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Use of 2′-Spirocyclic Ethers in HCV Nucleoside Design

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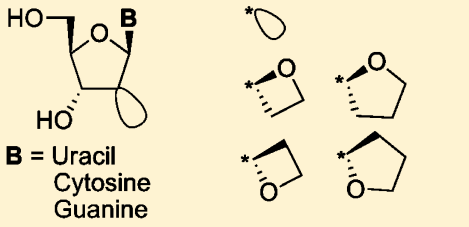
ABSTRACT: Conformationally restricted 2′-spironucleosides and their prodrugs were synthesized as potential anti-HCV agents. Although the replicon activity of the new agents containing pyrimidine bases was modest, the triphosphate of a 2′-oxetane cytidine analogue demonstrated potent intrinsic biochemical activity against the NS5B polymerase, with IC_{50} = 8.48 μM. Activity against NS5B bearing the S282T mutation was reduced. Phosphoramidate prodrugs of a 2′-oxetane 2-amino-6-*O*-methyl-purine nucleoside demonstrated potent anti-HCV activity in vitro, and the corresponding triphosphate retained similar potent activity against both wild-type and S282T HCV NS5B polymerase.



INTRODUCTION

Approximately 180 million individuals are infected with hepatitis C virus (HCV) worldwide.[1] Options comprising pegylated interferon-α (PEG-IFN) in combination with ribavirin (RBV) had been the treatment of choice for HCV infection for many years; however, this treatment had demonstrated limited efficacy and generally intolerable side effects.[2,3] Two newly approved drugs, Telaprevir and Boceprevir, have demonstrated improved efficacy in combination with PEG-IFN and RBV, but their use is associated with added side effects including rash, resistance development, and complex treatment regimens. In addition, these new agents are only approved for treating genotype 1 HCV-infected patients, thus excluding a large patient population infected with genotypes 2 to 6 virus. Therefore, there is still an urgent need to develop alternative direct acting antiviral agents (DAAs) that are more efficacious, have an improved safety profile, a high barrier to resistance, and are pan-genotypic.[4,5]

The RNA-dependent RNA polymerase (RdRp)[6−8] is essential to viral replication and thus represents a valid target for therapeutic intervention by design of specific inhibitors. Nucleoside inhibitors (NIs) of HCV polymerase are attractive due to their high potency and high generic barrier to resistance.[9] Several classes have been identified as potent anti-HCV agents in vitro, including the β-D-2′-deoxy-2′-α-F-2′-β-*C*-methyl-*ribo*-nucleosides,[10] the β-D-2′-β-*C*-methyl-*ribo*-nucleosides,[11,12] the 4′-azido-nucleosides,[13] and the 2′-*O*-methyl-nucleosides.[14] These nucleosides or their phosphate prodrugs have demonstrated broad genotype coverage. Among them, sofosbuvir (GS-7977)[15] demonstrated promising anti-HCV efficacy in vitro and in humans, and a new drug application has been filed recently in the U.S. In the same class, **I**, a prodrug of 2′-α-F-2′-β-*C*-methyl-guanosine, also showed potent anti-HCV activity with excellent safety and resistance profiles (Figure 1).[16] The superior anti-HCV efficacy, pan-genotypic coverage, and high genetic barrier to viral resistance of nucleosides makes

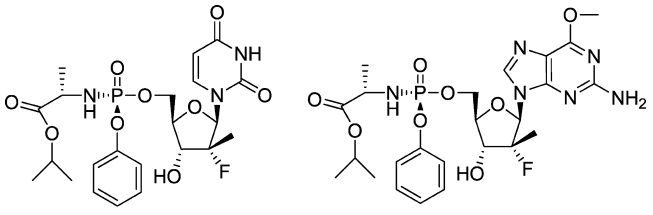


Figure 1. Prodrugs of β-D-2′-α-F-2′-β-*C*-methyl-nuclesides.

them of great interest in the search for novel analogues or their phosphate prodrugs.

As a part of our effort to identify novel nucleoside NS5B inhibitors, we explored 2′-spiro analogues (Figure 2). This investigation was based on the hypothesis that 2′-disubstituted nucleosides such as 2′-α-F-2′-β-*C*-methylcytidine (**II**),[10] 2′-methyl cytidine (**III**),[11,12] and the 2′-monosubstituted nucleoside 2′-*O*-methyl-cytidine[14] are active as inhibitors of the NS5B polymerase, and therefore, sufficient space might exist within the NS5B active-site to accommodate an additional atom especially if it resides within a constrained ring system. In addition, the 2′-*O*-methylcytidine derivative showed that a 2′-α oriented ether substituent was able to confer NS5B inhibitory activity. Further support for this hypothesis was obtained when the low energy conformations of both **II** and its 2′-spiro oxetane derivative were compared (Figure 3). Overlay of these low energy conformations demonstrated that they exhibited identical ring conformations and afforded good alignment of their respective 2′-substituents. We herein report the synthesis

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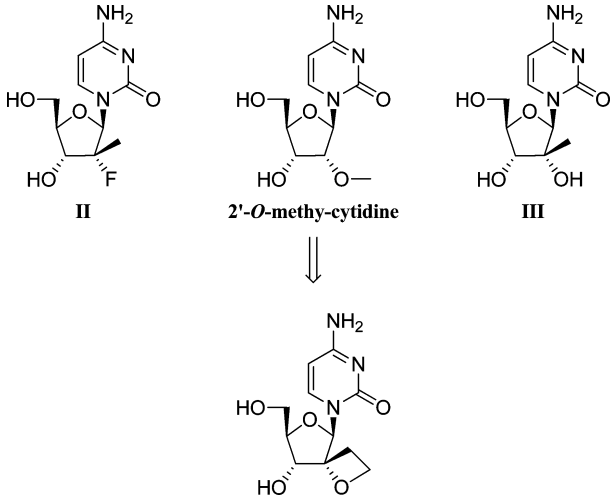

Figure 2. Design of 2′-spironucleosides.

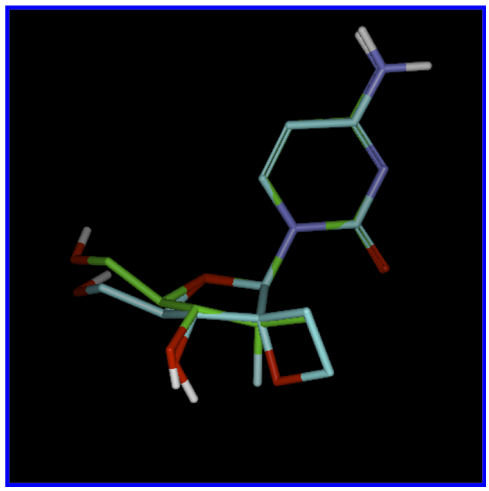

Figure 3. Superposition of the structures of the crystallographically determined compound **II** (green C's) and modeled 2′-oxetane cytidine (cyan C's).

and biological evaluation of 2′-spironucleosides and their phosphate prodrugs as anti-HCV agents.

CHEMISTRY

2′-Spirouridine analogues **2a−d** were synthesized from the corresponding protected intermediates **1a−d** prepared according to literature methods.[17] The ara-configuration of **1a** and **1b** was established stereoselectively by reaction of allylmagnesium chloride with 3′,5′-TIPS-protected 2′-oxouridine, while **1c** and **1d** having the ribo-configuration were prepared by coupling of 2-*β*-allyl-1,2,3,5-tetrabenzoyl-D-ribofuranoside with silylated uracil in the presence of $SnCl_4$. 2′-Spirocytidine analogues **4a−d** were also prepared from **1a−d** by treatment with TsCl/Et_3N in the presence of 4-dimethylaminopyridine (DMAP), followed by amination with ammonium hydroxide and desilylation with TBAF (Scheme 1).

The preparation of nucleotide phosphoramidate derivatives is depicted in Scheme 2 and relied upon coupling with reagents already bearing the desired amino acid ester and phenol substituents. Reaction of uridine analogues **2a−d** with **X1** (as a diastereomeric mixture at P, prepared in situ by the reaction of phenyl phosphorus dichloride with the requisite amino acid methyl ester in the presence of triethylamine) in the presence of *N*-methylimidazole (NMI) gave the corresponding prodrugs **5a−d** as mixtures of diasteromers. A diastereomerically pure phosphoramidate prodrug **6c** was prepared from **4c** and the homochiral phosphorus reagent (**X2**)[18] in the presence of *t*-butylmagnesium chloride. Nucleoside triphosphates **7b−c** and **8a−d** from **2b**, **2c**, and **4a−d** were prepared by reaction with phosphoryl chloride followed by treatment of the resulting intermediate with tributylammonium pyrophosphate in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DTP) (Scheme 2).

