Synthesis and Antiviral Activity of Pyranosylphosphonic Acid Nucleotide Analogues

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Pyranosyl nucleotide analogues have been designed so that the intramolecular base to phosphorus distance closely approximates that of natural nucleotides. This was achieved by attaching the phosphorus directly at the anomeric position and the base at the 4-position of the carbohydrate. A series of compounds incorporating natural bases and having this novel structure were made via a short synthesis starting from commercially available glycals. Addition of triisopropyl phosphite to the glycals furnished α- and β-2-enopyranosylphosphonates which were then substituted with the heterocycle using Mitsunobu chemistry. Deprotection afforded the 2′,3′-unsaturated isonucleotide analogue. In some cases the deprotection sequence induced double-bond migration leading to the 1′,2′-unsaturated derivative. NMR spectroscopic structural analysis established an axial preference for the base and an equatorial preference for the β-phosphorus which results in intramolecular base to phosphorus distances within 1 Å of that of natural nucleotides. All of the deprotected compounds were screened for inhibition of HCMV, HSV-2, and HIV replication. Several compounds inhibited HCMV and HSV-2, the most potent of which was the unsaturated cytosine analogue **18** (HCMV $IC_{50} = 10\ \mu M$, HSV-2 $IC_{50} = 85\ \mu M$). None of the compounds were cytotoxic at the highest dose (1 mM) tested. None of the compounds were inhibitory to HIV.

Biologically active pyranosyl nucleosides have been known for over 40 years.[1] Early examples such as gougerotin, blasticidin S, amicetin, and their relatives are elaborated by *Streptomyces*, inhibit protein synthesis, and have antibacterial activity. However, examples of pyranosyl nucleosides with significant antiviral activity are much rarer. This lack of activity might be considered surprising in light of the structural diversity of nucleoside antiviral agents. For instance, the most frequently utilized target for the intervention of herpes virus and human immunodeficiency virus (HIV) replication is their DNA polymerases. Some inhibitors of these enzymes are the triphosphate derivatives that are generated intracellularly from a wide structural range of nucleoside analogues.[2] This range includes analogues in which the furanose has been replaced by an acyclic moiety, cyclopentane, cyclobutane, oxetane, carbon-branched furanose, dioxolane, oxathiolane, and tetrahydrothiophene. The fact that pyranosyl nucleosides are not on this list demonstrates an obvious incompatibility of the pyranose. On the other hand, pyranosyl nucleosides have recently been reported that have the heterocyclic base transposed from the 1′- to the 2′-position on the pyranose which results in significant antiherpes activity.[3] Thus, the presence of a pyranose may be a detriment to activity only when the base and sugar are oriented in the traditional sense, i.e., a β-D-purinyl- or -pyrimidinylpyranoside.

Our antiviral design strategy was based on this premise. We chose to use the pyranose ring as a scaffold to position the key recognition elements advantageously, with slight regard to their natural positions. The primary criterion was that the distance between the heterocyclic base and the position at which the triphosphate is eventually appended be similar to that of natural furanose nucleosides. This distance is expected to be an important determinant in successful phosphorylation as well as in subsequent binding to the polymerase.[4] A simple modeling comparison of energy-minimized structures[5] showed a good fit (rms = 0.35 Å for phosphate and heterocyclic moieties) with the natural nucleotides if a pyranose was substituted with a phosphonic acid at the 1-position and with the heterocyclic base at the 4-position. Figure 1 is the superimposition of dCMP (gray) with one such example, 4-cytosinyl-2-enopyranosylphosphonic acid (black). Thus, these isonucleotides became the target of our synthetic endeavors. To our knowledge, nucleotide analogues in which the phosphorus occupies an anomeric position are unprecedented.

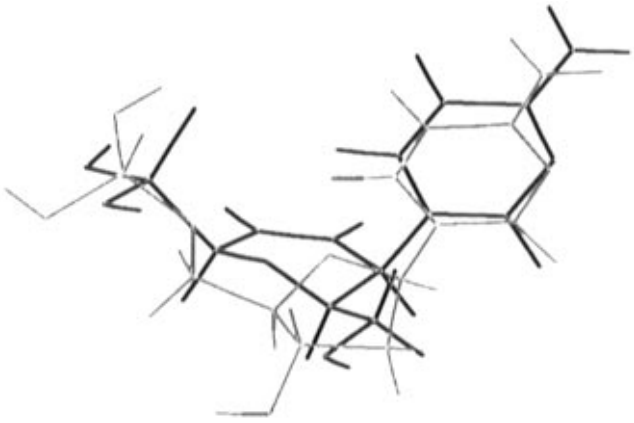

Figure 1. (4-Cytosinyl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonic acid (black) superimposed on dCMP (gray).

Additional incentive for the synthesis of such compounds was provided by a number of advantages we predicted to be inherent in these structures. For instance, glycosidic bond cleavage is a frequently en-

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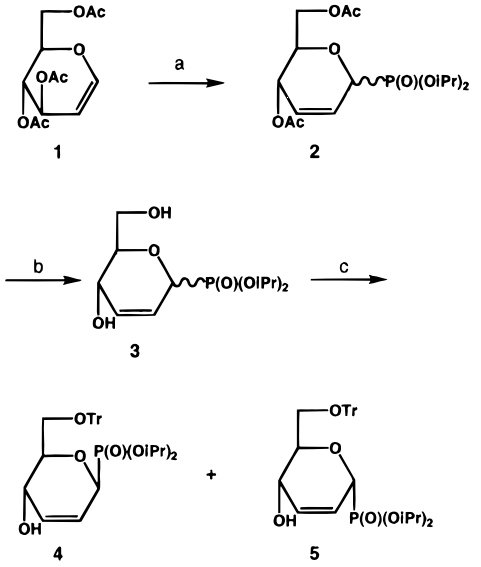
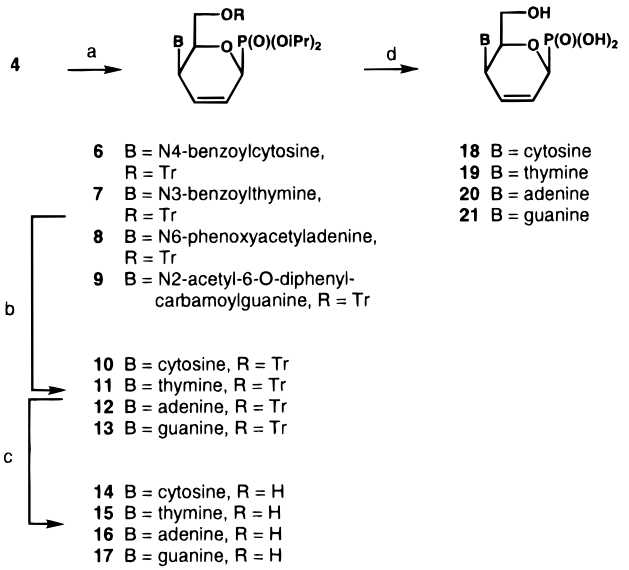
Scheme 1[a]



[a] Reagents: (a) $P(OiPr)_3$, $BF_3 \cdot Et_2O$, toluene; (b) NH_3/MeOH; (c) TrCl, pyridine.

Scheme 2[a]



[a] Reagents: (a) B, Ph_3P, DEAD, THF; (b) NH_3/MeOH or NEt_3/MeOH; (c) 80% AcOH; (d) TMSBr, lutidine, CH_3CN.

countered, degradative pathway of nucleoside antivirals, particularly the 2′,3′-dideoxynucleosides.[6] The placement of the heterocyclic base at the 4-position of the pyranose rather than at the anomeric position would alleviate this deficiency. Nucleoside analogues require stepwise phosphorylation to their 5′-triphosphate derivative before they can interact with viral polymerases. The *a priori* inclusion of the phosphonate would allow these new analogues to bypass the first, often rate-limiting, phosphorylation step. The presence of the pyran ring oxygen β to the phosphorus would be expected to increase the pK_a of the phosphonic acid making it isoelectronic with a phosphate.[7] Lastly, a number of acyclic, phosphonate, nucleotide analogues exemplified by 9-[2-(phosphonylmethoxy)ethyl]adenine (PMEA) and 1-[3-hydroxy-2-(phosphonomethoxy)propyl]-cytosine (HPMPC), have significant antiviral activity.[8] In the proposed pyranosyl nucleotides, the linkage between the base and phosphorus along the sequence 4′,5′,$O^{5'}$,1′ is the same as is present in HPMPC and PMEA.

In this paper we describe an efficient synthesis of these new analogues from commercially available glycals. Some of the compounds were inhibitors of HSV-2 and CMV. None showed cytotoxicity at 1 mM concentrations, the highest dose tested.

