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Nucleosides of 2-Azapurines. 7*H*-Imidazo[4,5-*d*]-1,2,3-triazines. 2†

John A. Montgomery,* Anne G. Laseter, Anita T. Shortnacy, Sarah J. Clayton, and H. Jeanette Thomas

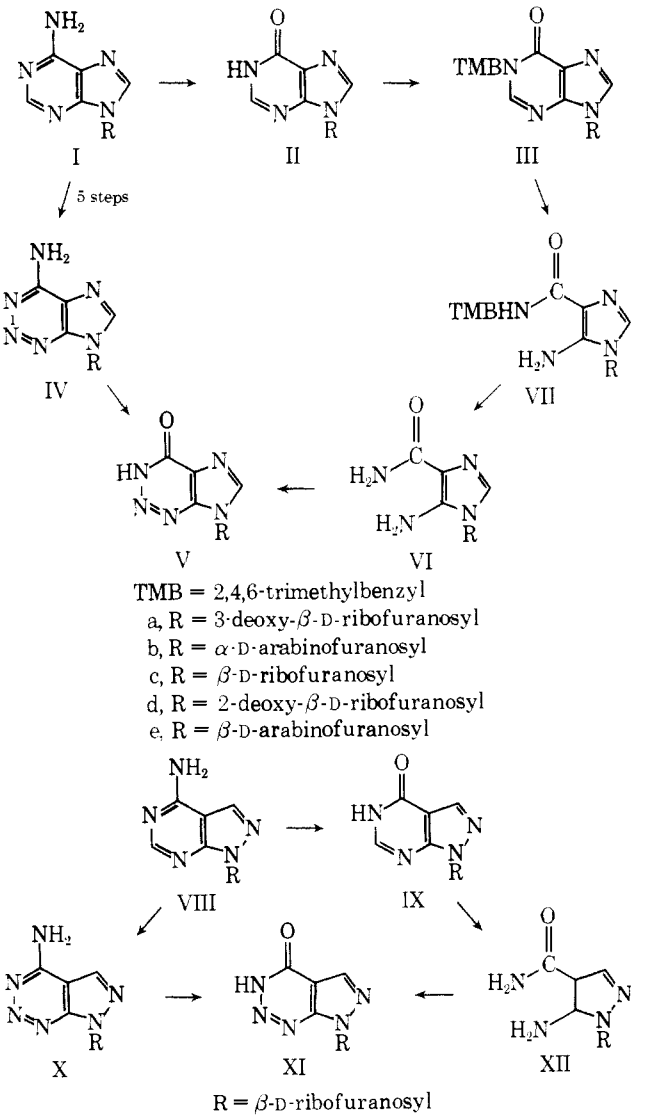
Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205. *Received January 6, 1975*

A number of nucleosides of 2-azaadenine (4-amino-7*H*-imidazo[4,5-*d*]-1,2,3-triazine) were prepared by a previously described route, and some of these were deaminated by means of adenosine deaminase. Alternatively, nucleosides of 2-azahypoxanthine (7*H*-imidazo[4,5-*d*]-1,2,3-triazin-4(3*H*)-one) were prepared from hypoxanthine nucleosides by a ring-opening and reclosure sequence. The cytotoxicity of these compounds to human epidermoid carcinoma No. 2 cells in culture and to certain resistant sublines thereof was determined. 2-Azaadenine nucleosides can be metabolized to nucleotides, the cytotoxic agents, by two pathways, but the activity of the 2-azahypoxanthine nucleosides appears to result only from cleavage back to 2-azahypoxanthine.

