of 6-chloro-2-fluoropurine (I, 1.5 g, 8.6 mmoles) in 25% methanolic Me_3N was kept at 25°. After a few min a cryst ppt appeared. The mixt was kept at 25° for 48 hr; the ppt was collected and washed with EtOH to yield colorless plates (0.45 g, 26%), mp 300°, of 2-fluoro-6-trimethylaminopurine betaine (XX): uv_{max} (pH 1.0) 270.5 (ϵ 7.9 × 10^3), (pH 6) 273 (7.3 × 10^3), (pH 12) 273 nm (7.6 × 10^3). *Anal.* ($C_8H_{11}N_5F$) C, H, N, F.

The filtrate of the above reaction, after evapn to dryness under reduced pressure, gave a cryst residue consisting of 2-fluoro-6-dimethylaminopurine (XIX), 1.1 g (70%), mp 220°. *Anal.* ($C_7H_8N_5F$) C, H, N, F. This material was identical with the product prepared by Montgomery and Hewson.[6]

Reaction of 2-Fluoro-6-chloropurine (I) with Hydrazine.—A 10% hydrazine hydrate ethanolic soln (25 ml) was added to 2-fluoro-6-chloropurine (I, 1.5 g, 8.7 mmoles) dissolved in EtOH (25 ml) at 5°. After stirring at 5° for 1 hr, the resulting ppt was collected by filtration, and dried to yield 2-fluoro-6-hydrazinopurine (XXI), 1.3 g (87%) of thin needles, mp 142°. *Anal.* ($C_5H_6N_6F$) C, H, N, F.

When XXI (20 mg) was boiled in H_2O (5 ml) and Raney Ni (50 mg) for 2 hr, the resulting soln showed uv spectra and R_f values identical with those of 2-fluoroadenine (VIII).

6,6-Bis(2-fluoroadenine) (XXII).—Solns of 2-fluoro-6-chloropurine (I, 1.20 g, 6.9 mmoles) in EtOH (25 ml) and 2-fluoro-6-hydrazinopurine (XXI, 1.15 g, 6.9 mmoles) in 70% aq EtOH (25 ml) were combined. Anhyd NaOAc (0.67 g, 7.6 mmoles) was added, and the mixt was refluxed for 6 hr and kept at 25° overnight. The ppt which formed was collected by filtration and repeatedly washed with H_2O and EtOH to yield 1.03 g (quant) of a yellow microcryst product, mp >300°. *Anal.* ($C_{10}H_6N_{10}F_2$) C, H, N. F test was positive.

Treatment of XXII with Raney Ni.—A suspension of XXII (0.5 g, 1.6 mmoles) in H_2O (25 ml) and Raney Ni (3 g) was refluxed for 12 hr. The reaction mix was filtered when hot, the Ni was washed with boiling H_2O, and the combined filtrates were evapd to dryness under reduced pressure. The residue was suspended in H_2O (5 ml), filtered, and dried to yield 70 mg (14%) of 2-fluoroadenine (VIII).

2-Hydroxylamino-6-aminopurine (2-Hydroxylaminoadenine) (XXIII).—A suspension of VIII (0.30 g, 1.8 mmoles) in 0.6 *M* ethanolic $HONH_2$ (300 ml) and 0.3 ml of 30% aq soln of $HONH_2 \cdot HCl$[22] was refluxed for 6 hr and kept at 25° overnight. The resulting ppt was collected by filtration, washed with H_2O, and dried to yield 0.17 g (53%) of microneedles: mp 270° dec; uv_{max} (pH 3.0) 242 (shoulder) (ϵ 12.2 × 10^3), 280 (10.1 × 10^3), (pH 7.0) 253 (8.6 × 10^3), 274 (9.1 × 10^3); pK_a = 4.74 (±0.04). *Anal.* ($C_5H_6N_6O \cdot 0.33H_2O$) C, H, N. XXXIII gave blue-green color with $FeCl_3$ soln (HONH) and deep orange-brown color with 1 *N* NaOH (azoxy formation). Boiling of XXIII with 5% ethanolic hydrazine for 3 hr gave a soln with uv spectra and R_f values identical with those of 2,6-diaminopurine (XVIII).

2-Hydroxylaminopurine (XXV).—A soln of 2-fluoropurine[6] (XXIV, 1.2 g, 8.5 mmoles) in 1 *M* ethanolic $HONH_2$ was refluxed for 10 hr and kept at 25° overnight. The resulting ppt was collected and washed with cold H_2O to yield a pale yellow cryst material, 0.83 g, mp 260° (exploded when inserted at 250°). Upon concn of the filtrate to about 30 ml, a second crop (0.31 g, mp 260°, expl) was obtd (yield, 87%). An anal. sample was obtd by thorough washing of the first ppt with 90% aq MeOH at 25°: uv_{max} (pH 7.0) 233 (ϵ 8.6 × 10^3), 346 nm (13.6 × 10^3); pK_{a_1} = 2.08 (±0.05), pK_{a_2} = 8.52 (±0.1). *Anal.* ($C_5H_4N_5O \cdot 0.33H_2O$) C, H, N. A suspension of XXV gave an intense dark blue color with $FeCl_3$ soln. When XXV was dissolved in 2 *N* NaOH an orange soln was obtd, but in contrast to 6-hydroxylaminopurine,[2] no ppt of the corresponding azoxy derivative was observed. The uv spectra of this soln showed profound decompn. A sample of XXV (10 mg) was dissolved in 5% aq NH_3 (5 ml) and Raney Ni (50 mg) was added. After boiling for 15 min the resulting soln showed uv spectra and R_f values identical with those of 2-aminopurine.[13]

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(22) A. Giner-Sorolla, S. A. O'Bryant, J. H. Burchenal, and A. Bendich, *Biochemistry*, **5**, 3057 (1966).

