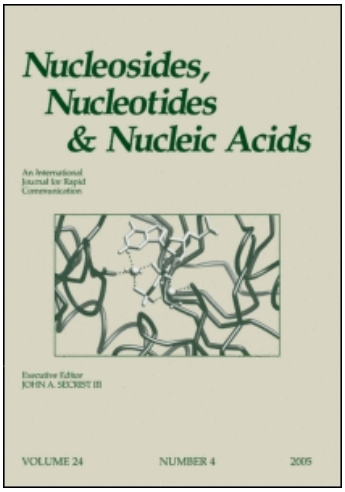

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In Vivo Antiviral Activity of 5-Amino-1-Methyl-3-β-D-Ribofuranosyl-Pyrazolo[4,3-*d*]Pyrimidin-7(6*H*)-One and Related Guanosine Analogues Prepared from Formycin

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IN VIVO ANTIVIRAL ACTIVITY OF 5-AMINO-1-METHYL-3-β-D-RIBOFURANOSYL-PYRAZOLO[4,3-*d*]PYRIMIDIN-7(6*H*)-ONE AND RELATED GUANOSINE ANALOGUES PREPARED FROM FORMYCIN

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Abstract: A short and simple synthesis of 5-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one, (**7**) was achieved from 7-amino-5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (**5**), in two steps, first deamination of **5** with NOCl, followed by amination of **6** with MeOH/NH_3. Also, an efficient synthesis of 5-amino-1(or 2)-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one was accomplished from the corresponding 1(or 2)-methyloxoformycin B in four steps by a sequence consisting of (i) 2′,3′,5′ acetylation with AC_2O, (*ii*) 5,7-chlorination with $PhP(O)Cl_2$, (*iii*) selective hydrolysis of the 7-chloro group with aqueous Na_2CO_3, (iv) followed by amination of the 5-chloro group with MeOH/NH_3. Single crystal X-ray analysis off **11** confirmed position 7 as the site of selective hydrolysis with Na_2CO_3. The three guanosine C-nucleosides prepared were evaluated for their ability to inhibit certain RNA and DNA viral replication in vitro and Semliki Forest virus infection in vivo. Only 5-amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**13**) provided protection (67% survivors, compared to 0% for placebo controls) against a lethal dose of Semliki Forest virus infection in mice. The antiviral effect of **13** is believed to be due to the enhancement of the host immune function.

Certain pyrazolo[4,3-*d*]pyrimidine C-nucleosides have been shown to exhibit a broad spectrum of biological effects[1] as antiviral and anticancer agents. The natural occurrence[2] and commercial availability[3] of formycin (**1**) has provided a continuing interest[4a-o] in certain functional group changes in the hope of achieving greater potency and selectivity towards therapeutic targets. We have synthesized and recently studied several guanosine analogues, 8-bromoguanosine, 8-mercaptoguanosine, 7-methyl-8-oxoguanosine, and 7-thia-8-oxoguanosine as modulators of immune responses.[5] Among the guanosine analogues tested, 7-thia-8-oxoguanosine appears to provide excellent protection against a broad spectrum of RNA and DNA viral infections in mice.[5b,c]

Townsend and Lewis first reported[6] the synthesis 5-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*] *pyrimidin*-7(6*H*)-one (**7**) prepared from formycin (**1**) in several steps and overall low yield. Acton and Ryan have reported[4i] a similar multistep synthesis of 7(6*H*)-thione derivative of **7** in poor yield.

