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Synthesis and biological evaluation of 9-(5′,5′-difluoro-5′-phosphonopentyl)guanine derivatives for PNP-inhibitors

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Abstract—9-(5′,5′-Difluoro-5′-phosphonopentyl)guanine (**DFPP-G**) and its hypoxanthine analogue (**DFPP-H**) were modified by introducing a methyl group to all possible positions of the linker connecting a purine and difluoromethylenephosphonic acid moiety to evaluate the effects of the methyl group on inhibition against purine nucleoside phosphorylase. The methyl group on the linker affected the inhibition in a positional-dependent manner. Inhibitory potency of α-methyl and β-methyl-substituted analogues of **DFPP-H** increased by about 600- to 1000-fold upon converting to cyclopropane nucleotide analogue (±)-**4**.
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1. Introduction

Purine nucleoside phosphorylase (PNP, purine nucleoside: orthophosphate ribosyl transferase EC 2.4.2.1) is the key enzyme of the purine salvage pathway.[1] It catalyzes the reversible phosphorylation of ribo- and 2′-deoxyribo-nucleosides of guanine and hypoxanthine in higher organisms, as well as of adenine in some prokaryotes. Potent inhibitors of human and parasitic PNPs may be available for the treatment of T-cell proliferative disease such as T-cell leukemia.[1]

Since PNP accomplishes the reversible phosphorylation of the purine nucleosides via a ternary complex of an enzyme, nucleoside, and orthophosphate, compounds that contain covalently linked elements of both substrates (nucleoside and orthophosphate) in their structure are expected to act as a 'multi-substrate analogue' inhibitor for PNP. Therefore, a number of metabolically stable acyclic nucleotides containing a purine and phosphate-like moiety connected by a linker have been synthesized.[2] Of the PNP inhibitors reported, 9-(5′,5′-difluoro-5′-phosphonopentyl)guanine (**DFPP-G**), developed by Halazy et al., is one of the most potent and structurally simple inhibitors of PNPs.[3]

During our previous studies directed toward the design and synthesis of a multi-substrate analogue inhibitor for PNP based on the use of difluoromethylenephosphonic acid as a phosphate mimic, we have examined structural modification of the linker and base moieties of **DFPP-G** (Fig. 1).[4] In these studies, the α,β-unsaturated analogue **1** was found to be 3-fold more potent than **DFPP-G** in comparison with their IC_{50} values.

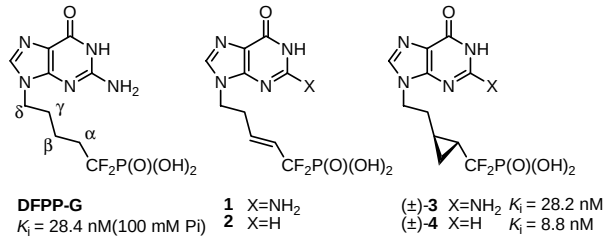

Figure 1. Multi-substrate analogue inhibitors of PNP based on a difluoro-methylenephosphonic acid as a phosphate mimic.

Keywords: PNP; Inhibitor; Multi-substrate analogues; Nucleotide analogues; Difluoromethylenephosphonic acid.
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The hypoxanthine modification of **1** leading to **2** was found to increase the binding affinity by 2-fold to PNP purified from *Cellulomonas* sp. We also found that while nucleotide analogue (±)-**3** (K_i = 28.2 nM, IC_{50} = 390 nM), incorporating a cyclopropane ring to the α,β-positions of the linker, shows approximately the same inhibition potency to **DFPP-G** (K_i = 28.4 nM, IC_{50} = 420 nM),[4a] the inhibition constants of (±)-**3** significantly decrease to K_i = 8.8 nM and IC_{50} = 70 nM, when the guanine of (±)-**3** is replaced by a hypoxanthine to give (±)-**4**.

In an effort to logically extend on the previous results, we have recently succeeded in crystallizing a binary complex with **DFPP-G** and calf-spleen PNP.[4d] High-resolution X-ray differentiation data indicated the following three important findings for structural futures of the complex. Thus, (1) **DFPP-G** acts as a multi-substrate analogue inhibitor as it binds to both nucleoside- and phosphate-binding sites (Fig. 2A). (2) The putative hydrogen bonds identified in the base-binding site indicate that the contact of guanine O^6 with Asn243 $O^{\delta 1}$ is not a direct one but is mediated by a water molecule (Fig. 2B). (3) The acyclic chain of the inhibitor is located in the ribose-binding site but makes no direct specific contacts with the protein. However, a hydrophobic amino acid residue (Phe 159) derived from the subunit of the PNP is located in a proximate position to the linker of **DFPP-G** (Fig. 2C).

On the basis of these findings, we were interested in modification of **DFPP-G** by introducing a hydrophobic functional group at the appropriate position of the linker to increase the inhibitory potency, since a hydrophobic group on the linker would be expected to interact with Phe 159 in the active site of PNP.[5] In the present study, we examined the synthesis and biological evaluation of analogous compounds of **DFPP-G** and its hypoxanthine congener (**DFPP-H**) that are modified by introducing a methyl group to all possible positions

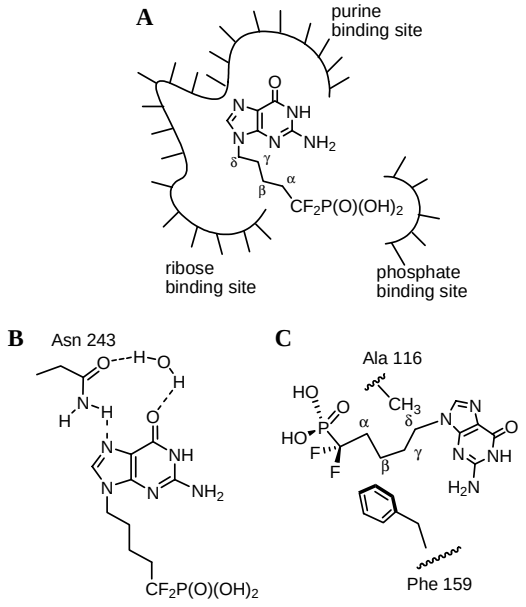
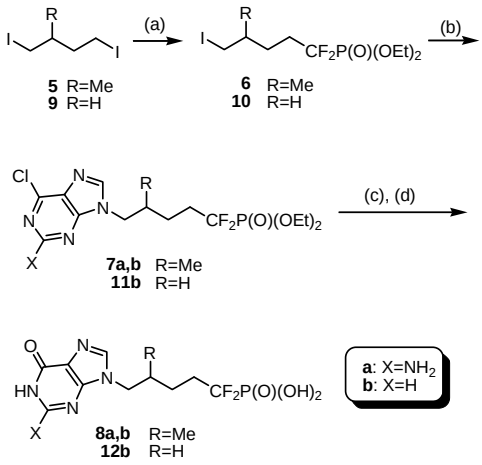


Figure 2. Interaction of **DFPP-G** with calf-spleen PNP.



Scheme 1. Reagents and conditions: (a) $LiCF_2P(O)(OEt)_2$/THF/−78 °C; (b) 2-amino-6-chloropurine or 6-chloropurine/K_2CO_3/DMF; (c) TMSBr/CH_2Cl_2; (d) 1 M HCl/reflux.

(α- to γ-position) of the linker to identify the proper position for the modifications of the linker (Fig. 1). We will now describe the results of our investigation in this paper.

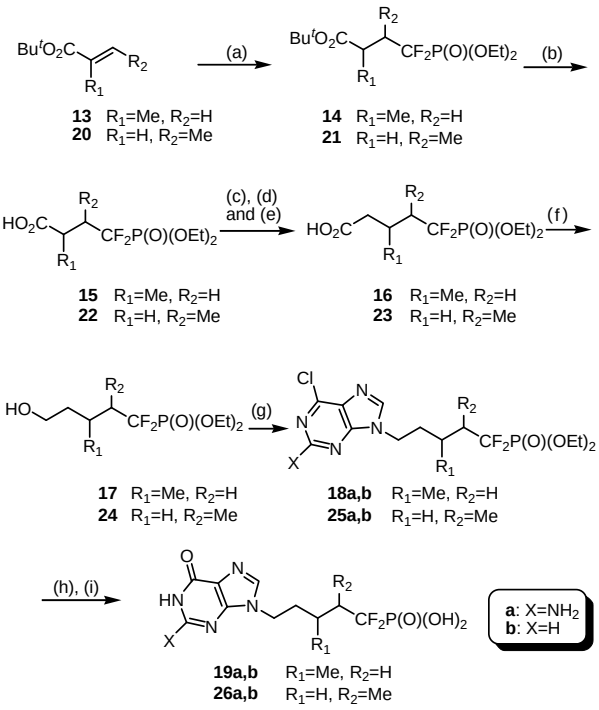
2. Results and discussion

2.1. Synthesis of novel nucleotide analogues having a methyl group at the γ-position

First, we examined the synthesis of the nucleotide analogues having a methyl group at the γ-position (Scheme 1). The reaction of 1,4-diiodo-2-methylbutane (**5**)[6] with lithium salts of diethyl difluoromethylphosphonate ($LiCF_2P(O)(OEt)_2$) occurred selectively at the sterically less-congested C-4 to give **6** in 30% yield along with small amounts (7%) of the corresponding bisphosphonate derivative. Treatment of **6** with 2-amino-6-chloropurine or 6-chloropurine in the presence of K_2CO_3 in DMF gave **7a** and **7b** in 37% and 52% yields, respectively. The removal of the ethyl-protecting group and hydrolysis of the 6-chloropurine moiety for **7a** and **7b** were performed by treatment with bromotrimethylsilane (TMSBr) in CH_2Cl_2, followed by reflux in 1 M HCl, to give the desired nucleotide analogues **8a** (**γ-Me-DFPP-G**) and **8b** (**γ-Me-DFPP-H**) as amorphous powders. A similar sequence was applied to the synthesis of **12b** (**DFPP-H**).

