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Attempt to reduce cytotoxicity by synthesizing the L-enantiomer of 4′-*C*-ethynyl-2′-deoxypurine nucleosides as antiviral agents against HIV and HBV

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We investigated the potential of 4′-*C*-substituted nucleosides for the treatment of HIV-1 and HBV. Of the nucleosides we prepared, several 4′-*C*-ethynyl-2′-deoxypurine nucleosides showed the most potent anti-HIV activity. However, two candidates, 4′-*C*-ethynyl-2′-deoxyguanosine and 9-(2-deoxy-4-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)-2,6-diaminopurine, were very toxic during *in vivo* study. On the other hand, lamivudine (3TC) is known to show remarkable activity against HIV and HBV with lower cytotoxicity. Therefore, we attempted to synthesize the L-enantiomer of 4′-*C*-ethynyl-2′-deoxypurine nucleosides in 20–21 steps. These methods consisted of preparing 4-*C*-ethynyl-L-sugar, starting from D-arabinose and then condensing the L-sugar derivative with 2,6-diaminopurine. 4′-*C*-Ethynyl-2′-deoxyguanosine was also prepared by enzymatic deamination from the 2,6-diaminopurine derivative. The compounds' antiviral activity against HIV and HBV was then evaluated. Unfortunately, they demonstrated no activity and no cytotoxicity.

Keywords: 4′-C-ethynyl-2′-deoxy nucleosides (4′-EdNs), L-enantiomer, lamivudine, anti-HIV-1 agent, anti-HBV agent

Introduction

A number of nucleosides with unnatural L-sugars that exhibit antiviral activity and no cytotoxicity have recently been discovered by Chu *et al.* They have reported on one of the most potent antiviral drugs, lamivudine (3TC) (Beach *et al.*, 1992). Consequently, these L-nucleosides are expected to lead to the development of more potent and less toxic antiviral nucleoside drugs.

We synthesized 4′-*C*-ethynyl-2′-deoxycytidine **1** and reported its anti-HIV activity to have an EC_{50} value of 0.0048 μM. However, this compound also showed potent cytotoxicity (IC_{50}=0.92 μM)(Ohrui *et al.*, 2000) (Figure 1). These results prompted us to synthesize the L-enantiomer of 4′-*C*-ethynyl-2′-deoxycytidine **2**; unfortunately, it did not show any activity against human immunodeficiency virus (HIV)-1 up to 100 μM (Kohgo *et al.*, 2001) (Figure 1).

During an exploration of novel nucleoside reverse transcriptase inhibitors (NRTIs), we selected 9-(2-deoxy-4-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)-2,6-diaminopurine **3** and 4′-*C*-ethynyl-2′-deoxyguanosine **5** as anti-HIV agents because of their high biological activity. Interestingly, compound **3** was also active against hepatitis B virus (HBV), with an excellent EC_{50} value. However, they showed high levels of toxicity in mice (Ashida, Yamasa Corporation, personal comunication). Therefore, we attempted to synthesize the L-enantiomers of 4′-EdNs (**3** and **5**), which were designated as compounds **4** and **6**, in order to reduce cytotoxicity without losing biological activity (Figure 2).

As mentioned above, the L-enantiomers of **1** completely lost biological activity. However, we are still interested in making L-enantiomers of 4′-EdNs (**3** and **5**) in order to elucidate the structure-activity relationship of these nucleosides.

Materials and methods

General method for chemistry

All melting points were determined on a Yanagimoto MP-500 D micro melting point apparatus and are uncorrected.

Figure 1. The structures of lamivudine (3TC), 4′-*C*-ethynyl-2′-deoxycytidine **1**, and L-enantiomer of 4′-*C*-ethynyl-2′-deoxycytidine **2**

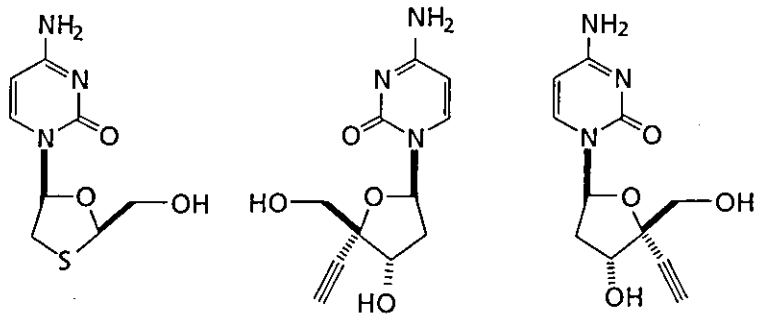
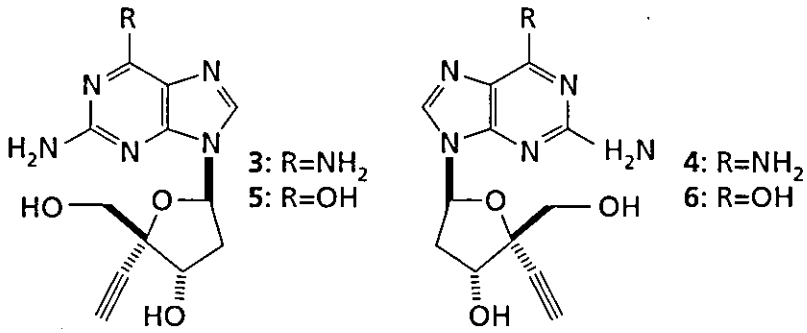


Figure 2. The structures and antiviral activities of 9-(2-deoxy-4-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)-2,6-diaminopurine **3**, L-enantiomer **4**, 4′-*C*-ethynyl- 2′-deoxyguanosine **5**, L-enantiomer **6**



HIV (EC_{50}) **3**: 0.00034 μM, **5**: 0.0015 μM
HBV (EC_{50}) **3**: 0.17 μM

The ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX-400 spectrometer or a Bruker Avance 500 spectrometer, with $CDCl_3$ or DMSO-d_6 as the solvent and tetramethylsilane (TMS) as reference. UV spectra were recorded with a Shimazu UV-160A spectrophotometer. Low-resolution mass spectra (MS) were taken on a JEOL JMS-AX500 spectrometer. Merck precoated plates (Kieselgel 60F254) were used for thin-layer chromatography (TLC) and spots were examined with ultraviolet light and sulphuric acid/anisaldehyde solution. Merck Kieselgel 60 was used for column chromatography. Reversed-phase column chromatography was performed on Fuji Silysia DM1020T. The obtained compounds' purity was verified by TLC or high-performance liquid chromatography (HPLC). Identity was verified by MS, ^{1}H-NMR and elementary analysis.

Antiviral evaluation

Compounds

3'-Azido-3'-deoxythymidine (AZT or zidovudine) was purchased from Sigma (St Louis, Mo., USA). (-)-2',3'-Dideoxy-3'-thiacytidine (3TC or lamivudine) was a generous gift from Dr RF Schinazi (Atlanta, Ga., USA).

Anti-HIV activity

For determination of anti HIV-1 activity, MT-4 cells were suspended at 10^5 cells/ml and exposed to HIV-1_{LAI} at 100 $TCID_{50}$. Immediately after viral exposure, the cell suspension (10^4 cells in 100 µl) was brought into each well of a 96-well flat microtitre plate (Coster, Cambridge, Mass., USA) containing various concentrations of test compounds. After incubation for 5 days, the number of viable cells was determined by the MTT method as previously described (Kodama *et al.*, 1996).

Anti-HBV activity

The 2.2.15 cells that secrete hepatitis B virions were kindly provided by Dr S Shigeta (Fukushima Medical University, Fukushima, Japan). The 2.2.15 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 µg/ml streptomycin, 100 U/ml penicillin G and 0.5 mg/ml genecitin. They were incubated at 37°C in a moist atmosphere containing 5% CO_2/95% air. The 2.2.15 cells were seeded at a density of 5×10^4 cells per 5 ml in 25 cm^2 flask (Becton Dickinson, NJ, USA). Compounds were added to the medium 3 days after the seeding. Cells were grown in the presence of drugs for 12 days, with the medium changed every 3 days.

After incubation, the medium was centrifuged (5 min, 190×g) and polyethylene glycol 6000 was added to the supernatant to a final concentration of 10%. The virus was pelleted at 1420×G for 30 min. The pellet was resuspended in 1 ml of 0.3% SDS and proteinase K at 0.2 mg/ml in TNE (10 mM Tris-HCl, pH 7.5 /150 mM NaCl/10 mM EDTA). The suspension was incubated for 2 h at 55°C. Digest was then extracted with phenol/chloroform, 1:1 (vol./vol.), and DNA was precipitated with ethanol. The DNA pellet was dissolved in 40 ml of TE (10 mM Tris-HCl, pH 8.0/1 mM EDTA). The HBV DNA was amplified with polymerase chain reaction (PCR). PCR amplification was carried out using a TaKaRa PCR amplification kit with AmpliTaq™ DNA polymerase and HBV primers (Takara Bio Inc., Ohtsu, Japan). For a PCR reagent, 5 µl of DNA specimen was added to 45 µl mixture.

