Chem. Pharm. Bull. **46**(3) 458—461 (1998) Vol. 46, No. 3

Design and Racemic Synthesis of Conformationally Restricted Carbocyclic Pyrimidine Nucleoside Analogs Based on the Structure of the L-Nucleoside Residue in Heterochiral DNA

Hidehito URATA,*[,a] Hidetaka MIYAGOSHI,[a] Hitoshi KAKUYA,[a] Hideki TOKUMOTO,[a] Takuya KAWAHATA,[b] Toru OTAKE,[b] and Masao AKAGI*[,a]

Osaka University of Pharmaceutical Sciences,[a] 4–20–1 Nasahara, Takatsuki, Osaka 569–11, Japan, Osaka Prefectural Institute of Public Health,[b] 1–3–69 Nakamichi, Higashinari-ku, Osaka 537, Japan.

Received September 22, 1997; accepted October 20, 1997

Carbocyclic pyrimidine nucleoside analogs which have restricted glycosidic conformation at $\chi \approx 180°$ were designed, based on the conformational features of the L-nucleotide residue in heterochiral DNA, and synthesized. The synthesis of (±)-carbocyclic 6,6′-*O*-cyclo-2′-deoxyuridine was achieved *via* bromination and subsequent intramolecular cyclization of carbocyclic 6′β-hydroxy-2′-deoxyuridine. (±)-Carbocyclic 6,6′-*O*-cyclo-2′-deoxycytidine was synthesized from protected carbocyclic 6,6′-*O*-cyclo-2′-deoxyuridine *via* the 4-triazole intermediate.

Key words carbocyclic nucleoside; *O*-cyclonucleoside; restricted glycosidic conformation

Most anti-retroviral nucleoside analogs are incorporated into the growing DNA strand by a viral reverse transcriptase after phosphorylation to triphosphate derivatives by cellular kinases, and prevent further elongation of the DNA strand.[1] Recently, a novel anti-human immunodeficiency virus (HIV) agent, 2′,3′-dideoxy-3′-thiacytidine was reported.[2–4] Surprisingly, it was found that the (−)-enantiomer (3TC) with an unnatural nucleoside configuration was biologically more potent than the (+)-enantiomer with a natural nucleoside configuration, and the (−)-enantiomer was 20- to 100-fold less toxic than the (+)-enantiomer. This unusual finding opens new approaches for the treatment of viral infections with unnatural L-nucleosides. However, unnatural L-nucleoside analogs might be disadvantageous for incorporation into the template–primer complex, since structural constraints would be required for proper alignment of the L-deoxynucleoside triphosphate (L-dNTP) to the complex.[5] The observation that L-oligodeoxynucleotides consisting of L-deoxyribose do not form duplex structure with complementary D-DNA or RNA sequences[6] may support the above hypothesis.

We have investigated the structure of the heterochiral oligonucleotide, which contains an unnatural L-nucleotide[7] residue in the natural sequence, and have found that the L-nucleotide residue of the heterochiral oligonucleotide forms stable Watson–Crick base-pairing with the complementary natural residue,[8] while the overall duplex stability is slightly decreased.[9] The decrease of the duplex stability suggests the structural unsuitability of L-nucleotides for incorporation into B-form DNA. Two-dimensional ^{1}H-NMR studies suggested that the unnatural L-nucleotide residue adopts an unusual low *anti* glycosidic conformation ($\chi \approx 180°$),[8] although typical double-stranded B-form DNA has a glycosidic torsion angle of $\chi = -90$—$-135°$.[10] This result suggests that low *anti* glycosidic conformation would be a critical conformational feature for the L-nucleotide to form Watson–Crick base-pairing in the right-handed double helix. Thus, we designed the carbocyclic pyrimidine nucleoside analogs **1** and **2** whose glycosidic conformation is fixed at $\chi \approx 180°$ by a oxygen-bridge between the base and the cyclopentane ring (*O*-cyclocarbanucleosides), in order to reduce the structural disadvantage of L-nucleosides for incorporation into the template–primer complex. Although various cyclonucleosides have been reported,[11–13] the synthesis of cyclonucleosides with low-*anti*-fixed glycosidic conformation has not been reported. The triphosphate derivatives of these carbocyclic nucleoside analogs might have optimal geometry for incorporation into the template–primer complex. We herein report the racemic synthesis of **1** and **2**.

