Purine Nucleosides. IV. The Synthesis of 6-Halogenated 9-β-D-Ribofuranosylpurines from Inosine and Guanosine[1]

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The present work is a study of the introduction of various halogens into purine ribosides at position 6. Under carefully controlled conditions the sugar moiety may be retained. These compounds provide many new and useful synthetic purine nucleoside intermediates which are now readily available from natural sources.

The synthesis of most purine nucleosides has been accomplished by the coupling of an appropriately blocked halo sugar with a metallic salt of a selected purine derivative.[2] It is general practice to couple the sugar moiety with the desired purine as one of the final steps. This procedure, however, suffers from the disadvantage that not all purine derivatives will undergo coupling in the prescribed manner. Thus, hydroxy-, mercapto-, and aminopurines have not been employed successfully in this reaction. This difficulty has been overcome in part by acylation or benzoylation of the amino group[3] or by the use of alkylthiopurines.[4-6] In many instances however[7-12] this difficulty has been surmounted only by coupling with a chloropurine which then was later changed to the desired nucleoside by stepwise replacement of the halogens. The inherent disadvantages of the chloromercury coupling procedure, such as low yields, contamination by mercury,[13] etc., suggest that alternative procedures for the synthesis of the important halopurine ribosides would be highly desirable. A preliminary report of our efforts in this direction has been published.[14] The present work describes the synthesis of many new halopurine riboside which are now readily accessible for further synthetic work.

The commercial availability of the naturally occurring purine nucleosides, inosine and guanosine, provided a good starting point for this study. The only published work utilizing these natural nucleosides for the synthesis of other purine ribosides is that of Fox and co-workers[15] who prepared 9-β-D-ribofuranosyl-6-purinethiol (VIII) and 2-amino-9-β-D-ribofuranosyl-6-purinethiol (X) from inosine and guanosine, respectively. The preparation of 6-chloro-9-β-D-ribofuranosylpurine (V) from VIII with chlorine gas in methanol[14] suggested the similar synthesis of 2-amino-6-chloro-9-β-D-ribofuranosylpurine (VI) from X. Although VI could be readily prepared by this method from 2-amino-6-methylthio-9-β-D-ribofuranosylpurine,[14] the synthesis of VI from X was at first unsuccessful. However, when *concentrated hydrochloric acid*, methanol, and chlorine were employed[16] at −20°, 2-amino-6-chloro-9-β-D-ribofuranosylpurine (VI) was obtained in good yield. Probably the most surprising thing about this reaction is that concentrated acid *did not* result in cleavage of ribose at temperatures below −20°. Similar treatment of 2-amino-9-β-D-ribofuranosyl-6-purinethiol (X) and 9-β-D-ribofuranosyl-6-purinethiol (VIII) with bromine and methanol in the presence of concentrated aqueous hydrobromic acid[17] at low temperature gave 6-bromo-9-β-D-ribofuranosylpurine (XI) and 2-amino-6-bromo-9-β-D-ribofuranosylpurine, respectively, in good yields. These reactions were executed without recourse to blocking of the hydroxyl groups and were accomplished without detectable hydrolysis of the sugar. This low temperature stability of these purine nucleosides to strong acid suggested that hydriodic acid might be employed to prepare iodopurine ribosides. This indeed proved to be the case. 2-Amino-6-chloro-9-β-D-ribofuranosylpurine (VI) and 47% hydriodic acid at −5° gave 2-amino-6-iodo-9-β-D-ribofuranosylpurine (XIV) in excellent yield. 6-Iodo-9-β-D-ribofuranosylpurine (XIII) was similarly prepared from 6-chloro-9-β-D-ribofuranosylpurine (V). The use of hydriodic acid to replace chlorine by iodine on the purine ring was first introduced by Fischer.[18,19] The possible direct synthesis of chloropurine ribosides from the corresponding hydroxypurine ribosides was also investigated. 2′,3′,5′-Triacetylguanosine (II)[20,21] was treated with N,N-diethylaniline and refluxing phosphorus oxychloride to give 2-amino-6-chloro-9-(2′,3′,5′-triacetyl-β-D-ribofuranosyl)purine (IV) in 57% yield. When the diethylaniline was omitted, cleavage of the nucleoside occurred. 2′,3′,5′-Triacetylinosine (I)[20,22] was similarly converted to the important 6-chloro-9-(2′,3′,5′-triacetyl-β-D-ribofuranosyl)purine (III) previously available only from 6-chloropurine and chlorotriacetylribofuranose.[23] Thiourea in refluxing ethanol readily converted III

(1) Supported by research grants CY-4008 (C3) and (C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) For a review of general purine nucleoside syntheses, see J. Baddiley, in "Chemistry of Carbon Compounds," IVc, E. H. Rodd, ed., Elsevier Publishing Co., New York, N. Y., 1960, Chap. XXI, p. 1707.

