Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: https://www.tandfonline.com/loi/tbbb20

Synthesis of nucleotide analogues, EFdA, EdA and EdAP, and the effect of EdAP on hepatitis B virus replication

Mai Kamata, Toshifumi Takeuchi, Ei Hayashi, Kazane Nishioka, Mizuki Oshima, Masashi Iwamoto, Kota Nishiuchi, Shogo Kamo, Shusuke Tomoshige, Koichi Watashi, Shinji Kamisuki, Hiroshi Ohrui, Fumio Sugawara & Kouji Kuramochi

To cite this article: Mai Kamata, Toshifumi Takeuchi, Ei Hayashi, Kazane Nishioka, Mizuki Oshima, Masashi Iwamoto, Kota Nishiuchi, Shogo Kamo, Shusuke Tomoshige, Koichi Watashi, Shinji Kamisuki, Hiroshi Ohrui, Fumio Sugawara & Kouji Kuramochi (2019): Synthesis of nucleotide analogues, EFdA, EdA and EdAP, and the effect of EdAP on hepatitis B virus replication, Bioscience, Biotechnology, and Biochemistry, DOI: 10.1080/09168451.2019.1673696

To link to this article: https://doi.org/10.1080/09168451.2019.1673696

BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY
https://doi.org/10.1080/09168451.2019.1673696

Synthesis of nucleotide analogues, EFdA, EdA and EdAP, and the effect of EdAP on hepatitis B virus replication

Mai Kamata[a], Toshifumi Takeuchi[a], Ei Hayashi[a], Kazane Nishioka[a,b], Mizuki Oshima[a,b], Masashi Iwamoto[a,b], Kota Nishiuchi[a], Shogo Kamo[a], Shusuke Tomoshige[a], Koichi Watashi[a,b], Shinji Kamisuki[c], Hiroshi Ohrui[d], Fumio Sugawara[a] and Kouji Kuramochi[a]

[a]Department of Applied Biological Science, Tokyo University of Science, Chiba, Japan; [b]Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan; [c]School of Veterinary Medicine, Azabu University, Sagamihara, Japan; [d]Faculty of Pharmacy, Yokohama University of Pharmacy, Yokohama, Japan

ABSTRACT

4′-Ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) and 4′-ethynyl-2′-deoxyadenosine (EdA) are nucleoside analogues which inhibit human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. EdAP, a cyclosaligenyl (cycloSal) phosphate derivative of EdA, inhibits the replication of the influenza A virus. The common structural feature of these compounds is the ethynyl group at the 4′-position. In this study, these nucleoside analogues were prepared by a common synthetic strategy starting from the known 1,2-di-*O*-acetyl-D-ribofuranose. Biological evaluation of EdAP revealed that this compound reduced hepatitis B virus (HBV) replication dose-dependently without cytotoxicity against host cells tested in this study.

ARTICLE HISTORY
Received 29 August 2019
Accepted 23 September 2019

KEYWORDS
Nucleotide analogue; EdAP; synthesis; hepatitis B virus

4′-Ethynyl-2-fluoro-2′-deoxyadenosine (EFdA, **1**) inhibits human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (Figure 1) [1–9]. This compound inhibits the replication of a wide spectrum of HIV-1 strains resistant to nucleoside reverse transcriptase inhibitors (NRTIs) [10–14]. Furthermore, EFdA has a long half-life *in vivo* and is currently in human clinical trials [15–20]. 4′-Ethynyl-2′-deoxyadenosine (EdA, **2**) also inhibits HIV-1 reverse transcriptase [9,21]. However, this compound is susceptible to degradation by adenosine deaminase (ADA) [22–24]. To overcome the sensitivity against ADA, the fluorine atom was introduced at the 2-positon in **2** to generate **1** [3,4,7,20,22]. The susceptibility of **1** to ADA was drastically decreased compared to that of **2**. EdAP (**3**) is a cyclosaligenyl (cycloSal) phosphate derivative of EdA [25,26]. EdAP inhibits the replication of the influenza virus A both *in vivo* and *in vitro*. The cycloSal moiety generally serves as a lipophilic masking unit to increase membrane-permeability of the nucleoside or nucleoside 5′-monophosphate [27–29]. The cycloSal moiety is hydrolyzed in cells to release the nucleoside 5′-monophosphate.

EFdA (**1**) was designed and first synthesized beginning with 2-amino-2′-deoxyadenosine via 4′-ethynyl -2-amino-2′-deoxyadenosine by Ohrui [1,2]. Other previous syntheses of **1** and **2** included the *N*-glycosylation between an electrophilic sugar and nucleophilic base (Scheme 1). Kuwahara and Ohrui synthesized **1** through a direct *N*-glycosylation between **4** and 2-fluoroadenine (**5**) (Scheme 1(a)) [30]. Ohrui also used **4** as the glycosyl donor for the synthesis of **2** (Scheme 1(b)) [21]. The silyl-Hilbert-Johnson reaction [31,32] between **4** and adenine (**8**) afforded **9**. The *N*-glycosylation reactions between **4** and the bases proceeded selectively. The high β-selectivity is explained by the neighboring group effect of the acetyl group at the C-2 position [31]. However, the syntheses of **1** and **2** included the deprotection of the benzyl groups using hazardous ammonia and highly reactive lithium metal. In the synthesis of **2**, the deprotection of the benzyl groups in **10** under the Birch reduction conditions gave **1**, together with the deaminated product **11** as a byproduct [21]. The use of 2-deoxyribose derivatives as glycosyl donors decreased the diastereoselectivity of the *N*-glycosylations due to the lack of neighboring group participation at the C-2 position (Scheme 1(c,d)). Kuwahara and Ohrui reported that the silyl-Hilbert-Johnson reaction between 2-deoxyribose derivative **12** and **5** gave the desired β-anomer **13** and undesired α-anomer in 46% and 25% yields, respectively (Scheme 1(c)) [33,34]. After separation of **12** by silica gel chromatography, this compound was converted into **1**. MacLaughlin used 3,5-di-*O*-(*p*-toluoyl)-2-deoxyribose derivative **14** as the glycosyl donor [35]. The coupling between **14** and **5** gave a 1.8:1 mixture of β- and α-anomers

CONTACT Kouji Kuramochi kuramoch@rs.tus.ac.jp
Supplemental data for this article can be accessed here.

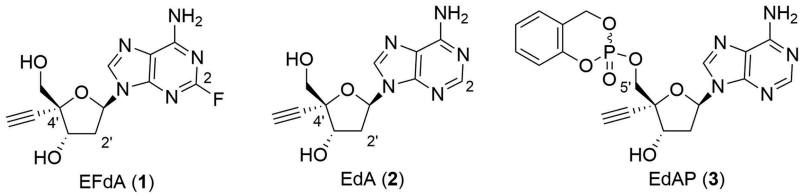
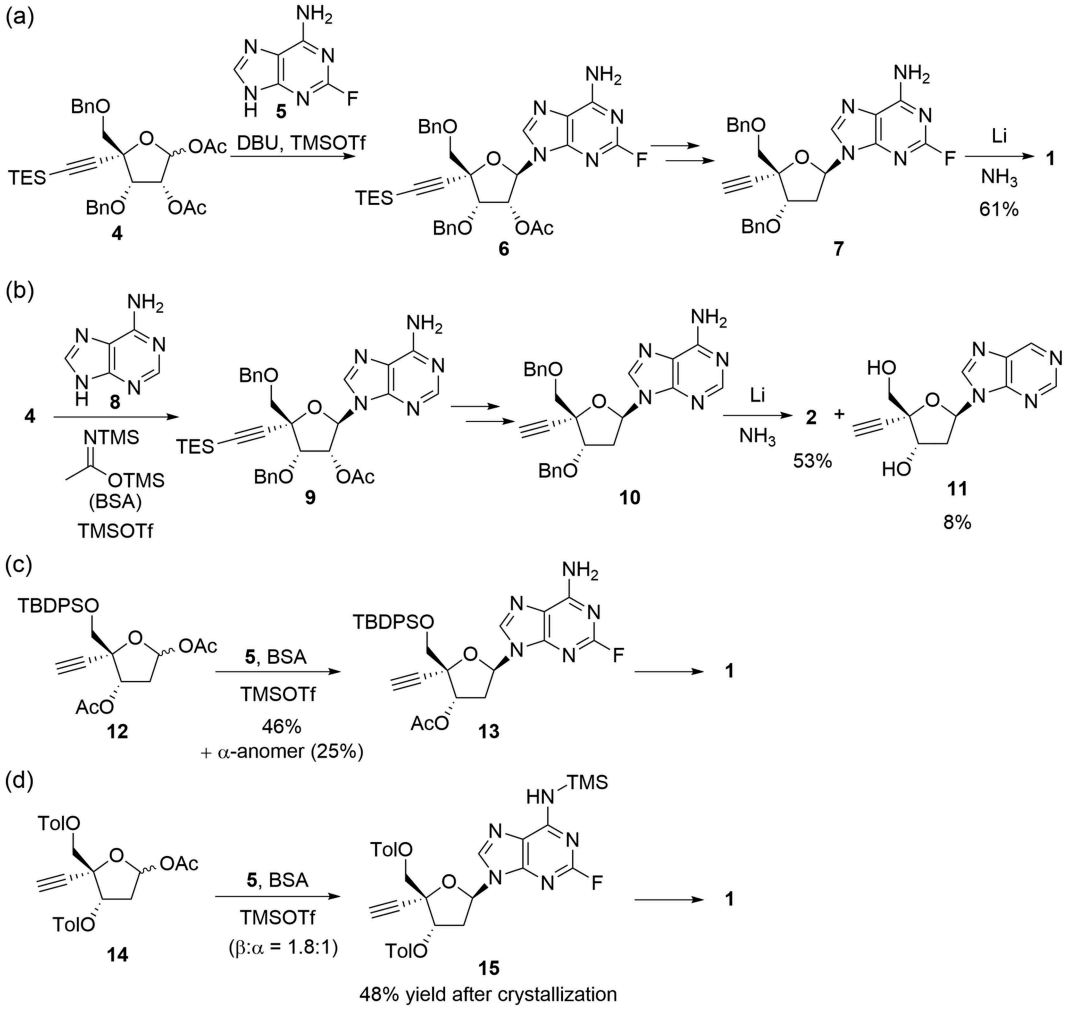