Xylo- and Arabinofuranosylthioguanine and Related Nucleosides Derived from 2-Acetamido-6-chloropurine[1]

WILLIAM W. LEE,* ABELARDO P. MARTINEZ, ROBERT W. BLACKFORD, VICTOR J. BARTUSKA, ELMER J. REIST, AND LEON GOODMAN[1]

Life Sciences Research, Stanford Research Institute, Menlo Park, California 94025

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9-(β-D-Xylo- and 9-(α- and β-D-arabinofuranosyl)thioguanine (**1**, α-**5**, and β-**5**) have been synthesized. The Hg derivative of 2-acetamido-6-chloropurine gave 9-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)- and 9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-2-acetamido-6-chloro-9*H*-purine (**9** and **10**, respectively) on reaction with the appropriate halo sugar. Treatment of **9** and **10** with NaSH and deacylation gave α-**5** and **1**, respectively. Compd **10** was converted to the xyloside of 2-amino-6-chloropurine (**11**) and guanine (**2**). Both of these could be converted through several intermediates to the 2′,3′-anhydronucleoside intermediate **15g**. Cleavage with NaOAc in aq DMF afforded 9-(β-D-arabinofuranosyl)guanine (β-**6**). Appropriate acylation, followed by thiation and deacylation, gave β-**5**, which was active against leukemia L1210 in mice; the other nucleosides tested were inactive.

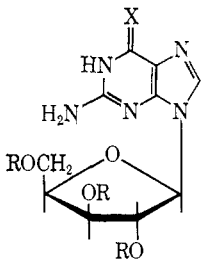
Many compounds with antitumor activity have been found among purines and nucleosides. Thioguanine and thioguanosine, for example, are useful carcinostatic agents.[2] Certain thioguanine nucleosides synthesized in these laboratories—*e.g.*, 3′-deoxythioguano-

(1) This work was performed under the auspices of Chemotherapy, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed here are those of the authors and do not necessarily reflect those of Chemotherapy.

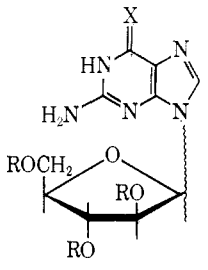
(2) J. A. Stock in "Experimental Chemotherapy," Vol. IV, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, 1966, pp 134–35.

sine,[3,4a,b] 3′-*O*-methylthioguanosine,[3,4a,c] and both anomers of 2′-deoxythioguanosine[4a,b,5]—have shown antitumor activity. Particularly interesting is α-2′-deoxythioguanosine, which is the first α-nucleoside with antitumor activity. This α-nucleoside is incorporated into the DNA of murine tumor cells.[6]

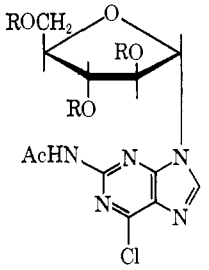
Other nucleosides of fraudulent sugars have shown antitumor activity. Thus, the β-D-arabinofuranosides of cytosine,[7] 6-mercaptopurine,[7a] and adenine[8a] have demonstrated antitumor activity, as have the β-D-xylofuranosides[8b] of adenine and 6-mercaptopurine.[9] These facts suggest that 9-(β-D-xylofuranosyl)thioguanine (**1**) and the α and β anomers of 9-(D-arabinofuranosyl)thioguanine (**5**) be synthesized for antitumor evaluation. The synthesis and some bioassay results are reported here. Other biological data have been published already for **1**;[10] further studies of **2** and β-**5** are in progress in other laboratories.



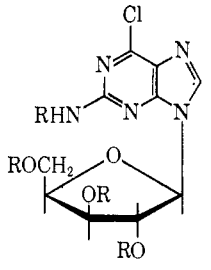
1, X = S; R = H
2, X = O; R = H
3, X = S; R = Ac
4, X = O; R = Ac



5, X = S; R = H
6, X = O; R = H
7, X = S; R = Ac
8, X = O; R = Ac



9, R = Bz



10, R = Ac
11, R = H

For the synthesis of **1** and α-**5**, use of the Hg derivative of 2-acetamido-6-chloropurine[5] seemed an attractive route.[3] Its application to the synthesis of α-**5** was straightforward. Thus, the reaction of the Hg derivative of 2-acetamido-6-chloropurine and 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl chloride[11] gave the blocked α-nucleoside **9**, which was treated with 2-mercaptoethanol and base[3] to afford 9-(α-D-arabinofuranosyl)guanine (α-**6**), and with NaSH in MeOH to give 9-(α-D-arabinofuranosyl)thioguanine (α-**5**).

