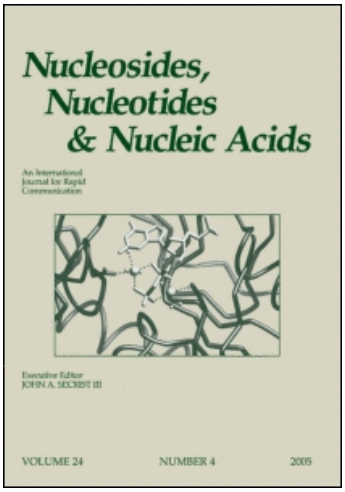

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Synthesis and Biological Activity of the Novel Adenosine Analogs; 3-Amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole and 3-Amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole

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Abstract

Chemical modification of the 4-nitrile group in 5-amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole-4-carbonitrile (**1**) afforded 5-amino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole (**3**). The methylation of **3**, via a three step procedure, gave 5-methylamino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole (**3a**). The mononuclear heterocyclic rearrangement (m.h.r) of **3** and **3a**, provided a convenient route to the novel azapentalene adenosine analogs 3-amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6**) and 3-amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6a**), respectively. Compound **6** exhibited no cytotoxicity when screened *in vitro* against either mouse L1210 leukemic cells or human foreskin fibroblasts. Nor was it active against human cytomegalovirus. Compound **6a** was designed and prepared to investigate the possibility that the lack of biological activity of **6** might be due to annular tautomerization limiting the ability of **6** to serve as a substrate for the activating enzyme adenosine kinase. This hypothesis was neither supported nor disproved by the results, as compound **6a** was also inactive in both the antiproliferative and antiviral test systems.

INTRODUCTION

Over the past several decades, naturally occurring nucleosides have provided medicinal chemists with the basis for the design of numerous agents displaying biological and chemotherapeutic activity. Historically, adenosine analogs have shown a wide range of biological activity[1] and have received considerable attention as neuromodulators.[2]

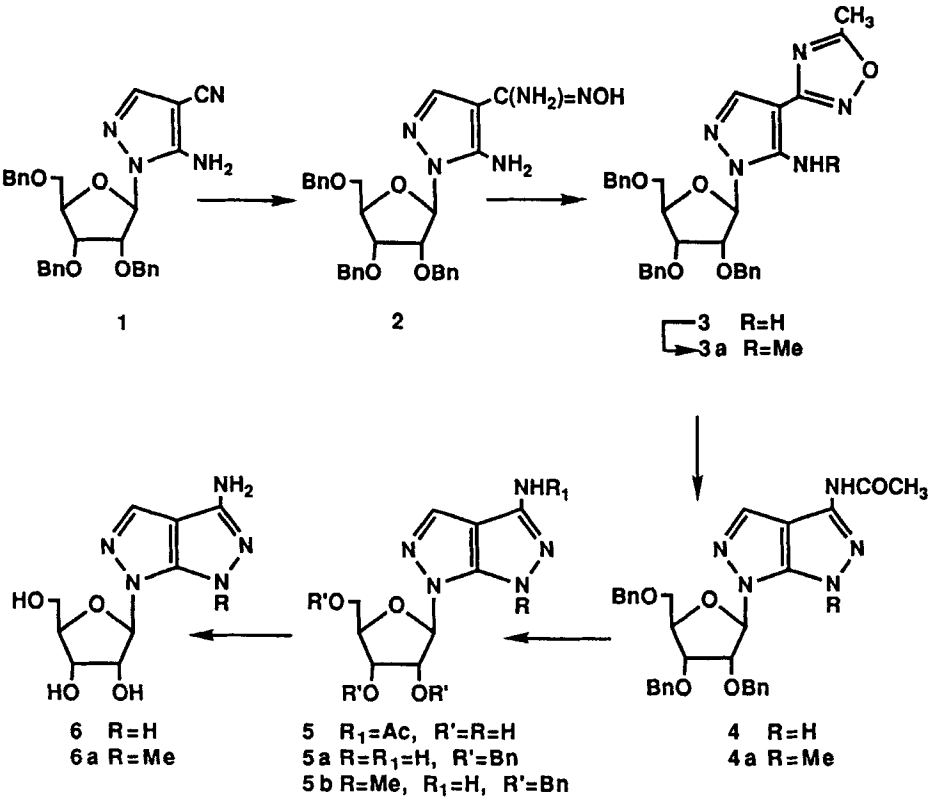
This manuscript is dedicated to the memory of Roland K. Robins

