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Synthesis of Thioinosine and Thio-AICA-riboside Analogs

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As the analog of thioinosine, 2-methyl-, 2-ethyl-, 2-methylthio-, and 2-methylamino-6-mercapto-9-β-D-ribofuranosylpurine were synthesized. From thio-AICA-riboside, 5-formamido-1-(2′,3′,5′-tri-O-formyl-β-D-ribofuranosyl)-4-imidazolethiocarboxamide and 5-acetamido-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)-4-imidazolethiocarboxamide were also prepared.

Since the discovery of 6-mercaptopurine (XII) as a clinically useful antitumor agent, a number of structually similar derivatives have been synthesized and evaluated for antitumor activity. Thioinosine (6-mercapto-9-β-D-ribofuranosylpurine) (X) and thioguanosine (9-β-D-ribofuranosyl-2-amino-6-mercaptopurine) are well known to show significant antitumor activity.[2] It is also known that the latter is very toxic.[3] From these facts, it appeared possible that the introduction of some group to 2-position of thioinosine might result in reduction in toxicity or increase in activity. As the analogs of thioinosine and thioguanosine, 6-thioxanthosine[4] (2-hydroxy-6-mercapto-9-β-D-ribofuranosylpurine), 2-fluoro-6-mercapto-9-β-D-ribofuranosylpurine,[5] 2-chloro-6-mercapto-9-β-D-ribofuranosylpurine,[5] and 2-dimethylamino-6-mercapto-9-β-D-ribofuranosylpurine[5] were synthesized, the first of which had no antitumor activity.[6] In previous studies, it was shown that thio-AICA-riboside(5-amino-1-β-D-ribofuranosyl-4-imidazolethiocarboxamide) (VIII)[4] had antitumor activity[3,7,8] because of the structural similarity to

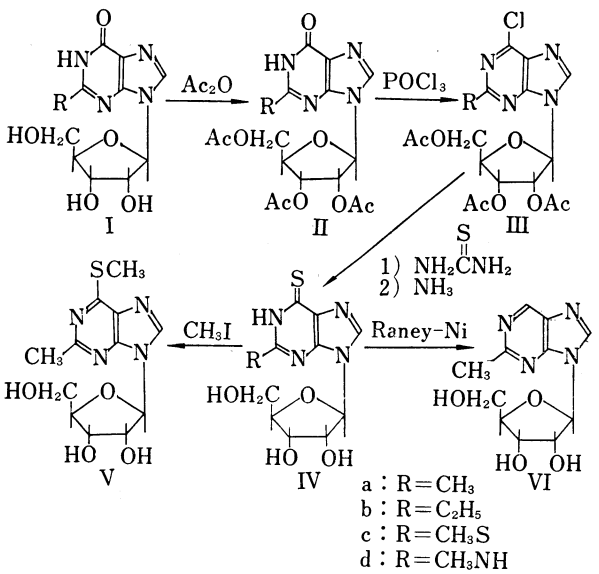
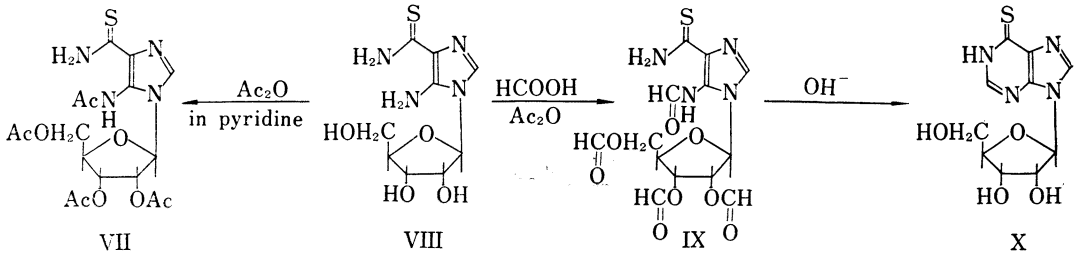


