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The Design, Synthesis, and Antiviral Activity of Monofluoro and Difluoro Analogues of 4′-Azidocytidine against Hepatitis C Virus Replication: The Discovery of 4′-Azido-2′-deoxy-2′-fluorocytidine and 4′-Azido-2′-dideoxy-2′,2′-difluorocytidine

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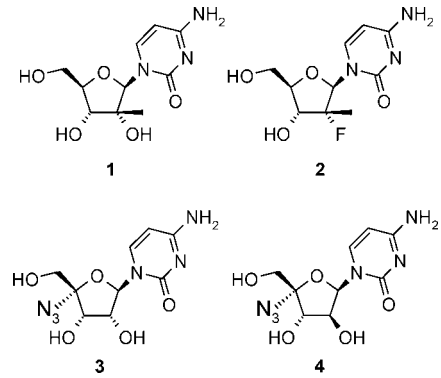
The discovery of 4′-azidocytidine (**3**) (R1479) (*J. Biol. Chem.* 2006, *281*, 3793; *Bioorg. Med. Chem. Lett.* 2007, *17*, 2570) as a potent inhibitor of RNA synthesis by NS5B (EC_{50} = 1.28 μM), the RNA polymerase encoded by hepatitis C virus (HCV), has led to the synthesis and biological evaluation of several monofluoro and difluoro derivatives of 4′-azidocytidine. The most potent compounds in this series were 4′-azido-2′-deoxy-2′,2′-difluorocytidine and 4′-azido-2′-deoxy-2′-fluoroarabinocytidine with antiviral EC_{50} of 66 nM and 24 nM in the HCV replicon system, respectively. The structure−activity relationships within this series were discussed, which led to the discovery of these novel nucleoside analogues with the most potent compound, showing more than a 50-fold increase in antiviral potency as compared to 4′-azidocytidine (**3**).

Introduction

Hepatitis C virus (HCV) is a causative agent of chronic liver disease, estimated to affect over 170 million people worldwide.[3] Although often asymptomatic, HCV infection can progress to fibrosis, reduced liver function, and hepatocellular carcinoma. Currently, the standard treatment for HCV infection involves treatment with pegylated α interferon in combination with the nucleoside analogue ribavirin. This treatment regimen effects a response in approximately 40−60% of the genotype-1 (GT-1) population.[4] Considering the limitations in efficacy and the adverse event profile associated with the current standard of care treatment, there remains a significant unmet clinical need for the development of new antiviral medication, specific for HCV. Recently, a number of nucleoside analogues have been reported to inhibit HCV replication. Among these, 2′-deoxy-2′-*C*-methylcytidine (**1**),[5] 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**2**),[6] and 4′-azido-cytidine (**3**)[1,2] have shown most promise and progressed into clinical development for the treatment of HCV infection. The identification of 4′-substituted ribonucleosides as potential inhibitors of HCV replication culminating in the selection of **3** as a clinical candidate suggested that further exploration of the SAR may allow further optimization of phosphorylation efficiency and antiviral potency.[1,2] Indeed, 4′-azido-arabinocytidine (**4**) (RO-9187) was later identified as an inhibitor of HCV replication with improved potency as compared to **3**, related to improved phosphorylation efficiency (NS5B EC_{50} = 171 nM).[7,8]

Further work focused on the assessment of fluorinated derivatives, on the carbohydrate moiety, of 4′-azidocytidine. Fluoronucleosides have a history of being well phosphorylated by cellular kinases and can be good substrates for RNA and DNA polymerases.[6,9−11] Here we discuss the synthesis and anti-



HCV activity of monofluoro and difluoro analogues of 4′-azidocytidine.

Chemistry

The synthesis of 4′-azido-2′-deoxy-2′-fluorocytidine (**10**) is outlined in Scheme 1. Thus, treatment of 4′-azidouridine (**5**)[8] with diphenylcarbonate, according to Reese's protocol, yielded **6** in 85%.[12] Protection with 4-methoxy-3,6-dihydro-2*H*-pyran, followed by hydrolysis with NaOH in EtOH, provided the arabino-derivative **8** in 56% yield over two steps. Fluorination with (diethylamino)sulfur trifluoride (DAST) furnished **9** as a single isomer in 56% yield. Standard conversion from uridine to cytidine was conducted with 1*H*-tetrazole and 4-chlorophenyldichlorophosphate followed by ammonia. Deprotection with acetic acid provided **10** in 26% yield over two steps.

4′-Azido-2′-deoxy-2′-fluoroarabinocytidine (**17**) was prepared as illustrated in Scheme 2. Thus, treatment of **11** with iodine and triphenylphosphine and imidazole in THF gave the iodide **12** in 76% yield. Elimination of the hydrogen iodide was performed by sodium methoxide to furnish **13** in 82%. Treatment of **13** with iodine and benzyltriethylammonium azide, prepared from the corresponding chloride and sodium azide in 4-methylmorpholine (MMP) and THF, provided **14** in 93% yield as a single isomer. Oxidative nucleophilic substitution of the

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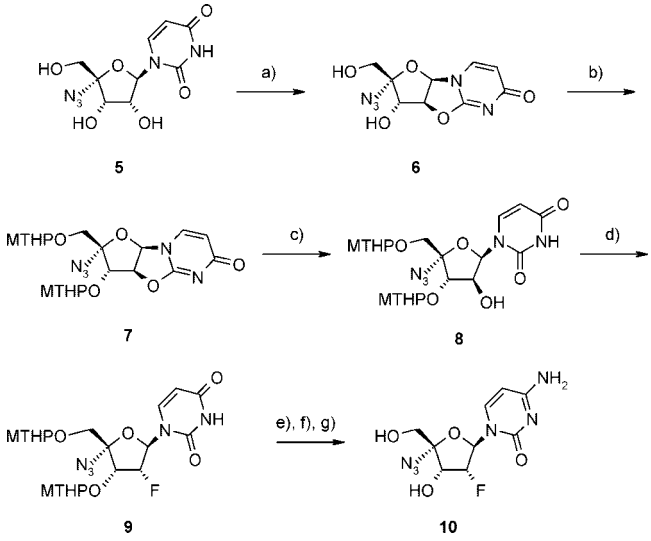
Scheme 1[a]



[a] (a) $(PhO)_2CO$, $NaHCO_3$, DMF, Δ; (b) CSA, MTHP; (c) NaOH, EtOH; (d) DAST, pyridine, CH_2Cl_2; (e) tetrazole, $Cl_2P(O)C_6H_4Cl$, Et_3N, MeCN; (f) NH_3, dioxane; (g) AcOH.

5′-iodine with *m*-chlorobenzoic acid/*m*-chloroperoxybenzoic acid, followed by deprotection and acetylation, gave **15** in 53% yield. Protection of the 3′,5′-hydroxy groups followed by standard conversion from uridine to cytidine, via the method developed by Divakar and Reese, furnished **17** in 88% yield over two steps.[12]

Synthesis of 3′-deoxy-3′-fluoroxylocytidine (**20**) is outlined in Scheme 3. Herein, protection of **5** by treatment with triphenylmethyl chloride in pyridine at 100 °C for 3 days gave **18** as the single product in 55% after chromatographic separation. Fluorination of **18** was achieved by heating with DAST and pyridine in dichloromethane for 18 h to furnish **19** in 31% yield. Conversion of **19** to cytidine via the triazole method,[12] followed by deprotection with Amberlyst 15 (H^+) ion-exchange resin in MeOH, provided **20** in 47% yield.

