, methyl acrylate (390 μL, 4.34 mmol) was added

in one portion, followed immediately by 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol (**3**) (800 mg, 2.17 mmol). The stirred mixture was refluxed, an additional portion of triethylamine (160 μL, 1.15 mmol) was added, and gentle refluxing was maintained for 1 h. The warm reaction mixture was filtered over Celite and washed with hot dioxane. The filtrate was concentrated and purified by flash chromatography (CH_2Cl_2–MeOH, 98:2 to 95:5). Crystallization in dioxane yielded 500 mg of **25** (1.6 mmol, 75% yield) as a white powder.

The methyl ester **25** (312 mg, 1 mmol) was stirred with 3 mL of a 2 N NaOH solution for 90 min at room temperature. The solution was cooled to 10 °C, and the pH was adjusted to 1.0 with concentrated HCl. The resulting white solid was filtered and washed with cold water to yield 220 mg (0.704 mmol, 70% yield) of the free acid **26** as a white powder.

A solution of this powder in 10 mL of DMF was stirred at room temperature for 15 min. Then potassium bicarbonate (235 mg, 2.35 mmol) was added, and the mixture was stirred at room temperature for a further 20 min. A solution of *N*-bromosuccinimide (151 mg, 0.85 mmol) in 10 mL of DMF was added over a period of 60 min. A smooth evolution of CO_2 gas occurred during the addition. The resulting yellow mixture was stirred for 90 min at room temperature. The solid was filtered and washed with DMF. The filtrate was concentrated to give a yellow oil, which was purified by preparative thin layer chromatography (CH_2Cl_2–MeOH, 90:10). The resulting oil (154 mg, 0.444 mmol, 63% yield) was dissolved in water, and after standing overnight at 4 °C, the crystalline mass was filtered and washed with ice-cold water to give 120 mg of **5** as white crystals. These crystals were slightly contaminated with *N*-bromosuccinimide and therefore further purified by HPLC on a Rogel column (MeOH–H_2O, 80:20) to afford after lyofilisation 95 mg of **5** as a white powder: UV (MeOH) λ_{max} 252 (ϵ 13 500), 297 nm (ϵ 11 200); CIMS (iC_4H_{10}) *m/e* 347 $[M + H]^+$; ^1H NMR (DMSO-d_6) δ 1.50–2.38 (m, 2H, H-3′, H-3″), 3.18 (m, 1H, H-5′), 3.30–4.17 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.58 (m, 2H, 6′-OH, H-2′), 4.94 (m, 1H, 4′-OH), 6.64 (d, 1H, J = 13.4 Hz, vinylic H), 7.26 (d, 1H, J = 13.4 Hz, vinylic H), 8.19 (s, 1H, H-6), 11.12 (br s, NH); ^{13}C NMR (DMSO-d_6) δ 34.8 (C-3′), 50.8 (C-2′), 60.0, 60.2 (C-6′, C-4′), 66.8 (C-1′), 82.2 (C-5′), 106.2 (vinylic C), 109.1 (C-5), 130.3 (vinylic C), 142.3 (C-6), 149.8 (C-2), 161.7 (C-4). Anal. ($C_{12}H_{15}N_2O_5Br·3H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-fluorocytosin-1-yl)-D-*arabino*-hexitol (6). 1,5-Anhydro-2,3-dideoxy-2-(5-fluorouracil-1-yl)-D-*arabino*-hexitol **2** (295 mg, 1.13 mmol) was first acetylated and then converted into its 5-fluorocytosine analogue, using the same procedure as described for 7. The resulting oil was first purified by preparative thin layer chromatography (CH_2Cl_2–MeOH, 9:1) and then crystallization from MeOH/Et_2O to yield 100 mg (0.39 mmol, 35% overall yield) of **6** as white crystals: mp 158 °C; UV (MeOH) λ_{max} 285 nm (ϵ 7200); CIMS (iC_4H_{10}) *m/e* 260 $[M + H]^+$, 130 $[B + H]^+$; ^1H NMR (DMSO-d_6) δ 1.48–1.90 (m, 1H, H-3′), 1.91–2.27 (m, 1H, H-3″), 3.20 (m, 1H, H-5′), 3.38–4.10 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.48 (m, 2H, 6′-OH, H-2′), 4.85 (d, 1H, J = 5.0 Hz, 4′-OH), 7.50 (br s, 2H, NH_2), 8.11 and 8.20 (J = 7.3 Hz, H-6); ^{13}C NMR (DMSO-d_6) δ 35.2 (C-3′), 51.4 (C-2′), 60.5, 60.7 (C-4′, C-6′), 67.5 (C-1′), 82.8 (C-5′), 127.5 (J = 30 Hz, C-6), 135.0 (J = 240 Hz, C-5), 154.0 (C-2), 157.1 (J = 12 Hz, C-4). Anal. ($C_{10}H_{14}N_3O_4F·H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-chlorocytosin-1-yl)-D-*arabino*-hexitol (7). 1,5-Anhydro-2,3-dideoxy-2-cytosin-1-yl-D-*arabino*-hexitol[1] (120 mg, 0.498 mmol) in 10 mL of pyridine–acetic anhydride (8:2) was stirred for 2 h at room temperature. The mixture was concentrated, dissolved in 30 mL of ethyl acetate, and washed twice with a saturated aqueous $NaHCO_3$ solution. The organic layer was dried, evaporated, and co-evaporated with pyridine. The oily residue was dissolved in 10 mL of anhydrous pyridine, and *N*-chlorosuccinimide (NCS) (87 mg, 0.652 mmol) was added. The mixture was stirred for 30 min at 100 °C and poured into 50 mL of a 5% aqueous $NaHCO_3$ solution containing 2% sodium thiosulfate. The solution was extracted with EtOAc (2 × 50 mL), and the organic layer was dried and evaporated. Chromatographic purification of the dark brown