Figure 1. Structures of EFdA (**1**), EdA (**2**), and EdAP (**3**).



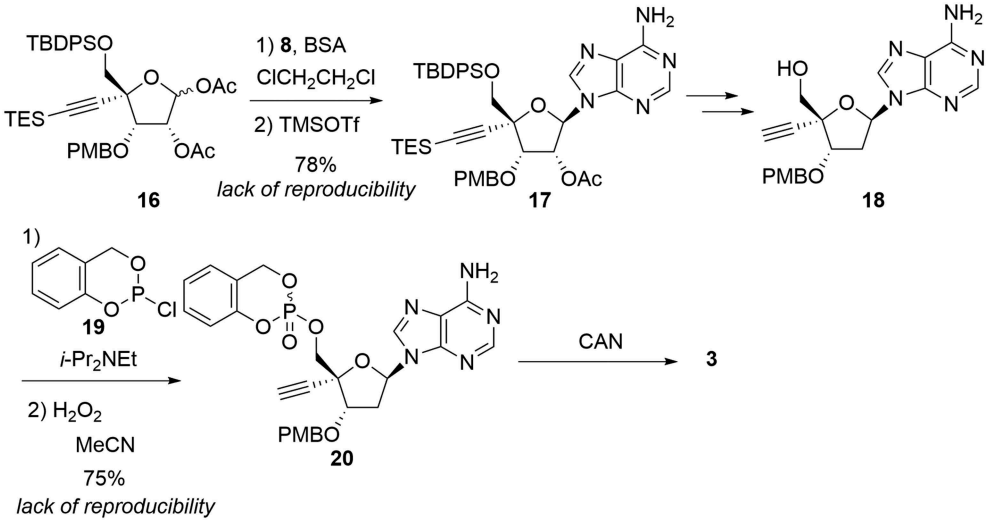
Scheme 1. Previous syntheses of EFdA (**1**) and EdA (**2**). (a) synthesis of **1** reported by Kuwahara and Ohrui [30]. (b) synthesis of **2** reported by Ohrui [21]. (c) alternative synthesis of **1** reported by Kuwahara and Ohrui [33,34]. (d) synthesis of **1** reported by MacLaughlin [35].

(Scheme 1(d)). After separation of the desired β-anomer **15** by crystallization, deprotection of the protective groups in **15** afforded **1**.

Takeuchi, Sugawara and Ohrui designed **16** as a glycosyl donor and used this compound for the synthesis of EdAP (**3**) (Scheme 2) [25,26]. The silyl-Hilbert-Johnson reaction between **16** and **8** afforded **17** in 78% yield. After conversion of **17** into **18**, the introduction of the cycloSal group into the hydroxy group in **18**, followed by deprotection of the *p*-methoxybenzyl (PMB) group gave **3**. However, we suffered from the lack of the reproducibility of the silyl-Hilbert-Johnson reaction between **16** and **8**, and the introduction of the cycloSal group into **18**. The yields were not reproducible, and the reactions were often accompanied by the formations of unidentified byproducts.

Chronic infection of hepatitis B virus (HBV) affects approximately 257 million people worldwide and is a risk factor for developing liver cirrhosis and hepatocellular carcinoma [36]. To solve this public health problem, antiviral agents that eliminate the infection are needed. Because EFdA (**2**) shows anti-HBV activity with IC_{50} value of 0.16 μM [37], we have been interested in the anti-HBV activity of EdA and EdAP.

In this paper, we report the syntheses of EFdA (**1**), EdA (**2**), and EdAP (**3**) starting from the 1,2-di-*O*-acetyl-D-ribofuranose **16** and the anti-HBV activity of EdAP. We optimized the conditions of the silyl-Hilbert-Johnson



Scheme 2. Synthesis of EdAP (**3**) reported by Takeuchi, Sugawara and Ohrui.

reaction and the cycloSal-introduction. The utility of **16** as a glycosyl donor has been demonstrated by the present synthesis of these nucleoside analogues.

Materials and methods

General information

Melting point (Mp) data were determined using a Micro Melting Point Determination Apparatus Type MM-2 instrument (Shimadzu Seisakusyo, Ltd) and were uncorrected. IR spectra were recorded on a Horiba FT-720 spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 and 100 MHz, respectively) spectrometer, using chloroform-*d* ($CDCl_3$), methanol-d_4 (CD_3OD) or dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. Chemical shift values are expressed in δ (ppm) relative to tetramethylsilane (TMS, δ 0.00 ppm) or the solvent resonance ($CDCl_3$, δ 7.26 ppm for ^{1}H NMR and δ 77.0 ppm for ^{13}C NMR; CD_3OD, δ 3.30 ppm for ^{1}H NMR and δ 49.0 ppm for ^{13}C NMR; DMSO-d_6, δ 2.49 ppm for ^{1}H NMR). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (*J*; Hz), and integration. Mass spectra were obtained by on a JEOL high-resolution double-focusing mass spectrometer (JMS-700, "MStation") using fast atom bombardment (FAB). Specific rotations were recorded on a JASCO polarimeter (P-1010), and recorded as $[\alpha]_D$ values (concentration in g/100 mL).

Synthesis

*(2R,3R,4S,5R)-2-(6-Amino-9H-purin-9-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-[(triethylsilyl)ethynyl]tetrahydrofuran-3-yl acetate (**17**) and (2R,3R,4S,5R)-2-(6-amino-3H-purin-3-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-[(triethylsilyl)ethynyl]tetrahydrofuran-3-yl acetate (**21**)*

N,O-Bis(trimethylsilyl)acetamide (BSA) (198 μL, 0.810 mmol) was added to a solution of adenine (**8**, 54.7 mg, 0.405 mmol) in 1,2-dichloroethane (810 μL). The mixture was stirred under an argon atmosphere at 70°C for 5.5 h. A solution of **16** [25,26] (98.8 mg, 0.135 mmol) in 1,2-dichloroethane (1.9 mL) was added to the mixture at room temperature. Trimethylsilyl triflate (TMSOTf) (73.3 μL, 0.405 mmol) was added to the resultant mixture at 0°C. The mixture was stirred under an argon atmosphere at 90°C for 19.5 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution. The mixture was diluted with $CHCl_3$ at 0°C to give a biphasic solution. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4, and concentrated to give a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/1, then MeOH) to afford **17** (49.9 mg, 46%) as a pale yellow oil and **21** (38.2 mg, 35%) as a pale yellow oil. The NMR spectroscopic data for **17** were identical with the reported data [25,26]. Compound **21**: $[\alpha]_D^{23}$ – 17.1 (*c* 0.34, $CHCl_3$). IR (KBr) ν_{max} = 3438, 3363, 3072, 3049, 2956, 2933, 2873, 2858, 2171, 1749, 1658, 1614, 1589, 1556, 1514, 1471 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$; relative to TMS) δ 8.43 (s, 1H), 7.95 (s, 1H), 7.64 (m, 4H), 7.46–7.40 (m, 2H), 7.38–7.32 (m, 4H), 7.22 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.50 (d, *J*= 4.0 Hz, 1H), 5.85 (dd, *J*= 6.0, 4.0 Hz, 1H), 4.75 (d, *J*= 6.0 Hz, 1H), 4.60 (d, *J* = 11.0 Hz, 1H), 4.52 (d, *J* = 11.0 Hz, 1H), 4.14 (d, *J*= 11.4 Hz, 1H), 3.85 (d, *J* = 11.4 Hz, 1H), 3.80 (s, 3H), 2.05 (s, 3H), 1.07 (s, 9H), 0.94 (t, *J*= 7.9 Hz, 9H), 0.58 (q, *J* = 7.9 Hz, 6H).

