[Chem. Pharm. Bull. 24(9)2089—2093(1976)]

UDC 547.456'783.04 : 546.185.221.04

Synthesis of 5-Amino-1-(3'-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide

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(Received November 18, 1975)

As an interesting analog of thio-AICA-riboside (5-amino-1-β-D-ribofuranosyl-4-imidazolethiocarboxamide), 3'-deoxy-thio-AICA-riboside (XV) was prepared. 5'-Acetyl-AICA-riboside (X) synthesized from Ip-AICA-riboside (VIII) was allowed to react with triphenylphosphine and carbon tetrachloride to give, after ammoniacal treatment, the 3'-chloro derivative (XI). Treatment of the compound XI with alkali gave a epoxide derivative (XVI). Hydrogenation of XI followed by acetylation gave 2',5'-diacetyl derivative (XIII). When XIII was thiolated with phosphorus pentasulfide, 2',5'-di-O-acetyl-thio-AICA-riboside (XIV) formed. Deacetylation of XIV with ammonia furnished XV, which did not show any significant antitumor activity.

The AICA-riboside analogs, thio-AICA-riboside (5-amino-1-β-D-ribofuranosyl-4-imidazolethiocarboxamide),[3] was synthesized in our laboratories in expectation of its inhibition of *de novo* purine ribonucleotide synthesis and demonstrated a moderate antitumor activity.[4] In this connection, a closely related compound, 5-formamido-1-(2',3',5'-tri-O-formyl-β-D-ribofuranosyl)-4-imidazolethiocarboxamide,[5] was prepared and proved to show a strong antitumor activity.[6] The compound, however, lacks water solubility, so that it is not satisfactory for intravenous administration. Interestingly, pyrazomycin[7] and virazole[8] reported in other laboratories are also structually similar to AICA-riboside and have a significant biological activity.

The impressive biological activity of the above AICA-riboside analogs encouraged a more extensive synthetic effort. It seemed desirable to prepare some derivatives of thio-AICA-riboside that would have both a strong antitumor activity and sufficient water solubility. As such compounds, 2'-deoxy-thio-AICA-riboside(5-amino-1-(2'-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide) and 3'-deoxy-thio-AICA-riboside (XV, 5-amino-1-(3'-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide) were selected. The present paper reports the synthesis of the latter.

Our initial approach to 2'-deoxy- or 3'-deoxy-thio-AICA-riboside involved a synthetic route *via* the imidazole cyclonucleoside having 2,2'- or 2,3'-anhydro linkage. The synthesis of such anhydro nucleosides was begun with the previously reported bromination[9] of 5-amino-

1) This work was presented at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Sendai, April, 1974.
2) Location: *Suzuki-cho, Kawasaki, 210, Japan.*
3) A. Yamazaki, I. Kumashiro, T. Takenishi, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **16**, 2172 (1968).
4) A. Hoshi, Y. Ohsaka, T. Nishimoto, and K. Kuretani, *Pharmacometrics*, **4**, 1(1970); A. Hoshi, K. Kumagai, and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **16**, 2028 (1968); A. Hoshi, Y. Ohsaka, and K. Kuretani, *ibid.*, **17**, 1720 (1969).
5) A. Yamazaki, T. Furukawa, M. Akiyama, M. Okutsu, I. Kumashiro, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **21**, 692 (1973).
6) A. Hoshi, Y. Ohsaka, T. Nishimoto, F. Kanzawa, and K. Kuretani, *Gann*, **61**, 383 (1970); A. Hoshi, F. Kanzawa, and K. Kuretani, *Cancer Chemother. Rep. Part* **1**, **55**, 229 (1971).
7) *Chem. Eng. News*, 43 (Sept 15, 1969).
8) J.T. Witkowski, R.K. Robins, R.W. Sidwell, and L.N. 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).
9) Dr. Suzuki, and M. Ozaki in our laboratories reported the bromination at the 23rd Annual meeting of the Chemical Society of Japan, Tokyo, (1970).