2.2. Synthesis of novel nucleotide analogues having a methyl group at the α- or β-position

Introduction of a methyl group into either the α- or β-position of the linker was started by the Michael reaction of $LiCF_2P(O)(OEt)_2$ to the substituted acyrylate derivatives[7] (Scheme 2). Treatment of *tert*-butyl methacrylate (**13**) with $LiCF_2P(O)(OEt)_2$ at −78 °C gave the Michael adduct **14** in 50% yield. Compound **14** was deprotected by TFA to give the free acid **15** in 87% yield. The Arndet–Eistert reaction of **15** in the usual manner gave the homologous acid **16** in 63% yield. Reduction

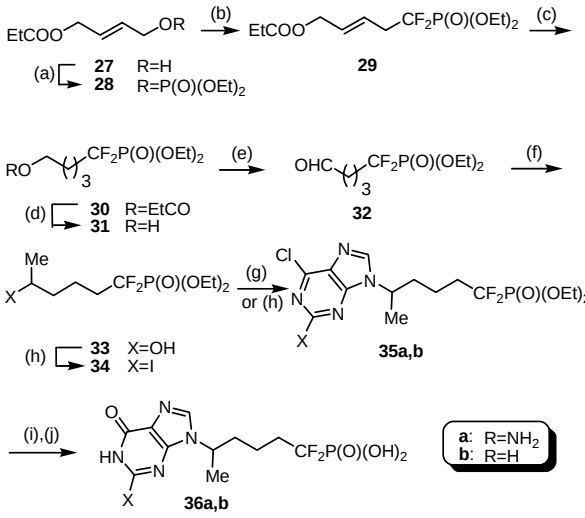


Scheme 2. Reagent and conditions: (a) $LiCF_2P(O)(OEt)_2$/THF/−78 °C; (b) TFA; (c) $SOCl_2$/reflux; (d) CH_2N_2/ether; (e) aq $AgNO_3$/1,4-dioxane; (f) $LiBH_4$/ether; (g) 6-chloropurine or 2-amino-6-chloropurine/DIAD/PPh_3/THF; (h) TMSBr/CH_2Cl_2; (g) 1 M HCl/reflux.

of **16** with $LiBH_4$ in ether gave **17** in 50% yield.[8] Compound **17** was condensed with 2-amino-6-chloropurine or 6-chloropurine under the Mitsunobu conditions to give **18a** and **18b** in 69% and 80% yields, respectively. The removal of the ethyl-protecting group and hydrolysis of the 6-chloropurine moiety for **18a** and **18b** were performed in the usual way to give the desired nucleotide analogues **19a** (**β-Me-DFPP-G**) and **19b** (**β-Me-DFPP-H**) as amorphous powders. The regioisomers **26a** (**α-Me-DFPP-G**) and **26b** (**α-Me-DFPP-H**), having a methyl group at the α-position, were synthesized from *tert*-butyl crotonate **20** in a similar sequence as shown in Scheme 2.

2.3. Synthesis of nucleotide analogues having a methyl group at the δ-position

Grignard reaction of the aldehyde **32** was applied to the synthesis of **DFPP-G** and **DFPP-H** analogues having a methyl group at the δ-position (Scheme 3). Treatment of (2*E*)-but-2-ene-1,4-diol[9] with propionic anhydride in refluxing acetone gave **27** in 60% yield.[10] After transforming to the phosphate **28**, it was coupled with $BrZnCF_2P(O)(OEt)_2$ in the presence of CuBr in DMF to give **29** in 94% yield.[11] Sequential hydrogenation (H_2/10% Pd–C) and reductive deprotection of an EtCO group with $LiBH_4$ in THF gave **31** in 45% overall yield for the two steps. Oxidation of **31** with the Dess–Martin periodinane gave the aldehyde **32**, which was alkylated with MeMgBr in THF to give **33** in 82% yield. The alcohol **33** readily reacted with 6-chloropurine under the Mitsunobu conditions to give **35b** in 53% yield. However, 2-amino-6-chloropurine was totally inert to **33** under



Scheme 3. Reagent and conditions: (a) $ClPO(OEt)_2$/pyridine; (b) $BrZnCF_2P(O)(OEt)_2$/CuBr/DMF; (c) H_2/Pd–C/ MeOH; (d) $LiBH_4$/ether; (e) Dess–Martin periodinane, CH_2Cl_2; (f) MeMgBr/THF; (g) **33**/6-chloropurine/DIAD/PPh_3/THF; (h) **34**/2-amino-6-chloropurine/K_2CO_3/DMF; (i) TMSBr/CH_2Cl_2; (j) 1 M HCl/reflux.

the Mitsunobu conditions. Then, the required 2-amino-6-chloropurine derivative **35a** was prepared in 44% yield via the reaction with iodide **34** and 2-amino-6-chloropurine in the presence of K_2CO_3 in DMF. Compounds **35a** and **35b** were transformed to the free acids **36a** (**δ-Me-DFPP-G**) and **36b** (**δ-Me-DFPP-H**) in the usual manner.

2.4. Inhibitory activity

Inhibitory activity of all compounds prepared as above against PNP purified from *Cellulomonas* sp. was determined by the method of Stoeckler et al.[12] with a minor modification and evaluated by IC_{50} and/or K_i values in comparison with that of **DFPP-G**.[13] As shown in Table 1, **α-**, **β-**, and **δ-Me-DFPP-G** have IC_{50} values ca 3- to 20-fold higher than that of **DFPP-G**. However, **γ-Me-DFPP-G** possesses a slightly lower IC_{50} value than **DFPP-G** and the inhibition is retained in the nanomolar range. The binding affinity (K_i) of **γ-Me-DFPP-G** was determined to be 13.9 nM by Dixon plot analysis. Therefore, **γ-Me-DFPP-G** was found to bind ca. 2-fold more tightly than **DFPP-G** (K_i = 28.4 nM) against the PNP. The above results suggest that the methyl group introduced at the α-, β- or δ-position of the linker of **DFPP-G** unfavorably interacted with the active site of PNP, while modification at the γ-position with an alkyl substituent may be more favorable.

As described in the introduction, cyclopropane nucleotide analogue (±)-**4** having a hypoxanthine base binds more tightly than the guanine analogue (±)-**3** against *Cellulomonas* sp. PNP. Similar substitution effects of hypoxanthine were also observed in the series of α,β-unsaturated derivatives (**1** and **2**) of **DFPP-G** and **DFPP-H**.[4a] Therefore, we first expected that **DFPP-H** would be a potent inhibitor of PNP. However, **DFPP-H** was found to be inactive at 0.1 mM concentration against *Cellulomonas* sp. PNP. While the exact reasons are unclear at present, introducing a methyl group onto the

Table 1. IC_{50} values of Me-substituted derivatives of **DFPP-G** and **DFPP-H** against PNP from *Cellulomonas* sp.[a]

Compound	IC_{50} (μM)[b]
DFPP-G	0.42 ± 0.07
α-Me-DFPP-G (26a)	9.16 ± 0.34
β-Me-DFPP-G (19a)	5.33 ± 0.24
γ-Me-DFPP-G (8a)	0.36 ± 0.01
δ-Me-DFPP-G (36a)	1.42 ± 0.02
DFPP-H (12b)	IA[c]
α-Me-DFPP-H (26b)	82.70 ± 3.25
β-Me-DFPP-H (19b)	41.65 ± 0.35
γ-Me-DFPP-H (8b)	10.62 ± 1.28
δ-Me-DFPP-H (36b)	25.76 ± 0.65

[a] Purchased from TOYOBO biochemicals.
[b] IC_{50} was determined in the presence of 0.1 mM inosine and 100 mM Pi (pH 7.5).
[c] IA indicates the sample is inactive at 0.1 mM concentration.

linker of **DFPP-H** resulted in modest PNP inhibitory activity. The trend of inhibitory activities for methyl-substituted derivatives of **DFPP-H** is approximately the same as that of **DFPP-G**. Thus, **γ-Me-DFPP-H** was found to be the most potent in the series of methyl-substituted derivatives of **DFPP-H**.

2.5. Effects of conformational restriction

As was described in the previous section, the inhibitory potency of **DFPP-H** was found to increase dramatically upon introducing a methyl group to the linker. In this section, we describe the effects of conformational restriction of **α-** and **β-Me-DFPP-H** with a cyclopropane ring on their inhibitory potency (Fig. 3).

The cyclopropane nucleotide analogue (±)-**4**, which was previously prepared and determined to inhibit *Cellulomonas* sp. PNP with IC_{50} = 70 nM and K_i = 8.8 nM, is considered to be a conformationally constrained molecule derived from **α-** and **β-Me-DFPP-H** by connecting the methyl group with β- and α-carbon of the linker, respectively (Fig. 3). Therefore, a direct comparison of (±)-**4** with **α-** and **β-Me-DFPP-H** in their IC_{50} and K_i values would be invaluable in evaluating how the cyclopropane ring affects the inhibition potency and the binding affinity against the PNP. This inspection revealed that (±)-**4** is about 600- to 1000-fold more potent than **α-** and **β-Me-DFPP-H** in their IC_{50} values. A direct comparison of **α-Me-DFPP-H** and (±)-**4** with their K_i values also revealed that (±)-**4** (K_i = 8.8 nM) binds ca 15-fold more tightly to the PNP than **α-Me-DFPP-H** (K_i = 126 nM). Thus, while real interaction of the cyclopropane moiety with the active site of the PNP remains unsolved, the high inhibitory potency of (±)-**4** would be estimated mainly by the result of conformational restriction at the α- to β-bond of **DFPP-H**.

