Synthesis of Dihydroisoxazole Nucleoside and Nucleotide Analogs

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The dihydroisoxazole nucleosides as well as their phosphonate derivatives were efficiently prepared *via* 1,3-dipolar cycloaddition reactions of nitrile oxides with corresponding vinyl nucleoside bases for antiviral studies.

Introduction

Naturally occurring nucleosides provide structural insight for the development of potent antiviral agents.[1] The unique feature of the known antiviral drugs, derived from nucleoside analogs, consists of a hydroxymethylated five-membered ring that is *cis* connected to a nucleoside base generally *via* a glycosidic bond. Chemically, the parent class belongs to the five-carbon cyclopentane compounds (C_5), which are occasionally found in nature,[2] and some of the derivatives show interesting antiviral or anticancer activities. From the cyclopentane system, a simple one-carbon replacement with oxygen atom generates furanose (C_4O) systems that are widely distributed in nature. Actually, the most studied antiviral drugs derive from the furanose-constituted 2′,3′-dideoxynucleosides **1** (Figure 1), and the modification of the furanose substituents has resulted in several excellent anti-HIV drugs such as AZT, ddC, d4T, and ddI.[3]

The introduction of a second heteroatom into the furanose ring would result in several new classes of nucleoside analogs. The presence of multihetero atoms in the five-membered ring is desired to retain the required conformation for the recognition between the nucleoside and the target enzyme (reverse transcriptase).[2a,3c] Indeed, the use of oxygen or sulfur atoms has produced several diheteroatom-substituted analogs **2** (**2a**, X = O; **2b**, X = S), and some of the dioxolanyl (C_3O_2) or oxathiolanyl (C_3OS) nucleosides are potent virus inhibitors.[4] Of note is that the newly approved antiviral agent **3** (B = cytosine) is a nucleoside analog, but its ring constitution is markedly different from the furanose of nucleoside. The conserved structural features of **3** include the glycosidic bond and the hydroxymethyl-group *cis* relationship with the nucleoside base.

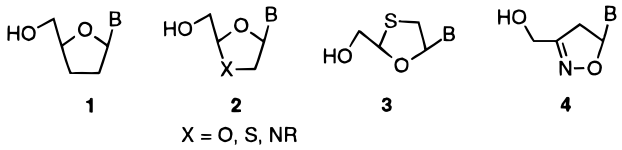

Figure 1.

It has been synthetically challenging to introduce both oxygen and nitrogen into the five-membered ring (C_3ON) while keeping a glycosidic bond.[5] For example, the classic problem of using system **2c** (X = NR) is the unstable nature of the aminal moiety probably due to the easy protonation of the nitrogen atom under physiological conditions. We are interested in the *N*−*O* compounds[6−8] such as dihydroisoxazolidine **4** and the mutual induction effect of two heteroatoms exerting several desired properties. Firstly, the nitrogen atom becomes less basic to prevent its protonation; therefore, the compounds **4** are expected to be stable at physiological pH. Secondly, the oxygen atom may also become less nucleophilic, making the glycosidic bond of **4** more resistant to acidic hydrolysis. In our previous paper,[8] we had reported the first preparation of anti-HIV-active dihydroisoxazolidinyl 6-chloropurine and adenine *via* 1,3-dipolar addition. This present paper reports the recent studies on other base-modified dihydroisoxazolidine nucleosides **4** as well as their corresponding phosphonate derivatives.

Results and Discussion

Nitrile Oxide Addition to Vinyl 6-Chloropurine. Dipolar cycloaddition reactions of nitrile oxides to olefins provide efficient means for the preparation of dihydroisoxazole.[8] The olefins, which are substituted with alkyl groups, react smoothly with nitrile oxides.[9] The rate of reactions can be increased by substituting olefins with either electron-withdrawing groups (e.g., CH_2=

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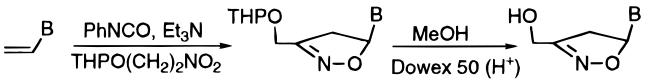
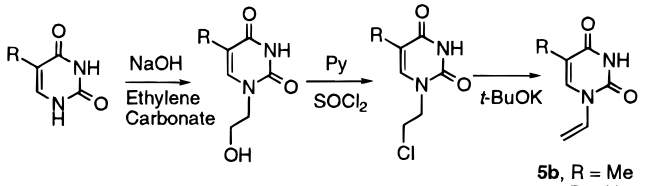
Scheme 1



5a: B = 6-chloropurine **6a**: B = 6-chloropurine **7a**: B = adenine
5b: B = thymine **6b**: B = thymine **7b**: B = thymine
5c: B = uracil **6c**: B = uracil **7c**: B = uracil

For **7a**, (a) NaN_3 ; (b) Ph_3P; (c) AcOH.

