Supporting Information

Discovery of PSI-353661, a Novel Purine Nucleotide Prodrug for the Treatment of HCV Infection

Wonsuk Chang, Donghui Bao, Byoung-Kwon Chun, Devan Naduthambi, Dhanapalan Nagarathnam, Suguna Rachakonda, P. Ganapati Reddy, Bruce S. Ross, Hai-Ren Zhang, Shalini Bansal, Christine L. Espiritu, Meg Keilman, Angela M. Lam, Congrong Niu, Holly Micolochick Steuer, Phillip A. Furman, Michael J. Otto, Michael J. Sofia*

Pharmasset, Inc.

303A College Road East

Princeton, New Jersey 08540-6608

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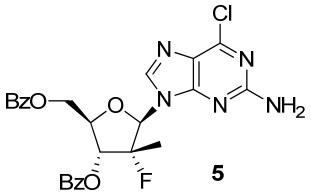
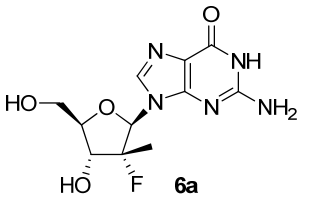
1. Chemistry general methods. Reactions were monitored by thin layer chromatography with Analtech Uniplate 250 micron and visualized by UV light or by charring in 5% sulfuric acid in methanol. All solvents and reagents were used as received from Sigma-Aldrich or Acros organics. NMR spectra were recorded with a Varian Mercury*plus* 400 MHz spectrometer. Infrared spectra were obtained on a Perkin Elmer Spectrum 100 FT-IR through a universal reflectance accessory. Optical rotations were measured using a Perkin Elmer Model 341 Polarimeter at ambient temperature and 589 nm. Low resolution mass spectra were recorded on a Waters Micromass QuattroMicro API. High resolution mass spectra were performed at the Wuxi AppTec in Shanghai, China using ESI method. HPLC were obtained on a Waters Alliance 2695 HPLC using a Waters Atlantis C18 column and a gradient of 50 mM triethylamine-acetic acid buffer and acetonitrile.

2. Synthesis of new compounds.



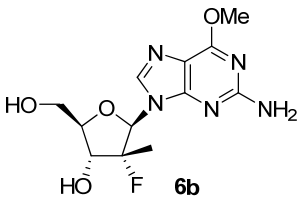
5

Chloride 5. To a solution of ribonolactol **3** (0.80 g, 2.1 mmol), 2-amino-6-chloropurine **4** (0.45 g, 2.5 mmol) and Ph_3P (0.56 g, 2.1 mmol) in anhydrous THF (20 mL) under N_2 was added diethyl azodicarboxylate (1.8 mL, 11 mmol). It was stirred at rt overnight. Then, the reaction was concentrated under reduce pressure. The residue was separated with flash column chromatography eluted with 25% EtOAc in Hex to give chloride **4 (**0.12 g, 11%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 8.05-8.02 (m, 2H), 7.94-7.92 (m, 2H), 7.88 (s, 1H), 7.62-7.57 (m, 1H), 7.52-7.42 (m, 3H), 7.34-7.30 (m, 2H), 6.43 (dd, *J* = 9.2, 13.6 Hz, 1H), 6.12 (d, *J* = 18.4 Hz, 1H), 5.38 (bs, 2H), 5.00 (dd, *J* = 4.4, 7.6 Hz, 1H), 4.75 (dt, *J* = 9.2, 4.8 Hz, 1H), 4.60 (dd, *J* = 5.2, 6.8 Hz, 1H), 1.33 (d, *J* = 22.8 Hz, 3H); LRMS calcd for $C_{25}H_{22}ClFN_5O_5$ $(M+H)^+$ 526.9, found 526.8.



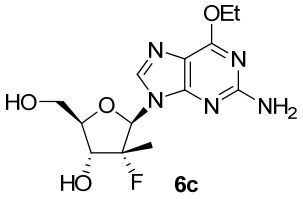
6a

2′-Fluoro-2′-*C*-methylguanosine 6a. To the solution of chloride **5** (400 mg, 0.76 mmol) in anhydrous MeOH (10 ml) was added 2-mercaptoethanol (600 mg, 7.6 mmol) and NaOMe (165 mg, 3.1 mmol). The solution was refluxed overnight under Ar. Then, the reaction was quenched with water (0.5 ml), and the solution was neutralized to pH 7 by addition of 1 N HCl. The solvent was removed under reduced pressure and the residue was purified by column chromatography eluted with 0 to 15% MeOH in CH_2Cl_2 to give guanosine **6a** (110 mg, 48%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 10.65 (s, 1H), 7.97 (s, 1H), 6.57 (s, 2H), 5.91 (d, *J* = 18.0 Hz, 1H), 5.62 (d, *J* = 7.2 Hz, 1H), 5.18 (t, *J* = 4.8 Hz, 1H), 4.05-4.15 (m, 1H), 3.79-3.86 (m, 2H), 3.63-3.65 (m, 1H), 1.04 (d, *J* = 22.4 Hz, 3H); LRMS calcd for $C_{11}H_{15}FN_5O_4$ $(M+H)^+$ 300.3, found 300.2.



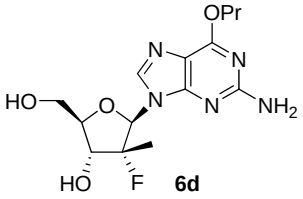
6b

Ether 6b. To a solution of chloride **5** (500 mg, 0.95 mmol) in anhydrous MeOH (10 ml) was added NaOMe (25% in MeOH, 0.90 ml, 3.9 mmol). The reaction was heated to 50 ^{o}C for 3 h. Then, it was neutralized to pH 7 by addition of AcOH. It was concentrated, and EtOAC (30 ml) was added. Insoluble solid was filtered off. The filtrate was concentrated, and purified by flash column chromatography on silica gel using 0 to 15% MeOH in CH_2Cl_2 as eluents. Nucleoside **6b** (203 mg, 68.3%) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.17 (s, 1H), 6.61 (s, 2H), 6.05 (d, J = 17.6 Hz, 1H), 5.67 (d, J = 7.2 Hz, 1H), 5.25 (t, J = 4.8 Hz, 1H), 4.18 (dt, J = 25.6, 8.0 Hz, 1H), 3.96 (s, 3H), 3.91 (dm, J = 9.2 Hz, 1H), 3.85 (ddd, J = 2.0, 5.2, 12.8 Hz, 1H), 3.69 (ddd, J = 2.8, 3.2, 12.0 Hz, 1H), 1.06 (d, J = 22.8 Hz, 3H); LRMS calcd for $C_{12}H_{17}FN_5O_4$ $(M+H)^+$ 314.3, found 314.2.



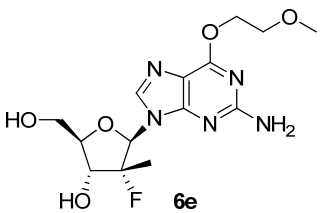
6c

Ether 6c. To a solution of chloride **5** (2.0 g, 3.8 mmol) in anhydrous EtOH (20 ml) was added NaH (60% in mineral oil, 0.61 g, 15.3 mmol). The reaction was heated to 50 ^{o}C for 3 h. Then, it was neutralized to pH 7 by addition of AcOH. It was concentrated, and EtOAC (30 ml) was added. Insoluble solid was filtered off. The filtrate was concentrated, and purified by column chromatography eluted with 0 to 10% MeOH in CH_2Cl_2 to give nucleoside **6c** (203 mg, 68%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.12(s, 1H), 6.49(s, 1H), 6.01 (d, J = 18 Hz, 1H), 5.62 (s, 1H), 5.19 (t, J = 4.6 Hz, 1H), 4.22 (dd, J = 6.4, 13.6 Hz, 2H), 4.10-4.11 (m, 1H), 3.79-3.88 (m, 2H), 3.64-3.67(m, 1H), 1.30-1.34 (m, 3H), 1.02 (d, J = 22.4 Hz, 3H); LRMS calcd for $C_{13}H_{19}FN_5O_4$ $(M+H)^+$ 328.3, found 328.1.



