Synthesis and Evaluation of 2- or 6- Modified Purine 2'-*C*-Methyl Ribonucleosides as Inhibitors of HCV Replication

Longhu Zhou,[†] *Hongwang Zhang,*[†] *Sijia Tao,*[†] *Maryam Ehteshami,*[†] *Jong Hyun Cho,*[†] *Tamara R. McBrayer,*[§] *Philip Tharnish,*[§] *Tony Whitaker,*[§] *Franck Amblard,*[†] *Steven J. Coats,*[§] *Raymond F. Schinazi*[†]*

[†]Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, and Veterans Affairs Medical Center, Atlanta, Georgia 30322, USA, [§]CoCrystal Pharma, Inc, Tucker, Georgia 30084, USA.

Corresponding Authors: rschina@emory.edu

EXPERIMENTAL SECTION:

HCV Replicon Assay: Huh 7 Clone B cells containing HCV Replicon RNA were seeded in a 96-well plate at 3000 cells/well, and the compounds were added in dose response in triplicate immediately after seeding.[1] Following five days incubation (37 ^{o}C, 5% CO_2), total cellular RNA was isolated by using the RNeasy96 well extraction kit from Qiagen. Replicon RNA and an internal control (TaqMan rRNA control reagents, Applied Biosystems) were amplified in a single step multiplex Real Time RT-PCR Assay. The antiviral effectiveness of the compounds was calculated by subtracting the threshold RT-PCR cycle of the test compound from the threshold RT-PCR cycle of the no-drug control

(ΔCt HCV). A ΔCt of 3.3 equals a 1-log reduction (equal to 90% less starting material) in Replicon RNA levels. The cytotoxicity of the compounds was also calculated by using the ΔCt rRNA values. 2'-*C*-Me cytidine was used as the positive control. To determine EC_{90} and CC_{50} values ΔCt values were first converted into fraction of starting material and then were used to calculate the % inhibition.

Cellular Pharmacology: Huh-7 cells and fresh plated human primary hepatocytes (BioreclamationIVT, Baltimore, MD) were seeded at 1×10^6 per well in 12-well plates. After attachment (Huh-7 cells) or acclimate overnight (hepatocytes), cells were exposed to 50 µM compounds respectively. At 4 h, medium was removed from the cell layers and cells were washed twice with ice-cold phosphate buffered saline (PBS) to remove any residual medium. Cells were resuspended in 70% MeOH containing 20 nM ddATP overnight at -20°C. The supernatants were dried under a flow of air and dried samples stored at -20°C until LC-MS/MS analysis.

NS5B-mediated RNA polymerization assay. C-terminal his-tagged NS5BΔ21 enzyme was purified as previously described (Powdrill et al. 2010). 1 µM of NS5BΔ21 was incubated at 30 ^oC with a synthetic 1 µM of 20-mer RNA templates (IDT) and 1 µM ^{32}P-radiolabeled GpG primer (Trilink) in a buffer containing 40 mM Tris pH 7.5, 6 mM NaCl and 2 mM $MgCl_2$. Reactions were initiated with the addition of 10 µM NTP mix, 1 µM of competing NTP, and varying concentrations of inhibitor ranging from (0 to 100 µM). Reactions were allowed to proceed for 120 min and subsequently stopped with the addition of 10 mM EDTA and formamide. Samples were visualized on 20% denaturing

polyacrylamide gel and quantified using QuantityOne software. IC_{50} values were calculated using KaleidaGraph software. Ki value is the average of two independent experiments.

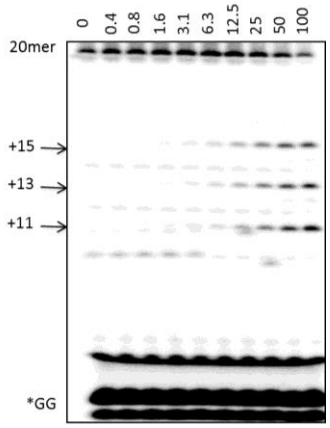
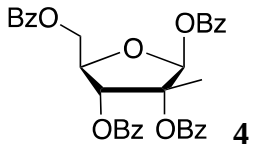


Figure 2: Enzymatic incorporation of **28**-TP by HCV NS_5B polymerase.

Synthesis:

Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. (Milwaukee). Reagents were purchased from commercial sources. Unless noted otherwise, the materials used in the examples were obtained from readily available commercial suppliers or synthesized by standard methods known to one skilled in the art of chemical synthesis. ^1H and ^{13}C NMR spectra were taken on a Varian Unity Plus 400 spectrometer at rt and reported in ppm downfield from internal tetramethylsilane. Deuterium exchange, decoupling experiments to confirm proton assignments. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). All J-values are in Hz. Purity of final compounds was determined to be > 95%, using an analytical HPLC analyses performed on a Hewlett-Packard 1100 HPLC with a Phenomenex Gemini-NX column (2 mm x 50 mm, 3 μm, C18, 110 Å) and further supported by clean NMR

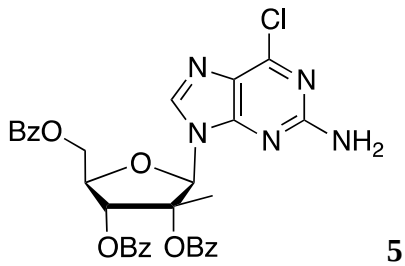
spectra. Mobile phase flow was 0.5 mL/min with a 3.5 min initial hold, a 6.5 min gradient from 96% aqueous media (0.05% formic acid) to 96% CH_3CN (0.05% formic acid), and a 15 min total acquisition time. Photo diode array detection was from 190 to 360 nm. Mass spectra were determined on a Micromass Platform LC spectrometer using electrospray ionization. Analytic TLC was performed on Whatman LK6F silica gel plates, and preparative TLC on Whatman PK5F silica gel plates. Column chromatography was carried out on Silica Gel or via reverse-phase high performance liquid chromatography.



4

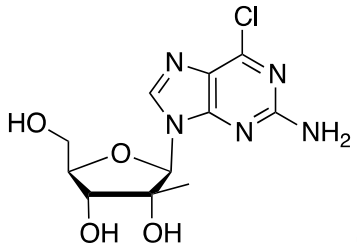
1,2,3,5-Tetrabenzoyl-2-C-methyl-β-D-ribofuranose **4**. To a suspension of 2-methyl-ribonolactone (16.2 g, 100 mmol) in 370 mL of dry CH_2Cl_2 and 66.7 mL of Et_3N was added benzoyl chloride (52.2 mL, 450 mmol). The mixture was stirred at ambient temperature overnight and 50 mL of MeOH was added to quench the reaction. After evaporation of the solvents, the residue was portioned between EtOAc and water. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. The residue was crystallized from EtOAc and hexane to provide 2-methyl-2,3,5-tribenzoyl-ribonolactone (45.0 g) in 95% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14-8.04 (m, 15H), 5.51 (d, *J* = 6.0 Hz, 1H), 5.15-5.19 (m, 1H), 4.80 (dd, *J* = 12.8 Hz, *J* = 3.2 Hz, 1H), 4.69 (dd, 1H, *J* = 12.0 Hz, *J* = 3.6 Hz), 1.95 (s, 3H). To a solution of 2-methyl-2,3,5-tribenzoyl-ribonolactone (34 g, 71.7 mmol) in 140 mL of dry THF was

added 1M lithium tri-*t*-butoxyaluminium hydride ($LiAl(O^tBu)_3H$) (100 mL, 100 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After the reduction was complete, the reaction was quenched by addition of 100 mL of 10% NH_4Cl and 100 mL of EtOAc. The reaction mixture was filtered and the aqueous layer was extracted with EtOAc (300 mL x 3). The combined organic layers were dried over Na_2SO_4 and evaporated to give the desired 2-methyl-2,3,5-tribenzoyl-ribonolactol as a white foam. The 2-methyl-2,3,5-tribenzoyl-ribonolactol was then dissolved in 300 mL of dry CH_2Cl_2 and 15.8 mL of Et_3N, and benzoyl chloride (9.4 mL, 79.5 mmol) was added dropwise at 0 ^oC. The mixture was stirred overnight at room temperature and then quenched with 10 mL of MeOH. The reaction mixture was portioned between 200 mL of CH_2Cl_2 and 200 mL of water. The aqueous layer was extracted with CH_2Cl_2 (300 ml x 2), and the combined organic layer was dried and concentrated. The residue was recrystallized from CH_2Cl_2 and hexane. The collected solid was washed with hexane to give compound **4** (32 g) in 77% yield. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.15-8.16 (m, 20H), 7.08 (s, 1H), 5.96 (d, *J* = 8.0 Hz, 1H), 4.80 (m, 1H), 4.70 (dd, *J* = 12.0 Hz, *J* = 4.0 Hz, 1H), 4.55 (dd, *J* = 12.0 Hz, *J* = 4.8 Hz, 1H), 1.96 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 16.9, 63.8, 76.1, 78.5, 86.6, 97.8, 128.1, 128.5, 128.6, 128.8, 129.2, 129.4, 129.6, 129.7, 129.9, 130.2, 132.9, 133.5, 133.6, 133.8, 164.5, 164.8, 176.6, 166.1.



