Purine Nucleosides. X. The Synthesis of Certain Naturally Occurring 2-Substituted Amino-9-β-D-ribofuranosylpurin-6(1H)-ones (N^2-Substituted Guanosines)[1]

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The synthesis of the naturally occurring guanosine nucleoside derivatives 2-methylamino- and 2-dimethylamino-9-β-D-ribofuranosylpurin-6-ones (VI and VII) has been accomplished in several steps via 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) or 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX). The N^2-methyl and N^2-dimethylguanosines prepared by this route were found to be identical with the nucleosides previously isolated from RNA.[3,4] This procedure was successfully extended to include the synthesis of N-(9-β-D-ribofuranosylpurin-6-on-2-yl)glycine (XIV) and N-(9-β-D-ribofuranosylpurin-6-on-2-yl)alanine (XVII). The latter derivative (XVII) has been previously isolated from the mold Fusarium sp. and the aglycon has been detected in Eremothecium ashbyii. The present work, in effect, provides a good indirect synthetic route for the preparation of N^2-substituted guanosines from guanosine.

A number of N-methyl derivatives of guanine, guanosine, and guanylic acid have been detected in various biological materials in recent years. 1-Methylguanosine, isolated from different sources of RNA,[3,4] has recently been prepared synthetically by methylation of guanosine.[5] 7-Methylguanylic acid has been detected in RNA by Dunn.[6] 7-Methylguanosine has also been prepared synthetically by direct methylation procedures.[7,8] 2-Methylamino-9-β-D-ribofuranosylpurin-6-one (VI, N^2-methylguanosine) and 2-dimethylamino-9-β-D-ribofuranosylpurin-6-one (VII, N^2-dimethylguanosine) were first isolated from RNA by Smith and Dunn[3] and later by Bergquist and Matthews.[4] The unidentified guanine nucleotide reported by Davis and co-workers[9] from yeast RNA has been shown to be N^2-dimethylguanylic acid[10] identical with the product of Smith and Dunn.[3] 2-Methylaminopurin-6-one (N^2-methylguanine) has been detected in human urine[11,12] and yeast RNA.[13] 2-Dimethylaminopurin-6-one (N^2-dimethylguanine) has been identified in human urine.[14] Both of these compounds have been detected in the ribonucleotide form in soluble RNA.[15-22] N^2-Dimethylguanosine (VII) has also recently been detected in human urine.[14]

As part of a continuing program designed to synthesize the methylated purine nucleosides which occur in nucleic acid, the chemical preparation of N^2-methyl- and N^2-dimethylguanosine (VI and VII) was investigated in our laboratory. Levene and Tipson[23] treated a solution of tri-O-acetylguanosine in acetone with aqueous sodium hydroxide and dimethyl sulfate and claimed to obtain N^2-2′,3′,5′-tetra-O-methylguanosine, although no characterization of the purine moiety was presented. Bredereck and co-workers[24] claimed to have obtained N^2-methylguanosine by methylation of guanosine at pH 13–14. These methylations[23,24] were carefully repeated in our laboratory; however, in each case the products exhibited a sharp maximum in the region of $\lambda^{pH\ 11}$ 270 mμ which is strongly indicative of opening of the imidazole ring.[25] No derivative of guanine could be detected from either the procedure of Levene and Tipson[23] or that of Bredereck, Haas, and Martini.[24] This is not unexpected since methylation of guanosine under these conditions probably results in alkylation at position 7 which would be followed by imidazole ring opening under the alkaline conditions.[7,8,25] Since the preparation of VI and VII could not be accomplished by the direct methylation[5,7,8] of guanosine it became clear that the methylamino and dimethylamino groups would have to be introduced indirectly before attachment of the D-ribose moiety or

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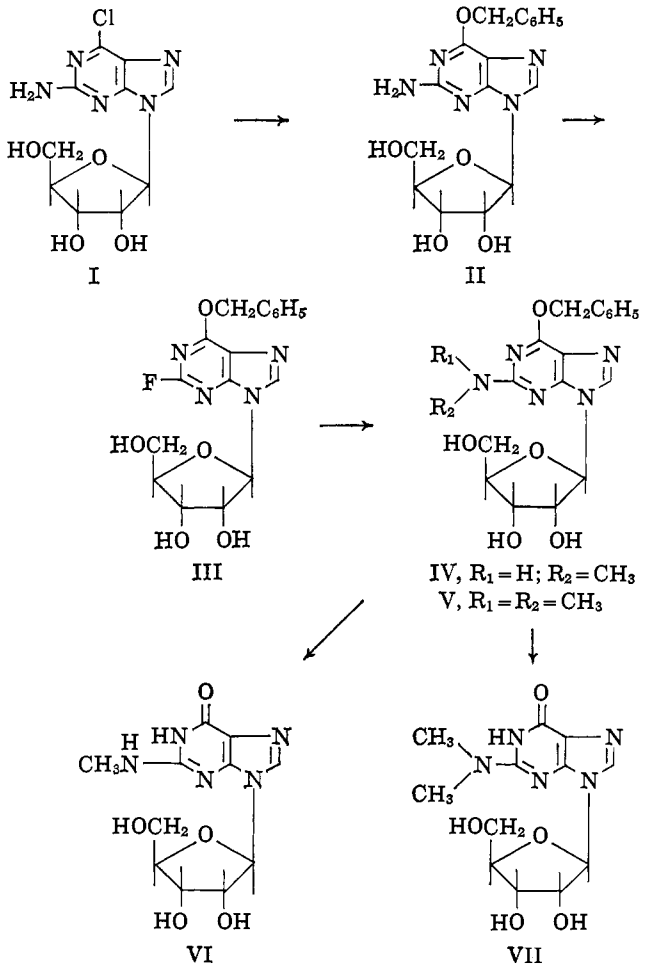
Scheme I



directly into the 2-position of a purine nucleoside derivative. The latter approach appeared to be the most promising and provides the major subject of the present study.

