Synthesis and Antiherpes Virus Activity of 1,5-Anhydrohexitol Nucleosides

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The synthesis of 1,5-anhydrohexitol nucleosides is described. These nucleoside analogues were obtained by alkylation of the heterocyclic bases with the tosylate **10** or by alkylation of the bases with the alcohol **12** under Mitsunobu conditions. The compounds were evaluated for antiviral and cytostatic activity. Highly selective activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) was noted for 1,5-anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol **4b** at a concentration of 0.07 μg/mL. This activity must be dependent on a specific phosphorylation by the virus-encoded thymidine kinase (TK), since compound **4b** was inactive against TK-deficient mutants of HSV-1. The corresponding cytosine **4c** and guanine **4e** analogues showed activity against HSV-1, HSV-2, and other herpes viruses (i.e. cytomegalovirus, varicella-zoster virus) at concentrations well below the cytotoxicity threshold (2 and 20 μg/mL, respectively). At these concentrations, compounds **4c** and **4e** proved also inhibitory to the growth of human T-cells (i.e. MT-4, CEM, MOLT-4).

Introduction

Modification in the sugar part of nucleosides has led to the development of several nucleoside analogues with antiviral properties. Well-known examples are acyclic nucleoside analogues (e.g. ACV),[1] pentose-fluorinated nucleosides (e.g. FMAU, FIAC),[2] carbocyclic nucleosides (e.g. carbovir),[3] nucleosides with a four-membered ring (e.g. oxetanocin),[4] and phosphonylated analogues of acyclic nucleosides (e.g. PMEA).[5] These compounds all have in common that they have to be phosphorylated by cellular or viral enzymes in order to exert their biological activity. A series of nucleoside analogues from which, until now, no congener has been accredited with significant antiviral activity are those containing a pyranose sugar moiety. During our work on the structure–activity relationship of nucleoside analogues active against the human immunodeficiency virus (HIV), we synthesized several 2,3,4-trideoxy-D-*glycero*-hex-3-enopyranosyl nucleosides[6] (**1**), several 2,3,4-trideoxy-D-*glycero*-hex-2-enopyranosyl nucleosides[7] (**2**), and nucleoside analogues with a 1,4-dioxane, 1,4-oxathiane, or 1,4-oxazine ring structure[8] (**3**) (Figure 1). Hex-2-enopyranosyl and hex-3-enopyranosyl analogues were also synthesized by Hansen et al.[9] These compounds, however, are devoid of antiviral activity. This inactivity, most probably, results from the poor intracellular phosphorylation of these compounds. These hexose nucleosides are apparently not recognized as substrates by cellular or viral kinases, and hence no antiviral activity is found.

A common feature of hexose nucleosides is the equatorial orientation of the base moiety.[10] This orientation could be altered by removing the 5′-oxygen function in the six-membered ring. By removing this oxygen function, the partial double bond character of the C(1′)–O(5′) bond, due to electron delocalization from the O(5′) lone pair p-type orbital into the antibonding σ* orbital of the C(1′)–N(1) bond (anomer effect), is removed. As a consequence

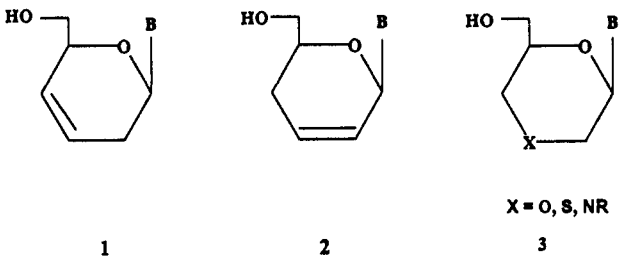

Figure 1.

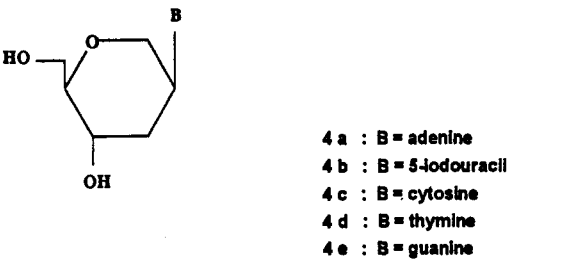

Figure 2.

the whole geometry of the molecule changes. Besides, the resulting compounds are chemically and enzymatically stabilized as compared to the original nucleosides. By a simple molecular overlap a good fit can be observed between the structure of 2′-deoxyuridine and 1,5-anhydrohexitol nucleosides, where the base moiety is moved to the 2′-position. The compounds **4a–e** (Figure 2) can be considered as ring-expanded 2′-deoxynucleoside analogues where a methylene group is inserted between the ring oxygen and the carbon atom linked to the base moiety. These compounds could have the advantage of carbocyclic nucleosides (i.e. stability), while the ring oxygen atom is still present, should this be necessary for recognition by anabolic enzymes.

In our synthetic strategy we started from glucose which was converted to a 3-deoxy-1,5-anhydro-D-hexitol, which was coupled at its 2-position to heterocyclic bases either by nucleophilic displacement or under Mitsunobu reaction conditions to afford the desired nucleoside analogues.

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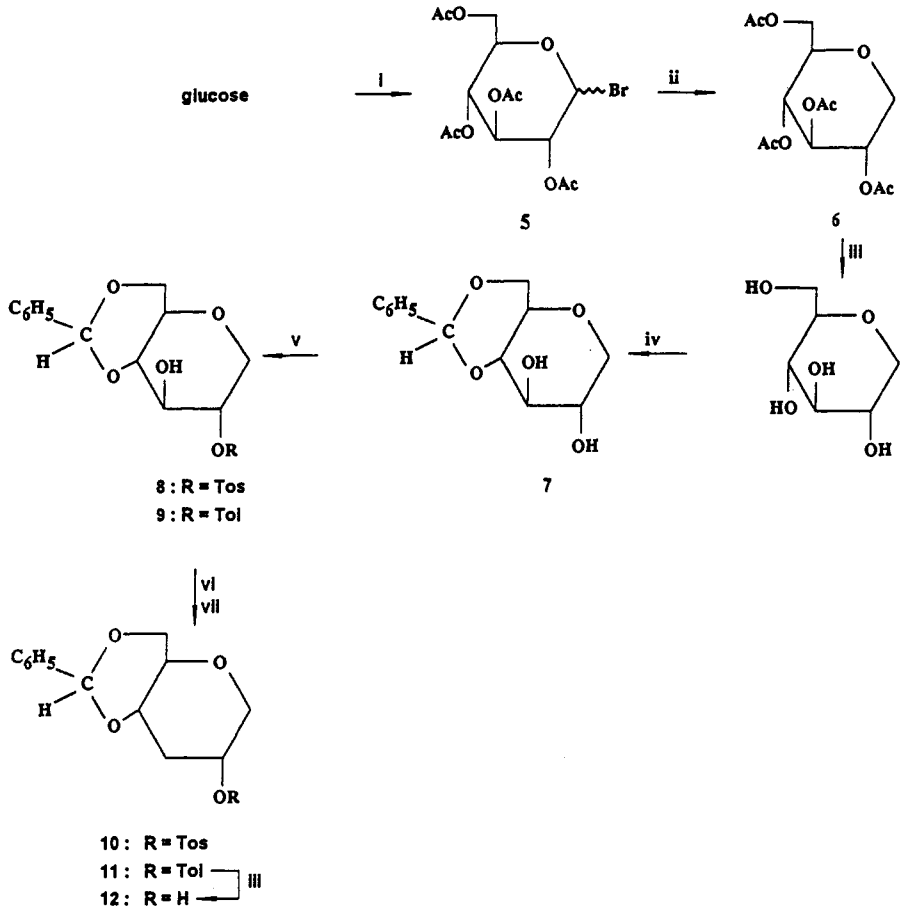
Scheme I[a]



[a] (i) Ac_2O, HBr/HOAc; (ii) Bu_3SnH, Et_2O; KF/H_2O; (iii) NaOMe (iv) C_6H_5COH, $ZnCl_2$; (v) Bu_2SnO, benzene, $CH_3C_6H_4SO_2Cl$ or $CH_3C_6H_4COCl$ resp. dioxane; (vi) $CSCl_2$, DMAP, 2,4-$Cl_2C_6H_3OH$, CH_2Cl_2; (vii) Bu_3SnH, AIBN, toluene.

