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Bismethylene Triphosphate Nucleotides of Uridine 4-Phosphate Analogues: A New Class of Anionic Pyrimidine Nucleotide Analogues

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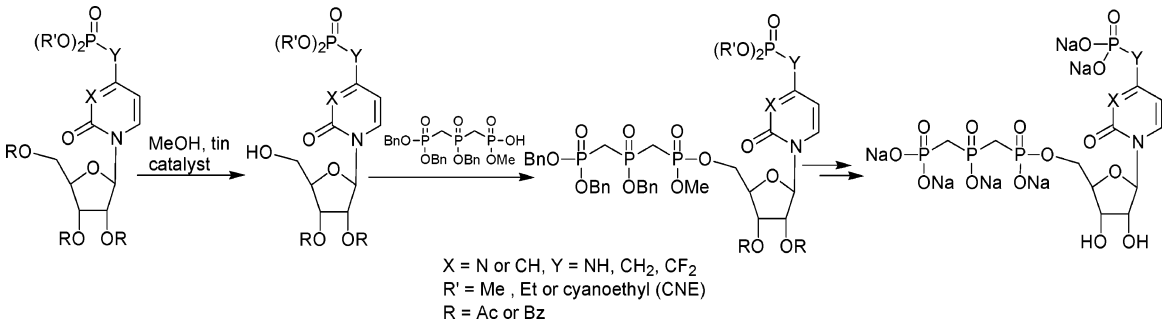

Cytidine-5′-triphosphate synthase (CTPS) catalyzes the formation of cytidine triphosphate (CTP) from glutamine, uridine 5′-triphosphate (UTP), and adenosine 5′-triphosphate (ATP). This reaction proceeds via formation of the high-energy intermediate UTP-4-phosphate (UTP-4-P). Stable analogues of UTP-4-P may be potent inhibitors of CTPS and useful as lead structures for the development of anticancer and antiviral agents. Several bismethylene triphosphate (BMT) nucleotides of uridine 4-phosphate (U-4-P) analogues have been prepared. A key step was the selective methanolysis, with the aid of a tin catalyst, of the 5′ ester moiety of 2′,3′,5′-tri-*O*-acetyl or tri-*O*-benzoyl U-4-P analogues. We believe this represents the first general approach to the selective cleavage of 5′ benzoyl esters in benzoylated nucleosides. Mitsunobu coupling of these 5′-deprotected U-4-P analogues to an unsymmetrical, protected BMT bearing a free phosphonic acid moiety at one of the terminal positions gave fully protected BMT-U-4-P analogues. Global deprotection of these species was achieved using TMSBr followed by treatment with $NH_4OH-MeOH$ or NH_4OH-pyridine. The resulting BMT nucleotides represent a new class of anionic pyrimidine nucleotide analogues.

Introduction

Nucleoside analogues have now been in use as antiviral and anticancer agents for several decades.[1a,b] Consequently, their synthesis continues to be an area of considerable importance to the pharmaceutical industry. Our interest in nucleoside and nucleotide analogues is a result of our desire to prepare inhibitors of cytidine 5′-triphosphate synthase (CTPS, EC 6.3.4.2). CTPS catalyzes the adenosine 5′-triphosphate (ATP)-dependent formation of cytidine 5′-triphosphate (CTP) from uridine 5′-triphosphate (UTP) using either ammonia or L-glutamine as the nitrogen source (Scheme 1). Numerous lines of evidence[2a−h] suggest that CTPS performs this reaction by transferring the γ-phosphate from ATP to the oxygen at position 4 of UTP to form UTP-

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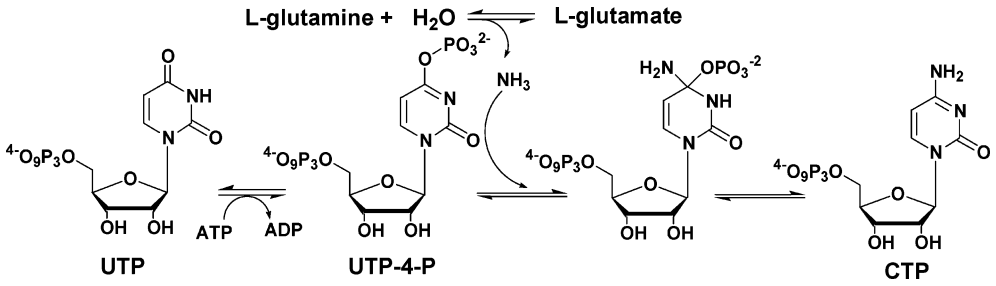
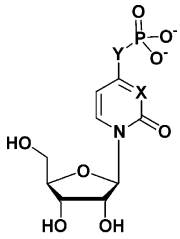
SCHEME 1. Reaction Catalyzed by CTPS



4-phosphate (UTP-4-P) as an intermediate and ADP. Ammonia, derived from the hydrolysis of glutamine or free in solution, then displaces the phosphate at *O*-4 of UTP-4-P to give CTP.

CTPS activity is elevated in some forms of leukemia[3a,b] and some solid tumors.[4] Because CTP plays a central role in the biosynthesis of phospholipids[5] and nucleic acids,[6] CTPS is a recognized target for the development of antineoplastic agents.[6,7] For example, the nucleoside derivative, 3-deazauridine (3-DaU), has been shown to induce apoptosis in myeloid leukemia cells in a dose-dependent manner.[8] 3-DaU, as well as another nucleoside analogue, cyclopentenylcytosine (CPEC), enhances the effects of various anticancer and antiviral agents suggesting that they could be employed in combination drug therapy.[9a−f] Studies suggest that 3-DaU and CPEC are triphosphorylated intracellularly and it is most likely that it is the triphosphorylated forms that are biologically active.[10,11] In addition, CTPS is a target for the development of antiviral[7,12] and antiprotozoal agents.[13]

One method of generating potent inhibitors of enzymes is to use analogues of either the transition state of the reaction or a high-energy intermediate formed during catalysis.[14a,b] We, therefore, anticipated that phosphonate and phosphoramidate nucleosides **1**−**6** (Figure 1), upon conversion to their respective nucleotide triphosphates, would serve as structural and electronic mimics of UTP-4-P and, consequently, be potent inhibitors of CTPS (Figure 1a). Recently, we described the synthesis of nucleosides **1**−**4**.[15]



1, X = N, Y = CH_2
2, X = N, Y = CF_2
3, X = CH, Y = CH_2
4, X = CH, Y = CF_2
5, X = N, Y = NH
6, X = CH, Y = NH

FIGURE 1. Structures of compounds **1**−**6**.

Although there are a few reports of phosphonate[16] and phosphoramidate[17] derivatives of the nucleosides uridine and cytidine, to our knowledge, there is only a single report of a nucleotide analogue bearing anionic groups on a pyrimidine.[18] Conversion of nucleosides to their corresponding 5′-triphosphates is often difficult because of the low yields, difficulties involved in large-scale purification, and the inherent instability of the compounds to hydrolysis. This transformation is especially challenging when a phosphorus-based substituent, such as a phosphonate or phosphoramidate moiety, is already present in the nucleoside precursor. However, nucleoside analogues are often converted into hydrolytically stable nucleotides by the attachment of nonhydrolyzable di- or triphosphate mimics for in vitro characterization of some of their biological properties and the methylene group has been widely used to replace the labile pyrophosphate oxygens.[19a−k] Expanding on the pioneering work of Mioskowski and co-workers,[20a−c] we recently reported the synthesis of bismethylene triphosphate (BMT) analogues of nucleosides by coupling compound **7** to the nucleoside followed by global deprotection of the resulting protected BMT nucleotide **8** (Scheme 2).[21] This methodology was used to

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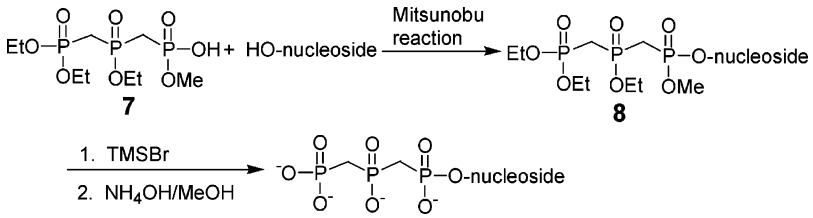
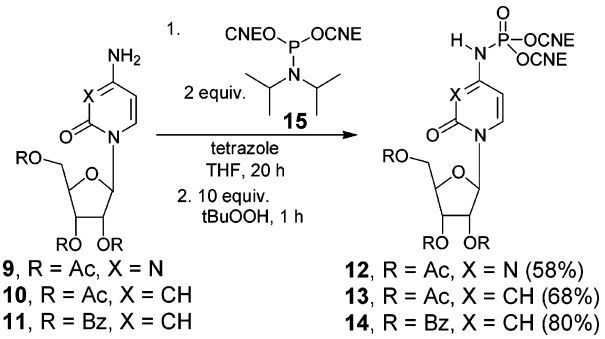
SCHEME 2. General Approach to the Synthesis of BMT Nucleotides



