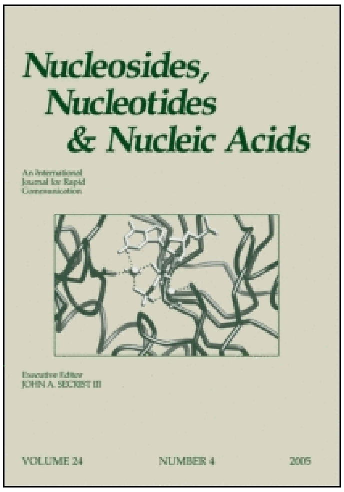

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Regioselective Metalation of 6-Methylpurines: Synthesis of Fluoromethyl Purines and Related Nucleosides for Suicide Gene Therapy of Cancer

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REGIOSELECTIVE METALATION OF 6-METHYLPURINES: SYNTHESIS OF FLUOROMETHYL PURINES AND RELATED NUCLEOSIDES FOR SUICIDE GENE THERAPY OF CANCER

Abdalla E. A. Hassan, William B. Parker, Paula W. Allan, and John A. Secrist III

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□ *Metalation of 6-methyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (**10**) with lithiating agents of varying basicities such as* n*-BuLi and LiHMDS in THF at* $-78°$ *C resulted in metalation at both of the 6-*CH_3 *moiety and the 8-CH position, irrespective of the molar equivalence of the base. On the other hand, a regioselective metalation at the 6-*CH_3 *moiety of **10** was observed with NaHMDS or KHMDS, under similar conditions. Treatment of the potassium salts of **10** and of the protected riboside derivative 6-methyl-9-(β-D-2,3,5-tri-*O*-*tert*-butyldimethylsilylribofuranosyl)purine (**22**) with* N*-fluorobenzenesulfonamide (NFSI) at* $-78°$ *C gave the corresponding 6-fluoromethylpurine derivatives **11** and **23**, respectively, in good yields. Deprotection of **11** and **23** under standard conditions gave 6-fluoromethylpurine (6-FMeP, **3**) and 6-fluoromethyl-9-(β-D-ribofuranosyl)purine (6-FMePR, **4**), respectively, in high yield. Both **3** and **4** demonstrated cytotoxic activity against CCRF-CEM cells in culture. 6-FMePR is a good substrate for* E. coli *purine nucleoside phosphorylase (*E. coli *PNP) with a comparable substrate activity to that of the parent nucleoside, 6-methyl-9-(β-D-ribofuranosyl)purine (6-MePR, **21**). The cytotoxic activity of 6-FMeP along with the substrate activity of 6-FMePR with* E. coli *PNP meet the fundamental requirements for using 6-FMeP as a potential toxin in PNP/prodrug based cancer gene therapy.*

Keywords Metalation; electrophilic fluorination; purine nucleoside phosphorylase; suicide gene therapy of cancer

INTRODUCTION

We have developed a cancer gene therapy strategy that is based on the activation of a non-toxic purine nucleoside (prodrug) to a highly

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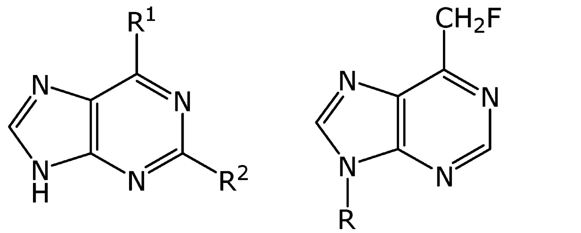


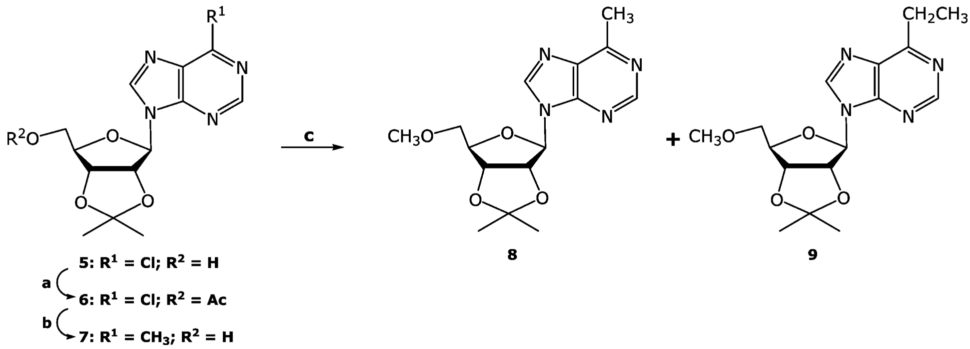
CHART 1

toxic purine analog by a non-human gene, *E. coli* purine nucleoside phosphorylase (*E. coli* PNP), which can be selectively expressed in tumor cells.[1–6] *E. coli* PNP differs from human PNP in its ability to accept not only 6-oxopurine nucleosides, but also 6-aminopurine and certain other 6-substituted purine nucleoside analogs as substrates.[7,8] This property has been used to cleave relatively non-toxic purine nucleoside analogs to very toxic purine analogs, which readily diffuse across cell membranes and have high bystander activity.[1,3] The toxic adenine analogs of most interest to date are 6-methylpurine (6-MeP, **1**) and 2-fluoroadenine (**2**)[4] (Chart 1); however, we still continue to search for the optimal toxin/prodrug combination that would have the desired biological properties. The small size and powerful electron-withdrawing properties of fluorine dramatically affect the physical (e.g., lipophilicity), chemical, and biological properties of organic compounds. Substitution of hydrogen atoms for fluorine at the nucleobase[9–17] and at the sugar moiety[18–20] of nucleosides have produced several anticancer and antiviral drugs as well as other molecules that are undergoing clinical investigation. Herein, we report on selective metalation at the 6-CH_3 moiety of 6-methylpurine derivative **10** and its application for the synthesis of 6-FMeP (**3**) as a potential toxin for application in PNP-based cancer gene therapy (Chart 1). We have also applied the methodology to a nucleoside precursor to prepare 6-FMePR (**4**). A preliminary account of this research has previously been published.[21] In addition, another very useful approach to 6-fluoromethylpurine derivatives has appeared.[22]