In selecting a suitable 2,6-disubstituted 9-β-D-ribofuranosylpurine as a synthetic intermediate it was desirable that the 2-substituents be readily susceptible to nucleophilic substitution and the substituent at the 6-position readily convertible to a keto function. The order of nucleophilic substitution of identical groups at positions 2 and 6 of the purine ring (2,6-dichloropurine as an example) is replacement of the 6-substituent followed by the 2-position.[26] Although 2-chloropurin-6-one has been readily prepared from 2,6-dichloropurine,[26] attempts in our laboratory to prepare 2-chloro-9-β-D-ribofuranosylpurin-6-one from 2,6-dichloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribo-furanosyl)purine[27] by similar treatment resulted in isolation of very little desired product, and a substantial amount of highly insoluble material which was not further identified. The synthesis of 2-methylaminopurin-6-one and 2-dimethylaminopurin-6-one has previously been reported[28] from 2-methylthiopurin-6-one and solutions of methylamine or dimethylamine at 140° in a sealed vassel. A superior procedure for preparation of these purines has now been developed in our laboratory using 2-chloropurin-6-one.[26] However, a steel bomb and 130° were required for reaction of the 2-chloro group with methanolic methylamine or methanolic dimethylamine. Therefore, an effort was made to select a substituent at position 2 which would be a better leaving group and allow introduction of a 2-substituted amino group to occur under milder conditions in the nucleoside. Several 2-fluoro-6-substituted 9-β-D-ribofuranosylpurines were selected for study. A number of fluoropurine ribonucleoside derivatives have previously been reported[29,30] and the greater reactivity of the fluoro over the chloro group has been noted.[30,31] In all previous nucleophilic substitution reactions of purine involving a choice of ready replaceable groups at positions 2 and 6, nucleophilic substitution has occurred preferentially[32,33] at position 6. When nucleophilic substitution occurs first at position 6 this introduces a relatively electron-rich group at that position which subsequently hinders the introduction of a second nucleophile at position 2. In order to overcome this difficulty, the possibility was considered of choosing substituents to reverse the order of usual nucleophilic substitution and thus provide for replacement of the substituent (fluorine) at the 2 position *first*. The synthesis of a number of 2-fluoro-6-substituted 9-β-D-ribofuranosylpurines which possessed a substituent at position 6 that could eventually be converted to a keto (hydroxyl) group then became a primary synthetic goal. The most useful intermediate of this type proved to be 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (1lI). 2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) was prepared in 24% yield by low-temperature diazotization of 2-amino-6-benzyloxy-9-β-D-ribofuranosylpurine (II) in the presence of fluoroboric acid. The preparation of II was accomplished by the reaction of sodium benzyloxide on 2-amino-6-chloro-9-β-D-ribofuranosylpurine[34] (I). Replacement of the 2-fluoro group was accomplished with considerable ease. Methanolic methylamine gave 2-methyamino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV) in excellent yield. Similarly, methanolic dimethylamine gave 2-dimethylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (V). The conversion of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) to IV and V, respectively, occurred essentially quantitatively in 5–10 min. at room temperature. This reactivity is to be compared with 2-chloro-9-β-D-ribofuranosylpurine[35] which required aqueous dimethylamine in a bomb at 87° for 15 hr. for similar replacement.[35] It is interesting to note that 2-chloro-6-methoxy-9-β-D-ribofuranosylpurine with methanolic ammonia at 83° gave 6-amino-2-chloro-9-β-D-ribofuranosylpurine[32] instead of the 2-amino-6-methoxypurine nucleoside.

Thus it is clear that the 2-fluoro group is so reactive toward nucleophilic substitution in this system that re-

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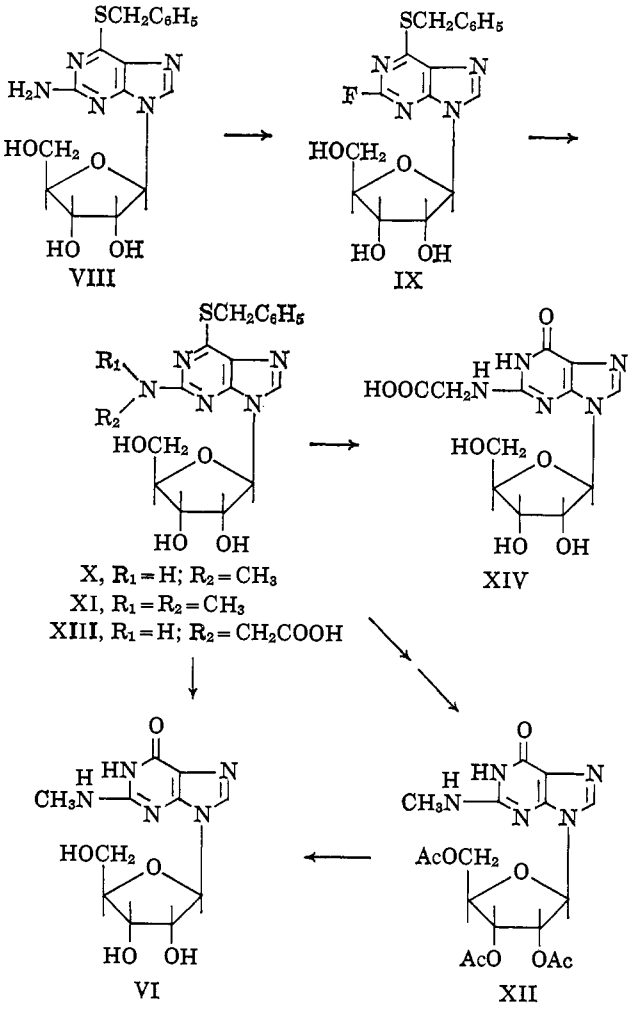
placement does indeed take place preferentially at position 2. Catalytic debenzylation of 2-methylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV) and 2-dimethylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (V) with palladium on carbon in the presence of hydrogen[36] readily gave pure, crystalline, 2-methylamino-9-β-D-ribofuranosylpurin-6-one (VI, N^2-methylguanosine) and 2-dimethylamino-9-β-D-ribofuranosylpurin-6-one (VII, N^2-dimethylguanosine), respectively, in excellent yields. The products VI and VII were chromatographically homogeneous and were found to possess the same R_f values and ultraviolet absorption spectra recorded for the N^2-methyl- and N^2-dimethylguanosines isolated by Smith and Dunn.[3] Compounds VI and VII were heated in 1 *N* hydrochloric acid and cleaved to the corresponding purines and D-ribose which were rigorously compared with authentic samples and found to be identical. The p.m.r. spectrum of N^2-methylguanosine (VI) in dimethyl-d_6 sulfoxide showed a doublet (3 H) at δ 3.24 due to the methyl group split by the NH proton. The p.m.r. spectrum of VII under similar conditions showed a sharp singlet (6 H) at δ 3.51 due to the two methyl groups in the same environment. Another 2-fluoro-6-substituted 9-β-D-ribofuranosylpurine investigated as a potential synthetic intermediate in this work was 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX). The readily available 2-amino-6-benzylthio-9-β-D-ribofuranosylpurine[37] (VIII) was diazotized in fluoroboric acid to give a 33% yield of 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX). The conversion of 2-amino-6-methylthiopurine to 2-fluoro-6-methylthiopurine has previously been reported in yields of 23%[31] and 15%[38] under similar conditions. 2-Methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X) and 2-dimethylamino-6-benzylthio-9-β-D-ribofuranosylpurine (XI) were readily prepared from IX being treated with methanolic methylamine and methanolic dimethylamine at room temperature. The conversion of the 6-benzylthio group to the 6-keto (hydroxy) function was next investigated. The synthesis of inosine from 6-methylthio-9-β-D-ribofuranosylpurine has recently been reported[39] by oxidation with N-chlorosuccinimide. The 6-methylsulfonyl group is a presumed intermediate in this reaction. It was found in the present study that hydrogen peroxide in glacial acetic acid and X or XI gave approximately 70% yield of N^2-methyl and N^2-dimethylguanosine, respectively. Although the products VI and VII proved to be identical with those prepared *via* 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III), considerable difficulty was encountered in obtaining pure crystalline samples by this route. When 2-methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X) was first acetylated and then oxidized with hydrogen peroxide in glacial acetic acid, 2-methylamino-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl) purin-6-one (XII) was isolated as a pure crystalline product. Deacetylation of XII gave N^2-methylguanosine (VI), chromatographically homogeneous, which readily recrystallized from water. It was quite