The biologic activity of 2-azaadenosine[1] led us to prepare other nucleosides of 2-azaadenine and also nucleosides of 2-azahypoxanthine. One of the 2-azaadenine derivatives was 2-azacordycepin (IVa) and another the α-arabinonucleoside (IVb). Interest in the latter nucleoside was based on the biologic activity of the α-arabinofuranosides of adenine[2] and 8-azaadenine.[2,3] These compounds were prepared from the adenine nucleosides by the route previously described: oxide formation at N-1, O-benzylation of the *N*-oxide, ring opening and deformylation with methanolic ammonia, and nitrosative ring closure (Scheme I).[1]

One approach to the synthesis of nucleosides of 2-azahypoxanthine is the deamination of the corresponding nucleosides of 2-azaadenine. Chemical deamination of 2-azaadenosine (IVc) failed, but deamination with adenosine deaminase was rapid and complete (uv and TLC). 2-Azainosine (Vc)[4,5] was isolated through its lead salt. This method was also successful for the conversion of 2-aza-2′-deoxyadenosine (IVd) to 2-aza-2′-deoxyinosine (Vd), but this compound was so sensitive to acidic cleavage that the procedure used for 2-azainosine had to be modified to eliminate the use of acetic acid, and, even so, the yield was low albeit deamination was complete (uv and TLC). Although the deamination of nucleosides of 2-azaadenine is quite a satisfactory procedure for substrates of adenosine deaminase, its usefulness is limited by the procedure required to prepare the 2-azaadenine nucleosides.[1] Alternatively, adenine nucleosides can be deaminated—by adenosine deaminase when feasible and chemically when the nucleosides are not substrates for the enzyme—and the resulting hypoxanthine nucleosides converted to the 2-aza derivatives. Unfortunately, however, the 1-oxide of these hypoxanthine nucleosides cannot be prepared directly,[6] and other 1-substituents have not proven completely satisfactory.[7-9] Alkylation of inosine (IIc) with α[2]-chloroisodurene proceeded in good yield to give 1-(2,4,6-trimethylbenzyl)inosine (IIIc), the pyrimidine ring of which was readily cleaved by aque-

Scheme I

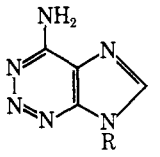


TMB = 2,4,6-trimethylbenzyl
a, R = 3-deoxy-β-D-ribofuranosyl
b, R = α-D-arabinofuranosyl
c, R = β-D-ribofuranosyl
d, R = 2-deoxy-β-D-ribofuranosyl
e, R = β-D-arabinofuranosyl

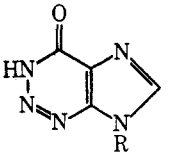
R = β-D-ribofuranosyl

†'Chemical Abstracts' name: 7*H*-imidazo[4,5-*d*]-1,2,3-triazine. The trivial 2-azapurine names and the purine numbering system have been used throughout to emphasize the relationship of this ring system to the purine ring system.

Table I. Cytotoxicity of 2-Azapurine Nucleosides



IV



V

Compd no.	R	ED_{50}, μM[a] H.Ep.-2/S	Degree of resistance, $(ED_{50}/R)/(ED_{50}/S)$[b] MP	FA	MeMPR	FA/PAR	MP/MeMPR
	H	10	1	>20	1–2	>20	1–2
IVc	β-D-Ribofuranose	0.2	1	1	1–2	5	>600
IVa	3-Deoxy-β-D-ribofuranose	80					
IVb	α-D-Arabinofuranose	>200[c]					
	H	0.3	>1000	2	2	2	>1000
Vc	β-D-Ribofuranose	0.7	>200	1	≤1	1	>200
Vd	2-Deoxy-α-ribofuranose	1.0	>100		1–2	1–2	
Ve	β-D-Arabinofuranose	40					
Va	3-Deoxy-β-D-ribofuranose	60					

[a]The concentration required to inhibit the growth of treated cells to 50% of that of untreated controls. [b]MP, resistant to 6-mercaptopurine, lacks hypoxanthine-guanine phosphoribosyltransferase; FA, resistant to 2-fluoroadenine, lacks adenine phosphoribosyltransferase; MeMPR, resistant to 6-(methylthio)purine ribonucleoside, lacks adenosine kinase; FA/FAR, resistant to 2-fluoroadenine and 2-fluoroadenosine, lacks adenine phosphoribosyltransferase and adenosine kinase; MP/MeMPR, resistant to 6-mercaptopurine and 6-(methylthio)-purine ribonucleoside, lacks hypoxanthine-guanine phosphoribosyltransferase and adenosine kinase (see ref 14). [c]No inhibition of the highest level tested.