1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-imidazolecarboxamide (VIII)[10] with N-bromosuccinimide in refluxing chloroform to afford 5-amino-2-bromo-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-imidazolecarboxamide (I) in 52% yield. The compound I was then treated with an excess of potassium sulfide in a sealed tube at 100° for 5—6 hr, giving 5-amino-2-mercapto-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-imidazolecarboxamide(II) in 58% yield. In this case, the use of thiourea was not effective. Acetylation of II with acetic anhydride in pyridine followed by deacetonation in 98% formic acid furnished 5-amino-2-mercapts-1-

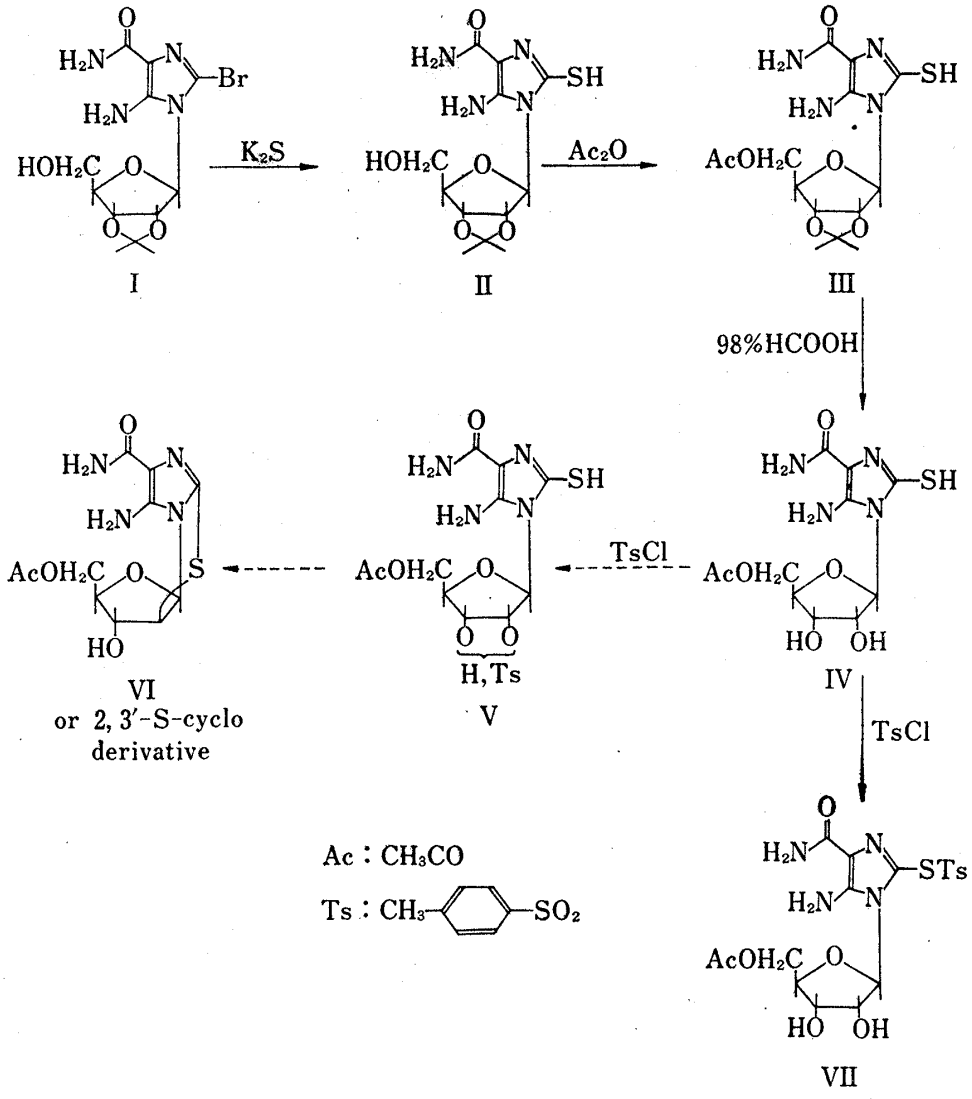

Chart 1

(5′-acetyl-β-D-ribofuranosyl)-4-imidazolecarboxamide (IV). Reaction of IV with tosyl chloride in pyridine gave the tosylated product which was not crystallized but was desulfurized with Raney nickel to yield, after ammoniacal treatment, the starting AICA-riboside. This suggests that the above tosylated derivative was not the desired intermediate (V) for cyclonucleoside synthesis. Clearly, tosylation occurred at the 2-mercapto group, giving a S-tosylated compound (VII).

A second approach to the key intermediate 3′-deoxy-AICA-riboside (XII, 5-amino-1-(3′-deoxy-β-D-ribofuranosyl)-4-imidazolecarboxamide) was based on the synthesis of 3′-

10) T. Mori, T. Saito, T. Kato, and T. Takenishi, Japan Patent 6465 (1966) [*Chem. Abstr.*, 63, 5731*b* (1966)].

