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Synthesis of Methylene- and Difluoromethylenephosphonate Analogues of Uridine-4-phosphate and 3-Deazauridine-4-phosphate

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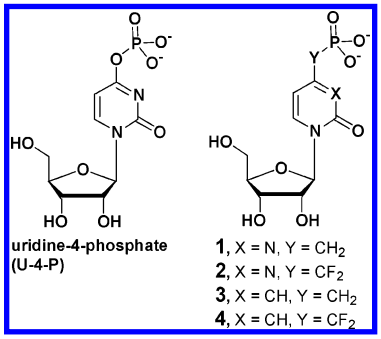

Cytidine triphosphate synthetase (CTPS) catalyzes the formation of cytidine triphosphate from glutamine, uridine-5′-triphosphate (UTP), and adenosine-5′-triphosphate. Inhibitors of CTPS are of interest because of their potential as therapeutic agents. One approach to potent enzyme inhibitors is to use analogues of high energy intermediates formed during the reaction. The CTPS reaction proceeds via the high energy intermediate UTP-4-phosphate (UTP-4-P). Four novel analogues of uridine-4-phosphate (U-4-P) and 3-deazauridine-4-phosphate (3-deazaU-4-P) were synthesized in which the labile phosphate ester oxygen was replaced with a methylene and difluoromethylene group. The methylene analogue of U-4-P, compound **1**, was prepared by a reaction of the sodium salt of *tert*-butyl diethylphosphonoacetate with protected, 4-*O*-activated uridine followed by acetate deprotection and decarboxylation. It was found that this compound undergoes relatively facile dephosphonylation presumably via a metaphosphate intermediate. The difluoromethylene derivative, compound **2**, was prepared by electrophilic fluorination of protected **1**. This compound was stable and did not undergo dephosphonylation. Synthesis of the methylene analogue of 3-deazaU-4-P, compound **3**, was achieved by ribosylation of protected 4-(phosphonomethyl)-2-hydroxypyridine. Electrophilic fluorination was also employed in the preparation of protected 4-(phosphonodifluoromethyl)-2-hydroxypyridine which was used as the key building block in the synthesis of difluoro derivative **4**. These compounds represent the first examples of a nucleoside in which the base has been chemically modified with a methylene or difluormethylenephosphonate group.

Introduction

The synthesis of chemically modified nucleosides is a very active area of chemical research. This is mainly due to their therapeutic potential as evidenced by the considerable number of nucleoside analogues that are now on the market as antiviral and anticancer agents.[1] Our interest in nucleoside analogues stems from our desire to prepare inhibitors of cytidine triphosphate synthetase (CTPS, E. C. 6.4.3.2). CTPS catalyzes the formation of cytidine triphosphate (CTP) from glutamine, uridine-5′-triphosphate (UTP), and adenosine-5′-triphosphate (ATP; Scheme 1). It has been proposed, on the basis of numerous mechanistic investigations,[2] that CTPS performs this reaction by transferring the γ-phosphate from ATP to the oxygen at position 4 of UTP to form UTP-4-phosphate (UTP-4-P) as an intermediate and adenosine-5′-diphosphate (ADP). Ammonia, which is generated at a separate site via rate-limiting glutamine

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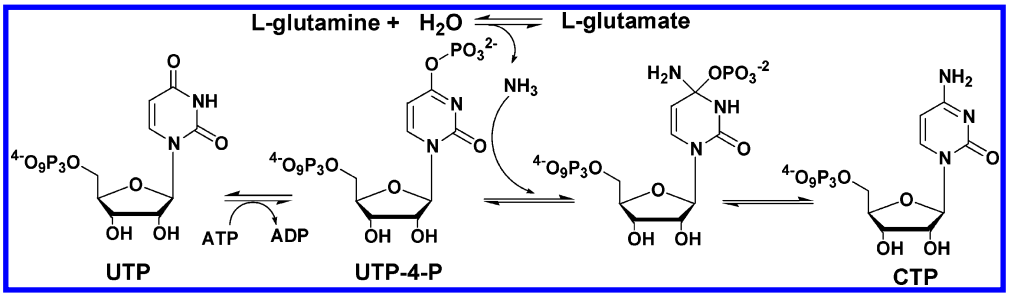
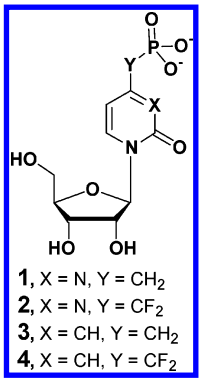
SCHEME 1. Reaction Catalyzed by CTPS



hydrolysis, then displaces the phosphate at the O-4 of UTP-4-P to give CTP. The reaction catalyzed by CTPS is the rate-limiting step in the biosynthesis of all cytosine nucleotides and provides one of the crucial building blocks for DNA synthesis. Therefore, it is hardly surprising that inhibitors of CTPS are being examined as a means of decreasing the viability of certain cancers and pathogenic organisms. For example, the nucleoside analogues 3-deazauridine (3-DaU) and cyclopentenylcytosine (CPEC) are triphosphorylated intracellularly which inhibits CTPS and reduces intracellular CTP levels.[3,4] Studies with several cancer cell lines and viruses indicate that 3-DaU and CPEC potentiate the effects of various anticancer and antiviral agents which suggests that they could be employed in combination drug therapy.[5] More recently, 3-DaU alone has been shown to induce apoptosis in myeloid leukemia cells in a dose-dependent manner.[6] One method of generating potent inhibitors of enzymes is to use analogues either of the transition state of the reaction or of a high energy intermediate formed during catalysis.[7] UTP-4-P is a high energy intermediate formed during the CTPS reaction. Consequently, a hydrolytically stable analogue of this species may exhibit potent inhibition of CTPS. Analogues of phosphorylated compounds which contain the isosteric and/or isoelectronic methylene or difluoromethylene phosphonate moiety in place of the phosphate group often inhibit enzymes which act upon phosphorylated substrates.[8] Here we report the synthesis of four novel analogues of uridine-4-phosphate (U-4-P) and 3-deazauridine-4-phosphate (3-DaU-4-P), compounds **1**−**4** (Chart 1), in which the labile phosphate group is replaced with a methylene- or difluoromethylenephoshonate moiety.

CHART 1. Structures of Target Analogues of U-4-P



Results and Discussion

There are only a limited number of reports of phosphonopyrimidine ribonucleosides. Honjo et al. have reported the synthesis of 2′,3′,5′-tri-*O*-benzoyl-4-diethylphosphonouridine by reacting 1-(2′,3′,5′-tri-*O*-benzoyl-*β*-D-ribofuranosyl)-4-chloro-2(*H*)-pyrimidinone with triethyl phosphite.[9] These workers also reported the synthesis of 5- and 6-phosphonouridine by reaction of lithiated 2′,3′,5′-tri-O-protected uridine with diethylchlorophosphate followed by deprotection.[9] Maruyama and co-workers have reported the synthesis of phosphonopyrimidine ribonucleosides by photolysis of 2′,3′-di-O- or 2′,3′,5′-tri-O-protected 5-bromouridine and triethylphosphite.[10] In all of these cases, the phosphonate group is attached directly to the pyrimidine ring. To our knowledge, nucleosides where the base bears a methylene or difluormethylenephosphonic acid group have never been reported.

We began our studies with the synthesis of compound **1**, in which the O-4 of U-4-P was replaced with a methylene unit. Synthetic approaches to C-4 substituted pyrimidine nucleosides include ribosylation of C-4 substituted pyrimidines,[11] sulfur extrusion of S-alkylated 4-thiouridines,[12] palladium-catalyzed cross-coupling of 4-chloropyrimidines with terminal alkynes,[13] or Bischofberger's approach which involves activation of the

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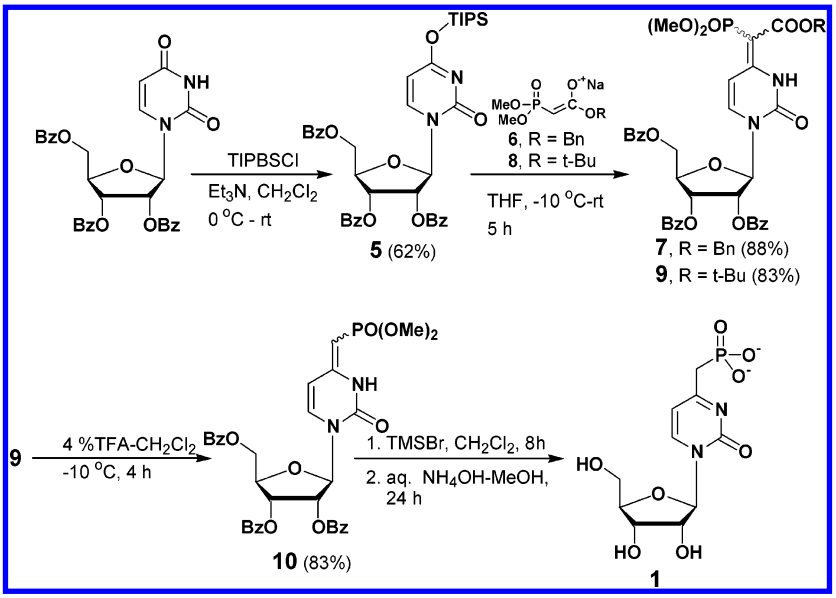
SCHEME 2. Synthesis of Compound 1



4-position of protected uridines followed by reaction with malonyl-type nucleophiles.[14] We anticipated that Bischofberger's approach would be applicable to the synthesis of **1** by reacting a protected phosphonoacetate derivative with protected, 4-O-activated uridine followed by acetate deprotection and decarboxylation. Thus, 2′,3′,5-tri-*O*-benzoyluridine was activated at the 4-position by reaction with triisopropylbenzenesulfonylchloride (TIPBSCl) in the presence of triethylamine which gave compound **5** in a 62% yield (Scheme 2). The reaction of **5** with 2.1 equiv of benzyl dimethylphosphonoacetate (**6**)[15] gave compound **7** in an 88% yield. We anticipated that upon hydrogenolysis of the benzyl group in **7** the resulting carboxylic acid would then spontaneously decarboxylate to give phosphonate **10**.[12b] However, the yield of this reaction was surprisingly low, and although some of the desired product was isolated, it was contaminated with a considerable amount of the tetrahydrouridine derivative which resulted from hydrogenation of the pyrimidine ring at C-5 and C-6; we were unable to obtain **10** in pure form. Therefore, the sodium salt of *tert*-butyl dimethylphosphonoacetate **8**[16] was used, which gave compound **9** in an 83% yield. Treatment of **9** with TFA in CH_2Cl_2 at −10 °C resulted in the removal of the *tert*-butyl group and decarboxylation, which gave compound **10** in an 83% yield. ^{1}H NMR revealed that, in $CDCl_3$, compound **10** existed exclusively as the tautomer in which the double bond is exocyclic to the pyrimidine ring.

