Supporting Information

Discovery and synthesis of C-nucleosides as potential new anti-HCV agents

Alistair G. Draffan,[*1] Barbara Frey,[1] Brett Pool,[1] Carlie Gannon,[1] Edward Tyndall,[1] Michael Lilly,[1] Paula Francom,[1] Richard Hufton,[1] Rosliana Halim,[1] Saba Jahangiri,[1] Silas Bond,[1] Van T. T. Nguyen,[1] Tyrone P. Jeynes,[1] Veronika Wirth,[1] Angela Luttick,[1] Danielle Tilmanis,[1] Jesse D. Thomas,[1] Melinda Pryor,[1] Kate Porter,[1] Craig J. Morton,[1] Bo Lin,[1] Jianmin Duan,[2] George Kukolj,[2] Bruno Simoneau,[2] Ginette McKercher,[2] Lisette Lagacée,[2] Ma'an Amad[2], Richard C. Bethell,[2] Simon P. Tucker.[1]

[1]Biota Scientific Management Pty. Ltd., 10/585 Blackburn Road, Notting Hill, Victoria 3168, Australia

[2]Boehringer Ingelheim (Canada) Ltd. Research and Development, 2100 rue Cunard, Laval (Québec) Canada, H7S 2G5

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1. Chemistry general methods

All solvents and reagents were purchased from Sigma-Aldrich or Acros organics used as received or dried further using a Glass Contour Solvent Purification System (Pure Process Technology). Reactions were monitored by thin layer chromatography with TLC Silicagel 60 F_{254} (Merck) and visualized by UV light. NMR spectra were recorded with a Bruker Ultrashield Plus 400 MHz spectrometer. Flash chromatography was performed using Grace Reveleris Silica or Grace Reveleris C18 RP pre-packed columns using mobile phases specified. Low resolution mass spectra were recorded using a Thermo Finnigan Surveyor HPLC system coupled with a Thermo Finnigan LCQ Advantage ion trap system, a Thermo Finnigan Surveyor HPLC system coupled with a Thermo Finnigan LCQ Deca XP Plus ion trap mass spectrometer, or a Thermo Fisher Scientific Accela High Speed LC system coupled with LCQ Fleet ion trap mass spectrometer (all using ESI mode). High resolution mass spectra were performed at Monash University, Melbourne using ESI method. HPLC traces were obtained using an Agilent 1200 series HPLC system with a Varian Pursuit XRs C18 column and a gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Preparatory HPLC was performed using an Agilent 1200 series Preparative HPLC system using a Varian Pursuit XRs C18 column (21.2x100 mm id 5µm) coupled with an Agilent 6120 Single Quadrupole mass spectrometer (ESI mode) controlled by Chemstation software. Nucleoside triphosphates were synthesized at Trilink Biotechnologies, Inc. using proprietary methods.

2. Synthesis of new compounds

Scheme 1, Preparation of 4-amino-7-(2-C-methyl-β-D-ribofuranosyl)-pyrrolo[2,1-f][1,2,4]triazine (Compound **1**).

Step a: A solution of **7**,[1] 3,5-bis-O-(2,4-dichlorophenylmethyl)-1-O-methyl-2-C-methyl-D-ribofuranose (19.2 g), in anhydrous THF (200 mL) was chilled to 0˚C under Ar in an ice:water bath then treated with NaH (60% dispersion in oil, 2.6 g) in 4 batches at 10 min intervals. The suspension was warmed to RT then treated dropwise with 2,4-dichlorobenzyl chloride (11.2 mL). The reaction was then heated to 70˚C under Ar for 16-24 h. The cooled reaction mixture was filtered, and the filtrate was eluted on silica with 10 – 30% EtOAc:hexane to form Compound **8** as a mixture of α and β anomers (16.68 g): 1H NMR ($CDCl_3$) major anomer: δ 1.42 (s, 3H); 3.46 (s, 3H); 3.64 (d, 1H, J = 4.8 Hz); 3.73 (dd, 1H, J = 3.9, 10.7 Hz); 3.67 (dd, 1H, J = 4.4, 10.7 Hz); 4.29 (q, 1H, J = 4.3 Hz); 4.56 – 4.85 (m, 7H); 7.11 – 7.25 (m, 3H, J = 2.0, 8.4 Hz); 7.31 – 7.41 (m, 5H); 7.58 (d, 1H, J = 8.4 Hz).

Step b: Compound **8** (6.91 g) in TFA (70 mL) was chilled to 0˚C then treated dropwise with water (7 mL). The mixture was warmed to RT then stirred for 4 h. The resulting solution was concentrated to 10 mL and added slowly to a 50:50 mixture of Et_2O:$NaHCO_3$ (sat.) (80 mL total). The mixture was neutralized with $NaHCO_3$ (aq), and the aqueous layer was extracted with ether (2 x 50 mL), and then the combined organic extracts were washed with $NaHCO_3$ (aq). The organic extract was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude material was eluted on a silica column with 5 – 40% EtOAc:hexane to afford Compound **9** (5.2 g) as a mixture of α and β anomers: ^{1}H NMR (d_6-acetone) both anomers: δ 1.52 (s, 6H); 3.79 (t, 4H, J = 4.8 Hz); 3.99 (d, 1H, J = 5.6 Hz); 4.10 (d, 1H, J = 7.3 Hz); 4.21 – 4.27 (m, 1H); 4.37 (dd, 1H, J = 4.5, 10.0 Hz); 4.62 – 4.95 (m, 12H); 5.10 (d, 1H, J = 9.1 Hz); 5.20 (d, 1H, J = 4.6 Hz); 5.57 (d, 1H, J = 4.8 Hz); 5.62 (s, 1H); 7.25 – 7.70 (m, 18H).

Step c: Compound **9** (5.52 g) in anhydrous DCM (40 mL) under Ar was treated with trichloroacetonitrile (1.95 mL) and Cs_2CO_3 (254 mg) and stirred at RT for 3 h. The solution was diluted with DCM (50 mL), washed with water (100 mL), and the aqueous layer extracted with DCM (2 x 50 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), filtered, and concentrated. The resulting residue was eluted through a silica column with 15% EtOAc:hexane to give the target compound **10** (5.58 g) as a mixture of α and β anomer: ^{1}H NMR ($CDCl_3$) major anomers: δ 1.56 (s, 3H); 3.71 (dd, 1H, J = 4.8, 10.8 Hz); 3.80 (dd, 1H, J = 4.0, 10.8 Hz); 4.15 (d, 1H, J = 8.1 Hz); 4.42 – 4.91 (m, 7H); 6.25 (s, 1H); 7.15 – 7.53 (m, 9H); 8.55 (s, 1H).

