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Synthesis and anti-HIV-1 evaluation of phosphonates which mimic the 5′-monophosphate of 4′-branched 2′,3′-didehydro-2′,3′-dideoxy nucleosides

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ABSTRACT

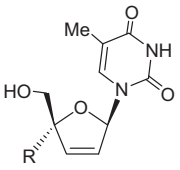
Synthesis of the 4′-ethynyl and 4′-cyano phosphonates **8**–**11**, which mimic the 5′-monophosphate of 4′-branched 2′,3′-didehydro-2′,3′-dideoxy nucleosides, was investigated by employing the 3′,4′-unsaturated nucleosides (**13** and **28**) as the starting material. The synthesis was initiated by the electrophilic addition of NIS/$(EtO)_2P(O)CH_2OH$ to these unsaturated nucleosides. After introduction of the 2′,3′-double bond, the 4′-hydroxylmethyl group of the resulting adducts was transformed into the ethynyl or cyano group. While the 4′-cyano phosphonates **9** and **11** were not sufficiently stable to be isolated, the 4′-ethynyl counterparts (**8** and **10**) were obtained as their mono-ammonium salts. The adenine derivative **8** showed almost comparable anti-HIV-1 activity to that of d4T.

1. Introduction

2′,3′-Didehydro-3′-deoxythymidine (**1**: d4T) is a well known anti-HIV drug (Fig. 1).[1] Synthesis of d4T analogues[2] has provided a various types of compounds, including branched-sugar derivatives.[3–5] In the course of our studies on the reaction of epoxy-sugar nucleosides with organoaluminum and organo-silicon reagents,[6] the 4′-branched d4T analogues were synthesized.[7] Although the 4′-methyl (**2**) and 4′-vinyl (**3**) analogues were not inhibitory to proliferation of HIV, the 4′-ethynyl derivative (**4**, Ed4T) was found to be more potent than d4T and much less toxic to various cells as well as to mitochondrial DNA synthesis.[8] Furthermore, its activity improves in the presence of a major mutation, K103N, a clinically significant mutation with decreased sensitivity to non-nucleoside reverse transcriptase inhibitor resistant HIV.[8] The 4′-cyano analogue (**5**) synthesized later by a different method[9] was almost as active as d4T.

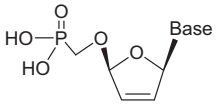
On the other hand, synthesis and anti-HIV activity of the phosphonates **6** and **7** have been reported (Fig. 2).[10,11] These compounds were designed as an isosteric and isoelectronic analogue of the 5′-monophosphate derived from the 2′,3′-didehydro-2′,3′-dideoxy nucleoside. The adenine derivative **6** was reported to show anti-HIV-1 activity comparable to that of d4T (**1**).

This situation motivated us to carry out synthesis of the phosphonates **8**–**11** (Fig. 3) having an ethynyl or cyano group at the



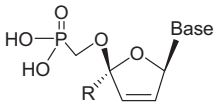
1 R = H (d4T)
2 R = Me
3 R = CH=CH_2
4 R = C≡CH (Ed4T)
5 R = C≡N

Figure 1. Structure of d4T (**1**) and its 4′-carbon-substituted derivatives (**2**–**5**).



6 Base = adenin-9-yl
7 Base = thymin-1-yl

Figure 2. Structure of the phosphonates **6** and **7**.



8 Base = adenin-9-yl, R = C≡CH
9 Base = adenin-9-yl, R = C≡N
10 Base = thymin-1-yl, R = C≡CH
11 Base = thymin-1-yl, R = C≡N

Figure 3. Structure of the 4′-carbon-substituted phosphonates **6** and **7**.

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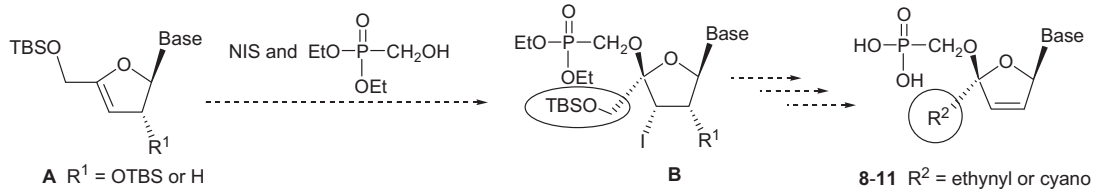


Scheme 1. Synthetic strategy for the 4′-carbon-substituted phosphonates **8**–**11**.

4′-position, which is the subject of this paper. The anti-HIV-1 activity of these compounds is also described.[12]

2. Results and discussion

Our synthetic strategy for the phosphonates **8**–**11** is shown in Scheme 1. The 3′,4′-unsaturated nucleoside **A** is expected to undergo electrophilic addition upon reaction with *N*-iodosuccinimide (NIS)/$(EtO)_2P(O)CH_2OH$ to give the *anti*-adduct **B**. The 4′-CH_2OTBS in **B** serves as a building block to construct the 4′-substituent R^2 (ethynyl or cyano group). Introduction of the 2′,3′-double bond could be achieved by simple elimination of HI in the case of R^1 = H; in the case of R^1 = OTBS several steps are envisioned including deiodination at the 3′-position, removal of the TBS group, sulfonylation of the 2′-hydroxyl group, and finally elimination of a sulfonyloxy group.

Synthesis of the adenine derivatives was first examined. It has already been reported from our laboratory that the 3′,4′-unsaturated adenosine **12** undergoes electrophilic addition by reacting with NIS/MeOH to give **14** in quantitative yield (Scheme 2).[13] Just by replacing the MeOH with $(EtO)_2P(O)CH_2OH$, the adduct **15** was formed, but it was difficult to separate **15** from $(EtO)_2P(O)CH_2OH$ by chromatography. Therefore, the N^6-pivaloyl derivative **13**[14] was used for the above electrophilic addition to give the *anti*-adduct **16** in 82% yield as a single isomer. The depicted stereochemistry of **16** was confirmed by NOE experiment: H-8/PCH_2O (1.3%). The stereochemical outcome observed here is consistent with that reported for electrophilic addition to the furanoid glycal of adenine.[10]

Radical reduction of **16** with Bu_3SnH/AIBN in refluxing benzene gave the 3′-deoxy derivative **17** in 99% yield (Scheme 3). When **17** was subjected to desilylation with Bu_4NF in THF, the spiro derivative **19** was obtained as the major product (33%) along with the desired diol **18** (18%). The presence of AcOH in the above reaction medium avoided the intramolecular nucleophilic attack of the 5′-alkoxide to the phosphorus center, and led to the sole formation of **18** (87%).

