Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 5578–5583
Bioorganic & Medicinal Chemistry

4′-Fluorinated carbocyclic nucleosides: Synthesis and inhibitory activity against *S*-adenosyl-L-homocysteine hydrolase

Yukio Kitade,[a,b,c,*] Takayuki Ando,[b] Tsuyoshi Yamaguchi,[a] Ayumi Hori,[a] Masayuki Nakanishi[a] and Yoshihito Ueno[a,b]

[a]*Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[b]*Center for Emerging Infectious Diseases, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[c]*Center for Advanced Drug Research, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*

Received 11 November 2005; revised 14 April 2006; accepted 14 April 2006
Available online 6 May 2006

Abstract—4′-Fluorinated analogue of 9-[(1′*R*,2′*S*,3′*R*)-2′,3′-dihydroxy-cyclopentan-1′-yl]adenine (DHCaA) and their related analogues were systematically synthesized under the Mitsunobu and palladium(0) coupling conditions followed by fluorination with inversion of the configuration by using diethylaminosulfur trifluoride, respectively. 4′-β-Fluoro DHCaA and 2-amino-4′-α-fluoro DHCaA demonstrated slight inhibitory activity against human and *Plasmodium falciparum S*-adenosyl-L-homocysteine hydrolase, respectively.

1. Introduction

Carbocyclic nucleosides[1] are a group of compounds structurally analogous to natural and synthetic nucleosides in which the furanose oxygen has been replaced by a methylene group. This replacement changes the furanose ring into a cyclopentane. Conformationally, the expected similarity in bond lengths and bond angles of the tetrahydrofuran and cyclopentane rings allows these analogues to behave as substrates or inhibitors of the enzymes in living cells. Therefore, it has been reported that the carbocyclic nucleosides possess a wide range of biological activities such as antiviral,[2,3] antitumor,[2,3] antiparasitic,[4] antiarthritic[5], and immunosuppressive[5,6] effects.

In the meantime, the partial fluorination of carbocyclic nucleoside has attracted our attention because of the remarkable fact that the introduction of a fluorine atom into the parent compound increases the biological activity.[7–10] For example, 2′- or 6′-fluorocarbocyclic guanosine (**1** and **2**) had antiviral activity against herpes simplex virus types I and II.[9,10]

Based on this information, we designed fluorinated analogues of carbocyclic nucleoside as *S*-adenosyl-L-homocysteine (SAH) hydrolase inhibitor, especially focusing on the modification at the 4′-position of the cyclopentane ring due to the necessity of *cis*-configuration of the 2′,3′-hydroxyl group for the inhibition. Although the synthesis of 4′-modified carbocyclic nucleoside, such as amino,[11]epimeric,[12] deoxy (DHCaA, **3**)[13], and β-fluoro (**4**)[14] analogues, has been reported previously, their inhibitory activities against SAH hydrolase were not investigated.

In view of these facts, we described herein a systematic synthesis of 4′-β-fluoro DHCaA **4** and the related analogues **5**–**7** as well as the inhibitory activities against SAH hydrolase (Fig. 1).

2. Chemistry

The synthetic routes for the preparation of 4′-fluoro DHCaA analogues are shown in Scheme 1. The Mitsunobu coupling is the most useful and common method for the direct substitution of the hydroxyl group with inversion of the configuration. 4′-α-Acetoxy carbocyclic nucleoside **9**[15,16] was prepared by the coupling reaction of allylic alcohol **8** with adenine under the Mitsunobu coupling[17] conditions (61%). Treatment of **9** with methanolic ammonia readily gave the corresponding

Keywords: Carbocyclic nucleoside; Fluoro carbocyclic nucleoside; Enzyme inhibitor.
* Corresponding author. Tel./fax: +81 58 293 2640; e-mail: kitade@biomol.gifu-u.ac.jp

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.04.023

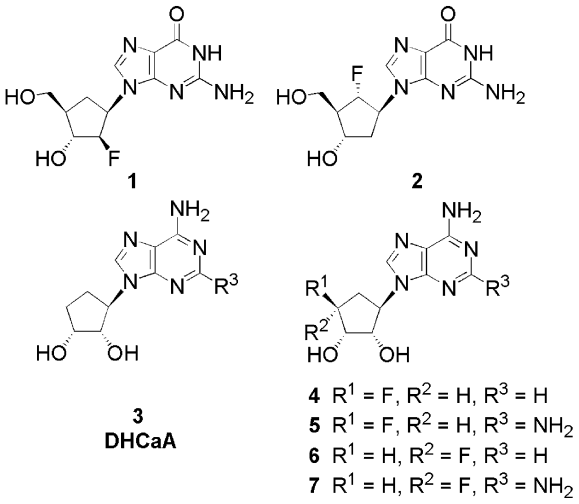


Figure 1. Carbocyclic nucleosides.