Chemistry

The synthesis starts from glucose (Scheme I) which was converted to tetra-*O*-acetylglucopyranosyl bromide **5** by acetic anhydride and HBr in glacial acetic acid.[11] The acetobromoglucose **5** thus obtained was reductively dehalogenated to **6** by tributyltin hydride in dry ether at room temperature.[12] Tributyltin hydride could also be generated *in situ* from bis(tributyltin) oxide and polymethylhydrosiloxane. The reaction involves a photochemical, rather than a thermal, initiation of the reduction, thereby avoiding the thermal instability of **5**.[12] Tributyltin bromide formed during the reaction was converted to the in ether highly insoluble tributyltin fluoride.

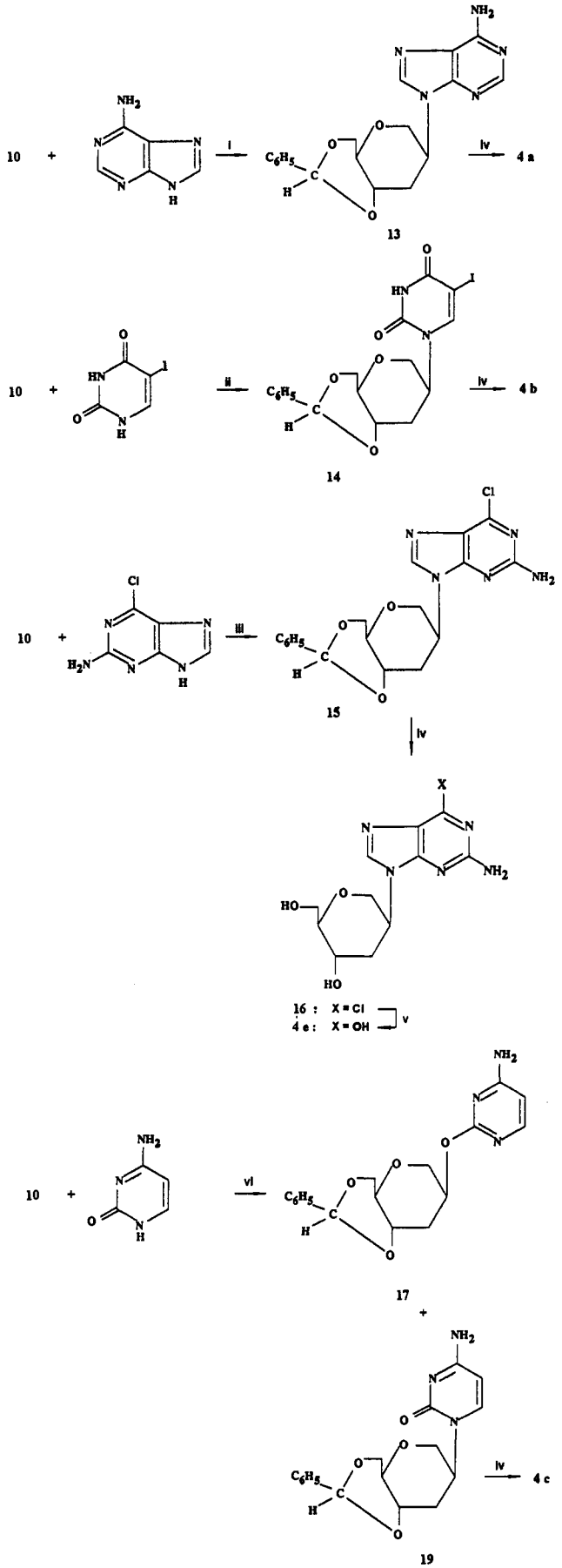
The acetyl groups were removed with sodium methoxide. A benzylidene protecting moiety was introduced by reaction with benzaldehyde in the presence of zinc chloride,[13] thus affording 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol, **7**.

Selective reaction of the hydroxyl function at position 2 was feasible following activation with dibutyltin oxide.[14] Position 2 either was selectively protected as an ester with toluoyl chloride or was functionalized with a leaving group by tosyl chloride, affording 1,5-anhydro-4,6-*O*-benzylidene-2-*O*-*p*-toluoyl-D-glucitol (**9**) or 1,5-anhydro-4,6-*O*-benzylidene-2-*O*-*p*-(tolylsulfonyl)-D-glucitol (**8**) in yields of 78% and 82%, respectively. The hydroxyl group in position 3 of **8** and **9** was removed by conversion to the 2,4-dichlorophenylthiocarbonate derivatives with thiophosgene, 4-(dimethylamino)pyridine (DMAP), and 2,4-dichlorophenol, followed by a Barton-type reduction (Bu_3SnH, AIBN) yielding **10** and **11** in 64% and 75% yield, respectively.[15] These reactions gave lower yields when smaller amounts of DMAP were used because of the hydrolysis of the benzylidene moiety as a side reaction.

Nucleophilic displacement of the tosylate[16] (Scheme II) of **10** with adenine/NaH in DMF worked well, affording **13** in 56% yield; deprotection with acetic acid gave the adenine analogue **4a**. Reaction of the sodium salt of 5-iodouracil[17] and **10** afforded **14** in 42% yield; **14** was then deprotected to **4b** with 80% acetic acid (22% overall yield). 2-Amino-6-chloropurine was introduced in 19% yield in the presence of potassium carbonate and a catalytic amount of 18-crown-6 to afford **15**. Deprotection with 80% acetic acid (giving **16**), followed by enzymatic hydrolysis of the 6-chlorine with adenosine deaminase[18] afforded the guanine analogue **4e** (11% overall yield). Direct alkylation of the other purines or pyrimidines with the tosylate **10** was low yielding. Reaction of **10** with thymine or cytosine gave only traces of the desired coupling product. Moreover, reaction with cytosine, in the presence of potassium carbonate as well as in the presence of potassium *tert*-butoxide[19] afforded mainly an O^2-coupled product **17** (in addition to the desired product **19**). Analogous results were recently reported by Nair and Nuesca during the synthesis of a series of isodideoxynucleoside.[20] We therefore introduced these base moieties starting from the alcohol **12** (obtained from **11**), using Mitsunobu reaction conditions[21] (Scheme III).