Step d: Compound **10** (5.0 g) in anhydrous DCM (200 mL) was treated with powdered 4 Å molecular sieves under Ar for 2 h. The solution was then chilled to -70˚C (internal temperature), treated with freshly distilled pyrrole (1.46 mL) then dropwise with $BF_3.OEt_2$ (2.76 mL). The mixture was stirred for 40 min, while maintaining the internal temperature below -55ºC. The mixture was then cooled to -70ºC and treated with 7 M NH_3 in methanol (20 mL) before being warmed to RT. The mixture was diluted with DCM and washed with water. The organic extract was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a grey gum. The crude material was applied to a silica flash column and eluted with 15% EtOAc:hexane to afford the pyrrole nucleoside **11** (2.72 g) as a mixture of α:β anomers in a ratio of about 2:3: ^{1}H NMR ($CDCl_3$) β-anomer only: δ 9.53 (br s, 1H), 7.60-7.14 (m, 9H), 6.36-6.35 (m, 1H), 6.11-6.10 (m, 1H), 6.04 (br s, 1H), 5.13 (s, 1H), 4.89-4.61 (m, 6H), 4.37-4.36 (m, 1H), 4.21 (d, J = 8.4 Hz, 1H), 4.10 (dd, J = 2.6 Hz, 10.5 Hz, 1H), 3.85 (dd, J = 1.9 Hz, 10.5 Hz, 1H), 1.16 (s, 3H).

Step e: Compound **11** (1.37 g, mixture of anomers) was suspended in anhydrous acetonitrile (6 mL). Anhydrous DMF was added until a homogeneous solution was observed (~1.0 mL), and the solution was chilled under Ar in an ice:acetone bath to -12ºC. After 10 min, the solution was treated dropwise with chlorosulphonyl isocyanate (0.17 mL), and the resulting mixture was stirred below 0ºC for 45 min, during which time a dark red colour was observed. The mixture was poured onto ice (100 g), diluted with EtOAc (100 mL), and stirred until the ice melted. The organic extract was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a dark pink oil. The crude material was suspended in 20 mL 50:50 DCM:hexane, applied to a silica flash column, and eluted with 15-50% EtOAc:hexane to render compound **12** as the β-anomer (**12b**, 0.69 g) with the corresponding α-anomer (**12a**, 0.28 g) collected separately: ^{1}H NMR ($CDCl_3$) β-anomer: δ 10.44 (s, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.47 (d, J = 2.0 Hz, 1H), 7.42-7.37 (m, 3H), 7.32-7.21 (m, 4H), 6.79 (dd, J = 2.6, 3.8 Hz, 1H), 6.10 (dd, J = 2.6 Hz, 3.5 Hz, 1H), 5.11 (s, 1H), 4.90 (d, J = 12.5 Hz, 1H), 4.85 (d, J = 13.1 Hz, 1H), 4.74 (d, J = 13.1 Hz, 1H), 4.73 (d, J = 12.5 Hz, 1H), 4.68 (d, J = 12.4 Hz, 1H), 4.59 (d, J = 12.4 Hz, 1H), 4.35 (ddd, J = 1.9 Hz, 2.4 Hz, 8.4 Hz, 1H), 4.13 (d, J = 8.4 Hz, 1H), 4.05 (dd, J = 2.8 Hz, 10.6 Hz, 1H), 3.76 (dd, J = 1.7 Hz, 10.6 Hz, 1H), 1.20 (3H, s). NOE observed between 1'-H (5.11 ppm) and 4'-H (4.35 ppm), consistent with the structure of the target compound as a single ß-anomer. **12a**: ^{1}H NMR ($CDCl_3$) α-anomer: δ 9.75 (s, 1H), 7.42 – 7.12 (m, 8H), 6.90 – 6.85 (m, 1H), 6.77 (dd, J = 2.6, 3.8 Hz, 1H), 6.19 (dd, J = 2.4, 3.8 Hz, 1H), 4.85 (s, 1H), 4.81 (d, J = 12.2 Hz, 1H), 4.75 (s, 2H), 4.62 (q, J = 12.8 Hz, 2H), 4.42 (d, J = 12.2 Hz, 1H), 4.29 (dt, J = 3.6, 6.5 Hz, 1H), 4.11 (d, J = 6.6 Hz, 1H), 3.81 – 3.66 (m, 2H), 1.50 (s, 3H).

Step f: Isomerisation procedure. To a solution of **12a** (4.5 g, 6.3 mmol) in dichloromethane (40 mL) was added $BF_3.OEt_2$ (10 mL, 86 mmol) and the solution stirred at reflux for 3 h. Further $BF_3.OEt_2$ (10 mL), 86 mmol) was added and the mixture stirred at reflux for another 3h. The mixture was poured onto wa-

ter and extracted with DCM. The organic extract was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude material was purified by flash chromatography to afford the β-anomer, **12b** (1.6 g, 36%) and the recovered α-anomer, **12a** (1.2 g, 26%).

Step g: NaH (72.5 mg, 60% dispersion in mineral oil) in dry THF (13 mL) was cooled to -5ºC for 10 min. Compound **12b** (0.6 g, β-anomer) in THF (2 mL) was added dropwise followed by portionwise addition of $Ph_2P(O)ONH_2$ (0.39 g) before stirring at 0ºC for 30 min. The mixture was partitioned between toluene and water, and the organic extracts were collected, dried ($MgSO_4$), filtered, and concentrated in vacuo to afford Compound **13**. The product was stored in the freezer and used in subsequent reactions without further purification: ^{1}H NMR (d_6-DMSO): δ 7.63 (br d, J = 1.9 Hz, 1H), 7.58-7.54 (m, 4H), 7.45-7.33 (m, 4H), 6.76 (d, J = 4.4 Hz, 1H), 6.14 (d, J = 4.4 Hz, 1H), 6.13 (br s, 2H), 5.36 (s, 1H), 4.76-4.62 (m, 6H), 4.22-4.19 (m, 1H), 4.0 (d, J = 7.0 Hz, 1H), 3.83 (dd, J = 3.5 Hz, 10.9 Hz, 1H), 3.76 (dd, J = 4.4 Hz, 10.9 Hz, 1H), 1.15 (s, 3H).