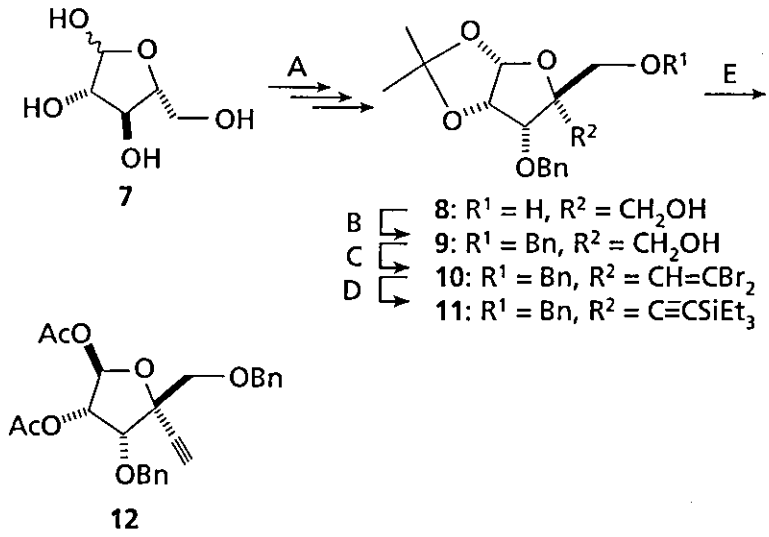
The final volume of 50 µl contained 10 mM Tris-HCl, pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 6.25 pmol of each primer and 1.25 U of AmpliTaq™ DNA polymerase. The 25 cycles PCR consisted of denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min and extension at 72°C for 3 min. The PCR product was separated by electrophoresis in a 4% NuSieve agarose and stained with ethidium bromide. The intensity of the photographic bands was quantitated by an image-analysing software, Photoshop (Adobe Systems Inc., Calif., USA). Fifty percent effective concentration (EC_{50}) was defined as the drug concentration that inhibited HBV viral DNA yield in the PCR product by 50%. The values were obtained by plotting percentage inhibition compared with the control concentration versus that of the drug.

Synthesis of key intermediate **12** (Figure 3)

3,5-Di-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene-α-L-ribo-pentofuranose ***(9)***. To a solution of **8** (4.66 g, 15 mmol) in DMF (39 ml) at 0°C was added 60% NaH (0.66 g, 16.5 mmol), and the mixture was stirred for 30 min at 0°C. Benzyl bromide (2.3 ml, 19.5 mmol) was added dropwise at 0°C. After being stirred for 1 h at the same temperature, 20 ml of water was added. The mixture was stirred for 15 min at room temperature and extracted with AcOEt. The organic phase was washed with water twice and sat NaCl (×1) and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel column chromatography eluting with 17–25% AcOEt in hexane to yield **9** (3.23 g, 54%) as a syrup.

^{1}H -NMR ($CDCl_3$) δ 7.24–7.36 (10H, m, 2×Ph), 5.79 (1H, d, *J*=3.9 Hz, H-1), 4.77, 4.49 (each 1H, d, *J*=11.7 Hz, CH_2Ph), 4.64 (1H, dd, *J*=5.4, 3.9 Hz, H-2), 4.53, 4.45 (each 1H, d, *J*=11.7 Hz, CH_2Ph), 4.28 (1H, d, *J*=5.4 Hz, H-3), 3.94 (1H, dd, *J*=11.7, 5.9 Hz, H-6), 3.83 (1H, dd, *J*=11.7, 7.8 Hz, H-6), 3.59, 3.54 (each 1H, d, *J*=10.7 Hz, H-5), 2.37 (1H, dd, *J*=7.8, 5.9 Hz, OH), 1.63, 1.34 (each 3H, s, acetonide); FAB-MS m/z: 401 (M^++H). Anal. Calcd for $C_{23}H_{28}O_6$: C; H, 7.05. Found: C, 68.77; H, 7.01.

Figure 3. Synthetic scheme for sugar key intermediate **12**



A: eight steps from 7, 26%. B: BnBr, NaH, DMF, 54%. C: 1) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; 2) CBr_4, Ph_3P, CH_2Cl_2, 84%; D: MeLi, Et_3SiCl, THF. E: 1) AcOH, TFA, H_2O; 2) Ac_2O, pyridine, 88%.

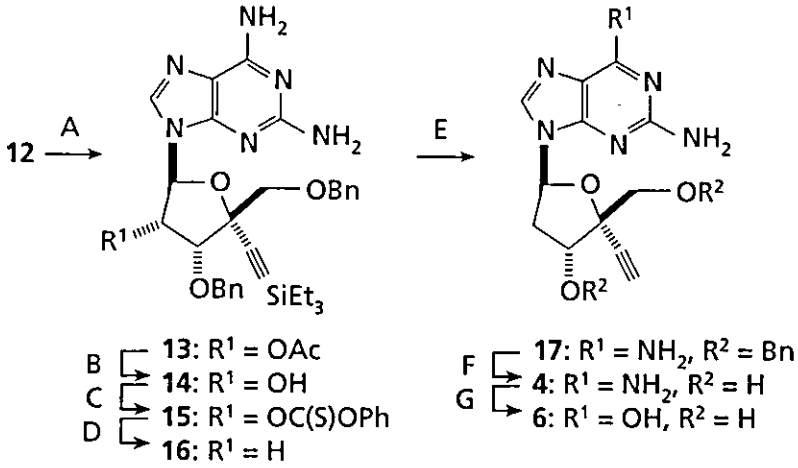
3,5-Di-O-benzyl-4-C-(2,2-dibromoethenyl)-1,2-O-isopropylidene-α-L-ribo-pentofuranose ***(10)***. To a solution of oxalyl chloride (1.1 ml, 12.1 mmol) in dichloromethane (19.4 ml) at −78°C, DMSO (1.2 ml, 16.1 mmol) was added dropwise. The mixture was stirred for 15 min at the same temperature, and a solution of **9** (3.23 g, 8.07 mmol) in CH_2Cl_2 (19.4 ml) was added dropwise. After stirring for 30 min at −78°C, Et_3N (3.6 ml, 24.2 mmol) was added. The mixture was stirred for 2 h at room temperature. The reaction was quenched with water and extracted with $CHCl_3$. The organic layer was washed with water and sat NaCl and dried over $MgSO_4$. After evaporation of the solvent, CBr_4 (5.38 g, 16.1 mmol) and Ph_3P (8.51 g, 32.3 mmol) were added to a solution of the residue in CH_2Cl_2 (68 ml) at 0°C. The mixture was stirred for 1 h at room temperature and washed with water; sat. $NaHCO_3$ and sat. NaCl were added, and the solution dried over Na_2SO_4. After evaporation of the solvent, AcOEt was added to the residue. After an insoluble precipitate was removed by filtration, the filtrate was evaporated. The residue was purified by silica gel column chromatography eluting with 9% AcOEt in hexane to yield **10** (3.76 g, 84%) as a syrup.

^{1}H-NMR ($CDCl_3$) δ 7.23–7.35 (10H, m, 2×Ph), 7.11 (1H, s, Br_2C=CH), 5.76 (1H, d, *J*=3.9 Hz, H-1), 4.72, 4.60 (each 1H, d, *J*=12.2 Hz, CH_2Ph), 4.59, 4.42 (each 1H, d, *J*=12.2 Hz, CH_2Ph), 4.53 (1H, t, *J*=4.4 Hz, H-2), 4.21 (1H, d, *J*=4.4 Hz, H-3), 3.82, 3.40 (each 1H, d, *J*=11.2 Hz, H-5), 1.58, 1.30 (each 3H, s, acetonide).

1,2-Di-O-acetyl-3,5-di-O-benzyl-4-C-triethysilylethynyl-β-L-ribo-pentofuranose ***(12)***. To a solution of **10** (3.76 g, 6.79 mmol) and Et_3SiCl (2.2 ml, 13.2 mmol) in THF (41 ml) at −10°C was added methyl lithium (9.0 ml of 2.2 M *n*-Et_2O solution, 19.8 mmol) under an argon atmosphere, and the mixture was stirred for 30 min at the same temperature. After addition of water, the mixture was stirred for 5 min at room temperature. After partition of the mixture, the organic layer was washed with sat NaCl and dried over Na_2SO_4. After evaporation of the solvent, water (20 ml) and trifluoroacetic acid (6.7 ml) was added to a solution of the residue **11** in AcOH (45 ml), and the mixture was stirred for 1.5 h at 40°C. The mixture was concentrated, and AcOEt was added to the residue. The solution was washed with sat. $NaHCO_3$ (×2) and dried over Na_2SO_4. After evaporation of the solvent, Ac_2O (6.7 ml) was added to the solution of the residue in pyridine (31 ml), and the mixture was stirred overnight at room temperature. The mixture was concentrated, and AcOEt was added to the residue. The solution was washed with water once, sat. $NaHCO_3$ twice, and sat. NaCl once and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel column chromatography eluting with 5–9% AcOEt in hexane to yield **12** (3.3 g, 88%) as a syrup.

^{1}H –NMR ($CDCl_3$) δ 7.25–7.34 (10H, m, 2×Ph), 6.20 (1H, s, H-1), 5.32 (1H, d, *J*=4.4 Hz, H-2), 4.65, 4.60 (each 1H, d, *J*=11.7 Hz, CH_2Ph), 4.56, 4.47 (each 1H, d, *J*=11.7 Hz, CH_2Ph), 4.48 (1H, d, *J*=4.9 Hz, H-3), 3.68, 3.61 (each

Figure 4. Synthetic scheme for the L-enantiomer of 4'-C-ethynyl derivatives **4** and **6**



A: silylated DAP, TMSOTf, ClC_2H_4Cl. B: Et_3N, MeOH. C: ClC(S)OPh, MeCN, DMAP. D: Bu_3SnH, AIBN, toluene. E: TBAF, THF, 44% from **12**. F: Li, naphthalene, THF, 57%. G: adnosine deaminase, potassium phosphate buffer, 54%.