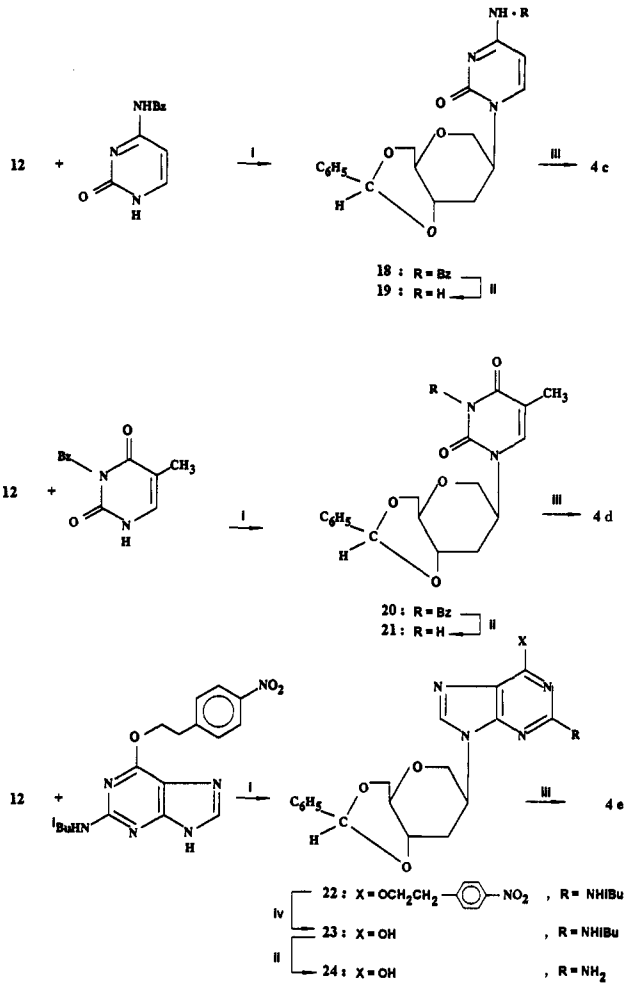
Compound **4c** was obtained in 29% yield from **12**, using N^4-benzoylcytosine,[22] triphenylphosphine (Ph_3P), and

Scheme II[a]



[a] (i) NaH, DMF; (ii) Na^+ salt of 5-iodouracil, DMF; (iii) K_2CO_3, 18-crown-6, DMF; (iv) 80% HOAc; (v) adenosine deaminase, phosphate buffer; (vi) K_2CO_3, 18-crown-6, DMF or tBuOK, DMSO.

Scheme III[a]



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

Table I. 400-MHz ^1H NMR Data of Compound **4a**

chemical shifts (ppm)		coupling constants (Hz)	
proton	DMSO-d_6	H,H	J(H,H)
H1′	3.87	$1'_{ax},1'_{eq}$	12.5
H1″	4.21	$1'_{ax},2'$	2
H2′	4.78	$1'_{eq},2'$	2.5
H3′	1.89	$1'_{eq},3'_{eq}$	1.5
H3″	2.29	$2',3'_{ax}$	4
H4′	3.52	$3'_{ax},3'_{eq}$	13.5
H5′	3.20	$3'_{ax},4'$	11
H6′	3.59	$3'_{eq},4'$	5
H6″	3.70	4′,HO4′	5
HO4′	4.92	4′,5′	9
HO6′	4.67	5′,6′	2.5
H2;H8	8.15;8.30	6′,6″	12
NH_2	7.25	6′,HO6′	5

diethyl azodicarboxylate (DEAD). Also, N^3-benzoylthymine[23] was reacted with **12** in the presence of Ph_3P and DEAD, which upon deprotection afforded **4d** in 50% yield. Partial loss of the *N*-benzoyl group of the protected pyrimidine during the purification of **20** was avoided by direct removal of the benzoyl group by NH_3/MeOH before isolation and purification.

The guanine analogue **4e** likewise was prepared by reaction of N^2-isobutyryl-O^6-[2-(*p*-nitrophenyl)ethyl]guanine[24] with **12** in the presence of Ph_3P and DEAD in THF. The (4-nitrophenyl)ethyl group of **22** was removed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and the isobutyryl

Table II. Activity against Human Herpes Viruses and Cytotoxicity of Compounds 4a–e

		minimal inhibitory concentration,[a] (μg/mL)									
virus (strain)	cell	**4a**	**4b**	**4c**	**4d**	**4e**	IDU	HPMPA	BVDU	GCV	ACV
HSV-1 (KOS)	ESM	7	0.07	0.7	40	0.2	0.2	0.2	0.004	0.004	0.02
HSV-1 (F)	ESM	4	0.07	1	40	0.2	0.2	0.2	0.004	0.007	0.4
HSV-1 (Mc Intyre)	ESM	20	0.07	2	7	0.7	1	2	0.02	0.001	0.7
HSV-2 (G)	ESM	7	0.07	0.04	150	0.1	0.2	0.02	10	0.004	0.02
HSV-2 (196)	ESM	0.04	0.07	0.02	40	0.02	0.2	0.07	300	0.02	2
HSV-2 (Lyons)	ESM	1	0.07	0.02	20	0.01	0.2	0.1	70	0.02	0.7
TK-HSV-1 (B2006)	ESM	150	>200	4	>400	20	70	2	>400	2.0	70
VZV (YS)	HEL	4	>100	0.27	>100	0.1	0.4	ND[b]	0.0015	0.072	0.15
VZV (OKA)	HEL	4	>100	0.04	>100	0.9	0.17	ND	0.0007	0.02	0.04
TK-VZV (YS-R)	HEL	3.2	>100	0.64	100	2.8	4	ND	30	0.9	5
TK-VZV (07-1)	HEL	10	>100	0.75	>100	4	4	ND	8	0.1	3
CMV (Davis)	HEL	>10	>10	0.75	>10	1.2	ND	ND	>100	6.2	ND
CMV (AD169)	HEL	>10	>10	0.64	>10	1.5	ND	ND	>100	3	ND
VSV	ESM	>200	>200	>400	>400	>200	>400	>400	>200	>400	>400
VV	ESM	40	>200	2	>400	7	0.7	0.7	2	70	>400
morphologic alteration	ESM	>400	>400	>400	>400	>400	>400	>400	>400	ND	ND
cell growth	HEL	>50	>50	2	>50	20	>50	ND	>200	85	>200

[a] Concentration required to reduce virus-induced cytopathicity (HSV, VSV, VV) plaque formation (VZV, CMV), or cell growth by 50%; for morphologic alteration, it corresponds to the minimal concentration required to cause a microscopically detectable change in normal cell morphology. The results listed are representative of a single experiment. The experiments were repeated several times. [b] Not determined.

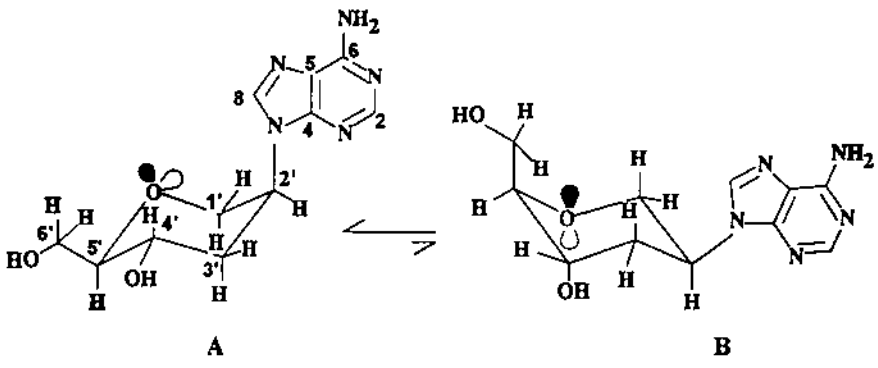


Figure 3.

group of **23** was removed with NH_3/MeOH to afford **24** in 47% overall yield from **12**.

