The Synthesis of 2-Azainosine and Related Derivatives by Ring Annulation of Imidazole Nucleosides (1)

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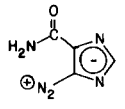
The synthesis of 7-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**6b**, 2-azainosine) and 5-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**4b**) have been achieved for the first time by direct diazotization of AICA riboside (**5b**) and *iso*-AICA riboside (**3b**), respectively. The conditions required for cyclization of the model methyl bases, **3a** and **5a**, as well as the nucleosides **3b**, **5b**, and **7** are described.

The chemotherapeutic and biological properties of certain azapurines are well documented and have been recently discussed in an excellent review (2). 8-Azainosine (3), a highly cytotoxic nucleoside, has been reported (4) to function as a substrate for a kinase and to undergo some of the anabolic reactions of IMP. This prompted us to initiate an investigation involving the closely related 2-azapurine nucleosides. A survey of the literature revealed that only two 2-azapurine nucleosides were known (5a,b) and, in fact, only a limited amount of research had been conducted on the parent imidazo[4,5-*d*]-*v*-triazine ring system, *per se* (6a,b). In view of these findings, we elected to conduct our preliminary work on the two model methyl compounds; 4-amino-1-methylimidazole-5-carboxamide (**3a**) (7) and 5-amino-1-methylimidazole-4-carboxamide (**5a**) (8) rather than the relatively inaccessible and expensive nucleosides.

Treatment of **3a** or **5a** with 2.4 equivalents of sodium nitrite furnished the imidazo[4,5-*d*]-*v*-triazines **4a** and **6a** in good yield. Evidence for complete ring closure was established by elemental analyses and pmr spectroscopy. The absence of absorption peaks for the amino and carboxamide protons which are found in the pmr spectra of **3a** and **5a** indicated that ring closure had occurred. The appearance of an NH singlet was also observed between δ 15.0-15.2 in the spectra of **4a** and **6a** which confirmed that ring closure had indeed occurred. The extreme downfield shift of the NH proton appears to be a characteristic trait of the 2-azapurin-6-ones. In all experiments, sodium nitrite was added to an acidic solution of the starting compound in order to eliminate the possible formation of undesirable side products (9). The possibility of isolating a stable diazo intermediate from the reaction mixture was excluded, since with ring *N*-substituted *o*-aminoimidazolecarboxamides the formation of an internally compensated zwitterion (**1**) (10) is not feasible. This single factor is probably the primary reason that DIC riboside (**2**) (11) has not been obtained *via* a coupling reaction (10). It is interesting to note that the infrared spectra of all diazotized reaction products in this investigation did not exhibit the characteristic diazo absorption band in the region of 2200 cm^{-1}.



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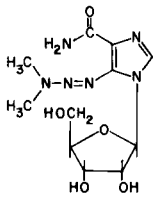

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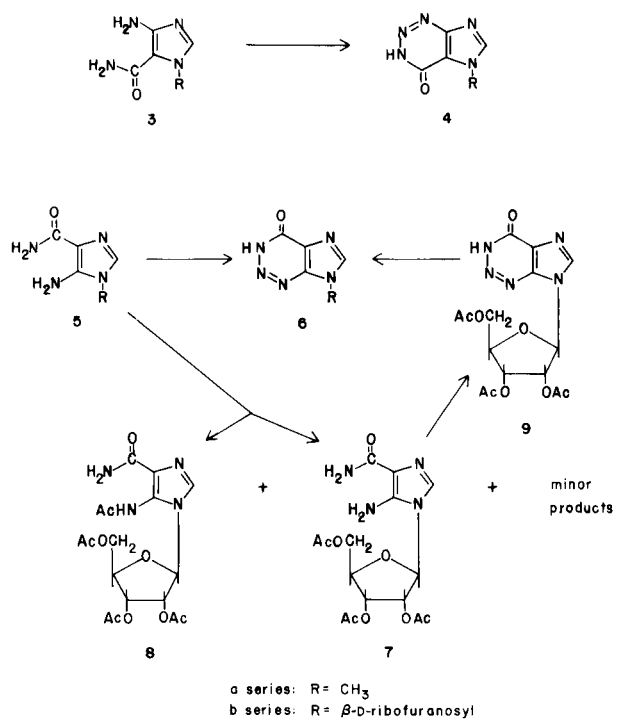
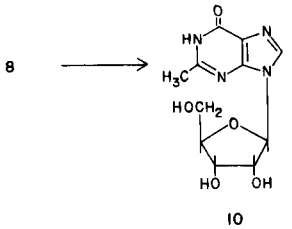
A striking similarity was observed in certain physical properties, *e.g.*, m.p., pmr and chromatographic mobilities, of the isomeric 5- and 7-methylimidazo[4,5-*d*]-*v*-triazin-4-ones (**4a** and **6a**) with the only major exception being their ultraviolet spectra. This same trend was subsequently observed with the corresponding 2-azapurine nucleosides, **4b** and **6b**.