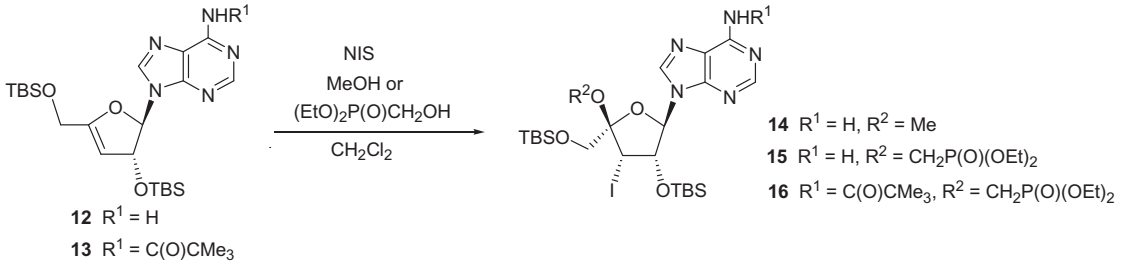
Regioselective O-silylation of **18** was carried out by reaction with TBDPSCl in pyridine to give **20** in 65% yield (Scheme 4). To introduce the 2′,3′-double bond, **20** was first reacted with triflic anhydride (Tf_2O) in pyridine (0 °C to rt, for 3 h). Subsequent addition of DBN to the reaction mixture (rt, for 22 h) gave **21** in 81% yield. Desilylation of **21** with Bu_4NF was carried out again in the presence of AcOH to give **22** in 92% yield.

Attempted oxidation of the 4′-CH_2OH of **22** with Dess–Martin periodinane resulted in recovery of the starting material. When **22** was oxidized with IBX (2-iodoxybenzoic acid)[15] in refluxing CH_3CN for 1.5 h, silica gel column chromatography of the reaction mixture gave the desired aldehyde **23** containing the hemiacetal **24** (Scheme 5). This mixture was converted to the 4′-ethynyl derivative **25** (57% yield from **22**) by reaction with $CH_3C(O)(N_2)PO(OEt)_2$[16] in EtOH in the presence of K_2CO_3 at rt for 1 week. For the preparation of the 4′-cyano derivative **27**, the mixture of **23** and **24** was first converted to the oxime **26** (53% from **22**). When **26** was treated with CH_3SO_2Cl in pyridine, spontaneous elimination occurred to give **27** in 74% yield.

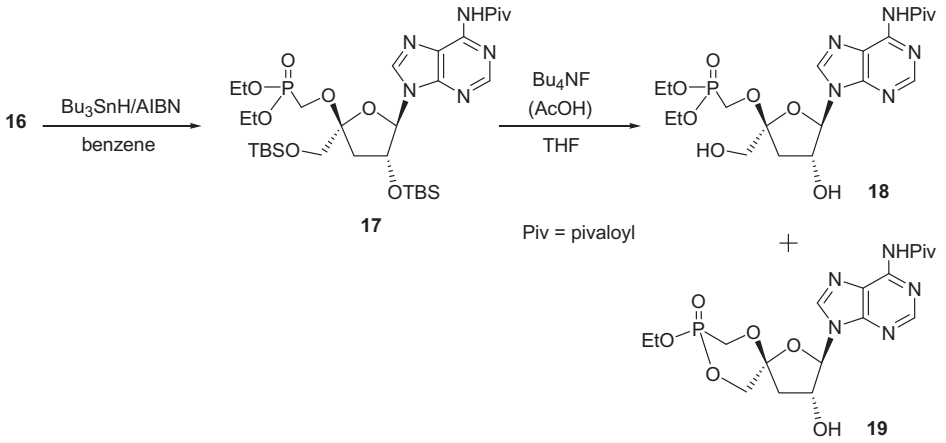
Synthesis of the thymine derivatives was next examined. When the 3′,4′-unsaturated thymine nucleoside **28**[17] was reacted with NIS/MeOH as a model experiment, the two *anti*-adducts **29** and **30** were formed in 91% and 5% yields, respectively (Scheme 6). However, quite unexpectedly, reaction of **28** with NIS/$(EtO)_2P(O)CH_2OH$ under the same conditions gave a complex mixture of products as evidenced by TLC. HPLC purification ($CHCl_3$/MeOH = 50:1) of the mixture enabled us to isolate the desired *anti*-adduct **31**, but the yield was only 21%. At the present time, structures of other products are not known. The stereochemistry of **31** was confirmed on the basis of NOE experiments: H-1′/H-5′ (1.2%) and H-6/PCH_2O (1.9%).

The aldehyde **34** (Fig. 4), a common precursor of the target compounds **10** and **11**, was prepared by the following series of reactions: (1) DBN-treatment of **31** to give **32** (90%), (2) desilylation of **32** to form **33** (91%), and (3) oxidation of the 4′-CH_2OH of **33** with IBX, the product of which was obtained as a mixture of **34** and the hemiacetal **35** as was seen in the case of **23** in Scheme 5. Preparation of the 4′-ethynyl derivative **36** (41% yield from **33**) was carried out using the same procedure as that for **25**. The 4′-cyano derivative **38** was prepared in 95% yield by way of the oxime **37** (obtained in 74% yield from **33**).

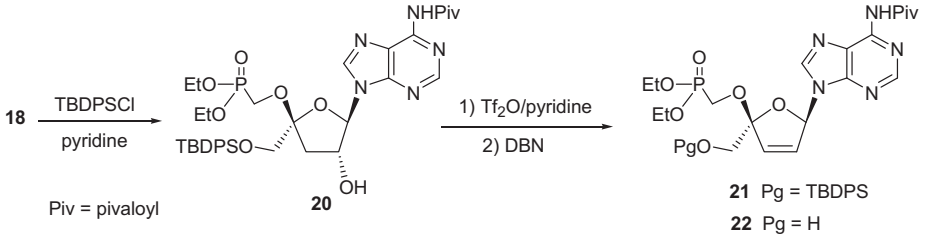
Finally, reaction of the 4′-carbon-substituted phosphonates (**25**, **27**, **36**, and **38**) prepared above with Me_3SiBr was examined. The adenine derivative of 4′-ethynyl phosphonate (**25**) was reacted with Me_3SiBr in DMF at rt for 9.5 h. The reaction mixture was evaporated to dryness, treated with ammonium hydroxide, and then washed with CH_2Cl_2, After reverse-phase column chromatography, the desire 4′-ethynyl phosphonate **8** was obtained as mono-ammonium salt in 70% yield. Likewise, from the thymine derivative **36**, the 4′-ethynyl phosphonate **10** was isolated as mono-ammonium



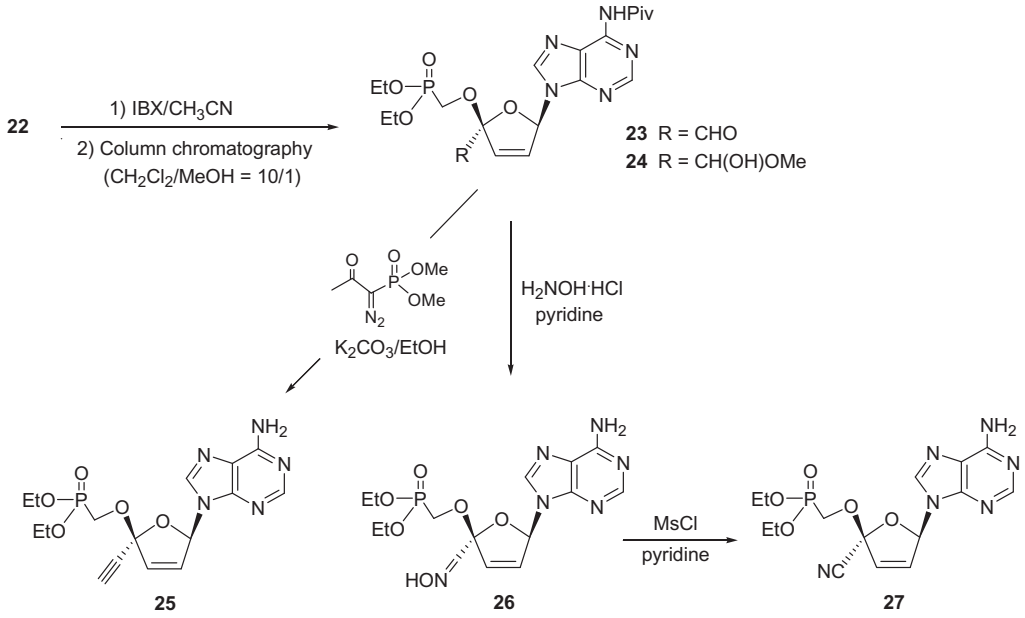
Scheme 2. Electrophilic addition to **12** and **13** by using NIS and ROH.



