(45) T. A. Khwaja, A. M. Mian, and L. Kigwana, *Int. Cancer Congr., Abstr., 11th, 1974*, p 433 and 434.
(46) In animal model studies we have shown that 5-fluoroanhydro-*ara*-C (**5**) caused an 80% increase in the life span of DBA/2 mice bearing intraperioneal transplants (1×10^5 cells) of leukemia L1210 (200 mg/kg, qd 1–5). During the progress of this work, Fox et al.[27] independently described the antileukemic activity of compound **5**.
(47) Further details of the antileukemic activity of these compounds will be published elsewhere.

Improved Antitumor Effects in 3′-Branched Homologues of 2′-Deoxythioguanosine. Synthesis and Evaluation of Thioguanine Nucleosides of 2,3-Dideoxy-3-(hydroxymethyl)-D-*erythro*-pentofuranose

Edward M. Acton,* Richard N. Goerner, Hong S. Uh, Kenneth J. Ryan, David W. Henry,[1]

Life Sciences Division, SRI International, Menlo Park, California 94025

Carol E. Cass, and Gerald A. LePage

The University of Alberta Cancer Research Unit, McEachern Laboratory, Edmonton, Alberta, Canada.
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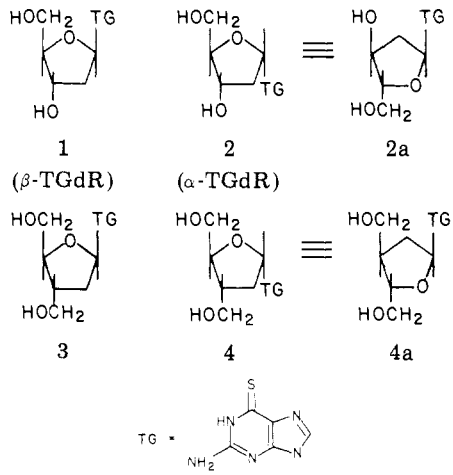
The 3-(hydroxymethyl) branched homologue of 2-deoxyribofuranose was synthesized from the corresponding branched ribofuranose 2-*O*-(*S*-methyl dithiocarbonate) with tributyltin hydride in the first direct, one-step deoxygenation at C-2 of a ribofuranose. Nucleoside coupling afforded the corresponding 3′-branched 2′-deoxyribonucleosides of thioguanine. The α- and β-nucleosides were equally inhibitory to growth of WI-L2 human lymphoblastoid cells, were phosphorylated and incorporated into the DNA of Mecca lymphosarcoma in mice to the same degree, and were more effective in these tests than the parent analogue α-2′-deoxythioguanosine. These results indicate that the hydroxy functions at the 3′ and 5′ positions of 2′-deoxynucleosides are interchangeable on the tumor enzymes, that the furanose ring oxygen and 2′-methylene are correspondingly interchangeable, and that acceptance by the enzymes is improved if primary hydroxyls are provided at both the 3′ and 5′ positions.

A number of thioguanine nucleosides have been synthesized[2-6] and screened for antitumor properties. Almost all of them are active.[6-9] The most important of them are the anomeric pair β- (β-TGdR, 1) and α-2′-deoxythioguanosine (α-TGdR, **2**),[4,10,11] which are currently undergoing clinical trial.[12] The β anomer is a potent and useful agent, but like most of the thioguanine (TG) nucleosides it suffers rapid enzymatic cleavage to TG, which frustates the potential advantage of the nucleoside over TG.[10] In studies on the biological properties of nucleosides in general, it has been found that the β anomeric configuration is almost invariably required for activity. α-2′-Deoxythioguanosine (**2**) is unique as an α anomer with sufficient activity against experimental tumors to be selected for clinical trial. It is also unique among nucleosides of TG in its resistance to cleavage, as indicated by the absence of thioxanthine and thiouric acid in urine of patients receiving α-TGdR and by the fact that much of the α-TGdR is excreted unchanged.[13] As a result, α-TGdR is especially notable for its low toxicity. It is also less potent that the β anomer, but the low toxicity means that patients can be treated with high doses of α-TGdR without side effects.



The low toxicity may be also, in part, the result of an unusual selectivity of α-TGdR for tumor tissue that has been observed.[10,11] Both α- and β-TGdR are phosphorylated and incorporated into DNA of mouse and human tumor tissues; the extent of incorporation varies with the tissue or enzyme source and appears to be proportional to the resultant carcinostatic effects. Extracts of various normal tissues were tested as well, and β-TGdR was found to be phosphorylated by most of them. However, α-TGdR was not phosphorylated to a significant level in bone marrow cells, an evident explanation for low host toxicity.

Further studies showed that in tumors α-TGdR is incorporated into DNA at the terminal nucleoside position of short fragments, terminating chain growth, while β-TGdR is incorporated into the internal nucleoside positions of DNA.[14] Nevertheless, it seems clear that the α-nucleoside is accepted by many of the enzymes that are responsible for processing β-TGdR and presumably the natural substrate, of β configuration, 2′-deoxyguanosine. It is highly unlikely that there is a separate set of kinases for processing the α anomer, and this suggests that the common assumption of gross structural dissimilarity of anomers, illustrated by structures 1 and **2**, be reexamined in the case of α- and β-TGdR. For either anomer, the purine moiety must occupy the same site on the enzyme. This suggested that 1 be compared with structure **2a** for the α anomer, and it required that the 5′-CH_2OH of 1 be replaced on the enzyme by the 3′-OH of **2a** and vice versa. This also required that the furanose oxygens and 2′-deoxy carbons exchange when one anomer replaces the other on the enzyme.[15] It appeared from this comparison that the α anomer as structure **2a** can simulate the β-anomer 1 reasonably well in terms of shape and placement of key

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functional groups.[16] Furthermore, phosphorylation of the α anomer must then take place on the 3′-OH of **2a** rather than on the 5′-OH of 1 in the normal β configuration.

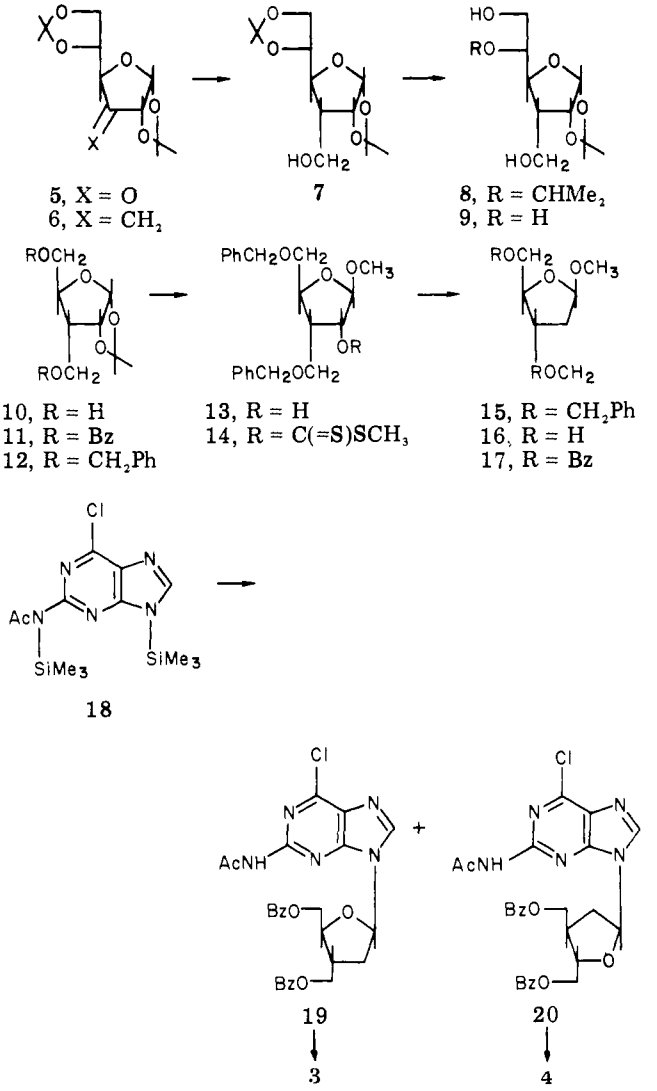
The comparison of structures **2a** and 1 suggests that the α anomer would better simulate the natural β configuration if the 3′-OH of **2a** was replaced by a 3′-CH_2OH. Such a structure, **4a**, would be the 3′-branched analogue **4** of α-TGdR. If this is a valid explanation for the activity of α-2′-deoxynucleosides, compound **4** should be a better enzyme inhibitor and thus an improved analogue of α-TGdR, possibly of clinical utility. We now report the synthesis and evaluation of **4** and its β-anomer **3**.

