4 $N_2\beta$ in all respects: yield 8%. Anal. ($C_{27}H_{23}N_5O_7$) C, H, N.

5-Amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (5) and 5-Amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (6). The deprotected ribonucleosides **5** and **6** were obtained by treating the tribenzoylated precursors **3** and **4** with methanolic ammonia for 3 days at 5°. After evaporation of the solvent the oil was taken up in water and extracted with ether. The aqueous layer was concentrated to an oil. Whereas compound **5** was crystallized easily from acetone (yield 90%), compound **6** (after one preparative TLC purification) crystallized extremely slowly when the oil was allowed to stand at −30° (yield 25%). Compound **5**, mp 160–161°, and compound **6**, mp 98–99°, were assigned $N_1\beta$ and $N_2\beta$ configuration, respectively (by NMR and x-ray crystallography). Anal. ($C_6H_{11}N_5O_4$) C, H, N.

Acknowledgment. We are grateful to Dr. T. Williams for discussions on the proton and ^{13}C NMR spectra and to Dr. F. Scheidl for microanalysis.

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Synthesis and Antiviral and Antimicrobial Activity of Certain 1-β-D-Ribofuranosyl-4,5-Disubstituted Imidazoles

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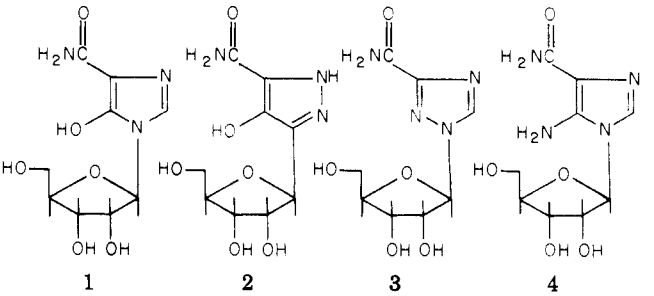
Starting with AICA ribonucleoside the following nucleosides were prepared. Methyl 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**5**) was converted into methyl 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**6**) via diazotization in the presence of cuprous chloride. Similarly, 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**9**) was converted into 5-chloro-, 5-bromo-, and 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile derivatives. These 5-halogenated imidazole nucleosides were treated with several nucleophiles such as ammonia, hydroxylamine, and hydrogen sulfide to provide, respectively, 5-haloimidazole-4-carboxamide, 5-haloimidazole-4-carboxamidoxime, and 5-haloimidazole-4-thiocarboxamide ribonucleosides. 5-Chloro- or 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile was treated with potassium hydrosulfide to yield 5-mercapto-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**16**). The catalytic reduction of 5-chloro- or 5-bromo-1-β-D-ribofuranosylimidazole-4-carboxamidoxime provided 1-β-D-ribofuranosylimidazole-4-carboxamidines as their hydrochloride and hydrobromide salts, respectively. These nucleosides were tested for in vitro antiviral, antifungal, and antibacterial activity. The 5-halo analogues of 1-β-D-ribofuranosylimidazole-4-carboxamide showed significant antiviral activity whereas compound **16** was found inhibitory to fungi.

The nucleosides of certain five-membered heterocycles such as pyrazomycin,[1,2] showdomycin,[3] and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[4] have exhibited a broad spectrum of biological activities. These properties have directed our attention to the synthesis of various imidazole nucleosides, especially in view of the potent antiviral activity of Virazole.[4] The synthesis of certain novel imidazole nucleosides has previously been reported from this laboratory.[5-8] The recently reported nucleoside antibiotic bredinin[9] (**1**) and the structural similarity of pyrazomycin (**2**) and Virazole (**3**) have suggested further modifications at the 5 position of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide[10] (AICA ribonucleoside, **4**). Modifications reported to date involve the 5-amino,[10] 5-nitro,[11] and 5-triazino[12] groups. Substituents such as chlorine, bromine, iodine, and sulfur at position 5 have not yet been reported. The synthesis of these novel analogues via diazotization of AICA ribonucleoside derivatives and

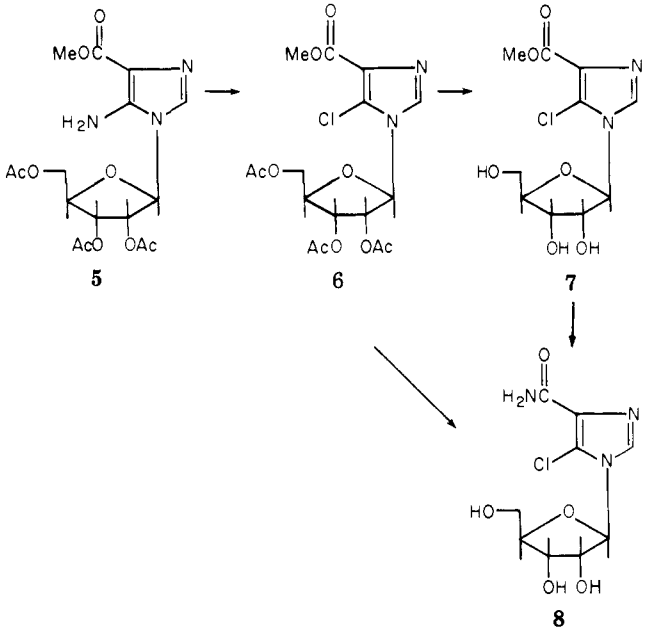


1 **2** **3** **4**

their biological evaluation is the subject of the present study.

