

## DESIGN AND SYNTHESIS OF 4'-CYANO DIDEOXY ISONUCLEOSIDES AND THEIR ACTIVITY AGAINST HIV-1 AND HBV

**Kengo Onitsuka,<sup>a</sup> Kakeru Yamaguchi,<sup>a,b</sup> Ryoh Tokuda,<sup>a</sup> Nobuyo Higashi-Kuwata,<sup>b</sup> Shunsuke Kuwahara,<sup>c</sup> Hiroki Kumamoto,<sup>d</sup> Kenji Maeda,<sup>b</sup> Kazuhiro Haraguchi,<sup>d</sup> Hiroaki Mitsuya,<sup>b,e,f</sup> and Shuhei Imoto<sup>a\*</sup>**

<sup>a</sup>Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan.

<sup>b</sup>Department of Refractory Viral Infections, National Center for Global Health and Medicine Research Institute, Tokyo, Japan. <sup>c</sup>Department of Chemistry, Faculty of Science and Research Center for Materials with Integrated Properties, Toho University, Chiba, Japan. <sup>d</sup>Nihon Pharmaceutical University, Saitama, Japan. <sup>e</sup>Experimental Retrovirology Section, HIV & AIDS Malignancy Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. <sup>f</sup>Departments of Infectious Diseases and Hematology, Kumamoto University School of Medicine, Kumamoto, Japan

e-mail address: simoto@ph.sojo-u.ac.jp

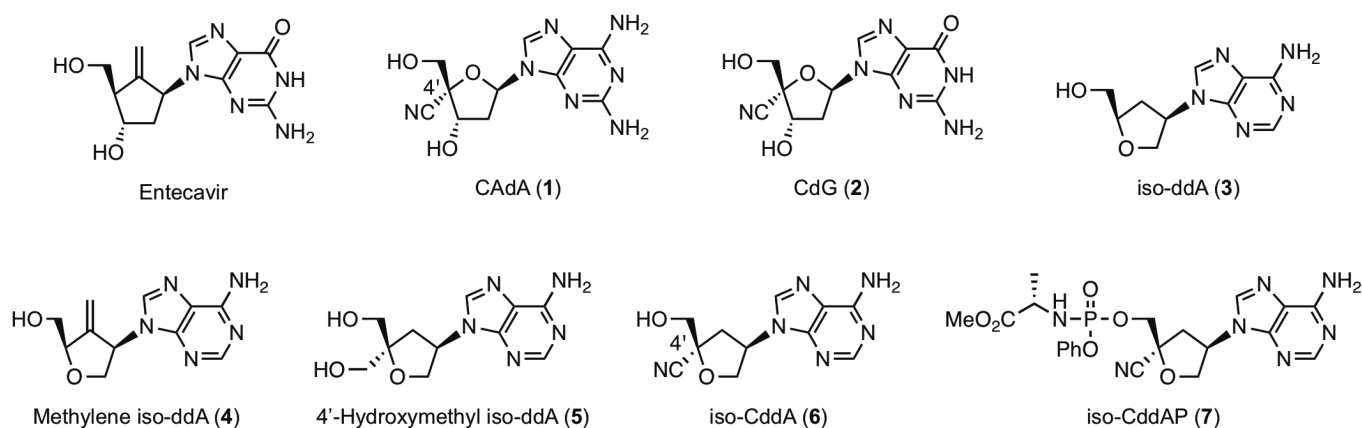
**Abstract** – Nucleoside reverse-transcriptase inhibitors are major antiviral agents against HIV-1 and HBV. Here, we describe the synthesis of a novel 4'-cyano dideoxy isonucleoside **6** and its phosphoramidate prodrug **7**. Intermediate **16** is a promising precursor for the preparation of isonucleosides bearing substituents at the 4' position with various nucleobases. In addition, **6** and **7** were evaluated for their antiviral properties against HIV-1 and HBV. Compound **7** displayed weak anti-HIV activity ( $EC_{50} = 61.4 \mu\text{M}$ ) and had no observed cytotoxicity in two different cell systems.

## INTRODUCTION

Viruses pose a major threat to human health, in part, because they readily acquire drug resistance by genetic mutation.<sup>1-3</sup> Thus, the discovery of new potent antiviral agents is an urgent priority for modern medicine. The synthesis of nucleoside/nucleotide analogs (NAs) has been an effective strategy for generating antiviral agents, and some have been used as antiviral medications in clinical practice.<sup>4-6</sup> For example, nucleoside reverse-transcriptase inhibitors (NRTIs), including abacavir and tenofovir disoproxil

fumarate, have been used in the treatment of human immunodeficiency virus 1 (HIV-1) infection. In addition, NRTIs including entecavir and lamivudine have been used to treat hepatitis B virus (HBV) infection. To date, various structural modifications have been introduced into nucleosides to develop antiviral medications with high bioactivity, low toxicity, and excellent pharmacokinetic features.<sup>7-9</sup> Our research group studies the antiviral effects of nucleosides bearing a substituent group at the position 4' carbon of ribose.<sup>10-12</sup> For instance, nucleosides bearing a cyano group at the 4' position, such as 4'-C-cyano-2-amino-2'-deoxyadenosine (CAAdA, **1**) and 4'-C-cyano-2'-deoxyguanosine (CdG, **2**), display potent antiviral activity against HIV-1 and HBV.<sup>10</sup> Moreover, CAAdA (**1**) is also effective against HBV strains that have acquired resistance to entecavir. However, glycosidic linkages are generally susceptible to enzymatic cleavage and hydrolysis under acidic conditions. To address this issue, a new class of nucleoside analogue known as isonucleosides have been developed,<sup>13-16</sup> in which the nucleobase at the position 1' carbon is moved to another position in the ribose moiety. One such isonucleoside, iso-dideoxyadenosine (iso-ddA, **3**),<sup>17</sup> reported by Huryn *et al.*, shows promising anti-HIV-1 activity ( $EC_{50} = 5-20 \mu\text{M}$ ). Furthermore, methylene iso-ddA (**4**),<sup>18</sup> which was designed based on the structure of highly potent entecavir and developed by introducing an exocyclic methylene into the structure of iso-ddA, shows potent anti-HBV activity ( $EC_{50} = 1.5 \mu\text{M}$ ).

The aforementioned findings are intriguing in relation to the antiviral activity of nucleoside derivatives with a cyano group at the position 4' carbon of isonucleosides. However, to our best knowledge, isonucleoside **5** with a hydroxymethyl group are the only examples bearing modifications at the position 4' carbon.<sup>19</sup> In this study, we designed iso-CddA (**6**), containing a cyano group at the position 4' carbon of iso-ddA, as well as its phosphate prodrug, iso-CddAP (**7**). Herein, we report the synthesis of compounds **6** and **7** together with an evaluation of their antiviral activities against HIV-1 and HBV.