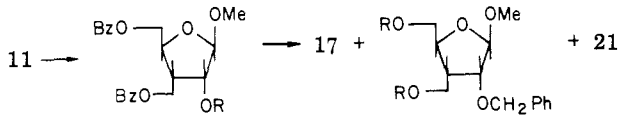
Chemistry. The synthesis of **4** required a 3-branched 2-deoxyribofuranoside to be coupled with the purine base. Despite considerable previous work on the synthesis of 3-branched ribofuranose derivatives, the only reported synthesis of a 2-deoxy analogue was by a method that gave a mixture of epimers at the C-3 branch point.[17] Furthermore, despite the widespread interest in the chemistry of 2-deoxyribose, no generally applicable chemical method has been available for the direct conversion of ribofuranose derivatives to the corresponding 2-deoxyribofuranose derivatives. For instance, the recently described[18] treatment of ribofuranose 2,3-thionocarbonates with tributyltin hydride gave mixtures of 2- and 3-deoxyribosides. On the other hand, the related tributyltin hydride treatment of thionobenzoates or *S*-methyl dithiocarbonates[19] to effect deoxygenation seemed especially applicable to this problem. The method was used in deoxygenations at C-3 of a furanose ring and at C-2 of pyranose rings. These seemed favorable precedents for deoxygenation at C-2 of a furanose ring, where reactivity of functional groups is known to be difficult.[20,21] (For example, the direct nucleophilic displacement of a furanose 2-*O*-sulfonyl group with a charged nucleophile was not attained until the highly reactive *O*-trifluoromethanesulfonyl group was used.[22]) We now report the use of the Barton–McCombie deoxygenation in the successful conversion of methyl 3-deoxy-3-(hydroxymethyl)-β-D-ribofuranoside derivative **9** to the 3-branched 2-deoxyriboside **11**. This synthesis demonstrates a direct method for the synthesis of 2-deoxyribosides that should be of general interest.

On the assumption that deoxygenation at C-2 could be accomplished, a logical starting material was the known[23] 3-deoxy-3-(hydroxymethyl)-D-allofuranose derivative **9**. Neutral periodate cleavage of **9** and borohydride reduction of the intermediate aldehyde in situ, as described[24] for a related 3-branched furanose, gave the 3-deoxy-3-(hydroxymethyl)ribose derivative **10** and allowed selective blocking at C-3 and C-5 for subsequent transformations at C-2.

Ruthenium tetroxide was the preferred oxidant[25] of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose to the precursor ketone **5**, thus avoiding the 3-*O*-(methylthio)-methyl ether contaminant[26] from the oxidation with dimethyl sulfoxide–acetic anhydride. The Wittig reaction of anhydrous[27] **5** to form the methylene compound **6** was best carried out in ether solution, using butyllithium[28] in hexane to generate the ylide. Hydroboration–oxidation of **6** afforded the 3-deoxy-3-(hydroxymethyl)-D-allose derivative **7**, unavoidably accompanied by a byproduct **8** (in a 3:1 ratio of **7**/**8**). Separation could be accomplished by dry column chromatography, and the impurity **8** was identified as the isopropyl ether rather than the unlikely acetone hemiacetal of **9** that had been proposed.[23] It appeared likely that the formation of **8** was caused by the excess of borane that was required, and, in fact, if the molar ratio of borane/**6** was increased much beyond 2:1 the amount of **8** increased. The cleavage of ketals to hydroxy ethers by borane is known;[29] either **8** or the 6-*O*-isopropyl isomer might have been formed, but cleavage at the primary C–O bond of ketals like **7** appears to be favored. The presence of the impurity **8** did not interfere with the subsequent selective hydrolysis of **7** to give **9** or the chain-shortening of **9** to **10**, so that it was more efficient to delay chromatographic purification until **10** was isolated. Overall from ketone **6**, the yield of **10** was 40%, characterized as a homogeneous syrup and converted to the crystalline di-*O*-benzoate **11**. That the $HOCH_2$ branch at C-3 was in the D-*allo* configuration was readily verified from the ^1H NMR spectra of **10** and **11** and purified samples of **7** and **9**. The consistent appearance of H-1 as a doublet and H-2 as a triplet with $J_{1,2}$ and $J_{2,3}$ both about 4 Hz was diagnostic for the cis,cis relationship of H-1, -2, and -3.

For the deoxygenation[19] at C-2, it appeared that thionobenzoate would be the more easily employed functional group, with benzoate protection of the primary OH's. Therefore, dibenzoate **11** was converted via the methyl furanoside **21** to thionobenzoate **22**, as a chromatographically purified yellow syrup that was characterized in the ^1H NMR by the downfield shift for H-2 [δ 6.3 (d, $J_{2,3}$ = 4.7 Hz)] relative to H-1 [δ 5.16 (s)]. However, when **22** was subjected to the deoxygenation conditions by treatment with 5 molar equiv of tributyltin hydride in refluxing toluene for 2 h, the expected 2-deoxy compound





21, R = H
22, R = C(=S)Ph

23, R = Bz
24, R = H

(identical to 17, below) was only a minor product (11%). The product 21 of hydrogen capture[19] was also observed, but the major product was the 2-*O*-benzyl compound **23**, suggesting that reduction of C=S to CH_2 had occurred. This product was identified by IR and ^1H NMR, which showed a strong upfield shift of H-2 [δ 4.10 (d, $J_{2,3}$ = 5.0 Hz] and little change in H-1 [δ 5.02 (s)] and indicated the presence of a benzyl group. The presence of the 2-*O*-benzyl group was clearly observed after saponification to **24** [δ 7.37 (s, C_6H_5), 4.92 (s, H-1), 4.61 (d, $PhCH_2$), 4.3 (m, H-4), 4.05 (d, H-2, $J_{2,3}$ = 5.5 Hz), 3.35 (s, OCH_3), 2.47 (rough q, H-3, $J \approx 5.5$ Hz)]. These structures were confirmed by mass spectral analysis of **23**, which showed diagnostic peaks for M - OCH_3 (*m/e* 445), M - $BzOCH_2$ (341), and M - CH_2Ph - $CH_3OCH{=}O$ (325), and of the mono(trimethylsilyl) derivative of **24**, which showed peaks for M - OCH_3 (309), M - CH_2PH (249), and CH_2Ph (91). This unusual reaction of thionobenzoate is being studied further, but it was not applicable to the synthesis of 2-deoxyribosides. Consequently, the alternative deoxygenation through the *S*-methyl dithiocarbonate **14** was explored.