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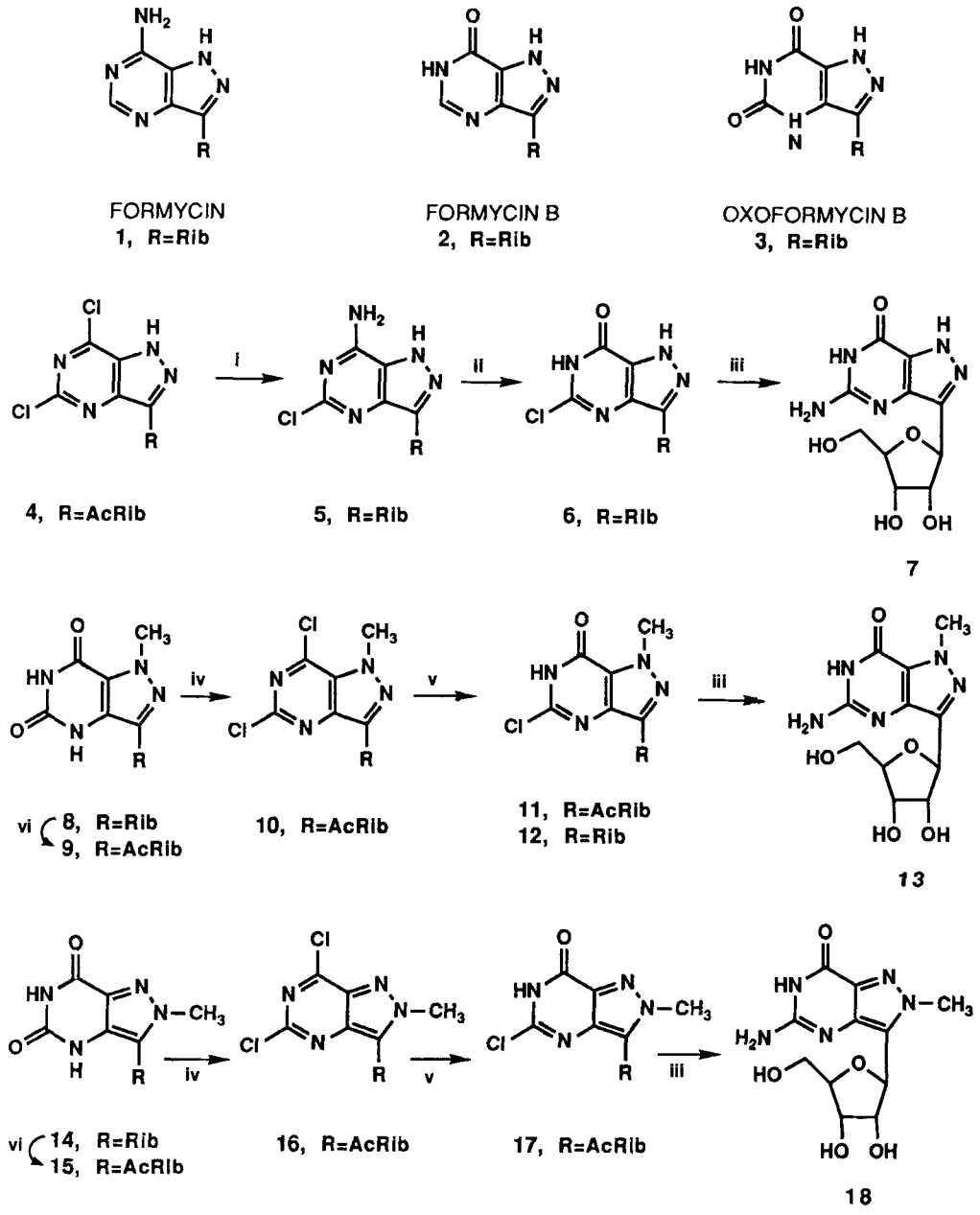
Earlier reports[7] from our group have suggested that N-methylation of pyrazole ring of formycin could enhance *syn* conformation and thereby reducing the deamination by adenosine deaminase. In a recent report[5], 7-methyl-8-oxoguanosine was shown to be superior to 8-bromo and 8-mercaptoguanosine in providing 75% survivors in a Semliki Forest viral infection in mice, which may be attributed to the presence of a 7-methyl group since 8-oxoguanosine is negative in this model under the same conditions. These results prompted us to synthesize for evaluation the novel guanosine analogs **13** and **18** in which the respective N-1 and N-2 positions of the pyrazole ring are methylated.

Benner and co-workers[8] recently incorporated 1-methyloxoformycin B into duplex DNA and RNA by DNA and RNA polymerases, thereby expanding the 'genetic alphabet' which enabled them to incorporate functionalized monomers into oligonucleotides with extended catalytic capacity. In this context the guanosine analogues **13** and **18** could have similar useful applications in the construction of more stable RNA molecules. Herein we report an alternate synthesis of the guanosine C-nucleoside **7** and report for the first time the synthesis of the N-1 and N-2 mono methylated guanosine analogues, **13** and **18**, respectively.

