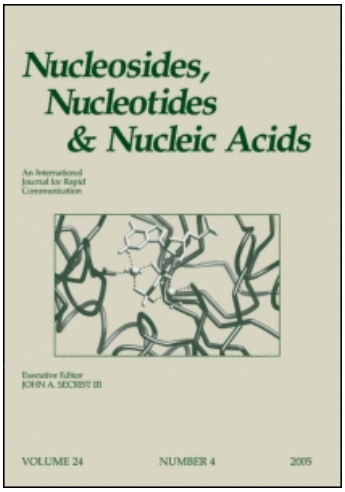

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Synthesis of Certain 4-Substituted-1-β-D-Ribofuranosyl-3-Hydroxypyrazoles Structurally Related to the Antibiotic Pyrazofurin

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SYNTHESIS OF CERTAIN 4-SUBSTITUTED-1-β-D-RIBOFURANOSYL-3-HYDROXYPYRAZOLES STRUCTURALLY RELATED TO THE ANTIBIOTIC PYRAZOFURIN

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ABSTRACT: Several 4-substituted-1-β-D-ribofuranosyl-3-hydroxypyrazoles were prepared as structural analogs of pyrazofurin. Glycosylation of the TMS derivative of ethyl 3(5)-hydroxypyrazole-4-carboxylate (3) with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose in the presence of TMS-triflate gave predominantly ethyl 3-hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (4a), which on subsequent ammonolysis furnished 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (5). Benzylation of 4a with benzyl bromide and further ammonolysis gave 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (8a). Catalytic (Pd/C) hydrogenation of 8a afforded yet another high yield route to 5. Saponification of the ester function of ethyl 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (7b) gave the corresponding 4-carboxylic acid (6a). Phosphorylation of 8a and subsequent debenzylation of the intermediate 11a gave 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide 5′-phosphate (11b). Dehydration of 3-benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carboxamide (8b) with $POCl_3$ provided the corresponding 4-carbonitrile derivative (10a), which on debenzylation with Cl_3SiI gave 3-hydroxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (13). Reaction of 13 with H_2S/pyridine and subsequent deacetylation gave 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (12b). Similarly, treatment of 13 with NH_2OH afforded 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamidoxime (14a), which on catalytic (Pd/C) hydrogenation gave the corresponding 4-carboxamidine derivative (14b). The structural assignment of these pyrazole ribonucleosides was made by single-crystal X-ray analysis of 6a. None of these compounds exhibited any significant antitumor or antiviral activity in cell culture.

INTRODUCTION: Pyrazofurin (4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide, 1) is a naturally occurring azolecarboxamide C-nucleoside

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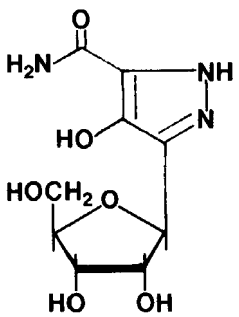
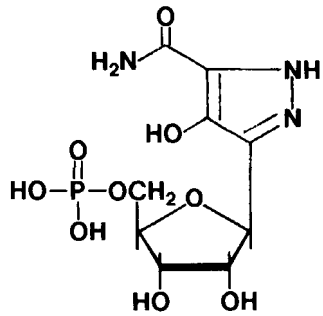
antibiotic[1] possessing significant antiviral activity in cell culture against a broad spectrum of DNA and RNA viruses.[2-4] Pyrazofurin is particularly active against a host of arena-, bunya-, myxo-, picorna-, pox-, retro-, rhabdo-, and togaviruses in vitro[4-6] at concentrations as low as 0.01 μg/mL.[5] Although pyrazofurin has a high degree of selectivity in its antiviral effects and shows a rather broad safety margin in cell culture, the LD_{50} dose in mice is about 5 mg/kg per day,[3,5] and has a low chemotherapeutic index against MLV and vaccinia virus.[3] De Clercq and Torrence[4] suggest that this unexpected toxicity is probably not associated with the structural features of the molecule responsible for the antiviral potency. However, the toxicity of pyrazofurin is such that it cannot readily be separated from its antiviral efficacy in animals.[7,8] It has been suggested that the toxicity of pyrazofurin in animals may be due to its inhibition of orotidylate decarboxylase.[9,10]



1



2

pyrazofurin

Pyrazofurin inhibited the growth of a number of different experimental tumor cell lines both in vitro and in vivo,[11,12] and has been studied clinically as an anticancer agent in man.[2,10,13,14] Although pyrazofurin is well tolerated by most patients at doses of 100 mg/m^2 following iv administration, infusion of 1 to leukemic patients resulted in severe toxicity.[13]

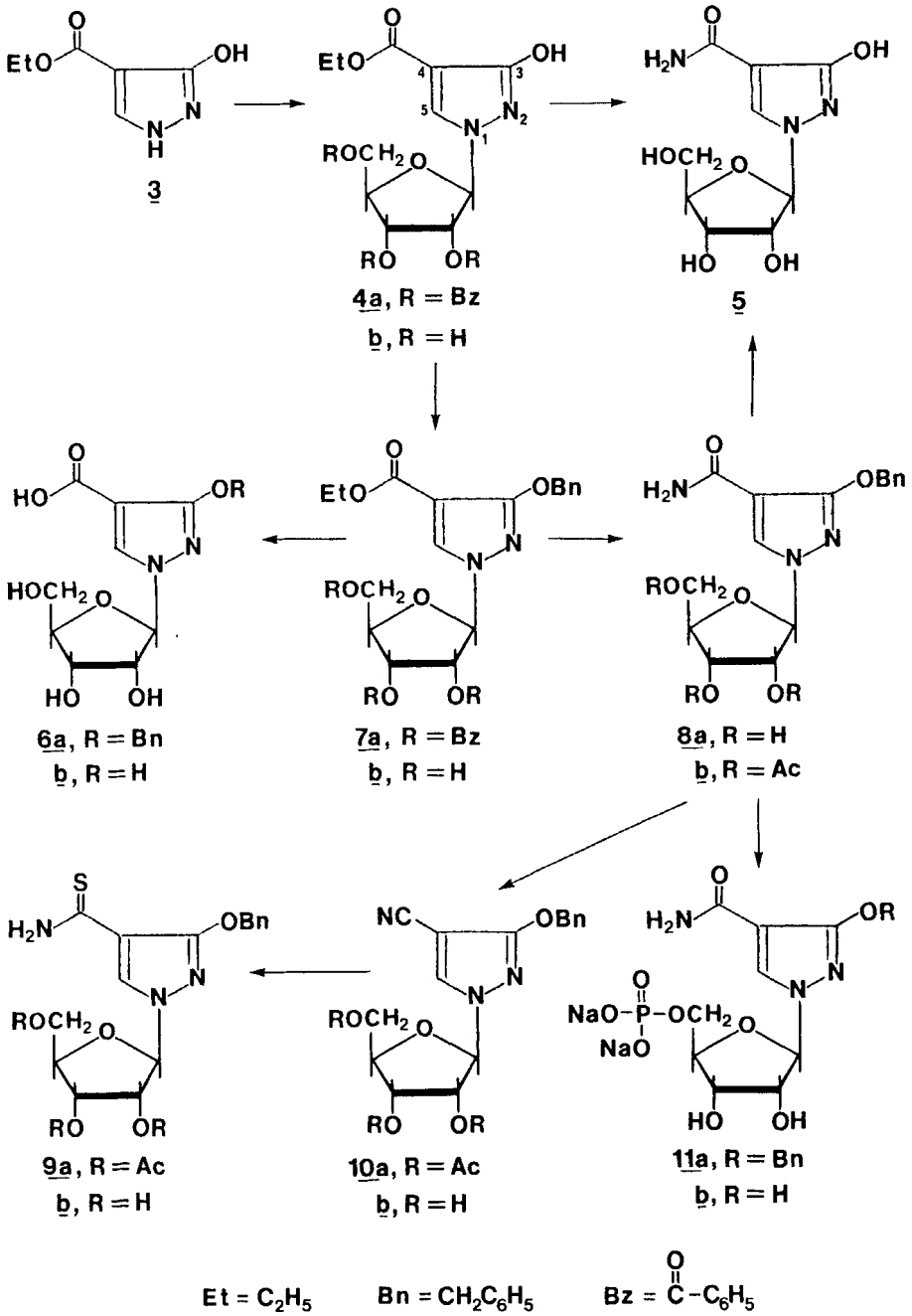
Pyrazofurin is converted to the 5′-triphosphate in human blood cells[15] and to the 5′-monophosphate (2) by the cellular adenosine kinase.[16] Pyrazofurin 5′-monophosphate (2) exhibited a broad spectrum antiviral activity in cell culture at concentrations very similar to 1 but is less toxic to the cells than pyrazofurin.[17] Compound 2 also exhibited significant inhibitory effects on the growth of L1210 and P388 leukemias and Lewis lung carcinoma cells in vitro.[17] In an effort to