Step h: To a solution of Compound **13** (60 mg) in anhydrous DMA (2 mL) was added formamidine acetate (700 mg), and the suspension heated under Ar at 140°C for 1.5 h. The mixture was cooled to RT and more formamidine acetate (700 mg) was added, and the mixture was heated for another 1.5 h at 140°C. The mixture was cooled to RT overnight, whereupon precipitation occurred. The supernatant was removed, and the precipitate washed with DCM. The supernatant and combined washings were concentrated in vacuo. Residual DMA was distilled off (Kugelrohr), and the residue taken up in DCM (5 mL), washed with water (1 mL), dried with $MgSO_4$, filtered, and concentrated. Residual DMA was removed by distillation, and the residue was purified on flash silica gel (50% EtOAc in hexane) to afford Compound **14** (32 mg) as a colourless syrup: ^{1}H NMR ($CDCl_3$): δ 7.89 (s, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.38-7.19 (m, 6H), 7.15 (dd, J = 2.1 Hz, 8.3 Hz, 1H), 6.82 (d, J = 4.5 Hz, 1H), 6.56 (d, J = 4.5 Hz, 1H), 5.88 (s, 1H), 5.76 (br s, 2H), 4.86 (s, 2H), 4.72 (s, 2H), 4.73-4.62 (m, 2H), 4.40-4.35 (m, 1H), 4.09 (d, J = 8.3 Hz, 1H), 3.97 (dd, J = 2.7 Hz, 10.9 Hz, 1H), 3.81 (dd, J = 3.7 Hz, 10.8 Hz, 1H), 1.14 (s, 3H); ^{13}C NMR ($CDCl_3$): δ 155.47, 147.18, 136.17, 134.65, 134.60, 134.25, 134.10, 133.79, 133.54, 133.30, 132.66, 130.42, 130.24, 130.17, 129.72, 129.39, 129.24, 128.84, 127.29, 127.26, 127.20, 114.43, 110.86, 100.48, 85.06, 83.76, 79.43, 78.67, 70.22, 70.19, 70.10, 62.88, 18.00.

Step i: to a solution of Compound **14** (240 mg) in dry methanol (30 mL) was added, with stirring, anhydrous sodium acetate (192 mg) and glacial acetic acid (670 μL). The mixture was degassed, purged with Ar, and 10% Palladium on charcoal was added (150 mg). The mixture was stirred at 45°C under H_2 for 66 h. The reaction mixture was degassed, purged with Ar, filtered through a pad of celite with MeOH, and concentrated to obtain the crude product. Column chromatography on flash silica gel (17% MeOH in EtOAc) rendered Compound **1** (70.7 mg). ESI-MS m/z 281 ($[M+H]^+$); ^{1}H NMR (d_6-DMSO) δ 7.81 (s, 1H), 7.61 (br s, 2H), 6.84 (d, J = 4.4 Hz, 1H), 6.70 (d, J = 4.4 Hz, 1H), 5.39 (s, 1H), 4.90 (br s, 1H), 4.75 (br t, J = 5.4 Hz, 1H), 4.66 (s, 1H), 3.78-3.55 (m, 3H), 3.62-3.55 (m, 1H), 0.78 (s, 3H). HRMS calcd for $C_{12}H_{17}N_4O_4$ $(M+H)^+$ 281.1244, found 281.1267

Scheme 2, Synthesis of 4-amino-7-(2-C-methyl-β-D-ribofuranosyl)-imidazo[2,1-f][1,2,4]triazine (Compound **2**)

Step a: To a solution of 6-methylthio nucleobase **15**[2] (526 mg) in anhydrous DMF (20 mL) under argon was added NBS (4000 mg) and the mixture was heated at 86°C for 1 h. The reaction mixture was distilled to dryness (Kugelrohr, 50°C) and the residue was taken up in ethyl acetate (150 mL), washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The residue (2.27 g) was purified on silica (Biotage, Graceresolv, 80 g) using ethyl acetate in hexane (10 to 50%). The desired 9-bromo product **16** (295 mg, 38%) was isolated as a slightly orange solid. ESI-MS m/z 245/247 ($[M+H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H), 7.74 (s, 1H), 2.72 (s, 3H).

Preparation of lactone **17**: To a solution of riboside **9** (5.3 g, 8.3 mmol) in dry dichloromethane (100 mL) was added pyridinium chlorochromate (PCC, 3.5 g, 16 mmol). The mixture was stirred at RT for 2d. The solvent was removed and the residue applied to a plug of silica in a solution of diethyl ether/DCM (1:1, 100 mL), and eluted with diethyl ether (300 mL). The filtrate was evaporated to dryness and the residue purified by flash chromatography (ethyl acetate in hexane) to afford target compound **17** as a colourless oil (3.9 g, 74%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.56 (ddt, *J* = 18.5, 8.3, 0.8 Hz, 3H), 7.52 – 7.42 (m, 3H), 7.43 – 7.28 (m, 3H), 4.93 (dd, *J* = 12.5, 0.7 Hz, 1H), 4.86 – 4.77 (m, 1H), 4.77 – 4.74 (m, 2H), 4.73 – 4.65 (m, 3H), 4.34 (d, *J* = 7.3 Hz, 1H), 4.01 (dd, *J* = 11.5, 2.6 Hz, 1H), 3.88 (dd, *J* = 11.5, 4.3 Hz, 1H), 1.70 (s, 3H).

Step b: A solution of bromonucleobase **16** (100 mg) in anhydrous tetrahydrofuran (6 mL) was cooled to -78 °C under nitrogen. *n*-BuLi (500 uL, 1.6 M in *n*-hexane) was added dropwise over 1 min. (The colour of the reaction mixture changed to orange-brown and the solution cleared.) The reaction mixture was stirred at -78 °C. After 30min at -78°C a solution of lactone **17** (500 mg) in anhydrous tetrahydrofuran (2 mL) was added dropwise over a period of 5min. The mixture was stirred for 30min at -78°C then warmed to 10°C within 2.5 h. The reaction was worked up at ~10°C as follows. Glacial acetic acid (50 µL) and methanol (20 mL) were added (to pH 3). The mixture was evaporated to dryness and the residue purified on silica (Biotage, Graceresolv, 40g) using ethyl acetate in hexane (20 to 100%). The target compound **18** (2 anomers 70:30) was isolated as an oil (155 mg, 47%). ESI-MS m/z 805 ($[M+H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 0.7H), 8.43 (s, 0.3H), 7.96 (s, 0.3H), 7.89 (s, 0.7H), 7.50-7.09 (m, 9H), 5.49 (s, 0.3H), 5.34 (s, 0.7H), 5.00-4.43 (m, 7.7H), 4.17 (d, J = 6.3, 0.3H), 3.95-3.85 (m, 1H), 3.76 (dd, J = 3.9Hz, 10.5 Hz, 1H), 2.700, 2.699 (2 s, 3H), 1.68 (s, 2.1H), 1.25 (s, 0.9H).