2′-Oxetane 2-amino-6-*O*-methyl-purine nucleoside **17** was prepared from 2-*C*-allyl-1,2,3,5-tetrabenzoyl-riboside (Scheme 3). Coupling of the sugar **9**[17] with 2-amino-6-chloropurine in the presence of DBN/TMSOTf gave exclusively the *β*-anomer **10**.[19] Installation of the desired purine 6-substituent and global deprotection was achieved in a single step by treatment with sodium methoxide. Selective protection of **11** followed by installation of the 2′-oxetane ring[17] and deprotection provided the target nucleoside **17**.

The free guanosine nucleoside **18** was prepared from **17** by treatment with adenosine deaminase (ADA) in water. Compound **18** was then converted to its 5′-triphosphate **19** by the method used for the preparation of compound **7b**. Phosphoramidate prodrugs of **17** were synthesized as diastereomeric mixtures by the method used for the preparation of compounds **5a−d**. A series of diastereomerically pure phosphoramidate prodrugs (**20b−o**) were obtained by SFC separation.[18] For the assignment of stereochemistry at phosphorus, the stereospecific method used for the preparation of **6c** was applied with **X2** of known absolute stereochemistry[18] to generate the single *Sp* isomer **20c**. The ^{31}P NMR resonance frequency of **20c** appeared at higher field (4.16 ppm) than that (4.74 ppm) of its *Rp* diastereomer **20d**, which is consistent with similar assignments in the literature.[20] The absolute stereochemistry at phosphorus in other diastereomers were assigned by analogy.

BIOLOGICAL ACTIVITY

The anti-HCV activities of the uridine **2a−d** and cytidine analogues **4a−d** were evaluated in the genotype 1b replicon assay (Table 1).[21] Among them, compounds **2b** and **4c** displayed weak activity, with the rest inactive when tested up to 100 *μ*M concentration.

Nucleosides must be phosphorylated in cells to their corresponding nucleoside triphosphates by kinases to exert their biological activity. Phosphoramidate prodrugs of nucleosides have been shown to deliver the nucleoside monophosphate directly into tissues, particularly into the liver, bypassing the rate-limiting first step of phosphorylation.[15] The phosphoramidate prodrugs **5a−d** and **6c** of the nucleosides **2a−d** and **4c** were prepared but resulted in modest levels of cellular activity (Table 2), suggesting that inefficient initial phosphorylation in the first step is not the primary reason for the weak activity of these pyrimidine nucleosides and their prodrugs.

The triphosphates of a range of the parent nucleosides were also prepared and their intrinsic activity against wild-type and S282T NS5B polymerase compared to that of **II** (Table 3). The 2′-oxetane uridine triphosphate **7c** demonstrated measurable inhibition of the wild-type enzyme. The intrinsic activity of the triphosphates (**8a−8d**) of each of the possible 4- and 5-membered spirocyclic cytidine variants **4a−d** was also

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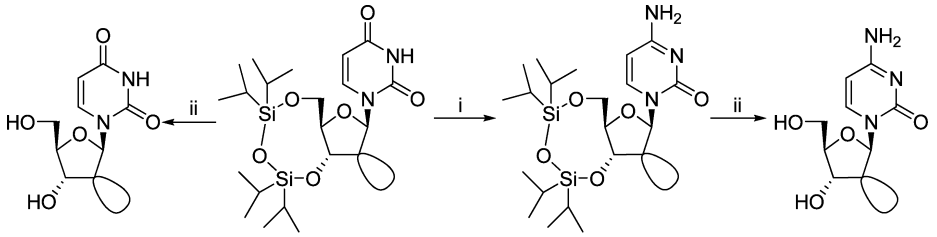
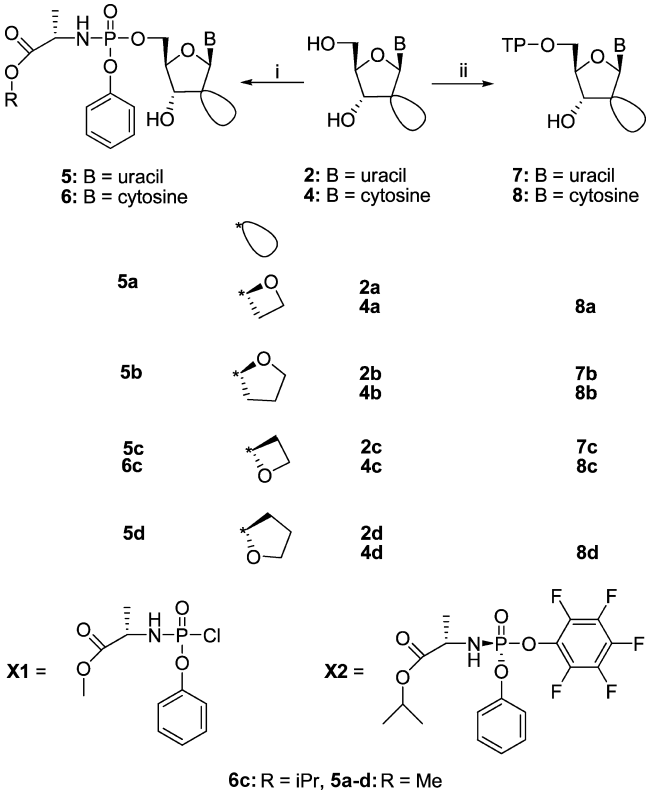
Scheme 1. Preparation of Pyrimidine Nucleosides[a]



2a	**1a**		**3a**	**4a**
2b	**1b**		**3b**	**4b**
2c	**1c**		**3c**	**4c**
2d	**1d**		**3d**	**4d**

[a]Reaction and conditions: (i) (1) TsCl/Et_3N/DMAP, (2) NH_4OH; (ii) TBAF.

Scheme 2. Preparation of Phosphoramidates and Triphosphates[a]



[a]Reaction and conditions: (i) **X1**/NMI for **5a−d** and **X2**/*t*-BuMgCl for **6C**; (ii) $POCl_3$/Bu_3N/Bu_3N·pyrophosphate/DTP.

evaluated, with the *ribo*-analogues **8c** and **8d** exhibiting greater activity than their *xylo*-counterparts. The activity of the 2′-oxetane analogue **8c** was greater than that of the 2′-tetrahydrofuran **8d** and was comparable to that of the 2′-*α*-F-2′-*C*-methyl cytidine derivative **II**. Both triphosphates **8c** and **8d** showed a similar resistance profile to the triphosphate of **II**, in that significant potency was lost against the S282T mutant enzyme.[10]

A good resistance profile is desirable for the next generation of nucleoside or nucleotide anti-HCV agents. Because phosphate prodrugs of 2′-*α*-F-2′-*β*-*C*-methylguanosine analogues such as compound **I** (PSI-353661) and PSI-352938 have demonstrated excellent resistant profiles,[16] we next turned to explore 2′-oxetane guanosine analogues. Although the 2′-oxetane-2-amino-6-*O*-methyl-purine **17** and 2′-oxetane guanosine (**18**) nucleosides were inactive in the whole cell replicon assay up to 100 μM (data not shown), it was encouraging that the triphosphate **19** of guanosine analogue **18** demonstrated comparable potencies against wild-type NS5B polymerase and its S282T mutant, like 2′-F-2′-*β*-*C*-methylguanosine (**I**, Table 4).

To optimize cellular potency in this series, a variety of prodrugs were prepared (Table 5). Most of the prodrugs demonstrated moderate activity without significant cytotoxicity up to 20 μM. The *n*-butyl ester **20e** demonstrated the best anti-HCV potency, with EC_{50} of 0.27 μM. Generally, as in the GS-7977 series, prodrugs with *Sp*-chirality on the phosphorus demonstrated greater anti-HCV potency than those with *Rp*,[15] but the activities of both isomers of the cyclopentyl esters (**20i** and **20j**) and benzyl esters (**20n** and **20o**) were similar. An enhancement in activity was observed when the phenyl group in **20k** was replaced with naphthyl group in **20p**. Nevertheless, even the most active compound in the series was still significantly less potent than compound **I**.

