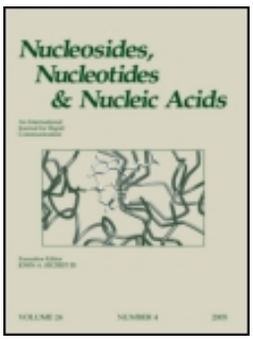

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Synthesis and Antiviral Activity Evaluation of 2′,5′,5′-Trifluoro-Apiosyl Nucleoside Phosphonic Acid Analogs

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ABSTRACT

Racemic synthesis of novel 2′,5′,5′-trifluoro-apiose nucleoside phosphonic acid analogs were performed as potent antiviral agents. Phosphonation was performed by direct displacement of triflate intermediate with diethyl (lithiodifluoromethyl) phosphonate to give the corresponding (α,α-difluoroalkyl) phosphonate. Condensation successfully proceeded from a glycosyl donor with persilylated bases to yield the nucleoside phosphonate analogs. Deprotection of diethyl phosphonates provided the target nucleoside analogs. An antiviral evaluation of the synthesized compounds against various viruses such as HIV, HSV-1, HSV-2, and HCMV revealed that the pyrimidine analogues have significant anti-HCMV activity.

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antiviral agents; 2′,5′,5′-trifluoro-apiose nucleoside phosphonic acid analogues; Vorbrüggen reaction

Introduction

The modification of the nucleosides and/or sugar moiety of a natural nucleoside is an obvious choice for developing new antiviral compounds, and apiose-based nucleoside could serve this purpose.

Recently, apiose 5′-nor nucleoside phosphonate,[1] such as, PMDTA (**1**), has been synthesized and has shown promising anti-HIV properties. Herdewijn *et al* reported the synthetic procedure of 3′-*C*-ethynyl analogue of PMTA (**2**).[2] This absence of a 4′-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases.

Phosphonates and structurally modified phosphonatesisosters can mimic phosphates in biological system.[3] The resistance of the phosphorus-carbon phosphonate linkage to hydrolysis by chemical agents or esterases is one of the features responsible for their increasing popularity. Fluoro-substitution at the α-carbon of phosphonates may increase the effectiveness of these phosphate mimetics as a result both geometric and electronic factors.[4] The replacement of phosphonates by fluorophosphonates has provided a number of analogues showing significant biological activity.[5]

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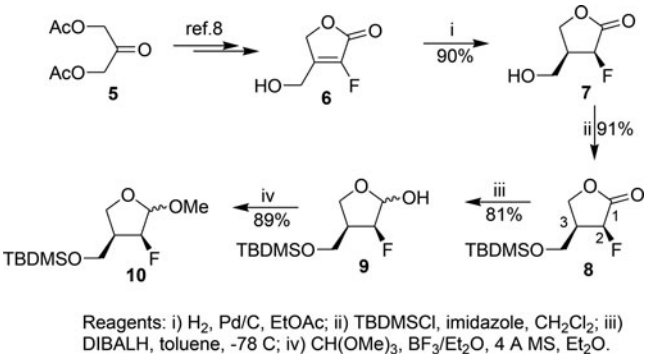


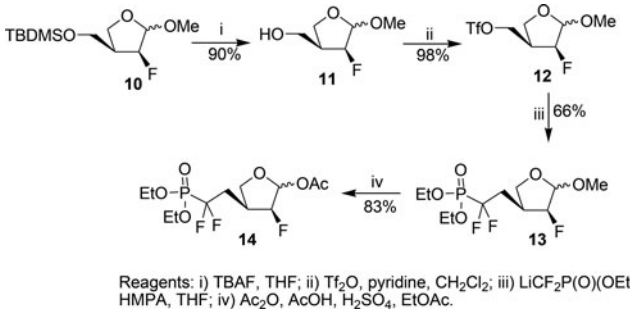
Scheme 1. Synthesis of fluorinated apioseglycosyl donor intermediate **10**.

9-(5,5-Difluoro-5-phosphonopentyl)guanine (**3**) has been utilized as a substrate analogue inhibitor of purine nucleoside phosphorylase.[6] 2-Chloro-2′,5′-dideoxy-5′-difluoromethylphosphinyl adenosine (2CDPA, **4**), the nonhydrolyzable analogue of 2-chlorodeoxyadenosine monophosphate was prepared for the treatment of refractory chronic leukemia and hairy cell leukemia to overcome the undesired metabolic pathway of 2CDA.[7] However, biological testing performed on various T cells showed that 2CDPA does not exhibit expected cytotoxic effect. The lack of cytotoxicity is probably caused by an insufficient level of phosphorylation inside T cells.

On the basis of the above encouraging results, we undertook the synthesis of isosteric and isopolar 5′-difluoromethylphosphonatederivatives of apiosyl 5′-nornucleosidephosphonateto find more effective antiviral agents.

Results and Discussion

Target compounds were synthesized from lactone derivative **6**, which was readily obtained from 1,3-dihydroxyacetone, as previously described (Scheme 1).[8] Hydrogenation of 2-fluoro-butenolide **6** to 2-fluorolactone **7** was achieved with 5% Pd/C under H_2 treatment with a yield of 90%. Protection[9] of **7** with TBDMSCl in methylene chloride at 25°C furnished the desired *O*-silyl ether **8**, which was converted to lactol**9** by DIBALH reduction in toluene at −78°C for 1.0 h in 74% two step yield. Protection of anomeric position was needed prior to phosphonation. Hence, methoxylation of anomeric position furnished glycoside **10** as anomeric mixture in a 89% yield using the conditions [$CH(OMe)_3$, BF_3/Et_2O] even in the presence of acid labile silyl protection group.[10] Removal of the TBDMS group of glycoside **10** by TBAF furnished alcohol **11** as anomeric mixture with a 90% yield which was converted to anomeric mixture of difluorophosphonate**13** using triflation followed by a triflate displacement according to the procedure of Berkowitz *et al.*[11] The preparation of a suitable glycosylating agent **14** was attempted by direct acetolysis of **13** under acidic conditions (Ac_2O, AcOH, H_2SO_4, EtOAc, 0°C)[12] to afford an anomeric mixture of 1-*O*-acetyl-furanoside **14** in a 83% yield (Scheme 2). The synthesis of adenine nucleoside was performed using a Vorbrüggencondensation[13] of



Scheme 2. Synthesis of fluorinated apioseglycosyl donor intermediate **14**.

compound **14** with silylated 6-chloropurine and trimethylsilyltriflate(TMSOTf) as a catalyst in dichloroethane (DCE) to yield the protected 6-chloropurine derivatives, **15**α and **15**β, respectively. A complete nuclear overhauser effect (NOE) study between proximal hydrogens verified their relative stereochemistry (Figure 2). NOE experiments of both products showed that glycosylation in α-direction is isomer **15**α(NOE: $H_{1\beta}/H_{3\alpha}$, 0.9%), and glycosylation of β-direction is isomer **15**β(NOE: $H_{1\alpha}/H_{3\alpha}$, 1.7%). The chlorine group from purine analog **15**β was then converted to an amine with methanolic ammonia at 68°C to produce the adenosine phosphonate derivative **16** in 67%yield. Hydrolysis of the diethyl phosphonate functional groups of **16** with bromotrimethylsilane treatments in CH_3CN in the presence of 2,6-lutidine yielded adenosine phosphonic acid derivative **17** (Scheme 3).[14] The structure of **17** was unambiguously determined by spectroscopical analysis: ^{1}H NMR, ^{13}NMR, ^{31}P NMR, UV, HRMS.