Scheme II



clear by comparison that the synthesis *via* 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) was a superior route for the preparation of N^2-methyl- and N^2-dimethylguanosine.

Synthesis of N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)alanine. The investigation of the acid-soluble nucleotides occurring in a *Fusarium* sp. grown in submerged culture revealed a new guanosine derivative[40,41] which on the basis of preliminary degradation studies was assigned the structure N-(9-β-D-ribofuranosylpurin-6-on-2-yl)alanine (XVII), "guanosine propionic acid." The isolation of the aglycon of this nucleoside, N-(purin-6-on-2-yl)alanine, "guanine propionic acid" has been reported from extracts of *Eremothecium ashbyii*.[42] It has been postulated that this derivative may be an intermediate in the interconversion of xanthine and guanine in this latter organism.[42] Acid hydrolysis gave L-lactic acid[43] which suggests that the alanine residue possesses the L-configuration. Since the nucleoside XVII is an N^2-substituted guanosine it seemed worthwhile to attempt the synthesis by the general procedure devised for the N^2-methylguanosines VI and VII.

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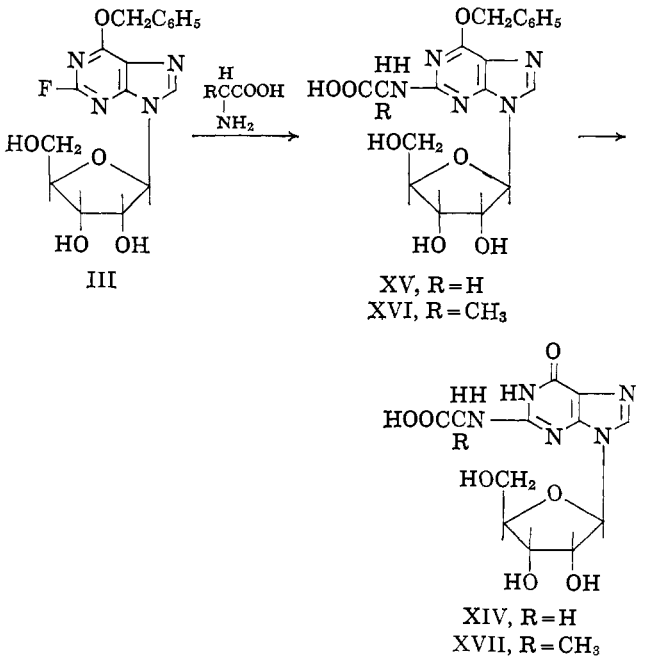
Scheme III



2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) and L-alanine in the presence of aqueous potassium carbonate and dimethylformamide gave an 80% yield of N-(6-benzyloxy-9-β-D-ribofuranosylpurin-6-on-2-yl)-L-alanine (XVI). Catalytic reduction of XVI with palladium on carbon in the presence of hydrogen gave an 86% yield of N-(9-β-D-ribofuranosylpurin-6-on-2-yl)-L-alanine (XVII). The corresponding N-(9-β-D-ribofuranosylpurin-6-on-2-yl)-D-alanine was similarly prepared. Comparison of the ultraviolet absorption spectra and other data recorded for the natural product[40] with that obtained for the synthetic material (XVII, both D- and L-forms) revealed that the compounds were similar in these respects. Chromatographic comparison of the synthetic product (XVII) with the natural nucleoside[44] revealed no differences. Establishment of the optical isomer of the amino acid moiety, whether derived from D-or L-alanine, awaits isolation of sufficient natural product to determine the optical rotation. N-(Purin-6-on-2-yl)-L-alanine was prepared according to the procedure of Ballio and co-workers.[36] The generality of this sequence of reactions *via* 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) was further demonstrated by the synthesis of N-(9-β-D-ribofuranosylpurin-6-on-2-yl)glycine (XIV). The preparation of XIV *via* 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX) was also studied. Oxidation of N-(6-benzylthio-9-β-D-ribofuranosylpurin-2-yl)glycine (XIII) to XIV was achieved with hydrogen peroxide and glacial acetic acid. The crude product XIV was purified by chromatography on Whatman No. 3MM paper to give 170 mg. of a homogeneous product identical with N-(9-β-D-ribofuranosylpurin-6-on-2-yl)glycine (XIV) prepared *via* 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III). 2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) was especially useful for the synthesis of purine nucleosides with an amino acid moiety at position 2. The benzyloxy group provided not only a function readily converted to oxy but also conveyed solubility properties to the molecule which allowed the nucleoside amino acid derivative to be readily recovered from aqueous solution. The high degree of water solubility of nucleoside amino acid presents an especially difficult problem in the isolation and purification of these materials, particularly when these substances become contaminated with inorganic salts. In the present procedure the N-(6-benzyloxy-9-β-D-ribofuranosylpurin-2-yl)amino acids were readily isolated and purified. The remaining step involved catalytic debenzylation which left toluene as the only contaminant in the final step.