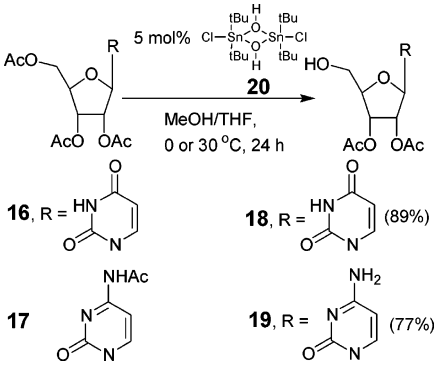
SCHEME 3. Synthesis of Phosphoramidates 12−14



9, R = Ac, X = N
10, R = Ac, X = CH
11, R = Bz, X = CH

12, R = Ac, X = N (58%)
13, R = Ac, X = CH (68%)
14, R = Bz, X = CH (80%)

SCHEME 4. Selective 5′-Deacylation of 16 and 17 by Orita et al.



prepare the first BMT analogues of UTP and CTP.[21] Herein, we report the first synthesis of phosphonate− and phosphoramidate−pyrimidine BMT analogues of the UTP-4-P intermediate formed during catalysis by CTPS. We also describe what we believe is the first general approach to achieve selective 5′-*O*-debenzoylations of perbenzoylated pyrimidine nucleosides.

Results and Discussion

Previously, we described the synthesis of the U-4-P analogues **1**−**4** with their hydroxyl groups protected as either benzoate or acetate esters and the phosphonate groups protected as methyl or ethyl esters.[15] We have also prepared 3-deaza phosporamidate analogues of U-4-P in which 4-*O* is replaced with an NH (i.e., **12**−**14**). Phosphoramidate **12**, in which the phosphoramidite moiety is protected with a cyanoethyl (CNE) (Scheme 3), was prepared by using a procedure similar to that developed by Wada et al. for the synthesis of the *O*-benzoylated derivative.[17] Thus, 2′,3′,5′-tri-*O*-acetyl cytidine (**9**) was reacted with reagent **15** in the presence of tetrazole and the resulting nucleoside phosphoramidite was oxidized in situ to give phosphoramidate **12** in a 58% yield. The acetyl- and benzoyl-protected 3-deaza derivatives of **12**, compounds **13** and **14**, were prepared in 68% and 80% yields, respectively, using a similar approach starting from compounds **10** and **11**.[22]

We previously reported the synthesis of compounds **1**−**4** with the phosphonate moiety protected as alkyl esters and the 2′, 3′, and 5′ hydroxyl groups protected as acetyl or benzoyl esters. To make the corresponding BMT nucleotides from these protected nucleosides, as well as from nucleosides **12**−**14**, it was necessary to deprotect the 5′-position and then follow the route outlined in Scheme 2. The selective cleavage of 5′-acetates in acetylated nucleosides has been achieved by using enzymes though this has yet to be shown to be of general utility.[23] Relatively recently, Ren et al. reported selective cleavage of 5′-acetates in acetylated nucleosides in yields ranging from 55% to 69% using 1% iodine in methanol at reflux.[24] Orita et al. described the selective deprotection of the 5′-acetate ester moieties in 2′,3′,5′-tri-*O*-acetyl uridine (**16**) and 2′,3′,5′-*O*,N^4-tetraacetyl cytidine (**17**) in 89% and 77% yields, respectively, by subjecting these compounds to methanol in the presence of 5 mol % tin catalyst **20** at 0−30 °C for 24 h (Scheme 4).[25] The good yields reported by Orita et al. prompted us to examine this approach for the deacetylation of some of our protected U-4-P analogues.

We first examined selective 5′-deacetylations starting with model compound **21**, which was readily obtained (Table 1, entry 1).[26] Conducting the reaction in MeOH at room temperature with 6.5 mol % catalyst, the reaction was complete within 18 h and compound **24** was obtained in a 75% yield. Applying

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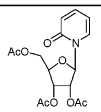
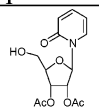
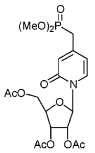
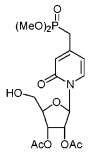
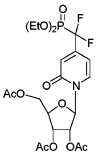
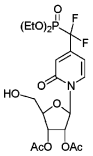
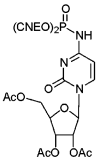
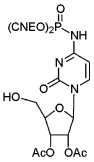
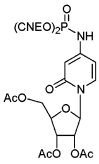
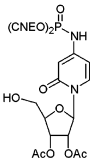
TABLE 1. 5′-*O*-Deacetylation of Nucleosides 12, 13, and 21−23 with Catalyst 20[a]

entry	reactant	time (h)	product	% yield[b]
1	 **21**	18	 **24**	75
2	 **22**	18	 **25**	87
3	 **23**	9	 **26**	81
4	 **12**	8	 **27**	84
5	 **13**	6.5	 **28**	85

[a] All reactions were conducted in dry MeOH at room temperature with 6.5 mol % catalyst **20**. [b] Percent yield of 5′-*O*-deacetylated product after chromatography.

these conditions to U-4-P analogues **12**, **13**, **22**, and **23**, the desired deacetylated products **25**−**28** were obtained in 81−87% yield. It appears that the presence of the nitrogen at the 3-position of the base and the type of substituent at the 4-position, whether electron donating or withdrawing, have little effect on the yield; however, the reaction proceeded faster with compounds **12**, **13**, and **23** (6.5−9 h) compared to compounds **21** and **22**, which required 18 h for the reaction to go to completion.

Some of our U-4-P analogues were prepared with the OH groups protected as benzoyl esters. The success we obtained with the deacetylations prompted us to examine whether we could also use Orita's catalyst to perform selective debenzoylations of these protected U-4-P analogues. To our knowledge, only one report has appeared describing a selective cleavage of the 5′-*O*-benzoyl ester of a benzoylated nucleoside. This was achieved by Chavis et al., who subjected 2′,3′,5′-tri-*O*-benzoyluridine to $NaBH_4$ in anhydrous EtOH for 3 h and obtained a 56% yield of the 5′-*O*-debenzoylated product.[27] We also performed this reaction using 2′,3′,5′-tri-*O*-benzoyluridine. However, in our hands this reaction proceeded in only a 34% yield and purification was difficult. Although Orita et al. did not report the selective cleavage of the primary benzoates over that of the secondary benzoyl esters, they did report that subjecting the benzoate ester of phenethyl alcohol to 5 mol % catalyst **20** at 30 °C for 24 h resulted in 15% cleavage.[25] This suggested to us that selective 5′-*O*-debenzoylation of nucleosides might be achieved by increasing the amount of catalyst, and/or reaction time, and/or temperature. Unfortunately, debenzoylation of 2′,3′,5′-tri-*O*-benzoyluridine (**29**) with 5 mol % **20** in refluxing anhydrous methanol proceeded very slowly and increasing the amount of catalyst to 17 mol % resulted in a complex mixture of products. However, by using 17 mol % catalyst and by maintaining the temperature at 66 °C, an 81% yield of the 5′-*O*-debenzoylated product **34** was obtained after 48 h (Table 2, entry 1). Applying these conditions to compound **30**, we were able to obtain an 84% yield of the debenzoylated product **35** (entry 2). Studies were conducted with nucleoside **30** to determine if the amount of catalyst could be reduced and it was found that compound **35** could also be obtained in good yield by using 10 mol % catalyst though the reaction took 75 h (entry 3). Using 17 mol % catalyst at 66 °C, the 5′-*O*-benzoate esters of compounds **31**[15] and **32**[15] were cleaved in excellent yields (entries 4 and 5). Indeed, the yields for debenzoylation of **30**−**32** (entries 2−5, Table 2) were superior to the yields obtained for their acetylated counterparts (entries 1−3, Table 1). Subjecting compound **14** to 17 mol % catalyst in MeOH at 66 °C resulted in cleavage of the acid-sensitive phosphoramidate group. Nevertheless, the desired debenzoylated product **38** was obtained in 56% yield by conducting the reaction in MeOH−THF (1:1), increasing the amount of catalyst to 47 mol %, lowering the temperature to 48 °C, and letting the reaction proceed for 72 h (entry 6). Subjecting compound **33**[15] to the conditions employed for compounds **29**−**32** or compound **14** did not yield the desired product **39**. The ^{1}H NMR spectrum of the crude reaction mixture suggested that depyrimidinylation occurred indicating that this debenzolyation procedure may not be suitable when the pyrimidine bears an electron-withdrawing group at the 4-position.[28]

We began our studies on the synthesis of the BMT derivatives of the U-4-P analogues by coupling compound **7** to nucleoside **36** using a Mitsonobu reaction (Scheme 5). However, due to the highly polar nature of the protected BMT product **40**, it proved quite challenging to purify the product and the yield was only 15%. To decrease the polarity of the coupled products and thereby facilitate their purification, we replaced the ethyl groups in **7** with benzyl groups using the procedure outlined in Scheme 6. Compound **41**[21] was treated with excess TMSBr for 30 h followed by MeOH to give the crude acid. Treatment of this crude acid with excess tribenzylorthoformate at 150 °C for 2.5 h gave compound **42** in excellent yield (91%). Reaction of **42** with trimethylphosphite gave compound **43** in a 61% yield. Treatment of compound **43** with KCN[29] followed by exchange of the potassium salt with H^+ using a strong ion exchange matrix gave acid **44** in 66% yield.