Results and Discussion

We planned to synthesize the target compounds from the racemic epoxide **3**, which is readily prepared from cyclopentadiene in three steps.[14,15] Ring opening of the epoxide **3** by uracil proceeded in a regioselective manner to give the 6′α-hydroxy derivative **4** (Chart 1).[16,17]

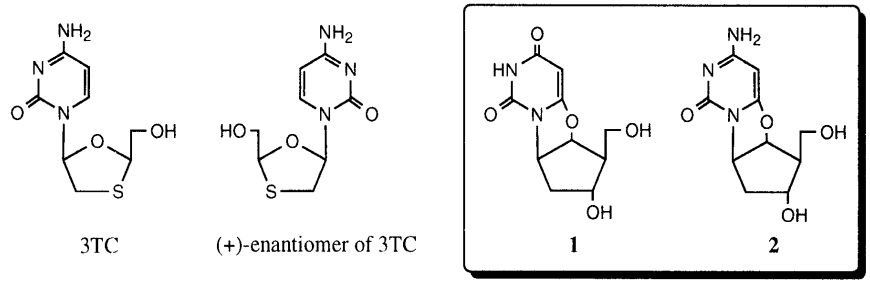

Fig. 1

* To whom correspondence should be addressed.

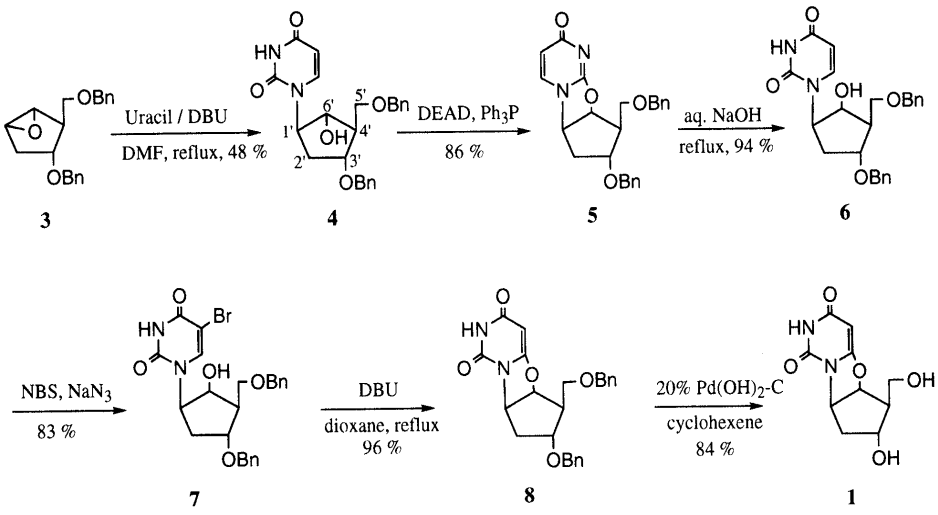

Chart 1

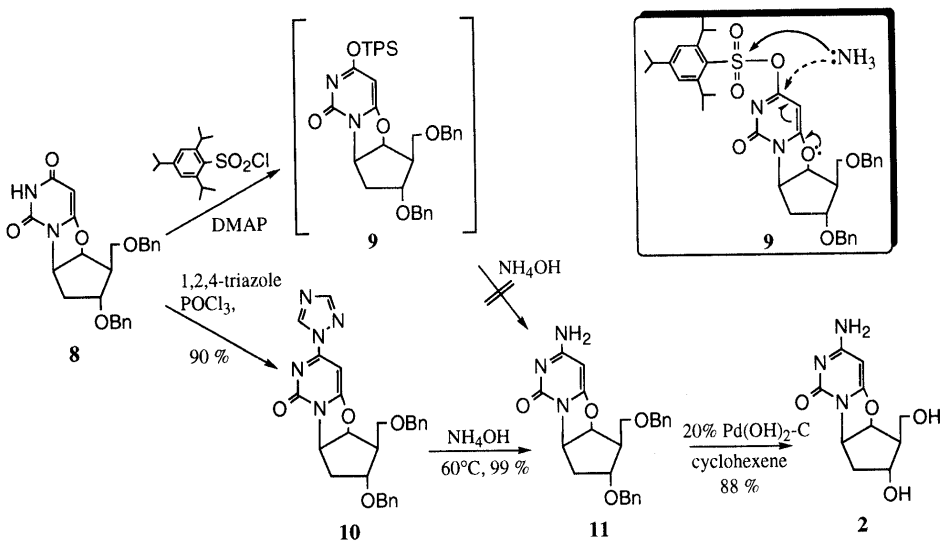

Chart 2

Treatment of the alcohol **4** with diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran (THF) afforded the 2,6′-*O*-cyclonucleoside **5**, which showed λ_{max} at 258 and 226 nm in EtOH. Alkaline hydrolysis of the anhydro derivative **5** provided the 6′β-hydroxy derivative **6**. The C5 position of **6** was brominated by treatment with *N*-bromosuccinimide (NBS) in the presence of sodium azide.[18] The resulting 5-bromo derivative **7** was found to undergo a facile intramolecular cyclization[19] on treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dioxane at refluxing temperature to give the novel 6,6′-*O*-cyclonucleoside **8** in 96% yield. The *O*-cyclic structure of **8** was confirmed by observation of the H5 signal as a singlet and the downfield shift (about 1 ppm) of the H6′ signal in the ^{1}H-NMR spectrum. Finally, compound **8** was treated with 20% $Pd(OH)_2$/C–cyclohexene in EtOH at refluxing temperature for 3.5 h to afford (±)-carbocyclic 6,6′-*O*-cyclo-2′-deoxyuridine (**1**) in 84.2% isolated yield.

The cytidine analog **2** was synthesized by the uracil-to-cytosine conversion method (Chart 2). The uridine analog **8** was first treated with triisopropylbenzenesulfonyl chloride (TPS-Cl) in the presence of 4-dimethylaminopyridine (DMAP) to give the O^4-TPS derivative **9**. However, ammonolysis