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Synthesis of Tetrazole Ribonucleosides and Their Evaluation as Antiviral Agents. 2.[1] 5-Amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole and 5-Amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole

Mohindar S. Poonian,* Eugene F. Nowoswiat, John F. Blount, and Michael J. Kramer

Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received January 26, 1976*

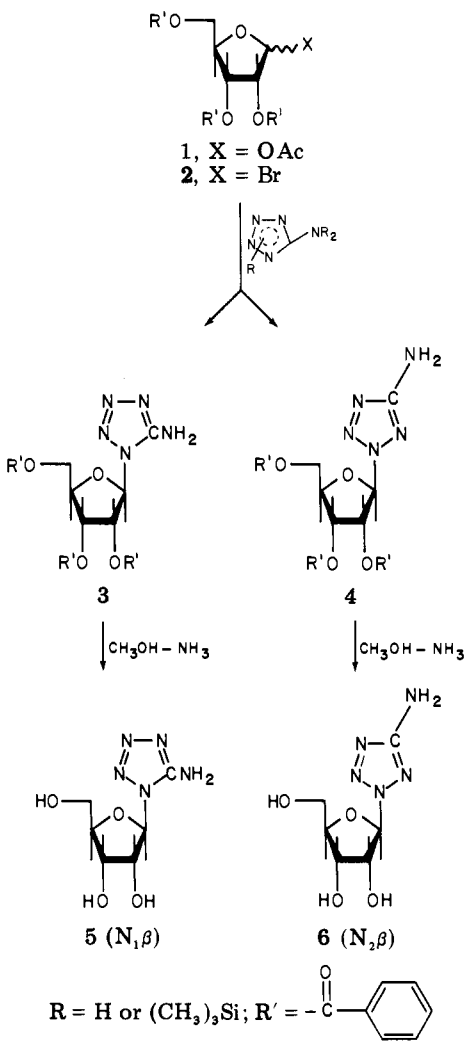
Synthesis of 5-amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole and 5-amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole is described. X-Ray crystallography was first used to establish the stereochemical configuration of the two isomers. By conducting ^{13}C NMR analysis on these isomers with known structures, i.e., $N_1\beta$ and $N_2\beta$, a correlation is developed for determining the *N*-ribosyl attachment site of tetrazole ribonucleosides. Results are also presented on antiviral testing of these synthetic 5-aminotetrazole ribonucleosides against influenza A2/Asian/J305 virus infection in mice.

The recent discoveries[2,3] of potent antiviral activities in several classes of nucleosides have given impetus to acceleration of research in this area. Our own interest in the synthesis of tetrazole ribonucleosides was stimulated by the discovery of antiviral activity of Virazole[2a] (ribavirin). In a previous report[4] we have described the synthesis, structure elucidation, and antiviral testing of the parent tetrazole ribonucleoside and two of its 5-substituted derivatives. This paper presents the synthesis, structural assignment, and antiviral evaluation of 5-amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (**5**) and 5-amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (**6**). Although ^{13}C spectroscopy was utilized in the assignment of the site of N-glycosylation of tetrazoles in our previous work,[4] the conclusions were based on analogy to the parent 1-(β-D-ribofuranosyl)tetrazole and N_1-methyl- and N_2-methyltetrazole derivatives. This work provides us with an isomeric pair of 5-aminotetrazole ribonucleosides which possess similar (β) anomeric configuration but differ in the site of *N*-ribosyl substitution. The availability of these compounds has enabled us to develop a correlation between ^{13}C spectroscopy and the *N*-ribosyl attachment site.

