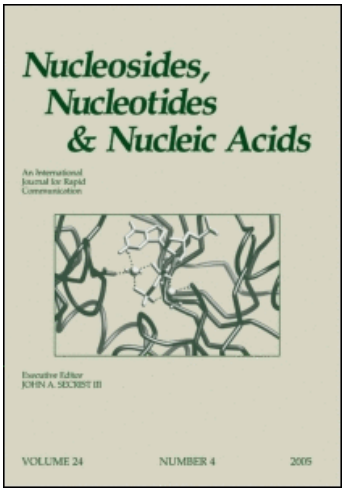

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5′,9-ANHYDRO-3-PURINE-*ISO*NUCLEOSIDES AS POTENTIAL ANTI-HEPATITIS C VIRUS AGENTS

Byoung-Kwon Chun, Peiyuan Wang, Abdalla Hassan, Jinfa Du, Phillip M. Tharnish, Eisuke Murakami, Lieven Stuyver, and Michael J. Otto □ *Pharmasset, Inc., Princeton, New Jersey, USA*

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□ *In order to study structure-activity relationships among the derivatives and congeners of 5′,9-anhydro-3-(β-D-ribofuranosyl)xanthine for anti-hepatitis C virus activity, a series of 5′,9-anhydro-purine-*iso*nucleosides with a substituent (s) at 6- or/and 8-position of the purine moiety were synthesized, and their anti-hepatitis C virus activity and cytotoxicity were evaluated and discussed.*

Keywords 5′,9-Anhydro-purine-*iso*nucleoside; nucleoside; hepatitis C virus; HCV

INTRODUCTION

Hepatitis C virus (HCV)-induced chronic hepatitis with concomitant cirrhosis and hepatocellular carcinoma is now the leading cause of liver transplant in the United States. There are about 3 million HCV carriers (2% of the population) in the United States and an estimate 170 million people worldwide. In up to 80% of the infected patients, the virus causes a chronic infection that can progress to chronic active hepatitis with cirrhosis and/or hepatocellular carcinoma.[1,2]

HCV is a 9.6 Kb positive strand RNA virus of the flaviviridae, genus *Hepacivirus*. It contains a single open reading frame coding for a 3,000 amino acid polyprotein, which is further processed by host and viral proteases

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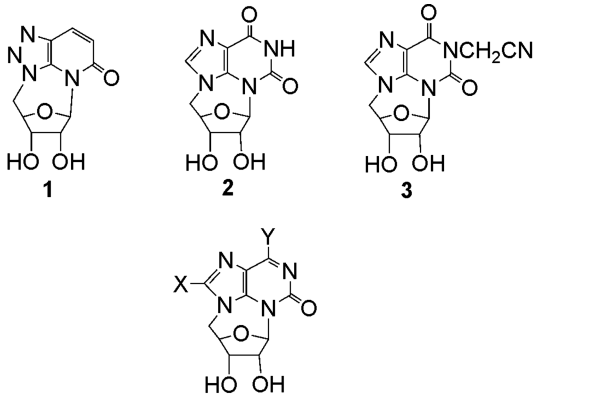
into various structural (core, E1, and E2) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) viral proteins.[3] The NS3 protease/helicase and the NS5B RNA dependent RNA polymerase are probably the most well studied targets for anti-HCV therapy since they are crucial for the viral replication.[4–6] However, the only approved therapies for chronic hepatitis C are interferon-α (INF-α), or pegylated-interferon-α either alone or in combination with ribavirin.[7] Combination therapy with ribavirin and pegylated-IFN for 6 to 12 months is currently the treatment of choice for chronic HCV infection. Although patients infected with HCV genotype 2 or -3 show high overall sustained response rate to treatment, defined as loss of HCV from serum 6 months after completion of treatment, those chronically infected with the genotype1 show only between 46% and 65% of response rate. Anemia and neuropsychiatric effects are the common adverse events of the combination therapy, which lead to premature cessation of therapy in 10–20% of patients.[7]

In light of these problems, we initiated a search for anti-HCV agents and discovered anti-HCV activity with 3,5′-cyclo-4-(β-D-ribofuranosyl)-*vic*-triazolo[4,5-*b*]pyridin-5-one (**1**).[8] Compound **1** showed no specific inhibitory activity against purified HCV RNA-dependent RNA polymerase (NS5B), which excluded the notion that the anti-HCV activity may be derived from the allosteric inhibition of the polymerase like that of non-nucleoside reverse transcriptase inhibitors (NNRTI) in HIV. Nevertheless, it was believed that this class of compounds might have a very unique mode of action worth further exploration. Therefore, as a part of structure-activity relationship study, the triazolopyridinone moiety of the lead compound **1** was replaced by a purine base, xanthine, which resulted in the discovery of 5′,9-anhydro-*iso*xanthosine (**2**) with improved anti-HCV activity in a replicon system.[9] Encouraged by this result, the derivatives with various substitutions on the purine moiety were prepared in search for more potent and less toxic anti-HCV agents. Herein, we report the synthesis and anti-HCV activity of 5′,9-anhydro-purine-*iso*nucleoside derivatives (**3–14**) (Figure 1).

RESULTS AND DISCUSSION

The starting material for **3–7** was 2′,3′-*O*-isopropylidene-5′,9-anhydro-*iso*xanthosine (**15**).[9] For preparation of 5′,9-anhydro-2-hydroxy-3-*iso*adenosine or 5′,9-anhydro-*iso*crotonoside (**4**), **15** was treated with Lawesson's reagent to give a 6-thio derivative **16** in 47% yield. Then **16** was heated with methanolic ammonia to afford a 6-amino derivative **17** in 63% yield, which was hydrolyzed with 0.5 N HCl to afford the target compound **4** as the HCl salt in 88% yield.

