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Synthesis of 5′-Amino-5′-phosphonate Analogues of Pyrimidine Nucleoside Monophosphates

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The 5′-amino-5′-phosphono derivatives of cytidine, cytosine arabinoside (ara-C), and uridine have been prepared via the corresponding nucleoside aldehydes. Phosphite addition to imines derived from the nucleoside aldehydes and *p*-methoxybenzylamine was efficient, and use of this amine allowed cleavage of the products to the parent amino phosphonic acids. The phosphite additions proved to be diastereoselective, with the cytidine and uridine derivatives favoring the 5′S stereochemistry and the ara-C derivative favoring the 5′R isomer. The stereochemistry of one cytidine derivative was established by single-crystal diffraction analysis, and detailed comparisons of the ^{13}C NMR data allowed assignments of the other amino phosphonates.

Cytosine arabinoside (**1**, ara-C, Figure 1) is an effective agent for treatment of myelogenous and other leukemias. Unfortunately remissions obtained through ara-C treatments tend to be short-lived, and ultimately patients relapse with highly resistant disease refractory to all subsequent forms of therapy.[1] We have prepared ara-C hydroxy phosphonates (e.g., **2** and **3**) designed to mimic ara-C monophosphate (ara-CMP, **4**) and perhaps circumvent resistance on the basis of current understanding of its metabolism.[2] Formal replacement of the hydroxyl group in a 5′-hydroxy-5′-phosphonate with an amino functionality would create a new series of nucleoside derivatives, 5′-amino-5′-phosphonates (**5**), which also could serve as analogues of ara-CMP. Because the amino group is inductively electron-withdrawing, the nucleoside α-amino phosphonates might be expected to have similar advantages to the α-hydroxy phosphonates in structure, acidity, and metabolic stability. In addition, the basicity of the amino group may lead to a zwitterionic structure at physiological pH and partially balance the negative charge of the phosphonic acid.

Although there are no reports on synthesis and bioactivity of nucleoside α-amino phosphonates, α-amino phosphonic acids are commonly viewed as analogues of the natural amino acids,[3] and an interesting spectrum of biological activities has been observed in peptidomimics that incorporate an α-amino phosphonic acid.[4] As a result, synthesis of phosphonate analogues of α-amino acids has been investigated extensively. The more general methods for preparation of chiral, nonracemic α-amino phosphonic acids include enantioselective addition of phosphite to achiral cyclic imines,[5] addition of phosphite to nonracemic chelating imines[3] (Figure 2, eq 1) and nitrones,[6] alkylation of phosphonamides with a stereogenic center either on the phosphonate ester[7a] or on an imine functionality,[7b] preparation from chiral, nonracemic α-hydroxy phosphonates via α-azidophosphonates[8]

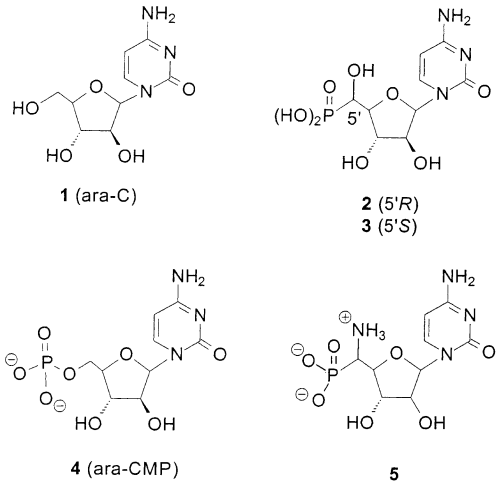

FIGURE 1.

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(6) De Risi, C.; Dondoni, A.; Perrone, D.; Pollini, G. P. *Tetrahedron Lett.* **2001**, *42*, 3033−3036.

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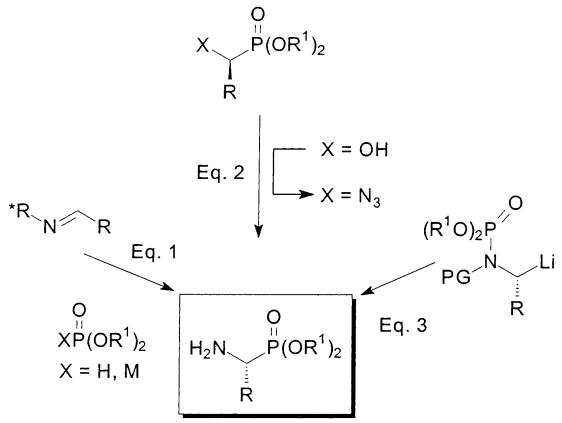
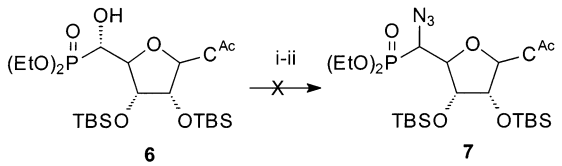


FIGURE 2.

SCHEME 1[a]



[a] (i) Tf_2O, pyr, CH_2Cl_2, −20 °C to rt; (ii) NaN_3, DMF, 70 °C.

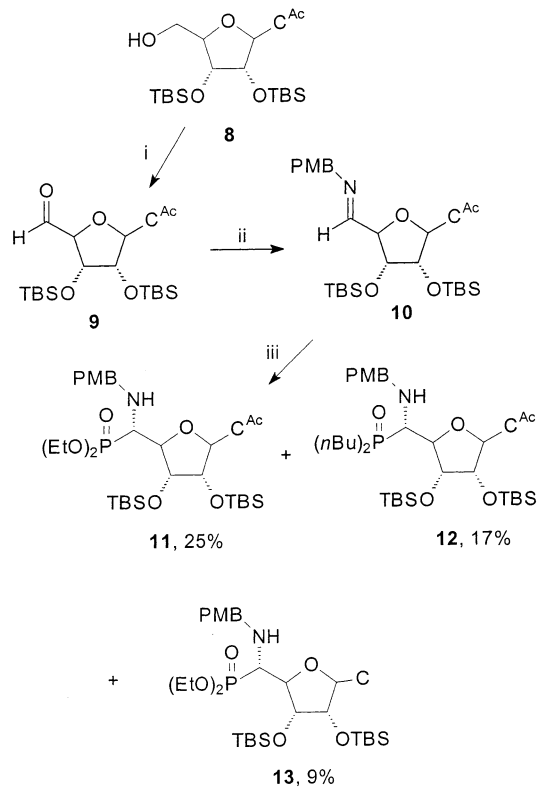
(eq 2), and phosphoramidate-amino phosphonate rearrangements[9] (eq 3).