Results and Discussion

Ribosylation of 5-Aminotetrazole. The synthesis of 5-amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-tetrazole (**3**) and 5-amino-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2*H*-tetrazole (**4**) was achieved by using two well-documented procedures (Scheme I): (a) by a reaction between fully silylated 5-aminotetrazole and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**2**) in acetonitrile at room temperature; (b) by an acid-catalyzed fusion[5] of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**1**) and 5-aminotetrazole at 165°. The resulting nucleoside mixtures differed dramatically in both yields and relative ratios of the $N_1\beta$ (**3**) and $N_2\beta$ (**4**) isomers in the two methods. From the silyl method the yield of the total ribosylated 5-aminotetrazole was 72% with a ratio of 55:45 between $N_1\beta$ and $N_2\beta$ isomers. The acid-catalyzed fusion method, on the other hand, resulted in quite poor yield of the coupled product which contained $N_2\beta$ as the predominant component, $N_1\beta$ isomer being present only in trace amounts (TLC). The tri-*O*-benzoylated nucleosides **3** and **4** were then deblocked by the standard methanolic-ammonia treatment to obtain 5-amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (**5**) and 5-amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (**6**), respectively.

Scheme I



Structural Determination of 5-Aminotetrazole Ribonucleosides. Although a number of exceptions to

Table I

Compound	^{1}H NMR,[a] $J_{1'-2'}$, Hz	^{13}C NMR,[a] C-5 of tetrazole moiety
5-Amino-tetrazole		157.0
3	2.5	
5	5.0	155.7
4	1.5	
6	4.0	167.7

[a] Spectra were recorded in Me_2SO-d_6 with tetramethylsilane as internal standard. The chemical shift values of ^{13}C spectra are in parts per million downfield from Me_4Si.

the "trans rule" are now known,[6] a predominant formation of the β anomer on condensation of a tri-*O*-benzoylated ribofuranosyl derivative with nitrogen-containing heterocycles is generally assumed. The initial indication that both isomers of 5-aminotetrazole ribonucleosides **5** and **6** obtained in our work might possess a β configuration was obtained from the similarity of their NMR coupling constant ($J_{1'-2'}$ = 5 and 4 Hz, Table I) to those of the previously described tetrazole ribonucleosides ($J_{1'-2'}$ = 4 Hz)[4] which had been assigned β configuration. The difference of 1 Hz in the $J_{1'-2'}$ values of compounds **5** and **6** could conceivably be a consequence of the distortion in the dihedral angle between $H_{1'}$ and $H_{2'}$ produced by intramolecular hydrogen bonding observed in the x-ray crystallography of compound **6** although we do not know whether such a conformation will persist in solution as well. The β configuration for compounds **5** and **6** was established unequivocally by x-ray crystallographic studies (details in the following section) which additionally confirmed N_1 and N_2, respectively, as the sites of ribonucleoside attachment in compounds **5** and **6**.

The usefulness of ^{13}C NMR spectroscopy in the assignment of the *N*-glycosyl site has been demonstrated in several systems. Among other systems it has been used in structural studies on ribonucleosides of 1,2,4-triazoles,[7] 1,2,3-triazoles,[8] and pyrazolo[3,4-*d*]pyrimidines.[9] In the case of triazoles, N-substitution by a ribosyl moiety always produces a significant upfield chemical shift in the ^{13}C signal of a carbon α to the substituted nitrogen and a downfield shift in signal of the carbon β to that nitrogen. More often than not, the magnitude of the α-carbon upfield shift is greater than the β-carbon downfield shift. The results from similar ^{13}C NMR studies on pyrazolo[3,4-*d*]pyrimidines were qualitatively consistent with the trends in triazoles. In the present studies, however, we have found that carbon-5 of 5-aminotetrazole upon ribosylation undergoes a small (1.3 ppm) upfield ^{13}C signal shift in compound **5** ($N_1\beta$ carbon-5 is α to the site of ribosylation) and a much larger (10.7 ppm) downfield ^{13}C signal shift in compound **6** ($N_2\beta$, carbon-5 is β to the site of ribosylation) (Table I). These results taken together with our previous ^{13}C studies on other derivatives of tetrazole ribonucleosides[4] and the ^{13}C studies on *N*-alkyltetrazole[10] derivatives lead to the conclusion that carbon-5 of tetrazoles upon N-ribosylation or N-alkylation undergoes a small or no ^{13}C signal shift when situated α to the site of substitution but suffers a large downfield ^{13}C signal shift when located β to that site. It is clear that these results are diametrically opposed to those observed in several other heterocyclic systems, some of which are referred to above. It is therefore appropriate to sound a warning for being extremely careful in interpreting ^{13}C NMR shifts in assigning sites of N-glycosidation or N-alkylation in heterocycles especially if the tautomeric structure of the parent base is ambiguous. However, it is our view that the correlations developed in this paper would permit the use of ^{13}C NMR spectroscopy as a powerful tool for N-glycosylation site determinations in tetrazole nucleosides.

