2.94–2.90 (m, 2 H), 1.77–1.68 (m, 5 H), 1.35 (m, 1 H); IR (KBr, cm^{-1}) 3450, 2500, 1500; MS m/z 329 (M^+, 39), 246 (16), 244 (15), 212 (61), 207 (100), 122 (22), 108 (34), 84 (38), 78 (20). Anal. ($C_{18}H_{21}ClFN_3S$) C, H, N.

Ethanol-Induced Gastric Lesions.[21] Charles River male albino rats (190–219 g) were deprived of food but not water for 18–24 h prior to use. Rats were dosed orally with drug or vehicle 1 h before ethanol administration (1 mL per rat po). One hour after ethanol administration, the rats were sacrificed by CO_2 asphyxiation. The stomachs were removed and kept moist with saline until the lesions were scored by an investigator unaware of the treatment groups (single blind). The grading of gastric ulcers took into account the size of the ulcers in mm^2 and the number of ulcers in each size category. The mean ulcer score for each treatment group was compared to the mean score of the vehicle-treated group, and the percent inhibition of ulcer formation was calculated.

Gastric Acid Secretion in the Pylorus-Ligated Rat.[22] One hour after oral administration of test compounds or vehicle, each rat was anesthetized with methohexital (30 mg/kg ip). A midline incision was made and the pylorus was ligated. The incision was closed with wound clips and the rat was allowed to recovery from anesthesia. Four hours after surgery, the rats were sacrificed by CO_2 asphyxiation. Gastric contents were collected, volumes were measured, and acid concentrations were determined by titration to pH 7.0 with 0.1 N NaOH with an automatic titrator. Total acid output (in microequivalents per 4 h) was determined by multiplying volume (in milliliters) by acid concentration (in microequivalents per milliliter).

Stress-Induced Gastric Ulcers.[23] Rats were immobilized in plastic restrainers and placed in a cold room at 4–5 °C for 3 h. Then, the rats were euthanized, and the gastric ulcers were graded as above. Test drugs or vehicle were administered po 30 min prior to immobilization.

Acute Lethality. The rats were orally dosed with drug and observed for lethality over a 7-day period.

Statistics. The mean score of each treatment group (±SEM) was compared with that of the control group and expressed as a percentage of inhibition. Statistical significance was determined by Dunnett's multiple comparison technique. Values for ED_{50} with 95% confidence limits were calculated by standard regression analysis of the dose–response data.

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Registry No. **4**, 39122-38-8; **5**, 13225-84-8; **6**, 123207-16-9; **9**, 123207-17-0; **10**, 123207-18-1; **11**, 108921-63-7; **12**, 123207-19-2; **13**, 123207-20-5; **14**, 123207-21-6; **15**, 119284-09-2; **16**, 119284-08-1; **17**, 119284-07-0; **18**, 119284-14-9; **19**, 119284-06-9; **20**, 119284-16-1; **21**, 119284-13-8; **22**, 119284-15-0; **23**, 119284-05-8; **24**, 119284-17-2; **25**, 123207-22-7; **26**, 123207-23-8; **27**, 123207-24-9; **28**, 123207-25-0; **29**, 87014-29-7; **30**, 119284-10-5; **31**, 119284-11-6; **32**, 119284-12-7; **34**, 123207-26-1; **35**, 123207-27-2; **36**, 123207-28-3; **37**, 123207-29-4; **38**, 123207-30-7; **39**, 123207-31-8; **40**, 123207-32-9; **41**, 123207-33-0; **42**, 123207-34-1; **43**, 123207-35-2; **44**, 123207-36-3; **45**, 123207-37-4; **46**, 123207-38-5; **47**, 123207-39-6; **48**, 119284-19-4; **49**, 123207-40-9; **50**, 123207-41-0; **51**, 123207-42-1; **52**, 123207-62-5; **52**·2HCl, 123207-43-2; **53**, 123207-44-3; **54**, 123207-45-4; **55**, 123207-46-5; **56**, 123207-63-6; **56**·2HCl, 123207-47-6; **57**, 123207-48-7; **58**, 123207-64-7; **58**·2HCl, 123207-49-8; **59**, 56643-75-5; **60**, 120107-85-9; **61**, 123207-50-1; **62**, 123207-60-3; **62**·HCl, 123207-51-2; **64**, 63878-73-9; **65**, 84832-00-8; **66**, 123207-52-3; **67**, 123207-53-4; **68**, 123207-54-5; *m*-$NO_2C_6H_4CHO$, 99-61-6; $C_6H_5NH_2$, 62-53-3; *m*-$CF_3CH_2OC_6H_4NH_2$, 123207-61-4; picolinic acid, 98-98-6; 3,5-difluoroaniline, 372-39-4; picolinic acid chloride hydrochloride, 39901-94-5; 2-fluoro-5-nitrobenzaldehyde, 27996-87-8; *N*-(3,5-difluorophenyl)-2-pyridinecarboxamide, 123207-55-6; lead cyanamide, 20890-10-2; morpholine, 110-91-8; 3-[(4-morpholinyl)methyl]nitrobenzene hydrochloride, 123207-56-7; 3-[(4-morpholinyl)methyl]nitrobenzene, 123207-57-8; *N*-sodioimidazole, 5587-42-8; *N*-[4-fluoro-3-(1*H*-imidazol-1-ylmethyl)phenyl]-2-pyridinecarboxamide, 123207-58-9; piperidine, 110-89-4; *N*-[4-fluoro-3-(piperidinylmethyl)phenyl]-2-pyridinecarboxamide, 123207-59-0; picoline, 1333-41-1; 2-methylpyrazine, 109-08-0; 2-aminopyridine, 504-29-0; ethyl 2-fluorobenzoate, 443-26-5; ethyl 2-fluoro-5-nitrobenzoate, 367-79-3.

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Growth Inhibition and Induction of Cellular Differentiation of Human Myeloid Leukemia Cells in Culture by Carbamoyl Congeners of Ribavirin[1]