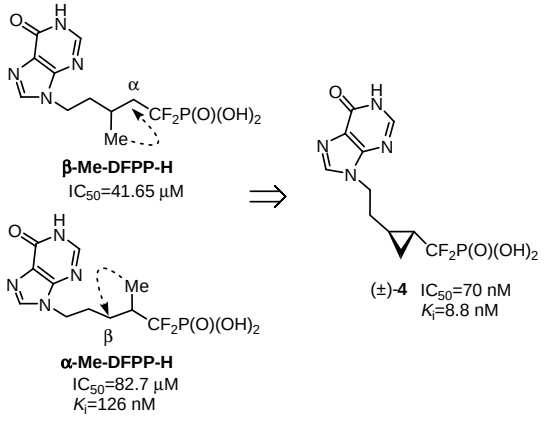


Figure 3. Comparison of (±)-**4** with **α-** and **β-Me-DFPP-H** in IC_{50} and K_i values against *Cellulomonas* sp. PNP in the presence of inosine (0.1 mM) and Pi (100 mM).

3. Conclusion

In conclusion, we have synthesized a new series of analogous compounds for **DFPP-G** and **DFPP-H**, which are modified by introducing a methyl group to the linker. The inhibitory potency of these analogues was found to be highly dependent upon the position of the methyl group. **γ-Me-DFPP-G** and **γ-Me-DFPP-H**, γ-methyl-substituted analogues of **DFPP-G** and **DFPP-H**, were found to be the most potent in the series of analogues prepared. In addition, we have shown that cyclopropane nucleotide analogue (±)-**4** is about 600- to 1000-fold more potent than **α-** and **β-Me-DFPP-H**. The information described herein may be used to design multi-substrate analogues with optimized interaction in the active sites of PNP, eventually leading to new inhibitors of PNP with potential medicinal applications.

4. Experimental

4.1. General

All reactions were carried out under a nitrogen atmosphere. NMR data were obtained on a Bruker DPX 400 using $CDCl_3$ or CD_3OD as a solvent. ^{13}C NMR (100 MHz) and ^{31}P NMR (162 MHz) were taken with broad-band ^1H decoupling. The chemical shift data for each signal on ^1H NMR (400 MHz) are expressed as relative ppm from $CHCl_3$ (δ 7.26) or CH_3OH (δ 3.30). The chemical shifts of ^{13}C are reported relative to $CDCl_3$ (δ 77.0) or CD_3OD (δ 49.0). 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 JASCO FTIR-620 spectrometer. Mass spectra were recorded on a VG Auto Spec. or a Micromass LCT using either electron impact ionization (EI) or electrospray ionization (ESI) techniques.

4.2. Diethyl 1,1-difluoro-5-iodo-4-methylpentylphosphonate (6)

To a stirred solution of diisopropylamine (2.2 mL, 15.7 mmol) in THF (22 mL) was added *n*-BuLi (10 mL of 1.58 M solution in hexane, 15.8 mmol) under ice cooling and the mixture was stirred for 30 min at 0 °C. To this solution was added at −78 °C a solution of diethyl difluoromethylphosphonate (3.0 g, 15.7 mmol) in THF

(10 mL) over 30 min. After being stirred at the same temperature for 40 min, a solution of 1,4-diiodo-2-methylbutane (**5**)[6] (4.9 g, 15.7 mmol) in THF (10 mL) was added. The mixture was stirred at −78 °C for 4 h and slowly warmed to 20 °C. The reaction mixture was then poured into saturated aqueous NH_4Cl and extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc = 5:1) to give **6** (1.82 g, 30%) as an oil. ^{1}H NMR ($CDCl_3$) δ 4.32–4.24 (4H, m), 3.24–3.14 (2H, m), 1.61–1.47 (1H, m), 1.39 (6H, t, J = 7.1 Hz), 1.01 (3H, d, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 121.2 (dt, J_{CP} = 215.8 Hz, J_{CF} = 259.6 Hz), 64.7 (d, J_{CP} = 6.6 Hz, 2C), 34.3, 31.8–31.2 (m), 20.2, 16.3 (2C, d, J_{CP} = 5.3 Hz), 16.1; ^{31}P NMR ($CDCl_3$) δ 6.65 (t, J_{PF} = 108.7 Hz); ^{19}F NMR ($CDCl_3$) δ −49.0 ∼ −49.8 (2F, m); IR (neat) 1269, 1024 cm^{-1}. ESIMS m/z 385 (MH^+). HRMS (ESI) calcd for $C_{10}H_{21}F_2PI$ (MH^+): 385.0214. Found: 385.0212.

4.3. Diethyl 5-(2-amino-6-chloro-9*H*-purin-9-yl)-1,1-difluoro-4-methylpentylphosphonate (7a)

To a stirred solution of **6** (500 mg, 1.4 mmol) and 2-amino-6-chloropurine (300 mg, 2.0 mmol) in DMF (3 mL) was added anhydrous potassium carbonate (400 mg, 3.0 mmol). The mixture was stirred at 20 °C for 23 h and poured into cold water. The mixture was extracted with ether. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel ($CHCl_3$/MeOH = 300:1) to give **7a** (210 mg, 37%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.71 (1H, s), 5.29 (2H, br s, NH_2), 4.30–4.23 (4H, m), 4.01–3.93 (2H, m), 2.32–2.19 (1H, m), 2.13–2.08 (1H, m), 1.67–1.55 (2H, m), 1.52–1.43 (1H, m), 1.37 (6H, t, J = 7.1 Hz), 0.97 (3H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 161.5, 155.4, 151.4, 144.8, 124.7, 121.9 (dt, J_{CP} = 217.4 Hz, J_{CF} = 258.7 Hz), 66.1 (2C, d, J_{CP} = 6.8 Hz), 50.0, 34.3, 32.6–32.0 (m), 25.8, 17.4, 16.9 (2C, d, J_{CP} = 5.1 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.45 (t, J_{PF} = 108.4 Hz); ^{19}F NMR ($CDCl_3$) δ −49.8 ∼ −50.2 (2F, m); IR (neat) 1614, 1557, 1264, 1028 cm^{-1}. ESIMS m/z 426 (MH^+). Anal. Calcd for $C_{15}H_{23}ClF_2N_5O_3P$: C, 42.31; H, 5.44; N, 16.45. Found: C, 42.26; H, 5.74; N, 16.90.

4.4. Diethyl 5-(6-chloro-9*H*-purin-9-yl)-1,1-difluoro-4-methylpentylphosphonate (7b)

Prepared as an oil from **6** (600 mg, 1.5 mmol) and 6-chloropurine (300 mg, 2.0 mmol) in an analogous manner for preparation of **7a**. ^{1}H NMR ($CDCl_3$) δ 8.89 (1H, s), 4.30–4.22 (4H, m), 4.13 (1H, d, J = 7.9 Hz), 4.10 (1H, d, J = 7.9 Hz), 2.26–2.16 (1H, m), 2.13–2.04 (1H, m), 1.75–1.66 (2H, m), 1.55–1.45 (1H, m), 1.38 (6H, t, J = 7.1 Hz), 0.96 (3H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 152.9, 152.7, 152.5, 146.4, 131.9, 121.0 (dt, J_{CP} = 215.6 Hz, J_{CF} = 259.8 Hz), 64.7 (d, J_{CP} = 6.3 Hz), 64.6 (d, J_{CP} = 6.5 Hz), 50.0, 33.3, 31.4–30.8 (m), 24.6, 16.9, 16.2 (2C, d, J_{CP} = 5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 6.41 (t, J_{PF} = 107.2 Hz); ^{19}F NMR ($CDCl_3$) δ −49.8 ∼ −49.6 (2F, m); IR (neat) 1614, 1557, 1264, 1028 cm^{-1}. ESIMS m/z 411 (MH^+). Anal. Calcd for $C_{15}H_{22}ClF_2N_4O_3P$: C, 43.86; H, 5.40; N, 13.64. Found: C, 44.36; H, 5.47; N, 14.08.

4.5. 5-(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-1,1-difluoro-4-methylpentylphosphonic acid (8a)

To a stirred solution of **7a** (500 mg, 1.3 mmol) in CH_2Cl_2 (8.5 mL) was added bromotrimethylsilane (0.8 mL, 6.3 mmol) at room temperature. The mixture was stirred for 44 h and evaporated under reduced pressure. The residue was treated with 1 M HCl (18 mL) under reflux. The volatile component of the mixture was removed in vacuo. Crystalline material was collected and washed with EtOAc to give **8a** (388 mg, 85%) as an amorphous powder. ^{1}H NMR (CD_3OD) δ 9.17 (1H, s), 4.27–4.22 (1H, m), 4.17–4.14 (1H, m), 2.30–2.23 (1H, m), 2.23–2.13 (2H, m), 1.74–1.64 (1H, m), 1.59–1.50 (1H, m), 1.01 (3H, d, J = 6.7 Hz); ^{13}C NMR (CD_3OD) δ 157.1, 155.0, 151.9, 138.4, 122.2 (dt, J_{CP} = 209.1 Hz, J_{CF} = 258.5 Hz), 108.7, 42.0, 34.1, 32.3–31.7 (m), 25.9, 17.1. ^{31}P NMR (CD_3OD) δ 4.77 (t, J_{PF} = 103.5 Hz); ^{19}F NMR (CD_3OD) δ −49.7 ∼ −51.5 (2F, m), IR (KBr) 3336, 1684, 1167 cm^{-1}. ESIMS m/z 352 (NH^+). HRMS (ESI) calcd for $C_{11}H_{17}N_5O_4F_2P$ (MH^+): 352.0986. Found: 352.0975.

4.6. 1,1-Difluoro-4-methyl-5-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)pentylphosphonic acid (8b)

Prepared as an amorphous powder in 85% yield from **7b** in an analogous manner for preparation of **8a**. ^{1}H NMR ($CDCl_3$) δ 9.56 (1H, s), 8.34 (1H, s), 4.47–4.32 (2H, m), 2.33–2.29 (1H, m), 2.28–2.09 (2H, m), 1.78–1.72 (1H, m), 1.57–1.53 (1H, m), 1.04 (3H, d, J = 6.7 Hz); ^{13}C NMR (CD_3OD) δ 154.6, 150.1, 149.0, 141.0, 140.3, 122.8 (dt, J_{CP} = 205.6 Hz, J_{CF} = 105.9 Hz), 42.9, 34.4, 32.1–32.0 (m), 26.0, 17.1; ^{31}P NMR (CD_3OD) δ 4.93 (t, J_{PF} = 105.9 Hz); ^{19}F NMR (CD_3OD) δ −49.2 ∼ −50.8 (2F, m); IR (KBr) 1699, 1569, 1541, 1167 cm^{-1}. ESIMS m/z 337 (MH^+). HRMS (ESI) calcd for $C_{11}H_{16}N_4O_4F_2P$ (MH^+): 337.0877. Found: 337.0866.

