determined in aqueous solution with a Cary Model 14 spectrophotometer; the infrared spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 221 spectrophotometer. The paper chromatograms were run on Whatman No. 1 paper by the descending technique.

3H-Imidazo[4,5-*b*]pyridine-7(4H)-thione (Ia) and 1H-Imidazo[4,5-*c*]pyridine-4(5H)-thione (IIa).—A suspension of 7-aminoimidazo[4,5-*b*]pyridine hydrochloride and 4-aminoimidazo[4,5-*c*]pyridine hydrochloride (3.2 g., 18.8 mmoles) in concentrated HCl (50 ml.) was cooled to 0°. With continuous stirring, solid $NaNO_2$ (2.7 g., 39.2 mmoles) was added over a 3-hr. period, and the mixture was stirred until a negative Bratton–Marshall test was obtained (18 hr.). The reaction was filtered to remove inorganic solid, and the filtrate was evaporated to dryness *in vacuo* with several additions of alcohol. Unreacted 4-aminoimidazo[4,5-*c*]pyridine hydrochloride was removed by filtration from a hot ethanol solution of the crude product. Two additional ethanol triturations followed by recrystallization of the product from ethanol removed essentially all of the starting compound; yield 2.1 g. (59%).

A solution of the mixture of 7-chloroimidazo[4,5-*b*]pyridine and 4-chloroimidazo[4,5-*c*]pyridine (540 mg., 2.9 mmoles) and thiourea (218 mg., 2.9 mmoles) in ethanol (20 ml.) was allowed to stand at room temperature, under anhydrous conditions, overnight. The reaction mixture was evaporated to dryness and the residue was chromatographed on a cellulose column using butanol–water (86:14) as the eluent. The green fluorescent product that was eluted from the column first was identified as essentially pure 1H-imidazo[4,5-*c*]pyridine-4(5H)-thione (77 mg.). The yellow fluorescent material that was eluted second was the desired 3H-imidazo[4,5-*b*]pyridine-7(5H)-thione (152 mg.). This almost pure product was dissolved in 1 *N* NaOH (3 ml.), the solution was filtered through dry Celite, and the filtrate was acidified with acetic acid. The crystals that precipitated on standing were collected by filtration and recrystallized from water to give the pure product: yield 62 mg.; m.p. dec. above 200°; R_f (water-saturated butanol) 0.44; neut. equiv., 167 (calcd., 165); λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—244 (7.9), 285 (9.7), 339 (17.9); pH 7—285 (10.9), 336 (12.3); pH 13—228 (14.3), 302 (17.0); $\bar{\nu}_{max}$ in cm.$^{-1}$, 3000–2500 (acidic H), 1600, 1510 (C=C, C=N).

Anal. Calcd. for $C_6H_5N_3S \cdot 0.75H_2O$: C, 43.67; H, 3.94; N, 25.47. Found: C, 43.99; H, 4.18; N, 25.82.

1H-Imidazo[4,5-*c*]pyridine-4(5H)-thione (IIa). B.—A mixture of 1H-imidazo[4,5-*c*]pyridin-4-ol (0.5 g., 2.7 mmoles) and phosphorus pentasulfide (3 g., 13.7 mmoles) in pyridine (50 ml.) was refluxed for 4 hr. under anhydrous conditions. The cooled reaction mixture was poured into ice water (300 ml.) and the resulting solution was acidified with glacial acetic acid and concentrated to one-third volume *in vacuo*. The sulfur that precipitated was removed by filtration, and the filtrate was concentrated to one-third volume and extracted with butanol. Evaporation of the butanol extract gave 320 mg. of crude product, which was dissolved in 1 *N* NaOH (5 ml.), and the solution was filtered through dry Celite. The purified product that precipitated on acidification of the filtrate with glacial acetic acid was collected by filtration, washed with water, and dried *in vacuo*; yield 149 mg. (26%). A second precipitation from base gave the pure product: 97 mg. (17%); m.p. dec. above 350°; R_f (water-saturated butanol) 0.51; λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—222 (12.7), 285 (sh), 338 (14.0); pH 7—224 (12.5), 283 broad (3.5), 325 (15.3); pH 13—225 (15.2), 246 (11.5), 316.5 (14.5), 325 (sh); $\bar{\nu}_{max}$ in cm.$^{-1}$, 3170, 3000, 2910 (NH, CH), 2800–2500 (acidic H), 1605, 1600, 1580, 1545 (C=C, C=N).

