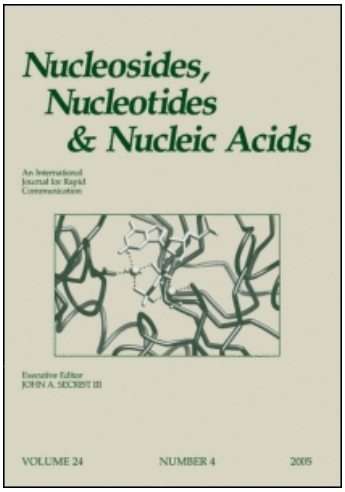

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Deamination of 9-(Hydroxymethylated cycloalkyl)-9*H*-adenines (Carbocyclic Adenine Nucleosides) by Adenosine Deaminase: Effect of High-Pressure Upon Deamination Rate and Enantioselectivity

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DEAMINATION OF 9-(HYDROXYMETHYLATED CYCLOALKYL)-9*H*-ADENINES (CARBOCYCLIC ADENINE NUCLEOSIDES) BY ADENOSINE DEAMINASE: EFFECT OF HIGH-PRESSURE UPON DEAMINATION RATE AND ENANTIOSELECTIVITY[1),#]

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Tokumi Maruyama[b)], Yoshiko Sato[b)], and Chikara Kaneko[a)]

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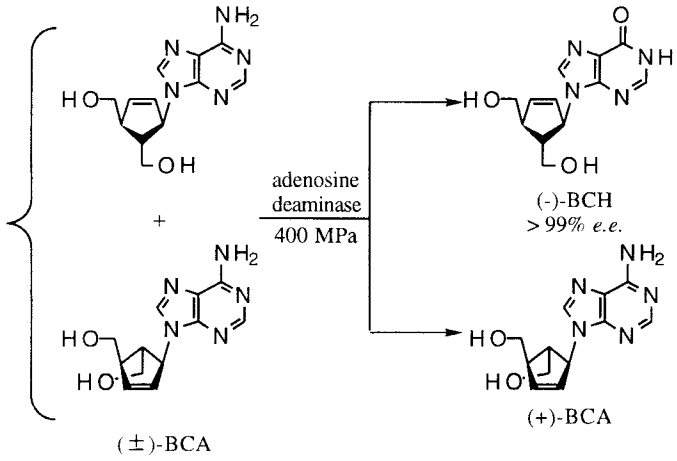
Abstract: The deamination of eight kinds of racemic carbocyclic adenine nucleosides by adenosine deaminase under high-pressure (400 MPa) was examined and the result was compared with that obtained from the reaction under atmospheric pressure. The deamination of all carbocyclic nucleosides irrespective to their ring size of carbocycles was facilitated remarkably by high-pressure. The reaction of three and five membered carbocyclic nucleosides resulted in the very high enantioselectivity both under high- and atmospheric pressure whereas the enantioselectivity of six membered carbocyclic nucleosides was suppressed under high-pressure. However, the enantioselectivity of four membered nucleosides was low under both conditions.

Adenosine deaminase (ADA), one of the most important enzyme in purine metabolism, catalyzes the hydrolysis of adenosine to inosine and ammonia.[2)] Lack of ADA causes severe combined immunodeficiency disease (SCID)[3)] and the levels of ADA change in the patients of acquired immunodeficiency syndrome (AIDS), anemia, various lymphomas, and leukemias.[4)] Recently, Quiocho and his coworkers[5)] have proposed an elegant mechanism for the deamination by ADA on the basis of the X-ray crystallographical analysis of the complex of 6*R*-hydroxy-1,6-dihydropurine ribonucleoside (HDPR) with ADA. On the other hand, ADA has recently been used for the preparation of pharmacologically active substances. For example, oxetanocin A is hydrolyzed to oxetanocin H having anti-HIV activity by ADA[6)] and the same enzymatic hydrolysis was applied to the resolution of (±)-

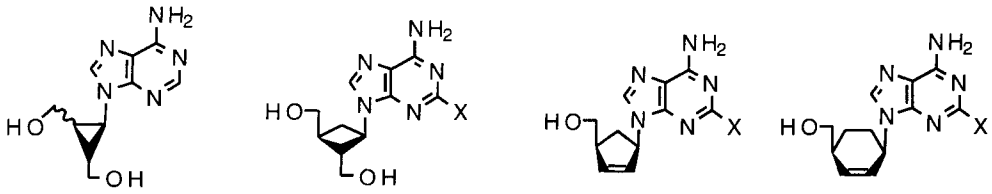
This paper is dedicated to the celebration of the 75th birthday of Dr. Yoshihisa Mizuno, Emeritus Professor of Hokkaido University.



Scheme 1

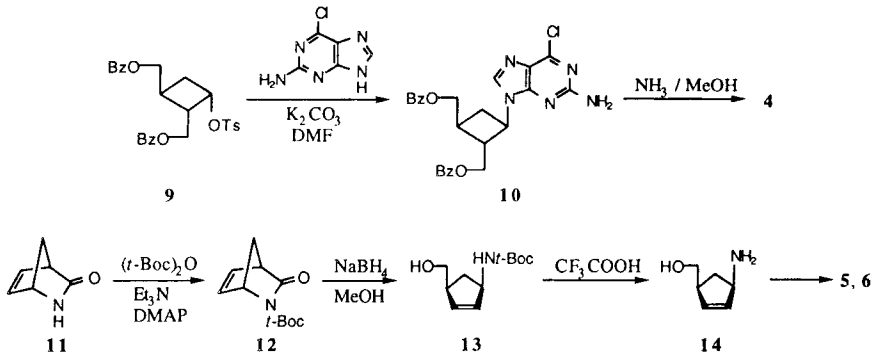
carbovir to each enantiomers.[7] Previously, we reported the kinetic resolution of racemic BCA (9-[*c*-4,*t*-5-(bishydroxymethyl)cyclopent-2-en-*r*-1-yl]-9*H*-adenine) using ADA.[8] The deamination of (±)-BCA by ADA did not proceed under ordinary conditions. However, use of high-pressure (400 MPa) for the reaction overcame this problem, and only (-)-BCA showing anti-HIV activity was deaminated to give the hypoxanthine derivative [(-)-BCH] with very high enantioselectivity. In this paper, we report the generality and limitation of deamination of 9-(hydroxymethylated cycloalkyl)-9*H*-adenines and their 2-amino derivatives (carbocyclic adenine nucleosides) by ADA under high-pressure, which would provide an efficient resolution of the racemates. The substrates used for the deamination are eight kinds of carbocyclic nucleosides **1**~**8** shown in Fig. 1.

