Synthesis of β-D-Ribo- and 2'-Deoxy-β-D-ribofuranosyl Derivatives of 6-Aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one by a Ring Closure of Pyrazole Nucleoside Precursors

Pranab K. Gupta, N. Kent Dalley, Roland K. Robins and Ganapathi R. Revankar*

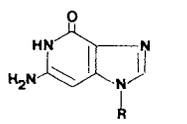
Cancer Research Center, Department of Chemistry,
Brigham Young University, Provo, Utah 84602

Received June 27, 1985

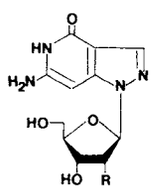
6-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**5**), as well as 2-(β-D-ribofuranosyl)- and 2-(2-deoxy-β-D-ribofuranosyl)- derivatives of 6-aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**18** and **22**, respectively) have been synthesized by a base-catalyzed ring closure of pyrazole nucleoside precursors. Glycosylation of the sodium salt of methyl 3(5)-cyanomethylpyrazole-4-carboxylate (**6**) with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**8**) provided the corresponding N-1 and N-2 glycosyl derivatives (**9** and **10**, respectively). Debenzoylation of **9** and **10** with sodium methoxide gave deprotected nucleosides **14** and **16**, respectively. Further ammonolysis of **14** and **16** afforded 5(or 3)-cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxamide (**15** and **17**, respectively). Ring closure of **15** and **17** in the presence of sodium carbonate gave **5** and **22**, respectively. By contrast, glycosylation of the sodium salt of **6** with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**11**) or the persilylated **6** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose gave mainly the N-2 glycosylated derivative **13**, which on ammonolysis and ring closure furnished **18**. Phosphorylation of **18** gave 6-amino-2-β-D-ribofuranosylpyrazolo[4,3-*c*]pyridin-4(5*H*)-one 5'-phosphate (**19**). The site of glycosylation and the anomeric configuration of these nucleosides have been assigned on the basis of ^{1}H nmr and uv spectral characteristics and by single-crystal X-ray analysis of **16**.

J. Heterocyclic Chem., **23**, 59 (1986).

Since our original report [1,2], the broad-spectrum antiviral [2,3] as well as potent antitumor activity [4-10] of 3-deazaguanine (6-aminoimidazo[4,5-*c*]pyridin-4(5*H*)-one, **1**) have been confirmed independently in a number of laboratories. Both 3-deazaguanine and 3-deazaguanosine exhibit significant antiviral activity against RNA and DNA viruses in cell culture [2]. 3-Deazaguanine is also active against influenza types A and B, and parainfluenza type I virus infection in mice [3]. 3-Deazaguanine exhibits cytotoxicity *in vitro* against a variety of cell types, which include L1210 [4,6,7], HeLa [4], human KB cells [8], Ehrlich ascites tumor cells [8], Chinese hamster ovary (CHO) cells [9], primary Chinese hamster embryo cells [5] and mammary carcinoma EMT-6 cells [10]. In animals, both **1** and **2** show inhibitory action against a broad spectrum of breast tumors, including mammary adenocarcinomas [5,6], and slow- and fast-growing mammary tumors [11]. 3-Deazaguanine is active *in vivo* against P388 leukemia, B16 melanoma and Ridgeway Osteogenic sarcoma [12]. 3-Deazaguanine is also highly active against MX-1 human mammary carcinoma xenograft with 100% tumor weight inhibition [12] and 97% tumor growth inhibition against mammary adenocarcinoma R3230AC [11]. 3-Deazaguanosine (**2**) has been found to be even more potent than **1** in the inhibition of CHO cells [9]. 2'-Deoxy-3--deazaguanosine (**3**), recently reported from our laboratory [13], was shown to be more active against L1210 and P388 leukemia in cell culture than **1**. 3-Deazaguanosine (*2*) was found to be a potent anti-leishmanial agent [14], which is at least 20 times more active than **1** or allopurinol ribonucleoside against *Leishmania tropica in vitro*. These observations provide a good rationale for the synthesis of other congeners of 3-deazaguanosine. In this paper we report the chemical synthesis of β-D-ribo-(**4**) and 2-deoxy-β-D-ribofuranosyl (**5**) derivatives of a guanine analog 6-aminopyrazolo[4,3-*c*]pyridin-4(5*H*)-one.



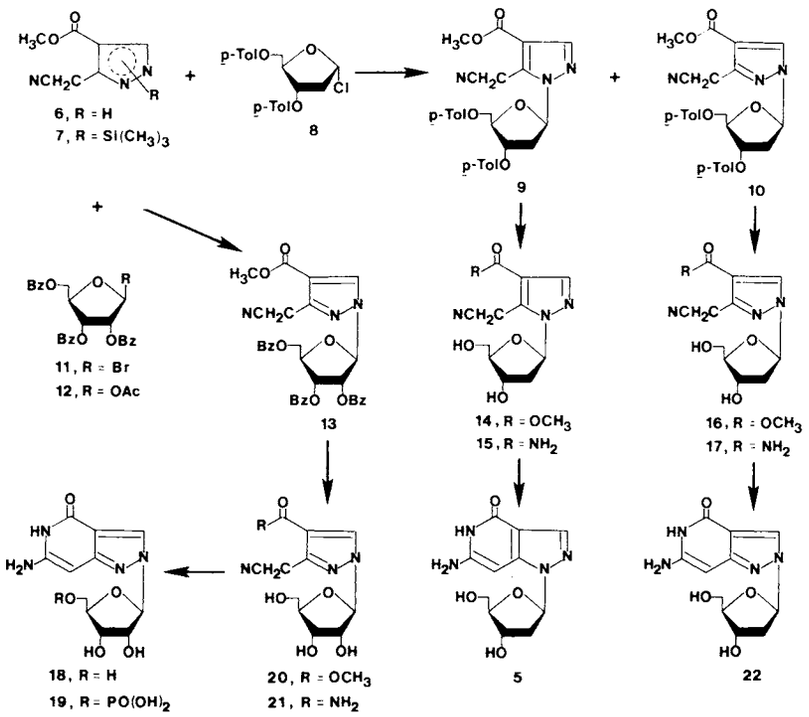
1, R = H
2, R = β-D-ribofuranose
3, R = 2-deoxy-β-D-ribofuranose