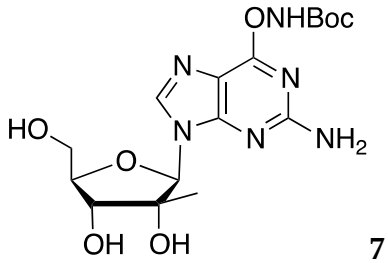
5

2-Amino-6-chloro-9H-(2-C-methyl-2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine (5). To a precooled (-40 °C) suspension of **4** (2.9 g, 5.0 mmol) and 2-amino-6-chloropurine (0.93 g, 5.5 mmol) in anhydrous acetonitrile (50 ml) was added 1,8-diazabicycl[5.4.0]undec-7-ene (DBU) (2.3 ml, 15 mmol) and trimethylsilyl triflate (3.8 ml, 20 mmol) dropwise. The reaction mixture was stirred at this temperature for 20 min and then the temperature was raised to 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was heated gradually to 80 °C and left at this temperature for 5 h. The reaction was then cooled down to room temperature, poured into a saturated aqueous solution of sodium bicarbonate (150 ml), and extracted with CH_2Cl_2 (100 ml x 3). The combined organic phase was dried over sodium sulfate and evaporated in vacuo. The residue was purified over silica gel (hexane/EtOAc; 0 to 50% EtOAc) to give the desired compound **5** (2.9 g) in 92% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33-8.14 (m, 16H), 6.64 (s, 1H), 6.40 (d, *J* = 6.8 Hz, 1H), 5.33 (s, 2H), 5.09 (dd, *J* = 11.6 Hz, *J* = 4.0 Hz, 1H), 4.73−4.81 (m, 2H), 1.60 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.7, 63.5, 75.99, 79.4, 85.6, 88.8, 125.9, 128.4, 128.5, 128.6, 128.7, 129.3, 129.7, 129.8, 129.9, 133.3, 133.7, 133.8, 141.4, 152.0, 152.8, 159.1, 165.3, 165.3, 166.3; LRMS Calcd for $C_{32}H_{27}ClN_5O_7$ $(M+1)^+$ 628.16, found 628.20.



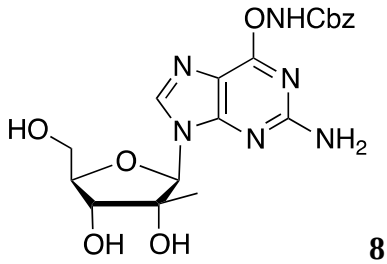
6

2-Amino-6-chloro-9H-(2-C-methyl-β-D-ribofuranosyl)-purine (6). A solution of **5** (2.7 g, 4.3 mmol) in saturated methanolic ammonia (60 ml) was stirred in a sealed tube for 8 h. The solvent was removed by evaporation and 100 ml of CH_2Cl_2 was added to the residue. The solid was filtered was filtered to give compound **6** (1.2 g) in 90% yield as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.56 (s, 1H), 6.01 (s, 1H), 4.20 (d, *J* = 8.8 Hz, 1H), 4.07-3.98 (m, 2H), 3.85 (dd, *J* = 12.6 Hz, *J* = 3.0 Hz, 1H), 0.97 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 61.0, 73.4, 80.3, 84.3, 92.7, 124.9, 142.6, 151.7, 154.7, 161.6; LRMS Calcd for $C_{11}H_{15}ClN_5O_4$ $(M+1)^+$ 316.08, found 316.21.



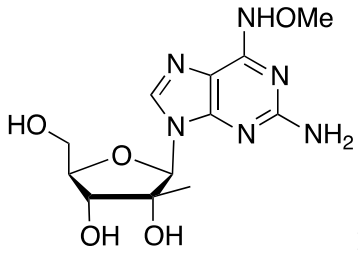
7

2-Amino-6-[N-(t-butyloxycarbonyl)aminoxy]-9H-(2'-C-methyl-β-D-ribofuranosyl)-purine (7). To a suspension of *N*-Boc-hydroxylamine (27 mg, 0.2 mmol) and NaH (8 mg, 60%, 0.2 mmol) in dry THF (1 ml), stirred at 0 °C for 10 min, was added compound **6** (28 mg, 0.09 mmol). The reaction mixture was stirred at ambient temperature for 3 h. After evaporation of volatiles under reduced pressure, the residue was purified over silica gel ($MeOH/CH_2Cl_2$; 0 to 50% MeOH) to give the desired compound **7** (25.5 mg, 69%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.36 (s, 1H), 5.99 (s, 1H), 4.20 (d, *J* = 9.2 Hz, 1H), 3.83 - 4.03 (m, 3H), 1.46 (s, 9H), 0.93 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 28.5, 61.1, 73.5, 80.4, 83.4, 84.2, 92.9, 113.5, 140.5, 155.7, 158.6, 161.5, 162.6. LRMS Calcd for $C_{16}H_{25}N_6O_7$ $(M+1)^+$ 413.18, found 413.30.



8

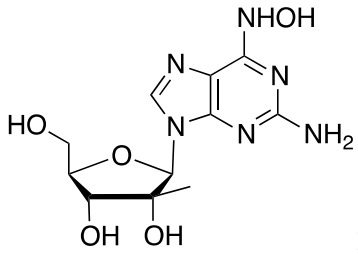
2-Amino-6-[N-(benzyloxycarbonyl)aminoxy]-9H-('2'-C-methyl-β-D-ribofuranosyl)-purine (8). The desired compound **8** was prepared in 88% yield using the same procedure as for compound **7** by replacing *N*-Boc-hydroxylamine by *N*-Cbz-hydroxylamine. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.36 (s, 1H), 7.30 (m, 5H), 5.98 (s, 1H), 5.19 (s, 2H), 4.19 (d, 1H, *J* = 8.8 Hz), 3.98-4.03 (m, 2H), 3.84 (dd, *J* = 12.4 Hz, *J* = 2.8 Hz, 1H), 0.93 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 60.9, 68.8, 73.2, 80.5, 84.6, 93.4, 120.6, 129.2, 129.4, 129.6, 137.3, 144.4, 152.9, 153.8, 159.2, 162.0. LRMS Calcd for $C_{19}H_{23}N_6O_7$ $(M+1)^+$ 447.16, found 447.27.



10

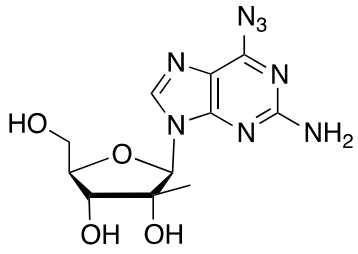
2-Amino-6-(N-methoxyamino)-9H-(2'-C-methyl-β-D-ribofuranosyl)purine (10). A solution of compound **6** (105 mg, 0.33 mmol), methoxyamine hydrochloride (560 mg, 6.6 mmol) and triethylamine (1.5 ml, 10.0 mmol) in ethanol/H_2O (1:1, 1 ml) was stirred in a sealed tube at 70 °C for 24 h. After removal of the volatiles under reduced pressure, the residue was purified over silica gel (MeOH/CH_2Cl_2; 0 to 20% MeOH) to give **10** (85 mg,

79%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.25 (s, 1H), 5.90 (s, 1H), 4.15 (d, 1H, *J* = 9.2 Hz), 3.65 – 4.01 (m, 3H), 0.96 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 18.9, 59.8, 60.3, 72.1, 82.5, 91.3, 111.3, 134.1, 142.2, 143.7, 152.2; LRMS Calcd for $C_{12}H_{19}N_6O_5$ $(M+1)^+$ 327.14, found 327.25.



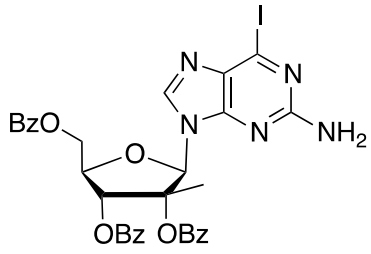
11

2-Amino-6-(N-hydroxylamino)-9H-(2'-C-methyl-β-D-ribofuranosyl)purine (11). A solution of compound **6** (19 mg, 0.06 mmol) and hydroxylamine (39.6 mg, 1.2 mmol) in ethanol/H_2O (1/1, 0.5 ml) was stirred at 35 °C for 24 h. After removal of the volatiles under reduced pressure, the residue was purified over silica gel ($MeOH/CH_2Cl_2$; 0 to 50% MeOH) to give compound **10** (9.5 mg, 51%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.22 (s, 1H), 5.91 (s, 1H), 4.17 (d, 1H, *J* = 8.0 Hz), 4.03-3.99 (m, 2H), 3.88-3.83 (m, 1H), 0.97 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 61.0, 73.4, 80.3, 84.1, 92.7, 111.3, 138.3, 149.3, 149.7, 155.5; LRMS Calcd for $C_{11}H_{17}N_6O_5$ $(M+1)^+$ 312.1, found 313.2.