The carbocyclic nucleosides **1**[9], **2**[9], and **3**[10] were synthesized according to the method previously reported from our laboratory. Although the nucleosides **4**[11], **5**[12], and **6**[7] have already been synthesized by other groups, we synthesized these compounds using slightly different routes from the previously reported ones. Thus, the known compound **9**[13] was condensed with 2-amino-6-chloropurine in the presence of potassium carbonate in dimethylformamide to give **10** in 26% yield. Ammonolysis of **10** with ammonia in methanol afforded carbocyclic oxetanocin D[11] (D designates 2,6-diaminopurine) in 77% yield. A common intermediate **14** of **5** and **6** was obtained from the reductive amido bond cleavage reaction[14] of bicycloamide **12**, prepared by the reaction of **11** with di-*t*-butyl dicarbonate,[15] followed by acid treatment. Transformation of **14** to **5** and **6** was carried out by usual purine ring construction.[7,12] 9-[*c*-4-(Hydroxymethyl)cyclohex-2-en-*r*-1-yl]-9*H*-adenine (**7**) and its



1 : 2,3-*trans* **3** : X = H (carbocyclic oxetanocin A) **5** : X = H **7** : X = H
2 : 2,3-*cis* **4** : X = NH_2 (carbocyclic oxetanocin D) **6** : X = NH_2 **8** : X = NH_2

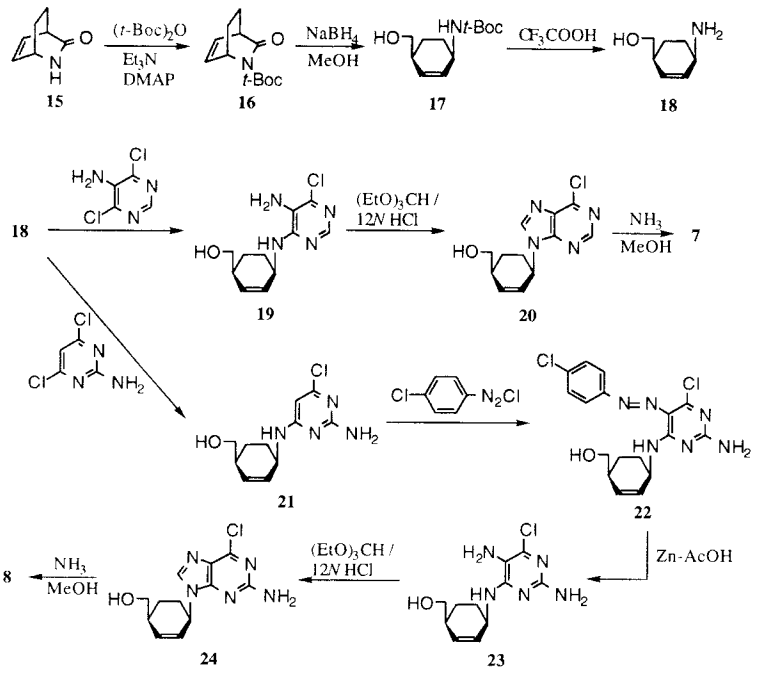
Fig. 1 Carbocyclic Nucleosides Used for the Deamination.



Scheme 2

2-amino derivative **8** have not been synthesized so far. Since **8-G** derived from **8** by deamination corresponds to homocarbovir (Table 2), its biological activity is quite interesting. The cyclohexenylamine **18** corresponding to the starting material of compound **7** and **8** was synthesized from the bicycloamide **15**.[16] To cleave the amido bond, *t*-butoxycarbonyl group was introduced to the 2-position of **15** by the method previously reported[15]. The resulting compound **16** was then treated with sodium borohydride to give the cyclohexene derivative **17**. Treatment of **17** with trifluoroacetic acid gave a cyclohexenylamine **18**, which was a starting material of **7** and **8**. Purine ring construction from **18** was carried out in a usual manner as shown in Scheme 3.

Deamination of the carbocyclic nucleosides thus obtained by ADA was carried out under high pressure and the result was compared with that obtained from the reaction under atmospheric pressure. Since it was clarified that the optimum pressure was 400 MPa for the deamination of (±)-BCA[8], this pressure was used for all deamination reactions under high-



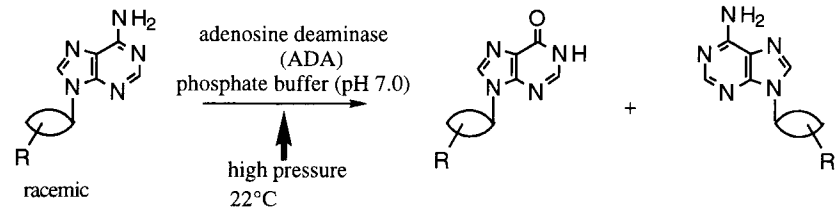
Scheme 3

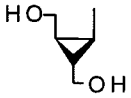
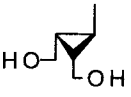
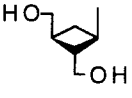
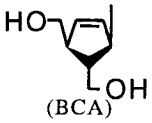
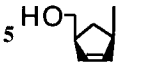
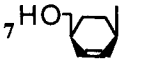
pressure. The results are shown in Tables 1 and 2. As a reference result, the deamination of (±)-BCA was also included in Table 1.

First, we examined the deamination of 9-[*t*-2,*c*-3-(bishydroxymethyl)cycloprop-*r*-1-yl)]-9*H*-adenine (**1**)[9] corresponding to a lower methylene homologue of carbocyclic oxetanocin A. Compound **1** was treated with ADA (1 unit/μmol) at 25 °C under 0.1 MPa (*ca.* 1 atm) for 8 d to give the hypoxanthine derivative **1-H** in 50% yield. The optical purity (enantiomeric excess: *e.e.*) of **1-H** was determined to be more than 99% by HPLC analysis. Although the absolute structure of **1-H** has not been determined as yet, it would be considered to be 1*R*,2*R*,3*R* on the basis of the deamination of (±)-BCA.[8] This deamination was remarkably facilitated by high-pressure (400 MPa) to give **1-H** having highly optical purity in 35% conversion yield in 12 h.

The deamination of 9-[*t*-2,*t*-3-(bishydroxymethyl)cycloprop-*r*-1-yl]-9*H*-adenine (**2**)[9] having anti-BLV (bovine leukemia virus) was faster than that of **1**. Thus, **2** was hydrolyzed to **2-H** in 46% by treatment with ADA (1 unit/μmol) at 25 °C under 0.1 MPa for 5 d whereas under 400 MPa the reaction was completed in 7 h to give **2-H** in quantitative yield.

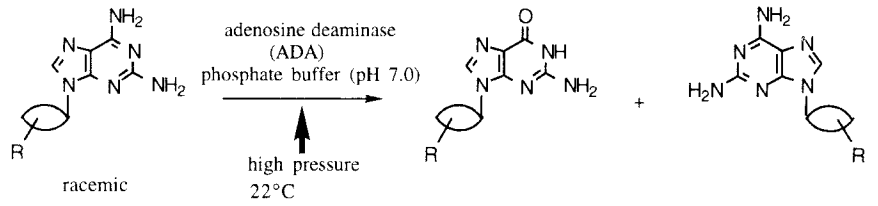
Table 1. Deamination of Carbocyclic Adenine Nucleosides by Adenosine Deaminase (ADA) under High-Pressure

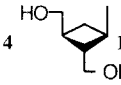
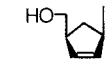
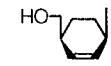