Scheme 2

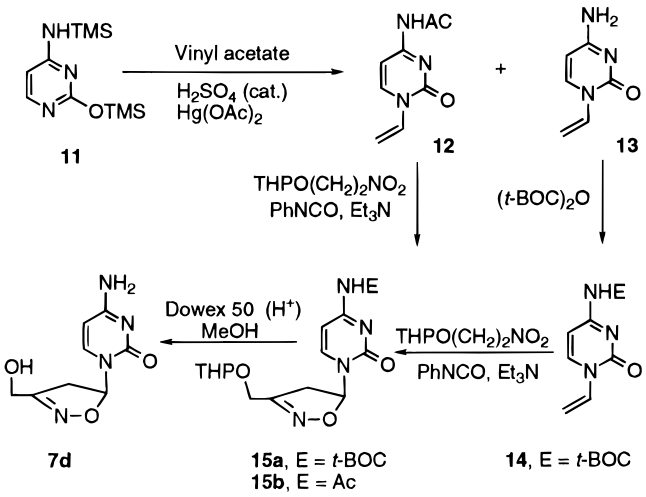


8 **9** **10** **5b**, R = Me **5c**, R = H

$CHCO_2Et$) or electron-donating groups (e.g., CH_2=CHOAc). However, the preparation of nucleoside analogs from vinyl nucleoside bases has not been well investigated (Scheme 1). We have recently disclosed that vinyl-6-chloropurine (**5a**), which was prepared by using a modified procedure of Pitha and Ts'O,[10] behaves similarly to other vinyl compounds to undergo the 1,3-dipolar cycloaddition. The phenyl isocyanate-promoted[11] reaction of $THPOCH_2CH_2NO_2$ with vinyl bases **5a** at room temperature produced a pair of enantiomers **6a**. The THP-protected product **6a**, which existed as two diastereomers due to the presence of the THP group, was converted to the hydroxymethyl derivative by using Dowex 50 (H^+) in methanol. The adenosine analog **7a** was obtained through steps of azide displacement, triphenylphosphine reduction, and acetic acid hydrolysis. The survival of the glyosidic bond of **7a** under these acidic conditions (acidic Dowex in methanol and aqueous acetic acid at 100 °C), which was atypical, confirmed our original expectation that compound **7a** could be acid-stable due to the mutual induction effect through the oxygen−nitrogen bond.

Nitrile Oxide Addition to Vinylpyrimidines. Several procedures have been developed for the preparation of vinylpyrimidines, which are the common intermediates for the constructions of nucleoside polymers. The direct exchange of the acetyl group of vinyl acetate with uracil or thymine was not very successful in our hands, presumably due to the presence of four nucleophilic centers. A multistep procedure was adapted to prepare vinylthymine (**5b**) and vinyluracil (**5c**) (Scheme 2). The *N*-monoalkylated thymine **9a** (R = Me) was obtained by the reaction of thymine **8a** with ethylene carbonate in the presence of a catalytic amount of sodium hydroxide, and a small amount of the catalyst is the key to avoid a large amount of *N*,*N*-dialkylated product.[10,12] Chlorination of **9a** with excess thionyl chloride gave 1-(2-chloroethyl)-uracil **10a** in 89% yield, followed dehydrochlorination by potassium *tert*-butoxide in dimethyl sulfoxide at room temperature, provided **5b** (R = Me) in 83% yield. The 1,3-dipolar addition of **5b** with $THPOCH_2CH_2NO_2$ gave **6b** (Scheme 1), which was deprotected to give the desired

Scheme 3



7d **15a**, E = *t*-BOC **15b**, E = Ac **14**, E = *t*-BOC

nucleoside analog **7b** in 60% overall yield. The uridine analog **7c** was prepared by using a similar procedure.