Step c: To compound **18** (150 mg) was added ammonia in methanol (10 mL, 7 N) and the reaction was stirred at room temperature overnight. The reaction mixture was heated at reflux for 2 h to reach completion. The solvents were evaporated and the residue was purified on silica (Biotage, Graceresolv, 12 g) using ethyl acetate in hexane (50 to 100%) to afford the target compound **19** (2 anomers 70:30) as an oil (91 mg, 63%). ESI-MS m/z 774 ($[M+H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 0.7H), 8.08 (s, 0.3H), 7.81 (s, 0.3H), 7.75 (s, 0.7H), 7.56-7.13 (m, 11H), 6.11 (s, 0.7H), 5.55 (s, 0.3H), 5.04-3.66 (m, 10H), 1.61 (s, 2.1H), 1.25 (s, 0.9H).

Step d: Compound **19** (90 mg) was dissolved in anhydrous dichloromethane (8 mL) under argon and cooled to -78°C. Triethylsilane (90 µL) was added, followed by $BF_3.OEt_2$ (74 µL) and the reaction mixture was warmed to 0°C. The reaction mixture was cooled to -78°C, more triethylsilane (180 µL) and $BF_3.OEt_2$ (155 µL) were added and the reaction mixture was warmed to room temperature for 3 h. Ammonia in methanol (20 mL, 1 N) was added to the reaction mixture and it was evaporated to dryness. The residue was suspended in dichloromethane and filtered. The filtrate was evaporated to afford crude product (60 mg, 68%). The crude material was purified by flash chromatography (ethyl acetate in hexane, 50% to 80%) to afford target compound **20** as a wax (41 mg, 47%) after co-evaporation methanol. ESI-MS m/z 758 ($[M+H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.71 (s, 1H), 7.61-7.15 (m, 10H), 6.54 (br s, 1H), 5.75 (s, 1H), 4.88-4.61 (m, 6H), 4.41-4.38 (m, 1H), 4.13 (d, J = 8.0Hz, 1H), 3.98

(dd, J = 2.8 Hz, 10.8 Hz, 1H), 3.81 (dd, J = 3.6 Hz, 10.8 Hz, 1H), 1.22 (s, 3H). NOESY (400 MHz, $CDCl_3$): NOE observed between 1'-H (5.75ppm) and 4'-H (4.41-4.38ppm), consistent with the structure of the target compound as a single ß-anomer.

Step e: A solution of nucleoside **20** (20mg) in dry methanol (2mL) containing anhydrous dichloromethane (0.2mL) was treated with anhydrous sodium acetate (16mg) and glacial acetic acid (120uL), then evacuated and purged with argon. Palladium on carbon (12mg, 10%) was added to the reaction mixture which was then evacuated and purged with hydrogen. The reaction mixture was stirred vigorously under a hydrogen balloon at 45°C for 18 h, then cooled to RT, evacuated and purged with argon 3 times before the vessel was opened. This process was repeated at 60°C overnight. The reaction mixture was filtered through celite with methanol (15 mL) and evaporated. The residue was purified on silica using methanol in ethyl acetate (0 to 20%) to afford the desired product, 4-amino-7-(2-C-methyl-β-D-ribofuranosyl)-imidazo[2,1-f][1,2,4]triazine, compound **2** (0.70 mg) as a colorless solid. ESI-MS m/z 282 ($[M+H]^+$). ^1H NMR (400 MHz, CD_3OD): δ 8.05 (s, 1H), 7.69 (s, 1H), 5.45 (s, 1H), 3.98-3.92 (m, 3H), 3.80 (dd, J = 4.0 Hz, 12.1 Hz, 1H), 1.00 (s, 3H). HRMS calcd for $C_{11}H_{16}N_5O_4$ $(M+H)^+$ 282.1197, found 282.1208.

Scheme 3 – Synthesis of **4** and **5**

Step a: Heterocycle **21** (550 mg, 3.7 mmol) was suspended in acetonitrile (20 mL) and triethylamine (3.0 mL, 22 mmol). DMAP (30 mg, 0.20 mmol) and Boc_2O (4.8 g, 5 mL, 37 mmol) were added and the mixture stirred at RT for 46 h. Additional Boc_2O (3.2 g, 3.4 mL, 15 mmol) was added and the mixture stirred for a further 6 d at RT. The solvent was removed to afford a crude product, which was purified using flash chromatography (EtOAc/Heptane) to afford compound **22** (560 mg, 38%). ESI-MS m/z 835.19 ($[2M+Na]^+$).

Compound **27** (1.7 g, 49%) was prepared similarly from heterocycle **26** (1.3 g, 8.6 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 1.1 Hz, 1H), 7.71 (d, J = 1.1 Hz, 1H), 1.76 (s, 9H), 1.42 (s, 18H).

Step b: To a solution of compound **22** (430 mg, 1.1 mmol) in DCE (10 mL) was added a solution of NBS (200 mg, 1.1 mmol) in DCE (10 mL) at -9°C. The mixture was stirred and warmed to 0°C over 1 h, then $NaHCO_3$ (sat.) added. The organic layer was separated and washed with further $NaHCO_3$ (sat.). The organic layer was separated, the solvent removed and the crude product purified by reverse phase chromatography (Grace Reveleris 40 g C18 column using a 0-100% $MeCN/H_2O$ gradient) to afford compound **23** as a white solid (220 mg, 43%). ESI-MS m/z 992.93 ($[2M+Na]^+$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.81 (d, J = 4.5 Hz, 1H), 6.76 (d, J = 4.5 Hz, 1H), 1.69 (s, 9H), 1.43 (s, 18H).

Compound **28** (1.63 g, 88%) was prepared similarly from compound **27** (1.7 g, 4.2 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 1.74 (s, 9H), 1.43 (s, 18H).

