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Stereoselective Synthesis of the 5′-Hydroxy-5′-phosphonate Derivatives of Cytidine and Cytosine Arabinoside

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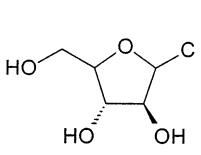
Both the (*R*)- and (*S*)-5′-hydroxy 5′-phosphonate derivatives of cytidine and cytosine arabinoside (ara-C) have been prepared via phosphite addition or a Lewis acid mediated hydrophosphonylation of appropriately protected 5′-nucleoside aldehydes. Phosphite addition to a cytosine aldehyde protected as the 2′,3′-acetonide gave predominately the 5′*R* isomer, while phosphite addition to the corresponding 2′,3′-bis TBS derivative favored the 5′*S* stereochemistry. In contrast, phosphite addition to the 2′,3′-bis TBS protected aldehyde derived from ara-C gave only the 5′*R* adduct. However, $TiCl_4$-mediated hydrophosphonylation of the same ara-C aldehyde favored the 5′*S* stereoisomer by a 2:1 ratio. Once all four of the diastereomers were in hand, the stereochemistry of these compounds could be assigned based on their spectral data or that obtained from their *O*-methyl mandelate derivatives. After hydrolysis of the phosphonate esters and various protecting groups, the four α-hydroxy phosphonic acids were tested for their ability to serve as substrates for the enzyme nucleoside monophosphate kinase and for their toxicity to K562 cells.

Introduction

Cytosine arabinoside (ara-C, **1**) is an important antileukemia agent, especially for treatment of acute myelogenous leukemias.[1] While the mechanisms responsible for the lethal effect of ara-C are not entirely clear, substantial evidence indicates that interference with DNA synthesis via its triphosphate derivative (ara-CTP) represents a major mode of ara-C-mediated lethality.[2] However, upon extended clinical use, leukemia cells may become resistant to ara-C resulting in recurrence of the disease. Decreased activity of deoxycytidine kinase, the enzyme that normally converts ara-C to ara-C monophosphate (ara-CMP, **2**) and the first step in metabolic formation of ara-CTP, represents a principal cause of this resistance.[3]

Our interest in development of new antileukemia agents for treatment of ara-C-resistant leukemias has led to preparation of new 5′-hydroxy-5′-phosphono derivatives of this nucleoside, compounds which can be viewed as analogues of ara-CMP. These phosphonates would be expected to display substantial similarity with ara-CMP in both structure and acidity, but enhanced metabolic stability as a result of the carbon−phosphorus bond.



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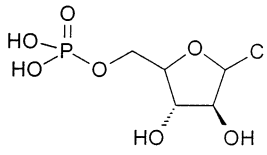

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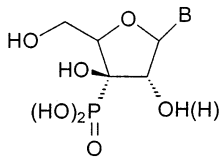
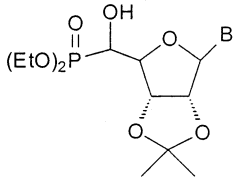


3a-c

a B = U
b B = A
c B = T



4a-d

a B = U^{MOM}
b B = C^{Bz}
c B = A^{Bz}
d B = G^{Bz}

Nucleoside 3′-hydroxy-3′-phosphonates (**3**) have been prepared through the nucleophilic addition of dialkyl phosphite to the 3′-ketones,[4] although in that series of compounds the limited conformational flexibility of the cyclic ketone governed the stereochemical outcome of the addition. A similar procedure was later applied to preparation of some 5′-hydroxy-5′-phosphono nucleosides (**4**), but the products were obtained as mixtures of

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(4) (a) McEldoon, W. L.; Wiemer, D. F. *Tetrahedron* **1995**, *51*, 7131−7148 and references therein. (b) Kralikova, S.; Budesinsky, M.; Masojidkova, M.; Rosenberg, I. *Nucleosides, Nucleotides, Nucleic Acids* **2000**, *19*, 1159−1183.

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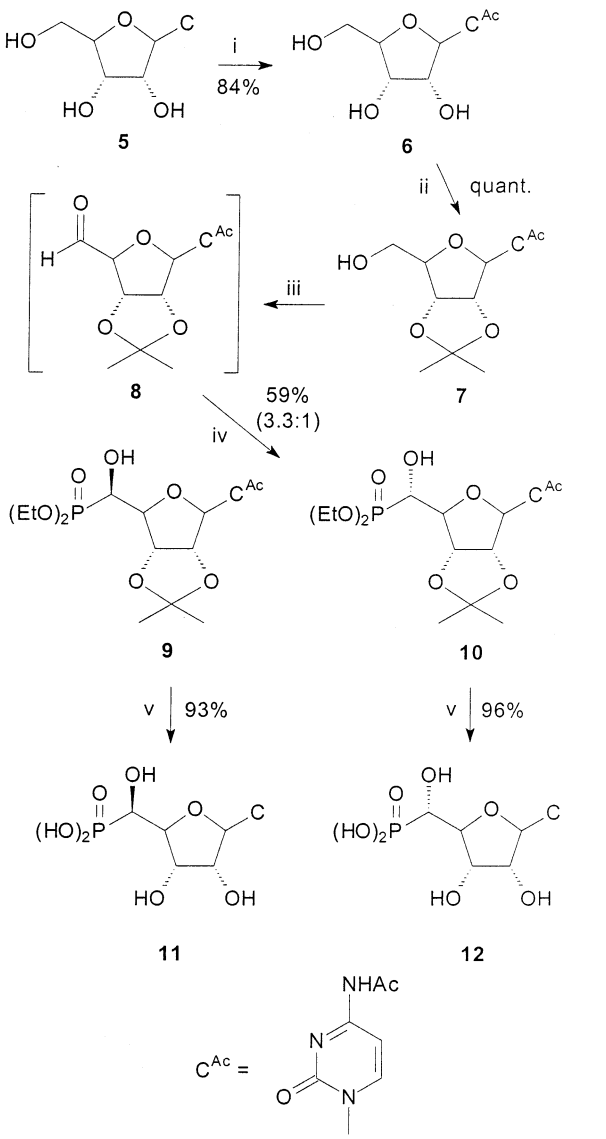
epimers, and no biological evaluations have been reported.[5] Furthermore, prior to this work there were no reports on such phosphonate derivatives of ara-C.[6] In this paper, we describe the stereoselective synthesis of 5′-hydroxy 5′-phosphonate derivatives of ara-C and cytidine through phosphite addition and $TiCl_4$-mediated hydrophosphonylation, as well as the results of the initial biological investigations of these new compounds.

Results and Discussion

Cytidine Derivatives. To develop the synthetic transformations with a less costly starting material, and to obtain samples for later biological comparisons, the initial investigations were conducted with commercial cytidine (**5**) rather than ara-C (Scheme 1). After acetylation of the free amine to give the amide **6**, the two secondary hydroxy groups initially were protected as the acetonide derivative **7**.[7] Because nucleoside aldehydes may be prone to isomerization,[8] oxidation of the free primary hydroxyl group always was followed immediately by reaction with phosphite. When a Collin's oxidation[9] was employed, the aldehyde **8** was obtained in very low yield and the overall yield for the oxidation and phosphite addition was unacceptable. When a modified Pfitzner−Moffatt oxidation[10] was employed, the crude aldehyde **8** could be extracted in reasonable yield and purity, and the subsequent phosphonylation was realized in good yield to give an epimeric mixture of 5′-hydroxy phosphonates **9** and **10** in a ratio of 3.3:1. The two epimers were separated by preparative RP-HPLC with a water and acetonitrile gradient. Separate reaction of phosphonates **9** and **10** with TMSBr and workup with MeOH gave the two parent phosphonic acids **11** and **12** in excellent yield.