Scheme 3. Preparation of the phosphonate **18** from **16**.



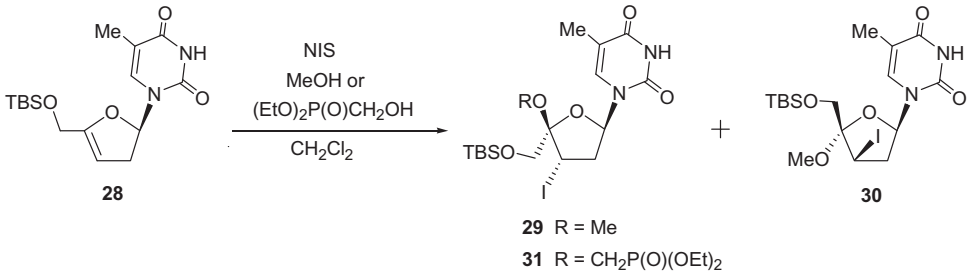
Scheme 4. Preparation of compound **22**.



Scheme 5. Preparation of compounds **25** and **27**.

salt in 75% yield. In contrast to these, the 4′-cyano derivatives **27** and **38** were found to decompose during purification by column chromatography eluted with H_2O as well as during evaporation of the solvent, liberating the respective nucleobase. Therefore, we were unable to isolate **9** and **11**.

The anti-HIV-1 activity of the 4′-ethynyl phosphonates of adenine (**8**) and thymine (**10**) was evaluated by the published procedures[18] and the results are summarized in Table 1. The data for **6**, **7**, and **1** (d4T) taken from Ref. 10 are also included in this Table. A similar trend was seen to the reported data, in that the adenine derivative (**8** or **6**) is more potent than the thymine derivative (**10** or **7**). It was rather discouraging that the potency of **8** was not improved compared with that of **6**, which lacks the 4′-ethynyl group. Also seen here is that the thymine derivative **10** synthesized in this study is devoid of activity, whereas the reported phosphonate **7** shows some activity against HIV-1.



Scheme 6. Electrophilic addition to **28** by using NIS and ROH.

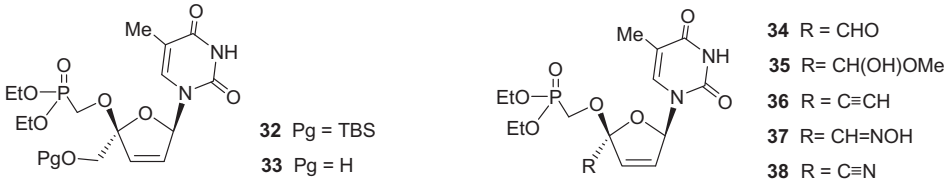


Figure 4. Structure of compounds **32–38**.

Table 1
Anti-HIV-1 activity of compounds **1**, **6**, **7**, **8**, and **10** in MT-4 cells

Compd	IC_{50}[a] (μM)	CC_{50}[b] (μM)
8	1.69	>100
10	>100	>100
6[c]	1.5	>600
7[c]	12.0	>600
1 (d4T)[c]	1.2	>600

[a] Inhibitory concentration required to achieve 50% protection of MT-4cells against the cytopathic effect of HIV-1.
[b] Cytotoxic concentration required to reduce the viability of mock-infected MT-4 cells by 50%.
[c] Data were taken from Ref. 10.

3. Conclusion

We planned to synthesize the adenine and thymine phosphonates (**8**–**11**) bearing an ethynyl or cyano group at the 4′-position. The synthetic sequence employed involves the following three characteristics. The first is face-selective electrophilic addition to the 3′,4′-unsaturated nucleosides (**13** and **28**) by reaction with NIS/$(EtO)_2P(O)CH_2OH$. The second is utilization of the 4′-hydroxymethyl group of the resulting adduct to construct the ethynyl or cyano group. The third is the fact that the starting material (**13** and **28**) can be prepared from naturally occurring nucleosides.

Due to the instability of the 4′-cyano phosphonates (**9** and **11**), only the 4′-ethynyl phosphonates **8** and **10** were obtained in pure form. Anti-HIV-1 evaluation of these compounds revealed that the adenine derivative **8** has almost comparable anti-HIV-1 activity to that of d4T (**1**).

4. Experimental section

4.1. General methods

NMR spectra were recorded either at 400 MHz (JNM-GX400) or at 500 MHz (JNM-LA500). Chemical shifts are reported relative to Me_4Si. Mass spectra (MS) were taken in FAB mode with *m*-nitrobenzyl alcohol as a matrix on a JNS-SX 102A. UV spectra were measured on a JASCO Ubest-55 spectrophotometer. Column chromatography was carried out on silica gel (Micro Bead Silica Gel PSQ 100B, Fuji Silysia Chemical Ltd) or ODS (Wakosil® 40C 18, Wako Pure Chemical Industries, Ltd). Thin later chromatography (TLC) was performed on silica gel (pre-coated silica gel plate F_{254}, Merck) or ODS (pre-coated TLC plate RP-8 F_{254}, Merck). High performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-6AD with a Shim-Pack PREP-SIL (H) KIT column (2 × 25 cm).

