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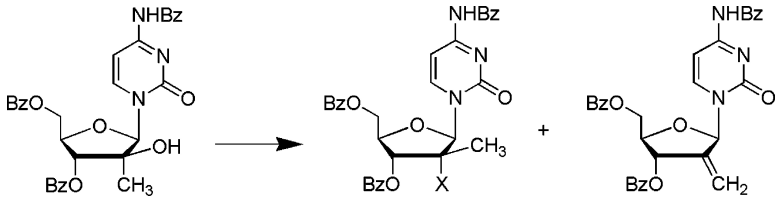

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Design, Synthesis, and Antiviral Activity of 2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine, a Potent Inhibitor of Hepatitis C Virus Replication

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The pyrimidine nucleoside beta-D-2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**1**) was designed as a hepatitis C virus RNA-dependent RNA polymerase (HCV RdRp) inhibitor. The title compound was obtained by a DAST fluorination of N^4-benzoyl-1-(2-methyl-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl]cytosine (**6**) to provide N^4-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl-β-D-ribofuranosyl]cytosine (**7a**). The protected 2′-*C*-methylcytidine (**7c**) was obtained as a byproduct from the DAST fluorination and allowed for the preparation of two biologically active compounds from a common precursor. Compound **1** and 2′-*C*-methylcytidine were assayed in a subgenomic HCV replicon assay system and found to be potent and selective inhibitors of HCV replication. Compound **1** shows increased inhibitory activity in the HCV replicon assay compared to 2′-*C*-methylcytidine and low cellular toxicity.

Introduction

Hepatitis C virus infection is a major health problem that leads to chronic liver disease, such as cirrhosis and hepatocellular carcinoma, in a substantial number of infected individuals. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to 20% of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The current standard of care for chronic hepatitis C is combination therapy with an interferon-α and ribavirin. Studies have shown that more patients with hepatitis C respond to pegylated interferon-α/ribavirin combination therapy than to combination therapy with unpegylated interferon-α. The overall response rate to treatment, defined as loss of HCV from serum 6 months after completion of treatment, is 40%. Because of the low response rates as well as toxic side effects and unsustained viral load reductions, these therapies are inadequate. Moreover, there is no established vaccine for HCV, and there is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection.[1]

The nonstructural protein NS5B has been characterized as an RNA-dependent RNA polymerase (RdRp) that is required for viral replication. This polymerase is considered to be an essential component in the HCV replication complex and therefore is an ideal target for drug discovery. Recently, several 2′-modified nucleoside analogues with potent inhibitory activity against the HCV NS5B polymerase have been identified. Among the most potent compounds in this class are 2′-deoxy-2′-fluorocytidine (2′-FdCyd)[2] and 2′-*C*-methyl nucleosides (Figure 1).[3−5] Despite the potent HCV inhibition of 2′-FdCyd, its therapeutic potential as an antiviral agent is diminished due to a lack of selectivity between host cells and the viral target. 2′-FdCyd triphosphate has been demonstrated to be a substrate for both RNA and DNA polymerases.[6,7] Here we describe the synthesis and biological activity of 2′-deoxy-2′fluoro-2′-*C*-methyl cytidine (**1**) as a potent anti-HCV agent.

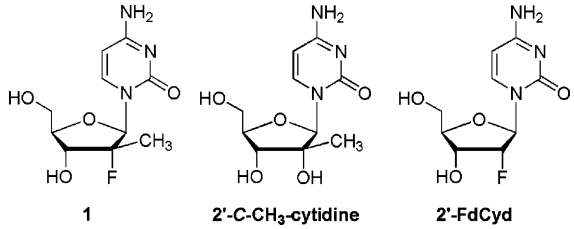

Figure 1. Structures of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**1**), 2′-*C*-methylcytidine, and 2′-deoxy-2′-fluorocytidine (2′-FdCyd).

Chemistry

For the synthesis of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**1**), N^4-benzoyl-1-(2-methyl-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl]cytosine (**6**) was chosen as the key intermediate and was prepared in approximately 20% yield in six steps from cytidine (Scheme 1).[8] Briefly, selective benzoylation of cytidine with benzoic anhydride in DMF,[9] followed by treatment with $TIDPSCl_2$ in pyridine, afforded N^4-benzoyl-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)cytidine (**2**).[10] Oxidation of the 2′-alcohol to the 2′-ketone derivative (**3**) was achieved with