4′-Azido-3′-deoxy-3′-fluorocytidine (**28**) was prepared in an analogous manner to **17** and is outlined in Scheme 4. Thus, 3′-deoxy-3′-fluorouridine (**21**), treated with iodine and triphenylphosphine and imidazole in THF, gave the iodide **22** in 93% yield. Subsequent elimination by sodium methoxide provided **23** in 92% yield. Treatment of **23** with iodine and benzyltriethylammonium azide furnished a 1:1 mixture of diastereomers that were separated by column chromatography to give the desired isomer **24** in 31% yield. This lack of selectivity has previously been observed with a 3′-deoxyuridine substrate.[8] Oxidative nucleophilic substitution of 5′-iodine with *m*-chlorobenzoic acid/*m*-chloroperoxybenzoic acid, followed by benzoylation, gave **26** in 98% yield. Standard conversion from uridine to cytidine furnished **28** in a yield of 53% over two steps.[12]

Treatment of *N*-benzoylgemcitabine, prepared according to the literature[13] with iodine and triphenylphosphine, yielded **30** in 90% yield. Elimination followed by protection with 4-methoxybenzoyl chloride and treatment with iodine and benzyltriethylammonium azide gave **32** 60% yield over three steps. Nucleophilic substitution of 5′-iodine **32** with benzoate, followed by deprotection, led to **34** in 35% yield (Scheme 5).

Results and Discussion

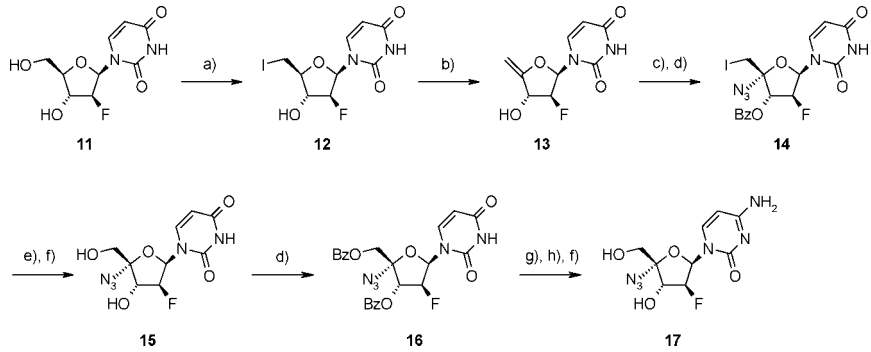
Compounds were characterized as inhibitors of HCV replication in the subgenomic replicon assay system using the 2209-23 cell line as described previously.[2,8] It was also determined if any of the compounds were cytotoxic or inhibited cell proliferation, an indication of potentially reduced selectivity against human polymerases. Cell viability was measured using the WST-1 assay (Roche), and cell proliferation was measured by quantification of the incorporation of tritiated thymidine into cellular DNA using the [^{3}H]-thymidine incorporation scintillation proximity assay (Amersham Biosciences).[5−8] Results are summarized in Table 1.

None of the compounds tested showed significant cytotoxic or cytostatic properties with the exception of the control compound, 2′deoxy-2′-fluoroarabinocytidine (dFAraC), which was a potent inhibitor of cell proliferation inhibiting tritiated thymidine incorporation with an IC_{50} value of 174 nM. Introduction of the 4′-azido moiety into dFAraC resulted in 4′-azido-2′-deoxy-2′-fluoroarabinocytidine (**17**), which did not show any measurable inhibition of cell proliferation. The introduction of the 4′-azido moiety therefore increased the selectivity of **17** by over 4000-fold as compared to dFAraC. In addition, **17** showed exceptionally high antiviral potency in the HCV replicon system. With an antiviral IC_{50} value of 24 nM, the potency of **17** was improved more than 50-fold as compared to 4′-azido-cytidine and 7-fold as compared to 4′-azido-arabinocytidine **4**.[7] Unexpectedly, the lack of a 2′-hydroxy group, in the ribo configuration, was not only acceptable for binding and incorporation by the HCV RNA polymerase, but such 2′-deoxy-nucleoside analogues could become highly potent without apparent effects on cell viability and proliferation in the HCV replicon system. Interestingly, inversion of the 2′-fluoro group to form 4′-azido-2′-deoxy-2′-fluorocytidine (**10**) reduced potency by >150-fold as compared to **17**. It has not yet been determined if this reduction in potency is due to reduced phosphorylation efficiency of **10** or whether incorporation efficiencies by HCV polymerase are different between **10** and **17** triphosphates. The related compound 2′-deoxy-2-fluorocytidine (FdC) has previously been characterized in the HCV replicon system. FdC was found to be a potent inhibitor of cell proliferation with an IC_{50} of 0.8 μM.[6] Therefore the introduction of the 4′-azido moiety into FdC to form **10** also abolished cell proliferation inhibition and increased selectivity by more than 125-fold.

Gemcitabine is a highly cytotoxic cytidine analogue and approved for the treatment of several cancer indications, including nonsmall cell lung cancer and pancreatic cancer. CC_{50} values for gemcitabine in human hepatoma cells have been reported to be in the nM range.[14,15] As the 2′-difluoro motif was accepted by kinases and polymerases, it was of interest to evaluate the effect of 4′-substitutions on the antiviral and cytotoxicity profile of such compounds. As shown in Table 1, the introduction of the 4′-azido moiety (**34**) resulted in a highly interesting compound with significant antiviral potency in the HCV replicon system (EC_{50} = 66 nM) and no apparent cytotoxicity, suggesting a substantial increase in selectivity as compared to gemcitabine. The effect of the 4′-azido moiety to confer high selectivity has therefore been consistent through **3**, **4**, **10**, **17**, and **34**.

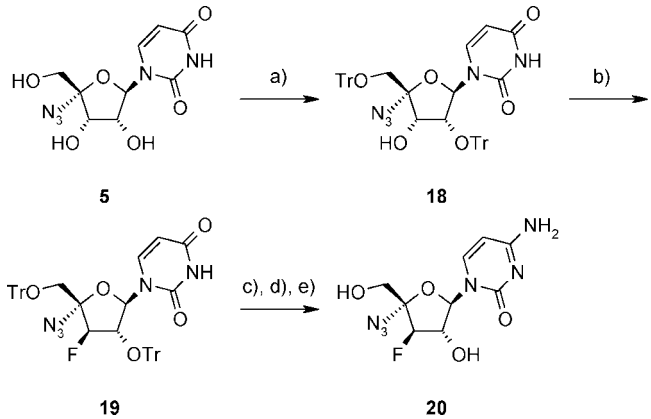
3′-Fluoro analogues of 4′-azidocytidine, **20** and **28**, were completely inactive as well as nontoxic in the replicon system. The 3′-hydroxyl group was suggested from structural studies with deoxycytidine kinase to be important for productive binding to the kinase, as hydrogen bonds are formed with E197 and Y86 in the human deoxycytidine kinase active site.[16] Removal of the 3′-hydroxy group in **20** and **28** may prevent these

Scheme 2[a]



[a] (a) I_2, PPh_3, imidazole, THF; (b) NaOMe, MeOH; (c) $[Bn(Et)_3N]N_3$, I_2, MMP, THF; (d) BzCl, DMAP, MMP, THF; (e) $(Bu_4N)HSO_4$, K_2HPO_4, mCPBA, mCBA, CH_2Cl_2; (f) NH_3, MeOH; (g) 1*H*-tetrazole, $Cl_2P(O)OC_6H_4Cl$, pyridine; (h) NH_3, dioxane.

