Pyrazolo[4,3-*d*]pyrimidine Nucleosides. Synthesis and Antiviral Activity of 1-β-D-Ribofuranosyl-3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones

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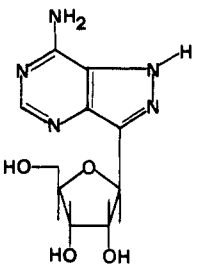
N_1 analogues of formycin B, with substituents at the 3 and 6 positions of the pyrazolo[4,3-*d*]pyrimidine moiety were synthesized by the direct $SnCl_4$-catalyzed ribosylation method. The site of the glycosidic linkage and the anomeric configurations were established on the basis of X-ray crystallography, as well as ^1H and ^{13}C nuclear magnetic resonance spectroscopy. Preliminary results of the antiviral testing of these derivatives in vitro are described.

The interesting biological properties[1] of formycin A (1)



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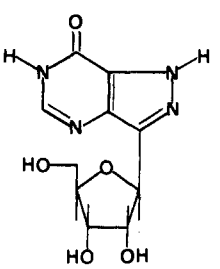

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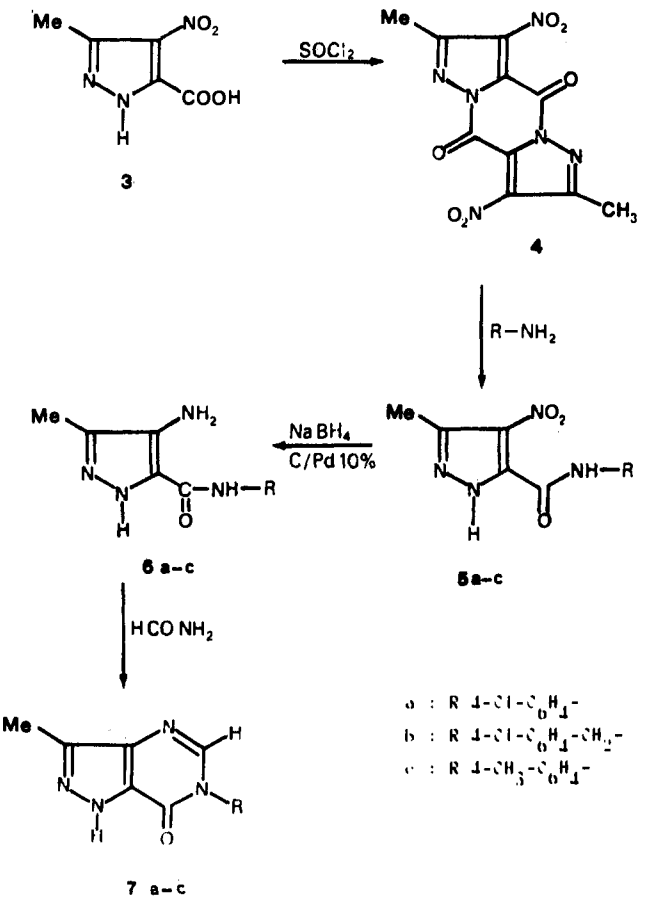
and B (2), two C-nucleosides containing the pyrazolo-[4,3-*d*]pyrimidine ring system, have stimulated a variety of studies both on their total synthesis[2] as well as on the preparation of more potent analogues.[3] A number of derivatives of 1 and 2 modified in the substituents at the 5 and 7 positions of the heterocycle have been reported,[4] as well as many derivatives with a modified ribofuranosyl moiety and attachment at the aglycon portion.[5]

Only a few reports[6] have dealt with the preparation of N-nucleoside analogues of 1 and 2. Recently, we have reported[7] a new method for the synthesis of a series of 3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones and the results of their biological evaluation.[8] In pursuit of our studies on this ring system, we have now prepared a series of 1-β-D-ribofuranosyl-3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one derivatives as potential antiviral agents.

Chemistry. The synthesis of the aglycon portion was approached with a procedure already described by us.[7,8] The key intermediate, diketopiperazine (4), was prepared starting from 3-methyl-4-nitropyrazole-5-carboxylic acid (3) according to the procedure of Musante.[9] The diketopiperazine showed an excellent reactivity toward nucleophylic reagents, such as aromatic, benzylic, and aliphatic amines, a behavior similar to that of imidazolide reagents, allowing us to obtain the nitro amides **5a–c** in high yield. Reduction of **5a–c** with sodium borohydride in the presence of 10% Pd/C[10] proceeded smoothly to give the corresponding amino amides **6a–c** in good yield. Pyrimidine cyclization of **6a–c** was finally achieved with formamide in an open vessel at 180 °C for 5 h to give the cyclic compounds **7a–c**, as depicted in Scheme I.