Our initial effort to synthesize a 2-azapurine nucleoside was *via* 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**7**). The nucleoside **7** was selected as starting material because the acetylated ribosyl moiety would allow a facile extraction of the final product away from residual salts. The majority of nucleosides with a *C-N* glycosyl bond are labile under strongly acidic conditions (12) and therefore, acetylation of **5b** was especially desirable since this should impart an additional degree of stability to the glycosyl bond of **7** during

REACTION SCHEME



diazotization. AICA riboside (**5b**) (11) was acetylated according to the reported procedure (13). We carefully repeated this reaction 4 times, adhering very closely to the available experimental details (14). However, tlc of each reaction mixture in several solvent systems showed them to be a mixture of 5 components (15) with **7** being the major constituent. A slower moving band furnished a nucleoside which was assigned the structure 5-acetamido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**8**) on the basis of pmr spectroscopy and elemental analysis. This assignment was corroborated by the conversion of **8** to 2-methylinosine (**10**) (15). In an effort to exclude the formation of **8** and increase the yield of **7**, we modified the acetylation procedure. It was found that **7** could be prepared in 79% yield by treating **5b** with acetic anhydride and pyridine at room temperature. Although this procedure was more inconvenient, **7** was the only product isolated from the reaction mixture.



The diazotization of **7** to afford 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**9**) was first attempted using very mild methods of diazotization. Ring closure of **7** with sodium nitrite and acetic acid (Method B for **4a**), sodium nitrite and Amberlite IR-120 (H^+) (Method A for **6a**) and various procedures using isoamyl nitrite (17) all proved fruitless. A successful ring closure was finally attained when **7** was diazotized with 3 equivalents of sodium nitrite in 6 *N* hydrochloric acid at -30°. The reaction mixture was carefully neutralized with concentrated ammonium hydroxide and purified by silica gel column chromatography to afford a crystalline product. The ultraviolet spectrum revealed that ring annulation had occurred and the pmr spectrum established that the solid was nucleoside material. Therefore, the solid was assigned the structure **9**. Deacetylation of **9** with methanolic ammonia at room temperature provided 7-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**6b**, 2-azainosine).

The successful synthesis of **9** from **7** at -30° under strongly acidic conditions prompted us to attempt the synthesis of 2-azainosine (**6b**) directly from AICA riboside (**5b**). A facile synthesis of **6b** from **5b** was accomplished using the above reaction conditions which eliminated two steps and increased the overall yield. The procedure differed only in the final method of purification where a Sephadex G-10 column was used for the removal of salts formed during neutralization. A similar reaction sequence, using 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (18) (**3b**, *iso*-AICA riboside) and 4 *N* hydrochloric acid, furnished 5-(β-D-ribofuranosyl)imidazo-[4,5-*d*]-*v*-triazin-4-one (**4b**) in good yield. We have now established that certain *o*-aminoimidazolecarboxamide nucleosides can be diazotized and ring closed to their respective imidazo[4,5-*d*]-*v*-triazine nucleoside counterparts using strongly acidic conditions. It would appear that the low reaction temperatures employed in this study have prevented any significant cleavage of the glycosyl bond without affecting the success of ring annulation. This diazotization procedure should find wide application in the nucleoside area, especially with other *o*-aminocarboxamide nucleosides, and thus lead to a variety of new and interesting bicyclic-*v*-triazine nucleosides.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. The proton magnetic resonance spectra were obtained on a Varian A-56/60 spectrometer using DSS as an internal standard. The infrared spectra were determined in pressed potassium bromide disks with a Beckman IR-8 spectrophotometer. The ultraviolet absorption spectra were recorded on a Beckman DK-2 spectrometer. The optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital read-out polarimeter. Thin layer chromatography was run on glass plates coated (250-μ)