Chemistry

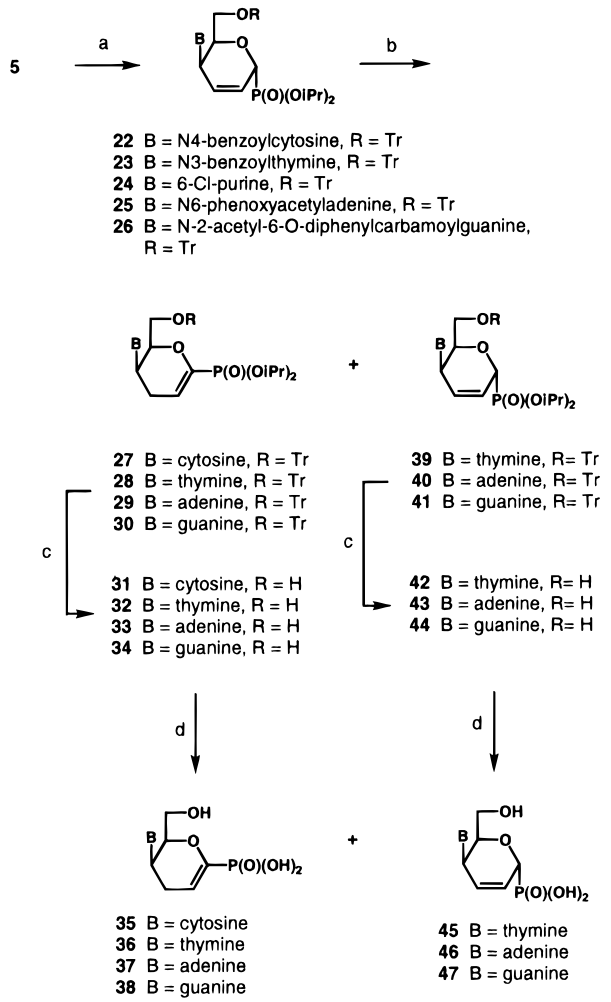
Construction of the requisite nucleotide analogues followed a common strategy. Readily available glycals were converted to 2,3-unsaturated glycopyranosylphosphonates which were then substituted at the 4-position with the purine or pyrimidine base. These intermediates were either directly deprotected or further modified in order to provide the 1,2-unsaturated isomer or fully saturated analogues.

The synthesis of the first key intermediate, the differentially protected (2,3-dideoxyhex-2-enopyranosyl)-phosphonate, is illustrated in Scheme 1. Paulsen has reported the addition of dialkyl phosphites to peracetylated glycals in the presence of boron trifluoride to efficiently furnish 2-enopyranosylphosphonates via a Ferrier rearrangement.[9] Trialkyl phosphites have also been added to both acyclic and cyclic enol ethers, but this reaction is performed with HCl and proceeds via an Arbuzov type mechanism.[10] We have found that trialkyl phosphites also are effective participants in the Ferrier rearrangements, affording unsaturated pyranosylphosphonates in high yields under mild conditions. Thus, commercially available tri-*O*-acetyl-D-glucal (**1**) on treatment with triisopropyl phosphite in the presence of catalytic boron trifluoride diethyl etherate furnished the 2,3-unsaturated pyranose **2** in 93% yield. The product, isolated as an approximately equal mixture of anomers, was deacetylated using either potassium carbonate in methanol or ammonia-saturated methanol. Compounds **2** and **3** β have been previously described but as the dimethyl rather than the diisopropyl esters.[9] After selective tritylation of the primary hydroxyl group, chromatographic separation on silica gel afforded an overall yield from **1** of 46% for the β-anomer **4** and 39% for the α-anomer **5**. The assignment of anomeric configuration was based on NMR spectroscopic methods and is discussed below.

Having the pyranosylphosphonates in hand, the heterocyclic bases were next appended at the 4-position. Scheme 2 illustrates the synthetic pathway starting from the β-anomer. Unsuccessful attempts to displace a methanesulfonate at the 4-position with the heterocycles prompted trials using Mitsunobu chemistry.[11] Protected derivatives of cytosine, thymine, adenine, and guanine were reacted with the 4-hydroxy sugar in the presence of triphenylphosphine and DEAD. In all cases the desired compound, having the inverted configuration at the sugar 4-position, was the major reaction component.

The deprotection of the compounds proceeded through a common sequence of reactions. The purine or pyrimidine moieties were first deacylated with ammonia-saturated methanol or a solution of triethylamine in methanol. The latter method sometimes afforded fewer side products. The trityl group was removed next by heating in aqueous acetic acid, and lastly, the phosphonate esters were cleaved with bromotrimethylsilane in

Scheme 3[a]



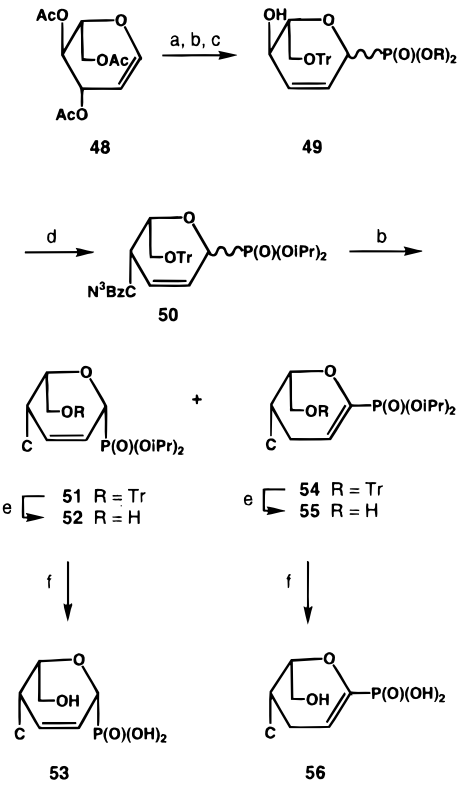
[a] Reagents: (a) B, Ph_3P, DEAD, THF; (b) NH_3/MeOH or NEt_3/MeOH; (c) 80% AcOH; (d) TMSBr, lutidine, CH_3CN.

the presence of lutidine. The final enopyranosylphosphonic acids were purified by a combination of ion exchange and reverse phase chromatography.

The key to the success of this reaction sequence was the proper selection of the heterocyclic protecting group. Without protection, the Mitsunobu coupling proceeded very poorly or not at all. Acyl protection of the heterocycle facilitated the reaction, but the subsequent basic cleavage conditions in some cases led to complex product mixtures, the purine derivatives being particularly vulnerable. Therefore, removal of the benzoyl group at N^3 of the thymine derivative was unsuccessful with ammonia/methanol but satisfactory with 10% triethylamine in methanol. Adenine and guanine required the use of the labile phenoxyacetyl and diphenylcarbamoyl protecting groups, respectively.

In order to investigate the effect of the anomeric configuration on antiviral activity, the same sequence of reactions was carried out starting from the α-pyranosylphosphonate **5** (Scheme 3). However, working with the α-anomers was problematic because it was discovered that this series was much more susceptible to base-induced, double-bond migration than was the *β*-series. The basic conditions required for the removal of acyl protecting groups was often enough to completely drive the 2′-position double bond to the 1′-position.[12] Such rearrangements of allylphosphonates to vinylphosphonates under basic conditions are well precedented[13] and, in this case, are presumably initiated by the abstraction of the anomeric proton.

Scheme 4[a]

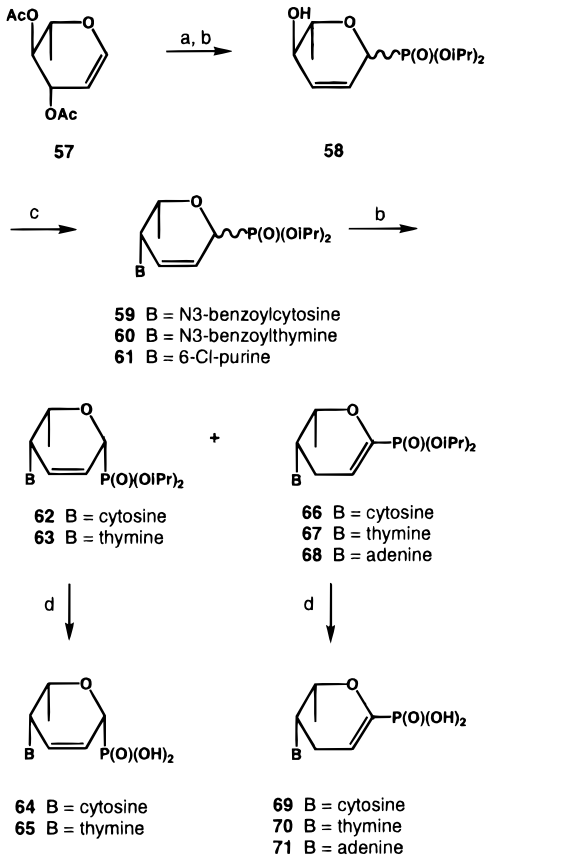


[a] Reagents: (a) $P(OiPr)_3$, $BF_3{\cdot}Et_2O$, toluene; (b) NH_3/MeOH; (c) TrCl, pyridine; (d) N^3BzC, Ph_3P, DEAD, THF; (e) 80% AcOH; (f) TMSBr, lutidine, CH_3CN.