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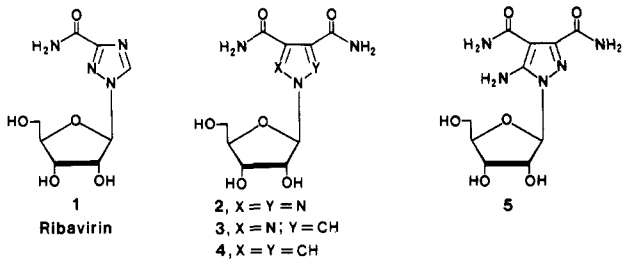
A series of 1,2,3-triazole (**2**), pyrazole (**3** and **5**), and pyrrole (**4**) ribonucleosides with two adjacent carbamoyl groups have been synthesized and evaluated for cell growth inhibition and induction of cellular differentiation of HL-60 cells in culture. Glycosylation of the TMS derivatives of dimethyl 1,2,3-triazole-4,5-dicarboxylate (**6**) and diethyl pyrazole-3,4-dicarboxylate (**7**) with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**) in the presence of TMS triflate gave predominantly the β-nucleosides **9** and **14**, respectively. Ammonolysis of **9** and **14** furnished 2-β-D-ribofuranosyl-1,2,3-triazole-4,5-dicarboxamide (**2**) and 1-β-D-ribofuranosylpyrazole-3,4-dicarboxamide (**3**), respectively. Stereoselective ring annulation of 1-deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose (**16**) with tetracyanoethylene (**15**) gave 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-3,4-dicarbonitrile (**17**). Deisopropylidenation of **17**, followed by oxidative hydrolysis of the reaction product (**18**), gave the 5-amino derivative of **3** (**5**). Stereospecific glycosylation of the sodium salt of preformed diethyl pyrrole-3,4-dicarboxylate (**22**) with 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose (**23**) was accomplished to furnish blocked nucleoside **24**, which on ammonolysis and deisopropylidenation gave 1-β-D-ribofuranosylpyrrole-3,4-dicarboxamide (**4**). The structures of **2** and **3** were assigned by single-crystal X-ray diffraction studies, which showed extensive inter- and intramolecular hydrogen bonding. Nucleosides **2–5** are devoid of significant cytotoxic properties against L1210 and WI-L2 leukemia cells in culture. However, these compounds were found to be inducers of cellular differentiation of HL-60 cells in the range of 30–60 μM and were comparable to ribavirin in this regard.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, **1**),[2] a synthetic azole nucleoside analogue of guanosine[3,4] synthesized and reported from our laboratory,[5] is singular in its broad-spectrum antiviral activity.[6,7]

0022-2623/90/1833-0336$02.50/0

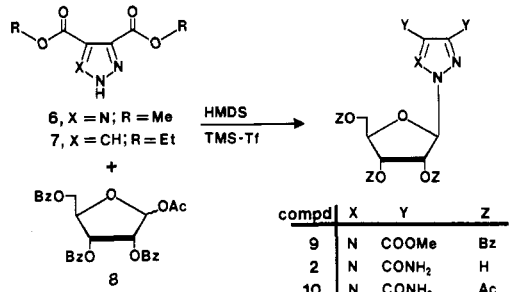
Chart I



Ribavirin has been developed clinically[8] and approved by the FDA for human use in an aerosol form for the treatment of lower respiratory disease caused by respiratory syncytial virus.[9-12] Ribavirin also exhibited weak antitumor effects against L1210 leukemia.[13] Recently ribavirin has been shown to induce the differentiation of human promyelocytic leukemia HL-60,[14] as well as murine erythroleukemia MEL[15] cells, in culture. The 5′-monophosphate of ribavirin, a form produced intracellularly, is a potent competitive inhibitor of inosine monophosphate dehydrogenase (IMPD),[16-18] which significantly reduces levels of GTP in cells.[19]

Our analysis of various antiviral ribonucleosides using a computer-aided receptor-modeling procedure[20] demonstrated the paramount importance of a hydrogen-bonding carbamoyl group[21] in drug–receptor interactions. This observation was further supported by the loss or decrease in antiviral potency of ribavirin when the carbamoyl functionality was altered and/or replaced.[22,23] Thus, the apparent importance of the hydrogen-bonding carbamoyl group prompted us to examine the effect that dual carbamoyl groups in certain ribavirin congeners might have on antitumor properties, as well as on the induction of differentiation in HL-60 cells. This added hydrogen-bonding potential might allow them to recognize the key enzymes in a selective way by either of the two carbamoyl groups. The enhanced hydrogen-bonding characteristics within and between the molecules[24] could contribute significantly in organizing the drug receptor sites in their native three-dimensional structures. The present work, therefore, describes the synthesis of certain 1,2,3-triazole (**2**), pyrazole (**3** and **5**), as well as pyrrole (**4**) ribonucleosides (Chart I) containing two adjacent carbamoyl groups and their cell growth inhibition and induction of cellular differentiation of human myeloid leukemia cells HL-60 in culture.

Scheme I



compd	X	Y	Z
9	N	COOMe	Bz
2	N	$CONH_2$	H
10	N	$CONH_2$	Ac
11	N	CN	Ac
12	N	$C(NOH)NH_2$	H
13	N	$C(S)NH_2$	H
14	CH	COOEt	Bz
3	CH	$CONH_2$	H

Results and Discussion

Chemistry. The cycloaddition of various glycosyl azides with substituted acetylenes has provided a route to the preparation of 1(or 3)-glycosyl-1,2,3-triazoles.[25-38] How-

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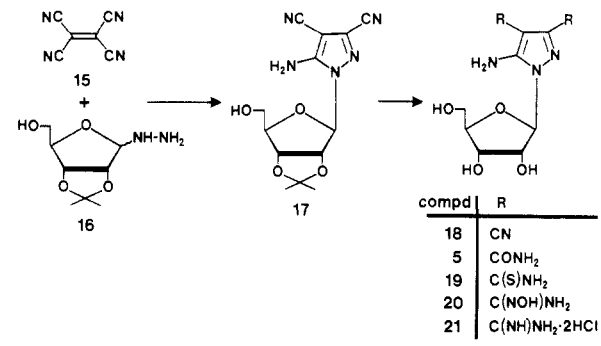
ever, the isomeric 2-glycosyl-1,2,3-triazoles are not accessible by the ring-closure of the azido sugars. Although direct glycosylation of ethyl 1,2,3-triazole-4-carboxylate with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride in the presence of mercuric cyanide has been reported[39] to give only the N-1 glycosylated product, acid catalyzed fusion reaction[40-42] or the glycosylation of the trimethylsilyl derivative of the triazole with protected halogenose[41] was found to furnish a mixture of isomeric N-1 and N-2 glycosylated 1,2,3-triazoles. In view of these findings, the synthesis of the desired N-2 glycosylated 4,5-disubstituted 1,2,3-triazoles seemed propitious by the Lewis acid catalyzed glycosylation procedure.[43] The starting aglycon dimethyl 1,2,3-triazole-4,5-dicarboxylate (**6**) required for the glycosylation studies was readily prepared as reported[44] and silylated with hexamethyldisilazane in the presence of $(NH_4)_2SO_4$ to give the silylated product. Condensation of the silylated **6** with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**) in anhydrous CH_3CN in the presence of 1.4 molar equiv of the Lewis acid catalyst trimethylsilyl trifluoromethanesulfonate (TMS triflate) at ambient temperature gave a nucleoside product, which after purification was identified as dimethyl 2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxylate (**9**) (Scheme I). The isolated yield of crystalline **9** was 94%. Careful investigation of the reaction mixture furnished chromatographic evidence of the formation of another nucleoside material in less than 2% yield; presumably the positional N-1 isomer. No attempt was made to isolate this minor product. Ammonolysis of **9** with $MeOH/NH_3$ (saturated at 0 °C) at 95 °C resulted in the debenzoylation of the carbohydrate moiety with concomitant conversion of the ester functions to amides to give one of the target nucleosides 2-β-D-ribofuranosyl-1,2,3-triazole-4,5-dicarboxamide (**2**) in excellent yield. The structural assignment of **2** was made on the basis of single-crystal X-ray diffraction studies, which showed very extensive intramolecular hydrogen-bonding in the solid state.