Step c: Freshly titrated *n*BuLi (1.51 M in hexanes, 270 µL, 0.42 mmol) was added to a solution of compound **23** (184 mg, 0.38 mmol) in THF (5 mL) at -100°C. Lactone **17** (242 mg, 0.38 mmol) in THF (3 mL) was added keeping the internal T < -100°C. The mixture was stirred for 15 min then allowed to warm to RT over 30 min before the addition of NH_4Cl (sat.). The mixture was extracted with EtOAc and the combined extracts dried with brine, then $MgSO_4$, and concentrated to dryness. The crude product was purified by flash chromatography (EtOAc/heptane) to afford the desired product **24** as a mixture of anomers (390 mg, 60%). ESI-MS m/z 1026.87 ($[M-H_2O]^+$, oxonium).

Compound **29** (0.44 g, 51%) was prepared similarly from compound **28** (0.40 g, 0.82 mmol) and lactone **17** (1.0 g, 1.6 mmol).

Step d: To a solution of compound **24** (260 mg, 0.25 mmol) in acetonitrile (5 mL) and triethylsilane (0.63 mL, 5.0 mmol) was added $BF_3.OEt_2$ (0.800 mL, 5.0 mmol) at -10°C. The mixture was stirred at this temperature for 2h then warmed to RT and stirred for a further 20 min. The mixture was diluted with chloroform then quenched with $NaHCO_3$(sat.). The mixture was stirred vigorously until the evolution of gas ceased. The mixture was extracted with $CHCl_3$ and the combined extracts dried with brine, then $MgSO_4$, and concentrated to dryness. The crude product was purified by flash chromatography (EtOAc/heptane) to afford compound **25** (140 mg, 74%, β-anomer), and a small amount of the α-anomer (13 mg, 7%). (Note: the use of DCM as a solvent gives a similar yield for the reduced product but with an anomeric ratio of α:β ~ 1:1). Compound **25** (β-anomer). ESI-MS m/z 772.91 ($[M+H]^+$), ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.75 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.56 – 7.47 (m, 3H), 7.45 (dd, J = 2.1, 6.8 Hz 3H), 7.39 (td, J = 2.1, 8.1 Hz, 2H), 7.30 (dd, J = 2.1, 8.3 Hz, 1H), 6.75 (d, J = 4.4 Hz, 1H), 6.50 (d, J = 4.5 Hz, 1H), 5.66 (s, 1H), 5.52 (d, J = 5.5 Hz, 1H), 4.84 (s, 2H), 4.81 (s, 2H), 4.74 (d, J = 4.9 Hz, 2H), 4.30 (dt, J = 3.8, 7.5 Hz, 1H), 4.15 (d, J = 7.3 Hz, 1H), 3.98 (dd, J = 3.5, 10.9 Hz, 1H), 3.91 (dd, J = 4.3, 10.8 Hz, 1H), 1.28 (d, J = 3.6 Hz, 3H). NOESY (400 MHz, $CDCl_3$): NOE observed between 1'-H (5.66 ppm) and 4'-H (4.30 ppm), consistent with the structure of the target compound as a single ß-anomer.

Compound **30** (0.35 g, 50%) was prepared similarly from compound **29** (0.95 g, 0.92 mmol). ESI-MS m/z 774.1 ($[M+H]^+$)

Step e: To a solution of compound **25** (55 mg, 0.07 mmol) in MeOH (13 mL) and EtOAc (2 mL) was added NH_4OAc (0.28 g, 3.6 mmol) and 10% Pd/C (55 mg). The mixture placed under an atmosphere of H_2 (60 psi) and stirred at RT for 24 h. The mixture was filtered and the filtrate evaporated to dryness. The crude product was purified by reverse phase chromatography (MPLC, MeCN/H_2O) to afford compound **4**. (13 mg, 62%). ^{1}H NMR (d_6-DMSO) δ: 10.55 (s, 1H), 6.67 (d, J = 4.4 Hz, 1H), 6.41 (d, J = 4.5 Hz, 1H), 5.87 (s, 2H), 5.23 (s, 1H), 4.94 (d, J = 6.4 Hz, 1H), 4.75 (t, J = 5.4 Hz, 1H), 4.50 (s, 1H), 3.77 – 3.68 (m, 2H), 3.68 – 3.62 (m, 1H), 3.60 – 3.51 (m, 1H), 0.84 (s, 3H); ESI-MS m/z 297.0 $(M+H)^+$; HRMS calcd for $C_{12}H_{17}N_4O_5$ $(M+H)^+$ 297.1193, found 297.1197.

Step f: To a solution of **30** (11 mg, 0.014 mmol) in DCM/MeCN (1:1, 2 mL) was added 1,1-dimethoxy-N,N-dimethyl-methanamine (0.14 mL, 1.05 mmol) and the solution stirred at RT overnight then at 60°C for 3 h. The solvent was removed and the crude product purified by flash chromatography to afford **31** as the main product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 8.19 (s, 1H), 7.61 – 7.54 (m, 2H), 7.47 – 7.14 (m, 7H), 5.59 (d, J = 0.6 Hz, 1H), 4.80 – 4.62 (m, 6H), 4.33 (dt, J = 3.4, 7.2 Hz, 1H), 4.06 (d, J = 7.7 Hz, 1H), 3.92 (dd, J = 3.0, 10.9 Hz, 1H), 3.80 (dd, J = 3.8, 10.8 Hz, 1H), 3.07 (d, J = 0.6 Hz, 3H), 3.05 (d, J = 0.5 Hz, 3H), 1.26 (s, 3H). NOE observed between 1'-H (5.59 ppm) and 4'-H (4.33 ppm), consistent with the structure of the target compound as a single ß-anomer.

Step e: to a solution of **30** (0.20 g, 0.26 mmol) in dichloromethane (25 mL) was added boron tribromide (3.0 mL, 2.6 mmol; 1 M solution in DCM) at -78°C. The mixture was allowed to warm to -30°C over 10min then quenched with a solution of methanol in DCM (1:1) then brought to RT and neutralized with ammonia (28%). The mixture was extracted with DCM and the aqueous layer freeze dried. The crude product was purified by HPLC to afford **5** as a white solid. (23 mg, 40%). HRMS calcd for $C_{11}H_{16}N_5O_5$ $(M+H)^+$ 298.1146, found 298.1213.^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 7.37 (s, 1H), 6.20 (s, 2H), 5.11 (s, 1H), 5.01 (d, *J* = 5.7 Hz, 1H), 4.78 (t, *J* = 5.4 Hz, 1H), 4.61 (s, 1H), 3.79 – 3.65 (m, 3H), 3.61 – 3.52 (m, 1H), 0.90 (s, 3H).