4.2. Chemical synthesis

4.2.1. 9-[2,5-Bis-*O*-*tert*-butyldimethylsilyl-3-deoxy-4-(diethoxyphosphinyl)methoxy-3-iodo-α-L-lyxofuranosyl]-*N*^{6}-pivaloyladenine (16)

To a CH_2Cl_2 (5.0 mL) solution containing **13** (100 mg, 0.267 mmol) and NIS (120 mg, 0.535 mmol) was added diethyl hydroxymethylphosphonate (0.131 mL, 0.891 mmol) at rt. The reaction mixture was stirred for 5 h, evaporated, and partitioned between saturated aqueous $Na_2S_2O_3$ and AcOEt. Column chromatography (CH_2Cl_2/MeOH = 70:1) of the organic layer gave **16** (121 mg, 79%) as a pale yellow foam. UV (MeOH) λ_{max} 272 nm (ε 18 500), λ_{min} 232 nm (ε 3 400); ^{1}H NMR ($CDCl_3$) δ −0.31, −0.01, 0.12 and 0.17 (12H, each as s), 0.77 and 0.94 (18H, each as s), 1.38 and 1.41 (6H, each as t, J = 7.1 Hz), 1.41 (9H, s), 3.85 (1H, t, J = 12.1 Hz), 3.94 (1H, t, J = 12.1 Hz), 4.02 (1H, d, J = 11.8 Hz), 4.16–4.32 (4H, m), 4.19 (1H, d, J = 11.8 Hz), 4.53 (1H, dd, J = 5.3 and 6.5 Hz), 4.63 (1H, d, J = 5.3 Hz), 6.34 (1H, d, J = 6.5 Hz), 8.63 (1H, br), 8.80 (1H, s), 8.82 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.51, −5.24, −5.15, −4.99, 16.42, 16.44, 16.46, 16.50, 17.82, 18.10, 25.43, 25.73, 27.33, 37.87, 40.60, 56.62, 58.34, 62.50, 62.56, 62.96, 63.02, 64.21, 75.21, 87.39, 109.74, 109.88, 122.12, 141.77, 149.65, 152.02, 153.14, 175.72; FAB-MS (*m*/*z*) 856 (M^++H). Anal. Calcd for $C_{32}H_{59}IN_5O_8PSi_2$: C, 44.91; H, 6.95; N, 8.18. Found: C, 44.61; H, 6.81; N, 7.99.

4.2.2. 9-[2,5-Bis-*O*-*tert*-butyldimethylsilyl-3-deoxy-4-(diethoxyphosphinyl)methoxy-α-L-lyxofuranosyl]-*N*^{6}-pivaloyladenine (17)

To a benzene (5.0 mL) solution containing **16** (100 mg, 0.13 mmol) and AIBN (31.8 mg, 0.19 mmol) was added Bu_3SnH (0.10 mL, 0.39 mmol). The mixture was stirred under reflux for 1.5 h. Column chromatography (CH_2Cl_2/MeOH = 80:1) of the reaction mixture gave **17** (90 mg, 99%) as a pale yellow foam. UV (MeOH) λ_{max} 272 nm (ε 16,800), λ_{min} 232 nm (ε 3200); ^{1}H NMR ($CDCl_3$) δ −0.11, 0.03, 0.09 and 0.10 (12H, each as s), 0.81 and

0.92 (18H, each as s), 1.34 and 1.36 (6H, each as t, J = 7.1 Hz), 1.39 (9H, s), 2.36 (1H, dd, J = 3.9 and 13.9 Hz), 2.40 (1H, dd, J = 5.9 and 13.9 Hz), 3.75 (1H, d, J = 11.1 Hz), 3.83 (1H, d, J = 11.1 Hz), 3.96 (1H, dd, J = 11.8 Hz), 4.12–4.24 (4H, m), 4.88 (1H, dt, J = 3.9 and 5.9 Hz), 6.10 (1H, d, J = 3.9 Hz), 8.50 (1H, s), 8.62 (1H, br s), 8.74 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.12, −5.06, −4.78, −4.74, 16.73, 16.79, 18.12, 18.46, 25.89, 26.06, 27.71, 40.90, 41.05, 56.22, 57.93, 62.77, 63.02, 63.09, 64.15, 75.96, 91.51, 111.16, 111.30, 123.09, 142.32, 149.84, 151.64, 153.08, 175.98; FAB-MS (m/z) 730 (M^++H). Anal. Calcd for $C_{32}H_{60}N_5O_8PSi_2$: C, 52.65; H, 8.28; N, 9.59. Found: C, 52.46; H, 8.37; N, 9.62.

4.2.3. 9-[3-Deoxy-4-(diethoxyphosphinyl)methoxy-α-L-lyxofuranosyl]-N^6-pivaloyladenine (18)

To a mixture of **17** (459 mg, 0.629 mmol) and AcOH (0.08 mL, 1.57 mmol) in THF (8.0 mL) was added Bu_4NF (1 M THF solution, 1.57 mL, 1.57 mmol). The reaction mixture was stirred at rt for 24 h, evaporated to dryness, and subjected to silica gel column chromatography (CH_2Cl_2/MeOH = 20:1). This gave **18** (305 mg, 97%) as a colorless foam. UV (MeOH) λ_{max} 272 nm (ε 16,500), λ_{min} 232 nm (ε 3100); ^{1}H NMR ($CDCl_3$) δ 1.32 and 1.33 (6H, each as t, J = 7.1 Hz), 1.41 (9H, s), 2.47 (1H, dd, J = 7.7 and 13.7 Hz), 2.72 (1H, dd, J = 7.1 and 13.7 Hz), 3.54 (1H, dd, J = 8.3 and 14.4 Hz), 3.67 (1H, d, J = 12.9 Hz), 3.94 (1H, dd, J = 8.9 and 14.4 Hz), 3.96 (1H, d, J = 12.9 Hz), 4.09–4.21 (4H, m), 5.05 (1H, ddd, J = 4.4, 7.1 and 7.7 Hz), 6.09 (1H, d, J = 4.4 Hz), 8.24 (1H, s), 8.47 (1H, br s), 8.67 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.32, 16.35, 16.37, 16.41, 27.27, 40.49, 41.19, 55.93, 57.64, 62.77, 62.84, 63.25, 63.32, 74.79, 91.64, 111.68, 111.77, 122.44, 141.24, 149.23, 151.17, 152.40, 175.97; FAB-MS (m/z) 502 (M^++H). Anal. Calcd for $C_{20}H_{32}N_5O_8P{\cdot}1/2H_2O$: C, 47.08; H, 6.52; N, 13.73. Found: C, 46.90; H, 6.42; N, 13.51.

4.2.4. Desilylation of 17 with Bu_4NF in the absence of AcOH: formation of the spiro derivative (19)

A mixture of **17** (100 mg, 0.137 mmol) and Bu_4NF (1 M THF solution, 0.343 mL, 0.343 mmol) in THF (5.0 mL) was stirred at rt for 5 h. The reaction mixture was evaporated to dryness and the residue was partially purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1). HPLC separation ($CHCl_3$/MeOH = 10:1) of the resulting crude products gave analytically pure **19** (21 mg, 33%, t_R = 8.3 min, foam) and **18** (12 mg, 18%, t_R = 9.8 min, foam).

Physical data for **19**: ^{1}H NMR ($CDCl_3$) δ 1.40 (6H, t, J = 8.4 Hz), 1.41 (9H, s), 2.18 (1H, dd, J = 7.1 and 13.4 Hz), 2.57 (1H, dd, J = 6.8 and 13.4 Hz), 3.88 (1H, dd, J = 12.0 and 14.6 Hz), 4.13 (1H, dd, J = 1.0 and 14.9 Hz), 4.20 and 4.24 (4H, each as dq, J = 7.1 and 8.4 Hz), 4.38 (1H, dd, J = 12.0 and 16.5 Hz), 5.00 (1H, dd, J = 2.9 and 14.9 Hz), 5.13 (1H, ddd, J = 4.4, 6.8 and 7.1 Hz), 5.54 (1H, s), 6.21 (1H, d, J = 4.4 Hz), 8.12 (1H, s), 8.51 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.37, 16.42, 27.30, 40.18, 40.50, 56.69, 58.12, 62.47, 62.54, 73.71, 73.79, 74.09, 91.61, 104.37, 104.41, 122.38, 140.79, 149.13, 151.02, 152.44, 175.97; FAB-HRMS (m/z) Calcd for $C_{18}H_{27}N_5O_7P$: 456.1648, found: 456.1637 (M^++H).