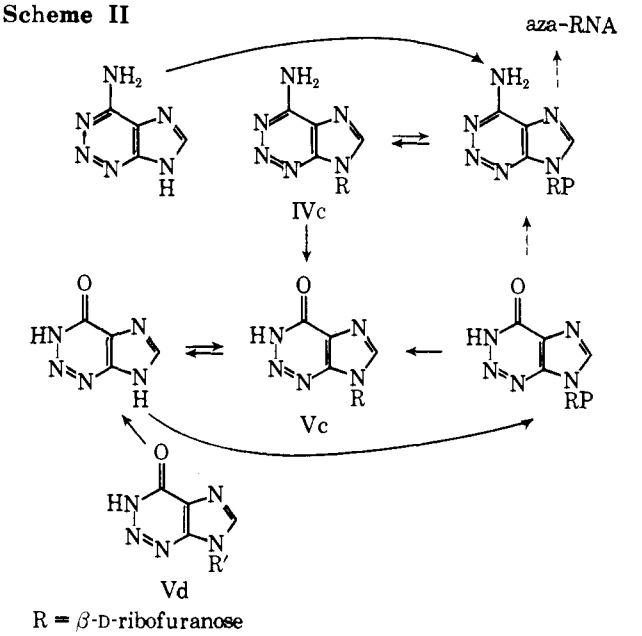
ous–alcoholic base to 5-amino-1-β-D-ribofuranosyl-*N*-(2,4,6-trimethylbenzyl)imidazole-4-carboxamide (VIIc), the 2,4,6-trimethylbenzyl group of which was removed by treatment with anhydrous trifluoroacetic acid at room temperature. Since the overall yield of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (VIc) was 46%, this route appeared satisfactory for the preparation of other nucleosides of 5-aminoimidazole-4-carboxamide for nitrosative ring closure to nucleosides of 2-azahypoxanthine. In this manner, 3′-deoxyinosine[10] (IIa), 9-β-D-arabinofuranosylhypoxanthine[11] (IIe), and 4-hydroxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine[12] (IX), all prepared by means of adenosine deaminase from Ia, Ie, and VIII, respectively, were converted into the imidazole nucleosides VIa and VIe and the pyrazole nucleoside XII, which, however, can be prepared more conveniently directly from IX by base treatment.[13] Nitrosation of these nucleosides with sodium nitrite in dilute hydrochloric acid solution at −30°[4,5] gave the desired nucleosides of 2-azahypoxanthine (Va and Ve) and 4-hydroxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (XI). The latter compound was also obtained by the deamination of 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]-*v*-triazine (X) with adenosine deaminase.

Biologic Evaluation. The cytotoxicities of the 2-azapurine nucleosides to human epidermoid carcinoma cells No. 2 in culture are given in Table I.[14] 2-Azaadenine itself is active against all H.Ep.-2 cell lines except those lacking adenine phosphoribosyltransferase; it must be activated by conversion to 2-azaadenylic acid. 2-Azaadenosine is active against all cell lines except those lacking both adenosine kinase and hypoxanthine–guanine phosphoribosyltransferase. Thus, it appears that 2-azaadenosine can be activated by phosphorylation to 2-azaadenylic acid, which may be deaminated or further phosphorylated and perhaps incorporated into nucleic acids. It can also be deaminated to 2-azainosine, which in turn can be cleaved to 2-azahypoxanthine, a substrate for hypoxanthine–guanine phosphoribosyltransferase. The 2-azainosinic acid formed by that enzyme could be aminated to 2-azaadenylic acid. It is not known whether 2-azaadenylic or 2-azainosinic acid is the cytotoxic agent, but a guanine nucleotide cannot be formed, as it is in the case of 8-azainosine. The analog of 2-azaadenosine, 2-aza-3′-deoxyadenosine (IVa), is only moderately cytotoxic, whereas 9-α-D-arabinofuranosyl-2-azaadenine (IVb), in contrast to its 8-aza isomer,[15] is not cytotoxic at all.