6d

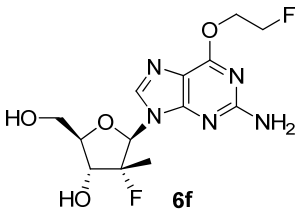
Ether 6d. This compound was prepared from **5** and PrOH following a similar procedure as the preparation of **6d** in 77% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (s, 1H), 6.54 (s, 2H), 6.01-6.05 (d, J = 16 Hz, 1H), 5.65-5.67 (d, J = 6.8 Hz, 1H), 5.23-5.25 (t, J = 4.8 Hz, 1H), 4.31-4.35 (t, J = 6.8 Hz, 2H), 4.12-4.22 (m, 1H), 3.82-3.90 (m, 2H), 3.69 (s, 1H), 1.73-1.78 (m, 2H), 1.02-1.07 (d, J = 22.4 Hz, 3H), 0.94-0.98 (t, J = 7.4 Hz,3H); LRMS calcd for $C_{14}H_{21}FN_5O_4$ $(M+H)^+$ 342.3, found 342.1.



6e

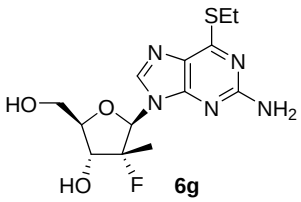
Ether 6e. This compound was prepared from **5** and 2-methoxyethanol following a similar procedure as the preparation of **6e** in 100% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 6.57 (bs, 2H), 6.03 (d, J = 18.0 Hz, 1H), 5.66 (d, J = 7.2 Hz, 1H), 5.24 (t, J = 4.8 Hz, 1H), 4.52 (dd, J = 4.4, 11.6

Hz, 1H), 4.47 (dd, *J* = 4.4, 11.6 Hz, 1H), 4.16 (dt, *J* = 24.8, 8.8 Hz, 1H), 3.89 (dm, *J* = 9.6 Hz, 1H), 3.83 (ddd, *J* = 2.0, 5.2, 12.4 Hz, 1H), 3.68 (m, 1H), 3.67 (t, *J* = 4.4 Hz, 2H), 3.29 (s, 3H), 1.04 (d, *J* = 22.4 Hz, 3H); LRMS calcd for $C_{14}H_{21}FN_5O_5$ $(M+H)^+$ 358.3, found 358.3.



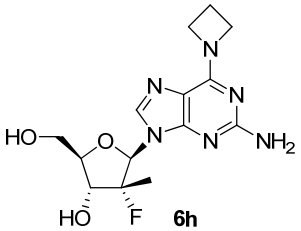
6f

Ether 6f. This compound was prepared from **5** and 2-fluoroethanol following a similar procedure as the preparation of **6f** in 100% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (s, 1H), 6.63 (bs, 2H), 6.05 (d, *J* = 18.0 Hz, 1H), 5.68 (d, *J* = 7.2 Hz, 1H), 5.26 (t, *J* = 4.8 Hz, 1H), 4.85 (t, *J* = 4.0 Hz, 1H), 4.73 (t, *J* = 4.0 Hz, 1H), 4.68 (q, *J* = 3.6 Hz, 1H), 4.61 (q, *J* = 4.0 Hz, 1H), 4.18 (dt, *J* = 25.2, 7.6 Hz, 1H), 3.91 (dm, *J* = 9.2 Hz, 1H), 3.85 (ddd, *J* = 2.0, 4.8, 12.4 Hz, 1H), 3.69 (ddd, *J* = 3.6, 4.4, 12.0 Hz, 1H), 1.07 (d, *J* = 22.4 Hz, 3H); LRMS calcd for $C_{14}H_{21}FN_5O_5$ $(M+H)^+$ 358.3, found 358.3.



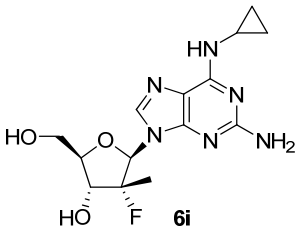
6g

Sulfane 6g. To a solution of chloride **5** (0.50 g, 0.95 mmol) in 1,2-dimeththoxyethane (10 ml) and MeOH (3 ml) was added K_2CO_3 (0.66 g, 4.8 mmol). The reaction was heated to 85 ^{o}C for 12 h. Then, it was neutralized to pH 7 by addition of 1 N HCl. The solution was concentrated and the crude was purified by column chromatography eluted with 0 to 10% MeOH in CH_2Cl_2 to give nucleoside **6g** (183 mg, 56%). ^{1}H NMR (DMSO-d_6) δ 8.25 (s, 1H), 6.65 (s, 1H), 6.05 (d, *J* = 18.8 Hz, 1H), 5.68 (d, *J* = 7.6 Hz, 1H), 5.25 (t, *J* = 4.0 Hz, 1H), 4.19 (dt, *J* = 25.6, 7.6 Hz, 2H), 3.91 (dm, *J* = 9.2 Hz, 1H), 3.85 (dd, *J* = 4.8, 12.0 Hz, 1H), 3.72-3.65 (m, 1H), 3.25 (q, *J* = 7.6 Hz, 2H), 1.32 (dt, *J* = 1.2, 7.2 Hz, 3H), 1.09 (d, *J* = 22.4 Hz, 3H); LRMS calcd for $C_{13}H_{19}FN_5O_3S$ $(M+H)^+$ 344.4, found 344.2.



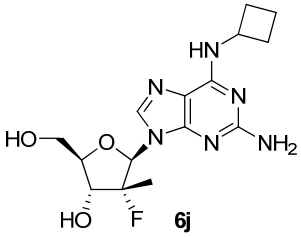
6h

Amine 6h. A solution of chloride **5** (80 mg, 0.15 mmol) and azetidine (1.0 mL, 15 mmol) in ethanol (10 mL) was heated to 70 ^{o}C for 16 h. Then, the solution was concentrated to give the crude product. The crude was purified by column chromatography eluted with 0 to 10% MeOH in CH_2Cl_2 to give nucleoside **6h** (17 mg, 33%). ^{1}H NMR (DMSO-d_6) δ 7.87 (s, 1H), 5.84-5.89 (m, 3H), 5.84-5.89 (m, 3H), 5.84-5.89 (m, 3H), 5.84-5.89 (m, 3H), 5.51 (s, br, 1H), 5.10 (s, br, 1H), 4.12-4.25 (m, 5H), 3.65-3.77 (m, 2H), 3.51-3.59 (m, 1H), 2.19-2.26 (m, 2H), 0.92 (d, *J* = 22.4 Hz, 3H); LRMS calcd for $C_{14}H_{20}FN_6O_3$ $(M+H)^+$ 339.3, found 339.1.



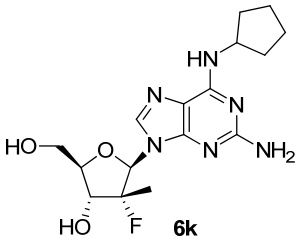
6i

Amine 6i. This compound was prepared from **5** and cyclopropanamine following a similar procedure as the preparation of **6h** in 99% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (s, 1H), 7.44 (s, 1H), 6.08 (m, 1H), 5.99 (s, 2H), 5.63 (d, J = 4.8 Hz, 1H), 5.25 (s, 1H), 4.16-4.22 (m, 1H), 3.82-3.90 (m, 2H), 3.68 (d, J = 12.0 Hz, 1H), 3.01(s, 1H), 1.06 (d, J = 22.4 Hz, 3H), 0.53-0.67 (m, 4H); LRMS calcd for $C_{14}H_{20}FN_6O_3$ $(M+H)^+$ 339.3, found 339.2.



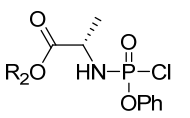
6j

Amine 6j. This compound was prepared from **5** and cyclobutanamine following a similar procedure as the preparation of **6h** in 22% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.99 (s, 1H), 7.59 (s, 1H), 6.00(d, J = 18.0 Hz, 1H), 5.93(s, 2H), 5.63 (s, 1H), 5.24 (s, 1H), 4.62-4.67 (m, 1H), 4.11-4.22 (m, 1H), 3.80-3.91 (m, 2H), 3.65-3.70 (m, 1H), 1.02-1.25 (m, 4H), 1.52-1.70 (m, 2H), 1.05 (t, J = 22.0 Hz, 3H); LRMS calcd for $C_{15}H_{22}FN_6O_3$ $(M+H)^+$ 353.3, found 353.2.



