Notes

Synthesis of Hypoxanthine, Guanine, and 6-Thiopurine Nucleosides of 6-Deoxy-D-allofuranose

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Hypoxanthine, guanine, and 6-thiopurine nucleosides of 6-deoxy-D-allofuranose have been prepared as potential antitumor agents. Thus, reaction of 6-deoxy-β-D-allofuranosyl bromide (1) with the trimethylsilyl derivatives of hypoxanthine and guanine afforded mixtures of the 9- and the 7-substituted bases, which were separated and deblocked with ammonia to give 9-(6′-deoxy-β-D-allofuranosyl)hypoxanthine (6), 7-(6′-deoxy-β-D-allofuranosyl)hypoxanthine (7), 9-(6′-deoxy-β-D-allofuranosyl)guanine (8), and 7-(6′-deoxy-β-D-allofuranosyl)guanine (9). The two nucleosides with the purine joined at the N-9 position, namely, 6 and 8, are easily distinguished from the other two nucleosides (7 and 9), having N-7 junctions, by their NMR spectra. Reaction of 1 with the trimethylsilyl derivative of 6-chloropurine afforded 10, which upon treatment with thiourea and deblocking gave 9-(6′-deoxy-β-D-allofuranosyl)-6-thiopurine (12). The hypoxanthine and guanine nucleosides showed no inhibition of mouse leukemia L1210 when tested in vivo, but the thiopurine nucleoside 12 showed strong inhibition of growth of L1210 both in vivo and in vitro. Compound 7 strongly inhibited purine nucleoside phosphorylase ($K_I = 8.8 \times 10^{-5}$ M), while compounds 8, 9, 6, and 12 were inactive.

Much effort has gone into the synthesis of nucleoside analogues modified in the sugar moiety because of the promise that these compounds could inhibit purine biosynthesis and act as anticancer agents. Thus, 9-(6′-deoxy-β-D-allofuranosyl)adenine, in which one of the hydrogen atoms on the 5′-position of adenosine[1] is replaced by a methyl group, was shown to inhibit the enzyme adenine phosphoribosyltransferase.[2]

In view of the substrate activity of other sugar-modified analogues of inosine and guanosine toward purine nucleoside phosphorylase (PNPase),[3] we have synthesized hypoxanthine, 6-thiopurine, and guanine nucleosides of 6-deoxy-D-allofuranose as potential inhibitors of this enzyme. PNPase converts the cytotoxic agent 6-thiopurine in some drug-resistant tumor strains into the nucleoside, nullifying its cytotoxic activity.[4] Accordingly, inhibitors of this enzyme may prove useful in combination with 6-thiopurine in cancer therapy. One inhibitor of this enzyme, namely, 8-aminoguanosine, is already known to be toxic to tumor cells.[5]

The starting point of our syntheses was 6-deoxy-2,3,5-tris-*O*-(*p*-nitrobenzoyl)-D-allofuranosyl bromide (1),[6] which was coupled with bis(trimethylsilyl)hypoxanthine and with *O*,9-bis(trimethylsilyl)-N^2-acetylguanine in the presence of tin tetrachloride to yield, in the first case, a mixture of blocked hypoxanthine nucleoside isomers 2 and 3, bearing links to positions 9 and 7 of the base, respectively, and in the second case a mixture of blocked guanine nucleosides 4 and 5 also linked at positions 9 and 7, respectively (Scheme I). A combination of column chromatography and fractional crystallization resulted in the isolation of each isomer in the pure form.

