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Synthesis of (2'S,3'S)-9-(4'-Phosphono-4',4'-difluoro-2',3'-methanobutyl)guanine and its Enantiomer. Evaluation of the Inhibitory Activity for Purine Nucleoside Phosphorylase

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Abstract: Conformationally constrained analogues **2a** and *ent*-**2a** of 9-(difluorophosphonopentyl)guanines **1**, a multi-substrate analogue inhibitor of PNP, were prepared from optically active *trans*-1-(diethoxyphosphinyl)difluromethy-2-hydroxymethylcyclopropanes (+)-**6** and (-)-**6**. Enzymatic double resolution was applied to obtain (+)-**6** and (-)-**6** with high enantiomeric purity. Inhibitory activity of **2a** and *ent*-**2a** were found to be 2400-fold less potent than **1**.
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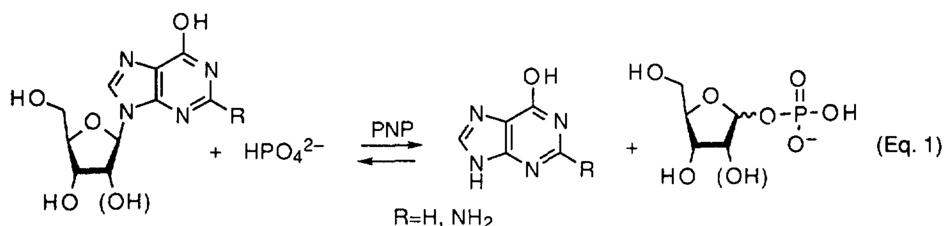
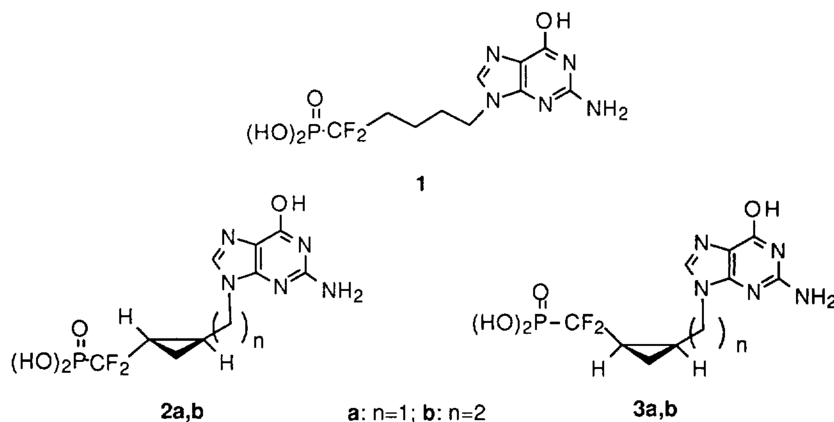
INTRODUCTION

Purine nucleoside phosphorylase (PNP: EC. 2.4.2.1) catalyzes the reversible phosphorolysis of nucleosides such as inosine, 2'-deoxyinosine, guanosine and 2'-deoxyguanosine to their respective free base and ribose- or 2-deoxyribose-α-1-phosphate (Eq. 1).[1] Individuals who are genetically deficient of PNP suffer from impairment of the T-cell component of their immune system but have normal B-cell function.[2] These observations led to the proposal that a PNP inhibitor should be useful as an immunosuppressive agent as well as in the treatment of T-cell proliferative disease such as T-cell leukaemia.[3] In addition, PNP inhibitors may protect purine nucleosides used as chemotheraputic agents such as 2',3'-dideoxyinosine(ddI) against PNP metabolism.[4] Consequently, extensive drug-discovery research has been devoted to the design and synthesis of inhibitors of PNP during the last ten years.



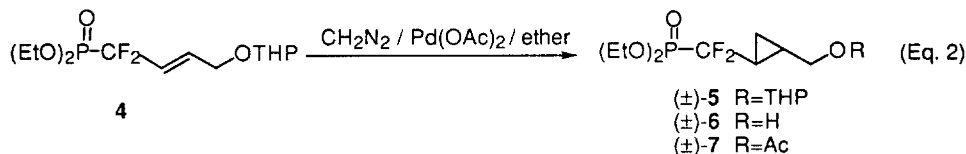
The reversible phosphorylation of the purine nucleosides catalyzed by PNP proceeds *via* a ternary complex of enzyme, nucleoside, and orthophosphate.[5a] Based on this fact, a number of metabolically stable acyclic nucleotide analogues containing a purine and a phosphate-like moiety have been designed and synthesized as "multi-substrate" analogue inhibitors for PNP.[5] Despite the large number of PNP inhibitors that have been designed and synthesized to date, no compound has yet reached the stage of clinical trial. Among the existing PNP inhibitors, 9-(5',5'-difluoro-5'-phosphonopentyl)guanine **1** developed by Halazy *et al.*,[6] is one of the most potent and simple PNP inhibitors. We have been interested in synthesizing conformationally constrained molecules of **1**, such as **2a,b**, **3a,b** and their enantiomers. These molecules having asymmetric carbons would be useful probes to elucidate the active conformation of **1** for developing a PNP inhibitor with significant activities. To the best of our knowledge, no compounds having asymmetric carbons such as **2** and **3** have yet been synthesized for PNP inhibitors. In this paper, we describe enantiodivergent synthesis of **2a** and *ent*-**2a**, in addition to evaluation of their inhibitory activity towards PNP.



