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Synthesis and anti-hepatitis C virus (HCV) activity of 3′-C-substituted-methyl pyrimidine and purine nucleosides

Won Jun Choi [a,b], Yu Min Kim [a], Hea Ok Kim [a], Hyuk Woo Lee [a,b], Dong-Eun Kim [c], Kwang-su Park [c], Youhoon Chong [c], Lak Shin Jeong [a,b,*]

[a] Department of Bioinspired Science, Ewha Womans University, 11-1 Seodaemun-gu, Daehyun-dong, Seoul 120-750, Republic of Korea
[b] Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, 11-1 Seodaemun-gu, Daehyun-dong, Seoul 120-750, Republic of Korea
[c] Department of Bioscience & Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

On the basis of potent anti-hepatitis C virus (HCV) activity of 2′-C-hydroxymethyladenosine, 3′-C-substituted-methyl-ribofuranosyl pyrimidine and purine nucleosides were designed and synthesized from D-xylose. Among compounds tested, all adenine analogues, **4a**, **4d**, and **4g** showed significant anti-HCV activity in a replicon-based cell assay irrespective of the substituent (Y = OH, N_3, or F) at the 3′-C-substituted methyl position, among which **4g** (Y = N_3) was the most potent, but it is also cytotoxic. This study guarantees the 3′-C-substituted-methyl nucleoside serves as a new template for the development of new anti-HCV agents.

1. Introduction

The disease infected with hepatitis C virus (HCV) is lethal in that its chronic infection is gradually developed to liver cirrhosis and hepatocellular carcinoma (HCC).[1,2] Since about 170 million people worldwide suffer from this disease, much efforts have been made to cure HCV infection, but only ribavirin in combination with interferon-α or pegylated interferon-α is being used in the treatment of HCV infection.[3] Interferon-α increases the nonadaptive antiviral response in cells.[4]

Ribavirin (Fig. 1) alone inhibits HCV subgenomic replicon replication in HuH6 cells with an EC_{50} of 87 ± 22 μM.[5] Ribavirin is converted to the mono-, di-, and triphosphate inside of cells.[6] This triphosphate is incorporated into a HCV RNA chain by a viral RNA polymerase, mutagenizing the genome and decreasing the yield of infectious virus.[7] Ribavirin also shows anti-HCV activity by inhibiting inosine-5′-monophosphate (IMP) dehydrogenase, resulting in depleting purine nucleotide pools.[8]

The HCV specific NS5b RNA dependent RNA polymerase[9,10] replicating a single strand genomic RNA into double strand RNA is one of the key enzymes in the life cycle[11] of HCV. Inhibition of this enzyme makes the HCV unable to replicate. Thus, it has been a promising therapeutic target for the development of new anti-HCV agents.

Many compounds have been synthesized to inhibit the HCV specific RNA polymerase.[12] Non-nucleoside derivatives[13] were discovered as the inhibitors binding at the allosteric site, while nucleoside analogues[14] are inhibitors acting at the catalytic site, inhibiting viral RNA synthesis. 2′-C-Methylcytidine (**1**, EC_{50} = 0.27 ± 0.04 μM)[5] and 2′-C-methyladenosine (**2**, EC_{50} = 0.60 ± 0.07 μM[5] or 0.3 μM[15]) are the representatives binding at the catalytic site of HCV, showing potent and selective anti-HCV activity (Fig. 2). They are incorporated into proviral RNA after being converted to their triphosphates, resulting in the viral RNA chain termination.[16] The 2′-methyl group prevents the incorporation of the natural substrate, nucleoside triphosphate (NTP), leading to the viral RNA chain termination.[16] On the basis of potent anti-HCV activity of **1** and **2**, we reported the synthesis and anti-HCV activity of 2′-C-hydroxymethyladenosine (**3**).[17] Compound **3** also showed potent anti-HCV activity in a

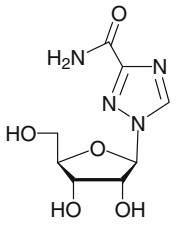

Figure 1. Structural formula of ribavirin.

* Corresponding author. Tel.: +82 2 3277 3466; fax: +82 2 3277 2851.
E-mail address: lakjeong@ewha.ac.kr (L.S. Jeong).

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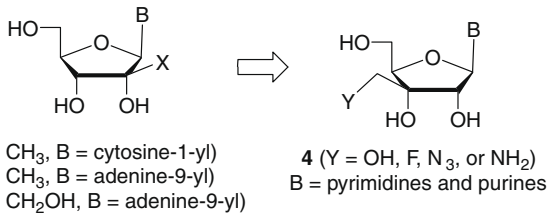


Figure 2. The rationale for the design of the target nucleoside **4**.

cell-based replicon assay, indicating that 2′-*C*-hydroxymethyl group might induce the hydrogen bonding interaction with HCV RNA polymerase and/or the steric repulsion like the 2′-methyl group. Based on these findings, we designed and synthesized bioisosteric compound **4** (Y = OH)[18] shifting the hydroxymethyl substituent from 2′-position to 3′-position and compared the conformation of **4** (Y = OH) with that of **3**. We also synthesized the analogues **4** (Y = N_3, NH_2, or F) in bioisosteric relationship with compound **4** (Y = OH). Herein, we report the synthesis, anti-HCV activity, and conformational analysis of 3′-C-substituted pyrimidine and purine nucleosides **4** from D-xylose.

2. Results and discussion

2.1. Chemistry

Our synthetic strategy to the final nucleosides was first to synthesize the glycosyl donor and then to condense with pyrimidine or purine base. The 3′-*C*-hydroxymethyl-ribofuranosyl pyrimidine and purine nucleosides **4a**–**c** were synthesized from D-xylose via the key intermediate **5** according to our previously reported procedure[18] (Scheme 1).

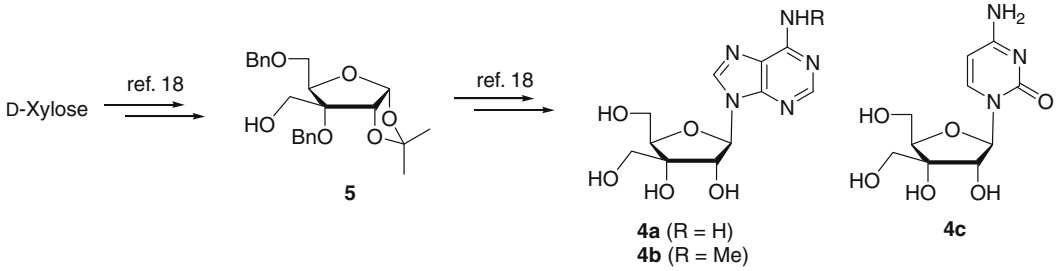
For the synthesis of 3′-*C*-fluoromethyl nucleosides, the glycosyl donor **8** was synthesized from the key intermediate **5**, as shown in Scheme 2. 3-*C*-Hydroxymethyl derivative **5** was converted to the mesylate **6** which was heated with tetra-*n*-butylammonium fluoride (TBAF) in DMF at 100 °C to give the 3-*C*-fluoromethyl derivative **7**. Acid-catalyzed hydrolysis of **7** followed by acetylation of resulting diol yielded the diacetate **8** in good yield, which is ready for the condensation with pyrimidine or purine bases.

For the synthesis of the adenine derivatives **4d** and **4e**, the glycosyl donor **8** was condensed with silylated 6-chloropurine to give the 6-chloropurine derivative **9** as a single diastereomer, due to the neighboring group participation by 2′-acetoxy group (Scheme 3). The 6-chloropurine derivative **9** was treated with methanolic ammonia and aqueous methylamine to give adenine derivative **10** and N^6-methyladenine derivative **11**, respectively, along with concomitant removal of the acetyl protecting group. Treatment of **10** and **11** with palladium black in 5% formic acid in methanol afforded **4d** and **4e**, respectively. For the synthesis of the cytosine derivative **4f**, the glycosyl donor **8** was condensed with silylated N^4-benzoylcytosine to give the N^4-benzoylcytosine derivative **12** as a single stereoisomer. Removal of the benzyl group of **12** with palladium black following the treatment with methanolic ammonia produced the final cytosine derivative **4f**.

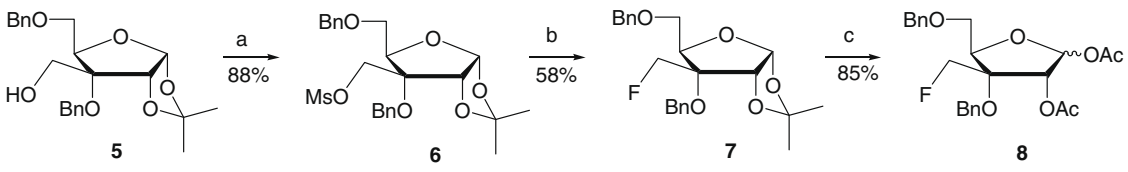
3′-*C*-Azidomethyl nucleosides were also synthesized from the same intermediate **5** via the glycosyl donor **15** (Scheme 4). Treatment of **5** with diphenylphosphoryl azide (DPPA), triphenylphosphine (PPh_3), and diisopropyl azodicarboxylate (DIAD) in THF produced the 3-*C*-azidomethyl derivative **14**.[19] Compound **14** was converted into the diacetate **15** by the same method used in Scheme 2.