12

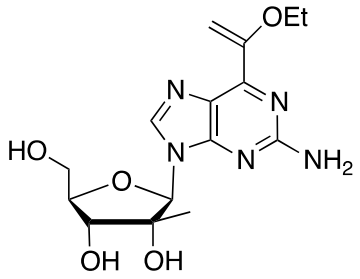
2-Amino-6-Azido-9H-(2'-C-methyl-β-D-ribofuranosyl)purine (12). A mixture of **6** (1.63 g, 5.17 mmol) and sodium azide (1.0 g, 15.4 mmol) in DMF (30 ml) was stirred at 95 °C under N_2 for 2 h. After removal of volatiles under reduced pressure, the residue was purified by column chromatography on silica gel ($MeOH/CH_2Cl_2$; 0 to 10% MeOH) to give **12** (1.03 g, 62%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.61 (s, 1H), 6.15 (s, 1H), 4.22 (d, *J* = 9.2 Hz, 1H), 4.07-4.02 (m, 2H), 3.88 (dd, *J* = 12.4 Hz, *J* = 2.8 Hz, 1H), 0.97 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 60.9, 73.2, 80.5, 84.3, 93.0, 113.2, 139.7, 145.7, 145.9, 147.3; LRMS calcd for $C_{11}H_{16}N_5O_5$ $(M+1)^+$ 323.12, found 323.10.



13

(2-Amino-6-iodo-9H-(2'-C-methyl-β-D-ribofuranosyl)purine (13). To a solution of **5** (500 mg, 0.79 mmol) in CH2Cl2 (5 ml) was added TMSI (223 mg, 1.11 mmol) at room temperature. After 2 h, more TMSI (669 mg) was added and the reaction mixture was stirred for 4 h until completion. The reaction mixture was then poured into a saturated solution of sodium bicarbonate and extracted with EtOAc (20 ml x 2). The combined organic layer was washed with a sodium thiosulfate solution and concentrated under reduced pressure. The residue was purified by silica gel chromatography column (EtOAc:hexane; 1:1 to 2:1) to give **13** (360 mg, 63%) as a white solid, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09-8.11 (m, 2 H), 7.92-7.98 (m, 3 H), 7.97 (s, 1 H), 7.44-7.62 (m, 6 H), 7.30-7.35 (m, 4 H), 6.59 (s, 1 H), 6.37-6.38 (d, *J* = 6.4 Hz, 1 H), 5.31 (s, 2 H), 5.03-5.07

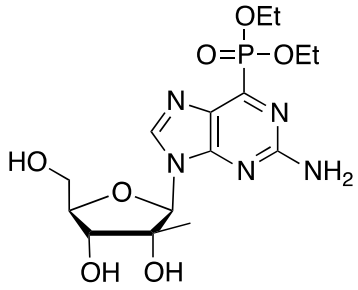
(dd, *J* = 4 Hz, *J* = 11.6 Hz, 1 H), 4.70-4.79 (m, 2 H), 1.58 (s, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.7, 63.5, 75.9, 79.5, 85.4, 88.8, 123.4, 128.3, 128.4, 128.5, 128.7, 129.3, 129.6, 129.7, 129.8, 132.8, 133.2, 133.6, 133.7, 140.7, 148.9, 158.8, 165.2, 165.3, 166.2; LRMS m/z calcd for $C_{32}H_{27}IN_5O_7$ $(M+1)^+$ 720.09, found 719.72.



14

(2*R*,3*R*,4*R*,5*R*)-2-(2-amino-6-(1-ethoxyvinyl)-9*H*-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (14). To a solution of iodo derivative **13** (600 mg, 0.83 mmol) and $Pd(Ph_3P)_2Cl_2$ (60 mg, 0.085 mmol) in THF (20 ml) under nitrogen atmosphere was added tributyl (1-ethoxyvinyl) stannane (0.9 ml, 2.66 mmol). The reaction mixture was stirred at 80 °C for 24 h and then poured into water. After extraction with EtOAc, the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on silica gel column chromatography (EtOAc:hexane = 1:1 v/v) to give the desired compound (300 mg, 3.32 mmol) in 54% yield. ^{1}HNMR (CD_3Cl_3, 400 MHz) δ 8.10-8.12 (m, 2 H), 7.92-7.98 (m, 3 H) 7.97 (s, 1 H), 7.43-7.59 (m, 6 H), 7.30-7.36 (m, 4 H), 6.64 (s, 1 H), 6.35-6.36 (d, *J* = 6.4 Hz, 1 H), 5.90-5.91 (d, *J* = 2.4 Hz, 1 H), 5.90 (s, 2 H), 5.00-5.04 (dd, *J* = 4.0 Hz, *J* = 12.0 Hz, 1 H), 4.87 (d, *J* = 2.8 Hz, 1 H), 4.79-4.83 (dd, *J* = 6.8 Hz, *J* = 12.0 Hz, 1 H), 4.68-4.70 (m, 1 H), 4.03-4.09 (q, *J* = 7.2 Hz, 2 H), 1.58 (s, 3 H), 1.46-1.50 (t, *J* = 6.8 Hz, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.5, 18.4, 64.8, 64.9, 77.7, 81.3, 86.2, 89.7, 94.4, 125.1, 129.6, 129.6, 129.8,

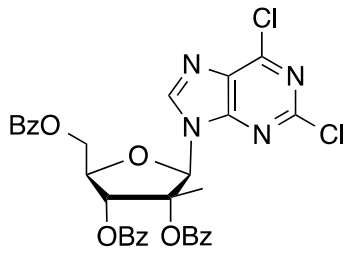
130.3, 130.7, 130.7, 130.9, 131.0, 131.1, 134.5, 134.8, 142.5, 154.5, 155.2, 156.7, 161.6, 166.6, 166.7, 167.8. LRMS m/z calcd for $C_{36}H_{34}N_5O_8$ $(M+1)^+$ 664.24, found 664.02. To a solution of protected vinyl compound (40 mg, 0.06 mmol) in MeOH (2 ml) was added a catalytic amount of sodium methoxide (50 μL, 25 % solution). The reaction mixture was stirred for 12 h and neutralized with acetic acid. After evaporation of the volatils, the residue was purified by column chromatography on silica gel (MeOH/CH_2Cl_2; 0 to 10% MeOH) to give **14** (15 mg, 79%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.52 (s, 1 H), 6.03 (s, 1 H), 5.74 (d, *J* = 2.8 Hz, 1 H), 4.84-4.85 (d, *J* = 2.8 Hz, 1 H), 4.18-4.20 (d, *J* = 9.2 Hz, 1 H), 3.97-4.03 (m, 3 H), 3.82-3.85 (dd, *J* = 2.8 Hz, *J* = 12.0 Hz, 1 H), 1.41-1.45 (t, *J* = 7.2 Hz, 3 H), 0.94 (s, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.5, 20.3, 61.0, 64.8, 73.5, 80.3, 84.2, 92.5, 94.0, 124.8, 142.4, 154.2, 155.2, 156.8, 161.3. LRMS m/z calcd for $C_{15}H_{22}N_5O_5$ $(M+1)^+$ 352.16, found 352.15.



15

Diethyl (2-amino-9-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetra-hydrofuran-2-yl)-9H-purin-6-yl)phosphonate **(15)** A mixture of chloro derivative **5** (0.2 g, 0.3 mmol) and triethylphosphite (4 ml, 23.3 mmol) was stirred at 130 °C overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (acetate/hexane/MeOH : 60/20/4) to give the desired protected phosphonate (0.18 g, 78%). ^{1}HNMR ($CDCl_3$, 400 MHz) δ

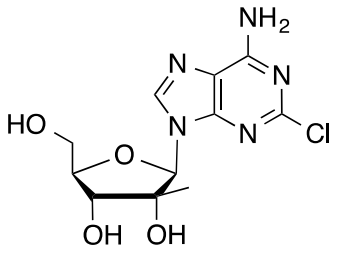
8.09-8.11 (m, 2 H), 8.05 (s, 1 H), 7.92-7.99 (m, 3 H), 7.43-7.61 (m, 6 H), 7.30-7.35 (m, 4 H), 6.64 (s, 1 H), 6.32 (d, *J* = 6.4 Hz, 1 H), 5.51 (brs, 2 H), 5.00-5.04 (dd, *J* = 4.0 Hz, *J* = 12.0 Hz, 1 H), 4.69-4.81 (m, 2 H), 4.31-4.38 (m, 4 H), 1.58 (s, 3 H), 1.36 (t, *J* = 6.8 Hz, 6 H). ^{31}P NMR (162 MHz, CD_3OD): 8.49. LRMS calcd for $C_{36}H_{37}N_5O_{10}P$ $(M+1)^+$ 730.22, found 730.30. A solution of the protected phosphonate (120 mg, 0.16 mmol) in NH_3/C_2H_5OH (10 ml) was stirred at room temperature for 4 days. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc:MeOH; 60:10) to give pure compound **15** 30 mg (44%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.63 (s, 1 H), 6.04 (s, 1 H), 4.27-4.34 (m, 4 H), 4.18 (d, *J* = 9.2 Hz, 1 H), 3.97-4.03 (m, 2 H), 3.81 (dd, *J* = 3.2 Hz, *J* = 12.8 Hz, 1 H), 1.33 (t, *J* = 7.2 Hz, 6 H), 0.94 (s, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 15.2, 15.3, 18.8, 59.6, 63.8 (dd, *J* = 4.0 Hz, *J* = 2.0 Hz), 72.0, 78.9, 82.9, 91.0, 127.9 (d, *J* = 22.0 Hz), 142.7, 149.0, 151.2, 154.0 (d, *J* = 13.0 Hz), 160.3 (d, *J* = 24.0 Hz). LRMS calcd for $C_{15}H_{25}N_5O_7P$ $(M+1)^+$ 418.14, found 418.13.