RESULTS AND DISCUSSION

Enzyme-catalyzed kinetic resolution of alcohol (±)-6.

The synthesis of **2a** and its enantiomer *ent*-**2a** began with α,α-difluroallylphosphonate **4**, a facile synthetic method of which has been recently developed by us.[7] Cyclopropanation of **4** with excess CH_2N_2 in ether in the presence of $Pd(OAc)_2$[8] gave (±)-**5** in 89% yield. Deprotection of (±)-**5** with *p*-TsOH in MeOH afforded racemic *trans*-1-(diethoxyphosphinyl)difluoromethyl-2-hydroxymethycyclopropane (±)-**6** in 79% yield (Eq. 2).



Lipase-catalyzed transesterification with vinyl acetate was attempted to resolve (±)-**6** under a variety of conditions using several kinds of lipase (Eq. 3).[9,10] The representative results of these reactions are summarized in Table 1. Upon treatment of (±)-**6** with vinyl acetate in THF in the presence of lipase PS (*Pseudomonas cepacia*)[11a] at room temperature, rapid transesterification occurred to give (±)-**7** without enantioselectivity (entry 1). Upon reducing the temperature to 0 °C, a very low enantiodiscrimination (E = 1.31) was observed (entry 2). The virtually same results were obtained with the use of lipase AK (*Pseudomonas fluorescens*)[11b] (entry 3). Modest enantioselectivity (E = 12.3) was observed when the transesterification was carried out at 37 °C in the presence of *Porcine Pancreas* lipase (PPL). Upon terminating the reaction at conversion of 54.8%, (-)-**6** with 81.2% *ee* and (+)-**7** with 67% *ee* were obtained in 45.6 and 52.1% isolated yield, respectively (entry 4).

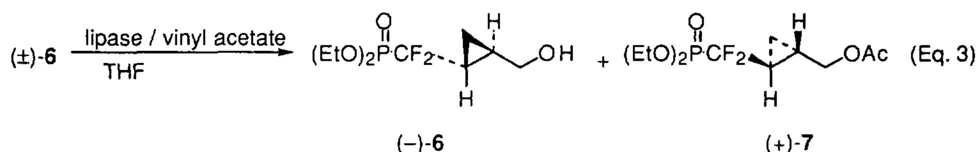


Table 1. Lipase-catalyzed transesterification of (±)-**6** with vinyl acetate in THF.

Entry	Conditions[a]			Conv[b] (%)	Alcohol (-)-**6**		Acetate (+)-**7**		E value[d]
	Lipase	Temp (°C)	Time (h)		Yield (%)	Ee (%)[c]	Yield (%)	Ee (%)[c]	
1	lipase PS	20	2		—	—	100	0	—
2	lipase PS	0	2.5	57.5	33.3	11.6	50.1	8.58	1.31
3	lipase AK	0	2.5	63.3	30.3	27.4	65.0	15.9	1.74
4	PPL[e]	37	32	54.8	45.6	81.2	52.1	67.0	12.3

[a] All reactions were carried out on 1 g scale in the presence 1.0 equiv. of vinyl acetate and 1 g of the lipase. [b] Calculated by the expression $c = ee_s / (ee_s + ee_p)$. [c] Determined by HPLC analysis after converting the (*S*)-MTPA ester. [d] Determined as reported by Sih (ref. 12). [e] Purchased from Sigma.

In an effort to obtain (+)-**6** with high enantiomeric purity, enzyme-catalyzed enantioselective hydrolysis of the acetate (±)-**7** was next carried out in an *i*-Pr_2O saturated phosphate buffer (pH 7.3) (Eq. 4 and Table 2). While lipase PS and PLE (pig liver esterase) were totally ineffective for the enantioselective hydrolysis (entries 1 and 2), PPL-catalyzed hydrolysis was found to proceed with moderate enantioselectivities (E = 15.7-21.0) at 0 °C as expected (entries 3-5). The enantioselectivity of the PPL-catalyzed hydrolysis slightly depended on the amount of the lipase. The best results were obtained when the hydrolysis was carried out using 50 mg of PPL per 1 mmol of the substrate. Upon terminating the reaction at conversion of 30.5%, alcohol (+)-**6** with 87.1% *ee* and acetate (-)-**7** with 38.2% *ee* were obtained in 35.3 and 62.9% yields, respectively (entry 5).

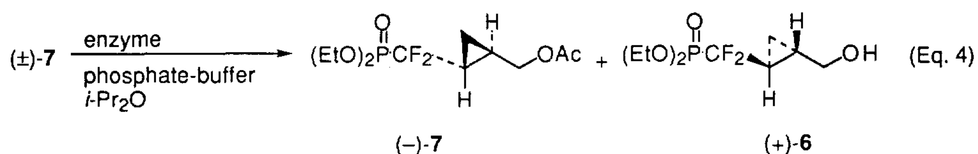


Table 2. Lipase- or esterase-catalyzed hydrolysis of (±)-**7** in an *i*-Pr_2O saturated phosphate buffer.