The only N-ribosylation studies[6] reported on pyrazolo-[4,3-*d*]pyrimidines, involving condensation between the heterocycle in its free form and fully acylated ribose in the presence of $(p\text{-}O_2N\text{-}C_6H_4O)_2P(O)OH$ at 165 °C, occurred at both ring nitrogens of the pyrazole, producing a mixture

Scheme I



of positional isomers with α and β configuration. In order to overcome this difficulty, we have chosen the stannic

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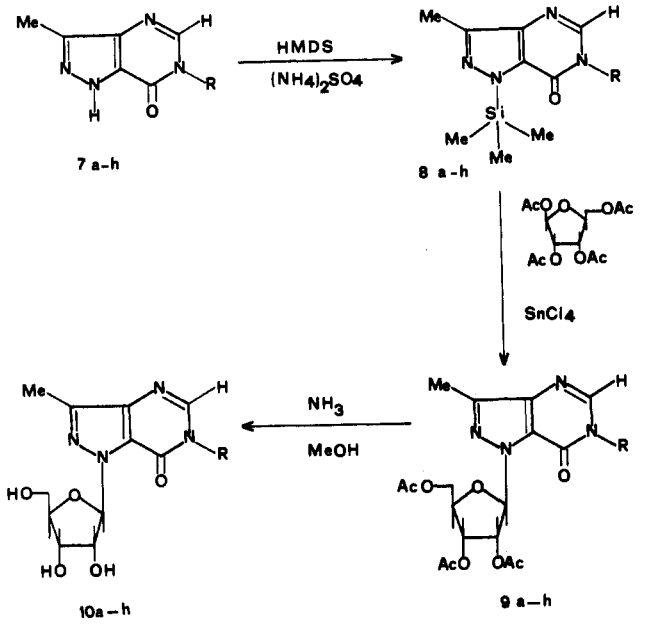
Scheme II



chloride catalyzed N-ribosylation method of silylated derivatives **8a–h**, taking advantage of the recently reported[11] regiospecific N_1-silylation of the pyrazolo[4,3-*d*]pyrimidine nucleus. The required silylated heterocycles **8a–h**, prepared by treatment with hexamethyldisilazane (HMDS) under reflux, were condensed without purification with peracetyl-β-D-ribofuranose in the presence of stannic chloride in acetonitrile. However, careful studies of the ribosylation process revealed that the procedure described by Vorbruggen,[12] adapted to low temperature, invariably gave the N^1-ribosylated products **9a–h** contaminated with a little starting material (5–10%), which was readly separated by ordinary silica gel column chromatography to afford the desired products in 65–80% yield. A number of factors were found to be critical to the success of the condensation, among them the following: (1) the purity of the silylated derivatives **8a–h**, which had to be free of solvents and Me_3Si reagents; (2) an order of addition of reagents in which an equimolecular amount of the peracetylated ribofuranose was added to the sylilated compound at −20 °C, followed by addition of 1.3 equiv of anhydrous stannic chloride and subsequent elevation of the temperature at 25 °C (Scheme II).

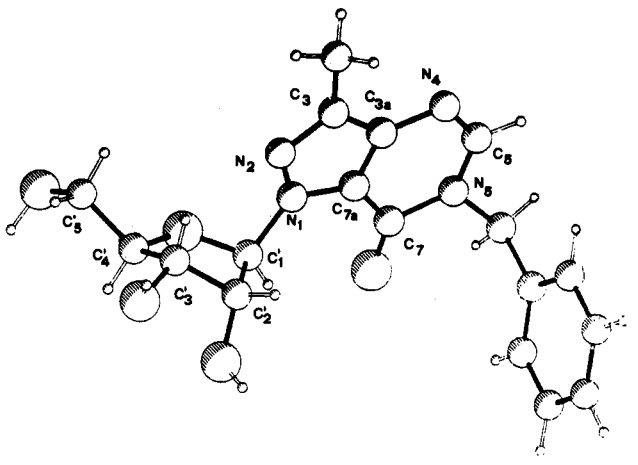


Figure 1. Perspective drawing **10d**.