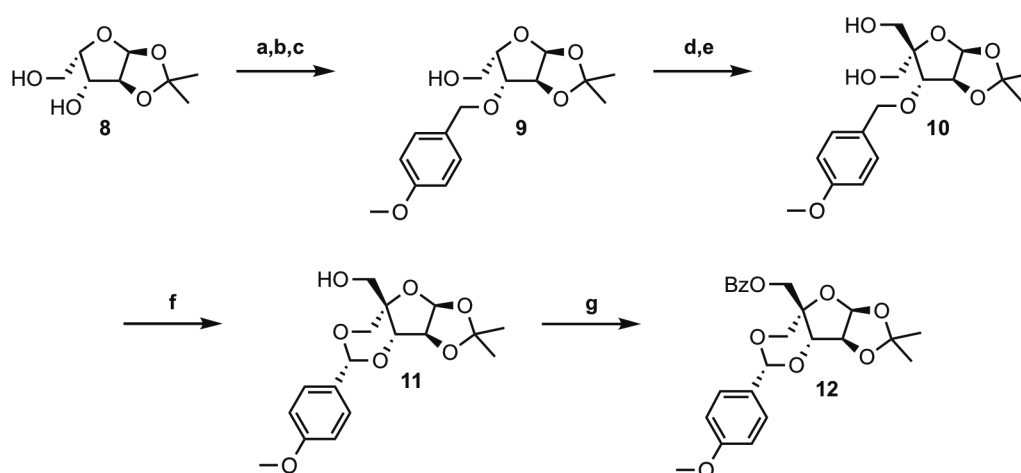
**Figure 1.** Structures of entecavir and compound 1-7

## RESULTS AND DISCUSSION

### Chemistry

#### Synthesis of 4'-cyano dideoxy isonucleoside

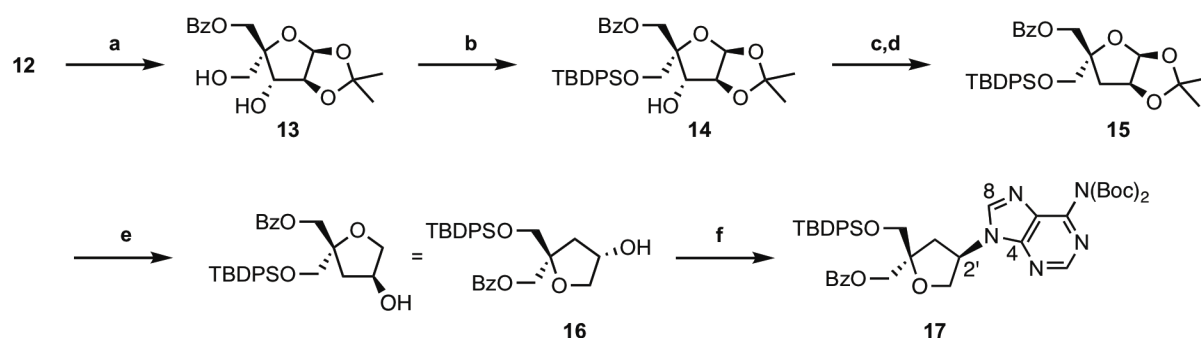
The synthesis of iso-CddA **6** and iso-CddAP **7** are illustrated in Schemes 1-3. 1,2-*O*-Isopropylidene-L-xyloside **8** was synthesized from L-xylose following a literature procedure.<sup>20</sup> A three step transformation of **8** gave the 3-*O*-*p*-methoxybenzyl protected derivative **9** in 81% yield. Compound **9** was converted to the diol **10** via Pfitzner-Moffatt oxidation and Cannizzaro reaction. Stereoselective acetalization of compound **10** was next examined. Initially, **10** was reacted with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) under anhydrous conditions at room temperature to give **11** with inseparable impurities. After modifying the reaction conditions, **11** was isolated in 64% yield when the reaction was carried out at 0 °C with 0.9 equivalents of DDQ. The structure of **11** was confirmed by X-ray crystallography (Figure S1 of Supporting Information). The remaining hydroxy group was protected with a benzoyl moiety to afford **12**.



**Scheme 1.** Synthesis of intermediate **12**; **Reagents and conditions:** (a) DMTrCl, pyridine, rt; (b) NaH, PMBCl, THF, DMF, 0 °C to rt; (c) TsOH·H<sub>2</sub>O, DCM, 81% (over 3 steps); (d) EDC·HCl, pyridine, TFA, toluene, DMSO, rt; (e) HCHOaq., NaOHaq., dioxane, rt, 82% (over 2 steps); (f) DDQ, DCM, 0 °C, 64%; (g) BzCl, pyridine, DCM, 93%.

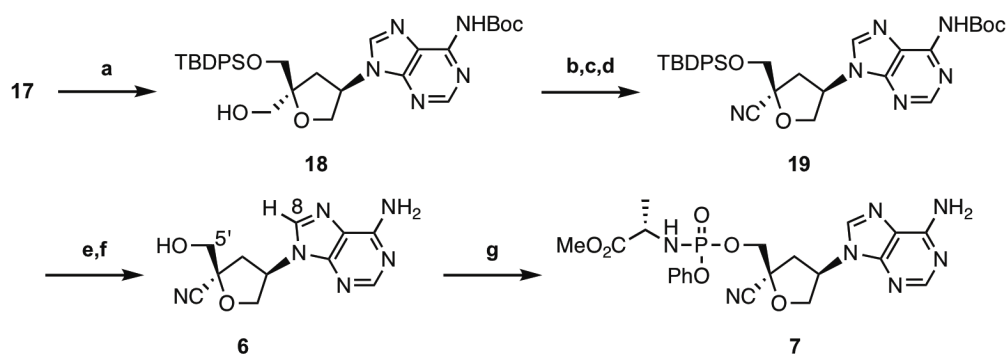
Selective deprotection of the 1,3-diol moiety of **12** using 80% acetic acid in aqueous solution afforded the monoacetal **13**. The primary alcohol of **13** was selectively protected as TBDPS ether to give **14**. The resulting secondary alcohol of **14** was reduced using TTMSS and AIBN via the formation of methyl xanthate to provide **15** in excellent yield. The reductive deoxygenation of 1,2-*O*-isopropylidene derivatives with triethylsilane in the presence of BF<sub>3</sub>·Et<sub>2</sub>O, as a Lewis acid catalyst,<sup>21</sup> gave **16**. Next, compound **16** was reacted with *N*<sup>6</sup>-di-(*tert*-butoxycarbonyl)adenine under Mitsunobu conditions using bis(2-methoxyethyl) azodicarboxylate and PPh<sub>3</sub> in THF. The desired product **17** was obtained in good

yield. In the Heteronuclear Multiple-Bond Correlation (HMBC) spectrum of **17**, a correlation between sugar H-2'/purine C-4 and sugar H-2'/purine C-8 was observed. Thus, **17** was assigned as the *N*<sup>9</sup>-isomer.