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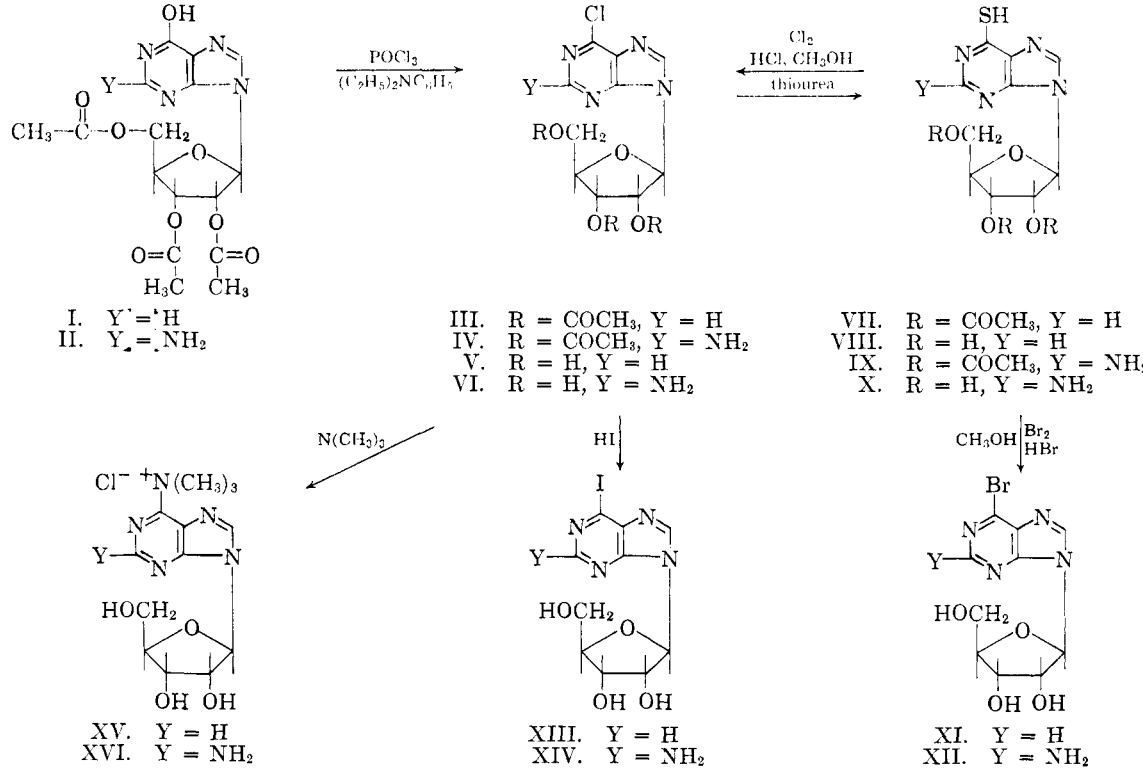

I. Y = H
II. Y = NH2

III. R = COCH3, Y = H
IV. R = COCH3, Y = NH2
V. R = H, Y = H
VI. R = H, Y = NH2

VII. R = COCH3, Y = H
VIII. R = H, Y = H
IX. R = COCH3, Y = NH2
X. R = H, Y = NH2

XV. Y = H
XVI. Y = NH2

XIII. Y = H
XIV. Y = NH2

XI. Y = H
XII. Y = NH2

Reaction Scheme

and IV to 9-(2′,3′,5′-triacetyl-β-D-ribofuranosyl)-6-purinethiol (VII) and 2-amino-9-(2′,3′,5′-triacetyl-β-D-ribofuranosyl)-6-purinethiol (IX), respectively. These chloropurine nucleosides have been utilized for the synthesis of 9-β-D-ribofuranosylpurine-6-trimethylammonium chloride (XV) and 2-amino-9-β-D-ribofuranosylpurine-6-trimethylammonium chloride (XVI). These derivatives, XV and XVI, are of interest since purine-6-trimethylammonium chloride[24] and 9-(tetrahydro-2-furyl)purine-6-trimethylammonium chloride[25] recently have been reported to possess antitumor activity against various animal tumors.