The preparation of *S*-methyl dithiocarbonates requires prolonged use of alkali, so stable *O*-benzyl ethers were used as protecting groups for **14**, even though previously this preparation was carried out successfully in the presence of a benzoate ester.[18] The di-*O*-benzyl derivative (**12**) of **10** was converted in one step[30] with methanolic hydrogen chloride to the methyl β-D-furanoside **13**, with no detectable α anomer. The *S*-methyl dithiocarbonate **14** as a chromatographically purified yellow syrup was best converted by slow addition of a toluene solution to excess tributyltin hydride (5 molar equiv) in refluxing toluene (20 and 10% solutions, respectively). The 2-deoxy compound **15** was the major product (67%), always accompanied by some of the product **13** (23%) of hydrogen capture. The heavily contaminating, nonpolar alkyltin compounds were nicely removed by simple chromatography on silica gel in petroleum ether, prior to the resolution of **15** and **13**. Debenzylation with sodium–liquid ammonia and benzoylation of **16** afforded the crystalline dibenzoate **17**, ready for nucleoside coupling.

Treatment of **17** in dichloromethane solution with hydrogen chloride readily afforded the 1-chloro sugar, but the conversion did not go beyond 65–75% conversion, even with prolonged treatment. Coupling with the bis(trimethylsilyl) derivative **18** of 2-acetamido-6-chloropurine as in the improved synthesis[31] of **1** and **2** afforded the nucleoside intermediates **19** and **20** in a combined yield of 45%. The products were separated by preparative thin-layer chromatography (TLC) or by liquid chromatography (LC) on scale up. The unreacted methyl glycoside was recovered (about 30%) as a mixture of **17** with its α anomer, judging from the NMR; this suggested that anomerization had occurred by breaking and re-forming the bond from C-1 to the ring O. There was also about 7% of the hydrolyzed 1-OH sugar. As expected, the anomers **19** and **20** were present in equal amounts. Each was obtained effectively free of the other by LC. The anomeric identities of **19** and **20** were determined from their NMR spectra. The patterns of the H-1 signals followed the convention observed[32] with other 2′-deoxyribonucleosides, triplet for the β anomer and quartet for the α anomer. Thus, as a β anomer, **19** exhibited an uneven triplet for H-1′ at δ 6.34, and **20** exhibited a quartet for H-1 at δ 6.30 that was equally diagnostic for the α anomer. Peak widths were about the same (8–10 Hz) and provided no basis for distinction. However, the H-2′ protons of **19** appeared as a strong multiplet compressed into a narrow range (along with H-3′) at δ 2.80–3.05, whereas the H-2′ protons of **20** gave multiplets spread over a wide range from δ 2.5 to 3.2 (and distinct from H-3′ at δ 3.4). This was in agreement with a previously noted[33,34] differentiation of anomeric 2′-deoxyribonucleosides. Anomers **19** and **20** were also distinguished by CD spectra which showed Cotton effects of opposite sign. The observed relationship, positive = 2-deoxy-α-D-ribo (**20**), negative = 2-deoxy-β-D-ribo (**19**), was previously observed[33,34] for some nucleosides containing 2-deoxy-D-ribose. This relationship has not been rationalized in the 2-deoxy series, and its generality is unclear as yet, but it does correspond to the relationship, positive = α-D-ribo, negative = β-D-ribo, that was observed[35] in a study and interpretation of the CD spectra of all the D-pentofuranosyladenines. The conclusion was that the cis or trans relationship between the C-1′ and C-2′ substituents was the major determinant for the sign of the Cotton effect, but that the C-1′, C-3′ relationship was also significant. The present results suggest that in the absence of any 2′ substituent the 1′,3′ relationship may become determining.

Conversion of **19** and **20** to the thioguanine nucleosides **3** and **4** in 60–70% yields was accomplished by treatment with anhydrous methanolic sodium hydrogen sulfide.[4] Excess hydrogen sulfide was used to avoid the presence of any methoxide as a competing nucleophile for the 6-Cl. In the isolation of the free nucleosides, it is essential to conduct the evaporation of an aqueous solution at a weakly acidic pH, to avoid the cleavage to TG that occurred at pH 8–9. In this respect, **3** and **4** appeared to be more labile than **1** and **2**. Sometimes partial cleavage occurred in the heating required for recrystallization of **3** and **4** from water. Purification was most reliably accomplished by reverse-phase LC. Ultraviolet maxima observed at 341 (neutral) and 319 nm (base) were diagnostic for TG nucleosides.

Biological Tests. Compounds **3** and **4** were tested along with **2** as a comparison for their incorporation into the DNA of murine tumor cells. Test methods were based on previous studies[36] of **1** and **2** using Mecca lymphosarcoma, because this cell line had a very limited stability to reutilize any TG that might be formed from nucleoside cleavage. Tumor-bearing mice were injected with radiosulfur-labeled samples of the nucleoside. Cells from the peritoneal cavity were removed, the acid-insoluble DNA was isolated and degraded, and the extent of incorporation of TG nucleosides was determined by counting for ^{35}S. Table I shows that both anomers **3** and **4** underwent phosphorylation and incorporation into DNA. The extent was essentially the same for **3** and **4** (2.36 and 2.42 nmol/mg) and was considerably better than for **2** (0.84). The ^{35}S was detected in both nucleoside and nucleotide fractions after degradation. The amount detected as nucleoside indicates the extent of incorporation at the terminal nucleoside position of DNA chains. Table I shows that the incorporation of both **3** and **4** was highly selective for the terminal position of DNA (90 and 91%, respectively), even more selective than incorporation of **2** (60% terminal). This indicates that **3** and **4**, when once incorporated, terminate chain elongation[14] more strongly than **2**. The type of nucleotide phosphate linkages was determined by treatment of the acid-soluble nucleotide fraction with 3′- and 5′-nucleotidases. Table I shows there

Table I. Incorporation into DNA of Mecca lymphosarcoma in Mice

A. packed cell volumes obtained from groups of 3 mice and the corresponding content of DNA

group	nucleoside tested	cell vol, mL	DNA content, mg
I	2	1.30	7.936
II	4	0.80	4.732
III	3	1.20	7.375

B. extent of incorporation of the [^{35}S]thioguanine nucleosides in DNA

nucleoside tested	nmol/mg of DNA
2	0.84
4	2.42
3	2.36

C. distribution of ^{35}S in degraded nucleic acids

nucleoside tested	^{35}S detected as nucleoside, %	^{35}S detected as nucleotide, %
2	60	40
4	91	9
3	90	10

D. extent of incorporation of the [^{35}S]thioguanine nucleosides in acid-soluble nucleotides (AS)

nucleoside tested	^{35}S detected as nucleoside, nmol/mg of DNA	^{35}S detected as nucleotide, nmol/mg of DNA
2	10.4	6.4
4	15.6	29.8
3	29.1	30.3

E. measurement of 3'- vs. 5'-phosphate linkages in acid-soluble nucleotides (AS) by extent of enzymatic cleavage

nucleoside tested	3'-nucleotidase, % nucleotide hydrolyzed	5'-nucleotidase, % nucleotide hydrolyzed
2	2	95.7
4	0	95.2
3	0	94.8

was a complete lack of response of **3** and **4** to 3'-nucleotidase and a high response (94.8 and 95.2%) to 5'-nucleotidase. This was predictable, since **3** and **4** have no secondary ring OH. For **2**, the similar lack of response to 3'-nucleotidase and high response (95.7%) to 5'-nucleotidase requires further consideration, since we believe that **2**—in conformation **2a**—must be phosphorylated and linked at the 3' position. However, this is on the basis that the 3'-OH of **2** is accepted and processed at the enzyme site normally for a 5'-OH. This therefore suggests that the site specificities of the 3'- and 5'-nucleotidases are uniquely reversed in the case of **2**.