RESULTS AND DISCUSSION

Chemistry

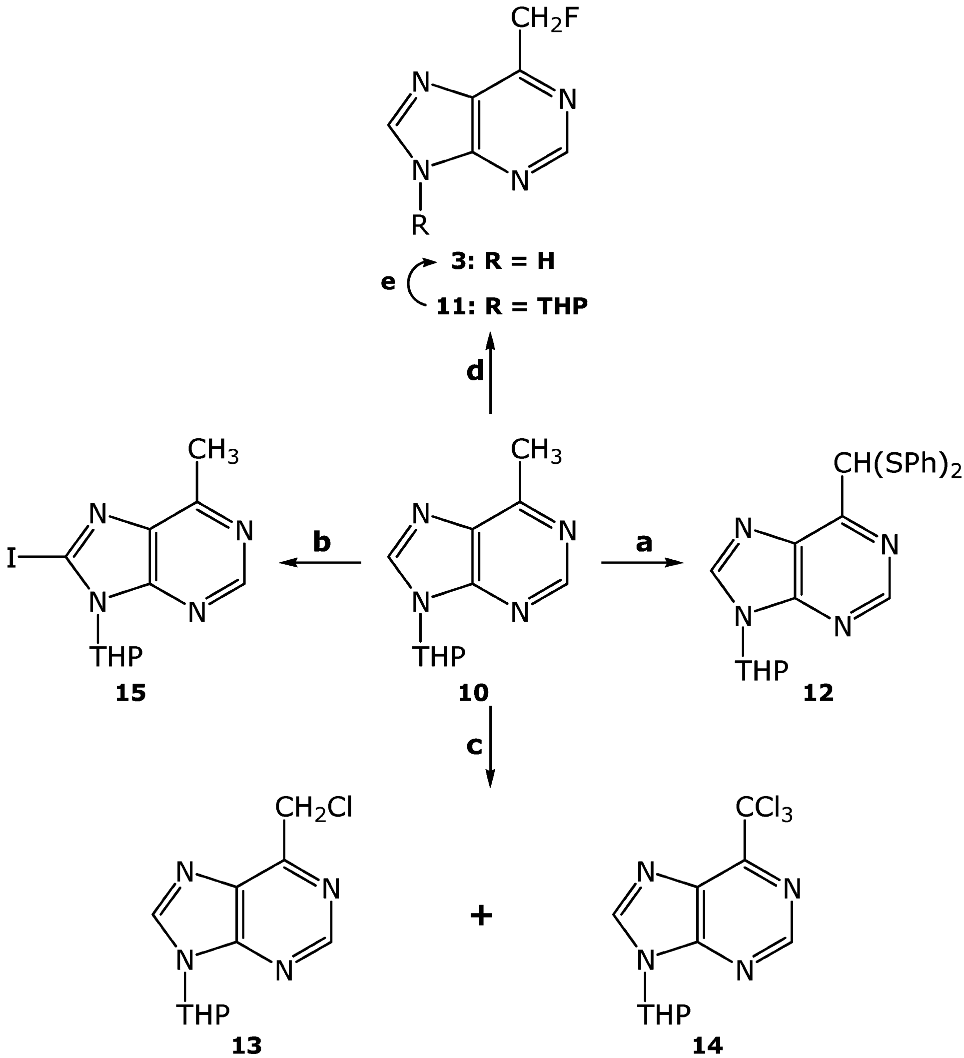
During the course of the synthesis of 5′-alkoxy derivatives of 6-methyl-9-(β-D-ribofuranosyl)purine as potential prodrugs for 6-MeP, we have observed under basic conditions a C-alkylation at the 6-methyl along with the desired *O*-alkylation at the 5′-hydroxyl. The precursor for the alky-



SCHEME 1 a) Ac_2O, pyridine, 4 hours, room temperature., 95%; b) CH_3ZnBr, Pd $(PPh_3)_4$, THF, 1 hour, 55°C, then $MeOH/NH_3$, 4 hours, room temperature, 77%; c) *t*-BuOK, CH_3I, THF, 30 minutes, 0°C, 87% for **8** and 7% for **9.**

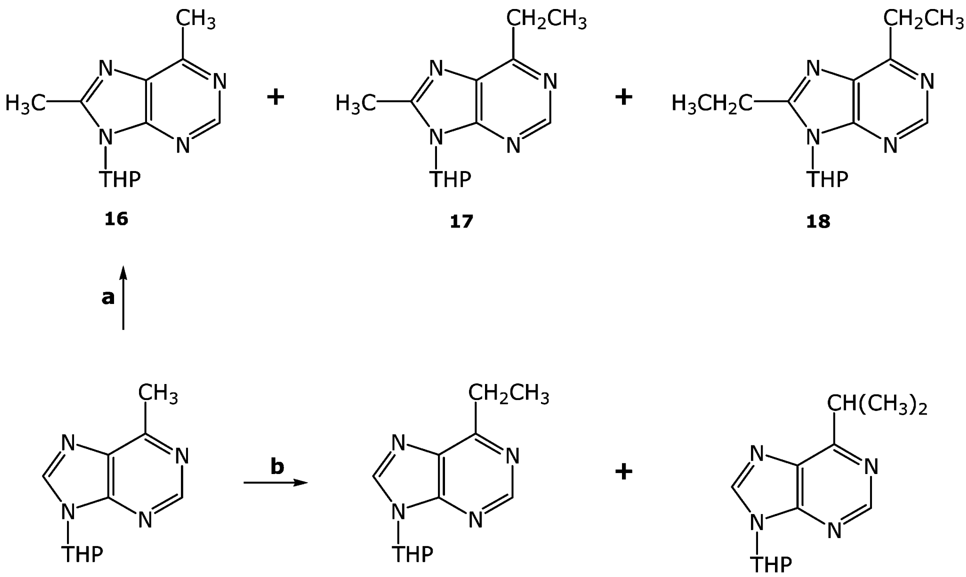
lation reaction, 6-methyl-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine (**7**),[23] was synthesized in good yield (Scheme 1) following our reported procedure.[24] Treatment of **7** with *t*-BuOK (1.2 equiv.) in THF in the presence of MeI gave the *O,C*-bisalkylated derivative **9** in 7% yield along with the desired 5′-*O*-methyl product **8** in 87% yield (Scheme 1). When the above reaction was carried out with the stronger base NaH (1.5 equiv.) under similar conditions, the amount of **9** was increased at the expense of the monoalkylated product **8** (**9**:**8**, 1.1:1). These results led us to examine the feasibility of the synthesis of 6-fluoromethylpurines utilizing a selective metalation-electrophilic fluorination of the 6-CH_3 moiety of 6-methylpurine derivatives.

We first attempted deprotonation of 6-methylpurine derivative **10**[25] using *t*-BuOK in THF and quenching with *N*-fluorobenzenesulfonimide (NFSI) at −40°C or at −78°C. At both temperatures a complex mixture was obtained and none of the desired monofluorinated product **11** was isolated (Scheme 2). This result was attributed to the higher acidity of the fluoromethyl protons as compared to the starting material. This conclusion was supported by conducting the reaction in the presence of electrophiles with similar electronic characteristics under similar conditions. Treatment of **10** with NaHMDS in the presence of diphenyl disulfide at −78°C gave 6-[bis(phenylthio)methyl] derivative **12** in 78% yield as a sole product and neither the 8-phenylthio or 6-phenylthiomethyl derivatives were isolated. Similar results were also obtained upon treatment of **10** with NaHMDS at −78°C and quenching with TsCl to give a mixture of 6-chloromethylpurine derivative **13** and 6-trichloromethyl derivative **14** in good yields. To our surprise, lithiation[26] of **10** with 5 equivalents of *n*-BuLi or LDA in THF at −78°C in the presence of I_2 gave 8-iodo-6-methylpurine derivative **15** in 82% yield based on recovered starting material.



SCHEME 2 a) NaHMDS, $(PhS)_2$, THF, 30 minutes, −78°C—room temperature, 78%; b) LDA, THF, I_2, 1 hour, −78°C, 48% c) NaHMDS, TsCl, THF, 20 minutes, −78°C, 41% for **13** and 8% for **14**; d) KHMDS, NFSI, THF, −80°C, 57%. e) 1*N* HCl, THF, 72 hours, room temperature, 95%.

These results led us to examine the role of the basicity, the counter cation, and the molar equivalents of the base on the regioselectivity of the metalation of **10** in the presence of CH_3I as electrophile (Scheme 3). Lithiation of **10** with *n*-BuLi (5 equiv.) in THF at −78°C, and quenching with CH_3I resulted in alkylation at the 6-CH_3 and the C-8 positions to give the 6-ethyl-8-methyl purine derivative **17** in 75% isolated yield (Table 1, entry 1). The use of LDA (1 equiv.) under similar conditions gave the C-8 alkylated product 6,8-dimethylpurine **16**[27] as the major product along with a mixture of the starting material **10**, 6-ethyl-8-methylpurine **17**, and



SCHEME 3 a) Lithiating agent Table 1, CH_3I, 30 minutes, −78°C; b) NaHMDS or KHMDS, CH_3I, 30 minutes, −78°C.

