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Original article

Synthesis and evaluation of the substrate activity of C-6 substituted purine ribosides with *E. coli* purine nucleoside phosphorylase: Palladium mediated cross-coupling of organozinc halides with 6-chloropurine nucleosides [1]

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ABSTRACT

A series of C-6 alkyl, cycloalkyl, and aryl-9-(β-D-ribofuranosyl)purines were synthesized and their substrate activities with *Escherichia coli* purine nucleoside phosphorylase (*E. coli* PNP) were evaluated. $(Ph_3P)_4Pd$-mediated cross-coupling reactions of 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (**6**) with primary alkyl (Me, Et, *n*-Pr, *n*-Bu, isoBu) zinc halides followed by treatment with NH_3/MeOH gave the corresponding 6-alkyl-9-(β-D-ribofuranosyl)purine derivatives **7**–**11**, respectively, in good yields. Reactions of **6** with cycloalkyl(propyl, butyl, pentyl)zinc halides and aryl (phenyl, 2-thienyl)zinc halides gave under similar conditions the corresponding 6-cyclopropyl, cyclobutyl, cyclopentyl, phenyl, and thienyl -9-(β-D-ribofuranosyl)purine derivatives **12**–**16**, respectively in high yields. *E. coli* PNP showed a high tolerance to the steric and hydrophobic environment at the 6-position of the synthesized purine ribonucleosides. Significant cytotoxic activity was observed for **8**, **12**, **15**, and **16**. Evaluation of **12** and **16** against human tumor xenografts in mice did not demonstrate any selective antitumor activity. In addition, 6-methyl-9-(β-D-arabinofuranosyl)purine (**18**) was prepared and evaluated.

1. Introduction

Suicide gene therapy of cancer is an approach that is being evaluated as a potential treatment for solid tumors. We have developed a cancer gene therapy strategy that is based on the activation of a non-toxic purine nucleoside analog (prodrug) to a highly toxic purine analog by *Escherichia coli* PNP selectively expressed in tumor cells [1–5]. *E. coli* PNP differs from human PNP in its ability to accept not only 6-oxopurine nucleosides, but also 6-aminopurine and certain adenine nucleoside analogs as substrates (Fig. 1). This property has been used to cleave non-toxic adenine nucleoside analogs such as 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine (MeP-dR, **1**) and 2-fluoro-2′-deoxyadenosine (F-dAdo, **3**), to the very toxic adenine analogs, 6-methylpurine (MeP, **2**) and 2-fluoroadenine (F-Ade, **4**) [6–8].

One of our goals has been to get more information about the *E. coli* PNP substrate structural requirements. We have reported on the correlation between various modifications at the sugar moiety of adenine nucleoside analogs and the substrate activity with *E. coli* PNP [5]. Crystal structures of a number of complexes of *E. coli* PNP with various compounds of varied substrate activities such as adenosine, MeP-dR, F-dAdo, and 2-fluoro-9-(β-D-arabinofuranosyl) adenine (F-araA, **5**) showed unique positioning of MeP-dR at the active site [9]. The nucleoside base moiety of MeP-dR was shown to be fitted into a hydrophobic pocket at the active site, resulting in a 2.6 Å shift of the sugar moiety of MeP-dR from the phosphate binding site when compared with adenosine. Although the binding of MeP-dR was significantly different from that of the natural substrate, it was still an excellent substrate for this enzyme [5]. It has been postulated that in the mechanism of the phosphorolysis reaction catalyzed by *E. coli* PNP, the glycosidic bond breaking occurs ahead of the phosphate bond formation and that the transition state has a considerable oxocarbenium character that is stabilized and subsequently attacked by one of the phosphate oxygens [10]. These observations suggested that an increase of the hydrophobic interaction at the C6 position would have a positive impact on the cleavage activity. If it is the case, an enhancement of

Abbreviations: E. coli PNP, *Escherichia coli* purine nucleoside phosphorylase; MeP-dR, 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine; F-dAdo, 2-fluoro-2′-deoxyadenosine; F-araA, 9-(β-D-arabinofuranosyl)-2-fluoroadenine; MeP, 6-methylpurine; F-Ade, 2-fluoroadenine.

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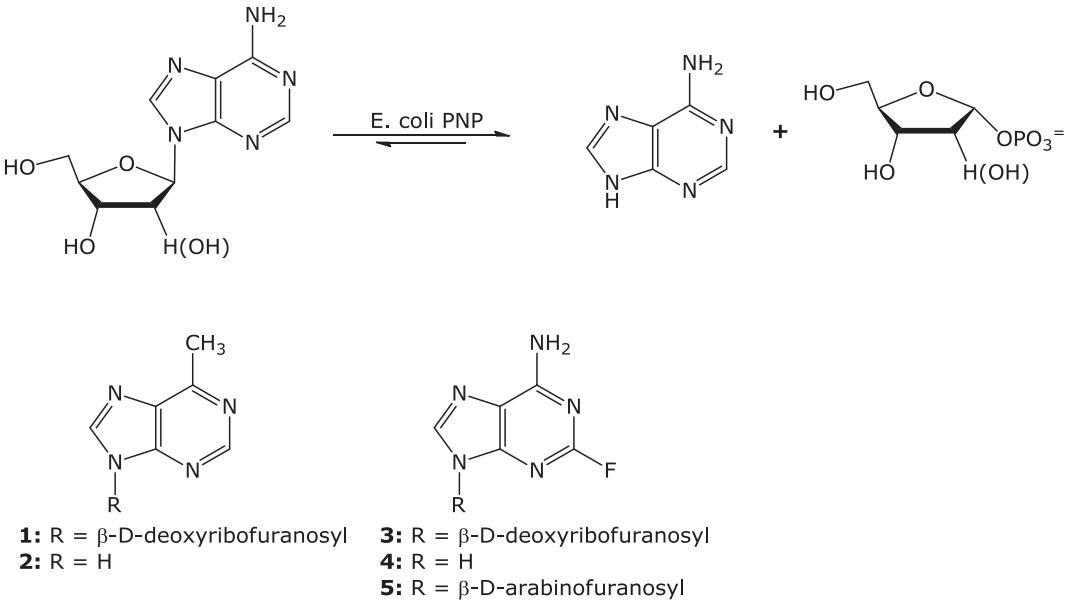


Fig. 1. The cleavage of adenosine by *E. coli* PNP; other selected substrates for this enzyme.

the cleavage activity of certain poor substrates such as arabinofuranosyladenine analogs might be observed. Herein, we report on the synthesis of selected C-6 alkyl and arylpurine nucleosides, their cleavage activity by *E. coli* PNP, and their evaluations both *in vitro* and *in vivo*.