These compounds were each deblocked with methanolic ammonia to yield 9- and 7-(6′-deoxy-β-D-allofuranosyl)-hypoxanthine (6 and 7) and 9- and 7-(6′-deoxy-β-D-allofuranosyl)guanine (8 and 9). The structural assignments were based on ultraviolet and NMR spectral data and on circular dichroism measurements, as described in the next section, and by synthesizing compound 6 by an independent route to verify its structure. Thus, bromide 1 was coupled with 9-(trimethylsilyl)-6-chloropurine in the presence of mercuric bromide and mercuric cyanide to yield blocked nucleoside 10 (Scheme II). 6-Chloropurine is known to couple to glycosyl halides only at the N-9 position. Deblocking with methanolic ammonia caused concomitant replacement of the chlorine atom by an amino group to yield the known 6-deoxy-β-D-allofuranosyladenine (11).[1] The NMR spectrum of this material was similar to that of adenosine, verifying the β configuration. This material was deaminated with barium nitrite and acetic acid to yield a single isomer of a hypoxanthine nucleoside identical with 6.

Compound 10 was also reacted with thiourea and deblocked to yield 6′-deoxy-β-D-allofuranosyl-6-thiopurine (12) (Scheme II).

Structural Assignments. The ultraviolet spectrum of hypoxanthine nucleoside 6 at three pH values exhibited a λ_{max} identical with that of inosine (see Table I), and guanine nucleoside 8 exhibited a λ_{max} close to that of 9-methylguanine.[7] Hence, the purine moieties of these nucleosides are joined to the sugars at the N-9 position. Hypoxanthine nucleoside 7 exhibited a λ_{max} similar to that of 7-ribofuranosylhypoxanthine,[8] different from that of the N-1 or N-9 substituted isomer, and guanine nucleoside 9 had a λ_{max} close to that of 7-methylguanine.[7] Thus, these

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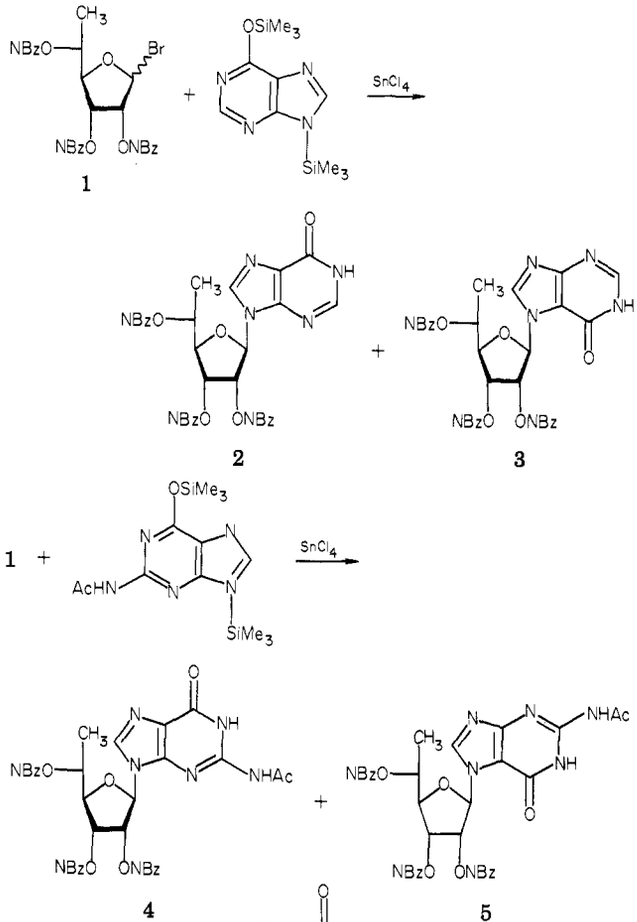
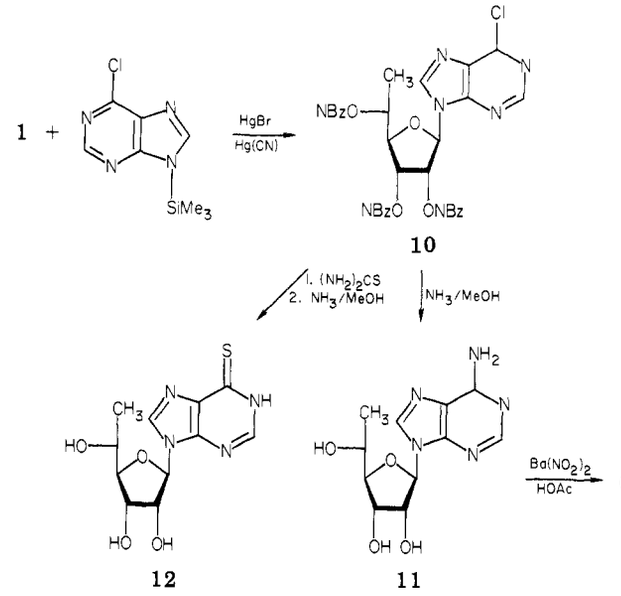
Scheme I[a]