The synthesis of 9-(β-D-arabinofuranosyl)thioguanine (β-**5**) seemed feasible either by (a) transformation of β-D-xylofuranosylthioguanine (**1**) *via* the epoxide **15t**[12] to β-**5**, or (b) by thiation of β-arabinofuranosylguanine (β-**6**)[13] that was suitably protected (*e.g.*, as β-**8**). The required amounts of β-**6**, not attainable by the original synthesis,[13] seemed available by the transformation of 9-(β-D-xylofuranosyl)guanine (**2**) *via* the epoxide **15g** to **6**. Either route (a or b) required that large amounts of **10** be accessible.

Reaction of the Hg derivative of 2-acetamidochloropurine with 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide[14] afforded the blocked nucleoside **10**, which required purification by Florisil column chromatography. Nucleoside **10** was converted with $HS(CH_2)_2OH$ and base to 9-(β-D-xylofuranosyl)guanine (**2**);[15] with NaSH and base to the xylofuranosylthioguanine **1**; or with methanolic NH_3 to **11**. Large amounts of these intermediates were available by this route.

Attempts to convert **1** to β-**5** proceeded satisfactorily through the initial steps. Because of good solubility in acetone, **1** could be smoothly acetonated to the isopropylidene derivative **12t** (see Scheme I) using $EtSO_3H$ rather than the less available di-*p*-nitrophenyl hydrogen phosphate.[19] Mesylation of **12t** resulted in the uptake of more than one mesyl group, but, after base treatment with NaHS or in the presence of mercaptoethanol the mesyl derivative **13t** was obtained. CF_3CO_2H converted **13t** to **14t**, which yielded the epoxide **15t** on treatment with NaOMe. Efforts to convert **15t** to β-**5** with $PhCO_2Na$ in 95% aq DMF gave an unidentified product that, although not a disulfide, had lost the properties of a thioguanine. An authentic disulfide (**17**) derived from xylofuranosylthioguanine (**1**) was prepared for comparison.

The desired β-**5** was finally obtained by route b. The aminochloropurine nucleoside **11** was readily acetonated (to **12c**; Scheme I) and then mesylated to give **13c**. Reaction with $HS(CH_2)_2OH$ and base afforded **13m**, which, when refluxed several hours in 70% HOAc, gave the mesyl guanine nucleoside **14g**. Treatment with methanolic NaOMe yielded the epoxide **15g**, which was readily isolated as the Na salt. Either NaOAc or NaO_2CPh in hot 95% aq DMF opened the epoxide **15g** at C-3′ to give β-**6**. No C-2′ opening (<3% detectable) was found. Starting with xylofuranosylguanine (**2**), the same series of transformations (**2** → **12g** → **13g** → **14g**) could be carried out. The deacetonation step (**13** → **14**) with both **13m** and

(3) G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **32**, 859 (1967).

(4) (a) These compds were screened for antitumor activity in the mouse leukemia L1210 systems under the auspices of Chemotherapy, National Cancer Institute, according to the protocol in *Cancer Chemother. Rep.*, **25**, 1 (1962); (b) A. Goldin, H. B. Wood, Jr., and R. R. Engle, *ibid.*, *Suppl.*, **1**, 1 (1968); (c) unpublished result.

(5) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963).

(6) G. A. LePage, *Can. J. Biochem.*, **46**, 655 (1968).

(7) (a) A. P. Kimball, B. Bowman, P. S. Bush, J. Herrior, and G. A. LePage, *Cancer Res.*, **26**, 1337 (1966); (b) P. J. Burke, A. H. Owens, Jr., J. Colsky, B. I. Shnider, John H. Edmonson, A. Schilling, Harvey S. Brodovsky, H. J. Wallace, Jr., and T. C. Hall, *ibid.*, **30**, 1512 (1970).

(8) (a) J. J. Brink and G. A. LePage, *Can. J. Biochem.*, **43**, 1 (1965); (b) D. B. Ellis and G. A. LePage, *Mol. Pharmacol.*, **1**, 231 (1965).

(9) K. Sato, G. A. LePage, and A. P. Kimball, *Cancer Res.*, **26**, 741 (1966).

(10) T. Kaneko and G. A. LePage, *ibid.*, **30**, 699 (1970).

(11) A. K. Bhattacharya, R. K. Ness, and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 428 (1963).

(12) In Scheme I, R = SH and OH are written as a matter of convenience although they actually exist in the thiolactam and lactam forms.

(13) E. J. Reist and L. Goodman, *Biochemistry*, **3**, 15 (1964).

(14) O. P. Crews, Jr., and L. Goodman in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1968, p 139.

(15) Other routes to **2** are less suitable for large amounts. Thus, the Friedel-Crafts method of nucleoside synthesis[16] has been used for **2**;[17] 2,6-dichloropurine has also been converted to **2** in a multistep synthesis.[18]

(16) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.*, **16**, 1076 (1968).

(17) W. W. Lee, A. P. Martinez, and L. Goodman, *J. Org. Chem.*, **36**, 842 (1971).

(18) S. Suzaki, A. Yamazaki, A. Kamimura, K. Mitsugi, and I. Kumashiro, *Chem. Pharm. Bull.*, **18**, 172 (1970).

(19) A. Hampton, *J. Amer. Chem. Soc.*, **83**, 3640 (1961).