2. Results and discussions

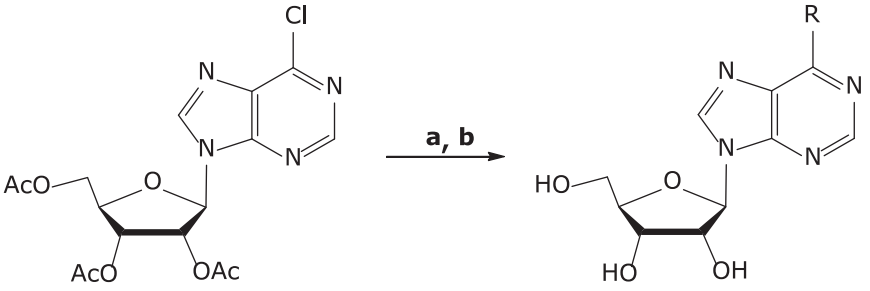
2.1. Chemistry

Transition metal catalyzed cross-couplings of organometallics [11] such as arylmagnesium halides [12], alkyl/arylzinc halides [13], alkyl/aryltin [14], trialkylaluminium [15], alkylcuprates reagents [16] and arylboronic acids [17] with 6-halopurines and 6-halopurine nucleoside analogs have been effectively used for C–C bond formations at the C-6 position of purine nucleosides. We have previously reported on the application of the palladium mediated cross-coupling of methylzinc bromide with N^9-protected-6-chloropurine and suitably protected 6-chloro (9-β-D-ribo- and deoxyribofuranosyl) purines for the synthesis of MeP and the corresponding nucleosides [18]. The mildness of the reaction conditions as well as the stability, safety, and the ease of the preparation of the organozinc reagents prompted us to utilize the same chemistry for the introduction of different carbon substituents at the C-6 position. Treatment of 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**6**) [19,20] with MeZnBr, EtZnBr, *n*-PrZnBr, *n*-BuZnBr, and isoBuZnBr in THF in the presence of *ca.* 0.05 equivalents of $(Ph_3P)_4Pd$ at 55 °C, followed by treatment with NH_3/MeOH gave the corresponding 6-alkyl-9-(β-D-ribofuranosyl)purines (**7**–**11**, Scheme 1, Table 1) [18] in good yields. Treatment of **6** with *sec*-alkylzinc halides under the same conditions, however gave mainly the corresponding 6-primary alkyl derivatives with the 6-*sec*-alkyl derivatives as minor products, which were not isolated. Cyclopropyl [21], cyclobutyl, and cyclopentylzinc halides were cross-coupled with **6** in the presence of $(Ph_3P)_4Pd$ efficiently to give, after deprotection of the sugar hydroxyl groups, the corresponding 6-cycloalkylpurine ribonucleosides **12**–**14** in good yields (Table 1). Phenyl and 2-thienylzinc bromides were also cross-coupled with **6** under similar conditions to give after removal of the acetyl groups by NH_3/MeOH treatment, 6-phenyl and 6-(2-thienyl)-9-(β-D-ribofuranosyl)purine derivatives (**15**) [12a,17c,26] and (**16**) [26–29], respectively, in good yields.

6-Methyl-9-(β-D-arabinofuranosyl)purine (**18**) was prepared by $(Ph_3P)_4Pd$ catalyzed cross-coupling of 6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)purine **17** [22] with MeZnBr under similar conditions. The arabinofuranosyl-6-chloropurine derivative **17** was prepared from 9-(β-D-arabinofuranosyl) hypoxanthine in two steps with minor modification of the literature procedure [20,22]. Cross-coupling of **17** with CH_3ZnBr followed by removal of the acetyl groups under the standard conditions furnished **18** in high yield (Scheme 2). NOE studies showed a distinct glycosidic torsional angle preference for the arabinofuranosyl purine derivatives **7** and **18** compared with the corresponding ribofuranosyl derivatives. Irradiation at the H-8 proton of **18** resulted in 2% and 5% NOE enhancements at the H-1′ and H-3′ signals, respectively. Irradiation at H-3′ gave enhancements of 4–5% and 2% of the signals at H-8 and at H-4′, respectively. These results are very similar to those reported for 9-(β-D-arabinofuranosyl) adenine [23] and suggest a more *syn* conformational preference for **18**.

2.2. Biology

Substrate characteristics with E. coli PNP. The rate of cleavage of 100 μM adenosine (the natural substrate) and its analog, MeP-R were 398,000 and 84,000 nmol/mg/h, respectively. The cleavage activities of 6-ethyl, *n*-propyl, *n*-butyl, isobutyl, cyclopropyl, cyclobutyl, and cyclopentylpurine ribonucleosides (**8**–**14**) by *E. coli* PNP were as good as the 6-methyl derivative (**7**) (Table 2). Surprisingly, a considerable cleavage activity was also observed with 6-phenyl and 6-thienyl ribonucleosides **15** and **16**. These results show the tolerance of *E. coli* PNP to the steric and hydrophobic effects at the C-6 position of the purine nucleosides and reflect a good accommodation of these hydrophobic substituents in the enzyme's active site. On the other hand, the observed poor substrate activity of 6-methyl-9-(β-D-arabinofuranosyl)purine (**18**) reflects the impact of the conformation around the glycosidic bond as a determinant factor in placing the molecule in the proper position at the active site. It is of interest that arabinofuranosyl-MeP (**18**) was much worse as a substrate than F-araA (**5**).



6 **7-16**

[a]Reaction conditions: a) RZnX, $(Ph_3P)_4Pd$, THF, 55 °C; b) NH_3, MeOH

Scheme 1. The synthesis of various 6-substituted purine nucleosides.

2.3. Cytotoxicity

Because compound **7** is known to be very toxic to human cells, the 6-substituted purine nucleoside analogs were evaluated in a standard assay for cytotoxicity against CEM cells (Table 3). All of the compounds tested, except **11** and **18**, were able to inhibit the growth of the CEM cells, but at greater concentrations than those required for compound **7**. Because phosphorylation of compound **7** by adenosine kinase is required for its cytotoxicity, it is likely that this same activation is needed for the other compounds in Table 3. Thus, these results suggest that these compounds may be relatively poor substrates for this enzyme or that the phosphorylated metabolites are less active. Three of the most potent inhibitors (**12**,**15**,**16**) were also tested against a panel of solid tumor cell lines (Table 4), where they were generally quite active.

