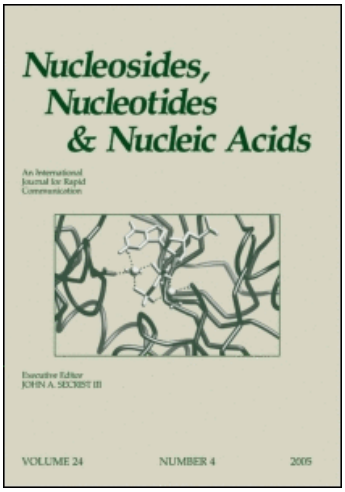

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Synthesis and Anti-Hcv Activity of N^9, 5'-Cyclo-3-(β-D-Ribofuranosyl)-8-Azapurin-2-One Derivatives

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SYNTHESIS AND ANTI-HCV ACTIVITY OF N^9,5′-CYCLO-3-(β-D-RIBOFURANOSYL)-8-AZAPURIN-2-ONE DERIVATIVES

Abdalla E. A. Hassan, Peiyuan Wang, Tamara McBrayer, Philip Tharnish, Lieven Stuyver, Michael J. Otto, and Kyoichi A. Watanabe □ *Pharmasset, Inc., Tucker, Georgia, USA*

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□ *A number of 1- or 6-substituted N^9,5′-cyclo-3-(β-D-ribofuranosyl)-8-azapurin-2-one derivatives were synthesized in multi-step reactions. Their anti-hepatitis C virus activities were evaluated and some structure–activity relationship is discussed.*

Keywords N^9,5′-Cyclo-3-(β-D-ribofuranosyl)-8-azapurine nucleosides; N^9,5′-Anhydro-isonucleosides; Potential anti-HCV nucleosides

INTRODUCTION

The flaviviridae family of viruses includes the causative agents of dengue, West Nile virus (WNV), yellow fever, and hepatitis C viruses. Hepatitis C virus (HCV) is one of the most serious flaviviridae infections in humans and is responsible for the second most common cause of viral hepatitis. HCV-induced chronic hepatitis with concomitant cirrhosis and hepatocellular carcinoma is now the leading cause of liver transplantations. Currently, nearly 2% of the U.S. population, and an estimated 170 million people worldwide, are HCV carriers.[1] The only approved therapy for chronic hepatitis C is alpha interferon (INF-α), either alone or in combination with ribavirin. However, its therapeutic value has been compromised largely due to its adverse effects[2,3] and additional new therapies are needed.

This article is dedicated to the memory of Dr. John A. Montgomery.

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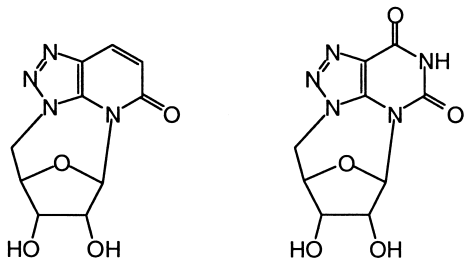


1 **2**

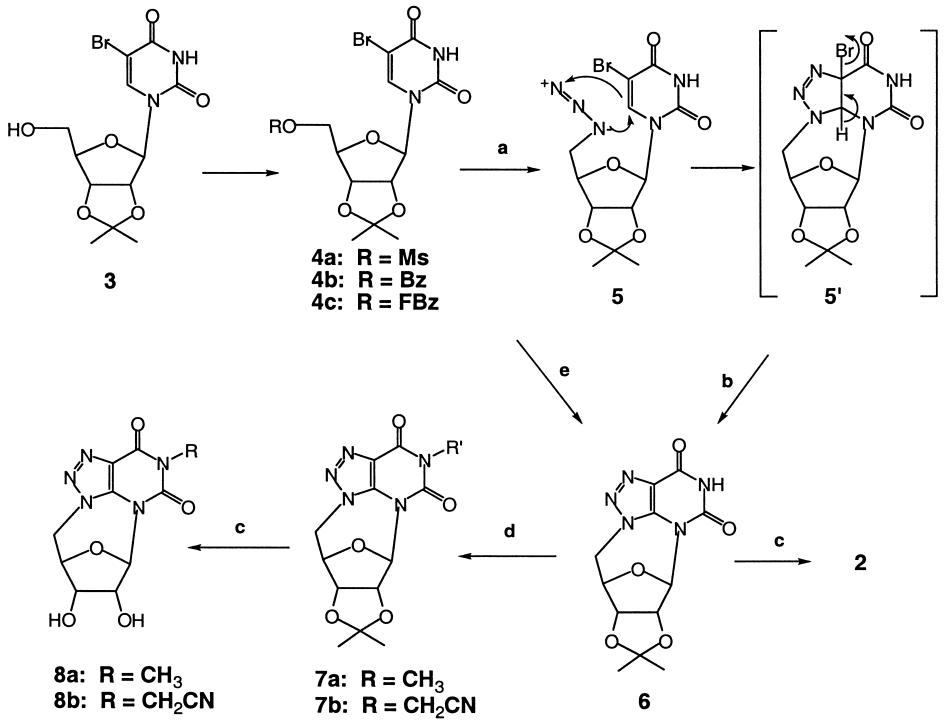
FIGURE 1 Structures of potential anti-HCV nucleosides, N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]-pyridine-2-one (**1**) and N^9,5′-cyclo-3-(β-D-ribofuranosyl)-8-azaxanthine (**2**).

