Pyrazolopyrimidine Nucleosides. 13. Synthesis of the Novel *C*-Nucleoside 5-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one, a Guanosine Analogue Related to the Nucleoside Antibiotic Formycin B

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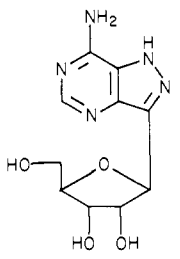
Abstract: The novel guanosine derivative 5-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one [5-aminoformycin B] has been prepared from the adenosine-type *C*-nucleoside antibiotic formycin. The synthetic route has used an initial ring opening followed by a series of chemical transformations and subsequent ring closure to afford 5-aminoformycin B. This synthetic route was also used to prepare the heterocyclic aglycon, 5-aminopyrazolo[4,3-*d*]pyrimidin-7-one, of the guanosine derivative.

Isolation and structure elucidation[1] of the antibiotics formycin



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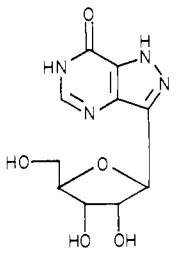

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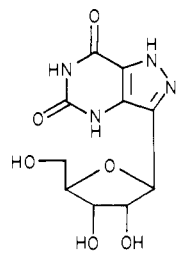

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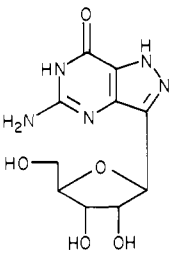

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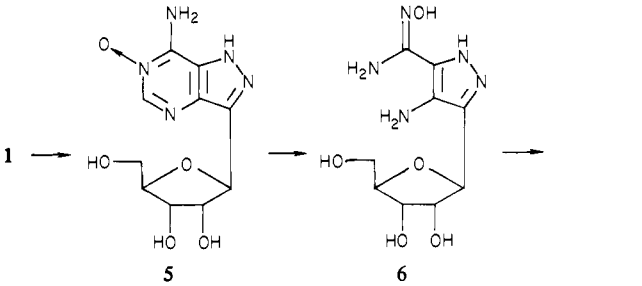
(**1**) and formycin B (**2**) as *C*-nucleosides was followed by the synthesis and study of a variety of *C*-nucleosides. The diverse biological activity which has been reported[2] for the *C*-nucleoside adenosine analogue formycin (**1**) and certain analogues of **1** has provided a continuing interest in *C*-nucleosides. The ability of formycin to mimic adenosine in many biological reactions has generated additional interest in this specific area and especially in 7-substituted derivatives. However, only a few derivatives of **1** and/or **2** with a substituent in the 5-position have been reported.[3] With the exception of the inactive catabolite of **2**, oxoformycin (**3**), only limited data are available on the biological activities of 5-substituted derivatives of **1** or **2** since the synthesis of such derivatives requires a suitable and easily attainable intermediate. We have communicated[4] on the synthesis of 4-amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (**7**) as a potential intermediate for the synthesis of certain bicyclic *C*-nucleosides. We would now like to report the detailed synthesis of **7** and the conversion of **7** into the *C*-nucleoside guanosine analogue 5-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (5-aminoformycin B, **4**).

Scheme I



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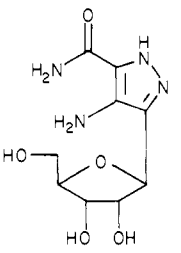

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Derivatives of guanosine have been widely studied as potential antitumor agents and as probes in biological reactions.[5] For a nucleoside to exhibit biological activity, it is generally necessary that the nucleoside first be converted to a nucleotide (at least the 5′-monophosphate derivative). To obtain the 5′-monophosphate derivative of a guanosine derivative or analogue in a mammalian

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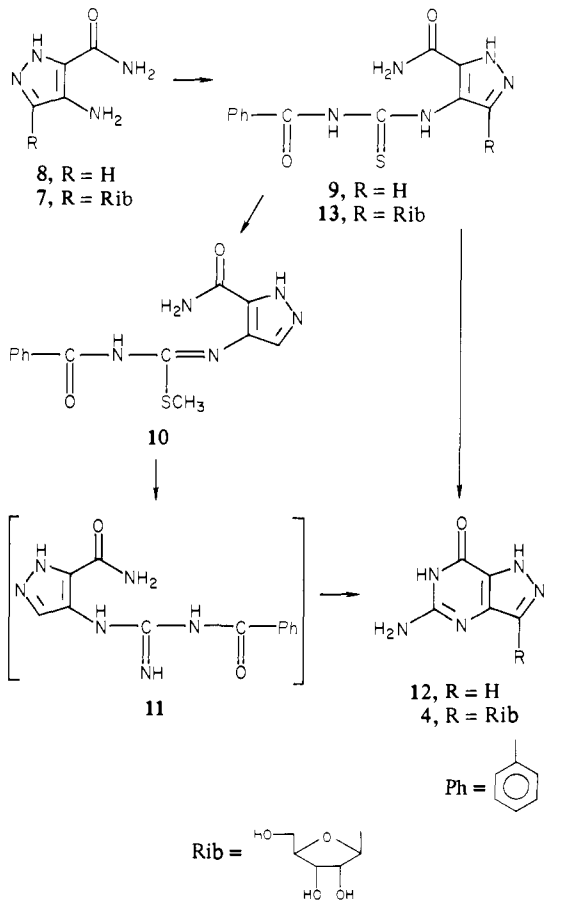
Scheme II



