Pyrazolopyrimidine Nucleosides. Part VII (1) The Synthesis of Certain Pyrazolo[3,4-*d*]pyrimidine Nucleosides Related to the Nucleoside Antibiotics Toyocamycin and Sangivamycin

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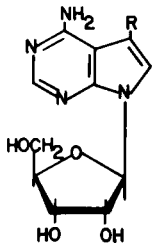
The condensation of 4-acetamido-3-cyanopyrazolo[3,4-*d*]pyrimidine (**5**) with crystalline 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl chloride (**6**) has furnished a good yield of nucleoside material (**7**) which on treatment with sodium methoxide in methanol provided a high yield of nucleoside which was subsequently established as methyl 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]-pyrimidine-3-formimidate monohydrate (**11**). The formimidate function of **11** was found to be highly reactive and **11** was readily converted into the corresponding carboxamidine (**8**), carboxamidoxime (**14**) and carboxamidrazone (**15**) when treated with the appropriate nucleophiles. Treatment of the imidate (**11**) with sodium hydrogen sulfide gave a high yield of the thiocarboxamide (**12**) which was then readily converted into 4-amino-3-cyano-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**16**). Aqueous base transformed **11** into 4-amino-1-(β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**10**) while more vigorous basic hydrolysis provided the corresponding carboxylic acid (**9**) in nearly quantitative yield. Decarboxylation of **9** proceeded smoothly in hot sulfolane to provide the known 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**13**) in 68% yield which unequivocally established the site of ribosylation and anomeric configuration for all nucleosides reported in this investigation.

The isolation and structural elucidation of tubercidin (**1**), toyocamycin (**2**), and sangivamycin (**3**) as pyrrolopyrimidine nucleosides was followed by their total synthesis (2), which stimulated considerable research in this