In a previous report[4,5] we described the chemical synthesis of N^3,5′-cyclo-4-(β-D-ribofuranosyl)-*v*-triazolo[4,5-*b*]pyridin-2-one (**1**), which inhibits the HCV-RNA in the HCV-subgenomic replicon cell line (Huh 7 cells) with an EC_{90} of 79.8 μM. As a part of our program for discovery of more potent anti-HCV agents, we have synthesized the xanthine analogue **2** (Figure 1) and several related purine derivatives[6] and found some of the compounds to be rather active in the replicon system. We report herein the chemical synthesis of the analogues and structure-activity relationship studies from anti-HCV screening test results.

RESULTS AND DISCUSSION

Chemistry

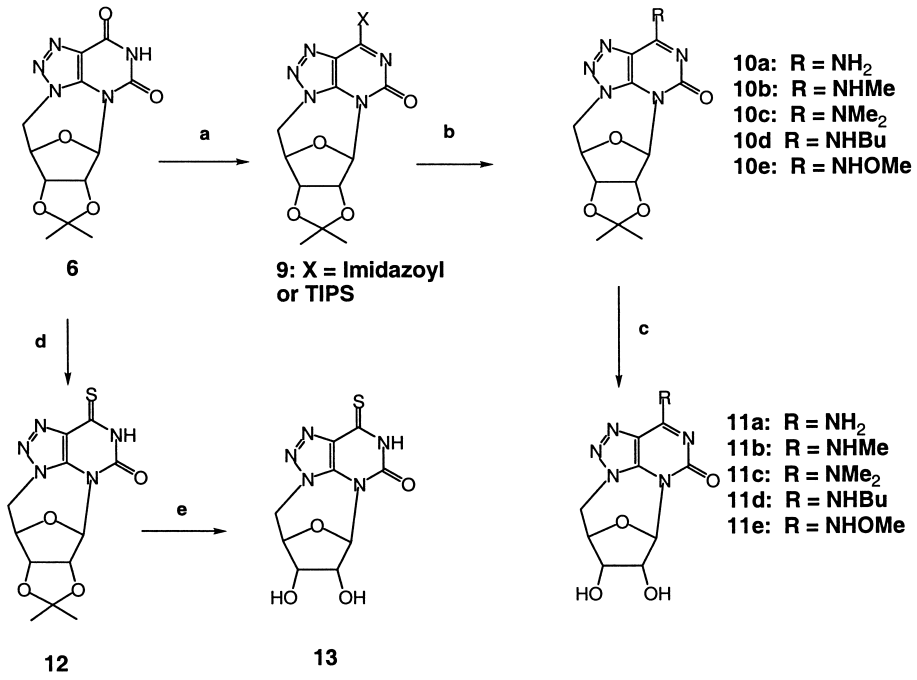
5-Bromo-2′,3-*O*-isopropylidene nucleoside derivative **3** was prepared from uridine by reported procedures.[7] Mesylation of **3** to **4a** (Scheme 1), followed by sodium azide treatment in DMF afforded the 5′-azido-5′-deoxy derivative **5**, which was obtained in 86% yield from **3** after crystallization from ethanol. Upon heating **5** in DMF at 110–120°C for 30 h, [2,3]-dipolar addition of the 5′-azido group to the 5,6-double bond occurred with concomitant elimination of HBr from the adduct **5′** resulting in the formation of 9,5′-cyclo-nucleoside **6**, which was obtained as colorless crystals in 76% yield after recrystallization from methanol and ethyl acetate. Compound **3** was acylated with benzoyl chloride or *p*-fluorobenzoyl chloride in pyridine at 0°C to give **4b** and **4c** in 84–87% yield. Upon treatment of **4b** or **4c** with sodium azide in DMF at 110–120°C for 3–5 days, the cyclonucleoside **6** was obtained directly in 75–80% yield. Acid hydrolysis of **6** in a 1:1 mixture of tetrahydrofuran and 1*M* hydrochloric acid at 90°C afforded 9,5′-cyclo-3-(β-D-ribofuranosyl)-8-azaxanthine (**2**) in quantitative yield as colorless crystals (Scheme 1).



SCHEME 1 Reagent: (a) NaN_3/DMF, 80°C, from **4a** to **5**; (b) DMF, 110–120°C, 30 h; (c) 1*N*HCl/THF, 90°C; (d) MeI/K_2CO_3/DMF for **7a**; 60% NaH/LiBr/$BrCH_2CN$/DMF for **7b**; (e) NaN_3/DMF, 110–120°C, from **4b** or **4c**.

