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Guanine, Thioguanine, and Related Nucleosides by the Mercuric Cyanide–Silyl Method. An Improved Synthesis of α-2′-Deoxythioguanosine[1]

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The silyl derivatives of 2-amino-6-chloropurine and 2-acetamido-6-chloropurine react readily with halo sugars in the presence of mercuric cyanide to afford nucleosides in high yields. These can be converted to guanine, thioguanine, and other related nucleosides by standard procedures. By this new and improved method and through a column chromatographic separation described here, α-2′-deoxythioguanosine has been obtained in excellent yield and high anomeric purity, and 9-β-D-xylofuranosylguanine has been obtained in almost twice the yield previously attainable.

α-2′-Deoxythioguanosine (α-TGdR, α-**5**[2a]) is the only α anomer of a nucleoside known to have antitumor activity.[3] The β anomer (β-TGdR, β-**5**) is also an interesting antitumor agent.[3] Studies have shown that both β- and α-TGdR can be phosphorylated and incorporated into the DNA of some murine tumors *in vivo*[4] and of some murine and human tumor extracts *in vitro*.[5] Unlike the β anomer, α-TGdR is not phosphorylated to a significant extent by extracts of normal bone marrows;[5] hence, α-TGdR is less toxic than the β anomer. For these reasons, there is great interest in α-TGdR, and large amounts are needed for additional studies. This article reports a new, improved synthesis of α-TGdR and a means of separating its precursor from that of the β anomer. The new method of synthesis seems generally useful for guanine, thioguanine, and other 2-amino 6-substituted purine nucleosides.[6]

Of the general methods of guanine nucleoside synthesis,[6] none could assure a better yield of α-**5** and a more favorable α:β anomer ratio than the original.[2a] However, the silyl method of nucleoside synthesis merited consideration. It has been extremely useful for pyrimidine nucleosides.[7] Although the silyl method was originally less attractive for purine nucleosides, a recent modification has given improved yields of adenine nucleosides.[8] The silyl method also afforded the possibility of altering the ratio of α:β anomers,[9] at least with pyrimidine nucleosides. On this basis, we investigated the silyl method for the synthesis of α-**5**.

In our first experiments with the silyl derivatives (**1b**) of 2-acetamido-6-chloropurine (**1a**) and 2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl chloride (**2**) we followed the method of Kotick, Szantay, and Bardos,[9a] to see whether the absence or presence of trimethylchlorosilane (TMCS) would allow stereospecific synthesis of α or β nucleosides from purines as well as from pyrimidines.[9a] Our experiments with TMCS are given in Table I. The main conclusion was that the yield of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-*erythro*-pentofuranosyl)-9*H*-purine (**3**) was not sufficient for our purposes, although some variation in α:β ratio (generally *ca.* 1) can be achieved. The yields of **3** represented the anomeric mixture freed of unreacted base and sugar products by column chromatography.

Other pertinent data from Table I show that the use of solvents other than benzene offer no advantages.

(1) (a) This work was carried out under the auspices of Drug Research and Development, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. NIH-71-2070. The opinions expressed in this paper are those of the authors and not necessarily those of Drug Research and Development. (b) Part of this work was presented at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 12–17, 1971.

(2) (a) R. H. Iwamoto, E. M. Acton, and L. Goodman, *J. Med. Chem.*, **6**, 684 (1963), synthesized α-**5**, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-thioguanine, *via* the mercury derivative of 2-acetamido-6-chloropurine. (b) Recently, the L isomers were prepared by the fusion method. See M. J. Robins, T. A. Khwaja, and R. K. Robins, *J. Org. Chem.*, **35**, 636 (1970).

(3) G. A. LePage, I. G. Junga, and B. Bowman, *Cancer Res.*, **24**, 835 (1964).

(4) (a) G. A. LePage and I. G. Junga, *Mol. Pharmacol.*, **3**, 37 (1967); (b) G. A. LePage, *Can. J. Biochem.*, **46**, 655 (1968).

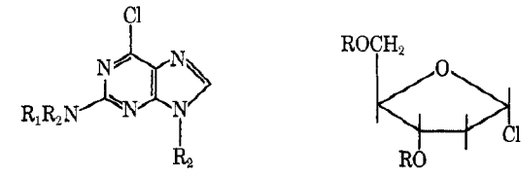
(5) A. Peery and G. A. LePage, *Cancer Res.*, **29**, 617 (1969).

(6) For general methods of guanine nucleoside synthesis, see discussion in G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **32**, 859 (1967).

(7) C. A. Dekker and L. Goodman in "The Carbohydrates Chemistry and Biochemistry," 2nd ed, Vol. 2A, W. Pigman and D. Horton, Ed., Academic Press, New York, N. Y., 1970, p 1.