To assign the configuration of the newly generated stereogenic center at C-5′, *O*-methyl mandelate esters were prepared from phosphonates **9** and **10**. Mandelate diastereomers have been employed by Spilling and coworkers[11] for determination of the enantiomeric purity and absolute configuration of other α-hydroxy phosphonates. Their approach also has been used to determine the configuration of a nucleoside 5′,6′-dihydroxy phosphonate,[7] and that assignment was confirmed by X-ray analysis.[6] In the present case, the *O*-methyl mandelate ester **13** was prepared by treatment of phosphonate **9** with (*S*)-*O*-methyl mandelic acid in the presence of EDC and DMAP (Scheme 2), while the isomeric **14** was obtained through parallel esterification of a mixture of phosphonates **9** and **10**. Partial ^{1}H and ^{31}P NMR data

SCHEME 1[a]



[a] Reagents and conditions: (i) MeOH, Ac_2O, reflux; (ii) 2,2-dimethoxypropane, $HClO_4$ (cat.), acetone, rt; (iii) DMSO, EDC, Pyr, TFA, C_6H_6, rt; (iv) LHMDS, $HP(O)(OEt)_2$, THF, −78 °C; (v) TMSBr, CH_2Cl_2, 0 °C to rt, then MeOH.

for the mandelates are listed in Table 1. In the ^{1}H NMR spectrum of ester **13**, the ethoxy groups showed upfield shifts of ∼0.2 and 0.4 ppm relative to the parent α-hydroxy phosphonate **9**, while in the spectrum of ester **14** the ethoxy groups signals were not significantly different from those of the parent compound **10**. The resonances for H-2′ and H-3′ and one methyl in the acetonide group were shifted in the opposite sense. For ester **14,** these three resonances were shifted upfield ∼1.5, 1.1, and 0.2 ppm, respectively, while the resonances of the H-2′ and H-3′ hydrogens and one methyl group in ester **13** were shifted only slightly upfield. On the basis of the chemical shifts observed with compounds **13** and **14**, and on analysis of the extended Newman projections between C-5′ and the stereogenic center in the mandelate, the absolute stereochemistry of C-5′ could be assigned. The *O*-methyl mandelate ester derived from the major 5′-hydroxy 5′-phosphonate **9** and (*S*)−(+)-*O*-methyl man-

(5) (a) Kralikova, S.; Budesinsky, M.; Masojidkova, M.; Rosenberg, I. *Tetrahedron Lett.* **2000**, *41*, 955−958. (b) These authors also assigned the major phosphonate derived from phosphite addition to a cytidine aldehyde as the 5′*R* isomer, although no data for that compound were included in their preliminary report.

(6) Presented in part at the International Conference on Phosphorus Chemistry, Sendai, Japan, August 2001. Chen, X.; Jung, K.; Wiemer, D. F.; Wiemer, A. J.; Hohl, R. J. *Phosphorus, Sulfur, Silicon Relat. Elem.* **2002**, *177*, 1783−1786.

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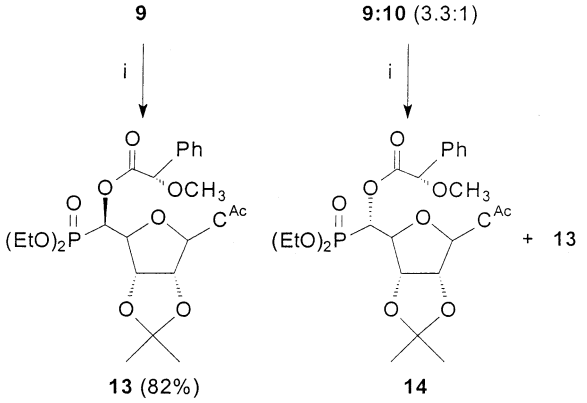
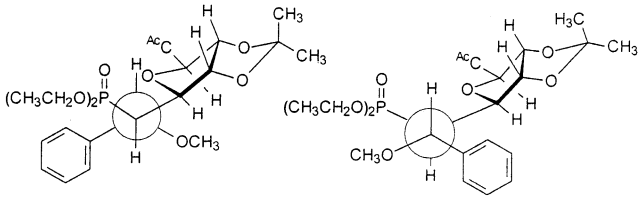
SCHEME 2[a]



[a] Reagents and conditions: (i) EDC, DMAP, (*S*)-*O*-methyl mandelic acid, CH_2Cl_2, rt.

TABLE 1. ^{1}H and ^{31}P NMR Chemical Shifts of Phosphonates and *O*-Methyl Mandelate Esters



	phosphonate **9**	ester **13**	phosphonate **10**	ester **14**
H-2′	5.20	4.85	5.20	3.72
H-3′	5.05	4.99	5.15	4.02
OCH_2	4.23−4.13	3.98−3.65	4.20−4.00	4.20−4.10
C-CH_3	1.35	1.32	1.35	1.17
OCH_2CH_3	1.37−1.30	1.16−1.06	1.35−1.23	1.35−1.30
^{31}P	21.61	15.92	21.60	16.17

delic acid was assigned as the 5′*R* isomer **13**.[5b] The mandelate ester derived from the minor 5′-hydroxy 5′-phosphonate was assigned as the 5′*S* isomer **14**.[12]

While the 5′*S*-hydroxy-5′-phosphonate **10** was available as the minor product from addition of phosphite to the acetonide-protected aldehyde **8**, the necessity of an HPLC separation made that approach an unattractive route for preparation of the 5′*S* phosphonic acid **12**. A more efficient preparation was suggested by a report of an asymmetric synthesis of a chiral α,*β*-dihydroxy phosphonate by addition of trimethylsilyl diethyl phosphite to α-silyloxy aldehydes.[13] In that work, a chelated transition state was postulated, in which an attractive electrostatic interaction between the silicon and the carbonyl oxygen favored a cyclic conformation of the α-silyloxy aldehyde. By analogy with that research, it was possible that a chelation factor might be introduced if the acetonide group in aldehyde **8** were replaced by two TBS groups.

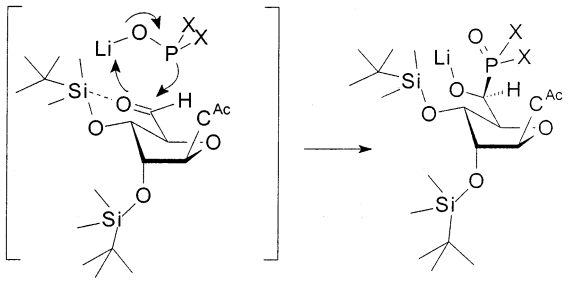
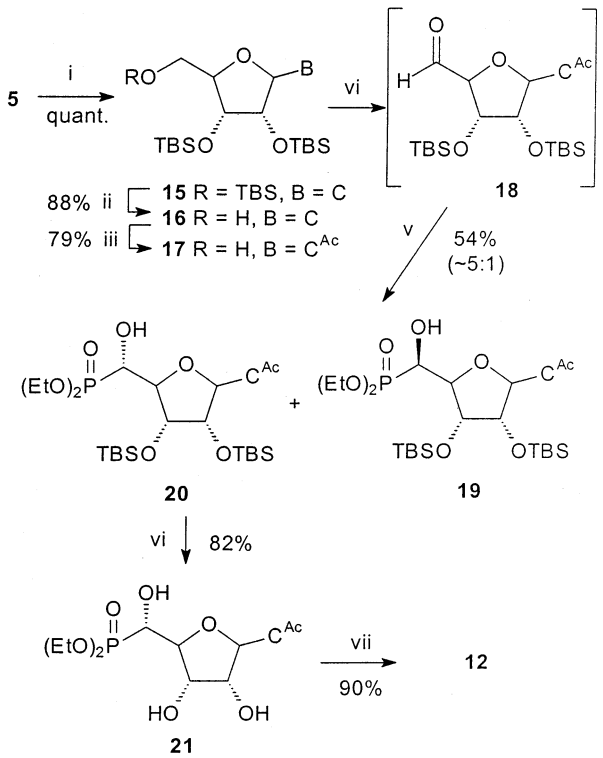


FIGURE 1. Possible transition state for phosphite addition to cytidine aldehyde **18**.

SCHEME 3[a]



[a] Reagents and conditions: (i) TBDMSCl, imid, pyr, rt; (ii) THF:TFA:H_2O (4:1:1), 0 °C; (iii) MeOH, Ac_2O, reflux; (iv) DMSO, EDC, pyr, TFA, toluene, rt; (v) $HP(O)(OEt)_2$, LHMDS, THF, −78 °C; (vi) TBAF, THF, rt; (vii) TMSBr, CH_2Cl_2, 0 °C to rt, then MeOH.

According to this premise, an addition reaction between tricoordinate diethyl phosphite and a chelated *β*-silyloxy aldehyde[14] would be expected to favor the 5′*S* hydroxy phosphonate (Figure 1).