De-O-acetylation of the peracetates **9a-h** performed with methanolic ammonia in the usual manner afforded the deblocked N_1-ribosides **10a–h** in good yield. The site of ribosylation and the β-configuration of **10a–h** could not be assigned unambiguously by ^1H and ^{13}C NMR spectroscopy, owing to the unavailability of both N_1 and N_2 isomers for comparison. Therefore, we submit the derivative **10d**, which furnished the more suitable crystals of this series of compounds, to an X-ray analysis in order to establish unequivocally its structure. Figure 1 is a perspective drawing of **10d** showing the conformation in the solid state. The main conformational features of the crystal structure of **10d** are as follows: (a) the two rings of the base are almost planar with χ^2 = 22.6 and 4.6 for C_3–C_{3a}–C_{7a}–N_1–N_2 (ring A) and C_{7a}–C_{3a}–N_4–C_5–N_6–C_7 (ring B), respectively, and their mean planes makes an interplanar angle of 1.6°; (b) the phenyl ring C is planar (χ^2 = 4.3) and is pratically perpendicular to the mean plane of the base (angle A–C = 93.4° and B–C = 94.7°); (c) the ribose ring adopts an envelope conformation ^3E ($C_{3'\text{,endo}}$) with phase angle of pseudorotation P = 12.2° and amplitude of Pucker τ_m = 41.5°;[13] (d) if the glycoside torsional angle χ_{CN} = O_1–C_1–N_1–N_2 is 66.4 (4)°, then the orientation of the base with respect to the ribose is anti;[14] (e) the conformation about the $C_{4'}$–$C_{5'}$ bond is gauche–trans, where $C_{1'}$–$C_{4'}$–$C_{5'}$–$O_{5'}$ = 69.4 (5)° and C_3–$C_{4'}$–$C_{5'}$–$O_{5'}$ = 173.9 (4)°. The ^1H NMR spectra of **10d** revealed that the anomeric proton ($H_{1'}$) appeared as a doublet centered at δ 6.52, with a coupling constant of 4.2 Hz. This downfield shift of the anomeric proton of **10d** can be attributed to the close proximity of the anisotropic C_7 carbonyl group; the same effect could not be observed in the N_2 isomer. This method has been used successfully to ascertain the ribosylation site in various types of nucleosides.[15] The ^{13}C NMR spectra of **10d** furnished additional support to the assignment of its structure. The interpretation of ^{13}C NMR spectra of isomeric N-ribosylated heterocycles was usually made by considering that a carbon adjacent (α) to a ribosylated nitrogen resonates upfield of the signal of that same carbon in other isomers.[11] Thus, for an N_1-ribosylated pyrazolo[4,3-*d*]pyrimidin-7-one, the ^{13}C NMR absorption of C_{7a} will be upfield and that of C_3 will be

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Table I. ^{13}C NMR Parameters of Nucleosides 10a–h[a]

compd	C-CH_3	C_3	C_{3a}	C_5	C_7	C_{7a}	$C_{1'}$	$C_{2'}$	$C_{3'}$	$C_{4'}$	$C_{5'}$
10a	10.46	143.15	137.93	145.35	152.47	125.52	91.09	73.51	70.78	85.03	62.29
10b	10.46	143.08	138.15	146.04	152.40	125.49	90.86	73.36	70.78	85.10	62.29
10c	10.46	143.27	138.61	145.66	152.48	125.52	91.02	73.51	70.78	85.03	62.37
10d	10.46	142.93	138.23	146.11	152.48	125.50	90.86	73.43	70.78	85.10	62.29
10e	10.46	142.85	138.15	146.03	152.48	125.42	90.86	73.43	70.78	84.95	62.29
10f	10.46	143.00	138.15	145.96	152.40	125.50	90.79	73.36	70.78	85.03	62.29
10g	10.46	143.01	138.16	146.11	152.47	125.50	90.86	73.44	70.78	85.03	62.29
10h	10.46	142.93	138.23	146.03	152.55	125.50	90.94	73.51	70.78	85.10	62.29

[a] All ^{13}C NMR spectra were recorded on a Bruker WP80 spectrometer with Me_2SO as solvent.

Table II. Comparative ED_{50} Values of Nucleosides 10a–h

compd	ED_{50}, μM	compd	ED_{50}, μM
10a	50	**10f**	350
10b	220	**10g**	174
10c	280	**10h**	49.5
10d	245	IDU	1.2
10e	152		

downfield of the corresponding signals in the spectrum of the N_2 isomer. Since we did not have both of the N-ribosylated isomers, the comparison of the ^{13}C NMR data of the free base and **10d** can still be used to ascertain the structure as suggested by Townsend to al. [16] The assignments of the resonances to the individual carbons of the aglycon portion of **10d** were made by comparison to the reported[17] ^{13}C NMR data for 3-methyl-pyrazolo[4,3-*d*]pyrimidin-7-one. Thus, this compound showed ^{13}C chemical shifts for C_{7a} and C_3 of 129.5 and 139.4 ppm, respectively, while for the same carbons, **10d** showed values of 125.5 (upfield shift) and 142.9 ppm (downfield shift) (Table I).