4.2.5. 9-[5-*O*-*tert*-Butyldiphenylsilyl-3-deoxy-4-(diethoxyphosphinyl)methoxy-α-L-lyxofuranosyl]-N^6-pivaloyladenine (20)

To a solution of **18** (298 mg, 0.595 mmol) in pyridine (8.0 mL) was added *tert*-butyldiphenylsilyl chloride (0.61 mL, 2.38 mmol). The mixture was stirred at rt for 4 days, and partitioned between AcOEt/saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 40:1) of the organic layer gave **20** (288 mg, 65%) as a foam. UV (MeOH) λ_{max} 272 nm (ε 18,700), λ_{min} 236 nm (ε 6100); ^{1}H NMR ($CDCl_3$) δ 1.11 (9H, s), 1.24 and 1.26 (6H, each as t, J = 7.1 Hz), 1.41 (9H, s), 2.58 (1H, dd, J = 7.5 and 13.8 Hz), 2.69 (1H, dd, J = 7.1 and 13.8 Hz), 3.67 (1H, dd, J = 10.3 and 12.7 Hz), 3.78 (1H, dd, J = 12.6 and 12.7 Hz), 3.80 and 3.83 (2H, each as d, J = 11.1 Hz), 4.00–4.13 (4H, m), 4.95 (1H, ddd, J = 4.6, 7.1 and 7.5 Hz), 6.09 (1H, d, J = 4.6 Hz), 7.39–7.47 (6H, m), 7.48–7.71 (4H, m), 8.41 (1H, s), 8.64 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.31, 16.37, 16.43, 19.23, 26.79, 27.32, 40.29, 40.57, 55.78, 57.49, 62.35, 62.41, 62.78, 62.85, 64.26, 74.77, 77.32, 91.10, 110.42, 110.56, 121.88, 127.89, 127.91, 130.06, 132.39, 132.49, 135.56, 135.59, 141.65, 148.93, 150.96, 152.48, 175.78; FAB-MS (m/z) 740 (M^++H). Anal. Calcd for $C_{36}H_{50}N_5O_8PSi$: C, 58.44; H, 6.81; N, 9.46. Found: C, 58.17; H, 6.81; N, 9.41.

4.2.6. (2*S*,5*R*)-9-[2-*tert*-Butyldiphenylsilyl-2-(diethoxyphosphinyl)methoxy-2,5-(dihydro)furan-5-yl]-N^6-pivaloyladenine (21)

To a pyridine (15 mL) solution of **20** (300 mg, 0.405 mmol) was added trifluoromethanesulfonic anhydride (0.08 mL, 0.486 mmol) at 0 °C under Ar atmosphere. After stirring this mixture at rt for 3 h, DBN (0.1 mL, 0.810 mmol) was added. The resulting reaction mixture was stirred at rt for 22 h and partitioned between AcOEt/saturated aqueous $NaHCO_3$. Column chromatography (CH_2Cl_2/MeOH = 60:1) of the organic layer gave **21** (237 mg, 81%) as colorless foam. UV (MeOH) λ_{max} 272 nm (ε 19,400), λ_{min} 235 nm (ε 7000); ^{1}H NMR ($CDCl_3$) δ 1.08 (9H, s), 1.24 and 1.27 (6H, each as t, J = 7.1 Hz), 1.41 (9H, s), 3.67 (1H, dd, J = 10.1 and 13.2 Hz), 3.77 (1H, dd, J = 11.3 and 13.2 Hz), 3.86 (1H, d, J = 10.9 Hz), 3.93 (1H, d, J = 10.9 Hz), 4.00–4.13 (4H, m), 6.34 (1H, dd, J = 1.8 and 5.9 Hz), 6.49 (1H, dd, J = 1.3 and 5.9 Hz), 6.97 (1H, dd, J = 1.3 and 1.8 Hz), 7.38–7.48 (6H, m), 7.65–7.70 (4H, m), 8.13 (1H, s), 8.52 (1H, br s), 8.78 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.33, 16.39, 19.21, 26.80, 27.39, 40.50, 56.51, 58.21, 62.35, 62.41, 62.65, 65.71, 65.75, 86.50, 115.20, 115.33, 122.78, 127.83, 129.94, 130.00, 130.31, 132.69, 132.74, 134.09, 135.55, 135.60, 141.08, 149.62, 151.52, 153.03, 175.66; FAB-MS (m/z) 722 (M^++H). Anal. Calcd for $C_{36}H_{48}N_5O_7PSi{\cdot}1/5H_2O$: C, 59.60; H, 6.72; N, 9.65. Found: C, 59.48; H, 6.67; N, 9.65.

4.2.7. (2*S*,5*R*)-9-[2-(Diethoxyphosphinyl)methoxy-2,5-dihydro-2-(hydroxymethyl)furan-5-yl]-N^6-pivaloyladenine (22)

To a THF (15 mL) solution containing **21** (1.26 g, 1.75 mmol) and acetic acid (0.112 mL, 2.09 mmol) was added Bu_4NF (1 M THF solution, 2.09 mL, 2.09 mmol). The mixture was stirred at rt for 10 h and evaporated to dryness. Silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) of the residue gave **22** (785 mg, 93%) as a colorless foam. UV (MeOH) λ_{max} 272 nm (ε 17,900), λ_{min} 236 nm (ε 5600); ^{1}H NMR ($CDCl_3$) δ 1.28 and1.34 (6H, each as t, J = 7.1 Hz), 1.41 (9H, s), 3.60 (1H, dd, J = 8.8 and 12.6 Hz), 3.66 (1H, dd, J = 8.5 and 14.3 Hz), 3.84 (1H, dd, J = 8.8 and 14.3 Hz), 4.01 (1H, dd, J = 5.7 and 12.6 Hz), 4.06–4.22 (4H, m), 4.54 (1H, dd, J = 5.7 and 8.8 Hz), 6.40 (1H, dd, J = 1.5 and 5.9 Hz), 6.56 (1H, dd, J = 1.7 and 5.9 Hz), 7.06 (1H, dd, J = 1.5 and 1.7 Hz), 8.16 (1H, s), 8.54 (1H, br), 8.76 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.28, 16.33, 16.39, 27.32, 40.39, 56.44, 58.14, 62.68, 62.75, 63.10, 63.16, 86.11, 114.71, 114.79, 122.81, 128.64, 134.96, 140.98, 149.57, 151.62, 152.91, 175.73; FAB-MS (m/z) 484 (M^++H). Anal. Calcd for $C_{20}H_{30}N_5O_7P{\cdot}1/2H_2O$: C, 48.77; H, 6.34; N, 14.22. Found: C, 48.64; H, 6.24; N, 14.06.

