Experimental

A. Bromination.—A 15% molar excess of N-bromosuccinimide was added to a cold concd. sulfuric acid solution of the tetracycline, stirred in an ice bath, and the reaction solution was then poured into cold anhydrous ethyl ether. The resulting precipitate was purified by dissolving in alcohol and precipitating with ether.

B. Nitration.—One equivalent of potassium nitrate was added with stirring to a cold concd. sulfuric acid solution of the tetracycline, and the solution was stirred for the required time. The product, isolated as the sulfate salt by pouring into cold anhydrous ether, was purified by dissolving it in water and precipitating the amphoteric form by adjusting the pH to about 4–5.

C. Nitration.—One equivalent of a 10% (by vol.) solution of concd. nitric acid in sulfuric acid was added to a cold concd. sulfuric acid solution of the tetracycline. This solution was stirred for 3 to 5 min., poured into cold anhydrous ethyl ether, and the precipitate collected. Purification was accomplished by dissolving in alcohol, filtering, and precipitating the product with ether.

D. Catalytic Reduction.—A solution of tetracycline in methanol (5 mg./ml.) containing a molar excess of concd. sulfuric acid and 10% by weight of platinum oxide was shaken in an atmosphere of hydrogen. When the theoretical amount of hydrogen necessary for reduction of the nitro group was absorbed the hydrogenation was stopped. The reduction solution was filtered from the catalyst and the filtrate was concentrated to about 20% of the original volume and added to cold anhydrous ethyl ether. In cases where the amphoteric form is reported, the sulfate was dissolved in water, the solution adjusted to pH 5–6, and the product collected.

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Analogs of the Aminonucleoside Derived from Puromycin. The Synthesis of 3′-Amino-3′-deoxyguanosine and 3′-Amino-3′-deoxycrotonoside

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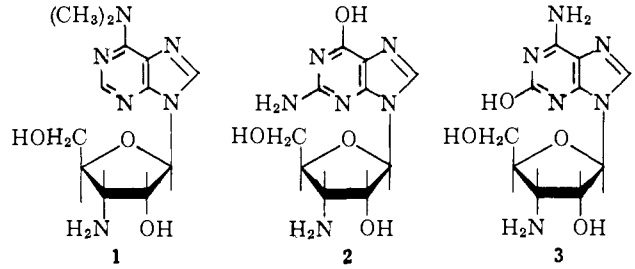
The title compounds were prepared by adaptations of known procedures. These nucleosides were inactive in several standard antitumor assays in mice.

Analogs of the aminonucleoside (**1**)[1] derived from puromycin are of interest because of the trypanocidal[2] and carcinostatic[3] activities exhibited by **1** in experimental animals. It has already been shown that replacement of the 6-dimethylaminopurine moiety of **1** by adenine[4] and hypoxanthine[5] as well as by various 6-alkylamino- and 6-dialkylaminopurines[6] gives nucleosides having considerable biological activity. On the other hand, replacement of the 3-aminoribofuranosyl moiety with the natural ribofuranosyl sugar results in a loss of biological effectiveness.[7] Therefore, we have been interested in the preparation of 3-aminoribofuranosyl derivatives of other naturally-occurring bases[8] and in this paper we wish to report the synthesis of 3′-amino-3′-deoxyguanosine (**2**) and 3′-amino-3′-deoxycrotonoside (**3**).

3′-Amino-3′-deoxyguanosine (**2**) was prepared by an adaptation of the procedure reported by Davoll and Lowy[9] for the synthesis of guanosine. Condensation