further decrease the toxic properties of pyrazofurin and hopefully retain the antiviral and antitumor potency, we have now synthesized certain N-nucleoside congeners of both 1 and 2.

CHEMISTRY: For the synthesis of such N-nucleoside analogs structurally related to 1 and 2, ethyl 3(5)-hydroxypyrazole-4-carboxylate[18] (3) was found to be a viable starting material. Glycosylation of the bis-trimethylsilyl derivative of 3 with 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose in anhydrous CH_3CN in the presence of 1.4 molar equivalent of trimethylsilyl trifluoromethanesulfonate at ambient temperature gave predominantly ethyl 3-hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-pyrazole-4-carboxylate (4a) (Scheme I). Careful investigation furnished chromatographic evidence of the formation of another nucleoside material in very small amount (<2%); presumably the positional N-2 isomer. No attempt was made to isolate this minor product. Debenzoylation of 4a with NaOMe/MeOH at room temperature furnished ethyl 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (4b) in good yield. Ammonolysis of 4b with $MeOH/NH_3$ (saturated at 0°C) at elevated temperature and pressure gave the pyrazofurin analog 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (5). A low yield synthesis of 4b and 5 has been reported recently by Preobrazhenskaya and coworkers[19] from ethyl 3-hydroxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carboxylate, which in turn was obtained from the iodine catalyzed fusion of 3 and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose.

In analogy to the work on pyrazofurin reported recently from our laboratory,[17] it was visualized that the synthesis of the desired nucleoside and nucleotide derivatives of 4-substituted-3-hydroxypyrazoles might be realized by protecting the acidic hydroxyl group of the aglycon moiety. Thus, benzylation of the sodium salt of 4a, produced in situ by NaH in CH_3CN, with benzyl bromide gave ethyl 3-benzyloxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (7a) in excellent yield, which was found to be a useful intermediate for subsequent reactions. Debenzoylation of 7a by the treatment of NaOMe/MeOH at room temperature afforded ethyl 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (7b) in 71% yield, whereas ammonolysis of 7a with $MeOH/NH_3$ furnished 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (8a) in over 80% yield. Catalytic (Pd/C) hydrogenation of 8a provided a convenient route to the

Scheme I

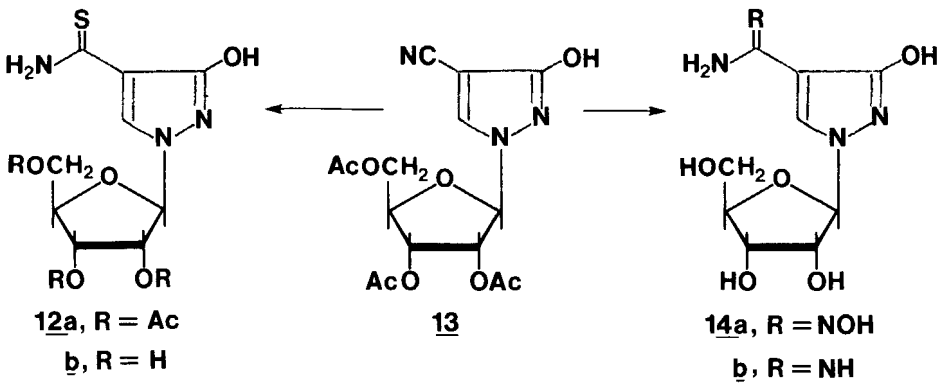


pyrazofurin analog 5, in which the yield of the desired product was 85%. Saponification of the ester function of 7b by the treatment of 6N NaOH at room temperature gave 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (6a), which on catalytic hydrogenation readily provided 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (6b). The absolute structural assignment of 6a was made on the basis of single-crystal X-ray crystallographic studies, which provided the structural proof of the pyrazole nucleosides prepared during this study.

Compound 8a also served as a versatile starting material for phosphorylation studies (Scheme I). Thus, phosphorylation of 8a with $POCl_3$ in trimethylphosphate at 0-5°C, according to the general procedure of Yoshikawa et al.,[20] provided a 74% yield of 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide 5'-phosphate (11a), isolated as the disodium salt. The purity and structure of 11a was confirmed by elemental and ^1H NMR analysis. Catalytic debenzylation of 11a in the presence of Pd/C in an atmosphere of hydrogen furnished an almost quantitative yield of 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide 5'-phosphate (11b), the N-nucleoside analog of 2.

In an effort to develop a synthetic procedure that would lead to 3-hydroxypyrazole ribonucleosides modified at the 4-position, the synthesis of 3-benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (10a) was considered. The synthesis of such derivatives are of particular interest since 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine reported from our laboratory[21] has been shown to be a competitive, reversible inhibitor of inosine phosphorolysis by human lymphoblast purine nucleoside phosphorylase.[22] Acetylation of 8a with acetic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) at ambient temperature gave 3-benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carboxamide (8b), which on dehydration with $POCl_3$ in the presence of N,N-diethylaniline at room temperature provided 10a. The yields of 8b and 10a are consistently good. Deacetylation of 10a with liquid NH_3 gave crystalline 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carbonitrile (10b). Compound 10b revealed a strong C≡N stretching at 2210 cm^{-1} in the IR spectrum. Further treatment of 10a with H_2S in a pyridine solution at room temperature, and subsequent deacetylation of the reaction product (9a) with $MeOH/NH_3$ furnished 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (9b). Attempted reductive cleavage of the benzyl ether

Scheme II



of either 9a or 9b with Pd/C, Na/NH_3[23] or sodium naphthalene[24] in THF resulted in an intractable reaction mixture from which the desired debenzylated product of 9b could not be isolated. Thus, in this instance initial cleavage of the benzyl ether group of 10a was found to be desirable. Although a number of ether-cleaving reagents are reported in the literature,[25-28] the use of monoiodotrichlorosilane (Cl_3SiI)[29] appears to be the reagent of choice for our specific needs. Monoiodotrichlorosilane, generated *in situ* by the reaction of $SiCl_4$ with NaI in CH_3CN/CH_2Cl_2, cleaves a wide variety of ethers of different structural types in good yields and under relatively (compared to iodotrimethylsilane) mild conditions.[25] Treatment of 10a with Cl_3SiI in CH_3CN at reflux temperature in an inert atmosphere furnished 3-hydroxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (13) in more than 87% yield (Scheme II). Reaction of 13 with H_2S in pyridine at 90°C for 14 h, and subsequent deacetylation of the reaction product 12a with NaOMe/MeOH gave the desired 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (12b) in good yield. Similarly, when free hydroxylamine was reacted with 13 in absolute EtOH, 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamidoxime (14a) was formed, which on hydrogenation in the presence of Pd/C furnished the corresponding carboxamidine (14b).