Scheme II



compounds have their purine moieties joined at the N-7 position. A compound reported in the literature[9] to be 7-β-D-ribofuranosylhypoxanthine and later shown to be the N-1 isomer[8] had a λ_{max} quite different from our compound **7**.

In order to determine the anomeric configuration of nucleosides, NMR coupling constant of the H-1′ proton is commonly used. However, all four of our compounds show a doublet for this proton with J = 6–7 Hz, consistent with either an α or β configuration.[10] A rule by Baker[11] states that sugar derivatives with an ester group at C-2′ will give rise to only 1,2-trans nucleosides. Although exceptions are known,[12] this rule seems to have held true in our case. We have assigned the β configuration to all four compounds for the following reasons: 9-α-D-Ribofuranosylguanine[13] exhibits a coupling constant of 4.5 Hz for the anomeric proton, quite different from the values for guanine nucleosides **8** and **9**. Moreover, in **8** the signals due to the purine protons at C-2 and C-8 and the anomeric proton H-1′ are superimposable with those of guanosine. Thus, both **8** and **9** have the β configuration. The NMR of compound **6** shows a similar resemblance to that of inosine and, therefore, also has the β configuration. Since it would be difficult to explain all but one of these compounds having the same configuration, **7** must also have the β configuration.

The Cotton effects of **6** and **8** are negative, consistent with a β configuration. In **7** and **9**, however, the Cotton effects are positive. This is, however, not surprising, since interaction between the sugar moiety and the downward-pointing carbonyl group of the purine could prevent the molecule from assuming the anti configuration necessary for a negative Cotton effect.[14]

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(10) L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W. W. Zorbach and R. S. Tipson, Eds., 1973, Wiley-Interscience, New York, pp 330–331.
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(14) T. L. V. Ulbricht in ref 10, p 200.

[a] NBz = $O_2NC_6H_4CO$.

Table I. UV Spectral and Optical Rotatory Data of Nucleosides

compd	λ_{max} (H_2O), nm at pH 1-2	at pH 5-7	at pH 11-13	$[\alpha]^{25}_D$ (H_2O), deg	Cotton effect
inosine	249	248	253		
6	248	248	252	−45	negative
7-ribosylhypoxanthine[a]	252	256	263		
1-ribosylhypoxanthine[a]	249	251	261		
7	252	256	262	−29	positive
9-methylguanine[b]		252, 270[c]			
8		253, 270[c]			negative
7-methylguanine[b]		248, 283[c]			
9		245, 287[c]			positive

[a] Reference 8. [b] Reference 7. [c] Inflection.

A noteworthy feature of the NMR spectra of these compounds is the signal due to the H-5′ proton. In Me_2SO as solvent this is obscured by an OH absorption, but upon addition of D_2O it appears as a quartet at 3.48 and 3.51 ppm in the N-9 isomers **6** and **8**, respectively. In the N-7 isomers **7** and **9**, this signal remains masked by the multiplet due to H-3′ or H-4′ centered at 3.8 ppm. Thus, in the N-9 isomers, the H-5′ signal is 0.3–0.4 ppm further upfield than it is in the N-7 isomers. In the N-9 compound **11** this quartet also appears (3.40 ppm). In ribose nucleosides this fact is not very useful, but in these 6-deoxyallofuranose nucleosides the H-5′ signal is a readily discernible quartet (J = 6 Hz) in the N-9 isomers. Thus, at least for guanine and hypoxanthine nucleosides of this sugar, the H-5′ NMR signal is a convenient way to distinguish N-9 from N-7 isomers. It would be profitable to examine other nucleosides of this sugar in order to test the scope of this rule. Recently published syntheses of nucleosides of 6-deoxyallofuranose[15] indicate an interest in such nucleosides.