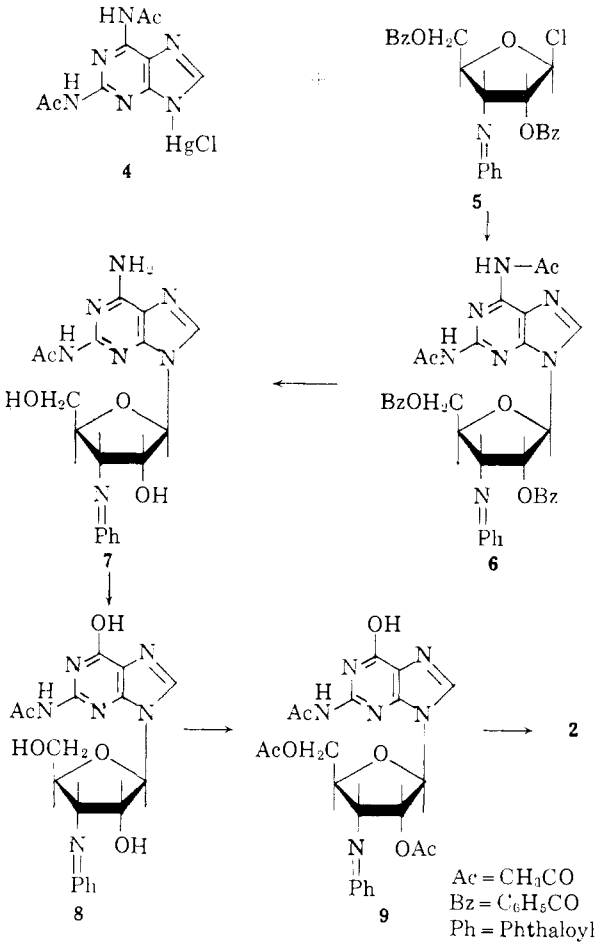


1 **2** **3**

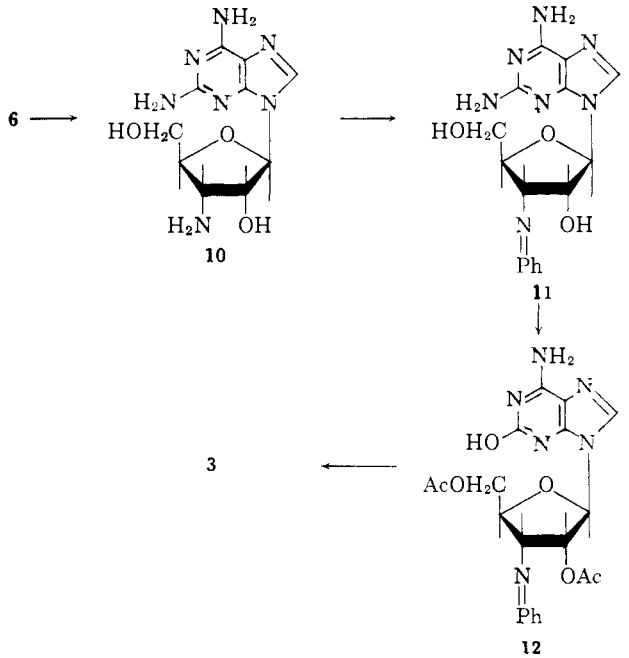
of chloromercuri-2,6-diacetamidopurine (**4**)[9,10] with the blocked 1-chloro-3-aminoribofuranose **5**[11] gave the blocked nucleoside **6** in 56% yield. Preferential cleavage of the 6-N-acetyl group (and of the sugar benzoates) was accomplished by heating with diisopropylamine[5,12] in refluxing methanol. The resulting **7** was obtained in 36% yield. Nitrous acid deamination of this partially blocked intermediate then gave the guanosine derivative **8** in 63% yield. In practice, however, it was found more convenient to isolate the deaminated product as the 2,5-di-*O*-acetate **9**. Finally, complete removal of the blocking groups by treatment of **9** with butylamine[6] in refluxing methanol furnished the desired 3′-amino-3′-deoxyguanosine (**2**) in 44% over-all yield from **7**.