Though a vast number of synthetic modifications have been performed on the adenine and sugar moieties of adenosine, few reports describing an aromatic azapentalene analog of adenosine can be found in the literature.[3,4] Azapentalene analogs of adenosine, iso-π-electronic with the electron rich pentalene dianion,[5] present interesting synthetic targets since the "adenine" moiety would possess significant differences in the distribution of ring electron densities and also result in spatial changes of atoms within the heterocycle. Changes of this nature could potentiate alterations in binding to biologically important enzymes and in doing so foster a significant biological response. We have already shown that pyrrolo[2,3-*d*]pyrimidine[6,7,8,9] and pyrrolo[2,3-*d*]pyridazine[10,11] nucleoside analogs related to adenosine have significant antiproliferative and antiviral activity.

In order to investigate the hypothesis that azapentalene analogs of adenosine could be biologically active, we report on the synthesis, and antiproliferative and antiviral evaluation of 3-amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6**) and its methylated analog 3-amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6a**).

Results and Discussion

Chemistry. The synthetic route utilized for the preparation of 3-amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6**) and 3-amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6a**) is outlined in Scheme I. 5-Amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole-4-carbonitrile (**1**) was selected as the starting material for the synthesis of both target compounds and was prepared in four steps from D-ribose by the method described by R.R. Schmidt and coworkers.[12] That the anomeric configuration in **1** is exclusively beta was established by a conversion of **1** to 4-amino-1-(ß-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (4-APPR) and a comparison of this nucleoside with an authentic sample.[4] The treatment of **1** with hydroxylamine gave 5-



Scheme I

amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole-4-carboxamidoxime (**2**). When **2** was treated with ethyl acetate (EtOAc) under basic conditions, ring closure[13] to give 5-amino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole (**3**) occurred. Annulation of the second pyrazole ring was conveniently accomplished by the mononuclear heterocyclic rearrangement (m.h.r.)[14] of **3** in dimethylformamide (DMF) in the presence of sodium hydride (NaH) to give 3-acetamido-6-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**4**).

The ^{1}H NMR spectrum of **2** had single resonances at δ 8.95 (OH), 6.22 (NH_2) and δ 5.73 (NH_2), indicative of the 4-carboxamidoxime and 5-amino groups, respectively. The ^{1}H NMR spectrum of **3** revealed the absence of the downfield (deshielded)

exchangeable protons attributed to the carboxamidoxime group. The signal for the 5-amino group at δ 5.4 was still present, as well as a singlet at δ 2.58 for the methyl group. The bicyclic product **4** exhibited two adjacent singlets centered at δ 10.8 which exchanged on deuterium oxide wash. These two peaks could be assigned to the newly formed amide proton and N-1 ring proton of the annulated pyrazole ring. In addition, the chemical shift of the methyl group was shifted upfield from the δ 2.58 value typical of a methyl group located between two heteroatoms of an heteroaromatic, to a position at δ 2.08 typical of an exocyclic acetamido group.

Our initial approach to the target compound **6** was to debenzylate **4** by hydrogenolysis[15] and then to remove the acetyl group from the 3-amino moiety. The debenzylation was accomplished with Pd/C in anhydrous 3% hydrogen chloride (HCl) in methanol to give 3-acetamido-6-(ß-D-ribofuranosyl)pyrazolo[3,4,-*c*]pyrazole hydrochloride (**5**). However, hydrolysis of the sugar and acetyl groups occurred to varying extents, and made it difficult to obtain reproducible results. Hydrogenolysis attempts of **5** over Pd/C in the absence of hydrogen chloride gave only starting material. Therefore, the acetyl group was first cleaved by the treatment of **4** with sodium methoxide in methanol to give 3-amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**5a**). Removal of the benzyl groups from **5a** was accomplished cleanly and in good yield by using the phase transfer hydrogenation[16] conditions of ammonium formate in methanol in the presence of Pd/C[17] to give **6**. The ^{1}H NMR spectrum of **6** was consistent with the desired product and supported by elemental analysis, IR, and U.V. spectral data.

