Supporting Information

Synthesis and Anti-HCV Activity of a Novel 2',3'-Dideoxy-2'-α-fluoro-2'-β-C-methyl Guanosine Phosphoramidate Prodrug

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1. Chemistry. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz (100 MHz for ^{13}C NMR) spectrometer. Chemical shift values are given in parts per million (ppm) relative to the internal standard, tetramethylsilane (TMS). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; hept, heptet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; tt, triplet of triplets. The coupling constants (*J*) are reported in Hertz (Hz). MS was obtained on a Thermo Fisher Scientific mass spectrometer equipped with an electrospray ion source (ESI) and operated in the positive mode. Flash column chromatography was performed over 200–300 mesh silica gel and the solvents were distilled prior to use.

*1.1. Preparation of (2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-fluoro-4-methyltetrahydrofuran-3-ol (**5**).* Starting material **4** was prepared according to a previously reported method.[1] ^{1}H NMR (400 MHz, CD_3OD) δ 8.20 (s, 1H), 6.14 (d, J = 17.6 Hz, 1H), 4.37 (dd, J = 24.4, 9.2 Hz, 1H), 4.04-3.99 (m, 5H), 3.84 (dd, J = 12.8, 3.2 Hz, 1H), 1.14 (d, J = 22.0 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 160.5, 152.9, 137.6, 113.9, 100.8 (d, $J_{C–F}$ = 179.3 Hz), 88.9 (d, $J_{C–F}$ = 39.1 Hz), 82.2, 71.1 (d, $J_{C–F}$ = 17.8 Hz), 59.4, 52.8, 15.4 (d, $J_{C–F}$ = 25.2 Hz); MS (m/z) $[M + H]^+$ calcd for $C_{12}H_{17}FN_5O_4$ 314.13, found 314.21.

To a stirred solution of compound **4** (250 mg, 0.8 mmol) in anhydrous DMF (10 mL) was added TBDMSCl (241 mg, 1.6 mmol) and imidazole (217 mg, 3.2 mmol) in sequence at room temperature. After 12 h, the reaction was quenched with water (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated, and purified through silica gel column chromatography [EtOAc/petroleum ether (PE) 50:50] to afford product **5** (276 mg, 81%) as a colorless semi-solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H), 6.14 (d, J = 17.2 Hz, 1H), 4.26 (dd, J = 24.4, 9.2 Hz, 1H), 4.13 (dd, J = 12.0 Hz, 1H), 4.04 (m, 1H), 4.03 (s, 3H), 3.96 (dd, J = 12.0, 2.8 Hz, 1H), 1.15 (d, J = 22.0 Hz, 3H), 0.95 (s, 9H), 0.14 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 160.6, 153.1, 137.0, 113.8, 100.8 (d, $J_{C–F}$ = 179.5 Hz), 88.3 (d, $J_{C–F}$ = 38.7 Hz), 82.0, 70.7 (d, $J_{C–F}$ = 17.9 Hz), 61.0, 52.8, 25.1, 18.1, 15.3 (d, $J_{C–F}$ = 25.0 Hz), -6.65, -6.69; MS (m/z) $[M + H]^+$ calcd for $C_{18}H_{31}FN_5O_4Si$ 428.21, found 428.30.

*1.2. Preparation of O-((2R,3R,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-2-(((tert-butyldimethylsilyl)oxy)methyl)-4-fluoro-4-methyltetrahydrofuran-3-yl)1H-imidazole-1-carbothioate (**6**).* A solution of compound **5** (276 mg, 0.65 mmol) in anhydrous MeCN (10 mL) was treated with 1,1-thiocarbonyldiimidazole (576 mg, 3.2 mmol) and stirred under nitrogen atmosphere at room temperature for 24 h. The reaction mixture was concentrated, and then purified through silica gel column chromatography (MeOH/CH_2Cl_2 2:98) to afford product **6** (239 mg, 69%) as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.56 (s, 1H), 8.18 (s, 1H), 7.87 (s, 1H), 7.12 (s, 1H), 6.94 (dd, J = 21.4, 8.4 Hz, 1H), 6.31 (dt, J = 17.6 Hz, 1H), 4.62-4.59 (m, 1H), 4.16-4.12 (m, 1H), 4.09 (s, 3H), 4.01 (dt, J = 11.6, 3.2 Hz, 1H), 1.34 (d, J = 22.4 Hz, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 183.6, 161.4, 160.5, 152.9, 137.4 (d, $J_{C–F}$ = 29.8 Hz), 130.0, 118.5, 114.2, 104.4 (d, $J_{C–F}$ = 174.0 Hz), 88.9 (d, $J_{C–F}$ = 40.7 Hz), 79.4, 61.4, 52.9, 25.0, 17.8, 16.5 (d, $J_{C–F}$ = 24.9 Hz), -6.7, -6.8; MS (m/z) $[M + H]^+$ calcd for $C_{22}H_{33}FN_7O_4SSi$ 538.21, found 538.26.