2-Azahypoxanthine is active against all cell lines except those lacking the hypoxanthine–guanine phosphoribosyltransferase. Both 2-azainosine (Vc) and 2-aza-2′-deoxyinosine (Vd), in contrast to 8-azainosine,[16] are also inactive against cell lines lacking the hypoxanthine–guanine phosphoribosyltransferase, indicating that their activity is due simply to their conversion back to 2-azahypoxanthine by inosine phosphorylase.

These results are summarized in Scheme II. They indi-

Scheme II



R = β-D-ribofuranose
R′ = 2-deoxy-β-D-ribofuranose
P = phosphate

cated that, despite the low cytotoxicity of IVa and IVb, other 2-azaadenine nucleosides are of potential interest because of the alternative pathways by which they can be metabolized. On the other hand, the 2-azahypoxanthine nucleosides appear far less promising since they are apparently simply "latent" forms of 2-azahypoxanthine. Other 2-azahypoxanthine nucleosides that are not substrates for inosine phosphorylase may be more interesting, but the ones prepared so (Va and Ve) are not very cytotoxic, including the ring analog 4-hydroxy-7-β-D-ribofuranosylpyrazolo[3,4-*d*]-*s*-triazine, which showed no inhibition at 75 μ*M*.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. The uv absorption spectra were determined in aqueous media with a Cary Model 17 spectrophotometer. The ^{1}H NMR spectra were obtained with a Varian XL-100-15 spectrometer in the solvents indicated with tetramethylsilane as an internal reference. Chemical shifts quoted in the case of multiplets are measured from the approximate centers. Evaporations were carried out under reduced pressure on a rotary evaporator. Thin-layer chromatography was performed on Analtech precoated (250 μmol) silica gel plates using the indicated solvent systems for development, and the spots were detected by irradiation with a Mineralight uv lamp after spraying with Ultraphor, by charring after spraying with saturated $(NH_4)_2SO_4$, or where applicable by ninhydrin spray. The preparative separations were carried out on Brinkmann 2-mm silica gel plates.

9-α-D-Arabinofuranosyladenine[17] (Ib). A suspension of Celite and chloromercuri-*N*-benzoyladenine (62.2 g from 60.7 mmol of the purine) in 1.6 l. of xylene was azeotropically dried by the distillation of 400 ml of xylene. Then tri-*O*-benzoyl-D-arabinofuranosyl bromide (32.0 g, 60.7 mmol in 400 ml of xylene) was added to the stirred, boiling mixture and after 1 hr the mixture was cooled, filtered, and evaporated to dryness. A solution of the residue in $CHCl_3$ (400 ml) was combined with the $CHCl_3$ washings of the filter cake and then washed twice with 30% KI solution (1200 ml), followed by H_2O (2.4 l.), and dried over $MgSO_4$ before evaporation to dryness. A suspension of the residue in MeOH (440 ml) containing NaOMe (17.8 ml, 1 *N* in MeOH) was refluxed for 0.5 hr. On cooling a solid precipitated from the resulting solution and was removed by filtration. The residue from evaporation of the filtrate was dissolved in H_2O and the solution evaporated to dryness. This procedure was repeated twice, and the residue was crystallized from MeOH. The combined solids were recrystallized from MeOH: yield 7.7 g (47%); mp 213°; $[\alpha]^{26}D$ +68.7 ± 0.5° (*c* 1.02 in H_2O) [lit.[17] mp 208°; $[\alpha]^{17}D$ + 69° (*c* 1.1 in H_2O)]. Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

3'-Deoxyinosine[10] (IIa). 3'-Deoxyadenosine (2.2 g, 8.76 mmol) was added to 160 ml of phosphate buffer (pH 7.5) containing 0.1 ml of adenosine deaminase.[3] After 2 hr the solution was heated at 100° for 5 min, filtered, and concentrated to 30 ml. The product crystallized on cooling: yield 1.96 g (82.7%); mp 203–205°; uv max in nm (ε × 10^{-3}) (0.1 *N* HCl and pH 7) 249 (11.9); uv max (0.1 *N* NaOH) 254 (12.2) [lit.[10] mp 197–199°; uv max (pH 1) 251 (10.9); uv max (pH 7) 250.5 (11.3); uv max (pH 13) 255.5 (13.3)]. Anal. ($C_{10}H_{12}N_4O_4 \cdot H_2O$) C, H, N.