of compound **9** with concentrated aqueous NH_4OH in dioxane afforded the cytidine derivative **11** in unsatisfactory yield, and the uridine derivative **8** was recovered. This result is explained by the preferential attack of the NH_3 molecule on the sulfur atom rather than the C4 position of the sulfonate **9**, because of the electron-donating effect of the oxygen atom at the C6 position. Thus, the uridine derivative **8** was converted to the triazolide **10** by treatment with tri(1*H*-1,2,4-triazol-1-yl)phosphine oxide prepared from phosphoryl chloride and 1,2,4-triazole in the presence of triethylamine at refluxing temperature.[20] Ammonolysis of the triazolide **10** successfully furnished the cytidine derivative **11** in 99.4% yield. Deprotection of **11** with 20% $Pd(OH)_2$/C–cyclohexene afforded (±)-carbocyclic 6,6′-*O*-cyclo-2′-deoxycytidine (**2**) in 87.6% isolated yield.

The characteristics of the UV absorption of **1** and **2** are very similar to those of the corresponding 6,2′-*O*-cyclopyrimidine nucleosides rather than 6,3′-*O*- or 6,5′-*O*-cyclonucleosides (Table 1). This result strongly suggests that these compounds have the 5-membered *O*-cyclic

Table 1. UV Data for 6-*O*-Cyclopyrimidine Nucleoside Analogs [λ_{max} nm (ε)]

Compound	0.1 N HCl	H_2O	0.1 N NaOH
1	254.8 (16500)	254.8 (16500)	254.0 (11600)
6,2′-*O*-Cyclouridine[a]	251.0 (16700)	251.5 (16400)	253.5 (12100)
6,3′-*O*-Cyclouridine[a]	259.5 (15300)	259.5 (15100)	262.0 (12200)
6,5′-*O*-Cyclouridine[a]	262.0 (12900)	261.5 (13000)	264.0 (9900)
2	267.2 (22800)	261.8 (14400)	261.6 (14200)
6,2′-*O*-Cyclocytidine[a]	265.0 (22900)	261.0 (14500)	262.0 (15100)
6,3′-*O*-Cyclocytidine[a]	273.0 (21000)	269.5 (14000)	270.0 (14300)
6,5′-*O*-Cyclocytidine[a]	278.0 (17300)	271.5 (11600)	272.5 (11800)

a) Data from reference 21.

structure.