Biological Results

When tested in vivo against L1210 compounds, **6–9** were found to be inactive, showing maximum T/C values of 109, 114, 112, and 101, respectively. Compound **12** showed significantly higher inhibitory action against L1210. In vivo it exhibited a T/C value of 148, and in vitro it caused a 50% inhibition of growth at 0.5 μg/mL.

The activity of **12** could conceivably be due to hydrolysis to 6-thiopurine. For this possibility to be tested, L1210 cells were treated with **12** for various times, and the broth was extracted with ethanol. Analysis of the extracts showed a gradual disappearance of **12** with time (80% in 90 min), indicating that the compound was being metabolized. However, no 6-thiopurine was detected. If the activity of compound **12** is not due to the liberation of the base, the corresponding 6-deoxy-L-talofuranosyl nucleoside, which is being synthesized, should exhibit a different activity than **12**.

All the compounds were screened as inhibitors of PNPase, and kinetic constants were determined for the analogues showing good inhibition. The PNPase-catalyzed conversion of inosine to hypoxanthine was significantly inhibited by compound **7** ($K_I = 8.8 \times 10^{-5}$ M), and from the analysis of replots, inhibition appears to be of the uncompetitive type. Compounds **6**, **8**, **9**, and **12** were not inhibitors of this reaction.

It is interesting to note that compound **6** with the "natural" N-9 linkage to the purine is inactive with PNPase, whereas the "unnatural" N-7 isomer **7** is active. Also surprising is the fact that **12**, which was active against L1210 tumor cells, is inactive against PNPase, suggesting that this activity is not related to PNPase inhibition.

Table II. Antitumor Screening Results[a]

compd	dose, mg/kg	MST, days (% T/C)	av. wt. change	survivors, day 5
12	200	148	−4.4	6/6
	100	146	−3.6	6/6
	50	142	−1.7	6/6
	25	116	−1.4	6/6
	12.5	107	−1.0	6/6

[a] Tests were done on L1210 by the NCI according to the protocol described in Instruction 14, Screening Data Summary Interpretation and Outline of Current Screen, Drug Evaluation Branch, Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Tumor Inoculum: 10^5 ascites cells implanted into BDF_1 mice. Each mouse was inoculated once with the indicated dose and observed for 30 days. Evaluation: MST = median survival time in days; % T/C = MST treated/MST control × 100. Criteria: % T/C = 125 considered moderate; % T/C = 150 considered significant antitumor effect.

Experimental Section

Ultraviolet spectra were taken on a Perkin-Elmer Lambda 3 spectrophotometer. Infrared spectra were taken on a Perkin-Elmer 735B spectrophotometer. Nuclear magnetic resonance spectra were taken on a Varian EM360A 60-MHz spectrometer with tetramethylsilane as standard. Optical rotations were measured with a Bendix Series 1100 automatic polarimeter. Circular dichroism measurements were performed by the Michigan Macromolecular Institute, Midland, MI.

The newly synthesized purine nucleoside analogues were tested for inhibitory activity on calf spleen purine nucleoside phosphoylase (EC 2.4.2.1) from Sigma Chemical Co. by using a coupled assay following the method of Hoffee et al.[17] Kinetic constants were determined for purine nucleoside analogues that showed good inhibition by using statistical methods developed by Cleland.[18] The type of inhibition was assessed by examining replots of K_m vs. I, slope vs. I, and v_{max} vs. I. In vitro screening was performed by Dr. David Kessel, Department of Oncology, Wayne State University, Detroit, MI. Analysis of metabolites of compound **12** treated with tumor cells was performed by Dr. A. C. Sartorelli, Yale University School of Medicine, New Haven, CT. In vivo tests were performed at the National Cancer Institute, Silver Springs, MD. Microanalyses were performed by the Spang Microanalytical Laboratory, Eagle Harbor, MI.

