http://dx.doi.org/10.5012/bkcs.2013.34.12.3621

Synthesis of Novel 2'-Fluoro-5'-deoxyphosphonic Acids and Bis(SATE) Adenine Analogue as Potent Antiviral Agents

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Received August 27, 2013, Accepted September 11, 2013

Novel 5'-deoxythreosyl purine phosphonic acid analogues containing a 2'-electropositive moiety such as fluorine atom, were designed and synthesized from commercially available 1,3-dihydroxy acetone. Condensation successfully proceeded from a glycosyl donor **6** under Vorbrüggen conditions and cross-metathesis gave the desired phosphonate analogues **7a**, **7b**, **17a** and **17b**. The synthesized nucleoside phosphonic acid analogues **13**, **16**, **23**, **26**, **28** were subjected to antiviral screening against HIV-1. The bis(SATE) adenine analogue **28** exhibited significant *in vitro* activities against HIV-1.

Key Words : anti-HIV agents, 2'-Fluoro-5'-deoxyphosphonic acid analogue, Vorbrüggen reaction

Introduction

As mimics of nucleoside monophosphates, phosphonate analogues exert their antiviral effect following sequential activation by cellular kinases to their diphosphate derivatives (nucleoside triphosphate analogues) which act as potent inhibitors of viral polymerases.[1] The selective inhibition of viral polymerases, as opposed to host cell DNA polymerases, is critical for the therapeutic use of such compounds. Various attempts to improve selectivity indices have led to nucleoside analogues with a modified furanose ring system due to the introduction of an electropositive moiety at the 2'-position of the furanose ring,[2] and recently, a nucleoside analogue with a fluorine atom at this position, GS-9148 (**1**), was reported to show excellent anti-HIV activity (Figure 1).[3]

Threose phosphonate nucleosides,[4] such as, PMDTA (**2**) and PMDTT (**3**), have been assembled from natural precursor molecules. Furthermore, it has been demonstrated threose nucleic acids (TNA) form thermal stable duplexes with DNA and RNA that are reminiscent of the natural associations of nucleic acids.[5] Moreover, diphosphates of threose nucleosides are accepted as substrates by several polymerases, and can be enzymatically incorporated into DNA.[6] In addition, these nucleosides are also accepted as substitutes for ribonucleosides at the catalytic site of hammerhead ribozyme, although the catalytic efficiency of ribozyme is then significantly reduced.[7] PMDTA has a phosphonomethoxy group at the 3'-position of its furanose ring and no substituent at the 4'-position.[8] This absence of a 4'-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases. To study the influences of different substituents on anti-HIV activity further, we undertook to synthesize 2'-fluorinated analogues of PMDTA.

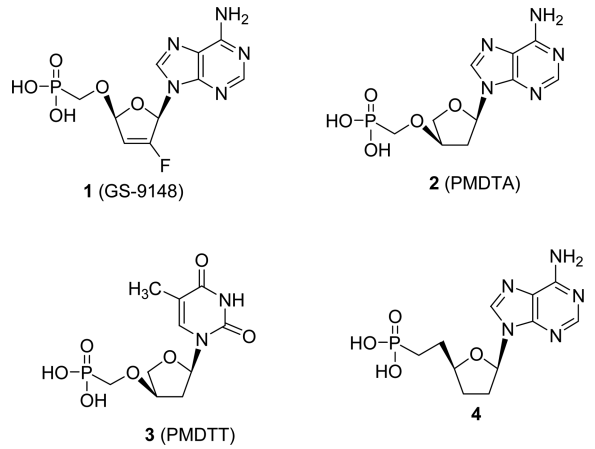


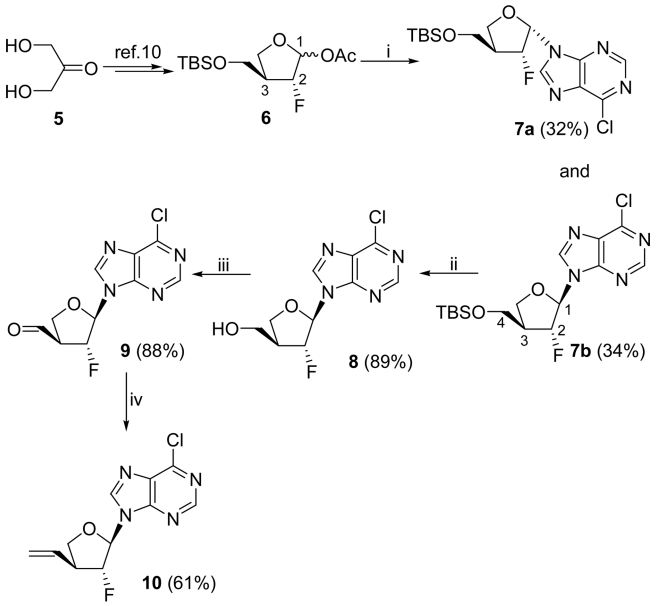
Figure 1. Synthesis rationale of threosyl 5'-deoxyphosphonic acid nucleosides as potent antiviral agents.

Several 5'-phosphate isosteres have been used to prepare nucleoside phosphonates. As shown in Figure 1, compound **4**[9] is a simple 5'-deoxynucleoside phosphonate, in which the 5'-oxygen of a nucleoside phosphate is replaced by methylene group. Importantly, all resultant phosphonates mimic the overall shape and geometry of nucleoside monophosphates.

Stimulated by the findings that 2'-electropositive nucleoside analogues and 5'-deoxyphosphonic acid nucleosides have excellent antiviral activities, we sought to synthesize a novel class of nucleosides consisting of 2'-fluorinated-5'-deoxythreosylphosphonic acid analogues in order to find therapeutics that are more effective against HIV.