chloro-9-β-D-xylofuranosylhypoxanthine by the procedure of Haga, *et al.*[11] Compound VIII was acetylated with acetic anhydride in pyridine and the product was deacetonated in 30% formic acid, affording 5-amino-1-(5′-acetyl-β-D-ribofuranosyl)-4-imidazolecarboxamide (X). When the reaction of X with triphenylphosphine and carbon tetrachloride at 110° for 6 hr was carried out, the major product (45%) was 5-amino-1-(5′-acetyl-3′-chloro-3′-deoxy-β-D-xylofuranosyl)-4-imidazolecarboxamide and the minor product (15%) was the starting material, 5′-acetyl-AICA-riboside. After the reaction mixture was treated with ammonia and purified by alumina column chromatography, the desired 5-amino-1-(3′-chloro-3′-deoxy-β-D-xylofuranosyl)-4-imidazolecarboxamide (XI) was isolated in a poor yield (10%). The structure of XI was demonstrated by alkaline treatment and catalytic hydrogenation to give 5-amino-1-(2′,3′-anhydro-β-D-ribofuranosyl)-4-imidazolecarboxamide (XVI) and 3′-deoxy-AICA-riboside, respectively, whose structures were confirmed by the nuclear magnetic resonance (NMR) spectra. The 100-MHz NMR spectrum of 3′-deoxy-AICA-riboside in D_2O showed two protons (3′-2H) at δ 2.24 as multiplet, two protons (5′-2H) at δ 3.76 as eight line multiplet, a proton (4′-H) at δ 4.57 as multiplet, a proton (2′-H) at δ 4.77 as multiplet, a proton (1′-H) at δ 5.62 as a doublet and a proton (2-H) at δ 7.46 as a singlet. The above assignment was confirmed by spin decoupling experiments: irradiation of the 2′-protons reduced the multiplet of the

