

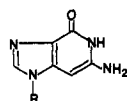
Synthesis and Antiviral/Antitumor Activities of Certain 3-Deazaguanine Nucleosides and Nucleotides

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A new procedure for the preparation of the antiviral and antitumor agent 3-deazaguanine (1) and its metabolite 3-deazaguanosine (2) has been developed by reacting methyl 5(4)-(cyanomethyl)imidazole-4(5)-carboxylate (4) and 5-(cyanomethyl)-1-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)imidazole-4-carboxylate (6), respectively, with hydrazine. The 3-deazaguanosine 3',5'-cyclic phosphate (13) was prepared from 5-(cyanomethyl)-1- β -D-ribofuranosyl-imidazole-4-carboxamide 5'-phosphate. Glycosylation of the trimethylsilyl trifluoromethanesulfonate gave the corresponding N-1 and N-3 glycosyl derivatives with α -configuration (18 and 20) as the major products, along with minor amounts of the β -anomers (19 and 21). However, glycosylation of the sodium salt of 4 with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythro-pentofuranose (17) gave exclusively the β -anomers (19 and 21) in good yield. Base-catalyzed ring closure of these imidazole nucleosides gave 2'-deoxy-3-deazaguanosine (29), the α -anomer 28, and the corresponding N-3 positional isomers 27 and 26. The site of glycosylation and the anomeric configuration of these nucleosides have been assigned on the basis of ^1H NMR and UV spectral characteristics and by single-crystal X-ray analysis for 27-29. In a preliminary screening, several of these compounds have demonstrated significant broad-spectrum antiviral activity against certain DNA and RNA viruses in vitro, as well as moderate activity against L1210 and P388 leukemia in cell culture.

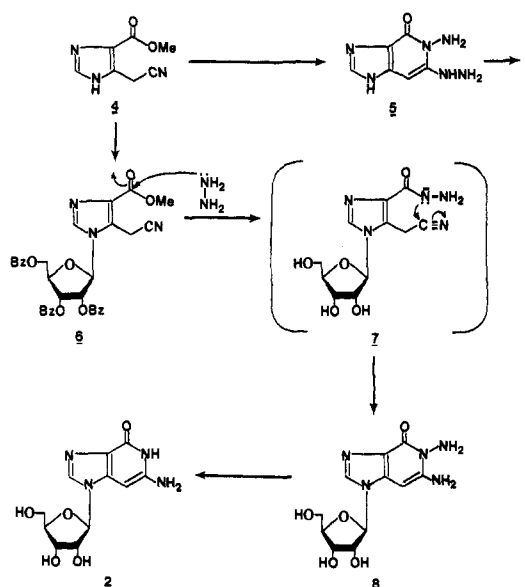
6-Amino-1,5-dihydroimidazo[4,5-*c*]pyridin-4-one (3-deazaguanine, 1) is a structural analogue of guanine in which the pyrimidine ring nitrogen in the 3-position is replaced by CH and was first reported by us¹ in 1976. Since that report,¹ the broad-spectrum antiviral activity against a variety of DNA and RNA viruses,² as well as the potent antitumor activity against L1210 leukemia and mammary adenocarcinomas in mice³⁻⁵ of 1 and its metabolites 3-deazaguanosine (2, 6-amino-1,5-dihydro-1- β -D-ribofuranosylimidazo[4,5-*c*]pyridin-4-one) and 3-deazaguanic acid (3, 6-amino-1,5-dihydro-1- β -D-ribofuranosylimidazo[4,5-*c*]pyridin-4-one 5'-phosphate), have been confirmed independently in a number of laboratories. 3-Deazaguanine (1) exhibited potent cytotoxic activity in vitro against a variety of tumor cells, which include L1210,^{3,5,6} HeLa,³ human KB cells,⁷ Ehrlich ascites tumor cells,⁷ Chinese hamster ovary cells,⁸ primary Chinese hamster embryo cells,⁴ and mammary carcinoma EMT-6 cells.⁹



1. R = H
2. R = β -D-ribofuranose
3. R = β -D-ribofuranosyl 5'-phosphate

3-Deazaguanine has shown inhibitory action against a broad spectrum of animal breast tumor models, including rat mammary adenocarcinomas, mouse mammary adenocarcinomas,^{4,5} slow- and fast-growing mammary tumors in mice, and the human breast xenograft subrenal capsule implant system. These observations are very interesting, since mammary adenocarcinoma (e.g., R3230AC) is a nonhormone-dependent estrogen-sensitive slow-growing tumor model for postoperative breast carcinomas and is not particularly susceptible to conventional cancer chemotherapeutic agents. Mammary adenocarcinoma 13762 is a fast-growing nonhormone-dependent tumor which is not responsive to estrogen treatment.¹⁰ These results suggest a possible use of 3-deazaguanine in chemotherapy

Scheme I



of human breast cancer. 3-Deazaguanine also inhibited the growth of *Escherichia coli* B3 in vitro but did not

- (1) Cook, P. D.; Rousseau, R. J.; Mian, A. M.; Dea, P.; Meyer, R. B., Jr.; Robins, R. K. *J. Am. Chem. Soc.* 1976, 98, 1492.
- (2) Allen, L. B.; Huffman, J. H.; Cook, P. D.; Meyer, R. B., Jr.; Robins, R. K.; Sidwell, R. W. *Antimicrob. Agents Chemother.* 1977, 12, 114.
- (3) Khwaja, T. A.; Kigwana, L.; Meyer, R. B., Jr.; Robins, R. K. *Proc. Am. Assoc. Cancer Res.* 1975, 16, 162.
- (4) Khwaja, T. A.; Varven, J. C. *Proc. Am. Assoc. Cancer Res.* 1976, 17, 200.
- (5) Schwartz, P.; Hammond, D.; Khwaja, T. A. *Proc. Am. Assoc. Cancer Res.* 1977, 18, 153.
- (6) Rivest, R. S.; Irwin, D.; Mandel, H. G. *Proc. Am. Assoc. Cancer Res.* 1980, 21, 279.
- (7) Streeter, D. G.; Koyama, H. H. P. *Biochem. Pharmacol.* 1976, 25, 2413.
- (8) Saunders, P. P.; Chao, L.-Y.; Loo, T. L.; Robins, R. K. *Biochem. Pharmacol.* 1981, 30, 2374.
- (9) Khwaja, T. A.; Momparler, L.; Varven, J. C.; Mian, A. M. *Proc. Am. Assoc. Cancer Res.* 1979, 20, 152.

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Scheme II

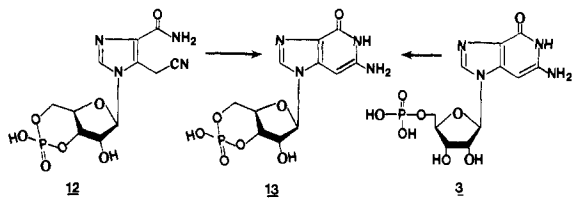


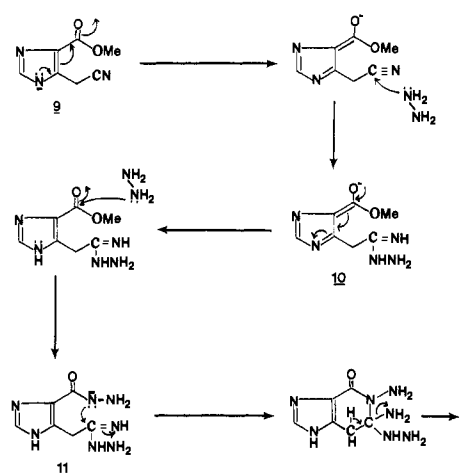
exhibit any antibacterial activity in vivo.^{11,12} The 7-ribose derivative of 1 shows antibacterial activity against several Gram-negative strains in vivo without any appreciable toxicity to the host.¹¹ The antibacterial action of 7-ribose-3-deazaguanine has been ascribed to its cleavage to 1 in *E. coli* B infected cells.¹³ Recently, 3-deazaguanosine (2) was shown to be a potent antileishmanial agent,¹⁴ which is at least 20 times more active than 1 or allopurinol ribonucleoside against *Leishmania tropica* in vitro but less active than formycin B.