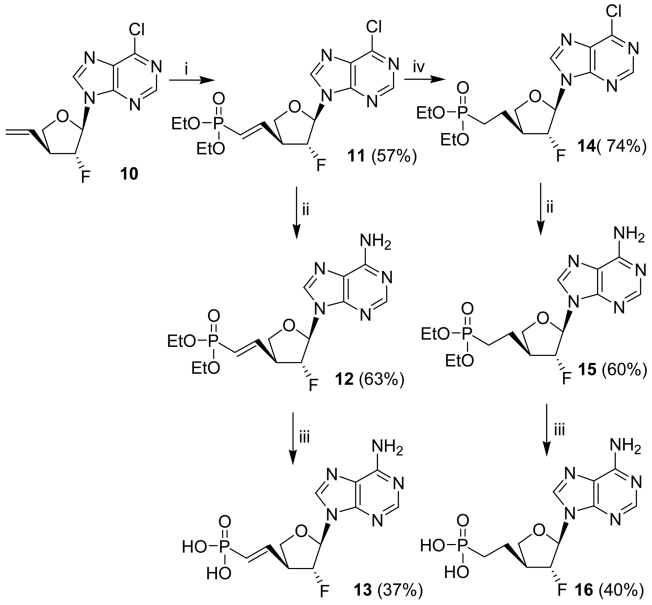
Results and Discussion

As depicted in Scheme 1, target compounds were prepared from the fluorinated glycosyl donor **6**, which was readily prepared from 1,3-dihydroxyacetone **5**, as previously described.[10] The synthesis of adenine nucleoside was carried out by Vorbrüggen condensation[11] of compound **6** with silylated 6-chloropurine using TMSOTf as a catalyst in DCE to give the protected 6-chloropurine derivatives **7a** and **7b**,



Reagents: i) Silylated 6-chloropurine, TMSOTf, DCE; ii) TBAF, THF; iii) Dess-Martin, CH_2Cl_2; iv) *n*-BuLi, Ph_3PCH_3I, PPh_3, THF.

Scheme 1. Synthesis of threosyl-2'-fluoro-3'-vinylidene 6-chloropurine analogue.



Reagents: i) Vinyldiethylphosphonate, Grubbs cat.(II) CH_2Cl_2; ii) NH_3, MeOH, 62 °C; iii) TMSBr, 2,6-lutidine, CH_3CN; iv) Pd/C, cyclohexene, MeOH.

Scheme 2. Synthesis of threosyl-2'-fluoro-5'-deoxyphosphonic acid adenine analogues.

respectively. Strong NOE (0.8%) of H-1' ↔ CH-3', which showed a 1',3'-*cis* relationship, was observed. According to this result, the 3'-hydroxymethyl group and the 1'-purine base of **7b** were located on the β face. On the other hand, for **7a** compound, weak NOE (0.4%) of H-1' ↔ CH-3', demonstrated a 1',3'-*trans* relationship (Figure 2).

For the homologation, removal of the silyl protecting group of **7b** using tetra *n*-butylammonium fluoride (TBAF) gave the primary alcohol **8**. Dess-Martin oxidation[12] of the alcohol of **8** gave the aldehyde **9**, which was subjected to Wittig olefination[13] to give compound **10** without loss of the 3'-stereochemistry. Cross-metathesis[14] of **10** with vinyl diethylphosphonate using a 2nd generation Grubbs catalyst[15] gave the vinylidene phosphonate nucleoside analogue **11** in 57% yield. The chlorine group of the purine analogue **11** was then converted to amine with methanolic ammonia at 62 °C to give the corresponding adenosine phosphonate derivative **12** at in 63% yield. Hydrolysis of the diethyl phosphonate functional groups of **12** by treatment with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine then gave the adenosine phosphonic acid derivative **13**.[16] When the vinylidene phosphonate was saturated under transfer catalytic hydrogenation conditions[17] it produced the ethyl phosphonate nucleoside analogue **14** in 74% yield. The adenine phosphonic acid analogue **16** was prepared using conditions similar to the ammonolysis and hydrolysis described to produce **13** (Scheme 2).

The guanine analogues, 2-fluoro-6-chloropurine[18] was condensed with the glycosyl donor **6** using conditions similar to those used for the preparation of **7a** and **7b** to give the analogues **17a** (31%) and **17b** (32%) from 6-chloropurine (Scheme 3). A complete NOE study allowed the unambiguous determination of the relative stereochemistries of purine analogues as described for **7a** and **7b**. Homologation was performed using reactions similar to those used to produce **10**, such as desilylation, Dess-Martin oxidation and Wittig olefination. Cross-metathesis of **20** with diethylvinylphos-

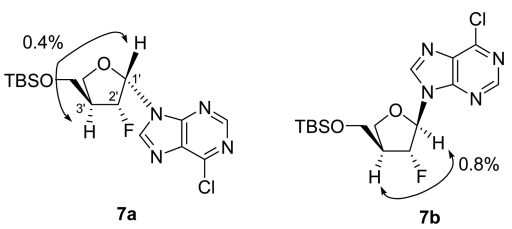
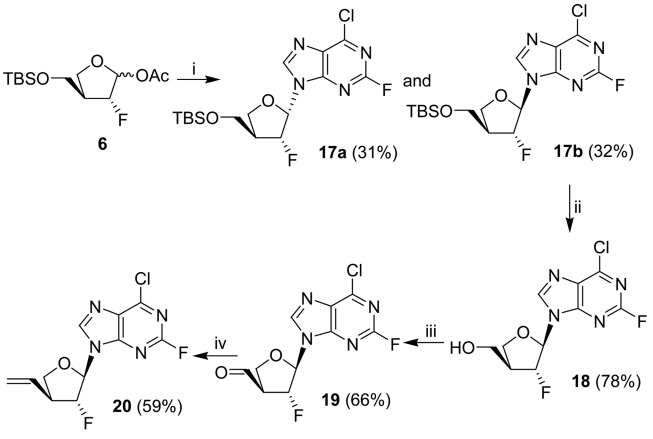
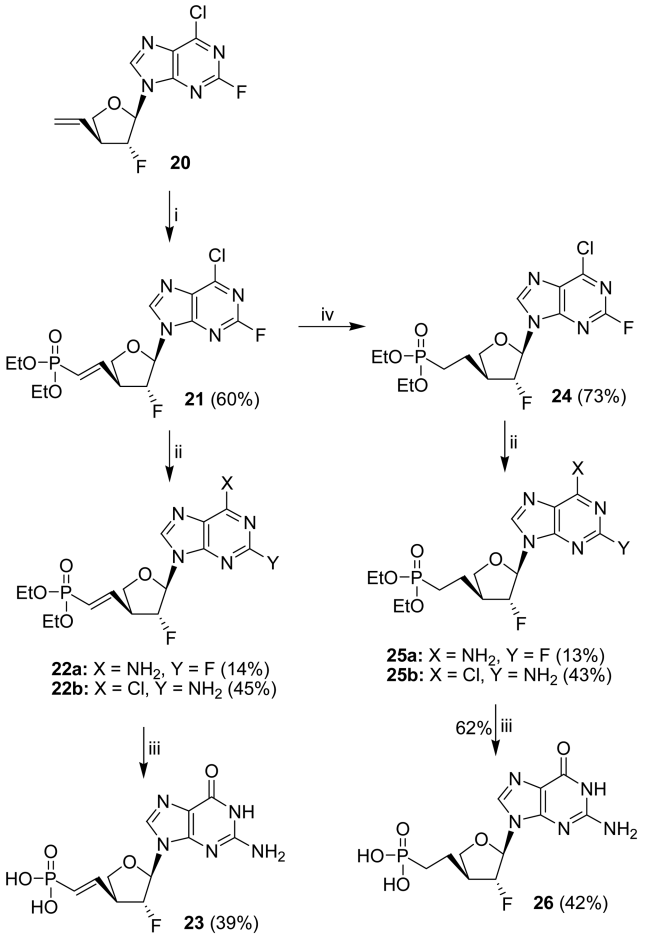