Adenosine analogs often require conversion to their 5'-phosphates by adenosine kinase in order to elicit a biological effect. Adenosine kinase will accept many different nucleosides as a substrate. However, it does not efficiently phosphorylate 1-methyladenosine or 6-methyl-7-amino-3-(ß-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (N^6-methylformycin).[18] A possible tautomer of compound **6** exists where the proton resides on N^2. This tautomer should not, based on the examples cited, be an efficient

substrate for adenosine kinase. We desired to eliminate the possible *in vitro* existence of the N^2-H tautomer in order to examine the effect of the tautomerization, if any, on the biological actvity of **6**.

One plausible approach which seemed appropriate was to prepare the N^1-methyl analog of **6**. This compound would have the desired sp^2 N^2 (comparable to N^1 of adenosine) and would be expected to behave as a more efficient substrate towards adenosine kinase. Thus, the preparation and biological evaluation of 3-amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6a**) may provide an indication of whether the lack of biological activity of **6** (see below) is due to its failure to serve as a substrate for adenosine kinase.

The preparation of **6a** began with the methylation of **3** to give 5-methylamino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(ß-D-ribofuranosyl)pyrazole (**3a**). This methylation was carried out in three steps. Compound **3** was first reacted with trifluoroacetic anhydride in pyridine to give the 5-trifluoroacetamide derivative. Alkylation of the anion of trifluoroacetamides has been shown to be an efficient route to N-substituted trifluoroacetamides.[19] Consequently, successive treatment of the trifluoroacetamide derivative of **3** with sodium hydride in the presence of dimethyl sulfate, followed by methanolic ammonia to remove the trifluoroacetyl group from the 5-amino group gave **3a**. Treatment of **3a** with sodium hydride in N,N-dimethylformamide, followed by neutralization with acetic acid, gave the protected bicyclic pyrazolo[3,4-*c*]pyrazole riboside derivative **4a**. Compound **4a** was treated with sodium methoxide in methanol to give 3-amino-1-methyl-6-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**5b**). The combined yield for the rearrangement (**3a** to **4a**) and deacetylation (**4a** to **5b**) steps was 70%. Initial attempts to debenzylate **5b** were performed utilizing the conditions we had used for the debenzylation of **5a**, ammonium formate and Pd/C. These conditions gave unsatisfactory results, as debenzylation was quite sluggish, and even after an extended time period (2 d) the reaction was incomplete. However, heating a solution of **5b** in an ethanol/cyclohexene mixture in the presence of $Pd(OH)_2/C$ gave complete

debenzylation in 6 h to afford the desired product 3-amino-1-methyl-6-(ß-D-ribofuranosyl)-pyrazolo[3,4-*c*]pyrazole (**6a**). The ^{1}H NMR spectrum of **6a** was consistent with the desired product and supported by elemental analysis, IR, mass and U.V. spectral data.

Biological Studies 3-Amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (**6**) was tested at a concentration of 100 μM against L1210 mouse leukemic cells in vitro and displayed no significant inhibition of cell proliferation. This lack of biological activity for **6** might be due to its being deaminated by cellular adenosine deaminase, as are many other adenosine analogs.[20] To test this possibility, the adenosine deaminase inhibitor deoxycoformycin[21] (1 μM) was added to the L1210 cell cultures along with **6**. In these experiments, cell proliferation was not inhibited, in either the presence or the absence of deoxycoformycin, which by itself also did not affect cell proliferation. These results indicate that deamination did not play a major role in limiting the biological activity of **6**.

Another possible explanation for the lack of biological activity for **6** is that it may not be a good substrate for the activating enzyme adenosine kinase, as discussed above. The N^1-methyl derivative **6a**, designed to favor substrate activity with this enzyme, also did not significantly inhibit proliferation of L1210 cells when added to the cultures at 100 μM. This result may be interpreted to indicate that neither **6** nor **6a** was a substrate for adenosine kinase. Alternatively, these compounds may lack biological activity for other reasons. In particular, the N^1-methyl substituent on **6a** may prevent it from being cytotoxic, even though it may be phosphorylated by adenosine kinase as proposed above.

Compounds **6** and **6a** were also evaluated at concentrations up to 100 μM against human cytomegalovirus (HCMV) and were inactive against HCMV and non-toxic to uninfected human foreskin fibroblasts (HFF cells) used to propagate the virus. The intermediates **3** and **4** also did not inhibit proliferation of L1210 cells *in vitro*.