CONCLUSION

We have synthesized and evaluated 2′-spironucleosides and their prodrugs as potential anti-HCV agents. The triphosphate of 2′-oxetane cytidine demonstrated comparable intrinsic

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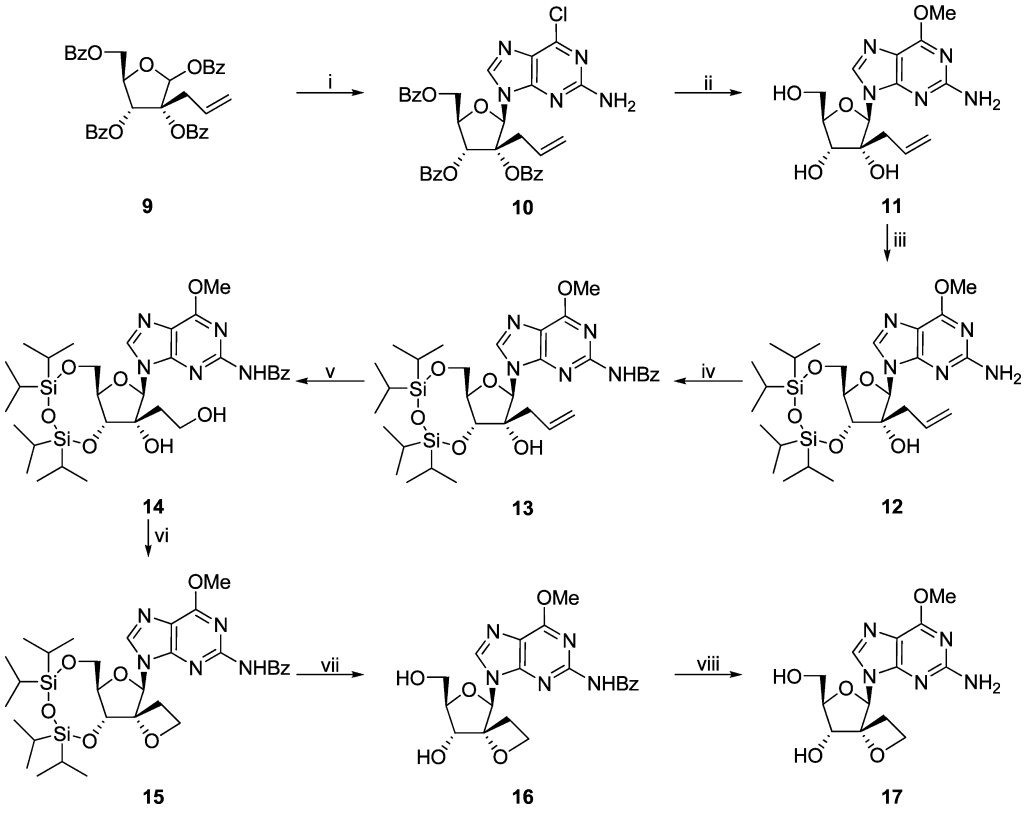
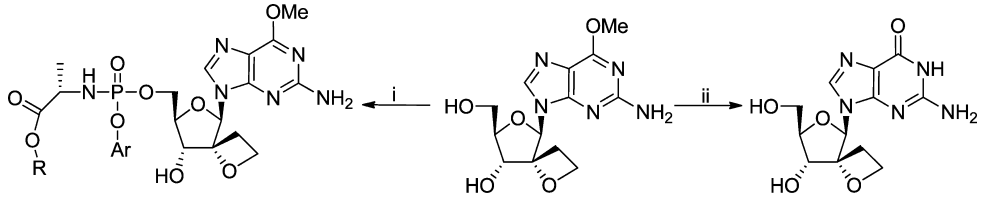
Scheme 3. Preparation of 2′-Oxetane 2-Amino-6-*O*-methyl-purine Nucleoside[a]



[a]Reaction and conditions: (i) 2-amino-6-chloropurine/DBN/TMSOTf/ACN; (ii) NaOMe/MeOH; (iii) $TIPSCl_2$/Pyr; (iv) BzCl/Pyr; (v) (1) OsO_4/NMO/tBuOH/H_2O, (2) $NaIO_4$/THF, (3) $NaBH_4$/EtOH/EtOAc; (vi) (1) MsCl/Pyr, (2) NaH/THF; (vii) NH_4F/MeOH; (viii) NaOMe/MeOH.

Scheme 4. Preparation of 2′-Oxetane Guanosine and its Prodrugs[a]



20a: R = Me, Ar = Ph (*Sp*/*Rp*)
20b: R = Et, Ar = Ph (*Sp*)
20c: R = iPr, Ar = Ph (*Sp*)
20d: R = iPr, Ar = Ph (*Rp*)
20e: R = n-Bu, Ar = Ph (*Sp*)
20f: R = n-Bu, Ar = Ph (*Rp*)
20g: R = i-Bu, Ar = Ph (*Sp*)
20h: R = i-Bu, Ar = Ph (*Rp*)
20i: R = c-pentyl, Ar = Ph (*Sp*)
20j: R = c-pentyl, Ar = Ph (*Rp*)
20k: R = neopentyl, Ar = Ph (*Sp*)
20m: R = neopentyl, Ar = Ph (*Rp*)
20n: R = Bn, Ar = Ph (*Sp*)
20o: R = Bn, Ar = Ph (*Rp*)
20p: R = neopentyl, Ar = naphthyl (*Sp*/*Rp*)

[a]Reagents and conditions: (i) phosphorus agent/NMI; (ii) ADA/H_2O; (iii) $POCl_3$/Bu_3N/Bu_3N·pyrophosphate/DTP.

activity against NS5B polymerase to that of compound **II**. For the 2′-oxetane cytidine analogues activity against NS5B bearing the S282T amino acid change was reduced, as was observed for other pyrimidine anti-HCV nucleosides or their phosphate prodrugs. However, the triphosphate of 2′-oxetane-guanosine, like those of the 2′-*α*-F-2′-*β*-*C*-methylguanosine series, retained comparable activity against both S282T and wild-type NS5B. Although 2′-oxetane guanosine analogues did not show significant replicon activity, their phosphoramidate prodrugs demonstrated potent anti-HCV activity in vitro and an excellent resistance profile.

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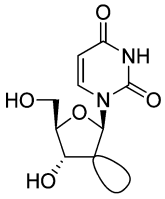
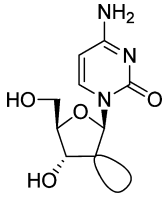
Table 1. Anti-HCV Activity and Cytotoxicity of Pyrimidine Nucleosides



2a-d



4a-d

compound		[a]EC_{50} (μM)	CC_{50} (μM)
2a		>100	>100
2b		14.9	>100
2c		>100	>100
2d		>100	>100
4a		>100	>100
4b		>100	>100
4c		56.6	>100
4d		>100	>100

[a]Effective concentration at which 50% inhibition occurs as determined by luciferase based genotype 1b replicon assays in Lunet cells.

EXPERIMENTAL SECTION

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker advance II 400 MHz and a Varian Unity Plus 400 MHz spectrometers at room temperature, with tetramethylsilane as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). Purities of the final compounds were determined by HPLC and were greater 95%. HPLC conditions to assess purity were as follows: Shimadzu HPLC 20AB Sepax HP-C18 4.6 mm × 50 mm (5 μm); flow rate, 3.0 mL/min; acquisition time, 6 min; wavelength, UV 220 nm; oven temperature, 40 °C. Chiral HPLC was conducted on Shimadzu HPLC 20A. The preparative HPLC system includes two sets of Gilson 306 pumps, a Gilson 156UV/vis detector, and a Gilson 215 injector and fraction collector, with Unipoint control software. A YMC 25 mm × 30 mm × 2 mm column was used. The mobile phase was HPLC grade water (A) and HPLC grade acetonitrile (B) system. SFC was conducted on Berger Multi-Gram SFC from Mettler Toledo Co, Ltd. LC/MS was conducted on Shimadzu LCMS 2010EV using electrospray positive [ES + ve to give MH^+] equipped with a Shim-pack XR-ODS 2.2 μm column (3.0 mm × 30 mm, 3.0 mm i.d.), eluting with 0.0375% TFA in water (solvent A) and 0.01875% TFA in acetonitrile solvent B). High-resolution mass spectra were obtained on a Agilent G1969a spectrometer.

General Procedure for the Preparation of Compounds 2a–d. To a solution of compound **1a**[17] (300 mg, 0.59 mmol) in anhydrous THF (10 L) was added $Et_3N{\cdot}3HF$ (0.15 mL), and the reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated to dryness under reduced pressure, and the residue was purified by flash silica gel column chromatography (0–15% MeOH in CH_2Cl_2) to give compound **2a** (61.26 mg, 38.8%).

2a: δ_H (DMSO-d_6): 11.42 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 6.08 (s, 1H), 5.87 (d, J = 5.2 Hz, 1H), 5.60 (d, J = 8.0 Hz, 1H), 5.04–5.06 (m, 1H), 4.30–4.35 (m, 1H), 4.19–4.24 (m, 1H), 3.95–3.98 (m, 1H), 3.50–3.61 (m, 3H), 3.01–3.08 (m, 1H), 2.39–2.45 (m, 1H). HRMS (TOF-ESI): calcd for $C_{11}H_{15}N_2O_6$, 271.0925; found, 271.0917.

2b: δ_H (DMSO-d_6): 11.32 (s, 1H), 7.80 (d, J = 8.0 Hz, 1H), 5.83 (s, 1H), 5.63 (d, J = 4.2 Hz, 1H), 5.59 (d, J = 8.0 Hz, 1H), 5.03–5.05 (m, 1H), 3.83–3.86 (m, 1H), 3.64–3.70 (m, 3H), 3.47–3.60 (m, 2H), 2.27–2.29 (m, 1H), 1.74–1.81 (m, 3H). HRMS (TOF-ESI): calcd for $C_{12}H_{17}N_2O_6$, 285.1087; found, 285.1070.