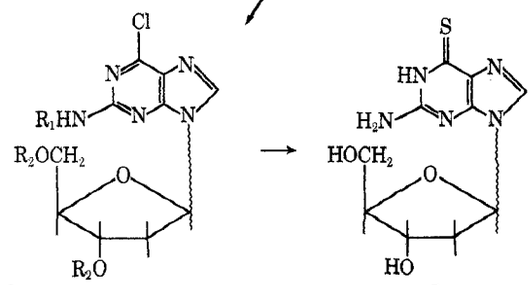
(8) (a) B. Shimizu and A. Saito, *Agr. Biol. Chem. (Tokyo)*, **33**, 119 (1969). (b) K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **36**, 2646 (1971). (c) After the portion of this work on α-**3** was completed, W. Hutzenlaub, R. L. Tolman, and R. K. Robins reported the use of the silyl procedure in the preparation of 8-azaguanosine at the 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 28–April 2, 1971.

(9) (a) M. P. Kotick, C. Szantay, and T. J. Bardos, *J. Org. Chem.*, **34**, 3806 (1969); (b) E. Wittenburg, *Chem. Ber.*, **101**, 1095 (1968).



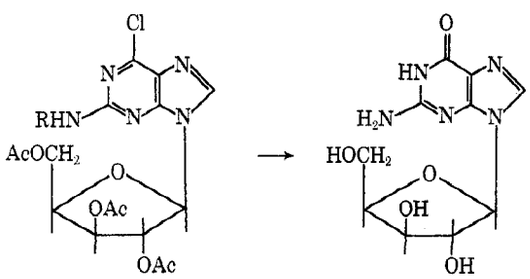
1a, R_1 = Ac; R_2 = H
b, R_1 = Ac; R_2 = $SiMe_3$
c, R_1 = R_2 = H
d, R_1 = R_2 = $SiMe_3$
e, R_1 = H; R_2 = $SiMe_3$

2, R = tolyl



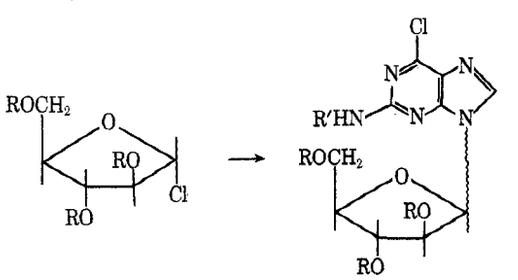
3, R_1 = Ac; R_2 = tolyl
4, R_1 = H; R_2 = tolyl

5



6a, R = Ac
b, R = H

7



8, R = $CH_2C_6H_5$

9, R = $CH_2C_6H_5$; R′ = Ac
10, R = $CH_2C_6H_5$; R′ = H

Additives such as mercuric acetate or mercuric cyanide in the presence of excess TMCS promoted decomposition, probably because the salts could not function as HCl acceptors under these conditions.[10] Excess base **1b** was harmless, but excess halo sugar **2** gave lower yields. A long reaction time (expt 7) resulted in some N-deacylation to 2-amino-6-chloro-9-(2-deoxy-3,5-di-*O*-toluoyl-D-*erythro*-pentofuranosyl)-9*H*-purine (**4**), contaminated by a second nucleoside that was not investigated. Initially the mixture of **4** and its contaminant was thought to be the two **7** isomers of **3** on the basis of our experience with N^2-acylguanine nucleosides[11a] and the observations of Miyaki and Shimizu[11b] on 7-substituted and 9-substituted N^2-acetylguanines. However, the **4** in this mixture was later found to be identical with the authentic **4**.

When no excess TMCS was used (expt 5) the yield was lower. Experiment 16 confirmed the considerable stability of **2** in the presence of TMCS.[9a] While TMCS had no adverse effect on the stability of **2**, hydrogen, chloride did; the same decomposition products, *e.g.*, furfuryl *p*-toluate, observed by previous investigators[12] were found.

In another series of experiments, we examined the condensation of **1b** and **2** under other conditions. Wittenburg[13] had successfully altered the $\alpha:\beta$ ratio of anomers by the use of added salts in the synthesis of pyrimidine nucleosides by the silyl method. His studies included some 3,5-di-*O*-acyl-2-deoxyribofuranosyl chlorides.[9b] Pyrstas, *et al.*,[12,14] have altered the yields of $\alpha:\beta$ ratio of anomers in the Hilbert–Johnson reaction of pyrimidines with **2** through proper choice of solvents. We found that with no excess TMCS, but with mercuric cyanide present, **1b** and **2** condensed to give high yields of **3**. The results are given in Table II. The crude yields included the by-products (**4** and others) as well as **3**. In some of the larger runs, there may be several per cent of solvent left in the product. Again, for **3**, the ratio of $\alpha:\beta$ anomers was slightly greater than one. It is noteworthy that the yields were high and reproducible on scaling up; this consistency has not been always observed with some other methods of nucleoside synthesis, *e.g.*, the fusion method.[15]