oil on 25 g of silica (CH_2Cl_2–MeOH, 98:2 to 95:5) yielded 90 mg of a foam. This foam was treated for 4 h at room temperature with 20 mL of MeOH saturated with ammonia and was evaporated. The residue was dissolved in 50 mL of H_2O and washed twice with 50 mL of Et_2O. The water layer was concentrated and coevaporated with dioxane to yield 63 mg (0.230 mmol, 46% overall yield) of the title compound **7**. An analytical sample (31 mg) was crystallized from MeOH: mp 197 °C; UV (MeOH) λ_{max} 290 nm (ϵ 7800); CIMS (iC_4H_{10}) *m/e* 276 $[M + H]^+$, 146 $[B + 2H]^+$; ^1H NMR (DMSO-d_6) δ 1.45–1.90 (m, 1H, H-3′), 1.92–2.30 (m, 1H, H-3″), 3.18 (m, 1H, H-5′), 3.38–4.10 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.42–4.77 (m, 2H, 6′-OH, H-2′), 4.90 (d, 1H, J = 5.0 Hz, 4′-OH), 7.48 (br s, 2H, NH_2), 8.37 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 35.0 (C-3′), 51.5 (C-2′), 60.4, 60.7 (C-6′, C-4′), 67.3 (C-1′), 82.7 (C-5′), 97.9 (C-5), 141.8 (C-6), 154.0 (C-2), 161.1 (C-4). Anal. ($C_{10}H_{14}N_3O_4Cl·0.75H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-iodocytosin-1-yl)-D-*arabino*-hexitol (8). 1,5-Anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol (**3**) (570 mg, 1.55 mmol) was dissolved in 20 mL of pyridine, and 4 mL of acetic anhydride was added. After stirring for 2 h at room temperature, the mixture was evaporated and coevaporated twice with toluene. TLC analysis (CH_2Cl_2–MeOH, 95:5) showed that the transformation into the 4,6-di-*O*-acetyl analogue was complete. After chromatographic purification (CH_2Cl_2–MeOH, 98:2 to 95:5), the resulting white solid (640 mg, 1.42 mmol) was coevaporated with anhydrous pyridine and a premixed solution of phosphorus oxychloride (265 μL, 2.84 mmol) and 1,2,4-triazole (785 mg, 11.36 mmol) in 30 mL of anhydrous pyridine was added. This mixture was stirred for 3 days at room temperature, evaporated, and coevaporated with toluene. The residue was dissolved in CH_2Cl_2 (30 mL), and the solution was successively washed with water (60 mL) and a saturated $NaHCO_3$ solution (60 mL). The organic layer was dried and evaporated to yield 790 mg of a foam. This foam was taken up in 50 mL of NH_4OH–1,4-dioxane (1:3) and stirred for 4 h at room temperature. The solvent was evaporated, and the residue was purified by column chromatography (CH_2Cl_2–MeOH, 99:1 to 97:3) to yield 473 mg (1.05 mmol) of the acetylated 5-iodocytosine derivative as a white solid. The protective groups were removed by treatment with 75 mL of methanol saturated with ammonia. After concentration, the oil was taken up in water and washed twice with Et_2O. The water layer was concentrated, and crystallization from MeOH yielded 339 mg (0.90 mmol, 58% overall yield) of **8** as white crystals: mp 218 °C; UV (MeOH) λ_{max} 296 nm (ϵ 7900); CIMS (iC_4H_{10}) *m/e* 368 $[M + H]^+$, 237 $[B + 2H]^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 1.77 (m, 1H, H-3′), 2.09 (m, 1H, H-3″), 3.18 (m, 1H, H-5′), 3.40–4.21 (m, 5H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.41–4.76 (m, 2H, 6′-OH, H-2′), 4.90 (d, 1H, J = 5.1 Hz, 4′-OH), 7.16 (br s) and 7.72 (br s) (2H, NH_2), 8.33 (s, 1H, H-6); ^{13}C NMR (200 MHz, DMSO-d_6) δ 35.2 (C-3′), 51.3 (C-2′), 55.5 (C-5), 60.6, 60.9 (C-6′, C-4′), 67.3 (C-1′), 82.7 (C-5′), 149.7 (C-6), 154.3 (C-2), 163.5 (C-4). Anal. ($C_{10}H_{14}N_3O_4I$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(6-hydroxypurin-9-yl)-D-*arabino*-hexitol (9). To a solution of 1,5-anhydro-2,3-dideoxy-2-adenin-9-yl-D-*arabino*-hexitol[1] (200 mg, 0.754 mmol) in 100 mL of phosphate buffer (pH 7.5) was added 0.5 mL of adenosine deaminase. The reaction mixture was incubated for 12 h at 30 °C and evaporated. The residue was diluted with 8 mL of H_2O, and the precipitate was filtered and recrystallized from H_2O, yielding 64 mg (0.24 mmol, 32% yield) of the analytically pure **9**. The filtrates were put on top of a XAD column which was eluted with water followed by MeOH–water (3:1). Evaporation afforded the title compound **9** as a white product (140 mg, 0.654 mmol, 86% yield): mp 267 °C; UV (MeOH) λ_{max} 250 nm (ϵ 10 100); CIMS (iC_4H_{10}) *m/e* 267 $[M + H]^+$, 137 $[B + 2H]^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 1.85 (m, 1H, H-3′ax), 2.18 (m, 1H, H-3′eq), 3.25 (m, 1H, H-5′), 3.35–3.74 (m, 3H, H-4′, H-6′, H-6″), 3.81–4.21 (m, 2H, H-1′ax, H-1′eq), 4.22–4.72 (br s and m, 2H, 6′-OH, H-2′), 4.82 (br s, 1H, 4′-OH), 8.05 (s, 1H), 8.30 (s, 1H, H-2, H-8), 12.2 (br s, 1H, NH); ^{13}C NMR (200 MHz , DMSO-d_6) δ 36.2 (C-3′), 50.6 (C-2′), 60.6, 60.7 (C-6′, C-4′), 68.1 (C-1′), 83.1 (C-5′), 123.6 (C-