The ultraviolet absorption spectral data and R_f values of the nucleosides prepared in the present study are listed in Tables I and II.

(44) The authors wish to thank Dr. A. Ballio for a small sample of the natural nucleoside for comparison with XVII.

Table I. R_f Values of Some N^2-Substituted Guanosines[a]

Compd.	Solvent systems A	B	C
N^2-Methylguanosine (VI)	0.65	0.48	0.59
N^2-Dimethylguanosine (VII)	0.77	0.56	0.64
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-L-alanine (XVII)	0.81	0.37	0.48
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-D-alanine (XVII)	0.82	0.37	0.46
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-glycine (XIV)	0.82	0.22	0.39

[a] The chromatograms were run on Whatman No. 1 paper using descending chromatography. The nucleosides were detected on the chromatograms with the aid of ultraviolet light: A, 5% aqueous ammonium bicarbonate; B, isopropyl alcohol–water–concentrated aqueous ammonia, 65:25:5 (v./v.); C, ethanol–water, 70:30 (v./v.).

Experimental

2-Amino-6-benzyloxy-9-β-D-ribofuranosylpurine (II). 2-Amino-6-chloro-9-β-D-ribofuranosylpurine (I,[34] 14.0 g., 0.048 mole) was added to a solution of 3.3 g. of sodium in 100 ml. of benzyl alcohol. The mixture was heated with stirring to 110–115° and stirred at that temperature for 5 min. The resulting mixture was cooled for 30 min. at room temperature and then poured with vigorous stirring into 2 l. of ethyl ether. To the mixture was added 5.4 ml. of glacial acetic acid. The ethereal mixture was stirred for 30 min. and then filtered. The solid was dried and then slurried and pulverized in 100 ml. of water. The mixture was neutralized with glacial acetic acid and filtered. The solid was dried at approximately 0.1 mm. over calcium chloride to give 12.0 g. of crude product. This product was dissolved in 200 ml. of boiling water and the solution filtered. The hot filtrate was poured over 200 g. of ice and the solution further cooled. The solid was filtered and dried to give 8.6 g. (47% yield). For analysis the product was dried at 80° (*ca.* 0.1 mm.) for 6 hr. The product could be recrystallized from isopropyl alcohol to give needles, m.p. 158–160°.

Anal. Calcd. for $C_{11}H_{19}N_5O_5$: C, 54.7; H, 5.13; N, 18.8. Found: C, 54.7; H, 4.89; N, 19.00.

2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III). Finely divided crude 2-amino-6-benzyloxy-9-β-D-ribofuranosylpurine (II, 10.0 g., 0.023 mole) was slowly added to 100 ml. of vigorously stirring 48% aqueous fluoroboric acid previously cooled to −20°. A solution of 2.8 g. (0.04 mole) of sodium nitrite in 10 ml. of water was gradually added to the mixture over a

Table II. Ultraviolet Absorption Spectra of Some N^2-Substituted Guanosines

Compd.	$\lambda_{max}^{pH\ 1}$, mμ (ε)	$\lambda_{min}^{pH\ 1}$, mμ (ε)	$\lambda_{max}^{pH\ 11}$, mμ (ε)	$\lambda_{min}^{pH\ 11}$, mμ (ε)
N^2-Methylguanosine (VI)	258 (14,300), 281[a] (7,900)	231 (4,100)	254 (14,800), 270[a] (11,400)	228 (3,100)
N^2-Dimethylguanosine (VII)	264 (12,800), 293[a] (5,900)	236 (3,200)	262 (12,200), 273[a] (10,600)	240 (7,500)
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-L-alanine (XVII)	258 (14,600), 277[a] (9,200)	229 (4,500)	258 (12,800), 270[a] (11,000)	234 (7,100)
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-D-alanine (XVII)	258 (14,500), 277[a] (9,400)	229 (4,900)	257 (12,800), 270[a] (10,600)	234 (7,400)
N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)glycine (XIV)	257 (14,700), 277[a] (9,500)	228 (3,700)	257 (13,600), 270[a] (11,500)	234 (7,200)

[a] Shoulder.

Table III. Ultraviolet Absorption Spectra of Some 2,6-Disubstituted Purine Ribonucleosides

