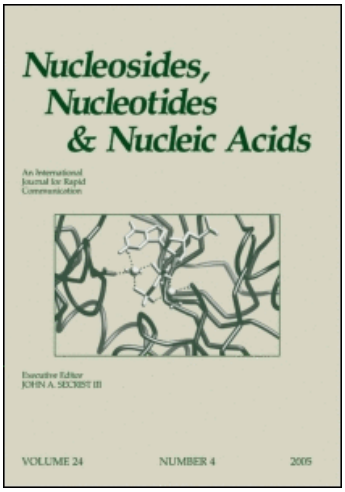

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SYNTHESIS OF PYRAZOLO[3,4-d]PYRIMIDINE RIBONUCLEOSIDE 3',5'-CYCLIC PHOSPHATES RELATED TO cAMP, cIMP AND cGMP[1]

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ABSTRACT: The synthesis of pyrazolo[3,4-d]pyrimidine ribonucleoside 3',5'-cyclic phosphates related to cAMP, cIMP and cGMP has been achieved for the first time. Phosphorylation of 4-amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (1) with $POCl_3$ in trimethyl phosphate gave the corresponding 5'-phosphate (2a). DCC mediated intramolecular cyclization of 2a gave the corresponding 3',5'-cyclic phosphate (3a), which on subsequent dethiation provided the cAMP analog 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 3',5'-cyclic phosphate (3b). A similar phosphorylation of 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (5), followed by cyclization with DCC gave the 3',5'-cyclic phosphate of 5 (9a). Dethiation of 9a with Raney nickel gave the cIMP analog 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-cyclic phosphate (9b). Oxidation of 9a with m-chloroperoxybenzoic acid, followed by ammonolysis provided the cGMP analog 6-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-cyclic phosphate (7). The structural assignment of these cyclic nucleotides was made by UV and ^1H NMR spectroscopic studies.

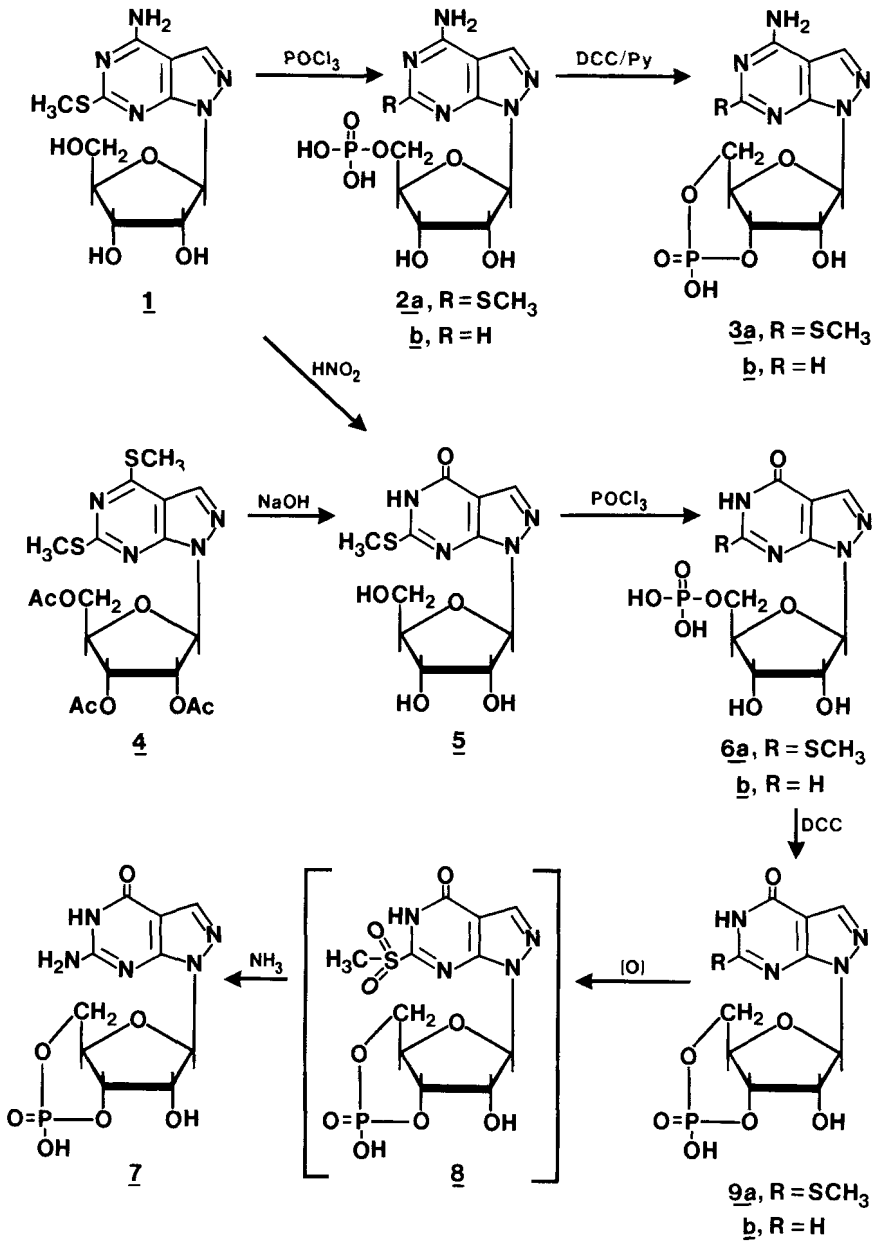
INTRODUCTION: In connection with our antiparasitic program directed towards the preparation of compounds related to allopurinol (pyrazolo-[3,4-d]pyrimidin-4(5H)-one) ribonucleosides,[2-7] we became interested in the synthesis of 3',5'-cyclic phosphates of pyrazolo[3,4-d]pyrimidine nucleosides. Despite the considerable effort expended on the chemistry of cyclic nucleotides and cyclic nucleotide analogs,[8] the synthesis of pyrazolo[3,4-d]pyrimidine ribonucleoside 3',5'-cyclic phosphates has not been realized to date. It is of particular interest that adenosine 3',5'-cyclic phosphate (cAMP) has been implicated in the differentiation of Trypanosoma lewisi from a rapidly reproducing to a nonreproductive form.[9] Epimastigote forms of Trypanosoma cruzi are known to contain a