Alternatively, **17** also was prepared from known tri-*O*-benzoyl *iso*crotonoside **18**.[10,11] Debenzoylation of **18** with *n*-butylamine, followed

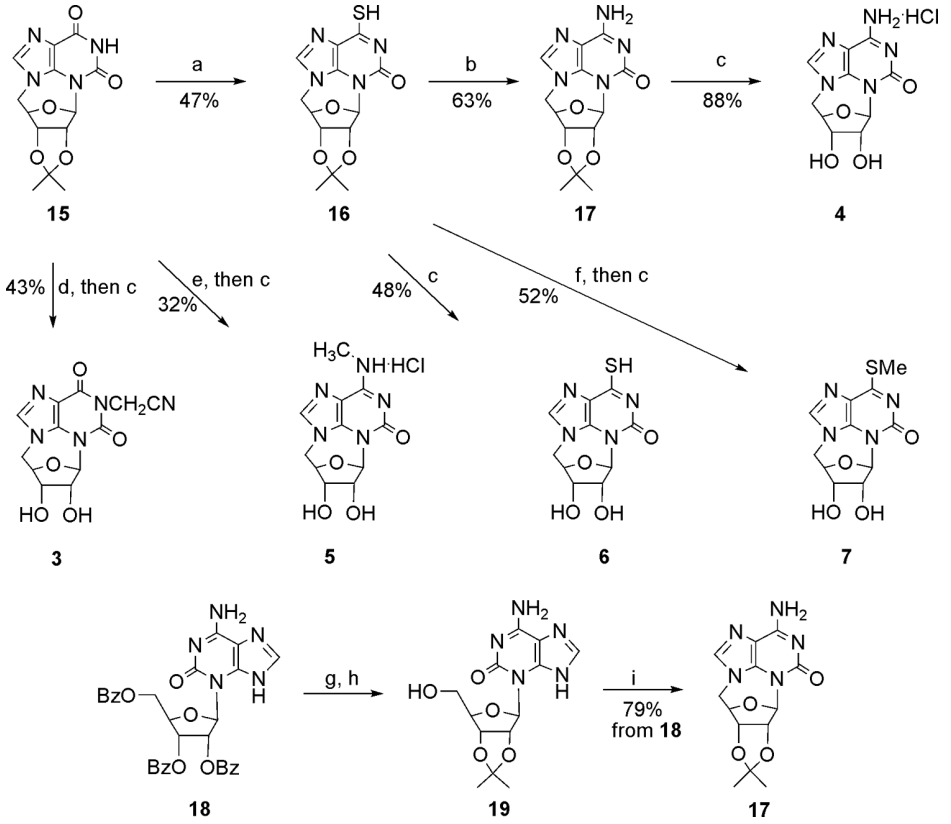


4: X = H, Y = NH_2 **8**: X = OH, Y = OH **12**: X = Br, Y =OH
5: X = H, Y = NCH_3 **9**: X = CH_3, Y =OH **13**: X = I, Y =OH
6: X = H, Y = SH **10**: X = Ph, Y =OH **14**: X = Br, Y = NH_2
7: X = H, Y = SCH_3 **11**: X= Cl, Y =OH

FIGURE 1 5′,9-Anhydro-3-purine-*iso*nucleosides.

by selective protection using 2,2-dimethoxypropane/TsOH afforded **19**, which was subjected to a Mitsunobu reaction to give **17** in 79% yield from **18**. Treatment of **15** with sodium hydride and LiBr in a mixture of 1,2-dimethoxyethane and *N*,*N*-dimethylformamide, and then with bromoacetonitrile gave an *N*1-cyanomethyl intermediate, which was deprotected with 0.5 N HCl to afford **3** in 43% yield from **15**. The N^6-methylamino purine derivative **5** was obtained by 6-*O*-sulfonylation of **15** with 2,4,6-triisopropylbenzenesufonyl chloride followed by treatment with methylamine and then acidic hydrolysis with 0.5 N HCl. The 6-thio derivative **6** was obtained simply by acidic hydrolysis of **16** in 48% yield. The S^6-methyl derivative **7** was obtained in 52% yield by treatment of **16** with methyl iodide in presence of aqueous NaOH followed by acidic hydrolysis with 75% trifluoroacetic acid (Scheme 1).

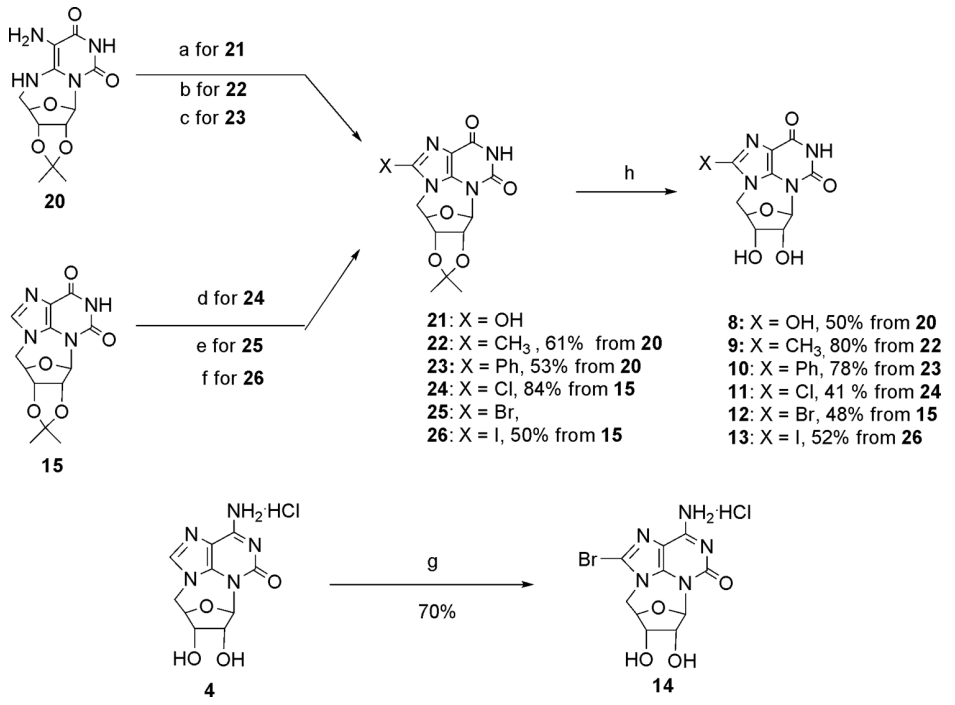
In order to prepare the 8-substiuted anhydro-purine-*iso*nucleosides, two different strategies were employed (Scheme 2). Cyclization of a diamine intermediate **20** with an appropriate reagent was employed for preparation of **8**, **9**, and **10**, while electrophilic substitution on the anhydro-xanthosine **15** and **4** was used for **11**, **12**, **13**, and **14**. For preparation of **8**, the diamime **20** was treated with carbonyl diimidazole in a mixture of dioxane and *N*,*N*-dimethylformate to give 5′,9-anhydro-8-hydroxyl-2′,3′-*O*-isopropylidene-3-*iso*xanthosine (**21**),[12] which was hydrolyzed with aqueous trifluoroacetic acid to afford the target compound **8** in 50% yield from **20**. For the preparation of **9**, compound **20** was treated with triethyl orthoacetate and *p*-toluenesulfonic acid in *N*,*N*-dimethylformate to give 5′,9-anhydro-2′,3′-*O*-isopropylidene-8-methyl *iso*xanthosine (**22**) in 61% yield,[13] which was treated with 0.5 N HCl to afford the target compound **9** in 80% yield. For



