# Synthesis and Biological Evaluation of Iodinated and Fluorinated 9-(2-Hydroxypropyl) and 9-(2-Hydroxyethoxy)methyl Purine Nucleoside Analogues

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The novel fluorinated and iodinated purine derivatives containing 9-(2-hydroxypropyl) (**1a**−**7a** and **9a**−**13a**) and 9-(2-hydroxyethoxymethyl) (**1b**−**3b, 5b**, and **7b**−**12c**) side chains were synthesized by a multistep synthetic route involving Baltz−Schiemann's fluorination and diazotation/iodination as key reactions. An unequivocal proof for the stereostructure of **5b** was obtained by X-ray structure analysis. New compounds were evaluated for their cytostatic activity against murine leukemia (L1210); mammary carcinoma (FM3A); and human T-lymphocytes (Molt4/C8 and CEM), melanoma (HBL), cervical carcinoma (HeLa), colon carcinoma (HT29 and SW620), laryngeal carcinoma (Hep2), and pancreatic carcinoma (MiaPaCa2) as well as diploid fibroblasts (WI38). Of all the compounds, the 2-aminopurin-6-thione derivative **9a** showed the most pronounced inhibitory activity against human SW620 cells. The 2-aminopurin-6-thione derivative **9b** exhibited the most selective inhibitory activity against human HeLa, Hep2, SW620, and murine L1210 cell proliferation as compared to normal fibroblast (WI38) cell proliferation. None of the compounds showed inhibitory activities against HIV-1, HIV-2, HSV-1, and HSV-2, vaccinia, vesicular stomatitis, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, or respiratory syncytial virus. The new purine derivatives, and particularly **9a** and **9b**, appear to demonstrate sufficient cytostatic potency and selectivity to justify further evaluation of their potential.

## Introduction

Interest in the synthesis and biological evaluation of purine nucleosides and their analogues has continued in recent years as new structures have been found to have clinical activity as both anticancer[1−4] and antiviral agents.[5] The 2-bromo-, 2-chloro-, and 2-fluoropurine nucleosides have shown outstanding activity in murine leukemia models, and the 2-chloro compound, known as cladribine, has been used for the treatment of hairy cell leukemia.[6] Furthermore, acyclic nucleoside analogues, aciclovir, penciclovir, and ganciclovir, are the therapeutic compounds of choice to interfere with severe herpes virus infections. These molecules act as fraudulent substrates of herpes simplex virus 1 thymidine kinase (HSV-1 TK),[7−9] blocking virus proliferation by complexes with the viral DNA. Moreover, acyclic nucleoside analogues have been used in combination with suicide enzymes in gene therapy of cancer[10−12] and AIDS.[13] The acyclonucleosides labeled with positron-emitting radioisotope $^{18}F$ have also been employed as tracer molecules for noninvasive positron emission tomography (PET) imaging of HSV-1 TK gene expression.[14−16]

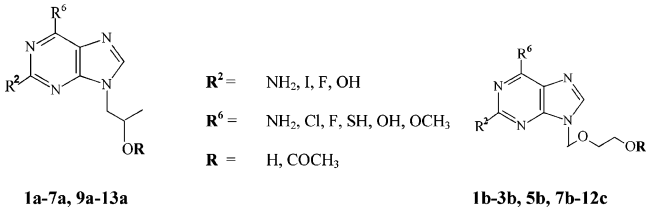


**Figure 1.** Purine acyclic nucleoside analogues with 2-hydroxypropyl (**1a**−**7a**, **9a**−**13a**) and 2-hydroxyethoxymethyl (**1b**−**3b**, **5b**, **7b**−**12c**) side chains.

We have found on the basis of binding affinity assays and molecular docking that 9-(2-hydroxypropyl)purine nucleoside analogues act as fraudulent substrates of herpes simplex virus (HSV) and varicella zoster virus (VZV) thymidine kinases.[17]

Taking into account the pharmacological potential of those classes of compounds, we have undertaken this study with the primary aim to evaluate the newly synthesized iodinated and fluorinated purine acyclic nucleoside analogues (Figure 1) on their cytostatic and antiviral activities.

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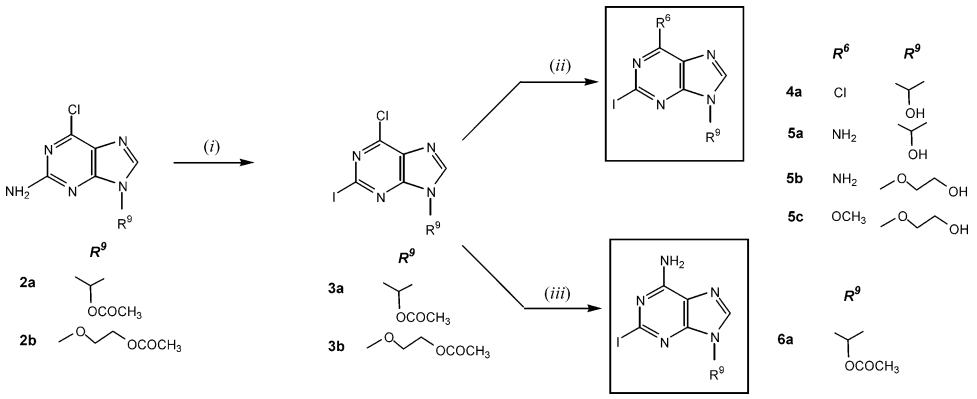
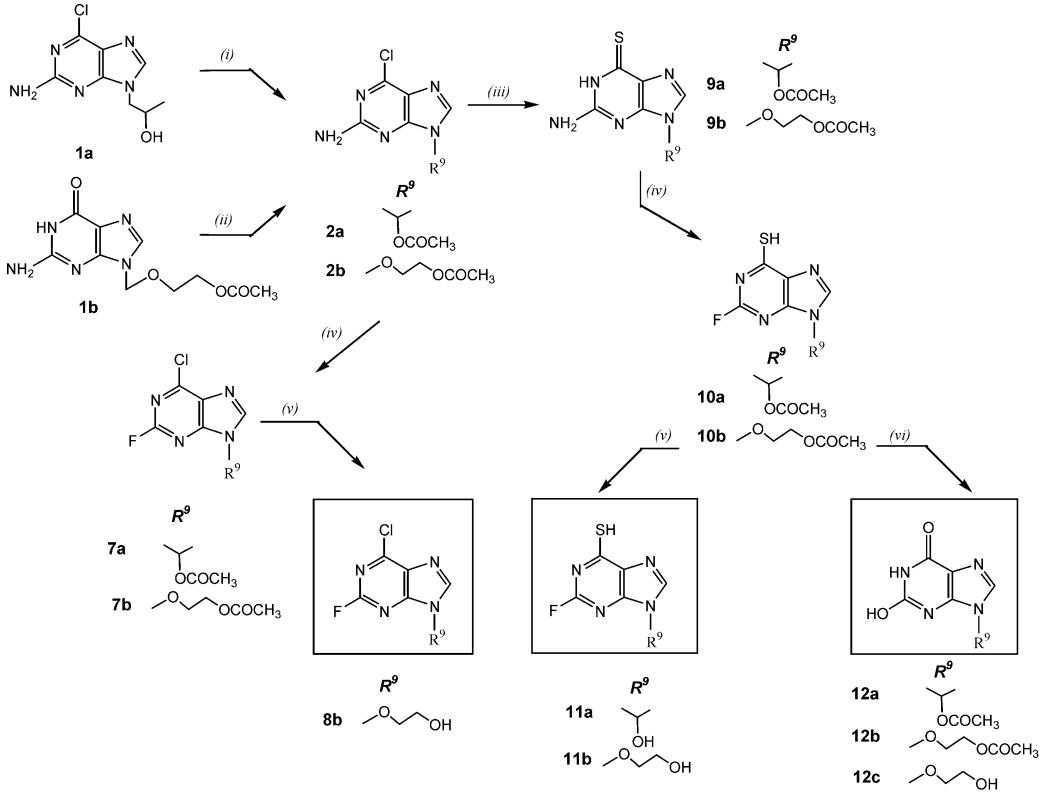
**Scheme 1**[a]



[a] Reagents and conditions: (i) $CH_2Cl_2$, isopentyl nitrite; (ii) methanolic $NH_3$; (iii) 1,2-dimethoxyethane (dry), $NH_3$.

**Scheme 2**[a]



[a] Reagents and conditions: (i) 4-DMAP, $CH_3CN$, TEA, acetic anhydride; (ii) $POCl_3$, $Et_4NCl$, $CH_3CN$, *N*,*N*-DEA; (iii) $(NH_2)_2CS$/EtOH; (iv) 60% HF/pyridine, *tert*-butyl nitrite (TBN); (v) LiOH, $CH_3CN$:$H_2O$ = 1:1; (vi) 30% $H_2O_2$, $NH_4OH$, $H_2O$.