Thus, a series of 1′-ene derivatives were produced along with the targeted 2′-ene compounds. The cytosine analogue was the most troublesome, affording exclusively the 1′-olefin during any attempt to remove the N^4-benzoyl protecting group. Similarly, attempts to remove the N^3-benzoyl group on thymine analogue **23** with methanolic ammonia caused complete 2′- to 1′-double-bond migration, although this could be mitigated by substituting triethylamine for the ammonia. Predictably, all efforts to convert the 6-chloropurine substituent on **24** to adenine, using ammonia, led to double-bond migration. However, the adenine analogue **40** could eventually be derived using N^6-phenoxyacetyl protection (**25**) which successfully succumbed to removal with methanolic triethylamine. In the case of the guanine analogue, an approximately equal amount of the rearranged olefin **30** was produced along with the deacylated 2′-unsaturated **41**. Fortunately, the remaining hydroxyl and phosphonic acid deprotections required in this series proceeded uneventfully as before.

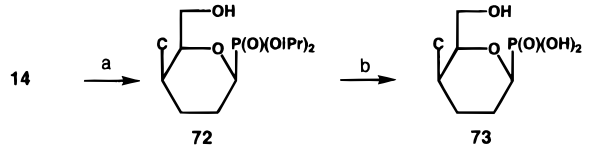
Other analogues were made starting from commercially available tri-*O*-acetyl-L-glucal (**48**) and di-*O*-acetyl-L-rhamnal (**57**). From L-glucal, only the cytosine analogue was made as shown in Scheme 4. The synthesis paralleled that used in the D-series with the exception that the mixture of phosphonyl sugar anomers were not separated but, instead, carried through the ammonia and acetic acid deprotection steps. Product separation at this point provided the *β*-diisopropylphosphonate **52** and the glycal diisopropylphosphonate **55**. Apparently, all of the α-anomer had again undergone rearrangement to the glycal under the basic conditions.

Scheme 5[a]



[a] Reagents: (a) $P(OiPr)_3$, $BF_3{\cdot}Et_2O$, toluene; (b) NH_3/MeOH; (c) B, Ph_3P, DEAD, THF; (d) TMSBr, lutidine, CH_3CN.

Scheme 6[a]



[a] Reagents: (a) Pd/C, H_2, MeOH; (b) TMSBr, lutidine, CH_3CN.

Removal of the phosphonate esters with bromotrimethylsilane afforded the (cytosinyl-L-pyranosyl)phosphonic acids **53** and **56**.

Scheme 5 illustrates the reaction sequence used to synthesize analogues from L-rhamnal. In general, yields were higher than those obtained with the 6-hydroxyl sugars. As had been done with L-glucal, phosphonate anomers were carried as a mixture into the ammonia treatment step. Different enopyranosyl isomers were obtained depending on the heterocycle present. The conversion of 6-chloropurine to adenine led entirely to rearrangement and the glycal phosphonate **68**. The debenzoylation of the cytosine analogue **59** gave the desired β-enopyranosylphosphonate **62** plus the glycal phosphonate **66**, while benzoyl removal from thymine analogue **60** also led to the glycal phosphonate **67** as well as the β-enopyranosylphosphonate **63**. This varied collection of intermediate phosphonates were all deesterified with bromotrimethylsilane to furnish the phosphonic acids shown.

Also for biological comparison purposes, the fully saturated (cytosinylpyranosyl)phosphonic acid **73** was made (Scheme 6). Hydrogenation of **14** over 10% palladium on carbon followed by deesterification with bromotrimethylsilane afforded the saturated product **73** in 88% overall yield.

Structural Elucidations

With the compounds in hand, NMR was first relied upon to confirm structures and deduce the configurations of the substituents on the pyranose ring. Using this information, a computer model was constructed from which intramolecular distances were measured and compared with those of natural nucleosides. In this way, the three-dimensional similarity of the target compounds and natural nucleotides could be assessed.

Structural assignments of the products were based on 1D and 2D NMR spectra. In general, the molecular skeleton was first established using $^1H-^1H$ spin network as determined by COSY spectra and carbon connectivity based on one-bond and two/three-bond $^1H-^{13}C$ correlation from HMQC[14] and HMBC[15] spectra, respectively. Analysis of the COSY spectra established, in addition to the proton-coupling network in the pyranose ring, the position of the double bond (2′−3′ or 1′−2′) in the ring. The ^{13}C chemical shifts of the protonated carbons were first assigned from the $^1H-^{13}C$ HMQC spectra; then the HMBC spectra were analyzed to establish the molecular skeleton. The following diagnostic three-bond correlations were observed. (i) H1′−C5′ and H5′−C1′ correlations established the ether linkage between the 1′- and 5′-carbons. (ii) The correlation between the base proton (H8 in the case of guanine and adenine and H6 in the case of cytosine and thymine) and C4′ established the location of the base at the 4′-position. (iii) In the case of purine derivatives, the C4 carbon of the base shows a correlation to H8 of the base and H4′ of the sugar, establishing the sugar−base connectivity through N9 and not N7.

Next, ring conformation and relative stereochemistry of the base and the anomeric center were determined by examination of either 1D NOE difference spectra or 2D $^1H-^1H$ NOESY spectra. In all cases, the base proton (H6 in the case of cytosine and thymine and H8 in the case of guanine and adenine) shows a NOE to H6′ (in addition to H4′) and not to H5′. However, H4′ shows a NOE to both H5′ and H6′. This indicates that while the base is *syn* with respect to C6′ and *anti* with respect to H5′, H4′ is *syn* with respect to both C6′ and H5′. Therefore, C6′ and H4′ are in an equatorial position, and the base and H5′ are in axial positions.

With the conformation of the base and H5′ established, the anomeric configuration of the 2,3-unsaturated pyranose derivatives was determined based on NOE interactions with H1′. In one set of isomers, strong NOE was observed between H1′ and H5′. Since H5′ had been earlier assigned an axial position, this strong interaction of H5′ with H1′ establishes the anomeric configuration to be β (with the H1′ in the axial and the phosphorus in the equatorial positions). In the other set of isomers, a weak, long range NOE is observed between the base proton (H6 in the case of cytosine and thymine and H8 in the case of guanine and adenine) and H1′ and no NOE is observed between H5′ and H1′. Since the base had been earlier assigned to the axial position (and C1′−C2′−C3′−C4′ is coplanar), its interaction with H1′ establishes the anomeric configuration to be α with H1′ in the equatorial and the phosphorus in the axial positions.

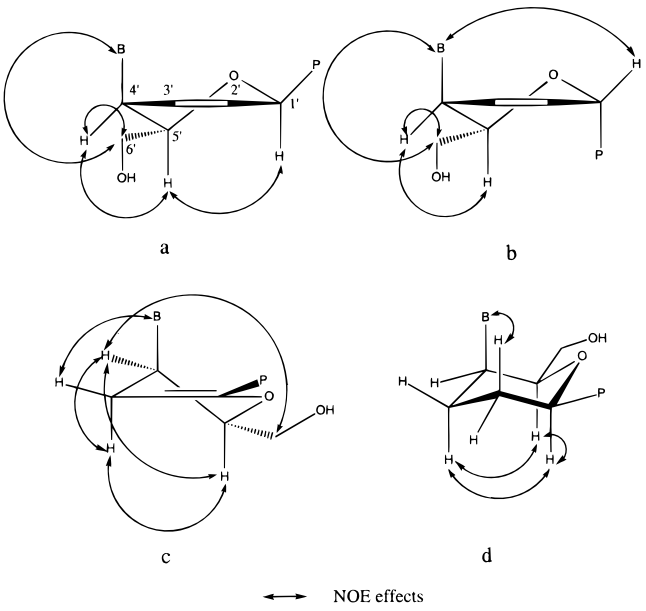


Figure 2. NMR-indicated conformations of the pyranosyl isonucleotides: (a) β-2′-ene, (b) α-2′-ene, (c) 1′-ene, and (d) β-saturated.

In the case of 1,2-unsaturated pyranose derivatives, one of the 3′-protons shows a NOE to the base proton (H8 purines, H6 pyrimidines) and H4′, while the other shows an interaction with H4′ and H5′. Hence, they are assigned to the equatorial and axial H3′ protons, respectively. These NOE interactions of the H3′ protons are again consistent with the heterocyclic base being in the axial position.

As determined by the NMR studies, the substituents on the pyranoses consistently adopted the same configurational pattern. The purine or pyrimidine base was always axial, the hydroxymethyl or methyl substituent at 5′ was equatorial, and the phosphorus was equatorial when β and axial when α. The generalized structures are depicted in Figure 2. Thus, the spatial positioning of substituents was the same for all β-anomers and likewise for all the α-anomers.