4.2.8. Oxidation of 22: formation of 23 and its hemiacetal 24

A CH_3CN (5.0 mL) solution containing **22** (100 mg, 0.206 mmol) and 2-iodoxybenzoic acid (70 mg, 1.20 mmol) was stirred at refluxing temperature for 1.5 h. The reaction mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. Silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) of the residue gave a mixture (84 mg) of **23** and the hemiacetal **24** as a pale yellow foam.

4.2.9. (2*S*,5*R*)-9-[2-(Diethoxyphosphinyl)methoxy-2-ethynyl-2,5-(dihydro)furan-5-yl]adenine (25)

To an EtOH (10 mL) solution containing a mixture of **23** and **24**, prepared from IBX-oxidation of **22** (100 mg, 0.206 mmol), were

added dimethyl 1-diazo(2-oxopropyl)phosphonate (100 mg, 0.521 mmol) and K_2CO_3 (84 mg, 0.609 mmol). The reaction mixture was stirred at rt for 1 week and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) of the organic layer gave **25** (45 mg, 57%) as a pale yellow foam. UV (MeOH) λ_{max} 260 nm (ε 14,700), λ_{min} 226 nm (ε 2000); ^{1}H NMR ($CDCl_3$) δ 1.30 and 1.34 (6H, each as t, J = 7.1 Hz), 2.86 (1H, s), 3.92 (1H, dd, J = 10.8 and 13.1 Hz), 4.08–4.20 (5H, m), 6.07 (2H, br), 6.32 (1H, dd, J = 1.5 and 5.6 Hz), 6.42 (1H, dd, J = 1.6 and 5.6 Hz), 7.16 (1H, dd, J = 1.5 and 1.6 Hz), 8.00 (1H, s), 8.37 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.33, 16.39, 57.56, 59.28, 62.56, 62.62, 62.69, 62.75, 76.29, 76.69, 86.31, 105.43, 105.59, 119.19, 129.81, 133.72, 138.75, 149.41, 153.49, 155.86; FAB-MS (m/z) 394 (M^++H). Anal. Calcd for $C_{16}H_{20}N_5O_5P\cdot1/2H_2O$: C, 47.77; H, 5.26; N, 17.40. Found: C, 48.02; H, 5.15; N, 17.07.

4.2.10. (2*R*,5*R*)-9-[2-(Diethoxyphosphinyl)methoxy-2,5-dihydro-2-(hydroxyiminomethyl)furan-5-yl]adenine (26)

To a pyridine (5.0 mL) solution containing a mixture of **23** and **24**, prepared from IBX-oxidation of **22** (100 mg, 0.206 mmol), was added hydroxylamine hydrochloride (104 mg, 1.65 mmol). The reaction mixture was stirred at 60 °C for 4 h and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) of the organic layer gave **26** (45 mg, 53%) as colorless foam. UV (MeOH) λ_{max} 260 nm (ε 14,800), λ_{min} 228 nm (ε 3200); ^{1}H NMR (CD_3OD) δ 1.23 and 1.24 (6H, each as t, J = 7.1 Hz), 3.82 (1H, dd, J = 10.7 and 13.4 Hz), 3.89 (1H, dd, J = 10.5 and 13.4 Hz), 3.99–4.09 (4H, m), 6.53 (1H, dd, J = 1.8 and 5.9 Hz), 6.63 (1H, dd, J = 1.5 and 5.9 Hz), 6.96 (1H, dd, J = 1.5 and 1.8 Hz), 7.53 (1H, s), 8.20 (1H, s), 8.22 (1H, s); ^{13}C NMR (CD_3OD) δ 16.61, 16.63, 16.67, 16.69, 57.18, 58.87, 64.18, 64.25, 64.27, 64.34, 87.97, 112.97, 113.12, 120.21, 131.36, 134.39, 140.94, 147.92, 150.60, 154.26, 157.48; FAB-MS (m/z) 413 (M^++H). Anal. Calcd for $C_{15}H_{21}N_6O_6P\cdot1/5H_2O$: C, 43.31; H, 5.14; N, 20.20. Found: C, 43.25; H, 5.14; N, 19.92.

4.2.11. (2*S*,5*R*)-9-[2-Cyano-2-(diethoxyphosphinyl)methoxy-2,5-(dihydro)furan-5-yl]adenine (27)

To a pyridine (10 mL) solution of **26** (255 mg, 0.618 mmol) was added CH_3SO_2Cl (0.14 mL, 1.9 mmol) at rt. The mixture was stirred for 12 h and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) of the organic layer gave **27** (180 mg, 74%) as a colorless foam. UV (MeOH) λ_{max} 259 nm (ε 16,200), λ_{min} 228 nm (ε 5800); ^{1}H NMR ($CDCl_3$) δ 1.32 and 1.33 (6H, each as t, J = 7.1 Hz), 3.98–4.08 (2H, m), 4.01–4.20 (4H, m), 6.10 (2H, br), 6.50 (1H, dd, J = 1.8 and 5.7 Hz), 6.56 (1H, dd, J = 1.5 and 5.7 Hz), 7.26 (1H, dd, J = 1.5 and 1.8 Hz), 7.89 (1H, s), 8.38 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.32, 16.34, 16.37, 16.40, 59.02, 60.73, 62.86, 62.93, 63.05, 63.11, 87.07, 103.58, 103.74, 112.91, 119.16, 130.59, 132.78, 138.07, 149.41, 153.77, 155.89; FAB-MS (m/z) 391 (M^++H). Anal. Calcd for $C_{15}H_{19}N_6O_5P\cdot3/4H_2O$: C, 44.18; H, 5.07; N, 20.61. Found: C, 44.35; H, 4.91; N, 20.23.

4.2.12. 1-[5-*O*-*tert*-Butyldimethylsilyl-2,3-dideoxy-4-(diethoxyphosphinyl)methoxy-3-iodo-α-L-*threo*-pentofuranosyl]thymine (31)

A CH_2Cl_2 (10 mL) solution containing of **28** (100 mg, 0.295 mmol), NIS (199 mg, 0.885 mmol) and diethyl hydroxymethylphosphonate (0.217 mL, 1.48 mmol) was stirred at rt for 5 h. After evaporation of the solvent, the residue was dissolved in AcOEt and washed with saturated aqueous $Na_2S_2O_3$ and then with saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 70:1) of the organic layer gave the crude product, which was purified by HPLC ($CHCl_3$/MeOH = 50:1) to give **31** (40 mg, 21%, t_R = 6.2 min) as a pale yellow foam. UV (MeOH) λ_{max} 265 nm (ε 10,300), λ_{min} 233 nm (ε 2400); ^{1}H NMR ($CDCl_3$) δ 0.02 and 0.14 (6H, each as s), 0.92 (9H, s), 1.33 and 1.35 (6H, each as t, J = 6.9 Hz), 2.05 (3H, s), 2.60 (1H, dd, J = 5.7 and 14.5 Hz), 3.02 (1H, ddd, J = 5.9, 8.9 and 14.5 Hz), 3.77 (1H, t, J = 12.0 Hz), 3.89 (1H, t, J = 12.0 Hz), 4.06 (2H, s), 4.16 and 4.18 (4H, each as dq, J = 6.9 and 14.6 Hz), 4.53 (1H, d, J = 5.9 Hz), 6.79 (1H, dd, J = 5.7 and 8.9 Hz), 7.64 (1H, s), 8.88 (1H, br); ^{13}C NMR ($CDCl_3$) δ −5.56, −5.32, 11.79, 16.43, 16.49, 18.05, 24.86, 25.71, 41.15, 56.39, 58.11, 62.35, 62.40, 62.96, 63.02, 63.79, 85.42, 109.88, 110.03, 113.06, 135.82, 150.72, 163.54; FAB-MS (m/z) 633 (M^++H). Anal. Calcd for $C_{21}H_{38}IN_2O_8PSi$: C, 39.88; H, 4.43; N, 6.06. Found: C, 39.93; H, 4.58; N, 6.04.

