D- and L-2′,3′-Didehydro-2′,3′-dideoxy-3′-fluoro-carbocyclic Nucleosides: Synthesis, Anti-HIV Activity and Mechanism of Resistance

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Introducing 2′-fluoro substitution on the 2′,3′-double bond in carbocyclic nucleosides has provided biologically interesting compounds with potent anti-HIV activity. As an extension of our previous works in the discovery of anti-HIV agents, D- and L-2′,3′-unsaturated 3′-fluoro carbocyclic nucleosides were synthesized and evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells. Among the synthesized L-series nucleosides, compounds **18**, **19**, **26** and **28** exhibited moderate antiviral activity (EC$_{50}$ 7.1 μM, 6.4 μM, 10.3 μM, and 20.7 μM, respectively), while among the D-series, the guanosine analogue (**35**, D-3′-F-C-d4G) exhibited the most potent anti-HIV activity (EC$_{50}$ 0.4 μM, EC$_{90}$ 2.8 μM). However, the guanosine analogue **35** was cross-resistant to the lamivudine-resistant variants (HIV-1$_{M184V}$). Molecular modeling studies suggest that hydrophobic interaction as well as hydrogen-bonding stabilize the binding of compound **35** in the active site of wild type HIV reverse transcriptase (HIV-RT). In the case of L-nucleosides, these two effects are opposite which results in a loss of binding affinity. According to the molecular modeling studies, cross-resistance of D-3′-F-C-d4G (**35**) to M184V mutant may be caused by the realignment of the primer and template in the HIV-RT$_{M184V}$ interaction, which destabilizes the RT-inhibitor triphosphate complex, resulting in a significant reduction in anti-HIV activity of the D-guanine derivative **35**.

Introduction

Nucleoside reverse transcriptase inhibitors (NRTI) have played an important role in the treatment of HIV infections.[1] However, major drawbacks of NRTI include the emergence of drug resistant variants and toxicity.[2−5] Therefore, conservative efforts have been made to improve the antiviral efficacy as well as to reduce the toxicity by modifying the structure.

The structures of several potent NRTIs, such as stavudine (d4T), abacavir, reverset (D-d4FC), and elvucitabine (L-d4FC) highlight the important role of a 2′,3′-double bond to enhance the antiviral activity (Figure 1).[1] Additionally, carbocyclic nucleosides such as abacavir have attracted considerable attention due to their potent antiviral activity as well as the stability toward metabolic degradation.[6] In view of these facts, it was of interest to incorporate these structural features into nucleoside analogues. Several interesting compounds with potent anti-HIV activity have been discovered as the result of this concept.[7−13] In connection to these efforts, our laboratory recently reported the stereoselective synthesis and anti-HIV activity of D- and L-2′,3′-didehyhydro-2′,3′-dideoxy-2′-fluoro-carbocyclic nucleosides.[14] Among the series, the adenosine analogue with L-configuration showed the most potent anti-HIV activity (EC$_{50}$ 0.77 μM). On the basis of molecular modeling studies, it was found that both the double bond on the carbocyclic ring as well as the 2′-fluoro substitution contribute to the favorable binding affinity between the inhibitor and the HIV-RT. Hence, further exploration of the antiviral activity of 3′-fluorine congeners was of interest to expand our knowledge on the structure−activity relationships of the same class of nucleosides.

For the synthesis of 2′-fluoro carbocyclic nucleosides, the fluorine substituted unsaturated carbocyclic ring was first constructed followed by coupling with various heterocyclic bases. However, this method was found to be unsuccessful in the synthesis for the 3′-fluoro congeners due to the instability of the final products under the same conditions used. Therefore, condensation of a *gem*-3′,3′-difluoro sugar **8** or **29** with base moieties followed by an elimination reaction in the last step was successful to obtain the target purine nucleosides. Pyrimidine nucleosides were, however, synthesized via the linear method using the intermediate **11** or **30**. Herein, synthesis, anti-HIV activity, and molecular modeling studies of D- and L-2′,3′-didehyhydro-2′,3′-dideoxy-3′-fluoro-carbocyclic nucleosides are reported.

Results and Discussion

Chemistry. Both D- and L-nucleosides were synthesized; however, the following descriptions are mainly based on the L-series according to Schemes 1−3, unless otherwise indicated. The epoxide **1** was synthesized from D-ribose by the known method in our laboratory.[14] The desired regioisomer was obtained by reductive ring opening of the epoxide with judicious selection of reducing reagents (Scheme 1). The preliminary study suggested that compound **4** was the major product when epoxide **2** was treated with LAH. The selective opening of the epoxide by the hydride may be due to the steric hindrance of the bulky trityl group adjacent to the 3′-position. On the basis of this result, several other reducing reagents were investigated (Table 1). Among them, Super-Hydride gave predominantly compound **4** (**4**:**3** = 15.3:1) in excellent yield (98%). Oxidation of the alcohol **4** with PDC gave the ketone **5** as an unstable compound. It was interesting to note that introducing a difluorine group at the 3′-position to compound **5** was quite difficult in comparison to the 2′-fluoro isomer.[14] A harsh condition of neat diethylaminosulfur trifluoride (DAST) with reaction temperature at 40 °C for 36 h was needed to convert the ketone **5** to difluoro compound **6** in 68% yield. The following elimination reaction also gave significant problems: Treating compound **6** with

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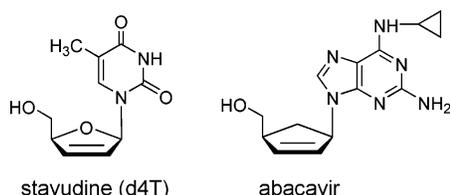
Figure 1. Several potent NRTIs with 2′,3′-double bond.
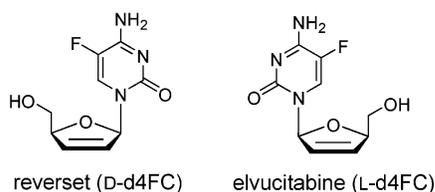
Scheme 1[a]
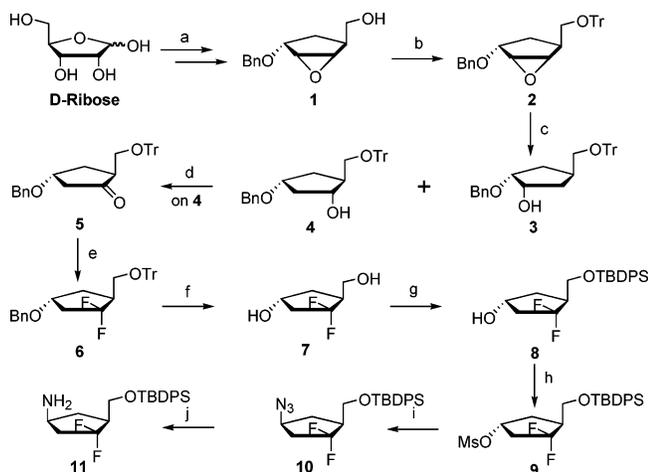
[a] Reagents and conditions: (a) (i) ref 14, (ii) α-AIBBr, CH₃CN, (iii) K₂CO₃, MeOH; (b) TrCl, DMAP, Et₃N, CH₂Cl₂; (c) Super-Hydride (1.0 M in THF), 0 °C to rt; (d) PDC, AcOH, 4 Å molecular sieve, CH₂Cl₂; (e) neat DAST, 40 °C; (f) TMSI, CH₂Cl₂; (g) TBDPSCl, imidazole, CH₂Cl₂; (h) MsCl, Et₃N, CH₂Cl₂, rt; (i) NaN₃, DMF, 130 °C; (j) H₂/Pd/C, 30 psi, rt.

Table 1. Ring-Opening Reaction of Epoxide **2**

entry	reducing agent	4:3	yield (%)
1	Super-Hydride	15.3:1	98
2	LAH	5.1:1	88
3	DIBAL-H	—	0
4	Red-Al	—	0
5	LiAl(*t*-butoxy)₃	—	0

potassium *tert*-butoxide (*t*BuOK) in THF at 50 °C did not produce the desired 3′-fluorovinyl moiety, and only the starting material was recovered. Hence, a modified synthetic sequence was adopted as illustrated in Scheme 1 (**6** to **11**). Both trityl and benzyl groups were removed using iodotrimethylsilane (TMSI) in 70% yield. The resulting diol **7** was selectively protected by *tert*-butyldiphenylsilyl chloride (TBDPSCl) to give the key intermediate **8** which was converted to amine **11** in three steps. For the pyrimidine nucleosides, the linear synthetic methodology reported by Shealy et al.[15,16] was used as a direct coupling reaction using the alcohol **8** under the Mitsunobu conditions resulting in the decomposition of the starting material. The amine **11** was coupled with substituted isocynate to give the corresponding urea **12** or **16** (Scheme 2). Reaction of **12** with concentrated ammonium hydroxide/ethanol/1,4-dioxane in a steel bomb gave the uridine analogue **13** in 41% yield. Amination of **13** followed by deprotection afforded the cytidine analogue **15**. For the thymidine analogue **17**, ring closure and deprotection were accomplished in one step under acidic condition from compound **16**. Last, the 2′,3′-double bond was obtained under basic elimination conditions to afford the target

Scheme 2[a]
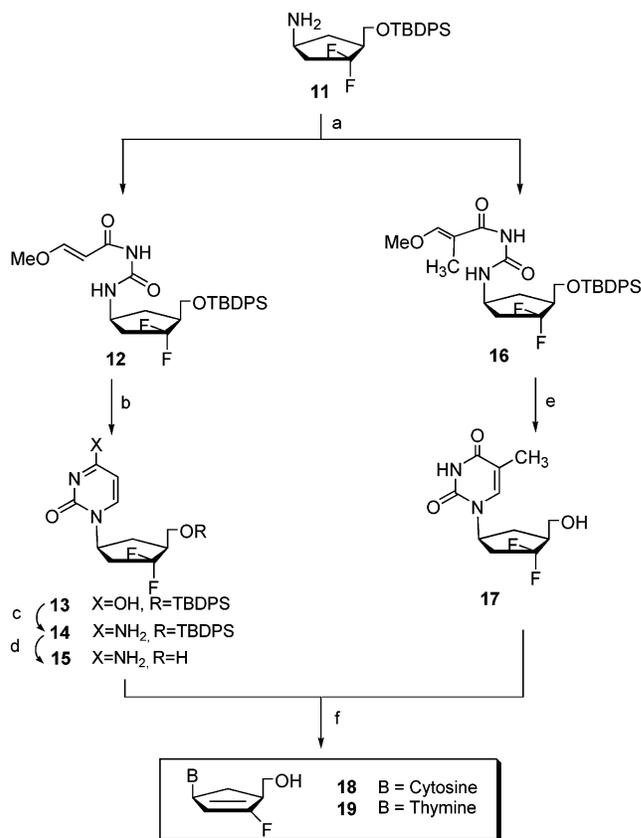
[a] Reagents and conditions: (a) (i) β-methoxyacryloyl isocyanate, THF, −30 °C to rt (for **12**) or β-methoxy-α-methacryloyl isocyanate, THF, −30 °C to rt (for **16**); (b) NH₄OH, 1,4-dioxane/EtOH, steel bomb, 90−100 °C; (c) (i) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et₃N, CH₃CN, rt, (ii) NH₄OH or NH₃/MeOH, rt; (d) HCl/MeOH, rt; (e) 3 N HCl/1,4-dioxane, reflux 3 h; (f) *t*BuOK, THF/1,4-dioxane, 90 °C.