deacetylated compound **10**[16] (91%). The fluorination by using diethylaminosulfur trifluoride (DAST)[18] with inversion of the configuration gave 4′-β-fluorinated compound **11** (40%). The following treatment with osmium tetraoxide (OsO_4) in the presence of 4-methylmorpholine *N*-oxide (NMO)[19] afforded the desired target compound **4**[14] (78%). Similarly, coupling reaction of **8** with 2-amino-6-chloropurine instead of adenine afforded **12** (52%); subsequent treatment of **12** with methanolic ammonia for the deprotection of the acetyl group and the amination of the 6-chloro group gave **13** (72%). 4′-Fluorination of **13** with DAST afforded **14** (32%), followed by osmium oxidation to give 2-amino-4′-β-fluoro DHCaA **5** (63%).

Alternatively, tetrakis(triphenylphosphine)palladium [$(PPh_3)_4Pd/PPh_3$] catalyst[20] was used to substitute the allylic ester with retention of the configuration. The coupling reaction of **8** with N^6-benzoyladenine by using the palladium catalyst gave **15** (52%); subsequent deprotection by methanolic ammonia afforded **16**[15] (95%). The 4′-β-hydroxyl group of **16** was converted to a fluorine substituent by DAST with inversion of the configuration to give 4′-α-fluoro compound **17** (49%); the following oxidation led to 4′-α-fluoro DHCaA **6** (71%). Likewise, 2-amino-4′-α-fluoro DHCaA **7** was obtained from the coupling product **18**[21] which was aminated, fluorinated, and oxidized in moderate yield as described.

The structures of these compounds were supported by spectral data (^1H NMR, differential NOE, ^{13}C NMR, MS, and HRMS) and microanalytical data.

3. Biological assay

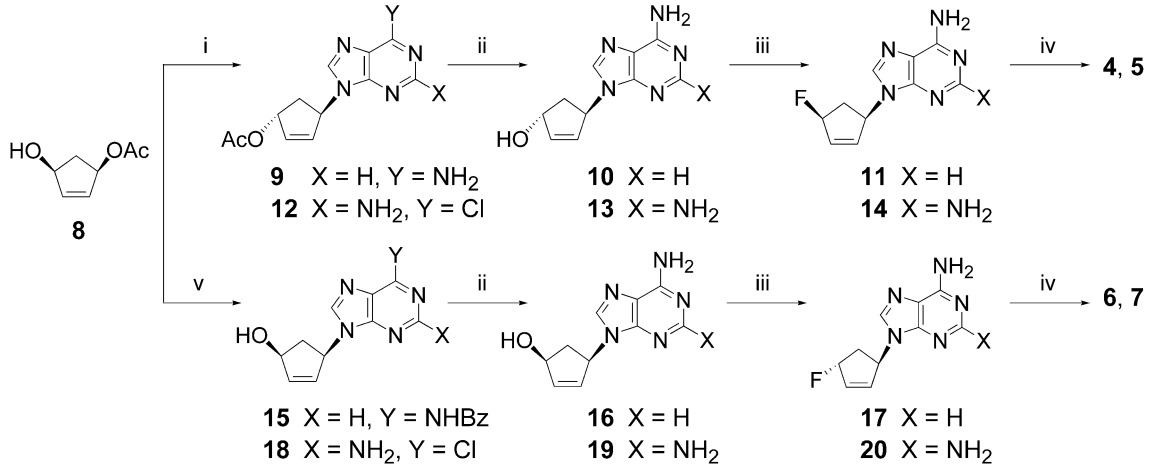
Inhibitory activities of 4′-fluoro carbocyclic nucleosides against human SAH hydrolase (HsSAHH) and *Plasmodium falciparum* SAH hydrolase (PfSAHH) are summarized in Table 1. Compounds **4** and **7** showed inhibitory activity against HsSAHH with IC_{50} value of 200 μM and PfSAHH with that of 220 μM, respectively. Other compounds did not display any inhibitory activity up to 1000 μM.