^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ 169.9, 159.2, 154.4, 154.2, 149.5, 140.4, 135.7 (2C), 135.5 (2C), 132.4, 132.3, 130.0, 129.9, 129.7, 129.2 (2C), 127.9 (2C), 127.8 (2C), 120.7, 113.5 (2C), 101.2, 92.1, 91.4, 84.2, 76.3, 74.4, 73.3, 66.5, 55.2, 26.9 (3C), 20.7, 19.2, 7.4 (3C), 4.1 (3C). HRMS (FAB) *m*/*z* calcd. for $C_{44}H_{56}N_5O_6Si_2$ ([M + H]$^+$) 806.3769, found 806.3768.

*Isomerization of **21** to **17**.*

p-Toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) (2.75 mg, 14.5 μmol) was added to a solution of **21** (38.9 mg, 48.3 μmol) in chlorobenzene (1.0 mL). The reaction mixture was refluxed for 50 min. The mixture was diluted with $CHCl_3$, and concentrated to a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2/1) to afford **17** (32.5 mg, 84%).

*(2R,3R,4S,5R)-2-(6-Amino-9H-purin-9-yl)-5-{[(tert-butyldiphenylsilyl)oxy]methyl}-4-(4-methoxybenzyloxy)-5-[(triethylsilyl)ethynyl]tetrahydrofuran-3-ol (**22**)*

A solution of **17** (1.32 g, 1.64 mmol) in a 2:5 mixture of Et_3N and MeOH (6.3 mL) was stirred at 45°C for 21 h. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by silica gel column chromatography (EtOAc only) to afford **22** (1.23 g, 98%) as a pale yellow oil. The NMR spectroscopic data for **22** were identical with the reported data [25,26].

*O-((2R,3R,4S,5R)-2-(6-Amino-9H-purin-9-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-{[(triethylsilyl)ethynyl]tetrahydrofuran-3-yl} O-phenyl carbonothioate (**23**)*

Phenyl chlorothionoformate (37.3 μL, 0.276 mmol) was added to a solution of **22** (0.211 mg, 0.276 mmol) and 4-dimethylaminopyridine (DMAP) (67.4 mg, 0.552 mmol) in CH_2Cl_2 (1.8 mL) at 0°C. The reaction mixture was stirred under an argon atmosphere at room temperature for 5 h. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/1) to afford **23** (0.215 g, 87%) as a colorless oil. The NMR spectroscopic data for **23** were identical with the reported data [25,26].

*9-((2R,4S,5R)-5-(tert-Butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-{[(triethylsilyl)ethynyl]tetrahydrofuran-2-yl}-9H-purin-6-amine (**24**)*

Azobisisobutyronitrile (AIBN) (9.84 mg, 59.9 μmol) was added to a solution of **23** (0.216 g, 0.236 mmol) and tri-(*n*-butyl)tin hydride (387 μL, 1.44 mmol) in toluene (6.0 mL). The reaction mixture was refluxed under an argon atmosphere for 3 h. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by column chromatography using silica gel containing 10% w/w anhydrous potassium carbonate (hexane/EtOAc = 1/2) to afford **24** (0.163 g, 91%) as a pale yellow oil. The NMR spectroscopic data for **24** were identical with the reported data [25,26].

*[(2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-2-ethynyl-3-(4-methoxybenzyloxy)tetrahydrofuran-2-yl]methanol (**18**)*

A 1.0 M solution of tetra-*n*-butylammonium fluoride (TBAF) in THF (1.2 mL, 1.20 mmol) was added to a solution of **24** (0.41 g, 0.548 mmol) in THF (5.5 mL). The mixture was stirred under an argon atmosphere at room temperature for 2 h. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc to give a biphasic solution. The layers were separated. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4, and concentrated to a residue. The residue was purified by silica gel column chromatography (EtOAc/MeOH = 15/1) to afford **18** (0.196 g, 95%) as a white solid. Mp = 135–136°C. The NMR spectroscopic data for **18** were identical with the reported data [25,26].

*EdA, (2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol (**2**)*

Cerium ammonium nitrate (CAN) (122.7 mg, 0.224 mmol) was added to a solution of **18** (35.4 mg, 89.5 μmol) in a 5:1 mixture of MeCN and water (0.9 mL) at 0°C. The mixture was stirred at 0°C for 5.5 h. The mixture was diluted with MeOH, and concentrated to a residue. The residue was purified by silica gel column chromatography (EtOAc/MeOH = 10/1) to afford EdA (**2**) (20.3 mg, 82%) as a white solid. Mp = 154–155°C. $[\alpha]_D^{23}$ + 13.7 (*c* 0.14, MeOH). IR (KBr) ν_{max} = 3392, 3273, 2927, 2112, 1649, 1604, 1506, 1479, 1421 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6; relative to the solvent resonance) δ 8.31 (s, 1H), 8.12 (s, 1H), 7.32 (br s, 2H), 6.34 (t, *J* = 6.3 Hz, 1H), 5.58–5.55 (2H, overlapped), 4.57 (q, *J* = 6.1 Hz, 1H), 3.66 (dd, *J* = 12.0, 5.3 Hz, 1H), 3.55 (dd, *J* = 11.8, 7.4 Hz, 1H), 3.51 (s, 1H), 2.78–2.72 (m, 1H), 2.43–2.38 (m, 1H). ^{1}H NMR (400 MHz, CD_3OD; relative to the solvent resonance) δ 8.51 (s, 1H), 8.34 (s, 1H), 6.49 (dd, *J* = 7.2, 4.5 Hz, 1H), 4.75 (t, *J* = 7.1 Hz, 1H), 3.85 (d, *J* = 12.2 Hz, 1H), 3.77 (d, *J* = 12.2 Hz, 1H), 3.11 (s, 1H), 2.84–2.78 (m, 1H), 2.70–2.63 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD; relative to the solvent resonance) δ 153.3, 149.6, 147.6, 143.3, 120.4, 87.0, 85.0, 80.6, 78.9, 71.5, 65.7, 40.1. HRMS (FAB) *m*/*z* calcd. for $C_{12}H_{14}N_5O_3$ ([M + H]$^+$) 276.1097, found 276.1095.

*2-{[(2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-2-ethynyl-3-(4-methoxybenzyloxy)tetrahydrofuran-2-yl]methoxy}-4H-benzo[d] [1,3,2]dioxaphosphinine 2-oxide (**20**)*

N,N-Diisopropylethylamine (61.5 μL, 0.353 mmol) and **19** (66.5 μL, 0.353 mmol) were added to a solution of **18** (69.7 mg, 0.176 mmol) in THF (2.7 mL). The mixture was stirred under an argon atmosphere at –40°C for 1 h. An aqueous 30% hydrogen peroxide solution (39.9 μL, 0.353 mmol) was added at – 40°C. The mixture was stirred at room temperature for 50

min. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by silica gel column chromatography (EtOAc/MeOH = 15/1) to afford a crude product. This purification was repeated three time to afford pure **20** (40.4 mg, 41%) as a 1.43:1 diastereomeric mixture as a pale yellow oil. The NMR spectroscopic data for **20** were identical with the reported data [25,26].

