Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 10223–10230

TETRAHEDRON

Synthesis of acyclic nucleotide analogues possessing a difluoromethylene phosphonyl group at the side chain

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Received 4 October 2003; revised 22 October 2003; accepted 22 October 2003

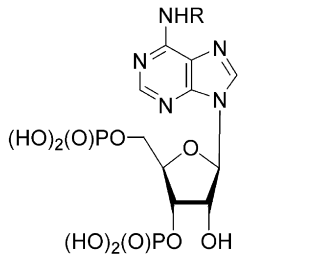
Abstract—A synthetic approach to a new type of acyclic nucleotide analogues **8** and **9** was examined. The design was based on acyclic modification of MRS 2179, a $P2Y_1$-antagonist, and replacement of one of two phosphate groups characterized by MRS 2179 with an isosteric difluoromethylenephosphonyl group. The nucleotide analogues **8** and **9** were enantio-divergently prepared as their ester-protecting derivatives from a highly differentiated 1,5-pentanediol derivative possessing a difluoromethylenephosphonyl group at the 3-position.

1. Introduction

Numerous kinds of nucleoside and nucleotide analogues have been synthesized based on the modification of naturally occurring nucleosides and nucleotides by an application of bioisosteres to develop biologically active compounds which are more resistant to enzymatic hydrolysis and degradation. The incorporation of a biomimetic functional group to the parent compounds confers on them a promising usefulness in the search for new therapeutic agents.[1] We are interested in a synthesis of P2Y receptor agonists and antagonists by modification of adenosine bisphophates, which have been known to show affinity to P2Y receptors.

Agonist binding at $P2Y_1$ receptors is known to result in activation of phospholipase C (PLC), which generates inositol phosphates and diacylglycerol from phosphatidyl-inositol 4,5-bisphosphate.[2] The $P2Y_1$ receptor is present in heart, skeletal and various smooth muscles, prostate, ovary, and brain.[3] A $P2Y_1$ receptor in platelets is involved in ADP-promoted aggregation.[4] Thus, a selective $P2Y_1$ receptor antagonist may have potential as an antithrombotic agent, while a selective receptor agonist may have potential as an antihypertensive or antidiabetic agent.[5] The bis-phosphate derivatives of naturally occurring nucleosides including adenosine 3′,5′-bisphosphate **1** (A3P5P) and adenosine 2′,5′-bisphosphate **2** (A2P5P) were reported to act as competitive antagonists or partial agonists of $P2Y_1$ receptors.[6] A new series of deoxyadenosine bisphosphates modified in the base and ribose moieties have been designed and synthesized.[7] For instance, the introduction of chlorine atom at the 2-position and the N^6-methyl modification resulted in the enhancement of antagonistic activity. N^6-Methyl-2′-deoxyadenosine 3′,5′-bisphosphate **3** greatly enhanced competitive antagonistic activity more than the parent A3P5P (Fig. 1).

A variety of modified cyclic and acyclic nucleotide



1 R=H
3 R=CH_3

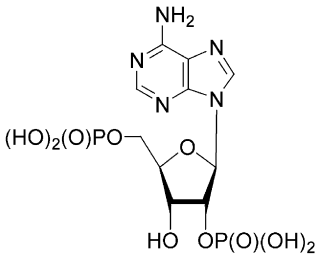


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Figure 1.

Keywords: acyclic nucleotide analogues; difluoromethylenephosphonates; adenosine bisphosphates; MRS 2179; P2Y receptors.
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doi:10.1016/j.tet.2003.10.064

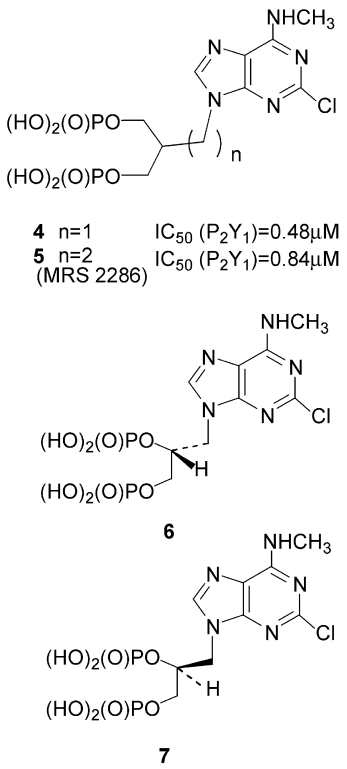


Figure 2.

analogues were synthesized to discuss the structure–activity relationships to $P2Y_1$ receptor antagonists and agonists.[7] The synthetic study of pharmacological probes for the selective $P2Y_1$ agonists and antagonists with high affinity is an interesting subject not only for the discovery of new therapeutic drugs but also for the investigation of the role of this receptor in vivo from the points of view of medicinal chemistry. Acyclic nucleotide analogues **4**–**7**, designed by modification of adenosine bisphosphates, were reported to show antithrombotic activity (Fig. 2).[7]

The properties of these acyclic analogues have stimulated us to develop a synthetic method directed toward novel acyclic nucleotide analogues **8** and **9** in which a difluoromethylenephosphonyl is incorporated as an isosteric group for one of two phosphate groups characterized by adenosine bisphosphates and their acyclic analogues. Herein, we wish to report an enantio-divergent synthesis of a new class of nucleotide analogues **24** and **28**, protecting derivatives of **8** and **9** (Fig. 3).

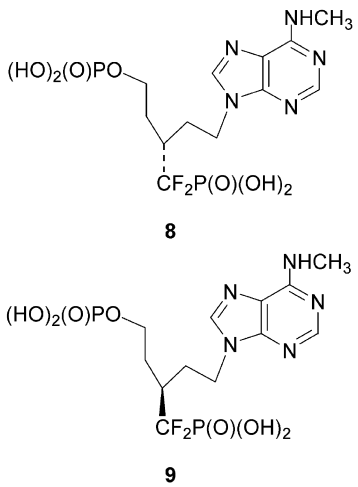


Figure 3.

2. Results and discussion

2.1. Synthesis of protected 1,5-pentanediol derivatives possessing a difluoromethylenephosphonate moiety

The concept of the synthetic approach to the nucleotide analogues **8** and **9** is based on the following two points. One is the modification of 2′-deoxyadenosine bisphosphate derivative **10** (MRS 2179) to an acyclic structure by removal of the oxygen atom from the 2-deoxyfuranose ring. Since bisphosphate **10** was reported to show potentially antagonistic activity to $P2Y_1$, investigation on the synthesis of the acyclic analogues is of considerable interest from the study aimed at synthesis of a biological probe for this receptor and its ligand (Fig. 4).