1H, d, *J*=10.7 Hz, H-5), 2.09, 1.84 (each 3H, s, Ac), 0.96 (9H, t, *J*=7.8 Hz, $3\times CH_2CH_3$,), 0.57 (6H, q, *J*=7.8 Hz, $3\times CH_2CH_3$,); FAB-MS m/z: 493 (M^+-AcOH); Anal. Calcd for $C_{31}H_{40}O_7Si$: C, 67.36; H, 7.29. Found: C, 67.16; H, 7.27.

Synthesis of 6

9-(3,5-Di-O-benzyl-2-deoxy-4-C-ethynyl-β-L-ribo-pentofuranosyl)-2,6-diaminopurine ***(17)***. A mixture of **12** (3.29 g, 5.96 mmol), 2,6-diaminopurine (1.35 g, 8.94 mmol) and *N, O*-bis(trimethylsilyl)acetamide (13.2 ml, 53.6 mmol) in 1,2-dichloroethane (49 ml) was refluxed for 3 h. After being kept for 1 h at room temperature, TMSOTf (0.77 mL, 4.0 mmol) was added dropwise to the mixture at 0°C under an argon atmosphere. The mixture was stirred at room temperature for 10 min, and then it was refluxed for 16 h. The mixture was quenched with sat $NaHCO_3$ at 0°C and filtered through a pad of Celite. The filtrate was extracted with $CHCl_3$, and the organic phase was washed with sat NaCl and dried over Na_2SO_4. After evaporation of the solvent, Et_3N (27.4 ml) was added to a solution of the residue **13** in MeOH (117 ml), and the mixture was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was co-distilled with MeCN (×2). Phenyl chlorothionoformate (0.83 ml, 5.96 mmol) was added to an acetonitrile solution (69 ml) of the residue **14** and 4-dimethylaminopyridine (1.47 g, 11.9 mmol) at room temperature under an argon atmosphere, and the mixture was stirred at the same temperature for 1 h. After the solvent was evaporated, the residue was partitioned between AcOEt and water. The organic phase was washed with water and sat NaCl and dried over Na_2SO_4. The filtrate was evaporated under reduced pressure and the residue passed through a short silica gel column (eluted with AcOEt:hexane:EtOH, 30:20:1) to give **15**. To a solution of **15** in toluene (126 ml) was added 2,2'-azobis(isobutylonitrile) (0.206 g, 1.19 mmol) and tri-*n*-butyltin hydride (3.3 ml, 11.9 mmol), and the mixture was stirred at 80°C under an argon atmosphere for 1 h. After being kept for 1 h at room temperature, the mixture was concentrated. To a solution of the residue **16** in THF (77 ml) at room temperature was added TBAF (3.3 ml of 1.0 M THF solution, 3.3 mmol). After stirring at the same temperature for 1 h, the solvent was evaporated. After the residue was dissolved in AcOEt, water was added. The mixture was stirred for 5 min at room temperature and filtered through a pad of Celite. The filtrate was partitioned, and the organic phase was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel column chromatography eluting with 5% EtOH in AcOEt to yield **17** (1.24 g, 44%) as a foam.

^{1}H-NMR ($CDCl_3$) δ 7.70 (1H, s, H-8), 7.25–7.36 (10H, m, 2×Ph), 6.30 (1H, dd, *J*=7.3, 4.9 Hz, H-1'), 5.31 (2H, br s, NH_2), 4.71 (1H, d, *J*=12.2 Hz, CHH'Ph), 4.52–4.65 (5H, m, CH_2Ph, NH_2, H-3'), 4.64 (1H, d, *J*=12.2 Hz, CH*H*'Ph), 3.82 (1H, d, *J*=10.7 Hz, H-5'), 3.72 (1H, d, *J*=10.7 Hz, H-5"), 2.61-2.76 (2H, m, H-2'); FAB-MS m/z 471 (M^++H); Anal. Calcd for $C_{26}H_{26}N_6O_3$: C, 66.37; H, 5.57; N, 17.86. Found: C, 66.17; H, 5.53; N, 17.74.

9-(2-Deoxy-4-C-ethynyl-β-L-ribo-pentofuranosyl) -2,6-diaminopurine ***(4)***. To a solution of naphthalene (5.2 g, 42.1 mmol) in THF (42 ml) at room temperature was added lithium metal (212 mg, 31.6 mmol) in several pieces.

After the mixture was stirred at the same temperature for 3 h, a solution of **17** (1.24 g, 2.63 mmol) in THF (15 ml) was added at −78 °C. The mixture was vigorously stirred at the same temperature for 2 h, and a solution of NH_4Cl (4.9 g) in water (15 ml) was added. After being stirred at room temperature for 30 min, the mixture was partitioned between AcOEt and water. The aqueous phase was concentrated to a volume of about 10 ml and filled up with water to 50 ml. The solution was purified by reverse-phased ODS column chromatography eluting with 10% ethanol in water to yield **4** (443 mg, 57%) as a crystal.

mp 200–205°C (decomposition, crystallized from water); $[\alpha]^{25}/_{D}$−39.0° (c=0.6, MeOH); UV (H_2O) λ_{max}=280 nm (ε 9427), 256 nm (ε 8649); ^{1}H −NMR (DMSO-d_6) δ 7.90 (1H, s, H-8), 6.73 (2H, br s, NH_2), 6.20 (1H, t, *J*=6.3 Hz, H-1′), 5.75 (2H, br s, NH_2), 5.60 (1H, t, *J*=4.9 Hz, OH), 5.51 (1H, d, *J*=5.4 Hz, OH), 4.49 (1H, q, *J*=5.9 Hz, H-3′), 3.65 (1H, dd, *J*=11.7, 5.4 Hz, H-5′), 3.56 (1H, dd, *J*=11.7, 7.3 Hz, H-5′), 3.46 (1H, s, ethynyl-H), 2.64 (1H, dt, *J*=13.2, 6.4 Hz, H-2′), 2.33 (1H, dt, *J*=13.2, 6.8 Hz, H-2′); FAB-MS m/z 291 (M^++H); Anal. Calcd for $C_{12}H_{14}N_6O_3$•0.2 H_2O: C, 49.08; H, 4.87; N, 28.62. Found: C, 48.77; H, 4.91; N, 28.95.

9-(2-Deoxy-4-C-ethynyl-β-L-ribo-pentofuranosyl)guanine (6). A solution of **4** (50 mg, 0.172 mmol) and adenosine deaminase (1.55 ml, 1548 unit) in 25 mM potassium phosphate buffer (90 ml, pH 7.0) was stirred at 40°C for 10 d. The solution was concentrated to a volume of about 10 ml and filled with water to 50 ml. The solution was purified by reverse-phased ODS column chromatography eluting with 5% ethanol in water. In order to remove salts, the ODS column chromatography was performed to yield **6** (27 mg, 54%) as a crystal.

mp 242–248°C (decomposition, crystallized from water); $[\alpha]^{32}/_{D}$−7.7°(c=0.1, H_2O); UV (H_2O) λ_{max}=253 nm (ε 14063); ^{1}H–NMR (DMSO-d_6) δ 10.62 (1H, br s, NH), 7.89 (1H, s, H-8), 6.47 (2H, br s, NH_2), 6.14 (1H, dd, *J*=6.8, 5.4 Hz, H-1′), 5.49 (1H, d, *J*=5.4 Hz, OH), 5.29 (1H, t, *J*=5.9 Hz, OH), 4.48 (1H, dt, *J*=6.8, 5.4 Hz, H-3′), 3.61 (1H, dd, *J*=11.7, 5.9 Hz, H-5′), 3.55 (1H, dd, *J*=12.2, 6.4 Hz, H-5′), 3.45 (1H, s, ethynyl-H), 2.56 (1H, dt, *J*=12.2, 6.4 Hz, H-2′), 2.36 (1H, dt, *J*=13.2, 6.8 Hz, H-2′); FAB-MS m/z 292 (M^++H); Anal. Calcd. for $C_{12}H_{13}N_5O_{40}$•0.6 H_2O:C, 47.71; H, 4.74; N, 23.18. Found: C, 47.31; H, 4.22; N, 22.78.

Results

Synthesis of the L-ribose unit bearing an ethynyl group at the 4-*C*-position is shown in Figure 3 and condensation, deoxygenation and deamination in Figure 4.

3,5-Di-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-L-*ribo*-pentofuranose **9**, a key intermediate, was obtained from D-arabinose **7** in nine steps (Yamada, Yamasa Corporation, personal comunication). Silylation and isopropylidenation of D-arabinose **7** gave 5-*O*-(tert-butyldiphenylsilyl)-1,2-*O*-isopropylidene-α-D-*arabino*-pentofuranose in two steps. The 3-hydroxyl group in the compound was inverted by oxidation with acetic anhydride in DMSO; the resulting ketone was then reduced with $NaBH_4$. The resulting alcohol was protected with a benzyl group and then the *tert*-butyldiphenylsilyl (TBDPS) group was removed by tetrabutylammonium fluoride (TBAF) in THF to give alcohol. The 4-*C*-hydroxymethyl group was introduced in two steps (by oxidation and Cannizzaro reaction) and then the β-hydroxymethyl group was protected with a benzyl group to afford key intermediate **9**. Swern oxidation of the 4-*C*-hydroxymethyl group in **9**, followed by dibromovinylation, gave **10** in 84% yield. Dehydrobromination and triethylsilylation of **10** were done simultaneously and hydrolysis with acetic acid and trifluoroacetic acid (TFA), followed by acetylation, gave **12** as the only β-anomer in 88% yield.

