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5′-O-Masked 2′-deoxyadenosine analogues as lead compounds for hepatitis C virus (HCV) therapeutic agents

Masahiro Ikejiri,[a] Takayuki Ohshima,[a] Keizo Kato,[a] Masaaki Toyama,[a]
Takayuki Murata,[b] Kunitada Shimotohno[b] and Tokumi Maruyama[a,*]

[a]Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan
[b]Department of Viral Oncology, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

Abstract—On the basis of our previous study on antiviral agents against the severe acute respiratory syndrome (SARS) coronavirus, a series of nucleoside analogues whose 5′-hydroxyl groups are masked by various protective groups such as carboxylate, sulfonate, and ether were synthesized and evaluated to develop novel anti-hepatitis C virus (HCV) agents. Among these, several 5′-O-masked analogues of 6-chloropurine-2′-deoxyriboside (e.g., 5′-O-benzoyl, 5′-O-p-methoxybenzoyl, and 5′-O-benzyl analogues) were found to exhibit effective anti-HCV activity. In particular, the 5′-O-benzoyl analogue exhibited the highest potency with an EC_{50} of 6.1 μM in a cell-based HCV replicon assay. Since the 5′-O-unmasked analogue (i.e., 6-chloropurine-2′-deoxyriboside) was not sufficiently potent ($EC_{50} = 47.2$ μM), masking of the 5′-hydroxyl group seems to be an effective method for the development of anti-HCV agents. Presently, we hypothesize two roles for the 5′-O-masked analogues: One is the role as an anti-HCV agent by itself, and the other is as a prodrug of its 5′-O-demasked (deprotected) derivative.
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1. Introduction

The hepatitis C virus (HCV), a member of the family *Flaviviridae*, is an enveloped, positive-sense, single-strand RNA virus.[1] The virus is a major causative agent of non-A, non-B hepatitis and infects an estimated 170 million people worldwide.[2] In most cases, acute phase infection with HCV is asymptomatic; however, the virus frequently establishes chronic hepatitis in up to 80% of the infected individuals and persists for decades with a substantially high risk of developing liver cirrhosis and hepatocellular carcinoma.[3] Currently, there is no vaccine available against HCV, and the approved drugs are combinations of interferon-α (IFN-α) and ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide). Although the use of pegylated IFN-α instead of the unmodified one has resulted in improved therapeutic effectiveness, the sustained virological response is still poor (41–55%); moreover, in some cases, significant side effects can be caused.[4] Therefore, more efficacious therapies are urgently required for ensuring public health.[5]

To date, a number of nucleoside analogues, including ribavirin, have been synthesized and tested for anti-HCV activity.[6,7] Among these, some 2′-modified ribonucleoside analogues such as 2′-C-methyl analogues 1,[6a] 2′-fluoro analogues 2,[6b] and 2′-O-methyl analogue 3[6c] exhibit potent anti-HCV activities in a cell-based HCV replicon assay (Fig. 1). Particularly, Valopicitabine, a prodrug of 1c, is currently in phase II clinical trials.[8] Recently, the anti-HCV properties of 4′-substituted ribonucleosides such as 4 have also been reported by the Roche group.[6d] Concerning the antiviral mechanism of common nucleoside analogues, it is known that most of these analogues are converted to their corresponding 5′-triphosphates by cellular and/or viral enzymes and then compete with natural triphosphates as substrates for
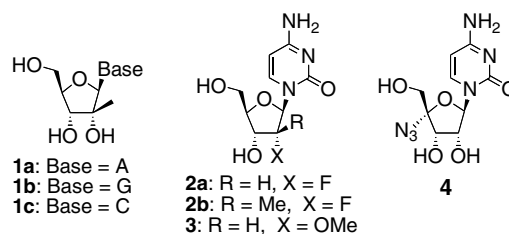
Figure 1. Examples of anti-HCV nucleoside analogues.

1a: Base = A
1b: Base = G
1c: Base = C

2a: R = H, X = F
2b: R = Me, X = F
3: R = H, X = OMe

4

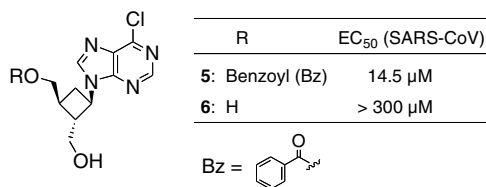| R	EC$_{50}$ (SARS-CoV)
5: Benzoyl (Bz)	14.5 μM
6: H	> 300 μM

Bz =

Figure 2. Anti-SARS-coronavirus (CoV) activities of carbocyclic oxetanocin analogues.

incorporation into viral nucleic acids during viral replication.[9] Therefore, the 5′-hydroxyl group is indispensable to the anti-HCV activity of these nucleoside analogues.[10]

Recently, we have reported that a carbocyclic oxetanocin analogue **5**, one of whose hydroxyl groups (that corresponds to the 5′-hydroxyl group of the ribonucleoside) was masked by a benzoyl group, showed antiviral activity against the severe acute respiratory syndrome (SARS) coronavirus (Fig. 2).[11] Interestingly, the antiviral activity of **5** (EC$_{50}$ = 14.5 μM) was much more efficacious than that of the unmasked **6** (almost no activity, EC$_{50}$ > 300 μM), which seems to indicate an inconsistency with the necessity of the 5′-hydroxyl group to this compound.

This result attracted our interest and led us to expect that this unique trend could contribute to developing anti-HCV agents as well. Thus, several nucleoside analogues, including **5** and **6**, were designed as candidate compounds for the agents (Fig. 3). In this paper, we report the syntheses of these nucleoside analogues and their biological evaluations as anti-HCV agents.

2. Results and discussion

2.1. Chemistry

Compounds **7**–**9**, **11**, **13**, **14**, **22**, and **23** were prepared based on the previous reports.[12] The syntheses of compounds **10**, **12**, and **15**–**19** were carried out by the treatment of diol **9** or **11** with the corresponding acyl or sulfonyl chloride (i.e., benzoyl chloride for **10** and **12**; pivaloyl chloride, butyryl chloride, *p*-methoxybenzoyl chloride, 2,4,6-trimethylbenzoyl chloride, and benzenesulfonyl chloride for **15**–**19**, respectively), as shown in Scheme 1. In the case of compound **18**, conventional conditions (trimethylbenzoyl chloride, DMAP, and pyridine) required a long reaction time and led to the gradual decomposition of **9** and **18**, probably because the 6-chloropurine moiety reacted with the amine bases by degrees due to its electrophilic nature. This side reaction was slightly suppressed by the use of a low-nucleophilic base, *N*,*N*-diisopropylethylamine instead of pyridine and DMAP.

Compound **10** was also prepared via an alternative route illustrated in Scheme 2A. Regioselective protection of the 5′-hydroxyl group of 2′-deoxyadenosine (**26**) yielded the benzoate **23** in 65% yield,[12c] which was subsequently converted to the silyl ether **27** in 93% yield. After transformation to the 6-chloropurine derivative **28** with a 58% yield,[13] the TBS group was cleaved to afford **10** in 72% yield. Compound **28** was also used as an intermediate in the synthesis of **25**, as shown in Scheme 2B. Exposure of **28** to sodium methylthiolate resulted in the formation of the debenzoylated 6-SMe analogue **29** with a 67% yield, which was reacylated into **30** in 97% yield. Unfortunately, the use of thiourea in place of sodium methylthiolate led to the decomposition of the resultant products. Finally, the removal of the TBS group afforded **25** in 88% yield. The synthesis of **24** is illustrated in Scheme 2C. The diacetate **31**, which was prepared as described in a previous report,[11] was treated with dimethylamine to furnish **32** in 62% yield.[14] Subsequently, regioselective benzoylation of the 5′-hydroxyl group in **32** afforded **24** in 77% yield.