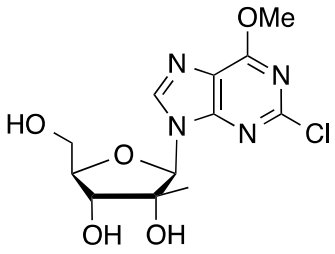
16

2,6-Dichloro-9H-(2-C-methyl-2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine (16). To a solution of tetra-*O*-benzoyl-2-methyl-β-D-ribofuranose **4** (7.0 g, 12.05 mmol) and 2,6-dichloropurine (2.5 g, 13.2 mmol) in acetonitrile (40 ml) at 0 °C was added DBU (5.6 ml) followed by TMSOTf (9.5 ml, 0.05 mol). The reaction mixture was stirred at 0 °C for 15

min, and gradually heated to 65 °C for 5 h. The reaction mixture was extracted with CH2Cl2 and the organic layer was washed with sodium bicarbonate, dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc:hexane; 1:1 to 2:1) to give 7 g of product **16** (94%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.40 (s, 1H), 8.16 (dd, *J* = 1.6 Hz, *J* = 8.4 Hz, 2H), 8.06 (dd, *J* = 1.6 Hz, *J* = 8.4 Hz, 2H), 7.85 (dd, *J* = 1.2 Hz, *J* = 8.4 Hz, 2H), 7.41 (m, 5H), 7.54 (m, 2H), 6.71 (s, 1H), 7.24 (m, 2H), 5.88 (d, *J* = 4 Hz, 1H), 4.97 (dd, *J* = 6.4 Hz, *J* = 12.4 Hz, 1H), 4.91 (dd, *J* = 3.6 Hz, *J* = 12.4 Hz, 1H), 1.57 (s, 3H), 4.66 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 17.7, 63.2, 75.7, 82.0, 83.4, 89.0, 128.4, 128.4, 128.5, 128.6, 128.6, 129.0, 129.3, 129.7, 129.7, 130.1, 131.3, 133.5, 133.7, 133.8, 144.2, 152.3, 152.3, 153.4, 164.9, 165.2, 166.3; LRMS calcd for $C_{32}H_{25}Cl_2N_4O_7$ $(M+1)^+$ 647.10, found 646.98.



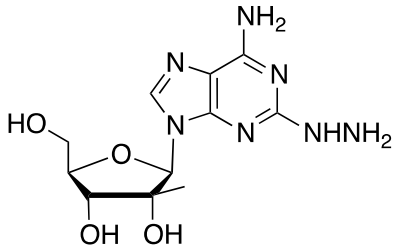
17



18

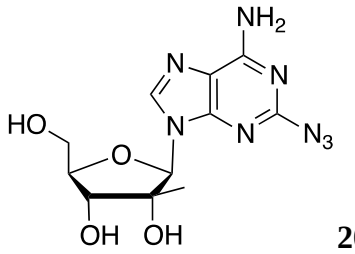
6-Amino-2-chloro-9H-(2-C-methyl-β-D-ribofuranosyl)-purine (17) and 2-chloro-6-methoxyl-9H-(2-C-methyl-β-D-ribofuranosyl)purine (18). A solution of **16** (0.5 g, 0.77 mmol) in NH_3/CH_3OH (30 ml) and THF (10 ml) was stirred for 3 days at room temperature. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 6:0.5) to afford **18**, 52 mg (21%) and **17**, 110 mg (46%). **17**: ^{1}HNMR (CD_3OD) δ 8.49 (s, 1H),

6.02 (s, 1H), 4.21 (d, *J* = 9.2 Hz, 1H), 4.06-4.01 (m, 2H), 3.89 (dd, *J* = 12.0, 3.2 Hz, 1H), 0.94 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 61.1, 73.4, 80.3, 84.4, 93.2, 119.1, 141.2, 151.5, 155.4, 158.1; LRMS calcd for $C_{11}H_{15}ClN_5O_4$ $(M+H)^+$ 316.08, found 316.10. **18**: ^{1}HNMR (CD_3OD) δ 8.78 (s, 1H), 6.10 (s, 1H), 4.20 (d, *J* = 9.2 Hz, 1H), 4.18 (s, 3H), 4.09-4.01 (m, 2H), 3.88 (dd, *J* = 12.0, 3.2 Hz, 1H), 0.93 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.1, 55.6, 61.0, 73.3, 80.3, 84.6, 93.3, 118.5, 122.1, 143.0, 153.3, 161.0; LRMS calcd for $C_{12}H_{16}ClN_4O_5$ $(M+1)^+$ 331.08, found 331.10.



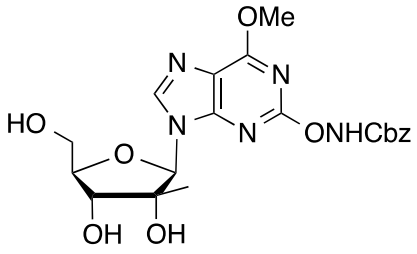
19

(2*R*,3*R*,4*R*,5*R*)-2-(6-amino-2-hydrazinyl-9*H*-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (19). A solution of 2-chloropurine **17** (100 mg, 0.31 mmol) and hydrazine (2 ml) in 2-methoxyethanol (5 ml) was heated at 110 °C for 5 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2: MeOH; 5:1 to 5:3) to give compound **19** (40 mg, 40%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.18 (s, 1 H), 5.99 (s, 1 H), 4.20 (d, *J* = 8.8 Hz, 1 H), 3.97-4.02 (m, 2 H), 3.81 (dd, *J* = 3.2 Hz, *J* = 12.8 Hz, 1 H), 0.95 (s, 3 H); ^{13}C NMR DMSO-d_6, 100 MHz) δ 20.1, 59.7, 72.0, 78.5, 82.4, 90.3, 113.6, 135.8, 151.0, 160.0, 161.9; LRMS calcd for $C_{11}H_{18}N_7O_4$ $(M+1)^+$ 312.14, found 312.06.



20

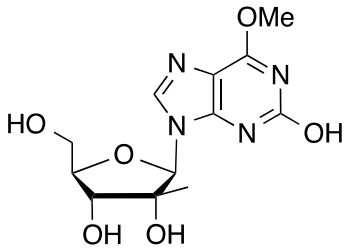
(2*R*,3*R*,4*R*,5*R*)-2-(6-amino-2-azido-9*H*-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (20). To a solution of 2-hydrazinylpurine **19** (500 mg, 1.55 mmol) in aqueous acetic acid (5%, 24 ml) was added sodium nitrite (0.17 g, 2.4 mmol). The reaction mixture was stirred for 1 h. A white solid was collected by filtration and washed with water to give **20** (400 mg, 77%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.70 (s, 0.2 H), 8.42 (s, 0.8 H), 6.15 (s, 0.2 H), 5.96 (s, 0.8 H), 4.17 (d, *J* = 8.8 Hz, 1 H), 3.97-4.04 (m, 2 H), 3.82 (dd, *J* = 2.8 Hz, *J* = 12.4 Hz, 1 H), 0.99 (s, 0.6 H), 0.91 (s, 2.4 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 61.0, 61.2, 73.5, 80.2, 80.3, 84.3, 84.4, 92.8, 93.1, 117.6, 140.5, 144.2, 151.7, 158.1, 158.2; LRMS calcd for $C_{11}H_{15}N_8O_4$ $(M+1)^+$ 323.12, found 323.08.