The preparation of cytidine analog **7d**, based on the similar procedure, requires the 4-amine protection of cytosine (Scheme 3). Bis(trimethylsilyl)cytosine (**11**) was prepared from cytosine by treatment with excess hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate.[13] Kaye and co-worker reported that the N-vinylation of **11** with vinyl acetate,[14] mercuric acetate, and sulfuric acid for 2 days gave **12** in 14% yield, wherein vinyl acetate behaved as both vinylating and acetylating agent. Besides, the acetylation proceeded more slowly than the vinylation because of the steric hindrance. It was of interest to find that the amino derivative 1-vinylcytosine **13** can be directly obtained in 65% yield by increasing the amounts of two catalysts and decreasing the reaction time, and in addition, this condition produced the acetate **12** in 9% yield, which was useful for the subsequent reactions. Although the direct 1,3-dipolar reaction of **12** with corresponding nitrile oxide gave the *N*-acetylated cytidine **15b** (E = Ac), the final step of deacetylation failed under basic conditions.[15] Alternatively, we used the *N*-BOC-protected derivative **14**, derived from **13** by refluxing with di-*tert*-butyl dicarbonate in (50%) THF/CH_2Cl_2 for 48 h, in the 1,3-dipolar cycloaddition, and the final treatment of **15a** (E = *t*-BOC) with 44% formic acid in methanol removed both protecting groups to achieve the synthesis of **7d** in 29% overall yield.

Preparation of Isoxazole Nucleotide Phosphonates. Nucleoside phosphonates have been considered as isosteric alternatives for nucleoside phosphates as antiviral agents.[2,3a] The potential value of nucleoside phosphonates makes it attractive to investigate the limit of the cycloaddition reactions of vinyl bases with a phosphonate starting material. Since the isocyanate−dehydrate method requires the 3-nitropropyl phosphonate derivatives that are difficult to prepare, we used the well-known NCS-oxidation method of oximes,[16] and the required starting material is the readily available oxime

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(15) Thymine was the only product isolated upon treatment of thymidine analog **7b** with methanolic ammonia at room temperature for 4 h. Cytosine was similarly obtained by the use of **7d**.
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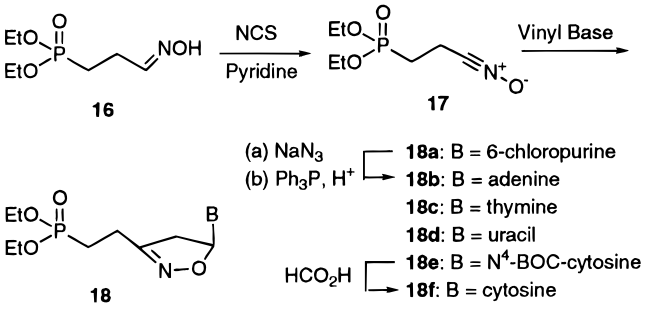
Scheme 4



16 (Scheme 4). The nitrile oxide **17** can be prepared by the NCS oxidation of **16** in the presence of pyridine and reacts smoothly with vinyl nucleoside bases to produce **18** in good yield. In the case of cytosine analog, the deprotection was achieved by using formic acid in methanol to afford the desired phosphonate **18f** in 34% overall yield. The nucleosides **7** and their phosphonate derivative **18** are being evaluated for anticancer and antiviral activities.

Conclusion

Reaction of (*β*-alkoxyethyl)nitrile oxides with vinylpyrimidines or -purines, *via* 1,3-dipolar cycloaddition reactions, allows the efficient synthesis of the hydroxymethylated isoxazolidine nucleosides **7** in which the furanose ring is replaced by an *N*−*O*-containing heterocycle. The usefulness of this reaction for the related analogs has also been demonstrated by the easy access to the corresponding phosphonate derivatives **18**. The connection of oxygen and nitrogen atoms provides a possibility to introduce the nucleophilic nitrogen into the furanose ring with a minimum of steric manipulation, providing opportunities to modify the furanose ring of nucleoside analogs with novel structural features. Consequently, it becomes possible to design other related *N*−*O*-containing derivatives for biological studies. Future direction should also include addressing the important problem of enantiostereoselective preparation of these new and related analogs.

Experimental Section

General Procedures. All reagents and solvents were purchased from Aldrich, Fisher, or Acros Chimica and, except when otherwise stated, were used without further purification. All reactions involving air-sensitive agents were conducted under an argon atmosphere. The drying of an organic solvent over Na_2SO_4 or $MgSO_4$ followed by filtration is referred to simply as "dried". Removal of solvents under reduced pressure on a rotary evaporator is referred to simply as "concentrated". Thin layer chromatography (TLC) was done on E. Merck silica gel (0.25 mm thickness) 60 F_{254} glass plates. Plates were viewed under UV light (254 nm) or developed in an iodine chamber. Column chromatography was performed on Mallinckrodt silica gel 60 (230−400 mesh). Melting points were recorded in open capillary tubes and are uncorrected. The 200 MHz proton NMR data were collected on a Varian instrument.