Compounds **1** and **2** were evaluated for anti-HIV-1 activity in MT-4 cells. However, neither of the compounds showed any significant antiviral activity. The lack of activity may be due to any of several reasons, among which low efficiency of phosphorylation by the nucleoside kinase might be the major factor.

In conclusion, the racemic synthesis of **1** and **2** was achieved. Although these compounds have no anti-HIV-1 activity, such conformationally restricted nucleoside analogs as **1** and **2** might be useful tools for evaluating the effects of the glycosidic conformation on the structure and function of nucleic acids.

Experimental

Melting points were measured on a Yanagimoto apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a Varian Gemini-200 or a Varian XL-300 spectrometer. Chemical shifts were measured relative to internal tetramethylsilane for $CDCl_3$ and DMSO-d_6, or internal *tert*-butyl alcohol, 1.23 ppm from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), for D_2O. Thin-layer chromatography was carried out on Merck $60F_{254}$ coated plates. The racemic epoxide **3** was synthesized according to a literature procedure.[14,15] Anti-HIV-1 activity assays were carried out according to reported methods.[22]

(±)-(1β,2α,3β,4α)-1-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)uracil (4) DBU (199 μl, 1.33 mmol) was added to a suspension of uracil (149 mg, 1.33 mmol) and the epoxide **3** (315 mg, 1.01 mmol) in dry dimethylformamide (DMF) (5 ml), and the mixture was refluxed for 7 h under an argon atmosphere. After cooling, the solvent was evaporated off and the residue was dissolved in ethyl acetate. The solution was washed with distilled water. The organic layer was dried with Na_2SO_4 and concentrated, then the residue was purified with silica gel column chromatography to give 206 mg (48.0%) of **4** as a colorless foam. ^{1}H-NMR ($CDCl_3$) δ: 2.00—2.20 (m, 2H, H-2′, H-2″), 2.27 (m, 1H, H-4′), 3.52—3.77 (m, 2H, H-5′, H-5″), 3.82 (br, 1H, OH), 3.92 (m, 1H, H-3′), 4.18 (t, 1H, J=9.1 Hz, H-6′), 4.40—4.55 (m, 4H, Ar-CH_2), 4.72 (q, 1H, J=9.1 Hz, H-1′), 5.63 (d, 1H, J=8.2 Hz, H-5), 7.15 (d, 1H, J=8.2 Hz, H-6), 7.20—7.40 (m, 10H, Ar-H), 8.92 (br s, 1H, NH). MS (EI) *m/z*: 423 (M^++1). HR-MS *m/z*: 423.1912 (Calcd for $C_{24}H_{27}N_2O_5$: 423.1918).

(±)-(5aα,7α,8β,8aα)-7-Benzyloxy-8-benzyloxymethyl-5a,7,8,8a-tetrahydro-2*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*a*]pyrimidin-2-one (5) A solution of **4** (1.12 g, 2.65 mmol) in THF (20 ml) was treated with triphenylphosphine (1.04 g, 3.97 mmol) and diethyl azodicarboxylate (625 μl, 3.97 mmol), and the mixture was stirred at room temperature under an argon atmosphere. After 2 h, the solvent was evaporated off. The residue was coevaporated with $CHCl_3$ and purified by silica gel column chromatography to give 925 mg (86.4%) of **5**. An analytical sample was recrystallized from ethyl acetate to afford colorless needles of **5**, mp 178—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.07 (m, 1H, H-2″), 2.33 (ddd, 1H, J=13.9, 5.8, 2.2 Hz, H-2′), 2.58 (m, 1H, H-4′), 3.73 (m, 2H, H-5′, H-5″), 3.93 (m, 1H, H-3′), 4.48 (s, 4H, Ar-CH_2), 4.87 (m, 1H, H-1′), 5.44 (dd, 1H, J=8.1, 5.8 Hz, H-6′), 5.97 (d, 1H, J=8.1 Hz, H-5), 7.16 (d, 1H, J=8.1 Hz, H-6), 7.20—7.40 (m, 10H, Ar-H), 8.92. MS (EI) *m/z*: 405 (M^++1). HR-MS *m/z*: 405.1812 (Calcd for $C_{24}H_{25}N_2O_4$: 405.1815). *Anal.* Calcd for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93. Found: C, 71.30; H, 5.85; N, 6.91.