	R	Reaction condition: Pressure (Reaction time)	Reaction condition: ADA	Product	Yield (%)	Enantio excess (%)
1		0.1 MPa (8 d) 400 MPa (12 h)	1 unit / μmol	**1-H***)	50 35	≈100 ≈100
2		0.1 MPa (5 d) 400 MPa (7 h)	1 unit / μmol	**2-H**	46 100	— —
3		0.1 MPa (2 h) 400 MPa (20 min)	0.2 unit / μmol	**3-H** (1-*R*)	19 35	38 38
	 (BCA)	400 MPa (90 h)	1 unit / μmol	BCH (1-*R*)	34	>99
5		0.1 MPa (6 h) 400 MPa (20 min)	0.2 unit / μmol	**5-H** (1-*R*)	46 48	>99 >99
7		0.1 MPa (17 d) 400 MPa (20 h)	0.1 unit / μmol	**7-H**	53 59	77 49

*) H designates a hypoxanthine derivative

Table 2. Deamination of Carbocyclic 2,6-Diaminopurine Nucleosides by Adenosine Deaminase (ADA) under High-Pressure



	Reaction condition		Product	Yield (%)	Enantio excess (%)
R	Pressure / Reaction time	ADA			
4 	0.1 MPa (12 d) 400 MPa (25 h)	1 unit / μmol	**4-G***) (carbocyclic oxetanocin-G) (1-*S*)	49 37	27 27
6 	0.1 MPa (5 h) 400 MPa (5 h)	0.2 unit / μmol	**6-G** ((-)-carbovir) (1-*R*)	4 40	— >99
8 	0.1 MPa (22 d) 400 MPa (2 d)	0.2 unit / μmol	**8-G**	33 33	52 19

*) G designates a guanine derivative.

(±)-Carbocyclic oxetanocin A (**3**) itself was much faster deaminated to (-)-carbocyclic oxetanocin H (**3-H**) by ADA (0.2 unit/μmol) under high-pressure. Thus, the deamination of **3** under 0.1 MPa for 2 h gave **3-H** in only 19% yield whereas the reaction under 400 MPa gave **3-H** in 35% yield within 20 min. However, though the 1*R*-isomer of **3-H** was the major product, the enantioselectivity was low (38% *e.e.*).

The cyclopentenyladenine **5**, on treatment with ADA under atmospheric pressure for 6 h, was hydrolyzed to the cyclopentenylhypoxanthine **5-H** in 46% yield, whose *e.e.* was determined to be more than 99% by 500 MHz ^{1}H-NMR spectrum of its (+)-MTPA [(*R*)-(+)-2-methoxy-2-(trifluoromethyl)phenylacetic acid] ester. The reaction was accelerated by high-pressure (400 MPa) to give 48% yield of **5-H** having 99≥% *e.e.* in 20 min. The absolute structure of **5-H** should be 1*R* by analogy with that of carbovir (**6-G**) as described below.

Deamination of 9-[*c*-4-(hydroxymethyl)cyclohex-2-en-*r*-1-yl]-9*H*-adenine (**7**) with ADA under 0.1 MPa for 17 d gave **7-H** in 53% yield. The enantiomeric excess of the unreacted **7** was determined to be 77% by 500 MHz ^{1}H-NMR spectrum of its (-)-MTPA ester. The deamination under 400 MPa for 20 h gave **7-H** in 59% yield. In this case, the *e.e.*

of unreacted **7** was 49%. This means that though high pressure remarkably accelerates the deamination, it decreases the enantioselectivity.

Next, we examined the deamination of carbocyclic 2,6-diaminopurine nucleosides **4**, **6** and **8** under high-pressure. Carbocyclic oxetanocin G (**4-G**) is more interesting carbocyclic nucleoside than **3**, because the 1*R*-isomer of **4-G** has significant anti-viral activities such as anti-HIV and anti-HSV.[17] If we can use this deamination for the resolution of 1*R*-isomer, it would provide an efficient method for the preparation of 1*R*-isomer. Therefore, we examined the deamination of carbocyclic oxetanocin D (**4**) by ADA. The deamination of compound **4** proceeded in a much slower rate than that of the corresponding adenine derivative **3**. Thus, the deamination of **4** proceeded in 12 d at 0.1 MPa to give **4-G** in 49% yield while the reaction under high-pressure gave a 37% yield of **4-G** within 25 h. The **4-G** thus obtained showed negative optical rotation and hence corresponded to carbocyclic oxetanocin G having anti-viral activities. The *e.e.* was determined by comparison its optical rotation with that of the known compound, (1*R*,2*R*,3*S*)-9-[2,3-(bishydroxymethyl)cyclobut-1-yl]-9*H*-guanine.[17] However, in each reaction the enantioselectivity was low (27%).

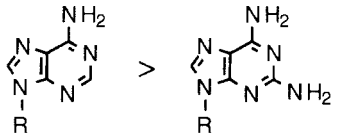
Next, we applied this high-pressure technique to the preparation of (-)-carbovir (**6-G**) having anti-HIV activity from racemic diamino-carbovir (**6**). The reaction proceeded within 5 h under 400 MPa to give (-)-carbovir with high enantiomeric excess (>99%) in 40% yield. The *e.e.* of (-)-carbovir was determined by ^{1}H-NMR spectrum analysis of its (-)-MPA [(*R*)-(-)-2-methoxyphenylacetic acid] ester. This method is superior to the deamination at atmospheric pressure, which requires an excess enzyme and a much longer reaction time.[7]

The diamino derivative **8** resisted most to the deamination, and 3 weeks were needed for its conversion of 33% to **8-G** under 0.1 MPa. The enantiomeric excess (52%) of **8-G** was determined by ^{1}H-NMR spectrum of its (+)-MTPA ester. The similar reaction under 400 MPa for 2 d gave **8-G** in 33% yield, whose *e.e.* was only 19%. The absolute structure of major product of **8-G** has not been determined as yet.

In conclusion, the deamination of all carbocyclic aminopurine nucleosides irrespective to their ring size of carbocycles was facilitated remarkably by high-pressure. However, their enantioselectivities were suppressed under high-pressure except for three and five membered carbocyclic nucleosides **1**, **5** and **6**. This means that **5** and **6** behave as the better substrates for ADA because their structures resemble more to normal nucleosides having pentose moiety than other carbocyclic nucleosides. However, it is not clear that why **1**, on the deamination, gives **1-H** with very high enantioselectivity.

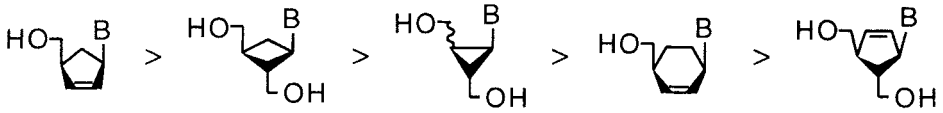
The deamination of carbocyclic nucleosides by ADA under high-pressure is summarized as follows:

1) The reaction rate of the adenine nucleosides is faster than that of the 2,6-diaminopurine derivatives.

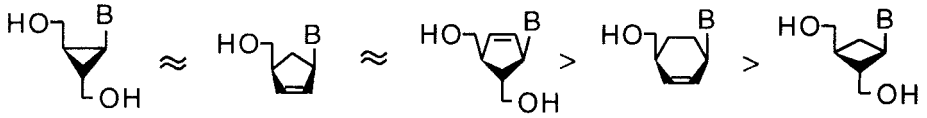


adenine 2,6-diaminopurine

The reaction rate decreases in order of:



2) The enantioselectivity decreases in order of:



Today, an enzyme has been extensively used for the preparation of chiral chemicals in the field of chemical industry. Although temperature is the most important factor in the enzymatic reaction, the present study has revealed that the pressure also plays a significant role.