Scheme 3[a]



[a] (a) TrCl, pyridine; (b) DAST, pyridine, CH_2Cl_2; (c) triazole, $POCl_3$, Et_3N, MeCN; (d) NH_4OH, dioxane; (e) Amberlyst 15, MeOH.

compounds from binding productively to human dCK and thus from being phosphorylated to their triphosphate derivatives.

Conclusions

We have presented data that shows both 4′-azido-2′-deoxy-2′,2′-difluorocytidine (**34**) and 4′-azido-2′-deoxyfluoroarabinocytidine (**17**) to be highly potent and selective inhibitors of HCV replication. Potency in this series was increased more than 50-fold as compared to 4′-azidocytidine (**3**), which has shown efficacy in HCV infected patients. Considerable potency is also observed with 4′-azido-2′-fluorocytidine **10**, although ∼3-fold lower as compared to 4′-azidocytidine (**3**). Further characterization of these compounds is therefore warranted as potential antiviral agents with low cytotoxicity in human cells. Derivatives at the 3′ position of 4′-azidocytidine did not show antiviral potency in cell culture, presumably due to inefficient phosphorylation.

Experimental Section

All starting materials, solvents and reagents were reagent grade and used as purchased. Chromatography solvents were HPLC grade and used without further purification. Flash column chromatography was performed on Merck KGaA Silica Gel 60 F_{254} (particle size 0.040−0.063 mm) unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) analysis using precoated glass plates (Silica Gel 60 F_{254}). The purity of compounds **10**, **17**, **20**, **28**, and **34** was determined by high pressure liquid chromatography and were found to be greater than 95%, apart from compound **20** (94%).

2,2′-Anhydro-1-(4′-azido-β-D-arabinofuranosyl)uracil (6). A mixture containing **5** (1.00 g, 3.50 mmol), diphenyl carbonate (0.83 g, 3.85 mmol), sodium hydrogen carbonate (0.030 g, 0.35 mmol), and *N*,*N*-dimethylformamide (1 mL) was heated at 100 °C under N_2. After 14 h, the reaction mixture was cooled to room temperature and diluted with CH_3CN (5 mL). The resulting precipitate was collected by filtration to provide **6** (0.80 g, 85%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.94 (d, J = 7.5 Hz, 1H), 6.62 (d, J = 5.6 Hz, 1H), 6.46 (d, J = 6 Hz, 1H), 5.92 (d, J = 7.5 Hz, 1H), 5.54 (t, J = 5.7 Hz, 1H), 5.35 (dd, J = 2.4 and 6 Hz, 1H), 4.50 (br s, 1H), 3.53−3.41 (m, 2H).

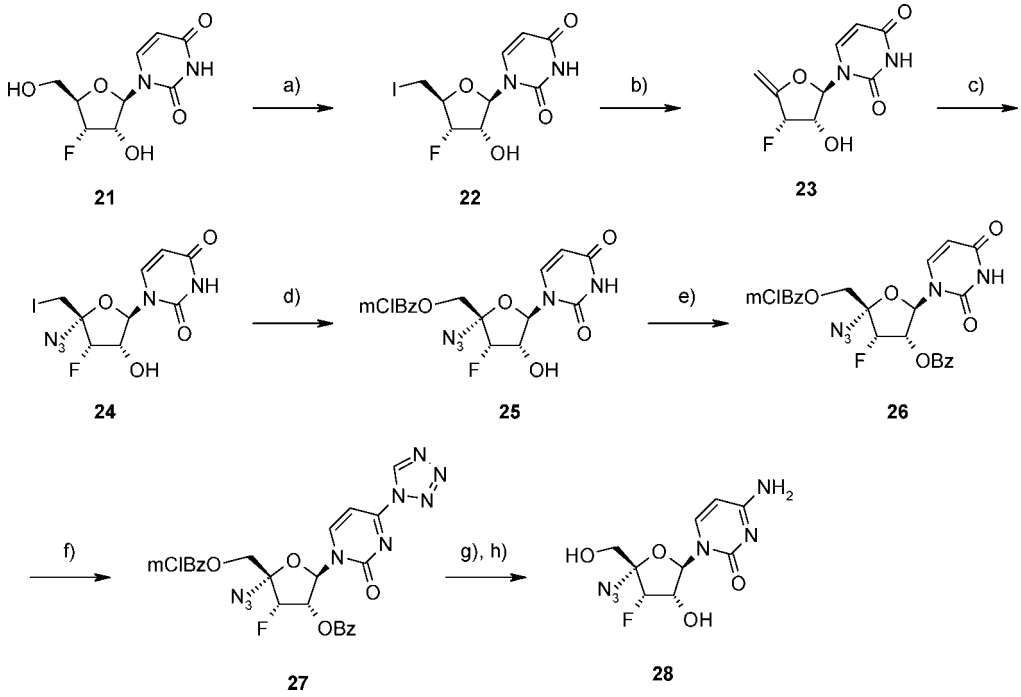
2,2′-Anhydro-1-(4′-azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-β-D-arabinofuranosyl)uracil (7). To a solution of **6** (0.77 g, 2.88 mmol) in dry *N*,*N*-dimethylformamide (15 mL) was added 4-methoxy-3,4-dihydro-2*H*-pyran (2.6 mL, 23 mmol), followed by camphorsulfonic acid (0.040 g, 0.17 mmol). The reaction mixture was stirred for 2 days at room temperature, neutralized with solid K_2CO_3, and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (10−15% MeOH in $CHCl_3$) gave **7** as an off-white solid (0.87 g, 61%). ^{1}H NMR (400 MHz, CD_3Cl): δ 7.36 (d, J = 7.5 Hz, 1H), 6.29 (d, J = 6.1 Hz, 1H), 6.09 (d, J = 7.5 Hz, 1H), 5.34 (dd, J = 2.0 and 6.1 Hz, 1H), 4.88 (d, J = 2.0 Hz, 1H), 3.89−3.79 (m, 2H), 3.69−3.56 (m, 6H), 3.45 (q, J = 10.5 Hz, 2H), 3.32 (s, 3H), 3.14 (s, 3H), 2.10−1.70 (m, 8H).

1-(4′-Azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-β-D-arabinofuranosyl)uracil (8). A solution of **7** (0.87 g, 1.76 mmol) and KOH (0.070 g, 1.25 mmol) in 9:1 mixture of EtOH/H_2O (10 mL) was refluxed for 3 h. The reaction mixture was cooled, neutralized with Amberlyst 15 ion-exchange resin, filtered, and evaporated to dryness under reduced pressure to give **8** (0.83 g, 92%) as a foam. ^{1}H NMR (400 MHz, CD_3Cl): δ 7.52 (d, J = 7.9 Hz, 1H), 6.36 (d, J = 5.1 Hz, 1H), 5.58 (d, J = 7.9 Hz, 1H), 4.53 (t, J = 5.1 Hz, 1H), 4.47 (d, J = 5.1 Hz, 1H), 3.80−3.55 (m, 8H), 3.48 (s, 2H), 3.30 (s, 3H), 3.24 (s, 3H), 1.95−1.70 (m, 8H).