In order to estimate intramolecular distances, we relied on a minimization program[5] to construct a low-energy model. Compound **18**, having the greatest antiviral activity of the series, was chosen as the example. The completed minimization qualitatively matched the NMR results in configuring the base, phosphorus, and hydroxymethyl substituents axially, equatorially, and equatorially, respectively, on the pyranose (as shown in both Figures 1 and 2a). This concurrence provided confidence that the minimized structure was a reasonable approximation of the actual solution conformation. Using the computer-generated model, intramolecular atomic distances were measured and found to compare favorably with the analogous distances in the natural nucleotides. The calculated distance from the N1 of the base to phosphorus is 5.0 Å. This is similar to the N9−P distances of 5.45 and 4.36 Å reported for two different crystal forms of AMP.[16,17] Also, the measured distance between N1 and C1′, which is the carbon that would correspond to the position of C5′ of natural nucleotides, is 3.8 Å. The distance between N1 and C5′ of CMP in the solid state is reported to be 4.2 Å.[18]

Table 1. Inhibitory Effects of Compounds on HCMV Replication, HSV-Induced Cytopathogenicity, and Host Cellular Growth

Compound	HCMV IC_{50}[a] (μM)	HSV-2 IC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d]
 **18**	10	85	> 1000	>100
 **35**	40	90	> 1000	> 25
 **73**	80	—	> 1000	> 12
 **37**	90	—	> 1000	> 11
 **64**	200	> 400	> 1000	> 5
 **69**	300	> 400	> 1000	> 3.3
 **21**	> 300	275	>1000	—
 **46**	> 300	300	> 1000	—
19, 20, 36, 38, 45, 47, 53, 56, 65, 70, 71	not active	not active	not active	—
HPMPC	0.5	10	200	400

[a] Concentration of compound required to achieve a 50% reduction in the replication of HCMV (Towne) in NHDF cells. [b] Concentration of compound required to achieve 50% protection of MRC-5 cells against cytopathic effect of HSV-2 (E 194). [c] Concentration of compound required to reduce NHDF cell growth by 50%. [d] Selectivity index = CC_{50} (NHDF)/IC_{50} (HCMV).

Biological Results

Having met the primary synthetic objective of positioning the base and phosphorus to closely approximate the spatial relationship present in natural nucleotides, the compounds were then tested for biological activity. The in vitro antiherpes activities of the nucleotide analogues are shown in Table 1. Compounds were tested for their inhibition of the replication of HCMV (Towne strain) in NHDF cells and their inhibition of the cytopathogenicity of HSV-2 (E 194 strain) in MRC-5 cells. Estimates of toxicity were obtained by determining the 50% cytotoxic concentration for mock-infected NHDF cells. HPMPC was included as a control.

Some of the compounds in this series displayed moderate inhibition of HCMV and HSV-2. The most active example, (4-cytosinyl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonic acid **(18)**, was ca. 20-fold less active than the control compound HPMPC. However, no toxicity was observed for **18** up to the highest concentration tested of 1 mM.

The results elucidated some general trends in structure−activity relationships. Analogues containing cytosine were the most active. D-Isomers were more active than L-isomers. Compounds having 2,′3′-unsaturation were more active than the analogous 1′,2′-unsaturated compounds which, in the single example shown, were more active than the fully saturated analogue. None

of the compounds were toxic to NHDF cells at the highest concentrations tested.

The compounds were also screened for inhibition of HIV (III b) in MT2 cells. None had activity.

Conclusion

This study has shown that a variety of pyranosylphosphonic acid nucleotide analogues can be readily constructed from simple glycals. Placement of the phosphorus at the 1-position and the base at the 4-position approximates the spatial positioning of these groups in natural nucleotides and in some cases leads to selective inhibition of herpes virus replication in cell culture.

Experimental Section

General Methods. Nuclear magnetic resonance spectra were recorded on a Varian Unity Plus 500 (^{1}H NMR, 500 MHz) spectrometer, and chemical shifts are reported in parts per million downfield from external tetramethylsilane. High-resolution mass spectra (HRMS) were provided by Galbraith Laboratories, Knoxville, TN, using FAB$^+$. UV spectra of the samples dissolved in pH 7.5, 5 mM Tris/HCl buffer were recorded on a Hewlett Packard 8452 spectrophotometer. Elemental analyses were provided by Galbraith Laboratories. Column chromatography utilized 70−230 mesh silica gel 60 from E. Merck, while preparative reverse phase HPLC was performed on a Rainin system using a 2.14 × 25 cm, C-18 column. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected.

Diisopropyl [4,6-Di-*O*-acetyl-2,3-dideoxy-α(and *β*)-D-*erythro*-hex-2-enopyranosyl]phosphonate (2). Boron trifluoride etherate (2.4 mL, 20 mmol) was added to a solution of 3,4,6-tri-*O*-acetyl-D-glucal (**1**) (27.2 g, 100 mmol) and triisopropyl phosphite (41.6 g, 200 mmol) in 500 mL of dry toluene under nitrogen. After heating at 100 °C for 4 h, the mixture was concentrated in vacuo to an oil which was diluted with toluene (125 mL) and reconcentrated. The concentration from toluene was repeated thrice more before the residue was taken up in toluene (800 mL) and extracted with saturated, aqueous $NaHCO_3$ and water. The organic phase was dried ($MgSO_4$), filtered, and evaporated affording **2** as a pale yellow oil (35.2 g, 93%): ^{1}H NMR ($CDC1_3$) δ 1.1−1.4 (m, 12H), 2.02 (m, 6H), 4.15 (m, 2H), 4.37 (m, 1H), 4.48 (m, 1H), 4.6−4.8 (m, 2H), 5.15 (m, 1H), 5.9−6.0 (m, 2H). Anal. ($C_{16}H_{28}O_8P$) C, H.

Diisopropyl [2,3-Dideoxy-*β*(and α)-D-*erythro*-hex-2-enopyranosyl]phosphonate (3). A solution of **2** (18.95 g, 64.4 mmol) in MeOH saturated with NH_3 at 0 °C (300 mL) was stirred at room temperature for 6 h. The solvent was removed in vacuo and the residue diluted with toluene and reconcentrated affording **3** as a crude, syrupy, 1:1 mixture of *β*- and α-anomers (14.7 g): ^{1}H NMR ($CDC1_3$) δ 1.2−1.4 (m, 12H), 3.3 (m, 1H), 3.84 (m, 1H), 4.11 (m, 1H), 4.23 (m, 1H), 4.5 (m, 1H), 4.6−4.8 (m, 2H), 5.8−6.0 (m, 2H). Anal. ($C_{12}H_{23}O_6P\cdot 0.25H_2O$) C, H.

Diisopropyl [2,3-Dideoxy-6-(triphenylmethyl)-*β*-D-*erythro*-hex-2-enopyranosyl]phosphonate (4) and Diisopropyl [2,3-Dideoxy-6-*O*-(triphenylmethyl)-α-D-*erythro*-hex-2-enopyranosyl]phosphonate (5). Triphenylmethyl chloride (13.95 g, 500 mmol) and pyridine (15 mL) were added to a solution of diol **3** (14.7 g, 50.0 mmol) in toluene (500 mL). After stirring at room temperature for 24 h, additional toluene (500 mL) was added and the mixture was extracted sequentially with 0.1 M hydrochloric acid, a saturated aqueous solution of $NaHCO_3$ (300 mL), and water (300 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to an oil which was chromatographed on a column of silica gel using CH_2Cl_2−MeOH (95:5) as eluent. **4** (*β*-anomer, 12.30 g, 46%) and **5** (α-anomer, 10.45 g, 39%) were isolated as pale yellow oils.

4: ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 3.2−3.4 (m, 2H), 4.07 (br s, 1H), 4.21 (m, 1H), 4.42 (br d, J = 16.8 Hz, 1H), 4.7−4.9 (m, 2H), 5.88 (m, 1H), 6.02 (m, 1H), 7.2−7.5 (m, 15H). Anal. ($C_{31}H_{37}O_6P\cdot 0.5\ CH_2Cl_2$) C, H.

5: ^{1}H NMR ($CDCl_3$) δ 1.2−1.3 (m, 12H), 3.3−3.5 (m, 2H), 3.53 (m, 1H), 4.22 (m, 1H), 4.53 (d, J = 17.6 Hz, 1H), 4.76 (m, 2H), 5.9−6.0 (m, 2H), 7.2−7.4 (m, 15H). Anal. ($C_{31}H_{37}O_6P\cdot 0.5\ CH_2Cl_2$) C, H.

Diisopropyl [4-(N^4-Benzoylcytosin-1-yl)-2,3,4-trideoxy-6-*O*-(triphenylmethyl)-*β*-D-*threo*-hex-2-enopyranosyl]phosphonate (6). Diethyl azodicarboxylate (0.522 g, 3.0 mmol) was added dropwise over 5 min to a 0 °C stirred suspension of **4** (1.8 g, 2.0 mmol), N^4-benzoylcytosine[19] (0.645 g, 3.0 mmol), and triphenylphosphine (0.786 g, 3 mmol) in anhydrous THF (20 mL) under nitrogen. The mixture was stirred at 0 °C for 4 h and then at room temperature for 20 h before being diluted with EtOH (5 mL) and concentrated in vacuo. The residue was partitioned between ethyl acetate (50 mL) and water (50 mL), and the aqueous layer was extracted further with ethyl acetate (50 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo affording a viscous orange-brown oil. Chromatography of the oil on silica gel (CH_2Cl_2−MeOH, 98:2) afforded phosphonate **6** (0.557 g, 44%) as a viscous, pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 3.1−3.3 (m, 2H), 3.92 (m, 1H), 4.62 (m, 1H), 4.8−4.9 (m, 2H), 5.53 (m, 1H), 5.9−6.1 (m, 2H), 6.42 (d, J = 7.2, 1H), 7.10−7.60 (m, 20H), 7.92 (d, J = 7.2, 1H).