*EdAP, 2-{[(2R,3S,5R)-5-(6-Amino-9H-purin-9-yl)-2-ethynyl-3-hydroxytetrahydrofuran-2-yl]methoxy}-4H-benzo[d] [1,3,2]dioxaphosphinine 2-oxide (**3**)*

CAN (80.7 mg, 0.108 mmol) was added to a solution of **20** (30.4 mg, 53.9 μmol) in a 5:1 mixture of MeCN and water (0.9 mL) at 0°C. The mixture was stirred at 0°C for 4.5 h. The mixture was diluted with MeOH, and concentrated to a residue. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10/1) to afford **3** (15.5 mg, 65%) as a 1.25:1 diasteromeric mixture as a pale yellow solid. Mp = 128–130°C (dec.). $[\alpha]_D^{22}$ – 2.67 (*c* 0.54, MeOH). The NMR spectroscopic data for **3** were identical with the reported data [25,26].

*(2R,3R,4S,5R)-2-(6-Amino-2-fluoro-9H-purin-9-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-[(triethylsilyl)ethynyl]tetrahydrofuran-3-yl acetate (**25**)*

BSA (128 μL, 0.524 mmol) was added to a solution of 2-fluoroadenine (**5**, 40.1 mg, 0.262 mmol) in 1,2-dichloroethane (550 μL). The mixture was stirred under an argon atmosphere at 70°C for 7 h. A solution of **16** (63.9 mg, 87.4 μmol) in 1,2-dichloroethane (1.2 mL) was added to the mixture at room temperature. TMSOTf (47.5 μL, 0.262 mmol) was added to the resultant mixture at 0°C, and the resultant mixture was stirred under an argon atmosphere at 90°C for 13 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution. The mixture was diluted with $CHCl_3$ to give a biphasic solution. The layers were separated. The aqueous layer was extracted with $CHCl_3$. The combined organic layer was dried over Na_2SO_4, and concentrated to a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 2/1) to afford **25** (61.6 mg, 86%) as a white solid. Mp = 144–146°C. $[\alpha]_D^{15}$ – 33.3 (*c* 0.28, $CHCl_3$). IR (KBr) ν_{max} = 3324, 3176, 3072, 2955, 2933, 2873, 2859, 1747, 1647, 1612, 1587, 1559, 1515, 1472 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$; relative to TMS) δ 7.86 (s, 1H), 7.64–7.60 (m, 4H), 7.43–7.39 (m, 2H), 7.36–7.31 (m, 4H), 7.27 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.22 (d, *J* = 5.0 Hz, 1H), 5.97 (br s, 2H), 5.74 (dd, *J* = 6.1, 5.0 Hz, 1H), 4.74 (d, *J* = 6.1 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.53 (d, *J* = 11.2 Hz, 1H), 3.97 (d, *J* = 11.2 Hz, 1H), 3.802 (d, *J* = 11.2 Hz, 1H), 3.797 (s, 3H), 2.04 (s, 3H), 1.03 (s, 9H), 0.96 (t, *J* = 7.9 Hz, 9H), 0.59 (q, *J* = 7.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ 169.9, 159.2, 159.1 (d, $J_{C\text{-}F}$ = 209.9 Hz), 157.1 (d, $J_{C\text{-}F}$ = 19.9 Hz), 151.1 (d, $J_{C\text{-}F}$ = 19.5 Hz), 139.5 (d, $J_{C\text{-}F}$ = 2.3 Hz), 135.6 (2C), 135.5 (2C), 132.6, 132.4, 129.9, 129.8, 129.7, 129.4 (2C), 127.8 (4C), 118.1 (d, J_{CF} = 4.0 Hz), 113.6 (2C), 101.6, 91.8, 86.3, 83.5, 76.3, 73.8, 73.3, 66.7, 55.2, 26.7 (3C), 20.6, 19.2, 7.41 (3C), 4.13 (3C). HRMS (FAB) *m/z* calcd. for $C_{44}H_{55}FN_5O_6Si_2$ ([M + H]$^+$) 824.3675, found 824.3676.

*(2R,3R,4S,5R)-2-(6-Amino-2-fluoro-9H-purin-9-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-[(triethylsilyl)ethynyl]tetrahydrofuran-3-ol (**26**)*

A solution of **25** (61.6 mg, 74.8 μmol) in a 2:5 mixture of Et_3N and MeOH (1.4 mL) was stirred at 45°C for 19 h. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by silica gel column chromatography (EtOAc only) to afford **26** (53.5 mg, 91%) as a pale yellow oil. $[\alpha]_D^{21}$ – 2.1 (*c* 0.27, $CHCl_3$). IR (KBr) ν_{max} = 3333, 3183, 3072, 3049, 2998, 2955, 2933, 2912, 2874, 2858, 2169, 1648, 1611, 1588, 1559, 1515, 1490 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$; relative to TMS) δ 7.81 (s, 1H), 7.62–7.60 (m, 4H), 7.44–7.39 (m, 2H), 7.36–7.32 (m, 6H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.22 (br s, 2H), 6.00 (d, *J* = 5.0 Hz, 1H), 4.89 (d, *J* = 11.2 Hz, 1H), 4.75 (ddd, *J* = 8.2, 6.0, 5.0 Hz, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 4.55 (d, *J* = 6.0 Hz, 1H), 3.97 (d, *J* = 11.0 Hz, 1H), 3.83 (d, *J* = 11.0 Hz, 1H), 3.80 (s, 3H), 3.45 (br s, 1H), 1.03 (s, 9H), 0.97 (t, *J* = 7.8 Hz, 9H), 0.62 (q, *J* = 7.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ159.6, 158.9 (d, $J_{C\text{-}F}$ = 209.9 Hz), 156.9 (d, $J_{C\text{-}F}$ = 20.0 Hz), 151.1 (d, $J_{C\text{-}F}$ = 19.5 Hz), 139.9, 135.6 (2C), 135.4 (2C), 132.6, 132.4, 129.93, 129.90, 129.8 (2C), 128.9, 127.8 (4C), 118.3, 113.9 (2C), 102.2, 91.2, 89.4, 83.0, 77.2, 73.1, 72.9, 66.8, 55.3, 26.7 (3C), 19.2, 7.42 (3C), 4.10 (3C). HRMS (FAB) *m/z* calcd. for $C_{42}H_{53}FN_5O_5Si_2$ ([M + H]$^+$) 782.3569, found 782.3568.

*O-((2R,3R,4S,5R)-2-(6-Amino-2-fluoro-9H-purin-9-yl)-5-(tert-butyldiphenylsilyloxymethyl)-4-(4-methoxybenzyloxy)-5-{[(triethylsilyl)ethynyl]tetrahydrofuran-3-yl} O-phenyl carbonothioate (**27**)*

Phenyl chlorothionoformate (9.3 μL, 68.4 μmol) was added to a solution of **26** (53.5 mg, 68.4 μmol) and DMAP (16.7 mg, 0.137 mmol) in dichloromethane (1.0 mL) at 0°C. The reaction mixture was stirred under an argon atmosphere at room temperature for 2 h. The mixture was diluted with $CHCl_3$, and concentrated to a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/1) to afford **27** (56.9 mg, 91%) as a pale yellow oil. $[\alpha]_D^{21}$ – 37.5 (*c* 0.30, $CHCl_3$). IR (KBr) ν_{max} = 3327, 3176, 3070, 2999, 2955, 2932, 2873, 2858, 2171, 1722, 1649, 1610, 1590, 1514, 1490 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$; relative to TMS) δ 7.87 (s, 1H), 7.64–7.61 (m, 4H), 7.42–7.27 (m, 11H), 6.93 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 6.35 (d, *J* = 4.9 Hz, 1H), 6.22 (dd, *J* = 6.0, 4.9 Hz, 1H), 6.13 (br s, 2H), 5.01

(d, J = 6.0 Hz, 1H), 4.83 (d, J = 11.1 Hz, 1H), 4.62 (d, J = 11.1 Hz, 1H), 4.02 (d, J = 11.2 Hz, 1H), 3.86 (d, J = 11.2 Hz, 1H), 3.80 (s, 3H), 1.03 (s, 9H), 0.97 (t, J = 7.9 Hz, 9H), 0.61 (q, J = 7.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ 194.4, 159.3, 159.0 (d, $J_{C\text{-}F}$ = 210.5 Hz), 157.0 (d, $J_{C\text{-}F}$ = 19.8 Hz), 153.4, 151.2 (d, $J_{C\text{-}F}$ = 19.5 Hz), 140.0 (d, $J_{C\text{-}F}$ = 2.7 Hz), 135.6 (2C), 135.5 (2C), 132.5 (2C), 129.9, 129.8, 129.7 (2C), 129.6, 129.5 (2C), 127.8 (2C), 127.7 (2C), 126.7, 121.7 (2C), 118.3 (d, $J_{C\text{-}F}$ = 4.0 Hz), 113.7 (2C), 101.3, 92.4, 86.1, 83.6, 80.8, 76.0, 73.8, 66.5, 55.3, 26.7 (3C), 19.2, 7.44 (3C), 4.10 (3C). HRMS (FAB) m/z calcd. for $C_{49}H_{57}FN_5O_6SSi_2$ ([M + H]$^+$) 918.3552, found 918.3552.