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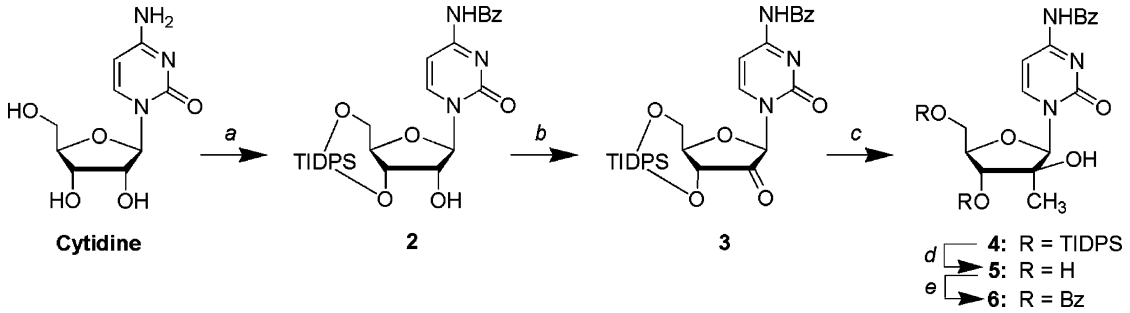
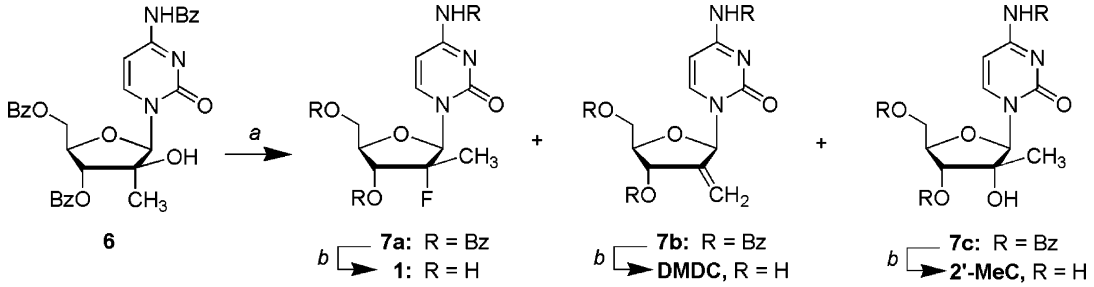
Scheme 1[a]



[a] Reagents and conditions: (a) (i) Bz_2O, DMF, room temp, (ii) $TIDPSCl_2$, DMF; (b) DMSO, TFAA, TEA, −15 °C; (c) MeLi, −78 °C; (d) 1 M TBAF, concd HOAc, rt; (e) BzCl, pyridine, rt.

Scheme 2[a]



[a] Reagents and conditions: (*a*) DAST, toluene, −20 °C to rt; (*b*) $MeOH/NH_3$, rt.

Table 1. Flourine-Coupled ^1H and ^{13}C NMR Chemical Shifts, Multiplicities, and *J* Values for Compounds **1**, **7a**, **8**, and **9**[a]

compd	$2CH_3$	H-1/C-1	C-2	H-3/C-3
1	δ 1.17, d, $^3J_{H-F} = 22.3$	δ 6.07, d, $^3J_{H-F} = 18.9$	δ 101.2, d, $^1J_{C-F} = 180.1$	overlapping mult.
	δ 16.6, d, $^2J_{C-F} = 25.9$	δ 88.6, d, $^2J_{C-F} = 37.4$		δ 70.5, d, $^2J_{C-F} = 18.3$
7a	δ 1.49, d, $^3J_{H-F} = 22.4$	δ 6.52, d, $^3J_{H-F} = 18.0$	δ 100.2, d, $^1J_{C-F} = 187.7$	δ 5.56, dd, $^3J_{H-F} = 20.7$
	δ 17.4, d, $^2J_{C-F} = 25.2$	δ 91.2, d, $^2J_{C-F} = 42.0$		δ 72.4, d, $^2J_{C-F} = 16.0$
8	δ 1.39, d, $^3J_{H-F} = 22.3$	δ 6.17, d, $^3J_{H-F} = 19.3$	δ 99.9, d, $^1J_{C-F} = 186.2$	δ 5.49, d, $^3J_{H-F} = 21.2$
	δ 17.3, d, $^2J_{C-F} = 25.1$	δ 90.7, d, $^2J_{C-F} = 44.2$		δ 72.7, d, $^2J_{C-F} = 16.1$
9	δ 1.35, d, $^3J_{H-F} = 22.3$	δ 6.13, d, $^3J_{H-F} = 18.9$	δ 102.1, d, $^1J_{C-F} = 180.1$	overlapping mult.
	δ 16.9, d, $^2J_{C-F} = 25.2$	δ 90.6, d, $^2J_{C-F} = 44.0$		δ 72.5, d, $^2J_{C-F} = 17.6$