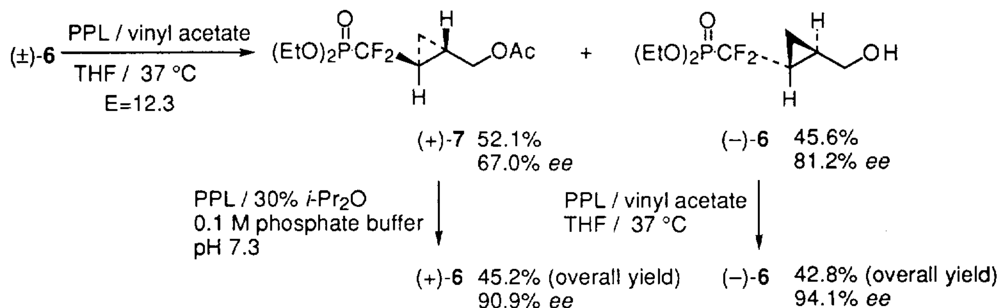
Entry	Conditions[a]		Conv[c] (%)	Alcohol (+)-**6**		Acetate (-)-**7**		E value[e]
	Enzyme (amounts)[b]	Time (h)		Yield (%)	Ee (%)[d]	Yield (%)	Ee (%)[d]	
1	lipase PS (300 mg)	2	—	100	—	—	—	—
2	PLE (150 units)[f]	12	67.5	61.9	10.3	34.2	21.4	1.47
3	PPL (300 mg)[f]	2	17.0	14.3	85.7	64.7	17.6	15.7
4	PPL (100 mg)[f]	8	24.9	32.5	86.1	63.7	28.6	17.9
5	PPL (50 mg)[f]	26	30.5	35.3	87.1	62.9	38.2	21.0

[a] All reactions were carried out at 0 °C in the presence of phosphate buffer (pH = 7.3). [b] Amounts used for 1 mmol of (±)-**7**. [c] Calculated by the expression $c = ee_S / (ee_S + ee_P)$. [d] Determined by HPLC analysis after converting (*S*)-MTPA ester. [e] Determined as reported by Sih (ref. 12). [f] Purchased from Sigma.

Enzymatic double resolution of (±)-6.

On the basis of results obtained from the above experiments with PPL-catalyzed transesterification and hydrolysis, enzymatic double resolution[13] of (±)-**6** with PPL was applied to obtain both optically active alcohols (+)-**6** and (-)-**6** with high enantiomeric purities. The acetate (+)-**7** (67.0% *ee*) and the alcohol (-)-**6** (81.2% *ee*), obtained from the first resolution through the PPL-catalyzed transesterification according to the conditions of entry 4 in Table 1, were separately submitted to the second kinetic resolution with PPL as shown in Scheme 1.[14] The hydrolysis of the acetate (+)-**7** was carried out under the best conditions (entry 5 in Table 2) to give the alcohol (+)-**6**, $[\alpha]_D^{25}+29.4$ (c 1.04, MeOH), with 90.9% *ee* in 86.8% yield (overall yield: 45.2%). An alcohol (-)-**6**, $[\alpha]_D^{25}-29.6$ (c 1.12, MeOH), with high enantiomeric purity (94.1% *ee*) could be prepared in 93.9% yield [overall yield: 42.8%] by re-treatment of the alcohol (–)-**6** of 81.2% *ee* with vinyl acetate in THF under the same conditions as above.[14] Using the enzymatic double resolution, 10 g of (±)-**6** were readily resolved.

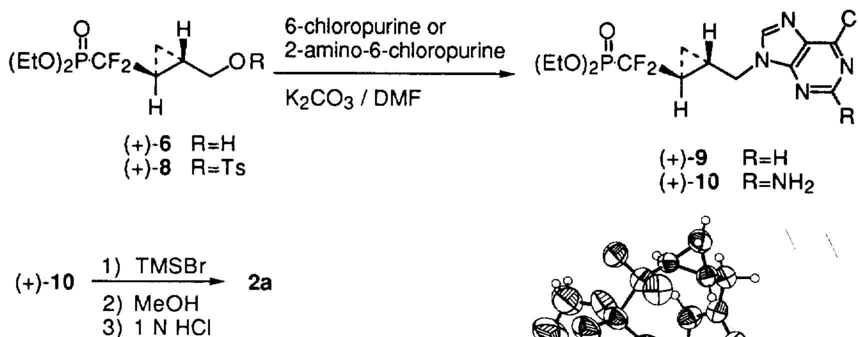
Scheme 1. Overall strategy for the resolution of (±)-**6**



Transformation of (+)-6 and (-)-6 to 9-(difluorophosphonoalkyl)guanines 2a and ent-2a.

