Springer-Verlag, New York, N.Y., 1976, p 179.
(32) R. W. Coburn, L. K. Y. Ng, L. Lemberger, and L. L. Kopin, *Biochem. Pharmacol.*, **23**, 873 (1974).
(33) This work was supported in part by the Non-Medical Use of Drugs Directorate, National Health and Welfare Canada, whom we thank for assistance in obtaining cannabinoids. The technical assistance of Ian Davie is gratefully acknowledged.

Synthesis and Antiviral and Enzymatic Studies of Certain 3-Deazaguanines and Their Imidazolecarboxamide Precursors

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Due to the varied and potent biological activity of 3-deazaguanine (**20**), 3-deaza-7-β-D-ribofuranosylguanine, 3-deazaguanosine (**22**), and 3-deazaguanylic acid (**24**), several 3-deazaguanines, mainly with modification in the 7 and 9 positions, were prepared. 7-(5-Deoxy-β-D-ribofuranosyl)- and 7-(tetrahydropyran-2-yl)-3-deazaguanine (**12** and **13**) were obtained by ammonolysis of the corresponding 1-substituted methyl 4-(cyanomethyl)imidazole-5-carboxylates, **6** and **8**, and subsequent in situ cyclization. 9-(5-Deoxy-β-D-ribofuranosyl)- and 9-(tetrahydropyran-2-yl)-3-deazaguanine (**14** and **15**) were obtained by ammonolysis of the corresponding 1-substituted methyl 5-(cyanomethyl)imidazole-4-carboxylates, **5** and **7**, to provide 1-(5-deoxy-β-D-ribofuranosyl)- and 1-(tetrahydropyran-2-yl)-5-(cyanomethyl)imidazole-4-carboxamides (**9** and **10**, respectively), which were subsequently cyclized with aqueous potassium carbonate. Methyl 4-(cyanomethyl)-1- or -3-(5-deoxy-2,3-di-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylates, **5** and **6**, were obtained from the stannic chloride catalyzed condensation of methyl 5(4)-(cyanomethyl)-1-(trimethylsilyl)imidazole-4(5)-carboxylate (**2**) and 5-deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**3**). Methyl 4(5)-(cyanomethyl)imidazole-5(4)-carboxylate (**1**) and dihydropyran in the presence of acid provided the tetrahydropyran-2-yl derivatives **7** and **8**. The in vitro antiviral and antibacterial activity of these 3-deazaguanines, their imidazolecarboxamide precursors, and several acetylated derivatives were compared with 3-deazaguanine (**20**), 3-deazaguanosine (**22**), and 3-deazaguanylic acid (**24**), their imidazolecarboxamde precursors, 4(5)-(cyanomethyl)imidazole-5(4)-carboxamide (**19**), 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (**21**), and 5-(cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide 5′-phosphate (**23**), and ribavirin. The most active compounds, **19**, **21**, and **23**, possessed an in vitro antiviral spectrum similar to, but generally less potent than, the corresponding ring-closed compounds **20**, **22**, and **24**. Compound **23** was found to be a potent, specific inhibitor of IMP dehydrogenase. Data are presented which support the antiviral activity of **19**, **21**, and **23** independent of the possible enzymatic cyclization to the corresponding imidazo[4,5-*c*]pyridine.

The recently synthesized[2] 3-deazaguanine [6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one, **20**] and its probable metabolites, 3-deazaguanosine [6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one, **22**] and 3-deazaguanylic acid [6-amino-1-β-D-ribofuranosylimidazo-[4,5-*c*]pyridin-4(5*H*)-one 5′-phosphate, **24**], are potent inhibitors of biosynthesis of purine nucleotides[3] and possess marked antiviral[4] and anticancer activity.[3b,5] Furthermore, 3-deaza-7-β-D-ribofuranosylguanine [6-amino-3-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one[2]] has highly significant antibacterial activity[6] against Gram-negative bacteria.

We have continued the study of 3-deazaguanine and its derivatives and now wish to report the synthesis, structure–activity relationships, and biochemical studies of certain 3-deazaguanines and their imidazole precursors which have sugar modifications in the 7 or 9 positions. We have included for comparison the biological activity of several related nucleosides which have been modified in the 6 position, such as the recently prepared[7] 6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine-4(5*H*)-thione (3-deaza-6-thioguanosine) and 4,6-diamino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine (2-amino-3-deazaadenosine).

Synthesis. The most useful synthetic approach to 3-deazaguanine (**20**) is that reported[2] utilizing the unique ring closure of 4(5)-(cyanomethyl)imidazole-5(4)-carboxamide (**19**) under basic conditions. Thus, we have extended this approach to the synthesis of the 9-substituted 3-deazaguanines **14** and **15** (Scheme I) through their corresponding imidazolecarboxamides **9** and **10**. Unfortunately, liquid ammonia treatment or other milder treatment of 1-substituted methyl 4-(cyanomethyl)-imidazole-5-carboxylates **6** and **8** did not provide the corresponding imidazolecarboxamides which, if formed,[8] cyclized in situ to the 7-substituted 3-deazaguanines **12** and **13**. The difference in reactivity of positional isomers of imidazole **1** can be attributed initially to steric hindrance by the 5-(cyanomethyl) group and the N_3 substituent of imidazoles **6** and **8** toward ammonolysis of the ester group in the 4 position as compared to the ammonolysis of the relatively unhindered ester group in the 5 position of imidazoles[8] **5** and **7**. A similar difference in the reactivity of the 1- and 3-β-D-ribofuranosides of 4(5)-cyano-5(4)-(cyanomethyl)imidazole toward cyclization has previously been noted.[7]

The imidazole carboxylates **5**–**8** were prepared by glycosylation of **1** or its silylated derivative **2**. The initial reaction, **2** → **5** and **6**, involves an anhydrous stannic chloride catalyzed condensation of 5-deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**3**) and methyl 1-(trimethylsilyl)-5(4)-(cyanomethyl)imidazole-4(5)-carboxylate (**2**) to provide the positional isomers **5** and **6**. Imidazole **1** and 2,3-dihydropyran (**4**) were allowed to react in the presence of acid to afford tetrahydropyran-2-yl derivatives **7** and **8**.