The synthesis and the utility of methyl 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**5**) in diazotization reactions have been reported earlier.[8] A cold (−25 ± 3°) solution of **5** in 6 N hydrochloric acid

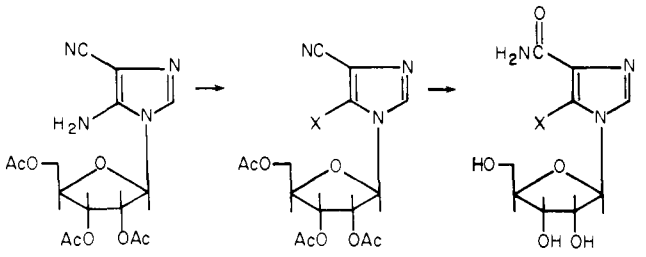
Scheme I



diazotized with sodium nitrite in the presence of cuprous chloride at −22° gave a 40% yield of methyl 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (**6**) as a syrup purified by silica gel column chromatography. Treatment of **6** with methanolic ammonia readily provided a crystalline compound which was characterized as methyl 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxylate (**7**) on the basis of elemental analysis. The prolonged ammonolysis (4–5 days) of **6** or **7** at ambient temperature yielded the desired product 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (**8**) in 70% yield (Scheme I).

The electronic character of the substituent at the 4 position should have a marked effect on the chemical reactivity of the diazonium group and, therefore, similar studies with 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile[13] (**9**) were also investigated. A concentrated solution of **9** in methanol was treated with sodium nitrite at −22° in the presence of 6 N hydrochloric acid. Addition of cuprous chloride was followed by immediate evolution of nitrogen gas to provide 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**10**) in 42% yield. The synthesis of compound **10** was also achieved in comparable yields (40–45%) when cuprous chloride was replaced by cuprous iodide in the above experiment. The corresponding 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**11**) was synthesized similarly when **9** was diazotized using sodium nitrite in 47–49% hydrobromic acid and the resulting solution was treated with cuprous bromide or cuprous iodide. In these reactions the significant formation (10–15%) of 1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**12**) as a side product was also observed. This hydrogenodediazoniation probably arises via the formation of an aryl (imidazolyl) radical which can abstract a proton from the solvent. The low-temperature diazotization reactions of the carbonitrile **9** were also studied in 48–50% fluoboric acid using sodium nitrite. Decomposition of the diazonium fluoborate salt of **9** by the addition of cuprous iodide provided 5-iodo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**13**) in 10% yield. When cuprous iodide was replaced by cuprous chloride or cuprous bromide, the corresponding chloro (**10**) and bromo (**11**) derivatives were

Scheme II



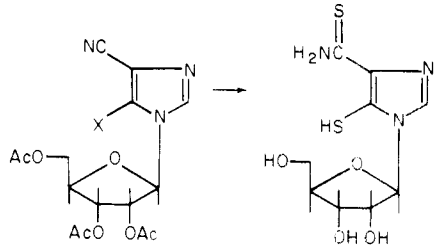
formed respectively in 10–15% yields.

The structure of compound **12** was determined by rigorous comparison with an authentic sample synthesized earlier in this laboratory.[8] Hydrolysis of the chloro derivative **10** with hydrogen peroxide in ammonium hydroxide provided 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (**8**) which was identical with that obtained from **6**. The corresponding bromo and iodo derivatives (**11** and **13**) were hydrolyzed similarly to provide 5-bromo-1-β-D-ribofuranosylimidazole-4-carboxamide (**14**) and 5-iodo-1-β-D-ribofuranosylimidazole-4-carboxamide (**15**), respectively (Scheme II).

It could be expected in 5-haloimidazole-4-carbonitrile nucleosides that the electron-withdrawing character of halo and nitrile groups would activate the 5-carbon and the nitrile bond toward nucleophilic attack. In view of the ready availability of chloro and bromo derivatives (**10** and **11**), the reactions of these nucleosides with various nucleophiles were also studied. Thus, the treatment of **10** or **11** with an excess of hydrogen sulfide and potassium hydrosulfide in methanol at 90° provided 5-mercapto-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**16**) in 70–75% yield. The structure of **16** was evident from its ultraviolet spectrum, ^{1}H NMR, spray test,[14] and elemental analysis. When compounds **10** and **11** were treated respectively with anhydrous potassium hydrosulfide in boiling ethanol, compound **16** was isolated as the only reaction product. These results suggest the simultaneous attack of the sulfide ion at the 5-carbon and the carbon of the nitrile group. However, under mild and more carefully controlled reaction conditions, when the chloro or bromo derivative (**10** or **11**) was treated with hydrogen sulfide in pyridine in the presence of triethylamine at room temperature, the 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**17**) and 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**18**) were obtained, respectively. The presence of 5-mercapto-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**19**) was also observed as detected by TLC and uv absorption studies. Compounds **17** and **18** were isolated by silica gel column chromatography and identified on the basis of uv and spray test.[14] The deacetylation of **17** and **18** in the presence of ammonium hydroxide provided 5-chloro-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**20**) and 5-bromo-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**21**), respectively (Scheme III).

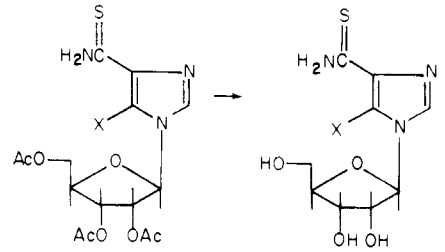
Hydroxylamine is another nucleophile which reacted readily with **10** in boiling ethanol to provide 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamidoxime (**22**) as a crystalline product. Though some deacetylation was observed, there was no evidence for the replacement of chlorine. Under similar conditions the bromo derivative **11** was converted into the corre-