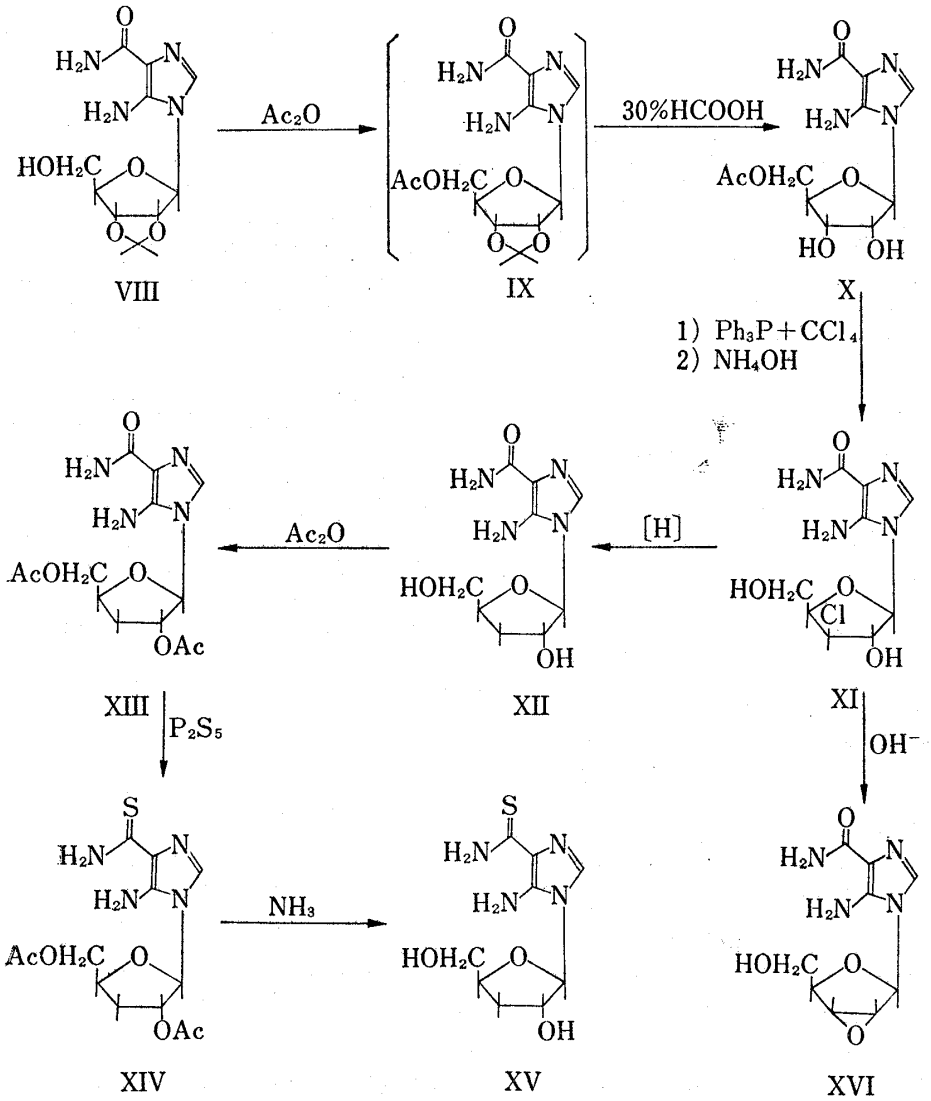

Chart 2

11) K. Haga, M. Yoshikawa, and T. kato, *Bull. Chem. Soc. Japan*, **43**, 3922 (1970).

3′-proton to a doublet of doublets, and further irradiation of the 4′-proton collapsed the multiplet of the 5′-proton to a doublet of doublets, which was characteristic of methylene protons adjacent to carbon possessing three kinds of different substituents. The spectrum of XVI was also informative; the C-1′ proton gave a singlet at δ 5.95 which would indicate a *trans* relationship between 1′-H and 2′-H protons, typical of 2′,3′-anhydro compounds of the ribofuranosyl type.

After acetylation of 3′-deoxy-AICA-riboside with acetic anhydride in pyridine, the resulting diacetyl derivative (XIII) was thiolated with phosphorus pentasulfide in refluxing pyridine to afford 5-amino-1-(2′,5′-diacetyl-3′-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide (XIV) in 32% yield from XIII. Deacetylation of XIV gave the final product, 3′-deoxy-thio-AICA-riboside. This compound proved to be quite unstable in acidic solution in common with deoxypurine nucleosides. Thus the attempted formylation of 3′-deoxy-thio-AICA-riboside with acetic anhydride and formic acid led to the isolation of 5-amino-4-imidazolethiocarboxamide.

Our success in preparing the tetraformyl derivative of thio-AICA-riboside has prompted us to investigate structures related to thio-AICA-riboside. However, the newly synthesized compounds, XI, XII, XV, and XVI, did not show any significant antitumor activity.[12]

The synthesis of 2′-deoxy-thio-AICA-riboside is now under investigation.