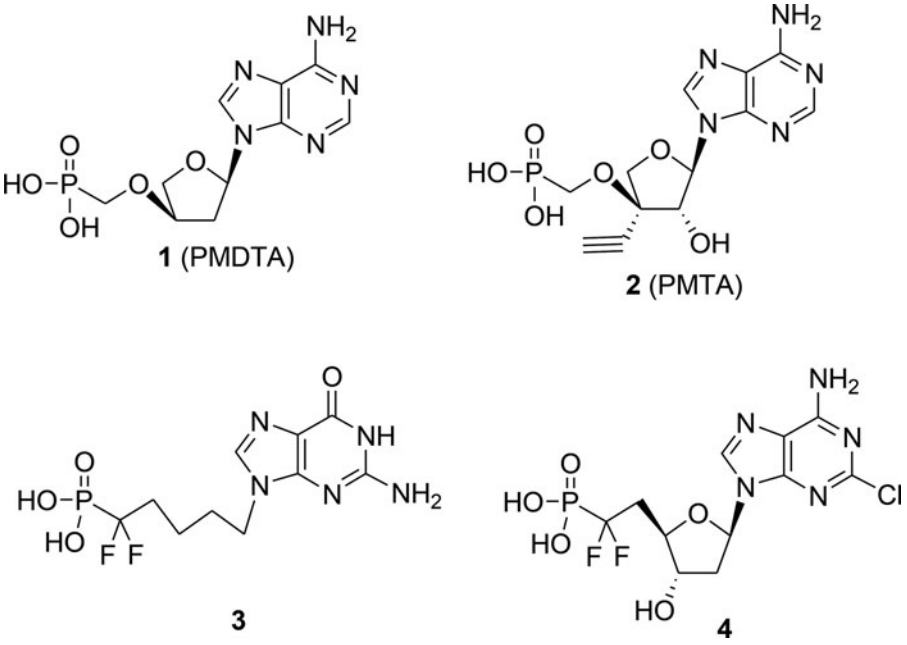
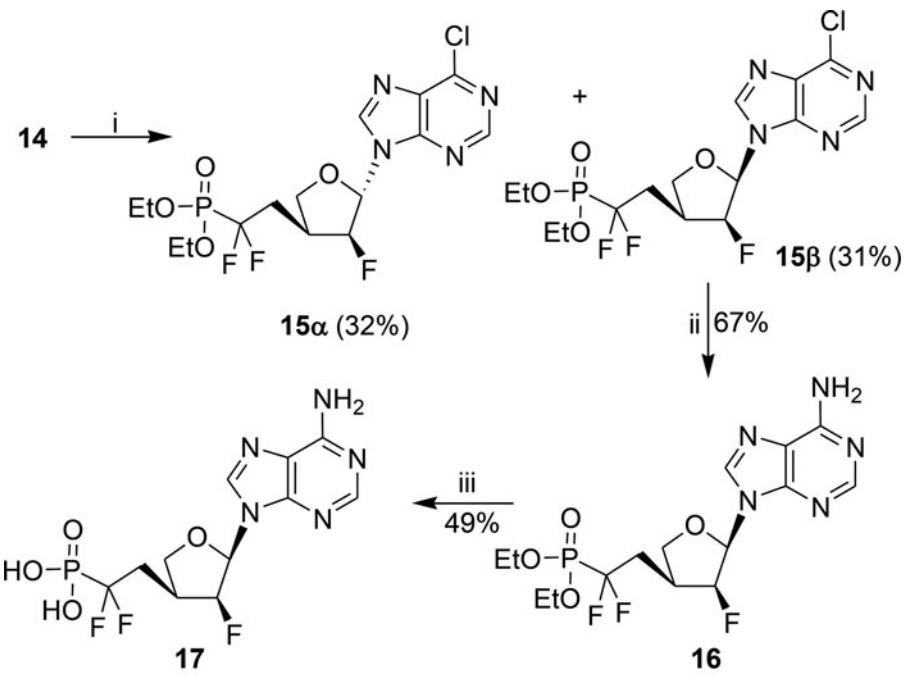


Figure 1. Synthesis rationale of 5′,5′-difluoro and apiose nucleoside phosphonic acids showing potent biological activity.



Scheme 3. Synthesis of 2′,5′,5′-trifluoro-apiosyl adenosine phosphonic acid analogues.

Condensation of 2-fluoro-6-chloropurine[15] with glycosyl donor **14** proceeded under conditions similar to those used for synthesis of analogues **15**α and **15**β to yield **18**α (35%) and **18**β (36%), respectively. The relative stereo chemistries of purine analogs**18**α and **18**β were determined by the study of NOE experiments between proximal diastereotropichydrogens.

Mild bubbling ammonia into compound **18**β in DME yielded 2-fluoro-6-aminopurine[16] analogue **19** (11%) and 2-amino-6-chloropurine analogue **20**(41%), respectively. In nucleophilic aromatic substitution, fluorine atom is better leaving group than chlorine atom. The 2-amino-6-chloropurine derivative **20** was treated with TMSBr and 2,6-lutidine to yield phosphonic acid and was then treated with sodium methoxide and 2-mercaptoethanol in methanol to produce guanosinephosphonic acid **21** (Scheme 4).[17]

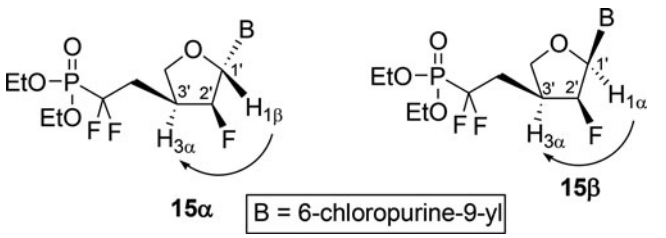
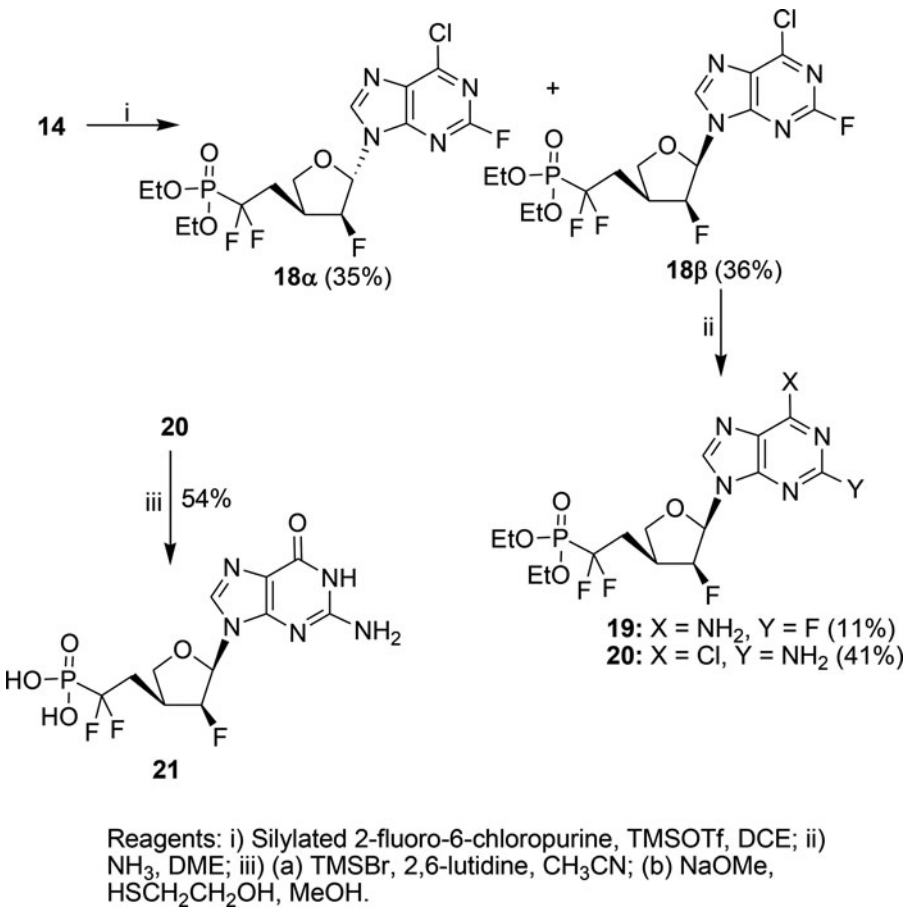