with SilicAR 7 GF (Mallinckrodt). Silica gel suitable for column chromatography was purchased from J. T. Baker Chemical Co. All solvent proportions were by volume. Paper chromatography was conducted on Whatman No. 1 chromatographic paper and the descending technique was used. Shortwave ultraviolet light (254 nm) was used to detect the spots and the chromatographic solvent systems used were: A, 5% aqueous ammonium bicarbonate (w/w); B, 1-butanol saturated with water; C, 1-propanol-ammonium hydroxide (sp gr 0.90)-water, 6:3:1 (v/v); D, ethanol-water, 7:3 (v/v); E, 1-butanol-acetic acid-water, 4:1:1 (v/v). Sephadex G-10 (Pharmacia) was allowed to stand for 12 hours in a 1% sodium chloride solution. The gel was then washed and equilibrated with distilled water before being used for desalting. The Sephadex G-10 column (2 x 37 cm) had a void volume of 70 ml. (Blue Dextran 2000) and a flow rate of 0.6 ml./minute. Elemental analyses were performed by Heterocyclic Chemical Corporation, Harrisonville, Missouri.

5-Methylimidazo[4,5-*d*]-*v*-triazin-4-one (**4a**).

Method A.

4-Amino-1-methylimidazole-5-carboxamide (**3a**) (7) (2.0 g., 14.3 mmoles) was dissolved in 2 *N* hydrochloric acid (40 ml.) and the solution cooled to 0° (Dry Ice-ethanol). To this solution was added, in one portion, a solution of sodium nitrite (2.4 g., 34.8 mmoles) in water (24 ml.). The stirred reaction mixture was maintained at 0° for 15 minutes. The cooling bath was then removed and as the reaction solution gradually warmed to room temperature (*ca.* 10-15 minutes) a light yellow solid separated from solution. The micro-crystalline solid was collected by filtration and washed with cold water (2 x 10 ml.) to provide 1.5 g. of pure **4a**, m.p. 199-200° (explodes). The filtrate was concentrated to *ca.* 15 ml. and after standing at 4° for 18 hours afforded an additional 0.25 g. of **4a** (total yield, 81%), m.p. 199-200° (explodes). A small sample was recrystallized from water, m.p. 199-200° (explodes); uv (ϵ x 10^{-3}) λ max (*p*H 1) 277 nm (3.46), 252 (4.56); λ min (*p*H 1) 267 nm (2.33), 236 (3.48); λ max (*p*H 11) sh 285 nm (5.27), 264 (6.05); λ min (*p*H 11) 238 nm (1.92); λ max (methanol) 275 nm (3.51), 253 (4.46); λ min (methanol) 269 nm (3.46), 235 (3.05); pmr (DMSO-d_6) δ 4.10 (s, 3, 5-CH_3), 8.50(s, 1, 6-H), 15.07 (bs, 1, NH); R_f values A, 0.69; B, 0.36; C, 0.50; D, 0.71; E, 0.54.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.74; H, 3.33; N, 46.34. Found: C, 39.49; H, 3.45; N, 46.51.

Method B.

To a cold solution (12°) of **3a** (0.25 g., 1.79 mmoles) in glacial acetic acid (10 ml.) was added, in one portion, a solution of sodium nitrite (0.3 g., 4.34 mmoles) in water (3 ml.). After 10 minutes, the ice bath was removed and the reaction solution allowed to warm to room temperature. The solution was stirred for 30 minutes at room temperature and then evaporated to dryness *in vacuo* (40°). The residue was triturated with cold water (5 ml.) and taken to dryness *in vacuo*. This procedure was repeated twice. The solid was dissolved in water (15 ml.) and then allowed to stand at 4° for 6 hours. The yellow solid was collected by filtration and air dried to furnish 0.18 g. (66.7%) of **4a**, m.p. 197-198° (explodes); uv, ir, pmr and chromatographic mobilities were identical with those of **4a** obtained by Method A.

7-Methylimidazo[4,5-*d*]-*v*-triazin-4-one (**6a**).

Method A.