Several other prodrug-type modifications were obtained by acetylation of **20**–**22**, according to standard procedures, to provide 6-acetamidoimidazo[4,5-*c*]pyridin-4(5*H*)-one (**17**), 5-(cyanomethyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribo-

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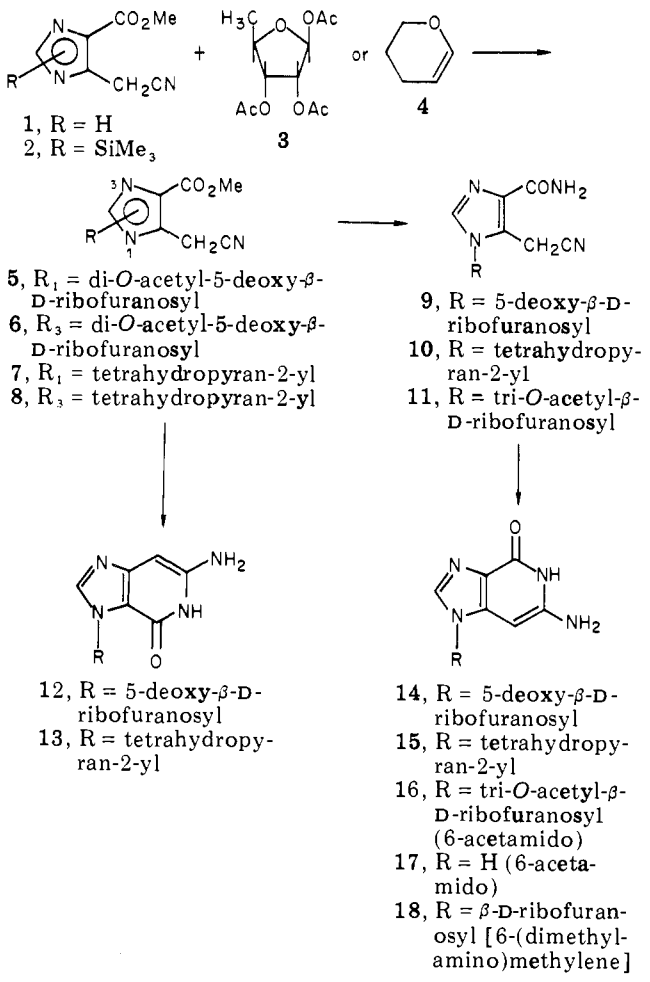
Scheme I



furanosyl)imidazole-4-carboxamide (11), and 6-acetamido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (**16**), respectively. 6-[*N*,*N*-(Dimethylamino)methyleneamino]-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5*H*)-one (**18**) was obtained from **20** and dimethylformamide dimethyl acetal following a previously described procedure.[9]

Replacement of the lactam–lactim function in the 6 position of **22** and 3-deaza-7-β-D-ribofuranosylguanine with a mercapto, amino, or bromo group or hydrogen (via the mercapto intermediate) has recently been reported[7] by ring closure of the β-D-ribofuranosyl derivatives of 5(4)-cyano-4(5)-(cyanomethyl)imidazole with appropriate reagents.

Antiviral Evaluation. Inhibition of the virus-induced cytopathic effect (CPE) was used as the initial indicator of antiviral activity. CPE was observed in human carcinoma of the nasopharynx (KB) cells after infection with adenovirus, type 3 (AV/3); type 1 (HSV/1) or type 2 (HSV/2) herpes simplex virus; vaccinia virus (VV); parainfluenza virus, type 3 (PIV/3); and rhinovirus, type 13 (RV/13). In these experiments, monolayers (18–24 h) of cells were exposed to 320 $CCID_{50}$ of virus and concentrations of each compound ranging in one-half log dilutions from 1000 to 1 μg/mL were added within 15 min. The degree of CPE inhibition and compound cytotoxicity were observed microscopically after 72 h of incubation at 37 °C and scored numerically in order to calculate a virus rating (VR) as previously described.[10] Significance of antiviral activity in terms of VR's has been assigned as follows: <0.5, slight or no activity; 0.5–0.9, moderate activity; and ≥1.0 marked activity.

Table I. Comparative in Vitro Antiviral Activity (Virus Rating)[a] of 3-Deazaguanines, Imidazole Precursors, and Ribavirin against RNA and DNA Viruses

compd no.	AV/3	HSV/1	HSV/2	VV	PIV/3	RV/13
20	1.1	1.3	1.1	1.3	1.2	1.2
22	0.8	0.9	0.9	1.1	0.9	1.0
24	0.8	1.0	0.8	1.0	0.9	1.1
14	–[b]	0.9	–	–	0.0	0.2
13	–	0.8	–	–	0.1	0.8
19	0.8	0.9	–	–	0.8	0.7
21	–	0.7	1.3	0.4	0.4	0.3
23	–	0.8	0.7	0.3	0.0	0.0
ribavirin	0.6	0.8	0.6	0.9	0.8	0.7

[a] The 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 any toxicity was evident at a 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. [b] Not determined.

The results of these experiments and corresponding data for ribavirin,[11] a known broad-spectrum antiviral agent, are included in Table I. Of the 5′-deoxynucleosides **9**, **12**, or **14**, only **14** exhibited any antiviral activity. Only compounds having moderate activity (VR ≥ 0.5) against at least one virus are indicated. Compounds **20**, **22**, and **24** have highly significant in vitro antiviral activity against all six viruses. The in vivo antiviral activity of these derivatives has recently been reported.[4a] It is interesting to note that **19**, 4-(cyanomethyl)imidazole-5-carboxamide,[2] exhibits significant in vitro antiviral activity against both RNA and DNA viruses which is comparable to ribavirin (Table I). Compounds **21** and **23** show reasonably good antiherpes activity but negligible activity against RNA viruses. Compound **13** showed significant activity against the RNA virus, RV/13, and the DNA virus, HSV/1. Notably inactive compounds in this series are **10**, **15**, and 3-deaza-7-β-D-ribofuranosylguanine. Modifications of the 6 position of 3-deazaguanosine and 3-deaza-7-β-D-ribofuranosylguanine result in complete loss of in vitro antiviral activity.

An in vivo experiment against influenza B viral infections in male Swiss mice is recorded in Table II. The compounds studied, **19**, **21**, **22**, and **7**, were compared with the in vivo antiviral activity of 3-deazaguanine (**20**) (see Scheme II). The compounds were administered orally in a protocol as previously described[4a] for **20**. 5-(Cyanomethyl)imidazole-4-carboxamide (**19**) appeared as active as 3-deazaguanine in this test. Similarly, 5-cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (**21**) appeared equally as active as the corresponding ring-closed derivative, 3-deazaguanosine (**22**), in increasing survival time and survival number. The 1-tetrahydropyranyl derivative of 3-deazaguanine (**7**) appeared to be inactive. These data parallel the in vitro data of Table I.

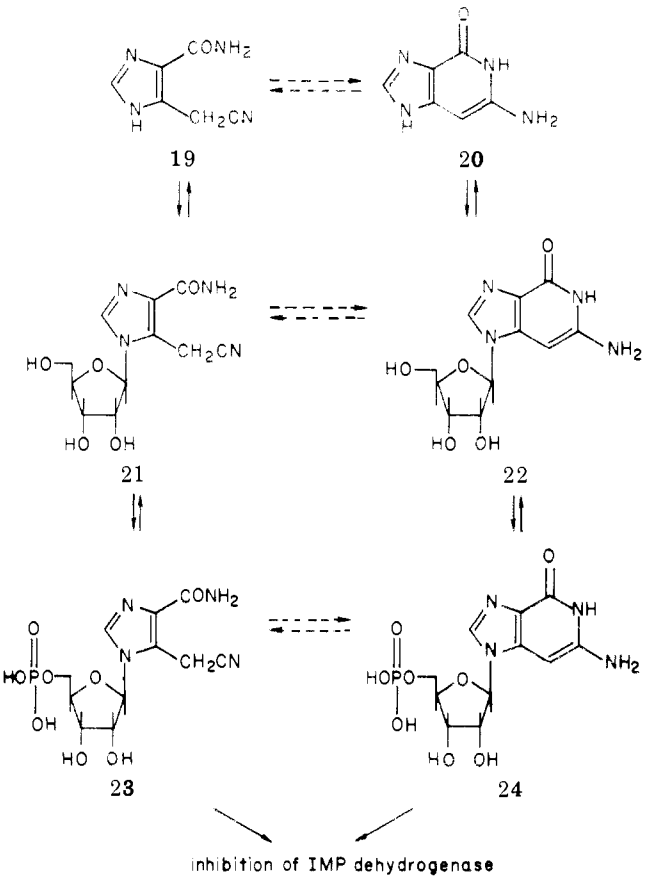
Biochemical Studies. Compounds **20**, **22**, and **24** and their corresponding imidazole precursors were tested as potential inhibitors of purine nucleotide biosynthesis in Ehrlich ascites tumor cell suspensions as described by Snyder et al.[12] In vitro suspensions of Ehrlich ascites tumor cells are incubated with [^{14}C]hypoxanthine (0.1 mM) with and without test compounds (1.0 mM). The incorporation of radioactivity into adenine and guanine nucleotides was then measured by thin-layer chromatography of the acid-soluble cell fraction. The inhibition of this incorporation was determined by comparison of the radioactivity recovered in the untreated and compound-