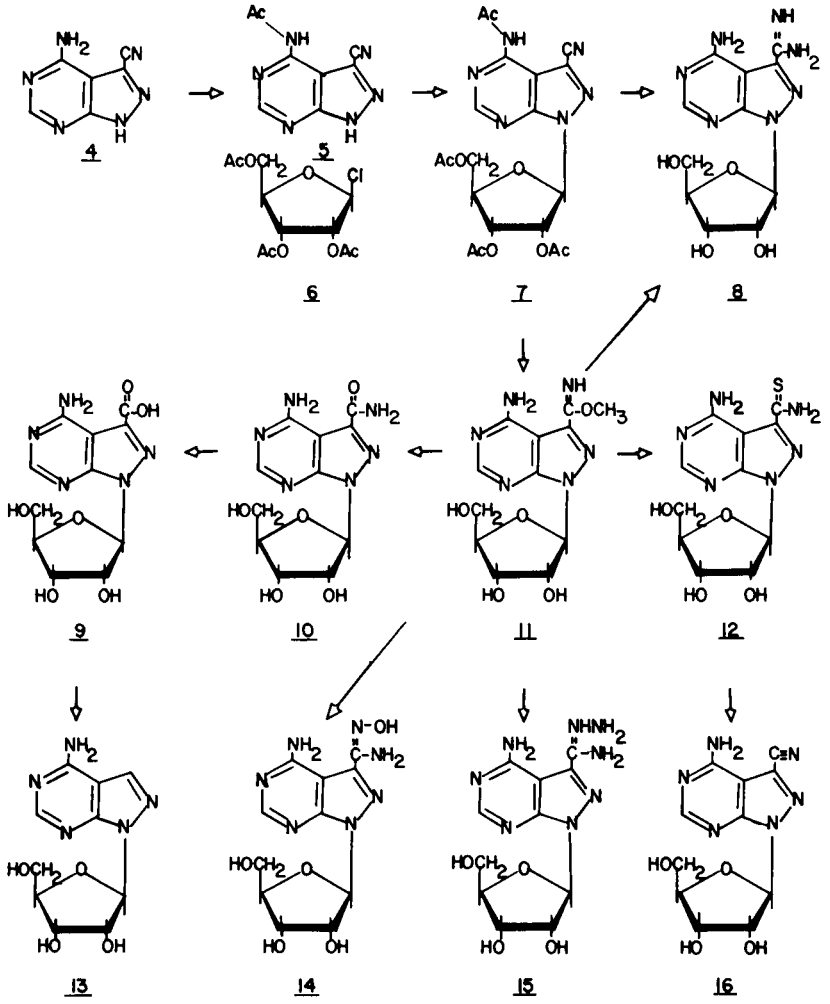
1 R=H

2 R=CN

3 R=CONH$_2$

area (3). These pyrrolopyrimidine nucleosides were reported (4,5) to possess significant biological and chemotherapeutic activity which has stimulated interest in the synthesis of some closely related nucleosides. A visual examination of the pyrrolopyrimidine nucleosides revealed that a nitrogen atom could be inserted in place of the C6 atom which would furnish "6-aza" derivatives of the above pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics. There have been some 4- and 4,6-disubstituted pyrazolo-[3,4-*d*]pyrimidine nucleosides reported (6,7) but we now wish to report the first synthesis of some 3,4-disubstituted derivatives of 1-(β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine.

The reaction of 4-acetamido-3-cyanopyrazolo[3,4-*d*]-pyrimidine (**5**) with crystalline (8) 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl chloride (**6**) in boiling nitromethane [utilizing potassium cyanide as the acid acceptor (9)] provided a good yield (tlc) of nucleoside material. Column chromatography provided a 56% yield of a chromatographically homogeneous syrup that was established *(vide infra)* as being 4-acetamido-3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**). The pmr spectrum of **7** displayed a doublet at δ 6.66 that was assigned to H_1' of the sugar moiety. However, the large coupling constant for H_1' of **7** and all derivatives prepared from **7** (see Table I), precluded (10) a direct assignment of anomeric configuration. Treatment of **7** with sodium methoxide in methanol resulted in the formation of a crystalline nucleoside in 82% yield. The crystalline nucleoside was examined by pmr spectroscopy which revealed a peak at δ 3.87 which suggested the presence of a methyl group. Elemental analysis indicated that if this was the desired product [4-amino-3-cyano-1-(β-D-ribofuranosyl)-



pyrazolo[3,4-*d*]pyrimidine (**16**)] then it had been isolated as the monohydrate plus 1 mole of methanol. However, the ir spectrum revealed the absence of an absorption band in the 2270 cm^{-1} region for a cyano group [a moderate absorption at 2254 cm^{-1} is present in the ir spectrum of **7**] which indicated that some modification of the 3-cyano group had occurred. The nucleoside was subsequently identified as methyl 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-formimidate monohydrate (**11**) based on the above data and on the observation that 4-amino-3-cyano-2-methylpyrazolo[3,4-*d*]pyrimidine readily forms a formimidate on treatment with methanolic ammonia (11). It is noteworthy that the 3-cyano group of **7** is so reactive towards nucleophilic attack, since it has been shown (11) that the cyano group of 4-amino-3-cyano-1-methylpyrazolo[3,4-*d*]pyrimidine does not react under similar conditions (methanolic ammonia, 24 hours at 25°). These results clearly demonstrate the differences in the effects exerted by a glycosyl group as compared to a simple alkyl group and suggest that the reactions of a model methyl compound should not always be interpreted as being fully representative of the chemical behavior expected for the corresponding nucleoside. It was subsequently found that methyl 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-formimidate monohydrate (**11**) could be prepared directly (no chromatography), in yields of 50-60%, by treatment of crude 4-acetamido-3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**) with sodium methoxide in methanol.

Further evidence of the highly reactive nature of the cyano group in **7** was obtained when it was found that the reaction of **7** with liquid ammonia at room temperature (40 hours) produced a good yield (76%) of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**8**). A nearly identical yield (77%) of the crystalline amidine (**8**) was obtained on treatment of **11** with liquid ammonia under these conditions. The imidate **11** can be considered as an activated form of 4-amino-3-cyano-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine

Table I

Physical Constants for Certain Pyrazolo[3,4-*d*]pyrimidine Ribosides

Compound	M.p. (°C)	$[\alpha]_D^{27°}$	Pmr parameters [δ(J/Hz)] H-1′($J_{1,2}$)	H-6	Other
7	Syrup	-43.5 (*c* 1.0, DMF)	6.66 (3.9) (a)	8.75	2.46 ($NHCOCH_3$)
8	188-190	-59.9 (*c* 1.0, DMF)	6.17 (5.0) (b)	8.24	
9	305 dec.	-94.0 (*c* 1.0, DMSO)	6.07 (4.1) (b)	8.05	
10	268-269.5 dec.	-64.8 (*c* 1.0, DMF)	6.22 (4.8) (b)	8.32	
11	132	-69.9 (*c* 1.0, DMF)	6.19 (5.0) (b)	8.26	3.87 (OCH_3)
12	252-253 dec.	-39.2 (*c* 1.0, DMF)	6.25 (5.0) (b)	8.29	
13	247.5-249 dec.	-77.0 (*c* 0.993, DMF)	6.15 (4.8) (b)	8.23	8.23 (H-3)
14	242-244	-65.6 (*c* 1.0, DMF)	6.18 (4.8) (b)	8.22	10.22 (=N-OH)
15	220-221	-58.8 (*c* 1.0, DMF)	6.16 (4.8) (b)	8.20	
16	238-240 dec.	-84.9 (*c* 1.0, DMF)	6.23 (4.9) (b)	8.38	

(a) Deuteriochloroform. (b) DMSO-d_6.

(**16**) (*vide infra*) which prompted us to use **11** as an intermediate for the synthesis of the other desired 3-substituted-4-aminopyrazolo[3,4-*d*]pyrimidine nucleosides. Both hydroxylamine and hydrazine hydrate reacted readily with **11** to yield the corresponding 3-carboxamidoxime (**14**) (86%) and 3-carboxamidrazone (**15**) (81%) derivatives. Sodium hydrosulfide in methanol reacted very rapidly at room temperature with **11** and in *ca.* 3 minutes 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**12**) began to precipitate from solution. The thiocarboxamide **12** was obtained initially as a low melting solid (m.p. 132-135°) which upon recrystallization from an ethanol-water mixture gave a higher melting form (m.p. 250.5-251.5°). A similar behavior was noted for the 3-carboxamidoxime derivative (**14**). Treatment of **12** with mercuric chloride and triethylamine in dimethylformamide furnished the evasive 4-amino-3-cyano-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**16**) ("6-azatoyocamycin"). It was subsequently established (tlc) that no appreciable amount of **16** is accumulated during the deblocking of 4-acetamido-3-cyano-1-(2,-3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**) with either sodium methoxide in methanol (25°) or methanolic ammonia (5°).

A catalytic amount of sodium hydroxide in water effected a smooth conversion of the imidate **11** into the more insoluble (water) carboxamide, 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**10**). The uv spectral data for **10** [λ max (*p*H 1) 265.2; λ max (methanol) 283.0; λ max (*p*H 11) 237.0, 283.0 nm] was found to be in excellent agreement with the uv spectral data (11) for 4-amino-1-methylpyrazolo[3,4-*d*]pyrimidine-3-carboxamide [λ max (*p*H 1) 229.2, 265.0; λ max (methanol) 239.5, 282.3; λ max (*p*H 11) 241.8, 283.7 nm] and considerably different from the uv spectral data for the 2-methyl isomer (11) which unequivocally established N1 as the actual site of glycosylation for **10**. The nucleosides **9** and **16** also displayed uv spectral data that were in good agreement with the data found (11) for the corresponding N1 methylated heterocyclic bases. Treatment of **10** with a slight excess of hot aqueous sodium hydroxide furnished a high yield (89%) of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxylic acid (**9**). The addition of an equivalent amount of mineral acid furnished the carboxylic acid **9** in the form of a gelatinous precipitate which eventually crystallized. The carboxylic acid **9** was surprisingly stable to heat (sinters at *ca.* 305°) since the analogous sangivamycic acid [4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid] was reported (2) to melt with a simultaneous decarboxylation at 238°. We had assumed that a carboxylic acid derivative (**9**) of the more electron deficient pyrazolo[3,4-*d*]pyrimidine ring system would decarboxylate (12) more readily than the corresponding carboxylic acid derivative of the pyrrolo[2,3-*d*]pyrimidine ring system.