(±)-(1β,2β,3β,4α)-1-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)uracil (6) A solution of **5** (859 mg, 2.12 mmol) in EtOH (50 ml) was treated with 1 M NaOH (5 ml) and the mixture was heated under reflux for 45 min. After cooling, the solution was neutralized with 2 M HCl and then concentrated to about one-tenth the original volume. The residue was extracted with ethyl acetate and washed with brine. The organic phase was dried with Na_2SO_4 and concentrated, then the residue was purified by means of silica gel column chromatography to give 840 mg (93.8%) of **6** as a colorless foam. ^{1}H-NMR ($CDCl_3$) δ: 1.95—2.30 (m, 2H, H-2′, H-2″), 2.37 (m, 1H, H-4′), 3.77 (d, 2H, J=5.5 Hz, H-5′, H-5″), 3.86 (d, 1H, J=3.0 Hz, OH), 4.11 (m, 1H, H-3′), 4.39—4.58 (m, 5H, Ar-CH_2, H-6′), 5.11 (m, 1H, H-1′), 5.60 (d, 1H, J=7.6 Hz, H-5), 7.23—7.40 (m, 10H, Ar-H), 7.48 (d, 1H, J=7.6 Hz, H-6), 9.64 (br s, 1H, NH). MS (EI) *m/z*: 423 (M^++1). HR-MS *m/z*: 423.1924 (Calcd for $C_{24}H_{27}N_2O_5$: 423.1918).

(±)-(1β,2β,3β,4α)-1-(4-Benzyloxy-3-benzyloxymethyl-2-hydroxycyclopentyl)-5-bromouracil (7) A solution of **6** (840 mg, 1.99 mmol) in 1,2-dimethoxyethane (100 ml) was treated with an aqueous solution of NaN_3 (516 mg, 7.95 mmol) in 2 ml of water and *N*-bromosuccinimide (389 mg, 2.19 mmol), and the mixture was stirred at room temperature for 24 h. The solvent was removed *in vacuo*, and the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), evaporated, and purified by means of silica gel column chromatography to give 831 mg (83.3%) of **7** as a colorless foam. ^{1}H-NMR ($CDCl_3$) δ: 2.05—2.30 (m, 2H, H-2′, H-2″), 2.38 (m, 1H, H-4′), 3.78 (d, 2H, J=5.0 Hz, H-5′, H-5″), 3.87 (d, 1H, J=3.5 Hz, OH), 4.12 (m, 1H, H-3′), 4.39—4.59 (m, 5H, Ar-CH_2, H-6′), 5.12 (m, 1H, H-1′), 7.23—7.40 (m, 10H, Ar-H), 7.87 (s, 1H, H-6), 9.69 (br s, 1H, NH). MS (EI) *m/z*: 500 (M^+). HR-MS *m/z*: 500.0938 (Calcd for $C_{24}H_{25}Br_1N_2O_5$: 500.0945).

(±)-(5aα,7α,8β,8aα)-7-Benzyloxy-8-benzyloxymethyl-5a,7,8,8a-tetrahydro-2*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*c*]pyrimidine-2,4(3*H*)-dione (8) DBU (495 μl, 3.31 mmol) was added to a solution of **7** (831 mg, 1.65 mmol) in dioxane (20 ml), and the mixture was refluxed for 1 h under an argon atmosphere. After cooling, the solvent was evaporated, and the residue was partitioned between ethyl acetate and aqueous 0.5 M KH_2PO_4. The organic phase was dried (Na_2SO_4), evaporated, and purified by means of silica gel column chromatography to give 669 mg (96.0%) of **8**. Analytical sample was recrystallized from EtOH to afford colorless leaflets of **8**, mp 179—181 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.01 (m, 1H, H-2″), 2.52 (m, 1H, H-4′), 2.62 (ddd, 1H, J=15.0, 5.8, 1.7 Hz, H-2′), 3.60—3.90 (m, 3H, H-3′, H-5′, H-5″), 4.38—4.60 (m, 4H, Ar-CH_2), 4.89 (m, 1H, H-1′), 5.05 (s, 1H, H-5), 5.41 (dd, 1H, J=7.6, 5.5 Hz, H-6′), 7.20—7.40 (m, 10H, Ar-H), 9.40 (br s, 1H, NH). MS (EI) *m/z*: 420 (M^+). HR-MS *m/z*: 420.1687 (Calcd for $C_{24}H_{24}N_2O_5$: 420.1683). *Anal.* Calcd for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75; N, 6.66. Found: C, 68.35; H, 5.71; N, 6.49.