SCHEME 1 a) Lawesson's reagent, 1,2-dichloroethane, reflux, 15 h; b) NH_3, MeOH, 90°C, 24h; c) H^+ $_3O$; d) NaH, DME-DMF, 0°C, 10 min, then $BrCH_2CN$, LiBr, 65°C, 2 h; e) 2,4,6-triisopropylbenzenesulfonyl chloride, DMAP, Et_3N, CH_3CN, rt, 7 h, then aqueous CH_3NH_2, rt, 15 h; f) MeI, 0.5 N NaOH, rt, 30 min; g) *n*- i) DEAD, PPh_3, DMF, rt, 30 min.

preparation of **10**, compound **20** was treated with benzaldehyde and acetic acid to generate an imine intermediate, which was then oxidatively cyclized using thionyl chloride to give 5′,9-anhydro-2′,3′-*O*-isopropylidene-8-phenyl-3-xanthosine (**23**) in 53% yield,[14] which was similarly hydrolyzed to afford the target compound **10** in 78% yield. To prepare the target compounds, **11**, **12**, and **13**, the 8-halo intermediates, **24**, **25**, and **26** were obtained by treating **15** with a corresponding *N*-halosuccinimide.[15] 8-Chlorination of **15** was effected by *N*-chlorosuccinimide (NCS) in presence of acetic acid to give 5′,9-anhydro-8-chloro-2′,3′-*O*-isopropylidene-3-xanthosine (**24**) in 84% yield,[16] which was hydrolyzed with aqueous trifluoroacetic acid to afford target compound **11** in 41% yield.

It is noteworthy that the chlorination of **15** with NCS did not significantly progress without acetic acid-catalysis. The 8-bromination of **15** with *N*-bromosuccinimide (NBS) occurred readily without acid catalyst in dioxane at room temperature to give a protected 5′,9-anhydro-8-bromo-2′,3′-*O*-isopropylidene-3-xanthosine (**25**),[17] which was then hydrolyzed with



SCHEME 2 a) $CO(Im)_2$, dioxane-DMF, rt, 15h; b) $CH_3(OEt)_3$, TsOH, DMF, 90°C, 1 hr; c) PhCHO, AcOH, MeOH, rt, 30 min, then $SOCl_2$, rt, 30 min; d) NCS-AcOH, dioxane, rt, 15h; e) NBS, dioxane, rt, 2h; f) NIS, dioxane, 90°C, 24h; g) NBS, dioxane-H_2O, rt, 2h; h) H^+ $_3O$.

trifluoroacetic acid to afford the target compound **12** in 48% yield from **15**. Another target compound **13** was obtained similarly from **15** in 26% yield using *N*-iodosuccinimide (NIS). Finally, the target compound **14** was prepared by bromination of compound **4** using NBS in 70% yield. The structure of the intermediates and final compounds were characterized by NMR (^{1}H, ^{13}C, COSY, NOE), UV, and high-resolution mass spectroscopy.

The antiviral activity of compounds **1–14** was evaluated in a HCV subgenomic RNA replicon system,[18] along with cytotoxicity in the replicon cell and several other cell lines (Table 1). A 6-thiopurine analogue **6**, its S^6-methyl derivative **7**, 8-methyl (**9**), and 8-chloro-(**11**) analogues exhibited the more potent anti-HCV activity than the lead compound **2**[9] while all the newly synthesized compounds except **10** were more potent than the original lead **1**[8]. An *iso*xanthosine analogue **2** and 6-aminopurine analogue **4** are less active than **6** and **7**, but more active than its *N*1-cyanomethyl derivative **3** and N^6-methyl-6-aminopurine analogue **5**. However, the anti-HCV activity of these nucleosides paralleled the cytotoxicity (CC_{50}) in the replicon system. To further evaluate the specific antiviral effect over a longer exposure time, replicon cells were kept in culture for 7 days, either in the presence or in absence of the compound **1** (at 100 μM), **2** (at 40 μM), **4** (at 100 μM), or Interferon α (100 IU/ml) (Figure 2).

TABLE 1 Anti-HCV activity and cytotoxicity of the prepared compounds in the HCV replicon system

Compounds	EC_{90} (μM)[1]	CC_{50} (μM)[2]	IC_{50} (μM)[3]				
	Replicon (Huh7)	Replicon (Huh7)	Huh7	HepG2	PBM	CEM	Vero
1	79.8	ND	49.6	>81	>100	13.8	>100
2	13.0	19.5	57.4	49.2	6.8	0.33	>100
3	36.7	50.5	42.2	>100	72.1	13.5	>100
4	11.9	14.5	28.0	>100	22.1	4.4	3.5
5	41.8	35.5	22.3	16.1	ND[4]	ND	ND
6	3.5	3.3	ND	ND	ND	ND	ND
7	4.0	3.7	66.7	33.5	ND	ND	ND
8	67.3	>100	ND	>100	ND	ND	ND
9	8.4	2.9	ND	ND	ND	ND	ND
10	>100	>100	ND	ND	ND	ND	ND
11	8.7	9.4	ND	50.2	ND	ND	ND
12	52.3	37.5	ND	ND	ND	ND	ND
13	74.6	37.1	ND	ND	ND	ND	ND
14	24.8	22.4	ND	ND	ND	ND	ND

[1]EC^{90} (μM) is a concentration that reduces 90% of the viral RNA in the replicon system cell.
[2]CC^{50} (μM) is a concentration that reduces 50% of cellular mRNA in the replicon system cell.
[3]IC^{50} (μM) is a concentration that reduces 50% of cellular growth.
[4]Not determined.