21

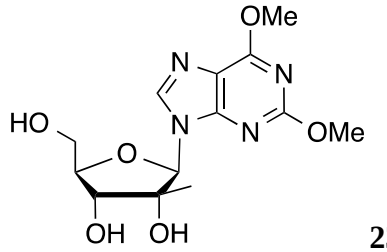
Benzyl ((9-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-6-methoxy-9H-purin-2-yl)oxy)carbamate (21). To a solution of **18** (340 mg, 2 mmol) in THF (5 ml) at 0 °C was added sodium hydride (48 mg, 2 mmol) and N-Cbz hydroxylamine (100 mg, 0.32 mmol). The reaction mixture was gradually warmed up to

50 °C and stirred at this temperature for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH 0 to10% MeOH) to give product **21** (108 mg, 78%). ^{1}HNMR (CD_3OD) δ 8.63 (s, 1H), 7.28 (m, 5H), 6.04 (s, 1H), 5.20 (s, 2H), 4.20 (d, *J* = 8.8 Hz, 1H), 4.08-4.01 (m, 2H), 3.99 (s, 3H), 3.88 (dd, *J* = 12.4, 2.8 Hz, 1H), 0.90 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 55.2, 61.04, 68.6, 73.3, 80.3, 84.4, 93.1, 118.9, 129.3, 129.4, 129.5, 137.3, 142.3, 154.0, 159.4, 163.3, 163.8; LRMS calcd for $C_{20}H_{24}N_5O_8$ $(M+1)^+$ 462.16, found 462.07.



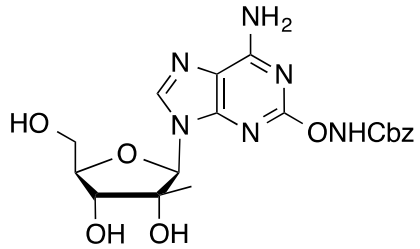
22

(2R,3R,4R,5R)-2-(2-hydroxy-6-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (22). A suspension of **21** (20 mg, 0.043 mmol) and Pd/C (5 mg) in MeOH (1 ml) was stirred under hydrogen atmosphere at room temperature overnight. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH 0 to 30% MeOH) to give product **22** (11 mg, 83%). ^{1}HNMR (CD_3OD) δ 8.28 (s, 1H), 6.00 (s, 1H), 4.14 (d, *J* = 4.8 Hz, 1H), 4.09 (s, 3H), 4.04-4.02 (m, 2H), 3.86 (dd, *J* = 12.4, 3.2 Hz, 1H), 0.93 (s, 3H); LRMS calcd for $C_{12}H_{18}N_4O_6$ $(M+1)^+$ 314.12, found 314.07.



23

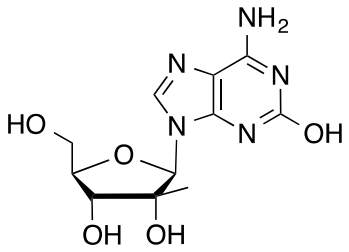
(2R,3R,4R,5R)-2-(2,6-dimethoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (23). A suspension of nucleoside of **16** (150 mg) and K_2CO_3 (300 mg) in MeOH (5 ml) was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH 0 to 10% MeOH) to give product product **23** (65.8 mg, 87%). ^{1}HNMR (CD_3OD) δ 8.56 (s, 1H), 6.07 (s, 1H), 4.23 (d, *J* = 8.8 Hz, 1H), 4.14 (s, 3H), 4.07-4.00 (m, 5H), 3.86 (dd, *J* = 12.4, 3.2 Hz, 1H), 0.95 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 54.9, 55.8, 61.1, 73.4, 80.3, 84.3, 93.0, 117.7, 141.5, 154.2, 163.3, 163.3; LRMS calcd for $C_{13}H_{19}N_4O_6$ $(M+1)^+$ 327.13, found 327.05.



24

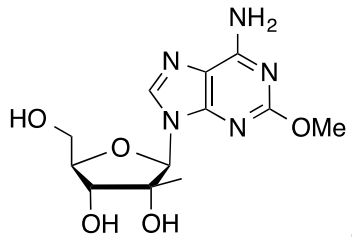
Benzyl ((6-amino-9-((2R,3R,4R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)-9H-purin-2-yl)oxy)carbamate (24). To a solution of **17** (340 mg, 2 mmol) in THF (5 ml) was added sodium hydride (48 mg, 2 mmol) and N-Cbz hydroxylamine (100 mg, 0.32 mmol) at 0 °C. The reaction mixture was gradually warmed up to 50 °C and stirred at this temperature for 24 h. The reaction mixture was

then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 10% MeOH) to give product **24** (114 mg, 81%). ^{1}HNMR (CD_3OD) δ 8.43 (s, 1H), 7.33-7.29 (m, 5H), 5.97 (s, 1H), 5.20 (s, 2H), 4.18 (d, *J* = 9.2 Hz, 1H), 4.09-4.01 (m, 2H), 3.87 (dd, *J* = 12.4, 3.2 Hz, 1H), 0.91 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 61.2, 68.55, 73.5, 80.3, 84.3, 92.9 117.4, 129.1, 129.2, 129.5, 137.3, 140.1, 151.8, 158.3, 159.5, 164.3; LRMS calcd for $C_{19}H_{23}N_6O_7$ $(M+1)^+$ 447.16, found 447.11.



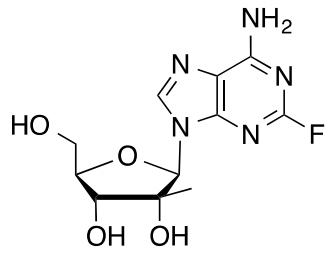
25

(2R,3R,4R,5R)-2-(6-amino-2-hydroxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (25). A suspension of **24** (25 mg, 0.056 mmol) and Pd/C (5 mg) in MeOH (1.5 ml) was stirred under hydrogen atmosphere at room temperature overnight. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 30% MeOH) to give compound **25** (17 mg, 97%). ^{1}HNMR (MDSO-d_6) δ 8.07 (s, 1H), 7.80 (bs, 3H), 5.72 (s, 1H), 5.21(bs, 3H), 3.97 (d, *J* = 8.0 Hz, 1H), 3.85-3.78 (m, 2H), 3.64 (d, *J* = 12.0 Hz, 1H), 0.83 (s, 3H); ^{13}C NMR DMSO-d_6, 100 MHz) δ 20.5, 59.8, 73.2, 78.9, 82.8, 90.7, 109.4, 126.9, 128.5, 137.6, 156.6; LRMS calcd for $C_{11}H_{16}N_5O_5$ $(M+1)^+$ 298.12, found 298.10.



26

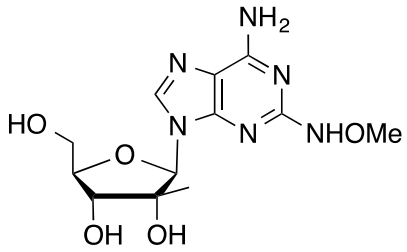
(2R,3R,4R,5R)-2-(6-amino-2-methoxy-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (26). A solution of **17** (72 mg, 0.23 mmol) and sodium methoxide (0.4 ml, 1.84 mmol) in MeOH (3 ml) was stirred at 65 °C overnight. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 15% MeOH) to give compound **26** (65.2 mg, 92%). ^{1}HNMR (CD_3OD) δ 8.35 (s, 1H), 6.00 (s, 1H), 4.24 (d, *J* = 8.8 Hz, 1H), 4.07-3.99 (m, 2H), 3.94 (s, 3H), 3.86 (dd, *J* = 12.4, 2.8 Hz, 1H), 0.97 (s, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 55.2, 61.3, 73.6, 80.3, 84.2, 92.9, 116.3, 139.6, 152.2, 158.2, 163.8; LRMS calcd for $C_{12}H_{18}N_5O_5$ $(M+H)^+$ 312.13, found 312.08.



28

(2R,3R,4R,5R)-2-(6-amino-2-fluoro-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (28). To a suspension of **4** (760 mg, 1.25 mmol) and 2-fluoroadenine (210 mg, 1.37 mmol) in dry acetonitrile (15 ml) at -40 °C was added DBU (0.58 ml, 3.75 mmol) dropwise and TMSOTf (0.95 ml, 3.75 mmol). The mixture was stirred for 20 min at -40 °C, then warm up to room temperature. After 30 min at this

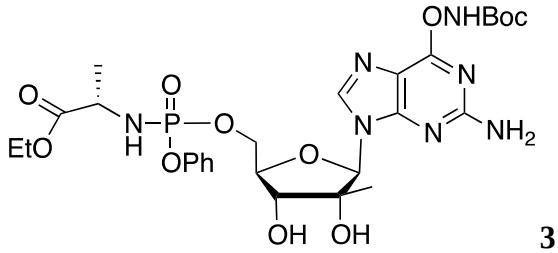
temperature, the reaction mixture was stirred at 65 °C for 5 h. The reaction was cooled down to room temperature, poured into aqueous solution of sodium bicarbonate (100 ml), and extracted with CH_2Cl_2 (30 ml x 2). The combined organic phase was dried over sodium sulfate and evaporated in vacuo. The residue was purified over silica gel (hexane/EtOAc, 0 to 50% EtOAc) to give intermediate **27** which was stirred in a saturated solution of methanolic ammonia (60 ml) in a sealed tube for 2 days at room temperature. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 12% MeOH) to give compound **28** (269 mg, 72%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.50 (s, 1 H), 5.98 (s, 1 H), 4.19 (d, *J* = 9.2 Hz, 1 H), 4.07-4.00 (m, 2 H), 3.87 (dd, *J* = 2.8 Hz, *J* = 12.4 Hz, 1 H), 0.94 (s, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.2, 61.1, 73.4, 80.3, 84.3, 93.1, 141.0, 159.0, 159.2, 159.6, 161.6. LRMS calcd for $C_{11}H_{15}FN_5O_4$ $(M+1)^+$ 300.27, found 300.05.