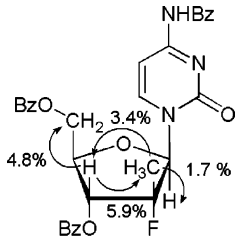
[a] NMR spectra were recorded at 30 °C (400 MHz) in DMSO-d_6 for compound **1**, $CDCl_3$ for compound **7a,** CD_3OD + $CDCl_3$ for **8**, and CD_3OD for **9** with concentrations of ~40 mg/0.75 mL. *J* values are in Hz.

trifluoroacetic anhydride/DMSO under Swern oxidation conditions.[11] Purification of compound **3** by silica gel chromatography followed by crystallization from petroleum ether−CH_2Cl_2 provided a white solid that was stable when stored at room temperature with minimal atmospheric exposure.[12] Treatment of the 2′-ketone (**3**) with methyllithium at −78 °C in diethyl ether gave exclusively the protected 1-[2-*C*-methyl-3,5-O−(tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]cytosine (**4**).[13] The 3′,5′-silyl protecting group was removed with TBAF/acetic acid and replaced with benzoyl protecting groups to provide compound **6**.

The fluorination of tertiary alcohols using DAST has been reported, but the stereochemistry of such transformations is substrate-specific and often unpredictable. For instance, Yang et al. reported that the DAST fluorination of a tertiary alcohol in 2-bromomethyl-DL-*myo*-inositol proceeds with retention of configuration.[14] Wachtmeister et al. obtained a 4-fluoro-1-cyclopentanol containing a tertiary fluorine in 25% yield using DAST as a fluorinating reagent, and this transformation proceeded with inversion of configuration.[15] Furthermore, dehydrations or eliminations, rearrangements, and ring contractions are often pervading problems in the DAST fluorination of highly functionalized molecules.[16]

Upon treating **6** with DAST in toluene or dichloromethane, a clean mixture of three products (**7a**−**7c**) in 15−20% yield for each compound was obtained (Scheme 2). The desired transformation of **6** to N^4-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl]cytosine (**7a**) proceeded with inversion of configuration and was stereospecific; no diastereomeric N^4-benzoyl-1-[2-fluoro-2-methyl-3,5-di-*O*-benzoyl-β-D-arabinofuranosyl]cytosine was detected in the crude reaction mixture. The presence of the tertiary fluorine at the 2′ position in **7a** was confirmed by the ^1H and ^{13}C NMR multiplicities and coupling constants (Table 1), whereas the stereochemistry of the fluorination was determined by nuclear Overhauser enhancement ^1H NMR difference spectroscopy (Figure 2).

Analysis of the ^1H NMR spectrum of compound **7a** revealed three distinct multiplicities due to H−F coupling: a doublet at δ 1.49 (2′-CH_3), a doublet of doublets at δ 5.56 (H-3′), and a doublet at δ 6.52 (H-1′). Irradiation of the H-3′ resonance resulted in a relatively large enhancement of both the H-5′ signal (4.8%) and the 2′-methyl signal (5.9%), while irradiation of the 2′-methyl signal resulted in an enhancement of both H-3′ (3.4%) and, to a lesser extent, H-1′ (1.7%). Deprotection of **7a** using methanolic ammonia provided the title compound,



7a

Figure 2. ^{1}H NMR NOE correlations of compound **7a**.

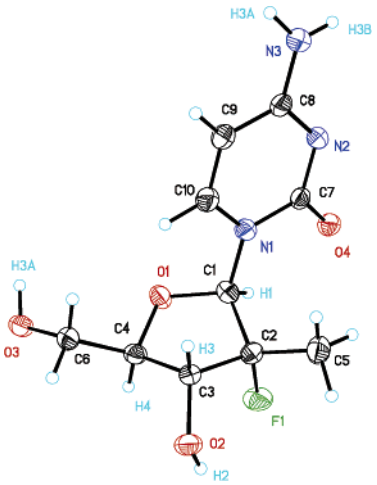
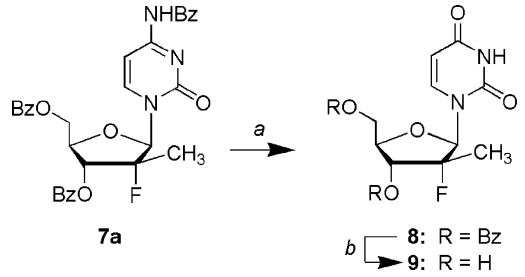


Figure 3. ORTEP drawing of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**1**).