The versatility of the 2-amino-6-halopurine ribosides is illustrated by the synthesis of 2-amino-6-methoxy-9-β-D-ribofuranosylpurine from VI and sodium methoxide. The isomeric 6-amino-2-methoxy-9-β-D-ribofuranosylpurine (spongosine) has been isolated from the carribean sponge *Cryptotethia crypta*.[9,26] 2-Amino-6-dimethyl aminopurine was readily prepared from 2-amino-6-iodo-9-β-D-ribofuranosylpurine (XIV) and aqueous dimethylamine. β-Hydroxyethylamine and 6-chloro-9-β-D-ribofuranosylpurine (V) gave 6-β-hydroxyethylamino-9-β-D-ribofuranosylpurine. This latter compound has recently been reported[27]; it was prepared by rearrangement of 1-(β-hydroxyethyl)adenosine in alkali.

Experimental[28]

2-Amino-6-chloro-9-β-D-ribofuranosylpurine (VI). Method 1.—Finely divided 2-amino-9-β-D-ribofuranosyl-6-purinethiol[15] (20.0 g.) was added to a solution of 120 ml. of aqueous concentrated hydrochloric acid and 100 ml. of methanol cooled to −20°. Chlorine gas then was bubbled into the suspension for 30 min. (Excess chlorine then was detected in the solution since it rapidly bleached pH test paper.) By this time all solid had dissolved. The temperature was maintained between −20 and −30° at all times. The yellow solution was stirred for an additional 30 min. at −20° and then carefully neutralized to pH 9 with methanolic ammonia (saturation at 0°). During this process the temperature was kept at −20°. The resulting suspension was diluted with 100 ml. of methanol and the ammonium chloride filtered. The filtrate was evaporated to dryness *in vacuo* and the yellow solid recrystallized from water. After two additional recrystallizations from methanol, a yield of 45% of white product was obtained, m.p. 171–172°. This product was found to be identical to that prepared from 2-amino-6-methylthio-9-β-D-ribofuranosylpurine.[14]

Method 2.—2-Amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (IV) (4.3 g.) was dissolved in 50 ml. of methanolic ammonia (saturated at 0°) at room temperature and the solution stirred for 6 hr. The solution then was evaporated *in vacuo* and several milliliters of acetone was slurried with the residual oil. Addition of 100 ml. of ethyl ether converted the oil to a white solid. The product was filtered, washed with ether, and dried to yield 2.7 g. (90%) of a white solid, m.p. 171–172° dec. Ultraviolet absorption spectra,[14] mixture melting point data, and chromatography established the product as pure VI.

2-Amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (IV).[29]—2′,3′,5′-Tri-*O*-acetylguanosine[20] (100 g.) was added with stirring to a solution of phosphorus oxychloride (750 ml.) and N,N-diethylaniline (38 ml.) at room temperature. The suspension was heated rapidly to reflux and refluxed for 3 min. The excess phosphorus oxychloride (500 ml.) was removed *in vacuo* on the steam bath. The resulting syrup was poured over excess ice with stirring, and the acidic ice and water mixture was stirred vigorously for several minutes. Using five 200-ml. portions of methylene chloride, the cold solution was extracted thoroughly, and the combined extracts were washed with five 300-ml. portions of cold (0–5°) *N* hydrochloric acid. The methyl-

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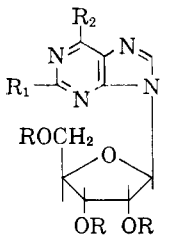
(28) All melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected unless otherwise indicated.

(29) Australian Patent 55,387, dated July 12, 1959, to the Wellcome Foundation Ltd., describes the synthesis of 2-amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine, and the ultraviolet absorption maxima are listed as 252 and 285 mμ in ethanol. Our product (IV) exhibited λ_{max} 249 and 310 mμ in ethanol similar to those recorded for 2-amino-6-chloro-9-β-ribofuranosylpurine.[14]

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN PURINE RIBOSIDE DERIVATIVES