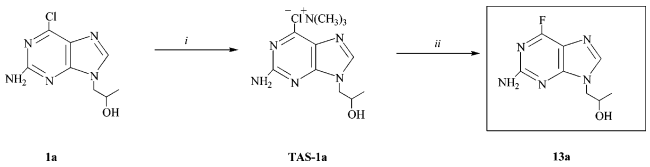
## Chemistry

2-Halogenated acyclic purine nucleosides were synthesized via Baltz−Schiemann reaction from the corresponding 2-amino purine derivatives as starting compounds (Schemes 1 and 2).[18]

The *N*-9-(2-hydroxypropyl)-2-amino-6-chloropurine (**1a**) was prepared by alkylation of 2-amino-6-chloropurine with propylene carbonate in the presence of a catalytic amount of sodium hydroxide.[19] Since the racemic propylene carbonate has been used for condensation with 2-amino-6-chloropurine, the 2-hydroxypropyl purine derivatives (**1a**−**12a**), containing an asymmetric carbon atom at the position 2′ of the exocyclic chain, have been obtained as racemates. The acetylation of **1a** was performed by a procedure analogous to that described for the corresponding guanine nucleosides to give the acetylated product **2a**[20] (Scheme 2). *O*-Acetylated aciclovir **1b** was prepared from guanosine by chemical transpurination.[21−23] Subsequent chlorination of this compound using an excess of phosphoryl chloride, tetraethylammonium chloride, and *N*,*N*-dialkylaniline as a base gave 2-amino-6-chloropurine derivative **2b**[22,24] (Scheme 2).

The iodine in the 2-position of the purine ring in **3a** and **3b** was introduced by diazotation/halogenation of **2a** and **2b** using isopentyl nitrite as nitrosating agent and diiodomethane as iodine source[25] (Scheme 1). The 6-chloro-2-iodopurine derivatives **3a** and **3b** were deacety-

**Scheme 3**[a]




[a] Reagents and conditions: (i) $N(CH_3)_3$/dry DMF; (ii) KF/dry DMF.

lated with methanolic ammonia, which caused both ammonolysis of the ester group (**4a**) and amination at the 6-position of the purine ring (**5a** and **5b**) (Scheme 1). Treatment of **3b** with methanolic ammonia also gave the 6-methoxy-2-iodopurine derivative **5c**. We have found that prolonged duration of the reaction at higher temperature gave mainly 2,6-diamino purine acyclic analogues, which is in accord with what has been found previously.[26] When the reaction was carried out with anhydrous ammonia in dry 1,2-dimethoxyethane,[24] the nucleophilic replacement of the 6-chloro by an amino group gave the 6-amino-2-iodopurine derivative **6a** (Scheme 1).

Introduction of fluorine in the 2-position of the purine ring in **7a**, **7b**, **10a**, and **10b** was performed using 60% (w/w) HF/pyridine and *tert*-butyl nitrite (TBN) in nonaqueous media[27,28] (Scheme 2). Deacetylation of **7a**, **10a**, and **10b** performed by LiOH in $CH_3CN$:$H_2O$ (1:1)[13] gave the corresponding 2-hydroxyethoxymethyl (**8b** and **11b**) and 2-hydroxypropyl (**11a**) purine derivatives. The purine-6-thione derivatives **9a** and **9b** were prepared from the corresponding chloro derivatives **2a** and **2b** by reaction with thiourea in absolute ethanol[29] (Scheme 2). An attempt to transform the 6-thio to a 6-keto group with the remaining fluorine substituted at 2 of the purine moiety, using hydrogen peroxide in the presence of a small amount of dilute, aqueous ammonia, failed. The xanthine derivatives **12a**−**c** were isolated in this reaction as products of hydrolysis. As reported previously, fluoropurine compounds were susceptible to hydrolysis in acid solution, due to a strong inductive effect of the fluorine atom.[30] Even heating of an aqueous solution of 6-fluoropurine gave hypoxanthine.[31]

The 6-fluoro-substituted purine derivative **13a** was prepared from the 6-chloro derivative **1a** using trimethylamine, which gave the trimethylammonium salt **TAS-1a**. Subsequent reaction of **TAS-1a** with anhydrous potassium fluoride[31,32] yielded the 6-fluoropurine nucleoside **13a** (Scheme 3).

**$^1$H and $^{13}$C NMR Spectra.** Assignment of $^1$H and $^{13}$C NMR spectra was performed on the basis of chemical shifts, signal intensities, magnitude, and multiplicity of H−H and C−F coupling constants as well as connectivities in HMBC (heteronuclear multiple-bond correlation) and HSQC (heteronuclear single-quantum coherence) spectra. The chemical shifts pattern of the C-2- and C-6-substituted purine moiety in both $^1$H and $^{13}$C NMR spectra are consistent with those observed for structuraly related nucleoside analogues.[33,34] The general feature of the $^1$H NMR spectra of 9-(2-hydroxypropyl)purine derivatives (**1a**−**7a, 9a**−**13a**) is that, besides the H-8 and $NH_2$ protons of the purine skeleton, the $CH_2$-1′, CH-2′, and $CH_3$-3′ protons of N-9 side chain were observed (Table 1). H−H coupling patterns for these protons are two doublets of doublets or a multiplet corresponding to methylene ($CH_2$-1′) protons, a multiplet for methane (CH-2′), and a doublet for methyl ($CH_3$-3′) protons. The chemical shifts of the methylene protons in aciclovir derivatives (**1b**−**3b**, **5b**, and **7b**−**12c**) are in the order $\delta$ ($CH_2$-1′) > $\delta$ ($CH_2$-3′) > $\delta$ ($CH_2$-4′), corresponding to enhanced shielding with increasing distance of $CH_2$ protons from the purine $\pi$-system.

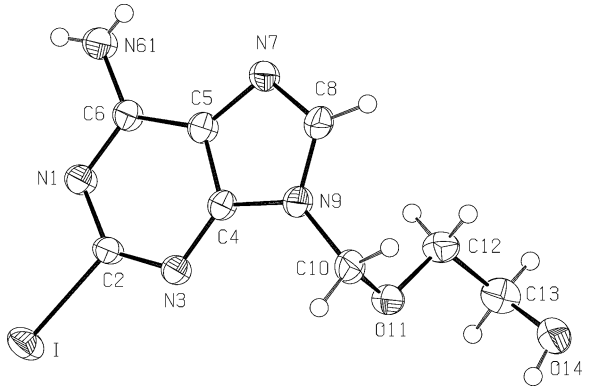


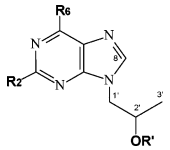

**Figure 2.** The molecular structure of **5b** with the atom-numbering system. Ellipsoids drawn at the 40% probability level and hydrogen atoms are shown with arbitrary radii.

The assignation of the $^{13}$C NMR spectra is given in the Experimental Section. The most significant C-2 substituent effect in the purine ring was found for 2-iodo-substituted derivatives **3**−**6**. In these compounds C-2 was shifted (ca. 43 ppm) upfield with respect to the 2-amino purine derivatives. Fluoro substitution at position 2 caused small changes in chemical shifts for C-2. This carbon is shielded (ca. 3 ppm) compared to the corresponding one in the 2-aminopurine derivatives. The magnitude of the one-bond C−F coupling constant at C-2 (212.0−221.2 Hz) is in agreement with those found for related fluorinated compounds.[35,36]

Introduction of a fluorine atom at the 2 position in the purine-6-thione compound **9a** caused a change to the tautomeric thiol form **10a**. Thus, C-6 is more deshielded (ca. 17 ppm) in **9a** than in **10a**. Chemical shifts of this carbon are in agreement with literature data for corresponding carbon in the related thione and thiol derivatives.[37] Summing up, both $^1$H and $^{13}$C NMR spectra corroborate the structure of the newly prepared compounds.

**X-ray Crystal Structure Analysis.** A perspective view of the 6-amino-2-iodopurine derivative **5b** with the labeling of its atoms is shown in Figure 2. The main structural features of **5b** (Table 3) are in accord with those found in structuraly related adenine,[38] 2,6-diaminopurine,[39] and 2-methoxy-6-aminopurine[40] derivatives. Atoms of the purine ring are coplanar within ±0.024(4) Å with the acyclic chain positioned almost orthogonally to the heterocyclic ring [the torsion angle C8−N9−C10−O11 amounts to −91.7(5)°]. Significant intermolecular hydrogen-bonding contacts were found in the crystal lattice (Table 4, Figure 3). The 2-amino group and atom N1 of the purine ring form centrosymmetric dimers, designated as $R_2^2(8)$ ring.[41] Translation of molecules along the *b* axis form $R_2^2(10)$ rings through O14−H···N7$^{ii}$ and O11···H−N61$^{ii}$ hydrogen bonds, [ii = $x$, $y + 1$, $z$].