Because of the sensitivity of the NCI-H23 tumor cell line to these compounds, the *in vivo* efficacy of compounds **12** and **16** were determined. NCI-H23 tumors were grown on the flanks of nude mice. When the tumors reached approximately 200 mg, the animals were treated with 33, 50, or 75 mg/kg of compound **12** or **16** (given ip daily for 9 consecutive days). Neither compound at any dose had any effect of tumor growth. Seventy-five mg/kg of compound **12** killed 5 of 6 mice, whereas with compound **16** this dose caused a 14% decrease in weight. Therefore, at maximally tolerated doses neither compound exhibited selective antitumor activity in mice.

Table 1
$Pd(PPh_3)_4$ catalyzed cross-coupling of **6** with organozinc halides followed by treatment with NH_3/MeOH.

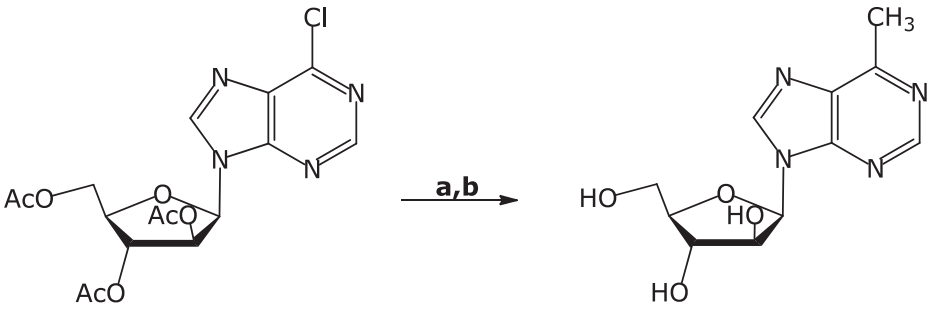
Entry	RZnX	Product, R	Yield %
1	MeZnBr	**7**, CH_3	95
2	EtZnBr	**8**, CH_2CH_3	82
3	*n*–PrZnBr	**9**, $CH_2CH_2CH_3$	86
4	*n*–BuZnBr	**10**, $CH_2(CH_2)_2CH_3$	89
5	isoBuZnBr	**11**, 	88
6	cycloPrZnBr	**12**, 	92
7	cycloBuZnBr	**13**, 	78
8	cyclopentylZnBr	**14**, 	60
9	PhZnBr	**15**, 	78
10	2-ThienylZnBr	**16**, 	80

3. Experimental

^1H NMR and ^{13}C NMR spectra were recorded on a Nicolet NT 300 NB spectrometer operating at 300.635 MHz (^1H) or 75.6 MHz (^{13}C). Chemical shifts are expressed in parts per million from tetramethylsilane. The hydrogen-decoupled ^{13}C NMR spectra were assigned by comparison of the J_{CH} values obtained from hydrogen-coupled ^{13}C NMR spectra. When necessary, selective hydrogen decoupling was performed in order to confirm the assignments. Ultraviolet absorption spectra were determined on Perkin–Elmer Lambda 19 spectrometer by dissolving each compound in methanol or water and diluting appropriately with 0.1 *N* HCl, pH 7 buffer, or 0.1 *N* NaOH. Values are in nanometers, and numbers in parentheses are extinction coefficients ($\varepsilon \times 10^{-3}$). Mass spectra were recorded on a Varian/MAT 311A double-focusing mass spectrometer in the fast atom bombardment (FAB) mode (glycerol matrix). CHN elemental analysis was carried out on Perkin–Elmer 2400 elemental analyzer. HPLC analysis was carried out on a Hewlett–Packard 1100 series liquid chromatograph with a Phenomenex Sphenclone 5 μM 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 pre-coated (250 μm) silica gel (GF) plates.

3.1. 6-Ethyl-9-(β-D-ribofuranosyl)purine (**8**) [24]

A solution of EtZnBr (1.2 mmoL) was generated by dropwise addition of $ZnBr_2$ (1.13 M, 1.1 mL, 1.2 mmol) in THF to 2 M solution of EtMgBr (1.1 mmol, 0.3 mL) in THF (6 mL) for 1 h at −78 °C. The solution was allowed to warm gradually to room temperature and then $(Ph_3P)_4Pd$ (27 mg, 0.02 mmol) in THF (2 mL) was added to the mixture. A solution of compound **6** (0.197 g, 0.477 mmol) in dry THF (4 mL) was added and the mixture was heated under argon for 1 h at 55 °C. The mixture was then cooled down to room temperature and quenched with saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (15 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 5% EtOH in $CHCl_3$) to give (0.11 g, 82%) **8** as a white solid, m.p. 98–100 °C, 1:1H_2O-EtOH (lit [24]. 104–106 °C): HPLC [99.5%; RT, 10.64 min, 0.01 M $NH_4H_2PO_4$:



17 18

[a]Reaction conditions: a) CH_3ZnBr, $(Ph_3P)_4Pd$, THF, 55 °C; b) NH_3, MeOH

Scheme 2. The synthesis of 9-(β-D-arabinofuranosyl)-6-methylpurine.

MeOH, 20 min linear gradient]; MS *m/z* 281 $(M + 1)^+$, UV λ_{max} pH 1, 265.3 (7.4); pH 7, 260.6 (8.0); pH 13, 261.2 (8.1); ^{1}H NMR (Me_2SO-d_6) δ 8.84 (1H, s, H-2, $^1J_{C,H}$ = 204.4 Hz), 8.76 (1H, s, H-8, $^1J_{C,H}$ = 214.3 Hz), 6.02 (1H, d, H-1′, $J_{1',2'}$ = 5.8 Hz), 5.54 (1H, d, 2′−OH, *J* = 5.9 Hz), 5.26 (1H, d, 3′−OH, *J* = 4.9 Hz), 5.14 (1H, t, 5′−OH, *J* = 5.6 Hz), 4.64 (1H, ddd, H-2′, $J_{2'3'}$ = 4.7 Hz), 4.19 (1H, ddd, H-3′, $J_{3'4'}$ = 3.4 Hz), 3.98 (1H, ddd, H-4′), 3.70 (1H, ddd, H-5′a, $J_{4',5'a}$ = 3.7 Hz, $J_{5'a,5'b}$ = 12.1 Hz), 3.64 (1H, ddd, H-5′b, $J_{4',5'b}$ = 4.4 Hz), 3.12 (2H, q, 6-C*H*$_2$CH$_3$), 1.35 (3H, t, 6−CH$_3$); ^{13}C NMR (Me_2SO-d_6) δ 162.64 (C-6), 151.78 (C-2), 150.22 (C-4), 143.97 (C-8), 132.19 (C-5), 87.59 (C-1′), 85.67 (C-4′), 73.58 (C-2′), 70.33 (C-3′), 61.30 (C-5′), 25.70 (6-*C*H$_2$CH$_3$), 12.20 (6−CH$_3$); Anal. Calcd. for $C_{12}H_{16}N_4O_4$; C, 51.42; H, 5.75; N, 19.99. Found C, 51.22; H, 5.65; N 20.89.