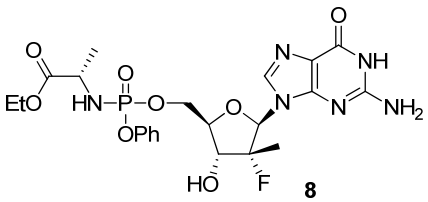
6k

Amine 6k. This compound was prepared from **5** and cyclopentanamine following a similar procedure as the preparation of **6h** in 53% yield (white solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.17 (s, 1H), 6.01 (d, J = 18.4 Hz, 1H), 5.90 (s, 2H), 5.62 (d, J = 6.8 Hz, 1H), 5.24 (t, J = 4.8 Hz, 1H), 4.46-4.51 (m, 1H), 4.14-4.25 (m, 1H), 3.82-3.90 (m, 2H), 3.67-3.70 (m, 1H), 1.82-1.93 (m, 2H), 1.61-1.75 (m, 2H), 1.45-1.60 (m, 4H), 1.06 (d, J = 22.4 Hz, 3H); LRMS calcd for $C_{16}H_{24}FN_6O_3$ $(M+H)^+$ 367.4, found 367.2.



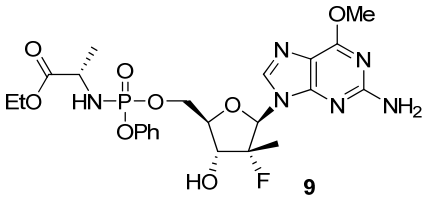
7a, R_2 = Et
7b, R_2 = Me
7c, R_2 = iPr
7d, R_2 = cyclopentyl
7e, R_2 = cyclohexyl

Phosphorochloridate 7a-e were prepared from the reported procedure[1] and used in the next steps without further purification.



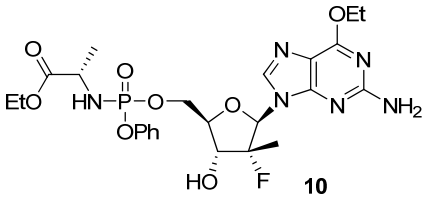
8

Prodrug 8. To a solution of nucleoside **6a** (90 mg, 0.30 mmol) and N-methylimidazole (500 mg, 6.17 mmol) in THF (25 ml) at rt was added phosphorochloridate **7a** (0.876 g, 3.01 mmol). The reaction was stirred overnight. Then, volatile component was evaporated under reduced pressure and the crude was purified by prep-HPLC give solid phosphoramidate **8** (17 mg, 11%) as a white solid. Purity (LCMS): 100%; ^{1}H NMR (DMSO-d_6) δ 11.57 (s, 1H), 8.49 (s, 1H), 7.77-7.80 (m, 2H), 7.15-7.22 (m, 3H), 6.62 (s, 2H), 6.02-6.09 (m, 2H), 5.80 (s, 1H), 4.27-4.37 (m, 4H), 3.97-4.16 (m, 2H), 3.85-3.87 (m, 1H), 0.85-1.27 (m, 9H); HRMS calcd for $C_{22}H_{29}FN_6O_8P$ $(M+H)^+$ 555.1763, found 555.1756.



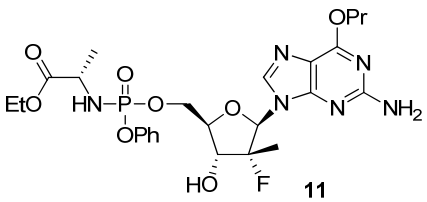
9

Prodrug 9. To a solution of nucleoside **6b** (50 mg, 0.16 mmol) and *N*-methylimidazole (0.10 ml, 1.3 mmol) in THF (1.5 ml) at 0 ^{o}C was added phosphorochloridate **7a** in THF (1.0 M, 0.48 ml, 0.48 mmol) dropwise. The reaction was slowly warmed to rt and stirred for 1 h. Then, water (0.1 ml) and EtOAc (5 ml) was added. The organic solution was washed with sat. aq. sodium citrate mono basic (2 × 2 ml) and sat. aq. $NaHCO_3$ (1 × 2 ml), dried ($MgSO_4$) and concentrated. The crude oil was purified by flash column chromatography on silica gel using 0 to 8% iPrOH in CH_2Cl_2 as eluents to give phosphoramidate **9** (28 mg, 31%) as a white solid. Purity (LCMS): 99.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 0.66H), 7.95 (s, 0.34H), 7.39-7.30 (m, 2H), 7.23-7.12 (m, 3H), 6.64 (bs, 2H), 6.14-6.01 (m, 2H), 4.48-4.24 (m, 3H), 4.16-3.98 (m, 3H), 3.96 (s, 3H), 3.86-3.76 (m, 2H), 1.24-1.05 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.88, 4.73; HRMS calcd for $C_{23}H_{31}FN_6O_8P$ $(M+H)^+$ 569.1920, found 569.1912.



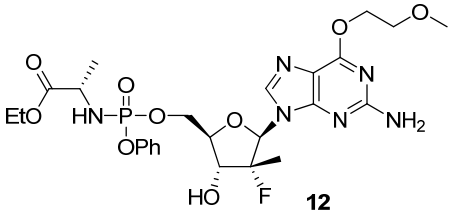
10

Prodrug 10. This compound was prepared from **6c** and **7a** following a similar procedure as the preparation of **9** in 75% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.5H), 7.94 (s, 0.5H), 7.39-7.30 (m, 2H), 7.25-7.13 (m, 3H), 6.59 (bs, 2H), 6.13-6.03 (m, 2H), 5.87-5.79 (m, 1H), 4.45 (q, J = 7.2 Hz, 2H), 4.40-4.24 (m, 2H), 4.16-3.96 (m, 3H), 3.40-3.35 (m, 1H), 3.83-3.75 (m, 1H), 1.36 (t, J = 7.2 Hz, 3H), 1.26-1.02 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.87, 4.74; HRMS calcd for $C_{24}H_{33}FN_6O_8P$ $(M+H)^+$ 583.2076, found 583.2070.



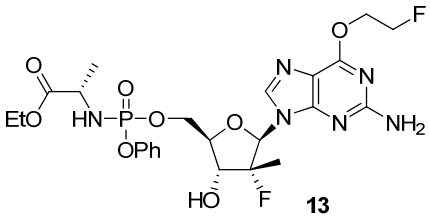
11

Prodrug 11. This compound was prepared from **6d** and **7a** following a similar procedure as the preparation of **9** in 66% yield (white solid). Purity (LCMS): 99.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.5H), 7.95 (s, 0.5H), 7.38-7.31 (m, 2H), 7.23-7.13 (m, 3H), 6.58 (bs, 2H), 6.13-6.00 (m, 2H), 5.87-5.79 (m, 1H), 4.46-4.24 (m, 2H), 4.36 (t, J = 7.2 Hz, 2H), 4.16-3.94 (m, 4H), 3.88-3.74 (m, 1H), 1.77 (sextet, J = 6.8 Hz, 2H), 1.24-1.04 (m, 9H), 0.97 (t, J = 7.6 Hz, 3H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.87, 4.75; HRMS calcd for $C_{25}H_{35}FN_6O_8P$ $(M+H)^+$ 597.2233, found 597.2235.



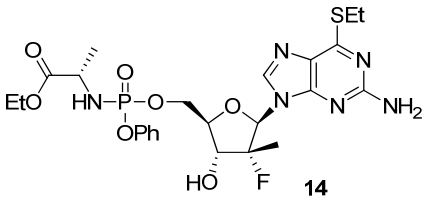
12

Prodrug 12. This compound was prepared from **6e** and **7a** following a similar procedure as the preparation of **9** in 39% yield (white solid). Purity (LCMS): 97.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (s, 0.5H), 7.96 (s, 0.5H), 7.38-7.32 (m, 2H), 7.23-7.11 (m, 3H), 6.62 (bs, 2H), 6.13-6.00 (m, 2H), 5.90-5.79 (m, 1H), 4.57-4.50 (m, 2H), 4.46-4.24 (m, 2H), 4.16-3.94 (m, 4H), 3.86-3.78 (m, 1H), 3.69 (t, J = 4.4 Hz, 2H), 3.31 (s, 3H), 1.28-1.03 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.87, 4.75; HRMS calcd for $C_{25}H_{35}FN_6O_9P$ $(M+H)^+$ 613.2182, found 613.2182.