**Table 1.** $^1$H Chemical Shifts ($\delta$/ppm)[a] and H−H Coupling Constants ($J$/Hz)[b] in $^1$H NMR Spectra for Compounds **1a**−**7a**, **9a**−**13a** (cf. Schemes 1−3)




| compd | H-8 | $NH_2$ | $COCH_3$ | OH | H-1 | H-2 | H-3 |
|---|---|---|---|---|---|---|---|
| **1a** $R_2 = NH_2$, $R_6 = Cl$ R = H | 8.27 (1H, s) | 7.00 (2H, s) | − | 5.36 (1H, d) $J^3 = 4.9$ | 4.30−4.13 (2H, m) | 4.01 (1H, m) | 1.24 (3H, d) $J^3 = 5.9$ |
| **2a** $R_2 = NH_2$, $R_6 = Cl$ R = Ac | 8.07 (1H, s) | 6.94 (2H, s) | 1.88 (3H, s) | − | 4.23 (1H) $J^3 = 3.46$; $J^2 = 14.48$ (dd) 4.28 (1H) $J^3 = 7.17$; 14.61 (dd) | 5.14 (1H, m) | 1.15 (3H, d) $J^3 = 6.3$ |
| **3a** $R_2 = I$, $R_6 = Cl$ R = Ac | 8.04 (1H, s) | − | 1.88 (3H, s) | − | 4.52 (1H) $J^3 = 3.15$; $J^2 = 14.96$ (dd) 4.28 (1H) $J^3 = 7.06$; 14.71 (dd) | 5.25 (1H, m) | 1.32 (3H, d) $J^3 = 6.4$ |
| **4a** $R_2 = I$, $R_6 = Cl$ R = H | 8.18 (1H, s) | − | − | 4.27 (1H, b) | 4.42−4.07 (2H, m) | 4.26 (1H, m) | 1.31 (3H, d) $J^3 = 6.4$ |
| **5a** $R_2 = I$, $R_6 = NH_2$ R = H | 7.95 (1H, s) | 7.60 (2H, s) | − | 5.05 (1H, d) $J^3 = 3.7$ | 4.02−3.95 (2H, m) | 3.90 (1H, m) | 1.04 (3H, d) $J = 5.3$ |
| **6a** $R_2 = I$, $R_6 = NH_2$ R = Ac | 8.04 (1H, s) | 7.68 (2H, b) | 1.92 (3H, s) | − | 4.31 (1H) $J^3 = 2.59$; $J^2 = 14.56$ (dd) 4.19 (1H) $J^3 = 6.65$; 14.55 (dd) | 5.13 (1H, m) | 1.16 (3H, d) $J^3 = 6.3$ |
| **7a** $R_2 = F$, $R_6 = Cl$ R = Ac | 8.68 (1H, s) | − | 1.88 (3H, s) | − | 4.47 (1H) $J^3 = 3.33$; $J^2 = 14.63$ (dd) 4.38 (1H) $J^3 = 6.65$; 14.63 (dd) | 5.25 (1H, m) | 1.32 (3H, d) $J^3 = 6.4$ |
| **9a**[c] $R_2 = NH_2$, $R_6 = S$ R = Ac | 7.82 (1H, s) | 6.78 (2H, b) | 1.89 (3H, s) | − | 4.13 (1H) $J^3 = 3.49$; $J^2 = 14.46$ (dd) 4.04 (1H) $J^3 = 6.98$; $J^2 = 14.62$ (dd) | 5.10 (1H, m) | 1.13 (3H, d) $J^3 = 6.3$ |
| **10a** $R_2 = F$, $R_6 = SH$ R = Ac | 8.06 (1H, s) | − | 2.05 (3H, s) | − | 4.46 (1H) $J^3 = 3.28$; $J^2 = 14.46$ (dd) 4.29 (1H) $J^3 = 6.90$; 14.69 (dd) | 5.27 (1H, m) | 1.31 (3H, d) $J^3 = 6.4$ |
| **11a** $R_2 = F$, $R_6 = SH$ R = H | 8.54 (1H, s) | − | − | 5.05 (1H, d) $J^3 = 4.4$ | 4.10−4.01 (2H, m) | 4.19 (1H, m) | 1.13 (3H, d) $J^3 = 5.7$ |
| **12a**[d] $R_2 = OH$, $R_6 = O$ R = Ac | 7.61 (1H, s) | − | 1.91 (3H, s) | − | 4.21 (1H) $J^3 = 3.39$; $J^2 = 14.88$ (dd) 4.14 (1H) $J^3 = 8.14$; $J^2 = 14.89$ | 5.07 (1H, m) | 1.18 (3H, d) $J^3 = 6.4$ |
| **13a** $R_2 = NH_2$, $R_6 = F$ R = H | 7.62 (1H, s) | 5.76 (2H, s) | − | 5.02 (1H, d) $J^3 = 5$ Hz | 3.93−3.86 (2H, m) | 3.82 (1H, m) | 1.02 (3H, d) $J^3 = 7$ Hz |

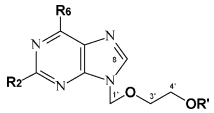
[a] DMSO-$d_6$ as solvent for all compounds, except for **3a, 7a**, and **10a**, which were recorded in $CDCl_3$; chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in parentheses: s, singlet; d, doublet; m, complex multiplet; b, broad. [b] Digital resolution, ±0.28 Hz. [c] Signal for −NH−, 11.97 ppm (1H, s). [d] Signal for −NH−, 11.93 ppm (1H, s); OH, 10.81 ppm (1H, s).

## Biological Results

**Cytostatic Activity.** Compounds **2a**−**7a**, **9a**−**13a**, **3b**, **5b**, and **9b**−**11b** were evaluated for their cytostatic activity against malignant tumor cell lines: murine leukemia (L1210) and mammary carcinoma (FM3A) and human T-lymphocyte (Molt4/C8, CEM), colon carcinoma (HT29 and SW620), cervical carcinoma (HeLa), melanoma (HBL), laryngeal carcinoma (Hep2), and pancreatic carcinoma (MiaPaCa2) cells as well as diploid fibroblasts (WI38) (Table 5).

Of all the compounds evaluated, 2-aminopurin-6-thione derivative **9a** showed the most pronounced inhibition of human SW620 cell lines (0.16 ± 0.1 $\mu$M). Replacement of the amino group (**2a** and **2b**) by iodine (**3a** and **3b**) at position 2 of the purine ring had considerable influence on antitumor activity. Thus, 2-iodopurine derivatives (**3a** and **3b**) showed rather pronounced cytostatic activities compared to the corresponding 2-aminopurine derivatives (**2a** and **2b**). It is interesting to note that deacetylation of **3a** caused a significant decrease of the antitumor activity that might be due to the greater lipophility of the acetylated derivative. Thus, the deacetylated analogue **4a** did not inhibit the growth of the examined cell lines. The most significant inhibitory effects of the 2-fluorine-substituted purine derivatives were observed with the 6-chloro-2-fluoropurine derivative **7a**. This compound showed inhibitory activity against all examined cell lines, with the best effect on human Hep2 (10 ± 0.6 $\mu$M) and SW620 (8.9 ± 0.6 $\mu$M) cell lines. Replacement of $NH_2$ group in **2a** by fluorine at position 2 in **7a** caused enhanced antitumor activity. On the contrary, compounds **10a** and **10b** containing 2-fluoropurin-6-thiol were less active than the corresponding 2-aminopurin-