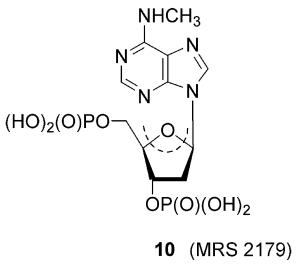


Figure 4.

The other is the replacement of one of two phosphate functional groups ($R\text{-}OPO_3H_2$) in **10** by α,α-difluoromethylenephosphonic acid ($R\text{-}CF_2PO_3H_2$, α,α-DFMPA) functionality. α,α-DFMPA is considered to act as an essentially non-hydrolyzable and metabolically stable bioisostere of monophosphates due to their closely similar physical properties. Recently, various kinds of α,α-DFMPA derivatives have been prepared for the development of potential enzyme inhibitors and useful probes to elucidate biochemical processes.[8] Nucleotide analogues containing an α,α-DFMPA moiety have also received much attention

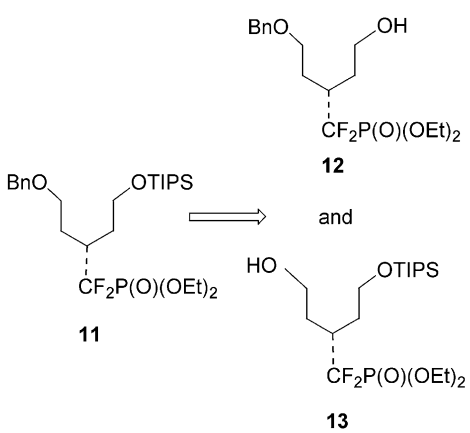
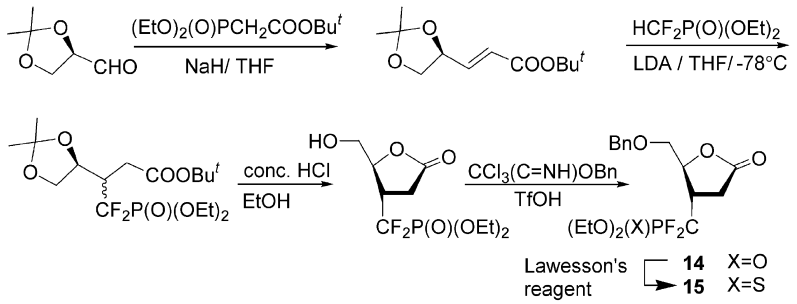
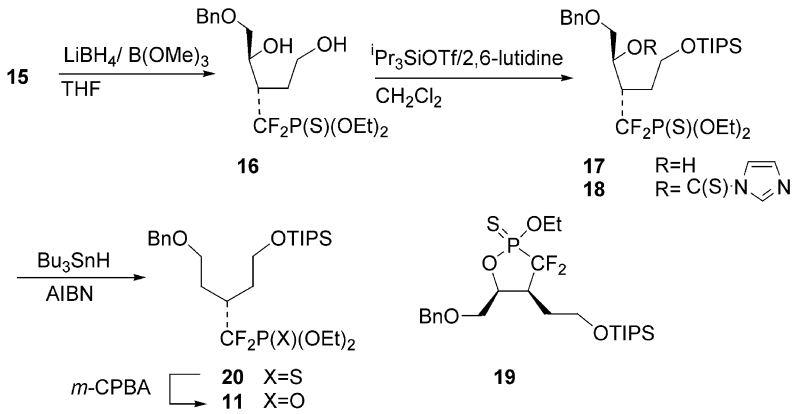


Figure 5.



Scheme 1.



Scheme 2.

in chemical and biological areas owing to the enhancement of chemical and enzymatic stability relative to that of the parent nucleotide.[9] Thus, acyclic nucleotide analogues **8** and **9** would be expected to show antithrombotic activity. The protected 1,5-pentanediol derivative **11** was considered as the common key intermediate for enantio-divergent synthesis of both enantiomers **8** and **9** via the mono-protected derivatives **12** and **13** through the selective removal of the protective groups of two primary hydroxyl groups (Fig. 5).

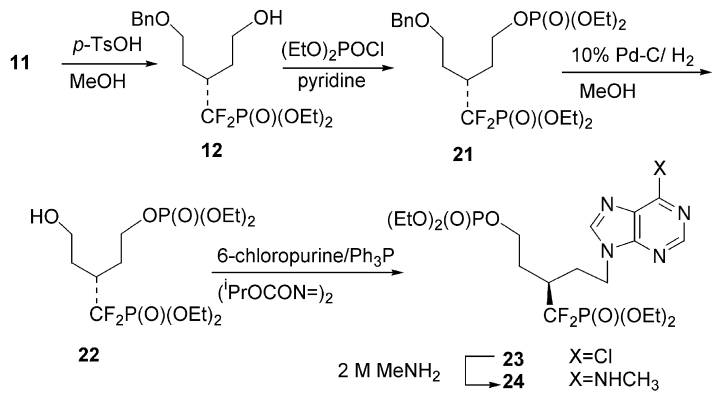
The chiral synthesis of **11** was examined from *trans*-lactone **14**[10] through reductive ring opening-deoxygenation sequence (vide infra). The enantiomerically pure *trans*-lactone **14** was prepared from 1,2-*O*-isopropylidene-(*R*)-glyceraldehyde through the previously described method[10] as outlined in Scheme 1.

Reductive ring-opening reaction of **14**[10] with $LiBH_4$/$B(OMe)_3$ resulted in decomposition and no desired reduction product was obtained. Then reduction of phosphonothioate analogue **15**,[10] a base-resistant congener of **14**, was examined. Reduction of **15** with $LiBH_4$/$B(OMe)_3$ under the same condition afforded the corresponding diol **16** in 95% yield. Selective protection of the primary hydroxyl group with TIPS was successfully achieved by treatment with triisopropylsilyl triflate (TIPSOTf) in the presence of 2,6-lutidine to provide TIPS ether **17** in 81% yield. To remove the hydroxyl group, **17** was converted to thiocarbonylimidazolide **18** by treatment with thiocarbonyldiimidazole in THF. In this reaction, careful control of the reaction temperature (65°C) was necessary to minimize formation of the cyclization product **19**. Deoxygenation of **18** with *n*-Bu_3SnH afforded **20** in 91% yield. Oxidation of **20** with *m*-CPBA in CH_2Cl_2, followed by aqueous quenching gave the requisite phosphonate derivative **11** in 75% yield (Scheme 2).