In summary, we can infer that all the derivatives (**10a–c**, **10e–h**) possess a N_1-ribosydic linkage and β configuration, since they show very similar spectroscopic properties to **10d**.

Antiviral Evaluation. The antiviral activity of N_1-pyrazolo[4,3-*d*]pyrimidine nucleosides **10a–h** was assayed in vitro by means of plaque-inhibition tests with monolayers of Vero cells infected with the MP strain of HSV-1. The ED_{50} values obtained with this assay are shown in Table II. Compounds **10a** and **10h** proved to have a similar degree of activity (ED_{50} = 50 μM) and were somewhat more active than the other compounds (ED_{50} ranging from 152 to 350 μM). Furthermore, the typical cytopathology of the MP strain, i.e., polycaryocytes, was partially inhibited by compounds **10a** and **10h**. All the drugs exhibited a low toxicity against unifected Vero cells at a 200 μM concentration.

Experimental Section

^1H NMR spectra were recorded on a Perkin-Elmer R32 instrument. ^{13}C NMR spectra were recorded on Bruker WP80 spectrometer; the chemical shift values are reported with respect to internal Me_4SI. IR spectra were obtained with Perkin-Elmer 297 spectrometer. Melting points were determined on a Tottoli-Buchi apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter (d 1 in DMF, unless otherwise stated) at a temperature in the range 22–23 °C.

Starting Materials. The 6-substituted 3-methyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones (**7d–h**) were prepared as described.[7,8]

N-Substituted 3-Methyl-4-nitropyrazole-5-carboxamides (5a–c). General Procedure. To an ice-cooled suspension of the diketopiperazine[9] **4** (10 mmol) in dioxane (30 mL), the appropriate amine (20 mmol) was added under efficient stirring, and the resulting solution was stirred overnight at room temperature. The solvent was removed in vacuo to give a solid, which was purified by crystallization from 1:1 ethanol/water.

Compound 5a: 88% yield; mp 238–239 °C; IR (KBr) ν_{max} 3380, 3240, 3130, 1670, 1600, 1580, 1550, 1510 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.6 (s, 3 H), 7.35 (d, 2 H, J = 9 Hz), 7.8 (d, 2 H, J = 9 Hz), 10.7 (br s, 1 H), 13.9 (br s, 1 H). Anal. ($C_{11}H_9ClN_4O_3$) C, H, Cl, N.

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Compound 5b: 84% yield; mp 220–221 °C; IR (KBr) ν_{max} 3280, 3120, 1660, 1630, 1560, 1510 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.5 (s, 3 H), 4.4 (d, 2 H, J = 4.5 Hz), 7.35 (s, 4 H), 9.05 (br s, 1 H); 13.5 (br s, 1 H). Anal. ($C_{12}H_{11}ClN_4O_3$) C, H, Cl, N.

Compound 5c: 93% yield; mp 239–240 °C; IR (KBr) ν_{max} 3380, 3240, 3140, 1670, 1600, 1550, 1500 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.3 (s, 3 H), 2.55 (s, 3 H), 7.15 (d, 2 H, J = 9 Hz), 7.6 (d, 2 H, J = 9 Hz), 9.5 (br s, 1 H), 13.4 (br s, 1 H). Anal. ($C_{12}H_{12}N_4O_3$) C, H, N.

N-Substituted 4-Amino-3-methylpyrazole-5-carboxamides (6a–c). General Procedure. A solution of the appropriate nitro amide (**5a–c**; 10 mmol) in methanol was added dropwise to a well-stirred, ice-cooled suspension prepared by adding an aqueous solution (15 mL) of sodium borohydride (20 mmol) to 10% palladium on carbon (50 mg) in water (10 mL). The mixture was stirred for 2–3 h (TLC control), the suspension was filtered, and the filtrate was precipitated with water. The crystalline compounds were collected by filtration and purified by crystallization from methanol.

Compound 6a: 72% yield; mp 250–251 °C; IR (KBr) ν_{max} 3400, 3270, 1645, 1600, 1540, 1530 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H), 4.5 (br s, 2 H), 7.4 (d, 2 H, J = 9 Hz), 7.8 (d, 2 H, J = 9 Hz), 9.9 (br s, 1 H), 12.6 (br s, 1 H). Anal. ($C_{11}H_{11}ClN_4O$) C, H, Cl, N.

Compound 6b: 67% yield; mp 190–191 °C; IR (KBr) ν_{max} 3400, 3200, 1650, 1550, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.1 (s, 3 H), 4.4 (m, 4 H), 7.3 (s, 4 H), 8.35 (br s, 1 H), 12.4 (br s, 1 H). Anal. ($C_{12}H_{13}ClN_4O$) C, H, Cl, N.