Acetylation of **2** with acetic anhydride in the presence of 4-(dimethylamino)pyridine (DMAP) at ambient temperature gave 2-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide (**10**). Subsequent dehydration of the carboxamide groups of **10** with phosgene (20% solution in toluene) at 0 °C provided 2-(2,3,5-tri-*O*-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarbonitrile (**11**). The yields of **10** and **11** were consistently good. Treatment of **11** with free hydroxylamine in absolute EtOH gave a

Scheme II



compd	R
18	CN
5	$CONH_2$
19	$C(S)NH_2$
20	$C(NOH)NH_2$
21	$C(NH)NH_2 \cdot 2HCl$

93% yield of 2-β-D-ribofuranosyl-1,2,3-triazole-4,5-dicarboxamidoxime (**12**), whereas reaction of **11** with anhydrous H_2S in the presence of triethylamine afforded the corresponding 4,5-bisthiocarboxamide derivative (**13**).

Although a number of pyrazole nucleosides in which the carbohydrate moiety is attached to a pyrazole nitrogen have been described,[45-49] the pyrazole nucleosides structurally related to ribavirin are reported[49-51] to have been prepared by the acid catalyzed fusion procedure. However, the synthesis of 1-β-D-ribofuranosylpyrazole-3,4-dicarboxamide (**3**) has not been reported. In the present study, the synthesis of **3** was accomplished by the trimethylsilyl procedure. Thus, glycosylation of the trimethylsilyl derivative of diethyl pyrazole-3,4-dicarboxylate[52] (**7**) with **8** in the presence of 1.4 molar equiv of TMS triflate gave an 83% yield of diethyl 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazole-3,4-dicarboxylate (**14**) (Scheme I). No detectable amount of the N-2 glycosyl isomer was formed under these conditions. Treatment of **14** with $MeOH/NH_3$ resulted in crystalline **3**, which was isolated in an 82% yield. The structure of **3** was assigned by single-crystal X-ray diffraction analysis.

A simple and straightforward synthesis of the 5-amino-substituted derivative of **3** was accomplished by the ring-annulation procedure (Scheme II). 1-Deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose[53] (**16**) was reacted regio- and stereoselectively with tetracyanoethylene (**15**) in EtOH to give a single nucleoside product, which was isolated and characterized as 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)pyrazole-3,4-dicarbonitrile (**17**). Similar ring-annulation of methylhydrazine with tetracyanoethylene has been reported to give 5-amino-1-methylpyrazole-3,4-dicarbonitrile.[54] The initial step involved in this reaction was the replacement of a cyano group of **15** to give a (tricyanovinyl)hydrazine intermediate, which eventually cyclized to furnish **17**. The ^1H NMR spectrum of **17** revealed a $\Delta\delta$ value of 0.17 ppm for the isopropylidene methyl signals indicating the β-configura-

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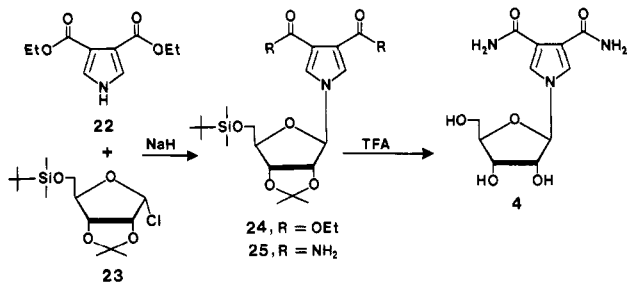
Scheme III



tion.[55] Deisopropylidenation of **17** with 90% aqueous trifluoroacetic acid at room temperature gave a 95% yield of crystalline 5-amino-1-β-D-ribofuranosylpyrazole-3,4-dicarbonitrile (**18**). The fact that compound **18** was indeed the C-5 amino derivative and not the 3-amino isomer was established by comparison of the ultraviolet absorption spectrum of **18** (λ_{max} 275 nm in pH 1, 7, and 11) with that of the model methyl compound 5-amino-1-methylpyrazole-3,4-dicarbonitrile[56] (λ_{max} 275 nm in pH 1, 7, and 11), which are identical. This assignment was further corroborated by ^{1}H NMR studies with **18** in which the C-5 NH_2 resonance appeared at δ 7.58 (in Me_2SO-d_6) and was comparable to the C-5 NH_2 resonance of 5-amino-1-methylpyrazole-3,4-dicarbonitrile (δ 7.06)[56] versus that of the 3-amino isomer (δ 6.14).[56] Treatment of an ammoniacal solution of **18** with H_2O_2 at room temperature for 12 h, followed by purification of the reaction product by silica gel column chromatography, gave yet another target compound, 5-amino-1-β-D-ribofuranosylpyrazole-3,4-dicarboxamide (**5**), in good yield. When compound **18** was allowed to react with anhydrous H_2S in pyridine containing triethylamine at 100 °C, 5-amino-1-β-D-ribofuranosylpyrazole-3,4-dithiocarboxamide (**19**) was formed, which was isolated as crystalline material of mp 164–165 °C. Similarly, treatment of **18** with free NH_2OH in absolute EtOH gave 5-amino-1-β-D-ribofuranosylpyrazole-3,4-dicarboxamidoxime (**20**), which was readily reduced by hydrogenation in the presence of Raney nickel and NH_4Cl to furnish the corresponding 3,4-dicarboxamidine derivative (**21**), isolated as the dihydrochloride salt.

Recently we reported a convenient procedure for the direct attachment of a glycon moiety to a preformed fully aromatic pyrrole derivative by the stereospecific sodium salt glycosylation method.[57–60] Use of this simple procedure for the preparation of the target 1-β-D-ribofuranosylpyrrole-3,4-dicarboxamide (**4**) has now been found to be very fruitful. In the present work, we selected diethyl pyrrole-3,4-dicarboxylate[61,62] (**22**) as the aglycon for the glycosylation studies (Scheme III). The sodium salt of **22**, generated in situ by NaH in anhydrous CH_3CN, was treated with 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose[63] (**23**) at room temperature. A smooth reaction was observed, and the desired diethyl 1-[2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]pyrrole-3,4-dicarboxylate (**24**) was isolated after column chromatography in 52% yield. Compound **24** was the only nucleoside product which could be isolated by chromatography procedures. Treatment of **24** with $MeOH/NH_3$ in a pressure vessel at 130 °C for 24 h converted the ester functions into a dicarboxamide (**25**), which on deisopropylidenation with 90% aqueous trifluoroacetic acid at room temperature furnished the target compound **4** as crystalline material. The anomeric configuration of the isolated **4** was assigned as β on the basis of ^{1}H NMR studies with **25**, which revealed the difference in proton chemical shifts between the methyl signals of the isopropylidene group to be 0.22 ppm, a difference characteristic of β-configuration.[55]

During the course of ^{1}H NMR studies of these target azole dicarboxamide nucleoside derivatives **2–5**, we observed that these nucleosides exhibit intramolecular hydrogen bonding and that the chemical shifts of carbamoyl protons show substantial change when engaged in such hydrogen-bonding. The salient features of the proton spectra are (1) the significant difference in the chemical shift (0.70–2.66 ppm) of the two $CONH_2$ protons indicates that one of the amide protons is hydrogen bonded with the oxygen atom of the adjacent $CONH_2$ group,[64] the feature which was corroborated by single-crystal X-ray diffraction studies, and (2) compounds **2** and **4** reveal two broad singlets for the amide protons, indicating intramolecular hydrogen bonding,[64] whereas compounds **3** and **5** exhibit