2c: δ_H (CD_3OD): 7.93 (d, J = 8.0 Hz, 1H), 6.17 (s, 1H), 5.67 (d, J = 8.0 Hz, 1H), 4.53 (m, 2H), 3.95 (m, 2H), 3.72 (m, 2H), 2.60 (m, 2H). LC-MS (ESI): 270 $[M + H]^+$.

2d: δ_H (CD_3OD): 8.09 (d, J = 8.4 Hz, 1H), 5.91 (s, 1H), 5.68 (d, J = 8.4 Hz, 1H), 3.90 (m, 6H), 1.95 (m, 4H). LC-MS (ESI): 284 $[M + H]^+$.

General Procedure for the Preparation of Cytidine Analogues 4a–d. To a solution of compound **1d** (0.08 g, 0.14 mmol) in MeCN (10 mL) was added DMAP (0.02 g, 0.14 mmol) and Et_3N (0.07 g, 0.71 mmol) followed by addition of TsCl (0.08 g, 0.43 mmol). The solution was stirred at room temperature for 1 h. To the solution was added NH_4OH (30%, 2 mL), and the mixture was stirred at room temperature for 1 h. Solvent was evaporated to dryness, and the residue was coevaporated with toluene twice to give crude cytosine analogue, which was dissolved in CH_2Cl_2 (10 mL) and pyridine (1 mL). To the solution was added BzCl (0.1 mL, 0.86 mmol), and the solution was stirred at room temperature for 2 h. Water (5 mL) was added, and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in EtOAc (100 mL), and the solution was washed with water and brine and dried over Na_2SO_4. Solvent was evaporated, and the residue was purified by flash silica gel column chromatography (0–60% EtOAc in hexanes) to give *N*-benzoylcytosine analogue, which was dissolved in THF (10 mL). To the solution was added TBAF (0.12 g, 0.48 mmol), and the solution was stirred at room temperature for 1 h. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0–8% MeOH in CH_2Cl_2) to give *N*-benzoyl nucleoside, which was dissolved in 7N NH_3 in MeOH (8 mL). The solution was stirred at room temperature for 20 h. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0–30% MeOH in CH_2Cl_2) to give product **4d** (0.02 g, 56% from **1d**).

4a: δ_H (DMSO-d_6): 7.55 (d, J = 7.2 Hz, 1H), 7.12–7.20 (m, 2H), 6.16 (s, 1H), 5.76 (d, J = 5.2 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 4.91–4.94 (m, 1H), 4.24–4.29 (m, 1H), 4.06–4.11 (m, 1H), 3.93–3.96 (m, 1H), 3.46–3.63 (m, 3H), 2.87–2.94 (m, 1H), 2.42–2.47 (m, 1H). LC-MS(ESI): m/z 269.9 $[M + 1]^+$. HRMS (TOF-ESI): calcd For $C_{11}H_{16}N_3O_5$, 270.1084; found, 270.1081.

4b: δ_H (DMSO-d_6): 7.65 (d, J = 7.2 Hz, 1H), 7.05–7.19 (m, 2H), 5.98 (s, 1H), 5.68 (d, J = 7.2 Hz, 1H), 5.57 (d, J = 5.6 Hz, 1H), 4.86–4.92 (m, 1H), 3.74–3.77 (m, 1H), 3.54–3.70 (m, 4H), 3.35–3.38 (m, 1H), 2.17–2.24 (m, 1H), 1.66–1.85 (m, 3H). LC-MS (ESI): m/z 283.9 $[M + 1]^+$. HRMS(TOF-ESI): calcd for $C_{12}H_{18}N_3O_5$, 284.1241; found, 285.1235.

4c: δ_H (CD_3OD): 8.04 (d, J = 7.6 Hz, 1H), 6.26 (s, 1H), 5.87 (d, J = 7.6 Hz, 1H), 4.55 (m, 2H), 3.96 (m, 2H), 3.74 (m, 2H), 2.54 (m, 2H). LC-MS (ESI): 270 $[M + H]^+$.

4d: δ_H (CD_3OD): 8.09 (d, J = 7.6 Hz, 1H), 5.99 (s, 1H), 5.87 (d, J = 7.6 Hz, 1H), 3.95 (m, 6H), 2.80 (m, 4H). LC-MS (ESI): 284 $[M + H]^+$.

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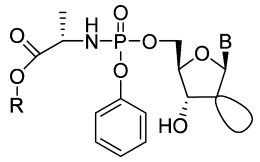
Table 2. Anti-HCV Activity and Cytotoxicity of Prodrugs of Pyrimidine Nucleosides



5: B = uridine
6: B = cytidine

compound		R	EC_{50} (μM)	CC_{50} (μM)
5a		Me	28.5	>100
5b		Me	20.6	>100
5c		Me	16.7	>100
5d		Me	28.3	>100
6c		iPr	16.7	>100

General Procedure for the Preparation of Prodrugs As a Mixtures of Diastereomers. To a precooled solution (−78 °C) of phenyl dichlorophosphate (2.1 g, 9.96 mmol) in CH_2Cl_2 (40 mL) was added L-alanine methyl ester hydrochloride (1.39 g, 9.96 mmol) followed by addition of Et_3N (2.02 g, 19.92 mmol) in CH_2Cl_2 (5 mL) slowly, and the mixture was stirred at −78 °C for 1 h then at room temperature for 16 h. Solvent was evaporated, and the residue was filtered with Et_2O (20 mL). Solvent was evaporated to dryness to give crude monochlorophosphate reagent, which was dissolved in CH_2Cl_2 (10 mL, 10.0 M) for the next reaction. To a mixture of compound **2a** (20.0 mg, 0.07 mmol) in CH_2Cl_2 (15 mL) were added *N*-methylimidazole (0.2 mL) and a solution of above monochlorophosphate reagent (0.5 mL, 0.50 mmol), and the resulting mixture was stirred at room temperature for 3 h. EtOAc (100 mL) was added and the mixture was washed with water, 1N HCl, aqueous $NaHCO_3$, and brine, sequentially. Organic solution was dried over Na_2SO_4 and evaporated, and the residue was purified by flash silica gel column chromatography (0−8% MeOH in CH_2Cl_2) to give compound **5a** (22 mg, 58.0%).

5a: δ_H ($CDCl_3$): 8.18 (s, 1H), 7.30 (m, 6H), 6.12 (ss, 1H), 5.62 (m, 1H), 4.07, 4.11, 3.80 (m, 8H), 3.74, 3.72 (ss, 3H), 3.17 (m, 1H), 2.60 (m, 1H), 1.37 (d, J = 7.2 Hz, 3H). ^{31}P NMR (162 MHz): 4.84, 4.26. LC-MS (ESI): 512 $[M + H]^+$.

5b: δ_H ($CDCl_3$): 8.75 (s, 1H), 7.60, 7.52 (dd, J = 8.0 Hz, 1H), 7.24 (m, 5H), 6.05, 6.04 (ss, 1H), 5.65, 5.58 (d, J = 8.0, 1H), 4.35 (m, 2H), 4.00 (m, 4H), 3.80 (m, 4H), 3.72, 3.70 (ss, 3H), 2.39 (m, 1H), 1.90 (m, 2H), 1.72 (m, 1H), 1.36 (m, 3H). ^{31}P NMR (162 MHz): 4.32, 4.01. LC-MS (ESI): 526 $[M + H]^+$.

5c: δ_H ($CDCl_3$): 9.15, 9.07 (ss, 1H), 7.26 (m, 7H), 6.19, 6.15 (ss, 1H), 5.65 (m, 1H), 4.50 (m, 4H), 3.95 (m, 4H), 3.72, 3.70 (ss, 3H), 3.42 (s, 1H), 2.75 (m, 1H), 2.46 (m, 1H), 1.35 (m, 3H). ^{31}P NMR (162 MHz): 4.21, 3.83. LC-MS (ESI): 512 $[M + H]^+$.

5d: δ_H ($CDCl_3$): 8.51, 8.40 (ss, 1H), 7.48, 7.42 (d, 8.0 Hz, 1H), 7.29 (m, 5H), 5.98 (s, 1H), 5.62 (m, 1H), 4.48 (m, 2H), 3.95 (m, 6H), 3.73, 3.72 (ss, 3H), 2.83 (m, 1H), 1.95 (m, 2H), 1.69 (m, 1H), 1.37 (m, 3H). ^{31}P NMR (162 MHz): 4.20, 3.79. LC-MS (ESI): 526 $[M + H]^+$.