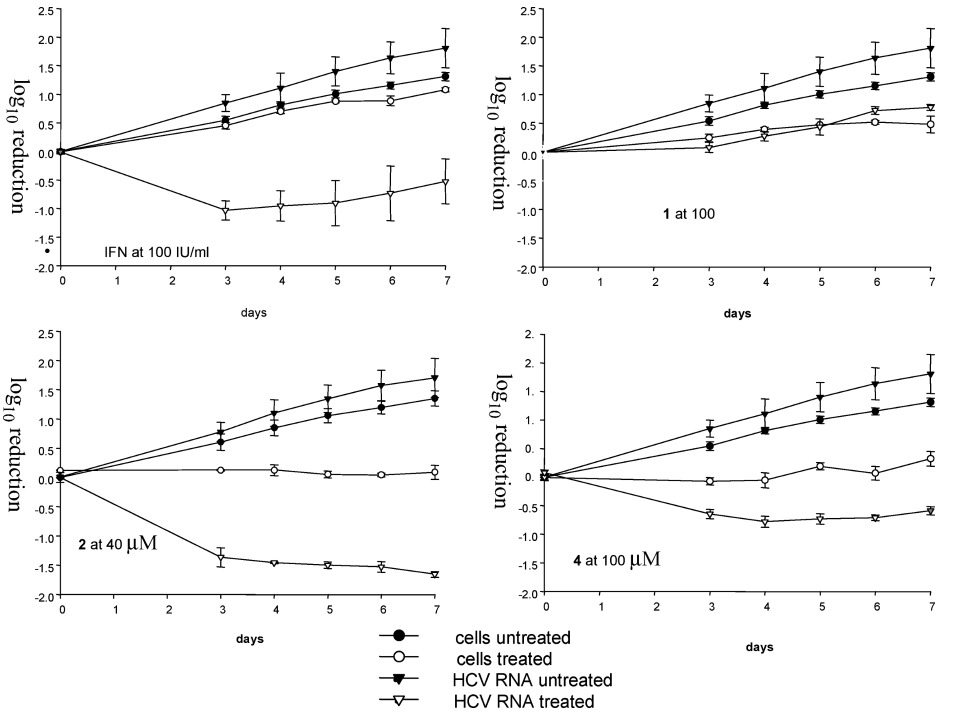


FIGURE 2 Comparison of the effect of compounds **1**, **2**, **4**, and interferon on HCV RNA and cellular growth in a HCV replicon system over 7 days.

Compounds **2** achieved more than 1 log reduction in HCV RNA in the replicon system which sustained for 7 days while interferon α also achieved a similar viral RNA reduction but failed to sustain the suppression. However, this experiment also showed that the compounds tested were cytostatic at the given concentration, which is less apparent for interferon. Similar results were obtained with other synthesized compounds (data not shown). These observations suggested that the apparent anti-HCV activity might be associated with the cytostatic effects of these compounds in the replicon system. Most of the compounds showed certain degree of cytotoxicity in other cell lines (Table 1).

In conclusion, we synthesized 5′,9-anhydro-purine-*iso*nucleosides (**3–14**), and evaluated their anti-HCV activity and cytotoxicity in a replicon cell system and other cell lines. It appears that the reduction of the HCV RNA in the replicon system by the synthesized compounds (**2–14**) may be attributed to their cytostatic effects.

EXPERIMENTAL

General

Nuclear magnetic resonance spectra were recorded on a Varian Unity Plus 400 spectrometer (Palo Alto, CA, USA) 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 (Palo Alto, CA, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (Newark, DE, USA), and column chromatography was performed using silica gel (60 Å) from Sorbent Technologies (Atlanta, GA, USA).

6-Amino-5′,9-anhydro-3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-9*H*-purine-2(3*H*)-one (17) Method 1: A mixture of **15** (5.0 g, 16.33 mmol) and Lawesson's reagent (10.0 g, 24.72 mmol) in anhydrous 1,2-dichloroethane (250 mL) was refluxed for 15 hours and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 30:1 to 10:1) to give **16** (2.5 g, 47%), which was dissolved in methanolic ammonia (50 mL), heated at 90°C in a sealed reaction flask for 24 hours and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **17** (1.5 g, 63% from **16**). **Method 2**: A mixture of **18**[11] (3.7 g, 6.21 mmol) and *n*-butylamine (10 mL) in methanol (30 mL) was stirred at rt for 2 days and concentrated to dryness. The residue was stirred in ethyl acetate (30 mL) for 30 minutes, filtered,

and the obtained solid refluxed in 2,2-dimethoxypropane (10 mL)-DMF (50 mL) in the presence of catalytic amount of TsOH for 1 hour. After cooling and concentration in vacuo, the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1 to 5:1) to give **19** (2.0 g, 99%, crude). To a solution of compound **19** (2.0 g, 6.19 mmol) and triphenylphosphine (2.4 g, 9.15 mmol) in anhydrous DMF (10 mL) was added diethyl azodicarboxylate (1.5 mL, 9.28 mmol) slowly. The reaction mixture was stirred at rt for 30 minutes, concentrated after the reaction was quenched with water (0.5 mL), and purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **17** (1.5 g, 79% from **18**): ^{1}H NMR (DMSO-d_6) δ 7.71 (s, 1H, H-8), 7.56 (d, 2H, J = 6.8 Hz, H_2N), 6.42 (s, 1H, H-1′), 4.84 (d, 1H, J = 6.0 Hz, H-2′ or 3′), 4.76 (d, 1H, J = 2.8 Hz, H-4′), 4.71 (d, 1H, J = 14.0 Hz, H-5′), 4.56 (d, 1H, J = 5.6 Hz, H-2′ or 3′), 4.19 (dd, 1H, J = 3.6, 13.6 Hz, H-5″), 1.45 (s, 3H, CH_3), 1.24 (s, 3H, CH_3).