Table 2. Elimination Reactions Using Traditional or Microwave (MW)-Assisted Methods

entry	substrate	temp (°C)	reaction time	isolated yield (%)	methods
1	L-cytidine analogue	90	9 h	35	traditional oil bath heating
2	D-cytidine analogue	70	6 min	84	MW-assisted
3	L-guanosine analogue	70	24 h	45	traditional oil bath heating
4	D-guanosine analogue	70	10 min	72	MW-assisted

cytidine **18** and thymidine **19** analogues in 35% and 46% yield, respectively (Table 2). To synthesize adenosine analogues, triphenylphosphine (TPP) and diisopropyl azodicarboxylate (DIAD) were first mixed in the THF:1,4-dioxane cosolvent at

Scheme 3[a]
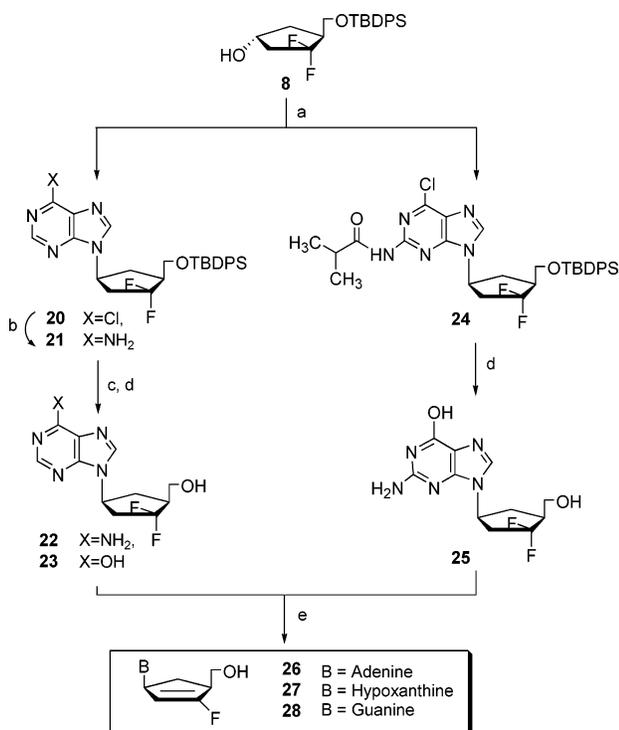
[a] Reagents and conditions: (a) DIAD, Ph₃P, purines, THF or THF/1,4-dioxane; (b) NH₃/MeOH, steel bomb, 110 °C; (c) 3 N HCl, MeOH; (d) (i) formic acid, (ii) NH₄OH or NH₃/MeOH, rt; (e) ᵗBuOK, THF/1,4-dioxane, 90 °C or ᵗBuOK, DMF, 70 °C.

0 °C and then further cooled to −78 °C. The key intermediate **8** and the 6-chloropurine were added sequentially, and the reaction was allowed to gradually warm up to room temperature until all the starting material was consumed (Scheme 3). The crude product **20**, which was contaminated with reduced DIAD species, was directly treated with methanolic ammonia in a steel bomb at 100 °C to give the adenosine analogue **21** in 37% yield in two steps. After the silyl group was removed under acidic condition, compound **22** was treated with ᵗBuOK in THF at 90 °C to furnish the adenine derivative **26** in 50% yield. The compound **21** could also be converted to inosine analogue **23** by treating with formic acid followed by ammonium hydroxide in 39% yield in two steps. After the elimination reaction similar to the method described for the adenosine analogue, the final inosine analogue **27** was obtained in 49% yield. Condensation of alcohol **8** with 2-amino-6-chloropurine or 6-chloro-N^2-isobutyrylpurine, under the Mitsunobu condition as described above, failed to give the corresponding nucleoside. However, when a mixture of TPP, 6-chloro-N^2-isobutyrylpurine and alcohol **8** in dry THF was treated with DIAD at 0 °C, the desired product **24** was able to be isolated. The compound **24** was converted to the guanosine analogue **25** using formic acid followed by ammonium hydroxide in 18% yield from **8** in two steps. The nucleoside **25** was further subjected to an elimination reaction using ᵗBuOK in DMF at 70 °C to give the guanosine analogue **28** in 45% yield.

Similar procedures were conducted to synthesize the D-series (Scheme 4). As the difluoro-substituted nucleosides on the carbohydrate moiety exhibit interesting biological activity,[17] the difluoro-nucleosides **31**−**33**, **37** and **38** in the D-series were also evaluated against HIV-1 (Table 3). It is noteworthy that the elimination reaction in the last step proceeded with significant difficulties. First of all, the starting material and product have almost identical R_f values on a silica gel plate as well as on a

column, which makes it extremely difficult to identify and separate. Second, the difluoro compounds are inert to the conventional methods of elimination using ᵗBuOK in aprotic solvent, and therefore, the reaction mixture has to be heated for long periods of time to convert all the starting material to the product (Table 3, entries 1 and 3). Unfortunately, under these conditions, the newly formed target nucleosides decomposed, which resulted in low yields. Fortunately, in the search for better conditions for the elimination step during the synthesis of the D-compounds, it was found that the microwave-assisted method had several advantages over the traditional method. Upon irradiation of the difluoro-nucleosides in the microwave synthesizer with maximum output power of 300 W, the elimination reaction was completed within 5−10 min at 70 °C and gave 70−80% yield, while a lower yield was obtained after a longer time in the traditional thermally assisted conditions (Table 2). This methodology may provide an efficient way for preparing molecules which have a fluorovinyl moiety. Assignment of the structures of newly synthesized nucleosides was accomplished by NMR, elemental analysis, mass, UV, and IR spectroscopy.

Anti-HIV Activity. All the synthesized pyrimidine (**18**, **19**, and **37**−**40**) and purine (**26**−**28**, **31**−**36**) nucleosides were evaluated against HIV-1 in human PBM cells. The EC₅₀, EC₉₀, and toxicity data are listed in Table 3. In the D-3′,3′-difluoro series, none of the compounds showed any antiviral activity nor cytotoxicity when tested up to 100 μM. Among the D- and L-3′-fluoro-2′,3′-unsaturated nucleosides, some of them exhibited moderate to potent anti-HIV activity. The cytidine **18**, thymidine **19**, adenosine **26** and guanosine **28** analogues in the L-series inhibited HIV-1 in PBM cells with EC₅₀ that ranged from 6.4 to 20.7 μM. The guanosine analogue **35**, bearing D-configuration, is the most active compound among all the synthesized nucleosides (EC₅₀ 0.41 μM, EC₉₀ 2.8 μM), although it exhibited moderate cytotoxicity in PBM cells (IC₅₀ 21.1 μM). The antiviral activities of 3′-fluoro-2′,3′-unsaturated carbocyclic nucleosides in the current studies were generally maintained or enhanced, in comparison to that of 2′-fluoro congeners.[14] Thus, the role of the fluorine substitution on the 3′-position of the double bond may have significant effects (vide infra for molecular modeling studies).

Antiviral Activity against Lamivudine-Resistant (HIV-1$_{M184V}$) Mutant Strain. One of the drawbacks of the NRTI is the emergence of drug-resistant mutant strains during the extended treatment period, which may significantly compromise the clinical efficacy. Lamivudine, an important component of the highly active antiretroviral therapy (HAART), confers a single mutation at residue 184 (M184V), which caused at least a 1000-fold decrease in its antiviral activity.[18,19] Discovery of novel NRTI agents against lamivudine-resistant mutant strain is of great interest. Unfortunately, all the potent NRTI with L-configuration against wild type HIV-1 are always cross-resistant to the lamivudine resistant mutant (M184V), which may be due to the steric hindrance between the bulky side chain of Val184 and the adjacent NRTI's sugar ring.[20] The situation is generally better in D-nucleosides, as their sugar rings project far away from the residue 184. However, the M184V mutant has also been isolated after using the abacavir, a prodrug of carbovir, which is a D-nucleoside.[21−26] In view of the structural similarity between the compound **35** and carbovir, it was of interest to understand its resistance profile. Hence, we further evaluated compound **35** against HIV$_{M184V}$ using carbovir (Table 4) as well as 3TC/AZT (data not shown) as control. On the basis of this study, compound **35** appears to confer resistance

Scheme 4[a]
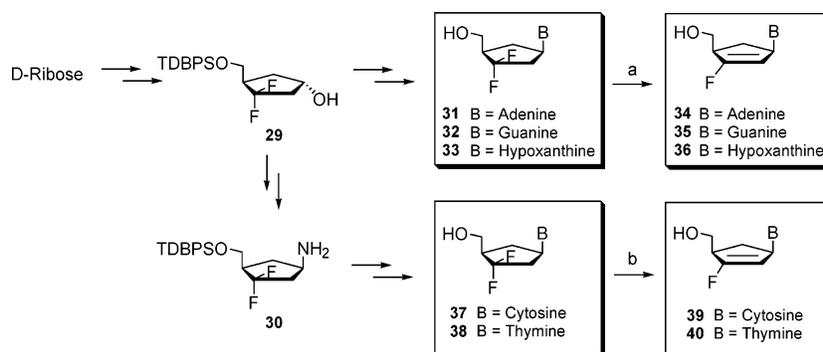
[a] Reagents and conditions: (a) ᵗBuOK, THF/1,4-dioxane, 90 °C, conventional oil bath heating for **34**, **36**; ᵗBuOK, DMF, 70 °C, microwave-assisted for **35**; (b) ᵗBuOK, THF/1,4-dioxane, 60 °C, traditional oil bath heating for **40**; ᵗBuOK, DMF, 70 °C, microwave-assisted for **39**.

Table 3. In Vitro Anti-HIV-1 Activity and Toxicity of D-3′,3′-Difluoro-2′,3′-dideoxy-carbocyclic Nucleosides and D- and L-3′-Fluoro-2′,3′-didehydro-carbocyclic Nucleosides

		anti-HIV-1 activity (μM)[a]		cytotoxicity (μM)		
B	configuration	EC_{50}	EC_{90}	PBM	CEM	Vero
adenine **31**	D	>100	>100	95.1	>100	>100
guanosine **32**	D	>100	>100	>100	>100	>100
hypoxanthine **33**	D	>100	>100	>100	>100	>100
cytosine **37**	D	>100	>100	>100	>100	>100
thymine **38**	D	>100	>100	>100	>100	>100
cytosine **18**	L	7.1	72.0	>100	>100	>100
thymine **19**	L	6.4	>100	>100	>100	>100
adenine **26**	L	10.3	33.5	>100	>100	>100
hypoxanthine **27**	L	>100	>100	>100	>100	>100
guanine **28**	L	20.7	>100	>100	>100	>100
adenine **34**	D	14.8	40.6	>100	>100	>100
guanine **35**	D	0.41	2.8	21.1	>100	>100
hypoxanthine **36**	D	>100	>100	>100	>100	>100
cytosine **39**	D	>100	>100	>100	>100	>100
thymine **40**	D	68.8	>100	>100	>100	>100
carbovir	D	0.087[b]	0.27[b]	N/A	N/A	N/A

[a] Anti-HIV activity evaluated in PBM cells against HIV-1$_{LAI}$ unless otherwise indicated. [b] Anti-HIV activity evaluated in PBM cells against HIV-1$_{xxBRU}$.

Table 4. Activity of D-3′-F-C-d4G against Lamivudine-Resistant Virus (HIV-1$_{M184V}$) in Human PBM Cells Using Carbovir as Control

	xxBRU		M184V		
compd	EC_{50} (μM)	EC_{90} (μM)	EC_{50} (μM)	EC_{90} (μM)	FI[a]
D-3′-F-C-d4G **35**	0.098	0.58	3.8	14.9	38.8
carbovir[b]	0.087	0.27	0.20	1.1	2.3

[a] FI is the fold increase (EC_{50} HIV-1$_{M184V}$/EC_{50} HIV-1$_{xxBRU}$). [b] We also performed the experiment using AZT and 3TC as control, in which AZT is not but 3TC is highly cross-resistant to M184V mutant, and compound **35** is cross-resistant to M184V mutant (data not shown).