6-ethylpurine **19** as a minor product (Table 1, entry 2). Increasing the molar equivalence of LDA (2 equiv.) increased the yield of the bisalkylation product **17** at the expense of the C-8 alkylation product **16** (Table 1, entry 3). Similar results were also obtained with Et_2NLi (Table 1, entries 4, 5), with additional equivalents resulting in significant formation of the trialkylated product **18**. When the relatively weak base LiHMDS was used, a moderately regioselective lithiation at the 6-CH_3 moiety was observed (Table 1, entry 7). These results demonstrate that lithiation of 6-methylpurine derivative **10** with the strong bases *n*-BuLi, LDA, or $LiNEt_2$ is poorly selective regardless of the molar equivalence, while the weaker base LiHMDS induces a selective metalation at the 6-CH_3 moiety. We next examined metalation of **10** with bases NaHMDS and KHMDS under the same conditions as used for the lithium salt. With NaHMDS metalation at the 6-CH_3 occurred regioselectively to give a mixture of 6-ethylpurine derivative **19** and 6-isopropylpurine derivative **20**[28,29] in a ratio of (**19**:**20**, 10.5:1; Table 1, entry 7). No 8-methylpurine was formed, as confirmed from the HPLC analysis of the crude product. Similar regioselective metalation at the 6-CH_3 moiety was obtained with KHMDS (Table 1, entry 8). These results as presented in Table 1 correlate the metalation regioselectivity with the nature of the metal ion as well as strength and steric aspects of the base.

We next attempted the metalation of **10** using KHMDS (5 equiv.) in THF at −78°C, followed by treatment with NSFI (1.1 equiv.). A complex mixture containing the desired product **11** was obtained, as judged by

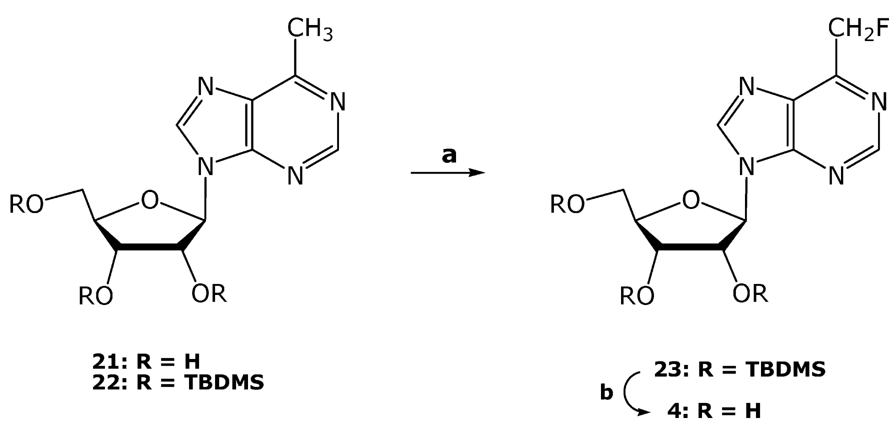
TABLE 1 Effect of the base on the regioselectivity of the metallation of **10**

		Ratio[b]					
Entry	Base[a] (molar equivalents)	10	16	17	18	19	20
1	*n*-Bu Li (5 eq.)	0.5	2.9	83.8	2.3	3.6	—
2	LDA (1 eq.)	22.6	61.5	8.6	—	3.7	—
3	LDA (2 eq.)	2.3	33.0	48.6	—	3.1	—
4	$LiNEt_2$ (1.2 eq.)	51.6	23.5	3.5	—	18.6	—
5	$LiNEt_2$ (5 eq.)	—	16.6	31.3	36.5	12.7	—
6	LiHMDS (5 eq.)	2.0	—	14.0	10.6	54.1	11.9
7	NaHMDS (5 eq.)	0.04	0.45	—	—	89.4	8.5
8	KHMDS (5 eq.)	0.02	0.35	—	—	92.1	5.5

[a] Metallation reactions were performed in THF −78°C for 30 minutes at and quenched with CH_3I (10 equiv.).

[b] The ratio of the products was determined by the% area of the HPLC chromatograms after a flash silica gel column chromatography, not normalized to 100%.

mass spectral analysis of the crude product. After examination of several ratios of KHMDS and NSFI as well as variations in the reaction time of the fluorination step, we found that the use of two equivalents of both KHMDS and NSFI followed by quenching the fluorination reaction in less than 5 minutes at −78°C, produced the desired 6-FMeP derivative **11** in 58% yield. The ^{1}H-NMR spectrum of **11** shows the 6-CH_2F signals as two sets of dd with the characteristic large fluorine-proton coupling constant; $J_{F,H}$ = 46.7 Hz. Applying the same sequence of metalation-fluorination (KHMDS/NSFI/THF/−78°C) reactions to the ribonucleoside derivative **22** produced the corresponding 6-fluoromethylpurine derivative **23** in 48% yield (Scheme 4). Deprotection of **11** and **23** under conventional conditions gave 6-FMeP (**3**) and 6-FMePR (**4**) in high yields.



21: R = H
22: R = TBDMS

23: R = TBDMS
4: R = H

SCHEME 4 a) KHMDS, NFSI, THF, −80°C, 47%; b) $Et_4NF{\cdot}xH_2O$, CH_3CN, 2 hours, room temperature, 95%.

TABLE 2 Cleavage of 6-FMePR (**4**) and 6-MePR (**21**) by *E. coli* PNP

Entry	Compound	Mean ± SD (nmoles/mg/hr)[a]
1	6-FMePR	66, 000
2	6-MePR	98, 000
3	Adenosine	398, 000

[a]Compounds (100 μM) were incubated with *E. coli* PNP and the substrates and products were separated by HPLC as described.[6] Number is the average of at least two determinations.

Biology

The newly synthesized compounds, **3** and **4** were evaluated for their cytotoxic activity against CCRF-CEM cells as described previously.[4] Cells were incubated with various concentrations of compound for 72 hours, and the concentration of 6-FMeP (**3**) that inhibited cell growth by 50% (IC_{50}) was 20 μM (N = 2), which was greater than the IC_{50} of 6-methylpurine (1.2 μM).[4] The ribonucleoside derivative **4** also showed potent cytotoxic activity with an IC_{50} of 0.07 μM, which was similar to that of 6-MePR (0.05 μM). These results suggested that 6-FMeP and 6-FMePR were substrates for the activating enzymes (adenine phosphoribosyltransferase and adenosine kinase, respectively) and that 6-FMeP nucleotides formed from these analogs were toxic to human cells, as are the MeP nucleotides. 6-FMeP-R was also a good substrate for *E. coli* PNP. The specific activity of 66,000 nmoles/mg-hr was similar to 98,000 nmoles of 6-MeP-R cleaved/mg-hr (Table 2), which indicated that the fluorine atom did not affect enzyme activity.