These unusual biological activities of 1 and 2 necessitated larger quantities of these compounds for further studies. We now report a convenient synthesis of 1 and 2 by a new ring-closure procedure along with the synthesis of 3-deazaguanosine 3',5'-cyclic phosphate (13), 2'-deoxy-3-deazaguanosine (6-amino-1,2-dihydro-1-(2-deoxy-β-D-erythro-pentofuranosyl)imidazo[4,5-c]pyridin-4-one, 29), and its positional isomer 7-(2-deoxy-β-D-erythro-pentofuranosyl)-3-deazaguanine (27).

Chemistry. The starting material, methyl 5-(4-(cyanomethyl)imidazole-4(5H)-carboxylate) (4) needed for this reaction, was prepared as reported earlier.¹ Treatment of 4 with hydrazine hydrate in ethanol at reflux temperature gave an excellent yield of 5-amino-6-hydrazino-1,5-dihydroimidazo[4,5-c]pyridin-4-one (5) (Scheme I), which, on hydrogenation in the presence of Raney nickel (W-4) catalyst, provided 1 in 80% yield, which is a considerable improvement over a previously reported procedure¹⁵ (52%). The advantage of this procedure over that previously reported¹⁵ is that 1 could be readily obtained in good yield without the use of a high-pressure reaction vessel. A similar treatment of methyl 5-(cyanomethyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (6)¹ with hydrazine hydrate gave crystalline 1-amino-3-deazaguanosine (8). Hydrogenation of 8 in the presence of Raney nickel (W-4) catalyst provided a new synthesis of 3-deazaguanosine (2), which was obtained in good yield.

A plausible mechanism for the ring closure of 6 to form 8 may be visualized simply as occurring by the direct attack of hydrazine on the ester carbonyl carbon to give the intermediate 7, which cyclizes immediately to form 8. However, in the case of 4 (mechanism A), abstraction of the ring NH proton by hydrazine gives the anion 9, which

Mechanism A



is followed by addition of hydrazine across the nitrile bond to give the intermediate 10. Further nucleophilic attack of hydrazine on the ester carbonyl carbon gives the imidazole precursor 11, which on ring closure and aromatization yields 5.

For the synthesis of 3-deazaguanosine 3',5'-cyclic phosphate (13) (Scheme II), 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide 5'-phosphate¹ was found to be a versatile starting material. Treatment of the *N,N*-dicyclohexylmorpholinecarboxamide salt of 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide 5'-phosphate in anhydrous pyridine with dicyclohexylcarbodiimide (DCC) according to the general procedure of Smith et al.¹⁶ gave 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide 3',5'-cyclic phosphate (12). The appearance of a singlet for the anomeric proton in the ¹H NMR spectrum of 12 at δ 5.76 ppm indicated the formation of the cyclic phosphate ring. Ring closure of 12 in the presence of aqueous Na₂CO₃ provided 13, which was isolated as the free acid.¹⁷ Direct DCC-mediated cyclization of 3-deazaguanosine (3)¹ to obtain 13, under the conditions of Smith et al.,¹⁶ provided a complex reaction mixture from which the isolation of pure 13 was very difficult.

The antitumor mode of action of 3-deazaguanosine has been postulated to be due to the consequence of its incorporation into tumor cell DNA.^{5,9} To evaluate this hypothesis we undertook the synthesis of the 2'-deoxy derivatives of 3-deazaguanosine (26–29). While this work was in progress, a paper appeared¹⁸ in which the syntheses of 26–29 were described. However, the physicochemical data reported for these compounds differ from the data we observed. As depicted in Scheme III, compound 4 was again proved to be a useful starting material. Silylation of 4 with hexamethyldisilazane gave the silylated product 14. Reaction of 14 with 1 molar equiv of 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-ribofuranose (15)¹⁹ in CH₃CN in the presence of 1.44 molar equiv of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) according to the procedure of Vorbrüggen et al.²⁰ gave a complex reaction

- (10) Hilf, R. In "Prediction of Response in Cancer Therapy, Will the Best Model of Breast Cancer Please Come Forward", NCI Monograph No. 34, 1971, p 43.
 (11) Matthews, T. R.; Yotter, D. W.; Cook, P. D.; Sidwell, R. W.; Robins, R. K.; Dougherty, P. F. 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Oct 27–29, 1976, American Society for Microbiology, Washington, DC, Abstr 425.
 (12) Saunders, P. P.; Chao, L.-Y.; Robins, R. K.; Loo, T. L. *Mol. Pharmacol.* 1979, 15, 691.
 (13) Streeter, D. G.; Miller, M.; Matthews, T. R.; Robins, R. K.; Miller, J. P. *Biochem. Pharmacol.* 1980, 29, 1791.
 (14) Berman, J. D.; Lee, L. S.; Robins, R. K.; Revankar, G. R. *Antimicrob. Agents Chemother.* 1983, 24, 283.
 (15) Srivastava, P. C.; Robins, R. K. *J. Heterocycl. Chem.* 1979, 16, 1063.

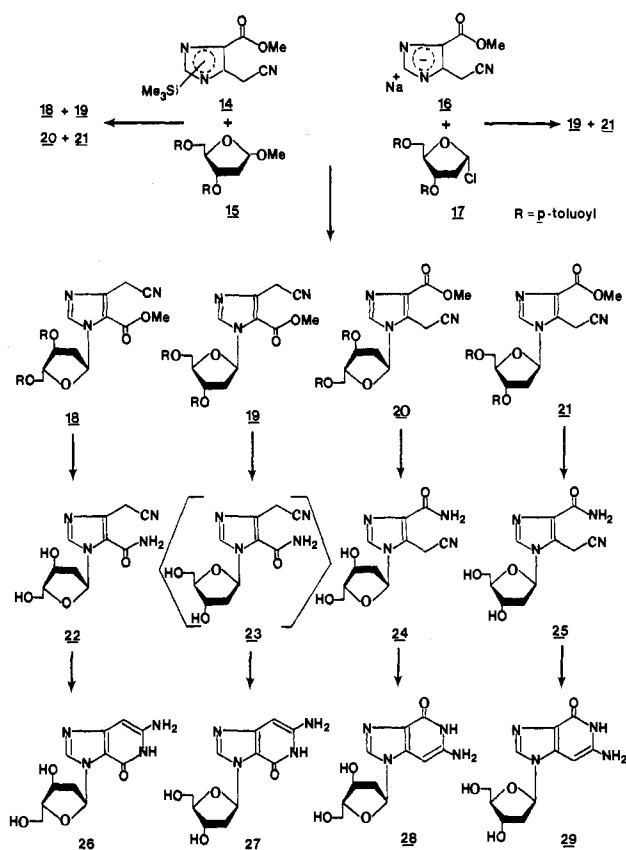
- (16) Smith, M.; Drummond, G. I.; Khorana, H. G. *J. Am. Chem. Soc.* 1961, 83, 698.
 (17) Robins, R. K.; Rousseau, R. J.; Mian, A. M. U.S. Patent 4056674, Nov 1977.
 (18) Mian, A. M.; Khwaja, T. A. *J. Med. Chem.* 1983, 26, 286.
 (19) Bhat, C. C. In "Synthetic Procedures in Nucleic Acid Chemistry"; Zorbach, W. W., Tipson, R. S., Eds.; Wiley-Interscience: New York, 1968; Vol. 1, p 521.
 (20) Vorbrüggen, H.; Krolkiewicz, K.; Benua, B. *Chem. Ber.* 1981, 114, 1234.

mixture of two major and two minor nucleosidic products along with trace amounts of unreacted sugar and the heterocycle. The separation of these products was achieved on a silica gel column by preparative LC techniques using acetone:hexane (1:4, v/v) as the solvent. The two major products were isolated as pure crystalline compounds and were identified as methyl 4-(cyanomethyl)-1-(2-deoxy-3,5-di-*O-p*-toluoyl- α -D-*erythro*-pentofuranosyl)imidazole-5-carboxylate (18, 20% yield) and methyl 5-(cyanomethyl)-1-(2-deoxy-3,5-di-*O-p*-toluoyl- α -D-*erythro*-pentofuranosyl)imidazole-4-carboxylate (20, 22% yield). The corresponding β -anomers 19 and 21 were isolated in less than 7% yield. The anomeric assignment of these isomers was determined by ^1H NMR wherein the β -isomers (19 and 21) exhibited the characteristic triplet for the anomeric proton and the α -isomers (18 and 20) the quartet²¹ (see the Experimental Section). Although deoxyribosylation had occurred with equal facility at both imidazole ring nitrogens, this reaction condition favored the formation of α -anomers 18 and 20. It has been well documented that the anomeric ratio of a Lewis acid mediated condensation of a silylated aglycon with a blocked 2-deoxy-D-*erythro*-pentofuranose is influenced by a number of factors, such as temperature, solvent, blocking groups, etc.²²⁻²⁴ The apparent preponderance of the α -anomers in the present study seems to be influenced by the participation of the cyclic glycon acyloxonium ion involving 5'-*O*-toluoyl group.^{25,26}