**Table 2.** $^1$H Chemical Shifts ($\delta$/ppm)[a] and H−H Coupling Constants ($J$/Hz)[b] in $^1$H NMR Spectra for Compounds **3b**, **5b**, **5c**, and **7b**−**12c** (cf. Schemes 1−3)




| compd | H-8 | $NH_2$ | $COCH_3$ | OH | H-1 | H-3 | H-4 |
|---|---|---|---|---|---|---|---|
| **3b** $R_2$ = I, $R_6$ = Cl R = Ac | 8.19 (1H, s) | − | 2.03 (3H, s) | − | 5.68 (2H, s) | 4.19 (2H, t) $J^3$ = 4.19 | 3.74 (2H, t) $J^3$ = 4.48 |
| **5b** $R_2$ = I, $R_6$ = $NH_2$ R = H | 8.22 (1H, s) | 7.73 (2H, s) | − | 4.71 (1H, t) $J$ = 5.35 | 5.50 (2H, s) | 3.49 (4H, m) | |
| **5c** $R_2$ = I, $R_6$ = $OCH_3$ R = H | 8.46 (1H, s) | − | − | 4.69 (1H, t) $J$ = 5.35 | 5.59 (2H, s) | 3.48 (4H, m) | |
| **7b** $R_2$ = F, $R_6$ = Cl R = Ac | 8.28 (1H, s) | − | 2.04 (3H, s) | − | 5.69 (2H, s) | 4.21 (2H, m) | 3.81 (2H, m) |
| **8b** $R_2$ = F, $R_6$ = Cl R = H | 8.83 (1H, s) | − | − | 4.69 (1H, m) | 5.65 (2H, s) | 3.54 (2H, m) | 3.45 (2H, m) |
| **9b** $R_2$ = $NH_2$, $R_6$ = S R = Ac | 8.02 (1H, s) | 6.87 (2H, b) | 1.93 (3H, s) | − | 5.37 (2H, s) | 4.10 (2H, m) | 3.67 (2H, m) |
| **10b** $R_2$ = F, $R_6$ = SH R = Ac | 8.20 (1H, s) | − | 2.04 (3H, s) | − | 5.66 (2H, s) | 4.21 (2H, m) | 3.80 (2H, m) |
| **11b** $R_2$ = F, $R_6$ = SH R = H | 8.78 (1H, s) | − | − | 4.65 (1H, t) $J$ = 4.95 | 5.65 (2H, s) | 4.53 (2H, m) | 3.46 (2H, m) |
| **12b** $R_2$ = OH, $R_6$ = O R = Ac | 7.81 (1H, s) | − | 1.96 (3H, s) | − | 5.44 (2H, s) | 4.08 (2H, m) | 3.65 (2H, m) |
| **12c** $R_2$ = OH, $R_6$ = O R = H | 7.71 (1H, s) | − | − | − | 5.39 (2H, s) | 3.49 (4H, m) | |

[a] DMSO-$d_6$, chemical shifts referred to TMS. Multiplicity of coupling and number of protons are given in parentheses: s, singlet; t, doublet; m, complex multiplet; b, broad. [b] Digital resolution, ±0.28 Hz. [c] Signal for $-OCH_3$, 4.03 ppm (3H, s). [d] Signal for −NH−, 11.98 ppm (1H, s). [e] Signal for −NH−, 10.80 ppm (1H, s). [f] Signal for −NH−, 10.39 ppm (1H, s).

**Table 3.** Selected Geometric Parameters (Å, deg) for **5b**

| | | | |
|---|---|---|---|
| I−C2 | 2.113(4) | C2−N1−C6 | 117.4(3) |
| N1−C2 | 1.343(5) | C2−N3−C4 | 109.8(3) |
| N1−C6 | 1.358(5) | C5−N7−C8 | 103.5(4) |
| N3−C2 | 1.319(5) | C4−N9−C8 | 105.3(3) |
| N3−C4 | 1.347(6) | N1−C2−N3 | 130.8(4) |
| N7−C5 | 1.387(6) | N3−C2−I | 115.9(3) |
| N7−C8 | 1.308(6) | N1−C6−C5 | 117.8(4) |
| N9−C8 | 1.380(7) | C4−N9−C10 | 128.6(3) |
| N9−C4 | 1.377(5) | C4−N9−C10−O11 | 79.7(5) |
| N9−C10 | 1.450(5) | C8−N9−C10−O11 | −91.7(5) |
| N61−C6 | 1.345(5) | N3−C4−N9−C10 | 9.1(7) |
| | | C10−O11−C12−C13 | 156.9(4) |

**Table 4.** Hydrogen-Bonding Geometry (Å, deg) for **5b**

| D−H···A[a] | D−H | H···A | D···A | D−H···A |
|---|---|---|---|---|
| N61−H61A···N1$^{i}$ | 0.71(9) | 2.42(9) | 3.034(8) | 148(9) |
| N61−H61B···O11$^{ii}$ | 0.69(9) | 2.43(8) | 3.060(6) | 154(8) |
| O14−H14···N7$^{iii}$ | 0.820(4) | 2.051(4) | 2.849(6) | 164.5(3) |

[a] Symmetry codes: (i) $-x$, $-y+1$, $-z+2$; (ii) $x$, $y-1$, $z$; (iii) $x$, $y+1$, $z$.

6-thione derivatives **9a** and **9b**. 2-Aminopurine-6-thione with the acetoxyethoxymethyl side chain (**9b**) exhibited marked selectivity in its cytostatic activity. This compound inhibited specifically the growth of human HeLa (5 ± 0.3 $\mu$M), Hep2 (4.9 ± 0.5 $\mu$M), SW620 (4.2 ± 0.3 $\mu$M), Molt4/C8 (8.9 ± 0.5 $\mu$M), CEM (7.5 ± 1.3 $\mu$M), and murine L1210 (3.2 ± 1.2 $\mu$M), FM3A (2.0 ± 0.6 $\mu$M) cells but not the other cells including normal fibroblasts (WI38). Compound **13a** containing fluorine at position 6 of the purine ring showed slight inhibitory activity against human HBL (25 ± 2.8 $\mu$M) and HeLa (63 ± 3.5 $\mu$M) cell lines.

**Antiviral Activity.** The compounds were evaluated against HIV-1($III_B$), HIV-2(ROD), HSV-1, HSV-2, vaccinia, vesicular stomatitis, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and respiratory syncytial virus. All compounds were antivirally inactive at subtoxic concentrations.

## Conclusions

New types of acyclic nucleoside analogues containing a 2-fluoro-, 2-iodo-, and 6-fluoropurine moiety were prepared. The introduction of halogen (F and I) in position 2 was performed by Baltz−Schiemann's reaction, while for the iodination the diazotization/halogenation procedure was applied. 6-Fluoropurine nucleoside analogue **13a** was obtained by the nucleophilic replacement of the quaternary ammonium salt as intermediate with potassium fluoride.

The structures of the newly synthesized compounds were deduced by one- and two-dimensional $^1$H and $^{13}$C NMR spectroscopy. The stereostructure of compound **5b** was unambiguously confirmed by its X-ray structural analysis.

The novel compounds were evaluated for their cytostatic and antiviral activity. Of all compounds, 2-aminopurin-6-thione derivative **9a** showed the most pronounced inhibitory activity against human colon

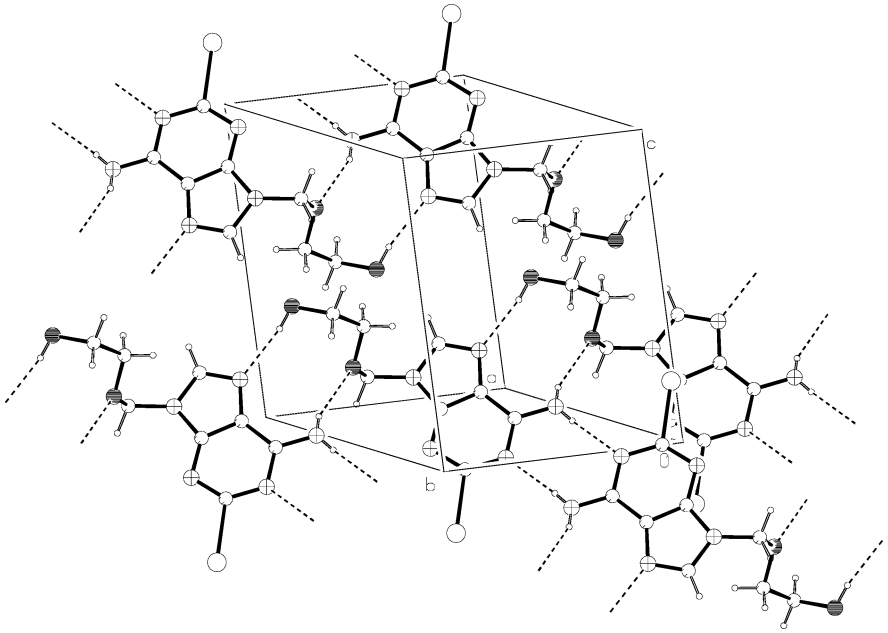


**Figure 3.** Packing diagram of **5b**.

carcinoma (SW620) cells. Among the fluorinated compounds, 6-chloro-2-fluoropurine derivative **7a** containing an acetylated hydroxy group showed the strongest inhibitory effect, particularly on the growth of human laryngeal carcinoma (Hep2) and colon carcinoma (SW620) cell lines. 2-Aminopurine-6-thione with an acetoxyethoxymethyl side chain (**9b**) showed the most selective inhibitory activity against human HeLa, Hep2, SW620, and murine L1210 cell proliferation as compared to normal fibroblast (WI38) cell proliferation.