4, R = OH
5, R = H

It was envisaged that the synthesis of **4** and **5** might be realized from the cyclization of the appropriate glycosyl derivatives of a pyrazole precursor (Scheme I). The synthesis of such a substituted pyrazole, methyl 3(5)-cyanomethylpyrazole-4-carboxylate (**6**) was accomplished as described in our previous report [15]. Since that report [15], the isolated percentage yield of the starting material methyl 3(5)-methoxycarbonylmethylpyrazole-4-carboxylate, needed for the synthesis of **6**, was increased three fold (from 6.2 to 18%) by prolonging the reaction time. Ammonolysis of methyl 3(5)-methoxycarbonylmethylpyrazole-4-carboxylate and subsequent dehydration according to

SCHEME I



the published procedure [15] gave **6**. Application of the sodium salt glycosylation procedure, developed recently in our laboratory [13, 16-19], for the preparation of the nucleosides of **6** was found to be very successful. The sodium salt of **6** (generated *in situ* by the treatment of sodium hydride in acetonitrile) was glycosylated with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose [20] (**8**) at ambient temperature. Deoxyribosylation had occurred at both ring nitrogens of the pyrazole and produced a mixture of two nucleoside products. These products were separated on a silica gel column and identified as methyl 5-cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**9**) and the positional isomer methyl 3-cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**10**) on the basis of ^{1}H nmr characteristics (triplets for the anomeric protons). No formation of α-anomers was detected. Since the starting chlorosugar **8** has the α-configuration [21] in the solid state, the exclusive formation of the protected 2′-deoxy-β-D-ribofuranosides **9** and **10** in this study is believed to be due to a direct Walden inversion (S_N2 mechanism) of the anionic pyrazole nitrogen on the C_1 carbon of the blocked 2-deoxy sugar [17].

Subsequent treatment of **9** with methanolic sodium methoxide at room temperature, followed by silica gel column chromatography of the reaction residue, provided methyl 5-cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**14**). The ir spectrum of **14** revealed nitrile and ester carbonyl absorption bands at 2260 and 1700 cm^{-1}, respectively. In the ^{1}H nmr spectrum of **14**, in addition to all the protons at appropriate positions, the methyl ester protons appeared as a sharp singlet (3*H*) at δ 3.82 ppm. A similar reaction of **10** with sodium methoxide in methanol gave methyl 3-cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**16**), the structure of which was confirmed by single crystal X-ray analysis. Further ammonolysis of **14** and **16** (or **10**) each with liquid ammonia gave 5-cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxamide (**15**) and 3-cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxamide (**17**), respectively. When **15** was heated under reflux in aqueous sodium carbonate solution containing ethanol, 6-amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**5**) was formed in good yield. The ^{1}H nmr signal for the anomeric proton of **5** followed the triplet (centered at δ 5.91) splitting pattern [22] for 2′-deoxy-β-D-ribofuranosyl nucleosides. The observed peak width of the anomeric proton of 14.0 Hz is also consistent with the usual values for the β-configuration [22], which provided evidence for the structural assignment of **5**. Similarly base-catalyzed cyclization of **17** proceeded smoothly to yield 6-amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**22**). Like **5**, the pattern of the anomeric proton signal of **22** (triplet centered at δ 6.10 Hz with peak width 13.5 Hz) followed the convention observed with other 2′-deoxyribonucleosides [22].