5), 139.1 (C-8), 145.7 (C-2), 148.3 (C-4), 156.9 (C-6). Anal. ($C_{11}H_{14}N_4O_4 \cdot 2H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(2,6-diaminopurin-9-yl)-D-*arabino*-hexitol (10). A solution of 1,5-anhydro-4,6-*O*-benzylidene-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol[1] (**28**) (200 mg, 0.52 mmol) in 100 mL of ethanol saturated with ammonia is heated in a Parr pressure reactor for 8 h at 100 °C. After evaporation, the obtained residue was purified by flash chromatography (CH_2Cl_2–MeOH, 95:5 to 90:10) to give 180 mg of **29** as a white solid. The benzylidene moiety was hydrolyzed with 80% acetic acid at 80 °C. Evaporation and coevaporation with toluene left an oil, which was taken up in water and washed twice with CH_2Cl_2. The water layer was concentrated, and crystallization from MeOH afforded 108 mg (0.39 mmol, 74% overall yield) of analytically pure **10**: UV(MeOH) λ_{max} 257 nm (ϵ 8200), 283 nm (ϵ 9600); EIMS *m/e* 280 $[M]^+$, 150 $[B + H]^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 1.82 (m, 1H, H-3'ax), 2.11–2.32 (m, 1H, H-3'eq), 3.18 (m, 1H, H-5'), 3.40–3.75 (m, 3H, H-4', H-6', H-6''), 3.80–3.92 (m, 1H, H-1'ax), 4.12–4.20 (m, 1H, H-1'eq), 4.20–4.75 (br s and m, 3H, 6'-OH, H-2', 4'-OH), 5.83 (s, 2H, NH_2), 6.70 (s, 2H, NH_2), 7.93 (s, 1H, H-8); ^{13}C NMR (200 MHz, DMSO-d_6) δ 36.0 (C-3'), 49.8 (C-2'), 60.7, 60.9 (C-6', C-4'), 68.3 (C-1'), 83.0 (C-5'), 112.5 (C-5), 136.9 (C-8), 151.6 (C-4), 156.1 (C-6), 160.3 (C-2). Anal. ($C_{11}H_{16}N_6O_3 \cdot 0.5H_2O$) C, H, N.