The requirement for strong base to induce the phosphoramidate rearrangement was not attractive in the presence of the extensive functionality of the nucleoside system, and so this approach was not explored. Because the 5′-hydroxy phosphonates of cytidine and ara-C can be prepared in a stereoselective fashion,[2] the method described in eq 2 initially was most attractive. Preparation of 5′-azido phosphonate **7** was attempted via synthesis of the corresponding triflate from the 5′-hydroxy phosphonate **6** followed by nucleophilic displacement of triflate with NaN_3 (Scheme 1), but unfortunately the desired compound **7** was not obtained. When this attempted transformation of a 5′-hydroxy phosphonate to a 5′-azido phosphonate was not immediately successful, efforts to synthesize the 5′-amino phosphonate nucleosides were focused on addition of phosphite to nucleoside imines. Addition of the lithium salt of diethyl phosphite to nucleoside aldehydes readily affords α-hydroxy phosphonates, which also encouraged exploration of the reactivity of nucleoside imines with phosphite anion.

As reported by Smith,[3] a nonracemic imine prepared by condensation of a nonracemic amine with an aldehyde can give high stereocontrol to lithium-mediated phosphonylation of the corresponding imine. However, given the several stereogenic centers inherently contained in nucleoside aldehydes, in this case chirality in the amine may not be required for stereochemical control. Among achiral amines, 4-methoxybenzylamine ($PMBNH_2$) appeared to offer real advantage because well-studied PMB deprotection methods allow choices for removal of the PMB group in the later stage of the synthesis.

Condensation of $PMBNH_2$ with the nucleoside aldehyde **9**, prepared from the protected cytidine **8** (Scheme

SCHEME 2[a]



[a] (i) DMSO, EDC, pyr, TFA, benzene; (ii) $PMBNH_2$, benzene, reflux with Dean−Stark apparatus; (iii) *n*BuLi, $HP(O)(OEt)_2$, THF, 0 °C to rt.

2), initially was not as straightforward as precedents suggested.[10] In the presence of different dehydrating agents, such as freshly dried Na_2SO_4, $MgSO_4$, or 4 Å molecular sieves, the reaction of aldehyde **9** with $PMBNH_2$ at room temperature did not afford the desired nucleoside imine **10**. However, when the aldehyde **9** was treated with $PMBNH_2$ in benzene and the reaction mixture was heated at reflux with a Dean−Stark apparatus, condensation was apparent. After removal of the solvent, the ^1H NMR spectrum of the resulting residue showed a resonance at 7.9 ppm as a broad single peak corresponding to the imine hydrogen. Without further purification, this nucleoside imine (**10**) was treated with the lithium salt of diethyl phosphite prepared as described[3] by reaction of diethyl phosphite and *n*-butyllithium. In addition to the desired PMB-protected amino phosphonate **11** and the corresponding deacylated product **13**, compound **12** (^{31}P resonance at 51.5 ppm) also was obtained in a substantial amount and could not be separated readily from phosphonate **11**. Instead of pursuing purification and characterization at this stage, it was assumed that separation might be achieved more readily after removal of the PMB group and that a structure determination could be completed at that time.

Oxidative cleavage of a PMB group through reaction with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)[11] or ceric ammonium nitrate (CAN)[12] is attractive for depro-

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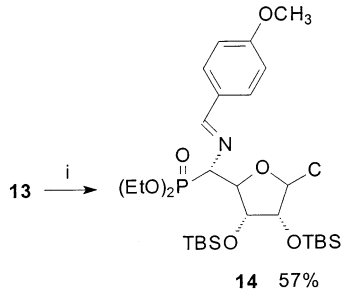
SCHEME 3[a]



[a] (i) DDQ, CH_2Cl_2/H_2O (20:1).

tection in the presence of the other aromatic moieties that may be labile to Pd-catalyzed hydrogenolysis. These two oxidants have been widely used for deprotection of PMB groups from alcohol,[11] tertiary amine,[13] and amide functionalities.[12] Reports of oxidative cleavage from secondary amines are more rare[14] and may require hydrolysis of an imine intermediate[14b] or addition of a reagent to trap the benzaldehyde byproduct.[14a]

As expected, attempted deprotection of secondary amine **13** through reaction with DDQ provided imine **14** instead of complete removal of the 4-methoxybenzyl group (Scheme 3). The mixture of phosphonate **11** and compound **12** also was treated with DDQ to give imines **15** and **16**, which could not be separated readily by flash chromatography (Scheme 4). Hydrolysis of these two conjugated imines led to a mixture of amino phosphonate **17** and compound **18** and the corresponding deacylated compounds **19** and **20**. The ^{1}H, ^{13}C (with ^{1}H decoupled, and with both ^{1}H and ^{31}P decoupled), and ^{31}P NMR spectra and the HR mass spectrum of compound **20** allowed its assignment as an α-amino phosphine oxide derivative of cytidine. The structure assignment for compound **20** also allowed identification of compounds **12**, **16**, and **18**, as the PMB-protected amino phosphine oxide, the conjugated imine phosphine oxide, and the deprotected amino phosphine oxide, respectively (Schemes 2 and 4).

To clarify the reaction pathway to formation of the phosphine oxide, parallel experiments have been conducted by treatment of diethyl phosphite (2 equiv) with *n*-BuLi or with LHMDS in THF from 0 °C to room temperature. The ^{31}P NMR spectra of the two reaction mixtures were recorded both during the reaction and after the reaction was quenched by addition of NH_4Cl. For the experiment with LHMDS, a broad single resonance at 7.6 ppm was observed during the reaction, and a sharp singlet at 8.0 ppm was observed after the reaction was quenched, which corresponds to recovered phosphite. For the parallel reaction with *n*-BuLi, four broad resonances were detected at 49.2, 38.9, 35.6, and 6.3 ppm during the reaction, and three sharp resonances were found after NH_4Cl was added, at 49.4, 35.2, and 8.0 ppm in a ratio of 1.5:4.6:1.0. This result indicated that exchange between the *n*-butyl and ethoxy groups can occur when *n*-butyllithium and diethyl phosphite are allowed to react and suggests that this exchange occurs prior to addition of phosphorus to the nucleoside imine. When diethyl phosphite is treated with LHMDS under the same conditions, there is no evidence of exchange with the ethoxy groups.[15]