EXPERIMENTAL

All melting points were determined on a micro-hot stage (Yanagimoto) and are not uncorrected. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer and proton nuclear magnetic resonance (^{1}H-NMR) spectra on a JEOL JNM-PMX 60 SI, Hitachi R-300, or JEOL JNM-FX 500 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were taken with a JEOL JMS-01SG-2 or JEOL JMS-DX 303 spectrometer. Column chromatography was performed on silica gel (Wakogel C-200) and TLC on Merck Kieselgel 60F_{254}. The ratios of mixtures of solvents for chromatography are shown as volume/volume. Adenosine deaminase type VI from calf intestinal mucosa was purchased from Sigma Chemical Co. Compounds **1**[9], **2**[9], and **3**[10]were synthesized as previously reported.

(±)-2-Amino-6-chloro-9-[*t*-2,*c*-3-(bisbenzoyloxymethyl)-cyclobut-*r*-1-yl]-9*H*-purine (10). To a solution of 2-amino-6-chloropurine (1.2 g, 7.08 mmol) in DMF (80 ml) was added anhydrous K_2CO_3 (0.5 g, 3.6 mmol). After the mixture was stirred at 110 °C for 30 min, *c*-2,*t*-3-bisbenzoyloxymethyl-*r*-1-cyclobutyl tosylate[13] (**9**) (2 g, 4.04

mmol) was added to the mixture. The further stirring was continued at 110 °C for 7 h. After removal of the solvent, the residue was dissolved in benzene (300 ml). The benzene layer was washed with water (150 ml x 2), dried over anhydrous $MgSO_4$, and condensed *in vacuo*. The residue was subjected to silica gel column chromatography (ϕ 2.8 x 30 cm). Elution with a linear gradient of ethyl acetate-benzene from 0~66% (1.6 l) gave **10** (525 mg, 26%) (mp 156~158 °C from benzene). Physical data for **10**: *Anal.* Calcd for $C_{25}H_{22}ClN_5O_4$: C, 61.04; H, 4.51; N, 14.24. Found: C, 60.99; H, 4.59; N, 14.41. UV: λ_{max} (MeOH) 311 nm, λ_{max} (0.05 *N* HCl) 315 nm. ^{1}H-NMR ($CDCl_3$) δ: 2.5-2.8 (3H, m, $C_{3'}$-, $C_{4'a}$-, $_{4'b}$-H), 3.40 (1H, m, $C_{2'}$-H), 4.5-4.7 (4H, m, 2'-CH_2-OBz, 3'-CH_2-OBz), 4.72 (1H, q, J = 8.8 Hz, $C_{1'}$-H), 5.06 (2H, br s, NH_2), 7.84 (1H, s, C_8-H), 7.22-8.15 (10H, m, 2'-OCOC_6H_5, 3'-OCOC_6H_5).

(±)-2,6-Diamino-9-[*t*-2,*c*-3-(bishydroxymethyl)-cyclobut-*r*-1-yl]-9*H*-purine (4). To a solution of **10** (450 mg, 0.916 mmol) in methanolic ammonia (8 ml) was heated in sealed tube at 100 °C for 1 day. After removal of the solvent, the residue was dissolved in water (30 ml). The solution was washed twice with chloroform (10 ml). The aqueous layer was condensed *in vacuo* to give a residue, to which methanol was added to give **4** (186 mg, 77%) (mp 211~213 °C from MeOH). Physical data for **4**: *Anal.* Calcd for $C_{11}H_{16}N_6O_2 \cdot 0.5H_2O$: C, 48.34; H, 6.27; N, 30.75. Found: C, 48.43; H, 6.08; N, 30.92. UV: λ_{max} (MeOH) 282, 257 nm, λ_{max} (0.05 *N* HCl) 295, 256 nm, λ_{max} (0.05 *N* NaOH) 282 nm. ^{1}H-NMR ($CDCl_3$) δ: 2.04 (1H, m, $C_{3'}$-H), 2.11 (1H, q, J = 9.8 Hz, $C_{4'b}$-H), 2.37 (1H, dt, J = 10.5, 7.9 Hz, $C_{4'a}$-H), 2.67 (1H, tt, J = 8.6, 5.5 Hz, $C_{2'}$-H), 3.3-3.5 (4H, m, 2'-CH_2OH, 3'-CH_2OH), 4.42 (1H, q, J = 8.6 Hz, $C_{1'}$-H), 4.61 (1H, br s, OH), 4.83 (1H, br s, OH), 5.75 (2H, br s, 2-NH_2), 6.69 (2H, br s, 6-NH_2), 7.84 (1H, s, C_8-H).

(±)-2-*tert*-Butoxycarbonyl-2-azabicyclo[2.2.1]hept-5-en-3-one (12). To a solution of 2-azabicyclo[2.2.1]hept-5-en-3-one (**11**)[18] (5.46 g, 50 mmol) in CH_2Cl_2 (100 ml) were added successively triethylamine (7 ml, 50 ml), di-*tert*-butyl dicarbonate (21.83 g, 100 mmol), and 4-(dimethylamino)pyridine (6.11 g, 50 mmol). The mixture was stirred for 24 h at room temperature. After removal of the solvent *in vacuo*, to the residue were added water (30 ml) and ether (30 ml). The organic layer was dried over anhydrous $MgSO_4$, and condensed *in vacuo* to give a oily substance, which was subjected to flash chromatography. Elution with hexane-AcOEt (5:1) gave **12** (10.4 g, 99%) as colorless prisms (mp 55~57 °C from pentane). Physical data for **12**: *Anal.* Calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 6.74; N, 6.69. Found: C, 63.38; H, 6.70; N, 6.55. IR ($CHCl_3$): 3025, 2995, 1790, 1755, 1705 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.51 (9H, s, *t*-butyl), 2.14 and 2.35 (each 1H, AB type, J = 8 Hz, C_7-H_2), 3.39 (1H, br s, C_4-H), 4.96 (1H, m, C_1-H), 6.66 (1H, dm, J = 5.5 Hz, C_5-H), 6.89 (1H, dd, J = 5.5, 2 Hz, C_6-H).

(±)-*cis*-(4-*tert*-Butoxycarbonylaminocyclopent-2-en-1-yl)carbinol (13). To a solution of **12** (1.05 g, 5 mmol) in MeOH (10 ml) added $NaBH_4$ (567 mg, 15 mmol) with stirring under ice cooling. The stirring was continued for 30 min, and then at room temperature for 1 h. The mixture was neutralized with 10% aq. HCl, and then condensed *in vacuo*. The residue was subjected to flash chromatography. Elution with hexane-AcOEt (1:1) gave **13** (847 mg, 79%) as colorless prisms (mp 54~56 °C from hexane-AcOEt). Physical data for **13**: High-resolution MS *m/z* Calcd for $C_{11}H_{19}NO_3$ (M^+): 213.1363. Found: 213.1320. IR ($CHCl_3$): 3420, 1705 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.40 (1H, m, C_5-H), 1.44 (9H, s, *t*-butyl), 2.50 (1H, ddd, J = 17, 9, 8 Hz, C_5-H), 2.84 (1H, m, C_1-H), 3.57 (1H, dd, J = 10, 4 Hz, C*H*HOH), 3.67 (1H, dd, J = 10, 4 Hz, CH*H*OH), 4.70 (1H, m, C_4-H), 4.86 (1H, br s, NH), 5.77 (1H, m, C_3-H), 5.83 (1H, m, C_2-H).