Table I. Crystal Data for Compounds **2** and **3**

	2	**3**
formula	$C_9H_{13}N_5O_6 \cdot 2H_2O$	$C_{10}H_{14}N_4O_6 \cdot 0.5H_2O$
molecular weight	323.26	295.25
crystal system	orthorhombic	monoclinic
space group	$P2_12_12_1$	$C2$
a, Å	6.6968 (6)	18.477 (3)
b, Å	9.8426 (12)	9.7716 (15)
c, Å	20.899 (4)	6.8821 (8)
β, deg	90.0	95.483 (12)
final R	0.031	0.040

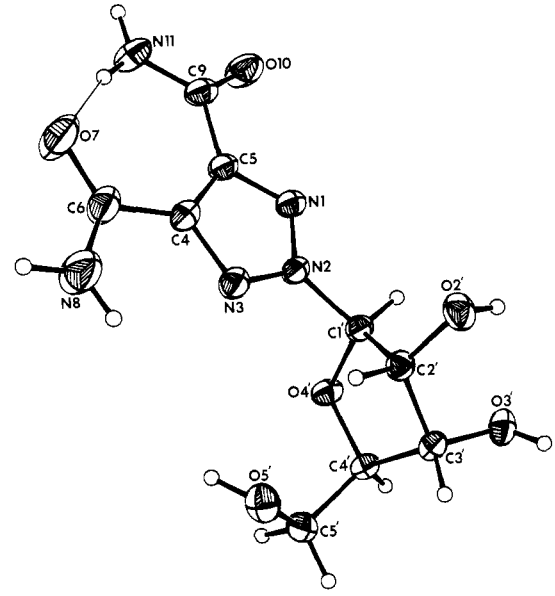


Figure 1. ORTEPII drawing of compound **2**.

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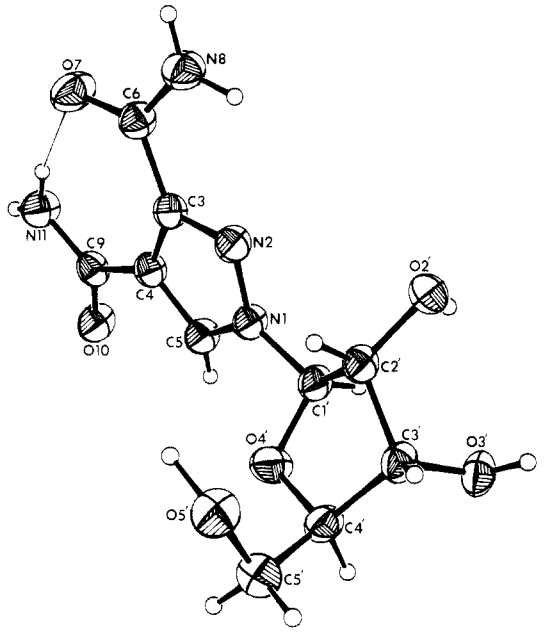

Figure 2. ORTEPII drawing of compound 3.

four broad singlets for the two $CONH_2$ groups, one for each of its N*H* proton. At this time we made no attempt to assign the individual amide protons in the ^1H NMR spectra.

Single-Crystal X-ray Diffraction Analysis of Compounds 2 and 3. Crystal data for compounds **2** and **3** are given in Table I. Compound **2** crystallized as the dihydrate from an 8:2 ethanol/water solution in the form of flat, colorless prisms. Compound **3** crystallized as the hemihydrate from a slowly cooled hot 1-propanol solution containing a trace of water. Crystals grew as clusters of needles.

Figures 1 and 2 are ORTEPII[65] drawings of compounds **2** and **3**, respectively. The carboxamide groups are not coplanar with the pyrazole ring in **3**. The groups at C-3 and C-4 are rotated 11.49 (11)° and 6.53 (11)° out of the pyrazole plane in opposite directions. Thus, the intramolecular hydrogen bond between O-7 and N-11 of 1.91 (4) Å [d(O-7···N-11) = 2.742 (3) Å] is entirely on one side of the plane. In **2**, on the other hand, the carboxamide group at C-5 is rotated 6.57 (7)° out of the triazole plane whereas the group at C-4 makes a dihedral angle with the plane of only 1.29 (6)°. In this case, O-7 and N-11 are on opposite sides of the heterocyclic ring, forming an intramolecular hydrogen bond of 1.87 (3) Å [d(O-7···N-11) =2.728 (2) Å].

The sugar conformations are very similar. Compound **3** is C-2′-endo/C-1′-exo with P= 146.6° and τ_m = 43.0° while **2** is C-2′-endo with P = 166.4° and τ_m = 39.4°.[66] The side chains of each are in the gauche$^-$–gauche$^+$ orientation. The glycosidic torsion angles, however, are very distinct: O-4′–C-1′–N-1–N-2 = 124.2 (2)° for **3** and O-4′–C-1′–N-2–N-1 = 71.22 (14)° for **2**. The glycosidic bonds are identical: 1.458 (3) Å [C-1′–N-1 in **3**] and 1.4593 (15) Å [C-1′–N-2 in **2**].

The extensive intermolecular hydrogen bonding in both structures is illustrated in Figure 3. Each carbamoyl oxygen acts as an acceptor of two protons: every amide proton participates in hydrogen bonding. In contrast, the carbonyl oxygen in ribavirin is an acceptor of only one proton and the amide nitrogen donates only one proton. Thus, it is clear that the possibilities for hydrogen bonding have been augmented 4-fold in the solid state by the addition of the second carbamoyl group. A more extensive

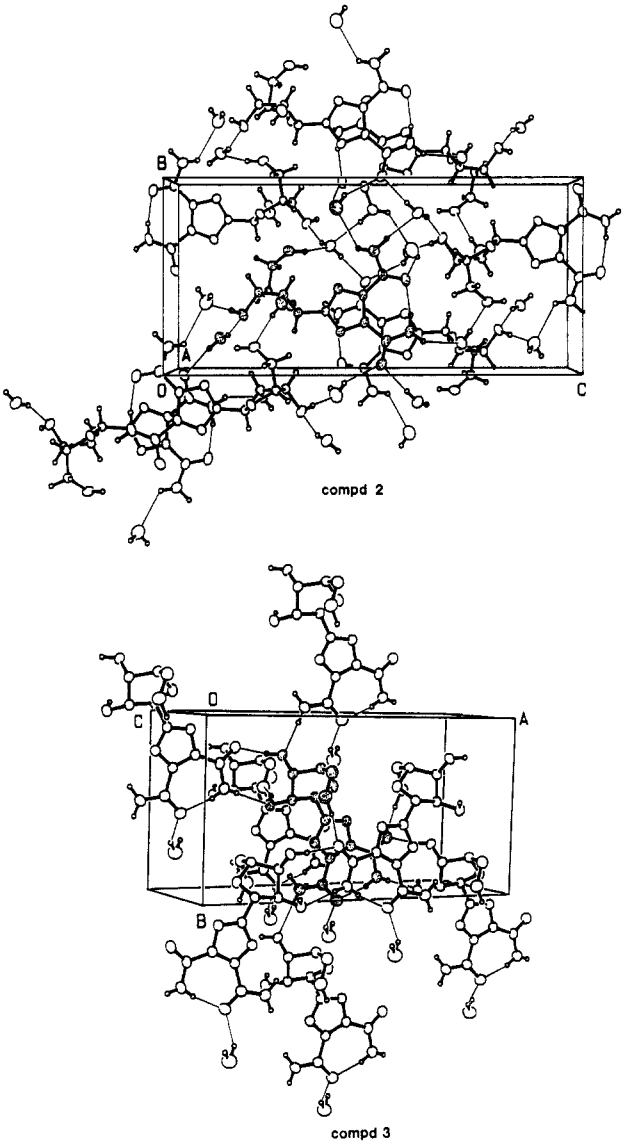

Figure 3. Molecular packing diagram of compounds **2** and **3** showing hydrogen-bonding networks in each (hydrogen bonds are drawn as thin lines).