Table II. Effect of 6-Amino-4,5-dihydroimidazo[4,5-*c*]pyridin-4(5*H*)-one (20), 6-Amino-1-β-ribofuranosylimidazo[4,5-c]pyridin-4(5*H*)-one (22), 5-(Cyanomethyl)imidazole-4-carboxamide (19), 5-(Cyanomethyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (21), and 6-Amino-1-(tetrahydropyran-2-yl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (7) on Influenza B Induced Deaths in Mice[a]

compd no.	dose, (mg/kg)/day	toxicity control, surv/total	infected, treated, surv/total	survivor increase, *P*[b]	infected, treated mean survival time, days	mean survival time increase, *P*[c]
saline	10[d]		3/20		7.5	
20	40		3/10	0.23	7.6	>0.05
	10		1/10	>0.3	9.3	<0.05
	2.5		0/10		9.5	<0.001
19	100	5/5	4/10	0.118	8.3	>0.05
	50	5/5	0/10	>0.3	8.5	>0.05
	12.5	5/5	1/10	>0.3	8.1	>0.05
	3.1		3/10	0.23	8.1	>0.05
22	40		2/10	>0.3	8.4	>0.05
	10		1/10	>0.3	8.9	>0.05
	2.5		4/10	0.118	7.7	>0.05
21	100	5/5	2/10	>0.3	6.9	
	50	5/5	4/10	0.118	8.5	>0.05
	12.5	5/5	2/10	>0.3	8.5	>0.05
	3.1		0/10		7.9	>0.05
7	100	5/5	2/10	>0.3	7.1	
	50	5/5	0/10		7.7	>0.05
	12.5	5/5	1/10	>0.3	7.8	>0.05
	3.1		0/10		8.4	>0.05

[a] Host, 20-22-g male Swiss-Webster mice; virus, influenza B (Lee); virus dose, ~LD_{90}; virus inoculation route, intranasal; treatment route, oral; treatment schedule, twice daily for 9 days starting 2 h previrus inoculation; observation period, 21 days. [b] *P* = probability (Fisher exact test). [c] *P* = probability (*t* test). [d] mL/kg per injection.

Scheme II. Suggested Pathways for the Biosynthetic Activation of 5-(Cyanomethyl)-4-imidazolecarboxamide (19) and 3-Deazaguanine (20)



treated incubations. 3-Deazaguanosine 5′-phosphate (24, 3-DGMP) and ribavirin are potent inhibitors of the biosynthesis of guanine nucleotides[3a] as indicated in Table III. Compounds **20** and **22** also possess significant inhibition of guanine nucleotide biosynthesis but at a lower level than 3-DGMP and ribavirin. Adenine nucleotide biosynthesis is inhibited to a lesser extent. The imidazole precursors **19**, **21**, and **23** exhibit a spectrum of inhibition of guanine nucleotide biosynthesis quite comparable to the

Table III. Comparative Inhibition of Purine Nucleotide Biosynthesis in Ehrlich Ascites Tumor Cells in Vitro[a] by 3-Deazaguanines, Imidazole Precursors, and Ribavirin

compd no.	% inhibition: adenine nucleotides (AMP + ADP + ATP)	% inhibition: guanine nucleotides (GMP + GDP + GTP)
20	20	40
22	24	38
24	18	68
19	8	39
21	6	11
23	15	55
ribavirin	14	65

[a] Approximately 6 × 10^6 cells/mL were incubated at 37 °C, 20 min, with and without 1 mM of the test compounds. [^{14}C]Hypoxanthine (55 μCi/μmol) was then added to a final concentration of 0.1 mM and the incubation continued for 60 min. Adenine and guanine nucleotides were separated on PEI-cellulose as previously described.[17]

Table IV. Comparative in Vitro Inhibition of IMP Dehydrogenase,[a] Adenylosuccinate Synthetase,[b] and IMP Cyclohydrolase[c] by **23**, **24**, and Ribavirin 5′-Phosphate

compd no.	I_{50}/[S][d]: IMP → XMP	I_{50}/[S][d]: IMP → SAMP	I_{50}/[S][d]: FAICARP → IMP
24	0.068	20	
23	0.10	58	1.5
ribavirin 5′-phosphate	0.016	160	0.46

[a] Partially purified from *E. coli*.[3a] [b] See ref 16a. [c] See ref 16b. [d] Ratio of compound concentration for 50% inhibition to substrate concentration. Substrate concentrations in these assays were 1.7-3.0 × 10^{-5} M.

corresponding purine analogues **20**, **22**, **24**, and ribavirin. Most notable is the 55% inhibition by **23**. 3-DGMP and **23** were assayed directly against IMP dehydrogenase (IMP:NAD oxidoreductase, E.C. 1.2.1.14, IMP → XMP)[3a] and adenylosuccinate synthetase [IMP:L-aspartate ligase (GDP), E.C. 6.3.4.4, IMP → AMP(SAMP)]. These were partially purified enzymes isolated from *Escherichia coli* B as previously described.[3a,16a] These experiments demonstrate (Table IV) that 3-DGMP and its synthetic imidazole precursor, **23**, are potent inhibitors of the important enzyme, IMP dehydrogenase. Ribavirin 5′-phosphate, which was assayed in parallel, is also a potent inhibitor of this same enzyme.[3a,13,14] As indicated in Table IV, compound **24** (3-DGMP), **23**, and ribavirin 5′-phosphate do *not* inhibit adenylosuccinate synthetase, the alternate branching enzyme of de novo purine nucleotide biosynthesis. Finally, **23** and ribavirin 5′-phosphate were found to be weak inhibitors of IMP cyclohydrolase (Table IV).

Antimicrobial Evaluation. All compounds listed in Schemes I and II and from previous work[2,7] were tested against various microorganisms in defined medium using the broth dilution technique.[15] Clinical isolates of *Candida albicans*, *Escherichia coli*, *Epidermophyton floccosum*, *Microsporum canis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Trichophyton mentagrophytes* were used for this study. 3-Deaza-7-β-D-ribofuranosylguanine and 3-deazaguanine had in vitro activity against *E. coli* at concentrations of 0.0025 and 0.125 μmol/mL,[6] respectively. None of the other compounds were active at 0.4 μmol/mL or less.