Figure 2. NOE differences between the proximal hydrogens of **7a** and **7b**.



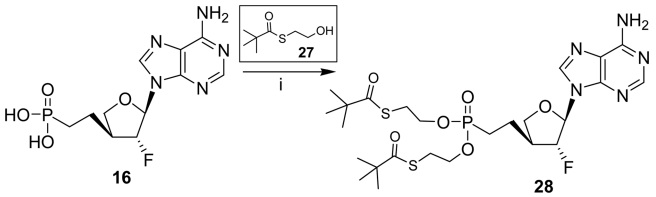
Reagents: i) Silylated 2-fluoro-6-chloropurine, TMSOTf, DCE; ii) TBAF, THF; iii) Dess-Martin, CH_2Cl_2; iv) *n*-BuLi, Ph_3PCH_3I, PPh_3, THF.

Scheme 3. Synthesis of threosyl-2'-fluoro-3'-vinylidene 2-fluoro-6-chloropurine analogue.



Reagents: i) vinyldiethylphosphonate, Grubbs cat.(II) CH_2Cl_2; ii) NH_3, DME, rt; iii) (a) TMSBr, 2,6-lutidine, CH_3CN; (b) NaOMe, $HSCH_2CH_2OH$, MeOH; iv) Pd/C, cyclohexene, MeOH.

Scheme 4. Synthesis of threosyl-2'-fluoro-5'-deoxyphosphonic acid guanine analogue.



Reagents: i) thioester, **27**, 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole, pyridine.

Scheme 5. Synthesis of target bis(SATE) prodrug of adenine analogue **16**.

Table 1. Median effective (EC_{50}) and cytotoxic (CC_{50}) concentrations of the synthesized nucleoside analogues

Compound No.	Anti-HIV-1 EC_{50} (μM)[c]	Cytotoxicity CC_{50} (μM)[d]
13	34.2	95
16	8.8	80
23	66.8	98
26	47.1	98
28	**2.2**	80
AZT[a]	0.003	>100
PMEA[b]	>10	>10
bis(SATE)PMEA	0.81	>10

[a]**AZT:** azidothymidine. [b]**PMEA**: 9-[2-(phosphonomethoxy)ethyl]adenine. [c]EC_{50} (μM): EC_{50} values are for 50% inhibition of virus production as indicated by supernatant RT levels. [d]CC_{50} (μM): CC_{50} values indicate 50% cytotoxic concentration.

phonate provided **21** in 60% yield.

Bubbling ammonia into the compound **21** gave separable 2-fluoro-6-aminopurine[19] **22a** (14%) and 2-amino-6-chloropurine **22b** (45%) analogues, respectively. The 2-amino-6-chloropurine derivative **22b** was treated with TMSBr to provide phosphonic acid and sequentially treated with sodium methoxide and 2-mercaptoethanol in methanol to give the desired guanine phosphonic acid **23** (Scheme 4).[20] Furthermore, the guanine phosphonic acid analogue **26** was synthesized from **21** *via* transfer catalytic hydrogenation, ammonolysis, and hydrolysis using conditions similar to those described for the synthesis of the adenine 6, line derivative **16**. To synthesize the thioester-protected analogue, compound **16** was reacted with thioester **27**[21] in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[22] to provide the bis(SATE) derivative as a target compound **28** (Scheme 5).

Antiviral Activity. The antiviral activities of phosphonate nucleosides are explained by their intracellular metabolism to diphosphates, subsequent incorporation into the viral genome, and chain termination.[23] MT-4 cells (1×10^5 cell/mL) were infected with HIV-1 ($HTLV\text{-}III_B$ strain) at a multiplicity of infection (MOI) of 0.02, and then cultured in the presence of various concentrations of the test compounds. After a 4-day incubation at 37 °C, numbers of viable cells were determined using the 3-(4,5-di-methylthiazole-2-yl)-2,5-diphenyltetrazolium bromide method. The cytotoxicities of the compounds were evaluated in parallel with their antiviral activities, by determining the viabilities of mock-infected cells.[24] Compounds **13**, **16**, **23**, **26** and **28** were tested against HIV-1, and the adenine analogue **16** showed moderate antiviral activity (Table 1). However, other three 5'-deoxyphosphonic acid nucleoside analogues showed weak or no anti-HIV activity at concentrations up to 100 μM.

In summary, based on the potent anti-HIV activities of 2'-electropositive nucleosides and 5'-deoxyphosphonic acid nucleoside analogues, we designed and successfully synthesized novel 2'-fluoro-5'-deoxyphosphonic acid nucleoside analogues starting from 1,3-dihydroxy acetone. The synthesized bis(SATE) adenine analogue **28** showed significant activity in a cell-based assay than the 2'-modified guanine phosphonic acid analogues **13**, **23** and **26**. Since 2'-fluorinated guanine nucleoside derivatives are not perfect mimics of the ribofuranose moiety, mechanisms of virus inhibition, that is, phosphorylation or the inhibition of RNA synthesis, might be impaired for these compounds. For the discovery of improved antiviral nucleoside derivatives, bis(SATE) analogue **28** was synthesized and assayed for anti-HIV activity using an *in vitro* assay system, It showed much improved