Chemistry. We have previously reported[9] the synthesis and conversion of 1- and 2-methyloxoformycins into the corresponding 1- and 2-methyloxoformycin B, **8** and **14**, respectively, by a nitrosyl chloride (NOCl) deamination procedure. In view of the facile deamination of formycin analogues by NOCl, we decided to explore the possibility of deaminating 7-amino-5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (**5**) to furnish 5-chloro-3-β-D-ribo furanosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**6**). Compound **6** could prove to be a useful precursor for the direct synthesis of the desired 5-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*] pyrimidin -7(6*H*)-one (**7**). Ammonolysis of 5,7-dichloro-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo [4,3-*d*]pyrimidine (**4**) with liquid NH_3 gave **5** in 89% yield.[10] The gaseous NOCl used for the present work was generated in situ by a modified procedure described by Doyle et al.[11] Therefore, excess of anhydrous NOCl was directly condensed into a stirred solution of compound **5**, in dry DMF at 0° C, which effected deamination to produce **6** in 71% isolated yield. Treatment of **6** with methanolic NH_3 (saturated at 0° C) in a pressure bomb at 135° C gave compound **7** along with minor impurities. The mixture was purified by preparative HPLC to furnish pure **7**. The structural identity of the product **7** was confirmed by UV, ^1H NMR and HPLC data obtained[12,13] on our product and an authenic sample prepared by Townsend and Lewis.[6]

Attempted methylation of compound **7** to obtain **13** and **18** were unsuccessful due to reaction products under a variety of reaction conditions. It was, therefore, decided to begin with 1-methy loxoformycin B to prepare 5-amino-1-methyl-3 -β-D-ribofur anosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one, (**13**).



Reagents: i, liq.NH_3 ; ii, NOCl/DMF ; iii, MeOH/NH_3 ; iv, NaH/THF, $C_6H_5P(O)Cl_2$; v, Aq.Na_2CO_3 /1,4-Dioxane ; vi, DMAP/Ac_2O .

Rib= β-D-ribofuranosyl ; **AcRib=** 2,3,5-tri-O-acetyl-β-D-ribofuranosyl.

1-Methyloxoformycin B (**8**) was protected as its 2′,3′,5′-tri-O-acetate **9**, obtained in 94% yield from **8**. Treatment of **9** with NaH in anhydrous THF gave the sodium salt of **9**, which in turn was treated with $PhP(O)Cl_2$ at 160° C to furnish the 5,7-dichloro-1-methyl-3- (2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,5-*d*]pyrimidine (**10**) in 78% isolated yield. After several attempts to standerize the selective conditions for mild hydrolysis, careful treatment of compound **10** with Na_2CO_3 in aqueous 1,4-dioxane at 120° C for 1 h furnished pure 5-chloro-1- methyl -3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**11**) in 60% isolated yield. The structure of **11** was unequivocally established by a single crystal X-ray study thereby confirming hydrolysis at position **7**. Amination of **11** with methanolic NH_3 at 135° C gave 5-amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**13**) as a crystalline compound in 80% yield.

This general procedure was also utilized for the synthesis of 5-amino-2-methyl-3-β-D-ribo furan osylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**18**) starting with 2-methyl-3-β-D- ribofurano sylpyrazolo[4,3-*d*]pyrimidin-5,7(4*H*,6*H*)-dione[9], **14** which was converted to the corresponding 2′,3′,5′-tri-O-acetate **15** which was chlorinated to give compound **16**, which on subsequent hydrolysis furnished 5-chloro-2-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo [4,3-*d*] pyrimidin-7 (6*H*) -one (**9**). Finally, **9** was converted into the corresponding guanosine analogue **18** via amination. The sequences of above reaction proceeded in satisfactory yield. The structure of compound **18** was based on UV and ^1H NMR data. Earlier it has been observed that UV spectra of N-1 and N-2 positional isomers of pyrazolo[4,3-*d*]pyrimidine derivatives[9] resemble rather closely and a similar pattern has emerged from the new compounds **13** and **18**. The UV spectra of these guanosine analogs **7**, **13** and **18**, exhibit a consistent bathochromic shift compared to the UV spectra of their precursors **6**, **11** and **9**, respectively, which could be a useful tool in following the amination reactions.

X-ray Diffraction Analysis. Crystallization of 5-chloro-1- methyl-3-(2,3,5-tri-O-acetyl -β-D- ribo furanosyl)pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (**11**) by slow evaporation of an ethanolic solution produced colorless, octagonal plates. A summary of crystal data is given in Table 1. An ORTEPII[14] plot of **11** is shown in Figure 1.