Scheme 3[a]



[a] Reagents and conditions: (*a*) 80% HOAc, reflux; (*b*) MeOH/NH_3, rt.

1, whose structure was unambiguously confirmed by X-ray crystallography and revealed the expected 3′-endo conformation (Figure 3).

The degradation enzymes cytidine deaminase (CDA) and deoxycytidine monophosphate deaminase (dCMP-DA) are responsible for the in vivo metabolic conversion of cytidine or cytidine monophosphate to uridine. To facilitate future in vivo studies of compound **1**, 2′-deoxy-2′-fluoro-2′-*C*-methyluridine (**9**) was prepared from **7a** by deamination in refluxing 80% acetic acid followed by debenzoylation using methanolic ammonia (Scheme 3).

Results and Discussion

The novel pyrimidine nucleoside analogues 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine (**1**) and 2′-deoxy-2′-fluoro-2′-*C*-methyluridine (**9**) were tested for anti-HCV activity in both a cell-based quantitative real-time RT-PCR assay and surrogate bovine viral diarrhea virus (BVDV) assays as previously described (Table 2).[17] The activity and cytotoxicity profiles of 2′-*C*-methylcytidine and 2′-deoxy-2′-fluorocytidine (2′-FdCyd) are included for comparison and indicate that compound **1** demonstrated a similar potency as 2′-FdCyd in the HCV replicon assay. Dynamic profiling of the cell growth in this replicon assay revealed no cytostasis for compound **1** at the HCV replicon EC_{90} value. As previously reported, 2′-FdCyd, although not cytotoxic, induced cytostasis at the EC_{90} value.[2] Additionally, much like 2′-FdCyd, but unlike 2′-*C*-MeCyd, compound **1** was inactive in the BVDV assays. Compound **9** demonstrated no activity or cytoxicity in any assay.

Table 2. Anti-HCV Activity and Cellular Toxicity of Compounds **1**, **9**, 2′-*C*-Methylcytidine (2′-*C*-MeCyd), and 2′-Deoxy-2′-fluorocytidine (2′-FdCyd)

	cpBVDV[a] (MDBK cells)		HCV replicon[b]	
compound	EC_{90} (μM)[b]	CC_{50} (μM)	EC_{90} (μM)	CC_{50}[c] (μM)
1	>100	>100	5.40 ± 2.6	>100
9	>100	>100	>100	>100
2-*C*-MeCyd	2.30 ± 0.1	>100	19.0 ± 5.7	>100
2-FdCyd	>100	>100	6.50 ± 1.6	>100

[a] cpBVDV = cytopathic BVDV. [b] 96 h, average of at least four experiments. [c] MTS CC_{50} was determined in a 4-day assay using the Celltiter 96 nonradioactive cell proliferation assay from Promega (Madison, WI).

Experimental Section

All reagents and anhydrous solvents were purchased from Aldrich or Acros and were used as received. ^{1}H, ^{19}F, and ^{13}C NMR spectra were obtained with a Varian Unity Plus 400 spectrometer at 400, 376, and 100 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts are reported as δ (ppm) downfield with respect to an internal standard of tetramethylsilane, while ^{19}F chemical shifts are reported downfield from an external standard of hexafluorobenzene. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter at the sodium D line (589 nm) in a 1-dm cell. Melting points were determined using an electrothermal digital melting point apparatus and are uncorrected. Atlantic Microlab, Inc. of Norcross, GA provided the elemental analysis.

***N*^{4}-Benzoyl-1-[2-*C*-methyl-3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*β*-D-arabinofuranosyl]cytosine (4).** Compound **3** (37.6 g, 64 mmol) was dissolved in anhydrous Et_2O (800 mL) under argon and cooled to −78 °C with stirring. To this solution was added MeLi (103 mL, 1.6 M in Et_2O) dropwise over 1 h. After stirring for an additional 2 h, the reaction mixture was quenched by dropwise addition of 1 M NH_4Cl (165 mL). Upon warming to room temperature, the mixture was diluted with EtOAc (600 mL) and H_2O (130 mL). The organic phase was separated, washed with H_2O (1 × 130 mL), dried (Na_2SO_4), and the concentrated to dryness to give a brown foam (42.5 g, >100%) that was used without further purification. An analytical sample was obtained by silica gel chromatography eluting with 2:1 Et_2O−petroleum ether: $[\alpha]^{25}_D$ +52.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.96−1.15 (m, 24H), 1.60 (s, 3H), 3.81 (dt, 1H, J = 1.9, 9.2 Hz), 4.02 (dd, 1H, J = 2.5, 13.7 Hz), 4.17−4.23 (m, 2H), 5.85 (s, 1H), 7.50−7.54 (m, 2H), 7.60−7.64 (m, 2H), 7.91 (d, 2H, J = 7.3 Hz), 8.38 (d, 1H, J = 7.3 Hz), 8.89 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 12.5, 13.0, 13.1, 13.6, 16.9, 17.1, 17.2, 17.4, 17.5, 17.6, 17.7, 20.9, 60.4, 72.9, 81.7, 91.2, 96.5, 127.8, 129.0, 133.2, 145.2, 156.6, 162.5, 166.7. Anal. Calcd ($C_{29}H_{45}N_3O_7Si_2$): C, 57.68; H, 7.51; N, 6.96. Found: C, 57.63; H, 7.55; N, 6.82.