cell, the glycosidic bond must first be enzymatically cleaved by purine nucleoside phosphorylase (PNPase) and the base or aglycon must then be a substrate for hypoxanthine-guanine phosphoribosyl transferase (HGPRTase).[5] Formycin derivatives, by virtue of their stable carbon–carbon glycosidic bond, are not substrates for phosphorylases.[6] Thus, an analogue such as **4** should not be converted to a biologically active nucleotide due to the absence of guanosine kinase. It is of some interest that formycin B has been reported to be an inhibitor of PNPase and, in fact, a more recent study has established that certain guanine and guanosine analogues are better inhibitors of PNPase than the corresponding hypoxanthine and inosine analogues. Therefore, **4** should be a better inhibitor of PNPase than formycin B and thus be a powerful probe in the study of nucleic acid metabolism and cell kinetics. The aglycon (**12**, guanine analogue) of **4** may also prove to be an effective inhibitor of PNPase and/or HGPRTase. Direct phosphorylation of inosine, which is very closely related to guanosine, has been reported;[7] however, this report has not been confirmed. Therefore, it is tempting to postulate that a guanosine analogue (e.g., **4**) which is not a substrate for PNPase might be used as a probe for the existence in mammalian cells of the so far unreported and elusive enzyme, guanosine kinase.

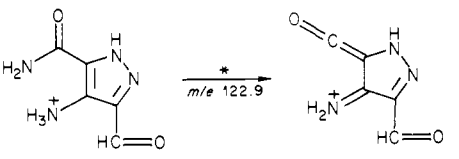
The synthesis of 4-amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (**7**), which has been given the sobriquet of Ψ APCA-riboside, was first described by Long.[3] However, this method provided a low yield of impure **7** and prompted us to investigate alternate routes of synthesis. Studies[8] on the pyrimidine ring fission of adenine and adenosine *N*-oxides suggested that formycin N^6-oxide[3] (**5**) might be a viable precursor of **7**.

A study of the stability of **5** showed that it reacted very rapidly with dilute hydrochloric acid, even at ambient temperature. A time course study on the reaction of **5** with acid, by UV analysis, revealed that the initial rapid loss of long wavelength absorption for **5** was followed by the slow development of a maximum at 267 nm (pH 11). Chromatographic analysis of these reactions showed that a mixture of products were obtained under a variety of conditions. We were unable to isolate a pure single product from these mixtures. Surprisingly, formycin N^6-oxide (**5**) was found to be relatively stable to basic conditions. Ultraviolet analysis of a 1 N sodium hydroxide solution of **5** showed no change after 11 days at room temperature. In fact, **5** was only changed very slowly by basic solutions of even higher normality or elevated temperatures.

The extreme ease of pyrimidine ring fission resulting from the treatment of **5** under acidic conditions suggested the use of an acidic ion-exchange resin as a mild acid reagent. An aqueous solution of formycin N^6-oxide (**5**) was stirred with Dowex 50 (H^+) for several hours. These acidic conditions effected a fission of the pyrimidine ring and furnished 4-amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamidoxime (**6**). The reaction time for several preparations of **6** was varied from 13 to 24 h with little effect being observed on the yield of the product. The carboxamidoxime **6** was identified by spectral comparisons with 4-amino-3-methylpyrazole-5-carboxamidoxime[9] and by its elemental analysis.

Based on preliminary experiments, we found that it was advantageous to synthesize **7** by a concurrent reduction and hydrolysis of the amidoxime function of **6**. These early investigations also showed that a conversion of the amidoxime into the amide was a slow reaction and proceeded through an unidentified intermediate. Subsequent to these initial experiments, we elected to treat formycin N^6-oxide (**5**) with Dowex 50 (H^+) to give **6** which, without purification, was then subjected to Raney nickel-catalyzed hydrogenation conditions for 4 days. An approximate 17% yield of **7** (from **5**) was obtained from this reaction after dry column chromatography. The 4-amino-3-(β-D-ribofuranosyl)-pyrazole-5-carboxamide (**7**) obtained in this manner was homogenous on thin-layer chromatograms. However, liquid chromatographic analysis indicated that **7** was actually about 90% pure. Pure **7** was obtained only by preparative, reverse-phase, liquid chromatography with a recovery of approximately 50%. A more productive preparation of **7** was subsequently achieved by purification of the intermediate amidoxime, **6**, prior to the conversion into **7**. When purified, **6** was used in the basic hydrogenation reaction to provide a product which was sufficiently pure for use in the subsequent syntheses. The nucleoside **7** was obtained in 31% yield (from **6**) (19% from **5**, 15.5% from **1**), without extensive chromatography.