Compd.	$\lambda_{max}^{pH\ 1}$, mμ (ε)	$\lambda_{max}^{pH\ 11}$, mμ (ε)	$\lambda_{max}^{methanol}$, mμ (ε)
2-Amino-6-benzyloxy-9-β-D-ribofuranosylpurine (II)	288 (10,700)	282 (10,600)	
	243 (10,300)	246 (11,600)	
2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III)			256 (13,800)
2-Fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX)	297 (25,500)	297 (26,200)	296 (27,500)
			291[a] (25,200)
2-Methylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV)	300 (8,500)	291 (8,500)	
	247 (10,500)	252 (12,700)	
2-Dimethylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (V)	312 (8,100)	298 (8,400)	
	257 (14,300)	258 (14,900)	
2-Methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X)	330 (9,100)	330 (9,900)	320 (10,400)
	256 (12,900)	(18,400)	253 (19,900)
2-Dimethylamino-6-benzylthio-9-β-D-ribofuranosylpurine (XI)	340 (5,600)	330 (6,000)	327 (6,300)
	263 (13,200)	260 (15,700)	258 (15,800)
	222 (13,100)	228 (11,000)	
N-(6-Benzyloxy-9-β-D-ribofuranosylpurin-2-yl)glycine (XV)	295 (9,100)	290 (9,100)	
	246 (11,000)	250 (12,600)	
N-(6-Benzyloxy-9-β-D-ribofuranosylpurin-2-yl)-L-alanine (XVI)	294 (10,000)	209 (10,000)	
	247 (12,300)	251 (13,500)	
N-(6-Benzylthio-9-β-D-ribofuranosylpurin-2-yl)-D-alanine (XVI)	295 (9,200)	290 (9,200)	
	247 (11,700)	251 (13,800)	
N-(6-Benzylthio-9-β-D-ribofuranosylpurin-2-yl)glycine (XIII)	322 (12,000)	320 (11,200)	
	252 (14,400)	253 (18,800)	
2-Methylamino-6-hydroxy-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (XII)	259 (14,000)	257 (13,900)	252 (17,900)
	282[a] (8,500)	268[a] (11,000)	275 (10,200)
N^2-Methylaminopurin-6-one	280 (13,900)	278 (7,200)	
	250 (6,300)	244 (9,500)	
N^2-Dimethylaminopurin-6-one	256 (19,000)	282 (7,500)	
	288 (6,500)	245[a] (12,900)	
N-(2-Aminopurin-6-on-2-yl)-L-alanine	249 (15,900)	244 (9,700)	
	277 (7,200)	277 (7,200)	

[a] Shoulder.

period of 30 min. while the temperature was maintained at −20°. After the solution was added, the mixture was stirred an additional 15 min. at −20°. To the mixture 100 ml. of 28% ammonium hydroxide then was added dropwise. The temperature of the mixture was maintained as low as possible during the neutralization. As the mixture thickened the temperature gradually rose from −20 to −5°. (It is important to maintain the temperature at least below −15° while the pH of the mixture is below 4.) After neutralization, the mixture was diluted with 100 ml. of water and the solid was filtered. The tan solid was then slurried in 100 ml. of water, filtered, and dried *in vacuo* over calcium chloride. The crude solid was dissolved in 50 ml. of boiling methanol and the boiling mixture was poured into 1500 ml. of boiling isopropyl ether. The hot mixture was filtered and the solid was discarded. The filtrate was evaporated under reduced pressure to a thick slurry. The slurry was filtered and the solid dried to give 2.4 g. (24% yield) of white product. A small amount of product was recrystallized from acetone–petroleum ether (b.p. 60–110°) and dried at 80° (*ca.* 0.1 mm.) for 6 hr. to give an analytically pure sample.

Anal. Calcd. for $C_{17}H_{17}FN_5O_5$: C, 54.3; H, 4.56; N, 14.88. Found: C, 54.31; H, 4.82; N, 15.01.

2-Fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX). Finely divided 2-amino-6-benzylthio-9-β-D-ribofuranosylpurine[37] (VIII, 46.8 g., 0.12 mole) was added to 200 ml. of 48% aqueous fluoroboric acid at 0° and the mixture then was cooled to −5°. A white solid precipitated, forming a thick suspension. An additional 100 ml. of cold (0°) fluoroboric acid was added to break up the suspension. A solution of sodium nitrite (13.8 g., 0.20 mole) in 40 ml. of water was slowly added over a period of 1.25 hr. while the temperature was maintained at −5°. The resulting solution was stirred at −5° for an additional 15 min. and then diluted with 200 ml. of ice water. A gummy solid formed which coagulated upon dropwise addition of 28% ammonium hydroxide. The acidic aqueous portion then was decanted and discarded. The gum immediately was mixed with 200 ml. of ice water and pulverized with a glass rod. The gum solidified and the aqueous mixture then was neutralized at 0 to 5° with 28% ammonium hydroxide. The solid was filtered and dried *in vacuo* over calcium chloride to give approximately

34 g. of crude product. A solution of 17 g. of the crude product dissolved in boiling methanol (100 ml.) was poured into 1500 ml. of boiling isopropyl ether. The resulting solution was filtered and the solid was discarded. The filtrate was evaporated to a volume of 200–300 ml. The white solid which formed then was filtered and dried to give 8 g. (33% yield) of monohydrate.

Anal. Calcd. for $C_{17}H_{17}FN_4O_4S \cdot H_2O$: C, 49.8; H, 4.68; F, 4.63; N, 13; H_2O, 4.40. Found: C, 49.9; H, 5.01; F, 4.60; N, 13.4; H_2O, 4.2.

To obtain an anhydrous sample the product was recrystallized from ethanol. The sample had a melting point of 144–146°.

Anal. Calcd. for $C_{17}H_{17}FN_4O_4S$: C, 52.1; H, 4.37; N, 14.3. Found: C, 52.4; H, 4.54; N, 14.3.

2-Methylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV). 2-Fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III, 500 mg., 1.3 mmoles) was dissolved in 20 ml. of 20% methanolic methylamine and the solution was allowed to stand at room temperature for 1 hr. The solution was then evaporated to a foam *in vacuo* and the foam was mixed with 25 ml. of water. The pH of the mixture was adjusted to 6 and the solid was extracted three times with 10-ml. portions of ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous magnesium sulfate and filtered and the filtrate was evaporated *in vacuo* at 60° to give a white foam. When dried at 80° (*ca.* 0.1 mm.) over P_2O_5 a yield of 370 mg. (72% yield) of analytically pure sample was obtained. The product was recrystallized from toluene.

Anal. Calcd. for $C_{18}H_{21}N_5O_4$: C, 55.8; H, 5.44; N, 18.1. Found: C, 55.9; H, 5.62; N, 17.8.

2-Dimethylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (V). A sample of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III, 350 mg., 0.9 mmole) was dissolved in 15 ml. of 20% methanolic dimethylamine and the solution was allowed to stand at room temperature for 30 min. The solution was then evaporated to dryness and the residue was mixed with 20 ml. of water. After acidification of the mixture to pH 6 the solid was extracted three times with 10-ml. portions of ethyl acetate. The combined extracts were dried with anhydrous magnesium sulfate and filtered. The filtrate was evaporated *in vacuo* at 60° to give a white foam. The foam was dried at 80° (*ca.* 0.1 mm.) over P_2O_5 to give an analytically pure sample (270 mg., 73% yield). The product was recrystallized from toluene to give a solid, m.p. 164–166°.

