Synthesis of Novel 3′-*C*-Methylene Thymidine and 5-Methyluridine/Cytidine H-Phosphonates and Phosphonamidites for New Backbone Modification of Oligonucleotides

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Novel 5′-*O*-DMT- and MMT-protected 3′-*C*-methylene-modified thymidine, 5-methyluridine, and 5-methylcytidine H-phosphonates **1**−**7** with *O*-methyl, fluoro, hydrogen, and *O*-(2-methoxyethyl) substituents at the 2′-position have been synthesized by a new effective strategy from the corresponding key intermediates 3′-*C*-iodomethyl nucleosides and intermediate BTSP, prepared in situ through the Arbuzov reaction. The modified reaction conditions for the Arbuzov reaction prevented the loss of DMT- and MMT-protecting groups, and directly provided the desired 5′-*O*-DMT- and/or MMT-protected 3′-*C*-methylene-modified H-phosphonates **1**−**6** although some of them were also prepared through the manipulation of protecting groups after the P−C bond formation. The modified Arbuzov reaction of 3′-*C*-iodomethyl-5-methylcytidine **53**, prepared from its 5-methyluridine derivative **42**, with BTSP provided the 5-methylcytidine H-phosphonate **54**, which was further transferred to the corresponding 4-*N*-(*N*-methylpyrrolidin-2-ylidene)-protected H-phosphonate monomer **7**. 5′-*O*-MMT-protected 3′-*C*-methylene-modified H-phosphonates **5**, **3**, and **7** were converted to the corresponding cyanoethyl H-phosphonates **50**, **51**, and **56** using DCC as a coupling reagent. One-pot three-step reactions of **50**, **51**, and **56** provided the desired 3′-*C*-methylene-modified phosphonamidite monomers **8**−**10**. Some of these new 3′-methylene-modified monomers **1**−**10** have been successfully utilized for the synthesis of 3′-methylene-modified oligonucleotides, which have shown superior antisense properties including nuclease resistance and binding affinity to the target RNA.

Introduction

Extensive investigation[1] of antisense oligonucleotides as therapeutics with antiviral, anticancer, antibacterial, antiinflammatory, and other indications[2] has resulted in 19 clinical candidates, and the first antisense drug, Vitravene, has been launched on the market.[3] Most of these candidates are phosphorothioate oligonucleotides in which one of the nonbridging oxygen atoms of the phosphate ester linkage of natural DNA (see structure I, Figure 1) is replaced with sulfur. This modification provides improved nuclease resistance to endonucleases and exonucleases and retains the ability to activate RNase H to cleave the target RNA.[4,5] However, phosphorothioate oligonucleotides have limitations of poor oral bioavailability and nonspecific binding to proteins. Recently, methylphosphonate,[6] phosphoramidate,[7] morpholino,[8] amide,[9] boranophosphate,[10] methylene(methylimino) (MMI),[11] 5′-methylphosphonate,[12] and other[13]

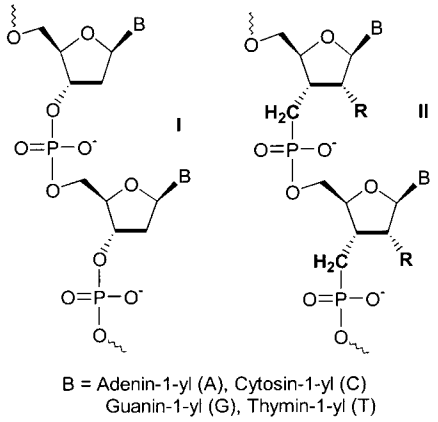

Figure 1. Chemical Structures of Natural DNA (I) and 2′-Substituted 3′-Methylene-Modified Oligonucleotides (II).

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backbone-modified oligonucleotides have received attention for their potential usefulness as antisense drugs with various limitations. Nevertheless, the search for new modified antisense oligonucleotides is still essential to increase nuclease resistance, biochemical stability, and binding affinity to the complementary RNA. It is also necessary to improve oral bioavailability and to alter distribution for antisense therapeutics.[3a]

3′-Methylene-modified oligonucleotide (see structure II, Figure 1), in which the phosphodiester linkage of DNA is replaced by a 3′-*C*-methylene phosphonate linkage, is expected to increase the enzymatic stability against endo/exo-nucleases and improve the target-binding affinity (Tm) to the complementary RNA based on the initial X-ray analysis of an octamer having a single 3′-*C*-methylene phosphonate linkage.[14] In addition, 3′-methylene-modified oligonucleotides may enhance membrane permeability, improve bioavailability and cellular uptake, and also alter distribution, because they have higher lipophilicity than the phosphodiester groups of natural DNA. However, 3′-methylene-modified oligonucleotide has received little attention because of the synthetic difficulty encountered in the preparation of appropriate monomers and oligonucleotides. To meet the challenge, we decided to study the 3′-methylene-modified oligonucleotides (structure II, Figure 1) with different 2′-modifications to investigate the additive improvements of backbone and carbohydrate modifications on their properties. The synthesis of 3′-*C*-methylene phosphonates (P^V) has been explored.[15] The 3′-*C*-methylene phosphonate dimer[16] and trimer[17] have been prepared, and the dimer has been incorporated into an octamer by a solution-phase approach.[14] However, the 3′-*C*-methylene phosphonate (P^V) monomer could not be utilized in the oligonucleotide synthesis by a solid-phase approach. Historically, phosphodiester and phosphotriester approaches can only be utilized for the synthesis of short oligonucleotides in solution, and they are not adaptable to the automated synthesizer. Therefore, these approaches do not meet the requirements for antisense drug discovery although the synthesis of those corresponding P^V monomers is relatively easier. The dimer approaches have been utilized for the synthesis of different backbone-modified oligonucleotides,[9−14] but they cannot be used for the synthesis of oligonucleotides with full length or consecutive modifications. These approaches also require at least sixteen essential dimers, instead of four monomers, leading to an extensive synthetic effort and high cost. Therefore, we decided to search for an approach to the synthesis of 3′-methylene-modified H-phosphonate (P^{III}) and phosphonamidite (P^{III}) monomers, which can be easily utilized for the synthesis of 3′-methylene-modified oligonucleotides without sequence limitation and are adaptable to the automated solid-phase synthesizer.

In this paper, we describe a new effective strategy for the synthesis of novel 5′-*O*-DMT- and MMT-protected 3′-*C*-methylene H-phosphonate thymidines, 5-methyluridines, and 5-methylcytidines **1**−**7** with 2′-*O*-methyl, fluoro, hydrogen, and *O*-(2-methoxyethyl) substituents (Figure 2) through an Arbuzov reaction as a key step. 3′-*C*-Methylene H-phosphonates **5**, **3**, and **7** were then successfully converted to the corresponding 3′-methylene-modified phosphonamidites **8**−**10** by a four-step two-pot procedure. Some of these 3′-methylene-modified H-phosphonates **1**−**7** and phosphonamidites **8**−**10** have been utilized as new monomers for the synthesis of 3′-methylene-modified oligonucleotides by the solid-phase automated approach.[18] Some preliminary biophysical properties and target-binding affinity are also described.

Results and Discussion

The syntheses of 3′-*C*-methylene nucleoside phosphonates (P^V) and phosphinic acid (P^V) have been reported.[15] 3′-Methylene-modified dinucleotide[16] and trinucleotide[17] were synthesized by the phosphotriester and phosphodi-

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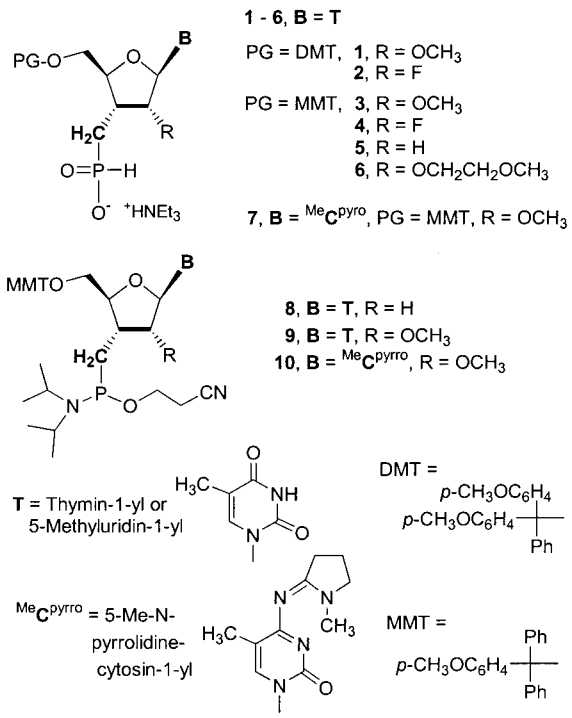
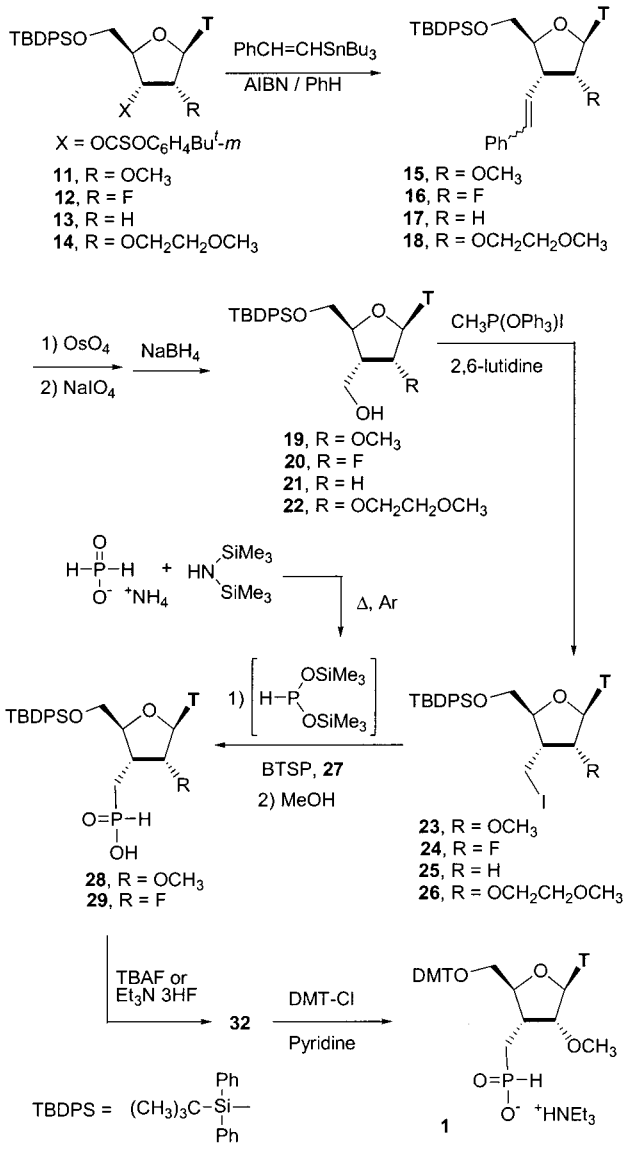


Figure 2. Novel 3′-*C*-Methylenethymidine, 5-Methyluridine and 5-Methylcytidine H−Phosphonates **1**−**7** and Phosphonamidites **8**−**10** with Different Substituents at the 2′-Position.

ester approaches in solution. One G*p*G dimer, containing one 3′-*C*-methylene-substituted phosphonate linkage, was incorporated into an octamer by a phosphotriester approach in solution for conformational studies.[14] This method can only be used for the synthesis of short oligonucleotides in solution. It is impossible to use this historic method for the synthesis of long oligonucleotides by the automated solid-phase approach. Only 3′-*C*-methylene H-phosphonate and phosphonamidite monomers (P^{III}) can be utilized for the synthesis of the desired 3′-methylene-modified oligonucleotides on solid support, therefore, adaptable to automation. Collingwood and coworkers[19] outlined the synthesis of 5′-*O*-DMT-3′-deoxy-3′-*C*-methylenethymidine H-phosphonate without detail. Recently, we have disclosed the synthesis of 5′-*O*-DMT- and MMT-protected 3′-*C*-methylene H-phosphonate thymidine/5-methyluridines with different 2′-substituents by a new efficient strategy.[20] The increasing interest in antisense therapeutics and the great importance for improving enzymatic stability, binding affinity, and other properties of antisense drugs prompted us to report the full details of our discovery on the 3′-methylene backbone modification.

The 3′-methylene-modified thymidine, 5-methyluridine, and 5-methylcytidine H-phosphonates **1**−**7** were synthesized through the Arbuzov reaction as a key step, and the 3′-methylene-modified phosphonamidites **8**−**10** were successfully synthesized from their corresponding H-phosphonate derivatives (Figure 2). Scheme 1 shows the synthesis of key intermediates 3′-*C*-iodomethyl nucleoside derivatives **23**−**26**. The 3′-*O*-(3-*tert*-butylphenoxythiocarbonate) nucleosides **11**−**14** were prepared by

Scheme 1



the reaction of 3′-*tert*-butylphenyl chlorothionoformate with the corresponding 5′-protected 2′-substituted nucleoside derivatives following the similar procedure for the preparation of 3′-*O*-phenoxythiocarbonyl derivatives.[21,22] The radical reactions of **11**−**14** with *β*-tributylstannyl styrene[23] were initiated by 2,2′-azobisisobutyronitrile (AIBN) providing the corresponding 3′-*C*-styrene derivatives **15**−**18**. The oxidation reactions of **15**−**18** were catalyzed by osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide. Thus resulted diols were oxidatively cleaved using sodium periodate as an oxidizing agent in a mixture of dioxane and water. Thus obtained corresponding 3′-*C*-aldehyde derivatives were directly reduced by sodium borohydride without further purification in order to avoid their decomposition. The 3′-*C*-hydroxymethyl nucleosides **19**−**22** were obtained in 36−80% overall yields in three steps from compounds

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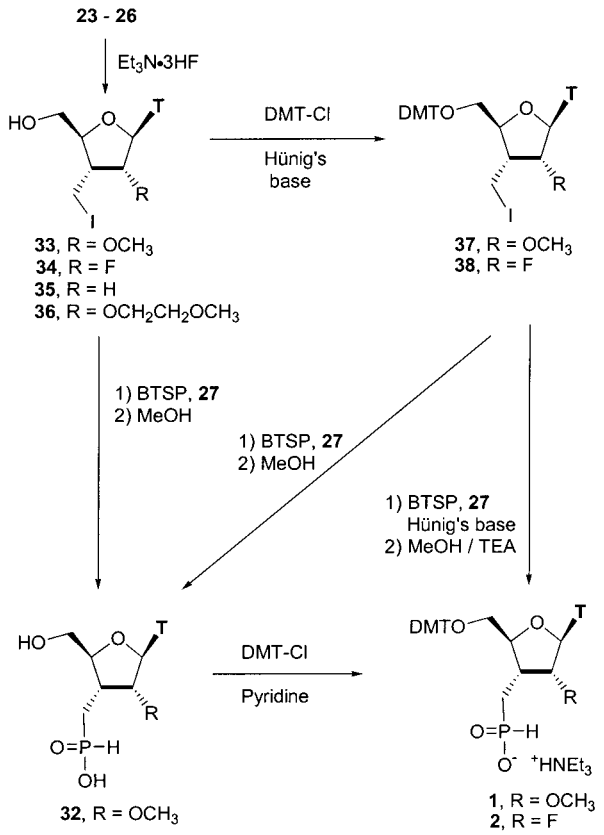
15−**18**, respectively, after flash chromatographic purification. 3′-*C*-Hydroxymethyl derivatives **19**−**22** were iodinated by methyl triphenoxyphosphonium iodide using 2,6-lutidine as a base in anhydrous DMF. 3′-*C*-Iodomethyl-2′-substituted nucleosides **23**−**26** were obtained as key intermediates in 80−92% yields.