29

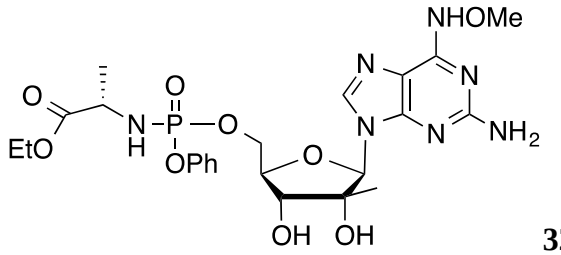
(2R,3R,4S,5R)-2-(6-amino-2-(methoxyamino)-9H-purin-9-yl)-5-(hydroxymethyl)-3-methyltetrahydrofuran-3,4-diol (29). A solution of **28** (57 mg, 0.19 mmol), O-methylhydroxylamine hydrochloride (168 mg, 2 mmol) and triethylamine (0.45 ml, 3 mmol) in EtOH/H_2O (1 ml, 1:1) was stirred at 110 °C for 15 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column

chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 20% MeOH) to give compound **29** (41 mg, 66%). ^{1}HNMR (CD_3OD, 400 MHz) δ 8.25 (s, 1 H), 6.03 (s, 1 H), 4.22 (d, *J* = 8.8 Hz, 1 H), 4.06-3.99 (m, 2 H), 3.88 (dd, *J* = 3.2 Hz, *J* = 12.4 Hz, 1 H), 3.78 (s, 3H), 0.96 (s, 3 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 20.3, 61.3, 64.0, 73.7, 80.2, 84.2, 92.9, 115.9, 138.9, 152.0, 157.6, 162.8; LRMS calcd for $C_{12}H_{19}N_6O_5$ $(M+1)^+$ 327.14, found 326.94.



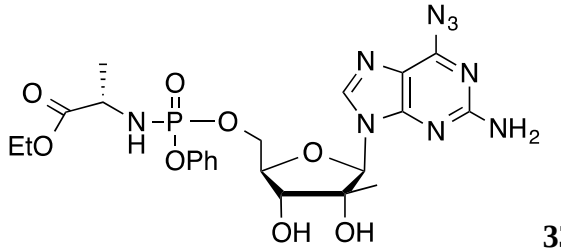
31

Ethyl (((((2R,3S,4R,5R)-5-(2-amino-6-(((tert-butoxycarbonyl)amino)oxy)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (31). To a solution of nucleoside **7** (41.2 mg, 0.1 mmol) and phosphorochloridate (88mg, 0.3 mmol) in THF (1 ml) and CH_3CN (1 ml) was added *N*-methylimidazole (25 μl, 0.3 mmol) dropwise at room temperature. The reaction mixture was stirred 3 h for completion and quenched with MeOH (0.1 ml). The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 10% MeOH) to give phosphoramidate **31** (48 mg, 72%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.02 (s, 0.5H), 8.01 (s, 0.5H), 7.35-7.15 (m, 5H), 6.03 (s, 0.5H), 6.00 (s, 0.5H), 4.60-4.47 (m, 2H), 4.27-3.92 (m, 5H), 1.47 (s, 6H), 1.34-1.26 (m, 6H), 1.21-1.13 (m, 3H), 0.96 (s, 1.5H), 0.93 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 4.96, 4.84; LRMS calcd for $C_{27}H_{39}N_7O_{11}P$ $(M+1)^+$ 668.24, found 668.45.



32

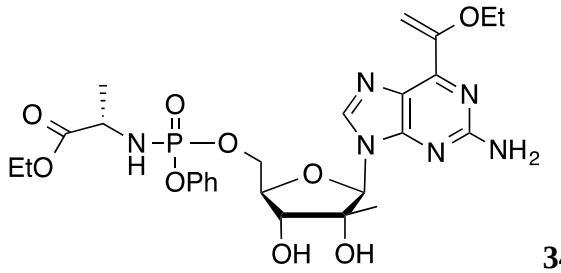
Ethyl ((((2R,3S,4R,5R)-5-(2-amino-6-(methoxyamino)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (32). A procedure similar to that used for **31** was employed for the synthesis of prodrug **32** (21%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 7.65 (s, 1H), 7.36-7.15 (m, 5H), 5.84 (s, 0.5H), 5.82 (s, 0.5H), 5.04 (s, 2H), 4.61-4.46 (m, 2H), 4.20-3.90 (m, 6H), 3.83 (s, 1.5H), 3.82 (s, 1.5H), 1.36-1.28 (m, 3H), 1.20 (t, *J* = 7.2 Hz, 1.5H), 1.18 (t, *J* = 7.2 Hz, 1.5H), 0.98 (s, 1.5H), 0.95 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.12, 5.07; LRMS calcd for $C_{23}H_{33}N_7O_9P$ $(M+1)^+$ 582.21, found 582.18.



33

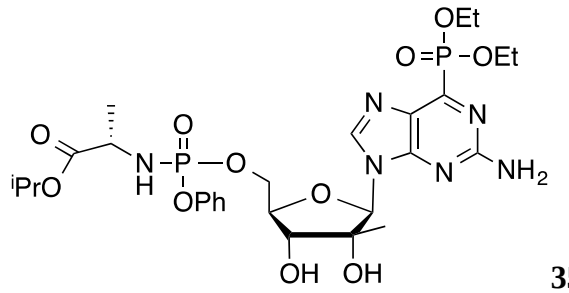
Ethyl ((((2R,3R,4R,5R)-5-(2-amino-6-azido-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (33). A procedure similar to that used for **31** was employed for the synthesis of prodrug **33** (79%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.22 (s, 0.5H), 8.21 (s, 0.5H), 7.33-7.12 (m, 5H), 6.15 (s, 0.5H), 6.13 (s, 0.5H), 4.61-4.51 (m, 2H), 4.30-3.81 (m, 5H), 1.32-

1.29 (m, 3H), 1.18 (t, *J* = 7.2 Hz, 1.5H), 1.17 (t, *J* = 7.2 Hz, 1.5H), 1.00 (s, 1.5H), 0.97 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.13, 5.03; LRMS calcd for $C_{22}H_{29}N_9O_8P$ $(M+1)^+$ 578.19, found 578.03; HRMS $(M+1)^+$ 578.1877, found 578.1877.



34

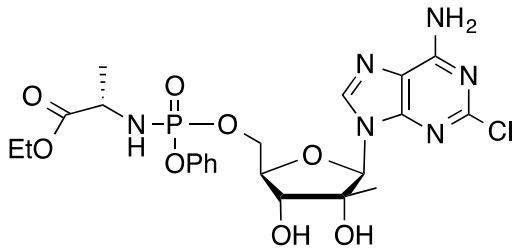
Ethyl ((((2R,3R,4R,5R)-5-(2-amino-6-(1-ethoxyvinyl)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (34). A procedure similar to that used for **31** was employed for the synthesis of prodrug **34** (32%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.13 (s, 1H), 7.30-7.12 (m, 5H), 6.03 (s, 0.5H), 6.00 (s, 0.5H), 5.84 (s, 0.5H), 5.80 (s, 0.5H), 4.59-4.20 (m, 5H), 4.09-3.89 (m, 5H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.29-1.25 (m, 3H), 1.18-1.11 (m, 3H), 0.97 (s, 1.5H), 0.94 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.11, 4.98; LRMS calcd for $C_{26}H_{36}N_6O_9P$ $(M+1)^+$ 607.23, found 607.12.



35

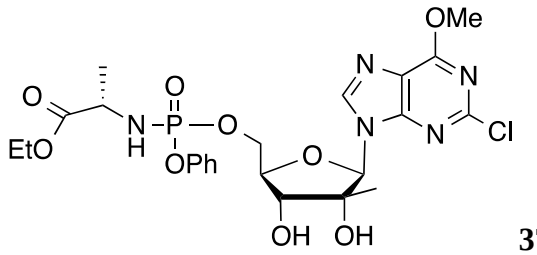
Isopropyl ((((2R,3R,4R,5R)-5-(2-amino-6-(diethoxyphosphoryl)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate

(35). A procedure similar to that used for **31** was employed for the synthesis of prodrug **35** (77%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.23 (s, 0.5H), 8.22 (s, 0.5H), 7.32-7.12 (m, 5H), 6.03 (s, 0.5H), 6.01 (s, 0.5H), 4.92-4.78 (m, 1H), 4.56-4.46 (m, 2H), 4.35-4.27 (m, 5H), 4.20-4.18 (m, 1H), 3.91-3.86 (m, 1H),1.37-1.33 (m, 6H), 1.28-1.26 (m, 3H), 1.21-1.10 (m, 6H), 0.97 (s, 1.5H), 0.94 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 8.54, 8.52, 5.15, 5.06; LRMS calcd for $C_{26}H_{39}N_6O_{11}P_2$ $(M+1)^+$, 687.23, found 687.01.