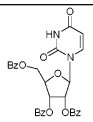
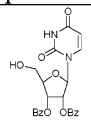
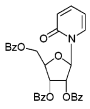
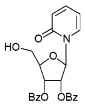
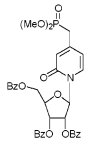
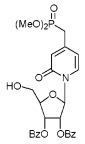
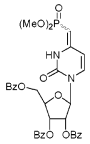
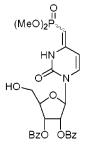
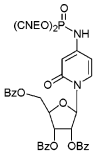
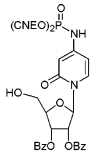
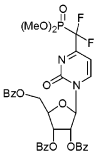
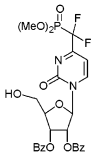
Compounds **26** and **35**−**37** were coupled to compound **44** using a Mitsunobu reaction in 50−72% yield and the products,

(27) Chavis, C.; Dumont, F.; Wightman, H.; Zeigler, H. C.; Imbach, J. L. *J. Org. Chem.* **1982**, *47*, 202.

(28) We were unable to develop conditions that would allow us to selectively deprotect **33**. The fluorines in **33** are introduced by electrophilic fluorination of **32** (see ref 15). Consequently, acetyl protection of the hydroxyl groups was not an option. We also prepared compound **33**, albeit in low yield, by electrophilic fluorination of compound **32** except the 5′OH was protected with a dimethoxytrityl (DMT) group. Surprisingly, all attempts to remove the DMT group resulted in the formation of two inseparable products in modest yield. ^{1}H, ^{19}F, ^{31}P NMR suggested that one of these compounds was compound **39** but we could not obtain it in pure form.

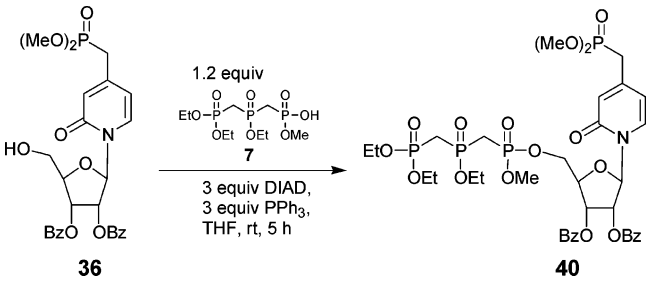
(29) Saady, M.; Lebeau, L.; Mioskowski, C. *Helv. Chim. Acta* **1995**, *78*, 670.

TABLE 2. 5′-*O*-Debenzoylation of Nucleosides 14 and 29−33 with Catalyst 20

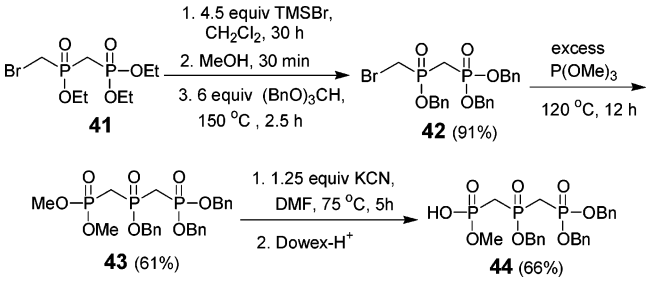
entry	reactant	mol % **20**	time (h)	temp (°C)	product	% yield[a]
1[b]	 **29**	17	48	66	 **34**	81
2[b]	 **30**	17	48	66	 **35**	84
3[b]	**30**	10	75	66	**35**	86
4[b]	 **31**	17	48	66	 **36**	90
5[b]	 **32**	17	48	66	 **37**	93
6[c]	 **14**	46	72	48	 **38**	56
						
7[b]	**33**	17	48	66	**39**	0
8[c]	**33**	40	48	48	**39**	0

[a] Percent yield of 5′-*O*-debenzoylated product after chromatography. [b] Reaction performed in anhydrous MeOH. [c] Reaction performed in anhydrous MeOH−THF (1:1).

SCHEME 5. Coupling of 7 to Nucleoside 36



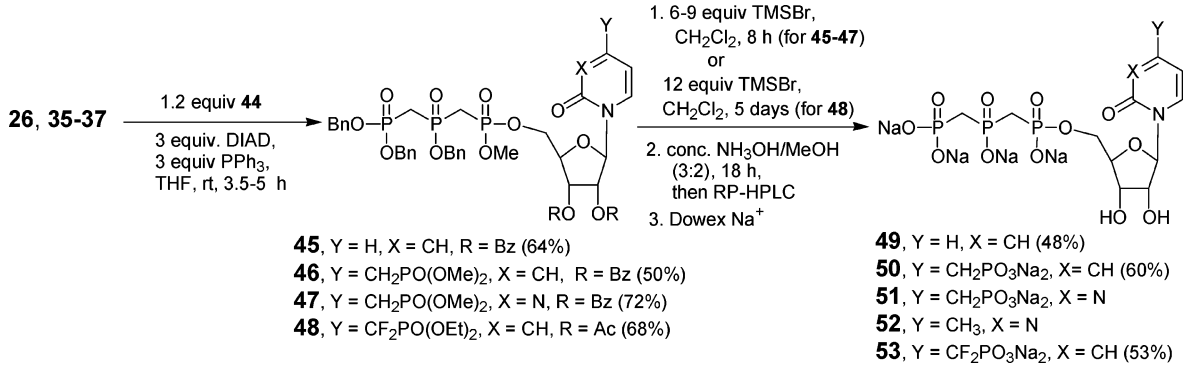
SCHEME 6. Synthesis of Compound 44



45−**48**, were readily purified by column chromatography (Scheme 7). Global deprotection of compounds **45**−**47** was accomplished by treatment with 6−9 equiv of TMSBr for 8 h followed by ammonium hydroxide in methanol. The resulting ammonium salts were purified using HPLC and then converted to their corresponding sodium salts **49**−**51**. BMT's **49** and **50** were obtained cleanly in a 60% yield. We previously reported that nucleoside **1** undergoes a slow dephosphonylation reaction to give 1-(β-D-ribofuranosyl)-4-methyl-2-pyrimidinone and we were unable to obtain **1** in pure form even after HPLC.[15]

Similarly, we were unable to obtain **51** in pure form and the dephosphonylated product **52** consisted of about 15% of the total product (∼54% overall yield). Due to the presence of the ethyl groups in fluorophosphonate **48**, 12 equiv of TMSBr and 5 days were required for complete phosphonate deprotection. Surprisingly, after treatment of the crude product with ammonium hydroxide, HPLC purification, and ion exchange chromatography, the yield of BMT **53** (53%) was similar to that for **49**−**51** indicating that, even after 5 days of being

SCHEME 7. Synthesis of Compounds 49−51 and 53



subjected to excess TMSBr, loss of product due to cleavage at C-5′ was not a significant problem.

Coupling of phosphoramidates **27** and **28** to **44** using the same conditions as described above for the synthesis of **45**−**48** led almost exclusively to the formation of byproducts whose structures were assigned as 2−5′ anhydro compounds **54** and **55** (Figure 2).[30] Only small amounts of the desired BMT nucleotides were formed and these were inseparable from the byproducts by silica gel chromatography.