Discussion

It is interesting that 3-deaza-7-β-D-ribofuranosylguanine[2] which has marked in vitro and in vivo antibacterial activity[6] was completely inactive in the in vitro antiviral tests. The presently described antiviral results and biological activity of the imidazole derivatives could be explained by assuming chemical or biochemical cyclization of one or more of the imidazoles to the corresponding imidazo[4,5-*c*]pyridine which would be related to 3-DGMP (Scheme II). To examine this possibility, compounds **19** and **21** were treated in cell culture as in the in vitro antiviral assay (except for the presence of viruses) and then examined for conversion to 3-deazaguanine metabolites. This study definitely showed no interconversions between **19** → **20** and **21** → **22** in the supernatant fluid and no detectable conversion in the KB cell lysates. It should be noted, however, that these data do not rule out the possibility of a virus-infected cell population converting one or more of the imidazoles to a 3-deazaguanosine metabolite.

It would thus appear that the imidazole derivatives here described possess antiviral activity in their own right and appear to inhibit the de novo synthesis of purines after conversion to the nucleotide, **23**. This would appear to be most likely in view of the inactivity of the 5′-deoxy derivatives **9**, **12**, and **14**. In considering this possibility, **21**, ribavirin, and aminoimidazolecarboxamide ribonucleoside (AICAR) have been assayed for direct phosphorylation by isolated rat liver nucleoside kinases.[17] The rate of phosphorylation of **21** (0.15 nmol/min) was considerably less than that of ribavirin (1.6 nmol/min) and AICAR (4.5 nmol/min) and may point to possible phosphorolysis of **21** to **19** and subsequent phosphoribosylation of **19** to its 5′-phosphate **23** via PRPP as the major route of activation (Scheme II). Although phosphoribosylation of AICA to AICAR phosphate is not considered a major route in the de novo or salvage pathways to IMP,[18] adenine phosphoribosyl transferase purified from beef liver catalyzed this reaction[19] and a similar transferase in Ehrlich ascites tumor cells might activate **19** to **23**.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a Varian A-60 spectrometer and a Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 using DSS as an internal reference. Ultraviolet spectra were recorded on a Cary Model 15 spectrophotometer and infrared spectra on a Perkin-Elmer 257 spectrophotometer (KBr pellets). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Evaporations were carried out under reduced pressure with the bath temperature below 40 °C unless otherwise noted. Detection of components on silica gel (ICN Life Sciences Group, Woelm F254) was made by ultraviolet light and with anisaldehyde-methanol-sulfuric acid (1:10:100) spray followed by heating. ICN Life Sciences Group Woelm silica gel (0.063-0.2 mm) was used for column chromatography.

Methyl 5-(Cyanomethyl)-1-(5-deoxy-2,3-di-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (5).[2] Methyl 5-(4)-(cyanomethyl)imidazole-4-carboxylate (1, 2.22 g, 13.4 mmol) was refluxed under anhydrous conditions for 12 h with hexamethyldisilazane (25 mL) and ammonium sulfate (50 mg). The excess hexamethyldisilazane was removed by distillation under reduced pressure, providing the trimethylsilyl derivative **2** as a yellowish brown oil. This was dissolved in 1,2-dichloroethane (35 mL). 5-Deoxy-1,2,3-tri-*O*-acetyl-β-D-ribofuranose (**3**, 3.4 g, 13.4 mmol) was added to the solution, followed by addition of anhydrous stannic chloride (2.25 mL, 19.3 mmol). The solution was stirred at room temperature for 9 h. Sodium hydrogen carbonate solution (7%, 100 mL) was added and the suspension was extracted twice with chloroform. The dried ($MgSO_4$) organic layer was evaporated in vacuo and the residue dissolved in chloroform and placed on a column of silica gel (200 g, packed in chloroform). Elution with ethyl acetate provided **5** (3.3 g, 68%) as a colorless syrup: UV λ_{max}^{pH1} 225 nm (ε 10250), λ_{max}^{pH7} 235 (10750), λ_{max}^{pH11} 238 (10250); ^{1}H NMR (Me_2SO-d_6) δ 1.45 (d, 3, CH_3), 2.08, 2.13 (s, 3, $COCH_3$), 3.85 (s, 3, CO_2CH_3), 4.45 (s, 2, CH_2), 6.05 (d, 1, $H_{1'}$, J = 5 Hz), 8.25 (s, 1, C_2H). Anal. ($C_{16}H_{19}N_3O_7$) C, H, N.

5-(Cyanomethyl)-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (9). A mixture of **5** (2.20 g, 6.02 mmol) and liquid ammonia (30 mL) was placed in a steel bomb (40 mL) and heated at 100 °C for 7 h. The ammonia was allowed to evaporate and the residue was subjected to a vacuum overnight to remove the last traces. The residue was absorbed on silica gel (5 g) and placed on a column of silica gel (100 g, packed in chloroform). Elution with chloroform–methanol (4:1) provided pure **9**. Recrystallization from methanol provided colorless cubes (1.42 g, 88%): mp 173–174 °C after drying at 100 °C for 3 h; $[\alpha]^{25}_D$ −47.8° (*c* 1, H_2O); UV λ_{max}^{pH1} 218 nm (ε 10000), λ_{max}^{pH7} 233 (10200), λ_{max}^{pH11} 234 (9730); ^{1}H NMR (Me_2SO-d_6) δ 1.35 (d, 3, CH_3), 5.66 (d, 1, $H_{1'}$, J = 5 Hz), 7.45 (d, 2, NH_2), 8.02 (s, 1, C_2H). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

6-Amino-1-(5-deoxy-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (14). A mixture of **9** (1.1 g, 4.13 mmol), aqueous sodium carbonate (5%, 7 mL), and ethanol (6 mL) was stirred under reflux for 0.75 h, treated with charcoal, and filtered through Celite. The light yellow, flocculant crystals were filtered and washed with water and then ethanol. Drying at 100 °C for 5 h provided 0.9 g (82%) of **14**: mp >310 °C; $[\alpha]^{25}_D$ −65.6° (*c* 1, 0.1 N NaOH); UV λ_{max}^{pH1} 284 nm (ε 13000), 308 sh (6920), λ_{max}^{pH7} 272 (12200), 299 (9150), λ_{max}^{pH11} 272 (12200), 295 sh (9150); ^{1}H NMR (Me_2SO-d_6) δ 1.36 (d, 3, CH_3, J = 6 Hz), 5.52 (s, 1, C_7H), 5.55 (d, 1, $H_{1'}$, J = 5 Hz), 5.75 (br s, 2, NH_2), 7.90 (s, 1, C_2H). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