3.2. 6-n-Propyl-9-(β-D-ribofuranosyl)purine (**9**) [24]

A solution of $(Ph_3P)_4Pd$ (25 mg, 0.02 mmol) in THF (1 mL) was added to a solution of *n*-PrZnCl [generated as above from a 2 M solution *n*-PrMgCl (0.53 mL) and a 1.13 M solution of $ZnBr_2$ (1 mL) at −78 °C to r.t., for 1 h] in THF (5 mL) at room temperature. A solution of **6** (0.175 g, 0.424 mmol) in THF (2 mL) was added and the mixture was heated for 5 h at 55 °C. The mixture was then cooled down to room temperature and quenched with a saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and stirred for 2 h at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 5% EtOH in $CHCl_3$) to give (0.107 g, 86%) **9** as a pale yellow waxy solid, which was recrystallized from EtOH, m.p. 104–106 °C (lit [24]. foam): HPLC [99%; RT 12.64 min; 0.01 *M* $NH_4H_2PO_4$: MeOH; 20 min linear gradient from 10 to 90%]; MS *m/z* 295 $(M + 1)^+$, UV λ_{max} pH 1, 266.0 (8.0); pH 7, 261.2 (8.3); pH 13, 261.6 (8.2); ^{1}H NMR (Me_2SO-d_6) δ 8.83 (1H, s, H-2), 8.76 (1H, s, H-8), 6.02 (1H, d, H-1′, $J_{1'2'}$ = 5.9 Hz), 5.54 (1H, d, 2′−OH, *J* = 6.0 Hz), 5.26 (1H, d, 3′−OH, *J* = 4.9 Hz), 5.14 (1H, t, 5′−OH, *J* = 5.6 Hz), 4.65 (1H, ddd, H-2′, $J_{1'2'}$ = 4.7 Hz), 4.19 (1H, ddd, H-3′, $J_{3',4'}$ = 3.5 Hz), 3.98 (1H, ddd, H-4′), 3.70 (1H, ddd, H-5′a, $J_{4',5a'}$ = 3.7 Hz, $J_{5'a,5'b}$ = 12.1 Hz), 3.59 (1H, ddd, H-5′b, $J_{4',5'b}$ = 4.1 Hz), 3.10 (2H, t, 6-C*H*$_2$CH$_2$CH$_3$), 1.85 (2H, m, 6-CH$_2$*C*H$_2$CH$_3$), 0.94 (3H, t, 6−CH$_2$CH$_2$*C*H$_3$); ^{13}C NMR (Me_2SO-d_6) δ 161.55 (C-6), 151.72 (C-2), 150.22 (C-4), 144.00 (C-8), 132.64 (C-5), 87.54 (C-1′), 85.68 (C-4′), 73.54 (C-2′), 70.34 (C-3′), 61.30 (C-5′), 34.29 (6-*C*H$_2$CH$_2$CH$_3$), 20.96 (6-CH$_2$*C*H$_2$CH$_3$), 13.78 (6−CH$_2$CH$_2$CH$_3$). Anal. Calcd. for $C_{13}H_{18}N_4O_4 \cdot 0.5H_2O$: C, 51.48; H, 6.31; N, 18.47. Found: C, 51.82; H, 6.25; N, 18.37.

Table 2
Substrate activities of 6-substituted purine nucleoside analogs with *E. coli* PNP.

Substrate	C6-substituent	Specific activity (nmoles/mg/hr)	(N)
Adenosine[a]	Amino	398,000	5
MeP-dR[a]	Methyl	461,000	10
F-araA[a]	Amino	1300	4
7	Methyl	84,000	8
8	Ethyl	69,000	3
9	*n*−Propyl	72,000	3
10	*n*-Butyl	86,000	3
11	Isobutyl	79,000	3
12	Cyclopropyl	51,000	3
13	Cyclobutyl	63,000	3
14	Cyclopentyl	42,000	3
15	Phenyl	21,000	3
16	Thienyl	9900	2
18	Methyl	14	2

Purified *E. coli* PNP (obtained from Dr. Steve Ealick, Cornell University, Ithaca, NY) was incubated at 25 °C with 100 μM of each nucleoside analog in the presence of 100 mM HEPES (pH 7.4), 50 mM phosphate (pH 7.4), 4% glycerol, 0.2 mM dithiothreitol, and an appropriate amount of enzyme to give a linear reaction. Samples were collected at various times after addition of substrate and the substrates and products were determined by monitoring UV absorbance as they eluted from a 150 × 4.6 mm, 5 μm BDS hypersil C-18 column (Keystone Scientific Inc. Bellfonte, PA) using a 30-min linear gradient of 5%–50% acetonitrile in 50 mM $NH_3H_2PO_4$ buffer pH 4.5 at a flow rate of 1 mL/min. Each number is the average of at least 2 separate measurements (N).

[a] From reference [5].

Table 3
Inhibition of CEM cell growth by 6-substituted purine nucleoside analogs.

Substrate	C6-substituent	IC_{50} (μM)	(N)
7	Methyl	0.02	2
8	Ethyl	0.72	3
9	*n*−Propyl	6.8	3
10	*n*-Butyl	49	2
11	Isobutyl	>130	2
12	Cyclopropyl	1	3
13	Cyclobutyl	7	2
14	Cyclopentyl	16	2
15	Phenyl	0.62	3
16	Thienyl	0.09	3
18	Methyl	>130	2

CCRF-CEM cells (American Type Culture Collection) were incubated at 37 °C with various concentrations of the 6-substituted purine nucleoside analogs. Cell numbers were determined 72 h after the addition of compound using a Coulter Counter and the amount of compound that resulted in 50% inhibition of cell growth was determined (IC_{50}). Each number is the average of 2 or 3 separate measurements (N).

Table 4
Inhibition of the growth of various solid tumor cell lines.

Cell line	IC_{50} of 6-substituted purine nucleoside analog (μM)		
	12	**15**	**16**
SNB7 (CNS)	33	31	10
DLD-1 (colon)	170	6	0.9
NCI-H23 (lung)	1	1.2	0.6
ZR-75-1 (mammary)	23	1.2	0.3
LOXIMVI (melanoma)	>200	6.2	0.3
PC-3 (prostate)	67	1.2	0.9
CAKI-1 (renal)	>200	31	30

The above cells were plated in 96-well microtiter plates and incubated with various concentrations of compound number **12**, **15**, or **16** at 37 °C. Cell viability was measured after 72 h of continuous incubation with compound using the sulforhodamine B assay (absorbance read at 570 nm), and the concentration of compound that inhibited cell growth by 50% was determined. The results shown are the result of one experiment.