*9-((2R,4S,5R)-5-(tert-Butyldiphenylsilyloxymethyl) -4-(4-methoxybenzyloxy)-5-{[(triethylsilyl)ethynyl]tetrahydrofuran-2-yl}-2-fluoro-9H-purin-6-amine (**28**)*

AIBN (4.38 mg, 26.7 μmol) was added to a solution of **27** (98.1 mg, 0.107 mmol) and tri-(*n*-butyl)tin hydride (172 μL, 0.641 mmol) in toluene (2.7 mL). The reaction mixture was refluxed under an argon atmosphere for 6 h. The mixture was diluted with EtOAc, and concentrated to a residue. The residue was purified by column chromatography using silica gel containing 10% w/w anhydrous potassium carbonate (hexane/EtOAc = 2/1) to afford **28** (65.4 mg, 80%) as a white solid. Mp = 163–165°C. $[\alpha]_D^{19}$ – 21.4 (c 0.13, $CHCl_3$). IR (KBr) ν_{max} = 3437, 3317, 3166, 3072, 2954, 2932, 2873, 2858, 2165, 1674, 1648, 1613, 1585, 1514, 1498 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$; relative to TMS) δ 7.94 (s, 1H), 7.64–7.60 (m, 4H), 7.43–7.39 (m, 2H), 7.36–7.31 (m, 4H), 7.28 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 6.36 (dd, J = 6.7, 5.2 Hz, 1H), 6.00 (br s, 2H), 4.68 (d, J = 11.6 Hz, 1H), 4.61 (d, J = 6.7 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 4.00 (d, J = 11.1 Hz, 1H), 3.87 (d, J = 11.1 Hz, 1H), 3.81 (s, 3H), 2.73–2.61 (m, 2H), 1.04 (s, 9H), 0.97 (t, J = 7.8 Hz, 9H), 0.61 (q, J = 7.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ 159.2, 158.9 (d, $J_{C\text{-}F}$ = 209.3 Hz), 156.9 (d, $J_{C\text{-}F}$ = 20.1 Hz), 150.7 (d, $J_{C\text{-}F}$ = 19.6 Hz), 139.5 (d, $J_{C\text{-}F}$ = 2.6 Hz), 135.6 (2C), 135.5 (2C), 132.7, 132.5, 129.90, 129.86 (2C), 129.1 (2C), 127.8 (4C), 118.2, 113.7 (2C), 102.3, 91.2, 84.6, 83.4, 77.1, 72.1, 66.3, 55.3, 37.3, 26.8 (3C), 19.2, 7.45 (3C), 4.21 (3C). HRMS (FAB) m/z calcd. for $C_{42}H_{53}FN_5O_4Si_2$ ([M + H]$^+$) 766.3620, found 766.3619.

*[(2R,3S,5R)-5-(6-Amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-3-(4-methoxybenzyloxy)tetrahydrofuran-2-yl]methanol (**29**)*

A 1.0 M solution of TBAF in THF (377 μL, 0.377 mmol) was added to a solution of **28** (0.131 g, 0.171 mmol) in THF (1.7 mL). The mixture was stirred under an argon atmosphere at room temperature for 2 h. The reaction was quenched by the addition of water. The mixture was diluted with EtOAc to give a biphasic solution. The layers were separated. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4, and concentrated to a residue. The residue was purified by silica gel column chromatography (hexane/EtOAc = 1/3) to afford **29** (66.4 mg, 94%) as a pale yellow amorphous. $[\alpha]_D^{23}$ – 24.8 (c 0.49, $CHCl_3$). IR (KBr) ν_{max} = 3294, 3180, 3012, 2935, 2836, 2114, 1685, 1614, 1514, 1442 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD; relative to the solvent resonance) δ 8.20 (s, 1H), 7.33 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 6.33 (dd, J = 7.0, 5.6 Hz, 1H), 4.69 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 6.4 Hz, 1H), 4.58 (d, J = 11.3 Hz, 1H), 3.85 (d, J = 13.3 Hz, 1H), 3.76 (s, 3H), 3.74 (d, J = 13.3 Hz, 1H), 3.11 (s, 1H), 2.80–2.74 (m, 1H), 2.63–2.57 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$; relative to the solvent resonance) δ 159.4, 158.5 (d, $J_{C\text{-}F}$ = 212.3 Hz), 157.3 (d, $J_{C\text{-}F}$ = 20.0 Hz), 149.9, 140.3, 129.53, 129.49 (2C), 119.1, 113.9 (2C), 86.4, 86.1, 79.6, 79.5, 77.2, 72.6, 67.9, 55.3, 38.3. HRMS (FAB) m/z calcd. for $C_{20}H_{21}FN_5O_4$ ([M + H]$^+$) 414.1578, found 414.1576.

*EFdA, (2R,3S,5R)-5-(6-Amino-2-fluoro-9H-purin-9-yl)-2-ethynyl-2-(hydroxymethyl)tetrahydrofuran-3-ol (**1**)*

CAN (96.3 mg, 0.176 mmol) was added to a solution of **29** (24.2 mg, 58.5 μmol) in a 5:1 mixture of MeCN and water (0.9 mL) at 0°C. The mixture was stirred at 0°C for 3 h. The mixture was diluted with MeOH, and concentrated to a residue. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 8/1) to afford EFdA (**1**) (10.0 mg, 58%) as a white solid. Mp = 219–220°C (dec.). $[\alpha]_D^{19}$ + 12.7 (c 0.50, MeOH). IR (KBr) ν_{max} = 3321, 3167, 3001, 2116, 1695, 1618, 1496 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD; relative to the solvent resonance) δ 8.25 (s, 1H), 6.34 (dd, J = 7.2, 4.9 Hz, 1H), 4.73 (t, J = 6.9 Hz, 1H), 3.85 (d, J = 12.2 Hz, 1H), 3.76 (d, J = 12.2 Hz, 1H), 3.08 (s, 1H), 2.81–2.74 (m, 1H), 2.63–2.56 (m, 1H). ^{13}C NMR (100 MHz, CD_3OD; relative to the solvent resonance) δ 160.4 (d, $J_{C\text{-}F}$ = 207.7 Hz), 159.1 (d, $J_{C\text{-}F}$ = 19.9 Hz), 151.5 (d, $J_{C\text{-}F}$ = 19.3 Hz), 141.4, 118.7, 86.8, 84.8, 80.7, 78.8, 71.7, 65.9, 39.9. HRMS (FAB) m/z calcd. for $C_{12}H_{13}FN_5O_3$ ([M + H]$^+$) 294.1002, found 294.1000.

Cell culture

Hep38.7-Tet cells were cultured at 37°C, 5% CO_2 as described previously [38,39]. The Hep38.7-Tet cells produce HBV replication under depletion of tetracycline from medium.

Quantification of cell viability

At 3 days after seeding, Hep38.7-Tet cells were incubated in the absence of tetracycline to induce HBV replication and treated with or without the compound for 6 days. 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxyanilide salt (XTT) cell

viability assays were performed with XTT cell proliferation kit II (Roche), according to the manufacturer's instructions [40]. The absorbance values were then measured at 450 nm with a 96-well plate reader. Cell growth inhibition was evaluated as the ratio of the absorbance of the sample to that of the control.