calcium independent soluble form of cAMP phosphodiesterase.[10] Therefore, it is logical to implicate cyclic nucleotides in the differentiation of epimastigotes to trypomastigotes of T. cruzi.[10] Although the synthesis of 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (4-APP riboside) has been reported[11] as early as 1964, the synthesis of the 3′,5′-cyclic phosphates of neither 4-APP riboside (3b), nor allopurinol riboside (9b) are documented in the literature. These cyclic phosphates would serve as important biochemical tools to study the exact mode of action of pyrazolo[3,4-d]pyrimidine nucleosides in biological systems. Herein we report the synthesis of 3b, 9b and also the cGMP analog 7.

CHEMISTRY: 5-Phosphoribosyl-1-pyrophosphate (PRPP) has long been shown to react enzymatically with pyrazolo[3,4-d]pyrimidines to give the corresponding nucleoside 5′-phosphates.[12,13] Subsequently, Japanese workers[14-18] have described a procedure for the production of the ribonucleotides of 4-amino-, 4-hydroxy- and 6-amino-4-hydroxy-pyrazolo[3,4-d]pyrimidines, using a wide variety of microorganisms, particularly Brevibacterium ammoniagenes (ATCC 6872) through a salvage pathway, without the addition of PRPP. In some cases crystalline nucleotide was isolated directly from the cultured broth. However, reports on the chemical phosphorylation of pyrazolo[3,4-d]pyrimidine nucleosides are sparse.[19,20] The improvements in chemical phosphorylation procedures[21] enabled us to prepare the target nucleoside phosphates in good yield.