Table 5. In Vitro Anti-HIV Activity of Selected 3′-FC-d4Ns and Carbovir against HIV Wild Type Virus and Correlation with Calculated Energy of Complex (Inhibitor-TP)/HIV-RT

compd	EC_{50} (μM)[a]	E_{rel} (kcal/mol)[b]
D-3′-F-C-d4G **35**	0.41	−24.4
L-3′-F-C-d4G **28**	20.7	+18.8
carbovir	0.087[c]	−10.8

[a] EC_{50} in PBM cells against HIV-1$_{LAI}$ unless otherwise indicated. [b] E_{rel} = (binding energy of inhibitor-TP) − (binding energy of natural 2′-dNTP). [c] EC_{50} in PBM cells against HIV-1$_{xxBRU}$.

to HIV-1$_{M184V}$ (Table 4). Molecular modeling was thus performed to understand the potential mechanism of the cross-resistance (vide infra).

Molecular Modeling Studies. Among all the synthesized nucleosides, D-3′-F-C-d4G is the most active compound, while its L-form exhibited only marginal activity against HIV-1. From the studies of drug resistant mutant, it was found that the anti-HIV activity of D-3′-F-C-d4G to HIV-RT$_{M184V}$ significantly decreased in comparison to the wild type virus. To understand the molecular basis of antiviral activity as well as the drug-resistance, molecular modeling studies were conducted on the interactions between the NRTI and HIV-RT. Our previous modeling studies have qualitatively demonstrated the relation-

ship between the binding affinity and the antiviral activity.[10−14] From present studies, the most active compound D-3′-F-C-d4G **35** has the most favorable relative binding energy (−24.4 kcal/mol, Table 5), which is significantly higher than that of its lower activity L-counterpart **28** (+18.8 kcal/mol, Table 5).

The minimized structure showed that D-3′-F-C-d4G was bound tightly in the well-defined binding pocket inside the wild type HIV-RT (Figure 2a). The triphosphate moiety is stabilized by the extensive hydrogen bonds with amino acids Arg65, Lys70, Lys72, Asp113 and Ala114. The carbocyclic ring stacks right over the phenyl ring of Tyr115 forming a favorable hydrophobic $\pi-\pi$ interaction, which has been observed in our previous reports.[12−14] Also, the 3′-fluorine is strongly interacting
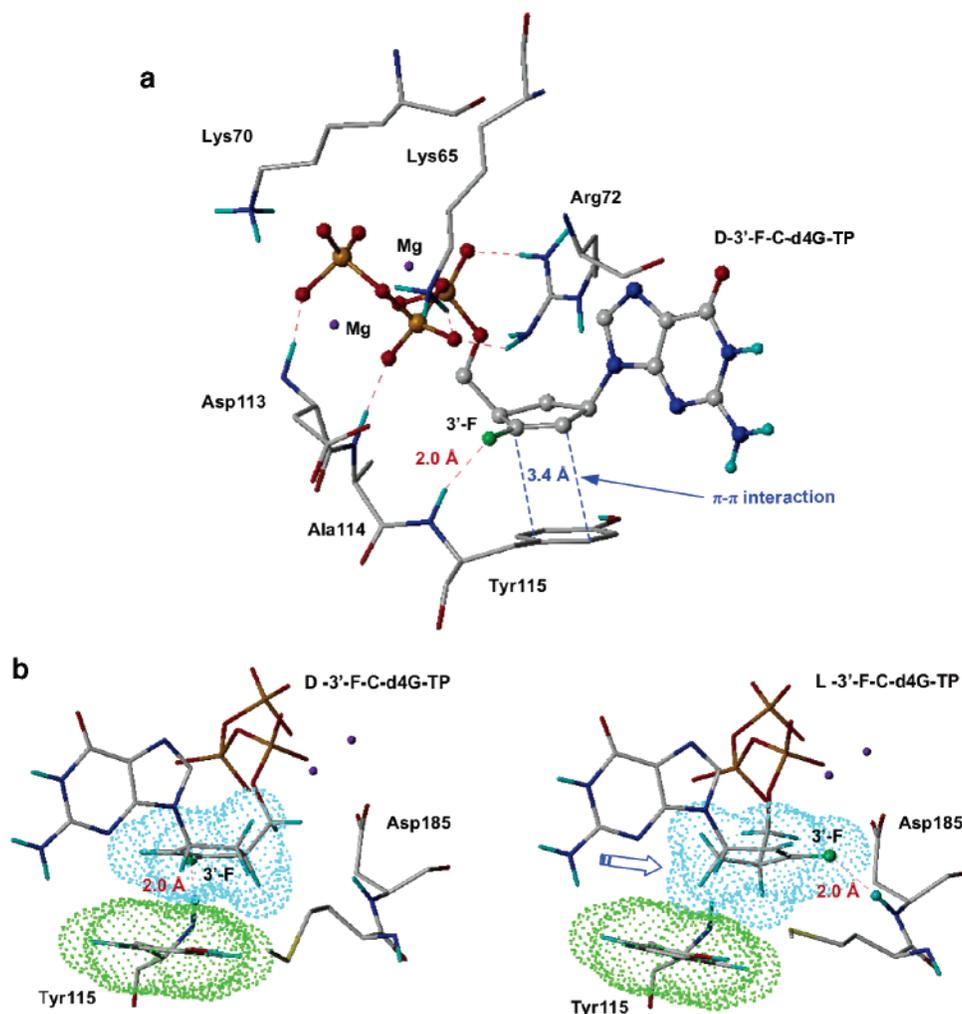
Figure 2. (a) Binding mode of D-3′-F-C-d4G-TP/HIV-RT$_{WT}$ complex. The triphosphate moiety is stabilized by hydrogen bonding with residues Lys65, Arg72, Lys70, Asp113, and Ala114. The other strong hydrogen bond is detected between the 3′-fluoro and the backbone amide of Tyr115. Also, the sugar ring is located right over the phenyl ring of Tyr115, forming a favorable hydrophobic interaction. (b) Comparing the binding mode of D- and L-3′-F-C-d4G-TP, a decreased hydrophobic interaction is observed for the latter due to the hydrogen bond of its 3′-fluoro with backbone amide of Asp185 pulling the sugar ring away from the top of Tyr115 (indicated by the blue arrow).

with the backbone amide of Tyr115 (Figure 2a). Stabilized by the combined effects of hydrophobic interaction and hydrogen bonding with Tyr115, D-3′-F-C-d4G is thus bound tightly with HIV-RT, reflecting a higher level of anti-HIV activity, although the initial kinase might have also played a significant role in determining the observed anti-HIV potency. In the case of L-3′-F-C-d4G, the 3′-fluorine is at a reasonable distance (2.0 Å) to interact in a hydrogen bond with backbone amide of Asp185 in lieu of Tyr115. However, we noticed that this interaction pulls the carbocyclic ring away from the Tyr115, which decreases the hydrophobic $\pi-\pi$ interaction (Figure 2b) and leads to a lower binding affinity (Table 5).

It has been well understood that the M184V mutation causes serious problems in positioning the L-nucleoside triphosphate at the active site by interfering the sugar ring with the bulky side chain of Val184. However, reports for the D-nucleosides, which confer significant cross-resistant to M184V, are rare. The antiviral activity of D-3′-F-C-d4G in the current report showed a marked decrease in the HIV-1$_{M184V}$ in comparison to the HIV-1$_{WT}$ (Tables 4 and 6). To understand the underlying mechanism, we further conducted the molecular dynamics studies of D-3′-F-C-d4G-TP, carbovir-TP and dGTP binding with the wild type HIV-RT as well as M184V mutant. According to our model, it is unlikely that the resistance of M184V mutant is caused by

Table 6. In Vitro Anti-HIV-1 Activity of D-3′-F-C-d4G against Wild Type (WT) and M184V Virus in Human PBM Cells Using Carbovir as Positive Control and Correlation with Calculated Energy of Complex (Inhibitor-TP)/HIV-RT after Molecular Dynamics Simulations

	xxBRU (WT)		M184V			
compd	EC$_{50}$ (μM)	E_{rel}[a] (kcal/mol)	EC$_{50}$ (μM)	E_{rel}[a] (kcal/mol)	FI[b]	ΔE_{rel}[c]
D-3-F-C-d4G **35**	0.098	−270.2[d]	3.8	84.9[d]	38.8	−355.1[d]
carbovir	0.087	−310.0[d]	0.20	−63.4[d]	2.3	−246.6[d]

[a] E_{rel} = (binding energy of inhibitor-TP) − (binding energy of natural 2′-dNTP). [b] FI is the fold increase (EC$_{50}$ HIV-1$_{M184V}$/EC$_{50}$ HIV-1$_{xxBRU}$). [c] $\Delta E_{rel} = E_{rel}(WT) − E_{rel}(M184V)$. [d] These values were calculated based on the molecular dynamics results (refer to Experimental Section).

the steric hindrance as we observed for the L-nucleosides. However, a steric clash was noticed between the Val184 side chain and the sugar ring of the final residue of the primer. In the D-3′-F-C-d4G/HIV-RT$_{M184V}$ complex, Val184 pushes the last residue of primer away and changes the conformation of adjacent Asp185. The conformational change of Asp185 propagates to magnesium atoms, Asp110, and triphosphate moiety of D-3′-F-C-d4G, through the strong electrostatic interactions between two magnesium atoms and nearby negatively charged residues such as Arg72 (Figure 3a). Although the D-3′-
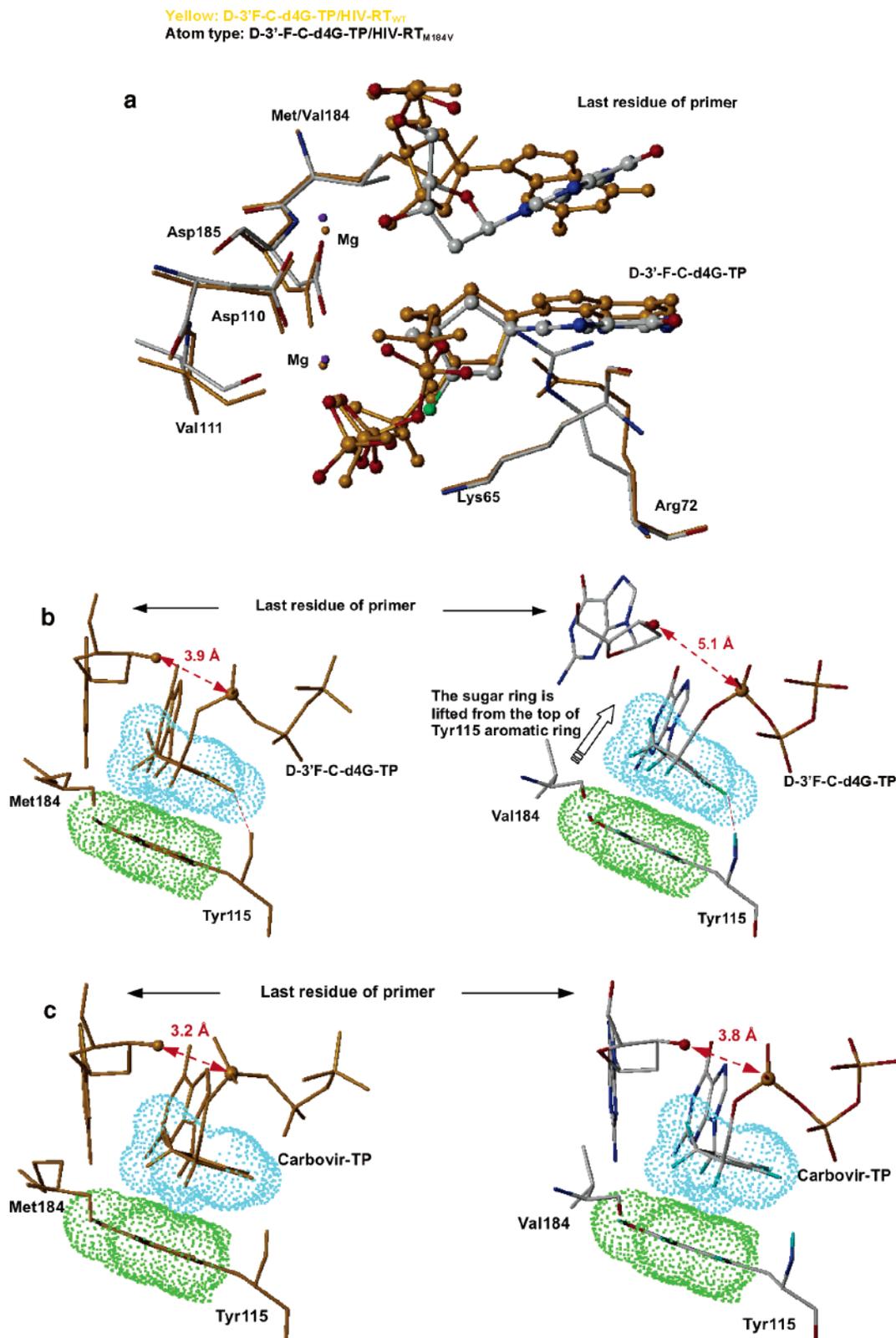
Figure 3. (a) Mutation of methionine to valine at position 184 induces conformational changes of the key residues inside the active site, such as Asp185, Asp110, Val111, and Arg72. (b) Comparison of the bind modes of D-3′-F-d4G-TP with HIV-RT$_{WT}$ (yellow color, left) and HIV-RT$_{M184V}$ (atom type, right). The propagated effect from the mutation on the codon 184 lifted the D-3′-F-d4G-TP from the surface of Tyr115 and causes a decrease of the hydrophobic interaction, resulting in a loss of binding affinity. Furthermore, the catalytic distance lengthened significantly (3.9 to 5.1 Å). (c) The comparison of the bind modes of carbovir-TP with HIV-RT$_{WT}$ (yellow color, left) and HIV-RT$_{M184V}$ (atom type, right). The binding mode is almost maintained in the mutant enzyme compared with the wild type enzyme.