Single-Crystal X-ray Diffraction Analysis of Compound 6a. A suitable crystal of the compound was mounted on a Nicolet P3 autodiffracto-

TABLE I
Crystal and Structure Data of Compd 6a

Formula	$C_{16}H_{18}N_2O_7$
Molecular Weight	350.32
F(000)	368
μ, cm^{-1}	9.18
Crystal size(mm)	0.3 x 0.3 x 0.1
Space group	P1
a, Å	4.857(2)
b, Å	10.071(9)
c, Å	16.889(6)
α, deg	97.48(4)
β, deg	95.56(3)
γ, deg	90.19(4)
V, $Å^3$	815(1)
Z	2
d, gcm^{-1}	1.43
radiation	Cu (1.54178 Å)
sin θ/λ max	0.54
unique observed data	2395
unobserved data	96
R	0.049
R_w	0.067
largest peaks in Δ F map, $eÅ^{-3}$	0.37, -0.32

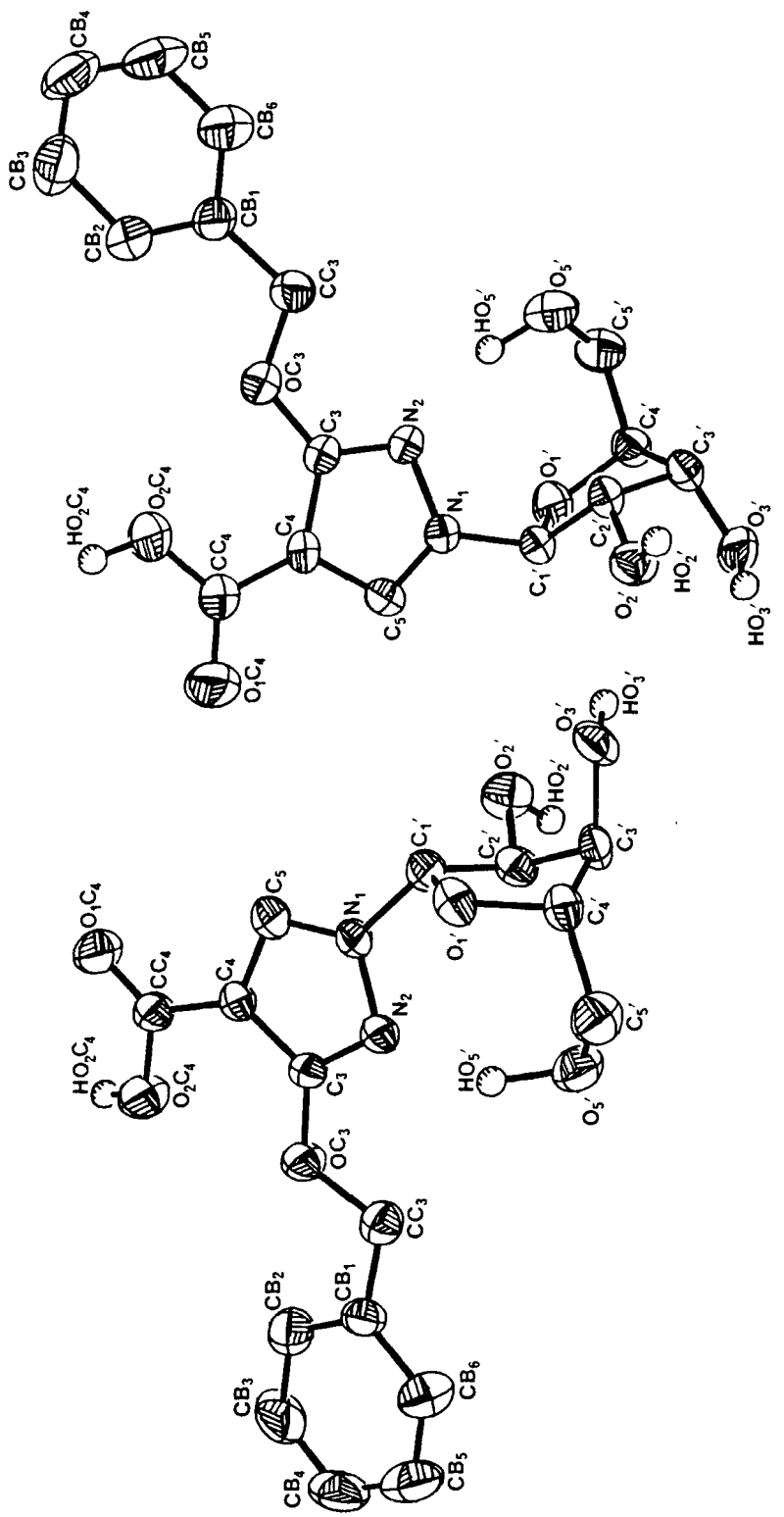
meter which utilized graphite monochromated Cu radiation (λ = 1.54178 Å). An attempt to calculate lattice parameters and an orientation matrix from 25 centered reflections failed suggesting that the crystal was twinned. It was possible to select fifteen reflections of one orientation suitable for lattice parameters and an orientation matrix was calculated from these values. Crystal and structure data are shown in Table I. Single crystal intensity data were obtained using a variable scan speed θ-2θ procedure. Sixty-one data were rejected as the backgrounds were measured on peaks of the twin, however a large majority of data was acceptable. Three check reflections were measured every 97 reflections. There was no systematic change in these data indicating crystal and electronic stability. The data were merged to 2491 independent reflections, 96 of which were considered unobserved as $I < 2\sigma(I)$. The structure was solved using direct methods and refined using a blocked cascading least squares procedure. Density calculations as well as the structure determination indicated that there were two molecules in the asymmetric unit. All nonhydrogen atoms were refined anisotropically. Positions for hydrogen atoms bonded to carbon atoms were calculated based on stereochemical considera-

tions. These atoms were allowed to ride on their neighboring carbon atoms during refinement. The thermal parameter of each of these hydrogen atoms was fixed at 1.2 times the initial equivalent isotropic thermal parameter of the neighboring atom. All hydrogens bonded to oxygen atoms were located in difference maps. The positional parameters of these atoms were not refined but the atoms were refined isotropically. An empirical extinction correction was made as several of the low angle reflections had larger F calculated than F observed. The resulting R values were R=0.049 and R_w=0.067 with weights based on counting statistics. The largest peaks in the final difference maps were 0.37eÅ^{-3} and -0.32eÅ^{-3}. Scattering factors were obtained from the "International Tables for X-ray Crystallography."[30] All computer calculations and the computer drawing were performed using SHELXTL.[31]

A computer drawing of the two crystallographically independent molecules of 6a with atom labels and conformation is shown in Figure 1. The bond lengths are listed in Table II. The positional and thermal parameters of the atoms are listed in Table III. There is good agreement between chemically similar bonds. It is quite evident that both the molecules are in the β conformation. The aglycon portions of both molecules are planar. The largest deviation of a base ring atom from the least-squares plane of the base is 0.002Å for N_2 in molecule A and 0.010Å for C_3 in molecule B. The carboxylic acid group deviates more from the plane of its base in molecule A than in molecule B as the dihedral angle between the planes of the base and carboxylic acid group is 15.2° in molecule A compared to 5.4° in molecule B.

The carbohydrate moiety of the two molecules are nearly identical. Both exist in the ^3_2T conformation with corresponding torsion angles of the two carbohydrate moieties differing by no more than 2.1°. In both glycon moieties there is an intramolecular hydrogen bond O_5,-HO_5,N_2 (see Figure 1 and Table IV). This hydrogen bond causes the respective molecules to exist in a rather compact form. The glycosidic torsion angles N_2-N_1-C_1,-C_2, of the two molecules are very similar with a value of 60.1° in molecule A and 61.1° in molecule B.