With pure (+)-**6** and (-)-**6** in hand, (+)-**6** was condensed with 6-chloropurine and 2-amino-6-chloropurine *via* the tosylate (+)-**8** to give 9-(difluorophosphonoalkyl)purine derivatives (+)-**9**, mp 91-92 °C, and (+)-**10** in 55 and 43% yield, respectively, by a conventional method [1) TsCl / Et_3N; 2) purine derivatives / K_2CO_3 / DMF]. X-ray crystallographic analysis suggests that (+)-**9** has the absolute configuration shown in Scheme 2. This conclusion coincided with the stereochemical outcome deduced from a prediction for lipase-catalyzed

transesterification of (±)-**6** on the basis of an empirical rule for enantiodiscrimination of chiral primary alcohols with lipases from *Pseudomonas cepacia*.[15] Transformation of (+)-**10** into the final product **2a** was achieved through removal of the phosphonate ethyl group [1) TMSBr / CH_2Cl_2, 2) MeOH] and aqueous acid hydrolysis [1 N HCl / 100 °C]. By exactly the same procedure as above, (-)-**6** was also converted to *ent*-**2a**.



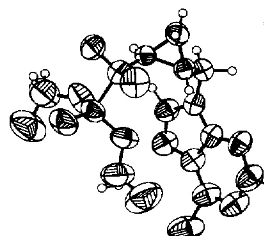


Fig. 1 ORTEP drawing of (+)-**9**

Inhibitory effects of 2a and ent-2a on PNP.

Inhibitory activity of **2a** and *ent*-**2a** toward PNP purified from a microorganism (*Cellulomonas sp.*)[16] was determined by the method of Stoeckler *et al*,[17] and evaluated in comparison with that of 9-(difluorophosphonopentyl)guanine **1**[6] (Table 3). The Ki values (69.5 μM) obtained for **2a** and *ent*-**2a** were identical. However, these compounds were 2400-fold less potent than **1** as a PNP inhibitor (Ki = 28.7 nM). IC_{50} values for **2a** and *ent*-**2a** were identical and substantially higher than the Ki value(s). Also, when analyzed by using a Lineweaver-Burk plot, the two compounds, as well as **1**, showed a mixted-type inhibition manner (data not shown). These results imply that they act as a multi-substrate analogue inhibitor.

Table 3. Comparison of inhibition constants of **1**, **2a**, and *ent*-**2a** for PNP from *Cellulomonas sp.*

Compound	IC_{50} (μM)[a]	Ki (M)
1	0.54	28.7×10^{-9}
2a	332	69.5×10^{-6}
ent-**2a**	332	69.5×10^{-6}

[a] Determined in the presence of 0.1 mM inosine and 100 nM Pi (pH 7.5).[18]

In conclusion, we have developed a method for the synthesis of both enantiomers of *trans*-1-[(diethoxyphosphinyl)difluoromethy]-2-hydroxymethylcyclopropane **6** through a technology of enzyme double resolution. Furthermore, the resolved (+)-**6** and (-)-**6** were transformed to conformationally constrained multi-substrate analogue inhibitors **2a** and *ent*-**2a** for PNP, respectively. Since **2a** and *ent*-**2a** are considered to be conformationally constrained analogues of an extend conformation of **1**, significantly weak inhibitory activity of **2a** and *ent*-**2a** suggest that **1** might not be an inhibitor of PNP when it takes an extend conformation. However,

the details must await further evaluation of molecules such as **2b** and **3a,b** in the inhibitory activities. Study on further synthetic uses of (+)-**6** and (–)-**6** for the compounds **2b**, **3a,b** and their enantiomers is in progress and will be the subject of future reports.

EXPERIMENTAL

General. All reactions were carried out under nitrogen atmosphere, unless otherwise specified. NMR data were obtained on a Bruker AM 400 or a Varian Gemini 300. The chemical shift data for each signal on ^{1}H NMR (400 or 300 MHz) are given in units of δ relative to TMS, $CHCl_3$ (δ 7.26) unless otherwise specified. ^{13}C NMR (100 or 75 MHz) and ^{31}P NMR (162 MHz) were taken with broad-band ^{1}H decoupling. The chemical shifts of ^{13}C are reported relative to $CDCl_3$ (δ 77.0) or acetone (δ 216.5). The chemical shifts of ^{31}P are recorded relative to external 85% H_3PO_4. ^{19}F NMR spectra (376 MHz) were measured using benzotrifluoride (BTF) as an internal reference. IR spectra were recorded on a Perkin-Elmer 1710 FTIR spectrometer. Mass spectra were measured on a Hitachi M-80 or a VG Auto Spec spectrometer.