F-C-d4G still maintains the hydrogen bond between the 3′-fluorine and backbone amide of Tyr115, inhibitor's sugar ring was lifted away from the surface of the Ty115 aromatic ring,

resulting in the loss of hydrophobic stacking interaction which may decrease the relative binding energy (Figure 3b right, Table 6). Furthermore, the primer/template reposition results in the

significant increase of the catalytic distance (3.9 Å in the wild type vs 5.1 Å in the M184V mutant) between the 3′-OH (last residue of the primer) and α-phosphate (D-3′-F-C-d4G-TP) (Figure 3b). Consequently, the incorporation of D-3′-F-C-d4G-MP into viral DNA chain in HIV-1$_{M184V}$ would be expected to be more difficult than in the HIV$_{WT}$, reflecting its decreased antiviral activity against the mutant. In the case of carbovir-TP, the relative binding energy to the M184V mutation also decreased, but to a less extent than the D-3′-F-C-d4G-TP, which is in accordance with the biological data (Table 6). Binding mode analysis of carbovir-TP/HIV-RT$_{M184V}$ complex revealed that the hydrophobic interaction was almost maintained and catalytic distance experienced only a small increase (3.2 Å in wild type vs 3.8 Å in M184V mutant, Figure 3c). These changes may not significantly affect the incorporation of carbovir-MP into viral DNA.

In summary, molecular modeling studies illustrated the important roles of $\pi-\pi$ interaction and the additional hydrogen bond in the binding affinity of D- and L-nucleosides in the HIV-RT active site. The cross-resistance of compound **35** to HIV-RT$_{M184V}$ may be partially due to the primer/template repositioning and resultant increased catalytic distance and the loss of hydrophobic interaction.

Experimental Section

General Methods. Melting points were determined on a Mel-temp II apparatus and were uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Mercury 400 spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR or Varian Inova 500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethylsilane as the internal standard. Chemical shifts (δ) are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or bs (broad singlet). UV spectra were recorded on a Beckman DU-650 spectrophotometer. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. High-resolution mass spectra were recorded on a Micromass Autospec high-resolution mass spectrometer. TLC was performed on Uni-plates (silica gel) purchased from Analtech Co. Column chromatography was performed using either silica gel-60 (220−440 mesh) for flash chromatography or silica gel G (TLC grade, >440 mesh) for vacuum flash column chromatography. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA.

Microwave-Assisted Synthesis. Reactions were run in the Discover reactor module (CEM Corporation) of focused microwaves with a magnetron operating at a frequency at 2.45 GHz and a maximum power output of 300 W. The thick-wall tube was heated in a closed cavity located inside the instrument with continuous stirring. The temperature was measured by an IR pyrometer inside the reactor.

(+)-(1R,2S,3S,4R)-2,3-Anhydro-1-O-benzyloxy-4-(O-triphenylmethyloxymethyl)-cyclopentane (2). To a suspension of epoxide **1**[14] (16.0 g, 72.6 mmol) in anhydrous CH$_2$Cl$_2$ were added DMAP (4.4 g, 36.3 mmol), triethylamine (15.1 mL, 109 mmol), and trityl chloride (30.4 g, 109 mmol) at room temperature. The reaction mixture was stirred for 24 h at room temperature and concentrated in vacuo. EtOAc/H$_2$O was added to the residue, and the organic layer was collected, dried over MgSO$_4$, and concentrated in vacuo. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:40 to 1:20) to give **2** as a white solid (30.6 g, 91%): mp 102−103 °C; [α]$^{24}_D$ +46.71° (c 1.47, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 7.39−7.24 (m, 20H), 4.59 (d, J = 2.5 Hz, 2H), 4.17 (t, J = 7.5 Hz, 1H), 4.19−4.16 (m, 1H), 3.54 (s, 1H), 3.45 (s, 1H), 3.14−3.15 (m, 1H), 2.96−2.99 (m, 1H), 2.57 (d, J = 7.0 Hz, 1H), 1.66−1.55 (m, 2H); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 143.9, 138.4, 128.7, 128.4, 127.9, 127.8, 127.7, 127.1, 86.8, 79.1, 71.7, 64.2, 57.9, 56.7, 39.2, 28.7. Anal. calcd for (C$_{32}$H$_{30}$O$_3$) C, H.

(+)-(1R,2S,4R)-1-O-Benzyloxy-2-hydroxyl-4-(O-triphenylm-ethyloxymethyl)-cyclopentane (3) and (+)-(1R,3S,4R)-1-O-Ben- zyloxy-3-hydroxyl-4-(O-triphenylmethyloxymethyl)-cyclopen-tane (4). Epoxide **2** (26.0 g, 56.2 mmol) was dissolved in a 1.0 M THF solution of Super-Hydride (180 mL, 180 mmol) at 0 °C. The suspension was allowed to warm up to room temperature and stirred for 30 min, and EtOAc/H$_2$O was added to quench the reaction. The organic layer was collected, and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:8) to give **3** (1.5 g, 6%) as a colorless oil and **4** (24.0 g, 92%) as a colorless oil. Compound **3**: ^{1}H NMR data is identical to the literature.[14] Compound **4**: [α]$^{25}_D$ +18.98° (c 0.93, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.43−7.22 (m, 20H), 4.48 (s, 2H), 4.01−3.96 (m, 2H), 3.25−3.22 (m, 1H), 2.96 (t, J = 8.5 Hz, 1H), 2.82 (d, J = 6.0 Hz, 1H), 2.49−2.44 (m, 1H), 2.17−2.04 (m, 2H), 1.94−1.89 (m, 1H), 1.44−1.25 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 144.1, 138.4, 128.7, 128.5, 127.9, 127.7, 127.6, 127.1, 86.8, 79.1, 76.6, 70.7, 66.2, 46.9, 40.7, 34.0. Anal. calcd for (C$_{32}$H$_{32}$O$_3$) C, H.

(−)-(2R,4R)-4-O-Benzyloxy-2-(O-triphenylmethyloxymethyl)-cyclopentan-1-one (5). To a solution of alcohol **4** (23.5 g, 50.6 mmol) in anhydrous CH$_2$Cl$_2$ (300 mL) were added 4 Å molecular sieve (40.5 g), pyridinium dichromate (37.6 g, 101.2 mmol), and acetic acid (4.4 mL, 76.0 mmol). After the mixture was stirred at room temperature for 3 h, Celite was added and stirred for another 30 min. The resulting brown slurry mixture was filtered over a Celite pad. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:50 to 1:20) to give ketone **5** (19.0 g, 81%) as a white solid: mp 106−108 °C; [α]$^{25}_D$ −59.32° (c 0.62, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.38−7.20 (m, 20H), 4.52 (s, 2H), 4.35 (t, J = 5.0 Hz, 1H), 3.48−3.45 (m, 1H), 3.21−3.19 (m, 1H), 2.67−2.62 (m, 1H), 2.57−2.38 (m, 3H), 2.17−2.10 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 184.7, 143.9, 138.1, 128.7, 128.5, 127.8, 127.7, 127.6, 127.0, 86.6, 74.8, 70.6, 62.1, 46.6, 45.6, 33.0. Anal. calcd for (C$_{32}$H$_{30}$O$_3$) C, H.

(+)-(2R,4R)-4-O-Benzyloxy-1,1-difluoro-2-(O-triphenylmethyloxymethyl)-cyclopentane (6). Ketone **5** (19.0 g, 41.1 mmol) was dissolved in neat diethyl aminosulfur trifluoride (DAST, 86.0 mL, 656.2 mmol) at room temperature. After being stirred at 40 °C for 36 h, the reaction mixture was diluted with 300 mL of CH$_2$Cl$_2$ and then slowly added into saturated NaHCO$_3$ (600 mL) solution. The organic layer was collected, and the aqueous layer was extracted with CH$_2$Cl$_2$. The combined organic layer was dried over MgSO$_4$ and concentrated in vacuo. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:100) to give **6** (13.5 g, 68%) as a pale yellow syrup: [α]$^{27}_D$ +3.00° (c 0.90, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.48−7.21 (m, 20H), 4.47 (s, 2H), 4.05 (s, 1H), 3.37−3.34 (m, 1H), 3.11−3.08 (m, 1H), 2.83−2.78 (m, 1H), 2.44−2.32 (m, 2H), 2.22−2.18 (m, 1H), 1.75−1.69 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 144.0, 138.1, 128.9, 130.7 (t, J = 252.5 Hz), 128.8, 128.5, 127.9, 127.8, 127.7, 127.6, 127.0, 86.8, 75.0, 70.8, 61.0 (d, J = 7.6 Hz), 44.7 (t, J = 21.5 Hz), 43.0 (t, J = 11.2 Hz), 34.3 (d, J = 6.1 Hz). Anal. calcd for (C$_{32}$H$_{30}$F$_2$O$_2$) C, H.