1-(4′-Azido-3′,5′-*O*-bis-(4-methoxy-tetrahydropyran-4-yl)-2′-deoxy-2′-fluoro-β-D-ribofuranosyl)uracil (9). To a solution of **8** (0.41 g, 0.8 mmol) in CH_2Cl_2 (5 mL) was added pyridine (1.2 mL) followed by DAST (0.64 mL, 4.8 mmol). The reaction mixture was stirred at room temperature for 24 h, diluted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (10−15% MeOH in $CHCl_3$) to give **9** (0.23 g, 56%) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54 (d, J = 7.4 Hz, 1H), 5.83 (d, J = 21.0 Hz, 1H), 5.80 (d, J = 7.4 Hz, 1H), 5.25 (dd, J = 5.5 and 53.9 Hz, 1H), 4.82 (dd, J = 5.5 and 22.0 Hz, 1H), 3.80−3.55 (m, 10H), 3.33 (s, 3H), 3.21 (s, 3H), 2.00−1.75 (m, 8H). MS (ES^+) m/z 516 (MH^+).

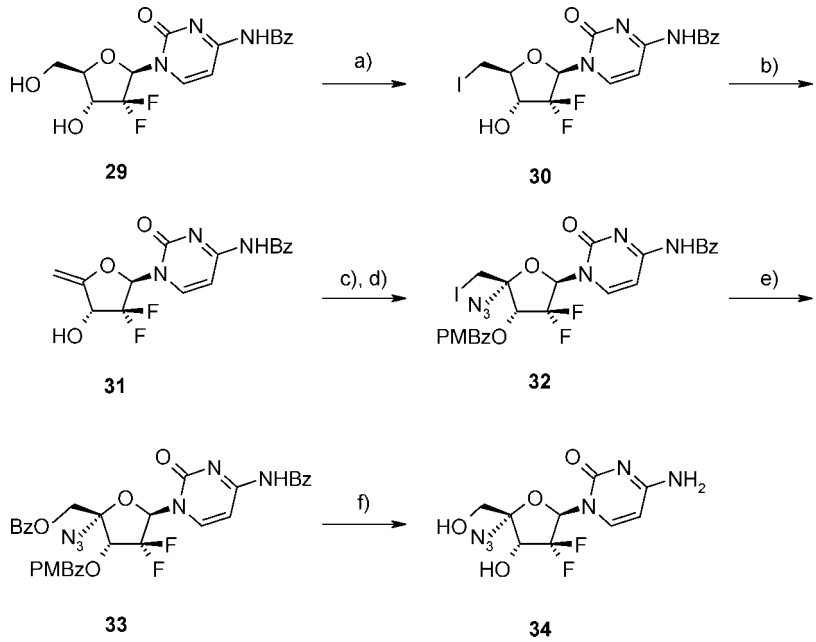
1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-ribofuranosyl)cytosine (10). To a ice−water bath cooled solution of **9** (0.21 g, 0.407 mmol) and 1*H*-tetrazole (0.086 g, 1.22 mmol) in dry pyridine (10 mL) was added chlorophenyldichlorophosphate (0.1 mL, 0.61 mmol) over 5 min under stirring. The reaction mixture was stirred at room temperature for 6 h and evaporated to dryness under reduced pressure (<30 °C). The residue was partitioned between cold CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was

Scheme 4[a]



[a] (a) I_2, PPh_3, Pyr, MeCN; (b) NaOMe, MeOH, 65 °C; (c) I_2, $[Bn(Et)_3N]N_3$, MeCN, THF, 5 °C; (d) $(Bu_4N)HSO_4$, K_2HPO_4, mCPBA, mCBA, CH_2Cl_2; (e) Bz_2O, Pyr; (f) 4-ClPhOP(O)Cl_2, 1*H*-tetrazole, Pyr, 5 °C; (g) 0.5 M, NH_3/dioxane; (h) 2 M NH_3/MeOH.

Scheme 5[a]



[a] (a) I_2, PPh_3, MeCN, Pyr; (b) tBuOK, DMF; (c) I_2, $[Bn(Et)_3N]N_3$, THF, MeCN; (d) *p*-MeOBzCl, MMP, DMAP, MeCN, THF; (e) NaBz, 15-crown-5, DMF, 60 °C; (f) 2 M NH_3/MeOH.

washed with brine and then dried over $MgSO_4$, filtered, and evaporated. The residue was treated with 0.5 M NH_3 in 1,4-dioxane (20 mL), and the resulting solution was stirred for 4 h at room temperature and evaporated to dryness under reduced pressure. The residue was dissolved in AcOH/MeOH/H_2O [4:2:1 (20 mL)], heated at 50 °C for 5 h, cooled, and evaporated to dryness under reduced pressure. Purification by preparative HPLC (Hypercarb column, H_2O in CH_3CN) provided **10** (0.03 g, 26%) as a white solid. ^1H NMR (400 MHz, methanol-d_4): δ 7.87 (d, J = 7.5 Hz, 1H), 6.12 (dd, J = 1.3 and 19.4 Hz, 1H), 5.89 (d, J = 7.5 Hz, 1H), 5.13 (ddd, J = 1.4, 5.3, and 53.9 Hz, 1H), 4.57 (dd, J = 5.3 and 22.1 Hz, 1H), 3.80 (dd, J = 12.1 and 39.3 Hz, 2H). HRMS (ES^+) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0908.

1-(2′-Deoxy-2′-fluoro-5′-iodo-β-D-arabinofuranosyl)uracil (12). A suspension of **11** (1.61 g, 6.54 mmol) and pyridine (7 mL) in anhydrous CH_3CN (142 mL) was cooled on an ice−water bath. Iodine (2.00 g, 7.88 mmol) and triphenylphosphine (2.23 g, 8.50 mmol) were added, and the reaction mixture was stirred at 0 °C for 30 min. After stirring at room temperature for 24 h, the reaction mixture was again cooled on an ice−water bath and more iodine (0.40 g, 1.58 mmol) and triphenylphosphine (0.45 g, 1.71 mmol) were added. The reaction mixture was stirred at room temperature

Table 1. EC_{50} and CC_{50} Determination in the HCV Replicon System

compd	GT-1b stable replicon (2209-23) EC_{50} (μM)	cytotoxicity (2209-23) CC_{50} (μM)	proliferation (^{3}H-Thy incorporation) IC_{50} (μM)
3	1.28	>2000	>100
4	0.171	>1000	>100
10	4.02	>100	>100
17	0.024	>100	>100
20	>100	>100	nd
28	>100	>100	nd
34	0.066	>100	nd
dFAraC	0.114	>100	0.174

for a further 41 h and then concentrated to 1/4 of the volume under reduced pressure, diluted with CH_2Cl_2 (200 mL), and washed with 0.5 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ solution. The aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), treated with activated carbon, and filtered through celite. The filter cake was then washed with CH_2Cl_2 (200 mL), followed by azeotropic distillation of the filtrate with toluene. Chromatography (1−5% MeOH in CH_2Cl_2) gave **12** as an off-white solid (1.78 g, 76%). ^{1}H NMR (400 MHz, methanol-d_4): δ 7.71 (dd, J = 1.8 and 8.2 Hz, 1H), 6.21 (dd, J = 3.3 and 19.9 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 5.02 (ddd, J = 1.8, 3.4, 51.9, 1H), 4.29 (ddd, J = 1.7, 3.3, 17.5, 1H), 3.99 (td, J = 3.4, 6.3, 1H), 3.53−3.45 (m, 2H). MS (ES^+) $[M + H]^+$ m/z 357.