CONCLUSIONS

We have demonstrated that a regioselective metalation at the 6-CH_3 of 6-methylpurine derivative **10** is achieved with bases NaHMDS and KHMDS, while less selective metalation at both C-8 and at the alkyl side chain(s) is observed with various lithium bases. We have utilized this observation for the synthesis of the novel purine derivative 6-FMeP, **3** and its ribonucleoside derivative 6-FMePR, **4** via metalation-fluorination. 6-FMeP (**3**) was found to have moderate cytotoxic activity against CCRF-CEM cells in vitro, and its ribonucleoside derivative **4** was found to have a good substrate activity with *E. coli* PNP. These biological properties suggest that 6-FMeP could be considered as a toxin in the PNP-based gene therapy of cancer, and that a 6-FMeP-containing nucleoside suitably blocked to prevent phosphorylation could be a suitable prodrug cleavable to 6-FMeP.

EXPERIMENTAL

Melting points were determined on a Mel-temp apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Nicolet

NT 300NB spectrometer (Madison, WI, USA) operating at 300.635 MHz (^{1}H) or 75.6 MHz (^{13}C). Chemical shifts were expressed in parts per million from tetramethylsilane. The hydrogen-decoupled ^{13}C NMR was assigned by comparison of the J_{CH} values obtained from hydrogen-coupled ^{13}C NMR spectra, and when necessary, selective hydrogen decoupling was performed in order to confirm the assignments. The NOE experiments were conducted in degassed $CDCl_3$. To minimize the effects of magnetic perturbations with the sample nonspinning, eight FIDs were recorded with the decoupler set to a desired frequency and eight FID's were recorded with the decoupler off-resonance. Ultraviolet absorption spectra were determined on Perkin-Elmer Lambda 9 spectrometer (Norwalk, CT, USA) by dissolving each compound in methanol or water and diluting 10-fold with 0.1 *N* HCl, pH 7 buffer, and 0.1 *N* NaOH. Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer (Palo Alto, CA, USA) in the fast atom bombardment (FAB) mode (glycerol matrix). HPLC analysis were carried out on a Hewlett-Packard 1100 series liquid chromatograph (Wilmington, DE, USA) with a Phenomenex Sphereclone 5 μ ODS (1) column (4.6 mm × 25 cm) with UV monitoring (254 nm). All flash column chromatography used 230–400 mesh silica gel from E. Merck. TLC was done on Analtech (Newark, DE, USA) precoated (250 μm) silica gel (GF) plates. Compounds **5**[10] and **10**[11] have been previously fully characterized. The 6-fluoromethylpurine derivatives **3** and **4** have full characterization data that agrees with the literature.[22] The various other 6- and 8-substituted purines are characterized with spectral data alone.

6-Chloro-9-(5-*O*-acetyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine (6)

Acetic anhydride (90 μL, 0.92 mmol) was added to a solution of **5**[10] (150 mg, 0.46 mmol) in dry pyridine (5 mL) at 0°C. The mixture was stirred for 4 hours at room temperature, EtOH (1 mL) and the solvents were evaporated in vacuo. Water work up and flash silica gel column chromatography (eluate: 30% EtOAc in hexanes) gave (160 mg, 95%) of **6** as a white solid: MS *m/z* 369 $(M+1)^+$, ^{1}H NMR ($CDCl_3$) δ 8.79 (1H, s, H-2), 8.25 (1H, s, H-8), 6.20 (1H, d, H-1′, $J = 2.2$ Hz), 5.43 (1H, dd, H-2′, $J = 2.2$, $J = 6.4$ Hz), 5.03 (1H, dd, H-3′, $J = 3.5$, $J = 6.4$ Hz), 4.54 (1H, ddd, H-4′, $J_{3',4'} = 3.5$, $J_{4',5a'} = 4.2$, $J_{4',5b'} = 5.9$ Hz), 4.36 (1H, dd, H5′a, $J_{4',5a'} = 4.2$, $J_{5'a,5'b} = 12.1$ Hz), 4.24 (1H, dd, H5′b, $J_{4',4b'} = 5.9$, $J_{5'a,5'b} = 12.1$ Hz), 1.99 (3H, s, Ac), 1.65 (3H, s, CMe_2), 1.41 (3H, s, CMe_2).

6-Methyl-9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)purine (7)[23,24]

Pd $(PPh_3)_4$ (46 mg, 0.04 mmol) in THF (1 mL) was added to a solution of CH_3ZnBr (0.23 mL, 0.95 mmol) in THF (4 mL) at room temperature.

Compound **6** (0.14 g, 0.38 mmol) in THF (1 mL) was added and the mixture was stirred for 1 hour at 55°C. The solvent was evaporated *in vacuo* and the residue was partitioned between EtOAc and H_2O. The organic phase was dried ($MgSO_4$) and evaporated. The residue was ′dissolved in MeOH saturated with NH_3 (5 mL) and stirred for 4 hours at room temperature. The solvent was evaporated and the residue was purified by silica gel chromatography (eluate; 1% MeOH in $CHCl_3$) to give (89 mg, 77%) of **7** as a white solid; HPLC 100% (RT 5.297, 0.01 *M* $NH_4H_2PO_4$: MeOH 60: 40); MS *m/z* 307.1 $(M+1)^+$; UV λ_{max} (pH 1) 264.2, λ_{max} (pH 7) 260.3, λ_{max} (pH 13) 260.5 nm; ^1H NMR ($CDCl_3$) δ 8.84 (1H, s, H-2), 8.09 (1H, s, H-8), 5.92 (1H, d, H-1′, $J = 4.8$ Hz), 5.86 (1H, dd, 5′-OH, $J = 2.0$, $J = 11.7$ Hz), 5.23 (1H, br dd, H-2′), 5.13 (1H, br dd, H-3′), 4.56 (1H, br d, H-4′), 3.99 (1H, m, H-5′a), 3.82 (1H, m, H-5′b), 2.88 (3H, s, 6-CH_3), 1.66 (3H, s, CMe_2), 1.39 (3H, s, CMe_2); Anal. Calcd. for $C_{14}H_{18}N_4O_4$.: C 54.89, H 5.92, N 18.29; Found: C 54.52, H 5.96, N 18.41.

6-Methyl-9-(2,3-*O*-isopropylidene-5-*O*-methyl-β-D-ribofuranosyl)purine (8) and 6-ethyl-9-(2,3-*O*-isopropylidene-5-*O*-methyl-β-D-ribofuranosyl)purine (9)