In an effort to improve the yield of the desired β -anomers 19 and 21, the sodium salt glycosylation procedure, developed recently in our laboratory,²⁷⁻²⁹ was employed. Application of this simple single-phase glycosylation procedure to the synthesis of 19 and 21 was found to be remarkably successful. Glycosylation of the sodium salt of 4 (16, generated in situ by the treatment of NaH in CH_3CN) with 1-chloro-2-deoxy-3,5-di-*O-p*-toluoyl- α -D-*erythro*-pentofuranose³⁰ (17) at ambient temperature gave a mixture of two nucleoside products (Scheme III). These products were separated on a silica gel column and identified as 19 (45% yield) and 21 (48.3% yield) on the basis of ^1H NMR characteristics (triplets for the anomeric protons). No formation of α -anomers was detected. Since the starting halosugar 17 has the α -configuration³¹ in the solid state, the exclusive formation of the blocked 2'-deoxy- β -nucleosides in the present study is believed to be due to a direct Walden inversion ($\text{S}_{\text{N}}2$) at the C_1 carbon by the anionic heterocyclic nitrogen.

Subsequent treatment of each of the blocked imidazole nucleosides with liquid NH_3 provided the corresponding 7- and 9-substituted 3-deazaguanine 2'-deoxyribonucleosides. Thus, treatment of 18 with liquid NH_3 at 100 $^\circ\text{C}$ for 10 h, followed by silica gel column chromatography of the reaction residue, gave 6-amino-3,5-dihydro-3-(2-

Scheme III



deoxy- α -D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (26) as a crystalline solid in a 69.5% yield, along with a minor amount of uncyclized 4-(cyanomethyl)-1-(2-deoxy- α -D-*erythro*-pentofuranosyl)imidazole-5-carboxamide (22). Similarly, treatment of 19 with $\text{CH}_3\text{OH}/\text{NH}_3$ at 100 $^\circ\text{C}$ for 18 h gave exclusively the cyclized product 6-amino-3,5-dihydro-3-(2-deoxy- β -D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (27). The ^1H NMR signal for the anomeric proton of 26 and 27 followed the "quartet-triplet" splitting pattern²¹ for the α - and β -anomers, respectively. The observed peak width of the anomeric proton of 10.2 and 13.0 Hz for 26 and 27 was also consistent with the usual α and β values.²¹ The UV absorption spectra of both 26 and 27 are virtually identical with that of 6-amino-3,5-dihydro-3- β -D-ribofuranosyl-imidazo[4,5-*c*]pyridin-4-one.¹

When the blocked N-1 glycosyl derivatives of the imidazole (20 and 21) were treated with liquid NH_3 at 100 $^\circ\text{C}$ in a pressure vessel, crystalline 5-(cyanomethyl)-1-(2-deoxy- α -D-*erythro*-pentofuranosyl)imidazole-4-carboxamide (24) and the corresponding β -anomer 25 were obtained in 75.0% and 67.7% yield, respectively. It is of particular interest that the N-3 glycosyl isomers 18 and 19, under similar reaction conditions, are mostly converted to 26 and 27, which is in analogy to the direct cyclization of methyl 4-(cyanomethyl)-1-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)imidazole-5-carboxylate to 7-ribosyl-3-deazaguanine.¹ Base-catalyzed cyclization of 24 and 25 proceeded smoothly to yield 6-amino-1,5-dihydro-1-(2-deoxy- α -D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (28) and 2'-deoxy-3-deazaguanosine (29), respectively. Unlike the previous report,¹⁸ both 28 and 29 were crystallized from water as needles. The UV absorption spectra of 28 and 29 [λ_{max} (pH 1) 283 and 310 (sh) nm; λ_{max} (pH 7) 269 and 295 (sh) nm; λ_{max} (pH 11) 271 nm] are virtually identical with that of 3-deazaguanosine.¹ The ^1H NMR spectrum of 29 in $\text{Me}_2\text{SO}-d_6$ displayed a pseudo-

(21) Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* 1965, 87, 4934.

(22) Ryu, E. K.; Bardos, T. J. *J. Heterocycl. Chem.* 1979, 16, 1049.

(23) Kotick, M. P.; Szantay, C.; Bardos, T. J. *J. Org. Chem.* 1969, 34, 3806.

(24) Skulnick, H. *J. Org. Chem.* 1978, 43, 3188.

(25) Prystas, M.; Farkas, J.; Sorm, F. *Coll. Czech. Chem. Commun.* 1965, 30, 3123.

(26) Wierenga, W.; Skulnick, H. I. *Carbohydr. Res.* 1981, 90, 41.

(27) Kazimierczuk, Z.; Revankar, G. R.; Robins, R. K. *Nucleic Acids Res.* 1984, 12, 1179.

(28) Gupta, P. K.; McKernan, P. A.; Robins, R. K.; Revankar, G. R. *J. Med. Chem.*, submitted.

(29) Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. *J. Am. Chem. Soc.*, in press.

(30) Hoffer, M. *Chem. Ber.* 1960, 93, 2777.

(31) Bhattacharya, A. K.; Ness, R. K.; Fletcher, H. G., Jr. *J. Org. Chem.* 1963, 28, 428.

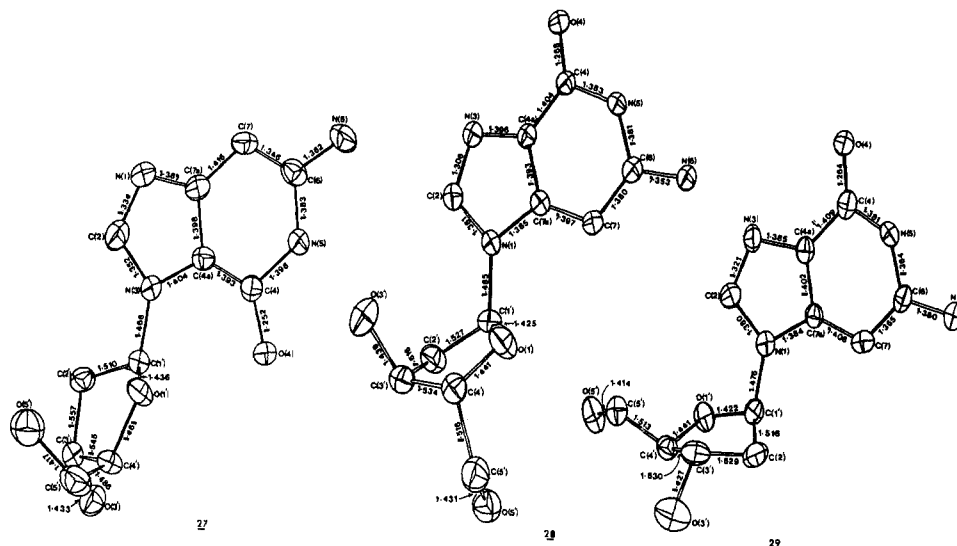


Figure 1. ORTEP drawing with the atom labels and bond lengths of compounds 27–29.

triplet for the anomeric proton centered at δ 5.94 ppm (peak width 13.5 Hz), indicating the β -configuration. Similarly, compound 28 revealed a quartet for the anomeric proton centered at δ 5.95 (peak width 10.5 Hz), indicating the α -configuration. The observed 0.73 and 0.68 ppm downfield shifts of the anomeric proton of 26 and 27 as compared to those of 28 and 29 can be attributed to the anisotropic effect of the carbonyl group on the anomeric proton in 26 and 27, respectively, thereby providing additional support for the assigned site of glycosylation.

Since there were several discrepancies between our observed melting point data and that reported¹⁸ for the same compounds (e.g., observed mp for 18, 146–147 °C (lit. mp 133–135 °C); 19, 132–133 °C (lit. mp 144–147 °C); 21, 82–83 °C (lit. mp 60–62 °C); 25, 156–158 °C (lit. mp 164–167 °C); 24, 169–170 °C (not isolated); 27, 147–149 °C (lit. mp >250 °C); 29, 230–231 °C (lit. mp >250 °C), no melting point and elemental analysis for 26; and no elemental analysis for 28, etc.), it was deemed desirable to unequivocally substantiate our structural assignments by single-crystal X-ray analyses for 2'-deoxy-3-deazaguanosine (29), the α -anomer 28, and 7-(2-deoxy- β -D-erythro-pentofuranosyl)-3-deazaguanine (27).