Diisopropyl [4-Cytosin-1-yl-2,3,4-trideoxy-6-*O*-(triphenylmethyl)-*β*-D-*threo*-hex-2-enopyranosyl]phosphonate (10). A solution of **6** (0.557 g, 0.76 mmol) in MeOH saturated at 0 °C with NH_3 (20 mL) was kept at room temperature for 16 h. After concentrating the mixture in vacuo, the residue was chromatographed on silica gel (CH_2Cl_2−MeOH, 95:5) affording phosphonate **10** (0.38 g, 80%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 3.3−3.5 (m, 2H), 4.04 (m, 1H), 4.72 (d, J = 17.5 Hz, 1H), 5.12 (m, 2H), 5.65 (m, 1H), 5.82 (d, J = 7.1 Hz, 1H), 6.1−6.3 (m, 2H), 7.1−7.6 (m, 15H), 7.83 (d, J = 7.1 Hz, 1H).

Diisopropyl (4-Cytosin-1-yl-2,3,4-trideoxy-*β*-D-*threo*-hex-2-enopyranosyl)phosphonate (14). A solution of **10** (0.38 g, 0.6 mmol) in 80% aqueous acetic acid (20 mL) was stirred at room temperature for 16 h and then concentrated in vacuo. The residual acetic acid was azeotropically removed with 5% ethanol in toluene (2 × 50 mL) to give 0.4 g of an oil which was chromatographed on silica gel (CH_2Cl_2) to provide **14** (0.195 g, 83%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 3.3−3.6 (m, 2H), 3.88 (m, 1H), 4.58 (d, J = 18 Hz, 1H), 4.7−4.9 (m, 2H), 5.40 (m, 1H), 5.79 (d, J = 7.2 Hz, 1H), 6.04 (m, 1H), 6.34 (m, 1H), 7.98 (d, J = 7.2 Hz, 1H).

(4-Cytosin-1-yl-2,3,4-trideoxy-*β*-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (18). Bromotrimethylsilane (0.66 mL, 5.0 mmol) was added dropwise to a solution of diisopropyl ester **14** (0.195 g, 0.5 mmol) and 2,4-lutidine (0.6 mL, 5 mmol) in dry acetonitrile (10 mL) at room temperature under nitrogen. After stirring for 14 h, the solvents were removed in vacuo and the residual, yellow oil was first taken in dry acetonitrile (30 mL) and reconcentrated and then in methanol (30 mL) and reconcentrated. A solution of the residue in water (5 mL) was applied on a column of Dowex 1 (AcO^- form) (20 mL) and eluted first with water (300 mL) and then with 0.5 M acetic acid. Product-containing fractions were concentrated in vacuo, purified by reverse phase chromatography (C-18, water), and crystallized from H_2O/acetonitrile to give white crystals of **18** (0.136 g, 90%): mp 250−300 °C dec; UV_{max} 276 nm (ϵ 10 532); ^{1}H NMR ($CDCl_3$) δ 3.5−3.7 (m, 2H), 4.01 (m, 1H), 4.58 (dd, J = 18.0, 1.83 Hz, 1H), 5.29 (m, 1H), 5.96 (m, 1H), 6.18 (d, J = 7.9 Hz, 1H), 6.53 (m, 1H), 8.26 (d, J = 7.9 Hz, 1H); HRMS 304.0692 (M + H − glycerol), calcd for $C_{10}H_{15}N_3O_6P$ 304.0698. Anal. ($C_{10}H_{14}N_3O_6P\cdot 0.5H_2O$) C, H, N.

Diisopropyl (4-Thymin-1-yl-2,3,4-trideoxy-*β*-D-*threo*-hex-2-enopyranosyl)phosphonate (15). In a manner similar to that described for **6**, **4** was coupled to N^3-benzoylthymine[20] to furnish crude intermediate **7**. **7** was deprotected first to **11** and then to **15** following the procedures used to make **10** and **14**, respectively. **15** was isolated as a white solid in 35% yield: ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 1.90 (s, 3H), 3.3−3.5 (m, 2H), 4.02 (m, 1H), 4.3−4.6 (m, 3H), 5.9−5.6 (m, 2H), 7.62 (s, 1H), 9.02 (s, 1H).

(4-Thymin-1-yl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (19). In a manner similar to that described for **18, 15** was converted to **19** which was isolated as white crystals in 40% yield: mp 200−250 °C dec; UV_{max} 270 nm (ε 7925); ^1H NMR ($CDCl_3$) δ 1.87 (d, J = 1.2 Hz, 3H), 3.46 (m, 1H), 3.67 (m, 1H), 3.99 (m, 1H), 4.55 (br d, J = 17.7 Hz, 1H), 5.18 (m, 1H), 5.96 (m, 1H), 6.50 (m, 1H), 7.93 (d, J = 1.2 Hz, 1H). Anal. ($C_{11}H_{15}N_2O_7P \cdot 1.1H_2O$) C, H, N.

Diisopropyl (4-Adenin-9-yl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonate (16). In a manner similar to that described for **6**, **4** and N^6-(phenoxyacetyl)adenine[21] were coupled to form intermediate **8**. **8** was directly deprotected first to **12** and then to **16** via the same procedures used to make **10** and **14**, respectively. **16** was obtained as a white solid in 12% overall yield: ^1H NMR ($CDCl_3$) δ 1.3−1.5 (m, 12H), 3.82 (m, 1H), 3.95 (m, 1H), 4.2−4.3 (m, 2H), 4.6−4.9 (m, 3H), 5.32 (m, 1H), 6.2−6.5 (m, 4H), 7.78 (s, 1H), 8.32 (s, 1H).

(4-Adenin-9-yl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (20). In a manner similar to that described for **18**, **16** was converted to **20** which was isolated as white crystals in 73% yield: mp 250−300 °C dec; UV_{max} 262 nm (ε 13 804); ^1H NMR ($CDCl_3$) δ 3.1−3.3 (m, 1H), 4.71 (d, J = 17.2 Hz, 1H), 4.83 (m, 1H), 5.28 (m, 1H), 6.1−6.5 (m, 2H), 8.30 (s, 1H), 8.39 (s, 1H). Anal. ($C_{11}H_{14}N_5O_5 \cdot 0.8H_2O$) C, H, N.

Diisopropyl (4-Guanin-9-yl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonate (17). In a manner similar to that described for **6**, **4** and N^2-acetyl-O^6-(diphenylcarbamoyl)guanine[22] were coupled to afford crude intermediate **9** which, in a manner similar to that described for **10**, was converted to crude **13**. **13** was then deprotected in a similar fashion as for **14** to give **17** as a white solid in 51% yield from **4**: ^1H NMR (D_2O) δ 1.4−1.5 (m, 12H), 3.1−3.4 (m, 2H), 4.58 (m, 1H), 4.84 (m, 2H), 4.9−5.1 (m, 2H), 6.2−6.4 (m, 2H), 7.76 (s, 1H).

(4-Guanin-9-yl-2,3,4-trideoxy-β-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (21). In a manner similar to that described for **18**, **17** was converted to **21** which was isolated as white crystals in 32% yield: mp 250−300 °C dec; UV_{max} 254 nm (ε 14 392); ^1H NMR ($CDCl_3$) δ 3.2−3.6 (m, 2H), 4.11 (br s, 1H), 4.68 (d, J = 17.7 Hz, 1H), 5.24 (m, 1H), 6.17 (m, 1H), 6.53 (m, 1H), 8.97 (s, 1H). Anal. ($C_{11}H_{14}N_5O_6P \cdot 0.5H_2O$) C, H, N.

Diisopropyl [4-(N^4-Benzoylcytosin-1-yl)-2,3,4-trideoxy-6-*O*-(triphenylmethyl)-α-D-*threo*-hex-2-enopyranosyl]phosphonate (22). In a manner similar to that described for **6**, **5** was converted to **22** which was isolated as a yellow oil in 48% yield: ^1H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 3.0−3.3 (m, 2H), 3.95 (m, 1H), 4.62 (m, 1H), 4.7−4.9 (m, 2H), 5.52 (m, 1H), 6.0−6.2 (m, 2H), 6.40 (d, J = 7.8 Hz, 1H), 7.1−7.4 (m, 20H), 7.92 (d, J = 7.8 Hz, 1H).

Diisopropyl [4-Cytosin-1-yl-2,3,4-trideoxy-6-*O*-(triphenylmethyl)-D-*threo*-hex-1-enopyranosyl]phosphonate (27). In a manner similar to that described for **10**, **22** was converted to **27** which was isolated as a white solid in 82% yield: ^1H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 2.1−2.2 (m, 1H), 2.7−2.8 (m, 1H), 3.2−3.4 (m, 2H), 4.21 (m, 1H), 4.6−4.9 (m, 2H), 5.41 (br d, J = 7.2 Hz, 1H), 5.44 (d, J = 7.3 Hz, 1H), 5.99 (m, 1H), 7.25 (m, 15H), 7.82 (d, J = 7.3 Hz, 1H).