Anal. Calcd. for $C_{19}H_{23}N_5O_5$: C, 57.0; H, 5.75; N, 17.5. Found: C, 57.2; H, 5.64; N, 17.2.

2-Methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X). To a solution of 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX, 10.0 g., 0.024 mole) in 100 ml. of methanol was added 25 ml. of a 20% solution of methanolic methylamine. The solution was allowed to stand at room temperature for 30 min. and then was evaporated to an oil *in vacuo*. Upon trituration with a 50% petroleum ether (b.p. 60–110°)–acetone mixture the oil solidified and the product was filtered from the mixture. The solid was slurried and pulverized in acetone, filtered, and dried to yield 8.1 g. (82% yield) of pure product as shown by chromatography. An analytically pure sample, m.p. 119–120°, was obtained by recrystallization of the product from petroleum ether (b.p. 60–110°)–acetone.

Anal. Calcd. for $C_{18}H_{21}N_5O_4S$: C, 53.6; H, 5.25; N, 17.35. Found: C, 53.7; H, 5.23; N, 17.0.

2-Dimethylamino-6-benzylthio-9-β-D-ribofuranosylpurine (XI). To a solution of 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX, 5.0 g., 0.012 mole) in 100 ml. of methanol was added 25 ml. of a 20% solution of methanolic dimethylamine. The solution then was evaporated to an oil *in vacuo* and acetone was added to the residue. A solid formed when the mixture was allowed to stand. The solid was pulverized, filtered from the mixture, washed thoroughly with acetone, and dried to yield 4.9 g. (96% yield) of product. An analytically pure sample, m.p. 153–154°, was obtained by recrystallization of the product from a mixture of petroleum ether–acetone.

Anal. Calcd. for $C_{19}H_{23}N_5O_4S$: C, 54.6; H, 5.52; N, 16.8. Found: C, 54.3; H, 5.52; N, 16.8.

N-(6-Benzyloxy-9-β-D-ribofuranosylpurin-2-yl)glycine (XV). A solution of 800 mg. (2.3 moles) of glycine and 138 mg. (1.0 mmole) of K_2CO_3 in 4 ml. of water was diluted with 4 ml. of dimethylformamide. To the solution was added 500 mg. (1.3 mmoles) of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III) which dissolved on slight warming. The solution was heated on the steam bath (*ca.* 80°) for 3 hr. during which time the pH dropped to about 8. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in 15 ml. of water. The resulting solution was acidified to pH 5 with 25% aqueous formic acid. A white solid formed and was filtered from the mixture and washed with water. The solid was dried over calcium chloride *in vacuo* to yield 470 mg. (81% yield) of product judged to be over 95% pure by ultraviolet absorption spectral data. A small amount was recrystallized from a water–acetone mixture and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 to yield an analytically pure sample. When heated the compound began to brown at *ca.* 100° and melted with decomposition at 162–164°.

Anal. Calcd. for $C_{19}H_{21}N_5O_7$: C, 52.9; H, 4.92; N, 16.3. Found: C, 52.6; H, 5.03; N, 16.1.

N-(6-Benzyloxy-9-β-D-ribofuranosylpurin-2-yl)-L-alanine (XVI). A solution of 480 mg. (5.4 moles) of L-alanine and 280 mg. (2.0 mmoles) of potassium carbonate in 8 ml. of water was diluted with 8 ml. of dimethylformamide. To the solution was added 1.0 g. (2.7 mmoles) of 2-fluoro-6-benzyloxy-9-β-D-ribofuranosylpurine (III). The resulting solution was heated on the steam bath (*ca.* 80°) for 3 hr. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in 30 ml. of water. A gummy solid formed when the solution was acidified to pH 4–5 with 25% aqueous formic acid. When the mixture was allowed to stand overnight, the gum solidified and was pulverized and filtered from the mixture and the product was washed thoroughly with water. The solid was dried at 80° (*ca.* 0.1 mm.) over calcium chloride to give 960 mg. (80% yield) of product which was quite acceptable for further synthetic use. A small amount of product was recrystallized from a water–acetone mixture and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 to give an analytically pure sample, m.p. 197–199° dec.

Anal. Calcd. for $C_{20}H_{23}N_5O_7$: C, 53.9; H, 5.21; N, 15.7. Found: C, 53.8; H, 5.04; N, 15.7.

N-(6-Benzyloxy-9-β-D-ribofuranosylpurin-2-yl)-D-alanine (XVI). This product was prepared as for the L-isomer and recrystallized from water containing a small amount of acetone to give a compound which melted with decomposition from 183 to 185°.

Anal. Calcd. for $C_{20}H_{23}N_5O_7$: C, 53.9; H, 5.21; N, 15.7. Found: C, 53.7; H, 5.29; N, 15.5.

N-(6-Benzylthio-9-β-D-ribofuranosylpurin-2-yl)glycine (XIII). Glycine (0.99 g., 13.2 mmoles) and potassium carbonate (0.66 g., 4.8 mmoles) were dissolved in 20 ml. of water and the solution was diluted with 20 ml. of dimethylformamide. To the resulting solution was added 2-fluoro-6-benzylthio-9-β-D-ribofuranosylpurine (IX, 2.6 g., 6.6 mmoles). The solid dissolved upon heating and the solution was heated on the steam bath (*ca.* 80°) for 3 hr. The solution was then evaporated to dryness *in vacuo* and the residue was dissolved in 50 ml. of water. The aqueous solution was acidified with 25% aqueous formic acid to pH 4–5 and the precipitated solid was filtered from the mixture, washed thoroughly with water, and dried over calcium chloride at room temperature. A yield of 2.5 g. (85% yield) of chromatographically homogeneous, white product was obtained. The product was recrystallized from acetone–water and dried *in vacuo* over calcium chloride to give an analytically pure sample, m.p. 209–210° dec.

Anal. Calcd. for $C_{19}H_{21}N_5O_6S \cdot 0.5H_2O$: C, 50.1; H, 4.85; N, 15.4. Found: C, 50.3; H, 4.83; N, 15.2.