3.3. 6-n-Butyl-9-(β-D-ribofuranosyl)purine (**10**) [24]

A solution of $(Ph_3P)_4Pd$ (23 mg, 0.02 mmol) in THF (1 mL) was added to a solution of *n*-BuZnCl [generated as above from a 2 M solution *n*-BuMgCl (0.5 mL) and 1.13 M solution $ZnBr_2$ (1 mL) at −78 °C to r.t., for 1 h] in THF (5 mL) at room temperature. A solution of **6** (0.166 g, 0.4 mmol) in THF (2 mL) was added and the mixture was heated for 2 h at 55 °C. The mixture was then cooled down to room temperature and quenched with a saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and kept for 3 h at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 5% EtOH in $CHCl_3$) to give (0.11 g, 89%) **10** as a pale yellow waxy solid, which was recrystallized from H_2O-EtOH, m.p. 98–100 °C (lit [24]. foam): HPLC [99%; RT 12.64 min; 0.01 M $NH_4H_2PO_4$: MeOH; 20 min linear gradient from 10 to 90%]; MS *m/z* 309 $(M + 1)^+$, UV λ_{max} pH 1, 267.0 (8.1); pH 7, 260.9 (8.4); pH 13, 261.1 (8.7); ^1H NMR (Me_2SO-d_6) δ 8.83 (1H, s, H-2), 8.75 (1H, s, H-8), 6.03 (1H, d, H-1′, $J_{1',2'} = 5.9$ Hz), 5.55 (1H, d, 2′–OH, $J = 5.9$ Hz), 5.27 (1H, d, 3′–OH, $J = 4.8$ Hz), 5.13 (1H, t, 5′–OH, $J = 5.6$ Hz), 4.64 (1H, ddd, H-2′, $J_{2',3'} = 5.0$ Hz), 4.18 (1H, ddd, H-3′, $J_{3',4'} = 3.4$ Hz), 4.00 (1H, ddd, H-4′), 3.69 (1H, ddd, H-5′a, $J_{4',5'a} = 3.5$ Hz, $J_{5'a,5'b} = 12.1$ Hz), 3.59 (1H, ddd, H-5′b, $J_{4',5'b} = 4.1$ Hz), 3.12 (2H, t, 6-$CH_2CH_2CH_2CH_3$), 1.81 (2H, m, 6-$CH_2CH_2CH_2CH_3$), 1.33 (2H, m, 6–$CH_2CH_2CH_2CH_3$), 0.91 (3H, t, 6–$CH_2CH_2CH_2CH_3$); ^{13}C NMR (Me_2SO-d_6) δ 161.76 (C-6), 151.72 (C-2), 150.21 (C-4), 143.98 (C-8), 132.56 (C-5), 87.53 (C-1′), 85.68 (C-4′), 73.54 (C-2′), 70.33 (C-3′), 61.30 (C-5′), 31.94 (6-$CH_2CH_2CH_2CH_3$), 29.74 (6-$CH_2CH_2CH_2CH_3$), 21.87 (6–$CH_2CH_2CH_2CH_3$), 13.64 (6–$CH_2CH_2CH_2CH_3$); Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found: C, 54.17; H, 6.35; N, 18.04.

3.4. 6-Isobutyl-9-(β-D-ribofuranosyl)purine (**11**)

A solution of $(Ph_3P)_4Pd$ (73 mg, 0.06 mmol) in THF (2 mL) was added to a 0.5-*M* solution of isoBuZnCl (10 mL) in THF (5 mL) at room temperature. A solution of **6** (0.520 g, 1.26 mmol) in THF (5 mL) was added and the mixture was heated for 2 h at 55 °C. The mixture was then cooled down to room temperature and quenched with a saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and kept for 4 h at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 6% EtOH in $CHCl_3$) to give (0.34 g, 88%) of a white solid: MS m/*z* 309 $(M + 1)^+$; UV λ_{max} pH 1, 266.8 (8.8); pH 7, 261.6 (9.2); pH 13, 261.7 (9.1); ^1H NMR (Me_2SO-d_6) δ 8.84 (1H, s, H-2), 8.75 (1H, s, H-8), 6.02 (1H, d, H-1′, $J_{1',2'} = 5.8$ Hz), 5.52 (1H, d, 2′–OH, $J = 6.0$ Hz), 5.24 (1H, d, 3′–OH, $J = 4.8$ Hz), 5.12 (1H, t, 5′–OH, $J = 5.2$ Hz), 4.67 (1H, ddd, H-2′, $J_{2',3'} = 5.0$ Hz), 4.19 (1H, ddd, H-3′, $J_{3',4'} = 3.4$ Hz), 3.98 (1H, ddd, H-4′), 3.69 (1H, ddd, H-5′a, $J_{4',5'a} = 4.0$ Hz, $J_{5'a,5'b} = 11.9$ Hz), 3.57 (1H, ddd, H-5′b, $J_{4',5'b} = 4.1$ Hz), 2.97 (2H, d, 6-$CH_2CH(CH_3)_2$), 2.34 (1H, m, 6-$CH_2CH(CH_3)_2$), 0.93 and 0.90 (3H, d, 6–$CH_2CH(CH_3)_2$); ^{13}C NMR (Me_2SO-d_6) δ 161.02 (C-6), 151.69 (C-2), 150.28 (C-4), 144.08 (C-8), 133.01 (C-5), 87.50 (C-1′), 85.72 (C-4′), 73.54 (C-2′), 70.37 (C-3′), 61.33 (C-5′), 41.34 (6-$CH_2CH(CH_3)_2$), 27.69 (6-$CH_2CH(CH_3)_2$), 22.49 and 22.47 (6–$CH_2CH(CH_3)_2$); Anal. Calcd. for $C_{14}H_{20}N_4O_4$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.58; H, 6.32; N, 18.06.