The structure assigned to nucleoside **7** was confirmed by UV spectral comparisons to the UV spectral data reported[9,10] for the known 4-amino-3-methylpyrazole-5-carboxamide, by the ^1H NMR spectra and by mass spectral analysis. The appearance of a predominate ion at *m/e* 138 in the mass spectrum of **7** indicates that ammonia is readily eliminated from the *b* + 30 ion and supports the *vic*-aminocarboxamide structure assignment. A similar elimination from the *vic*-hydroxycarboxamide function of pyrazofurin has been reported.[11]



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The cytotoxicity of **7** toward L-1210 cell cultures was evaluated in a study which also included pyrazofurin and AICA riboside. The findings of this study have been previously communicated.[4]

The facile, acid-catalyzed pyrimidine ring fission observed for formycin N^6-oxide (**5**) prompted us to improve on the published[3] method for the synthesis of **5**. Formycin (**1**) was treated with *m*-chloroperbenzoic acid in methanol at reflux and formycin N^6-oxide (**5**) was isolated by pouring the reaction mixture into a large volume of ethyl acetate. Although the yield of **5** obtained was not significantly improved by this modification, the isolation was greatly simplified and the evaporation of acidic solutions of **5** was avoided.

The conversion of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA riboside) into guanosine using mild methods which involved reactions with benzoyl isothiocyanate have been described.[12] A versatile anhydronucleoside was described which could be converted into a variety of 2-substituted inosines. These procedures were basically used as a design for the conversion of **7** into 5-aminoformycin B (**4**).

Before the synthesis of **4** was begun, a resynthesis of 5-aminopyrazolo[4,3-*d*]pyrimidin-7-one[14] (**12**) using like methods was completed.

A condensation of 4-aminopyrazole-3-carboxamide[13] (**8**) with benzoyl isothiocyanate resulted in the formation of 4-((*N*-benzoylthiocarbamoyl)amino)pyrazole-3-carboxamide (**9**). The structure **9** was confirmed by an examination of its ^{1}H NMR spectrum. A pattern of three acidic, exchangeable protons was observed between δ 11 and δ 14 with no signal being observed between δ 4 and δ 7. Treatment of **9** with methyl iodide under basic conditions gave 4-((*N*-benzoyl-*S*-methylisothiocarbamoyl)amino)pyrazole-3-carboxamide (**10**). The ^{1}H NMR spectra of **10** established that methylation of the pyrazole ring had not occurred. The only signal which could be attributed to a methyl group was observed at δ 2.67, which indicated that only the sulfur atom of the thiocarbamoyl side chain of **9** had been methylated.

Compound **10** was treated with a saturated solution of ammonia in dimethylformamide to obtain a mixture of two new products. This mixture was dissolved in a dilute sodium hydroxide solution, and this solution was heated at reflux to produce a single compound which was identified as 5-aminopyrazolo[4,3-*d*]pyrimidin-7-one (**12**) by its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of **12** exhibited a broad, two proton singlet at δ 6.24 which disappeared on the addition of D_2O. Several recrystallizations and/or reprecipitations were required to obtain pure **12**. A reexamination of the mixture resulting from the treatment of **10** with ammonia revealed that one of the two products was **12** and presumably the other compound was the guanidino derivative **11**. Thus, the synthesis of **12** was achieved, in part, directly from **10** by the use of a more concentrated ammonia solution than that used in the studies[12] on imidazole derivatives.