Experimental[13]

5-Amino-2-mercapto-1-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-imidazolecarboxamide (II)——To 60 ml of EtOH was added 25 g of K_2S and the solution was refluxed for 10 min. After filtration, the 2-bromo derivative (I, 9 g, 23.9 mmole) was added to the hot filtrate, and the mixture was heated in a sealed tube at 110° for 6 hr. An insoluble material was filtered, the filtrate was adjusted to pH 6.0, and the solution was concentrated to dryness. A small amount of H_2O was added and the mixture was triturated, giving a yellow crystal. Recrystallization from EtOH afforded 4.6 g (58%) of II, mp 237—238° (decomp.); *Rf* 0.69 (solvent A); 0.75 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 299 (14700); $\lambda_{max}^{pH\ 7}$ nm (ε): 298 (15200); $\lambda_{max}^{pH\ 13}$ nm (ε): 268 (sh), 298 (9300). *Anal.* Calcd. for $C_{12}H_{18}O_5N_4S$: C, 43.63; H, 5.49; N, 16.96. Found: C, 43.70; H, 5.57, N, 16.96.

5-Amino-2-mercapto-1-(5′-acetyl-2′,3′-O-isopropylidene-β-D-ribofuranosyl)-4-imidazolecarboxamide (III)——To a stirred solution of II (1.0 g, 3.0 mmole) in pyridine (25 ml), Ac_2O (408 mg, 4.0 mmole) was added portionwise. The compound dissolved gradually and, after 30 min, a clear solution was obtained. This was stirred at room temperature overnight, during which time crystallization occurred. The resulting crystals were filtered and recrystallized from EtOH to give 850 mg (75%) of white crystals, mp 231° (decomp.); *Rf* 0.76 (solvent A), 0.81 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 298 (15300); $\lambda_{max}^{pH\ 7}$ nm (ε): 296 (17500); $\lambda_{max}^{pH\ 13}$ nm (ε): 270 (sh), 298 (8500). *Anal.* Calcd. for $C_{14}H_{20}O_6N_4S$: C, 45.15; H, 5.41; N, 15.05. Found: C, 45.21; H, 5.53; N, 14.99.

2-Mercapto-1-(5′-acetyl-β-D-ribofuranosyl)-4-imidazolecarboxamide (IV)——A solution of III (1.4 g) in 98% HCOOH (20 ml) was stirred at room temperature for 1 hr. Evaporation and subsequent co-distillation with EtOH were repeated several times, giving a gum. This was triturated in AcOEt to give an amorphous powder (1.0 g), which was homogeneous by thin-layer chromatography and paper chromatography; *Rf* 0.39 (solvent A); 0.37 (solvent B).

5-Amino-1-(5′-acetyl-β-D-ribofuranosyl)-4-imidazolecarboxamide (X)——Compound VIII (5.96 g, 20 mmole) was added to a solution of triethylamine (5 ml) and $CHCl_3$ (2.86 g, 27.8 mmole) was added portionwise. The mixture was stirred at 55—56° for 5 hr. Within about 2 hr, it became clear. The solution was then poured into stirred ice-water to decompose Ac_2O. Evaporation of the solvent *in vacuo* left a gum (IX), 300 ml of 30% HCOOH was added, and the solution was allowed to stand at room temperature for 3 days to remove the isopropylidene group. The solvent was removed *in vacuo* at below 30° to afford a gum. Addition of EtOH and evaporation of the solvent were repeated several times until the smell of HCOOH disappeared. Finally, water (20 ml) was added, the solution was extracted with $CHCl_3$ (3 × 5 ml), and the water layer was concentrated *in vacuo*. The residue was crystallized from EtOH to give 3.8 g (63%) of white crystals; mp 168—169°;

12) Private communication by Drs. Hoshi and Kuretani of National Cancer Center Research Institute.