A 1*M* solution of *t*-BuOK in THF (0.22 mL, 0.26 mmol) was added to a solution of **7** (66 mg, 0.22 mmol) in THF (2 mL) at 0°C and the mixture was stirred for 5 minutes. CH_3I (50 μL, 0.52 mmol) was added and the mixture was stirred for 30 minutes at 0°C. The volatiles were evaporated and the residue was purified by a flash silica gel chromatography (eluate: 40%-20% hexanes in EtOAc) to give (64 mg, 87%) of **8** as a colorless syrup and (5 mg, 7%) of **9** as a colorless syrup: Spectroscopic parameters for **8**: MS *m/z* 321 $(M+1)^+$; UV λ_{max} (pH 1) 264.0, λ_{max} (pH 7) 260.2, λ_{max} (pH 13) 260.5 nm; ^1H NMR ($CDCl_3$) δ. 8.87 (1H, s, H-2), 8.23 (1H, s, H-8), 6.28 (1H, d, H-1′, $J = 2.5$ Hz), 5.27 (1H, dd, H-2′, $J = 2.4$, $J = 6.2$ Hz), 4.98 (1H, dd, H-3′, $J = 2.3$, $J = 6.2$ Hz), 4.52 (1H, m, H-4′), 3.64 (1H, dd, H-5′a, $J = 3.2$, $J = 10.4$ Hz), 3.57 (1H, dd, H-5′b, $J = 4.2$, $J = 10.4$ Hz), 3.33 (3H, s, 5′-*O*-CH_3), 2.87 (3H, s, 6-CH_3), 1.65 (3H, s, CMe_2), 1.40 (3H, s, CMe_2); spectroscopic parameters for **9**: MS *m/z* 335 $(M+1)^+$; UV λ_{max} (pH 1) 265.4, λ_{max} (pH 7) 260.8, λ_{max} (pH 13) 261.3 nm; ^1H NMR ($CDCl_3$) δ. 8.91 (1H, s, H-2), 8.28 (1H, s, H-8), 6.28 (1H, d, H-1′, $J = 2.4$ Hz), 5.27 (1H, dd, H-2′, $J = 2.5$, $J = 6.3$ Hz), 4.98 (1H, dd, H-3′, $J = 2.3$, $J = 6.2$ Hz), 4.51 (1H, m, H-4′), 3.64 (1H, dd, H-5′a, $J = 3.3$, $J = 10.4$ Hz), 3.57 (1H, dd, H-5′b, $J = 4.2$, $J = 10.4$ Hz), 3.34 (3H, s, 5′-*O*-CH_3), 3.23 (2H, q, 6-$C\underline{H_2}CH_3$, $J = 7.6$ Hz), 1.65 (3H, s, CMe_2), 1.44 (3H, t, 6-$CH_2C\underline{H_3}$, $J = 7.6$ Hz), 1.40 (3H, s, CMe_2).

6-[Bis(phenylthio)methyl]-9-(tetrahydro-*2H*-pyran-2-yl)purine (12)

1*M* solution of NaHMDS in THF (1.9 mL) was added dropwise to a solution of **10** (100 mg, 0.46 mmol) in THF (3 mL) at −78°C and the mixture was stirred for 5 minutes. A solution of diphenyl disulfide (0.23 g, 2.3 mmol) in THF (3 mL) was added and the mixture was further stirred for 30 minutes at room temperature. NH_4Cl solution (1*M*, 3 mL) was added and the solvents were evaporated under reduced pressure. The residue was partitioned between $CHCl_3$ and H_2O, the organic phase was dried over ($MgSO_4$), evaporated, and purified by short flash silica gel column (eluate; 1% MeOH in $CHCl_3$) to (155 mg, 78%) of **12** as a pale yellow solid: MS *m/z* 435 $(M+1)^+$, ^1H NMR ($CDCl_3$) δ 8.91 (1H, s, H-2), 8.22 (1H, s, H-8), 7.95–7.20 (10H, m, 6-CHS$\underline{Ph_2}$), 6.21 (1H, s, 6-$\underline{C}$HSPh$_2$), 5.77 (1H, dd, H-1′, J = 2.6, J = 9.9 Hz), 4.18 (1H, m, H-5′$_a$), 3.79 (1H, m, H-5′$_b$), 2.12–1.68 (6H, m, H-2′$_{a,b}$, H-3′$_{a,b}$, and H-4′$_{a,b}$).

6-Fluoromethyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (11)[21,22]

A pre-cooled solution of KHMDS (1.17 g, 5.85 mmol) in THF (20 mL) was added dropwise to a solution of **10** (1.06 g, 4.87 mmol) in THF (20 mL) over 5 minutes at −80°C. The mixture was stirred for 30 minutes, and then treated with a cold solution of NFSI (1.9 g, 5.85 mmol) in THF (5 mL) at −80°C. The mixture was stirred for 5 minutes at the same temperature, then quenched with NH_4Cl (1*N*, 10 mL). The mixture was partitioned between EtOAc and H_2O, the organic phase was dried over ($MgSO_4$), and evaporated. The residue was purified by a flash silica gel column (eluate 1% MeOH in $CHCl_3$) to give (0.513 g, 57.8%, based on a recovered 0.24 g, 23% of **10**) of **11** as a pale yellow solid: MS *m/z* 237.1 $(M+1)^+$, UV λ_{max} (pH 1) 261.1, λ_{max} (pH 7) 265.6, λ_{max} (pH 13) 274.9 nm; ^1H NMR ($CDCl_3$) δ 9.00 (1H, s, H-2, $^1J_{C,H}$ = 206.3 Hz), 8.34 (1H, s, H-8, $^1J_{C,H}$ = 212.3 Hz), 5.97 (1H, dd, 6-$CH_{2a}F$, J = 12.6, $J_{F,H}$ = 46.7 Hz), 5.87 (1H, dd, 6-$CH_{2b}F$, J = 12.6, $J_{F,H}$ = 46.7 Hz), 5.83 (1H, dd, H-1′, J = 2.2, J = 5.1 Hz), 4.20 (1H, m, H-5′$_a$), 3.81 (1H, m, H-5′$_b$), 2.15–1.69 (6H, m, H-2′$_{a,b}$, H-3′$_{a,b}$, and H-4′$_{a,b}$).

6-Fluoromethylpurine (3)[21,22]

1 *N* HCl (4 mL) was added to a solution of **11** (0.2 g, 0.85 mmol) in THF (5 mL) and the mixture was stirred for 72 hours at room temperature. The volatiles were evaporated in vacuo and the concentrated aqueous solution was applied to Dowex 50 W (H^+) column. The column was washed with H_2O until no UV absorbing fractions were observed, then eluted with 2.5% NH_4OH. The collected fractions were evaporated and the residue was purified by flash silica gel column (eluate; 15% MeOH in $CHCl_3$) to give (123 mg, 95%) of **3** as a pale yellow solid: m.p. 204–206°C; MS *m/z* 153. 1 $(M+1)^+$, ^1H NMR (DMSO-d_6) δ 13.65 (1H, br s, 9-NH), 8.93 (1H, s, H-2), 8.68 (1H, s, H-8), 5.83 (2H, d, 6-CH_2F, J = 46.6 Hz); ^{13}C NMR (DMSO-d_6) δ 156.13 (C-4), 151.63 (C-2), 150.81 (C-6), 146.64 (C-8), 127.07 (C-5), 81.33

(C-6, J = 165.9 Hz); Anal. Calcd. for $C_6H_5N_4F$, C 47.37, H 3.31, N 36.53; found C 47.25, H 3.42, N 36.32.

6-Chloromethyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (13) and 6-trichloromethyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (14)

1 *M* solution of NaHMDS in THF (0.45 mL) was added dropwise to a solution of **10** (0.1 g, 0.45 mmol) in THF (3 mL) at −78°C and the mixture was stirred for 0.5 hours at −78°C. A pre-cooled solution of TsCl (0.35 g, 1.84 mmol) in THF (2 mL) was added at −80°C and the mixture was stirred for 20 minutes at the same temperature. A 1 *N* solution of NH_4Cl (4 mL) was added and the mixture was warmed to room temperature, partitioned between EtOAc and H_2O, and the organic phase was dried over ($MgSO_4$) and evaporated. The residue was purified by a flash silica gel column (eluate: 40% EtOAc in hexanes) to give (45 mg, 41%) of **13** as a pale yellow syrup and (11 mg, 8%) of **14** as a pale yellow syrup: spectroscopic parameters for **13**; UV λ_{max} (pH 1) 267.1, λ_{max} (pH 7) 268.6, λ_{max} (pH 13) 268.3; ^{1}H NMR ($CDCl_3$) δ 8.99 (1H, s, H-2), 8.34 (1H, s, H-8), 5.82 (1H, dd, H-1′, J = 2.6, J = 10.0 Hz), 5.07 (1H, d, 6-CH_{2a}Cl, J = 11.7 Hz), 5.02 (1H, d, 6-CH_{2b}Cl, J = 11.7 Hz), 4.20 (1H, m, H-5′$_a$), 3.80 (1H, m, H-5′$_b$), 2.19–1.68 (6H, m, H-2′$_{a,b}$, H-3′$_{a,b}$, and H-4′$_{a,b}$); spectroscopic parameters for **14**: H NMR ($CDCl_3$) δ 9.06 (1H, s, H-2), 8.40 (1H, s, H-8), 5.84 (1H, dd, H-1′, J = 2.5, J = 10.1 Hz), 4.20 (1H, m, H-5′$_a$), 3.80 (1H, m, H-5′$_b$), 2.208–1.68 (6H, m, H-2′$_{a,b}$, H-3′$_{a,b}$, and H-4′$_{a,b}$).