Synthesis of the 2′,3′-TBS protected aldehyde **18** was realized via a sequence including tri-*O*-silylation (**15**),[15] deprotection of the primary hydroxyl group (**16**),[16] acylation of the primary amine (**17**),[7] and final oxidation of the primary alcohol to the aldehyde **18** (Scheme 3). Phosphonylation of aldehyde **18** proceeded in good yield and favored the desired *S* hydroxy phosphonate **20** over the *R* isomer **19** by a ∼5:1 ratio. The configuration of C-5′

(12) These assignments also are consistent with the stereochemical outcome of allylation reactions conducted with aldehydes **8** and **18**. Reaction of aldehyde **8** with allyltrimethylsilane gave only the 5′*R* adduct while reaction of aldehyde **18** under the same conditions gave only the 5′*S* product, as shown by single-crystal diffraction analysis. Furthermore, allylation of aldehyde **23** with allyltrimethylsilane gave only the 5′*R* adduct, as established by diffraction analysis and also parallel to the course of phosphite addition. Unpublished results from X. Chen and D. F. Wiemer.

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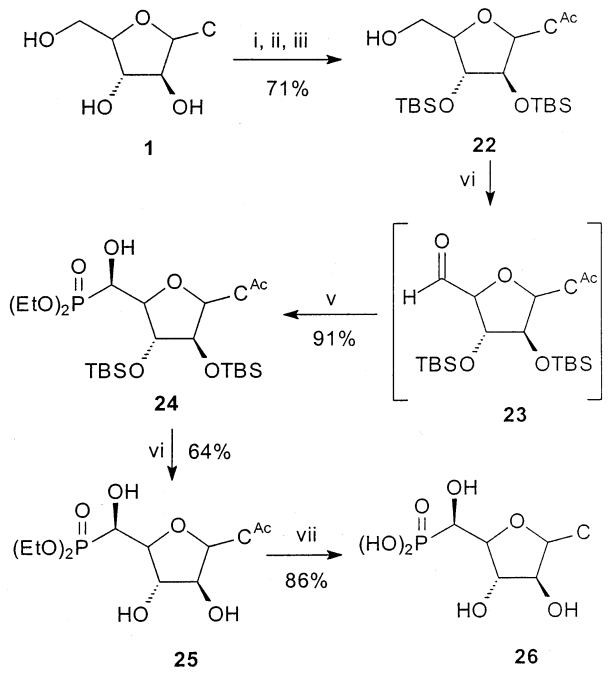
SCHEME 4[a]



[a] Reagents and conditions: (i) TBDMSCl, imid, pyr, rt; (ii) THF:TFA:H_2O (4:1:1), 0 °C; (iii) MeOH, Ac_2O, reflux; (iv) DMSO, EDC, pyr, TFA, C_6H_6, rt; (v) $HP(O)(OEt)_2$, LHMDS, THF, −78 °C; (vi) TBAF, THF, rt; (vii) TMSBr, CH_2Cl_2, 0 °C to rt, then MeOH.

was determined by comparison of the NMR spectra of the fully deprotected phosphonic acid **12** prepared this way with the authentic compound prepared as shown in Scheme 1. The diastereomers **19** and **20** can be separated by careful flash chromatography and the *S* isomer is favored by this approach, which makes the synthetic route shown in Scheme 3 the more efficient way to prepare the 5′*S* hydroxy phosphonic acid **12**.

Ara-C Derivatives. Because the trans 2′- and 3′-hydroxyl groups of ara-C would be difficult to protect as an acetonide, preparation of these phosphonic acids was pursued through the TBS-protected 5′-nucleoside aldehyde **23** (Scheme 4). After Pfitzner−Moffatt oxidation of the alcohol **22**,[7] the aldehyde **23** easily can be obtained in pure form and high yield by simple extraction with ether, and the subsequent phosphonylation also proceeded in excellent yield. Perhaps surprisingly, phosphonylation gave exclusively one isomer (**24**). In contrast to the cytidine series, where the acetonide limits conformations of the furan ring, it is possible that the two bulky trans TBS groups favor a different envelope conformation (Figure 2). While the distance between the carbonyl group and the silicon of the C-3′ silyl ether may be too great to allow association, the TBS group on the C-2′ oxygen may block the *si* face of the carbonyl group. Thus diethyl phosphite can only approach from the relatively open *re* face, resulting in formation of a single 5′ isomer. On the basis of this analysis, the configuration at C-5′ of phosphonate **24** tentatively was assigned as *R*.[12]

Several strategies were explored to obtain the 5′*S*-epimer corresponding to compound **24**. A Mitsunobu reaction was attempted with phosphonate **24**, but only the starting material was recovered under standard reaction conditions. An alternative strategy based on

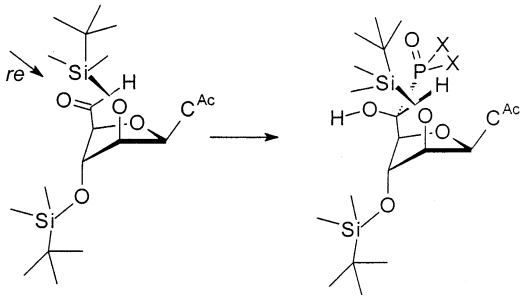
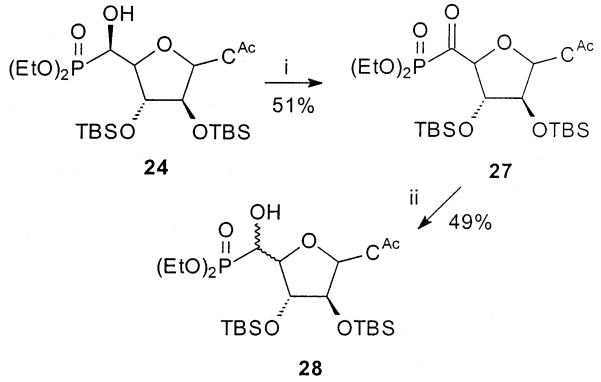


FIGURE 2. Facial selectivity for phosphite addition to ara-C aldehyde **23**.

SCHEME 5[a]



[a] Reagents and conditions: (i) PDC, Ac_2O, CH_2Cl_2, reflux; (ii) $NaBH_3CN$, THF; rt.

oxidation−reduction also was explored. With phosphonate **24** in hand, oxidation appeared to be the shortest route to the acyl phosphonate **27**. Several oxidation conditions were examined with limited success, including a solvent-free reaction with Al_2O_3-supported CrO_3[17] or $ZnCr_2O_7$,[18] oxidation with NMO catalyzed by TPAP,[19] and Swern oxidation, but either no reaction or just trace amounts of the acyl phosphonate were observed under these conditions. Fortunately when phosphonate **24** was treated with PDC and Ac_2O in CH_2Cl_2[20] (Scheme 5), most of the substrate was converted to the desired acyl phosphonate as monitored by ^{31}P NMR, but isolation was difficult because compound **27** undergoes hydrolysis to diethyl phosphite and the corresponding acid upon exposure to moisture.[21] After partial purification of the reaction mixture by flash chromatography, the acyl phosphonate **27** was obtained in moderate yield. However, reduction of this acyl phosphonate with $NaBH_3CN$ did not give the 5′*S*-epimer as a major product. Instead a mixture of three α-hydroxy phosphonates was obtained in a ratio of 5:1:1 and the major product was compound **24**. The two minor products included the desired 5′*S*-epimer and a 4′-diastereomer, which may be produced via an enol form of the acyl phosphonate.

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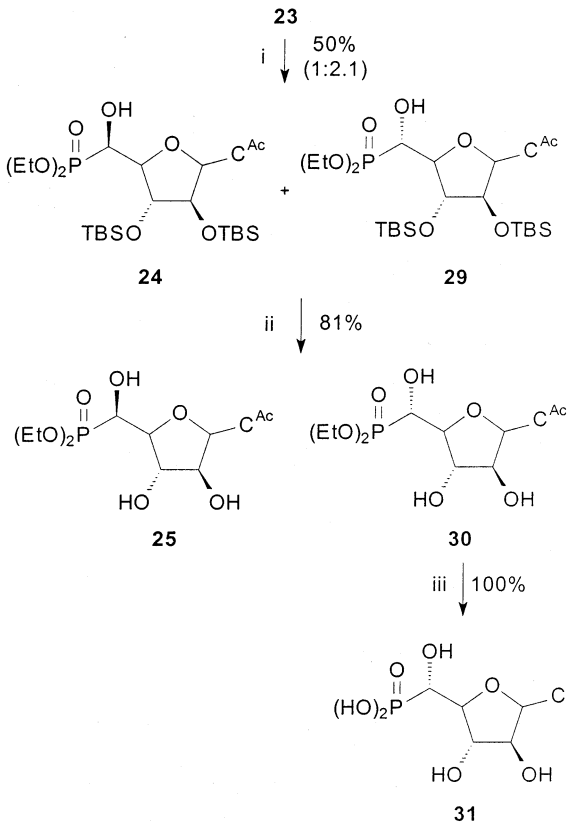
SCHEME 6[a]



[a] Reagents and conditions: (i) $(EtO)_2POSiMe_3$, $TiCl_4$, CH_2Cl_2, −78 °C to rt; (ii) TBAF, THF, rt; (iii) TMSBr, CH_2Cl_2, 0 °C to rt, then MeOH.