(±)-*cis*-4-(Hydroxymethyl)cyclopent-2-en-1-ylamine (14). To trifluoroacetic acid (2 ml) was added **13** (426 mg, 2 mmol) under ice-cooling. The mixture was stirred at room temperature for 1 h, and then condensed *in vacuo*. The residue was used without further purification for the preparation of **5**[12] and **6**[7].

(±)-2-Azabicyclo[2.2.2]oct-5-en-3-one (15). A mixture of 1,3-cyclohexadiene (4.3 g, 59.4 mmol) and benzenesulfonyl cyanide[18] (11.9 g, 71.3 mmol) was stirred under argon atmosphere at room temperature for 48 h. To the mixture were added silica gel (4 g) and $CHCl_3$ (40 ml). After being stirred for 6 h, silica gel was filtered off by suction. The filtrate was condensed *in vacuo*, and the residue was subjected to silica gel (120 g) column chromatography. Elution with AcOEt-hexane (2:3) gave **15** (3.2 g, 43%) as colorless powder (mp 125~127 °C from Et_2O) (lit.[16] mp 124-125.5 °C). Physical data for **15**: High-resolution MS *m/z* Calcd for C_7H_9NO (M^+): 123.0684. Found: 123.0676. IR ($CHCl_3$): 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.47 (2H, br s, 7-C*H*$_2$), 1.74-1.98 (2H, m, 8-C*H*$_2$), 3.35 (1H, s, C_4-H), 4.28 (1H, s, C_1-H), 6.36 (1H, dd, J = 9, 6 Hz, C_5-H), 6.42 (1H, dd, J = 9, 6 Hz, C_6-H), 8.07 (1H, br s, NH).

(±)-2-(*N*-*tert*-Butoxycarbonyl)-2-azabicyclo[2.2.2]oct-5-en-3-one (16). To a solution of **15** (2.5 g, 16 mmol) in dry CH_2Cl_2 (40 ml) were added successively di-*tert*-butyl dicarbonate (7.0 g, 32 mmol), triethylamine (2.24 ml) and DMAP (2.0 g, 16 mmol) with stirring under argon atmosphere. After being stirred for 7 h, the mixture was dissolved in AcOEt. The solution was washed with water, and the organic layer was dried over anhydrous $MgSO_4$ and condensed *in vacuo*. The residue was subjected to silica gel (60 g) column chromatography. Elution with AcOEt-hexane (1:6) gave **16** (3.5 g, 97%) as colorless needles (mp 101~103 °C from Et_2O). Physical data for **16**: High-resolution MS *m/z* Calcd for $C_{12}H_{17}NO_3$ (M^+): 223.1207. Found: 223.1245. IR ($CHCl_3$): 1720, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.53 (9H, s, *t*-butyl), 1.61 (2H, br s, 8-C*H*$_2$), 1.86-2.06

(2H, m, 7-CH_2), 3.46 (1H, dd, J = 4, 2 Hz, C_4-H), 5.27 (1H, br s, C_1-H), 6.37-6.48 (2H, m, C_5-, C_6-H).

(±)-*cis*-(4-*tert*-Butoxycarbonylaminocyclohex-2-en-1-yl)carbinol (17). To a solution of **16** (300 mg, 1.3 mmol) in absolute MeOH (4 ml) was added portionwise $NaBH_4$ (235 mg, 6.5 mmol) with stirring under ice-cooling. After being stirred for 5 h at room temperature, the mixture was dissolved in AcOEt (10 ml). The solution was washed with water, dried over anhydrous $MgSO_4$, and condensed *in vacuo*. The residue was subjected to silica gel (15 g) column chromatography. Elution with AcOEt-hexane (1:1) gave **17** (297 mg, 98%) as a colorless oil. Physical data for **17**: High-resolution MS *m/z* Calcd for $C_{12}H_{21}NO_3$ (M^++1): 228.1598. Found: 228.1613. IR ($CHCl_3$): 3500, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.45 (9H, s, *tert*-butyl), 1.55-1.82 (4H, m, C_5-, C_6-H_2), 2.26 (1H, m, C_1-H), 3.57 (2H, q, J = 10 Hz, CH_2OH), 4.14 (2H, br s, NH and OH), 4.62 (1H, br s, C_4-H), 5.78 (2H, s, C_2-, C_3-H).

(±)-*cis*-4-(Hydroxymethyl)cyclohex-2-en-1-ylamine (18). The alcohol **17** (238 mg, 1.05 mmol) was added to trifluoroacetic acid (2 ml) under ice-cooling. The mixture was stirred at room temperature for 1 h, and then condensed *in vacuo*. The residue was used without further purification for the preparation of **19** or **21**.

(±)-5-Amino-4-chloro-6-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl) aminopyrimidine (19). To a solution of **18** (2.0 g, 15.7 mmol) in 1-butanol (80 ml) were added 5-amino-4,6-dichloropyrimidine (3.0 g, 18.3 mmol) and *N*,*N*-diisopropylethylamine (27 ml). The mixture was heated under reflux for 31.5 h. After evaporation of the solvent, the residue was subjected to flash chromatography on silica gel. Elution with hexane-AcOEt (1:2) gave **19** (2.0 g, 50%) as an yellow oil. Physical data for **19**: High-resolution MS *m/z* Calcd for $C_{11}H_{15}ClN_4O$ (M^+), $C_{11}H_{15}Cl^*N_4O$ (M^++2): 254.0934, 256.0907. Found: 254.0951, 256.0920. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.71-1.91 (4H, m, $C_{5'}$-, $C_{6'}$-H_2), 2.28-2.39 (1H, m, $C_{4'}$-H), 3.46 (2H, br s, NH and OH), 3.60-3.67 (1H, m, $C_{1'}$-H), 4.12 (2H, dd, J = 14.3, 7.0 Hz, CH_2OH), 4.66-4.74 (1H, m, $C_{3'}$-H), 4.98 (1H, d, J = 7.3 Hz, $C_{2'}$-H), 5.88 (2H, s, NH_2), 8.07 (1H, s, C_2-H).