Scheme 1 shows the synthesis of 3′-*C*-methylene H-phosphonates through the Arbuzov reaction. A mixture of ammonium phosphinate[24] and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was heated neat at 110 °C under argon atmosphere for 2 h to generate the intermediate bis(trimethylsilyl)phosphonite (BTSP) (**27**).[25] BTSP has been utilized for the synthesis of other small molecule H-phosphonates through nucleophilic substitution and addition reactions,[25,26] but it has never been used to form the C−P bond for the synthesis of nucleoside H-phosphonates in nucleotide chemistry. We utilized BTSP as a phosphorus source to form a C−P bond of 3′-*C*-methylene H-phosphonates in one step from the corresponding iodomethyl nucleosides. 3′-*C*-Iodomethyl-2′-*O*-methyl-5-methyluridine **23** was reacted in anhydrous CH_2Cl_2 under argon atmosphere with BTSP **27**, prepared in situ. The resulted silyl intermediate was hydrolyzed with methanol providing the desired 2′-*O*-methyl-3′-*C*-methylene-5-methyluridine H-phosphinic acid **28**. The flash chromatographic purification provided the H-phosphinic acid **28** as its salt-free acid form in 31% yield. The 2′-fluoro-3′-*C*-methylene-5-methyluridine H-phosphinic acid **29** was also synthesized from the corresponding iodomethyl compound **24** by the same procedure. The reduced products, 3′-*C*-methyl nucleosides **30** and **31**, were also obtained as byproducts from the above reactions (see the Supporting Information). The mechanism for the formation of 3′-*C*-methyl derivatives is still not clear.

H-Phosphonates **28** and **29** with the 5′-*O*-*tert*-butyldiphenylsilyl (TBDPS)-protecting group cannot be used directly for the solid-phase oligonucleotide synthesis; therefore, the TBDPS group needs to be changed to the automation-adaptable protecting group DMT. 3′-*C*-Methylene H-phosphinic acid **28** was treated with tetrabutylammonium fluoride to provide H-phosphonate **32** as its tetrabutylammonium salt. This salt did not react well with 4,4′-dimethoxytrityl chloride (DMT-Cl) for the synthesis of the desired final monomer, and the pure phosphinic acid **32** could not be isolated from this salt. Therefore, an alternative method was then used to remove the TBDPS-protecting group. Compound **28** was treated with triethylamine trifluoride in a mixed solvent of DMF and THF providing the salt-free H-phosphinic acid **32** in 97% yield. Compound **32** was reacted with DMT-Cl in anhydrous pyridine to give the desired product 5′-*O*-DMT-2′-*O*-methyl-3′-*C*-methylene-5-methyluridine H-phosphonate **1** as its triethylamine salt in 65% yield after flash chromatographic purification using $CHCl_3$−MeOH−Et_3N as an eluent.

Scheme 2



On the basis of our experience, changing protecting group at the H-phosphinic acid stage is more difficult than that at the general nucleoside stage; therefore, the 5′-*O*-TBDPS-protecting group was exchanged to the DMT-protecting group prior to the formation of the C−P bond. The 5′-*O*-TBDPS-protecting group was removed by the treatment of compounds **23**−**26** with triethylamine trifluoride (Scheme 2). The deprotected 3′-*C*-iodomethyl nucleosides **33**−**36** were obtained in high yields. Compounds **33** and **34** were reacted with DMT-Cl in the presence of diisopropylethylamine (Hünig's base) providing the 5′-*O*-DMT-protected products **37** and **38**, respectively, in 88−99% yields. Compound **37** was reacted with the intermediate BTSP **27** under the same reaction conditions as described above for the synthesis of **28**. The desired product **1** was not obtained, while the deprotected product **32** was isolated instead. The acid-labile DMT-protecting group of compound **1** was removed by the resultant H-phosphinic acid as soon as it formed. The direct reaction of the unprotected nucleoside **33** with BTSP **27** also gave compound **32**. These results confirmed the structure of product **32** and also indicated that the 5′-hydroxyl group does not effect the C−P bond formation in the Arbuzov reaction. The samples of compound **32** obtained from **28**, **37**, and **33** by the different methods showed the identical chromatographic and spectroscopic properties. Protection of the 5′-hydoxyl group by DMT for 3′-*C*-methylene H-phosphinic acid **32** to give **1** was not as efficient as that for nucleoside **33** to form **37**; therefore, direct conversion from **37** to **1** without losing the DMT-protecting group would be critical for the efficient synthesis of 3′-*C*-methylene H-phosphonate monomers. We discovered later that the addition of Hünig's base in the Arbuzov reaction prevented losing the DMT-protecting group because the Hünig's base

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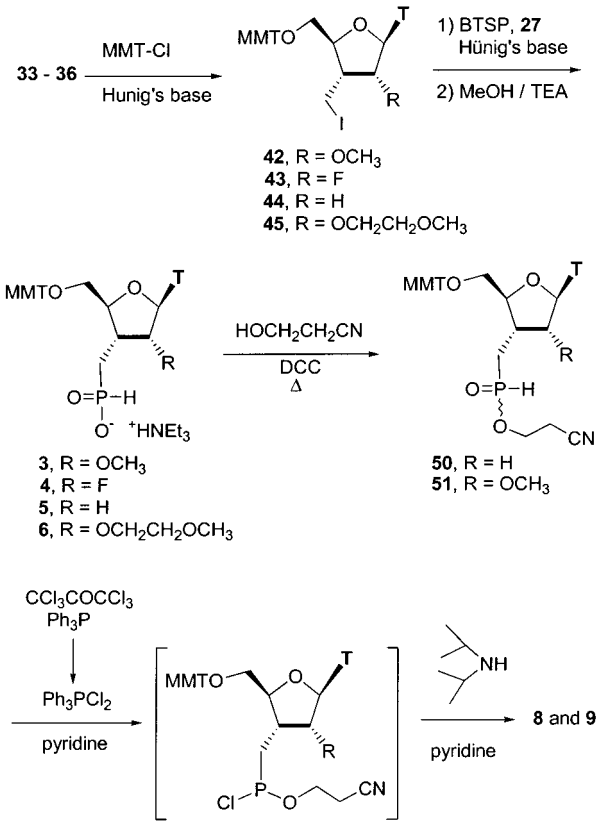
Scheme 3



neutralized the resultant H-phosphinic acid. Therefore, 5′-*O*-DMT-3′-*C*-iodomethyl compounds **37** and **38** were reacted with BTSP **27** in the presence of Hünig's base. The desired 5′-*O*-DMT-3′-*C*-methylene-5-methyluridine H-phosphonate monomers **1** and **2** with the different 2′-substituents were obtained as their triethylamine salts after chromatographic purification using $CHCl_3$−MeOH−Et_3N as an eluent.

The H-phosphonate monomers **1** and **2** have been successfully utilized for the synthesis of 3′-methylene-modified oligonucleotides on solid support by the H-phosphonate chemistry.[18] However, the DMT-protecting group was very easily removed by a trace amount of acid in solvent or air during purification, handling, and storing; therefore, the coupling efficiency was affected. Then, we decided to use the less acid-labile MMT-protecting group, yet still adaptable to the automated synthesis, to protect the 5′-position (Scheme 3). Compounds **33**−**36** were reacted with 3 equiv of *p*-anisylchlorodiphenylmethane (4′-methoxytrityl chloride, MMT-Cl) in the presence of Hünig's base for 48 h using a mixed solvent of THF and ethyl acetate. The 5′-*O*-MMT-3′-*C*-iodomethyl nucleoside derivatives **42**−**45** were obtained in 84−99% yields. The reaction of **42**−**45** with BTSP **27** in the presence of Hünig's base, followed by their hydrolysis with a mixture of methanol and triethylamine, provided the desired products 5′-*O*-MMT-3′-methylene-substituted nucleoside H-phosphonates **3**−**6** with *O*-methyl, fluoro, hydrogen, and *O*-(2-methoxyethyl) substituents at the 2′-position. This new synthetic strategy with the modified Arbuzov reaction as the key step paved a new way for the synthesis of 3′-*C*-methylene H-phosphonates directly from nucleosides.

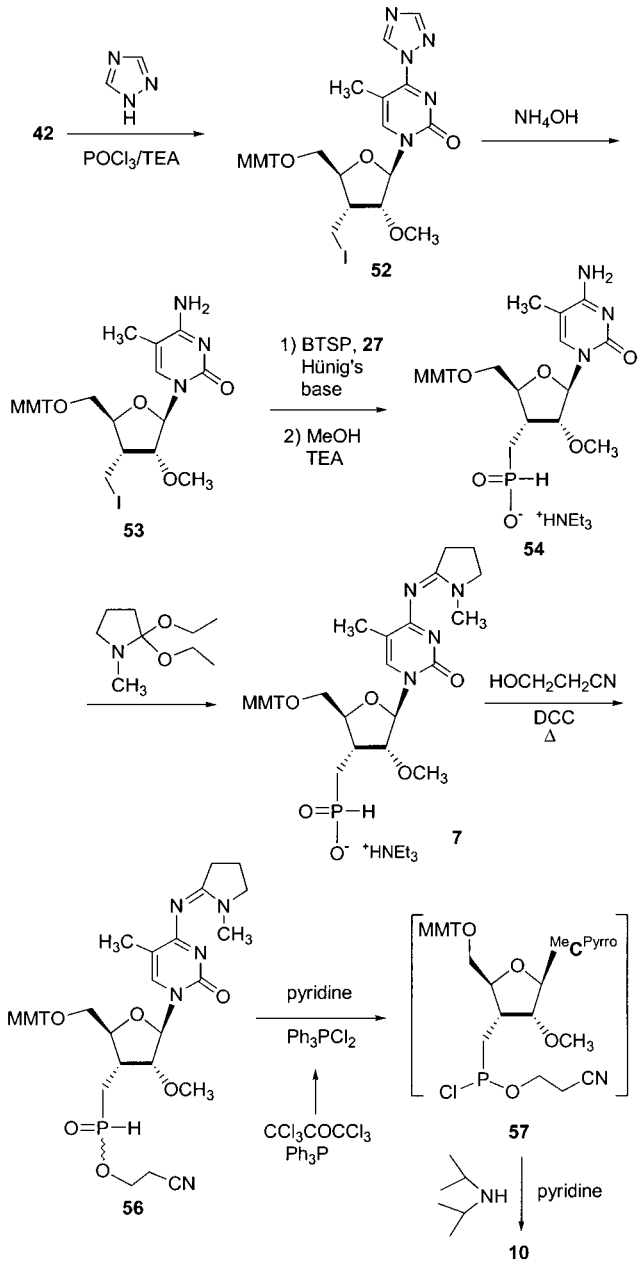
The automated synthesis of natural DNA (structure I, Figure 1) is carried out by 3′-*O*-H-phosphonate and/or 3′-*O*-phosphoramidite approaches, and the later approach is more efficient. 3′-*C*-H-phosphonate nucleotides **1**−**6** have been utilized for the synthesis of 3′-methylene-modified antisense oligonucleotides (structure II, Figure 1) by a *C*-H-phosphonate approach.[18] We expected that 3′-*C*-phosphonamidite monomers would also work more efficiently than the corresponding 3′-*C*-H-phosphonate monomers although they would be more synthetically challenging. Therefore, the transformation of 3′-*C*-H-phosphonate to 3′-*C*-phosphonamidite was investigated (Scheme 3). 2′-Deoxy- and 2′-*O*-methyl-3′-*C*-methylene nucleoside H-phosphonates **5** and **3** were condensed at an elevated temperature with 3-hydroxypropionitrile using dicyclohexylcarbodiimide (DCC) as a coupling agent. The hexanes extraction of the excess DCC, hydroxypropionitrile, and other impurities from an acetonitrile solution was proved to be an effective way for the purification of the resulted cyanoethyl H-phosphonates **50** and **51** without chromatographic purification. The cyanoethyl H-phosphonate thymidine **50** and 5-methyluridine **51** were obtained as their mixtures of two diastereoisomers at phosphorus as indicated by the ^{31}P NMR spectra. The commercial available chlorinating agent triphenylphosphine dichloride (Ph_3PCl_2) did not work for the transformation of **50** to phosphonamidite **8** through the 3′-*C*-methylene chlorocyanoethyloxyphosphine intermediate. The chlorinating agent triphenylphosphine dichloride was prepared in situ from triphosgene (Note: toxic) and triphenylphosphine based on the literature procedure.[27] The cyanoethyl H-phosphonate **50** was then reacted with the freshly prepared Ph_3PCl_2 in the presence of pyridine to give the chlorocyanoethyloxyphosphine intermediate, which was then reacted in situ with diisopropylamine in dichloromethane at low temperature. The reaction mixture was directly purified by flash chromatography on a silica gel column using hexanes−EtOAc-Et_3N and then hexanes−THF-Et_3N as eluents to give the desired product 3′-deoxy-3′-*C*-methylenethymidine phosphonamidite **8** in 56% overall yield. The 3′-deoxy-2′-*O*-methyl-3′-*C*-methylene-5-methyluridine phosphonamidite **9** was also synthesized from the corresponding cyanoethyl H-phosphonate **51** by the similar procedure in 68% yield. 3′-*C*-Methylene phosphonamidite monomers **8** and **9** were obtained as mixtures of their two diastereoisomers at phosphorus.