3.5. 6-Cyclopropyl-9-(β-D-ribofuranosyl)purine (**12**)

A mixture of magnesium turnings (74 mg, 3.0 mmol) and cyclopropyl bromide (0.24 mL, 3.02 mmol) in anhydrous THF (6 mL) was heated for 1 h at 60 °C until complete dissolution of the magnesium. The solution was cooled to room temperature and was treated with a 1.13 M solution of $ZnBr_2$ (2.7 mL, 3.0 mmol) and the resulting white suspension was stirred for 1 h at room temperature. $(Ph_3P)_4Pd$ (50 mg, 0.04 mmol) in THF (1 mL) was added to the mixture, followed by the addition of **6** (0.22 g, 0.53 mmol) in THF (2 mL) and the mixture was heated for 4 h at 45 °C. The mixture was then cooled down to room temperature and quenched with saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and kept for 2 h at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 7% EtOH in $CHCl_3$ to give (0.148 g, 92%) as a pale yellow solid which was crystallized from MeOH-heptane, m.p. 154–156 °C (lit [25]. 158–160 °C); HPLC [99.8%; RT 12.62 min; 0.01 M $NH_4H_2PO_4$: MeOH; 20 min linear gradient from 10 to 90%]; MS *m/z* 293.1 $(M + 1)^+$; UV λ_{max} pH 1, 276.5 (12.5); pH 7, 265.3 (12.4); pH 13, 265.6 (12.7); ^1H NMR (Me_2SO -d_6) δ. 8.74 (1H, s, H-2), 8.72 (1H, s, H-8), 6.00 (1H, d, H-1′, $J_{1',2'} = 5.9$ Hz), 5.52 (1H, d, 2′–OH, $J = 5.9$ Hz), 5.25 (1H, d, 3′–OH, $J = 5.1$ Hz), 5.15 (1H, dd, 5′–OH, $J_{5'a',5'-OH} = 5.0$ Hz, $J_{5'b',5'-OH} = 6.1$Hz), 4.61 (1H, ddd, H-2′, $J_{2',3'} = 4.9$ Hz), 4.17 (1H, ddd, H-3′, $J_{3',4'} = 3.5$ Hz), 3.99 (1H, ddd, H-4′), 3.70 (1H, ddd, H-5′a, $J_{4',5'a} = 4.0$ Hz, $J_{5',5'b} = 12.1$ Hz), 3.58 (1H, dd, H-5′b, $J_{4'5'b} = 4.1$ Hz), 2.70 (1H, m, 6-cycloPr-*CH*), 1.27–1.23 (4H, m, 6-cycloPr-CH_2-CH_2); ^{13}C NMR (Me_2SO-d_6) δ 162.88 (C-6), 151.85 (C-2), 149.54 (C-4), 143.73 (C-8), 132.14 (C-5), 87.59 (C-1′), 85.62 (C-4′), 73.58 (C-2′), 70.27 (C-3′), 61.26 (C-5′), 12.69 (6-cycloPr-*CH*), 10.94 (6-cycloPr-$CHCH_2CH_2$); Anal. Calcd. for $C_{13}H_{16}N_4O_4$: C, 53.42; H, 5.52; N, 19.18. Found: C, 53.30; H, 5.23; N 19.20.

3.6. 6-Cyclobutyl-9-(β-D-ribofuranosyl)purine (**13**)

A mixture of magnesium turnings (45 mg, 1.8 mmol) and cyclobutyl bromide (0.25 mg, 1.85 mmol) in anhydrous THF (5 mL) was heated for 3 h at 60 °C until complete dissolution of the magnesium. The solution was cooled to −78 °C and treated with $ZnBr_2$ (1.13 M, 1.6 mL, 1.8 mmol) in THF and the resulting white suspension was warmed gradually to room temperature and was stirred further for 1 h at room temperature. $(Ph_3P)_4Pd$ (27 mg) in THF (1 mL) was added, followed by addition of **3** (0.15 g, 0.36 mmol) in THF (2 mL) and the mixture was heated for 4 h at 55 °C. The mixture was then cooled down to room temperature and quenched with a saturated solution of NH_4Cl. The solvent was concentrated

under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 5% MeOH in $CHCl_3$) to give (88 mg, 78%) as a pale yellow solid which was crystallized from EtOH in hexanes, m.p. 82–84 °C; HPLC [98%; RT 13.70 min; 0.01 M $NH_4H_2PO_4$: MeOH; 20 min linear gradient from 10 to 90%]; MS *m/z* 307 $(M + 1)^+$; UV λ_{max} pH 1, 270.2 (9.9); pH 7, 263.1 (10.0); pH 13, 263.3 (10.0); ^{1}H NMR (Me_2SO-d_6) δ 8.89 (1H, s, H-2), 8.74 (1H, s, H-8), 6.03 (1H, d,H-1′, $J_{1',2'} = 5.7$ Hz), 5.52 (1H, d, 2′–OH, $J = 5.2$ Hz), 5.26 (1H, d, 3′–OH, $J = 4.8$ Hz), 5.14 (1H, t, 5′–OH, $J = 5.6$ Hz), 4.64 (1H, ddd, H-2′, $J_{2',3'} = 4.9$ Hz), 4.29–4.18 (2H, m, 6-cycloBu; *CH*$CH_2CH_2CH_2$ and H-3′), 4.01 (1H, ddd, H-4′, $J_{3',4'} = 3.2$ Hz), 3.72 (1H, ddd, H-5′a, $J_{4',5'a} = 3.5$ Hz, $J_{5'a,5'b} = 12.1$ Hz), 3.60 (1H, dd, H-5′b, $J_{4',5'b} = 4.1$ Hz), 2.60–2.47 (2H, m, 6-cycloBu CHC*H*$_a$CH$_b$CH$_2$), 2.41–2.31 (2H, m, 6-cycloBu CHCH$_a$C*H*$_b$CH$_2$), 2.20–1.93 (2H, m, HCH$_a$CH$_b$C*H*$_2$); ^{13}C NMR (Me_2SO-d_6) δ 163.14 (C-6), 151.87 (C-2), 150.33 (C-4), 143.90 (C-8), 131.51 (C-5), 87.64 (C-1′), 85.68 (C-4′), 73.64 (C-2′), 70.33 (C-3′), 61.31 (C-5′), 36.77 (6-cycloBu *C*HCH$_2$CH$_2$CH$_2$), 26.96 (6-cycloBu CH*C*H$_2$CH$_2$CH$_2$), 26.88 (6-cycloBu CHCH$_2$*C*H$_2$CH$_2$), 18.17 (6-cycloBu CHCH$_2$CH$_2$*C*H$_2$); Anal. Calcd. for $C_{14}H_{18}N_4O_4 \cdot 0.5H_2O$: C, 53.33; H, 6.07; N, 17.77. Found: C, 53.00; H, 5.95; N, 17.53.