The unfavorable stereoselectivity observed in reduction of acyl phosphonate **27** encouraged exploration of another approach to the 5′*S*-epimer. On the basis of the observation of chelation-controlled allylation of a 5′-nucleoside aldehyde mediated by $TiCl_4$[22] it was reasonable to consider whether a $TiCl_4$-mediated phosphonylation of aldehyde **23** would reverse the 5′*R* stereoselectivity observed above. This Lewis acid may not be compatible with the basic phosphonylation conditions used previously, but conditions that would allow use of $TiCl_4$ have been reported for addition of diethyl trimethylsilyl phosphite to an aldehyde.[23,4b] For example, a stereoselective synthesis of a β-oxygenated α-hydroxy phosphonate by $TiCl_4$-mediated hydrophosphonylation of α-benzyloxy aldehydes was reported by the Shibuya group.[24] Therefore a similar strategy was examined for preparation of the 5′*S*-hydroxy phosphonate (Scheme 6). When the 5′-aldehyde **23** was treated with $TiCl_4$ at low temperature followed by addition of diethyl trimethylsilyl phosphite and then water, varying amounts of recovered aldehyde and the *S* and the *R* products were obtained.[25] The best yield of the desired 5′*S*-epimer **29** was obtained when the reaction was quenched by addition of water at room temperature, which gave the *S* and *R* adducts **29** and **24** in a 2.1:1 ratio and a total yield of 50%. The two epimers were not readily separated at this stage, but when desilylation was conducted on this mixture the deprotected α-hydroxy phosphonates **25** and **30** were obtained in good yield and these compounds were readily separated. Phosphonates **25** and **30** separately were treated with TMSBr followed by workup with MeOH to provide the target phosphonic acids **26** and **31**.

TABLE 2. ^{13}C Chemical Shifts (ppm) and $^{13}C-^{31}P$ Coupling Constants (Hz) for Cytidine, Ara-C, and Their Phosphonic Acid Derivatives in D_2O[a,b]

	C-1′	C-2′	C-3′	C-4′	C-5′	$J_{4'-CP}$	$J_{5'-CP}$
cytidine	91.6	75.3	70.6	85.0	62.1		
11	91.6	72.5	77.6	88.3	71.5	13.3	152.6
12	93.6	77.2	86.4	72.7	71.3	7.2	152.3
ara-C	87.2	76.8	76.8	84.5	62.1		
26	86.6	74.8	77.1	84.5	70.2	11.7	148.8
31	88.5	78.0	85.5	77.7	70.2	7.4	156.2

[a] Data for cytidine and ara-C measured relative to internal dioxane at 67.86 ppm.[26] [b] Data for compounds **11**, **12**, **26**, and **31** measured relative to 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt at 0 ppm.

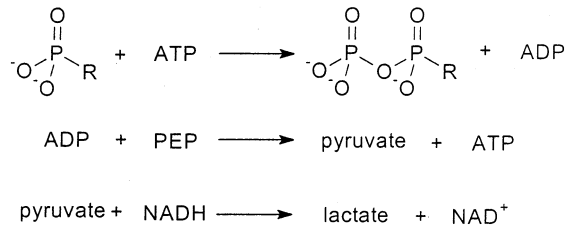


FIGURE 3. Enzymatic phosphorylation of synthetic nucleoside phosphonates.

The chemical shifts for the carbohydrate carbons and the C−P coupling constants of cytidine, ara-C, and their corresponding 5′*R*- and 5′*S*-hydroxy-5′-phosphonic acids are listed in Table 2. It is interesting that the assigned *R*- and *S*-phosphonic acids of ara-C show the same pattern as the cytidine derivatives in chemical shifts for C-3′ and C-4′, and in the C−P coupling constants for C-4′. On the basis of these data, phosphonic acid **26** was assigned as the 5′*R*-isomer while phosphonic acid **31** was assigned as the 5′*S*-isomer.

Bioactivity. The four 5′-hydroxy phosphonic acids **11**, **12**, **26**, and **31** were tested for their ability to serve as substrates for nucleotide monophosphate (NMP) kinase. The in vitro enzyme assay was designed to use NMP kinase with adenosine triphosphate (ATP) as cofactor and linkage to pyruvate kinase and lactic dehydrogenase as shown in Figure 3.[27] The degree of phosphorylation of the phosphonic acid in each test correlates with the conversion of nicotinamide adenine dinucleotide (NADH) to oxidized nicotinamide adenine dinucleotide (NAD^+), and can be monitored spectrophotometrically by a reduction in absorbance at 340 nm. As shown in Table 3, the three tested phosphonic acids were phosphorylated very

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(25) The reaction of aldehyde **21** with diethyl trimethylsilyl phosphite in the absence of $TiCl_4$ in CH_2Cl_2 from −78 °C to room temperature gave the R adduct as its trimethylsilyl derivative in 64% yield. After hydrolysis of the silyoxy groups, the C-5′ stereochemistry was confirmed by comparison with compound **23a.**

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TABLE 3. Relative Phosphorylation[a] and Toxicity[b] of Nucleoside Phosphonates to K562 Cells

compd	rate of phosphorylation, %	LC_{50}
11	1.1	0.5 μM
12	1.0	>10 μM
26	1.2	6 μM
31		50 μM
ara-C		5 nM

[a] Relative phosphorylation of nucleoside phosphonates expressed as a percentage relative to UMP utilization. [b] Measured by ^{3}H thymidine incorporation.

slowly, with not more than ~1% of the activity of the natural substrate uridine monophosphate (UMP).

These phosphonic acids also were tested for their effect on phosphorylation of UMP with a variation of the enzyme assay as described above. While the ara-C derived phosphonic acids **26** and **31** have no effect on phosphorylation of UMP, the cytidine-derived phosphonic acids **11** and **12** affect phosphorylation of UMP in opposite directions. The rate of UMP phosphorylation in the presence of compound **11** increased to ~1.6× that observed in the absence of compound **11**. Surprisingly the rate of UMP phosphorylation in the presence of compound **12** dropped dramatically, to about 7% of the rate observed in the absence of compound **12**. The enzyme kinetics of compound **12** suggested that it may be a competitive or noncompetitive inhibitor of NMP kinase.[28]

All four phosphonic acids also were tested for their toxicity to the human-derived leukemia cell line K562 by measurement of ^{3}H-thymidine incorporation in precipitable DNA from cells treated with various concentrations of the four phosphonic acids.[29] Of these four compounds, phosphonic acid **11** showed the lowest LC_{50} value at 0.5 μM (Table 3). Moreover, comparison of the LC_{50} values of the cytidine-derived phosphonic acids **11** and **12** and the ara-C-derived phosphonic acids **26** and **31** revealed that the configuration at C-5′ is important in their effect on the K562 cell line. The phosphonic acids with the 5′*R* configuration both showed lower LC_{50} values than those with the 5′*S* configuration, with a much greater than 20-fold difference in the toxicities of diastereomers **11** and **12** in the K562 cell line.

Summary

The 5′-hydroxy-5′-phosphonate derivatives of ara-C and cytidine can be prepared stereoselectively in high yield through nucleophilic addition of phosphite or $TiCl_4$-mediated hydrophosphonylation. The configuration of the newly generated stereocenter at C-5′ can be assigned based on NMR analysis of *O*-methyl mandelate ester derivatives of the cytidine phosphonates, and the NMR data of the ara-C phosphonic acids. Because the 5′ configuration appears to have a significant impact on the bioactivity of these phosphonates, future studies on structure−activity relationships will require stereoselective synthesis.

Experimental Section

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Pyridine, CH_2Cl_2, and Et_3N were distilled from CaH_2 prior to use. All reactions were conducted in oven-dried glassware under an atmosphere of argon with magnetic stirring. Silica gel 60 (particle size 40−63 μm) was employed for flash chromatography. Standard ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, unless otherwise noted, with $CDCl_3$ as solvent and $(CH_3)_4Si$ (^{1}H, 0.0 ppm) or $CDCl_3$ (^{13}C, 77.2 ppm) as internal standards. ^{31}P NMR chemical shifts are recorded in ppm relative to 85% H_3PO_4 (external standard). High-resolution ESI mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Elemental analyses were performed by Atlantic Microlab, Inc.