Chart 1

1) Location: *a) Suzuki-cho, Kawasaki; b) Toneyama, Toyonaka, Osaka.*
2) H.E. Skipper, J.A. Montgomery, J.R. Thomson, and F.M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959).
3) A. Hoshi, Y. Ohsaka, T. Nishimoto, and K. Kuretani, *Pharmacometrics*, **4**, 1 (1970).
4) A. Yamazaki, I. Kumashiro, T. Takenishi, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **16**, 2172 (1968).
5) J.F. Gerster and R.K. Robins, *J. Org. Chem.*, **31**, 3258 (1966).
6) A. Hoshi, K. Kumagai, and K. Kuretani, *Pharmacometrics*, **2**, 368 (1968).
7) A. Hoshi, K. Kumagai, and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **16**, 2080 (1968).
8) A. Hoshi, Y. Ohsaka, and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **17**, 1720 (1969).

AICA-riboside (5-amino-1-β-D-ribofuranosyl-4-imidazolecarboxamide) playing an important role in purine biosynthesis. Our interest in such thioinosine and thio-AICA-riboside had led us to undertake the preparation of 2-substituted thioinosines and in particular of thio-AICA-riboside derivatives. To this end, AICA-riboside was used as the starting material.[9]



VII VIII IX X

Chart 2

Previously, reaction of AICA-riboside with ethyl acetate in the presence of sodium ethoxide was reported to give 2-methylinosine (Ia)[10] in good yield. Acetylation of Ia with acetic anhydride followed by chlorination of the resulting gummy acetate (IIa) with phosphoryl chloride[11,12] afforded 6-chloro compound (IIIa). This was converted by reaction with thiourea and subsequent deacetylation to 2-methyl-6-mercapto-9-β-D-ribofuranosylpurine (IVa). Compound IVa was methylated with methyl iodide to furnish 2-methyl-6-methylthio-9-β-D-ribofuranosylpurine (Va), while desulfrization of IVa with active Raney nickel gave 2-methyl-9-β-D-ribofuranosylpurine (VI), which was an analog of the antibiotic nebularin.[13] 2-Ethyl-6-mercapto-9-β-D-ribofuranosylpurine (IVb) was obtained in a similar manner, starting with 2-ethylinosine (Ib).[10] When 2-methylthioinosine (Ic)[14] was acetylated and then chlorinated, 6-chloro compound (IIIc) was obtained. Subsequent treatment of IIIc with thiourea afforded 2-methylthio-6-mercapto-9-β-D-ribofuranosylpurine (IVc). Acetylation of N^2-methylguanosine (Id)[14] followed by chlorination gave the crystalline 6-chloro compound (IIId), which was allowed to react with thiourea, giving 2-methylamino-6-mercapto-9-(2′, 3′, 5′-tri-O-acetyl-β-D-ribofuranosyl) purine. Deblocking of the acetate resulted in 2-methylamino-6-mercapto-9-β-D-ribofuranosylpurine (IVd).

Thio-AICA-riboside, obtained by reaction of 5-amino-4-cyano-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl) imidazole with hydrogen sulfide followed by acidic treatment,[4] was allowed to react with formic acid in acetic anhydride to give 5-formamido-1-(2′,3′,5′-tri-O-formyl-β-D-ribofuranosyl)-4-imidazolethiocarboxamide (IX) very easily. When thio-AICA-riboside was acetylated with acetic anhydride in pyridine, 5-acetamido-1-(2′,3′,5′-tri-O-acetyl-β-D-ribo furanosyl)-4-imidazolethiocarboxamide (VII) was formed. On heating in alkaline solution, IX gave thioinosine and VII did IVa.