5-Amino-1-methylimidazole-4-carboxamide (**5a**) (8) (2.00 g., 14.3 mmoles) was dissolved in 6 *N* hydrochloric acid (60 ml.) and the solution cooled to 0° (Dry Ice-ethanol). To this solution was added, in one portion, a solution of sodium nitrite (2.4 g., 34.8 mmoles) in water (24 ml.). The stirred reaction mixture was maintained at 0° for 10 minutes and then allowed to warm to room temperature. After stirring for 1 hour at room temperature, the reaction solution was evaporated to dryness *in vacuo* (40°). The residue was triturated with water (15 ml.) and evaporated to dryness *in vacuo*. The solid was then dissolved in hot water (40 ml.) and allowed to stand at room temperature for 16 hours. The crystalline solid was collected by filtration and washed with cold water (10 ml.) to furnish 1.58 g. of **6a**, m.p. 202-203° (explodes). The combined wash and filtrate was concentrated to *ca.* 15 ml. and allowed to stand at 4° for 16 hours to provide a second crop (0.26 g.) of **6a** (total yield, 84%), m.p. 203° (explodes). An analytical sample was prepared by recrystallization from water, m.p. 205-206° (explodes); uv (ϵ x 10^{-3}) λ max (*p*H 1) 286 nm (5.17), sh 245 (4.45); λ min (*p*H 1) 258.5 nm (2.77); λ max (*p*H 11) 292 nm (7.41), 250.5 (5.88); λ min (*p*H 11) 264 nm (3.24), 235.5 (3.64); λ max (methanol) 287 nm (5.48), sh 245 (4.32); λ min (methanol) 257.5 nm (2.66); pmr (DMSO-d_6) δ 4.03 (s, 3, 7-CH_3), 8.50 (s, 1, 6-H), 15.10 (bs, 1, NH); R_f values A, 0.74; B, 0.30; C, 0.49; D, 0.69; E, 0.50.

Anal. Calcd. for $C_5H_5N_5O \cdot 0.5H_2O$: C, 37.50; H, 3.78; N, 43.73. Found: C, 37.78; H, 3.74; N, 43.59.

Method B.

5-Amino-1-methylimidazole-4-carboxamide (**5a**) (0.50 g., 3.58 mmoles) was suspended in water (20 ml.) containing Amberlite IR-120 (H^+) (15 ml.). The mixture was stirred until **5a** had dissolved and then cooled to 3°. A solution of sodium nitrite (0.60 g., 8.70 mmoles) in water (6 ml.) was then added in one portion. The reaction mixture was stirred and maintained at 5° for 20 minutes and then allowed to warm to room temperature. The mixture was stirred for 30 minutes at room temperature, the resin was then removed by filtration and washed with water (5 x 15 ml.). The combined wash and filtrate were evaporated to dryness *in vacuo* (40°) and the solid was dissolved in hot water (20 ml.), treated with charcoal, and filtered. The filtrate was allowed to stand for 18 hours at room temperature and the solid which had separated was collected by filtration to provide 0.20 g. (37%) of **6a**: m.p. 200-201° (explodes); uv, ir, pmr and chromatographic mobilities were identical with those of **6a** obtained by method A.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**7**) and 5-Acetamido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxamide (**8**).

AICA riboside (**5b**) (11) was acetylated according to the procedure of Suzuki and Kumashiro (13). The syrups from three acetylations (2 x 10.3 g. and 1 x 3.09 g.) were dissolved in chloroform, combined and evaporated *in vacuo* (water bath 50°) to a hard foam (35.1 g., tlc, ethyl acetate-chloroform-methanol 7:2:1, 5 components). This material was divided into 3 equal portions and each portion applied to a silica gel column (2.7 x 85 cm., slurry packed in chloroform and prewashed with eluent). The columns were eluted with ethyl acetate-chloroform-methanol (7:2:1, 1.5 l.) and 50 ml. fractions were collected. In a typical chromatographic run, fractions 10-13 contained three minor fast moving bands, 15-17 contained pure **7** and 18-22 contained a mixture of **7** and the slower moving **8**. Fractions 15-17 were combined and evaporated to afford a hard colorless foam (20.2 g.). In a similar manner, fractions 10-13 containing the three minor components were combined and evaporated *in vacuo* (50°, 2 hours) to provide 4.8 g. of a hard foam. The fractions 18-22 containing a mixture of **7** and **8** were combined, dissolved in