9- and 7-[6′-Deoxy-2′,3′,5′-tris-*O*-(*p*-nitrobenzoyl)-β-D-allofuranosyl]hypoxanthine (2 and 3). 6-Deoxy-2,3,5-tris-*O*-(*p*-nitrobenzoyl)-D-allofuranosyl bromide (1; 0.6 g, 0.0009 mol)

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(18) W. W. Cleland, *Methods Enzymol.*, 63A, 103–138 (1979).

was stirred in dry dichloroethane (20 mL) with *O*,9-bis(trimethylsilyl)hypoxanthine (0.3 g; 0.001 mol) as a solution of tin tetrachloride in dichloroethane (0.0013 mol) was added. The mixture was stirred at room temperature overnight and then refluxed for 2 h. A solution of 0.6 mL of triethylamine in ethanol (10 mL) was added, and the mixture was evaporated to dryness. The residue was dissolved in chloroform, washed with water, dried over magnesium sulfate, filtered through Celite, and evaporated to yield a glassy foam. The mixture was then chromatographed on silica gel. Ethyl acetate eluted an amorphous tar, which was discarded. Further elution with ethyl acetate yielded the two nucleoside isomers without separation, which were rechromatographed. Ethyl acetate–ether (1:1) yielded a fraction enriched in the faster-moving isomer and upon recrystallization yielded **3** (0.24 g, 37%), mp 269–272 °C dec. Anal. ($C_{32}H_{23}N_7O_{14}$) C, H, N.

The crude product from a second synthesis was chromatographed on alumina. Elution with ethyl acetate–ethanol–acetic acid (50:50:4) yielded a fraction enriched in the slower-moving isomer, which was chromatographed on silica gel with ethyl acetate to yield pure **2** (36%), mp 162–167 °C. Anal. ($C_{32}H_{23}N_7O_{14}$) C, H, N.

***O*,9-Bis(trimethylsilyl)-*N*^{2}-acetylguanine.** *N*^{2}-Acetylguanine[16] was refluxed in hexamethyldisilazane overnight. The volatile material was removed under vacuum, and the residue was distilled [150–160 °C (0.05 mmHg)] to yield a thick tar, which was used without further purification.

9- and 7-[6′-Deoxy-2′,3′,5′-tris-*O*-(*p*-nitrobenzoyl)-β-D-allofuranosyl]-*N*^{2}-acetylguanine (4 and 5). 6-Deoxy-D-allofuranosyl bromide (1) was coupled with *O*,9-bis(trimethylsilyl)-*N*^{2}-acetylguanine by the usual procedure. The crude product was chromatographed on silica gel to remove nonpolar material. The fraction eluting with acetone–chloroform (1:1) was recrystallized from acetone to yield **5** in 22% yield: mp 276–277 °C. Anal. ($C_{34}H_{26}N_8O_{15}$) C, H, N.

The mother liquor from the above crystallization was evaporated and dissolved in ethyl acetate. Addition of ether precipitated **4**: mp 160–170 °C. Anal. ($C_{34}H_{26}N_8O_{15}$) C, H, N.

9-(Trimethylsilyl)-6-chloropurine. 6-Chloropurine (2 g, 0.013 mol) was stirred for 2 days in dry benzene (50 mL) with trimethylsilyl chloride (1.6 g, 0.014 mol) and triethylamine (1.5 g, 0.014 mol). The mixture was filtered, and the filtrate was evaporated to yield the product as a solid.