Experimental Section

Proton magnetic resonance (^{1}H NMR) spectra were obtained with a Bruker WP270SY spectrometer (solutions in dimethylsulfoxide-d_6 or deuteriochloroform

with tetramethylsilane as internal standard), with chemical shift values reported in δ, parts per million, relative to the internal standard. Ultraviolet spectra were recorded on a Hewlett-Packard model 8450A UV/Vis spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Thin layer chromatography was run on glass plates coated (0.25 mm) with silica gel. Compounds of interest were detected by either ultraviolet lamp (254 nm), iodine vapors, or treatment with sulfuric acid followed by heating. Evaporations were performed under reduced pressure at 40 °C with a rotary evaporator. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

5-Amino-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazole-4-carboxamidoxime (**2**). A solution of **1** (17.1 g, 33.5 mmol) in ethanol (50 mL) was added dropwise with stirring to a refluxing solution of hydroxylamine (9.3 g, 134 mmol) and triethylamine (13.56 g, 134 mmol) in ethanol (500 mL). After 5 h at reflux the solution was cooled and concentrated in vacuo to a viscous syrup which was slurried with ethyl acetate (200 mL) and filtered to remove triethylamine hydrochloride. The filtrate was washed successively with water and aqueous saturated sodium chloride, dried over magnesium sulfate and then concentrated in vacuo to give **2**, which was slightly contaminated by a minor product. Final purification via chromatography (10 x 10 cm filter funnel, 500 g silica gel, 230-400 mesh, cyclohexane: ethyl acetate (1:5)) gave **2** (10.2 g, 58%, viscous syrup): IR (KBr) 3500-2720, 2924, 2854, 1651-1567, 1497, 1455, 1363, 1321, 1265, 1208, 1166-990 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.43-3.6 (m, 2H, H-5'); 4.18 (m, 1H); 4.32 (t, 1H); 4.47-4.7 (m, 8H); 5.65 (s, 2H, NH_2); 5.95 (d, 1H, 1'-H); 6.22 (s, 2, NH_2); 7.2-7.5 (m, 15H, Ph H's); 7.63 (s, 1H, H-3); 8.96 (s, 1H, OH). Peaks at δ 5.65, 5.95 and 8.96 exchange with D_2O. Anal. ($C_{30}H_{33}N_5O_5$) C, H, N.

5-Amino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)-1H-pyrazole (**3**). Compound **2** (10.3 g, 18.95 mmol) was added to an ethanolic solution of sodium ethoxide (430 mg sodium metal, 18.6 mmol, in 125 mL ethanol) followed by the addition of ethyl acetate (7.3 mL). The mixture was heated at

reflux for 8 h, neutralized with acetic acid and then concentrated in vacuo (40 °C, 0.5 torr) and the resultant residue partitioned between ethyl acetate (100 mL) and water (100 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo (40 °C, 0.5 torr) affording **3** as a syrup. The crude product was chromatographed (10 x 10 cm filter funnel, silica gel, 230-400 mesh, 300 g, toluene:ethyl acetate (8:3)) to give **3** (8.18 g, 76%, syrup): IR (thin layer smear) 3437, 3332, 3064, 3028, 2924, 2868, 1630, 1595, 1539, 1497, 1455, 1363, 1314, 1251, 1132-1018, 934, 892 and 737 cm^{-1}; UV λ_{max} nm (ε): methanol, 239 (6375), 266 (6668); ^1H NMR ($CDCl_3$) δ 2.58 (s, 3H, CH_3); 3.50 (d, 1H, 5'-H); 3.54 (d, 1H, 5'-H); 4.20-4.24 (t, 1H); 4.26-4.29 (m, 1H); 4.4-4.7 (m, 8H); 5.41 (s, 2H, NH_2); 5.95 (d, 1H, 1'-H); 7.16-7.36 (m, 15, Ph H's); 7.76 (s, 1H, C_3H). Peak at δ 5.41 exchanges with D_2O. Anal. ($C_{32}H_{33}N_5O_5$) C, H, N.

