Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 2495–2505

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of 1,1-Difluoro-5-(1*H*-9-purinyl)-2-pentenylphosphonic Acids and the Related Methano Analogues. Remarkable Effect of the Nucleobases and the Cyclopropane Rings on Inhibitory Activity Toward Purine Nucleoside Phosphorylase

Tsutomu Yokomatsu,[a] Hiroshi Abe,[a] Mutsumi Sato,[a] Kenji Suemune,[a] Taro Kihara,[b] Shinji Soeda,[b] Hiroshi Shimeno[b] and Shiroshi Shibuya[a,*]

[a]*School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*
[b]*Faculty of Pharmaceutical Science, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan*

Received 8 July 1998; accepted 9 September 1998

Abstract—A series of 1,1-difluoro-5-(1*H*-9-purinyl)-2-pentenylphosphonic acids, (*E*)-**2a,b** and (*Z*)-**2a,b**, as well as the related methano analogues (±)-**3a,b** and (±)-**4a,b** were prepared for evaluation of their PNP inhibitory activities. The cyclopopane ring and the hypoxanthine residue were found to increase the profile of inhibitory activity. The IC_{50} and K_i values of difluoro{(1*R**,2*S**)-2-[2-(6-oxo-6,9-dihydro-1*H*-9-purinyl)ethyl]cyclopropyl}methylphosphonic acid (±)-**3b** toward PNP purified from *Cellulomonas sp.* were determined to be 70 nM and 8.8 nM, respectively.

Introduction

Purine nucleoside phosphorylase (PNP: EC. 2.4.2.1), a key enzyme of the anabolic and catabolic pathways of purine nucleosides, catalyzes the reversible phosphorolysis of ribo- and 2′-deoxyribonucleosides of guanine and hypoxanthine in higher organisms, as well as of adenine in some prokaryotes.[1] Individuals who genetically lack 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 chemotherapeutic agents such as 2′,3′-dideoxyinosine against PNP metabolism.[4] Consequently, extensive drug-discovery research has been devoted to the design and synthesis of inhibitors of PNP during the last 10 years.

The reversible phosphorylation of the purine nucleosides catalyzed by PNP proceeds via a ternary complex of enzyme, nucleoside, and orthophosphate as shown in the schematic drawing (Fig. 1).[5] Based on these facts, a number of the 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 synthesized to date, no compound has yet reached the stage of clinical trial. Among the existing PNP inhibitors, 5-(2-amino-6-oxo-6,9-dihydro-1*H*-9-purinyl)-1,1-difluoropentylphosphonic acid **1**, simply derived by connection of the purine base and the difluoromethylphosphonic acid for a mimetic of the orthophosphate with *n*-butyl spacer, are reported to possess relatively high inhibitory activity (Ki = 18 nM) against human erythrocyte PNP and act as a 'multi-substrate' analogue inhibitor.[6]

Key words: enzyme inhibitors; mimetics; nucleotides; phosphonic acids and derivs.
*Corresponding author. Tel: & Fax: +81-426-76-3239; E-mail: shibuyas@ps.toyaku.ac.jp

0968-0896/98/$ - see front matter
PII: S0968-0896(98)00199-0

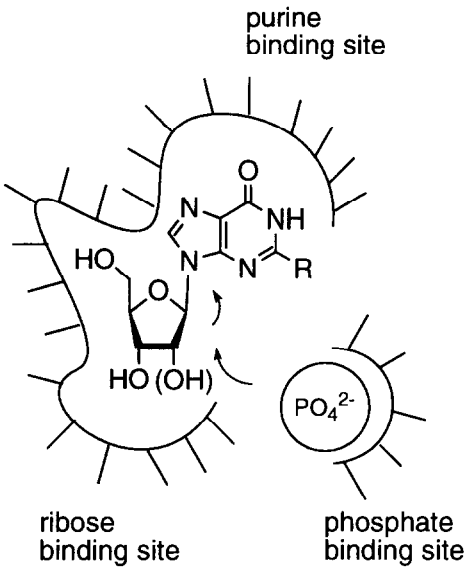


Figure 1. Schematic drawing of ternary complex of PNP and substrates.

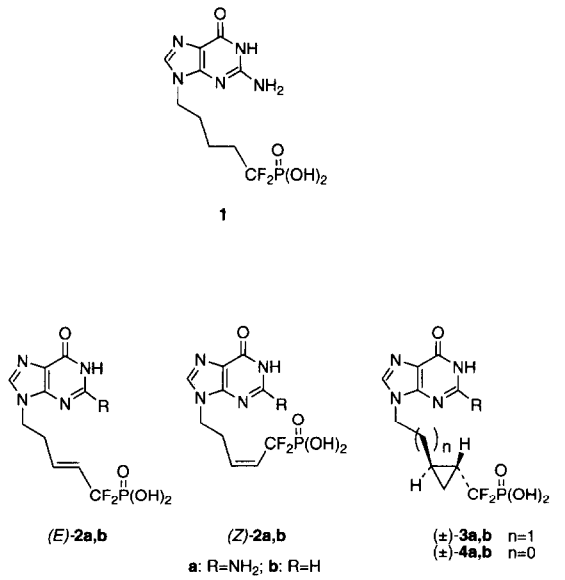


Figure 2. Novel nucleotide analogues for PNP inhibitors.

In order to develop new PNP inhibitors with significant activity, it would be valuable to investigate the structural requirements which enhance the PNP inhibitory activity of the compound **1**. From consideration of structure **1** as well as a functional feature of PNPs, it is apparent that conformational and toporogical flexibility of **1** should be modified for improving the inhibitory potency. Therefore, we sought to replace the *n*-butyl spacer with structually rigid linkers that are capable of binding more strongly in the restricted binding pockets (purine binding site and phosphate binding site)[5c–e] of PNPs. We also considered that hypoxanthine substitution for guanine of **1** would be useful for improving the binding affinity to PNPs, since, among natural purine nucleosides, inosine is reported to be the best substrate toward various PNPs.[7c,8b] In this context, capitalizing on recent methodology for the synthesis of ally-α,α-difluorophosphonates[8a] and the corresponding methano analogues developed in our laboratories,[8b] we newly synthesized a series of novel nucleotide analogues illustrated in Figure 2 for a PNP inhibitor, wherein a nucleobase and the difluoromethylphosphonic acid are linked with an alkyl spacer having either a double bound or cyclopropane ring. Herein, we describe effects of the double bond and the cyclopropane rings as well as the nature of nucleobases on the PNP inhibitory activities. The results indicate that among the nucleotide analogues prepared, the compound (±)-**3b** is the most potent inhibitor for *Cellulomonas sp.* PNP,[7] suggesting that the substitution of **1** with a cyclopropylalkyl spacer and a hypoxanthine nucleobase is a better inhibitory motif.

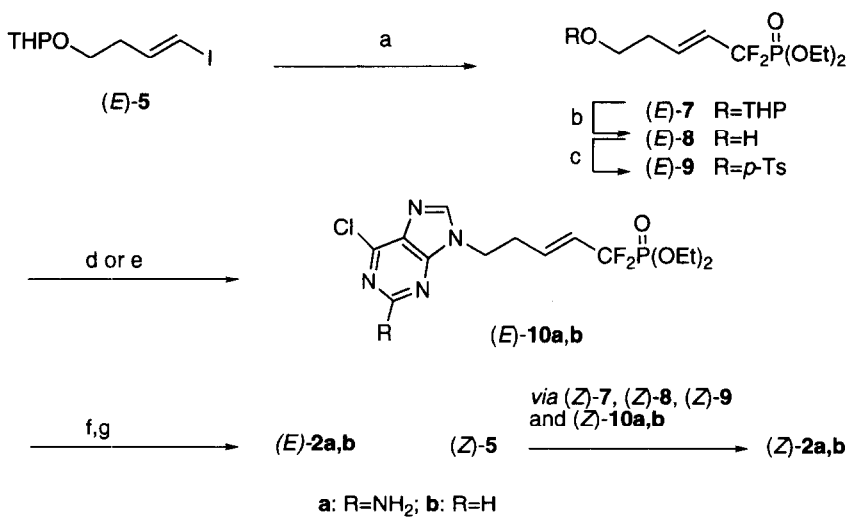
Results and Discussion

Synthesis of 1,1-difluoro-5-(1*H*-9-purinyl)-2-pentenylphosphonic acids (*E*)-2a,b and (*Z*)-2a,b