1-(2′,5′-Dideoxy-2′-fluoro-erytho-β-D-pent-4-enofuranosyl)uracil (13). Sodium methoxide (0.76 g, 14.1 mmol) was added to a suspension of compound **12** (1.00 g, 2.81 mmol) in anyhdrous methanol (21 mL), and the reaction mixture was stirred at 65 °C for 5 h. The reaction mixture was cooled to room temperature and diluted with methanol (10 mL). Pyridinium form DOWEX-H^+ (4 g) was added, and the mixture stirred until a clear solution was obtained (ca. 20 min). The mixture was filtered and the resin washed with methanol and then evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (10% MeOH in CH_2Cl_2 in) to give **13** (0.52 g, 82%) as a white solid. ^{1}H NMR (methanol-d_4): δ 7.46 (dd, J = 2.1 and 8.2 Hz, 1H), 6.50 (dd, J = 3.3 and 19.4 Hz, 1H), 5.71 (d, J = 8.2 Hz, 1H), 5.03 (ddd, J = 1.9, 3.1, and 51.5 Hz, 1H), 4.67 (d, J = 2.3 Hz, 1H), 4.66 (dd, J = 1.7 and 11.7 Hz, 1H), 4.46 (d, J = 2.3 Hz, 1H). MS (ES^+) $[M + H]^+$ m/z 229.

1-(4′-Azido-3′-*O*-benzoyl-2′-deoxy-2′-fluoro-5′-iodo-β-D-arabinofuranosyl)uracil (14). Benzyltriethylammonium chloride (0.98 g, 4.29 mmol) and sodium azide (0.28 g, 4.29 mmol) were suspended in anhydrous CH_3CN (22 mL). The suspension was stirred vigorously for 15 h and was then filtered. The filtrate containing quarternary ammonium azide was added to a solution of compound **13** (0.48 g, 2.10 mmol) and 4-methylmorpholine (0.07 mL, 0.64 mmol) in anhydrous THF (15 mL). A solution of iodine (0.90 g, 3.58 mmol) in anyhdrous THF (19 mL) was added dropwise over 1 h under stirring at 0 °C. The reaction mixture was stirred at 0 °C for a further 4 h. *N*-Acetyl-L-cysteine (0.034 g, 0.21 mmol), 4-methylmorpholine (1.16 mL, 10.5 mmol), and DMAP (0.26 g, 2.10 mmol) were added, followed by a dropwise addition of benzoyl chloride (0.54 mL, 4.63 mmol). Stirring at 0 °C continued for 1 h. A solution of 0.1 M Na_2SO_3 in saturated aqueous $NaHCO_3$ (50 mL) was added, and the mixture was shaken. The mixture was diluted with CH_2Cl_2 (150 mL) and washed with saturated aqueous $NaHCO_3$ and water. The organic layer was separated and the aqueous layer washed with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (3% MeOH in CH_2Cl_2) to give **14** (0.98 g, 93%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (br s, 1H), 8.10−7.50 (m, 6 H), 6.59 (dd, J = 4.3 and 17.9 Hz, 1H), 5.87 (dd, J = 2.1, 11.3 Hz, 1H), 5.84 (t, J = 1.9 Hz, 1H), 5.43 (ddd, J = 2.1, 3.9, and 51.7 Hz, 1H), 3.88−3.79 (m, 2H). MS (ES^+) $[M + H]^+$ m/z 501.9.

1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)uracil (15). 3-Chloroperoxybenzoic acid [55% in balance with 3-chlorobenzoic acid (10%) and water (35%)] (4 × 3.33 g) was added portion-wise over 20 h to a two-phase suspension containing **14** (0.97 g, 1.94 mmol), tetra-*n*-butylammonium hydrogensulfate (0.70 g, 2.05 mmol), and 3-chlorobenzoic acid (0.33 g, 2.13 mmol) in CH_2Cl_2 (125 mL) and 1.7 M aqueous K_2HPO_4 (45 mL) under stirring at rt. A solution of Na_2SO_3 (7.5 g, 59.5 mmol) in saturated aqueous $NaHCO_3$ (150 mL) was added. The mixture was shaken for 5 min. The organic layer was separated and the aqueous layer washed with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. The residue was treated with saturated ammonia in MeOH (150 mL) for 19 h at room temperature, after which time the solution was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) followed by reversed phase column chromatography (5−10% CH_3CN in H_2O) to give **15** (0.31 g, 56%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.64 (dd, J = 1.7 and 8.1 Hz, 1H), 6.40 (d, J = 6.0 Hz, 1H), 6.34 (dd, J = 5.7 and 10.6 Hz, 1H), 5.71 (t, J = 6.0 Hz, 1H), 5.67 (d, J = 8.1 Hz, 1H), 5.25 (dt, J = 5.5 and 53.9 Hz, 1H), 4.45 (dt, J = 5.6 and 23.1 Hz, 1H), 3.78−3.69 (m, 2H). ^{13}C NMR (DMSO-d_6): δ 163.5 (C-4), 150.8 (C-2), 141.8 (C-6), 102.5 (C-5), 97.34 and 97.26 (C-4′), 95.9 and 94.4 (C-2′), 81.7 (C-1′), 75.3 and 75.1 (C-3′), 62.8 (C-5′). MS (ES^+) $[MH]^+$ m/z 288.1.

1-(4′-Azido-2′-deoxy-3′,5′-*O*-dibenzoyl-2′-fluoro-β-D-arabinofuranosyl)uracil (16). A solution of compound **15** (0.244 g, 0.850 mmol), 4-methylmorpholine (0.65 mL, 5.95 mmol), and DMAP (0.05 g, 0.425 mmol) in anhydrous THF (25 mL) was cooled to 0 °C and benzoyl chloride (0.247 mL, 2.12 mmol) was added dropwise over 5 min. The reaction mixture was stirred at 0 °C for a further 1 h and was then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The aqueous layer was washed with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by silica gel column chromatography (2−5% MeOH in CH_2Cl_2) to give compound **16** (0.406 g, 96%) as a white solid. ^{1}H NMR (methanol-d_4): δ 8.10−7.39 (m, 11H), 6.63 (dd, J = 5.1 and 14.4 Hz, 1H), 6.08 (dd, J = 3.1 and 20.3 Hz, 1H), 5.74−5.72 and 5.61−5.59 (m, 1H), 5.67 (d, J = 8.2 Hz, 1H), 4.96−4.84 (m, 2H). MS (ES^+) $[M + H]^+$ m/z 496.0.