***N*^{4}-Benzoyl-2′-*C*-methyl-*β*-D-arabinofuranosylcytidine (5).** Crude **4** (128.0 g, 0.212 mol) was dissolved in THF (1.28 L) and treated with glacial HOAc (23.0 mL, 0.401 mol). To this solution was added tetrabutylammonium fluoride (384 mL, 1 M in THF) at room temperature and stirred for 0.75 h. The mixture was treated with silica gel (750 g) and concentrated to dryness in vacuo, and the tan-colored residue was placed onto a silica gel column. Eluting with 1:7 EtOH−CH_2Cl_2 afforded a waxy solid that was preadsorbed onto silica gel

(300 g) and chromatographed as before to give an off-white solid (46.4 g, 61%). Crystallization from aqueous acetone afforded an analytical sample: mp 197−200 °C; $[\alpha]^{22}_D$ +132.0° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.20 (s, 3H), 3.62−3.69 (m, 2H), 3.73−3.78 (m, 2H), 5.19 (t, 1H, *J* = 5.4 Hz), 5.25 (s, 1H), 5.52 (d, 1H, *J* = 5.0 Hz), 5.99 (s, 1H), 7.32 (d, 1H, *J* = 5.8 Hz), 7.50 (Ψt, 2H, *J* = 7.7 Hz), 7.62 (Ψt, 1H, *J* = 7.3 Hz), 8.00 (d, 2H, *J* = 7.3 Hz), 8.14 (d, 1H, *J* = 6.9 Hz), 11.22 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 19.6, 61.3, 77.5, 78.5, 85.2, 88.9, 95.2, 128.5, 132.8, 133.3, 147.3, 154.9, 162.9, 167.4. Anal. Calcd ($C_{17}H_{19}N_3O_6$·0.5H_2O): C, 55.14; H, 5.41; N, 11.35. Found: C, 55.21; H, 5.47; N, 11.33.

***N*^{4}-Benzoyl-1-(2-*C*-methyl-3,5-di-*O*-benzoyl-*β*-D-arabino-furanosyl]cytosine (6).** Compound **5** (46.0 g, 0.127 mol) was dissolved in anhydrous pyridine (200 mL) and the solvent was removed in vacuo. The resulting syrup was dissolved in anhydrous pyridine, cooled to 0 °C under argon with stirring, and treated with BzCl (30.0 mL, 0.250 mol) dropwise over 10 min. After the addition was complete, the ice bath was removed and stirring was continued for 1.5 h. Water (5 mL) was added and the mixture was concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ (1 × 500 mL) and water (1 × 500 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to dryness to afford a syrup that was purified by silica gel chromatography eluting with 1:1 EtOAc−hexanes. Compound **6** was isolated as an off-white solid (48.5 g, 67%): $[\alpha]^{22}_D$ +9.6° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.64 (s, 3H), 4.50 (m, 1H), 4.78−4.85 (m, 2H), 5.50 (d, 1H, *J* = 3.4 Hz), 6.42 (s, 1H), 7.44−7.54 (m, 7H), 7.57−7.66 (m, 3H), 7.94 (d, 2H, *J* = 7.8 Hz), 8.05−8.09 (m, 4H), 8.21 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR ($CDCl_3$) δ 19.9, 64.4, 79.2, 80.6, 81.6, 90.3, 96.7, 128.1, 128.6, 128.8, 128.9, 129.5, 129.9, 130.0, 133.2, 133.5, 134.0, 147.5, 156.0, 162.7, 165.9, 166.6. Anal. Calcd ($C_{31}H_{27}N_3O_8$): C, 65.37; H, 4.78; N, 7.38. Found: C, 65.59; H, 4.79; N, 7.16.