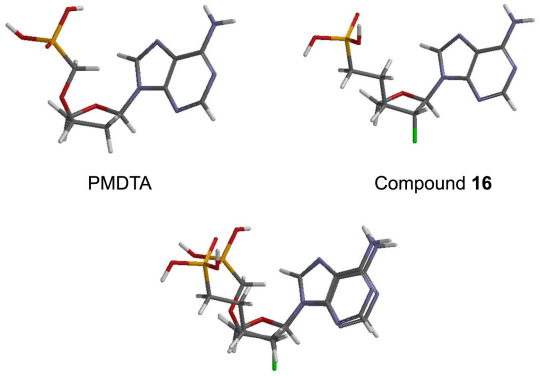


Figure 3. Superimpose of PMDTA and **16**.

anti-HIV activity than adenine nucleoside phosphonic acid **16** (Table 1). As shown in the superimposition model of PMDTA (**2**) and the corresponding analogue **16** (Figure 3), discrepancies of phosphonic acid regions are more pronounced than those of the base moiety. Note the furanose puckering of PMDTA (**2**) is closer to that of the adenine analogue **16**.[25]

Experimental Section

Uncorrected melting points were determined on a Meltemp II laboratory device. NMR spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). MS spectra were collected in electrospray ionization (ESI) mode. Elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out in a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

(*rel*)-(1'*S*,2'*R*,3'*S*)-9-(3'-*t*-Butyldimethylsilanyloxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (7a) and (*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-*t*-butyldimethylsilanyloxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (7b): 6-Chloropurine (216 mg, 1.4 mmol), anhydrous HMDS (10 mL), and a catalytic amount of ammonium sulfate (14 mg) were refluxed to a clear solution, and the solvent was then distilled off under anhydrous conditions. The residue obtained was dissolved in anhydrous 1,2-dichloroethane (8 mL), and to this mixture, a solution of **6** (175 mg, 0.6 mmol) in dry DCE (10 mL) and TMSOTf (311 mg, 1.4 mmol) was added, and stirred for 8 h at rt. The reaction mixture was quenched with 5.0 mL of saturated $NaHCO_3$, stirred for 1 h, filtered through a Celite pad, and the filtrate obtained was then extracted twice with CH_2Cl_2 (80 mL). Combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 4:1:0.01) to give compounds **7a** (74 mg, 32%) and **7b** (79 mg, 34%). Data for **7a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 (s, 1H), 8.34 (s, 1H), 6.23 (dd, J = 18.6, 5.8 Hz, 1H), 3.77-3.67 (m, 5H), 2.39-2.28 (m, 1H), 0.88-0.86 (m, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 151.4, 151.1, 144.8, 132.5, 92.2 (d, J = 172.0 Hz), 88.5 (d, J = 23.2 Hz), 60.5, 57.4, 39.3 (d, J = 22.2 Hz), 25.4, 18.3, −5.1; Anal. Calc. for $C_{16}H_{24}ClFN_4O_2Si$: C, 49.67; H, 6.25; N, 14.48. Found: C, 49.71; H, 6.23; N, 14.50; MS *m/z* 387 $(M+H)^+$. Data for **7b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.70 (s, 1H), 8.31 (s, 1H), 6.19 (dd, J = 18.2, 4.8 Hz, 1H), 3.77-3.63 (m, 5H), 2.38-2.27 (m, 1H), 0.83 (m, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.6, 151.3, 151.0, 144.7, 132.3, 91.6 (d, J = 170.8 Hz), 87.2 (d, J = 21.2 Hz), 59.4, 56.2, 38.2 (d, J = 21.4 Hz), 25.5, 18.4, −4.8; Anal. Calc. for $C_{16}H_{24}ClFN_4O_2Si$ (+0.5 MeOH): C, 49.18; H, 6.50; N, 13.90. Found: C, 49.21; H, 6.52; N, 13.88; MS *m/z* 387 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-Hydroxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (8). To a solution of **7b** (2.54 g, 6.56 mmol) in THF (12 mL), TBAF (7.7 mL, 1.0 M solution in THF) was added at 0 °C. The mixture was stirred overnight at rt and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/EtOAc/MeOH, 2:1:0.05) to give **8** (1.59 g, 89%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.69 (s, 1H), 8.29 (s, 1H), 6.15 (dd, J = 16.8, 5.4 Hz, 1H), 3.79-3.64 (m, 5H), 2.36-2.24 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8, 151.4, 151.1, 144.5, 132.6, 92.5 (d, J = 166.8 Hz), 86.4 (d, J = 20.4 Hz), 60.2, 56.2, 38.2 (d, J = 21.4 Hz); Anal. Calc. for $C_{10}H_{10}ClFN_4O_2$ (+1.0 MeOH): C, 43.36; H, 4.62; N, 18.39. Found: C, 43.32; H, 4.64; N, 18.41; MS *m/z* 273 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*R*)-9-(3'-Carbaldehyde-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (9). Compound **8** (290 mg, 1.066 mmol) was dissolved in anhydrous CH_2Cl_2 (8 mL), and to this solution was added Dess-Martin reagent (588 mg, 1.38 mmol). The mixture was stirred for 3 h at ambient temperature, concentrated and the residue was purified by silica gel column chromatography using Hexane/EtOAc (1:4) as eluent. A second column, which was also eluted with EtOAc, was necessary to remove traces of Dess-Martin reagent-related impurities to give **9** (253 mg, 88%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.69 (s, 1H), 8.70 (s, 1H), 8.28 (s, 1H), 6.19 (dd, J = 18.0, 5.6 Hz, 1H), 4.05-3.94 (m, 3H), 2.93-2.85 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.7, 151.5, 151.0, 150.7, 144.1, 133.4, 92.5 (d, J = 18.4 Hz), 88.5 (d, J = 164.4 Hz), 55.7, 51.1 (d, J = 18.8 Hz); MS *m/z* 271 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-Vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (10). To ylide solution [methyltriphenylphosphonium iodide (188 mg, 0.462 mmol), triphenylphosphine (14.25 mg, 0.055 mmol), 1.6 M *n*-butyllithium solution (0.289 mL, 0.462 mmol) in dry tetrahydrofuran (5.0 mL) at −78 °C, was added dropwise to a solution of olefin **9** (125 mg, 0.462 mmol) in dry THF (7 mL). The reaction mixture was warmed to room temperature, stirred for 4 h,