Scheme III



10, X = Cl
11, X = Br

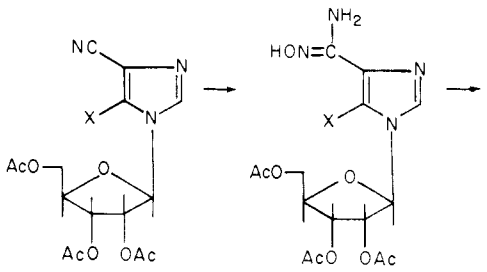
16



17, X = Cl
18, X = Br
19, X = SH

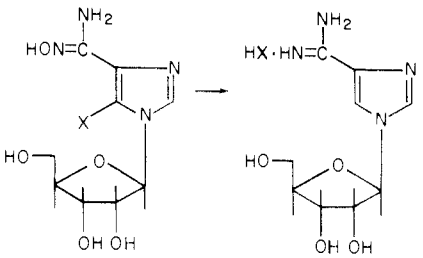
20, X = Cl
21, X = Br

Scheme IV



10, X = Cl
11, X = Br

22, X = Cl
23, X = Br



24, X = Cl
25, X = Br

26, X = Cl
27, X = Br

sponding 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamidoxime (**23**). The deacetylation of **22** and **23** in ammonium hydroxide readily provided 5-chloro-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (**24**) and 5-bromo-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (**25**), respectively, as crystalline products in 70–75% yield. Compound **24** was also subjected to hydrogenation under pressure in the presence of Raney nickel catalyst. In this experiment the reduction of amidoxime group and dehalogenation occurred simultaneously and 1-β-D-ribofuranosylimidazole-4-carboxamidine hydrochloride (**26**) was isolated as the final product. The dehalogenation was confirmed by ^{1}H NMR which showed the presence of two (C_2 and C_5) protons in the aromatic region. The structure of **26** was also confirmed by comparison with an authentic sample which was previously

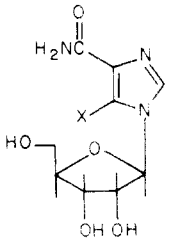
Table I. Comparative in Vitro Antiviral Activity

Compd	Virus rating			
	HV/1	VV	RV/13	PIV/3
3[a]	0.8-1.2	0.6-1.0	0.6-1.0	0.8-1.2
8	0.6-0.8	0.5-0.6	0.4-0.9	0.2
14	0.7-0.8	0.5	0.4-0.5	0.1
15	0.6-0.7	0.3-0.4	0.0	0.1
28	0.9		0.4	0.3
29[a]	0.7-1.1	0.3-0.6	0.3-0.6	0.2-0.4

[a] The antiviral data of these compounds were recorded in this study under similar conditions for comparison purposes.

reported from this laboratory.[8] Similar results were obtained when the bromo derivative **25** was similarly hydrogenated and the corresponding 1-β-D-ribofuranosylimidazole-4-carboxamidine hydrobromide (**27**) was isolated (Scheme IV).

Biological Activity. This series of imidazole nucleosides including 1-β-D-ribofuranosylimidazole-4-carboxamide[8] (**28**) has exhibited interesting in vitro activity against DNA and RNA viruses and demonstrates an excellent structure–activity relationship with Virazole and the recently reported 5-fluoro-1-β-D-ribofuranosylimidazole-4-carboxamide[15] (**29**) where the presence of a



28, X = H
29, X = F

carboxamide group at the 4 position appeared necessary and certain substitutions at the 5 position further aided antiviral efficacy. Hydrogen (compound **28** in which there is an isosteric replacement of N-2 of the triazole ring in Virazole) and fluorine substitutions particularly exhibited the most broad-spectrum efficacy but were less active as compared to Virazole. It is known that halogen in the 5 position of certain pyrimidines is responsible for the specific physical and biological properties of those compounds; the increased efficacy of fluorine over the other halogens, similar to what is observed in our studies, has been seen in those pyrimidines as well.[16]

It is interesting to note that 5-mercapto-1-β-D-ribofuranosylimidazole-4-thiocarboxamide inhibited, in vitro, the growth of certain microorganisms and, unlike antiviral activity, the replacement of the 4-thiocarboxamide group with a carboxamide and/or 5-mercapto with a halogen resulted in reduced or loss of in vitro antimicrobial activity.

Antiviral Evaluation. Inhibition of virus-induced cytopathic effects (CPE) was used as the indictor of antiviral activity. Cultures of human carcinoma of nasopharynx (KB) cells were grown in disposable plastic microplates.[17] All monolayers (24 h) were exposed to 320 $CCID_{50}$ of virus and a concentration of each compound ranging from 1000 to at least 1 μg/ml was added within 15 min. CPE development was observed after a 72-h incubation at 37°. As described previously,[17] the degree of CPE inhibition and compound cytotoxicity were scored numerically and used in calculating a virus rating (VR). In terms of VR's, the degree of activity is as follows: <0.5, slight or no activity; 0.5–0.9, moderate activity; and ≥1.0, marked activity. Viruses used in this study were type 1

Table II. Inhibition of Purine Nucleotide Biosynthesis in Ehrlich Ascites Tumor Cells in Vitro[a]

Compd	% inhibition: Adenine nucleotides (AMP + ADP + ATP)	% inhibition: Guanine nucleotides (GMP + GDP + GTP)
3	14	65
8	23	53
14	15	60
15	0	21
16	0	34
28	16	63
29	0	40

[a] Approximately 6×10^6 cells/ml were incubated at 37°, 20 min, with and without 1 mM of the test compounds. Hypoxanthine-^{14}C (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.[20]

herpes virus (HV/1), type 13 rhino virus (RV/13), type 3 parainfluenza virus (PIV/3), and vaccinia virus (VV). In vitro antiviral activity of certain imidazole nucleosides and comparative VR values of Virazole and compound **29** are shown in Table I. Of the compounds tested, only **8**, **14**, **15**, and **28** had activity against the DNA viruses, herpes and vaccinia. Two of these four, **8** and **14**, had slight activity against the RNA virus RV/13, but none inhibited PIV/3. Although the herpes activity was moderate, the compounds were not considered potent enough for clinical development.