2-Methylamino-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purin-6-one (XII). A sample of 2-methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X, 3.0 g., 7.0 mmoles) was dissolved in a solution of 15 ml. of acetic anhydride and 5 ml. of pyridine. The solution was stirred at room temperature for 6 hr. and then evaporated to an oil *in vacuo* at 40°. The oil was dissolved in 10 ml. of glacial acetic acid and 2.5 ml. of 30% hydrogen peroxide was added to the solution. The solution was stirred for 24 hr. at room temperature and then evaporated to an oil *in vacuo*. The oil was mixed with 50 ml. of water and the mixture was extracted five times with 20-ml. portions of ethyl acetate. The combined extracts were evaporated until a slurry of white solid remained. This was filtered and dried to give 800 mg. (25% yield) of product. This product was recrystallized from methanol (25 ml.) to give 680 mg. of analytically pure product, m.p. 265–268° dec.

Anal. Calcd. for $C_{17}H_{21}N_5O_8$: C, 48.3; H, 4.97; N, 16.6. Found: C, 48.5; H, 5.03; N, 16.6.

2-Methylamino-6-hydroxy-9-β-D-ribofuranosylpurine (VI). Method A. A sample of 2-methylamino-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purin-6-one (XII, 600 mg., 0.7 mmole) was dissolved in a methanolic solution of methylamine (15 ml. of methylamine in 40 ml. of methanol) and the solution was allowed to stand at room temperature for 16 hr. The solution was then evaporated to dryness and the residue was slurried in acetone. The white solid was filtered and dried to give 420 mg. (94% yield) of product which was chromatographically homogeneous. The product was shown to be over 95% pure by ultraviolet absorption data. The product was recrystallized from water and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. to give an analytically pure sample of the monohydrate. When heated the compound began to turn brown at about 200° and decomposed without melting upon further heating.

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot H_2O$: C, 42.0; H, 5.45; N, 22.2. Found: C, 41.9; H, 5.50; N, 22.2.

Method B. To a solution of 300 mg. (0.78 mmole) of 2-methylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (IV) in 80 ml. of 50% ethanol–water was added 150 mg. of 5% palladium on carbon. The mixture was then hydrogenated on a Parr shaker under 46 p.s.i. of hydrogen for 8 hr. Upon completion of the hydrogenation the mixture was evaporated to dryness *in vacuo* and the residue was boiled in 15 ml. of water. The boiling mixture was filtered through a Celite pad and the palladium on carbon was washed three times with 5-ml. portions of boiling water. The filtrate was then cooled and the product was filtered from the mixture and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. to give 140 mg. (55% yield) of analytically pure monohydrate, $[\alpha]^{26}D$ −34.6° (*c* 1.01, 50% dimethyl sulfoxide–ethanol).

Anal. Calcd. for $C_{11}H_{15}N_5O_5 \cdot H_2O$: C, 42.0; H, 5.45; N, 22.2. Found: C, 42.25; H, 5.44; N, 22.05.

Method C. To a solution of 2 ml. of 30% hydrogen peroxide in 5 ml. of glacial acetic acid was added 1.5 g. (0.6 mmole) of 2-methylamino-6-benzylthio-9-β-D-ribofuranosylpurine (X). The solution was stirred at room temperature for 24 hr. and then poured slowly into 200 ml. of vigorously stirring acetone. The white solid that formed was filtered from the mixture, washed thoroughly with acetone, and dried *in vacuo* over calcium chloride to give 800 mg. (73% yield) of product. The product was chromatographically homogeneous and identical with products prepared by methods A and B as judged by ultraviolet absorption spectra. This product, however, was not crystalline.

2-Dimethylamino-9-β-D-ribofuranosylpurin-6-one (VII). A sample of 2-dimethylamino-6-benzyloxy-9-β-D-ribofuranosylpurine (V, 200 mg., 0.5 mmole) was dissolved in 50 ml. of 50% ethanol–water, and 100 mg. of 5% palladium on carbon was added to the solution. The mixture was then shaken for 8 hr. under 46 p.s.i. of hydrogen. The mixture was then filtered through a Celite pad and the palladium on carbon was washed five times with 5-ml. portions of boiling water. The filtrate was evaporated to dryness and the white residue was slurried with acetone. The white solid was filtered from the mixture and dried to give 130 mg. (84% yield) of product which was chromatographically homogeneous. The product was recrystallized from 3.5 ml. of water to yield 100 mg. (65% yield) of analytically pure, colorless needles, m.p. 242° dec., $[\alpha]^{26}D$ −35.6° (*c* 1.07, 50% dimethyl sulfoxide–ethanol).

N-(9-β-D-ribofuranosylpurin-6-on-2-yl)glycine (XIV). Method A. A sample of N-(6-benzyloxy-9-β-D-ribofuranosylpurin-2-yl)glycine (XV, 300 mg., 0.7 mmole) was dissolved in 50 ml. of 50% ethanol–water and 150 mg. of 5% palladium on carbon was added to the solution. The mixture was shaken in a Parr bottle under 46 p.s.i. of hydrogen gas for 6.5 hr. and filtered. The filtrate was evaporated to dryness *in vacuo* and the white residue was triturated with acetone and filtered. A yield of 180 mg. (77% yield) of product was obtained. The product at this point was

chromatographically homogeneous. The product was recrystallized from 5 ml. of water and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. to give 130 mg. (55% yield) of analytically pure N-(9-β-D-ribofuranosylpurin-6-on-2-yl)glycine. The compound turned brown when heated to 235° and slowly decomposed without melting, $[\alpha]^{24}D$ −28.9° (*c* 0.738, 0.844 *N* NaOH).

Anal. Calcd. for $C_{12}H_{15}N_5O_7 \cdot H_2O$: C, 40.2; H, 4.78; N, 19.52. Found: C, 40.48; H, 4.80; N, 19.95.