*1.3. Preparation of 9-((2R,3R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-fluoro-3-methyltetrahydrofuran-2-yl)-6-methoxy-9H-purin-2-amine (**7**).* A reaction mixture of compound **6** (220 mg, 0.41 mmol), tri-*n*-butyltin hydride (549 μL, 2.05 mmol) and α,α-azobisbutyronitrile (34 mg, 0.21 mmol) in anhydrous toluene (7 mL) was stirred under nitrogen atmosphere at 80 °C for 4 h. Then, it was concentrated and purified through silica gel column chromatography (MeOH/CH_2Cl_2 1:99) affording product **7** (126 mg, 75%) as a colorless semi-solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 6.16 (d, J = 16.4 Hz, 1H), 4.48-4.53 (m, 1H), 4.16 (dd, J = 11.6, 2.0 Hz, 1H), 4.07 (s, 3H), 3.91 (dd, J = 11.6, 2.8 Hz, 1H), 2.45 (ddd, J = 25.2, 14.0, 11.2 Hz, 1H), 2.25 (td, J = 14.0, 4.8 Hz,1H), 1.22 (d, J = 21.6 Hz, 3H), 0.98 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 160.7, 153.2, 137.0, 113.9, 104.2 (d, $J_{C–F}$ = 174.0 Hz), 90.1 (d, $J_{C–F}$ = 40.7 Hz), 80.7, 62.8, 52.8, 36.0 (d, $J_{C–F}$ = 22.2 Hz), 25.2, 18.1, 17.8 (d, $J_{C–F}$ = 24.9 Hz), -6.6, -6.7; MS (m/z) $[M + H]^+$ calcd for $C_{18}H_{31}FN_5O_3Si$ 412.22, found 412.33.

*1.4. Synthesis of ((2S,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-4-fluoro-4-methyltetrahydrofuran-2-yl)methanol (**8**).* A solution of compound **7** (111 mg, 0.27 mmol) in anhydrous THF (5 mL) was treated with TBAF (1 M in THF, 0.41 mL, 0.41

mmol) and stirred at room temperature for 2 h. It was then concentrated and purified through silica gel column chromatography (MeOH/CH_2Cl_2 2:98) giving guanosine **8** (51 mg, 64%) as a colorless semi-solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.28 (s, 1H), 6.12 (d, J = 17.2 Hz, 1H), 4.41-4.47 (m, 1H), 4.03 (s, 3H), 3.99 (dd, J = 12.4, 2.8 Hz, 1H), 3.73 (dd, J = 12.4, 3.2 Hz, 1H), 2.48 (ddd, J = 25.6, 14.0, 11.6 Hz, 1H), 2.21 (ddd, J = 16.4, 14.4, 5.2 Hz, 1H), 1.17 (d, J = 22.0 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 160.5, 153.0, 137.5, 113.8, 104.2 (d, $J_{C–F}$= 174.1 Hz), 90.6 (d, $J_{C–F}$ = 40.9 Hz), 80.8, 61.0, 52.8, 37.9 (d, $J_{C–F}$ = 22.2 Hz), 17.8 (d, $J_{C–F}$ = 24.8 Hz); MS (m/z) $[M + H]^+$ calcd for $C_{12}H_{17}FN_5O_3$ 298.13, found 298.07.

*1.5. Preparation of (S)-cyclopentyl 2-(((S)-(perfluorophenoxy)(phenoxy)phosphoryl) amino) propanoate (**12**).* To a solution of phenyl dichlorophosphate (**10**, 3.07 g, 20 mmol) in CH_2Cl_2 (40 mL) was added L-alanyl cyclopentyl ester hydrochloride (**9**, 3.87 g, 20 mmol) then a solution of NEt_3 (5.5 mL, 40 mmol) in CH_2Cl_2 (5 mL) at -78 °C. The mixture was stirred at room temperature overnight, and further treated with a solution of pentafluorophenol (3.68 g, 20 mmol) and NEt_3 (5.5 mL, 40 mmol) in CH_2Cl_2 (5 mL). After stirred at room temperature for another 4 h, the solid was filtered off and washed with CH_2Cl_2 (10 mL). The filtrate was concentrated and redissolved in EtOAc (100 mL). The resulting solution was washed with sat. $NaHCO_3$ (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, concentrated, and purified through silica gel chromatography (EtOAc/PE 5:95 to 50:50). The pure single S_P-isomer **12** (3.78 g, 39%) was obtained as a white solid by further recrystallization from a mixture of EtOAc and hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, J = 8.0 Hz, 2H), 7.24-7.19 (m, 3H), 5.22-5.18 (m, 1H), 4.18-4.08 (m, 1H), 4.00-3.95 (m, 1H), 1.89-1.83 (m, 2H), 1.72-1.59 (m, 6H), 1.45 (d, J = 6.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7 (d, $J_{C–P}$ = 8.7 Hz), 150.2 (d, $J_{C–P}$ = 7.2 Hz), 129.9, 125.7, 120.1 (d, $J_{C–P}$ = 5.0 Hz), 78.9, 50.6 (d, $J_{C–P}$ = 1.8 Hz), 32.6 (d, $J_{C–P}$ = 11.9 Hz), 23.6, 21.0 (d, $J_{C–P}$ = 4.3 Hz); MS (m/z) $[M + H]^+$ calcd for $C_{20}H_{20}F_5NO_5P$ 480.10, found 480.15.