Table II. Crystal Data for 5-Amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (5) and for 5-Amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (6)

	5	6
Formula	$C_6H_{11}N_5O_4$	$C_6H_{11}N_5O_4$
Formula wt	217.18	217.18
Space group	$P2_1$	$P2_12_12_1$
a, Å	8.170 (3)	6.904 (3)
b, Å	6.736 (6)	11.700 (4)
c, Å	8.507 (3)	11.803 (4)
β, deg	103.98 (3)	
Z	2	4
d_{calcd}, g cm^{-3}	1.586	1.512
μ (Cu Kα), cm^{-1}	11.7	11.1

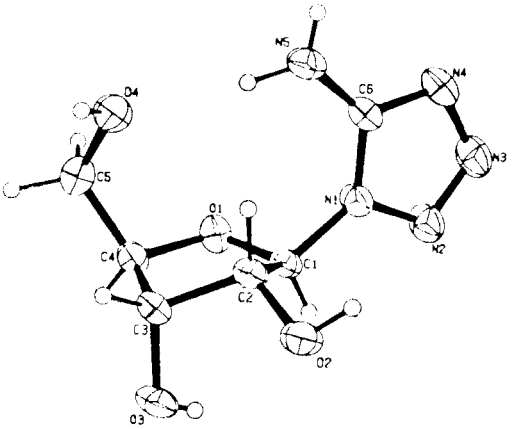


Figure 1. Stereodrawing of 5-amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (5), showing its conformation in the crystalline state.

X-Ray Crystallographic Studies. 5-Amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (5). The crystal data are summarized in Table II. The intensity data were collected on a Hilger-Watts four-circle diffractometer from a crystal which was approximately 0.12 × 0.25 × 0.40 mm in size. Of 1017 accessible reflections with $\theta < 76°$, 996 had intensities which were significantly greater than background. The structure was solved by a multiple solution procedure[11] and was refined by full-matrix least squares. For the final refinement anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atom parameters were refined. The final discrepancy index is $R = 0.045$ for the 996 observed reflections. The analysis established that 5-aminotetrazole ribonucleoside (**5**) has the $N_1\beta$ structure, as can be seen in the stereodiagram (Figure 1).

5-Amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (6). The crystal data for compound **6** are given in the Table II. Two sets of intensity data, both measured on a Hilger-Watts diffractometer, were recorded for this compound. Nickel-filtered Cu Kα radiation was used for the first set and zirconium-filtered Mo Kα radiation for the second set. The same crystal was used for both data sets; its size was approximately 0.3 × 0.3 × 0.6 mm. For the Cu data, 1035 of the 1056 accessible reflections with $\theta < 76°$ were considered observed [$I > 2.5\sigma$ (1)], and for the Mo data, 1729 of the 2574 reflections with $\theta < 36°$ were observed.