4.7. Diethyl 5-(6-chloro-9*H*-purine-9yl)-1,1-difluoropentylphosphonate (11b)

To a stirred solution of **10**[3] (800 mg, 2.1 mmol) and 6-chloropurine (400 mg, 2.7 mmol) in DMF (4 mL) was added anhydrous potassium carbonate (600 mg, 4.2 mmol). The mixture was stirred at 20 °C for 23 h and poured into cold water. The mixture was extracted with ether. The combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel ($CHCl_3$/MeOH = 300:1) to give **11b** (500 mg, 60%) as an oil. ^{1}H NMR ($CDCl_3$) δ 8.75 (1H, s), 8.13 (1H, s), 4.34–4.31 (2H, m), 4.29–4.22 (4H, m), 2.19–2.06 (2H, m), 2.04–1.99 (2H, m), 1.68–1.62 (2H, m), 1.37 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 152.5, 151.6, 149.3, 146.0, 132.1, 120.9 (dt, J_{CP} = 215.6 Hz, J_{CF} = 259.9 Hz), 64.7 (d, J_{CP} = 6.8 Hz, 2*C*), 44.1, 34.4–32.9 (m), 29.4, 17.9, 16.2 (d, J_{CP} = 5.3 Hz, 2C); ^{31}P NMR ($CDCl_3$) δ 7.05 (t, J_{PF} = 107.1 Hz); ^{19}F NMR ($CDCl_3$) δ

−48.9 ~ −49.3 (2F, m); IR (neat) 1593, 1560, 1266, 1024 cm^{-1}. ESIMS *m/z* 397 (MH^+). Anal. Calcd for $C_{14}H_{20}ClF_2N_4O_3P$: C, 42.38; H, 5.08; N, 14.12. Found: C, 41.37; H, 4.93; N, 13.62.

4.8. 1,1-Difluoro-5-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)pentylphosphonic acid (12b)

To a stirred solution of **11b** (500 mg, 1.3 mmol) in CH_2Cl_2 (8.5 mL) was added bromotrimethylsilane (0.8 mL, 6.3 mmol) at room temperature. The mixture was stirred for 44 h and evaporated under reduced pressure. The residue was treated with 1 M HCl (18 mL) under reflux. The volatile component of the mixture was removed in vacuo. Crystalline material was collected and washed with EtOAc to give **12b** (356 mg, 85%) as an amorphous powder. ^1H NMR (CD_3OD) δ 9.20 (1H, s), 8.27 (1H, s), 4.48–4.44 (2H, m), 2.20–2.13 (2H, m), 2.12–2.04 (2H, m), 1.73–1.63 (2H, m); ^{13}C NMR (CD_3OD) δ 155.4, 151.3, 149.8, 141.5, 127.1–120.5 (m), 117.9, 47.3, 34.7–34.4 (m), 30.3, 19.4; ^{31}P NMR (CD_3OD) δ 4.91 (t, J_{PF} = 105.0 Hz); ^{19}F NMR (CD_3OD) δ −49.7 ~ −50.3 (2F, m); IR (KBr) 1684, 1559, 1541 cm^{-1}. ESIMS *m/z* 323 (MH^+); HRMS (ESI) calcd for $C_{10}H_{14}N_4O_4F_2P$ (MH^+): 323.0721. Found: 323.0714.

4.9. *tert*-Butyl 4-(diethoxyphosphoryl)-4,4-difluoro-2-methylbutanoate (14)

To a solution of LDA [prepared from 11.8 mL (84.4 mmol) of diisopropylamine and 54 mL (84.4 mmol) of 1.58 M hexane solution of *n*-BuLi at 0 °C] in THF (140 mL) was added diethyl difluoromethylphosphonate (13.0 g, 70.3 mmol) in THF (20 mL) at −78 °C. After stirring was continued for 40 min at the same temperature, to this solution was added *tert*-butyl methacrylate (**13**) (10.0 g, 70.3 mmol) in THF (20 mL) at −78 °C. After the stirring was continued for 4 h at the same temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The resulting residue was chromatographed on silica gel (hexane/AcOEt = 5:1) to give **14** (11.7 g, 50%) as an oil. ^1H NMR ($CDCl_3$) δ 4.32–4.22 (4H, m), 2.82–2.77 (1H, m), 2.69–2.51 (1H, m), 2.08–2.01 (1H, m), 1.44 (9H, s), 1.38 (6H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 175.2, 120.7 (dt, J_{CP} = 215.9 Hz, J_{CF} = 260.3 Hz), 80.7, 64.7 (2C, d, J_{CP} = 6.0 Hz), 37.2–36.6 (m), 33.9–33.8 (m), 27.8 (3C), 18.3, 16.2 (d, J_{CP} = 5.3 Hz, 2*C*); ^{31}P NMR ($CDCl_3$) δ 7.02 (t, J_{PF} = 107.5 Hz); ^{19}F NMR ($CDCl_3$) δ −48.5 ~ −49.0 (2F, m); IR(neat) 1731, 1275, 1159, 1024 cm^{-1}. ESIMS *m/z* 353 (MNa^+). HRMS (ESI) calcd for $C_{13}H_{25}O_5F_2NaP$ (MNa^+): 353.1305. Found: 353.1296.

4.10. 4-(Diethoxyphosphoryl)-4,4-difluoro-2-methylbutanoic acid (15)

A mixture of **14** (10.0 g, 30.2 mmol) and trifluoroacetic acid (23.1 mL, 302.1 mmol) in CH_2Cl_2 (150 mL) was stirred at room temperature for 16 h and evaporated under reduced pressure. The residue was chromatographed on silica gel (hexane/acetone = 1:1) to give **15** (7.2 g, 87%) as an oil. ^1H NMR ($CDCl_3$) δ 4.32–4.25 (4H, m), 3.00–2.96 (1H, m), 2.74–2.60 (1H, m), 2.18–2.04 (1H, m), 1.39 (6H, t, J = 7.1 Hz), 1.33 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$) δ 180.6, 120.7 (dt, J_{CP} = 217.7 Hz, J_{CF} = 260.4 Hz), 65.2 (d, J_{CP} = 6.2 Hz), 62.9 (d, J_{CP} = 6.2 Hz), 37.0–36.5 (m), 32.9, 18.2, 16.2 (2C, d, J_{CP} = 5.4 Hz); ^{31}P NMR ($CDCl_3$) δ 6.74 (t, J_{PF} = 107.0 Hz); ^{19}F NMR ($CDCl_3$) δ −48.0 (1F, dddd, J_{FF} = 297.4 Hz, J_{FP} = 107.0 Hz, J_{FH} = 28.6, 12.8 Hz), −49.3 (1F, dddd, J_{FF} = 297.4 Hz, J_{FP} = 107.0 Hz, J_{FH} = 27.1, 11.7 Hz); IR (neat) 1732, 1255, 1025 cm^{-1}. ESIMS *m/z* 297 (MNa^+). Anal. Calcd for $C_9H_{17}F_2O_5P$: C, 39.42; 6.25. Found: C, 39.26; H, 6.30.

4.11. 5-(Diethoxyphosphoryl)-5,5-difluoro-3-methylpentanoic acid (16)

A mixture of **15** (2 g, 7.3 mmol) and $SOCl_2$ (0.8 mL, 10.9 mmol) was heated under reflux for 2 h. The volatile component of the mixture was removed in vacuo to give an oil: ^1H NMR ($CDCl_3$) δ 4.33–4.25 (4H, m), 3.38–3.33 (1H, m), 2.80–2.71 (1H, m), 2.21–2.14 (1H, m), 1.44 (3H, d, J = 7.1 Hz), 1.39 (6H, t, J = 7.1 Hz). The residue was dissolved in Et_2O (5 mL) and treated with a large excess of ethereal solution of CH_2N_2, prepared from *N*-methyl-*N*-nitrosourea (5 g, 48.5 mmol) in a biphasic mixture of 50% aqueous KOH solution (30 mL) and ether (32 mL). The mixture was stirred at room temperature for 12 h and evaporated. The residue was dissolved in 1,4-dioxane (10 mL) and 10% aqueous $AgNO_3$ solution (10 mL), and heated under reflux for 2 h. After cooling to room temperature, the reaction was quenched with 1 M aqueous HCl solution. The resulting precipitate was filtered through a pad of Celite and the filtrate was extracted with EtOAc. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc = 2:1) to give **16** (2 g, 63%) as an oil. ^1H NMR ($CDCl_3$) δ 4.31–4.24 (4H, m), 2.88–2.49 (2H, m), 2.32–2.11 (1H, m), 2.06–1.97 (2H, m), 1.38 (6H, t, J = 7.1 Hz), 1.12 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 177.9, 121.6 (dt, J_{CP} = 216.8 Hz, J_{CF} = 260.4 Hz), 65.2 (d, J_{CP} = 6.4 Hz), 65.0 (d, J_{CP} = 6.8 Hz), 41.4, 39.6–39.1 (m), 24.2, 20.7, 16.3 (2C, d, J_{CP} = 5.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.12 (t, J_{PF} = 107.8 Hz); ^{19}F NMR ($CDCl_3$) δ −46.9 (1F, dddd, J_{FF} = 312.8 Hz, J_{FP} = 107.8 Hz, J_{FH} = 27.9, 14.7 Hz), −48.2 (1F, dddd, J_{FF} = 312.8 Hz, J_{FP} = 107.8 Hz, J_{FH} = 25.6, 12.8 Hz); IR (neat) 1731, 1254, 1025 cm^{-1}. ESIMS *m/z* 311 (MNa^+). HRMS (ESI) calcd for $C_{10}H_{19}O_5F_2NaP$ (MNa^+): 311.0836. Found: 311.0829.