For the synthesis of 3b, 7 and 9b, it seemed propitious to start with 4-amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine (1), reported recently from our laboratory.[2] Phosphorylation of unprotected 1 with $POCl_3$ in trimethyl phosphate, according to the general procedure of Yoshikawa and coworkers[22] gave the corresponding 5′-monophosphate (2a) in 51% yield (Scheme I). Compound 2a crystallized out from aqueous acidic solution. Desulfurization of 2a with Raney nickel in aqueous alkaline media at reflux temperature afforded a convenient route to the preparation of 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidine 5′-monophosphate (2b), isolated as the free acid. Compound 2b was found to be identical with the one reported by Hecht and coworkers.[19] When 2a was allowed to react with N,N′-dicyclohexylcarbodiimide (DCC) in anhydrous pyridine in the presence of 4-morpholino-N,N′-dicyclohexylcarboxamidine under high-dilution conditions,[23] smooth

Scheme 1



cyclization occurred to provide 4-amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 3',5'-cyclic phosphate (3a), which was isolated in 79% yield. As in the case of 2a, the ultraviolet (UV) spectrum of 3a was very similar to that of the starting nucleoside 1. In the ^{1}H NMR spectrum of 3a, the characteristic[8] singlet for the anomeric proton was observed at δ 6.14. Further dethiation of 3a with Raney nickel furnished the cAMP analog 4-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 3',5'-cyclic phosphate (3b). The identity of 3b was established by UV spectral analysis, which was very similar to that of 4-APP riboside,[11,24] and the purity was assured by the elemental analysis.

For the synthesis of the cIMP and cGMP analogs (9b and 7, respectively), 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (5) was chosen as a suitable precursor. Compound 5 was obtained by two different routes. Deamination of 1 with aqueous nitrous acid gave a rather low yield of 5. Alternatively, the sodium hydroxide treatment of 4,6-bis(methylthio)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine[25] (4) gave a 56% yield of crystalline 5. As in the case of 2a, treatment of 5 at -5°C with $POCl_3$ in trimethyl phosphate furnished 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-monophosphate (6a) in 57% yield (Scheme I). Dethiation of 6a with Raney nickel readily provided the allopurinol ribotide, 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-monophosphate (6b). All the physicochemical properties of 6b agreed in all respects to that of allopurinol riboside 5'-phosphate as reported in the literature.[17] High dilution, DCC mediated intramolecular cyclization of 6a was then carried out in refluxing pyridine containing 4-morpholino-N-N'-dicyclohexylcarboxamidine to give a 35% yield of pure 6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-cyclic phosphate (9a). The anomeric singlet at δ 6.06 in the ^{1}H NMR spectrum indicated the formation of 3',5'-cyclic nucleotide. The preparation of the cIMP analog 1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-cyclic phosphate (9b) was performed by dethiation of 9a using Raney nickel. The identity of the resulting product (9b) was confirmed by UV and ^{1}H NMR spectroscopy, as well as by elemental analysis.

Compound 9a proved to be an excellent precursor for the synthesis of the cGMP analog 6-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrim-

idin-4(5H)-one 3',5'-cyclic phosphate (7). Treatment of 9a with m-chloroperoxybenzoic acid in an ethanolic solution gave a mixture containing one major product, assumed to be the 6-methylsulfone derivative (8). Without isolation of this intermediate, the mixture was treated with liquid NH_3 in a steel reaction vessel at 90°C. Compound 7 was isolated in 66% overall yield by ion-exchange chromatography. Absence of the S-methyl protons and appearance of a signal for the amino protons in the ^1H NMR spectrum of 7 at δ 6.80 confirmed that nucleophilic substitution had indeed taken place. The presence of the anomeric proton singlet at δ 5.92 also indicated that the phosphodiester ring was still intact. Moreover, the UV spectrum of 7 was very similar to that of the guanosine analog 6-amino-1-β-D-ribofuranosylpyrazolo[3,4-d]-pyrimidin-4(5H)-one.[2]

Thus, the chemical synthesis of cAMP, cIMP and cGMP analogs in the pyrazolo[3,4-d]pyrimidine ring system has been achieved by the conventional methods.

EXPERIMENTAL SECTION: Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H 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 analyses was verified by ^1H NMR. Infrared spectra (IR in KBr) 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 Robertson Laboratory Inc., Madison, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. All solvents used were reagent grade. Detection of nucleoside components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30°C.