quenched with saturated sodium bicarbonate solution, and then partitioned between saturated sodium bicarbonate solution and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. Combined extracts were dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo*, and chromatographed (Hexane-EtOAc, 1:2) to afford **10** (76 mg, 61%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.31 (s, 1H), 6.21 (dd, *J* = 19.2, 5.2 Hz, 1H), 5.77-5.75 (m, 1H), 5.05-4.96 (m, 2H), 3.74-3.68 (m, 3H), 2.85-2.81 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 151.3, 150.9, 144.6, 143.6, 132.8, 112.3, 96.1 (d, *J* = 168.4 Hz), 88.3 (d, *J* = 16.8 Hz), 62.1, 39.4 (d, *J* = 18.4 Hz); Anal. Calc. for $C_{11}H_{10}ClFN_4O$: C, 49.17; H, 3.75; N, 20.85; Found: C, 49.21; H, 3.78; N, 20.82; MS *m/z* 269 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine} phosphonate (11). To a solution of the 6-chloropurine derivative **10** (174 mg, 0.650 mmol) and diethyl vinylphosphonate (426 mg, 2.60 mmol) in CH_2Cl_2 (8.0 mL), 2nd-generation Grubbs catalyst (22.10 mg, 0.026 mmol) was added. The reaction mixture was refluxed for 26 h under dry argon and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/*n*-Hexane/MeOH, 2:1:0.05) to give **11** (150 mg, 57%) as a foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 (s, 1H), 8.32 (s, 1H), 6.67 (dd, *J* = 17.2, 21.4 Hz, 1H), 6.20 (dd, *J* = 19.2, 5.2 Hz, 1H), 6.11 (dd, *J* = 17.2, 19.8. Hz, 1H), 4.12-4.06 (m, 4H), 3.73-3.66 (m, 3H), 2.82-2.78 (m, 1H), 1.33-1.30 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8, 151.4, 151.0, 149.5, 144.2, 132.6, 117.2, 95.6 (d, *J* = 168.4 Hz), 87.6 (d, *J* = 17.0 Hz), 62.4, 61.8, 60.6, 40.7 (d, *J* = 16.4 Hz), 15.8; Anal. Calc. for $C_{15}H_{19}ClFN_4O_4P$ (+0.5 MeOH): C, 44.24; H, 5.03; N, 13.31; Found: C, 44.26; H, 5.02; N, 13.29; MS *m/z* 405 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-vinyl-2'-fluoro-tetrahydrofuran-1'-yl) adenine} phosphonate (12). A solution of **11** (188 mg, 0.464 mmol) in saturated methanolic ammonia (7 mL) was stirred overnight at 66 ºC in a steel bomb, and volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to give **12** (112 mg, 63%) as a white solid: UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.36 (s, 1H), 8.17 (s, 1H), 6.65 (dd, *J* = 17.4, 20.8 Hz, 1H), 6.23 (dd, *J* = 18.0, 5.4 Hz, 1H), 6.10 (dd, *J* = 17.2, 19.8. Hz, 1H), 4.10-4.05 (m, 4H), 3.76-3.68 (m, 3H), 2.83-2.77 (m, 1H), 1.32-1.30 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.5, 152.7, 150.4, 148.8, 141.3, 119.0, 115.6, 94.8 (d, *J* = 167.8 Hz), 88.4 (d, *J* = 16.6 Hz), 63.3, 62.7, 61.5, 41.2 (d, *J* = 16.6 Hz), 15.3; Anal. Calc. for $C_{15}H_{21}FN_5O_4P$ (+1.0 MeOH): C, 46.04; H, 6.04; N, 16.78; Found: C, 46.08; H, 6.02; N, 16.80; MS *m/z* 386 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-[(3'-Vinyl-2'-fluoro-tetrahydrofuran-1'-yl) adenine]phosphonic acid (13). To a solution of the phosphonate **12** (161 mg, 0.419 mmol) in anhydrous CH_3CN (10 mL) and 2,6-lutidine (0.898 mL, 8.38 mmol) was added trimethylsilyl bromide (641 mg, 4.19 mmol). The mixture was heated overnight at 75 ºC under nitrogen and then concentrated *in vacuo* to give a brown residue, and then co-evaporated from conc-aqueous NH_4OH (2 × 22 mL). The resultant purified by twice triturating the residue in acetone (8 mL) and removing the acetone by evaporation. The residue so obtained was then purified by preparative reverse-phase chromatography. Lyophilization of the appropriate fraction provided the phosphonic acid salt **13** (53.65 mg, 37%) as a white salt (ammonium salt): mp 157-159 ºC; UV (H_2O) λ_{max} 261.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 8.34 (s, 1H), 8.16 (s, 1H), 6.66 (dd, *J* = 17.6, 21.0 Hz, 1H), 6.21 (dd, *J* = 17.6, 5.2 Hz, 1H), 6.12 (dd, *J* = 17.4, 19.4. Hz, 1H), 3.72-3.65 (m, 3H), 2.85-2.78 (m, 1H); ^{13}C NMR (D_2O, 75 MHz) δ 155.3, 152.4, 150.1, 148.5, 141.5, 118.8, 114.7, 93.6 (d, *J* = 165.8 Hz), 89.6 (d, *J* = 16.8 Hz), 61.6, 40.6 (d, *J* = 16.8 Hz); HPLC t_R = 10.67; HRMS $[M-H]^+$ req. 328.0678, found 328.0679.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-ethyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine} phosphonate (14). A solution of vinyl phosphonate nucleoside analogue **13** (265 mg, 0.655 mmol) in methanol (8 mL) was added 10% Pd/C (10 mg) and cyclohexene (4 mL) under Ar. The reaction mixture was refluxed for 24 h. The reaction mixture was filtered through a pad of Celite, evaporated, and purified by silica gel column chromatography using methanol and methylene chloride (10:1) to give ethyl phosphonate analogue **14** (197 mg, 74%) as a white solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.75 (s, 1H), 8.34 (s, 1H), 6.19 (dd, *J* = 18.6, 5.4 Hz, 1H), 4.11-4.06 (m, 4H), 3.76-3.68 (m, 3H), 2.28-1.86 (m, 5H), 1.31-1.28 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8, 151.4, 150.0, 142.9, 132.8, 94.8 (d, *J* = 162.8 Hz), 89.1 (d, *J* = 16.8 Hz), 61.4, 38.9 (d, *J* = 16.8 Hz), 28.7, 18.8, 14.9; Anal. Calc. for $C_{15}H_{21}ClFN_4O_4P$ (+1.0 MeOH): C, 43.79; H, 5.74; N, 12.77; Found: C, 43.83; H, 5.72; N, 12.79; MS *m/z* 407 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-ethyl-2'-fluoro-tetrahydrofuran-1'-yl) adenine} phosphonate (15). The adenine derivative **15** was prepared from the 6-chloropurine analogue **14** using an ammonolysis procedure similar to that described for **12**: yield 60%; mp 172-174 ºC; UV (MeOH) λ_{max} 260.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.38 (s, 1H), 8.19 (s, 1H), 6.16 (dd, *J* = 18.8, 5.5 Hz, 1H), 4.15-4.09 (m, 4H), 3.73-3.65 (m, 3H), 2.28-2.27 (m, 2H), 2.01-1.88 (m, 3H), 1.30-1.26 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.9, 152.3, 150.2, 141.4, 118.7, 93.5 (d, *J* = 162.4 Hz), 88.4 (d, *J* = 16.3 Hz), 60.6, 37.6 (d, *J* = 16.2 Hz), 28.3, 18.5, 15.1; Anal. Calc. for $C_{15}H_{23}FN_5O_4P$ (+1.0 MeOH): C, 45.82; H, 6.49; N, 16.69; Found: C, 45.78; H, 6.51; N, 16.71; MS *m/z* 388 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-{9-(3'-Ethyl-2'-fluoro-tetrahydrofuran-1'-yl) adenine} phosphonic acid (16). Phosphonic acid **16** was synthesized from **15** using hydrolysis conditions identical to that for **13**: yield 40%, mp 160-162 ºC; UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 8.36 (s, 1H), 8.17 (s, 1H), 6.14 (dd, *J* = 17.6, 5.4 Hz, 1H), 3.70-3.64 (m, 3H), 2.25-2.21 (m, 2H), 2.03-1.89 (m, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 155.2, 152.5, 150.6, 141.9, 119.4, 95.2 (d, *J* = 160.8 Hz), 87.7 (d, *J* = 16.4 Hz), 61.4, 38.2 (d, *J* = 16.5 Hz),