(±)-(5aα,7α,8β,8aα)-5a,7,8,8a-Tetrahydro-7-hydroxy-8-hydroxymethyl-2*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*c*]pyrimidine-2,4(3*H*)-dione (1) A mixture of **8** (100 mg, 0.23 mmol), 20% $Pd(OH)_2$ (25 mg), and cyclohexene (1 ml) in EtOH (5 ml) was refluxed for 3.5 h. The catalyst was removed by filtration and was washed with hot 70% EtOH. The filtrate was concentrated to dryness and the residue was recrystallized from EtOH to give pure **1** (46 mg, 84.2%), mp 259—261 °C (dec). ^{1}H-NMR (D_2O) δ: 1.95 (m, 1H, H-2″), 2.29 (m, 1H, H-4′), 2.53 (dd, 1H, J=13.9, 6.5 Hz, H-2′), 3.80—3.93 (m, 2H, H-5′, H-5″), 4.06 (m, 1H, H-3′), 4.98 (t, 1H, J=7.6 Hz, H-1′), 5.20 (s, 1H, H-5), 5.54 (dd, 1H, J=7.6, 5.0 Hz, H-6′). MS (SI-MS) *m/z*: 241 (M^++1). HR-MS *m/z*: 241.0843 (Calcd for $C_{10}H_{13}N_2O_5$: 241.0823). *Anal.* Calcd for $C_{10}H_{12}N_2O_5 \cdot 1/6H_2O$: C, 49.38; H, 5.11; N, 11.52. Found: C, 49.59; H, 5.02; N, 11.37.

(±)-(5aα,7α,8β,8aα)-7-Benzyloxy-8-benzyloxymethyl-5a,7,8,8a-tetrahydro-2-(1,2,4-triazol-1-yl)-4*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*c*]pyrimidin-4-one (10) A mixture of 1,2,4-triazole (1.23 g, 17.8 mmol) and phosphorus oxychloride (472 μl, 5 mmol) in acetonitrile (10 ml) was stirred at 0 °C. After 10 min, triethylamine (2.69 ml, 19.3 mmol) was added at 0 °C, and the mixture was stirred at room temperature for 1.5 h. The resulting crystalline precipitate was filtered off, and the precipitate was washed with acetonitrile (5 ml). The filtrate and washings were combined. This solution (5 ml) was added to **8** (294 mg, 0.7 mmol) and 4-dimethylaminopyridine (85 mg, 0.7 mmol), and the mixture was refluxed for 1.5 h. After cooling, the reaction was quenched by adding

water (1 ml) and the whole was evaporated. The residue was extracted with $CHCl_3$, and the organic phase was washed with saturated aqueous $NaHCO_3$, then 0.5 M KH_2PO_4, dried (Na_2SO_4), and evaporated. The residue was purified by means of silica gel column chromatography to give 298 mg (90.3%) of **10** as a pale yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 2.24 (m, 1H, H-2″), 2.67 (m, 1H, H-4′), 2.75 (m, 1H, H-2′), 3.65—3.90 (m, 3H, H-3′, H-5′, H-5″), 4.37—4.60 (m, 4H, Ar-CH_2), 5.11 (m, 1H, H-1′), 5.62 (dd, 1H, J=8.0, 6.3 Hz, H-6′), 6.45 (s, 1H, Ar-H), 7.23—7.40 (m, 10H, Ar-H), 8.08 (s, 1H, Ar-H), 9.18 (s, 1H, Ar-H). MS (EI) *m/z*: 471 (M^+). HR-MS *m/z*: 471.1902 (Calcd for $C_{26}H_{25}N_5O_4$: 471.1904).