R	R_1	R_2	λ_{max}^{pH}		$\lambda_{max}^{pH\ 11}$	ϵ	λ_{max}^{EtOH}	ϵ
$COCH_3$	NH_2	Cl					310	7,900
							249	8,600
$COCH_3$	NH_2	SH					346	27,600
							275	7,000
							258	7,300
$COCH_3$	H	SH	320	24,400	308	24,400		
					231	16,200		
$COCH_3$	NH_2	I	317	10,100	312	10,800		
			245 (s)	10,600	241 (s)	14,200		
			223	27,100				
H	H	I					274	11,600
$COCH_3$	NH_2	OCH_3	287	10,500	278	10,500		
			244	8,500	248	11,100		
H	NH_2	$[N^+(CH_3)_3Cl^-]$					316	6,570
							247	6,100
							225	23,700
H	H	$[N^+(CH_3)_3Cl^-]$					264	7,850
H	NH_2	$N(CH_3)_2$	291	11,400	282	14,700		
			256	12,500	265 (s)	10,600		
					229	18,300		
$COCH_3$	H	Cl					264	9,540
H	NH_2	Cl					310	7,200
							246	6,900
							222	24,200
H	H	Br	265	7,613	265	8,275		
H	NH_2	Br	247	7,960	247	8,650		
			310	7,890	308.5	8,130		

ene chloride solution was washed with distilled water until the water extracts were neutral to pH paper. After drying the methylene chloride extract with anhydrous sodium sulfate, the excess solvent was evaporated *in vacuo* to leave a thin oil. To the oil was added 300 ml. of fresh methylene chloride, and the resulting solution was poured slowly with vigorous stirring into 4 l. of anhydrous diethyl ether. A pale yellow solid gradually precipitated and was filtered to yield 30 g. of solid, m.p. 145–148°. The filtrate was evaporated *in vacuo* at room temperature, and as the volume was reduced, a white precipitate gradually formed and was filtered to yield an additional 30 g., m.p. 145–148°. Total yield was 60 g. (59%). Recrystallization from isopropanol gave an analytically pure sample, m.p. 147.5–148.5°.

Anal. Calcd. for $C_{16}H_{18}ClN_5O_7$: C, 45.0; H, 4.2; N, 16.4. Found: C, 44.7; H, 4.3; N, 16.0.

9-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinethiol (VII).—6-Chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (III, 10.0 g.) was dissolved in 100 ml. of absolute ethanol containing 2.5 g. of thiourea. The solution was refluxed for 1 hr. The white solid was filtered and washed with absolute ethanol to yield 7.2 g. (73%) of VIII. Recrystallization was accomplished from acetone containing a small amount of water to give a pure product, m.p. 255–256°.

Anal. Calcd. for $C_{16}H_{18}N_4O_7S$: C, 46.8; H, 4.4; N, 13.7. Found: C, 46.6; H, 4.4; N, 13.8.

6-(β-Hydroxyethylamino)-9-β-D-ribofuranosylpurine.—6-Chloro-9-β-D-ribofuranosylpurine (V)[14] was added to 150 ml. of isopropyl alcohol containing 3.7 g. of 2-aminoethanol. The solution was refluxed for 6 hr. and finally chilled overnight at 15°. The product that separated (9 g.) was filtered, washed with isopropyl alcohol, and dried at room temperature for 5 hr. The crude product melted at 182–186°. After recrystallization from a mixture of methanol and water (2:1), the pure product melted at 197–199°.[30]

Anal. Calcd. for $C_{12}H_{17}N_5O_5 \cdot H_2O$: C, 43.9; H, 5.5; N, 21.4. Found: C, 44.1; H, 5.7; N, 21.4.

2-Amino-6-bromo-9-β-D-ribofuranosylpurine (XII).—Thioguanosine[15] (20 g.) was added to a cold solution (−10°) of 75 ml. of aqueous hydrobromic acid (47–49%) and 50 ml. of absolute methanol. To the vigorously stirred mixture at −15 to −10° was added 20 ml. of bromine in 1-ml. portions over a period of 30 min. The reaction mixture was stirred for 1.5 hr. after the final addition of bromine, during which time a brown-red solution resulted. The solution was then poured over 500 g. of ice and the pH adjusted to 8 with concentrated aqueous ammonia. The temperature of the solution was kept below −10° at all times by external cooling. The resulting suspension was stirred at −5° for 30 min. and was then filtered and washed with 100 ml. of cold water. The precipitate was immediately recrystallized from 400 ml. of hot water which had been adjusted to pH 7.5 with concentrated aqueous ammonia. A colorless product separated at room temperature and was filtered and washed first with water (50 ml.) and finally with ethanol (50 ml.) to yield 6.5 g. of pure product, m.p. 163–165°. The filtrate was chilled at 5° for 24 hr. to give an additional 6 g. of product.

Anal. Calcd. for $C_{10}H_{12}BrN_5O_4$: C, 34.7; H, 3.5; N, 20.2. Found: C, 34.7; H, 3.8; N, 20.3.