1-(4′-Azido-2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)cytosine (17). 4-Chlorophenyldichlorophosphate (0.20 mL, 1.22 mmol) was added dropwise over 5 min to a solution, cooled on an ice−water bath of **16** (0.40 g, 0.815 mmol) and 1*H*-tetrazole (0.17 g, 2.45 mmol) in anhydrous pyridine (15 mL). After stirring for 5 min, the reaction mixture was allowed to warm to room temperature. After 5 h, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and water, dried (4 Å molecular sieves), and evaporated to dryness under reduced pressure. Azeotropic distillation with toluene removed the remaining traces of pyridine from the product. The residue was then treated with 0.5 M ammonia in 1,4-dioxane (40 mL) for 5.5 h at room temperature. The solution was evaporated to dryness under reduced pressure, and the residue treated with saturated NH_3 in methanol (55 mL) for 15 h at rt. The mixture was evaporated to dryness under reduced pressure and the residue purified by silica gel column chromatography [saturated NH_3 in MeOH (0.5%), MeOH (19.5%), CH_2Cl_2 (80%)] to give **17** as an off-white solid (0.21 g, 93%); mp 99.0−100.0 °C. ^{1}H NMR (methanol-d_4): δ 7.75 (dd, J = 1.2 and 7.6 Hz, 1H), 6.48 (dd, J = 5.0 and 12.4 hz, 1H), 5.91 (d, J = 7.4 Hz, 1H), 5.19 (dt, J = 4.3 and 56.0 Hz, 1H), 4.47 (dd, J = 4.3 and 21.6 Hz, 1H), 3.83 (s, 2H). ^{13}C NMR (DMSO-d_6): δ 166.3 (C-4), 155.3 (C-2), 142.3 (C-6), 97.5 and 97.4 (C-4′), 94.8 (C-5), 96.2 and 94.3 (C-2′), 82.9 (C-1′), 75.8 and 75.6 (C-3′), 63.0 (C-5′). MS (ES^+) $[2 M + Na]^+$ m/z 595.2. HRMS (ES^+) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0911.

1-(4′-Azido-2′,5′-di-*O*-trityl-β-D-ribofuranosyl)uracil (18). Triphenylmethyl chloride (19.30 g, 69.23 mmol) was added to a solution

of **5** (5.00 g, 17.53 mmol) in pyridine (50 mL). After stirring at 100 °C under N_2 for 72 h, the reaction mixture was cooled to room temperature and then evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2, washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Chromatography (EtOAc in hexanes) provided **18** (7.43 g, 55%) as an off-white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.02−8.97 (br s, 1H), 7.32−7.25 and 7.54−7.45 (m, 30H), 6.23 (d, J = 6 Hz, 1H), 5.18 (d, J = 8 Hz, 1H), 4.72 (t, J = 5.5 Hz, 1H), 3.36 (t, J = 4.5 Hz, 1H), 3.10 (s, 2H), 2.79 (d, J = 4.1 Hz, 1H). MS (ES^-) $[M - H]^-$ m/z 768.2.

1-(4′-Azido-3′-deoxy-3′-fluoro-2′,5′-di-*O*-trityl-β-D-xylofuranosyl)uracil (19). (Diethylamino)sulfur trifluoride (1.6 mL, 12.40 mmol) was carefully added to a flask containing a solution of **18** (6.40 g, 8.31 mmol) in pyridine (6 mL) and CH_2Cl_2 (90 mL). Once complete, the reaction mixture was heated at 50 °C for 18 h under N_2. The cooled reaction mixture was poured onto ice−water and extracted with CH_2Cl_2. The organics were washed with brine, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. Chromatography (EtOAc in hexanes) provided **19** (2.0 g, 31%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16−8.14 (br s, 1H), 7.47−7.16 (m, 30H), 6.85 (dd, J = 1.6 and 8 Hz, 1H), 6.66 (d, J = 3.1 Hz, 1H), 5.60 (dd, J = 2.2 and 8 Hz, 1H), 4.14 (dd, J = 3.2 and 21.1 Hz, 1H), 3.72 (d, J = 49 Hz, 1H), 3.41−3.33 (m, 2H). MS (ES^-) $[M - H]^-$ m/z 770.2.

1-(4′-Azido-3′-deoxy-3′-fluoro-β-D-xylofuranosyl)cytosine (20). $POCl_3$ (1 mL, 11.82 mmol) was slowly added to a flask, cooled to 0 °C, containing **19** (2.28 g, 2.95 mmol), triazole (3.06 g, 44.31 mmol), triethylamine (8.2 mL, 59.08 mmol), and CH_3CN (90 mL). The resulting mixture was stirred at 0 °C for 15 min and then allowed to warm to room temperature. After 2.5 h, the crude reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The resulting solid was dissolved in dioxane (60 mL) and treated with NH_4OH (10 mL) and left to stir at room temperature for 20 h. The crude reaction mixture was evaporated to dryness under reduced pressure. Chromatography (15% MeOH in CH_2Cl_2) provided 1-(4′-azido-3′-deoxy-3′-fluoro-2′,5′-di-*O*-trityl-β-D-xylofuranosyl)cytosine (1.78 g, 2.39 mmol) as a light-brown solid. This material was dissolved in MeOH (60 mL) and treated with 2 M NH_3 in EtOH (7.2 mL). After stirring for 3 h, the precipitate was removed by filtration and the filtrate evaporated to dryness under reduced pressure. Chromatography (0−10% MeOH in CH_2Cl_2) provided **20** (0.40 g, 47%) as an off-white solid; mp 171.8−172.9 °C. ^{1}H NMR (300 MHz, methanol-d_4): δ 7.66 (d, J = 7.5 Hz, 1H), 6.26 (d, J = 4.2 Hz, 1H), 5.95 (d, J = 7.5 Hz, 1H), 4.77 (dd, J = 2.5 and 51 Hz, 1H), 4.44 (ddd, J = 2.5, 4, and 21 Hz, 1H), 3.99−3.87 (m, 2H). HRMS (ES^+) calcd for $C_9H_{12}FN_6O_4$ $[M + H]^+$ 287.0904; found 287.0896.

1-(3′-Deoxy-3′-fluoro-5′-iodo-β-D-ribofuranosyl)uracil (22). 3′-Deoxy-3′-fluorouridine[17] **21** (0.31 g, 1.28 mmol) and triphenylphosphine (0.47 g, 1.79 mmol) were dissolved in CH_3CN/pyridine [95:5 (13 mL)]. Iodine (0.422 g, 1.66 mmol) was added, and the reaction mixture was stirred for 18 h at room temperature. Water (5 mL) was added, and the solvents were evaporated to dryness under reduced pressure. Azeotropic distillation with CH_3CN and then with $CHCl_3$ was performed to remove the water. The residue was purified by silica gel column chromatography (2−6% EtOH in CH_2Cl_2) to give **22** (0.426 g, 93%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 11.43 (br s, 1H), 7.70 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 6.1 Hz, 1H), 5.85 (d, J = 8.0 Hz, 1H), 5.72 (d, J = 8.1 Hz, 1H), 4.90 (dd, J = 4.6 and 53.0 Hz, 1H), 4.55−4.40 (m, 1H), 4.25 (dt, J = 7.5 and 13.7 Hz, 1H), 3.48 (ddd, J = 6.9, 10.4, 16.5, 2H). MS (ES^+) $[M + H]^+$ m/z 357.1.