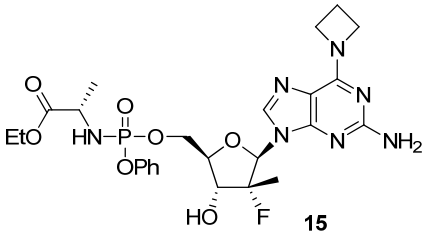
13

Prodrug 13. This compound was prepared from **6f** and **7a** following a similar procedure as the preparation of **9** in 59% yield (white solid). Purity (LCMS): 95.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.00 (s, 0.5H), 7.98 (s, 0.5H), 7.39-7.32 (m, 2H), 7.23-7.12 (m, 3H), 6.66 (bs, 2H), 6.14-6.00 (m, 2H), 5.90-5.79 (m, 1H), 4.85 (t, J = 4.0 Hz, 1H), 4.73 (t, J = 4.0 Hz, 1H), 4.69-4.67 (m, 1H), 4.63-4.59 (m, 1H), 4.46-4.26 (m, 3H), 4.17-3.94 (m, 3H), 3.86-3.76 (m, 1H), 1.28-1.03 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.87, 4.75; HRMS calcd for $C_{24}H_{32}F_2N_6O_8P$ $(M+H)^+$ 601.1982, found 601.1983.



14

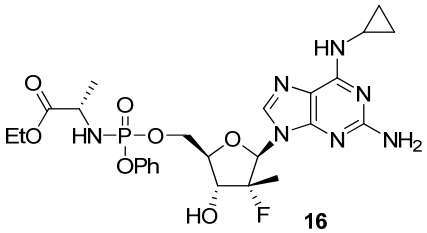
Prodrug 14. This compound was prepared from **6g** and **7a** following a similar procedure as the preparation of **9** in 95% yield (white solid). Purity (LCMS): 98.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, J = 4.0 Hz, 0.5H), 8.01 (d, J = 4.0 Hz, 0.5H), 7.39-7.26 (m, 2H), 7.25-7.10 (m, 3H), 6.69 (bs, 2H), 6.13-6.03 (m, 2H), 5.87-5.79 (m, 1H), 4.45-4.22 (m, 2H), 4.18-3.90 (m, 4H), 3.86-3.72 (m, 1H), 3.28-3.24 (m, 2H), 1.39-1.02 (m, 12H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.85, 4.73; HRMS calcd for $C_{24}H_{33}FN_6O_7PS$ $(M+H)^+$ 599.1848, found 599.1847.



15

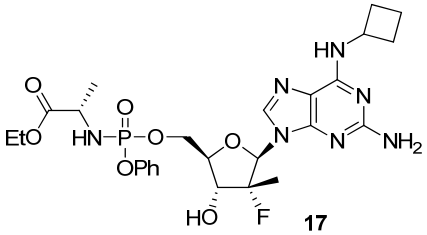
Prodrug 15. This compound was prepared from **6h** and **7a** following a similar procedure as the preparation of **9** in 52% yield (white solid). Purity (LCMS): 99.0%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 0.5H), 7.48 (s, 0.5H), 7.32-7.26 (m, 2H), 7.23-7.11 (m, 3H), 5.98 (dd, J = 5.6, 19.2 Hz, 1H), 4.98 (s, 2H), 4.74-4.62 (m, 2H), 4.45-4.34 (m, 4H), 4.22-3.90 (m, 6H), 2.45 (quintet, J = 6.8 Hz, 2H), 2.34 (bs,

1H), 1.33-1.14 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.93, 4.34; HRMS calcd for $C_{25}H_{34}FN_7O_7P$ $(M+H)^+$ 594.2236, found 594.2238.



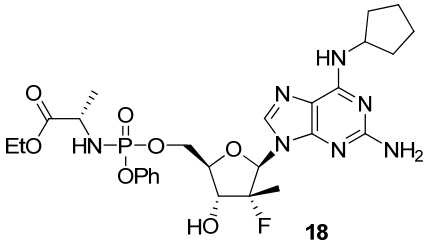
16

Prodrug 16. This compound was prepared from **6i** and **7a** following a similar procedure as the preparation of **9** in 62% yield (white solid). Purity (LCMS): 98.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 0.5H), 7.80 (s, 0.5H), 7.46 (bs, 1H), 7.39-7.32 (m, 2H), 7.25-7.13 (m, 3H), 6.14-6.00 (m, 3H), 5.86-5.74 (m, 1H), 4.48-4.24 (m, 3H), 4.14-3.94 (m, 4H), 3.86-3.75 (m, 1H), 1.28-1.12 (m, 10H), 0.68-0.56 (m, 4H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.85, 4.80; HRMS calcd for $C_{25}H_{34}FN_7O_7P$ $(M+H)^+$ 594.2236, found 594.2235.



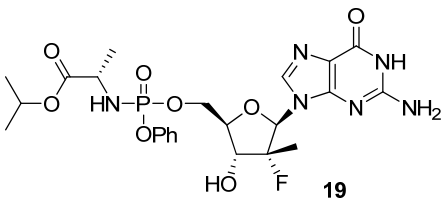
17

Prodrug 17. This compound was prepared from **6j** and **7a** following a similar procedure as the preparation of **9** in 67% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82 (s, 0.5H), 7.81 (s, 0.5H), 7.64 (bs, 1H), 7.39-7.26 (m, 2H), 7.26-7.10 (m, 3H), 6.10-5.96 (m, 3H), 5.86-5.74 (m, 1H), 4.67 (bs, 1H), 4.46-4.16 (m, 3H), 4.14-3.94 (m, 4H), 3.86-3.74 (m, 1H), 2.23-2.02 (m, 4H), 1.64-1.54 (m, 2H), 1.26-1.03 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.86, 4.80; HRMS calcd for $C_{26}H_{36}FN_7O_7P$ $(M+H)^+$ 608.2392, found 608.2394.



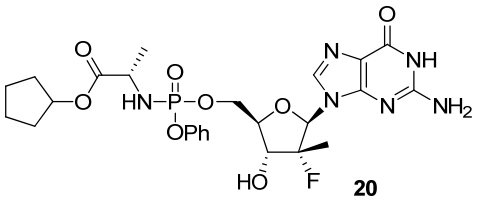
18

Prodrug 18. This compound was prepared from **6k** and **7a** following a similar procedure as the preparation of **9** in 57% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 0.5H), 7.80 (s, 0.5H), 7.39-7.30 (m, 2H), 7.26-7.13 (m, 3H), 6.10-5.92 (m, 3H), 5.86-5.72 (m, 1H), 4.52-4.24 (m, 4H), 4.14-3.92 (m, 3H), 3.86-3.74 (m, 1H), 1.92-1.84 (m, 2H), 1.74-1.62 (m, 2H), 1.58-1.44 (m, 4H), 1.24-1.04 (m, 9H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.85, 4.80; HRMS calcd for $C_{27}H_{38}FN_7O_7P$ $(M+H)^+$ 622.2549, found 622.2547.



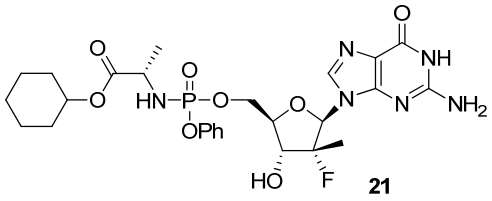
19

Prodrug 19. This compound was prepared from **6a** and **7c** following a similar procedure as the preparation of **8** in 19% yield (white solid). Purity (LCMS): 99.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H), 7.79 (d, J = 7.6 Hz, 1H), 7.32-7.37 (m, 2H), 7.13-7.22 (m, 3H), 6.59 (s, 2H), 5.97-6.03 (m, 2H), 5.80 (d, J = 6.4 Hz, 1H), 4.78-4.84 (m, 1H), 4.24-4.40 (m, 3H), 4.06-4.12 (m, 1H), 3.74-3.79 (m, 1H), 1.05-1.21 (m, 12H); HRMS calcd for $C_{23}H_{31}FN_6O_8P$ $(M+H)^+$ 569.1920, found 569.1918.