All of the synthesized compounds were examined by Hoshi, *et al.*,[3,6] who reported that IVa, IVb, and IVc were inactive against Nakahara-Fukuoka sarcoma. Compound IVd was found to be less active than thioguanosine.[3]

6-Mercaptopurine and 5-fluorouracil (XIV) are potent antimetabolites and both are in clinical use today. The former is an analog of hypoxanthine, its ribonucleotide (inosine-5′-phosphate) being the intermediate in purine biosynthesis. In view of these facts, it seemed

9) T. Shiro, A. Yamanoi, S. Konishi, S. Okumura, and M. Takahashi, *Agr. Biol. Chem.* (Tokyo), **26**, 785 (1962).
10) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3258 (1967).
11) J.F. Gerster, J.W. Jones, and R.K. Robins, *J. Org. Chem.*, **28**, 945 (1963).
12) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **33**, 2583 (1968).
13) K. Isono and S. Suzuki, *J. Antibiotics* (Japan), **13A**, 270 (1960).
14) A. Yamazaki, I. Kumashiro, and T. Takenishi, *J. Org. Chem.*, **32**, 3032 (1967).

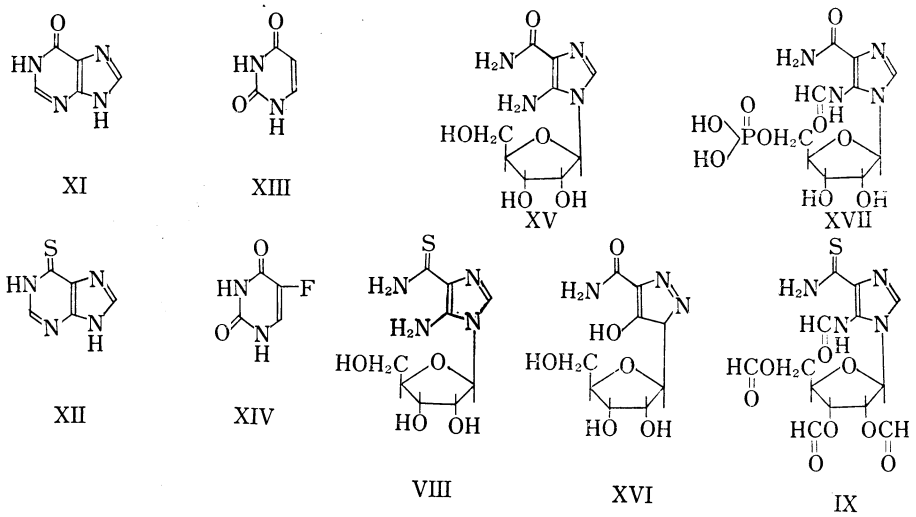


Chart 3

highly probable that some imidazole derivatives should also be antimetabolites because of the existence of AICAR (5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide-5′-phosphate) and SAICAR (5-amino-4-imidazole-N-succinocarboxamide ribonucleotide) in metabolic pathways. Thus, thio-AICA-riboside, which was structually similar to AICA-riboside, was synthesized[4] and found to have moderate antitumor activity.[7] Interestingly, the antibiotic pyrazomycin (XVI), which was extremely similar to AICA-riboside, was isolated and proved to have antivirus activity in 1969.[15] The compound IX is also an analog of 5-formamido-1-β-D-ribofuranosyl-4-imidazolecarboxamide-5′-phosphate (XVII), which is a precursor of purine nucleotide, and has been found to have stronger antitumor activity[16,17] than that of thio-AICA-riboside. This was to be expected, since thio-AICA (5-amino-4-imidazolethiocarboxamide) was completely inactive but its formyl derivative, 5-formamido-4-imidazolethiocarboxamide, was as active as 6-mercaptopurine.[7] Hoshi, *et al.* [16,17] have reported that IX was active against a variety of tumors and, moreover, active against L-1210 leukemia. Therapeutically, IX has been considered to be more advantageous[16] than 6-mercaptopurine which is in clinical use. On the other hand, the introduction of acetyl group to 5-amino group led to loss of activity.[16] These findings indicate that the activity of IX might be a result of its cyclization *in vivo* to thioinosine as in the case of 5-formamido-4-imidazolethiocarboxamide.