4.2.13. (2*S*,5*R*)-1-[2-(*tert*-Butyldimethylsiloxy)methyl-2-(diethoxyphosphinyl)methoxy-2,5-(dihydro)furan-5-yl]thymine (32)

An CH_3CN (10 mL) solution containing **31** (250 mg, 0.40 mmol) and DBN (0.24 mL, 1.98 mmol) was stirred for 1.5 h at refluxing temperature. The reaction mixture was partitioned between AcOEt and saturated aqueous NH_4Cl. Silica gel column chromatography (CH_2Cl_2/MeOH = 50:1) of the organic layer gave **32** (182 mg, 90%) as a pale yellow foam. UV (MeOH) λ_{max} 264 nm (ε 9300), λ_{min} 234 nm (ε 2300); ^{1}H NMR ($CDCl_3$) δ 0.02 and 0.03 (6H, each as s), 0.84 (9H, s), 1.28 and 1.29 (6H, each as t, J = 6.9 Hz), 1.89 (3H, d, J = 1.2 Hz), 3.62 (1H, d, J = 11.0 Hz), 3.75–3.85 (2H, m), 3.87 (1H, d, J = 11.0 Hz), 4.09 and 4.11 (4H, each as q, J = 6.9 Hz), 6.07 (1H, dd, J = 1.5 and 5.8 Hz), 6.25 (1H, dd, J = 1.9 and 5.8 Hz), 6.83 (1H, dd, J = 1.5 and 1.9 Hz), 7.16 (1H, d, J = 1.2 Hz), 9.67 (1H, br); ^{13}C NMR ($CDCl_3$) δ −5.65, −5.63, 12.26, 16.28, 16.34, 16.39, 18.05, 25.62, 56.29, 58.00, 62.34, 62.40, 62.64, 62.70, 64.18, 87.88, 111.73, 113.65, 113.78, 130.05, 134.10, 135.50, 150.73, 163.94; FAB-MS (m/z) 505 (M^++H). Anal. Calcd for $C_{21}H_{37}N_2O_8PSi$: C, 49.99; H, 5.55; N, 7.39. Found: C, 49.75; H, 5.69; N, 7.39.

4.2.14. (2*S*,5*R*)-1-[2-(Diethoxyphosphinylmethoxy)-2,5-dihydro-2-(hydroxymethyl)furan-5-yl]thymine (33)

To a THF (8.0 mL) solution containing **32** (181 mg, 0.396 mmol) and AcOH (0.025 mL, 0.479 mmol) was added Bu_4NF (1 M solution in THF, 0.48 mL, 0.48 mmol) at rt. The reaction mixture was stirred for 41 h and evaporated to dryness. Silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) of the residue gave **33** (131 mg, 91%) as a colorless foam. UV (MeOH) λ_{max} 264 nm (ε 8900), λ_{min} 235 nm (ε 2300); ^{1}H NMR ($CDCl_3$) δ 1.34 and 1.37 (6H, each as t, J = 7.1 Hz), 1.92 (3H, d, J = 1.1 Hz), 3.50 (1H, dd, J = 8.5 and 12.9 Hz), 3.71 (1H, dd, J = 8.2 and 14.4 Hz), 3.98–4.03 (2H, m), 4.13–4.27 (4H, m), 4.48 (1H, dd, J = 8.2 and 8.5 Hz), 6.07 (1H, dd, J = 1.5 and 5.9 Hz), 6.52 (1H, dd, J = 1.7 and 5.9 Hz), 6.98 (1H, dd, J = 1.5 and 1.7 Hz), 7.15 (1H, d, J = 1.1 Hz), 9.57 (1H, br); ^{13}C NMR ($CDCl_3$) δ 12.38, 16.31, 16.34, 16.36, 16.40, 56.36, 58.06, 62.67, 62.73, 62.80, 63.14, 63.20, 77.32, 87.99, 111.79, 113.78, 113.87, 128.95, 134.78, 135.27, 150.90, 163.83; FAB-MS (m/z) 391 (M^++H). Anal. Calcd for $C_{15}H_{23}N_2O_8P\cdot1/4H_2O$: C, 45.63; H, 6.00; N, 7.09. Found: C, 45.36; H, 6.15; N, 7.02.

4.2.15. Oxidation of 33: formation of 34

To an CH_3CN (10 mL) solution of **33** (226 mg, 0.579 mmol) was added IBX (178 mg, 0.637 mmol). The resulting suspension was stirred at refluxing temperature for 2 h and then filtered through a Celite pad. Silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) of the filtrate gave a mixture (210 mg) of **34** and its hemiacetal **35** as a pale yellow foam.

4.2.16. (2*S*,5*R*)-1-[2-(Diethoxyphosphinyl)methoxy-2-ethynyl-2,5-(dihydro)furan-5-yl]thymine (36)

To an ethanol (5.0 mL) solution containing a mixture of **34** and **35**, prepared from IBX-oxidation of **33** (80 mg, 0.205 mmol), were

added dimethyl 1-diazo(2-oxopropyl)phosphonate (81 mg, 0.420 mmol) and K_2CO_3 (48 mg, 0.61 mmol). The reaction mixture was stirred at rt for 29 h and then partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 50:1) of the organic layer gave **36** (32 mg, 41%) as a colorless foam. UV (MeOH) λ_{max} 264 nm (ε 9200), λ_{min} 235 nm (ε 2800); ^{1}H NMR ($CDCl_3$) δ 1.34 and 1.35 (6H, each as t, J = 7.1 Hz), 1.75 (1H, s), 1.95 (3H, s), 3.98 (1H, dd, J = 10.5 and 13.2 Hz), 4.12–4.25 (5H, m), 6.12 (1H, dd, J = 1.5 and 5.6 Hz), 6.32 (1H, dd, J = 1.7 and 5.6 Hz), 7.16 (1H, dd, J = 1.5 and 1.7 Hz), 7.16 (1H, s), 8.84 (1H, br); ^{13}C NMR ($CDCl_3$) δ 12.23, 16.27, 16.31, 16.36, 57.64, 59.35, 62.38, 62.44, 62.55, 62.61, 76.21, 77.32, 88.18, 105.13, 105.30, 112.10, 129.81, 133.76, 135.07, 150.63, 163.85; FAB-MS (m/z) 385 (M^++H). Anal. Calcd for $C_{16}H_{21}N_2O_7P{\cdot}1/2H_2O$: C, 48.86; H, 5.64; N, 7.12. Found: C, 49.09; H, 5.66; N, 7.14.