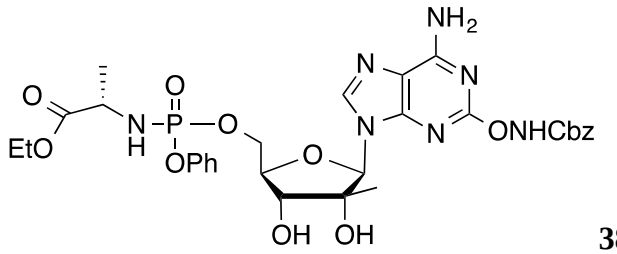
36

Ethyl ((((2R,3R,4R,5R)-5-(6-amino-2-chloro-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (36). A procedure similar to that used for **31** was employed for the synthesis of prodrug **36** (69%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.20 (s, 0.5H), 8.19 (s, 0.5H), 7.35-7.15 (m, 5H), 6.03 (s, 0.5H), 6.00 (s, 0.5H), 4.60-4.50 (m, 2H), 4.27-3.89 (m, 5H), 1.32-1.27 (m, 3H), 1.20 (t, *J* = 7.2 Hz, 1.5H), 1.17 (t, *J* = 7.2 Hz, 1.5H), 0.96 (s, 1.5H), 0.94 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 4.95, 4.82; LRMS calcd for $C_{22}H_{29}ClN_6O_8P$ $(M+1)^+$ 571.15, found 571.07; HRMS $(M+1)^+$ 571.1473, found 571.1475.



37

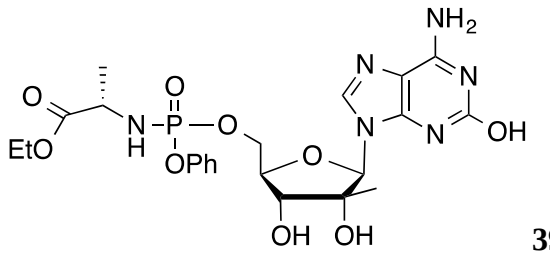
Ethyl ((((2R,3R,4R,5R)-5-(2-chloro-6-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (37). A procedure similar to that used for **31** was employed for the synthesis of prodrug **37** (75%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.39 (s, 0.5H), 8.38 (s, 0.5H), 7.33-7.12 (m, 5H), 6.11 (s, 0.5H), 6.09 (s, 0.5H), 4.62-4.50 (m, 2H), 4.29-3.89 (m, 8H), 1.33-1.28 (m, 3H), 1.20 (t, *J* = 7.2 Hz, 1.5H), 1.17 (t, *J* = 7.2 Hz, 1.5H), 0.96 (s, 1.5H), 0.93 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.01, 4.86; LRMS calcd for $C_{23}H_{30}ClN_5O_9P$ $(M+H)^+$ 586.15, found 586.01; HRMS $(M+1)^+$ 586.1470, found 586.1475.



38

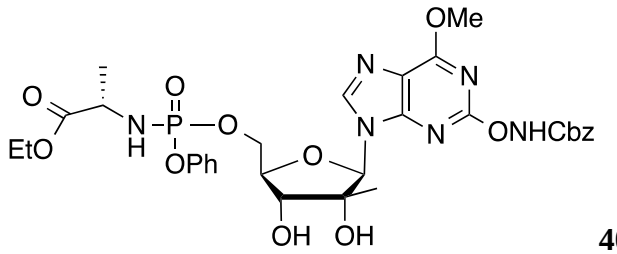
Ethyl ((((2R,3S,4R,5R)-5-(6-amino-2-((((benzyloxy)carbonyl)amino)oxy)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (38). A procedure similar to that used for **31** was employed for the synthesis of prodrug **38** (69%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.10 (s, 0.5H), 8.09 (s, 0.5H), 7.32-7.14 (m, 10H), 5.98 (s, 0.5H), 5.96 (s, 0.5H), 5.21 (s, 2H), 4.57-4.50 (m, 2H), 4.26-3.90 (m, 5H), 1.29 (t, *J* = 6.0 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 1.5H), 1.15 (t, *J* = 7.2

Hz, 1.5H), 0.94 (s, 1.5H), 0.91 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.04, 4.92; LRMS calcd for $C_{30}H_{37}N_7O_{11}P$ $(M+1)^+$ 702.23, found 702.10; HRMS $(M + 1)^+$ 702.2289, found 702.2298.



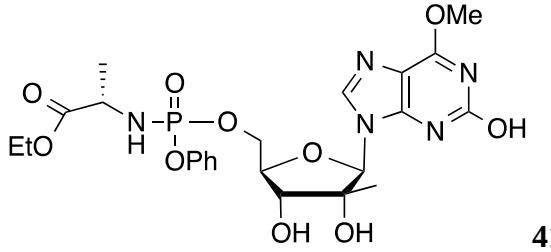
39

Ethyl ((((2R,3R,4R,5R)-5-(6-amino-2-hydroxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (39). A suspension of prodrug **38** (19 mg, 0.027 mmol) and Pd/C (5 mg) in MeOH (1 ml) was stirred under hydrogen atmosphere at room temperature for 15 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 12% MeOH) to give phosphoramidate **39** (9.5 mg, 63%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 7.91 (s, 1H), 7.37-7.16 (m, 5H), 5.91 (s, 0.5H), 5.89 (s, 0.5H), 4.59-4.41 (m, 2H), 4.21-3.90 (m, 5H), 1.35-1.28 (m, 3H), 1.25-1.17 (m, 3H), 1.01 (s, 1.5H), 0.98 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.01, 4.88; LRMS calcd for $C_{22}H_{30}N_6O_9P$ $(M+1)^+$ 553.18, found 553.07; HRMS $(M+1)^+$ 553.1812, found 553.1811.



40

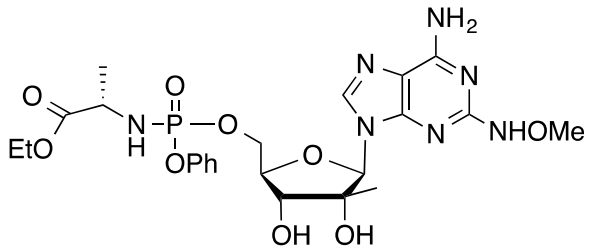
Ethyl ((((2R,3S,4R,5R)-5-(2-((((benzyloxy)carbonyl)amino)oxy)-6-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (40). A procedure similar to that used for **31** was employed for the synthesis of prodrug **40** (67%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.26 (s, 0.5H), 8.25 (s, 0.5H), 7.33-7.13 (m, 10H), 6.04 (s, 0.5H), 6.02 (s, 0.5H), 5.22 (s, 1H), 5.21 (s, 1H), 4.62-4.50 (m, 2H), 4.29-3.90 (m, 8H), 1.30-1.28 (m, 3H), 1.18 (t, *J* = 7.2 Hz, 1.5H), 1.17 (t, *J* = 7.2 Hz, 1.5H), 0.94 (s, 1.5H), 0.91 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.07, 4.94; LRMS calcd for $C_{31}H_{38}N_6O_{12}P$ $(M+1)^+$ 717.23, found 717.10; HRMS $(M+1)^+$ 717.2285, found 717.2287.



41

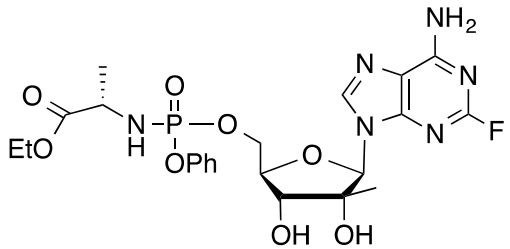
Ethyl ((((2R,3R,4R,5R)-3,4-dihydroxy-5-(2-hydroxy-6-methoxy-9H-purin-9-yl)-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (41). A suspension of **40** (20 mg, 0.028 mmol) and Pd/C (5 mg) in MeOH (1 ml) was stirred under hydrogen atmosphere at room temperature for 15 h. The reaction mixture was then concentrated under reduced pressure and the residue was purified by column

chromatography on silica gel (CH_2Cl_2:MeOH; 0 to 12% MeOH) to give phosphoramidate **41** (12.1 mg, 76%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.09 (s, 0.5H), 8.08 (s, 0.5H), 7.36-7.13 (m, 5H), 6.03 (s, 0.5H), 6.01 (s, 0.5H), 4.61-4.45 (m, 2H), 4.21-3.90 (m, 8H), 1.33-1.28 (m, 3H), 1.20 (t, *J* = 7.2 Hz, 1.5H), 1.18 (t, *J* = 7.2 Hz, 1.5H), 0.96 (s, 1.5H), 0.93 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.01, 4.88; LRMS calcd for $C_{23}H_{31}N_5O_{10}P$ $(M+1)^+$ 568.18, found 568.07; HRMS $(M+1)^+$ 568.1809, found 568.1812.