In a separate series of experiments, **3** and **4** were compared with **2** for their effects on the proliferation of human lymphoblastoid cells (WI-L2) in suspension culture. The cells were grown in RPMI 1640 containing 10% fetal calf serum. Exposures to **2–4** were conducted at several concentrations. Table II shows that **3** and **4** were equally effective in the inhibition of the proliferation of WI-L2 cells and were considerably more effective than **2**. These experiments were also conducted with **2** and **4** in RPMI 1640 containing 10% dialyzed horse serum. The horse serum was found to have no detectable capacity for cleavage of **2–4** to TG in a 24-h incubation in the absence of cells, whereas fetal calf serum produced some cleavage of all three nucleosides. The results with horse serum were similar to those in Table II, except that both agents exhibited somewhat greater antiproliferative activity; **4** continued to be more effective than **2**.

Discussion

The new nucleosides **3** and **4** were active, and equally active, in each of the tests. Furthermore, in each test they were considerably more effective than the parent analogue **2**. These findings support the rationale for the design of **3** and **4** that the OH functions at 3' and 5' of 2'-deoxyribosides are essentially interchangeable on the enzyme, that the furanose ring O and CH_2 at 2' are correspondingly interchangeable, and that acceptability on the enzyme is improved if primary CH_2OH functions are provided at both the 3' and 5' positions. Thus, the common assumption that α- and β-nucleosides are inherently dissimilar is inaccurate and must be revised, at least for 2'-deoxyribosides. That **3** and **4** are equally active is a logical consequence of the above rationale and of the improved interchangeability achieved in **3** and **4**. Now that identical CH_2OH functions are provided at 3' and 5' in **3** and **4**, the only difference between these anomers depends on the distinction between the ring O and CH_2, and this distinction may not be made on the enzymes involved in tumor anabolism. It appears that the unusual structures of **2–4** may retain their antitumor effects simply because the important enzymes in the cancer cells are insensitive to the structural changes. It may be because the tumor enzymes are less discriminatory that **3** and **4** appear to be more effective than **2**, since these 3'-branched analogues do not require discrimination between the OH's at the 3' and 5' positions. On the other hand, structures **2–4** may acquire selectivity of action because the normal enzymes involved in the toxic effects of β-TGdR and other TG nucleosides do not tolerate these changes. For example, **2–4** showed no cleavage to TG in lymphoblastoid cells WI-L2, suggesting that **3** and **4** may retain the nontoxic properties of **2**. It also remains to be seen if **3** and **4** share the unusual resistance to phosphorylation in bone marrow that is exhibited by **2**.

Further studies on **3** and **4** and the applicability of these structural concepts are in progress.

Experimental Section

Solutions in organic solvents were dried over Na_2SO_4 and filtered. Evaporations were carried out in vacuo on a rotary evaporator. Melting points are uncorrected.

Table II. Inhibition of Proliferation of WI-L2 Cells by 3 and 4 Compared to α-TGdR (2)[a]

	number of cells/mL ($\times 10^{-5}$)								
		2, μM			4, μM			3, μM	
time, h	control (0 drug)	100	200	300	100	200	300	100	200	300
0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
23.5	3.4	3.2	3.0	2.9	2.4	2.2	2.0	2.6	2.3	2.2
47.5	10.7	7.6	6.0	5.2	3.8	2.9	2.3	3.7	2.8	2.6
71	16.0	9.6	7.1	5.8	4.0	2.9	2.4	4.0	3.0	2.6

[a] Each value represents the average of four determinations, using two separate cultures for each condition.

Spectra. IR spectra were determined with a Perkin-Elmer 735B spectrometer on liquid film or, for solids, on Nujol mull. Proton magnetic resonance (^{1}H NMR) was determined on $CDCl_3$ solutions (Me_4Si internal reference, δ 0.0) with Varian T-60, EM390, and XL-100 spectrometers; signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). We thank Mr. L. W. Cary for the spectra at 100 MHz. UV spectra were determined on a Cary 11 or 14 recording spectrophotometers. Circular dichroism was determined on a Jasco ORD/UV-5 spectropolarimeter equipped with a Sproul Scientific SS-20 CD modification and a programmed 15-Å slit width control. Mass spectra were recorded on an LKB Model 9000 spectrometer at 12 eV or, for high resolution, on a CEC double-focusing spectrometer. We thank Dr. D. W Thomas for the spectra and interpretations.

Chromatography. The R_f values are for TLC analyses, which were done on 2 × 8 in. plastic plates precoated with 0.25-mm layers of silica gel (Polygram Sil G/UV_{254}). Conventional column chromatography was done on silica gel 60 (E. Merck). Dry column chromatography was done on ICN dry column silica gel (activity III). Analytical LC was done on a Spectra-Physics Model 3500 liquid chromatograph using a Waters 0.39 × 30 cm column (P/N 27477) of 10-μm silica gel. For **3** and **4**, a reverse-phase column (0.39 × 25 cm) of 10-μm Spherisorb silica ODS 10 from Spectra-Physics was used. Reverse-phase semipreparative LC was done using in series two Waters columns (0.78 × 61 cm) of Bondapak C_{18} (37–60 μm). Preparative LC was performed on a Waters Associates Prep LC/system 500 instrument, using a Prep Pak-500/silica cartridge column and a refractive index detector.

3-Deoxy-3-methylene-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (6). In a modification of a previous procedure,[28] a stirred suspension of 32.5 g (0.0902 mol) of methyltriphenylphosphonium bromide in 300 mL of dry ethyl ether was treated at room temperature with 64 mL (0.10 mol) of 1.6 M butyllithium in hexane. After stirring the solution for 2 h, a solution of 20.0 g (0.0769 mol) of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (**5**) as the anhydrous[27] ketone in 300 mL of dry ether was added dropwise. The mixture was stirred at room temperature for 2 h, refluxed for 1 h, cooled, and treated with 300 mL of water. The layers were separated, the aqueous layer was saturated with sodium chloride and extracted with ether, and the combined organic layers were dried and evaporated. The product after short-path distillation (82–90 °C, 0.18–0.25 mm; Kugelrohr) was a homogeneous syrup (63% yield): TLC R_f (benzene–ethyl acetate, 20:1) 0.42. The ^{1}H NMR data agreed with the assignments[37] corrected from the initial report.[38]

3-Deoxy-3-(hydroxymethyl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (7). An anhydrous tetrahydrofuran (THF) solution (229 mL, 0.217 mol) of 0.95 M borane–THF complex was stirred at 0–5 °C under a nitrogen atmosphere and was treated dropwise with 28.1 g (0.109 mol) of **6** in 350 mL of anhydrous THF. The solution was stirred at ambient temperature for 3 h, cooled again, and treated with 135 mL of THF–H_2O (1:1), 168 mL (0.340 mol) of 2 N NaOH, and 135 mL (0.135 mol) of 30% H_2O_2; each addition required 10 min. The turbid mixture was stirred at room temperature for 2.5 h, chilled again, and treated portionwise with 450 mL of saturated aqueous $Na_2S_2O_3$ to decompose the excess peroxide. Sodium chloride was added to saturate the aqueous layer, which was separated and extracted with ethyl acetate (3 × 500 mL).

The organic layers were combined, dried, and evaporated to yield 31.3 g of syrup containing the product, R_f (benzene–ethyl acetate, 1:1) 0.5, and an impurity, R_f 0.2 (lit.[23] R_f 0.5 and 0.2). The syrup was used directly in the next step.