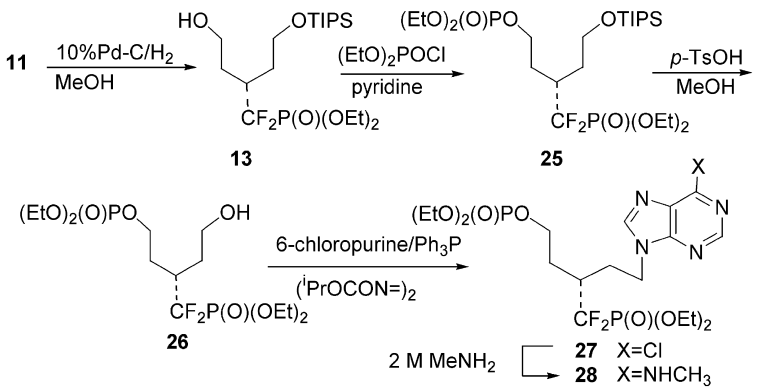
2.2. Synthesis of acyclic nucleotide analogues

Selective deprotection of either the TIPS- or benzyl group of **11** could yield the requisite **12** and **13**. Compound **12** was obtained in quantitative yield by of **11** exposure to *p*-TsOH in MeOH. Conversion of **12** to the phosphate **21** was easily achieved by treatment with $(EtO)_2POCl$ in 72% yield. Successively, hydrogenolysis of **24** over 10% Pd–C in MeOH yielded the required alcohol **22** in 94% yield. Condensation of **22** with 6-chloropurine under the Mitsunobu conditions (PPh_3, DIAD, THF) gave **23** in 88% yield. The methylamination of **23** with 2 M $MeNH_2$ provided **24** in 89% yield (Scheme 3).

On the other hand, the benzyl group of **11** was removed by hydrogenolysis in the presence of 10% Pd–C to give **13** in quantitative yield. Treatment of **13** with $(EtO)_2POCl$, followed by deprotection of the TIPS group with *p*-TsOH provided **26**, the enantioisomer of **22**, in 77% yield. The condensation of **26** with 6-chloropurine by the Mitsunobu reaction (PPh_3, DIAD, THF) afforded **27** in 85% yield. The methylamination of **27** with 2 M $MeNH_2$ gave **28** in 86%



Scheme 3.



Scheme 4.

yield. The biological evaluation of novel nucleotide analogues **8** and **9** will be examined after hydrolysis of **24** and **28** (Scheme 4).

3. Experimental

3.1. General

All reactions were carried out under a nitrogen atmosphere unless otherwise specified. Optical rotations were recorded on a JASCO DIP-360 digital polarimeter under standard conditions. NMR data were obtained on a Bruker DPX 400 or a Varian Mercury-300 BB 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 ^{1}H decoupling. The chemical shift data for each signal on ^{1}H 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 measured on a Micromass LCT using electrospray ionization (ESI) techniques.

3.1.1. 5-*O*-Benzyl-2,3-dideoxy-3-[(diethoxyphosphorothioyl)(difluoro)methyl]-D-*erythro*-pentitol (16). To a stirred solution of lactone **15**[10b] (5.22 g, 12.8 mmol) in THF was added $LiBH_4$ (840 mg, 38.4 mmol) and $B(OMe)_3$ (0.14 mL, 1.3 mmol) at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. Then the reaction mixture was poured into saturated aqueous NH_4Cl solution and the whole mixture was extracted with ether three times. The combined organic layer was dried over $MgSO_4$, and concentrated in vacuo. The remaining residue was chromatographed on silica gel. Elution with hexane/AcOEt (5:1) afforded **16** (5.02 g, 95%) as an oil: $[\alpha]_D^{25}=+1.1$ (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.24 (5H, m), 4.59 (1H, d, J=11.9 Hz), 4.55 (1H, d, J=11.9 Hz), 4.51–4.44 (1H, m), 4.33–4.18 (4H, m), 3.84–3.72 (1H, m), 3.71–3.50 (3H, m), 2.99 (1H, b), 2.73–2.56 (1H, m), 2.38 (1H, b), 2.08–1.95 (2H, m), 1.35 (3H, t, J=7.0 Hz), 1.35 (3H, t, J=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.6, 128.4, 127.8, 127.7, 123.0 (dt, J_{CP}=171.9 Hz, J_{CF}=270.0 Hz), 73.3, 72.5, 66.9 (d, J_{CP}=3.5 Hz), 64.6 (d, J_{CP}=6.5 Hz), 64.5 (d, J_{CP}=6.5 Hz), 43.0 (dt, J_{CP}=16.7 Hz, J_{CF}=17.7 Hz), 26.0 (d, J_{CP}=1.6 Hz), 16.0 (d, J_{CP}=6.3 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −45.9 (1F, ddd, J_{HF}=18.7 Hz, J_{FF}=292.5 Hz, J_{PF}=111.4 Hz), −46.8 (1F, ddd, J_{HF}=18.7 Hz, J_{FF}=292.5 Hz, J_{PF}=111.4 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 75.6 (t, J_{PF}=111.4 Hz); IR (neat) 3398, 2981, 2904, 1455, 1390, 1020 cm^{-1}; ESIMS *m/z* 435 (M^++Na), 413 (M^++H), 395 (M^+−OH). Anal. calcd for $C_{17}H_{27}O_5F_2PS$: C, 49.51; H, 6.60. Found: C, 49.60; H, 6.56.

3.1.2. 5-*O*-Benzyl-2,3-dideoxy-3-[(diethoxyphosphorothioyl)(difluoro)methyl]-1-*O*-(triisopropylsilyl)-D-*erythro*-pentitol (17). To a stirred solution of **16** (5.02 g, 12.2 mmol) in CH_2Cl_2 (70 mL) was added 2,6-lutidine (2.1 mL,