This result is not unprecedented in that Shibuya and co-workers,[32a] Kimura and co-workers,[32b] and Li and Miller[32c] have shown that 2-5′ anhydro pyrimidine nucleosides can be readily formed from the corresponding pyrimidine nucleosides using triphenylphosphine and DIAD in dioxane or THF. Saady and co-workers reported the formation of N^3-5′ anhydro purine nucleosides when attempting to couple phosphoric or phosphonic acid derivatives to the 5′-OH of purine nucleosides via the Mitsunobu reaction in THF.[33] They found that formation of this byproduct could be minimized by conducting the reaction in dry pyridine.[33] We found that Mitsunobu coupling of **27** and **44** in dry pyridine led to the formation of the desired compound **56** in 55% yield (Scheme 8). However, even when pyridine was used as solvent, Mitsunobu coupling of **28** and **44** still gave mainly compound **55**. We also attempted this reaction using the modified Mitsunobu reaction conditions that we described previously for the synthesis of the BMT derivatives of uridine and cytidine;[21] however, this also proved unsuccessful. Clearly, the cyclization of **28** upon activation of its 5′-OH occurs very rapidly, much faster than it does for the corresponding aza analogue **27**.

At present, the reason for this difference in reactivity is not clear. So far, we have been unable to couple acid **44** to nucleoside **28** using a Mitsunobu reaction or any other reaction conditions.[34] Global deprotection of compound **56** was achieved by using 6 equiv of TMSBr followed by concd NH_4OH−pyridine.[17] After RP-HPLC and conversion to the sodium salt, the desired product **57** was obtained in approximately 40% yield. We found that some dephosphorylation occurred during this process and about 3−4% of the product consisted of compound **58** (by ^{1}H NMR), which we were unable to remove.[35]

Conclusions

Several bismethylene triphosphate (BMT) nucleotides of uridine 4-phosphate (U-4-P) analogues were prepared. A key step in this process was the use of the tin catalyst of Orita et al. to effect the selective cleavage of both 5′-acetyl and 5′-benzoyl esters in 2′,3′,5′-tri-*O*-acetyl or tri-*O*-benzoyl U-4-P analogues. To the best of our knowledge, this represents the first general approach to the selective cleavage of 5′-benzoyl esters from benzoyl-protected nucleosides. Further studies on the generality of this methodology, such as its application to selective debenzoylations of benzoylated carbohydrates, are in progress.

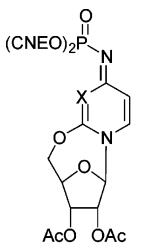


FIGURE 2. Structures of byproducts **54** and **55**.

(30) The structures of **54** and **55** were assigned based on the ^{31}P NMR, ^{1}H NMR, and mass spectral data of partially purified material. For example, the LREIMS of the crude product that was isolated from the attempted Mitsunobu reaction of phosphoramidate **28** and **44** exhibited a *m*/*z* of 494, which is consistent with compound **55**. In the ^{31}P NMR this product exhibited a chemical shift of 8.1 ppm, which is a downfield shift of approximately 6.5 ppm compared to compound **28**. This is consistent with a phosphoramidate to phosphoryl imine conversion (see ref 31). The ^{1}H NMR of the product that was isolated from the attempted Mitsunobu reaction of phosphoramidate **28** and **44** exhibited significant changes in comparison to the ^{1}H NMR of compound **28**. These include the following: (a) the doublet corresponding to phosphoramidate N-H proton in **28** is no longer evident, (b) downfield shifts with increased geminal coupling for the two diastereotopic protons attached to C-5′, (c) an upfield shift of about 0.4 ppm for the H-1′ proton, and (d) a downfield shift of approximately 0.5 ppm for the H-5 proton. Similar differences were also found between compound **27** and the product that was isolated from the attempted Mitsunobu reaction of phosphoramidate **27** and **44**. All of these changes are consistent with the conversion of a nucleoside into a 1,5-anhydro nucleoside (see refs 32a−c).

(31) Napierzaj, A.; Zaeadzki, S.; Zwierzak, A. *Tetrahedron* **2000**, *56*, 6299.

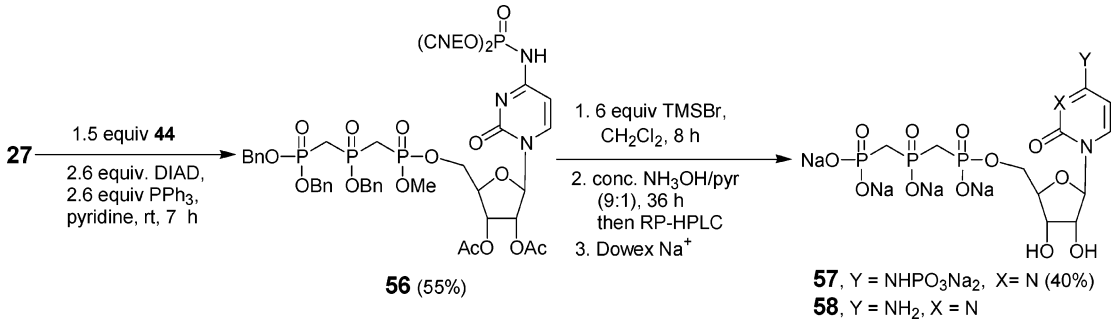
(32) (a) Shibuya, S.; Kuninaka, A.; Hiroshi, Y. *Chem. Pharm. Bull.* **1974**, *22*, 719. (b) Kimura, *J*.; Fujisawa, Y.; Sawada, T. *Chem. Lett*. **1974**, 691. (c) Li., H.; Miller, M. J. *J. Org. Chem.* **1999**, *64*, 9289.

(33) Saady, M.; Lebeau, L.; Mioskowski, C. *Tetrahedron Lett.* **1995**, *36*, 2239.

(34) We have also attempted to convert phosphonic acid **44** to the corresponding phosphonyl chloride in situ and then react the crude phosphonyl chloride with **28**. However, only trace amounts of the coupled product were obtained using this procedure.

(35) Wada et al. reported the synthesis of unprotected **12** and found the compound to be stable under basic conditions (0.1 M NaOH, concd ammonia) unstable under acidic conditions ($t_{1/2}$ = 8 h in 0.1 N HCl) and have a $t_{1/2}$ of 60 min at 70 °C in 0.1 M ammonium acetate, pH 7.0. No studies were performed under neutral conditions at room temperature (see refs 17 and 18). We have found that its BMT nucleotide, compound **55**, undergoes very slow dephosphorylation in D_2O at room temperature. The dephosphorylation is presumed to proceed by a metaphosphate intermediate.

SCHEME 8. Synthesis of Compound 57



Several of these 5′-deprotected U-4-P analogues were converted into their fully protected BMT nucleotides by coupling an unsymmetrical, protected BMT analogue bearing a free phosphonic acid moiety at one of the terminal positions using a Mitsunobu reaction. Global deprotection of these species was achieved by using TMSBr followed by treatment with NH_4OH−MeOH or NH_4OH−pyridine, which afforded the UTP-4-P intermediate analogues. These analogues are currently being examined as inhibitors of purified CTPS and the results of these studies will be reported in due course.

Experimental Section

2′,3′,5′-Tri-*O*-acetylcytidine 4-*N*-[*O*,*O*-Bis(2-cyanoethyl)phosphoramidate] (12). To a solution of 2′,3′,5′-tri-*O*-acetylcytidine[36] (1.5 g, 4.06 mmol) and tetrazole (0.569 g, 8.12 mmol) in dry THF (20 mL) was added a solution of phosphoramidite **15**[37] (2.20 g, 8.12 mmol) in dry THF (10 mL). After 20 h, the solution was cooled to −10 °C and *tert*-butyl hydroperoxide (2.43 mL, 24.36 mmol) was added and the solution stirred for 1 h. The reaction was diluted with EtOAc and washed with sat. $NaHCO_3$ and the aqueous layer was back extracted with EtOAc. The combined organic layers were dried and concentrated and the residue purified by flash chromatography (2% MeOH−98% EtOAc to 4% MeOH−96% EtOAc) to give **12** as a white foam (1.298 g, 58%). ^{1}H NMR (300 MHz) δ 10.25 (br s, 1H), 7.43 (d, J = 7.7 Hz, 1H), 6.13 (d, J = 8.5 Hz, 1H), 5.94 (d, J = 4.9 Hz, 1H), 5.39 (m, 2H), 4.20−4.34 (m, 7H), 2.75 (t, J = 6.1 Hz, 4H), 2.07−2.11 (three overlapping singlets, 9H); ^{13}C NMR (75 MHz) δ 170.2, 169.6, 160.4, 148.9, 140.1, 117.2, 101.9 (d, J = 14.8 Hz), 88.4, 79.9, 72.9, 69.9, 62.9, 61.47 (d, J = 5.4 Hz), 20.74, 20.42, 20.39, 19.64 (d, J = 7.2 Hz); ^{31}P NMR (121 MHz) δ 6.53; LR$^+$ESIMS *m*/*z* (rel intensity) 556.1 (M + 1, 100), 298.1 (52); HR$^+$ESIMS *m*/*z* calculated for $C_{21}H_{27}N_5O_{11}P$ (M + 1) 556.1453, found 556.1445.