6-Chloro-9-vinylpurine (5a). A solution of concentrated sulfuric acid (0.3 mL) in ethyl acetate (2 mL) was added to a suspension of mercuric acetate (0.64 g, 2 mmol) in vinyl acetate (15 mL) to form a clear solution. This procedure avoids the coloring of acetate by the direct addition of acid. The powdered 6-chloropurine (3.1 g, 20 mmol) was then added to the reaction mixture, followed by an additional portion of vinyl acetate (10 mL) under argon. The reaction mixture was refluxed for 2 h before it was filtered. The filtrate was concentrated, and the residue was chromatographed on silica gel with 60% ethyl acetate/petroleum ether to give **5a** (2.98 g, 82%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.78 (s, 1H), 8.32 (s, 1H), 7.26 (dd, J = 9.1, 16.0 Hz, 1H), 6.02 (dd, J = 1.8, 16.8 Hz, 1H), 5.30 (dd, J = 1.8, 9.2 Hz, 1H).

6-Chloro-9-[3-[(tetrahydropyranyl-2-oxy)methyl]-4,5-dihydro-1,2-isoxazol-5-yl]purine (6a). A mixture of **5** (0.72 g, 4 mmol), $THPO(CH_2)_2NO_2$ (0.95 g, 6 mmol), triethylamine (0.2 mL), and phenyl isocyanate (1.12 mL, 10 mmol) in benzene (20 mL) was stirred overnight at room temperature. To the mixture was added water (20 mL), and the resulting solution was stirred for 1 h. After filtration, the filtrate was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give **6a** (0.92 g, 68%) as a clear oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.74 (1 H, s), 8.29 (s, 0.5 H), 8.21 (s, 0.5 H), 6.86 (m, 1H), 4.64 (m, 3H), 3.52−3.87 (m, 4H), 1.53−1.79 (m, 6H). Anal. Calcd for $C_{14}H_{16}ClN_5O_3$: C, 49.78; H, 4.77; N, 20.73. Found: C, 49.29; H, 4.84; N, 20.47.

9-[3-(Hydroxymethyl)-4,5-dihydro-1,2-isoxazol-5-yl]adenine (7a). The mixture of **6a** (0.50 g, 1.48 mmol), Dowex 50 (H^+) (0.50 g), and methanol (20 mL) was stirred at room temperature for 1 h and then filtered. The filtrate was concentrated and chromatographed on silica gel with 10% MeOH/$CHCl_3$ to give the corresponding alcohol (0.37 g, 99%) as a white solid: mp 188−189 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.83 (s, 1H), 8.76 (s, 1H), 7.01 (dd, J = 4.1, 8.5 Hz, 1H), 5.50 (t, J = 5.9 Hz, 1H), 4.44−4.47 (m, 2H), 3.79−3.83 (m, 2H). Anal. Calcd for $C_9H_8ClN_5O_2$: C, 42.62; H, 3.17; N, 27.61. Found: C, 42.66; H, 3.23; N, 27.62.

The mixture containing 6-chloro-9-[3-(hydroxymethyl)-4,5-dihydro-1,2-isoxazol-5-yl]purine (0.25 g, 1 mmol), sodium azide (0.32 g, 5 mmol), ethanol (10 mL), and water (0.5 mL) was refluxed for 1 h. After the reaction mixture was cooled to room temperature, triphenylphosphine (0.40 g, 1.5 mmol) was added and the resulting solution was stirred at room temperature for 30 min. Concentration of the mixture gave a residue that was dissolved in acetic acid (5 mL) and water (2 mL) and refluxed for 30 min. The mixture was concentrated and coevaporated with toluene (2 × 5 mL) to dryness. The crude product was chromatographed on silica gel with 10% MeOH/$CHCl_3$ to yield **7a** (0.12 g, 51%) as a white solid: mp 126−127 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.19 (s, 1H), 8.18 (s, 1H), 7.34 (s, 2H), 6.85 (dd, J = 8.8, 4.3 Hz, 1H), 5.48 (t, J = 6.1 Hz, 1H), 4.43 (d, J = 3.2 Hz, 1H), 4.40 (d, J = 3.2 Hz, 1Hz), 3.72 (d, J = 9.1 Hz, 1H), 3.69 (d, J = 4.3 Hz, 1H). Anal. Calcd for $C_9H_{10}N_6O_2 \cdot 0.25H_2O$: C, 45.28; H, 4.43; N, 35.20. Found: C, 45.43; H, 4.37; N, 35.24.