Figure 2. NOE differences between the proximal hydrogens of **15**α and **15**β.

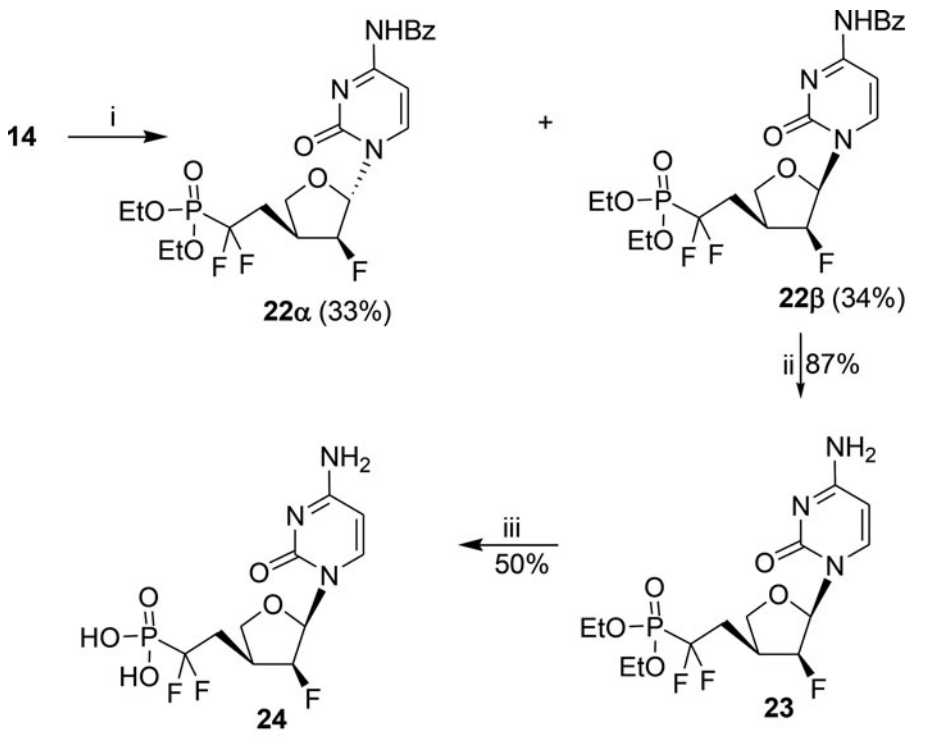


Scheme 4. Synthesis of 2′,5′,5′-trifluoro-apiosyl guanosinephosphonic acid analogues.

Condensation of N^4-benzoyl cytosine with glycosyl donor **14** proceeded under conditions similar to those used for the synthesis of adenine analogues to yield **22***α* (33%) and **22***β* (34%), respectively. Ammonolysis of **22***β* followed by deprotection of diethyl phosphonate furnished the target cytosine phosphonic acid **24** (Scheme 5). Also, uracil and thymine nucleoside analogues **27** and **28** were also prepared from **14** *via* condensation and deprotection procedures (Scheme 6).

Biological activity evaluation

The antiviral assay against several viruses such as the human immunodeficiency virus 1 (HIV-1), herpes simplex virus-1,2 (HSV-1,2) and human cytomegalovirus (HCMV) was performed. As shown in Table 1, compound pyrimidine analog **28** exhibited moderate or weak antiviral activity against HCMV in the Davis cell without any cytotoxicity up to 100 μmol.[18] The reason for the lower antiviral activity of final phosphonic acids than their ester analogies might be the result of poor cell membrane diffusion of phosphonate ions *in vitro* conditions. This suggests that this



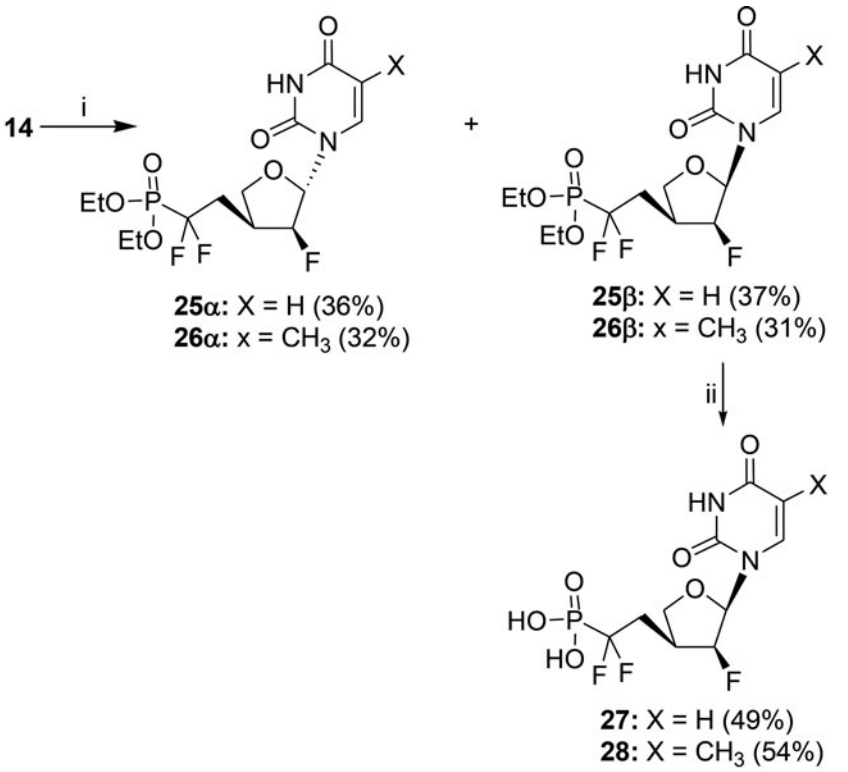
Scheme 5. Synthesis of 2′,5′,5′-trifluoro-apiosyl cytosine phosphonic acid analogues.

class of apiosyl nucleoside, which has fluorine group in the 2′-position, can be a novel structural template for the development of new anti-HCMV agents.