4.12. Diethyl 1,1-difluoro-5-hydroxy-3-methylpentylphosphonate (17)

To a stirred solution of **16** (2.4 g, 8.4 mmol) in ether (84 mL) was added $LiBH_4$ (200 mg, 9.2 mmol) at 0 °C. The mixture was stirred at room temperature for 12 h

and quenched with water. The mixture was extracted with $CHCl_3$. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc = 1:1) to give **17** (1.2 g, 50%) as an oil. ^{1}H NMR ($CDCl_3$) δ 4.31–4.23 (4H, m), 3.74–3.64 (2H, m), 2.22–2.14 (2H, m), 1.98–1.86 (1H, m), 1.68–1.63 (1H, m), 1.54–1.49 (1H, m), 1.36 (6H, t, J = 7.1 Hz), 1.06 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 121.8 (dt, J_{CP} = 215.4 Hz, J_{CF} = 260.3 Hz), 64.6 (2C, d, J_{CP} = 6.8 Hz), 60.0, 40.4–39.9 (m), 40.1, 23.7, 16.2 (2C, d, J_{CP} = 5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 7.61 (t, J_{PF} = 109.0 Hz); ^{19}F NMR ($CDCl_3$) δ −44.5 (1F, dddd, J_{FF} = 297.0 Hz, J_{FP} = 109.0 Hz, J_{FH} = 27.9, 13.6 Hz), −48.0 (1F, dddd, J_{FF} = 297.0 Hz, J_{FP} = 109.0 Hz, J_{FH} = 27.1, 14.7 Hz); IR (neat) 3447, 1262, 1026 cm^{-1}. ESIMS *m/z* 275 (MH^+). Anal. Calcd for $C_{10}H_{21}F_2O_4P$: C, 43.8; H, 7.72. Found: C, 43.63; H, 7.66.

4.13. Diethyl 5-(2-amino-6-chloro-9*H*-purin-9-yl)-1,1-difluoro-3-methylpentylphosphonate (18a)

To a stirred solution of **17** (900 mg, 3.4 mmol), 2-amino-6-chloropurine (700 mg, 4.4 mmol), and triphenylphosphine (1.1 g, 4.4 mmol) in THF (33 mL) was added diisopropyl azodicarboxylate (1.0 mL, 5.0 mmol) at 20 °C. The mixture was stirred at 20 °C for 12 h. The volatile components of the mixture were evaporated. The residue was chromatographed on silica gel (EtOAc) to give **18a** (1.0 g, 60%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.76 (1H, s), 5.22 (2H, br s, NH_2), 4.30–4.23 (4H, m), 4.19–4.06 (2H, m), 2.46–2.32 (1H, m), 2.04–1.85 (3H, m), 1.83–1.78 (1H, m), 1.38 (6H, t, J = 7.1 Hz), 1.11 (3H, d, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 161.5, 155.3, 151.4, 144.4, 124.8, 122.4 (dt, J_{CP} = 217.4 Hz, J_{CF} = 259.5 Hz), 66.1 (2C, d, J_{CP} = 6.9 Hz), 42.9, 41.1–40.8 (m), 38.0, 25.9, 20.6, 16.7 (2C, d, J_{CP} = 5.1 Hz); ^{31}P NMR ($CDCl_3$) δ 7.30 (t, J_{PF} = 107.0 Hz); ^{19}F NMR ($CDCl_3$) δ −45.3 (1F, dddd, J_{FF} = 298.1 Hz, J_{FP} = 107.0 Hz, J_{FH} = 28.2, 10.5 Hz), −48.0 (1F, dddd, J_{FF} = 298.1 Hz, J_{FP} = 107.0 Hz, J_{FH} = 29.4, 15.1 Hz); IR (neat) 3326, 1614, 1559, 1262, 1024 cm^{-1}. ESIMS *m/z* 426 (MH^+). HRMS (ESI) calcd for $C_{15}H_{24}N_5O_3F_2PCl$ (MH^+): 426.1273. Found: 426.1286.

4.14. Diethyl 5-(6-chloro-9*H*-purin-9-yl)-1,1-difluoro-3-methylpentylphosphonate (18b)

To a stirred solution of **17** (900 mg, 3.1 mmol), 6-chloropurine (600 mg, 4.0 mmol), and triphenylphosphine (1.1 g, 4.0 mmol) in THF (31 mL) was added diisopropyl azodicarboxylate (0.9 mL, 4.7 mmol) at room temperature. The mixture was stirred at 20 °C for 12 h and the volatile components of the mixture were removed in vacuo. The residue was chromatographed on silica gel (EtOAc) to give **18b** (1.0 g, 80%) as an oil. ^{1}H NMR ($CDCl_3$) δ 8.74 (1H, s), 8.16 (1H, s), 4.36–4.32 (2H, m), 4.29–4.22 (4H, m), 2.18–1.99 (4H, m), 1.90–1.85 (1H, m), 1.37 (6H, t, J = 7.1 Hz), 1.14 (3H, d, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$) δ 152.6, 152.5, 151.5, 145.9, 132.1, 121.5 (dt, J_{CP} = 215.3 Hz, J_{CF} = 260.4 Hz), 64.8 (2C, d, J_{CP} = 4.6 Hz), 42.3, 40.2–39.6 (m), 37.0, 24.6, 20.5, 16.3 (2C, d, J_{CP} = 5.1 Hz); ^{31}P NMR ($CDCl_3$) δ 7.05 (t, J_{PF} = 107.1 Hz); ^{19}F NMR ($CDCl_3$) δ −47.1 ∼ −47.8 (2F, m); IR (neat) 1593, 1558, 1267, 1026 cm^{-1}. ESIMS *m/z* 411 (MH^+). HRMS (ESI) calcd for $C_{15}H_{23}N_4O_3F_2Cl$ (MH^+): 411.1164. Found: 411.1169.

4.15. 5-(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-1,1-difluoro-3-methylpentylphosphonic acid (19a)

Prepared as an amorphous powder from **18a** in 87% yield in an analogous manner for preparation of **8a**. ^{1}H NMR (CD_3OD) δ 9.10 (1H, s), 4.32 (2H, t, J = 7.0 Hz), 2.31–2.22 (1H, m), 2.12–1.87 (4H, m), 1.17 (3H, d, J = 6.0 Hz); ^{13}C NMR δ 156.8, 154.8, 151.3, 138.1, 122.4 (dt, J_{CP} = 210.6 Hz, J_{CF} = 258.4 Hz), 108.5, 44.5, 40.8–40.2 (m), 37.4, 25.8, 20.8; ^{31}P NMR δ 4.92 (CD_3OD) (t, J_{PF} = 106.4 Hz); ^{19}F NMR (CD_3OD) δ −47.1 (1F, dddd, J_{FF} = 294.0 Hz, J_{FP} = 106.4 Hz, J_{FH} = 29.0, 12.0 Hz), −49.5 (1F, dddd, J_{FF} = 294.0 Hz, J_{FP} = 106.4 Hz, J_{FH} = 27.5, 12.0 Hz); IR (KBr) 3365, 1685, 1169 cm^{-1}. ESIMS *m/z* 352 (MH^+). HRMS (ESI) calcd for $C_{11}H_{17}N_5O_4F_2P$ (MH^+): 352.0986. Found: 352.0985.

4.16. 1,1-Difluoro-3-methyl-5-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)pentylphosphonic acid (19b)

Prepared as an amorphous powder from **18b** in 86% yield in an analogous manner for preparation of **8a**. ^{1}H NMR (CD_3OD) δ 8.27 (1H, s), 4.47 (2H, t, J = 7.2 Hz), 2.20–1.90 (5H, m), 1.18 (3H, d, J = 6.1 Hz); ^{13}C NMR (CD_3OD) δ 154.7, 150.0, 148.8, 140.8, 122.7 (dt, J_{CP} = 208.6 Hz, J_{CF} = 258.9 Hz), 117.5, 45.2, 41.0–40.5 (m), 37.6, 25.9, 20.9; ^{31}P NMR (CD_3OD) δ 4.69 (t, J_{PF} = 104.8 Hz); ^{19}F NMR (CD_3OD) δ −46.9 (1F, dddd, J_{FF} = 293.2 Hz, J_{FP} = 104.8 Hz, J_{FH} = 27.1, 12.8 Hz), −48.8 (1F, dddd, J_{FF} = 293.2 Hz, J_{FP} = 104.8 Hz, J_{FH} = 26.7, 13.9 Hz); IR (KBr) 1705, 1569, 1170 cm^{-1}. ESIMS *m/z* 337 (MH^+). HRMS (ESI) calcd for $C_{11}H_{16}N_4O_4F_2P$ (MH^+): 337.0877. Found: 337.0866.

4.17. *tert*-Butyl 4-(diethoxyphosphoryl)-4,4-difluoro-3-methylbutanoate (21)

Prepared as an oil from *tert*-butyl crotonate **20** in 94% yield in an analogous manner for preparation of **14**. ^{1}H NMR ($CDCl_3$) δ 4.32–4.21 (4H, m), 2.79 (1H, dd, J = 3.6, 15.7 Hz), 2.75–2.63 (1H, m), 2.17 (1H, dd, 10.0, 15.7 Hz), 1.45 (9H, s), 1.38 (6H, t, J = 7.1 Hz), 1.17 (3H, d, J = 6.9 Hz), ^{13}C NMR ($CDCl_3$) δ 171.9, 122.7 (dt, J_{CP} = 211.9 Hz, J_{CF} = 262.9 Hz), 29.1, 17.5 (d, J_{CP} = 5.4 Hz), 14.0; ^{31}P NMR ($CDCl_3$) δ 7.22 (t, J_{FP} = 109.5 Hz); ^{19}F NMR ($CDCl_3$) δ −50.7 (1F, ddd, J_{FF} = 300.1 Hz, J_{FP} = 109.5 Hz, J_{FH} = 14.6 Hz), −54.1 (1F, ddd, J_{FF} = 300.1 Hz, J_{FP} = 109.5 Hz, J_{FH} = 18.5 Hz); IR (film) 2982, 1732, 1369, 1272, 1169, 1023 cm^{-1}. EIMS *m/z* 274 (MH^+−*t*-Bu). Anal. Calcd for $C_{13}H_{25}F_2O_5P$: C, 47.27; H, 7.63. Found: C, 47.26; H, 7.41.