TABLE I

FORMATION OF **3** FROM **1b**[a] AND **2** WITH TMCS PRESENT

Expt	Mole ratio of 1b:2	Solvent	Temp, °C	Time, hr	Yield, %
1	1	B	80	0.5	48
2	2.2	B	80	6	45
3	1	B	80	18	44
4	1	B	80	7.5	44[b]
5	1	B	80	5	33[c]
6	1	B	80	12	31[d]
7	1	B	30, 45	20, 24	13[d,e]
8	1	DMF	55	60	*f*
9	1	CH_3CN	82	5	*g*
10	1	CH_3CN	25	60	*c, h*
11	1	PhCl	80	18	*c*
12	1	B	80, 25	2, 18	18[i]
13	1	B	50	1	18[j]
14	0.67	B	80	1	23[k,l]
15	0.67	B	80	0.5	22[l]
16	0	B	80	0.5	X[m]

[a] 5–10 mmol. Procedure in Experimental Section. Solvent B is benzene. [b] 30-mmol scale. [c] No excess TMCS used. [d] $Et_3N \cdot HCl$ not removed. [e] Also 6.5% of **4** that contained another unidentified component. [f] $Hg(OAc)_2$ added; black decomposition products and **1a**. [g] Decomposition of sugar. [h] Trace of **4**. [i] $Hg(CN)_2$ added. [j] Reduced pressure. Ratio of $\alpha:\beta \sim 2$. [k] Distilled and added 15% TMCS solution simultaneously. [l] Ratio of $\alpha:\beta \sim 1.5$. [m] No decomposition of **2**.

(10) The excess trimethylchlorosilane could negate the mercury salts as HCl acceptors by these reactions: $Hg(OAc)_2 + 2HCl \rightarrow HgCl_2 + 2HOAc$; $2HOAc + 2Me_3SiCl \rightarrow 2Me_3SiOAc + 2HCl$.

(11) (a) W. W. Lee, A. P. Martinez, and L. Goodman, *J. Org. Chem.*, **36**, 842 (1971); (b) M. Miyaki and B. Shimizu, *Chem. Pharm. Bull.*, **18**, 1446 (1970).

(12) M. Prystaš and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 1900 (1965).

(13) E. Wittenburg, *Chem. Ber.*, **101**, 1614 (1968), and prior work; *e.g.*, ref 9b.

(14) M. Prystaš, J. Farkaš, and F. Šorm, *Collect. Czech. Chem. Commun.*, **30**, 3123 (1965).

(15) W. W. Lee, A. P. Martinez, G. L. Tong, and L. Goodman, *Chem. Ind. (London)*, 2007 (1963).

TABLE II

FORMATION OF 3[a] FROM 1b AND 2 WITH MERCURIC CYANIDE PRESENT

Expt	Millimoles 1b	Mole ratio of 1b:2	$Hg(CN)_2$, mmol	Solvent	Time, min	Yield,[b] %
1	5.0	1	4.8	B	120	84
2	2.5	0.97	6.0	B	45	75
3	5.0	1.2	12.0	B	60	~100
4	17.2	1.2	41	B	60	~100
5	2.4	1.5	4.0	CH_3CN[c]	60	~100
6	42.6	0.83	79	B	60	~100
7	63.8	0.85	127	B	50/60	~100

[a] Procedure in Experimental Section; solvent B is benzene. [b] Crude yield after rapid Florisil columning. Larger runs not always free of solvent. [c] Less by-product (by tlc) than with benzene.

Separation of the anomers at **3** rather than at **5** seemed more feasible from our past experience with similar nucleosides.[11a,16] Also, LePage[4b] had demonstrated that the anomers of **3** could be separated to give α-**3** free of β-**3** (<0.1%) by thick plate chromatography. The method, though laborious and unsuitable for large scale preparative work, does suggest that a practical chromatographic method may be found by further study. After considerable experimentation, the following process was deemed the best. The crude product containing **3** and some nucleoside by-products was crystallized from chloroform–carbon tetrachloride to remove most of the relatively insoluble β-**3**. The mother liquors were then rapidly chromatographed through a short Florisil column to separate sugar by-products and the other nucleoside by-products from **3**. A final separation using a higher ratio of adsorbent to nucleoside afforded the pure α anomer in one fraction and a fraction containing unseparated α- and β-**3** that could be recycled. The yields of pure α-**3** and β-**3** each ranged between 25 and 40% in several runs, tending toward the higher side as the separation process was improved. The anomeric purity of α-**3** obtained by this process was found to be at least 99.5% (the limits of detection) by quantitative chromatography and uv measurement.[4b] The α-**3** and β-**3** were converted to α-**5** and β-**5** by the literature procedure,[2a] thus providing further confirmation of their structures.

The anomeric purity of the α-**3** and β-**3** fractions was monitored during separations by tlc and nmr. The H-8 protons of α-**3** and β-**3** were separable in the nmr obtained with $DCCl_3$ or DMSO-d_6–$DCCl_3$ mixtures as solvent, but not in DMSO-d_6 alone.