General Procedure for the Deacetylation of 12, 13, and 21−23 To Give Nucleosides 24−28. To a solution of the nucleoside (**12**, **13**, **21**−**23**) in dry MeOH (0.133 mmol of nucleoside/mL of MeOH) was added catalyst **20**[38] (6.5 mol %). This solution was stirred for 6.5−18 h (see Table 1 for reaction times) followed by concentration and purification of the product using flash chromatography.

[Difluoro{1-[3,4-bis(acetoxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-yl}methyl]phosphonic Acid Diethyl Ester (26). Compound **26** was prepared from compound **23**[8] as a white foam in 81% yield (80% EtOAc−20% hexane) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 7.90 (d, J = 7.4 Hz, 1H), 6.67 (s, 1H), 6.38 (d, J = 7.2 Hz), 6.21 (d, J = 4.8 Hz, 1H), 5.49 (overlapping dd, J = 4.9 Hz, 1H), 5.42 (overlapping dd, J = 4. 9 Hz, 1H), 4.14−4.26 (m, 4 H), 3.92 (d, J = 11.8 Hz, 1H), 3.76 (d, J = 12.3 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.31 (t, J = 7.3 Hz, 6 H); ^{13}C NMR (75 MHz) δ 169.8, 169.3, 161.4, 144.5 (dt, J = 22.3, 14.0 Hz), 134.5, 118.3 (dt, J = 6.5, 3.1 Hz), 116.2 (dt, J = 264, 214 Hz), 103.2, (t, J = 4.2 Hz), 87.8, 83.3, 74.0, 70.5, 65.3 (d, J = 5.6 Hz), 60.9, 20.4, 20.2, 16.1 (d, J = 5.5 Hz); ^{31}P NMR (121 MHz) δ 6.1 (t, J = 108 Hz); ^{19}F NMR (282 MHz) δ −112.61 (d, J = 110 Hz), −112.63 (d, J = 110 Hz); LREIMS *m*/*z* (rel intensity) 497.1 (M$^+$, 9), 321.0 (46), 282.1 (33), 217.1 (100), 127.1 (44); HREIMS *m*/*z* calculated for $C_{19}H_{26}F_2NO_{10}P$ 497.1262, found 497.1273

2′,3′-Di-*O*-acetylcytidine 4-*N*-[*O*,*O*-Bis(2-cyanoethyl)phosphoramidate] (27). Compound **27** was prepared from compound **12** as a white foam in 84% yield (5% MeOH−95% EtOAc) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 10.25 (br s, 1H), 7.94 (d, J = 8.0 Hz, 1H), 6.29 (d, J = 7.9 Hz), 6.01 (s, 1H), 5.39 (s, 1H), 4.15−4.31 (m, 5H), 3.85 (d, J = 11.3 Hz, 1H), 3.74 (d, J = 11.8 Hz, 1H), 2.75 (t, J = 5.9 Hz, 4H), 2.06 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (75 MHz) δ 170.0, 169.8, 160.8, 149.3, 141.3, 117.3, 101.53 (d, J = 11.9 Hz), 87.3, 83.8, 73.4, 71.2, 61.5, 61.41 (d, J = 20.9 Hz), 20.6, 20.4, 19.6 (d, J = 6.6 Hz); ^{31}P NMR (121 MHz) δ 6.5; LR$^+$ESIMS *m*/*z* (rel intensity) 514.1 (M + 1, 100); HR$^+$ESIMS *m*/*z* calculated for $C_{19}H_{25}N_5O_{10}P$ (M + 1) 514.1339, found 514.1346.

General Procedure for the Debenzoylation of 29−32 To Give Nucleosides 34−37. Catalyst **20** (17 mol %) was added to a solution of the nucleoside (**29**−**32**) in dry MeOH (0.133 mmol of nucleoside/mL of dry MeOH) and the resulting solution was stirred for 48 h at 66 °C (oil bath). The solution was concentrated and the product purified by flash chromatography (**34**, **36**, and **37**) or recrystalization (**35**).

{1-[3,4-Bis(benzoyloxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-ylmethyl}phosphonic Acid Dimethyl Ester (36). Compound **36** was prepared from compound **31**[15] as a white foam in 90% yield (95% EtOAc−5% EtOH then 90% EtOAc−10% EtOH) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 7.90 (d, J = 7.9 Hz, 4H), 7.77 (d, J = 7.3 Hz, 1H), 7.45−7.52 (m, 2H), 7.28−7.35 (m, 4H), 6.40 (s, 1H), 6.39 (d overlapping with s, J = 5.1 Hz, 1H), 6.28 (d, J = 7.3 Hz, 1H), 5.84−5.93 (m, 2H), 4.45 (s, 1H), 3.91−4.09 (m, 3H), l 3.71 (d, J = 10.9 Hz, 6H), 2.96 (d, J = 22.4 Hz, 2H); ^{13}C NMR (75 MHz) δ 165.5, 165.0, 162.0, 145.5 (d, J = 8.6 Hz), 134.0, 133.42, 133.36, 129.73, 129.66, 128.86, 128.76, 128.34, 128.30, 121.3 (d, J = 8.7 Hz), 108.62 (d, J = 3.2 Hz), 89.8, 83.9, 74.5, 71.4, 61.5, 53.0 (d, J = 4.1 Hz), 32.3 (d, J = 136.3 Hz); ^{31}P NMR (121 MHz) δ 27.9; LR$^+$ESIMS *m*/*z* (rel intensity) 558.1 (M + 1, 100), 341.1 (32); HR$^+$ESIMS *m*/*z* calculated for $C_{27}H_{29}NO_{10}P$ 558.1529, found 558.1534.

{1-[3,4-Bis(benzoyloxy)-5-hydroxymethyltetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidenemethyl}-phosphonic Acid Dimethyl Ester (37). Compound **37** was prepared from compound **32**[15] as a white foam in 93% yield (90% EtOAc−10% EtOH) by using the general procedure described above. ^{1}H

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NMR (300 MHz) δ 10.40 (s, 1H), 7.93 (d, J = 7.3 Hz, 4H), 7.49−7.56 (m, 2H), 7.23−7.39 (m, 4H), 7.01 (d, J = 6.8 Hz, 1H), 6.20 (d, J = 4.7 Hz, 1H), 5.75−5.80 (m, 2H), 5.62 (d, J = 6.7 Hz, 1H), 4.38 (s, 1H), 3.85−3.40 (m, 3H), 3.67 (d, J = 11.3 Hz, 6H), 2.68 (s, 1H); ^{13}C NMR (75 MHz) δ 165.5, 165.2, 152.3 (d, J = 22.7 Hz), 148.5, 133.4, 131.9, 129.7, 129.6, 128.9, 128.5, 128.4, 128.3, 104.2 (d, J = 22.7 Hz), 86.5, 83.5, 73.4 (d, J = 196.5 Hz), 61.9, 52.1 (dd, J = 4.0, 1.8 Hz); ^{31}P NMR (121 MHz) δ 27.7; LR$^+$-ESIMS m/z (rel intensity) 559.2 (M + 1, 100), 341.1 (17), 219.1 (28); HR$^+$ESIMS m/z calculated for $C_{26}H_{28}N_2O_{10}P$ (M + 1) 559.1482, found 559.1494.