Anal. Calcd. for $C_6H_5N_3S$: C, 47.68; H, 3.34; N, 27.81; S, 21.21. Found: C, 47.64; H, 3.34; N, 27.40; S, 21.13.

7-Aminoimidazo[4,5-*b*]pyridine (IX) Hydrochloride and 4-Aminoimidazo[4,5-*c*]pyridine (VIII) Hydrochloride.[3,4]—A mixture of 2,4-diamino-3-nitropyridine (1 g., 6.5 mmoles) in ethanol (125 ml.) was hydrogenated at atmospheric pressure in the presence of platinum dioxide catalyst (100 mg.). After reduction was complete, the catalyst was removed by filtration in a nitrogen atmosphere, and the filtrate was evaporated to dryness *in vacuo*. The oily residue was dissolved in diethoxymethyl acetate (10 ml.), and the resulting solution was allowed to stand at room temperature under anhydrous conditions for 1 hr. before it was evaporated to dryness *in vacuo* with several additions of ethanol. The residue was dissolved in ethanol and diluted to cloudiness with water. The product that precipitated from the solution was collected by filtration and identified by its spectra as the 7-acetamidoimidazopyridine: yield 278 mg. (24%). A sample did not melt but sublimed above 260°; R_f (water-saturated butanol) 0.63; λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—286 (21.7), pH 7—273 (17.3), pH 13—282 (16.2); $\bar{\nu}_{max}$ in cm.$^{-1}$, 3200–2600 (CH, acidic H), 1710 (C=O), 1630, 1585 (C=C, C=N), 1450 (CH).

Deacetylation was effected by dissolving the acetamidoimidazopyridine in hot 6 *N* HCl. The product crystallized on cooling the acid solution and the crystals were collected by filtration and recrystallized from ethanol–water to give the 7-aminoimidazo[4,5-*b*]pyridine as the hydrochloride salt: yield 120 mg. (11%); m.p. 330–335°; R_f (water-saturated butanol) 0.48; λ_{max} in mμ ($\epsilon \times 10^{-3}$),[12] pH 1—263 (12.0), 280 (18.4), 287 (sh); pH 7—263 (12.0), 277 (13.2), 286 (sh); pH 13—275 (13.9); $\bar{\nu}_{max}$ in cm.$^{-1}$, 3300, 3100 (NH, CH), 2860–2400 (acidic H), 1660–1610 (NH, C=C, C=N), 1540, 1510 (C=C, C=N).

Anal. Calcd. for $C_6H_6N_4 \cdot HCl$: C, 42.27; H, 4.14; N, 32.88. Found: C, 42.07; H, 4.16; N, 32.51.

Deacetylation of the filtrate from the isolation of the 7-acetamidoimidazopyridine gave a mixture of both the 7- and 4-aminoimidazopyridine hydrochloride. A pure sample of the 4-aminoimidazo[4,5-*c*]pyridine hydrochloride was isolated as the hemihydrate after several fractional recrystallizations from water: m.p. >300°; R_f (water-saturated butanol) 0.35; λ_{max} in mμ ($\epsilon \times 10^{-3}$),[12] pH 1—260 (8.5), 275 (8.9); pH 7—258 (8.9), 267 (8.9); pH 13—274 (7.5); $\bar{\nu}_{max}$ in cm.$^{-1}$, 3350–2600 (NH, CH, acidic H), 1685, 1630 (NH, C=C, C=N) (absence of strong 1510 band found in 7-aminoimidazo[4,5-*b*]pyridine·HCl).

Anal. Calcd. for $C_6H_5N_3 \cdot HCl \cdot 0.5H_2O$: C, 40.26; H, 4.51; N, 31.30. Found: C, 40.52; H, 4.40; N, 31.32.

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical Section of Southern Research Institute who performed the spectral and microanalytical determinations reported and to Dr. G. J. Dixon under whose direction the cell culture cytotoxicity tests were done.