Another glycosyl donor **15** was condensed with silylated 6-chloropurine and N^4-benzoylcytosine using the same conditions used in Scheme 3 to give the 6-chloropurine derivative **16** and N^4-benzoylcytosine derivative **18**, respectively (Scheme 5). Palladium black in 5% formic acid in methanol used to remove the benzyl protecting group in Scheme 3 could not be used for compounds **16** and **18** as the azido group was reduced to the amino group under this condition. Alternatively, boron trichloride and boron tribromide were used, but tertiary benzyl group was inert under these conditions, only giving mono debenzylated products, **17** and **19** as major products. To solve this problem, another strategy to oxidize the benzyl group to the benzoyl group was employed, as shown in Scheme 6.

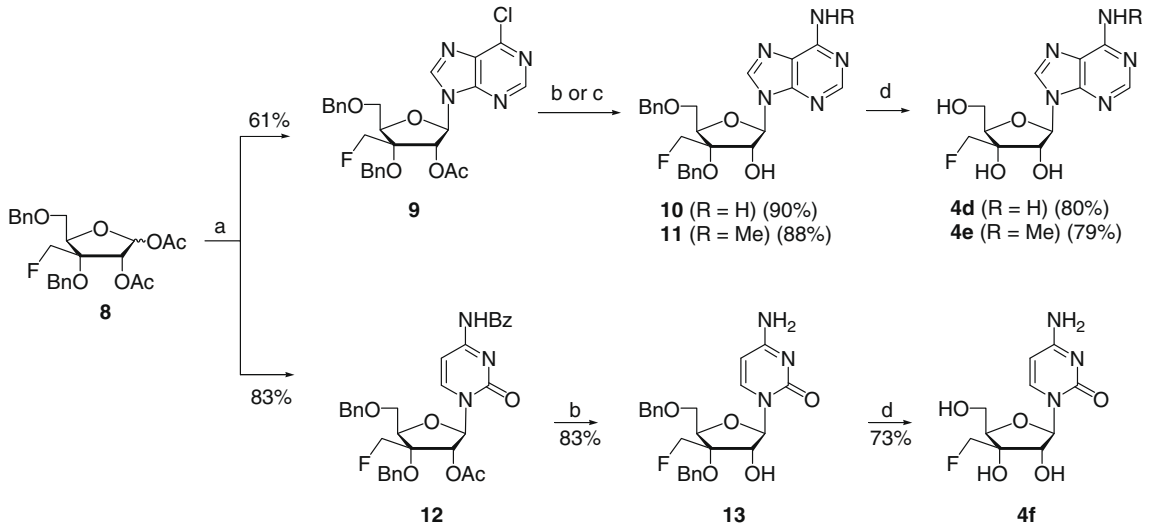
Oxidation of **16** with catalytic $RuCl_3$ and excess $NaIO_4$ in co-solvents ($CH_3CN/CCl_4/H_2O$ = 2:2:3) afforded **20** as a mixture of the desired dibenzoyl and partially oxidized monobenzoyl derivatives.[20] Treatment of the mixture **20** with methanolic ammonia yielded the final adenine derivative **4g** and its 3′-benzyl derivative **21**. Similarly compound **20** was converted to the final N^6-methyladenine derivative **4h** and its 3′-benzyl derivative **22**. However, the same oxidation on the cytosine derivative **18** resulted in the decomposition instead of giving the desired dibenzoate **23**. Thus, we employed alternative procedure to synthesize the cytosine derivative **4j** as depicted in Scheme 7.



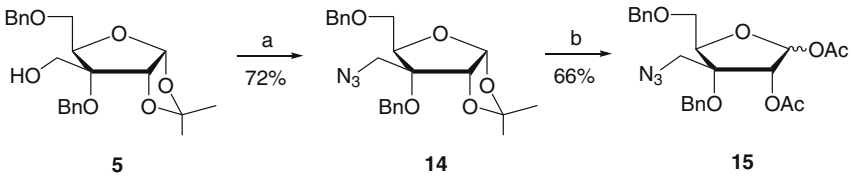
Scheme 1.



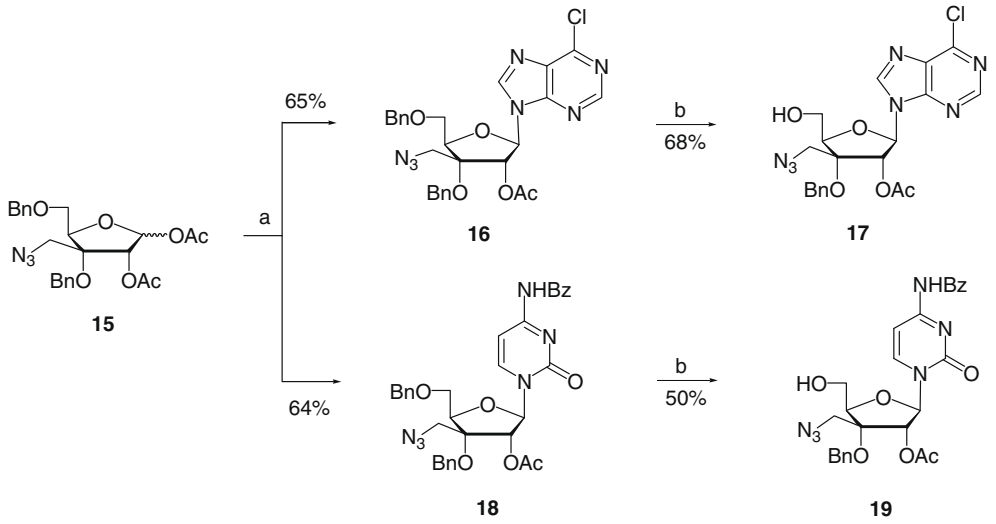
Scheme 2. Reagents and conditions: (a) MsCl, pyridine, rt, overnight; (b) TBAF, DMF, 100 °C, overnight; (c) (i) 80% AcOH, 90 °C, overnight; (ii) Ac_2O, pyridine, rt, overnight.



Scheme 3. Reagents and conditions: (a) persilylated bases, TMSOTf, DCE, rt; (b) NH_3/MeOH, 80 °C, 1 d; (c) 40% NH_2Me in H_2O, 80 °C, 1 d; (d) Pd black, 5% HCO_2H in MeOH, reflux, overnight.



Scheme 4. Reagents and conditions: (a) Ph_3P, DIAD, DPPA, THF, rt, 10 h; (b) (i) 80% AcOH, 90 °C, overnight; (ii) Ac_2O, pyridine, rt, 7 h.

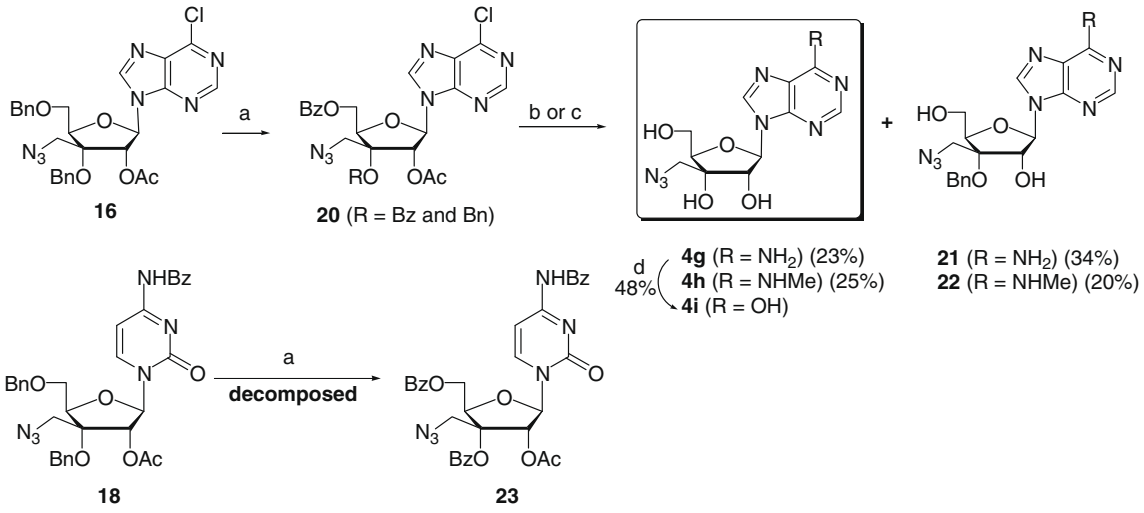


Scheme 5. Reagents and conditions: (a) persilylated bases, TMSOTf, DCE, rt, 2 h; (b) BCl_3 or BBr_3, CH_2Cl_2, −78 °C, 1.5 h to −40 °C, 3 h.