(±)-9-(4'ß-Hydroxymethylcyclohex-2'-en-1'ß-yl)-6-chloropurine (20). To a suspension of **19** (2.0 g, 7.9 mmol) in triethyl orthoformate (42 ml) was added dropwise 12 *N* HCl (2.3 ml) with stirring under ice-cooling. After being stirred for 13 h at room temperature, the solution was condensed *in vacuo*. The residue was dissolved in THF (45 ml). To the solution was added 0.5 *N* HCl (45 ml) with stirring under ice-cooling. After being stirred at room temperature for 3.5 h, the solution was neutralized with 1 *N* NaOH. The mixture was condensed *in vacuo*, and the residue was subjected to flash chromatography on silica gel. Elution with hexane-AcOEt (1:1) gave **20** (2.0 g, 95%) as colorless needles (mp 149~150 °C from AcOEt). Physical data for **20**: High-resolution MS *m/z* Calcd for

$C_{12}H_{13}ClN_4O$ (M^+), $C_{12}H_{13}Cl^*N_4O$ (M^++2): 264.0778, 266.0752. Found: 264.0781, 266.0734. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.77-1.88 (2H, m, $C_{5'}$-H_2), 2.10-2.17 (2H, m, $C_{6'}$-H_2), 2.44-2.55 (1H, m, $C_{4'}$-H), 3.69-3.85 (2H, m, CH_2OH), 3.29-3.36 (1H, m, $C_{1'}$-H), 5.97 (1H, ddd, J = 9.9, 4.4, 2.2 Hz, $C_{3'}$-H), 6.30 (1H, d, J = 9.9 Hz, $C_{2'}$-H), 8.28, 8.75 (2H, each s, purine-H).

(±)-9-(4'ß-Hydroxymethylcyclohex-2'-en-1'ß-yl)-9*H*-adenine (7). NH_3 gas was passed in a solution of **20** (439 mg, 1.79 mmol) in absolute MeOH (25 ml) under ice-salt cooling. The solution was heated in a sealed tube at 83 °C for 18 h. After evaporation of excess NH_3 and the solvent, the residue was subjected to flash chromatography on silica gel. Elution with AcOEt-MeOH (8:1) gave **7** (324 mg, 74%) as colorless needles (mp 201~203 °C from AcOEt-MeOH). Physical data for **7**: High-resolution MS *m/z* Calcd for $C_{12}H_{15}N_5O$ (M^+): 245.1277. Found: 245.1287. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.66-1.70 (2H, m, $C_{5'}$-H_2), 1.92-1.96 (2H, m, $C_{6'}$-H_2), 2.25-2.31 (1H, m, $C_{4'}$-H), 3.17 (2H, d, J = 5.5 Hz, CH_2OH), 4.76 (1H, t, J = 5.5 Hz, OH), 5.08-5.13 (1H, m, $C_{1'}$-H), 5.87 (1H, ddd, J = 10.0, 4.3, 2.8 Hz, $C_{3'}$-H), 6.12 (1H, d, J = 10.0 Hz, $C_{2'}$-H), 8.00, 8.15 (2H, each s, purine-H).

(±)-2-Amino-4-chloro-6-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl)-aminopyrimidine (21). A mixture of **18** (1.8 g, 14.1 mmol), 2-amino-4,6-dichloropyrimidine (2.0 g, 12.2 mmol), and triethylamine (2.24 g, 24.4 mmol) in EtOH (20 ml) was heated in a sealed tube at 100 °C for 40 h. After evaporation of the solvent, the residue was subjected to silica gel (60 g) column chromatography. Elution with AcOEt-hexane (2:1) gave **21** (1.23 g, 40%) as a colorless oil. Physical data for **21**: High-resolution MS *m/z* Calcd for $C_{11}H_{15}ClN_4O$ (M^+), $C_{11}H_{15}Cl^*N_4O$ (M^++2): 254.0933, 256.0904. Found: 254.0926, 256.0926. IR ($CHCl_3$): 3650, 3540, 3450, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 1.10-2.41 (5H, m, $C_{4'}$-H, $C_{5'}$-, $C_{6'}$-H_2), 3.53 (2H, d, J = 6 Hz, CH_2OH), 4.21-4.45 (1H, m, $C_{1'}$-H), 5.31-5.64 (2H, m, $C_{2'}$-, $C_{3'}$-H), 5.73 (1H, s, C_5-H).

(±)-2-Amino-4-chloro-5-[(4-chlorophenyl)azo]-6-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl)aminopyrimidine (22). To a mixture of **21** (125 mg, 0.5 mmol), AcONa (1.0 g), AcOH (2.6 ml), H_2O (2.6 ml), and MeOH (1.5 ml) was added an aqueous solution of 4-chlorobenzene diazonium hydrochloride [prepared by the reaction of *p*-chloroaniline (77 mg, 0.6 mmol)-3 *N* HCl (1.3 ml) with $NaNO_2$ (45 mg, 0.65 mmol)-H_2O (0.6 ml)] with stirring under ice-cooling. After being stirred for 6 h at room temperature, the mixture was condensed *in vacuo*. To the residue was added H_2O to give a crystalline substance (**22**), which was collected by suction, dried under reduced pressure, and used without further purification for the preparation of **23**.

(±)-2,5-Diamino-4-chloro-6-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl)aminopyrimidine (23). To a solution of **22** (0.5 mmol) in EtOH-H_2O (1:1, 15 ml)

and AcOH (0.16 ml) was added Zn powder (325 mg, 5 mmol). After being stirred for 3 h, the mixture was filtered, and the filtrate was condensed *in vacuo* to give a residue, which was subjected to silica gel (20 g) column chromatography. Elution with AcOEt-hexane (3:1) gave **23** (88 mg, 65%) as colorless amorphism (mp 73~78 °C from AcOEt). Physical data for **23**: High-resolution MS *m/z* Calcd for $C_{11}H_{16}ClN_5O$ (M^+), $C_{11}H_{16}Cl^*N_5O$ (M^++2): 269.1042, 271.1013. Found: 269.1040, 271.1024. IR (Nujol): 3680, 3400 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 1.26-1.92 (6H, m, C*H*$_2$OH, $C_{5'}$-, $C_{6'}$-H_2), 2.00-2.43 (1H, m, $C_{4'}$-H), 4.33-4.50 (1H, m, $C_{1'}$-H), 5.79 (2H, s, $C_{2'}$-, $C_{3'}$-H).

(±)-2-Amino-6-chloro-9-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl)-9*H*-purine (24). To a suspension of **23** (88 mg, 0.33 mmol) in triethyl orthoformate (1.9 ml) was added portionwise 12 *N* HCl (0.1 ml) with stirring at 0 °C. After being stirred for 13 h at room temperature, the mixture was condensed *in vacuo* to give a residue, which was again dissolved in THF (3 ml). To the solution was added 0.5 *N* HCl (2.4 ml) with stirring under ice-cooling. After being stirred at room temperature for 6 h, the solution was adjusted to pH 8.0 with 1 *N* NaOH. The mixture was condensed *in vacuo* to give a residue, which was subjected to silica gel (20 g) column chromatography. Elution with AcOEt-MeOH (100:1) gave **24** (56 mg, 61%) as colorless prisms (mp 156~158 °C from MeOH-AcOEt). Physical data for **24**: High-resolution MS *m/z* Calcd for $C_{12}H_{14}ClN_5O$ (M^+), $C_{12}H_{14}Cl^*N_5O$ (M^++2): 279.0886, 281.0856. Found: 279.0865, 281.0852. IR (Nujol): 3400 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 60 MHz) δ: 1.30-2.69 (5H, m, $C_{4'}$-H, $C_{5'}$-, $C_{6'}$-H_2), 3.68 (2H, d, J = 7 Hz, C*H*$_2$OH), 4.83-5.35 (1H, m, $C_{1'}$-H), 5.68-6.43 (2H, m, $C_{2'}$-, $C_{3'}$-H), 8.00 (1H, s, purine-H).