4.18. 4-(Diethoxyphosphoryl)-4,4-difluoro-3-methylbutanoic acid (22)

Prepared from **21** in 94% yield as an oil in an analogous manner for preparation of **15**. ^{1}H NMR ($CDCl_3$) δ 4.33–4.25 (4H, m), 2.95 (1H, dd, J = 3.8 Hz, J = 16.4 Hz), 2.84–2.74 (1H, m), 2.32 (1H, dd, J = 9.7, 16.5 Hz), 1.39 (6H, t, J = 7.1 Hz), 1.22 (3H, d, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 177.6, 121.8 (dt, J_{CP} = 215.6 Hz, J_{CF} = 263.0 Hz), 65.6 (d, J_{CP} = 7.3 Hz), 65.6 (d, J_{CP} = 7.4 Hz), 35.6–35.0 (m), 34.3, 16.2 (2C, d, J_{CP} = 5.4 Hz), 13.0; ^{31}P NMR ($CDCl_3$) δ 6.64 (t, J_{PF} = 109.1 Hz); ^{19}F NMR ($CDCl_3$) δ $-50.5 \sim -54.5$ (2F, m). IR (neat) 1732, 1253, 1024 cm^{-1}. ESIMS m/z 275 (MH^+). Anal. Calcd for $C_9H_{17}F_2O_5P$: C, 39.42; H, 6.25. Found: C, 39.47; H, 6.17.

4.19. 5-(Diethoxyphosphoryl)-5,5-difluoro-4-methylpentanoic acid (23)

Prepared from **22** in 58% yield as an oil in an analogous manner for preparation of **16**. ^{1}H NMR ($CDCl_3$) δ 4.31–4.26 (4H, m), 2.52–2.46 (1H, m), 2.43–2.46 (1H, m), 2.43–2.35 (1H, m), 2.35–2.15 (2H, m), 1.69–1.64 (1H, m), 1.38 (6H, t, J = 7.1 Hz), 1.15 (3H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 178.0, 122.9 (dt, J_{CP} = 212.5 Hz, J_{CF} = 263.0 Hz), 64.6 (d, J_{CP} = 6.3 Hz), 64.4 (d, J_{CP} = 6.4 Hz), 37.8–37.2 (m), 31.3, 24.1, 16.3 (d, J_{CP} = 5.5 Hz, 2C), ^{31}P NMR ($CDCl_3$) δ 7.12 (t, J_{PF} = 109.4 Hz); ^{19}F NMR ($CDCl_3$) δ -50.8 (1F, ddd, J_{FF} = 301.1 Hz, J_{FP} = 109.4 Hz, J_{FH} = 15.1 Hz), -52.3 (1F, ddd, J = 301.1 Hz, J_{FP} = 109.4 Hz, J_{FH} = 18.4 Hz); IR (film) 1731, 1254, 1025 cm^{-1}. ESIMS m/z 289 (MH^+). Anal. Calcd for $C_{10}H_{19}F_2O_5P$: C, 41.67; H, 6.64. Found: C, 41.75; H, 6.70.

4.20. Diethyl 1,1-difluoro-5-hydroxy-2-methylpentylphosphonate (24)

Prepared from **23** in 34% yield as an oil in an analogous manner for preparation of **17**. ^{1}H NMR ($CDCl_3$) δ 4.31–4.23 (4H, m), 3.65 (2H, t, J = 6.3 Hz), 2.24–2.18 (1H, m), 1.93–1.70 (3H, m), 1.55–1.48 (1H, m), 1.38 (6H, t, J = 7.1 Hz), 1.13 (3H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 123.0 (dt, J_{CP} = 211.5 Hz, J_{CF} = 262.8 Hz), 64.7 (d, J_{CP} = 6.6 Hz), 64.5 (d, J_{CP} = 6.5 Hz), 62.2, 38.3–37.7 (m), 29.8, 25.2, 16.2 (d, J_{CP} = 5.4 Hz), ^{19}F NMR ($CDCl_3$) δ -51.0 (1F, ddd, J_{FF} = 298.5 Hz, J_{FP} = 110.9 Hz, J_{FH} = 16.2 Hz), -52.6 (1F, ddd, J_{FF} = 298.5 Hz, J_{FP} = 110.9 Hz, J_{FH} = 17.7 Hz); IR (film) 3462, 1265, 1028 cm^{-1}. ESIMS m/z 297 (MNa^+). Anal. Calcd for $C_{10}H_{21}F_2O_4P$: C, 43.80; H, 7.72. Found: C, 43.94; H, 7.64.

4.21. Diethyl 5-(2-amino-6-chloro-9*H*-purin-9-yl)-1,1-difluoro-2-methylpentylphosphonate (25a)

Prepared from **24** in 61% yield as an oil in an analogous manner for preparation of **18a**. ^{1}H NMR ($CDCl_3$) δ 7.88 (1H, s), 5.16 (2H, br s, NH_2), 4.28–4.20 (4H, m), 4.14–4.03 (2H, m), 2.35–2.28 (1H, m), 2.03–1.99 (2H, m), 1.89–1.80 (2H, m), 1.36 (3H, t, J = 7.1 Hz), 1.35 (3H, t, J = 7.1 Hz), 1.12 (3H, d, J = 7.0 Hz); ^{13}C NMR (CD_3OD) δ 161.5, 155.2, 151.4, 144.5, 124.8, 123.4 (dt, J_{CP} = 213.5 Hz, J_{CF} = 262.0 Hz), 66.0 (2C, d, J_{CP} = 7.1 Hz), 44.6, 39.4–38.9 (m), 27.9, 27.1, 16.7 (2C, d, J_{CP} = 5.2 Hz), 12.5; ^{31}P NMR ($CDCl_3$) δ 7.16 (t, J_{PF} = 109.8 Hz); ^{19}F NMR ($CDCl_3$) δ -50.9 (1F, ddd, J_{FF} = 300 Hz, J_{FP} = 109.8 Hz, J_{FH} = 14.3 Hz), -52.8 (1F, ddd, J_{FF} = 300 Hz, J_{FP} = 109.8 Hz, J_{FH} = 18.8 Hz); IR (film) 3327, 1614, 1558, 1263, 1024 cm^{-1}. ESIMS m/z 426 (MH^+). HRMS(ESI) calcd for $C_{15}H_{24}N_5O_3F_2PCl$ (MH^+): 426.1273. Found: 426.1271.

4.22. Diethyl 5-(6-chloro-9*H*-purin-9-yl)-1,1-difluoro-2-methylpentylphosphonate (25b)

Prepared from **24** in 86% yield as an oil in an analogous manner for preparation of **18a**. ^{1}H NMR ($CDCl_3$) δ 8.74 (1H, s), 4.41–4.21 (6H, m), 2.27–2.04 (2H, m), 2.00–1.82 (2H, m), 1.45–1.41 (1H, m), 1.36 (6H, t, J = 7.2 Hz), 1.13 (3H, d, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 152.7 (2C), 146.0, 132.3, 129.3, 122.7 (dt, J_{CP} = 211.1 Hz, J_{CF} = 263.2 Hz), 64.9 (d, J_{CP} = 6.2 Hz), 64.7 (d, J_{CP} = 6.3 Hz), 44.6, 38.2–37.7 (m), 27.3, 26.2, 16.3 (d, J_{CP} = 5.4 Hz); ^{31}P NMR ($CDCl_3$) δ 7.04 (t, J_{PF} = 110.2 Hz); ^{19}F NMR ($CDCl_3$) δ $-50.4 \sim -52.1$ (2F, m); IR (film) 1592, 1558, 1267, 1027 cm^{-1}. ESIMS m/z 411 (MH^+). HRMS (ESI) calcd for $C_{15}H_{23}N_4O_3F_2PCl$ (MH^+): 411.1164. Found: 411.1163.

4.23. 5-(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-1,1-difluoro-2-methylpentylphosphonic acid (26a)

Prepared as an amorphous powder from **25a** in 82% yield in an analogous manner for preparation of **8a**. ^{1}H NMR ($CDCl_3$) δ 9.09 (1H,s), 4.29–4.26 (2H, m), 3.80–2.08 (2H, m), 2.03–1.81 (2H, m), 1.47–1.36 (1H, m), 1.18 (3H, d, J = 7.0 Hz); ^{13}C NMR (CD_3OD) δ 156.9, 154.8, 151.4, 138.1, 123.2 (dt, J_{CP} = 207.1 Hz, J_{CF} = 260.9 Hz), 108.6, 46.5, 39.0–38.4 (m), 27.5, 27.0, 12.8; ^{31}P NMR (CD_3OD) δ 5.04 (t, J_{PF} = 108.3 Hz); ^{19}F NMR (CD_3OD) δ $-51.8 \sim -53.9$ (2F, m); IR (KBr) 3124, 1684 cm^{-1}. ESIMS m/z 352 (NH^+). HRMS (ESI) calcd for $C_{11}H_{17}N_5O_4F_2P$ (MH^+): 352.0986. Found: 352.0966.