Method B. To a solution of 3 ml. of 30% hydrogen peroxide in 20 ml. of glacial acetic acid was added 2.7 g. (6.0 mmoles) of N-(6-benzylthio-9-β-D-ribofuranosylpurin-2-yl)glycine (XIII). The solid slowly dissolved and the solution was stirred at room temperature for 24 hr. The solution was then added dropwise to 200 ml. of vigorously stirring acetone and a yellow-white solid formed. The solid was filtered from the mixture and immediately dried *in vacuo* over calcium chloride. The dried product was then dissolved in 15 ml. of water by the addition of 1 *N* sodium hydroxide. Upon acidification of the solution to pH 4 with 25% formic acid a solid precipitated from the solution. The mixture was allowed to stand at 5° for 48 hr. and then filtered. The solid was washed with 2 ml. of water and then slurried with acetone, filtered from the solution, and dried to give 430 mg. of crude product. The product was purified in 75-mg. batches using paper chromatography. The 75 mg. was dissolved in methanol–water and the solution was streaked lengthwise along a 46 × 57 cm. sheet of Whatman 3MM paper. The sample was then chromatographed using 3% ammonium chloride solution and descending chromatography. The product was contained in the fastest moving strip and the darkest portion (under ultraviolet light) of this strip was cut out and extracted with boiling water. The aqueous solution was evaporated to dryness and the residue was dissolved in 3 ml. of water. To the solution was added 0.5 drop of 25% formic acid. The solid which formed, was filtered from the solution and washed with water. A yield of 32 mg. of chromatographically homogeneous product was obtained. A total of 170 mg. of product was obtained from combined runs. The product was recrystallized from water and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. to give an analytically pure sample. When heated the compound turned brown at 235° and slowly decomposed upon further heating.

Anal. Calcd. for $C_{12}H_{15}N_5O_7 \cdot H_2O$: C, 40.2; H, 4.78; N, 19.52. Found: C, 40.42; H, 4.66; N, 19.95.

N-(9-β-D-Ribofuranosylpurin-6-on-2-yl)-L-alanine (*XVII*). To a solution of 1.8 g. (4.0 mmoles) of N-(6-benzyloxy-9-β-D-ribofuranosylpurin-2-yl)-L-alanine (XVI) in 75 ml. of 50% ethanol–water was added 900 mg. of 5% palladium on carbon. The mixture was shaken for 6 hr. under a pressure of 46 p.s.i. of hydrogen. The solution was filtered and evaporated to dryness *in vacuo*. The white residue was slurried in acetone, filtered, and dried to give 1.2 g. (86% yield) of chromatographically homogeneous product. A small amount was recrystallized from ethanol and dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. to give an analytically pure sample. When heated the compound began to turn brown after 200° and effervesced at 246°, $[\alpha]^{25}D$ −9.2° (*c* 1.10, H_2O), R_f 0.48 (ethanol–water, 7:3), R_f 0.79 (water–isopropyl alcohol, 2:1). These R_f values (Whatman No. 1 paper) were identical with those found for the natural nucleoside.[44]

Anal. Calcd. for $C_{13}H_{17}N_5O_7 \cdot 0.25H_2O$: C, 43.3; H, 4.90; N, 19.4. Found: C, 43.3; H, 5.03; N, 19.2.

N-(9-β-D-Ribofuranosyopurin-6-on-2-yl)-D-alanine (*XVII*). A solution of 750 mg. (1.7 mmoles) of N-(6-benzyloxy-9-β-D-ribofuranosylpurin-2-yl)-D-alanine in 75 ml. of 50% ethanol–water was mixed with 370 mg. of 5% palladium on carbon and hydrogenated as for the L-isomer. The product was treated as for the L-isomer to yield 520 mg. (87% yield) of chromatographically homogeneous, white product. Recrystallization of the product from methanol–acetone gave a pure sample which was dried at 80° (*ca.* 0.1 mm.) over P_2O_5 for 6 hr. for analysis, $[\alpha]^{25}D$ +9.9° (*c* 1.0.0, H_2O). The R_f values (Whatman No. 1 paper) were identical with those found for the natural nucleoside.[44]

Anal. Calcd. for $C_{13}H_{17}N_5O_7 \cdot 0.25H_2O$: C, 43.3; H, 4.90; N, 19.4. Found: C, 43.5; H, 4.92; N, 19.6.

2-Methylaminopurin-6-one.[28] A sample of 4.5 g. (26.4 mmoles) of 2-chloropurin-6-one[26] was dissolved in 130 ml. of 25% methanolic methylamine and heated in a steel bomb at 130° for 18 hr. The excess methylamine was evaporated *in vacuo* and the product was filtered from the methanol. A yield of 3.4 g. (77% yield) of white product was obtained. An analytically pure sample was obtained when the product was reprecipitated from hot, dilute hydrochloric acid with ammonium hydroxide. The sample was dried at 100° (*ca.* 24 mm.) over calcium chloride for analysis.

Anal. Calcd. for $C_6H_7N_5O$: C, 43.6; H, 4.28; N, 42.4. Found: C, 43.5; H, 4.67; N, 42.5.

2-Dimethylaminopurin-6-one.[28] 2-Chloropurin-6-one[26] (9.0 g., 0.053 mole) was dissolved in 100 ml. of 20% methanolic dimethylamine and heated in a steel bomb at 130° for 18 hr. The solution was cooled and then evaporated to dryness. The residue was recrystallized from 2 *N* hydrochloric acid to give 8.2 g. (62% yield) of the hydrochloride. This was recrystallized from ethanol and dried to give an analytically pure sample of the monohydrate of the hydrochloride.

Anal. Calcd. for $C_7H_9N_5O \cdot HCl$: C, 36.0; H, 5.18; N, 30.0. Found: C, 35.8; H, 5.17; N, 29.8.

N-(Purin-6-on-2-yl)-L-alanine.[40] A solution of 6.0 g. (0.035 mole) of 2-chloropurin-6-one,[26] 5.5 g. of potassium carbonate, and 6.4 g. of L-alanine in 25 ml. of water was heated in a glass-lined, steel bomb at 135° for 18 hr. The resulting solution was cooled and acidified to pH 3 with concentrated formic acid. After the solution was allowed to stand at 5° for 12 hr. the solid which had formed was filtered from the mixture, washed with 5 ml. of ice water, and dried to give 3.5 g. (42% yield) of product. Recrystallization of the product from water gave a chromatographically pure sample.

Anal. Calcd. for $C_8H_9N_5O_3 \cdot 0.5H_2O$: C, 41.4; H, 4.77; N, 30.2. Found: C, 41.8; H, 4.69; N, 29.9.