On the basis of the above observations, the lithium salt of diethyl phosphite, prepared in situ by treatment of diethyl phosphite with LHMDS, was used in phosphonylation of the nucleoside imines below. The imines **10** and **22** (Scheme 5), prepared from protected cytidine **8**[2] and uridine **21**,[16] respectively, via oxidation of the primary alcohol and condensation of the resulting aldehyde with $PMBNH_2$, were treated with the lithium salt of diethyl phosphite to afford two sets of epimeric amino phosphonates in very good yield. In the cytidine series, the amino phosphonates **11** and **24** were obtained in a ratio of 6:1. In the uridine series, the major amino phosphonate **23** was preferred by ratio of 2:1 to the minor isomer **25**, and a third isomer (**26**) was observed with a ^{31}P NMR resonance at 26.4 ppm and a significant downfield shifted resonance for C-4′ compared to phosphonates **23** and **25**. Extensive NMR experiments have been done to clarify the stereochemistry of the third isomer. The C-4′ resonance is easily identified in the ^{13}C NMR spectrum on the basis of C−P coupling constants. Therefore assignment of the H-4′ resonance could be made through an HMQC experiment. Once this resonance was identified, NOE experiments revealed a significant correlation between H-4′ and H-6 in isomer **26**, whereas no NOE effect was observed in isomer **23**. On the basis of these data, the uridine derivatives **23** and **25** were assigned as 4′β isomers and phosphonate **26** was assigned as a 4′α isomer.

A similar phosphonylation procedure was employed to synthesize amino phosphonate derivatives **29** and **30** from a protected ara-C (**27**), and the 5′ isomers were obtained in a ratio of ∼ 5:2 (Scheme 6). The two possible 4′α-isomers also were observed in the reaction mixture with ^{31}P NMR resonance at 25.2 and 24.7 ppm. However, only trace amounts of these products were generated and their isolation was not pursued.

To remove the PMB protecting groups from these amino phosphonates, a CAN oxidation was employed on the basis of the premise that this acidic reagent might also cleave the imine bond in the intermediate benzyl imines. Therefore, the 5′-amino phosphonate **23** was treated with CAN in a mixture of acetonitrile and water (Scheme 7). After several hours, a very polar spot was

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(15) (a) McEldoon, W. L.; Wiemer, D. F. *Tetrahedron* **1995**, *51*, 7131−7148. (b) Lefebvre, I. M.; Evans, S. A., Jr. *J. Org. Chem.* **1997**, *62*, 7532−7533. (c) Davis, F. A.; Lee, S.; Yan, H.; Titus, D. D. *Org. Lett.* **2001**, *3*, 1757−1760.

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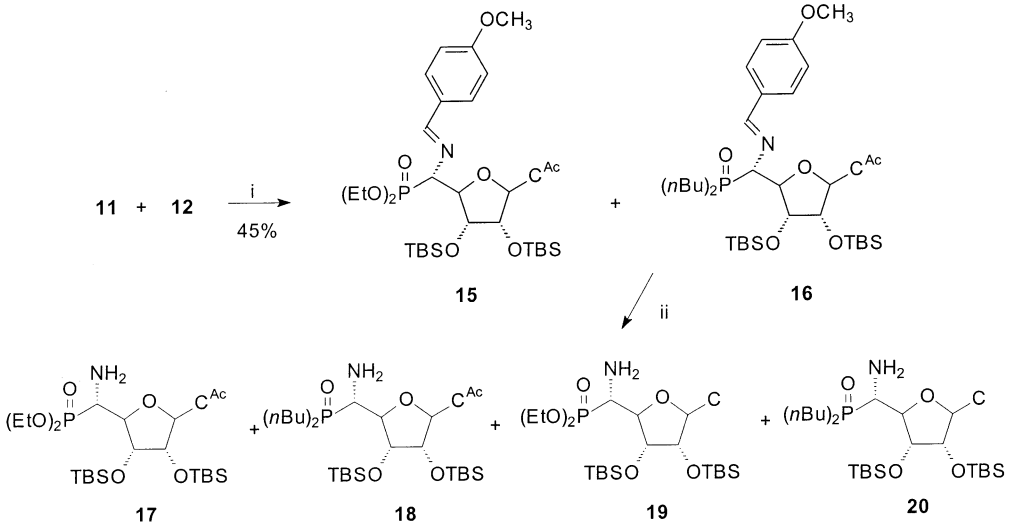
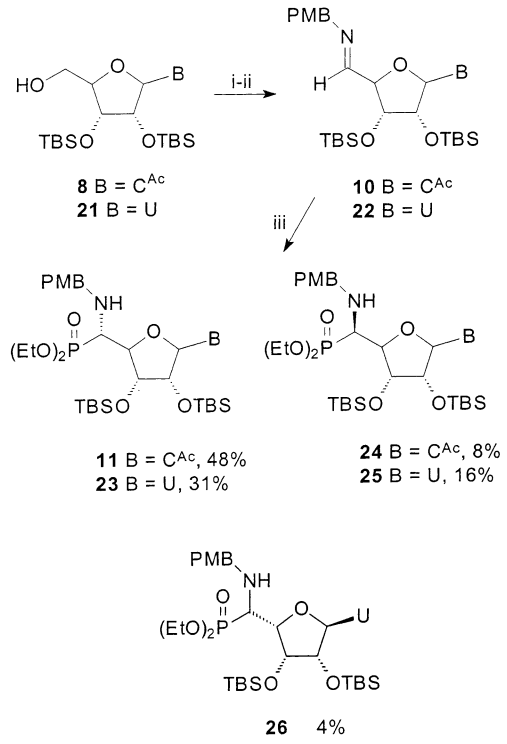
SCHEME 4[a]



[a] (i) DDQ, CH_2Cl_2/H_2O (20:1); (ii) 1 N HCl aq, MeOH.