It would appear that the assignment of the anomeric configuration of the N-3 glycosylated imidazoles 18 and 19 has been erroneously reversed by Mian and Khwaja.¹⁸ The melting point of our β -blocked nucleoside 19 matches that of the α -anomer described by Mian and Khwaja.¹⁸ Similarly, the melting point for our α -anomer 18 corresponds to that of the β -anomer assigned by these authors.¹⁸ These data also suggest the erroneous assignments may have been carried over to the ring-closed 7-(2-deoxy-D-ribofuranosyl)-3-deazaguanines 26 and 27. Since the melting point and the chemical shift (in $\text{Me}_2\text{SO}-d_6$) of the anomeric proton in ^1H NMR spectrum of our α -anomer 26 correspond to those reported for the β -anomer of Mian and Khwaja,¹⁸ we confirmed our structural assignment of the β -anomer 27 by single-crystal X-ray analysis. Unfortunately, Mian and Khwaja¹⁸ did not report either a melting point or elemental analysis for the corresponding α -anomer, 26.

Single-Crystal X-ray Diffraction Analysis of 27–29

Slow crystallization of 27–29 from water gave X-ray quality crystals. Data for the determination of lattice parameters and for the structural studies were collected by utilizing a Nicolet P3 autodiffractometer using graphite

Table I

	compd 27	compd 28	compd 29
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
<i>a</i>	6.987 (1) Å	8.165 (8) Å	4.754 (1) Å
<i>b</i>	9.237 (2) Å	10.933 (9) Å	11.199 (3) Å
<i>c</i>	19.626 (5) Å	13.483 (8) Å	11.993 (3) Å
β	90°	90°	91.54°
<i>Z</i>	4	4	2
sin θ/λ limit	0.70	0.65	0.65
unique reflectns	1942	1554	1425
reflectns used in refinement	1483 ^a	1399 ^a	1274 ^b

^aThose reflections with $F < 4\sigma(F)$ were not used in refinement.

^bThose reflections with $F < 2\sigma(F)$ were not used in refinement.

monochromated Mo $K\alpha$ radiation ($\lambda = 0.71069$ Å). The lattice parameters for the three nucleosides under study were obtained by using a least-squares technique of 15 centered 2θ values and the space groups were determined by using systematic extinction data. Single-crystal X-ray data were collected by using θ - 2θ scan technique with variable scan rates, and the crystal data are summarized in Table I.

The structure of 27 was solved by using a combination of Patterson and direct method techniques, while the structures of 28 and 29 were solved by using direct methods. All structures were refined by using a full-matrix least-squares procedure. The crystallographic calculations were done by using the SHELX-76 program package.³² The final residual values are as follows: for 27, $R = 0.062$, $R_w = 0.034$; for 28, $R = 0.042$ and $R_w = 0.032$ (weights for both 27 and 28 were based on counting statistics), and for 29, $R = 0.058$ (unit weights were used). A perspective view of the molecules, drawn with the aid of the ORTEP program,³³ is shown in Figure 1 along with numbering of the atoms and bond lengths (Å).

The results of this structure-determination study unequivocally confirmed the β -anomeric configuration and the site of glycosyl attachment as N-1 in 29, whereas 28 has the α -anomeric configuration and the glycosylation site as N-1. However, compound 27 has β -anomeric configuration and the glycosyl attachment on N-3.

(32) Sheldrick, G. M. SHELX-76, "A Program for X-Ray Crystal Structure Determination", University of Cambridge, England, 1976.

(33) ORTEP Program: Johnson, C. K. ORTEP Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965.

Table II. Comparative in Vitro Antiviral Activity of Ribavirin and 3-Deazaguanine and Its Derivatives in Vero Cells

compound	toxic level ^a	virus ratings ^b			
		HSV1	VV	Para 3	VSV
3-deazaguanine (1)	2.2	1.40	1.55	0.98	1.55
5-amino-1,5-dihydro-6-hydrazinoimidazo[4,5-c]pyridin-4-one (5)	240.0	0.05	0.15	0.03	0
3-deazaguanosine (2)	4.3	1.40	1.50	1.18	1.50
1-amino-3-deazaguanosine (8)	450.0	0.25	1.25	0.30	0.45
3-deazaguanosine 3',5'-cyclic phosphate (13)	5.3	1.02	1.15	0.62	1.00
7-(2-deoxy- α -D-ribofuranosyl)-3-deazaguanine (26)	400.0	0.30	1.18	0.22	0.35
7-(2-deoxy- β -D-ribofuranosyl)-3-deazaguanine (27)	none	0.80	1.42	0.92	1.06
9-(2-deoxy- α -D-ribofuranosyl)-3-deazaguanine (28)	none	1.00	1.65	0.70	0.80
2'-deoxy-3-deazaguanosine (29)	none	1.28	1.72	1.45	1.03
1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin)	none	0.60	1.20	1.30	0.80

^aIn micrograms per milliliter. ^bThe virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0-4) assigned to T for each drug level was subtracted from that of C, and the differences (C - T) were totaled. If partial toxicity was evident at any drug level, the C - T of that level was divided by 2. The sum of all C - T values was then divided by 10 times the number of test cups used per drug level.

Table III. In Vitro Antitumor Activity of 3-Deazaguanine and Its Derivatives

compound	ID ₅₀ ^a	
	L1210	P388
3-deazaguanine (1)	6.2 × 10 ⁻⁵ M	39% inhibtn at 10 ⁻⁴ M
5-amino-1,5-dihydro-6-hydrazinoimidazo[4,5-c]pyridin-4-one (5)	3.5 × 10 ⁻⁵ M	6.2 × 10 ⁻⁵ M
3-deazaguanosine (2)	1.8 × 10 ⁻⁵ M	1.6 × 10 ⁻⁵ M
1-amino-3-deazaguanosine (8)	<i>b</i>	<i>b</i>
3-deazaguanosine 3',5'-cyclic phosphate (13)	4.1 × 10 ⁻⁶ M	4.5 × 10 ⁻⁵ M
7-(2-deoxy- α -D-ribofuranosyl)-3-deazaguanine (26)	8.0 × 10 ⁻⁵ M	31% inhibtn at 10 ⁻⁵ M
7-(2-deoxy- β -D-ribofuranosyl)-3-deazaguanine (27)	<i>b</i>	<i>b</i>
9-(2-deoxy- α -D-ribofuranosyl)-3-deazaguanine (28)	45% inhibtn at 10 ⁻⁵ M	<i>b</i>
2'-deoxy-3-deazaguanosine (29)	2.0 × 10 ⁻⁵ M	4.2 × 10 ⁻⁵ M

^aInhibitory dose 50 (ID₅₀) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. ^bInactive at 10⁻⁴ M.

Biological Studies

Antiviral. 3-Deazaguanine and most of its derivatives synthesized during this study were tested against herpes simplex type 1 (HSV1), vaccinia (VV), parainfluenza type 3 (Para 3), and vesicular stomatitis (VSV) viruses in vitro in parallel with ribavirin (Table II). 3-Deazaguanine (1), 3-deazaguanosine (2), and 2'-deoxy-3-deazaguanosine (29), all exhibited potent activity against all the above viruses. 1-Amino-3-deazaguanosine (8) and 7-(2-deoxy- β -D-ribofuranosyl)-3-deazaguanine (27) showed potent activity only against VV. However, compounds 13 and 28 were found to inhibit the growth of HSV1 and VV significantly, in vitro. Compound 13 also showed marked activity against VSV. All other compounds are devoid of significant antiviral activity against these viruses in vitro.