1-(2-Hydroxyethyl)uracil (9b). Uracil (9.9 g, 88 mmol), dried previously in *vacuo* at 120 °C for 4 h, was heated in dry DMF (200 mL) with a catalytic amount of NaOH until all uracil was dissolved. To this reaction mixture was added ethylene carbonate (8.8 g, 100 mmol), and the resulting mixture was allowed to reflux for 1.5 h. After cooling, the solution was concentrated and the residue was diluted with 300 mL of water. Unreacted uracil formed a precipitate and was filtered. After neutralization with Dowex 50 (H^+), the water was evaporated *in vacuo*. The residue was chromatographed on silica gel with 20% methanol/$CHCl_3$ to give **9b** (8.54 g, 54%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.23 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 5.54 (d, J = 8.1 Hz, 1H), 4.91 (t, J = 5.3 Hz, 1H), 3.85−3.44 (m, 4H).

1-(2-Chloroethyl)uracil (10b). Pyridine (0.2 mL) was added to a solution of **9b** (1.87 g, 12 mmol) in dry, hot dioxane (30 mL). Thionyl chloride (5 mL) in dioxane (5 mL) was then added dropwise, and the solution was refluxed for 1 h. The solvent was evaporated *in vacuo*, and the residue was redissolved in 100 mL of $CHCl_3$. The solution was partitioned over water and extracted with $CHCl_3$ to collect the organics, which were dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give **10b** (1.80 g, 86%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.35 (s, 1H), 7.67 (d, J = 7.8 Hz, 1H), 5.59 (d, J = 7.9 Hz, 1H), 4.02 (t, J = 5.9 Hz, 2H), 3.86 (t, J = 5.5 Hz, 2H).

1-Vinyluracil (5c). The chloride **10b** (1.5 g, 8.6 mmol) dissolved in dry dimethyl sulfoxide (20 mL) was added drop-

wise to the stirred solution of potassium *tert*-butoxide (3.26 g, 29 mmol) in dimethyl sulfoxide (20 mL). After the reaction mixture was stirred for 1 h at room temperature, 60 mL of cold water was added to it, and the solution was made slightly acidic by adding Dowex 50 (H^+). The solution was filtered and evaporated *in vacuo* (0.2 mm) at 60 °C to give a residue. The residue was chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give **5c** (1.06 g, 74%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.51 (s, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.09 (dd, J = 9.3, 16.0 Hz, 1H), 5.73 (d, J = 8.0 Hz, 1H), 5.36 (dd, J = 2.3, 16.1 Hz, 1H), 4.91 (dd, J = 2.3, 9.2 Hz, 1H).

1-[3-(Hydroxymethyl)-4,5-dihydro-1,2-isoxazol-5-yl)]-uridine (7c). To a solution of $THPO(CH_2)_2NO_2$ (0.40 g, 2.3 mmol) in a mixture of THF (15 mL) and benzene (6 mL) under an argon atmosphere were added phenyl isocyanate (0.80 mL, 7 mmol) and triethylamine (0.1 mL). The reaction mixture was stirred for 1 h at room temperature, and 1-vinyluracil (**5c**) (0.34 g, 2.5 mmol) was added to this solution. The resulting mixture was stirred overnight at room temperature, filtered, and dried. After evaporation of solvent, the crude product was chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give crude **6c** (0.40 g). A mixture of **6c** (0.40 g) and Dowex 50 (H^+) (1.16 g) in methanol (20 mL) was stirred for 2 h. The filtered solution was evaporated under reduced pressure, and the residue was chromatographed on silica gel with 10% methanol/CH_2Cl_2 to give **7c** (0.33 g, 68%) as a white solid: mp = 191−192 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.41 (s, 1H), 7.30 (d, J = 8.1 Hz, 1H), 6.59 (dd, J = 3.4, 9.7 Hz, 1H), 5.66 (d, J = 8.1 Hz, 1H), 5.42 (bs, 1H), 4.28 (d, J = 3.7 Hz, 2H), 3.61 (dd, J = 10.2, 19.1 Hz, 1H), 3.28 (dd, J = 10.4, 13.7 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 41.0, 56.1, 84.5, 102.8, 140.6, 150.3, 160.7, 163.2; HRMS (EI) m/z calcd for ($C_8H_9N_3O_4$) 211.1786, found 211.0589.

1-(2-Hydroxyethyl)thymine (9a). Thymine (12.6 g, 100 mmol), dried previously in vacuum at 120 °C for 4 h, was heated in dry DMF (200 mL) with a small amount of NaOH until all uracil was dissolved. To this solution was added ethylene carbonate (8.8 g, 100 mmol), and the mixture was refluxed for 1.5 h. Upon cooling, the mixture was concentrated and the residue was dissolved in 300 mL of water. Unreacted uracil formed a precipitate and was filtered off. After neutralization with Dowex 50 (H^+), the water was evaporated *in vacuo*. The residue was chromatographed on silica gel with 20% methanol/chloroform to give **9a** (13.1g, 70%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.19 (s, 1H), 7.43 (d, J = 1.1 Hz, 1H), 4.89 (t, J = 5.3 Hz, 1H), 3.69 (t, J = 5.1 Hz, 2H), 3.58 (t, J = 4.8 Hz, 2H), 1.75 (s, 3H).