Diethyl [1′-(5′(*R*)-Hydroxy-2′,3′-*O*-isopropylidene-β-D-ribo-penta-1′,4′-furanosyl)-N^4-acetylcytosyl]-5′-phosphonate (9) and Diethyl [1′-(5′(*S*)-Hydroxy-2′,3′-*O*-isopropylidene-β-D-ribo-penta-1′,4′-furanosyl)-N^4-acetylcytosyl]-5′-phosphonate (10). DMSO (3 mL) was added to alcohol **7**[7] (650 mg, 2.0 mmol) and EDC (1.19 g, 6.2 mmol) suspended in benzene (6 mL). Pyridine (0.24 mL, 3.0 mmol) and then TFA (0.19 mL, 2.5 mmol) were added, and the reaction mixture was stirred at room temperature for 4 h. The reaction was diluted by addition of ethyl acetate (100 mL), the organic layer was washed with cold, dilute acid (pH 3), and the aqueous layer was extracted with ethyl acetate. The combined organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo to give crude aldehyde **8**.

To a solution of diethyl phosphite (0.75 mL, 8.2 mmol) in THF (10 mL) at −78 °C was added dropwise via syringe LHMDS (8 mL, 1.0M in THF). After 15 min, a solution of aldehyde **8** in THF (10 mL) was added and the reaction mixture was alowed to stir at −78 °C for 2 h. The reaction was quenched by slow addition of acetic acid in diethyl ether and the resulting mixture was filtered through Celite. After the filtrate was concentrated in vacuo, the residue was purified by flash chromatography using a gradient solvent system (MeOH/EtOAc) to give recovered alcohol **7** (132 mg, 0.4 mmol, 20%) and an epimeric 3:1 mixture of α-hydroxy phosphonates **9** and **10** (434 mg, 59% yield from **7** to **9** and **10** based on recovered alcohol **7**) as white solids, which were separated by RP-HPLC (H_2O−25% CH_3CN/H_2O, 5 min; 25% CH_3CN/H_2O, 25 min; C_{18} column i.d. 21.4 mm, UV detection at 325 nm).

For compound **9**: ^{1}H NMR δ 10.26 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 5.76 (d, J = 1.8 Hz, 1H), 5.21 (dd, J = 6.4, 1.8 Hz, 1H), 5.05 (dd, J = 6.4, 2.5 Hz, 1H), 4.65 (m, 1H), 4.37 (dd, J = 9.9, 2.2 Hz, 1H), 4.26−4.14 (m, 4H), 2.26 (s, 3H), 1.58 (s, 3H), 1.34 (m, 9H); ^{13}C NMR δ 171.4, 163.5, 155.5, 147.3, 113.8, 97.1 (d, J_{CP} = 3.6 Hz), 97.1, 87.6 (d, J_{CP} = 10.4 Hz), 84.8, 80.5, 68.6 (d, J_{CP} = 161.7 Hz), 63.4 (d, J_{CP} = 7.2 Hz), 63.2 (d, J_{CP} = 7.1 Hz), 27.3, 25.3, 25.1, 16.6, 16.5; ^{31}P NMR δ 21.61; HRMS (ESI$^+$) m/z calcd for $C_{18}H_{29}N_3O_9P$ $(M + H)^+$ 462.1641, found 462.1651.

For compound **10**: ^{1}H NMR δ 9.48 (s, 1H), 7.85 (d, J = 7.4 Hz, 1H), 7.40 (d, J = 7.4 Hz, 1H), 5.61 (d, J = 2.0 Hz, 1H), 5.19 (dd, J = 6.4, 3.6 Hz, 1H), 5.15 (dd, J = 6.4, 2.0 Hz, 1H), 4.63 (m, 1H), 4.23−4.10 (m, 5H), 2.26 (s, 3H), 1.57 (s, 3H), 1.35 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H); ^{13}C NMR δ 170.9, 163.4, 155.4, 148.1, 114.7, 98.7, 97.1, 86.8, 83.7, 81.2 (d, J_{CP} = 13.7 Hz), 68.8 (d, J_{CP} = 163.9 Hz), 63.3 (d, J_{CP} = 6.8 Hz), 62.9 (d, J_{CP} = 7.2 Hz), 27.4, 25.5, 25.2, 16.7, 16.6; ^{31}P NMR δ 21.6. Anal. Calcd for $C_{18}H_{28}N_3O_9P$: C, 46.86; H, 6.12; N, 9.11. Found: C, 46.82; H, 6.19; N, 9.07.

(*S*)-*O*-Methyl Mandelate Ester 13. To a solution of phosphonates **9** (22 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) were added EDC (14 mg, 0.08 mmol) and DMAP (3 mg, 0.02 mmol) at room temperature. (*S*)-(+)-*O*-Methyl mandelic acid (10 mg, 0.06 mmol) was added and the reaction was monitored by TLC. After 5 min at room temperature, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layers were combined and dried over $MgSO_4$, and then concentrated in vacuo. Flash chromatography of the residue with gradient elution (MeOH in EtOAc) gave the *O*-methyl mandelic acid ester **13** (18 mg) in 82% yield based on recovered

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compound **9** (5 mg). For compound **13**: ^{1}H NMR δ 10.04 (s, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.46 (m, 2H), 7.36 (m, 4H), 5.81 (d, J = 2.5 Hz, 1H), 5.70 (dd, J = 10.2, 5.0 Hz, 1H), 4.99 (dd, J = 6.7, 4.0 Hz, 1H), 4.87 (s, 1H), 4.85 (dd, J = 6.7, 2.5 Hz, 1H), 4.50 (ddd, J = 9.7, 5.0, 4.0 Hz, 1H), 4.00−3.64 (m, 4H), 3.43 (s, 3H), 2.30 (s, 3H), 1.56 (s, 3H), 1.32 (s, 3H), 1.14 (t, J = 7.2 Hz, 3H), 1.08 (t, J = 7.4 Hz, 3H); ^{13}C NMR δ 171.3, 169.2 (d, J_{CP} = 3.8 Hz), 163.6, 154.9, 146.4, 135.7, 129.2, 128.8 (2C), 127.7 (2C), 115.0, 97.3, 94.9, 85.6, 84.6, 82.4, 81.4 (d, J_{CP} = 5.9 Hz), 68.1 (d, J_{CP} = 164.4 Hz), 63.3 (d, J_{CP} = 7.2 Hz), 63.1 (d, J_{CP} = 6.4 Hz), 57.6, 27.3, 25.4, 25.2, 16.4 (d, J_{CP} = 4.1 Hz), 16.3 (d, J_{CP} = 4.1 Hz); ^{31}P NMR δ 15.9. Anal. Calcd for $C_{27}H_{36}N_3O_{11}P$: C, 53.20; H, 5.95; N, 6.89. Found: C, 53.00; H, 6.16; N, 6.62.

***(S)*-*O*-Methyl Mandelate Ester 14.** A solution of epimeric 5′-hydroxy phosphonates **9** and **10** (3:1, 143 mg, 0.31 mmol) in CH_2Cl_2 was treated with EDC (61 mg, 0.32 mmol), DMAP (11 mg, 0.09 mmol), and (*S*)-(+)-*O*-methyl mandelic acid (41 mg, 0.25 mmol), as described above for compound **13**, to obtain the *O*-methyl mandelic acid esters **13** (34 mg) and **14** (35 mg) in a total yield of 79% based on recovered starting materials **9** and **10** (77 mg, 54%). For compound **14**: ^{1}H NMR δ 10.25 (s, 1H), 8.26 (d, J = 7.5 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.30 (m, 3H), 7.23 (m, 2H), 5.72 (d, J = 2.0 Hz, 1H), 5.61 (dd, J = 13.4, 3.2 Hz, 1H), 4.73 (s, 1H), 4.72 (m, 1H), 4.27−4.10 (m, 4H), 4.02 (dd, J = 6.5, 2.8 Hz, 1H), 3.72 (dd, J = 6.5, 2.0 Hz, 1H), 3.36 (s, 3H), 2.35 (s, 3H), 1.49 (s, 3H), 1.32 (m, 6H), 1.17 (s, 3H); ^{13}C NMR δ 172.0, 169.6 (d, J_{CP} = 3.6 Hz), 163.9, 155.6, 146.5, 136.0, 130.3, 129.8 (2C), 128.1 (2C), 114.7, 96.8, 95.8, 86.7, 86.0, 82.8, 81.5 (d, J_{CP} = 10.9 Hz), 68.9 (d, J_{CP} = 168.1 Hz), 64.1 (d, J_{CP} = 7.8 Hz), 64.0 (d, J_{CP} = 6.7 Hz), 57.8, 27.4, 25.8, 25.4, 17.1 (d, J_{CP} = 6.0 Hz), 17.0 (d, J_{CP} = 6.1 Hz); ^{31}P NMR δ 16.2. Anal. Calcd for $C_{27}H_{36}N_3O_{11}P$: C, 53.20; H, 5.95; N, 6.89. Found: C, 52.94; H, 5.98; N, 6.82.