Antitumor. The 3-deazaguanine nucleosides and nucleotides synthesized during this study were also tested against L1210 and P388 leukemia in vitro (Table III). All the compounds except 8, 27, and 28 were found to be moderate inhibitors of the growth of leukemia L1210 in vitro. 3-Deazaguanosine (2), the cyclic phosphate 13, 5-amino-6-hydrazino-1,5-dihydroimidazo[4,5-c]pyridin-4-one (5), and 2'-deoxy-3-deazaguanosine (29) are also significantly active against P388 leukemia in vitro. The other compounds under study were inactive. Thus, only 3-deazaguanine (1), 3-deazaguanosine (2), 2'-deoxy-3-deazaguanosine (29), and 3-deazaguanosine 3',5'-cyclic phosphate (13) exhibited a broad spectrum of antiviral and antitumor activity in vitro.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (¹H NMR) spectra were determined at 89.6 MHz with a JEOL FX-90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of water as indicated by elemental analysis was verified by ¹H NMR. Infrared

spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and Robertson Labs, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM Reagents). J.T. Baker silica gel (70-230 mesh) was used for column chromatography. Preparative liquid chromatography (LC) was run by utilizing the Waters Prep 500 LC system. All solvents used were reagent grade. Detection of components on TLC was by UV light and with 10% H₂SO₄ in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C.

5-Amino-6-hydrazino-1,5-dihydroimidazo[4,5-c]pyridin-4-one (5). To a suspension of methyl 5(4)-(cyanomethyl)imidazole-4(5)-carboxylate¹ (4; 4.95 g, 30 mmol) in absolute EtOH (40 mL) was added hydrazine hydrate (99-100%, 5.0 g, 100 mmol) and the mixture was heated under reflux with the exclusion of moisture. Within a few minutes a clear solution was obtained and after 1 h solid started precipitating out. Heating was continued for further 15 h, after which time the reaction mixture was cooled to room temperature. The crystalline solid was collected, washed with EtOH (5 × 25 mL), followed by ether (3 × 50 mL), and air-dried to yield 5.35 g of the title compound. Recrystallization from water using decolorizing carbon provided an analytical sample (5.0 g, 92.6%) as light yellow needles: mp >300 °C (after drying at 80 °C for 15 h); IR (KBr) ν 1560 (NHNH₂), 1660 (C=O), 3120-3320 (NH, NH₂) cm⁻¹; UV λ_{max} (pH 1) 271 nm (ϵ 7700), 292 (sh) (5400); UV λ_{max} (pH 7) 262 nm (ϵ 6900), 299 (4800); UV λ_{max} (pH 11) 262 nm (ϵ 7400), 298 (3800); ¹H NMR (Me₂SO-*d*₆) δ 4.27 (br s, 2, NHNH₂), 5.35 (s, 2, N₅ NH₂), 5.93 (s, 1, C₇ H), 7.12 (br s, 1, NHNH₂), 7.76 (s, 1, C₂ H); MS, *m/e* 181 (M⁺). Anal. (C₆H₈N₆O·0.25 H₂O) C, H, N.

6-Amino-1,5-dihydroimidazo[4,5-c]pyridin-4-one (3-Deazaguanine, 1). To a solution of 5 (1.80 g, 10 mmol) in boiling water (100 mL) was added freshly prepared Raney nickel catalyst (W-4, wet weight ~5 g), and the mixture was heated under reflux for 30 min. An additional amount of the catalyst (~3 g) was added, and refluxing was continued further for 30 min. The mixture was filtered hot through a Celite pad to remove the catalyst, which was washed well with boiling water (5 × 25 mL).

The combined filtrate and washings were decolorized with charcoal and concentrated to ~25 mL. It was allowed to stand at room temperature overnight in the dark. The light yellow needles that separated were collected and dried (at 100 °C for 12 h) to yield 1.20 g (80%): mp >320 °C [lit.¹ mp >350 °C]; IR (KBr) ν 1665 (C=O), 2950–3400 (NH₂) cm⁻¹; UV λ_{\max} (pH 1) 274 nm (ϵ 11500), 312 (6500); UV λ_{\max} (pH 7) 262 nm (ϵ 10500), 298 (8100); UV λ_{\max} (pH 11) 262 nm (ϵ 10000), 298 (7800); ¹H NMR (Me₂SO-*d*₆) δ 5.55 (s, 1, C₇ H), 5.65 (s, 2, NH₂), 7.82 (s, 1, C₂ H), 10.70 (br s, 1, NH), 12.35 (br s, 1, NH). Anal. (C₆H₆N₄O) C, H, N.

This compound was identical in all respects with 3-deazaguanine previously reported from our laboratory.¹

5,6-Diamino-1,5-dihydro-1- β -D-ribofuranosylimidazo[4,5-c]pyridin-4-one (1-Amino-3-deazaguanosine, 8). To a solution of methyl 5-(cyanomethyl)-1-(2,3,5-tri-*O*-benzoyl- β -D-ribofuranosyl)imidazole-4-carboxylate¹ (6; 12.19 g, 20 mmol) in absolute EtOH (125 mL) was added hydrazine hydrate (99–100%, 10.0 g, 200 mmol) and the mixture was heated under reflux. Within 2 h solid started precipitating out. Heating was continued for further 20 h. After cooling (ice bath), the solid was collected, washed with EtOH (5 \times 40 mL) followed by ether (2 \times 50 mL), and air-dried to yield 4.80 g of off-white solid. Crystallization from water (charcoal) provided analytically pure 8 as white needles: yield 4.50 g (75.7%); mp 254–255 °C dec; IR (KBr) ν 1680 (C=O), 3100–3400 (OH, NH₂) cm⁻¹; UV λ_{\max} (pH 1) 287 nm (ϵ 11600), 312 (sh) (5600); UV λ_{\max} (pH 7) 276 nm (ϵ 11300), 297 (sh) (8900); UV λ_{\max} (pH 11) 276 nm (ϵ 11000), 297 (sh) (8500); ¹H NMR (Me₂SO-*d*₆) δ 5.35 (s, 2, N₅ NH₂), 5.45 (d, 1, *J* = 5 Hz, C₁ H), 5.60 (s, 1, C₇ H), 6.20 (s, 2, C₆ NH₂), 7.90 (s, 1, C₂ H), and other sugar protons. Anal. (C₁₁H₁₅N₅O₅) C, H, N.

6-Amino-1,5-dihydro-1- β -D-ribofuranosylimidazo[4,5-c]pyridin-4-one (3-Deazaguanosine, 2). To a solution of 8 (1.48 g, 5 mmol) in boiling water (50 mL) was added Raney nickel catalyst (W-4, wet weight ~3 g) and the mixture was heated under reflux for 45 min. After removal of the catalyst, the filtrate was worked up as described for 1, and 3-deazaguanosine was obtained as white crystals after crystallization from water: yield 1.03 g (73%); mp 256–257 °C dec (lit.¹ mp 255–257 °C); IR (KBr) ν 1665 (C=O), 2920–3500 (OH, NH₂) cm⁻¹; UV λ_{\max} (pH 1) 284 nm (ϵ 13600), 309 (sh) (6500); UV λ_{\max} (pH 7) 270 nm (ϵ 10000), 298 (8000); UV λ_{\max} (pH 11) 272 nm (ϵ 10000), 295 (sh) (8000); ¹H NMR (Me₂SO-*d*₆) δ 5.50 (d, 1, *J* = 5.5 Hz, C₁ H), 5.53 (s, 1, C₇ H), 5.66 (s, 2, NH₂), 7.93 (s, 1, C₂ H), 10.48 (s, 1, NH), and other sugar protons. Anal. (C₁₁H₁₄N₄O₅) C, H, N.

5-(Cyanomethyl)-1- β -D-ribofuranosylimidazole-4-carboxamide 3',5'-Cyclic Phosphate (12). To 5-(cyanomethyl)-1- β -D-ribofuranosylimidazole-4-carboxamide 5'-phosphate ammonium salt¹ (3.96 g, 10 mmol) in anhydrous pyridine (150 mL) was added *N,N*-dicyclohexylmorpholinecarboxamidine³⁴ (2.93 g, 10 mmol), and the resulting solution was evaporated several times with dry pyridine to an anhydrous syrup. The dry syrup was dissolved in pyridine (750 mL) and added dropwise (over 1-h period) through a reflux condenser into a refluxing anhydrous solution of dicyclohexylcarbodiimide (DCC, 10.30 g, 50 mmol) in dry pyridine (2 L). The resulting solution was refluxed for an additional 15 h before water (125 mL) was added dropwise. After allowing to stand at room temperature for another 15 h, the solution was evaporated to dryness. To the residue were added water (125 mL) and ethyl ether (100 mL). The suspension was stirred vigorously and then filtered. The aqueous phase was separated and extracted with ethyl ether (3 \times 100 mL). The aqueous layer was passed through a Dowex 50-X8 resin column (Na⁺ form, 100–200 mesh, 2.5 \times 25 cm), and the eluent was evaporated to a syrup. Ethanol (100 mL) was added to the syrup, and the resulting mixture was kept at room temperature for 24 h. The mixture was filtered and the clear filtrate was allowed to stand at 0–5 °C for a week. The resulting precipitate (1.5 g) was collected and dissolved in water (~5 mL), and the product was precipitated by adding ethanol (50 mL). The light brown product was dissolved in water (~5 mL) and silica gel (5 g) was added. The suspension was evaporated to a powder. Coevaporation with EtOH (3 \times 50 mL) gave a dry residue, which was added on top of a silica gel column (2.5 \times 30 cm, packed in CHCl₃), and