However, the introduction of a fluorine atom to 4′-α position of cyclopentane ring (**7**) caused the reduction of each inhibitory activity, it increased the selective index (IC_{50} of HsSAHH/IC_{50} of PfSAHH), more than

Table 1. Inhibitory activities of carbocyclic nucleosides against human and *Plasmodium falciparum* SAH hydrolases (SAHH)

Entry	Human SAHH IC_{50} (μM)	*P. falciparum* SAHH IC_{50} (μM)	Selective index
3	9.0	18	0.5
4	200	ND[a]	< 0.2
5	ND[a]	ND[a]	—
6	ND[a]	ND[a]	—
7	ND[a]	220	> 4.5

[a] No inhibitory activity showed at 1000 μM.



Scheme 1. Reagents and conditions: (i) adenine (for **9**) or 2-amino-6-chloropurine (for **12**), Ph_3P, DEAD, THF, rt; (ii) NH_3, MeOH, rt (for **10**), 55 °C (for **16**), 100 °C (for **13** and **19**); (iii) DAST, CH_2Cl_2, 0 °C; (iv) OsO_4, NMO, THF–H_2O, rt; (v) N^6-benzoyladenine (for **15**) or 2-amino-6-chloropurine (for **18**), NaH, $(Ph_3P)_4Pd$, Ph_3P, DMSO, THF, 55 °C.

4.5. In the meantime, 4′-β-fluorine substituent of **4** had an effect for the inhibitory activity against only HsSAHH, and the selective index was less than 0.2.

4. Discussion

The inhibitory activity of **4** against HsSAHH and that of **7** against PfSAHH were less than one-tenth of that of DHCaA **3**, however, both 4′-α- and 4′-β-fluorine substituents were effective for selective inhibition against PfSAHH and HsSAHH, respectively. In our previous work[23] on the crystallization of each SAHH holding noraristeromycin analogue, we clarified that Thr60 of HsSAHH and Cys59 of PfSAHH, which located around 2-position of adenine moiety, were the key residues for selective inhibition. The important amino acid residue around 4′-hydroxyl group of sugar moiety is still unclear. Based on these notions, it is suggested that there is a difference of the specific binding site, or characteristic depression.

In this paper, we demonstrated the synthesis of fluorinated carbocyclic nucleosides and their inhibitory activities against HsSAHH and PfSAHH. These results will contribute greatly to the design of potent inhibitors against PfSAHH.

5. Experimental

5.1. General procedure

Melting points were recorded on a Yanaco Micro Melting Point Apparatus. Elemental analyses were carried out at the microanalytical laboratory of Gifu Pharmaceutical University. ^{1}H and ^{13}C spectra were recorded at 400 MHz on a JEOL JNM α400 (operated at 400 and 100 MHz, respectively) using $CDCl_3$ with TMS as internal standard or DMSO-d_6. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet), and br (broad). Coupling constants (*J*) are expressed in hertz. The differential nuclear overhauser effect (DIFNOE) data were described; the irradiated proton (the correlated proton: % of enhancement value). Mass spectra (MS and HRMS) were recorded at a 70 eV on JEOL JMS-D300 spectrometer and Shimadzu QP 1000 A. Reactions were monitored by thin-layer chromatography (TLC) using MERCK silica gel 60 F_{254}. Column chromatography was carried out on silica gel (Wako gel C-300).

5.1.1. 9-[(1′*R*,4′*S*)-4′-Fluoro-2′-cyclopenten-1′-yl]-9-*H*-adenine (11). To a stirred solution of compound **10** (264 mg, 1.22 mmol) in CH_2Cl_2 (53 mL) at 0 °C, DAST (0.78 mL, 5.89 mmol) was added and the mixture was stirred for 3 h. The mixture was poured into saturated aqueous $NaHCO_3$ and then the aqueous phase was extracted with $CHCl_3$, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography eluting with hexane/EtOAc (45:1) and then the fractions were evaporated under reduced pressure. Compound **11** was obtained (107 mg, 40%): mp 160–162 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.38 (s, 1H, H-2), 7.85 (s, 1H, H-8), 6.41 (m, 1H, H-3′), 6.30 (m, 1H, H-2′), 5.79–5.67 (m, 4H, H-1′, H-4′ and NH_2), 3.04 (m, 1H, H-5′), 2.13 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.38, 152.69, 138.75, 138.32, 137.00, 136.10 (d, *J* = 8.6), 135.06 (d, *J* = 17.2), 95.10 (d, *J* = 174.4), 39.20 (d, *J* = 21.4), 29.51 (d, *J* = 33.8); mass (EI) *m*/*z*: 219 (M^+), 199, 198, 173, 151; HRMS (EI) calcd for $C_{10}H_{10}FN_5$: 219.0920, found: 219.0928; Anal. Calcd for $C_{10}H_{10}FN_5$: C, 54.79; H, 4.60; N, 31.95. Found: C, 54.63; H, 4.61; N, 31.78.