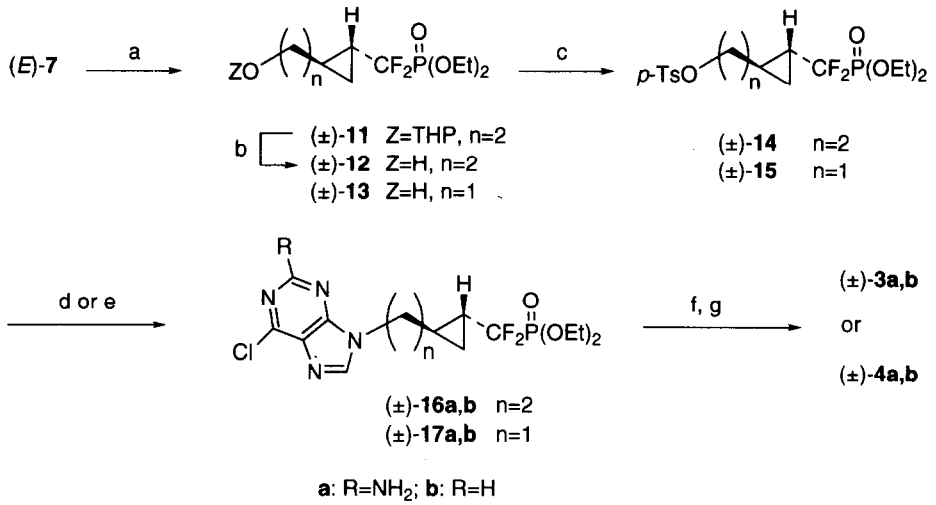
The syntheses of (*E*)-**2a,b** and (*Z*)-**2a,b** are summarized in Scheme 1. According to the method reported previously,[8a] the zinc reagent **6**, prepared from diethyl bromodifluoromethylphosphonate and zinc powder in *N*,*N*-dimethylformamide (DMF), was treated with alkenyl iodide (*E*)-**5** in the presence of a stoichiometric amount of CuBr to give stereospecifically ally-α,α-difluorophosphonate (*E*)-**7** in 93% yield. After converting to the tosylate (*E*)-**9** in the usual manner (71% yield for two steps via (*E*)-**8**), it was coupled with either 2-amino-6-chloropurine or 6-chloropurine to give (*E*)-**10a** and (*E*)-**10b** in 50% and 61% yield, respectively.[9,10] Treatment of (*E*)-**10a,b** with bromotrimethylsilane (TMSBr) in dichloromethane, followed by hydrolysis with water in one pot afforded (*E*)-**2a** and (*E*)-**2b** in 85% and 75% yield.[11] To obtain the corresponding *Z*-isomers (*Z*)-**2a,b**, a series of reactions under exactly the same conditions as described above was followed starting from (*Z*)-**5**. It is worth noting that the coupling reaction of (*Z*)-**5** with the zinc reagent **6** gave (*Z*)-**7** stereospecifically in 93% yield, and all other steps proceeded to give comparative yield to that of a series of *E*-isomers.

Synthesis of difluorophosphonoalkylpurine derivatives (±)-3a,b and (±)-4a,b having cyclopropane rings

The racemic methano analogues (±)-**3a,b** were synthesized from (*E*)-**7** via cyclopropanation as outlined in



Scheme 1. Reagents: (a) $BrZnCF_2P(O)(OEt)_2$ (**6**), CuBr, DMF, 25 °C; (b) *p*-TsOH, MeOH; (c) *p*-TsCl, Et_3N, CH_2Cl_2; (d) 2-amino-6-chloropurine, K_2CO_3, DMF; (e) 6-chloropurine, K_2CO_3, DMF; (f) TMSBr, CH_2Cl_2; (g) H_2O.



Scheme 2. Reagents: (a) CH_2N_2, $Pd(OAc)_2$, ether; (b) *p*-TsOH, MeOH; (c) *p*-TsCl, Et_3N, CH_2Cl_2; (d) 2-amino-6-chloropurine, K_2CO_3, DMF; (e) 6-chloropurine, K_2CO_3, DMF; (f) TMSBr, CH_2Cl_2; (g) H_2O.

Scheme 2. While (*E*)-**7** was totally inert to cyclopropanation with the Simmons–Smith reagents under a variety of conditions, the desired cyclopropane derivative (±)-**11** was obtained in good yield upon treatment of (*E*)-**7** with an excess amount of CH_2N_2 in ether in the presence of $Pd(OAc)_2$.[8b,12,13] The tetrahydropyranyl (THP) group of (±)-**11** was removed to give the alcohol (±)-**12** in virtually quantitative yield. The alcohol (±)-**12** was converted to (±)-**3a,b** via (±)-**14** and (±)-**16a,b** by exactly the same procedure as a series of (*E*)-**2a,b**. The nor-derivatives (±)-**4a,b** were manipulated via (±)-**15** and (±)-**17a,b** from the alcohol (±)-**13**, which was previously prepared by similar synthetic sequences.[8b]

Inhibitory activity

Inhibitory activity of the propenyl derivatives (*E*)-**2a,b** and (*Z*)-**2a,b** as well as the methano derivatives (±)-**3a,b**, and (±)-**4a,b** toward PNPs purified from *Cellulomonas sp.*[7] and human erythrocytes was determined by the method of Stoeckler et al.[14] with minor modification and evaluated in comparison with that of parental **1**[6] (Table 1).

As shown in Table 1, all compounds except (±)-**4a** synthesized in this study were found to retain higly potent inhibitory activity against both PNPs at the

Table 1. Comparison of inhibition constants of **1**, (*E*)-**2a,b**, (*Z*)-**2a,b**, (±)-**3a,b** and (±)-**4a,b** for PNPs from *Cellulomonas sp.* and human erythrocytes

Compound	PNP from *Cellulomonas sp.*[a]		PNP from human erythrocytes[b]	
	IC_{50} (nM)[c]	Ki (nM)	IC_{50} (nM)[c]	Ki (nM)
1	540	28.7	380	53.7
(*E*)-**2a**	170	20.4	400	30.7
(*Z*)-**2a**	200	29.1	310	27.6
(±)-**3a**	390	28.2	330	43.4
(±)-**4a**	291,000	53,200.0	391,000	39,067.0
(*E*)-**2b**	280	11.4	320	32.1
(*Z*)-**2b**	240	16.2	360	32.2
(±)-**3b**	70	8.8	340	17.3
(±)-**4b**	190	5.4	380	23.4

[a]Purchased from TOYOBO biochemicals.
[b]Purchased from Sigma.
[c]Determined in the presence of 0.1 mM inosine and 100 nM Pi (pH 7.5).

range of nM order with respect to IC_{50} and Ki values. The Ki values of a series of guanine derivatives (*E*)-**2a**, (*Z*)-**2a**, and (±)-**3a** against the PNP from *Cellulomonas sp.* range from 20.4 nM to 29.1 nM and show that the binding affinities are almost identical to that of **1**. However, the IC_{50} values for this series of inhibitors are 1.4 to 3.2-fold more potent than that of **1**. Of the three derivatives, (*E*)-**2a** showed the smallest IC_{50} value, suggesting that it may most strongly bind to the active site of the PNP. The inhibition of the PNP by (*E*)-**2a** was determined to be in a mixed-type inhibition manner as analyzed by the Lineweaver–Burk plot, while **1** inhibited the enzyme in a competitive inhibition manner[15] (Fig. 3). The results suggest that such modification of pentylphosphonic acid residue of **1** alters the inhibition mode and the interaction of (*E*)-**2a** to a complex of enzyme/nucleoside and/or orthophosphate might be crucial for the improved inhibitory potency (IC_{50}).[15]

Inhibition of *Cellulomonas sp.* PNP by hypoxanthine derivatives (*E*)-**2b**, (*Z*)-**2b**, (±)-**3b** and (±)-**4b** resulted in a significant decrease in Ki value compared to the corresponding guanine derivatives. This suggests that the hypoxanthine substitution for guanine could improve the binding affinity for the PNP or PNP/substrate complex.[15] While no significant difference in Ki and IC_{50} values between configurational isomers of alkenylphosphonates (*E*)-**2b** and (*Z*)-**2b** was observed, these compounds (Ki = 11.4 and 16.2 nM) were apparently more potent than **1**. The Ki and IC_{50} values of the cyclopropane derivative (±)-**3b** having a hypoxanthine nucleobase were determined to be 8.8 and 70 nM, respectively. The results show that not only the binding affinity (Ki) but also the inhibitory potency (IC_{50}) of the inhibitor toward *Cellulomonas. sp.* PNP is significantly improved by introducing a cyclopropane ring to the inhibitor molecule and replacement of the guanine residue with a hypoxanthine nucleobase.