The differences shown in the antitumor cell activity spectrum by **9a** versus **9b**, which may be considered as statistically significant ($p < 0.001$), are intriguing and suggest that unique mechanistic differences may underlie their cytostatic activity. Further evaluation of the mechanism of antitumor activity for these agents appears to be warranted.

## Experimental Protocols

**General.** Melting points (uncorrected) were determined with a Kofler micro hot-stage (Reichert, Wien). Precoated Merck silica gel 60F-254 plates were used for thin-layer chromatography (TLC), and the spots were detected under UV light (254 nm). Column chromatography (CLC) was performed using Kemika silica gel (0.063−0.2 mm), and the glass column was slurry-packed under gravity. Solvent systems used for the TLC and CLC were $CH_2Cl_2$:$CH_3OH$ 12:1 (S1), 10:1 (S2), 9:1 (S3), and 5:1 (S4). The electron impact mass spectra were recorded with an EXTREL FT MS 2001 instrument with ionizing energy 70 eV. Elemental analyses were performed in the Central Analytic Service, Ruđer Bošković Institute, Zagreb, Croatia. $^1$H and $^{13}$C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 75.46 MHz for the $^{13}$C resonance. The samples were dissolved in $CDCl_3$ or DMSO-$d_6$ and measured in 5 mm NMR tubes. The $^1$H and $^{13}$C NMR chemical shift values ($\delta$) are expressed in ppm referred to TMS and coupling constants ($J$) in hertz.

**2-Amino-6-chloro-9-(2-hydroxypropyl)-9*H*-purine (1a).**[17] A mixture of 2-amino-6-chloropurine (3.0 g, 17.7 mmol), propylene carbonate (3.0 mL, 35.4 mmol), and pulverized sodium hydroxide (67 mg) in dry DMF (30 mL) was heated under reflux with stirring for 5.5 h. The unreacted 2-amino-6-chloropurine was filtered off and the filtrate evaporated to dryness. The residue was purified by CLC with solvent system S4 yielding crude **1a**. Recrystallization from 2-propanol afforded pure **1a**: 1.750 g, 43%; mp = 99−101 °C; MS *m*/*z* 227 $M^+$; UV (methanol) $\lambda_{max}$ 310, 248, 224 (log $\epsilon$ 3.77, 3.62, 4.35); $^{13}$C NMR (DMSO) $\delta$ 162.00 (C-2), 154.48 (C-6), 151.38 (C-4), 145.72 (C-8), 125.60 (C-5), 66.48 (C-2′), 52.07 (C-1′), 21.96 (C-3′).

**9-[(2-Acetoxyethoxy)methyl]-2-amino-1,9-dihydro-6*H*-purine-6-one (1b)[21−23] and 9-(2-Acetoxypropyl)-2-amino-6-chloro-9*H*-purine (2a).** To a suspension of **1a** (2.0 g, 8.8 mmol), 4-(dimethylamino)pyridine (81 mg, 0.67 mmol), acetonitrile (120 mL), and triethylamine (1.6 mL, 11.7 mmol) was added the acid anhydride (1.0 mL, 10.6 mmol). After stirring for 40 min at room temperature, methanol (1.0 mL) was added to the mixture and stirring was continued for a further 5 min. The mixture was evaporated to dryness under reduced pressure and the resulting oil crystallized from 2-propanol. The obtained crystals were washed well with ethanol and ether. Recrystallization gave pure **2a** (1.72 g, 72%): mp = 167−169 °C; UV (methanol) $\lambda_{max}$ 309, 248, 223 (log $\epsilon$ 4.02, 3.88, 4.59); $^{13}$C NMR (DMSO) $\delta$ 159.96 (C-2), 154.54 (C-6), 149.46 (C-4), 143.66 (C-8), 123.16 (C-5), 68.16 (C-2′), 46.92 (C-1′), 17.22 (C-3′), 169.83 (*C*OCH$_3$), 20.89 (CO*C*H$_3$).

**9-[(2-Acetoxyethoxy)methyl]-2-amino-6-chloro-9*H*-purine (2b).** Compound **2b** was prepared according to a procedure given in the literature.[22] Evaporated organic extracts were purified by CLC with solvent system $S_3$. Crystallization from 2-propanol afforded the yellow crystals of **2b** (352 mg, 60%): mp = 121−124 °C (lit.[22] mp = 125−126 °C); UV (methanol) $\lambda_{max}$ 308, 248, 223 (log $\epsilon$ 3.44, 3.55, 4.08); $^1$H NMR data are in accord with the data reported in the literature.

**9-(2-Acetoxypropyl)-6-chloro-2-iodo-9*H*-purine (3a).** A stirred solution of **2a** (163 mg, 0.605 mmol) in 5 mL of diiodomethane was treated with *n*-pentyl nitrite (1.6 mL, 12.12 mmol). Stirring was continued at 85 °C for 1 h with exclusion of moisture. Volatile materials and diiodomethane were evaporated in vacuo to give an oily product, which was purified by CLC with solvent system $S_1$. Fractions containing the product were combined and evaporated to give crude material of **3a**. Recrystallization from ethanol afforded the yellow crystals of **3a** (117 mg, 51%): mp = 150−155 °C; MS *m*/*z* 380.1 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 282 (log $\epsilon$ 3.84); $^{13}$C NMR ($CDCl_3$) $\delta$ 153.24 (C-6), 151.06 (C-4), 145.44 (C-8), 131.73 (C-5), 116.74 (C-2), 68.24 (C-2′), 47.88 (C-1′), 17.10 (C-3′), 170.34 (*C*OCH$_3$), 20.83 (CO*C*H$_3$). Anal. ($C_{10}H_{10}ClIN_4O_2$) C, H, N.

**9-[(2-Acetoxyethoxy)methyl]-6-chloro-2-iodo-9*H*-purine (3b).** A mixture of compound **2b** (202 mg, 0.71 mmol), *n*-pentyl nitrite (1.92 mL, 14.2 mmol), and diiodomethane (5

**Table 5.** Inhibitory Effects of Acyclic Purine Nucleosides on the Growth of Malignant Tumor Cell Lines and Diploid Fibroblasts (WI38)

| | tumor cell growth [$IC_{50}$[a] ($\mu$M)] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | L1210 | FM3A | Molt4/C8 | CEM | HT29 | HeLa | HBL | Hep2 | SW620 | MiaPaCa2 | WI38 |
| **2a** $R_2 = NH_2$, $R_6 = Cl$ R = Ac | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **2b** $R_2 = NH_2$, $R_6 = Cl$ R = Ac | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **3a** $R_2 = I$, $R_6 = Cl$ R = Ac | 13.0 ± 0.8 | 47 ± 2 | 12.3 ± 0.7 | 15.9 ± 3.7 | 6.42 ± 0.4 | 5.13 ± 0.3 | 5.25 ± 0.2 | 6.31 ± 0.5 | 3.55 ± 0.3 | 14.5 ± 1.6 | 14.1 ± 1.8 |
| **3b** $R_2 = I$, $R_6 = Cl$ R = Ac | 8.6 ± 0.7 | 34 ± 2 | 9.4 ± 1.2 | 11 ± 1.0 | >500 | 398 ± 26 | 19.9 ± 7.7 | 56.2 ± 11 | 15.8 ± 1.7 | >500 | 63.1 ± 8.4 |
| **4a** $R_2 = I$, $R_6 = Cl$ R = H | >500 | 479 ± 30 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **5a** $R_2 = I$, $R_6 = NH_2$ R = H | 490 ± 14 | 452 ± 67 | 500 | 465 ± 49 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **5b** $R_2 = I$, $R_6 = NH_2$ R = H | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **5c** $R_2 = I$, $R_6 = OCH_3$ R = H | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **6a** $R_2 = I$, $R_6 = NH_2$ R = Ac | 421 ± 38 | 462 ± 54 | 436 ± 90 | 381 ± 47 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **7a** $R_2 = F$, $R_6 = Cl$ R = Ac | 37 ± 8 | 93 ± 34 | 41 ± 0 | 44 ± 5 | 75.9 ± 3.5 | 50 ± 1.4 | 44.7 ± 9.5 | 10 ± 0.63 | 8.9 ± 0.6 | 93.3 ± 6.3 | 100 ± 7.3 |
| **9a** $R_2 = NH_2$, $R_6 = S$ R = Ac | 128 ± 6 | 366 ± 189 | 370 ± 70 | 381 ± 45 | >500 | 89 ± 2.3 | 630 ± 42 | 10 ± 1.5 | 0.16 ± 0.1 | 25 ± 2.2 | 0.4 ± 0.1 |
| **9b** $R_2 = NH_2$, $R_6 = S$ R = Ac | 3.2 ± 1.2 | 2.0 ± 0.6 | 8.9 ± 0.5 | 7.5 ± 1.3 | >500 | 5.0 ± 0.3 | 15.8 ± 0.7 | 4.9 ± 0.48 | 4.2 ± 0.3 | >500 | >500 |
| **10a** $R_2 = F$, $R_6 = SH$ R = Ac | 226 ± 20 | 368 ± 186 | >500 | >500 | >500 | >500 | >500 | 200 ± 17.6 | >500 | >500 | >500 |
| **10b** $R_2 = F$, $R_6 = SH$ R = Ac | 40 ± 1 | 136 ± 11 | 35 ± 6 | 37 ± 10 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **11a** $R_2 = F$, $R_6 = SH$ R = H | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **11b** $R_2 = F$, $R_6 = SH$ R = H | 170 ± 5 | 155 ± 5 | 164 ± 9 | 117 ± 53 | >500 | >500 | >500 | >500 | >500 | >500 | >500 |
| **13a** $R_2 = NH_2$, $R_6 = F$ R = H | >500 | >500 | >500 | >500 | >500 | >500 | 25 ± 2.8 | 63 ± 3.5 | >500 | >500 | >500 |