13) All melting points are uncorrected. Ultraviolet (UV) absorption spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer, and the NMR spectra were measured with a Varian HA-100 using DSS as an internal standard. All chromatographies were performed on Toyo No. 51 filter paper by the ascending technique. Solvent systems: A, *n*-PrOH–NH_3 (28%)–H_2O (20: 12: 3, v/v); B, *n*-BuOH–AcOH–H_2O (4: 1: 1, v/v).

Rf 0.36 (solvent A); 0.38 (solvent B); *Anal.* Calcd. for $C_{11}H_{16}O_6N_4$: C, 44.00; H, 5.37, N, 18.66. Found: C, 44.12; H, 5.41; N, 18.53.

5-Amino-1-(3′-chloro-3′-deoxy-β-D-xylofuranosyl)-4-imidazolecarboxamide (XI)——To 160 ml of triethylphosphate, triphenylphosphine (23.5 g, 0.09 mole), CCl_4 (66 ml), and compound X (9.09 g, 0.03 mole) were added and the solution was heated under reflux with stirring for 6 hr. After cooling, the mixture was poured into 500 ml of ether and the ether layer was removed by decantation. The residual black gum was dissolved in $CHCl_3$ (100 ml), the solution was extracted with H_2O until the extract became free from ultraviolet absorption, and the extracts were combined and evaporated *in vacuo* to give a gum. This was then dissolved in a mixture of MeOH (100 ml) and conc. NH_4OH (30 ml) and stirred at 0° for 5 hr. The solvent was removed *in vacuo*, H_2O (100 ml) was added, and, after removing a small amount of insoluble material, the solution was concentrated to dryness. A solution of the residue in 30 ml of MeOH was applied to an alumina column (2.5×100 cm). After washing with benzene (150 ml), ether (150 ml), and MeOH–ether (1: 4, v/v, 200 ml), the column was eluted with MeOH-ether (1: 1, v/v). The combined fractions containing the desired product were concentrated to dryness, giving a gum, which was crystallized from EtOH. The resulting crystals were collected and dried. Yield, 0.85 g (10%); mp 180—181°; *Rf* 0.83 (solvent A); 0.87 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 266 (10300); $\lambda_{max}^{pH\ 7}$ nm (ε): 265 (11000); $\lambda_{max}^{pH\ 13}$ nm (ε): 266 (12000). *Anal.* Calcd. for $C_9H_{13}O_4N_4Cl$: C, 39.07; H, 4.74; N, 20.25. Found: C, 39.57; H, 5.00; N, 20.13.

5-Amino-1-(3′-deoxy-β-D-ribofuranosyl)-4-imidazolecarboxamide (3′-Deoxy-AICA-riboside, XII)——To a solution of XI (2 g, 7.2 mmole) in aqueous 50% MeOH (200 ml), Raney nickel (7.2 g, wet volume) was added and the mixture was refluxed with stirring for 5 hr. The catalyst was filtered off, and the filtrate was concentrated to dryness under reduced pressure. The residue was crystallized from MeOH to give 0.9 g (51%) of XII; mp 218—219°; *Rf* 0.40 (solvent A); 0.60 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε); 267 (11500); $\lambda_{max}^{pH\ 7}$ nm (ε): 266 (12000); $\lambda_{max}^{pH\ 13}$ nm (ε): 267 (12200). *Anal.* Calcd. for $C_9H_{14}O_4N_4$: C, 44.62; H, 5.83; N, 23.13. Found: C, 44.39; H, 6.00; N, 22.96.