5-Methylamino-4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)-1H-pyrazole (3a). To a solution of **3** (20 g, 35.23 mmol) in cold (0-5 °C) pyridine (200 mL) was added trifluoroacetic anhydride (5.7 mL) dropwise with stirring. The ice bath was removed and the mixture was stirred at room temperature for 1.5 h. The mixture was concentrated in vacuo (40 °C, 0.5 torr) to give a residue. To this residue was added ethyl acetate (250 mL) and water (75 mL) and the mixture shaken vigorously. The organic layer was dried ($MgSO_4$), filtered by gravity, and the filtrate concentrated in vacuo (40 °C, 10 torr) to give 21 g of a syrup. This syrup was then dried by heating at 0.5 torr and 40 °C for 14 h. This material was dissolved in acetonitrile (300 mL) and added to a flask containing sodium hydride (1.13 g, from 2.27 g of 50% sodium hydride/mineral oil suspension, washed with pentane). To this mixture was added dimethylsulfate (5.4 mL) and the mixture stirred at room temperature for 6 h. The mixture was concentrated in vacuo (40 °C, 10 torr) and the resultant residue partitioned between ethyl acetate (250 mL) and water (75 mL). The organic layer was washed with water (75 mL) and then with saturated aqueous sodium chloride solution (75 mL) and then dried over magnesium sulfate. The drying agent was removed by filtration and the filtrate concentrated to a dark syrup. This material was purified by column chromatography (13 x

17 cm filter funnel, 350 g silica gel, 70-230 mesh, toluene:ethyl acetate (6:1)) and the product fractions concentrated to give 13.25 g of the 5-trifluoroacetyl-methylamino intermediate. This material was dissolved in methanol (250 mL) and the solution cooled (ice bath). Ammonia gas was bubbled into the methanolic solution until saturated and the ice bath was removed and a slow continuous stream of ammonia was bubbled into the solution. After 16 h, the solution was concentrated (40 °C, 10 torr) to a syrup. This material was coevaporated two times with 100 mL portions of cyclohexane and then left on the rotary evaporator at 40 °C and 0.5 torr for 2 h to give **3a** (10.88 g, 53%, syrup): IR (thin film) 3360, 3065, 3022, 2931, 2868, 1609, 1588, 1455, 1427, 1251, 1082, 941, 829, 737 and 695 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.57 (s, 3H, CH_3), 2.88 (d, 3H, NH$\underline{CH_3}$), 3.44-3.58 (m, 2H, 5'-CH_2), 4.19-4.25 (m, 1H, 3' or 4'-H), 4.33- 4.37 (m, 1H, 3'or 4'-H), 4.44-4.76 (m, 7H, Ph$\underline{CH_2}$'s and 2'-H), 5.90-5.98 (q, 1H, $\underline{NH}CH_3$), 5.99 (d, 1H, 1'-H), 7.23-7.34 (m, 5H, Ph's), 7.61 (s, 1H, C3H). Anal. ($C_{33}H_{35}N_5O_4$) C, H, N.

3-Acetamido-6-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (4). A mixture of **3** (8.0 g, 8.8 mmol) and sodium hydride (1.17 g of 50% mineral oil suspension, washed with pentane) in dimethylformamide (100 mL) was heated under nitrogen with stirring at 100 °C for 1.5 h. The mixture was concentrated in vacuo (40 °C, 0.5 torr) and the residue dissolved in methanol. The mixture was neutralized with acetic acid and concentrated in vacuo (40 °C, 0.5 torr). The resultant residue was purified by chromatography (4.5 x 45 cm column, 200 g silica gel, 230-400 mesh, toluene:ethyl acetate (7:3)) to give **4** as a sticky solid. The solid was slurried with ethyl ether and the resultant cream white solid collected, washed with ethyl ether and dried in vacuo (40 °C, 0.5 torr) for 24 h to give **4** (5.2 g, 65%, mp 124-128 °C): IR (KBr) 3458-2500, 3325, 3023, 2924, 2861, 1687, 1602, 1553, 1518, 1455, 1363, 1258, 1173, 1131, 1075, 836, 737 and 695 cm^{-1}; UV λ_{max} nm (ε): methanol, 232 (9,196), 267 (15,667); ^1H NMR ($CDCl_3$) δ 2.19 (s, 3H, CO$\underline{CH_3}$); 3.62 (d, 2H, 5'-H); 4.34-4.36 (m, 2H); 4.47 (d, 2H); 4.54 (s, 2H); 4.63 (d, 2H); 4.80-4.83 (t, 1H); 6.13 (d, 1H, 1'-H); 7.22-7.32 (m, 15H, Ph

H's); 7.39 (s, 1H, 3-H); 10.78-10.82 (d, 2H, NH's). Peaks at δ 10.78-10.82 exchange with D_2O. Anal. ($C_{32}H_{33}N_5O_5$) C, H, N.