An excellent yield of 4-((*N*-benzoylthiocarbamoyl)amino)-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (**13**) was obtained from the condensation of benzoyl isothiocyanate with ΨAPCA-riboside (**7**). The ^{1}H NMR spectrum of **13** showed a pattern of acidic, exchangeable protons very similar to the pattern which had been observed in the ^{1}H NMR spectrum of **9**. Nucleoside **13** was treated under basic conditions with methyl iodide. A pure product was not obtained from the aqueous reaction mixture. Evaporation of the solvent furnished a residue which was then stirred with dilute sodium hydroxide for 2 days. Attempts to isolate an anhydronucleoside analogous to the anhydronucleoside described[12] in the corresponding imidazole studies were unsuccessful. Thin-layer chromatographic analysis indicated that four or more components were present in this reaction mixture. The mixture was then heated with concentrated ammonium hydroxide to obtain another mixture composed of two major and several minor products. Pure samples of the two major products were obtained by silica gel column chromatography of the mixture. The UV spectra of one compound was very similar to the spectra recorded for the guanine analogue **12**. The second major product was assigned the structure of 5-(methylthio)-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (5-(methylthio)formycin B) from the close similarity of its UV spectra to the UV spectra of 3-methyl-5-(methylthio)pyrazolo[4,3-*d*]pyrimidin-7-one.[9,10] Additional support for this assignment was provided by a mass spectrum; M^+ = 674 (M + 5Me_4Si), *m/z* = 355 (*b* + 30 + 2Me_4Si). Thus, evidently a ring closure of the methylthiocarbamoyl intermediate competes, in basic solution, with the formation of an anhydronucleoside.

Subsequently, **13** was reacted with methyl iodide and the crude reaction products were then treated with a saturated solution of ammonia in dimethylformamide. After evaporation of the solvent, the mixture was then heated at reflux with dilute sodium hydroxide. The target compound, 5-amino-3-(β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidin-7-one (5-aminoformycin B, **4**) was isolated from this reaction after extensive chromatography. The structural assignment was supported by elemental analysis and by spectral comparisons with the guanine analogue **12**.

As was the case with the synthesis of 5-aminopyrazolo[4,3-*d*]pyrimidin-7-one (**12**), 5-amino-3-(β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidin-7-one (5-aminoformycin B, **4**) was also obtained directly from the reaction between ammonia and the methylthiocarbamoyl intermediate. Following a methylation of the nucleoside **13**, the reaction products were treated with a saturated solution of ammonia in dimethylformamide. The solvent and excess ammonia were removed by evaporation, and the residue was stirred with water to afford impure **4**. Pure **4** was obtained by solvent (ethanol) extraction of the impure product followed by a recrystallization of the remaining solid. An approximate yield of 7% of **4** (from **7**) was achieved. However, considerable quantities of impure **4** were recovered from the solvent extraction and recrystallization filtrates. An additional quantity of pure **4** was obtained by repeating the above purification procedure.

Studies designed to established the ability of both **4** and **12** as substrates and/or inhibitors of PNPase and/or HGPRTase are currently under active investigation in our laboratories.

Experimental Section

Elemental analyses were obtained from Heterocyclic Chemical Corp, Harrisonville, MO., or M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ultraviolet spectra were recorded on a Beckman ACTA C-III spectrophotometer. The ^{1}H NMR spectra were obtained for both Me_2SO-d_6 and Me_2SO-d_6/D_2O solutions of the compounds and were recorded on a Varian EM-390 instrument. Chemical shifts are expressed as δ values from an internal standard (DSS). Mass spectra were recorded on a Varian MAT 112S instrument and are electron-impact spectra. HPLC was conducted on a Waters Associates instrument.

Thin-layer chromatography was performed on glass plates coated (0.25 mm) with SilicAR-7GF (Mallinckrodt). The chromatogram components were visualized with a shortwave (254-nm) UV mineralight. Dry column chromatography used silica gel (J. T. Baker #5-3405) to which 0.5% of a phosphor (Du Pont #609) had been added, packed in nylon columns of required diameter, and sealed at one end. The sealed end was perforated, and the solvent and products were allowed to elute from the column. The progress of the UV absorbing components were monitored with a shortwave (254-nm) UV mineralight. HPLC analyses used a 4 × 300 mm μBondapak C_{18} (Waters) column. A Porasil B-C_{18} (Waters) column (8 × 1800 mm) was used for the preparative separations. Water was used as the mobile phase.

Chromatographic solvent systems used were as follows: A, acetonitrile/0.1 M ammonium chloride, 9:2, v/v; B, chloroform/methanol, 3:1, v/v; C, methanol; D, water; E, ethyl acetate; F, ethyl acetate/water/1-propanol, 4:2:1, v/v/v, upper phase; G, chloroform/methanol, 9:2, v/v.

Evaporations were conducted on a rotary evaporator using a hot water bath as a heat source and under water aspirator vacuum for low boiling (bp ≤ ethanol) solvents or high vacuum for higher boiling solvents, unless otherwise specified.

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Benzoyl isothiocyanate was purchased from Trans-World Chemicals, redistilled and stored at 5 °C until needed. **Formycin** was purchased from Meiji Seika Kasha Co., Tokyo, Japan.