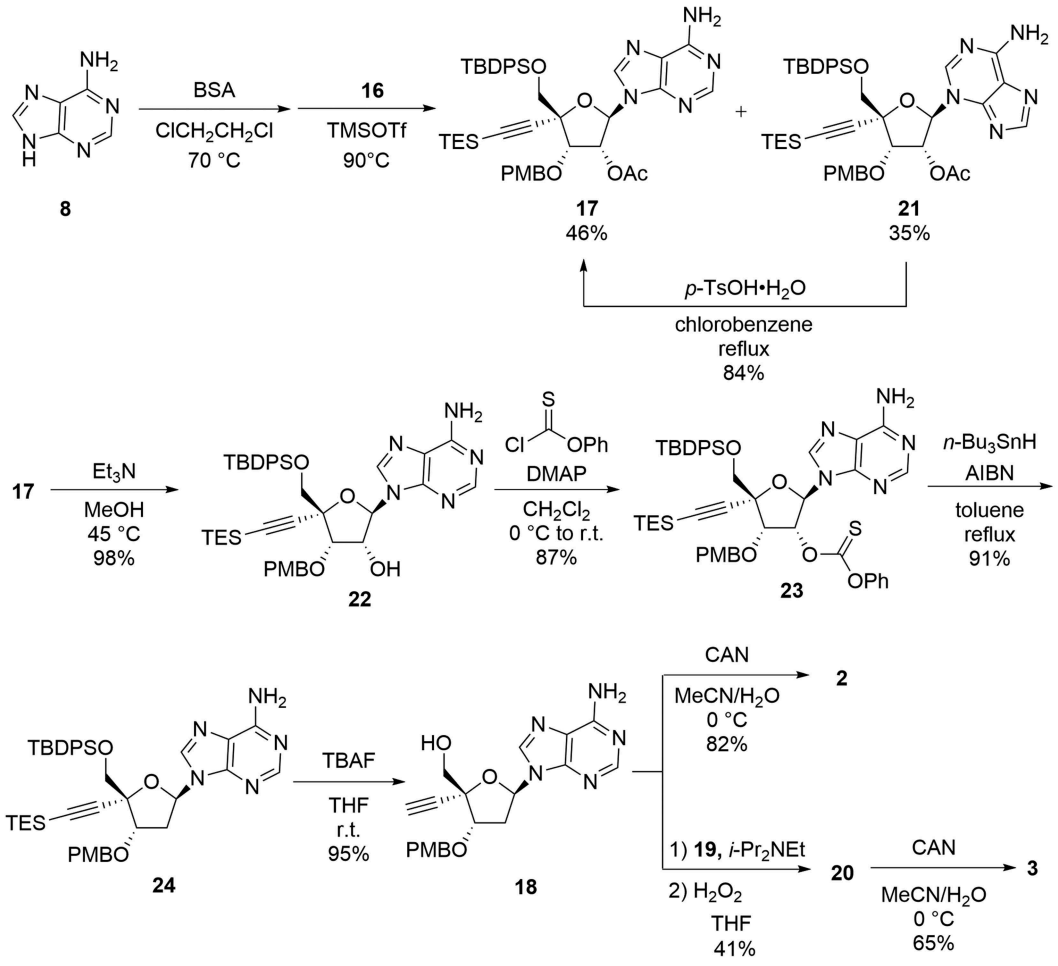
HBV replication assay

At 3 days after seeding, Hep38.7-Tet cells were incubated in the absence of tetracycline to induce HBV replication and treated with or without the compound for 6 days. HBV DNA in the culture supernatant were recovered and quantified by real-time PCR using 5′-AAGGTAGGAGCTGGAGCATTCG-3′ (Forward) and 5′-AGGCGGATTTGCTGGCAAAG-3′ (Reverse) as a primer set and 5′-AGCCCTCAGGCTCAGGGC ATAC-3′ as a probe [41].

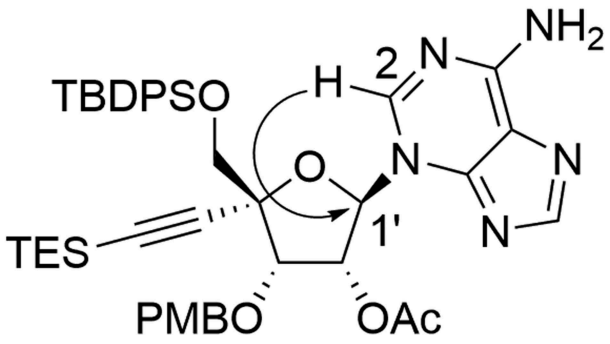
Results

Synthesis of EdA and EdAP

The syntheses of EdA (**2**) and EdAP (**3**) are depicted in Scheme 3. The silyl-Hilbert-Johnson reaction consists of the following two reactions: silylation of a base and glycosylation between the silyated base and an electrophilic sugar. In the previous protocol [25,26], the silylation of adenine (**8**) with BSA was performed in the presence of 1,2-di-*O*-acetyl-D-ribofuranose **16**. Following this protocol, we frequently observed decomposition of **16** during the silylation step. Thus, we considered that the silylation of **8** with BSA in the absence of **14** will improve the reproducibility of the results. Actually, we obtained reproducible results according to the following procedure. First, **8** was reacted with an excess amount of BSA in 1,2-dicloroethane at 70°C. Then, a mixture of the silylated adenine and **16** (α:β = 7.7:1) was treated with TMSOTf and heated at 90°C. This reaction afforded the desired *N*-glycoside **17** in 46% yield, together with isoadenine derivative **21** in 35% yield [42,43]. The structure of **21** was confirmed by HMBC correlation from H-2 (δ 8.42) at the adenine moiety to C-1′ (δ 91.5) at the ribose moiety (Figure 2 and Figure S10 in Supplementary material). Although compound **21** was obtained, this compound easily isomerized to **17** by heating with a catalytic amount of *p*-TsOH·H_2O in refluxing chlorobenzene [44]. Deprotection of the acetyl group in **17** under basic conditions gave alcohol **22**. The hydroxy group in **22** was replaced with a hydrogen atom by a Barton-McCombie



Scheme 3. Synthesis of EdA (**2**) and EdAP (**3**).



21

Figure 2. A selected HMBC correlation in compound **21.**

deoxygenation reaction [45,46]. The alcohol **22** was converted into phenyl thionocarbonate **23**, which was heated with tri-(*n*-butyl)tin hydride and AIBN to give **24**. Removal of the *tert*-butyldiphenylsilyl (TBDPS) and triethylsilyl (TES) groups in **24** with TBAF gave **18** in 95% yield. Deprotection of the PMB group in **18** with CAN gave EdA (**2**) in 82% yield. We found that the lack of reproducibility in the introduction of the cycloSal into **18** was due to the low solubility of **18** in acetonitrile. The use of THF as a solvent instead of acetonitrile improved the reproducibility of the cycloSal-introduction. Coupling of **18** with chlorophosphite **19** [27] in THF, followed by oxidation of the resultant phosphite ester with 30% hydrogen peroxide gave **20** as a 1.43:1 diastereomeric mixture in 41% yield. The difficulty in removal of trace impurities from the product by silica gel chromatography caused the low-isolated yield. Deprotection of the PMB group in **20** with CAN gave **3** as a 1.25:1 diastereomeric mixture in 65% yield.

Synthesis of EFdA

The glycosyl donor **16** was also used for the synthesis of EFdA (**1**) (Scheme 4). The silyl-Hilbert-Johnson reaction between **5** and **16** afforded the desired β-anomer **25** as a sole product in 86% yield. In the synthesis of EdA, the *N*-glycoside **17** and isoadenine derivative **21** were obtained in the glycosylation step. Because the nucleophilicity of the nitrogen at the three-position in 2-fluoroadenine will be suppressed by the electron withdrawing fluorine at the C-2 position, the silylated 2-fluoroadenine will selectively react with **16** at the nitrogen at the nine-position (Figure 3). According to the same protocol for the synthesis of **2**, compound **25** was converted into compound **29**. Deprotection of the PMB group in **29** with CAN gave **1** in 58% yield.