3.7. 6-Cyclopentyl-9-(β-D-ribofuranosyl)purine (**14**)

A solution of $(Ph_3P)_4Pd$ (46 mg, 0.04 mmol) in THF (1.5 mL) was added to a solution of cyclopentylZnCl [generated as above from a 2 M Et_2O solution cyclopentylMgCl (0.5 mL) and a 1.13 M THF solution of $ZnBr_2$ (1 mL) at −78 °C to r.t., for 1 h] in THF (5 mL) at room temperature. A solution of **3** (0.224 g, 0.543 mmol) in THF (3 mL) was added and the mixture was heated for 45 min at 55 °C. The mixture was then cooled down to room temperature and quenched with saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 7% EtOH in $CHCl_3$) to give (0.1 g, 60%) **14** as a pale yellow foam: HPLC [99%; RT 12.64 min; 0.01 M $NH_4H_2PO_4$: MeOH; 20 min linear gradient from 10 to 90%]; MS *m/z* 321.2 $(M + 1)^+$; UV λ_{max} pH 1, 238.2 (6.4); pH 7, 262.2 (6.4); pH 13, 262.2 (6.4); ^{1}H NMR (Me_2SO-d_6) δ 8.83 (1H, s, H-2), 8.74 (1H, s, H-8), 6.01 (1H, d, H-1′, $J_{1',2'.} = 5.7$ Hz), 5.53 (1H, d, 2′–OH, $J = 5.6$ Hz), 5.25 (1H, d, 3′–OH, $J = 4.6$ Hz), 5.13 (1H, t, 5′–OH, $J = 5.5$ Hz), 4.64 (1H,ddd, H-2′, $J_{2',3'} = 4.4$ Hz), 4.18 (1H, ddd, H-3′, $J_{3',4'} = 3.1$ Hz), 4.02 (1H, ddd, H-4′), 3.79 (1H, m, 6-C*H*CH$_2$CH$_2$CH$_2$CH$_2$), 3.68 (1H, ddd, H-5′a, $J_{4',5'a} = 3.6$ Hz, $J_{5'a,5'b} = 12.0$ Hz), 3.56 (1H, dd, H-5′b, $J_{4',5'b} = 4.6$ Hz), 2.07–1.69 (8H, m, 6-CH*H*$_2$C*H*$_2$C*H*$_2$CH*H*$_2$), ^{13}C NMR (Me_2SO-d_6) δ 165.59 (C-6), 152.36 (C-2), 150.76 (C-4), 144.36 (C-8), 132.57 (C-5), 87.59 (C-1′), 85.69 (C-4′), 73.57 (C-2′), 70.37 (C-3′), 61.33 (C-5′), 42.01 (6-*C*HCH$_2$CH$_2$CH$_2$CH$_2$), 32.09 and 32.06 (6-CH*C*H$_2$CH$_2$CH$_2$CH$_2$), 25.81 (6–CHCH$_2$*C*H$_2$*C*H$_2$CH$_2$); Anal. Calcd. for $C_{15}H_{20}N_4O_4 \cdot 0.4H_2O$: C, 54.96; H, 6.40; N, 17.17. Found: C, 55.11; H, 6.34; N, 16.84.

3.8. 6-Phenyl-9-(β-D-ribofuranosyl)purine (**15**) [12a,17c,26]

A solution of PhZnBr (16.94 mmoL) was generated by dropwise addition of a 1.13 M solution of $ZnBr_2$ (15 mL) in THF to a 3 M solution of PhMgBr (19.64 mmol, 5.64 mL) in THF (75 mL) at −0 °C for 1 h. After the solution was allowed to warm to room temperature, a solution of $(Ph_3P)_4Pd$ (0.5 g, 0.4 mmoL) in THF (10 mL) was added to it. A solution of compound **6** (3.55 g, 8.6 mmol) in dry THF (10 mL) was then added and the mixture was heated under argon for 4 h at 55 °C. The mixture was then cooled down to room temperature and quenched with a saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (30 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 6% MeOH in $CHCl_3$) to give (2.2 g, 78%) of **15** as a white solid which was crystallized from EtOH/toluene, m.p. 224–226 °C (lit [17c]. 228–230 °C): MS *m/z* 329 $(M + 1)^+$; UV λ_{max} pH 1, 303.0 (18.3); pH 7, 288.4 (18.7); pH 13, 288.4 (18.4); ^{1}H NMR (Me_2SO-d_6) δ 9.03 (1H, s, H-2), 8.92 (1H, s, H-8), 8.83–8.80 (2H, m, 6-Ph), 7.65–7.58 (3H, m, 6-Ph), 6.11 (1H, d, H-1′, $J_{1',2'} = 5.6$ Hz), 5.60 (1H, d, 2′–OH, $J = 5.9$ Hz), 5.28 (1H, d, 3′–OH, $J = 4.9$ Hz), 5.16 (1H, t, 5′–OH, $J = 5.5$ Hz), 4.67 (1H, ddd, H-2′, $J_{2',3'} = 4.8$ Hz), 4.23 (1H, ddd, H-3′, $J_{3',4'} = 3.9$ Hz), 4.00 (1H, ddd, H-4′), 3.75 (1H, ddd, H-5′a, $J_{4',5'a} = 3.6$ Hz, $J_{5'a,5'b} = 11.9$ Hz), 3.63 (1H, ddd, H-5′b, $J_{4',5'b} = 4.1$ Hz); ^{13}C NMR(Me_2SO -d_6) δ 157.17(C-6 or C-4), 152.93 (C-4 or C-6), 151.85 (C-2), 144.86 (C-8), 135.19 (ipso C-6Ph), 131.08 (para C-6Ph), 130.82 (C-5), 129.33 and 128.63 (meta and ortho C-6Ph), 87.64 (C-1′), 85.63 (C-4′), 73.72 (C-2′), 70.21 (C-3′), 61.17 (C-5′); Anal. Calcd. for $C_{16}H_{16}N_4O_4 \cdot 0.2H_2O$: C, 57.85; H, 4.98; N, 16.94. Found: C, 57.78; H, 4.81; N, 16.99.