Methyl 4-(Cyanomethyl)-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)imidazole-5-carboxylate (6) and Methyl 5-(Cyanomethyl)-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxylate (5). Compound 1 (3.0 g, 18.2 mmol) was refluxed under anhydrous conditions for 12 h with hexamethyldisilazane (50 mL) and ammonium sulfate (25 mg). The excess hexamethyldisilazane was removed by distillation

under reduced pressure, providing the trimethylsilyl derivative **2** as a yellowish brown oil. The oil was dissolved in dry 1,2-dichloroethane (100 mL). Blocked sugar **3** (4.73 g, 18.2 mmol) was added to the solution, followed by the addition of stannic chloride (1.06 mL, 9.1 mmol) in one portion. The reaction solution was stirred at room temperature for 48 h and then poured into a 7% sodium hydrogen carbonate solution (200 mL). The mixture was filtered through Celite and extracted with chloroform (3 × 50 mL), and the combined, dried ($MgSO_4$) extracts were evaporated under reduced pressure (50 °C) to a colorless oil (6.6 g). Column chromatography (250 g of silica gel packed in chloroform, eluted with ethyl acetate) provided, as the first isomer off the column, methyl 4-(cyanomethyl)-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)imidazole-5-carboxylate (**6**, 3.2 g 48%). Recrystallization from ethanol provided colorless needles: mp 123–124 °C after drying at 65 °C for 5 h; UV λ_{max}^{pH1} 234 nm (ε 8430), $\lambda_{max}^{pH7,11}$ 242 (11 000); ^1H NMR (Me_2SO-d_6) δ 1.45 (d, 3, C_5CH_3, J = 6 Hz), 2.12, 2.15 (s, 3, $COCH_3$), 3.85 (s, 3, CO_2CH_3), 4.2 (s, 2, CH_2), 6.39 (d, 1, $H_{1'}$, J = 3 Hz), 8.34 (s, 1, C_2H). Anal. ($C_{16}H_{19}N_3O_7$) C, H, N.

Further elution of the column with ethyl acetate provided methyl 5-(cyanomethyl)-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxylate (**5**, 1.7 g, 26%). This material was identical with the material prepared above.

6-Amino-3-(5-deoxy-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (12). A mixture of **6** (2.6 g, 7.13 mmol) and liquid ammonia (25 mL) was heated in a steel bomb (40 mL) for 2.5 h at 100 °C. The ammonia was allowed to evaporate at room temperature and the last trace removed by application of a vacuum overnight. The greenish brown residue was recrystallized from water (charcoal) to provide 1.3 g (68%) of **12** as light yellow microcrystals: mp decomposes >200 °C; $[\alpha]^{25}_D$ +29.3° (*c* 1.06, water); UV λ_{max}^{pH1} 277 nm (ε 11 700), 317 (5980), λ_{max}^{pH7} 258 (6500), 317 (7530), λ_{max}^{pH11} 258 (6500), 317 (7280); ^1H NMR (Me_2SO-d_6) δ 1.35 (d, 3, C_5CH_3, J = 6 Hz), 5.38 (s, 2, NH_2), 5.5 (s, 1, C_7H), 6.24 (d, 1, $H_{1'}$, J = 4 Hz), 10.62 (br s, 1, NH). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

Structure Proof for 9: Conversion of Imidazole Ribonucleoside 21 into Imidazole 5′-Deoxynucleoside 9. A. 5-(Cyanomethyl)-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide. A solution of **21** (1.41 g, 5 mmol), dry acetone (20 mL), and 2,2-dimethoxypropane (10 mL) was cooled in an ice bath and stirred as perchloric acid (70%, 280 mg) was added. The ice bath was removed and the reaction was allowed to stir at room temperature for 3 h. The reddish orange solution was adjusted to ca. pH 7 with 10% aqueous potassium hydroxide and evaporated in vacuo to a syrup which was dissolved in chloroform, filtered, and placed on a column of silica gel (45 g). Elution with chloroform–methanol (20:1) provided the isopropylidene derivative (1.35 g, 84%) as a white foam: ^1H NMR (Me_2SO-d_6) δ 1.39, 1.58 (s, 3, CH_3), 4.48 (s, 2, CH_2), 6.00 (d, 1, $H_{1'}$, J = 2 Hz), 7.35, 7.53 (s, 1, NH), 8.14 (s, 1, C_2H). Anal. ($C_{14}H_{18}N_4O_5$) C, H, N.

B. 5-(Cyanomethyl)-1-(2,3-*O*-isopropylidene-5-tosyl-β-D-ribofuranosyl)imidazole-4-carboxamide. To a cooled (5 °C) solution of the isopropylidene (0.6 g, 1.86 mmol) and dry pyridine (10 mL) was added, with stirring, *p*-toluenesulfonyl chloride (390 mg, 2.05 mmol). TLC [silica gel, chloroform–methanol (4:1)] indicated that some starting material was left after stirring 36 h at 5 °C. Additional *p*-toluenesulfonyl chloride (100 mg) was added and stirring continued further for 12 h. Crushed ice (0.5 g) was added and the solution evaporated in vacuo to a syrup. The syrup was coevaporated three times with 25 mL each of ethanol. Water was added (25 mL) and the mixture was extracted three times with 25 mL each of chloroform. The chloroform extracts were washed with 1 N H_2SO_4, dried ($MgSO_4$), and placed on a column of silica gel (20 g). Elution with chloroform–methanol (20:1) provided the tosyl derivative (0.62 g, 70%) as a white foam: ^1H NMR (Me_2SO-d_6) δ 1.37, 1.58 (s, 3, CH_3), 2.44 (s, 3, CH_3), 4.45 (s, 2, CH_2), 5.99 (d, 1, $H_{1'}$, J = 2.5 Hz), 8.04 (s, 1, C_2H). Anal. ($C_{21}H_{24}N_4O_7S$) C, H, N.

C. 5-(Cyanomethyl)-1-(5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carboxamide. A solution of the tosylate (1.40 g, 2.94 mmol), sodium iodide (1.5 g, 10.1 mmol), and 2-butanone (100 mL) was refluxed 2 h under a nitrogen atmosphere. The reaction mixture was cooled, filtered, and evaporated in vacuo to an orange amorphous solid. The residue was dissolved in chloroform, extracted with water (2 × 25 mL), and dried ($MgSO_4$). Removal of the solvent provided a syrup (1.2 g) which was dissolved in ether, treated with charcoal, and diluted with hexane until the cloud point was obtained. Cooling at 5 °C for 12 h provided 1.0 g (79%) of small white crystals: mp 124–125 °C (after drying at 80 °C for 5 h); ^1H NMR ($CDCl_3$) δ 1.40, 1.65 (s, 3, CH_3), 3.48 (d, 2, CH_2), 5.9 (d, 1, $H_{1'}$, J = 4 Hz), 7.83 (s, 1, C_2H). Anal. ($C_{14}H_{17}N_4O_4I$) C, H, N.

D. 5-(Cyanomethyl)-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (9). A mixture of the iodo compound (4.3 g, 10 mmol), sodium acetate (902 mg, 11 mmol), 10% Pd/C catalyst (1.5 g), and ethanol (200 mL) was hydrogenated at 50 psi at room temperature for 3 h. The mixture was filtered and the catalyst washed with hot ethanol. The filtrate was evaporated in vacuo, dissolved in ethyl acetate, and extracted with water (50 mL), saturated sodium hydrogen carbonate (50 mL), and water (50 mL). The dried ($MgSO_4$) ethyl acetate fraction was evaporated in vacuo to provide the deoxyisopropylidene-blocked nucleoside as a colorless foam (2.75 g, 90%). This foam was dissolved in formic acid (88%, 40 mL) and stirred at room temperature for 2 h. The solvent was evaporated in vacuo at 30 °C and the last traces of acid were removed by repeated coevaporation with water (30 °C). The residue was dissolved in methanol and treated with concentrated ammonium hydroxide until neutral. The solution was evaporated in vacuo and the residue crystallized from aqueous methanol to provide **9** (1.5 g, 63%) which is identical with **9** prepared by the alternate synthesis above.