The glycosidation of **12** with pertrimethylsilylated 2,6-diaminopurine (DAP) gave nucleoside **13** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf), and deacetylation of **13** was carried out with triethylamine in methanol. Thionocarbonylation of the 2′-hydroxy group in **14** was followed by short silica gel column purification. After radical reduction with tri-*n*-butyltin hydride in the presence of 2,2′-azobis(isobutylonitrile) (AIBN), TBAF treatment provided sugar-protected derivative **17** in 44% yield. In contrast to the synthesis of D-enantiomers, debenzylation was done with lithium naphthalenide (Liu *et al.*, 1997) to give **4** in 57% yield. The rate of deamination in **4** was very slow, but conversion of **4** to **6** was achieved by utilizing an excess of adenosine deaminase (54% yield).

The results of antiviral evaluation against HIV and HBV are shown in Table 1.

Discussion

9-(2-Deoxy-4-*C*-ethynyl-β-L-*ribo*-pentofuranosyl)-2,6-diaminopurine **4** was synthesized from D-arabinose in 20 steps by condensing the protected 4-*C*-ethynyl sugar derivative **12** with 2,6-diaminopurine. The compound was then converted to the L-enantiomer of 4′-*C*-ethynyl-2′-deoxyguanosine **6** through hydrolysis by adenosine deaminase (ADA). The rate of this enzymatic hydrolysis was very slow (10 d) in comparison with that of the D-enantiomer (2 h). All spectral data from both enantiomers were identical, except for the specific rotation.

These nucleosides were evaluated for anti-HIV activity toward MT-4 cells (by an MTT assay) and for anti-HBV activity toward 2.2.15 cells. Unfortunately, they were found to be inactive against both viruses.

Table 1. The antiviral activities of L-enantiomers (**4** and **6**)

Compound	EC_{50} (μM) HIV-1 *,†	HBV‡	CC_{50} (μM)
4	>350	>35	>350
6	>100	>35	>100
AZT	0.0032	ND§	29.4
3TC	0.1	1.57	>100

*Determined with MTT assay; †MT-4 cells were employed; ‡2.2.15 cells were employed; §not determined.

Acknowledgements

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Design, Efficient Synthesis, and Anti-HIV Activity of 4′-*C*-Cyano- and 4′-*C*-Ethynyl-2′-deoxy Purine Nucleosides

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ABSTRACT

Some 4′-*C*-ethynyl-2′-deoxy purine nucleosides showed the most potent anti-HIV activity among the series of 4′-*C*-substituted 2′-deoxynucleosides whose 4′-*C*-substituents were methyl, ethyl, ethynyl and so on. Our hypothesis is that the smaller the substituent at the *C*-4′ position they have, the more acceptable biological activity they show. Thus, 4′-*C*-cyano-2′-deoxy purine nucleosides, whose substituent is smaller than the ethynyl group, will have more potent antiviral activity. To prove our

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hypothesis, we planned to develop an efficient synthesis of 4′-*C*-cyano-2′-deoxy purine nucleosides (4′-CNdNs) and 4′-*C*-ethynyl-2′-deoxy purine nucleosides (4′-EdNs). Consequently, we succeeded in developing an efficient synthesis of six 2′-deoxy purine nucleosides bearing either a cyano or an ethynyl group at the *C*-4′ position of the sugar moiety from 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside. Unfortunately, 4′-*C*-cyano derivatives showed lower activity against HIV-1, and two 4′-*C*-ethynyl derivatives suggested high toxicity in vivo.

Key Words: NRTIs; Efficient synthesis; 4′-CNdNs; 4′-EdNs; Anti-HIV-1 agents.

INTRODUCTION

There are mainly two ways for the preparation of our 4′-*C*-substituted-2′-deoxynucleosides (4′-SdNs). One is a condensation method and the other is modification of nucleosides. During our exploration of novel nucleoside reverse transcriptase inhibitors (NRTIs), we have prepared many 4′-SdNs by the use of glycosidation reaction of 4-*C*-substituted sugar derivatives with nucleobases. This method was effective to make various kinds of 4′-SdNs. However, this synthetic route incurs some problems in making 4′-*C*-cyano-2′-deoxy purine nucleosides, because 4-*C*-substituted sugars have low reactivity in glycosidation reaction, especially when its substituent is an electron withdrawing group like a cyano group (data not shown). These problems encouraged us to develop a preparation method of 4′-*C*-cyano-2′-deoxy purine nucleosides from natural 2′-deoxynucleosides, which were so far difficult to be synthesized by condensation of sugars with nucleobases. The synthetic routes of 4′-SdNs from the corresponding nucleosides as starting materials were already reported. Matsuda et al. synthesized various 4′-*C*-substituted pyrimidine nucleosides by intramolecular radical cyclization reaction of 3′-*O*-diphenylvinylsilyl nucleosides with their 4′-radical and then conversion of 4′-*C*-hydroxyethyl nucleosides.[1] O-Yang et al. also reported an interesting article, which described the preparation of 4′-cyanothymidine (4′-CNT) from thymidine via 5′-aldehyde.[2] Haraguchi et al. recently reported ring opening reaction of 4′,5′-epoxy-nucleosides to make 4′-*C*-branched nucleosides.[3] These methods were useful for the preparation of 4′-SdNs, but there were few cases of the preparation of 4′-*C*-cyano purine nucleosides.

In this report, we describe the design and efficient synthesis of 4′-*C*-substituted 2′-deoxy purine nucleosides bearing a cyano and an ethynyl group at the *C*-4′ position of the sugar moiety from purine 2′-deoxynucleosides, and their anti-HIV activity. Furthermore, a preliminary toxicity test using 4′-*C*-ethynyl derivatives in vivo will be reported.

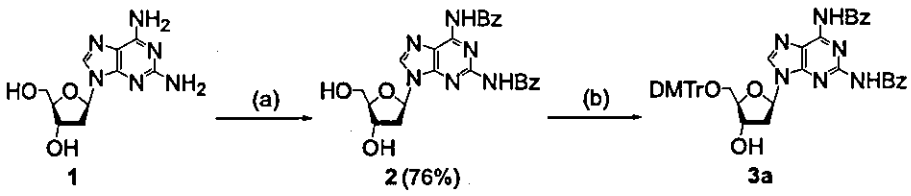
RESULTS AND DISCUSSIONS

From previous studies on structure-activity relationships of 4′-*C*-substituted nucleosides,[1,2,4–10] it is expected that a smaller substituent at the *C*-4′ position will give more acceptable biological activity against HIV-1. Actually, 4′-*C*-cyano-2′-deoxycytidine showed highest activity compared to 4′-*C*-ethynyl and 4′-*C*-methyl derivatives. From these results, we speculated that there was relationship between the

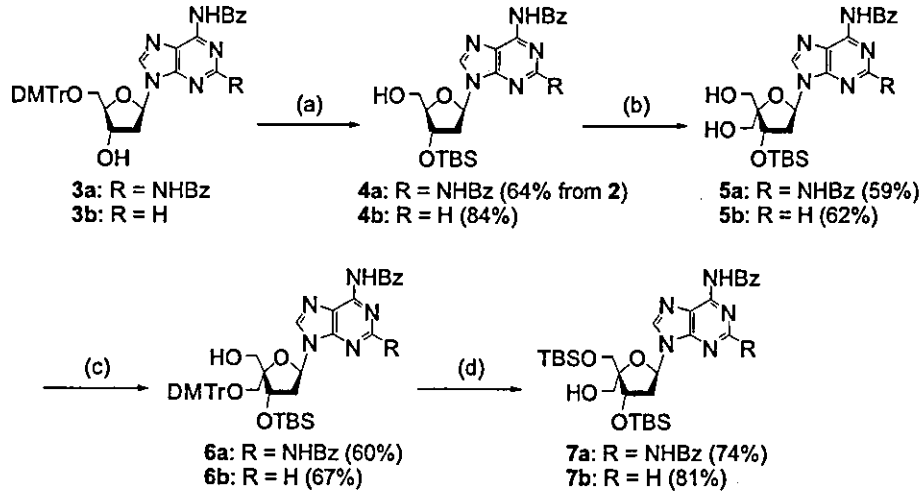
biological activity and a parameter, namely, $-\Delta G^0$ values[11] between equatorial and axial substituents on a cyclohexane rings (CN < F < C ≡ CH < CH = CH_2 < Me ≦ Et <*tert*-Bu). On the other hand, we have already found that 4′-*C*-ethynyl-2′-deoxy purine nucleosides exhibited potent anti-HIV activity and moderate cytotoxicity compared to the corresponding pyrimidine derivatives.[6,7,10] Therefore, we were interested in the synthesis and biological activity of 2′-deoxy purine nucleosides bearing a cyano group at *C*-4′ position. However, the glycosidation reaction will have drawbacks as described in the above introduction on synthetic problems. These problems prompted us to develop a preparation method of 4′-*C*-substituted purine nucleosides from the corresponding nucleosides, and this approach would enable us to synthesize 4′-*C*-cyano purine nucleoside derivatives, which were so far difficult to be synthesized by condensation of sugars with nucleobases.