the title compound was eluted with CHCl₃:MeOH (3:2, v/v). Evaporation of the appropriate homogeneous fractions gave 1.0 g (26%) of 12: mp >220 °C dec; IR (KBr) ν 1670 (C=O), 2220 (weak, CN), 3400 (OH) cm⁻¹; UV λ_{\max} (pH 1) 218 nm (ϵ 7200); UV λ_{\max} (pH 7) 233 nm (sh) (ϵ 7500); UV λ_{\max} (pH 11) 235 nm (sh) (ϵ 9200); ¹H NMR (D₂O) δ 4.48 (s, 2, CH₂), 5.76 (s, 1, C₁ H), 7.98 (s, 1, C₂ H). Anal. (C₁₁H₁₂N₄PNaO₇·H₂O) C, H, N, P.

6-Amino-1,5-dihydro-1- β -D-ribofuranosylimidazo[4,5-c]pyridin-4-one 3',5'-Cyclic Phosphate (3-Deazaguanosine 3',5'-Cyclic Phosphate, 13). A solution of 12 (0.77 g, 2 mmol) in water (30 mL) was adjusted to pH 10.0 with 10% aqueous Na₂CO₃ solution and heated under reflux for 1 h. The light brown solution was concentrated to ~15 mL and then adjusted to pH 6.5 with Dowex-50 (H⁺) resin before it was placed on a column of Bio-Rad AG-1 X 8 (formate form, 100–200 mesh, 30 mL). The column was first washed with water (500 mL) and then with water to 0.1 M formic acid gradient (2 L each). The product appeared after about 1.5 L of gradient had passed through the column. The fractions containing the homogeneous product were pooled, evaporated to a small volume, freeze-dried, and lyophilized to provide 0.40 g (50%) of 13 as a light brown powder: mp >200 °C dec; UV λ_{\max} (pH 1) 285 nm (ϵ 13500), 309 (sh) (8300); UV λ_{\max} (pH 7) 270 nm (ϵ 11300), 297 (sh) (8500); UV λ_{\max} (pH 11) 270 nm (ϵ 10300), 296 (sh) (8300); ¹H NMR (D₂O) δ 5.90 (s, 1, C₁ H), 8.80 (s, 1, C₂ H). Anal. (C₁₁H₁₃N₄PO₇·2H₂O) C, H, N, P.

Methyl 5-(Cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D- and - β -D-erythro-pentofuranosyl)imidazole-4-carboxylate (18 and 19) and Methyl 4-(Cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D- and - β -D-erythro-pentofuranosyl)imidazole-5-carboxylate (20 and 21). Method A. A mixture of dry 4 (4.95 g, 30 mmol), hexamethyldisilazane (HMDS, 60 mL), and ammonium sulfate (0.10 g) was heated under reflux for 15 h with the exclusion of moisture. Excess HMDS was removed by distillation to provide the trimethylsilyl derivative 14 as a yellowish brown oil. To the solution of 14 in dry CH₃CN (200 mL) was added 1-*O*-methyl-2-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribofuranose¹⁹ (15; 11.53 g, 30 mmol), followed by TMS-triflate³⁵ (9.60 g, 43.2 mmol). The clear reaction mixture was stirred at ambient temperature for 45 h. TLC (silica gel, acetone:hexane, 1:4) of an aliquot (treated with EtOH) indicated almost complete conversion of the sugar and the heterocycle to a multiple of products. The reaction mixture was evaporated to dryness, and the residue was dissolved in CHCl₃ (250 mL) and poured into a 5% aqueous NaHCO₃ solution (500 mL). The organic layer was separated and washed with 5% NaHCO₃ solution (2 \times 100 mL), followed by water (3 \times 50 mL). The dried (Na₂SO₄) organic extracts were evaporated, and the residual syrup was purified on a silica gel column by preparative LC techniques using acetone:hexane (1:4, v/v) as the solvent. The following four nucleosides were isolated in the order listed. **Methyl 4-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythro-pentofuranosyl)imidazole-5-carboxylate (18):** white needles (MeOH), 3.1 g (20%); mp 146–147 °C; IR (KBr) ν 1720 (C=O), 2250 (CN) cm⁻¹; UV λ_{\max} (pH 1) 247 nm (ϵ 20200); UV λ_{\max} (MeOH) 247 nm (ϵ 19500); UV λ_{\max} (pH 11) 254 nm (ϵ 17800); ¹H NMR (CDCl₃) δ 2.38, 2.44 (2 s, 6, 2 CH₃), 3.95 (s, 3, CO₂CH₃), 4.60 (s, 2, CH₂), 6.71 (q, 1, C₁ H, peak width 10.5 Hz), 7.26 (m, 4, Ph), 7.52 (s, 1, C₂ H), 7.96 (m, 4, Ph), and other sugar protons. Anal. (C₂₈H₂₇N₃O₇) C, H, N.

Methyl 4-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- β -D-erythro-pentofuranosyl)imidazole-5-carboxylate (19) was obtained as white needles (MeOH): 1.0 g (6.5%); mp 132–133 °C; IR (KBr) ν 1715 (C=O), 2240 (CN) cm⁻¹; UV λ_{\max} (pH 1) 247 nm (22200); UV λ_{\max} (MeOH) 247 nm (ϵ 19600); UV λ_{\max} (pH 11) 255 nm (ϵ 18800); ¹H NMR (CDCl₃) δ 2.40, 2.44 (2 s, 6, 2 CH₃), 3.95 (s, 3, CO₂CH₃), 4.67 (s, 2, CH₂), 6.75 (t, 1, C₁ H, peak width 13.5 Hz), 7.26 (m, 4, Ph), 7.84 (s, 1, C₂ H), 8.02 (m, 4, Ph), and other sugar protons. Anal. (C₂₈H₂₇N₃O₇) C, H, N.

Methyl 5-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythro-pentofuranosyl)imidazole-4-carboxylate (20) was isolated as a crystalline (MeOH) solid: 3.4 g (22%); mp 63–65 °C; IR (KBr) ν 1715 (C=O), 2260 (CN) cm⁻¹; UV λ_{\max} (pH 1) 243

(34) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* 1961, 83, 649.(35) Marsmann, H. C.; Horn, H. G. *Z. Naturforsch., B* 1973, 27, 1448.

nm (ϵ 33600); UV λ_{\max} (pH 7) 243 nm (ϵ 36000); UV λ_{\max} (pH 11) 243 nm (ϵ 21400); $^1\text{H NMR}$ (CDCl_3) δ 2.41 (s, 6, 2 CH_3), 3.92 (s, 3, CO_2CH_3), 4.62 (s, 2, CH_2), 6.32 (q, 1, C_1H , peak width 10.0 Hz), 7.24 (m, 4, Ph) 7.76 (s, 1, C_2H), 7.92 (m, 4, Ph), and other sugar protons. Anal. ($\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_7\text{H}_2\text{O}$) C, H, N.

Methyl 5-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- β -D-erythro-pentofuranosyl)imidazole-4-carboxylate (21) was isolated as an amorphous solid: 0.75 g (4.8%); mp 82–83 °C; IR (KBr) ν 1720 (C=O), 2240 (CN) cm^{-1} ; UV λ_{\max} (pH 1) 244 nm (ϵ 30600); UV λ_{\max} (pH 7 and 11) 243 nm (ϵ 22000); $^1\text{H NMR}$ (CDCl_3) δ 2.40, 2.44 (2 s, 6, 2 CH_3), 3.91 (s, 3, CO_2CH_3), 4.64 (s, 2, CH_2), 6.22 (t, 1, C_1H , peak width 14.0 Hz), 7.25 (m, 4, Ph), 7.73 (s, 1, C_2H), 7.90 (m, 4, Ph), and other sugar protons. Anal. ($\text{C}_{28}\text{H}_{27}\text{N}_3\text{O}_7$) C, H, N.