Effect of EdAP on hepatitis B virus replication

The inhibitory effect of EdAP on the replication of HBV is depicted in Figure 4. EdAP did not show significant cytotoxic activity against Hep38.7-Tet

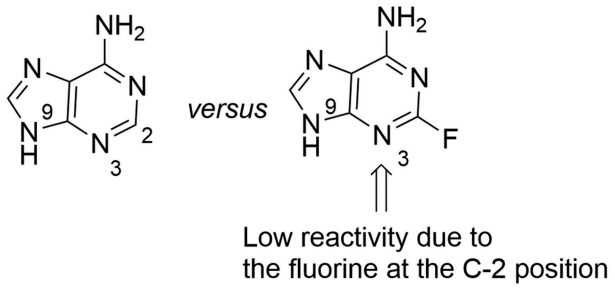
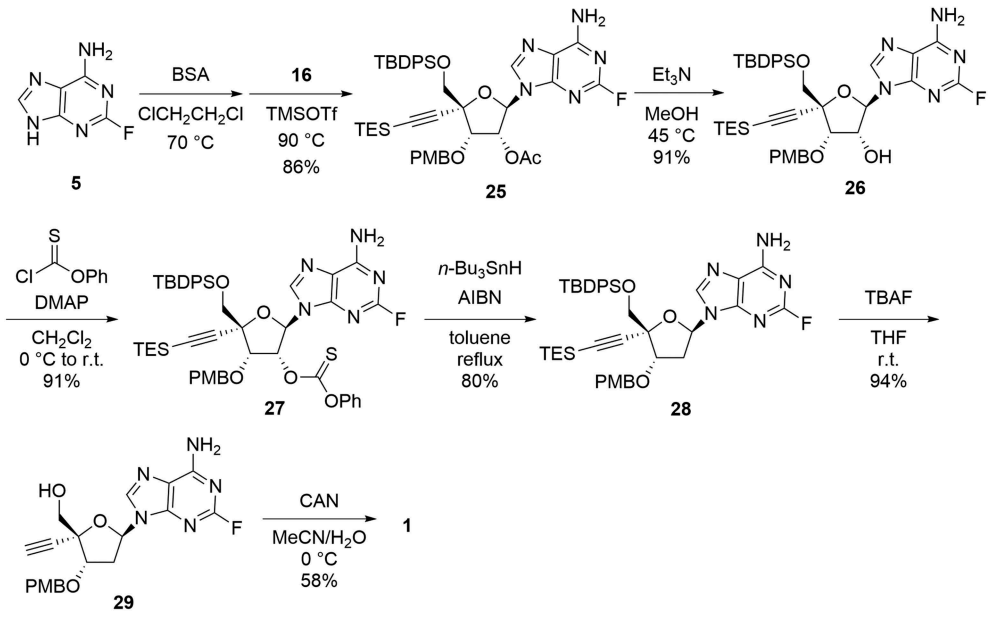


Figure 3. Comparison of the reactivity between adenine and 2-fluoroadenine.



Scheme 4. Synthesis of EFdA (**1**).

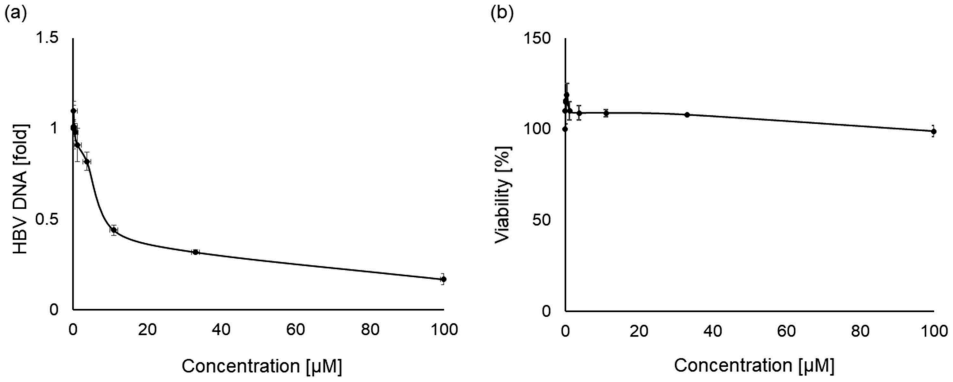


Figure 4. Effect of EdAP on the replication of HBV. (a) Cytotoxicity of EdAP against Hep38.7-Tet cells after 6 days was determined by XTT assay [40]. (b) Anti-HBV activity of EdAP was evaluated by real-time PCR quantifying HBV DNA in the culture supernatant of Hep38.7-Tet cells treated with or without the compound for 6 days [41]. The data indicate the means ± SD of five samples from an experiment.

cells at a concentration below 100 μM (Figure 4(a)). EdAP reduced the HBV DNA secreted from the cells in a dose-dependent manner (Figure 4(b)). The half-maximal inhibitory concentration (IC_{50}) value of EdAP was determined to be 14.5 μM. On the other hand, the effect of EdA on the replication of HBV is weak (Figure S21 in Supplementary material). EdA at 30 μM reduced HBV DNA to only 78%. These results suggest that the cycloSal moiety installed in EdAP improved the anti-HBV activity of EdA.

Conclusion

The syntheses of EFdA, EdA, and EdAP were achieved using a common synthetic strategy starting from known 1,2-diacetylribose derivative **16**. The present method provides easy and reproducible access to these nucleoside analogues. The present biological studies indicate that EdAP reduced HBV replication with an IC_{50} value of 14.5 μM, whereas this compound did not influence the proliferation of the host cells. The present findings provide valuable information for the design and development of novel nucleoside/nucleotide drugs against HBV.

Author contributions

S.Kamisuki., H.O., F.S., and K.K. designed the research; M. K., T.T., E.H., K.N., M.O., M.I., and K.N. and K.K. performed the research. S.Kamo., S.T., K.W., S.Kamisuki., and K. K. analyzed the data; S.T. and K.K. wrote the article.

Acknowledgments

This study was supported by the Japan Society for the Promotion of Science KAKENHI (JP17H04085, JP66K T0111); the JST CREST program (JPMJCR1413); the Japan Agency for Medical Research and Development, AMED (JP18fk0310114j0002, JP18fk0310101j1002, JP18fk0310103j0 202, JP18fm0208019j0002, JP18fk0210036j0001).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Core Research for Evolutional Science and Technology [JPMJCR1413]; Japan Agency for Medical Research and Development [JP18 fk0210036j0001, JP18fk0310101j1002, JP18fk0310103j0202, JP18fk0310114j0002, JP18fm0208019j0002]; Japan Society for the Promotion of Science [KAKENHI/JP17H04085, KAKENHI/JP66KT0111].

ORCID

Toshifumi Takeuchi http://orcid.org/0000-0002-5096-0857
Shogo Kamo http://orcid.org/0000-0002-5799-4937
Shusuke Tomoshige http://orcid.org/0000-0002-4948-5809
Shinji Kamisuki http://orcid.org/0000-0001-8773-3909
Fumio Sugawara http://orcid.org/0000-0002-5419-2955
Kouji Kuramochi http://orcid.org/0000-0003-0571-9703

References

[1] Kohgo S, Ohrui H, Kodama E, et al. 4′-*C*-substituted-2-haloadenosine derivative. Can Pat CA. 2005:2502109.
[2] Kohgo S, Yamada K, Kitano K, et al. Design, efficient synthesis, and anti-HIV activity of 4′-C-cyano- and 4′-C-ethynyl-2′-deoxy purine nucleosides. Nucleosides Nucleotides Nucleic Acids. 2004;23:671–690.
[3] Ohrui H. 2′-Deoxy-4′-C-ethynyl-2-fluoroadenosine, a nucleoside reverse transcriptase inhibitor, is highly potent against all human immunodeficiency viruses type 1 and has low toxicity. Chem Rec. 2006;6:133–143.
[4] Ohrui H, Hayakawa H, Kohgo S, et al. Creation of low toxic reverse-transcriptase inhibitory nucleosides that prevent the emergence of drug-resistant HIV variants. J Synth Org Chem Jpn. 2006;64:716–723.
[5] Ohrui H, Kohgo S, Hayakawa H, et al. 2′-Deoxy-4′-*C*-ethynyl-2-fluoroadenosine: a nucleoside reverse transcriptase inhibitor with highly potent activity