For the synthesis of 3′-amino-3′-deoxycrotonoside (**3**),[13] the intermediate nucleoside **6** was treated with

(1) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 1 (1955).
(2) R. I. Hewitt, A. R. Gumble, W. S. Wallace, and J. H. Williams, *Antibiot. and Chemotherapy*, **4**, 1222 (1954).
(3) P. L. Bennett, S. L. Halliday, J. J. Oleson, and J. H. Williams, "Antibiotics Annual 1954–1955," Medical Encyclopedia, Inc., New York, N. Y., 1955, pp. 766–769.
(4) B. R. Baker, R. E. Schaub, and H. M. Kissman, *J. Am. Chem. Soc.*, **77**, 5911 (1955).
(5) L. Goldman, J. W. Marsico, and M. J. Weiss, *J. Med. Chem.*, **6**, 410 (1963).
(6) L. Goldman, J. W. Marsico, and R. B. Angier, *J. Am. Chem. Soc.*, **78**, 4173 (1956).
(7) H. M. Kissman, C. Pidacks, and B. R. Baker, *ibid.*, **77**, 18 (1955).
(8) H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 2575 (1958).
(9) J. Davoll and B. A. Lowy, *ibid.*, **73**, 1650 (1951).
(10) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 959 (1957).
(11) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Am. Chem. Soc.*, **77**, 5905 (1955).
(12) L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 413 (1963).
(13) Crotonoside has been synthesized by J. Davoll, *J. Am. Chem. Soc.*, **73**, 3174 (1951).



methanolic butylamine to give the previously prepared[5] 2,6,3′-triaminonucleoside **10**. Preferential phthaloylation of the 3′-amino group gave **11**, which was submitted to treatment with nitrous acid for the preferential deamination of the 2-amino group.[13] The resulting isoguanine derivative was then isolated as a crude 3,5-di-*O*-acetate **12**, which on complete removal of the blocking groups with butylamine in refluxing methanol gave the required 3′-amino-3′-deoxycrotonoside (**3**) as



an amorphous solid. A completely satisfactory analysis for this product could not be obtained although the analysis for the flavianate salt was essentially satisfactory.

The anomeric configuration of **6** and of all the nucleosides derived from **6** is reasonably assumed to be β on the basis of the C_1–C_2 *trans* rule of Baker, Joseph, Schaub, and Williams[14] and the relatively high (for nucleoside condensations) yield of 56% by which **6** was obtained.

Biological Evaluation.—The two nucleosides, 3′-amino-3′-deoxyguanosine (**2**) and 3′-amino-3′-deoxycrotonoside (**3**) were completely inactive when tested against sarcoma 180, the 6C3HED lymphosarcoma and the mammary adenocarcinoma 72J in the C3H mouse at doses of 50 mg./kg. and 62.5 mg./kg., respectively, administered once daily for 6 days by the interperitoneal route.

Experimental

Melting points were taken on a Kofler micro hot-stage and are corrected. Ultraviolet spectra were determined in methanol (unless stated otherwise) on a Cary recording spectrophotometer. Aliquots were diluted 1:10 with 0.1 *N* aqueous hydrochloric acid for the acid spectrum and 1:10 with 0.1 *N* aqueous sodium hydroxide for the base spectrum. Rotations were taken at 24–25°. Solutions were dried over magnesium sulfate and evaporations were carried out *in vacuo*. The material used for partition chromatography was Celite 545 (Johns-Manville Corp.) which had been washed with 6 *N* hydrochloric acid, with distilled water until neutral, and finally with methanol. The material was dried at 50°. Karl Fischer moisture determinations gave excessively high values with most of the hydrated nucleosides of this series.

2,6-Diacetamido-9-(2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (6).—To an azeotropically dried suspension of 5 g. (about 11 mmoles) of chloromercuri-2,6-diacetamidopurine (**4**),[9,10] which had been deposited on 5 g. of Celite, in 200 ml. of xylene was added 5.05 g. (10 mmoles) of crystalline chloro sugar (**5**), derived[11] from 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-deoxy-3-phthalimido-β-D-ribofuranoside, in 20 ml. of xylene, and the mixture was stirred under reflux for 3 hr. The cooled mixture was filtered and the filtrate evaporated. The Celite precipitate was washed well with chloroform and the washings were added to the residue from the xylene solution. The chloroform solution (120 ml.) was washed with two 30 ml. portions of a 30% aqueous potassium iodide solution and then with water. The dried organic phase was evaporated and the residue was crystallized and recrystallized from ethyl acetate to afford 3.9 g. (56%) of **6**, m.p. 138–140°; $[\alpha]_D$ 14.9° (*c* 2.018, chloroform); λ_{max} 267 mμ (ϵ 21,800 in acid), 273 mμ (ϵ 21,330 on methanol) 270 mμ (ϵ 19,920 in base).