Reaction of the silyl derivative **1b** with other halo sugars was examined. Reaction with 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide gave 2-acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-9*H*-purine (**6a**) in consistently higher yields and higher purity than were obtainable from the mercury derivative of 2-acetamido-6-chloropurine.[16] Reaction of **1b** with 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl chloride (**8**) gave a 67% yield of an anomeric mixture of 2-acetamido-6-chloro-9-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)-9*H*-purine (**9**). This crude product required immediate column purification; otherwise, extensive darkening took place. The anomeric ratio, α:β, of **9** was about 1, although nmr suggests it to be slightly richer in the β anomer. By contrast, the reaction of **1b** with the above arabinofuranosyl chloride (**8**) in the *absence* of mercuric cyanide by the method of Glaudeman and Fletcher method[17] gave less than 20% yield of **9**, but with the β anomer predominating. The reaction of unsilylated **1a** with **8** under the same conditions[17] gave negligible amounts of **9** because of poor solubility of **1a**.

The results immediately raised another question: Is the acetyl group in the silyl derivative of **1a** necessary? If not, this would eliminate the need for **1a**. Its preparation from **1c** proceeds only in moderate yield (small scale, 64%;[2a] many larger runs, about 50%) and requires the elimination of overacetylated product.

Treatment of **1c** with hot hexamethyldisilazane afforded a silyl derivative of undetermined structure (probably **1d**, **1e**, or both) which was combined immediately with **2** in hot benzene containing mercuric cyanide. A nucleoside product was formed and gave, after purification, an 80% yield of analytically pure **4** (α:$\beta \sim 1$). A comparison on the same tlc plate showed that the anomeric mixture of **4** was more difficult to separate than that of **3**. However, **4** should be reacetylatable to **3** for the separation. Furthermore, there is the possibility that treatment of **4** with a different acylating agent might give an anomeric mixture easier to separate than **3**.

The silyl derivative of **1c** also reacted with 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide to give, after column purification, an 84% yield of **6b**. That this was a 9-substituted nucleoside was shown by its conversion to authentic **7** in 51% overall yield from **1c**. This represents double the yield of **7** previously obtained *via* the mercury derivative of **1a** (23% from **1c**).[16] Furthermore, the **6b** prepared by this route is so pure that in larger scale runs no column purification was needed before conversion to **7** (see Acknowledgments).

In the same way, the silyl derivative of **1c** reacted with **8** to afford 2-amino-6-chloro-9-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)-9*H*-purine (**10**) in excellent yield after purification. The ratio of β:α was about 1, though slightly richer in β, according to nmr results. The crude **10** was more stable than the crude **9** (obtained *via* TMCS). An interesting side observation is that **1c** itself can be used for nucleoside condensation if it can be dissolved. Thus, heating **1c** in DMF with **2** and triethylamine afforded a 15% yield of **4**, which was identical with an authentic sample, together with another 15% of product assumed to be a nucleoside (by ir and tlc), which was not investigated.

These experiments with several halo sugars show that the silyl derivatives of both **1a** and **1c** are suitable for the preparation of nucleoside intermediates that lead to guanine, thioguanine, and other 2-amino 6-substituted nucleosides in high yields. The use of the silyl derivative of **1a** may be advantageous in cases where anomer separation is required. However, in other cases, the silyl derivative of **1c** is advantageous; it is simpler to obtain. Silylation with hexamethyldisilazane gives cleaner nucleoside products than TMCS. The silyl method is a new, general, and superior method for synthesizing such nucleosides. By this method and

(16) W. W. Lee, A. P. Martinez, R. W. Blackford, V. J. Bartuska, E. J. Reist, and L. Goodman, *J. Med. Chem.*, **14**, 819 (1971).

(17) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 3004 (1963).

the separation technique described, α-2′-deoxythioguanosine can be prepared in quantity.

Experimental Section

Optical rotations were measured with a Perkin-Elmer Model 141 automatic polarimeter. Thin layer chromatograms were run in cyclohexane–ethyl acetate (6:4) on silica gel HF (E. Merck AG Darmstadt) with multiple development (usually five times). All spots were detected by uv light. All solutions were dried with anhydrous magnesium sulfate and were concentrated in a rotatory spin evaporator *in vacuo* with a bath temperature of <50° unless otherwise noted. Celite is a diatomaceous earth product of Johns-Manville. Florisil, an activated magnesium silicate product of the Floridin Co., of 100–200 mesh was used in the column chromatography.

Formation of 3 with TMCS Present.—The general procedure for the preparation of 2-acetamido-6-chloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribofuranosyl)-9*H*-purine is illustrated by expt 4 in Table I. A solution of 10.0 ml (72 mmol) of triethylamine in 30 ml of benzene was added dropwise over 30 min to a stirred mixture of 6.33 g (30 mmol) of 2-acetamido-6-chloropurine (**1a**),[2a] 6 g of 3A molecular sieves, 80 ml of dry (over 3A sieves) benzene, and 8.0 g (74 mmol) of TMCS. The mixture was stirred for 24 hr at room temperature while protected from moisture. [In some cases, this mixture containing the silyl derivative (**1b**) of **1a** and the triethylamine hydrochloride was used directly in the nucleoside condensation. See expt 6 and 7, Table I.] The mixture was filtered, the residue was washed with 80 ml of dry benzene, and the combined filtrate and wash were evaporated to dryness. (In some experiments, this solution was not evaporated, but was was used directly.)