[a] 50% inhibitory concentration, or compound concentration required to inhibit tumor cell proliferation by 50%.

mL) was heated at 85 °C for 1 h with exclusion of moisture. The reaction mixture was worked up in the same way as described for the compound **3a**. Crystallization from ethanol afforded the yellow crystals of **3b** (169 mg, 60%): mp = 147−149 °C; MS *m*/*z* 396 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 261 (log $\epsilon$ 4.15); $^{13}$C NMR ($CDCl_3$) $\delta$ 152.74 (C-6), 150.80 (C-4), 144.98 (C-8), 131.38 (C-5), 117.38 (C-2), 68.16 (C-3′), 73.21 (C-1′), 62.52 (C-4′), 170.73 (*C*OCH$_3$), 20.56 (CO*C*H$_3$). Anal. ($C_{10}H_{10}ClIN_4O_3$) C, H, N.

**6-Chloro-9-(2-hydroxypropyl)-2-iodo-9*H*-purine (4a) and 6-Amino-9-(2-hydroxypropyl)-2-iodo-9*H*-purine (5a).** A solution of compound **3a** (920 mg, 2.418 mmol) in methanolic ammonia (30 mL, saturated at −5 °C) was heated carefully to 60 °C for 5 h in a tightly stoppered flask. Two products detected by TLC were separated by CLC (eluent system $S_3$). Evaporation of fractions and recrystallization of crude product yielded **4a** [180 mg, 22%; mp = 147−150 °C; MS *m*/*z* 338.1 ($M^+$); UV (methanol) $\lambda_{max}$ 264 (log $\epsilon$ 4.13). Anal. ($C_8H_8ClIN_4O$) C, H, N.] and **5a** [536 mg, 70%; mp = 168−171 °C; MS *m*/*z* 319.1 ($M^+$); UV (methanol) $\lambda_{max}$ 267, 222 (log $\epsilon$ 4.17, 4.41); $^{13}$C NMR (DMSO) $\delta$ 155.97 (C-6), 150.31 (C-4), 141.61 (C-8), 120.74 (C-5), 118.58 (C-2), 64.53 (C-2′), 50.33 (C-1′), 20.96 (C-3′). Anal. ($C_8H_{10}IN_5O$) C, H, N.].

**6-Amino-9-[(2-hydroxyethoxy)methyl]-2-iodo-9*H*-purine (5b) and 9-[(2-Hydroxyethoxy)methyl]-2-iodo-6-methoxy-9*H*-purine (5c).** A solution of compound **3b** (195 mg, 0.49 mmol) in methanolic ammonia (10 mL, saturated at −5 °C) in a tightly stoppered flask was heated carefully to 55 °C for 6 h. The solvent was removed in vacuo to give a white solid. Two products, detected by TLC, were separated by CLC (eluent system $S_3$). Combined fractions were evaporated in vacuo to give crude products **5b** and **5c**. Crystallization from ethanol gave **5b** (78 mg, 48%) and **5c** (43 mg, 24%).

**5b:** mp = 174−175 °C; MS *m*/*z* 335.1 ($M^+$); UV (methanol) $\lambda_{max}$ 276 (log $\epsilon$ 4.09), 266, 221 (log $\epsilon$ 4.39, 4.63); $^{13}$C NMR (DMSO) $\delta$ 155.70 (C-6), 150.87 (C-4), 141.13 (C-8), 121.05 (C-5), 118.86 (C-2), 72.91 (C-2′), 71,30 (C-1′), 59.82 (C-3′), 62.52 (C-4′). Anal. ($C_8H_{10}IN_5O_2$), C, H, N.

**5c:** mp = 181−184 °C; MS *m*/*z* 350.1 ($M^+$); UV (methanol) $\lambda_{max}$ 262, 214 (log $\epsilon$ 4.25, 4.41); $^{13}$C NMR (DMSO) $\delta$ 159.43 (C-

6), 152.90 (C-4), 144.13 (C-8), 120.62 (C-5), 118.84 (C-2), 72.91 (C-2′), 70.94 (C-1′), 59.82 (C-3′), 62.52 (C-4′), 54.79 ($OCH_3$). Anal. ($C_9H_{11}IN_4O_3$) C, H, N.

**9-(2-Acetoxypropyl)-6-amino-2-iodo-9*H*-purine (6a).** To a suspension of **5a** (170 mg, 0.55 mmol), 4-(dimethylamino)-pyridine (5 mg, 0.04 mmol), acetonitrile (15 mL), and triethylamine (0.1 mL, 0.72 mmol) was added the acid anhydride (0.06 mL, 0.66 mmol). After stirring for 1 h at room temperature, methanol (0.1 mL) was added to the mixture and stirring was continued for a further 5 min and the reaction mixture was then processed as described for **2a** to give **6a (**121 mg, 62%): mp = 173−175 °C; MS *m*/*z* 272 ($M^+$); UV (methanol) $\lambda_{max}$ 269, 223 (log $\epsilon$ 4.25, 4.38); $^{13}$C NMR (DMSO) $\delta$ 155.92 (C-6), 150.40 (C-4), 141.42 (C-8), 121.73 (C-5), 118.40 (C-2), 68.18 (C-2′), 47.70 (C-1′), 17.09 (C-3′), 169.82 (*C*$OCH_3$), 20.85 ($COCH_3$). Anal. ($C_{10}H_{12}IN_5O_2$) C, H, N.

**9-(2-Acetoxypropyl)-6-chloro-2-fluoro-9*H*-purine (7a).** HF (70%) in pyridine (w/w), in a HF-resistant flask, was cooled at −50 °C by dry ice/acetone bath. The solution was then diluted to 60% (w/w, 10 mL) by careful addition of dry pyridine. The compound **2a** (400 mg, 1.49 mmol) was added to the flask and the temperature of the bath was allowed to rise to −30 °C. At that temperature, *tert*-butyl nitrite (0.26 mL, 2.23 mmol) was added to the stirred mixture and stirring was continued for 10 min with exclusion of moisture. The solution was rapidly poured into 100 g of crushed ice/$H_2O$. The aqueous mixture was extracted with 5 × 30 mL of $CH_2Cl_2$, and the combined organic phase was washed with 3 × 10 mL of $H_2O$ and 5% $NaHCO_3/H_2O$ to pH ∼ 7, dried over $Na_2SO_4$, and filtered off, and the solvent was evaporated in vacuo. The oily residue was purified by CLC (solvent system $S_1$). Fractions containing the product were combined and evaporated to give an oily material. The crude oily product was dissolved in 2-propanol and after cooling to 4 °C gave crystalline product **7a** (175 mg, 43%): mp = 84−86 °C; MS *m*/*z* 272 ($M^+$); UV (methanol) $\lambda_{max}$ 271 (log $\epsilon$ 3.53); $^{13}$C NMR ($CDCl_3$) $\delta$ 156.37 (d $J$ = 214.45, C-2), 154.46 (d $J$ = 17.45, C-6), 152.94 (d $J$ = 17.66, C-6), 148.87 (C-8), 130.09 (C-5), 68.19 (C-2′), 47.70 (C-1′), 17.10 (C-3′), 169.82 (*C*$OCH_3$), 20.85 ($COCH_3$). Anal. ($C_{10}H_{10}ClFN_4O_2$) C, H, N.