(−)-(2R,4R)-1,1-Difluoro-4-hydroxy-2-hydroxymethyl-cyclopentane (7). To a solution of **6** (12.7 g, 26.2 mmol) in anhydrous CH$_2$Cl$_2$ (300 mL) was added iodotrimethylsilane (11.2 mL, 78.6 mmol) at −20 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 6 h. An additional portion of iodotrimethylsilane (5.6 mL, 39.3 mmol) was added, and the mixture was stirred for another 8 h. The reaction was quenched with MeOH at −20 °C, and the mixture was carefully neutralized with solid NaHCO$_3$. The resulting brown mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:100 to 1:10) to give diol **7** (2.8 g, 70%) as a pale brown oil: [α]$^{25}_D$ −21.53° (c 0.90, MeOH); ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.46−4.43 (m, 1H), 3.83−3.76 (m, 2H), 2.82−2.70 (m, 1H), 2.48−2.38 (m, 1H), 2.28−2.19 (m, 1H), 2.02−1.90 (m, 2H), 1.77 (bs, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 131.8 (dd, J = 252.4 and 248.0 Hz) 68.6 (t, J = 5.2 Hz), 60.5 (d, J = 8.6 Hz), 46.2 (t, J = 21.5

Hz), 45.7 (t, $J = 23.9$ Hz), 36.3 (d, $J = 5.8$ Hz). Anal. calcd for ($C_6H_{10}F_2O_2$) C, H.

(−)-(2R,4R)-2-(O-tert-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-hydroxy-cyclopentane (8). To a solution of diol **7** (2.6 g, 17.1 mmol) and imidazole (1.9 g, 27.4 mmol) in anhydrous CH_2Cl_2 (100 mL) was slowly added *tert*-butyldiphenylsilane chloride (4.8 mL, 18.8 mmol) at 0 °C during 1 h. The resulting mixture was stirred at 0 °C for another 45 min and quenched with MeOH. The mixture was concentrated in vacuo, and the residue was dissolved in EtOAc/H_2O. The organic layer was collected, and the aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:20 to 1:4) to give **8** (4.9 g, 74%) as a colorless oil: $[\alpha]^{27}_d$ −14.62° (*c* 0.90, $CHCl_3$); ¹H NMR (500 MHz, $CDCl_3$) δ 7.67−7.37 (m, 10H), 4.44 (s, 1H), 3.76 (ddd, $J = 450.0$, 10.5 and 5.5 Hz, 2H), 2.81−2.69 (m, 1H), 2.50−2.40 (m, 1H), 2.24−2.16 (m, 1H), 2.04−1.95 (m, 2H), 1.05 (s, 9H); ¹³C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.4, 133.4, 131.1, 129.7, 127.7, 68.7 (d, $J = 6.7$ Hz), 61.5 (d, $J = 8.1$ Hz), 46.4 (t, $J = 22.4$ Hz), 45.8 (t, $J = 23.9$ Hz), 37.1 (d, $J = 5.2$ Hz), 26.8, 19.2. Anal. calcd for ($C_{22}H_{28}F_2O_2Si$) C, H.

(−)-(2R,4R)-2-(O-tert-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-[(methylsulfonyl)oxy]cyclopentane (9). Methanesulfonyl chloride (0.4 mL, 5.12 mmol) in 10 mL of anhydrous CH_2Cl_2 (10 mL) was slowly added to a solution of alcohol **8** (1.00 g, 2.56 mmol) and triethylamine (1.4 mL, 10.2 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C. The reaction mixture was allowed to warm up to room temperature and kept for 4 h. After the solvent was removed in vacuo, the residue was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:20 to 1:10) to give **9** (1.17 g, 98%) as a colorless oil: $[\alpha]^{25}_D$ −19.66° (*c* 0.32, $CHCl_3$); ¹H NMR (500 MHz, $CDCl_3$) δ 7.67−7.38 (m, 10H), 5.26 (m, 1H), 3.76 (d, $J = 5.5$ Hz, 2H), 3.03 (s, 3H), 2.74−2.50 (m, 3H), 2.36−2.32 (m, 1H), 2.18−2.12 (m, 1H), 1.05 (s, 9H); ¹³C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.0 (d, $J = 2.0$ Hz), 129.9, 129.8 (t, $J = 250.9$ Hz), 127.8, 77.8 (dd, $J = 3.75$ and 7.6 Hz), 61.0 (dd, $J = 2.9$ and 4.8 Hz), 46.4 (dd, $J = 21.5$ and 23.9 Hz), 43.7 (t, $J = 26.8$ Hz), 34.8, 34.7 (t, $J = 4.8$ Hz), 26.8, 19.2. HR-MS calcd for ($C_{23}H_{30}NF_2O_4$-SSi + H)⁺ 469.1680, found 469.1662.

(+)-(2R,4S)-4-Azido-2-(O-tert-butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentane (10). Compound **9** (1.17 g, 2.5 mmol) was dissolved in anhydrous DMF (45 mL) and heated at 130 °C for 1.5 h. After the solvent was removed in vacuo, the residue was dissolved in EtOAc and washed with water. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:200 to 1:100) to give **10** (0.97 g, 93%) as a colorless oil: $[\alpha]^{26}_D$ +4.2° (*c* 0.51, $CHCl_3$); ¹H NMR (500 MHz, $CDCl_3$) δ 7.67−7.38 (m, 10H), 3.96 (m, 1H), 3.78 (ddd, $J = 38.0$, 11.0 and 5.5 Hz, 2H), 2.56−2.34 (m, 1H), 2.21−2.10 (m, 1H), 1.82−1.75 (m, 1H), 2.04−1.95 (m, 2H), 1.06 (s, 9H); ¹³C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.2 (d, $J = 3.4$ Hz), 129.8, 128.9 (dd, $J = 248.5$ and 254.2 Hz), 127.8, 61.2 (dd, $J = 7.1$ and 2.9 Hz), 56.4 (dd, $J = 6.1$ and 3.8 Hz), 47.2 (dd, $J = 23.4$ and 21.0 Hz), 41.9 (t, $J = 24.8$ Hz), 32.9 (t, $J = 1.5$ Hz), 26.8, 19.2. HR-MS calcd for ($C_{22}H_{27}F_2N_3OSi$ + H)⁺ 416.1970, found 416.2022.

(−)-(2R,4S)-2-(O-tert-Butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentanamine (11). A suspension of azido compound **10** (0.97 g, 2.33 mmol) and 10% Pd/C (330 mg) in absolute EtOH was shaken under 30 psi of H_2 at room temperature for 2.0 h. Celite was added into the solution, and the slurry was filtered through a Celite pad. The volatile was removed in vacuo, and the residue was purified by column chromatography on a silica gel (MeOH: CH_2Cl_2 = 1:50 to 1:10) to give **11** (0.86 g, 95%) as a colorless oil: $[\alpha]^{26}_D$ −9.98° (*c* 0.79, MeOH); ¹H NMR (500 MHz, $CDCl_3$) δ 7.68−7.36 (m, 10H), 3.78 (ddd, $J = 55.0$, 10.0 and 5.0 Hz, 2H), 3.46−3.39 (m, 1H), 2.51−2.28 (m, 3H), 1.93−1.82 (m, 1H), 1.45−1.39 (m, 3H), 1.05 (s, 9H); ¹³C NMR (125 MHz, $CDCl_3$) δ 135.6

(d, $J = 2.4$ Hz), 133.4 (d, $J = 1.2$ Hz), 130.0 (dd, $J = 252.8$ and 3.2 Hz), 129.7, 127.8, 61.6 (dd, $J = 7.6$ and 1.9 Hz), 48.0 (dd, $J = 23.4$ and 20.2 Hz), 47.9 (dd, $J = 6.1$ and 4.2 Hz), 45.8 (t, $J = 22.4$ Hz), 37.3 (d, $J = 4.8$ Hz) 26.8, 19.3. HR-MS calcd for ($C_{22}H_{29}F_2NOSi$ + H)⁺ 390.2065, found 390.2030. Anal. calcd for ($C_{22}H_{29}F_2NOSi$) C, H, N.

(+)-(1S,4R)-9-[6-(O-tert-Butyldiphenylsilyloxymethyl)-2,3-dideoxy-3,3-difluoro-cyclopentanyl]uracil (13). To a suspension of silver cyanate (810 mg, 5.4 mmol) in anhydrous benzene (20 mL) was added β-methoxyacryloyl chloride (650 mg, 5.4 mmol). The mixture was heated under reflux for 30 min and cooled to room temperature. The supernatant solution was added into the solution of amine **11** (700 mg, 1.8 mmol) in anhydrous THF (30 mL) at −30 °C during 15 min. The mixture was allowed to gradually warm up to room temperature and kept overnight. After the solvent was removed in vacuo, the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:3 to 1:1) to give crude **12** (600 mg) as a yellow syrup which was directly used for the next step. Crude compound **12** (600 mg) was dissolved in 1,4-dioxane/ethanol (20 mL/20 mL) and treated with 28% solution of ammonium hydroxide (20 mL) in a steel bomb at 90−100 °C for 17 h. After the solvent was removed in vacuo, the residue was purified by column chromatography on a silica gel (MeOH: CH_2Cl_2 = 1:200 to 1:100) to give **13** (360 mg, 41%) as a pale yellow syrup: $[\alpha]^{26}_d$ +13.12° (*c* 0.31, $CHCl_3$); UV (H_2O) λ_{max} 266.0 (MeOH); ¹H NMR (500 MHz, $CDCl_3$) δ 9.22 (s, 1H), 7.67−7.38 (m, 10H), 7.22 (d, $J = 8.5$ Hz, HzH1H), 5.68 (d, $J = 8.0$ Hz, 1H), 5.26−5.19 (m, 1H), 3.88 (dtd, $J = 11.0$, 10.0 and 5.0 Hz, 2H), 2.70−2.60 (m, 1H), 2.252−2.44 (m, 2H), 2.27−2.16 (m, 1H), 1.79−1.70 (m, 1H), 1.07 (s, 9H); ¹³C NMR (125 MHz, CD_3OD) δ 162.8, 150.9, 140.0 (d, $J = 2.0$ Hz), 135.6, 133.1, 133.0, 129.9, 128.3 (t, $J = 250.9$ Hz), 127.9, 103.6, 61.0 (d, $J = 5.2$ Hz), 49.6, 47.0 (t, $J = 21.9$ Hz), 40.7 (t, $J = 24.9$ Hz), 32.3 (d, $J = 4.2$ Hz), 26.8, 19.3. HR-MS calcd for ($C_{26}H_{30}F_2N_2O_3Si$ + H)⁺ 485.2072, found 485.2169 Anal. calcd for ($C_{26}H_{30}F_2N_2O_3Si$) C, H, N.

(+)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-(O-tert-butyldiphenylsilyloxymethyl)-cyclopentanyl]cytosine (14). To a solution of uracil derivative **13** (360 mg, 0.74 mmol) in anhydrous acetonitrile (25 mL) were added 2,4,6-triisopropyl benzenesulfonyl chloride (450 mg, 1.48 mmol), 4-(dimethylamino)pyridine (90.4 mg, 0.74 mmol), and triethylamine (0.42 mL, 3.0 mmol) at 0 °C. After the mixture was stirred at room temperature for 12 h, a 28% solution of ammonium hydroxide (15 mL) was added to the brown mixture and stirred at room temperature for another 12 h. The reaction mixture was concentrated in vacuo, and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:30) to give **14** (300 mg, 84%) as a white solid: mp 250−252 °C; $[\alpha]^{24}_D$ +15.86° (*c* 0.31, MeOH); UV (MeOH) λ_{max} 272.0 nm; ¹H NMR (500 MHz, CD_3OD) δ 7.59−7.30 (m, 10H), 7.44 (d, $J = 7.0$ Hz, 1H), 5.75 (d, $J = 7.5$ Hz, 1H), 5.00−4.92 (m, 1H), 3.88 (ddd, $J = 56.0$, 10.5 and 6.0 Hz, 2H), 2.59−2.45 (m, 2H), 2.30−2.19 (m, 2H), 1.78−1.71 (m, 1H), 0.96 (s, 9H); ¹³C NMR (125 MHz, CD_3-OD) δ 165.8, 157.4, 142.0, 135.4, 133.1, 133.0, 129.7, 128.8 (t, $J = 250.9$ Hz), 127.5, 95.2, 61.1 (d, $J = 8.1$ Hz), 52.0 (d, $J = 5.8$ Hz), 40.2 (t, $J = 24.8$ Hz), 32.0 (d, $J = 4.2$ Hz), 25.9, 18.6. HR-MS calcd for ($C_{26}H_{31}F_2N_3O_2Si$ + H)⁺ 484.2232, found 484.2212. Anal. calcd for ($C_{26}H_{31}F_2N_3O_2Si$) C, H, N.