29.1, 18.8; HPLC t_R = 10.92; HRMS $[M-H]^+$ req. 330.0677, found 330.0679.

(*rel*)-(1'*S*,2'*R*,3'*S*)-9-(3'-*t*-Butyldimethylsilanyloxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 6-chloropurine (17a) and (*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-*t*-Butyldimethylsilanyloxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine (17b). Condensation of **6** with 2-fluoro-6-chloropurine under Vorbruggen condensation conditions similar to those described for **7a** and **7b** gave **17a** and **17b**, respectively. Data for **17a**: yield 31%; UV (MeOH) λ_{max} 267.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (s, 1H), 6.21 (dd, *J* = 18.4, 5.4 Hz, 1H), 3.75-3.68 (m, 5H), 2.23-2.19 (m, 1H), 0.90-0.88 (m, 9H), 0.02-0.01 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.1 (d, *J* = 219 Hz), 153.3, 145.6, 136.2, 120.6, 92.2 (d, *J* = 166.7 Hz), 89.1 (d, *J* = 16.4 Hz), 60.5, 57.6, 38.7 (d, *J* = 16.2 Hz), 25.5, 18.7, −4.6; Anal. Calc. for $C_{16}H_{23}ClF_2N_4O_2Si$: C, 47.46; H, 5.73; N, 13.84; Found: C, 47.48; H, 5.75; N, 13.86; MS *m/z* 405 $(M+H)^+$. data for **17b**: yield 32%; UV (MeOH) λ_{max} 268.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (s, 1H), 6.19 (dd, *J* = 18.5, 5.5 Hz, 1H), 3.74-3.67 (m, 5H), 2.22-2.18 (m, 1H), 0.88-0.86 (m, 9H), 0.02-0.01 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.3 (d, *J* = 219.6 Hz), 153.5, 145.8, 135.8, 120.8, 91.6 (d, *J* = 167.8 Hz), 88.5 (d, *J* = 16.6 Hz), 59.6, 57.4, 38.4 (d, *J* = 16.4 Hz), 25.4, 18.4, −5.2; Anal. Calc. for $C_{16}H_{23}ClF_2N_4O_2Si$: C, 47.46; H, 5.73; N, 13.84; Found: C, 47.42; H, 5.72; N, 13.81; MS *m/z* 405 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-Hydroxymethyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine (18). Desilylation of **17b** was performed using a procedure similar to that described for **8**: yield 78%; UV (MeOH) λ_{max} 269.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.46 (s, 1H), 6.21 (dd, *J* = 18.4, 5.5 Hz, 1H), 3.74-3.67 (m, 3H), 3.51-3.49 (m, 2H), 2.21-2.15 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.9 (d, *J* = 217.8 Hz), 154.0, 144.6, 134.8, 121.6, 91.6 (d, *J* = 169.4 Hz), 89.4 (d, *J* = 16.8 Hz), 57.6, 56.2, 37.2 (d, *J* = 16.6 Hz); Anal. Calc. for $C_{10}H_9ClF_2N_4O_2$ (+1.0 MeOH): C, 40.94; H, 4.06; N, 17.36; Found: C, 40.97; H, 4.05; N, 17.34; MS *m/z* 291 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-Carbaldehyde-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine (19). Oxidation of **18** was performed using the Dess-Martin reaction conditions described for **9**: yield 66%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.45 (s, 1H), 6.20 (dd, *J* = 18.6, 5.6 Hz, 1H), 3.72-3.68 (m, 3H), 3.48-3.42 (m, 2H), 2.23-2.16 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.6, 157.4 (d, *J* = 218.6 Hz), 154.2, 144.7, 133.9, 122.3, 91.3 (d, *J* = 167.8 Hz), 88.5 (d, *J* = 16.6 Hz), 58.4, 57.4, 38.4 (d, *J* = 16.4 Hz).