Only trace amounts of apparent decarboxylation products were found (tlc) when **9** was fused neat or in the presence of copper powder or cupric oxide. It was also found that heating in quinoline [copper catalyst (13)] did not seem to promote decarboxylation. The recent observation (14) that certain decarboxylations proceed better in nonbasic solvents, such as sulfolane, prompted us to try these conditions for the decarboxylation of **9**. Indeed, decarboxylation occurred very smoothly when **9** was heated in deoxygenated (15) sulfolane at *ca.* 213°. Removal of the solvent left a crude product which was examined by tlc and revealed the presence of a new compound and the absence of any starting carboxylate (**9**). The uv spectra, of the crude product, showed the same uv λ max's and λ min's as found in the uv spectra of a pure sample of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]-

Table II

Uv Spectral Data [λ/nm (10^{-3} ε)]for Certain Pyrazolo[3,4-*d*]pyrimidine Ribosides

Compound	λ pH 1 max	λ pH 1 min	λ Methanol max	λ Methanol min	λ pH 11 max	λ pH 11 min
7	281.0 (14.0)	248.0 (6.28)	282.0 (14.0)	248.7 (6.15)	318.5 (11.0) 235.1 (11.7)	262.0 (7.71) 230.6 (11.3)
8	267.0 (10.2)	251.0 (8.29)	282.8 (11.6) 236.0 (10.2)	256.0 (7.36) 220.0 (8.56)	281.5 (11.3) 235.0 (10.6)	255.0 (7.42) 225.3 (8.35)
9	265.0 (12.2)	248.1 (9.64)	280.8 (12.4) 232.1 (11.0)	250.5 (6.54) 226.2 (10.7)	280.0 (13.4) 233.6 (10.7)	249.5 (6.13) 232.0 (10.7)
10	265.2 (9.33)	251.0 (8.18)	283.0 (10.9)	255.0 (6.10)	283.0 (9.75) 237.0 (9.42)	254.8 (5.90) 227.7 (7.99)
11	267.0 (11.1) 224.0 (15.7)	250.5 (8.57)	284.0 (12.6) 242.2 (10.2)	257.0 (6.93) 222.0 (6.93)	283.0 (12.4) 238.5 (10.5)	255.5 (6.75) 226.0 (7.88)
12	275.0 (12.8)	261.2 (12.6)	296.5 (15.2) 236.0 (15.4)	253.3 (11.9) 225.0 (14.3)	280.0 (15.4) 236.0 (15.4)	254.0 (13.0) 229.3 (13.9)
13	257.7 (12.0)	239.2 (8.03)	285.0 (a) 275.0 (12.3) 259.0 (11.0)	263.0 (10.9) 237.2 (5.56)	284.0 (a) 273.0 (12.3) 259.0 (11.0)	263.0 (10.9) 239.5 (5.10)
14	263.8 (10.6)	248.0 (9.35)	281.5 (15.6)	245.8 (8.70)	281.0 (12.5) 231.8 (11.8)	251.0 (8.40) 227.2 (11.3)
15	262.0 (11.9)	250.0 (11.2)	283.5 (12.8)	249.5 (7.10)	281.3 (14.6)	248.0 (7.98)
16	268.2 (11.6)	249.0 (8.35)	285.0 (12.8) 236.3 (9.79)	252.5 (5.29) 230.0 (9.55)	282.3 (8.92) 239.0 (6.44)	255.0 (4.29) 227.3 (4.23)

(a) Shoulder.

pyrimidine (**13**) prepared (6b) by another route. The crude product was recrystallized from water to provide fine, white needles, m.p. 247.5-249° dec., mixed m.p. with authentic **13** (6b) (m.p. 253-255°) was undepressed. The optical rotation of the product $[\alpha]_D^{27°}$ -77.0° (DMF) was very similar to the value previously reported (6b) for **13** ($[\alpha]_D$ -81.7° in DMF) and the uv and pmr spectra were virtually superimposable on those of an authentic sample of **13**, prepared in this laboratory. Since the structure of 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**13**) has been unequivocally proven (16), this established the site of ribosyl attachment (N1) and anomeric configuration (β) for all nucleosides prepared in this investigation.

This facile synthesis of 1-β-D-ribofuranosyl derivatives of 3,4-disubstituted pyrazolo[3,4-*d*]pyrimidines offers

an interesting new class of nucleosides for biological evaluation and should offer impetus for research in the synthesis of other similar polysubstituted pyrazolo[3,4-*d*]-pyrimidine nucleosides. Further investigations in this area are in progress in our laboratory.