Deprotection of the phosphonate moiety in **10** was achieved by a reaction with TMSBr in CH_2Cl_2 for 8 h. After removal of the solvent and unreacted TMSBr, the residue was subjected to NH_4OH/MeOH for 24 h to remove the benzoyl-protecting groups, after which the reaction was concentrated and then lyophilized several times to remove excess NH_4OH. A ^{31}P NMR spectrum of the crude product in D_2O showed that, in addition to a large peak at 15.8 ppm which corresponded to compound **1**, a very small peak at 1.7 ppm, which was approximately 2% of the intensity of the peak at 15.8 ppm, was also present. Spiking the sample with sodium phosphate resulted in an increase in the size of the minor peak, which suggested that the minor peak was inorganic phosphate. Attempts to purify nucleoside **1** by RP-HPLC using a variety of different eluents at different pH values proved unsuccessful, in that the minor peak was always present in varying amounts after HPLC and conversion to the sodium salt. Additional ^{31}P NMR studies in D_2O showed that compound **1** decomposed over time as the peak at 15.8 ppm decreased and the peak at 1.7 ppm increased. After 48 h, the peak at 1.7 ppm exhibited 25% of the intensity of the peak at 15.8 ppm. Decomposition of **10** occurred faster in a 3:2 DMSO-d_6−D_2O mixture. A ^{31}P NMR spectrum of the compound taken within the first 20 min of dissolution showed a major peak at 13.0 ppm and a small peak at 0.8 ppm. After just 48 h, the peak at 13.0 ppm had almost disappeared, and the peak at 0.8 ppm was by far the major peak (Figure 1). The negative ESI mass spectrum revealed a strong peak with a *m*/*z* of 321 which corresponds to compound **1**. Among the peaks in the positive ESI mass spectrum was a peak with a *m*/*z* of 243. This peak increased in intensity during storage of **1** in water or DMSO−water. The ^{1}H NMR of this material revealed that compound **1** exists mainly as the C═N tautomer in D_2O as evidenced by a doublet at approximately 3.0 ppm, which corresponds to that of the methylene protons adjacent to the phosphorus. This doublet almost completely disappeared after 9 h in D_2O indicating that these protons are readily exchanged with the solvent's deuterons. A doublet at 6.6 ppm corresponding to the H-5 proton of **1** slowly decreased with time while another doublet at 6.5 ppm, which initially constituted 5% of these two doublets, increased over time and, after 48 h, then constituted 25% of these doublets. In the 3:2 DMSO-d_6−D_2O mixture, the doublet at 6.5 ppm constituted approximately 87% of these two doublets after 48 h. Finally, a small broad singlet at 2.3 ppm, which was present within the first 15 min after dissolution in D_2O, was barely evident after 48 h.

These studies reveal that **10** dephosphonylates in solution to give 1-(β-D-ribofuranosyl)-4-methyl-2-pyrimidinone (**11**; Scheme 3) and inorganic phosphate. The new doublet at 6.5 ppm and the singlet at 2.3 ppm correspond to the H-5 proton and the

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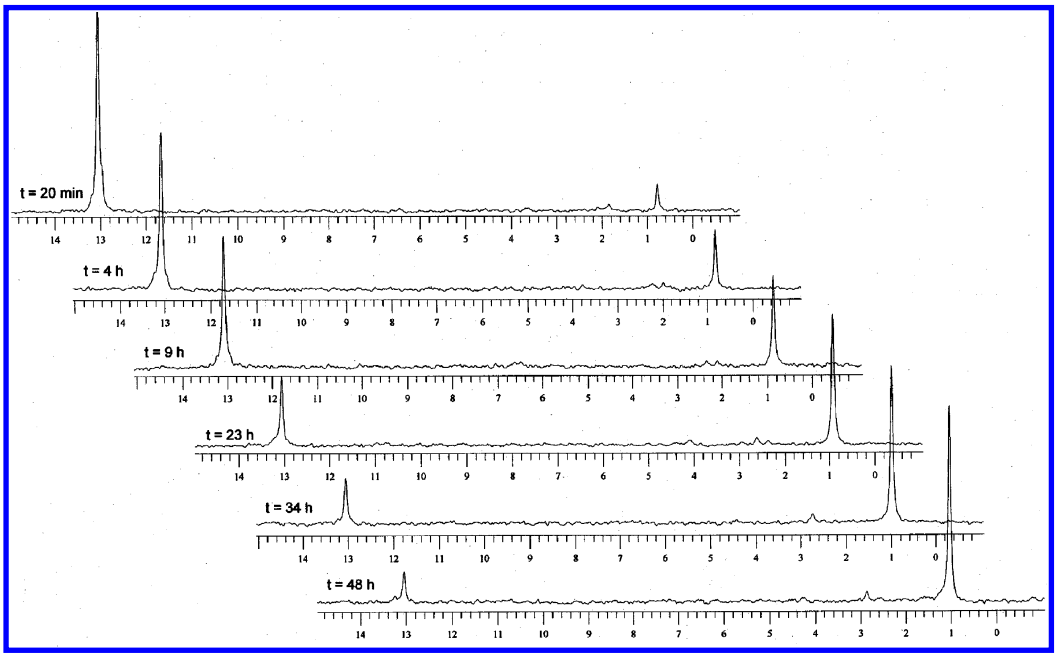
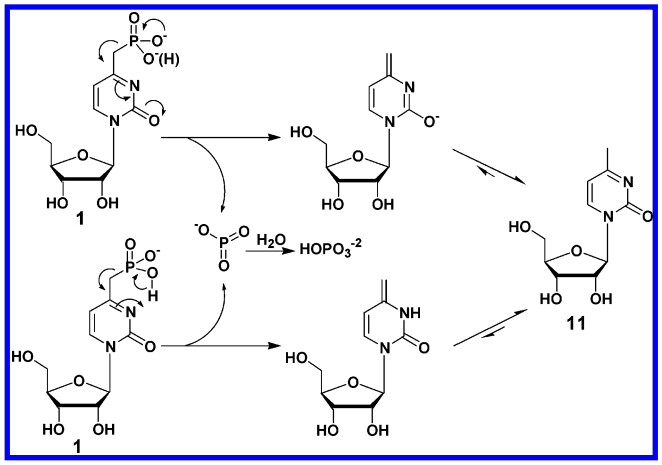


FIGURE 1. ^{31}P NMR spectra of compound **1** in 3:2 DMSO-d_6:D_2O taken at various time intervals over a period of 48 h.

SCHEME 3. Possible Mechanisms for the Dephosphonylation of Compound 1



methyl protons in compound **11**.[17] The methyl protons exchange slowly with the solvent deuterons. The high-resolution positive ion ESI mass spectrum of the peak with a *m*/*z* of 243 was also consistent with the decomposition product being compound **11**. Carbon−phosphorus bonds are usually highly resistant to hydrolysis. Nevertheless, some phosphonates are unstable toward C−P bond cleavage giving inorganic phosphate and/or polyphosphates. This has been well documented for β-carbonyl phosphonates, though usually these reactions require elevated temperatures and prolonged reaction times at a neutral pH.[18] It has been suggested that these reactions proceed by a dissociative mechanism involving metaphosphate.[18] The dephosphonylation of **10** occurs with considerable ease and probably proceeds via a similar mechanism involving metaphosphate or a metaphosphate-like transition state (Scheme 3). We are not aware of any studies demonstrating this phenomenon with 4-phosphonomethyl-2-pyrimidinones; however, to our knowledge, this is the first synthesis of this class of compounds. Nevertheless, Warren has shown that aminomethylphosphonic acid reacts instantaneously at room temperature with ninhydrin in aq ethylene glycol and triethylamine to give a mixture of inorganic phosphate and 2-hydroxyethylphosphate.[19] It was suggested that this reaction proceeds by formation of imine **12** followed by rapid decomposition via formation of metaphosphate (Scheme 4).[19] Martell and Langohr have suggested that the facile dephosphonylation of the metal chelate of the imine resulting from the reaction of 2-amino-3-phosphonopropionic acid with pyridoxal proceeds by the unimolecular dissociation of metaphosphate.[20]

To prepare the fluorinated analogue of **1**, phosphonate **10** was subjected to electrophilic fluorination[21] using potassium hexamethyldisilazane (KMHDS) and *N*-fluorobenzenesulfonimide (NFSi) which gave compound **13** in a 45% yield (Scheme 5). Complete deprotection of **13**, as described above for compound **10**, gave compound **2** in a 51% yield after purification by HPLC and conversion to its sodium salt. Unlike compound **1**, compound **2** did not undergo any detectable dephosphonylation even when stored for days in D_2O or DMSO. Very little has appeared in the literature concerning the influence of α-fluorines on the rates of decarboxylation of β-keto carboxylic acid, and, to our knowledge, no studies concerning their effect on the dephosphonylation of β-ketophosphonates or β-iminophosphonates have been reported. Bartlett et al. have noted that difluorooxaloacetic acid is extraordinarily resistant to decarboxylation in an aqueous solution. This was attributed to the difficulty in cracking the hydrate which dominates in aqueous solutions as well as to the destabilizing influence of

(17) 1-(β-D-Ribofuranosyl)-4-methyl-2-pyrimidinone (**11**) is a known compound. See: Holy, A.; Ludzisa, A.; Votruba, I.; Sediva, K.; Pischel, H. *Collect. Czech. Chem. Commun.* **1985**, *50*, 393. Its ^{1}H NMR spectrum was not reported.