There is a rather extensive hydrogen bonding network which links the two molecules (Table IV). All of the hydrogen atoms bonded to oxygen atoms of the two molecules, with the exception of HO_2, in molecule B, are involved in hydrogen bonds. Oxygen of O_1C_4 of molecule A interacts with



Molecule A Molecule B

Computer drawing of twinned crystal of 6a
(The hydrogen atoms bonded to carbon atoms are omitted for clarity)

Figure I

TABLE II

Bond lengths (Å) with e.s.d.[a] values in parenthesis

	Molecule A	Molecule B
N_1-N_2	1.375(4)	1.378(5)
N_2-C_3	1.310(4)	1.315(5)
C_3-OC_3	1.336(5)	1.332(6)
OC_3-CC_3	1.426(6)	1.434(5)
CC_3-CB_1	1.501(6)	1.499(7)
C_3-C_4	1.427(6)	1.429(6)
C_4-CC_4	1.450(6)	1.456(6)
CC_4-O_1C_4	1.211(6)	1.218(6)
CC_4-O_2C_4	1.323(6)	1.289(7)
O_2C_4-HO_2C_4	0.98[a]	0.86
C_4-C_5	1.379(6)	1.386(6)
C_5-N_1	1.331(6)	1.326(5)
N_1-$C_{1'}$	1.458(5)	1.442(5)
$C_{1'}$-$C_{2'}$	1.537(6)	1.525(6)
$C_{2'}$-$O_{2'}$	1.404(5)	1.411(5)
$O_{2'}$-$HO_{2'}$	0.95	0.92
$C_{2'}$-$C_{3'}$	1.511(6)	1.529(6)
$C_{3'}$-$O_{3'}$	1.430(5)	1.425(6)
$O_{3'}$-$HO_{3'}$	0.94	0.97
$C_{3'}$-$C_{4'}$	1.497(6)	1.508(7)
$C_{4'}$-$C_{5'}$	1.516(6)	1.511(7)
$C_{5'}$-$O_{5'}$	1.411(6)	1.410(7)
$O_{5'}$-$HO_{5'}$	1.17	1.05
$C_{4'}$-$O_{1'}$	1.450(5)	1.499(5)
$O_{1'}$-$C_{1'}$	1.383(6)	1.408(5)
average C-C in benzene	1.379(13)	1.378(18)

[a] e.s.d. value on bond lengths involving H atoms is estimated at 0.03Å. The positioned parameters of these atoms were not refined.

two hydrogen atoms, while the corresponding oxygen of molecule B does not participate in any hydrogen bonding. This may account for the large dihedral angle between the plane of the carboxylic acid group and the aglycon in molecule A, as mentioned above.

The 4-substituted-1-β-D-ribofuranosyl-3-hydroxypyrazoles synthesized during this study were tested (for experimental details, see ref. 32) in parallel with pyrazofurin for their inhibitory effects on the growth of L1210 murine lymphocytic leukemia, WIL2 human B lymphoblastic leukemia, and CCRF-CEM human T lymphoblastic leukemia in cell culture. Only compounds 14a and 14b exhibited very slight (ID_{50} values[33] ranging from 30 to 45 μM) inhibitory effects against these cell lines, whereas pyrazofurin was active at 0.02 μM. The remaining compounds were inactive. Although pyrazofurin exhibited pronounced antiviral activity[17] against parainfluenza type 3, measles, vaccinia, and herpes simplex type 2 viruses, the 4-substituted-3-hydroxypyrazole ribonucleosides were inactive against these cell lines in culture.

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 90 MHz with a JEOL FX-90Q and at 300 MHz with IBM NR/300 FTNMR spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of solvent 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, Florham Park, NJ. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. J. T. Baker silica gel (70-230 mesh) was used for column chromatography. All solvents used were reagent grade. Tetrahydrofuran, dioxane and DMF were stored over molecular sieves (4A) prior to use. 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.

Large scale synthesis of Ethyl 3(5)-hydroxypyrazole-4-carboxylate (3). Hydrazine hydrate (85%, 48 mL) was added dropwise to a solution of di-

TABLE III
Positional (x 10^4) and Thermal Parameters (x 10^3) of the Atoms with e.s.d. values in parenthesis

	Molecule A			
	x	y	z	Ueq
N_1	5040(7)	4362(3)	5532(2)	38(1)
N_2	3220	4118	4852	38(1)
C_3	2045(9)	2967(4)	4912(2)	34(1)
OC_3	94(7)	2362(3)	4373(2)	47(1)
CC_3	-713(10)	3034(4)	3696(3)	43(1)
H_1CC_3	-1358	3913	3873	47[a]
H_2CC_3	832	3107	3390	47[a]
CB_1	-3001(10)	2221(4)	3194(3)	41(1)
CB_2	-4213(12)	1124(5)	3434(3)	57(2)
HCB_2	-3583	839	3940	61[a]
CB_3	-6328(14)	427(5)	2945(4)	76(2)
HCB_3	-7203	-321	3123	78[a]
CB_4	-7205(13)	791(6)	2210(4)	68(2)
HCB_4	-8633	281	1867	83[a]
CB_5	-6019(12)	1883(7)	1963(3)	65(2)
HCB_5	-6653	2151	1453	61[a]
CB_6	-3932(11)	2599(6)	2445(3)	55(2)
HCB_6	-3115	3364	2268	66[a]
C_4	3099(10)	2449(4)	5627(3)	38(1)
CC_4	2299(10)	1234(4)	5928(3)	42(1)
O_1C_4	3370(8)	846(3)	6532(2)	56(1)
O_2C_4	273(8)	557(3)	5466(2)	62(1)
HO_2C_4	-693	-38	5771	166(24)[a]
C_5	5020(10)	3396(4)	5999(3)	41(1)
HC_5	6132	3366(4)	6498	47[a]
$C_{1'}$	6469(9)	5659(4)	5703(3)	35(1)
$HC_{1'}$	7636	5654	6195	38[a]
$C_{2'}$	4460(9)	6831(4)	5802(3)	36(1)
$HC_{2'}$	2633	6664	5533	39[a]
$O_{2'}$	3917(7)	7156(3)	6605(2)	48(1)
$HO_{2'}$	2206	7602	6526	112(19)[a]
$C_{3'}$	6018(9)	7898(4)	5466(3)	36(1)
$HC_{3'}$	4841	8608	5318	38[a]
$O_{3'}$	8192(7)	8462(3)	6044(2)	44(1)
$HO_{3'}$	7457	8830	6520	69(14)[a]
$C_{4'}$	7334(9)	7123(4)	4788(3)	35(1)
$HC_{4'}$	8906	7625	4679	40[a]
$C_{5'}$	5640(10)	6915(5)	3978(3)	50(2)
$H_1C_{5'}$	6592	6291	3625	58[a]
$H_2C_{5'}$	5524	7761	3774	58[a]
$O_{5'}$	2923(7)	6418(3)	3986(2)	50(1)
$HO_{5'}$	3300	5288	4068	138(21)[a]
$O_{1'}$	7945(6)	5842(3)	5064(2)	39(1)