18.3 mmol) and TIPSOTf (3.6 mL, 13.4 mmol) at −78°C. After being stirred for 1 h at the same temperature, the reaction was quenched with methanol (3 mL) at −78°C. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with saturated aqueous Na_2CO_3 solution and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The remaining residue was chromatographed on silica gel. Elution with hexane/AcOEt (20:1) yielded **17** (5.6 g, 81%) as an oil: $[\alpha]_D^{25}=-3.5$ (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.24 (5H, m), 4.59 (1H, d, J=12.0 Hz), 4.53 (1H, d, J=12.0 Hz), 4.52–4.44 (1H, m), 4.31–4.19 (4H, m), 3.83 (1H, dt, J=9.9, 6.0 Hz), 3.71 (1H, dt, J=9.9, 6.5 Hz), 3.56 (1H, dd, J=7.6, 9.7 Hz), 3.49 (1H, dd, J=5.2, 9.7 Hz), 2.97 (1H, d, J=5.0 Hz), 2.74–2.57 (1H, m), 2.06–1.90 (2H, m), 1.35 (3H, t, J=7.1 Hz), 1.34 (3H, t, J=7.1 Hz), 1.13–0.97 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.3, 127.6 (2C), 123.2 (dt, J_{CP}=171.8 Hz, J_{CF}=269.8 Hz), 73.1, 72.6, 67.1 (d, J_{CP}=1.9 Hz), 64.6 (d, J_{CP}=7.0 Hz), 64.4 (d, J_{CP}= 7.1 Hz), 62.0, 42.2 (dt, J_{CP}=15.6 Hz, J_{CF}=18.4 Hz), 25.9 (d, J_{CP}=2.4 Hz), 17.9, 16.0 (d, J_{CP}=6.3 Hz), 11.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −44.9 (1F, ddd, J_{HF}=16.9 Hz, J_{FF}= 292.9 Hz, J_{PF}=112.5 Hz), −47.7 (1F, ddd, J_{HF}=20.1 Hz, J_{FF}=292.9 Hz, J_{PF}=112.5 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 76.0 (t, J_{PF}=112.5 Hz); IR (neat) 3063, 1463, 1390, 1098, 1022 cm^{-1}; ESIMS *m*/*z* 591 (M^++Na). Anal. calcd for $C_{26}H_{47}O_5F_2PSSi$: C, 54.91; H, 8.33. Found: C, 55.24; H, 8.45.

3.1.3. 5-*O*-Benzyl-2,3-dideoxy-3-[(diethoxyphosphorothioyl)(difluoro)methyl]-4-*O*-(1*H*-imidazol-1-ylcarbonothioyl)-1-*O*-(triisopropylsilyl)-D-*erythro*-pentitol (18) and (4*S*,5*S*)-5-[(benzyloxy)methyl]-2-ethoxy-3,3-difluoro-4-{2-[(triisopropylsilyl)oxy]ethyl}-1,2-oxaphospholane 2-sulfide (19). A mixture of **17** (15.91 g, 28.0 mmol) and 1,1′-thiocarbonyldiimidazole (14.97 g, 84.0 mmol) in THF (170 mL) was heated at 65°C for 15 h. The reaction mixture was evaporated and the remaining residue was chromatographed on silica gel. Elution with hexane/AcOEt (15:1) gave cyclization product **19** (5.85 g, 40%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.24 (5H, m), 4.83–4.70 (1H, m), 4.63–4.52 (2H, m), 4.35–4.22 (2H, m), 3.87–3.63 (4H, m), 3.25–3.01 (1H, m), 1.99–1.87 (1H, m), 1.83–1.71 (1H, m), 1.38–1.31 (3H, m), 1.15–0.99 (21H, m); ^{19}F NMR (376 MHz, $CDCl_3$) δ −49.5 (0.7F, ddd, J_{HF}=13.3 Hz, J_{FF}=269.2 Hz, J_{PF}=115.9 Hz), −53.7 (0.3F, ddd, J_{HF}=26.8 Hz, J_{FF}=269.1 Hz, J_{PF}=120.2 Hz), −54.0 (0.7F, ddd, J_{HF}=23.6 Hz, J_{FF}=262.9 Hz, J_{PF}=82.9 Hz), −58.7 (0.3F, ddd, J_{HF}=11.1 Hz, J_{FF}=269.1 Hz, J_{PF}= 91.9 Hz); ^{31}P NMR(162 MHz, $CDCl_3$) δ 82.2 (0.7P, dd, J_{PF}=82.9, 115.9 Hz), 79.6 (0.3P, dd, J_{PF}=91.9, 120.2 Hz); ESIMS *m*/*z* 523 (M^++H). Successive elution with hexane/AcOEt (5:1) afforded **18** (6.71 g, 35%) as an oil: $[\alpha]_D^{25}$= +19.8 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (1H, s), 7.60 (1H, s), 7.34–7.22 (5H, m), 7.02 (1H, s), 6.27 (dt, J=1.7, 6.0 Hz), 4.60 (1H, d, J=12.2 Hz), 4.56 (1H, d, J=12.2 Hz), 4.28–4.13 (4H, m), 3.94 (1H, dd, J=6.2, 10.8 Hz), 3.90–3.81 (2H, m), 3.77 (1H, dd, J=5.8, 10.8 Hz), 3.24–3.08 (1H, m), 2.28–2.17 (1H, m), 1.97–1.85 (1H, m), 1.30 (6H, t, J=7.1 Hz), 1.13–0.98 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 137.5, 136.8, 130.7, 128.3, 127.7, 127.5, 122.0 (dt, J_{CP}=174.5 Hz, J_{CF}= 270.5 Hz), 118.0, 79.2 (d, J_{CP}=2.8 Hz), 73.1, 67.7, 64.7 (d, J_{CP}=6.8 Hz), 64.6 (d, J_{CP}=6.8 Hz), 61.6, 39.8 (dt, J_{CP}=16.5 Hz, J_{CF}=19.4 Hz), 27.6, 18.0, 16.1 (d, J_{CP}= 6.2 Hz), 11.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.4 (1F, ddd, J_{HF}=16.6 Hz, J_{FF}=294.4 Hz, J_{PF}=111.8 Hz), −50.0 (1F, ddd, J_{HF}=19.2 Hz, J_{FF}=294.4 Hz, J_{PF}=111.8 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 75.5 (t, J_{PF}=111.8 Hz); IR (neat) 3047, 3016, 2829, 1464, 1390, 1326, 1285, 1230, 1104 cm^{-1}; ESIMS *m*/*z* 551 (M^+−OC(S)$C_3H_3N_2$). Anal. calcd for $C_{30}H_{49}N_2O_5F_2PS_2Si$: C, 53.08; H, 7.28; N, 4.13. Found: C, 53.31; H, 7.35; N, 4.45.