1-(2-Chloroethyl)thymine (10a). Pyridine (1.0 mL) was added to a solution of **9a** (6.91 g, 41 mmol) in dry, hot dioxane (150 mL). Thionyl chloride (30 mL) dissolved in dioxane (20 mL) was then added dropwise to this solution, and the resulting solution was refluxed for 1 h. The solvent was evaporated *in vacuo* and was redissolved in $CHCl_3$ (500 mL). The mixture was extracted over water (50 mL), and all the organics were collected and dried over Na_2SO_4, filtered, concentrated, and chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give **10a** (7.52 g, 89%) as a white solid: ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.33 (s, 1H), 7.58 (s, 1H), 3.97 (dd, J = 1.2, 5.1 Hz, 2H), 3.87 (dd, J = 1.7, 5.2 Hz, 2H), 1.76 (s 3H).

1-Vinylthymine (5b). The chloride **10a** (6.00 g, 32 mmol) dissolved in dry dimethyl sulfoxide (40 mL) was added dropwise to the stirred solution of potassium *tert*-butoxide (13.0 g, 116 mmol) in dimethyl sulfoxide (40 mL). After the mixture was stirred at room temperature for 1 h, 80 mL of cold water was added, and the mixture was made slightly acidic by adding Dowex 50 (H^+). After filtration, the solution was evaporated *in vacuo* (0.2 mm) at 60 °C and the residue was chromatographed on silica gel with 50% ethyl acetate/petroleum ether to give **5c** (4.0 g, 83%) as a white solid:[10] ^{1}H NMR (DMSO-d_6, 200 MHz) δ 9.21 (s, 1H), 7.34 (s, 1H), 7.21 (dd, J = 9.1, 16.0 Hz, 1H), 5.07 (dd, J = 2.1, 16.1 Hz, 1H), 4.91 (dd, J = 2.2, 9.1 Hz, 1H′), 1.98 (s, 3H).

1-[3-(Hydroxymethyl)-4,5-dihydro-1,2-isoxazol-5-yl)]-thymidine (7b). To a solution of $THPO(CH_2)_2NO_2$ (0.45 g, 2.5 mmol) in a mixture of THF (15 mL) and benzene (6 mL) under argon atmosphere were added phenyl isocyanate (0.80 mL, 7 mmol) and triethylamine (0.1 mL). The reaction mixture was stirred for 1 h at room temperature, and 1-vinylthymine (**5b**) (0.39 g, 2.5 mmol) was added. The mixture was stirred overnight at room temperature, filtered, and dried. After evaporation of solvent, the crude product was chromatographed on a silica gel column with 50% ethyl acetate/petroleum ether to give crude **6b** (0.45 g). A mixture of **6b** (0.45 g) and Dowex 50 (H^+) (1.16 g) in methanol (20 mL) was stirred for 2 h at room temperature. The filtered solution was evaporated *in vacuo*, and the residue was chromatographed on silica gel with 10% methanol/CH_2Cl_2 to give **7b** (0.37 g, 66%) as a white solid: mp 170−171 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.42 (s, 1H), 7.12 (1s, 1H), 6.62 (dd, J = 3.6, 10.0 Hz, 1H), 5.44 (t, J = 6.1 Hz, 1H), 4.28 (dd, J = 3.1, 5.8 Hz, 1H), 3.60 (dd, J = 10.0, 18.8 Hz, 1H), 3.21 (dd, J = 3.8, 18.9 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 12.5, 40.7, 56.2, 84.2, 110.7, 135.8, 150.4, 160.5, 163.9; HRMS (EI) m/z calcd for ($C_9H_{11}N_3O_4$) 225.2057, found 225.0754.