6-Amino-5′,9-anhydro-3-(β-D-ribofuranosyl)-9*H*-purine-2(3*H*)-one-HCl salt (4) 17 (1.5 g, 4.91 mmol) was dissolved in 0.5 N HCl (20 mL), stirred at rt for 15 hours, concentrated, and co-evaporated with toluene to dryness. The obtained solid was triturated with methanol and the crystalline compound **4** precipitated was collected by filtration and dried under high vacuum (1.3 g, 88%): UV λ_{max} 245 (shoulder), 287 nm (peak) (MeOH); ^{1}H NMR (DMSO-d_6) δ 13.00 (br s, 1H, HN), 9.77 (s, 1H, HN), 8.68 (s, 1H, HN), 8.04 (s, 1H, H-8), 6.00 (s, 1H, H-1′), 5.77 (br s, 1H, HO-2′), 5.43 (br s, 1H, HO-3′), 4.73 (d, 1H, J = 14.0 Hz, H-5′), 4.55 (t, 1H, J = 4.0 Hz, H-4′), 4.48 (dd, 1H, J = 3.6, 13.6 Hz, H-5″), 4.14 (t, 1H, J = 4.8 Hz, H-3′), 4.11 (d, 1H, J = 4.8 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 152.25, 146.52, 141.53, 141.25, 111.55, 94.08, 82.52, 73.86, 70.96, 52.81; HRFABMS estimated 300.0500 for $C_{10}H_{12}N_5O_4Cl$ $(M-H)^-$, observed 300.0487.

5′,9-Anhydro-1-cyanomethyl-3-(β-D-ribofuranosyl)-9*H*-purine-2,6(1*H*, 3*H*)-dione (3) To a solution of **15** (140 mg, 0.46 mmol) in DME (4 mL)-DMF (4 mL) was added NaH (28 mg, 0.69 mmol) at 0°C. The mixture was stirred at 0°C for 10 minutes. Then LiBr (80 mg, 0.92 mmol) and bromoacetonitrile (64 μL, 0.92 mmol) were added in 10-minute interval. The resulting mixture was then heated at 65°C for 2 hours and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 30:1) to give a *N*1-cyanomethyl intermediate as a white solid, which was dissolved in 0.5 N HCl (3 mL), stirred for 2 hours, concentrated, and co-evaporated with toluene to dryness. Upon triturating the residue with MeOH, the crystalline compound **3** precipitated was collected by filtration and dried under high vacuum (60 mg, 43%): UV λ_{max} 236, 266 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 7.84 (s, 1H, H-8), 6.08 (s, 1H, H-1′), 5.73 (d, 1H, J = 4.4 Hz, HO-2′), 5.36 (d, 1H, J = 7.2 Hz, HO-3′), 4.83 (d, 2H, J = 2.0 Hz, CH_2CN), 4.67 (d, 1H, J = 14.0 Hz, H-5′), 4.53 (t, 1H, J = 3.6 Hz, H-4′), 4.42 (dd, 1H, J = 3.6, 13.6 Hz, H-5″), 4.16–4.10 (m,

2H, H-2′, 3′); ^{13}C NMR (DMSO-d_6) δ 155.75, 148.08, 139.11 (2C), 116.67, 116.24, 94.40, 82.74, 74.28, 71.09, 52.34, 28.78.

5′,9-Anhydro-6-methylamino-3-(β-D-ribofuranosyl)-9*H*-purine-2(3*H*)-one-HCl salt (5) To a solution of **15** (250 mg, 0.82 mmol), 4-(dimethylamino)pyridine (200 mg, 1.64 mmol), and Et_3N (2 mL) in anhydrous CH_3CN (4 mL) was added 2,4,6-triisopropylbenzenesulfonyl chloride (223 mg, 0.74 mmol) at rt. The reaction mixture was stirred at rt for 7 hours and then 40% aqueous CH_3NH_2 (2 mL) was added. The resulting mixture was stirred at rt for 15 hours, concentrated, and co-evaporated with toluene to dryness. The obtained residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 30:1 containing 1% Et_3N) to give a N^6-methyl derivative, which was dissolved in 0.5 N HCl (2 mL), stirred at rt for 15 hours, concentrated, and co-evaporated with toluene to dryness. Upon triturating the residue with methanol, the crystalline compound **5** precipitated was collected by filtration and dried under high vacuum (82 mg, 32% from **15**): UV λ_{max} 290 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 9.87 (s, 1H, HN), 7.59 (s, 1H, H-8), 5.98 (s, 1H, H-1′), 5.58 (d, 1H, J = 4.4 Hz, HO-2′), 5.31 (d, 1H, J = 6.8 Hz, HO-3′), 4.57 (d, 1H, J = 13.6 Hz, H-5′), 4.44 (m, 1H, H-4′), 4.29 (dd, 1H, J = 3.2, 13.2 Hz, H-5″), 4.11 (m, 1H, H-3′), 3.98 (m, 1H, H-2′), 3.60 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 151.06, 147.11, 140.48, 139.32, 111.81, 93.92, 82.44, 73.89, 70.88, 52.54, 29.16; HRFABMS estimated 316.1900 for $C_{11}H_{15}N_5O_4Cl$ $(M+H)^+$, observed 316.2051.

5′,9-Anhydro-3-(β-D-ribofuranosyl)-6-thio-9*H*-purine-2(3*H*)-one (6) **16** (34 mg, 0.11 mmol) was dissolved in 0.5 N HCl (1 mL), stirred at rt for 2 hours, concentrated, and co-evaporated with toluene to dryness. Upon triturating the residue with MeOH, the crystalline product **6** precipitated was collected by filtration and dried under high vacuum (15 mg, 48%): UV λ_{max} 256, 341 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 12.39 (s, 1H, HN or HS), 7.76 (s, 1H, H-8), 6.01 (s, 1H, H-1′), 5.68 (br s, 1H, HO-2′), 5.35 (br s, 1H, HO-3′), 4.58 (d, 1H, J = 13.6 Hz, H-5′), 4.48 (m, 1H, H-4′), 4.40 (dd, 1H, J = 3.6, 14.0 Hz, H-5″), 4.15 (t, 1H, J = 4.8 Hz, H-3′), 4.04 (d, 1H, J = 5.2 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 157.33, 148.73, 139.33, 138.28, 117.51, 93.12, 82.60, 74.41, 71.06, 52.05; HRFABMS estimated 289.0583 for $C_{10}H_{10}N_4O_4SLi$ $(M+Li)^+$, observed 289.0595.