4-Amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 5'-Monophosphate (2a). To a precooled (ice-salt bath, -5°C) solution of $POCl_3$ (5 mL, 54 mmol) in freshly distilled trimethyl phosphate (TMP, 90 mL) was added dry 4-amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-

d]pyrimidine[2] (1, 4.0 g, 12.8 mmol) and the reaction mixture was stirred with the exclusion of moisture for 5 h until phosphorylation was complete, as shown by TLC of a hydrolyzed aliquot on silica gel with CH_3CN:0.1N NH_4Cl (7:3) as developer. After the phosphorylation was complete, the reaction mixture was poured over crushed ice (200 mL) and the solution was adjusted to pH 2 with 2N NaOH. Following extraction with $CHCl_3$ (4 x 75 mL) to remove the TMP, the aqueous solution was applied to a column of activated charcoal[26] (120 g, acid washed Au-4). The column was washed with water until the eluate was salt free (negative $AgNO_3$ test). The nucleotide was eluted with a solution of EtOH: H_2O:NH_4OH (10:10:1, v/v) using a UV monitor. The homogeneous fractions were combined and concentrated down to a volume of approximately 100 mL and then treated with 1N HCl to a pH of 3. Upon cooling to 5°C for 18 h, a white solid separated out. The yield after filtration and drying was 2.6 g (51%); mp 206°C (discolors at 195°C); IR ν 3100-3300 (NH_2, OH)cm^{-1}; UV λ_{max} (pH 1) 242 nm (ε 26,700), 275 (18,500); UV λ_{max} (pH 7) 268 nm (ε 22,800); UV λ_{max} (pH 11) 242 nm (ε 28,100), 275 (19,500); ^1H NMR (Me_2SO-d_6): δ 2.48 (s, 3, SCH_3), 5.20 (br s, 2, $PO[OH]_2$), 6.08 (d, 1, J=4.3 Hz, C_1,H), 7.72 (br s, 2, NH_2), 8.15 (s, 1, C_3H). Anal. Calcd for $C_{11}H_{16}N_5O_7SP.1/4H_2O$: C, 33.21; H, 4.18; N, 17.60; S, 8.06; P, 7.79. Found: C, 33.32; H, 4.20; N, 17.38; S, 8.30; P, 7.65.

4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 5′-Monophosphate (2b). To a solution of 2a (1.5 g, 3.8 mmol) in water (50 mL) containing NH_4OH (0.5 mL) was added Raney nickel (W-4, 2.5 g, wet weight) and the mixture was refluxed for 1 h. The reaction mixture was filtered while hot through a Celite pad, washed with 10% NH_4OH solution (2 x 25 mL) and the combined filtrates concentrated to one-half the volume. After acidification to pH 3 with Dowex-50 (H^+) resin, the solution was lyophilized. For further purification, an aqueous solution of the phosphate was applied to a column of activated charcoal (50 g, acid washed Au-4) and washed with H_2O until the eluate was salt free. The nucleotide was eluted with EtOH:H_2O:NH_4OH (10:10:1, v/v). The homogeneous fractions containing the desired product were pooled, evaporated to a volume of 50 mL, acidified to pH 3 with Dowex-50 (H^+) resin and lyophilized. The product, 0.93 g (70%), was found to be identical in all respects with the one reported in the literature[19]; mp > 180°C (dec.); IR ν 3100-3250 (NH_2, OH)cm^{-1}; UV λ_{max} (pH 1) 217 nm (ε 23,700), 256 (9,500); UV λ_{max}

(pH 7 and 11) 260 sh, nm (ε 8,800), 273 (10,000); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 6.14 (d, 1, J = 5.0 Hz, C_1,$\underline{H}$), 7.84 (br s, 2, $N\underline{H}_2$), 8.22 and 8.24 (2s, 2, $C_3\underline{H}$ and $C_6\underline{H}$).