Compound 6c: 71% yield; mp 244–245 °C; IR (KBr) ν_{max} 3400, 3260, 1650, 1610, 1540 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.15 (s, 3 H), 2.3 (s, 3 H), 4.5 (br s, 2 H), 7.10 (d, 2 H, J = 9 Hz), 7.65 (d, 2 H, J = 9 Hz), 9.55 (br s, 1 H), 12.4 (br s, 1 H). Anal. ($C_{12}H_{14}N_4O$) C, H, N.

6-Substituted 3-Methyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones (7a–c). General Procedure. A suspension of the appropriate amino amide (**6a–c**; 5 mmol) in formamide (5 mL) was heated on an oil bath at 180 °C for 5 h in an open vessel. The residue was diluted with DMF (5 mL), treated with charcoal, and filtered over a small pad of Celite 503. Cyclic compounds were precipitated by the addition of water (~20 mL) to the filtrate and crystallized from 1:1 DMF/H_2O.

Compound 7a: 62% yield; mp >320 °C; IR (KBr) ν_{max} 3160, 3060, 1700, 1600, 1570, 1540, 1500 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.45 (s, 3 H), 7.6 (s, 4 H), 8.1 (s, 1 H), 13.1 (br s, 1 H). Anal. ($C_{12}H_9ClN_4O$) C, H, Cl, N.

Compound 7b: 73% yield; mp 292–293 °C; IR (KBr) ν_{max} 3150, 3070, 1690, 1580, 1530 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 5.25 (s, 2 H), 7.4 (s, 4 H), 8.3 (s, 1 H), 13.9 (br s, 1 H). Anal. ($C_{13}H_{11}ClN_4O$) C, H, Cl, N.

Compound 7c: 64% yield; mp >300 °C; IR (KBr) ν_{max} 3160, 3070, 1710, 1580, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 2.45 (s, 3 H), 7.38 (s, 4 H), 8.0 (s, 1 H), 13.6 (br s, 1 H). Anal. ($C_{13}H_{12}N_4O$) C, H. N.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones (9a–h). General Procedure. A mixture of the appropriate 3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (7a–h; 3 mmol), hexamethyldisilizane (15 mL), and ammonium sulfate (0.1 g) was refluxed for 8 h under anhydrous conditions. Excess hexamethyldisilazane was removed under reduced pressure to provide the trimethylsilyl derivative (**8a–h**) as a brownish oil. The oil was suspended in acetonitrile (50 mL), and to this magnetically stirred suspension was added 1,2,3,5-tetraacetylribose (0.95 g, 3 mmol).

To the suspension, cooled at −20 °C, was added a solution of anhydrous $SnCl_4$ (0.46 mL, 3.9 mmol) in acetonitrile (10 mL), and the mixture was then left at 25 °C for 18 h. Chromatography (TLC, silica gel, AcOEt–toluene (1:1)] of an aliquot (treated with MeOH) revealed almost complete conversion of the starting materials. The reaction mixture was evaporated, and the residue was redissolved in $CHCl_3$ (100 mL) and treated with a cold saturated $NaHCO_3$ solution (2 × 50 mL). The mixture was filtered through Celite, and the residue was washed with $CHCl_3$ (3 × 50 mL). The combined extract was washed with H_2O (50 mL), dried (Na_2SO_4), and filtered, and the filtrate was evaporated under reduced pressure to obtain a semisolid product. A solution of the crude product in ethyl acetate was evaporated with silica gel (10 g), and the residue was added on a dry silica gel (100 g) column. Elution with AcOEt–toluene solutions gave the pure product as colorless oil.

Compound 9a: 65% yield; oil; IR ($CHCl_3$) ν_{max} 1750, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H), 2.10 (s, 3 H), 2.12 (s, 3 H), 2.5 (s, 3 H), 4.0–4.6 (m, 3 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.85 (d, 1 H, J = 3.5 Hz), 7.35 (d, 2 H, J = 9 Hz), 7.55 (d, 2 H, J = 9 Hz), 7.9 (s, 1 H).

Compound 9b: 70% yield; oil; IR ($CHCl_3$) ν_{max} 1750, 1695, 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3 H), 2.1 (s, 3 H), 2.12 (s, 3 H), 2.48 (s, 3 H), 4.1–4.5 (m, 3 H), 5.15 (s, 2 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.9 (d, 1 H, J = 3.5 Hz), 7.3 (s, 4 H), 7.95 (s, 1 H).