Experimental[18]

2-Methyl-6-chloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (IIIa)——2-Methylinosine (Ia, 30 g)[10] was dissolved in a solution of pyridine (360 ml) and acetic anhydride (300 ml), and the solution was allowed to stand at room temperature overnight. After the reaction, the solvent was removed *in vacuo*, about 80 ml of ethanol was added, and the solution was concentrated. This procedure was repeated five times to decompose acetic anhydride completely. To a stirred suspension of the above gummy 2′,3′,5′-tri-

15) H.A. Sober, "Handbook of Biochemistry," 2nd ed., Chemical Rubber Co., Cleveland, Ohio, 1970, p. G–194.
16) A. Hoshi, Y. Ohsaka, T. Nishimoto, F. Kanzawa, and K. Kuretani, *Gann*, **61**, 383 (1970).
17) A. Hoshi, F. Kanzawa, and K. Kuretani, *Cancer Chemother. Rep. Part* **1**, **55**, 229 (1971).
18) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer.

O-acetyl-2-methylinosine (IIa, 5 g) in phosphoryl chloride (30 ml), N,N-dimethylaniline (2 ml) was added and the mixture was refluxed for 3 min, during which the color of the solution turned to yellowish green. After cooling, the reaction mixture was poured into an excess of ice-water with stirring. The product was extracted six times with 50 ml-portions of chloroform. The combined chloroform extracts were washed with 180 ml of cold 1N hydrochloric acid to remove N,N-dimethylaniline, cold water, and 5% sodium hydrogen carbonate. After being dried over anhydrous sodium sulfate, the chloroform extracts were evaporated *in vacuo* to give a gummy product, which was crystallized from ethanol. Recrystallization from ethanol gave 2.3 g of pure crystals. The yield was 44% based on I. mp 111° UV $\lambda_{max}^{pH\ 1}$ mμ: 249.5, 271; $\lambda_{max}^{pH\ 13}$ mμ: 271. *Anal.* Calcd. for $C_{17}H_{19}O_7N_4Cl$: C, 47.84; H, 4.49; N, 13.13. Found: C, 48.27; H, 4.85; N, 13.20.

2-Methyl-6-mercapto-9-β-D-robifuranosylpurine (IVa)——The compound (IIIa, 4 g, 9.4 mmoles) and thiourea (924 mg, 12.1 mmoles) were refluxed in 40 ml of ethanol for 1 hr. After the solvent was removed *in vacuo*, the residue was suspended in 50 ml of water and the product was extracted three times with 40 ml-portions of chloroform. The chloroform extracts were dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, the residue was added to 50 ml of 2N ammonium hydroxide, and the solution was refluxed for 10 min to remove protecting groups. Evaporation of the solvent gave a gummy residue, which was dissolved in a small amount of water. The resulting acetamide was extracted with chloroform. The water layer was concentrated to afford a crystalline mass. The crude product were filtered and recrystallized from water, affording 1.8 g (64%) of pure compounds. The sample was dried at 60° over phosphorus pentoxide for analysis. mp 139—142°. $[\alpha]_D^{25}$ −70.7° (c=1.00, 0.1N NaOH). UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 230 (11600), 330 (22100); $\lambda_{max}^{pH\ 6}$ mμ (ε): 230 (10400), 328 (25500); $\lambda_{max}^{pH\ 13}$ mμ (ε): 239 (s), 315 (19400). *Anal.* Calcd. for $C_{11}H_{14}O_4N_4S \cdot H_2O$: C, 41.77; H, 5.10; N, 17.84. Found: C, 41.68; H, 4.99; N, 17.82.