5′,9-Anhydro-6-methylthio-3-(β-D-ribofuranosyl)-9*H*-purine-2(3*H*)-one (7) To a mixture of **16** (100 mg, 0.31 mmol) and 0.5 N NaOH (1 mL) was added MeI (0.1 mL) at rt. The resulting mixture was stirred at rt for 30 minutes and concentrated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 30:1) to give a S^6-methyl thioxanthine derivative (54 mg, 52%): UV λ_{max} 271, 316 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 7.86 (s, 1H, H-8), 6.43 (s, 1H, H-1′), 4.86 (d, 1H, J = 6.4 Hz, H-2′ or 3′), 4.84 (d, 1H, J = 3.6 Hz, H-4′), 4.77 (d, 1H, J = 14.0 Hz, H-5′), 4.74 (d, 1H, J = 6.0 Hz, H-2′ or 3′), 4.28 (dd, 1H, J = 4.0, 14.0 Hz,

H-5″), 2.51 (s, 3H, CH_3S), 1.47 (s, 3H, CH_3), 1.24 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 169.00, 151.54, 139.98, 139.90, 121.93, 112.49, 91.69, 84.96, 84.50, 81.21, 51.92, 26.14, 24.59, 11.35. The intermediate was dissolved in 75% trifluoroacetic acid (2 mL) and stirred at rt for 24 hours. Upon concentration, the residue was co-evaporated with toluene and purified by silica gel column chromatography ($CHCl_3$:MeOH = 10:1) to give **7** (37 mg, 40% from **16**): UV λ_{max} 223, 270, 316 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 7.87 (s, 1H, H-8), 6.05 (s, 1H, H-1′), 5.75 (d, 1H, J = 4.8Hz, HO-2′), 5.30 (d, 1H, J = 7.6 Hz, HO-3′), 4.63 (d, 1H, J = 13.6 Hz, H-5′), 4.49 (t, 1H, J = 4.0 Hz, H-4′), 4.42 (dd, 1H, J = 4.0, 13.6 Hz, H-5″), 4.12 (dt, 1H, J = 4.8, 7.2 Hz, H-3′), 4.00 (d, 1H, J = 4.4 Hz, H-2′), 2.48 (s, 3H, CH_3S); ^{13}C NMR (DMSO-d_6) δ 168.19, 151.32, 139.38 (2C), 121.74, 93.90, 82.33, 73.95, 71.08, 52.02, 11.09; HRFABMS estimated 297.0658 for $C_{11}H_{13}N_4O_4S$ $(M+H)^+$, observed 297.0643.

5′,9-Anhydro-8-hydroxy-3-(β-D-ribofuranosyl)xanthine (8) To a suspension of compound **20** (50 mg, 0.17 mmol) in anhydrous dioxane (1 mL)-DMF (0.5 mL) was added carbonyl diimidazole (100 mg, 0.62 mmol) at rt. The mixture was stirred for 1 hour and additional carbonyl diimidazole (100 mg, 0.62 mmol) added. Then the mixture was stirred for 15 hours at rt, concentrated, and co-evaporated with toluene. Th residue was washed with methanol to remove imidazole and dried to give compound **21** as a white solid. Compound **21** was dissolved in 70% aqueous trifluoroacetic acid (1 mL), stirred for 15 hours, concentrated, co-evaporated with toluene, and the residue was triturated with methanol. The precipitated crystals (**8**) were collected by filtration and dried to give *in vacuo* (24 mg, 50%): UV λ_{max} 289 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.27 (s, 1H, NH or OH), 11.19 (s, 1H, NH or OH), 5.99 (s, 1H, H-1′), 5.59 (s, 1H, HO-2′), 5.33 (s, 1H, HO-3′), 4.44 (s, 1H, H-4′), 4.18 (m, 1H, H-3′), 4.06 (d, 1H, J = 14.0 Hz, H-5′), 4.01 (d, 1H, J = 4.4 Hz, H-2′), 3.78 (d, 1H, J = 14.0 Hz, H-5″); ^{13}C NMR (DMSO-d_6) δ 152.89, 151.47, 148.18, 135.64, 127.87, 93.49, 82.58, 74.46, 71.08, 50.06. HRFABMS estimated 283.0679 for $C_{10}H_{11}N_4O_6$ $(M+H)^+$, found 283.0678.

5′,9-Anhydro-8-methyl-3-(β-D-ribofuranosyl)xanthine (9) A mixture of compound **20** (300 mg, 1.01 mmol), *p*-toluenesulfonic acid monohydrate (30 mg, 0.16 mmol), and triethyl orthoformate (3 mL, 17.91 mmol) in *N,N*-dimethylformamide (6 mL) was heated at 90°C for 1 hour. After cooling to rt, the reaction mixture was treated with sodium bicarbonate powder (30 mg), concentrated, and the residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 10:1) to give compound **22** (197 mg, 61%). **22** was then treated with 0.5 N HCl (2 mL), stirred for 15 hours, concentrated in vacuo, and the residue was co-evaporated with toluene to give a solid. The solid was triturated with methanol, filtered, and the collected crystal dried under high vacuum to give compound **9** (138 mg, 80% from **22**): UV λ_{max} 237, 272 nm (H_2O); ^{1}H NMR (DMSO-d_6) δ 11.14

(s, 1H, NH), 6.02 (s, 1H, H-1′), 5.71 (br s, 1H, HO-2′), 5.31 (br s, 1H, HO-3′), 4.49 (m, 1H, H-4′), 4.43 (d, 1H, J = 13.2 Hz, H-5′), 4.30 (dd, 1H, J = 3.6, 13.2Hz, H-5″), 4.19 (m, 1H, H-3′), 4.02 (d, 1H, J = 4.8, H-2′), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 157.12, 148.69, 145.76, 139.76, 115.63, 93.47, 82.27, 74.18, 71.34, 51.79, 14.02.