9-β-D-Arabinofuranosylhypoxanthine[11] (IIe). In the same manner, 9-β-D-arabinofuranosyladenine (2.0 g dissolved in 350 ml of 0.02 *N* H_3PO_4) was converted to IIe: total yield 1.60 g (80%); mp 245–246°; uv max in nm (ε × 10^{-3}) (pH 7) 249 (11.7) [lit.[11] mp 243–245°; uv max 249 (12.0)].

5-Amino-1-(3-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamidine. A mixture of 3'-deoxyadenosine 1-oxide[18] (1.62 g, 6.08 mmol) and benzyl bromide (4.15 g, 24.3 mmol) in DMF (20 ml) was stirred for 24 hr (solution occurred) before it was evaporated to dryness in vacuo. The semisolid residue (1-benzyloxy-3'-deoxyadenosine bromide), after trituration with ether, was dissolved in 400 ml of methanolic ammonia (saturated at 0°) and the solution heated at 80° for 2 days before it was evaporated to dryness. A solution of the dark, gummy residue in a mixture of H_2O (200 ml) and MeOH (50 ml) was treated with an equivalent of aqueous picric acid. The picrate was collected by filtration, washed, and dissolved in methanol (300 ml) and the solution treated with Dowex 1-X8 (CO_3^{2-}) to regenerate the base. The solution of 5-amino-*N*-benzyloxy-1-(3-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamidine was hydrogenated in the presence of Raney nickel catalyst (ca. 1 g) for 3 days at room temperature and atmospheric pressure. The catalyst was removed by filtration before the solution was evaporated to dryness. To a solution of the residue in water was added picric acid (465 mg in H_2O). The picrate that crystallized was removed by filtration and dried. A small portion was recrystallized for analysis, mp 158–160°. Anal. ($C_{17}H_{18}N_8O_{10}$) C, H, N.

The remainder of the picrate was converted back to the free amidine for the next step by treatment with Dowex 1-X8.

2-Aza-3'-deoxyadenosine (IVa). To a solution of 5-amino-1-(3-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamidine in H_2O (5 ml) acidified with acetic acid and chilled in an ice bath was added $NaNO_2$ (109 mg) in 5 min. After being allowed to stand overnight in the refrigerator, the solution was filtered. The orange solid was recrystallized from H_2O (charcoal): yield 55 mg (28%); mp 251–252° dec; uv max in nm (ε × 10^{-3}) (0.1 *N* HCl) 253, 293 (8.4, 4.4); uv max (pH 7) 256, 297 (8.26, 6.35); uv max (0.1 *N* NaOH) 256, 298 (8.32, 6.38). Anal. ($C_9H_{12}N_6O_3$) C, H, N.

5-Amino-1-α-D-arabinofuranosylimidazole-4-carboxamidine. A solution of 9-α-D-arabinofuranosyladenine (4.5 g, 16.8 mmol) in glacial acetic acid (225 ml) containing 30% H_2O_2 (22.5 ml) was allowed to stand at room temperature for 8 days before evaporation to dryness. Recrystallization of the residue (9-α-D-arabinofuranosyladenine 1-oxide) from EtOH gave a white solid (3.3 g) that was dissolved in dry DMF (50 ml) containing benzyl bromide (8.0 g, 46.7 mmol). After 3 days, the solution was evaporated to dryness to give an orange syrup (9-α-D-arabinofuranosyl-1-benzyloxyadenine bromide) that was washed with ether before it was dissolved in 400 ml of methanolic ammonia (saturated at 0°). The solution was heated at 88° for 2 days before it was evaporated to a dark syrup that was dissolved in H_2O (100 ml) and MeOH (25 ml) to give a clear solution that was treated with an equivalent of aqueous picric acid. The dark picrate that formed was removed by filtration and dissolved in MeOH. The solution was treated with Dowex 1-X8 (CO_3^{2-}) to regenerate the free amidine. The solution of 5-amino-1-(α-D-arabinofuranosyl)-*N*-benzyloxyimidazole-4-carboxamidine containing Raney nickel catalyst (ca. 1 g) was hydrogenated for 7 days at room temperature and atmospheric pressure. The catalyst was then removed by filtration with washing and the filtrate evaporated to dryness: yield 815 mg. To a solution of this material in H_2O was added picric acid (3.17 mmol in H_2O): yield of picrate 1.89 g. A 100-mg portion of the picrate was recrystallized (charcoal) from H_2O for analysis: mp 206–207°. Anal. ($C_{17}H_{18}N_8O_{11}$) C, H, N.