The solution conformation of **4a** was deduced from its NMR spectra. Proton chemical shifts and coupling constants are depicted in Table I. The low values for $J(H1'_{ax},H2')$, $J(H1'_{eq},H2')$, and $J(H2',H3'_{ax})$ indicate an equatorial position of the hydrogen in position 2′ and an axial position of the heterocyclic moiety. The coupling constants found for H4′ suggest an axial orientation of this proton. The coupling between hydroxyl protons and the protons on adjacent carbon atoms in DMSO-d_6 differ significantly for axial and equatorial hydroxyls (OH_{eq}: J = 4.2–5.7 Hz and OH_{ax}: J = 3.0–4.2 Hz). The observed value for J(H4′,HO4′) confirms that HO4′ is an equatorial hydroxyl. The chemical shifts of the carbon atoms of compound **4a**, measured in DMSO-d_6, are given in the experimental section. The orientation of the base can be estimated from ^3J(C8,H2′) and ^3J(C4,H2′) couplings. A significantly higher value for the former (4.5 vs 1.5 Hz) was found. From Lemieux's graph[25] the corresponding dihedral angles are approximately ±50° [^3J(C4,H2′)] and ±140° [^3J(C8,H2′)], which shows that this compound exists predominantly in an anti conformation. This confirms a preference of the trans orientation of the fragment C8–N7–C2′–H2′. These results can be explained by the presence of more unfavorable 1,3-diaxial interactions in conformer B than in conformer A (Figure 3).

Biological Activity

Compounds **4a–e** were evaluated for their inhibitory effect on the cytopathicity 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), vaccina virus (VV) and vesicular stomatitis virus (VSV) in human embryonic skin muscle (ESM) fibroblast cell cultures.[26,27] As reference compounds were included (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 5-iodo-2′-deoxyuridine (IDU), (*S*)-9-(3-hydroxy-2-(phosphonylmethoxy)propyl)-adenine (HPMPA) (Table II).

All compounds **4a–e** demonstrate a marked activity against HSV-1 and HSV-2. Compound **4b** effected a 50% reduction of the cytopathicity induced by HSV-1 and HSV-2 at a concentration of 0.07 μg/mL. However, compound **4b** was not active against TK⁻ HSV-1. This indicates that the anti-HSV activity of **4b** depends on phosphorylation by the virus-encoded thymidine kinase. Also the cytosine **4c** and guanine **4e** analogues proved active when evaluated for their inhibitory effect on the cytopathicity of HSV-1 [minimal inhibitory concentration (MIC): 0.7–2, 0.2–0.7 μg/mL, respectively] and HSV-2 (MIC = 0.2–0.04, 0.01–0.1 μg/mL, respectively). Compounds **4d** and **4a** were less active against HSV-1 and HSV-2 than were **4b**, **4c**, and **4e**.

Compounds **4c** and **4e** were also inhibitory to VV, but less so than to HSV. None of the compounds was active against VSV.

None of the test compounds proved cytotoxic at a concentration up to 400 μg/mL, as monitored by a microscopically detectable alteration of normal cell morphology (ESM). However, the cytosine **4c** and guanine (**4e**) analogues were found to inhibit the growth of the cells [human embryonic lung (HEL)] at a concentration of 2 and 20 μg/mL, respectively.

The five anhydrohexitol nucleosides were also evaluated for their inhibitory effect on the cytopathicity of varicella-zoster virus (VZV, strains OKA and YS), TK⁻ VZV (strains O7–1 and YS-R), and cytomegalovirus (CMV) (strains AD 169 and Davis) in HEL cells (Table II). In this case, acyclovir (ACV) and ganciclovir (GCV) were included as the reference compounds.

Compounds **4a**, **4c**, and **4e** exhibited activity against VZV at a concentration that was 10–20-fold lower than the concentration required to inhibit cell growth. They were equally active against TK⁺ and TK⁻ VZV, which means that the virus-encoded TK is not required for their antiviral activity. In contrast, compound **4b**, although

Table III. Activity against Human Immunodeficiency Viruses and Tumor Cell Lines of Compounds 4a–e

virus (strain)/cell	50% inhibitory concentration[a] (μg/mL)				
	4a	4b	4c	4d	4e
HIV-1 (III_B)	>4	>100	>0.8	36 ± 10	>4
HIV-2 (ROD)	>4	>100	>0.8	>100	>4
MT-4	12.5 ± 5.5	>100	1.1 ± 0.5	>100	21 ± 13
MOLT-4	>100	>100	0.87 ± 0.01	>100	14 ± 1.1
CEM	>100	>100	1.4 ± 0.2	>100	21 ± 3.7
FM3A	97.5 ± 3.5	>100	28 ± 1.4	>100	12.3 ± 0.9
L1210	≥100	>100	29 ± 3	>100	5.4 ± 0.3

[a] Concentration required to acheive 50% protection of MT-4 cells against cytopathicity of HIV-1 or HIV-2 or concentration required to reduce the growth (viability) of MT-4, MOLT-4, CEM, FM3A, or L1210 cells by 50%.

very active against HSV-1 and HSV-2, proved inactive against VZV. This is in agreement with the fact that **4b** has no affinity for VZV TK (50% inhibitory concentration >50 μM), while for BVDU the IC_{50} is 0.09 μM (when evaluated under the same conditions).

The guanosine analogue **4e** proved active against CMV at a concentration that was about 15-fold lower than the concentration required to inhibit cell growth. Compound **4c** also inhibited CMV plaque formations but only at a concentration that was 3-fold lower than the concentration required to inhibit host cell proliferation (Table II).

Compounds **4a–e** were also evaluated for their cytostatic activity against murine and human tumor cell lines (Table III). Compound **4b** was not inhibitory to the growth of any of the tumor cell lines, at a concentration of 100 μg/mL, which further underscores the selectivity of its anti-HSV effect (MIC: 0.07 μg/mL). Compound **4e** was cytostatic at a 50% inhibitory concentration (IC_{50}) ranging from 5.4 to 21 μg/mL; **4e** did not discriminate in its cytostatic activity against murine and human tumor cell lines. In contrast, **4c** proved markedly more inhibitory to human MT-4, Molt-4, and CEM cell proliferation (IC_{50}: 0.9–1.4 μg/mL) than murine tumor cell lines (IC_{50}: 28 μg/mL). When **4c** was evaluated against CEM/dCK$^-$ (2′-deoxycytidine kinase deficient CEM) cells, the IC_{50} was 75 μg/mL. These findings suggest that **4c** needs to be phosphorylated by the cellular dCK to be metabolically active.

Compounds **4a–e** were also evaluated for their inhibitory effects on the cytopathicity of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) in MT-4 cells. The compounds did not inhibit the cytopathicity of HIV-1 or HIV-2 at concentrations below the cytotoxicity threshold for the host (MT-4) cells (Table III). Compounds **4c** and **4e** effected a 50% reduction in the growth (viability) of MT-4 cells at a concentration of 1.1 and 21 μg/mL, respectively.

Conclusion

Of the 2-substituted 1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol derivatives that were the subject of the present study, three compounds appear particularly interesting for further follow up: (i) the 5-iodouracil derivative **4b** which is a highly selective, viral TK-dependent, inhibitor of HSV-1 and HSV-2 replication but has no activity whatsoever against VZV. This behavior is different from that of other vial TK-dependent agents as BVDU which are active against both HSV-1 and VZV.[28,29] Apparently, compound **4b** discriminates in its affinity to the HSV- and VZV-thymidine kinases; (ii) the cytosine **4c** and guanine **4e** derivatives, which were found active against a wide range of herpesviruses, including HSV-1, HSV-2, VZV, and CMV at concentrations that were not toxic to the host cells. Cellular deoxycytidine kinase seems to be involved in the phosphorylation of **4c**. Other enzymes involved in the metabolism of compounds **4b**, **4c**, and **4e** remain to be identified. Also their mechanism (i.e. target) of action remains subject of further investigation.