Treatment of **6** with MeI and K_2CO_3 in DMF afforded the N-1 methyl derivative **7a** in high yield. The N-1 cyanomethyl derivative **7b** was obtained in quantitative yield by treatment of **6** with NaH and bromoacetonitrile in a 1:1 mixture of DME and DMF. Upon acid hydrolysis of **7a** and **7b**, the corresponding 2′,3′-diols **8a** and **8b** were obtained in high yield.

Compound **6** was converted to the 6-imidazolyl derivative **9** in 81% yield by treatment with triphenyl phosphine, imidazole, N,N-diisopropylethylamine and I_2 in toluene at 95–100°C for 2 h (Scheme 2). When **9** was treated with NH_4OH for 24 h at room temperature, the 8-aza-isoguanine derivative **10a** was obtained. Various 6-(substituted amino) derivatives **10b-e** were prepared directly from **6** by treatment with triisopropylbenzenesulfonyl chloride and DAMP, followed by reaction with the corresponding amines. Compounds **10a-e** were then deprotected to the corresponding diols (**11a-e**) by acid hydrolysis. Some of the mono-substituted amino derivatives of **10** and **11** exist as a mixture of amino-imino tautomers as evidenced by ^1H NMR showing a pair of signals, which merged to simple spectra when heated.



SCHEME 2 Reagent: (a) Ph_3P/imidazole/iPr_2NEt/I_2/toluene; (b) Et_3N/DMAP/TIPSCI/amine; (c) 1N HCl/THF, 90°C; (d) Lawesson's reagent/DCE, reflux; (e) CF_3CO_2H/H_2O, 50–55°C.

Compound **6** was treated with Lowesson's reagent in dichloroethane at reflux to obtain the 6-thio derivative **12** in 67.5% as a pale yellow solid. Acid treatment of **12** afforded 63% yield of 9,5′-cyclo-(3-β-D-ribofuranosyl)-8-aza-6-thioxanthine **13**.

Anti-HCV Activity

Compounds **2**, **8a-b**, **11a-e**, and **13** were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon Huh7 cell line as described previously.[8] Table 1 summarizes the HCV replicon activity and cytotoxicity of these compounds. As shown, 6-amino (**11a**), 6-methylamino (**11b**) and 6-methoxyamino (**11e**) derivatives were found to exhibit interesting anti-HCV activities with the EC_{90} values of 10.9, <6.25, and 9.7 μM, respectively. But they were also toxic with the CC_{50} values of 11.0, 6.7, and 1.9 μM, respectively. The 8-aza-xanthine **2** and 8-aza-6-thio-xanthine **13** showed moderate anti-HCV activities with the EC_{90} values of 23.7 and 43.2 μM, respectively. Methyl substitution on N-1 position intensifies the activity (e.g., **8a**, $EC_{90} = 1.5$ μM), but causes toxicity. Cyanomethyl substitution on N-1 (e.g., **8b**) did not show significant anti-HCV activity.

TABLE 1 Anti-HCV Activity of Synthesized Compounds in the Huh 7 Replicon Cells

Compounds	EC_{90} (μM)	CC_{50} (μM)
1	79.8	30.6
2	23.7	24.4
8a	1.5	1.8
8b	>100	>100
11a	10.9	11.0
11b	<6.25	6.7
11c	>100	>100
11d	>100	>100
11e	9.7	1.9
13	43.2	32

From the biological results, the most active compound among C-6-amino substituted derivatives was the methylamino analogue **(11b)**. Methyl substitution on N-1 position intensifies the activity, but unfortunately it was also toxic. It seems that the activity would be reduced as the size of substituent on the amino group at C-6 or alkyl group at N-1 grows larger.

This class of compounds did not inhibit purified the HCV RNA-dependent RNA polymerase (NS5B) in vitro when tested in cell-free systems.[4] It is not surprising that they do not appear to inhibit HCV-RNA-dependent RNA polymerase since there is no 5′-OH in these compounds. The mode of anti-HCV action is now the subject of further studies.

It should be noted that the difference between antiviral activity and cell toxicity (IC_{90} and CC_{50} values) are rather close in most cases. Roughly, the concentration of drug required to inhibit cell growth by 50% reduces 90% of viral RNA production. Thus, it is unclear whether the extent of apparent reduction of HCV RNA is derived from antiviral activity of the test compounds or is due to reduction of viable cells. Nevertheless, these data still warrant further chemical synthesis and biological studies of this class of molecules to explore the possibility of discovering compounds with reduced cytotoxicity and retained or enhanced anti-HCV activity.

EXPERIMENTAL

Chemical Synthesis

General. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Unity Plus 400 spectrometer 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). Values given for coupling constants are first order, UV spectra were recorded on a Varian CARY 50 Bio UV-Visible spectrophotometer. Fast atom bombardment mass spectroscopy was performed by the Emory University Mass Spectrometry Center. TLC was performed on Uniplates (silica gel) purchased from Analtech Co., and column chromatography was performed using silica gel (60 Å) from Sorbent Technologies, Atlanta, Georgia. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, Georgia.