absolute ethanol (50 ml.) and allowed to stand at room temperature for 48 hours. The long, transparent needles that separated were collected by filtration and washed with ethanol to furnish 2.16 g. (5.5%) of pure **8**. The filtrate and washings were combined and evaporated to dryness. The residue was dissolved in ethyl acetate (25 ml.) and an equal volume of benzene added. After standing for 18 hours at room temperature, crystalline **7** (8.4 g.) was collected by filtration. The two crops of **7** were combined and recrystallized from ethyl acetate-benzene (1:1) to afford 24.7 g. (69.8%) of **7**, m.p. 88-90° (lit. (13) 130-131°), picrate 146-148° (lit. (13) 146-147°); $[\alpha]_D^{27}$ -30.5 (*c* 1.00, ethanol); uv (ϵ x 10^{-3}) λ max (*p*H 1) 266.5 nm (10.88), 245 (10.19); λ min (*p*H 1) 253 nm (9.80); λ max (*p*H 11) 266 nm (13.15), sh 240 (8.84) λ min (*p*H 11) 225.5 nm (3.34); λ max (methanol) 263.5 nm (13.61) [lit. (13) λ max (*p*H 1) 270 nm, sh 247; λ max (*p*H 13) 270 nm]; pmr (DMSO-d_6) δ 7.40 (s, 1, 2-H), 6.74 (bs, 1, $CONH_2$), 2.05, 2.10 (2s, 9, $COCH_3$); (DMSO-d_6/deuterium oxide) δ 5.89 (d, 1, $J_{1',2'}$ = 5.3 Hz, 1'-H); R_f values A, 0.78; C, 0.54; D, 0.81; E, 0.73 (lit. (13) 0.64).

Recrystallization of **8** from ethanol provided an analytical sample: m.p. 208-209°; $[\alpha]_D^{26}$ + 2.9 (*c* 0.51, chloroform); uv (ϵ x 10^{-3}) λ max (*p*H 1) sh 240 nm (8.19); λ max (*p*H 11) 240 nm (12.84); λ max (methanol) sh 240 nm (9.38); pmr (DMSO-d_6) δ 9.80 (bs, 1, NH), 8.00 (s, 1, 2-H), 7.24 (bd, 2, $CONH_2$), 2.06, 2.02, (2s, 12, $COCH_3$).

Anal. Calcd. for $C_{17}H_{22}N_4O_9$: C, 47.89; H, 5.20; N, 13.14. Found: C, 47.95; H, 5.24; N, 13.09.

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**7**).

AICA riboside (**5b**) (11) (2.00 g., 7.74 mmoles) was added to a mixture of acetic anhydride (3.0 ml., 31.7 mmoles) and pyridine (20 ml.) and the mixture stirred at room temperature for 10 hours under anhydrous conditions. The reaction mixture was poured over cracked ice (100 ml.) and stirred until solution had been effected. The aqueous solution was extracted with chloroform (3 x 35 ml.) and the extracts were washed with a cold saturated aqueous solution of sodium bicarbonate (2 x 40 ml.), water (40 ml.), cold 0.1 *N* hydrochloric acid (2 x 20 ml.), and water (2 x 40 ml.). The chloroform phase was dried over anhydrous magnesium sulfate and then evaporated *in vacuo* (2 hours, 50°) to provide 2.35 g. (79%) of **7** as a colorless foam. The material was homogeneous on tlc (chloroform-methanol, 16:1 and ethyl acetate-chloroform-methanol, 7:2:1). Recrystallization from ethyl acetate-benzene (1:1) afforded 2.10 g. of crystalline **7**, m.p. 88-90°, picrate 146-148° (water); $[\alpha]_D^{27}$ -30.5 (*c* 1.00, ethanol); uv, ir, pmr and chromatographic mobilities were identical with **7** prepared by the preceding method.

2-Methyl-9-(β-D-ribofuranosyl)purin-6-one (**10**, 2-Methylinosine).