3.1.4. *O*,*O*-Diethyl (2*R*)-4-(benzyloxy)-1,1-difluoro-2-{2-[(triisopropylsilyl)oxy]ethyl}butylphosphonothioate (20). To a stirred solution of **19** (1.66 g, 2.45 mmol) in toluene (18 mL) was added *n*-Bu_3SnH (0.99 mL, 3.68 mmol) and AIBN (0.1 g) in small portions over 1 h at 100°C. After the reaction mixture had been stirred for 4 h at the same temperature, the reaction mixture was evaporated. The resulting residue was chromatographed on silica gel. Elution with hexane/AcOEt (20:1) afforded **20** (1.23 g, 91%) as an oil: $[\alpha]_D^{25}$=+3.6 (*c* 1.1, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.23 (5H, m), 4.50 (2H, s), 4.28–4.16 (4H, m), 3.76 (2H, t, J=6.7 Hz), 3.57 (2H, t, J=7.1 Hz), 2.67–2.47 (1H, m), 2.23–2.04 (2H, m), 1.79–1.68 (1H, m), 1.66–1.56 (1H, m), 1.33 (6H, t, J=7.1 Hz), 1.11–0.98 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 128.3, 127.5, 127.4, 123.4 (dt, J_{CP}=173.2 Hz, J_{CF}=268.7 Hz), 72.8, 68.2, 64.2 (d, J_{CP}=6.8 Hz), 61.1, 36.6 (dt, J_{CP}=16.3 Hz, J_{CF}= 19.4 Hz), 31.1 (d, J_{CP}=2.9 Hz), 28.0 (d, J_{CP}=2.5 Hz), 18.0, 16.1 (d, J_{CP}=6.3 Hz), 11.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.8 (1F, ddd, J_{HF}=17.7 Hz, J_{FF}=289.1 Hz, J_{PF}=114.5 Hz), −49.0 (1F, ddd, J_{HF}=18.5 Hz, J_{FF}= 289.1 Hz, J_{PF}=114.5 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 76.8 (t, J_{PF}=114.5 Hz); IR (neat) 2942, 2866, 1098, 1024 cm^{-1}; ESIMS *m*/*z* 575 (M^++Na), 553 (M^++H), 379 (M^+−OTIPS). Anal. calcd for $C_{26}H_{47}O_4F_2PSSi$: C, 56.49; H, 8.57. Found: C, 56.20; H, 8.49.

3.1.5. Diethyl (2*R*)-4-(benzyloxy)-1,1-difluoro-2-{2-[(triisopropylsilyl)oxy]ethyl}butylphosphonate (11). To a stirred solution of **20** (4.25 g, 7.69 mmol) in CH_2Cl_2 (230 mL) was added *m*-CPBA (6.64 g, 38.45 mmol) at 0°C. After being stirred for 15 min at room temperature, the reaction mixture was washed with saturated aqueous Na_2CO_3 solution and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. Elution with hexane/AcOEt (15:1) afforded **11** (3.11 g, 75%) as an oil. $[\alpha]_D^{25}$=+0.20 (*c* 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.22 (5H, m), 4.50 (2H, s), 4.31–4.18 (4H, m), 3.77 (2H, t, J=6.6 Hz), 3.58 (2H, t, J=6.9 Hz), 2.52–2.32 (1H, m), 2.24–2.03 (2H, m), 1.78–1.67 (1H, m), 1.66–1.54 (1H, m), 1.37 (3H, t, J=7.0 Hz), 1.35 (3H, t, J=7.0 Hz), 1.13–0.97 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 128.3, 127.5, 127.4, 122.8 (dt, J_{CP}=211.4 Hz, J_{CF}=263.0 Hz), 72.8, 68.2, 64.3 (d, J_{CP}=6.9 Hz), 61.1, 37.0 (dt, J_{CP}= 15.1 Hz, J_{CF}=19.3 Hz), 31.0 (d, J_{CP}=2.7 Hz), 28.0 (d, J_{CP}=2.0 Hz), 18.0, 16.3 (d, J_{CP}=5.4 Hz), 11.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.8 (1F, ddd, J_{HF}=17.2 Hz, J_{FF}= 301.2 Hz, J_{PF}=111.0 Hz), −49.1 (1F, ddd, J_{HF}=18.8 Hz, J_{FF}=301.2 Hz, J_{PF}=111.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.55 (t, J_{PF}=111.0 Hz); IR (neat) 2942, 2866, 1099, 1027 cm^{-1}; ESIMS *m*/*z* 537 (M^++H). Anal. calcd for

$C_{26}H_{47}O_5F_2PSi$: C, 58.19; H, 8.83. Found: C, 57.93; H, 8.55.

3.1.6. Diethyl (2*R*)-4-(benzyloxy)-1,1-difluoro-2-(2-hydroxyethyl)butylphosphonate (12). To a stirred solution of **11** (100 mg, 0.19 mmol) in MeOH (6 mL) was added *p*-TsOH·H_2O (10 mg) and stirred for 15 h at room temperature. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution. The organic layer was removed in vacuo. The oily residue was suspended with H_2O and then extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The remaining residue was chromatographed on silica gel. Elution with AcOEt afford **12** (72 mg, 100%) as an oil: $[\alpha]_D^{25}$=+0.35 (*c* 1.2, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.23 (5H, m), 4.52 (2H, s), 4.33–4.20 (4H, m), 3.72 (2H, t, *J*=6.0 Hz), 3.68–3.53 (2H, m), 2.57–2.38 (1H, m), 2.25–2.05 (2H, m), 1.79–1.67 (1H, m), 1.66–1.50 (1H, m), 1.37 (3H, t, *J*=7.0 Hz), 1.36 (3H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 128.4, 127.7, 122.6 (dt, J_{CP}=211.4 Hz, J_{CF}=263.2 Hz), 73.0, 68.5, 64.5 (d, J_{CP}=7.0 Hz), 60.0, 37.4 (dt, J_{CP}=15.3 Hz, J_{CF}=19.2 Hz), 30.7 (d, J_{CP}=1.7 Hz), 27.4 (d, J_{CP}=3.3 Hz), 16.3 (d, J_{CP}=5.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −48.7 (1F, ddd, J_{HF}=17.0 Hz, J_{FF}=300.1 Hz, J_{PF}=110.3 Hz), −50.4 (1F, ddd, J_{HF}=19.2 Hz, J_{FF}=300.1 Hz, J_{PF}=110.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.40 (t, J_{PF}=110.3 Hz); IR (neat) 3444, 2938, 2871, 1261, 1026 cm^{-1}; ESIMS *m/z* 403 (M^++Na), 381 (M^++H), 363 (M^+−OH). Anal. calcd for $C_{17}H_{27}O_5F_2P$: C, 53.68; H, 7.16. Found: C, 53.53; H, 7.09.