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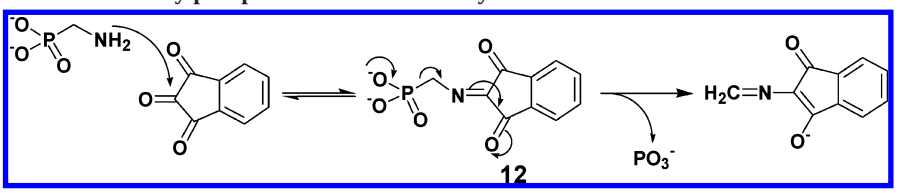
SCHEME 4. Reaction of Aminomethylphosphonic Acid with Ninhydrin



SCHEME 5. Synthesis of Compound 2

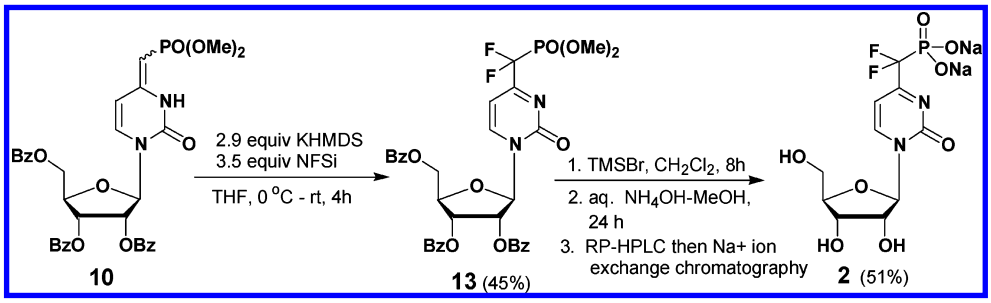
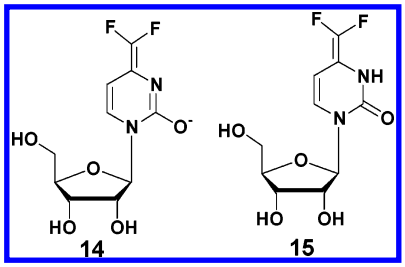
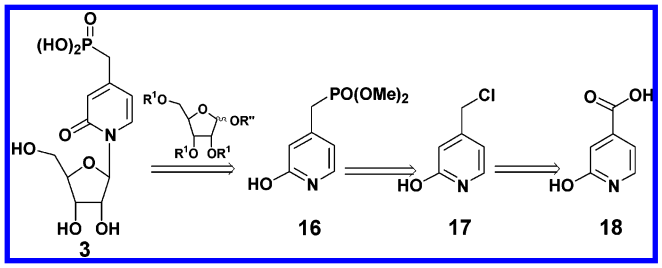


CHART 2. Eneamine-Type Compounds 14 and 15



SCHEME 6. Retrosynthesis of Compound 3



the fluorines on enol formation.[22] It is possible that the presence of the fluorines may have a destabilizing influence on the formation of the enamine-type compounds **14** and **15** (Chart 2), and this may also account for the stability of **2** toward dephosphonylation.

3-DeazaUTP is an inhibitor of CTP synthetase.[3,4] CTPS exhibits the same affinity for 3-deazaUTP as it does for the substrate UTP which indicates that the enzyme does not require the nitrogen at the 3-position for binding, at least in the ground state.[2] We anticipated that deaza nucleotides **3** and **4** would exhibit an affinity for CTPS which is similar to that of aza compounds **1** and **2**, yet neither would undergo dephosphonylation because of the lack of a nitrogen at the 3-position. Our approach to compound **3**, as illustrated in Scheme 6, was to couple phosphonate **16** to an appropriately protected ribofuranose derivative. Our initial route to phosphonate **16** was via alkyl chloride **17** which has been prepared by Uchida et al. by $LiAlH_4$ reduction of the 4-carbomethoxy-2-hydroxypyridine (**18**) followed by a reaction of the crude alcohol product with $SOCl_2$.[23] To this end, compound **18** was prepared in a 29% yield from 4-carbomethoxypyridine using the method of Boekelheide and Lehn which involved oxidation of 4-carbomethoxypyridine to the *N*-oxide (**19**) followed by a reaction with refluxing Ac_2O (Scheme 7).[24,25] In our hands, Uchida's procedure for the synthesis of **17** from **18** proceeded with a yield of only 21%. Finally, we were unable to obtain phosphonate **16** from **17** in yields greater than 35% which translates to an overall yield of **16** from 4-carbomethoxy-2-pyridinone of just 2.1%.

Because of the low yields using the approach outlined in Scheme 7, alternative routes to **16** were examined (Scheme 8). A reaction of 2-chloroisonicotinic acid (**20**) with thionyl chloride followed by a reaction of the crude acid chloride with benzyl alcohol gave the corresponding 2-*O*-benzyl derivative **21**.[26] The crude ester was reacted with the sodium salt of benzyl alcohol which gave the dibenzyl-protected compound **21** in an 80% yield (three steps).[26] Reduction of the ester with $LiBH_4$ in the presence of a small amount of methanol in refluxing ether gave compound **22** in an 86% yield. Reaction of compound **22** with $POBr_3$ in DMF/CH_2Cl_2 gave alkyl bromide **23** in a 77% yield. A Michaelis−Arbuzov reaction between **23** and trimethylphosphite performed either neat or in benzene resulted in reactions at both the methylbromide moiety and the benzylic carbon of the benzyl-protecting group. This afforded a mixture of the desired product **24** and dimethyl benzylphosphonate in an overall low yield, and we were unable to separate **24** from the contaminating dimethyl benzylphosphonate. Hydrogenolysis of crude **24** gave **16** in a 24% yield from **23** (two steps). The reaction of **23** with the sodium salt of dimethylphosphite also proceeded very poorly. Although this route was an improvement over the approach taken in Scheme 7, the difficulties encountered with the Michaelis−Arbuzov reaction prompted us to devise an alternative route to **16** (Scheme 8). Compound **20** was converted to 2-hydroxyisonicotinic acid (**25**) in an 89% yield by heating it in concd HCl for 48 h. Reduction of the acid moiety using BH_3−THF gave alcohol **26** in a 54% yield. The reaction of **26** with

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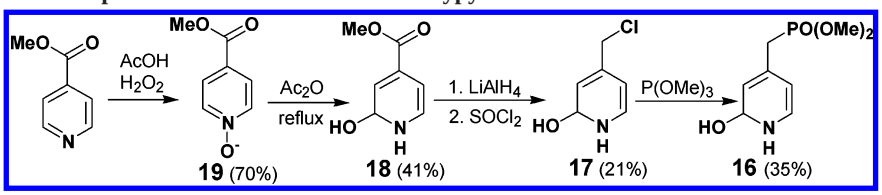
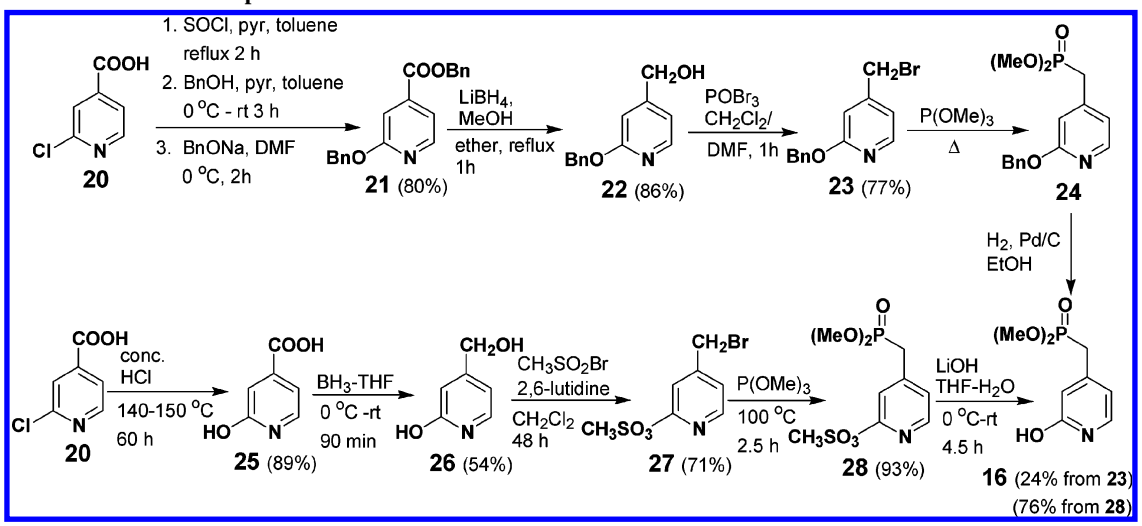
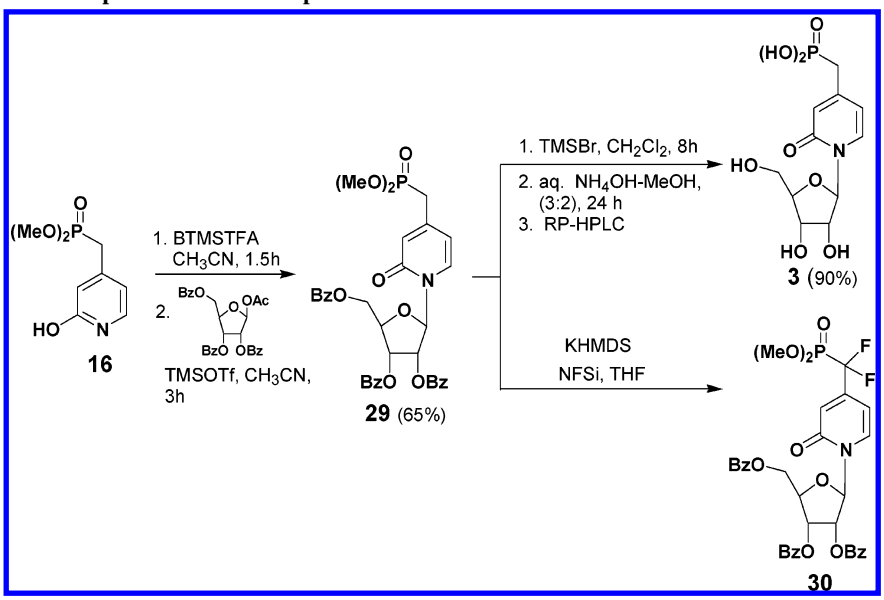
SCHEME 7. Synthesis of Phosphonate 16 from 4-Carbomethoxypyridine



SCHEME 8. Formation of Phosphonate 16 from 2-Chloroisonicotinic Acid



SCHEME 9. Synthesis of Compound 3 from Phosphonate 16

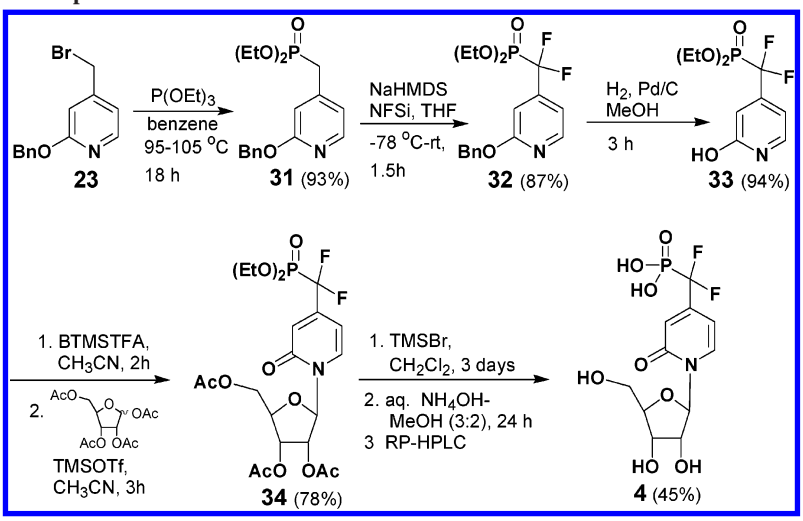


methane sulfonylbromide in the presence of 2,6-lutidine resulted both in bromination of the hydroxylmethyl functionality and in sulfonation at the 2-position to give compound **27** in a 71% yield. The Michaelis−Arbuzov reaction between **27** and trimethylphosphite proceeded smoothly to give phosphonate **28** in a 93% yield. Finally, the removal of the sulfonate group was achieved using LiOH in THF−H_2O which gave compound **16** in a 76% yield. Using this approach, we found that the overall yield of **16** improved to 24%.