trans-1-(Diethoxyphosphinyl)difluoromethyl-2-hydroxymethylcyclopropane tetrahydropyranyl ether (±)-5. To a stirred suspension of **4**[7] (6.56 g, 20 mmol) and $Pd(OAc)_2$ (449 mg, 2 mmol) in ether (30 mL) was slowly added an ethereal solution (50 mL) of CH_2N_2 (prepared from 10 g of *N*-methyl-*N*-nitrosourea). Vigorous gas evolution was observed. The mixture was stirred at room temperature for 1 h, then filtered. The filtrate was concentrated to give crude products. These procedures were repeated to consume the starting material. Purification of the crude products by column chromatography on silica gel (hexane:EtOAc=5:1) gave (±)-**5** (6.56 g, 89% yield) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.67-4.60 (1H, m), 4.36-4.20 (4H, m), 3.88-3.78 (1H, m), 3.73-3.65 (0.5H, m), 3.58-3.43 (2H, m), 3.40-3.30 (0.5H, m), 1.87-1.40 (6H, m), 1.37 (6H, t, J = 7.1 Hz), 1.06-0.97 (1H, m), 0.81-0.69 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 118.8 (dt, J_{CP}=221.0, J_{CF}=259.7 Hz), 98.2, 68.3, 64.2 (d, J_{CP}=6.3 Hz), 62.1, 30.4, 25.3, 19.4, 18.2 (dt, J_{CP} = 18.7, J_{CF} = 24.4 Hz), 16.3 (d, J_{CP} = 5.3 Hz), 14.7, 6.7; ^{31}P NMR ($CDCl_3$) δ 6.84 (t, J_{PF} = 115.6 Hz); IR (neat) 1274, 1030 cm^{-1}; EIMS *m/z* 343 (MH^+); HRMS calcd for $C_9H_{18}O_4F_2P$ (MH^+-C_5H_8O): 259.0911. Found: 259.0902.

trans-1-(Diethoxyphosphinyl)difluoromethyl-2-hydroxymethylcyclopropane (±)-6. A stirred solution of (±)-**5** (5.13 g, 15 mmo) in MeOH (50 mL) was treated with *p*-TsOH•H_2O (57 mg, 0.3 mmol) at room temperature for 5 h. The reaction was quenched with *sat.* $NaHCO_3$. The volatile component of the mixture was removed *in vacuo* and the residue was extracted with $CHCl_3$. The extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (hexane:EtOAc=1:2) to give (±)-**6** (3.06 g, 79%) as an oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.35-4.22 (4H, m), 3.75 (1H, ddd, J = 11.2, 5.5, 2.8 Hz), 3.31 (1H, dd, J =11.2, 7.8 Hz), 2.36-2.10 (1H, broad s, -OH), 1.51-1.46 (1H, m), 1.41-1.35 (7H, m), 1.11-1.03 (1H, m), 0.77-0.69 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 118.8 (dt, J_{CP} = 221.7, J_{CF} = 260 Hz), 64.4 (d, J_{CP} = 6.5 Hz), 64.0, 18.2 (dt, J_{CP} = 19.8, J_{CF} = 23.8 Hz), 17.6, 16.2 (d, J_{CP} = 4.8 Hz), 6.0; ^{19}F NMR ($CDCl_3$) δ -48.5 (1F, ddd, J_{FF} = 284.0, J_{HF} = 10.1, J_{FP} = 115.4 Hz), -54.6 (1F, ddd, J_{FF} = 290.4, J_{HF} = 17.6, J_{FP} = 115.4 Hz); ^{31}P NMR ($CDCl_3$) δ 7.11 (t, J_{PF} = 115.4 Hz); IR (neat) 3437, 1263, 1029 cm^{-1}; EIMS *m/z* 258 (M^+), 241 (M^+-OH); Anal. Calcd for $C_9H_{17}OF_2P$: C, 41.85; H, 6.64. Found: C, 42.03; H, 6.56.

trans-2-Acetoxymethyl-1-(diethoxyphosphinyldifluoromethyl)cyclopropane (±)-7. A stirred solution of (±)-**6** (1.29 g, 5 mmol) in pyridine (5.0 mL) was treated with Ac_2O (2.0 mL) at room temperature for 3 h. The mixture was diluted with *sat.* $KHSO_4$ and extracted with ether. The extracts were washed with brine and

sat. $NaHCO_3$, dried ($MgSO_4$), concentrated to give (±)-**7** (1.49 g, 99%): an oil; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.27 (2H, q, J = 7.3 Hz), 4.25 (2H, q, J = 7.2 Hz), 4.00 (1H, dd, J = 6.8, 11.6 Hz), 3.95 (1H, dd, J = 7.1, 11.6 Hz), 2.05 (3H, s), 1.61-1.40 (2H, m), 1.37 (6H, t, J = 7.1 Hz), 1.07-1.02 (1H, m), 0.77-0.72 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 118.9 (dt, J_{PC} = 222.3, J_{FC} = 259.4 Hz), 65.8, 65.4 (d, J_{PC} =5.44 Hz), 20.6, 18.9-18.3 (m), 16.1 (d, J_{PC} = 4.6 Hz), 13.9, 6.4; ^{19}F NMR ($CDCl_3$) δ -51.2 (ddd, J_{FF} = 296.2, J_{HF} = 13.3 Hz, J_{FP} = 116.6 Hz), -52.2 (ddd, J_{FF} = 296.2, J_{HF} = 13.2, J_{FP} = 116.6 Hz); ^{31}P NMR ($CDCl_3$) δ 6.55 (t, J_{PF} = 116.6 Hz); EIMS *m/z* 301 (M^+), 241 (M^+-OAc); IR (neat) 1742, 1272, 1239, 1033 cm^{-1}. Anal. Calcd for $C_{11}H_{19}O_5F_2P$: C, 43.86; H, 6.69; Found: C, 43.95; H, 6.38.