Efforts were then initiated to synthesize the β-D-ribofuranosyl nucleoside **4** (Scheme I). 2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl bromide (**11**) was freshly prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose according to the method of Fletcher and co-workers [23]. The sodium salt of **6**, produced *in situ* by sodium hydride in acetonitrile, was treated with the ribofuranosyl bromide **11** at ambient temperature. A complex reaction mixture was obtained, and the product was purified on a silica gel column to provide the blocked nucleoside identified as methyl 3-cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazole-4-carboxylate (**13**). The isolated yield of **13** by this method was 66%. Debenzoylation of **13** with liquid ammonia at 100° for 3 hours gave methyl 3-cyanomethyl-1-β-D-ribofuranosylpyrazole-4-carboxylate (**20**), the uv absorption spectrum of which was virtually identical to that of **16**. Ammonolysis of **13** (or **20**) with liquid ammonia for a prolonged period of time gave 3-cyanomethyl-1-β-D-ribofuranosylpyrazole-4-carboxamide (**21**). Ring closure of **21** in the presence of aqueous sodium carbonate gave a 75% yield of 6-amino-2-β-D-ribofuranosylpyrazolo[4,3-*c*]-pyridin-4(5*H*)-one (**18**). The essentially identical uv absorption spectra of **18** and **22** (see experimental) provided evidence for the site of glycosylation in **18** as N-2. The observed small coupling constant (J = 3.0 Hz) of the anomeric proton in **18** lends support for the assignment of the β-configuration [24]. Direct phosphorylation of the unprotected **18** with phosphorus oxychloride in the presence of trimethyl phosphate at low temperature, according to the general procedure of Yoshikawa *et al.* [25], provided a rather low yield of 6-amino-2-β-D-ribofuranosylpyrazolo-[4,3-*c*]pyridin-4(5*H*)-one 5′-phosphate (**19**). The purity and structure of **19** was confirmed by elemental and ^{1}H nmr analyses.

In an effort to obtain the desired N-1 glycosyl isomer **4**, two additional glycosylation procedures were attempted. First, the attachment of the β-D-ribofuranosyl moiety to **6** using the general trimethylsilyl-Lewis acid procedure [26] was employed. Silylation of **6** with hexamethyldisilazane in the presence of ammonium sulfate gave the silylated product **7**. Reaction of **7** with one molar equivalent of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**) in the presence of 1.44 molar equivalent of stannic chloride (or trimethylsilyl trifluoromethanesulfonate) furnished a 79% yield of a blocked nucleoside identical with **13** as the major product. Secondly, the high temperature glycosylation procedure [27] using nonsilylated **6** and **12** in nitromethane with boron-trifluoride etherate as the catalyst gave similar results.

It has previously been found in our laboratory [2] that the yield and ratio of positional isomers in the trimethylsilyl-Lewis acid procedure markedly depends on the ratio of stannic chloride to aglycon and the carbohydrate employed. In the case of the imidazole counterpart of **6**, glycosylation in the presence of 1.44 molar equivalent of stannic chloride gave exclusively the N-1 glycosyl isomer [2]. The intermediacy of a stannic chloride heterocyclic complex, which provides the regiospecificity of glycosylation, was presumed to be a plausible explanation for this observation [2]. However, in the present case, a ring-nitrogen is not adjacent to the pyrazole ester group and thus cyclic complexation with stannic chloride cannot take place. Therefore, in this case the site of ribosylation is determined probably by steric reasons; the acyloxonium form of the carbohydrate would acylate the least sterically hindered N-2 ring-nitrogen. The deoxyribosylation via the sodium salt procedure had occurred with equal facility at both pyrazole ring nitrogens where the alkylation is not selective and affords both N-1 and N-2 deoxyribonucleosides.

Single Crystal X-ray Diffraction Analysis of **16**.

Since the crystals of **15**, **17** or **21** suitable for single-crystal X-ray analysis were not available, the methyl ester derivative **16** was used. Slow crystallization of **16** from water provided X-ray quality crystals. A suitable crystal (0.30 x 0.25 x 0.20 mm) was mounted on a Nicolet P3 auto-

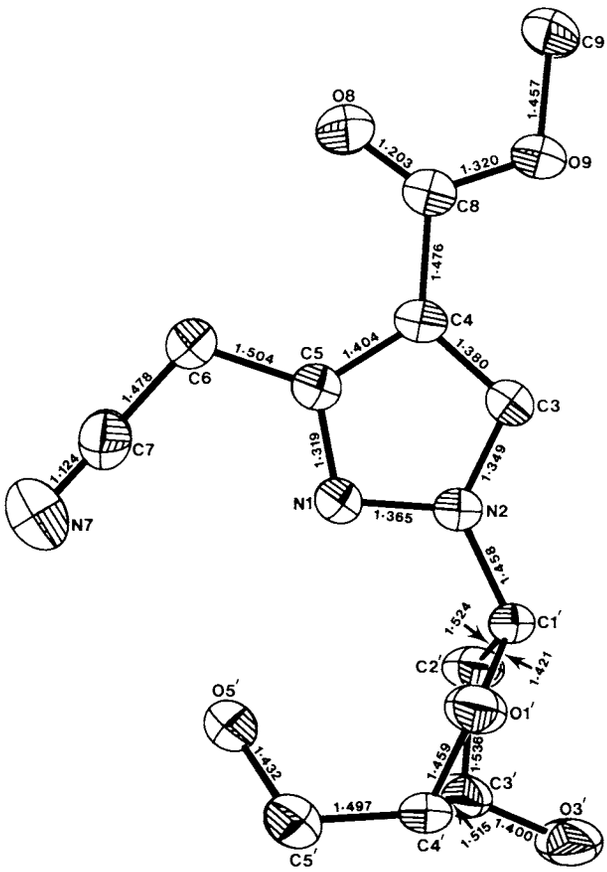


FIGURE I Computer drawing of compound 16

diffractometer and the diffration data for the determination of both the lattice parameters and the structural study were collected using Mo Kα graphite monochromated radiation (λ = 0.71069 Å). Compound **16** crystallized in the monoclinic space group $P2_1$ with a = 6.310 (2) Å, b = 12.602 (5) Å, c = 8.633 (4) Å, β = 105.27 (3)°, volume = 662.2 (5) Å^3 with Z = 2. A total of 2396 non-zero unique reflections were measured to a sin θ/λ limit of a 0.76 using a θ-2θ variable speed scan technique. There were 314 reflections less than 2σ(I) and these data were considered unobserved. The structure was solved by using direct methods and refined by using a full-matrix least-squares technique to a final R value of 0.053 and R_w = 0.036. All non-hydrogen atoms were refined anisotropically while hydrogen atoms, which were located in difference maps were refined isotropically. All computer calculations performed in this study were made using SHELX-76 program [28].