A 2.10-g sample of the mixture was resolved by dry column (3.8 × 75 cm) chromatography on silica gel, developed with benzene–ethyl acetate (1:1). The faster moving zone, after elution with CH_2Cl_2–CH_3OH (4:1), yielded 1.54 g (76%) of product; NMR data were as reported.[23] The slower zone yielded 0.46 g (23%) of syrup that appeared to be the same as the impurity previously assigned[23] an unlikely hemiacetal structure, but it did not undergo hydrolysis as reported, and we identify the compound as **3-deoxy-3-(hydroxymethyl)-5(or 6)-*O*-isopropyl-1,2-*O*-isopropylidene-α-D-allofuranose (8)**: ^{1}H NMR δ 5.75 (d, H-1, $J_{1,2}$ = 4 Hz), 4.75 (t, H-2, J = 4 Hz), 2.0–2.4 (m, H-3), 1.48 and 1.34 (2 s, CMe_2), 1.25 (d, 2 CH_3 of *O*-isopropyl, J = 6 Hz); MS m/e 261 ($M^+ - CH_3$), 173 ($M^+ - HOCH_2CHO$-*i*-Pr); high-resolution MS m/e 261.1333 ($M^+ - CH_3$ calcd 261.1338) confirmed $C_{13}H_{24}O_6$ for **8**.

3-Deoxy-3-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-allofuranose (9). Selective hydrolysis[38] of the di-*O*-isopropylidene compound with 0.1 N H_2SO_4 for 3.5 h yielded the crude product[23] (95%): TLC R_f (benzene–CH_3OH, 3:1) 0.48; ^{1}H NMR δ 5.80 (d, H-1, J = 4 Hz), 4.76 (rough t, H-2, $J \approx$ 4.5 Hz), 1.48 and 1.28 (2 s, CMe_2). The unchanged isopropyl ether impurity (**8**) was observed, R_f 0.61.

3-Deoxy-3-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-ribofuranose (10). Successive periodate cleavage of **9** at neutral pH and borohydride reduction of the intermediate aldehydo sugar was carried out as for the 3-(cyanomethyl) analogue,[24] starting with 62.0 g (0.263 mol) of **9**. After the decomposition of excess borohydride, the reaction mixture was filtered, the solids were washed with 500 mL of ethyl acetate, and the combined filtrates were evaporated. The residual mixture was partitioned between 1.5 L of ethyl acetate and 600 mL of brine. The aqueous layer was washed with ethyl acetate, and the combined organic layers were dried and evaporated. The residual syrup (54 g) showed two components, R_f 0.43 (product) and 0.61 (**8**) in ethyl acetate, with trace spots at R_f 0.51, 0.79, and 0.96. A 13.2-g portion was purified by column (3.8 × 64 cm) chromatography on silica gel with ethyl acetate as eluent, collecting 25-mL fractions, to yield 8.72 g (66%) of homogeneous syrup: ^{1}H NMR δ 5.78 (d, H-1, J = 4 Hz), 4.73 (t, H-2, J = 4 Hz), 2.7–1.9 (m, H-3), 1.48 and 1.24 (2 s, CMe_2). Efficient purification was also accomplished by preparative LC. The syrup (7 g) was diluted with ethyl acetate to a 10-mL volume and chromatographed with ethyl acetate (solvent pressure 10 atm, column pressure 22.5 atm, solvent flow rate 200 mL/min) using a refractive index detector. Fractions were monitored by TLC, which showed that homogeneous **10** was eluted between 8.5 and 20 min to give 4.5 g in 45 min of operation. Although the precursors to **10** could be purified at each step, that offered no overall advantage.

5-*O*-Benzoyl-3-deoxy-3-[(benzoyloxy)methyl]-1,2-*O*-isopropylidene-α-D-allofuranose (11): yield 94%; mp 90–91 °C. Anal. ($C_{23}H_{24}O$) C, H.

5-*O*-Benzyl-3-deoxy-3-[(benzyloxy)methyl]-1,2-*O*-isopropylidene-α-D-allofuranose (12). With exclusion of moisture under a nitrogen atmosphere, a suspension of 4.08 g (0.0851 mol) of a 50% sodium hydride–mineral oil dispersion in 50 mL of dimethylformamide (DMF) was stirred at 0–5 °C and treated with a solution of 8.00 g (0.0387 mol) of **10** in 100 mL of DMF, added in portions over a period of 30 min. After 30 min at 0–5 °C, the stirred gelatinous mixture was treated with 22.4 mL (0.194 mol) of benzyl chloride over a period of 15 min. The mixture was stirred overnight at room temperature and then treated with 100 mL of H_2O in portions, with cooling. After 20 min of stirring, the mixture was extracted with ethyl acetate (3 × 400 mL). The combined organic layers were dried and evaporated (≤65 °C). The remaining DMF and benzyl chloride were removed at 0.1 mm and 60–65 °C to yield 14.6 g (98%) of syrup: TLC R_f (benzene–ethyl acetate, 19:1) 0.42; ^{1}H NMR δ 7.28 (s, 2 C_6H_5), 5.98 (d, H-1, J = 4 Hz), 4.68 (t, H-2, $J \approx$ 5 Hz), 4.57 and 4.53 (2 s, 2 OCH_2Ph), 2.6–2.0 (m, H-3), 1.44 and 1.29 (2 s, CMe_2); no OH absorption near 3.0 μm in the IR.

Methyl 5-*O*-Benzyl-3-[(benzyloxy)methyl]-3-deoxy-β-D-ribofuranoside (13). Methanolysis directly[30] on the isopropylidene compound was accomplished by treating a stirred solution of 110 g (0.286 mol) of **12** in 3 L of absolute CH_3OH with 130 mL of saturated methanolic hydrogen chloride prepared at 0 °C. The stoppered solution was stirred for 60 h, chilled in ice, and treated with solid $NaHCO_3$ in portions with vigorous stirring until it was neutral. The solution was filtered and evaporated. The residue was dissolved in 1.5 L of $CHCl_3$, filtered, dried, and evaporated to yield 96.5 g (94%) of syrup: TLC R_f (benzene–ethyl acetate, 1:1) 0.64 with a trace impurity of R_f 0.3; ^{1}H NMR δ 7.31 (s, 2 C_6H_5), 4.78 (s, H-1), 4.58 and 4.49 (2 s, 2 OCH_2Ph), 3.27 (s, OCH_3), 2.6–2.1 (m, H-3); IR (film) 2.95 μm (OH). No evidence of the α anomer was observed.

Methyl 5-*O*-Benzyl-3-[(benzyloxy)methyl]-3-deoxy-2-*O*-[(methylthio)thionocarbonyl]-β-D-ribofuranoside (14). With exclusion of moisture under a nitrogen atmosphere, 2.97 g (0.061 mol) of a 50% sodium hydride–mineral oil dispersion and 20 mg of imidazole (catalyst) were treated at 0–5 °C with a solution

of 11.6 g (0.0330 mol) of **13** in 220 mL of THF added dropwise with stirring. The resultant suspension was stirred for 30 min, refluxed for 2 h, cooled again to 0–5 °C, stirred, and treated with 15 mL of CS_2 dropwise over a period of 15 min. After 2 h of stirring the mixture at room temperature, 15 mL of methyl iodide was added dropwise with cooling. The mixture was stirred overnight, refluxed for 2 h, cooled, and poured into 600 mL of H_2O. The layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 500 mL). The combined organic layers were washed with 500 mL of brine, dried, and concentrated to 13.6 g of brown syrup, which consisted of one major product, R_f (benzene–ethyl acetate, 19:1) 0.52, with several minor contaminants. Purification by dry column (3.8 × 130 cm) chromatography on silica gel developed with benzene–ethyl acetate (19:1) and using ethyl acetate to elute the fractions afforded 12.1 g (82%) of homogeneous yellow syrup: IR (film) 8.20 and 9.25 μm (C=S); ^{1}H NMR δ 7.37 and 7.33 (2 s, 2 C_6H_5), 5.98 (d, H-2, $J_{2,3}$ = 3.5 Hz), 4.98 (s, H-1), 4.62 and 4.50 (2 s, 2 OCH_2Ph), 4.5–4.1 (m, H-4), 3.73–3.53 (rough d, 2 H-5, 3-CH_2), 3.32 (s, OCH_3), 3.0–2.6 (m, H-3), 2.47 (s, SCH_3).