5.1.2. 9-[(1′*R*,2′*S*,3′*R*,4′*R*)-2′, 3′-Dihydroxy-4′-fluorocyclopentan-1′-yl]-9-*H*-adenine (4). To a stirred solution of compound **11** (113 mg, 514 μmol) and *N*-methylmorpholine *N*-oxide (NMO, 152 mg, 1.3 mmol) in THF (7.4 mL) and H_2O (0.74 mL), 2% OsO_4 solution (1.3 mL, 0.10 mmol) was added and stirred at room temperature for 21 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (30:1–10:1). Compound **4** was obtained (102 mg, 78%): mp 128–130 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (s, 1H, H-2), 8.12 (s, 1H, H-8), 7.21 (s, 2H, NH_2), 5.35 (d, 1H, OH-2′), 5.24 (d, 1H, OH-3′), 4.92 (m, 1H, H-4′), 4.70 (m, 1H, H-1′), 4.59 (m, 1H, H-2′), 4.00 (dd, 1H, H-3′), 2.73 (m, 1H, H-5′$_\alpha$), 2.27 (m, 1H, H-5′$_\beta$); DIFNOE: H-1′ (H-5′$_\alpha$: 5.8%), H-3′ (H-2′: 2.9%), H-4′ (H-5′$_\alpha$: 4.6%), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 156.06, 152.26, 149.70, 140.42, 119.37, 95.12 (d, *J* = 191.7), 73.82 (d, *J* = 4.9), 72.05 (d, *J* = 7.4), 57.95 (d, *J* = 3.3), 33.36 (d, *J* = 22.3); mass (EI) *m*/*z*: 253 (M^+), 236, 216, 204, 192; HRMS (EI) calcd for $C_{10}H_{12}FN_5O_2$: 253.0975, found: 253.0969; Anal. Calcd for $C_{10}H_{12}FN_5O_2$: C, 47.43; H, 4.78; N, 27.66. Found: C, 47.49; H, 4.74; N, 27.46.

5.1.3. 9-[(1′*R*,4′*R*)-4′-Acetoxy-2′-cyclopenten-1′-yl]-9-*H*-2-amino-6-chloropurine (12). This compound was prepared by an analogous method for the preparation of compound **9**. To a solution of **8** (402 mg, 2.83 mmol), Ph_3P (1480 mg, 5.65 mmol), and 2-amino-6-chloropurine (968 mg, 5.71 mmol) in THF, 40% diethyl azodicarboxylate solution (DEAD, 2.6 mL, 5.5 mmol) was added and stirred at room temperature for 21 h. The reaction mixture was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (200:1–100:1). The fractions were evaporated under reduced pressure. Compound **12** was obtained (428 mg, 52%): mp 147–148 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.70 (s, 1H, H-8), 6.34 (m, 1H, H-3′), 6.17 (m, 1H, H-2′), 5.97 (m, 1H, H-1′), 5.75 (m, 1H, H-4′), 5.10 (s, 2H, NH_2), 2.56 (m, 1H, H-5′), 2.40 (m, 1H, H-5′), 1.63 (s, 3H, $COCH_3$); Anal. Calcd for $C_{12}H_{12}ClN_5O_2 \cdot 1/4\ H_2O$: C, 48.33; H, 4.22; N, 23.48. Found: C, 48.46; H, 4.10; N, 23.29.

5.1.4. 9-[(1′*R*,4′*R*)-4′-Hydroxy-2′-cyclopenten-1′-yl]-9-*H*-2-aminoadenine (13). Compound **12** (204 mg, 695 μmol) was treated with methanolic ammonia (50 mL) at 100 °C in a sealed tube for 63 h. The reaction mixture was evaporated under reduced pressure and purified by

silica gel column chromatography eluting with $CHCl_3$/MeOH (25:1–10:1). Compound **13** was obtained (117 mg, 72%): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.50 (s, 1H, H-8), 6.58 (s, 2H, NH_2-6), 6.13 (m, 1H, H-3′), 5.96 (m, 1H, H-2′), 5.74 (m, 2H, OH and H-1′), 5.49 (m, 1H, H-4′), 5.01 (s, 2H, NH_2-2), 2.20–2.09 (m, 2H, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.84, 157.57, 152.6, 140.99 137.27, 132.83, 114.49, 76.64 60.10, 42.48; mass (EI) *m/z*: 232 (M^+), 214, 151, 134, 108; HRMS (EI) calcd for $C_{10}H_{12}N_6O$ 232.1073. Found: 232.1079.