**Scheme 2.** Synthesis of intermediate **17**; **Reagents and conditions:** (a) AcOH, H<sub>2</sub>O, DCM, rt, 82%; (b) TBDPSCl, Imidazole, DMF, 30 °C, 87%; (c) CS<sub>2</sub>, MeI, NaH, THF, 0 °C; (d) TTMSS, AIBN, toluene, 80 °C, 91% (over 2 steps); (e) Triethylsilane, BF<sub>3</sub>·Et<sub>2</sub>O, DCM, 0 °C, 43%; (f) PPh<sub>3</sub>, *N*<sup>6</sup>-Boc<sub>2</sub>-adenine, Bis(2-methoxyethyl) azodicarboxylate, THF, rt, 78%.

The benzoyl moiety, protecting the hydroxyl group of compound **17**, was removed with ammonia in methanol to give **18**, which was also cleaved to a single *tert*-butoxycarbonyl from di(*tert*-butoxycarbonyl)-amine. The primary alcohol was oxidized to aldehyde with Dess-Martin reagent, then converted into the corresponding oxime. Further dehydration of this moiety to a cyano group gave **19**. Deprotection of the *tert*-butoxycarbonyl group of **19** with 80% HCO<sub>2</sub>H, followed by deprotection of the silyl group with NH<sub>4</sub>F furnished target compound **6**. The configuration of **6** was confirmed by the presence of cross peaks between H-5' and purine H-8 in the NOESY experiment. Finally, compound **6** was transformed into phosphoramidate prodrug **7**. Compound **7** was obtained as a mixture of two diastereomers at the phosphorus center. The purities of the tested compounds were at least 95% as determined by analytical RP-HPLC.



**Scheme 3.** Synthesis of 4'-cyano dideoxy isonucleosides **6** and **7**; **Reagents and conditions:** (a) 5M NH<sub>3</sub>/MeOH, rt, 82%; (b) DMP, DCM, rt; (c) NH<sub>2</sub>OH·HCl, pyridine, rt; (d) CDI, MeCN, rt, 62% (over 3 steps); (e) 80% HCO<sub>2</sub>H, rt; (f) Ammonium hydrogen fluoride, MeOH, 60 °C, 68% (over 2 steps); (g) Phenyl (methoxy-*L*-alanyl) phosphorochloridate, THF, rt, 9%.

## Biological Evaluation

Anti-HIV-1 activity was determined using MT-2 cells, whereas anti-HBV activity was determined using HepG2.2.15 cells. Darunavir and entecavir were used as controls for anti-HIV-1 activity and anti-HBV activity, respectively. Cytotoxicity was determined using MT-2 cells and HepG2.2.15 cells. The results of these experiments are summarized in Table 1. Neither compound showed significant cytotoxicity at concentrations up to 100  $\mu\text{M}$  against both the MT-2 and HepG2.2.15 cell lines. Compound **7** showed weak antiviral activity against HIV-1 ( $\text{EC}_{50} = 61.4 \mu\text{M}$ ). The potency of the anti-HIV-1 activity of **7** was about three to ten times less than that of the parent compound, iso-ddA ( $\text{EC}_{50} = 5\text{--}20 \mu\text{M}$ ). By contrast, compound **6** showed no anti-HIV-1 activity up to 100  $\mu\text{M}$ . These results suggest that introduction of a cyano group at the position 4' carbon of iso-ddA leads to a decrease in anti-HIV-1 activity caused by a conformation change of the tetrahydrofuran ring. In addition, **6** might not be phosphorylated by cellular kinases, because the parent compound (iso-ddA) has a low efficiency of phosphorylation.<sup>22, 23</sup> Neither compound **6** nor **7** showed antiviral activity against HBV up to 100  $\mu\text{M}$ .

**Table 1.** Antiviral activity and cytotoxicity of **6** and **7**

Compound	$\text{EC}_{50}$ ( $\mu\text{M}$ ) Against		$\text{CC}_{50}$ ( $\mu\text{M}$ ) in	
	Anti-HIV-1	Anti-HBV	MT-2	Hep G2.2.15
<b>6</b>	>100	>100	>100	>100
<b>7</b>	61.4	>100	>100	>100
Darunavir	0.0034	N.D.	>100	>100
Entecavir	N.D.	0.003	>100	>100

Abbreviations:

$\text{EC}_{50}$ , 50% effective concentration;  $\text{CC}_{50}$ , 50% cytotoxic concentration; N.D., not determined.

## CONCLUSION

In summary, the novel 4'-cyano dideoxy isonucleoside **6** was designed and synthesized and its phosphoramidate prodrug **7** prepared. Compound **7** was synthesized in 21 steps from L-xylose with an overall yield of 0.24%. In the established synthetic route, the intermediate **16** is a promising precursor for preparing dideoxy isonucleosides bearing substituents (e.g. ethynyl, vinyl, ethyl) at the 4' position with various nucleobases. Compounds **6** and **7** were evaluated for their antiviral properties against HIV-1 and HBV *in vitro*. Compound **7** had no observed cytotoxicity in the two cell systems tested and displayed weak anti-HIV-1 activity ( $\text{EC}_{50} = 61.4 \mu\text{M}$ ). It was found that a 4'-cyano substitution on the dideoxy isonucleoside **3** leads to a decrease in anti-HIV-1 activity. The biological inactivity of **6** might be due to a conformation change of the tetrahydrofuran moiety caused by introducing the cyano group. We believe the structural information presented here will prove useful in the development of potent but safe antiviral

agents. Further synthesis of other 4'-substituted dideoxy isonucleosides are currently underway in our research group and will be reported in due course.

## EXPERIMENTAL

### *General methods*

Reagents and anhydrous solvents were purchased and used without further purification. TLC was performed using Merck precoated TLC plates (Silica gel 60 F<sub>254</sub>, 0.25 mm). Flash column chromatography was performed using pre-packed cartridges (Yamazen Hi-flash or Biotage ZIP sphere) on a Yamazen AI-580S automated flash chromatography system. <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P NMR spectra were recorded on a JEOL ECA 500 spectrometer operating at room temperature. Chemical shifts were reported in parts per million ( $\delta$ ) relative to the residual solvent peak. Multiplicities are described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), or doublet of triplets (dt), multiplet (m). Coupling constants ( $J$ ) are reported in hertz (Hz). ESI or APCI Mass spectral analysis was performed on a JEOL JMS-T100LP system. Analytical HPLC was performed on a JASCO instrument equipped with a YMC Hydrosphere C18 column (6.0  $\times$  150 mm) with UV detection at 254 nm.