5′,9-Anhydro-3-(β-D-2,3-*O*-isopropylidene ribofuranosyl)-8-phenylxanthine (23) A mixture of compound **20** (100 mg, 0.34 mmol), benzaldehyde (50 μL, 0.50 mmol), and acetic acid (30 μL, 0.524 mmol) in methanol (5 mL) was stirred at rt for 30 minutes and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 10:1) to give an imine intermediate, which was then dissolved in thionyl chloride (3 mL). The solution was stirred for 30 minutes and concentrated to dryness. The residue was dissolved in 2,2-dimethoxypropane (3 mL), heated at 90 °C for 1 hour for reprotection, and cooled to rt. The precipitated crystals (**23**) were collected by filtration (69 mg, 53%): UV λ_{max} 273 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H, NH), 7.66 (m, 2H, phenyl), 7.52 (m, 3H, phenyl), 6.37 (s, 1H, H-1′), 5.04 (d, 1H, J = 6.0 Hz, H-2′ or 3′), 4.87 (d, 1H, J = 6.0 Hz, H-2′ or 3′), 4.74 (m, 1H, H-4′), 4.54 (d, 1H, J = 14.4 Hz, H-5′), 4.13 (dd, 1H, J = 2.8, 14.4 Hz, H-5″), 1.43 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 157.09, 149.09, 148.19, 141.67, 129.71, 129.49, 129.18, 128.68, 117.22, 111.83, 90.56, 84.56, 83.33, 80.99, 52.96, 25.82, 24.26; HRFABMS estimated 383.1355 for $C_{19}H_{19}N_4O_5$ $(M+H)^+$, found 383.1360.

5′,9-Anhydro-8-phenyl-3-(β-D-ribofuranosyl)xanthine (10) To a solution of compound **23** (60 mg, 0.157 mmol) in methanol (5 mL) was added c-HCl (30 μL). The resulting solution was evaporated under reduced pressure. The residue was dissolved methanol (5 mL) and the resulting solution was concentrated under reduced pressure, which was repeated additional two times. The resulting residue was triturated with methylene chloride to give a precipitated crystals (**10**), which were collected by filtration and dried in vacuo (42 mg, 78%): UV λ_{max} 272 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.33 (s, 1H, NH), 7.71–7.69 (m, 2H, phenyl), 7.55–7.53 (m, 3H, phenyl), 6.11 (s, 1H, H-1′), 4.55 (dd, 1H, J = 3.6, 13.6 Hz, H-5′), 4.47 (m, 1H, H-4′), 4.33 (d, 1H, J = 13.2Hz, H-5″), 4.24 (t, 1H, J = 4.8 Hz, H-3′), 4.09 (d, 1H, J = 5.2 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 157.16, 148.74, 146.98, 140.50, 129.69, 129.49, 129.45, 128.61, 117.01, 93.27, 82.64, 74.47, 70.67, 53.48; HRFABMS estimated 343.1042 for $C_{16}H_{15}N_4O_5$ $(M+H)^+$, found 343.1055.

5′,9-Anhydro-8-chloro-3-(β-D-2,3-O-isopropylidene ribofuranosyl) xanthine (24) A mixture of compound **15** (100 mg, 0.33 mmol), *N*-chlorosuccinimide (44 mg, 0.33 mmol), and acetic acid (2 mL) in dioxane (10 mL) was stirred at rt for 15 hours, concentrated, co-evaporated with toluene, and the residue was purified by silica gel column chromatography (chloroform:methanol = 30:1) to give compound **24** (93 mg, 84%) as a white solid: UV λ_{max} 230, 268 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.09

(br s, 1H, NH), 6.32 (s, 1H, H-1′), 4.94 (d, 1H, $J = 6.0$ Hz, H-2′or 3′), 4.84 (d, 1H, $J = 6.0$ Hz, H-2′ or 3′), 4.82 (d, 1H, $J = 2.8$ Hz, H-4′), 4.63 (d, 1H, $J = 13.6$ Hz, H-5′), 4.18 (dd, 1H, $J = 3.6$, 14.0 Hz, H-5″), 1.44 (s, 3H, CH_3), 1.24 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 156.23, 148.70, 140.75, 133, 50, 115.78, 112.23, 91.08, 84.41, 83.47, 81.23, 52.83, 25.97, 24.43; HRFABMS estimated 347.0728 for $C_{13}H_{13}N_4O_5ClLi$ $(M+Li)^+$, found 347.0735.

5′,9-Anhydro-8-chloro-3-(β-D-ribofuranosyl)xanthine (11) Compound **24** (70 mg, 0.21 mmol) was treated with 80% trifluoroacetic acid at rt for 3 hours, concentrated, and co-evaporated with toluene to give a white solid, which was then triturated with methanol. The precipitated crystals (**11**) were collected by filtration and dried under high vacuum (25 mg, 41%): ^{1}H NMR (DMSO-d_6) δ 11.38 (s, 1H, NH), 6.00 (s, 1H, H-1′), 4.50 (m, 1H, H-4′), 4.43 (d, 1H, $J = 13.6$ Hz, H-5′), 4.34 (dd, 1H, $J = 3.2$, 13.6 Hz, H-5″), 4.25 (t, 1H, $J = 5.2$ Hz, H-3′), 4.04 (d, 1H, $J = 5.2$ Hz, H-2′), 3.80 (br s, 2H, 2HO); ^{13}C NMR (DMSO-d_6) δ 156.37, 148.29, 139.98, 132.47, 115.66, 93.55, 81.83, 73.97, 71.03, 53.19.

5′,9-Anhydro-8-bromo-3-(β-D-ribofuranosyl)xanthine (12) A mixture of compound **15** (36 mg, 0.12 mmol) and NBS (32 mg, 0.18 mmol) in dioxane (2 mL) was stirred at rt for 2 hours, concentrated, and the residue purified by silica gel column chromatography (chloroform:methanol = 30:1) to give compound **25**: ^{1}H NMR (DMSO-d_6) δ 11.42 (s, 1H, NH), 6.33 (s, 1H, H-1′), 4.92 (d, 1H, $J = 5.6$ Hz, H-2′or 3′), 4.84 (d, 1H, $J = 6.0$ Hz, H-2′ or 3′), 4.81 (d, 1H, $J = 2.4$ Hz, H-4′), 4.62 (d, 1H, $J = 14.0$ Hz, H-5′), 4.17 (dd, 1H, $J = 3.6$, 14.0 Hz, H-5″), 1.44 (s, 3H, CH_3), 1.23 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 156.09, 148.60, 140.89, 122.44, 117.58, 111.95, 90.88, 84.26, 83.26, 81.09, 53.74, 25.81, 24.27; HRFABMS estimated 391.0229 for $C_{13}H_{13}N_4O_5BrLi$ $(M+Li)^+$, observed 391.0245.