5.1.5. 9-[(1′*R*,4′*S*)-4′-Fluoro-2′-cyclopenten-1′-yl]-9-*H*-2-aminoadenine (14). This compound was prepared by an analogous method for the preparation of compound **11**. To a stirred solution of compound **13** (190 mg, 817 μmol) in CH_2Cl_2 (36 mL) at 0 °C, DAST (500 μL, 3.77 mmol) was added with stirring for 1 h. The mixture was poured into saturated aqueous $NaHCO_3$ and then the aqueous phase was extracted with $CHCl_3$, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (50:1–35:1). The fractions were evaporated under reduced pressure. Compound **14** was obtained (60.4 mg, 32%): mp 226–227 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.57 (s, 1H, H-8), 6.35 (m, 1H, H-3′), 6.25 (m, 1H, H- 2′), 5.58-5.46 (m, 4H, H-1′, H-4′ and NH_2-6), 4.76 (s, 2H, NH_2-2), 3.04–2.93 (m, 1H, H-5′), 2.15–2.03 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.95, 156.02, 151.46, 136.45, 136.42, 135.94 (d, J = 64.2), 134.47 (d, J = 18.1), 95.17 (d, J = 174.4), 55.70 (d, J = 2.4), 39.11 (d, J = 21.4); mass (EI) *m/z*: 234 (M^+), 214, 213, 188, 150; HRMS (EI) calcd for $C_{10}H_{11}FN_6$ 234.1029. Found: 234.1024; Anal. Calcd for $C_{10}H_{11}FN_6 \cdot 6/13\ H_2O$: C, 49.84; H, 4.91; N, 34.87. Found: C, 50.09; H, 4.75; N, 34.52.

5.1.6. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′-Dihydroxy-4′-fluorocyclopentan-1′-yl]-9-*H*-2-aminoadenine (5). This compound was prepared by an analogous method for the preparation of compound **4**. To a stirred solution of compound **14** (44 mg, 0.164 mmol) and NMO (48 mg, 411 μmol) in THF (2.4 mL) and H_2O (0.24 mL), 2% OsO_4 solution (416 μL, 32 μmol) was added and stirred at room temperature for 19 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (20:1–8:1). The fractions were evaporated under reduced pressure. The crystallization from EtOAc gave compound **5** (27.7 mg, 63%): mp 124–126 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.67 (s, 1H, H-8), 6.54 (s, 2H, NH_2-6), 5.62 (s, 2H, NH_2-2), 5.16 (m, 2H, OH), 4.71 (m, 1H, H-4′), 4.41 (q, 1H, H-1′), 4.37(m, 1H, H-2′), 3.96 (m, 1H, H-3′), 2.55 (m, 1H, H-5′$_\alpha$), 2.03 (m, 1H, H-5′$_\beta$); DIFNOE: H-1′ (H-5′$_\alpha$: 4.8%), H-3′ (H-2′: 3.4%), H-4′ (H-5′$_\alpha$: 4.3%), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.98, 156.14, 152.06, 136.19, 113.59, 95.37 (d, J = 176.9), 73.79 (d, J = 1.8), 73.59, 56.76, 33.65 (d, J = 22.2); mass (EI) *m/z*: 268 (M^+), 251, 231, 214, 192; HRMS (EI) calcd for $C_{10}H_{13}FN_6O_2$ 268.1084, found: 268.1089; Anal. Calcd for $C_{10}H_{13}FN_6O_2 \cdot 4/15$ EtOAc: C, 45.56; H, 5.23; N, 28.81. Found: C, 45.26; H, 5.50; N, 28.84.

5.1.7. 9-[(1′*R*,4′*R*)-4′-Fluoro-2′-cyclopenten-1′-yl]-9-*H*-adenine (17). This compound was prepared by an analogous method for the preparation of compound **11**. To a stirred solution of compound **16** (185 mg, 850 μmol) in CH_2Cl_2 (39 mL) at 0 °C, DAST (550 μL, 4.2 mmol) was added, and the mixture was stirred for 1 h. The mixture was poured into aqueous saturated $NaHCO_3$ and then the aqueous phase was extracted with $CHCl_3$, dried with Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography eluting with hexane/EtOAc (45:1) and then the fractions were evaporated under reduced pressure. Compound **17** was obtained (91 mg, 49%): mp 199–200 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37 (s, 1H, H-2), 7.71 (s, 1H, H-8), 6.41 (m, 1H, H-3′), 6.33 (m, 1H, H-2′), 5.97–5.87 (m, 2H, H-1′ and H-4′), 5.53 (s, 2H, NH_2), 2.80 (m, 1H, H-5′), 2.40 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 156.01, 152.38, 149.18, 139.23, 138.27 (d, J = 4.2), 133.71 (d, J = 14.8), 119.14, 97.07 (d, J = 166.2), 58.59, 38.17 (d, J = 23.0); mass (EI) *m/z*: 219 (M^+), 211, 199, 183, 173; HRMS (EI) calcd for $C_{10}H_{10}FN_5$: 219.0920, found: 219.0930; Anal. Calcd for $C_{10}H_{10}FN_5$: C, 54.79; H, 4.60; N, 31.95. Found: C, 54.74; H, 4.52; N, 31.84.