8-Iodo-6-methyl-9(tetrahydro-*2H*-pyran-2-yl)purine (15)

LDA (0.5*M,* 4.2 mL) was added dropwise to a solution of **10** (90 mg, 0.41 mmol) in THF (3 mL) at −78°C and the mixture was stirred for 30 minutes. I_2 (0.5 4 g, 2.1 mmol) in THF (1 mL) was and the mixture was stirred for 1 hour at −78°C. 10% Aqueous $Na_2S_2O_3$ (10 mL) was added and the mixture was stirred for 30 minutes at room temperature. EtOAc was added to the mixture and the organic phase was separated, dried over $MgSO_4$ and evaporated. The residue was purified by a flash silica gel column (eluate: 3% MeOH in $CHCl_3$) to give (41 mg, 48%) of **10** and (60 mg, 43%) of **15** as a white solid: UV λ_{max} (pH 1) 282.9 and 217.2, λ_{max} (pH 7) 271.5, λ_{max} (pH 13) 271.7; ^{1}H NMR ($CDCl_3$) δ 8.77 (1H, s, H-2,$^1J_{C,H}$ = 204.8 Hz,), 5.65 (1H, dd, H-1′, J = 2.4, J = 11.4 Hz), 4.23 (1H, m, H-5′$_a$), 3.75 (1H, m, H-5′$_b$), 3.16 (1H, m, H-2′), 2.82 (3H, s, 6-CH_3), 2.18–1.61 (5H, m, H-2′$_b$, H-3′$_{a,b}$, and H-4′$_{a,b}$).

6,8-Dimethyl-9(tetrahydro-*2H*-pyran-2-yl)purine (16)

LDA (2*M*, 0.21 mL) was added dropwise to a solution of **10** (92 mg, 0.42 mmol) in THF (3 mL) at −78°C. The mixture was stirred for 30 minutes, and then MeI (0.25 mL, 4.2 mmol) was added and the mixture was stirred for further 1 hour at −78°C. A solution of NH_4Cl (1*N*, 3 mL) was added and the whole was partitioned between EtOAc and H_2O. The organic phase was separated, dried over ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by a flash silica gel column (eluate: 3% MeOH in $CHCl_3$) to give 69 mg as a mixture of **10** and **15**: HPLC [eluate; H_2O:CH_3CN, 20 minutes linear gradient from 10–90%; **10** (22.59%, RT = 12.49 minutes); **16** (61.47%, RT = 14.77 minutes)]. An analytical sample of **16** was obtained by preparative TLC (eluate 1.5% MeOH in $CHCl_3$): MS *m/z* 233 $(M+1)^+$; ^1H NMR ($CDCl_3$) δ 8.77 (1H, s, H-2), 5.79 (1H, dd, H-1′, $J = 2.5$, $J = 5.9$ Hz), 4.21 (1H, m, H-5′$_a$), 3.75 (1H, m, H-5′$_b$), 2.81 (3H, s, 6-CH_3), 2.79 (3H, s, 8-CH_3), 2.48 (1H, m, H-2′$_a$), 2.08 (1H, m, H-2′$_b$), 1.92–1.66 (6H, m, H-3′$_{a,b}$, and H-4′$_{a,b}$).

6-Ethyl-8-methyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (17)

A 1.6 *M* solution of *n*-BuLi in hexanes (1.07 mL, 1.72 mmol) was added to a solution of **10** (75 mg, 0.34 mmol) in THF (5 mL) at −78°C. The mixture was stirred for 30 minutes under −70°C, then CH_3I (0.2 mL) was added and the mixture was stirred for 30 minutes. A solution of NH_4Cl (1*N*, 3 mL) was added and the mixture was partitioned between EtOAc and H_2O. The organic phase was separated, dried over ($MgSO_4$) and evaporated. The residue was purified by a flash silica gel column (eluate: 3% MeOH in $CHCl_3$) to give (62 mg, 75%) of **17** as a pale yellow syrup: MS *m/z* 247.2 $(M+1)^+$, ^1H NMR ($CDCl_3$) δ 8.81 (1H, s, H-2), 5.80 (1H, dd, H-1′, $J = 2.4$, $J = 8.9$ Hz), 4.20 (1H, m, H-5′$_a$), 3.75 (1H, m, H-5′$_b$), 3.18 (2H, q, 6-$C\underline{H_2}CH_3$, $J = 7.5$ Hz),.2.78 (3H, s, 8-CH_3), 2.49 (1H, m, H-2′a), 2.08–1.63 (5H, m, H-2′$_b$, H-3′$_{a,b}$, and H-4′$_{a,b}$), 1.41 (3H, t, 6-$CH_2C\underline{H_3}$, $J = 7.5$ Hz).

6,8-Diethyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (18)

MS *m/z* 262.2 $(M+1)^+$, ^1H NMR ($CDCl_3$) δ 8.81 (1H, s, H-2), 5.73 (1H, dd, H-1′, $J = 2.3$, $J = 11.2$ Hz), 4.21 (1H, m, H-5′$_a$), 3.73 (1H, m, H-5′$_b$), 3.19 (2H, q, 6-$C\underline{H_2}CH_3$, $J = 7.7$ Hz),.3.11 (2H, q, 8-$C\underline{H_2}CH_3$, $J = 7.6$ Hz), 2.65 (1H, m, H-2′a), 2.08–1.62 (5H, m, H-2′$_b$, H-3′$_{a,b}$, and H-4′$_{a,b}$), 1.47 (3H, t, 6-$CH_2C\underline{H_3}$, $J = 7.5$ Hz), 1.47 (3H, t, 8-$CH_2C\underline{H_3}$, $J = 7.6$ Hz).