2′,3′-*O*-Isopropylidine-5-bromouridine (3). 5-Bromouridine (130 g, 0.403 mol) was suspended in acetone (1 L) and treated with 1*M* HCl in Et_2O (25 mL) for 48 h at room temperature with stirring. The mixture was neutralized with 1*N* NH_4OH to pH = 7 and the solvent was evaporated in vacuo. The residue was crystallized from EtOH to give (137 g, 94%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ 1.28 (3H, s, CH_3), 1.48 (3H, s, CH_3), 3.59 (2H, m, H-5′a and H-5′b), 4.12 (1H, brq, H-4′), 4.75 (1H, dd, H-3′, J = 3.6, J = 6.4 Hz), 4.92 (1H, dd, H-2′, J = 2.4, J = 6.0 Hz), 5.32 (1H, t, J = 5.2 Hz), 5.83 (1H, d, H-1′, J = 2.8 Hz), 8.38 (1H, s, H-6), 11.92 (1H, s, NH).

5′-*O*-Benzoyl-2′,3′-*O*-isopropylidine-5-bromouridine (4b). Benzoyl chloride (7.7 mL, 66.1 mmol) was added dropwise to a solution of **3** (12 g, 33.0 mmol) in pyridine (50 mL) at 0°C. The mixture was stirred at room temperature for 3 h then the reaction was quenched with ice-H_2O and stirred for further 30 min. The solvent was evaporated and co-evaporated with toluene. The residue was partitioned between EtOAc and H_2O. The organic phase was washed with sat. $NaHCO_3$, H_2O, dried ($MgSO_4$), and the solvent was evaporated to give **4b** as a white solid (13.0 g, 84%), which was crystallized, from MeOH. ^{1}H-NMR ($CDCl_3$) δ 8.70 (1H, br s, NH), 8.01 (2H, m, Bz), 7.65–7.45 (3H, m, Bz), 5.74 (1H, d, H-1′), 4.99 (1H, dd, 5′a), 4.91 (1H, dd, 5′b), 4.65–4.53 (3H, m, H2′, H-3′ and H-4′), 1.60 (3H, s, CH_3), 1.39 (3H, s, CH_3).

5′-*O*-(4-Fluorobenzoyl)-2′,3′-*O*-isopropylidine-5-bromouridine (4c). *p*-Fluoro-benzoyl chloride (0.9 mL, 7.6 mmol) was added dropwise to a solution of **3** (2.5 g, 6.88 mmol) in pyridine (20 mL) at 0°C and the mixture was stirred at room temperature for 1 h. The reaction was quenched with ice-H_2O and stirred for further 30 min. The solvent was evaporated and co-evaporated with toluene. The residue was partitioned between EtOAc and H_2O. The organic phase was washed with sat. $NaHCO_3$, H_2O, dried ($MgSO_4$), and evaporated, and the residue was purified by a flash silica gel column (eluate; 20% EtOAc in hexanes to give **4c** as a white solid (2.91 g, 87%). ^{1}H-NMR (DMSO-d_6) δ 11.93 (1H, br s, NH), 8.14 (1H, s, H-6), 8.03 (2H, dd, F-Bz), 7.35 (2H, dd like t, F-Bz), 5.79 (1H, d, H-1′), 5.14 (1H, d,

H-2′), 4.92 (1H, br dd, H-5′a), 4.52 (1H, dd, 5′b, J = 3.6, J = 11.6 Hz), 4.46–4.39 (2H, m, H-3′, and H-4′), 1.50 (3H, s, CH_3), 1.31 (3H, s, CH_3).

5′-Azido-5′-deoxy-2′,3′-*O*-isopropylidine-5-bromouridine (5). Method A. To a solution of **3** (3 g, 8.3 mmol) in dry pyridine (20 mL) was added MsCl (0.7 mL, 9.1 mmol) at 0°C. The mixture was stirred for 1 h at 0°C, then another (0.12 mL) MsCl was charged, and the stirring continued for 1 h. The solvent was evaporated and coevaporated with toluene 2 times, and the residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was separated, dried ($MgSO_4$), and the solvent was removed in vacuo to give **4a** as a pale yellow foam, which, without purification was dissolved in DMF (20 mL). NaN_3 (2.15 g, 33.2 mmol) was added to the solution, and the mixture was heated for 2 h at 80°C. After cooling to room temperature, the mixture was concentrated in vacuo, and the residue was flash chromatographed on a silica gel column (eluate: 5% MeOH in $CHCl_3$) to give **5** (2.78 g, 86%) as a colorless solid, which was crystallized, from EtOH in hexanes. ^{1}H-NMR (DMSO-d_6) δ 11.96 (1H, s, NH), 8.27 (1H, s, H-6), 5.81 (1H, s, H-1′), 5.13 (1H, br dd, H-2′), 4.76 (1H, dd, H-3′, J = 4.4, J = 6.0 Hz), 4.15 (1H, q, H-4′), 3.60 (2H, br d, H-5′a and H-5′b), 1.49 (3H, s, CH_3), 1.29 (3H, s, CH_3).