Biochemical Evaluation. Virazole, which has been used in these studies for comparative antiviral evaluation, is a potent inhibitor of the biosynthesis of guanine nucleotides.[18,19] Furthermore, this inhibition has been linked to the possible mechanism of antiviral action of this compound. It was, therefore, of interest to test the closely related compounds reported in these studies as possible inhibitors of purine nucleotide biosynthesis utilizing the procedure of Snyder et al.[20] In vitro suspensions of Ehrlich ascites tumor cells are incubated with [^{14}C]hypoxanthine (0.1 mM) without and with test compounds (1.0 mM). The incorporation of radioactivity into adenine and guanine nucleotide is then measured by thin-layer chromatography of the acid-soluble cell fraction. The inhibition of this incorporation is determined by comparing the radioactivity recovered in the untreated and compound-treated incubations. Table II demonstrates that Virazole (**3**) is a specific inhibitor of guanine nucleotide biosynthesis as previously reported.[19] Of the four compounds demonstrating significant antiviral activity in Table I (**8**, **14**, **15**, and **28**), three of these compounds showed significant inhibition (>50%) of guanine nucleotide biosynthesis in this assay (**8**, **14**, and **28**, Table II). These results alone do not by any means define the mode of action of these compounds but do point out that regulation of this important pathway to nucleic acid biosynthesis may have chemotherapeutic value.

Antimicrobial Evaluation. These imidazole nucleosides were tested against various microorganisms in defined medium using broth dilution technique.[21] In vitro, 5-mercapto-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (**16**) had broad-spectrum antifungal activity inhibiting not only *Candida albicans* (av MIC, 40 μg/ml) but also isolates of *Trichophyton*, *Microsporum*, and *Epidermophyton* (av MIC, 20 μg/ml). In vitro inhibition of these pathogens by compound **16** was not reversed by the presence of serum, protein, or nucleosides in the growth medium, but organisms grown on medium containing whole blood were much less sensitive to this compound (Table III).

Table III. Comparative in Vitro Antimicrobial Activity MIC,[a] μmol/ml

Compd	*Staph. aureus*	*C. albicans*	*T. mentagrophytes*
8	Not active	0.3	Not active
14	Not active	0.15	Not active
16	0.05	0.05	0.02
20	Not active	Not active	0.3
21	0.3	Not active	Not active

[a] Activity MIC = minimal inhibitory concentration, range 0.4–0.005 μmol/ml.

To evaluate the in vivo therapeutic effect of **16**, a guinea pig skin infection model was used.[22] This nucleoside was formulated in either polyethylene glycol "300" or in propylene glycol (20%), 70% sorbitol (3%), and ethyl alcohol to 100 ml at a 5% (w/v) concentration.

Topical treatment of skin infections caused by *Trichophyton mentagrophytes* twice daily for 8 days with either preparation of **16** resulted in only a slight visible reduction of lesion intensity as compared with placebo-treated lesions. Cultures of infected skin removed from treated lesions showed that viable *T. mentagrophytes* cells remained in these tissues.

When skin infected with *Trichophyton* (placebo treated) was placed in broth concentrations of **16** was high as 100 μg/ml, outgrowth of the infecting organisms still occurred. The physiological state of the pathogen may be an important factor in sensitivity or resistance to compound **16** since this organism is inhibited when not growing in tissue.

Experimental Section

The physical properties were determined with the following instruments: melting points, Thomas-Hoover apparatus (uncorrected); ir, Perkin-Elmer Model 257 spectrophotometer (KBr); uv spectra, Cary 15 uv spectrophotometer (pH 1 and 11); specific rotation, Perkin-Elmer Model 141 polarimeter. The ^{1}H NMR spectra were determined on a Hitachi Perkin-Elmer Model R-20A spectrometer using DSS as an internal standard. The presence of exchangeable protons was confirmed by ^{1}H NMR spectroscopy in absolute Me_2SO-d_6 by exchange with D_2O followed by reintegration. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., or Heterocyclic Chemical Corp., Harrisonville, Mo. Where analyses are indicated by only symbols of the elements, the analytical results for those elements were within ±0.4% of the theoretical value.

Woelm silica gel (0.063–0.2 mm) purchased from ICN Life Science Group, Cleveland, Ohio, was used for column chromatography and Woelm TLC plates (silica gel F_{254}) were used to check the homogeneity of the compounds. Short-wave ultraviolet light (mineralite UVS 11) was used to detect the spots and the chromatographic solvent systems used were A, 40% (v/v) chloroform in ethyl acetate; B, ethyl acetate–chloroform–acetone, 5:3:2 (v/v); C, ethyl acetate–1-propanol–water (4:1:2, v/v; top layer). The spray test[14] was used to detect the presence of the thioamide group. The thin-layer chromatograms were charred after spraying with a solution of anisaldehyde–methanol–sulfuric acid (1:35:3.9). A dark brown to black color indicated the presence of sugar.

Methyl 5-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate (6). Methyl 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate[5,8] (4.0 g, 10 mmol) was dissolved in a precooled (−22 to −25°) and mechanically stirred solution of 6 N hydrochloric acid (100 ml). To this was added, dropwise (5 min), a solution of sodium nitrite (3.45 g, 50 mmol) in water (10 ml). After 5 min of additional stirring, a mixture of cuprous chloride (5.0 g) in 6 N hydrochloric acid (10 ml) was added cautiously. Immediate evolution of nitrogen occurred. The stirring was continued for 75–90 min at ca. −25°. The reaction mixture was adjusted to pH 6–7 by gradual addition

of a saturated solution of sodium bicarbonate while stirring the reaction mixture vigorously and not allowing the temperature to rise above −20°. Ethyl acetate (200 ml) and Celite (20 g) were added to the stirred reaction mixture and filtered over suction through a Celite pad. The ethyl acetate layer was separated and the water portion was extracted with ethyl acetate (100 ml). The ethyl acetate portions were combined, washed with a saturated solution of $NaHCO_3$, followed by water, and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo gave the crude residue (syrup) which was passed through a column of silica gel (ca. 200 g) and eluted with 40% (v/v) ethyl acetate in $CHCl_3$. The major uv-absorbing fractions were collected and evaporated in vacuo to provide 1.70 g (40%) of pure product **6** as a syrup: ^1H NMR ($CDCl_3$) δ 2.15 (s, br, 9, $C_{2'}$, $C_{3'}$, $C_{5'}$ acetyls), 3.93 (s, 3, CH_3 of methyl carboxylate), 4.40 (s, 3, $C_4'H$ and $C_5'H_2$), 5.50 (m, 2, $C_2'H$ and $C_3'H$), 5.96 (d, 1, J = 4.5 Hz, $C_1'H$), 7.87 (s, 1, C_2H). Anal. ($C_{16}H_{19}N_2O_9Cl$) C, H, N, Cl.