To the residue was added a warm solution of 11.7 g (30.0 mmol) of the chloro sugar **2**[18] in 310 ml of benzene containing 15% TMCS and 10 g of 3A molecular sieves. The mixture was heated at reflux for 7.5 hr (some experiments were heated with distillation of some TMCS and benzene; see expt 13 and 14) and then evaporated to dryness *in vacuo*. The residue was stirred for 30 min in 175 ml of methanol and 250 ml of ethyl acetate and filtered; the filtrate was evaporated to dryness, leaving 16.4 g of residue. This was dissolved in chloroform–ethyl acetate (4:1) and chromatographed through a 3.2 × 61 cm column containing 237 g of Florisil. After the sugar by-products were eluted with 600 ml of chloroform, further elution with 725 ml of ethyl acetate afforded the anomeric mixture of nucleosides (7.2 g, 44%). Recrystallization from 59 ml of chloroform afforded 2.94 g (17.5%) of the β anomer of **3** (which contains a trace of the α anomer), R_f 0.30 and 0.20 (trace). The mother liquors were concentrated and rechromatographed through a column of the same size, eluting with 4.5 l. of chloroform and then 1.2 l. of 5% ethyl acetate in chloroform, to obtain 3.20 g (19%) of the α anomer of **3**, R_f 0.20.

Preparation of 3 with Mercuric Cyanide Present.—The general procedure is illustrated by expt 7 in Table II. The 2-acetamido-6-chloropurine (**1a**, 13.5 g, 63.8 mmol) was silylated as described above. The filtered solution of **1b** and benzene washes (total, about 850 ml) and 32 g of mercuric cyanide were stirred and heated (oil bath, temperature 120°) to 60–65°. The 29.0 g (74.5 mmol) of the chloro sugar **2** was added in one portion, and the stirred mixture was rapidly brought to reflux (about 5 min) and maintained at reflux temperature for 50 min.

The reaction mixture was evaporated to dryness. The residual amber gums were dissolved in 500 ml of methylene chloride, filtered to remove the mercuric cyanide, washed successively with 250 ml of 30% potassium iodide solution and 150 ml of water, dried, and evaporated.

The crude **3** (contains solvent; over 36 g, the theoretical yield) was dissolved in a hot solution of 500 ml of chloroform and 800 ml of carbon tetrachloride, left at room temperature overnight, then chilled for 4 hr in Dry Ice and filtered. The white crystalline **β-3** was thoroughly washed with 800 ml of cold (chilled over Dry Ice) chloroform–carbon tetrachloride (2:3) and dried for 3 hr at 56° (1 mm) to afford 16.4 g (45%) of **β-3** (this contains a little **α-3**). Some amber gums on the walls of the crystallization flask were discarded.

The filtrate from the crystallization was passed through a 5.4 × 32.5 cm column containing 300 g of Florisil, eluting with chloroform–carbon tetrachloride. The first 2.87 l. of eluate contained 3.78 g of sugar products, the next 1.20 l., about 0.27 g of sugar plus other material. When product began to appear in the next 0.375 l., the solvent was changed to ethyl acetate–chloroform–carbon tetrachloride (5:2:3) and the product was rapidly stripped off with 2.13 l., affording fraction A, 21.5 g of mainly crude **α-3** (contains solvent; theoretical yield is 19.6 g). This chromatography operation took about 3.5 hr.

A 9.0-g portion of fraction A was chromatographed through 300 g of Florisil on a 5.4-cm diameter column eluting with chloroform (1.0 l.) and 5% ethyl acetate in chloroform (3.7 l.). Traces of **α-3** appeared toward the end of that fraction. The next two fractions, 10 l. of 7% ethyl acetate in chloroform and 6.0 l. of 10–13% ethyl acetate in chloroform, afforded 6.0 g (equivalent to 14.3 g of fraction A) of **α-3**, which was at least 99.5% anomerically pure by the quantitative chromatography and uv measurement techniques employed by LePage.[4b] The properties of **α-3** agreed with literature values except the rotation: $[\alpha]^{21}D$ −62° (*c* 0.5, $CHCl_3$) and lit.[2a] $[\alpha]^{24}D$ −55° ($CHCl_3$); uv max (EtOH) 225 nm (ε 30,600), 245 (sh, 37,400) and 284 (10,600);[19] nmr ($DCCl_3$–DMSO-d_6) δ 10.20 (s, 1, NHAc), 8.38 (s, 1, H-8), other features compatible with structure. For comparison, into the same tube was added some **β-3**: nmr (above tube) δ 10.32 (s, 1, NHAc), 8.32 (s, 1, H-8). For **β-3** alone: nmr (DMSO) δ 10.65 (s, 1, NHAc), 8.42 (s, 1, H-8), 7.9–6.95 (4 d, 8, 2 COC_6H_4-Me), 6.42 (t, 1, $J_{1'-2'}$ = 7 Hz, H-1′), 5.75 (m, 1, H-3′), 4.50 (m, 3, H-4′, 2 H-5′), 3.15 (m, 2, H-2′), 2.25 and 2.21 (both s, 6, 2 $C_6H_4CH_3$), 2.04 (s, 3, $COCH_3$). Other properties agreed with literature values.[2] In the succeeding fractions, **β-3** began to appear, so that all the remaining nucleosides were rapidly stripped off with ethyl acetate to afford 1.33 g (equivalent to 2.86 g of fraction A) of **α-3** and **β-3**. Total material recovered, 7.33 g. The overall yields are **β-3** (contains little **α-3**), 45%; **α-3**, 40%; mixture of **α-3** and **β-3**, 8%.