The structure of the nucleobase of the nor-derivatives (±)-**4a,b** has significant effect on the inhibitory activities; the hypoxanthine derivative (±)-**4b** (Ki = 5.4 nM) is ca. 10,000-fold more potent than the guanine derivative (±)-**4a**.[15] The results clearly show that the amine functionality of the purine residue on the inhibitor affects unfavorably interactions between the inhibitor and the active site of the enzyme. This trend was also observed in a series of assays for PNP from human erythrocytes. Comparison of the Ki value (23.4 nM) of hypoxanthine derivative (±)-**4b** with that of the corresponding guanine derivative (±)-**4a** revealed that (±)-**4b** is ca. 1600-fold more potent than (±)-**4a**, suggesting that the two derivatives could bind to human erythrocytes PNP in the same fashion as that to *Cellulomonas sp.*

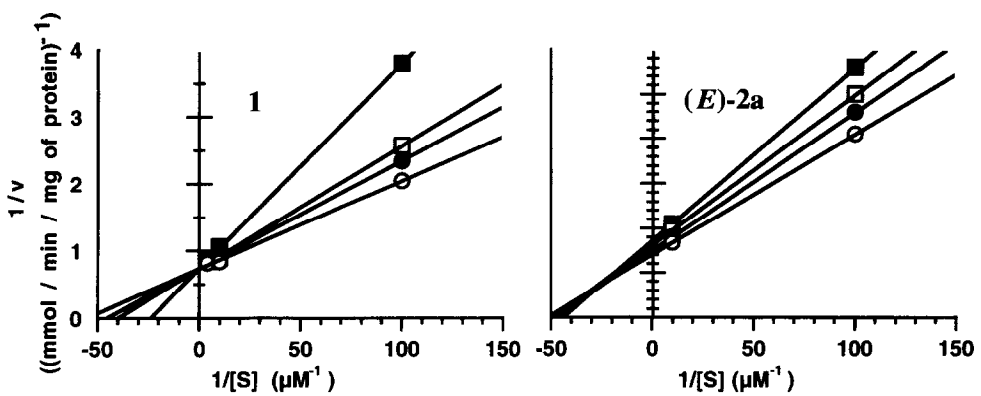


Figure 3. Linweaver–Burk plots for inhibition of *Cellulomonas sp.* PNP activity by compound **1** or (*E*)-**2a**. Inhibitor concentration: ○, 0 μM; ●, 0.01 μM; □, 0.1 μM; △, 0.5 μM; ■, 1.0 μM.

PNP, although the binding affinity of (±)-**4b** for human erythrocytes PNP considerably decreased (Ki = 5.4 versus 23.4 nM). However, unlike the inhibition to *Cellulomonas sp.* PNP, there was no significant difference in IC_{50} value between **1** and the other derivatives. The different recognition of the two species of PNP for these derivatives may be due to the different amino acid sequence in their active site.[7c] Therefore, this finding implies a possible creation of a species-specific PNP inhibitor based on the difference between the amino acid sequences of active sites. The creation of species-specific PNP inhibitor may provide useful clues for the development of an improved immunosuppressive agent and chemotherapeutic with a high degree of safety and effectiveness.

Conclusion

In conclusion, a novel series of nucleotide analogues (*E*)-**2a,b**, (*Z*)-**2a,b**, (±)-**3a,b** and (±)-**4a,b** has been synthesized using efficient sequences involving the coupling reaction of the zinc reagent **6** and iodoalkenes as well as the cyclopropanation of the resulting ally-α,α-difluorophosphonates. The evaluation of PNP inhibitory activities reveals that the most potent compounds in this set are analogues (±)-**3b** and (±)-**4b**. A comparison of the inhibitory potencies of (±)-**4a**, (±)-**4b**, and **1** demonstrated a clear preference for the cyclopropane ring and the hypoxanthine nucleobase on the binding affinities for PNP purified from *Cellulomonas sp.* Further refinements in the structures of the compounds related to (±)-**3b** and (±)-**4b** are a logical extension of this work.

Experimental

General. All reactions were carried out under nitrogen atmosphere. NMR data were obtained on a Bruker DPX 400 for a solution in the indicated solvent unless otherwise specified. ^{13}C NMR (100 MHz) and ^{31}P NMR (162 MHz) were taken with broad-band ^{1}H decoupling. The chemical shift data for each signal on ^{1}H NMR (400 MHz) are given in units of δ relative to $CHCl_3$ (δ 7.26) for $CDCl_3$ solution or 3-(trimethylsilyl)propionic-2,2,3,3,-d_4-acid sodium salt (TSP, δ 0) for D_2O solution. The chemical shifts of ^{13}C are reported relative to $CDCl_3$ (δ 77.0) or TSP (δ 0) unless otherwise stated. The chemical shifts of ^{31}P are recorded relative to external 85% H_3PO_4. ^{19}F NMR spectra (376 MHz) were measured using either benzotrifluoride (BTF) as an internal reference for $CDCl_3$ solution or trifluoroacetic acid as an external standard for D_2O solution. 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.

(*E*)-4-Iodo-1-(tetrahydro-2*H*-2-pyranoyloxy)but-3-ene (*E*)-5. To a stirred suspension of bis(cyclopentadienyl)zirconium dichloride (Cp_2ZrCl_2) (10.4 g, 35.7 mmol) in THF (95 mL) was added a solution of Red-Al® (5.26 g of 70% solution of sodium bis(2-methoxyethoxy)-aluminum hydride in toluene, 17.9 mmol) in THF (30 mL). The mixture was stirred for 1 h at room temperature. The resulting Schwartz's reagent (Cp_2Zr(H)Cl) in THF was transferred to a solution of 1-(tetrahydro-2*H*-2-pyranoyloxy)but-3-yne (5 g, 32.5 mmol) in ether (90 mL) at room temperature. The mixture was stirred for 12 h and treated with a solution of I_2 (5 g, 20 mmol) in THF (25 mL) for 2 h. *n*-Hexane was added and the resulting precipitate was filtered by suction. The filtrate was diluted with sat. $Na_2S_2O_3$. The aqueous layer was extracted with *n*-hexane. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 100:1) to give (*E*)-**5** (6.2 g, 68%) as an oil. ^{1}H NMR ($CDCl_3$) δ 6.57 (1H, dt, *J* = 7.1, 14.4 Hz), 6.12 (1H, d with small splits, *J* = 14.4 Hz), 4.58 (1H, dd, *J* = 4.1, 2.8 Hz), 3.87–3.74 (2H, m), 3.53–3.41 (2H, m), 2.37–2.32 (2H, m), 1.85–1.75 (1H, m), 1.74–1.68 (1H, m), 1.61–1.50 (4H, m); ^{13}C NMR ($CDCl_3$) δ 142.7, 98.3, 76.3, 65.2, 61.8, 36.0, 30.2, 25.1, 19.1; EIMS *m/z* 283 ($M^+ + 1$). Anal. calcd for $C_9H_{15}IO_2$: C, 38.31; H, 5.36. Found: C, 38.60; H, 5.45.

(*Z*)-4-Iodo-1-(tetrahydro-2*H*-2-pyranoyloxy)but-3-ene (*Z*)-5. A mixture of 4-iodo-1-(tetrahydro-2*H*-2-pyranoyloxy)but-3-yne[16] (17.5 g, 62.5 mmol), *p*-toluenesulfonyl hydrazide (24.5 g, 125 mmol) and sodium acetate (15.4 g, 187 mmol) in 50% aqueous THF (400 mL) was heated under reflux for 12 h. After being cooled to room temperature, the mixture was extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography on silica gel (hexane:EtOAc = 100:1) to give (*Z*)-**5** (11.8 g, 67%) as an oil. ^{1}H NMR ($CDCl_3$) δ 6.32–6.27 (2H, m), 4.61 (1H, dd, *J* = 4.0, 2.9 Hz), 3.89–3.71 (2H, m), 3.54–3.47 (2H, m), 2.47–2.42 (2H, m), 1.88–1.79 (1H, m), 1.75–1.69 (1H, m), 1.62–1.50 (4H, m); ^{13}C NMR ($CDCl_3$) δ 137.9, 98.3, 83.8, 64.9, 61.9, 35.2, 30.4, 25.2, 19.2; EIMS *m/z* 283 ($M^+ + 1$). Anal. calcd for $C_9H_{15}IO_2$: C, 38.31; H, 5.36. Found: C, 38.22; H, 5.51.