1-Vinylcytosine (13). Cytosine (2.3 g, 21 mmol) was heated at 140−150 °C with hexamethyldisiliazane (16 mL) and a trace of ammonium sulfate for 24 h. When a clear solution was formed, the solution was cooled to room temperature and concentrated *in vacuo*. The resulting residue was refluxed with vinyl acetate (30 mL), mercuric acetate (0.25 g, 0.8 mmol), and concd sulfuric acid (0.1 mL) under argon atmosphere for 24 h. The solvent was removed under reduced pressure, and methanol (50 mL) was added, followed by addition of enough ammonium hydroxide to make the solution slightly basic. The insoluble residue was extracted twice with boiling methanol (15 mL), and the methanol solutions were combined and concentrated. The residue was chromatographed on silica gel with 10% methanol/$CHCl_3$ to give **13** (1.86 g, 65%) and **12** (0.34 g, 9%) as white solids:[14] ^{1}H NMR (DMSO-d_6, 200 MHz) **13** δ 7.92 (d, J = 7.4 Hz, 1H), 7.40 (s, 2H), 7.24 (dd, J = 9.2, 16.2 Hz, 1H), 5.82 (d, J = 7.3 Hz, 1H), 5.22 (dd, J = 1.2, 16.2 Hz, 1H), 4.78 (dd, J = 1.3, 9.2 Hz, 1H); **12** δ 11.00 (s, 1H), 8.36 (d, J = 7.3 Hz, 1H), 7.77−7.30 (m, 3H), 5.55 (d, J = 16.1 Hz, 1H), 5.10 (d, J = 9.1 Hz, 1H).

***N*^{4}-(*tert*-Butyloxycarbonyl)-1-vinylcytosine (14).** Di-*tert*-butyl dicarbonate (1.70 g, 8 mmol) was added to the solution of 1-vinylcytosine (**13**) (1.0 g, 7 mmol) in THF (20 mL) and CH_2Cl_2 (20 mL), and the reaction mixture was refluxed for 48 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel with 10% methanol/$CHCl_3$ to give **14** (1.43 g, 83%): ^{1}H NMR (DMSO-d_6, 200 MHz) δ 10.52 (s, 1H), 8.31 (d, J = 7.6 Hz, 1H), 7.23 (dd, J = 9.0, 16.2 Hz, 1H), 7.07 (d, J = 7.3 Hz, 1H), 5.52 (d, J = 16.0 Hz, 1H), 5.07 (d, J = 9.1 Hz, 1H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 28.6, 83.3, 96.3, 104.5, 132.6, 143.1, 151.7, 154.3, 163.5; HRMS (EI) m/z calcd for ($C_{11}H_{15}N_3O_3$) 237.2605, found 237.1110.

1-[3-(Hydroxymethyl)-4,5-dihydro-1,2-isoxazol-5-yl)]-cytidine (7d). To a solution of $THPO(CH_2)_2NO_2$ (0.25 g, 1.4 mmol) in a mixture of THF (15 mL) and benzene (6 mL) under argon atmosphere were added phenyl isocyanate (0.50 mL, 4.6 mmol) and triethylamine (0.05 mL). The reaction mixture was stirred for 1 h at room temperature, and *N*^{4}-(*tert*-butyloxycarbonyl)-1-vinylcytosine (**14**) (0.30 g, 1.3 mmol) was added. The mixture was stirred overnight at room temperature, filtered, and dried. After evaporation of solvent, the crude product was chromatoghaphed on silica gel column with 60% ethyl acetate/petroleum ether to give crude **15a** (0.34 g). **15a** (0.34 g) was dissolved in 44% formic acid/methanol (20 mL), and the reaction mixture was stirred for 30 min at 40 °C. The solution was evaporated under reduced pressure, and the residue was chromatographed on silica gel with 20% methanol/$CHCl_3$ to give **7d** (80 mg, 29%) as a white solid: mp 176−177 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.28 (m, 3H), 6.60 (dd, J = 3.3, 9.5 Hz, 1H), 5.75 (d, J = 7.3 Hz, 1H), 5.41 (bs, 1H), 4.26 (bs, 2H), 3.58 (dd, J = 9.7, 18.8 Hz, 1H), 3.08 (dd, J = 3.4, 18.5 Hz, 1H); HRMS (FAB) m/z calcd for ($C_8H_{10}N_4O_3P$) 211.1778, found 211.0822.

General Procedure for the Dihydroisoxazole Nucleotides 18a−d. To a stirring suspension of *N*-chlorosuccin-

imide in dry CH_2Cl_2 (10 mL) was added a solution of *O,O*-diethyl 3-hydroiminopropyl phosphate **16** in dry CH_2Cl_2 (10 mL) in one portion. As the suspended *N*-chlorosuccinimide completely disappeared, the vinyl base in dry $CHCl_3$ (10 mL) was added dropwise and the temperature was raised to 40−50 °C. After 10 min of stirring at this temperature, triethylamine was added dropwise over 15 min, and the mixture was stirred for another 30 min. The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel with methanol/methylene chloride to give **18**. **18a** was converted to **18b** using the same procedures as for **7a**. The final step of deprotection of **18e** was achieved by 44% formic acid in methanol to give **18f**.