7-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (Formycin N^6-oxide) (5). A mixture of Formycin (**1**, 5 g), *m*-chloroperbenzoic acid (5 g), and methanol (250 mL) was stirred and heated at reflux for 2.5 h. An additional portion of *m*-chloroperbenzoic acid (5 g) was then added, the stirring and heating being continued for another 2.5 h. The hot reaction solution was poured into ethyl acetate (1.5 L) with rapid stirring and then allowed to stand at room temperature for 14 h. The yellow solid was collected by filtration, washed with ethyl acetate (100 mL), and then air-dried to give 4 g of impure **5**. The solid was suspended in boiling ethanol (700 mL), treated with charcoal, and filtered and the filter cake washed with boiling ethanol (100 mL). The ethanol solutions were combined and evaporated to dryness in vacuo. The solid residue was washed with ethyl acetate (50 mL) and air-dried to yield 3.55 g (67%) of **5**. The product was homogeneous on TLC (solvent system A), and the UV spectra were identical with the spectra reported[3] for **5**: UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 292 (6.65), 230 (12.20); pH 11, 310 (4.80), 236 (35.20); MeOH, 299 (4.25), 245 (13.87), 230 (23.35). Anal. Calcd for $C_{10}H_{13}N_5O_5$: C, 42.40; H, 4.59; N, 24.73. Found: C, 42.60; H, 4.69; N, 24.45.

4-Amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamidoxime (6). Formycin N^6-oxide (**5**) (670 mg) was dissolved in 30 mL of water. Dowex 50-X4 (H^+) (8 g) was added and the mixture stirred at room temperature for 15 h. The Dowex was removed by filtration and washed with water (50 mL), and the filtrate and wash were discarded. The resin was then stirred with a water:concentrated ammonium hydroxide (2:1, v/v) mixture for 0.5 h. The resin was removed by filtration, washed with water (30 mL), and again treated with dilute ammonia as previously described. The ammonia solutions and water washes were combined and evaporated to dryness in vacuo. The residue was coevaporated with water (5 × 20 mL) and then dissolved in boiling water (40 mL). The boiling solution was treated with charcoal, filtered, and again evaporated to dryness in vacuo. The residue was coevaporated with methanol (3 × 10 mL) and then stirred with ethanol (20 mL). This produced a white solid which was collected by filtration and air-dried to yield 270 mg (42%) of **6**, mp 180–185 °C dec. Pure **6** was obtained by recrystallization from methanol and drying at 110 °C in vacuo: mp 190–192 °C dec. UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 11, 270 (6.15), 237 (6.29). TLC solvent system A, R_1 = 1.17; solvent system B, R_1 = 0.71. Anal. Calcd for $C_9H_{15}N_5O_5$ (273.3): C, 39.56; H, 5.53; N, 25.53. Found: C, 39.59; H, 5.56; N, 25.77.

4-Amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (ΨAPCA-riboside) (7). Method A. Formycin N^6-oxide (**5**) (2 g) was dissolved in water (100 mL). Dowex 50-X4 (H^+) (20 g) was added and the mixture stirred at room temperature for 18 h. The resin was removed by filtration and washed with water (200 mL). The resin was then stirred with a mixture of water/concentrated ammonium hydroxide (1:1, v/v) (120 mL) for 1 h. The mixture was filtered, the resin was washed with water (100 mL), and the wash was combined with the ammonia filtrate. The solution was then evaporated to dryness in vacuo to give a brown foam, which was dissolved in 0.2 N sodium hydroxide (80 mL). Water wet Raney nickel (3 g) was added and the mixture shaken under hydrogen (2.8 Kg/cm^2) for 4 days. The reaction mixture was filtered, the catalyst was washed with boiling water (200 mL), and the wash was added to the filtrate. The basic solution was then stirred with Amberlite IRC-50 (80 g) until neutral (approximately 20 min). The resin was then removed by filtration and washed with water (3 × 100 mL). The washes were combined with the filtrate, and the solution was evaporated to dryness in vacuo. The residue was dissolved in boiling water (50 mL), the solution treated with charcoal, filtered, and then freeze-dried to give a light yellow solid (880 mg). The solid was dissolved in water (50 mL), silica gel (J. T. Baker #5-3405) (2 g) was added, and the mixture was evaporated to dryness in vacuo. The dry mixture was placed on top of a silica gel dry column (2 in. × 8 in.). The column was eluted with solvent system C, and the first 50 mL of eluate was collected. The methanolic solution was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in water (20 mL) and the solution freeze-dried to obtain a white solid (300 mg). Reverse phase LC analysis showed that this material was approximately 90% pure. Samples (3 × 60 mg) of this material were chromatogramed on a Porasil B-C_{18} column (8 × 1800 mm) using solvent system D as the mobile phase. The major product (K' = 0.52) for each sample was collected, and then the three solutions were combined, concentrated to 20 mL in vacuo, and then freeze-dried to give 90 mg of pure **7**: mp wide range >85 °C. $[\alpha]_D^{24.5} = -56.5°$ (c = 1, H_2O); UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) H_2O, 282 (4.8), 232.5 sh (4.5); pH 11, 282 (4.9), 232.5 sh (4.9); MS M^+, m/z 258; ^1H NMR δ 4.73 (d, $H_{1'}$, $J_{1',2'}$ = 6.5 Hz). TLC solvent system A, R_1 = 1.07, R_6 = 0.91; solvent system B, R_1 = 0.11; solvent system D, R_1 = 1.11. Anal. Calcd for $C_9H_{14}N_4O_5$ (258.2): C, 41.86; H, 5.46; N, 21.70. Found: C, 41.64; H, 5.52, N, 21.63.