5.1.8. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′-Dihydroxy-4′-fluorocyclopentan-1′-yl]-9-*H*-adenine (6). This compound was prepared by an analogous method for the preparation of compound **4**. To a stirred solution of compound **17** (28.6 mg, 130 μmol) and NMO (40 mg, 342 μmol) in THF (1.9 mL) and H_2O (190 μL), 2% OsO_4 solution (0.33 mL, 26 μmol) was added and stirred at room temperature for 22 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (30:1–8:1). Compound **6** was obtained (25.7 mg, 78%): mp 126 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.18 (s, 1H, H-2), 8.12 (s, 1H, H-8), 7.18 (s, 2H, NH_2), 5.47 (d, J = 5.6, 1H, OH), 5.35 (d, J = 4.0, 1H, OH), 5.10 (m, 1H, H-1′), 5.04 (m, 1H, H-4′), 4.98 (m, 1H, H-1′), 4.15 (dt, 1H, H-3′), 4.07 (dd, 1H, H-4′), 2.74 (m, 1H, H-5′$_\alpha$), 2.31 (m, 1H, H-5′$_\beta$); DIFNOE: H-1′ (H-5′$_\alpha$: 5.8%), H-3′ (H-2′: 4.8%, H-4′: 4.8%), H-4′ (H-5′$_\beta$: 4.6%), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 155.89, 152.11, 149.60, 140.40, 118.41, 97.68 (d, J = 176.0), 77.04 (d, J = 22.2), 71.99 (d, J = 7.4), 52.31, 33.77 (d, J = 23.0); mass (EI) *m/z*: 253 (M^+), 236, 218, 204, 192; HRMS (EI) calcd for $C_{10}H_{12}FN_5O_2$ 253.0975. Found: 253.0984; Anal. Calcd for $C_{10}H_{12}FN_5O_2 \cdot 9/10H_2O$: C, 44.58; H, 5.16; N, 25.99. Found: C, 44.54; H, 5.20; N, 25.94.

5.1.9. 9-[(1′*R*,4′*S*)-4′-Hydroxy-2′-cyclopenten-1′-yl]-9-*H*-2-aminoadenine (19). This compound was prepared by an analogous method for the preparation of compound **13**. Compound **18** (166 mg, 660 μmol) was treated with NH_3 in 2-propanol (50 mL) at 100 °C in a sealed tube for 48 h. The reaction mixture was evaporated under reduced pressure and purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (30:1–8:1). Compound **19** was obtained (150 mg, 98%): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.63 (s, 1H, H-8), 6.67 (s, 2H, NH_2-6), 6.13 (m, 1H, H-3′), 5.92 (m, 1H, H-2′), 5.74 (m, 2H, NH_2-2), 5.45 (m, 1H, OH), 5.22 (m, 1H, H-

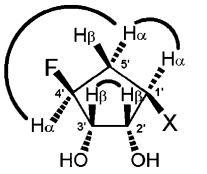
1′), 4.09 (m, 1H, H-4′), 2.81 (m, 1H, H-5′), 1.62 (m, 1H, H-5′); Anal. Calcd for $C_{10}H_{12}N_6O\cdot 11/10H_2O$: C, 47.65; H, 5.68; N, 33.34. Found: C, 47.80; H, 5.51; N, 33.19.