The anomeric configuration of **11** is β. The sugar conformation is ^3E (C3′ *endo*) with a pseudo-rotation angle (P) of 17.9° and amplitude of pucker (τ_m) of 33.5°.[15] The glycosidic torsion angle (χ_{CN} = 04′-Cl′-C3-C9) of 54.8(3)° corresponds to a *syn* conformation of the base with respect to the blocked ribofuranose moiety. The C5′-05′ side chain has the *tg* orientation. The only available proton for hydrogen bonding is H6; a nearly linear N6-H6...010′ hydrogen bond is seen in the crystal structure with an H...O distance of 1.97(3)Å. Details of these structural studies will be published elsewhere.[16]

TABLE I. Crystal Data for compound **11**

Formula	$C_{17}H_{19}N_4O_8Cl$	Crystal System	monoclinic
Formula wt.	442.81	space group	$P2_1$
a, (Å)	8.8313(9)	*Z*	2
b, (Å)	12.633(2)	wavelength (Å)	1.5498
c, (Å)	8.9941(10)	*R*	0.0286
β, (°)	90.776(7)	reflections ($F \geq 4\sigma_F$)	3838
V, (Å^3)	1003.4(2)	total unique reflections	4104

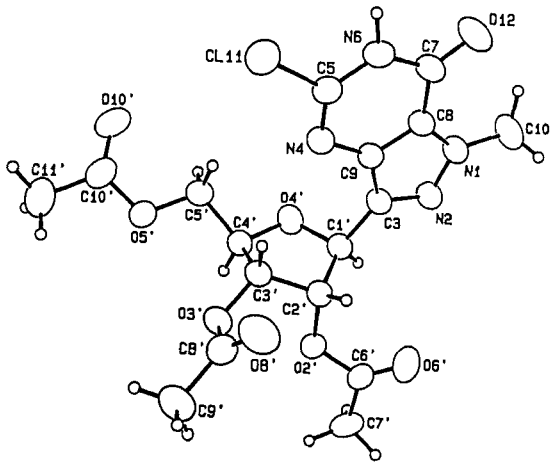


FIGURE 1. Perspective drawing of **11** illustrating atom labeling and molecular conformation.

Antiviral Studies. The guanosine-type C-nucleoside analogues prepared in this study (**7**, **13**, and **18**) were tested for activity against representative RNA and DNA viruses in cell cultures (for experimental details see ref 17). At a concentration up to 100μg mL^{-1}, none of these nucleosides inhibited the replication of Herpes simplex type 2 in Vero cells, Adeno 2 in HeLa cells, Rhino 1-A in HeLa cells, Parainfluenza type 3 in Vero cells, Semliki Forest virus in Vero cells or vaccinia in HeLa cells.

Recently, 7-thia-8-oxoguanosine, synthesized in our laboratory was found[5] to be devoid of significant in vitro antiviral activity, but in vivo prophylaxis provided excellent protection against

TABLE II. Effects of Guanosine Analogs against Semliki Forest Virus Infection in Mice

compd	dose[a] mg/kg	survivors/total (%)	mean survival time,[b] days
Placebo[c]		0/12 (0)	7.1 ± 1.6[d]
7-Thia-8-oxo-guanosine	100	11/12(92)[e]	8.0 ± 0.0
13	200	7/12(58)	7.0 ± 0.8
	100	8/12(67)[e]	8.0 ± 0.8
	50	8/12(67)[e]	6.0 ± 0.8
	30	6/12(50)[e]	9.2 ± 2.9
	10	1/12(8)	9.6 ± 2.4
7	50	0/12(0)	7.4 ± 1.8
18	50	1/12(8)	6.0 ± 0.8

[a] Half-daily doses were administered intraperitoneally at -24 and -18 hours relative to virus inoculation. [b] Of mice that died. Survivors lived through 21 days. [c] 2% Sodium bicarbonate used as a placebo control. [d] Standard deviation. [e] Statistically significant ($p < 0.025$), determined by the two-tailed Fisher exact test.

Semliki Forest virus. Considering these results, compounds **7**, **13** and **18** were tested in vivo for their ability to provide protection against a lethal Semliki Forest virus infection in mice. The results of this study are shown in Table II and indicate that at a dose of 100 mg per kg per day given intraperitoneally in half-daily doses for one day, compound **13** protected 67% of mice, while 7-thia-8-oxoguanosine at the same dosages provided 92% protection as a standard.

