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Synthesis and evaluation of 5′-modified 2′-deoxyadenosine analogues as anti-hepatitis C virus agents

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ABSTRACT

In order to study the effect of 5′-modification of 2′-deoxynucleoside on its anti-HCV activity, several analogues were synthesized and evaluated. Among the analogues, a 5′-deoxy-5′-phenacylated analogue exhibited a good anti-HCV activity with an EC_{50} of 15.1 μM. This compound is expected to operate via a type of mechanism that does not involve a generally known 5′-*O*-triphosphorylation process.

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Hepatitis C virus (HCV)[1] is a major causative agent of non-A and non-B hepatitis. It is estimated to have infected >170 million individuals, that is, 3.5% of the world's population. HCV infection is a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Current therapy based on pegylated interferon and ribavirin is often poorly tolerated and is effective in only 50% of patients. Therefore, the development of further effective therapeutic agents against HCV is an urgent public health requirement.

In our previous study,[2] we revealed that several 5′-*O*-masked analogues of 6-chloropurine-2′-deoxyriboside, such as benzoate **1** and benzyl ether **2**, exhibit an effective anti-HCV activity in a subgenomic replicon cell line and are more potent than the corresponding unmasked analogue **3** (Fig. 1). Since it is generally accepted that most nucleoside antivirals exhibit their potency after being converted to the corresponding 5′-triphosphates,[3] the unmasked (or phosphorylated) 5′-hydroxyl group is indispensable for the antiviral activity. Accordingly, our result that the 5′-*O*-masking leads to an improvement in the anti-HCV activity appears to be inconsistent with the common understanding, interestingly.

We presume that the anti-HCV activity of certain 5′-*O*-masked analogues would arise from a new type of mechanism that does not involve the 5′-*O*-triphosphorylation process. However, there is still room for the discussion on the 5′-*O*-masking effect because certain carbon–oxygen bonds, for example, the carboxylic ester bond of compound **1** (i.e., the benzoate moiety in compound **1**), are often hydrolyzed in cultured cells; in other words, there is a possibility that compound **1** simply operates as a prodrug of **3**.[4,5] Therefore, in order to confirm the effectiveness of 5′-*O*-masking groups, particularly that of the benzoyl group of compound **1**, we planned the syntheses and anti-HCV evaluations of ketone analogues **4** and **5**, in which the 5′-oxgen atom was replaced with a methylene group to prevent the hydrolytic removal of the benzoyl group.

The synthesis of **4** began with readily available 3′-*O*-TBS-2′-deoxyadenosine (**6**)[6] (Scheme 1). First, we attempted to subject

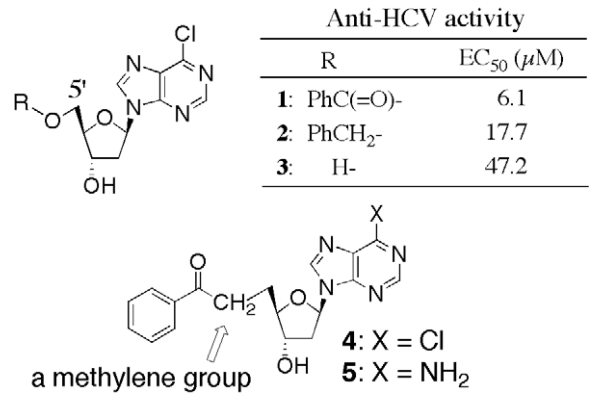

Figure 1. Structures of 5′-modified analogues.

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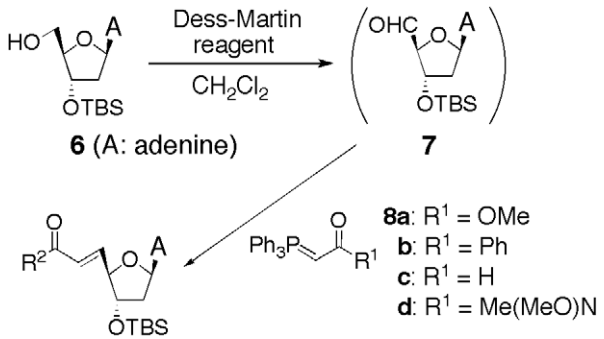


compd	R^2	yield (%)	*E*:*Z*
9	MeO–	90	*E* only
10	Ph–	60	13:1
11	H–	—	
12	Me(MeO)N–	—	