3.1.7. Diethyl (2*R*)-4-(benzyloxy)-2-{2-[(diethoxyphophoryl)oxy]ethyl}-1,1-difluorobutylphosphonate (21). To a stirred solution of **12** (100 mg, 0.26 mmol) in CH_2Cl_2 (1 mL) was added pyridine (8.4 μL, 1.04 mmol) and $(EtO)_2POCl$ (42 μL, 0.29 mmol) at 0°C and then further stirred for 18 h at the same temperature. A saturated aqueous $KHSO_4$ solution was then added and extracted with EtOAc. The reaction mixture was washed with saturated aqueous Na_2CO_3 and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The remaining residue was chromatographed on silica gel. Elution with EtOAc gave **21** (96 mg, 72%) as an oil: $[\alpha]_D^{25}$=−2.9 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.21 (5H, m), 4.52 (1H, d, *J*=12.1 Hz), 4.48 (1H, d, *J*=12.1 Hz), 4.33–4.18 (4H, m), 4.17–4.02 (6H, m), 3.58 (2H, t, *J*=6.5 Hz), 2.56–2.35 (1H, m), 2.29–2.16 (2H, m), 1.92–1.79 (1H, m), 1.75–1.63 (1H, m), 1.37 (3H, t, *J*=6.9 Hz), 1.35 (3H, t, *J*=7.0 Hz), 1.31 (3H, dt, *J*=0.6, 7.1 Hz), 1.30 (3H, dt, *J*=0.6, 7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 128.0, 127.2, 122.1 (dt, J_{CP}=211.4 Hz, J_{CF}=263.2 Hz), 72.5, 67.3, 65.1 (d, J_{CP}=4.9 Hz), 64.2 (d, J_{CP}=6.9 Hz), 63.4 (d, J_{CP}=5.7 Hz), 36.9 (dt, J_{CP}=15.8 Hz, J_{CF}=19.3 Hz), 28.3, 27.6, 16.0 (d, J_{CP}=4.9 Hz), 15.8 (d, J_{CP}=6.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.6 (1F, ddd, J_{HF}=16.6 Hz, J_{FF}=302.5 Hz, J_{PF}=109.3 Hz), −49.4 (1F, ddd, J_{HF}=18.6 Hz, J_{FF}=302.5 Hz, J_{PF}=109.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.03 (1P, t, J_{PF}=109.3 Hz), −0.76 (1P, s); IR (neat) 2985, 1271, 1029 cm^{-1}; ESIMS *m/z* 539 (M^++Na). Anal. calcd for $C_{21}H_{36}O_8F_2P_2$: C, 48.84; H, 7.03. Found: C, 48.78; H, 6.92.

3.1.8. Diethyl (2*R*)-4-[(diethoxyphophoryl)oxy]-1,1-difluoro-2-(2-hydroxyethyl)butylphosphonate (22). A mixture of **21** (850 mg, 1.65 mmol), 10% Pd–C (170 mg) and MeOH (12 mL) was stirred under a hydrogen atmosphere at room temperature for 15 h. After removal of Pd–C by filtration, the filtrate was concentrated under reduced pressure to give an oily residue, which was chromatogaraphed on silica gel. Elution with $CHCl_3$/MeOH (30:1) afforded **22** (660 mg, 94%) as an oil: $[\alpha]_D^{25}$=−8.6 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34–4.23 (4H, m), 4.22–4.05 (6H, m), 3.80–3.66 (2H, m), 2.63–2.44 (1H, m), 2.31–2.20 (1H, m), 2.19–2.08 (1H, m), 1.85–1.74 (1H, m), 1.68–1.56 (1H, m), 1.38 (6H, t, *J*=7.1 Hz), 1.34 (6H, t, *J*=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.3 (dt, J_{CP}=211.5 Hz, J_{CF}=263.5 Hz), 65.5, 64.4 (d, J_{CP}=5.9 Hz), 63.7, 59.5 (d, J_{CP}=7.2 Hz), 36.6 (dt, J_{CP}=15.6 Hz, J_{CF}=19.1 Hz), 30.5, 28.5, 16.2 (d, J_{CP}=4.7 Hz), 15.9 (d, J_{CP}=5.9 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.7 (1F, ddd, J_{HF}=15.6 Hz, J_{FF}=301.8 Hz, J_{PF}=109.3 Hz), −49.9 (1F, ddd, J_{HF}=19.2 Hz, J_{FF}=301.8 Hz, J_{PF}=109.3 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.21 (1P, t, J_{PF}=109.3 Hz), −0.39 (1P, s); IR (neat) 3431, 2986, 1265, 1029 cm^{-1}; ESIMS *m/z* 449 (M^++Na). Anal. calcd for $C_{14}H_{30}O_8F_2P_2$: C, 39.44; H, 7.09. Found: C, 39.32; H, 6.89.

3.1.9. Diethyl (2*R*)-4-(6-chloro-9*H*-purin-9-yl)-2-{2-[(diethoxyphophoryl)oxy]ethyl}-1,1-difluorobutylphosphonate (23). To a stirred solution of **22** (660 mg, 1.55 mmol) in THF (3 mL) was added a solution of triphenylphosphine (490 mg, 1.86 mmol) and 6-chloropurine (290 mg, 1.86 mmol) in THF (5 mL) at 0°C. To this solution was added dropwise a solution of diisopropylazodicarboxylate (0.37 mL, 1.86 mmol) in THF over a period of 5 min. The reaction mixture was warmed to room temperature and stirred for 18 h. The solution was concentrated under reduced pressure, and the residue was chromatographed on silica gel. Elution with $CHCl_3$/MeOH (30:1) to afford **23** (770 mg, 88%) as an oil: $[\alpha]_D^{25}$=+5.9 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (1H, s), 8.26 (1H, s), 4.45 (2H, t, *J*=7.6 Hz), 4.34–4.06 (10H, m), 2.54–2.28 (3H, m), 2.21–2.08 (1H, m), 1.93–1.80 (1H, m), 1.38 (3H, t, *J*=7.1 Hz), 1.37 (3H, t, *J*=7.0 Hz), 1.33 (6H, t, *J*=7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6, 145.5, 131.5, 121.7 (dt, J_{CP}=211.3 Hz, J_{CF}=263.8 Hz), 64.7 (2C), 64.6, 63.8 (d, J_{CP}=5.6 Hz), 42.0, 37.6 (dt, J_{CP}=15.6 Hz, J_{CF}=19.7 Hz), 28.1, 27.9, 16.2 (d, J_{CP}= 4.6 Hz), 15.9 (d, J_{CP}=6.3 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.4 (1F, ddd, J_{HF}=15.4 Hz, J_{FF}=303.9 Hz, J_{PF}= 107.0 Hz), −49.3 (1F, ddd, J_{HF}=17.9 Hz, J_{FF}=303.9 Hz, J_{PF}=107.0 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.42 (1P, t, J_{PF}=107.0 Hz), −0.34 (1P, s); IR (neat) 2985, 1561, 1263, 1029 cm^{-1}; ESIMS *m/z* 585 (M^++Na), 563 (M^++H). Anal. calcd for $C_{19}H_{31}N_4O_7ClF_2P_2$: C, 40.54; H, 5.55; N, 9.95. Found: C, 40.50; H, 5.64; N, 9.98.