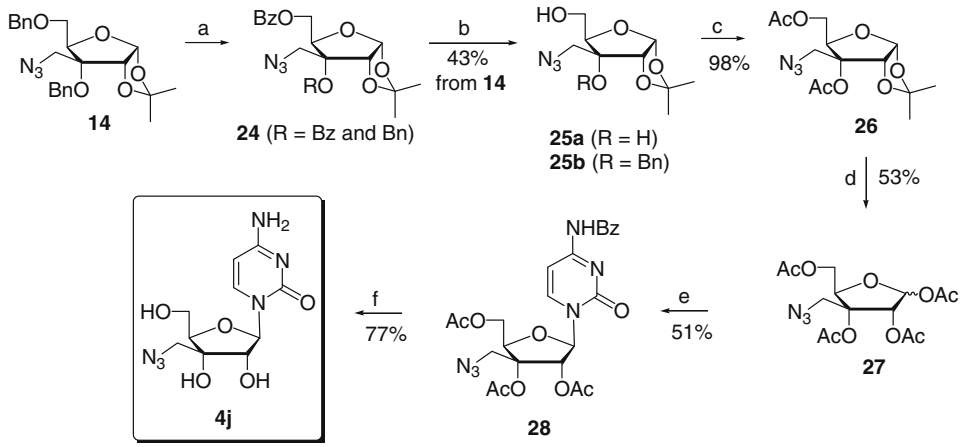
Oxidation of the dibenzylate **14** under the same oxidation conditions used in Scheme 6 gave an inseparable mixture **24** of the desired dibenzoate and partially oxidized monobenzoyl derivative. Treatment of **24** with sodium methoxide produced diol **25a** (R = H) and 3′-benzyl derivative **25b** (R = Bn), which were easily separated by silica gel chromatography. The diol **25a** was treated with acetic anhydride in pyridine at 80 °C to give the diacetate **26**. Hydrolysis of 1,2-acetonide of **26** under the same condition used in previous Schemes 2 and 4 followed by acetylation of resulting diol afforded the undesired pyranosyl acetate as a major product and the desired furanosyl acetate **27** as a minor product in 9:1 ratio. However, treatment of **26** with acetic acid, acetic anhydride, and *c*-H_2SO_4 gave the desired glycosyl donor **27** and the undesired pyranosyl acetate in 4.5:1 ratio. The glycosyl donor **27** was condensed with persilylated N^4-benzoylcytosine to give the N^4-benzoylcytosine derivative **28** as the single β-anomer in 51% yield. The 3′-C-azidomethyl-substituted cytosine derivative **4j** was obtained by treating **28** with methanolic ammonia.

2.2. Anti-HCV activity

All synthesized final nucleosides **4a**–**j** were tested for anti-HCV activity in a replicon-based cell assay.[21] Antiviral activity and cytotoxicity of **4a**–**j** are shown in Table 1. All adenine analogues, **4a**, **4d**,



Scheme 6. Reagents and conditions: (a) $RuCl_3 \times H_2O$, $NaIO_4$, rt, 2 d; (b) NH_3/MeOH, rt, overnight; (c) 40% NH_2Me in H_2O, 80 °C, 1 d; (d) $NaNO_2$, AcOH,H_2O, rt to 40 °C, 2.5 h.



Scheme 7. Reagents and conditions: (a) $RuCl_3 \times H_2O$, $NaIO_4$, rt, 2 d; (b) NaOMe, MeOH, rt, 18 h; (c) Ac_2O, pyridine, 80 °C, 1 d; (d) c-H_2SO_4, AcOH, Ac_2O, 30 °C, 5 h; (e) persilylated N^4-Bz-cytosine, TMSOTf, DCE, rt, 4 h; (f) NH_3/MeOH, 80 °C, overnight.

and **4g** showed significant anti-HCV activity irrespective of the substituent (Y = OH, N_3, or F) at the 3′-C-substituted methyl position, among which **4g** (Y = N_3) was the most potent, but they are also cytotoxic. This result indicates that a hydrogen bonding acceptor such as oxygen, nitrogen or fluorine at the 3′-C-substituted methyl position may be essential for anti-HCV activity. In case of N^6-methyladenine derivatives, 3′-*C*-hydroxymethyl derivative **4b** (Y = OH) also showed toxicity-dependent anti-HCV activity, while 3′-*C*-azido- and 3′-*C*-fluoro-methyl derivatives (Y = N_3 and F) were inactive up to 100 μM. The hypoxanthine derivative **4i** (Y = N_3) and the cytosine derivatives **4f** (Y = F) and **4j** (Y = N_3) did not show any significant anti-HCV activity up to 100 μM.

2.3. Conformational analysis

The relative energies of Northern- and Southern-type conformers were calculated for 2′-*C*-hydroxymethyladenosine (**3**) and 3′-*C*-hydroxymethyladenosine (**4a**). As shown in Table 2, 2′-*C*-hydroxymethyladenosine (**3**) displayed a strong preference for a Northern (C3′-*endo*) conformation similar to the ribo configuration of adenosine, whereas 3′-*C*-hydroxymethyladenosine (**4a**) showed to prefer a Southern (C2′-*endo*) conformation. These results are in agreement with those of calculation of conformational preference for 2′-*C*-methyladenosine (**2**) and 3′-*C*-methyladenosine.[15] It was reported that only 2′-*C*-methyladenosine showing a Northern (C3′-*endo*) conformation exhibited anti-HCV activity among 2′- and 3′-*C*-methyladenosine series, but the compounds, **4a**, **4b**, **4d**, and **4g** synthesized in this study adopting Southern (C2′-*endo*) conformations also showed significant anti-HCV activity, indicating that the substituent such as OH, F, or N_3 at the 3′-C-substituted methyl position might induce the hydrogen bonding interaction with HCV RNA polymerase and/or the steric repulsion[15] like the 2′-*C*-methyl group.

3. Conclusion

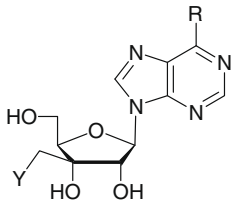
In this work, we have accomplished the synthesis of 3′-C-substituted methyl pyrimidine and purine nucleosides from D-xylose as potent anti-HCV agents. Although highly active anti-HCV agents could not be discovered in this study, finding of 3′-*C*-substituted-methyl nucleosides showing significant anti-HCV activity guarantees that they can be utilized as new templates for the development of other anti-HCV agents.

4. Experimental section

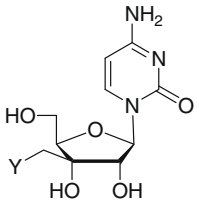
4.1. General methods

^{1}H NMR Spectra ($CDCl_3$, CD_3OD or DMSO-d_6) were recorded on Varian Unity Invoa 400 MHz. The ^{1}H NMR data are reported as

Table 1
Anti-HCV activity of the 3′-C-substituted-methyl nucleosides (**4a–j**)[a]



4a,b,d,e,g-i



4c,f,j

Compounds	EC_{50}[b] (μM)	CC_{50}[c] (μM)	SI[d] (CC_{50}/EC_{50})
4a (Y = OH, R = NH_2)	18.6 ± 10.8	37.3 ± 11.3	2.0
4b (Y = OH, R = NHMe)	37.8 ± 11.2	58.8 ± 11.9	1.6
4c (Y = OH)	ND	ND	—
4d (Y = F, R = NH_2)	15.3 ± 5.0	18.9 ± 9.8	1.2
4e (Y = F, R = NHMe)	>100	>100	—
4f (Y = F)	>100	>100	—
4g (Y = N_3, R = NH_2)	11.8 ± 3.8	12.3 ± 2.8	1.0
4h (Y = N_3, R = NHMe)	>100	>100	—
4i (Y = N_3, R = OH)	>100	>100	—
4j (Y = N_3)	>100	>100	—
2[e]	0.3	ND	—

[a] Each experiment was repeated three times and data are expressed as mean values ± standard deviation.
[b] Inhibitor concentration needed to reduce viral replication to 50%. Interferon α-2b was used as a (positive) reference compound (10000 Units/well) and reduced the signal in the viral RNA (luciferase) assay to background levels without any cytotoxic activity.
[c] Inhibitor concentration that inhibits cell growth by 50%.
[d] Selectivity index.
[e] Ref. 15.

peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet and m for multiplet. Coupling constants are reported in hertz. ^{13}C NMR ($CDCl_3$, CD_3OD or DMSO-d_6) were recorded on Varian Unity Inova 100 MHz. ^{19}F NMR ($CDCl_3$, CD_3OD) were recorded on Varian Unity Inova 376 MHz. The chemical shifts were reported as parts per million (δ) relative to the solvent peak. Optical rotations were determined on Jasco III in methanol. UV spectra were recorded on U-3000 made by Hitachi in methanol. Infrared spectra were recorded on FT-IR (FTS-135) made by Bio-Rad. The IR data were reported as s for sharp. Elementary analyses were measured on EA1110. Melting points were measured on B-540 made by Buchi. Reactions were checked with TLC (Merck precoated 60F254 plates). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid, sulfuric acid and methanol. Column chromatography was performed on Silica Gel 60 (230–400 mesh, Merck). Reverse column chromatography was performed on YMC-GEL ODS-A (12 nm S-150 μm, AA12SA5). All the anhydrous solvents were distilled over CaH_2, P_2O_5 or sodium/benzophenone prior to the reaction.