(12) Although numerical values for λ and ε for the ultraviolet spectra of VIII and IX are not given,[5,6] our curves agree reasonably well with the published curves.[5,6]

Enzyme Inhibitors. X. A Reinvestigation of the Alkylation of 6-Chloropurine by 3-Bromo-1-propanol[1]

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Recently, we described the alkylation of 6-chloropurine by 3-bromo-1-propanol and reported that the major product of reaction was the corresponding 9-isomer (61% yield).[2] In addition, a second alkylated 6-chloropurine was obtained[2] as a minor product (14% yield), and it was considered to be the corresponding 7-isomer by analogy with the results of Montgomery and Temple[3] who originally developed this method of synthesis. Because of some recent work on the position

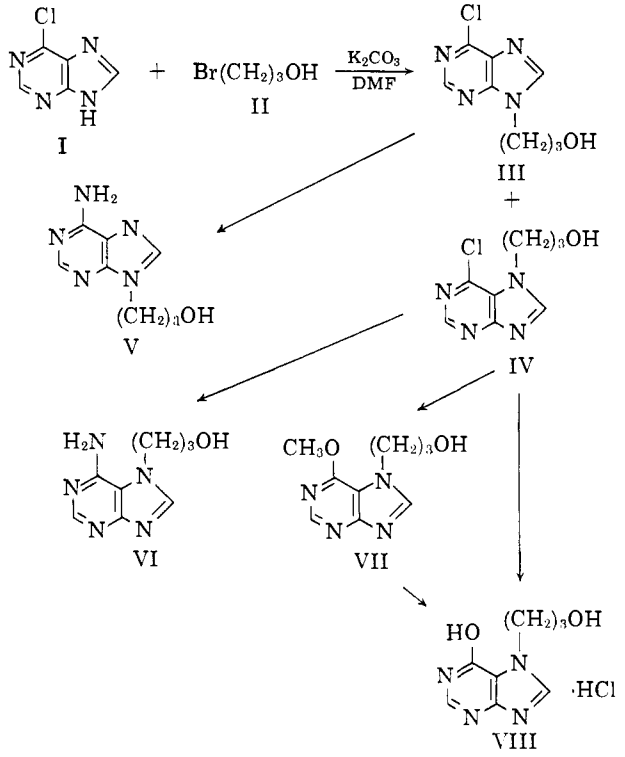
(1) This investigation was supported by Public Health Service Research Grant CA-06388-03 from the National Cancer Institute, by a Public Health Service research career program award 5-K3-CA-18718 from the National Cancer Institute, by Training Grant 5-T1-JM-555 from the Division of General Medical Sciences, and by Grant T-337 from the American Cancer Society.

(2) H. J. Schaeffer and R. Vince, *J. Med. Chem.*, **8**, 33 (1965).

(3) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

of glycosidation of some 6-dimethylaminopurines,[4] we decided to prepare some 3-hydroxypropyl positional isomers by alternate routes for the purpose of establishing the structure of the minor alkylation product and to determine the effect of these positional isomers on the enzyme, adenosine deaminase. Thus, when 6-chloropurine (I) was allowed to react with 3-bromo-1-propanol (II) in dimethylformamide in the presence of anhydrous potassium carbonate, a mixture of alkylated 6-chloropurines was obtained from which the 9-isomer (III) and the 7-isomer (IV) were separated by chromatography on alumina (see Chart I). It has previously