While the 3′-*C*-methylene H-phosphonate and phosphonamidite thymidine/5-methyluridine monomers **1**−**6**, **8**, and **9** are available, our research moved to the 5-methylcytidine derivatives. Various thymidine/uridine nucleosides derivatives have been successfully converted to the corresponding 5-methylcytidine/cytidine analogues under $POCl_3$/triazole/NH_4OH or other similar reaction conditions.[28] 3′-*C*-methylene H-phosphonate nucleotides **1**−**6** could not be directly converted to their 5-methylcytidine derivatives because $POCl_3$ would also activated the H-phosphonate group. 3′-*C*-methylene phosphonamidite thymidine **8** was converted to the corresponding 5-methylcytidine derivative (data not shown); however, the yield was very low, and it was difficult to manage the reaction conditions, because the ammonium hydroxide,

(27) Rivero, I. A.; Somanathan, R.; Hellberg, L. H. *Synth. Commun.* **1993**, *23*, 711−714.

(28) (a) Divakar, K. J.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1171−1176. (b) Perbost, M.; Sanghvi, Y. S. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2051−2052. (c) Jones, A. S.; Sayers, J. R.; Walker, R. T.; De Clercq, E. *J. Med. Chem.* **1988**, *31*, 268−271.

Scheme 4



essential for the second step, also reacted with the phosphonamidite group of the resultant product. Therefore, we decided to convert 3′-*C*-iodomethyl-2′-*O*-methyl-5-methyluridine **42** to the corresponding 5-methycytidine derivative **53** which was further used for the synthesis of 3′-*C*-methylene H-phosphonate and phosphonamidite 5-methylcytidine monomers. Scheme 4 depicts the synthesis of the protected 2′-*O*-methyl-3′-*C*-methylene-5-methylcytidine H-phosphonate **7** and phosphonamidite **10**. The 3′-*C*-iodomethyl nucleoside **42** was reacted with 1,2,4-triazole in the presence of phosphorus oxychloride to give the triazole compound **52**. After general workup, compound **52** was treated with aqueous ammonium hydroxide providing the 5-methylcytidine derivative **53** in a quantitative yield. Compound **53** without N^4-protecting group was reacted with BTSP under the modified reaction conditions as described above to give 2′-*O*-methyl-5′-*O*-MMT-3′-*C*-methylene-5-methylcytidine H-phosphonate **54** as its triethylamine salt. H-phosphonate

58: 5'-TTT TTT TTT TTT TTT **T*T*T* T***-3' (2'-OMe)

59: 5'-**T***CC AGG **T*GT*** CCG CAT* C-3' (2'-OMe)

60: 5'-CTC GTA C**T*T* T*T***C CGG TCC-3' (2'-OMe)

61: 5'-**T***CC AGG **T*GT*** CCG CA**T*** C-3' (2'-F)

62: 5'-CTC GTA C**T*T* T*T***C CGG TCC-3' (2'-F)

63: 5'-**T***CC AGG **T*GT*** CCG CA**T*** C-3' (2'-H)

Figure 3. New 3′-Methylene-Modified Oligonucleotides (all P=O).

54 was reacted with *N*-methyl-2,2-diethoxypyrrolidine[29] in the presence of triethylamine to generate the N^4-(*N*-methylpyrrolidin-2-ylidene)-protected H-phosphonate **7**, which can be used for the synthesis of oligonucleotides directly by the H-phosphonate chemistry. Compound **7** was condensed with 3-hydroxypropionitrile under the same reaction conditions as described above for **50** and **51** to give the corresponding cyanoethyl H-phosphonate 5-methylcytidine **56**. The one-pot three-step transformation of **56** to the final phosphonamidite **10** was carried out under the similar reaction conditions as described above for compound **8**. The N^4-(*N*-methylpyrrolidin-2-ylidene)-5′-*O*-MMT-2′-*O*-methyl-3′-*C*-methylene-5-methylcytidine phosphonamidite **10** was obtained in 72% yield as a white foam and a mixture of two diastereoisomers at phosphorus. New compounds **1**−**10**, **16**, **18**−**20**, **22**−**26**, **28**−**51** and **53**−**56** were all characterized by the essential ^{1}H, ^{13}C, ^{31}P, and ^{19}F NMR spectroscopic as well as high-resolution mass spectrometric analyses. The structures of most of the new compounds were further confirmed by their combustion analysis.

3′-Methylene-modified H-phosphonate and phosphonamidite monomers **1**−**10** with different substituents at the 2′-position are available, and **1**−**5** have been utilized for the synthesis of 3′-methylene-modified oligonucleotides by the automated solid-phase approach.[18] Figure 3 shows some representative examples of the 3′-*C*-methylene phosphonate-substituted oligonucleotides **58**−**63** for biophysical studies. The oligonucleotides **58**−**63** exhibited an excellent target-binding affinity to the complementary RNA with the melting temperature of hybridized duplex up to 3.64 °C increase per nucleotide modification relative to the phosphorothioate DNA. The oligonucleotide **58** is completely resistant without degradation to the enzyme snake venom phosphodiesterase (SVPD).[18] One octamer oligonucleotide with a 2′-*O*-methyl-3′-*C*-methylene phosphonate-substituted linkage, synthesized in our lab, was studied by X-ray crystallographic analysis.[30]

In conclusion, we have developed a new and useful strategy for the synthesis of various 2′-substituted 3′-*C*-methylene H-phosphonate nucleotides through the Arbuzov reaction under modified reaction conditions. New 5′-*O*-DMT- and MMT-3′-*C*-methylenethymidine, 5-methyluridine, and 5-methylcytidine H-phosphonates **1**−**7** with four different substituents OCH_3, F, H, OCH_2CH_2-

(29) (a) Heerwein, H.; Borner, P.; Fuchs, O.; Sasse, H. J.; Schrodt, H.; Spille, J. *Chem. Ber.* **1956**, *89*, 2060−2079. (b) Bredereck, H.; Effenberger, F.; Beyerlin, H. P. *Chem. Ber.* **1964**, *97*, 3081−3087. (c) McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040−2048.

(30) Egli, M.; Tereshko, V.; Teplova, M.; Minasov, G.; Joachimiak, A.; Sanishvili, R.; Weeks, C. M.; Miller, R.; Maier, M. A.; An, H.; Cook, P. D.; Manoharan, M. *Biopolymers* **2000**, *48*, 234−252.

OCH_3 at the 2′-position were successfully synthesized by this strategy. Two types of protecting groups, DMT and MMT, at the 5′-position provided more flexibility, and the corresponding monomers have been successfully utilized for the synthesis of 3′-*C*-methylene-modified oligonucleotides by the automated solid-phase approach. 3′-*C*-Methylenethymidine and 5-methyluridine/cytidine phosphonamidites **8**−**10**, the more reactive monomers, have been synthesized in high yields by a four-step two-pot procedure through the chlorocyanoethoxyphosphine intermediates. This overall strategy from 3′-*C*-iodomethyl nucleosides to H-phosphonates and finally to phosphonamidites can also be used to other nucleobases. Initial biophysical studies of the 3′-methylene-modified oligonucleotides provided very promising results regarding their chemical and enzymatic stability as well as their target-binding affinity. The synthesis of 3′-*C*-methylene H-phosphonates/phosphonamidites of other nucleobases and oligonucleotides, as well as their antisense properties studies, is in progress and will be reported in due course.

Experimental Section

^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded at 199.97, 50.29, 188.15, and 80.96 MHz unless otherwise indicated. Chemical shifts are expressed relative to the added tetramethylsilane. High-resolution FAB or MALDI TOF mass spectra were recorded. Combustion analyses were performed by M−H−W Laboratories, Phoenix, AZ. 5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-*O*-(3-*tert*-butylphenoxythio-carbonyl)-2′-*O*-methyl-5-methyluridine (**11**), 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-(3-*tert*-butylphenoxythiocarbonyl)-2′-deoxy-2′-*β*-fluoro-5-methyluridine (**12**), 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-(3-*tert*-butylphenoxythiocarbonyl)thymidine (**13**), and 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-*O*-(3-*tert*-butylphenoxythiocarbonyl)-2′-*O*-(2-methoxyethyl)-5-methyluridine (**14**) were synthesized by the reaction of the corresponding 5′-protected 2′-substituted nucleosides with 3′-*tert*-butylphenyl chlorothionoformate following the similar procedure for the preparation of 3′-*O*-phenoxythiocarbonyl derivatives.[21] 5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(2-phenylethenyl)thymidine (**17**) was prepared according to the published procedure.[21] Ammonium phosphinate was prepared based on the reported procedure.[24] *N*-Methyl-2,2-diethoxypyrrolidine was prepared based on the literature procedure with modification.[29] Other starting materials and reagents were purchased from Aldrich and used directly. *β*-Tributylstannyl styrene was prepared based on the literature procedure.[23]

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-2′-*β*-fluoro-3′-*C*-(2-phenylethenyl)-5-methyluridine (16). To a solution of compound **12** (13.0 g, 18.8 mmol) in 150 mL of benzene was added *β*-tributylstannyl styrene (PhCH=CHSnBu$_3$)[23] (17.5 g, 47 mmol, 2.5 equiv). The resulted solution was degassed three times with argon at room temperature and 45 °C. After 2,2′-azobisisobutyronitrile (AIBN) (1.0 g, 6.1 mmol) was added, the resulted solution was refluxed for 2 h. Another part of AIBN (1.0 g, 6.1 mmol) was added after cooling the reaction mixture to 40 °C. The reaction mixture was then refluxed for 2 h. This procedure was repeated until the starting material disappeared. The solvent was evaporated, and the residue was purified by flash chromatography on a silica gel column using 10:1 and 5:1 hexanes−EtOAc as eluents to give 6.31 g (57%) of compound **16** as a white foam: silica gel TLC R_f 0.60 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9H), 1.43 (s, 3H), 3.19−3.50 (m, 1H), 3.84 (dd, 1H, J = 12.0, 2.6 Hz), 4.22−4.30 (m, 2H), 5.13 (dd, 1H, J = 52.0, 4.0 Hz), 6.06 (d, 1H, J = 18.6 Hz), 6.08−6.20 (m, 1H), 6.67 (d, 1H, J = 17.8 Hz), 7.24−7.68 (m, 16H), 8.12 (s, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 12.1, 19.6, 27.3, 31.3, 45.4, 45.8, 61.9, 84.0, 89.8, 90.6, 96.5, 100.2, 111.0, 120.0, 120.1, 125.6, 127.7, 128.1, 129.8, 130.2, 132.7, 133.2, 135.4, 135.5, 136.2, 136.4, 150.6; HRMS (FAB) m/z 585.260 $(M + H)^+$ ($C_{34}H_{38}FN_2O_4Si$ requires 585.258). Anal. Calcd for $C_{34}H_{37}FN_2O_4Si$: C, 69.81; H, 6.38; N, 4.78. Found: C, 70.00; H, 6.50; N, 5.04.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-2′-*O*-(2-methoxyethyl)-3′-*C*-(2-phenylethenyl)-5-methyluridine (18). Compound **18** was prepared by the similar procedure as described above for compound **16** from compound **14** (15 g, 20 mmol), AIBN and PhCH=CHSnBu$_3$ (18.7 g, 50 mmol, 2.5 equiv). The crude product was purified by flash chromatography on a silica gel column using 10:1 and 5:1 hexanes−EtOAc as eluents to give 1.74 g (14%) of compound **18** as a white foam: silica gel TLC R_f 0.30 (1:1 hexanes−EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H), 1.43 (s, 3H), 3.18−3.30 (m, 1H), 3.37 (s, 3H), 3.58−3.62 (m, 2H), 3.79−3.80 (m, 2H), 4.06−4.37 (m, 4H), 4.95 (s, 1H), 6.25−6.40 (m, 1H), 6.62 (d, 1H, J = 16 Hz), 7.27−7.71 (m, 16 H), 9.21 (s, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 11.9, 19.6, 27.2, 45.3, 59.0, 62.1, 70.2, 72.0, 84.6, 87.1, 90.2, 110.4, 122.8, 126.4, 127.8, 128.0, 128.3, 128.6, 130.0, 132.7, 133.5, 134.7, 135.3, 135.4, 136.9, 150.3, 154.1; HRMS (FAB) m/z 641.302 $(M + H)^+$ ($C_{37}H_{45}N_2O_6Si$ requires 641.304). Anal. Calcd for $C_{37}H_{44}N_2O_6Si\cdot{}^1/_2H_2O$: C, 68.38; H, 6.82. Found: C, 68.72; H, 6.81.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-2′-*O*-methyl-3′-*C*-(2-phenylethenyl)-5-methyluridine (15). Compound **15** was synthesized and purified by the similar procedure as described above for compound **16** from compound **11**: silica gel TLC R_f 0.51 (97:3 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 1.47 (s, 3H), 3.15−3.30 (m, 1H), 3.63 (s, 3H), 3.78−3.95 (m, 2H), 4.19−4.31 (m, 2H), 6.01 (s, 1H), 6.19−6.34 (m, 1H), 6.63 (d, 1H, J = 16.2 Hz), 7.20−7.50 (m, 11H), 7.62−7.75 (m, 5H), 10.10 (s, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 11.9, 19.6, 27.2, 45.3, 59.6, 62.2, 84.5, 88.1, 89.2, 110.6, 122.5, 126.4, 127.8, 128.0, 128.7, 130.1, 132.7, 133.3, 134.8, 135.3, 135.4, 136.8, 150.6, 164.5; HRMS (FAB) m/z 619.261 $(M + Na)^+$ ($C_{35}H_{40}N_2O_5SiNa$ requires 619.260).