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 spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer or Bruker AMX 400 MHz with tetramethylsilane as internal standard for the ^1H 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 signal, m = multiplet, ddd = double doublet of doublet, dm = double 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). Anhydrous solvents were obtained as follows: methanol was refluxed on magnesium methoxide (I_2, Mg, MeOH) overnight and then distilled; water was removed from DMF by distillation with benzene followed by distillation *in vacuo*; dichloromethane was stored on calcium hydride, refluxed and distilled; tetrahydrofuran and dioxane were refluxed overnight on lithium aluminum hydride and distilled; diethyl ether was refluxed overnight on sodium and distilled.

Elemental analyses were obtained from Dr. W. Rozdzinski, Institut für Organische Chemie, Biochemie und Isotopenforschung, D-7000 Stuttgart 80, Germany.

1,5-Anhydro-4,6-*O*-benzylidene-D-glucitol (7). Removal of the protecting groups of **6**[12] was accomplished by treatment of 30.06 g (90.5 mmol) of **6** with 400 mL of 0.1 N NaOMe for 2 h at room temperature. The mixture was neutralized with acetic acid and evaporated. After coevaporation with toluene, 12.4 g (91 mmol) of freshly dried $ZnCl_2$ and 46.5 mL (455 mmol) of benzaldehyde were added and the suspension was stirred vigorously for 2 days at room temperature.

The mixture was poured into cold water and extracted three times with ethyl acetate. The combined organic layer was dried on anhydrous Na_2SO_4. After filtration and removal of the solvent, the excess benzaldehyde was partially removed *in vacuo* at 70 °C (oil pump). The solid residue was purified further by washing on a glass funnel with *n*-hexane, followed by chromatographic purification [(1) hexane–CH_2Cl_2, 1:1; (2) CH_2Cl_2; (3) CH_2Cl_2–MeOH, 98:2], affording 17.1 g (68 mmol, 75% yield) of **7**. CIMS (iC_4H_{10}): *m/e* 253 (MH^+). ^1H NMR (DMSO-d_6): δ 3.00–3.90 (m, 7H) and 4.10–4.30 (m, 1H) (H-1′, H-1″, H-2′, H-3′, H-4′, H-5′, H-6′, H-6″), 5.03–5.31 (dd, 2H, 2′-OH, 3′-OH), 5.55 (s, 1H, PhCH), 7.20–7.57 (m, 5H, aromatic H) ppm. ^{13}C NMR (DMSO-d_6: δ 68.0 (C-6′), 70.2, 70.4 (C-1′, C-5′), 71.0 (C-2′), 74.4 (C-3′), 81.1 (C-4′), 100.7 (PhCH), 126.3, 127.9, 128.7, 137.8 (aromatic C) ppm.

1,5-Anhydro-4,6-*O*-benzylidene-2-*O*-(*p*-tolylsulfonyl)-D-glucitol (8). The glucitol derivative **7** (8.5 g, 33.67 mmol) and dibutyltin oxide (8.38 g, 33.67 mmol) were suspended in 250 mL of benzene. The mixture was refluxed for 16 h with azeotropic removal of water. After the volume had been reduced to approximately 100 mL, the residue was dissolved in 150 mL of anhydrous dioxane, and 7.06 g (37.04 mmol) of *p*-toluenesulfonyl chloride was added. The mixture was heated at 50 °C for 6 h, resulting in a quantitative conversion to a less polar product. The mixture was concentrated, absorbed on Celite, and purified by column chromatography (CH_2Cl_2–hexane, 1:1; CH_2Cl_2) to yield 11.22 g (27.6 mmol, 82%) of **8** as a white powder. EIMS; *m/e* 406 (M^+). 400-MHz ^1H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 3.35–3.42 (m, 2H, H-4′, H-5′), 3.49 (t, J = 11 Hz, 1H, H-1′α), 3.61 (m, 1H, H-6′), 3.67 (m, 1H, H-3′), 3.87 (dd, J = 5.5 and 11 Hz, 1H, H-1′β), 4.14–4.25 (m, 2H, H-2, H-6″), 5.05 (s, 1H, PhCH), 5.12

(d, J = 5.5 Hz, 1H, OH), 7.35–7.50 (m, 7H, aromatic H), 7.85 (m, 2H, aromatic H) ppm. 90-MHz ^{13}C NMR (DMSO-d_6): δ 21.0 (CH_3), 66.9, 67.6 (C-1, C-6), 70.7, 70.8 (C-3, C-5), 79.2, 80.4 (C-2, C-4), 100.7 (PhCH) ppm + aromatic C.

1,5-Anhydro-4,6-*O*-benzylidene-2-*O*-*p*-toluoyl-D-glucitol (9). A mixture of the sugar derivative **7** (8.5 g, 33.67 mmol) and dibutyltin oxide (8.38 g, 33.67 mmol) in 250 mL of benzene was refluxed for 16 h with azeotropic removal of water. The solution was concentrated, and 150 mL of dry dioxane was added. *p*-Toluoyl chloride (4.44 mL, 33.67 mmol) was added dropwise, and the mixture was stirred at room temperature for 5 h. The mixture was concentrated, absorbed on Celite, and purified by column chromatography to afford 9.73 g (26.30 mmol, 78%) of **9** as a white powder. CIMS (iC_4H_{10}): m/e 371 (MH^+). ^1H NMR ($CDCl_3$): δ 2.40 (s, 1H, CH_3), 3.19–4.51 (m, 8H, H-1′, H-1″, H-2′, H-3′, H-4′, H-5′, H-6′, H-6″), 4.93–5.50 (br s, 1H, 3′-OH), 5.55 (s, 1H, PhCH), 7.05–8.03 (m, 9H, aromatic H) ppm. ^{13}C NMR ($CDCl_3$): δ 21.5 (CH_3), 67.2, 68.4 (C-1′, C-6′), 70.9, 71.9 (C-3′, C-5′), 72.6 (C-2′), 80.9 (C-4′), 101.9 (PhCH), aromatic C, 165.9 (C=O) ppm.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-2-*O*-(*p*-tolylsulfonyl)-D-*ribo*-hexitol (10). (A) Tosylated sugar **8** (11.22 g, 27.6 mmol) and 23.60 g (193 mmol) of 4-(dimethylamino)pyridine (DMAP) were dissolved in 400 mL of dry CH_2Cl_2. The reaction mixture was cooled to −40 °C, and 2.53 mL of thiophosgene (33.12 mmol) was added with vigorous stirring. The mixture was brought to room temperature. After the mixture was stirred for 1 h, 6.30 g (38.64 mmol) 2,4-dichlorophenol was added and stirring was continued for 2 h more. The mixture was poured into 300 mL of 1 M KH_2PO_4 and extracted twice with CH_2Cl_2. The organic layers were dried, the volatiles were removed *in vacuo*, and the residue was purified by flash chromatography (hexane–CH_2Cl_2, 8:2, to CH_2Cl_2).