Preparation of Prodrug 6c. To a suspension of nucleoside **4c** (50 mg, 0.45 mmol) and corresponding diastereomerically pure phosphoramidate reagent[18] (0.15 g, 0.33 mmol) in THF (5 mL) was added a solution of *t*-butylmagnesium chloride (1M, 0.45 mL, 0.45 mmol) in THF at 0 °C slowly. The reaction mixture was stirred at 0 °C for 3 h. EtOAc (100 mL) was added, and the solution was washed with aqueous NH_4Cl and brine and dried over Na_2SO_4. Solvent was removed and the residue was purified by flash silica gel column chromatography (0−5% MeOH in CH_2Cl_2) to give compound **6c** (40 mg, 40%). δ_H ($CDCl_3$): 7.11−7.43 (m, 6H), 6.18 (s, 1H), 5.76 (d, J = 7.6 Hz, 1H), 4.96 (m, 1H), 4.63 (m, 1H), 4.33−4.50 (m, 4H), 4.04 (m, 1H), 3.94 (m, 1H), 3.79 (m, 1H), 2.63, 2.43 (mm, 2H), 1.33 (d, J = 7.2 Hz, 3H), 1.24 (d, J = 6.4 Hz, 6H). ^{31}P NMR (162 MHz): 4.03. LC-MS (ESI): 539 $[M + H]^+$.

Preparation of Nucleoside Triphosphates. Triphosphates (**7b**, **7c** and **8a−d** and others) of the nucleosides were prepared by Nublocks, LLC, San Diego, CA.

Preparation of 6-Chloropurine Nucleoside 10. To a precooled solution (0 °C) of compound **9**[17] (4.00 g, 6.59 mmol) and 6-chloroguanine (1.68 g, 9.89 mmol) in MeCN (80 mL) were added DBN (2.46 g, 19.78 mmol) then TMSOTf (5.86 g, 26.38 mmol), and the solution was heated at 65 °C for 5 h then room temperature for 16 h. The solution was poured into a mixture of EtOAc (300 mL) and excess $NaHCO_3$ with ice. Organic solution was washed with $NaHCO_3$ and brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (5−60% EtOAc in hexanes) to give compound **10** (3.20 g, 74%). δ_H (CD_3OD): 8.18−7.25 (m, 16 Hz), 6.73 (s, 1H), 5.40 (m, 3H), 5.12 (m, 2H), 4.82 (m, 1H), 4.74 (m, 3H), 3.04 (m, 1H), 2.52 (m, 1H). LC-MS (ESI): 654 $[M + H]^+$.

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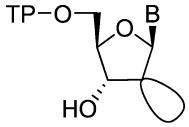
Table 3. Anti-HCV Activity of the Nucleoside Triphosphates against NS5B Polymerase and Its S282T Mutant



7: B = uracil
8: B = cytosine

triphosphate		IC_{50} (NS5B)	IC_{50} (S282T)
		(μM)	(μM)
7b		>100	NA
7c		39.4	>100
8a		>100	NA
8b		>100	NA
8c		8.48	>100
8d		45.3	>100
II		8.41	56.7

Table 4. Intrinsic Anti-HCV Activity of Triphosphate of Synthesized Guanosine Analogue against Wild-Type NS5B Polymerase and Its S282T Mutant

triphosphate	IC_{50} (wild type) (μM)	IC_{50} (S282T) (μM)
19	9.5	10.4
I-TP	4.2	5.31

Preparation of 6-*O*-Methylnucleoside 11. To a mixture of compound **10** (3.20 g, 4.89 mmol) in MeOH (80 mL) was added 25% NaOMe in MeOH (1.86 g, 48.92 mmol), and the solution was stirred at room temperature for 24 h. Solvent was evaporated, and the residue was purified by flash silica gel column chromatography (0−15% MeOH in CH_2Cl_2) to give compound **11** (1.33 g, 57%) as white solid. δ_H (CD_3OD): 8.09, 8.13 (s, 1H), 5.97 (s, 1H), 5.67 (m, 1H), 4.77 (m, 1H), 4.56 (m, 1H), 4.45 (d, J = 8.8 Hz, 1H), 4.13−3.83 (m, 6H), 2.25 (m, 1H), 2.05 (m, 1H). LC-MS (ESI): 338 $[M + H]^+$.

Preparation of Compound 13. To a solution of compound **11** (1.33 g, 3.94 mmol) in pyridine (20 mL) was added TIPSCl (1.37 g, 4.34 mmol) slowly, and the solution was stirred at room temperature for 16 h. Solvent was evaporated, and the residue was dissolved in EtOAc (400 mL). The organic solution was washed with brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−5% MeOH in CH_2Cl_2) to give intermediate **12** (1.30 g, 73%). δ_H (CD_3OD): 7.757 (s, 1H), 5.93 (s, 1H), 5.66 (m, 1H), 4.88 (m, 1H), 4.82 (s, 2H), 4.73 (d, J = 7.6 Hz, 1H), 4.64 (m, 1H), 4.20 (m, 1H), 4.08 (m, 7H), 2.20 (m, 2H), 1.07 (m, 28H). LC-MS (ESI): 450 $[M + H]^+$.

To a solution of the above intermediate in pyridine (10 mL) and CH_2Cl_2 (20 mL) was added benzoyl chloride (0.63 g, 4.48 mmol), and the solution was stirred at room temperature for 5 h. Water (10 mL) was added and the solution was evaporated to give a residue which was dissolved in EtOAc (200 mL). Organic solution was washed with brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−5% MeOH in CH_2Cl_2) to give compound **13** (1.50 g, 76%) as foam. δ_H (CD_3OD): 8.46 (s, 1H), 7.78 (m, 6H), 5.98 (s, 1H), 5.72 (m, 1H), 5.00 (d, J = 8.0 Hz, 1H), 4.83 (d, J = 10.4 Hz, 1H), 4.50 (m, 1H), 4.35 (m, 1H), 4.10 (m, 5H), 2.32 (m,1H), 2.20 (m, 1H), 1.05 (m, 28H). LC-MS (ESI): 684 $[M + H]^+$.

Preparation of Compound 14. To a mixture of compound **13** (0.30 g. 0.44 mmol) in THF (6 mL), *t*-butanol (6 mL) and water (1 mL) was added OsO_4 in *t*-butanol (0.25% 0.5 mL), followed by addition of 50% *N*-methylmorpholine *N*-oxide (NMO, 0.2 mL, 0.85 mmol). The solution was stirred at room temperature for 16 h. Solvent was evaporated and the residue was coevaporated with toluene twice to give diol as a mixture of diastereomers which was dissolved in THF (10 mL). To the solution was added water (1 mL) followed by addition of $NaIO_4$ (excess) portionwise until starting material disappeared at room temperature for 3 h. EtOAc (100 mL) was added, and the solution was washed with brine and dried over Na_2SO_4. Solvent was evaporated, and the residue was dissolved in a solution of EtOAc (10 mL) and EtOH (10 mL). To the precooled solution of the resulting aldehyde at 0 °C was added $NaBH_4$ (50.16 mg, 1.32 mmol), and the mixture was stirred at 0 °C for 1 h. EtOAc (100 mL) was added, and the organic solution was washed with brine and dried over Na_2SO_4. The solvent was evaporated to dryness, and the residue was purified by flash silica gel column chromatography (0−10% MeOH in CH_2Cl_2) to give compound **14** (0.14 g, 43% from **3**). δ_H ($CDCl_3$): 8.57 (s, 1H), 8.48 (s, 1H), 7.70 (m, 5H), 6.26 (s, 1H), 4.15 (m, 9H), 1.28 (m, 2H), 1.15 (m, 28H). LC-MS (ESI): 688 $[M + H]^+$.

Preparation of Compound 17. To a solution of compound **14** (0.33 g, 0.47 mmol) in CH_2Cl_2 (30 mL) and pyridine (3 mL) was added MsCl (0.11 g, 0.94 mmol), and the solution was stirred at room temperature for 3 h. Water (10 mL) was added, and the mixture was extracted with EtOAc (100 mL). The organic solution was washed with brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−100% EtOAc in hexanes) to give a mesylate (0.30 g, 82%). δ_H ($CDCl_3$): 8.53 (s, 1H), 8.21 (s, 1H), 7.65 (m, 5), 6.14 (s, 1H), 4.80 (s, 1H), 4.54 (m, 2H), 4.33 (m, 1H), 4.16 (s, 3H), 4.10 (m, 3H), 2.95 (s, 3H), 2.05 (m, 2H), 1.05 (m, 28H). LC-MS (ESI): 766 $[M + H]^+$.

To a solution of the mesylate (0.20 g, 0.26 mmol) in THF (10 mL) was added NaH (60% mineral oil, 110 mg, 2.75 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was poured into EtOAc (100 mL), and the solution was washed with brine and dried over Na_2SO_4. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−80% EtOAc in hexanes) to give 2′-oxetanyl intermediate **15** (0.15 g, 84%). δ_H ($CDCl_3$): 8.47 (s, 1H), 8.23 (s, 1H), 7.65 (m, 5H), 6.38 (s, 1H), 7.74 (m, 1H), 4.59 (m, 1H), 4.46 (d, J = 9.2 Hz, 1H), 4.26 (d, J = 13.2 Hz, 1H), 4.15 (s, 3H), 4.00 (m, 2H), 2.56 (m, 2H), 1.09 (m, 28H). LC-MS (ESI): 686 $[M + H]^+$.