Synthesis of 4′-*C*-cyano-2′-deoxy purine nucleosides starting from 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside (dDAP) is shown in Schemes 1–3. To begin with, 9-(2-deoxy-5-*O*-dimethoxytrityl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine **3a** was prepared from dDAP **1** by way of *N*-benzoylation and 5′-*O*-dimethoxytritylation (Scheme 1). Similarly, N^6-benzoyl-2′-deoxy-5′-*O*-dimethoxytrityladenosine **3b**, which was commercially available from Yamasa Corporation, was also used as the starting material for the synthesis of the target compounds.

The key intermediates **7a,b** were obtained according to Scheme 2. In the process for the synthesis of 3′-protected nucleosides **4b**, we initially expected that protection of the 3′-hydroxyl group as *tert*-butyldimethylsilyl ether (TBS) and the following deprotection of the 5′-*O*-dimethoxytrityl group (DMTr) would give the compound **4b** in good yield. However, when deprotection of the dimethoxytrityl group with 80% AcOH was tried at room temperature, the isolated yield of compound **4b** was very low because of acid sensitivity of *N*-acylated 2′-deoxyadenosine. On the contrary, when 2% *p*-toluenesulphonic acid (TsOH) in $CHCl_3$–MeOH was utilized for removal of DMTr group, this treatment gave 3′-TBS-derivative **4b** in 84% yield. To give 4′-*C*-hydroxymethyl derivative **5b**, compound **4b** was oxidized to the corresponding 5′-carboaldehyde by Moffatt oxidation, and then the aldehyde was treated under aldol reaction conditions with formaldehyde. The 4′-α-hydroxyl group of **5b** was selectively protected to give DMTr ether **6b** in 67% yield. Compound **6b** was converted to the key intermediate **7b** in 81% yield by *tert*-butyldimethylsilylation of the 5′-hydroxyl group and the following deprotection of the 4′-hydroxymethyl group. These reactions were also effective to prepare protected dDAP derivative **7a**. The overall yields were 12.7% in 6 steps.

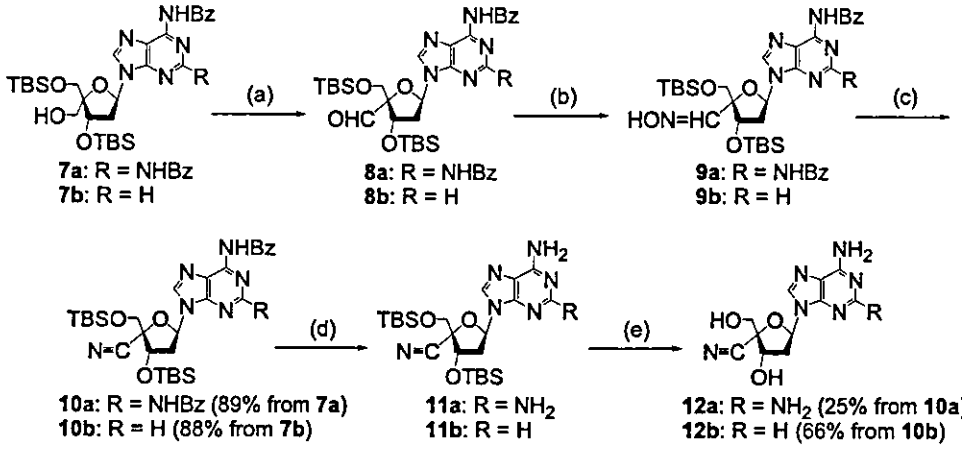


Scheme 1. *Reagents and conditions:* dDAP **1** was obtained by enzymatic base exchange reaction from 2′-deoxyuridine. (a) 1. TMSCl, pyridine, 0°C, 30 min; 2. BzCl, pyridine, 0°C, 2 h; 3. NH_4OH, H_2O, 0°C, 30 min; (b) DMTrCl, pyridine, rt., 3 h.

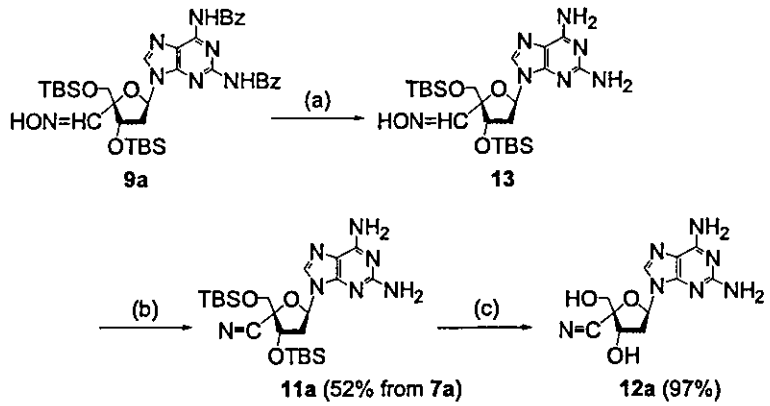


Scheme 2. Reagents and conditions: (a) 1. TBSCl, imidazole, DMF, rt., overnight; 2. TsOH · H_2O, MeOH, $CHCl_3$, 0°C, 15–30 min; (b) 1. EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 2 h; 2. aq CH_2O, 1N NaOH, dioxane, rt., 1–3 h; 3. $NaBH_4$, EtOH, 0°C, 30 min; (c) DMTrCl, Et_3N, DMF, rt., 1 h, for **5a**; DMTrCl Et_3N, CH_2Cl_2, 0°C, 30 min, for **5b**; (d) 1. TBSCl, imidazole, DMF, rt., overnight; 2. TsOH · H_2O, MeOH, $CHCl_3$, 0°C, 20–30 min.

Conversion of key intermediates **7a,b** to the corresponding 4′-*C*-cyano derivatives **12a,b** is shown in Scheme 3. 4′-*C*-Formyl derivatives **8a,b**, which were obtained by Moffatt oxidation of 4′-*C*-hydroxymethyl derivatives **7a,b**, were derived to 4′-*C*-aldoxime derivatives **9a,b** by treatment with hydroxylamine hydrochloride in pyridine, and were further dehydrated by methanesulfonyl chloride and triethylamine in CH_2Cl_2



Scheme 3. Reagents and conditions: (a) EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 1 h; (b) NH_2OH · HCl, pyridine, rt., 30 min; (c) MsCl, Et_3N, CH_2Cl_2, 0°C, 30 min; (d) aq $MeNH_2$, MeOH, rt., overnight, for **10a**; NH_4OH, MeOH, rt., overnight, for **10b**; (e) TBAF, THF, rt., 15 min.

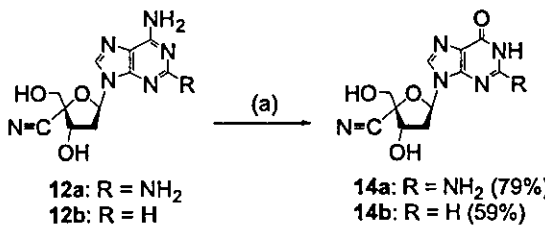


Scheme 4. Reagents and conditions: (a) aq $MeNH_2$, MeOH, rt., 46 h; (b) 1,1′-carbonyldiimidazole, MeCN, rt., 4 h; (c) TBAF, THF, rt., 10 min.

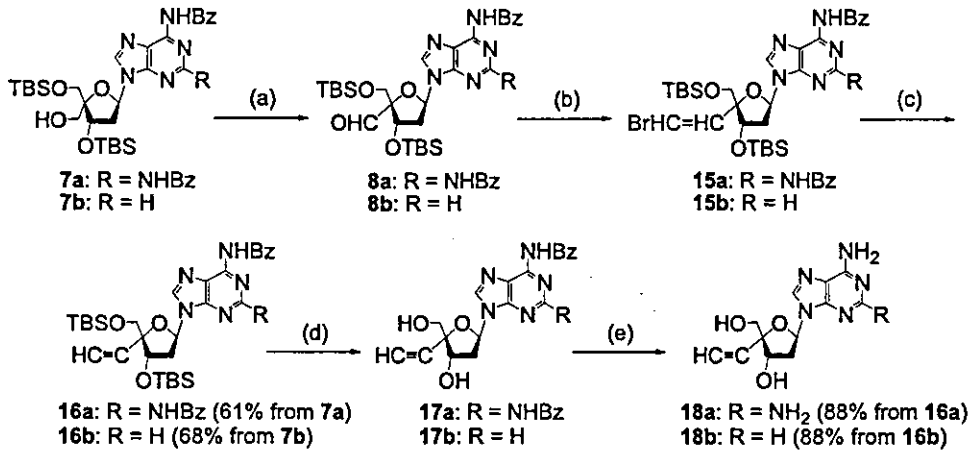
to give 4′-*C*-cyano derivatives **10a,b** in good yields through 3 steps. These 4′-*C*-cyano derivatives **10a** and **10b** were deblocked under aq $MeNH_2$ or NH_4OH in MeOH, respectively, to give crude debenzoylated products **11a** and **11b**. Treatment of **11a** and **11b** with tetra-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave 4′-*C*-cyano-2′-deoxy-2,6-diaminopurine-ribonucleoside **12a** and 2′-deoxyadenosine nucleoside **12b** in 25% and 66% yield, respectively.

Next, an alternative route was investigated because the isolated yield of **12a** was very low (Scheme 4). The cyano group might be unstable under $MeNH_2$ in MeOH for debenzoylation. 4′-*C*-Aldoxime **9a**, which was similarly derived from **7a**, was deblocked by treatment with aq $MeNH_2$ in MeOH to give the debenzoylated aldoxime derivative **13**. Crude **13** was then dehydrated with 1,1′-carbonyldiimidazole in MeCN to give **11a** in 52% yield from **7a**. In the case of compound **13** whose amino group is not protected, when dehydration of oxime was performed with MsCl, sulfonylation of the amino groups also occurred. Finally, desilylation of **11a** gave the desired nucleosides **12a** in 97% yield. The isolated yield of **12a** from **7a** was improved to 50% (as compared to 22% yield in Scheme 3).