Scheme 4 Improved synthesis of **4**

Step a: To a solution of benzyl alcohol (2.6 mL, 26 mmol) in THF (20 mL) was added *n*BuLi (12 mL, 1.95M, 24 mmol) dropwise at -10°C. The mixture was warmed to RT then cooled -10°C, whereupon 2,4-dichloropyrrolo[2,1-f][1,2,4]triazine,[3] **32** (3.0 g, 16 mmol), was added portionwise. The mixture was stirred at RT for 3h before the addition of NH_4Cl (sat.) then extracted with EtOAc. The combined extracts were washed with brine, then dried ($MgSO_4$) and concentrated to dryness. The crude product was purified by flash chromatography (MeOH/DCM) then recrystallization from heptane to afford **33** (3.0 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, J = 2.6, 1.5 Hz, 1H), 7.53 – 7.49 (m, 2H), 7.45 – 7.37 (m, 3H), 6.87 (dd, J = 4.5, 2.1 Hz, 1H), 6.72 (dd, J = 4.5, 2.5 Hz, 1H), 5.6 (s, 2H).

Step b: To a stirred solution of **33** (1.0 g, 3.9 mmol) in DCM (8 mL) was added NBS (0.69 g, 3.9 mmol) at -10 °C. The resulting suspension was stirred at this temperature for 10 min then at RT overnight before DMF (0.25 mL) was added to form a solution. The solution was stirred at RT for 10 min then washed three times with $NaHCO_3$ (dil.). The organic layer was evaporated to dryness to afford **35** along with C7 Br isomer and SM in ratio of 6.7:1:1 (1.1 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.47 (m, 2H), 7.45 – 7.35 (m, 3H), 6.92 (d, J = 4.8 Hz, 1H), 6.77 (d, J = 4.6 Hz, 1H), 5.62 (s, 2H); (C7-Br non-overlapping peaks) δ 7.57 (d, J = 2.8 Hz, 1H), 6.76 (d, J = 2.9 Hz, 1H), 5.64 (s, 1H).

Step c: *n*-Butyllithium (2.5 M,3.6 mmol) was added dropwise to a solution of **35** (0.62 g, 1.8 mmol) in 2-methyltetrahydrofuran (20 mL) at -100°C, ensuring T < -95°C throughout the addition. The mixture was stirred at -100°C before a solution of **34**[4] (0.79 g, 1.8 mmol) in 2-methyltetrahydrofuran (2 mL) was added quickly, ensuring T < -90°C throughout the addition. The mixture was stirred for 35min, then NH_4Cl(sat.) added and the mixture allowed to warm to RT. After the addition of ethyl acetate, the organic layer was separated, dried ($MgSO_4$) and the solvent removed. The crude product was purified by flash chromatography (EtOAc/heptane) to afford the desired product **36** as a mixture of anomers (0.57 g, 45%). ESI-MS m/z 674.02 ($[M\text{-}H_2O]^+$, oxonium).

Step d: $BF_3.OEt_2$ (0.75 mL, 5.8 mmol) was added to a solution of **36** (0.58 g, 0.84 mmol) and triethylsilane (2.5 mL, 16 mmol) in DCM (5mL) at -78°C. The mixture was stirred for 15 min at -78°C then $NaHCO_3$ (aq) added and the mixture warmed to RT. After the evolution of gas ceased, the mixture was extracted with DCM and the combined organic layers combined and dried with brine, then $MgSO_4$, and concentrated to dryness to afford **37** (0.56g, 99%). ESI-MS m/z 675.81 ($[M+H]^+$).

Step e: Under an atmosphere of Ar, a solution of **37** (0.39 g, 0.57 mmol) in 1,4-dioxane (3 mL) was added to a mixture of, Cs_2CO_3 (0.37 g, 1.14 mmol), acetamide (0.068 g, 1.14 mmol), tris(dibenzylideneacetone)dipalladium (0) (0.013 g, 0.014 mmol) and 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene ("Xantphos", 0.020 g, 0.030 mmol). The mixture was stirred at RT for 1h then heated at 130°C for 30 min in a microwave reactor. The cooled mixture was diluted with water and extracted with EtOAc. The combined extracts were washed with $NaHCO_3$(sat.), dried ($MgSO_4$) and the solvent removed. The crude product was purified by flash chromatography (EtOAc/heptane) to afford a mixture of **38** and **39** (127 mg). ESI-MS m/z 698.96 ($[M+H]^+$); m/z 608.89 ($[M+H]^+$)

Step f: To a suspension of **38** and **39** (117 mg) in methanol (5 mL) was added 10% Pd/C (0.035g) under Ar. The mixture was placed under hydrogen (1 atm) and stirred at RT for 4 h, 50°C for 6 h then at RT for a further 70 h. The catalyst was removed by filtration and the filtrate evaporated to dryness. The crude product was purified by reverse phase chromatography ($MeCN/H_2O$) to afford **40** (39 mg, 20% from **37**), ESI-MS m/z = 338.95.

Step g: A solution of **40** (39 mg) in sodium methoxide (2 mL, 1M) was stirred at RT for 30 min, then at 50 °C for a further 10 min, then at 80 °C for a further 1 h. The mixture was cooled to RT and glacial acetic acid (0.2 mL) added. The solvent was removed and the crude product was purified using revers phase chromatography (MeCN/H_2O) to afford **4** (24 mg, 71%). Data agree with initial preparation of this compound.

Scheme 5 – Synthesis of pyrazolo-nucleoside **3**

Step a: To a suspension of NaH (1.0 g, 25 mmol) in dry DME (80 mL) under Ar was added diethoxyphosphorylacetonitrile, **41**, (5.5 mL, 34 mmol) at 0°C over a period of 20 min. To this mixture was added a solution of 2,3,5-tri-O-benzyl-2-C-methyl-D-ribofuranose (**9**) (10.9 g, 17 mmol) in DME (80 mL) at 0°C over a period of 30 min. The mixture was allowed to warm to RT and stirred for a further 1.5 h. Water was added and the resulting mixture extracted with EtOAc. The organic layers were combined and dried with brine, then $MgSO_4$, and concentrated to dryness. The crude product was purified by flash chromatography (EtOAc/heptane) to afford the desired product **42** as a mixture of anomers (3.7 g, 72 %), α:β ~ 3:2 by integration of 2'CMe signal in ^{1}H NMR (400 MHz, d_6-acetone): 1.61 (2'CMe, α-anomer), 1.48 (2'CMe, β-anomer). ESI-MS m/z 663.9 ($[M+H]^+$).