[1′-(5′(*R*)-Hydroxy-β-D-ribo-penta-1′,4′-furanosyl)cytosyl]-5′-phosphonic Acid (11). To a stirred solution of phosphonate **9** (75 mg, 0.16 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added TMSBr (0.65 mL, 4.92 mmol) dropwise via syringe and then the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred at room temperature for 2 days, and then the volatiles were removed by rotary evaporation. The residue was coevaporated with MeOH three times and then washed with diethyl ether and ethyl acetate to give a dark yellow solid. This solid was dissolved in water and coevaporated with water three times to provide a white solid that precipitated from cold water to afford a white solid (49 mg, 93%). This white solid was dissolved in 100 mM ammonium bicarbonate (1.5 mL) and lyophilized to give a white solid. This material was dissolved in distilled water and lyphoilized, and the process was repeated twice to give the phosphonic acid **11**: ^{1}H NMR (D_2O, DSS standard) δ 8.24 (d, J = 7.9 Hz, 1H), 6.25 (d, J = 7.9 Hz, 1H), 5.95 (d, J = 5.2 Hz, 1H), 4.45 (m, 2H), 4.37 (dd, J = 5.1 Hz, 1H), 4.14 (dd, J = 12.5, 2.5 Hz, 1H); ^{13}C NMR (D_2O) δ 162.1, 151.5, 147.2, 98.1, 91.6, 88.3 (d, J_{CP} = 13.2 Hz), 77.6, 71.7, 71.5 (d, J_{CP} = 152.6 Hz); ^{31}P NMR (D_2O) δ 16.2; HRMS (ESI) *m*/*z* calcd for $C_9H_{13}N_3O_8P$ $(M - H)^-$ 322.0440, found 322.0427.

1-(2′, 3′-Di-*O*-*tert*-butyldimethylsilyl-β-D-ribo-penta-1′, 4′-furanosyl)-*N*^{4}-acetylcytosine (17). To a solution of protected cytidine **16**[16] (9.86 g, 20.9 mmol) at reflux in MeOH (200 mL) was added Ac_2O (29.0 mL, 307 mmol) dropwise in 5 batches over 1 h. The reaction mixture was heated for 3 h, allowed to cool to room temperature, and then stirred overnight. The volatile materials were removed in vacuo and the residue was partitioned between EtOAc and water. The aqueous phase was neutralized by $NaHCO_3$ and extracted with EtOAc. The combined EtOAc extract was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (MeOH gradient in CH_2Cl_2) to afford alcohol **17** (8.08 g, 75%) as a white solid, along with recovered compound **16** (0.52 g, 5%). For the alcohol **17**: ^{1}H NMR δ 9.89 (br s, 1H), 8.08 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 7.4 Hz, 1H), 5.45 (d, J = 4.3 Hz, 1H), 4.68 (dd, J = 4.3, 4.3 Hz, 1H), 4.19 (dd, J = 4.5, 4.3 Hz, 1H), 4.15 (m, 1H), 4.00 (br d, J = 11.8 Hz, 1H), 3.72 (br d, J = 12.2 Hz, 1H), 3.70 (br s, 1H, exchanges with CD_3OD), 2.29 (s, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.08 (s, 6H), 0.06 (s, 3H), 0.03 (s, 3H);^{13}C NMR δ171.4, 163.2, 155.4, 147.9, 96.9, 96.2, 86.3, 73.5, 71.4, 61.5, 26.0 (6C), 25.2, 18.3, 18.2, −4.2, −4.5, −4.6, −4.7. Anal. Calcd for $C_{23}H_{43}N_3O_6Si_2$: C, 53.77; H, 8.44; N, 8.18. Found: C, 53.40; H, 8.59; N, 8.09.

Diethyl [1′-(5′(*S*)-Hydroxy-2′,3′-di-*O*-*tert*-butyldimethylsilyl-β-D-ribo-penta-1′,4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (20). DMSO (15 mL) was added to alcohol **17** (2.01 g, 3.9 mmol) and EDC (2.25 g, 11.7 mmol) suspended in toluene (15 mL). Pyridine (0.48 mL, 5.9 mmol) and then TFA (0.39 mL, 5.0 mmol) were added, and the reaction mixture was allowed to stirr at room temperature for 6 h. After the reaction was diluted by addition of diethyl ether (100 mL) and washed with dilute acid (pH 3), the aqueous layer was extracted with ether, and the combined organic layer was dried ($MgSO_4$) and concentrated in vacuo to give the crude aldehyde **18** (2.10 g).

To a stirred solution of diethyl phosphite (0.35 mL, 3.8 mmol) in THF (15 mL) at −78 °C was added LHMDS (1 M THF solution, 3.6 mL) dropwise. After 15 min, a solution of aldehyde **18** (925 mg, 1.8 mmol) in THF (5 mL) was added via cannula. The reaction mixture was stirred at −78 °C for 2 h, then allowed to warm to −20 °C and quenched by addition of acetic acid in ether. The reaction mixture was first partitioned between ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate. The combined ethyl acetate layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography with EtOAc to give **20** (403 mg), **19** (91 mg), and mixture of **20** and **19** (105.8 mg) in a total 54% yield.

For compound **20**: ^{1}H NMR δ 10.21 (s, 1H), 8.34 (d, J = 7.4 Hz, 1H), 7.42 (d, J = 7.4 Hz, 1H), 5.60 (d, J = 3.6 Hz, 1H), 4.51 (br t, J = 3.0 Hz, 1H), 4.43 (br d, J = 4.5 Hz, 1H), 4.27−4.07 (m, 6H), 2.30 (s, 3H), 1.34 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.2 Hz, 3H), 0.89 (s, 18H), 0.10 (s, 3H), 0.08 (s, 6H), 0.07 (s, 3H); ^{13}C NMR δ 171.5, 163.3, 155.3, 147.2, 96.7, 94.6, 83.7, 73.8, 71.5 (d, J_{CP} = 13.1 Hz), 76.1 (d, J_{CP} = 163.1 Hz), 63.7 (d, J_{CP} = 7.5 Hz), 62.8 (d, J_{CP} = 7.5 Hz), 26.0 (6C), 25.1, 18.2, 18.2, 16.7 (d, J_{CP} = 5.2 Hz), 16.6 (d, J_{CP} = 5.7 Hz), −4.2, −4.3, −4.8 (2C); ^{31}P NMR δ 21.5; HRMS (ESI$^+$) *m*/*z* calcd for $C_{27}H_{53}N_3O_9PSi_2$ $(M + H)^+$ 650.3058, found 650.3063.

Diethyl [1′-(5′(*S*)-Hydroxy-β-D-ribo-penta-1′,4′-furanosyl)-*N*^{4}-acetyl cytosyl]-5′-phosphonate (21). TBAF (1 M solution in THF, 2.75 mL, 2.75 mmol) was added to a solution of phosphonate **20** (357 mg, 0.55 mmol) in THF (5 mL) at room temperature. After the solution was stirred for 4 h, the solvent was removed in vacuo and the residue was purified by flash chromatography (15% MeOH in EtOAc) to give compound **21** (189 mg, 82%) as a white solid: ^{1}H NMR (CD_3OD) δ 8.70 (d, J = 7.4 Hz, 1H), 7.41 (d, J = 7.4 Hz, 1H), 5.87 (d, J = 1.7 Hz, 1H), 4.39 (m, 1H), 4.25−4.15 (m, 7H), 2.18 (s, 3H), 1.35 (t, J = 6.9 Hz, 3H), 1.31 (t, J = 6.9 Hz, 3H); ^{13}C NMR (CD_3OD) δ 173.2, 164.5, 158.1, 147.0, 97.8, 93.8, 84.6, 76.0, 70.6 (d, J_{CP} = 12.1 Hz), 67.3 (d, J_{CP} = 168.7 Hz), 64.6 (d, J_{CP} = 7.2 Hz), 64.2 (d, J_{CP} = 6.6 Hz), 24.7, 17.0, 16.9; ^{31}P NMR (CD_3OD) δ 23.3; HRMS (ESI$^+$) *m*/*z* calcd for $C_{15}H_{25}N_3O_9P$ $(M + H)^+$ 422.1328, found 422.1330.