To the solution of the 2′-oxetanyl intermediate in MeOH (10 mL) was added NH_4F (1.3 mmol, 46.8 mg), and the mixture was heated at 60 °C for 5 h. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−10% MeOH in CH_2Cl_2) to give compound **16** (0.05 g, 43% from **14**) as white solid. LC-MS (ESI): 428 $[M + H]^+$.

A solution of compound **16** (0.20 g, 0.45 mmol) in MeOH (10 mL) was added NaOMe (4.8 M, 0.8 mL), and the solution was stirred at room temperature for 20 h. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−15% MeOH in CH_2Cl_2) to give compound **17** (0.10 g, 69%). δ_H (CD_3OD): 815 (s, 1H), 6.26 (s, 1H), 4.50 (m, 3H), 4.05 (s, 3H),

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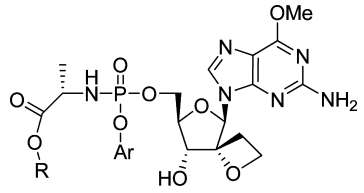
Table 5. Anti-HCV Activity and Cytotoxicity of Prodrugs of 6-*O*-Methylguanosine Analogues



compd	Ar	R	*Sp*/*Rp*	alogP	EC_{50} (1b) (μM)	CC_{50} (μM)
20a	Ph	Me	*Sp*/*Rp* = 1/1	0.27	7.16	>20
20b	Ph	Et	*Sp*	0.62	0.86	>20
20c	Ph	iPr	*Sp*	1.0	1.49	>20
20d	Ph	iPr	*Rp*	1.0	4.52	>20
20e	Ph	*n*-Bu	*Sp*	1.6	0.27	>20
20f	Ph	*n*-Bu	*Rp*	1.6	3.1	>20
20g	Ph	i-Bu	*Sp*	1.46	0.57	>20
20h	Ph	*i*-Bu	*Rp*	1.46	4.42	>20
20i	Ph	*c*-pentyl	*Sp*	1.67	0.45	>20
20j	Ph	*c*-pentyl	*Rp*	1.67	0.67	>20
20k	Ph	neopentyl	*Sp*	1.74	1.02	>20
20m	Ph	neopentyl	*Rp*	1.74	9.31	>20
20n	Ph	Bn	*Sp*	1.85	8.65	>20
20o	Ph	Bn	*Rp*	1.85	11.7	>20
20p	naphthyl	neopentyl	*Sp*/*Rp* = 1/1	2.64	0.31	>20
I	Ph	iPr	*Sp*	2.0	<0.0045	>100

3.96 (m, 1H), 3.80 (m, 2H), 2.57 (m, 1H), 2.27 (m, 1H). LC-MS (ESI): 324 $[M + H]^+$.

Preparation of Compound 18. To a solution of compound **17** (0.04 g, 0.12 mmol) in MOPS buffer (0.1 M, 10 mL) was added adenosine deaminase (2.0 mg), and the solution was kept at 37 °C for 2 days. Solvent was evaporated and the residue was purified by flash silica gel column chromatography (0−30% MeOH in CH_2Cl_2) to give a crude compound **18** containing MOPS. Crystalline MOPS was removed by filtration after heating the crude **18** in MeOH. The mother liquor containing **18** was concentrated to dryness and the residue was purified by flash silica gel column chromatography (0−30% MeOH in CH_2Cl_2) to give compound **18** as white solid (0.02 g, 53%). δ_H (CD_3OD): 8.04 (s, 1H), 6.21 (s, 1H), 4.54 (m, 2H), 4.36 (d, *J* = 8.8 Hz, 1H), 4.94 (m, 1H), 3,78 (m, 2H), 2.60 (m, 1H), 2,33 (m, 1H). LC-MS (ESI): 310 $[M + H]^+$.

Preparation of Triphosphate 19 Was Accomplished As for 7b and 8a−d. Diastereomerically pure **20c** was prepared by method used for compound **6c**. **20c**: δ_H ($CDCl_3$) 7.69 (s, 1H), 7.32−7.12 (m, 5H), 6.16 (s, 1H), 5.09 (s, 2H), 4.96 (m, 1H), 4.73 (dd, 1H, *J* = 8.8, 10.4 Hz), 4.61−4.53 (m, 3H), 4.33 (m, 1H), 4.06 (s, 3H), 3.98−3.90 (m, 2H), 3.70 (dd, 1H, *J* = 9.2, 11.2 Hz), 3.24 (d, 1H, *J* = 10.0 Hz), 2.68 (m, 1H), 2.17 (m, 1H), 1.30 (d, 3H, *J* = 7.2 Hz), 1.18 (2d, 6H, *J* = 6.4 Hz). ^{31}P NMR (162 MHz): 4.16. LC-MS (ESI): 593 $[M + H]^+$.

Phosphoramidate prodrugs (**20a** and **20p**) were prepared as diastereomeric mixtures from the corresponding amino acid esters according to method used for the preparation of compounds **5a−d**. Diastereomerically pure prodrugs (**20b−o**) were obtained by SFC separation of the mixtures. Samples of diastereomeric mixtures were subjected to SFC using a chiral column (2 × 15 cm) and eluted with 35% isopropyl alcohol in carbon dioxide at 100 bar. An injection loading of 4 mL of sample at a concentration of 17 mg/mL in methanol was used. According to the deastereomerically pure **20c**, the *Rp* isomer eluted first. The appropriate fractions of the multiple runs were combined and concentrated under reduced pressure to give product.

20a: δ_H ($CDCl_3$): 7.69, 7.61 (ss, 1H), 7.25 (m, 5H), 6.18 (ss, 1H), 5.08 (ss, 2H), 4.60 (m, 3H), 4.35 (m,1H), 4.06 (ss, 3H), 3.90 (m, 3H), 3.60 (ss, 3H), 3.35 (m, 1H), 2.66 (m, 1H), 2.18 (m, 1H), 1.32 (m, 3H). ^{31}P NMR (162 MHz): 4.61, 3.97. LC-MS (ESI): 565 $[M + H]^+$.

20b: δ_H ($CDCl_3$): 7.70 (s, 1H), 7.32−7.14 (m, 5H), 6.17 (s, 1H), 5.10 (s, 2m), 4.72 (t, 1H, *J* = 9.2 Hz), 4.59−4.54 (m, 3H), 4.35 (m, 1H), 4.15−4.09 (m, 5H), 4.00−3.91 (m, 2H), 3.77 (dd, 1H, *J* = 9.6, 11.2 Hz), 3.36 (d, 1H, *J* = 10.4 Hz), 2.67 (m, 1H), 2.18 (m, 1h), 1.31 (d, 3H, *J* = 7.2 Hz), 1.20 (t, 3H, *J* = 7.2 Hz). ^{31}P NMR (162 MHz): 4.08. LC-MS (ESI): 579 $[M + H]^+$.

20d: δ_H ($CDCl_3$): 7.62 (s, 1H), 7.32−7.12 (m, 5H), 6.20 (s, 1H), 5.11 (s, 2H), 4.97 (m, 1H), 4.6−4.53 (m, 4H), 4.38 (m, 1H), 4.07 (s, 3H), 3.99−3.92 (m, 2H), 3.85 (t, 1H, *J* = 11.2 Hz), 3.61 (br s, 1H), 2.62 (m, 1H), 2.15 (m, 1H), 1.98 (bs, 1H), 1.29 (d, 3H, *J* = 6.8 Hz), 1.20 (t, 3H, *J* = 6.4 Hz). ^{31}P NMR (162 MHz): 4.74. LC-MS (ESI): 593 $[M + H]^+$.

20e: δ_H ($CDCl_3$): 7.70 (s, 1H), 7.32−7.12 (m, 5H), 6.17 (s, 1H), 5.11 (s, 2H), 4.71 (t, 1H, *J* = 6.8 Hz), 4.59−4.52 (m, 3H), 4.35 (m, 1H), 4.11−3.91 (m, 7H), 3.81 (dd, 1H, *J* = 9.6, 11.6 Hz), 3.41 (d, 1H, *J* = 10.0 Hz), 2.67 (m, 1H), 2.17 (m, 1H), 1.54 (m, 2H), 1.36−1.27 (m, 5H), 0.87 (t, 3H, *J* = 7.6 Hz). ^{31}P NMR (162 MHz): 4.12. LC-MS (ESI): 607 $[M + H]^+$.

20f: δ_H ($CDCl_3$): 7.62 (s, 1H), 7.33−7.13 (m, 5H), 6.20 (s, 1H), 5.07 (s, 2H), 4.65−4.52 (m, 4H), 4.37 (m, 1H), 4.12−3.88 (m, 6H), 3.93 (m, 1H), 3.79 (t, 1H, *J* = 11.2 Hz), 3.43 (br s, 1H), 2.63 (m, 1H), 2.15 (m, 1H), 1.57 (m, 2H), 1.38−1.29 (m, 5H), 0.96 (t, 3H, *J* = 7.2 Hz). ^{31}P NMR (162 MHz): 4.70. LC-MS (ESI): 607 $[M + H]^+$.