Step b: To a solution of **42** (11 g, 17 mmol) in DMF (70 mL) was added *tert*-butoxy bis(dimethylamino)methane (35 mL, 170 mmol) at 60 °C. The mixture was stirred at 60 °C for 20 h then cooled to RT. The solution was diluted with EtOAc (300 mL) and the resulting mixture washed with water (2 x 100 mL). The organic layer was dried with brine then $MgSO_4$ then concentrated to dryness to afford **43** as a mixture of anomers, (13 g, crude), α:β ~ 1:1 by integration of 2'CMe signal in ^{1}H NMR (400 MHz, d_6-acetone): 1.52 (2'CMe, α-anomer), 1.50 (2'CMe, β-anomer). ESI-MS m/z 718.9 ($[M+H]^+$). The crude product was used in the next step without further purification.

Step c: To a solution of **43** (crude from above) in EtOH (100 mL) and water (20 mL) was added hydrazine.HCl (8.9 g, 130 mmol). The mixture was stirred at 105 ºC for 2 h then cooled to RT and water added (5 mL). The pH was adjusted to basic using Na_2CO_3 (sat.) then extracted with EtOAc. The organic extracts were combined, dried with brine and $MgSO_4$, and concentrated to dryness to afford **44** as a crude oil. ESI-MS m/z 706.18 ($[M+H]^+$). The crude product was used in the next step without further purification.

Step d: A solution of **44** in toluene (20 mL) was deoxygenated by bubbling Ar through it for 10 min. To this solution was added ethyl N-cyanoformimidate (14 mL, 150 mmol) at 85 ºC and the mixture stirred at this temperature for 45 min, cooled to RT and concentrated to dryness. The crude product was purified using flash chromatography (ethyl acetate/heptane) to afford the desired β-nucleoside **45** (2.4 g, β anomer, 19% from **42**). ESI-MS m/z 758.0 ($[M+H]^+$). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.16 (s, 1H), 8.10 (s, 1H), 7.77 – 7.22 (m, 9H), 5.45 (s, 1H), 4.85 – 4.68 (m, 6H), 4.31 (dt, J = 6.3, 4.3 Hz, 1H), 4.21 (d, J = 6.1 Hz, 1H), 3.94 (dd, J = 4.3, 2.7 Hz, 2H), 1.33 (s, 3H). NOE observed between 1'-H (5.45 ppm) and 4'-H (4.31 ppm), consistent with the structure of the target compound as a single ß-anomer.

Step e: To a solution of **45** (2.3 g, 3.0 mmol) in MeOH (100 mL) and sodium acetate (1.2 g, 15 mmol) was added 10% Pd on charcoal added (500 mg) under Ar. The mixture was purged with H_2 and stirred at 45 ºC under H_2 for 3.5 h. The mixture was again degassed, purged with Ar and filtered through a celite pad, washing with further MeOH. The filtrate was concentrated to dryness and the crude product purified by reverse phase chromatography (C18, MeCN/water) to afford the desired compound **3** (0.66 g, 78 %). ESI-MS m/z 282.05 ($[M+H]^+$); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.66 (br s, 1H), 8.34 (br s,

1H), 8.16 (s, 1H), 8.03 (s, 1H), 4.99 (s, 1H), 4.94 (d, J = 6.2 Hz, 1H), 4.86 (dd, J = 6.1, 4.9 Hz, 1H), 4.57 (s, 1H), 3.80 – 3.69 (m, 3H), 3.64 – 3.53 (m, 1H), 0.87 (s, 3H). HRMS calcd for $C_{11}H_{16}N_5O_4$ $(M+H)^+$ 282.1197, found 282.1202.

3. Biological assays

Cell-Based HCV RNA Replication Luciferase Reporter Assay

Cell-based assays were performed as described previously[5]. Briefly, Huh-7 cells harboring stable sub-genomic HCV replicons[6] were incubated at 37ºC and 5% CO_2 with serial dilutions of test compounds in DMEM supplemented with 10% FBS and a final DMSO concentration of 0.5%. After 72 h the medium was removed and cells were lysed, incubated with luciferase substrate and the luminescence measured. A non-linear curve fit of the luminescence data to the Hill model was used to determine the 50% effective concentration (EC_{50}) for inhibition of HCV replication.

HCVcc JFH1 viral replication assay

Inhibition of HCV replication was carried out as described previously[7]. Briefly, *in vitro*-transcribed RNAs was transfected into human hepatoma replicon-cured Huh-7.5 cells to produce infectious virus[8]. Huh-7.5 cells were seeded in a 96-well plate in DMEM and incubated for 24 h at 37°C and 5% CO_2. For assay, the medium was removed and 100 µl of virus was added, with or without compounds, in fresh medium with a final DMSO concentration of 0.5%. After 72 h the medium was removed, total RNA was extracted and quantified by real-time RT-PCR.

Inhibitory potency (EC_{50}) of 1 on HCV RNA replication in replicon assays of various genotypes, and in the HCVcc JFH1 2a whole virus assay; NS5B inhibitory potency (IC_{50}) of 1-NTP on NS5B polymerase enzymes of various genotypes.

Genotype	EC_{50} (µM)	IC_{50} (µM)
1a	0.76	0.57
2a	0.63	0.39
2b	0.41	NT
3a	0.42	0.50
4a	0.88	NT
5a	0.82	0.48
6a	1.0	NT
7a	NT	0.81
HCVcc JFH1 2a	0.58	NT

NT = not tested

HCV Polymerase inhibition assay

The G1b(Con1) NS5b enzyme was cloned into a protein expression plasmid with an additional N-terminal His_6-tag and a C-terminal deletion of either 21 or 55 amino acids. The G1a(H77), G2a(JFH-1), G3a(VRL69), G4a(ED43), G5a(SA13) and G7a(QC69) NS5b enzymes were cloned into protein expression plasmids with an additional C-terminal His_6-tag and a C-terminal deletion of 21 amino acids. The G3a enzyme was provided by P. White (University of New South Wales). All enzymes were purified by a three-step purification method, using affinity (NiNTA), ion exchange and gel filtration chromatography. The kinetic constant, K_m, was determined for the Poly(U)-ddC template and rATP using a non-linear, least squares fit of initial rates as a function of substrate concentration assuming Michaelis-Menten kinetics.

The polymerase inhibition of A-analogues was assessed in standard HCV NS5b Δ55 RNA-dependent RNA polymerase (RdRp) assays or HCV NS5b Δ21 RNA-dependent RNA polymerase (RdRp) assays using a polyU RNA template. The polymerase inhibition of G-analogues was assessed in HCV NS5b Δ55 RNA-dependent RNA polymerase (RdRp) assays which employed a polyC RNA template.