TABLE III (Cont'd)
Molecule B

	x	y	z	Ueq
N_1	8275(7)	7860(3)	8654(2)	36(1)
N_2	10226(8)	7907(3)	9307(2)	37(1)
C_3	11440(9)	6740(4)	9214(2)	37(1)
OC_3	13479(7)	6369(3)	9721(2)	49(1)
CC_3	14282(10)	7318(4)	10415(3)	43(1)
H_1CC_3	14930	8131	10251	44[a]
H_2CC_3	12726	7507	10719	44[a]
CB_1	16552(10)	6730(4)	10920(3)	42(1)
CB_2	17782(11)	5535(5)	10681(3)	56(2)
HCB_2	17162	5032	10171	57[a]
CB_3	19925(13)	5043(6)	11174(4)	72(2)
HCB_3	20812	4224	10993	76[a]
CB_4	20755(13)	5731(7)	11911(4)	73(2)
HCB_4	22178	5377	12256	79[a]
CB_5	19564(12)	6907(7)	12150(3)	65(2)
HCB_5	20183	7400	12662	69[a]
CB_6	17466(12)	7413(6)	11688(3)	57(2)
HCB_6	16628	8244	11851	59[a]
C_4	10364(10)	5926(4)	8493(3)	40(1)
CC_4	11115(11)	4590(4)	8149(3)	50(2)
O_1C_4	9948(10)	4008(4)	7530(3)	78(2)
O_2C_4	13131(9)	4089(3)	8562(2)	67(1)
HO_2C_4	13478	3253	8447	78(15)[a]
C_5	8310(10)	6710(4)	8174(3)	40(1)
HC_5	7710	6462	7691	44[a]
$C_{1'}$	6890(9)	9072(4)	8487(3)	34(1)
$HC_{1'}$	5626	8819	8018	39[a]
$C_{2'}$	8861(9)	10161(4)	8320(2)	34(1)
$HC_{2'}$	10727	10137	8564	37[a]
$O_{2'}$	9115(7)	10033(3)	7488(2)	44(1)
$HO_{2'}$	10685	10350	7306	108(19)[a]
$C_{3'}$	7401(9)	11433(4)	8641(3)	39(1)
$HC_{3'}$	8605	12207	8746	42[a]
$O_{3'}$	5140(7)	11694(3)	8083(2)	49(1)
$HO_{3'}$	5793	11463	7559	59(13)[a]
$C_{4'}$	6194(9)	11024(4)	9365(3)	40(1)
$HC_{4'}$	4665	11602	9464	44[a]
$C_{5'}$	7998(11)	11211(5)	10153(3)	52(2)
$H_1C_{5'}$	7068	10795	10535	59[a]
$H_2C_{5'}$	8209	12154	10330	59[a]
$O_{5'}$	10659(7)	10660(4)	10127(2)	53(1)
$HO_{5'}$	10587	9622	9954	92(17)[a]
$O_{1'}$	5539(7)	9609(3)	9155(2)	43(1)

Ueq is defined as one-third of the trace of the orthogonalised Uij tensor. [a] Value is the isotropic U.

TABLE IV
Hydrogen bond data

D———H·····A			H·····A(Å)	D·····A(Å)	D—H···A(deg)	translation of A
			intramolecular			
O_5,A[a]	HO_5,A	N_2A	1.886(2)	2.893(5)	140.5(2)[b]	x, y, z
O_5,B	HO_5,B	N_2B	1.918(4)	2.931(5)	161.6(2)	x, y, z
			intermolecular			
O_3,A	HO_3,A	O_2,B	2.003(3)	2.726(5)	132.0(2)	x, y, z
O_2C_4A	HO_2C_4A	O_3,A	1.735(3)	2.672(5)	157.5(5)	x-1, y-1, z
O_2C_4B	HO_2C_4B	O_3,B	1.830(4)	2.662(5)	162.3(3)	x+1, y-1, z
O_2,B	HO_2,B	O_1C_4A	2.038(4)	2.921(6)	160.0(2)	x+1, y+1, z
O_3,B	HO_3,B	O_1C_4A	2.024(4)	2.703(5)	125.3(2)	x, y+1, z

[a]The letter A or B is added to the atom label to designate the molecule to which atom belongs. [b]The e.s.d. values on H·····A and D—H···A are underestimated as the positional parameters of the H atoms were not refined. Based on past experience an uncertainty of about 0.03 Å on H·····A distance and 2 to 4 degrees on D——H·····A angle would be reasonable.

ethyl ethoxymethylenemalonate (86.4 g, 0.4 mol) in absolute EtOH (600 mL) and the mixture was heated under reflux for 1 h. Upon cooling, the hydrazine salt that crystallized was collected, washed with cold EtOH (2 x 25 mL) and dried. An aqueous solution of the salt was acidified with 15% HCl to pH 3. The product that precipitated was collected, washed with cold water (2 x 25 mL) and crystallized from aqueous EtOH as needles, 40 g (64%); mp 181-182°C [Lit.[18] mp 180-181°C].

Ethyl 3-hydroxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (4a). A mixture of dry 3 (3.12 g, 20 mmol), hexamethyldisilazane (HMDS, 25 mL), and $(NH_4)_2SO_4$ (0.10 g) was heated under reflux for 8 h with the exclusion of moisture. Excess HMDS was removed by distillation to provide the Me_3Si derivative of 3, which was dissolved in anhydrous CH_3CN (150 mL). To the solution was added 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribofuranose (10.09 g, 20 mmol) and stirred for 10 min before trimethylsilyl trifluoromethanesulfonate (Me_3Si triflate, 5.10 mL, 28 mmol) was added. The reaction mixture was stirred for 25 h at ambient temperature. The CH_3CN was evaporated and the residue was dissolved in EtOAc (250 mL). The organic layer was washed successively with aqueous

saturated $NaHCO_3$ solution (3 x 50 mL), saturated brine solution (2 x 50 mL), water (3 x 50 mL), and dried over Na_2SO_4. The solvent was evaporated and the residue was purified on a silica gel column (3.5 x 50 cm) using chloroform:acetone (8:1, v/v) as eluent to yield 10.22 g (85%) of the title compound as homogeneous foam; mp ~ 71°C; IR ν 1680, 1720 (C=O), 3000 (OH)cm^{-1}; UV λ_{max} (MeOH) 231 nm (ε 38,000); ^1H NMR (Me_2SO-d_6) δ 1.21 (t, 3, $CO_2CH_2CH_3$), 4.14 (m, 2, $CO_2CH_2CH_3$), 6.28 (d, 1, J = 2.5 Hz, C_1,H), 7.50-7.98 (m, 15, $3COC_6H_5$), 8.32 (s, 1, C_5H), 10.86 (br s, 1, ring NH). Anal. Calcd for $C_{32}H_{28}N_2O_{10}$: C, 64.00; H, 4.70; N, 4.66. Found: C, 64.13; H, 4.90; N, 4.40.

Ethyl 3-hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (4b). To a solution of 4a (6.0 g, 10 mmol) in MeOH (100 mL) was added NaOMe till the pH of the solution was between 9-10, and the mixture was stirred at room temperature for 18 h with the exclusion of moisture. The white precipitate that separated was collected by filtration, dissolved in water, acidified with Dowex-50 (H^+) resin to pH 2 and evaporated to dryness. The residue was purified on a reverse phase column (C-18) by prep LC techniques using water → MeOH gradient. The fractions containing the homogeneous product were evaporated to dryness and the residue was crystallized from aqueous EtOH to yield 1.80 g (62.5%); mp 117-118°C [Lit.[19] mp 115-116°C]; IR ν 1685 (C=O), 2930-3480 (OH) cm^{-1}; UV λ_{max} (pH 1) 243 nm (ε 8,700); UV λ_{max} (pH 7) 275 nm (ε 6,000); UV λ_{max} (pH 11) 277 nm (ε 7,800); ^1H NMR (Me_2SO-d_6) δ 1.30 (t, 3, $CO_2CH_2CH_3$), 4.10 (m, 2, $CO_2CH_2CH_3$), 5.35 (d, 1, J = 4.0 Hz, C_1,H), 8.10 (s, 1, C_5H). Anal. Calcd for $C_{11}H_{16}N_2O_7$: C, 45.84; H, 5.59; N, 9.72. Found: C, 45.87; H, 5.68; N, 9.62.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (5). Method 1. Compound 4b (2.90 g, 10 mmol) and MeOH/NH_3 (MeOH saturated with NH_3 at 0°C, 100 mL) were placed in a steel bomb (250 mL). The bomb was three-quarters submerged in a steam bath and heated for 20 h. The bomb was cooled and the NH_3 was allowed to evaporate at room temperature. The residue was subjected to a vacuum overnight to remove the last traces of NH_3. The residue was dissolved in water (25 mL), adjusted to pH 4 with Dowex-50 (H^+), adsorbed on a C-18 column, washed with water and then eluted with 5% MeOH in H_2O using preparative LC techniques. The homogeneous product was crystallized from water to yield 1.50 g (48%); mp 184-185°C [Lit.[19] mp 177-178°C]; IR ν 1670 (C=O), 3180-3440 (OH,