Diethyl [(*E*)-1,1-difluoro-5-(tetrahydro-2*H*-2-pyranoyloxy)-2-pentenyl]phosphonate (*E*)-7. To a stirred suspension of Zn dust (2.4 g, 37 mmol) in dry DMF (50 mL) was slowly added a solution of **6** (9.9 g, 37 mmol) in DMF (10 mL). During the addition, exothermic reaction occurred. The addition was controlled so that the internal temperature was maintained at 50–60 °C. After addition was completed, the solution was stirred at

room temperature for an additional 3 h, then CuBr (5.3 g, 37 mmol) was added in one portion. The mixture was stirred at the same temperature for 30 min. A solution of (*E*)-**5** (5.2 g, 19 mmol) was added dropwise at room temperature (exothermic reaction occurred). After the mixture was stirred at room temperature for 15 h, water was added to quench the reaction. Biphasic mixture was passed through Celite, and extracted with Et_2O. The extract was washed with brine, and dried over $MgSO_4$. Evaporation of the solvent, followed by chromatographic purification on silica gel (hexane:EtOAc = 5:1 to 3:1) gave (*E*)-**7** (5.9 g, 93%) as an oil. ^{1}H NMR ($CDCl_3$) δ 6.36–6.25 (1H, m), 5.81–5.67 (1H, m), 4.58 (1H, dd, J = 4.2, 2.8 Hz), 4.31–4.16 (4H, m), 3.86–3.76 (2H, m), 3.51–3.42 (2H, m), 2.50–2.39 (2H, m), 1.89–1.62 (2H, m), 1.61–1.43 (4H, m), 1.36 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 136.7 (dt, J_{CP} = 6.1 Hz, J_{CF} = 10.2 Hz), 122.5 (dt, J_{CP} = 13.0 Hz, J_{CF} = 21.2 Hz), 116.7 (dt, J_{CP} = 220.1 Hz, J_{CF} = 258.8 Hz), 98.7, 65.6, 64.5 (d, J_{CP} = 6.5 Hz), 62.2, 32.4, 30.5, 25.3, 19.4, 16.3 (d, J_{CP} = 5.2 Hz); ^{31}P NMR ($CDCl_3$) δ 7.07 (t, J_{PF} = 114.5 Hz); ^{19}F NMR ($CDCl_3$) δ −45.75 (2F, dddt, J_{FP} = 114.5 Hz, J_{FH} = 12.4, 6.4, 3.4 Hz), IR (film) 1672, 1271 cm^{-1}; EIMS m/z 343 ($M^+ + 1$). Anal. calcd for $C_{14}H_{25}F_2O_5P$: C, 49.12; H, 7.36. Found: C, 48.74; H, 7.38.

Diethyl [(*Z*)-1,1-difluoro-5-(tetrahydro-2*H*-2-pyranoyloxy)-2-pentenyl]phosphonate (*Z*)-7. Prepared from (*Z*)-**5** in an analogous manner to that for the preparation of (*E*)-**7**. Yield: 93%; an oil; ^{1}H NMR ($CDCl_3$) δ 6.13–6.04 (1H, m), 5.68–5.55 (1H, m), 4.60 (1H, dd, J = 2.7, 4.3 Hz), 4.32–4.22 (4H, m), 3.88–3.78 (2H, m), 3.53–3.45 (2H, m), 2.69–2.60 (2H, m), 1.86–1.78 (1H, m), 1.74–1.68 (1H, m), 1.59–1.50 (4H, m), 1.38 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 138.4, 121.0 (dt, J_{CP} = 12.9 Hz, J_{CF} = 21.7 Hz), 117.5 (dt, J_{CP} = 219.1 Hz, J_{CF} = 259.5 Hz), 98.1, 65.7, 64.0, 61.6, 30.1, 28.6, 24.9, 19.0, 15.9; ^{31}P NMR ($CDCl_3$) δ 7.36 (t, J_{PF} = 114.0 Hz); ^{19}F NMR ($CDCl_3$) δ −42.24 (2F, dddt, J_{FP} = 114.0 Hz, J_{FH} = 15.8, 2.9, 2.9 Hz); IR (film) 1658, 1273 cm^{-1}; EIMS m/z 343 ($M^+ + 1$). Anal. calcd for $C_{14}H_{25}F_2O_5P$: C, 49.12; H, 7.36. Found: C, 48.85; H, 7.15.

Diethyl [(*E*)-1,1-difluoro-5-hydroxy-2-pentenyl]phosphonate (*E*)-8. A stirred solution of (*E*)-**7** (3.7 g, 11 mmol) in MeOH (35 mL) was treated with *p*-TsOH·H_2O (102 mg, 0.5 mmol) at room temperature for 18 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 = 2:1) to give (*E*)-**8** (2.3 g, 82%) as an oil. ^{1}H NMR ($CDCl_3$) δ 6.35–6.26 (1H, m) 5.84–5.73 (1H, m), 4.31–4.21 (4H, m), 3.74 (2H, q, J = 6.0 Hz), 2.46–2.40 (2H, m), 1.37 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 137.0 (dt, J_{CP} = 5.8 Hz, J_{CF} = 10.2 Hz), 122.2 (dt, J_{CP} = 13.2 Hz, J_{CF} = 21.2 Hz), 116.5 (dt, J_{CP} = 220.5 Hz, J_{CF} = 258.9 Hz), 64.5 (d, J_{CP} = 6.7 Hz), 60.4, 35.1, 16.0 (d, J_{CP} = 5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 7.09 (t, J_{PF} = 114.1 Hz); ^{19}F NMR ($CDCl_3$) δ −46.11 (2F, dddt, J_{FP} = 114.1 Hz, J_{FH} = 12.4, 2.6, 2.6 Hz); IR (film) 3437, 1671, 1260 cm^{-1}; EIMS m/z 259 ($M^+ + 1$). Anal. calcd for $C_9H_{17}F_2O_4P$: C, 41.86; H, 6.64. Found: C, 41.63; H, 6.64.

Diethyl [(*Z*)-1,1-difluoro-5-hydroxy-2-pentenyl]phosphonate (*Z*)-8. Prepared from (*Z*)-**7** in an analogous manner to that for the preparation of (*E*)-**8**. Yield: 68%; an oil; ^{1}H NMR ($CDCl_3$) δ 6.13–6.04 (1H, m), 5.72–5.60 (1H, m), 4.33–4.24 (4H, m), 3.76 (2H, t, J = 5.8 Hz), 2.69–2.64 (2H, m), 1.39 (6H, t, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 139.9, 121.8–121.3 (m), 117.6 (dt, J_{CP} = 219.0 Hz, J_{CF} = 260.0 Hz), 64.7 (d, J_{CP} = 6.5 Hz), 61.2, 31.8, 16.1 (d, J_{CP} = 4.4 Hz); ^{31}P NMR ($CDCl_3$) δ 7.51 (t, J_{PF} = 114.2 Hz); ^{19}F NMR ($CDCl_3$) δ −40.59 (2F, ddd with small splits, J_{FP} = 114.2 Hz, J_{FH} = 14.7, 2.7 Hz); IR (film) 3443, 1658, 1261 cm^{-1}; EIMS m/z 259 ($M^+ + 1$). Anal. calcd for $C_9H_{17}F_2O_4P$: C, 41.86; H, 6.64. Found: C, 41.61; H, 6.58.

(*E*)-5-(Diethoxyphosphoryl)-5,5-difluoro-3-pentenyl 4-methyl-1-benzenesulfonate (*E*)-9. To a stirred solution of (*E*)-**8** (1.7 g, 4.8 mmol) in CH_2Cl_2 (18 mL) was added successively *p*-toluenesulfonyl chloride (1.2 g, 6.3 mmol) and triethylamine (1.4 mL, 9.7 mmol) at room temperature. The mixture was stirred for 40 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 = 4:1) gave (*E*)-**9** (2.4 g, 87%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.76 (2H, d, J = 8.2 Hz), 7.33 (2H, d, J = 8.2 Hz), 6.17–6.09 (1H, m), 5.71 (1H, q with small splits, J = 13.9 Hz), 4.28–4.16 (4H, m), 4.08 (2H, t, J = 6.3 Hz), 2.51–2.48 (2H, m), 2.42 (3H, s), 1.34 (6H, t, J = 7.1 Hz); ^{13}C NMR δ 144.7, 133.7, 132.4, 129.6, 127.6, 124.2–123.6 (m), 116.1 (dt, J_{CP} = 219.4 Hz, J_{CF} = 259.3 Hz), 67.9, 64.4, 31.1, 21.2, 16.0; ^{31}P NMR ($CDCl_3$) δ 6.63 (t, J_{PF} = 112.7 Hz); ^{19}F NMR ($CDCl_3$) δ −46.44 (2F, dddt, J_{FP} = 112.7 Hz, J_{FH} = 12.8, 3.3, 2.7 Hz); IR (film) 1361, 1271 cm^{-1}; EIMS m/z 412 (M^+). Anal. calcd for $C_{16}H_{23}F_2O_6PS$: C, 46.60; H, 5.62. Found: C, 46.56; H, 5.68.