The remainder of the picrate was converted to the base by treatment of its aqueous solution with Dowex 1-X8 (CO_3^{2-}) for use in the next step.

9-α-D-Arabinofuranosyl-2-azaadenine (IVb). Amino-1-(α-D-arabinofuranosyl)imidazole-4-carboxamidine (300 mg, 1.1 mmol) was nitrosated as described above for the preparation of IVa. The dark red solid obtained was purified by chromatography on a silica gel plate using 3:1 $CHCl_3$–MeOH as the developer. The major band was eluted with MeOH to give an orange glass that crystallized from water: yield 60 mg; mp 232–233°; uv max in nm (ε × 10^{-3}) (0.1 *N* HCl) 253, 293 (8.20, 4.34); uv max (pH 7 and 0.1 *N* NaOH) 256, 298 (7.90, 6.05). Anal. ($C_9H_{12}N_6O_4$) C, H, N.

2-Aza-3'-deoxyinosine (Va). To a solution of 5-amino-1-(3-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (178 mg, 0.735 mmol) in a mixture of EtOH (2.5 ml) and 6 *N* HCl (25 ml) at −30° was added dropwise $NaNO_2$ (232 mg, 3.36 mmol in 1.1 ml of H_2O). After 30 min at −30°, EtOH (25 ml) was added and the solution neutralized. The dry residue from evaporation of the reaction mixture was extracted with absolute EtOH (3 × 30 ml) and the extracts were evaporated to dryness. An aqueous solution of the residue was treated with $Pb(OAc)_2$ and the resulting white precipitate was removed by filtration, washed with H_2O, and dried. H_2S was bubbled into a suspension of the salt in H_2O. The PbS was removed by filtration and the solution evaporated to dryness: yield 102 mg (55%). A portion of this material was recrystallized from water for analysis: uv max in nm (ε × 10^{-3}) (0.1 *N* HCl) 244 and 250 sh, 285 (9.73); uv max (pH 7) 248, 291 (4.30, 6.06); uv max (pH 13) 250 (5.73), 255 sh, 293 (7.08). Anal. ($C_9H_{11}N_5O_4 \cdot 1.25H_2O$) C, H, N.

2-Azainosine[4,5] (Vc). To a solution of 2-azaadenosine (148.6 mg, 0.56 mmol) in phosphate buffer at pH 7.5 (100 ml) was added adenosine deaminase (0.1 ml). The next day the solution was heated for 3 min in a boiling water bath, chilled, filtered, and acidified with HOAc and $Pb(OAc)_2$ was added until no further precipitation

[3] Crystalline suspension in 3.2 *M* $(NH_4)_2SO_4$ solution (Sigma Chemical Co., St. Louis, Mo.).

occurred. The precipitate was removed by filtration, and the pH of the filtrate was raised to 10 with concentrated NH_4OH giving a light pink precipitate which was dissolved in 28% HOAc. The solution was treated with H_2S and PbS removed by filtration. The filtrate was evaporated to a glass that was dissolved in H_2O which was evaporated to dryness, then several times in EtOH, each time evaporating the solution to dryness. The white residue was recrystallized from EtOH–H_2O: yield 91 mg (61%); mp 175° dec; uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 248 sh, 285 (3.32, 4.84); uv max (pH 7) 248, 291 (5.09, 6.15); uv max (0.1 *N* NaOH) 249, 293 (5.96, 7.14) [lit.[4] mp 173–175°; uv max (pH 1) 285 (4.5)]. Anal. ($C_9H_{11}N_5O_5$) C, H, N.