Methyl 5-Chloro-1-β-D-ribofuranosylimidazole-4-carboxylate (7). A solution of **6** (1.04 g, 2.5 mmol) in dry methanol (20 ml) was saturated with ammonia at 0° and stored in a pressure bottle at room temperature for 15 h. On cooling the white needles separated out which were filtered[23] and washed with cold methanol, followed by anhydrous ether, to provide 290 mg (40%) of an analytically pure sample of **7**: mp 84°. Anal. ($C_{10}H_{13}N_2O_6Cl$) C, H, N.

5-Chloro-1-β-D-ribofuranosylimidazole-4-carboxamide (8). Method A. A solution of **6** (2.09 g, 5 mmol) in methanolic ammonium hydroxide (1:1, v/v, 20 ml) was stored in a pressure bottle at room temperature for 4–5 days. The solvent was evaporated under reduced pressure and the residue was left under vacuum (0.1–0.2 mm) at 40° to remove most of the acetamide formed. The residue free from acetamide was crystallized from ethanol to yield 975 mg (70%) of pure product **8**: mp 112°; $[\alpha]^{25}D$ −38.7° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 237 nm (ε 8150); $\lambda_{max}^{pH\ 11}$ 241 nm (ε 8500); ^1H NMR (Me_2SO-d_6) δ 5.65 (d, 1, J = 5 Hz, $C_1'H$), 7.25 and 7.4 (pair of s, br, 2, $CONH_2$), 8.2 (s, 1, C_2H). Anal. ($C_9H_{12}N_3O_5Cl$) C, H, N, Cl.

Method B. A solution of **7** (293 mg, 1 mmol) in methanolic ammonium hydroxide (1:1, v/v, 10 ml) was stored in a pressure bottle at room temperature for 4 days. The solvent was evaporated and the residue was crystallized from water to provide 205 mg (75%) of compound **8**: mp 112°; uv and chromatographic mobilities were identical with those of **8** obtained by method A.

Method C (General Procedure A). A mixture of **10** (1.50 g, 3.88 mmol) and ammonium hydroxide (50 ml) was cooled to 0°. An ice-cold solution of hydrogen peroxide (30%, 5 ml) was added and the reaction mixture was stirred at 0–4° for 20 h. The solvent was concentrated in vacuo (ca. 5 ml) and allowed to stand at 4° overnight. The separated crystalline product was filtered off and dried to provide 735 mg (68.24%) of **8** which on recrystallization from water yielded an analytical sample: mp 112–113°; uv, ir, ^1H NMR, and chromatographic mobilities were identical with those of **8** obtained by method A.

5-Chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (10). Method A (General Procedure B). A solution of 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carbonitrile[13] (**9**, 18.3 g, 50 mmol) in methanol (ca. 25 ml) was added to a mechanically stirred and precooled (−25 to −28°) solution of 6 N hydrochloric acid (250 ml). After 5–10 min of stirring a solution of sodium nitrite (11.31 g, 164 mmol) in water (15 ml) was slowly (5 min) added and the stirring was continued at −25 ± 3° for 20 min. A mixture of cuprous chloride or cuprous iodide (16.0 g) in water (5 ml) was added to the reaction mixture and the stirring was continued for 90 min. After this period, chloroform (200 ml) was added and the reaction mixture was adjusted to pH 7 by careful addition of a concentrated solution of sodium hydroxide while the stirring was continued to maintain a reaction temperature of −20°. A mixture of Celite (20 g) and chloroform (200 ml) was added and the reaction mixture was allowed to gain the room temperature. It was filtered through a Celite pad. The organic layer was separated and the aqueous layer was extracted with chloroform (200 ml). The combined chloroform portion was washed with a saturated solution of sodium bicarbonate, followed by water, and dried ($MgSO_4$). The solvent was evaporated in vacuo and the dark residue was passed through a column of silica gel packed in chloroform. The column was eluted with 40% (v/v) ethyl acetate in chloroform which separated the desired chloro compound **10** from the corresponding slightly slower moving, deaminated by-product **12**. The fast-moving fractions, containing major product, were collected. The evaporation of solvent in vacuo provided a light yellow syrup which was crystallized from methanol to yield 8.0 g (42%) of **10** as a white crystalline product: mp 106°; $[\alpha]^{25}D$ −12.02° (*c* 1, MeOH); γ_{max} (KBr) 2236 cm^{-1} (C≡N); $\lambda_{max}^{pH\ 1}$ 227 nm (ε 11 600); $\lambda_{max}^{pH\ 11}$ 232 nm (ε 9920); ^1H NMR ($CDCl_3$) δ 2.15 (s, br, 9, $C_{2'}$, $C_{3'}$, $C_{5'}$ acetyls), 4.4 (s, 3, $C_4'H$ and $C_5'H_2$), 5.14 (m, 2, $C_2'H$ and $C_3'H$), 5.87 (d, 1, J = 4.5 Hz, $C_1'H$), 7.83 (s, 1, C_2H). Anal. ($C_{15}H_{16}N_3O_7Cl$) C, H, N, Cl.