Diisopropyl (4-Cytosin-1-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonate (31). In a similar manner to that described for **14**, **27** was converted to **31** which was isolated as a white solid in 83% yield: ^1H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 2.23 (m, 1H), 2.93 (m, 1H), 3.33 (m, 1H), 3.73 (m, 1H), 4.04 (m, 1H), 4.5−4.7 (m, 2H), 5.36 (m, 1H), 5.74 (d, J = 7.5 Hz, 1H), 6.12 (m, 1H), 7.31 (d, J = 7.5 Hz, 1H).

(4-Cytosin-1-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonic Acid (35). In a manner similar to that described for **18**, **31** was converted to **35** which was isolated as white crystals in 57% yield: mp 250−300 °C dec; UV_{max} 278 nm (ε 10 292); ^1H NMR ($CDCl_3$) δ 2.12 (m, 1H), 2.84 (m, 1H), 3.5−3.7 (m, 2H), 4.02 (br s, 1H), 5.07 (d, J = 7.3 Hz, 1H), 5.69 (d, J = 9.5 Hz, 1H), 6.06 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H); HRMS 304.0697 (M + H − glycerol), calcd for $C_{10}H_{10}N_3O_6P$ 306.0698. Anal. ($C_{10}H_{14}N_3O_6P \cdot 0.4H_2O$) C, H, N.

Diisopropyl (4-Thymin-1-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonate (32). In a manner similar to that described for **6**, **5** and N^3-benzoylthymine[20] were reacted to furnish crude intermediate **23** which was converted to **28** using the same procedure as described for **10**. **28** was then deprotected in a manner similar to that described for **14**, to furnish **32** in 35% overall yield from **5**: ^1H NMR ($CDCl_3$) δ 1.32 (m, 12H), 1.86 (s, 3H), 2.28 (m, 1H), 2.95 (m, 1H), 3.4−3.6 (m, 2H), 3.7 (m, 1H), 4.00 (m, 1H), 4.70 (m, 2H), 4.85 (br d, J = 8.1 Hz, 1H), 6.15 (d, J = 10.2 Hz, 1H), 7.05 (s, 1H), 9.71 (br s, 1H).

(4-Thymin-1-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonic Acid (36). In a manner similar to that described for **18**, **32** was converted to **36**. The final compound was obtained as a white solid in 40% yield after precipitation from H_2O by acetonitrile: mp 200−250 °C dec; UV_{max} 272 nm (ε 2857); ^1H NMR ($CDCl_3$) δ 1.82 (d, J = 1.2 Hz, 3H), 2.21 (m, 1H), 2.88 (m, 1H), 3.6 (m, 1H), 3.75 (m, 1H), 4.12 (m, 1H), 5.02 (m, 1H), 5.78 (m, 1H), 7.35 (d, J = 1.2 Hz, 1H). Anal. ($C_{11}H_{15}N_2O_7P \cdot H_2O$) C, H, N.

Diisopropyl (4-Thymin-1-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)phosphonate (42). A solution of the crude **23** (see preparation of **32**) in 10% NEt_3/MeOH was kept at room temperature for 8 h and then concentrated in vacuo. The residue was diluted with toluene and reconcentrated to furnish intermediate **39**. The deprotection of **39** was carried out in a manner similar to that described for **14**, to afford **42** as a white solid in 29% overall yield from **5**: ^1H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 1.98 (s, 3H), 3.2−3.4 (m, 2H), 3.83 (m, 1H), 4.2−4.4 (m, 4H), 6.04 (m, 1H), 6.22 (m, 1H), 7.26 (s, 1H), 9.52 (s, 1H).

(4-Thymin-1-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (45). In a manner similar to that described for **18**, **42** was converted to **45**. The final purification was a precipitation from H_2O by acetonitrile affording **45** as a white solid in 35% yield: mp 200−250 °C dec; UV_{max} 274 nm (ε 7949); ^1H NMR ($CDCl_3$) δ 1.88 (d, J = 1.2 Hz, 3H), 3.47 (m, 1H), 3.69 (m, 1H), 4.63 (m, 1H), 4.70 (br d, J = 20.1 Hz, 1H), 5.18 (m, 1H), 5.96 (m, 1H), 6.53 (m, 1H), 7.46 (d, J = 1.2 Hz, 1H). Anal. ($C_{11}H_{15}N_2O_7P \cdot 1.2H_2O$) C, H, N.

Diisopropyl (4-Adenin-9-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonate (33). In a manner similar to that described for **6**, **5** was reacted with 6-chloropurine to afford crude intermediate **24** which was isolated as a foam (∼41% yield). A solution of the foam in methanol saturated at 0 °C with NH_3 was heated at 100 °C for 8 h in a sealed vessel. After the removal of solvent in vacuo, the residue was chromatographed on a column of silica gel (CH_2Cl_2−MeOH, 95:5) to give partially purified glycal phosphonate **29** as a white solid. In a manner similar to that described for **14**, **29** was then converted to **33** which was isolated as a white solid in 53% yield: ^1H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 2.55 (m, 1H), 3.03 (m, 2H), 3.69 (m, 1H), 4.18 (m, 1H), 4.73 (m, 2H), 5.24 (m, 2H), 5.89 (br s, 2H), 6.20 (m, 1H), 7.86 (s, 1H), 8.33 (s, 1H).

(4-Adenin-9-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonic Acid (37). In a manner similar to that described for **18**, **33** was converted to **37** which was isolated as a white solid in 83% yield: mp 250−300 °C dec; UV_{max} 262 nm (ε 12 481); ^1H NMR ($CDCl_3$) δ 2.43 (m, 1H), 3.08 (m, 1H), 3.33 (m, 1H), 3.61 (m, 1H), 4.34 (m, 1H), 5.34 (m, 1H), 5.35 (d, J = 6.84 Hz, 1H), 5.87 (m, 1H), 8.27 (s, 1H), 8.45 (s, 1H). Anal. ($C_{11}H_{14}N_5O_5P \cdot 1.1H_2O$) C, H, N.

Diisopropyl (4-Adenin-9-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)phosphonate (43). In a manner similar to that described for **6**, **5** was coupled to N^6-(phenoxyacetyl)-adenine[21] to furnish intermediate **25** as an oil. A solution of this oil in 10% NEt_3/MeOH was kept at room temperature for 30 min. After dilution with toluene (100 mL), the mixture was concentrated in vacuo. The residue was taken up in toluene and reconcentrated to give crude **40**. Treatment with 80% aqueous acetic acid (30 mL) at 80 °C for 6 h, followed by concentration in vacuo, left an oil which was chromatographed on a column of silica gel (CH_2Cl_2−MeOH, 95:5) to furnish **43** as a white solid in 6.8% overall yield from **5**: ^1H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 3.2−3.5 (m, 2H), 4.10 (m, 1H), 4.54 (m,

1H), 4.8−4.9 (m, 2H), 5.18 (m, 1H), 6.08 (br s, 2H), 6.1−6.3 (m, 2H), 8.30 (s, 1H), 8.42 (s, 1H).

(4-Adenin-9-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (46). In a manner similar to that described for **18**, **43** was converted to **46** which was isolated as a crystalline solid in 58% yield: mp 250−300 °C dec; UV_{max} 262 nm (ϵ 13 484); ^{1}H NMR ($CDCl_3$) δ 3.1−3.4 (m, 2H), 4.78 (m, 1H), 4.86 (d, *J* = 20.4 Hz, 1H), 5.34 (m, 1H), 6.24 (m, 1H), 6.59 (br d, *J* = 10.4 Hz, 1H), 8.34 (s, 1H), 8.45 (s, 1H). Anal. ($C_{11}H_{14}N_5O_5P\cdot1.5H_2O$) C, H; N: calcd, 19.77; found, 18.59.

Diisopropyl (4-Guanin-9-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonate (34) and Diisopropyl (4-Guanin-9-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)-phosphonate (44). In a manner similar to that described for **6, 5** was coupled to N^2-acetyl-O^6-(diphenylcarbamoyl)-guanine[22] to furnish the crude intermediate **26**. **26** was converted to a mixture of **30** and **41** following the deprotection procedure used for **10** and then deprotected further using the procedure described for **14**. The resulting mixture of **34** and **44** was separated by silica gel chromatography (CH_2Cl_2−MeOH, 95:5) affording **34** in a 18% overall yield and **44** in a 12% overall yield.

34: ^{1}H NMR (D_2O) δ 1.3−1.4 (m, 12H), 2.42 (m, 1H), 3.01 (m, 1H), 3.2−3.4 (m, 2H), 4.19 (m, 1H), 4.70 (m, 2H), 5.06 (m, 1H), 6.08 (m, 1H), 7.56 (s, 1H).

44: ^{1}H NMR (D_2O) δ 1.2−1.4 (m, 12H), 3.5−3.7 (m, 3H), 4.48 (m, 1H), 4.66 (m, 2H), 5.0−5.2 (m, 2H), 5.95 (m, 1H), 6.16 (m, 1H), 6.48 (s, 2H), 7.84 (s, 1H).

(4-Guanin-9-yl-2,3,4-trideoxy-D-*threo*-hex-1-enopyranosyl)phosphonic Acid (38). In a manner similar to that described for **18,** glycal **34** was converted to **38** which was isolated as white crystals in 96% yield: mp 250−300 °C dec; UV_{max} 254 nm (ϵ 12 500); ^{1}H NMR ($CDCl_3$) δ 2.4−2.5 (m, 1H), 3.0−3.1 (m, 1H), 3.4−3.7 (m, 2H), 4.32 (m, 1H), 5.26 (d, *J* = 6.7 Hz, 1H), 5.83 (m, 1H), 8.50 (s, 1H). Anal. ($C_{11}H_{14}N_5O_6P\cdot0.5H_2O$) C, H, N.