(*Z*)-5-(Diethoxyphosphoryl)-5,5-difluoro-3-pentenyl 4-methyl-1-benzenesulfonate (*Z*)-9. Prepared from (*Z*)-**8** in an analogous manner to that for the preparation of (*E*)-**9**. Yield: 84%; an oil; ^{1}H NMR ($CDCl_3$) δ 7.79 (2H, d, J = 8.3 Hz), 7.35 (2H, d, J = 8.3 Hz), 5.96–5.89 (1H, m), 5.69–5.57 (1H, m), 4.30–4.20 (4H, m), 4.09 (2H, t,

J=6.3 Hz), 2.75–2.68 (2H, m), 2.45 (3H, s), 1.36 (6H, t, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 144.8, 135.6 (dt, J_{CP}=6.6 Hz, J_{CF}=7.0 Hz), 132.7, 129.7, 127.7, 123.1 (dt, J_{CP}=12.8 Hz, J_{CF}=22.5 Hz), 117.5 (dt, J_{CP}=218.8 Hz, J_{CF}=259.7 Hz), 69.0, 64.5 (d, J_{CP}=6.7 Hz), 27.9, 21.4, 16.2 (d, J_{CP}=5.2 Hz); ^{31}P NMR ($CDCl_3$) δ 6.99 (t, J_{PF}=112.9 Hz); ^{19}F NMR ($CDCl_3$) δ −42.55 (2F, dddt, J_{FP}=112.9 Hz, J_{FH}=15.4, 3.0, 2.6 Hz); IR (film) 1361, 1271 cm^{-1}; EIMS m/z 412 (M^+). Anal. calcd for $C_{16}H_{23}F_2O_6PS$: C, 46.60; H, 5.62. Found: C, 46.46; H, 5.64.

Diethyl [(*E*)-5-(2-amino-6-chloro-9*H*-9-purinyl)-1,1-difluoro-2-pentenyl]phosphonate (*E*)-10a. To a stirred solution of (*E*)-**9** (1.0 g, 2.5 mmol) in DMF (11 mL) was added successively 2-amino-6-chloropurine (540 mg, 3.2 mmol) and K_2CO_3 (676 mg, 4.9 mmol) at room temperature. The mixture was stirred at the same temperature for 24 h and was partitioned between $CHCl_3$ and H_2O. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give a residue. Column chromatography of the residue on silica gel eluted with $CHCl_3$:MeOH = 300:1 gave (*E*)-**10a** (500 mg, 50%): mp 95–97 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (1H, s, C8-H), 6.34–6.20 (1H, m), 5.73 (1H, dt with small splits, J=13.1, 12.0 Hz,), 5.11 (2H, broad s), 4.28–4.14 (6H, m), 2.73–2.72 (2H, m), 1.35 (6H, t, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 159.2, 153.6, 151.1, 142.1, 134.6 (dt, J_{CP}=5.6 Hz, J_{CF}=10.2 Hz), 125.0, 124.5 (dt, J_{CP}=13.2 Hz, J_{CF}=30.4 Hz), 116.2 (dt, J_{CP}=220.1 Hz, J_{CF}=259.3 Hz), 64.6 (d, J_{CP}=6.8 Hz), 42.4, 32.0, 16.2 (d, J_{CP}=5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 6.60 (t, J_{PF}=113.0 Hz); ^{19}F NMR ($CDCl_3$) δ −46.26 (2F, dddt, J_{FP}=113.0 Hz; J_{FH}=12.0, 3.0, 2.6 Hz); IR (KBr) 3328, 1264 cm^{-1}; EIMS m/z 409 (M^+). Anal. calcd for $C_{14}H_{19}ClF_2N_5O_3P$: C, 41.03; H, 4.67; N, 17.09. Found: C, 40.70; H, 4.77; N, 16.94. Further elution with $CHCl_3$:MeOH = 100:1 gave the corresponding *N*-7 isomer (200 mg, 20%). The physical data of the *N*-7 isomer was not steadily collected except ^{1}H NMR (300 MHz, $CDCl_3$). The signal due to the C8-proton of the *N*-7 isomer was observed at δ 8.02.[10]

Diethyl [(*Z*)-5-(2-amino-6-chloro-9*H*-9-purinyl)-1,1-difluoro-2-pentenyl]phosphonate (*Z*)-10a. Prepared from (*Z*)-**9** as colorless crystals (mp 116–117 °C) along with the corresponding *N*-7 isomer in an analogous manner to that for the preparation of (*E*)-**10a**. Yield: 34%; ^{1}H NMR ($CDCl_3$) δ 7.83 (1H, s, C8-H), 5.94–5.86 (1H, m), 5.72–5.61 (1H, m), 5.06 (2H, broad s), 4.30–4.19 (4H, m), 4.21 (2H, t, J=7.4 Hz), 2.97–2.91 (2H, m), 1.37 (6H, t, J=7.1 Hz); ^{13}C NMR δ 159.1, 153.8, 151.1, 142.4, 136.4, (dt, J_{CP}=6.2 Hz, J_{CF}=7.4 Hz), 125.1, 123.9 (dt, J_{CP}=12.8 Hz, J_{CF}=22.8 Hz), 117.6 (dt, J_{CP}=218.0 Hz, J_{CF}=260.0 Hz), 64.8 (d, J_{CP}=6.8 Hz), 42.8, 28.7, 16.3 (d, J_{CP}=5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 6.91 (t, J_{PF}=111.0 Hz); ^{19}F NMR ($CDCl_3$) δ −42.25 (2F, ddd with small splits, J_{FP}=111.0 Hz, J_{FH}=13.2, 2.6 Hz); IR (KBr) 3328, 1264 cm^{-1}; EIMS m/z 409 (M^+). Anal. calcd for $C_{14}H_{19}ClF_2N_5O_3P$: C, 41.03; H, 4.67; N, 17.09. Found: C, 40.94; H, 4.79; N, 16.86. The signal due to the C8 proton of the *N*-7 isomer was observed at δ 8.04 in the ^{1}H NMR spectrum (300 MHz, $CDCl_3$).[10]

Diethyl [(*E*)-5-(6-chloro-9*H*-9-purinyl)-1,1-difluoro-2-pentenyl]phosphonate (*E*)-10b. To a stirred solution of (*E*)-**9** (992 mg, 2.4 mmol) in DMF (10 mL) was added successively 6-chloropurine (484 mg, 3.1 mmol) and K_2CO_3 (666 mg, 4.8 mmol) at room temperature. The mixture was stirred at the same temperature for 30 h. Work-up as above gave a residue. Column chromatography of the residue on silica gel eluted with $CHCl_3$:MeOH = 500:1 gave (*E*)-**10b** (579 mg, 61%) as colorless crystals (mp. 68–69 °C). ^{1}H NMR ($CDCl_3$) δ 8.76 (1H, s, C2-H), 8.13 (1H, s, C8-H), 6.31–6.23 (1H, m), 5.74–5.63 (1H, m), 4.43 (2H, t, J=6.9 Hz), 4.28–4.12 (4H, m), 2.82–2.80 (2H, m), 1.35 (6H, t, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 151.9, 151.6, 151.0, 145.1, 134.1 (dt, J_{CP}=5.6 Hz, J_{CF}=10.3 Hz), 131.5, 125.1 (dt, J_{CP}=13.2 Hz, J_{CF}=21.4 Hz), 116.2 (dt, J_{CP}=219.9 Hz, J_{CF}=259.2 Hz), 64.6 (d, J_{CP}=6.8 Hz), 43.1, 32.2, 16.3 (d, J_{CP}=5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 6.55 (t, J_{PF}=112.6 Hz); ^{19}F NMR ($CDCl_3$) δ −46.56 (2F, ddd with small splits, J_{PF}=112.6 Hz, J_{FH}=12.4, 2.6 Hz); IR (KBr) 1266 cm^{-1}; EIMS m/z 394 (M^+). Anal. calcd for $C_{14}H_{18}ClF_2N_4O_3P$: C, 42.59; H, 4.60; N, 14.19. Found: C, 42.53; H, 4.69; N, 14.24. Further elution with $CHCl_3$:MeOH = 300:1 gave the corresponding *N*-7 regioisomer (100 mg, 11%). The physical data of the *N*-7 isomer was not steadily collected except ^{1}H NMR (300 MHz, $CDCl_3$). The signals due to the C2 and C8 protons of the *N*-7 isomer were observed at δ 8.91 and 8.24, respectively.[10]