Method B. 4-Amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamidoxime (**6**) (5.1 g) was dissolved in 0.2 N sodium hydroxide (500 mL). Water wet Raney nickel (15 g) was added and the mixture shaken under hydrogen (2.8 Kg/cm^2) for 4.5 days. The catalyst was removed by filtration and washed with boiling water (500 mL). The wash and filtrate were combined and stirred with Amberlite IRC-50 (70 g) until neutral (approximately 30 min). The resin was removed by filtration and then washed with water (2 × 200 mL). The washes were combined with the filtrates, and the resulting solution was evaporated to dryness in vacuo. The brown residue was stirred with boiling methanol (200 mL). After the mixture was left standing at room temperature for 12 h, a brown insoluble solid was removed from the mixture by filtration and discarded. The methanol filtrate was evaporated to dryness in vacuo and the residue redissolved in boiling methanol (100 mL). The boiling solution was treated with charcoal, and the charcoal was washed with boiling methanol (40 mL). The two alcoholic solutions were combined and evaporated to dryness in vacuo. The residue was stirred with hot methanol (50 mL), and the mixture was evaporated to dryness in vacuo. This coevaporation with methanol was repeated two more times, then the residue was extracted with boiling methanol (100 mL) and the mixture filtered to remove insoluble material. After the mixture was left standing at room temperature for 12 h, the crystalline solid, which had deposited from the methanol filtrate, was collected by filtration and air-dried to yield 1.5 g (31%) of **7** which was sufficiently pure for our subsequent syntheses.

4-((*N*-Benzoylthiocarbamoyl)amino)pyrazole-3-carboxamide (9). 4-Aminopyrazole-3-carboxamide (**8**)[13] (7.6 g) was dissolved, by warming, in water (200 mL). A mixture of benzoyl isothiocyanate (10.4 g) and ethanol (20 mL) was added slowly (approximately 2 min) to the rapidly stirred solution of **8**. The mixture was stirred at room temperature for 1 h, during which time the sides of the reaction flask were frequently scraped clean of adhering solid. The solid was then collected, washed with hot ethanol (250 mL), and air-dried to yield 11.95 g of product. The solid was recrystallized from a methanol/acetone mixture to obtain 8.7 g (50%) of **9**. Pure **9** was obtained by recrystallization of a sample (1 g) from a methanol/acetone mixture and drying in vacuo at 110 °C: 550 mg; mp 254–257 °C, melts, then resolidifies, and then decomposes at 338–340 °C; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 302 (11.7), 268 (24.0), 236 sh (14.5); pH 11, 307 (9.26), 262 sh (14.9), 236.5 (17.8); methanol, 308 (12.0) 265 (23.0), 238.5 sh (12.6); ^1H NMR δ 7.4–8.1 (m, 7 H, C_6H_5 and amide NH_2), 9.14 (s, 1 H, H_3), 11.3 (s, 1 H, NH), 13.35 (s, 1 H, NH), 13.72 (s, 1 H, NH). TLC solvent system C, R_f = 0.83; solvent system E, R_f = 0.68; solvent system F, R_f = 0.86. Anal. Calcd. for $C_{12}H_{11}N_5O_2S$ (289.3): C, 49.82; H, 3.83; N, 24.21. Found: C, 49.89; H, 3.62; N, 24.42.

4-((*N*-Benzoyl-*S*-methylisothiocarbamoyl)amino)pyrazole-3-carboxamide (10). 4-((*N*-Benzoylthiocarbamoyl)amino)pyrazole-3-carboxamide (**9**) (500 mg) was dissolved in 0.1 N sodium hydroxide (75 mL). Methyl iodide (0.15 mL) was then added and the mixture stirred at room temperature for 1 h. The reaction mixture was acidified (approximately pH 6) with glacial acetic acid. The solid was collected by filtration, washed with water (20 mL), and then air-dried to yield 500 mg (95%) of product. Pure **10** was obtained by recrystallizaiton from methanol with charcoal treatment and drying at 110 °C in vacuo to yield 260 mg of **10**: mp 196–197 °C; UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 241 (17.1); pH 11, 305 sh (9.25), 232 (16.1); methanol 305 (17.0), 283 (16.2), 241 (13.3); ^1H NMR δ 2.67 (s, 3 H, SCH_3), 7.4–8.3 (m, 7 H, C_6H_5 and amide NH_2), 8.44 (s,, 1 H, H_3), 10.95 (s, 1 H, NH), 13.42 (s, 1 H, NH); TLC solvent system E, R_f = 0.59, R_9 = 0.86; solvent system F, R_f = 0.86. Anal. Calcd for $C_{13}H_{13}N_5O_2S$ (303.3): C, 51.48; H, 4.32; N, 23.09. Found: C, 51.54; H, 4.23; N, 23.25.