NH_2)cm^{-1}; UV λ_{max} (pH 1) 244 nm (ε 8,300); UV λ_{max} (pH 7) 275 nm (ε 6,600); UV λ_{max} (pH 11) 275 nm (ε 7,200); ^{1}H NMR (Me_2SO-$\underline{d}_6$) δ 5.43 (d, 1, J = 4.5 Hz, C_1,$\underline{H}$), 7.08 (br s, 2, CON$\underline{H}_2$), 8.10 (s, 1, $C_5\underline{H}$). Anal. Calcd for $C_9H_{13}N_3O_6$: C, 41.70; H, 5.05; N, 16.21. Found: C, 41.43; H, 5.28; N, 16.47.

Method 2. To a solution of 8a (1.75 g, 5 mmol) in 95% EtOH (100 mL) was added 10% Pd/C (0.10 g) and the mixture was shaken in a Parr hydrogenator for 5 h at 45 psi of H_2. The mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The residue was crystallized from aqueous EtOH as colorless needles, 1.10 g (85%); mp 185°C. This product was found to be identical with that prepared by Method 1.

Ethyl 3-benzyloxy-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)pyrazole-4-carboxylate (7a). To a solution of 4a (2.40 g, 4 mmol) in dry CH_3CN (100 mL) was added NaH (60% in oil, 160 mg) and the mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. Benzyl bromide (0.68 g, 4 mmol) was added and the mixture was stirred for 24 h. Evaporation of the solvent gave an oily residue, which was dissolved in EtOAc (200 mL). The EtOAc solution was washed successively with aqueous $NaHCO_3$ solution (5%, 2 x 50 mL), saturated brine solution (2 x 50 mL), water (2 x 50 mL), and dried over Na_2SO_4. The solvent was evaporated and the residual foam was purified on a silica gel column (2.5 x 40 cm) using $CHCl_3$ as eluent. Evaporation of the solvent and crystallization of the homogeneous residue from hexanes:EtOAc (1:1, v/v) gave 2.0 g (72.4%) of 7a as colorless needles; mp 75°C; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3, $CO_2CH_2\underline{CH}_3$), 4.24 (m, 2, $CO_2\underline{CH}_2CH_3$), 5.30 (s, 2, $\underline{CH}_2C_6H_5$), 5.92 (d, 1, J = 3.5 Hz, C_1,$\underline{H}$), 7.40 (m, 15, $3COC_6\underline{H}_5$), 7.96 (m, 6, $CH_2C_6\underline{H}_5$ and $C_5\underline{H}$). Anal. Calcd for $C_{39}H_{34}N_2O_{10}$: C, 67.81; H, 4.96; N, 4.05. Found: C, 67.52; H, 5.11; N, 3.78.

Ethyl 3-benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylate (7b). To a solution of 7a (6.90 g, 10 mmol) in MeOH (100 mL) was added NaOMe till the pH of the solution was between 9-10, and the mixture was stirred at room temperature for 16 h with the exclusion of moisture. The reaction mixture was neutralized with Dowex-50 (H^+) resin and the resin was removed by filtration. Evaporation of the filtrate and crystallization of the residue from EtOH gave 2.68 g (71%) of 7b as needles; mp 98°C, IR ν 1660, 1700 (C=O), 2950, 3380 (OH)cm^{-1}; UV λ_{max} (pH 1) 255 nm (ε 6,100); UV λ_{max} (pH 7) 255 nm (ε 7,600); UV λ_{max} (pH 11) 255 nm (ε 8,000); ^{1}H NMR

(Me_2SO-d_6) δ 1.20 (t, 3, $CO_2CH_2CH_3$), 4.18 (m, 2, $CO_2CH_2CH_3$), 5.22 (s, 2, $CH_2C_6H_5$), 5.55 (d, 1, J = 4.5 Hz, C_1,H), 7.40-7.90 (m, 5, $CH_2C_6H_5$), 8.40 (s, 1, C_5H). Anal. Calcd for $C_{18}H_{22}N_2O_7$: C, 57.14; H, 5.86; N, 7.40. Found: C, 57.35; H, 6.10; N, 7.46.

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide (8a). A mixture of 7a (6.90 g, 10 mmol) and MeOH/NH_3 (MeOH saturated with NH_3 at 0°C, 100 mL) was heated in a steel bomb at 100°C for 5 days. After cooling, the solvent was evaporated, the residue was dissolved in MeOH (20 mL), adsorbed on silica gel (20 g) and placed on top of a silica gel column (4 x 40 cm). The column was eluted with $CHCl_3$:MeOH (10:1, v/v). The homogeneous fractions were pooled, evaporated and the residue was crystallized from EtOH to yield 2.79 g (80%) of 8a; mp 175°C; IR ν 1630, 1650 (C=O), 2839-3480 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1 and 7) 245 nm (ε 10,500); UV λ_{max} (pH 11) 245 nm (ε 10,000); ^1H NMR (Me_2SO-d_6) δ 5.25 (s, 2, $CH_2C_6H_5$), 5.48 (d, 1, J = 3.5 Hz, C_1,H), 6.56 and 7.12 (2 br s, 2, $CONH_2$), 7.38 (m, 5, $CH_2C_6H_5$), 8.22 (s, 1, C_5H). Anal. Calcd for $C_{16}H_{19}N_3O_6$: C, 55.00; H, 5.48; N, 12.02. Found: C, 54.75; H, 5.45; N, 12.00.

3-Benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carboxamide (8b). A mixture of 8a (3.49 g, 10 mmol), acetic anhydride (100 mL), and DMAP (0.2 g) was stirred for 15 h at room temperature. Acetic anhydride was evaporated and the residue was dissolved in EtOAc (200 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ (2 x 50 mL), followed by water (2 x 50 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent, and purification of the residue on a silica gel column (4 x 40 cm) using $CHCl_3$ as the eluent gave 4.30 g (90.4%) of 8b as syrup; IR (neat) ν 1650 (C=O), 3400-3500 (NH_2)cm^{-1}; UV λ_{max} (pH 1 and 7) 243 nm (ε 25,700); UV λ_{max} (pH 11) 243 nm (ε 17,400); ^1H NMR ($CDCl_3$) δ 1.34 (m, 9, $3COCH_3$), 4.56 (s, 2, $CH_2C_6H_5$), 4.92 (d, 1, J = 3.5 Hz, C_1,H), 5.28 and 5.86 (2 br s, 2, $CONH_2$), 6.63 (m, 5, $CH_2C_6H_5$), 7.22 (s, 1, C_5H). Anal. Calcd for $C_{22}H_{25}N_3O_9$: C, 55.57; H, 5.30; N, 8.84. Found: C, 55.84; H, 5.37; N, 8.69.