against wide spectrum of HIV-1 strains, favorable toxic profiles, and stability in plasma. Nucleosides Nucleotides Nucleic Acids. 2007;26:1543–1546.
[6] Michailidis E, Marchand B, Kodama EN, et al. Mechanism of inhibition of HIV-1 reverse transcriptase by 4′-Ethynyl-2-fluoro-2′-deoxyadenosine triphosphate, a translocation-defective reverse transcriptase inhibitor. J Biol Chem. 2009;284:35681–35691.
[7] Ohrui H. Development of modified nucleosides that have supremely high anti-HIV activity and low toxicity and prevent the emergence of resistant HIV mutants. Proc Jpn Acad Ser B Phys Biol Sci. 2011;87:53–65.
[8] Michailidis E, Huber AD, Ryan EM, et al. 4′-Ethynyl -2-fluoro-2′-deoxyadenosine (EFdA) inhibits HIV-1 reverse transcriptase with multiple mechanisms. J Biol Chem. 2014;289:24533–24548.
[9] Kodama EI, Kohgo S, Kitano K, et al. 4′-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants *in vitro*. Antimicrob Agents Chemother. 2001;45:1539–1546.
[10] Kawamoto A, Kodama E, Sarafianos SG, et al. 2′-Deoxy-4′-*C*-ethynyl-2-halo-adenosines active against drug-resistant human immunodeficiency virus type 1 variants. Int J Biochem Cell Biol. 2008;40:2410–2420.
[11] Michailidis E, Ryan EM, Hachiya A, et al. Hypersusceptibility mechanism of Tenofovir-resistant HIV to EFdA. Retrovirology. 2013;10:65.
[12] Maeada K, Desai DV, Aoki M, et al. Delayed emergence of HIV-1 variants resistant to 4′-ethynyl -2-fluoro-2′-deoxyadenosine: comparative sequential passage study with lamivudine, tenofovir, emtricitabine and BMS-986001. Antivir Ther. 2014;19:179–189.
[13] Wu VH, Smith RA, Masoum S, et al. MK-8591 (4′-ethynyl-2-fluoro-2′-deoxyadenosine) exhibits potent activity against HIV-2 isolates and drug-resistant HIV-2 mutants in culture. Antimicrob Agents Chemother. 2017;61:e00714–17.
[14] Njenda DT, Aralaguppe SG, Singh K, et al. Antiretroviral potency of 4′-ethnyl-2′-fluoro-2′-deoxyadenosine, tenofovir alafenamide and second-generation NNRTIs across diverse HIV-1 subtypes. J Antimicrob Chemother. 2018;73:2721–2728.
[15] Takamatsu Y, Das D, Kohgo S, et al. The high genetic barrier of EFdA/MK-8591 stems from strong interactions with the active site of drug-resistant HIV-1 reverse transcriptase. Cell Chem Biol. 2018;25:1268–1278.
[16] Murphey-Corb M, Rajakumar P, Michael H, et al. Response of simian immunodeficiency virus to the novel nucleoside reverse transcriptase inhibitor 4′-ethynyl-2-fluoro-2′-deoxyadenosine *in vitro* and *in vivo*. Antimicrob Agents Chemother. 2012;56:4707–4712.
[17] Hachiya A, Reeve AB, Marchand B, et al. Evaluation of combinations of 4′-ethynyl-2-fluoro-2′-deoxyadenosine with clinically used antiretroviral drugs. Antimicrob Agents Chemother. 2013;57:4554–4558.
[18] Zhang W, Parniak MA, Mitsuya H, et al. Preformulation studies of EFdA, a novel nucleoside reverse transcriptase inhibitor for HIV prevention. Drug Dev Ind Pharm. 2014;40:1101–1111.
[19] Stoddart CA, Galkina SA, Joshi P, et al. Oral administration of the nucleoside EFdA (4′-ethynyl-2-fluoro-2′-deoxyadenosine) provides rapid suppression of HIV viremia in humanized mice and favorable pharmacokinetic properties in mice and the rhesus macaque. Antimicrob Agents Chemother. 2015;59:4190–4198.
[20] Markowitz M, Sarafianos SG. 4′-Ethynyl-2-fluoro-2′-deoxyadenosine, MK-8591: a novel HIV-1 reverse transcriptase translocation inhibitor. Curr Opin HIV AIDS. 2018;13:294–299.
[21] Ohrui H, Kohgo S, Kitano K, et al. Syntheses of 4′-*C*-ethynyl-β-D-*arabino*- and 4′-*C*-ethynyl-2′-deoxy -β-D-*ribo*-pentofuranosylpyrimidines and -purines and evaluation of their anti-HIV activity. J Med Chem. 2000;43:4516–4525.
[22] Ohrui H. A new paradigm for developing antiviral drugs exemplified by the development of supremely high anti-HIV active EFdA. J Antivir Antiretrovir. 2014;6:32–39.
[23] Nakata H, Amano M, Koh Y, et al. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4′-ethynyl-2-fluoro-2′-deoxyadenosine. Antimicrob Agents Chemother. 2007;51:2701–2708.
[24] Kirby KA, Michailidis E, Fetterly TL, et al. Effects of substitutions at the 4′ and 2 positions on the bioactivity of 4′-ethynyl-2-fluoro-2′-deoxyadenosine. Antimicrob Agents Chemother. 2013;57:6254–6264.
[25] Sugawara F, Takeuchi T, Kamisuki S, et al. Jpn Kokai Tokkyo Koho. JP. 2015;151346A.
[26] Takeuchi T, Sriwilaijaroen N, Sakuraba A, et al. Design, synthesis, and biological evaluation of EdAP, a 4′-ethynyl-2′-deoxyadenosine 5′-monophosphate analog, as a potent influenza A inhibitor. Molecules. 2019;24:2603.
[27] Meier C. 2-Nucleos-5′-*O*-yl-4*H*-1,3,2-benzodioxa phos-phinin-2-oxides—A new concept for lipophilic, potential prodrugs of biologically active nucleoside monophosphate. Angew Chem Int Ed Engl. 1996;35:70–72.
[28] Meier C, Knispel T, Clercq ED, et al. *cyclo*Sal-pronucleotides of 2′,3′-dideoxyadenosine and 2′,3′-dideoxy-2′,3′-didehydroadenosine: synthesis and antiviral evaluation of a highly efficient nucleotide delivery system. J Med Chem. 1999;42:1604–1614.
[29] Meier C, Balzarini J. Application of the cycloSal-prodrug approach for improving the biological potential of phosphorylated biomolecules. Antiviral Res. 2006;71:282–292.
[30] Fukuyama K, Ohrui H, Kuwahara S. Synthesis of EFdA via a diastereoselective aldol reaction of a protected 3-keto furanose. Org Lett. 2015;17:828–831.
[31] Vorbrüeggen H. Adventures in silicon-organic chemistry. Acc Chem Res. 1995;28:509–520.
[32] Niedballa U, Vorbrüggen H. A general synthesis of pyrimidine nucleosides. Angew Chem Int Ed Engl. 1970;9:461–462.
[33] Kageyama M, Nagasawa T, Yoshida M, et al. Enantioselective total synthesis of the potent anti-HIV nucleoside EFdA. Org Lett. 2011;13:5264–5266.
[34] Kageyama M, Miyagi T, Yoshida M, et al. Concise synthesis of the anti-HIV nucleoside EFdA. Biosci Biotechnol Biochem. 2012;76:1219–1225.
[35] McLaughlin M, Kong J, Belyk KM, et al. Enantioselective synthesis of 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA) via enzymatic desymmetrization. Org Lett. 2017;19:926–929.
[36] Geneva: World Health Organization. Global hepatitis report 2017; 2017. Available from: https://www.who.

int/hepatitis/publications/global-hepatitis-report2017/en/

[37] Takamatsu Y, Tanaka Y, Kohgo S, et al. 4′-Modified nucleoside analogs: potent inhibitors active against entecavir-resistant hepatitis B virus. Hepatology. 2015;62:1024–1036.

[38] Ogura N, Watashi K, Noguchi T, et al. Formation of covalently closed circular DNA in Hep38.7-Tet cells, a tetracycline inducible hepatitis B virus expression cell line. Biochem Biophys Res Commun. 2014;452:315–321.

[39] Iwamoto M, Watashi K, Tsukuda S, et al. Evaluation and identification of hepatitis B virus entry inhibitors using HepG2 cells overexpressing a membrane transporter NTCP. Biochem Biophys Res Commun. 2014;443:808–813.

[40] Roehm NW, Rodgers GH, Hatfield SM, et al. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J Immunol Methods. 1991;142:257–265.

[41] Passioura T, Watashi K, Fukano K, et al. De novo macrocyclic peptide inhibitors of hepatitis B virus cellular entry. Cell Chem Biol. 2018;25:906–915.

[42] Leonard NJ, Laursen RA. The synthesis of 3-β-D-ribofuranosyladenine. J Am Chem Soc. 1963;85:2026–2028.

[43] Leonard NJ, Laursen RA. Synthesis of 3-β-D-ribofuranosyladenine and (3-β-D-ribofuranosyladenine)-5′-phosphate. Biochemistry. 1965;4:354–365.

[44] Framski G, Gdaniec Z, Gdaniec M, et al. A reinvestigated mechanism of ribosylation of adenine under silylating conditions. Tetrahedron. 2006;62:10123–10129.

[45] Barton DHR, McCombie SW. A new method for the deoxygenation of secondary alcohols. J Chem Soc Perkin Trans. 1975;1:1574–1585.

[46] Crich D, Quintero L. Radical chemistry associated with the thiocarbonyl group. Chem Rev. 1989;89:1413–1432.