Reaction of 6 with DAST. To a stirred solution of **6** (2.96 g, 5.20 mmol) in anhydrous toluene (50 mL) was added DAST (1.0 mL, 7.8 mmol) at −20 °C under argon. After the addition was complete, the cooling bath was removed and stirring was continued for 1 h. The reaction mixture was poured into saturated $NaHCO_3$ (50 mL) and washed until gas evolution ceased. The organic phase was dried (Na_2SO_4), concentrated to dryness, and purified by silica gel chromatography eluting with 1:1:1 EtOAc−$CHCl_3$−hexanes to afford compound **7a** (0.55 g, 19%) as a white solid, followed by compound **7b** (0.39 g, 14%) as an off-white solid. Elution was continued with 1:1:1 EtOH−EtOAc−$CHCl_3$−hexanes to afford compound **7c** (0.451 g, 15%) as an off-white solid. Analytical samples were obtained by recrystallization from the indicated solvents.

***N*^{4}-Benzoyl-3′,5′-di-*O*-benzoyl-2′-fluoro-2′-*C*-methyl-cytidine (7a)**: mp 241 °C (CH_2Cl_2−hexanes); $[\alpha]^{22}_D$ +82.0° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.49 (d, 3H, *J* = 22.4 Hz), 4.64 (dd, 1H, *J* = 3.44, 12.9 Hz), 4.73 (d, 1H, *J* = 9.5 Hz), 4.90 (dd, 1H, *J* = 2.4, 12.7 Hz), 5.56 (dd, 1H, *J* = 8.6, 20.7 Hz), 6.52 (d, 1H, *J* = 18.0 Hz), 7.47−7.57 (m, 7H), 7.62−7.71 (m, 3H), 7.89 (d, 2H, *J* = 6.9 Hz), 8.07−8.11 (m, 5H), 8.67 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 17.4 (d, *J* = 25.2 Hz), 62.1, 72.4 (d, *J* = 16.0 Hz), 77.7, 91.2 (d, *J* = 42.0 Hz), 97.5, 100.2 (d, *J* = 187.7 Hz), 127.8, 128.6, 128.8, 128.9, 129.2, 129.6, 129.7, 130.3, 133.2, 133.4, 133.8, 134.1, 143.8, 154.6, 162.6, 165.6, 166.1; ^{19}F NMR ($CDCl_3$) δ 3.9 (m). Anal. Calcd ($C_{31}H_{26}FN_3O_7$·0.7H_2O): C, 63.74; H, 4.73; N, 7.19. Found: C, 63.71; H, 4.54; N, 7.20.

***N*^{4}-Benzoyl-1-[2-deoxy-2-methylidene-3,5-di-*O*-benzoyl-*β*-D-*glycero*-pentofuranosyl]cytosine (7b)**: mp 173.4−174.4 (EtOH); $[\alpha]^{22}_D$ −40.4° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.58 (dd, 1H, *J* = 3.7, 5.0 Hz), 4.70−4.81 (m, 2H), 5.55 (s, 1H), 6.09−6.11 (m, 1H), 7.03 (d, 1H, *J* = 1.3 Hz), 7.40−7.66 (m, 10H), 7.85 (d, 1H, *J* = 7.3 Hz), 7.91 (d, 2H, *J* = 7.7 Hz), 8.03 (dd, 2H, *J* = 0.9, 8.3 Hz), 8.03 (dd, 2H), 8.86 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 63.9, 73.3, 80.7, 85.9, 97.8, 117.3, 127.8, 128.0, 128.7, 128.9, 129.1, 129.4, 129.6, 129.9, 130.2, 133.0, 133.6, 133.8, 144.1, 144.9, 155.1, 162.5, 165.9, 166.1. Anal. Calcd ($C_{31}H_{25}N_3O_7$): C, 67.51; H, 4.57; N, 7.62. Found: C, 67.21; H, 4.51; N, 7.66

***N*^{4}-Benzoyl-2′-*C*-methyl-3′,5′-di-*O*-benzoylcytidine (7c)**: mp 176.7−179.1 °C (EtOH); $[\alpha]^{22}_D$ +46.2° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3H), 4.67−4.80 (m, 3H), 4.82−4.87 (m, 1H), 5.30 (d, 1H, *J* = 5.8 Hz), 6.09 (s, 1H), 7.47−7.56 (m, 4H), 7.89 (d, 2H, *J* = 7.3 Hz), 8.07−8.14 (m, 4H), 8.68 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.4, 62.7, 75.8, 78.5, 79.0, 93.4, 97.3, 127.8, 128.5, 128.7, 128.9, 129.0, 129.4, 129.6, 130.0, 132.9, 133.2, 133.6, 144.3, 156.0, 162.8, 165.8 166.2, 166.8. Anal. Calcd ($C_{31}H_{27}N_3O_8$·0.4H_2O): C, 64.56; H, 4.86; N, 7.29. Found: C, 64.54; H, 4.81; N, 7.32.