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (6a). To a solution of 7b (1.89 g, 5 mmol) in H_2O (5 mL) was added 6N NaOH (3 mL) and the mixture was stirred at room temperature for 24 h. Water was evaporated and the residue was triturated with EtOH (3 x 10 mL). The solid was dissolved in H_2O (10 mL) and the solution neutralized with

Dowex-50 (H^+) resin. The resin was removed by filtration, the filtrate evaporated to dryness and the residue was crystallized from H_2O to yield 1.50 (85.6%) of the title compound; mp 138°C; IR ν 1675 (C=O), 3000-3500 (OH)cm^{-1}; UV λ_{max} (pH 1) 242 nm (ε 18,200); UV λ_{max} (pH 7) 238 nm (ε 11,900); UV λ_{max} (pH 11) 232 nm (ε 11,200); ^1H NMR (Me_2SO_6-$\underline{d}_6$) δ 5.22 (s, 2, $C\underline{H}_2C_6H_5$), 5.52 (d, 1, J = 4.0 Hz, C_1,$\underline{H}$), 7.41 (m, 5, $CH_2C_6\underline{H}_5$), 8.31 (s, 1, $C_5\underline{H}$). Anal. Calcd for $C_{16}H_{18}N_2O_7$: C, 54.85; H, 5.18; N, 7.99. Found: C, 54.70; H, 5.20; N, 7.90.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxylic acid (6b). In a similar manner as for 5 (Method 2), hydrogenation of 6a (1.75 g, 5 mmol) in the presence of Pd/C (10%, 0.20 g) gave 1.06 g (81.5%) of 6b; mp > 80°C (dec.); IR ν 1690 (C=O), 3000-3400 (OH)cm^{-1}; UV λ_{max} (pH 1) 240 nm (ε 14,800); UV λ_{max} (pH 7) 238 nm (ε 16,400); UV λ_{max} (pH 11) 264 nm (ε 13,700); ^1H NMR (Me_2SO_6-$\underline{d}_6$) δ 5.46 (d, 1, J = 4.5 Hz, C_1,$\underline{H}$), 8.16 (s, 1, $C_5\underline{H}$). Anal. Calcd for $C_9H_{12}N_2O_7 \cdot 1/2H_2O$: C, 40.15; H, 4.86; N, 10.40. Found: C, 40.40; H, 5.05; N, 10.49.

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carboxamide 5'-phosphate Disodium Salt (11a). A solution of $POCl_3$ (1.80 mL, 19.5 mmol) in freshly distilled trimethyl phosphate (36 mL) was cooled to 0°C, and dry 8a (2.15 g, 6 mmol) was added with stirring. The mixture was protected from moisture and was stirred for 7 h at 0°C 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 into cold water (75 mL), and the pH was adjusted to 2.0 with 2N NaOH. The aqueous solution was extracted with $CHCl_3$ (2 x 100 mL) to remove trimethyl phosphate, and then applied to a column of activated charcoal (75 g, acid washed Au-4). The column was washed with water until the eluate was salt free. The nucleotide was eluted with a solution of EtOH:H_2O:NH_4OH (10:10:1, v/v) using a UV monitor. On evaporation of the solvent, the residue indicated a mixture of two components, one of which is probably the dehydrated carbonitrile derivative. The mixture was dissolved in NH_4OH (25 mL) containing H_2O_2 (30%, 2 mL) and stirred for 2 h, after which TLC showed a single product. Evaporation of the solvent gave a residue, which was dissolved in a small amount of water and passed through a column of Dowex-50 (H^+) resin (25 mL). The column was washed with water, and the fractions containing the nucleotide were collected. The solution was concentrated to a small

volume (~20 mL) and passed through a column of Dowex-50W-X8 (20-50 mesh, Na^+ form, 25 mL). The column was washed with water, and the fraction containing the sodium salt of the nucleotide was evaporated to dryness. The residue was dissolved in water (25 mL) and lyophilized to provide 2.10 g (74%) of the title compound; mp 158°C (dec.); IR ν 1650 (C=O), 3380-3460 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1 and 7) 245 nm (ε 8,000); UV λ_{max} (pH 11) 245 nm (ε 7,600). Anal. Calcd for $C_{16}H_{18}N_3O_9Na_2P.H_2O$: C, 39.12; H, 4.10; N, 8.55. Found: C, 39.27; H, 4.38; N, 8.50.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamide 5'-phosphate Disodium Salt (11b). To a solution of 11a (2.36 g, 5 mmol) in H_2O:EtOH (1:1, 100 mL) was added 20% Pd/C (0.10 g) and the mixture was hydrogenated at 45 psi for 30 min. The catalyst was removed by filtration on a Celite pad, and the filtrate evaporated to dryness. The residue was triturated with EtOH (2 x 20 mL) and the solid was collected by filtration. An aqueous solution of the solid was lyophilized to yield 1.85 g (96.5%) of 11b; mp >165°C (dec.); IR ν 1650 (C=O), 3200-3420 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1) 245 nm (ε 7,300); UV λ_{max} (pH 7) 275 nm (ε 5,000); UV λ_{max} (pH 11) 275 nm (ε 5,800). Anal. Calcd for $C_9H_{12}N_3O_9Na_2P.H_2O$: C, 27.56; H, 3.34; N, 10.71; P, 7.89. Found: 27.85; H, 3.40; N, 10.43; P, 7.80.

3-Benzyloxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (10a). To a mixture of $POCl_3$ (30 mL) and N,N-diethylaniline (15 mL) was added 8b (4.75 g, 10 mmol), and stirred at room temperature for 2 h. The solution was evaporated to dryness and the residue was dissolved in cold water (50 mL). The aqueous solution was extracted with $CHCl_3$ (3 x 75 mL). The combined organic phase was washed successively with cold 0.05 N HCl (2 x 25 mL), 5% $NaHCO_3$ solution (3 x 25 mL), water (2 x 50 mL) and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a syrup, which was purified on a silica gel column (4 x 45 cm) using $CHCl_3$:acetone (8:1, v/v) as the eluent. Removal of the solvent from homogeneous fractions gave 3.80 g (83.1%) of 10 as syrup; IR (neat) 1740 (C=O), 2200 (C≡N)cm^{-1}; UV λ_{max} (MeOH) 240 nm (ε 19,700); ^1H NMR ($CDCl_3$) δ 2.04 and 2.10 (2s, 9, $3COCH_3$), 5.25 (s, 2, $CH_2C_6H_5$), 5.60 (d, 1, J = 4.5 Hz, C_1,H), 7.32 (m, 5, $CH_2C_6H_5$), 7.70 (s, 1, C_5H). Anal. Calcd for $C_{22}H_{23}N_3O_8$: C, 57.76; H, 5.07; N, 9.18. Found: C, 57.48; H, 5.36; N, 9.06.

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-carbonitrile (10b). Compound 10a (4.47 g, 10 mmol) was combined with liquid NH_3 (50 mL) in a steel

reaction vessel and allowed to stand at room temperature for 15 h, after which NH_3 was evaporated. The dry residue was purified on a flash silica gel column (4 x 30 cm) using $CHCl_3$:MeOH (9:1) as the eluent, and crystallized from aqueous EtOH to yield 1.60 g (48.3%) of 10b; mp 138°C; IR ν 2210 (C≡N), 3440 (OH)cm^{-1}; UV λ_{max} (pH 1 and 7) 240 nm (ε 19,200); UV λ_{max} (pH 11) 238 nm (ε 17,900); ^1H NMR (Me_2SO-d_6) δ 5.27 (s, 2, $CH_2C_6H_5$), 5.56 (d, 1, J = 4.0 Hz, C_1,H), 7.40 (m, 5, $CH_2C_6H_5$), 8.52 (s, 1, C_5H). Anal. Calcd for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68. Found: C, 57.76; H, 5.13; N, 12.44.