3.9. 6-(2-Thienyl)-9-(β-D-ribofuranosyl)]purine (**16**) [26–29]

A mixture of magnesium turnings (466 mg, 19.2 mmol) and 2-thienyl bromide (1.8 mL, 19.2 mmol) in anhydrous THF (5 mL) was stirred under argon for 3 h at 37 °C. The resulting red color solution was cooled to 0 °C and treated with a 1 M THF solution of $ZnBr_2$ (19.2 mL) and the thick suspension was stirred further for 1 h at room temperature. $(Ph_3P)_4Pd$ (277 mg, 0.24 mmol) in THF (5 mL) was added followed by the addition of a solution of **6** (2 g, 4.85 mmol) in THF (20 mL) and the mixture was heated for 2 h at 45 °C. The mixture was then cooled down to room temperature and quenched with saturated solution of NH_4Cl. The solvent was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (20 mL) and kept overnight at room temperature. The solvent was evaporated and the residue was purified by a flash silica gel chromatography (elution with 5% MeOH in $CHCl_3$) to give (1.3 g, 89%) as a yellow solid; MS *m/z* 307 $(M + 1)^+$; UV λ_{max} pH 1, 338.8 (19.3); pH 7, 325.2 (24.1); pH 13, 324.8 (24.7); ^{1}H NMR (Me_2SO-d_6) δ 8.91 (1H, s, H-2), 8.87 (1H, s, H-8), 8.84 (1H, dd, 6-C-S-C*H*CHCH, $J = 1.1$ Hz, $J = 3.7$ Hz), 7.94 (1H, dd, 6-C-S-CHCHC*H*, $J = 1.1, J = 5.0$ Hz), 7.36 (1H, dd, 6-C-S-CHC*H*CH, $J = 5.0, J = 3.7$ Hz), 6.06 (1H, d, H-1′, $J_{1'2'} = 5.5$ Hz), 5.57 (1H, d, 2′–OH, $J = 5.9$ Hz), 5.05 (1H, d, 3′–OH, $J = 5.6$ Hz), 5.15 (1H, t, 5′–OH, $J = 5.5$ Hz), 4.64 (1H, ddd, H-2′, $J_{2',3'} = 5.1$ Hz), 4.21 (1H, ddd, H-3′, $J_{3',4'} = 3.2$ Hz), 4.00 (1H, dd, H-4′), 3.71 (1H, ddd, H-5′a, $J_{4',5'a} = 3.7$, $J_{5'a,5'b} = 11.9$ Hz), 3.60 (1H, dd, H-5′b, $J_{4',5'b} = 4.6$ Hz); Anal. Calcd. for $C_{14}H_{14}N_4O_4S \cdot 0.3H_2O$: C, 49.49; H, 4.33; N, 16.49. Found: C, 49.40; H, 4.03; N, 16.27.

3.10. 6-Chloro-9-(tri-O-acetyl-β-D-arabinofuranosyl)purine (**17**) [22,29]

To a solution of **23** (1 g, 2.66 mmol) in anhydrous $CHCl_3$ (25 mL) was added *N,N*-dimethylformamide (0.2 mL) and $SOCl_2$ (4.5 mL, 30 mmol) dropwise over 10 min. The mixture was heated for 4 h at reflux temperature, then cooled down to room temperature and the

solvent was evaporated. The residue was dissolved in EtOAc (50 mL) and neutralized with cold aqueous $NaHCO_3$ solution. The organic phase was washed with H_2O, dried over ($MgSO_4$) and evaporated. The residue was purified by a flash silica gel chromatography (elution with; 1% MeOH in $CHCl_3$) to give (1.05 g, 96%) of **17** as a colorless foam: MS m/z 413 $(M + 1)^+$; UV λ_{max} pH 1, 263.2; pH 7, 263.2; pH 13, 261.6; ^1H NMR ($CDCl_3$) δ 8.78 (1H, s, H-2), 8.34 (1H, s, H-8), 6.66 (1H, d, H-1′, $J_{1',2'} = 4.6$ Hz), 5.55 (1H, dd, H-2′, $J_{2',3'} = 3.1$ Hz), 5.47 (1H, dd, H-3′, $J_{3',4'} = 4.5$ Hz), 4.51 (1H, dd, H-5′a, $J_{4',5'a} = 5.8$ Hz, $J_{5'a,5'b} = 12.0$ Hz), 4.49 (1H, dd, H-5′ b, $J_{4',5'b} = 4.4$ Hz), 4.41 (1H, dt, H-4′), 2.19 (3H, s, Ac), 2.15 (3H, s, Ac), 1.90 (3H, s, Ac).

3.11. 6-Methyl-9-(β-D-arabinofuranosyl)purine (**18**)

A solution of $(Ph_3P)_4Pd$ (36 mg, 0.03 mmol) in THF (1 mL) was added to a solution of CH_3ZnBr (1 mmol, generated as above) in THF (5 mL) at room temperature. A solution of **17** (0.167 g, 0.39 mmol) in THF (3 mL) was added at room temperature and the mixture was stirred for 5 h at 55 °C. After an aqueous work up, the residue obtained by evaporation of the dried organic phase was dissolved in MeOH saturated with NH_3 (10 mL) and stirred for 3 h at room temperature. The solvent was evaporated and the residue was purified by silica gel chromatography (elution with 6% EtOH in $CHCl_3$) to give (83 mg, 78%) of **18** as a colorless solid that was crystallized from hot ethanol, m.p. 220–222 °C; MS *m/z* 267.1 $(M + 1)^+$; UV λ_{max} pH 1, 263.6 (7.4); pH 7, 260.3 (8.0); pH 13, 261.0 (8.3); ^1H NMR (Me_2SO-d_6) δ 8.77 (1H, s, H-2), 8.56 (1H, s, H-8), 6.38 (1H, d, H-1′, $J_{1',2'} = 5.1$ Hz), 5.66 (1H, br s, 2′–OH), 5.58 (1H, d, 3′–OH, $J = 4.4$ Hz), 5.11 (1H, br t, 5′–OH), 4.27 (1H, m, H-2′, $J_{2',3'} = 5.2$ Hz), 4.20 (1H,ddd, H-3′, $J_{3',4'} = 5.2$ Hz), 3.82 (1H, ddd, H-4′), 3.72–3.63 (2H, m, H-5′a,b), 2.72 (1H, s, 6–CH_3); NOE: Irradiation at H-1′ an enhancements of 2%, 1–2% and 10% were observed at H-8, at H-4′ and H-2′, respectively. Irradiation at H-3′ gave enhancements of 4–5% and 2% of the signals at H-8 and at H-4′, respectively. ^{13}C NMR(Me_2SO-d_6) δ 157.69 (C-6), 151.51 (C-2, $^1J_{CH} = 203.3$ Hz), 150.09 (C-4), 144.55 (C-8, $^1J_{CH} = 215.5$ Hz), 132.11 (C-5), 84.20 (C-4′), 83.71 (C-1′, $^1J_{CH} = 164.8$ Hz), 75.64 (C-2′), 74.67 (C-3′), 60.65 (C-5′), 19.01 (6–CH_3); Anal. Calcd. for $C_{11}H_{14}N_4O_4$; C 49.62, H 5.30, N 21.04; found C 49.45, H 5.15, N 21.00.

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