Meanwhile, the syntheses of **20** and **21** turned out to be unexpectedly difficult because of the instability of these
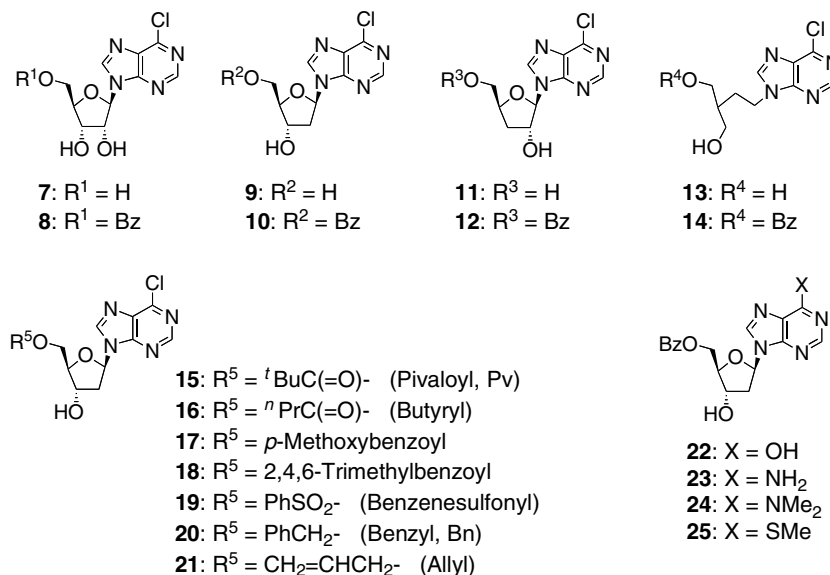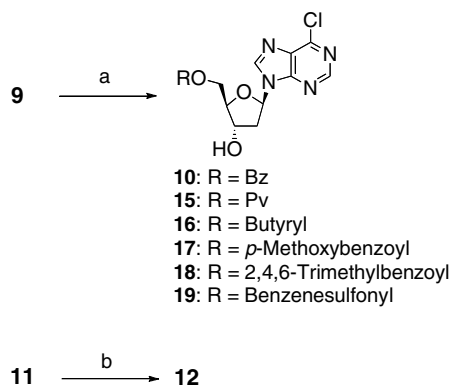

7: R^1 = H
8: R^1 = Bz

9: R^2 = H
10: R^2 = Bz

11: R^3 = H
12: R^3 = Bz

13: R^4 = H
14: R^4 = Bz

15: R^5 = ^{t}BuC(=O)- (Pivaloyl, Pv)
16: R^5 = ^{n}PrC(=O)- (Butyryl)
17: R^5 = *p*-Methoxybenzoyl
18: R^5 = 2,4,6-Trimethylbenzoyl
19: R^5 = PhSO$_2$- (Benzenesulfonyl)
20: R^5 = PhCH$_2$- (Benzyl, Bn)
21: R^5 = CH$_2$=CHCH$_2$- (Allyl)

22: X = OH
23: X = NH$_2$
24: X = NMe$_2$
25: X = SMe

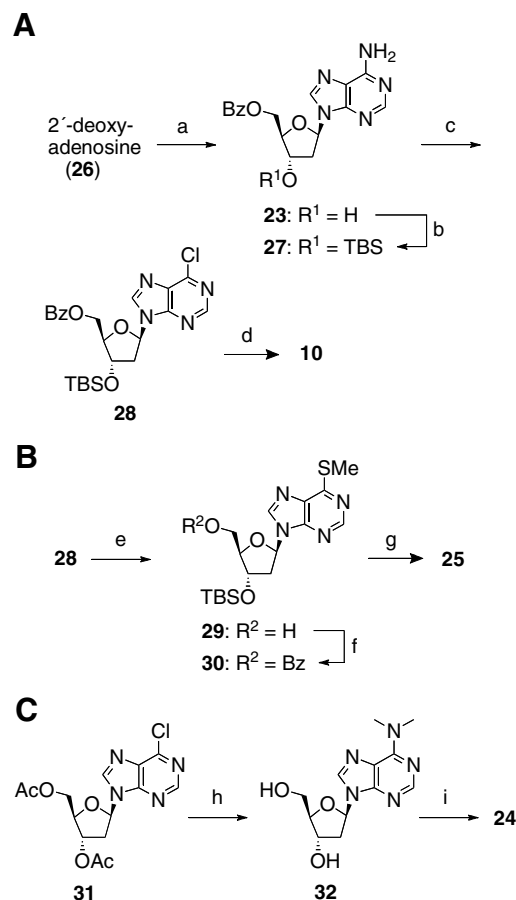**Figure 3.** The candidate compounds for anti-HCV agents.

Scheme 1. Reagents and conditions: (a) for **10**, BzCl, pyr, 0 °C, 85%; for **15**, PvCl, DMAP, pyr, 0 °C to rt, 81%; for **16**, butyryl chloride, pyr, 0 °C to rt, 68%; for **17**, *p*-methoxybenzoyl chloride, pyr, 0 °C, 72%; for **18**, 2,4,6-trimethylbenzoyl chloride, *N,N*-diisopropylethylamine, MeCN, rt, 21%; for **19**, benzenesulfonyl chloride, pyr, 0 °C to rt, 40%; (b) BzCl, pyr, CH₂Cl₂, 0 °C, 59%.


Scheme 2. Reagents and conditions: (a) BOPDC, BzOH, pyr, rt, 65% (Ref. [12c]); (b) TBSCl, imidazole, pyr, rt, 93%; (c) ′BuONO, Et₄NCl, CCl₄–CH₂Cl₂, 0–50 °C, 58%; (d) TBAF, AcOH, THF, rt, 72%; (e) MeSNa, water–DMF, rt, 67%; (f) BzCl, Et₃N, DMAP, CH₂Cl₂, 0 °C, 97%; (g) TBAF, THF, 0 °C, 88%; (h) Me₂NH, water–dioxane, rt, 62%; (i) BzCl, pyr, 0 °C to rt, 77%.

molecules under both strongly basic and acidic conditions; for example, the exposure of **9** to BnBr–NaH or BnOC(=NH)CCl₃–CF₃SO₃H afforded a complex mixture. Thus, a stepwise protection–deprotection process was required, which is illustrated in Scheme 3 (3A: synthesis of **20**, 3B: synthesis of **21**). The monoacetate **33**, which was prepared from **26** by employing a partly modified Santaniello's procedure,[15] was converted to the silyl ether **34** in 94% yield. The use of a mixed solvent (pyridine–DMF) effectively reduced the competitive silylation of the 6-amino group. Protection of the amino group with pivaloyl chloride followed by the removal of the acetyl group in **35** afforded **36** with a 98% yield in two steps. The addition of DMAP and MeOH in the *N*-pivaloylation step was effective in converting the *N,N*-dipivaloylated by-product to the desired **35**. Chemoselective benzylation of the 5′-hydroxyl group in **36** was successfully carried out by using 2.5 equiv of potassium *tert*-butoxide and 1.1 equiv of benzyl bromide in THF to provide **37** in 97% yield. After the removal of the pivaloyl group (76% yield), the resultant amino group of **38** was substituted with a chloro group to provide **39**, which was subsequently deprotected to afford **20** with a 34% yield in two steps. Compound **21** was synthesized employing almost the same method as that for **20** described above (Scheme 3B, 20% overall yield from **36**).

2.2. Biological evaluation

The above synthesized nucleoside analogues were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon Huh7 cell line (LucNeo#2),[16] and the result is shown in Table 1. These cells contain a HCV subgenomic replicon RNA encoding a luciferase reporter gene as a marker. The potency of the analogues against the HCV replicon is expressed as EC_{50}, which was quantified by a luciferase assay after a two-day incubation period with the corresponding compound. In addition, the associated cytotoxicity, which is expressed as CC_{50} in Table 1, was evaluated in a

tetrazolium (XTT)-based assay according to the manufacturer's protocol.

As expected, the benzoylated carbocyclic oxetanocin analogue **5** exhibited a stronger anti-HCV activity than that shown by the unprotected **6** ($EC_{50} = 6.4$ μM and 46.1 μM, respectively). This trend seems to be consistent with that of the anti-SARS-coronavirus activity, as mentioned above (Fig. 2; $EC_{50} = 14.5$ μM and >300 μM, respectively). However, compound **5** displayed a cytotoxic effect at a level close to its EC_{50} value ($CC_{50} = 20.0$ μM; antiviral index = 3.1); therefore, further evaluation of this compound was not undertaken.