Methyl 4-(Cyanomethyl)-1-(tetrahydropyran-2-yl)imidazole-5-carboxylate (8) and Methyl 5-(Cyanomethyl)-1-(tetrahydropyran-2-yl)imidazole-4-carboxylate (7). A mixture of **1** (12.4 g, 75.2 mmol), freshly distilled dihydropyran (15 mL), bis(*p*-nitrophenyl) phosphate (75 mg), and dry ethyl acetate (250 mL) was refluxed for 10 h. Additional dihydropyran (10 mL) and bis(*p*-nitrophenyl) phosphate (25 mg) were added, and reflux was continued for 5 h. Complete dissolution was obtained at 3 h of reflux. The light tan solution was evaporated in vacuo to a syrup which was dissolved in chloroform and placed on a column of silica gel (500 g) packed in chloroform. Elution with chloroform–ethyl acetate (1:1) provided 11.1 g (59%) of methyl 4-(cyanomethyl)-1-(tetrahydropyran-2-yl)imidazole-5-carboxylate (**8**) as a colorless syrup which crystallized on standing at room temperature overnight. Recrystallization from ligroine (bp 30–60 °C)–ether provided white rosettes: mp 82–83 °C; IR (KBr) 1720 (s, C=O), 2260 cm^{-1} (m, C≡N); UV λ_{max}^{pH1} 222 nm (ε 11 020), λ_{max}^{pH5} 242 (12 100), λ_{max}^{pH11} 242 (12 100); ^1H NMR (Me_2SO-d_6) δ 3.88 (s, 3, CH_3), 4.14 (s, 2, CH_2), 5.90 (m, 1, H_2), 8.22 (s, 1, C_2H). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

Further elution with chloroform–acetone (1:1) provided 6.2 g (33%) of methyl 5-(cyanomethyl)-1-(tetrahydropyran-2-yl)imidazole-4-carboxylate (**7**) as a clear syrup which crystallized on standing at room temperature overnight. Recrystallization from ligroine (bp 30–60 °C)–ether provided white needles: mp 98–99 °C; IR (KBr) 1718 (s, C=O), 2240 cm^{-1} (w, C≡N); UV λ_{max}^{pH1} 220 nm (ε 11 080), λ_{max}^{pH7} 240 (10 050), λ_{max}^{pH11} 242 (9820); ^1H NMR (Me_2SO-d_6) δ 3.85 (s, 3, CH_3), 4.40 (s, 2, CH_2), 5.51 (m, 1, H_2), 8.08 (s, 1, C_2H). Anal. ($C_{12}H_{15}N_3O_3$) C, H, N.

5-(Cyanomethyl)-1-(tetrahydropyran-2-yl)imidazole-4-carboxamide (10). Compound **7** (0.60 g, 2.41 mmol) and liquid ammonia (10 mL) were placed in a steel bomb (40 mL). The bomb was three-quarters submerged in a steam bath and heated for 3 h. At this point, TLC [chloroform–methanol (9:1), silica gel] indicated complete conversion of the starting material to the carboxamide and a trace of 3-deaza-9-(tetrahydropyran-2-yl)-guanine (**15**). The ammonia was allowed to evaporate at room temperature, and the residue was subjected to a vacuum overnight to remove the last traces of ammonia. The greenish brown residue was recrystallized from methanol (charcoal) to provide 0.4 g (71%) of **10** as faint yellow needles: mp 198–199 °C after drying at 100 °C for 3 h; IR (KBr) 1685 (s, C=O), 2250 (w, C≡N), 3140 and 3300 cm^{-1} (2 s, NH_2); ^1H NMR (Me_2SO-d_6) δ 4.50 (s, 1, CH_2), 5.45 (m, 1, H_2), 7.35 (s, 1, NH), 7.53 (s, 1, NH), 8.03 (s, 1, C_2H). Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.

6-Amino-1-(tetrahydropyran-2-yl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (15). A mixture of **10** (702 mg, 3 mmol), aqueous sodium carbonate solution (5%, 6 mL), and ethanol (10 mL) was

refluxed 20 min. Complete dissolution was obtained as reflux began. The product precipitated as reflux was continued. The suspension was cooled and filtered, and the residue was washed thoroughly with water and then ethanol (690 mg, 97%). Recrystallization from a large volume of ethanol–water (1:1) provided white needles: mp 268–269 °C dec after drying at 100 °C for 5 h; UV λ_{max}^{pH1} 285 nm (ϵ 13450), 313 sh (6950), λ_{max}^{pH7} 270 (12600), 300 (9350), λ_{max}^{pH11} 272 (12400), 295 (9130); ^{1}H NMR (Me_2SO-d_6–NaOD) 5.22 (m, 1, H_2), 5.58 (s, 1, C_6H), 7.68 (s, 1, C_2H). Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.

6-Amino-3-(tetrahydropyran-2-yl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (13). A mixture of 8 (2.49 g, 10 mmol) and liquid ammonia (20 mL) was placed in a steel bomb (40 mL) and heated at 100 °C (oil bath) for 5 h. The ammonia was allowed to evaporate at room temperature and the residue subjected to a vacuum overnight. Recrystallization from methanol (charcoal) provided 13 (1.1 g, 47%) as small off-white needles: mp 208 °C dec after drying at 100 °C for 2 h; UV λ_{max}^{pH1} 275 nm (ϵ 12300), 317 (6140), λ_{max}^{pH7} 257 (6800), 315 (7680), λ_{max}^{pH11} 257 (6580), 315 (7450); ^{1}H NMR (Me_2SO-d_6) δ 5.38 (s, 2, NH_2), 5.58 (s, 1, C_7H), 6.05 (m, 1, H_2), 8.22 (s, 1, C_2H), 10.62 (s, 1, NH). Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.

5-(Cyanomethyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (11). A suspension of 21 (1.41 g, 5 mmol), acetic anhydride (15 mL), and *p*-(dimethylamino)pyridine (20 mg) was stirred at room temperature for 8 h. TLC [silica gel, $CHCl_3$–MeOH (4:1)] indicated three products. Additional *p*-(dimethylamino)pyridine (20 mg) and acetic anhydride (5 mL) were added and stirring was continued at room temperature for 36 h. The solution was evaporated in vacuo to a syrup which was dissolved in chloroform and placed on a column of silica gel (60 g). Elution with chloroform–methanol (20:1) provided the triacetate (2.0 g, 95%) as a white foam: ^{1}H NMR (Me_2SO-d_6) δ 2.08, 2.10, 2.16 (s, 3, $COCH_3$), 4.40 (s, 2, CH_2), 6.13 (d, 1, $H_{1'}$, J = 5 Hz), 7.39, 7.58 (s, 2, NH), 8.19 (s, 1, C_2H). Anal. ($C_{17}H_{20}N_4O_8$) C, H, N.