5.1.10. 9-[(1′*R*,4′*R*)-4′-Fluoro-2′-cyclopenten-1′-yl]-9-*H*-2-aminoadenine (20). This compound was prepared by an analogous method for the preparation of compound **19**. To a stirred solution of compound **19** (204 mg, 880 μmol) in CH_2Cl_2 (25 mL) at 0 °C, DAST (590 μL, 4.47 mmol) was added and stirred for 1 h at rt. The mixture was poured into saturated aqueous $NaHCO_3$ and then the aqueous phase was extracted with $CHCl_3$, dried with Na_2SO_4, filtered and evaporated. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (40:1–15:1) and then fractions were evaporated under reduced pressure. Compound **20** was obtained (126 mg, 61%): mp 191–192 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.58 (s, 1H, H-8), 6.67 (s, 1H, NH_2-6), 6.32 (m, 2H, H-2′ and H-3′), 6.06 (m, 1H, H-1′), 5.78 (s, 1H, NH_2-2), 5.61 (s, 1H, H-4′), 2.91 (m, 1H, H-5′), 1.94 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.08, 156.07, 151.46, 138.25 (d, J = 9.8), 135.42, 133.65 (d, J = 14.8), 113.41, 97.15 (d, J = 166.2), 57.66, 38.29 (d, J = 23.9); mass (EI) *m*/*z*: 234 (M^+), 214, 213, 188, 171; HRMS (EI) calcd for $C_{10}H_{11}FN_6$ 234.1029. Found: 234.1025; Anal. Calcd for $C_{10}H_{11}FN_6\cdot 1/2\ H_2O$: C, 49.38; H, 4.97; N, 34.55. Found: C, 49.18; H, 4.62; N, 34.48.

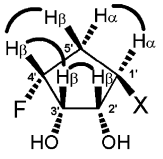
5.1.11. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′-Dihydroxy-4′-fluorocyclopentan-1′-yl]-9-*H*-2-aminoadenine (7). This compound was prepared by an analogous method for the preparation of compound **4**. To a stirred solution of compound **20** (97.5 mg, 416 μmol) and NMO (85.6 mg, 750 μmol) in THF (25 mL) and H_2O (2.5 mL), 2% OsO_4 solution (491 μL, 41.3 μmol) was added and stirred at room temperature for 22 h. The mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (20:1–12:1). The fractions were evaporated under reduced pressure. The crystallization from AcOEt gave compound **7** (106 mg, 95%): mp 143–144 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.31 (s, 1H, H-8), 6.63 (s, 2H, NH_2-6), 5.74 (s, 2H, NH_2-2), 5.39 (m, 2H, OH), 5.02 (m, 1H, H-4′), 4.86 (m, 1H, H-1′), 4.09 (m, 1H, H-3′), 4.02 (m, 1H, H-2′), 2.65 (m, 1H, H-5′$_\alpha$), 2.22 (m, 1H, H-5′$_\beta$); DIFNOE: H-1′ (H-5′$_\alpha$: 5.8%), H-3′ (H-2′: 4.8%, H-4′: 4.8%), H-4′ (H-5′$_\beta$: 4.6%), ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.94, 155.99, 151.77, 137.03, 112.74, 97.88 (d, J = 175.3), 77.29 (d, J = 22.2), 72.04 (d, J = 7.4), 51.87, 33.88 (d, J = 23.0); mass (EI) *m*/*z*: 268 (M^+), 251, 231, 207, 192; HRMS (EI) calcd for $C_{10}H_{13}FN_6O_2$ 268.1084, found: 268.1090; Anal. Calcd for $C_{10}H_{11}FN_6\cdot 9/20$ EtOAc: C, 46.03; H, 5.43; N, 27.30. Found: C, 46.28; H, 5.67; N, 27.41.

5.2. NOE correlation

NOE analysis was performed to assign the configuration of each substituent on cyclopentane ring. For 4′-β-fluorinated compounds, a strong NOE, [H-1′ and H-5′$_\alpha$] and [H-4′ and H-5′$_\alpha$] which showed 1′,4′-*cis* relationship, was observed. Furthermore, we did not find any significant NOE [H-1′ and H-2′] and [H-3′ and H-4′], and find that of [H-2′ and H-3′]. According to this result, 2′- and 3′-hydroxyl groups located α face. On the other hand, for 4′-α-fluorinated compounds, strong NOE, [H-1′ and H-5′$_\alpha$], [H-2′ and H-3′], [H-3′ and H-4′], and [H-4′andH-5′$_\beta$] were assigned to 1′,4′-*trans* relationship and α face stereochemicals of 2′- and 3′-hydroxyl groups and 4′ fluorine substituent.