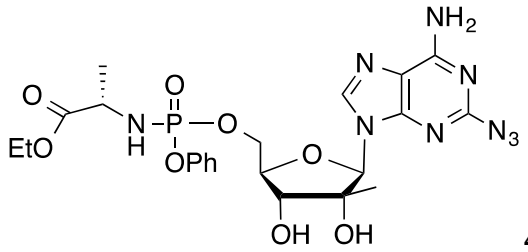
42

Ethyl ((((2R,3S,4R,5R)-5-(6-amino-2-(methoxyamino)-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (42). A procedure similar to that used for **31** was employed for the synthesis of prodrug **42** (19%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 7.98 (s, 0.5H), 7.97 (s, 0.5H), 7.34-7.17 (m, 5H), 6.03 (s, 0.5H), 5.99 (s, 0.5H), 4.62-4.45 (m, 2H), 4.23-3.66 (m, 8H), 1.43-1.11 (m, 6H), 0.98 (s, 1.5H), 0.95 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.13, 5.07; LRMS calcd for $C_{23}H_{33}N_7O_9P$ $(M+1)^+$ 582.21, found 582.33.



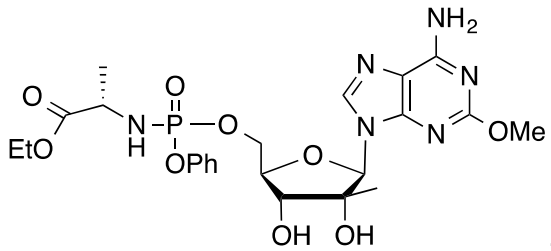
43

Ethyl ((((2R,3R,4R,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (43). A procedure similar to that used for **31** was employed for the synthesis of prodrug **43** (85%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.18 (s, 0.5H), 8.17 (s, 0.5H), 7.35-7.15 (m, 5H), 6.00 (s, 0.5H), 5.98 (s, 0.5H), 4.60-4.47 (m, 2H), 4.26-3.96 (m, 5H), 1.33-1.27 (m, 3H), 1.19 (t, *J* = 7.2 Hz, 1.5H), 1.17 (t, *J* = 7.2 Hz, 1.5H), 0.97 (s, 1.5H), 0.95 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.00, 4.87; LRMS calcd for $C_{22}H_{29}FN_6O_8P$ $(M+1)^+$ 555.18, found 555.05; HRMS $(M+1)^+$ 555.1769, found 555.1777.



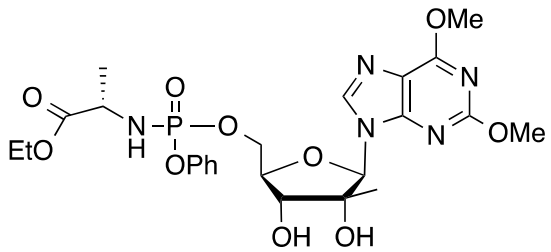
44

Ethyl ((((2R,3R,4R,5R)-5-(6-amino-2-azido-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (44). A procedure similar to that used for **31** was employed for the synthesis of prodrug **44** (20%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.33 (s, 0.1 H), 8.32 (s, 0.1 H), 8.11 (s, 0.4H), 8.10 (s, 0.4H), 7.33-7.12 (m, 5H), 6.16 (s, 0.1 H), 6.12 (s, 0.1 H), 5.98 (s, 0.4H), 5.95 (s, 0.4H), 4.56-4.44 (m, 2H), 4.28-4.17 (m, 2H), 4.10-3.85 (m, 3H), 1.31-1.25 (m, 3H), 1.19-1.12 (m, 3H), 1.02 (s, 0.3 H), 0.99 (s, 0.3 H), 0.95 (s, 1.2H), 0.93 (s, 1.2H); ^{31}PNMR (162 MHz, CD_3OD) δ 4.98, 4.82; LRMS calcd for $C_{22}H_{29}N_9O_8P$ $(M+1)^+$ 578.18, found 578.05.



45

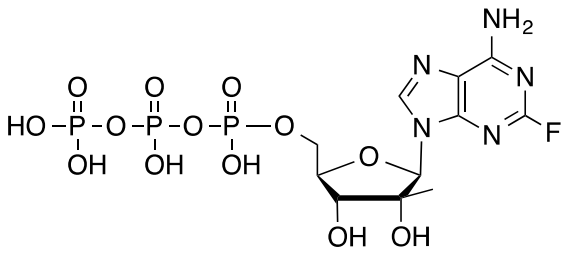
Ethyl ((((2R,3R,4R,5R)-5-(6-amino-2-methoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (45). A procedure similar to that used for **31** was employed for the synthesis of prodrug **45** (71%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.06 (s, 0.5H), 8.05 (s, 0.5H), 7.36-7.15 (m, 5H), 6.01 (s, 0.5H), 5.98 (s, 0.5H), 4.62-4.44 (m, 2H), 4.35-4.18 (m, 2H), 4.12-3.86 (m, 6H), 1.31-1.27 (m, 3H), 1.19 (t, *J* = 7.2 Hz, 1.5H), 1.15 (t, *J* = 7.2 Hz, 1.5H), 1.00 (s, 1.5H), 0.98 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.06, 4.89; LRMS calcd for $C_{23}H_{32}N_6O_9P$ $(M+1)^+$ 567.20, found 567.10; HRMS $(M+1)^+$ 567.1968, found 567.1973.



46

Ethyl ((((2R,3R,4R,5R)-5-(2,6-dimethoxy-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (46). A procedure similar to that used for **31** was employed for the synthesis of prodrug **46** (81%). ^{1}HNMR (400 MHz, CD_3OD) (1:1 mixture) δ 8.20 (s, 0.5H), 8.19 (s, 0.5H), 7.34-7.12 (m, 5H), 6.07 (s, 0.5H), 6.04 (s, 0.5H), 4.62-4.46 (m, 2H), 4.36-4.20 (m, 2H), 4.13 (s, 1.5H), 4.12 (s, 1.5H), 4.17-3.85 (m, 6H), 1.31-1.26 (m, 3H), 1.18 (t, *J* = 7.2 Hz,

1.5H), 1.15 (t, *J* = 7.2 Hz, 1.5H), 0.99 (s, 1.5H), 0.97 (s, 1.5H); ^{31}PNMR (162 MHz, CD_3OD) δ 5.10, 4.93; LRMS calcd for $C_{24}H_{33}N_5O_{10}P$ $(M+1)^+$ 582.20, found 582.06; HRMS $(M+1)^+$ 582.1965, found 582.1965.



TP of 28

((2R,3R,4R,5R)-5-(6-amino-2-fluoro-9H-purin-9-yl)-3,4-dihydroxy-4-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate (Triphosphate of compound 28). In a dry 5 ml of round bottom flask containing a small stirring bar is added the nucleoside **28** (5 mg, 16.7 µmol), 6 accounts 4Å MS and $PO(OMe)_3$ (0.2 ml). After stirring overnight, the mixture is cooled drown in an ice-bath. Collidine is added (4 µl) and the reaction stirred for 10 min at 0 °C. 4 µl of $POCl_3$ is then added and the reaction stirred for 1 h at 0 °C (a white precipitate be seen). A 1M DMF solution of TBAP (120 µM) and tributylamine (20 µl) are added subsequently, and the reaction is stirred for 30 min at room temperature. The reaction is then quenched by a 0.2 M solution of TEAB and stirred for 45 min at ambient. The mixture is then transferred in a simple HPLC tube, and washed three times by DCM (3 x 1 ml). The resulting aqueous phase is then purified HPLC using ionexchange column. The collected fraction was checked by LC-MS-MS. LC-MS-MS calcd for $C_{11}H_{16}FN_5O_{13}P_3$ $(M-H)^+$ 537.99, found 538.0.

---

(1) Stuyver L. J.; Whitaker T.; McBrayer T. R.; Hernandez-Santiago B. I.; Lostia S.; Tharnish P. M.; Ramesh M.; Chu C. K.; Jordan R.; Shi J.; Rachakonda S.; Watanabe K. A.; Otto M. J.;

Schinazi R. F. Ribonucleoside analogue that blocks replication of bovine viral diarrhea and hepatitis C viruses in culture*. Antimicrob. Agents Chemother*. **2003**, *47*, 244-254.