We have already reported the synthesis of 4′-*C*-ethynyl-2′-deoxyinosine and 4′-*C*-ethynyl-2′-deoxyguanosine from the corresponding 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside derivatives by enzymatic deamination with adenosine



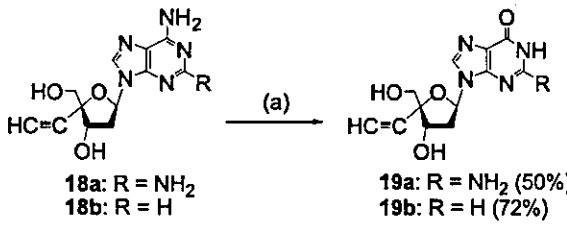
Scheme 5. Reagents and conditions: (a) adenosine deaminase, Tris-HCl buffer, 40°C, 1–2 h.



Scheme 6. *Reagents and conditions:* (a) EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 90 min–3 h; (b) PPh_3 = CHBr, THF, −40°C, 90 min–2 h; (c) *tert*-BuOK, THF, −40°C, 1–2 h; (d) TBAF, THF, rt., 30 min; (e) aq $MeNH_2$, MeOH, rt., overnight, for **17a**; NH_4OH, MeOH, rt., overnight, for **17b**.

deaminase.[6] This procedure was utilized to synthesize 4′-*C*-cyano-2′-deoxyguanosine **14a** and 4′-*C*-cyano-2′-deoxyinosine **14b** (Scheme 5). 4′-*C*-cyano nucleosides **12a** and **12b** were readily hydrolyzed to give **14a** and **14b**, respectively, by treatment with adenosine deaminase in Tris-HCl buffer (pH 7.5).

The 4′-*C*-hydroxymethyl derivatives **7a,b** are versatile intermediates for the synthesis of various 4′-*C*-substituted nucleosides. Therefore, we also utilized these intermediates **7a,b** to synthesize 4′-*C*-ethynyl-2′-deoxy purine nucleosides **18a,b**. Scheme 6 shows the synthesis of 4′-*C*-ethynyl derivatives **18a,b** from the key intermediates **7a,b**. Matsuda et al. synthesized 4′-*C*-ethynyl-2′-deoxycytidine by treatment of the corresponding 4′-*C*-chlorovinyl derivative with *n*-BuLi in THF in good yield.[5] However, conversion of N^6-benzoyl-4′-*C*-chlorovinyl-2′-deoxyadenosine to the corresponding 4′-*C*-ethynyl derivative **16b** under this condition resulted in lower isolated yield than that of 4′-*C*-ethynyl-2′-deoxycytidine derivative because of decomposition of desired product. After investigating reaction conditions, we obtained 4′-*C*-ethynyl derivatives **16a,b** by conversion of the 4′-*C*-formyl group of **8a,b** to a more reactive 4′-*C*-bromovinyl group with bromomethylenetriphenylphosphorane[12] in



Scheme 7. *Reagents and conditions:* (a) adenosine deaminase, Tris-HCl buffer, 40°C, 1–2 h.

Table 1. Anti-HIV activity of 4′-*C*-ethynyl and 4′-*C*-cyano 2′-deoxy purine nucleosides.

Compound no.	Anti-HIV activity[a]		
	EC_{50} (μM)	CC_{50} (μM)	Selectivity index
4′-*C*-ethynyl dAdo **18b**	0.0098	16	1633
4′-*C*-ethynyl dDAP **18a**	0.00034	0.9	2647
4′-*C*-ethynyl dIno **19b**	0.13	137	1054
4′-*C*-ethynyl dGuo **19a**	0.0015	1.4	933
4′-*C*-cyano dAdo **12b**	0.051	12	235
4′-*C*-cyano dDAP **12a**	0.00079	>0.034	>43
4′-*C*-cyano dIno **14b**	0.051	23	451
4′-*C*-cyano dGuo **14a**	0.000188	>0.034	>181
3′-azidothymidine (AZT)	0.0032	29.4	9188

[a]Anti-HIV activity was determined by MTT assay.[13,14] MT-4 cells and HIV-1_{LAI}[15] were employed.

THF, and then the following dehydrobromination of bromoolefin **15a,b** with *tert*-BuOK. After removal of the TBS groups of **16a,b** by treatment with TBAF in THF, benzamide groups at the base moiety of **17a,b** were cleaved by aq $MeNH_2$ or NH_4OH in MeOH, respectively, to give 4′-*C*-ethynyl-2′-deoxy-2,6-diaminopurine 2′-deoxyriboside **18a** and 2′-deoxyadenosine derivative **18b**.

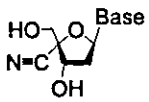
The preparation of 4′-*C*-ethynyl-2′-deoxyguanosine **19a** and 4′-*C*-ethynyl-2′-deoxyinosine **19b** (Scheme 7) was already reported.[6]

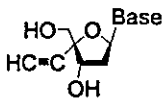
Consequently, we were able to synthesize four 4′-*C*-cyano- and two 4′-*C*-ethynyl-2′-deoxy- purine ribonucleosides by a more useful procedure using 2′-deoxyadenosine and 2, 6-diaminopurine 2′-deoxyriboside as the starting materials.

Antiviral Evaluation

Table 1 shows a summary of anti-HIV activity of 4′-*C*-cyano-2′-deoxy purine nucleosides together with that of 4′-*C*-ethynyl-2′-deoxy purine nucleosides. The structures are summarized in Scheme 8.

4′-*C*-Ethynyl dAdo **18b**, 4′-*C*-ethynyl dDAP **18a**, and 4′-*C*-ethynyl dGuo **19a** were highly potent against HIV-1, with subnanomolar to nanomolar EC_{50}s. 4′-*C*-Ethynyl dIno **19b** was only moderately active against the virus. It is noteworthy that both 4′-*C*-





12a: Base = 2,6-diaminopurin-9-yl
12b: Base = adenin-9-yl
14a: Base = guanin-9-yl
14b: Base = hypoxanthin-9-yl

18a: Base = 2,6-diaminopurin-9-yl
18b: Base = adenin-9-yl
19a: Base = guanin-9-yl
19b: Base = hypoxanthin-9-yl

Scheme 8. The structures of 4′-*C*-cyano- and 4′-*C*-ethynyl-2′-deoxy purine nucleosides.

Table 2. Anti-HIV activity of 4′-*C*-ethynyl and 4′-*C*-cyano 2′-deoxy purine nucleosides against drug-resistant infectious clones.

Compound no.	Anti-HIV activity[a], EC_{50} (μM)		
	Wild type (HXB2)	MDR[b]	M184V[c]
4′-*C*-ethynyl dAdo **18b**	0.008	0.0062	0.047
4′-*C*-ethynyl dDAP **18a**	0.0014	0.001	0.0059
4′-*C*-ethynyl dIno **19b**	0.81	0.51	16.6
4′-*C*-ethynyl dGuo **19a**	0.007	0.0048	0.008
4′-*C*-cyano dAdo **12b**	0.043	0.083	2.28
4′-*C*-cyano dIno **14b**	0.242	0.296	6.06
3′-azidothymidine(AZT)	0.022	15.3	0.01
3′-thiacytidine (3TC)	0.71	1.1	>100

[a]Anti-HIV activity was determined by MAGI assay.[16-18] HeLa-CD4-LTR-β-gal cells were employed.
[b]An infectious molecular clone (HIV-1_{MDR}) which contains five amino acid substitutions (Ala62Val, Val75Leu, Phe77Leu, Phe116Tyr and Gln115Met) and shows a high level of resistance against a variety of 2′,3′-dideoxy nucleoside analogues (AZT, ddI, ddC, d4T).[19]
[c]An infectious molecular clone (HIV-1_{M184V}) which contains an amino acid substitution (Met184-Val) and shows a high level of resistance against 3TC.

ethynyl dDAP **18a** and 4′-*C*-ethynyl dAdo **18b** had a favorable toxicity profile, with selectivity indices of 2647 and 1633, respectively. On the contrary, 4′-*C*-cyano dDAP **12a** and 4′-*C*-cyano dGuo **14a** showed remarkable cytotoxicity. Additionally, anti-HIV activity of 4′-*C*-cyano dAdo **12b** and 4′-*C*-cyano dIno **14b** decreased in comparison to that of 4′-*C*-ethynyl derivatives. Interestingly, 4′-*C*-cyano dIno **14b** was as potent as 4′-*C*-cyano dAdo **12b** against HIV-1 in spite of showing the low activity of 4′-*C*-ethynyl dIno **19b**.