6-Acetamido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (16). A mixture of 22[2] (1.4 g, 4.97 mmol), acetic anhydride (13 mL), and dry pyridine (20 mL) was stirred at room temperature for 3.5 h. The solution was evaporated in vacuo and the residue coevaporated three times with water. Recrystallization of the residues from ethanol provided beige needles (1.5 g, 67%): mp 241–242 °C dec after drying at 100 °C for 5 h; UV λ_{max}^{pH1} 277 nm (ϵ 12700), 297 (13400), λ_{max}^{pH7} 268 (13200), 299 (12300), λ_{max}^{pH11} 223 (13700), 285 (10800); ^{1}H NMR (Me_2SO-d_6) δ 1.15 (s, 9, $COCH_3$), 6.19 (d, 1, $H_{1'}$, J = 5 Hz), 6.58 (s, 1, C_7H), 8.25 (s, 1, C_2H), 10.70 (br s, 1, NH), 11.5 (br s, 1, NH). Anal. ($C_{19}H_{22}N_4O_9$) C, H, N.

6-Acetamidoimidazo[4,5-*c*]pyridin-4(5*H*)-one (17). A mixture of 20[2] (0.5 g, 3.33 mmol), acetic anhydride (12 mL), and concentrated phosphoric acid (1 drop) was refluxed 0.5 h. The solution was evaporated in vacuo to provide a green residue. This was coevaporated with toluene (2 × 25 mL each), treated with ice (~50 mL), and stirred for 1 h. The precipitate was filtered and washed with water and then ether. TLC of the yellow residue indicated two products [silica gel, chloroform–methanol (4:1)]. The residue was dissolved in methanol (25 mL) and concentrated ammonium hydroxide (2.5 mL) and stirred for 15 min. The suspension was filtered and washed with water, methanol, and then ether. The beige residue was crystallized from aqueous methanol to provide 17 (0.5 g, 78%) as off-white microcrystals: mp >340 °C (after drying at 100 °C for 5 h); UV λ_{max}^{pH1} 278 nm (ϵ 13700), λ_{max}^{pH7} 261 (12410), 272 sh (12040), 292 (9150), λ_{max}^{pH11} 276 (11110); ^{1}H NMR (CF_3CO_2D) δ 2.43 (s, 3, CH_3), 6.98 (s, 1, C_7H), 9.18 (s, 1, C_8H). Anal. ($C_8H_8N_4O_2$) C, H, N.

6-[*N*,*N*-(Dimethylamino)methyleneamino]-1-(β-D-ribofuranosyl)imidazo[4,5-*c*]pyridin-4(5*H*)-one (18). A suspension of 22[2] (564 mg, 2 mmol), dimethylformamide dimethyl acetal (1.19 g, 10 mmol), and dimethylformamide (10 mL) was heated with stirring at 80 °C for 6 h. The deep red solution was evaporated in vacuo to a thin syrup, and ethanol (15 mL) was added, followed by ether, until the cloud point was obtained. Cooling overnight at 0 °C provided 450 mg of brown microcrystals (washed with cold ethanol). An additional 200 mg of brown microcrystals of the same purity was obtained from a second crop. The total yield of the (dimethylamino)methylene derivative of 3-deazaguanosine was 650 mg (96%). An analytical sample was obtained by dissolving a portion in hot dimethylformamide and adding ether to the cooled solution until the cloud point was obtained. Cooling at 0 °C overnight provided 18 as yellow microcrystals: mp 243–245 °C dec (after drying at 100 °C for 5 h); $[\alpha]^{25}_D$ −78.9° (*c* 1, DMF); UV λ_{max}^{pH1} 275 nm sh (ϵ 13100), 290 (14100), λ_{max}^{pH7} 230 (14100), 312 (18650), λ_{max}^{pH11} 233 (13450), 312 (18650); ^{1}H NMR (Me_2SO-d_6) δ 2.98 (s, 3, CH_3), 3.08 (s, 3, CH_3), 5.70 (d, 1, $H_{1'}$, J = 6 Hz), 6.15 (s, 1, C_7H), 8.05 (s, 1), 8.11 (s, 1), 10.7 (br s, 1, NH). Anal. ($C_{14}H_{19}N_5O_5$) C, H, N.

Possible Interconversion between 19, 20, and 21 and 22 in Cell Culture. TLC Study. Compounds 19, 20, 22, and 21 were dissolved separately in cell culture growth media (minimum essential medium plus 1090 fetal bovine serum) at concentrations of 1000 μg/mL, placed on monolayers of KB cells, and incubated at 37 °C for 18 h. Samples of the supernatant fluid and lysates of the washed KB cells were concentrated 10-fold in vacuo (30 °C) and spotted on 5 × 10 cm silica gel plates.[20] Development was done in three systems: methanol–ethyl acetate (4:1); isopropyl alcohol–ammonium hydroxide–water (7:1:2); and acetonitrile–ammonium chloride (0.2 M) (3:1). Each sample of supernatant fluid contained only a single detectable compound which was identical with the original standard.[20] There were no detectable intracellular compounds as determined by TLC analysis of the KB cell lysates.

References and Notes

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(2) P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr., and R. K. Robins, *J. Am. Chem. Soc.*, **98**, 1492 (1976); P. D. Cook, R. J. Rousseau, A. M. Mian, R. B. Meyer, Jr., P. Dea, G. Ivanovics, D. G. Streeter, J. T. Witkowski, M. G. Stout, L. N. Simon, R. W. Sidwell, and R. K. Robins, *ibid.*, **97**, 2916 (1975).

(3) (a) D. G. Streeter and H. H. Koyama, *Biochem. Pharmacol.*, **25**, 2413 (1976); (b) P. Schwartz, D. Hammond, and T. J. Khwaja, *Proc. Am. Assoc. Cancer Res.*, **18**, 153 (1977).

(4) (a) L. B. Allen, J. H. Huffman, P. D. Cook, R. B. Meyer, Jr., R. K. Robins, and R. W. Sidwell, *Antimicrob. Agents Chemother.*, **12**, 114 (1977); (b) R. W. Sidwell, L. B. Allen, J. H. Huffman, J. T. Witkowski, P. D. Cook, R. L. Tolman, G. R. Revankar, L. N. Simon, and R. K. Robins, *Chemotherapy*, **6**, 279 (1976).

(5) T. A. Khwaja, L. Kigwana, R. B. Meyer, Jr., and R. K. Robins, *Proc. Am. Assoc. Cancer Res.*, **16**, 162 (1975); T. A. Khwaja and J. Varven, *ibid.*, **17**, 200 (1976); A. M. Mian and T. A. Khwaja, 2nd Joint Conference of CIC/ACS, Medicinal Chemistry Division, Montreal, Canada, May 1977, Abstract No. 15.

(6) (a) T. R. Matthews, D. W. Yotter, P. D. Cook, R. W. Sidwell, R. K. Robins, and P. F. Dougherty, 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 1976, Abstract No. 425; (b) T. R. Matthews, P. F. Dougherty, P. D. Cook, R. W. Sidwell, R. K. Robins, and D. W. Yotter, in ref 6a, Abstract No. 426.

(7) P. D. Cook and R. K. Robins, *J. Org. Chem.*, **43**, 289 (1978).