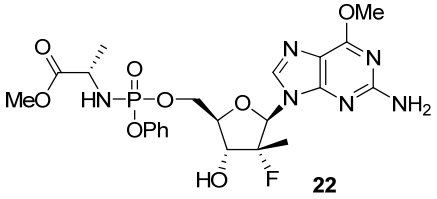
20

Prodrug 20. This compound was prepared from **6a** and **7d** following a similar procedure as the preparation of **8** in 10% yield (white solid). Purity (LCMS): 97.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 1H), 7.79 (d, J = 8.4Hz, 1H), 7.32-7.38 (m, 2H), 7.15-7.22 (m, 3H), 6.60 (s, 2H), 5.97-6.03 (m, 2H), 5.80-5.95 (m, 1H), 4.95-4.96 (m, 1H), 4.25-4.39 (m, 2H), 4.05-4.07 (m, 1H), 3.77-3.79 (m, 1H), 1.71-1.76 (m, 2H), 1.48-1.56 (m, 7H), 1.19 (d, J = 7.2Hz, 3H), 1.08 (dd, J = 4.8, 22.8Hz, 3H); HRMS calcd for $C_{25}H_{33}FN_6O_8P$ $(M+H)^+$ 595.2076, found 595.2077.



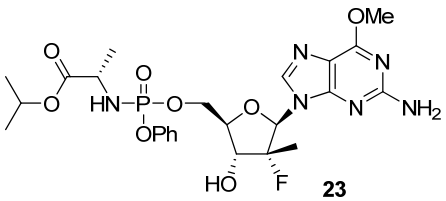
21

Prodrug 21. This compound was prepared from **6a** and **7e** following a similar procedure as the preparation of **8** in 16% yield (white solid). Purity (LCMS): 95.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.79 (d, J = 6.0 Hz, 1H), 7.32-7.38 (m, 2H), 7.15-7.22 (m, 3H), 6.62 (s, 2H), 6.01-6.04 (m, 2H), 5.97 (d, J = 8.4 Hz, 1H), 4.57-4.60 (m, 1H), 4.26-4.41 (m, 3H), 4.00-4.12 (m, 1H), 3.76-3.81 (m, 1H), 1.61-1.67 (m, 4H), 1.20-1.44 (m, 9H), 1.13 (d, J = 12.8 Hz, 3H); HRMS calcd for $C_{26}H_{35}FN_6O_8P$ $(M+H)^+$ 609.2233, found 609.2230.



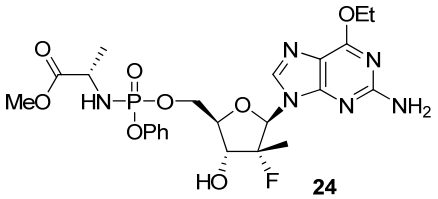
22

Prodrug 22. This compound was prepared from **6b** and **7b** following a similar procedure as the preparation of **9** in 45% yield (white solid). Purity (LCMS): 99.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.66H), 7.95 (s, 0.34H), 7.40-7.32 (m, 2H), 7.24-7.14 (m, 3H), 6.64 (bs, 2H), 6.11 (d, J = 19.2 Hz, 0.34H), 6.10 (d, J = 19.2 Hz, 0.66H), 5.87 (d, J = 6.8 Hz, 0.34H), 5.80 (d, J = 6.8 Hz, 0.66H), 4.48-4.24 (m, 3H), 4.16-4.05 (m, 2H), 3.961 (s, 1H), 3.958 (s, 2H), 3.90-3.78 (m, 1H), 3.56 (s, 1H), 3.53 (s, 2H), 1.21 (t, J = 6.8 Hz, 3H), 1.08 (dd, J = 2.8, 22.4 Hz, 3H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.86, 4.75; HRMS calcd for $C_{22}H_{29}FN_6O_8P$ $(M+H)^+$ 555.1763, found 555.1758.



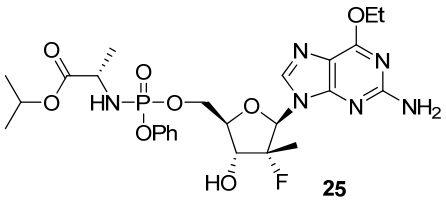
23

Prodrug 23. This compound was prepared from **6b** and **7c** following a similar procedure as the preparation of **9** in 39% yield (white solid). Purity (LCMS): 97.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.66H), 7.95 (s, 0.34H), 7.39-7.30 (m, 2H), 7.25-7.13 (m, 3H), 6.63 (bs, 2H), 6.11 (d, J = 18.8 Hz, 0.34H), 6.09 (d, J = 19.2 Hz, 0.66H), 6.06-5.88 (m, 1H), 5.90-5.78 (m, 1H), 4.80 (septet, J = 6.8 Hz, 1H), 4.45-4.24 (m, 3H), 4.16-4.05 (m, 1H), 3.96 (s, 3H), 3.84-3.70 (m, 1H), 1.28-1.03 (m including d at 1.20 with J = 7.2 Hz, 12H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.91, 4.72; HRMS calcd for $C_{24}H_{33}FN_6O_8P$ $(M+H)^+$ 583.2076, found 583.2081.



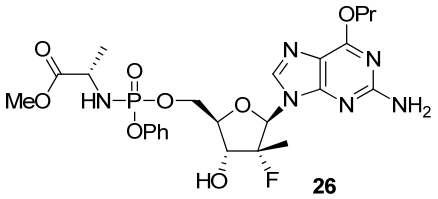
24

Prodrug 24. This compound was prepared from **6c** and **7b** following a similar procedure as the preparation of **9** in 22% yield (white solid). Purity (LCMS): 99.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, J = 8 Hz, 1H), 7.29-7.32 (m, 2H), 7.12-7.18 (m, 3H), 6.53 (s, 2H), 6.03-6.09 (m, 1H), 5.30 (dd, J = 5.2, 20.4 Hz, 1H), 4.41-4.45 (m, 2H), 4.28-4.39 (m, 2H), 4.05-4.11 (m, 1H), 3.77-3.82 (m, 1H), 3.51(d, J = 10.8 Hz, 1H), 3.51(d, J = 11.6 Hz 3H), 1.33 (t, J =7.2 Hz, 3H), 1.16-1.22(m, 3H), 1.05 (dd, J = 3.2, 22.4 Hz, 3H); HRMS calcd for $C_{23}H_{31}FN_6O_8P$ $(M+H)^+$ 569.1920, found 569.1916.



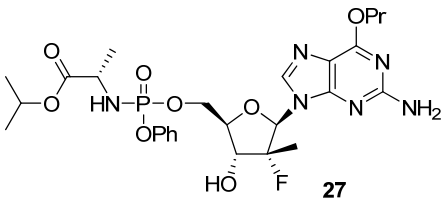
25

Prodrug 25. This compound was prepared from **6c** and **7c** following a similar procedure as the preparation of **9** in 68% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.66H), 7.94 (s, 0.34H), 7.39-7.30 (m, 2H), 7.25-7.10 (m, 3H), 6.59 (bs, 2H), 6.14-5.97 (m, 2H), 5.88-5.76 (m, 1H), 4.80 (septet, J = 6.4 Hz, 1H), 4.45 (q, J = 7.2 Hz, 2H), 4.41-4.20 (m, 3H), 4.18-4.04 (m, 1H), 3.82-3.70 (m, 1H), 1.36 (t, J = 6.8 Hz, 3H), 1.28-1.02 (m, 12H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.90, 4.73; HRMS calcd for $C_{25}H_{35}FN_6O_8P$ $(M+H)^+$ 597.2233, found 597.2231.