5-Amino-1-(2′,5′-diacetyl-3′-deoxy-β-D-ribofuranosyl)-4-imidazolecarboxamide (XIII)——Compound XII (0.95 g, 3.93 mmole) was dissolved in a solution of pyridine (23 ml) and Ac_2O (1 ml), and the solution was allowed to stand at room temperature overnight. After the reaction, the solution was poured into ice-water (30 ml) and extracted three times with 30 ml portions of $CHCl_3$. The combined extracts, dried over anhydrous Na_2SO_4, were evaporated to dryness under reduced pressure. The resulting residue was crystallized from a small amount of EtOH, giving a crude product. Recrystallization from MeOH afforded 895 mg (70%) of colorless crystals; mp 139—140°; *Rf* 0.68 (solvent A); 0.63 (solvent B); $\lambda_{max}^{pH\ 1}$ nm (ε): 268 (10500); $\lambda_{max}^{pH\ 7}$ nm (ε): 266 (12100); $\lambda_{max}^{pH\ 13}$ nm (ε): 267 (12000). *Anal.* Calcd. for $C_{13}H_{18}O_6N_4 \cdot 1/2\ H_2O$: C, 46.57; H, 5.71; N, 16.71. Found: C, 46.61; H, 5.73; N, 16.51.

5-Amino-1-(2′,5′-diacetyl-3′-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide (XIV)——To a solution of XIII (326 mg, 1 mmole) in pyridine (5 ml) was added P_2S_5 (220 mg) with stirring. The solution was heated under reflux for 7 hr. The resulting brown mixture was poured into ice-water (50 ml) and extracted three times with 50 ml portions of $CHCl_3$. The combined extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness. The residue was crystallized from EtOH, giving 110 mg (32%) of XIV, mp 214—215°; *Rf* 0.61 (solvent A); 0.62 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 278 (9600), 324 (16500); $\lambda_{max}^{pH\ 7}$ nm (ε): 271 (9800), 325 (15400); $\lambda_{max}^{pH\ 13}$ nm (ε): 270 (8900), 326 (15100). *Anal.* Calcd. for $C_{13}H_{18}O_5N_4S$: C, 45.60; H, 5.30; N, 16.37. Found: C, 45.86; H, 5.35; N, 16.10.

5-Amino-1-(3′-deoxy-β-D-ribofuranosyl)-4-imidazolethiocarboxamide (3′-Deoxy-thio-AICA-riboside, XV)——Compound XIV (369 mg) was dissolved in 30 ml of MeOH saturated with NH_3 at 0° and the solution was allowed to stand at room temperature overnight. The solvent was removed *in vacuo* to afford a gum, which was crystallized from MeOH. The product was dried and weight 160 mg (59%) of needles; mp 179—180°; *Rf* 0.48 (solvent A); 0.69 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 279 (9400), 325 (15900); $\lambda_{max}^{pH\ 7}$ nm (ε): 271 (9000), 325 (15500); $\lambda_{max}^{pH\ 13}$ nm (ε): 271 (9200), 326 (15700). *Anal.* Calcd. for $C_9H_{14}O_3N_4S$: C, 41.85; H, 5.46; N, 21.69. Found: C, 41.63; H, 5.46; N, 21.82.

5-Amino-1-(2′,3′-anhydro-β-D-ribofuranosyl)-4-imidazolecarboxamide (XVI)——A solution of XI (1.0 g) in 30 ml of conc. NH_4OH was allowed to stand at room temperature overnight. The solution was then concentrated to dryness *in vacuo*. The residue was then crystallized from H_2O to give 1.0 g (86%) of white crystals; mp 205—206°; *Rf* 0.56 (solvent A); 0.56 (solvent B); UV $\lambda_{max}^{pH\ 1}$ nm (ε): 267 (11000); $\lambda_{max}^{pH\ 7}$ nm (ε): 265 (11700); $\lambda_{max}^{pH\ 13}$ nm (ε): 265 (11400). *Anal.* Calcd. for $C_9H_{12}O_4N_4$: C, 45.00; H, 5.04; N, 23.32. Found: C, 45.14; H, 5.32; N, 23.07.

Acknowledgement The authors express their gratitude to Dr. I. Kumashiro of Ajinomoto Co., Inc. for his encouragement throught the course of this work and Professor M. Ikehara of the Osaka University for his helpful suggestions.