In summary, based on the potent biological activities of the fluorinated phosphonate nucleosides and apiosylnucleoside phosphonic acid analogues, we designed and successfully synthesized novel 5′,5′-difluoro-2′-fluoroapiosyl nucleosidephosphonic acid analogues from 1,3-diacetylacetone. Among them, pyrimidine analog **28** showed significant anti-HCMV activity.

Experimental Section

Uncorrected melting points were determined using a Mel-temp II laboratory device. Nuclear magnetic resonance (NMR) spectra were recorded using 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). Ultraviolet (UV) spectra were obtained using a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). Mass spectra (MS) were collected in electrospray ionization (ESI) mode. Elemental analyses were performed using a Perkin–Elmer 2400 analyzer (Perkin–Elmer, Norwalk, CT, USA). Thin layer chromatography (TLC) was performed on



Scheme 6. Synthesis of 2′,5′,5′-trifluoro-apiosyl uracil and thymine phosphonic acid analogues.

Table 1. The antiviral activity of the synthesized compounds

	HIV-1EC_{50}(μM)	HSV-1EC_{50}(μM)	HSV-2EC_{50}(μM)	HCMVEC_{50}(μM)	CytotoxicityCC_{50}(μM)
16	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100
19	79.0	>100	>100	>100	>100
20	72.4	>100	>100	>100	>100
21	66.3	>100	>100	>100	>100
23	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100
25β	>100	>100	>100	42.4	>100
26β	>100	>100	>100	20.1	95
27	>100	>100	>100	40.1	>100
28	>100	>100	>100	15.3	90
AZT	0.007	ND	ND	ND	2.56
GCV	ND	ND	ND	0.45	>10
ACV	ND	0.3	ND	ND	>100

AZT: Azidothymidine; GCV: Ganciclovir; ACV: Acyclovir
ND: Not Determined
EC_{50}(μM): Concentration required to inhibit 50% of the virus induced cytopathicity
CC_{50}(μM): Concentration required to reduce the cell viability by 50%

Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were performed in a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry tetrahydrofuran (THF) was obtained by distillation from Na and benzophenone immediately prior to use.

(rel)-(2S,3S)-2-Fluoro-dihydro-3-(hydroxymethyl)furan-1(3H)-one (7)

To a solution of lactone **6** (3.168 g, 24mmol) in 120 mL of EtOAc, 1.2 g of Pd/C (5% w/w) was added under H_2 atmosphere; the mixture was stirred for 6 h. After filtration of the reaction mixture through a celite pad, the filtrate was concentrated and purified using silica gel column chromatography (EtOAc/hexane, 1:3) to yield compound **7** (2.89 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.41 (dd, J= 8.8, 7.2 Hz, 1H), 4.25–4.08 (m, 2H), 3.64 (dd, J=9.2, 6.8 Hz, 1H), 3.35 (dd, J=9.2, 8.2 Hz, 1H), 2.47–2.39 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.6 (d, J= 22.4 Hz), 104.9 (d, J= 208.6 Hz), 64.7, 63.2, 34.6 (d, J= 21.8 Hz); Anal. Calcd. for $C_5H_7FO_3$: C, 44.78; H, 5.26; found: C, 44.65; H, 5.34; MS m/z135 $(M + H)^+$.

(rel)-(2S,3R)-2-Fluoro-dihydro-3-(t-butyldimethylsilyloxymethyl)furan-1(3H)-one (8)

t-Butyldimethylsilyl chloride (TBDMSCl) (1.54 g, 10.24 mmol) was added slowly at 0°C to a solution of **7** (1.15 g, 8.62 mmol) and imidazole (1.17 g, 17.24 mmol) in CH_2Cl_2 (40 mL), and stirred for 6 h at room temperature. The solvent was evaporated under reduced pressure. The residue was diluted with H_2O (100 mL) and extracted twice with ethyl acetate (EtOAc) (100 mL $\times$2). The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:3) to yield compound **8** (1.94 g, 91%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.40–4.35 (dd, J= 11.4, 8.8 Hz, 1H), 4.26–4.12 (m, 2H), 3.86 (dd, J= 10.8, 6.6 Hz, 1H), 3.65 (dd, J= 10.8, 8.2 Hz, 1H), 2.48–2.35 (m, 1H), 0.87 (m, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.4 (d, J= 23.6 Hz), 103.7 (d, J= 202.6 Hz), 65.4, 63.9, 35.5 (d, J= 20.4 Hz), 25.6, 18.4, −4.6; Anal. Calcd. for $C_{11}H_{21}FO_3Si$: C, 53.19; H, 8.52; found: C, 53.24; H, 8.59; MS m/z 249 $(M + H)^+$.

(rel)-(1R/1S,2S,3R)-[(2-Fluoro-tetrahydro-1-hydroxyfuran-3-yl)methoxy](t-butyl) dimethylsilane (9)

A solution of compound **8** (1.05 g, 4.26mmol) in toluene (50 mL) was treated with 8.52 mL of 1 M DIBAL-H in hexane at −78°C for 1 h. The reaction was quenched with 2 mL of methanol (MeOH) and warmed to room temperature for 1 h before aqueous (aq) $NaHCO_3$ (4 mL) and EtOAc (50 mL) were added to the mixture.

The resulting mixture was filtered and the filtrate was concentrated to dryness. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:6) to yield compound **9** (863mg, 81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (d, J = 16.4 Hz, 0.5H),5.68 (d, J =14.8 Hz, 0.5H), 3.89–3.84 (m, 2H), 3.67–3.51 (m, 3H), 2.31–2.24 (m, 1H), 0.89 (m, 9H), 0.02 (m, 6H); Anal. Calcd. for $C_{11}H_{23}FO_3Si$: C, 52.77; H, 9.26; found: C, 52.90; H, 9.34.

(rel)-(1R/1S,2S,3R)-[(2-Fluoro-tetrahydro-1-methoxyfuran-3-yl)methoxy](t-butyl) dimethylsilane (10)

Lactol**9** (1.84 g, 7.38 mmol) was dissolved in anhydrous diethyl ether (25 mL), and powdered anhydrous molecular sieves (4 Å, 0.18 g) were added. With stirring, methyl orthoformate (1.62 mL, 14.4 mmol) and $BF_3 \cdot OEt_2$ (224 μL) were added, and stirred for 40 min. The reaction mixture was quenched with Et_3N and brine until neutral. The mixture was extracted with diethyl ether, dried over anhydrous $MgSO_4$, and concentrated to give a residue. The residue was purified by using silica gel column chromatography (EtOAc/hexane, 1:20) to yield compound **10** (1.73 g, 89%) as diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.46 (d, J=17.7Hz, 0.5H), 5.37 (d, J=15.6 Hz, 0.5H), 3.97–3.82 (m, 3H), 3.73–3.56 (m, 2H), 2.25–2.18 (m, 1H), 0.89 (m, 9H), 0.01 (m, 6H); Anal. Calcd. for $C_{12}H_{25}FO_3Si$: C, 54.51; H, 9.53. Found: C, 54.39; H, 9.47; MS m/z265 $(M + H)^+$.