Method B (General Procedure C). A solution of **9** (3.6 g, 10 mmol) in methanol (15 ml) was added to a cold (−25°) and mechanically stirred solution of fluoroboric acid (48–50%, 100 ml). A solution of sodium nitrite (1.0 g, 14.5 mmol) in water (5 ml) was added and the stirring at −25° was continued for 10 min. A mixture of cuprous chloride (2.7 g) in water (ca. 4.0 ml) was added and immediate evolution of N_2 was observed. The stirring was continued for 45 min after which the reaction mixture was adjusted to pH 7 by addition of a concentrated solution of sodium hydroxide while maintaining the reaction temperature to −20°. A mixture of Celite (10 g) in chloroform (200 ml) was added. The stirring was continued until the reaction mixture gained the room temperature. The pure product **10** was isolated by silica gel column chromatography as described in general procedure B: yield, 400 mg (10%); mp, uv, NMR, and TLC mobilities were identical with those of **10** obtained by method A.

5-Bromo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (11). Method A. The synthesis of compound **11** was achieved following general procedure B with the only difference that in this case hydrobromic acid (47–49%) and cuprous bromide (or cuprous iodide) were used instead of 6 N hydrochloric acid and cuprous chloride, respectively. The quantities of the reagents used were similar. A crystalline compound was obtained after column chromatography which was recrystallized from methanol to provide 9.5 g (45%) of **11** as a crystalline product: mp 108–109°; $[\alpha]^{25}D$ +4.95° (*c* 1, MeOH); γ_{max} (KBr) 2237 cm^{-1} (C≡N); $\lambda_{max}^{pH\ 1}$ 228 nm (ε 10 500); $\lambda_{max}^{pH\ 11}$ 235 nm (ε 9825); ^1H NMR (Me_2SO-d_6) δ 2.12 (t, 9, $C_{2'}$, $C_{3'}$, $C_{5'}$ acetyls), 4.40 (s, 3, $C_4'H$ and $C_5'H_2$), 5.58 (m, 2, $C_2'H$ and $C_3'H$), 6.03 (d, 1, J = 5 Hz, $C_1'H$), 8.43 (s, 1, C_2H). Anal. ($C_{15}H_{16}N_3O_7Br$) C, H, N.

Method B. General procedure C was followed using cuprous bromide instead of cuprous chloride to provide 500 mg (12%) of pure product which was identical in all respects (melting point, uv, ir, and TLC mobility) with **11** isolated by method A.

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (12). This compound was isolated as a by-product from general procedure B used for the synthesis of **10** or **11**. After column chromatography, the slightly slower moving (as compared to **10** or **11**) and major product containing sugar positive (invisible under uv light) fractions were collected. Evaporation of the solvent in vacuo gave a crystalline product which was recrystallized from methanol to yield 10–15% (1.7–2.5 g) of the crystalline product **12**: mp 121–122° (lit.[8] 122–123°); $[\alpha]^{25}D$ −54.63° (*c* 1, MeOH); λ_{max} 2234 cm^{-1} (C≡N). The chromatographic mobilities and ^1H NMR were identical with those of an authentic sample.[8]

5-Iodo-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (13). The product was isolated following general procedure C, using cuprous iodide instead of cuprous chloride. The compound obtained after column chromatography was crystallized from ethanol to provide 475 mg (10%) of **13**: mp 140–141°; γ_{max} (KBr) 2240 cm^{-1} (C≡N); $\lambda_{max}^{pH\ 1}$ 238 nm (ε 11 320); $\lambda_{max}^{pH\ 11}$ 239 nm (ε 11 030); ^1H NMR (Me_2SO-d_6) δ 2.10 (s, br, 9, $C_{2'}$, $C_{3'}$, $C_{5'}$ acetyls), 4.35 (s, 3, $C_4'H$ and $C_5'H_2$), 5.50 (m, 2, $C_2'H$ and $C_3'H$), 5.75 (d, 1, J = 3 Hz, C_1H), 8.22 (s, 1, C_2H). Anal. ($C_{15}H_{16}N_3O_7I$) C, H, N, I.

5-Bromo-1-β-D-ribofuranosylimidazole-4-carboxamide (14). A mixture of **11** (2.00 g, 4.64 mmol) and ammonium hydroxide (50 ml) was treated with hydrogen peroxide (30%, 5 ml) as described in general procedure A. The product thus obtained was recrystallized from water to provide 1.21 g (80.5%) of **14** in the crystalline form: mp 176–177°; $[\alpha]^{25}D$ −25.2° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 239 nm (ε 8030); $\lambda_{max}^{pH\ 11}$ 244 nm (ε 9130); ^1H NMR (Me_2SO-d_6) δ 5.69 (d, 1, J = 5 Hz, $C_1'H$), 7.25 and 7.40 (pair of

s, br, 2, $CONH_2$), 8.29 (s, 1, C_2H). Anal. ($C_9H_{12}N_3O_5Br$) C, H, N.

5-Iodo-1-β-D-ribofuranosylimidazole-4-carboxamide (15). A mixture of **13** (300 mg, 0.6 mmol) and ammonium hydroxide (7 ml) was treated with hydrogen peroxide (30%, 0.7 ml) as described in general procedure A. The isolated product was recrystallized from water to yield 174 mg (75%) of **15** in the form of needles: mp 176–177°; $[\alpha]^{25}D$ −11.9° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 241 nm (ε 9000); $\lambda_{max}^{pH\ 11}$ 247 nm (ε 10040). Anal. ($C_9H_{12}N_3O_5I$) C, H, N.

5-Mercapto-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (16). Method A. A mixture of **10** or **11** (10 mmol), methanol (100 ml), and KOH (2.50 g) was saturated with hydrogen sulfide at 0°. The clear solution was heated in a bomb at 90° for 5 h. The solvent was evaporated in vacuo; the residue was dissolved in water and adjusted to pH 5 by gradual addition of cold dilute hydrochloric acid. The mixture was cooled in ice and the separated crystalline compound was filtered over suction. The crude product was recrystallized from water to provide 2.15 g (74%) of **16** in the form of yellow shining needles: mp 211–212° dec; $[\alpha]^{25}D$ −81.2° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 278 nm (ε 6520) and 355 (19400); $\lambda_{max}^{pH\ 11}$ 260 nm (sh) (ε 7400) and 354 (19690); ^1H NMR (Me_2SO-d_6) δ 6.02 (s, br, 1, $C_1'H$), 9.02 (s, 1, C_2H), 9.43 and 9.5 (s, pair, 2, $CSNH_2$). Anal. ($C_9H_{13}N_3O_4S_2$) C, H, N, S.