Method B. To a suspension of 4 (4.95 g, 30 mmol) in anhydrous CH_3CN (100 mL) was added NaH (50% in oil, 1.60 g, 33 mmol), and the mixture was stirred at ambient temperature in an atmosphere of dry argon for 30 min, during which time a clear solution of 16 was obtained. Finely powdered dry (over KOH) 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythro-pentofuranose³⁰ (17; 12.83 g, 33 mmol) was added portionwise and the resulting mixture was stirred further for 3 h under argon. The reaction mixture was filtered and to the filtrate was added silica gel (~30 g). The mixture was evaporated to dryness. Coevaporation with benzene (5 \times 75 mL) gave dry residue, which was placed on top of a silica gel column (5 \times 55 cm), prepacked in benzene. The column was eluted first with benzene:ethyl acetate (4:1.5, v/v, 2.5 L), followed by benzene:ethyl acetate (4:2, v/v). After the initial elution of the unreacted sugar 17 (0.50 g), methyl 4-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- β -D-erythro-pentofuranosyl)imidazole-5-carboxylate (19; 7.0 g, 45.1%), followed by methyl 5-(cyanomethyl)-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl- β -D-erythro-pentofuranosyl)imidazole-4-carboxylate (21; 7.5 g, 48.3%) was obtained. Compounds 19 and 21 were found to be identical with that obtained by method A.

6-Amino-3,5-dihydro-3-(2-deoxy- α -D-erythro-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (26). A mixture of 18 (1.03 g, 2 mmol) and liquid NH_3 (20 mL) was placed in a steel bomb (50 mL). The bomb was three-quarters submerged in a steam bath and heated for 10 h. The NH_3 was allowed to evaporate at room temperature, and the residue was subjected to a vacuum overnight to remove the last traces of NH_3 . The residue was extracted several times with boiling ether (5 \times 100 mL) to remove *p*-toluamide. The ether-insoluble material was purified on a silica gel column (2 \times 40 cm, packed in EtOAc), using EtOAc:H₂O:1-PrOH, 4:2:1, upper layer, as the eluent. A small amount of 4-(cyanomethyl)-1-(2-deoxy- α -D-erythro-pentofuranosyl)imidazole-5-carboxamide (22; 30 mg, mp 115 °C) was isolated from the initial fractions. The later homogeneous fractions were pooled and evaporated to dryness, and the residue was crystallized from water (charcoal) to provide 26 as colorless needles, which, on exposure to light and air, developed color: 0.37 g (69.5%); mp >245 °C dec; IR (KBr) ν 1660 (C=O), 3200–3330 (OH, NH₂) cm^{-1} ; UV λ_{\max} (pH 1) 276 nm (ϵ 8800), 317 (5200); UV λ_{\max} (pH 7) 260 nm (ϵ 5800), 316 (5500); UV λ_{\max} (pH 11) 258 nm (ϵ 5800), 316 (5200); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 5.30 (br s, 2, C_6NH_2), 5.50 (s, 1, C_7H), 6.68 (q, 1, C_1H , peak width 10.2 Hz), 8.20 (s, 1, C_2H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4\text{H}_2\text{O}$) C, H, N.

6-Amino-3,5-dihydro-3-(2-deoxy- β -D-erythro-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (27). A mixture of 19 (5.17 g, 10 mmol), liquid NH_3 (80 mL), and anhydrous MeOH (20 mL) was heated in a steel bomb at 100 °C for 18 h. MeOH/ NH_3 was evaporated and the brown residue was purified on a silica gel column (3.5 \times 70 cm) using CHCl_3 :MeOH (95:15, v/v) as the solvent. Crystallization of the pure material from water gave 1.55 g (58.2%) of the title compound: mp 147–149 °C dec; IR (KBr) ν 1625, 1660 (C=O), 3250–3360 (OH, NH₂) cm^{-1} ; UV λ_{\max} (pH 1) 277 nm (ϵ 13300), 317 (6500); UV λ_{\max} (pH 7) 258 nm (ϵ 8100), 317 (8400); UV λ_{\max} (pH 11) 258 nm (ϵ 8000), 316 (7800); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 5.38 (br s, 2, C_6NH_2), 5.49 (s, 1, C_7H), 6.62 (t, 1, C_1H , peak width 13.0 Hz), 8.22 (s, 1, C_2H), 10.53 (br s, 1, N_5H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4$) C, H, N.

5-(Cyanomethyl)-1-(2-deoxy- α -D-erythro-pentofuranosyl)imidazole-4-carboxamide (24). Compound 20 (5.17

g, 10 mmol) and liquid NH_3 (100 mL) were placed in a steel bomb (250 mL). The bomb was three-quarters submerged in a steam bath and heated for 10 h. At this point the TLC (silica gel, EtOAc:H₂O:1-PrOH, 4:2:1, upper phase) indicated almost complete conversion of the starting material to 24 and a minor amount of 28. The ammonia was allowed to evaporate at room temperature, and the residue was subjected to a vacuum overnight to remove the last traces of NH_3 . The residue was extracted several times with boiling ether (5 \times 100 mL, to remove *p*-toluamide) and the ether-insoluble material was dissolved in MeOH, adsorbed on silica gel (~5 g), and placed on top of a silica gel column (4 \times 40 cm, packed in EtOAc). The column was eluted with EtOAc:H₂O:1-PrOH (4:2:1, upper phase) and the appropriate homogeneous fraction were pooled and evaporated to dryness. Crystallization of the residue from water gave white needles: 2.0 g (75%); mp 169–170 °C; IR (KBr) ν 1660 (C=O), 2240 (CN), 3170–3450 (OH) cm^{-1} ; UV λ_{\max} (pH 1) 217 nm (ϵ 10500); UV λ_{\max} (pH 7 and 11) 235 nm (ϵ 9700); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 4.10 (m, 2, C_2H_2) 4.45 (s, 2, CH_2), 6.10 (q, 1, C_1H , peak width 10.0 Hz), 7.24 and 7.42 (2 br s, 2, CONH_2), 8.00 (s, 1, C_2H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4$) C, H, N.

6-Amino-1,5-dihydro-1-(2-deoxy- α -D-erythro-pentofuranosyl)imidazo[4,5-*c*]pyridin-4-one (28). A mixture of 24 (2.66 g, 10 mmol), 5% aqueous Na_2CO_3 solution (29 mL), and EtOH (15 mL) was heated under gentle reflux with stirring for 30 min. Complete dissolution was obtained as reflux started. The slightly brown solution was filtered, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. The residue was dissolved in MeOH, adsorbed on silica gel (~10 g), and placed on top of a silica gel column (4 \times 40 cm, packed in EtOAc). The column was eluted with EtOAc:H₂O:1-PrOH (4:2:1, upper phase), and the homogeneous fractions were pooled and evaporated to dryness. The residue was crystallized from water as off-white needles: 2.2 g (83%); mp >240 °C dec; IR (KBr) ν 1650, 1670 (C=O), 3150–3320 (OH, NH₂) cm^{-1} ; UV λ_{\max} (pH 1) 283 nm (ϵ 13000), 310 (sh) (6900); UV λ_{\max} (pH 7) 270 nm (ϵ 13200), 298 (9800); UV λ_{\max} (pH 11) 271 nm (ϵ 12500), 297 (sh) (9300); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 5.48 (s, 1, C_7H), 5.60 (br s, 2, C_6NH_2), 5.95 (q, 1, C_1H , peak width 10.5 Hz), 7.89 (s, 1, C_2H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4$) C, H, N.

5-(Cyanomethyl)-1-(2-deoxy- β -D-erythro-pentofuranosyl)imidazole-4-carboxamide (25). A mixture of 21 (5.17 g, 10 mmol) and liquid NH_3 (100 mL) was heated for 20 h at 100 °C, as described for 24. After the removal of NH_3 , the residue was purified on a silica gel column (5 \times 50 cm, packed in EtOAc) using EtOAc:H₂O:1-PrOH (4:2:1, upper layer) as the eluent. The appropriate homogeneous fractions were pooled and evaporated to dryness. Crystallization of the residue from water (charcoal) gave white needles: 1.80 g (67.7%); mp 156–158 °C; IR (KBr) ν 1660 (C=O), 2260 (CN), 3350 (OH) cm^{-1} ; UV λ_{\max} (pH 1) 214 nm (ϵ 13300); UV λ_{\max} (pH 7) 233 nm (ϵ 24500); UV λ_{\max} (pH 11) 230 nm (ϵ 14900); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 4.35 (s, 2, CH_2), 6.12 (t, 1, C_1H , peak width 13.5 Hz), 7.32 and 7.50 (2 br s, 2, CONH_2), 8.10 (s, 1, C_2H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4$) C, H, N.