### **1,2-*O*-Isopropylidene-3-*O*-(4-methoxybenzyl)- $\alpha$ -L-xylofuranose (9)**

A mixture of **8** (11.1 g, 58.4 mmol), 4, 4'-dimethoxytrityl chloride (21.8 g, 64.3 mmol) in dry pyridine (290 mL) was stirred at room temperature for 1 h. Then, MeOH was added to the solution for quenching. After removal of the solvent, the residue was diluted with EtOAc, washed with saturated NaHCO<sub>3</sub> aqueous solution, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated and coevaporated with dioxane. To a stirred solution of the residue in DMF (146 mL) and THF (146 mL) was added NaH (2.80 g, 70.0 mmol) at 0 °C, and the mixture was stirred for 1 h. After adding 4-methoxybenzyl chloride (7.95 mL, 58.9 mmol), the reaction mixture was stirred 19 h at room temperature. The reaction was quenched with MeOH and the solvent was evaporated to dryness. The residue was diluted with EtOAc, washed with saturated NaCl aqueous solution, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated and the residue was used to the next step without further purification. To a stirred solution of the residue in CH<sub>2</sub>Cl<sub>2</sub> (281 mL) was added *p*-toluenesulfonic acid monohydrate (3.80 g, 22.6 mmol) at 0 °C. After being stirred at 0 °C for 20 min, the reaction was quenched with saturated NaHCO<sub>3</sub> aqueous solution. The organic layer was dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel (Hexane/EtOAc, 75:25 to 50:50, *v/v*) to give compound **9** (14.7 g, 47.2 mmol, 3 steps 81% yield). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.25 (d,  $J$  = 8.6 Hz, 2H), 6.91 (dt,  $J$  = 8.6, 2.3 Hz, 2H), 5.83 (d,  $J$  = 3.4 Hz, 1H), 4.72 (t,  $J$  = 5.7 Hz, 1H), 4.65 (d,  $J$  = 3.4 Hz, 1H), 4.57 (d,  $J$  = 11.5 Hz, 1H), 4.42 (d,  $J$  = 11.5 Hz, 1H), 4.09-4.06 (m, 1H), 3.85 (d,  $J$  = 3.4 Hz, 1H), 3.74 (s, 3H),

3.64-3.53 (m, 2H), 1.39 (s, 3H), 1.25 (s, 3H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 158.82, 129.91, 129.29, 113.65, 110.45, 104.39, 81.67, 80.95, 80.79, 70.60, 58.65, 55.06, 26.57, 26.08; MS (ESI) *m/z* (M+Na)<sup>+</sup> calcd. 333.1314; found 333.1326.

#### **4-C-(Hydroxymethyl)-1,2-O-isopropylidene-3-O-(4-methoxybenzyl)-α-L-xylofuranose (10)**

To a stirred solution of **9** (12.0 g, 38.6 mmol) in toluene (72.0 mL) and DMSO (107 mL) was added pyridine (6.20 mL, 76.8 mmol), EDC·HCl (22.1 g, 115 mmol) and TFA (3.72 mL, 48.6 mmol). After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, washed with water and brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, and evaporated to dryness. The residue was dissolved in dioxane (82.0 mL) and added 37% formaldehyde aqueous solution (28.6 mL) and 2 mol/L sodium hydroxide aqueous solution (57.9 mL). After being stirred for overnight, the reaction mixture was neutralized with 12 mol/L HCl aqueous solution (9.60 mL). After removal of the solvent, the residue was diluted with EtOAc, washed with water, brine, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel (Hexane/EtOAc, 60:40 to 0:100, *v/v*) to give compound **10** (10.8 g, 31.8 mmol, 2 steps 82% yield). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.27 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 5.89 (d, *J* = 4.6 Hz, 1H), 4.80 (t, *J* = 5.7 Hz, 1H), 4.75 (dd, *J* = 4.6, 2.3 Hz, 1H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.45 (t, *J* = 5.7 Hz, 1H), 4.41 (d, *J* = 11.5 Hz, 1H), 4.02 (d, *J* = 2.3 Hz, 1H), 3.74 (s, 3H), 3.53-3.38 (m, 4H), 1.46 (s, 3H), 1.28 (s, 3H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 158.73, 129.99, 129.09, 113.60, 111.91, 104.09, 89.75, 85.83, 83.65, 71.10, 61.13, 60.91, 55.06, 27.31, 27.03; MS (ESI) *m/z* (M+Na)<sup>+</sup> calcd. 363.1420; found 363.1462.

#### **4-C-(Hydroxymethyl)-1,2-O-isopropylidene-3,5-O-[(*R*)-(4-methoxyphenyl)methylene]-α-L-xylofuranose (11)**

A mixture of **10** (2.50 g, 7.34 mmol), DDQ (1.50 g, 6.61 mmol), dry 3Å molecular sieves (1.40 g) in dry CH<sub>2</sub>Cl<sub>2</sub> (130 mL) was stirred at 0 °C for overnight. The reaction mixture was filtered through a celite pad. The organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solution, brine, dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel (Hexane/EtOAc, 70:30 to 30:70, *v/v*) to give compound **11** (1.58 g, 4.67 mmol, 64% yield). <sup>1</sup>H NMR (500 MHz, Chloroform-*d*) δ 7.40 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.11 (d, *J* = 4.0 Hz, 1H), 5.41 (s, 1H), 4.70 (d, *J* = 4.0 Hz, 1H), 4.39 (d, *J* = 13.2 Hz, 1H), 4.38 (s, 1H), 4.02 (d, *J* = 13.2 Hz, 1H), 3.97 (dd, *J* = 12.0, 3.4 Hz, 1H), 3.80 (s, 3H), 3.61 (dd, *J* = 12.0, 9.7 Hz, 1H), 2.09 (dd, *J* = 9.7, 3.4 Hz, 1H), 1.56 (s, 3H), 1.31 (s, 3H); <sup>13</sup>C NMR (126 MHz, Chloroform-*d*) δ 160.27, 129.98, 127.50, 113.75, 112.05, 105.97, 98.99, 85.44, 82.48, 78.79, 70.25, 63.52, 55.40, 26.07, 25.36; MS (ESI) *m/z* (M+Na)<sup>+</sup> calcd. 361.1263; found 361.1260.

#### **4-C-(Benzoyloxymethyl)-1,2-O-isopropylidene-3,5-O-[(R)-(4-methoxyphenyl)methylene]- $\alpha$ -L-xylofuranose (12)**

A mixture of **11** (1.09 g, 3.21 mmol), pyridine (6.00 mL), benzoyl chloride (0.410 mL, 3.53 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (30.0 mL) was stirred at room temperature for 3.5 h. The reaction was quenched with water and the water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel (Hexane/EtOAc, 85:15, v/v) to give compound **12** (1.31 g, 2.97 mmol, 93% yield). <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  8.07 (d, *J* = 7.5 Hz, 2H), 7.61 (t, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 2H), 7.43 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 8.6 Hz, 2H), 6.15 (d, *J* = 4.0 Hz, 1H), 5.45 (s, 1H), 4.74 (d, *J* = 4.0 Hz, 1H), 4.63 (s, 1H), 4.62 (d, *J* = 11.5 Hz, 2H), 4.47 (d, *J* = 11.5 Hz, 1H), 4.38 (d, *J* = 13.2 Hz, 1H), 4.18 (d, *J* = 13.2 Hz, 1H), 3.81 (s, 3H), 1.65 (s, 3H), 1.32 (s, 3H); <sup>13</sup>C NMR (126 MHz, Chloroform-*d*)  $\delta$  165.93, 160.35, 133.53, 129.84, 129.64, 128.69, 127.53, 113.81, 112.48, 106.36, 99.05, 85.31, 80.65, 79.17, 69.41, 64.73, 55.46, 26.08, 25.43; MS (ESI) *m/z* (M+Na)<sup>+</sup> calcd. 465.1525; found 465.1658.