Compound 9c: 66% yield; oil; IR ($CHCl_3$) ν_{max} 1755, 1705, 1585, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 9 H), 2.42 (s, 3 H), 2.52 (s, 3 H), 4.1–4.5 (m, 3 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.92 (d, 1 H, J = 3.5 Hz), 7.3 (m, 4 H), 7.9 (s, 1 H).

Compound 9d: 72% yield; mp 69–70 °C (MeOH); IR ($CHCl_3$) ν_{max} 1750, 1695, 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3 H), 2.1 (s, 6 H), 2.5 (s, 3 H), 4.1–4.6 (m, 3 H), 5.2 (s, 2 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.92 (d, 1 H, J = 3.6 Hz), 7.3 (s, 5 H), 7.95 (s, 1 H).

Compound 9e: 75% yield; oil; IR ($CHCl_3$) ν_{max} 1750, 1695, 1585 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3 H), 2.08 (s, 3 H), 2.10 (s, 6 H), 2.5 (s, 3 H), 3.9–4.5 (m, 3 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.95 (d, 1 H, J = 3.5 Hz), 7.85 (s, 1 H).

Compound 9f: 80% yield; oil; IR ($CHCl_3$) ν_{max} 1750, 1695, 1590, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 6 H, J = 7 Hz), 1.65 (m, 3 H), 2.05 (s, 3 H), 2.1 (s, 6 H), 2.5 (s, 3 H), 4–4.6 (m, 5 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.9 (d, 1 H, J = 3.7 Hz), 7.95 (s, 1 H).

Compound 9g: 73%; oil; IR ($CHCl_3$) ν_{max} 1750, 1695, 1580, 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3 H), 2.1 (s, 6 H), 2.5 (s, 3 H), 4.05–4.5 (m, 5 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.9 (d, 1 H, J = 3.7 Hz), 7.35 (m, 5 H), 7.95 (s, 1 H).

Compound 9h: 75% yield; oil; IR ($CHCl_3$) ν_{max} 1755, 1695, 1620, 1585 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.05, (s, 3 H), 2.1 (s, 6 H), 2.45 (s, 3 H), 3.75 (s, 3 H), 4.05–4.5 (m, 3 H), 5.1 (s, 2 H), 5.8 (m, 1 H), 5.95 (m, 1 H), 6.9 (d, 1 H, J = 3.5 Hz), 7.0 (d, 2 H, J = 9 Hz), 7.3 (d, 2 H, J = 9 Hz), 7.95 (s, 1 H).

1-β-D-Ribofuranosyl-3-methyl-6-substituted-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-ones (10a–h). General Procedure. The appropriate acetylated nucleoside (**9a–h**; 2 mmol) was dissolved in methanolic NH_3 (100 mL), and the solution was maintained at 0 °C for 18 h. The solvent was evaporated, and the residue was dissolevd in CH_2Cl_2. The solution was washed with water, and the dried (Na_2SO_4) layer was evaporated to dryness. The crude product was purified by crystallization from methanol/diethyl ether (1:1).

Compound 10a: 82% yield; mp 124–125 °C; $[\alpha]^{22}_D$ −74.55° (*c* 1.006, DMF); IR (KBr) ν_{max} 3350, 1700 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.45 (s, 3 H), 3.7 (m, 1 H), 4.2 (m, 1 H), 4.3–4.8 (m, 3 H), 6.65 (d, 1 H, J = 4.2 Hz), 7.3 (d, 2 H, J = 9 Hz), 7.5 (d, 2 H, J = 9 Hz), 7.9 (s, 1 H). Anal. ($C_{17}H_{17}ClN_4O_5 \cdot 0.5H_2O$) C, H, Cl, N.

Compound 10b: 78% yield; mp 102–104 °C; $[\alpha]^{22}_D$ −62.01° (*c* 1.024, DMF); IR (KBr) ν_{max} 3350, 1690, 1590 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 3.6 (m, 1 H), 4.0 (m, 1 H), 4.3 (m, 1 H), 4.7 (m, 1 H), 5.3 (m, 3 H), 6.52 (d, 1 H, J = 4.25 Hz), 7.4 (s, 4 H), 8.45 (s, 1 H). Anal. ($C_{18}H_{19}ClN_4O_5 \cdot H_2O$) C, H. Cl, N.

Compound 10c: 83% yield; mp 157–159 °C; $[\alpha]^{22}_D$ −78.7° (*c* 1.0, DMF) IR (KBr) ν_{max} 3350, 1690 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 2.45 (s, 3 H), 3.9 (m, 1 H), 4.25 (m, 1 H), 4.7 (m, 1 H), 5.15 (m, 1 H), 5.35 (m, 1 H), 6.5 (d, 1 H, J = 4.3 Hz), 7.4 (m, 4 H), 8.1 (s, 1 H). Anal. ($C_{18}H_{20}N_4O_5 \cdot 0.7H_2O$) C, H, N.