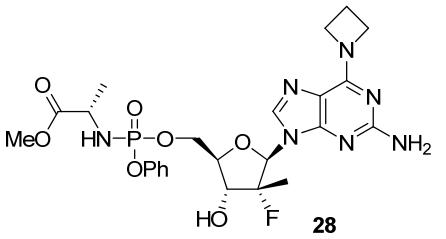
26

Prodrug 26. This compound was prepared from **6d** and **7b** following a similar procedure as the preparation of **9** in 19% yield (white solid). Purity (LCMS): 95.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (d, J = 7.6 Hz, 1H), 7.33-7.38 (m, 2H), 7.12-7.22 (m, 3H), 6.58 (s, 2H), 6.03-6.13 (m, 2H), 5.85 (dd, J = 7.2, 18.6 Hz, 1H), 4.27-4.48 (m, 5H), 4.07-4.15 (m, 1H), 3.79-3.84 (m, 1H), 3.56 (s, 3H), 1.72-1.81 (m, 2H), 1.19-1.21 (m, 3H), 1.08 (d, J = 22.8 Hz, 3H), 0.97 (t, J = 7.2 Hz, 3H); HRMS calcd for $C_{24}H_{33}FN_6O_8P$ $(M+H)^+$ 583.2076, found 583.2085.



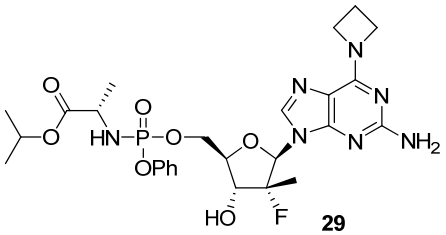
27

Prodrug 27. This compound was prepared from **6d** and **7c** following a similar procedure as the preparation of **9** in 56% yield (white solid). Purity (LCMS): 99.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.97 (s, 0.5H), 7.94 (s, 0.5H), 7.39-7.28 (m, 2H), 7.25-7.12 (m, 3H), 6.58 (bs, 2H), 6.12 (d, J = 8.0 Hz, 0.5H), 6.07 (d, J = 8.0 Hz, 0.5H), 6.05-5.97 (m, 1H), 5.87 (dm, J = 6.0 Hz, 0.5H), 5.80 (dm, J = 7.6 Hz, 0.5H), 4.86-4.76 (m, 1H), 4.46-4.24 (m including t at 4.35 with J = 7.6 Hz, 5H), 4.16-4.06 (m, 1H), 3.80-3.72 (m, 1H), 1.77 (sextet, J = 7.2 Hz, 2H), 1.23-1.02 (m, 12H), 0.97 (t, J = 7.6 Hz, 3H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.90, 4.74; HRMS calcd for $C_{26}H_{37}FN_6O_8P$ $(M+H)^+$ 611.2389, found 611.2390.



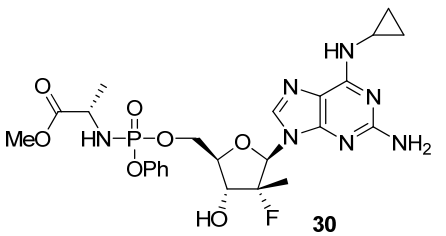
28

Prodrug 28. This compound was prepared from **6h** and **7b** following a similar procedure as the preparation of **9** in 30% yield (white solid). Purity (LCMS): 96.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 4.0 Hz, 1H), 7.32-7.35 (m, 2H), 7.15-7.21 (m, 3H), 6.00-6.07 (m, 4H), 5.75-5.85 (m, 1H), 4.22-4.40 (m, 6H), 4.01-4.13 (m, 2H), 3.75-3.82 (m, 1H), 3.65 (s, 1H), 3.59-3.62 (m, 2H), 3.54 (d, J = 9.2 Hz, 3H), 2.35-2.38 (m, 2H), 1.07 (d, J = 22.4 Hz, 3H); HRMS calcd for $C_{24}H_{32}FN_7O_7P$ $(M+H)^+$ 580.2079, found 580.2075.



29

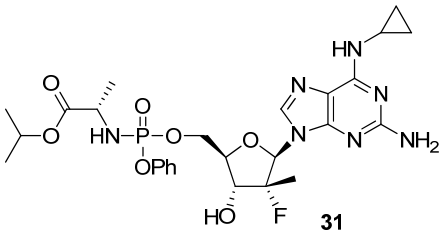
Prodrug 29. This compound was prepared from **6h** and **7c** following a similar procedure as the preparation of **9** in 52% yield (white solid). Purity (LCMS): 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 0.5H), 7.47 (s, 0.5H), 7.32-7.21 (m, 5H), 7.16-7.11 (m, 1H), 5.98 (d, J = 19.2 Hz, 0.5H), 5.96 (d, J = 18.8 Hz, 0.5H), 5.02-4.92 (m including bs at 4.99, 3H), 4.75-4.63 (m, 2H), 4.45-4.36 (m, 4H), 4.20 (sextet, J = 4.0 Hz, 1H), 4.01-3.86 (m, 3H), 2.49-2.41 (m, 2H), 1.32-1.14 (m, 12 H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 5.02, 4.44; HRMS calcd for $C_{26}H_{36}FN_7O_7P$ $(M+H)^+$ 608.2392, found 608.2391.



30

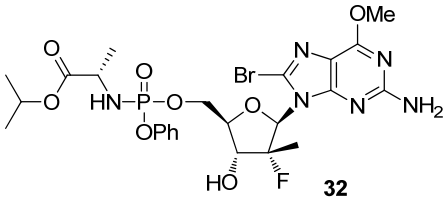
Prodrug 30. This compound was prepared from **6i** and **7b** following a similar procedure as the preparation of **9** in 42% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ

7.79 (s, 0.5H), 7.80 (s, 0.5H), 7.44 (bs, 1H), 7.38-7.31 (m, 2H), 7.22-7.13 (m, 3H), 6.10-5.98 (m, 3H), 5.86-5.70 (m, 1H), 4.46-4.22 (m, 3H), 4.12-4.00 (m, 2H), 3.86-3.75 (m, 1H), 3.54 (s, 1.5H), 3.51 (s, 1.5H), 1.22 (m, 1H), 1.19 (d, J = 4.4 Hz, 1.5H), 1.17 (d, J = 4.4 Hz, 1.5H), 1.07 (dd, J = 4.0, 22.4 Hz, 3H), 0.63 (q, J = 7.2 Hz, 2H), 0.60-0.54 (m, 2H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.84, 4.81; HRMS calcd for $C_{24}H_{32}FN_7O_7P$ $(M+H)^+$ 580.2079, found 580.2070.



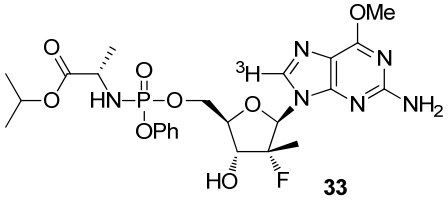
31

Prodrug 31. This compound was prepared from **6i** and **7c** following a similar procedure as the preparation of **9** in 78% yield (white solid). Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 0.66H), 7.80 (s, 0.34H), 7.45 (bs, 1H), 7.38-7.31 (m, 2H), 7.24-7.13 (m, 3H), 6.09-5.96 (m, 3H), 5.86-5.74 (m, 1H), 4.81 (septet, J = 6.8 Hz, 1H), 4.46-4.26 (m, 3H), 4.14-4.03 (m, 1H), 3.82-3.72 (m, 1H), 3.02 (bs, 1H), 1.28-1.05 (m including d at 1.19 with J = 6.8 Hz, 13H), 0.70-0.56 (m, 4H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.89, 4.80; HRMS calcd for $C_{26}H_{36}FN_7O_7P$ $(M+H)^+$ 608.2392, found 608.2397.



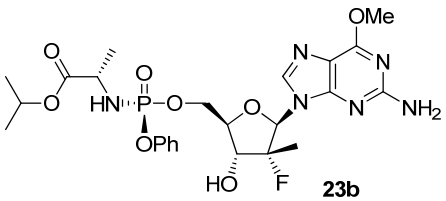
32

Bromide 32. To a solution of **23** (100 mg, 0.172 mmol) in dioxane (1 ml) was added *N*-bromosuccinimide (61 mg, 0.343 mmol) and it was stirred at rt for 5 h. Then, the solution was concentrated and purified by column chromatography eluted with 0 to 5% iPrOH in CH_2Cl_2 to give the bromide **32** (83 mg, 73%) as a white solid. Purity (LCMS): 98.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.34-7.27 (m, 2H), 7.18-7.09 (m, 3H), 6.78 (bs, 2H), 6.02-5.84 (m, 2H), 5.73-5.62 (m, 1H), 4.83-4.69 (m, 2H), 4.46-4.30 (m, 2H), 4.17-4.05 (m, 1H), 3.93 (s, 3H), 3.77-3.65 (m, 1H), 1.28-1.03 (m, 12H); ^{31}P NMR (162 MHz, DMSO-d_6) δ 4.67, 4.48; LRMS calcd for $C_{24}H_{32}BrFN_6O_8P$ $(M+H)^+$ 661.1, 663.1, found 661.2, 663.2.