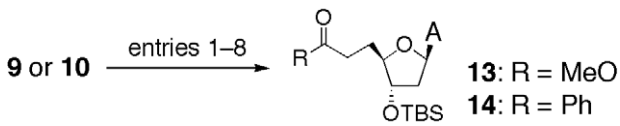
Scheme 1.

the isolated aldehyde **7** obtained via the oxidation of **6** to the following Wittig reaction; however, it was unsuccessful due to the instability of **7**. This issue was overcome by using a one-pot oxidation–Wittig reaction with Dess–Martin periodinane (DMP) and stabilized phosphorus ylide.[7] Among the four types of ylides examined (**8a**–**d**), two of them (**8a** and **8b**) successfully afforded the desired compounds **9** and **10** in 90% (*E*-isomer only) and 60% (*E*:*Z* = 13:1) yields, respectively, while the others (**8c** and **8d**) yielded complex mixtures. Since the Dess–Martin oxidation is not very suitable for the large-scale synthesis of **9** and **10** because of the explosive nature of DMP (and also its precursor, 2-iodoxybenzoic acid[8]), several other one-pot protocols such as Moffatt oxidation–Wittig,[9] PCC–Wittig,[10] TEMPO–BAIB–Wittig,[11] and TPAP–NMO–Wittig[12] were examined with **6** and **8a**. However, the TLC analyses of all the attempts revealed low yields and/or the formation of by-products.

With the thus-obtained products, the reduction of the C–C double bond was examined (Table 1). Compound **9** was converted to **13** under standard hydrogenation conditions (Pd/C, H_2, THF) with an excellent yield although the reaction required a long reaction time (~2 days) and comparatively large quantities of the catalyst (50 wt.%) (entry 1). In contrast, the conjugate reduction of **9** by sodium borohydride–transition metal salt (e.g., $NiCl_2$ and CuCl) systems[13] furnished **13** in a short time (1–3 h), but the yield was moderate (entries 2 and 3). The use of elemental magnesium in methanol led to a complex mixture (entry 4). In the case of **10**, chemoselective hydrogenations by Sajiki's procedures (Pd/fibroin–H_2 or Pd/C–Ph_2S–H_2)[14] were ineffective, resulting in the recovery of a large amount of the starting material (entries 5 and 6), while the 1,4-reduction with indium hydride generated in situ by using $PhSiH_3$–$In(OAc)_3$ or Bu_3SnH–$InCl_3$[15] efficiently afforded the desired product **14** in good yields (entries 7 and 8). Consequently, the conditions in the case of entries 1 and 7 were employed for routine syntheses of **13** and **14**, respectively, in view of their simple experimental procedures as well as their good yields.

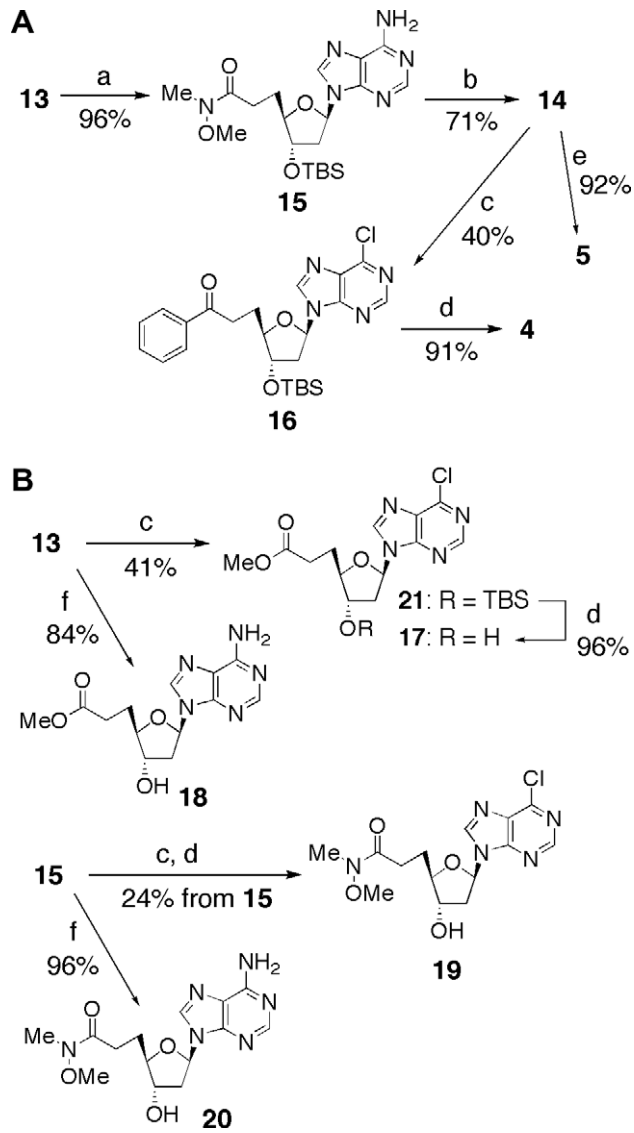
Compound **13** was readily converted to **14** with a two-step sequence, that is, via a Weinreb amide **15**, as illustrated in Scheme 2-A. Using a Grignard reagent (PhMgBr) led to a better yield (71%) than when phenyl lithium was used (58% yield). This two-step conversion will effectively serve for the synthesis of various analogues because the phenyl moiety of **14** can be easily replaced with other groups by changing the type of Grignard reagent. The amino group of **14** was substituted by a chloro group to afford **16** (40% yield),