5-Chloro-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (20). The hydrogen sulfide gas was bubbled through a solution of **10** (5.00 g, 12.96 mmol) in anhydrous pyridine (120 ml) and triethylamine (4 ml) at room temperature for 3 h. The reaction solution, thus saturated with hydrogen sulfide, was stirred in a tightly stoppered flask for 12 h. The solvent was evaporated in vacuo and the residue was dissolved in chloroform (200 ml). It was washed with water (100 ml × 2) and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was passed through a column of silica gel packed in chloroform. It was eluted with 40% (v/v) ethyl acetate in chloroform which separated the desired 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**17**) from the faster moving starting material **10** and a slower moving product, presumably the 5-mercapto-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**19**). The latter product had similar uv ($\lambda_{max}^{pH\ 1}$ 279 and 358 nm; $\lambda_{max}^{pH\ 11}$ 259 and 356 nm) as reported for **16**. The fractions containing the major, desired product were evaporated in vacuo to provide the intermediate compound **17** in crude crystalline form: yield, 2.32 g (42.7%); mp 148–149°; $\lambda_{max}^{pH\ 1}$ 262 and 300 nm; $\lambda_{max}^{pH\ 11}$ 263 and 301 nm. The intermediate product **17** was taken in ammonium hydroxide (50 ml) and the reaction mixture was stirred for 16 h at 0–4°. The solvent was evaporated in vacuo and the residue was passed through a column of silica gel (100 g) packed in chloroform. It was eluted with ethyl acetate–chloroform–acetone (5:3:2, v/v). The major, uv and sugar positive fractions were collected and the solvent was evaporated in vacuo to provide 760 mg (20%) of the pure product **20** as a foam; mp 169–170° dec; $\lambda_{max}^{pH\ 1}$ 262 nm (ε 11200), 300 (11800); $\lambda_{max}^{pH\ 11}$ 263 nm (ε 12600), 301 (12100). Anal. ($C_9H_{12}N_3O_4ClS$) C, H, N.

5-Bromo-1-β-D-ribofuranosylimidazole-4-thiocarboxamide (21). The synthesis of compound **21** was achieved starting from **11** via the formation of intermediate, 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (**18**, syrup), following exactly similar procedure as for the synthesis of the corresponding compound **20**. The product was obtained in 20–22% yield after column chromatography and was crystallized from ethanol to provide **21** as a crystalline compound: mp 160–161° dec; $\lambda_{max}^{pH\ 1}$ 267 nm (ε 10040), 300 (10630); $\lambda_{max}^{pH\ 11}$ 267 nm (ε 11510), 302 (10960). Anal. ($C_9H_{12}N_3O_4BrS$) C, H, N, S.

5-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamidoxime (22). A mixture of **10** (5.07 g, 13.14 mmol), absolute ethanol (25 ml), and hydroxylamine (0.48 g) was refluxed over a steam bath for 1 h. More hydroxylamine (0.48 g) was added and the clear reaction solution was refluxed for a further 2 h. It was left at room temperature overnight. The separated crystalline product was filtered off and washed with cold ethanol followed by ether. The crude product was recrystallized from methanol to provide 2.95 g (53.8%) of **22** as a crystalline compound: mp 173–174°; $\lambda_{max}^{pH\ 1}$ 247 nm (ε 10470); $\lambda_{max}^{pH\ 11}$ 236 nm (ε 7680); ^1H NMR (Me_2SO-d_6) δ 2.13 (s, br, 9, C_2', C_3', C_5' acetyls), 5.53 (s, br, 2, NH_2), 5.98 (d, 1, *J* = 5 Hz, $C_1'H$), 8.19 (s, 1, C_2H), 9.63 (s, 1, NOH). Anal. ($C_{15}H_{19}N_4O_8Cl$) C, H, N.

5-Bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamidoxime (23). A mixture of 5-bromo-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (**11**, 6.62 g, 14.30 mmol), absolute ethanol (50 ml), and hydroxylamine (0.590 g) was refluxed over a steam bath for 1 h. Additional hydroxylamine (0.590 g) was added and the refluxing was continued for a further period of 2 h. The reaction mixture was allowed to cool and the separated crystalline product was collected by filtration and washed with cold ethanol and ether. The crude product was recrystallized from methanol to yield 5.55 g (77.8%) of crystalline compound **23**: mp 154–155°; $\lambda_{max}^{pH\ 1}$ 249 nm (ε 8800); $\lambda_{max}^{pH\ 11}$ 232 nm (ε 8100); ^1H NMR (Me_2SO-d_6) δ 2.1 and 2.13 [s (2), 9, C_2', C_3', C_5' acetyls], 5.51 (s, br, 2, NH_2), 5.99 (d, 1, *J* = 5.5 Hz, $C_1'H$), 8.28 (s, 1, C_2H), 9.62 (s, 1, NOH). Anal. ($C_{15}H_{19}N_4O_8Br$) C, H, N.

5-Chloro-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (24). A mixture of **22** (0.401 g, 0.96 mmol) and ammonium hydroxide (9 ml) was stirred at 0–4° for 10 h. The solvent was evaporated in vacuo and the residue was crystallized from ethanol to provide 0.241 g (86%) of crystalline compound **24**: mp 155–156° dec; $[\alpha]^{25}D$ −25.9° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 248 nm (ε 10280); $\lambda_{max}^{pH\ 11}$ 232 nm (ε 7550); ^1H NMR (Me_2SO-d_6–D_2O) δ 5.68 (d, 1, *J* = 5 Hz, $C_1'H$), 8.17 (s, 1, C_2H). Anal. ($C_9H_{13}N_4O_5Cl$) C, H, N, Cl.