Diethyl [(*Z*)-5-(6-chloro-9*H*-9-purinyl)-1,1-difluoro-2-pentenyl]phosphonate (*Z*)-10b. Obtained from (*Z*)-**9** along with the corresponding *N*-7 isomer in an analogous manner to that for the preparation of (*E*)-**10b**. Yield: 39%; an oil; ^{1}H NMR ($CDCl_3$) δ 8.73 (1H, s, C2-H), 8.22 (1H, s, C8-H), 5.96-5.87 (1H, m), 5.72–5.60 (1H, m), 4.43 (2H, t, J=7.1 Hz), 4.30–4.17 (4H, m), 3.04–2.96 (2H, m), 1.35 (6H, t, J=7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 151.7, 150.7, 145.4, 136.0 (dt, J_{CP}=6.3 Hz, J_{CF}=7.3 Hz), 131.4, 124.3 (dt, J_{CP}=12.9 Hz, J_{CF}=22.7 Hz), 117.4 (dt, J_{CP}=217.9 Hz, J_{CF}=259.9 Hz), 77.2, 64.7 (d, J_{CP}=6.8 Hz), 43.2, 28.9, 16.3 (d, J_{CP}=5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 6.80 (t, J_{PF}=112.6 Hz); ^{19}F NMR ($CDCl_3$) δ −42.19 (2F, ddd with small splits, J_{FP}=112.6 Hz, J_{FH}=15.4, 2.3 Hz); IR (film) 1267 cm^{-1}; EIMS m/z 394 (M^+). Anal. calcd for $C_{14}H_{18}ClF_2N_4O_3P$: C, 42.59; H, 4.60; N, 14.19. Found: C, 42.57; H, 4.74; N, 14.67. The signal due to the C2 and C8

protons of the *N*-7 isomer were observed at δ 8.89 and 8.35, respectively, in the ^{1}H NMR spectrum (300 MHz, $CDCl_3$).[10]

(*E*)-5-(2-Amino-6-oxo-6,9-dihydro-1*H*-9-purinyl)-1,1-difluoro-2-pentenylphosphonic acid (*E*)-2a.[17] To a stirred solution of (*E*)-**10a** (374 mg, 0.9 mmol) in CH_2Cl_2 (5 mL) was added bromotrimethylsilane (0.6 mL, 4.6 mmol) at room temperature. The mixture was stirred for 38 h and evaporated under reduced pressure. The residue was treated with H_2O (3 mL) at room temperature for 33 h. The volatile component of the mixture was removed in vacuo. A crystalline material was collected, and washed with cold $CHCl_3$ to give (*E*)-**2a** (261 mg, 85%). Mp >300 °C; ^{1}H NMR (D_2O) δ 8.76 (1H, s), 6.12–6.04 (1H, m), 5.62–5.52 (1H, m), 4.28 (2H, t, J=6.5 Hz), 2.66–2.61 (2H, m); ^{31}P NMR (D_2O) δ 4.17 (t, J_{PF}=101.3 Hz); ^{19}F NMR (D_2O) δ −34.01 (2F, ddd, J_{FP}=101.3 Hz, J_{FH}=12.4, 2.3 Hz); IR (KBr) 3445, 1677, 1179 cm^{-1}; UV (H_2O) λ_{max} 253 nm (ε=7023); ESIMS *m/z* 336 (MH^+). High resolution FABMS *m/z* calcd for $C_{10}H_{13}F_2N_5O_4P$ (MH^+): 336.0673. Observed: 336.0660.

(*Z*)-5-(2-Amino-6-oxo-6,9-dihydro-1*H*-9-purinyl)-1,1-difluoro-2-pentenylphosphonic acid (*Z*)-2a.[17] Prepared from (*Z*)-**10a** in an analogous manner to that for the preparation of (*E*)-**2a**.; Yield: 75%; mp >300 °C; ^{1}H NMR (D_2O) δ 8.78 (1H, s), 5.85–5.75 (1H, m), 5.66–5.53 (1H, m), 4.23 (2H, t, J=6.7 Hz), 2.78–2.70 (2H, m); ^{31}P NMR (D_2O) δ 4.26 (t, J_{PF}=100.7 Hz); ^{19}F NMR (D_2O) δ −30.0 (2F, dddd, J_{FP}=100.7 Hz, J_{FH}=24.1, 16.9, 1.9 Hz); IR (KBr) 3317, 1635, 1162 cm^{-1}; UV (H_2O) λ_{max} 251 nm (ε=11238); FABMS *m/z* 336 (MH^+). High resolution FABMS *m/z* calcd for $C_{10}H_{13}F_2N_5O_4P$ (MH^+): 336.0673. Observed: 336.0666.

(*E*)-1,1-Difluoro-5-(6-oxo-6,9-dihydro-1*H*-9-purinyl)-2-pentenylphosphonic acid (*E*)-2b. Prepared from (*E*)-**10b** in an analogous manner to that for the preparation of (*E*)-**2a**. Yield: 93%; amorphous powder; ^{1}H NMR (D_2O) δ 9.10 (1H, s with small splits), 8.31 (1H, s), 6.16–6.12 (1H, m), 5.65–5.55 (1H. m), 4.52 (2H, t, J=6.4 Hz), 2.78–2.71 (2H, m); ^{13}C NMR (D_2O) δ 154.5, 149.5 (with small splits), 147.4, 139.9, 133.1, 126.5 (dt, J_{CP}= 12.2 Hz, J_{CF}=21.3 Hz), 118.2 (dt, J_{CP}=201.9 Hz, J_{CF}= 257.2 Hz), 115.9, 45.3, 31.6; ^{31}P NMR (D_2O) δ 4.12 (t, J_{PF}=101.3 Hz); ^{19}F NMR (D_2O) δ −34.16 (2F, dd, J_{FP}=101.3 Hz, J_{FH}=12.4 Hz); IR (KBr) 1708, 1180 cm^{-1}; UV (H_2O) λ_{max} 250 nm (ε=13050); FABMS *m/z* 321 (MH^+). High resolution FABMS *m/z* calcd for $C_{10}H_{12}F_2N_4O_4P$ (MH^+): 321.0564. Observed: 321.0564.

(*Z*)-1,1-Difluoro-5-(6-oxo-6,9-dihydro-1*H*-9-purinyl)-2-pentenylphosphonic acid (*Z*)-2b. Prepared from (*Z*)-**10b** in an analogous manner to that for the preparation of (*E*)-**2a**. Yield: 81%; amorphous powder; ^{1}H NMR (D_2O) δ 9.18 (1H, s with small splits), 8.38 (1H, s), 6.01–5.94 (1H, m), 5.80–5.69 (1H, m), 4.58 (2H, t, J=6.5 Hz), 2.98–2.91 (2H, m); ^{13}C NMR (D_2O) δ 154.2, 149.6–149.1 (m), 147.3, 139.6, 134.6, 125.6–125.0 (m), 119.1 (dt, J_{CP}=202.8 Hz, J_{CF}=257.3 Hz), 115.5, 45.5, 28.3; ^{31}P NMR (D_2O) δ 4.14 (t, J_{PF}=102.8 Hz); ^{19}F NMR (D_2O) δ −30.06 (2F, dd, J_{FP}=102.8 Hz, J_{FH}=16.2 Hz); IR (KBr) 1711, 1171 cm^{-1}; UV (H_2O) λ_{max} 251 nm (ε=6786); FABMS *m/z* 321 (MH^+). High resolution FABMS *m/z* calcd for $C_{10}H_{12}F_2N_4O_4P$ (MH^+): 321.0564. Observed: 321.0564.