(±)-2,6-Diamino-9-(4'ß-hydroxymethylcyclohex-2'-en-1'ß-yl)-9*H*-purine (8). NH_3 gas was introduced into a solution of **24** (274 mg, 0.98 mmol) in absolute MeOH (15 ml) under ice-salt cooling. The solution was warmed in sealed tube at 80 °C for 25 h. After evaporation of excess NH_3 and the solvent, the residue was subjected to flash chromatography on silica gel. Elution with AcOEt-MeOH (8:1) gave **8** (133 mg, 52%) as colorless amorphism (mp 235~236 °C from MeOH-AcOEt). Physical data for **8**: High-resolution MS *m/z* Calcd for $C_{12}H_{16}N_6O$ (M^+): 260.1385. Found: 260.1398. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.60-1.68 (2H, m, $C_{5'}$-H_2), 1.82-1.91 (2H, m, $C_{6'}$-H_2), 2.21-2.27 (1H, m, $C_{4'}$-H), 2.63 (1H, s, OH), 4.56 (1H, t, J = 6.0 Hz, C*H*HOH), 4.70 (1H, t, J = 6.0 Hz, CH*H*OH), 4.85-4.89 (1H, m, $C_{1'}$-H), 5.76 (2H, s, NH_2), 5.83 (1H, ddd, J = 10.0, 4.5, 2.5 Hz, $C_{3'}$-H), 6.08 (1H, d, J = 10.0 Hz, $C_{2'}$-H), 6.62 (2H, s, NH_2), 7.56 (1H, s, purine-H).

General Procedure for the Deamination of 9-(Hydroxymethylated Cycloalkyl)-9*H*-adenines (1~8) by Adenosine Deaminase (ADA) under High-Pressure. High-pressure reactions were carried out by using a piston-cylinder apparatus

equipped with a K. P. 15. B pump (Hikari Kouatsu Kiki Ltd., Co., Japan). A solution of cycloalkyladenines (**1**~**8**, 20 μM) and ADA (0.1~1 unit/μM) in phosphate buffer (pH 7.0, 4.7 ml) was placed in a Teflon tube (4.7 ml) with a Teflon stopper. The tube was placed in a high-pressure reactor and pressurized to 400 MPa at 22 °C. The pressure was released and the reaction mixture was monitored by high-performance liquid chromatography (HPLC) (Waters; column, μPoracil C_{18}; solvent, dioxane:water=1:7; UV 254 nm) to estimate the conversion yields of hypoxanthine (**1-H**~**3-H**, **5-H**, and **7-H**) and guanine derivatives (**4-G**, **6-G**, and **8-G**). The reaction mixture was then concentrated under reduced pressure to give a residue, which was submitted to silica gel column chromatography. Elution with $CHCl_3$-MeOH (5:1) gave hypoxanthine (**3-H**, **5-H**, and **7H**) or guanine derivatives (**4-G**, **6-G**, and **8-G**) which were immediately converted to the MTPA esters in order to determine the enantiomeric excess (*e.e.*). Hypoxanthine derivatives (**1-H** and **2-H**) were not isolated and the *e.e.* of **1**-H was determined by HPLC on CHIRALCEL OD using hexane-EtOH (1:9) as an eluent.

9-[*t*-2,*c*-3-(Bishydroxymethyl)cyclobut-*r*-1-yl]-1*H*,9*H*-hypoxanthine (*R*)-(+)-2-Methoxy-2-(trifluoromethyl)phenylacetic Acid Ester [3-H (+)-MTPA Ester]. The (+)-MTPA ester of **3-H** was obtained in a usual manner, from the reaction of **3-H** [$[\alpha]_D^{22}$ -3.66 ° (*c* = 0.6, MeOH)] (3.5 mg, 13.9 μmol) with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (9.9 mg, 42.3 μmol) in the presence of 4-dimethylaminopyridine (DMAP) (2.8 mg, 23.3 μmol) and *N,N'*-dicyclohexylcarbodiimide (DCC) (8.7 mg, 42.3 μmol) in CH_2Cl_2 (1.5 ml) at room temperature for 3 h. The ester was isolated by preparative TLC using $CHCl_3$-MeOH (10:1) as a developing solvent. Major product: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (*interalia*): 2.94 (1H, m, C_3'-H), 3.46 (3H, s, OMe), 3.54 (3H, s, OMe), 4.30 (1H, AB type dd, *J* = 11.4, 5.3 Hz, C*H*HOMTPA), 4.34 (2H, d, *J* = 4.5 Hz, C*H*$_2$OMTPA), 4.49 (1H, AB type dd, *J* = 11.4, 4.5 Hz, CH*H*OMTPA), 4.68 (1H, q, *J* = 8.3 Hz, C_1'-H), 7.56 and 8.23 (each 1H, s, purine-H x 2).

Minor product: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ (*interalia*): 3.05 (1H, m, C_3'-H), 3.46 (3H, s, OMe), 3.52 (3H, s, OMe), 4.26 (1H, AB type dd, *J* = 11.4, 5.3 Hz, C*H*HOMTPA), 4.34 (2H, d, *J* = 4.5 Hz, C*H*$_2$OMTPA), 4.44 (1H, AB type dd, *J* = 11.4, 4.5 Hz, CH*H*OMTPA), 4.58 (1H, q, *J* = 8.3 Hz, C_1'-H), 7.56 and 8.23 (each 1H, s, purine-H x 2).

9-[*c*-4-(hydroxymethyl)cyclopent-2-en-*r*-yl]-1*H*,9*H*-hypoxanthine (*R*)-(+)-2-Methoxy-2-(trifluoromethyl)phenylacetic Acid Ester [5-H (+)-MTPA Ester]. The (+)-MTPA ester of **5-H** was obtained, from the reaction of **5-H** (2.2 mg, 9.5 μmol) with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (3.5 mg, 14.9 μmol) in the presence of DMAP (1.1 mg, 9.0 μmol) and DCC (3.0 mg, 14.5 μmol) in CH_2Cl_2 (0.5 ml) at room temperature for 4.5 h. The ester was isolated by

preparative TLC using ethyl acetate-MeOH (10:1). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.25-1.28 (1H, m, $C_{5'}$β-H), 2.88 (1H, dt, *J* = 13.8, 8.0 Hz, $C_{5'}$α-H), 3.21-3.28 (1H, m, $C_{4'}$-H), 3.53 (3H, d, *J* = 1.0 Hz, OMe), 4.25 (1H, AB type dd, *J* = 10.8, 6.3 Hz, C*H*HOMTPA), 4.55 (1H, AB type dd, *J* = 10.8, 6.5 Hz, C*H*HOMTPA), 5.68 (1H, tt, *J* = 6.8, 2.5 Hz, $C_{1'}$-H), 5.87 (1H, dt, *J* = 5.8, 2.5 Hz, $C_{3'}$-H), 6.04 (1H, dt, *J* = 5.8, 2.5 Hz, $C_{2'}$-H), 7.40-7.43 (3H, m, Ar-H x 3), 7.51-7.55 (2H, m, Ar-H x 2), 7.65 and 8.08 (each 1H, s, purine-H x 2).