To obtain compound **3**, phosphonate **16** was treated with bis(trimethylsilyl)trifluoroacetamide (BTMSTFA) followed by a reaction with acetyl 2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose in the presence of trimethylsilyl triflate (TMSOTf; Scheme 9). This gave nucleoside **29** in a 65% yield. Complete deprotection of **29** was achieved by treatment with 3 equiv of TMSBr for 8 h followed by a reaction with aq NH_4OH in methanol for 24 h. HPLC purification of the resulting material using TFA (0.1%) in H_2O−CH_3CN as the eluent gave **3** as its free acid in a 90% yield. Nucleoside **3** did not exhibit any decomposition even after storage for several weeks in water or DMSO indicating that the ring nitrogen is essential for dephosphonylation.

We initially attempted to construct compound **4** by electrophilic fluorination of compound **29** followed by deprotection in a manner analogous to that described for the synthesis of compound **2** (Scheme 9). Although ^{19}F NMR spectra of the crude product suggested that electrophilic fluorination of **29** gave the desired compound **30**, a number of byproducts were also formed, and we were unable to obtain **30** in pure form.

SCHEME 10. Synthesis of Compound 4



Therefore, for the synthesis of compound **4**, we envisaged a route similar to that taken for nucleoside **29** where the difluoromethylenephosphonate analogue of **16** would be prepared and then coupled to the protected ribose. Unfortunately, electrophilic fluorination of **16** gave a complex mixture of products. Nevertheless, we reasoned that a hydroxyl-protected version of **16** would undergo electrophilic fluorination more cleanly. Phosphonate **28** was not considered to be a viable candidate for electrophilic fluorination because of the presence of the methylsulfonate moiety. Nonetheless, we reasoned that phosphonate **24**, which contained a benzyl-protected hydroxyl group, would readily undergo electrophilic fluorination. Unfortunately, preparing this compound was problematic because of the low yield of the Michaelis−Arbuzov reaction. However, we were surprised to find that when performing the Michaelis−Arbuzov reaction on **23** with triethylphosphite in benzene at 95−105 °C, phosphonate **31** could be obtained in a 93% yield, and no diethyl benzylphosphonate was formed (Scheme 10).[27] The reason for the difference in yield and product distribution when using the two different phosphites is not clear. One possible explanation is that the benzylic carbon may be slightly more sterically hindered than the alkyl bromide carbon and, therefore, might be less susceptible to an attack by the more bulky triethylphosphite. Fluorination of **31** using 2.2 equiv of NaHMDS and 2.5 equiv of NFSi gave compound **32** in an 87% yield. The benzyl group in **32** was removed by hydrogenolysis using catalyst Pd/C to give compound **33** in a 95% yield. Coupling of phosphonate **33** to acetyl 2,3,5-tri-*O*-acetyl-*β*-D-ribofuranose using the procedure described above for the synthesis of **29** gave the protected nucleoside **34** in a 78% yield. Complete deprotection of **34** was achieved in the usual manner, except removal of the ethyl-protecting groups from **34** required 8 equiv of TMSBr added over a period of 3 days. It has been reported that anomerization can occur when subjecting 2′-deoxy nucleotide analogues to TMSBr.[28] However, ^{31}P and ^{1}H NMR analysis of the crude reaction mixture revealed that anomerization had not taken place. This could be due to the presence of the benzoyl-protecting group at the 2′-position. After HPLC purification using TFA (0.1%) in H_2O-CH_3CN as the eluent, nucleoside **4** was obtained as its free acid in a 45% yield. This deprotection yield is somewhat lower than that of its nonfluorinated analogue **3**, and this is probably due to the prolonged exposure of **34** to TMSBr. As with nucleoside **3**, compound **4** was stable and did not exhibit any dephosphonylation even after prolonged storage in various solvents.

In summary, several unique analogues of U-4-P and 3-deazaU-4-P were prepared in which the labile phosphate ester oxygen was replaced with either a methylene or a difluoromethylene group. The methylene analogue of U-4-P, compound **1**, was readily prepared by a reaction of the sodium salt of a *tert*-butyl diethylphosphonoacetate with protected, 4-O-activated uridine followed by acetate deprotection and decarboxylation. However, we were not able to isolate **1** in pure form because of its tendency to undergo dephosphonylation, possibly via a metaphosphate intermediate. The difluoromethylene analogue of **1**, nucleoside **2**, was prepared by electrophilic fluorination of protected **1**. This compound did not undergo dephosphonylation. The synthesis of the methylene analogue of 3-deazaU-4-P, compound **3**, was achieved by ribosylation of protected 4-(phosphonomethyl)-2-hydroxypyridine followed by deprotection. Electrophilic fluorination was also employed in the preparation of protected 4-(phosphonodifluoromethyl)-2-hydroxypyridine which was ribosylated and then deprotected to give difluoro derivative **4**. To our knowledge, compounds **1**−**4** represent the first examples of ribonucleosides in which the base has been chemically modified with a methylene or difluoromethylenephosphonate group. The antiproliferative properties of these compounds will be examined, and the results will be reported in due course. Finally, it has not escaped our attention that the cell permeability of these compounds may be compromised because oftheir highly anionic nature. If necessary, more cell permeable phosphonate diester or phosphoramidate prodrug derivatives[29] of these compounds will be prepared and examined as antiproliferative agents.

(27) This was discovered after compound **3** was prepared, and so phosphonate **31** was not used for the synthesis of compound **3**. In any case, we preferred methyl-protecting groups as opposed to ethyl-protecting groups for phosphonate protection since methyl groups are removed more readily by TMSBr than ethyl groups; this reduces the compounds exposure to TMSBr which can cause side reactions and lower yields. We would have preferred methyl protection for the synthesis of compound **4**; however, this was not practical.

(28) Brossette, T.; Le Faou, A.; Goujon, L.; Valleix, A.; Creminon, C.; Grassi, J.; Mioskowski, C.; Lebeau, L. *J. Org. Chem.* **1999**, *64*, 5083.

(29) Kurz, T.; Schluter, K.; Kaula, U.; Bergmann, B.; Walter, R. D.; Geffken, D. *Bioorg. Med. Chem.* **2006**, *14*, 5121.

Experimental Section

2′,3′,5′-Tris-(*O*-benzoyl)-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]uridine (5). To a solution of 2,3,5-tri-*O*-benzoyluridine (7.00 g, 12.6 mmol) and DMAP (153 mg, 1.26 mmol) in dry CH_2Cl_2 (70 mL) at 0 °C (ice bath) was added triethylamine (10.5 mL, 75.6 mmol), and the solution was stirred for 30 min. A solution of TIPBSCl (6.46 g, 21.4 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise over 5 min, and the mixture was stirred for 1 h at 4 °C; then the ice bath was removed, and the mixture was stirred for a further 6 h. A 1:1 mixture of EtOAc−hexane (400 mL) was added, and the mixture was filtered. The filtrate was concentrated to about 50 mL, 400 mL of ether was added, and the mixture was filtered again. The filtrate was concentrated. Purification of the residue by flash chromatography (70% hexane−30% EtOAc) gave pure **5** as a white foam (6.6 g, 64% yield). ^{1}H NMR (300 MHz) δ 8.04 (d, J = 7.1 Hz, 2H), 7.92 (d, J = 5.7 Hz, 3H), 7.87 (d, J = 7.2 Hz, 2H), 7.30−7.62 (m, 11H), 7.19 (s, 2H), 6.28 (bs, 1H), 5.91 (d, J = 6.9 Hz, 1H), 5.82 (d, J = 5.4 Hz, 1H), 5.75 (bs, 1H), 4.62−4.84 (m, 3H), 4.20−4.25 (m, 2H), 2.81−2.93 (m, 1H), 1.25 (d, J = 7.6 Hz, 18H);^{13}C NMR (75 MHz) δ 167.2, 165.9, 165.1, 165.0, 154.6, 153.3, 151.2, 145.3, 133.6, 130.3, 129.9, 129.7, 129.5, 129.1, 128.7, 128.4, 124.0, 95.7, 89.9, 80.4, 74.6, 70.4, 63.2, 34.2, 29.6, 24.5, 23.3; LR$^+$ESIMS *m*/*z* (relative intensity) 823.5 (M + 1, 100), 445 (57). HR$^+$ESIMS *m*/*z*: calcd for $C_{45}H_{47}N_2O_{11}S$, 823.2901; found, 823.2920.