CHART I

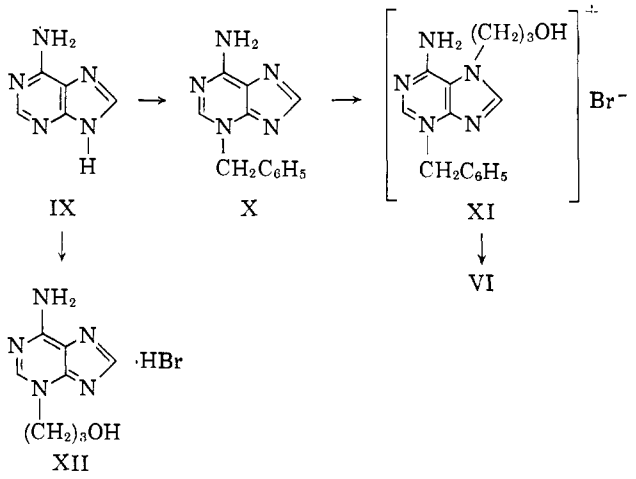


been shown that the major product of the reaction is the 9-isomer (III) by comparison[2] of this material with a sample of 9-(3-hydroxypropyl)-6-chloropurine which had been prepared by an alternate unambiguous synthesis.[5] Further confirmation of the identity of the two differently prepared samples of III was obtained when it was found that they gave the same 9-(3-hydroxypropyl)adenine (V). In addition, it was reported[2] that when IV was allowed to react with methanolic ammonia, the corresponding 6-amino analog (VI) was obtained which was purified by preparation of the hydrochloride salt. We now find that the minor product of alkylation of 6-chloropurine is, in fact, the 7-substituted isomer IV, but that the product which was previously reported as the hydrochloride salt of V is actually the hydrochloride salt of 9-(3-hydroxypropyl)-6-hydroxypurine (VIII). The reported elemental analysis for C, H, and Cl is in agreement with both structures.

It has now been found that when IV is allowed to react with liquid ammonia at 70°, an excellent yield of 7-(3-hydroxypropyl)adenine (VI) is obtained. Repetition of the experiment in which IV was treated with methanolic ammonia led again to a noncrystalline product which on conversion to a hydrochloride salt gave 7-(3-hydroxypropyl)-6-hydroxypurine hydrochloride (VIII). Examination of the ultraviolet spectrum of the noncrystalline material revealed absorption at 262 mμ which is characteristic of the 6-methoxy analog (VII).[6] Attempted preparation of the hydrochloride salt of VII resulted in the cleavage of the ether and produced the hydrochloride salt of 7-(3-hydroxypropyl)-6-hydroxypurine (VIII).[7]

Finally, the synthesis of VI by an alternative route and the synthesis of 3-(3-hydroxypropyl)adenine (XII) was accomplished by the procedures outlined in Chart II. Treatment of adenine (IX) with benzyl bromide

CHART II



gave the known 3-benzyladenine (X).[8] When X was allowed to react with 3-bromo-1-propanol, a moderate yield of 3-benzyl-7-(3-hydroxypropyl)adenine hydrobromide (XI) was obtained.[9] Catalytic hydrogenolysis of the benzyl group of XI using a palladium-on-charcoal catalyst gave a 49% yield of VI,[9] which was identical with the sample of VI which had been prepared by treating IV with liquid ammonia. When adenine (IX) was allowed to react with 3-bromo-1-propanol in dimethylacetamide, an isomerically alkylated adenine was obtained which has been assigned the structure 3-(3-hydroxypropyl)-adenine hydrobromide (XII) on the basis of its ultraviolet spectrum, its pK_a', and its method of preparation[10] (see Table I for a comparison of the ultra-

(4) L. B. Townsend, R. K. Robins, R. N. Loeppky, and N. J. Leonard, *J. Am. Chem. Soc.*, **86**, 5320 (1964).

(5) H. J. Schaeffer and P. S. Bhargava, *Biochemistry*, **4**, 71 (1965).

(6) R. N. Prasad and R. K. Robins [*J. Am. Chem. Soc.*, **79**, 6401 (1957)] have reported that 6-methoxy-7-methylpurine has λ_{max} at 256 (pH 1) and 258 mμ (pH 11). Professor Robins has informed me that in Table III of their paper a typographical error is present in that the pH value for column 4 should read pH 11.

(7) Recently we have isolated 6-methoxy-7-(*m*-nitrobenzyl)purine from the reaction of 6-chloro-7-(*m*-nitrobenzyl)purine with 20% methanolic NH_3. The 6-methoxy analog underwent rapid cleavage under the influence of hydrogen chloride in methanol to give the 6-hydroxy derivative. These and other results on the *m*-nitrobenzylpurines will be published at a later time.

(8) J. A. Montgomery and H. J. Thomas, *J. Am. Chem. Soc.* **85**, 2672 (1963).

(9) Related alkylations of 3-benzyladenine have been shown to yield the 3,7-disubstituted compounds from which the benzyl group could be removed by catalytic hydrogenolysis; see, for example, ref. 8 and N. J. Leonard and T. Fujii, *ibid.*, **85**, 3719 (1963).

(10) (a) B. C. Pal, *Biochemistry*, **1**, 558 (1962); (b) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 1914 (1962); (c) N. J. Leonard and J. H. Deyrup, *ibid.*, **84**, 2148 (1962).