4.24. 1,1-Difluoro-2-methyl-5-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)pentylphosphonic acid (26b)

Prepared as an amorphous powder from **25b** in 85% yield in an analogous manner for preparation of **8a**. ^{1}H NMR (CD_3OD) δ 9.24 (1H, s), 8.28 (1H, s), 4.44 (2H, t, J = 7.0 Hz), 2.27–1.95 (2H, m), 1.93–1.89 (2H, m), 1.46–1.42 (1H, m), 1.17 (3H, d, J = 6.9 Hz); ^{13}C NMR (CD_3OD) δ 155.1, 149.8, 149.0, 140.9, 123.5 (dt, J_{CP} = 206.1 Hz, J_{CF} = 260.5 Hz), 118.2, 47.0, 39.1–38.6 (m), 27.9, 27.3, 12.7; ^{31}P NMR (CD_3OD) δ 4.97 (t, J_{PF} = 106.7 Hz); ^{19}F NMR (CD_3OD) δ -51.9 (1F, ddd, J_{FF} = 297.0 Hz, J_{FP} = 106.7 Hz, J_{FH} = 15.8 Hz), -53.6 (1F, ddd, J_{FF} = 297.0 Hz, J_{FP} = 106.7 Hz, J_{FH} = 15.8 Hz); IR (KBr) 1716, 1569, 1542, 1172 cm^{-1}. ESIMS m/z 337 (MH^+). HRMS (ESI) calcd for $C_{11}H_{16}N_4O_4F_2P$ (MH^+): 337.0877. Found: 337.0863.

4.25. (2*E*)-4-Hydroxybut-2-enyl propionate (27)

A solution of (2*E*)-but-2-ene-1,4-diol (2.67 g, 30 mmol) and $(EtCO)_2O$ (3.9 mmol) in acetone (12 mL) was heated at reflux for 10 h. After cooling to room temperature, volatile components of the mixture were evaporated in vacuo. The residue was diluted with ether and washed with brine. The ethereal layer was dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel (hexane/EtOAc = 5:1) to give **27** (2.6 g, 60%) as an oil. ^{1}H NMR ($CDCl_3$) δ 5.95–5.89 (1H, m), 5.86–5.80 (1H, m), 4.59 (2H, d, J = 5.7 Hz), 4.17 (2H, d, J = 4.9 Hz), 2.37–2.32 (2H, m), 1.59 (1H, br s), 1.15 (3H, t, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 173.9, 133.4, 124.1, 63.8. 61.6, 27.0, 8.5; IR (film) 3290, 1734, 1650 cm^{-1}. MS (EI) m/z 144 (M^+), 126 (M^+-H_2O).

4.26. (2*E*)-4-[(Diethoxyphosphoryl)oxy]but-2-enyl propionate (28)

To a stirred solution of **27** (6.55 g, 45.5 mmol) in pyridine (7.4 mL) was added $ClPO(OEt)_2$ (6.6 mL, 45.5 mmol) at 0 °C. The mixture was stirred at the same temperature for 12 h and then poured onto cold water. The mixture was extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (hexane/EtOAc = 2:1) to give **28** (11.25 g, 88%) as an oil. ^{1}H NMR ($CDCl_3$) δ 5.89–5.81 (2H, m), 4.59 (2H, d, J = 4.0 Hz), 4.54–4.51 (2H, m), 4.14–4.07 (4H, m), 2.37–2.31 (2H, m), 1.35–1.31 (9H, m), 1.14 (3H, t, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 173.3, 127.7–127.5 (2C, m), 66.1 (d, J_{CP} = 5.1 Hz), 63.2 (2C, d, J_{CP} = 5.6 Hz), 62.9, 26.8, 15.5 (2C, d, J_{CP} = 6.5 Hz), 8.5; ^{31}P NMR ($CDCl_3$) δ −12.8; IR (film) 1738, 1457, 1273, 1030 cm^{-1}. ESIMS m/z 303 (MNa^+). HRMS (ESI) calcd for $C_{11}H_{21}O_6NaP$ (MNa^+): 303.0973. Found: 303.0937.

4.27. (2*E*)-5-(Diethoxyphosphoryl)-5,5-difluoropent-2-enyl propionate (29)

To a stirred solution of the zinc reagent, prepared from diethyl bromomethyldifluoromethylphosphonate (5.55 g, 20.8 mmol) and zinc (1.35 g, 20.8 mmol) in DMF (20 mL), were successively added CuBr (2.97 g, 20.8 mmol) and a solution of **28** (2.9 g, 10.4 mmol) in DMF (10 mL) at room temperature.[11,14] The mixture was stirred at the same temperature for 12 h and poured onto water under ice-cooling. The resulting mixture was extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc = 3:1) to give **29** (3.08 g, 94%) as an oil. ^{1}H NMR ($CDCl_3$) δ 5.79–5.76 (2H, m), 4.56 (2H, d, J = 7.0 Hz), 4.29–4.21 (4H, m), 2.89–2.76 (2H, m), 2.36–2.30 (2H, m), 1.36 (6H, t, J = 7.0 Hz), 1.13 (3H, t, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 173.4, 130.4, 122.6, 119.0 (dt, J_{CP} = 215.5 Hz, J_{CF} = 260.4 Hz), 64.0 (2C, d, J_{CP} = 6.6 Hz), 63.5, 36.8 (dt, J_{CP} = 15.5 Hz, J_{CF} = 24.4 Hz), 26.9, 15.8 (2C, d, J_{CP} = 5.0 Hz), 8.5; ^{31}P NMR ($CDCl_3$) δ 7.04 (t, J_{PF} = 107.0 Hz); ^{19}F NMR ($CDCl_3$) δ −48.4 (dt, J_{PF} = 107.0 Hz, J_{HF} = 19 Hz); IR (film) 1738, 1540, 1271, 1027 cm^{-1}. ESIMS m/z 337 (MNa^+). HRMS (ESI) calcd for $C_{12}H_{21}O_5F_2NaP$ (MNa^+): 337.0992. Found: 337.0979.

4.28. 5-(Diethoxyphosphoryl)-5,5-difluoropentylpropionate (30)

A mixture of **29** (2 g, 0.93 mmol), 10% Pd–C (100 mg), and MeOH (100 mL) was stirred under a hydrogen atmosphere for 12 h at room temperature. The mixture was filtered and the filtrate was concentrated under reduced pressure. The remaining residue was chromatographed on silica gel. Elution with hexane/EtOAc (4:1) gave **30** (1.9 g, 96%) as an oil. ^{1}H NMR ($CDCl_3$) δ 4.31–4.23 (4H, m), 4.10–4.08 (2H, m), 2.35–2.30 (2H, m), 2.08–2.04 (2H, m), 1.69–1.65 (4H, m), 1.38 (6H, t, J = 7.1 Hz), 1.16–1.12 (3H, m); ^{13}C NMR ($CDCl_3$) δ 173.4, 121.2 (dt, J_{CP} = 215.8 Hz, J_{CF} = 259.4 Hz), 64.7 (2C, J_{CP} = 6.9 Hz), 64.0, 34.0–33.3 (m), 28.3, 27.5, 17.4, 16.3 (2C, d, J_{CP} = 5.4 Hz), 9.0; ^{31}P NMR ($CDCl_3$) δ 7.46 (t, J_{PF} = 108.9 Hz); ^{19}F NMR ($CDCl_3$) δ −48.8 ∼ −49.2 (2F, m); IR (film) 1737, 1270, 1025 cm^{-1}. ESIMS m/z 339 (MNa^+). Anal. Calcd for $C_{12}H_{23}F_2O_5P$: C, 45.57; H, 7.33. Found: C, 44.65; H, 7.24.

4.29. Diethyl 1,1-difluoro-5-hydroxypentylphosphonate (31)

To a stirred solution of **30** (1.26 g, 4 mmol) in ether (40 mL) was added $LiBH_4$ (131 mg, 6 mmol) under ice cooling. The mixture was stirred at room temperature for 12 h and poured onto ice water. The biphasic mixture was extracted with $CHCl_3$. The extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (hexane/EtOAc = 1:1) to give **31** (572 mg, 55%). ^{1}H NMR ($CDCl_3$) δ 4.30–4.23 (4H, m), 3.67 (2H, t, J = 6.2 H), 2.15–2.04 (2H, m), 1.38 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 120.6 (dt, J_{CP} = 215.5 Hz, J_{CF} = 259.3 Hz), 64.3 (2C, d, J_{CP} = 6.9 Hz), 60.2, 33.8–33.4 (m), 32.0, 17.1, 16.2 (2C, d, J_{CP} = 5.4 Hz); ^{31}P NMR ($CDCl_3$) δ 7.57 (t, J_{PF} = 109.0 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −48.8 ∼ −49.2 (2F, m); IR (film) 3468, 1262, 1024 cm^{-1}. ESIMS m/z 261 (MH^+). Anal. Calcd for $C_9H_{19}F_2O_4P$: C, 41.54; H, 7.36. Found: C, 41.55; H, 7.26.

4.30. Diethyl 1,1-difluoro-5-oxopentylphosphonate (32)

A solution of **31** (700 mg, 2.7 mmol) in CH_2Cl_2 (28 mL) was treated with Dess–Martin periodinane (1.7 g, 4.1 mmol) at room temperature for 1 h. The mixture was poured on cold 10% $Na_2S_2O_3$ and extracted with ether. The extracts were washed with satd aq $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated to give **32** (650 mg) as an oil: ^{1}H NMR ($CDCl_3$) δ 9.78 (1H, d, J = 2.0 Hz), 4.31–4.23 (4H, m), 2.55 (2H, J = 7.2 Hz), 2.24–2.00 (2H, m), 1.98–1.90 (2H, m), 1.38 (6H, t, J = 7.1 Hz). This oil was used for the next reaction without purification.