**9-[(2-Acetoxyethoxy)methyl]-6-chloro-2-fluoro-9*H*-purine (7b).** Cooled (−50 °C) 70% HF in pyridine (w/w, 7.8 mL) was diluted to 60% (w/w) by careful addition of dry pyridine (1.3 mL). The compound **2b** (720 mg, 2.53 mmol) and *tert*-butyl nitrite (0.427 mL, 3.79 mmol) were added to the mixture at −30 °C and stirring was continued for 10 min. The solution was rapidly poured into 100 g of crushed ice/$H_2O$. The aqueous mixture was extracted with 5 × 30 mL of $CH_2Cl_2$ and the combined organic phase was washed with 3 × 10 mL of $H_2O$ and 5% $NaHCO_3/H_2O$ to pH ∼ 7, dried over $Na_2SO_4$, filtered, and evaporated in vacuo. The residue was purified by CLC (solvent system $S_1$). The oil product, dissolved in 2-propanol, gave pure **7b** (284 mg, 39%): mp = 59−61 °C; MS *m*/*z* 288 ($M^+$); UV (methanol) $\lambda_{max}$ 267 (log $\epsilon$ 3.99); $^{13}$C NMR ($CDCl_3$) $\delta$ 157.62 (d $J$ = 221.45, C-2), 153.21 (d $J$ = 17.45, C-4), 153.87 (d $J$ = 17.13, C-6), 145.63 (C-8), 130.09 (d $J$ = 4.65, C-5), 68.26 (C-3′), 73.30 (C-1′), 62.65 (C-4′), 170.70 (*C*$OCH_3$), 20.74 ($COCH_3$). Anal. ($C_{10}H_{10}ClFN_4O_3$) C, H, N.

**6-Chloro-2-fluoro-9-[(2-hydroxyethoxy)methyl]-9*H*-purine (8b).** A solution of **7b** (200 mg, 0.69 mmol) in $CH_3CN$:$H_2O$ (1:1, 30 mL) was treated in one portion with solid lithium hydroxide monohydrate (43 mg, 1.03 mmol), which was completely dissolved for 15 min. The reaction was continued for a further 3 h. Glacial acetic acid (∼0.3 mL) was added and the solution was evaporated to dryness. The solid residue was purified by CLC with solvent system $S_3$. Crystallization of crude product from ethanol afforded the white crystals **8b** (51 mg, 30%): mp = 125−127 °C; MS *m*/*z* 246 ($M^+$); UV (methanol) $\lambda_{max}$ 282, 237 (log $\epsilon$ 3.99, 3.57); $^{13}$C NMR (DMSO) $\delta$ 156.51 (d $J$ = 214.28, C-2), 154.19 (d $J$ = 17.47, C-6), 150.59 (d $J$ = 18.16, C-4), 148.55 (d $J$ = 2.58, C-8), 130.08 (C-5), 71.33 (C-3′), 73.41 (C-1′), 59.88 (C-4′). Anal. ($C_8H_8ClFN_4O_2$) C, H, N.

**9-(2-Acetoxypropyl)-2-amino-1,9-dihydro-6*H*-purin-6-thione (9a).** To a suspension of **2a** (470 mg, 1.74 mmol) in absolute ethanol (15 mL) was added thiourea (530 mg, 6.94 mmol), followed by 3 drops of formic acid. The mixture was heated under reflux for 1 h and then allowed to cool at room temperature. The white precipitate was collected by filtration and washed with a small amount of absolute ethanol to yield **9a** (350 mg, 75%): mp = 255−258 °C; MS *m*/*z* 267 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 346 (log $\epsilon$ 4.34); $^{13}$C NMR (DMSO) $\delta$ 174.95 (C-6), 153.10 (C-2), 148.30 (C-4), 141.07 (C-8), 128.07 (C-5), 68.20 (C-2′), 46.62 (C-1′), 20.91 (C-3′), 169.80 (*C*$OCH_3$), 17.20 ($COCH_3$). Anal. ($C_{10}H_{13}N_5O_2S$) C, H, N.

**9-[(2-Acetoxyethoxy)methyl]-2-amino-1,9-dihydro-6*H*-purin-6-thione (9b).** In a similar manner as described for **9a**, treatment of **2b** (250 mg, 0.88 mmol) with thiourea (277 mg, 3.50 mmol) in absolute ethanol (10 mL) in the presence of formic acid (2 drops) yielded **9b** (180 mg, 72%): mp = 217−221 °C; MS *m*/*z* 283 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 346 (log $\epsilon$ 4.32); $^{13}$C NMR (DMSO) $\delta$ 170.36 (CO), 153.98 (C-2), 147.47 (C-4), 143.87 (C-5), 174.34 (C-6), 140.71 (C-8), 72.72 (C-1′), 67.01 (C-3′), 62.81 (C-4′), 20.69 ($CH_3$). Anal. ($C_{10}H_{13}N_5O_3S$) C, H, N.

**9-(2-Acetoxypropyl)-2-fluoro-9*H*-purin-6-thiol (10a).** A stirred solution of **9a** (150 mg, 0.56 mmol) in 60% HF/pyridine (5.0 mL) was treated at −30 °C with *tert*-butyl nitrite (0.1 mL, 0.84 mmol). Stirring was continued for 10 min and the reaction mixture was processed as described for **7a** to give **10a** (125 mg, 83%): mp = 159−160 °C; MS *m*/*z* 270 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 295, 294 (sh) (log $\epsilon$ 4.32, 4.32); $^{13}$C NMR ($CDCl_3$) $\delta$ 158.28 (d $J$ = 217.8 Hz, C-2), 152.05 (d $J$ = 16.19 Hz, C-4), 144.82 (C-8), 130.05 (C-5), 68.50 (C-2′), 47.85 (C-1′), 21.03 (C-3′), 169.93 (*C*$OCH_3$), 17.35 ($COCH_3$). Anal. ($C_{10}H_{11}FN_4O_2S$) C, H, N.

**9-[(2-Acetoxyetoxy)methyl]-2-fluoro-9*H*-purin-6-thiol (10b).** A stirred solution of **9b** (70 mg, 0.25 mmol) in 60% HF/pyridine (2.0 mL) was treated at −30 °C with TBN (0.04 mL, 0.84 mmol). Stirring was continued for 10 min and the reaction mixture was processed as described for **7a** to give **10b** (45 mg, 63%): mp = 151−157 °C; MS *m*/*z* 286 ($M^{+\bullet}$); UV (methanol) $\lambda_{max}$ 295, 294 (sh) (log $\epsilon$ 4.22, 4.22); $^{13}$C NMR (DMSO) $\delta$ 170.11 (*C*$OCH_3$), 157.43 (d $J$ = 213.01 Hz, C-2), 157.83 (d $J$ = 15.93 Hz, C-6), 152.16 (d $J$ = 17.11 Hz, C-4), 147.36 (C-8), 129.86 (d $J$ = 3.62 Hz, C-5), 72.98 (C-1′), 67.29 (C-3′), 62.68 (C-4′), 20.42 ($COCH_3$). Anal. ($C_{10}H_{11}FN_4O_3S$) C, H, N.

**2-Fluoro-9-(2-hydroxypropyl)-9*H*-purin-6-thiol (11a).** In a similar manner as described for **8b**, treatment of **10a** (400 mg, 1.48 mmol) with solid lithium hydroxide monohydrate (186 mg, 4.4 mmol) in $CH_3CN$:$H_2O$ (1:1, 30 mL) for 1 h yielded **11a** (210 mg, 59%): mp = 205−208 °C; MS *m*/*z* 228 ($M^+$); UV (methanol) $\lambda_{max}$ 296, 295 (sh) (log $\epsilon$ 4.06, 4.06); $^{13}$C NMR (DMSO) $\delta$ 157.30 (d $J$ = 212.0, C-2), 157.29 (d $J$ = 15.87, C-6), 152.32 (d $J$ = 16.99, C-4), 134.45 (C-8), 129.82 (C-5), 68.50 (C-2′), 47.85 (C-1′), 21.03 (C-3′). Anal. ($C_8H_9FN_4OS$) C, H, N.

**9-[(2-Hydroxyetoxy)methyl]-2-fluoro-9*H*-purin-6-thiol (11b).** In a similar manner as described for **11a**, **10b** (70 mg, 0.26 mmol) was treated with solid lithium hydroxide monohydrate (32.6 mg, 0.77 mmol) in $CH_3CN$:$H_2O$ (1:1, 5.5 mL) for 1 h. Glacial acetic acid (0.2 mL) was added and the solution was evaporated to dryness. The solid residue was purified by CLC with solvent system $S_3$. Recrystallization of crude product from ethanol afforded the white crystals **11b** (15 mg, 23.6%): MS *m*/*z* 244 ($M^+$); UV (methanol) $\lambda_{max}$ 296, 295 (sh) (log $\epsilon$ 4.15, 4.15). Anal. ($C_8H_9FN_4O_2S$) C, H, N.