5-Aminopyrazolo[4,3-*d*]pyrimidin-7-one (12). A mixture of **10** (5.56 g) and *N*,*N*-dimethylformamide (150 mL), which had been previously saturated with ammonia at 0 °C, was placed in a sealed reaction vessel and heated at 125 °C for 2 h. After being cooled, the reaction mixture was evaporated to dryness on a steam bath in the hood. The solid residue was washed with water (25 mL) and air-dried to yield 3.42 g; mp >300 °C. TLC in solvent system G showed that this product was composed of two major components. By TLC comparison with the product from a previous small scale reaction and by UV analysis of the mixture, it was determined that one of the components was the desired **12** and it was assumed that the other product was the intermediate **11**. The solid mixture (3.42 g) was stirred and heated at reflux in a 1 N sodium hydroxide (70 mL) solution for 3.5 h. The solution was acidified (approximately pH 6) with concentrated hydrochloric acid and the mixture allowed to stand at 5 °C for 14 h. The solid which had separated was collected by filtration, washed with water (25 mL) and then acetone (25 mL), and air-dried. The dry solid was powdered and then extracted with boiling ethanol (3 × 50 mL). The ethanol insoluble solid was recrys-

tallized twice from water to obtain 1.14 g (41%) of impure **12**. Pure **12** was obtained by reprecipitation (at approximately pH 6) from hot sodium hydroxide with dilute hydrochloric acid, then recrystallization from water, and then reprecipitation (at approximately pH 6) from a hot dilute sodium hydroxide solution with glacial acetic acid to yield 380 mg of **12**; mp >300 °C dec. TLC: solvent systems B, D, and F all showed single elongated spots. The solid was dried in vacuo at 110 °C. Anal. Calcd for $C_5H_5N_5O$ (151.1): C, 39.74; H, 3.33; N, 46.34. Found: C, 38.46, 38.24; H, 3.52, 3.44; N, 45.57, 45.47. The elemental analyses indicated that **12** was slowly being hydrated. Anal. Calcd for $C_5H_5N_5O \cdot 0.25H_2O$ (155.6): C, 38.59; H, 3.56; N, 45.00. A sample of **12** was exposed to the atmosphere for several days and then analyzed. Found: C, 38.05; H, 3.44; N, 45.15. UV: λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 279 (4.36), pH 11, 299 (5.06); methanol, 300 (4.67), 243.5 sh (5.68). ^{1}H NMR: δ 6.24 (b s, 2 H, NH_2), 7.67 (s, 1 H, H_3).

4-((*N*-Benzoylthiocarbamoyl)amino)-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide Monohydrate (13). 4-Amino-3-(β-D-ribofuranosyl)-pyrazole-5-carboxamide (**7**) (1.3 g) was mixed with benzoyl isothiocyanate (830 mg) and *N*,*N*-dimethylformamide (25 mL). The mixture was stirred at room temperature for 2 h and then evaporated to a heavy oil in vacuo. The oil was triturated with hot toluene (2 × 25 mL) and then dissolved in methanol (20 mL). The methanolic solution was added slowly to chloroform (200 mL) with rapid stirring. The volume of the resulting mixture was reduced to approximately 100 mL by heating on a steam bath. The solution was then allowed to stand at room temperature for 14 h. The white solid which had separated was collected by filtration, washed with chloroform, and then air-dried to yield 1.93 g (91%); mp wide range >120 °C. TLC in solvent system G showed that the product contained trace impurities. Pure **13** was obtained by recrystallization of a small sample from a methanol/ethyl acetate mixture: UV λ_{max} (nm) ($\epsilon \times 10^{-3}$) pH 1, 283.5 sh (12.7), 245 (19.8); pH 11, 236 (20.0); methanol, 282 sh (9.01), 242 (16.0); ^{1}H NMR δ 4.92 (d, 1 H, $H_{1'}$), δ 7.0–8.3 (m, C_6H_5 and amide NH_2), 11.5 (s, 1 H, NH), 11.94 (s, 1 H, NH), 12.99 (s, 1 H, NH). TLC solvent system B, R_1 = 1.17, R_7 = 2.40; solvent system D, R_1 = 0.86, R_7 = 0.78. The hydration of **13** was confirmed by the ^{1}H NMR spectrum. Anal. Calcd for $C_{17}H_{19}N_5O_6S \cdot H_2O$ (439.5): C, 46.46; H, 4.82; N, 15.94. Found: C, 46.40; H, 4.91; N, 15.51.