33

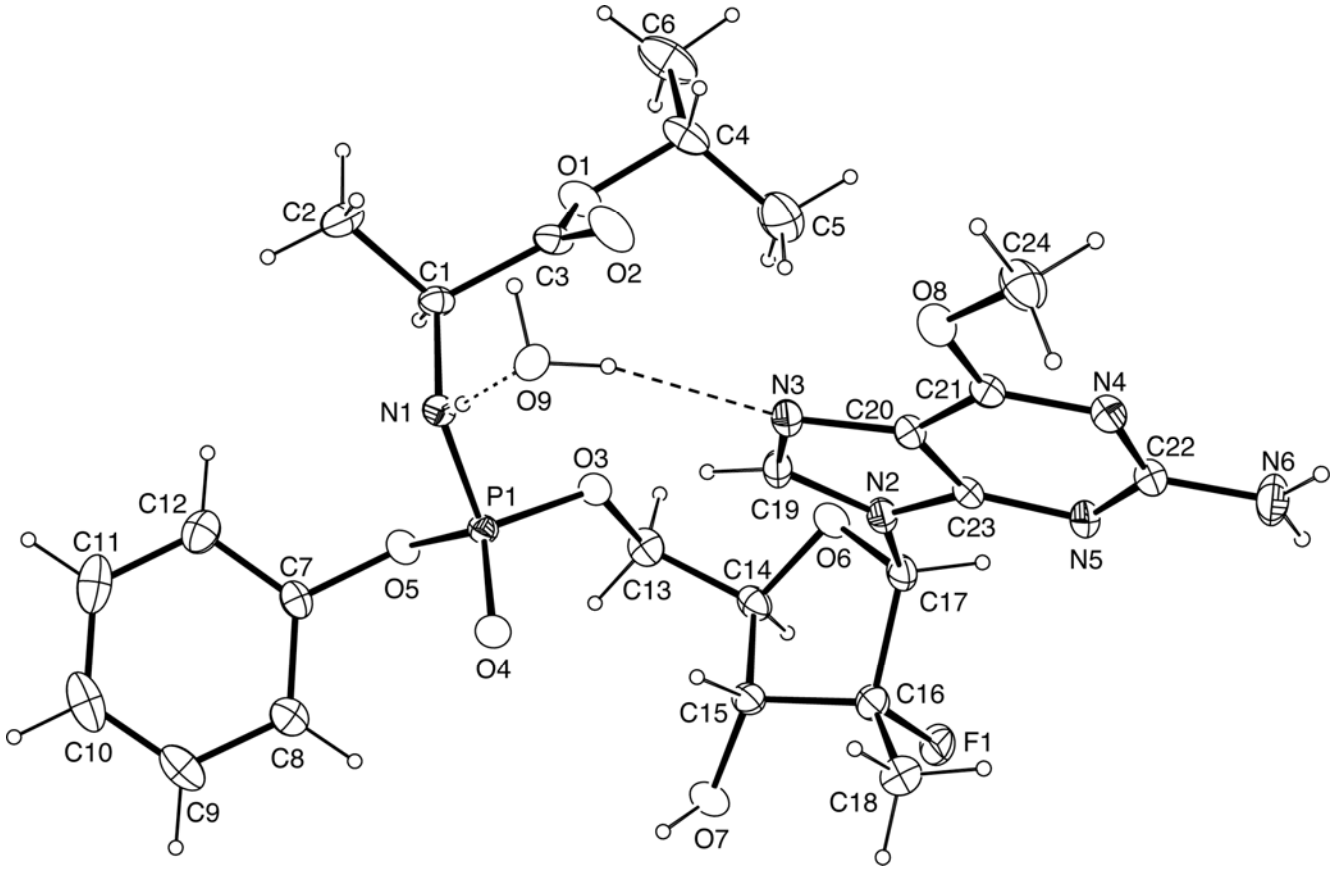
Tritide 33. An 1 ml rbf was charged with bromide **32** (1.5 mg, 0.0023 mmol), MeOH (0.15 ml), Et_3N (0.01 ml), and Pd on C (10%, 1 mg). The reaction flask was attached to Tri-Sober®, degassed with He, and evacuated with rapid agitation. The mixture was treated with 10 Ci tritium gas. After 1 h, the tritium uptake was completed and residual tritium gas was absorbed in the secondary uranium bed. The residue was filtered and the filtrate was concentrated. The total activity was measured at 80 mCi. It was purified by HPLC to give the 8-tritide **33** in an EtOH/water (1/1) solution. Concentration: 1 mCi/ml; Radiochemical purity: 99.7%; Specific activity: 21.1 Ci/mmol; ^{3}H NMR (300 MHz, MeOD) δ 8.00 (s, 0.5H), 7.97 (s, 0.5H); LRMS calcd for $C_{24}H_{32}TBrFN_6O_8P$ $(M+H)^+$ 585.2, found 585.17.



23b

Prodrug 23b. Compound **23** was purified by simulated moving bed chromatography (Chiral Technologies, Inc.) to give **23b** as a white solid. Purity (LCMS): 98.9%; $[\alpha]_D^{20}$ = +22.8 (*c* 1.27, MeOH); IR (thin flim) 3504, 3386, 1732, 1584, 1476, 1390, 1327, 1255, 1203, 1136, 1093, 1071, 1043, 1120, 971, 936, 901, 855, 827, 773, 691 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.33-7.28 (m, 2H), 7.25-7.22 (m, 2H), 7.17-7.13 (m, 1H), 6.05 (d, *J* = 18.8 Hz, 1H), 5.24 (bs, 2H), 4.95 (septet, *J* = 6.0 Hz, 1H), 4.70-4.64 (m, 2H), 4.44 (m, 1H), 4.26-4.22 (m, 1H), 4.19-4.11 (m, 1H), 4.05 (s, 3H), 3.95 (doublet-sextet, *J* = 2.0, 9.2 Hz, 1H), 2.42 (bs, 1H), 1.33 (d, *J* = 6.8 Hz, 1H), 1.20 (d, *J* = 22.6 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (*J* = 6.9 Hz), 161.7, 159.6, 152.9, 150.5 (*J* = 6.1 Hz), 137.6, 129.8, 125.1, 120.2, 120.1, 116.0, 101.3 (*J* = 179.7 Hz), 89.6 (*J* = 38.7 Hz), 80.2 (*J* = 6.8 Hz), 73.2 (*J* = 17.4 Hz), 69.4, 65.5 (*J* = 3.8 Hz), 53.9 50.3, 21.62, 21.57, 20.8 (*J* = 5.3 Hz), 16.5 (*J* = 25.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.42; ^{19}F NMR (376 MHz, $CDCl_3$) δ -161.7; HRMS calcd for $C_{24}H_{33}FN_6O_8P$ $(M+H)^+$ 583.2076, found 583.2072.