Initial attempts to obtain the structure from the Cu data by the usual multiple solution procedure[11] were fruitless. Analysis of the Mo data by the multiple solution procedure

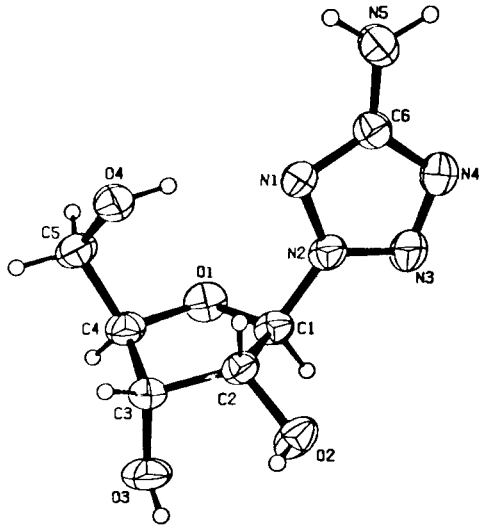


Figure 2. Stereodrawing of 5-amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (**6**), showing its conformation in the crystalline state.

immediately yielded the structure. The Cu data were used for the refinement, which was carried out by full-matrix least squares. For the final refinement, anisotropic thermal parameters were used for the heavier atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy index is $R = 0.040$ for the 1035 observed reflections. The analysis established the structure of compound **6** to be $N_2\beta$. The conformation of the molecule in the crystal is shown in Figure 2. From Figure 2 it can be seen that there is an intramolecular hydrogen bond from O(4) to N(1). The O···N distance is 2.89 Å and the O–H···N angle is 162°.

Biological Results. Procedure. Swiss albino mice weighing 9–12 g were lightly anesthetized with ether and infected intranasally with approximately 3.2 LD_{50} of influenza A2/Asian/J305 virus. The test substances were dissolved in water and mice received 0.5 ml intraperitoneally (ip) immediately before virus infection and at 1, 5, 24, 30, 48, and 72 h after virus infection for a total of seven treatments. Control mice received 0.5 ml of water (ip) according to the above schedule. Animals were observed for 21 days following infection and the number of survivors recorded on the 21st day. The results of antiviral testing are presented in the Table III.

Ribavirin produced a statistically significant effect against influenza A2/Asian/J305 virus infection in mice but 5-amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (**5**) and 5-amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (**6**) were without effect at the doses tested.

Experimental Section

General. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton magnetic resonance and ^{13}C spectra were recorded on a Varian XL-100 and Bruker HFX-10 spectrometer, respectively, with tetramethylsilane as internal reference. The values are given in parts per million downfield from Me_4Si. Thin-layer chromatographic analysis was performed on 0.25-mm silica gel plates (60F-254) purchased from Brinkman Instruments, Westbury, N.Y. In the case of tribenzoylated tetrazole nucleosides, solvent system A (chloroform–acetone, 13:1, v/v) was used in developing the analytical TLC plates. These compounds were visualized under a uv lamp. For the TLC development of debenzoylated tetrazole nucleosides, however, solvent system B (toluene–methanol, 1:1, v/v) was found useful. These materials were visualized by spraying the TLC plates with a solution of glacial acetic acid–sulfuric acid–*p*-anisaldehyde (50:1:0.5, v/v) and then heating the plates at 110° for about 5–15 min. Components containing the ribonucleoside moiety gave yellowish green or green color.

Table III. Effect of Ribavirin, 5-Amino-1-(β-D-ribofuranosyl)-1*H*-tetrazole (**5**), and 5-Amino-2-(β-D-ribofuranosyl)-2*H*-tetrazole (**6**) against Influenza A2/Asian/J305 Virus Infection in Mice[a]

Compound	Dose,[b] mg/kg ip × 7	No. of mice surviving/no. tested at 21 days: Treated	Control	p[c]
Ribavirin	50	11/24	1/24	<0.001
5	50	0/8	0/8	>0.05
6	50	0/8	0/8	>0.05

[a] Swiss albino mice weighing 9–12 g were lightly anesthetized with ether and infected intranasally with approximately 3.2 LD_{50} of influenza A2/Asian/J305 virus. [b] The test substances were dissolved in H_2O and mice received 0.5 ml intraperitoneally (ip) immediately before virus infection and at 1, 5, 24, 30, 48, and 72 h after virus infection for a total of seven treatments. Control mice received 0.5 ml of water intraperitoneally. [c] Fisher exact test.