6-Amino-1-(2-deoxy- β -D-erythro-pentofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (2'-Deoxy-3-deazaguanosine, 29). A mixture of 25 (2.66 g, 10 mmol), 5% aqueous Na_2CO_3 solution (28 mL), and EtOH (30 mL) was heated under gentle reflux for 30 min. The reaction mixture was worked up and purified as described for 28 to yield 1.73 g (65%). Crystallization from water gave an analytical sample: mp 230–231 °C; IR (KBr) ν 1625, 1665 (C=O), 3220–3420 (OH, NH₂) cm^{-1} ; UV λ_{\max} (pH 1) 283 nm (ϵ 13000), 310 (sh) (6700); UV λ_{\max} (pH 7) 269 nm (ϵ 11700), 295 (sh) (8500); UV λ_{\max} (pH 11) 275 nm (ϵ 12600); $^1\text{H NMR}$ ($\text{Me}_2\text{SO}-d_6$) δ 5.44 (s, 1, C_7H), 5.59 (br s, 2, C_6NH_2), 5.94 (t, 1, C_1H , peak width 13.5 Hz), 7.86 (s, 1, C_2H), 10.32 (br s, 1, N_5H), and other sugar protons. Anal. ($\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_4$) C, H, N.

Antiviral Evaluation. Inhibition of the virus-induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed in African green monkey kidney (Vero, V) cells after infection with herpes simplex type 1 (HSV 1, KOS), vaccinia (VV), parainfluenza type 3 (Para 3), and vesicular stomatitis (VSV) viruses. In this system, monolayers (18 h) of cells were exposed to the following TCID₅₀ (tissue culture 50% infective

dose) units of virus: HSV 1 (63), VV (200), Para 3 (56), VSV (3), and concentrations of each compound in one-half log dilutions ranging from 1000 to 1 $\mu\text{g}/\text{mL}$ were added within 15-30 min. The degree of CPE inhibition was observed microscopically after 72 h of incubation at 37 °C in 5% CO₂ and scored numerically in order to calculate a virus rating (VR) as previously reported.³⁶ Significance of antiviral activity in terms of VRs has been assigned as follows: 0.5, slight or no activity; 0.5-0.9, moderate activity; ≥ 1.0 , marked activity.

Antitumor Evaluation. L1210 leukemia and P388 lymphoid neoplasm were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum. For determination of cell growth inhibition, L1210 and P388 cells were seeded in 13 \times 100 tubes at 5 \times 10⁴ cells/mL (2 mL/tube). Cells were grown in the presence of the compound of interest, at 4-5 log doses, for 48 h at 37 °C. Cell growth was assessed by cell count, using a Coulter cell counter. Cell growth at each dose level was expressed

as a percentage of growth in control tubes and dose resulting in 50% inhibition of growth was determined.

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Registry No. 1, 41729-52-6; 2, 56039-11-3; 3, 56039-13-5; 4, 56039-06-6; 5, 91713-21-2; 6, 58931-20-7; 8, 91713-22-3; 12, 91713-23-4; 13, 62190-71-0; 14 (isomer 1), 58459-35-1; 14 (isomer 2), 56596-91-9; 15, 4330-34-1; 16, 91713-28-9; 17, 4330-21-6; 18, 91713-24-5; 19, 91713-25-6; 20, 91713-26-7; 21, 91713-27-8; 22, 91713-29-0; 26, 83587-64-8; 27, 83587-63-7; 24, 91741-79-6; 25, 83587-61-5; 28, 83587-62-6; 29, 87202-41-3; hydrazine hydrate, 7803-57-8; 5-(cyanomethyl)-1- β -D-ribofuranosylimidazole-4-carboxamide, 91796-78-0.

(36) Sidwell, R. W.; Huffman, J. H. *Appl. Microbiol.* 1971, 22, 797.

(1,3-Dialkyl-5-amino-1H-pyrazol-4-yl)arylmethanones. A Series of Novel Central Nervous System Depressants

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A series of novel (1,3-dialkyl-5-amino-1H-pyrazol-4-yl)arylmethanones was synthesized. Pharmacological evaluation of these compounds demonstrated central nervous system depressant activity, potential anticonvulsant properties, and a low order of acute toxicity. In addition, selected compounds showed potential antipsychotic effects. This report focuses on the synthesis and structure-activity relationships of these compounds. (5-Amino-1-ethyl-3-methyl-1H-pyrazol-4-yl)(2-chlorophenyl)methanone (21) was the most active compound against pentylene-tetrazole-induced convulsions. (5-Amino-1,3-dimethyl-1H-pyrazol-4-yl)(3-chlorophenyl)methanone (4) also has a favorable anticonvulsant depression ratio. (5-Amino-1,3-dimethyl-1H-pyrazol-4-yl)(3-trifluoromethylphenyl)methanone (8), (5-amino-1,3-dimethyl-1H-pyrazol-4-yl)(3-thienyl)methanone (13), and (5-amino-3-ethyl-1-methyl-1H-pyrazol-4-yl)phenylmethanone (14) are very potent depressants. (5-Amino-1,3-dimethyl-1H-pyrazol-4-yl)(2-thienyl)methanone (12) possessed marked central depressant activity without anticonvulsant activity and without impairment of motor functioning. (5-Amino-1,3-dimethyl-1H-pyrazol-4-yl)(2-fluorophenyl)methanone (2) has a behavioral profile suggestive of antipsychotic activity and gave a positive Ames test result.

Epilepsy is a continuing medical problem despite the discovery and successful marketing of a number of drugs for its treatment;¹⁻³ thus the search continues for useful anticonvulsant agents.

A short series of novel (1,3-dialkyl-5-amino-1H-pyrazol-4-yl)arylmethanones had been prepared as intermediates in the synthesis of a series of compounds with antianxiety activity.^{4,5} When these were submitted for pharmacological evaluation, some were found to possess interesting central nervous system depressant activity, potential anticonvulsant properties, plus a low order of acute toxicity. The series was expanded to attempt to maximize the level of activity and to study the structure-activity relationships. Encouraging activity was also observed in selected compounds when evaluated for po-

tential antipsychotic activity.

Chemistry. Three general methods were developed for the synthesis of the title compounds. These are summarized in Chart I.

Route A. Many of the (1,3-dialkyl-5-amino-1H-pyrazol-4-yl)methanones were prepared by reaction of the (1,3-dialkyl-5-chloro-1H-pyrazol-4-yl)methanones⁶ with concentrated ammonium hydroxide under heat and pressure.

Route B. A series of 1,3-dialkyl-5-amino-1H-pyrazoles were prepared by a variation of the method of Taylor and Hartke⁷ in which 3-amino-2-butenitrile was condensed with methylhydrazine. Larger alkylhydrazines do not ring close under neutral or basic conditions, the reaction stopping at the open alkylhydrazone stage. However, Koike et al.⁸ used hydrogen chloride to effect this type of ring closure with phenylhydrazine. In our case, the addition of more than 1 equiv of concentrated hydrochloric acid to the larger alkylhydrazones resulted in high yields

(1) Millichop, J. G. *Postgrad. Med.* 1965, 37, 22.

(2) Berger, J. G.; Iorio, L. C. *Annu. Rep. Med. Chem.* 1979, 14, 27-28.

(3) Effland, R. C.; Forsch, M. F. *Annu. Rep. Med. Chem.* 1982, 17, 16-17.

(4) DeWald, H. A.; Lobbestael, S.; Butler, D. E. *J. Med. Chem.* 1977, 20, 1562.

(5) DeWald, H. A.; Butler, D. E. U.S. Patent 3660425, May 2, 1972.

(6) Butler, D. E.; DeWald, H. A. *J. Org. Chem.* 1971, 36, 2542.

(7) Taylor, E. C.; Hartke, K. S. *J. Am. Chem. Soc.* 1959, 81, 2456.

(8) Koike, E.; Iida, H.; Okawa, M.; Kashioka, A. *J. Chem. Soc. Jpn., Ind. Chem. Sect.* 1954, 57, 56; *Chem. Abstr.* 1955, 49, 11629c.