4.2.17. (2*S*,5*R*)-1-[2-(Diethoxyphosphinyl)methoxy-2,5-dihydro-2-(hydroxyiminomethyl)furan-5-yl]thymine (37)

To a pyridine (10 mL) solution containing a mixture of **34** and **35**, prepared from IBX-oxidation of **33** (226 mg, 0.579 mmol), was added hydroxylamine hydrochloride (188 mg, 2.70 mmol). The reaction mixture was stirred at rt for 20 h and partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) of the organic layer gave **37** (172 mg, 74%) as a colorless foam. UV (MeOH) λ_{max} 264 nm (ε 9 600), λ_{min} 237 nm (ε 4700); ^{1}H NMR ($CDCl_3$) δ 1.34 (6H, t, J = 7.1 Hz), 1.94 (3H, s), 3.90 (2H, d, J = 10.7 Hz), 4.12–4.21 (4H, m), 6.15 (1H, dd, J = 1.2 and 5.9 Hz), 6.47 (1H, dd, J = 1.5 and 5.9 Hz), 7.07 (1H, dd, J = 1.2 and 1.5 Hz), 7.23 (1H, s), 7.56 (1H, s), 9.58 (1H, br), 10.3 (1H, br); ^{13}C NMR ($CDCl_3$) δ 12.32, 16.39, 16.41, 16.44, 16.46, 56.94, 58.64, 62.78, 62.85, 62.94, 63.00, 88.47, 110.48, 110.62, 112.01, 130.00, 133.42, 135.73, 146.34, 150.67, 164.13; FAB-MS (m/z) 404 (M^++H). Anal. Calcd for $C_{15}H_{22}N_3O_8P{\cdot}1/2H_2O$: C, 43.69; H, 5.62; N, 10.19. Found: C, 44.01; H, 5.47; N, 9.96.

4.2.18. (2*S*,5*R*)-1-[2-Cyano-2-(diethoxyphosphinyl)methoxy-2,5-(dihydro)furan-5-yl]thymine (38)

To a pyridine (3.0 mL) solution of **37** (59 mg, 0.146 mmol) was added CH_3SO_2Cl (0.068 mL, 0.876 mmol) at rt. The reaction mixture was stirred for 20 h and partitioned between CH_2Cl_2/saturated aqueous $NaHCO_3$. Silica gel column chromatography (CH_2Cl_2/MeOH = 40:1) of the organic layer gave **38** (54 mg, 95%) as a colorless foam. UV (MeOH) λ_{max} 262 nm (ε 8700), λ_{min} 234 nm (ε 2900); ^{1}H NMR ($CDCl_3$) δ 1.35 and 1.36 (6H, each as t, J = 7.1 Hz), 1.95 (3H, d, J = 1.3 Hz), 4.05–4.14 (2H, m), 4.15–4.24 (4H, m), 6.35 (1H, dd, J = 1.3 and 5.9 Hz), 6.40 (1H, dd, J = 1.8 and 5.9 Hz), 6.47 (1H, d, J = 1.3 Hz), 7.27 (1H, dd, J = 1.3 and 1.8 Hz), 9.14 (1H, br); ^{13}C NMR ($CDCl_3$) δ 12.35, 16.39, 16.44, 16.49, 59.40, 61.12, 62.83, 62.90, 63.05, 63.12, 89.08, 103.39, 103.55, 112.87, 113.10, 130.87, 133.02, 134.14, 150.23, 163.22; FAB-MS (m/z) 386 (M^++H). Anal. Calcd for $C_{15}H_{20}N_3O_7P$: C, 46.76; H, 5.23; N, 10.42. Found: C, 46.88; H, 5.26; N, 10.75.

4.2.19. (2*S*,5*R*)-9-[2-Ethynyl-2,5-dihydro-2-(phosphonomethoxy)-furan-5-yl]-adenine mono-ammonium salt (8)

To a DMF (5.0 mL) solution of **25** (100 mg, 0.254 mmol) was added Me_3SiBr (0.329 mL, 2.54 mmol) at 0 °C. The reaction mixture was stirred for 9.5 h at rt, concentrated in vacuo, and neutralized with NH_4OH (1.0 mL). The resulting solution was washed with CH_2Cl_2. Reverse phase column chromatography (H_2O) of the aqueous layer gave **8** (63 mg, 70%) as a pale yellow foam. UV (H_2O) λ_{max} 260 nm (ε 15,700), λ_{min} 226 nm (ε 2500); ^{1}H NMR (D_2O) δ 3.18 (1H, s), 3.37 (1H, dd, J = 10.2 and 12.4 Hz), 3.65 (1H, dd, J = 11.0 and 12.4 Hz), 6.38 (1H, dd, J = 1.2 and 5.9 Hz), 6.42 (1H, dd, J = 1.5 and 5.9 Hz), 6.81 (1H, dd, J = 1.2 and 1.5 Hz), 7.97 (1H, s), 7.98 (1H, s); ^{13}C NMR (D_2O) δ 60.99, 62.26, 78.39, 78.45, 85.91, 105.47, 105.59, 118.27, 130.11, 133.65, 139.20, 148.83, 152.90, 155.99; FAB-MS (m/z) 338 (M^++H). Anal. Calcd for $C_{12}H_{15}N_6O_5P{\cdot}9/5H_2O$: C, 37.27; H, 4.45; N, 21.73. Found: C, 37.57; H, 4.45; N, 21.48.

4.2.20. (2*S*,5*R*)-1-[2-Ethynyl-2,5-dihydro-2-(phosphonomethoxy)furan-5-yl]-thymine mono-ammonium salt (10)

To a DMF (5.0 mL) solution of **36** (50 mg, 0.130 mmol) was added Me_3SiBr (0.252 mL, 1.95 mmol) at 0 °C. The reaction mixture was stirred for 9.5 h at rt, concentrated in vacuo, and neutralized with NH_4OH (2.0 mL). The resulting solution was washed with CH_2Cl_2. Reverse phase column chromatography (H_2O) of the aqueous layer gave **10** (34 mg, 75%) as a colorless foam. UV (H_2O) λ_{max} 267 nm (ε 9700), λ_{min} 235 nm (ε 3000); ^{1}H NMR (D_2O) δ 1.71 (3H, d, J = 1.1 Hz), 3.19 (1H, s), 3.53 (1H, dd, J = 10.2 and 12.3 Hz), 3.81 (1H, dd, J = 11.0 and 12.3 Hz), 6.13 (1H, dd, J = 1.6 and 5.7 Hz), 6.34 (1H, dd, J = 1.7 and 5.7 Hz), 6.87 (1H, dd, J = 1.6 and 1.7 Hz), 7.10 (1H, d, J = 1.1 Hz); ^{13}C NMR (DMSO-d_6) δ 11.97, 61.29, 62.53, 78.16, 78.50, 87.74, 105.09, 105.21, 110.82, 129.85, 133.91, 135.55, 150.42, 163.90; FAB-MS (m/z) 346 (M^++H). Anal. Calcd for $C_{12}H_{16}N_3O_7P{\cdot}H_2O$: C, 39.68; H, 4.99; N, 11.57. Found: C, 39.91; H, 4.84; N, 11.71.

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