(4-Guanine-9-yl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosyl)phosphonic Acid (47). In a manner similar to that described for **18, 44** was converted to **47** which was isolated as white crystals in 86% yield: mp 250−300 °C dec; UV_{max} 254 nm (ϵ 13 805); ^{1}H NMR ($CDCl_3$) δ 3.2−3.5 (m, 2H), 4.74 (br s, 1H), 4.82 (d, *J* = 20.7 Hz, 1H), 5.16 (br s, 1H), 6.2 (m, 1H), 6.58 (d, *J* = 9.7 Hz, 1H), 8.26 (s, 1H). Anal. ($C_{11}H_{14}N_5O_6P\cdot0.5H_2O$) C, H, N.

Diisopropyl [2,3-Dideoxy-6-*O*-(triphenylmethyl)-β(and α)-L-*erythro*-hex-2-enopyranosyl]phosphonate (49). In a manner similar to that described for **2**−**4** (and **5**) tri-*O*-acetyl-L-glucal (**48**) was converted to **49** which was isolated as a pale yellow oil in 94% overall yield: ^{1}H NMR ($CDCl_3$) δ 1.3−1.4 (m, 12H), 3.2−3.3 (m, 2H), 4.07 (m, 1H), 4.21 (m, 1H), 4.2−4.4 (m, 1H), 4.7−4.9 (m, 2H), 5.8−6.0 (m, 2H), 7.2−7.5 (m, 15H).

Diisopropyl (4-Cytosin-1-yl-2,3,4-trideoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonate (52) and Diisopropyl (4-Cytosin-1-yl-2,3,4-trideoxy-L-*threo*-hex-1-enopyranosyl)-phosphonate (55). In a manner similar to that described for **6**, **49** was converted to crude **50** which was isolated as a yellow oil (48% yield). The oil was treated with NH_3/MeOH as described for **10**, to afford a mixture of partially deprotected intermediates **51** and **54** which was deprotected further using acetic acid as was done to prepare **14**. The resulting mixture of **52** and **55** was separated by silica gel chromatography (CH_2Cl_2−MeOH, 95:5) to furnish each as a white solid.

52: 32% yield from **50**; ^{1}H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 3.2−3.6 (m, 2H), 3.90 (m, 1H), 4.57 (d, *J* = 18 Hz, 1H), 4.6−4,9 (m, 2H), 5.41 (m, 1H), 5.78 (d, *J* = 7.2 Hz, 1H), 6.05 (m, 1H), 6.32 (m, 1H), 8.00 (d, *J* = 7.2 Hz, 1H).

55: 53% yield from **50**; ^{1}H NMR ($CDCl_3$) δ 1.2−1.3 (m, 12H), 2.25 (m, 1H), 2.98 (m, 1H), 3.36 (m, 1H), 3.40 (m, 1H), 4.06 (m, 1H), 4.5−4.7 (m, 2H), 5.39 (m, 1H), 5.76 (d, *J* = 7.4 Hz, 1H), 6.12 (m, 1H), 7.32 (d, *J* = 7.4 Hz, 1H).

(4-Cytosin-1-yl-2,3,4-trideoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonate (53). In a manner similar to that described for **18**, **52** was converted to **53** which was isolated as white crystals in 83% yield: mp 250−300 °C dec; ^{1}H NMR ($CDCl_3$) δ 3.4−3.6 (m, 2H), 3.93 (m, 1H), 4.50 (d, *J* = 18.1 Hz, 1H), 5.22 (m, 1H), 5.88 (m, 1H), 6.06 (d, *J* = 7.8 Hz, 1H), 6.45 (m, 1H), 8.12 (d, *J* = 7.8 Hz, 1H). Anal. ($C_{10}H_{14}N_3O_6P\cdot0.5H_2O$) C, H, N.

(4-Cytosin-1-yl-2,3,4-trideoxy-L-*threo*-hex-1-enopyranosyl)phosphonic Acid (56). In a manner similar to that described for **18**, **55** was converted to **56** which was isolated as a white solid in 53% yield: mp 250−300 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.14 (m, 1H), 2.91 (m, 1H), 3.53 (m, 1H), 3.72 (m, 1H), 4.08 (br s, 1H), 5.12 (d, *J* = 8.1 Hz, 1H), 5.76 (m, 1H), 6.05 (d, *J* = 7.5 Hz, 1H), 7.56 (d, *J* = 7.5 Hz, 1H). Anal. ($C_{10}H_{14}N_3O_6P\cdot0.4H_2O$) C, H, N.

Diisopropyl [2,3,6-Trideoxy-α(and β)-L-*erythro*-hex-2-enopyranosyl]phosphonate (58). In a manner similar to that described for **2** and **3**, 3,4-di-*O*-acetyl-6-deoxy-L-glucal (**57**) was converted to **58** which was isolated as a yellow oil in 91% yield: ^{1}H NMR ($CDCl_3$) δ 1.2−1.4 (m, 15H), 3.2−3.4 (m, 1H), 3.82 (m, 1H), 4.38 (m, 1H), 4.72 (m, 2H), 5.8−6.0 (m, 2H).

Diisopropyl (4-Cytosin-1-yl-2,3,4,6-tetradeoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonate (62) and Diisopropyl (4-Cytosin-1-yl-2,3,4,6-tetradeoxy-L-*threo*-hex-1-enopyranosyl)phosphonate (66). In a manner similar to that described for **6**, **58** was converted to crude intermediate **59** which was isolated as a yellow oil (∼48% yield). The oil was then deprotected as in the preparation of **10** to furnish a mixture of **62** and **66** which was separated on a column of silica gel (CH_2Cl_2−MeOH, 95:5). **62** and **66** were isolated as white solids in 38% and 30% yields, respectively.

62: ^{1}H NMR ($CDCl_3$) δ 1.10 (d, *J* = 6.3 Hz, 3H), 1.2−1.4 (m, 12H), 3,88 (m, 1H), 4.52 (d, *J* = 18.3 Hz, 1H), 4.7−4.9 (m, 2H), 5.37 (br s, 1H), 5.70 (d, *J* = 7.2 Hz, 1H), 5.97 (m, 1H), 6.30 (m, 1H), 7.92 (d, *J* = 7.2 Hz, 1H).

66: ^{1}H NMR ($CDCl_3$) δ 1.21 (d, *J* = 6.3 Hz, 1H), 1.3−1.5 (m, 12H), 2.14 (m, 1H), 2.80 (m, 1H), 4.13 (m, 1H), 4.6−4.8 (m, 2H), 5.23 (br d, *J* = 7.3 Hz, 1H), 5.72 (d, *J* = 7.5 Hz, 1H), 5.98 (m, 1H), 7.27 (d, *J* = 7.5 Hz, 1H).

(4-Cytosin-1-yl-2,3,4,6-tetradeoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonic Acid (64). In a manner similar to that described for **18**, **62** was converted to **64** which was isolated as white crystals in 56% yield: mp 250−300 °C dec; UV_{max} 276 nm (ϵ 10 735); ^{1}H NMR ($CDCl_3$) δ 1.03 (d, *J* = 6.5 Hz, 3H), 4.03 (m, 1H), 4.50 (d, *J* = 17.6 Hz, 1H), 5.12 (m, 1H), 5.94 (m, 1H), 6.04 (d, *J* = 7.3 Hz, 1H), 6.43 (m, 1H), 8.05 (d, *J* = 7.3 Hz, 1H); HRMS 288.0756 (M + H − glycerol), calcd for $C_{10}H_{15}N_3O_5P$ 288.0749. Anal. ($C_{10}H_{14}N_3O_5P\cdot H_2O$) C, H; N: calcd, 12.99; found, 13.77.

(4-Cytosin-1-yl-2,3,4,6-tetradeoxy-L-*threo*-hex-1-enopyranosyl)phosphonic Acid (69). In a manner similar to that described for **18**, glycal **66** was converted to **69** which was isolated as white crystals in 87% yield: mp 250−300 °C dec; UV_{max} 276 nm (ϵ 10 323); ^{1}H NMR ($CDCl_3$) δ 1.16 (d, *J* = 6.3 Hz, 3H), 2.20 (m, 1H), 2.88 (m, 1H), 4.21 (m, 1H), 5.04 (d, *J* = 7.8 Hz, 1H), 5.76 (m, 1H), 6.06 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H); HRMS 288.0754 (M + H − glycerol), calcd for $C_{10}H_{15}N_3O_5P$ 288.0749. Anal. ($C_{10}H_{14}N_3O_5P\cdot H_2O$) C, H, N.