To a stirred solution of ethanolic sodium ethoxide, prepared from metallic sodium (0.4 g., 17.45 mg.-atoms) and absolute ethanol (25 ml.), was added 5-acetamido-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**8**) (0.853 g., 2.0 mmoles). The reaction mixture was heated at reflux temperature on a steam bath for 4 hours, allowed to cool to room temperature, and then diluted with water (25 ml.). The aqueous solution was neutralized to *p*H 3 with Amberlite IR-120 (H^+) (19). The resin was removed by filtration and washed well with water. The filtrate and washings were combined and concentrated *in vacuo* to a colorless residue. The residue was dissolved in hot 95% ethanol (20 ml.) and let stand at room temperature for 18 hours. The crystalline solid was removed by filtration to provide 0.36 g. (65%) of **10**, m.p. 204-206°. A small sample was dried over phosphorus pentoxide at 110° for 24 hours, m.p. 207-208° (20); $[\alpha]_D^{27}$ -50.0 (*c* 1.02, water) (20); uv (ϵ x 10^{-3}) λ max (*p*H 1) 250 nm (12.64); λ min (*p*H 1) 221.5 nm (4.38); λ max (*p*H 11) 255 nm (13.63); λ min (*p*H 11) 225 nm (1.41); λ max (methanol) sh 270 nm (5.64), 249.5 (12.14); λ min (methanol) 223 nm (4.29); pmr (DMSO-d_6) same as the authentic sample (20) except, δ 12.33 (bs, 1, NH); ir and chromatographic mobilities were identical with the sample prepared by the reported (16,20) procedure.

7-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**9**).

5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**7**) (3.84 g., 10 mmoles) was dissolved in 6 *N* hydrochloric acid (50 ml.) at -20°. The solution was cooled to -30° and then sodium nitrite (2.1 g., 30 mmoles) dissolved in water (10 ml.) was added dropwise over a 10 minute period while maintaining the temperature between -30° and -25°. After the addition was complete, the reaction solution was stirred for an additional 30 minutes at -25°. The reaction solution was diluted with cold (-30°) ethanol (50 ml.) and the *p*H adjusted to 6.8 with concentrated ammonium hydroxide (*ca.* 29 ml.) while keeping the temperature below -20°. The reaction mixture was filtered through a Celite pad and the Celite pad washed with ethanol (3 x 50 ml.). The combined wash and filtrate was evaporated *in vacuo* (water bath temperature ≤ 35°) to a thick residue. The dark residue was suspended in chloroform (50 ml.), filtered, and the filter cake washed with chloroform (2 x 25 ml.). The washings and filtrate were combined and evaporated to dryness (water bath 45°) to provide a dark brown foam. The foam was dissolved in ethyl acetate-chloroform-methanol (7:2:1, 15 ml.) and applied to a silica gel column (2.7 x 85 cm., slurry packed in chloroform and prewashed with eluent). The column was eluted with ethyl acetate-chloroform-methanol (7:2:1, 1.5 l.) with 50 ml. fractions being collected. Fractions 11-14 were pooled and evaporated to a foam. This material was dissolved in hot, 50% aqueous ethanol (75 ml.) treated with charcoal and filtered through a Celite pad. The filtrate was evaporated to dryness *in vacuo* (45°) to provide **9** as a colorless foam (*ca.* 2.9 g.). The foam was dissolved in ethanol (30 ml.) and allowed to stand for 18 hours at room temperature. The crystalline solid was collected by filtration and air dried to provide 2.69 g. (68%) of **9** (21). A small sample was dried 24 hours over phosphorus pentoxide at 65°, m.p. (sinters 77°) 84-86°; $[\alpha]_D^{26}$ -40.9 (*c* 1.00, ethanol); ir (cm^{-1}) 1718 (C=O), 1745 (ester C=O); pmr (DMSO-d_6) δ 2.02, 2.05, 2.12 (3s, 9, $COCH_3$), 4.02 (s, 2, water), 6.42 (d, 1, $J_{1',2'}$ = 5.0 Hz, 1'-H), 8.74 (s, 1, 6-H), 15.31 (bs, 1, NH); R_f values, A, 0.86; B, 0.87; C, 0.49; D, 0.88; E. 0.78.

Anal. Calcd. for $C_{15}H_{17}N_5O_8 \cdot H_2O$: C, 43.59; H, 4.63; N, 16.94. Found: C, 43.61; H, 4.62; N, 16.91.

7-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**6b**).

Method A.

A solution of **5b** (2.0 g., 7.74 mmoles) was diazotized with sodium nitrite (1.67 g., 24 mmoles) in 6 *N* hydrochloric acid (35 ml.) in the same manner as that described for **9**. The diluted reaction solution (35 ml. of ethanol) was neutralized to *p*H 7.0 with concentrated ammonium hydroxide (*ca.* 21 ml.). The cold reaction mixture (≤ -20°) was filtered and the filter cake washed with 95% aqueous ethanol (4 x 25 ml.). The filter cake was then dissolved in water-ethanol (9:1, 20 ml.) and allowed to stand for 48 hours at 4°. The crystalline material which separated from solution was collected by filtration and air dried to give 0.66 g. of