(8) Steric hindrance to the 5-carboxymethoxy group in **6** and **8** might be such that, alternatively, the 4-(cyanomethyl) group is first *aminated* to the amidinomethyl group which may be a better nucleophilic cyclizing moiety than the 4-carboxamide moiety in **9**, **10**, and **21**.

(9) J. Zemlicka and J. Holy, *Collect. Czech. Chem. Commun.*, **32**, 3159 (1967).

(10) (a) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971); (b) R. W. Sidwell in "Chemotherapy of Infectious Diseases", H. H. Gadebusch, Ed., CRC Press, Cleveland, Ohio, 1976, p 31.

(11) J. T. Witkowski, R. K. Robins, R. W. Sidwell, and L. B. Simon, *J. Med. Chem.*, **15**, 1150 (1972); R. W. Sidwell, J.

H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, 177, 705 (1972). Ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, is the name approved by the U.S. Adopted Names Council for this drug; Virazole is the ICN Pharmaceuticals, Inc., trademark.
(12) F. F. Snyder, J. F. Henderson, and D. A. Cook, *Biochem. Pharmacol.*, 21, 2351 (1972); J. F. Henderson, C. M. Smith, F. F. Snyder, and G. Zombor, *Ann. N.Y. Acad. Sci.*, 255, 489 (1975).
(13) (a) D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 1174 (1973); (b) D. G. Streeter, J. P. Miller, R. K. Robins, and L. N. Simon, *Ann. N.Y. Acad. Sci.*, 284, 201 (1977); (c) J. P. Miller, L. J. Kigwana, D. G. Streeter, R. K. Robins, L. N. Simon, and J. Roboz, *ibid.*, 284, 211 (1977).
(14) C. M. Smith, L. J. Fontenelle, H. Muzik, A. R. Paterson, H. Unger, L. W. Brox, and J. F. Henderson, *Biochem. Pharmacol.*, 23, 2727 (1974).
(15) G. R. Revanker, T. R. Matthews, and R. K. Robins, *J. Med. Chem.*, 18, 1253 (1975).
(16) (a) F. B. Rudolph and H. J. Fromm, *J. Biol. Chem.*, 244, 3832 (1969); (b) J. G. Flaks, M. J. Erwin, and J. M. Buchanan, *ibid.*, 229, 603 (1957).
(17) J. T. Witkowski, D. G. Streeter, and R. K. Robins, *J. Med. Chem.*, in press.
(18) S. C. Hartman in "Metabolic Pathways", Vol. IV, 3rd ed., D. M. Greenberg, Ed., Academic Press, New York, N.Y., 1970, p 1.
(19) L. N. Lukens and J. Flaks, *Methods Enzymol.*, 6, 696 (1963).
(20) Merck high-performance thin-layer chromatography plates (HP-TLC silica gel 10 F-254) which detect ca. nanogram quantities via UV were employed. One microliter of a millimole solution of **19**, **20**, **22**, and **21** was used as standard.

Prodrugs of 9-β-D-Arabinofuranosyladenine. 1. Synthesis and Evaluation of Some 5′-(*O*-Acyl) Derivatives[1]

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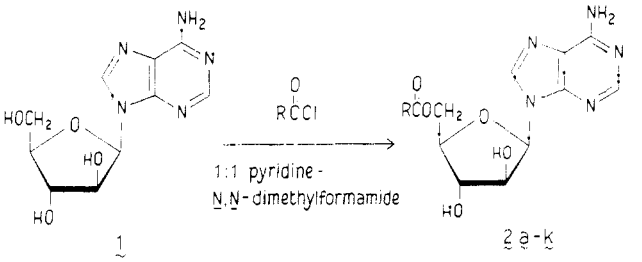
A number of 5′-(*O*-acyl) derivatives of 9-β-D-arabinofuranosyladenine (*ara*-A, VIRA-A) (**2a–k**) were prepared by direct acylation of the parent nucleoside **1** in pyridine–*N*,*N*-dimethylformamide. These compounds, designed as prodrugs for **1**, offer a range of solubilities and lipophilicities indicating for several examples improved solubility and the potential for improved membrane transport over **1**. All are resistant to deactivation by adenosine deaminase. Of special interest is the 5′-(*O*-valeryl) derivative **2d** that shows a marked increase in antiviral activity over **1**.

The potent antiviral nucleoside 9-β-D-arabinofuranosyladenine (**1**) (*ara*-A, vidarabine, VIRA-A) has shown activity[2] against certain DNA viruses such as herpes, varicella, and cytomegaloviruses and has demonstrated clinical utility as a topical agent for herpes keratitis of the eye.[3] Most dramatically, however, the drug has been shown useful as a highly effective, relatively nontoxic, systemic agent to treat the usually fatal herpes encephalitis.[4] In an impressive, placebo-controlled study of biopsy-proven cases of the illness, the mortality rate was lowered[4] from 70 to 28%. Despite its proven efficacy, **1** does suffer from a number of limitations: (1) a low aqueous solubility[5] of ca. 0.4 mg/mL at 25 °C that poses a severe restraint to parenteral administration of the drug; (2) a ready deamination by adenosine deaminase to give 9-β-D-arabinofuranosylhypoxanthine (*ara*-Hx), the chief metabolite which possesses low-level antiviral activity;[6] and (3) a low lipophilicity that precludes the use of **1** as a topical agent for treating genital, oral, and other cutaneous herpes infections.

In an attempt to overcome these problems which manifest themselves as difficulties with formulation, delivery, and topical application, as well as with a lack of resistance to enzymic deactivation in vivo, a series of 5′-monoesters of **1** (**2a–k**) was synthesized. It was anticipated that such acyl derivatives would serve to counteract the various intra- and/or intermolecular hydrogen-bonding forces that are presumably responsible for rendering the arabino nucleoside far less soluble than adenosine, its ribo counterpart.[7] Compounds **2a–k** would be predicted to have a less compact crystal structure and thereby show lower melting points. The result is an increase in free energy within the crystalline matrix that would give rise to an expected increase in solubility that is desired for a parenteral drug. The gains in lipophilicity would be of an advantage in obtaining compounds that would be more prone to traverse biological membranes and skin layers, and thereby one might possibly gain insight into designing drugs that would be suited for topical application for treatment of cutaneous herpes infections. Such rationale has been followed in prodrug design for a variety of pharmaceuticals,[8,9] and this concept has been applied to prodrugs for the nucleoside antileukemic 3-β-D-arabinofuranosylcytosine (*ara*-C)[10,11] and, albeit with limited success, to **1**, as shown in certain 5′-esters,[12,13] as well as with tri-*O*-acyl derivatives[14] of **1**.

Scheme I



Chemistry. Direct acylation of the free nucleoside **1** was effected by adding 1.1 equiv of the appropriate acyl chloride to a suspension of the nucleoside in 1:1 pyridine–*N*,*N*-dimethylformamide (see Scheme I). It is to be pointed out that the solvent–base combination was found to greatly facilitate the selectivity of the acylation of the primary hydroxyl group over either of the secondary alcoholic groups. This result is apparently a consequence of having as the acylating reagent a charged species (i.e., *N*-acylpyridinium chloride) in an aprotic, nonpolar solvent such as *N*,*N*-dimethylformamide.[15,16] By maintaining ice-bath temperatures, the 5′-acylated nucleosides **2a–k**

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