A computer drawing showing structural formula, conformation, atom lables and interatomic bond distances of compound **16** is shown in Figure I. The estimated standard deviations on all bond lengths are between 0.003 and 0.005 Å. The hydrogens of the aglycon and the carbohydrate moiety are omitted for clarity. The result of this structure determination study confirmed that the nucleoside **16** exists in the β-anomeric configuration and the site of glycosyl attachment is N-2. Since **16** is N-2 glycosyl isomer, compound **14** must be the N-1 positional isomer. Consequently the ring closed products **5** and **22** are assigned as N-1 and N-2 glycosyl isomers, respectively with the β-anomeric configuration.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H nmr) spectra were determined at 89.6 MHz with a JEOL FX 90Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of water as indicated by elemental analysis was verified by ^{1}H nmr. Infrared spectra (ir) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (uv, sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee, and Robertson Labs, Florham Park, New Jersey. Thin-layer chromatography (tlc) was run on silica gel 60 F-254 plates (EM Reagents). J.T. Baker silica gel (70-230 mesh) was used for column chromatography. All solvents used were reagent grade. Acetonitrile was dried over molecular sieve (4A, 24 h) and freshly distilled before use. Detection of components on tlc was by uv light and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°.

Methyl 3(5)-Methoxycarbonylmethylpyrazole-4-carboxylate.

A mixture of diethoxymethyl acetate (23.30 g, 145 mmoles) and dimethyl acetonedicarboxylate (24.90 g, 145 mmoles) was heated under reflux for 6 hours. The mixture was evaporated to dryness and the residue was co-evaporated with benzene (3 x 75 ml). The residual oil was dissolved in methanol (25 ml) and cooled to 0°. With continued cooling, hydrazine (97%, 2.6 ml, 75 mmoles) in methanol (50 ml) was added, dropwise. The resulting solution was stirred at ambient temperature for 48 hours and then filtered. The filtrate was evaporated and the residue was purified on a silica gel column (3 x 60 cm), prepacked in benzene:ether (2:1, v/v). Elution of the column with the same solvent system gave the title compound as the major product. Evaporation of the solvent and crystallization of the residue from ethyl acetate gave 5.30 g (18%), mp 102-103° (Lit [15] mp 103-105°); ir (potassium bromide): ν 1720, 1730 (C = O), 3250 (NH) cm^{-1}; uv (ethanol): λ max 240 (sh), nm (ϵ 1,400); ^{1}H nmr (deuteriochloroform): δ 3.70 (s, 3, CO_2CH_3), 3.82 (s, 3, CO_2CH_3), 4.15 (s, 2, CH_2), 8.10 (s, 1, C_3H), 13.40 (br s, 1, NH).

Methyl 5-Cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**9**) and Methyl 3-Cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**10**).

To a solution of methyl 3(5)-cyanomethylpyrazole-4-carboxylate [15] (**6**, 1.65 g, 10 mmoles) in dry acetonitrile (25 ml) was added sodium hydride (50% in oil, 0.53 g, 11 mmoles) in a nitrogen atmosphere. The clear solution was stirred for 45 minutes before a solution of 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose [20] (**8**, 4.10 g, 10.5 mmoles) in dry acetonitrile (200 ml) was added dropwise. The mixture was stirred at ambient temperature for 4 hours and then filtered through a Celite pad. The residue was washed with acetonitrile (3 x 25 ml) and the combined filtrates evaporated to give a foam, which was purified on a silica gel column (3.5 x 75 cm) using toluene:ethyl acetate (8:1, v/v) as the solvent. The following two nucleosides were isolated in the order listed:

Methyl 3-Cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**10**).

This compound was obtained as white foam, 1.96 g (38%), mp 72°; ir (potassium bromide): ν 1605, 1725 (C = O), 2260 (C $\equiv$ N) cm^{-1}; uv: λ max (methanol) 237 nm (ϵ 34,600); ^{1}H nmr (deuteriochloroform): δ 2.39, 2.41 (2s, 6, $2CH_3$), 3.84 (s, 3, CO_2CH_3), 3.92 (s, 2, CH_2), 6.20 (t, 1, J = 7.5 Hz, $C_1'H$), 7.24 (m, 4, Ph), 8.00 (m, 4, Ph), 8.14 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{28}H_{27}N_3O_7$ (517.54): C, 64.98; H, 5.25; N, 8.11. Found: C, 65.24; H, 5.36; N, 7.81.

Methyl 5-Cyanomethyl-1-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**9**).