#### **4-C-(Benzoyloxymethyl)-1,2-O-isopropylidene- $\alpha$ -L-xylofuranose (13)**

To a stirred solution of **12** (1.31 g, 2.97 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.50 mL) was added 80% AcOH aqueous solution (12.5 mL). After being stirred at room temperature for 24 h, the reaction mixture was neutralized with saturated NaHCO<sub>3</sub> aqueous solution. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> and the combined organic layer was washed with saturated NaHCO<sub>3</sub> aqueous solution. The organic layer was dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel (Hexane/EtOAc, 50:50 to 35:65, v/v) to give compound **13** (788 mg, 2.43 mmol, 82% yield). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.04-7.90 (m, 2H), 7.75-7.61 (m, 1H), 7.61-7.48 (m, 2H), 5.90 (d, *J* = 4.0 Hz, 1H), 5.57 (d, *J* = 5.2 Hz, 1H), 4.72 (t, *J* = 6.3 Hz, 1H), 4.55 (d, *J* = 5.2 Hz, 1H), 4.43 (d, *J* = 10.9 Hz, 1H), 4.36 (d, *J* = 10.9 Hz, 1H), 4.13 (d, *J* = 6.3 Hz, 1H), 3.62 (d, *J* = 6.3 Hz, 2H), 1.45 (s, 3H), 1.24 (s, 3H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  165.42, 133.45, 129.63, 129.23, 128.79, 111.29, 104.51, 88.66, 87.36, 75.54, 63.26, 60.11, 26.51, 25.99; MS (ESI) *m/z* (M+Na)<sup>+</sup> calcd. 347.1107; found 347.1076.

#### **4-C-(Benzoyloxymethyl)-5-O-(*tert*-butyldiphenylsilyl)-1,2-O-isopropylidene- $\alpha$ -L-xylofuranose (14)**

A mixture of **13** (788 mg, 2.43 mmol), imidazole (331 mg, 4.86 mmol) and TBDPSCl (0.749 mL, 2.92 mmol) in dry DMF (24.3 mL) was stirred at 30 °C for 72 h. Then, methanol was added to the solution for quenching. After removal of the solvent, the residue was diluted with EtOAc, washed with water, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated to dryness. The residue was purified by column chromatography on silica gel (Hexane/EtOAc, 80:20 to 50:50, v/v) to give compound **14** (1.19 g, 2.11



mmol, 87% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ )  $\delta$  7.95 (d,  $J = 8.0$  Hz, 2H), 7.72 (t,  $J = 6.9$  Hz, 1H), 7.65-7.55 (m, 6H), 7.41 (m, 2H), 7.34 (t,  $J = 7.5$  Hz, 2H), 7.28 (t,  $J = 7.5$  Hz, 2H), 5.95 (d,  $J = 4.0$  Hz, 1H), 5.73 (d,  $J = 5.2$  Hz, 1H), 4.65 (d,  $J = 10.9$  Hz, 1H), 4.57 (d,  $J = 4.0$  Hz, 1H), 4.45 (d,  $J = 10.9$  Hz, 1H), 4.23 (d,  $J = 5.2$  Hz, 1H), 3.93 (d,  $J = 10.3$  Hz, 1H), 3.78 (d,  $J = 10.3$  Hz, 1H), 1.48 (s, 3H), 1.25 (s, 3H), 0.92 (s, 9H);  $^{13}\text{C}$  NMR (126 MHz,  $\text{DMSO-}d_6$ )  $\delta$  165.31, 135.09, 135.02, 133.59, 132.63, 132.55, 129.85, 129.85, 129.38, 129.22, 128.85, 127.85, 127.79, 111.24, 104.91, 88.83, 87.12, 75.32, 63.12, 62.58, 26.38, 26.31, 25.72, 18.73; MS (ESI)  $m/z$  ( $\text{M}+\text{Na}$ ) $^+$  calcd. 585.2285; found 585.2220.

#### **4-C-(Benzoyloxymethyl)-5-O-(tert-butyldiphenylsilyl)-3-deoxy-1,2-O-isopropylidene- $\alpha$ -L-xylofuranose (15)**

A mixture of **14** (578 mg, 1.03 mmol),  $\text{CS}_2$  (373  $\mu\text{L}$ , 6.18 mmol) and MeI (385  $\mu\text{L}$ , 6.18 mmol) in THF (10.3 mL) was added at 0  $^\circ\text{C}$ . After 30 min, NaH (82.4 mg, 2.06 mmol) was added to the reaction mixture. After being stirred at 0  $^\circ\text{C}$  for 30 min, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$ , then the reaction was quenched with water. The organic layer was washed with water, brine, dried over  $\text{MgSO}_4$ , and filtered. The filtrate was concentrated and coevaporated with toluene. To a stirred solution of the residue in toluene (10 mL) was added AIBN (84.5 mg, 0.515 mmol) and TTMSS (949  $\mu\text{L}$ , 3.09 mmol), the mixture was stirred at 80  $^\circ\text{C}$  for 2 h. After being cooled to room temperature, the reaction mixture was directly applied to column chromatography on silica gel (Hexane/EtOAc, 91:9,  $v/v$ ) to give compound **15** (512 mg, 0.936 mmol, 91% yield).  $^1\text{H}$  NMR (500 MHz, Chloroform- $d$ )  $\delta$  7.92 (dd,  $J = 8.6, 1.1$  Hz, 2H), 7.72-7.60 (m, 4H), 7.55 (t,  $J = 7.4$  Hz, 1H), 7.47-7.27 (m, 8H), 5.91 (d,  $J = 4.0$  Hz, 1H), 4.82 (m, 1H), 4.60 (d,  $J = 11.5$  Hz, 1H), 4.47 (d,  $J = 11.5$  Hz, 1H), 3.71 (s, 2H), 2.31 (dd,  $J = 14.3, 6.3$  Hz, 1H), 2.20 (d,  $J = 14.3$  Hz, 1H), 1.60 (s, 3H), 1.33 (s, 3H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (126 MHz, Chloroform- $d$ )  $\delta$  166.20, 135.76, 135.71, 133.13, 133.08, 130.09, 129.90, 128.42, 127.89, 112.82, 106.96, 87.05, 81.40, 66.92, 66.27, 35.98, 27.39, 26.91, 26.34, 19.34; MS (ESI)  $m/z$  ( $\text{M}+\text{Na}$ ) $^+$  calcd. 569.2335; found 569.2394.