TABLE I

XYLO- AND ARABINOFURANOSYL NUCLEOSIDES

Compd	Yield, %[a]	Mp, °C[a]	Solv[b]	Chromatog[c]	Formula	Anal.[d]
1	(55)	205–208 (205–208)	W	0.61 TA	$C_{10}H_{13}N_5O_4S \cdot H_2O$	C, H, N, S
2	53 (80)	238–240	W	0.15 PB 0.60 PE	$C_{10}H_{13}N_5O_5 \cdot H_2O$	C, H, N
3	22 (64)	253–256 (238–240)	M	0.90 TA	$C_{16}H_{19}N_5O_7S$	C, H, N, S
4	52 (69)	233–234	C	0.58 TA	$C_{16}H_{19}N_5O_8$	C, H, N
α-5	27	219–221	W	0.35 PH 0.63 PE	$C_{10}H_{13}N_5O_4S$	C, H, N, S
β-5	38 (45)[e]	237–237.5	W	0.67 TA	$C_{10}H_{13}N_5O_4S \cdot 0.25H_2O$	C, H, N
α-6	(50)	>300	W		$C_{10}H_{13}N_5O_5 \cdot 1.5H_2O$	C, H, N
α-7	10	221–223 dec			$C_{16}H_{19}N_5O_7S$	C, H, N, S
β-7	33 (80)[f]	210–212	E	0.80 TB	$C_{16}H_{19}N_5O_7S \cdot 0.5H_2O$	C, H, N
9	(29)[g]	195–196	EA	0.56 TD	$C_{33}H_{26}ClN_5O_8$	C, H, N, Cl
10	(45)[g]				$C_{18}H_{20}ClN_5O_8$	C, H
11	25 (90)	97–112	C–DM	0.73 TA	$C_{10}H_{12}ClN_5O_4$	C, H, Cl
12c	37 (100)	*h*	F–S	0.66 TC	$C_{13}H_{16}ClN_5O_4 \cdot 0.25Et_2O$	C, H, N, Cl
12g	(78)	282–284 (278–280)	E–W	0.42 TA	$C_{13}H_{17}N_5O_5 \cdot H_2O$	C, H, N
12t	(77)	243–246 dec (243–246 dec)	W	0.77 TA	$C_{13}H_{17}N_5O_4S \cdot 1.75H_2O$	C, H, N
13c	(77)[g]	119–120	EA–S	0.84 TC	$C_{14}H_{18}ClN_5O_6S \cdot 0.33EtOAc$	C, H, N
13g	(86)	246–247 (242–244)	M	0.64 TA	$C_{14}H_{19}N_5O_7S$	C, H, N
13m	(85)	204–205		0.57 TA	$C_{16}H_{23}N_5O_7S_2 \cdot 0.4EtOH$	C, H,N
13t	(52)	229–231 (229–231)	E–W	0.60 TC	$C_{14}H_{19}N_5O_6S_2$	C, H, N, S
14g	68 (100)	*h*	EA	0.54 TA	$C_{11}H_{15}N_5O_7S$	C, H, S
14t	85 (91)	203–204	W	0.71 TA	$C_{11}H_{15}N_5O_6S_2 \cdot H_2O$	C, H, N, S
15g[i]	(80)		M	0.26 TA 0.56 PE	$C_{10}H_{10}N_5O_4Na \cdot 0.5MeOH \cdot 0.5H_2O$	C, H, N
15t	33 (52)	>300 dec	W	0.55 TA	$C_{10}H_{11}N_5O_3S \cdot 0.66H_2O$	C, H, S
17	93		W	0.45 TA	$C_{20}H_{24}N_{10}O_8S_2 \cdot 2H_2O$	C, H, N
18	60	207–208	M	0.57 TA	$C_{11}H_{15}N_5O_5$	C, H, N

[a] Yield data and melting point values are for anal. samples except values in parentheses, which are for homogeneous products suitable for the next reaction. [b] Crystn or trituration solvents are: DM, 1,2-dimethoxyethane; C, $CHCl_3$; D, DMF; E, EtOH; EA, EtOAc; F, Et_2O; M, MeOH; S, Skellysolve B (essentially hexane, bp 60–68°); W, H_2O. [c] Solvent systems for tlc are MeOH–EtOAc in various ratios: TA (30:70), TB (20:80), TC (10:90); TD is $CHCl_3$–EtOAc (65:35). Solvent systems for paper chromatography are: PB, *n*-BuOH–H_2O (saturated); PE, 5% Na_2HPO_4, pH 8.9; PH, *n*-BuOH–HOAc–H_2O (5:2:3). [d] Where analyses are indicated only by symbol of the elements, anal. results obtained were within ±0.4% of theoretical values. [e] Overall yield from **15g**. [f] Overall yield from β-**6**. [g] After column chromatography. [h] Solid foam. [i] As the Na salt.

13g was more satisfactory with hot aq AcOH than with CF_3CO_2H.

The β-arabinofuranosylguanine β-**6** could be acetylated (to β-**8**), then heated in pyridine with P_2S_5 to afford β-**7**, and deacylated with methanolic NaOMe to yield β-**5**. β-**5** has been obtained in 45% yield from the Na salt of the epoxide **15g** without isolating the intermediates. The route *via* **2** provided the best overall yield.

In the Experimental Section, only representative procedures are given. The properties of the compds and intermediates are summarized in Table I.

The antitumor activity of these compds was determined against L1210 mouse leukemia.[4a] The results for the target nucleosides, summarized in Table II, show that β-D-arabinofuranosylthioguanine (β-**5**) is active and has low toxicity. The other compds in Table I are not active against L1210; however, other biological activity has been reported for **1**,[10] and **2** is under study by other laboratories.