Diethyl difluoro{(1*R,2*S**)-2-[2-(tetrahydro-2*H*-2-pyranyloxy)ethyl]cyclopropyl}methylphosphonate (±)-11.** To a stirred suspension of (*E*)-**7** (4.58 g, 13 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. This procedure was repeated to consume the starting material. Purification of the crude products by column chromatography on silica gel (hexane:EtOAc=5:1) gave (±)-**11** (4.69 g, 98% yield) as a mixture of diastereoisomers. An oil; ^{1}H NMR ($CDCl_3$) δ 4.63–4.57 (1H, m), 4.37–4.19 (4H, m), 3.91–3.77 (2H, m), 3.56–3.41 (2H, m), 1.90–1.46 (8H, m), 1.37 (6H, t, J=7.2 Hz), 1.34–1.18 ((2H, m), 1.00–0.90 (1H, m), 0.64–0.54 (1H, m); ^{13}C NMR ($CDCl_3$) δ 118.6 (dt, J_{CP}=221.0 Hz, J_{CF}=257.2 Hz), 98.4 (for one of diastereomers), 98.3 (for one of diastereomers), 66.2, 63.7 (d, J_{CP}=6.1 Hz), 61.6 (with small splits), 62.1, 32.6 (with small splits), 30.2, 25.0, 19.4 (dt, J_{CP}=18.7 Hz, J_{CF}=24.0 Hz), 19.0 (with small splits), 19.5, 15.9 (d, J_{CP}=5.4 Hz), 12.1, 7.8; ^{31}P NMR($CDCl_3$) δ 7.55 (J_{PF}= 117.4 Hz); ^{19}F NMR ($CDCl_3$) δ −49.63 (1F, dddd, J_{FF}=294.4 Hz, J_{FP}=117.4 Hz, J_{FH}=62.4, 13.1 Hz), −52.28 (1F, dddd, J_{FF}=294.4 Hz, J_{FP}=117.4 Hz, J_{FH}=104.2, 14.7 Hz); EIMS *m/z* 273 (MH^+−THP). Anal. calcd for $C_{13}H_{27}F_2O_5P$: C, 50.54; H, 7.64. Found: C, 50.36; H, 7.58.

Diethyl difluoro[(1*R,2*S**)-2-(2-hydroxyethyl)cyclopropyl]methylphosphonate (±)-12.** A stirred solution of (±)-**11** (4.3 g, 12 mmol) in MeOH (40 mL) was treated with *p*-TsOH·H_2O (114 mg, 0.6 mmol) at room temperature for 21 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=5:1) to give (±)-**12** (3.1 g, 95%) as an oil. ^{1}H NMR ($CDCl_3$) δ 4.33–4.25 (4H, m), 3.77–3.66 (2H, m), 2.06–1.97 (1H, m), 1.79 (1H, broad s), 1.41 (3H, t, J=7.0 Hz), 1.39 (3H, t,

J = 7.0 Hz), 1.32–1.17 (2H, m), 1.10–0.98 (2H, m), 0.55–0.50 (1H, m); ^{13}C NMR ($CDCl_3$) δ 119.0 (dt, J_{CP} = 228.7 Hz, J_{CF} = 256.5 Hz), 65.0 (d, J_{CP} = 6.5 Hz), 64.5 (d, J_{CP} = 7.4 Hz), 61.8, 36.0, 19.5 (dt, J_{CP} = 25.6 Hz, J_{CF} = 20.4 Hz), 16.4 (d, J_{CP} = 4.7 Hz), 13.8 (d, J_{CP} = 6.1 Hz), 7.1; ^{31}P NMR ($CDCl_3$) δ 8.36 (dd, J_{PF} = 119.2 Hz, J_{PF} = 113.0 Hz); ^{19}F NMR δ −43.73 (1F, dd, J_{FF} = 292.8 Hz, J_{FP} = 113.0 Hz), −60.47 (1F, ddd, J_{FF} = 292.8 Hz, J_{FP} = 119.2 Hz, J_{FH} = 22.4 Hz); IR (film) 3439, 1262 cm^{-1}; EIMS m/z 273 ($M^+ + 1$). Anal. calcd for $C_{10}H_{19}F_2O_4P$: C, 44.12; H, 7.04. Found: C, 44.31; H, 6.87.

2-{(1*R,2*S**)-[(Diethoxyphosphoryl)(difluoro)methyl]cyclopropyl}ethyl 4-methyl-1-benzenesulfonate (±)-14.** This compound was prepared from (±)-**12** in an analogous manner to that for the preparation of (*E*)-**9**. Yield: 81%; an oil; ^{1}H NMR ($CDCl_3$) δ 7.79 (2H, d, J = 8.34 Hz), 7.34 (2H, d, J = 8.34 Hz), 4.30–4.22 (4H, m), 4.10 (2H, t, J = 6.7 Hz), 2.45 (3H, s), 1.71–1.57 (2H, m), 1.37 (6H, t, J = 7.0 Hz), 1.29–1.12 (2H, m), 0.96–0.92 (1H, m), 0.57–0.53 (1H, m); ^{13}C NMR δ 144.8, 133.2, 129.9, 127.9, 118.7 (dt, J_{CP} = 222.1 Hz, J_{CF} = 259.4 Hz), 69.6, 64.4 (d, J_{CP} = 6.4 Hz), 32.4, 21.6, 19.9 (dt, J_{CP} = 19.1 Hz, J_{CF} = 24.3 Hz), 16.4 (d, J_{CP} = 5.0 Hz), 11.7 (d, J_{CP} = 2.5 Hz), 8.2; ^{31}P NMR ($CDCl_3$) δ 7.49 (t, J_{PF} = 116.7 Hz); ^{19}F NMR ($CDCl_3$) δ −50.83 (1F, ddd, J_{FF} = 295.3 Hz, J_{FP} = 116.7 Hz, J_{FH} = 13.9 Hz), −52.10 (1F, ddd, J_{FF} = 295.3 Hz, J_{FP} = 116.7 Hz, J_{FH} = 14.5 Hz), IR (film) 1360, 1271 cm^{-1}; EIMS m/z 426 (M^+). Anal. calcd for $C_{17}H_{25}F_2O_6PS$: C, 47.88; H, 5.91. Found: C, 47.82; H, 6.03.

2-{(1*R,2*R**)-[(Diethoxyphosphoryl)(difluoro)methyl]cyclopropyl}methyl 4-methyl-1-benzenesulfonate (±)-15.** This compound was prepared as an oil from (±)-**13** in an analogous manner to that for the preparation of (*E*)-**9**. The spectroscopic data was identical to those of an authentic sample prepared previously.[8b]

Diethyl [{(1*R,2*S**)-2-[2-(2-amino-6-chloro-9*H*-9-purinyl)ethyl]cyclopropyl}(difluoro)methyl]phosphonate (±)-16a.** Prepared from (±)-**14** along with the corresponding *N*-7 isomer in an analogous manner to that for the preparation of (*E*)-**10a**. Yield: 64%; mp 97–98 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (1H, s, C8-H), 5.10 (2H, broad s), 4.32–4.13 (6H, m), 2.04–1.95 (1H, m), 1.75–1.66 (1H, m), 1.38 (6H, t with small splits, J = 7.0 Hz), 1.33–1.29 (1H, m), 1.20–1.15 (1H, m), 1.01–0.96 (1H, m), 0.57–0.52 (1H, m); ^{13}C NMR ($CDCl_3$) δ 159.1, 153.6, 150.8, 142.5, 124.9, 118.5 (dt, J_{CP} = 221.4 Hz, J_{CF} = 259.8 Hz), 64.4 (d, J_{CP} = 8.8 Hz), 43.4, 32.4, 20.1–19.4 (m), 16.2 (d, J_{CP} = 4.4 Hz), 12.5, 8.1; ^{31}P NMR ($CDCl_3$) δ 7.31 (t, J_{PF} = 115.8 Hz); ^{19}F NMR ($CDCl_3$) −50.67 (1F, ddd, J_{FF} = 294.8 Hz, J_{FP} = 115.8 Hz, J_{FH} = 13.9 Hz), −51.76 (1F, ddd, J_{FF} = 294.8 Hz, J_{FP} = 115.8 Hz, J_{FH} = 13.9 Hz), IR (KBr) 3331, 3208, 1280 cm^{-1}; EIMS m/z 423 (M^+). Anal. calcd for $C_{15}H_{21}ClF_2N_5O_3P$: C, 42.51; H, 4.99; N, 16.53. Found: C, 42.74; H, 5.05; N, 16.31. The signal due to the C8 proton of the *N*-7 isomer were observed at δ 7.98 in the ^{1}H NMR spectrum (300 MHz, $CDCl_3$).[10]