#### **1, 4-Anhydro-4-C-(benzoyloxymethyl)-5-O-(tert-butyldiphenylsilyl)-3-deoxy-L-xylitol (16)**

To a stirred solution of **15** (188 mg, 0.344 mmol) in  $\text{CH}_2\text{Cl}_2$  (3.44 mL) was added  $\text{Et}_3\text{SiH}$  (164  $\mu\text{L}$ , 1.03 mmol) and  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (131  $\mu\text{L}$ , 1.03 mmol) at 0  $^\circ\text{C}$ . After being stirred at 0  $^\circ\text{C}$  for 4 h, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with saturated  $\text{NaHCO}_3$  aqueous solution, brine, dried over  $\text{MgSO}_4$ , and filtered. After removal of the solvent, the residue was purified by flash column chromatography on silica gel (Hexane/EtOAc, 75:25,  $v/v$ ) to give compound **16** (72.7 mg, 0.148 mmol, 43% yield).  $^1\text{H}$  NMR (500 MHz, Chloroform- $d$ )  $\delta$  7.98 (dd,  $J = 8.0, 1.2$  Hz, 2H), 7.72-7.61 (m, 4H), 7.56 (t,  $J = 7.4$  Hz, 1H), 7.48-7.29 (m, 8H), 4.56 (d,  $J = 11.5$  Hz, 1H), 4.50 (d,  $J = 11.5$  Hz, 1H), 4.48-4.45 (m, 2H), 3.91 (dt,  $J = 9.7, 1.7$  Hz, 1H), 3.87 (dd,  $J = 9.7, 4.0$  Hz, 1H), 3.72 (d,  $J = 10.3$  Hz, 1H),

3.62 (d,  $J = 10.3$  Hz, 1H), 2.27 (dd,  $J = 14.3, 6.3$  Hz, 1H), 2.19 (d,  $J = 6.3$  Hz, 1H), 1.98 (dt,  $J = 14.3, 1.7$  Hz, 1H), 1.07 (s, 9H);  $^{13}\text{C}$  NMR (126 MHz, Chloroform- $d$ )  $\delta$  166.59, 135.74, 133.19, 133.08, 130.07, 129.89, 129.75, 128.56, 127.86, 84.23, 76.02, 72.74, 66.91, 66.20, 39.88, 26.91, 19.35; MS (ESI)  $m/z$  (M+Na) $^+$  calcd. 513.2073; found 513.2028.

**[(2*S*,4*R*)-4-[(6-*N,N*-Di-*tert*-Butoxycarbonyl-adenin)-9-yl]-2-(*tert*-butyldiphenylsilyloxymethyl)tetrahydrofuran-2-yl]methyl benzoate (17)**

A mixture of **16** (353 mg, 0.719 mmol), PPh<sub>3</sub> (415 mg, 0.719 mmol), *N*<sup>6</sup>-di(*tert*-butoxycarbonyl)adenine (531 mg, 1.58 mmol) and bis(2-methoxyethyl) azodicarboxylate (370 mg, 1.58 mmol) in dry THF (7.20 mL) was stirred at room temperature for 18 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, then washed with brine, dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by flash column chromatography on silica gel (Hexane/EtOAc, 75:25 to 50:50,  $v/v$ ) to give compound **17** (460 mg, 0.559 mmol, 78% yield).  $^1\text{H}$  NMR (500 MHz, Methanol- $d_4$ )  $\delta$  8.77 (s, 1H), 8.48 (s, 1H), 7.99 (d,  $J = 7.5$  Hz, 2H), 7.64-7.56 (m, 5H), 7.46 (t,  $J = 8.0$  Hz, 2H), 7.38-7.22 (m, 6H), 5.45-5.42 (m, 1H), 4.62 (d,  $J = 11.5$  Hz, 1H), 4.45 (d,  $J = 11.5$  Hz, 1H), 4.43-4.37 (m, 2H), 3.95 (1H,  $J = 10.3$  Hz, 1H), 3.77 (d,  $J = 3.8$  Hz, 1H), 2.70 (dd,  $J = 13.8, 8.0$  Hz, 1H), 2.57 (dd,  $J = 13.8, 5.7$  Hz, 1H), 1.38 (s, 18H), 0.99 (s, 9H);  $^{13}\text{C}$  NMR (126 MHz, Methanol- $d_4$ )  $\delta$  166.27, 153.35, 151.56, 150.33, 149.71, 145.00, 135.35, 133.16, 132.65, 129.82, 129.77, 129.38, 129.01, 128.42, 127.62, 84.60, 83.98, 70.20, 65.65, 65.09, 55.78, 36.47, 26.73, 25.99, 18.71; MS (ESI)  $m/z$  (M+H) $^+$  calcd. 808.3742; found 808.3758.

**[(2*S*,4*R*)-4-[(6-*N-tert*-Butoxycarbonyl-adenin)-9-yl]-2-(*tert*-butyldiphenylsilyloxymethyl)tetrahydrofuran-2-yl]methanol (18)**

Compound **17** (129 mg, 0.160 mmol) was dissolved in 5M ammonia in MeOH (30 mL) and stirred at room temperature for overnight. After removal of the solvent, the residue was purified by flash column chromatography on silica gel (Hexane/EtOAc, 25:75 to 0:100,  $v/v$ ) to give compound **18** (79.3 mg, 0.131 mmol, 82% yield).  $^1\text{H}$  NMR (500 MHz, Chloroform- $d$ )  $\delta$  8.71 (s, 1H), 8.03 (s, 1H), 7.89 (s, 1H), 7.67-7.57 (m, 4H), 7.51-7.28 (m, 6H), 5.26-5.23 (m, 1H), 4.37 (dd,  $J = 9.2, 6.3$  Hz, 1H), 4.19 (dd,  $J = 9.2, 6.3$  Hz, 1H), 3.85 (d,  $J = 10.3$  Hz, 1H), 3.78-3.65 (m, 3H), 2.52 (dd,  $J = 13.8, 8.0$  Hz, 1H), 2.44 (dd,  $J = 13.8, 6.9$  Hz, 1H), 2.20 (s, 1H), 1.57 (s, 9H), 1.04 (s, 9H);  $^{13}\text{C}$  NMR (126 MHz, Chloroform- $d$ )  $\delta$  152.97, 151.05, 150.04, 149.81, 140.40, 135.69, 132.86, 130.09, 130.07, 127.94, 121.93, 86.16, 82.42, 71.25, 66.55, 65.71, 55.11, 36.30, 28.29, 26.99, 19.35; MS (APCI)  $m/z$  (M+H) $^+$  calcd. 626.2775; found 626.2814.