[2-Oxo-2-{1-[3,4-bis-(benzoyloxy)-5-(benzoyloxymethyl)tetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidene}-2-(bis-(benzyloxy)phosphoryl)acetic Acid Benzyl Ester (7). NaH (75 mg, 1.86 mmol, 2.1 equiv, 60% dispersion in oil) was added to a solution of phosphonate **6**[15] (0.50 g, 1.93 mmol, 2.18 equiv) in dry THF (10 mL) at 0 °C (ice bath) under Ar. The ice bath was removed, and the mixture was stirred for 1 h. The mixture was cooled using a KCl ice bath (−10 °C), and a solution of nucleoside **5** (0.728 g, 0.886 mmol, 1 equiv) in dry THF (10 mL) was added. The mixture was stirred for 30 min; the ice bath was removed, and stirring was continued for an additional 4 h. The reaction was diluted with 200 mL of Et_2O and was washed with 10% NH_4Cl and saturated brine. The organic layer was dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography (60% hexane−40% EtOAc) gave pure **7** as a white foam (0.619 g, 88% yield). ^{1}H NMR (300 MHz) δ 8.08 (d, J = 7.3 Hz, 2H), 7.90−7.96 (m, 4H), 7.70 (d, J = 8.7 Hz, 0.5H), 7.22−7.59 (m, 14.5H), 7.15 (d, J = 8.3 Hz, 0.5H), 7.12 (d, J = 9.3 Hz, 0.5H), 6.29 (d, J = 5.8 Hz, 0.5H), 6.26 (d, J = 5.4 Hz, 0.5H), 5.84−5.88 (m, 1H), 5.69−5.72 (m, 1H), 5.22 (s, 1H), 5.17 (s, 1H), 4.64−4.70 (m, 3H), 3.59−3.66 (m, 6H); ^{13}C NMR (75 MHz) δ 169.2 (d, J = 8.8 Hz), 165.9 (C═O), 165.8 (d, J = 7.6 Hz), 165.2, 165.1, 160.1 (d, J = 8.7 Hz), 160.2 (d, J = 23.0 Hz), 147.4, 147.3 (d, J = 1.9 Hz), 136.3, 135.8, 135.8, 135.76, 133.7, 133.6, 133.5, 129.8, 129.7, 129.6, 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 102.4, 102.0 (d, J = 15.2 Hz), 88.4, 88.1, 81.3 (d, J = 205 Hz), 80.1 (d, J = 188 Hz), 73.6, 71.1, 66.2, 65.8, 63.7, 63.7, 52.7 (d, J = 5.4 Hz), 52.1 (d, J = 5.4 Hz); ^{31}P NMR (121 MHz) δ 27.3, 23.3; LR$^+$ESIMS *m*/*z* (relative intensity) 797 (M + 1, 100). HREIMS *m*/*z*: calcd for $C_{41}H_{38}N_2O_{13}P$, 797.2112; found, 797.2094.

2-{1-[3,4-Bis-(benzoyloxy)-5-(benzoyloxymethyl)tetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidene}-2-(bis-(*tert*-butyloxy)phosphoryl)acetic Acid Benzyl Ester (9). NaH (398 mg, 9.94 mmol, 2.1 equiv, 60% dispersion in oil) was added to a solution of phosphonate **8**[16] (2.32 g, 4.73 mmol, 2.2 equiv) in dry THF (60 mL) at 0 °C (ice bath) under Ar. The ice bath was removed, and the mixture was stirred for 1 h. The mixture was cooled using a KCl ice bath (−10 °C), and a solution of nucleoside **5** (3.9 g, 4.73 mmol, 1 equiv) in dry THF (60 mL) was added. The mixture was stirred for 30 min; the ice bath was removed, and stirring was continued for an additional 7 h. The reaction was diluted with 200 mL of saturated NH_4Cl and transferred to a separatory funnel containing 600 mL of ether. The layers were separated, and the organic layer was washed with 10% NH_4Cl and saturated brine. The combined aq layers were back extracted with 200 mL of ether. The combined organics were dried ($MgSO_4$) and concentrated, and the resulting foam was purified by flash chromatography (60% hexane−40% EtOAc to 50% hexane−50% EtOAc) to give pure **9** as a white foam (3.0 g, 83% yield). ^{1}H NMR (300 MHz) δ 12.89 (s, 0.5H), 12.51 (s, 0.5H), 8.03−8.09 (m, 2H), 7.85−7.95 (m, 4H), 7.64 (d, J = 8.2 Hz, 0.5H), 7.20−7.60 (m, 9.5H), 7.09 (d, J = 8.5 Hz, 0.5 H), 7.06 (d, J = 8.6 Hz, 0.5H), 6.28 (d, J = 5.8 Hz, 0.5H), 6.25 (d, J = 5.6 Hz, 0.5H), 5.83−5.88 (m, 1H), 5.70−5.75 (m, 1H), 4.60−4.79 (m, 3H), 3.60−3.70 (m, 6H), 1.45 (s, 4.5H), 1.42 (s, 4.5H); ^{13}C NMR (75 MHz) δ 168.8 (d, J = 7.5 Hz), 166.0, 165.2 (d, J = 14.2 Hz), 165.2, 165.2, 159.4 (d, J = 24 Hz), 158.8 (d, J = 8.6 Hz), 147.5, 147.5 (d, J = 2.1 Hz), 134.9, 134.7, 133.6, 133.6, 133.5, 129.8, 129.7, 129.6, 129.2, 129.1, 128.6, 128.5, 128.4, 128.3, 102.5, 102.0 (d, J = 15.4 Hz), 89.0, 88.6, 83.0 (d, J = 203 Hz), 82.5 (d, J = 186 Hz), 80.6, 80.4, 80.3, 73.4, 71.1, 71.0, 63.7, 52.4 (d, J = 5.6 Hz), 51.9 (d, J = 5.4 Hz), 28.1; ^{31}P NMR (121 MHz) δ 28.0, 24.2; LR$^+$ESIMS *m*/*z* (relative intensity) 763 (M + 1, 100), 707 (28). HR$^+$ESIMS *m*/*z*: calcd for $C_{38}H_{40}N_2O_{13}P$, 763.2268; found, 763.2262.

{1-[3,4-Bis-(benzoyloxy)-5-benzoyloxymethyltetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidenemethyl}-phosphonic Acid Dimethyl Ester (10). To a solution of nucleoside **9** (2.90 g, 3.81 mmol) in dry CH_2Cl_2 (60 mL) cooled in a KCl ice bath (−10 °C) under Ar was added a solution of TFA (3.5 mL, 46 mmol, 12 equiv) in CH_2Cl_2 (60 mL) over a period of 30 min. The bath was removed, and the reaction was stirred for 4 h. The mixture was diluted with 800 mL of ether and washed with saturated $NaHCO_3$, water, and saturated brine. The combined aq layers were back extracted with ether (2 × 200 mL). The combined organics were dried ($MgSO_4$) and concentrated, and the resulting oil was purified by flash chromatography (30% hexane−70% EtOAc) to give pure **10** as a colorless oil (2.1 g, 83% yield). ^{1}H NMR (300 MHz) δ 10.39 (s, 1H), 8.10 (d, J = 7.8 Hz, 2H), 7.93 (d, J = 7.8 Hz, 4H), 7.45−7.63 (m, 5H), 7.30−7.40 (m, 4H), 6.67 (d, J = 8.0 Hz, 1H), 6.34 (d, J = 6.5 Hz, 1H), 5.80−5.87 (m, 1H), 5.65 (overlapping dd, J = 6.4 Hz, 1H), 5.43 (d, J = 8.3 Hz, 1H), 4.70−4.84 (m, 1H), 4.50−4.65 (m, 2H), 3.60−3.69 (m, 6H); ^{13}C NMR (75 MHz) δ 165.9, 165.2, 151.8 (d, J = 4.3 Hz), 148.1, 133.6, 133.6, 133.4, 130.7, 129.8, 129.7, 129.5, 129.2, 128.6, 128.4, 104.3 (d, J = 12.9 Hz), 86.9, 79.8, 74.6 (d, J = 196 Hz), 72.9, 71.1, 63.9, 51.0 (d, J = 4.3 Hz); ^{31}P NMR (121 MHz) δ 27.6; LREIMS *m*/*z* (relative intensity) 662 (M, 5), 445 (100), 201 (45), 105 (73). HREIMS *m*/*z*: calcd for $C_{33}H_{31}O_{11}N_2P$, 662.1665; found, 662.1668.

General Procedure for the Deprotection of Nucleosides 1−4. To a solution of the protected nucleoside in dry CH_2Cl_2 (≈0.8 mmols of nucleoside/mL of solvent) was added TMSBr (3 equiv for compounds **1**−**3**, 8 equiv added over 3 days for compound **4**), and the reaction was stirred for 20 h for compounds **1**−**3** or for 3 days for compound **4**. The reaction was concentrated. Dry CH_2Cl_2 (5 mL) was added, and the solution was concentrated by rotary evaporation. This was repeated. The residue was subjected to high vacuum for 2 h. A solution of aq concd ammonium hydroxide−methanol (3:2, 5 mL) was added, and the mixture was stirred for 24 h. The reaction was concentrated, and the residue was dissolved in water and lyophilized. Lyophilization was repeated to give a white powder. Purification was achieved using preparative RP-HPLC (C-18 column). For compounds **1** and **2**, triethylammonium acetate (pH 8.8)−acetonitrile was used as the eluent. After removal of the solvent and after lyophilization from water (five times), compounds **1** and **2** were obtained as their triethylammonium salts. The triethylammonium salts are hygroscopic. Therefore, they were passed through a small Dowex 50 Na^+ ion exchange column which gave compounds **1** and **2** as their sodium salts and as easy-to-handle white powders after lyophilization. For compounds **3** and **4**, 0.1% TFA in water−acetonitrile was used as the eluent which gave compounds **3** and **4** as white powders after removal of the eluent and after lyophilization.

{1-[3,4-Bis-(hydroxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyrimidin-4-ylmethyl)phosphonic Acid Bis-sodium Salt (1). Compound **1** was prepared from 100 mg (0.150 mmol) of **10** using the general procedure described above. After RP-HPLC (99% triethylammonium acetate, 1% CH_3CN (pH 8.8), isocratic, t_R = 7.7 min) and Na^+ ion exchange chromatography, 41.0 mg of a mixture of compound **1** (≈95% of the total by ^1H NMR integration of H-5) and dephosphonylated compound **11** (≈5% of the total by ^1H NMR of H-5) was obtained. ^1H NMR (D_2O, 300 MHz) δ 8.18 (d, J = 6.9 Hz, 1H), 6.60 (d, J = 6.7 Hz, 1H), 6.50 (d, J = 7.5 Hz), 5.76 (s, 1H), 4.16 (s), 4.03 (m), 4.02 (d, J = 4.4 Hz), 3.70 (dd, J = 12.9, 3.9 Hz), 2.92 (m, exchange with solvent deuterons), 2.62 (bs, exchange with solvent deuterons); ^{31}P NMR (D_2O, 121 MHz) δ 15.8, 1.7; LR$^-$ESIMS *m*/*z* (relative intensity) 321 (M − 2Na + 1, 100); LR$^+$ESIMS *m*/*z* (relative intensity) 323 (M − 2Na + 3, 100), 243 (66), 191 (65). HR$^-$ESIMS *m*/*z*: calcd for $C_{10}H_{14}N_2O_8P$ (compound **1** − 2Na + 1), 321.0488; found, 321.0497. HR$^+$ESIMS *m*/*z*: calcd for $C_{10}H_{15}N_2O_5$ (compound **11** + 1), 243.0981; found, 243.0986.