(rel)-(1R/1S,2S,3S)-(2-Fluoro-tetrahydro-1-methoxyfuran-3-yl)methanol (11)

To a solution of compound**10** (620 mg, 2.34 mmol) in THF (15 mL), TBAF (3.52 mL, 1.0 M solution in THF) at 0°C was added. The mixture was stirred at room temperature for 5 h, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:5) to give compound**11** (316 mg, 90%) as diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.42–5.31 (m, 1H), 4.02–3.98 (m, 1H), 3.85–3.76 (m, 1H), 3.64–3.47 (m, 2H), 3.38–3.29 (m, 1H), 3.25, 3. 24 (s, s, 3H), 2.21–2.12 (m, 1H); Anal. Calcd. for $C_6H_{11}FO_3$: C, 48.00; H, 7.38. Found: C, 48.12; H, 7.45; MS m/z151 $(M + H)^+$.

(rel)-(1R/1S,2S,3R)-(2-Fluoro-tetrahydro-1-methoxyfuran-3-yl)methyl trifluoromethanesulfonate (12)

To a cooled solution (−78°C) of glycoside **11** (324 mg, 2.16 mmol) in pyridine (0.854 mL, 10.8 mmol) and CH_2Cl_2 (25 mL), triflic anhydride (730 mg, 2.59 mmol) was slowly added. After 3.5 h, the reaction mixture was poured onto a mixture of ice and sodium hydrogen carbonate. The aqueous layer was extracted with CH_2Cl_2 (3× 50 mL), and the combined CH_2Cl_2 solution were dried, and rapidly and repeatedly concentrated with toluene to remove any residual pyridine. The residue was extracted with light petroleum (3× 50 mL), and the combined extracted were filtered and cooled. After careful evaporation of additional solvent,

the crude residue **12** (597 mg, ~98%) was subjected to next reaction without further purification.

(rel)-Diethyl 5,5-difluoro-4-[(1R/1S,2S,3S)-2-fluoro-tetrahydro-1-methoxyfuran-3-yl] ethylphosphonate (13)

To a solution of diisopropylamine (471 μL, 3.36 mmol) and HMPA (584 μL, 3.36 mmol) at −78°C in THF (7 mL) under Ar was added *n*-butyllithium (2.1 mL of a 1.6 M solution in hexane, 3.36 mmol). The resulting solution was allowed to stir for 30 min at 0°C and then cooled to −78°C. To this solution of LDA at −78°C were added *via* cannula, a (−78°C) solution of diethyl (α,α-difluoromethyl) phosphonate (631 mg, 3.36 mmol) in THF (2.8 mL), and, 3 min later, a (−78°C) solution of triflate**12** (270 mg, 0.96 mmol) in THF (2.8 mL), dropwise, *via* cannula. After 10 min at −78°C, the reaction was quenched by adding aqueous NH_4Cl (16.0 mL) and Et_2O (16.0 mL). The aqueous layer was further extracted with EtOAc (2× 65 mL), and the combined organic extracts were dried, filtered, and evaporated. Silica gel flash chromatography (EtOAc/hexane, 1:2) gave **13** (202 mg, 66%) as a form. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.44–4.32 (m, 1H), 4.25–4.18 (m, 4H), 3.99–3.78 (m, 2H), 3.61–3.56 (m, 1H), 3.25, 3.24 (s, s, 3H), 2.35–2.16 (m, 3H), 1.24 (m, 6H); Anal. Calcd. for $C_{11}H_{20}F_3O_5P$: C, 41.26; H, 6.29. Found: C, 41.14; H, 6.32; MS *m/z*321 $(M + H)^+$.

(rel)-Diethyl 4-[(1R/1S,2S,3S)-1-acetoxy-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (14)

Glycoside **13** (505 mg, 1.58 mmol)was dissolved in EtOAc (13 mL), mixed with a solution of EtOAc (25 mL), acetic anhydride (14.5 mL), acetic acid (11.0 mL) and conc H_2SO_4 (0.066 mL) at −10°C, and stirred for 24 h at room temperature. The reaction was diluted with $CHCl_3$ (100 mL) and poured into cold 5% aqueous $NaHCO_3$ (165 mL). The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (3× 33 mL). The combined organic layers were washed with brine, dried, and evaporated to dryness. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:2) to yield compound **14** (456mg, 83%) as a form. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55–6.42 (m, 1H), 4.27–4.19 (m, 5H), 3.86–3.70 (m, 1H), 2.63–2.53 (m, 1H), 2.29–2.18 (m, 3H), 2.03, 2.01 (s, s, 3H), 1.26–1.21 (m, 6H); Anal. Calcd. for $C_{12}H_{20}F_3O_6P$: C, 41.39; H, 5.79. Found: C, 41.44; H, 5.86; MS *m/z*349 $(M + H)^+$.

(rel)-Diethyl 4-[(1S,2S,3S)-1-(6-chloro-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (15α) and (rel)-diethyl 4-[(1R,2S,3S)-1-(6-chloro-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (15β)

6-Chloropurine (266 mg, 1.72 mmol), anhydrous HMDS (12 mL), and a catalytic amount of ammonium sulfate (12 mg) were refluxed to a clear solution (18 h); the solvent was then distilled under anhydrous conditions. The residue obtained

was dissolved in anhydrous 1,2-dichloroethane (10 mL), and to this mixture, a solution of **14** (299 mg, 0.86 mmol) in dry DCE (10 mL) and TMSOTf (382 mg, 1.72mmol) was added, and stirred for 6 h at rt. The reaction mixture was quenched with 10.0 mL of saturated $NaHCO_3$, stirred for 2 h, filtered through a Celite pad, and the filtrate obtained was then extracted twice with CH_2Cl_2 (2×100 mL). Combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified using silica gel column chromatography (EtOAc/hexane/MeOH, 3:1:0.03) to yield compounds **15**α (121 mg, 32%) and **15**β (117 mg, 31%), respectively. Data for **15**α: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.78 (s, 1H), 8.26 (s, 1H), 6.23 (dd, J=16.4, 6.0 Hz, 1H), 4.27–4.23 (m, 4H), 3.87 (dd, J= 10.8, 6.2 Hz, 1H), 3.68–3.51 (m, 2H), 2.22–2.13 (m, 1H), 1.74–1.61 (m, 2H), 1.27 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.64 (t, $J_{P,F}$= 101.6 Hz); Anal. Calc. for $C_{15}H_{19}ClF_3N_4O_4P$: C, 40.69; H, 4.33; N, 12.65. Found: C, 40.82; H, 4.40; N, 12.78; MS m/z443 $(M + H)^+$; Data for **15**β: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.24 (s, 1H), 6.19 (dd, J=18.2, 6.8 Hz, 1H), 4.30–4.25 (m, 4H), 3.92 (dd, J= 11.0, 6.4 Hz, 1H), 3.71–3.53 (m, 2H), 2.25–2.14 (m, 1H), 1.80–1.67 (m, 2H), 1.29–1.28 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.70 (t, $J_{P,F}$= 104.2 Hz); Anal. Calc. for $C_{15}H_{19}ClF_3N_4O_4P$ (+0.5 MeOH): C, 40.64; H, 4.62; N, 12.23. Found: C, 40.55; H, 4.57; N, 12.32; MS m/z443 $(M + H)^+$.