1-(3′,5′-Dideoxy-3′-fluoro-erytho-β-D-pent-4-enofuranosyl)uracil (23). Compound **22** (0.426 g, 1.2 mmol) was dissolved into a solution of 0.4 M sodium methoxide in MeOH (27 mL). The reaction mixture was stirred for 5 h at 65 °C. Pyridinium form DOWEX H^+ was added portionwise until neutral pH (2−3 g), and the mixture was stirred at room temperature for 10 min. The resin was removed by filtration and washed with MeOH, and the filtrate was evaporated to dryness under reduced pressure. The residue was slurried in 2% EtOH in CH_2Cl_2 and purified by silica gel column chromatography (2−6% EtOH in CH_2Cl_2) to give **23** as a white solid (0.251 g, 92%). ^{1}H NMR (DMSO-d_6): δ 11.50 (br s, 1H), 7.75 (d, J = 8.1 Hz, 1H), 6.10 (d, J = 6.2, 1H), 6.04 (d, J = 7.6 Hz, 1H), 5.70 (dd, J = 2.2, 8.1 Hz, 1H), 5.26 (dd, J = 4.4 and 55.8 Hz, 1H), 4.68−4.55 (m, 1H), 4.55 (ddd, J = 2.2, 6.4, and 18.9 Hz, 2H). MS (ES^+) $[M + H]^+$ m/z 229.1.

1-(4′-Azido-3′-deoxy-3′-fluoro-5′-iodo-β-D-ribofuranosyl)uracil (24). Benzyltriethylammonium chloride (0.296 g, 1.3 mmol) and sodium azide (0.084 g, 1.3 mmol) were suspended in anhydrous CH_3CN (7 mL) and sonicated for several minutes. The resulting fine suspension was stirred for 3 h at room temperature and filtered under nitrogen into a dry THF solution (4.4 mL) of **23** (0.148 g, 0.65 mmol). 4-Methylmorpholine (0.021 mL, 0.19 mmol) was added, the resulting solution was cooled on an ice−water bath, and a solution of iodine (0.28 g, 1.1 mmol) in anhydrous THF (6 mL) was added dropwise over 60 min. The reaction mixture was stirred for 16 h at 0−9 °C. *N*-Acetyl-L-cysteine (0.011 g, 0.065 mmol) was added, and the solution was stirred until bubbling subsided. The solvent was concentrated under reduced pressure to half-volume, and then a solution of 0.1 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$/brine was added under stirring. The mixture was extracted with 10% EtOH in CH_2Cl_2, and the organic layers were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The 4′-epimeric mixture (∼1:1) was separated by silica gel column chromatography (2−3% EtOH in CH_2Cl_2) and then by semi-preparative Hyper Carb HPLC column purification (10−90% CH_3CN in H_2O) to give **24** as a white solid (0.081 g, 31%). ^{1}H NMR ($CDCl_3$): δ 8.50 (br s, 1H), 7.34 (d, J = 8.1 Hz, 1H), 5.81 (d, J = 8.1 Hz, 1H), 5.70 (d, J = 4.1 Hz, 1H), 5.38 (dd, J = 5.7 and 52.1 Hz, 1H), 4.84−4.76 (m, 1H), 3.55 (dd, J = 11.3 and 28.8 Hz, 2H). MS (ES^-) $[M - H]^-$ m/z 395.9.

1-(4′-Azido-5′-*O*-(4-chloro)benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)uracil (25). A solution of **24** (0.081 g, 0.2 mmol) in CH_2Cl_2 (12 mL) was combined with a mixture of tetrabutylammonium hydrogensulfate (0.073 g, 0.22 mmol) and *m*-chlorobenzoic acid (0.048 g, 0.31 mmol) in 1.75 M aqueous K_2HPO_4 (4.7 mL). The two-phase system was stirred vigorously at room temperature, and one portion of *m*-chloroperbenzoic acid [55% in balance with 3-chlorobenzoic acid (10%) and water, (35%)] (0.360 g) was added. After 1.5 h, 3 × 120 mg of this reagent mixture was added at 1.5 h intervals. After the last addition, the mixture was vigorously stirred for another 18 h at rt. A solution of 0.1 M Na_2SO_3 in saturated aqueous $NaHCO_3$ (10 mL) was added, and the mixture was stirred vigorously at rt for 15 min. The organic layer was separated, and the water layer was extracted with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (2−4% EtOH in CH_2Cl_2) to give **25** as a white solid (0.032 g, 37%). ^{1}H NMR ($CDCl_3$): δ 8.04 (t, J = 1.8 Hz, 1H), 7.96−7.91 (m, 1H), 7.57 (ddd, J = 1.1, 2.2, and 8.0 Hz, 1H), 7.42 (t, J = 7.9 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 5.75 (d, J = 8.1 Hz, 1H), 5.74 (d, J = 2.9 Hz, 1H), 5.52 (dd, J = 5.7 and 51.8 Hz, 1H), 4.83−4.75 (m, 1H), 4.61 (s, 2H). MS (ES^-) $[M - H]^-$ m/z 424.

1-(4′-Azido-2′-*O*-benzoyl-5′-*O*-(4-chloro)benzoyl-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)uracil (26). Benzoic anhydride (0.021 g, 0.09 mmol) was added to a solution of **25** (0.032 g, 0.076 mmol) in pyridine (0.75 mL). After stirring for 16 h at rt, MeOH (5 mL) was added. The solvents were evaporated to dryness under reduced pressure, and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (1% EtOH in CH_2Cl_2) gave **26** (0.040 g, 98%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 8.86 (br s, 1H), 8.12−8.08 (m, 3H), 8.00−7.96 (m, 1H), 7.66−7.40 (m, 5H), 7.22 (d, J = 8.1 Hz, 1H), 5.89−5.76 (m, 4H), 4.75−4−64 (m, 2H). MS (ES^+) $[M + H]^+$ m/z 530.6.

1-(4′-Azido-3′-deoxy-3′-fluoro-β-D-ribofuranosyl)cytosine (28). Compound **26** (0.040 g, 0.075 mmol) and 1*H*-tetrazole (0.016 g, 0.23 mmol) were azeotroped with pyridine to remove moisture and then redissolved in anhydrous pyridine (1.3 mL). The solution was cooled on an ice−water bath, and 4-chlorophenylphosphorodichloridate (0.018 mL, 0.11 mmol) was added. The reaction mixture was stirred at 0−5 °C for 5 min and then allowed to warm to room temperature. After 5 h, the reaction mixture was evaporated to dryness under reduced pressure and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The intermediate **27** was then treated with 0.5 M NH_3 in 1,4-dioxane (5 mL) for 4 h at rt and then evaporated to dryness under reduced pressure. The residue was dissolved in saturated NH_3 in MeOH (15 mL) and the mixture was stirred for 16 h at room temperature and then evaporated to dryness under reduced pressure. Purification by semipreparative Hyper Carb HPLC column purification (10−90% CH_3CN in H_2O) gave **28** (0.011 g, 53%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 7.71 (d, J = 7.5 Hz, 1H), 7.32 (br d, J = 4.8 Hz, 2H), 6.18 (d, J = 7.6 Hz, 1H), 5.98 (br s, 1H), 5.78 (d, J = 7.5 Hz, 1H), 5.72 (br s, 1H), 5.07 (dd, J = 4.5 and 53.6 Hz, 1H), 4.45 (ddd, J = 4.4, 7.3, and 22.2 Hz, 1H), 3.49 (q, J = 12.1 Hz, 2H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 287. HRMS (ES$^+$) calcd for $C_9H_{12}FN_6O_4$ [M + H]$^+$ 287.0904; found 287.0909.