Compound 10d: 78% yield; mp 171–172 °C; $[\alpha]^{22}_D$ −73.16° (*c* 1.02, DMF) IR (KBr) ν_{max} 3350, 1690, 1590 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 3.9 (m, 1 H), 4.25 (m, 1 H), 4.65 (m, 2 H), 5.2 (m, 3 H), 6.52 (d, 1 H, J = 4.2 Hz), 7.35 (s, 5 H), 8.42 (s, 1 H). Anal. ($C_{18}H_{20}N_4O_5$) C, H, N.

Compound 10e: 67% yield; mp 144–145 °C; $[\alpha]^{22}_D$ −75.28° (*c* 1.2, DMF) IR (KBr) ν_{max} 3350, 1690, 1585 cm^{-1}; UV λ_{max} (MeOH) 213 nm (ϵ 9500), 263 (12900), 280 (5000); ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, 3 H), 2.45 (s, 3 H), 3.7–4.8 (m, 5 H), 6.6 (d, 1 H, J = 4.1 Hz), 7.9 (s, 1 H). Anal. ($C_{15}H_{22}N_4O_5 \cdot 0.5H_2O$) C. H. N.

Compound 10f: 81% yield; mp 83–85 °C; $[\alpha]^{22}_D$ −62.12° (*c* 1.11, DMF); IR (KBr) ν_{max} 3350, 1690, 1595, 1520 cm^{-1}; UV λ_{max}

(MeOH) 213 nm (ε 10300), 264 (5300), 284 (5500); ^{1}H NMR (Me_2SO-d_6) δ 0.92 (d, 6 H, J = 7 Hz), 2.4 (s, 3 H), 3.5–4.8 (m, 5 H), 5.2 (m, 1 H), 6.55 (d, 1 H, J = 4 Hz), 8.25 (s, 1 H). Anal. ($C_{16}H_{24}N_4O_5 \cdot H_2O$) C, H, N.

Compound 10g: 75% yield; mp 163–165 °C; $[\alpha]^{22}_D$ −73.42° (c 1.05, DMF); IR (KBr) ν_{max} 3350, 1690, 1595, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.4 (s, 3 H), 3.5 (m, 1 H), 3.95 (m, 1 H), 4.3 (m, 1 H), 4.7 (m, 2 H), 5.15 (m, 1 H), 6.55 (d, 1 H, J = 4 Hz), 7.4 (s, 5 H), 8.55 (s, 1 H). Anal. ($C_{19}H_{22}N_4O_5$) C, H, N.

Compound 10h: 80% yield; mp 151–152 °C; $[\alpha]^{22}_D$ −77.12° (c 1, DMF) IR (KBr) ν_{max} 3350, 1695, 1615, 1585, 1520 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.35 (s, 3 H), 3.65 (s, 3 H), 3.7–4.2 (m, 3 H), 4.35 (m, 1 H), 4.6 (m, 1 H), 5.0 (s, 2 H), 6.65 (d, 1 H, J = 4.1 Hz), 6.9 (d, 2 H, J = 9 Hz), 7.2 (d, 2 H, J = 9 Hz), 7.85 (s, 1 H). Anal. ($C_{19}H_{22}N_4O_6$) C, H, N.

Cell Cultures. Vero cells were routinely grown in monolayers in Eagle's basal medium (EBM) supplemented with 10% bovine serum (BS) and maintained in a maintenance medium consisting of EBM containing 2% BS.

Virus and Virus Titration. MP strain of herpes simplex virus type 1 (HSV-1), a DNA virus that causes fusion of infected cells,[16] was propagated in Vero cells at 34 °C and stored at −80 °C.

Plaque titrations were performed on Vero cell monolayers that were maintained after infection in EBM medium containing 1% BS and 0.2% human γ-globulin. Foci of infected wells were counted after fixation of the monolayers with methanol and staining with Giemsa. The infectious titer was expressed in plaque-forming units (pfu) per millimeter.[17] Phosphate-buffered saline (PBS) supplemented with 1% serum was used for dilutions and culture washing.