6-Ethyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (19) and 6-isopropyl-9-(tetrahydro-*2H*-pyran-2-yl)purine (20)[13]

A solution of KHMDS (0.35 g. 1.65 mmol) in THF (3 mL) was added dropwise to a solution of **10** (72 mg, 0.33 mmol) in THF (3 mL) at −78°C. Upon the addition of the base, an orange red colored mixture developed. The mixture was stirred for 15 minutes at −78°C, and then quenched with MeI (0.2 mL, 3.3 mmol). The mixture was stirred for 5 minutes, 1 *N* NH_4Cl (3 mL) was added, and then was partitioned between EtOAc and H_2O. The organic phase was separated, dried over ($MgSO_4$) and evaporated. The residue was purified by a flash silica gel column (eluate: 3% MeOH in $CHCl_3$) to give 72 mg as a mixture of **19** and **20**: HPLC [eluate; H_2O:CH_3CN, 20 minutes linear gradient from 10–90%; **19** (89.4%, RT = 14.07 minutes); **20** (8.5%, RT = 15.08 minutes)]. Spectroscopic parameters for **19**: MS *m/z* 233 $(M+1)^+$, ^1H NMR ($CDCl_3$) δ 8.89 (1H, s, H-2), 8.24 (1H, s, H-8), 5.79 (1H, dd, H-1′, J = 3.7, J = 5.9 Hz), 4.19 (1H, m, H-5′$_a$), 3.80 (1H, m, H-5′$_b$), 3.24 (1H, q, 6-$C\underline{H_2}CH_3$, J = 7.5 Hz),.2.13–1.69 (6H, m, H-2′$_{a,b}$, H-3′$_{a,b}$, and H-4′$_{a,b}$), 1.45 (3H, t, 6-$CH_2C\underline{H_3}$, J = 7.5 Hz). Spectroscopic parameters for **20**: ^1H NMR ($CDCl_3$) δ 8.92 (1H, s, H-2), 8.24 (1H, s, H-8), 5.80 (1H, m, H-1′), 4.19 (1H, m, H-5′$_a$), 3.82 (1H, m, H-5′$_b$), 3.78 (1H, m, 6-$C\underline{H}Me_2$), 2.14–1.68 (6H, m, H-2′$_b$, H-3′$_{a,b}$, and H-4′$_{a,b}$), 1.45 (6H, d, 6-$CH\underline{Me_2}$, J = 7.1 Hz).

6-Fluoromethyl-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)purine (23)

A 1 *M* solution of NaHMDS in THF (4.4 mL) was added dropwise to a solution of **22** (2.23 g, 3.66 mmol) in THF (25 mL) at −78°C and the mixture was stirred for 25 minutes. A pre-cooled solution of NFSI (1.43 g, 4.4 mmol) in THF (15 mL) was added at −80°C and the mixture was stirred for 5 minutes at the same temperature. A solution of NH_4Cl (1*N*, 10 mL) was added and the mixture was warmed to room temperature, partitioned between EtOAc and H_2O, and the organic phase was dried over ($MgSO_4$), and evaporated. The residue was purified by a flash silica gel column (eluate: 10% EtOAc in hexanes) to give (1.1 g, 47.9%) of **21** as a white glassy solid: MS *m/z* 628 $(M+1)^+$; UV λ_{max} (pH 1) 276.1, 216.5, λ_{max} (pH 7) 268.0, λ_{max} (pH 13) 268.0; ^1H NMR ($CDCl_3$) δ 8.99 (1H, s, H-2), 8.50 (1H, s, H-8), 6.15 (1H, d, H-1′, J = 5.2 Hz), 5.91 (1H, dd, 6-$CH_{2a}F$, $J_{a,b}$ = 12.6, $J_{H,F}$ = 46.6 Hz), 5.89 (1H, dd, 6-$CH_{2b}F$, $J_{a,b}$ = 12.6, $J_{H,F}$ = 46.6 Hz), 4.65 (1H, dd, H-2′, $J_{1',2'}$ = 5.2, $J_{2',3'}$ = 4.3 Hz), 4.32 (1H, dd, H-3′, $J_{1',2'}$ = 5.2, $J_{3',4'}$ = 3.6 Hz), 4.16 (1H, ddd, H-4′, $J_{3',4'}$ = 3.6, $J_{4',5a'}$ = 2.6, $J_{4',5b'}$ = 3.7 Hz), 4.03 (1H, dd, H5′a, $J_{4',5a'}$ = 2.6, $J_{5'a,5'b}$ = 11.7 Hz), 3.81 (1H, dd, H5′b, $J_{4',4b'}$ = 2.6, $J_{5'a,5'b}$ = 11.7 Hz), 0.97–0.78 (27 H, m, *tert*-$\underline{Bu}SiMe_2$), 0.15- -0.25

(18H, 6s, *tert*-BuSi$\underline{Me_2}$); Anal. Calcd. for $C_{29}H_{25}N_4O_4Si_3F$; C 55.55, H 8.84; N 8.94, found C 55.38, H 8.65, N 8.97.

6-Fluoromethyl-9-(β-D-ribofuranosyl)purine (4)

Solid $Et_4NF{\cdot}x\ H_2O$ (1.2 g, 7.97 mmol) was added to a solution of **23** (1, 1.59 mmol) in CH_3CN (10 mL) at room temperature. The mixture was stirred for 2 hours and the solvent was evaporated under reduced pressure. The residue was purified by a flash silica gel column (eluate; 10% EtOH in $CHCl_3$) to give (430 mg, 95%) of **4** as a white solid: m.p. 185–187°C; MS *m/z* 285 $(M+1)^+$; UV λ_{max} (pH 1) 268.3, λ_{max} (pH 7) 264.2, λ_{max} (pH 13).264.7; ^{1}H NMR (DMSO-d_6) δ 8.99 (1H, s, H-2, $^1J_{C,H} = 206.3$ Hz), 8.91 (1H, s, H-8, $^1J_{C,H} = 215.9$ Hz), 6.07 (1H, d, H-1′, $J = 5.5$ Hz), 5.86 (2H, d, 6-$CH_{2a,b}$F, $J_{H,F} = 46.6$ Hz), 5.58 (1H, d, 2′-OH, $J = 5.9$ Hz), 5.28 (1H, d, 3′-OH, $J = 4.9$ Hz), 5.12 (1H, t, 5′-OH, $J = 5.3$ Hz), 4.64 (1H, ddd, H-2′, $J_{1',2'} = 5.5$, $J_{2',3'} = 5$, $J_{2',2'OH'} = 5.9$ Hz), 4.20 (1H, q, H-3′, $J_{2',3'} = 5.0$, $J_{3',4'} = 3.6$, $J_{3',3'OH} = 4.9$ Hz), 3.99 (1H, q, H-4′, $J_{3',4'} = 3.6$, $J_{4',5'a,} = 4.1$, $J_{4',5'a} = 4.0$ Hz), 3.70 (1H, ddd, H-5′a, $J_{4',5'a} = 4.0$, $J_{5'a,\ 5'\text{-OH}} = 5.3$, $J_{5'a,5'b} = 11.9$ Hz), 3.59 (1H, ddd, H-5′b, $J_{4',5'b} = 4.1$, $J_{5'b,\ 5'\text{-OH}} = 5.8$, $J_{5'a,5'b} = 11.9$ Hz), ^{13}C NMR (DMSO-d_6) δ 153.42 (C-6), 151.88 (C-2), 151.56 (C-4), 145.55 (C-8), 131.75 (C-5), 87.72 (C-1′), 85.72 (C-4′), 80.52 ($^1J_{CF} = 167.0$ Hz), 73.78 (C-2′), 70.24 (C-3′), 61.18 (C-5′); Anal. Calcd. for $C_{11}H_{13}N_4O_4F$, C 46.48, H 4.61, N 19.71; found C 46.38, H 4.62, N 19.66.