(Bromomethylbenzyloxyphosphinoylmethyl)phosphonic Acid Dibenzyl Ester (42). Trimethylsilyl bromide (2.94 mL, 22.3 mmol) was added to a solution of compound **41**[21] (1.5 g, 4.46 mmol) in dry dichloromethane (11 mL) and the solution was then stirred for 30 h. After removal of the solvent, the resulting residue was subjected to high vacuum for several hours. Dry MeOH (15 mL) was added and the solution stirred for 30 min. The solution was then concentrated and this process was repeated two more times and the final residue subjected to high vacuum overnight. Tribenzylorthoformate (8.88 g, 26.8 mmol) was added and the mixture heated to 150 °C for 2.5 h. The mixture was subjected to high vacuum rotary evaporation to remove the benzyl alcohol and the resulting residue was purified with use of flash chromatography (50% EtOAC−50% hexane then 70% EtOAC−30% hexane) to give compound **42** as a pale yellow oil that solidified as a white solid after being subjected to high vacuum overnight (2.12 g, 91%). Mp 50−51 °C; ^{1}H NMR (300 MHz) δ 7.20−7.40 (m, 15 H), 4.93−5.13 (m, 6H), 3.32−3.50 (m, H), 2.49−2.77 (m, 2H); ^{13}C NMR (75 MHz) δ 135.7, 135.62, 135.58, 135.54, 135.45, 128.64, 128.58, 128.15, 128.13, 128.06, 68.3 (d, J = 6.8 Hz), 68.0 (d, J = 6.4 Hz), 67.5 (d, J = 6.2 Hz), 25.9 (dd, J = 144.8, 90.0 Hz), 21.6 (d, J = 101.9 Hz); ^{31}P NMR (121 MHz) δ 40.1 (d, J = 2.3 Hz), 21.1 (d, J = 2.3 Hz); LREIMS m/z (rel intensity) 433.1 (M$^+$ − 91, 5), 327.0 (22), 91.1 (100); HREIMS m/z calculated for $C_{16}H_{18}BrO_5P_2$ (M$^+$ − 91) 432.9790, found 432.9783.

[(Dibenzyloxyphosphorylmethyl)benzyloxyphosphinoylmethyl]-phosphonic Acid Dimethyl Ester (43). A solution of compound **42** (0.300 g, 0.575 mmol) in freshly distilled trimethylphosphite (2.1 mL, 33 equiv) was heated to 120 °C for 12 h. The mixture was subjected to high vacuum rotary evaporation to remove the trimethylphosphite and dimethyl methylphosphonate. The resulting residue was purified with flash chromatography (5% MeOH−95% EtOAc) to give compound **43** as a pale yellow oil (0.203 g, 61%). ^{1}H NMR (300 MHz) δ δ7.20−7.40 (m, 15H), 4.95−5.17 (m, 6H), 3.75 (d, J = 9.5 Hz, 3H), 3.67 (d, J = 9.5 Hz, 3H), 2.60−2.90 (m, 4H); ^{13}C NMR (75 MHz) δ 135.8, 135.7, 135.6, 128.5, 128.44, 128.37, 128.01, 127.94, 127.91, 68.0 (d, J = 5.5 Hz), 67.8 (d, J = 5.3 Hz), 66.9 (d, J = 6.5 Hz), 53.0 (d, J = 6.5 Hz), 52.8 (d, J = 6.5 Hz), 28.5 (dd, J = 133.0, 89.0 Hz), 27.4 (dd, J = 134.0, 88.9 Hz); ^{31}P NMR (121 MHz) δ δ 39.8 (t, J = 4.7 Hz), 23.6 (d, J = 4.0 Hz), 21,9 (d, J = 3.6 Hz); LREIMS m/z (rel intensity) 552.1 (M$^+$, 4%), 355.0 (100), 279.0 (20), 91.0 (100); HREIMS m/z calculated for $C_{25}H_{31}O_8P$ 552.1232, found 552.1237.

[(Dibenzyloxyphosphorylmethyl)benzyloxyphosphinoylmethyl]-phosphonic Acid Monomethyl Ester (44). KCN (147 mg, 2.26 mmol) was added to a solution of compound **43** (1.00 g, 1.81 mmol) in dry DMF (20 mL). After the solution was heated to 75 °C over 5 h, the solution was concentrated and the product was purified by using flash chromatography (MeOH−CH_2Cl_2−NH_4OH, 2:10:0.5). The purified material was dissolved in MeOH−H_2O (7:3, 150 mL) and mixed with Dowex 50W×8 H$^+$ ion-exchange resin and stirred for 4 h. The mixture was filtered and the filtrate concentrated to give compound **44** as a pale yellow oil (644 mg, 66%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.34−7.41 (m, 15H), 4.90−5.36 (m, 6H), 3.65 (d, J = 11.4 Hz), 3.03 (overlapping dd, J = 20.2 Hz), 2.82 (overlapping dd, J = 19.5 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ 136.0, 135.88, 135.78, 135.74, 135.70, 135.66, 128.18, 128.13, 128.0, 127.78, 127.75, 127.69, 68.0 (d, J = 5.5 Hz), 67.95 (d, J = 5.4 Hz), 66.9 (d, J = 6.6 Hz), 51.7 (d, J = 6.1 Hz), 27.3 (dd, J = 133.2, 89.5 Hz); ^{31}P NMR (121 MHz, CD_3OD) δ 41.9, 22.5 (d, J = 7.6 Hz), 20.1; LR$^+$ESIMS m/z (rel intensity) 539.0 (M + 1, 100); HR$^+$ESIMS m/z calculated for $C_{24}H_{30}O_8P_3$ 539.1154, found 539.1159.

General Procedure for the Preparation of Compounds 45−48. To a solution of the nucleoside (**26**, **35**−**37**), compound **44** (1.2 equiv), and DIAD (3 equiv) in dry THF (0.10 mmol of nucleoside/mL of THF) was added a solution of triphenylphosphine (3 equiv) in dry THF (8 mL, 0.17 mmol of triphenylphosphine/mL of THF) over a period of 1.5−2 h by syringe pump. The reaction was stirred for 3.5 h then the solution was concentrated and the residue purified with use of flash chromatography.

{1-[3,4-Bis(benzoyloxy)-5-(bis[(benzyloxyphosphorylmethyl)-benzyloxyphosphorylmethyl]methyloxyphosphoryl) tetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4ylmethyl}phosphonic Acid Dimethyl Ester (46). Compound **46** was prepared from compound **36** as a white foam in 50% yield (100% EtOAc then 5% EtOH−95% EtOAc then 10% EtOH−90% EtOAc) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 7.82−7.89 (m, 4H), 7.61−7.69 (m, 1H), 7.42−7.46 (m, 2H), 7.15−7.37 (m, 17H), 6.52−6.64 (m, 1H), 6.35 (s, 1H), 6.22 (overlapping dd, J = 7.2 Hz, 1H), 5.90 (overlapping dd, J = 5.3 Hz, 0.5 H), 5.77 (overlapping dd, J = 5.3 Hz, 0.5 H), 5.63−5.70 (m, 1H), 4.85−5.15 (m, 6H), 4.40−4.69 (m, 3H), 3.63−3.79 (m, 9H), 2.63−3.17 (m, 6H); ^{13}C NMR (75 MHz) δ 165.3, 165.23, 165.17, 165.10, 161.79, 161.76, 161.75, 161.73, 145.54, 145.49, 145.42, 145.39, 145.32, 135.89, 135.83, 135.81, 135.79, 135.76, 135.74, 133.61, 133.59, 133.52, 133.06, 133.00, 132.8, 129.88, 129.87, 129.79, 129.78, 128.79, 128.73, 128.71, 128.68, 128.67, 128.61, 128.60, 128.56, 128.51, 128.49, 128.48, 128.44, 128.42, 128.20, 128.17, 128.12, 128.08, 128.02, 128.00, 120.91, 120.89, 120.84, 120.82, 120.78, 108.81, 108.78, 108.76, 108.62, 108.59, 87.6, 87.2, 86.9, 81.23, 81.17, 87.14, 81.09, 74.66, 74.61, 74.58, 70.8, 70.7, 70.6, 687.23, 68.18, 68.13, 68.11, 68.06, 68.02, 67.97, 67.21, 67.14, 67.12, 67.09, 67.08, 65.66, 65.62, 65.34, 65.30, 65.25, 53.68, 53.62, 53.41, 53.37, 53.10, 53.08, 53.05, 53.03, 32.5 (d, J = 134.5 Hz), 26.93−29.61 (m); ^{31}P NMR (121 MHz) δ 39.78−39.84 (m), 39.36−39.54 (m), 27.74, 27.72, 23.3−23.9 (m), 21.7−21.9 (m); LR$^+$ESIMS m/z (rel intensity) 1078 (M + 1, 100); HR$^+$ESIMS m/z calculated for $C_{51}H_{56}N_1O_{17}P_4$ 1078.2499, found 1078.2528.