3-Acetamido-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole hydrochloride (5). To a solution of **4** (428 mg, 0.75 mmol) in methanol (50 mL) was added 3% hydrogen chloride in methanol (15 mL) and 75 mg of 10% palladium on carbon. This mixture was hydrogenated (50 psi) for 16 h and then diluted with water (40 mL). The catalyst was removed by filtration through a bed of Celite and the filtrate concentrated in vacuo (first at 40 °C, 15 torr, then at 0.5 torr) to afford a white solid. The product was dissolved in 50 mL of hot methanol and then concentrated to 15 mL on a hot plate. On cooling the product crystallized as a fine white solid which was collected by filtration. The solid was washed with ether and dried in vacuo (40 °C, 100 °C, 0.5 torr) to give **5** (70 mg, 29%, mp 190 (sinter)-195 °C (dec)): IR (KBr) 3620-2207, 3304, 2945, 2861, 1714, 1630, 1602, 1553, 1419, 1370, 1230, 1173, 1103, 1089, 1047, 885 and 850 cm^{-1}; ^1H NMR (Me_2SO-*d* 6) δ 2.1 (s, 3, $COCH_3$), 3.38-3.44 (dd, 1H), 3.53-3.59 (dd, 1H), 3.83-3.88 (pseudo q, 1H), 4.12-4.15 (t, 1H) 4.61-4.65 (t, 1H), 5.85 (broad s, 6, OH's, NH_2, NH^+), 5.74 (d, 1, H-1'), 7.29 (d, 1, H-2), 11.59 (s, NH). Anal. ($C_{11}H_{16}N_5O_5Cl$) C, H, N, Cl.

3-Amino-6-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)pyrazolo-[3,4-*c*]pyrazole (5a). Compound **4** (1.0 g, 1.76 mmol) was dissolved in a solution of methanol (100 mL) containing 50 mg of dissolved sodium and the mixture heated at reflux for 24 h. The mixture was cooled to room temperature and neutralized with acetic acid and then concentrated in vacuo to give a viscous residue. This material was partitioned between ethyl acetate (75 mL) and water (75 mL). The aqueous layer was discarded and the organic layer dried over magnesium sulfate before being dissolved in a minimum amount of ethyl acetate. The solution of crude product was placed on a short silica gel column (3.5 x 20 cm, 30 g, 230-400 mesh, ethyl acetate) and the product fractions combined and concentrated in vacuo to give **5** as a hard glass (802 mg, 87%, hard glass): IR (thin layer smear) 3641-2860, 3057, 3029, 2924, 2868, 1630, 1425, 1499, 1454, 1370, 1244, 830

and 737 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.51-3.61 (m, 2H, 5'-H), 4.36-4.39 (m, 2H, 3' and 4'-H), 4.44 (s, 2H, $PhC\underline{H_2}$), 4.53 (s, 2H, $PhC\underline{H_2}$), 4.60 (s, 2H, $PhC\underline{H_2}$), 4.81 (t, 1H, 2'-H), 5.83 (d, 1H, H-1', $J_{1',2'}$= 4.9 Hz), 5.98 (s, 2H, NH_2), 7.09 (s, 1H, C_3H), 7.2-7.34 (m, 15 Ph H's), 10.2 (s, 1H, N_6H). Anal. ($C_{30}H_{31}N_5O_4$) C, H, N.