the desired product. The original filtrate and washings were combined, evaporated *in vacuo* (water bath $\leqslant 35°$) and the residue dissolved in 95% aqueous ethanol (100 ml.). After standing for 4 hours at 4°, the precipitated solid was collected by filtration and washed with 95% aqueous ethanol (2 x 20 ml.) followed by absolute ethanol (5 x 20 ml.). This solid which did not contain any nucleoside material (tlc and uv) was discarded and this procedure was repeated twice. The combined washings and filtrate, from this last step, were evaporated to dryness. The pink residue was dissolved in water (6 ml.) and applied to a Sephadex G-10 column. The column was eluted with water and 5 ml. fractions were collected. The ultraviolet absorbing fractions, 13-36, were combined and evaporated. The residual solid was dissolved in hot water (30 ml.), treated with Norit, and filtered through Celite. The filtrate was allowed to stand for 48 hours at 4° and the crystalline solid collected by filtration to provide an additional 0.88 g. of the desired product. The total product (1.54 g.) was dissolved in water (50 ml.) and adjusted to *p*H 4.2 (hydrion paper) with Dowex 50 (H^+, 100-200 mesh). The resin was removed by filtration and washed well with water. The combined washings and filtrate were evaporated to dryness. The light-yellow solid was crystallized from ethanol-water (8:2) to provide 1.39 g. (66%) of **6b**, m.p. 176-177° (explodes). An analytical sample was recrystallized from the same solvent to furnish **6b** as yellow crystals, m.p. 176-177° (explodes); $[\alpha]_D^{27}$ -36.8 (*c* 1.02, water); uv (ϵ x 10^{-3}) λ max (*p*H 1) 284.5 nm (5.38), sh 250 (4.60); λ min (*p*H 1) 258 nm (3.15); λ max (*p*H 11) 292 nm (7.43), 249 (6.62); λ min (*p*H 11) 264 nm (3.63); λ max (methanol) 285 nm (5.41), sh 250 (4.31); λ min (methanol) 257 nm (3.23); ir (cm^{-1}) 1698 (C=O); pmr (DMSO-d_6) δ 6.18 (d, 1, $J_{1',2'}$ = 5 Hz, 1'-H), 8.78 (s, 1, 6-H), 15.10 (bs, 1, NH); R_f values A, 0.75; B, 0.11; C, 0.44; D, 0.67; E, 0.24.

Anal. Calcd. for $C_9H_{11}N_5O_5$: C, 40.15; H, 4.12; N, 26.01. Found: C, 40.18; H, 4.10; N, 25.91.

Method B.

A solution of **9** (1.18 g., 3 mmoles) in methanol (40 ml., previously saturated at -5° with ammonia) was allowed to stand at room temperature for 7 hours in a sealed pressure bottle. After removal of the solvent, the syrup was triturated with chloroform (10 ml.) and the chloroform layer carefully decanted off. This procedure was repeated three times and the syrup was then triturated with methanol (15 ml.) and a yellow solid crystallized from the solution. The crystalline material (0.695 g.) was collected by filtration and washed with a small amount of methanol. The solid was dissolved in water (30 ml.) and the *p*H adjusted to 3.8 using Dowex 50 (H^+, 100-200 mesh). The washings and filtrate were combined, evaporated to dryness, and the resulting solid was crystallized from ethanol-water (8:2, 20 ml.) to provide 0.679 g. (84.1%) of **6b** as yellow rosettes, m.p. 175-176° (explodes). This nucleoside was identical (pmr, uv, ir, paper chromatography and mixture melting point) with **6b** prepared by method A.

5-(β-D-Ribofuranosyl)imidazo[4,5-*d*]-*v*-triazin-4-one (**4b**).