9-[*c*-4-(Hydroxymethyl)cyclohex-2-en-*r*-1-yl]-1*H*,9*H*-hypoxanthine (*S*)-(-)-2-Methoxy-2-(trifluoromethyl)phenylacetic Acid Ester [7-H (-)-MTPA Ester]. The (-)-MTPA ester of **7-H** was obtained from the reaction of **7-H** (4.0 mg, 16.3 μmol) with (*S*)-(-)-α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA) (5.7 mg, 24.4 μmol) in the presence of DMAP (1.8 mg, 14.7 μmol) and DCC (5.0 mg, 24.4 μmol) at room temperature for 2 h. The ester was purified by flash chromatography using ethyl acetate-MeOH (20:1) as an eluent. Major product: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.66-1.89 (2H, m, $C_{5'}$-H_2), 1.91-2.18 (2H, m, $C_{6'}$-H_2), 2.62-2.76 (1H, m, $C_{4'}$-H), 3.54 (3H, s, OMe), 4.35 (1H, AB type dd, *J*= 10.8, 5.1 Hz, C*H*HOMTPA), 4.44 (1H, AB type dd, *J* = 10.8, 5.9 Hz, C*H*HOMTPA), 5.22-5.31 (1H, m, $C_{1'}$-H), 5.95 (1H, dm, *J* = 9.9 Hz, $C_{3'}$-H), 6.18 (1H, d, *J* = 9.9 Hz, $C_{2'}$-H), 7.38-7.45 (3H, m, Ar-H x 3), 7.50-7.57 (2H, m, Ar-H x 2), 7.97 and 8.32 (each 1H, s, purine-H x 2). Minor product: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 1.66-1.89 (2H, m, $C_{5'}$-H_2), 1.91-2.18 (2H, m, $C_{6'}$-H_2), 2.62-2.76 (1H, m, $C_{4'}$-H), 3.56 (3H, d, *J* = 0.7 Hz, OMe), 4.27 (1H, AB type dd, *J* = 10.8, 4.5 Hz, C*H*HOMTPA), 4.53 (1H, AB type dd, *J* = 10.8, 5.5 Hz, C*H*HOMTPA), 5.22-5.31 (1H, m, $C_{1'}$-H), 5.85 (1H, m, $C_{3'}$-H), 6.09 (1H, dm, *J* = 9.9 Hz, $C_{2'}$-H), 7.38-7.45 (3H, m, Ar-H x 3), 7.50-7.57 (2H, m, Ar-H x 2), 7.86 and 8.32 (each 1H, s, purine-H x 2).

9-[*t*-2,*c*-3-(Bishydroxymethyl)cyclobut-*r*-1-yl]-9*H*-guanine (4-G): mp 247~248 °C (from MeOH), colorless needles, $[\alpha]_D^{26}$ -7.6 ° (*c* = 1.0, DMSO) [lit.[17] mp >270 °C, $[\alpha]_D^{26}$ -27.6 ° (*c* = 1.01, DMSO)]

(1*R*,5*S*)-9-[4-(Hydroxymethyl)cyclopent-2-en-1-yl]-9*H*-guanine [6-G: (-)-carbovir]: colorless amorphism (mp 269~271 °C from H_2O), $[\alpha]_D^{22}$ -67.3 ° (*c* = 0.24, MeOH) [lit.[7] mp 268~270 °C, $[\alpha]_D^{23}$ -62.1 ° (*c* = 0.3, MeOH)].

(1*R*,5*S*)-9-[4-(Hydroxymethyl)cyclopent-2-en-1-yl]-9*H*-guanine (*R*)-(-)-2-Methoxyphenylacetic Acid Ester [6-G (-)-MPA Ester]. The (-)-MPA ester of **6-G** [(-)-carbovir] was obtained from the reaction of **6-G** (15 mg, 0.06 mmol) with (*R*)-(-)-α-methoxyphenylacetic acid (MPA) (30 mg, 0.18 mmol) in the presence of DCC (37 mg, 0.18 mmol) and DMAP (6.3 mg, 0.052 mmol) in dry CH_2Cl_2 (2 ml) for 12 h at room temperature. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 1.20-1.56 (2H, m, $C_{5'}$-H_2), 2.58 (1H, dt, J = 7.5, 5.0 Hz, $C_{4'}$-H), 3.33 (3H, s, OCH_3), 4.10 (1H, dd, *J* = 14.2, 7.5 Hz, C*H*H'-O),

4.19 (1H, dd, J = 14.2, 7.5 Hz, C*H*H'-O), 4.87 (1H, s, -C*H* (OMe) Ph), 5.35 (1H, m, $C_{1'}$-H), 5.88-5.96 (2H, m, $C_{2'}$-, $C_{3'}$-H), 7.30-7.42 (5H, m, Ph), 7.56 (1H, s, C_8-H).

9-[*c*-4-(Hydroxymethyl)cyclohex-2-en-*r*-1-yl]-9*H*-guanine (*R*)-(+)-2-Methoxy-2-(trifluoromethyl)phenylacetic Acid Ester [8-G (+)-MTPA Ester]. The (+)-MTPA-ester of **8-G** was obtained from the reaction of **8-G** (1.2 mg, 4.8 μmol) with (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) (1.7 mg, 7.2 μmol) in the presence of DMAP (0.5 mg, 4.4 μmol) and DCC (1.5 mg, 7.2 μmol) in CH_2Cl_2 (2.5 ml) at room temperature for 13 h. The ester was purified by preparative TLC using ethyl acetate-MeOH (5:1) as a developing solvent. Major product: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.74-1.82 (2H, m, $C_{5'}$-H_2), 1.97-2.06 (2H, m, $C_{6'}$-H_2), 2.59-2.66 (1H, m, $C_{4'}$-H), 3.57 (3H, s, OMe), 4.26 (1H, AB type dd, J = 10.8, 5.9 Hz, C*H*HOMTPA), 4.43 (1H, AB type dd, J = 10.8, 6.6 Hz, C*H*HOMTPA), 4.78 (2H, s, NH_2), 4.96-5.03 (1H, m, $C_{1'}$-H), 5.38 (2H, s, NH_2), 5.85 (1H, ddd, J = 10.5, 4.0, 2.5 Hz, $C_{3'}$-H), 5.97 (1H, d, J = 10.5 Hz, $C_{2'}$-H), 7.40-7.43 (3H, m, Ar-H x 3), 7.44 (1H, s, purine-H), 7.52-7.56 (2H, m, Ar-H x 2). Minor product: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.74-1.82 (2H, m, $C_{5'}$-H_2), 1.97-2.06 (2H, m, $C_{6'}$-H_2), 2.59-2.66 (1H, m, $C_{4'}$-H), 3.56 (3H, s, OMe), 4.34 (2H, d, J = 6.2 Hz, CH_2OMTPA), 4.78 (2H, s, NH_2), 4.96-5.03 (1H, m, $C_{1'}$-H), 5.38 (2H, s, NH_2), 5.91 (1H, ddd, J = 9.8, 3.0, 1.5 Hz, $C_{3'}$-H), 6.02 (1H, d, J = 9.8 Hz, $C_{2'}$-H), 7.40-7.43 (3H, m, Ar-H x 3), 7.49 (1H, s, purine-H), 7.52-7.56 (2H, m, Ar-H x 2).

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