[1′-(5′(*S*)-Hydroxy-β-D-ribo-penta-1′,4′-furanosyl)cytosyl]-5′-phosphonic Acid (12). To a stirred solution of phosphonate **21** (162 mg, 0.38 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added TMSBr (0.76 mL, 5.8 mmol) dropwise via syringe. The reaction mixture was allowed to warm to room temperature and stirred for 24 h, and then the volatiles were removed by rotary evaporation. The residue was dissolved in MeOH, stirred for 40 min, and then concentrated in vacuo. The residue was coevaporated with MeOH three times. The residue was dissolved in a minimum of MeOH and then precipitated by addition of EtOAc, and this process was repeated three times.

The resulting white solid was dissolved in 50 mM NH_4HCO_3 and lyophilized. The phosphonic acid can be generated from the ammonium salt by lyophilizing from water twice to give a white solid **12** (112 mg, 90%): ^{1}H NMR (D_2O, DSS standard) δ 8.01 (d, J = 7.7 Hz, 1H), 6.04 (d, J = 7.6 Hz, 1H), 5.85 (d, J = 3.1 Hz, 1H), 4.34−4.28 (m, 3H), 3.94 (dd, J = 12.6, 3.4 Hz, 1H); ^{13}C NMR (D_2O) δ 168.9, 160.2, 145.1, 98.7, 93.6, 86.4, 77.1, 72.7 (d, J = 7.2 Hz), 71.3 (d, J = 152.3 Hz); ^{31}P NMR δ 16.2; HRMS (ESI$^+$) m/z calcd for $C_9H_{15}N_3O_8P$ $(M + H)^+$ 324.0597, found 324.0590.

Diethyl [1′-(5′(*R*)-Hydroxy-2′,3′-di-*O*-*tert*-butyldimethylsilyl-β-D-arabino- penta-1′,4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (24). DMSO (10 mL) was added to alcohol **22**[7] (2.05 g, 4.0 mmol) and EDC (2.30 g, 12.0 mmol) suspended in benzene (10 mL). After pyridine (0.48 mL, 6.0 mmol) and TFA (0.40 mL, 5.2 mmol) were added, the reaction mixture was allowed to stir at room temperature for 6 h. The reaction was diluted by addition of diethyl ether (100 mL) and extracted with cold water, and the aqueous layer was extracted with ether. The combined organic layers were dried ($MgSO_4$) and then concentrated in vacuo to give crude aldehyde **23** (2.23 g).

To a solution of diethyl phosphite (0.77 mL, 8.4 mmol) in THF (30 mL) at −78 °C was added dropwise via syringe LHMDS (8 mL, 1.0 M in THF). After 15 min, a solution of crude aldehyde **23** (2.21 g) in THF (10 mL) was added and the reaction mixture was allowed to stir at −78 °C for 3 h. The reaction then was quenched by slow addition of acetic acid in diethyl ether and the resulting mixture was partitioned between EtOAc and water. After the aqueous phase was extracted with EtOAc, the combined organic phase was dried ($MgSO_4$) and then concentrated in vacuo. The residue was purified by flash chromatography (EtOAc in hexane gradient) to give the 5′-hydroxy phosphonate **24** as a white solid (2.33 g, 91%): ^{1}H NMR (CD_3OD) δ 8.52 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 6.15 (d, J = 2.7 Hz, 1H), 4.43 (s, 1H), 4.32 (d, J = 2.7 Hz, 1H), 4.29−4.07 (m, 6H), 2.17 (s, 3H), 1.37 (t, J = 7.1 Hz, 6H), 0.95 (s, 9H), 0.79 (s, 9H), 0.19 (s, 3H), 0.18 (s, 3H), 0.08 (s, 3H), −0.19 (s, 3H); ^{13}C NMR (CD_3OD) δ 173.2, 164.5, 157.6, 148.8, 97.2, 91.4, 88.2, 80.6 (d, J_{CP} = 13.8 Hz), 77.1, 68.5 (d, J_{CP} = 162.1 Hz), 64.9 (d, J_{CP} = 7.4 Hz), 64.0 (d, J_{CP} = 7.0 Hz), 26.36 (3C), 26.33 (3C), 24.6, 18.9, 18.7, 17.1, 17.0, −4.30, −4.37, −4.56, −5.21; ^{31}P NMR (CD_3OD) δ 23.9. Anal. Calcd for $C_{27}H_{52}N_3O_9Si_2P$: C, 49.90; H, 8.07; N, 6.47. Found: C, 49.69; H, 8.10; N, 6.55.

Diethyl [1′-(5′(*R*)-Hydroxy-β-D-arabino-penta-1′,4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (25). Phosphonate **24** (742 mg, 1.14 mmol) was dissolved in THF (20 mL) at room temperature and TBAF (5.5 mL, 1.0 M in THF) was added. After 4 h, the solvent was removed in vacuo, and the residue was purified by flash chromatography (MeOH in EtOAc gradient) to obtain pure phosphonate **25** (277 mg, 64%): ^{1}H NMR (CD_3OD) δ 8.41 (d, J = 7.5 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 6.21 (d, J = 3.1 Hz, 1H), 4.44 (s, 1H), 4.29−4.20 (m, 7H), 2.18 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.34 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD) δ 173.2, 164.4, 157.9, 148.5, 97.0, 90.6, 87.0 (d, J_{CP} = 5.6 Hz), 78.4 (d, J_{CP} = 9.4), 75.9, 68.5 (d, J_{CP} = 164.8 Hz), 64.9 (d, J_{CP} = 7.4 Hz), 64.2 (d, J_{CP} = 7.8 Hz), 24.7, 17.0, 17.0; ^{31}P NMR (CD_3OD) δ 23.8; HRMS (ESI$^+$) m/z calcd for $C_{15}H_{25}N_3O_9P$ $(M + H)^+$ 422.1328, found 422.1330. Anal. Calcd for $C_{15}H_{24}N_3O_9P$: C, 42.74; H, 5.74; N, 9.98. Found: C, 42.97; H, 5.88; N, 10.01.

[1′-(5′(*R*)-Hydroxy-β-D-arabino-penta-1′,4′-furanosyl)-cytosyl]-5′-phosphonic Acid (26). To a stirred solution of phosphonate **25** (155 mg, 0.4 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added TMSBr (1.2 mL, 9.3 mmol) dropwise via syringe. The reaction mixture was stirred at room temperature for 1 days, and then concentrated. The residue was coevaporated with MeOH three times and then washed with diethyl ether and ethyl acetate to give a dark yellow solid. The solid was dissolved in a minimum of MeOH and precipitated by addition of EtOAc. After three repetitions of this process, the resulting solid was dissolved in NH_4HCO_3 (50 mM) and lyophylized. The resulting ammonium salt was lyophylized from water twice to give the phosphonic acid **26** as a white solid (94 mg, 78%): ^{1}H NMR (D_2O, DSS standard) δ 8.04 (d, J = 7.5 Hz, 1H), 6.18 (d, J = 4.3 Hz, 1H), 6.07 (d, J = 7.5 Hz, 1H), 4.43 (m, 2H), 4.22 (m, 1H), 4.06 (dd, J = 12.8, 3.0 Hz, 1H); ^{13}C NMR (D_2O) δ 168.1, 159.0, 146.1, 98.1, 88.2, 86.1 (d, J_{CP} = 11.6 Hz), 78.2, 76.6, 71.2 (d, J_{CP} = 151.9 Hz); ^{31}P NMR (D_2O) δ 15.8; HRMS (ESI) m/z calcd for $C_9H_{13}N_3O_8P$ $(M - H)^-$ 322.0440, found 322.0430.

Diethyl [1′-(5′(*S*)-Hydroxy-2′,3′-di-*O*-*tert*-butyldimethylsilyl-β-D-arabino-penta-1′,4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (29) and Phosphonate 24. To a solution of the unpurified aldehyde **23** (102 mg, 0.2 mmol) in CH_2Cl_2 at −78 °C was added $TiCl_4$ (1 M solution in CH_2Cl_2, 0.4 mL). After 15 min a solution of trimethylsilyl phosphite (0.09 mL) in CH_2Cl_2 was added dropwise. The reaction mixture was stirred at −78 °C for 3 h, and then allowed to warm to room temperature and stirred overnight. The reaction mixture then was partitioned between EtOAc and saturated NH_4Cl, and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by column chromatography (first 60% EtOAc in hexane and then 1% MeOH in EtOAc) to give recovered aldehyde **23** (7 mg, 7%) and a 1:2 mixture of phosphonates **24** and **29** (61 mg, 50% based on recovered aldehyde **23**).