4-Amino-6-methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 3',5'-Cyclic Phosphate (3a). To a suspension of 2a (2.84 g, 2.7 mmol) in anhydrous pyridine (40 mL) was added 4-morpholino-N,N'-dicyclohexylcarboxamidine[27] (2.1 g, 7.2 mmol) and sonicated to a clear solution. The solution was evaporated several times with dry pyridine to an anhydrous syrup. The residual syrup was dissolved in pyridine (350 mL) and added dropwise (over 2 h period), through a reflux condenser, into a refluxing solution of N,N'-dicyclohexylcarbodiimide (DCC: 8.0 g, 39 mmol) in dry pyridine (1500 mL). The solution was refluxed for an additional 2 h and then stirred overnight at room temperature. Water (100 mL) was added dropwise with stirring and the solution was evaporated to dryness. To the residue was added water (80 mL) and ethyl ether (100 mL). The suspension was stirred vigorously and then filtered. The aqueous phase was again extracted with ether (3 x 100 mL), evaporated to one-half the volume and filtered. The solution was acidified to pH 3 with 1N HCl and the initial precipitate was collected by filtration. Upon cooling, a second crop was obtained from the filtrate. Total yield of the title compound, 2.13 g (79%); mp > 260°C (discolors at 215°C); IR ν 1220 (P=O), 3100-3300 (NH_2, OH)cm^{-1}; UV λ_{max} (pH 1) 216 nm (ε 18,700), 229 sh (13,900), 271 (12,200); UV λ_{max} (pH 7 and 11) 242 nm (ε 18,000), 275 (10,500); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 2.48 (s, 3, $SC\underline{H}_3$), 6.14 (s, 1, C_1,$\underline{H}$), 8.14 (s, 1, $C_3\underline{H}$). Anal. Calcd for $C_{11}H_{14}N_5O_6SP.2\ H_2O$: C, 31.43; H, 4.56; N, 16.66; S, 7.63; P, 7.37. Found: C, 31.33; H, 4.54; N, 16.80; S, 7.97; P, 7.62.

4-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidine 3',5'-Cyclic Phosphate (3b). To a solution of 3a (0.2 g, 0.57 mmol) in water (20 mL) containing NH_4OH (0.2 mL) was added Raney nickel (W-4, 0.8 g, wet weight) and the mixture was refluxed for 45 min. Another 0.8 g of Raney nickel was then added and after 90 min of total reaction time, the hot mixture was filtered through a Celite pad and washed with hot water (2 x 10 mL). The combined filtrates were acidified with Dowex-50 (H^+) resin and lyophilized to yield 50 mg (29%) of 3b; mp > 260°C (discolors at 235°C); IR ν 3300-3420 (NH_2, OH)cm^{-1}; UV λ_{max} (pH 1) 216 nm (ε 26,300), 256 (10,200); UV λ_{max} (pH 7 and 11) 260 sh, nm (ε 9,400), 273 (11,000);

^{1}H NMR (Me_2SO-d_6): δ 6.19 (s, 1, C_1,H), 8.10 (br s, 2, NH_2), 8.26 and 8.27 (2s, 2, C_3H and C_6H). Anal. Calcd for $C_{10}H_{12}N_5O_6P$: C, 36.48; H, 3.67; N, 21.27; P, 9.41. Found: C, 36.44; H, 3.73; N, 21.07; P, 9.19.

6-Methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one (5). To a solution of 4,6-bis(methylthio)-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine[25] (4, 9.7 g, 20.6 mmol) in dioxane (150 mL) was added 1N NaOH (100 mL) and the mixture was refluxed for 3 h. The solution was cooled (0-5°C) and acidified (pH 4) with Dowex-50 (H^+) resin. As the solvent was evaporated, a solid product separated, which was collected and crystallized from MeOH to yield 3.65 g (56%) of 5; mp 229-230°C; IR ν 1670 (C=O), 3200-3400 (OH)cm^{-1}; UV λ_{max} (pH 1 and 7) 220 sh, nm (ε, 18,800), 268 (18,200); UV λ_{max} (pH 11) 235 nm (ε 24,500), 273 (17,500); ^{1}H NMR (Me_2SO-d_6): δ 2.52 (s, 3, SCH_3), 6.03 (d, 1, J = 4.0 Hz, C_1,H), 8.05 (s, 1, C_3H), 12.45 (s, 1, N_5H). Anal. Calcd for $C_{11}H_{14}N_4O_5S$: C, 42.03; H, 4.49; N, 17.83; S, 10.20. Found: C, 42.02; H, 4.41; N, 17.84; S, 10.47.