Antiviral Drugs: Determination of the ED_{50}. A stock solution of each drug was prepared by dissolving 10 mg of the compound in 1 mL of dimethyl sulfoxide, and appropriate dilutions were made in the maintenance medium. For 50% effective dose (ED_{50}) titrations, all titrations were done at least in duplicate. Confluent Vero cells in 25-cm^2 flasks were infected with approximately 200 pfu and incubated at 36 °C for 48 h in the maintenance medium containing 0.2% human immune serum globulin and graded concentration of the drugs. One set of control cultures were maintained without drug. Plaques were read after fixation and staining. The number of plaques emerging in the presence of each drug concentration was calculated as a percent of the control as follows: (average number of plaques with drug/average number of plaques without drug) × 100. Each drug titration was plotted on semilogarithmic graph paper to give dose–response lines from which ED_{50} values were calculated. The ED_{50} concentration was that amount of drug per milliliter of medium that inhibited the plaques numbers by 50% compared with the no-drug controls. 5-Iodo-2′-deoxyuridine (IDU), the compound most frequently used in clinical practice, was used as positive control of anti-herpes activity.

Crystal data: $C_{18}H_{20}N_4O_5$; space group $P2_12_12_1$; a = 5.442 (1), b = 13.387 (2), c = 23.759 (4) Å; Z = 4, d_{calcd} = 1.43 g cm^{-3}. Intensity data were collected on an Enraf-Nonius CAD-4 automatic four-circle diffractometer, Mo Kα radiation with $\omega/2\vartheta$ scan technique ($\vartheta \leq 26$). 1986 independent reflections were collected, out of which 1154 having $I > 2\sigma(I)$ were considered observed. The structure was solved by direct methods by means of MULTAN-82. The structure was refined by full-matrix least squares, assuming anisotropic temperature factors for all atoms, except H's, which were refined isotopically. Final values of the discrepancy indices were R = 0.039 and R_w = 0.041.

Registry No. **4**, 80030-73-5; **5a**, 89889-56-5; **5b**, 89889-57-6; **5c**, 89889-58-7; **6a**, 89889-59-8; **6b**, 89889-60-1; **6c**, 89889-61-2; **7a**, 89889-62-3; **7b**, 89889-63-4; **7c**, 89889-64-5; **7d**, 80030-90-6; **7e**, 80043-70-5; **7f**, 80030-87-1; **7g**, 80030-88-2; **7h**, 86927-78-8; **8a**, 89889-65-6; **8b**, 89889-66-7; **8c**, 89889-67-8; **8d**, 89908-13-4; **8e**, 89889-68-9; **8f**, 89889-69-0; **8g**, 89889-70-3; **8h**, 89889-71-4; **9a**, 89889-72-5; **9b**, 89889-73-6; **9c**, 89889-74-7; **9d**, 89889-75-8; **9e**, 89889-76-9; **9f**, 89889-77-0; **9g**, 89889-78-1; **9h**, 89889-79-2; **10a**, 89889-80-5; **10b**, 89889-81-6; **10c**, 89889-82-7; **10d**, 89889-83-8; **10e**, 89889-84-9; **10f**, 89889-85-0; **10g**, 89889-86-1; **10h**, 89889-87-2; *p*-chloroaniline, 106-47-8; *p*-chlorobenzylamine, 104-86-9; *p*-toluidine, 106-49-0; β-D-ribofuranosetetraacetyl, 13035-61-5.

β-Lapachone: Synthesis of Derivatives and Activities in Tumor Models

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In order to find a 3,4-dihydro-2*H*-naphtho[1,2-*b*]pyran-5,6-dione more potent than the naturally occurring 2,2-dimethyl derivative [β-lapachone (**10a**)], we synthesized a series of analogous compounds with modifications at position 2 of the pyran ring or at positions 8 and 9 of the benzene ring. Of the compounds tested in vitro for inhibition of RNA-dependent DNA polymerase and in mice infected with Rauscher leukemia, all retained good enzyme activity. Inhibition of the reverse transcriptase activity of the 2,2-substituted derivatives **10b–e** was as strong as **10a**. However, only the 2-methyl-2-phenyl derivative **10e** proved to be about as potent as the 2,2-dimethyl reference compound **10a** in prolonging the mean survival time of mice with Rauscher leukemia virus induced leukemia.

From a number of substances inhibiting the activity of retrovirus reverse transcriptase only β-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-naphtho[1,2-*b*]pyran-5,6-dione) was found to prolong the survival time of chickens infected intraperitoneally with Rous sarcoma virus.[1] Molecular biological studies have revealed that the drug inhibits reverse transcriptase, as well as eukaryotic DNA polymerase α.[2,3] In other studies, β-lapachone, which can be isolated from various tropical trees[4] or synthesized chemically from lapachol following the procedures of Hooker,[5] was shown to inhibit the growth of certain bacteria in vitro[4,6] and the cellular production of Friend virus.[7] The activity of β-lapachone in vitro and possibly in in vivo systems[1] prompted us to synthesize a number of derivatives in order to compare their activity to inhibit reverse transcriptase in vitro and to prolong the survival of mice

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