9-[6′-Deoxy-2′,3′,5′-tris-*O*-(*p*-nitrobenzoyl)-β-D-allofuranosyl]-6-chloropurine (10). 6-Deoxy-2,3,5-tris-*O*-(*p*-nitrobenzoyl)-D-allofuranosyl bromide (1; 7.5 g, 0.011 mol) and 9-(trimethylsilyl)-6-chloropurine (2.8 g, 0.012 mol) were stirred in dry 1,2-dichlorethane (100 mL) with molecular sieves. Mercuric cyanide (0.28 g, 0.001 mol) and mercuric bromide (4.41 g, 0.012 mol) were added, and the mixture was stirred overnight at room temperature. The mixture was diluted with chloroform, washed with aqueous 10% potassium iodide solution, dried, and filtered. Evaporation yielded a yellow foam, which was chromatographed on silica gel. Elution with ethyl acetate–benzene (1:1) and evaporation of solvent, followed by recrystallization from acetone–ethanol, yielded 7 g of product **10**: 85% yield; mp 120–125 °C. Anal. ($C_{32}H_{22}N_7O_{13}Cl$) C, H, N, Cl.

9-(6′-Deoxy-β-D-allofuranosyl)adenine (11). Blocked chlorpurine nucleoside **10** (2 g, 0.0026 mol) was treated with ammonia-saturated methanol for 5 days. The solution was evaporated to dryness, dissolved in distilled water, repeatedly washed with chloroform, and then evaporated to dryness. The solid was dissolved in ethanol, precipitated with ether, redissolved in ethanol, and again precipitated to yield 0.98 g (~100%) of compound **11**.

7-(6′-Deoxy-β-D-allofuranosyl)hypoxanthine (7). Blocked nucleoside **3** was deblocked with methanolic ammonia as described above to yield **7** as an amorphous white powder: yield 44%; NMR (Me_2SO-d_6/D_2O) δ 1.20 (d, *J* = 6 Hz, H′-6), 3.80 (m, H′-5), 4.00 (m, H′-4), 4.38 (m, H′-3), 4.55 (m, H′-2), 6.18 (d, *J* = 6 Hz, H′-1), 8.12 (s, H-8), 8.68 (s, H-2). Anal. ($C_{11}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H.

9-(6-Deoxy-β-D-allofuranosyl)hypoxanthine (6). A. From 2. Compound **2** was deblocked as above to yield **6** as an amorphous powder: yield 67%; NMR (Me_2SO-d_6/D_2O) δ 1.15 (d, *J* = 6 Hz, H′-6), 3.48 (q, *J* = 6 Hz, H′-5), 3.82 (m, H′-4), 4.30 (m, H′-3), 4.60 (m, H′-2), 5.94 (d, *J* = 6 Hz, H′-1), 8.20 (s, H-8), 8.43 (s, H-2); IR (KBr) ν_{max} 3600–2600 (OH, NH), 1690 (C=O), 1600, 1410, 1340, 1220, 1080 cm^{-1}. Anal. ($C_{11}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H, N.

B. From 11. Compound **11** (0.5 g, 0.0018 mol) was allowed to react with barium nitrite (1.03 g, 2.3 × 0.0018 mol) in a mixture of water (20 mL) and acetic acid (0.7 mL) for 1 week. An equivalent amount of dilute sulfuric acid was added, then the pH was adjusted to ~7 with barium hydroxide. Carbon dioxide was bubbled through, and the solid was removed by filtration. The filtrate was evaporated to dryness, suspended in ethanol, and filtered, and ether was added to precipitate the product. Filtration yielded 0.25 g of solid, which was shown to be identical with **6** by NMR, $[\alpha]_D$, and UV.

9-[6′-Deoxy-2′,3′,5′-tris-*O*-(*p*-nitrobenzoyl)-β-D-allofuranosyl]-6-thiopurine. Blocked chloropurine nucleoside **10** (1.5 g, 0.001 mol) was refluxed with excess thiourea (0.8 g) in 200 mL of ethanol for 4 h. The mixture was cooled, and the yellow solid was filtered off and recrystallized from ethyl acetate–acetone to give 1.5 g of yellow crystals (~100%), mp 165–172 °C.