2-Aza-2'-deoxyinosine (Vd). 2-Aza-2'-deoxyadenosine (120 mg, 0.48 mmol) was deaminated as described above for Vc, and the product was converted to its Pb salt, which was suspended in 50 ml of H_2O and treated with H_2S. The PbS was removed by filtration with washing, and the filtrate lyophilized. The residue was crystallized from EtOH containing H_2O: yield 24 mg; mp sinters at 110° and decomposes between 140 and 170°; uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 246, 277 (5.12, 4.25); uv max (pH 7) 248, 291 (5.84, 7.00); uv max (0.1 *N* NaOH) 250, 293 (6.68, 8.04). Anal. ($C_9H_{11}N_5O_4 \cdot 0.75H_2O$) C, H, N.

9-β-D-Arabinofuranosyl-2-azahypoxanthine (Ve). To a solution of 5-amino-1-β-D-arabinofuranosylimidazole-4-carboxamide (VIe, 497 mg, 1.92 mmol)[6] in 2.5 ml of EtOH and 25 ml of 6 *N* HCl at −30° was added a water solution of $NaNO_2$ (525 mg in 2.5 ml) over a period of 15 min. After an additional 30 min at −30° the solution was neutralized and evaporated to dryness. The residue was chromatographed on a Sephadex G-10 column using water as the eluent. To a solution of the material from this treatment in water was added $Pb(OAc)_2 \cdot 3H_2O$ (337 mg) and then concentrated NH_4OH. The white precipitate that formed was removed by filtration and suspended in water, and H_2S was passed into the suspension. The PbS was removed by filtration before the solution was evaporated to dryness: yield of white solid 196 mg (38%). This material was shown by TLC to contain only traces of impurities. Recrystallization from water gave the chromatographically homogeneous, analytical sample: uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 285 (4.76); uv max (pH 7) 247 (4.95), 290 (6.08); uv max (0.1 *N* NaOH) 249 (5.78), 293 (7.24); ^1H NMR (DMSO-d_6) 3.68 (m, $2H_{5'}$), 3.82 (m, $H_{4'}$), 4.13 (m, $H_{3'}$), 4.28 (m, $H_{2'}$), 6.45 (d, $J_{1'2'} = 5$ Hz, $H_{1'}$), 8.56 (s, H_8). Anal. ($C_9H_{11}N_5O_5$) C, H, N.

5-Amino-1-(3-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (VIa). A mixture of 3'-deoxyinosine (1.38 g, 5 mmol), K_2CO_3 (1.0 g), and α^2-chloroisodurene (1.0 g) in DMF was allowed to stir overnight at room temperature before it was evaporated to dryness. A suspension of the solid residue in a mixture of 5 *N* NaOH (10 ml) and EtOH (90 ml) was refluxed for 30 min before it was neutralized (ice bath) with 6 *N* HCl and evaporated to dryness. The residue was stirred overnight at room temperature with 25 ml of trifluoroacetic acid and then the solution was evaporated to dryness. The residue was evaporated several times with ethanol before it was chromatographed on a column of Bio Sil A (5:1 $CHCl_3$–MeOH): yield 480 mg (40%). A portion of this material was further purified by conversion to its picrate salt which was converted back to the free base by treatment with Dowex 1-X8 (CO_3^{2-}): mp 214–215°; uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 246 sh (8.35), 267 (10.2); uv max (pH 7 and 0.1 *N* NaOH) 267 (12.2); ^1H NMR (DMSO-d_6) 2.00 (complex m, $2H_{3'}$), 3.56 (m, $2H_{5'}$), 4.36 (m, $H_{2'}$ and $H_{4'}$), 5.07 (m, O_5·H), 5.43 (d, $J_{1'2'} = 2$ Hz, $H_{1'}$), 5.55 (d, O_2·H), 5.84 and 6.68 (2 br s, NH_2 and $CONH_2$), 7.32 (s, H_2). Anal. ($C_9H_{14}N_4O_4$) C, H, N.