(rel)-Diethyl 4-[(1R,2S,3S)-1-(6-amino-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (16)

A solution of **15**β (362 mg, 0.82mmol) in saturated methanolic ammonia (15 mL) was stirred overnight at 65°C in a steel bomb and the volatiles were evaporated. The residue was purified using silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:12) to yield **16** (232 mg, 67%) as a white solid: UV (MeOH) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ8.41 (s, 1H), 8.17 (s, 1H), 7.40 (br s, 2H, D_2O exchangeable), 6.25 (dd, J=17.8, 6.2 Hz, 1H), 4.26–4.22 (m, 4H), 3.87–3.70 (m, 2H), 3.58 (dd, J= 10.6, 6.4 Hz, 1H), 2.26–2.18 (m, 1H),2.03–1.84 (m, 2H), 1.31–1.28 (m, 6H);^{31}P (121.5 MHz, DMSO-d_6) δ7.54 (app t, $J_{P,F}$= 103.2 Hz); Anal. Calc. for $C_{15}H_{21}F_3N_5O_4P$ (+1.0 MeOH): C, 42.22; H, 5.53; N, 15.39; Found: C, 42.43; H, 5.65; N, 15.49; MS m/z424 $(M + H)^+$.

(rel)-4-[(1R,2S,3S)-1-(6-Amino-9H-purin-9-yl)-tetrahydrofuran-3-yl]-2-fluoro-5,5-difluoroethyl-phosphonic acid sodium salt (17)

To a solution of compound **16** (139 mg, 0.33 mmol) and 2,6-lutidine (2.3 mL, 19.8 mmol) in 24 mL of dry CH_3CN was added bromotrimethylsilane (1.01 g, 6.6 mmol) at room temperature under nitrogen. The reaction mixture was continuously refluxed for 24 h. The reaction mixture was concentrated under high vacuum at room temperature, and the residue was coevaporated with MeOH and 0.5

M TEAB solution. Purification by HPLC using reverse phase C_{18} and ion exchange with Dowex-Na^+ resin offered **17** (63 mg, 49%) as a colorless solid (sodium salt) after lyophilization. ^{1}H NMR (D_2O, 300 MHz) δ8.38 (s, 1H), 8.18 (s, 1H), 6.19 (dd, J=18.0, 6.2 Hz, 1H), 3.82–3.63 (m, 2H), 3.58 (dd, J= 10.2, 6.6 Hz, 1H), 2.61 (dd, J= 9.8, 6.4 Hz, 1H), 2.26–2.18 (m, 2H), 1.84–1.65 (m, 1H); ^{13}C NMR (D_2O, 75 MHz) δ 155.8, 153.1, 149.4, 140.2, 125.4 (dt, J=211.2,270.6 Hz), 119.7,96.2 (d, J= 202.6 Hz), 89.6, (d, J= 24.7 Hz), 68.4, 24.4 (d, J= 22.8 Hz), 19.2 (dd, J= 25.4, 19.7 Hz); ^{31}P (121.5 MHz, D_2O) δ5.82 (dd, $J_{P,F}$= 105.3, 90.2 Hz); HPLC t_R= 10.24; HRMS $[M\text{-}H]^+$ req. 366.0745, found 366.0743.

(rel)-Diethyl 4-[(1S,2S,3S)-1-(2-fluoro-6-chloro-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (18α) and (rel)-diethyl 4-[(1R,2S,3S)-1-(2-fluoro-6-chloro-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (18β)

Condensation of **14** with 2-fluoro-6-chloropurine under Vorbrüggen condensation conditions similar to those described for **15**α and **15**β yielded **18**α and **18**β, respectively. Data for **18**α: yield 35%; UV (MeOH) λ_{max} 268.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.26 (s, 1H), 6.25 (dd, J=18.8, 6.0 Hz, 1H), 4.29–4.27 (m, 4H), 3.87–3.66 (m, 2H), 3.57 (dd, J= 10.4, 7.2 Hz, 1H), 2.21–2.09 (m, 1H), 1.84–1.78 (m, 2H), 1.31 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.32 (t, $J_{P,F}$= 106.8 Hz); Anal. Calc. for $C_{15}H_{18}ClF_4N_4O_4P$: C, 39.10; H, 3.94; N, 12.16; Found: C, 39.17; H, 3.89; N, 12.18; MS m/z461 $(M + H)^+$.data for **18**β: yield 36%; UV (MeOH) λ_{max} 268.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.30 (s, 1H), 6.22 (dd, J=19.0, 6.2 Hz, 1H), 4.31–4.29 (m, 4H), 3.85 (dd, J= 10.4, 7.0 Hz, 1H), 3.60–3.43 (m, 2H), 2.28–2.14 (m, 1H), 1.78–1.62 (m, 2H), 1.29 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.25 (t, $J_{P,F}$= 105.6 Hz); Anal. Calc. for $C_{15}H_{18}ClF_4N_4O_4P$: C, 39.10; H, 3.94; N, 12.16; Found: C, 39.03; H, 3.98; N, 12.11; MS m/z461 $(M + H)^+$.

(rel)-Diethyl 4-[(1R,2S,3S)-1-(2-fluoro-6-amino-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (19) and (rel)-diethyl 4-[(1R,2S,3S)-1-(2-amino-6-chloro-9H-purin-9-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (20)

Dry ammonia gas was bubbled into a stirred solution of **18**β (450 mg, 0.98mmol) in DME (12.0 mL) at room temperature overnight. Salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue obtained was purified using silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:10) to produce **19** (47 mg, 11%) and **20** (183 mg, 41%). Data for **19**; UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.32 (s, 1H), 7.70 (br s, NH_2, 2H, D_2O exchangeable), 6.21 (dd, J=18.5, 6.2 Hz, 1H), 4.30–4.28 (m, 4H), 3.88–3.69 (m, 2H), 3.61–3.55 (dd, J= 9.8, 6.6 Hz, 1H), 2.26–2.12 (m, 1H),1.70–1.58 (m, 2H), 1.31 (m, 6H); ^{31}P (121.5

MHz, DMSO-d_6) δ 7.13 (t, $J_{P,F}$= 109.5 Hz); Anal. Calc. for $C_{15}H_{20}F_4N_5O_4P$ (+1.0 MeOH): C, 40.61; H, 5.11; N, 14.80; Found: C, 40.83; H, 5.19; N, 14.76; MS *m/z* 442 $(M + H)^+$. Data for **20**; UV (MeOH) λ_{max} 307.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.14 (s, 1H), 7.67 (br s, NH_2, 2H, D_2O exchangeable), 6.21 (dd, *J*=19.1, 6.4 Hz, 1H),4.30 (m, 4H), 3.86 (dd, *J*= 9.7, 7.4 Hz, 1H), 3.65–3.51 (m, 2H), 2.32–2.25 (m, 1H),1.82–1.76 (m, 2H), 1.30–1.28 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ7.11 (t, $J_{P,F}$= 107.2 Hz); Anal. Calc. for $C_{15}H_{20}ClF_3N_5O_4P$ (+1.0 MeOH): C, 39.29; H, 4.94; N, 14.32; Found: C, 39.44; H, 4.87; N, 15.22; MS *m/z* 458 $(M + H)^+$.