6-Methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-Monophosphate (6a). A solution of 5 (0.57 g, 1.8 mmol) in TMP (12 mL) and $POCl_3$ (0.6 mL, 6.6 mmol) was treated as described for 2a to give 0.5 g of slightly impure 6a, which was further purified by ion-exchange chromatography (DEAE Sephadex column, eluted with a gradient of 0.25 M NH_4HCO_3 → 0.8 M NH_4HCO_3, 1 L each). Product eluted from the column at a concentration of about 0.6 M. The homogeneous fractions containing the desired product were pooled and evaporated to dryness. An aqueous solution (25 mL) of the residue was acidified with Dowex-50 (H^+) resin to pH 3. After filtration, the aqueous solution was lyophilized to yield 0.41 g (57%) of 6a; mp 159-162°C (softens at 135°C); IR ν 1690 (C=O), 3200-3450 (OH)cm^{-1}; UV λ_{max} (pH 1) 220 sh, nm (ε 13,800), 266 (13,800); UV λ_{max} (pH 7) 220 sh, nm (ε 13,800), 269 (14,200); UV λ_{max} (pH 11) 236 nm (ε 18,500), 274 (13,600); ^{1}H NMR (Me_2SO-d_6): δ 2.58 (s, 3, SCH_3), 6.02 (d, 1, J=4.0 Hz, C_1,H), 8.08 (s, 1, C_3H). Anal. Calcd for $C_{11}H_{15}N_4O_8SP.3/4H_2O$: C, 32.40; H, 4.08; N, 13.74; S, 7.86; P, 7.60. Found: C, 32.24; H, 4.00; N, 14.03; S, 8.16; P, 7.62.

1-β-D-Ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 5'-Monophosphate (6b). Dethiation of 6a (0.47 g, 1.2 mmol) with Raney nickel (W-4, 2.0 g, wet weight), as described for 2b, gave 0.22 g (52.7%) of 6b; mp > 157°C; mp of Na salt of 6bm > 240°C (dec.) [Lit.[17] mp 235-250°C (dec.)];

IR ν 1060 (P=O), 1690 (C=O), 3150-3300 (OH)cm^{-1}; UV λ_{max} (pH 1) 248 nm (ε 4,800); UV λ_{max} (pH 7) 248 nm (ε 7,300); UV λ_{max} (pH 11) 269 nm (ε 6,300); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 6.07 (d, 1, J = 2.5 Hz, C_1,$\underline{H}$), 8.13 and 8.14 (2s, 2, $C_3\underline{H}$ and $C_6\underline{H}$), 12.32 (s, 1, $N_5\underline{H}$). Anal. Calcd for $C_{10}H_{13}N_4O_8P.H_2O$: C, 32.80; H, 4.13; N, 15.30; P, 8.46. Found: C, 32.52; H, 4.36; N, 15.45; P, 8.19.

6-Methylthio-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-Cyclic Phosphate (9a). In a similar manner as for 3a, ring closure of 6a (0.3 g, 0.76 mmol) in dry pyridine (500 mL) in the presence of 4-morpholino-N-N'-dicyclohexylcarboxamidine (1.4 g, 6.8 mmol) gave impure 9a. The product was further purified by ion-exchange chromatography (DEAE Sephadex column, eluted with a gradient of H_2O → 0.65 M NH_4HCO_3, 1 L each). Product eluted from the column at the NH_4HCO_3 concentration of 0.4 M. The homogeneous fractions containing the desired product were pooled and evaporated to dryness. An aqueous solution (20 mL) of the residue was acidified with Dowex-50 (H^+) resin to pH 3. After filtration, the aqueous solution was lyophilized to yield 0.10 g (35%) of 9a as white solid; mp >210°C (dec.); IR ν 1685 (C=O), 3150-3300 (OH)cm^{-1}; UV λ_{max} (pH 1) 220 sh, nm (ε 13,200), 267 (14,300); UV λ_{max} (pH 7) 220 sh, nm (ε 13,200), 269 (14,300); UV λ_{max} (pH 11) 235 nm (ε 18,900), 273 (14,300); ^{1}H NMR (Me_2SO-$\underline{d}_6$): δ 2.58 (s, 3, $SC\underline{H}_3$), 6.06 (s, 1, C_1,$\underline{H}$), 8.10 (s, 1, $C_3\underline{H}$), 12.64 (br s, 1, $N_5\underline{H}$). Since the free acid was too hygroscopic, the compound was analyzed as ammonium salt. Anal. Calcd for the ammonium salt, $C_{11}H_{16}N_5O_7SP.3/4H_2O$: C, 33.90; H, 3.75; N, 14.37; S, 8.22; P, 7.95. Found: C, 33.70; H, 3.90; N, 14.14; S, 8.48; P, 8.25.