[Difluoro-{1-[3,4-bis-(benzoyloxy)-5-benzoyloxymethyl-tetrahydrofuran-2-yl]-2-oxo-1,2-dihydro-pyrimidin-4-yl}-methyl]-phosphonic Acid Dimethyl Ester (13). To a solution of compound **10** (1.00 g, 1.51 mmol, 1 equiv) and NFSi (1.18 g, 3.78 mmol, 2.5 equiv) in dry THF (50 mL) cooled to 0 °C (ice bath) and under Ar was added dropwise a 0.5 M solution of KHMDS in toluene (6.64 mL, 3.32 mmol, 2.2 equiv) over a period of 70 min. The ice bath was removed, and stirring was continued further for 2 h at room temperature. NFSi (0.476 g, 1 equiv) was added, and the reaction was cooled to 0 °C. A 0.5 M solution of KHMDS in toluene (2.0 mL, 1 mmol, 0.66 equiv) was added over a period of 20 min. The reaction was stirred at 0 °C for 30 min; the ice bath was removed, and the reaction was stirred for an additional 1 h. NH_4Cl (10%, 2 mL) was added, the reaction was filtered through Celite, and the Celite was washed with 15 mL of THF. The filtrate was concentrated to ≈15 mL, diluted with CH_2Cl_2 (50 mL), and filtered again though filter paper. The filtrate was concentrated to ≈15 mL, diluted with toluene (50 mL), filtered through filter paper, and concentrated. The residue was subjected to flash chromatography (40% hexane−60% EtOAc) which gave pure **13** as a colorless oil (0.478 g, 45% yield). ^1H NMR (300 MHz) δ 8.18 (d, J = 6.9 Hz, 1H), 8.02 (d, J = 7.2 Hz, 2H), 7.92 (d, J = 7.4 Hz, 1H), 7.87 (d, J = 7.4 Hz, 2H), 7.20−7.58 (m, 9H), 6.53 (d, J = 7.1 Hz, 1H), 6.32 (s, 1H), 5.80−5.60 (m, 2H), 4.65−4.87 (m, 3H), 3.92 (d, J = 4.7 Hz, 6H); ^{13}C NMR (75 MHz) δ 168.4 (dt, J = 25.0, 15.3 Hz), 165.9, 165.1, 165.0, 153.7, 145.8, 133.6, 133.6, 129.8, 129.7, 129.5, 129.1, 128.6, 128.4, 114.7 (dt, J = 269, 227 Hz), 100.8, 90.8, 80.8, 74.8, 70.5, 63.2, 55.5 (d, J = 6.5 Hz); ^{31}P NMR (121 MHz) δ 7.5 (t, J = 100 Hz); ^{19}F NMR (282 MHz) δ −112.3 (dd, J = 312, 100 Hz), −113.5 (dd, J = 312, 100 Hz); LR$^+$ESIMS *m*/*z* (relative intensity) 699 (M + 1, 100), 445 (37), 371 (42). HR$^+$ESIMS *m*/*z*: calcd for $C_{33}H_{30}N_2O_{11}F_2P$, 699.1555; found, 699.1572.

[Difluoro-{1-[3,4-bis-(hydroxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydro-pyrimidin-4-yl}-methyl]phosphonic Acid Bis-sodium Salt (2). Compound **2** was prepared from 52 mg (0.0745 mmol) of **13** using the general procedure described above. After RP-HPLC (98% triethylammonium acetate, 2% CH_3CN (pH 8.8), isocratic, t_R = 9.2 min) and Dowex Na^+ ion exchange chromatography, compound **2** was obtained as a white powder (15.1 mg, 51% yield). ^1H NMR (D_2O, 500 MHz) δ 8.49 (d, J = 6.9 Hz, 1H), 6.91 (d, J = 6.9 Hz, 1H), 5.86 (s, 1H), 4.27 (d, J = 4.7 Hz, 1H), 4.05−4.17 (m, 2H), 3.96 (d, J = 12.8 Hz, 1H), 3.79 (dd, J = 12.8, 4.1 Hz, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 171.9 (dt, J = 22.9, 12.6 Hz), 156.3, 145.5, 118.4 (dt, J = 265.7, 166.7 Hz), 104.1, 92.2, 83.6, 74.5, 68.2, 60.0; ^{31}P NMR (D_2O, 121 MHz) δ 4.79 (t, J = 81 Hz); ^{19}F NMR (D_2O, 282 MHz) δ −114.2 (dd, J = 287, 81 Hz), −115.4 (dd, J = 287, 81 Hz); LR$^-$ESIMS *m*/*z* (relative intensity) 357 (M − 2Na + 1, 100), 224 (44); HREIMS *m*/*z*: calcd for $C_{10}H_{12}F_2N_2O_8P$, 357.0299; found, 357.0297.

2-Benzyloxyisonicotinic Acid Benzyl Ester (21). A suspension of 2-chloroisonicotinic acid (9.00 g, 57.0 mmol), pyridine (0.227 g, 2.87 mmol), and thionyl chloride (13.6 g, 114 mmol, 2 equiv) in dry toluene (60 mL) was heated to reflux for 2 h during which the solution became homogeneous. The reaction was concentrated by high vacuum rotary evaporation. The residue was dissolved in dry toluene (30 mL) and cooled to 0 °C (ice bath). Dry pyridine (13.8 mL) was added, which was followed by benzyl alcohol (9.3 g, 86 mmol), and the mixture was stirred at room temperature for 3 h. Water was added (34 mL), which was followed by concentrated HCl (13.5 g). The layers were separated, and the aq layer was extracted with toluene (60 mL). The organic layers were combined, washed with H_2O and saturated brine, then dried ($MgSO_4$), and concentrated to give a yellow oil; this was dried under high vacuum for several hours. ^1H NMR of the residue indicated that this consisted of the benzyl ester of 2-chloroisonicotinic acid and a small amount of benzyl alcohol (10%). To a solution of benzyl alcohol (6.46 mL, 59.7 mmol) in dry DMF (24 mL) at 0 °C (ice bath) was added NaH (2.39 g, 59.7 mmol). The ice bath was removed; the mixture was stirred at room temperature for 1 h and then cooled to 0 °C. To this was added a solution of the crude benzyl ester in dry DMF (24 mL), and the mixture was stirred for 2 h. Glacial acetic acid (710 mg) was added, and the mixture was stirred for 30 min. Toluene (150 mL) was added and was followed by a solution of saturated $NaHCO_3$ (75 mL). The layers were separated, and the organic layer was washed with water and saturated brine, dried ($MgSO_4$), and concentrated. The residue was subjected to flash chromatography (80% hexane−20% EtOAc) to give compound **21** as a pale yellow solid (14.7 g, 81%). Mp 42−43 °C; ^1H NMR (300 MHz) δ 8.26 (d, J = 5.5 Hz, 1H), 7.22−7.45 (m, 12H), 5.39 (s, 2H), 5.34 (s, 2H); ^{13}C NMR (75 MHz) δ 164.7, 164.2, 147.66, 140.2, 136.9, 135.3, 128.6, 128.4, 128.3, 127.9, 116.0, 111.6, 68.0, 67.3; LREIMS *m*/*z* (relative intensity) 319 (M, 100), 213 (35), 91 (65). HREIMS *m*/*z*: calcd for $C_{20}H_{17}NO_3$, 319.1208; found, 319.1203.

(2-Benzyloxy-pyridin-4-yl)-methanol (22). A mixture of compound **21** (3.45 g, 10.8 mmol), $LiBH_4$ (0.352 g, 16.2 mmol), and dry MeOH (0.519 g, 16.2 mmol) in dry ether (40 mL) was refluxed for 1 h. A 0.3 N HCl solution was added until the reaction was slightly acidic. The mixture was extracted with CH_2Cl_2 (3 × 60 mL), and the combined organics were dried ($MgSO_4$) and concentrated. The residue was subjected to flash chromatography (80% hexane−20% EtOAc to 70% hexane−30% EtOAc) to give pure **22** as a colorless liquid (2.0 g, 86%). ^1H NMR (300 MHz) δ 8.10 (d, J = 4.8 Hz, 1H), 7.20−7.45 (m, 5H), 6.84 (d, J = 4.8 Hz, 1H), 6.80 (s), 5.35 (s, 2H), 4.65 (s, 2H), 2.16 (s, 1H); ^{13}C NMR (75 MHz) δ 163.9, 153.6, 146.5, 137.0, 128.3, 127.8, 114.8, 108.0, 67.7, 63.0; LREIMS *m*/*z* (relative intensity) 215 (M, 100), 138 (14), 109 (47), 91 (89). HREIMS *m*/*z*: calcd for $C_{13}H_{13}NO_2$, 215.0946; found, 215.0944.

2-Benzyloxy-4-bromomethylpyridine (23). To a solution of $POBr_3$ (4.89 g, 17.1 mmol, 1.2 equiv) in dry CH_2Cl_2 (40 mL) at 0 °C was added dropwise dry DMF (19 mL) over a period of 30 min. A solution of compound **22** (3.0 g, 14 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise over a period of 30 min and stirred for 1 h. The mixture was transferred via canula to a cold solution of saturated $NaHCO_3$ (100 mL) keeping the temperature below 10 °C. The layers were separated, and the aq layer was extracted with CH_2Cl_2 (3 × 60 mL). The combined organics were washed with water and saturated brine, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography (95% hexane−5% EtOAc) gave pure **23** as a white solid (2.97 g, 77%). Mp 29−30 °C; ^1H NMR (300 MHz) 8.13 (d, J = 4.5 Hz, 1H), 7.20−7.47 (m, 5H), 6.88 (d, J = 4.5 Hz, 1H), 6.80 (s, 1H), 5.37 (s, 2H), 4.32 (s, 2H); ^{13}C NMR (75 MHz) 164.0, 148.78, 147.3, 137.1, 128.4, 127.9, 127.8, 117.1, 110.9, 67.7, 30.5; LREIMS *m*/*z* (relative intensity) 277 (M, 55), 198 (100), 91 (54); HREIMS *m*/*z*: calcd for $C_{13}H_{12}ONBr$, 277.0102; found, 277.0107.