4.2. Chemistry

4.2.1. 3β-*C*-Methanesulfonyloxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (6)

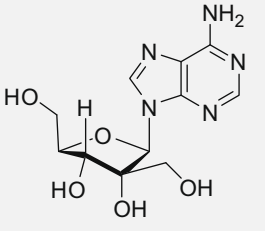
To a stirred solution of **5** (505 mg, 1.26 mmol) in pyridine (10 mL), methanesulfonyl chloride (0.19 mL, 2.52 mmol) was added and the mixture was stirred at room temperature for 18 h. The solvent was removed and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to give **6** (532 mg, 88%) as a white solid: mp 88–91 °C; $[\alpha]_D^{20}$ −42.63 (*c* 1.37, MeOH); ^1H NMR ($CDCl_3$) δ 7.34–7.26 (m, 10H), 5.87 (d, 1H, *J* = 4.0 Hz), 4.78 (dd, 2H, *J* = 10.8, 32.4 Hz), 4.76 (d, 1H, *J* = 4.0 Hz), 4.56 (d, 2H, *J* = 4.0 Hz), 4.42 (s, 2H), 4.34 (dd, 1H, *J* = 2.8, 4.8 Hz), 3.76 (dd, 1H, *J* = 3.2, 11.2 Hz), 3.66 (dd, 1H, *J* = 3.2, 11.2 Hz), 2.85 (s, 3H), 1.61 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.53, 137.93, 128.65, 128.50, 128.12, 128.00, 127.88, 127.79, 113.28, 104.18, 83.78, 79.73, 78.81, 73.97, 69.78, 69.07, 66.94, 37.40, 26.86, 26.79; Anal. Calcd for $C_{24}H_{30}O_8S$: C, 60.23; H, 6.32. Found: C, 60.45; H, 6.19.

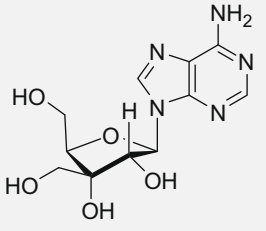
4.2.2. 3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (7)

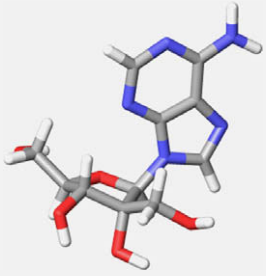
To a stirred solution of **6** (781 mg, 1.63 mmol) in DMF (50 mL), tetrabutylammonium fluoride (4.89 mL, 4.89 mmol, 1.0 M solution in THF) was added and the mixture was stirred at 100 °C for 18 h.

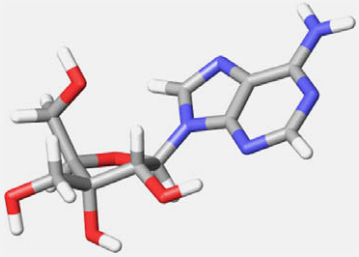
Table 2
Lowest-energy conformations of 2′-*C*-hydroxymethyladenosine (**3**) and 3′-*C*-hydroxymethyladenosine (**4a**)

Compound	2′-*C*-Hydroxymethyladenosine (**3**)	3′-*C*-Hydroxymethyladenosine (**4a**)
$[(E_N - E_S)/kcal\ mol^{-1}]$[a]	−2.92	+5.03
Lowest-energy conformation	Northern, C3′-*endo* conformer	Southern, C2′-*endo* conformer









[a] Calculated energy difference between North and South conformers. Conformational search algorithm implemented in MacroModel (v. 7.5.106, Schrödinger Inc.) was performed using the MMFFs force field in the presence of water as a solvent. Starting from both north and south conformers provided the same lowest-energy conformers.

The reaction mixture was evaporated and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8:1) to give **7** (380 mg, 58%) as a colorless syrup: $[\alpha]_D^{20}$ +13.92 (*c* 1.20, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.45–7.31 (m, 10H), 5.91 (d, 1H, *J* = 4.0 Hz), 4.81 (dd, 2H, *J* = 12.0, 22.4 Hz), 4.80 (d, 1H, *J* = 4.0 Hz), 4.69 (dd, 1H, *J* = 2.4, 11.2 Hz), 4.59–4.56 (m, 1H), 4.57 (s, 2H), 4.35–4.32 (m, 1H), 3.77 (dd, 1H, *J* = 2.8, 11.2 Hz), 3.63 (dd, 1H, *J* = 4.8, 11.2 Hz), 1.65 (s, 3H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.65, 137.98, 128.64, 128.47, 128.33, 128.08, 127.80, 127.74, 127.68, 127.63, 112.92, 104.31, 84.93, 83.18, 80.72, 79.95, 73.70, 68.90, 67.06, 26.76, 26.73; ^{19}F NMR ($CDCl_3$) δ −229.18 (t, *J* = 48.5 Hz); Anal. Calcd for $C_{23}H_{27}FO_5$: C, 68.64; H, 6.76. Found: C, 68.80; H, 6.87.

4.2.3. 3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-1,2-di-*O*-acetyl-α,β-D-ribofuranose (8)

A solution of **7** (619 mg, 1.53 mmol) in 80% aqueous acetic acid (10 mL) was stirred at 90 °C overnight and the solvents were removed. To a solution of crude residue in pyridine (8 mL), acetic anhydride (0.73 mL, 7.69 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h. The solvents were evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to give **8** (585 mg, 85%) as a colorless syrup.

Major isomer: ^{1}H NMR ($CDCl_3$) δ 7.37–7.27 (m, 10H), 6.24 (d, 1H, *J* = 2.4 Hz), 5.48 (d, 1H, *J* = 2.0 Hz), 4.89 (dd, 2H, *J* = 8.0, 47.2 Hz), 4.62–4.54 (m, 4H), 4.50–4.47 (m, 1H), 3.74 (s, 2H), 2.08 (s, 3H), 2.04 (s, 3H); Anal. Calcd for $C_{24}H_{27}FO_7$: C, 64.56; H, 6.10. Found: C, 64.31; H, 6.28.

4.2.4. 9-(3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-2-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (9)

A mixture of 6-chloropurine (423 mg, 2.74 mmol), $(NH_4)_2SO_4$ (5 mg, catalytic amount), and hexamethyldisilazane (15 mL) was refluxed overnight under N_2 atmosphere, and the mixture was evaporated. The resulting solid was dissolved in anhydrous 1,2-dichloroethane (10 mL). To this mixture was added a solution of **8** (612 mg, 1.37 mmol) in 1,2-dichloroethane (8 mL) and the reaction mixture was cooled to 0 °C. Trimethylsilyl trifluoromethanesulfonate (0.5 mL, 2.74 mmol) was added dropwise at the same temperature, and the reaction mixture was stirred at room temperature for 2 h. After quenched with aqueous $NaHCO_3$ solution, the mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to give **9** (450 mg, 61%) as a white foam: UV (MeOH) λ_{max} 263.5 nm; $[\alpha]_D^{20}$ −63.40 (*c* 2.05, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.50 (s, 1H), 7.41–7.31 (m, 10H), 6.54 (d, 1H, *J* = 7.2 Hz), 5.92 (d, 1H, *J* = 7.6 Hz), 4.97 (dd, 2H, *J* = 10.8, 47.6 Hz), 4.81 (dd, 2H, *J* = 2.4, 11.2 Hz), 4.64 (d, 2H, *J* = 2.4 Hz), 4.58 (dd, 2H, *J* = 2.0, 7.6 Hz), 3.83 (t, 1H, *J* = 2.4 Hz), 2.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.24, 152.32, 151.28, 143.89, 138.05, 136.48, 131.99, 129.01, 128.81, 128.69, 128.66, 128.45, 128.26, 128.18, 128.03, 127.93, 127.40, 85.93, 84.74, 83.53, 83.02, 76.66, 74.33, 69.31, 67.52, 20.69; ^{19}F NMR ($CDCl_3$) δ −226.90 (t, *J* = 47.0 Hz); Anal. Calcd for $C_{27}H_{26}ClFN_4O_5$: C, 59.95; H, 4.84; N, 10.36. Found: C, 60.11; H, 4.95; N, 10.29.