General procedure for lipase-catalyzed transesterification of (±)-6 with vinyl acetate. A mixture of (±)-**6** (1 g, 3.86 mmol), vinyl acetate (0.36 mL, 3.86 mmol) and enzyme (lipase PS, AK, or PPL) (1 g) in THF (15 mL) was stirred under the conditions indicated in Table 1. The reaction was terminated by filtering off the enzyme. After removal of the filtrate *in vacuo*, the residue was purified by column chromatography on silica gel (hexane:EtOAc=4:1 to 1:1) to give the acetate (+)-**7** and alcohol (-)-**6** as oils. Yields and E-values of the transesterification reaction are summarized in Table 1. Physical data of (+)-**7** and (-)-**6** are identical to those of the racemic samples except for specific rotations (see the text).

General procedure for lipase- or esterase-catalyzed enantioselective hydrolysis of acetate (±)-6. A mixture of (±)-7 (302 mg, 1 mmol) and enzyme (lipase PS, PPL or PLE) in 0.1 M phosphate buffer (pH 7.3) (7 mL) containing 3 mL of *i*-Pr_2O was stirred under the conditions indicated in Table 2. The mixture was filtered with a pad of Celite and the solid residue was washed with ether. After removal of the filtrate *in vacuo*, the residue was purified by column chromatography on silica gel (hexane:EtOAc=4:1 to 1:1) to give the acetate (-)-**7** and alcohol (+)-**6** as oils. Yields and E-values of the reaction are summarized in Table 2. Physical data of (-)-**7** and (+)-**6** are identical to those of the racemic samples except for specific rotations (see the text).

Determination of enantiomeric purity of alcohols (+)-6 and (-)-6. To a stirred solution of (2*S*)-2-methoxy-2-phenyl-3,3,3-trifluoropropanoic acid [(*S*)-MTPA] (91.5 mg, 0.36 mmol), *N*,*N*-dicyclohexylcarbodiimide (DCC) (74.2 mg, 0.36 mmol), and 4-dimethylaminopyridine (DMAP) (4.4 mg, 0.036 mmol) in CH_2Cl_2 (1 mL) was added a solution of (+)-**6** or (–)-**6** (0.18 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred at the same temperature for 30 min, and then kept at room temperature until the starting material disappeared on TLC (4 h). The reaction was quenched with *d.* HCl (6 mL) at 0 °C. The mixture was extracted with $CHCl_3$. The extracts were washed successively with *sat.* $NaHCO_3$ and brine, and then dried ($MgSO_4$). The solution was concentrated *in vacuo*, and diluted with ether. The resulting suspension was passed through silica gel (0.5 g). The filtrate was evaporated to leave (*S*)-MTPA esters of (+)-**6** or (–)-**6**, which were analyzed by HPLC [hexane:EtOAc=1:1, Fine pack (Jasco), flow rate = 1.0 mL/min, UV detector (254 nm), (*S*)-MTPA ester of (+)-**6**: Rt = 10.8 min, (*S*)-MTPA ester of (-)-**6**: Rt = 11.9 min].

(1S,2S)-1-(Diethoxyphosphinyldifluoro)methy-2-(4'-toluenesulfonyl)oxymethylcyclopropane (+)-8. To a stirred solution of (+)-**6** (2.58 g, 10 mmol) in $CHCl_3$ (25 mL) was added successively *p*-toluenesulfonyl chloride (2.10 g, 11 mmol) and triethylamine (2.8 g, 20 mmol) at room temperature. The mixture was stirred for 12 h at the same temperature and quenched with H_2O. The mixture was extracted with $CHCl_3$. The extracts were washed with brine, dried ($MgSO_4$), and evaporated to leave a residue. Purification by column chromatography on silica gel (hexane:EtOAc=2:1) gave (+)-**8** (3.42 g, 83%) as an oil. $[\alpha]_D^{25}$+22.0 (c 1.27, MeOH), ^1H NMR ($CDCl_3$, 300 MHz) δ 7.78 (2H, d, J = 8.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 4.33-4.18 (4H,

m), 2.45 (3H, s), 1.63-1.53 (1H, m), 1.50-1.40 (1H, m), 1.36 (6H, t, J = 7.0 Hz), 1.10-1.01 (1H, m), 0.78-0.68 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.8, 132.9, 129.8, 127.7, 118.0 (dt, J_{CP} = 222.5, J_{CF} = 259.7 Hz), 71.8, 64.4 (d, J_{CP} = 5.8 Hz), 64.3 (d, J_{CP} = 5.9 Hz), 21.5, 18.9 (dt, J_{CP} = 19.6, J_{CF} = 24.3 Hz), 16.3 (d, J_{CP} = 5.0 Hz), 13.8, 6.9; ^{19}F NMR ($CDCl_3$) δ -51.50 (1F, ddd, J_{FF} = 296.4, J_{HF} = 13.2, J_{FP} = 113.4 Hz), -52.42 (1F, ddd, J_{FF} = 296.4, J_{HF} = 13.4, J_{FP} = 113.4 Hz); ^{31}P NMR ($CDCl_3$) δ 6.30 (t, J_{PF} = 113.4 Hz); IR (neat) 1272, 1032 cm^{-1}; EIMS *m/z* 412 (M^+). Anal. Calcd for $C_{16}H_{23}O_6F_2PS$: C, 46.59; H, 5.62. Found: C, 46.80; H, 5.65.