1,5-Anhydro-2-(2-aminopurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol (11). 1,5-Anhydro-4,6-*O*-benzylidene-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol[1] **28** (400 mg, 1.03 mmol) was dissolved in 100 mL of ethyl acetate. Palladium on carbon catalyst (10%, 100 mg) was added, and the solution was shaken for 24 h in a Parr hydrogenation apparatus (40 psi). The mixture was heated to 50 °C and filtered. The filtrate, containing **30**, was purified by column chromatography (CH_2Cl_2–MeOH, 98:2 to 95:5). The resulting white solid (250 mg, 0.71 mmol) was treated with 80% acetic acid at 60 °C for 6 h to remove the benzylidene moiety. After evaporation and coevaporation with toluene, the resulting oil was taken up in water and washed twice with CH_2Cl_2. The water layer was concentrated, and crystallization from MeOH afforded 170 mg (0.64 mmol, 62% overall yield) of analytically pure **11**: UV (MeOH) λ_{max} 247 nm (ϵ 5600), 310 nm (ϵ 8100); EIMS *m/e* 265 $[M]^+$, 136 $[B + H]^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 1.68–2.09 (m, 1H, H-3'), 2.30 (d, ^2J = 13.0 Hz, 1H, H-3'eq), 3.17 (m, 1H, H-5'), 3.36–3.78 (m, 3H, H-4', H-6', H-6''), 3.82–4.05 (m, 1H, H-1'ax), 4.10–4.32 (m, 1H, H-1'eq), 4.50–5.19 (br s and m, 3H, 6'-OH, H-2', 4'-OH), 6.52 (s, 2H, NH_2), 8.24 (s, 1H), 8.61 (s, 1H) (H-6, H-8); ^{13}C NMR (200 MHz, DMSO-d_6) δ 36.3 (C-3'), 49.7 (C-2'), 60.7, 60.9 (C-6', C-4'), 68.2 (C-1'), 83.3 (C-5'), 126.6 (C-5), 141.6 (C-8), 149.3 (C-6), 153.1 (C-4), 160.7 (C-2). Anal. ($C_{11}H_{15}N_5O_3$) C, H, N.

Single-Crystal X-ray Analysis. The cell constants and the reflections were measured with a Siemens P4-PC diffractometer using the XSCANS software[24] (graphite monochromator, Mo Kα radiation (λ = 0.710 73 Å) for **3**, Cu Kα radiation (λ = 1.541 78 Å) for **9**, cell dimension by least-squares refinement of the 2θ values of 20 relections (11 ° < 2θ < 19° for **3**, 21° < 2θ < 36° for **9**). The structures were solved by DIRDIF[25] using a 5-iodouracil fragment as starting model (for **3**), and direct methods (for **9**) and refined by full-matrix least-squares to a final R = 0.031 (for **3**) and R = 0.057 (for **9**).