6-Chloro-9-[3-[(diethoxylphosphinyl)ethyl]-4,5-dihydro-1,2-isoxazol-5-yl]purine (18a): yield 84%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.83 (s, 2H), 7.00 (m, 1H), 4.06 (m, 4H), 3.76 (m, 1H), 3.19 (m, 1H), 2.75 (m, 2H), 2.16 (m, 2H), 1.27 (t, J = 7.0 Hz, 6H); HRMS (EI) m/z calcd for ($C_{14}H_{19}ClN_5O_4P$ and M + H) 387.7656 and 388.7736, found 387.0854 and 388.0969.

9-[3-[(Diethoxylphosphinyl)ethyl]-4,5-dihydro-1,2-isoxazol-5-yl]adenine (18b): yield 50%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 8.27 (s, 1H), 8.15 (s, 1H), 7.33 (s, 2H), 4.06 (m, 4H), 3.68 (m, 2H), 2.72 (m, 2H), 2.17 (m, 2H), 1.28 (t, J = 7.1 Hz, 6H); ^{13}C NMR (CD_3OD) δ 17.1, 17.2, 21.7, 22.2, 22.3, 24.6, 43.8, 63.8, 64.0, 85.5, 141.2, 150.4, 154.3, 157.5, 160.8, 161.1. Anal. Calcd ($C_{14}H_{20}N_6O_4P$): C, 45.66; H, 5.75; N, 22.82. Found: C, 46.43; H, 5.73; N, 22.90.

1-[3-(Diethoxylphosphinyl)-4,5-dihydro-1,2-isoxazol-5-yl]thymine (18c): yield 53%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.38 (s, 1H),7.30 (s, 1H), 6.59 (dd, J = 3.7, 10.3 Hz, 1H), 4.04 (m, 4H), 3.56 (dd, J = 10.3, 18.7 Hz, 1H), 3.24 (dd, J = 4.0, 19.1 Hz, 1H), 2.62 (m, 2H), 2.10 (m, 2H), 1.80 (s, 3H), 1.26 (t, J = 7.0 Hz, 6H); ^{13}C NMR (CD_3OD) δ 11.4, 15.7, 15.8, 20.2, 20.7, 20.8, 23.0, 42.6, 62.4, 62.5, 86.0, 111.1, 136.7, 150.8, 159.4, 159.7; HRMS (EI) m/z calcd for ($C_{14}H_{22}N_3O_6P$) 359.3129, found 359.1230.

1-[3-[(Diethoxylphosphinyl)ethyl]-4,5-dihydro-1,2-isoxazol-5-yl]uracil (18d): yield 57%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 11.39 (s, 1H), 7.42 (d, J = 8.1 Hz, 1H), 6.56 (dd, J = 3.7, 9.9 Hz, 1H), 5.63 (d, J = 8.1, 1H), 4.03 (m, 4H), 3.57 (dd, J = 9.5, 19.1 Hz, 1H), 3.18 (m, 1H), 2.60 (m, 2H), 2.07 (m, 2H), 1.26 (t, J = 7.0 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 16.6, 16.7, 20.4, 20.8, 20.9, 23.2, 42.3, 61.5, 61.6, 84.9, 102.6, 141.0, 150.4, 159.4, 159.8, 163.3; HRMS (EI) m/z calcd for ($C_{13}H_{20}N_3O_6P$ + H) 346.3028, found 346.1172.

1-[3-[(Diethoxylphosphinyl)ethyl]-4,5-dihydro-1,2-isoxazol-5-yl)]ytosine (18f): yield 34%; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.34 (d, J = 7.4 Hz, 1H), 7.25 (bs, 2H), 6.57 (dd, J = 4.0, 9.6 Hz, 1H), 5.73 (d, J = 8.1 Hz, 1H), 4.01 (m, 4H), 3.53 (dd, J = 7.5, 19.9 Hz, 1H), 3.19 (m, 1H), 2.62 (m, 2H), 2.06 (m, 2H), 1.25 (t, J = 7.0 Hz, 6H); ^{13}C NMR (CD_3OD) δ 17.1, 17.2, 21.6, 22.1, 22.2, 24.5, 45.3, 63.8, 63.9, 88.3, 96.8, 142.5, 158.0, 161.1, 161.4, 168.0. Anal. Calcd ($C_{13}H_{20}N_5O_5P$): C, 45.35; H, 6.15; N, 16.27. Found: C, 45.52; H, 6.13; N, 16.36.

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Supporting Information Available: Copies of NMR spectra (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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