(1R,2R)-1-(Diethoxyphosphinyldifluoro)methy-2-(4'-toluenesulfonyl)oxymethylcyclopropane (-)-8. This compound was obtained as an oil from (-)-**6** by the same procedure as above. The physical data was identical to those of (+)-**8** except for specific rotation: $[\alpha]_D^{25}$ -22.2 (c 1.27, MeOH).

(2'S,3'S)-6-Chloro-9-(4'-diethylphosphono-4',4'-difluoro-2',3'-methanobutyl)purine (+)-9. To a stirred solution of (+)-**6** (2.06 g, 5 mmol) in DMF (20 mL) was added successively 6-chloropurine (773 mg, 5 mmol) and K_2CO_3 (1.38 g, 10 mmol) at room temperature. The mixture was stirred at the same temperature for 24h and was partitioned between $CHCl_3$ and *sat.* $NaHCO_3$. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to give a residue. Purification of column chromatography on silica gel ($CHCl_3$:MeOH=200:1) gave (+)-**9** (1.02 g, 52%): mp 91-92 °C; $[\alpha]_{365}^{25}$ +0.90 (c 1.11, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.71 (1H, s), 8.26 (1H, s), 4.34 (1H, dd, J = 13.9, 7.0 Hz), 4.20-4.06 (4H, m), 1.79-1.62 (2H, m), 1.28 (3H, t, J = 6.6 Hz), 1.26 (3H, t, J = 6.7 Hz), 1.21-1.13 (1H, m), 1.00-0.91 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.9, 151.8, 150.9, 144.7, 131.4, 117.9 (dt, J_{CP} = 222.6, J_{CF} = 260.0 Hz), 64.5 (d, J_{CP} = 6.8 Hz), 64.4 (d, J_{CP} = 6.8 Hz), 46.7, 20.0 (dt, J_{CP} = 19.2, J_{CF} = 24.5 Hz), 16.2 (d, J_{CP} = 4.4 Hz), 15.3, 7.7; ^{19}F NMR ($CDCl_3$) δ -51.0 (1F, ddd, J_{FF} = 297.2, J_{HF} = 12.8, J_{FP} = 113.6 Hz), -53.3 (1F, ddd, J_{FF} = 297.2, J_{HF} = 14.7, J_{FP} = 115.2 Hz); ^{31}P NMR ($CDCl_3$) δ 6.09 (dd, J_{PF} = 113.6, 115.2 Hz). IR (KBr) 1258, 1034 cm^{-1}; EIMS *m/z* 394 (M^+). Anal. Calcd for $C_{14}H_{18}N_4O_3PF_2Cl$: C, 42.59; H, 4.60; N, 14.19. Found: C, 42.60; H, 4.61; N, 14.16.

Crystal data for compound (+)-9. $C_{14}H_{18}N_4O_3PF_2Cl$, M=394.00, tetragonal, space group = $P4_32_12$, a = 11.343(1), b = 11.343(1), c = 28.748(4) Å, V = 3699(1)Å^3, T =288 K, Z = 8, D_c=1.41 g cm^{-3}, μ = 28.85 cm^{-1}, Crystal dimensions = 0.30x0.25x0.25 mm^3, λ(Cu-Kα) = 1.54178 Å. Data collection was performed by a Mac-Science MXC 18 diffractometer. The structure was solved by a direct method using SHELXS86. Full-matrix least-squares refinement of atomic parameters (271) converged at R = 0.047, wR = 0.065 over 1788 independent reflections. The absolute configuration of (+)-**9** was determined by the R-value method[19] from the anomalous scattering due to the heavy atom and found to be 2'*S*,3'*S* (ΔR = 0.59%, ΔwR = 0.81%).

(2'S,3'S)-2-Amino-6-chloro-9-(4'-diethylphosphono-4',4'-difluoro-2',3'-methanobutyl) purine (+)-10. This compund was obtained as amorphous powder from (+)-**8** (1.1 g, 2.68 mmol) and 2-amino-6-chloropurine (414 mg, 2.68 mmol) in an analogous manner to that of (+)-**9**. $[\alpha]_D^{25}$+6.22 (c 1.0, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, s), 5.43 (2H, s), 4.20-4.03 (5H, m), 3.93 (1H, dd, J = 7.1 Hz), 1.75-1.59 (2H, m), 1.27 (6H, t, J = 7.1 Hz), 1.14-1.08 (1H, m), 0.89-0.83 (1H, m); ^{13}C NMR ($CDCl_3$, 100 MH) δ 160.2, 154.7, 152.0, 142.7, 125.7, 118.9 (dt, J_{PC} = 222.8, J_{FC} = 259.9 Hz), 64.8 (d, J_{CP} = 6.6 Hz), 46.3, 20.4-19.5 (m), 16.3, 15.3, 7.5; ^{19}F NMR ($CDCl_3$) δ -50.9 (1F, ddd, J_{FF} = 296.5, J_{HF} = 12.8, J_{FP} = 114.4 Hz), -53.0 (1F, ddd, J_{FF} = 296.5, J_{HF} = 13.7, J_{FP} = 114.4 Hz); ^{31}P NMR ($CDCl_3$) δ 6.25 (t, J_{FP} = 114.4 Hz), IR

(neat) 1263, 1029 cm^{-1}; EIMS *m/z* 409 (M^+); HRMS *m/z* calcd for $C_{14}H_{19}N_5O_3F_2PCl$ (M^+): 409.0882. Found: 409.0895.