3-Amino-1-methyl-6-(2,3,5-tri-O-benzyl-ß-D-ribofuranosyl)-pyrazolo[3,4-*c*]pyrazole (5b). A mixture of **3a** (10.75 g, 18.87 mmol) and sodium hydride (1.13 g of 50% sodium hydride/mineral oil dispersion) in N,N,-dimethylformamide (125 mL) was heated at 100 °C under a blanket of argon. After 30 minutes the reaction mixture was cooled (ice bath) to room temperature and acetic acid (1.34 mL) was added to neutralize the reaction mixture. The reaction mixture was then concentrated (40 °C, 0.5 torr) to give a viscous residue. This residue was partitioned between ethyl acetate (250 mL) and water (75 mL). The organic layer was washed with saturated aqueous sodium chloride (75 mL) and then dried (MgSO4). The drying agent was removed by gravity filtration and the filtrate concentrated in vacuo to give **4a** (11.0 g). This material was dissolved in methanol (300 mL) and to this solution was added a solution of sodium methoxide in methanol (500 mg sodium in 100 mL of methanol). This amber solution was heated at reflux for 15 h and then cooled to room temperature with an ice bath. Acetic acid (1.25 mL) was added and the resultant mixture concentrated (40 °C, 10 torr) to give a viscous residue. This residue was partitioned between ethyl acetate (250 mL) and water (75 mL). The organic layer was dried ($MgSO_4$) and concentrated to give a foam. This material was dissolved in ethyl acetate (30 mL) and purified over a column (13 x 17 cm filter funnel, silica gel, 300 g, 70-230 mesh, ethyl acetate/methanol 10:1) and the product containing fractions concentrated (40 °C, 10 torr) to give **5b** as hard glass (6.93 g, 70%): IR (thin layer film) 3325, 2924, 2868, 1735, 1630, 1560, 1490, 1455, 1405, 1307, 1208 and 1159-990 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.32-3.50 (m, 5'-$\underline{CH_2}$, $N\underline{CH_3}$ and H_2O), 4.22-4.24 (m, 1H, 3' or 4'-H), 4.36-4.39 (m, 3H, $PhC\underline{H_2}$ and 3' or 4'-H), 4.53-4.70 (m, 4H, $PhC\underline{H_2}$), 4.89-4.92 (m, 1H, 2'-H), 7.16-7.32 (m, 16 H, Ph's and C_3H). Anal. ($C_{31}H_{33}N_5O_4$) C, H, N.

3-Amino-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (6). A mixture of **5a** (5.0 g, 9.5 mmol), ammonium formate (10 g, 158 mmol) and 10% Pd/C (5.75 g) in methanol (350 mL) was stirred at room temperature for 2 d until a single product was evident by thin layer chromatography (silica gel, chloroform:methanol (10:1)). The Pd/C was removed by filtration through a bed of Celite and the filtrate concentrated in vacuo affording a viscous residue. This material was dissolved in water (150 mL) and lyophilized. The residue obtained from the lyophilization was again dissolved in water (150 mL) and the lyophilization repeated. The residue obtained was dissolved in methanol (50 mL) and adsorbed onto anhydrous powdered sodium sulfate (30 g). Chromatography (silica gel, 230-400 mesh, chloroform:methanol (4:1)) gave the product as an amorphous residue. This residue was dissolved in boiling ethanol (100 mL), and the solution decolorized with activated charcoal. The charcoal was removed by filtration and the filtrate concentrated to 30 mL on a hot plate . On cooling, crystallization occurred, and the product was collected by filtration and dried at 65 °C at 0.5 torr for 12 h to give **6** (1.24 g, 50%, mp 200-203.5 °C): IR (KBr) 3578-2361, 3472, 2903, 2868, 2825, 1651, 1546, 1504, 1447, 1356, 1300, 1208, 1060, 997, 906, 800 and 723 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.43-3.45 (dd, 1H), 3.56-3.61 (dd, 1H), 3.85 (pseudo d, 1H), 4.12 (s, 1H), 4.56 (s, 1H), 5.01 (s, 1H, OH), 5.26 (s, 1H, OH), 5.54 (s, 1H, OH), 5.54 (d, 1H, H-1'), 6.02 (s, NH_2), 7.02 (d, 1H, $CH3$,) 10.22 (s, 1H, NH). UV λ_{max} nm (ε): methanol, 256 (18,265). Anal. ($C_9H_{13}N_5O_4$) C, H, N.