**9-(2-Acetoxypropyl)xanthine (12a).** In a cooled flask (0 °C) was stirred a suspension of **10a** (230 mg, 0.93 mmol**)**, 30% $H_2O_2$ (0.8 mL), $NH_4OH$ (0.4 mL), and water (10 mL) at room temperature for 4 h. The reaction mixture was evaporated to dryness in vacuo and the yellow residue was purified by CLC (solvent system $S_3$). Fractions containing the product were combined and evaporated to give **12a** (166 mg, 71%): mp 301−304 °C; MS *m*/*z* 253 ($M^+$); UV (methanol) $\lambda_{max}$ 255 (log $\epsilon$ 3.80); $^{13}$C NMR (DMSO) $\delta$ 169.36 (*C*$OCH_3$), 157.81 (C-2), 150.72 (C-6), 140.56 (C-4), 132.27 (C-8), 115.22 (C-5), 68.06 (C-2′), 47.69 (C-1′), 20.58 ($COCH_3$), 16.70 (C-3′). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

**9-[(2-Acetoxyethoxy)methyl)]xanthine (12b) and 9-[(2-Hydroxyethoxy)methyl]xanthine (12c).** In a similar manner as described for **12a**, from the reaction mixture of **10b** (50

mg, 0.174 mmol) in $H_2O$ (0.9 mL), 30% $H_2O_2$ (0.8 mL), and $NH_4OH$ (0.4 mL) was isolated **12b** (38 mg, 81.5%) as an oily product and **12c** as a byproduct (10 mg, 25.4%).

**12b:** UV (methanol) $\lambda_{max}$ 256 (log $\epsilon$ 3.72); $^{13}$C NMR (DMSO) $\delta$ 170.18 ($COCH_3$), 157.95 (C-2), 150.98 (C-6), 140.69 (C-4), 137.14 (C-8), 115.69 (C-5), 72.97 (C-1′), 66.17 (C-3′), 62.66 (C-4′), 20.50 ($COCH_3$); MS *m*/*z* 269 ($M^+$). Anal. ($C_{10}H_{12}N_4O_5$) C, H, N.

**12c:** UV (methanol) $\lambda_{max}$ 258 (log $\epsilon$ 4.01); $^{13}$C NMR (DMSO) $\delta$ 158.36 (C-2), 152.76 (C-6), 136.63 (C-8), 115.26 (C-5), 72.74 (C-1′), 70.02 (C-3′), 59−87 (C-4′). Anal. ($C_8H_{10}N_4O_4$) C, H, N.

**2-Amino-6-fluoro-9-(2-hydroxypropyl)-9*H*-purine (13a).** Anhydrous trimethylamine (2.44 mL) was condensed at −78 °C and was added dropwise to a cooled suspension of **1a** (200 mg, 0.879 mmol) in a mixture of anhydrous THF (10 mL) and DMF (2.5 mL) at −78 °C under argon atmosphere. The resulting suspension was warmed to room temperature immediately after addition of trimethylamine and was stirred overnight. The reaction mixture was allowed settle, the supernatant was decanted off from the white precipitates, and the residual volatiles were removed completely in vacuo to afford the ammonium salt (100 mg). The resulting salt (50 mg, 0.174 mmol) was treated with anhydrous KF (101.09 mg, 1.74 mmol) in anhydrous DMF (10 mL) at 80 °C for 3 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to dryness in vacuo. The crude product was purified by column chromatography ($CH_2Cl_2$:MeOH = 4:1) to give **13a** (103 mg, 56%): UV (methanol) $\lambda_{max}$ 296, 295 (sh) (log $\epsilon$ 4.18, 4.17); $^{13}$C NMR (DMSO) $\delta$ 196.78 (C-6), 155.29 (C-2), 150.64 (C-4), 133.14 (C-8), 109.39 (C-5), 60.48 (C-2′), 45.67 (C-1′), 16.86 (C-3′). Anal. ($C_8H_{10}FN_5O$) C, H, N.

**X-ray Determination.** Diffraction-quality crystals were grown by slow evaporation at room temperature of a very dilute solution of ethanol. The intensities were collected at 293(2) K in the $\omega$ scan mode on a Philips PW1100 diffractometer updated by Stoe[42] using Mo K$\alpha$ radiation ($\lambda$ = 0.710 73 Å), and corrected only for Lorentz polarization factor. During the data collection, crystal decomposition of 18% was observed. The crystal structure was solved by direct methods. All nonhydrogen atoms were refined anisotropically by full-matrix least squares on $F^2$. Hydrogen atoms were treated using appropriate riding models, except for the H-atoms bonded to the amino group attached at C-6 position of the purine ring, which were located by subsequent isotropic refinement and difference electron-density synthesis. All calculations were performed on an IBM PC/AT compatible microcomputer using SHELXS97,[43] SHELXL97,[44] and ORTEPIII[45] programs. Additional crystallographic data (excluding structure factors) for the structure **5b** reported in this paper have been deposited at the Cambridge Crystallographic Data Centre as supplementary publications No. CCDC−206447.

Crystal data for **5b**: $C_8H_{10}IN_5O_2$, $M_r$ = 335.104, triclinic, space group *P*-1 (No. 2), $a$ = 7.928(1) Å, $b$ = 8.069(2) Å, $c$ = 10.027(2) Å, $\alpha$ = 88.76(2)°, $\beta$ = 73.58(1)°, $\gamma$ = 65.08(1)°, $V$ = 554.5(2) Å$^3$, $Z$ = 2, $F(000)$ = 324, $D_c$ = 2.007 gcm$^{-3}$, $\mu$(Mo K$\alpha$) = 2.882 mm$^{-1}$, $S$ = 1.019, $R/R_w$ = 0.0646/0.1579 for 153 parameters and 2407 reflections, and $R/R_w$ = 0.0663/0.1608 for all 2557 independent reflections measured in the range 3.08−$\Theta$−27.97°, $(\Delta\rho)_{max}$ = 4.021 e Å$^{-3}$ (0.80 Å from *I*), $(\Delta\rho)_{min}$ = −3.311 e Å$^{-3}$ (0.83 Å from *I*), $(\Delta/\sigma)_{max}$ = 0.001, extinction coefficient = 0.11(1) (SHELXL97[44]).

**Materials for Biological Tests. Cell Culturing.** The following human tumor cell lines were obtained from ATCC: HBL (melanoma), HeLa (cervical carcinoma) HT29 and SW620 (colon carcinoma), Hep2 (laryngeal carcinoma), and MiaPaCa2 (pancreatic carcinoma) as well as WI38 (diploid fibroblasts). The cells were cultured in medium (D-MEM or RPMI 1640) supplemented with 10% FBS, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified atmosphere with 5% $CO_2$ at 37 °C. All cells were grown as a monolayer culture. The cells were plated in 96-microwell plates at a concentration of 3 × 10$^4$/mL (HeLa, HT29, Hep2, MiaPaCa2) or at 5 × 10$^4$/mL (SW620, WI38). Twenty-four hours later, the test compounds at different concentrations were added and the cells were treated for an additional 72 h. Control cells were grown under the same conditions, but in the absence of test compounds. The number of cells was determined by the MTT test.[46] This method is based on reduction of MTT (yellow) with a mitochondrial dehydrogenase that is active only in live cells to yield a DMSO-soluble formazan product (red). Absorbance was measured at 570 nm. The number of living cells is linearly proportional to the amount of reduced MTT. The results were expressed as a percentage of growth. A compound concentration required to 50% inhibition of cell proliferation ($IC_{50}$) was calculated by a manual graphical fitting method. Each number represents the mean value ± standard deviation from four parallel samples in three individual experiments, and significance was adjudicated by Mann−Whitney and Student's *t*-test. The significant difference was accepted at $p \leq 0.05$. The compounds were dissolved in DMSO at a concentration of $10^{-1}$ M and diluted with medium to concentrations between $10^{-6}$ and $10^{-4}$ M. The concentration of DMSO was less than 0.1%, and at that concentration it did not effect the cell growth.

Antitumor activity against L1210 (murine leukemia), FM3A (murine mammary carcinoma), and Molt4/C8 and CEM (human T-lymphocytes) cell lines were measured essentially as originally described for the mouse leukemia (L1210) cell line.[47]

**Antiviral Activity Assays.** Antiviral activity against HIV-1, HIV-2, HSV-1, HSV-2, vaccinia, vesicular stomatitis, parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, or respiratory syncytial virus was determined as described previously.[48,49]

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