3.1.10. Diethyl (2*R*)-4-[(diethoxyphophoryl)oxy]-1,1-difluoro-2-{2-[6-(methylamino)-9*H*-purin-9-yl]ethyl}butylphosphonate (24). A mixture of **23** (670 mg, 1.19 mmol) in an aqueous $MeNH_2$ solution (2.0 M, 10 mL) was stirred for 4 h at room temperature. The solution was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$:MeOH 50:1) to afford **24** (590 mg, 89%) as an oil: $[\alpha]_D^{25}$=+6.6 (*c* 1.2,

$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (1H, s), 7.82 (1H, s), 5.71 (1H, b), 4.32 (1H, t, J=7.5 Hz), 4.29–4.20 (4H, m), 4.19–4.03 (6H, m), 3.21 (3H, b), 2.48–2.24 (3H, m), 2.18–2.03 (1H, m), 1.93–1.81 (1H, m), 1.37 (3H, t, J=7.1 Hz), 1.36 (3H, t, J=7.1 Hz), 1.33 (3H, t, J=7.1 Hz), 1.32 (3H, t, J=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.3, 152.8, 148.6, 139.5, 121.7 (dt, J_{CP}=210.4 Hz, J_{CF}=263.3 Hz), 119.6, 64.7 (d, J_{CP}=4.0 Hz), 64.5, 63.6 (d, J_{CP}=4.6 Hz), 41.2, 37.4 (dt, J_{CP}=17.5 Hz, J_{CF}=18.6 Hz), 28.0, 27.3, 16.1, 15.8 (d, J_{CP}=5.3 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.2 (1F, ddd, J_{HF}=16.2 Hz, J_{FF}=304.0 Hz, J_{PF}=107.8 Hz), −49.0 (1F, ddd, J_{HF}=17.7 Hz, J_{FF}=304.0 Hz, J_{PF}=107.8 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.59 (1P, t, J_{PF}=107.8 Hz), −0.53 (1P, s); IR (neat) 3417, 2984, 1625, 1263, 1028 cm^{-1}; ESIMS m/z 558 (M^++H). Anal. calcd for $C_{20}H_{35}N_5O_7F_2P_2$: C, 43.09; H, 6.33; N, 12.56. Found: C, 42.67; H, 6.29; N, 12.37.

3.1.11. Diethyl (2*R*)-1,1-difluoro-4-hydroxy-2-{2-[(triisopropylsilyl)oxy]ethyl}butylphosphonate (13). A mixture of **11** (500 mg, 0.93 mmol), 10% Pd–C (100 mg) and MeOH (7 mL) was stirred under hydrogen atmosphere for 15 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 (1:1) gave **13** (420 mg, 100%) as an oil: $[\alpha]_D^{25}$=−1.2 (*c* 1.0, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.33–4.21 (4H, m), 3.87–3.72 (4H, m), 2.59–2.42 (1H, m), 2.19–2.07 (2H, m), 1.76–1.58 (2H, m), 1.38 (3H, t, J=7.0 Hz), 1.38 (3H, t, J=7.1 Hz), 1.16–1.00 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.7 (dt, J_{CP}=211.7 Hz, J_{CF}=263.0 Hz), 64.4 (d, J_{CP}=7.0 Hz), 64.4 (d, J_{CP}=6.9 Hz), 61.5, 60.5, 37.0 (dt, J_{CP}=15.4 Hz, J_{CF}=19.0 Hz), 30.8 (d, J_{CP}=0.9 Hz), 30.4 (d, J_{CP}=3.7 Hz), 17.9, 16.3 (d, J_{CP}=5.4 Hz), 11.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ −48.0 (1F, ddd, J_{HF}=15.7 Hz, J_{FF}=300.3 Hz, J_{PF}=110.8 Hz), −50.8 (1F, ddd, J_{HF}=20.9 Hz, J_{FF}=300.3 Hz, J_{PF}=110.8 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.48 (t, J_{PF}=110.8 Hz); IR (neat) 3421, 2944, 2868, 1258, 1028 cm^{-1}; ESIMS m/z 469 (M^++Na), 273 (M^+−OTIPS); HRMS (ESI) calcd for $C_{19}H_{41}O_5F_2NaPSi$ (M^++Na): 469.2327. Found: 469.2345.

3.1.12. Diethyl (2*S*)-4-[(diethoxyphosphoryl)oxy]-1,1-difluoro-2-{2-[(triisopropylsilyl)oxy]ethyl}butylphosphonate (25). To a stirred solution of **13** (420 mg, 0.94 mmol) in pyridine (0.3 mL) was added $(EtO)_2POCl$ (0.15 mL, 1.03 mmol) at 0°C. The mixture was stirred for 18 h at the same temperature. Saturated aqueous $KHSO_4$ solution was added and extracted with EtOAc. The extracts were washed with saturated aqueous Na_2CO_3 and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The remaining residue was purified by silica gel column chromatography (EtOAc) to afford **25** (410 mg, 75%) as an oil: $[\alpha]_D^{25}$=+0.66 (*c* 1.2, MeOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.34–4.22 (4H, m), 4.18–4.06 (6H, m), 3.78 (2H, t, J=6.4 Hz), 2.53–2.35 (1H, m), 2.29–2.07 (2H, m), 1.93–1.81 (1H, m), 1.68–1.55 (1H, m), 1.38 (6H, t, J=7.1 Hz), 1.33 (6H, t, J=7.1 Hz), 1.14–1.00 (21H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 122.4 (dt, J_{CP}=211.4 Hz, J_{CF}=263.0 Hz), 65.3 (d, J_{CP}=5.5 Hz), 64.3 (d, J_{CP}=7.0 Hz), 63.6 (d, J_{CP}=5.8 Hz), 60.7, 36.7 (dt, J_{CP}=15.4 Hz, J_{CF}=19.5 Hz), 30.7 (d, J_{CP}=1.3 Hz), 28.5 (d, J_{CP}=4.0 Hz), 17.9, 16.2 (d, J_{CP}=5.4 Hz), 16.0 (d, J_{CP}=6.6 Hz), 11.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −47.8 (1F, ddd, J_{HF}=17.6 Hz, J_{FF}=302.2 Hz, J_{PF}=109.8 Hz), −48.8 (1F, ddd, J_{HF}=18.0 Hz, J_{FF}=302.2 Hz, J_{PF}=109.8 Hz); ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.05 (1P, t, J_{PF}=109.8 Hz), −0.84 (1P, s); IR (neat) 2943, 2868, 1272, 1030 cm^{-1}; ESIMS m/z 605 (M^++Na), 583 (M^++H); HRMS (ESI) calcd for $C_{23}H_{51}O_8F_2P_2Si$(M^++H): 583.2797. Found: 583.2794.