EXPERIMENTAL

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Pmr spectra were obtained on a Varian A-60 or a XL-100-12 spectrophotometer using [$^2H_6$]dimethylsulfoxide as solvent and sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) as an internal standard, unless otherwise noted, with the chemical shifts being expressed as δ parts per million from the standard. Uv spectra were determined on a Beckman DK-2 spectrophotometer. Ir spectra were determined (pressed potassium bromide discs) with a Beckman IR-8 spectrophotometer and are given in cm^{-1}. Optical rotations were obtained with a Perkin-Elmer model 141 automatic digital readout polarimeter. Tlc were run using chromatographic grade silica gel (SilicAR 7GF) purchased from J. T. Baker Company and a short range (254 nm) ultraviolet light was used for detection of compounds. Dry column chromatography was run using CC-7 (200-325 mesh) obtained from Mallinckrodt Company. Elemental analyses were performed by Heterocyclic Chemical Co. Unless otherwise noted, concentrations were carried out *in vacuo* at 35°.

4-Acetamido-3-cyanopyrazolo[3,4-*d*]pyrimidine (**5**).

A suspension of dry (1 hour/110° 0.5 torr) 4-amino-3-cyanopyrazolo[3,4-*d*]pyrimidine (12), (**4**), (20 g.), in a mixture of acetic anhydride (100 ml.) and dry pyridine (100 ml., which had been stored over potassium hydroxide pellets) was vigorously stirred and brought to reflux temperature during 5 minutes. The mixture was stirred vigorously and heated at reflux temperature for a total of 35 minutes. (Solution occurs in *ca.* 20 minutes). The solution was then cooled to room temperature (ice-bath) and concentrated *in vacuo* to give a dark, pasty solid. Methanol was added (100 ml.) and the solution allowed to stand for 0.5 hour followed by another concentration *in vacuo*. The solid was triturated with a mixture of ice and water (60 g. total) for 15 minutes and the solid was then collected by filtration. The dark colored filter cake was washed with cold water (0°) (2 x 20 ml.). The moist solid was then dissolved in 850 ml. of boiling water, treated with Norite (3 g.) and filtered while hot. The filtrate was cooled to 0° and the tan colored solid collected by filtration. The solid was then dissolved in the minimum amount of 1.25 *N* sodium hydroxide solution required to effect a solution (about 75 ml.), treated with another 2 g. of Norite and then filtered. The filtrate was cooled to 0° and stirred while the solution was adjusted to *p*H 5 by the dropwise addition of acetic acid. The white precipitate was collected by filtration, washed with cold (0°) water (2 x 15 ml.) and after drying *in vacuo* (25°, 0.5 torr Hg) furnished 13.4 g. (53%) of **5**, m.p. > 360°. A small sample was recrystallized twice from water to give small white needles m.p. > 360° ir: 2273 (w, CN), 1700 (s, C=O). Uv (ϵ x 10^{-3}): λ max (*p*H 1) 276.0 (11.0); λ min (*p*H 1) 247.0 (5.30); λ max (methanol) 278.0 (10.4); λ min (methanol) 253.2 (5.50); λ max (*p*H 11) 281.0 (6.86), 310.0 (8.44); λ min (*p*H 11) 258.5 (5.70), 290.0 (7.50).

Anal. Calcd. for $C_8H_8N_6O$: C, 47.40; H, 2.99; N, 41.58. Found: C, 47.38; H, 2.93; N, 41.49.

4-Acetamido-3-cyano-1-(2,3,5-tri-*o*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**).

ALL OPERATIONS FOR THIS PREPARATION WERE CARRIED OUT IN AN EFFICIENT FUME HOOD SINCE COPIUS AMOUNTS OF HYDROGEN CYANIDE ARE EVOLVED.