2-Acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-9*H*-purine (6a).—A 3.5-g (16.5 mmol) portion of **1a** was silylated as above and filtered. The filtrate and benzene washes (total, 350 ml) were treated with 10.0 g (39.5 mmol) of mercuric cyanide and 40 ml of a benzene solution containing 8.02 g (23.1 mmol) of 2,3,5-tri-*O*-acetyl-D-xylofuranosyl bromide.[20] After 2 hr at reflux temperature, the reaction was worked up as above. Purification through a 125 g column of Florisil gave, on elution with methylene chloride and then ethyl acetate, 5.76 g (62%) of **6a**, R_f 0.50 in methanol–ethyl acetate (2:8), identical by tlc, ir, uv, and other properties with **6a** prepared from the mercury derivative of **1a**.[16]

2-Acetamido-6-chloro-9-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)-9*H*-purine (9). A. Silylation with TMCS.—A small portion (0.53 g, 2.5 mmol) of **1a** was silylated as above. The filtered benzene solution was evaporated to dryness. The residue of silylated base was taken up in 75 ml of dry ethylene dichloride, and combined with 20 g of mercuric cyanide, 2.0 g of molecular sieves, and 3.0 mmol of 2,3,5-tri-*O*-benzyl-D-arabinosyl chloride (**8**).[17] The mixture was heated at reflux for 17 hr. The reaction mixture was filtered, and the filtrate was *immediately* chromatographed through 45 g of Florisil in a 1.6 × 60 cm column. After elution of by-products in 1.0 l. of ethylene dichloride–methylene chloride (1:9), the product was eluted in 800 ml of methylene chloride containing an increasing amount of ethyl acetate (initially, 10%; finally, 20%). Evaporation of the solvent left 1.0 g (67%) of an anomeric mixture of **9**: R_f 0.83 and 0.70 in ethyl acetate, after four passes.

B. Silylation with Hexamethyldisilazane.—A mixture of 4.23 g (20.0 mmol) of **1a** and 0.9 g of ammonium sulfate in 70 ml of hexamethyldisilazane was stirred and heated at reflux for 4 hr, with protection from moisture. The solution was evaporated to dryness at 50°, leaving the silylated **1a** as a colorless syrup. To this was added 6.72 g (26.5 mmol) of mercuric cyanide and 80 ml of dry benzene. This mixture was stirred and heated to incipient reflux; to this was rapidly added an 80-ml benzene solution of 20.7 mmol of **8** [from 13.85 g (20.7 mmol) of 2,3,5-tri-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-D-arabinose[17]]. The mixture was stirred and heated at reflux for 16 hr under a nitrogen atmosphere. The reaction mixture was worked up as for **3** (with mercuric

(18) (a) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960); (b) C. C. Bhat in "Synthetic Procedures in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1968, p 521.

(19) The literature[2a] values for the uv of **α-3** were determined in ethanol, not $CHCl_3$.

(20) O. P. Crews, Jr., and L. Goodman, ref 18b, p 139.

cyanide present). The residue in 125 ml of methylene chloride was chromatographed through 200 g of Florisil on a 3.5 × 32 cm column. The product **9** (11.2 g, 91%) was eluted in fractions totaling 1.4 l. of 10% ethyl acetate in methylene chloride. A portion of a central fraction was evaporated to give the analytical sample of **9**: R_f 0.85 (α anomer) and 0.72 (β anomer) in ethyl acetate, four passes; ir (neat) 3.05 (NH), 5.88 (NAc), 6.21, 6.32, 6.60 μ (characteristic of all 2-AcNH-6-Cl-9-R-purines, with areas under 6.21 and 6.32 peaks approximately equal); nmr ($DCCl_3$) δ 8.47 (broad, NHAc), 8.40 and 8.22 (both s, 1, H-8 of anomers), 7.38 and 7.26 (both s, 12, 3 C_6H_4Me), 6.43 (d, $J_{1',2'}$ = 4 Hz, H-1′ of β-**9**), 6.26 (d, $J_{1',2'}$ = 2 Hz, H-1′ of α-**9**), 4.68, 4.67, 4.62, 4.58, and 4.55 (all s, 6, $CH_2C_6H_5$), 4.3–3.80 (several m, 5, H-2′–H-5′), 2.52 (s, 3, $NCOCH_3$); uv max (pH 1) 225 nm (sh) (ϵ 24,800), 260 (sh) (17,100), 288 (15,100); (pH 7) 225 nm (sh) (ϵ 25,300), 260 (sh) (18,900), 291 (17,100); (pH 13) 267 nm (9300).