We finally summarized anti-HIV activity of 4′-CNdNs and 4′-EdNs against drug-resistant infectious clones as shown in Table 2. The 4′-EdNs analogs (**18a**, **18b**, and **19a**) were active against the infectious clones tested, including HIV-1_{M184V}. However, the activity of 2′-deoxyinosine derivatives (**14b** and **19b**) was low against, especially, clone HIV-1_{M184V}. Taken together, although both 4′-*C*-cyano and 4′-*C*-ethynyl derivatives were active against HIV-1, the 4′-*C*-ethynyl substitution appears to be more acceptable for activity against 3TC-resistant HIV-1_{M184V}. On the other hand, we have not explained any relationship between cytotoxicity and biological activity on 4′-SdNs including both 4′-CNdNs and 4′-EdNs, but these biological data were already reported.[6,7,10]

Preliminary Toxicity Test In Vivo

A preliminary toxicity test for three 4′-*C*-ethynyl derivatives (**18a**, **18b**, and **19a**) was conducted, and the results are summarized in Table 3. Two of the three candidates (**18a** and **19a**) were toxic, but 4′-*C*-ethynyl dAdo **18b** was not in mice.

We developed an efficient preparation method for purine 2′-deoxyribonucleoside derivatives bearing a cyano group at the *C*-4′ position from the corresponding

Table 3. Preliminary toxicity test of three 4'-*C*-ethynyl-2'-deoxy purine nucleosides.[a]

	Intravenous administration		Oral administration	
Compound no.	Dose (mg/kg)	Mortality (%)	Dose (mg/kg)	Mortality (%)
4'-*C*-ethynyl dAdo **18b**	100	0	100	0
	10	0	10	0
	3	0	3	0
	1	0	1	0
4'-*C*-ethynyl dDAP **18a**	100	100 (1 d)[b]	100	100 (1 d)[b]
	10	100 (2 d)[b]	10	100 (2 d)[b]
	3	0	3	100 (2 d)[b]
	1	0	1	0
4'-*C*-ethynyl dGuo **19a**	100	100 (1 d)[b]	100	100 (1 d)[b]
	10	100 (2 d)[b]	10	100 (4 d)[b]
	3	100 (4 d)[b]	3	100 (4 d)[b]
	1	0	1	0

[a]Six-week-old ICR male mice were employed.
[b]Numbers in parentheses represent survival days of mice after administration.

2'-deoxynucleosides as the starting materials. This method was also applicable to effective preparation of 4'-*C*-ethynyl-2'-deoxy purine nucleosides. The total yields of 4'-*C*-substituted derivatives were improved (3.4 ~ 17%) compared to that of the method by condensation of sugars with nucleobases (< 1%[6]) by the use of these routes. Subsequently, it became easy for us to make derivatives bearing an electron withdrawing substitutent like a cyano group.

We initially expected that the target derivatives having a smaller 4'-*C*-substituent would lead to potent anti-HIV activity. However, we found that 4'-*C*-ethynyl derivatives were superior to 4'-*C*-cyano derivatives in terms of anti-HIV activity. Additionally, it turned out that 4'-*C*-ethynyl dDAP **18a** and dGuo **19a** were toxic in the preliminary toxicity test, but 4'-*C*-ethynyl dAdo **18b** was not.

Further investigations to discover novel NRTIs are in progress in our laboratory.

EXPERIMENTALS

Chemistry

General Method for Chemistry. All melting points were determined on a Yanagimoto MP-500D micro melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX-400 spectrometer or a Bruker Avance 500 spectrometer in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane (TMS) as reference. UV spectra were recorded with a Shimazu UV-160A spectrophotometer. Low-resolution mass spectra (MS) were taken on a JEOL JMS-AX500 spectrometer. Merck precoated plates (Kieselgel 60F254) were used for thin-layer chromatography (TLC) and spots were examined with ultraviolet light and sulfuric acid/anisaldehyde solution. Merck Kieselgel 60 was used for column chromatography. Reversed-phase

column chromatography was performed on Fuji Silysia DM1020T. Purity of all compounds obtained was verified by TLC or high-performance liquid chromatography (HPLC), and identity was verified by MS, ^{1}H-NMR, and elementary analysis.

9-(2-Deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (2). 9-(2-Deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine **1** (26.6 g, 100 mmol) was coevaporated with pyridine and then it was suspended in pyridine (400 mL). Chlorotrimethylsilane (88.0 mL, 700 mmol) was added dropwise to the suspension at 0°C. After stirring for 30 min at 0°C, the mixture was added dropwise benzoyl chloride (82.0 mL, 700 mmol) and was stirred for 2 h. The reaction mixture was added ice-water. After stirring for 15 min, the mixture was added conc. NH_4OH and was stirred for 30 min. The mixture was evaporated under reduced pressure. The residue was added AcOEt (600 mL) and saturated $NaHCO_3$ (600 mL) and was stirred for 1 h at 0°C. The precipitate formed was collected by filtration to give **2** (36.2 g, 76.3 mmol, 76.3%).

^{1}H-NMR (DMSO-d_6) δ 11.21, 11.00 (each 1H, s, NH), 8.62 (1H, s, H-8), 8.08–7.51 (10H, m, aromatic), 6.42 (1H, t, H-1′, J = 6.5), 5.34 (1H, d, 3′-OH, J = 4.0), 4.94 (1H, t, 5′-OH, J = 6.0), 4.44 (1H, m, H-3′), 3.88 (1H, q, H-4′, J = 5.0), 3.62, 3.54 (each 1H, m, H-5′), 2.79, 2.34 (each 1H, m, H-2′). FABMS m/z: 475 (MH^+). Anal. Found: C, 57.82; H, 4.80; N, 16.76. Calcd. for $C_{24}H_{22}N_6O_5 \cdot 1.4H_2O$: C, 57.69; H, 5.00; N, 16.82.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (4a). Compound **2** (36.2 g, 76.4 mmol) which was dried by coevaporation with pyridine was dissolved in pyridine (300 mL). The solution was added dimethoxytrityl chloride (37.6 g, 111 mmol) and stirred for 3 h at room temperature. After addition of EtOH, the reaction mixture was evaporated. The residue was suspended to AcOEt and water and the precipitate formed was removed by filtration. The organic layer was washed successively water and brine. The organic layer was dried over Na_2SO_4 and was evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: AcOEt/*n*-hexane, 3:1–4:1–5:1) to give crude **3a** (69.9 g), which was used for the synthesis of **4a** without further purification.

To a solution of crude **3a** (69.9 g) in DMF (370 mL) was added imidazole (8.80 g, 129 mmol) and *tert*-butylchlorodimethylsilane (16.5 g, 109 mmol) and the solution was stirred at room temperature overnight. After addition of water, the reaction mixture was evaporated under reduced pressure. The residue was dissolved to AcOEt and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure.

The residue was dissolved in $CHCl_3$ (510 mL) and was added dropwise toluenesulfonic acid hydrate (14.6 g) in MeOH (220 mL) at 0°C. After addition, the solution was stirred for 15 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved to AcOEt and stirred for 30 min. The precipitate formed was collected by filtration to give **4a** (28.8 g, 48.9 mmol, 64.0%).

^{1}H-NMR ($CDCl_3$) δ 9.30, 9.13 (each 1H, s, NH), 7.95 (1H, s, H-8), 8.06–7.45 (10H, m, aromatic), 6.29 (1H, dd, H-1′, J = 6.5, 8.0), 4.96 (1H, dd, 5′-OH, J = 4.5, 9.0), 4.85 (1H, m, H-3′), 4.04 (1H, m, H-4′), 3.99–3.83 (2H, m, H-5′), 3.10, 2.29 (each 1H, m, H-2′), 0.91 (9H, s, *tert*-Bu), 0.12, 0.10 (each 3H, s, Me). FABMS m/z: 589

(MH^+). Anal. Found: C, 60.96; H, 6.14; N, 13.99. Calcd. for $C_{30}H_{36}N_6O_5Si$: C, 61.20; H, 6.16; N, 14.27.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*C*-hydroxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (5a). The solution of **4a** (50.0 g, 85.0 mmol) in toluene (190 mL) and DMSO (290 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (24.6 g, 128 mmol). Pyridine (6.90 mL) and trifluoroacetic acid (3.30 mL) were added and stirred for 2 h at room temperature. The reaction mixture was added AcOEt (750 mL) and ice-water (750 mL). The mixture was stirred and precipitate formed was collected by filtration. The filtrate was washed successively with water and brine and was dried over Na_2SO_4 and evaporated under reduced pressure. This residue was combined with the precipitate to give crude aldehyde.

The crude aldehyde was dissolved in dioxane (240 mL) and added 37% formaldehyde (45.0 mL) and 2 N NaOH (45.0 mL). After stirring for 3 h at room temperature, the reaction mixture was neutralized by addition of AcOH. The mixture was diluted with AcOEt and washed successively with water, saturated $NaHCO_3$ and brine. The solution was dried over Na_2SO_4 and evaporated under reduced pressure.

The residue was dissolved in EtOH (360 mL) and added $NaBH_4$ (3.20 g, 85.0 mmol) at 0°C. After stirring for 30 min, the reaction mixture was neutralized by addition of AcOH. The mixture was diluted with mixture of $CHCl_3$ and MeOH and washed successively with water and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. After coevaporation of the residue with AcOEt, it was dissolved to AcOEt (500 mL) and stirred at room temperature. Precipitate formed was collected by filtration to give **5a** (30.83 g, 49.83 mmol, 58.6%).