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(hydroxymethyl)-2′-*O*-methyl-5-methyluridine (19). To a solution of styrene **15** (30.0 g, 50.0 mmol) and *N*-methylmorpholine *N*-oxide (NMMO) (8.83 g, 75.0 mmol, 1.5 equiv) in 900 mL of dioxane was added a catalytic amount of osmium tetraoxide (4% aqueous solution, 12.75 mL, 0.51 g, 2.0 mmol, 0.04 equiv). The flask was covered by aluminum foil, and the reaction mixture was stirred at room temperature overnight. A solution of $NaIO_4$ (32.1 g, 150.0 mmol, 3.0 equiv) in 30 mL of water was added to the above stirred reaction mixture. The resulted reaction mixture was stirred for 1 h at 0 °C and 2 h at room temperature, followed by addition of 60 mL of ethyl acetate. The mixture was filtered through a Celite pad and washed with ethyl acetate. The filtrate was washed three times with 10% aqueous $Na_2S_2O_3$ solution until the color of aqueous phase disappeared. The organic phase was further washed with water and brine, dried (Na_2SO_4), and concentrated. Thus obtained aldehyde was dissolved in 800 mL of ethanol−water (4:1, v/v). Sodium borohydride ($NaBH_4$) (9.5 g, 0.25 mol, 5.0 equiv) was added in portions at 0 °C. The resulted reaction mixture was stirred at room temperature for 2 h and then treated with 1000 g of ice water. The mixture was extracted with ethyl acetate. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated. The resulted residue was purified by flash chromatography on a silica gel column using 50:1 to 20:1 CH_2Cl_2-MeOH as gradient eluents to give 17.8 g (68% from **15** for three steps) of product **19** as a white foam: silica gel TLC R_f 0.40 (15:1 CH_2Cl_2−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 9H), 1.50 (s, 3H), 2.45−2.60 (m, 1H), 2.62−2.72 (m, 1H), 3.60 (s, 3H), 3.65−3.95 (m, 3H, 1 OH), 4.00−4.40 (m, 3H), 5.94 (s, 1H), 7.30−7.50 (m, 6H), 7.52 (s, 1H), 7.60−7.74 (m, 4H), 9.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.1, 19.5, 21.1, 42.9, 59.6, 63.2, 81.7, 87.7, 88.5, 110.8, 127.9, 130.1, 130.2, 132.5, 133.2, 135.1, 135.3, 135.5, 150.5, 161.4; HRMS (FAB) m/z 657.137 $(M + Cs)^+$ ($C_{28}H_{36}N_2O_6SiCs$ requires 657.139). Anal. Calcd for $C_{28}H_{36}N_2O_6Si\cdot{}^1/_2H_2O$: C, 63.05; H, 6.99; N, 5.25. Found: C, 63.07; H, 7.16; N, 5.11.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-2′-*β*-fluoro-3′-*C*-(hydroxymethyl)-5-methyluridine (20). Compound **20** was prepared by a procedure similar to that described above for compound **19** from compound **16** (4.12 g, 7.05 mmol). The

crude product was purified by flash chromatography on a silica gel column using 200:1 and 50:1 CH_2Cl_2−MeOH as eluents to give 1.77 g (49%) of compound **20** as a white foam: silica gel TLC R_f 0.25 (20:1 $CHCl_3$−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 9H), 1.57 (s, 3H), 2.56−2.88 (m, 1H), 2.92 (bs, 1H, ex D_2O), 3.62−3.71 (m, 1H), 3.78−3.86 (m, 2H), 4.12−4.18 (m, 2H), 5.32 (dd, 1H, J = 52.0, 4.5 Hz), 5.97 (d, 1H, J = 18.0 Hz), 7.36−7.69 (m, 11H), 9.80 (bs, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 12.2, 19.1, 27.1, 44.2, 44.6, 57.4, 57.6, 63.4, 82.5, 89.6, 90.3, 95.1, 98.7, 110.1, 127.7, 128.0, 130.1, 132.5, 133.0, 135.4, 135.6, 150.4, 164.2; ^{19}F NMR ($CDCl_3$) δ −63.77 (ddd, J_1 = 55.2 Hz, J_2 = 35.5 Hz, J_3 = 19.8 Hz); HRMS (FAB) m/z 535.201 (M + Na)$^+$ ($C_{27}H_{33}FN_2O_5SiNa$ requires 535.204). Anal. Calcd for $C_{27}H_{33}FN_2O_5Si$: C, 63.24; H, 6.49; N, 5.47. Found: C, 63.40; H, 6.28; N, 5.37.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(hydroxymethyl)thymidine (21).[31] Compound **21** was prepared by a procedure similar to that described above for compound **19** from compound **17** (5.67 g, 10.0 mmol). The product was purified by flash chromatography on a silica gel column using 40:1 and 20:1 CH_2Cl_2−MeOH as eluents to give 3.99 g (80% overall) of compound **21** as a white foam.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(hydroxymethyl)-2′-*O*-(2-methoxyethyl)-5-methyluridine (22). Compound **22** was prepared by the similar procedure as described above for compound **19** from compound **18** (5.0 g, 7.8 mmol). The crude product was purified by flash chromatography on a silica gel column using 2:1, 1:1 and 1:2 hexanes−EtOAc as eluents to give 1.60 g (36%) of compound **22** as a white foam: silica gel TLC R_f 0.40 (20:1 CH_2Cl_2−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 9H), 1.50 (s, 3H), 2.25 (bs, 1H, ex D_2O), 2.52−2.78 (m, 1H), 3.38 (s, 3H), 3.52−4.25 (m, 10H), 5.86 (s, 1H), 7.38−7.70 (m, 11H), 9.95 (bs, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 12.1, 19.5, 27.1, 43.1, 58.2, 58.8, 63.1, 69.5, 71.6, 82.3, 86.1, 89.8, 110.5, 128.0, 130.2, 132.5, 133.2, 135.1, 135.3, 136.5, 150.5, 164.4; HRMS (FAB) m/z 569.268 (M + H)$^+$ ($C_{30}H_{41}N_2O_7Si$ requires 569.268). Anal. Calcd for $C_{30}H_{40}N_2O_7Si$: C, 63.33; H, 7.09; N, 4.93. Found: C, 63.13; H, 6.88; N, 5.12.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(iodomethyl)-2′-*O*-methyl-5-methyluridine (23). 2,6-Lutidine (10 mL, 9.2 g, 85.8 mmol, 1.94 equiv) and methyl triphenoxyphosphonium iodide (24.3 g, 53.7 mmol, 1.2 equiv) were sequentially added to a solution of compound **19** (23.3 g, 44.2 mmol) in 400 mL of anhydrous DMF under stirring at 0 °C. The resulted reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The reaction mixture was diluted with 100 mL of ethyl acetate and washed twice with 0.1 N $Na_2S_2O_3$ aqueous solution to remove iodine. The organic phase was further washed with aqueous $NaHCO_3$ solution, water, and brine. The aqueous phases were back extracted with ethyl acetate. The combined organic phase was dried (Na_2SO_4) and concentrated. The resulted residue was purified by flash chromatography on a silica gel column using 200:1 to 30:1 CH_2Cl_2-MeOH as gradient eluents to give 22.8 g (81%) of the iodo product **23** as a white foam: silica gel TLC R_f 0.54 (20:1 CH_2Cl_2−MeOH), R_f 0.63 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H), 1.61 (s, 3H), 2.60−2.84 (m, 2H), 3.18 (t, 3H, J = 9.0 Hz), 3.64 (s, 3H), 3.67−3.80 (m, 1H), 3.84−3.98 (m, 2H), 4.05−4.21 (m, 1H), 5.91 (s, 1H), 7.32−7.49 (m, 6H), 7.54−7.74 (m, 5H), 8.90 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.3, 19.4, 27.2, 44.9, 59.0, 62.5, 83.2, 86.8, 88.3, 110.6, 127.7, 128.0, 128.2, 130.2, 130.3, 132.4, 132.9, 134.9, 135.4, 135.6, 150.4, 164.3; HRMS (FAB) m/z 635.144 (M + H)$^+$ ($C_{28}H_{36}IN_2O_5Si$ requires 635.143). Anal. Calcd for $C_{28}H_{35}IN_2O_5Si$: C, 53.00; H, 5.55; N, 4.41. Found: C, 53.20; H, 5.53; N, 4.39.

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-2′-*β*-fluoro-3′-*C*-(iodomethyl)-5-methyluridine (24). Compound **24** was prepared as described above for compound **23** from compound **20** (1.3 g, 2.54 mmol), methyl triphenoxyphosphonium iodide (1.70 g, 3.8 mmol, 1.5 equiv) and 2,6-lutidine (0.59 mL, 0.54 g, 5.08 mmol). The product was purified by flash chromatography on a silica gel column using 200:1 CH_2Cl_2-MeOH as an eluent to give 1.45 g (92%) of compound **24** as a white foam: silica gel TLC R_f 0.33 (20:1 CH_2CH_2−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 9H), 1.63 (s, 3H), 2.76−3.18 (m, 3H), 3.76 (dd, 1H, J = 12.0, 2.8 Hz), 3.97 (d, 1H, J = 8.0 Hz), 4.17 (dd, 1H, J = 12.0, 2.0 Hz), 5.21 (dd, 1H, J = 52.0, 3.8 Hz), 5.98 (d, 1H, J = 19.4 Hz), 7.39−7.71 (m, 11H), 8.46 (bs, 1H, ex D_2O); HRMS (FAB) m/z 645.103 (M + Na)$^+$ ($C_{27}H_{32}FN_2O_4SiNa$ requires 645.105). Anal. Calcd for $C_{27}H_{32}FN_2O_4Si$: C, 52.07; H, 5.18; N, 4.50. Found: C, 52.28; H, 5.28; N, 4.28.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(iodomethyl)thymidine (25).[19] Compound **25** was prepared as described above for compound **23** from compound **21** (32.0 g, 64.6 mmol), methyl triphenoxyphosphonium iodide (34.3 g, 75.8 mmol, 1.17 equiv), and 2,6-lutidine (14.3 mL, 13.1 g, 122 mmol, 1.89 equiv) in 660 mL of DMF. The product was purified by flash chromatography on a silica gel column using 5:1 to 2:1 hexanes−EtOAc as eluents to give 33.2 g (85%) of compound **25** as a white foam: silica gel TLC R_f 0.54 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.12 (s, 9H), 1.69 (s, 3H), 2.15−2.40 (m, 2H), 2.60−2.80 (m, 1H), 3.06−3.28 (m, 2H), 3.75−3.89 (m, 2H), 3.98−4.10 (m, 1H), 6.18 (t, 1H, J = 5.8 Hz), 7.35−7.54 (m, 7H), 7.63−7.78 (4H), 9.52 (bs, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 6.9, 12.3, 19.4, 27.1, 40.2, 40.7, 63.9, 84.1, 85.2, 111.1, 127.7, 128.0, 130.1, 132.6, 133.0, 135.2, 135.4, 135.6, 150.7, 164.2; HRMS (MALDI) m/z 627.113 (M + Na)$^+$ ($C_{27}H_{33}IN_2O_4$-SiNa requires 627.115).

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-(iodomethyl)-2′-*O*-(2-methoxyethyl)-5-methyluridine (26). The iodo compound **26** was synthesized as described above for compound **23** from compound **22** (1.34 g, 2.35 mmol), 2,6-lutidine (0.547 mL, 503 mg, 4.69 mmol, 2.0 equiv), and methyl triphenoxyphosphonium iodide (1.28 g, 2.83 mmol, 1.2 equiv) in 25 mL of DMF. The crude product was purified by flash chromatography on a silica gel column. Gradient elution with 5:1, 3:1, and then 1:1 hexanes−EtOAc provided 1.24 g (78%) of the iodo product **26** as a white foam: silica gel TLC R_f 0.39 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H), 1.62 (s, 3H), 2.64−2.85 (m, 2H), 3.20−3.35 (m, 1H), 3.38 (s, 3H), 3.50−4.25 (m, 8H), 5.91 (s, 1H), 7.32−7.50 (m, 6H), 7.60 (s, 1H), 7.62−7.78 (m, 4H), 10.46 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.4, 19.5, 27.2, 45.0, 58.0, 62.5, 70.3, 71.9, 83.3, 85.6, 88.9, 110.5, 128.1, 128.2, 130.1, 130.3, 132.4, 132.9, 135.0, 135.4, 135.6, 150.7, 164.7; HRMS (FAB) m/z 679.172 (M + H)$^+$ ($C_{30}H_{40}IN_2O_6$-Si requires 679.170).

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methyl-5-methyluridine (28) and 5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-dexoy-3′-*C*-methyl-2′-*O*-methyl-5-methyluridine (30). A mixture of ammonium phosphinate (0.41 g, 5.0 mmol, 5.0 equiv)[24] and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (1.07 mL, 0.82 g, 5.05 mmol, 5.05 equiv) was heated neat at 100−110 °C for 2 h under argon atmosphere with a condenser. The resulted intermediate bis-(trimethylsilyl)phosphonite (BTSP) (**27**) was cooled to −5−0 °C. Anhydrous CH_2Cl_2 (5 mL) was injected, followed by a solution of iodo-compound **23** (0.64 g, 1.0 mmol) in 8 mL of CH_2Cl_2. The resulted reaction mixture was stirred at room temperature overnight, filtered, and concentrated. The clear oily residue was dissolved in 5 mL of CH_2Cl_2 and 5 mL of MeOH. The solution was stirred at room temperature for 2 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate. The solution was washed with water and brine. The organic phase was dried and concentrated. The residue was purified by flash chromatography on a silica gel column using 10:1, 2:1, and then 1:1 EtOAc−MeOH as eluents to provide 105 mg (31%) of the desired product H-phosphinic acid **28** and 150 mg (59%) of the reduced byproduct **30** as white foams. H-Phosphinic acid **28** (free acid): silica gel TLC R_f 0.30 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR (CD_3OD) δ 1.10 (s, 9H), 1.33 (s, 3H), 1.30−1.51 (m, 1H), 1.74−1.98 (m, 1H), 2.47−2.68 (m, 1H), 3.53 (s, 3H), 3.87−4.08 (m, 3H), 4.19 (d, 1H, J = 11.6 Hz), 7.07 (5.82, 8.32; d, 1H, J = 500 Hz, PH), 5.91 (s, 1H), 7.35−7.50 (m, 6H), 7.59 (s, 1H), 7.65−6.80 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 11.7, 18.9, 26.8, 36.1, 48.6, 57.3, 63.1, 84.8, 88.3, 108.9, 128.0, 129.9, 132.5, 133.1, 134.9, 135.0, 150.1,

(31) Haly, B.; Bellon, L.; Mohan, V.; Sanghvi, Y. S. *Nucleosides Nucleotides* **1996**, *15*, 1383−1395.

163.7; ^{31}P NMR (CD_3OD) δ 27.1; MS (ES) *m/z* 571 ($M - H)^-$; HRMS (FAB) *m/z* 595.200 ($M + Na)^+$ ($C_{28}H_{37}N_2O_7PSiNa$ requires 595.200).