4.2.5. 9-(3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl) adenine (10)

A solution of **9** (147 mg, 0.272 mmol) in saturated methanolic ammonia (5 mL) was stirred in a glass bomb at 80 °C for 1 d and the volatiles were evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 40:1–20:1) to give **10** (117 mg, 90%) as a white foam: UV (MeOH) λ_{max} 258.5 nm; $[\alpha]_D^{20}$ −75.87 (*c* 1.06, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H), 8.07 (s, 1H), 7.40–7.22 (m, 10H), 6.12 (d, 1H, *J* = 7.2 Hz), 6.05 (br s, 2H), 5.07 (s, 1H), 4.96 (s, 1H), 4.89 (dd, 2H, *J* = 11.2, 42.0 Hz), 4.73 (d, 1H, *J* = 7.2 Hz), 4.65 (t, 1H, *J* = 2.4 Hz), 4.57–4.55 (m, 2H), 3.84 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 155.59, 152.77, 150.09, 139.06, 137.88, 136.97, 128.84, 128.79, 128.68, 128.44, 128.36, 128.15, 128.10, 127.95, 127.87, 127.77, 119.57, 88.53, 85.58, 83.85, 83.47, 82.06, 74.23, 69.71, 67.72; ^{19}F NMR ($CDCl_3$) δ −230.04 (t, *J* = 47.0 Hz); Anal. Calcd for $C_{25}H_{26}FN_5O_4$: C, 62.62; H, 5.47; N, 14.61. Found: C, 62.95; H, 5.51; N, 14.73.

4.2.6. 9-(3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl) -N^6-methyladenine (11)

A solution of **9** (257 mg, 0.475 mmol) in 40% aqueous $MeNH_2$ (8 mL) was heated at 80 °C for 1 d and the reaction mixture was evaporated. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 40:1–20:1) to give **11** (205 mg, 88%) as a white foam: UV (MeOH) λ_{max} 264.5 nm; $[\alpha]_D^{20}$ −64.55 (*c* 2.35, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H), 7.98 (s, 1H), 7.41–7.19 (m, 10H), 6.11 (d, 1H, *J* = 6.8 Hz), 5.07 (s, 1H), 4.95 (s, 1H), 4.90 (dd, 2H, *J* = 11.2, 44.0 Hz), 4.73 (d, 1H, *J* = 7.2 Hz), 4.63 (t, 1H, *J* = 2.8 Hz), 4.55 (d, 1H, *J* = 7.2 Hz), 4.56–4.54 (m, 2H), 4.55 (d, 1H, *J* = 7.2 Hz), 3.82 (d, 2H, *J* = 2.0 Hz), 3.15 (br s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.39, 153.06, 138.27, 137.91, 137.01, 128.81, 128.72, 128.68, 128.38, 128.18, 128.13, 127.91, 127.82, 119.96, 88.82, 85.41, 82.33, 77.84, 74.23, 69.71, 67.78; ^{19}F NMR ($CDCl_3$) δ −230.12 (t, *J* = 47.4 Hz); Anal. Calcd for $C_{26}H_{28}FN_5O_4$: C, 63.27; H, 5.72; N, 14.19. Found: C, 63.40; H, 5.79; N, 14.27.

4.2.7. 9-(3β-*C*-Fluoromethyl-β-D-ribofuranosyl)adenine (4d)

To a stirred solution of **10** (110 mg, 0.229 mmol) in 5% formic acid in MeOH (10 mL) was added Pd black (98% Pd, 34 mg, catalytic amount) and the reaction mixture was refluxed overnight. The mixture was filtered through a pad of Celite and washed with MeOH several times. After evaporation of solvents, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1–10:1) to give **4d** (55 mg, 80%) as a white solid: mp 210–216 °C (dec); UV (MeOH) λ_{max} 259.0 nm; $[\alpha]_D^{20}$ −22.88 (*c* 1.04, MeOH); ^{1}H NMR (CD_3OD) δ 8.29 (s, 1H), 8.18 (s, 1H), 6.00 (d, 1H, *J* = 7.6 Hz), 4.93 (dd, 1H, *J* = 9.6, 46.4 Hz), 4.77 (d, 1H, *J* = 8.0 Hz), 4.64 (dd, 1H, *J* = 9.6, 46.4 Hz), 4.20 (t, 1H, *J* = 2.4 Hz), 3.90 (dd, 1H, *J* = 2.8, 13.2 Hz), 3.82 (dd, 1H, *J* = 2.8, 13.2 Hz); ^{13}C NMR (CD_3OD) δ 157.86, 153.54, 150.12, 142.72, 121.39, 91.03, 89.01, 87.15, 85.49, 79.87, 79.70, 75.49, 75.42, 62.65; ^{19}F NMR ($CDCl_3$) δ −238.02 (t, *J* = 47.4 Hz); Anal. Calcd for $C_{11}H_{14}FN_5O_4$: C, 44.15; H, 4.72; N, 23.40. Found: C, 43.92; H, 4.84; N, 23.56.

4.2.8. 9-(3β-*C*-Fluoromethyl-β-D-ribofuranosyl)-N^6-methyladenine (4e)

Compound **11** (160 mg, 0.324 mmol) was converted to N^6-methyladenine derivative **4e** (80 mg, 79%) as a white solid according to the same procedure used in the preparation of **4d**: mp 135–140 °C; UV (MeOH) λ_{max} 265.0 nm; $[\alpha]_D^{20}$ −50.60 (*c* 0.67 , MeOH); ^{1}H NMR (CD_3OD) δ 8.18 (s, 2H), 5.97 (d, 1H, *J* = 7.6 Hz), 4.94 (dd, 1H, *J* = 10.0, 46.8 Hz), 4.86 (d, 1H, *J* = 5.6 Hz), 4.75 (d, 1H, *J* = 7.6 Hz), 4.65 (dd, 1H, *J* = 10.0, 46.8 Hz), 4.20 (t, 1H, *J* = 2.0 Hz), 3.93–3.81 (m, 2H), 3.07 (br s, 3H); ^{13}C NMR (CD_3OD) δ 156.93, 153.45, 148.80, 142.13, 121.86, 91.01, 88.94, 87.17, 85.49, 75.38, 62.63, 27.80; ^{19}F NMR ($CDCl_3$) δ −238.12 (t, *J* = 47.5 Hz); Anal. Calcd for $C_{12}H_{16}FN_5O_4$: C, 46.01; H, 5.15; N, 22.35. Found: C, 46.13; H, 5.21; N, 22.18.

4.2.9. 1-(3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-2-*O*-acetyl-β-D-ribofuranosyl)-N^4-benzoylcytosine (12)

The glycosyl donor **8** (140 mg, 0.314 mmol) was condensed with silylated N^4-benzoylcytosine (134 mg, 0.690 mmol) according to the same procedure used in the preparation of **9**, to give **12**

(158 mg, 83%) as white foam: UV (MeOH) λ_{max} 308.0 nm; $[\alpha]_D^{20}$ +42.08 (*c* 1.15, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.56 (br s, 1H), 8.25 (d, 1H, *J* = 7.2 Hz), 7.89 (d, 2H, *J* = 6.8 Hz), 7.62–7.28 (m, 14H), 6.60 (d, 1H, *J* = 7.2 Hz), 5.40 (d, 1H, *J* = 7.2 Hz), 4.93 (dd, 1H, *J* = 11.2, 47.6 Hz), 4.85 (dd, 1H, *J* = 11.2, 47.6 Hz), 4.88–4.82 (m 2H), 4.69 (d, 1H, *J* = 11.6 Hz), 4.59 (d, 1H, *J* = 11.2 Hz), 4.55 (t, 1H, *J* = 2.4 Hz), 3.92–3.82 (m, 2H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.51, 162.26, 144.92, 138.09, 136.60, 133.28, 129.14, 129.05, 128.79, 128.75, 128.60, 128.55, 128.45, 128.37, 128.26, 127.88, 127.77, 127.36, 127.32, 86.99, 84.24, 83.73, 83.48, 82.51, 74.26, 74.08, 69.28, 67.50, 20.83; ^{19}F NMR ($CDCl_3$) δ −228.19 (t, *J* = 47.0 Hz); Anal. Calcd for $C_{33}H_{32}FN_3O_7$: C, 65.88; H, 5.36; N, 6.98. Found: C, 66.11; H, 5.42; N, 7.06.