6-Bromo-9-β-D-ribofuranosylpurine (XI).—9-β-D-Ribofuranosyl-6-purinethiol (VIII, 10 g.)[15] was added to a mixture of 30 ml. of hydrobromic acid (48%) and 30 ml. of methanol stirred and previously cooled to −15°. To the resulting suspension was added, dropwise, 8 ml. of bromine, in 50 ml. of methanol, over a period of 25 min. The solution was stirred for 1 hr. at −10° after the final addition and then poured into 100 g. of an ice and water mixture. The solution was then adjusted to pH 9 with concen-

(30) H. G. Windmueller and N. O. Kaplan (ref. 27) give 195–196° for the melting point of this compound.

trated aqueous sodium hydroxide previously cooled at −5°. After stirring for 10 min. at −5°, the solution was adjusted to pH 6 with hydrobromic acid (48%) and extracted four times with 250 ml. of ethyl acetate. The extract was washed twice with 35-ml. portions of water and then dried over sodium sulfate. The solvent was removed on a steam bath to yield 1.2 g. of product, and the filtrate from the initial extraction was extracted in a continuous extractor for 24 hr. at 15–20° to yield, on evaporation of the ethyl acetate, an additional 4 g. of product. A small sample was recrystallized from methanol to yield a pure sample which melted at 181–182°.

Anal. Calcd. for $C_{10}H_{11}BrN_4O_4$: C, 36.2; H, 3.3; N, 16.9. Found: C, 36.2; H, 3.1; N, 16.8.

6-Trimethylammonium-9-β-D-ribofuranosylpurine Chloride (XV).—6-Chloro-9-β-D-ribofuranosylpurine[14] (1.0 g.) was dissolved in 250 ml. of dry 1,2-dimethoxyethane and the solution cooled to room temperature. Trimethylamine (10 ml.), in 20 ml. of 1,2-dimethoxyethane, was added with stirring, and within seconds a white precipitate began to form. After 30 min. the white solid was filtered, washed with a small amount by dry ether, and immediately placed in a vacuum desiccator. It is extremely important that this be done rapidly since the product is very hygroscopic. A nearly quantitative yield of pure product was obtained, m.p. ≈ 160° (Kofler Heizbank). Recrystallization resulted in decomposition, and for analysis a small sample was dried *in vacuo* over phosphorus pentoxide at 60°.

Anal. Calcd. for $C_{13}H_{20}ClN_5O_4$: C, 45.2; H, 5.8; N, 20.3, Cl, 10.3. Found: C, 45.2; H, 5.6; N, 20.0; Cl, 10.1.

6-Iodo-9-β-D-ribofuranosylpurine (XIII).—6-Chloro-9-β-D-ribofuranosylpurine[14] (1.0 g.) was added to 10 ml. of aqueous hydriodic acid (47%) at −20°. The solid dissolved almost immediately, and the solution was stirred at −15 to −20° until a yellow solid precipitated. The yellow suspension was stirred for an additional 15 min. at the same temperature; crushed ice (10 cc.) was added and the mixture neutralized to pH 7 with cold concentrated aqueous ammonia. The temperature was maintained at −10 to −15° at all times during the neutralization. The solution was allowed to stand overnight, whereupon the 6-iodo-9-β-D-ribofuranosylpurine crystallized. A yield of 200 mg. was obtained. One recrystallization from water and methanol gave a pure product, m.p. 173° dec.

Anal. Calcd. for $C_{10}H_{11}IN_4O_4$: C, 32.1; H, 3.0; N, 14.8; I, 33.9. Found: C, 32.0; H, 3.0; N, 14.4; I, 33.5.

2-Amino-9-β-D-ribofuranosylpurine-6-trimethylammonium Chloride (XVI).—2-Amino-6-chloro-9-β-D-ribofuranosylpurine (VI, 3.0 g.) was dissolved in 400 ml. of 1,2-dimethoxyethane, and anhydrous trimethylamine (25 ml.) was added to the solution at 25°. A precipitate formed immediately, and after 30 min. the solid was filtered and washed with ether. The product was dried overnight to give a quantitative yield of XVI.

Anal. Calcd. for $C_{13}H_{21}ClN_6O_4 \cdot 1.5\ H_2O$: C, 40.3; H, 5.9; N, 21.6. Found: C, 40.4; H, 6.4; N, 20.8.