20g: δ_H ($CDCl_3$): 7.71 (s, 1H), 7.31−7.12 (m, 5H), 6.18 (s, 1H), 5.14 (s, 2H), 4.7 (t, 1H, *J* = 8.8 Hz), 4.60−4.53 (m, 3H), 4.35 (m, 1H), 4.05 (s, 3H), 4.05−3.85 (m, 4H), 3.78 (dd, 1H, *J* = 6.8, 10.4 Hz), 3.53 (d, 1H, *J* = 9.6 Hz), 2.66 (m, 1H), 2.17 (m, 1H), 1.87 (m, 1H), 1.33 (d, 3H, *J* = 6.8 Hz), 0.87 (d, 6H, *J* = 6.4 Hz). ^{31}P NMR (162 MHz): 4.15. LC-MS (ESI): 607 $[M + H]^+$.

20h δ_H ($CDCl_3$): 7.61 (s, 1H), 7.34−7.13 (m, 5H), 6.19 (s, 1H), 5.05 (s, 2H), 4.68−4.52 (m, 4H), 4.37 (m, 1H), 4.09−3.98 (m, 4H), 3.93−3.82 (m, 3H), 3.74 (t, 1H, *J* = 9.2 Hz), 3.34 (d, 1H, *J* = 9.6 Hz), 2.62 (m, 1H), 2.15 (m, 1H), 1.90 (m, 1H), 1.32 (d, 3H, *J* = 7.2 Hz), 0.89 (d, 6H, *J* = 6.4 Hz). ^{31}P NMR (162 MHz): 4.74. LC-MS (ESI): 607 $[M + H]^+$.

20i: δ_H ($CDCl_3$): 7.69 (s, 1H), 7.32−7.13 (m, 5H), 6.16 (s, 1H), 5.12 (m, 1H), 5.07 (s, 2H), 4.73 (m, 1H), 4.60−4.53 (m, 3H), 4.34 (m, 1H), 4.06 (s, 3H), 3.98−3.90 (m, 2H), 3.66 (dd, 1H, *J* = 9.6, 11.2 Hz), 3.17 (d, 1H), 2.68 (m, 1H), 2.17 (m, 1H), 1.80 (m, 2H), 1.68−1.52 (m, 6H), 1.29 (d, 3H, *J* = 6.8 Hz). ^{31}P NMR (162 MHz): 4.18. LC-MS (ESI): 619 $[M + H]^+$.

20j: δ_H ($CDCl_3$): 7.62 (s, 1H), 7.33−7.13 (m, 5H), 6.20 (s, 1H), 5.16−5.13 (m, 3H), 4.64−4.54 (m, 4H), 4.38 (m, 1H), 4.07 (s, 3H),

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3.99−3.91 (m, 2H), 3.83 (dd, 1H, J = 9.6, 11.6 Hz), 2.63 (m, 1H), 2.15 (m, 1H), 1.80 (m, 2H), 1.71−1.55 (m, 6H), 1.28 (d, 3H, J = 6.8 Hz). ^{31}P NMR (162 MHz): 4.76. LC-MS (ESI): 619 $[M + H]^+$.

20k: δ_H ($CDCl_3$): 7.69 (s, 1H), 7.32−7.12 (m, 5H), 6.16 (s, 1H), 5.07 (s, 2H), 4.72 (t, 1H, J = 10.0 Hz), 4.60−4.52 (m, 3H), 4.34 (m, 1H), 4.07−4.01 (m, 4H), 3.92 (m, 1H), 3.82 (d, 1H, J = 10.4 Hz), 3.70 (m, 1H), 3.15 (d, 1H, J = 10.0 Hz), 2.68 (m, 1H), 2.17 (m, 1H), 1.35 (d, 3H, J = 6.8 Hz), 0.89 (s, 9H). ^{31}P NMR (162 MHz): 4.11. LC-MS (ESI): 621 $[M + H]^+$.

20m: δ_H ($CDCl_3$): 7.62 (s, 1H), 7.34−7.13 (m, 5H), 6.19 (s, 1H), 5.07 (s, 2H), 4.66−4.52 (m, 4H), 4.38 (m, 1H), 4.11−4.03 (m, 4H), 3.93 (m, 1H), 3.85−3.72 (m, 3H), 3.42 (br s, 1H), 2.63 (m, 1H), 2.15 (m, 1H), 1.34 (d, 3H, J = 7.2 Hz), 0.91 (s, 9H). ^{31}P NMR (162 MHz): 4.76. LC-MS (ESI): 621 $[M + H]^+$.

20n: δ_H ($CDCl_3$): 7.73 (s, 1H), 7.37−7.11 (m, 10H), 6.17 (s, 1H), 5.09 (s, 2H), 5.07 (s, 2H), 4.75 (m, 1H), 4.58 (m, 3H), 4.40 (m, 1H), 4.09−4.01 (m, 4H), 3.92 (m, 1H), 3.74 (t, 1H, J = 2.6 Hz), 3.36 (br s, 1H), 2.68 (m, 1H), 2.19 (m, 1H), 1.28 (d, 3H, J = 7.2 Hz). ^{31}P NMR (162 MHz): 4.27. LC-MS (ESI): 641 $[M + H]^+$.

20o: δ_H ($CDCl_3$): 7.60 (s, 1H), 7.36−7.12 (m, 10H), 6.17 (s, 1H), 5.10 (d, 2H, J = 1.2 Hz), 5.05 (s, 2H), 4.66 (m, 1H), 4.62−4.51 (m, 3H), 4.34 (m, 1H), 4.13−4.03 (m, 4H), 3.89 (m, 1H), 3.74 (t, 1H J = 11.2 Hz), 3.31 (d, 1H, J = 7.6 Hz), 2.63 (m, 1H), 2.14 (m, 1H), 1.31 (d, 3H, J = 7.2 Hz). ^{31}P NMR (162 MHz): 4.64. LC-MS (ESI): 641 $[M + H]^+$.

20p: δ_H ($CDCl_3$): 8.10 (m, 1H), 7.82 (m, 1H), 7.69−7.63 (m, 2H), 7.49 (m, 3H), 7.36 (m, 1H), 6.15 (ds, 1H), 5.08 and 5.04 (s, 2H), 4.84−4.60 (m, 2H), 4.54 (t, 2H, J = 7.6 Hz), 4.40 (m, 1H), 4.11 (m, 1H), 4.04 (s, 3H), 3.93 (m, 2H), 3.79 (dd, 1H, J = 1.6, 10.8 Hz), 3.64 (dd, 1H, J = 6.4, 10.0 Hz), 3.37 (m, 1H), 2.66 (m, 1H), 2.16 (m, 1H), 1.31 and 1.28 (ss, 3H), 0.86 (s, 9H). ^{31}P NMR (162 MHz): 5.041, 4.47. LC-MS (ESI): 671 $[M + H]^+$.

Molecular Modeling. Initial models for a number of the 2′-spiro fused nucleosides were energy minimized to a convergence gradient of 0.001 using MMFFs[22] with a distance dependent dielectric model of 2r. Conformers were generated for each of these using torsional sampling (MCMM)[23] followed by energy minimization. An RMSD of 0.25 and a 20 kcal/mol energy window were used as criterion to limit the number of conformers output with an upper limit of 1000 being possible. None of the compounds resulted in more than 100 conformers given these limits (MacroModel, version 9.7, Schrödinger, LLC, New York, NY, 2009).

Biological Assays. *HCV Replicon Assays.* HCV replicon assays using ET-Lunet cells were performed as described previously.[21] Briefly, replicon-containing cells (3000 cells/well in a 96-well plate) were incubated for 4 days with serially diluted test compounds. Inhibition of HCV RNA replication was determined by measuring the levels of luminescence expressed via the firefly luciferase reporter gene using Bright-Glo reagents (Promega, Madison, WI). Effect of test compounds on the proliferation of ET-Lunet cells after 4 days incubation was determined by using Cell Titer-Glo (Promega, Madison, WI). The EC_{50} and CC_{50} values, the concentrations at which 50% inhibition were achieved, were determined using GraphPad Prism software (San Diego, CA).

NS5B Polymerase Assays. IC_{50} values were determined using recombinant HCV NS5B from wild-type or S282T genotype 1b NS5B. All reactions were performed in a 20 μL mixture containing varying concentrations of the test compound, 5 μM of the four natural ribonucleotides, [α-^{32}P]UTP, 20 ng/μL of genotype 1b (−) IRES RNA template, 1 unit/μL of SUPERase•In (Ambion, Austin, TX), 40 ng/μL of NS5B, 5 mM $MgCl_2$, and 2 mM DTT in 50 mM Hepes buffer (pH 7.5). The reaction was quenched by adding 80 μL of stock solution (12.5 mM EDTA, 2.25 M NaCl, and 225 mM sodium citrate) after incubating at 27 °C for 30 min. The radioactive RNA products were quantified as described previously.[16] The IC_{50} values were calculated using GraphFit program version 5 (Erithacus Software, Horley, Surrey, UK).