1-β-D-Ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-Cyclic Phosphate (9b). To a solution of 9a (0.42 g, 1.2 mmol) in water (100 mL) containing NH_4OH (0.5 mL) was added Raney nickel (W-4, 2.5 g, wet weight) and the mixture was heated under reflux for 30 min. The reaction mixture was filtered while hot through a glass fiber filter, washed with hot water (2 x 20 mL) and the combined filtrates evaporated to one-half the volume. After acidification to pH 3 with Dowex-50 (H^+) resin, the solution was filtered through a membrane filter unit (45 μ) and lyophilized to give 0.10 g (30%) of the title compound; mp 205-206°C (dec.); IR ν 1610 and 1690 (C=O), 3100-3300 (OH)cm^{-1}; UV λ_{max} (pH 1 and 7) 251 nm (ε 9,200); UV λ_{max} (pH 11) 254 sh, nm (ε 9,900), 269

(12,900); ^{1}H NMR (Me_2SO-d_6): δ 6.06 (s, 1, C_1,H), 8.14 and 8.16 (2s, 2, C_3H and C_6H). Anal. Calcd for $C_{10}H_{11}N_4O_7P.1\ H_2O$: C, 34.05; H, 3.86; N, 15.88; P, 8.78. Found: C, 34.09; H, 3.90; N, 16.11; P, 8.84.

6-Amino-1-β-D-ribofuranosylpyrazolo[3,4-d]pyrimidin-4(5H)-one 3',5'-Cyclic Phosphate (7). To a solution of 9a (0.58 g, 1.5 mmol) in EtOH (75 mL) was added m-chloroperoxybenzoic acid (0.8 g, 4.6 mmol) and the mixture was stirred at room temperature for 1 h. Water (10 mL) was added to bring the cloudy reaction mixture into solution. After stirring at room temperature overnight, the reaction mixture was evaporated to dryness. The residue was dissolved in water, extracted with ether (2 x 25 mL) and the aqueous phase was filtered. Lyophilization of the filtrate gave the methylsulfone (8) as the amorphous solid (0.5 g). Without further purification, compound 8 was heated at 90°C with liquid NH_3 (50 mL) in a stainless steel reaction vessel for 12 h. After cooling (-40°C), the reaction vessel was opened and excess NH_3 was allowed to evaporate. The residue was dissolved in water and acidified with Dowex-50 (H^+) resin. Upon ion-exchange chromatography (DEAE Sephadex column, eluted with a gradient of 0.25 → 0.35 M NH_4HCO_3) and further purification by HPLC (reverse-phase, C_{18}-column with water → 7% CH_3CN in H_2O gradient) gave 0.35 g (66%) of 7; mp > 320°C (discolors > 200°C); IR ν 1690 (C=O), 3200-3400 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1 and 7) 251 nm (ε 20,700); UV λ_{max} (pH 11) 262 nm (ε 17,900); ^{1}H NMR (Me_2SO-d_6): δ 5.92 (s, 1, C_1,H), 6.80 (s, 2, NH_2, exchanged with D_2O), 7.87 (s, 1, C_3H), 10.70 (s, 1, N_5H). Anal. Calcd for $C_{10}H_{12}N_5O_7P.2H_2O$: C, 31.51; H, 4.23; N, 18.37; P, 8.12. Found: C, 31.56; H, 4.08; N, 18.42; P, 8.13.

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