TABLE I

THE ULTRAVIOLET[a] AND pK_a' DATA OF SOME SUBSTITUTED ADENINES

Compd. (adenine derivative)	H+ λ_{max}, mμ	H+ $\epsilon \times 10^{-3}$	OH− λ_{max}, mμ	OH− $\epsilon \times 10^{-3}$	pK_a' 50% DMF	pK_a' H_2O
3-Methyl[b]	274	17.7	273	13.3	5.3	
3-γ,γ-Dimethylallyl[b]	277	18.3	273	12.5	5.4	
3-(3-Hydroxypropyl) (XII)	274	18.7	273	13.9		6.3[c]
7-Methyl[b]	272	15.0	270	10.5	3.6	
7-(3-Hydroxypropyl) (VI)	272	14.5	270	9.2		4.4[c]
1-Methyl[b]	250	11.7	270	14.4	6.95 11.9	

[a] Taken in aqueous solution. [b] Data taken from ref. 10c. [c] These data were determined by Dr. M. A. Schwartz of the State University of New York at Buffalo.

violet spectra and pK_a' of these and related compounds). Thus, our results are in agreement with those of Montgomery and Temple[3] who found that in the alkylation of 6-chloropurine under conditions similar to the ones which we employed a mixture of 7- and 9-substituted 6-chloropurines was formed in which the 9-isomer predominates.[11]

Enzymatic evaluation of 7-(3-hydroxypropyl)adenine (VI) against adenosine deaminase revealed that it was essentially noninhibitory, whereas 3-(3-hydroxypropyl)adenine was weakly inhibitory ($[I/S]_{0.5} \cong 11$). By comparison, 9-(3-hydroxypropyl)adenine has an $[I/S]_{0.5} = 0.70$.[5] In agreement with our previous suggestion, these results may be rationalized if it is assumed that the enzyme has little bulk tolerance for a group at the 7-position of the purine nucleus as in VI or that an essential binding group at the 9-position is absent.[2] The weakly inhibitory activity of XII may be explained by assuming that the 3-hydroxypropyl chain at the 3-position of the purine nucleus can bind to the binding site to which a group at the 9-position of the purine nucleus normally utilizes. However, further work is necessary to differentiate this concept from the one in which there is relatively little bulk tolerance at the 3-position of the purine nucleus.

Experimental[12]

7-(3-Hydroxypropyl)adenine (VI).—A mixture of 200 mg. (0.939 mmole) of IV and 15 ml. of liquid NH_3 was heated in a steel bomb at 70–75° for 18 hr. The volatile materials were allowed to evaporate at room temperature, and the residual solid was removed and dried *in vacuo*; yield 230 mg., m.p. 181° (Kofler Heizbank). An aqueous solution of the crude material was passed through a Dowex 50 W-X8 resin column (3.0 g.) and the column was washed with water. Fractions of 5 ml. were collected until all of the chloride had been removed from the column. The pure product was then eluted from the column with 10% NH_4OH (50 ml.). Evaporation of the ammonium hydroxide solution gave pure VI, yield 163 mg. (90%), m.p. 201–203°. A small sample was recrystallized from ethanol–hexane and gave the analytical sample: m.p. 201–203°; λ_{max} in ($\epsilon \times 10^{-3}$), pH 1—272 (11.5), pH 7—270 (9.30), pH 13—270 (9.20); ν in cm.$^{-1}$ (KBr), 3450 (OH), 3300 and 1650 (NH_2), 1600 and 1550 (C=N and C=C).

Anal.[13] Calcd. for $C_8H_{11}N_5O$: C, 49.73; H, 5.73; N, 36.14. Found: C, 49.52; H, 5.62; N, 36.12.

7-(3-Hydroxypropyl)-6-hydroxypurine Hydrochloride (VIII).—A solution of 150 mg. (0.705 mmole) if IV in 10 ml. of 1 *N* HCl was heated under reflux for 45 min. The reaction mixture was treated with charcoal to remove a slight yellow color. The clear filtrate was evaporated *in vacuo* and gave a clear oil (172 mg.). The oil was dissolved in anhydrous methanol saturated with HCl, and addition of ether followed by chilling gave a white solid product (105 mg.), m.p. 155–165°. Recrystallization of the crude material from methanol–ether gave 82 mg. (50.4%) of the analytical sample (VIII): m.p. 176–178°, 179–182° (oil bath); λ_{max} in mμ ($\epsilon \times 10^{-3}$), pH 1—254 (9.35), pH 7—257 (9.35), pH 13—263 (10.0); ν in cm.$^{-1}$ (KBr), 3400 (OH), 1700 (C=N$^+$H, C=O), 1570 and 1530 (C=N and C=C).