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-(3'-Vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine} phosphonate (20). Wittig olefination of the aldehyde **10** was performed using a procedure similar to that described for **10**: yield 59%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.46 (s, 1H), 6.21 (dd, *J* = 18.4, 5.4 Hz, 1H), 5.73 (m, 1H), 5.05-4.98 (m, 2H), 3.73-3.68 (m, 3H), 3.50 (m, 2H), 2.81-2.76 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.6 (d, *J* = 219.4 Hz), 154.5, 144.3, 142.5, 134.6, 123.6, 112.3, 95.6 (d, *J* = 167.8 Hz), 90.2 (d, *J* = 16.8 Hz), 61.6, 39.1 (d, *J* = 16.4 Hz); Anal. Calc. for $C_{11}H_9ClF_2N_4O$: C, 46.09; H, 3.16; N, 19.54; Found: C, 46.12; H, 3.15; N, 19.55; MS *m/z* 287$(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-chloropurine} phosphonate (21). Phosphonate nucleoside analogue **21** was prepared from **20** using a cross metathesis procedure similar to that described for **11**: yield 60%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.44 (s, 1H), 6.67 (dd, *J* = 20.2, 18.6 Hz, 1H), 6.21-6.15 (m, 2H), 4.15-4.10 (m, 4H), 3.72-3.66 (m, 3H), 2.80-2.75 (m, 1H), 1.35-1.33 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 157.3 (d, *J* = 218.8 Hz), 154.7, 149.5, 143.1, 133.9, 124.3, 115.3, 95.7 (d, *J* = 166.6 Hz), 89.4 (d, *J* = 16.4 Hz), 62.2, 61.5, 60.9, 40.4 (d, *J* = 16.2 Hz), 15.7; Anal. Calc. for $C_{15}H_{18}ClF_2N_4O_4P$ (+1.0 MeOH): C, 42.25; H, 4.87; N, 12.32; Found: C, 42.21; H, 4.89; N, 12.30; MS *m/z* 423 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-fluoro-6-aminopurine} phosphonate (22a) and (*rel*)-(1'*R*,2'*R*,3'*S*)-diethyl {9-(3'-vinyl-2'-fluoro-tetrahydrofuran-1'-yl) 2-amino-6-chloropurine} phosphonate (22b). Dry ammonia gas was bubbled into a stirred solution of **21** (180 mg, 0.426 mmol) in DME (8.0 mL) at room temperature overnight. Salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue obtained was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to give **22a** (24 mg, 14%) and **22b** (80 mg, 45%). Data for **22a**; UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.21 (s, 1H), 7.74 (br s, NH_2, 2H), 6.65 (dd, *J* = 19.8, 16.4 Hz, 1H), 6.21 (dd, *J* = 19.7, 18.2 Hz, 1H), 6.09 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.13-4.09 (m, 4H), 3.73-3.65 (m, 3H), 2.81-2.74 (m, 1H), 1.54-1.50 (s, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.7 (d, *J* = 268.8 Hz), 155.2, 152.3, 148.8, 142.3, 119.4, 115.4, 94.8 (d, *J* = 168.4 Hz), 87.2 (d, *J* = 17.2 Hz), 63.4, 62.8, 61.5, 39.6 (d, *J* = 16.6 Hz), 14.4; Anal. Calc. for $C_{15}H_{20}F_2N_5O_4P$ (+1.0 MeOH): C, 44.14; H, 5.55; N, 16.08; Found: C, 44.11; H, 5.57; N, 16.09; MS *m/z* 404 $(M+H)^+$. Data for **22b**; UV (MeOH) λ_{max} 308.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.14 (s, 1H), 7.71 (br s, NH_2, 2H), 6.62 (dd, *J* = 20.2, 17.8 Hz, 1H), 6.24 (dd, *J* = 19.6, 17.7 Hz, 1H), 6.12 (dd, *J* = 14.6, 5.0 Hz, 1H), 4.16-4.10 (m, 4H), 3.75-3.68 (m, 3H), 2.82-2.75 (m, 1H), 1.53-1.49 (s, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 158.5, 154.4, 151.7, 149.4, 144.0, 125.2, 114.6, 93.9 (d, *J* = 166.8 Hz), 88.7 (d, *J* = 16.8 Hz), 62.8, 62.2, 61.6, 41.2 (d, *J* = 16.8 Hz), 14.7; Anal. Calc. for $C_{15}H_{20}ClFN_5O_4P$ (+1.0 MeOH): C, 42.53; H, 5.35; N, 15.50; Found: C, 42.48; H, 5.36; N, 15.48; MS *m/z* 420 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-{(3'-Vinyl-2'-fluoro-tetrahydrofuran-1'-yl) guanine} phosphonic acid (23). To a solution of **22b** (159 mg, 0.379 mmol) in dry CH_3CN (15 mL) was added trimethylsilyl bromide (0.0873 mL, 6.62 mmol) at room temperature. The mixture was stirred for 24 h, and solvent was removed by co-evaporation with methanol three times. The residue was dissolved in MeOH (15.0 mL) and 2-mercaptoethanol (105.5 mL, 1.52 mmol), and then NaOMe (80.6 mg, 1.52 mmol) was added. The mixture was refluxed

for 18 h under N_2, cooled, neutralized with glacial AcOH, and evaporated to dryness *in vacuo*. The residue obtained was co-evaporated from conc NH_4OH (2 × 25 mL) and the resultant solid was triturated with acetone (2 × 10 mL). After evaporating the acetone, the residue was purified by preparative column chromatography using reverse-phase C18 silica gel and elution with water. Lyophilization of the appropriate fraction provided **23** (53.52 mg, 39%) as a yellowish salt (ammonium salt). mp 162-164 ºC; UV (H_2O) λ_{max} 254.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 10.8 (br s, NH, 1H), 8.12 (s, 1H), 7.03 (br s, NH_2, 2H), 6.63 (dd, J = 19.4, 17.2 Hz, 1H), 6.17-6.08 (m, 2H), 3.72-3.65 (m, 3H), 2.80-2.75 (m, 1H); ^{13}C NMR (D_2O, 75 MHz) δ 157.4, 154.6, 152.3, 149.4, 136.2, 119.1, 115.3, 96.0 (d, J = 168.1 Hz), 86.9 (d, J = 17.4 Hz), 63.0, 62.5, 61.8, 40.4 (d, J = 17.2 Hz); HPLC t_R = 9.82 min; HRMS $[M-H]^+$ req. 344.0678, found 344.0676.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-ethyl-2'-fluoro-tetrahydrofuran-1-yl) 2-fluoro-6-chloropurine} phosphonate (24). Compound **24** was synthesized from **21** by transfer catalytic hydrogenation similar to that described for **14**: yield 73%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.43 (s, 1H), 6.12 (dd, J = 15.8, 5.0 Hz, 1H), 4.12-4.09 (m, 4H), 3.73-3.66 (m, 3H), 2.81-2.76 (m, 1H), 2.14-2.02 (m, 4H), 1.52-1.50 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 157.1 (d, J = 218.8 Hz, 1H), 153.1, 145.3, 136.2, 121.2, 96.2 (d, J = 166.8 Hz), 88.5 (d, J = 16.7 Hz), 61.8, 41.1 (d, J = 17.2 Hz), 28.5, 18.4, 14.7; Anal. Calc. for $C_{15}H_{20}ClF_2N_4O_4P$ (+1.0 MeOH): C, 42.07; H, 5.29; N, 12.26; Found: C, 42.11; H, 5.31; N, 12.24; MS *m/z* 425 $(M+H)^+$.