5-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (5-Aminoformycin B) (4). Compound **13** (1.63 g) was dissolved in 0.1 N sodium hydroxide (40 mL). Methyl iodide (600 mg) was added, and the mixture was stirred at room temperature for 2.5 h. A few drops of glacial acetic acid were added to acidify (approximately pH 6) the mixture, which was then evaporated to dryness in vacuo. The residue was coevaporated with methanol (2 × 20 mL) to obtain a yellow foam. The foam was dissolved in *N*,*N*-dimethylformamide (20 mL), which had been previously saturated with ammonia at 0 °C. The resulting solution was placed in a sealed reaction vessel and heated, in an oil bath, at 130 ± 5 °C (bath temperature) for 3 h. The reaction mixture was then evaporated on a steam bath, in the hood, to give a brown oil. The oil was coevaporated, in vacuo, with water (2 × 20 mL) and then with ethanol (20 mL). The residue was recrystallized from water (10 mL) to obtain a brown solid (480 mg). The water filtrate was saved for later processing. UV analysis of the solid confirmed that **4** had been obtained. TLC analysis (solvent system B) showed that the solid consisted of one major product with R_f = 0.38 and several minor products. The solid (450 mg) was dissolved in a methanol/water (3:1, v/v) mixture (40 mL). Silica gel (J. T. Baker #5-3405) (10 g) was added and the mixture evaporated to dryness in vacuo. The dry mixture was coevaporated with methanol (3 × 30 mL) and then placed on top of a shallow bed (64 × 20 mm) of silica gel, which had been prewashed with methanol. The bed was washed with methanol (4 × 100 mL), and the combined washes were filtered and then evaporated to dryness in vacuo to obtain a solid residue. The solid residue was stirred with hot ethanol (25 mL) and, after being cooled to room temperature, the mixture was filtered and the solid washed with ethanol (20 mL). The ethanol extraction was repeated to obtain a total of 220 mg (20% yield) of **4** which showed only trace impurities on TLC (solvent system B) analysis. Pure **4** was obtained by recrystallization of a sample from a water/acetonitrile mixture; mp wide range >250 °C with decomposition. A sample of **4** was dried in vacuo at 110 °C just prior to elemental analysis. Anal. Calcd for $C_{10}H_{13}N_5O_5$ (283.2): C, 42.41; H, 4.63; N, 24.73. Found: C, 42.24; H, 4.56; N, 24.42. A sample of **4** which was not dried just prior to elemental analysis gave C, H, and N which corresponded to the hemihydrate: UV λ_{max} (nm), ($\epsilon \times 10^{-3}$) pH 1, 282 (7.02); pH 11, 301 (6.28), 249 sh (8.62); methanol, 300 (4.97), 249.5 sh (8.62); ^{1}H NMR δ 4.80 (d, 1 H, $H_{1'}$, $J_{1',2'}$ = 6 Hz), 5.97 (s, 2 H, NH_2), 10.8 (b s, 1 H, ring NH), 13.33 (b s, 1 H, ring NH). TLC solvent system A, R_1 = 1.09; solvent system B, R_1 = 0.91, R_{13} = 0.53.

The water filtrate from the first recrystallization of **4** was evaporated to dryness in vacuo. The residue was dissolved in 0.5 N sodium hydroxide (20 mL), and the solution was heated at reflux for 1 h. The solution was acidified (approximately pH 6) with formic acid and then evaporated to dryness in vacuo. The residue was stirred with water (5 mL) and the pH adjusted (pH >10) with concentrated ammonium hydroxide. The basic solution was applied to a column of Dowex 1-X8 (formate) (100 mL). The column was washed with water (1.2 L) and then eluted with a linear formic acid gradient, using water (1 L) in the mixing chamber and 0.5 N formic acid in the reservoir. Fractions of approximately 18 mL were collected at 6-min intervals and analyzed by UV and TLC (solvent system B). Fractions which contained only **4** (No. 46-55) were pooled and evaporated, in vacuo, to dryness. The residue was coevaporated with ethanol (3 × 25 mL) and then recrystallized from a methanol/ethanol mixture to obtain additional **4** (19 mg). The fractions from the formate column which were predominately **4**, but also contained small amounts of other products, were pooled and evaporated to dryness in vacuo. After coevaporation with ethanol (3 × 25 mL), 70 mg of slightly impure **4** was obtained.

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