The nucleoside **13** is equally effective (67% protection) at a dose of 50 mg per Kg per day and exhibited 50% survival at a dosage as low as 30 mg/Kg/day. The guanosine analogues **7** and **18** were, on the other hand, totally ineffective at 50 mg/Kg dosage level. We also tested compounds **7**, **13** and **18** for the effect on B-cell proliferative response, in human peripheral blood mononuclear cells with *staphylococcus aureus* cowan. The results (see reference 18 for experimental procedure) indicate that compound **13** at 1μM concentration induced an increase in lymphocyte proliferation of 12.6 times that seen in the absence of the drug, whereas compounds **7** and **18** had no effect.

In summary, the procedures described herein provided a simple and efficient means of gaining access to a variety of guanosine-type C-nucleosides. The "biomodulatory" activity[19] which has emerged from these studies provides new leads in the design of better agents for potential use against viral diseases.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus or on a Haake-Buchler digital melting point apparatus and are uncorrected. Nuclear magnetic resonance (1H NMR) spectra (b = broad singlet) were determined at 300.1 MHz with an IBM NR300AF spectrometer. The chemical shifts are expressed in d values (parts per million) relative to tetramethylsilane as internal standard. Ultraviolet spectra (UV: sh = shoulder) were recorded on a Beckman DU-50 spectrophotometer. Elemental analyses were performed by Robertson Laboratory, Madison, NJ. Evaporations were carried out under reduced pressure with the bath temperature below 40o C. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 plates (EM reagents). E. Merck silica gel (230-400 mesh) was used for flash column chromatography. HPLC purity determinations were done with a Waters 600 solvent delivery system equipped with a Waters 990 photodiode array detector and a Beckman ultrasphere 5 m reversed-phase column (4.6 x 250 mm).

5-Chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (5-chloroformycin B) (6). Freshly prepared NOCl[11] (~2 mL) was directly condensed (dropwise over a period of ~1 h) into a cooled (0° C) and stirred solution of 7-amino-5-chloro-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine (**5**[10], 2.10 g, 6.97 mmol) in dry DMF (50 mL). The stirring was continued for 3 h at 0° C when TLC (EtOAc:1-PrOH:H_2O, 4:1:2, v/v, upper phase) indicated deamination was complete. The reaction mixture was allowed to warm up to room temperature before it was purged with N_2 to remove excess NOCl. The contents of the flask were poured onto ice (~200 g) and the solution was stirred for 30 min before concentrating under vacuum to dryness. The residue was purified by flash chromatography using the same solvent system as above (TLC) for elution to give **6** as a hygroscopic solid: 1.50 g (71%); mp 109° C (foams); UV (MeOH) λ_{max} nm (ε x 10^{-3}) 278 (14.3); ^{1}H NMR (Me_2SO-d_6) δ 3.51 (m, 2, C_5-CH_2), 3.82 (m, 1, C_4-H), 3.99 (m, 1, C_3-H), 4.33 (m, 1, C_2-H), 4.87 (d, 1, $J_{1',2'}$ = 6.9 Hz, C_1-H), 13.2 and 14.2 (2 br s, 2, 2 NH). Anal. Calcd for $C_{10}H_{11}N_4O_5Cl$:C, 39.67; H, 3.66; N, 18.51; Cl, 11.71. Found:C, 39.50; H, 3.56; N, 18.44; Cl, 11.52.

5-Amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (5-aminoformycin B) (7). A mixture of **6** (0.428 g, 1 mmol) and MeOH/NH_3 (60 mL, saturated at 0° C) was stirred in a steel bomb at 135° C for 48 h. The bomb was cooled (0° C), opened and NH_3 allowed to evaporate at room temperature. The methanolic solution was concentrated under vacuum and the residue was isolated as straw colored powder after EtOH wash (20 mL). The product **7** was further purified by HPLC [performed with a Waters Associates Model 6000A pump, Partisil 5μ ODS column (12 x 120 mm, YMC, Inc.) using 20-75% aqueous MeOH as solvent system at a flow rate of 3 mL/min.] to furnish 0.15 g (64%) of off-white colored solid; mp >260° C (decomposed, lit.[6] mp >250° C, decomposes); UV and ^{1}H NMR spectral data are identical with the literature[6] values. Anal. Calcd for $C_{10}H_{13}N_5O_5$3/4 H_2O:C, 40.47; H, 4.92; N, 23.60. Found:C, 40.49; H, 4.79; N, 23.96.