At doses of 400 mg/kg, the intermediates **3**, α-**7** (200 mg/kg), **9**, **11**, **12c**, **12g**, **12t**, **13c**, **13m**, **13t**, **14c**, **14t**, **17**, and **18** were inactive in the L1210 test. Compound **4** was inactive against KB cells in tissue culture.

Experimental Section

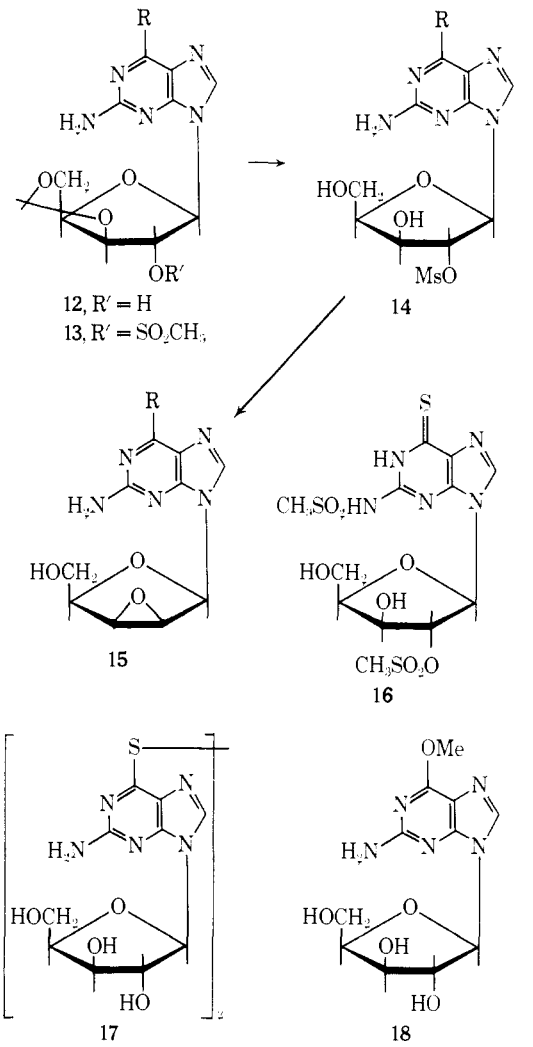
Melting points were determined in a Fisher-Johns apparatus and were not corrected. Optical rotations were measured with a Perkin-Elmer Model 141 automatic polarimeter. Paper chromatograms were run by the descending technique on Whatman No. 1 paper. Tlc were run on silica gel HF (E. Merck AG Darmstadt). The solvent systems are listed in Table I. All spots were detected by uv light. All solns were dried ($MgSO_4$) and were concd in a rotatory spin evaporator *in vacuo* with a bath temp of <50° unless otherwise noted. Celite is a diatomaceous earth product of Johns-Manville. Florisil is an activated Mg silicate product of the Floridin Co.

Nucleosides of 2-Acetamido-6-chloropurine. Nucleosides 9 and 10.—The general procedure[3] for the reaction of the Hg deriv[5] of 2-acetamido-6-chloropurine (deposited on Celite) with a halo sugar was followed. To prep 2-acetamido-6-chloro-9-(tri-*O*-benzoyl-α-D-arabinosyl)-9*H*-purine (**9**), 21 mmoles of 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl chloride[11] and 9.9 g (22 mmoles) of the Hg deriv were refluxed in xylene for 6 hr to give 8.2 g of a yellow foam. This was chromatographed through 300 g of silica on a 2.4 × 24 cm column, eluting with a solvent mixt changing from 100% $CHCl_3$ to 100% EtOAc to give 4.02 g (29%) of homogeneous **9**, which was crystd to give the anal. sample of **9**: uv max ($CHCl_3$) 238 nm (ε 39,300), 258 sh (13,000), 285 (12,100); other properties are shown in Table I. An attempt to prep **9** by the reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinofuranose with the Hg derivative of 2-acetamido-6-chloropurine in the presence of $TiCl_4$[20] gave a nucleoside product that was not the desired **9**.

In the same way, 2-acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-9*H*-purine (**10**) was obtained by the reaction of 2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl bromide[14] with an equimolar amt of the Hg deriv of 2-acetamido-6-chloropurine in refluxing xylene for 2.5–4.5 hr. Chromatog of the product through a column of 100–200 mesh Florisil (6 times weight of crude **10**) with an elution solvent changing from 100% $CHCl_3$

(20) D. H. Murray and J. Prokop, ref 14, p 193.

SCHEME I



c series, R = Cl; **g** series, R = OH;[12] **m** series, R = SCH_2CH_2OH; **t** series, R = SH[12]

to 100% EtOAc afforded the homogeneous **10** in yields of 25–45% as a solid foam. The 45% yield was obtd on a run that gave 27 g of chromatographically purified **10**: uv max (pH 7) 258 nm (ε 11,300), 286 nm (11,200).