Diethyl [{(1*R,2*S**)-2-[2-(6-chloro-9*H*-9-purinyl)ethyl]cyclopropyl}(difluoro)methyl]phosphonate (±)-16b.** Prepared from (±)-**14** along with the corresponding *N*-7 isomer in an analogous manner to that for the preparation of (*E*)-**10b**. Yield: 47%; an oil; ^{1}H NMR ($CDCl_3$) δ 8.75 (1H, s, C2-H), 8.30 (1H, s, C8-H), 4.52–4.36 (2H, m), 4.32–4.21 (4H, m), 2.18–2.10 (1H, m), 1.78–1.70 (1H, m), 1.39 (6H, t with small splits, J = 7.1 Hz), 1.35–1.26 (1H, m), 1.19–1.10 (1H, m), 1.03–0.98 (1H, m), 0.58–0.53 (1H, m); ^{13}C NMR ($CDCl_3$) δ 151.4, 151.3, 150.2, 145.6, 131.3, 118.3 (dt, J_{CP} = 221.6 Hz, J_{CF} = 259.3 Hz), 64.2 (d, J_{CP} = 5.7 Hz), 64.1 (d, J_{CP} = 6.6 Hz), 43.8, 32.3, 19.6 (dt, J = 23.8, 20.0 Hz), 16.0, 12.3, 7.8; ^{31}P NMR ($CDCl_3$) δ 7.18 (dd, J_{PF} = 117.1 Hz, J_{PF} = 111.7 Hz); ^{19}F NMR ($CDCl_3$) δ −50.49 (1F, ddd, J_{FF} = 294.8 Hz, J_{FP} = 111.7 Hz, J_{FH} = 12.8 Hz), −52.22 (1F, ddd, J_{FF} = 294.8 Hz, J_{FP} = 117.1 Hz, J_{FH} = 15.1 Hz); IR (film) 1594, 1562, 1268 cm^{-1}; EIMS m/z 408 (M^+). Anal. calcd for $C_{15}H_{20}ClF_2N_4O_3P$: C, 44.07; H, 4.93; N, 13.71. Found: C, 43.57; H, 5.30; N, 13.68. The signals due to the C2 and C8 protons of the *N*-7 isomer were observed at δ 8.89 and 8.47, respectively, in the ^{1}H NMR spectrum (300 MHz, $CDCl_3$).[10]

Diethyl [{(1*R,2*R**)-2-[2-(2-amino-6-chloro-9*H*-9-purinyl)methyl]cyclopropyl}(difluoro)methyl]phosphonate (±)-17a.** Prepared as crystals (mp 92–93 °C) from (±)-**15** in an analogous manner to that for the preparation of (*E*)-**10a**. The spectroscopic data was identical to those of an authentic sample prepared previously.[8b]

Diethyl [{(1*R,2*R**)-2-[2-(6-chloro-9*H*-9-purinyl)methyl]cyclopropyl}(difluoro)methyl]phosphonate (±)-17b.** Prepared as crystals (mp 63–65 °C) from (±)-**15** in an analogous manner to that for the preparation of (*E*)-**10b**. The spectroscopic data was identical to those of an authentic sample prepared previously.[8b]

{(1*R,2*S**)-2-[2-(2-Amino-6-oxo-6,9-dihydro-1*H*-9-purinyl)ethyl]cyclopropyl}(difluoro)methylphosphonic acid (±)-3a.** Prepared as an amorphous powder from (±)-**16a** in an analogous manner to that for the preparation of (*E*)-**2a**. Yield: 85%; ^{1}H NMR (D_2O) δ 8.87 (1H, s), 4.33–4.21 (2H, m), 1.93–1.86 (1H, m), 1.79–1.70 (1H, m), 1.15–1.01 (2H, m), 0.79–0.74 (1H, m), 0.43–0.38 (1H, m); ^{13}C NMR (D_2O, relative to δ 216.5 of acetone-d_6) δ 155.2, 154.6, 149.8, 137.5, 120.0 (dt, J_{CP} = 210.6 Hz, J_{CF} = 257.0 Hz), 107.6, 45.6, 31.3, 19.4 (dt, J_{CP} = 18.2 Hz, J_{CF} = 24.3 Hz), 11.8, 7.62; ^{31}P NMR (D_2O) δ 4.89 (t, J_{PF} = 104.0 Hz); ^{19}F NMR (D_2O) δ

−36.29 (1F, ddd, J_{FF} = 285.0 Hz, J_{FP} = 104.0 Hz, J_{FH} = 12.0 Hz), −40.91 (1F, ddd, J_{FF} = 285.0 Hz, J_{FP} = 104.0 Hz, J_{FH} = 17.0 Hz); IR (KBr) 3360, 1627, 1199 cm^{-1}; UV (H_2O) λ_{max} 253 nm (ε = 14757); FABMS *m/z* 350 (MH^+). High resolution FABMS *m/z* calcd for $C_{11}H_{15}F_2N_5O_4P$ (MH^+): 350.0830. Observed: 350.0830.

Difluoro{(1*R,2*S**)-2-[2-(6-oxo-6,9-dihydro-1*H*-9-purinyl)-ethyl]cyclopropyl}methylphosphonic acid (±)-3b.** Prepared from (±)-**16b** in an analogous manner to that for the preparation of (*E*)-**2a**. Yield: 83%; amorphous powder; ^1H NMR (D_2O) δ 9.11 (1H, s), 8.23 (1H, s), 4.48–4.36 (2H, m), 1.94–1.86 (1H, m), 1.82–1.73 (1H, m), 1.10–0.97 (2H, m), 0.74–0.69 (1H, m), 0.38–0.33 (1H, m); ^{13}C NMR (D_2O) δ 154.5, 149.4, 147.4, 140.0, 120.5 (dt, J_{CP} = 207.2 Hz, J_{CF} = 256.5 Hz), 115.9, 46.6, 31.7, 19.9–19.3 (m), 11.7, 7.69; ^{31}P NMR (D_2O) δ 4.86 (t, J_{PF} = 105.0 Hz); ^{19}F NMR (D_2O) δ −36.36 (1F, ddd, J_{FF} = 286.1 Hz, J_{FP} = 105.0 Hz, J_{FH} = 12.4 Hz), −40.99 (1F, ddd, J_{FF} = 286.1 Hz, J_{FP} = 104.7 Hz, J_{FH} = 16.2 Hz); IR (KBr) 3402, 1709, 1573, 1188 cm^{-1}; UV (H_2O) λ_{max} 250 nm (ε = 10460); FABMS *m/z* 335 (MH^+). High resolution FABMS *m/z* calcd for $C_{11}H_{14}F_2N_4O_4P$ (MH^+): 335.0721. Observed: 335.0721.

{(1*R,2*R**)-2-[2-(2-Amino-6-oxo-6,9-dihydro-1*H*-9-purinyl)methyl]cyclopropyl}(difluoro)methylphosphonic acid (±)-4a.** Prepared as an amorphous powder from (±)-**17a** in an analogous manner to that for the preparation of (*E*)-**2a**. The spectroscopic data of (±)-**4a** was identical to those of an authentic sample prepared previously.[8b]

Difluoro{(1*R,2*R**)-2-[2-(6-oxo-6,9-dihydro-1*H*-9-purinyl)-methyl]cyclopropyl}methylphosphonic acid (±)-4b.** Prepared from (±)-**17b** in an analogous manner to that for the preparation of (*E*)-**2a**. Yield: 81%; amorphous powder; ^1H NMR (D_2O) δ 9.22 (1H, s), 8.36 (1H, s), 4.55 (1H, dd, *J* = 14.5, 6.4 Hz), 4.25 (1H, dd, *J* = 14.5, 8.4 Hz), 1.86–1.77 (1H, m), 1.77–1.69 (1H, m), 1.21–1.16 (1H, m), 1.04–0.99 (1H, m); ^{13}C NMR (D_2O) δ 154.3, 149.6–149.1 (m), 147.3, 139.3, 119.3 (dt, J_{CP} = 205.5 Hz, J_{CF} = 257.3 Hz), 115.6, 48.9, 19.7 (dt, J_{CP} = 18.1 Hz, J_{CF} = 24.8 Hz), 14.5, 7.4; ^{31}P NMR (D_2O) δ 4.48 (t, J_{PF} = 102.6 Hz); ^{19}F NMR (D_2O) δ −38.97 (1F, ddd, J_{FF} = 287.6 Hz, J_{FP} = 102.6 Hz, J_{FH} = 13.2 Hz), −40.71 (1F, dd with small splits, J_{FF} = 287.6 Hz, J_{FP} = 102.6 Hz); IR (KBr) 3407, 1711, 1574, 1190 cm^{-1}; UV (H_2O) λ_{max} 251 nm (ε = 6615); FABMS *m/z* 321 (MH^+). High resolution FABMS *m/z* calcd for $C_{10}H_{12}F_2N_4O_4P$ (MH^+): 321.0564. Observed: 321.0564.

Assay and inhibition of PNP. PNP activity was measured by the xanthine oxidase couple assay of Stoeckler et al.[14] 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, or Sigma, St. Louis, 300 μL), 0.12 U/mL xanthine oxidase (Sigma, St. Louis, 300 μL), 3–600 mM inhibitor (1 mL), and distilled water (800 mL), 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 (1.25×10^4), 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. Inhibition mechanism of test compounds was evaluated by using a Lineweaver–Burk plot. It was verified that the compounds had no inhibitory activity toward xanthine oxidase in this assay.

Acknowledgements

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

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