This compound was obtained as an amorphous solid, 0.93 g (18%), mp 78°; ir (potassium bromide): ν 1610, 1715 (C = O), 2260 (C $\equiv$ N) cm^{-1}; uv (methanol): λ max 235 nm (ϵ 54,600); ^{1}H nmr (deuteriochloroform): δ 2.40, 2.44 (2s, 6, $2CH_3$), 3.90 (s, 3, CO_2CH_3), 4.16 (s, 2, CH_2), 6.40 (t, 1, J = 8.0 Hz, $C_1'H$), 7.30 (m, 4, Ph), 7.92 (m, 5, Ph and C_3H), and other sugar protons.

Anal. Calcd. for $C_{28}H_{27}N_3O_7$ (517.54): C, 64.98; H, 5.25; N, 8.11. Found: C, 65.22; H, 5.44; N, 7.95.

Methyl 5-Cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**14**).

To a solution of **9** (0.80 g, 1.54 mmoles) in dry methanol (25 ml was added 1M sodium methoxide in methanol (1.25 ml) and the mixture was stirred at room temperature for 1 hour. The reaction mixture was neutralized with Dowex-50 H$^+$ resin and filtered. The filtrate was evaporated to dryness. The residual semisolid was purified on a silica gel column (2.5 x 45 cm), prepacked in chloroform. Elution of the column with chloroform:methanol (19:1, v/v) gave a homogeneous residue, which was crystallized from aqueous ethanol to yield 0.17 g (37%), mp 118-120°; ir (potassium bromide): ν 1700 (C = O), 2260 (C $\equiv$ N), 3400 (OH) cm^{-1}; uv: λ max (*p*H 1) 218 nm (ϵ 11,000); λ max (*p*H 7) 218 nm (ϵ 11,100); λ max (*p*H 11) 224 nm (ϵ 10,200); ^{1}H nmr (DMSO-d_6): δ 3.82 (s, 3, CO_2CH_3), 4.40 (s, 2, CH_2), 6.16 (t, 1, peak width 14.5 Hz, $C_1'H$), 8.18 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{12}H_{15}N_3O_5$ (281.17): C, 51.24; H, 5.33; N, 14.94. Found: C, 50.99; H, 5.43; N, 14.80.

5-Cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxamide (**15**).

Compound **14** (0.50 g, 1.78 mmoles) and liquid ammonia (5 ml) were placed in a steel bomb (25 ml). The bomb was three-quarters submerged in a steam bath and heated for 4 hours. The ammonia was allowed to evaporate at room temperature and the residue was subjected to a vacuum overnight to remove the last traces of ammonia. The residual brown solid was purified on a silica gel column (2 x 30 cm) using chloroform:methanol (9:1, v/v) as the solvent. The homogeneous solid was crystallized from methanol as light yellow needles to yield 0.17 g (57%), mp 106-107°; ir (potassium bromide): ν 1655 (C=O), 2240 (C≡N), 3400 (OH, NH_2)cm^{-1}, uv: λ max (*p*H 1) 278 nm (ε 4,500); λ max (*p*H 7) 280 nm (ε 6,100): λ max (*p*H 11) 282 nm (ε 5,600); ^{1}H nmr (DMSO -d_6) δ 4.22 (s, 2, CH_2), 6.08 (t, 1, peak width 14.5 Hz, $C_1'H$), 7.16 and 7.60 (2 br s, 2, $CONH_2$), 8.46 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_4$ (266.26): C, 49.62; H, 5.30; N, 21.04. Found: C, 49.42; H, 5.42; N, 21.12.

6-Amino-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**5**).

A mixture of **15** (0.26 g, 1 mmole), 5% aqueous sodium carbonate (2.5 ml), and ethanol (2 ml) was heated under gentle reflux with stirring for 30 minutes. Complete dissolution was obtained as reflux started. The brown solution was filtered and the filtrate was stored at 0° for 24 hours. The crystalline product that separated was collected by filtration, washed with cold water (3 x 5 ml) and recrystallized from water as off-white needles to yield 0.14 g (53%), mp 146°; ir (potassium bromide): ν 1630, 1660 (C=O), 3200-3380 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 285 nm (ε 7,200); λ max (*p*H 7) 228 nm (ε 13,300), 271 (5,500); λ max (*p*H 11) 229 nm (ε 14,400), 282 (5,300); ^{1}H nmr (DMSO-d_6): δ 5.40 (s, 1, C_7H), 5.52 (s, 2, NH_2), 5.91 (t, 1, peak width 14.0 Hz, $C_1'H$), 7.80 (s, 1, C_3H), 10.20 (br s, 1, N*H*), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_4 \cdot \frac{1}{2}H_2O$ (275.26): C, 47.99; H, 5.49; N, 20.34. Found: C, 47.99; H, 5.50; N, 20.10.

Methyl 3-Cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxylate (**16**).

A mixture of **10** (0.80 g, 1.54 mmoles), absolute methanol (10 ml) and 1*M* sodium methoxide in methanol (1.2 ml) was stirred at room temperature for 1 hour. The reaction mixture was worked up and purified as described for **14**, and the product was crystallized from water to yield 0.20 g (46%), mp 106-107°; ir (potassium bromide): ν 1695 (C=O), 2260 (C≡N), 3370 (OH) cm^{-1}; uv: λ max (*p*H 1) 222 nm (ε 10,800); λ max (*p*H 7) 222 nm (ε 10,000; λ max (*p*H 11) 222 nm (ε 9,300); ^{1}H nmr (DMSO -d_6): δ 3.80 (s, 3, CO_2CH_3), 4.18 (s, 2, CH_2), 6.13 (t, 1, peak width 14.5 Hz, $C_1'H$), 8.62 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{12}H_{15}N_3O_5 \cdot \frac{1}{4}H_2O$ (285.77): C, 50.43; H, 5.34; N, 14.71. Found: C, 50.46; H, 5.47; N, 14.58.