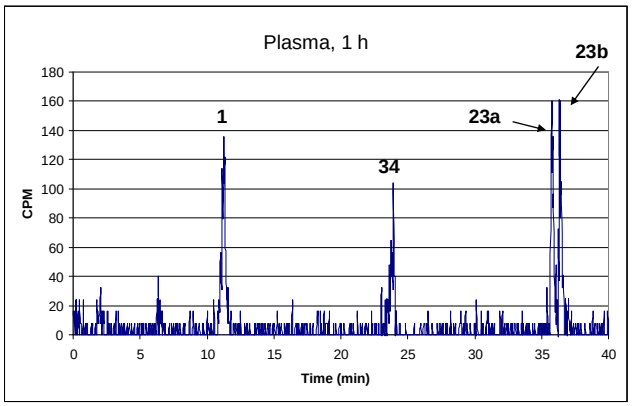
3. ORTEP drawing of 23b (PSI-353661)



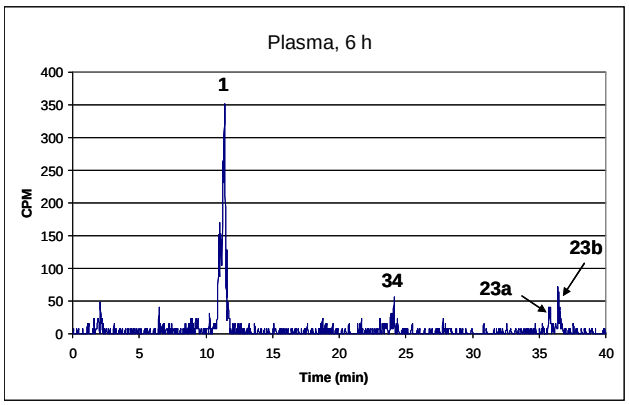
4. *In vivo* plasma and liver metabolism profile

Rats dosed orally with 60 µCi **33** in 2.9 ml of peanut oil. HPLC radioactive traces: rat plasma samples (a) at 1 h, and (b) at 6 h; rat liver samples (c) at 1 h, and (d) at 6 h. Peak designations refer to non-tritiated reference standards.

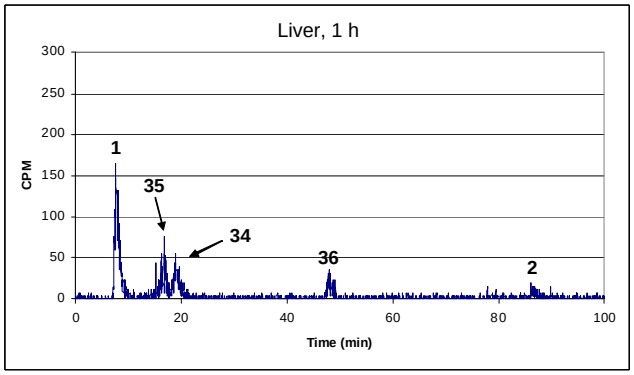
(a)



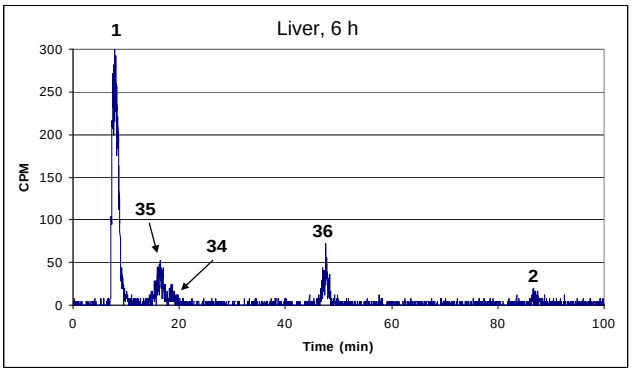
(b)



(c)



(d)



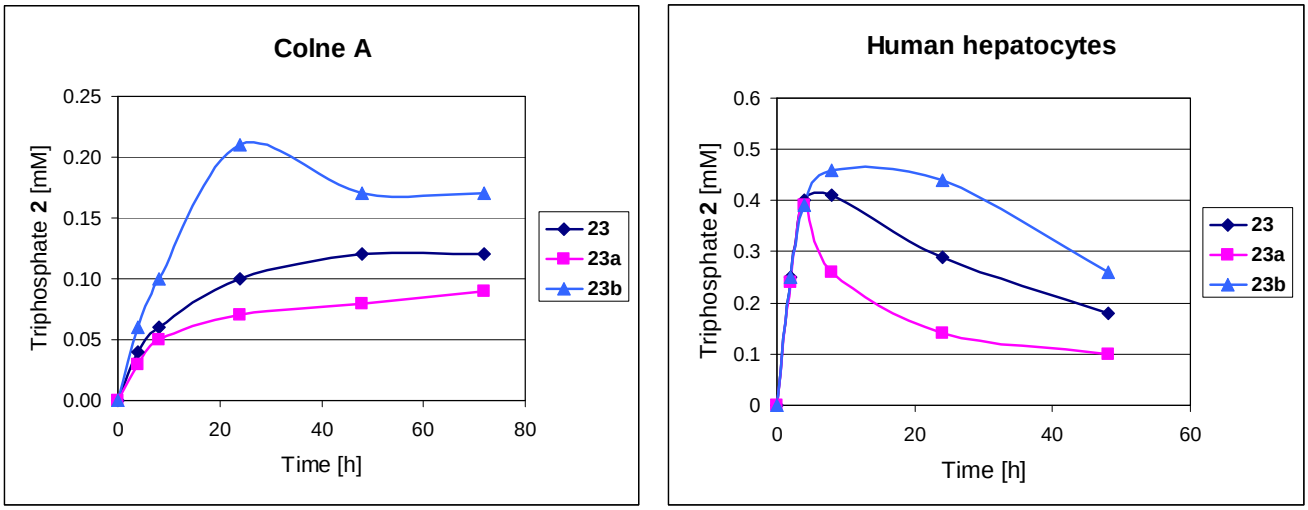
5. Measurement of cytostatic effect

Each compound (10, 50, and 100 µM) was added to Clone A cells (2 x 10^3 cells/well) and allowed to incubate for 5 days at 37°C. A medium only control was used to determine the minimum absorbance value and an untreated cell control was included. At the time of 0, 24, 48, 72 and 96 hours, MTS dye from the CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit (Promega) was added to each well and the plate was incubated for an additional 2 hours. The absorbance at 490 nm was read with a Victor3 plate reader (Perkin Elmer) using the medium only control wells as blanks. The cell growth curves were generated and the cell doubling times were calculated for treated and untreated cells. The cytostatic effect was determined by comparing the doubling time of treated cell with that of untreated one.

6. Measurement of triphosphate concentration

Clone A cells and primary hepatocytes were seeded (500,000 and 1,000,000 cells) into 6-well plates in DMEM containing 10% FBS and Primary Cell Plating Medium (CellzDirect, Inc), respectively. After overnight incubation to allow the cells to attach, Clone A cells and primary hepatocytes were incubated for up to 72 and 48 hours at 37°C in a 5% CO_2 atmosphere in the fresh medium containing up to 100 µM of test compound Clone respectively. At selected times, extracellular medium was removed and the cell

layer was washed with cold PBS. After trypsinization, cells were counted and centrifuged at 1,200 rpm for 5 min. The cell pellets were resuspended in 1mL of cold 60% methanol and incubated overnight at -20°C. The samples were centrifuged at 14,000 rpm for 5 min and the supernatants were collected and dried using a SpeedVac Concentrator, then stored at -20°C until they were to be analyzed by HPLC. Residues were suspended in 100 µL of water, and 50 µL aliquots were injected into HPLC. The test compound and metabolites were separated by ion exchange HPLC with a Whatman 10 µm SAX column (Whatman, Maidstone, England) using a Series 200 HPLC system (PerkinElmer, MA). The mobile phase consisted of buffer A (0.02 M KH_2PO_4, pH 3.5) and buffer B (1 M KH_2PO_4, pH 3.5). Elution was performed using a linear gradient of buffer B from 0 to 100% for 100 min. The test compound and its respective metabolites were identified based on the retention time of synthesized standards. The intracellular concentration (pmol/10^6 cells) of the metabolites was converted to µM based on a 3 µL cell volume for normal human liver parenchymal cells.[2]



[1] McGuigan, C.; Hassan-Abdallah, A.; Srinivasan, S.; Wang, Y. K.; Siddiqui, A.; Daluge, S. M.; Gudmundsson, K. S.; Zhou, H. Q.; McLean, E. W.; Peckham, J. P.; Burnette, T. C.; Marr, H.; Hazen, R.; Condreay, L. D.; Johnson, L.; Balzarini, J. *J. Med. Chem.* **2006**, *49*, 7215-7226.

[2] Duarte, M. I.; Andrade, H. F. Jr.; Mariano, O. N.; Corbett, C.E.; Sesso, A. Baseline volume data of human liver parenchymal cell. *J. Submicrosc. Cytol. Pathol.* **1989**, *21*, 275-279.