Methyl 5-*O*-Benzyl-3-[(benzyloxy)methyl]-2,3-dideoxy-β-D-*erythro*-pentofuranoside (15). Under anhydrous conditions and a nitrogen atmosphere, a stirred, refluxing solution of 27.0 g (0.0936 mol) of tributyltin hydride in 300 mL of toluene was treated with a solution of 8.50 g (0.0187 mol) of **14** in 40 mL of dry toluene dropwise over a period of 80 min. After continued stirring and reflux for 16 h, the mixture was concentrated (70 °C) to a residual yellow liquid, which was purified by conventional column chromatography on silica gel. An initial column (5 × 46 cm) was eluted with about 3.5 L of petroleum ether (bp 30–60 °C) until the malodor was gone, and TLC monitoring showed that nonpolar alkyltin compounds were all removed. The reaction products were then eluted with 2 L of ethyl acetate–CH_3OH (1:1) to yield 8.6 g of syrup. Fractionation on a second column (4.3 × 71 cm) by elution with benzene–ethyl acetate (1:1), collection of 22-mL fractions, monitoring by TLC, and evaporation afforded 4.29 g (67%) of a homogeneous syrup: TLC R_f (benzene–ethyl acetate, 85:15) 0.41; ^{1}H NMR δ 7.33 and 7.31 (2 s, 2 C_6H_5), 4.99 (q, H-1, on expansion of sweep width from 500 to 100 Hz, $J_{1,2;cis}$ = 4.6 Hz, $J_{1,2;trans}$ = 1.0 Hz), 4.59 and 4.49 (2 s, 2 OCH_2Ph), 4.10 (t × d, H-4, $J_{3,4}$ = 5.0 Hz, $J_{4,5}$ = 6.8 Hz), 3.55 (d, 5-H_2, J = 6.8 Hz), 3.46 (d, 3-CH_2, J = 8.2 Hz), 3.28 (s, OCH_3), 2.7–2.1 (m, H-3), 2.05 (m, H-2 trans to H-1), 1.82 (q × d, H-2 cis to H-1, $J_{1,2;cis}$ = 4.6 Hz, $J_{2,3;trans}$ = 10.6 Hz, J_{gem} = 13.0 Hz). Further elution afforded 1.54 g (23%) of **13**, identical to **13** above by TLC, NMR, and IR.

Methyl 2,3-Dideoxy-3-(hydroxymethyl)-β-D-*erythro*-pentofuranoside (16). A stirred solution of 3.00 g (8.80 mmol) of **15** in 200 mL of anhydrous NH_3 protected from H_2O under a reflux condenser cooled with dry ice–acetone was treated with 1.01 g (0.0438 mol) of sodium spheres (Matheson Coleman & Bell) with vigorous stirring over a 10-min period. The resulting deep-blue solution was stirred for 30 min, and then solid NH_4Cl was added in portions to discharge the blue color, followed by 5 mL of 95% ethanol to ensure complete decomposition of metallic sodium. The NH_3 was evaporated under a stream of N_2, and the residual damp gray solid was stirred with ethyl acetate (2 × 250 mL). The combined organic extracts were dried and evaporated. The residue from the extracts was freed of remaining solid by extraction with $CHCl_3$ (2 × 250 mL) to yield 1.42 g (100%) of homogeneous syrup: TLC R_f (two developments in ethyl acetate) 0.47; ^{1}H NMR δ 4.95 (d, H-1, J = 4.5 Hz), 3.35 (s, OCH_3); IR (film) 2.8–3.1 μm (OH, strong).

Methyl 5-*O*-Benzoyl-3-[(benzoyloxy)methyl]-2,3-dideoxy-β-D-*erythro*-pentofuranoside (17). In the customary benzoylation procedure, a cold, stirred solution of 1.42 g (8.77 mmol) of **16** in 28 mL of pyridine was treated with 4.2 mL of benzoyl chloride dropwise. After 30 h, the stirred suspension was hydrolyzed with 10 drops of H_2O for 30 min and poured into 40 mL of H_2O, and the mixture was extracted with ether (3 × 100 mL). The combined extracts were washed with H_2O and with 3 N H_2SO_4 to remove pyridine, and after washing with aqueous $NaHCO_3$ were dried and evaporated to yield 3.26 g (100%) of a syrup, R_f (benzene–ethyl acetate, 17:3) 0.60, which crystallized on standing. Recrystallization from hexene gave white needles: mp 53.5–55 °C; ^{1}H NMR (100 MHz with expansion of sweep width from 500 to 100 Hz and with decoupling) δ 8.16–7.94 and 7.60–7.25 (2 m, 2 OBz), 5.06 (d, H-1, $J_{1,2;cis}$ = 4.5 Hz, $J_{1,2;trans}$ = 0.7 Hz visible on expansion), 4.7–4.2 (m, H-4 plus 2 $BzOCH_2$), 3.36 (s, OCH_3), 2.86 (m, H-3), 2.24 (q, H-2 trans to H-1, $J_{2,3;cis}$ = 7.2 Hz, J_{gem} = 12.6 Hz; $J_{1,2;trans}$ = 0.7 Hz visible on expansion), 1.9 (d × q, H-2 cis to H-1, $J_{1,2;cis}$ = 4.5 Hz, $J_{2,3;trans}$ = 10.5 Hz, J_{gem} = 12.6 Hz); MS *m/e* 339 (M^+ – OCH_3), 235 (M^+ – OCH_3Bz + H) but no apparent M^+ peak near 370. Anal. ($C_{21}H_{11}O_6$) C, H.

5-*O*-Benzoyl-3-[(benzoyloxy)methyl]-2,3-dideoxy-D-*erythro*-pentofuranosyl Chloride. A stream of anhydrous HCl was bubbled through an anhydrous solution of 2.40 g (6.49 mmol) of **17** in 40 mL of CH_2Cl_2 at room temperature for 3 h. Further CH_2Cl_2 was added to replenish any losses. The solution was evaporated, toluene was added, and the solution was evaporated at 0.2 mm (≤48 °C). The residual amber syrup was used immediately; 65–75% conversion to the chloro sugar was estimated by comparing integrated ^{1}H NMR peaks for OCH_3, C_6H_5, and H-1 of the chloro sugar [δ 6.3 (m)]. Complete conversion was attained in glacial acetic acid solution, but the syrupy chloro sugar could not be completely freed of acetic acid without decomposition.