9-(6′-Deoxy-β-D-allofuranosyl)-6-thiopurine (12). The above blocked nucleoside (0.8 g) was deblocked in methanolic ammonia in the usual manner. Recrystallization from ethanol yielded the product as a white powder: mp 230–234 °C dec; NMR (Me_2SO-d_6) δ 1.10 (d, *J* = 6 Hz, H-6′), 3.80 (m, H-4′, H-5′), 4.28 (m, H-3′), 4.58 (m, H-2′), 5.93 (d, *J* = 6 Hz, H-1′), 6.00–4.50 (m, OH), 8.35 (s, H-2), 8.64 (s, H-8); IR (KBr) ν_{max} 3600–2800 (OH), 1590, 1540, 1415, 1335, 1195, 1080, 970 cm^{-1}. Anal. ($C_{32}H_{23}N_7O_{13}S$) C, H, N, S.

7-(6′-Deoxy-β-D-allofuranosyl)guanine (9). Compound **5** was deblocked in the usual manner to give **9** as an amorphous powder: yield 98%; decomposes without melting at 240 °C; NMR (Me_2SO-d_6/D_2O) δ 1.44 (d, *J* = 6 Hz, H′-6), 3.9 (m, H′-5), 4.12 (m, H′-4), 4.32 (m, H′-3), 4.45 (m, H′-2), 5.93 (d, *J* = 6 Hz, H′-1), 8.37 (s, H-8); IR (KBr) ν_{max} 3600–2400 (NH, OH), 1660 (C=O), 1465, 1380, 1220, 1075 cm^{-1}. Anal. ($C_{11}H_{15}N_5O_5$) C, H.

9-(6′-Deoxy-β-D-allofuranosyl)guanine (8). Compound **4** was deblocked to give **8** as an amorphous powder: yield 96%; decomposes without meeting at 238 °C; NMR (Me_2SO-d_6/D_2O) δ 1.13 (d, *J* = 6 Hz, H′-6), 3.51 (q, *J* = 6 Hz, H′-5), 3.87 (m, H′-4), 4.25 (m, H′-3), 4.60 (m, H′-2), 5.75 (d, *J* = 7 Hz, H′-1), 8.06 (s, H-8); IR (KBr) ν_{max} 3600–2800 (OH, NH), 1690, 1640 (C=O), 1520, 1370, 1080 cm^{-1}. Anal. ($C_{11}H_{15}N_5O_5 \cdot 0.5H_2O$) C, H.

In vitro testing was performed by incubation of L1210 cells with the compound for 24 h, followed by a 1:1 dilution with fresh medium and further incubation for 24 h. Total cell number was recorded as percent of control (untreated) growth.

Analysis of metabolites of compound **12** was performed by treating L1210 cells (1 × 10^6/mL) with the substrate (0.5 μg/mL) for various times up to 90 min. Ethanol was added, and after centrifugation, the supernatent liquid was evaporated. The residue was analyzed by HPLC by using a C8 reverse-phase column. Elution was with aqueous acentonitrile.

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Registry No. 1, 85505-01-7; **2**, 85421-81-4; **3**, 85421-82-5; **4**, 85421-83-6; **5**, 85421-84-7; **6**, 85421-86-9; **7**, 85421-85-8; **8**, 85421-89-2; **9**, 85421-88-1; **10**, 85421-90-5; 11, 3253-81-4; **12**, 2946-43-2; purine nucleoside phosphorylase, 9030-21-1; *O*,9-bis(trimethylsilyl)hypoxanthine, 17962-89-9; *O*,9-bis(trimethylsilyl)-*N*^{2}-acetylguanine, 54187-52-9; *N*^{2}-acetylguanine, 19962-37-9; 9-(trimethylsilyl)-6-chloropurine, 32865-86-4; 6-chloropurine, 87-42-3; 9-[6′-deoxy-2′,3′,5′-tris-*O*-(*p*-nitrobenzoyl)-β-D-allofuranosyl]-6-thiopurine, 85421-87-0.