Thioguanine Nucleosides (1) (α-5).—Treatment of chromatographically purified **9** and **10** with hot methanolic NaHS, then with NaOMe, according to the published procedure,[3] afforded the corresponding deacylated nucleosides, 9-(α-D-arabinofuranosyl)-guanine (**α-5**), $[\alpha]^{26}D$ + 85 (*c* 1.0, 0.1 *N* NaOH), and 9-(β-D-xylofuranosyl)guanine (**1**), $[\alpha]^{20}D$ − 82.4 (*c* 0.50, DMF); other properties are given in Table I. When crude unpurified **9** or **10** was used, it was difficult to isolate any cryst **α-5** or **1**.

A longer route from **10** to **1** (*via* **2** → **4** → **3** → **1**) offered no advantages except when using unpurified **10**. Then the org solvent sol **4** was separable from the inorg salts. The thiation of **4** with P_2S_5 in pyridine gave good yields of **3** at 80°; at reflux temp, darker prod in poorer yields were obtained. In contrast to this, the thiation of the arabinoside **β-8** (see preparation of **β-5** below) proceeded well at reflux temp, but not at 80°. When **10** was treated with methanolic NaOMe, **18** was readily obtained.

Guanine Nucleosides 2 and α-6.—Refluxing the nucleosides **9** and **10** with NaOMe and 2-mercaptoethanol[3] in MeOH afforded the corresponding guanine nucleosides 9-(α-D-arabinofuranosyl)-guanine (**α-6**) and 9-(β-D-xylofuranosyl)guanine (**2**), $[\alpha]^{21}D$ − 33.2 (*c* 0.25, H_2O). The xyloside **2** gelled readily, especially from solns contg salts or impurities. The initial anal. sample was obtained by acetylation to the cryst 9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)guanine (**4**) and then deacylation. Subsequently, cryst **2**, obtained through purification of its Na salt,[21] was used to seed all crystn. The properties of cryst **2** before recrystn were: mp 235–240°; $[\alpha]^{21}D$ − 33.2 (*c* 0.25, H_2O); $[\alpha]^{21}D$ − 54.4 (*c* 0.50, DMF). Recrystn gave the anal. pure **2**·H_2O: $[\alpha]^{21}D$ − 36.5 (*c* 0.25, H_2O) and other properties (see Table I) agreeing well with those for **2** obtd by other routes.[17,18]

TABLE II

ANTITUMOR ACTIVITY AGAINST LEUKEMIA L1210[a]

Compd	Dose, mg/kg	Survivors	Wt diff (*T*/*C*), g	Increase in survival (*T*/*C*), %
1	400[b]	6/6	−1.8	115
	400	6/6	−1.5	97
2	80[b]	5/6	−0.7	117
	400	0/6		
	200	1/6		
	100	5/6	−4.4	105
	80	6/6	−3.0	108
	40	6/6	−0.8	103
	20	6/6	−1.0	97
	10	6/6	−0.3	105
	5	6/6	0.4	102
β-5	400	6/6	−2.7	152
	600	5/6	−2.7	144
	400	6/6	−2.6	159
	267	6/6	−1.7	152
	178	6/6	−2.2	152
α-5	400[b]	6/6	−1.8	101
	200	4/4	−0.3	98
	100	4/4	−0.6	102
	50	4/4	−1.1	96
β-6[c]	400[d]	6/6	−3.0	100
	400	4/4	−1.0	97
	200	4/4	0.8	97
	100	4/4	0.2	100
α-6	200[d]	2/6	−4.8	
	40[d]	6/6	0.7	97
	400	2/4	0.1	
	200	4/4	−0.3	101
	100	4/4	0.5	101

[a] See ref 4a for screening protocol against lymphoid leukemia L1210 in BDF_1 mice. The doses are single injections unless otherwise noted. An increase in survival time (*T*/*C*) of 125% or more is considered a positive result. [b] Nine doses. [c] Compd **β-6** was inactive against Walker 256 (im) in rats at 400 mg/kg dose and inactive against KB cells in tissue culture tests. [d] Fifteen doses.

2-Amino-6-chloro-9-(β-D-xylofuranosyl)-9*H*-purine (11).—A soln of 6.87 g (14.6 mmoles) of crude **10** was kept in 110 ml of MeOH satd with NH_3 for 5 hr at 5°[22] to afford 1.1 g (25%) of **11**, mp 97–112°, $[\alpha]^{22}D$ − 14.4 (*c* 0.50, DMF); homogeneous by tlc, which was anal. From chromatographically purified **10**, yields up to 90% of **11** were attainable.

9-(3,5-*O*-Isopropylidene-β-D-xylofuranosyl)nucleosides (12).—To 1.75 g (5.51 mmoles) of **1** in 160 ml of Me_2CO was added 3 ml of 2,2-dimethoxypropane and 2.4 ml of $EtSO_3H$, and the mixt was stirred for 2 hr. Addn of another 2.0 ml of 2,2-dimethoxypropane and 1.0 ml of $EtSO_3H$ effected complete soln. After another 1.5 hr of stirring, the mixt was poured into 175 ml of satd $NaHCO_3$ soln. The inorg salts were removed, and the filtrate was evapd to dryness. The residue was stirred with 75 ml of H_2O for 3 hr to yield 1.45 g (73%) of white homogeneous 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)thioguanine (**12t**). 2-Amino-6-chloro-9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)-9*H*-purine (**12c**) and the corresponding guanine (**12g**) were obtained in a similar manner, except that **12g** required 24 hr for reaction. In the work-up of **12g**, vigorous bumping was encountered unless the aq acetone soln was cautiously evapd until most of the acetone was gone and then cooled to ppt the **12g**.