2.2.1. Structure–activity relationship (SAR) of the sugar moiety. To evaluate the effect of the sugar moiety, several 6-chloropurine derivatives that possessed the ribofuranosyl structure (**7** and **8**), or deoxyribofuranosyl structure (2′-deoxy: **9** and **10**; 3′-deoxy: **11** and **12**), or an acyclic backbone (**13** and **14**) were tested. Among them, the 2′-deoxyribonucleoside derivative **10** exhibited the highest potency against the HCV replicon with an EC_{50} of 6.1 μM, which is ninefold potent over that of ribavirin, and a CC_{50} of 111 μM. Additionally, the
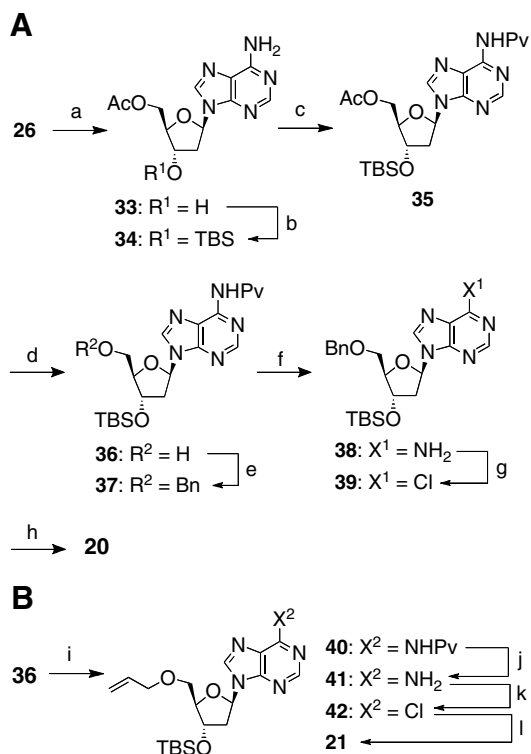
Scheme 3. Reagents and conditions: (a) lipase (CAL), vinyl acetate, MS4A, THF, 60 °C, 89%; (b) TBSCl, DMAP, pyr–DMF, rt, 94%; (c) PvCl, Et$_3$N, CH$_2$Cl$_2$, rt, and then DMAP, MeOH, rt; (d) K$_2$CO$_3$, MeOH, 0 °C, 98% from **34**; (e) BnBr, ^{t}BuOK, THF, 0 °C, 97%: (f) K$_2$CO$_3$, MeOH, rt, 76%, (g) ^{t}BuONO, Et$_4$NCl, CCl$_4$–CH$_2$Cl$_2$, 0–50 °C (h) TBAF, AcOH, THF, rt, 34% from **38**; (i) allyl bromide, ^{t}BuOK, THF, 0 °C; (j) K$_2$CO$_3$, MeOH, rt, 73% from **36**; (k) ^{t}BuONO, Et$_4$NCl, CCl$_4$–CH$_2$Cl$_2$, 0–50 °C (l) TBAF, AcOH, THF, rt, 28% from **41**.

Table 1. Inhibitory potency (EC$_{50}$) and cytotoxicity (CC$_{50}$) of compounds **5**–**25** and ribavirin (positive control) in HCV replicon assay[a]

Compound	EC$_{50}$[b] (μM)	CC$_{50}$[c] (μM)	Antiviral index[d]
5	6.4	20.0	3.1
6	46.1	>200	4.3
7	31.0	>200	>6.5
8	41.0	97.3	2.4
9	47.2	173	3.7
10	6.1	111	18.2
11	64.1	>200	>3.1
12	38.1	70.5	1.9
13	80.8	>200	>2.5
14	13.3	108	8.1
15	28.0	177	6.3
16	30.3	>200	>6.6
17	8.4	76.4	9.1
18	>50	91.0	<1.8
19	28.8	146	5.1
20	17.7	178	10.1
21	24.5	140	5.7
22	>100	—	—
23	105	>200	>1.9
24	22.3	>200	>9.0
25	>100	—	—
Ribavirin	54.8	>200	>3.7

[a] The same experiment was performed at least three times independently.
[b] Average of 50% effective concentrations.
[c] Average of 50% cytotoxic concentrations.
[d] Antiviral index was defined as the 50% toxic dose divided by the 50% effective dose.

5′-*O*-benzoyl analogue **10** was more potent than the corresponding unprotected analogue **9** (EC$_{50}$: 6.1 μM (5′-OBz, **10**) vs 47.2 μM (5′-OH, **9**)). This trend was also observed in the acyclic analogues **13** and **14** (EC$_{50}$: 13.3 μM (−OBz, **14**) vs 80.8 μM (−OH, **13**)). In contrast, in case of the ribofuranosyl structure, the 5′-hydroxyl analogue **7** was slightly more potent as compared with the 5′-*O*-benzoyl analogue **8** (EC$_{50}$ = 31.0 and 41.0 μM, respectively). These results indicate the importance of the masked 5′-hydroxyl group in the 2′-deoxyribofuranosyl structure. It is also interesting to note that the 2′-deoxyribonucleoside derivative **10** was more potent than the corresponding ribonucleoside derivatives such as **7** and **8** in spite of the fact that HCV is an RNA virus and most of the anti-HCV agents bear ribofuranosyl structures (or their bioisosteres).[6,7]

2.2.2. SAR of the 5′-*O*-moiety. As a next step, we evaluated the inhibitory activities of compounds **15**–**21**, whose 5′-hydroxyl groups were masked with various protective groups (i.e., pivaloyl, butyryl, trimethylbenzoyl, benzenesulfonyl, benzyl, and allyl groups) to examine the effect of the substituent group at the 5′-position. Among these compounds, the EC$_{50}$ value of the close structural analogue **17** (*p*-methoxybenzoyl analogue) was comparable to that of **10** (EC$_{50}$ = 8.4 and 6.1 μM, respectively); however, **17** led to an undesir-

able increase in cytotoxicity (CC$_{50}$ = 76.4 μM). Notably, other types of protective groups such as ether groups (compounds **20** and **21**) and a sulfonate group (compound **19**) also exhibited good anti-HCV activity (EC$_{50}$ = 17.7, 24.5, and 28.8 μM, respectively). Overall, it appears that protective groups containing a phenyl ring are preferable for the antiviral activity.

2.2.3. SAR at position 6 of the purine base. The effect of the substituents at the purine 6-position was investigated by the evaluation of compounds **22**–**25**. Among these analogues, the 6-dimethylamino derivative **24** exhibited a good potency with an EC$_{50}$ of 22.3 μM and low cytotoxicity (CC$_{50}$ > 200 μM), while the 6-amino derivative **23** showed a weak potency (EC$_{50}$ = 105 μM). Unfortunately, the others (i.e., 6-hydroxyl (hypoxanthine) analogue **22** and 6-methylthio analogue **25**) did not show any significant potency (EC$_{50}$ > 100 μM). Accordingly, the chloro group at the purine 6-position was considered to be important for the anti-HCV activity.[17]

The luciferase assay described above revealed that compound **10** exhibited the maximum potency among the analogues. Next, in order to confirm the anti-HCV activity of **10**, the replicon RNA levels were quantified by performing real-time RT-PCR analysis.[16] Fig. 4 shows the result obtained with **10** and ribavirin (positive control). Compound **10** reduced the replicon RNA amount up to approximately 45% at 12.5 μM and 6% at 25 μM, which is almost consistent with the result of the luciferase assay (EC$_{50}$ = 6.1 μM).
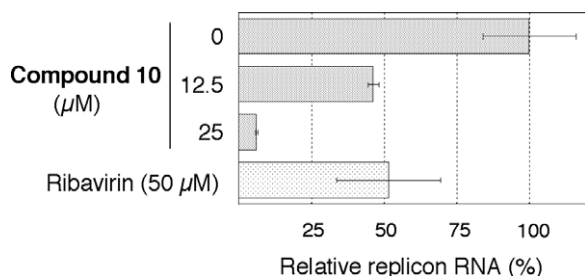
Figure 4. The quantity of HCV subgenomic replicon RNA. The RNA amounts were determined by real-time RT-PCR analysis after a two-day incubation period with compound **10** or ribavirin.

Among these molecules, two compounds can be hypothesized to be species with real antiviral activity. One is the 5′-*O*-masked 2′-deoxyadenosine analogue itself such as **10**, and the other is the deprotected **9** (or its activated form, 5′-triphosphate) since carboxylic ester bonds are often hydrolyzed in cultured cells, as reported earlier.[18] However, the chemically stable 5′-*O*-masked analogues **20** and **21** (Bn and allyl ether, respectively) showed anti-HCV potency to some extent. Moreover, the deoxyadenosine derivative **23**, which would be transformed to the inactive 2′-deoxyadenosine after the hydrolysis, also showed potency. Thus, taking these results into consideration, it would be reasonable to consider that some 5′-*O*-masked analogues themselves possess the anti-HCV potency. On the other hand, it would also be reasonable to consider that compound **9** (or its 5′-triphosphate) is one of the real active species since **9** also exhibited anti-HCV activity, though only moderately; in other words, compound **10** operates as a prodrug of **9**.[19] Therefore, at this stage, we believe that both compounds (e.g., **9** and **10**) would function as the species with antiviral activity in the cells.