1-(4-*N*-Benzoyl-2′-dideoxy-2′,2′-difluoro-5′-iodo-β-D-ribofuranosyl)cytosine (30). A solution of 4-*N*-benzoylgemcitabine (**29**)[15] (3.50 g, 9.54 mmol) in dry CH_3CN/pyridine [95:5 (150 mL)] was cooled on an ice−water bath. Iodine (3.15 g, 12.4 mmol) and triphenylphosphine (3.38 g, 12.9 mmol) were added, and the reaction mixture was stirred at 0 °C for 30 min. The ice−water bath was then removed, and stirring continued at room temperature for 64 h. The reaction mixture was diluted with CH_2Cl_2 (300 mL) and washed with 0.5 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ (200 mL). The aqueous layer was washed with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrate was diluted with toluene (150 mL) and concentrated under reduced pressure. Purification by silica gel column chromatography (1−5% MeOH in CH_2Cl_2) gave **30** (4.10 g, 90%) as a white solid. ^{1}H NMR (methanol-d_4): δ 8.20−7.53 (m, 7H), 6.34 (t, J = 8.2 Hz, 1H), 4.19−4.06 (m, 1H), 3.88−3.82 (m, 1H), 3.72−3.68 (m, 1H), 3.61−3.57 (m, 1H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 478.

1-(4-*N*-Benzoyl-2′,5′-dideoxy-2′,2′-difluoro-β-D-pent-4-enoribofuranosyl)cytosine (31). Compound **30** (4.10 g, 8.59 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (125 mL), and the solution was cooled to 0 °C. A solution of potassium *tert*-butoxide (1.93 g, 17.2 mmol) in anhydrous *N*,*N*-dimethylformamide (25 mL) was added dropwise over 20 min. The reaction mixture was stirred at 0 °C for another 60 min, after which time HPLC analysis showed some remaining compound **30**. A solution of potassium *tert*-butoxide (0.19 g, 1.72 mmol) in anhydrous *N*,*N*-dimethylformamide (2.5 mL) was added dropwise over 5 min, and stirring continued for another 5 min at 0 °C. Pyridinium form DOWEX H$^+$ (16 g) was added, and the mixture was stirred at room temperature for 10 min. The resin was removed by filtration and washed with DMF. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10% MeOH in CH_2Cl_2) to give **31** (2.37 g, 79%) as a white solid. ^{1}H NMR (methanol-d_4): δ 7.99−7.52 (m, 7H), 6.47 (t, J = 7.3 Hz, 1H), 4.89 (t, J = 9.7 Hz, 1H), 4.78 (s, 1H), 4.57 (s, 1H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 350.

1-(4′-Azido-4-*N*-benzoyl-2′-dideoxy-2′,2′-difluoro-5′-iodo-β-D-ribofuranosyl)cytosine (32). Benzyltriethylammonium chloride (3.08 g, 13.5 mmol) and NaN_3 (0.878 g, 13.5 mmol) were suspended in anhydrous CH_3CN (150 mL) and sonicated for 3 min. The resulting fine suspension was stirred for 3 h at room temperature and was then filtered under nitrogen into a dry THF solution (47 mL) of **31** (2.36 g, 6.76 mmol). 4-Methylmorpholine (0.223 mL, 2.03 mmol) was added and the resulting solution cooled on an ice−water bath. A solution of iodine (2.92 g, 11.5 mmol) in anhydrous THF (62 mL) was added dropwise over 60 min and then left to stir for 16 h at 0−9 °C. The mixture was concentrated to half-volume under reduced pressure and the remaining mixture left to stir for 24 h at 0−9 °C. *N*-Acetyl-L-cysteine (0.11 g, 0.68 mmol) was added, and the solution was stirred until bubbling subsided. The mixture was kept at 5 °C and 4-methylmorpholine (3.72 mL, 33.8 mmol) and DMAP (826 mg, 6.76 mmol) were added, followed by anisoylchloride (3.45 g, 20.3 mmol) dissolved in MeCN (14 mL). The resulting mixture was stirred for 1 h. MeOH (15 mL) was added and the solvents were concentrated to half-volume under reduced pressure, and then a solution of 0.1 M $Na_2S_2O_3$ in saturated aqueous $NaHCO_3$ (500 mL) was added and the mixture was stirred until colorless. The mixture was extracted with CH_2Cl_2, and the organic layer was separated and then washed with 5% citric acid, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was separated twice by silica gel column chromatography (0.5−2% EtOH in CH_2Cl_2) to give **32** (3.33 g, 76%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, J = 8.6 Hz, 2H), 7.95 (br s, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.70 (br s, 1H), 7.65 (t, J = 7.2 Hz, 1H), 7.55 (t, J = 8.0 Hz, 2H), 6.98 (d, J = 8.6 Hz, 2H), 6.72 (br s, 1H), 5.90 (br s, 1H), 3.91 (s, 3H), 3.87 (s, 2H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 484.

1-[4′-Azido-4-*N*-5′-*O*-dibenzoyl-3′-*O*-(4-methoxybenzoyl)-2′-dideoxy-2′,2′-difluoro-β-D-ribofuranosyl)cytosine (33). Compound **32** (3.33 g, 5.1 mmol) was dissolved in DMF (43 mL), together with 15-crown-5 (1.72 mL, 8.67 mmol) and sodium benzoate (1.25 g, 8.67 mmol), and the mixture was stirred for 65 h at 60 °C. Further sodium benzoate (2.5 g, 17.34 mmol) and 15-crown-5 (3.4 mL, 17.34 mmol) were added, and the mixture was stirred for 5 d. As the reaction was not complete, further quantities of sodium benzoate (1.25 g, 8.67 mmol), 15-crown-5 (1.72 mL, 8.67 mmol), and DMF (5 mL) were added. Stirring continued for a further 4 d. The thick suspension was filtered through celite and the filtrate evaporated to dryness under reduced pressure. The residue was treated with EtOAc, and the mixture was extracted with 5% aqueous $NaHCO_3$. The organic layer was separated, washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Purification by silica gel column chromatography (20−40% EtOAc in hexanes) provided **33** as an off-white solid (1.46 g, 44%). ^{1}H NMR ($CDCl_3$): δ 8.12−7.43 and 6.97−6.95 (m, 16 H), 6.77 (m, 1H), 5.86 (m, 1H), 4.87 (s, 2H), 3.89 (s, 3H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 647.

4′-Azido-2′-dideoxy-2′,2′-difluoro-β-D-ribofuranosyl)cytosine (34). Compound **33** (1.46 g, 2.26 mmol) was dissolved in dioxane (10 mL) and then saturated NH_3 in methanol (150 mL) was added and the mixture was stirred for 16 h at room temperature. The residue after evaporation of the solvents was subjected to purification by silica gel column chromatography (5−20% EtOH in CH_2Cl_2) and by semipreparative Hyper Carb HPLC column chromatography (10−90% CH_3CN in H_2O) to give **34** as a white solid (0.548 g, 80%); mp 143.0−144.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58 (d, J = 7.6 Hz, 1H), 7.46 (s, 2H), 6.73 (br s, 1H), 6.30 (br s, 1H), 5.81 (br s, 1H), 5.80 (d, J = 7.6 Hz, 1H), 4.50 (br s, 1H), 3.80 (s, 2H). MS (ES$^+$) [M + H]$^+$ *m*/*z* 305. HRMS (ES$^+$) calcd for $C_9H_{11}F_2N_6O_4$ [M + H]$^+$ 305.0810; found 305.0812.

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Supporting Information Available: Biology methods, analytical methods, and HPLC spectral data for compounds **10**, **17**, **20**, **28**, and **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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