4.2.10. 1-(3β-*C*-Fluoromethyl-3,5-di-*O*-benzyl-β-D-ribofuranosyl)cytosine (13)

Compound **12** (218 mg, 0.362 mmol) was converted to protected cytosine derivative **13** (137 mg, 83%) as a white solid according to the same procedure used in the preparation of **10**: mp 181–184 °C; UV (MeOH) λ_{max} 271.0 nm; $[\alpha]_D^{20}$ +55.43 (*c* 1.51, MeOH); ^{1}H NMR (CD_3OD) δ 7.91 (d, 1H, *J* = 7.6 Hz), 7.44–7.24 (m, 10H), 6.26 (d, 1H, *J* = 7.6 Hz), 5.66 (d, 1H, *J* = 7.6 Hz), 5.04 (s, 1H), 4.89 (dd, 2H, *J* = 11.6, 44.4 Hz), 4.64 (d, 1H, *J* = 10.4 Hz), 4.54 (d, 1H, *J* = 10.4 Hz), 4.50 (t, 1H, *J* = 2.8 Hz), 4.18 (d, 1H, *J* = 7.6 Hz), 3.88–3.81 (m, 2H); ^{13}C NMR (CD_3OD) δ 167.55, 159.11, 142.91, 142.24, 140.27, 138.98, 129.88, 129.67, 129.44, 129.39, 129.17, 128.89, 128.84, 128.67, 128.54, 96.73, 89.39, 86.05, 84.34, 83.18, 75.01, 70.95, 68.45; ^{19}F NMR (CD_3OD) δ −232.96 (t, *J* = 47.4 Hz); Anal. Calcd for $C_{24}H_{26}FN_3O_5$: C, 63.29; H, 5.75; N, 9.23. Found: C, 63.46; H, 5.89; N, 9.13.

4.2.11. 1-(3β-*C*-Fluoromethyl-β-D-ribofuranosyl)cytosine (4f)

To a stirred solution of **13** (79 mg, 0.173 mmol) in 5% formic acid in MeOH (8 mL) was added Pd black (98% Pd, 5 mg, catalytic amount) and the reaction mixture was refluxed overnight. The mixture was filtered through a pad of Celite and washed with MeOH several times. After evaporation of solvents, the resulting residue was purified by C_{18} reverse phase column chromatography (0–3% acetone in water) to give **4f** (35 mg, 73%) as a white solid: mp 230–235 °C (dec); UV (MeOH) λ_{max} 271.0 nm; $[\alpha]_D^{20}$ +88.91 (*c* 0.51, MeOH); ^{1}H NMR (CD_3OD) δ 7.99 (d, 1H, *J* = 7.2 Hz), 6.00 (d, 1H, *J* = 7.6 Hz), 5.91 (d, 1H, *J* = 7.2 Hz), 4.71 (dd, 1H, *J* = 10.0, 46.8 Hz), 4.54 (dd, 1H, *J* = 10.0, 48.0 Hz), 4.24 (d, 1H, *J* = 7.2 Hz), 4.08 (t, 1H, *J* = 2.8 Hz), 3.83–3.74 (m, 2H); ^{13}C NMR (CD_3OD) δ 167.47, 158.75, 144.22, 96.67, 91.20, 87.93, 86.80, 85.11, 79.62, 79.44, 75.94, 75.88, 62.06; ^{19}F NMR (CD_3OD) δ −236.47 (t, *J* = 47.4 Hz); Anal. Calcd for $C_{10}H_{14}FN_3O_5$: C, 43.64; H, 5.13; N, 15.27. Found: C, 43.69; H, 5.22; N, 15.31.

4.2.12. 3β-*C*-Azidomethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (14)

To a stirred solution of PPh_3 (1.57 g, 5.99 mmol) in THF (50 mL), were added diisopropyl azodicarboxylate (1.21 g, 5.99 mmol) and diphenylphosphoryl azide (1.65 g, 5.99 mmol) at 0 °C. The mixture was stirred for 10 min at the same temperature, and white precipitation was formed. The solution of **5** (2.01 g, 4.99 mmol) in THF (50 mL) was added to the mixture at the same temperature and the reaction mixture was stirred at room temperature for 18 h. The solvents were removed and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8:1–5:1) to give **14** (1.52 g, 72%) as a pale yellowish syrup: $[\alpha]_D^{20}$ +64.10 (*c* 1.46, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.41–7.21 (m, 10H), 5.80 (d, 1H, *J* = 4.0 Hz), 4.78 (dd, 2H, *J* = 2.4, 13.2 Hz), 4.61 (d, 1H, *J* = 4.0 Hz), 4.57 (dd, 2H, *J* = 6.8, 11.2 Hz), 4.53 (t, 1H, *J* = 2.4 Hz), 4.40 (dd, 1H, *J* = 3.2, 6.8 Hz), 3.76 (dd, 1H, *J* = 3.2, 11.2 Hz), 3.63 (dd, 1H, *J* = 6.8, 11.2 Hz), 1.61 (s, 3H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.49, 138.06, 128.59, 128.46, 128.02, 127.87, 127.79, 113.12, 104.07, 84.11, 80.24, 79.39, 73.80, 68.43, 67.72, 52.10, 26.89, 26.75; IR (KBr) 2101.27 cm^{-1} (s); Anal. Calcd for $C_{23}H_{27}N_3O_5$: C, 64.93; H, 6.40; N, 9.88. Found: C, 64.74; H, 6.56; N, 10.05.

4.2.13. 3β-*C*-Azidomethyl-3,5-di-*O*-benzyl-1,2-di-*O*-acetyl-α,β-D-ribofuranose (15)

A solution of **14** (2.0 g, 4.70 mmol) in 80% aqueous acetic acid (10 mL) was stirred at 90 °C overnight and the solvents were removed. To a solution of crude residue in pyridine (15 mL), acetic anhydride (2.22 mL, 13.5 mmol) was added, and the reaction mixture was stirred at room temperature for 18 h. The solvents were evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1) to give **15** (1.49 g, 66%) as a colorless syrup.

Major isomer: ^{1}H NMR ($CDCl_3$) δ 7.40–7.25 (m, 10H), 6.27 (d, 1H, *J* = 2.8 Hz), 5.49 (d, 1H, *J* = 2.8 Hz), 4.68 (s, 2H), 4.56 (dd, 2H, *J* = 3.2, 7.6 Hz), 4.54 (t, 1H, *J* = 2.4 Hz), 3.75 (d, 2H, *J* = 7.2, 13.2 Hz), 2.06 (s, 3H), 2.05 (s, 3H).

4.2.14. 9-(3β-*C*-Azidomethyl-3,5-di-*O*-benzyl-2-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (16)

The glycosyl donor **15** (430 mg, 0.916 mmol) was condensed with silylated 6-chloropurine (284 mg, 1.84 mmol) according to the same procedure used in the preparation of **9**, to give **16** (338 mg, 65%) as a white foam: UV (MeOH) λ_{max} 263.5 nm; $[\alpha]_D^{20}$ −76.92 (*c* 0.91, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.70 (s, 1H), 8.53 (s, 1H), 7.44–7.30 (m, 10H), 6.50 (d, 1H, *J* = 7.2 Hz), 5.99 (d, 1H, *J* = 7.2 Hz), 4.90 (d, 1H, *J* = 10.8 Hz), 4.80 (d, 1H, *J* = 10.8 Hz), 4.66 (d, 1H, *J* = 12.0 Hz) 4.57 (t, 1H, *J* = 2.4 Hz), 4.55 (d, 1H, *J* = 12.0 Hz), 3.87 (dd, 1H, *J* = 2.4, 12.0 Hz), 3.80 (s, 2H), 3.76 (dd, 1H, *J* = 2.4, 11.2 Hz), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.90, 152.05, 151.94, 150.98, 143.69, 137.56, 136.15, 131.82, 128.82, 128.55, 128.35, 128.20, 127.93, 127.30, 86.01, 84.04, 83.85, 73.99, 68.79, 67.03, 51.76, 20.61; Anal. Calcd for $C_{27}H_{26}ClN_7O_5$: C, 57.50; H, 4.65; N, 17.38. Found: C, 57.60; H, 4.81; N, 17.53.

4.2.15. 9-(3β-*C*-Azidomethyl-3,5-di-*O*-benzoyl-2-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (20, R = Bz) and 9-(3β-*C*-azidomethyl-5-*O*-benzoyl-3-*O*-benzyl-2-*O*-acetyl-β-D-ribofuranosyl)-6-chloropurine (20, R = Bn)

To a stirred heterogeneous solution of **16** (434 mg, 0.770 mmol) in co-solvents (17.5 mL, $CH_3CN/CCl_4/H_2O$ = 2:2:3) were added $RuCl_3 \times H_2O$ (16.6 mg, catalytic amount) and $NaIO_4$ (1.3 g, 6.16 mmol) at 0 °C and the mixture was stirred at room temperature for 2 d. The mixture was diluted with CH_2Cl_2 and extracted with CH_2Cl_2 (three times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was prepared for the next step without further purification.