1-Methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-5,7(4,6*H*)-dione(9). A mixture of 1-methyloxoformycin B (**8**)[9], (4.20 g, 14.09 mmol), acetic anhydride (75 mL) and 4-N,N-dimethylaminopyridine (DMAP) (0.2 g, 1.6 mmol) was stirred at 100° C for 30 min under an argon atmosphere to obtain a clear solution. The stirring was continued at room temperature for 16 h. Evaporation of the reaction mixture gave a syrupy residue which was dissolved in EtOH (100 mL) and refluxed for 1 h. The solvent was then evaporated and the residue was chromatographed on

a silica gel column (4 x 25 cm) with EtOAc:Hexanes (9:1, v/v) as the eluent. The homogeneous fractions were pooled, evaporated to dryness and the resulting white foam was crystallized from EtOH to yield 5.62 g (94%) of **9**; mp 150° C; ^{1}H NMR ($CDCl_3$) δ 2.07 and 2.15 (2 s, 9, 3 $COCH_3$), 4.12 (s, 3, N-CH_3), 4.28-4.52 (m, 3, C_4-H and C_5-CH_2), 5.18 (d, 1, $J_{1',2'}$ = 5.7 Hz C_1-H), 5.27-5.36 (m, 2, C_2-H and C_3-H), 8.91 and 9.10 (2 br s, 2, 2NH). Anal. Calcd for $C_{17}H_{20}N_4O_9$:C, 48.11; H, 4.75; N, 13.20. Found:C, 48.11; H, 4.75; N, 13.16.

5,7-Dichloro-1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine(10). To a solution of **9** (11 g, 25.9 mmol) in anhydrous THF (400 mL) was added NaH (60% dispersion in oil, 3.12 g, 78 mmol) with stirring under anhydrous (argon) conditions, furnishing a clear solution in 2 h at room temperature. Phenyl phosphonic dichloride (45 mL, 75 mmol) was added to the solution and stirring was continued for 30 min at room temperature. The THF was removed under vacuum while heating in an oil bath (60° C) and the reaction mixture was stirred at 160° C for 2.5 h. The reaction mixture was cooled and poured into a mixture of ice (~500 g) and EtOAc (500 mL) cooled at 0° C, while stirring vigorously. The aqueous mixture was stirred at 0° C for 1 h and the suspension was filtered. The organic layer was separated from the filtrate and washed with cold saturated $NaHCO_3$ solution (2 x 250 mL) and cold water (2 x 100 mL). The organic phase was dried over anhydrous Na_2SO_4, solvent evaporated and the residue purified by silica gel column (6 x 60 cm) chromatography using EtOAc:Hexanes (1:1, v/v) as the eluent. Compound **10** was obtained as a foam, 9.36 g (78%); UV (MeOH) λ_{max} nm (ε x 10^{-3}) 260 (7.6), 321 (13.6); ^{1}H NMR ($CDCl_3$) δ 2.01, 2.08 and 2.11 (3 s, 9, 3$COCH_3$), 4.33 (s, 3, N-CH_3), 4.20-4.42 (m, 3, C_4-H and C_5-CH_2), 5.43 (d, 1, $J_{1',2'}$ = 6.1 Hz, C_1-H), 5.54 (t, 1, C_3-H), 5.79 (t, 1, C_2-H). Anal. Calcd for $C_{17}H_{18}N_4O_7$ Cl_2:C, 44.26; H, 3.93; N, 12.14; Cl, 15.37. Found:C, 44.40; H, 4.03; N, 12.08; Cl, 15.13.

5-Chloro-1-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidin-7 (6*H*)-one (11). To a solution of **10** (9.22 g, 20 mmol) in 1,4-dioxane (200 mL) was added an aqueous solution of Na_2CO_3 (4.87 g, 46 mmol in 65 mL of H_2O) and the resulting solution was heated (120° C bath temperature) to reflux for 1 h with stirring. The solution was cooled to room temperature (salts separated) and neutralized with Dowex H^+ resin. The aqueous mixture was filtered and resin washed with MeOH (3 x 50 mL). The combined filtrates were concentrated in vacuo and the residue coevaporated with EtOH (3 x 50 mL). The straw colored residue was purified on a silica gel column (5 x 45 cm) with EtOAc/Hexanes (7:3, v/v) as the solvent. The homogeneous fractions were pooled, evaporated to dryness and the foam was crystallized from EtOH to yield 5.3 g (60%) of **11**; mp 149° C; UV (MeOH) λ_{max} nm (ε x 10^{-3}) 285 (15.1); ^{1}H NMR ($CDCl_3$) δ 2.04 and 2.09 (2 s, 9, 3$COCH_3$), 4.21 (s, 3, N-CH_3), 4.23-4.43 (m, 3, C_4-H and C_5-CH_2), 5.32 (d, 1, $J_{1',2'}$ = 5.85 Hz, C_1-H), 5.54 (t, 1, C_3-H), 5.74 (t, 1, C_2-H), and 10.21 (br s, 1, NH). Anal. Calcd for $C_{17}H_{19}N_4O_8Cl$:C, 46.10; H, 4.32; N, 12.65; Cl, 8.00. Found:C, 46.27; H, 4.31; N, 12.39; Cl, 7.81.