Compound **3** was crystallized from MeOH. Intensities from a crystal 0.30 × 0.20 × 0.15 mm, space group $P2_12_12_1$, orthorhombic, a = 7499(2) Å, b = 9.134(5) Å, c = 20.759(4) Å, V = 1421.9 (9) Å^3, ω scan, $(\sin\theta/\lambda)_{max}$ = 0.594, $-1 \le h \le 8$, $-1 \le k \le 10$, $-1 \le l \le 24$, 1996 reflections collected, 1825 independent reflections (R_{int} = 0.030), 1393 observed reflections ($F > 4.0\sigma(F)$), Lorentz-polarization corrections, semiemperical absorption corrections (μ = 2.279 mm^{-1}). Hydrogen atoms from methanol and O(3') from difference Fourier maps, rest placed at calculated positions (C-H 0.96 Å, N–H 0.90 Å, O–H 0.85 Å). Hydrogen atoms were refined isotropically in riding mode, except the hydrogens of C(1'), C(41'), O(41'), C(5'), and O(3'), which were refined with fixed isotropic temperature factors, 1.1 times that of the parent atom. Isotropic extinction parameter 0.000 16 was used for extinction correction as described by Larsen.[26]

Compound **9** was crystallized from MeOH. Intensities from a crystal 0.40 × 0.15 × 0.15 mm, space group $P2_1$, monoclinic, a = 6.891(1) Å, b = 25.601(4) Å, c = 7.715(1) Å, β = 94.37(1)°, V = 1357.1(3) Å^3, ω–2θ scan, $(\sin\theta/\lambda)_{max}$ = 0.500, $-1 \le h \le 6$, $-1 \le k \le 25$, $-7 \le l \le 7$, 2015 reflections collected, 1536 independent reflections (R_{int} = 0.078), 1346 observed reflections ($F > 4.0\sigma(F)$), Lorentz-polarization correction, no absorption correction (μ = 1.037 mm^{-1}).

Hydrogen atoms from difference Fourier maps, positioins refined with fixed isotropic temperature factor 1.2 times that of the parent atom. Isotropic extinction parameter 0.0103 was used for extinction correction as described by Larsen.[26] Program package used for calculations and drawing: SHELXTL PC.[27]

Transport Study: Thymidine Influx Competition Method. Male $B_6D_2F_1$ mice were asphyxiated with CO_2. Blood was immediately collected into a syringe containing 3.8% sodium citrate solution by cardiac puncture and used the same day. Mouse erythrocytes were obtained by centrifugation at 12 800 rpm for 1 min followed by four washes with cold buffered saline. The obtained mouse erythrocytes were suspended in the same medium to a 10% hematocrit. The transport study was performed at room temperature according to the literature method.[19,20] Nucleoside influx was initiated by the rapid addition of buffered saline containing [*methyl*-^3H]thymidine at four concentrations (0.1, 0.2, 0.5, and 1 mM) alone and together with various concentrations of 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol **3** (0.053, 0.26, 0.53, and 1.05 mM). After 3 s, transport was terminated by adding (nitrobenzyl)thioinosine (NBMPR). Cells were pelleted and washed once with NBMPR solution. Cell pellets were extracted with ice-chilled 5% perchloric acid. After 30 min of cooling in ice, aliquots were counted by liquid scintillation counting in Scinti Verse II. Radioactivity trapped in the extracellular space of the pellet was determined as a zero-time value by reversal of the order of addition of NBMPR and nucleoside to the cells. This value was subtracted from radioactivity in the influx samples. Duplicated samples were performed in each experiment.

Antiviral Assay Procedures. Assays for activity against herpesviruses were performed as described previously.[22] The origin of the viruses, herpes simplex virus type 1 (HSV-1) (strain KOS, F, and McIntyre), thymidine kinase deficient TK^- HSV-1 (strain B 2006), herpes simplex virus type 2 (HSV-2) (strains G, 196 and Lyons), varicella-zoster virus (VZV, strains Oka and YS), TK^- VZV (strains 07-1 and YS-R), vaccinia virus (VV), vesicular stomatitis virus (VSV), and cytomegalovirus (CMV, strains AD169 and Davis) have been described.[23] Cytotoxicity measurements were based on either microscopically detectable alteration of normal cell morphology or inhibition of cell growth. The antiviral activity and cytotoxicity assays were performed in human embryonic skin–muscle (E_6-SM) or human embryonic lung (HEL) cells seeded in 96-well microtiter trays. Assays for activity against human immunodeficiency virus type 1 (HIV-1) (strain III_B) and type 2 (HIV-2) (strain ROD) were performed as described previously.[28]

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Supplementary Material Available: Crystal data for **3** and **9** and tables listing atomic coordinates, bond lengths, bond angles, and torsion angles (27 pages). Ordering information is given on any current masthead page.

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