SCHEME 5[a]

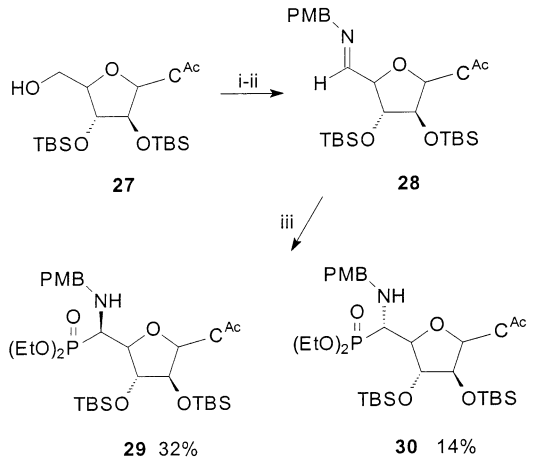


[a] (i) DMSO, EDC, pyr, TFA, benzene; (ii) $PMBNH_2$, benzene, reflux with Dean−Stark apparatus; (iii) LHMDS, $HP(O)(OEt)_2$, THF, 0 °C to rt.

observed by TLC corresponding to the free amino phosphonate **31**. To our surprise, after the reaction mixture was neutralized and extracted with EtOAc and the organic phase was dried over $NaSO_4$ and concentrated, the amino phosphonate **31** had condensed with benzaldehyde to form the corresponding imine. This adduct was observed by TLC as a less polar spot than phosphonate **31**. Acidic hydrolysis of the imine product and subsequent workup with isopropylamine as a benzaldehyde scavenger afforded the free amino phosphonate **31** in good yield.

Although removal of TBS groups from compound **31** could be realized by treatment of TBAF, the excess TBAF could not be completely removed by flash chromatography. Bubenik[17] reported that the TBS groups and ethyl groups in phosphonate ethyl esters could be removed by initial treatment with TMSBr and subsequent acidic workup at high temperature. Therefore phosphonate **31** was treated with TMSBr and the reaction was quenched by addition of MeOH to afford the phosphonic acid **32** directly in an excellent yield. Presumably, HBr generated in situ from the excess TMSBr was sufficient to remove the TBS groups.

SCHEME 6[a]



[a] (i) DMSO, EDC, pyr, TFA, benzene; (ii) $PMBNH_2$, benzene, reflux with Dean−Stark apparatus; (iii) LHMDS, $HP(O)(OEt)_2$, THF, 0 °C to rt.

These results encouraged efforts to simplify the deprotection procedure because the imine moiety is an acid-sensitive functionality. Accordingly after DDQ oxidation of the PMB-protected amino phosphonate **11**, complete deprotection transformed imine phosphonate **15** to the phosphonic acid **33** in excellent yield. In this reaction, the benzylic imine, the phosphonate esters, and the N^4-

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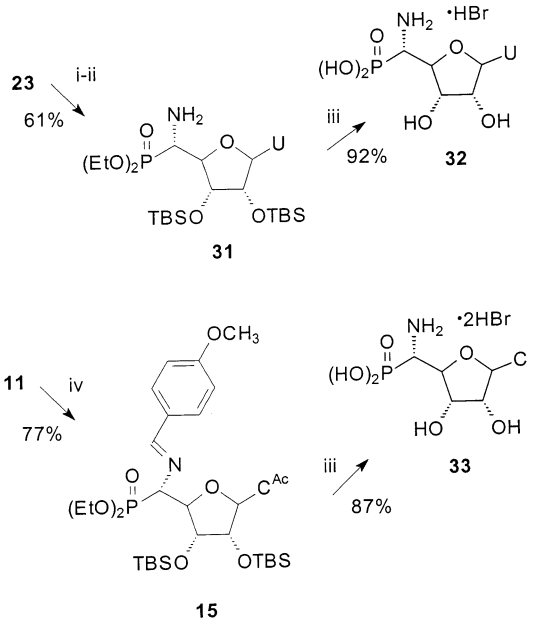
SCHEME 7[a]



[a] (i) CAN, CH_3CN/H_2O; (ii) 1 N HCl, MeOH; (iii) TMSBr, CH_2Cl_2, then MeOH; (iv) DDQ, CH_2Cl_2/H_2O (20:1).

acetyl group were cleaved cleanly in one flask. To our delight, the imine phosphonate **15** could be obtained as a single crystal and therefore the stereochemistry at the 5′ position was established as the *S* configuration through an X-ray diffraction analysis.[18]

Following parallel procedures, the minor amino phosphonate derivative of cytidine **24** and the uridine derivative **25** (Table 1) and the major and minor ara-C derivatives **29** and **30** were oxidized with DDQ to the corresponding imine adducts **34**−**37** in good yields. Complete deprotection of these imines provided, respectively, the phosphonic acids **38**−**41** as their hydrogen bromide salts in excellent yield.

The ^{13}C chemical shifts of uridine, cytidine, and ara-C and their amino phosphonic acids are listed in Table 2. For uridine and cytidine, the phosphonic acids **39** and **38** derived from the minor phosphite addition adducts of uridine and cytidine showed resonances for C-4′ with upfield shifts of 4.8 and 3.9 ppm, respectively, relative to their parent nucleosides, while the phosphonic acids **32** and **33** derived from the major phosphite products have no significant change in the resonances for C-4′ but appear with C-1′ shifted 5.3 and 5.1 ppm downfield respectively relative to their parent nucleosides. For ara-C, the stereochemical preference appeared in an opposite sense. The resonance for C-4′ in phosphonic acid **40**, generated from the major phosphite product, shifted upfield 5.7 ppm. The minor phosphonic acid **41** displayed a resonance for C-4′ with an upfield shift of 3.3 ppm, much less than the major phosphonic acid **40**, and the C-1′ resonance shifted downfield by 3.8 ppm. With the X-ray analyses for imine **15** and the above NMR data, the amino phosphonic acids **32** and **41** were assigned the same 5′S configuration as phosphonic acid **33**, and the amino phosphonic acids **39** and **40** were assigned as the 5′*R* isomers, the same configuration as acid **38**.