Diethyl [1′-(5′(*S*)-Hydroxy-β-D-arabinopenta-1′,4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (30). TBAF (1 M solution in THF, 2.75 mL) was added to a mixture of phosphonates **24** and **29** (650 mg, 1.28 mmol) in THF (8 mL) at room temperature. After the reaction was stirred for 5 h, the solvent was removed under vacuum and the residue was purified by flash chromatography (15% MeOH in EtOAc) to give compounds **25** (171 mg), **30** (157 mg), and a mixture of **25** and **30** (107 mg) as a white solid in a total yield of 81%. For compound **30**: ^{1}H NMR (CD_3OD) δ 8.44 (d, J = 7.5 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 6.22 (d, J = 3.8 Hz, 1H), 4.32 (m, 2H), 4.22 (m, 6H), 2.18 (s, 3H), 1.37 (t, J = 6.9 Hz, 3H), 1.34 (t, J = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD) δ 173.2, 164.4, 158.1, 148.1, 97.2, 89.6, 85.7 (d, J_{CP} = 2.0 Hz), 77.9 (d, J_{CP} = 9.5 Hz), 76.4, 68.2 (d, J_{CP} = 167.2 Hz), 64.7 (d, J_{CP} = 6.6 Hz), 64.3 (d, J_{CP} = 6.6 Hz), 24.7, 17.0 (d, J_{CP} = 3.4 Hz), 16.9 (d, J_{CP} = 2.8 Hz); ^{31}P NMR (CD_3OD) δ 21.1; HRMS (ESI$^+$) m/z calcd for $C_{15}H_{25}N_3O_9P$ $(M + H)^+$ 422.1328, found 422.1335.

[1′-5′(*S*)-Hydroxy-β-D-arabino-penta-1′,4′-furanosyl)cytosyl]-5′-phosphonic Acid (31). To a stirred solution of phosphonate **30** (149 mg, 0.35 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added TMSBr (0.70 mL, 5.29 mmol) dropwise via syringe. The reaction mixture was allowed to warm to room temperature and stirred for 24 h, and then the solvent was removed by rotary evaporation. The residue was dissolved in MeOH, stirred for 40 min, and then concentrated in vacuo. The residue was coevaporated with MeOH three times and then precipitated from a minimum amount of MeOH by addition of EtOAc. After this process was repeated three times, the resulting white solid was dissolved in 50 mM NH_4HCO_3 and freeze-dried. The phosphonic acid was generated from the ammonium salt by lyophilizing from water twice to give a white solid **31** (113 mg, 100%): ^{1}H NMR (D_2O, DSS standard) δ 8.18 (d, J = 7.8 Hz, 1H), 6.16 (d, J = 5.3 Hz, 2H), 4.46 (dd, J = 5.2, 5.2 Hz, 1H), 4.27 (dd, J = 5.9, 5.5 Hz, 1H), 4.19 (m, 1H), 4.02 (dd, J = 12.4, 3.5 Hz, 1H); ^{13}C NMR (D_2O) δ 164.0, 153.7, 147.8, 97.5, 88.5, 85.5, 78.0, 77.7 (d, J_{CP} = 7.4 Hz), 70.2 (d, J_{CP} = 156.2 Hz); ^{31}P NMR (D_2O) δ 16.4; HRMS (ESI$^+$) m/z calcd for $C_9H_{15}N_3O_8P$ $(M + H)^+$ 324.0597, found 324.0601.

Diethyl [1′-(5′-Keto-2′,3′-di-*O*-*tert*-butyldimethylsilyl-β-D-arabino-penta-1′, 4′-furanosyl)-*N*^{4}-acetylcytosyl]-5′-phosphonate (27). To PDC (132 mg, 0.35 mmol) and Ac_2O (0.14 mL, 1.5 mmol) in refluxing CH_2Cl_2 (6 mL) was added a solution of **24** (325 mg, 0.5 mmol) in CH_2Cl_2 (2 mL) via

cannula. After 3 h, the reaction mixture was allowed to cool to room temperature, diluted by addition of EtOAc, and filtered through a pad of silica gel and $MgSO_4$. The filtrate was concentrated in vacuo and purified by flash chromatography (EtOAc gradient in hexane) to obtain recovered **24** (39 mg, 12%) and the acyl phosphonate **27** (145 mg, 45%, accompanied by diethyl phosphite in a ratio of 1:1 based on ^{31}P NMR) as a sticky solid. This material was used in subsequent experiments without further purification. For compound **27**: ^1H NMR δ 10.05 (br s, 1H), 8.96 (d, $J = 7.5$ Hz, 1H), 7.48 (d, $J = 7.5$ Hz, 1H), 6.41 (d, $J = 2.5$ Hz, 1H), 4.87 (s, 1H), 4.79 (d, $J = 3.6$ Hz, 1H), 4.34−4.10 (m, 5H), 2.26 (s, 3H), 1.39 (t, $J = 7.1$ Hz, 3H), 1.36 (t, $J = 7.1$ Hz, 3H), 0.92 (s, 9H), 0.71 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H), −0.03 (s, 3H), −0.25 (s, 3H); ^{13}C NMR δ 205.4 (d, $J_{CP} = 168.4$ Hz), 170.9, 162.7, 154.8, 149.2, 95.7, 92.3 (d, $J_{CP} = 63.9$ Hz), 89.9, 79.2, 75.0, 64.6 (d, $J_{CP} = 8.0$ Hz), 64.2 (d, $J_{CP} = 7.4$ Hz), 25.8 (3C), 25.7 (3C), 25.0, 18.0, 17.8, 16.6 (d, $J_{CP} = 5.0$ Hz), 16.6 (d, $J_{CP} = 5.0$ Hz), −4.46, −4.74, −5.27, −5.42; ^{31}P NMR δ 7.35 (diethyl phosphite), −2.70; HRMS (ESI^+) *m*/*z* calcd for $C_{27}H_{51}N_3O_9Si_2P$ $(M + H)^+$ 648.2902, found 648.2908.

Cell Culture. K562 cells were grown in RPMI-1640 media supplemented with 10% heat inactivated fetal calf serum. Cells were maintained in T-75 vented culture flasks at 37 °C in humidified 5% CO_2 atmosphere. Cell viability was determined using trypan blue exclusion by incubating cells for 5 min with a trypan blue solution (1:1 0.4 mg/dL) and using a hemocytometer to count viable (clear) and nonviable (blue) cells.

[^{3}H]-Thymidine Incorporation. K562 cells were incubated in 96-well round-bottom plates and treated with varying concentrations of the test phosphonates. After 20 h, 20 μL of [^{3}H]-thymidine (0.1385 TBq/mmol, 3.75 Ci/mmol in media) was added to each well. After 24 h, the cells were filtered through 934AH filter paper (Reeves Angel) using a Brandel harvester. Filters were washed with water and dried overnight. The amount of ^{3}H-thymidine incorporated into cellular DNA was quantified by scintillation counting using 3A70 cocktail (Research Products).

NMP Kinase Assays. The conversion of phosphonates to monophosphate-phosphonates was measured with a method described previously.[27] The test phosphonates were added to a mixture of NMP kinase (0.015 units, Sigma), pyruvate kinase (35 units, Sigma), and lactic dehydrogenase (50 units, Sigma) in 1 mL reaction mixture (Triethanolamine (0.07 M), KCl (65 mM), $MgSO_4$ (16 mM), ATP (1.7 mM), PEP (1.2 mM), NADH (0.36 μM), pH 7.6). Control reactions contained UMP (0.87 mM) as substrate. The mixture was allowed to reach equilibrium, and the reactions were started by the addition of either the test phosphonates or UMP. A diode array spectrophotometer was used to follow the decrease in absorbance at 340 nm. The oxidation of NADH, and subsequent decline in absorbance at 340 nm, reflected the rate of phosphorylation either of UMP or of the synthetic phosphonate.

Enzyme Inhibition. The inhibition of NMP kinase by the test phosphonates was measured with the basic system as described above with slight modification. UMP (0.87 mM) was added to all the reaction mixtures to serve as the substrate. The test phosphonate was added in varying concentrations. The reactions were initiated by the addition of ATP. The oxidation of NADH, and subsequent decline in absorbance at 340 nm, represented the phosphorylation of UMP, and the reduction in the rate of decline was proportional to the concentration and inhibitory effect of the test phosphonates.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **9**, **11**, **12**, **20**, **21**, **26**, **27**, **30**, and **31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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