5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-dideoxy-2′-*β*-fluoro-3′-*C*-[(hydroxyphosphinyl)methyl]-5-methyluridine (29) and 5′-*O*-(*tert*-Butyldiphenylsilyl)-2′,3′-didexoy-2′-*β*-fluoro-3′-*C*-methyl-5-methyluridine (31). Compounds **29** and **31** were prepared by a procedure similar to that described above for compounds **28** and **30** from HMDS (18.0 g, 50 mmol), ammonium phosphinate (1.0 g, 12 mmol), and compound **24** (0.75 g, 1.2 mmol). The crude products were purified by flash chromatography on a silica gel column using 20:1 and 5:1 CH_2Cl_2−MeOH as eluents to give 0.10 g (15%) of compound **29** as a white powder and 0.30 g (50%) of compound **31** as a white foam. H-Phosphinic acid **29** (free acid): silica gel TLC R_f 0.30 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR (CD_3OD) δ 1.10 (s, 9H), 1.42 (s, 3H), 1.35−1.91 (m, 2H), 2.56−2.86 (m, 1H), 3.85−4.31(m, 3H), 5.38 (dd, 1H, J = 52.0, 4.2 Hz), 7.13 (5.88, 8.39; d, 1H, J = 502 Hz, PH), 6.01 (d, 1H, J = 20.0 Hz), 7.32−7.78 (m, 11H); ^{31}P NMR (CD_3OD) δ 25.01; ^{19}F NMR (CD_3OD) δ −62.62 (ddd, J_1 = 54.8 Hz, J_2 = 34.1 Hz, J_3 = 20.1 Hz); HRMS (FAB) *m/z* 583.180 ($M + Na)^+$ ($C_{27}H_{34}FN_2O_6PSiNa$ requires 583.180).

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methyl-5-methyluridine Tetrabutylammonium Salt (32). To a solution of 5′-TBDPS-protected H-phosphinic acid **28** (140 mg, 0.20 mmol) in 1 mL of DMF and 8 mL of THF was added tetrabutylammonium fluoride (1.0 M in THF, 0.31 mL, 0.31 mmol, 1.5 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 4 h and concentrated. The residue was purified by flash chromatography on a silica gel column using 20:1, 2:1, and then 1:1 EtOAc−MeOH as eluents to give 108 mg (94%) of the sticky oily product **32** as its tetrabutylammonium salt: silica gel TLC R_f 0.39 (1:1 EtOAc−MeOH); ^{1}H NMR (CD_3OD) δ 1.02 (t, 12H, J = 7.0 Hz), 1.30−1.55 (m, 8H), 1.57−1.78 (m, 8H), 1.85 (s, 3H), 1.80−2.00 (m, 1H), 2.34−2.65 (m, 1H), 3.18−3.45 (m, 9H), 3.55 (s, 3H), 3.70−4.06 (m, 4H), 7.06 (5.81, 8.31; d, 1H, J = 500 Hz, PH), 5.87 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (CD_3OD) δ 12.6, 14.0, 20.7, 24.8, 27.2, 28.9, 36.1, 58.3, 59.5, 60.4, 87.3, 87.7, 88.0, 89.7, 110.2, 138.3, 152.1, 166.7; ^{31}P NMR (CD_3OD) δ 26.9; MS (ES) *m/z* 333 ($M - H)^-$.

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methylthymidine (32) (Salt-Free). A mixture of **28** (500 mg, 0.87 mmol) and triethylamine trifluoride (1.15 mL, 1.14 g, 7.07 mmol, 8.1 equiv) in 10 mL of DMF and 15 mL of THF was stirred at room temperature for 48 h. The solvent was evaporated, and the residue was purified by flash chromatography on a silica gel column using 10:1, 2:1, and then 1:1 EtOAc−MeOH as eluents to give 370 mg (97%) of product **32** as a white foam: silica gel TLC R_f 0.39 (1:1 EtOAc−MeOH); ^{1}H NMR (CD_3OD) δ 1.30−1.80 (m, 2H), 1.86 (s, 3H), 2.24−2.55 (m, 1H), 3.55 (s, 3H), 3.74−4.06 (m, 4H), 7.06 (5.81, 8.31; d, 1H, J = 500 Hz, PH), 5.87 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (CD_3OD) δ 12.6, 27.2, 28.9, 36.1, 58.3, 60.4, 87.3, 87.7, 88.0, 89.8, 110.2, 138.3, 152.1, 166.7; ^{31}P NMR (CD_3OD) δ 27.0; ^{31}P NMR (CD_3OD + HCl) δ 36.9; MS (ES) *m/z* 333 ($M - H)^-$; HRMS (FAB) *m/z* 357.083 ($M + Na)^+$ ($C_{12}H_{19}N_2O_7PNa$ requires 357.082). Anal. Calcd for $C_{12}H_{19}N_2O_7P$: C, 43.12; H, 5.72; N, 8.38. Found: C, 43.37; H, 5.87; N, 8.25.

3′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methyl-5-methyluridine Triethylamine Salt (1). The unprotected H-phosphinic acid **32** (70 mg, 0.2 mmol) was coevaporated twice with anhydrous pyridine and then dissolved in 3 mL of pyridine. To this solution was added DMT-Cl (203 mg, 0.6 mmol, 3.0 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 24 h and concentrated. The residue was purified by flash chromatography on a silica gel column. Elution with 100:10:1 to 100:30:1 $CHCl_3$−MeOH−Et_3N provided 95 mg (65%) of triethylamine salt product **1** as a white foam: silica gel TLC R_f 0.39 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR (CD_3OD) δ 1.20−1.45 (m, 3 + 9H, Et_3N), 1.55−1.95 (m, 2H), 2.60−2.85 (m, 1H), 3.16 (q, 6H, J = 7.2 Hz, Et_3N), 3.57 (s, 3H), 3.76 (s, 6H), 3.90−4.15 (m, 3H), 5.81 (s, 1H), 6.86 (d, 4H, J = 10.0 Hz), 7.07 (5.83, 8.31; d, 1H, J = 496 Hz, PH), 7.20−7.53 (m, 9H), 7.91 (s, 1H); ^{13}C NMR (CD_3OD) δ 9.3, 12.3, 27.3, 29.1, 37.5, 47.7, 55.8, 58.2, 62.5, 86.1, 86.5, 86.7, 87.9, 90.4, 110.6, 114.3, 128.2, 129.0, 129.6, 131.5, 136.7, 136.8, 137.5, 145.9, 151.9, 160.3, 166.2; ^{31}P NMR (CD_3OD) δ 27.2; HRMS (FAB) *m/z* 659.212 ($M + Na)^+$ ($C_{33}H_{37}N_2O_9PNa$ requires 659.213).

3′-Deoxy-3′-*C*-(iodomethyl)-2′-*O*-methyl-5-methyluridine (33). A solution of **23** (6.35 g, 10.0 mmol) and triethylamine trifluoride (6.52 mL, 6.45 g, 40.0 mmol, 4.0 equiv) in 100 mL of THF was stirred at room temperature for 24 h. The reaction mixture was diluted with 200 mL of ethyl acetate and washed with water and brine. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column. Elution with 3:1, 1:1, and then 1:2 hexanes−EtOAc provided 3.76 g (95%) of product **33** as a white foam: silica gel TLC R_f 0.40 (1:2 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.87 (s, 3H), 2.56−2.74 (m, 1H), 3.17−3.30 (m, 2H), 3.61 (s, 3H), 3.70−4.04 (m, 4H), 5.88 (s, 1H), 8.18 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.6, 45.1, 59.2, 60.8, 85.2, 88.7, 89.1, 110.6, 138.0, 152.1, 166.6; HRMS (FAB) *m/z* 397.026 ($M + H)^+$ ($C_{12}H_{18}IN_2O_5$ requires 397.026). Anal. Calcd for $C_{12}H_{17}IN_2O_5$: C, 36.37; H, 4.32; N, 7.07. Found: C, 35.97; H, 4.17; N, 7.01.

2′,3′-Dideoxy-2′-*β*-fluoro-3′-*C*-(iodomethyl)-5-methyluridine (34). Compound **34** was prepared by the similar procedure as described above for compound **33** from compound **24** (0.62 g, 3.25 mmol) and triethylamine trifluoride (2.65 mL, 16.2 mmol, 5 equiv). The product was purified by flash chromatography on a silica gel column using 20:1 CH_2Cl_2-MeOH as an eluent to give 1.15 g (92%) of compound **34** as a white foam: silica gel TLC R_f 0.25 (20:1 CH_2Cl_2-MeOH); ^{1}H NMR ($CDCl_3$) δ 1.90 (s, 3H), 2.60 (t, 1H, J = 4.8 Hz, ex D_2O), 2.82−3.30 (m, 3H), 3.82−4.16 (m, 3H), 5.26 (dd, 1H, J = 52.0, 4.4 Hz), 5.88 (d, 1H, J = 19.6 Hz), 7.59 (s, 1H), 9.08 (bs, 1H, ex D_2O); ^{19}F NMR ($CDCl_3$) δ −63.23 (ddd, J_1 = 55.4 Hz, J_2 = 35.2 Hz, J_3 = 20.2 Hz); HRMS (FAB) *m/z* 385.006 ($M + H)^+$ ($C_{11}H_{15}FIN_2O_4$ requires 385.006). Anal. Calcd for $C_{11}H_{14}FIN_2O_4$: C, 34.37; H, 3.67; N, 7.29. Found: C, 34.31; H, 3.88; N, 7.03.

3′-Deoxy-3′-*C*-(iodomethyl)thymidine (35). The unprotected iodo compound **35** was synthesized as described above for compound **33** from compound **25** (33.3 g, 55.0 mmol) and triethylamine trifluoride (40.4 mL, 39.95 g, 247 mmol, 4.5 equiv) in 700 mL of THF. Recrystallization of the crude product from ethyl acetate provided 19.0 g (94%) of the deprotected iodo product **35** as white needles: mp 164−165 °C; silica gel TLC R_f 0.20 (1:2 hexanes−EtOAc); ^{1}H NMR (CD_3OD) δ 1.88 (s, 3H), 2.20−2.32 (m, 2H), 2.47−2.68 (m, 1H), 3.23−3.34 (m, 1H), 3.36−3.48 (m, 1H), 3.70−3.97 (m, 3H), 6.09 (t, 1H, J = 5.4 Hz), 7.93 (s, 1H); ^{1}H NMR (DMSO-d_6) δ 1.79 (s, 3H), 2.10−2.25 (m, 2H), 2.43−2.64 (m, 1H), 3.25−3.37 (m, 1H), 3.39−3.50 (m, 1H), 3.55−3.80 (m, 3H), 6.04 (t, 1H, J = 5.6 Hz), 7.83 (s, 1H), 11.27 (s, 1H, ex D_2O); ^{13}C NMR (DMSO-d_6) δ 9.5, 12.3, 39.5, 60.9, 83.3, 85.1, 108.9, 136.2, 150.4, 163.8; HRMS (MALDI TOF) *m/z* 388.997 ($M + Na)^+$ ($C_{11}H_{15}IN_2O_4Na$ requires 388.997). Anal. Calcd for $C_{11}H_{15}IN_2O_4$: C, 36.08; H, 4.12; N, 7.65. Found: C, 36.27; H, 3.94; N, 7.64.

3′-Deoxy-3′-*C*-(iodomethyl)-2′-*O*-(2-methoxyethyl)-5-methyluridine (36). The unprotected iodo-compound **36** was synthesized as described above for compound **33** from compound **26** (1.12 g, 1.65 mmol) and triethylamine trifluoride (1.1 mL, 1.08 g, 6.7 mmol, 4.0 equiv) in 20 mL of THF. The crude product was purified by flash chromatography on a silica gel column. Gradient elution with 2:1, 1:2, and then 1:3 hexanes−EtOAc provided 504 mg (69%) of the deprotected iodo-product **36** as a white foam: silica gel TLC R_f 0.27 (1:3 hexanes−EtOAc); ^{1}H NMR (CD_3OD) δ 1.87 (s, 3H), 2.47−2.75 (m, 1H), 3.18−3.37 (m, 2H), 3.40 (s, 3H), 3.59−3.70 (m, 2H), 3.71−3.90 (m, 2H), 3.92−4.17 (m, 4H), 5.87 (s, 1H), 8.17 (s, 1H); ^{13}C NMR (CD_3OD) δ 12.5, 45.2, 59.2, 60.9, 71.0, 72.9, 85.4, 87.3, 89.7, 110.5, 138.0, 152.1, 166.6; HRMS (FAB) *m/z* 441.053 ($M + H)^+$ ($C_{14}H_{22}IN_2O_6$ requires 441.052).

3′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′-*C*-(iodomethyl)-2′-*O*-methyl-5-methyluridine (37). A mixture of **33** (3.17 g, 8.0 mmol), Hünig's base (4.2 mL, 3.11 g, 24 mmol, 3.0 equiv), and DMT-Cl (5.42 g, 16.0 mmol, 2.0 equiv) in 80 mL of ethyl

acetate was stirred at 0 °C to room temperature for 7 h. The resulted reaction mixture was diluted with ethyl acetate, and washed with water and then brine. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column using 3:1 to 1:1 hexanes−EtOAc as gradient eluents to give 5.53 g (99%) of product **37** as a white foam: silica gel TLC R_f 0.35 (30:1 CH_2Cl_2−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 3H), 2.70−2.86 (m, 2H), 3.09−3.30 (m, 2H), 3.66 (s, 3H), 3.68−3.71 (m, 1H), 3.80 (s, 6H), 3.91 (d, 1H, J = 4.4 Hz), 3.99 (d, 1H, J = 9.8 Hz), 5.91 (s, 1H), 6.86 (d, 4H, J = 8.0 Hz), 7.20−7.45 (m, 9H), 7.80 (s, 1H), 9.10 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.2, 45.1, 55.3, 59.0, 61.5, 82.5, 86.8, 88.4, 110.6, 113.4, 127.3, 128.2, 130.2, 135.2, 135.3, 144.2, 150.6, 158.8, 164.6; HRMS (FAB) m/z 721.139 $(M + Na)^+$ ($C_{33}H_{35}IN_2O_7Na$ requires 721.138). Anal. Calcd for $C_{33}H_{35}IN_2O_7 \cdot H_2O$: C, 55.31; H, 5.20; N, 3.91. Found: C, 55.44; H, 5.19; N, 3.87.