Coupling of the Chloro Sugar with 18. 2-Acetamido-6-chloropurine (Het-Chem-Co.; 2.24 g, 10.6 mmol) was converted[31] to **18** by heating with hexamethyldisilazane and $(NH_4)_2SO_4$ under anhydrous N_2 at a bath temperature of 150–160 °C for 3 h and then evaporating the excess silazane at 0.5 mm (50–60 °C). The residual syrup sometimes contained a trace of solid. To the syrup under N_2 was added 3.80 g (15.1 mmol) of $Hg(CN)_2$ and 72 mL of dry benzene. The mixture was stirred and heated to incipient reflux (bath 81 °C), and a solution of the above chloro sugar (from 6.49 mmol of **17**) in 72 mL of dry benzene was added in one portion. After 2 h of heating, the solution was evaporated (bath ≤43 °C), and the residue was triturated and extracted with CH_2Cl_2 (2 × 300 mL). The combined and filtered extracts were washed with 200 mL of aqueous 30% KI and with brine, dried, and evaporated. The residual syrup was converted at 0.5 mm (20–25 °C) to a foamed glass: 3.10 g; TLC R_f (benzene–ethyl acetate, 1:1) 0.25 (**20**), 0.34 (**19**), and 0.95 (**17** and its α anomer), with impurities at R_f 0.75–0.81 (probably the α- and β-1-OH analogues of **17**). Integrated ^{1}H NMR signals for H-1 indicated the composition (w/w) was 45% of **19** and **20**, 31% of **17** and its α anomer [δ 5.13 (q, J = 1.4, J = 5.0 Hz), 3.40 (s, OCH_3)], and 7% of the 1-OH sugars [δ 5.5 (m, H-1 of α and β)]. The product was chromatographed on a dry column (1.26 × 100 cm) of 250 g of silica gel, using benzene–ethyl acetate (1:1) as solvent and eluent. The methyl glycoside fraction (1.05 g) was recycled into further couplings. The nucleoside fraction (1.66 g, 46%) contained equal amounts of **19** and **20** with only trace impurities (analytical LC with hexane–ethyl acetate, 1:1, gave retention constants of 7.3 for **19** and 10.0 for **20**, with a selectivity constant α of 1.36).

The anomers were separated by preparative LC in hexane–ethyl acetate (6:7), at a solvent pressure of 2.3 atm, a column pressure of 23 atm, and a solvent flow of 250 mL/min. The fractions were monitored by TLC in hexane–ethyl acetate (2:3) and quantitatively by analytical LC. A second cycling was required to yield 0.47 g (13%, 98% pure containing <0.1% of **20**) of β-anomer **19** and 0.63 g (17%, 98% pure containing 1.5% of **19**) of the α-anomer **20**. There was 0.30 g of the α,β mixture to be recycled. Alternatively, the initial dry column separation could be omitted, and the entire purification of the product from 5.29 g of **17** was completed in four LC separations. Further purification of small samples of **19** and **20** was accomplished on the instrument for analytical LC with two microPorasil columns (3.9 × 30 cm) in series. Twenty 1.5-mg samples were injected and the first and last 10% of the product peak were discarded. The collected products (14–16 mg) were reprecipitated from 2 mL of CH_2Cl_2 by adding 20 mL of hexane to give 12 mg of **19** and 6 mg of **20**.

2-Acetamido-6-chloro-9-[5-*O*-benzoyl-3-[(benzoyloxy)methyl]-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]-9*H*-purine (19) contained <0.01% of the α anomer: UV (CH_3OH) λ_{max} 259.5 nm (ε 10 480), 283 (10 070); CD (CH_3OH) 262 nm [[θ] −3300° L/(cm mol)]; ^{1}H NMR δ 8.17 (s, H-8), 6.34 (uneven t, H-1′), 2.92 (m, H-3′ plus 2 H-2′), 2.49 (s, $COCH_3$). Anal. ($C_{27}H_{24}ClN_5O_6 \cdot 0.33H_2O$) C, H, N.

2-Acetamido-6-chloro-9-[5-*O*-benzoyl-3-[(benzoyloxy)methyl]-2,3-dideoxy-α-D-*erythro*-pentofuranosyl]-9*H*-purine (20) contained <0.001% of the β anomer: UV (CH_3OH) λ_{max} 259.5

nm (ε 11 270), 283 (10 400); CD (CH_3OH) 259 nm [[θ] 7920° L/(cm mol)], 287 (12 500); ^1H NMR δ 8.21 (s, H-8), 6.30 (q, H-1′, J = 3.0, 7.2 Hz), 3.4 (m, H-3′), 3.2–2.5 (2 H-2′), 2.43 (s, $COCH_3$). Anal. ($C_{27}H_{24}ClN_5O_6 \cdot 0.5H_2O$) C, H, N.

2-Amino-9-[2,3-dideoxy-3-(hydroxymethyl)-α-D-*erythro*-pentofuranosyl]-9*H*-purine-6(1*H*)-thione (4). A stream of H_2S was bubbled for 30 min through a refluxing solution of 920 mg (1.67 mmol) of **20** (containing 1.5% of **19** by LC) in 50 mL of anhydrous CH_3OH. With continued heating (bath 70–75 °C) and introduction of H_2S, the solution was stirred and treated with 5.0 mL of 1 N NaSH in anhydrous CH_3OH (NaSH solution prepared by dissolving 1.25 g of Na in 54 mL of dry CH_3OH and saturation with H_2S). After 15 min, a precipitate formed. After 2 h, the stream of H_2S was removed, and stirring and heating were continued for 15 min under a blanket of dry N_2. Then, 2.7 mL of 1 N NaOH was added, and the clear solution was refluxed under N_2 for 1 h. The yellow solution was cooled to room temperature and neutralized to pH 5–6 with 9–10 mL of methanolic 1 N acetic acid. If even a mildly basic pH (8–9) was retained, nucleoside cleavage occurred in the following evaporation (<40 °C) to a semisolid. Partitioning of the residue between 25 mL of H_2O and 40 mL of CH_2Cl_2 produced 290 mg of white solid at the interface, which was collected by filtering the mixture. The aqueous layer was separated, washed with 25 mL of CH_2Cl_2, and freeze-dried. Crystallization of the residue from 10 mL of hot H_2O gave 65 mg. Another 20 mg was recovered from the filtrate by LC on a C-18 reverse-phase semipreparative column (3% CH_3CN in H_2O as eluent). A final purification of the combined product (375 mg, 76% yield, containing <0.1% TG) was accomplished by LC and freeze-drying the eluate: UV (pH 7) λ_{max} 226 nm (ε 15 160), 257 (8220), 341 (22 700); UV (0.1 N NaOH) λ_{max} 252 nm (ε 13 300), 270 (6900), 319 (19 300); ^1H NMR (Me_2SO-d_6, internal Me_4Si) δ 8.16 (s, H-8), 6.00 (m, H-1′). Anal. ($C_{11}H_{15}N_5O_3S \cdot 0.5H_2O$) C, H, N, S.

2-Amino-9-[2,3-dideoxy-3-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl]-9*H*-purine-6(1*H*)-thione (3). By the procedure for **4**, 420 mg (0.764 mmol) of **19** (98% pure by LC, containing <0.1% **20**) afforded 90 mg of precipitate that separated at the H_2O–CH_2Cl_2 interface (<0.1% TG present). Additional 60-mg and 6-mg portions were obtained from the H_2O layer. Final purification of the combined product (156 mg, 69% yield) was accomplished by LC and freeze-drying the eluate: UV (pH 7) 226 nm (ε 15 000), 257 (8040), 341 (23 600); UV (0.1 N NaOH) 252 nm (ε 13 900), 270 (7010), 319 (20 100); ^1H NMR (Me_2SO-d_6, internal Me_4Si) δ 8.05 (s, H-8), 6.07 (t, H-1′, J = 6 Hz). Anal. ($C_{11}H_{15}N_5O_3S \cdot 0.45H_2O$) C, H, N, S.

Biological Methods. (a) Tests for Incorporation into DNA of Mecca Lymphosarcoma in Mice. Radiosulfur-labeled samples of **3** and **4** were prepared by exchange with rhombic ^{35}S by the method[36] for labeled **1** and **2**. Labeled **2** was used as comparison. Nine AKD_2F_1 female mice bearing 4-day old Mecca lymphosarcoma ascites tumors were used in groups of three, as follows.

Group I. Each of three mice was injected ip with 2.6 μmol of ^{35}S-labeled α-TGdR (labeled **2**, 5.82 μCi); after 2 h the injection was repeated, and after an additional 2 h the cells were washed out of the peritoneal cavity with saline and centrifuged. The cells from the three mice were pooled. They were extracted twice with 5 volumes of cold 0.4 M perchloric acid. The perchloric acid extracts were neutralized with 2 M KOH to pH 7 and used as the acid-soluble solution (AS), after addition of dithioerythritol (DTE).