5-Amino-1-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (13). A mixture of **11** (2.21 g, 5 mmol) and MeOH/NH_3 (60 mL, saturated at 0° C) was stirred in a steel bomb at 135°

C (bath) for 48 h. The bomb was cooled (0° C), opened and NH_3 was allowed to evaporate at room temperature. The methanolic solution was concentrated under vacuum and the residue[20] was crystallized from water using decolorizing carbon to yield 1.20 g (80%) of **13**; mp 245° C; UV λ_{max} nm (ε x 10^{-3}) (pH 1), 227 (30.0), 287 (8.7), (pH 7) 224 (31.5), 253 (sh, 9.5), 301 (9.0), (pH 11) 208 (sh, 36.4) 250 (sh, 9.6), 305 (10.3); ^{1}H NMR (Me_2SO-d_6) δ 3.56 (m, 2, $C_{5'}CH_2$), 3.81 (m,1, $C_{4'}H$), 3.96 (m, 1, $C_{3'}H$), 4.03 (s, 3, N-CH_3), 4.36 (m, 1, $C_{2'}H$), 4.72 (d, 1, $J_{1',2'}$ = 7.0 Hz, $C_{1'}H$), 4.81 (d, 1, $C_{3'}O4H$), 4.94 (d, 1, $C_{2'}OH$), 5.30 (br s, 1, $C_{5'}OH$), 5.98 (br s, 2, NH_2). ms:298 (M+1), 194 (B+30). Anal. Calcd for $C_{11}H_{15}N_5O_5$1/2 H_2O:C, 43.13; H, 5.26; N, 22.86. Found:C, 43.50; H, 5.40; N, 22.66.

2-Methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimi-dine-5,7(4H,6*H*)-dione (15). The title compound was prepared in a similar manner as described for **9** by using **14**[9] (1.70 g, 5.70 mmol), acetic anhydride (30 mL) and DMAP (0.1 g, 0.8 mmol). The product was isolated as a foam 1.81 g (74.8%); ^{1}H NMR ($CDCl_3$) δ 2.08, 2.10 and 2.18 (3 s, 9, 3$COCH_3$), 3.96 (s, 3, N-CH_3), 4.29-4.63 (m, 3, $C_{4'}H$ and $C_{5'}CH_2$), 5.9 (s, 1, $C_{1'}H$), 5.15-5.33 (m, 2, $C_{2'}H$ and $C_{3'}H$), 8.81 and 8.98 (2 br s, 2, 2 NH). Anal. Calcd for $C_{17}H_{20}N_4O_9$:C, 48.11; H, 4.75; N, 13.20. Found:C, 48.02; H, 4.86; N, 13.18.

5,7-Dichloro-2-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo-[4,3-*d*]pyrimidine(16). The title compound was prepared in a similar manner as described for **10** by using **15** (1.69 g, 4 mmol), NaH (60% dispersion in oil, 0.48 g, 12 mmol), anhydrous THF (100 mL) and $PhP(O)Cl_2$ 15 mL, 25 mmol). The product was isolated as hygroscopic foam 1.38 g (75%); ^{1}H NMR ($CDCl_3$) δ 2.06, 2.07 and 2.9 (3 s, 9, 3$COCH_3$), 4.36 (s, 3, N-CH_3), 4.29-4.41 (m, 3, $C_{4'}H$ and $C_{5'}CH2$), 5.42 (d, 1, $J_{1',2'}$ = 6.9 Hz, $C_{1'}H$), 5.59 (t, 1, $C_{3'}H$), 6.05 (t, 1, $C_{3'}H$). Anal. Calcd for $C_{17}H_{18}N_4O_7Cl_2$:C, 44.26; H, 3.93; N, 12.14. Found:C, 44.46; H, 4.02; N, 11.92.