Anal. Calcd. for $C_{36}H_{29}N_7O_9$: C, 61.46; H, 4.15; N, 13.94. Found: C, 61.42; H, 4.44; N, 13.75.

2-Acetamido-6-amino-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (7).—A solution of 350 mg. (0.5 mmole) of the blocked nucleoside **6** in 35 ml. of methanol containing 1.75 ml. of diisopropylamine[12] was refluxed for 2 hr. and evaporated. The residue was collected with ether and dissolved with heating in 8 ml. of water[15] and 8 ml. of methanol. Most of the methanol was boiled off and the solid which crystallized out on cooling overnight was collected (100 mg.) and recrystallized from methanol to afford 81 mg. (36%) of **7**, m.p. 172–180°. Recrystallization from aqueous methanol gave a sample, m.p. 178–180°; $[\alpha]_D$ +142° (*c* 0.985, pyridine); λ_{max} 267 mμ (ϵ 20,350 in acid), 272 mμ (ϵ 17,900 in methanol), 269 mμ (ϵ 17,420 in base).

Anal. Calcd. for $C_{26}H_{23}N_7O_6 \cdot H_2O$: C, 50.95; H, 4.46; N, 20.80. Found: C, 50.95; H, 4.74; N, 19.82.[16]

(14) B. R. Baker, J. P. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(15) Treatment with water was necessary in order to remove a more polar impurity (R_f 0.2) from the desired product (R_f 0.6, 1-butanol/H_2O).

(16) Repeated analyses of samples having approximately the same melting point showed variable H_2O content (Karl Fischer).

2-Acetamido-6-hydroxy-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (8).—The 6-amino compound **7** (227 mg., 0.5 mmole) was dissolved in a warm mixture of 10 ml. of water and 6.5 ml. of acetic acid. Sodium nitrite (0.4 g., 5.8 mmoles) was added and the mixture was stirred at room temperature overnight. The solution was evaporated at 45° and the residue was triturated with water and filtered. From the filtrate there was obtained by total evaporation and crystallization from a small amount of water 143 mg. (63%) of **8** with m.p. 280–284° dec. A sample recrystallized from aqueous ethanol had m.p. 280–283° dec.; $[\alpha]_D$ 56° (*c*, 0.982, dimethylformamide); λ_{max} 242 mμ and 260 mμ (ε 18,160 and 18,720 in acid), 241 mμ, 255 mμ and 282 mμ (ε 17,840, 17,630 and 12,720 in methanol) 262 mμ (ε 15,440 in base).

Anal. Calcd. for $C_{20}H_{18}N_6O_7$: C, 52.86; H, 3.99; N, 18.50. Found: C, 53.10; H, 4.38; N, 18.10.

In subsequent preparations, it was found more practical to acetylate the carbohydrate moiety before isolation as shown in the following experiment.

2-Acetamido-6-hydroxy-9-(2,5-di-*O*-acetyl-3-deoxy-3-phthlaimido-β-D-ribofuranosyl)purine (9).—The 6-amino compound **7** (453 mg., partial hydrate) was deaminated with 795 mg. of sodium nitrite in 13.2 ml. of acetic acid and 20.5 ml. of water as described above. The crude residue was dried by several distillations with ethanol and was then dissolved in 14.6 ml. of pyridine and 1.4 ml. of acetic anhydride. After 18 hr. the mixture was poured into saturated aqueous bicarbonate solution and this was extracted with several portions of chloroform. The extracts were washed with water, dried, and evaporated. The residue was crystallized from ethyl acetate–acetone to afford 228 mg. (46%) of **9**, m.p. 242–246°. A sample recrystallized several times from ethanol–acetone showed m.p. 252–255°; $[\alpha]_D$ 69.5° (*c* 1.04, dimethylformamide); λ_{max} 242 mμ and 258 mμ (ε 19,140, 19,220 in acid), 233 mμ, 241 mμ, 255 mμ and 283 mμ (ε 18,840, 19,780, 18,640, 13,630 in methanol) 262 mμ (ε 16,160 in base).