Then compound **25** was dissolved in 70% trifluoroacetic acid (1 mL), stirred for 15 hours, and concentrated to dryness. The residue was co-evaporated with toluene and triturated with methanol. The precipitated crystals (**12**) were collected by filtration and dried in vacuo (20 mg, 48% from **15**). UVλ_{max} 244, 268 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H, NH), 6.00 (s, 1H, H-1′), 5.68 (br s, 1H, HO-2′), 5.35 (br s, 1H, HO-3′), 4.50 (t, 1H, $J = 3.6$ Hz, H-4′), 4.42 (d, 1H, $J = 13.2$ Hz, H-5′), 4.33 (dd, 1H, $J = 3.6$, 13.2 Hz, H-5″), 4.23 (t, 1H, $J = 4.4$ Hz, H-3′), 4.04 (d, 1H, $J = 4.8$ Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 156.30, 148.35, 140.30, 121.55, 117.57, 93.53, 81.83, 73.99, 71.02, 34.30; HRFABMS estimated 350.9916 for $C_{10}H_9N_4O_5BrLi$ $(M+Li)^+$, observed 350.9916 (the same as estimated).

5′,9-Anhydro-8-iodo-3-(β-D -2,3-*O*-isopropylidene ribofuranosyl)xanthine (26) A mixture of compound **15** (200 mg, 0.65 mmol) and NIS (300 mg, 1.27 mmol) in anhydrous dioxane (20 mL) was heated at 90°C for 24 hours, concentrated, and the residue purified by silica gel column chromatography (chloroform:methanol = 30:1) to give compound **26** (140 mg, 50%): UV

λ_{max} 245, 270 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.34 (s, 1H, NH), 6.31 (s, 1H, H-1′), 4.84 (d, 1H, J = 5.6 Hz, H-2′ or 3′), 4.80 (m, 2H, H-4′, 2′or 3′), 4.54 (d, 1H, J = 14.0 Hz, H-5′), 4.11 (dd, 1H, J = 3.6, 14.0 Hz, H-5″), 1.42 (s, 3H, CH_3), 1.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 155.61, 148.27, 140.29, 120.25, 111.56, 96.70, 90.46, 83.93, 82.87, 80.76, 55.15, 25.45, 23.89; HRFABMS estimated 439.0091 for $C_{13}H_{13}N_4O_5ILi$ $(M+Li)^+$, found 439.0098.

5′,9-Anhydro-8-iodo-3-(β-D-ribofuranosyl)xanthine (13) Compound **26** (140 mg, 0.32 mmol) was dissolved in 80% trifluoroacetic acid (1 mL), stirred for 15 hours, and concentrated to dryness. The resulting residue was co-evaporated with toluene and triturated with methanol. The precipitated crystals (**13**) were collected by filtration and dried in vacuo (35 mg, 28%). The another crop (30 mg, 24%) was obtained from mother liquor: UV λ_{max} 246, 270 nm (MeOH); ^{1}H NMR (DMSO-d_6) δ 11.29 (s, 1H, NH), 6.01 (s, 1H, H-1′), 4.50 (t, 1H, H-4′), 4.37 (d, 1H, J = 13.2 Hz, H-5′), 4.28 (dd, 1H, J = 4.0, 13.2 Hz, H-5″), 4.18 (t, 1H, J = 4.80 Hz, H-3′), 4.03 (d, 1H, J = 5.2 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 156.16, 148.34, 140.05, 120.50, 96.27, 93.45, 81.88, 74.02, 71.02, 56.10; HRFABMS estimated 398.9778 for $C_{10}H_9N_4O_5ILi$ $(M + Li)^+$, found 398.9785.

5′,9-Anhydro-8-bromo-3-(β-D-ribofuranosyl)-2-oxoadenine (14) A mixture of compound **4** (100 mg, 0.33 mmol) and NBS (89 mg, 0.498 mmol) in dioxane-water (10:4 mL) was stirred at rt for 2 hours, concentrated, and co-evaporated with toluene (5 mL × 2). The residue was triturated with chloroform-methanol (30:1) and filtered through filter paper. The white crystal was washed with chloroform-methanol (10:1), filtered, and dried in vacuo to afford compound **14** (67 mg, 53%). An additional amount of compound **14** (21 mg, 17%) was recovered from the filtrate: UV λ_{max} 287 nm (MeOH); ^{1}NMR (DMSO-d_6) δ 9.87 (s, 1H, NH), 8.71 (s, 1H, NH), 5.99 (s, 1H, H-1′), 4.54 (m, 1H, H-4′), 4.53 (dd, 1H, J = 5.6, 13.6 Hz, H-5′), 4.43 (dd, 1H, J = 4.4, 13.6 Hz, H-5″), 4.24 (m, 1H, H-3′), 4.08 (d, 1H, J = 5.2 Hz, H-2′); ^{13}C NMR (DMSO-d_6) δ 146.33, 142.05, 136.84, 125.26, 109.86, 94.50, 81.83, 73.50, 70.93, 53.95.

Anti-HCV evaluation. HCV-replicon RNA-containing Huh7 cells (Clone A cells; Apath, LLC, St. Louis, MO, USA) were kept in exponential growth in DMEM media (high glucose, no pyruvate) containing 10% fetal bovine serum, 1X nonessential amino acids, penicillin-streptomycin-glutamine (100 units/L, 100 g/L, and 2.92 mg/L, respectively) and G418 (500–1000 g/mL).[19] Antiviral assays were performed in the same media without G418. Cells were seeded in a 96-well plate at 1000 cells per well and test compounds were added immediately after seeding. After 96 hrs of incubation, total cellular RNA was isolated (Rneasy 96 kit, Qiagen, Valencia, CA, USA). HCV RNA and an internal control (TaqMan Ribosomal RNA control Reagents, Applied Biosystems, Foster City, CA, USA) were amplified in a single-step

multiplex RT-PCR protocol, as recommended by the manufacturer. The HCV primers and probe used have been described previously.[12]

To express the antiviral effectiveness of a compound, the threshold RT-PCR cycle of the test compound was subtracted from the average threshold RT-PCR cycle of the 'no drug' control and the concentrate generating a 1-log reduction (i.e., EC_{90}) in replicon RNA levels was calculated. The cytotoxicity of the test compound was also determined by calculating the effect on ribosomal RNA levels.[20] Cytotoxicity testing using MTS was performed as described previously.[21,22]

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