3. Conclusion

A series of nucleoside analogues **5**–**25** were synthesized and their abilities to inhibit HCV RNA replication were evaluated. Among these, several 5′-*O*-masked analogues of 6-chloropurine-2′-deoxyriboside, such as **10**, **17**, and **20**, exhibited effective anti-HCV activity. In particular, 5′-*O*-benzoyl analogue **10** exhibited the highest activity. Presently, we hypothesize two roles for these 5′-*O*-masked analogues: One is the role as an anti-HCV agent by itself, and the other is as a prodrug of its 5′-*O*-de-masked (deprotected) derivative.

There are two notable structural features of these potent compounds: one is the masked 5′-hydroxyl group, and the other is the 2′-deoxyribofuranosyl structure. In relation to the substituent group at position 6 of the purine base, the chloro group seems to be preferable. These unique features are rarely seen in common anti-HCV agents; therefore, although some issues such as improvement of the antiviral potency remain to be resolved, we hope that the present study will contribute to developing a new class of HCV therapeutic agents.

4. Experimental

4.1. Chemistry

4.1.1. General methods. The chemicals used were of commercial origin (Wako chemicals, TCI, Kanto Kagaku, nacalai tesque, and Aldrich) and were employed without further purification. The progress of all reactions was monitored by TLC (silica gel 60F$_{254}$, Merck). The column chromatography was performed with a Combi-*Flash* Companion system (Teledyne Isco, Inc.). The melting points were measured on a Yanaco melting point apparatus and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400M spectrometer. The solvent ^{1}H and ^{13}C signals were used as internal standards (DMSO-d_6: 2.50 and 39.5 ppm; acetone-d_6: 2.05 and 29.8 ppm; CD$_3$OD: 3.31 and 49.0 ppm; CDCl$_3$: 7.26 and 77.0 ppm, respectively). The mass spectra were recorded on a Bruker Apex-Qe FT-ICR MS spectrometer.

4.1.2. Synthesis of 9-(5-*O*-benzoyl-β-D-2-deoxyribofur-anosyl)-6-chloropurine (10). To a stirred solution of **9** (50 mg, 0.18 mmol) in pyridine (0.5 mL) was added benzoyl chloride (32 μL, 0.28 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1.5 h. Subsequent to the addition of saturated NaHCO$_3$ solution, the mixture was extracted with ethyl acetate. The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2:1 to 3:1) to give **10** (59 mg, 85%) as a white solid. Mp 111.0–111.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.47 (1H, m), 2.99 (1H, dt, J = 13.6, 6.4 Hz), 4.19 (1H, q-like, J = 4.4 Hz), 4.44 (1H, dd, J = 12.0, 6.0 Hz), 4.56 (1H, dd, J = 12.0, 4.0 Hz), 4.68 (1H, quintet-like, J = 4.8 Hz), 5.61 (1H, br d, J = 2.8 Hz), 6.50 (1H, t, J = 6.4 Hz), 7.48 (2H, br t, J = 7.6 Hz), 7.64 (1H, br t, J = 7.6 Hz), 7.85 (2H, br d, J = 7.6 Hz), 8.74 (1H, s), 8.86 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 38.4, 64.1, 70.2, 84.2, 84.3, 128.7, 129.1, 129.3, 131.5, 133.4, 146.2, 149.3, 151.2, 151.6, 165.4; HRMS (ESI) calcd for C$_{17}$H$_{15}$ClN$_4$NaO$_4$ (M+Na$^+$) 397.0680, found 397.0664.

4.1.3. 6-Chloro-9-(5-*O*-pivaloyl-β-D-2-deoxyribofurano-syl)purine (15). To a stirred solution of **9** (63 mg, 0.23 mmol) and DMAP (3 mg, 10 mol%) in pyridine (0.5 mL) was added pivaloyl chloride (43 μL, 0.35 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 30 min and then at room temperature for 15 min. The work-up process was performed in the same manner as described for **10**. The crude material was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 2:1) to give **15** (67 mg, 81%) as a white solid. Mp 111.5–112.0 °C. ^{1}H NMR (DMSO-d_6) δ: 1.06 (9H, s), 2.45 (1H, m), 2.91 (1H, dt, J = 13.6, 6.0 Hz), 4.04 (1H, m), 4.16 (1H, dd, J = 12.0, 6.0 Hz), 4.26 (1H, dd, J = 12.0, 4.4 Hz), 4.52 (1H, m), 5.54 (1H, br d, J = 2.8 Hz), 6.47 (1H, t, J = 6.4 Hz), 8.80 (1H, s), 8.84 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 26.7, 38.1, 38.4, 63.6, 70.1, 84.1,

84.2, 131.5, 146.0, 149.3, 151.2, 151.6, 177.2; HRMS (ESI) calcd for $C_{15}H_{19}ClN_4NaO_4$ (M+Na$^+$) 377.0993, found 377.0978.

4.1.4. 9-(5-*O*-Butyryl-β-D-2-deoxyribofuranosyl)-6- chloropurine (16).

To a stirred solution of **9** (100 mg, 0.37 mmol) in pyridine (1.0 mL) was added butyryl chloride (61 μL, 0.59 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 4.5 h. The work-up process was performed in the same manner as described for **10**. The crude material was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 3:2) to give **16** (97 mg, 68%) as a colorless oil. ^{1}H NMR (acetone-d_6) δ: 0.88 (3H, t, $J = 7.6$ Hz), 1.56 (2H, sextet, $J = 7.6$ Hz), 2.26 (2H, t, $J = 7.6$ Hz), 2.61 (1H, m), 3.03 (1H, dt, $J = 13.6$, 6.4 Hz), 4.21 (1H, q-like, $J = 4.4$ Hz), 4.31 (1H, dd, $J = 12.4$, 5.6 Hz), 4.34 (1H, dd, $J = 12.4$, 4.8 Hz), 4.70–4.75 (2H, m), 6.59 (1H, t, $J = 6.4$ Hz), 8.66 (1H, s), 8.73 (1H, s); ^{13}C NMR (acetone-d_6) δ: 13.7, 18.9, 36.2, 40.3, 64.3, 72.0, 85.8, 85.9, 133.0, 145.9, 150.9, 152.4, 173.4; HRMS (ESI) calcd for $C_{14}H_{17}ClN_4NaO_4$ (M+Na$^+$) 363.0836, found 363.0821.

4.1.5. 6-Chloro-9-(5-*O*-*p*-methoxybenzoyl-β-D-2-deoxyribofuranosyl)purine (17).

To a stirred solution of **9** (100 mg, 0.37 mmol) in pyridine (1.0 mL) was added *p*-methoxybenzoyl chloride (61 μL, 0.44 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1 h. The work-up process was performed in the same manner as described for **10**. The crude material was purified by silica gel column chromatography (ethyl acetate/hexane, 3:1 to 1:0) to give **17** (108 mg, 72%) as a white solid. Mp 148.5–149.0 °C. ^{1}H NMR (DMSO-d_6) δ: 2.47 (1H, m), 2.98 (1H, dt, $J = 13.2$, 6.4 Hz), 3.83 (3H, s), 4.17 (1H, dt, $J = 6.0$, 4.0 Hz), 4.38 (1H, dd, $J = 12.0$, 6.0 Hz), 4.52 (1H, dd, $J = 12.0$, 4.4 Hz), 4.67 (1H, quintet, $J = 5.2$ Hz), 5.59 (1H, br d, $J = 4.4$ Hz), 6.49 (1H, t, $J = 6.4$ Hz), 6.98 (2H, br d, $J = 8.8$ Hz), 7.79 (2H, br d, $J = 8.8$ Hz), 8.76 (1H, s), 8.84 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 38.4, 55.5, 63.7, 70.2, 84.3, 84.4, 113.9, 121.5, 131.2, 131.5, 146.1, 149.3, 151.2, 151.6, 163.2, 165.1; HRMS (ESI) calcd for $C_{18}H_{17}ClN_4NaO_5$ (M+Na$^+$) 427.0785, found 427.0767.

4.1.6. 6-Chloro-9-(5-*O*-(2,4,6-trimethylbenzoyl)-β-D-2-deoxyribofuranosyl)purine (18).