Method B. A mixture of 5′-azido-5′-deoxy-2′,3′-*O*-isopropylideneuridine (**5**, 2.30 g, 7.44 mmol) and ammonium cerium nitrate (6.52 g, 11.89 mmol), and LiBr (768 mg, 8.84 mmol) in anhydrous acetonitrile (120 mL) was heated at 80°C for 30 min, neutralized by triethylamine, and concentrated. The residue was partitioned between H_2O and $CHCl_3$. The aqueous layer is extracted with $CHCl_3$, and the combined extracts were concentrated and the residue is purified on a silica gel column ($CHCl_3$:MeOH = 30:1) to give **5** (2.23 g, 77%). ^{1}H NMR ($CDCl_3$) δ 8.80 (bs, 1H), 7.67 (s, 1H), 5.66 (d, 1H, J = 2.0 Hz), 4.86 (m, 1H), 4.73 (m, 1H), 4.21 (m, 1H), 3.59 (m, 2H), 1.51 (s, 3H), 1.29 (m, 3H).

9,5′-Cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-8-azaxanthine (6). Method A. A solution of **5** (1 g, 2.58 mmol) in DMF (10 mL) was heated for 30 h at 110–120°C. The solvent was removed in vacuo and the residue was partitioned between EtOAc and H_2O. The organic layer was dried ($MgSO_4$), and evaporated to leave a pale crystalline residue. Recrystallization from MeOH and EtOAc afforded **6** (602 mg, 76%) as colorless solid. ^{1}H-NMR (DMSO-d_6) δ 11.67 (1H, s, NH), 6.30 (1H, s, H-1′), 5.21 (1H, d, H-5′a), 4.95–4.89 (3H, m, H-2′, H-3′, and H-4′), 4.61 (1H, d, H-5′b), 1.45 (3H, s, CH_3), 1.24 (3H, s, CH_3).

Method B. A mixture of **4b** (3.82 g, 8.22 mmol) and NaN_3 (3.2 g, 49.2 mmol) in DMF (30 mL) was heated for 3 days at 110–120°C. The mixture was cooled to room temperature. Insoluble material precipitated and was

removed by filtration. The filtrate was concentrated to dryness and the residue was worked up as above to give (2.0 g, 79%) of **6**.

Method C. A mixture of **4c** (23 g, 47.4 mmol) and NaN_3 (4.6 g, 71.1 mmol) in DMF (500 mL) was heated for 5 days at 110–120°C. The mixture was cooled to room temperature and the insoluble material was removed by filtration. The filtrate was concentrated to dryness and the residue was partitioned between EtOAc and H_2O. The organic layer was evaporated and the residue was crystallized from MeOH and EtOAc to give **6** (9.04 g, 62.2%). The aqueous layer was evaporated to dryness and the residue was combined to that obtained from evaporation of the mother liquor of the organic layer and purified by flash silica gel column chromatography (hexanes:EtOAc, 1:1.5) to give 2.1 g (14.5%) of **6**.

9,5′-Cyclo-3-(β-D-ribofuranosyl)-8-azaxanthine (2). A solution of **6** (4.1 g, 13.35 mmol) in a 1:1 mixture of THF and 1*N* HCl (1:1, 40 mL) was heated at 90°C for 4 h. Upon cooling the mixture to room temperature, white crystals separated, which were collected, washed with cold water, and dried in vacuo to give 3.5 g (98%) of **2**. ^{1}H-NMR (DMSO-d_6) δ 11.59 (1H, s, NH), 5.98 (1H, s, H-1′), 5.74 (1H, d, 2′-OH, $J = 4.8$ Hz), 5.39 (1H, d, 3′-OH, $J = 7.2$ Hz), 4.98 (1H, d, H5′a, $J = 13.6$ Hz), 4.80 (1H, dd, H5′b, $J = 13.6$, $J = 4.0$ Hz), 4.56 (1H, dd like t, H-2), 4.16–4.06 (2H, m, H-3′ and H-4′).

9,5′-Cyclo-1-methyl-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-8-azaxanthine (7a). To a mixture of **6** (152 mg, 0.5 mmol) and K_2CO_3 (172 mg, 1.25 mmol) in DMF (5 mL) was added MeI (62 mL, 1 mmol), and the mixture was stirred at room temperature for 20 h. The solvent was removed in vacuo, and the residue was partitioned between EtOAc and H_2O. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified on a silica gel column (eluate; EtOAc:hexanes 2:1) to give (150 mg, 93%) of **7a** as a white solid which was crystallized from EtOAc in hexanes: ^{1}H-NMR ($CDCl_3$) δ 6.68 (1H, s, H-1′), 5.05 (1H, d, H5′a, $J = 14$ Hz), 4.92 (2H, m, H-2′, and H-3′), 4.74 (1H, d, H-4′, $J = 5.6$ Hz), 4.67 (1H, dd, H-5′b, $J = 14$, $J = 4$ Hz), 3.41 (3H, s, N-CH_3), 1.58 (3H, s, CH_3), 1.34 (3H, s, CH_3).