*1.6. Synthesis of (S)-cyclopentyl 2-(((S)-(((2S,4R,5R)-5-(2-amino-6-methoxy-9H-purin-9-yl)-4-fluoro-4-methyltetrahydrofuran-2-yl)methoxy)(phenoxy)phosphoryl)amino)propanoate (**1**).* To a mixture of compound **12** (72 mg, 0.15 mmol) and guanosine **8** (30 mg, 0.1 mmol) in THF (5 mL) was added a solution of ^{t}BuMgCl (1 M in THF,

0.3 mL, 0.3 mmol). The reaction was stirred at room temperature for 2 h, then diluted with EtOAc (10 mL), and washed with brine (10 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated and purified through silica gel chromatography (MeOH/CH_2Cl_2 2:98) to give the phosphoramidate prodrug **1** (49 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.96 (s, 1H), 7.32 (t, J = 8.0 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 7.17-7.13 (m, 1H), 6.11 (d, $J_{C\text{-}F}$ = 17.6 Hz, 1H), 5.04-5.00 (m, 1H), 4.60-4.53 (m, 1H), 4.50-4.45 (m, 1H), 4.41-4.35 (m, 1H), 4.02 (s, 3H), 3.91-3.84 (m, 1H), 2.72-2.56 (m, 1H), 2.33-2.24 (m, 1H), 1.82-1.52 (m, 8H), 1.26 (d, J = 7.2 Hz, 3H), 1.21 (d, $J_{C\text{-}F}$ = 22.0 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.2 (d, $J_{C–P}$ = 5.3 Hz), 161.3, 160.5, 153.0, 150.7 (d, $J_{C–P}$ = 6.8 Hz), 137.8, 129.3 (d, $J_{C–P}$ = 0.9 Hz), 124.7 (d, $J_{C–P}$ = 1.2 Hz), 120.0 (d, $J_{C–P}$ = 4.8 Hz), 114.1, 103.7 (d, $J_{C–F}$ = 174.2 Hz), 91.3 (d, $J_{C–F}$ = 43.3 Hz), 78.7 (d, $J_{C–P}$ = 8.5 Hz), 78.0, 66.7 (d, $J_{C–P}$ = 5.0 Hz), 52.8, 50.2 (d, $J_{C–P}$ = 1.2 Hz), 37.9 (d, $J_{C–F}$ = 22.4 Hz), 32.1, 32.0, 23.14, 23.13, 19.1 (d, $J_{C–P}$ = 6.5 Hz), 17.8 (d, $J_{C–F}$ = 24.8 Hz); MS (m/z) $[M + H]^+$ calcd for $C_{26}H_{35}FN_6O_7P$ 593.23, found 593.26.

2. Anti-HCV Assay. A human hepatoma cell line (Huh-7) containing replicating HCV subgenomic genotype 1b, 1a, 2a, or 1b S282T replicon with a luciferase reporter gene (luc-ubi-neo) was used to evaluate anti-HCV activity of the compounds. In this assay, the level of luciferase signal correlates with the viral RNA replication directly. The HCV replicon-reporter cell line (NK/luc-ubi-neo) was cultured in DMEM medium supplemented with 10% FBS and 0.5 mg/mL Geneticin (G418). Cells were maintained in a subconfluent state to ensure high levels of HCV replicon RNA synthesis. To evaluate the antiviral activity of compounds, serial dilutions were prepared with concentrations ranging from 0.14 to 300 μM. Diluted compounds were transferred to a 96-well plate followed by the addition of replicon cells (6000 cells per well). Cells were incubated with the compounds for 48 h after which luciferase activity was measured. Reduction of luciferase signal reflected the decrease of HCV replicon RNA in the treated cells and used to determine the EC_{50} value (concentration which yielded a 50% reduction in luciferase activity).

3. Cytotoxicity Assay. A Huh-7 cell line carrying a luciferase reporter gene (driven by a HIV LTR promoter) stably integrated into the chromosome was used to analyze

the cytotoxic effect of the compounds. This cell line (LTR-luc) was maintained in DMEM medium with 10% fetal bovine serum (FBS). Design of the cytotoxicity assay was similar to that of the HCV replicon assay. Reduction of luciferase activity in the treated cells correlated with the cytotoxic effect of the test compound and was used to calculate the CC_{50} value (concentration that inhibited cell growth by 50%).

4. References

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