(+)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcyclopentanyl]cytosine (15). To a solution of **14** (300 mg, 0.62 mmol) in MeOH (2 mL) was added 3 N HCl (2 mL). After being stirred at room temperature for 17 h, the resulting mixture was coevaporated with EtOH, and the residue was purified by column chromatography on a silica gel (MeOH:CH_2Cl_2 = 1:15) to give **15** (110 mg, 72%) as a white solid: mp 134−136 °C; $[\alpha]^{23}_d$ +4.07° (*c* 0.22, MeOH); UV (H_2O) λ_{max} 275.0 nm (MeOH); ¹H NMR (400 MHz, CD_3OD) δ 7.72 (d, $J = 8.0$ Hz, 1H), 5.95 (d, $J = 7.0$ Hz, 1H), 5.14−5.07 (m, 1H), 3.88 (ddd, $J = 47.0$, 11.5 and 5.5 Hz, 2H), 2.70−2.35 (m, 4H), 1.97−1.90 (m, 1H); ¹³C NMR (100 MHz, CD_3-OD) δ 165.8, 157.4, 142.0, 128.8 (t, $J = 249.2$ Hz), 95.2, 59.0 (d, $J = 7.6$ Hz), 52.0 (t, $J = 7.6$ Hz), 40.1 (t, $J = 25.1$ Hz), 32.0 (d,

J = 3.8 Hz). HR-MS calcd for $(C_{10}H_{13}F_2N_3O_2 + H)^+$ 246.1054, found 246.0975 Anal. calcd for $(C_{10}H_{13}F_2N_3O_2 \cdot 0.65H_2O)$ C, H, N.

(+)-(1S,4R)-9-[2,3-Dideoxy-2,3-didehydro-3,3-fluoro-6-hydroxymethylcyclopent-2-enyl]cytosine (18). To a suspension of **15** (110 mg, 0.45 mmol) in anhydrous THF:1,4-dioxane (10 mL: 10 mL) cosolvent was added potassium *tert*-butoxide (121 mg, 1.0 mmol). The reaction mixture was stirred at 90 °C for 9 h. The yellow suspension was filtered through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:30 to 1:15) to give **18** (35 mg, 35%) as a white solid: mp 244−248 °C; $[\alpha]^{25}_D$ +127.49° (*c* 0.36, MeOH); UV (H$_2$O) λ_{max} 284.0 nm (ϵ 17115, pH 2), 274.0 nm (ϵ 11975, pH 7), 274.0 nm (ϵ 11680, pH 11); ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.83 (d, J = 7.5 Hz, HzH1H), 5.90 (d, J = 7.5 Hz, 1H), 5.62−5.61 (m, 1H), 5.19 (s, 1H), 3.70 (ddd, J = 132.5, 11.5 and 3.5 Hz, 2H), 2.95−2.93 (m, 1H), 2.86−2.79 (m, 1H), 1.72−1.69 (m, 1H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 166.1, 166.0 (d, J = 282.8 Hz), 157.6, 142.5, 103.3 (d, J = 13.0 Hz), 94.3, 59.8, 55.9 (d, J = 12.2 Hz), 42.9 (d, J = 19.0 Hz), 31.7 (d, J = 6.1 Hz). Anal. calcd for $(C_{10}H_{12}F_1N_3O_2)$ C, H, N.

(+)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcyclopentanyl]thymine (17). To a suspension of silver cyanate (400 mg, 2.7 mmol) in anhydrous benzene (8 mL) was added β-methoxy-α-methacryloyl chloride (360 mg, 2.7 mmol). The mixture was heated under reflux for 30 min and cooled to room temperature. The supernatant solution was added into the solution of amine **11** (350 mg, 0.89 mmol) in anhydrous THF (8 mL) at −30 °C during 15 min. The mixture was allowed to gradually warm up to room temperature and kept overnight. After removing the solvent in vacuo, the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:3 to 1:1) to give crude **16** (360 mg) as a yellow syrup which was directly used for the next step. Crude compound **16** (360 mg) was dissolved in 1,4-dioxane (60 mL) and treated with 3 N HCl (15 mL) at the refluxed temperature for 2.5 h. After the solvent was removed in vacuo, the residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:50 to 1:20) to give **17** (145 mg, 63%) as a white solid: mp 140−142 °C; $[\alpha]^{23}_D$ +3.9° (*c* 0.15, MeOH); UV (H$_2$O) λ_{max} 270.0 nm; ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.52 (s, 1H), 5.07−4.98 (m, 1H), 3.76 (ddd, J = 33.2, 10.8 and 5.2 Hz, 2H), 2.63−2.32 (m, 4H), 1.98−1.89 (m, 4H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 164.8, 151.4, 137.6, 128.7 (dd, J = 250.8 and 248.5 Hz), 110.6, 58.9 (dd, J = 7.6 and 2.3 Hz), 50.8 (t, J = 6.8 Hz), 39.5 (t, J = 25.1 Hz), 31.3 (d, J = 3.8 Hz), 11.0. Anal. calcd for $(C_{11}H_{14}F_2N_2O_3)$ C, H, N.

(+)-(1S,4R)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxymethylcyclopent-2-enyl]thymine (19). Compound **17** (140 mg, 0.54 mmol) was converted to thymine derivative **19** (60 mg, 46%) as a white solid using the same procedure as for **18**: mp 182−184 °C (dec); $[\alpha]^{26}_D$ +23.99° (*c* 0.26, MeOH); UV (H$_2$O) λ_{max} 272.0 nm (ϵ 14258, pH 2), 272.0 nm (ϵ 14240, pH 7), 271.0 nm (ϵ 11651, pH 11); ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.69 (s, 1H), 5.58−5.53 (m, 1H), 5.15 (s, 1H), 3.69 (ddd, J = 121.6, 11.6 and 3.2 Hz, 2H), 2.91−2.90 (m, 1H), 2.78−2.70 (m, 1H), 1.76−1.70 (m, 1H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 165.2, 166.0 (d, J = 282.7), 151.6, 138.2, 109.7, 103.4 (d, J = 13.7 Hz), 59.6 (d, J = 1.5 Hz), 54.7 (d, J = 12.2 Hz), 42.7 (d, J = 18.3 Hz), 30.9 (d, J = 6.1 Hz), 11.0. Anal. calcd for $(C_{11}H_{13}FN_2O_3 \cdot 0.1H_2O)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-(O-tert-butyldiphenylsilyloxymethyl)-cyclopentanyl]adenine (21). Triphenylphosphine (2.12 g, 8.1 mmol) and diisopropyl azodicarboxylate (1.59 mL, 8.1 mmol)) were dissolved in anhydrous THF:1,4-dioxane (14 mL:7 mL) cosolvent and cooled to 0 °C. The resulting yellowish suspension was further cooled to −78 °C. 6-Chloropurine (1.25 g, 8.1 mmol) and a solution of alcohol **8** (630 mg, 1.61 mmol) in THF (14 mL) were added successively. The resulting mixture was kept at −78 °C for 0.5 h and then stirred at room temperature for 24 h. MeOH was added to quench the reaction, and the mixture was evaporated in vacuo. The residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:10) to give **20** as a crude product, which was treated with methanolic ammonia in a steel bomb at 100 °C for 24 h. After evaporation in vacuo, the

residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:50 to 1:20) to give **21** as a colorless syrup (305 mg, 37% from **8**): $[\alpha]^{25}_d$ −10.89° (*c* 0.45, CHCl$_3$); UV (MeOH) λ_{max} 260.0; ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.34 (s, 1H), 7.84 (s, 1H), 7.68−7.38 (m, 10H), 5.07 (m, 1H), 3.92 (ddd, J = 31.0, 10.5 and 5.0 Hz, 2H), 2.91−2.81 (m, 1H), 2.73−2.56 (m, 3H), 2.28−2.22 (m, 1H), 2.06 (bs, 1H), 1.08 (s, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 155.6, 153.0, 150.2, 138.2, 135.6, 135.6, 133.2, 133.1, 129.9, 128.4 (dd, J = 252.4 and 250.0 Hz), 127.8, 120.0, 61.1 (d, J = 5.8 Hz), 49.8 (t, J = 4.8 Hz), 47.5 (t, J = 21.5 Hz), 33.7 (d, J = 17.0 Hz), 26.8, 19.3. Anal. calcd for $(C_{27}H_{31}F_2N_5-OSi)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcyclopentanyl]adenine (22). Compound **21** (300 mg, 0.59 mmol) was dissolved in MeOH (5 mL) and treated with 3 N HCl (10 mL) at room temperature for 16 h. After the mixture was neutralized with solid NaHCO$_3$, the resulting suspension was filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:50 to 1:20) to give **22** (140 mg, 88%) as a white solid: mp 156−158 °C; $[\alpha]^{25}_d$ −9.86° (*c* 0.56, MeOH); UV (H$_2$O) λ_{max} 260.0 (MeOH); ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.28 (s, 1H), 8.24 (s, 1H), 5.13 (m, 1H), 3.86 (ddd, J = 50.0, 11.5 and 6.0 Hz, 2H), 2.89−2.77 (m, 2H), 2.71−2.57 (m, 2H), 2.36−2.30 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 156.0, 152.3, 149.4, 139.5, 128.9 (dd, J = 251.8 and 248.0 Hz), 119.0, 59.1 (dd, J = 8.1 and 1.9 Hz), 50.4 (dd, J = 7.6 and 4.4 Hz), 41.1 (t, J = 25.8 Hz), 33.0 (d, J = 3.9 Hz). Anal. calcd for $(C_{11}H_{13}F_2N_5O)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxymethylcyclopent-2-enyl]adenine (26). Compound **22** (60 mg, 0.22 mmol) was converted to adenine derivative **26** (27 mg, 49%) as a white solid using the same procedure as for **18**: mp 225 °C (dec); $[\alpha]^{29}_D$ −43.80° (*c* 0.18, DMSO); UV (MeOH) λ_{max} 261.0 nm (ϵ 17931, pH 2), 261.0 nm (ϵ 17780, pH 7.4), 261.0 nm (ϵ 18556, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.31 (s, 1H), 8.24 (s, 1H), 5.69−5.64 (m, 1H), 5.44 (m, 1H), 3.75 (ddd, J = 126.5, 11.0 and 4.0 Hz, 2H), 3.09−3.04 (m, 1H), 3.02−3.00 (m, 1H), 2.05−2.00 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 166.3 (d, J = 283.2 Hz), 156.0, 152.2, 148.8, 139.8, 118.9, 103.2 (d, J = 13.8 Hz), 60.1, 54.0 (d, J = 12.4 Hz), 43.2 (d, J = 18.6 Hz), 32.2 (d, J = 6.2 Hz). Anal. calcd for $(C_{11}H_{12}FN_5O)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-2,2-difluoro-6-hydroxymethylcyclopentanyl]hypoxanthine (23). A crude 6-chloropurine analogue **20** (850 mg) was treated with 85% formic acid (40 mL) at 90 °C for 3 h. After the volatile was completely removed in vacuo, the residue was dissolved in concentrated ammonium hydroxide (35%) and stirred at room temperature overnight. The solution was evaporated in vacuo, and the residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:30 to 1:15) to give **23** (93 mg, 39% from **8**) as a white solid: mp 244−246 °C; $[\alpha]^{24}_D$ −10.08° (*c* 0.23, MeOH); UV (MeOH) λ_{max} 248.0 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.22 (s, 1H), 8.09 (s, 1H), 5.18−5.10 (m, 1H), 3.84 (ddd, J = 53.5, 11.5 and 6.0 Hz, 2H), 2.93−2.76 (m, 2H), 2.70−2.58 (m, 2H), 2.36−2.29 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 157.6, 148.8, 145.0, 139.0, 128.8 (dd, J = 251.9 and 248.0 Hz), 124.3, 59.1 (d, J = 6.2 Hz), 50.7 (dd, J = 7.1 and 4.2 Hz), 41.3 (t, J = 25.8 Hz), 33.1 (d, J = 4.2 Hz). Anal. calcd for $(C_{11}H_{12}F_2N_4O_2)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxymethylcyclopent-2-enyl]hypoxanthine (27). Compound **23** (22 mg, 0.081 mmol) was converted to inosine derivative **27** (10 mg, 49%) as a white solid using the same procedure as for **18**: mp 226−230 °C; $[\alpha]^{27}_D$ −33.05° (*c* 0.20 MeOH); UV (H$_2$O) λ_{max} 249.0 nm (ϵ 12220, pH 2), 249.0 nm (ϵ 12820, pH 7), 255.0 nm (ϵ 13552, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.26 (s, 1H), 8.08 (s, 1H), 5.66−5.72 (m, 1H), 5.43 (s, 1H), 3.75 (ddd, J = 124.5, 11.5 and 4.0 Hz, 2H), 3.03−3.09 (m, 1H), 2.97 (dt, J = 14.0 and 9.5 Hz, 1H), 2.05−2.00 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 166.4 (d, J = 283.9 Hz), 157.6, 148.4, 145.1, 139.2, 123.9, 103.1 (d, J = 14.8 Hz), 60.0, 54.2 (d, J = 12.9 Hz), 43.3 (d, J = 18.6 Hz), 32.4 (d, J = 5.8 Hz). Anal. calcd for $(C_{11}H_{11}FN_4O_2 \cdot 0.1H_2O)$ C, H, N.