3-Benzyloxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (9b). A slow stream of H_2S was bubbled through a solution of 10a (4.57 g, 10 mmol) in dry pyridine (125 mL) containing triethylamine (4 mL) for 3 h. After stirring for 12 h at room temperature, the mixture was evaporated to dryness. The residue was co-evaporated with EtOH (3 x 50 mL) and the residual syrup (9a, 3.20 g) was deacetylated without further purification. The above syrup (9a) was dissolved in MeOH/NH_3 (saturated at 0°C, 100 mL) and stirred at room temperature for 16 h. MeOH/NH_3 was evaporated and the residue was purified on a silica gel column (2.5 x 50 cm) using $CHCl_3$:MeOH (6:1, v/v) as the eluent. The fractions containing the homogeneous product were pooled, evaporated and the residue was crystallized from EtOH to yield 0.80 g (21.9% overall yield); mp 132°C; IR ν 1150 (C=S), 3300-3450 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1) 243 nm (ε 5,500), 302 (10,200); UV λ_{max} (pH 7) 243 nm (ε 4,000), 302 (7,300); UV λ_{max} (pH 11) 243 nm (ε 5,500), 302 (9,500); ^1H NMR (Me_2SO-d_6) δ 5.28 (s, 2, $CH_2C_6H_5$) 5.52 (d, 2, J = 3.5 Hz, C_1,H), 7.40 (m, 5, $CH_2C_6H_5$), 8.35 (s, 1, C_5H), 8.13 and 9.33 (2br s, 2, $CSNH_2$). Anal. Calcd for $C_{16}H_{19}N_3O_5S.1/2H_2O$: C, 51.33; H, 5.38; N, 11.22; S, 8.56. Found: C, 51.38; H, 5.53; N, 11.26; S, 8.39.

3-Hydroxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-4-carbonitrile (13). To a mixture of $SiCl_4$ (0.34 mL, 3 mmol) and NaI (0.45 g, 3 mmol) in dry CH_3CN (25 mL) was added 10a (1.14 g, 2.5 mmol) and heated under reflux for 15 h in a nitrogen atmosphere. The cooled (0.5°C) reaction mixture was poured into ice-water (15 mL), stirred for 10 min and the aqueous solution was extracted with EtOAc (2 x 50 mL). The combined organic phase was washed with 10% $Na_2S_2O_4$ solution (2 x 25 mL), followed by water (50 mL) and dried over Na_2SO_4. After removal of the solvent, the residue was purified on a silica gel column (2.5 x 25 cm) using

$CHCl_3$:acetone (8:1, v/v) as the eluent to yield 0.80 g (87.1%) of 13 as gum; IR (neat) ν 1730 (C=O), 2220 (C≡N), 3000-3400 (OH)cm^{-1}; UV λ_{max} (pH 1) 238 nm (ε 14,700); UV λ_{max} (pH 7) 268 nm (ε 12,000); UV λ_{max} (pH 11) 268 nm (ε 10,300); ^{1}H NMR ($CDCl_3$) δ 2.04-2.08 (3s, 9, $3COCH_3$), 5.60 (d, 1, J = 3.5 Hz, C_1,H), 7.76 (s, 1, C_5H). Anal. Calcd for $C_{15}H_{17}N_3O_8$: C, 49.05; H, 4.66; N, 11.44. Found: C, 48.94; H, 4.65; N, 11.14.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-thiocarboxamide (12b). To a cooled (-20°C) solution of 13 (0.53 g, 1.44 mmol) in anhydrous pyridine containing triethylamine (1 mL) was bubbled H_2S for 2 h. The solution was later heated in a steel bomb at 90°C for 14 h. After cooling the bomb, the reaction mixture was purged with N_2 and evaporated to dryness. Co-evaporation with EtOH (4 x 50 mL), followed by toluene (4 x 25 mL) gave dry residue (12a), which was dissolved in absolute EtOH and to this was added 1N NaOMe solution (4.2 mL). The mixture was stirred at ambient temperature for 1.5 h, neutralized with Dowex-50 (H^+) resin, filtered and the filtrate evaporated to dryness. The residue was purified on a flash silica gel column (2.5 x 20 cm) using $CHCl_3$:MeOH (2:1, v/v) as the eluent and crystallized from aqueous EtOH to yield 0.26 g (65.6%) of the title compound; mp 154-155°C; IR ν 1200, 1490 [C(S)N], 3200-3370 (OH, NH_2)cm^{-1}; UV λ_{max} (pH 1) 246 nm (ε 6,600), 299 (10,000); UV λ_{max} (pH 7) 222 nm (ε 11,500), 248 (6,800), 322 (10,200); UV λ_{max} (pH 11) 221 nm (ε 11,000), 248 (5,500), 323 (8,800); ^{1}H NMR (Me_2SO-d_6) δ 5.44 (d, 1, J = 2.5 Hz, C_1,H), 8.29 (s, 1, C_5H), 8.90 and 9.37 (2br s, 2, $CSNH_2$). Anal. Calcd for $C_9H_{13}N_3O_5S$: C, 49.05; H, 4.66; N, 11.44. Found: C, 48.94; H, 4.65; N, 11.24.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamidoxime (14a). To a solution of 13 (0.28 g, 0.76 mmol) in absolute EtOH (10 mL) was added free NH_2OH (0.50 g, 15 mmol) and the mixture was stirred at room temperature for 4 h with the exclusion of moisture. The reaction mixture was evaporated to dryness and the residue was purified by short column chromatography using EtOAc:MeOH:H_2O:acetone (3:1:1:1, v/v) as the solvent. The appropriate fraction was evaporated, the residue dissolved in water and lyophilized to yield 0.11 g (52.8%) of 14a as pink colored powder; mp 150°C (dec.); UV λ_{max} (pH 1) 252 nm (ε 4,700); UV λ_{max} (pH 7) 286 nm (ε 4,700); UV λ_{max} (pH 11) 286 nm (ε 5,900); ^{1}H NMR (Me_2SO-d_6) δ 3.83 (m, 1, C_4,H), 4.03 (t, 1, C_2,H), 5.39 (d, 1, J = 4.5 Hz, C_1,H), 5.96 (br s, 2, NH_2), 7.98 (s, 1, C_5H). Anal. Calcd for $C_9H_{14}N_4O_6 \cdot 1/2H_2O$: C, 38.16; H, 5.33; N, 19.78. Found: C, 37.84; H, 5.21; N, 19.46.

3-Hydroxy-1-β-D-ribofuranosylpyrazole-4-carboxamidine (14b). To a solution of 14a (0.14 g, 0.51 mmol) in 95% EtOH (10 mL) was added 10% Pd/C (0.10 g) and the mixture was shaken in a Parr hydrogenator for 24 h at 50°C at 50 psi of H_2. The mixture was filtered through a Celite pad and the filtrate evaporated to dryness. The residue was crystallized from EtOH to yield 55 mg (41.7%) of the title compound as rosette; mp 223°C; UV λ_{max} (pH 1) 254 nm (ε 6,800); UV λ_{max} (pH 7) 234 nm (ε 5,300), 289 (5,700); UV λ_{max} (pH 11) 289 nm (ε 6,000); ^{1}H NMR (Me_2SO-d_6) δ 3.52 (m, 2, C_5,CH_2), 3.87 (m, 1, C_4,H), 4.04 (t, 1, C_2,H), 4.31 (t, 1, C_3,H), 5.25 (d, 1, J = 4.0 Hz, C_1,H), 7.96 (br s, 2, NH_2), 8.0 (s, 1, C_5H), 9.10 (br s, 1, NH); ms[FAB]: m/z 259 (M+1). Anal. Calcd for $C_9H_{14}N_4O_5 \cdot 3/4H_2O$: C, 39.77; H, 5.74; N, 20.61. Found: C, 40.07; H, 5.42; N, 20.37.

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33. Inhibitory dose 50 (ID_{50}) is the concentration of the compound in the culture media that produced 50% of the tumor cell growth as compared to the untreated controls.

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