(2'R,3'R)-2-Amino-6-chloro-9-(4'-diethylphosphono-4',4'-difluoro-2',3'-methanobutyl) purine (-)-10. This compound was prepared from (-)-**8** in an analogous manner to that of (+)-**9**. Physical data were identical to those of (+)-**10** except for the specific rotation: $[\alpha]_D^{25}$ -5.37 (c 6.5, MeOH).

(2'S,3'S)-9-(4'-Phosphono-4',4'-difluoro-2',3'-methanobutyl)guanine 2a. To a stirred solution of (+)-**8** (395 mg, 0.96 mmol) in CH_2Cl_2 (5 mL) was added bromotrimethylsilane (0.26 mL, 2.0 mmol) at room temperature. The mixture was stirred for 24 h and evaporated under reduced pressure. The residue was treated with MeOH (3 mL) at room temperature for 24 h. The volatile component of the mixture was removed *in vacuo* and residue was dissolved in 1 N HCl (2 mL). The solution was heated under reflux for 24 h. The reaction mixture was evaporated and the solid material was recrystallized from 50% MeOH-H_2O to give **2** (305 mg, 85%). Mp >300 °C; $[\alpha]_D^{25}$+5.40 (c 1.20, H_2O); ^1H NMR (D_2O, 300 MHz, relative to TSP) δ 8.27 (1H, s), 3.57 (1H, dd, J = 5.7, 14.7 Hz), 3.27 (1H, dd, J = 7.8, 14.7 Hz), 0.96 (2H, broad s), 0.45-0.30 (1H, m), 0.29-0.17 (1H, m); ^{13}C NMR (D_2O, 75 MHz, relative to acetone (δ 216)) δ 156.1, 155.5, 150.7, 137.9, 119.9 (dt, J_{PC} = 210.3, J_{FC} = 257.4 Hz), 108.1, 48.2, 19.5 (dt, J =17.7, 23.7 Hz), 14.4, 7.3; ^{19}F NMR (D_2O) δ -52.1 (1F, ddd, J_{HF} = 288.9, J_{HF} = 10.7, J_{FP} = 105.8 Hz), -54.3 (1F, ddd, J_{FF} = 288.9, J_{HF} = 14.1, J_{FP} = 105.8 Hz); ^{31}P NMR (D_2O) δ 6.84 (t, J_{PF} = 105.8 Hz); IR (KBr) 3358, 1638, 1053 cm^{-1}; UV (H_2O) λ_{max} 253 nm (11046); FABMS *m/z* 336 (MH^+), High resolution FABMS *m/z* calcd for $C_{10}H_{13}N_5O_4F_2P$ (MH^+): 336.0673. Found: 336.0643. Anal. Calcd for $C_{10}H_{12}N_5O_4F_2P \cdot HCl \cdot H_2O$: C, 30.82; H,3.88; N, 17.97. Found: C, 30.64; H, 4.14; N, 17.50.

(2'R,3'R)-9-(4'-Phosphono-4',4'-difluoro-2',3'-methanobutyl)guanine ent-2a. This compound (mp >300 °C) was prepared from (-)-**8** in an analogous manner to that of **2a**. The physical data was identical to those of **2a** except for the specific rotation: $[\alpha]_D^{25}$-5.71 (c 1.47, H_2O).

Assay and inhibition of PNP. PNP activity was measured by the xanthine oxidase couple assay of Stoeckler *et al.*[17] with minor modification. Briefly, the assay mixture contained 0.5 M potassium phosphate buffer (pH 7.5, 300 μL), 0.2 U/mL PNP (Toyobo, Tokyo, 300 μL), 0.12 U/mL xanthine oxidase (Sigma, 300 mL), 3-600 μM inhibitor (1 mL), and distilled water (800 μL), and was incubated at 30 °C for 5 min. To the reaction mixture was added 10 mM inosine (Wako Pure Chemical Co., Osaka, 300 μL), and the increase in absorbance at 293 nm based on the formation of uric acid was monitored for 2 min with a Shimadzu UV-1600 spectrophotometer. PNP activity was calculated by using the molecular extinction coefficient of uric acid (1.25 x 10^4), and the specific activity was expressed as μ mol of uric acid/min/mg of protein. IC_{50} was the concentration of compound giving 50% of enzyme inhibition. Ki values were determined by using a Dixon plot and a computer developed in-house for linear regression analysis. It was verified that the compounds had no inhibitory activity toward xanthine oxidase in this assay.

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