3-Cyanomethyl-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazole-4-carboxamide (**17**).

A mixture of **10** (5.17 g, 10 mmoles), liquid ammonia (50 ml) and anhydrous methanol (40 ml) was heated in a steed bomb at 100° for 14 hours. Methanolic ammonia was evaporated and the brown residue was purified on a silica gel column (3.5 x 70 cm) using chloroform:methanol (19:3, v/v) as the solvent. The homogeneous product was crystallized from methanol to yield 0.82 g (31%) as yellow needles, mp 102-104°; ir (potassium bromide): ν 1650 (C=O), 2240 (C≡N), 3400 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 270 nm (ε 2,100); λ max (*p*H 7) 274 nm (ε 3,300); λ max (*p*H 11) 282 nm (ε 2,900); ^{1}H nmr (DMSO-d_6): δ 4.16 (s, 2, CH_2), 6.06 (t, 1, peak width 14.5 Hz, $C_1'H$), 7.20 and 7.60 (2 br s, 2,$CONH_2$), 8.44 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_4 \cdot \frac{1}{2}H_2O$ (275.26): C, 47.99; H, 5.49; N, 20.35. Found: C, 47.72; H, 5.20; N, 20.09.

6-Amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**22**).

In the same manner as for **5**, the title compound was prepared using **17** (2.66 g, 10 mmoles), 5% aqueous sodium carbonate (25 ml) and ethanol (20 ml). The product was crystallized from water as off-white needles, to yield 1.86 g (70%), mp 108-109°; ir (potassium bromide): ν 1630, 1665 (C=O), 3350 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 245 nm (ε 10,400); λ max (*p*H 7) 228 nm (ε 19,900), 267 (7,200), 308 (sh) (2,900); λ max (*p*H 11) 229 nm (ε 24,00), 265 (4,000), 314 (3,700); ^{1}H nmr (DMSO-d_6) δ 5.24 (s, 1, C_7H), 5.32 (br s, 2, NH_2), 6.10 (t, 1, peak width 13.5 Hz, $C_1'H$), 8.41 (s, 1, C_3H), 9.92 (br s, 1, N*H*), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_4$ (266.26): C, 49.62; H, 5.30; N, 21.04. Found: C, 49.41; H, 5.29; N, 20.80.

Methyl 3-Cyanomethyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (**13**). Method A.

To a suspension of **6** (1.65 g, 10 mmoles) in anhydrous acetonitrile (25 ml) was added sodium hydride (60% in oil, 0.44 g, 11 moles), and the mixture was stirred at ambient temperature in an atmosphere of dry argon for 30 minutes during which time a clear solution was obtained. To the solution was added 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide [23] (**11**, prepared from 5.54 g, 11 mmoles of 1-*O*-acetyl-2,3,5-tri-*O*-benzoly-β-D-ribofuranose) in dry acetonitrile (40 ml), dropwise over a period of 15 minutes. The resulting mixture was stirred at room temperature for 4 hours under argon. The reaction mixture was filtered and to the filtrate was added silica gel (10 g). The mixture was evaporated to dryness. Co-evaporation with benzene (2 x 75 ml) gave dry residue, which was placed on top of a silica gel column (2.5 x 45 cm), prepacked in toluene. Elution of the column with toluene:chloroform (1:3, v/v) gave a light yellow colored solid, which was crystallized from benzene containing 5% methanol to yield 4.02 g (66%), mp 75°; ir (potassium bromide): ν 1600, 1720 (CO_2CH_3, C=O), 2260 (C≡N) cm^{-1}; uv (methanol): λ max 227 nm (ε 45,600); ^{1}H nmr (deuteriochloroform): δ 3.80 (s, 3, CO_2CH_3), 3.83 (s, 2, CH_2), 6.15 (d, 1, J = 3.5 Hz, $C_1'H$), 7.45 (m, 10, 2 COC_6H_5), 8.05 (m, 6, COC_6H_5 and C_3H), and other sugar protons.

Anal. Calcd. for $C_{33}H_{27}N_3O_9$ (609.6): C, 65.02; H, 4.46; N, 6.89. Found: C, 64.93; H, 4.63; N, 6.68.

Method B.

Compound **6** (1.65 g, 10 mmoles) was heated under reflux for 10 hours with hexamethyldisilazane (HMDS, 15 ml) and ammonium sulfate (0.10 g) with the exclusion of moisture. The excess HMDS was removed by distillation providing the trimethylsilyl derivative **7** as a yellowish brown solid. The solid **7** was dissolved in dry 1,2-dichloroethane (60 ml). 1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**12**, 5.04 g, 10 mmoles) was added to the solution, followed by the addition of stannic chloride (1.67 ml, 14.4 mmoles) in one portion. The reaction mixture was stirred at ambient temperature for 15 hours under anhydrous conditions. Tlc (silica gel, benzene:chloroform, 1:3, v/v) of an ethanolyzed aliquot indicated complete conversion of the carbohydrate and heterocycle to the title compound. The reaction mixture was poured into a 5% sodium bicarbonate solution (200 ml) and stirred for 1 hour. The mixture was filtered through a Celite pad, extrated with chloroform (3 x 100 ml) and the combined, dried (over sodium sulfate) organic phase was evaporated to dryness. The residual semi-solid was purified on a silica gel column (3.5 x 60 cm), prepacked in benzene. Elution of the column with benzene:chloroform (1:3, v/v) gave a homogeneous light yellow colored solid, which was crystallized from benzene/methanol to yield 4.80 g (79%), mp 75°. This compound was found to be identical to **13** prepared by Method A.