2′,3′-Dideoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*β*-fluoro-3′-*C*-(iodomethyl)-5-methyluridine (38). Compound **38** was prepared by the similar procedure for compound **37** from compound **34** (1.15 g, 3 mmol), Hünig's base (1.16 g, 9 mmol, 3 equiv) and DMT-Cl (2.03 g, 6 mmol, 2 equiv). The product was purified by flash chromatography on a silica gel column using 10:1, 5:1, and 2:1 hexanes−EtOAc as eluents to give 1.82 g (88%) of compound **38** as a white foam: silica gel TLC R_f 0.40 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 3H), 2.73−2.95 (m, 1H), 2.94−3.18 (m, 2H), 3.26−3.43 (m, 1H), 3.67−3.80 (m, 1H), 3.80 (s, 6H), 3.98−4.13 (m, 1H), 5.26 (dd, 1H, J = 52.0, 4.4 Hz), 6.01 (d, 1H, J = 18 Hz), 6.85−7.44 (m, 13H), 7.69 (s, 1H), 9.67 (bs, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 12.2, 45.4, 45.8, 55.3, 61.3, 82.3, 87.0, 88.9, 89.6, 95.6, 99.3, 111.1, 113.4, 127.3, 128.1, 130.1, 135.1, 135.2, 144.1, 150.2, 158.8, 164.2; HRMS (FAB) m/z 687.135 $(M + H)^+$ ($C_{32}H_{33}FIN_2O_6$ requires 687.136).

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methyl-5-methyluridine (32) and 3′-Deoxy-3′-*C*-methyl-2′-*O*-methyl-5-methyluridine (39) (from 37). A mixture of ammonium phosphinate (1.66 g, 20 mmol, 10 equiv) and HMDS (4.64 mL, 3.55 g, 22.0 mmol, 11.0 equiv) was heated at 100−110 °C for 2 h under argon atmosphere with a condenser. The intermediate BTSP **27** was cooled to 0 °C, and 8 mL of anhydrous CH_2Cl_2 was injected, followed by injecting a solution of iodo-compound **37** (1.40 g, 2.0 mmol) in 10 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature overnight, concentrated, and dissolved in 20 mL of THF−MeOH (2:1). The mixture was stirred at room temperature for 1 h, concentrated, and treated with water−ethyl acetate. The mixture was filtered through a Celite pad and washed with water and ethyl acetate. The layers were separated, and the organic phase was washed with water. The combined aqueous phase was concentrated and purified on a silica gel column using 100:10:1, 100:20:1, and then 100:30:1 $CHCl_3$−MeOH−Et_3N as eluents. The crude product obtained was further purified on a reversed-phase column eluting with water to remove all possible inorganic salts and then with 25:1 and 5:1 water−MeOH. The H-phosphinic acid product **32** was obtained as a white foam, yield 160 mg (24%): silica gel TLC R_f 0.40−0.50 (1:1 EtOAc−MeOH), R_f 0.38 (50:10:1 $CHCl_3$−MeOH−Et_3N). Compound **32** obtained by this method from **37** shows the identical chromatographic and spectroscopic properties as the same compound **32** obtained from compound **28** (Scheme 1). The organic phase obtained from above reaction mixture was dried (Na_2SO_4) and concentrated. The foam residue was purified by flash chromatography on a silica gel column. Elution with 2:1 to 1:1 hexanes−EtOAc provided 290 mg (53%) of the reduced product **39** as white needles.

Compounds 32 and 39 (from 33). Compound **33** (594 mg, 1.5 mmol) was reacted with BTSP **27** similarly as described above for **32** from **37**. Chromatographic purification provided 118 mg (23%) of product **32** as a white foam and 256 mg (63%) of the reduced byproduct **39** as white needles. Compounds **32** and **39** obtained by this way from **33** show identical chromatographic and spectroscopic properties as the same compounds **32** and **39** obtained from compound **37**.

3′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-methyl-5-methyluridine Triethylamine Salt (1) and 3′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-3′-*C*-methyl-2′-*O*-methyl-5-methyluridine (40) (from 37). A mixture of ammonium phosphinate (810 mg, 9.7 mmol, 4.0 equiv) and HMDS (2.08 mL, 1.59 g, 9.8 mmol, 4.04 equiv) was heated at 100−110 °C under argon atmosphere with a condenser. The resulted BTSP (**27**) was cooled to 0 °C and 10 mL of anhydrous CH_2Cl_2 was injected, followed by a solution of compound **37** (1.70 g, 2.43 mmol) and Hünig's base (860 μL, 638 mg, 4.9 mmol, 2.0 equiv) in 10 mL of CH_2Cl_2. The resulted reaction mixture was stirred at room temperature overnight and then treated with THF−MeOH−Et_3N (5:8:1). The reaction mixture was stirred at room temperature for 2 h, concentrated, and then treated with mixture of water and ethyl acetate. The layers were separated and concentrated. The residue obtained from the aqueous phase was purified by flash chromatography on a silica gel column using 200:100:1 $CHCl_3$-MeOH-Et_3N as an eluent to give 560 mg (31%) of final product **1** and 48 mg (6%) of the deprotected product **32** as white foams. Product **1** obtained by this way shows the identical chromatographic and spectroscopic properties as the same compound obtained from compound **32**. The organic phase of the reaction mixture was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column. Elution with 3:1 to 1:1 hexanes−EtOAc provided 750 mg (54%) of the reduced product **40** as a white foam.

2′,3′-Dideoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*β*-fluoro-3′-*C*-[(hydroxyphosphinyl)methyl]-5-methyluridine Triethylamine Salt (2) and 2′,3′-Dideoxy-5′-*O*-(4,4′-dimethoxytrityl)-2′-*β*-fluoro-3′-*C*-methyl-5-methyluridine (41) (from 38). Compounds **2** and **43** were prepared by the similar procedure for compounds **1** and **40** from compound **38** (0.75 g, 1.1 mmol), ammonium phosphinate (0.365 g, 4.4 mmol, 4.0 equiv), and HMDS (0.72 g, 4.46 mmol, 4.05 equiv). The product was purified by flash chromatography on a silica gel column using 200:5:1 CH_2Cl_2−MeOH−Et_3N as an eluent to give 175 mg (17%) of compound **2** as a white foam. The reduced compound was further purified by flash chromatography on a silica gel column using 3:1 and 2:1 hexanes−EtOAc as eluents to give 303 mg (49%) of compound **41** as a white foam. H-Phosphonate **2**: silica gel TLC R_f 0.40 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.25−1.45 (m, 3 + 9H, Et_3N), 3.02 (q, 6H, J = 7.4 Hz, Et_3N), 3.25 (dd, 1H, J = 12.0, 4.4 Hz), 3.61−3.66 (m, 1H), 3.76 (s, 6H), 3.02−4.15 (m, 1H), 5.46 (dd, 1H, J = 62.0, 4.5 Hz), 6.01 (d, 1H, J = 17.2 Hz), 6.81−7.43 (m, 14H), 7.25 (6.02, 8.48; d, 1H, J = 492 Hz, PH), 7.69-(s, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 8.6, 12.0, 25.86, 27.5, 36.5, 36.9, 45.6, 55.2, 61.1, 84.1, 84.4, 86.6, 89.1, 89.8, 94.9, 98.6, 110.2, 113.3, 127.0, 128.0, 128.2, 130.1, 134.9, 135.4, 144.2, 150.4, 158.6, 164.3; ^{31}P NMR ($CDCl_3$) δ 21.27; ^{19}F NMR ($CDCl_3$) δ −61.84 (ddd, J_1 = 55.2 Hz, J_2 = 35.0 Hz, J_3 = 18.7 Hz); MS (ES) m/z 623.2 $(M - H)^-$ ($C_{32}H_{33}FN_2O_8P$ requires 623.2).

3′-Deoxy-3′-*C*-(iodomethyl)-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methyluridine (42). A mixture of **33** (1.19 g, 3.0 mmol), Hünig's base (2.1 mL, 1.55 g, 12.0 mmol, 4.0 equiv), and *p*-anisylchlorodiphenylmethane (4′-methoxytrityl chloride, MMT-Cl) (2.78 g, 9.0 mmol, 3.0 equiv) in 40 mL of ethyl acetate and 10 mL of THF was stirred at room temperature for 48 h. The reaction mixture was diluted with ethyl acetate and washed with water, followed by brine. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column. Elution with 3:1 to 1:3 hexanes−EtOAc provided 1.90 g (95%) of the MMT-protected product **42** as a white foam: silica gel TLC R_f 0.48 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 3H), 2.69−2.86 (m, 2H), 3.09−3.31 (m, 2H), 3.67 (s, 3H), 3.60−3.70 (m, 1H), 3.80 (s, 3H), 3.90−4.09 (m, 2H), 5.91 (s, 1H), 6.87 (d, 2H, J = 8.0 Hz), 7.25−7.50 (m, 12H), 7.78 (s, 1H), 9.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.3, 45.1, 55.3, 59.0, 61.6, 82.4, 86.8, 87.1, 88.4, 110.6, 113.4, 127.4, 127.7, 128.2, 128.4, 130.4, 134.7, 135.3, 143.6, 143.7, 150.6, 158.9, 164.6; HRMS (FAB) m/z 691.128 $(M + Na)^+$ ($C_{32}H_{33}IN_2O_6Na$ requires 691.128). Anal. Calcd for $C_{32}H_{33}IN_2O_6$: C, 55.98; H, 5.13; N, 4.08. Found: C, 55.79; H, 5.10; N, 4.03.

2′,3′-Dideoxy-2′-*β*-fluoro-3′-*C*-(iodomethyl)-5′-*O*-(4-methoxytrityl)-5-methyluridine (43). Compound **43** was prepared by the similar procedure as described above for compound **42** from compound **34** (2.72 g, 7.08 mmol), MMT-Cl (6.6 g, 21.2 mmol, 3 equiv), and Hünig's base (3.72 mL, 2.75 g, 21.2 mmol, 3 equiv) in 75 mL of ethyl acetate and 15 mL of THF. The crude product was purified by flash chromatography on a silica gel column using CH_2Cl_2 and 200:1 CH_2Cl_2−MeOH as eluents to give 4.08 g (88%) of compound **43** as a pale yellow foam: silica gel TLC R_f 0.45 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.53 (s, 3H), 2.78−3.16 (m, 3H), 3.33 (dd, 1H, J = 12.0, 2.8 Hz), 3.69−3.74 (m, 1H), 3.81 (s, 3H), 4.02−4.07 (m, 1H), 5.26 (dd, 1H, J = 50.0, 3.2 Hz), 6.01 (d, 1H, J = 18.2 Hz), 6.86−7.45 (m, 14H), 7.67 (s, 1H), 9.52 (bs, 1H, ex D_2O); ^{13}C NMR ($CDCl_3$) δ 12.2, 45.5, 45.8, 55.3, 61.4, 82.2, 87.2, 88.9, 89.7, 95.6, 99.3, 111.1, 113.4, 127.4, 127.8, 128.2, 128.4, 130.4, 134.6, 135.2, 143.5, 143.7, 150.2, 159.0, 164.1; ^{19}F NMR ($CDCl_3$) δ −64.51 (ddd, J_1 = 54.1 Hz, J_2 = 33.9 Hz, J_3 = 19.8 Hz).

3′-Deoxy-3′-*C*-(iodomethyl)-5′-*O*-(4-methoxytrityl)thymidine (44). Compound **44** was prepared as described above for compound **42** from compound **35** (14.8 g, 40.4 mmol), Hünig's base (25.0 mL, 18.55 g, 143 mmol, 3.5 equiv), and MMT-Cl (37.5 g, 121 mmol, 3.0 equiv) in a mixture of THF (380 mL) and ethyl acetate (220 mL). The crude product was purified by flash chromatography on a silica gel column. Gradient elution with 4:1 to 1:3 hexanes−EtOAc provided 21.76 g (84.3%) of the MMT-protected product **44** as a white foam: silica gel TLC R_f 0.38 (1:1 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 3H), 2.25−2.38 (m, 2H), 2.56−2.74 (m, 1H), 2.99−3.20 (m, 2H), 3.30 (dd, 1H, J = 10.8, 3.4 Hz), 3.55 (dd, 1H, J = 10.8, 2.8 Hz), 3.80 (s, 3H), 3.80−3.94 (m, 1H), 6.15 (t, 1H, J = 5.8 Hz), 6.85 (d, 2H, J = 10.0 Hz), 7.20−7.54 (m, 12H), 7.60 (s, 1H), 8.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 6.4, 12.2, 40.4, 40.6, 55.3, 63.2, 84.4, 87.1, 110.9, 113.4, 127.3, 128.1, 128.4, 130.4, 134.9, 135.4, 143.9, 150.5, 158.9, 164.0; HRMS (MALDI TOF) m/z 661.117 (M + Na)$^+$ ($C_{31}H_{31}IN_2O_5Na$ requires 661.117). Anal. Calcd for $C_{31}H_{31}IN_2O_5$: C, 58.31; H, 4.89; N, 4.38. Found: C, 58.06; H, 4.63; N, 4.22.

3′-Deoxy-3′-*C*-(iodomethyl)-2′-*O*-(2-methoxyethyl)-5′-*O*-(4-methoxytrityl)-5-methyluridine (45). The 5′-MMT-2′-methoxyethyoxy compound **45** was prepared as described above for compound **42** from compound **36** (472 mg, 1.07 mmol), Hünig's base (0.79 mL, 586 mg, 4.5 mmol, 4.0 equiv), and MMT-Cl (1.32 g, 4.27 mmol, 4.0 equiv) in a mixture of THF (6 mL) and ethyl acetate (4 mL). The crude product was purified by flash chromatography on a silica gel column. Gradient elution with 3:1, 2:1, 1:1, and then 1:3 hexanes−EtOAc provided 690 mg (99%) of the MMT-protected product **45** as a white foam: silica gel TLC R_f 0.57 (1:2 hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 3H), 2.70−2.89 (m, 2H), 3.19−3.31 (m, 2H), 3.39 (s, 3H), 3.58−3.70 (m, 3H), 3.80 (s, 3H), 3.80−3.94 (m, 1H), 4.05−4.25 (m, 3H), 5.89 (s, 1H), 6.87 (d, 2H, J = 8.0 Hz), 7.24−7.48 (m, 12H), 7.78 (s, 1H), 9.69 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.3, 45.3, 55.3, 58.9, 61.6, 70.2, 71.9, 82.6, 85.6, 87.1, 89.1, 110.5, 113.4, 127.4, 128.2, 128.4, 130.5, 134.7, 135.3, 143.6, 143.7, 150.5, 158.9, 164.6; HRMS (FAB) m/z 735.155 (M + Na)$^+$ ($C_{34}H_{37}IN_2O_7Na$ requires 735.154).