(rel)-4-[(1R,2S,3S)-1-(2-Amino-6-oxo-9H-purin-9-yl)-tetrahydrofuran-3-yl]-2-fluoro-5,5-difluoroethyl-phosphonic acid sodium salt(21)

To a solution of **20** (278 mg, 0.61 mmol) and 2,6-lutidine (3.92 g, 36.6 mmol) in dry CH_3CN (24.4 mL), trimethylsilyl bromide (1.87 g, 12.2 mmol) was added at room temperature. The mixture was stirred for 24 h and the solvent was removed using evaporation with MeOH three times. The residue was dissolved in MeOH (24.4 mL) and 2-mercaptoethanol (190 mg, 2.44 mmol), and then NaOMe (131 mg, 2.44 mmol) was added. The mixture was refluxed for 18 h under N_2, cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue was purified by a C_{18} reverse-phase column chromatography with a gradient from 100% triethylammonium acetate buffer (TEAA buffer, 50 mM) to a mixture of 80% TEAA buffer and 20% CH_3CN (t_R 9.90 min), and ion exchange with Dowex-Na^+ resin yielded **21** (133 mg, 54%) as a colorless solid (sodium salt) after lyophilization. ^{1}H NMR (D_2O, 300 MHz) δ 7.95 (s, 1H), 6.18 (dd, *J*=17.8, 6.2 Hz, 1H), 3.90–3.75 (m, 2H), 3.60–3.56 (dd, *J*= 9.8, 7.2 Hz, 1H), 2.26–2.17 (m, 1H), 1.78–1.65 (m, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 157.8, 154.7, 152.5, 135.6, 128.4 (ddd, *J* = 208.2, 260.8, Hz), 118.3, 97.4 (d, *J* = 210.5 Hz),79.3 (d, *J* = 26.0 Hz), 65.9, 24.6 (d, *J* = 24.8 Hz), 19.6 (dd, *J* = 23,8, 18.6 Hz); ^{31}P (121.5 MHz, D_2O) δ5.89 (dd, $J_{P,F}$ = 110.2, 88.4 Hz); HPLC t_R= 9.90 min; HRMS $[M\text{-}H]^+$ req. 382.0756, found 382.0754.

(rel)-Diethyl 4-[(1S,2S,3S)-1-(N_4-benzoylamino-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (22α) and (rel)-diethyl 4-[(1R,2S,3S)-1-(N_4-benzoylamino-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (22β)

Condensation of **14** with N_4-benzoyl cytosine under Vorbrüggen condensation conditions similar to those described for **15**α and**15**β yielded **22**α and **22**β as solids. Data for **22**α: yield 33%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.18 (d, *J* = 7.0 Hz, 1H), 8.03–7.98 (m, 2H), 7.65–7.54 (m, 4H), 6.21 (dd, *J* =18.6, 6.2 Hz, 1H), 4.29–4.26 (m, 4H), 4.18–4.10 (m, 1H), 3.86 (dd, *J* = 10.2, 7.6 Hz, 1H), 3.59 (dd, *J* = 10.2, 6.8 Hz, 1H), 2.34–2.25 (m, 1H), 1.78–1.65 (m, 2H), 1.32 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.32 (t, $J_{P,F}$= 106.2 Hz); Anal. Calc. for $C_{21}H_{25}F_3N_3O_6P$: C, 50.10; H, 5.01; N, 8.35;

Found: C, 50.21; H, 4.95; N, 8.28; MS *m/z*504 $(M + H)^+$.data for **22**β: yield 34%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20 (d, $J = 7.2$ Hz, 1H), 8.05–7.99 (m, 2H), 7.63–7.55 (m, 4H), 6.19 (dd, $J = 18.4$, 6.4 Hz, 1H), 4.24–4.19 (m, 4.5H), 4.16–4.11 (m, 0.5H), 3.88 (dd, J= 10.4, 7.0 Hz, 1H), 3.65 (dd, $J = 10.4$, 6.2 Hz, 1H), 2.37–2.28 (m, 1H), 1.72–1.63 (m, 2H), 1.32–1.29 (m, 6H); ^{31}P (121.5 MHz, $CDCl_3$) δ7.27 (t, $J_{P,F} = 107.4$ Hz); Anal. Calc. for $C_{21}H_{25}F_3N_3O_6P$ (+0.5 MeOH): C, 49.74; H, 5.24; N, 8.09; Found: C, 49.87; H, 5.18; N, 8.12; MS *m/z*504 $(M + H)^+$.

(rel)-Diethyl 4-[(1R,2S,3S)-1-(4-amino-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (23)

Compound **22**β (442 mg, 0.88 mmol) was treated with saturated methanolic ammonia (12 mL) overnight at rt. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$/1:10) to give compound **23** (305 mg, 87%): UV (MeOH) λ_{max} 270.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.75 (d, $J = 7.0$ Hz, 1H), 7.27 (br d, 2H, D_2O exchangeable), 6.15 (dd, $J = 19.2$, 7.0 Hz, 1H), 5.75 (d, $J = 7.0$ Hz, 1H), 4.24–4.20 (m, 4.5H), 4.13–4.09 (m, 0.5H), 3.86 (dd, J= 10.8, 6.8 Hz, 1H), 3.70 (dd, $J = 10.8$, 7.2 Hz, 1H), 2.36–2.23 (m, 1H), 2.01–1.92 (m, 2H), 1.27–1.24 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ7.08 (t, $J_{P,F} = 108.2$ Hz); Anal. Calc. for $C_{14}H_{21}F_3N_3O_5P$ (+1.0 MeOH): C, 41.78; H, 5.84; N, 9.74; Found: C, 41.85; H, 5.77; N, 9.68; MS *m/z*400 $(M + H)^+$.

(rel)-4-[(1R,2S,3S)-1-(4-Amino-2-oxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl]-2-fluoro-5,5-difluoroethyl-phosphonic acid sodium salt(24)

Final cytosine analogue **24** was synthesized from **23** by the similar deprotection procedure as described for **17**: Yield 50%; UV (H_2O) λ_{max} 271.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.47 (d, J= 7.0 Hz, 1H), 6.11 (dd, J=18.4, 6.8 Hz, 1H), 5.56 (d, J= 7.0 Hz, 1H), 4.27–4.18 (m, 1H), 3.89 (dd, J= 10.4, 6.0 Hz, 1H), 3.67 (dd, J= 10.4, 8.0 Hz, 1H), 2.36–2.25 (m, 1H), 2.02–1.93 (m, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 165.7, 155.6, 141.5, 123.7 (dt, $J = 211.7$, 265.2 Hz), 96.2 (d, J= 211.6 Hz, 1H), 87.2 (d, $J = 24.7$ Hz), 66.2, 23.4 (d, $J = 20.2$ Hz), 19.2 (dd, $J = 25.8$, 20.8 Hz); ^{31}P (121.5 MHz, D_2O) δ5.76 (dd, $J_{P,F} = 113.4$, 88.4 Hz); HPLC $t_R = 9.42$ min; HRMS $[M\text{-}H]^+$ req. 342.0637, found 342.0635.