Anal. Calcd for $C_{33}H_{32}ClN_5O_5 \cdot 1.5\ H_2O$: C, 61.8; H, 5.50; N, 10.9. Found: C, 61.6; H, 5.41; N, 10.78.

Another portion of **9**, 2.43 g, was chromatographed through a 3.2 × 45 cm column of silica gel (110 g) eluting with 40% ethyl acetate–cyclohexane to afford first 0.75 g of pure α-**9**, R_f 0.29, then 1.04 g of anomeric mixture, R_f 0.18–0.29, and finally 0.53 g of pure β-**9**, R_f 0.18 (all R_f's on same tlc plate after two passes). CD results[21] as well as nmr confirmed the structural assignments of these anomers. α-**9** had nmr ($DCCl_3$) δ 8.20 (s, H-8), 6.21 (d, 1, $J_{1',2'}$ = 2 Hz, H-1′); uv, identical with that of the anomeric mixture. *Anal.* Calcd for $C_{33}H_{32}ClN_5O_5$: C, 64.5; H, 5.25; N, 11.4. Found: C, 64.6; H, 5.31; N, 11.1. β-**9** had nmr ($DCCl_3$) δ 8.37 (s, H-8), 6.37 (d, 1, $J_{1',2'}$ = 4 Hz, H-1′); uv, identical with that of the anomeric mixture. Found: N, 11.4%.

2-Amino-6-chloro-9-(2-deoxy-3,5-di-*O*-toluoyl-D-ribofuranosyl)-9*H*-purine (4).—Hexamethyldisilazane was used to silylate 2.00 g (11.6 mmol) of 2-amino-6-chloropurine (**1c**); and the product was treated for 1.5 hr at reflux with 4.65 g (12 mmol) of **2**, as described in procedure B for **9**, above. The reaction mixture was worked up as above to give 5.99 g (99%) of **4**. A portion (1.0 g) was chromatographed through 70 g of Florisil on a 3.2 × 27.5 cm column with ethyl acetate–methylene chloride (1:4). The first 500 ml of eluent was discarded. The next 400 ml afforded 0.85 g (84%) of the analytically pure **4**: R_f 0.39 and 0.48 (multiple passes); $[\alpha]^{21}D$ −41° (*c* 0.5, $CHCl_3$); ir (Nujol) 2.35 (sh), 3.00, 3.11 (NH_2), 5.78 (C=O, esters), 5.88 (sh, NHAc), 6.18, 6.36, and 6.58 μ (characteristic of all 2-NH_2-6-Cl-9-R-purines; area under 6.18 peak is much greater than that under 6.36); uv max (pH 1) 218 nm (ϵ 25,400), 242 (28,200), 317 (13,100); (pH 7) 219 nm (ϵ 24,200), 243 (27,500), 317 (14,500); (pH 13) 230 nm (sh) (ϵ 30,300), 307 (7000); nmr ($DCCl_3$) δ 8.20, 8.08 (both s, H-8 of α- and β-**4**; some overlapping with COC_6H_4Me), 7.5 (both m, 1, H-1′ anomers), 6.4 (2, NH_2), 2.44, 2.42 (both s, 6, $2C_6H_4CH_3$) with satisfactory integration for the eight aryl and five other furanose protons.

Anal. Calcd for $C_{26}H_{24}ClN_5O_5$: C, 59.8; H, 4.63; N, 13.4. Found: C, 59.8; H, 4.67; N, 13.2.

The formation of **4** from **3** by refluxing in ethanol containing sodium acetate was followed by tlc. The time for conversion of half of **3** to **4** was 30 min for the α anomer and 3 hr for the β anomer. Methanol solutions of **3** exposed to the atmosphere had formed detectable amounts of **4** (by tlc) after 2 or 3 days.

2-Amino-6-chloro-9-(1,3,5-tri-*O*-acetyl-β-D-xylofuranosyl)-9*H*-purine (6b).—A portion (0.43 g, 2.5 mmol) of **1c** was silylated with hexamethyldisilazane as above and treated with the xylosyl bromide [prepared from 1.00 g (3.14 mmol) of 1,2,3,5-tetra-*O*-acetyl-β-D-xylofuranose] for 2.3 hr at reflux temperature of benzene. This reaction gave a theoretical yield of **6b**, which was purified through a column of 60 g of Florisil, eluting with methanol–methylene chloride (1:9) to afford 0.89 g (84%) of **6b** as a white foam, R_f 0.45 in ether–ethyl acetate (4:6). The analytical sample of **6b** was from another run that had been chromatographed through Florisil and eluted with 2% methanol in benzene. This **6b** had $[\alpha]D$ +13.9° (*c* 0.5, $CHCl_3$); ir (Nujol) 2.86 (sh), 2.98, 3.10 (NH_2), 5.69 (CO, ester), 6.18, 6.36, 6.57 μ (like those in **4**); uv max (pH 1) 219 nm (ϵ 28,800), 247 (7700), 307 (8270); (pH 7) 221 nm (ϵ 29,100), 247 (7500), 307 (8400); (pH 13) 245 nm (sh) (ϵ 7100), 308 (7900); nmr ($DCCl_3$) δ 8.10 (s, 1, H-8), 6.12 (d, $J_{1',2'}$ = 2.5 Hz, H-1′), 6.4 (broad, NH_2), 2.15 and 2.10 (both s, 9, $3COCH_3$) with satisfactory integration for the other five furanose protons. Some benzene was also present.