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methyluridine Triethylamine Salt (3) and 3′-Deoxy-5′-*O*-(4-methoxytrityl)-3′-*C*-methyl-2′-*O*-methyl-5-methyluridine (46). A mixture of ammonium phosphinate (996 mg, 12.0 mmol, 4.0 equiv) and HMDS (2.56 mL, 1.95 g, 12.1 mmol, 4.05 equiv) was heated at 100−110 °C for 2 h under argon atmosphere with a condenser. The intermediate BTSP **27** was cooled to 0 °C, and 10 mL of CH_2Cl_2 was injected. To this mixture was injected a solution of **42** (2.0 g, 2.99 mmol) and Hünig's base (1.0 mL, 742 mg, 5.74 mmol, 1.92 equiv) in 15 mL of CH_2Cl_2. After the reaction mixture was stirred at room temperature overnight, a mixture of THF−MeOH−Et_3N (7/12/0.5 mL) was added, and the stirring was continued for 1 h. The reaction mixture was filtered through a Celite pad and washed with CH_2Cl_2. The solvent was evaporated, and the residue was purified by flash chromatography similar as described above for compounds **1** and **40** providing 750 mg (35%) of product **3** and 1.02 g (63%) of the reduced product **46** as white foams. H-Phosphonate **3**: silica gel TLC R_f 0.40 (50:10:1 $CHCl_3$-MeOH-Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 3 + 9H, Et_3N), 1.50−1.85 (m, 2H), 2.45−2.70 (m, 1H), 3.02 (q, 6H, J = 7.2 Hz, Et_3N), 3.10−3.25 (m, 1H), 3.42−3.60 (m, 1H), 3.49 (s, 3H), 3.70 (s, 3H), 3.90−4.01 (m, 2H), 5.85 (s, 1H), 6.77 (d, 2H, J = 8.0 Hz), 7.14 (5.90, 8.39; d, 1H, J = 498 Hz, PH), 7.10−7.50 (m, 12H), 7.58 (s, 1H), 10.58 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 8.6, 12.0, 18.0, 26.5, 27.2, 36.8, 42.1, 45.6, 53.6, 55.2, 57.7, 61.9, 84.5, 84.9, 85.2, 86.6, 89.2, 109.7, 113.3, 127.1, 128.0, 128.5, 130.4, 135.1, 135.3, 143.9, 150.4, 158.6, 164.4; ^{31}P NMR ($CDCl_3$) δ 22.2; HRMS (FAB) m/z 629.202 (M + Na)$^+$ ($C_{32}H_{35}N_2O_8PNa$ requires 629.202). Anal. Calcd for $C_{32}H_{35}N_2O_8P\cdot Et_3N\cdot H_2O$: C, 62.80; H, 7.16; N, 5.78. Found: C, 62.70; H, 7.38; N, 5.63.

2′,3′-Dideoxy-2′-*β*-fluoro-3′-*C*-[(hydroxyphosphinyl)-methyl]-5′-*O*-(4-methoxytrityl)-5-methyluridine Triethylamine Salt (4) and 2′,3′-Dideoxy-2′-*β*-fluoro-5′-*O*-(4-methoxytrityl)-3′-*C*-methyl-5-methyluridine (47). The H-phosphonate product **4** and the reduced product **47** were synthesized by the similar procedure as described above for compounds **3** and **46** from compound **43** (2.0 g, 3.05 mmol), ammonium phosphinate (1.01 g, 12.2 mmol, 4 equv), and HMDS (2.61 mL, 2.0 g, 12.35 mmol, 4.05 equiv), as well as Hünig's base. The products were purified by flash chromatography as described above for compounds **3** and **46** providing 451 mg (26%) of compound **4** and 690 mg (43%) of compound **47** as white foams. H-Phosphonate **4**: silica gel TLC R_f 0.40 (50:10:1 $CHCl_3$-MeOH-Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 9H, J = 7.4 Hz, Et_3N), 1.25−1.30 (m, 1H), 1.38 (s, 3H), 1.54−1.80 (m, 2H), 2.96 (q, 6H, J = 7.4 Hz, Et_3N), 3.27 (dd, 1H, J = 12.0, 3.2 Hz), 3.62−3.67 (m, 1H), 3.79 (s, 3H), 4.05−4.12 (m, 1H), 5.45 (dd, 1H, J = 50.0, 3.6 Hz), 6.02 (d, 1H, J = 17.2 Hz), 7.24 (5.99, 8.48; d, 1H, J = 498 Hz, PH), 6.82−7.45 (m, 14H), 7.70 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 21.36; HRMS (FAB) 696.319 (M + H)$^+$ ($C_{37}H_{48}FN_2O_7P$ requires 696.321).

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-5′-*O*-(4-methoxytrityl)thymidine Triethylamine Salt (5) and 3′-Deoxy-5′-*O*-(4-methoxytrityl)-3′-*C*-methylthymidine (48). Compounds **5** and **48** were synthesized as described above for compounds **3** and **48** from ammonium phosphinate (2.0 g, 24.0 mmol, 3.75 equiv), HMDS (5.1 mL, 3.9 g, 24.1 mmol, 3.76 equiv), compound **44** (4.10, 6.42 mmol), and Hünig's base (2.1 mL, 1.56 g, 12.0 mmol, 1.87 equiv) in CH_2Cl_2. The crude product was purified by flash chromatography on a silica gel column. Gradient elution with 400:20:1 to 200:60:1 $CHCl_3$-MeOH-Et_3N provided 1.58 (36%) of H-phosphonate product **5** as a white foam. The reduced product was collected and repurified using 2:1, 1:1, and then 1:2 hexanes−EtOAc as eluents providing 2.04 (62%) of the product **48** as a white foam. H-Phosphonate **5**: silica gel TLC R_f 0.45 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 9H, J = 7.2 Hz, Et_3N), 1.49 (s, 3H), 1.30−1.70 (m, 2H), 2.10−2.32 (m, 1H), 2.45−2.78 (m, 2H), 2.94 (q, 6H, J = 7.2 Hz, Et_3N), 3.22−3.34 (m, 1H), 3.38−3.51 (m, 1H), 3.65−3.85 (m, 1H), 3.78 (s, 3H), 6.09 (s, 1H), 6.12 (s, 1H), 6.82 (d, 2H, J = 8.0 Hz), 7.16 (5.94, 8.39; d, 1H, J = 490 Hz, PH), 7.15−7.50 (m, 12H), 7.57 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 22.2; ES MS m/z 575 (M − H)$^-$. Anal. Calcd for $C_{31}H_{33}N_2O_7P\cdot Et_3N$: C, 65.75; H, 7.13; N, 6.20. Found: C, 65.48; H, 7.17; N, 6.09.

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-2′-*O*-(2-methoxyethyl)-5′-*O*-(4-methoxytrityl)-5-methyluridine Triethylamine Salt (6) and 3′-Deoxy-2′-*O*-(2-methoxyethyl)-5′-*O*-(4-methoxytrityl)-3′-*C*-methyl-5-methyluridine (49). Compounds **6** and **49** were synthesized as described above for compounds **3** and **46** from ammonium phosphinate (410 mg, 5.06 mmol, 4.6 equiv), HMDS (1.18 mL, 902 mg, 5.59 mmol, 5.08 equiv), compound **45** (780 mg, 1.1 mmol), and Hünig's base (390 μL, 289 mg, 2.23 mmol, 2.0 equiv) in CH_2Cl_2. The crude product was purified by flash chromatography on a silica gel column. Elution with 200:40:1 and then 200:60:1 $CHCl_3$−MeOH−Et_3N provided 214 mg (26%) of H-phosphonate product **6** as a white foam. The reduced product was collected and repurified using 2:1, 1:1, and then 1:2 hexanes−EtOAc as eluents providing 380 mg (59%) of product **49** as a white foam. H−Phosphonate **6**: silica gel TLC R_f 0.40 (50:10:1 $CHCl_3$−

MeOH−Et_3N); ^1H NMR ($CDCl_3$) δ 1.21 (t, 9H, J = 7.2 Hz, Et_3N), 1.33 (s, 3H), 1.50−2.00 (m, 2H), 2.45−2.80 (m, 1H), 3.00 (q, 6H, J = 7.2 Hz, Et_3N), 3.11−3.26 (m, 1H), 3.31 (s, 3H), 3.42−3.60 (m, 3H), 3.73 (s, 3H), 3.74−3.83 (m, 1H), 4.00−4.16 (m, 2H), 4.18−4.25 (m, 1H), 5.87 (s, 1H), 6.81 (d, 2H, J = 10.0 Hz), 7.20 (5.95, 8.45; d, 1H, J = 500 Hz, PH), 7.10−7.35 (m, 8H), 7.36−7.47 (m, 4H), 7.60 (s, 1H), 11.0 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 8.5, 12.1, 37.0, 45.1, 55.2, 58.8, 62.0, 69.3, 71.8, 84.2, 86.6, 89.7, 109.6, 113.3, 127.0, 127.9, 128.5, 130.4, 135.1, 143.9, 150.6, 158.6, 164.6; ^{31}P NMR ($CDCl_3$) δ 22.2; HRMS (FAB) m/z 637.231 $(M + Na)^+$ ($C_{34}H_{39}N_2O_9PNa$ requires 673.229).

3′-Deoxy-3′-C-[[(2-cyanoethoxy)phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)thymidine (50). A solution of H-phosphonate **5** (1.75 g, 2.58 mmol), dicyclohexycarbodiimide (DCC) (1.25 g, 6.0 mmol, 2.3 equiv), and 3-hydroxypropionitrile (0.511 mL, 531 mg, 7.48 mmol, 2.9 equiv) in 35 mL of anhydrous THF was stirred at 65 °C for 24 h. The reaction mixture was filtered, and the solid was washed with ethyl acetate. The filtrate was concentrated, and the residue was dissolved in $CHCl_3$. The solution was washed with water, dried, and concentrated. The white foam was dissolved in acetonitrile and extracted with hexanes. Concentration of the acetonitrile phase provided 1.28 g (79%) of a white foam product **50** as a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.38 (15:1 EtOAc-MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H), 1.60−1.98 (m, 2H), 2.20−2.30 (m, 1H), 2.48−2.58 (m, 1H), 2.67−2.71 (m, 2H), 2.72−2.87 (m, 1H), 3.28 (dd, 1H, J = 10.8, 3.2 Hz), 3.54−3.62 (m, 1H), 3.79 (s, 3H), 3.75−3.90 (m, 1H), 4.10−4.20 (m, 2H), 4.21−4.32 (m, 1H), 6.11, 6.13 (2s, 1H), 6.85 (d, 2H, J = 8.0 Hz), 7.18, 7.20 (6.50, 7.87; 6.52, 7.89; 2d, 1H, J = 548 Hz, PH, 2 diastereoisomers), 7.20−7.32 (m, 8H), 7.38−7.47 (m, 4H), 7.58 (s, 1H), 9.50 (bs, 1H); ^{31}P NMR ($CDCl_3$) δ 38.0, 38.2; HRMS (FAB) m/z 636.245 $(M + Li)^+$ ($C_{34}H_{36}N_3PO_7$-Li requires 636.245). Anal. Calcd for $C_{34}H_{36}N_3PO_7 \cdot 3H_2O$: C, 59.73; H, 6.14; N, 6.14. Found: C, 60.01; H, 6.11; N, 6.17.

3′-Deoxy-3′-*C*-[[(2-cyanoethoxy)phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methyluridine (51). Compound **51** was synthesized by the similar procedure as described above for compound **50** from H-phosphonate **3** (1.58 g, 2.23 mmol), DCC (1.15 g, 5.62 mmol, 2.5 equiv), and 3-hydroxypropionitrile (0.458 mL, 476 mg, 6.69 mmol, 3.0 equiv). 1.40 g (95%) of a white foam product **51** was obtained as a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.30 (15:1 EtOAc−MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 3H), 1.40−1.95 (m, 2H), 2.00−2.18 (m, 1H), 2.62−2.71 (m, 2H), 2.73−2.88 (m, 1H), 3.16−3.26 (m, 1H), 3.60 (s, 3H), 3.66−3.75 (m, 1H), 3.79 (s, 3H), 3.99−4.30 (m, 3H), 5.91, 5.94 (2s, 1H), 6.85 (d, 2H, J = 12 Hz), 7.13, 7.22 (6.45, 7.82; 6.52, 7.91; 2d, 1H, J = 548, 556 Hz, PH, 2 diastereoisomers), 7.20−7.36 (m, 8H), 7.37−7.50 (m, 4H), 7.72, 7.76 (2s, 1H), 9.50 (bs, 1H); ^{31}P NMR ($CDCl_3$) δ 38.6, 38.9; HRMS (FAB) m/z 682.229 $(M + Na)^+$ ($C_{35}H_{38}N_3O_8PNa$ requires 682.229). Anal. Calcd for $C_{35}H_{38}N_3O_8P$: C, 63.67; H, 5.80; N, 6.37. Found: C, 63.46; H, 6.03; N, 6.27.

3′-Deoxy-3′-*C*-[[(2-cyanoethoxy)(diisopropylamino)-phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)thymidine (8). To a solution of triphosgene (0.21 g, 0.707 mmol, 1.08 equiv) (Note: toxic) in 2 mL of CH_2Cl_2 at 10 °C under argon atmosphere was added a solution of triphenylphosphine (0.54 g, 2.05 mmol, 3.1 equiv) in 3 mL of CH_2Cl_2. The resulted solution was warmed to room temperature, and pyridine (0.24 mL, 230 mg, 2.96 mmol, 4.5 equiv) was added. A solution of cyanoethyl H-phosphonate **50** (0.41 g, 0.65 mmol) and pyridine (0.24 mL) in 5 mL of CH_2Cl_2 was added at 5 °C. The resulting reaction mixture was stirred at room temperature for 2−3 h and re-cooled to −40 °C. A solution of diisopropylamine (0.524 mL, 378 mg, 3.73 mmol, 5.7 equiv) in 0.5 mL of dichloromethane was added dropwise. The reaction mixture was warmed to room temperature and applied onto a silica gel column. Gradient elution with 1:1 hexanes−EtOAc (containing 0.5% Et_3N) and then 1:1 hexanes−THF (0.5% Et_3N) provided 259 mg (56% overall yield) of 3′-*C*-methylene phosphonamidite product **8** as a white foam in a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.50 (1:1 hexanes−EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 0.95−1.20 (m, 2H), 1.05 (d, 6H, J = 6.8 Hz), 1.18 (d, 6H, J = 6.8 Hz), 1.55, 1.60 (2s, 3H), 2.05−2.13 (m, 1H), 2.17−2.27 (m, 1H), 2.37 (t, 2H, J = 6.0 Hz), 2.58−2.75 (m, 2H), 3.29−3.35 (m, 1H), 3.41−3.58 (m, 3H), 3.67−3.75 (m, 2H), 3.79 (s, 3H), 6.08, 6.09 (2s, 1H), 6.85 (d, 2H, J = 6.0 Hz), 7.20−7.40 (m, 8H), 7.42−7.51 (m, 4H), 7.72 (s, 1H), 8.23 (bs, 1H); ^{31}P NMR ($CDCl_3$) δ 128.0, 128.5; HRMS (FAB) m/z 713.346 $(M + H)^+$ ($C_{40}H_{50}N_4O_6P$ requires 713.346). Anal. Calcd for $C_{40}H_{49}N_4O_6P \cdot 1.5H_2O$: C, 64.88; H, 7.03; N, 7.57. Found: C, 64.73; H, 6.84; N, 7.68.