To a stirred solution of **9** (100 mg, 0.37 mmol) and *N,N*-diisopropylethylamine (478 μL, 2.96 mmol) in acetonitrile (4.0 mL) was added 2,4,6-trimethylbenzoyl chloride (303 μL, 1.85 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 3 h. The work-up process was performed in the same manner as described for **10**. The crude material was purified by preparative thin layer chomatography (Merck, 113895) (ethyl acetate/hexane, 2:1) to give **18** (33 mg, 21%) as a white semi-solid. ^{1}H NMR (acetone-d_6) δ: 2.14 (6H, s), 2.25 (3H, s), 2.62 (1H, ddd, $J = 13.6$, 6.8, 4.0 Hz), 3.11 (1H, dt, $J = 13.6$, 6.0 Hz), 4.34 (1H, m), 4.56 (1H, dd, $J = 12.0$, 4.4 Hz), 4.59 (1H, dd, $J = 12.0$, 6.0 Hz), 4.79–4.86 (2H, m), 6.61 (1H, t, $J = 6.4$ Hz), 6.84 (2H, br s), 8.60 (1H, s), 8.66 (1H, s); ^{13}C NMR (acetone-d_6) δ: 19.7, 21.1, 40.0,

65.1, 72.4, 85.9, 86.0, 129.0, 131.8, 133.0, 135.6, 140.0, 146.1, 150.9, 152.3, 152.4, 169.9; HRMS (ESI) calcd for $C_{20}H_{21}ClN_4NaO_4$ (M+Na$^+$) 439.1149, found 439.1127.

4.1.7. 9-(5-*O*-Benzenesulfonyl-β-D-2-deoxyribofuranosyl)-6-chloropurine (19).

To a stirred solution of **9** (100 mg, 0.37 mmol) in pyridine (1.0 mL) was added benzenesulfonyl chloride (94 μL × 4 times at intervals of 30 min, 0.74 × 4 mmol) at room temperature, and the mixture was stirred at the same temperature for 3 h in total. The work-up process was performed in the same manner as described for **10**. The crude material was purified by silica gel column chromatography (methanol/chloroform, 1:20) to give **19** (60 mg, 40%) as a white semi-solid. ^{1}H NMR (acetone-d_6) δ: 2.57 (1H, ddd, $J = 14.0$, 6.8, 4.4 Hz), 3.00 (1H, dt, $J = 14.0$, 6.4 Hz), 4.21 (1H, m), 4.33 (1H, dd, $J = 10.8$, 6.0 Hz), 4.40 (1H, dd, $J = 10.8$, 4.0 Hz), 4.73 (1H, m), 4.81 (1H, br d, $J = 4.0$ Hz), 6.56 (1H, t, $J = 6.8$ Hz), 7.56 (2H, t, $J = 8.0$ Hz), 7.68 (1H, t, $J = 8.0$ Hz), 7.85 (2H, d, $J = 8.0$ Hz), 8.57 (1H, s), 8.66 (1H, s); ^{13}C NMR (acetone-d_6) δ: 39.8, 70.8, 72.0, 85.5, 86.0, 128.5, 130.2, 133.1, 134.8, 136.7, 146.1, 150.9, 152.3, 152.3; HRMS (ESI) calcd for $C_{16}H_{15}ClN_4NaO_5S$ (M+Na$^+$) 433.0349, found 433.0330.

4.1.8. 9-(5-*O*-Benzoyl-β-D-3-deoxyribofuranosyl)-6-chloropurine (12).

To a stirred solution of **11** (600 mg, 2.22 mmol) in CH_2Cl_2 (15 mL) were added pyridine (3.5 mL) and benzoyl chloride (268 μL, 2.31 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 2 h. Subsequent to the addition of saturated NaHCO$_3$ solution, the mixture was extracted with ethyl acetate. The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 2:1) to give **12** (488 mg, 59%) as a white solid. Mp 160.5–161.0 °C. ^{1}H NMR (DMSO-d_6) δ: 2.12 (1H, ddd, $J = 13.2$, 6.0, 2.0 Hz), 2.47 (1H, m), 4.50 (1H, dd, $J = 12.0$, 5.6 Hz), 4.57 (1H, dd, $J = 12.0$, 2.8 Hz), 4.73 (1H, m), 4.87 (1H, m), 5.85 (1H, br s), 6.06 (1H, d, $J = 2.0$ Hz), 7.47–7.51 (2H, m), 7.66 (1H, m), 7.85–7.87 (2H, m), 8.75 (1H, s), 8.80 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 34.6, 65.4, 74.1, 78.3, 91.7, 128.7, 129.1, 129.3, 131.4, 133.4, 145.6, 149.2, 151.1, 151.6, 165.5; HRMS (ESI) calcd for $C_{17}H_{16}ClN_4O_4$ (M+H$^+$) 375.0860, found 375.0867.

4.1.9. 5′-*O*-Benzoyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (27).

To a stirred solution of **23** (915 mg, 2.45 mmol) in pyridine (12 mL) were added imidazole (1.00 g, 14.7 mmol) and *tert*-butyldimethylsilyl chloride (1.11 g, 7.36 mmol) at room temperature, and the mixture was stirred overnight at the same temperature. Subsequent to the addition of water, the mixture was stirred for 15 min. After concentration under reduced pressure, the residue was dissolved in ethyl acetate. The ethyl acetate solution was washed with water, 1 M aqueous HCl, saturated NaHCO$_3$ solution and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure.

The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 4:1 to 1:0) to give **27** (1.07 g, 93%) as a white solid. Mp 129.5–130.0 °C. ^{1}H NMR (CDCl$_3$) δ: 0.14 (6H, s), 0.93 (9H, s), 2.49 (1H, ddd, J = 13.2, 6.4, 4.8 Hz), 2.97 (1H, dt, J = 13.2, 6.0 Hz), 4.28 (1H, q-like, J = 4.0 Hz), 4.50 (1H, dd, J = 12.4, 4.8 Hz), 4.62 (1H, dd, J = 12.4, 4.4 Hz), 4.80 (1H, m), 5.75 (2H, br s), 6.39 (1H, t, J = 6.4 Hz), 7.42 (2H, t, J = 8.0 Hz), 7.56 (1H, t, J = 8.0 Hz), 7.94 (1H, s), 7.97 (2H, d, J = 8.0 Hz), 8.32 (1H, s); ^{13}C NMR (CD$_3$OD) δ: −4.7, −4.5, 18.8, 26.3, 40.5, 64.5, 73.4, 86.0, 86.2, 120.7, 129.6, 130.5, 130.9, 134.4, 141.5, 150.2, 153.8, 157.3, 167.6; HRMS (ESI) calcd for C$_{23}$H$_{31}$N$_5$NaO$_4$Si (M+Na$^+$) 492.2043, found 429.2021.

4.1.10. 9-[5-O-Benzoyl-3-O-(*tert*-butyldimethylsilyl)-β-D-2-deoxyribofuranosyl]-6-chloropurine (28). To a stirred solution of **27** (245 mg, 0.52 mmol) in CCl$_4$ (10 mL) were added a solution of tetraethylammonium chloride (346 mg, 2.08 mmol) in CH$_2$Cl$_2$ (2.5 mL) and then *tert*-butyl nitrite (313 μL, 2.60 mmol) at ice-water temperature. The mixture was stirred for 1 h at the same temperature, warmed to room temperature for 0.5 h, and stirred at 50 °C for 2 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:3 to 1:2) to give **28** (147 mg, 58%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6) δ: 0.12 (3H, s), 0.13 (3H, s), 0.90 (9H, s), 2.47 (1H, m), 3.09 (1H, dt, J = 13.2, 6.0 Hz), 4.19 (1H, q-like, J = 4.8 Hz), 4.42 (1H, dd, J = 12.0, 5.2 Hz), 4.57 (1H, dd, J = 12.0, 4.8 Hz), 4.92 (1H, q, J = 5.6 Hz), 6.50 (1H, t, J = 6.4 Hz), 7.46–7.50 (2H, m), 7.64 (1H, m), 7.82–7.85 (2H, m), 8.74 (1H, s), 8.87 (1H, s); ^{13}C NMR (acetone-d_6) δ: −4.7, −4.6, 18.5, 26.1, 40.1, 64.4, 73.1, 85.9, 85.9, 129.4, 130.1, 130.7, 133.2, 134.0, 146.5, 151.0, 152.3, 152.3, 166.4; HRMS (ESI) calcd for C$_{23}$H$_{29}$ClN$_4$NaO$_4$Si (M+Na$^+$) 511.1544, found 511.1517.