4.31. Diethyl 1,1-difluoro-5-hydroxyhexylphosphonate (33)

To a stirred solution of MeMgBr (1.1 mL of 3 M solution in ether) in THF (5.3 mL) was added a solution of the crude aldehyde **32** in THF (5.3 mL) at 0 °C. The mixture was stirred at the same temperature for 30 min and at room temperature for 5 h. The mixture was poured onto saturated aqueous NH_4Cl. The mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (hexane/EtOAc = 1:1) to give **33** (563 mg, 76%). ^{1}H NMR ($CDCl_3$) δ 4.30–4.23 (4H, m), 3.86–3.78 (1H, m), 2.12–2.04 (2H, m), 1.71–1.64 (2H, m), 1.53–1.48 (2H, m), 1.38 (6H, t, J = 7.1 Hz), 1.21 (3H, t, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 121.5 (dt, J_{CP} = 215.3 Hz, J_{CF} = 259.4 Hz), 67.7, 64.7 (2C, d, J_{CP} = 6.8 Hz), 38.8, 34.3–33.7 (m), 23.5, 17.1–17.0 (m), 16.4 (2C, d, J_{CP} = 5.2 Hz); ^{31}P NMR ($CDCl_3$) δ 7.59 (t, J_{PF} = 109.7 Hz); ^{19}F NMR ($CDCl_3$) δ −48.7 ∼ −49.2 (2F, m); IR (film) 3455, 1267, 1025 cm^{-1}. ESIMS m/z 297 (MNa^+). Anal. Calcd for $C_{10}H_{21}F_2O_4P$: C, 43.80; H, 7.72. Found: C, 43.76; H, 7.49.

4.32. Diethyl 1,1-difluoro-5-iodohexylphosphonate (34)

To a stirred solution of Ph_3P (4.49 g, 17.1 mmol), imidazole (1.24 g, 18.3 mmol), and I_2 (17.1 mmol) in CH_2Cl_2 (65 mL) was added a solution of **33** (3.13 g, 11.4 mmol) in CH_2Cl_2 (30 mL) at room temperature during a period of 2.5 h. After being stirred for 30 min, the mixture was diluted with CH_2Cl_2. The resulting precipitates were removed by filtration. The filtrates were washed with sat. $Na_2S_2O_3$ and brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (hexane/EtOAc = 5:1) to give **34** (4.16 g, 95%) as an oil. ^{1}H NMR ($CDCl_3$) δ 4.31–4.24 (4H, m), 4.20–4.14 (1H, m), 2.14–2.08 (2H, m).1.93 (3H, d, J = 6.9 Hz), 1.90–1.77 (2H, m), 1.76–1.66 (2H, m), 1.39 (6H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 120.6 (dt, J_{CP} = 215.4 Hz, J_{CF} = 259.4 Hz), 64.4 (2C, d, J_{CP} = 6.8 Hz), 42.2, 33.0 (dt, J_{CP} = 14.7 Hz, 20.9 Hz), 28.8, 28.7, 21.2–21.1 (m), 16.4 (2C, d, J_{CP} = 5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 8.34 (t, J_{PF} = 108.4 Hz); ^{19}F NMR ($CDCl_3$) δ −49.1 (2F, dt, J_{FP} = 108.4 Hz, J_{FH} = 19.8 Hz); IR (film) 1271, 1203 cm^{-1}. ESIMS m/z 385 (MH^+). HRMS(ESI) calcd for $C_{10}H_{21}F_2IO_3P$ (MH^+): 385.0241. Found: 385.0247.

4.33. Diethyl 5-(2-amino-6-chloro-9*H*-purin-9-yl)-1,1-difluorohexylphosphonate (35a)

To a stirred solution of **34** (4.15 g, 10.8 mmol) and 6-chloropurine (2.62 g, 15.5 mmol) in DMF (37 mL) was added anhydrous potassium carbonate (3.2 g, 23.2 mmol). The mixture was stirred at 20 °C for 24 h and diluted with $CHCl_3$. The solution was washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (0.5% MeOH in $CHCl_3$) to give **35a** (2.04 g, 44%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.80 (1H, s), 5.21 (2H, br s), 4.61–4.52 (1H, m), 4.28–4.20 (4H, m), 2.33–2.16 (1H, m), 2.13–1.97 (2H, m), 1.96–1.85 (1H, m), 1.59 (3H, d, J = 6.9 Hz), 1.55–1.46 (2H, m), 1.36 (3H, t, J = 7.1 Hz), 1.35 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 158.9, 153.6, 151.3, 140.2, 125.4, 120.5 (dt, J_{CP} = 215.5 Hz, J_{CF} = 259.6 Hz), 64.5 (d, J_{CP} = 7.5 Hz), 64.4 (d, J_{CP} = 7.5 Hz), 50.8, 36.0, 33.1 (dt, J_{CP} = 14.1 Hz, J_{CF} = 20.9 Hz), 20.6, 17.4 (m), 16.3 (2C, d, J_{CP} = 5.2 Hz); ^{31}P NMR ($CDCl_3$) δ 8.25 (t, J_{PF} = 107.9 Hz); ^{19}F NMR ($CDCl_3$) δ −48.9 (1F, dddd, J_{FF} = 295.9 Hz, J_{FP} = 107.9 Hz, J_{FH} = 24.5 Hz), −50.0 (1F, dddd, J_{FF} = 295.9 Hz, J_{FP} = 107.9 Hz, J_{FH} = 24.5 Hz); IR (film) 1652, 1559, 1259, 1027 cm^{-1}. ESIMS m/z 426 (MH^+), HRMS (ESI) calcd for $C_{15}H_{24}ClF_2N_5O_3P$ (MH^+): 426.1273. Found: 426.1310.

4.34. Diethyl 5-(6-chloro-9*H*-purin-9-yl)-1,1-difluorohexylphosphonate (35b)

Prepared from **33** in 53% yield as an oil in an analogous manner for preparation of **18b**. ^{1}H NMR ($CDCl_3$) δ 8.73 (1H, s), 8.15 (1H, s), 4.80–4.75 (1H, m), 4.27–4.19 (4H, m), 2.17–2.05 (2H, m), 2.04–1.96 (2H, m), 1.67 (3H, t, J = 7.1 Hz), 1.35 (3H, t, J = 7.1 Hz), 1.34 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 146.0, 145.0, 132.2, 129.0, 120.8 (dt, J_{CP} = 215.5 Hz, J_{CF} = 259.7 Hz), 64.5 (2C, d, J_{CP} = 6.7 Hz), 52.3, 35.6, 33.4–32.8 (m), 20.4, 17.5, 16.1 (2C, d, J_{CP} = 5.3 Hz); ^{31}P NMR ($CDCl_3$) δ 7.06 (t, J_{PF} = 107.2 Hz); ^{19}F NMR ($CDCl_3$) δ −48.9 ∼ −49.3 (2F, m); IR (film) 1591, 1557, 1267, 1024 cm^{-1}. ESIMS m/z 411 (MH^+). Anal. Calcd for $C_{15}H_{22}ClF_2N_4O_3P$: C, 43.86; H, 5.40; N, 13.64. Found: C, 43.82; H, 5.35; N, 13.91.

4.35. 5-(2-Amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)-1,1-difluorohexylphosphonic acid (36a)

Prepared as an amorphous powder from **35a** in 94% yield in an analogous manner for preparation of **8a**. ^{1}H NMR (CD_3OD) δ 9.21 (1H, s), 4.82–4.76 (1H, m), 2.20–2.03 (4H, m), 1.67 (3H, d, J = 6.8 Hz), 1.65–1.55 (2H, m); ^{13}C NMR (CD_3OD) δ 157.5, 155.7, 151.9, 137.8, 122.7 (dt, J_{CP} = 210.9 Hz, J_{CF} = 257.4 Hz), 109.7, 55.7, 36.9, 34.8 (dt, J_{CP} = 15.0 Hz, J_{CF} = 21.0 Hz), 21.1, 19.5 (m); ^{31}P NMR (CD_3OD) δ 6.98 (t, J_{PF} = 106.1 Hz); ^{19}F NMR (CD_3OD) δ −50.1 (ddt, J_{FP} = 106.1 Hz, J_{FH} = 18.0 Hz, J_{FH} = 5.0 Hz); IR (KBr) 3342, 1701, 1170 cm^{-1}. ESIMS m/z 352 (MH^+). HRMS (ESI) calcd for $C_{11}H_{17}F_2N_5O_4P$ (MH^+): 352.0986. Found: 352.0954.

4.36. 1,1-Difluoro-5-(6-oxo-1,6-dihydro-9*H*-purin-9-yl)hexylphosphonic acid (36b)

Prepared as an amorphous powder from **35b** in 82% yield in an analogous manner for preparation of **8a**. ^{1}H NMR (CD_3OD) δ 9.20 (1H, s), 7.90 (1H, s), 4.58–4.53 (1H, m), 1.80–1.62 (4H, m), 1.30 (3H, d, J = 6.8 Hz), 1.23–1.09 (2H, m); ^{13}C NMR (CD_3OD) δ 154.8, 150.1, 149.8, 148.7, 139.6, 122.2 (dt, J_{CP} = 209.6 Hz, J_{CF} = 254.6 Hz), 56.0, 36.4, 34.5–33.9 (m), 20.5, 18.9; ^{31}P NMR (CD_3OD) δ 4.77 (t, J_{PF} = 107.6 Hz); ^{19}F NMR (CD_3OD) δ −49.6 ∼ −50.0 (2F, m); IR (KBr) 1716, 1568, 1172 cm^{-1}. ESIMS m/z

337 (MH^+). HRMS (ESI) calcd for $C_{11}H_{16}N_4O_4F_2P$ (MH^+): 337.0877. Found: 337.0889.

4.37. Assay and inhibition of PNP

PNP activity was measured by the xanthine oxidase couple assay of Stoeckler et al.[12] with minor modification. Briefly, the assay mixture contained either 5 or 500 mM potassium phosphate buffer (pH 7.5, 300 μL), 0.2 U/mL PNP (Toyobo, Tokyo, 300 μL), 0.12 U/mL xanthine oxidase (Sigma, St. Louis, 300 mL), 3–600 mM inhibitor (1 mL), and distilled water was incubated at 30 °C for 5 min. To the reaction mixture was added 10 mM inosine (Wako Pure Chemical Co., Osaka, 300 μM), 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 spectrometer. PNP activity was calculated by using the molecular extinction coefficient of uric acid (1.24×10^4), and the specific activity was expressed as millimole of uric acid per minute per milligram of protein. IC_{50} was the concentration of compounds giving 50% enzyme inhibition. K_i 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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