(B) The obtained thiocarbonyl compound was dissolved in 300 mL of anhydrous toluene. After N_2 was bubbled through the solution for 10 min, 7.84 mL (29.15 mmol), of tri-*n*-butyltin hydride and 325 mg (2 mmol) of 2,2′-azobis(2-methylpropionitrile) were added, and the reaction mixture was heated at 80 °C overnight. The mixture was evaporated and purified on silica gel affording 6.90 g (17.67 mmol, 64%) of **10**. CIMS (NH_3): m/e 391 (MH^+). ^1H NMR ($CDCl_3$): δ 1.50–2.10 (m, 2H, H-3′, H-3″), 2.48 (s, 3H, CH_3), 3.06–4.84 (m, 7H, H-1′, H-1″, H-2′, H-4′, H-5′, H-6′, H-6″), 5.50 (s, 1H, PhCH), 7.04–7.98 (m, 9H, aromatic H) ppm. ^{13}C ($CDCl_3$): δ 21.4 (CH_3), 35.3 (C-3′) 68.7, 69.0 (C-1′, C-6′), 72.9, 73.1 (C-4′, C-5′), 75.7 (C-2′), 101.5 (PhCH) ppm + aromatic C.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-2-*O*-*p*-toluoyl-D-*ribo*-hexitol (11). The reaction was carried out as described for the synthesis of **10**. Treatment of 9.73 g (26.30 mmol) of the toluoylated hexitol **9** afforded 6.79 g (19.73 mmol, 75%) of **11** after chromatographic purification. CIMS (iC_4H_{10}): m/e 355 (MH^+). ^1H NMR ($CDCl_3$) δ 1.42–2.12 (m, 2H, H-3′, H-3″), 2.39 (s, 3H, CH_3), 3.12–3.92 (m, 4H) and 4.02–4.49 (m, 2H) (H-1′, H-1″, H-4′, H-5′, H-6′, H-6″), 4.95–5.43 (m, 1H, H-2), 5.54 (s, 1H, PhCH), 7.10–8.08 (m, 9H, aromatic H) ppm. ^{13}C NMR ($CDCl_3$): δ 21.5 (CH_3), 34.8 (C-3′), 66.9 (C-5′), 69.0, 69.1 (C-1′ and C-6′), 73.4 (C-2′), 76.0 (C-4′), 101.5 (PhCH), aromatic C, 165.3 (C=O) ppm.

1,5-Anhydro-4,6-*O*-benzylidene-3-deoxy-D-glucitol (12). Removal of the toluoyl group of **11** was accomplished by treatment of 6.79 g (19.73 mmol) with 300 mL of 0.1 M NaOMe for 4 h at room temperature. After neutralization and evaporation of the volatiles, the residue was purified by column chromatography (CH_2Cl_2–MeOH, 99:1), yielding 3.72 g (15.81 mmol, 80%) of the title compound. CIMS (iC_4H_{10}): m/e 237 (MH^+). ^1H NMR (DMSO-d_6): δ 1.20–1.66 (m, 1H, H-3′), 2.06–2.42 (m, 1H, H-3″), 2.99–3.98 (m, H6) and 4.05–4.30 (m, 1H) (H-1′, H-1″, H-2′, H-4′, H-5′, H-6′, H-6″), 5.08 (d, 1H, 2′-OH), 5.57 (s, 1H, PhCH), 7.17–7.67 (m, 5H, aromatic H) ppm. ^{13}C NMR ($CDCl_3$): δ 38.3 (C-3′), 65.4 (C-5′), 69.1 (C-6′), 72.3, 73.0 (C-1′ and C-2′), 76.3 (C-4′), 101.6 (PhCH), 126.1, 128.2, 129.0, 137.2 (aromatic C) ppm.

1,5-Anhydro-4,6-*O*-benzylidene-2-(adenin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol (13). A mixture of 1.35 g (10 mmol) of adenine, 400 mg of sodium hydride (60% dispersion, 10 mmol), and 529 mg (2 mmol) of 18-crown-6 in 60 mL of dry DMF was stirred at 80 °C for 1 h. After a solution of 1.95 g (5 mmol) of **10** in 30 mL of anhydrous DMF was added, stirring was continued for 16 h at 100 °C. The reaction mixture was cooled and evaporated. The residue was dissolved in ethyl acetate (100 mL), and the organic phase was washed with saturated $NaHCO_3$ solution (50 mL) and H_2O (2 × 25 mL), dried, and evaporated. The solid residue was purified by column chromatography (CH_2Cl_2–MeOH, 97:3), affording 989 mg (2.8 mmol, 56% yield) of **13**. An amount of 190 mg (0.49 mmol, 9%) of the unreacted tosylate **10** was recovered. UV (MeOH): λ_{max} 262 nm (ε = 11 300). EIMS: m/e 353 (M^+). 400-MHz ^1H NMR (DMSO-d_6) δ 2.17 (dt, ^2J = 1,3 Hz, $^3J_{3'4'}$ = 12 Hz, $^3J_{3'2'}$ = 4 Hz, 1H, H-3′ax), 2.46 (dm, 1H, H-3′eq), 3.53 (ddd, $^3J_{4'5'}$ = 9 Hz, $^3J_{5'6'}$ = 10 Hz and 5 Hz, 1H, H-5′), 3.73 (ddd, ^3J = 12, 9, and 4 Hz, 1H, H-4′), 3.80 (t, ^2J = 10 Hz, 1H, H-6′ax), 4.10 (dd, ^2J = 13 Hz, ^3J = 2.5 Hz, 1H, H-1′ax), 4.22 (dd, ^3J = 5 Hz, 1H, H-6′eq), 4.44 (d, ^2J = 13 Hz, 1H, H-1′eq), 4.90 (br s, 1H, H-2′), 5.62 (s, 1H, PhCH), 7.30–7.40 (m, 7H, NH_2, aromatic H), 8.18 (s, 1H), 8.27 (s, 1H) (H-2 and H-8) ppm. 400-MHz ^{13}C NMR (DMSO-d_6): δ 32.23 (C-3′), 50.47 (C-2′), 68.11, 68.88 (C-1′, C-6′), 73.42, 73.80 (C-4′, C-5′), 100.93 (PhCH), 118.56 (C-5), 126.21 (Co), 128.08 (Cm), 128.85 (Cp), 137.77 (Cx), 139.28 (C-8), 149.49 (C-4), 152.58 (C-2), 156.17 (C-6) ppm.

1,5-Anhydro-2-(adenin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol (4a). The benzylidene moiety of **13** was hydrolyzed by heating 989 mg (2.8 mmol) in 100 mL of 80% acetic acid at 80 °C for 3 h. After evaporation and coevaporation with toluene, the residue was dissolved in water and washed with diethyl ether. The water layer was evaporated, and the residue was crystallized from MeOH–Et_2O, affording 602 mg (2.27 mmol, 81% yield) of **4a**. Mp: 237–239 °C. UV (MeOH): λ_{max} 261 nm (ε = 13 500). CIMS (NH_3): m/e 266 (MH^+), 136 (BH_2^+). 400-MHz ^1H NMR (DMSO-d_6) δ 1.89 (ddd, ^2J = 13 Hz, $^3J_{3'4'}$ = 11 Hz, $^3J_{3'2'}$ = 4 Hz, 1H, H-3′ax) 2.29 (dm, ^2J = 13.5 Hz, 1H, H-3′eq), 3.20 (m, $^3J_{4'5'}$ = 9 Hz, 1H, H-5′), 3.52 (m, $^3J_{4'5'}$ = 9 Hz, 1H, H-4′), 3.59 (m, 1H, H-6′), 3.70 (ddd, ^2J = 12 Hz, $^3J_{OH}$ = 5 Hz, $^3J_{5'6'}$ = 2.5 Hz, 1H, H-6″), 3.87 (dd, ^2J = 12.5 Hz, ^3J = 2 Hz, 1H, H-1′ax), 4.21 (dt, ^2J = 12.5 Hz, ^3J = 2.5 Hz, ^4J = 1.5 Hz, 1H, H-1′eq), 4.67 (t, ^3J = 5.5 Hz, 1H, 6′-OH), 4.78 (m, $^3J_{2'3'ax}$ = 4 Hz, 1H, H-2′), 4.92 (d, ^3J = 5 Hz, 1H, 4′-OH), 7.25 (s, 2H, NH_2), 8.15 (s, 1H), 8.30 (s, 1H) (H-2, H-8) ppm. ^{13}C NMR (DMSO-d_6): δ 36.03 (C-3′), 50.18 (C-2′), 60.59 (C-6′), 60.85 (C-4′), 68.11 (C-1′), 83.11 (C-5′), 118.40 (C-5), 139.70 (C-8), ^3J (C8, H2′) = 4.5 Hz, 149.47 (C-4), ^3J (C4, H2′) = 1.5 Hz, 152.49 (C-2), 156.11 (C-6) ppm. Anal. ($C_{11}H_{15}N_5O_3$·0.35Et_2O) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(5-iodouracil-1-yl)-D-*arabino*-hexitol (4b). A mixture of 2.60 g (10 mmol) of the sodium salt of 5-iodouracil[17] and 1.95 g (5 mmol) of crude tosylate **10** in 80 mL of dry DMF was stirred at 100 °C for 16 h. The reaction mixture was cooled and evaporated. The residue was dissolved in 100 mL of EtOAc, and the organic layer was washed successively with saturated $NaHCO_3$ solution (50 mL) and water (3 × 50 mL), dried, and evaporated. Column chromatography (CH_2Cl_2–MeOH, 98:2) afforded 58 mg (2.1 mmol, 42% yield) of **14** as an oil, and 371 mg (0.95 mmol) of the unreacted tosylate was recovered. The obtained oil was heated in 100 mL of 80% acetic acid at 80 °C until complete hydrolysis of the benzylidene moiety. The mixture was evaporated and coevaporated with toluene and purified by column chroamtography (CH_2Cl_2–MeOH, 90:10), yielding 408 mg (1.11 mmol, 53%) of the title product **4b** which was crystallized from MeOH. Mp: 219–220 °C. UV (MeOH): λ_{max} 282 nm (ε = 15 800). CIMS (NH_3): m/e 369 (MH^+). ^1H NMR (DMSO-d_6): δ 1.50–1.90 (m, 1H, H-3′), 1.95–2.25 (m, 1H, H-3″), 2.18–4.20 (m, 6H, H-1′, H-1″, H-5′, H-4′, H-6′, H-6″), 4.50 (m, 1H, 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) ppm. ^{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) ppm. Anal. ($C_{10}H_{13}N_2O_5I$·0.5H_2O) C, H, N.