Table II. Cell Growth Inhibition (ID_{50})[a] in Culture by Certain Congeners of Ribavirin

compd	L1210[b]	WI-L2[c]	K562[d]	HL-60[d]
1 (ribavirin)	36	42	50	67
2	>100	41	26	32
3	>100	100	160	58
4	48	33	32	36
5	44	46	51	39
12	>200	>200	>200	>200
13	>200	>200	>200	>200
19	>200	154	>200	200
20	>200	>200	>200	>200
21	200	179	>200	130

[a] The inhibitory dose (ID_{50}) is the micromolar concentration of the compound that inhibits tumor cell growth by 50% as compared to that of the untreated controls. [b] Murine leukemia cell line. [c] Human B-lymphoblast cell line. [d] Human myeloid leukemia cell lines.

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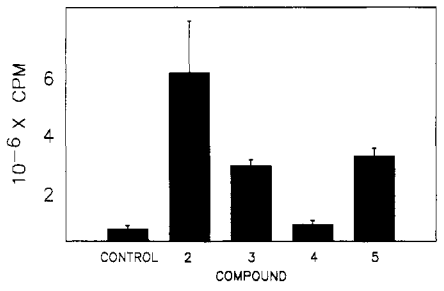

Figure 4. Chemiluminescence of reduced luminol measured in cultures of HL-60 cells following treatment with compounds and stimulation with phorbol ester. Cells were incubated in growth medium alone (control) or with 30 μM compound **2**, 60 μM compound **3**, 40 μM compound **4**, or 60 μM compound **5** for 4 days at 37 °C. Chemiluminescence was stimulated with tetradecanoylphorbol ester as described in the Experimental Section. The intensity of chemiluminescence (cpm) varies with time (cf. Figure 5). The maximum values of the intensity of chemiluminescence are shown here. The values shown are the mean and standard deviation for triplicate determinations in one experiment which is representative of three separate experiments that gave identical results.

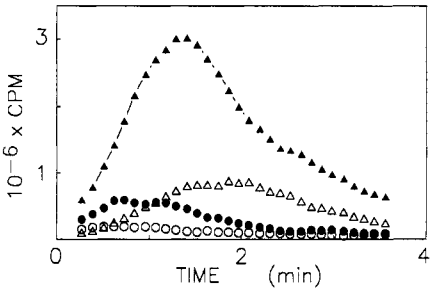

Figure 5. Chemiluminscence of HL-60 cells following incubation with compound **2**, GM-CSF-containing medium, and fMLP. Cells were incubated in growth medium with or without 30 μM compound **2** for 4 days, then cells were resuspended in a solution with or without 5% GCT-conditioned medium and incubated for 2 h, and chemiluminescence was measured after stimulation with fMLP as described in the Experimental Section: (○) control cells incubated without compound and without GCT medium, (●) cells incubated without compound and with GCT medium, (△) cells incubated with compound **2** and without GCT medium, (▲) cells incubated with compound **2** and with GCT-conditioned medium. The data shown are representative of similar results from three separate experiments.

treatment of the crystal structures will be reported elsewhere.[67]

Growth Inhibition and Induction of Cellular Differentiation of HL-60 Cells in Culture. The growth of HL-60 leukemia cells was inhibited to 50% of the control growth by compounds **2**–**5** at concentrations in the 30–60 μM range (Table II). Thus, the compounds exhibited relatively low cytotoxic activity in comparison with therapeutically useful, cytotoxic agents, such as cytosine arabinoside and 5-fluorouracil, which gave ID_{50} values of 0.002 and 0.01 μM, respectively. Alterations of the carbamoyl group greatly reduced the growth-inhibitory properties. Compounds **2**, **3**, and **5** induced the in vitro differentiation of myeloid leukemia cells HL-60. Differentiation of the HL-60 promyelocytic leukemia cell line was measured by the increased production of superoxide in response to a stimulus.[68,69] Experimentally, superoxide production was measured by the reduction of the dye luminol by superoxide and the subsequent chemiluminescence of reduced luminol. Therefore, the degree of differentiation of HL-60 cells was directly proportional to their ability to cause chemiluminescence of the reduced luminol in response to a specific stimulus. Untreated HL-60 cells produced only a small amount of superoxide when incubated with a phorbol ester which activates protein kinase C.[68] After HL-60 cells were incubated with compounds **2**, **3**, and **5**, the cells responded to the phorbol ester stimulus with increased superoxide production (Figure 4). Furthermore, incubation with compounds **2**, **3**, and **5** increased the response of HL-60 cells to GM-CSF, a cytokine in GCT cell conditioned medium,[70] and subsequently enabled the cells to produce superoxide in response to a stimulus by fMLP, a compound derived from the bacterial cell wall (Figure 5). From a therapeutic point of view, cell differentiating agents might cause rapidly proliferating, malignant cancer cells to develop into mature, nonproliferating forms, thus arresting tumor growth.[71] Since differentiating agents act by a mechanism that does not involve cell killing, they would produce fewer negative side effects compared to cytotoxic anticancer agents. In light of the therapeutic benefit of tiazofurin [2-β-D-ribofuranosylthiazole-4-carboxamide] for the differentiation of myeloid leukemia,[72] the compounds described here may be useful for the treatment of that disease.

In summary, a facile synthesis of 1,2,3-triazole (**2**), pyrazole (**3** and **5**), and pyrrole (**4**), ribonucleosides containing two carbamoyl groups has been developed. These nucleosides showed extensive inter- and intramolecular hydrogen bonding in solution as well as in the solid state. Although nucleosides **2**–**5** exhibited no significant cytotoxic properties against L1210 and WI-L2 leukemia cells in culture, these compounds were found to be inducers of cellular differentiation of HL-60 cells in the range of 30–60 μM.

Experimental Section

General Procedures. Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. Silica gel (E. Merck, 230–400 mesh) was used for flash chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light, and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR, in KBr) spectra were recorded with a Perkin-Elmer 1420 spectrophotometer and ultraviolet (UV, sh = shoulder) spectra were recorded on a Beckman DU-50 spectrophotometer. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard (key: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad). The presence of water as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy.