Anal. Calcd. for $C_{24}H_{22}N_6O_9$: C, 53.53; H, 4.12; N, 15.61. Found: C, 53.88; H, 4.43; N, 15.77.

2-Amino-6-hydroxy-9-(3-amino-3-deoxy-β-D-ribofuranosyl)purine (3′-aminoguanosine, 2).—A solution of 340 mg. of crude **9** (obtained as above from 309 mg., 0.683 mmole of **8**) in 30 ml. of methanol and 2 ml. of *n*-butylamine[6] was allowed to reflux for 24 hr. and was evaporated. The residue was mixed with 20 ml. each of ether and water and the layers were separated. The ether phase was washed several times with water and the combined water layers were washed once with ether. The aqueous solution was evaporated and the residue was recrystallized from water to afford 84 mg. (44% over-all from **7**), m.p. 220–224° dec. Two recrystallizations from large volumes of methanol gave a sample with m.p. 241–243° dec.; $[\alpha]_D$ 26.6° (*c* 0.45, 50% aqueous acetic acid,), 5.6° (*c*, 0.978, dimethylformamide; sample from a different preparation); λ_{max}[17] 255 mμ (ε 12,910 in acid), 254 mμ (ε 14,640) 262 mμ (ε 11,650 in base).

Anal. Calcd. for $C_{10}H_{14}N_6O_4 \cdot 0.25H_2O$: C, 41.90; H, 5.10; N, 29.30. Found: C, 41.98; H, 5.40; N, 28.90.

2,6-Diamino-9-(3-amino-3-deoxy-β-D-ribofuranosyl)purine (10).—A solution of 5.63 g. (8 mmole) of the blocked nucleoside **6** in 120 ml. of methanol and 24 ml. of *n*-butylamine was stirred under reflux for 25 hr. and at room temperature for 16 hr. The white precipitate was collected and washed with a little methanol. Concentration of the filtrate *in vacuo* afforded a second crop of solid. The material (1.58 g., 70%) had m.p. 227–232° dec. and was shown to be identical with material prepared by another route.[5]

2,6-Diamino-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (11).—A mixture of 1.3 g. (4.6 mmoles) of 2,6-diamino-9-(3-amino-3-deoxy-β-D-ribofuranosyl)purine (**10**), 680 mg. of powdered phthalic anhydride and 30 ml. of dimethylformamide was stirred under reflux for 1 hr. The dark orange solution was treated with activated charcoal and evaporated *in vacuo*. The residue was triturated with water and collected to give 1.9 g. of a tan solid, m.p. 175–186°. The material was crystallized with great loss from aqueous methanol to give a partially hydrated solid, m.p. 180–188°; λ_{max} 242 mμ and 292 mμ (ε 15,600 and 11,500 in acid), 242 mμ and 280 mμ (ε 12,900 and 11,300 in methanol) 220 mμ, 252 mμ and 280 mμ (ε 29,600, 10,000 and 9800 in base).

Anal. Calcd. for $C_{18}H_{17}N_7O_5 \cdot 0.5H_2O$: C, 51.41; H, 4.32; N, 23.32. Found: C, 51.13; H, 4.78; N, 22.37.

6-Amino-2-hydroxy-9-(2,5-di-*O*-acetyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (12).—To a solution of 822 mg. (2 mmoles) of 2,6-diamino-9-(3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (**11**) in 5 ml. of acetic acid and 10 ml. of water was added 900 mg. of sodium nitrite and the mixture was stirred in a 40–45° bath for 10 min. Urea (668 mg.) was then added, the mixture was stirred for 45 min. at room temperature, and was kept in the refrigerator overnight. Evaporation and reevaporation with benzene gave a residue which was mixed with 20 ml. of pyridine and 5 ml. of acetic anhydride, stirred for 24 hr., added to ice water, and neutralized with sodium bicarbonate. The mixture was extracted thoroughly with chloroform and the extracts were washed with water, dried, and evaporated. Trituration with ethanol afforded 478 mg. (49%) of **12** as tan solid, m.p. 150–170°, which was not purified further.