(21) Purification *via* the Na salt was successfully demonstrated with the pyranose form of **2**. See A. P. Martinez and W. W. Lee, *J. Org. Chem.*, **34**, 416 (1969).

(22) J. F. Gerster, B. C. Hinshaw, R. K. Robins, and L. B. Townsend, *ibid.*, **33**, 1070 (1968).

9-(3,5-*O*-Isopropylidene-2-*O*-mesyl-β-D-xylofuranosyl)-nucleosides (13).—A soln of 0.55 g (1.7 mmoles) of **12g** in 10.5 ml of dry pyridine was stirred and cooled in an ice bath while 1.0 ml (1.48 g; 13 mmoles) of MsCl was added over 15 min. After stirring in the ice bath for 1 hr and at room temp for 1 hr, the reaction mixt was replaced in the ice bath and treated with 15 ml of 2 *N* NaOH. The dark red soln, after standing 15 min at room temp, was neutralized (pH 7) with HOAc, and the amber soln was evapd. The residue was slurried with 50 ml of CH_2Cl_2 and 25 ml of PhMe and evapd. This process was repeated. The residue was taken up in 25 ml of H_2O to give a yellow solid that was collected and dried [3 hr at 56° (1 mm)] to afford 0.59 g (86% yield) of homogeneous 9-(3,5-*O*-isopropylidene-2-*O*-mesyl-β-D-xylofuranosyl)guanine (**13g**); recrystn afforded the colorless anal. sample of **13g**.

For the thioguanine series, after 0.40 g (1.08 mmoles) of **12t** had been treated with 0.80 ml (10 mmoles) of MsCl in 8 ml of pyridine, the soln was dild with 40 ml of PhMe and evapd at 30° to a residue R. The residue R was dissolved in 20 ml of a MeOH soln of NaHS (20 ml of 1 *N* NaOMe satd with H_2S at room temp), heated at reflux for 1 hr, brought to pH 7 with HOAc, and evapd. The residue was tritd with H_2O and then MeOH to give 0.20 g (45%) of **13t**. Boiling with EtOH–H_2O (2:1) gave the anal. sample of **13t**: uv max (pH 1) 263 nm (ε 8200), 342 nm (22,300); (pH 7) 257–265 nm (ε 7600), 341 nm (23,700); (pH 13) 252 nm (ε 14,300), 257 nm (sh) (7100), 318 nm (20,000); other properties are shown in Table I. In a second expt, mesylation product from **12t** was treated with $HS(CH_2)_2OH$ and NaOMe (instead of NaHS) to give a 53% yield of **13t**.

In a third expt, run like the first, the residue R was not converted to **13t** with base but was dissolved in CH_2Cl_2, washed with H_2O, and evapd. The residue R was unstable and not completely characterized, but it appears to have 2 mesyl groups by nmr and had R_f 0.80 by tlc in solvent TC (**12t** and **13t** had R_f 0.65 and 0.40, respectively, on the same plate). The mother liquors, after standing 2 months, afforded some light yellow crystals of incompletely characterized compd Q, mp 168–169°; uv max (pH 1) 274 nm (ε 7800), 336 nm (20,500); (pH 7) 263 nm (ε 14,300), 340 (24,800); (pH 13) 258 nm (ε 24,800), 313–319 nm (17,800); ir, no absorption of $MeSO_3^-$ at 9.4 μ; R_f 0.50 in TA. *Anal.* ($C_{12}H_{17}N_5O_8S_3 \cdot H_2O$): C, H, N, S. This lack of Ms^- absorption in the ir and the similarity of the uv to that of a thioguanine nucleoside suggest that Q may be **16**·H_2O.

For the 2-amino-6-chloropurine series, 1.1 g (3.16 mmoles) of **12c** was treated with 1.0 ml (12.9 mmoles) of MsCl in 20 ml of pyridine for 60 min at ice temp and 30 min at ambient temp, recooled, and combined with 25 ml of 1 *N* NaOH. The soln was evapd at 30°, and the residue was partitioned between 75 ml each of CH_2Cl_2 and H_2O. The org layer was washed and evapd. The residue, a solid foam, was chromatographed on a 2.2 × 37 cm column contg 40 g of Florisil with an elution solvent changing from 100% $CHCl_3$ to 100% EtOAc to give 1.10 g (77%) of homogeneous **13c**, which was recrystd to give the anal. sample: uv max (pH 1) 257 nm (ε 8500), 318 nm (9650); (pH 7) 257 nm (ε 8500), 317 nm (10,300). In subsequent runs, it was sometimes feasible to eliminate the chromatographic purification.

The 6-(2-mercaptoethanol) nucleoside (**13m**) was prepd by treating a soln of 0.24 g (0.57 mmole) of **13c** in 15 ml of MeOH with 0.60 ml (8.6 mmoles) of 2-mercaptoethanol and 2.0 ml of 1 *N* methanolic NaOMe. The soln was heated for 1 hr (oil bath temp 62–65°), acidified with HOAc, and evapd. The residue was tritd with H_2O and recrystd to afford the anal. sample of **13m** (see Table I). Treatment of **13m** with base did not give **13g** cleanly. When **13m** was treated with NaOH in MeOH–H_2O (3:2) at 70° (bath temp) for 1.5 hr, one of the products that could be isolated was **12g**, showing that a Ms group can be lost under these conditions.