Dimethyl 2-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxylate (9). A mixture of dry dimethyl 1,2,3-triazole-4,5-dicarboxylate[44] (**6**, 10.15 g, 54.5 mmol), hexamethyldisilazane (HMDS, 150 mL), and $(NH_4)_2SO_4$ (0.2 g) was

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heated under reflux (oil-bath temperature 140 °C) for 8 h with the exclusion of moisture. Excess HMDS was removed by distillation in vacuo to provide the Me_3Si derivative of **6**, which was dissolved in anhydrous CH_3CN (500 mL). To the above clear solution was added 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**8**, 27.7 g, 55 mmol) and the mixture was stirred for 10 min before trimethylsilyl trifluoromethanesulfonate (14.5 mL, 75 mmol) was added. The reaction mixture was stirred for 12 h at ambient temperature. The CH_3CN was evaporated and the residue was dissolved in CH_2Cl_2 (500 mL). The organic layer was washed successively with aqueous saturated $NaHCO_3$ solution (3 × 100 mL), saturated NaCl solution (3 × 100 mL), and water (3 × 50 mL) and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was crystallized from MeOH to furnish 32.2 g (94%) of **9**: mp 123 °C; UV (MeOH) λ_{max} nm ($\epsilon \times 10^{-3}$) 230 (6.7); ^{1}H NMR (Me_2SO-d_6) δ 3.88 (s, 6 H, 2 COOCH_3), 4.65 (m, 2 H, C$_{5'}CH_2$), 5.01 (m, 1 H, C$_{4'}H$), 6.10 (m, 1 H, C$_{3'}H$), 6.32 (m, 1 H, C$_{2'}H$), 6.88 (d, 1 H, $J_{1',2'}$ = 2.7 Hz, C$_{1'}H$), 7.45–7.95 (3 m, 15 H, 3 PhH). Anal. ($C_{32}H_{27}N_3O_{11}$) C, H, N.

2-β-D-Ribofuranosyl-1,2,3-triazole-4,5-dicarboxamide (2). Compound **9** (30 g, 47.7 mmol) and MeOH/NH_3 (MeOH saturated with anhydrous NH_3 at 0 °C, 250 mL) were placed in a steel reaction vessel (500 mL). The vessel was three-quarters submerged in an oil bath and heated at 95 °C for 16 h. The reaction vessel was cooled and opened carefully, and the NH_3 was allowed to evaporate at room temperature. The MeOH was evaporated to dryness and the residue was triturated with hot toluene (3 × 100 mL) and filtered, and the brown residue crystallized from aqueous EtOH (95%) to furnish 11.1 g (81%) of **2**: mp 213 °C; UV (pH 1, 7, and 11) λ_{max} nm ($\epsilon \times 10^{-3}$) 220 (9.7); ^{1}H NMR (Me_2SO-d_6) δ 3.51 (m, 2 H, C$_{5'}CH_2$), 3.97, 4.26, and 4.53 (3 m, 3 H, C$_{4',3',2'}H$), 4.78 (t, 1 H, C$_{5'}OH$), 5.27 and 5.67 (2 d, 2 H, C$_{2',3'}OH$), 5.90 (d, 1 H, $J_{1',2'}$ = 4.3 Hz, C$_{1'}H$), 8.0 and 9.07 (2 br s, 4 H, 2 CONH_2). Anal. ($C_9H_{13}N_5O_6$) C, H, N.

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarboxamide (10). A mixture of **2** (6.2 g, 21.6 mmol), acetic anhydride (100 mL), and 4-(dimethylamino)pyridine (DMAP, 0.2 g) was stirred for 15 h at room temperature. Acetic anhydride was removed under reduced pressure and the residue was dissolved in EtOAc (500 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ (100 mL), followed by water (2 × 50 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column (4 × 40 cm) chromatography using $CHCl_3$/MeOH (6:1, v/v) as the eluent. The homogeneous fractions were pooled and evaporated and the residue was crystallized from EtOH to yield 6.4 g (71%) of **10**: mp 174 °C; UV (MeOH) λ_{max} nm ($\epsilon \times 10^{-3}$) 222 (8.1); ^{1}H NMR (Me_2SO-d_6) δ 1.97, 2.10, and 2.11 (3 s, 9 H, 3 COCH_3), 4.25 (m, 2 H, C$_{5'}CH_2$), 4.48 (m, 1 H, C$_{4'}H$), 5.67 and 5.83 (2 m, 2 H, C$_{3',2'}H$), 6.39 (d, 1 H, $J_{1',2'}$ = 2.4 Hz, C$_{1'}H$), 8.05 and 9.06 (2 br s, 4 H, 2 CONH_2). Anal. ($C_{15}H_{19}N_5O_9$) C, H, N.

2-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,3-triazole-4,5-dicarbonitrile (11). To a cold (−5 °C) solution of **10** (4.7 g, 11.4 mmol) in anhydrous CH_2Cl_2 (75 mL) and pyridine (10 mL) was added a solution of 20% phosgene in toluene (14.7 mL, 30 mmol) dropwise with stirring. After the addition was complete (30 min), the reaction mixture was stirred at 0 °C for 2 h. The resulting brown solution was poured onto crushed ice (200 g) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed successively with cold 1 N HCl (100 mL), 10% aqueous $NaHCO_3$ (2 × 100 mL), and water (2 × 50 mL) and then dried (Na_2SO_4) and evaporated to dryness. The residue on crystallization from EtOH furnished 3.9 g (90%) of **11**: mp 121 °C; IR ν_{max} 2250 (weak, C≡N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.99, 2.07, and 2.09 (3 s, 9 H, 3 COCH_3), 4.25 (m, 2 H, C$_{5'}CH_2$), 4.55 (m, 1 H, C$_{4'}H$), 5.54 and 5.78 (2 m, 2 H, C$_{3',2'}H$), 6.64 (d, 1 H, $J_{1',2'}$ = 2.5 Hz, C$_{1'}H$). Anal. ($C_{15}H_{15}N_5O_7$) C, H, N.

2-β-D-Ribofuranosyl-1,2,3-triazole-4,5-dicarboxamidoxime (12). A solution of **11** (1.0 g, 2.65 mmol) and free NH_2OH (2 g) in absolute EtOH (100 mL) was stirred at ambient temperature for 16 h. The precipitated solid was collected by filtration, washed with EtOH (25 mL) followed by diethyl ether (50 mL), and dried to provide 0.78 g (93%) of **12**: mp 155 °C, foams; (202 °C, dec); UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 230 (7.2); (pH 7) 214 (7.7), 252 (6.3); (pH 11) 265 (8.3); ^{1}H NMR (Me_2SO-d_6) δ 3.50 (m, 2 H, C$_{5'}CH_2$), 3.95 (m, 1 H, C$_{4'}H$), 4.23 and 4.50 (2 m, 2 H, C$_{3',2'}H$), 4.78 (t, 1 H, C$_{5'}OH$), 5.26 and 5.63 (2 br s, 2 H, C$_{2',3'}OH$), 5.84 (d, 1 H, $J_{1',2'}$ = 4.3 Hz, C$_{1'}H$), 6.16 (br s, 4 H, 2 NH_2), 9.89 (br s, 2 H, 2 NOH). Anal. ($C_9H_{15}N_7O_6$) C, H, N.