4.1.11. An alternative synthesis of compound 10. To a stirred solution of **28** (330 mg, 0.67 mmol) in THF (5 mL) was added 1 M THF solution of TBAF–AcOH (1:1, 810 μL, 0.81 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 5 h and then at room temperature for 1 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 2:1 to 3:1) to give **10** (181 mg, 72%) as a white solid.

4.1.12. 3′-O-(*tert*-Butyldimethylsilyl)-2′-deoxy-6-S-methyl-6-thioinosine (29). To a stirred solution of **28** (225 mg, 0.46 mmol) in DMF (3.5 mL) was added a solution of methyl mercaptan sodium salt (15% in water, 860 μL, 1.84 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 3 h. After dilution of the mixture with ethyl acetate, the organic layer was washed with water, saturated NaHCO$_3$ solution and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane,

1:3 to 1:2) to give **29** (122 mg, 67%) as a white solid. Mp 96.0–96.5 °C. ^{1}H NMR (DMSO-d_6) δ: 0.12 (6H, s), 0.90 (9H, s), 2.33 (1H, ddd, J = 13.2, 6.4, 3.2 Hz), 2.67 (3H, s), 2.87 (1H, ddd, J = 13.2, 7.6, 6.0 Hz), 3.51 (1H, m), 3.61 (1H, dt, J = 11.6, 5.2 Hz), 3.88 (1H, m), 4.62 (1H, m), 5.04 (1H, t, J = 5.6 Hz), 6.42 (1H, t, J = 6.8 Hz), 8.67 (1H, s), 8.74 (1H, s); ^{13}C NMR (acetone-d_6) δ: −4.7, −4.6, 11.6, 18.5, 26.1, 41.5, 63.0, 74.0, 86.5, 90.0, 133.2, 143.7, 148.6, 152.0, 162.3; HRMS (ESI) calcd for C$_{17}$H$_{28}$N$_4$NaO$_3$SSi (M+Na$^+$) 419.1549, found 419.1558.

4.1.13. 5′-O-Benzoyl-3′-O-(*tert*-butyldimethylsilyl)-2′-deoxy-6-S-methyl-6-thioinosine (30). To a stirred solution of **29** (122 mg, 0.31 mmol) in CH$_2$Cl$_2$ (4 mL) were added triethylamine (255 μL, 1.86 mmol), DMAP (4 mg, 10 mol%), and benzoyl chloride (71 μL, 0.62 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 2 h. Subsequent to the addition of saturated NaHCO$_3$ solution, the mixture was extracted with ethyl acetate. The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:4) to give **30** (150 mg, 97%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ: 0.12 (3H, s), 0.12 (3H, s), 0.90 (9H, s), 2.40–2.55 (1H, overlapping with DMSO-d_6), 2.66 (3H, s), 3.10 (1H, dt, J = 13.2, 6.4 Hz), 4.17 (1H, q-like, J = 4.4 Hz), 4.41 (1H, dd, J = 12.0, 5.2 Hz), 4.55 (1H, dd, J = 12.0, 4.8 Hz), 4.91 (1H, m), 6.46 (1H, t, J = 6.4 Hz), 7.46–7.50 (2H, m), 7.65 (1H, m), 7.84–7.87 (2H, m), 8.64 (1H, s), 8.68 (1H, s); ^{13}C NMR (acetone-d_6) δ: −4.7, −4.6, 11.5, 18.5, 26.1, 40.1, 64.5, 73.2, 85.5, 85.7, 129.3, 130.2, 130.8, 133.0, 134.0, 143.7, 148.8, 152.3, 161.9, 166.5; HRMS (ESI) calcd for C$_{24}$H$_{32}$N$_4$NaO$_4$SSi (M+Na$^+$) 533.1811, found 533.1818.

4.1.14. 5′-O-Benzoyl-2′-deoxy-6-S-methyl-6-thioinosine (25). To a stirred solution of **30** (150 mg, 0.30 mmol) in THF (3 mL) was added 1 M THF solution of TBAF (360 μL, 0.36 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1.5 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 2:1) to give **25** (102 mg, 88%) as a white solid. Mp 148.0–148.5 °C. ^{1}H NMR (DMSO-d_6) δ: 2.46 (1H, m), 2.66 (3H, s), 2.99 (1H, dt, J = 13.6, 6.4 Hz), 4.17 (1H, m), 4.43 (1H, dd, J = 12.0, 6.0 Hz), 4.55 (1H, dd, J = 12.0, 4.4 Hz), 4.68 (1H, m), 5.58 (1H, br d, J = 4.4 Hz), 6.47 (1H, t, J = 6.4 Hz), 7.49 (2H, t, J = 7.6 Hz), 7.65 (1H, t, J = 7.6 Hz), 7.87 (2H, d, J = 7.6 Hz), 8.62 (1H, s), 8.69 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 11.5, 40.0, 65.1, 72.2, 85.5, 85.7, 129.4, 130.2, 130.9, 132.9, 134.0, 143.3, 148.9, 152.4, 161.9, 166.5; HRMS (ESI) calcd for C$_{18}$H$_{18}$N$_4$NaO$_4$S (M+Na$^+$) 409.0946, found 409.0929.

4.1.15. N^6,N^6-Dimethyl-2′-deoxyadenosine (32). To a stirred solution of **31** (200 mg, 0.56 mmol) in dioxane (1 mL) was added 50% dimethylamine–water solution (8 mL) at room temperature, and the mixture was stirred

overnight at the same temperature. The solvent was removed under reduced pressure, and the resultant residue was washed with ether several times, and purified by silica gel column chromatography (methanol/chloroform, 1:10) to give **32** (98 mg, 62%) as a white solid. The ^1H and ^{13}C NMR spectra and the mass spectrum were identical to the reported values.[14]

4.1.16. 5′-*O*-Benzoyl-*N*[6],*N*[6]-dimethyl-2′-deoxyadenosine (24). To a stirred solution of **32** (290 mg, 1.04 mmol) in pyridine (5 mL) was added benzoyl chloride (181 μL, 1.56 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 4 h. Subsequent to the addition of saturated NaHCO$_3$ solution, the mixture was extracted with ethyl acetate. The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 3:2 to 4:1) to give **24** (306 mg, 77%) as a white semi-solid. ^1H NMR (acetone-d_6) δ: 2.53 (1H, ddd, $J = 13.6$, 6.8, 4.0 Hz), 3.05 (1H, dt, $J = 13.6$, 6.8 Hz), 3.49 (6H, br s), 4.30 (1H, q-like, $J = 4.4$ Hz), 4.54 (1H, dd, $J = 12.0$, 5.6 Hz), 4.62 (1H, dd, $J = 12.0$, 4.4 Hz), 4.70 (1H, br d, $J = 4.0$ Hz), 4.85 (1H, m), 6.50 (1H, t, $J = 6.8$ Hz), 7.49 (2H, t, $J = 8.0$ Hz), 7.64 (1H, t, $J = 8.0$ Hz), 7.99 (2H, d, $J = 8.0$ Hz), 8.14 (1H, s), 8.20 (1H, s); ^{13}C NMR (acetone-d_6) δ: 38.4, 40.1, 65.3, 72.3, 72.4, 85.1, 85.5, 121.3, 129.4, 130.2, 130.9, 134.0, 138.4, 151.2, 152.9, 155.7, 166.6; HRMS (ESI) calcd for $C_{19}H_{21}N_5NaO_4$ (M+Na$^+$) 406.1491, found 406.1475.