Compound **20** *(R = Bz)*: ^{1}H NMR ($CDCl_3$) δ 8.59 (s, 1H), 8.23 (s, 1H), 8.14–8.05 (m, 2H), 7.69–7.38 (m, 8H), 6.46 (d, 1H, *J* = 7.2 Hz), 6.32 (d, 1H, *J* = 7.2 Hz), 5.08 (t, 1H, *J* = 4.0 Hz), 5.00 (dd, 1H, *J* = 4.0, 12.4 Hz), 4.63 (dd, 2H, *J* = 4.8, 12.8 Hz) 4.53 (d, 1H, *J* = 12.8 Hz), 4.11 (d, 1H, *J* = 7.2 Hz), 2.05 (s, 3H).

4.2.16. 9-(3β-*C*-Azidomethyl-β-D-ribofuranosyl)adenine (4g) and 9-(3β-*C*-Azidomethyl-3-*O*-benzyl-β-D-ribofuranosyl) adenine (21)

A solution of **20** (crude 116 mg, 0.211 mmol) in saturated methanolic ammonia (8 mL) was stirred in a glass bomb at 80 °C overnight and the volatiles were evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1–10:1) to give **4g** (28 mg, 23% for two steps) as a white solid and **21** (58 mg, 34% for two steps) as a slightly yellowish solid.

Compound **4g**: mp 213–217 °C (dec); UV (MeOH) λ_{max} 257.5 nm; $[\alpha]_D^{20}$ −25.66 (*c* 0.643, MeOH); ^{1}H NMR (CD_3OD) δ 8.27

(s, 1H), 8.16 (s, 1H), 5.95 (d, 1H, J = 7.6 Hz), 4.65 (d, 1H, J = 8.0 Hz), 4.17 (t, 1H, J = 2.0 Hz), 3.87 (dd, 1H, J = 2.4, 13.2 Hz), 3.81 (dd, 1H, J = 2.0, 13.2 Hz), 3.69 (s, 2H); ^{13}C NMR (CD_3OD) δ 157.87, 153.48, 150.04, 142.77, 129.50, 91.02, 89.02, 80.96, 76.62, 62.64, 55.53; IR (KBr) 2101.33 cm^{-1} (s); Anal. Calcd for $C_{11}H_{14}N_8O_4$: C, 40.99; H, 4.38; N, 34.77. Found: C, 41.13; H, 4.51; N, 34.67.

Compound **21**: mp 86–89 °C; ^{1}H NMR (CD_3OD) δ 8.28 (s, 1H), 8.16 (s, 1H), 7.48 (d, 2H, J = 7.6 Hz), 7.36 (t, 2H, J = 6.8 Hz), 7.29 (d, 1H, J = 7.6 Hz), 6.05 (d, 1H, J = 8.0 Hz), 5.02 (d, 1H, J = 11.2 Hz), 4.87 (d, 1H, J = 8.0 Hz), 4.84 (d, 1H, J = 11.2 Hz), 4.40 (t, 1H, J = 2.4 Hz), 4.10 (d, 2H, J = 2.4 Hz), 3.94 (dd, 1H, J = 2.4, 13.2 Hz), 3.85 (dd, 1H, J = 2.0, 13.2 Hz).

4.2.17. 9-(3β-*C*-Azidomethyl-β-D-ribofuranosyl)-*N*^{6}-methyladenine (4h) and 9-(3β-*C*-Azidomethyl-3-*O*-benzyl-β-D-ribofuranosyl)-*N*^{6}-methyladenine (22)

A solution of **20** (crude 143 mg, 0.260 mmol) in 40% aqueous $MeNH_2$ (8 mL) was heated at 80 °C overnight and the reaction mixture was evaporated. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give **4h** (20 mg, 25% for two steps) as a white solid and **22** (41 mg, 20% for two steps) as a slightly yellowish solid.

Compound **4h**: mp 222–226 °C (dec); UV (MeOH) λ_{max} 263.0 nm; $[\alpha]_D^{20}$ −51.93 (*c* 0.31, MeOH); ^{1}H NMR (CD_3OD) δ 8.20 (s, 2H), 5.94 (d, 1H, J = 7.6 Hz), 4.64 (d, 1H, J = 8.0 Hz), 4.57 (br s, 1H), 4.17 (t, 1H, J = 2.0 Hz), 3.87 (dd, 1H, J = 2.0, 13.2 Hz), 3.81 (dd, 1H, J = 2.0, 13.2 Hz), 3.69 (s, 2H), 3.10 (br s, 3H); ^{13}C NMR (CD_3OD) δ 157.08, 153.45, 148.82, 142.22, 121.98, 103.87, 91.05, 88.98, 80.94, 76.58, 62.66, 55.53; IR (KBr) 2104.58 cm^{-1} (s); Anal. Calcd for $C_{12}H_{16}N_8O_4$: C, 42.86; H, 4.80; N, 33.32. Found: C, 42.70; H, 4.67; N, 33.44.

Compound **22**: mp 94–96 °C; ^{1}H NMR (CD_3OD) δ 8.23 (s, 1H), 8.16 (s, 1H), 7.37–7.31 (m, 5H), 6.10 (d, 1H, J = 7.6 Hz), 4.57 (s, 2H), 4.54 (d, 1H, J = 7.6 Hz), 4.25 (t, 1H, J = 2.4 Hz), 3.77–3.65 (m, 2H), 3.50–3.47 (m, 2H), 3.12 (br s, 3H).

4.2.18. 9-(3β-*C*-Azidomethyl-β-D-ribofuranosyl)hypoxanthine (4i)

Compound **4g** (34 mg, 0.108 mmol) was dissolved in H_2O (3 mL) and $NaNO_2$ (254 mg, 3.69 mmol) and AcOH (0.3 mL) were added to this solution. The mixture was stirred at room temperature for 1 h, and at 40 °C for 1.5 h. The reaction mixture was evaporated and the resulting residue was purified by C_{18} reverse phase column chromatography (0–7% acetone in water) to give **4i** (17 mg, 48%) as a pale yellowish foam: UV (MeOH) λ_{max} 243.0 nm; $[\alpha]_D^{20}$ −70.88 (*c* 0.45, MeOH); ^{1}H NMR (DMSO-d_6) δ 12.41 (br s, 1H), 8.34 (s, 1H), 8.08 (s, 1H), 5.90 (d, 1H, J = 7.6 Hz), 5.72 (d, 1H, J = 6.4 Hz), 5.47 (s, 1H), 5.37 (dd, 1H, J = 4.8, 5.6 Hz), 4.34 (dd, 1H, J = 6.8, 7.6 Hz), 4.01 (t, 1H, J = 2.8 Hz), 3.62 (d, 1H, J = 13.2 Hz), 3.43 (d, 1H, J = 13.2 Hz); ^{13}C NMR (DMSO-d_6) δ 156.48, 148.45, 146.04, 138.90, 124.36, 86.74, 86.43, 79.03, 75.90, 60.51, 53.45; IR (KBr) 2103.01 cm^{-1} (s); Anal. Calcd for $C_{11}H_{13}N_7O_5$: C, 40.87; H, 4.05; N, 30.33. Found: C, 40.54; H, 4.16; N, 30.17.

4.2.19. 3β-*C*-Azidomethyl-3,5-di-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-ribofuranose (24, R = Bz) and 3β-*C*-Azidomethyl-5-*O*-benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (24, R = Bn)

To a stirred heterogeneous solution of **14** (120 mg, 0.282 mmol) in co-solvents (10.5 mL, $CH_3CN/CCl_4/H_2O$ = 2:2:3) were added $RuCl_3 \times H_2O$ (10 mg, catalytic amount) and $NaIO_4$ (603 mg, 2.82 mmol) at 0 °C. The mixture was stirred at room temperature for 2 d. The mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 8:1–5:1) to give a mixture of **24** (R = Bz and Bn).

Compound **24** *(R = Bz)*: ^{1}H NMR ($CDCl_3$) δ 8.12–8.09 (m, 2H), 8.05–8.01 (m, 2H), 7.61–7.52 (m, 2H), 7.45–7.28 (m, 4H), 5.89 (d, 1H, J = 3.6 Hz), 4.94 (d, 1H, J = 4.0 Hz), 4.63 (dd, 2H, J = 4.0, 8.0 Hz), 4.29 (d, 1H, J = 13.2 Hz), 3.62 (d, 1H, J = 13.2 Hz), 1.50 (s, 3H), 1.34 (s, 3H); Anal. Calcd for $C_{23}H_{23}N_3O_7$: C, 60.92; H, 5.11; N, 9.27. Found: C, 61.12; H, 5.03; N, 9.41.