6-Amino-2-hydroxy-9-(3-amino-3-deoxy-β-D-ribofuranosyl)-purine (3′-amino-3′-deoxycrotoniside, 3).—A solution of 809 mg. (1.63 mmoles) of 6-amino-2-hydroxy-9-(2,5-di-*O*-acetyl-3-deoxy-3-phthalimido-β-D-ribofuranosyl)purine (**12**) in 40 ml. of methanol and 6 ml. of butylamine was refluxed for 19 hr. and the solution was filtered and evaporated. The residue from the evaporation was partitioned in ether–water and the water phase was used to dissolve the original precipitate. The aqueous solution was evaporated and the residue was evaporated several times with ethanol to afford 416 mg. of glass which was purified by partition chromatography. The material was dissolved in 7.5 ml. of the lower and 8 ml. of the upper phase of the system ethyl acetate–methanol–water (4:1:2) and 15 g. of Celite was added. The moist mixture was packed on top of a column which had been prepared from 250 g. of Celite and 125 ml. of the lower phase. The column was eluted with the upper phase and the ultraviolet absorption of the effluent stream was monitored. There was eluted some 60 mg. of oil in the first column volume (486 ml.) and then nothing through the 5th column volume. The column was then washed with 1400 ml. of methanol and the washings were evaporated to afford 256 mg. of solid. This was purified, somewhat, by solution in a large volume of methanol and partial evaporation to give a light grey powder (203 mg.) with $\lambda_{max}^{H_2O}$ 247 mμ (ε 9040), 291 mμ (ε 7400). Literature values[13] for 6-amino-2-hydroxy-β-D-ribofuranosylpurine are 247 mμ (ε 9000) and 291 mμ (ε 11,000), indicating an 82% purity for our sample.

A sample obtained in a similar preparation and purified by partition chromatography on Celite from butanol–water was further purified by solution in methanol, treatment with charcoal and evaporation to a small volume. The resulting gel was cooled overnight and filtered. The amorphous solid was washed with ether and dried *in vacuo* at 110° for 2 hr. It decomposed at about 200° and showed $[\alpha]_D$ 15° (*c* 0.668, water); $\lambda_{max}^{H_2O}$ 235 mμ and 280 mμ (ε 6320 and 11,360 in acid), 247 mμ and 292 mμ (ε 8450 and 9900 in water), 249 mμ and 283 mμ (ε 6320 and 9340 in base), all calculated for the semihydrate.

Anal. Calcd. for $C_{10}H_{14}N_6O_4 \cdot 0.5H_2O$: C, 41.24; H, 5.20; N, 28.86. Found: C, 41.40; H, 5.36; N, 28.14.

A **picrate** was prepared from 86 mg. of chromatographed material which was dissolved in 4 ml. of water and treated with 3 ml. of saturated aqueous picric acid. The precipitate was collected, washed with water, and recrystallized from ethanol. The substance (14 mg.) showed m.p. 172–176° dec.

Anal. Calcd. for $C_{16}H_{17}N_9O_{11} \cdot H_2O$: C, 36.30; H, 3.61; N, 23.79. Found: C, 36.28; H, 3.51; N, 22.34.

A **flavianate** was prepared from unchromatographed material in water with aqueous flavianic acid. The orange precipitate was washed with water, ethanol, and ether and was dried *in vacuo;* it decomposed slowly above 235°.

Anal. Calcd. for $C_{20}H_{20}N_8O_{12}S \cdot 1.5H_2O$: C, 38.53; H, 3.56; N, 17.97; S, 5.15; H_2O 4.34. Found: C, 38.99; H, 3.88; N, 17.64; S, 5.19; H_2O (Karl Fischer), 7.80.[18]

Acknowledgment.—We wish to thank Mr. C. C. Pidacks and staff for the partition chromatography work reported herein, Mr. W. Fulmor and staff for the spectroscopic and polarimetric data and Mr. L. Brancone and staff for the microanalytical data. We also wish to thank Dr. A. Vogel and staff for the antitumor assays.

(17) Dissolved in 2-methoxyethanol and diluted with methanol.

(18) Karl Fischer moisture determinations gave excessively high values with most of the nucleosides of this series.