Methyl 3-Cyanomethyl-1-β-D-ribofuranosylpyrazole-4-carboxylate (**20**).

A mixture of **13** (1.20 g, 2 mmoles) and liquid ammonia (12 ml) was heated in a steel reaction vessel at 100° for 3 hours. The ammonia was allowed to evaporate at room temperature and the residue was subjected to a vacuum overnight to remove the last traces of ammonia. The brown residue so obtained was purified on a silica gel column (2.5 x 45 cm) using chloroform:methanol (9:1, v/v) as the solvent. Evaporation of the appropriate homogeneous fractions gave a light brown solid, which was crystallized from aqueous ethanol to yield 0.25 g (41%), mp 89°; ir

(potassium bromide): ν 1700 (C=O), 2260 (C≡N), 3400 (OH) cm^{-1}; uv: λ max (*p*H 1) 222 nm (ε 8,000); λ max (*p*H 7) 222 nm (ε 8,600); ^1H nmr (DMSO-d_6): δ 3.78 (s, 3, CO_2CH_3), 4.16 (s, 2, CH_2), 5.70 (d, 1, J = 4.0 Hz, $C_1'H$), and other sugar protons.

Anal. Calcd. for $C_{12}H_{15}N_3O_6$ (297.27): C, 48.48; H, 5.08; N, 14.13. Found: C, 48.11; H, 5.41; N, 13.98.

3-Cyanomethyl-1-β-D-ribofuranosylpyrazole-4-carboxamide (**21**).

Compound **13** (1.20 g, 2 mmoles), dry methanol (10 ml) and liquid ammonia (10 ml) were heated in a steel reaction vessel at 100° for 15 hours. The reaction mixture was worked up and purified as described for **17**, and the product was crystallized from aqueous ethanol to yield 0.18 g (33%), mp 88-90°; ir (potassium bromide): ν 1660 (C=O), 2260 (C≡N), 3360 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 270 nm (ε 5,900); λ max (*p*H 7) 274 nm (ε 7,600); λ max (*p*H 11) 282 nm (ε 6,500); ^1H nmr (DMSO-d_6): δ 4.15 (s, 2, CH_2), 5.70 (d, 1, J = 4.0 Hz, $C_1'H$), 7.20 and 7.62 (2 br s, 2, $CONH_2$), 8.50 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_5$ (282.25): C, 46.81; H, 5.00; N, 19.85. Found: C, 46.73; H, 4.90; N, 19.56.

6-Amino-2-β-D-ribofuranosylpyrazolo[4,3-*c*]pyridin-4(5*H*)-one (**18**).

In the same manner as for **5**, the title compound was prepared using **21** (0.23 g, 0.81 mmole), 5% aqueous sodium carbonate (3 ml) and ethanol (5 ml). The product was crystallized from water as colorless needles, to yield 0.17 g (75%), mp 158°; ir (potassium bromide); ν 1630, 1660 (C=O), 3240-3350 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 245 nm (ε 12,400); λ max (*p*H 7) 228 nm (ε 23,00), 267 (8,600), 309 (sh) (3,700); λ max (*p*H 11) 266 nm (ε 9,000), 312 (sh) (4,500); ^1H nmr (DMSO-d_6): δ 5.24 (s, 1, C_7H), 5.28 (br s, 2, NH_2), 5.64 (d, 1, J = 3.0 Hz, $C_1'H$), 8.45 (s, 1, C_3H), and other sugar protons.

Anal. Calcd. for $C_{11}H_{14}N_4O_5$ (282.25): C, 46.81; H, 5.00; N, 19.85. Found: C, 46.60; H, 5.15; N, 19.69.

6 Amino-2-β-D-ribofuranosylpyrazolo[4,3-*c*]pyridin-4(5*H*)-one 5'-phosphate (**19**).

To a solution of phosphorus oxychloride (2.82 g, 18.4 mmoles) in trimethyl phosphate (15 ml) (cooled to 0° with an ice bath) was added dried and powdered **18** (1.30 g, 4.6 mmoles). The suspension was stirred at 0° with the exclusion of moisture for 6 hours. The amber colored solution was added dropwise to vigorously stirred ether (300 ml). The ether was decanted and additional ether (150 ml) was added to the residual solid. After stirring for half an hour, the ether was decanted and the process was repeated once more with additional ether (150 ml). The solid was dissolved in water (20 ml) and the *p*H of the aqueous solution was adjusted to 8 with 1*N* sodium hydroxide. The alkaline solution was passed through a column of Dowex-50 H^+ resin (2 x 10 cm) and washed with water. The fractions containing the homogeneous product were pooled, concentrated, freezed and lyophilized to yield 0.30 g (18%) of the title compound, mp 165-168°; ir (potassium bromide): ν 1660 (C=O), 2900-3300 (OH, NH_2) cm^{-1}; uv: λ max (*p*H 1) 246 nm (ε 18,500); λ max (*p*H 7) 267 nm (ε 17,200), 309 (sh) (7,600); λ max (*p*H 11) 266 nm (ε 10,500), 317 (sh) (11,200); ^1H nmr (deuterium oxide): δ 6.00 (d, 1, J = 3.25 Hz, $C_1'H$), 8.54 (s, 1, C_3H).