2-Hydroxy-isonicotinic Acid (25). To 2-chloroisonicotinic acid (5.00 g, 31.7 mmol) was added 90 mL of concd HCl. The suspension was heated to 140−150 °C (oil bath). The reaction initially became a solution upon heating, though after heating for 24 h again it became a suspension. Another 20 mL of concd HCl was added, the reaction became clear, heating was continued for another 24 h, after which an additional 20 mL of concd HCl was added, and the reaction was heated for 12 h. The reaction was cooled in an ice bath during which compound **25** precipitated out of solution. The mixture was diluted with an equal volume of ice-cold water and filtered, and the filter cake was washed with cold water. The precipitate was dried under high vacuum to give pure **25** as a white solid (3.92 g, 89%). NMR spectra were identical to those reported.[30] ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.67 (bs, 2H), 7.43 (d, J = 6.6 Hz, 1H), 6.77 (s, 1H), 6.47 (d, J = 6.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 166.2, 162.9, 143.0, 136.7, 121.4, 104.0.

4-Hydroxymethyl-pyridin-2-ol (26). To a solution of compound **25** (4.80 g, 34.5 mmol) in dry THF (170 mL) at 0 °C (ice bath) was added a 1 M solution of BH_3−THF (150 mL, 150 mmol). The ice bath was removed, and the reaction was stirred for 90 min. The reaction was cooled with an ice bath, and MeOH (75 mL) was added slowly. The reaction was concentrated to dryness. MeOH (100 mL) was added to the solid residue, stirred for 15 min, and then concentrated to dryness. The residue was suspended in 20% MeOH−80% $CHCl_3$, stirred for 5 min, and then filtered. The precipitate was subjected to this again; the two filtrates were combined and concentrated to give crude **26** as a pale yellow solid. Recrystallization in absolute ethanol gave pure **26** as a white solid (1.52 g). The filtrate from the recrystallization was concentrated, and the residue was purified by flash chromatography (20% EtOH−80% EtOAc and then 20% MeOH−80% $CHCl_3$) to get an additional 0.7 g of pure **26** (total yield: 2.22 g, 54%). Mp 144−145 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.40 (d, J = 6.8 Hz, 1H), 6.60 (s, 1H), 6.40 (d, J = 6.8 Hz, 1H), 4.54 (s, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 164.6, 158.2, 133.8, 114.3, 105.5, 61.8; LREIMS *m*/*z* (relative intensity) 125 (M, 100), 96 (24), 80 (22). HREIMS *m*/*z*: calcd for $C_6H_7NO_2$, 125.0477; found, 125.0478.

Methanesulfonic Acid 4-Bromomethyl-pyridin-2-yl Ester (27). To a solution of compound **26** (0.125 g, 1.00 mmol) and 2,6-lutidine (0.670 g, 2.50 mmol, 2.50 equiv) in dry CH_2Cl_2 (4 mL) was added methanesulfonylbromide (0.397 g, 2.5 mmol, 2.5 equiv). The reaction was stirred for 48 h, diluted with CH_2Cl_2 (40 mL), and washed with 0.3 N HCl and H_2O. The organic layer was dried ($MgSO_4$) and concentrated, and the resulting residue was purified by flash chromatography (70% hexane−30% EtOAc) to give pure **27** as a colorless oil (187 mg, 71%). ^{1}H NMR (300 MHz) δ 8.26 (d, J = 5.0 Hz, 1H), 7.25 (d, J = 5.0 Hz, 1H), 7.07 (s, 1H), 4.36 (s, 1H), 345 (s, 1H); ^{13}C NMR (75 MHz) δ 157.6, 151.1, 148.3, 122.7, 115.1, 40.7, 29.2; LREIMS *m*/*z* (relative intensity) 267 (M (^{81}Br), 40), 265 (M (^{79}Br), 40), 187 (100), 108 (35), 80 (38); HREIMS *m*/*z*: calcd for $C_7H_8BrNO_3S$, 264.9408; found, 264.9408.

Methanesulfonic Acid 4-(Dimethoxyphosphorylmethyl)pyridin-2-yl Ester (28). A solution of compound **27** (1.38 g, 5.20 mmol) in $P(OMe)_3$ (20 mL) was heated at 100 °C for 2.5 h. The reaction was concentrated by high vacuum rotary evaporation, and the resulting residue was purified by flash chromatography (5% MeOH−95% $CHCl_3$) to give pure **28** as a pale yellow oil which solidified after we subjected the oil to high vacuum overnight (1.43 g, 93%). Mp 80−81 °C; ^{1}H NMR (300 MHz) δ 8.15 (d J = 4.9 Hz, 1H), 7.14 (bs, 1H), 6.94 (s, 1H), 3.61 (d, J = 11.4 Hz, 6H), 3.38 (s, 3H), 3.10 (d, J = 22.5 Hz, 2H); ^{13}C NMR (75 MHz) δ 157.4 (d, J = 2.7 Hz), 147.7 (d, J = 2.5 Hz), 146.0 (d, J = 8.6 Hz), 123.9 (d, J = 5.6 Hz), 116.2 (d, J = 6.2 Hz), 52.9 (d, J = 6.9 Hz), 40.4, 32.0 (d, J = 137 Hz); ^{31}P NMR (121 MHz) δ 27.2; LREIMS *m*/*z* (relative intensity) 295 (M, 73), 217 (100), 121 (21). HREIMS *m*/*z*: calcd for $C_9H_{14}NO_6PS$, 295.0279; found, 295.0285.

(2-Hydroxypyridin-4-ylmethyl)phosphonic Acid Dimethyl Ester (16). To a solution of phosphonate **28** (1.36 g, 4.61 mmol) in THF (35 mL) at 0 °C (ice bath) was added an ice cold solution of LiOH hydrate (386 mg, 9.22 mmol) in H_2O (35 mL) over a period of 10 min. After the addition, the solution was stirred for 5 min, and then the ice bath was removed and stirred for 4.5 h at room temperature. The reaction was neutralized with 0.3 N HCl and concentrated by high vacuum rotary evaporation, and the residue was purified by flash chromatography (20% MeOH−80% $CHCl_3$) to give compound **16** as a white solid (0.758 g, 76%). Mp 132−134 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.36 (d, J = 6.7 Hz, 1H), 6.47 (s, 1H), 6.38 (d, J = 6.7 Hz, 1H), 3.74 (d, J = 11.1 Hz, 6H), 3.21 (d, J = 22.8 Hz, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ 164.0, 148.2 (d, J = 9.1 Hz), 134.3, 119.8 (d, J = 8.3 Hz), 109.4 (d, J = 4.0 Hz), 52.6 (d, J = 7.1 Hz), 31.2 (d, J = 136.1 Hz); ^{31}P NMR (CD_3OD, 121 MHz) δ 29.4; LREIMS *m*/*z* (relative intensity) 217 (M, 100), 185 (15), 121 (44), 109 (37). HREIMS *m*/*z*: calcd for $C_8H_{12}NO_4P$, 217.0504; found, 217.0497.

{1-[3,4-Bis-(benzoyloxy)-5-benzoyloxymethyltetrahydro-furan-2-yl]-2-oxo-1,2-dihydro-pyridin-4-ylmethyl}phosphonic Acid Dimethyl Ester (29). Phosphonate **16** (0.300 g, 1.37 mmol, 1 equiv) and acetyl 2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (0.691 g, 1.37 mmol) were suspended in dry CH_3CN (6 mL) under Ar at 0 °C (ice bath). To this was added BTMSTFA (0.40 mL, 1.5 mmol, 1.1 equiv); the ice bath was removed, and the solution was stirred for 1.5 h. TMSOTf (0.247 mL, 1.37 mmol, 1 equiv) was added, and the solution was stirred for 3 h. The reaction was diluted with EtOAc (200 mL) and ether (100 mL) and washed with saturated $NaHCO_3^-$ and saturated brine. The organic layer was dried ($MgSO_4$) and concentrated, and the resulting residue was subjected to flash chromatography (5% EtOH−95% EtOAc) to give pure compound **29** as a white foam (0.59 g, 65%). ^{1}H NMR (300 MHz) δ 8.09 (d, J = 7.6, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 7.3 Hz, 2H), 7.30−7.63 (m, 9H), 6.56 (d, J = 3.9 Hz), 6.40 (s, 1H, 3H), 6.10 (d, J = 7.2 Hz, 1H), 5.89 (overlapping dd, J = 5.8 Hz), 5.78 (overlapping dd, J = 5.1 Hz, 1H), 4.60−4.90 (m, 3H), 3.72 (d, J = 11.2 Hz, 6H), 2.93 (d, J = 22.5 Hz, 2H); ^{13}C NMR (75 MHz) δ 166.0, 165.2, 165.0, 161.5 (d, J = 2.2 Hz), 145.2 (d, J = 8.6 Hz), 133.5, 133.4, 132.1, 129.8, 129.7, 129.6, 129.3, 128.6, 128.5, 128.3, 121.1 (d, J = 9.9 Hz), 108.4 (d, J = 4.2 Hz), 88.3, 80.0, 74.8, 70.8, 63.6, 53.0 (d, J = 7.1 Hz), 52.9 (d, J = 6.3 Hz), 32.5 (d, J = 137.5 Hz); ^{31}P NMR (121 MHz) δ 27.6; LREIMS *m*/*z* (relative intensity) 661 (M, 1), 539 (24), 445 (28), 282 (79), 105 (100). HREIMS *m*/*z*: calcd for $C_{34}H_{32}NO_{11}P$, 661.1717; found, 661.1707.

{1-[3,4-Bis-(hydroxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-ylmethyl}phosphonic Acid (3). Compound **3** was prepared from 115 mg (0.242 mmol) of **29** using the general procedure. After RP-HPLC (90% TFA (0.1%) in water−1% CH_3CN, isocratic, t_R = 9.0 min), compound **3** was obtained as a white powder (69.8 mg, 90% yield). ^{1}H NMR (D_2O, 300 MHz) δ 7.70 (d, J = 6.7 Hz, 1H), 6.36 (d, J = 6.7 Hz, 1H), 6.35 (s, 1H), 5.88 (s, 1H), 4.06 (s, 1H), 3.97 (s, 2H), 3.76 (d, J = 12.7 Hz, 1H), 3.63 (d, J = 12.7 Hz), 2.90 (d, J = 21.9 Hz, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 163.1, 150.1 (d, J = 7.2 Hz), 132.7, 118.4 (d, J = 6.5 Hz), 111.5, 90.2, 83.7, 74.8, 68.9, 60.4, 34.8 (d, J = 28.5 Hz); ^{31}P NMR (D_2O, 121 MHz) δ 23.0; LR$^-$ESIMS *m*/*z* (relative intensity) 320 (M − 1, 100). HR$^-$ESIMS *m*/*z*: calcd for $C_{11}H_{15}NO_8P$, 320.0535; found, 320.0540.