2-Methyl-6-methylthio-9-β-D-ribofuranosylpurine (V)——The compound (IVa, 1.8 g, 6.04 mmoles) was dissolved in 75 ml of 0.1N sodium hydroxide and, to this, methyl iodide (1.03 g, 7.3 mmoles) was added. The mixture was stirred vigorously at room temperature for 2 hr. The resulting crystals were filtered and recrystallized from water affording 1.3 g (69%) of V. mp 134—135°. $[\alpha]_D^{25}$ −69.6° (c=1.00, 0.1N NaOH). UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 227 (13300), 305 (13800); $\lambda_{max}^{pH\ 6}$ mμ (ε): 225 (13500), 294 (17900); $\lambda_{max}^{pH\ 13}$ mμ (ε): 294 (19600). *Anal.* Calcd. for $C_{12}H_{16}O_4N_4S$: C, 46.14; H, 5.16; N, 17.94. Found: C, 45.98; H, 5.16; N, 17.76.

2-Methyl-9-β-D-ribofuranosylpurine (VI)——To a solution of IVa (1 g) in 50 ml of water was added Raney nickel[19] (2 ml). The solution was refluxed for 3 hr with stirring. After the catalyst was filtered off and washed with water, the filtrate and washings were combined and evaporated to dryness *in vacuo*. The residue was crystallized from a small amount of ethanol. Recrystallization from ethanol gave 430 mg (49%). mp 177—180°. $[\alpha]_D^{25}$ −75.0° (c=1.00, 0.1N NaOH). UV $\lambda_{max}^{pH\ 1}$ mμ (ε): 269 (7200); $\lambda_{max}^{pH\ 6}$ mμ (ε): 246 (3700), 270 (8100); $\lambda_{max}^{pH\ 13}$ mμ (ε): 270 (8400). *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: C, 49.62; H, 5.30; N, 21.04. Found: C, 49.46; H, 5.16; N, 20.81.

2′,3′,5′-Tri-O-acetyl-2-ethylinosine (IIb)——2-Ethylinosine (10 g)[10] was dissolved in a solution of 200 ml of pyridine and 100 ml of acetic anhydride. The solution was allowed to stand at room temperature overnight. Ethanol was added and the solvent was removed *in vacuo*. This procedure was repeated several times to decompose acetic anhydride. The gummy product was added to a small amount of methanol and the solution was left at room temperature for 5 days. The resulting crystals were filtered and recrystallized from methanol to give 7.8 g (58%) of IIb, which was dried at 60° over phosphorus pentoxide. mp 123—125°. *Anal.* Calcd. for $C_{18}H_{22}O_8N_4 \cdot CH_3OH$: C, 50.22; H, 5.73; N, 12.34. Found: C, 50.41; H, 5.44; N, 12.12.

2-Ethyl-6-mercapto-9-β-D-ribofuranosylpurine (IVb)——The compound IIb (4 g) was chlorinated with 20 ml of phosphoryl chloride containing 0.9 ml of N,N-dimethylaniline. The refluxing time was 5 min. After the reaction, the mixture was treated in a manner similar to that described for IIIa to yield a crude product. An analytically pure sample was obtained by recrystallization from water to provide 890 mg (62%) of IVb. mp 171—173°. UV $\lambda_{max}^{pH\ 1}$ mμ (ε).: 229 (8100), 326 (19100); $\lambda_{max}^{pH\ 13}$ mμ (ε): 313 (18800). *Anal.* Calcd. for $C_{12}H_{16}O_5N_4$: C, 46.15; H, 5.13; N, 17.95. Found: C, 45.91; H, 5.29; N, 17.83.