4.1.17. 5′-*O*-Acetyl-2′-deoxyadenosine (33). 2′-Deoxyadenosine (**26**) (540 mg, 2.01 mmol), vinyl acetate (417 μL, 4.5 mmol), molecular sieves 4 A (500 mg), and lipase acrylic resin from *Candida antarctica* (300 mg) purchased from SIGMA were suspended in THF (20 mL), and the mixture was stirred at 60 °C for 1.5 h. The enzyme was filtered off and washed with MeOH, and the solvents were removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (methanol/chloroform, 1:20 to 1:10) to give **33** (523 mg, 89%) as a white solid. The ^1H NMR spectrum was identical to the reported values.[15]

4.1.18. 5′-*O*-Acetyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (34). To a stirred solution of **33** (670 mg, 2.29 mmol) in pyridine-DMF (1:1, 12 mL) were added *tert*-butyldimethylsilyl chloride (860 mg, 5.71 mmol) and DMAP (140 mg, 1.15 mmol) at room temperature, and the mixture was stirred overnight at the same temperature. Subsequent to the addition of water, the mixture was stirred for 20 min and then extracted with ethyl acetate. The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate) to give **34** (940 mg, 94%) as a white solid. Mp 145–146 °C. ^1H NMR (DMSO-d_6) δ: 0.12 (6H, s), 0.90 (9H, s), 1.98 (3H, s), 2.32 (1H, ddd, $J = 13.6$, 6.8, 4.0 Hz), 2.97 (1H, dt, $J = 13.6$, 6.4 Hz), 3.99 (1H, q-like, $J = 3.6$ Hz), 4.12 (1H, dd, $J = 12.0$, 6.0 Hz), 4.24 (1H, dd, $J = 12.0$, 5.2 Hz), 4.69 (1H, m), 6.34 (1H, t,

$J = 6.8$ Hz), 7.29 (2H, br s), 8.14 (1H, s), 8.32 (1H, s); ^{13}C NMR (DMSO-d_6) δ: −5.1, −4.9, 17.6, 20.5, 25.6, 38.4, 63.4, 72.4, 83.3, 84.1, 119.2, 139.7, 149.0, 152.5, 156.1, 170.0; HRMS (ESI) calcd for $C_{18}H_{30}N_5O_4Si$ (M+H$^+$) 408.2067, found 408.2082.

4.1.19. 3′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxy-*N*-(2,2-dimethyl-1-oxopropyl)adenosine (36). To a stirred solution of **34** (300 mg, 0.74 mmol) and triethylamine (408 μL, 2.94 mmol) in CH$_2$Cl$_2$ (6 mL) was added pivaloyl chloride (207 μL, 1.70 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 1 h. Subsequent to the addition of DMAP (9 mg, 10 mol%) and methanol (2 mL), the mixture was stirred overnight at room temperature. After dilution of the mixture with ethyl acetate, the organic layer was washed with water, 5% KHSO$_4$ solution, saturated NaHCO$_3$ solution and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure to leave the crude compound **35** (385 mg), which was employed in the next reaction without purification.

To a stirred solution of **35** (385 mg) in methanol (5 mL) was added potassium carbonate (203 mg, 1.48 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 0.5 h. Subsequent to the addition of acetic acid (150 μL) and water (3 mL), the organic solvent was removed under reduced pressure. The resultant mixture was extracted with ethyl acetate, and the organic layer was washed with saturated NaHCO$_3$ solution and brine, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 3:1 to 4:1) to give **36** (324 mg, 98% from **34**) as a white solid. *Compound 35*: ^1H NMR (DMSO-d_6) δ: 0.13 (6H, s), 0.91 (9H, s), 1.28 (9H, s), 1.98 (3H, s), 2.40 (1H, ddd, $J = 13.2$, 6.4, 4.0 Hz), 3.01 (1H, dt, $J = 13.2$, 6.4 Hz), 4.03 (1H, m), 4.14 (1H, dd, $J = 12.0$, 6.0 Hz), 4.25 (1H, dd, $J = 12.0$, 4.8 Hz), 4.73 (1H, m), 6.46 (1H, t, $J = 6.8$ Hz), 8.61 (1H, s), 8.69 (1H, s), 10.14 (1H, br s). *Compound 36*: Mp 86–87 °C. ^1H NMR (DMSO-d_6) δ: 0.12 (6H, s), 0.91 (9H, s), 1.28 (9H, s), 2.34 (1H, ddd, $J = 13.6$, 6.4, 3.6 Hz), 2.89 (1H, ddd, $J = 13.6$, 7.2, 6.0 Hz), 3.52 (1H, dt, $J = 12.0$, 5.2 Hz), 3.61 (1H, dt, $J = 12.0$, 5.2 Hz), 3.89 (1H, m), 4.63 (1H, m), 5.05 (1H, br t, $J = 5.2$ Hz), 6.45 (1H, dd, $J = 7.2$, 6.4 Hz), 8.64 (1H, s), 8.69 (1H, s), 10.14 (1H, br s); ^{13}C NMR (acetone-d_6) δ: −4.7, −4.6, 18.5, 26.1, 27.5, 40.7, 41.4, 63.0, 74.0, 86.5, 90.0, 126.1, 143.7, 151.3, 152.2, 176.0; HRMS (ESI) calcd for $C_{21}H_{35}N_5NaO_4Si$ (M+Na$^+$) 472.2356, found 472.2363.

4.1.20. 5′-*O*-Benzyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxy-*N*-(2,2-dimethyl-1- oxopropyl)adenosine (37). To a stirred solution of **36** (340 mg, 0.76 mmol) in THF (7.5 mL) was added potassium *tert*-butoxide (210 mg, 1.89 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1 min. Subsequent to the addition of benzyl bromide (99 μL, 0.83 mmol) at ice-water temperature, the mixture was stirred at the same temperature for further 1 h. After dilution of the mixture with water, the aqueous layer was extracted with ethyl acetate. The organic layer was washed with water and

brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 2:1) to give **37** (397 mg, 97%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ: 0.10 (3H, s), 0.10 (3H, s), 0.89 (9H, s), 1.28 (9H, s), 2.37 (1H, ddd, J = 13.2, 6.4, 4.0 Hz), 2.94 (1H, dt, J = 13.2, 6.4 Hz), 3.57 (1H, dd, J = 10.4, 4.8 Hz), 3.68 (1H, dd, J = 10.8, 4.8 Hz), 4.01 (1H, q-like, J = 4.4 Hz), 4.51 (2H, s), 4.68 (1H, m), 6.46 (1H, t, J = 6.4 Hz), 7.27–7.34 (5H, m), 8.58 (1H, s), 8.67 (1H, s), 10.14 (1H, br s); ^{13}C NMR (acetone-d_6) δ: −4.7, −4.6, 18.5, 26.1, 27.5, 40.7, 41.0, 70.6, 73.8, 73.8, 85.1, 87.4, 125.6, 128.3, 128.4, 129.1, 139.2, 143.0, 151.0, 152.5, 175.9; HRMS (ESI) calcd for $C_{28}H_{42}N_5O_4Si$ (M+H^{+}) 540.3006, found 540.3017.

4.1.21. 5′-*O*-Benzyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (38).

To a stirred solution of **37** (390 mg, 0.72 mmol) in methanol (7 mL) was added potassium carbonate (299 mg, 2.17 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 7 h. After dilution with CH_2Cl_2, the mixture was filtrated. Subsequent to the condensation of the filtrate in vacuum, the resultant residue was diluted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 3:2 to 3:1) to give **38** (250 mg, 76%) as a white solid. Mp 134–135 °C. ^{1}H NMR (DMSO-d_6) δ: 0.09 (3H, s), 0.10 (3H, s), 0.88 (9H, s), 2.30 (1H, ddd, J = 13.2, 6.8, 3.6 Hz), 2.88 (1H, dt, J = 13.2, 6.8 Hz), 3.57 (1H, dd, J = 10.8, 5.2 Hz), 3.67 (1H, dd, J = 10.8, 5.2 Hz), 3.98 (1H, q-like, J = 4.4 Hz), 4.51 (2H, s), 4.64 (1H, m), 6.33 (1H, t, J = 6.8 Hz), 7.26–7.35 (7H, m), 8.12 (1H, s), 8.26 (1H, s); ^{13}C NMR (CD$_3$OD) δ: −4.7, −4.6, 18.8, 26.3, 42.0, 70.7, 74.1, 74.5, 85.7, 88.1, 120.3, 128.9, 129.0, 129.5, 139.2, 140.8, 150.2, 153.8, 157.3; HRMS (ESI) calcd for $C_{23}H_{34}N_5O_3Si$ (M+H^{+}) 456.2431, found 456.2444.

4.1.22. 9-(5-*O*-Benzyl-β-ᴅ-2-deoxyribofuranosyl)-6-chloropurine (20).

To a stirred solution of **38** (250 mg, 0.55 mmol) in CCl_4 (10 mL) were added a solution of tetraethylammonium chloride (364 mg, 2.20 mmol) in CH_2Cl_2 (2.5 mL) and then *tert*-butyl nitrite (326 μL, 2.75 mmol) at ice-water temperature. The mixture was stirred for 1 h at the same temperature, warmed to room temperature for 0.5 h, and stirred at 50 °C for 2 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:3 to 1:2) to give **39** (108 mg) with inseparable by-products, which was employed in the next reaction without further purification.