4.2.20. 3β-*C*-Azidomethyl-1,2-*O*-isopropylidene-α-D-ribofuranose (25a)

To a stirred solution of **24** (crude 540 mg, 1.27 mmol) in MeOH (10 mL) was added NaOMe (171 mg, 3.10 mmol) and the mixture was stirred at room temperature for 18 h. The mixture was extracted with CH_2Cl_2 (two times) and ethyl acetate (two times). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1–1:1) to give **25a** (205 mg, 43% for two steps) as a white solid: mp 131–133 °C; $[\alpha]_D^{20}$ +1.78 (*c* 0.95, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.74 (d, 1H, J = 3.6 Hz), 4.44 (d, 1H, J = 4.0 Hz), 3.96 (dd, 1H, J = 2.8, 7.2 Hz), 3.79 (dd, 1H, J = 3.2, 12.4 Hz), 3.61 (dd, 1H, J = 3.2, 12.4 Hz), 3.44 (d, 1H, J = 13.2 Hz), 3.12 (d, 1H, J = 13.2 Hz), 1.55 (s, 3H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 113.78, 105.04, 83.22, 82.16, 60.69, 53.63, 26.90, 26.78; IR (KBr) 2096.08 cm^{-1} (s); Anal. Calcd for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13. Found: C, 43.94; H, 6.31; N, 17.09.

4.2.21. 3β-*C*-Azidomethyl-3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-α-D-ribofuranose (26)

To a stirred solution of **25a** (238 mg, 0.971 mmol) in pyridine was added acetic anhydride (1.0 mL, excess amount) and the mixture was stirred at 80 °C for 1 d. The solvents were evaporated, and the residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1–3:1) to give **26** (314 mg, 98%) as a colorless syrup: $[\alpha]_D^{20}$ −93.46 (*c* 1.47, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.78 (d, 1H, J = 3.6 Hz), 4.73 (d, 1H, J = 4.0 Hz), 4.51 (dd, 1H, J = 2.0, 11.2 Hz), 4.22 (dd, 1H, J = 2.0, 11.2 Hz), 4.19 (t, 1H, J = 2.8 Hz), 4.09 (d, 1H, J = 13.2 Hz), 3.31 (d, 1H, J = 13.2 Hz), 2.14 (s, 3H), 2.11 (s, 3H), 1.54 (s, 3H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.73, 169.86, 133.57, 130.15, 128.50, 112.88, 103.74, 83.74, 80.47, 62.18, 48.78, 26.56, 26.43; Anal. Calcd for $C_{13}H_{19}N_3O_7$: C, 47.41; H, 5.82; N, 12.76. Found: C, 47.63; H, 5.92; N, 12.65.

4.2.22. 3β-*C*-Azidomethyl-1,2,3,5-tetra-*O*-acetyl-α,β-D-ribofuranose (27)

Compound **26** (511 mg, 1.55 mmol) was dissolved in a solution of acetic acid (8 mL) and acetic anhydride (2 mL, excess amount). After the mixture was cooled to 0 °C, *c*-H_2SO_4 (0.04 mL) was added to this mixture. The reaction mixture was stirred at 30 °C for 5 h and poured into saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1–1:1) to give α,β-mixture of **27** (308 mg, 53%) as a colorless syrup and the corresponding pyranosyl derivative (65 mg, 12%).

Compound **27** (β-isomer, major): ^{1}H NMR ($CDCl_3$) δ 6.09 (d, 1H, J = 2.0 Hz), 5.43 (d, 1H, J = 2.0 Hz), 4.59 (dd, 1H, J = 2.8, 12.0 Hz), 4.48 (dd, 1H, J = 2.8, 6.4 Hz), 4.20 (d, 1H, J = 13.2 Hz), 4.14 (dd, 1H, J = 6.4, 12.0 Hz), 3.80 (d, 1H, J = 13.2 Hz), 2.14 (s, 3H), 2.11 (s, 3H), 2,10 (s, 3H), 2.09 (s, 3H); Anal. Calcd for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 44.98; H, 5.27; N, 11.33.

4.2.23. 1-(3β-*C*-Azidomethyl-2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-*N*^{4}-benzoylcytosine (28)

The glycosyl donor **27** (308 mg, 0.825 mmol) was condensed with silylated N^4-benzoylcytosine (355 mg, 1.65 mmol) according

to the same procedure used in the preparation of **9**, to give **28** (192 mg, 51%) as white foam: UV (MeOH) λ_{max} 308.0 nm; $[\alpha]_D^{20}$ −10.49 (*c* 1.01, MeOH); ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.87 (dd, 2H, *J* = 7.2, 7.6 Hz), 7.57 (tt, 1H, *J* = 6.8, 8.0 Hz), 7.47 (dd, 2H, *J* = 7.6, 8.0 Hz), 6.21 (d, 1H, *J* = 7.2 Hz), 5.50 (d, 1H, *J* = 6.8 Hz), 4.63 (dd, 1H, *J* = 3.2, 5.2 Hz), 4.49 (dd, 1H, *J* = 3.2, 12.4 Hz), 4.39 (dd, 1H, *J* = 5.2, 12.4 Hz), 4.06–3.99 (m, 2H), 2.17 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.25, 170.34, 169.99, 166.94, 165.19, 162.69, 154.98, 144.34, 133.39, 133.09, 129.14, 127.80, 97.70, 87.55, 83.36, 81.84, 75.97, 62.43, 60.49, 50.95, 21.64, 21.16, 21.02; Anal. Calcd for $C_{23}H_{24}N_6O_9$: C, 52.27; H, 4.58; N, 15.90. Found: C, 52.55; H, 4.71; N, 16.03.

4.2.24. 1-(3β-*C*-Azidomethyl-β-D-ribofuranosyl)cytosine (4j)

A solution of **28** (75 mg, 0.142 mmol) in saturated methanolic ammonia (6 mL) was stirred in a glass bomb at 80 °C overnight and the volatiles were evaporated. The resulting residue was purified by C_{18} reverse phase column chromatography (0–3% acetone in water) to give **4j** (33 mg, 77%) as a white solid: mp 199–206 °C (dec); UV (MeOH) λ_{max} 271.0 nm; $[\alpha]_D^{20}$ −15.15 (*c* 0.63, MeOH); ^{1}H NMR (CD_3OD) δ 8.03 (d, 1H, *J* = 7.6 Hz), 5.98 (d, 1H, *J* = 7.6 Hz), 5.91 (d, 1H, *J* = 7.2 Hz), 4.20 (d, 1H, *J* = 7.2 Hz), 4.07 (t, 1H, *J* = 2.4 Hz), 3.76 (dd, 2H, *J* = 1.6, 2.4 Hz), 3.61 (d, 1H, *J* = 12.8 Hz), 3.53 (d, 1H, *J* = 12.8 Hz); ^{13}C NMR (CD_3OD) δ 167.73, 159.16, 144.24, 96.64, 91.30, 83.96, 80.94, 77.23, 62.05, 55.55; IR (KBr) 2113.37 cm^{-1} (s); Anal. Calcd for $C_{10}H_{14}N_6O_5$: C, 40.27; H, 4.73; N, 28.18. Found: C, 40.11; H, 4.91; N, 28.28.

4.3. Antiviral assay

4.3.1. Anti-HCV assay

The human hepatoma cell line Huh-7, carrying the subgenomic HCV genotype 1 replicon with the luc-ubi-neo (reporter/selective) fusion gene,[22] was kindly provided by Dr. Ralf Bartenschlager (University of Heidelberg, Heidelberg, Germany). The cells were grown as described.[22,23] The conditions of the luminescence-based assay used to test the antiviral activity of the compounds were previously described.[23] Briefly, Huh-5–2 cells were seeded at a density of 5×10^3 per well in a tissue culture-treated white 96-well view plate in complete DMEM supplemented with 500 μg/mL G418. After incubation for 24 h at 37 °C (5% CO_2), medium was refreshed (with G418) and DMSO stock of test compounds were added. After four days of incubation at 37 °C, cell culture medium was removed and luciferase activity was determined using the Steady-Glo luciferase assay system (Promega, Leiden, The Netherlands).

4.3.2. Cytostatic effect

Huh-5–2 cells were seeded at a density of 5×10^3 per well of a 96-well plate in complete DMEM with the appropriate concentrations of G418. Serial dilutions of the test compounds in complete DMEM without G418 were added 24 h after seeding. Cells were allowed to proliferate for three days at 37 °C, after which the cell number was determined by WST-1 assay.

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