REFERENCES

1. Sorscher, E. J.; Peng, S.; Bebok, Z.; Allan, P.W.; Bennett, L.L. Jr.; Parker, W.B. Tumor cell bystander killing in colonic carcinoma utilizing the *Escherichia coli* DeoD gene to generate toxic purines. *Gene Ther.* **1994**, 1, 233–238.
2. Parker, W.B.; King, S.; Allan, P.W.; Bennett, L.L. Jr.; Secrist, J.A. III; et al. *In vivo* gene therapy of cancer with *E. coli* purine nucleoside phosphorylase. *Human Gene Ther.* **1997**, 8, 1637–1644.
3. Gadi, V.K.; Alexander, S.D.; Kudlow, J.E.; Allan, P.; Parker, W.B.; Sorscher, E.J. *In vivo* sensitization of ovarian tumors to chemotherapy by expression of *E. coli* purine nucleoside phosphorylase in a small fraction of cells. *Gene Ther.* **2000**, 7, 1738–1743.
4. Parker, W.B.; Allan, P.W.; Shaddix, S.C.; Rose, L.M.; Speegle, H.F.; et al. Metabolism and metabolic actions of 6-methylpurine and 2-fluoroadenine in human cells. *Biochem. Pharmacol.* **1998**, 55, 1673–1681.
5. Hughes, B.W.; King, S.A.; Allan, P.W.; Parker, W.B.; Sorscher, E.J. Cell to cell contact is not required for bystander cell killing by *Escherichia coli* purine nucleoside phosphorylase. *J. Biol. Chem.* **1998**, 273, 2322–2328.
6. Parker, W.B.; Allan, P.W.; Hassan, A.E.A.; Secrist, J.A. III, Sorscher, E.J.; Waud, W.R. Antitumor activity of 2-fluoro-2′-deoxyadenosine against tumors that express *Escherichia coli* purine nucleoside phosphorylase. *Cancer Gene Ther.* **2003**, 10, 23–29.
7. Zimmerman, T.P.; Gersten, N.B.; Ross, A.F.; Miech, R.P. Adenine as substrate for purine nucleoside phosphorylase. *Can J Biochem.* **1971**, 49, 1050–1054.
8. Jensen, K.F.; Nygaard, P. Purine nucleoside phosphorylase from *Escherichia coli* and *Salmonella typhimurium*. *Eur. J. Biochem.* **1975**, 51, 253–265.
9. Anderson, V.R.; Perry, C.M. Fludarabine: A review of its use in non-Hodgkin's lymphoma. *Drugs* **2007**, 67, 1633–1655.

10. Bonate, P.L.; Arthaud, L.; Cantrell, W.R. Jr.; Stephenson, K.; Secrist, J.A. III; Weitman, S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. *Nat. Rev. Drug Discov.* **2006**, 5, 855–863.
11. Comella, P. A review of the role of capecitabine in the treatment of colorectal cancer. *Ther. Clin. Risk Manag.* **2007**, 3, 421–431.
12. Joshi, S.C.; Pant, I.; Shukla, A.N.; Khan, F.A. Chemotherapy in advanced non-small cell lung cancer: a review. *J. Clin. Diagn. Res.* **2008**, 2, 780–785.
13. Kim, Y.J.; Bang, S.; Park, J.Y.; Park, S.W.; Chung, J.B.; Song, S.Y. Phase II study of 5-fluorouracil and paclitaxel in patients with gemcitabine-refractory pancreatic cancer. *Cancer Chemother. Pharmacol.* **2009**, 63, 529–533.
14. Koukourakis, G.V.; Kouloulias, V.; Koukourakis, M.J.; Zacharias, G.A.; Zabatis, H.; Kouvaris, J. Efficacy of the oral fluorouracil pro-drug capecitabine in cancer treatment: a review. *Molecules* **2008**, 13, 1897–1922.
15. Mealy, N.E.; Lupone, B. Drugs under development for the treatment of head and neck cancer. *Drugs of the Future* **2006**, 31, 627–639.
16. Parker, W.B.; Shaddix, S.C.; Rose, L.M.; Shewach, D.S.; Hertel, L.W.; et al. Comparison of the mechanism of cytotoxicity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine, 2-chloro-9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)adenine, and 2-chloro-9-(2-deoxy-2,2-difluoro-b-D-ribofuranosyl)adenine in CEM cells. *Mol. Pharmacol.* **1999**, 55, 515–520.
17. Secrist, J.A. III. Nucleosides as anticancer agents: from concept to the clinic. *Nucleic Acids Symp. Ser.* **2005**, 49, 15–16.
18. Walko, C.M.; Lindley, C. Capecitabine: A review. *Clin. Ther.* **2005**, 27, 23–44.
19. Peek, S.F.; Cote, P.J.; Jacob, J.R.; Toshkov, I.A.; Hornbuckle, W.E.; et al. Antiviral activity of clevudine [L-FMAU, (1-(2-fluoro-5-methyl-β- L-arabinofuranosyl) uracil)] against woodchuck hepatitis virus replication and gene expression in chronically infected woodchucks (*Marmota monax*). *Hepatology* **2001**, 33, 254–266.
20. Clark, J.L.; Hollecker, L.; Mason, J.C.; Stuyver, L.J.; Tharnish, P.M.; et al. Design, synthesis, and antiviral activity of 2′-deoxy-2′-fluoro-2′-C-methylcytidine, a potent inhibitor of hepatitis C virus replication. *J. Med Chem.* **2005**, 48, 5504–5508.
21. Hassan, A.E.A.; Parker, W.B.; Allan, P.W.; Montgomery, J.A.; Secrist, J.A. III. Selective metalation of 6-methylpurines: Synthesis of 6-fluoromethylpurines and related nucleosides. *Nucleosides, Nucleotides & Nucleic Acids* **2003**, 22, 747–749.
22. Silhar, Peter; Pohl, R.; Votruba, I.; Hocek, M. The first synthesis and cytostatic activity of novel 6-(fluoromethyl)purine bases and nucleosides. *Org. Biomol. Chem.* **2005**, 3, 3001–3007.
23. Woenckhaus, C.W. Syntheses and biochemical properties of hydrogen-transferring coenzyme models. *Chem. Ber.* **1964**, 97, 2439–2446.
24. Silamkoti, A.V.; Allan, P.W.; Hassan, A.E.A.; Fowler, A.T.; Sorscher, E.J.; et al. Synthesis and biological activity of 2-fluoroadenine and 6-methylpurine nucleoside analogs as prodrugs for suicide gene therapy of cancer. *Nucleosides, Nucleotides & Nucleic Acids* **2005**, 24, 881–885.
25. Hassan, A.E.A.; Abou-Elkair, R.A.I.; Montgomery, J.A.; Secrist, J.A. III. Convenient syntheses of 6-methylpurine and related nucleosides. *Nucleosides, Nucleotides & Nucleic Acids* **2000**, 19, 1123–1134.
26. Kumamoto, H.; Tanaka, H.; Tsukioka, R.; Ishida, Y.; Nakamura, A.; et al. First evident generation of purin-2-yllithium: Lithiation of an 8-silyl-protected 6-chloropurine riboside as a key step for the synthesis of 2-carbon-substituted adenosines. *J. Org. Chem.* **1999**, 64, 7773–7780.
27. Hocek, M.; Hockova, D.; Dvořáková, H. Dichotomy in regioselective cross-coupling reactions of 6,8-dichloropurines with phenylboronic acid and methylmagnesium chloride: Synthesis of 6,8-disubstituted purines. *Synthesis* **2004**, 889–894.
28. Dvořáková, H.; Dvořák, D.; Holý, A. Coupling of 6-chloropurines with organocuprates derived from Grignard reagents: A convenient route to *sec* and *tert* 6-alkylpurines. *Tetrahedron Lett.* **1996**, 37, 1285–1288.
29. Dvořáková, H.; Dvořák, D.; Holý, A. Synthesis of acyclic nucleotide analogues derived from 6-(*sec*- or *tert*-alkyl) purines *via* coupling of 6-chloropurine derivatives with organocuprates. *Collect. Czech. Chem. Commun.* **1998**, 63, 2065–2074.