5-Bromo-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (25). A mixture of **23** (3.576 g, 7.72 mmol) and ammonium hydroxide (50 ml) was stirred at 0–4° for 5 h. The solvent was evaporated in vacuo and the residue was crystallized from ethanol to yield 2.464 g (94.6%) of **25** as the crystalline product: mp 157–158° dec; $[\alpha]^{25}D$ −15.3° (*c* 1, DMF); $\lambda_{max}^{pH\ 1}$ 251 nm (ε 8410); $\lambda_{max}^{pH\ 11}$ 231 nm (ε 7010); ^1H NMR (Me_2SO-d_6–D_2O) δ 5.68 (d, 1, *J* = 3 Hz, $C_1'H$), 8.25 (s, br, 1, C_2H). Anal. ($C_9H_{13}N_4O_5Br$) C, H, N.

1-β-D-Ribofuranosylimidazole-4-carboxamidine Hydrochloride (26). This compound was obtained starting from **24** and following exactly similar procedure as reported for the synthesis of compound **27** described below. The product **26** was crystallized from ethanol to provide pure crystalline compound in 70% yields. Its mp 166° (212–213° dec), uv, and ^1H NMR data were similar to those of an authentic sample.[8]

1-β-D-Ribofuranosylimidazole-4-carboxamidine Hydrobromide (27). A solution of **25** (1.50 g, 4.45 mmol) in water (50 ml) was hydrogenated on a Parr apparatus in the presence of Raney nickel (wet, 1.50 g) at room temperature and 45 psi for 2 h. The insoluble material was discarded by filtration through a Celite pad. Solvent from the filtrate was evaporated and the residue was crystallized from ethanol to provide 1.174 g (81.7%) of hydrobromide compound **27**: mp 200–201°; $\lambda_{max}^{pH\ 1}$ 244 nm (ε 11470); $\lambda_{max}^{pH\ 11}$ 243 nm (ε 10640); ^1H NMR (Me_2SO-d_6–D_2O) δ 5.78 (d, 1, *J* = 4.5 Hz, $C_1'H$), 8.29 and 8.59 (s, pair, 2, C_2H and C_5H). Anal. ($C_9H_{14}N_4O_4HBr \cdot 1.5H_2O$) C, H, N.

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Synthesis and Anti-DNA Virus Activity of the 5′-Monophosphate and the Cyclic 3′,5′-Monophosphate of 9-(β-D-Xylofuranosyl)guanine

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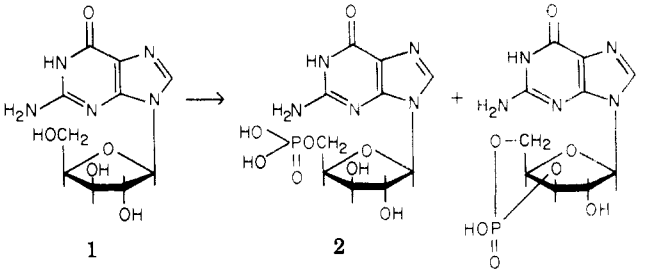
9-(β-D-Xylofuranosyl)guanine (xylo-G) was converted chemically to the 9-(β-D-xylofuranosyl)guanine 5′-monophosphate (xylo-GMP) and 9-(β-D-xylofuranosyl)guanine cyclic 3′,5′-monophosphate (c-xylo-GMP). These compounds were tested against a variety of DNA viruses in tissue culture in parallel with 9-(β-D-arabinofuranosyl)adenine (ara-A). This evaluation revealed that xylo-G, xylo-GMP, and c-xylo-GMP were all moderately active but less effective than ara-A. When the four compounds were administered intracerebrally as a treatment for herpes virus, type 1 induced encephalitis in mice, c-xylo-GMP exhibited superior activity to that shown by the other three. When administered intraperitoneally, c-xylo-GMP was found to have a therapeutic index of about 4, which is less than that for ara-A (~30) in the same system.

Nucleosides possessing either D-arabinofuranose[1] or D-xylofuranose[2] in place of D-ribofuranose moieties have received increasing attention in recent years as antimetabolites. Of particular importance in antiviral and antitumor studies of such nucleoside analogues are 1-(β-D-arabinofuranosyl)cytosine (ara-C),[3] 9-(β-D-arabinofuranosyl)adenine (ara-A),[4] and 9-(β-D-xylofuranosyl)-adenine.[5] The observation that most of the nucleoside analogues must be converted to nucleotides[1,6] before they are biologically active, coupled with the increased water solubility of such nucleotides over the corresponding nucleosides,[7] has prompted considerable activity toward the synthesis and biological evaluation of phosphorylated compounds of the above class.[8]

The importance of guanine nucleotide metabolism in a variety of microbiological and mammalian systems has been comprehensively reviewed.[9] Antimetabolites have proved to be unique biochemical tools in probing enzymatic transformations. The biological resistance to purine and pyrimidine antimetabolites is ascribed to high levels of a deaminase[4b] or lack of enzymatic phosphorylation of the nucleosides.[10] This problem could be overcome by using 5′-monophosphates of the nucleosides. However, the free nucleotides at physiological pH carry two negative charges and in general[11] penetrate the cell as an intact nucleotide in very small amounts. The exogenous adenosine cyclic 3′,5′-monophosphate (c-AMP)[12a] and guanosine cyclic 3′,5′-monophosphate (c-GMP)[13] may exert specific biological effects of c-AMP or c-GMP, respectively, on the cell membrane[12b] and the metabolic pathways inside the cell. The interesting biological activity reported for 9-(β-D-xylofuranosyl)purines[14] suggested the synthesis of 9-(β-D-xylofuranosyl)guanine 5′-monophosphate and the cyclic 3′,5′-monophosphate as potential antiviral agents.



1 2 3

For the synthesis of 9-(β-D-xylofuranosyl)guanine (1), the method reported by Lee and co-workers[15] via the