(±)-(5aα,7α,8β,8aα)-2-Amino-7-benzyloxy-8-benzyloxymethyl-5a,7,8,8a-tetrahydro-4*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*c*]pyrimidin-4-one (11) A solution of **10** (293 mg, 0.62 mmol) in dioxane (10 ml) was treated with concentrated ammonium hydroxide (2 ml) in a sealed tube at 60 °C for 14 h. The mixture was concentrated and coevaporated with EtOH and $CHCl_3$. The residue was purified by means of silica gel column chromatography to give 259 mg (99.4%) of **11**. An analytical sample was recrystallized from EtOH to afford colorless flakes of **11**, mp 234—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.79 (m, 1H, H-2″), 2.45 (m, 2H, H-2′, H-4′), 3.60—3.80 (m, 3H, H-3′, H-5′, H-5″), 4.37—4.60 (m, 4H, Ar-CH_2), 4.75 (t, 1H, J=7.1 Hz, H-1′), 5.03 (s, 1H, H-5), 5.30 (dd, 1H, J=6.9, 6.0 Hz, H-6′), 6.86 (br s, 2H, NH_2), 7.23—7.40 (m, 10H, Ar-H). MS (EI) *m/z*: 419 (M^+). HR-MS *m/z*: 419.1848 (Calcd for $C_{24}H_{25}N_3O_4$: 419.1843). *Anal.* Calcd for $C_{24}H_{25}N_3O_4$: C, 68.72; H, 6.01; N, 10.02. Found: C, 68.44; H, 6.10; N, 9.77.

(±)-(5aα,7α,8β,8aα)-2-Amino-5a,7,8,8a-tetrahydro-7-hydroxy-8-hydroxymethyl-4*H*,6*H*-cyclopent[4,5]oxazolo[3,2-*c*]pyrimidin-4-one (2) A mixture of **11** (100 mg, 0.238 mmol), 20% $Pd(OH)_2$ (25 mg), and cyclohexene (1 ml) in EtOH (5 ml) was refluxed for 18 h. (After 14 h, further 20% $Pd(OH)_2$ (5 mg) and cyclohexene (0.5 ml) were added.) The catalyst was removed by filtration and washed with hot 50% EtOH. The filtrate was concentrated to dryness and the residue was recrystallized from 90% EtOH to give pure **2** (50 mg, 87.6%), mp >300 °C (dec.). ^{1}H-NMR (D_2O) δ: 1.93 (m, 1H, H-2″), 2.27 (m, 1H, H-4′), 2.49 (dd, 1H, J=14.0, 6.6 Hz, H-2′), 3.97 (m, 2H, H-5′, H-5″), 4.02 (m, 1H, H-3′), 4.90 (t, 1H, J=7.3 Hz, H-1′), 5.39 (s, 1H, H-5), 5.46 (dd, 1H, J=7.3, 5.3 Hz, H-6′). MS (EI) *m/z*: 239 (M^+). HR-MS (EI) *m/z*: 239.0901 (Calcd for $C_{10}H_{13}N_3O_4$: 239.0905). *Anal.* Calcd for $C_{10}H_{13}N_3O_4$: C, 50.20; H, 5.48; N, 17.57. Found: C, 50.31; H, 5.45; N, 17.31.

Anti-HIV-1 Activity Assay in MT-4 Cells MT-4 cells were infected for 1 h with HIV-1 (LAV-1) at a 50%-tissue culture infective dose ($TCID_{50}$) of 0.001/cell. Then, the cells were washed and resuspended at 1×10^5 cells/ml in RPMI-1640 medium. The cell suspension (200 μl/well) was cultured for 5 d in a 96-well culture plate containing various concentrations (12 doses, maximum 1000 μg/ml and minimum 0.49 μg/ml) of the drugs. Control assays were performed, without drugs, with HIV-1-infected and -uninfected cultures. On day 5, the inhibitory concentration (IC) of the test sample required to prevent completely the HIV-1-induced cytopathic effect (CPE) was determined through an optical microscope and the cell growth was examined to obtain the cytotoxic concentration (CC) that reduces the viability of MT-4 cells.

Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

References and Notes

1) Mitsuya H., Yarchoan R., Broder S., *Science*, **249**, 1533—1544 (1990).
2) Soudeyns H., Yao X.-J., Gao Q., Belleau B., Kraus J.-L., Nguyen-Ba N., Spira B., Wainberg M. A., *Antimicrob. Agents Chemother.*, **35**, 1386—1390 (1991).
3) Coates J. A. V., Cammack N., Jenkinson H. J., Mutton I. M., Pearson B. A., Storer R., Cameron J. M., Penn C. R., *Antimicrob. Agents Chemother.*, **36**, 202—205 (1992).
4) Schinazi R. F., Chu C. K., Peck A., McMillan A., Mathis R., Cannon D., Jeong L.-S., Beach J. W., Choi W.-B., Yeola S., Liotta D. C., *Antimicrob. Agents Chemother.*, **36**, 672—676 (1992).
5) Furman P. A., Wilson J. E., Reardon J. E., Painter G. R., *Antiviral Chem. Chemother.*, **6**, 345—355 (1995).
6) Garbesi A., Capobianco M. L., Colonna F. P., Tondelli L., Arcamone F., Manzini G., Hilbers C. W., Aelen J. M. E., Blommers M. J. J., *Nucleic Acids Res.*, **21**, 4159—4165 (1993).
7) Urata H., Ogura E., Shinohara K., Ueda Y., Akagi M., *Nucleic Acids Res.*, **20**, 3325—3332 (1992).
8) Urata H., Ueda Y., Suhara H., Nishioka E., Akagi M., *J. Am. Chem. Soc.*, **115**, 9852—9853 (1993).
9) Urata H., Akagi M., *Tetrahedron Lett.*, **37**, 5551—5554 (1996).
10) *a*) Dickerson R. E., Drew H. R., *J. Mol. Biol.*, **149**, 761—786 (1981); *b*) Drew H. R., Wing R. M., Takano T., Broka C., Tanaka S., Itakura K., Dickerson R. E., *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 2179—2183 (1981).
11) *a*) Miller N., Fox J. J., *J. Org. Chem.*, **29**, 1772—1776 (1964); *b*) Falco E. A., Otter B. A., Fox J. J., *ibid.*, **35**, 2326—2330 (1970); *c*) Honjo M., Furukawa Y., Nishikawa M., Kamiya K., Yoshida Y., *Chem. Pharm. Bull.*, **15**, 1076—1079 (1967).
12) *a*) Ueda T., Usui H., Shuto S., Inoue H., *Chem. Pharm. Bull.*, **32**, 3410—3416 (1984); *b*) Yoshimura Y., Otter B. A., Ueda T., Matsuda A., *ibid.*, **40**, 1761—1769 (1992).
13) *a*) Ikehara M., Kaneko M., *Chem. Pharm. Bull.*, **15**, 1261—1262 (1967); *b*) Ikehara M., Maruyama T., *Tetrahedron*, **31**, 1369—1372 (1975).
14) Biggadike K., Borthwick A. D., Evans D., Exall A. M., Kirk B. E., Roberts S. M., Stephenson L., Youds P., *J. Chem. Soc., Perkin Trans. 1*, **1988**, 549—554.
15) Partridge J. J., Chadha N. K., Uskokovic M. R., *J. Am. Chem. Soc.*, **95**, 532—540 (1973).
16) The numbering system used for carbocyclic nucleosides in reference 17 is employed in the text and Experimental section to facilitate comparison of the NMR spectra. In this nomenclature, the carbon atom replacing the furanose ring oxygen of natural nucleosides is designated C-6′.
17) *a*) Biggadike K., Borthwick D., Exall A. M., Kirk B. E., Roberts S. M., Youds P., *J. Chem. Soc., Chem. Commun.*, **1987**, 1083—1084; *b*) Borthwick A. D., Biggadike K., *Tetrahedron*, **48**, 571—623 (1992).
18) Kumar R., Wiebe L. I., Knaus E. E., *Can. J. Chem.*, **72**, 2005—2010 (1994).
19) Sano T., Shuto S., Inoue H., Ueda T., *Chem. Pharm. Bull.*, **33**, 3617—3622 (1985).
20) Divakar K. J., Reese C. B., *J. Chem. Soc., Perkin Trans. 1*, **1982**, 1171—1176.
21) Maruyama T., Sato S., Honjo M., *Chem. Pharm. Bull.*, **30**, 2688—2697 (1982).
22) Kawahata T., Otake T., Mori H., Morimoto M., Ueba N., Kusumoto I. T., El-Mekkawy S., Hattori M., Namba T., *J. Traditional Med.*, **13**, 59—65 (1996).