{1-[3,4-Bis(benzoyloxy)-5-(bis[(benzyloxyphosphorylmethyl)-benzyloxyphosphorylmethyl]methyloxyphosphoryl)tetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidenemethyl}-phosphonic Acid Dimethyl Ester (47). Compound **47** was prepared from compound **37** as a white foam in 72% yield (95% EtOAc−5% MeOH) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 10.37 (s, 1H), 7.83−7.92 (m, 4H), 7.49−7.59 (m, 2H), 7.20−7.39 (m, 19H), 7.01−7.15 (m, 1H), 6.40−6.53 (m, 1H), 5.50−5.92 (m, 3H), 4.90−5.21 (m, 6H), 4.30−4.60 (m, 3 H), 3.61−3.89 (m, 9H), 2.65−3.23 (m, 4H); ^{13}C NMR (75 MHz) δ 165.30, 165.25, 165.18, 165.11, 152.14, 152.01, 151.96, 148.42, 148.33, 148.29, 135.80, 135.71, 135.64, 135.58, 135.55, 133.54, 133.51, 133.46, 131.28, 131.08, 129.77, 129.67, 128.70, 128.61, 128.53, 128.50, 128.47, 128.43, 128.40, 128.36, 104.65, 104.48, 104.35, 104.18, 85.42, 85.25, 85.17, 85.09, 80.91, 80.81, 80.76, 80.66, 75.47, 75.30, 73.00, 72.88, 72.71, 72.42, 72.37, 72.30, 71.00, 70.85, 68.17, 68.13, 68.06, 67.96, 67.87, 67.80, 67.26, 67.18, 67.08, 67.06, 67.00, 66.97, 66.88, 65.80, 65.73, 65.42, 65.34, 53.75, 53.66, 53.49, 53.42, 53.36, 53.29, 53.11, 53.06, 52.97, 52.06, 52.03, 52.00, 51.97, 26.22−30.02 (m); ^{31}P NMR (121 MHz) δ 39.20−39.84 (m), 27.95, 27.83, 27.81, 27.75, 23.28−24.16 (m), 21.66−21.90 (m); LR$^+$ESIMS m/z (rel intensity) 1079.2 (M + 1, 100), 540.1 (30), 219.1 (10); HR$^+$ESIMS m/z calculated for $C_{50}H_{55}N_2O_{17}P_4$ (M + 1) 1079.2451, found 1079.2461.

[Difluoro{1-[3,4-bis(acetoxy)-5-(bis[(benzyloxyphosphorylmethyl)benzyloxyphosphorylmethyl]methyloxyphosphoryl)-tetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-yl}methyl]-phosphonic Acid Diethyl Ester (48). Compound **48** was prepared

from compound **26** as a white foam in 68% yield (95% EtOAc−5% MeOH) by using the general procedure described above. ^{1}H NMR (300 MHz) δ 7.71−7.79 (m, 1H), 7.16−7.38 (m, 15H), 6.69 (s, 1H), 6.28−6.46 (m, 2H), 5.31−5.55 (m, 2H), 4.80−5.15 (m, 6H), 4.13−4.61 (m, 7H), 3.65−3.78 (m, 3H), 2.59−3.14 (m, 4H), 2.04 (s, 6H), 1.31 (t, J = 6.9 Hz); ^{13}C NMR (125 MHz) δ 169.43, 169.38, 169.18, 161.0, 144.1−147.8 (m), 135.70, 135.61, 135.53, 133.55, 133.47, 133.32, 128.49, 128.07, 128.00, 129.97, 127.90, 118.7, 116.0 (dt, J = 258, 208 Hz), 113.14−103.35 (m), 87.20, 87.09, 86.73, 80.52, 80.43, 80.33, 73.69−73.89 (m), 69.37, 67.82−68.10 (m), 66.91−67.26 (m), 64.67−65.16 (m), 52.77−53.50 (m), 26.08−30.30 (m), 20.44, 20.34, 16.26, 16.19; ^{31}P NMR (121 MHz) δ 39.27−40.06 (m), 23.50−24.14 (m), 21.63−21.77 (m), 6.26 (t, J = 108.1 Hz), 6.22 (108.1 Hz); ^{19}F NMR (283 MHz) δ −112.43 (d, J = 108.0 Hz), −112.44 (d, J = 107.4 Hz), −112.47 (d, J = 108.6 Hz), −112.53 (d, J = 107.5 Hz); LR$^+$ESIMS m/z (rel intensity) 1018.2 (M + 1, 100), 550.6 (28); HR$^+$ESIMS m/z calculated for $C_{43}H_{54}NO_{17}F_2P_4$ (M + 1) 1018.2310, found 1018.2324.

General Procedure for the Deprotection of 45−48. TMSBr (6 equiv for **45**, 9 equiv for **46** and **47**, 12 equiv for **48**) was added to a solution of **45**−**48** in dry CH_2Cl_2 (0.040 mmol/mL of CH_2Cl_2). The reaction mixture was stirred for 8 h (for **45**−**47**) or 5 days (for **48**). The solution was then concentrated, dry CH_2Cl_2 was added, and the mixture was concentrated again, then this was repeated and the residue was subjected to high vacuum for 1 h. A solution of NH_4OH−MeOH (5 mL, 3:2) was then added to the residue and the resulting solution was stirred for 24 h. The solution was concentrated and the resulting white residue was dissolved in water and lyophilized. The resulting white powder was dissolved in 100 mM triethylammonium acetate (TEAA) (pH 7.0 for crude **49**, **50**, and **53**) or pH 8.8 (for crude **51**) and purified by using semipreparative reversed-phase HPLC with 100 mM TEAA−CH_3CN as the eluent. Compounds obtained after HPLC purification were dissolved in water and repeatedly lyophilized until all of the TEAA was removed (by ^{1}H NMR). The resulting triethylammonium salts were converted into their penta- (compound **49**) or hexa- (compounds **50**, **51**, and **53**) sodium salts, using a Dowex 50W×8 Na^+ form ion exchange column.

{1-[3,4-Bis(hydroxy)-5-{[(dihydroxyphosphorylmethyl)-hydroxyphosphorylmethyl]hydroxyphosphoryl}tetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-ylmethyl}phosphonic Acid, Hexasodium Salt (50). Compound **50** was prepared from compound **46** (0.108 g, 0.0928 mmol) by using the general procedure described above. The following solvent systems were used for preparative HPLC: solvent A, 100 mM TEAA, pH 7.0; solvent B, CH_3CN. The following elution profile was used: 0−15 min, 99% A−1% B; 15−50 min, linear gradient of 99% A−1% B to 94% A−6% B. Flow rate = 8 mL/min. t_r = 30.04 min. Conversion of the compound to the hexasodium salt by using a Dowex 50W×8 Na^+ ion exchange column gave nucleotide **50** as a white powder (38.4 mg, 60%). ^{1}H NMR (300 MHz, D_2O) δ 7.85 (d, J = 5.5 Hz, 1H), 6.64 (d, J = 5.5 Hz, 1H), 6.43 (s, 1H), 6.13 (s, 1H), 4.12−4.30 (m, 5H), 2.86 (d, J = 21.3 Hz, 2H), 2.137−2.34 (m, 4H); ^{13}C NMR (75 MHz, D_2O) δ 163.2, 152.6 (d, J = 8.4 Hz), 132.3, 118.0 (d, J = 6.6 Hz), 111.9 (d, J = 2.7 Hz), 83.1, 82.8 (d, J = 7.5 Hz), 74.8, 69.1, 63.0 (d, J = 3.6 Hz), 36.4 (d, J = 121.9 Hz), 29.7−33.3 (m); ^{31}P NMR (121 MHz, D_2O) δ 27.2 (P_β), 18.3 (P_α), 16.8, 15.8 (P_γ); LR$^-$ESIMS m/z (rel intensity) 556 (M − 6Na + 5H$^+$, 100). HR$^-$ESIMS m/z calculated for $C_{13}H_{22}NO_{15}P_4$ (M − 6Na + 5H$^+$) 555.9940, found 555.9953. The analytical HPLC chromatogram of **50**, using the elution profile described above for the purification of **50**, indicated it to be greater than 98.8% pure (flow rate 1 mL/min, t_r = 12.94 min).

{1-[3,4-Bis(hydroxy)-5-{[(dihydroxyphosphorylmethyl)-hydroxyphosphorylmethyl]hydroxyphosphoryl}tetrahydrofuran-2-yl]-2-oxo-2,3-dihydro-1*H*-pyrimidin-4-ylidenemethyl}-phosphonic Acid, Hexasodium Salt (51). Compound **51** was prepared from compound **47** (0.108 g, 0.100 mmol) by using the general procedure described above. The following solvents were used for preparative HPLC: solvent A, 100 mM TEAA, pH 8.8; solvent B, CH_3CN. The following elution profile was used: 0−13 min, 99% A−1% B; 13−40 min, linear gradient of 99% A−1% B to 94% A−6% B. Flow rate = 8 mL/min. t_r = 27.36 min. Conversion of the compound to the hexasodium salt by using a Dowex 50W×8 Na^+ ion exchange column gave nucleotide **51** as a white powder (37.0 mg, 54%). Analysis of the NMR spectra revealed that the powder contained approximately 15% of compound **52**. ^{1}H NMR (300 MHz, D_2O) δ 8.37, 8.34 (overlapping singlets, 1H), 6.74, 6.66 (overlapping singlets, 1H), 5.87 (s, 1H), 4.42 (br s, 4H), 4.12 (s, 1H), 3.01 (d, J = 16.5 Hz, 0.4 H, exchange with solvent deuterons), 2.10−2.34 (m, 4H); ^{31}P NMR (121 MHz, D_2O) δ 27.6 (P_β), 18.3 (P_α), 15.8 (P_γ), 14.0, 1.05; LR$^-$ESIMS m/z (rel intensity) 557.0 (M − 6Na + 5H$^+$, 100), 477.0 (85). HR$^-$ESIMS m/z calculated for $C_{12}H_{21}N_2O_{15}P_4$ (M − 6Na + 5H$^+$) 556.9892, found 556.9896.