2-β-D-Ribofuranosyl-1,2,3-triazole-4,5-dithiocarboxamide (13). To a cold (−20 °C) solution of **11** (0.37 g, 1 mmol) in absolute EtOH (25 mL) was added triethylamine (2 mL). Anhydrous H_2S was bubbled through the solution for 2 h, and the solution was stirred at ambient temperature for 2 h before excess H_2S was purged off with N_2. The reaction mixture was evaporated to dryness. The residue was coevaporated with EtOH (4 × 50 mL), followed by toluene (3 × 25 mL). The anhydrous residue was dissolved in dry MeOH (20 mL) and to this was added 1 N NaOMe in MeOH solution (5 mL). The mixture was stirred at ambient temperature for 1 h, neutralized with Dowex-50 (H^+) resin, and filtered, and the filtrate was evaporated to dryness. The residue was purified on a flash silica gel column (2.5 × 25 cm) using $CHCl_3$/MeOH (6:1, v/v) as the eluent, and the homogeneous fractions were pooled and evaporated to furnish 0.19 g (59%) of **13** as an amorphous solid: mp 80 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.57 (m, 2 H, C$_{5'}CH_2$), 4.01, 4.24 and 4.52 (3 m, 3 H, C$_{4',3',2'}H$), 4.77 (t, 1 H, C$_{5'}OH$), 5.29 and 5.64 (2 d, 2 H, C$_{2',3'}OH$), 5.82 (d, 1 H, $J_{1',2'}$ = 4.3 Hz, C$_{1'}H$), 9.69 and 10.05 (2 br s, 4 H, 2 C(S)NH_2). Anal. ($C_9H_{13}N_5O_4S_2$) C, H, N, S.

Diethyl 1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazole-3,4-dicarboxylate (14). In a similar manner as for **9**, glycosylation of diethyl pyrazole-3,4-dicarboxylate[52] (**7**, 3.12 g, 14.7 mmol) with **8** (8.9 g, 17.6 mmol) gave syrupy **14** (8.0 g, 83%): UV λ_{max} ($\epsilon \times 10^{-3}$) (MeOH) 230 (44.7); ^{1}H NMR ($CDCl_3$) δ 1.20 and 1.31 (2 t, 6 H, 2 CH_2CH_3), 4.14 and 4.32 (2 q, 4 H, 2 CH_2CH_3), 4.55 (m, 3 H, C$_{5'}CH_2$ and C$_{4'}H$), 4.75 (m, 1 H, C$_{3'}H$), 6.01 (m, 1 H, C$_{2'}H$), 6.15 (d, 1 H, $J_{1',2'}$ = 3.2 Hz, C$_{1'}H$), 7.45 and 7.92 (2 m, 15 H, 3 PhH), 8.24 (s, 1 H, C$_5H$). Anal. ($C_{35}H_{32}N_2O_{11}$) C, H, N.

1-β-D-Ribofuranosylpyrazole-3,4-dicarboxamide (3). In a similar manner as for **2**, treatment of **14** (7.0 g, 7 mmol) with MeOH/NH_3 (50 mL) followed by crystallization of the product from aqueous EtOH furnished **3** (2.5 g, 82%): mp 188–190 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, C$_{5'}CH_2$), 3.93 (m, 1 H, C$_{4'}H$), 4.12 and 4.34 (2 m, 2 H, C$_{3',2'}H$), 4.95 (t, 1 H, C$_{5'}OH$), 5.15 and 5.51 (2 d, 2 H, C$_{2',3'}OH$), 5.70 (d, 1 H, $J_{1',2'}$ = 4.3 Hz, C$_{1'}H$), 7.38 (d, 1 H, J = 3.0 Hz, CON(H)H), 7.84 (s, 1 H, CON(H)H), 8.14 (s, 1 H, CON(H)H), 8.56 (s, 1 H, C$_5H$), 9.74 (d, 1 H, J = 3.0 Hz, CON(H)H). Anal. ($C_{10}H_{14}N_4O_6$) C, H, N.

5-Amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-pyrazole-3,4-dicarbonitrile (17). A solution of tetracyanoethylene (**15**, 36.0 g, 281 mmol) in absolute EtOH (300 mL) was added dropwise with stirring to a solution of 1-deoxy-1-hydrazinyl-2,3-*O*-isopropylidene-D-ribose[53] (**16**, 32.0 g, 156.7 mmol) in EtOH (300 mL), over a period of 30 min at 0 °C. The reaction mixture was stirred at ice-bath temperature for an additional 2 h and then stirred at room temperature for 15 h. The brown solution was filtered and evaporated to dryness. The residue was dissolved in MeOH (150 mL), decolorized with charcoal, and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (50 mL), adsorbed onto silica gel (90 g), and placed on top of a silica gel column (10 × 25 cm) packed with CH_2Cl_2. The column was eluted with CH_2Cl_2/EtOAc (10:1, v/v); the homogeneous fractions were pooled and evaporated to dryness. The residual yellow foam was crystallized from a CH_2Cl_2/MeOH mixture to yield 20.6 g (33%) of **17**: mp 155–156 °C; IR ν_{max} 2230 (C≡N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.31 and 1.48 (2 s, 6 H, C(CH_3)$_2$), 3.29 (m, 2 H, C$_{5'}CH_2$), 4.13 (m, 1 H, C$_{4'}H$), 4.83 (m, 1 H, C$_{3'}H$), 4.97 (t, 1 H, C$_{5'}OH$), 5.21 (m, 1 H, C$_{2'}H$), 6.11 (d, 1 H, $J_{1',2'}$ = 3.8 Hz, C$_{1'}H$), 7.58 (br s, 2 H, C$_5N H_2$). Anal. ($C_{13}H_{15}N_5O_4$) C, H, N.

5-Amino-1-β-D-ribofuranosylpyrazole-3,4-dicarbonitrile (18). A solution of **17** (7.12 g, 23.3 mmol) in TFA/water (60 mL, 51:9, v/v) was stirred at room temperature for 30 min. The solvent was evaporated and the residue was coevaporated with EtOH (3 × 50 mL). The light brown residue thus obtained was crystallized from a mixture of CH_2Cl_2/MeOH to yield 5.8 g (95%) of **18**: mp 194–195 °C dec; IR ν_{max} 2220 (C≡N) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1, 7, and 11) 275 (34.1); ^{1}H NMR (Me_2SO-d_6) δ 3.50 (m, 2 H, C$_{5'}CH_2$), 3.89 (m, 1 H, C$_{4'}H$), 4.09 (m, 1 H, C$_{3'}H$), 4.42 (m, 1 H, C$_{2'}H$), 5.01 (t, 1 H, C$_{5'}OH$), 5.19 and 5.48 (2 d, 2 H, C$_{2',3'}OH$), 5.72 (d, 1 H, $J_{1',2'}$ = 4.5 Hz, C$_{1'}H$), 7.58 (br s, 2 H, C$_5N H_2$). Anal. ($C_{10}H_{11}N_5O_4$) C, H, N.