3′,5′-Di-*O*-benzoyl-2′-deoxy-2′-fluoro-2′-methyluridine (8). Compound **7a** (0.225 g, 0.394 mmol) was suspended in 80% aqueous HOAc (15 mL) and heated under reflux with stirring for 12 h. The clear solution was cooled, concentrated to dryness in vacuo, and coevaporated with 50% MeOH−water (3 × 5 mL) to remove the residual HOAc. Purification by silica gel chromatography, eluting with 2% EtOH−CH_2Cl_2, gave 0.160 g of **8** (87%) as a white solid. Crystallization from 2-propanol afforded an analytical sample: mp 256.4−257.6 °C; $[\alpha]^{22}_D$ +71.7° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 1.39 (d, 3H, *J* = 22.3 Hz), 4.49 (dd, 1H, *J* = 3.9, 12.7 Hz), 4.57 (m, 1H,), 4.79 (dd, 1H, *J* = 2.7, 12.5 Hz), 5.42 (d, 1H, *J* = 8.1 Hz), 5.49 (dd, 1H, *J* = 9.20, 21.2 Hz), 6.17 (d, 1H, *J* = 19.3), 7.37−7.50 (m, 4H), 7.51−7.57 (m, 3H, H-6) 7.93−8.01 (m, 4H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 17.3 (d, *J* = 25.1 Hz), 62.1, 72.7 (d, *J* = 16.1 Hz), 90.7 (d, *J* = 44.2 Hz), 99.9 (d, *J* = 186.2 Hz), 103.1, 128.5, 128.6, 128.7, 129.4, 129.5, 130.1, 133.6, 134.0, 139.3, 150.4, 163.2, 165.7, 166.1; ^{19}F NMR ($CDCl_3$ + CD_3OD) δ 6.02 (m). Anal. Calcd ($C_{24}H_{21}FN_2O_7$): C, 61.54; H, 4.52; N, 5.98. Found: C, 61.42; H, 4.51; N, 5.96.

General Procedure for Deprotection. The free nucleosides were prepared by treating compounds **7a**−**c** and **8** with NH_3/MeOH (ca. 7 N, ∼12 mL/mmol) followed by stirring at room temperature overnight (8−12 h). The solvent was removed in vacuo, and the compounds were isolated as indicated.

2′-Deoxy-2′-fluoro-2′-*C*-methylcytidine (1). Compound **7a** (6.30 g, 0.011 mol) was deprotected to give **1** (2.18 g, 76%) as a white powder after column chromatography eluting with 9% EtOH in $CHCl_3$ and then 17% EtOH and finally 25% EtOH in $CHCl_3$: mp 216.4−218.0 °C (EtOH); $[\alpha]^{22}_D$ +125.6° (*c* 1, H_2O); ^{1}H NMR (DMSO-d_6) δ 1.17 (d, 3H, *J* = 22.3 Hz), 3.63 (dd, 1H, *J* = 2.7, 13.7 Hz), 3.70−3.84 (m, 3H), 5.24 (app s, 1H), 5.60 (d, 1H, *J* = 5.4 Hz), 5.74 (d, 1H, *J* = 7.71 Hz), 6.07 (d, 1H, *J* = 18.9 Hz), 7.31 (s, 1H, NH_2), 7.42 (s, 1H, NH_2), 7.90 (d, 1H, *J* = 7.3 Hz); ^{13}C NMR (DMSO-d_6) δ 16.6 (d, *J* = 25.9 Hz), 58.5, 70.5 (d, *J* = 18.3 Hz), 81.4, 88.6 (d, *J* = 37.4 Hz), 94.4, 101.2 (d, *J* = 180.1 Hz), 140.5, 154.8, 165.2; ^{19}F NMR (DMSO-d_6) δ 2.60 (m). Anal. Calcd ($C_{10}H_{14}FN_3O_4$·1.5H_2O): C, 41.96; H, 5.94; N, 14.69. Found: C, 42.24; H, 5.63; N, 14.54.