2-Methylthio-6-chloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (IIIc)——2-Methylthioinosine (IIIa, 3 g)[14] was acetylated with acetic anhydride (30 ml) in pyridine (36 ml) as described for IIa to give 2′,3′,5′-tri-O-acetyl-2-methylthioinosine (IIIb, 3.7 g), which failed to crystallize. This product, which was chromatographically homogeneous, showed $\lambda_{max}^{pH\ 1}$ 270, $\lambda_{max}^{pH\ 6}$ 260, and $\lambda_{max}^{pH\ 13}$ 273 mμ in the ultraviolet spectra. A solution of the above IIIb (3.7 g) in phosphoryl chloride (19 ml) and N,N-dimethylaniline (1.2 ml) was refluxed for 10 min. The solution turned to clear green color. After cooling, the mixture was poured into ice-water with vigorous stirring, and the product was extracted with chloroform. The extracts were washed with cold 1N hydrochloric acid, 5% sodium hydrogen carbonate, and cold water. The chloroform layer, dried with anhydrous sodium sulfate, was evaporated *in vacuo* to dryness. The residue was then triturated in a small amount of ethanol, giving a crystalline product. Recrystallization from ethanol gave 2.1 g (55%) of IIIc. mp 104—107°. $[\alpha]_D^{25}$ +9.1° (c=1.00, 0.1N NaOH). UV λ_{max}^{EtOH} mμ (ε): 235 (17500), 265 (12700),

19) A. Domingcez, I.C. Lorez, and R. Franco, *J. Org. Chem.*, **26**, 1625 (1961).

307.5 (9100). *Anal.* Calcd. for $C_{17}H_{19}O_7N_4SCl$: C, 44.50; H, 4.17; N, 12.21. Found: C, 44.39; H, 4.13; N, 12.21.

2-Methylthio-6-mercapto-9-β-D-ribofuranosylpurine (IVc)——To 25 ml of ethanol was added IIIc (2 g, 4.36 mmoles) and thiourea (0.66 g, 8.7 mmoles). The solution was refluxed for 1 hr and treated as described for IVa to give a crude product, which was crystallized from ethanol. After recrystallization from ethanol, 520 mg (36%) of pure crystals were obtained. The sample was dried at 60° over phosphorus pentoxide for 6 hr. mp 187° (decomp.). $[\alpha]_D^{25}$ −39.4° (c=1.00, 0.1N NaOH). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 224 (10000), 266 (9900), 337 (13400); $\lambda_{max}^{pH\,6}$ mμ (ε): 228 (12000), 264 (11100), 333 (16900); $\lambda_{max}^{pH\,13}$mμ (ε): 267 (16200), 321 (14700). *Anal.* Calcd. for $C_{11}H_{14}O_4N_4S\cdot\frac{1}{2}H_2O$: C, 38.94; H, 4.46; N, 16.52. Found: C, 39.24; H, 4.78; N, 16.43.

2′,3′,5′-Tri-O-acetyl-N²-methylguanosine (IId)——N^2-Methylguanosine (Id, 5 g)[14] was acetylated with acetic anhydride (50 ml) in pyridine (100 ml). The reaction mixture was then worked up in the same manner as described for IIa. A crude product was recrystallized from 50% aqueous ethanol, affordeing 6.6 g (93%) of a pure sample. mp 260—261° (decomp.). *Anal.* Calcd. for $C_{17}H_{21}O_8N_5$: C, 48.22; H, 5.00; N, 16.54. Found: C, 47.92; H, 4.98; N, 16.57.

2-Methylamino-6-chloro-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine (IIId)——A solution of IId (5 g) and N,N-dimethylaniline (1.6 ml) in phosphoryl chloride (25 ml) was refluxed for 8 min. The mixture was then worked up in the same manner as described for IIIc. The gummy residue obtained was dissolved in 10 ml of ethanol and the solution was allowed to stand in a refrigerator overnight. The precipitate was filtered and recrystallized from a small amount of ethanol, giving 3.7 g (71%) of a pure compound. mp 105—107°. UV λ_{max}^{EtOH} mμ (ε): 224, 255.5, 322.5. *Anal.* Calcd. for $C_{17}H_{20}O_7N_5Cl$: C, 46.21; H, 4.56; N, 15.85. Found: C, 46.37; H, 5.70; N, 15.68.