(*rel*)-(1'*R*,2'*R*,3'*S*)-Diethyl {9-(3'-ethyl-2'-fluoro-tetrahydrofuran-1-yl) 2-fluoro-6-aminopurine} phosphonate (25a) and (*rel*)-(1'*R*,2'*R*,3'*S*)-diethyl {9-(3'-ethyl-2'-fluoro-tetrahydrofuran-1-yl) 2-amino-6-chloropurine} phosphonate (25b). Ammonolysis of **24** using the same procedure described for **14** gave **25a** and **25b**. Data for **25a**; yield 13%; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.20 (s, 1H), 7.74 (br s, NH_2, 2H, D_2O exchangeable), 6.08 (d, J = 15.8, 5.2 Hz, 1H), 4.14-4.10 (m, 4H), 3.71-3.64 (m, 3H), 2.82-2.75 (m, 1H), 2.12-1.99 (m, 4H), 1.55-1.53 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 161.0 (d, J = 267.8 Hz, 1H), 155.3, 152.3, 142.6, 123.4, 98.6 (d, J = 168.4 Hz), 89.4 (d, J = 16.8 Hz), 60.6, 40.4 (d, J = 17.4 Hz), 29.4, 18.7, 15.4; Anal. Calc. for $C_{15}H_{22}F_2N_5O_4P$ (+1.0 MeOH): C, 43.94; H, 5.99; N, 16.01; Found: C, 43.90; H, 6.01; N, 16.00; MS *m/z* 406 $(M+H)^+$. Data for **25b**; yield 43%; UV (MeOH) λ_{max} 307.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.22 (s, 1H), 7.75 (br s, NH_2, 2H, D_2O exchangeable), 6.13 (d, J = 16.0, 5.0 Hz, 1H), 4.15-4.11 (m, 4H), 3.72-3.67 (m, 3H), 2.81-2.74 (m, 1H), 2.14-2.02 (m, 4H), 1.53-1.52 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 158.6, 154.8, 151.0, 143.8, 125.7, 99.2 (d, J = 167.8 Hz), 88.6 (d, J = 16.4 Hz), 61.7, 41.2 (d, J = 16.7 Hz), 28.7, 19.0, 14.8; Anal. Calc. for $C_{15}H_{22}ClFN_5O_4P$ (+1.0 MeOH): C, 42.34; H, 5.77; N, 15.43; Found: C, 42.30; H, 5.75; N, 15.45; MS *m/z* 422.

(*rel*)-(1'*R*,2'*R*,3'*S*)-9-{(3'-Ethyl-2'-fluoro-tetrahydrofuran-1-yl) guanine} phosphonic acid (26). Nucleoside phosphonic acid **26** was synthesized using the hydrolysis conditions used for **23**. Yield 42%; mp 159-162 ºC; UV (H_2O) λ_{max} 252.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.8 (br s, NH, H, D_2O exchangeable), 8.11 (s, 1H), 7.10 (br s, NH_2, 2H, D_2O exchangeable), 6.11 (d, J = 16.2, 5.2 Hz, 1H), 3.70-3.64 (m, 3H), 2.77-2.71 (m, 1H), 2.09-1.94 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) d 157.8, 154.4, 152.3, 136.5, 119.2, 97.2 (d, J = 168.0 Hz), 90.1 (d, J = 16.4 Hz), 60.5, 40.4 (d, J = 16.4 Hz), 28.5, 18.6, 15.3; HPLC t_R = 10.02 min; HRMS $[M-H]^+$ req. 346.0676, found 346.0675.

(*rel*)-(1'*R*,2'*R*,3'*S*)- Bis(SATE) phosphoester of [9-(3'-ethylphosphonate-2'-fluoro-tetrahydrofuran-1-yl)] adenine (28). A solution of adenine phosphonic acid (ammonium salt) derivative **16** (73.80 mg, 0.212 mmol) and tri-*n*-butylamine (117 mg, 0.636 mmol) in methanol (4.5 mL) was mixed for 30 min and concentrated under reduced pressure. The residue was thoroughly dried with anhydrous ethanol and toluene. The resulting foamy solid was dissolved in anhydrous pyridine (15 mL) to which thioester **27** (649 mg, 4.0 mmol) and 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (251 mg, 0.848 mmol) were added. The mixture was stirred overnight at room temperature and quenched with tetrabutylammonium bicarbonate buffer (12.0 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (70 mL) and extracted with $CHCl_3$ (80 mL) two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.05:4:1) to give **28** (48 mg, 37%) as a white solid: mp 131-133 ºC; UV (MeOH) λ_{max} 262.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.30 (s, 1H), 8.15 (s, 1H), 6.11 (d, J = 16.1, 5.0 Hz, 1H), 4.03-4.02 (m, 4H), 3.56 (d, J = 9.6 Hz, 2H), 3.16 (t, J = 6.4 Hz, 4H), 2.21-2.13 (m, 2H), 1.22-1.16 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.2, 157.1, 154.7, 152.8, 148.2, 145.6, 124.6, 119.4, 96.6 (d, J = 168.2 Hz), 88.8 (d, J = 18.4 Hz), 83.6, 67.5, 67.3, 62.4, 61.6, 53.4, 38.4 (d, J = 16.4 Hz), 30.2, 28.7, 28.5, 23.7, 14.6; Anal. Calc. for $C_{25}H_{39}FN_5O_6PS_2$ (+1.0 MeOH): C, 47.95; H, 6.65; N, 10.75. Found: C, 47.90; H, 6.61; N, 10.79; MS *m/z* 620 $(M+H)^+$.

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