Anal. Calcd for $C_{16}H_{18}ClN_5O_7 \cdot 0.2\ C_6H_6$: C, 46.6; H, 4.37; N, 15.2. Found: C, 46.1; H, 4.69; N, 15.2.

9-β-D-Xylofuranosylguanine (7). A. From 6b.—A solution of 0.53 g (1.24 mmol) of **6b** and 0.32 ml (4.5 mmol) of mercaptoethanol in 10 ml of MeOH was treated with 4.4 ml of 1 *N* sodium methoxide in methanol and 0.04 ml of water, and heated at reflux for 3.5 hr. After cooling at 5°, the crystalline sodium salt of **7** was collected and slurried with 5 ml of MeOH and 0.4 ml of glacial acetic acid to give, in two crops, 0.18 g (51%) of **7**: mp 234–240° (lit.[15] mp 238–240°); R_f 0.10 in methanol–ethyl acetate (2:8), identical with that of authentic **7**; $[\alpha]^{21}D$ −34° (*c* 0.25, H_2O) [lit.[15] $[\alpha]^{21}D$ −36.5° (*c* 0.25, H_2O)]; uv max (pH 1) 255 nm (ϵ 11,700), 275 (sh) (8000); (pH 7) 252 (ϵ 12,600), 275 (sh) (8700); (pH 13) 257 (ϵ 10,600), 264 (sh) (10,700), identical with that of authentic **7**; uv max (pH 1) 255 nm (ϵ 11,600), 275 (sh) (8000); (pH 7) 252 (ϵ 12,900), 270 (sh) (9200); (pH 13) 257 (ϵ 10,800), 265 (sh) (10,800); identical by ir with authentic **7**. The mother liquors still contained **7** by tlc.

The overall yield of **7** from **1c** was 43% as compared with 23% by the previous method.[15]

B. From 6a.—An 8.96-g (19.1 mmol) portion of **6a** was treated with mercaptoethanol and base, as above, to yield 3.05 g (56%) of **7**, identical with authentic **7**[15] by ir, tlc, uv, and rotation.

2-Amino-6-chloro-9-(2,3,5-tri-*O*-benzyl-D-arabinofuranosyl)-9*H*-purine (10).—The silyl derivative of **1c** [prepared from 1.04 g (5.81 mmol) of **1c** and hexamethyldisilazane] and 6.00 mmol of **8** [prepared from 4.00 g (6.00 mmol) of 2,3,5-tri-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-D-arabinofuranose][17] were heated in refluxing benzene for 2 hr, worked up as above, and chromatographed through 100 g of Florisil on a 3.4 × 29.5 cm column, with 10% ethyl acetate in Skellysolve B as eluent. From 850 ml of the eluent was obtained 3.05 g (92%) of analytically pure **10** as a gum: R_f 0.36 in cyclohexane–ethyl acetate (6:4); ir (Nujol) 2.85 (sh), 3.00, 3.11 (NH_2), 6.19, 6.37, and 6.59 μ (characteristic of 2-NH_2-6-Cl-9-R-purines); uv max (pH 1) 209 nm (ϵ 26,000), 237 (21,700), 260 (sh) (20,100), 321 (15,700); (pH 7) 215 nm (ϵ 26,000), 236 (25,000), 260 (22,400), 321 (17,100); (pH 13) 250 nm (sh) (ϵ 24,500), 314 (15,300); nmr ($DCCl_3$) δ 8.18 and 8.08 (both s, 1, H-8 of β- and α-**10**, respectively), 6.35 (d, ~0.5, $J_{1',2'}$ = 4 Hz, H-1′ of β), 6.17 (d, ~0.5, $J_{1',2'}$ = 2 Hz, H-1′ of α-**10**), 5.3 (broad, 2, NH_2), 4.60, 4.57, and 4.56 (all s, 6, 2 $C_6H_4CH_3$) with satisfactory integration for the 15 aryl and 5 other furanose protons. The ratios of anomers is about 1, but slightly richer in β. The H-8 and H-1′ protons are assigned by analogy to those of **9**.

Anal. Calcd for $C_{31}H_{30}ClN_5O_4$: C, 65.2; H, 5.29; N, 12.2. Found: C, 65.2; H, 5.50; N, 12.2.

Registry No.—**1b**, 35095-89-7; **2**, 4330-21-6; α-**3**, 35129-57-8; β-**3**, 7356-40-3; α-**4**, 35095-92-2; β-**4**, 35095-93-3; α-**5**, 35085-15-5; **6b**, 35085-16-6; α-**9**, 35085-17-7; β-**9**, 35085-18-8; α-**10**, 35085-19-9; β-**10**, 35085-24-6; guanine, 73-40-5; thioguanine, 154-42-7.

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(21) J. Ingwall, manuscript in preparation.