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Notes

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ABBREVIATIONS USED

TMSOTf, trimethylsilyl trifluoromethanesulfonate; SFC, supercritical fluid chromatography; MOPS, 3-(*N*-morpholino)-propanesulfonic acid

REFERENCES

(1) *Hepatitis C*; World Health Organization Fact Sheet No. 164;World Health Organization: Geneva, October 2000.

(2) Zeuzem, S.; Berg, T.; Moeller, B.; Hinrichsen, H.; Mauss, S.; Wedemeyer, H.; Sarrazin, C.; Hueppe, D.; Zehnter, E.; Manns, M. P. Expert opinion on the treatment of patients with chronic hepatitis C. *J. Viral Hepatitis* **2009**, *16*, 75−90.

(3) Fried, M. W.; Shiffman, M. L.; Reddy, K. R.; Smith, C.; Marinos, G.; Goncales, F. L.; Haussinger, D.; Diango, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* **2002**, *347*, 975−982.

(4) James, R.; Burton, M. D., Jr.; Gregory, T.; Everson, M. D. HCV NS5B polymerase inhibitors. *Clin. Liver Dis.* **2009**, *13*, 453−465.

(5) Beaulieu, P. L. Recent advances in the development of NS5B polymerase inhibitors for the treatment of hepatitis C virus. *Expert Opin. Ther. Pat.* **2009**, *19*, 145−164.

(6) Hwang, S. B.; Park, K. J.; Kim, Y. S.; Sung, Y. C.; Lai, M. M. Hepatitis C virus NS5B protein is a membrane-associated phosphoprotein with a predominantly perinuclear localization. *Virology* **1997**, *227*, 439−446.

(7) Behrens, S. E.; Tomei, L.; De Francesco, R. Identification and properties of the RNA-dependent RNA polymerase of hepatitis C virus. *EMBO J.* **1996**, *15*, 12−22.

(8) Takamizawa, A.; Mori, C.; Fuke, I.; Manabe, S.; Murakami, S.; Fujita, J.; Onishi, E.; Andoh, T.; Yoshida, I.; Okayama, H. Structure and organization of the hepatitis C virus genome isolated from human carriers. *J. Virol.* **1991**, *65*, 1105−1113.

(9) Brown, N. A. Progress towards improving antiviral therapy for hepatitis C with hepatitis C virus polymerase inhibitors. Part 1: Nucleoside analogues. *Expert Opin. Invest. Drugs* **2009**, *18*, 709−725.

(10) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. Design, synthesis, and antiviral activity of 2′-deoxy-2′-fluoro-2′-*C*-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med. Chem.* **2005**, *48*, 5504−5508.

(11) Pierra, C.; Amador, A.; Benzaria, S.; Cretton-Scott, E.; D'Amours, M.; Mao, J.; Mathieu, S.; Moussa, A.; Bridges, E. G.; Standring, D. N.; Sommadossi, J. P.; Storer, R.; Gosselin, G. Synthesis and pharmacokinetics of valopicitabine (NM283), an efficient prodrug of the potent anti-HCV agent 2′-*C*-methylcytidine. *J. Med. Chem.* **2006**, *49*, 6614−20.

(12) Bassit, L.; Grier, J.; Bennett, M.; Schinazi, R. F. Combinations of 2′-C-methylcytidine analogues with interferon-α2b and triple combination with ribavirin in the hepatitis C virus replicon system. *Antiviral Chem. Chemother.* **2008**, *19*, 25−31.
(13) Smith, D. B.; Kalayanov, G.; Sund, C.; Winqvist, A.; Maltseva, T.; Leveque, V. J.-P.; Rajyaguru, S.; Le Pogam, S.; Najera, I.; Benkestock, K.; Zhou, X.-X.; Kaiser, A. C.; Maag, H.; Cammack, N.; Martin, J. A.; Swallow, S.; Johansson, N. G.; Klumpp, K.; Smith, M. 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. *J. Med. Chem.* **2009**, *52*, 2971−2978.
(14) Carroll, S. S.; Tomassini, J. E.; Bosserman, M.; Getty, K.; Stahlhut, M. W.; Eldrup, A. B.; Bhat, B.; Hall, D.; Simcoe, A. I.; LaFemina, R.; Rutkowski, C. A.; Wolanski, B.; Yang, Z.; Migliaccio, G.; De Francesco, R.; Kuo, L. C.; MacCoss, M.; Olsen, D. B. Inhibition of hepatitis C virus RNA replication by 2′-modified nucleoside analogs. *J. Biol. Chem.* **2003**, *278*, 11979−11984.
(15) Sofia, M. J.; Bao, D.; Chang, W.; Du, J.; Nagarathnam, D.; Rachakonda, S.; Reddy, P. G.; Ross, B. S.; Wang, P.; Zhang, H.-R.; Bansal, S.; Espiritu, C.; Keilman, M.; Lam, A. M.; Steuer, H. M.; Niu, C.; Otto, M. J.; Furman, P. A. Discovery of a β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* **2010**, *53*, 7202−7218.
(16) Furman, P. A.; Muramami, E.; Niu, C.; Lam, A. M.; Espiritu, C.; Bansal, S.; Bao, H.; Tolstykh, T.; Sreuer, H. M.; Keilman, M.; Zennou, V.; Bourne, N.; Veselenak, R. L.; Chang, W.; Ross, B. S.; Du, J.; Otto, M. J.; Sofia, M. J. Activity and the metabolic activation pathway of the potent and selective hepatitis C virus pronucleotide inhibitor PSI-35661. *Antiviral Res.* **2011**, *91*, 120−132.
(17) Babu, B. R.; Keinicke, L.; Petersen, M.; Nielsen, C.; Wengel, J. 2′-Spiro *ribo*- and arabinonucleosides: synthesis, molecular modeling and incorporation into oligodoxynucleoties. *Org. Biomol. Chem.* **2003**, *1*, 3514−3526.
(18) Ross, B. S.; Reddy, P. G.; Zhang, H.-R.; Rachakonda, S.; Sofia, M. J. Synthesis of diastereomerically pure nucleotide phosphoramidates. *J. Org. Chem.* **2011**, *76*, 8311−8319.
(19) Eldrup, A. B.; Allerson, C. R.; Bennett, C. F.; Bera, S.; Bhat, B.; Bhat, N.; Bosserman, M. R.; Brooks, J.; Burlein, C.; Carroll, S. S.; Cook, P. D.; Getty, K. L.; MacCoss, M.; McMasters, D. R.; Olsen, D. B.; Prakash, T. P.; Prhavc, M.; Song, Q.; Tomassini, J. E.; Xia, J. Structure−activity relationship of purine ribonucleosides for inhibition of hepatitis C virus RNA-dependent RNA polymerase. *J. Med. Chem.* **2004**, *47*, 2283−2295.
(20) Ross, B. S.; Sofia, M. J.; Pamulapati, G. R.; Rachakonda, S.; Zhang, H.-R.; Chun, B.-K.; Wang, P. Nucleoside phosphoramidates. WO 2011/123645 A2, October 6, 2011.
(21) Lam, A. M.; Espiritu, C.; Murakami, E.; Zennou, V.; Bansal, S.; Steuer, H. M.; Niu, C.; Keilman, M.; Bao, H.; Bourne, N.; Veselenak, R. L.; Reddy, P. G.; Chang, W.; Du, J.; Nagarathnam, D.; Sofia, M. J.; Otto, M. J.; Furman, P. A. Inhibition of hepatitis C virus replicon RNA synthesis by PSI-352938, a cyclic phosphate prodrug of β-D-2′-deoxy-2′-α-fluoro-2′-β-C-methylguanosine. *Antimicrob. Agents Chemother.* **2011**, *55*, 2566−2575.
(22) (a) Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720−729. (b) Halgren, T. A. MMFF VII. Characterization of MMFF94, MMFF94s, and other widely available force fields for conformational energies and for intermolecular-interaction energies and geometries. *J. Comput. Chem.* **1999**, *20*, 730−748.
(23) (a) Chang, G.; Guida, W. C.; Still, W. C. An internal-coordinate Monte Carlo method for searching conformational space. *J. Am. Chem. Soc.* **1989**, *111*, 4379−4386. (b) Saunders, M.; Houk, K. N.; Wu, Y. D.; Still, C. W.; Lipton, M.; Chang, G.; Guida, W. C. Conformations of cycloheptadecane. A comparison of methods for conformational searching. *J. Am. Chem. Soc.* **1990**, *112*, 1419−1427.