iso-AICA riboside (**3b**) (18) (2.00 g., 7.74 mmoles) was dissolved in 4 *N* hydrochloric acid (35 ml.) at -15°. To this stirred solution was added a solution of sodium nitrite (1.67 g., 24 mmoles) in water (10 ml.) over a 10 minute period while maintaining the temperature between -15° and -12°. The solution was stirred an additional 30 minutes at -15° after the addition was complete. The reaction solution was diluted with cold (-25°) ethanol (35 ml.) and then neutralized to *p*H 6.4 with concentrated ammonium hydroxide (*ca.* 12 ml.) while keeping the temperature at -15°. The deep yellow solution was concentrated *in vacuo* (water bath 35°) and the residue dissolved in ethanol-water (7:3, 50 ml.). After standing for 2 hours at 4°, the precipitate which had formed was collected by filtration and washed with cold ethanol-water (7:3, 10 ml.) and then cold absolute ethanol (50 ml.). This procedure was repeated again using ethanol-water (8:2, 15 ml.) followed by ethanol (50 ml.). The respective filter cakes which did not contain any nucleoside material (tlc and uv) were discarded. The filtrate and washings, from the above procedure, were taken to dryness and the resulting yellow residue dissolved in water (6 ml.) and applied to a Sephadex G-10 column. The column was eluted with water and 5 ml. fractions were taken. The uv absorbing fractions, 19-34, were combined and evaporated *in vacuo* to a semisolid. The yellow residue was dissolved in hot ethanol-water (just enough water to effect solution), treated with Norit, and filtered through a Celite pad. The concentrated filtrate was allowed to stand for 24 hours at 4° and the crystalline material (1.51 g.) which had separated was collected by filtration and air dried. The filtrate was evaporated to dryness *in vacuo*, dissolved in water (5 ml.), and reapplied to the Sephadex G-10 column. The column was eluted with water and 5 ml. fractions were collected. Fractions 20-30 were combined and evaporated *in vacuo* (40°) to a semisolid. The semisolid was dissolved in hot 95% aqueous ethanol and allowed to stand at room temperature for 18 hours to afford 0.52 g. of additional product (total, 2.03 g.). The two crops were combined, dissolved in water (50 ml.) and the *p*H adjusted to 3.5 using Dowex 50 (H^+, 100-200 mesh) resin. The resin was removed by filtration and washed well with water. The filtrate and washings were combined and evaporated *in vacuo* to afford a solid. The solid was recrystallized from ethanol-water (enough water to effect solution) to afford 1.69 g. (81%) of **4b** as long yellow needles; m.p. 184-185° (explodes); $[\alpha]_D^{27}$ + 42.3 (*c* 1.025, water); uv (ϵ x 10^{-3}) λ max (*p*H 1) 276 nm (4.15), 254 (5.36); λ min (*p*H 1) 272 nm (4.09), 237.5 (4.36); λ max (*p*H 11) sh 285 nm (5.92), 265.5 (7.91); λ min (*p*H 11) 238.5 nm (3.93); λ min (methanol) sh 280 nm (3.96), 255 (5.17); λ min (methanol) 237.5 nm (3.98); ir (cm^{-1}) 1692 (C=O); pmr (DMSO-d_6) δ 6.25 (d, 1, $J_{1',2'}$ = 5 Hz, 1'-H) 8.92 (s, 1, 6-H), 15.06 (bs, 1, NH); R_f values, A, 0.75; B, 0.12; C, 0.50; D, 0.67; E, 0.27.

Anal. Calcd. for $C_9H_{11}N_5O_5$: C, 40.15; H, 4.12; N, 26.01. Found: C, 40.11; H, 4.07; N, 25.80.

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(19) A calibrated Corning Model 7 *p*H meter was used to determine the end point.

(20) An authentic sample of 2-methylinosine was prepared according to A. Yamazaki, *et al.*, ref. 16. We found it advantageous to lyophylize the diluted reaction solution after treatment with charcoal. This procedure furnished a fluffy, colorless product which contained a half-mole of water (pmr); m.p. (sinters 140°) 154-156° (lit. (16) 165-166°); $[\alpha]_D^{27}$ -50.05 (*c* 0.985, water) [lit. (16) $[\alpha]_D^{26}$ -50.0 (*c* 1.00,water)]; uv ($\epsilon \times 10^{-3}$) λ max (*p*H 1) 250 nm (12.50); λ max (*p*H 11) 255 nm (13.43); λ max (methanol) sh 270 nm (5.68), 249.5 (12.12); pmr (DMSO-d_6) δ 2.39 (s, 3, 2-CH_3), 5.92 (d, 1, $J_{1',2'}$ = 6.5 Hz, 1'-H), 8.31 (s, 1, 8-H); R_f values A, 0.77; C, 0.47; D, 0.73; E, 0.30 (lit. (16) 0.35).

(21) In subsequent runs, the combined fractions were evaporated to dryness, dissolved in ethanol and seeded. A second recrystallization from ethanol provided pure **9**.