^{1}H-NMR (DMSO-d_6) δ 11.20, 11.00 (each 1H, s, NH), 8.63 (1H, s, H-8), 8.08–7.51 (10H, m, aromatic), 6.44 (1H, t, H-1′, J = 6.5), 4.90 (1H, t, OH, J = 5.5), 4.78 (1H, t, H-3′, J = 5.5), 4.46 (1H, t, OH, J = 5.5), 3.65–3.53 (4H, m, H-5′ and H-6′), 2.96, 2.43 (each 1H, m, H-2′), 0.89 (9H, s, *tert*-Bu), 0.10, 0.097 (each 3H, s, Me). FABMS m/z: 619 (MH^+). Anal. Found: C, 58.99; H, 6.14; N, 13.08. Calcd. for $C_{31}H_{38}N_6O_6Si \cdot 0.8H_2O$: C, 58.81; H, 6.30; N, 13.27.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*C*-dimethoxytrityloxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (6a). To a solution of **5a** (24.8 g, 40.0 mmol) in DMF (200 mL) was added triethylamine (11.2 mL, 80.0 mmol) and dimethoxytrityl chloride (20.3 g, 60.0 mmol) and stirred for 1 h at room temperature. The reaction mixture was diluted with AcOEt and was washed with water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 2:1–1:1) to give **6a** (22.2 g, 24.1 mmol, 60.3%).

^{1}H-NMR ($CDCl_3$) δ 9.33, 9.23 (each 1H, s, NH), 8.07 (1H, s, H-8), 8.13–6.91 (23H, m, aromatic), 6.27 (1H, t, H-1′, J = 6.5), 5.14 (1H, dd, H-3′, J = 3.5, 6.0), 4.59 (1H, dd, 5′-OH, dd, J = 5.5, 8.5), 4.30 (1H, dd, H-5′a, J = 5.5, 12.5), 3.81 (1H, d, H-5′b, J = 9.0, 12.5), 3.59 (1H, d, H-6′a, J = 10.5), 3.28 (1H, m, H-2′a), 3.17 (1H, d, H-6′b, J = 11.0), 2.48 (1H, m, H-2′b), 0.84 (9H, s, *tert*-Bu), 0.09, 0.07 (each 3H, s, Me). FABMS m/z: 921 (MH^+). Anal. Found: C, 67.87; H, 6.35; N, 8.80. Calcd. for $C_{52}H_{56}N_6O_8Si$: C, 67.80; H, 6.13; N, 9.12.

9-(3,5-Di-*O-tert*-Butyldimethylsilyl-2-deoxy-4-*C*-hydroxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (7a). To a solution of **6a** (29.9 g, 32.5 mmol) in DMF (100 mL) was added imidazole (3.30 g, 48.8 mmol) and *tert*-butylchlorodimethylsilane (5.90 g, 39.0 mmol) and stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and was washed with water. The organic layer was dried over $MgSO_4$ and evaporated.

The residue was dissolved to $CHCl_3$ (620 mL) and was added dropwise TsOH hydrate (6.20 g) in MeOH (190 mL) at 0°C. After addition, the solution was stirred for 20 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 3:1) to give **7a** (17.5 g, 23.9 mmol, 73.5%).

^{1}H-NMR ($CDCl_3$) δ 9.14, 9.05 (each 1H, s, NH), 8.13 (1H, s, H-8), 8.02–7.53 (10H, m, aromatic), 6.45 (1H, t, H-1′, J = 6.5), 4.99 (1H, dd, H-3′, J = 4.5, 6.5), 3.91–3.74 (4H, m, H-5′ and H-6′), 3.12, 2.54 (each 1H, m, H-2′), 2.70 (1H, dd, 6′-OH, J = 5.0, 8.5), 0.93, 0.86 (each 9H, s, *tert*-Bu), 0.17, 0.15, 0.02, 0.01 (each 3H, s, Me). FABMS m/z: 733 (MH^+). Anal. Found: C, 60.51; H, 7.19; N, 11.37. Calcd. for $C_{37}H_{52}N_6O_6Si_2$: C, 60.63; H, 7.15; N, 11.46.

9-(3,5-Di-*O-tert*-Butyldimethylsilyl-4-*C*-cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (10a). The solution of **7a** (2.00 g, 2.73 mmol) in toluene (6.00 mL) and DMSO (12.0 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.57 g, 8.19 mmol). The mixture was added pyridine (0.22 mL) and trifluoroacetic acid (0.11 mL) and was stirred for 1 h at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8a**.

To a solution of crude aldehyde **8a** in pyridine (20.0 mL) was added hydroxylamine hydrochloride (0.28 g, 4.03 mmol) and stirred for 30 min at room temperature. The reaction mixture was evaporated and the residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated to give crude oxime **9a**.

To a solution of crude oxime **9a** in CH_2Cl_2 (20.0 mL) was added triethylamine (0.76 mL, 5.45 mmol) and methanesulfonyl chloride (0.32 mL, 4.13 mmol) at 0°C and stirred for 30 min at same temperature. The reaction mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-Hexane/AcOEt, 1:1) to give **10a** (1.77 g, 2.43 mmol, 89.0%).

^{1}H-NMR ($CDCl_3$) δ 9.54, 9.29 (each 1H, s, NH), 7.97 (1H, s, H-8), 8.03–7.49 (10H, m, aromatic), 6.43 (1H, t, H-1′, J = 6.5), 5.21 (1H, t, H-3′, J = 5.5), 4.22, 3.99 (each 1H, d, H-5′, J = 11.0), 3.42, 2.53 (each 1H, m, H-2′), 0.97, 0.86 (each 9H, s, *tert*-Bu), 0.24, 0.17, 0.054, 0.051 (each 3H, s, Me). FABMS m/z: 728 (MH^+). Anal. Found: C, 57.87; H, 6.97; N, 12.78. Calcd. for $C_{37}H_{49}N_7O_5Si_2 \cdot 2H_2O$: C, 58.17; H, 6.99; N, 12.83.

9-(4-*C*-Cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine (12a). The mixture of **10a** (1.00 g, 1.37 mmol) in MeOH (10.0 mL) and 40% $MeNH_2$ (10.0 mL)

was stirred at room temperature overnight. Precipitate formed was collected by filtration to give crude debenzoylated product **11a** (0.25 g).

To a solution of crude **11a** (0.25 g) in THF (9.00 mL) was added tetra-*n*-butylammonium fluoride (1M solution in THF, 1.00 mL, 1.00 mmol) and stirred for 15 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: AcOEt/MeOH,10:1). The residue was triturated with 2-propanol to give **12a** (0.10 g, 0.34 mmol, 24.8% from **10a**).

^{1}H-NMR (DMSO-d_6) δ 7.93 (1H, s, H-8), 6.77 (2H, s, NH_2), 6.34 (1H, t, H-1′, J = 7.0), 6.28 (1H, d, 3′-OH, J = 4.5), 5.87 (1H, t, 5′-OH, J = 6.5), 5.85 (2H, s, NH_2), 4.64 (1H, q, H-3′, J = 4.5), 3.78 (1H, dd, H-5′a, J = 6.0, 12.0), 3.65 (1H, dd, H-5′b, J = 6.5, 12.0), 2.87, 2.37 (each 1H, m, H-2′). FABMS m/z: 292 (MH^+). Anal. Found: C, 43.18; H, 4.62; N, 31.80. Calcd. for $C_{11}H_{13}N_7O_3 \cdot 0.8H_2O$: C, 43.22; H, 4.81; N, 32.07.

Improved Route for the Synthesis of 9-(4-*C*-Cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine (12a) from 7a. The solution of **7a** (1.00 g, 1.40 mmol) in toluene (3.00 mL) and DMSO (6.00 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.79 g, 4.10 mmol). Pyridine (0.11 mL) and trifluoroacetic acid (60.0 μL) were added and stirred for 90 min at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8a**.

To a solution of crude aldehyde **8a** in pyridine (10.0 mL) was added hydroxylamine hydrochloride (0.19 g, 2.70 mmol) and stirred for 1 h at room temperature. The reaction mixture was evaporated and the residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated to give crude oxime **9a**.

The mixture of crude oxime **9a** in MeOH (10.0 mL) and 40% $MeNH_3$ (10.0 mL) was stirred for 46 h at room temperature. Precipitate formed was collected by filtaration to give crude debenzoylated 4′-*C*-oxime derivative **13** (0.61 g).

To a suspension of crude **13** (0.61 g) in MeCN (5 mL) was added 1,1′-carbonyldiimidazole (0.40 g, 2.50 mmol) and stirred for 4 h at room temperature. The reaction mixture was diluted with AcOEt and washed successively with water and brine. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 100:1–50:1) to give **11a** (0.38 g, 0.73 mmol, 52.1% from **9a**).

^{1}H-NMR ($CDCl_3$) δ 7.62 (1H, s, H-8), 6.32 (1H, t, H-1′, J = 6.25), 5.35 (2H, s, NH_2), 4.93 (1H, t, H-3′, J = 5.75), 4.66 (2H, s, NH_2), 4.00, 3.87 (each 1H, d, H-5′, J = 11.0), 3.08, 2.50 (each 1H, m, H-2′), 0.96, 0.89 (each 9H, s, *tert*-Bu), 0.20, 0.17, 0.086, 0.046 (each 3H, s, Me). FABMS m/z: 520 (MH^+). Anal. Found: C, 52.50; H, 7.84; N, 18.39. Calcd. for $C_{23}H_{41}N_7O_3Si_2 \cdot 0.4H_2O$: C, 52.42; H, 7.99; N, 18.60.

To a solution of **11a** (0.272 g, 0.523 mmol) in THF (4.80 mL) was added tetra-*n*-butylammonium fluoride (1M solution in THF, 1.10 mL, 1.10 mmol) and stirred for 10 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: AcOEt/MeOH,10:1–5:1) to give **12a** (0.147 mg, 0.506 mmol, 96.7%). Total isolated yield of **12a** from **7a** by this procedure was 50.4%.