1,5-Anhydro-2-(cytosin-1-yl)-2,3-dideoxy-D-*arabino*-hexitol (4c). Method A. A mixture of 390 mg (1 mmol) of **10**, 167 mg (1.5 mmol) of cytosine, 276 mg (2 mmol) of K_2CO_3, and 396 mg (1.5 mmol) of 18-crown-6 in 20 mL of dry DMF was stirred at 100 °C for 21 h. The reaction mixture was cooled, evaporated, and coevaporated with *m*-xylene. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 98:2), affording 160 mg of the O^2-isomer **17** as a white powder (0.48 mmol, 48%).

Method B.[19] A mixture of 167 mg (1.5 mmol) of cytosine and 168 mg (1.5 mmol) of freshly sublimated potassium *tert*-butoxide in 5 mL of anhydrous DMSO was sonicated and occasionally heated until an homogeneous solution was formed. This solution was added to a solution of 390 mg (1 mmol) of **10** in 8 mL of DMSO. The reaction mixture was stirred overnight at 50 °C. After removal of the solvent *in vacuo*, the residue was dissolved in EtOAc (20 mL) and washed three times with water (3 × 20 mL). The organic layer was dried and evaporated. Purification by column chromatography (CH_2Cl_2–MeOH, 95:5) afforded 120 mg (0.36 mmol, 36%) of the O^2-isomer **17** and 50 mg (0.15 mmol, 15%) of the N^1-isomer **19**.

Method C. A suspension of 2.15 g (10 mmol) of N^4-benzoylcytosine,[22] 1.18 g (5 mmol) of the alcohol **12**, and 3.28 g (12.5 mmol) of triphenylphosphine in 100 mL of anhydrous dioxane was treated with 1.97 mL (12.5 mmol) of diethyl azodicarboxylate in 20 mL of anhydrous THF for 15 h at room temperature. After removal of the volatiles, the residue was taken up in 100 mL of EtOAc and washed twice with 50 mL of water.

The organic layer was dried on anhydrous Na_2SO_4, evaporated, and adsorbed on silica gel. Purification by column chromatography afforded 800 mg (1.85 mmol, 37%) of crude 1,5-anhydro-4,6-*O*-benzylidene-2,3-dideoxy-2-(N^4-benzoylcytosin-1-yl)-D-*arabino*-hexitol, **18**.

The benzoyl group was removed by treatment with 70 mL of NH_3/MeOH for 5 h at room temperature. Evaporation left an oil which was purified on silica gel (CH_2Cl_2–MeOH, 98:2) to afford 400 mg of the debenzoylated derivative **19** as an oil.

The obtained oil was treated with 50 mL of 80% acetic acid at 80 °C for 5 h. After evaporation and coevaporation with toluene, the residue was dissolved in water and washed with diethyl ether. The water layer was evaporated, and the precipitate was crystallized from MeOH–Et_2O, yielding 234 mg of the title compound **4c** (0.97 mmol, 80%). Mp: 278 °C. UV (MeOH): λ_{max} 276 nm (ϵ = 8200). CIMS (iC_4H_{10}): *m/e* 242 (MH^+). ^1H NMR (DMSO-d_6): δ 1.47–1.87 (m, H-3′), 1.91–2.28 (m, H-3″), 2.95–3.30 (m, 1H, H-5′), 3.35–4.10 (m, 4H, H-1′, H-1″, H-4′, H-6′, H-6″), 4.52 (m, 2H, 6′-OH, H-2′), 4.85 (d, *J* = 4.8 Hz, 1H, 4′-OH), 5.66 (d, *J* = 7.5 Hz, 1H, H-5), 6.99 (s, NH_2), 7.97 (d, *J* = 7.5 Hz, H-6) ppm. ^{13}C NMR (DMSO-d_6): δ 35.7 (C-3′), 51.5 (C-2′), 61.0, 61.2 (C-4′, C-6′), 67.9 (C-1′), 82.9 (C-5′), 93.7 (C-5), 144.3 (C-6), 156.3 (C-2), 165.7 (C-4) ppm. Anal. ($C_{10}H_{15}N_3O_4$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(thymin-1-yl)-D-*arabino*-hexitol (4d). The title compound was synthesized from **10** but in very moderate yield. Better results are obtained starting from the alcohol **12**.

A suspension of 2.40 g (10.46 mmol) of N^3-benzoylthymine,[23] 1.23 g (5.23 mmol) of the alcohol **12**, and 3,43 g (13.08 mmol) of triphenylphosphine in 100 mL of anhydrous dioxane was treated with 2.06 mL (13.08 mmol) of diethyl azodicarboxylate (DEAD) in 15 mL of anhydrous THF. The solution was stirred overnight at room temperature, after which the volatiles were removed *in vacuo*. The residue containing **20** was taken up 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, 98:2). This yielded 3.5 g of crude **21** which also contained hydrazine dicarboxylate.

The crude **21** was taken up in 50 mL 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, 93:7). Crystallization from MeOH–Et_2O afforded 671 mg of **4d** as white crystals (2.62 mmol, 50% overall yield). Mp: 169–171 °C. UV (MeOH): λ_{max} 272 nm (ϵ = 9500). CIMS (iC_4H_{10}): *m/e* 257 (MH^+). ^1H NMR (DMSO-d_6): δ 1.77 (s, CH_3), 1.6–2.5 (m, 2H, H-3′, H-3″), 3.05–3.30 (m, 1H, H-5′), 3.4–4.1 (m, 4H) and 3.7–4.1 (m, 2H) (H-1′, H-1″, H-4′, H-6′, H-6″), 4.52 (m, 1H, H-2′), 4.65 (t, *J* = 5.7 Hz, 6′-OH), 4.89 (d, *J* = 5 Hz, 4′-OH), 7.88 (s, H-6), 11.25 (br, NH) ppm. ^{13}C NMR (DMSO-d_6): δ 12.3 (CH_3), 35.2 (C-3′), 50.1 (C-2′), 60.3, 60.8 (C-4′, C-6′), 66.9 (C-1′), 82.4 (C-5′), 108.3 (C-5), 138.9 (C-6), 150.9 (C-2), 163.8 (C-4) ppm. Anal. ($C_{11}H_{16}N_2O_5 \cdot 0.5H_2O$) C, H, N.