1-Cyanomethyl-9,5′-cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-8-azaxanthine (7b). Sodium hydride dispersion in mineral oil (68 mg, 1.71 mmol) was added to a solution of 9,5′-cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-8-azaxanthine (500 mg, 1.63 mmol) in DME:DMF (4:1, 10 mL) at 0°C. After stirring for 10 min at 0°C, LiBr (283 mg, 3.26 mmol) was added and the suspension was stirred at room temperature for 15 min. $BrCH_2CN$ (0.23 mL, 3.26 mmol) was added and the mixture was heated for 2 h at 65°C. The solvent was evaporated and the residue was partitioned between EtOAc and H_2O. The organic phase was dried

($MgSO_4$) and evaporated. The residue was purified by a flash silica gel column chromatography (eluate; 2% MeOH in $CHCl_3$) to give (560 mg, 99%) as a white solid. ^{1}H-NMR (DMSO-d_6) δ 6.47 (1H, s, H-1′), 5.27 (1H, d, H5′a, $J = 14.4$ Hz), 5.01–4.87 (5H, m, H-2′, H-3′, and N-CH_2CN), 4.67 (1H, dd, H-5′b, $J = 14.4$, $J = 4.0$ Hz), 1.47 (3H, s, CH_3), 1.25 (3H, s, CH_3).

9,5′-Cyclo-1-methyl-3-(β-D-ribofuranosyl)-8-azaxanthine (8a). A solution of **7a** (99 mg, 0.31 mmol) in a 1:1 mixture of THF and 1*N* HCl (10 mL) was heated at 90°C for 3 h. The solvent was evaporated and the residue was purified by a flash silica gel column chromatography (eluate 12% MeOH in $CHCl_3$) to give **8a** (78 mg, 89%) as a white solid: ^{1}H-NMR (DMSO-d_6) δ 6.03 (1H, s, H-1′), 5.77 (1H, d, 2′-OH, $J = 4.8$ Hz), 5.42 (1H, d, 3′-OH, $J =$ 6.8 Hz), 5.03 (1H, br d, H5′a, $J = 15.2$ Hz), 4.81 (1H, dd, H-5′b, $J = 14$, $J = 4$ Hz), 4.58 (1H, br dd, H-4′), 4.10 (2H, m, H-2′, and H-3′), 3.20 (3H, s, N-CH_3).

In a similar manner, from 300 mg (0.87 mmol) of **7b**, 260 mg (98%) of 1-cyanomethyl-9,5′-cyclo-3-(β-D-ribofuranosyl)-8-azaxanthine (**8b**) was obtained after crystallization from MeOH. ^{1}H-NMR (DMSO-d_6+D_2O) δ 6.04 (1H, s, H-1′), 5.04 (1H, d, H-5′a, $J = 13.6$ Hz), 4.91–4.81 (2H, m, H-5′b, and H-4′), 4.62 (1H, m, H-3′ or H-2′), 4.38 (1H, m, H-3′ or H-2′), 4.20-4.06 (2H, m, N-CH_2CN).

9,5′-Cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-6-imidazolyl-8-azapurine-2-one (9). A mixture of **6** (565 mg, 1.84 mmol), PPh_3 (1.16 g, 4.42 mmol), imidazol (0.44 g, 6.51 mmol), diisopropylethylamine (1.6 mL, 9.2 mmol), and iodine (1 g, 3.81 mmol) in toluene (15 mL) was heated for 2 h at 95–100°C. After cooling to room temperature, the mixture was concentrated in vacuo, and the residue was mixed with EtOAc. Insoluble materials were filtered through a Celite pad, the filtrate was washed with water, brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified on a flash silica gel column (eluate; 7:3 EtOAc:hexanes) to give (0.536 g, 81%) of **9** as a pale yellow solid which was crystallized from EtOH in hexanes. ^{1}H-NMR (DMSO-d_6) δ 7.64 (1H, s, 6-Im), 7.11 (1H, br s, 6-Im), 6.92 (1H, br s, 6-Im), 6.37 (1H, s, H-1′), 5.23 (1H, d, H5′a, $J = 14$ Hz), 4.92–4.81 (3H, m, H-2′, H-3′, and H4′), 4.66 (1H, dd, H-5′b, $J = 4.4$, $J =$ 14 Hz), 1.47 (3H, s, CH_3), 1.26 (3H, s, CH_3).

9,5′-Cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-6-amino-8-azapurine-2-one (10a). Triethylamine (0.35 mL, 2.48 mmol) was added to a mixture of **6** (152 mg, 0.5 mmol), DMAP (150 mg, 1.23 mmol), and triisopropylbenzenesulfonyl chloride (373 mg, 1.23 mmol) in CH_3CN (10 mL) at ca. 0°C, and the mixture was stirred at room temperature for 24 h. To the mixture was added conc. NH_4OH (20 mL), and stirring continued

for further 12 h. The solvent was concentrated in vacuo, and the residue was chromatographed on a silica gel column (eluate: EtOAc:hexanes 3:1) to give **10a** (130 mg, 86%) as a pale yellow solid, which was used directly in the next step without further purification.