In summary, the 5′-amino-5′-phosphonate derivatives of uridine, cytidine, and ara-C can be prepared through addition of the lithium salt of diethyl phosphite to nucleoside imines protected with a PMB group. The configuration of the new stereogenic center at C-5′ of the major 5′-amino-5′-phosphonate cytidine derivative was

TABLE 1. Synthesis of 5′-Amino Phosphonic Acids of Cytidine, Uridine, and Ara-C

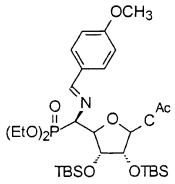
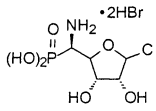
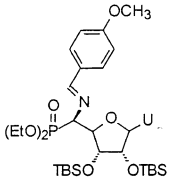
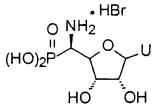
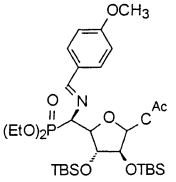
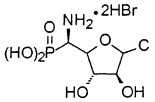
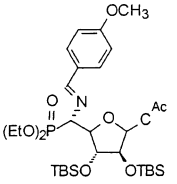
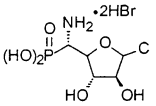
DDQ Oxidation Substrate	DDQ Oxidation Product and Yield	Deprotection Product
24	 **34**, 61%	 **38**, 78%
25	 **35**, 63%	 **39**, 86%
29	 **36**, 69%	 **40**, 81%
30	 **37**, 59%	 **41**, 85%

TABLE 2. ^{13}C Chemical Shifts (ppm) for Uridine, Cytidine, Ara-C, and Their 5′-Amino Phosphonic Acid Derivatives[a,b]

	C-1′	C-2′	C-3′	C-4′	C-5′
uridine	90.7	74.9	70.8	85.4	62.1
32	96.0	74.8	74.1	84.1	53.7
39	92.6	77.8	75.9	80.6	53.1
cytidine	91.6	75.3	70.6	85.0	62.1
33	96.7	75.2	73.9	84.4	53.8
38	93.8	78.4	75.9	81.1	53.0
ara-C	87.2	76.8	76.8	84.5	62.1
41	91.0	78.2	79.9	81.2	52.4
40	89.6	77.8	84.2	78.8	52.6

[a] Data for cytidine and ara-C measured in D_2O relative to internal dioxane at 67.86 ppm.[19] [b] Data for compounds **32**, **33**, and **38**−**41** measured in D_2O relative to DSS at 0 ppm.

(18) The single-crystal X-ray diffraction data for compound **15** is available from Cambridge Crystallographic Data Centre with reference number CCDC 197774.

(19) Hruska, F. E.; Blonski, W. J. P. *Can. J. Chem.* **1982**, *60*, 3026−3032.

determined as S through an X-ray diffraction analysis of a single crystal of the corresponding 5′-phenylmethylene amino phosphonate. The stereochemistry for the uridine and ara-C 5′-amino-5′-phosphonic acids could be assigned by comparison of their ^{13}C NMR data with that of the cytidine 5′-amino phosphonic acid. The stereoselectivity observed in phosphonylation of cytidine and uridine 5′-imines favored the 5′*S* isomers, whereas for ara-C the 5′*R* isomer was greatly favored. After separation of the diastereomeric adducts, the PMB group can be removed through reaction with DDQ and an acidic hydrolysis, conditions that also cleave all other protecting groups to afford the parent phosphonic acids.

Experimental Section

5′*S*-(*p*-Methoxybenzyl)amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl Cytidine (13). The aldehyde **9** was prepared from alcohol **8** (319 mg, 0.62 mmol) through a modified Moffatt oxidation.[2] Without further purification, the aldehyde **9** was dissolved in benzene and treated with 4-methoxybenzylamine (84 mg, 0.61 mmol). The reaction mixture was heated at reflux with a Dean−Stark apparatus for 4 h. After the reaction mixture was allowed to cool to room temperature, the solvent was removed under vacuum to give the crude imine **10**, which was used in the next step without further purification.

To a solution of diethyl phosphite (0.25 mL, 2.73 mmol) in THF at 0 °C was added dropwise *n*-BuLi (2.26 M in hexane, 0.74 mL). The mixture was stirred for 30 min, allowed to warm to room temperature, and added via a cannula to a solution of imine **10** in THF (8 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and was monitored by TLC. After the mixture was stirred for 5 h, water (5 mL) was added and THF was removed in vacuo. The aqueous layer was saturated with NaCl and extracted with EtOAc. The combined EtOAc extract was dried ($NaSO_4$) and filtered. The filtrate was concentrated and purified by flash chromatography (MeOH gradient in EtOAc) to give a mixture of amino phosphonate **11** and amino phosphine oxide **12** (ratio based on ^{31}P NMR is 1:0.7, 203 mg, 42% in total from alcohol **31**) and amino phosphonate **13** (40 mg, 9% from alcohol **31**). For phosphonate **11**: ^{31}P NMR δ 27.1. For phosphine oxide **12**: ^{31}P NMR δ 51.5. For phosphonate **13**: ^{1}H NMR δ 7.84 (d, J = 7.6 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 8.7 Hz, 2H), 5.90 (d, J = 7.6 Hz, 1H), 5.88 (d, J = 7.4 Hz, 1H), 4.45 (dd, J = 7.0, 5.2 Hz, 1H), 4.23−4.10 (m, 5H), 4.05 (d, J = 12.9 Hz, 1H), 3.81 (dd, J = 12.9, 3.5 Hz, 1H), 3.80 (s, 3H), 3.73 (dd, J = 5.1, 2.3 Hz, 1H), 3.00 (dd, J = 17.0, 2.1 Hz, 1H), 1.37 (t, J = 7.2 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H), 0.88 (s, 9H), 0.86 (s, 9H), 0.10 (s, 3H), 0.00 (s, 3H), −0.05 (s, 3H), −0.13 (s, 3H); ^{13}C NMR δ 167.5, 160.8, 158.3, 144.2, 133.0, 131.7 (2C), 115.2 (2C), 96.7, 90.9, 86.9, 75.7 (d, J_{CP} = 3.6 Hz), 75.0 (d, J_{CP} = 15.3 Hz), 64.1 (d, J_{CP} = 7.0 Hz), 63.8 (d, J_{CP} = 8.3 Hz), 55.9, 55.0 (d, J_{CP} = 136.9), 52.4, 26.6 (3C), 26.5 (3C), 19.0, 19.0, 17.1 (d, J_{CP} = 4.2 Hz), 17.0 (d, J_{CP} = 3.7 Hz), −3.9, −4.0, −4.2, −4.2; ^{31}P NMR δ 27.6; HRMS (ESI) m/z calcd for $C_{33}H_{60}N_4O_8PSi_2$ $(M + H)^+$ 727.3687, found 727.3688.