4-Acetamido-3-cyanopyrazolo[3,4-*d*]pyrimidine (**5**) (10.4 g., 51.3 mmoles) and potassium cyanide (3.41 g., 52.3 mmoles) were finely pulverized, dried separately (8 hours, 110°/0.5 torr Hg) and then placed in a dry flask containing nitromethane (384 ml., dried over 4 A° molecular sieves for 2 d.). The mixture was heated to reflux temperature while stirring vigorously and then 14.8 g. (50.3 mmoles) of crystalline (8) 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl chloride (**6**) (m.p. 46-50°) was added in one portion. Vigorous stirring and heating was continued for 2 hours and the mixture was then concentrated *in vacuo* to a brown syrup which contained some suspended solid. The syrup was dissolved in 300 ml. of ethyl acetate and washed in succession with water (100 ml.), saturated sodium bicarbonate solution (4 x 50 ml.) and then a saturated sodium chloride solution (2 x 20 ml.). The solution was dried (anhydrous sodium sulfate) and then passed through a layer of SilicAR CC-7 (5 cm x 7 cm). The pad of silica gel was washed with ethyl acetate (2 x 20 ml.) and the filtrates were then combined and concentrated *in vacuo* to yield a light brown foam (20 g.). The foam was examined by tlc and appeared to contain two nucleoside components; a major component with a R_f of 0.3 [chloroform-acetone-methanol (44:5:1) as solvent] and a minor component with a R_f of 0.25. Both components were nucleoside material since the spots charred when sprayed with 5% sulfuric acid and then heated and they also absorbed uv light. The foam from above was dissolved in chloroform (15 ml.) and applied to the top of a dry packed column (37 cm x 6.5 cm) of SilicAR CC-7 (607 g.) and the column eluted with a mixture of chloroform-acetone-methanol (44:5:1) with 20 ml. fractions being collected. Fractions 10-34 contained the faster running component (pale yellow foam, 12.9 g., 56%), fractions 34-45 contained 3.4 g. of a mixture (mostly the faster running component) and fractions 46-50 contained 730 mg. of the slower moving component (R_f 0.25). Examination of the slower moving component by pmr revealed that it was a mixture of two compounds and this material was not examined further. The faster moving, major component was assigned the structure **7** on the basis of the physicochemical data listed in Tables I and II and the subsequent chemical conversion of **7** into a nucleoside of established structure.

Methyl 4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-formimidate Monohydrate (**11**).

Sodium metal (*ca.* 10 mg.) was dissolved in 50 ml. of dry methanol (distilled from calcium hydride) and then 3.89 g. of chromatographically homogeneous 4-acetamido-3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**) (as a dry foam) was added (17). Solution occurred immediately and in *ca.* 70 minutes the reaction was essentially complete as shown by tlc (major new spot, R_f 0.6, chloroform-methanol, 7:3). The reaction mixture was adjusted to a "*p*H" of 7 by the addition of small portion of Dowex 50 (H^+ form, prewashed with anhydrous methanol). The solution was then quickly filtered to remove the ion-exchange resin followed by concentration *in vacuo*. Trituration of the residue with isopropanol (3 x 10 ml.) afforded a light yellow powder, 2.44 g. that was recrystallized from methanol to give 2.2 g. (82%) of **11** as a pale yellow powder, m.p. *ca* 127-131°. A sample was recrystallized once more (methanol-water) for analysis: shiny clusters, m.p. 132° (forms a highly viscous melt that bubbles at 145°)

Anal. Calcd. for $C_{12}H_{10}N_6O_5 \cdot H_2O$: C, 42.1; H, 5.3; N, 24.55. Found: C, 42.1; H, 5.4; N, 24.7.

Alternately, the crude product (light brown foam) obtained during the preparation of 4-acetamido-3-cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**7**) (after passing the solution through a SilicAR CC-7 pad followed by evaporation) could be treated as above to give the product in over all yields (based on the amount of **5** used in the reaction) of 50-60%.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxamidine (**8**).

Method 1.

Methyl 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-formimidate monohydrate (**11**) (1.5 g.) was added to 10 ml. of liquid ammonia and the sealed steel reaction vessel was allowed to stand for 40 hours at room temperature. The ammonia was then allowed to evaporate and the residue suspended in 10 ml. of boiling water. Methanol was added to the boiling mixture until a clear solution had occurred and the solution was then concentrated to a volume of 10 ml. by heating. Crystallization was allowed to take place at room temperature. The solid was collected by filtration and washed with a small amount of cold (0°) methanol to give 1.1 g. (77%) of **8** as small rosettes, m.p. 188-190°. A sample was recrystallized form methanol (needles) m.p. 189-190°.

Anal. Calcd. for $C_{11}H_{15}N_7O_4$: C, 42.7; H, 4.9; N, 31.7. Found: C, 42.7; H, 5.0; N, 31.6.

Method 2.

Chromatographically homogeneous **7** (908 mg. of foam) was treated as above. After evaporation of the ammonia, the solid residue was triturated with 13 ml. of ethanol-2-propanol (1:2) and then recrystallized from methanol-water to yield 500 mg. (76%) of **8** (in two crops) m.p. 188-190°.

4-Amino-1-(β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidoxime Hemihydrate (**14**).