Table 1
Chemoselective reduction of C–C double bond



Entry	Substrate	Conditions	Product	Yield (%)
1	**9**	Pd/C, H_2, THF, rt, 2 d	**13**	94
2	**9**	$NiCl_2$, $NaBH_4$, MeOH, 0 °C, 1 h	**13**	50
3	**9**	CuCl, $NaBH_4$, MeOH, 0 °C, 2.5 h	**13**	76
4	**9**	Mg, MeOH, reflux, 2 h	Complex mix.	
5	**10**	Pd/fibroin, H_2, MeOH, rt, 2 d	**14**	Trace[a]
6	**10**	Pd/C, Ph_2S, H_2, MeOH, rt, 2 d	**14**	31[b]
7	**10**	$PhSiH_3$, $In(OAc)_3$, EtOH, rt, over night	**14**	84
8	**10**	Bu_3SnH, $InCl_3$, *i*-PrOH −78 °C to rt, 2 h	**14**	93

[a] 93% of **10** was recovered.
[b] 56% of **10** was recovered.



Scheme 2. Reagents: (a) Me(MeO)NH·HCl, *n*-BuLi, THF; (b) PhMgBr, THF; (c) *t*-BuONO, Et_4NCl, CCl_4–CH_2Cl_2; (d) TBAF, AcOH, THF; (e) $Et_3N{\cdot}3HF$, THF; (f) TAS-F, MeCN.

which was subsequently treated with a mixture of tetrabutylammonium fluoride (TBAF) and acetic acid, giving the desired product **4** in 91% yield. A moderate yield of **16** was mainly obtained due to the competitive elimination of its nucleobase moiety. The other desired compound **5** was prepared in 92% yield by exposing **14** to triethylamine trihydrofluoride.

Since we are interested in the structure–activity relationship (SAR) of not only the benzoyl moiety but also the methyl ester and Weinreb amide moieties contained in the synthetic intermediates, we conducted syntheses of the corresponding analogues **17**–**20**, as shown in Scheme 2-B. 6-Chloropurine analogues **17** and **19** were prepared from **13** and **15**, respectively, under conditions almost identical to those used in the synthesis of **4** (i.e., *t*-BuONO–Et_4NCl and TBAF–AcOH). The conversion to **18** and **20** was effectively accomplished by the treatment of **13** and **15** with tris(dimethylamino)sulfonium difluorotrimethylsilicate (TAS-F),[16] while that with TBAF led to a mixture of the desired product and certain tertabutylammonium salts that were difficult to separate.

The synthesized nucleoside analogues mentioned above were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon Huh7 cell line (LucNeo#2),[17] and the result is presented in Table 2 and Figure 2. These cells contain an HCV subgenomic replicon RNA encoding a luciferase reporter gene as a marker. The antiviral 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 (expressed as CC_{50} in Table 2) was evaluated in a tetrazolium (XTT)-based assay according to the manufacturer's protocol.