Synthesis of 5-Amino-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1*H*-tetrazole (3) and 5-Amino-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2*H*-tetrazole (4). Method A. A commercially available sample of 5-aminotetrazole (25.75 g, 0.25 mol) was dissolved in dry pyridine and coevaporated (2 × 200 ml) to remove water from the sample. To this was added 201.8 g (1.25 mol, 300 ml) of 1,1,1,3,3,3-hexamethyldisilazane and the contents were heated at reflux for 3 h. Progress of the reaction was monitored by NMR analysis and when only minor NH signals were observed, the reaction was deemed to be completed. The mixture was cooled and concentrated to dryness, and the white residue was dissolved in anhydrous acetonitrile. This solution was added to the solution of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[12] (0.25 mol in 250 ml of acetonitrile) and the combined mixture was stirred at room temperature for 65 h. The reaction was quenched by the addition of methanol, followed by stirring for 5 min. A crop of colorless solid material fell out of the solution. It was filtered out and found to be a mixture of two compounds by TLC (system A). More of a similar mixture was obtained as solid material when the filtrate was concentrated to an oil and the oil was treated with anydrous methanol. On treatment of the mixture with ethanol, one of the components remained insoluble. The insoluble compound was characterized to be $N_1\beta$ (**3**) based on the assignment of $N_1\beta$ configuration to its debenzoylated product **5**. The filtrate was then fractionated by silica gel chromatography (5.0 × 80 cm column, 70 × 230 mesh silica). The material which was eluted by 93:7 (v/v) toluene–ether was identified to be compound **4** with $N_2\beta$ configuration (by virtue of its conversion to compound **6** which has been assigned the $N_2\beta$ structure; see Discussion). Further elution of the column [85:15 (v/v), toluene–ether] provided more of compound **3**: total coupling yield, 72%; compound **3**, $N_1\beta$, 54.4 g (41%); compound **4**, $N_2\beta$, 41.0 g (31%). Compound **3**, mp 193–195°, crystallized from acetone; compound **4**, mp 130–130.5°, crystallized from ethanol. Anal. ($C_{27}H_{23}N_5O_7$) C, H, N.

Method B. A sample of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (25.2 g, 50 mmol) was mixed with 5-aminotetrazole monohydrate (5.15 g, 50 mmol) and the mixture was heated in a conical flask at 165°. After the mixture had melted, bis(*p*-nitrophenyl) phosphate (50 mg) was added and the heating continued for another 15 min under diminished pressure (0.1 mm). The reaction mixture was cooled to room temperature and treated with ethanol. This resulted in deposition of a solid which was found to be a mixture of the starting ribofuranosyl derivative and a new product. Most of the starting material was removed as insoluble component on treatment of the mixture with toluene. The product was further purified (a) by silica gel chromatography (3.6 × 63 cm column) and (b) by preparative TLC. The resultant material was crystallized from ethanol to give colorless crystals, mp 130°. The product was found to be identical with compound

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)-1H-tetrazole (5) and 5-Amino-2-(β-D-ribofuranosyl)-2H-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.

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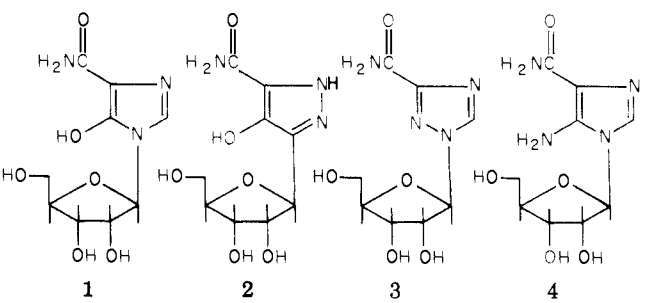
Synthesis and Antiviral and Antimicrobial Activity of Certain 1-β-D-Ribofuranosyl-4,5-Disubstituted Imidazoles

Prem C. Srivastava,* David G. Streeter, Thomas R. Matthews, Lois B. Allen, Robert W. Sidwell, and Roland K. Robins

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received December 16, 1975*

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