3.1.13. Diethyl (2*S*)-4-[(diethoxyphophoryl)oxy]-1,1-difluoro-2-(2-hydroxyethyl)butylphosphonate (26). A mixture of **25** (390 mg, 0.67 mmol), MeOH (20 mL) and *p*-TsOH·H_2O (60 mg) was stirred for 15 h at room temperature. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution. The organic layer was removed in vacuo. The oily residue was diluted with H_2O and then extracted with EtOAc. The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The crude materials were purified by silica gel column chromatography (EtOAc) to afford **26** (220 mg, 77%) as an oil. The spectroscopic data of **26** were identical to those of **22** except for the specific rotation: $[\alpha]_D^{25}$=+6.7 (*c* 1.3, $CHCl_3$).

3.1.14. Diethyl (2*S*)-4-(6-chloro-9*H*-purin-9-yl)-2-{2-[(diethoxyphophoryl)oxy]ethyl}-1,1-difluorobutylphosphonate (27). This compound (200 mg, 85%) was obtained from **26** (180 mg, 0.42 mmol), 6-chloropurine (78 mg, 0.5 mmol), triphenylphosphine (132 mg, 0.5 mmol) and diisopropyl azodicarboxylate (0.1 mL, 0.5 mmol) according to the same conditions as described in Section 3.1.9 for the synthesis of **23**. The spectral data of **27** were identical with those of **23** except for the specific rotation: $[\alpha]_D^{25}$=−3.6 (*c* 1.6, $CHCl_3$).

3.1.15. Diethyl (2*S*)-4-[(diethoxyphophoryl)oxy]-1,1-difluoro-2-{2-[6-(methylamino)-9*H*-purin-9-yl]ethyl}butylphosphonate (28). This compound (130 mg, 86%) was obtained from **27** (160 mg, 0.28 mmol) according to the same conditions as described in Section 3.1.10 for the synthesis of **24**. The spectral data of **28** were identical with those of **24** except for the specific rotation: $[\alpha]_D^{25}$=−9.4 (*c* 1.1, $CHCl_3$).

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (C) (to S. S. and T. Y.) and for Encouragement of Young Scientists (to T. M.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543. (b) Beaucage, L. S.; Iyer, P. L. *Tetrahedron* **1993**, *49*, 6123. (c) Porritt, G. M.; Reese, C. B. *Tetrahedron Lett.* **1989**, *30*, 4713. (d) Wendeborn, S.; Jouanno, C.; Wolf, R. M.; Mesmaeker, A. *Tetrahedron Lett.* **1996**, *37*, 5511. (e) Ahmad, A. *Tetrahedron Lett.* **1991**, *32*, 4483.

2. Harden, T. K.; Hawkins, P. T.; Stephens, L.; Boyer, J. L.; Downed, P. *Biochem. J.* **1988**, *252*, 583.
3. Jassens, R.; Communi, D.; Pirroton, S.; Samson, M.; Parmenthe, R.; Boeynaems, J. M. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 588.
4. Jin, J.; Daniel, J. L.; Kunapuri, S. P. *J. Biol. Chem.* **1998**, *273*, 2030.
5. Crowley, M. R. *J. Cardiovasc. Pharmacol.* **1997**, *30*, 102.
6. Boyer, J. L.; Romero-Avila, T.; Schachter, J. B.; Harden, T. K. *Mol. Pharmacol.* **1996**, *50*, 1323.
7. (a) Kim, H. S.; Barak, D.; Harden, T. K.; Boyer, J. L.; Jacobsen, K. A. *J. Med. Chem.* **2001**, *44*, 3092, and references cited therein. (b) Campaoini, E.; Boyer, J. L.; Mohanram, A.; Harden, T. K.; Jacobson, K. A. *J. Med. Chem.* **1998**, *41*, 183, and references cited therein. (c) Kim, H. S.; Ravi, R. G.; Marquez, V. E.; Maddileti, S.; Wihlborg, A.-K.; Erlinge, D.; Malmsjo, M.; Boyer, J. L.; Harden, T. K.; Jacobsen, K. A. *J. Med. Chem.* **2002**, *45*, 208, and references cited therein. (d) Jacobson, K. A.; Javis, M. F.; Williams, M. *J. Med. Chem.* **2002**, *49*, 4057, and references cited therein. (e) Boyer, J. L.; Mohanram, A.; Campoini, E.; Jacobson, K. A.; Harden, T. K.; Brit, J. *Pharmcology* **1998**, *124*, 1. (f) Xu, B.; Stephens, A.; Kirschenheuter, G.; Greslin, A. F.; Cheng, X.; Sennelo, J.; Cook, G.; Jacobson, K. A. *J. Med. Chem.* **2002**, *45*, 5694, and references cited therein.
8. For recent leading references, see: (a) Burke, T. R., Jr.; Ye, B.; Yan, X.; Wang, S.; Jia, Z.; Chen, L.; Zhang, Z.-Y.; Barford, D. *Biochemistry* **1996**, *35*, 15989. (b) Jia, Z.; Ye, Q.; Dinaut, A. N.; Wang, Q.; Waddleton, D.; Payette, P.; Ramachandran, C.; Kennedy, B.; Hum, G.; Taylor, S. D. *J. Med. Chem.* **2001**, *44*, 4584. (c) Yokomatsu, T.; Murano, T.; Umesue, I.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 529. (d) Halazy, S.; Ehrhard, A.; Eggenspiller, A.; Berges-Gross, V.; Danzin, C. *Tetrahedron* **1996**, *52*, 177. (e) Yokomatsu, T.; Hayakawa, Y.; Kihara, T.; Koyanagi, S.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem.* **2000**, *8*, 2571. (f) Yokomatsu, T.; Murano, T.; Akiyama, T.; Koizumi, J.; Shibuya, S.; Tsuji, Y.; Soeda, S.; Shimeno, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 229. (g) Yokomatsu, T.; Kato, J.; Sakuma, C.; Shibuya, S. *Synlett* **2003**, 1407.
9. (a) Matulic-Adamic, J.; Usman, N. *Tetrahedron Lett.* **1994**, *35*, 7193. (b) Matulic-Adamic, J.; Haeberli, P.; Usman, N. *J. Org. Chem.* **1995**, *60*, 2563. (c) Lopin, C.; Gautier, A.; Gouhier, G.; Piettre, S. R. *J. Am. Chem. Soc.* **2002**, *129*, 14668.
10. (a) Murano, T.; Muroyama, S.; Yokomatsu, T.; Shibuya, S. *Synlett* **2002**, 1657. (b) Murano, T.; Yuasa, Y.; Muroyama, S.; Yokomatsu, T.; Shibuya, S. *Tetrahedron* **2003**, *59*, 9059.