5′*S*-[(*p*-Methoxyphenyl)methylene]amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl Cytidine (14). DDQ (82 mg, 0.4 mmol) was added to a solution of the amino phosphonate **13** (218 mg, 0.3 mmol) in CH_2Cl_2 and water (19 mL, 20:1). The reaction mixture was stirred for 6 h, and EtOAc (10 mL) was added. The organic phase was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and filtered. Flash chromatography of the concentrated filtrate (10% MeOH in EtOAc) gave imine phosphonate **14** as a light yellow solid (124 mg, 57%): ^{1}H NMR ($CDCl_3$) δ 8.86 (d, J = 7.5 Hz, 1H), 8.36 (d, J = 4.6 Hz, 1H), 7.66 (d, J = 8.4 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 5.84 (d, J = 1.3 Hz, 1H), 5.69 (d, J = 7.3 Hz, 1H), 4.69 (ddd, J = 7.7, 4.0, 1.7 Hz, 1H), 4.17 (m, 5H), 3.95 (dd, J = 20.8, 1.5 Hz, 1H), 3.88 (s, 3H), 3.79 (dd, J = 7.6, 4.0 Hz, 1H), 1.30 (t, J = 7.0 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.84 (s, 9H), 0.25 (s, 3H), 0.09 (s, 3H), −0.04 (s, 3H), −0.18 (s, 3H); ^{13}C NMR δ 166.5 (d, J_{CP} = 12.0 Hz), 165.9, 162.6, 155.9, 143.3, 130.2 (2C), 128.6 (d, J_{CP} = 2.8 Hz), 114.5 (2C), 93.2, 91.4, 81.3 (d, J_{CP} = 6.8 Hz), 75.6, 71.2 (d, J_{CP} = 12.3 Hz), 67.0 (d, J_{CP} = 151.3 Hz), 63.1 (d, J_{CP} = 7.2 Hz, 2C), 55.7, 26.1 (3C), 26.0 (3C), 18.3, 18.1, 16.7 (d, J_{CP} = 6.1 Hz, 2C), −3.7, −3.8, −4.8, −5.1; ^{31}P NMR δ 21.1; HRMS (ESI) m/z calcd for $C_{33}H_{58}N_4O_8PSi_2$ $(M + H)^+$ 725.3531, found 725.3562.

5′*S*-[(*p*-Methoxyphenyl)methylene]amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-N^4-acetyl Cytidine (15). DDQ (20 mg, 0.09 mmol) was added to a solution of the protected amino phosphonate **11** (55 mg, 0.07 mmol) in CH_2Cl_2 and water (20:1). The reaction mixture was stirred for 7 h and then was diluted with EtOAc. The organic phase was washed with $NaHCO_3$ and brine, dried (Na_2SO_4), and filtered. The filtrate was concentrated and purified by flash chromatography (EtOAc) to give imine phosphonate **15** (43 mg, 77%) as a white solid: ^{1}H NMR δ 9.33 (d, J = 7.6 Hz, 1H), 8.44 (d, J = 4.6 Hz, 1H), 7.78 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 7.6 Hz, 1H), 7.07 (d, J = 8.9 Hz, 2H), 5.96 (d, J = 2.7 Hz, 1H), 4.70 (ddd, J = 6.2, 4.5, 1.6 Hz, 1H), 4.27 (m, 1H), 4.23−4.11 (m, 4H), 4.05 (dd, J = 20.1, 1.8 Hz, 1H), 3.90 (dd, J = 5.7, 3.7 Hz, 1H), 3.88 (s, 3H), 2.21 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H), 1.30 (t, J = 7.2 Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.13 (s, 3H), 0.03 (s, 6H), −0.05 (s, 3H); ^{13}C NMR δ 173.3, 169.0 (d, J_{CP} = 12.8 Hz), 164.6, 164.5, 158.2, 147.5, 131.5 (d, J_{CP} = 1.4 Hz, 2C), 129.6 (d, J_{CP} = 3.0), 115.7 (2C), 98.0, 92.4, 83.9 (d, J_{CP} = 6.5 Hz), 77.2, 73.2 (d, J_{CP} = 13.6 Hz), 68.4 (d, J_{CP} = 154.0 Hz), 64.8 (d, J_{CP} = 7.2 Hz), 64.6 (d, J_{CP} = 6.5 Hz), 56.2, 26.6 (3C), 26.5 (3C), 24.8, 19.1, 19.0, 17.0 (d, J_{CP} = 2.1 Hz), 16.9 (d, J_{CP} = 3.0 Hz), −3.7, −3.8, −4.5, −4.7; ^{31}P NMR δ 21.8; HRMS (ESI) m/z calcd for $C_{35}H_{60}N_4O_9PSi_2$ $(M + H)^+$ 767.3637, found 767.3620.

5′*S*-Amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl Cytidine (19) and 5′*S*-Amino-5′-di-*n*-butylphosphinyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl Cytidine (20). To a solution of phosphonate **11** and phosphine oxide **12** (1:0.7, 202 mg, 0.26 mmol) in CH_2Cl_2 and H_2O (20:1, 10 mL) was added DDQ (71 mg, 0.30 mmol). The reaction mixture was stirred at room temperature and monitored by TLC. After 6 h, the reaction mixture was filtered through filter paper and rinsed with CH_2Cl_2. The combined CH_2Cl_2 solution was washed with $NaHCO_3$ and brine, dried (Na_2SO_4), and filtered. The filtrate was purified by flash chromatography (EtOAc) to give a mixture of imine phosphonate **15** and phosphine oxide **16** (89 mg, ratio based on ^{31}P NMR was 1:0.5).