To a stirred solution of **39** (108 mg) in THF (2 mL) was added 1 M THF solution of TBAF–AcOH (1:1, 300 μL, 0.30 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1 h and then at room temperature for 5 h. The solvent was removed under reduced pressure, and the resultant residue was purified by preparative thin layer chromatography (Merck, 113895) (methanol/chloroform, 1:10) to give **20**

(181 mg, 34% from **38**) as a colorless oil. *Compound 39*: ^{1}H NMR (acetone-d_6) δ: 0.14 (6H, s), 0.93 (9H, s), 2.54 (1H, m), 2.97 (1H, m), 3.71 (1H, dd, J = 10.8, 4.0 Hz), 3.80 (1H, dd, J = 10.8, 4.0 Hz), 4.14 (1H, m), 4.59 (2H, s), 4.84 (1H, m), 6.58 (1H, t, J = 6.4 Hz), 7.27–7.36 (5H, m), 8.66 (1H, s), 8.70 (1H, s). *Compound 20*: ^{1}H NMR (DMSO-d_6) δ: 2.41 (1H, ddd, J = 13.2, 6.4, 4.0 Hz), 2.83 (1H, dt, J = 13.2, 6.4 Hz), 3.59 (1H, dd, J = 10.8, 5.2 Hz), 3.69 (1H, dd, J = 10.8, 4.4 Hz), 4.04 (1H, m), 4.47–4.52 (3H, m), 5.45 (1H, br s, J = 4.4 Hz), 6.48 (1H, t, J = 6.4 Hz), 7.23–7.33 (5H, m), 8.78 (1H, s), 8.80 (1H, s); ^{13}C NMR (acetone-d_6) δ: 41.2, 71.0, 72.6, 73.8, 85.7, 87.6, 128.4, 128.5, 129.1, 132.8, 139.2, 145.7, 150.7, 152.4, 152.5; HRMS (ESI) calcd for $C_{17}H_{18}ClN_4O_3$ (M+H^{+}) 361.1067, found 361.1077.

4.1.23. 5′-*O*-Allyl-3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (41).

To a stirred solution of **36** (300 mg, 0.67 mmol) in THF (20 mL) was added potassium *tert*-butoxide (188 mg, 1.68 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1 min. Subsequent to the addition of allyl bromide (203 μL, 2.35 mmol) at ice-water temperature, the mixture was stirred at the same temperature for further 2.5 h. After dilution of the mixture with saturated NaHCO$_3$ solution, the aqueous layer was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:2 to 1:1) to give **40** (300 mg) with inseparable by-products, which was employed in the next reaction without further purification.

To a stirred solution of **40** (300 mg) in methanol (7 mL) was added potassium carbonate (253 mg, 1.84 mmol) at ice-water temperature, and the mixture was stirred at room temperature for 7 h. After dilution with CH_2Cl_2, the mixture was filtrated. Subsequent to the condensation of the filtrate in vacuum, the resultant residue was diluted with ethyl acetate. The ethyl acetate layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:1 to 4:1) to give **41** (198 mg, 73% from **36**) as a white solid. *Compound 40*: ^{1}H NMR (acetone-d_6) δ: 0.16 (6H, s), 0.95 (9H, s), 1.38 (9H, s), 2.49 (1H, m), 2.94 (1H, m), 3.64–3.75 (2H, m), 4.05–4.10 (3H, m), 4.80 (1H, m), 5.15 (1H, d, J = 10.4 Hz), 5.28 (1H, d, J = 17.2 Hz), 5.93 (1H, m), 6.55 (1H, t, J = 6.4 Hz), 8.45 (1H, s), 8.60 (1H, s), 8.98 (1H, br s). *Compound 41*: Mp 100.0–100.5 °C. ^{1}H NMR (DMSO-d_6) δ: 0.11 (6H, s), 0.90 (9H, s), 2.30 (1H, ddd, J = 13.2, 6.4, 3.6 Hz), 2.87 (1H, dt, J = 13.2, 7.2 Hz), 3.52 (1H, dd, J = 10.8, 5.2 Hz), 3.61 (1H, dd, J = 10.8, 5.2 Hz), 3.94–3.98 (3H, m), 4.63 (1H, m), 5.14 (1H, dq, J = 10.4, 2.0 Hz), 5.23 (1H, dq, J = 17.2, 2.0 Hz), 5.87 (1H, ddt, J = 17.2, 10.4, 5.6 Hz), 6.33 (1H, t, J = 6.8 Hz), 7.26 (2H, br s), 8.14 (1H, s), 8.29 (1H, s); ^{13}C NMR (CD$_3$OD) δ: −4.7, −4.6, 18.8, 26.3, 42.0, 70.8, 73.3, 74.0, 85.7, 88.0, 117.5, 120.3, 135.7, 140.9, 150.2, 153.8, 157.3; HRMS (ESI) calcd for $C_{19}H_{32}N_5O_3Si$ (M+H^{+}) 406.2274, found 406.2289.

4.1.24. 9-(5-*O*-Allyl-β-D-2-deoxyribofuranosyl)-6-chloropurine (21). To a stirred solution of **41** (235 mg, 0.58 mmol) in CCl_4 (11 mL) were added a solution of tetraethylammonium chloride (384 mg, 2.32 mmol) in CH_2Cl_2 (2.6 mL) and then *tert*-butyl nitrite (345 μL, 2.90 mmol) at ice-water temperature. The mixture was stirred for 1 h at the same temperature, warmed to room temperature for 0.5 h, and stirred at 50 °C for 2 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane, 1:6) to give **42** (95 mg) with inseparable by-products, which was employed in the next reaction without further purification.

To a stirred solution of **42** (95 mg) in THF (1.5 mL) was added 1 M THF solution of TBAF–AcOH (1:1, 330 μL, 0.33 mmol) at ice-water temperature, and the mixture was stirred at the same temperature for 1 h and then at room temperature for 6 h. The solvent was removed under reduced pressure, and the resultant residue was purified by silica gel column chromatography (methanol/chloroform, 0:1 to 1:20) to give **21** (50 mg, 28% from **41**) as a colorless oil. *Compound 42*: ^1H NMR (acetone-d_6) δ: 0.16 (6H, s), 0.94 (9H, s), 2.54 (1H, m), 2.95 (1H, dt, $J = 13.2$, 6.0 Hz), 3.67 (1H, dd, $J = 10.8$, 4.0 Hz), 3.74 (1H, dd, $J = 10.8$, 4.0 Hz), 4.05 (2H, d, $J = 5.6$ Hz), 4.12 (1H, m), 4.82 (1H, m), 5.15 (1H, d, $J = 10.4$ Hz), 5.27 (1H, d, $J = 17.2$ Hz), 5.91 (1H, m), 6.59 (1H, t, $J = 6.4$ Hz), 8.69 (1H, s), 8.72 (1H, s). *Compound 21*: ^1H NMR (DMSO-d_6) δ: 2.40 (1H, ddd, $J = 13.6$, 6.8, 4.4 Hz), 2.81 (1H, dt, $J = 13.6$, 6.4 Hz), 3.54 (1H, dd, $J = 10.4$, 5.2 Hz), 3.63 (1H, dd, $J = 10.4$, 4.0 Hz), 3.95 (2H, dt, $J = 5.2$, 1.2 Hz), 4.00 (1H, q-like, $J = 4.8$ Hz), 4.47 (1H, m), 5.12 (1H, dq, $J = 10.4$, 2.0 Hz), 5.20 (1H, dq, $J = 17.2$, 2.0 Hz), 5.46 (1H, br d, $J = 4.4$ Hz), 5.84 (1H, ddt, $J = 17.2$, 10.4, 5.6 Hz), 6.48 (1H, t, $J = 6.4$ Hz), 8.81 (1H, s), 8.82 (1H, s); ^{13}C NMR (DMSO-d_6) δ: 39.0, 69.8, 70.5, 71.3, 84.1, 85.9, 116.5, 131.3, 134.9, 145.7, 149.2, 151.3, 151.6; HRMS (ESI) calcd for $C_{13}H_{16}ClN_4O_3$ (M+H$^+$) 311.0911, found 311.0918.

4.2. Biological evaluation

Cell culture, luciferase assay, and real-time RT-PCR analysis were performed as described previously.[16] The cytotoxicity was evaluated in a tetrazolium (XTT)-based assay according to the manufacturer's protocol (Cell Proliferation Kit II (XTT), Roche Diagnostics, Cat. No. 1465015).

Acknowledgment

Supplementary data

Supplementary information (^1H and ^{13}C NMR spectra of compounds **10**, **12**, **15–21**, **24**, **25**, **27–30**, **34**, **36–38**, and **41**) is available online at www.sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc. 2007.08.025.

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