3′-Deoxy-3′-*C*-[[(2-cyanoethoxy)(diisopropylamino)-phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methyluridine (9). 3′-*C*-Methylene phosphonamidite **9** was synthesized by the similar procedure as described above for compound **8** from cyanoethyl H-phosphonate **51** (1.40 g, 2.12 mmol), triphosgene (0.662 g, 2.23 mmol, 1.05 equiv), triphenylphosphine (1.73 g, 6.59 mmol, 3.1 equiv), and diisopropylamine (1.63 mL, 1.18 g, 11.6 mmol, 5.5 equiv). The crude product was purified by flash chromatography on a silica gel column using 1:1 hexanes−EtOAc (0.3% Et_3N) and then 1:1 hexanes−THF (0.3% Et_3N) as eluents to provide 1.13 g (68% overall yield) of a white foam product **9** as a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.37, 0.44 (1:1 hexanes−EtOAc); ^1H NMR (400 MHz, $CDCl_3$) δ 0.99−1.40 (m, 2H), 1.07 (d, 6H, J = 5.6 Hz), 1.20 (d, 6H, J = 5.6 Hz), 1.48 (s, 3H), 1.72−1.81 (m, 1H), 2.23−2.35 (m, 2H), 2.50−2.78 (m, 1H), 3.26 (d, 1H, J = 11.6 Hz), 3.40−3.55 (m, 2H), 3.61 (s, 3H), 3.65−3.78 (m, 2H), 3.79 (s, 3H), 4.00 (d, 1H, J = 10.4 Hz), 4.08−4.22 (m, 1H), 5.82, 5.88 (2s, 1H), 6.85 (d, 2H, J = 8.0 Hz), 7.18−7.40 (m, 8H), 7.41−7.55 (m, 4H), 7.75, 7.86 (2s, 1H), 8.62 (bs, 1H); ^{31}P NMR ($CDCl_3$) δ 128.9, 130.0; HRMS (FAB) m/z 743.356 $(M + H)^+$ ($C_{41}H_{52}N_4O_7P$ requires 743.357). Anal. Calcd for $C_{41}H_{51}N_4O_7P \cdot 0.5CHCl_3$: C, 62.05; H, 6.46; N, 6.98. Found: C, 61.87; H, 6.61; N, 6.93.

3′-Deoxy-3′-*C*-(iodomethyl)-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methylcytidine (53). Phosphorus oxychloride (2.20 mL, 3.62 g, 23.6 mmol, 2.5 equiv) was added dropwise to a stirred solution of 1,2,4-triazole (6.35 g, 92.0 mmol, 10.0 equiv) in 70 mL of anhydrous acetonitrile at −40 °C, and the resulted reaction mixture was stirred for 20 min. A solution of 3′-*C*-iodomethyl-5-methyluridine compound **42** (6.15 g, 9.20 mmol) in 50 mL of acetonitrile was added slowly to above reaction mixture. The reaction mixture was stirred for 4 h until starting material **42** was completely converted to triazole compound **52** (monitored by TLC, silica gel, R_f 0.46, 1:2 hexanes−EtOAc). The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The resulted solution was washed with saturated aqueous $NaHCO_3$ solution, water, and brine. The organic phase was dried (Na_2SO_4) and concentrated. The crude product **52** was obtained as a white foam:^1H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H), 2.70−2.80 (m, 2H), 3.17 (t, 1H, J = 11.6 Hz), 3.29 (dd, 1H, J = 11.2, 2.4 Hz), 3.73 (d, 1H, J = 11.2 Hz), 3.78 (s, 3H), 3.80 (s, 3H), 4.05 (d, 1H, J = 4.0 Hz), 4.12 (d, 1H, J = 10.4 Hz), 6.00 (s, 1H), 6.87 (d, 2H, J = 8.0 Hz), 7.20−7.38 (m, 8H), 7.39−7.50 (m, 4H), 8.08 (s, 1H), 8.44 (s, 1H), 9.28 (s, 1H). Thus-obtained compound **52** was dissolved in 100 mL of THF, and 60 mL of aqueous ammonium hydroxide was added at −30 °C. The sealed reaction mixture was stirred at room temperature overnight and concentrated. The residue was dissolved in ethyl acetate, and the solution was washed with saturated aqueous $NaHCO_3$, water, and brine. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column using 40:1 and 30:1 CH_2Cl_2−MeOH as eluents to give 6.14 g (99.8%) of 5-methylcytidine compound **53** as a white foam: silica gel TLC R_f 0.40, 0.44 (10:1 CH_2Cl_2-MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 3H), 2.67−2.79 (m, 2H), 3.16 (t, 1H, J = 11.6 Hz), 3.23 (dd, 1H, J = 11.2, 2.4 Hz), 3.67 (d, 1H, J = 11.2 Hz), 3.72 (s, 3H), 3.80 (s, 3H), 3.97 (d, 1H, J = 4.0 Hz), 4.00−4.08 (m, 1H), 5.94 (s, 1H), 6.86 (d, 2H, J = 8.0 Hz), 7.23−7.38 (m, 8H), 7.40−7.49 (m, 4H), 7.87 (s, 1H);^{13}C NMR ($CDCl_3$) δ 12.9, 45.0, 55.5, 59.1, 61.9, 82.4, 86.7, 87.2, 89.1, 101.6, 113.5, 127.5, 128.3, 128.7, 130.6, 134.9, 138.2, 143.8, 143.9, 156.1, 159.1, 166.4; HRMS (MALDI) m/z 690.144

$(M + Na)^+$ ($C_{32}H_{34}IN_3O_5Na$ requires 690.144). Anal. Calcd for $C_{32}H_{34}IN_3O_5 \cdot {}^1/_2CHCl_3$: C, 53.68; H, 4.87. Found: C, 53.74; H, 4.90.

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-5-methylcytidine Triethylamine Salt (54) and 3′-Deoxy-5′-*O*-(4-methoxytrityl)-3′-*C*-methyl-2′-*O*-methyl-5-methylcytidine (55). The H-phosphonate cytidine **54** and reduced product **55** were prepared by a procedure similar to that described above for compound **3** and **46** from iodomethyl compound **53** (0.47 g, 0.7 mmol), ammonium phosphinate (0.23 g, 2.8 mmol, 4.0 equiv), and HMDS (0.60 mL, 0.46 g, 2.85 mmol, 4.08 equiv). The product was purified by flash chromatography on a silica gel column using 150:50:1, 200:100:1 and then 100:100:1 $CHCl_3$−MeOH−Et_3N as eluents to give 143 mg (29%) of a white foam product **54** as its triethylamine salt. The reduced compound was further purified by flash chromatography on a silica gel column using 30:1, 20:1, and then 15:1 CH_2Cl_2-MeOH as eluents to give 160 mg (42%) of compound **55** as a white foam. H-Phosphonate **54**: silica gel TLC R_f 0.43 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 9H, J = 7.2 Hz, Et_3N), 1.36 (s, 3H), 1.65−2.00 (m, 2H), 2.37−2.60 (m, 1H), 2.94 (q, 6H, J = 7.2 Hz, Et_3N), 3.17−3.35 (m, 1H), 3.50−3.62 (m, 1H), 3.63 (s, 3H), 3.77 (s, 3H), 4.05−4.20 (m, 2H), 5.95 (s, 1H), 6.84 (d, 2H, J = 10.0 Hz), 7.14 (5.92, 8.36; d, 1H, J = 488 Hz, PH), 7.17−7.60 (m, 12H), 7.74 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 20.1; HRMS (FAB) m/z 606.236 $(M + H)^+$ ($C_{32}H_{37}N_3O_7P$ requires 606.237).

3′-Deoxy-3′-*C*-[(hydroxyphosphinyl)methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-4-*N*-(*N*-methylpyrrolidin-2-ylidene)-5-methylcytidine (7). *N*-Methyl-2,2-diethoxypyrrolidine[28] (1.06 g, 6.1 mmol, 5.0 equiv) was added to a stirred solution of H-phosphonate 5-methylcytidine **54** (0.86 g, 1.21 mmol) and Et_3N (1.0 mL) in 24 mL of anhydrous MeOH at 0 °C under argon atmosphere. The resulted reaction mixture was stirred at room temperature for 6 h and concentrated. The crude product was purified by flash chromatography on a silica gel column using 200:30:1, 200:60:1, and then 200:100:1 $CHCl_3$−MeOH−Et_3N as eluents to give 0.877 g (92%) of the protected H-phosphonate product **7** as a white foam: silica gel TLC R_f 0.43 (50:10:1 $CHCl_3$−MeOH−Et_3N); ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 9H, J = 7.2 Hz, Et_3N), 1.53 (s, 3H), 1.70−2.12 (m, 4H), 2.29−2.55 (m, 2H), 2.70−2.80 (m, 1H), 2.97 (q, 6H, J = 7.2 Hz, Et_3N), 3.02 (s, 3H), 3.18−3.60 (m, 4H), 3.66 (s, 3H), 3.78 (s, 3H), 4.00−4.18 (m, 2H), 6.03 (s, 1H), 6.83 (d, 2H, J = 8.0 Hz), 7.08 (5.84, 8.31; d, 1H, J = 494 Hz, PH), 7.15−7.40 (m, 8H), 7.44−7.53 (m, 4H), 7.75 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 22.6; HRMS (FAB) m/z 687.295 $(M + H)^+$ ($C_{37}H_{44}N_4O_7P$ requires 687.294).

3′-Deoxy-3′-*C*-[[(2-cyanoethoxy)phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-4-*N*-(*N*-methylpyrrolidin-2-ylene)-5-methylcytidine (56). Cyanoethyl H-phosphonate **56** was prepared by the similar procedure as described above for compound **50** from compound **7** (0.87 g, 1.1 mmol), DCC (0.681 g, 3.3 mmol, 3.0 equiv), and 3-hydroxypropionitrile (0.30 mL, 313 mg, 4.4 mmol, 4.0 equiv) in 22 mL of anhydrous THF. 0.81 g (99%) of a white foam product **56** was obtained as a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.48 (1:1 EtOAc−MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59, 1.61 (2s, 3H), 1.70−2.40 (m, 5H), 2.58−2.80 (m, 2H), 3.03 (s, 3H), 3.10−3.38 (m, 3H), 3.44 (t, 2H, J = 7.4 Hz), 3.60−3.75 (m, 1H), 3.68 (s, 3H), 3.79 (s, 3H), 3.94−4.28 (m, 4H), 6.04, 6.08 (2s, 1H), 6.85 (d, 2H, J = 10.0 Hz), 7.08, 7.16 (5.70, 8.45; 5.78, 8.54; 2d, 1H, J = 550, 552 Hz, PH, 2 diastereoisomers), 7.18−7.38 (m, 8H), 7.40−7.52 (m, 4H), 7.77, 7.85 (2s, 1H); ^{31}P NMR ($CDCl_3$) δ 37.6, 38.1; HRMS (FAB) m/z 740.323 $(M + H)^+$ ($C_{40}H_{47}N_5O_7P$ requires 740.321).

3′-Deoxy-3′-*C*-[[(2-cyanoethoxy)(diisopropylamino)-phosphinyl]methyl]-5′-*O*-(4-methoxytrityl)-2′-*O*-methyl-4-*N*-(*N*-methylpyrrolidin-2-ylene)-5-methylcytidine (10). 3′-*C*-Methylene phosphonamidite **10** was prepared by a procedure similar to that described above for compound **8** from the corresponding cytidine compound **56** (0.504 g, 0.68 mmol), triphosgene (0.224 g, 0.75 mmol, 1.1 equiv), tryiphenylphosphine (0.60 g, 2.28 mmol, 3.3 equiv), and diisopropylamine (0.526 mL, 379 mg, 3.75 mmol, 5.5 equiv). The crude product was purified by flash chromatography on a silica gel column using 1:2 hexanes−EtOAc (0.5% Et_3N), 1:2 hexanes−THF (0.5% Et_3N), and then 20:1 EtOAc−MeOH (0.5% Et_3N) as eluents to give 403 mg (72% overall yield) of a white foam product **10** as a mixture of two diastereoisomers at phosphorus: silica gel TLC R_f 0.42 (5:1 EtOAc−MeOH); ^{1}H NMR ($CDCl_3$) δ 1.07 (d, 6H, J = 6.4 Hz), 1.19 (d, 6H, J = 6.4 Hz), 1.25−1.60 (m, 2H), 1.62 (s, 3H), 1.95−2.32 (m, 4H), 2.40−2.70 (m, 1H), 3.03 (s, 3H), 3.12−3.31 (m, 3H), 3.35−3.58 (m, 4H), 3.60−3.90 (m, 3H), 3.69 (s, 3H), 3.79 (s, 3H), 3.99−4.20 (m, 2H), 5.98, 6.04 (2s, 1H), 6.85 (d, 2H, J = 8.0 Hz), 7.20−7.58 (m, 12H), 7.96 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 127.5, 129.2; HRMS (FAB) m/z 823.431 $(M + H)^+$ ($C_{46}H_{60}N_6O_6P$ requires 823.431).

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Supporting Information Available: Spectral data of compounds **30**, **31**, **39**−**41**, **46**−**49**, and **55**; NMR spectra of compounds **1**, **2**, **4**, **6**, **7**, **10**, **26**, **36**, **38**, **43**, **45**, and **56**. This material is available free of charge via the Internet at http://pubs.acs.org.

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