Compound **1** was converted to the HCl salt and crystallized from aqueous ethanol: mp 243 °C (dec); $[\alpha]^{22}_D$ +108.4° (*c* 1, H_2O); ^{1}H NMR (DMSO-d_6) δ 1.29 (d, 3H, *J* = 22.6 Hz), 3.65 (dd, 1H, *J* = 2.3, 12.7 Hz), 3.76−3.90 (m, 3H), 5.96 (d, 1H, *J* = 17.3 Hz), 6.15 (d, 1H, *J* = 7.9 Hz), 8.33 (d, 1H, *J* = 7.9 Hz), 8.69 (s, 1.5H), 9.78 (s, 1.5H); ^{13}C NMR (DMSO-d_6) δ 16.2 (d, *J* = 24.4 Hz), 58.2, 69.9 (d, *J* = 16.8 Hz), 82.1, 88.8 (d, *J* = 32.0 Hz), 94.6, 101.1 (d, *J* = 181.5 Hz), 143.2, 147.6, 159.6; ^{19}F NMR (DMSO-d_6) δ 1.69 (m). Anal. Calcd ($C_{10}H_{15}ClFN_3O_4$): C, 40.62; H, 5.11; N, 14.21. Found: C, 40.80; H, 5.09; N, 14.23.

2′-Deoxy-2′-fluoro-2′-*C*-methyluridine (9). Deprotection of **8** (0.120 g, 0.209 mmol) followed by column chromatography eluting with 5−10% acetone in diethyl ether provided **9** (0.054 g, 100%) as a white solid: mp 237.3−238.0 °C; $[\alpha]^{25}_D$ +83.2° (*c* 1, MeOH); ^{1}H NMR (CD_3OD) δ 1.35 (d, 3H, *J* = 22.3 Hz), 3.79 (dd, 1H, *J* = 2.1, 12.5 Hz), 3.94−4.02 (m, 3H, 5.70 (d, 1H, *J* = 8.1 Hz), 6.13 (d, 1H, *J* = 18.9 Hz), 8.09 (d, 1H); ^{13}C NMR (CD_3OD) δ 16.9 (d, *J* = 25.2 Hz), 60.1, 72.5 (d, *J* = 17.6 Hz), 83.5, 90.6 (d, *J* = 44.0 Hz), 102.1 (d, *J* = 180.1 Hz), 103.0, 142.0, 152.4, 166.0; ^{19}F NMR (CD_3OD) δ 4.07 (bs). Anal. Calcd ($C_{10}H_{13}FN_2O_5$): C, 46.16; H, 5.04; N, 10.77. Found: C, 45.96; H, 4.93; N, 10.49.

Isolation of 2′-*C*-methylcytidine. Compound **7c** (0.1 g, 0.176 mmol) upon deprotection and crystallization from MeOH

gave a white solid (0.032 g, 71%): mp 244.2−245.8 °C (lit.[13] mp 239.5−242 °C, lit.[18] mp 243−245 °C); $[\alpha]^{23}_D$ +135.7° (*c* 1, H_2O) {lit.[18] $[\alpha]_D$ +132° (*c* 0.5, H_2O), lit.[19] $[\alpha]^{20}_D$ +128° (*c* 1, H_2O)}; ^{1}H NMR (DMSO-d_6) δ 0.92 (s, 1H), 3.58−3.62 (m, 2H), 3.70−3.77 (m, 2H), 4.98 (s, 1H), 5.06 (d, 1H, J = 7.3 Hz), 5.11 (t, 1H, J = 5.0 Hz), 5.67 (d, 1H, J = 7.7 Hz), 5.87 (s, 1H), 7.11 (s, 1H), 7.17 (s, 1H), 7.94 (d, 1H, J = 7.2 Hz); ^{13}C NMR (DMSO-d_6) δ 20.0, 59.0, 71.8, 78.3, 81.8, 91.3, 93.6, 141.1, 155.5, 165.4.

Isolation of 2′-Deoxy-2′-methylidenecytidine (DMDC). Compound **7b** was deprotected and crystallized from H_2O: mp 190−194 °C (dec) (transition at 90−92 °C) (lit.[20] mp 89−90 °C); $[\alpha]^{22}_D$ −39.8° (*c* 1, H_2O); ^{1}H NMR (DMSO-d_6) δ 3.50−3.70 (m, 3H), 4.43 (broad t, 1H), 4.93 (t, 1H, J = 5.2 Hz), 5.13 (s, 1H), 5.29 (s, 1H), 5.61 (d, 1H, J = 6.4 Hz), 5.69 (d, 1H, J = 7.6 Hz), 6.51 (s, 1H), 7.20 (s, 1H), 7.23 (s, 1H), 7.47 (d, 1H, J = 7.6 Hz); ^{13}C NMR (DMSO-d_6) δ 60.5, 69.8, 84.1, 84.2, 94.6, 110.8, 142.0, 151.1, 155.3, 165.5.

Biological Methods. Antiviral assays with bovine viral diarrhea virus and the HCV replicon were performed as described previously.[17]

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