(2-Benzyloxypyridin-4-ylmethyl)phosphonic Acid Diethyl Ester (31). To a solution of bromide **23** (10.7 mmol) in dry benzene (130 mL) was added $P(OEt)_3$ (18.4 mL, 107 mmol), and the mixture was heated to 95 °C (oil bath) for 16 h. $P(OEt)_3$ (9.20 mL, 53.0 mmol) was added, the mixture was heated to 105 °C, and the benzene was slowly distilled off. When approximately 85 mL of benzene was removed, the temperature was lowered to 95 °C, and heating continued for another 2 h. The benzene was removed by

(30) Rollet, F.; Richard, C.; Pilichowski, J.-F.; Aboab, B. *Org. Biomol. Chem.* **2004**, *2*, 2253.

rotary evaporator, and excess phosphate and diethyl ethylphosphonate were removed using a high vacuum rotary evaporator. Purification of the residue by flash chromatography (20% hexane−80% EtOAc to 10% hexane−90% EtOAc) gave pure **31** as a colorless oil (3.34 g, 93%). ^{1}H NMR (300 MHz) δ 8.01 (d, J = 4.9 Hz), 7.20−7.40 (m, 5H), 6.79 (bs, 1H), 6.67 (s, 1H), 5.29 (s, 2H), 3.90−4.05 (m), 3.00 (d, J = 21.8 Hz), 1.18 (t, J = 7.9 Hz, 6H); ^{13}C NMR (75 MHz) δ 163.8 (d, J = 2.2 Hz), 146.7 (d, J = 3.7 Hz), 143.7 (d, J = 8.7 Hz), 137.1, 128.2, 127.7 (CH_{arom}), 127.6 (CH_{arom}), 118.4 (d, J = 5.4 Hz), 112.0 (d, J = 7.5 Hz), 67.4 ($PhCH_2$), 62.2 (d, J = 6.5 Hz), 33.2 (d, J = 137.4 Hz), 16.2 (d, J = 6.6 Hz); ^{31}P NMR (121 MHz) δ 25.9; LREIMS *m*/*z* (relative intensity) 335 (M, 100), 258 (28), 229 (25), 91 (51). HREIMS *m*/*z*: calcd for $C_{17}H_{22}NO_4P$, 335.1286; found, 335.1292.

[(2-Benzyloxypyridin-4-yl)difluoromethyl]phosphonic Acid Diethyl Ester (32). To a solution of phosphonate **31** (3.10 g, 9.25 mmol) in dry THF (75 mL) was added NFSi (7.29 g, 23.1 mmol). The solution was cooled to −78 °C, and KHMDS (40.7 mL of a 0.5 M solution in toluene, 20.3 mmol) was added dropwise over 2 min. The mixture was stirred for 30 min at −78 °C; the cold bath was removed, and the mixture was stirred for 1 h. The reaction was quenched with water (300 mL) and then extracted with CH_2Cl_2 (3 × 300 mL). The organics were dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography (30% hexane−70% EtOAc) gave pure **32** as a colorless oil (2.97 g, 87%). ^{1}H NMR (300 MHz) δ 8.24 (d, J = 5.2 Hz, 1H), 7.20−7.48 (m, 5H), 7.08 (d, J = 4.8 Hz), 7.01 (s, 1H), 5.38 (s, 2H), 4.10−4.30 (m, 4H), 1.30 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz) δ 163.7, 147.4, 143.8 (dt, J = 22.5, 13.9 Hz), 136.8, 128.3, 127.8, 126.1, 116.6 (dt, J = 264.3, 214.3 Hz), 113.6 (t, J = 6.1 Hz), 108.7 (dt, J = 2.2, 7.8 Hz), 67.9, 66.0 (d, J = 6.3 Hz), 16.2 (d, J = 5.4 Hz); ^{31}P NMR (121 MHz) δ 6.8 (t, J = 10.2 Hz); ^{19}F NMR (282 MHz) δ −110.7 (d, J = 90.3 Hz); LREIMS *m*/*z* (relative intensity) 371 (M, 100), 265 (20), 233 (19), 91 (59). HREIMS *m*/*z*: calcd for $C_{17}H_{20}F_2NO_4P$, 371.1098; found, 371.1100.

[Difluoro-(2-hydroxy-pyridin-4-yl)-methyl]-phosphonic Acid Diethyl Ester (33). To a solution of **32** (2.1 g, 5.7 mmol) in MeOH (30 mL) was added 10% Pd/C (200 mg), and the reaction was stirred for 3 h. The reaction was filtered through Celite, and the filtrate was concentrated. Purification of the residue by flash chromatography (5% MeOH−95% $CHCl_3$) gave pure **33** as a white solid (1.58 g, 99%). Mp 74−76 °C; ^{1}H NMR (300 MHz) δ 7.43 (d, J = 6.9 Hz, 1H), 6.79 (s, 1H), 6.50 (d, J = 6.9 Hz, 1H), 4.10−4.34 (m, 4H), 1.34 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz) δ 164.5, 146.4 (dt, J = 22.3, 13.8 Hz), 135.5, 118.2, 116.1 (dt, J = 214.0, 264.2 Hz), 103.9 (t, J = 5.0 Hz), 65.3 (d, J = 6.8 Hz), 16.3 (d, J = 5.0 Hz); ^{31}P NMR (121 MHz) δ 6.3 (t, J = 108.2 Hz); ^{19}F NMR (282 MHz) δ 112.4 (d, J = 108.2 Hz); LREIMS *m*/*z* (relative intensity) 281 (M, 90), 225 (90), 145 (100), 109 (64). HREIMS *m*/*z*: calcd for $C_{10}H_{14}F_2NO_4P$, 281.0629; found, 281.0628.

[Difluoro-{1-[3,4-bis-(acetoxy)-5-acetoxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydro-pyridin-4-yl}-methyl]phosphonic Acid Diethyl Ester (34). To a solution of phosphonate **33** (0.10 g, 0.36 mmol) and acetyl 2,3,5-tri-*O*-acetyl-β-D-ribofuranose (0.113 g, 0.36 mmol) in dry CH_3CN (2 mL) was added BTMSTFA (0.113 mL, 0.42 mmol), and it was stirred at rt for 2 h. To this was added TMSOTf (0.077 mL, 0.42 mmol), and that was stirred for 3 h. The reaction was diluted with EtOAc (40 mL), washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography (20% hexane−80% EtOAc) gave pure **34** as a white foam (0.15 g, 78%). ^{1}H NMR (300 MHz) δ 7.57 (d, J = 7.4 Hz, 1H), 6.65 (s, 1H), 6.36 (d, J = 7.4 Hz, 1H), 6.17 (d, J = 4.1 Hz, 1H), 5.20−5.35 (m, 2H), 4.10−4.38 (m, 6H), 2.05 (s, 3H), 2.30 (s, 3H), 2.00 (s, 3H), 1.3 (d, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz) δ 170.0, 169.3, 169.1, 160.9, 144.5 (dt, J = 22.6, 14.1 Hz), 132.8, 118.9 (dt, J = 5.6, 3.2 Hz), 115.9 (dt, J = 263.9, 214.4 Hz), 103.0 (t, J = 5.3 Hz), 88.1, 79.5, 73.8, 69.4, 65.1 (d, J = 6.8 Hz), 62.5, 20, 20.3, 16.1 (d, J = 5.4 Hz); ^{31}P NMR (121 MHz) δ 6.1 (t, J = 108 Hz); ^{19}F NMR (282 MHz) δ −112.7 (d, J = 108 Hz); LREIMS *m*/*z* (relative intensity) 539 (M, 10), 321 (45), 259 (100), 139 (73). HREIMS *m*/*z*: calcd for $C_{21}H_{28}F_2NO_{11}P$, 539.1368; found, 539.1371.

[Difluoro-{1-[3,4-bis-(hydroxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydro-pyridin-4-yl}-methyl]phosphonic Acid (4). Compound **4** was prepared from 88 mg (0.164 mmol) of **34** using the general procedure. However, the TMSBr was added in portions starting with 4 equiv of TMSBr at the beginning of the reaction. After 24 h, another 2 equiv of TMSBr was added followed by an additional 2 equiv after 48 h. After RP-HPLC (99% TFA (0.1%) in water−1% CH_3CN, isocratic, t_R = 9.4 min) and lyophilization, compound **4** was obtained as a white powder (26.3 mg, 45% yield). ^{1}H NMR (D_2O, 300 MHz) δ 7.88 (d, J = 7.4 Hz, 1H), 6.62 (s, 1H), 6.54 (d, J = 7.4 Hz, 1H), 5.96 (s, 1H), 4.12 (s, 1H), 4.03 (s, 2H), 3.83 (d, J = 12.8 Hz, 1H), 3.69 (d, J = 12.8 Hz, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 163.2, 148.3 (dt, J = 22.8, 12.0 Hz), 133.6, 117.9 (dt, J = 163.0, 71.4 Hz), 116.7 (t, J = 7.4 Hz), 105.6 (t, J = 5.3 Hz), 90.5, 83.7, 74.8, 68.8, 60.4; ^{31}P NMR (D_2O, 121 MHz) δ 4.0 (t, J = 96.0 Hz); ^{19}F NMR (D_2O, 282 MHz) δ −113.0 (d, J = 97.0 Hz); LR$^-$ESIMS *m*/*z* (relative intensity) 356 (M − 1, 100), 224 (20). HR$^-$EIMS *m*/*z*: calcd for $C_{11}H_{13}F_2NO_8P$, 356.0347; found, 356.0359.

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Supporting Information Available: NMR spectra (^{1}H, ^{13}C, ^{31}P (when applicable), and ^{19}F spectra (when applicable)) for compounds **1**−**5**, **7**, **9**, **10**, **13**, **16**, **21**−**23**, **25**−**29**, and **31**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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