HCV NS5b Δ55 RNA-dependent RNA polymerase (RdRp) assays consisted of 20 µg/mL polyU RNA template (Sigma) and 100 nM NS5b enzyme in a 50 µL reaction mixture containing 20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 mM DTT and 0.4 U/µL RNasin. Elongation reactions were initiated by the addition of rATP at K_m, with a 1:10,000 ratio of [α-^{33}P]ATP and proceeded for 60 min at 25°C. Reactions were quenched by the addition of 50 µL of 0.2 M EDTA, and product formation was collected by filtration through MultiScreen plates (Millipore), followed by washing with 0.5 M sodium phosphate buffer (pH 7.0). Quantification of product formation was read using a TopCount plate reader (Perkin Elmer).

HCV NS5b Δ21 RdRp assays consisted of 50 ug/mL polyU or polyC RNA template (Integrated DNA Technologies) and NS5b enzyme (100 nM or 400nM respectively) in a 50 µL reaction mixture containing 20 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 3 mM DTT, 0.05% BSA and 0.4 U/µl RNasin. Elongation reactions were initiated by the addition of rATP/rGTP at K_m, with a 1:15,000 ratio of [α-^{33}P]ATP/[α-^{33}P]GTP and proceeded for 60 min at 25°C. Reactions were quenched by the addition of 50 µL of 0.2 M EDTA, and product formation was collected by filtration through MultiScreen plates (Millipore), followed by washing with 0.5 M sodium phosphate buffer (pH 7.0). Quantification of product formation was read using a MicroBeta plate reader (Perkin Elmer).

The inhibitor concentration at which the enzyme catalysed rate is reduced by half (IC_{50}) was determined by a non-linear fit to the Hill model.

In vitro anabolism studies

In vitro anabolism of **1** and the intracellular accumulation of its triphosphate (**1**-NTP) were studied in adherent Huh-7 and plated primary human hepatocytes. Huh-7 cells were maintained in DMEM containing 10% FBS supplemented with 0.25 mg/ml G418 for cell lines carrying HCV subgenomic replicons. Primary human hepatocytes (Cellz Direct, NC, USA) were maintained in InVitroGro Medium (Celsis, In Vitro Technologies, MD USA). Both cell lines were incubated at 37ºC in 5% CO_2.

For intracellular anabolism studies, Huh-7 cells were incubated with fresh DMEM + 10% FBS containing 10 µM **1** for a period of 24 h at 37˚C. The cells were then washed three times with drug-free medium to remove extracellular **1** and incubated with fresh DMEM + 10% FBS for a specified time period. At the end of the incubation period cells were washed with cold phosphate buffered saline, pH 7.4 then

harvested and lysed by sonication at 4˚C. The resulting cell lysate was used to identify the metabolites of **1**. Isolation of **1** and the intracellular metabolites of **1** from plated primary human hepatocytes used similar experimental conditions, except that InVitroGro Medium was used as the cell culture medium.

Sample Analysis for intracellular nucleoside metabolites: The cell lysate samples were subjected to two freeze/thaw cycles in a dry ice/acetone bath. Cells extracts were centrifuged at 16000 g for 5 min at 4˚C and 40 µL of supernatant analysed using a API4000 triple quadrupole system. Metabolites were separated using a linear pH gradient (solvent A: 10 mM ammonium acetate in 30:70 MeCN/H_20 containing 0.008% glacial Acetic Acid, solvent B: 1 mM ammonium acetate in 30:70 MeCN/H_20 containing 0.225% ammonia hydroxide; gradient from 10 to 100% buffer B over 2.5 min, 0.5 ml/min) on a Biobasic AX, 3.0 x 50 mm column maintained at 40˚C. The mass spectrometer operated in positive ion electrospray mode utilizing a multiple reaction monitoring scan functionality at m/z m/z 361.1-> 227.1, 441.1-> 227.1 and 521.1-> 227.1 for Compound **1** mono-, di- and triphosphate, respectively. The respective metabolites were identified and quantified by comparing their chromatographic profiles and peak area response with those of authentic standards.

Intracellular concentrations of Compound 1 and its mono-, di- and triphosphate in replicon cells and primary hepatocytes (after 24 hour incubation with compound 1 at an initial concentration of 10 µM)

Species	**Replicon Huh-7 cells**	**Human hepatocytes**	**Rat hepatocytes**	**Dog hepatocytes**
	(pmol/million cells)			
Compound **1**	31.8	46.2	16.9	29.7
1-NMP	3.36	27.6	7.3	7.0
1-NDP	0.53	40.5	9.7	8.3
1-NTP	9.33	487	49.9	51.2

For experiments to investigate the decay half-life of Compound **1** triphosphate, the concentration of the triphosphate after 24-hr pre-incubation with **1** was set as time zero and the first order disappearing rate (k) of the triphosphate concentration in tested cells was used to calculate the decay t1/2 (t1/2=0.693/-k). The half-life data presented below represents the average for two experiments (two donors for human hepatocytes).

Intracellular decay half-lives in replicon cells and human hepatocytes (after 24 hour pre-incubation with compound 1 at an initial concentration of 10 µM)

1-NTP	**Huh-7 cells**	**Human Hepatocytes**
Triphosphate decay half-life, Initial phase (α,h)	0.7	3.3
Triphosphate decay half-life, Elimination phase (β,h)	60	35

4. Pharmacokinetics

The pharmacokinetic properties of **1** were determined in male Sprague-Dawley rats, beagle dogs and cynomolgus monkeys following an oral dose of 5 mg/kg and iv dose of 2 mg/kg. Pharmacokinetic parameters were analyzed using the WinNonlin non-compartmental model and are summarised below.

Pharmacokinetic properties of 1 following a single iv (2 mg/kg) or oral dose (5 mg/kg) in rats, monkeys and dogs (n=3 in each species).

	CL (mL/min/kg)	**V_{ss} (L/kg)**	**$t_{1/2}$ (h)**	**C_{max} (µM)**	**AUC (µM·h)**	**%F**
Rat	18.8	4.19	4.0	2.38	13.0	83
Monkey	20.5	2.43	4.3	0.82	5.0	33
Dog	11.4	5.22	7.6	8.36	24.9	96

Abbreviations: CL: total clearance; V_{ss}: steady state volume distribution; $t_{1/2}$: terminal elimination half-life; C_{max}: maximum concentration observed following the oral dose; AUC: area under the curve of plasma exposure; %F: oral bioavailability.

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