Diisopropyl (4-Thymin-1-yl-2,3,4,6-tetradeoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonate (63) and Diisopropyl (4-Thymin-1-yl-2,3,4,6-tetradeoxy-L-*threo*-hex-1-enopyranosyl)phosphonate (67). In a manner similar to that described for **6**, the anomeric mixture **58** was converted to crude intermediate **60** which was isolated as a yellow oil. This mixture of anomers was then treated with NH_3/MeOH as described for **10** to furnish a mixture of **63** and **67**. Chromatographic separation on silica gel (CH_2Cl_2−MeOH, 95:5) afforded **63** as a white solid in 20% yield and **67** as a white solid in 19% yield.

63: ^{1}H NMR ($CDCl_3$) δ 1.13 (d, *J* = 5.4 Hz, 3H), 1.3−1.5 (m, 12H), 1.95 (s, 3H), 3.88 (m, 1H), 4.53 (d, *J* = 18.9 Hz, 1H), 4.80 (m, 2H), 5.09 (m, 1H), 5.95 (m, 1H), 6.33 (m, 1H), 7.80 (s, 1H), 8.51 (s, 1H).

67: ^{1}H NMR ($CDCl_3$) δ 1.27 (d, *J* = 5.3 Hz, 3H), 1.3−1.4 (m, 12H), 1.88 (s, 3H), 2.18 (m, 1H), 2.85 (m, 1H), 4.11 (m, 1H), 4.74 (m, 2H), 5.04 (d, *J* = 7.8 Hz, 1H), 6.08 (m, 1H), 7.08 (s, 1H), 8.82 (s, 1H).

(4-Thymin-1-yl-2,3,4,6-tetradeoxy-β-L-*threo*-hex-2-enopyranosyl)phosphonic Acid Sodium Salt (65). In a manner similar to that described for **18**, **63** was converted to **65**. After

reverse phase chromatography, the product was dissolved in H_2O and the pH adjusted to 7 by the addition of a saturated aqueous solution of sodium perchlorate. After concentrating in vacuo to 2 mL, the product was precipitated by the addition of acetone. Isolation of the solid afforded pure **65** in 73% yield: mp 200−250 °C dec; UV_{max} 274 nm (ϵ 9973); ^1H NMR ($CDCl_3$) δ 1.06 (d, J = 5.86 Hz, 3H), 1.87 (s, 3H), 3.99 (m, 1H), 4.41 (m, 1H), 4.99 (m, 1H), 5.86 (m, 1H), 6.48 (m, 1H), 7.38 (s, 1H); HRMS 325.0578 (M + H − glycerol), calcd for $C_{11}H_{15}N_2O_6$-NaP 325.0565. Anal. ($C_{11}H_{14}N_2O_6PNa\cdot0.4H_2O$) C, H, N.

(4-Thymin-1-yl-2,3,4,6-tetradeoxy-L-*threo*-hex-1-enopyranosyl)phosphonic Acid (70). In a manner similar to that described for **18**, **67** was converted to **70**. After reverse phase chromatography, the product was dissolved in H_2O and the pH was adjusted to 7 by the addition of a saturated aqueous solution of sodium perchlorate. After concentrating in vacuo to 2 mL, the product was precipitated by the addition of acetone. Isolating the solid afforded pure **70** in 80% yield: mp 200−250 °C dec; UV_{max} 274 nm (ϵ 8306); ^1H NMR ($CDCl_3$) δ 1.17 (d, J = 1.17 Hz, 3H), 1.88 (d, J = 1.0 Hz, 3H), 2.16 (m, 1H), 2.83 (m, 1H), 4.16 (m, 1H), 4.91 (m, 1H), 5.61 (m, 1H), 7.42 (d, J = 1.0 Hz, 1H); HRMS 325.0573 (M + H − glycerol), calcd for $C_{11}H_{15}N_2O_6NaP$ 325.0578. Anal. ($C_{11}H_{14}N_2O_6$-$PNa\cdot0.5H_2O$) C, H, N.

Diisopropyl (4-Adenin-9-yl-2,3,4,6-tetradeoxy-L-*threo*-hex-1-enopyranosyl)phosphonate (68). In a manner similar to that for **6**, the anomeric mixture **58** was reacted with 6-chloropurine to afford intermediate **61** which was isolated as a yellow oil (~34% yield). Using NH_3/MeOH as described in the preparation of **29**, the oil was converted to **68** which was isolated as a white solid in 18% yield from **58**: ^1H NMR ($CDCl_3$) δ 1.13 (d, J = 6.9 Hz, 3H), 1.3−1.4 (m, 12H), 2.37 (m, 1H), 2.94 (m, 1H), 4.34 (m, 1H), 4.77 (m, 2H), 5.02 (br d, J = 6.9 Hz, 1H), 5.72 (br d, 2H), 6.06 (m, 1H), 7.98 (s, 1H), 8.35 (s, 1H).

(4-Adenin-9-yl-2,3,6-trideoxy-L-*threo*-hex-1-enopyranosyl)phosphonic Acid (71). In a manner similar to that described for **18**, **71** was prepared from glycal **68** and isolated as white crystals in 88% yield: mp 172 °C; UV_{max} 262 nm (ϵ 12 808); ^1H NMR ($CDCl_3$) δ 1.01 (d, J = 6.4 Hz, 3H), 2.37 (m, 1H), 2.99 (m, 1H), 4.37 (m, 1H), 4,99 (d, J = 6.8 Hz, 1H), 5.78 (m, 1H), 8.10 (s, 1H), 8.24 (s, 1H); HRMS 312.0862 (M + H − glycerol), calcd for $C_{11}H_{15}N_5O_4P$ 312.0861. Anal. ($C_{11}H_{14}N_5O_4P\cdot$ $0.4H_2O$) C, H, N.

Diisopropyl (4-Cytosin-1-yl-2,3,4-trideoxy-β-D-*threo*-hexopyranosyl)phosphonate (72). A solution of **14** (0.195 g, 0.5 mmol) in methanol (10 mL) was stirred under H_2 at 1 atm for 4 h in the presence of 10% Pd on carbon (0.05 g). The mixture was filtrated and concentrated in vacuo to give phosphonate **72** as a white solid (0.190 g, 98%): ^1H NMR ($CDCl_3$) δ 1.2−1.4 (m, 12H), 1.7−2.1 (m, 4H), 3.49 (m, 1H), 3.67 (m, 1H), 3.88 (m, 2H), 4.76 (m, 2H), 4.97 (m, 1H), 5.71 (d, J = 7.8 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H).

(4-Cytosin-1-yl-2,3,4-trideoxy-β-D-*threo*-hexopyranosyl)phosphonic Acid (73). A solution of **72** (0.195 g, 0.49 mmol), 2,4-lutidine (0.6 mL, 5 mmol), and bromotrimethylsilane (0.66 mL, 5 mmol) in acetonitrile (5 mL) was stirred at room temperature for 24 h. The solvent was removed in vacuo, and the residue was taken in methanol (10 mL), reconcentrated, and then dissolved in water (5 mL) and applied on a Dowex 1 (AcO^- form) column (20 mL). After eluting with water (300 mL) followed by 0.1 M acetic acid, product-containing fractions were evaporated and the residue was precipitated from H_2O/acetonitrile to give **73** as a white solid (0.136 g, 90%): mp 250−300 °C dec; UV_{max} 276 nm (ϵ 9503); ^1H NMR ($CDCl_3$) δ 1.7−1.9 (m, 2H), 2.0−2.2 (m, 2H), 3.5−3.7 (m, 2H), 3.82 (m, 1H), 3.91 (m, 1H), 4.71 (m, 1H), 6.05 (d, J = 8.0 Hz, 1H), 8.51 (d, J = 8.0 Hz, 1H); HRMS 306.0859 (M + H − glycerol), calcd for $C_{10}H_{17}N_3O_6P$ 306.0855. Anal. ($C_{10}H_{16}N_3O_6P\cdot1.2H_2O$) C, H, N.

Biological Methods: HCMV Assay.[23] Confluent monolayers of normal human dermal fibroblasts (NHDF) in a 24-well plate were infected with HCMV (Towne). After 1−2 h at 37 °C, the medium was replaced and serial dilutions of the drug were applied to the wells. Some wells were left free of drug as controls. After 7−10 days at 37 °C, the wells were stained with crystal violet and scored visually under a microscope. All inhibitors were assayed for anti-HCMV activity in duplicate. The data were plotted as percent inhibition of the average plaque number vs the concentration of drug. The IC_{50} value of the inhibitor is defined as the concentration of drug that decreased the number of plaques to 50% of the number present in the control wells with no drug.

The drugs' cytotoxic effect was assessed by incubating uninfected NHDF monolayers with serial dilutions of drug at 37 °C, 4% CO_2, for 7−10 days and then washing with sterile PBS, adding XTT medium, incubating at 37 °C for 45 min, and then reading absorbance at 450 nm.[24,25]

HSV-2 Assay.[24,25] Serial dilutions of drug in Earl's MEM with 10% FBS were added to wells of a 96-well plate containing MRC-5 cells. After incubation at 37 °C, 5% CO_2, for 15 min, ~100 PFU/well HSV-2 (E 194) was added to the wells and incubated at 37 °C, 5% CO_2, for 2.5−3 days until ca. 90% killing was present in the control (no drug) wells. The medium was then removed, the cells were washed with sterile PBS, and XTT medium was added. After incubating at 37 °C for 30−40 min, absorbance was read at 450−650 nm.

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