9-(2-*O*-Mesyl-β-D-xylofuranosyl)thioguanine (14t) and -guanine (14g).—A soln of 1.17 g of **13t** in 50 ml of 90% CF_3COOH (TFA) was stirred 10 min at room temp,[23] dild with 50 ml of PhMe and evapd to afford 1.25 g of the TFA salt of **14t**. The salt was dissolved in a min of H_2O, NH_4OH was added to pH 6, and the cryst **14t** was collected (Table I).

(23) J. E. Christensen and L. Goodman, *Carbohyd. Res.*, **7**, 510 (1968).

Compd **13m** was simultaneously deacetonated and converted to the guanine nucleoside **14g** in good yield by refluxing in 70% HOAc for 3.5 hr. The deacetonation of **13m** with TFA seemed satisfactory, but the product could not be cleanly converted to an epoxide in the next step. Likewise, **13c** was treated with 70% HOAc to afford the presumed **14c** but could not be cleanly converted to an epoxide in the next step.

The isopropylidene group of **13g** was removed by heating for 15 min in 70% HOAc to give **14g** in high yields. Reaction of **13g** with TFA gave a much poorer yield of **14g**.

Prepn of Epoxides 15.—A 28.0-ml portion of 1 *N* NaOMe in MeOH was added to 1.67 g (4.63 mmoles) of **14g** in 140 ml of MeOH in a stoppered flask. After stirring for 6 hr at room temp, the mixt was refrigerated overnight, and the homogeneous cryst Na salt of 9-(2,3-anhydro-β-D-lyxofuranosyl)guanine (**15g**) was collected (74% after drying). An overall yield of 26.7% from **10** was achieved in one run. This Na salt could be used directly in the next step. Attempts to convert the Na salt to the guanine epoxide **15g** gave intractable gels that could not be crystd.

The thioguanine **14t** was similarly treated with NaOMe in MeOH, then acidified to give the thioguanine epoxide **15t**.

9-(β-D-Arabinofuranosyl)guanine (β-6).—The Na salt of **15g** (1.0 g, 3.2 mmoles) and 1.0 g of anhyd NaOAc in 65 ml of 95% aq DMF were heated 7 hr at reflux and evapd. The residue was extd with 25 ml of DMF (dried over mol sieves), filtered to remove NaOAc, and evapd. The residue was tritd with 50 ml of boiling EtOH and then filtered to afford, after drying, 0.86 g (95%) of β-**6** contg a trace contaminant. This contaminant was apparently not **2**, for acetonation of the reaction mixt (see prepn of **12g**) gave less than 3% (the limit of detection) of **12g**. The β-**6** was recrystd; its properties were identical with the lit. values.[13] In subsequent expts the crude β-**6** was not isolated, but converted to the thioguanine nucleoside β-**5**.

9-(β-D-Arabinofuranosyl)thioguanine (β-5).—A 2.00-g portion of the Na salt of **15g** (6.40 mmoles, calcd Na **15g**·0.5MeOH·0.5-H_2O) was converted to β-**6** as described. This was allowed to react with 20 ml of Ac_2O in 150 ml of pyridine for 45 min at 65–70°. After decompn of excess Ac_2O with MeOH, the reaction mixt was evapd to dryness to afford crude tri-*O*-acetyl nucleoside β-**8**. This was redissolved in 250 ml of pyridine, combined with 11.3 g of P_2S_5, and heated 6.5 hr at reflux under N_2 to give 1.83 g of crude blocked thioguanine nucleoside β-**7**. The crude β-**7** was refluxed for 3 hr with an equiv amt of NaOMe in MeOH under N_2, acidified with HOAc, and evapd to afford crude β-**5**. The product was dissolved in 110 ml of boiling H_2O, and the soln was filtered. After concn to half-vol and cooling, the cryst, anal. pure β-**5** was collected (277 mg, 24.8% overall yield from the Na salt of **15g**). The β-**5** had these properties: $[\alpha]^{21}D$ − 7.2 (*c* 0.25, DMF); uv max (pH 1) 263 nm (ε 8400), 343 nm (21,700); (pH 7) 252 nm (ε 7000) to 268 nm (7200), 342 nm (21,900); (pH 13) 252 nm (ε 13,400), 272 nm (7500), 318 nm (18,800); other properties are listed in Table I. The best overall yield of β-**5** from the Na salt of **15g** was 45%. Attempts to prepare β-**5** from the epoxide **15t** with NaOBz in 95% aq DMF gave a material insol in H_2O and MeOH, which was initially thought to be the disulfide of β-**5**. However, comparison with an authentic disulfide (**17** prepared below) showed that the insol material was not reduced by dithiothreitol or $NaBH_4$ and was not a disulfide.

9-(β-D-Xylofuranosyl)thioguanine Disulfide (17).—Using the procedure of Doerr, *et al.*,[24] 80 mg of **1** in 25 ml of pH 8 phosphate buffer was treated with 0.25 ml of 0.5 *N* I_2 in aq NaI. The colorless **17** that crystd was collected, washed with H_2O, dried, and anal. The disulfide **17** was reduced cleanly to the thioguanine **1** in 2 min on treatment with methanolic dithiothreitol; reduction with $NaBH_4$ also gave some **1**.

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(24) I. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox, *J. Org. Chem.*, **26**, 3401 (1961).