[Difluoro{1-[3,4-bis(hydroxy)-5-(bis{[(dihydroxyphosphorylmethyl)hydroxylphosphorylmethyl]hydroxyphosphoryl})tetrahydrofuran-2-yl]-2-oxo-1,2-dihydropyridin-4-yl}methyl]phosphonic Acid, Hexasodium Salt (53). Compound **53** was prepared from compound **48** (0.110 g, 0.108 mmol) by using the general procedure described above. The following solvents were used for preparative HPLC: solvent A, 100 mM TEAA, pH 7.0; solvent B, CH_3CN. The following elution profile was used: linear gradient of 99% A−1% B to 93% A−7% B over 40 min. Flow rate = 8 mL/min. t_r = 28.24 min. Conversion of the compound to the hexasodium salt by using a Dowex 50W×8 Na^+ ion exchange column gave nucleotide **53** as a white powder (41.0 mg, 53%). ^{1}H NMR (300 MHz, D_2O) δ 7.95 (d, J = 6.1 Hz, 1H), 6.72 (s, 2H), 6.14 (s, 1H), 4.42−4.26 (m, 5H), 2.14−2.29 (m, 4H); ^{13}C NMR (75 MHz, D_2O) δ 163.4, 150.8 (dt, J = 22.7, 11.0 Hz), 133.1, 120.2 (dt, J = 264, 176 Hz), 116.2 (t, J = 7.0 Hz), 106.6 (t, J = 4.5 Hz), 89.6, 82.7 (d, J = 7.2 Hz), 74.9, 69.1, 63.0 (d, J = 4.2 Hz), 29.8−33.4 (m); ^{31}P NMR (121 MHz, D_2O) δ 27.7 (P_β), 18.3 (P_α), 15.9 (P_γ), 4.0 (t, J = 84.4 Hz); ^{19}F NMR (282 MHz, D_2O) δ −110.7 (d, J = 85.3 Hz); LR$^-$ESIMS m/z (rel intensity) 591.9 (M − 6Na + 5H$^+$, 100); HR$^-$ESIMS m/z calculated for $C_{13}H_{21}F_2NO_{15}P_4$ (M − 6Na + 5H$^+$) 591.9752, found 591.9742. The analytical HPLC chromatogram of **53**, obtained by using the elution profile described above for the purification of **53**, indicated that the product was greater than 99% pure (flow rate 1 mL/min, t_r = 17.42 min).

2′,3′-Di-*O*-acetyl-5′-(bis[(benzyloxyphosphorylmethyl)-benzyloxyphosphorylmethyl]methyloxyphosphoryl)cytidine 4-*N*-[*O*,*O*-Bis(2-cyanoethyl)phosphoramidate] (56). To a solution of nucleoside **27** (0.051 g, 0.10 mmol), compound **44** (0.087 g, 0.15 mmol), and DIAD (0.051 mL, 0.26 mmol) in dry pyridine (1 mL) was added a solution of triphenylphosphine (0.65 mg, 0.25 mmol) in dry pyridine (1 mL) over a period of 1.5 h by syringe pump. The reaction was stirred for 7 h then concentrated and the residue was purified with FC (10% MeOH−90% EtOAc) to give compound **56** as a white foam (0.057 g, 55%). ^{1}H NMR (300 MHz) δ 10.40 (br s, 1H), 7.68−7.81 (m, 1H), 7.21−7.38 (m, 15H), 6.08 (m, 2H), 5.30−5.57 (m, 2H), 4.83−5.39 (m, 6H), 4.11−4.59 (m, 7H), 3.66−3.79 (m, 3H), 2.55−3.18 (m, 8H), 2.02−2.08 (m, 6H); ^{13}C NMR (75 MHz) δ 169.7, 169.6, 169.5, 160.0, 148.4, 140.9, 140.4, 135.8, 135.7, 135.6, 128.6, 128.1, 128.07, 128.01, 116.8, 103.4, 103.2, 102.9, 86.7, 86.5, 86.3, 81.1, 80.98, 80.87, 72.8, 72.5, 70.1, 70.0, 68.24, 68.17, 68.08, 68.00, 67.5, 67.4, 67.2, 67.1, 65.22, 65.16, 65.0, 64.9, 64.8, 61.34, 61.26, 53.6, 53.5, 53.23, 53.16, 25.5−30.1 (m), 20.5, 20.4, 19.7, 19.6; ^{31}P NMR (121 MHz) δ 39.0−39.9 (m), 23.6−24.3 (m), 21.5−21.8 (m), 6.9; LR$^+$ESIMS m/z (rel intensity) 1034.2 (M + 1, 100), 944.1 (50), 536.6 (86); HR$^+$ESIMS m/z calculated for $C_{43}H_{52}N_5O_{17}P_4$ (M + 1) 1034.2309, found 1034.2280.

5′-(Bis[(dihydroxyphosphorylmethyl)hydroxyphosphorylmethyl]hydroxyphosphoryl)cytidine 4-*N*-Phosphoramidic Acid, Hexasodium Salt (57). TMSBr (0.063 mL, 0.52 mmol) was added to a solution of nucleotide **56** (0.90 mg, 0.087 mmol) in 2 mL of dry CH_2Cl_2 and the solution was stirred for 8 h. The solution was concentrated and the resulting residue was dissolved in dry CH_2-

Cl_2 and then concentrated again. This process was repeated and the resulting residue was subjected to high vacuum for 12 h. A solution of NH_4OH−pyridine (9:1, 5 mL) was added, followed by stirring for 36 h. The mixture was concentrated and the resulting residue was dissolved in water and lyophilized to give a white powder. The product was then purified by using semipreparative RP-HPLC with the following solvents: solvent A, 100 mM TEAA, pH 9.0; solvent B, CH_3CN. The following elution profile was used: 0−35 min, 100% A; 35−55 min, linear gradient of 100% A to 90% A−10% B. Flow rate = 8 mL/min. t_r = 49.2 min. Conversion of the compound to the hexasodium salt by using a Dowex 50W×8 Na^+ ion exchange column gave nucleotide **57** as a white powder (24.0 mg, 40%). ^{1}H NMR (300 MHz, D_2O) δ 7.94 (br s, 1H), 6.40 (br s, 0.6H), 5.86 (s, 1H), 4.00−4.26 (m, 5H), 2.07−3.30 (m, 4H); ^{31}P NMR (121 MHz, D_2O) δ 28.7 (P_β), 19.43 (P_α), 16.9 (P_γ), −2.3; LR$^-$ESIMS *m*/*z* (rel intensity) 557.9 (M − 6Na + 5H$^+$, 22), 478.0 (100); HR$^-$ESIMS *m*/*z* calculated for $C_{11}H_{20}N_3O_{15}P_4$ (M − 6Na + 5H$^+$) 557.9845, found 557.9835. We did not attempt to obtain a ^{13}C NMR due to a spontaneous dephosphorylation that occurs when in D_2O. The analytical HPLC chromatogram of **57**, using 100% solvent A as eluent, indicated it to be greater than 98.8% pure (flow rate 1 mL/min, t_r = 14.78 min) with compound **58** (t_r = 9.76 min) constituting about 1% of the product. In contrast, ^{1}H NMR indicated that approximately 3−4% of compound **58** was present.

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Supporting Information Available: Characterization data for compounds **13**, **14**, **22**, **24**, **25**, **28**, **34**, **35**, **38**, **45**, and **49**, and NMR spectra [(^{1}H, ^{13}C, ^{31}P (when applicable), ^{19}F (when applicable)] and HPLC chromatograms (when applicable) for compounds **12**−**14**, **22**, **24**−**28**, **34**−**38**, **42**−**51**, **53**, **56**, and **57**. This material is available free of charge via the Internet at http://pubs.acs.org.

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