Anal. Calcd. for $C_8H_{11}ClN_4O_2$: C, 41.65; H, 4.81; Cl, 15.36; N, 24.29. Found: C, 41.46; H, 4.85; Cl, 15.15; N, 24.17.

3-Benzyl-7-(3-hydroxypropyl)adenine Hydrobromide (XI).—A mixture of 1.20 g. (5.33 mmoles) of 3-benzyladenine[8,14] and 1.49 g. (10.7 mmoles) of 3-bromo-1-propanol in 75 ml. of N,N-dimethylacetamide was heated at 75° for 24 hr. Removal of the volatile materials *in vacuo* gave a bright yellow oil (3.88 g.) which was crystallized from absolute ethanol and gave 1.00 g. (53.2%) of the desired product, m.p. 219–222°. Recrystallization of the crude material from methanol–ether gave the analytical sample (707 mg.): m.p. 229–231°; λ_{max} mμ ($\epsilon \times 10^{-4}$), pH 1—277 (1.53), pH 7—279 (1.66), pH 13—282 (1.47); ν in cm.$^{-1}$ (KBr), 3350 (OH), 1660 (C=N$^+$H), 1620 and 1580 (C=N C=C).

Anal. Calcd. for $C_{15}H_{18}BrN_5O$: C, 49.16; H, 4.95; Br, 21.94. Found: C, 49.22; H, 5.07; Br, 21.85.

Hydrogenolysis of 3-Benzyl-7-(3-hydroxypropyl)adenine Hydrobromide.—A solution of 260 mg. (0.715 mmole) of XI in 100 ml. of absolute ethanol and 3 ml. of water was hydrogenated over 100 mg. of 5% palladium-on-charcoal catalyst at 80° and 3.8 kg./cm.^{2} for 48 hr. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo* to a thick oil (244 mg.). The crude mixture was dissolved in 25 ml. of water and made basic with 5% $NaHCO_3$. The aqueous solution was continuously extracted with ether for 24 hr. to remove unreacted starting material. The aqueous fraction was evaporated to 5 ml. and passed through a Dowex 50 W-X8 column (2.0 g.). The column was washed with water to remove all inorganic material. The column was eluted with 15% NH_4OH (50 ml.); evaporation of the eluate gave 73.0 mg. (49.2%) of VI, m.p. 202°. The melting point and ultraviolet and infrared spectra of a recrystallized sample were identical in all respects with an authentic sample.

3-(3-Hydroxypropyl)adenine Hydrobromide (XII).—A solution of 5.51 g. (41.1 mmoles) of adenine and 21.7 g. (156 mmoles) of 3-bromo-1-propanol in 125 ml. of dimethylacetamide was heated at 115° for 18 hr. The solvent was removed *in vacuo* and gave a dark oil which after crystallization from absolute ethanol gave a crude product, yield 4.93 g. (43.8%), m.p. 170–177°. Recrystallization of the crude material from methanol–ether gave the analytical sample (4.08 g.): m.p. 202–204°; λ_{max} in mμ ($\epsilon \times 10^{-4}$), pH 1—274 (1.87), pH 7—273 (1.52), pH 13—273 (1.39); ν in cm.$^{-1}$ (KBr), 3450 (OH), 3300 (NH_2), 1670 (C=N$^+$H), 1620 and 1590 (C=N and C=C).

Anal. Calcd. for $C_8H_{12}BrN_5O$: C, 35.05; H, 4.41; Br, 29.15. Found: C, 35.26; H, 4.38; Br, 29.40.

(11) It is possible that other isomeric alkylated purines were formed in minor yields but none could be isolated.

(12) The infrared spectra were determined on a Perkin-Elmer Model 137 spectrophotometer; the ultraviolet spectra and enzyme reactions were determined on a Perkin-Elmer 4000A spectrophotometer. The melting points, unless noted otherwise, were determined in capillary tubes on a Mel-Temp block and are corrected.

(13) The analyses reported in this paper were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

(14) J. A. Montgomery and H. J. Thomas, *J. Heterocyclic Chem.*, **1**, 115 (1964).