As shown in Table 2, the ketone analogue **4** exhibited an antiviral activity against the HCV replicon with an EC_{50} of 15.1 μM (entry 1), which is nearly comparable to that of benzoate analogue **1** (entry 7). The cytotoxicity of **4** was somewhat high (CC_{50}: 76.3 μM), but was not high enough to exert an influence on the EC_{50} value because the cytotoxicity at 15 μM was considerably low (ca. 0–2%) (Fig. 2A). Thus, the decrease in the luciferase activity with **4** results from its anti-HCV activity, not its cytotoxicity. Interestingly, compounds **17** and **19** also exhibited anti-HCV activities (entries 3 and 5, respectively). In contrast, the 6-amino analogues **5**, **18**, and **20** did not exhibit any significant anti-HCV activity (entries 2, 4, and 6).[18]

To confirm the anti-HCV potency of compound **4**, subgenomic replicon RNA levels were quantified by real-time RT-PCR analysis (Fig. 2B). Exposing the replicon cells to 12.5 and 25 μM of **4** reduced the replicon RNA amount up to approximately 60% and 25%, respectively. This result is almost consistent with that of the luciferase assay with **4**.

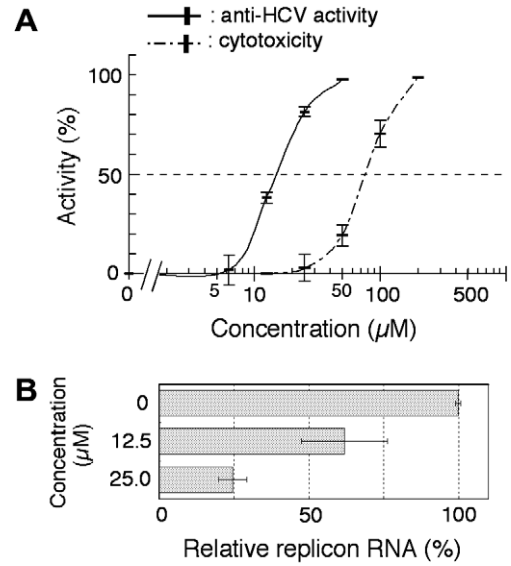
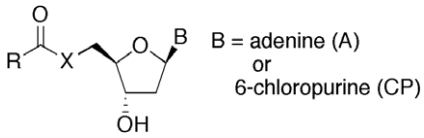


Figure 2. Anti-HCV activity and cytotoxicity of **4**: (A) result of luciferase assay and XTT assay; (B) result of real-time RT-PCR.

Taking these data into account, it appears that the phenacyl group ($BzCH_2$–) equipped at the C5′ position as well as the benzoyloxy group (BzO–) is effective functional group for anti-HCV activity; this should be noteworthy because the 5′-phenacyl group is expected to operate without being converted to the corresponding 5′-hydroxyl group (or 5′-triphosphate group). This result strongly supports our hypothesis that the 5′-*O*-masking group can contribute to the anti-HCV activity not only as a unit for the prodrug system but also as a part of the substrate. Although the detailed mechanism is unclear and the biological activity is still insufficient, the antiviral potency of such 5′-modified analogues is of great interest because they are likely to operate via a pathway that does not involve the 5′-*O*-phosphorylation process. We hope that the present study will contribute to developing a new class of HCV therapeutic agents.

Table 2
Inhibitory potency (EC_{50}) and cytotoxicity (CC_{50}) of the synthesized analogues in HCV replicon assay



Entry	Compound	R	X	B	EC_{50}[a] (μM)	CC_{50}[a] (μM)
1	**4**	Ph	CH_2	CP	15.1 ± 0.4	76.3 ± 5.2
2	**5**	Ph	CH_2	A	>200	—
3	**17**	MeO	CH_2	CP	32.9 ± 1.6	>200
4	**18**	MeO	CH_2	A	>200	—
5	**19**	Me(MeO)N	CH_2	CP	40.4 ± 1.4	>200
6	**20**	Me(MeO)N	CH_2	A	>200	—
7	**1**	Ph	O	CP	6.1[b]	111[b]

[a] EC_{50}: 50% effective concentration; CC_{50}: 50% cytotoxic concentration.
[b] Extracts obtained from our previous study (Ref. 2).

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Supplementary data

Supplementary data associated with this article (experimental details and spectroscopic data of new compounds **4**–**6**, **9**, **10**, **13**–**21**) can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.015.

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