The mixture of imine phosphonate **15** and phosphine oxide **16** (1:0.5, 85 mg) was dissolved in MeOH and treated with 1 N HCl (1 mL). The mixture was shaken for 5 min, and then TLC showed the hydrolysis was complete. After concentration under vacuum, the aqueous residue was extracted with EtOAc, and the organic phase was washed with saturated $NaHCO_3$ and dried (Na_2SO_4). After concentration, the residue was purified by flash chromatography (MeOH gradient in EtOAc) to afford a mixture of amino phosphonate **17** and phosphine oxide **18** (ratio based on ^{31}P NMR was 1:0.7, 43 mg), amino phosphonate **19** (15 mg), and phosphine oxide **20** (9 mg). For amino phosphonate **17**: ^{31}P NMR δ27.1. For phosphine oxide **18**: ^{31}P NMR δ56.9. For amino phosphonate **19**: ^{1}H NMR δ 7.86 (d, J = 7.5 Hz, 1H), 5.90 (d, J = 7.6 Hz, 1H), 5.71 (d, J = 5.7 Hz, 1H), 4.63 (dd, J = 5.7, 4.8 Hz, 1H), 4.36 (dd, J = 4.7, 3.7 Hz, 1H), 4.26 (ddd, J = 5.1, 3.7, 3.4 Hz, 1H), 4.21−4.09 (m, 4H), 3.31 (dd, J = 16.8, 3.4 Hz, 1H), 1.34 (t, J = 7.1 Hz, 3H), 1.30 (t, J = 7.1 Hz, 3H), 0.95 (s, 9H), 0.89 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H), 0.08 (s, 3H), 0.03 (s, 3H); ^{13}C NMR δ 167.8, 158.4, 145.2, 96.3, 94.0, 85.3, 75.0, 74.2 (d, J_{CP} = 11.7 Hz), 64.4 (d, J_{CP} = 7.1 Hz), 64.0 (d, J_{CP} = 7.1 Hz), 50.5 (d, J_{CP} = 168.0 Hz), 26.6 (6C), 19.1 (2C), 16.9 (J_{CP} = 5.2 Hz, 2C), −3.9, −4.2 (2C), −4.3; ^{31}P NMR δ 26.9; HRMS (ESI) m/z calcd for $C_{25}H_{52}N_4O_7PSi_2$ $(M + H)^+$ 607.3112, found 607.3117. Phos-

phine oxide **20**: ^{1}H NMR δ 7.84 (d, J = 7.4 Hz, 1H), 5.90 (d, J = 7.3 Hz, 1H), 5.54 (d, J = 5.4, 1H), 4.77 (dd, J = 5.2, 5.2, 1H), 4.40 (m, 2H), 3.28 (dd, J = 13.4, 1.4 Hz, 1H), 1.87 (m, 4H), 1.50 (m, 8H), 0.97 (s, 9H), 0.97 (t, J = 7.2 Hz, 3H), 0.91 (s, 9H), 0.91 (t, J = 7.2 Hz, 3H), 0.18 (s, 3H), 0.16 (s, 3H), 0.11 (s, 3H), 0.04 (s, 3H); ^{13}C NMR δ 167.9, 158.3, 146.2, 96.2, 96.1, 84.9, 74.2, 74.2 (d, J_{CP} = 10.9), 51.7 (d, J_{CP} = 71.1), 26.6 (6C), 26.4 (d, J_{CP} = 62.8 Hz), 25.9 (d, J_{CP} = 61.0), 25.4 (d, J_{CP} = 14.2), 25.4 (d, J_{CP} = 14.2 Hz), 24.7 (d, J_{CP} = 4.2 Hz), 24.6 (d, J_{CP} = 2.6 Hz), 19.1, 19.0, 14.1, 14.0, −3.9, −4.1 (2C), −4.3; ^{31}P NMR δ 57.1; HRMS (ESI) m/z calcd for $C_{29}H_{60}N_4O_5PSi_2$ $(M + H)^+$ 631.3840, found 631.3838.

5′*S*-(*p*-Methoxybenzyl)amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-*N*^{4}-acetyl Cytidine (11) and 5′*R*-(*p*-Methoxybenzyl)amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl-*N*^{4}-acetyl Cytidine (24). The aldehyde was prepared in situ from alcohol **8** (463 mg, 0.9 mmol) through a modified Moffatt oxidation. The crude aldehyde, without further purification, was dissolved in benzene and treated with 4-methoxybenzylamine (123 mg, 0.9 mmol). The reaction mixture was heated at reflux with Dean−Stark apparatus for 4 h. After the reaction mixture was allowed to cool to room temperature, the solvent was removed under vacuum to give the crude imine **10**.

Lithium diethyl phosphite was prepared from diethyl phosphite (0.41 mL, 4.5 mmol) and LHMDS (1.0 M in THF, 3.6 mL) in THF from −50 °C to room temperature. To a solution of compound **10** in THF at −78 °C was added the lithium diethyl phosphite dropwise through a cannula. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The reaction was stopped by addition of saturated NH_4Cl. The aqueous layer was extracted with EtOAc, and the combined organic layer was dried (Na_2SO_4) and filtered. The filtrate was concentrated under vacuum, and the residue was purified by flash chromatography (EtOAc gradient in hexane) to give amino phosphonates **11** (330 mg, 48% from compound **8**) and **24** (55 mg, 8% from compound **8**) as white solids. For amino phosphonate **11**: ^{1}H NMR δ 8.43 (d, J = 7.7 Hz, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 5.91 (d, J = 5.3 Hz, 1H), 4.45 (dd, J = 5.0, 5.0 Hz, 1H), 4.30 (m, 1H), 4.22−4.07 (m, 5H), 3.82 (m, 2H), 3.81 (s, 3H), 3.06 (dd, J = 17.5, 1.7 Hz, 1H), 2.18 (s, 3H), 1.38 (t, J = 6.9 Hz, 3H), 1.32 (t, J = 7.0 Hz, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.11 (s, 3H), 0.03 (s, 3H), −0.01 (s, 3H), −0.08 (s, 3H); ^{13}C NMR δ 173.1, 164.5, 160.8, 158.2, 147.7, 133.1, 131.5 (2C), 115.3 (2C), 98.5, 92.0, 86.4, 76.3 (d, J_{CP} = 3.7 Hz), 74.2 (d, J_{CP} = 13.6 Hz), 64.1 (d, J_{CP} = 7.6 Hz), 63.9 (d, J_{CP} = 7.7 Hz), 55.9, 54.9 (d, J_{CP} = 138.8 Hz), 52.9, 26.6 (3C), 26.5 (3C), 24.7, 19.0, 19.0, 17.1 (d, J_{CP} = 5.1 Hz), 17.0 (d, J_{CP} = 5.3 Hz), −3.9, −4.1, −4.1, −4.3; ^{31}P NMR δ 27.5; HRMS (ESI) m/z calcd for $C_{35}H_{62}N_4O_9PSi_2$ $(M + H)^+$ 769.3793, found 769.3805. Anal. Calcd for $C_{35}H_{61}N_4O_9PSi_2$: C, 54.66; H, 8.00; N, 7.29. Found: C, 54.66; H, 8.01; N, 7.14. For amino phosphonate **24**: ^{1}H NMR δ 8.09 (d, J = 7.6 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.26 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.95 (d, J = 6.3 Hz, 1H), 4.73 (m, 2H), 4.36 (dd, J = 3.0, 2.8 Hz, 1H), 4.20−4.06 (m, 4H), 3.98 (dd, J = 12.4, 2.2 Hz, 1H), 3.85 (dd, J = 12.4, 2.2 Hz, 1H), 3.77 (s, 3H), 3.71 (dd, J = 10.1, 10.1 Hz, 1H), 2.18 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H), 1.29 (t, J = 7.1 Hz, 3H), 0.94 (s, 9H), 0.89 (s, 9H), 0.20 (s, 3H), 0.15 (s, 3H), 0.02 (s, 3H), −0.05 (s, 3H); ^{13}C NMR δ 173.2, 164.6, 160.4, 158.3, 148.2, 134.1, 130.4 (2C), 114.9 (2C), 98.6, 93.5, 81.3, 77.8, 75.5 (d, J_{CP} = 10.2 Hz), 63.8 (d, J_{CP} = 7.4 Hz), 63.6 (d, J_{CP} = 7.2 Hz), 55.9 (d, J_{CP} = 143.2 Hz), 55.8, 51.5, 26.9 (3C), 26.8 (3C), 24.7, 19.6, 19.2, 17.1 (d, J_{CP} = 5.7 Hz), 17.0 (d, J_{CP} = 5.6 Hz), −3.2, −3.9, −4.1, −4.4; ^{31}P NMR δ 28.1; HRMS (ESI) m/z calcd for $C_{35}H_{62}N_4O_9PSi_2$ $(M + H)^+$ 769.3793, found 769.3775.