Methyl 4-amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-formimidate monohydrate (**11**) (1.5 g., 4.17 mmoles) was dissolved in anhydrous methanol (25 ml.) at reflux temperature and 0.5 g. (15 mmoles) of crystalline hydroxylamine added. Solution was almost complete in 0.5 hour when a solid other than **11** started to separate from solution. The suspension was heated at reflux temperature a total of 2 hours, cooled to 0°, the solid collected by filtration and washed with cold (0°) methanol (2 x 5 ml.). The solid (fine crystals) weighed 1.34 g. and had a m.p. of 151-152° (foams). Recrystallization from 1:1 methanol-water (40 ml.) gave pure **14**, 1.21 g. (86%), m.p. 242-244° (clear melt).

Anal. Calcd. for $C_{11}H_{15}N_7O_5 \cdot 0.5\ H_2O$: C, 39.55; H, 4.8; N, 29.3. Found: C, 39.8; H, 4.9; N, 29.2.

4-Amino-1-(β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-3-carboxamidrazone Hemihydrate (**15**).

To a suspension of **11** (1.5 g., 4.16 mmoles) in methanol (30 ml.) was added 2.5 ml. (42.5 mmoles) of 85% hydrazine hydrate. The mixture was warmed to effect a clear solution and the solution was then allowed to stand at 25° for 18 hours. The clusters of pale yellow needles that had formed were collected by filtration and washed with small amounts of methanol to yield 1.25 g. of **15**, m.p. 281-220°. A second crop (100 mg., m.p. 208-210°) was obtained from the supernatent. The crystals were combined and recrystallized from water (10 ml.) to give 1.12 g. (81.2%) of pure **15**, m.p. 220-221°.

Anal. Calcd. for $C_{11}H_{16}N_6O_4 \cdot 0.5\ H_2O$: C, 39.6; H, 5.1; N, 33.6. Found: C, 39.8; H, 5.2; N, 33.75.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-thiocarboxamide (**12**).

Sodium metal (140 mg., 6.4 mmoles) was dissolved in 45 ml. of anhydrous methanol and then anhydrous hydrogen sulfide was passed through the solution with magnetic stirring for 5 minutes. The nucleoside **11** (2.3 g., 6.4 mmoles) was added in one portion to the stirred solution of sodium hydrogen sulfide and in *ca.* 3 minutes a clear solution had occurred followed by the immediate appearance of a yellow solid. The mixture was stirred for 2 hours at 25°, cooled to 0° and the solid which had separated was then collected by filtration and washed with cold methanol (2 x 7 ml.). The solid (m.p. 132-135°, vigorous bubbling with no odor) was dissolved in 400 ml. of an ethanol-water mixture (1:1). The solution was concentrated to 200 ml. by heating and this was followed by cooling to room temperature. The solid which had separated from solution was collected (filtration) and washed in succession with cold ethanol (2 x 5 ml.) and then anhydrous ether (10 ml.). The light yellow solid weighed 1.69 g. (81.3%), m.p. 250-251.5° (vigorous dec., unpleasant odor). A small sample was recrystallized from a methanol-water mixture, m.p. 252-253° (dec. as above).

Anal. Calcd. for $C_{11}H_{14}N_6O_4S$: C, 40.5; H, 4.3; N, 25.6. Found: C, 40.6; H, 4.6; N, 25.4.

4-Amino-3-cyano-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine Monohydrate (**16**).

Dry (0.5 torr Hg, 25°, 1 hour) **12** (1 g., 3.06 mmoles) was dissolved in 70 ml. of warm (50°) dimethylformamide (DMF). Mercuric chloride (0.84 g., 3.10 mmoles) and triethylamine (1 ml., 7.24 mmoles) were added to the DMF solution and the mixture stirred at room temperature for 3 hours. The solution was then filtered through Celite to remove the black mercuric sulfide that had formed. The filter cake was washed with dry DMF (10 ml.) and the pale yellow filtrates were combined and concentrated *in vacuo*. The residue was triturated with cold (0°) methanol (10 ml.) to give 800 mg. of a pale yellow solid. The solid was suspended in 20 ml. of boiling methanol and then water was added dropwise to the hot suspension until solution had been effected. The solvent was removed by heating until crystallization started (final volume ca 15 ml.). Rapid stirring and cooling to 0° gave 580 mg. (61%) of a white solid, m.p. 235-238° dec. Repetition of the above crystallization procedure, with the aid of Norite, gave 500 mg. (42.6%) of pure **16**, m.p. 238-240° (sinters with preliminary darkening at 225°). The ir spectrum of **16** contained a moderate absorption for the cyano group at 2274 cm^{-1}.