**(2*S*,4*R*)-4-[(6-*N*-*tert*-Butoxycarbonyl-adenin)-9-yl]-2-(*tert*-butyldiphenylsilyloxymethyl)tetrahydrofuran-2-carbonitrile (19)**

A mixture of **18** (682 mg, 1.13 mmol) and Dess-Martin periodinane (717 mg, 1.69 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (22.6 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, then the reaction was quenched with 10% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> aqueous solution. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated and coevaporated with pyridine. The residue was used to the next step without further purification. To a stirred solution of the residue in pyridine (11.3 mL) was added NH<sub>2</sub>OH·HCl (177 mg, 2.54 mmol) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was diluted with EtOAc, then washed with saturated NaHCO<sub>3</sub> aqueous solution, dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated and coevaporated with MeCN. To a stirred solution of the residue in MeCN (11.3 mL) was added *N,N*-carbonyldiimidazole (366 mg, 2.26 mmol) at room temperature. After being stirred for 1.5 h, the reaction was diluted with EtOAc, washed with brine, dried over MgSO<sub>4</sub>, and filtered. After removal of the solvent, the residue was purified by flash column chromatography on silica gel (Hexane/EtOAc, 50:50, v/v) to give compound **19** (420 mg, 0.701 mmol, 62% yield). <sup>1</sup>H NMR (500 MHz, Chloroform-*d*) δ 8.71 (s, 1H), 7.91 (s, 1H), 7.90 (s, 1H), 7.66-7.62 (m, 4H), 7.47-7.34 (m, 6H), 5.50-5.48 (m, 1H), 4.46 (dd, *J* = 10.3, 6.3 Hz, 1H), 4.39 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.99 (d, *J* = 10.9 Hz, 1H), 3.93 (d, *J* = 10.9 Hz, 1H), 3.03 (dd, *J* = 14.3, 8.0 Hz, 1H), 2.69 (dd, *J* = 14.3, 5.2 Hz, 1H), 1.08 (s, 9H); <sup>13</sup>C NMR (126 MHz, Chloroform-*d*) δ 153.18, 150.78, 150.20, 149.74, 139.99, 135.69, 132.14, 130.33, 128.09, 121.80, 119.08, 82.54, 80.51, 72.95, 66.53, 54.02, 41.00, 28.27, 26.86, 19.38; MS (APCI) *m/z* (M+Na)<sup>+</sup> calcd. 621.2622; found 621.2579.

**4'-Cyano iso-dideoxy adenosine (6)**

Compound **19** (252 mg, 0.420 mmol) was dissolved in 80% HCO<sub>2</sub>H (5.00 mL) and stirred at room temperature for 5 h. The reaction mixture was neutralized with saturated NaHCO<sub>3</sub> aqueous solution and the mixture was extracted with EtOAc. The organic layer was dried over MgSO<sub>4</sub>, and filtered. The filtrate was concentrated to dryness. The residue was dissolved in MeOH (4.20 mL) and added ammonium hydrogen fluoride (238 mg, 4.18 mmol). After being stirred at 60 °C for 90 min, the reaction mixture was cooled to room temperature. The precipitate was collected and recrystallized from MeOH to give **6** (73.8 mg, 0.284 mmol, 68% yield). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.17 (s, 1H), 8.15 (s, 1H), 7.26 (s, 2H), 5.82 (t, *J* = 6.3 Hz, 1H), 5.42-5.37 (m, 1H), 4.38 (dd, *J* = 9.7, 4.0 Hz, 1H), 4.29 (dd, *J* = 9.7, 6.3 Hz, 1H), 3.72 (d, *J* = 6.3 Hz, 2H), 2.97 (dd, *J* = 14.3, 8.0 Hz, 1H), 2.65 (dd, *J* = 14.3, 5.7 Hz, 1H); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) δ 156.04, 152.45, 149.25, 138.91, 120.20, 118.84, 80.36, 72.09, 64.51, 53.52, 39.89; MS (ESI) *m/z* (M+H)<sup>+</sup> calcd. 261.1100; found 261.1051.

#### 4'-Cyano iso-dideoxy adenosine phosphoramidate (7)

To a stirred solution of **6** (70.0 mg, 0.269 mmol) and *N*-methylimidazole (354  $\mu$ L, 4.44 mmol) in THF (1.35 mL) was added 1.0 M phenyl (methoxy-*L*-alanyl) phosphorochloridate in THF solution (1.35 mL, 1.35 mmol). After being stirred at room temperature for 24 h, the reaction mixture was diluted with EtOAc. The organic layer was washed with 10% AcOH, water, saturated NaHCO<sub>3</sub> aqueous solution, then dried over MgSO<sub>4</sub> and filtered. After removal of the solvent, the residue was purified by flash column chromatography on silica gel (DCM/MeOH, 97/3 to 80/20, v/v) to give compound **7** (12.5 mg, 0.0249 mmol, 9% yield). <sup>1</sup>H NMR (500 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  8.10-8.07 (m, 2H), 7.25-7.18 (m, 2H), 7.11-7.05 (m, 3H), 5.40-5.37 (m, 1H), 4.46-4.30 (m, 4H), 3.90-3.86 (m, 1H), 3.58-3.56 (m, 3H), 3.02-2.97 (m, 1H), 2.72-2.61 (m, 1H), 1.26-1.22 (m, 3H); <sup>13</sup>C NMR (126 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  175.53, 175.49, 175.35, 175.31, 157.35, 153.83, 151.89, 151.85, 150.56, 150.48, 140.65, 140.60, 130.79, 130.74, 126.34, 126.22, 121.49, 121.45, 121.27, 121.23, 120.29, 119.39, 119.25, 79.97, 79.90, 73.82, 73.66, 69.21, 69.17, 68.60, 55.74, 55.71, 52.80, 51.60, 51.42, 41.38, 41.28, 20.36, 20.30, 20.25, 20.19; <sup>31</sup>P-NMR (202 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  3.02, 2.72; MS (ESI) *m/z* (M+H)<sup>+</sup> calcd. 502.1604; found 502.1588.

**Anti-HIV-1, Anti-HBV and cytotoxicity assay:** Anti-HIV-1 assays using wild-type HIV (HIV-1NL4-3) were conducted as previously described.<sup>24</sup> In brief, MT-4 cells were exposed to HIV-1NL4-3 at fifty 50% tissue culture infectious doses (TCID<sub>50</sub>). After viral exposure, the cell suspension (5  $\times$  10<sup>3</sup> cells in 100  $\mu$ L) was plated in each well of a 96-well flat microtiter culture plate containing various concentrations of a compound. After incubation for 5 days, the number of viable cells in each well was measured using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The potency of HIV-1 inhibition by a compound was determined based on its inhibitory effect on virally-induced cytopathicity in MT-4 cells. All assays were conducted in triplicates. Anti-HBV assays were conducted as previously described.<sup>25</sup> Briefly, HepG2 2.2.15 cells were seeded in 96-well cell culture plates at a density of 4  $\times$  10<sup>3</sup> cells in 200  $\mu$ L per well together with various concentrations of a compound. On days 3 and 7 after plating, culture medium was removed and fresh medium and a drug were replenished. On day 14, the cells were harvested for DNA collection and the DNA samples were extracted from the cells using QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and was re-suspended in 100  $\mu$ L Tris-EDTA buffer. Total cellular DNA samples were amplified using the genesig Advanced Kit for quantification of Hepatitis B Virus genomes (PrimerDesign<sup>TM</sup> Ltd., Southampton, UK) and Applied Biosystems<sup>®</sup> 7500 Fast Real-time PCR System (Life Technologies Japan Ltd., Tokyo Japan). PCR preparations were incubated at 95 °C for 10 min, followed by 50 cycles at 95 °C for 10 s and 60 °C for 1 min. The data were analyzed using 7500 Fast Software Version 2.0.6 (Life Technologies Japan Ltd., Tokyo Japan) and threshold cycle (CT) values were obtained. To derive the HBV copy numbers from the