3-Amino-1-methyl-6-(ß-D-ribofuranosyl)pyrazolo[3,4-*c*]pyrazole (6a). To a solution of **5b** (1.3 g, 2.46 mmol) in ethanol (75 mL) and cyclohexene (12 mL) was added 20% $Pd(OH)_2$/C (1.3 g) and the mixture heated at reflux for 6 h. The catalyst was removed by filtering the reaction mixture through a bed of Celite and the filtrate concentrated (40 °C, 10 torr) to give a semisolid residue. This material was coevaporated two times with 50 mL portions of acetonitrile to give 720 mg of a yellow/white solid. This crude material was dissolved in methanol (30 mL) and adsorbed onto powdered sodium sulfate (15 g). This powder was placed on a silica gel column (3.5

x 35 cm, 60 g, 70-230 mesh) and the column eluted with acetonitrile/methanol (6:1). The product-containing fractions were pooled and concentrated in vacuo to give a white solid. The solid was slurried with acetone (10 mL) and the product collected by filtration and then washed with acetone and ether (15 mL). This material was dried (65 °C, 0.5 torr) for 8 h to give **6a** (285 mg, 43%, mp 205-208 °C): IR (KBr) 3557-2200, 1637, 1567, 1455, 1398, 1363, 1300, 1110, 1103, 948, 934 and 807 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.29-3.34 (m, 1H, 5'-H), 3.49-3.51 (m, 5'-H and H_2O), 3.85-3.9 (dd, 1H, 3'-H), 4.12-4.14 (dd, 1H, 4'-H), 4.57-4.60 (dd, 1H, 2'-H), 4.86 (t, 1H, 5'-OH), 5.10 (d, 1H, 2' or 3' OH), 5.35 (d, 1H, 2' or 3' OH), 5.75 (d, 1H, 1'-H, $J_{1'2'}$ = 4.0 Hz), 5.84 (s, 2H, NH_2), 7.16 (s, 1H, C3H); mass spectrum FAB^+, m/z 269 (22.65% of base pk), 270 (base pk), 138 (MH^+ - sugar, 7.8% of base pk). UV λ_{max} nm (ε): methanol, 251 (8475). Anal. ($C_{10}H_{15}N_5O_4$) C, H, N.

In Vitro Antiproliferative Studies. The *in vitro* cytotoxicity against L1210 was evaluated as described previously.[22] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice with 10% heat-inactivated (56°, 30 min) horse serum. Cell number was determined once or twice daily for 4 days, in the continuous presence of the test compound at 100 μM.

***In Vitro* Antiviral Evaluation.** (a) **Cells and Viruses.** Diploid human foreskin fibroblasts (HFF cells) were grown in minimal essential medium (MEM) with Earle's salts [MEM(E)] supplemented with 10% fetal bovine serum. Cells were passaged according to conventional procedures as detailed previously.[6] A plaque-purified isolate, P_O, of the Towne strain of HCMV was used and was a gift of Dr. M. F. Stinski, University of Iowa.

(b) **Assays for Antiviral Activity.** HCMV plaque reduction experiments were performed using monolayer cultures of HFF cells by a procedure similar to that described previously[6], with the exceptions that the virus inoculum (0.2 mL) contained approximately

50-100 plaque forming units (pfu) of HCMV and the compounds to be assayed were dissolved in the overlay medium.

(c) **Cytotoxicity Assays.** Cytotoxicity produced in HFF cells was estimated by visual scoring of cells not affected by virus infection in the plaque reduction assays described above. Drug-induced cytopathology was estimated at 30-fold magnification and scored on a zero to four plus basis on the day of staining for plaque enumeration.[6]

(d) **Data Analysis.** Dose-response relationships were constructed by linearly regressing the percent inhibition of parameters derived in the preceding sections against $\log_{10}$ of drug concentration. Fifty-percent inhibitory (IC_{50}) concentrations were calculated from the regression lines. Samples containing positive controls (ganciclovir for HCMV) were used in all assays. Results from sets of assays were rejected if inhibition by the positive control deviated from its mean response by more than 1.5 standard deviations.

Elemental Analysis.

	CALCULATED			FOUND		
	C	H	N	C	H	N
2	66.28	6.12	12.88	66.08	6.19	12.64
3	67.71	5.86	12.34	67.51	6.00	12.12
3a	68.14	6.07	12.04	67.98	6.02	11.92
4	67.71	5.86	12.34	67.52	5.73	12.23
5	39.58	4.38	20.98	39.67	4.75	20.73
5a	68.55	5.94	13.32	68.69	6.04	13.12
5b	68.99	6.16	12.98	68.89	6.05	12.82
6	42.35	5.09	27.44	42.26	5.14	27.16
6a	44.61	5.62	26.01	44.50	5.73	25.83

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