1,5-Anhydro-2,3-dideoxy-2-(guanin-9-yl)-D-*arabino*-hexitol (4e). Method A. 1,5-Anhydro-4,6-*O*-benzylidene-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol (15). The tosylate **10** (1.56 g, 4 mmol) and 848 mg (5 mmol) of 2-amino-6-chloropurine were dissolved in 30 mL of anhydrous DMF to which was added 830 mg (6 mmol) of anhydrous potassium carbonate and 530 mg (2 mmol) of 18-crown-6. The mixture was stirred at 120 °C for 5 h, after which the volatiles were removed *in vacuo* and the residue was adsorbed on silica gel. Purification yielded 295 mg (0.76 mmol, 19%) of the title compound **15**. ^1H NMR ($CDCl_3$): δ 1.86–2.32 (m, 1H, H-3′), 2.45–2.75 (m, 1H, H-3″), 3.5–3.9 (m, 3H), 4.07 (dd, *J* = 2.6 and 13 Hz, 1H), 4.34 (m, 2H), 4.77 (m, 1H, H-2′), 5.30 (s, NH_2), 5.48 (s, 1H, PhCH), 7.2–7.5 (m, NH, aromatic H), 8.27 (s, 1H, H-8) ppm. ^{13}C NMR ($CDCl_3$): δ 32.8 (C-3′), 50.8 (C-2′), 68.8, 69.2 (C-6′, C-1′), 73.7, 74.6 (C-4′, C-5′), 101.9 (PhCH), 125.9, 128.1, 128.9, 137.0 (Ph), 126.1 (C-5), 141.1 (C-8), 151.5 (C-6), 153.5 (C-4), 159.0 (C-2) ppm.

1,5-Anhydro-2-(2-amino-6-chloropurin-9-yl)-2,3-dideoxy-D-*arabino*-hexitol (16). The obtained compound **15** (295 mg, 0.76 mmol) was heated in 50 mL of 80% acetic acid at 80 °C until complete hydrolysis of the benzylidene moiety. Evaporation and coevaporation with toluene left an oil which was purified on silica gel (CH_2Cl_2–MeOH, 9:1). The product precipitated on concentration of the eluate yielding 145 mg (0.48 mmol, 63%) of **16**. UV (MeOH): λ_{max} 224 nm (ϵ = 27 000), 249 (ϵ = 6100), 310 (ϵ = 8000). ^1H NMR (DMSO-d_6) δ 1.7–2.5 (m, 2H, H-3′, H-3″), 2.90–4.30 (m, 6H), 4.67 (m, 2H, 6′-OH), H-2′), 4.95 (d, *J* = 5.2 Hz, 1H, 4′-OH), 6.95 (s, 2H, NH_2), 8.30 (s, 1H, H-8) ppm. ^{13}C NMR (DMSO-d_6): δ 35.7 (C-3′), 50.3 (C-2′), 60.5, 60.7 (C-4′, C-6′), 67.8 (C-1′), 83.0 (C-5′), 123.0 (C-5), 141.9 (C-8), 149.5 (C-6), 154.0 (C-4), 159.8 (C-2) ppm.

1,5-Anhydro-2,3-dideoxy-2-(guanin-9-yl)-D-*arabino*-hexitol (4e). A mixture of 145 mg (0.48 mmol) of **16** and 0.5 mL of a suspension of adenosine deaminase in 100 mL of 0.05 M phosphate buffer, pH 7.5, was incubated for 4 h at 30 °C. The reaction mixture was concentrated to about 15 mL, and the precipitate was filtered off. Recrystallization from water afforded 50 mg of analytically pure **4e**. The filtrates were put on top of a XAD column (25 × 2 cm) which was eluted with water followed by MeOH–water (3:1). Evaporation gave an additional 70 mg of **4e** as a white product, totalling 0.43 mmol (89%). Mp: >300 °C. UV (H_2O): λ_{max} 253 (ϵ = 9100). CIMS (iC_4H_{10}): *m/e* 282 (MH^+). ^1H NMR (DMSO-d_6): δ 1.61–2.40 (m, 2H, H-3′, H-3″), 2.81–4.38 (m, 6H, H-1′, H-1″, H-4′, H-5′, H-6′, H-6″) 4.52 (br, 2H, 6′-OH, H-2′), 4.92 (br, 1H, 4′-OH), 6.56 (br, 2H, NH_2), 7.87 (s, 1H, H-8) ppm. ^{13}C NMR (DMSO-d_6): δ 36.3 (C-3′), 50.2 (C-2′), 61.0, 61.2 (C-4′, C-6′), 68.4 (C-1′), 83.2 (C-5′), 116.3 (C-5), 136.9 (C-8), 151.5 (C-4), 154.1 (C-2), 157.9 (C-6) ppm. Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

Method B. N^2-Isobutyryl-O^6-[2-(*p*-nitrophenyl)ethyl]guanine[24] (2.8 g, 7.5 mmol) was suspended in 100 mL of dry dioxane and refluxed for 30 min. After cooling to room temperature, the suspension was treated with triphenylphosphine (2.63 g, 10 mmol) and 1.18 g (5 mmol) of **12** in 70 mL dry THF. Diethyl azodicarboxylate (DEAD, 95%, 1.66 mL, 10 mmol) in 20 mL of dry THF was added over 30 min, and the reaction mixture was stirred overnight at room temperature to yield a clear solution. The reaction mixture was concentrated, and flash column chromatography ((1) CH_2Cl_2–hexane, 1:1; (2) CH_2Cl_2; (3) CH_2Cl_2–MeOH, 95:5) afforded **22** which was still contaminated with triphenylphosphine.

The obtained mixture was dissolved in 100 mL of dry pyridine and treated with 1.5 mL (10 mmol) of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) for 15 h at room temperature. The mixture was concentrated and coevaporated with toluene. The residue was purified by column chromatography ((1) CH_2Cl_2–MeOH, 99:1; (2) CH_2Cl_2–MeOH, 96:4) to yield 1.15 g (2.62 mmol, 53%) of the N^2-isobutyrylguanine derivative **23**. The isobutyryl group was removed by treating 1.10 g (2.51 mmol) of **23** with 70 mL of MeOH saturated with ammonia for 28 h at room temperature. After evaporation and coevaporation with toluene, purification on silica gel yielded 824 mg (2.23 mmol, 89%) of 1,5-anhydro-4,6-*O*-benzylidene-2,3-dideoxy-2-(guanin-9-yl)-D-*arabino*-hexitol, **24**. 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 with Et_2O. The water layer was concentrated, and crystallization from water afforded 315 mg (1.12 mmol, 50% yield) of analytically pure **4e**.

Antiviral and Cytostatic Activity Assay Procedure. Assays for activity against herpesviruses were performed as

described previously.[26] 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) has been described.[27] 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 (ESM) or human embryonic lung (HEL) cells seeded in 96-well microtiter trays. Assays for cytostatic activity, and activity against human immuno deficiency virus type 1 (HIV-1) (strain III_B) and type 2 (HIV-2) (strain ROD), were performed as described previously.[30]

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