5-Amino-(1-β-D-arabinofuranosyl)imidazole-4-carboxamide[6] (VIe) Picrate. To a solution of 9-β-D-arabinofuranosylhypoxanthine (134 mg, 0.5 mmol) in dry DMF (15 ml) was added K_2CO_3 (100 mg) and α^2-chloroisodurene (93 mg, 0.5 mmol), and the reaction mixture was stirred overnight at room temperature. The residue, after removal of the DMF, was suspended in a mixture of 9 ml of EtOH and 1 ml of 5 *N* NaOH and the mixture refluxed for 0.5 hr before it was neutralized (ice bath) and evaporated to dryness. A suspension of the dry residue in trifluoroacetic acid was allowed to stand at room temperature for 18 hr before evaporation to dryness. Picric acid (126 mg in 10 ml of H_2O) was added to an aqueous solution (15 ml) of the residue. The yellow solid that formed was recrystallized from MeOH: yield 105 mg (43%). Anal. ($C_{15}H_{17}N_7O_{12}$), C, H, N.

In a second run, VIe was isolated as the free base and converted to Ve.

4-Hydroxy-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine[12] (IX). 4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (2.78 g, 10.4 mmol) was added in small portions to 200 ml of phosphate buffer (pH 7.5) containing 0.1 ml of adenosine deaminase. The next day the solution was heated at 100° for 5 min, filtered, and concentrated. The chromatographically homogeneous product was collected in three crops: total yield 1.99 g (71%); uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl and pH 7) 252 (8.05); uv max (0.1 *N* NaOH) 272 (10.7) [lit.[12] uv max (pH 1) 252 (8.35); uv max (pH 12) 271 (11.2)].

4-Hydroxy-7-β-D-ribofuranosyl-7*H*-pyrazolo[3,4-*d*]-*v*-triazine (XI). **A.** To a solution of 5-amino-1-β-D-ribofuranosylpyrazole-4-carboxamide[13] (XII, 688 mg, 232 mmol) in 6 *N* HCl (44 ml) at −30° was added dropwise $NaNO_2$ (575 mg, 8.3 mmol in 2.8 ml of H_2O) and the slurry stirred for 30 min before it was diluted with EtOH (10 ml) and neutralized. The precipitated salts were removed by filtration and washed with EtOH. The filtrate and washings were evaporated to dryness. An aqueous solution of the residue was acidified to pH 4 with Amberlite IR-120 (H^+) before dilution with EtOH. The precipitated salts were removed by filtration and the solution was evaporated to dryness. Recrystallization from water gave 508 mg (81%). A portion was recrystallized again from water: uv max in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 279 (6.06), 293 sh; uv max (pH 7) 296 (7.05); uv max (0.1 *N* NaOH) 300 (8.54); ^1H NMR (DMSO-d_6) 3.57 (m, $2H_{5'}$), 4.95 (q, $H_{4'}$), 4.28 and 4.71 (2 t, $H_{2'}$ and $H_{3'}$), 6.23 (d, $J_{1'2'} = 4.5$ Hz, $H_{1'}$), 8.09 (H_5). Anal. ($C_9H_{11}N_5O_5$) C, H, N.

B. To a solution of 4-amino-7-β-D-ribofuranosyl-7*H*-pyrazolo[3,4-*d*]-*v*-triazine (X, 5.5 mg) in phosphate buffer (3.75 ml, 0.05 *M*, pH 7.5) was added adenosine deaminase (0.5 mg in 0.05 ml of H_2O). After a few minutes the uv spectrum of the solution was determined: uv max (pH 7.5) 296 (6.98).

Acknowledgments. This investigation was supported by Contract NO1-CM-43762 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. The authors wish to thank Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for the analytical and spectral data reported and Mrs. Martha Thorpe for her help in the interpretation of the NMR spectra. They also wish to thank Dr. L. L. Bennett, Jr., and Miss Jean Carpenter for the cytotoxicity data reported.

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