5′*S*-Amino-5′-diethylphosphonyl-2′,3′-di-*O*-*tert*-butyldimethylsilyl Uridine (31). CAN (1.9 g, 3.5 mmol) was added to a solution of amino phosphonate **23** (732 mg, 1.0 mmol) in acetonitrile (7 mL) and distilled water (7 mL). After the reaction mixture was stirred for 9 h, water (5 mL) was added and the aqueous layer was extracted with EtOAc. The combined organic extract was washed with Na_2SO_3, dried (Na_2SO_4), and filtered. The filtrate was concentrated, and the residue was dissolved in MeOH. To this solution was added 1 N HCl (3 mL), and the reaction mixture was shaken for 4 min. After dilution with EtOAc, isopropylamine (1 mL) was added to trap the resulting 4-methoxybenzylaldehyde and neutralize the reaction mixture. The aqueous layer was extracted with EtOAc, and the combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was concentrated, and the residue was purified by flash chromatography (MeOH gradient in EtOAc) to give amino phosphonate **31** (369 mg, 61%) as a light brown solid: ^{1}H NMR δ 7.91 (d, J = 7.8 Hz, 1H), 5.78 (d, J = 5.9 Hz, 1H), 5.73 (d, J = 7.8 Hz, 1H), 4.56 (dd, J = 5.9, 4.7 Hz, 1H), 4.35 (dd, J = 4.7, 3.0 Hz, 1H), 4.26 (ddd, J = 4.6, 3.1, 3.0, 1H), 4.20−4.11 (m, 4H), 3.30 (dd, J = 17.4, 3.1 Hz, 1H), 1.34 (t, J = 7.2 Hz, 3H), 1.31 (t, J = 7.1 Hz, 3H), 0.95 (s, 9H), 0.89 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), 0.09 (s, 3H), 0.04 (s, 3H); ^{13}C NMR δ 166.1, 152.4, 144.4, 103.1, 92.1, 85.9, 75.1 (d, J_{CP} = 2.4 Hz), 74.3 (d, J_{CP} = 12.4 Hz), 64.4 (d, J_{CP} = 6.1 Hz), 64.0 (d, J_{CP} = 7.2 Hz), 50.7 (d, J_{CP} = 147.3 Hz), 26.6 (3C), 26.5 (3C), 19.1, 19.0, 16.9 (d, J_{CP} = 5.9 Hz, 2C), −4.0, −4.1, −4.1, −4.4; ^{31}P NMR δ 26.7; HRMS (ESI) m/z calcd for $C_{25}H_{51}N_3O_8PSi_2$ $(M + H)^+$ 608.2952, found 608.2953.

5′-[1′-(5′(*S*)-Amino-*β*-D-ribo-penta-1′,4′-furanosyl)uracil] Phosphonic Acid (32). To a solution of amino phosphonate **31** (163 mg, 0.27 mmol) in CH_2Cl_2 (5 mL) was added TMSBr at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The volatile components were removed in vacuo, and the residue was coevaporated with MeOH and then with water. The resulting residue was dissolved in minimum amount of MeOH and precipitated with EtOAc. After centrifugation, the precipitate was separated from the supernant. The solid was dissolved in water and lyophilized to give amino phosphonic acid **32** as its hydrogen bromide salt (100 mg, 92%) as a white solid: ^{1}H NMR (D_2O, DSS standard) δ 7.66 (d, J = 7.8 Hz, 1H), 5.87 (d, J = 7.8 Hz, 1H), 5.69 (d, J = 5.1 Hz, 1H), 4.70 (dd, J = 5.1, 5.1 Hz, 1H), 4.50 (dd, J = 5.1, 4.6 Hz, 1H), 4.31 (ddd, J = 9.3, 4.7, 4.5 Hz, 1H), 3.61 (dd, J = 13.3, 9.3 Hz, 1H); ^{13}C NMR δ 169.2, 154.5, 147.3, 105.2, 96.0, 84.1 (d, J_{CP} = 3.1 Hz), 74.8, 74.1 (d, J_{CP} = 1.5 Hz), 53.7 (d, J_{CP} = 136.7 Hz); ^{31}P NMR δ 9.2; HRMS (ESI) m/z calcd for $C_9H_{14}N_3O_8PNa$ $(M + Na)^+$ 346.0416, found 346.0406.

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Supporting Information Available: General experimental protocols; additional examples of reactions described in the Experimental Section; ^{1}H and ^{13}C NMR spectra for compounds **13**−**15**, **19**, **20**, **24**−**26**, and **29**−**41**; and an ORTEP drawing for compound **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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