2-Methylamino-6-mercapto-9-β-D-ribofuranosylpurine (IVd)——To 50 ml of ethanol were added IIId (2.5 g, 5.67 mmoles) and thiourea (1.08 g, 14.2 mmoles). The mixture was refluxed for 1 hr. The solvent was removed *in vacuo* and, on addition of a small amount of water, the residue crystallized. Recrystallization from aqueous ethanol gave 2.2 g (87%) of 2-methylamino-6-mercapto-9-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)purine. mp>250°. UV λ_{max}^{EtOH} mμ (ε): 214, 265, 348.5. *Anal.* Calcd. for $C_{17}H_{21}O_7N_5S$: C, 46.47; H, 4.82; N, 15.93. Found: C, 46.58; H, 4.99; N, 15.96. To a solution of ethanolic ethoxide (prepared from 0.6 g of metallic sodium and 100 ml of ethanol) was added 2.2 g of the above compound. The mixture was then heated to reflux for 1 hr. After 100 ml of water was added, the solution was neutralized with Amberlite IR-120 (H^+ form). The resin was removed by filtration and washed with water. The filtrate and washings were combined and concentrated under reduced pressure. The resulting crystals were filtered and recrystallized from water to afford 0.85 g (52.5%) of IVd. The sample was dried at 60° for 5 hr. mp 140—142°. $[\alpha]_D^{25}$ −8.0° (c=1.00, 0.1N NaOH). UV $\lambda_{max}^{pH\,1}$ mμ (ε): 269 (9700), 348 (18500); $\lambda_{max}^{pH\,6}$ mμ (ε): 263 (10700), 345 (21100); $\lambda_{max}^{pH\,13}$mμ (ε): 257 (14400), 322 (15400). *Anal.* Calcd. for $C_{12}H_{17}O_4N_5S\cdot\frac{1}{2}H_2O$: C, 42.90; H, 5.40; N, 20.84. Found: C, 42.86; H, 5.67; N, 20.77.

5-Acetamido-1-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-thiocarboxamide (VII)——Thio-AICA-riboside (VIII, 274 mg, 1 mmole)[4] was dissolved in a solution of acetic anhydride (1 ml) and pyridine (4 ml) and the solution was refluxed for 5 min. After the reaction, addition and evaporation of ethanol were repeated several times to decompose acetic anhydride completely. The resulting residue was dissolved in a small amount of chloroform and the solution was applied to a column of alumina. The product was eluted with a solvent of benzene and ethyl acetate (50: 50, v/v). The eluate containing UV absorption was evaporated *in vacuo*, giving 73 mg (17%) of glassy compound. The sample was dried at 60° for 5 hr. $[\alpha]_D^{26}$ −4.0° (c=0.50, pyridine). UV λ_{max}^{EtOH} mμ (ε): 268 (8300), 313 (7400). *Anal.* Calcd. for $C_{17}H_{22}O_8N_4S\cdot\frac{1}{6}$benzene: C, 47.46; H, 5.09; N, 12.33. Found: C, 47.54; H, 5.18; N, 12.32.

5-Formamido-1-(2′,3′,5′-tri-O-formyl-β-D-ribofuranosyl)-4-imidazolethiocarboxamide (IX)——Ten grams of thio-AICA-riboside was dissolved in a mixture of formic acid (147 ml) and acetic anhydride (73 ml) with cooling, and the solution was kept at room temperature overnight. The resulting silky yellowish crystals were filtered, washed with methanol and dried *in vacuo*. The sample showed one spot on TLC and weighted 12.6 g (90%). mp 172° (decomp.). $[\alpha]_D^{25}$ +15.5° (c=1.00, pyridine). UV λ_{max}^{EtOH} mμ (ε): 289 (10200), 337.5 (5300). *Anal.* Calcd. for $C_{13}H_{14}O_8N_4S$: C, 40.41; H, 3.65; N, 14.50. Found: C, 40.60; H, 3.79; N, 14.20.