2-Amino-6-iodo-9-β-D-ribofuranosylpurine (XIV).—2-Amino-6-chloro-9-β-D-ribofuranosylpurine (VI, 3.0 g.) was added slowly with stirring to 18 ml. of aqueous hydriodic acid (47%) previously cooled to −5°. A yellow solid separated shortly, and the suspension was stirred at 0° for an additional 1.5 hr. Crushed ice (25 g.) was then added to the suspension, and the mixture was adjusted to pH 8 with iced concentrated aqueous ammonia. The resulting neutral solution was heated to dissolve all the solid and then allowed to stand. White crystals formed after standing overnight to yield 2.97 g. (76%) of crude product which was recrystallized from methanol to give a pure sample, m.p. 170° dec.

Anal. Calcd. for $C_{10}H_{12}IN_5O_4$: C, 30.6; H, 3.1; N, 17.8; I, 32.3. Found: C, 30.5; H, 3.1; N, 17.7; I, 32.1.

2-Amino-6-methoxy-9-β-D-ribofuranosylpurine.—2-Amino-6-chloro-9-β-D-ribofuranosylpurine (VI, 3.0 g.) was dissolved in a 50-ml. solution of sodium methoxide (6% in methanol), and the solution was refluxed for 1 hr. on a steam bath, cooled, and carefully neutralized with concentrated aqueous hydrochloric acid. The sodium chloride was filtered and the filtrate evaporated to dryness. Recrystallization of the product from methanol and ethyl acetate (1:4) gave a 55% yield of pure product, m.p. 133–135°.

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot {}^1/_2H_2O$: C, 43.1; H, 5.3; N, 22.8; H_2O: 2.9. Found: C, 43.2; H, 5.8; N, 22.8; H_2O, 2.9.

2-Amino-6-dimethylamino-9-β-D-ribofuranosylpurine.—2-Amino-6-iodo-9-β-D-ribofuranosylpurine (XIV, 1.0 g.) was added to 25 ml. of aqueous dimethylamine (25%). The solution was heated on the steam bath for 15 min. and evaporated to dryness *in vacuo*. Absolute ethanol was added and removed under reduced pressure. This process was repeated several times to give a yellow solid which was extracted several times with boiling acetone and filtered to give 550 mg. (70%) of solid. Recrystallization from methanol and acetone gave a pure sample, m.p. 209–211°.

Anal. Calcd. for $C_{12}H_{18}N_6O_4$: C, 46.5; H, 5.9; N, 27.1. Found: C, 46.7; H, 5.6; N, 26.8.

2-Amino-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-6-purinethiol.—2-Amino-6-chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (IV, 10.0 g.) was dissolved in 200 ml. of ethanol containing 5.0 g. of thiourea. The solution was refluxed for 1 hr. and then evaporated to dryness. The solid was suspended in water and filtered to give 7.5 g. (75%) of the desired product. One recrystallization from water provided a pure product, m.p. 253–255° dec.

Anal. Calcd. for $C_{16}H_{19}N_5O_7S$: C, 45.2; H, 4.5; N, 16.5. Found: C, 45.2; H, 4.6; N, 16.5.

6-Chloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (III).—2′,3′,5′-Tri-*O*-acetylinosine[20,22] (30 g.) was added with stirring to a solution of freshly distilled phosphorus oxychloride (150 ml.) and redistilled N,N-diethylaniline (10 ml.). The suspension was rapidly heated to reflux and refluxed for 3 min. with constant stirring. The resulting clear, yellow solution was poured over excess ice with vigorous stirring and the solution stirred for several minutes. The ice-cold solution was extracted with five 150-ml. portions of chloroform, and the combined chloroform solution was extracted with five 300-ml. portions of cold 1 *N* hydrochloric acid to remove the last traces of N,N-diethylaniline in the chloroform. The chloroform extract was then washed thoroughly with distilled water until the water extracts were neutral to pH paper. The chloroform solution then was dried with anhydrous sodium sulfate and excess chloroform removed *in vacuo* to yield a thick oil. The oil was dissolved in ether and the ether removed *in vacuo*. This process was repeated several times to yield a hygroscopic white powder (18 g., 56%). The product melts gradually at 40–50°.

Anal. Calcd. for $C_{16}H_{17}ClN_4O_7$: C, 46.6; H, 4.2; N, 13.6. Found: C, 46.5; H, 4.3; N, 13.5.

This product was readily deacylated to give 6-chloro-9-β-D-ribofuranosylpurine (V) according to the method of Brown and Weliky.[23]

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