(−)-(1S,4R)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcy-clopentanyl]guanine (25). A solution of alcohol **8** (600 mg, 1.54 mmol), tripheylphosphine (1.61 g, 6.1 mmol), and 6-chloro-N^2-isobutyrylpurine (1.47 g, 6.1 mmol) in anhydrous THF (20 mL) was cooled to 0 °C, and then diisopropyl azodicarboxylate (1.2 mL, 6.1 mmol) was slowly added during 2 h. The reaction mixture was allowed to warm up to room temperature and stirred for 4 h. The clear yellowish solution was concentrated in vacuo, and the residue was purified by column chromatography on a silica gel (EtOAc:hexanes = 1:10 to 1:1) to give the corresponding nucleoside **24** as a crude product, which was treated with 85% formic acid (30 mL) at 90 °C for 4 h. After the volatile was completely removed, the residue was further treated with methanolic ammonia at room temperature for 24 h. After the concentration in vacuo, the residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:20 to 1:10) to give **25** (80 mg, 18% from **8**) as a white solid: mp: 253 °C (dec); [α]$^{27}_d$ −9.30° (c 0.25, MeOH); UV (MeOH) λ_{max} 253.0 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.86 (s, 1H), 3.83 (ddd, J = 50.5, 11.5 and 5.5 Hz, 2H), 2.81−2.72 (m, 2H), 2.61−2.51 (m, 2H), 2.30−2.22 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 158.0, 153.7, 151.8, 136.4, 128.9 (dd, J = 252.2 and 248.0 Hz), 116.5, 59.1 (dd, J = 8.1 and 2.4 Hz), 50.0 (dd, J = 7.1 and 3.4 Hz), 41.1 (t, J = 25.8 Hz), 32.8 (d, J = 3.4 Hz). HR-MS calcd for (C$_{11}$H$_{13}$N$_5$F$_2$O$_2$ + H)$^+$ 286.1116, found 286.1137.

(+)-(1S,4R)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]guanine (28). To a suspension of **25** (70 mg, 0.25 mmol) in anhydrous DMF (8 mL) was added potassium *tert*-butoxide (120 mg, 1.0 mmol). The reaction mixture was stirred at 70 °C for 24 h. The yellow suspension was filtered through a short silica gel pad and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on a silica gel (MeOH:CH$_2$Cl$_2$ = 1:20 to 1:10) to give **28** (30 mg, 45%) as a white solid: mp 224−227 °C (dec); [α]$^{27}_d$ +40.13° (c 0.11, MeOH); UV (H$_2$O) λ_{max} 253.0 nm (ε 13343, pH 2), 251.0 nm (ε 13635, pH 7), 260.0 and 268.0 nm (ε 11470 and 11893, respectively, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.92 (s, 1H), 5.52−5.46 (m, 1H), 5.37 (s, 1H), 3.74 (ddd, J = 114.0, 11.0 and 4.0 Hz, 2H), 3.05−3.00 (m, 1H), 2.90 (dt, J = 14.0 and 9.0 Hz, 1H), 2.01−1.96 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 165.9 (d, J = 282.9 Hz), 158.0, 153.8, 151.2, 136.6, 116.2, 103.1 (d, J = 13.9 Hz), 60.1, 53.2 (d, J = 12.9 Hz), 43.2 (d, J = 19.1 Hz), 32.2 (d, J = 5.6 Hz). Anal. calcd for (C$_{11}$H$_{12}$FN$_5$O$_2$·1.1H$_2$O) C, H, N.

(+)-(2S,4S)-2-(O-tert-Butyldiphenylsilyloxymethyl)-1,1-difluoro-4-hydroxy-cyclopentane (29): [α]$^{24}_d$ +14.97° (c 0.83, CHCl$_3$); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.67−7.66 (m, 4H), 74.5−7.37 (m, 11H), 4.44 (bs, 1H), 3.80 (dd, J = 5.0 and 10.5 Hz, 1H), 3.72 (dd, J = 6.0 and 10.5 Hz, 1H), 2.75 (m, 1H), 2.45 (m, 1H), 2.20 (q, J = 14.0 Hz, 1H), 2.02−1.98 (m, 2H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 135.6, 133.3 (d, J = 3.9 Hz), 133.2, 131.2, 129.8 (d, J = 1.4 Hz), 129.1, 127.8 (d, J = 1.4 Hz), 68.8, 61.5, 46.4 (t, J = 21.4 Hz), 46.0 (t, J = 28.1 Hz), 37.1 (d, J = 5.3 Hz), 26.8, 19.3. Anal. calcd for (C$_{22}$H$_{28}$F$_2$O$_2$Si) C, H, N.

(+)-(2S,4R)-2-(O-tert-Butyldiphenylsilyloxymethyl)-1,1-difluoro-cyclopentanamine (30): [α]$^{26}_D$ +10.02° (c 0.68, MeOH); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.68−7.37 (m, 10H), 3.86−3.70 (m, 2H), 3.49−3.39 (m, 1H), 2.52−2.28 (m, 3H), 1.94−1.79 (m, 1H), 1.45−1.39 (m, 3H), 1.05 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 135.6 (d, J = 2.4 Hz), 133.4, 130.0 (dd, J = 253.1 and 249.2 Hz), 129.7, 127.7, 61.6 (dd, J = 7.7 and 2.3 Hz), 48.1 (m), 48.0 (dd, J = 22.9 and 21.3 Hz), 46.1 (t, J = 22.9 Hz), 37.3 (d, J = 4.6 Hz) 26.8, 19.2. Anal. calcd for (C$_{22}$H$_{29}$F$_2$NOSi·0.2H$_2$O) C, H, N.

(+)-(1R,4S)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcy-clopentanyl]adenine (31): mp 158−160 °C; [α]$^{25}_D$ +9.50° (c 0.33, MeOH); UV (H$_2$O) λ_{max} 260.0 (MeOH); ^{1}H NMR (500 MHz, CD$_3$-OD) δ 8.24 (s, 1H), 5.12 (m, 1H), 3.92 (ddd, J = 50.0, 11.5 and 5.5 Hz, 2H), 2.85 (m, 2H), 2.65 (m, 2H), 2.32 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 155.9, 152.3, 149.3, 139.5, 128.7 (dd, J = 251.6 and 248.0 Hz), 119.0, 59.2 (t, J = 5.8 Hz), 50.3, 41.1 (t, J = 25.8 Hz), 32.9. Anal. calcd for (C$_{11}$H$_{13}$F$_2$N$_5$O) C, H, N.

(+)-(1R,4S)-9-[2,3-Dideoxy-3-difluoro-6-hydroxymethylcyclo-pentanyl]guanine (32): mp 258 °C (dec); [α]$^{28}_d$ +9.29° (c 0.15,

MeOH); UV (H$_2$O) λ_{max} 254.0 nm (ε 10631, pH 2), 252.0 nm (ε 11008, pH 7), 256.0 nm (ε 9234, pH 11); mp >250 °C; ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.83 (s, 1H), 3.79 (ddd, J = 51.0, 14.5 and 6.5 Hz, 2H), 2.77−2.70 (m, 2H), 2.57−2.54 (m, 2H), 2.32−2.21 (m, 1H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 158.0, 153.7, 151.8, 136.4, 128.9 (dd, J = 251.5 and 246.9 Hz), 116.5, 59.1 (d, J = 8.4 Hz), 49.9 (d, J = 3.8 Hz), 41.0 (t, J = 25.9 Hz), 32.8 (d, J = 3.8 Hz). Anal. calcd for (C$_{11}$H$_{13}$F$_2$N$_5$O$_2$·0.3H$_2$O) C, H, N.

(+)-(1R,4S)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcy-clopentanyl]hypoxanthine (33): mp 227 °C; [α]$^{23}_D$ +9.97° (c 0.45, MeOH); UV (MeOH) λ_{max} 247.0 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.25 (s, 1H), 8.09 (s, 1H), 5.14 (m, 1H), 3.84 (ddd, J = 55.0, 11.5 and 6.0 Hz, 2H), 2.82 (m, 2H), 2.65 (m, 2H), 2.32 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 157.6, 148.8, 145.0, 139.0, 128.7 (dd, J = 251.5 and 247.5 Hz), 124.3, 59.0 (dd, J = 8.0 and 2.4 Hz), 50.7, 41.3 (t, J = 25.9 Hz), 33.1 (d, J = 4.8 Hz). Anal. calcd for (C$_{11}$H$_{12}$F$_2$N$_4$O$_2$) C, H, N.

(+)-(1R,4S)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]adenine (34): mp 218−220 °C (dec); [α]$^{22}_d$ +41.00° (c 0.13, DMSO); UV (MeOH) λ_{max} 260.0 nm (ε 10925, pH 2), 261.0 nm (ε 10807, pH 7), 261.0 nm (ε 11780, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.31 (s, 1H), 8.24 (s, 1H), 5.66 (br, 1H), 5.44 (s, 1H), 3.75 (ddd, J = 130.0, 11.0 and 3.5 Hz, 2H), 3.05 (br, 1H), 2.98 (m, 1H), 2.00 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 166.2 (d, J = 287.5 Hz), 155.9, 152.1, 148.8, 139.8, 118.8, 103.2 (d, J = 14.3 Hz), 60.0, 53.9 (t J = 12.9 Hz), 43.2 (d, J = 18.6 Hz), 32.2 (d, J = 5.8 Hz); MS: m/z 250 (M + 1). Anal. calcd for (C$_{11}$H$_{12}$FN$_5$O) C, H, N.