4 X = adenine
5 X = 2-aminoadenine



6 X = adenine
7 X = 2-aminoadenine

5.3. Enzyme assay

In the synthetic direction, the enzyme assay was a modification of an earlier method.[22] The enzyme was incubated with 100 mM adenosine, 5 mM DL-homocysteine, and inhibitors on 0.2 mL of 10 mM potassium phosphate, pH 7.2, buffer at 30 °C for 2 min in the standard assay system. The reaction was started by the addition of 3 mL SAH hydrolase (human: 0.43 μg, *P. falciparum*: 0.54 μg) and terminated by the addition of 20 μL of 0.67 N HCl. The reaction mixture was kept on ice until the HPLC analysis. The mixture was analyzed for SAH by a Shimadzu HPLC system. In the synthetic reaction, one unit of SAH hydrolase was defined as the amount synthesizing 1 mmol of SAH/min at 30 °C.

Acknowledgment

This research was in part supported by Grants-in-Aid for Scientific Research on Priority Area No. 16017239 (to Y. K).

References and notes

1. Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229.
2. Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, S. R.; Earl, R. A.; Guedj, R. *Tetrahedron* **1994**, *50*, 10611.
3. De Clercp, E. *Nucleosides Nucleotides* **1988**, *17*, 625.
4. Hiraoka, O.; Satake, H.; Iguchi, S.; Matsuda, A.; Ueda, T.; Wataya, Y. *Biochem. Biophys. Res. Commun.* **1986**, *3*, 1114.
5. Saso, Y.; Conner, E. M.; Teegarden, B. R.; Yuan, C. S. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 106.
6. Wolos, J. A.; Frondorf, K. A.; Babcock, G. F.; Stripp, S. A.; Bowlin, T. L. *Cell Immunol.* **1993**, *149*, 402.
7. Nakanishi, M.; Iwata, A.; Yatome, C.; Kitade, Y. *J. Biochem.* **2001**, *129*, 101.
8. Kitade, Y.; Kojima, H.; Zulfiqur, F.; Kim, H. S.; Wataya, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3963.
9. Borthwick, A. D.; Butt, S.; Biggadike, K.; Exall, A. M.; Roberts, S. M.; Youds, P. M.; Kirk, B. E.; Booth, B. R.; Cameron, J. M.; Cox, S. W.; Marr, C. L. P; Shill, M. D. *J. Chem. Soc., Chem. Commun.* **1988**, *10*, 656.

10. Borthwick, A. D.; Kirk, A. D.; Biggadike, K. B.; Exall, A. M.; Butt, S.; Roberts, S. M.; Knight, D. J.; Coates, J. A. V.; Ryan, M. *J. Med. Chem.* **1991**, *34*, 907.
11. Hedge, V. R.; Seley, K. L.; Schneller, S. W.; Elder, T. J. *J. Org. Chem.* **1998**, *63*, 7092.
12. Siddiqi, S. M.; Chen, X.; Schnellar, S. W.; Ikeda, S.; Snoeck, R.; Andrrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1994**, *37*, 1382.
13. Wolf, M. S.; Lee, Y.; Bartlett, W. J.; Borcherding, D. R.; Borchardt, R. T. *J. Med. Chem.* **1992**, *35*, 1782.
14. Siddiqi, S. M.; Oertel, F. P.; Chen, X.; Schneller, S. W. *J. Chem. Soc., Chem. Commun.* **1993**, *10*, 708.
15. (a) Kitade, Y.; Kozaki, A.; Yatome, Y. *Tetrahedron Lett.* **2001**, *42*, 433; (b) Kitade, Y.; Kozaki, A.; Miwa, T.; Nakanishi, M. *Tetrahedron* **2002**, *58*, 1271.
16. Siddiq, S. M.; Chen, X.; Schneller, S. W. *Nucleosides Nucleotides* **1993**, *12*, 267.
17. (a) Mitsunobu, O. *Synthesis* **1981**, 1; (b) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017.
18. Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574.
19. Poli, G. *Tetrahedron Lett.* **1989**, *30*, 7385.
20. Trost, B. M.; Kuo, G.-H.; Benneche, T. J. *J. Am. Chem. Soc.* **1988**, *110*, 621.
21. Rajappan, V.; Schneller, S. W.; Williams, S. L.; Kern, E. R. *Bioorg. Med. Chem.* **2002**, *10*, 883.
22. Gomi, T.; Date, T.; Osawa, H.; Fujioka, M.; Aksamit, R. R., ; Backlund, P. S., Jr.; Cantoni, G. L. *J. Biol. Chem.* **1989**, *264*, 16138.
23. Tanaka, N.; Nakanishi, M.; Kusakabe, Y.; Shiraiwa, K.; Yabe, S.; Ito, Y.; Kitade, Y.; Nakamura, KT. *J. Mol. Biol.* **2004**, *343*, 1007.