CT values, a standard curve was generated with 10-fold serial dilutions of an HBV plasmid (pHBVWT; 20 to  $2 \times 10^8$  copies per reaction). The amount of HBV DNA in each assay sample was compared to that in compound-free control samples. The cytotoxicities of the compounds in MT-2 cells and HepG2.2.15 cells were also determined. Cells were plated in a 96-well plate at a density of  $2 \times 10^3$  cells in 200  $\mu$ L per well and were continuously exposed to the compound at a certain concentration throughout the entire culturing period. The number of viable cells in each well was determined using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan).

## ACKNOWLEDGEMENTS

The present work was supported in part by the Japan Agency for Medical Research and Development (AMED) for research on innovative development and the practical application of new drugs for hepatitis B under Grant Numbers JP16fk0310501 and JP19fk0310113 (SI and HM); a grant from Japan Society for the Promotion of Sciences (HM); a grant from National Center for Global Health and Medicine Research Institute (HM); and the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, National Institutes of Health (HM). We are grateful to Mrs. Misato Yamakawa for assistance on NMR measurements.

## REFERENCES

1. L. Menéndez-Arias, *Antivir. Res.*, 2013, **98**, 93.
2. F. Morfin and D. Thouvenot, *J. Clin. Virol.*, 2003, **26**, 29.
3. F. Zoulim and S. Locarnini, *Gastroenterology*, 2009, **137**, 1593.
4. P. Merino, 'Chemical Synthesis of Nucleoside Analogues', Wiley & Sons Inc., Newjersey, 2013.
5. T. Cihlar and A. S. Ray, *Antivir. Res.*, 2010, **85**, 39.
6. L. Menéndez-Arias, M. Álvarez, and B. Pacheco, *Curr. Opin. Virol.*, 2014, **8**, 1.
7. H. Kumamoto, M. Fukano, T. Nakano, K. Iwagami, C. Takeyama, S. Kohgo, S. Imoto, M. Amano, N. Kuwata-Higashi, M. Aoki, H. Abe, H. Mitsuya, K. Fukuhara, and K. Haraguchi, *J. Org. Chem.*, 2016, **81**, 2827.
8. C. Liu, S. G. Dumbre, C. Pannecouque, C. Huang, R. G. Ptak, M. G. Murray, S. D. Jonghe, and P. Herdewijn, *J. Med. Chem.*, 2016, **59**, 9513.
9. W. Yang, Y. Peng, J. Wang, C. Song, W. Yu, Y. Zhou, J. Jiang, Q. Wang, J. Wu, and J. Chang, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 1291.
10. Y. Takamatsu, Y. Tanaka, S. Kohgo, S. Murakami, K. Singh, D. Das, D. J. Venzon, M. Amano, N. Kuwata, M. Aoki, N. S. Delino, S. Hayashi, S. Takahashi, Y. Sukenaga, K. Haraguchi, S. G. Sarafianos, K. Maeda, and H. Mitsuya, *Hepatology*, 2015, **62**, 1024.
11. S. Kohgo, S. Imoto, R. Tokuda, Y. Takamatsu, N. Higashi-Kuwata, M. Aoki, M. Amano, H. Kansui,

- K. Onitsuka, K. Maeda, and H. Mitsuya, *ChemistrySelect*, 2018, **3**, 3313.
12. K. Onitsuka, R. Tokuda, N. Kuwata-Higashi, H. Kumamoto, M. Aoki, M. Amano, S. Kohgo, D. Das, K. Haraguchi, H. Mitsuya, and S. Imoto, *Nucleosides Nucleotides Nucleic Acids*, in press.
  13. J. A. Montgomery, S. D. Clayton, and H. J. Thomas, *J. Org. Chem.*, 1975, **40**, 1923.
  14. V. Nair and Z. M. Nuesca, *J. Am. Chem. Soc.*, 1992, **114**, 7951.
  15. A. Kakefuda, S. Shuto, T. Nagahata, J. Seki, T. Sasaki, and A. Matsuda, *Tetrahedron*, 1994, **50**, 10167.
  16. Y. Yoshimura, K. Asami, T. Imamichi, T. Okuda, K. Shiraki, and H. Takahata, *J. Org. Chem.*, 2010, **75**, 4161.
  17. D. M. Huryn, B. C. Sluboski, S. Y. Tam, M. Weigele, I. Sim, B. D. Anderson, H. Mitsuya, and S. Broder, *J. Med. Chem.*, 1992, **35**, 2347.
  18. S. J. Yoo, H. O. Kim, Y. Lim, J. Kim, and L. S. Jeong, *Bioorg. Med. Chem.*, 2002, **10**, 215.
  19. L. B. Zintek, G. S. Jeon, and V. Nair, *Heterocycles*, 1994, **37**, 1853.
  20. B. Jiang, J. F. Liu, and S. Y. Zhao, *J. Org. Chem.*, 2003, **68**, 2376.
  21. S. Mukherjee, B. G. Roy, S. N. Das, and S. B. Mandal, *Tetrahedron Lett.*, 2012, **53**, 4929.
  22. P. Franchetti, L. Cappellacci, M. Grifantini, L. Messini, G. A. Sheikha, A. G. Loi, E. Tramontano, De M. Antonella, M. G. Spiga, and P. La Colla, *J. Med. Chem.*, 1994, **37**, 3534.
  23. S. Pal and V. Nair, *Biochem. Pharmacol.*, 2000, **60**, 1505.
  24. M. Sugiyama, Y. Tanaka, T. Kato, E. Orito, K. Ito, S. K. Acharya, R. G. Gish, A. Kramvis, T. Shimada, N. Izumi, M. Kaito, Y. Miyakawa, and M. Mizokami, *Hepatology*, 2006, **44**, 915.
  25. N. Higashi-Kuwata, S. Hayashi, D. Das, S. Kohgo, M. Murakami, S. Hattori, S. Imoto, D. J. Venzon, K. Singh, S. G. Sarafianos, Y. Tanaka, and H. Mitsuya, *Antimicrob. Agents Chemother.*, 2019, doi: 10.1128/AAC.02143-18.