5-Chloro-2-methyl-3-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]-pyrimidin-7(6*H*)-one (17). The title compound was prepared in a similar manner as described for **11** by using **16** (0.88 g, 2 mmol) in 1,4-dioxane (12 mL) and aqueous Na_2CO_3 (0.48 g, 4.6 mmol in 4 mL H_2O). The product was isolated as foam to yield 0.57 g (65%); UV (MeOH) λ_{max} nm (ε x 10^{-3}) 286 (14.9); ^{1}H NMR ($CDCl_3$) δ 2.06, 2.07 and 2.9 (3 s, 9, 3$COCH_3$), 4.19 (s, 3, N-CH_3), 4.12-4.38 (m, 3, $C_{4'}H$ and $C_{5'}CH2$), 5.30 (d, 1, $J_{1',2'}$ = 6.7 Hz, $C_{1'}H$), 5.56 (t, 1, $C_{3'}H$), 6.00 (t, 1, $C_{2'}H$), and 11.0 (br s, 1, NH). Anal. Calcd for $C_{17}H_{19}N_4O_8Cl$:C, 46.10; H, 4.32; N, 12.65; Cl, 8.00. Found:C, 46.33; H, 4.45; N, 12.77; Cl, 7.79.

5-Amino-2-methyl-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one (18). The title compound was prepared in a similar manner as described for **13** by using **9** (0.12 g, 0.27 g, 0.27 mmol) and MeOH/NH_3 (20 mL). The product [72 mg (90%)] was crystallized from 95% EtOH to give a crystalline product, mp 220° C (decomposes); UV λ_{max} nm (ε x 10^{-3}) (pH 1) 226 (27.0), 248 (sh, 7.6), 286 (9.3), (pH 7) 227 (28.1), 247 (sh, 8.0), 303 (9.4), (pH 11) 224 (29.5), 247 (sh, 8.1), 307 (9.6); ^{1}H NMR (Me_2SO-d_6) δ 3.55 (m, 2, $C_{5'}CH_2$), 3.85 (m, 1, $C_{4'}H$), 3.95 (s, 3, N-CH_3), 3.99 (m, 1, $C_{3'}H$), 4.49 (m, 1, $C_{2'}H$), 4.92 (d, 1, $J_{1',2'}$ = 7.3 Hz, $C_{1'}H$), 6.09 (br s, 2, NH_2), 10.80 (br s, 1 NH). Anal. Calcd for $C_{11}H_{15}N_5O_5$1/2 H_2O:C, 43.13; H, 5.26; N, 22.86 Found:C, 43.42; H, 5.11; N, 22.60.

Semliki Forest Virus Model. Swiss Webster female mice (Charles River Labs, Wilmington, MA), weighing about 20 g each at the beginning of the experiment, were inoculated intraperitoneally with test compounds (or placebo) in aqueous 2% sodium bicarbonate solution at -24 and -18 h relative to virus inoculation. The optimal dose of compound **13** (the most active compound of the present study) was established in preliminary experiments and the other two guanosines **7** and **18** were compared to **13** at these doses for relative potency. The dosing schedule indicated here was also found to be optimum for all guanosines tested. A lethal dose (10 x LD_{50}) of the Semliki Forest virus (original strain) was administered by ip injection to groups of 12 mice. In a few cases, however, some mice died within a day or two of drug administration and were eliminated from the final results, as indicated in Table II.

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(20) In a few experiments ~10% of compound **12** [UV λ_{max} nm (ε x 10^{-3}) (pH 1) 227 (25.5), 267 (11.0), 278 (11.2), (pH 7) 212 (37.2), 232 (sh, 15.0), 287 (14.5), (pH 11) 291 (14.3), 308 (sh, 7.1); ^{1}H NMR (Me_2SO-d_6) δ 3.60 (m, 2, C_5-CH2), 3.85 (m, 1, C_4-H), 4.0 (m, 1, C_3-H), 4.40 (m, 1, C_2-H), 4.87 (d, 1, $J_{1',2'}$ = 7.0 Hz, C_1-H), 13.2 (br s, 1, NH)] was obtained which can be recycled to furnish **13**. Anal. Calcd for $C_{11}H_{13}N_4O_5Cl$:C, 41.71; H, 4.13; N, 9.69. Found:C,, 42.03; H, 4.25; N, 9.40.

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