(−)-(1R,4S)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]guanine (35). To a suspension of **33** (60 mg, 0.21 mmol) in anhydrous DMF (5 mL) was added potassium *tert*-butoxide (82 mg, 0.69 mmol). The reaction mixture in the thick-walled tube was placed in a microwave synthesizer and irradiated at maximum output power of 300 W with air-cooling at 70 °C for 10 min. The brown suspension was filtered through a short silica gel pad, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on a silica gel (MeOH: CH$_2$Cl$_2$ = 1:20 to 1:10) to give **35** (40 mg, 72%) as a white solid: mp 220 °C (dec); [α]$^{25}_d$ −44.12° (c 0.11, MeOH); UV (H$_2$O) λ_{max} 253.0 nm (ε 13553, pH 2), 252.0 nm (ε 14393, pH 7), 256.0 nm and 268.0 (ε 11186 and 11829, respectively, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.92 (s, 1H), 5.49 (m, 1H), 5.37 (s, 1H), 3.74 (ddd, J = 113.5, 11.5 and 4.0 Hz, 2H), 3.02 (m, 1H), 2.90 (dt, J = 14.0 and 9.0 Hz, 1H), 2.00−1.97 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 165.9 (d, J = 282.0 Hz), 158.0, 153.8, 151.2, 136.6, 116.2, 103.1 (d, J = 13.7 Hz), 60.0, 53.2 (d, J = 12.9 Hz), 43.2 (d, J = 18.3 Hz), 32.2 (d, J = 5.4 Hz). Anal. calcd for (C$_{11}$H$_{12}$-FN$_5$O$_2$·0.9H$_2$O) C, H, N.

(+)-(1R,4S)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]hypoxanthine (36): mp 254−256 °C; [α]$^{27}_D$ +34.01° (c 1.51, MeOH); UV (H$_2$O) λ_{max} 249.0 nm (ε 10354, pH 2), 248.5 nm (ε 13925, pH 7), 254.0 nm (ε 10142, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.15 (s, 1H), 8.08 (s, 1H), 5.68 (m, 1H), 5.44 (s, 1H), 3.86 (dd, J = 10.5 and 4.5 Hz, 1H), 3.60 (m, 1H), 2.96−2.85 (m, 2H), 1.97 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$-OD) δ 166.4 (d, J = 283.9 Hz), 157.6, 148.4, 145.1, 139.2, 123.9, 103.1 (d, J = 14.8 Hz), 60.0, 54.2 (d, J = 12.9 Hz), 43.3 (d, J = 18.6 Hz), 32.4 (d, J = 5.8 Hz). Anal. calcd for (C$_{11}$H$_{11}$FN$_4$O$_2$) C, H, N.

(−)-(1R,4S)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcy-clopentanyl]cytosine (37): mp 132−134 °C; [α]$^{26}_D$ −4.62° (c 0.3, MeOH); UV (H$_2$O) λ_{max} 282.0 nm (ε 16381, pH 2), 274.0 nm (ε 10363, pH 7), 273.0 nm (ε 11099, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.72 (d, J = 7.5 Hz, 1H), 5.95 (d, J = 7.0 Hz, HzH1H), 5.14−5.07 (m, 1H), 3.81 (ddd, J = 47.0, 11.5 and 5.5 Hz, 2H), 2.70−2.35 (m, 4H), 1.97−1.90 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$-OD) δ 165.8, 157.5, 142.2, 128.9 (dd, J = 250.9 and 249.0 Hz), 95.2, 59.0 (t, J = 5.6 Hz), 52.0 (t, J = 6.2 Hz), 40.1 (t, J = 25.2 Hz), 32.0 (d, J = 4.4 Hz). Anal. calcd for (C$_{10}$H$_{13}$F$_2$N$_3$O$_2$·0.6H$_2$O) C, H, N.

(−)-(1*R*,4*S*)-9-[2,3-Dideoxy-3,3-difluoro-6-hydroxymethylcy-clopentanyl]thymine (38): $[\alpha]^{26}_D$ −3.2° (*c* 0.25, MeOH); mp 142−144 °C; UV (H$_2$O) λ_{max} 271.0 nm (ϵ 8478, pH 2), 271.0 nm (ϵ 8509, pH 7), 270.0 nm (ϵ 7587, pH 11); ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.56 (s, 1H), 5.08−5.02 (m, 1H), 3.80 (ddd, *J* = 33.2, 9.2 and 4.8 Hz, 2H), 2.65−2.37 (m, 4H), 2.01−1.92 (m, 4H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 164.9, 151.4, 137.7, 128.8 (dd, *J* = 250.9 and 248.0 Hz), 110.7, 58.9 (dd, *J* = 8.0 and 2.4 Hz), 50.8 (t, *J* = 4.2 Hz), 39.5 (t, *J* = 23.6 Hz), 31.4 (d, *J* = 3.8 Hz), 11.0. Anal. calcd for (C$_{11}$H$_{14}$F$_2$N$_2$O$_3$) C, H, N.

(−)-(1*R*,4*S*)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]cytosine (39). Compound **37** (60 mg, 0.24 mmol) was converted to cytosine derivative **39** (46 mg, 84%) as a white solid using the same procedure as for **35**: mp 220−230 °C; $[\alpha]^{25}_D$ −124.63° (*c* 0.33, MeOH); UV (H$_2$O) λ_{max} 283.0 nm (ϵ 15638, pH 2), 274.0 nm (ϵ 10805, pH 7), 274.0 nm (ϵ 10512, pH 11); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.83 (d, *J* = 7.5 Hz, HzH1H), 5.90 (d, *J* = 7.5 Hz, 1H), 5.63−5.59 (m, 1H), 5.19 (s, 1H), 3.70 (ddd, *J* = 132.5, 10.5 and 3.5 Hz, 2H), 2.95−2.93 (m, 1H), 2.82 (td, *J* = 14.0 and 9.0 Hz, 1H), 1.73−1.68 (m, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 166.1, 166.0 (d, *J* = 283.2 Hz), 157.5, 142.6, 103.3 (d, *J* = 13.4 Hz), 94.3, 59.8 (d, *J* = 1.9 Hz), 55.9 (d, *J* = 8.1 Hz), 42.9 (d, *J* = 18.6 Hz), 31.7 (d, *J* = 6.1 Hz). Anal. calcd for (C$_{10}$H$_{12}$FN$_3$O$_2$) C, H, N.

(−)-(1*R*,4*S*)-9-[2,3-Dideoxy-2,3-didehydro-3-fluoro-6-hydroxy-methylcyclopent-2-enyl]thymine (40): mp 184−186 °C (dec); $[\alpha]^{25}_D$ −24.17° (*c* 0.15, MeOH); UV (H$_2$O) λ_{max} 272.0 nm (ϵ 15633, pH 2), 273.0 nm (ϵ 15750, pH 7), 271.0 nm (ϵ 12548, pH 11); ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.69 (d, *J* = 1.2 Hz, 1H), 5.57−5.54 (m, 1H), 5.15 (s, 1H), 3.69 (ddd, *J* = 121.6, 11.6 and 3.2 Hz, 2H), 2.93−2.90 (m, 1H), 2.78−2.70 (td, *J* = 14.4 and 9.2 Hz, 1H), 1.76−1.70 (m, 1H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 165.2, 166.0 (d, *J* = 282.7), 151.6, 138.2, 109.7, 103.4 (d, *J* = 13.7 Hz), 59.6 (d, *J* = 2.3 Hz), 54.7 (d, *J* = 12.2 Hz), 42.7 (d, *J* = 18.3 Hz), 30.9 (d, *J* = 6.1 Hz), 11.0. Anal. calcd for (C$_{11}$H$_{13}$FN$_2$O$_3$) C, H, N.

Antiviral and Cytotoxicity Assay. HIV drug susceptibility assays were performed as previously described.[28] Cytotoxicity assays in PBM, CEM, and Vero cells were conducted as previously described.[29]

Molecular Modeling Study. (a) Conformational Analysis: The initial conformations of inhibitors were constructed by builder module in MACROMODEL, version 8.5 (Schrodinger, Inc.), based on the crystal structure of carbovir. The Monte Carlo conformational search was performed in 5000-step, in the presence of GB/SA water model using MMFFs force field in MACROMODEL.

(b) Binding affinity study to HIV-1 reverse transcriptase: All molecular modeling studies of the enzyme−substrate complexes were performed using Sybyl 7.0 (Tripos Associates, St. Louis, MO) on a Silicon Graphics Tezro workstation or a SGI Origin 300 workstation. The enzyme site of the enzyme−ligand complex was built based on the X-ray structure of the covalently trapped catalytic complex of HIV-1 RT with TTP and primer−template duplex (PDB entry 1rtd).[30] A model of the NRTI binding site was built, which consisted of residues between Lys1 and Pro243 in the p66 subunit and a 7:4 (template-primer) duplex. The conformationally optimized structures of carbocyclic nucleosides were used to define the initial Cartesian coordinates. The heterocyclic moiety of the *n* + 1th nucleotide in the template overhang was modified to the base complementary to the incoming NRTIs if needed, i.e., the adenine moiety which was in the original X-ray structure (1rtd)[30] was modified to guanine. The inhibitor triphosphates were manually docked to the active site of the enzyme by adjusting the torsional angles to those found in the X-ray structure.[30] Gästeiger-Hückel charges were then given to the nucleoside triphosphate with formal charges (+2) to the two Mg atoms in the active site, and Kollman-All-Atom charges were loaded to the enzyme site using the biopolymer module in Sybyl. Fluorine parameters were obtained from literature,[31,32] and MM2 parameters were entered into the parameter files. In order to eliminate local strains resulting from merging inhibitors and/or point mutations, residues inside 6 Å from the merged inhibitors and mutated residues were annealed until energy change from one iteration to the next was less than 0.05 kcal/mol. The annealed enzyme−inhibitor complexes were minimized by using Kollman-All-Atom force field until iteration number reached 5000.

The structures (D-3′-F-C-d4G-TP/HIV-RT$_{WT}$, D-3′-F-C-d4G-TP/HIV-RT$_{M184V}$, carbovir-TP/HIV-RT$_{WT}$, carbovir-TP/HIV-RT$_{M184V}$, GTP/ HIV-RT$_{WT}$, and GTP/HIV-RT$_{M184v}$) were further confirmed by the molecular dynamics studies using MACROMODEL, version 9.1 (Schrodinger, Inc.). The complex was minimized until there was no significant movement in atomic coordinates using MMFF94s force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. A conjugate gradient Polak−Ribiere first derivative method was used for energy minimization. Molecular dynamics simulations on nucleoside-TP/ RT complex was performed with MMFF94s in the presence of GB/ SA continuum water model on a SGI Origin 300 workstation running the IRIX 6.5 operating system by heating from 0 to 300 K over 5 ps and equilibrating at 300 K for an additional 10 ps. Production dynamics simulations were carried out for 500 ps with a step size of 1.5 fs at 300 K. A shake algorithm was used to constrain covalent bonds to hydrogen atoms. A distance constrain was used to constrained the two magnesium atoms with Asp110, Val111, Asp185, and α- and β-phosphate of nucleotide. For simulation of the nucleoside-TP/RT complex, the residues further away than 15 Å from the active site were not considered and the residues from 6 to 15 Å were constrained by harmonic constraints. Only residues inside the 6 Å sphere from the bound nucleoside-TP were allowed to move freely.

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