Anal. Calcd. for $C_{11}H_{12}N_6O_4 \cdot H_2O$: C, 42.6; H, 4.5; N, 27.1. Found: C, 42.8; H, 4.8; N, 27.2.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**10**).

The nucleoside **11** (2 g., 5.57 mmoles) was suspended in 30 ml. of water and 1.0 ml. of 1.25 *N* sodium hydroxide solution was then added. The mixture was stirred at room temperature for 18 hours and the finely suspended solid was then collected by filtration (after cooling to 0°). The white solid was washed with cold (0°) water (2 x 10 ml.) and then dried *in vacuo* (25° 0.5 torr Hg) to yield 1.78 g. of **10**, m.p. 262-265°. Recrystallization of this solid from water (230 ml. required) gave an analytically pure product, 1.43 g. (74.5%), m.p. 270-271° (brown melt, bubbling).

Anal. Calcd. for $C_{11}H_{14}N_6O_5$: C, 42.6; H, 4.5; N, 27.1. Found: C, 42.6; H, 4.7; N, 27.1.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxylic Acid (**9**).

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine-3-carboxamide (**10**) (730 mg., 2.14 mmoles) was dissolved in 10 ml. of water containing 2.0 ml. of 1.25 *N* sodium hydroxide solution. The solution was heated at reflux temperature for 18 hours (ammonia evolution ceased during this period of time). The clear solution was then acidified by the addition of 2.45 ml. of 1.02 *N* hydrochloric acid solution. A gelatinous precipitate formed immediately and after further stirring this turned into a white solid. The suspension was stirred at 0°for 1 hour and the solid collected by filtration. The solid was washed with cold (0°) water (2 x 5 ml.) and then dissolved in hot water (60 ml.). The temperature was allowed to drop slowly to 0° as crystallization proceeded. The solid was collected by filtration to yield 590 mg. (89%) of white needles, m.p. 296° (sinters with drakening at 240°). A small sample was recrystallized from water to afford an analytical sample of **9**, darkens at 245°, sinters at 305°, m.p. >360°.

Anal. Calcd. for $C_{11}H_{13}N_5O_6$: C, 42.45; H, 4.2; N, 22.5. Found: C, 42.4; H, 4.3; N, 22.4.

4-Amino-1-(β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**13**).

Powdered, dry **9** (330.3 mg., 1.06 mmoles) was suspended in 15 ml. of freshly distilled sulfolane (Phillips Petroleum Co.). Dry nitrogen was passed through the suspension for 0.5 hour and the suspension of **9** was then lowered into a preheated (213°) Woods metal bath. A smooth evolution of carbon dioxide started [evolved carbon dioxide was passed through a rubber tube to an inverted, filled (water) graduated cylinder (placed in a beaker of water) so as to allow monitoring of the reaction] and continued for 40 minutes (19 ml. collected). The reaction was 1/3 completed in 10 minutes. Most of the sulfolane (12.5 ml.) was then removed by distillation *in vacuo*. The residue was triturated with methylene chloride (200 ml.) and the off-white powder collected by filtration (250 mg., m.p. 220-235°) (the over-all appearance of the uv spectrum of this powder was the same as that of pure **13**). The solid was dissolved in water (40 ml.), treated with Norite (0.3 g.) and then the solution was concentrated to 4 ml. by heating. The pure product separated from solution was small white needles, 170 mg. (60%), m.p. 247.5-249° (dec., darkens at 225°). An additional 20 mg. (7.7%) of product was obtained by concentrating the filtrate. The uv and nmr spectra of this material were identical to those of an authentic sample of **13** prepared by a different route (6b), $[\alpha]_D^{27°}$ -77.0° (C, 0.993, DMF).

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(15) It was important that the reaction be run in freshly distilled sulfolane under a nitrogen atmosphere since failure to take these precautions lowered the yield of product from 77% to 11%.

(16) For establishment of anomeric configuration of (**13**) see ref. 6a and for assignmnet of the site of glycosylation see ref. 6b.

(17) If traces of water are present it may be necessary to add additional amounts of sodium methoxide solution to maintain a "*p*H" of about 10, throughout the reaction.