The extracted pellet was reextracted twice with cold 95% EtOH, partially dried in vacuo, and then suspended in 3.0 mL of 10% NaCl. The pH was adjusted to 7.0 with small additions of NaOH, DTE was added, and the precipitate was extracted for 40 min at 100 °C. The suspension was cooled and centrifuged. The 10% NaCl extract was treated with 3 volumes of cold 95% EtOH and centrifuged. The precipitated sodium nucleates were incubated for 18 h in 2.0 mL of 0.1 M NaOH at 37 °C and chilled, and 0.10 mL of 3 M HCl was added. The resultant DNA precipitate was separated by centrifugation, and the supernatant solution of degraded RNA was removed. The DNA was dissolved in 0.05 M Tris buffer (Sigma, St. Louis) to pH 7.0, and the solution was treated with DNase I (Sigma) for 1 h at 37 °C. Then, Tris buffer was added to pH 8.6, 30 units of phosphodiesterase I (ICN, Cleveland) was added, and incubation at 37 °C continued for another 1.75 h. This solution was counted in a scintillation system, and aliquots were chromatographed on Whatman 3MM paper in 5% KH_2PO_4 containing 0.01 M mercaptoethanol. TG, α-TGdR, and TGdR phosphate were used as markers. The spots cut from paper were counted directly in a polar scintillation system that gives counts equal to those obtained in solutions (Bray's).

The AS solution was used for similar chromatography, and an aliquot (2.0 mL) was treated with 5 units of phosphodiesterase (free of nucleotidase) at pH 8.6 for 1 h at 37 °C and then with 3′-nucleotidase (4 units) (wheat germ, ICN, Cleveland) at pH 5 for 1 h at 37 °C. Other aliquots were treated with crude snake venom (*Crotalus adamanteus*, Sigma, contained phosphodiesterase plus 5′-nucleotidase) for 2 h at 37 °C. They (250 μL) were then chromatographed on 3MM paper with 5% KH_2PO_4 containing mercaptoethanol, and the spots were counted.

Group II. Three mice were treated as in group I using 2.6 μmol of [^{35}S]**4** (5.5 μCi per injection).

Group III. Three mice were treated with 2.6 μmol of [^{35}S]**3** (3.3 μCi per injection).

Results from the three groups were compared in Table I.

(b) Tests on Growth of Human Lymphoblastoid Cells (WI-L2) in Culture. WI-L2 cells, obtained from Dr. J. E. Seegmiller, Department of Medicine, University of California, San Diego, were grown in RPMI 1640 containing 10% fetal calf serum or, as noted, in RPMI 1640 containing 10% dialyzed horse serum. The latter was found to have no detectable capacity for cleavage of **2**, **3**, or **4** to TG in a 24-h incubation in the absence of cells, whereas fetal calf serum produced some cleavage of all three nucleosides in 24 h, although none was detectable in short incubations (20 min). The nucleosides **2**–**4** were not cleaved to TG by the WI-L2 cells (incubated with labeled nucleoside and [^{35}S]TG measured).

For experiments, penicillin and streptomycin were included in the growth medium. Stock cultures, free of mycoplasma, were diluted with fresh growth medium at 2- to 3-day intervals to keep cells proliferating logarithmically. Cell culture reagents were obtained from Grand Island Biological Co., Calgary, Alta.

Exposures to **2**–**4** were conducted at several concentrations, using duplicate 20-mL cultures for each condition. Exposures were initiated by combining equal portions of growth medium containing logarithmically proliferating cells (2×10^5 cells/mL) and medium containing drug (at twice the concentration to be tested). Cell numbers were determined at 24-h intervals using an electronic particle counter (Coulter Electronics, Hialeah, Fl.).

The results in Table II are from the experiment with calf serum. Similar results with **2** and **4** were obtained using horse serum.

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Synthesis and Platelet Aggregation Inhibitory Activity of 6-Isobutyl-α-methyl-3-pyridineacetic Acid

Ronald H. Rynbrandt,* Burris D. Tiffany, Doris P. Balgoyen, Edward E. Nishizawa, and A. Rene Mendoza

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001. *Received August 31, 1978*

2-(4′-Isobutylphenyl)propionic acid, ibuprofen, is an antiinflammatory agent which possesses moderate platelet aggregation inhibitory activity. It was therefore of interest to determine what effect the replacement of the phenyl group of ibuprofen by a 3-pyridyl ring would have on platelet aggregation inhibitory activity. As a result, 6-isobutyl-α-methyl-3-pyridineacetic acid (**7**) and its 2-chloro analogue **13** were synthesized. The key step in the synthesis of **7** and **13** involved the oxidative rearrangement of enol ether **11** to the carboxylic ester **12** with thallium trinitrate. The entire sequences of reactions for the synthesis of compounds **7** and **13** are described in detail. Platelet aggregation inhibitory evaluation of **7** and **13** showed **7** to possess activity equivalent to ibuprofen; however, **13** was devoid of platelet aggregation inhibitory activity at an equivalent dose.

2-(4′-Isobutylphenyl)propionic acid, ibuprofen, is an antiinflammatory agent which possesses moderate platelet aggregation inhibitory activity.[1] It was therefore of interest to determine what effect the replacement of the phenyl group of ibuprofen by a 3-pyridyl ring would have on platelet aggregation inhibitory activity. As a result, 6-isobutyl-α-methyl-3-pyridineacetic acid (**7**) and its 2-chloro analogue, 2-chloro-6-isobutyl-α-methyl-3-pyridineacetic acid (**13**), were prepared and tested for platelet aggregation inhibitory activity.

Chemistry. The initial attempt to prepare **7** (Scheme I) was patterned to afford 3-acetyl-6-isobutylpyridine (**5**), which we anticipated could be converted to **7** by the method recently described by White and Wu.[2] This conversion failed; however, it is presented here in order to illustrate a limitation of this method for the preparation of carboxylic acids. 1,2-Dihydro-6-isobutyl-2-oxonicotinic acid (**2**)[3] was converted to methyl 2-chloro-6-isobutylnicotinate (**3**) by treatment with phosphorus oxychloride and phosphorus pentachloride, followed by absolute methanol. Dehalogenation of **3** with hydrogen and 5% palladium on charcoal catalyst afforded **4** in 68% yield. Conversion of **4** to the desired ketone **5** was accomplished by condensation with ethyl acetate followed by decarboxylation.

White and Wu[2] have recently described the conversion of 4-isobutylacetophenone to 2-(4′-isobutylphenyl)-propionic acid by treatment of the ketone with chloroacetonitrile to afford the glycidylnitrile, which was subsequently opened with lithium perchlorate followed by basic hydrolysis to afford the acid. Treatment of ketone **5** with chloroacetonitrile and sodium hydroxide in dimethylformamide afforded the glycidylnitrile **6** as a 50:50 mixture of isomers (NMR) in 57% yield. When **6** was treated with lithium perchlorate in xylene at 110 °C, the desired acid **7** was not obtained. NMR analyses of the reaction products showed the acidic component to be isobutyric acid and the neutral material to be a pyridineglycidylnitrile devoid of an isobutyl group. When the glycidylnitrile of 3-acetylpyridine was treated in a similar manner, the reaction afforded α-methyl-3-pyridineacetic acid in good yield. The apparent reason for the failure of this method with **6** is that the pyridyl nucleus activates the methylene carbon atom of the 6-isobutyl group such that it is oxidized and subsequently cleaved by lithium perchlorate.

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