9,5′-Cyclo-3-(β-D-ribofuranosyl)-6-amino-8-azapurine-2-one Hydrochloride (11a, HCl salt). A solution of **10a** (120 mg, 0.39 mmol) in a 1:1 mixture of THF and 1*N* HCl (1:1, 10 mL) was heated at 90°C for 1 h and cooled to room temperature. The solvent was evaporated and coevaporated with EtOH several times. The solid residue was crystallized from EtOH and H_2O to give (92 mg, 89%) of the HCl salt of **11a**. ^{1}H-NMR of the major isomer (DMSO-d_6 + D_2O) δ 5.99 (1H, s, H-1′), 5.09 (1H, d, H5′a, J = 13.6 Hz), 4.90 (1H, dd, H5′b, J = 13.6, J = 4.4 Hz), 4.61 (1H, t, H-4′, J = 4.4 Hz), 4.14–4.06 (2H, m, H-2′ and H-3′); ^{1}H-NMR of the minor isomer (DMSO-d_6 + D_2O) δ 5.97 (1H, s, H-1′), 4.98 (1H, d, H5′a, J = 14.0 Hz), 4.87 (1H, dd, H5′b, J = 14.0, J = 3.6 Hz), 4.56 (1H, t, H-4′, J = 4.4 Hz), 4.14–4.06 (2H, m, H-2′ and H-3′).

***N*^{9},5′-Cyclo-3-(2,3-*O*-isopropylidine-β-D-ribofuranosyl)-6-methylamino-8-azapurine-2-one (10b).** Triethylamine (0.4 mL, 2.83 mmol) was added to a mixture of **6** (200 mg, 0.65 mmol), DMAP (200 mg, 1.64 mmol), and triisopropylbenzenesulfonyl chloride (400 mg, 1.32 mmol) in CH_3CN (10 mL) at ca. 0°C, and the mixture was stirred at room temperature for 12 h. To the mixture was CH_3NH_2 (2 mL), and the stirring continued for 2 h. The solvent was removed in vacuo, and the residue purified on a silica gel column (eluate; EtOAc:hexanes 3:1) to give (181 mg, 91%, white solid) of **10b** as a mixture of tautomers (6-MeNH−: 6-MeN=; 20:1 at room temperature). ^{1}H-NMR (DMSO-d_6) δ 8.93 (1H, q, 6-NH, J = 4.8 Hz), 6.34 (1H, s, H-1′), 5.15 (1H, d, H-5′a, J = 14.0 Hz), 4.87–4.84 (2H, dd, H-4′, and H-3′, J = 4.4, J = 5.6 Hz), 4.70 (1H, d, H-2′, J = 6.0 Hz), 4.59 (1H, dd, H-5′b, J = 4.4, J = 14.0 Hz), 2.90 (3H, d, N-CH_3, J = 4.8 Hz), 1.46 (3H, s, CH_3), 1.24 (3H, s, CH_3).

In a similar manner, **10c-10e** were prepared.

10c obtained in 84% yield as a white powder: ^{1}H NMR (DMSO-d_6+D_2O) δ 6.05 (1H, s, H-1′), 4.96 (1H, d, H-5′a, J = 14.0 Hz), 4.76 (1H, dd, H-5′b, J = 4.4, J = 14.0 Hz), 4.57 (1H, dd like t, H-4′, J = 4.0 Hz), 4.11 (1H, t, H-3′, J = 5.2 Hz), 3.96 (1H, t, H-2′, J = 5.2 Hz), 3.75 (3H, s, N-CH_3), 3.71 (3H, s, N-CH_3).

10d (95%, white powder), ^{1}H-NMR (DMSO-d_6) δ 9.00 (1H, t, 6-NH, J = 5.6 Hz), 6.33 (1H, s, H-1′), 5.16 (1H, d, H-5′a, J = 14.0 Hz), 4.88–4.84 (2H, dd, H-4′, and H-3′, J = 4.0, J = 6.0 Hz), 4.71 (1H, d, H-2′, J = 6.0 Hz), 4.60 (1H, dd, H-5′b, J = 4.0, J = 14.0 Hz), 3.41 (2H, m, N-Bu), 1.59–1.52 (2H, m, N-Bu), 1.46 (3H, s, CH_3), 1.37–1.28 (2H, m, N-Bu), 1.24 (3H, s, CH_3), 0.90 (3H, t, N-Bu, J = 5.6 Hz).

10e (86%, white powder), ^{1}H-NMR (DMSO-d_6) δ 10.53 (1H, s, NH), 6.24 (1H, s, H-1′), 5.14 (1H, d, H5′a, $J = 14.4$ Hz), 4.92–4.83 (3H, m, H-2′, H-3′, and H4′), 4.55 (1H, dd, H-5′b, $J = 4.0$, $J = 14.4$ Hz), 3.78 (3H, s, NOCH3), 1.45 (3H, s, CH_3), 1.24 (3H, s, CH_3).