(rel)-Diethyl 4-[(1S,2S,3S)-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (25α) and (rel)-diethyl 4-[(1R,2S,3S)-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (25β)

Uracil analogues were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of 6-chloropurine analogues **15**α and **15**β. Data for **25**α: yield 36%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.19 (br s, 1H, D_2O exchangeable), 7.52 (d, $J = 7.4$ Hz, 1H), 6.17 (dd, J =18.2, 6.6 Hz, 1H), 5.58 (d, J

= 7.4 Hz, 1H), 4.28–4.25 (m, 4.5H), 4.18–4.14 (m, 0.5H), 3.88 (dd, J = 10.8, 6.8 Hz, 1H), 3.75 (dd, J = 10.8, 7.8 Hz, 1H), 2.23–2.15 (m, 1H), 1.98–1.85 (m, 1H), 1.30 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.54 (t, $J_{P,F}$ = 112.0 Hz); Anal. Calc. for $C_{14}H_{20}F_3N_2O_6P$: C, 42.01; H, 5.04; N, 7.00; Found: C, 42.13; H, 5.08; N, 7.09; MS m/z401 $(M + H)^+$. Data for **25**β: yield 37%;^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.21 (br s, 1H, D_2O exchangeable), 7.56 (d, J = 7.4 Hz, 1H), 6.19 (dd, J = 18.8, 6.8 Hz, 1H), 5.55 (d, J = 7.4 Hz, 1H), 4.29–4.18 (m, 5H), 3.91 (dd, J = 10.2, 7.0 Hz, 1H), 3.72 (dd, J = 10.2, 6.2 Hz, 1H), 2.21–2.13 (m, 1H), 1.95–1.81 (m, 1H), 1.32–1.29 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ7.59 (t, $J_{P,F}$ = 109.8 Hz); Anal. Calc. for $C_{14}H_{20}F_3N_2O_6P$ (+0.5 MeOH): C, 41.85; H, 5.33; N, 6.73; Found: C, 41.95; H, 5.28; N, 6.83; MS m/z401 $(M + H)^+$.

(rel)-Diethyl 4-[(1S,2S,3S)-1-(2,4-dioxo-5-methyl-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (26α) and (rel)-diethyl 4-[(1R,2S,3S)-1-(2,4-dioxo-5-methyl-3,4-dihydropyrimidin-1(2H)-yl)-2-fluoro-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (26β)

Thymine analogues were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of 6-chloropurine analogues **15**α and **15**β. Data for **26**α: yield 35%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.18 (br s, 1H, D_2O exchangeable), 7.69 (s, 1H), 6.19 (dd, J = 19.2, 7.0 Hz, 1H), 4.31–4.25 (m, 4.5H), 4.17–4.12 (m, 0.5H), 3.87 (dd, J = 10.4, 7.0 Hz, 1H), 3.60 (dd, J = 10.4, 7.6 Hz, 1H), 2.35–2.28 (m, 1H), 2.02–1.93 (m, 2H), 1.79 (s, 3H), 1.32–1.29 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.44 (t, $J_{P,F}$ = 109.6 Hz); Anal. Calc. for $C_{15}H_{22}F_3N_2O_6P$: C, 43.48; H, 5.35; N, 6.76; Found: C, 43.56; H, 5.43; N, 6.87; MS m/z415 $(M + H)^+$. Data for **27**β: yield 36%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.14 (br s, 1H, D_2O exchangeable), 7.71 (s, 1H), 6.21 (dd, J = 18.6, 7.2 Hz, 1H), 4.29–4.20 (m, 4.5H), 4.15–4.11 (m, 0.5H), 3.83 (dd, J = 10.2, 7.2 Hz, 1H), 3.62 (dd, J = 10.2, 7.2 Hz, 1H), 2.23–2.16 (m, 1H), 1.94–1.83 (m, 2H), 1.72 (s, 3H), 1.30–1.27 (m, 6H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.56 (t, $J_{P,F}$ = 111.3 Hz); Anal. Calc. for $C_{15}H_{22}F_3N_2O_6P$ (+1.0 MeOH): C, 43.07; H, 5.87; N, 6.28; Found: C, 43.16; H, 5.78; N, 6.36; MS m/z 415 $(M + H)^+$.

(rel)-4-[(1R,2S,3S)-1-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl]-2-fluoro-5,5-difluoroethyl-phosphonic acid sodium salt (27)

Uracil phosphonic acid analogue **27** was synthesized from **25**β using the similar hydrolysis conditions as described for **18**: Yield 49%; UV (H_2O) λ_{max} 260.5 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.81 (d, J = 7.0 Hz, 1H), 6.13 (dd, J =17.6, 6.8 Hz, 1H), 5.85 (d, J = 7.0 Hz, 1H), 4.28 (dd, J = 9.8, 6.8 Hz, 1H), 4.19 (dd, J = 9.8, 8.2 Hz, 1H), 3.89 (dd, J = 10.8, 7.2 Hz, 1H), 3.71 (dd, J = 10.8, 6.4 Hz, 1H), 2.25–2.16 (m, 1H), 1.96–1.82 (m, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 166.5, 152.5, 142.3, 126.4

(dt, J = 206.2, 268.4 Hz), 103.5, 94.6 (d, J = 205.8 Hz), 87.0 (d, J = 23.8 Hz), 67.3, 24.7 (d, J = 22.6 Hz), 18.5 (dd, J = 24.8, 19.4 Hz); ^{31}P (121.5 MHz, D_2O) δ 5.75 (dd, $J_{P,F}$ = 108.5, 86.7 Hz); HPLC t_R=10.38 min; HRMS [M-H]$^+$ req. 343.0637, found 343.0635.

(rel)-4-[(1R,2S,3S)-1-(2,4-Dioxo-5-methyl-3,4-dihydropyrimidin-1(2H)-yl)-tetrahydrofuran-3-yl]-2-fluoro-5,5-difluoroethyl-phosphonic acid sodium salt (28)

Thymine analogue **28** was synthesized from **26**β using the similar hydrolysis conditions as described for **18**: Yield 54%; UV (H_2O) λ_{max} 267.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.73 (s, 1H), 6.18 (dd, J=17.4, 7.6 Hz, 1H), 4.26 (dd, J = 8.8, 7.6 Hz, 1H), 4.18 (dd, J = 7.8, 6.8 Hz), 3.83 (dd, J = 10.4, 7.2 Hz, 1H), 3.75 (dd, J = 10.4, 6.2 Hz), 2.24–2.17 (m, 1H), 2.03–1.92 (m, 2H), 1.77 (s, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 163.5, 150.1, 135.9, 125.6 (dt, J = 204.4, 263.6 Hz), 109.3, 86.5 (d, J = 210.4 Hz), 66.1, 23.7 (d, J = 24.4 Hz), 18.2 (dd, J = 23.4, 19.6 Hz), 12.7; ^{31}P (121.5 MHz, D_2O) δ5.72 (t, $J_{P,F}$ = 109.4 Hz); HPLC t_R=10.78 min; HRMS [M-H]$^+$ req. 357.0635, found 357.0633.

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