Anal. Calcd. for $C_{11}H_{15}N_4O_8P \cdot 2\frac{1}{2}H_2O$ (407.23): C, 32.44; H, 4.95; N, 13.75; P, 7.60. Found: C, 32.23; H, 4.77; N, 13.63; P, 7.72.

Acknowledgement.

This investigation was supported in part by Contract DAMD 17-79-C-9046 with the U. S. Army Medical Research and Development Command, Washington, D. C. This is contribution No. 5001 to the Army Research Program on Antiparasitic Drugs.

REFERENCES AND NOTES

[1] P. D. Cook, R. J. Rousseau, A. M. Mian, R.B. Meyer, Jr., P. Dea, G. Ivanovics, D. G. Streeter, J. T. Witkowski, M. G. Stout, L.N. Simon, R. W. Sidwell and R. K. Robins, *J. Am. Chem. Soc.*, **97**, 2916 (1975).

[2] P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr. and R. K. Robins, *J. Am. Chem. Soc.*, **98**, 1492 (1976).

[3] L. B. Allen, J. H. Huffman, P. D. Cook, R. B. Meyer, Jr., R. K. Robins and R. W. Sidwell, *Antimicrob. Agents Chemother.*, **12**, 114 (1977).

[4] T. A. Khwaja, L. Kigwana, R. B. Meyer, Jr. and R. K. Robins, *Proc. Am. Assoc. Cancer Res.*, **16**, 162 (1975).

[5] T. A. Khwaja and J. C. Varven, *Proc. Am. Assoc. Cancer Res.*, **17**, 200 (1976).

[6] P. Schwartz, D. Hammond and T. A. Khwaja, *Proc. Am. Assoc. Cancer Res.*, **18** 153 (1977).

[7] R. S. Rivest, D. Irwin and H. G. Mandel, *Proc. Am. Assoc. Cancer Res.*, **21**, 279 (1980).

[8] D. G. Streeter and H. H. P. Koyama, *Biochem. Pharmacol.*, **25**, 2413 (1976).

[9] P. P. Saunders, L-Y. Chao, T. L. Loo and R. K. Robins, *Biochem. Pharmacol.*, **30**, 2374 (1981).

[10] T. A. Khwaja, L. Momparler, J. C. Varven and A. M. Mian, *Proc. Am. Assoc. Cancer Res.*, **20**, 152 (1979).

[11] T. A. Khwaja, *Cancer Treat. Rep.*, **66**, 1853 (1982).

[12] R. K. Robins and G. R. Revankar, *Med. Res. Rev.*, **5**, 273 (1985).

[13] G. R. Revankar, P. K. Gupta, A. D. Adams, N. K. Dalley, P. A. McKernan, P. D. Cook, P. G. Canonico and R. K. Robins, *J. Med. Chem.*, **27**, 1389 (1984).

[14] J. D. Berman, L. S. Lee, R. K. Robins and G. R. Revankar, *Antimicrob. Agents Chemother.*, **24**, 283 (1983).

[15] K. W. Ehler, R. K. Robins and R. B. Meyer., *J. Med. Chem.*, **20**, 317 (1977).

[16] Z. Kazimierczuk, G. R. Revankar and R. K. Robins, *Nucleic Acids Res.*, **12**, 1179 (1984).

[17] Z. Kazimierczuk, H. B. Cottam, G. R. Revankar and R. K. Robins, *J. Am. Chem. Soc.*, **106**, 6379 (1984).

[18] H. B. Cottam, Z. Kazimierczuk, S. Geary, P. A. McKernan, G. R. Revankar and R. K. Robins, *J. Med. Chem.*, **28**, 1461 (1985).

[19] P. K. Gupta, R. K. Robins and G. R. Revankar, *Nucleic Acids Res.*, **13**, 5341 (1985).

[20] M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

[21] A. K. Bhattacharya, R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 428 (1963).

[22] M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **87**, 4934 (1965).

[23] J. D. Stevens, R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **33**, 1806 (1968).

[24] L. B. Townsend, "Synthetic Procedures in Nucleic Acid Chemistry", Vol 2, W. W. Zorbach and R. S. Tipson, eds, Wiley-Interscience, New York, 1973, p 330.

[25] M. Yoshikawa, T. Kato and T. Takenishi, *Tetrahedron Letters*, 5065 (1967).

[26] H. Vorbruggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, **114**, 1234 (1981).

[27] H. B. Cottam, C. R. Petrie, P. A. McKernan, R. J. Goebel, N. K. Dalley, R. B. Davidson, R. K. Robins and G. R. Revankar, *J. Med. Chem.*, **27**, 1119 (1984).

[28] G. M. Sheldrick, SHELX-76 "A Program for X-ray Crystal Structure Determination (1976), University of Cambridge, England.