***N*^{9},5′-Cyclo-3-(β-D-ribofuranosyl)-6-methylamino-8-azapurine-2-one (11b).** A solution of **10b** (130 mg, 0.41 mmol) in THF:1*N* HCl (1:1, 5 mL) was heated at 90°C for 4 h. The reaction mixture was concentrated in vacuo, and the residue was dried by several azeotropic distillations with EtOH. The solid residue was recrystallized from EtOH and H_2O to give (97 mg, 82%) **11b**. ^{1}H-NMR of the major isomer (DMSO-d_6 + D_2O) δ 6.03 (1H, s, H-1′), 5.04 (1H, d, H5′a, $J = 13.6$ Hz), 4.86 (1H, dd, H-5′b, $J = 3.6$ Hz, $J = 14$ Hz), 4.61 (1H, dd like t, H-4′, $J = 4.8$, $J = 4$ Hz), 4.15 (1H, t, H-3′, $J = 4.8$ Hz), 4.06 (1H, d, H-2′, $J = 5.2$ Hz), 3.74 (3H, s, N-CH_3).

In a similar manner, **11c-e** were prepared from **10c-e**.

11c, 83% yield, white powder, (recrystallized from EtOH). ^{1}H-NMR (DMSO-d_6 + D_2O) δ 6.05 (1H, s, H-1′), 4.96 (1H, d, H-5′a, $J = 14.0$ Hz), 4.76 (1H, dd, H-5′b, $J = 4.4$, $J = 14.0$ Hz), 4.57 (1H, dd like t, H-4′, $J = 4.0$ Hz), 4.11 (1H, t, H-3′, $J = 5.2$ Hz), 3.96 (1H, t, H-2′, $J = 5.2$ Hz), 3.75 (3H, s, N-CH_3), 3.71 (3H, s, N-CH_3).

11d (85%, colorless powder), ^{1}H-NMR (DMSO-d_6 + D_2O) δ 6.00 (1H, s, H-1′), 4.94 (1H, d, H-5′a, $J = 13.6$ Hz), 4.76 (1H, dd, H-5′b, $J = 4.0$, $J = 13.6$ Hz), 4.54 (1H, dd like t, H-4′, $J = 3.6$), 4.12 (1H, dd like t, H-3′, $J = 5.2$ Hz), 3.96 (1H, d, H-2′, $J = 5.2$ Hz), 3.40 (2H, m, N-Bu), 1.59-1.52 (2H, m, N-Bu), 1.37-1.28 (2H, m, N-Bu), 0.90 (3H, t, N-Bu, $J = 7.2$ Hz).

11e (60%, white solid), ^{1}H-NMR (DMSO-d_6 + D_2O) δ 5.95 (1H, s, H-1′), 4.92 (1H, d, H5′a, $J = 14.0$ Hz), 4.74 (1H, dd, H-5′b, $J = 4.0$, $J = 14.0$ Hz), 4.61 (1H, dd like t, H-4′, $J = 4.4$ Hz), 4.13 (1H, t, H-3′, $J = 4.8$ Hz), 4.03 (1H, d, H-2′, $J = 5.2$ Hz), 3.78 (3H, s, N-OMe).

9,5′-Cyclo-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-8-aza-6-thioxanthine (12). A mixture of compound **6** (2.5 g, 8.1 mmol) and Lawesson's reagent (4.94 g) in anhydrous dichloroethane (160 mL) was heated under reflux for 12 h. The reaction mixture was concentrated to dryness and the residue was purified by silica gel column chromatography with 10–20% EtOAc in hexanes to give **12** (1.78 g, 67.5%) as a yellowish solid. ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 4.63 (m, 1H, H-5′a), 4.90 and 5.00 (m, 3H, 2′,3′,4′-H), 5.20 (d, 1H, $J = 14$ Hz, H-5′b), 6.28 (s, 1H, H-1′), 12.86 (s, 1H, NH).

9,5′-Cyclo-3-β-D-ribofuranosyl-8-aza-6-thioxanthine (13). A mixture of compound **12** (55 mg, 0.17 mmol) in 4 mL of CF_3CO_2H/H_2O (2:1, v/v) solution was stirred at 50–55°C for 3 h. A precipitate appears. The mixture

was filtered and the solid was washed with EtOH to give **13** (30 mg, 63%) as a yellowish solid. ^{1}H NMR (DMSO-d_6) δ 4.11 and 4.16 (m, 2H, H-2′, 3′), 4.55 (m, 1H, H-4′), 4.80 (dd, 1H, J = 4.4, 14 Hz, H-5′a), 4.97 (d, 1H, J = 14 Hz, H-5′b), 5.96 (s, 1H, H-1′).

Antiviral Assay

Briefly, HCV-replicon cells (Clone A cells; Apath LLC, St. Louis, Missouri) in log phase growth were exposed to various concentrations of the test compounds for 3 days, after which time the HCV RNA was extracted and the amount produced quantified by real-time PCR. The potency of the compounds against HCV replicon is expressed as EC_{90} (effective concentration to reduce the HCV RNA by 90%). MTS was utilized to determine the associated potential toxic side-effects (CC_{50}) as described previously.[9]

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