5-Amino-1-β-D-ribofuranosylpyrazole-3,4-dicarboxamide (5). A solution of **18** (0.80 g, 2.65 mmol) in MeOH (60 mL) and water (35 mL) was treated with NH_4OH (28%, 7.0 mL) and H_2O_2 (30%, 6.5 mL). The reaction mixture was stirred at room temperature in a pressure bottle for 12 h, and then evaporated to dryness. The residue was coevaporated with MeOH (3 × 50 mL), dissolved in MeOH, and adsorbed onto silica gel (10 g). The adsorbed material was placed on top of a silica gel column (2.5 × 20 cm) and eluted with CH_2Cl_2/MeOH (8:2, v/v). The homogeneous fractions were pooled and evaporated to give a white foam, which was crystallized from a mixture of CH_2Cl_2/MeOH to yield 0.60 g (75%) of **5**: mp 235 °C dec; IR ν_{max} 1650 (C=O), 3300–3400 (OH, NH_2) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1, 7, and 11) 280 (3.3); ^1H NMR (Me_2SO-d_6) δ 3.60 (m, 2 H, $C_{5'}CH_2$), 3.87 (m, 1 H, $C_{4'}H$), 4.18 (m, 1 H, $C_{3'}H$), 4.54 (m, 1 H, $C_{2'}H$), 4.91 (t, 1 H, $C_{5'}OH$), 5.03 and 5.38 (2 d, 2 H, $C_{2',3'}OH$), 5.69 (d, 1 H, $J_{1',2'}$ = 4.5 Hz, $C_{1'}H$), 6.99 (br s, 3 H, C_5NH_2 and CONH(H)), 7.72 and 7.78 (2 s, 2 H, $CONH_2$), 9.65 (d, 1 H, J = 3.0 Hz, CON(H)H). Anal. ($C_{10}H_{15}N_5O_6$) C, H, N.

5-Amino-1-β-D-ribofuranosylpyrazole-3,4-dithiocarboxamide (19). Anhydrous H_2S was bubbled through a stirred solution of **18** (0.20 g, 0.75 mmol) in anhydrous pyridine (30 mL) containing dry Et_3N (5 mL) for 3 h at room temperature. The reaction mixture was heated at 100 °C in a sealed steel reaction vessel for 48 h. The reaction vessel was cooled and opened carefully. The mixture was purged with argon to remove excess H_2S and then evaporated to dryness. The residue was dissolved in MeOH (10 mL), adsorbed onto silica gel (2.0 g), and placed on top of a silica gel column (2 × 15 cm). The column was eluted with CH_2Cl_2/MeOH (96:4, v/v). The homogeneous fractions were pooled and evaporated to dryness. The residual yellow foam was crystallized from a mixture of MeOH/CH_2Cl_2 to give 80 mg (32%) of **19**: mp 164–165 °C; IR ν_{max} 1220 (C=S), 3200–3400 (OH, NH_2) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 281 (15.1); (pH 7) 280 (15.7); (pH 11) 278 (14.5), 305 (13.5); ^1H NMR (Me_2SO-d_6) δ 3.57 (m, 2 H, $C_{5'}CH_2$), 3.86 (m, 1 H, $C_{4'}H$), 4.11 (m, 1 H, $C_{3'}H$), 4.51 (m, 1 H, $C_{2'}H$), 4.91 (t, 1 H, $C_{5'}OH$), 5.11 and 5.36 (2 d, 2 H, $C_{2',3'}OH$), 5.71 (d, 1 H, $J_{1',2'}$ = 4.2 Hz, $C_{1'}H$), 7.95 (br s, 2 H, C_5NH_2), 9.07 (br s, 2 H, C(S)NH_2), 9.91 and 10.42 (2 s, 2 H, C(S)NH_2). Anal. ($C_{10}H_{15}N_5O_4S_2$) C, H, N, S.

5-Amino-1-β-D-ribofuranosylpyrazole-3,4-dicarboxamidoxime (20). In a similar manner as for **12**, reaction of **18** (0.50 g, 1.88 mmol) with free NH_2OH (0.62 g, 18.8 mmol) in absolute EtOH (40 mL) and purification of the reaction product on a flash silica gel column (2.5 × 15 cm) using CH_2Cl_2/MeOH (9:1, v/v) as the eluent furnished 0.44 g (70%) of **20**: mp 164–165 °C; IR ν_{max} 3300–3400 (OH, NH_2) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 275 (sh) (3.5); (pH 7) 239 (sh) (9.6); (pH 11) 242 (sh) (9.9); ^1H NMR (Me_2SO-d_6) δ 3.57 (m, 2 H, $C_{5'}CH_2$), 3.87 (m, 1 H, $C_{4'}H$), 4.19 (m, 1 H, $C_{3'}H$), 4.53 (m, 1 H, $C_{2'}H$), 4.91 (t, 1 H, $C_{5'}OH$), 5.06 and 5.34 (2 d, 2 H, $C_{2',3'}OH$), 5.64 (d, 1 H, $J_{1',2'}$ = 4.4 Hz, $C_{1'}H$), 5.67 (br s, 2 H, C_5NH_2), 6.64 and 6.82 (2 br s, 4 H, 2 C(NOH)N(H)H), 9.04 and 9.84 (2 br s, 2 H, 2 C(NOH)N(H)H). Anal. ($C_{10}H_{17}N_7O_6 \cdot {}^1/_2H_2O$) C, H, N.

5-Amino-1-β-D-ribofuranosylpyrazole-3,4-dicarboxamidine Dihydrochloride (21). A mixture of **20** (0.10 g, 0.3 mmol), Raney nickel (1.0 g), and NH_4Cl (0.20 g, 3.7 mmol) in 50% aqueous EtOH (15 mL) was shaken on a Parr hydrogenator at 50 psi for 12 h. The mixture was filtered through a Celite pad and the filtrate was evaporated to dryness. The residue was dissolved in EtOH (10 mL), adsorbed onto silica gel (2 g), and placed on top of a silica gel column (2 × 15 cm). The column was eluted with CH_2Cl_2/MeOH (6:4, v/v). The homogeneous fractions were pooled and evaporated to dryness. The product was further purified by HPLC using C_{18} reverse phase column and water as the eluent to give 65 mg (58%) of **21** as white amorphous solid: mp 240 °C dec; IR ν_{max} 3100–3400 (OH, NH_2) cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 286 (20.4); (pH 7) 285 (20.4); (pH 11) 275 (20.3); ^1H NMR (Me_2SO-d_6) δ 3.55 (m, 2 H, $C_{5'}H$), 3.91 (m, 1 H, $C_{4'}H$), 4.20 (m, 1 H, $C_{3'}H$), 4.50 (m, 1 H, $C_{2'}H$), 5.04 (t, 1 H, $C_{5'}OH$), 5.20 and 5.53 (2 d, 2 H, $C_{2',3'}OH$), 5.81 (d, 1 H, $J_{1',2'}$ = 4.8 Hz, $C_{1'}H$), 6.79 (br s, 2 H, C_5NH_2), 8.94–9.29 (br s, 8 H, 2 C(NH)NH_2·HCl). Anal. ($C_{10}H_{17}N_7O_4 \cdot 2HCl \cdot {}^3/_4H_2O$) C, H, N, Cl.

Diethyl 1-[2,3-O-Isopropylidene-5-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]pyrrole-3,4-dicarboxylate (24). To a suspension of diethyl pyrrole-3,4-dicarboxylate[62] (**22**, 4.22 g, 20 mmol) in dry CH_3CN (150 mL) was added NaH (60% in oil, 0.8 g, 20 mmol) under anhydrous and inert (argon) conditions. The suspension was stirred at room temperature for 1 h. A freshly prepared solution of 1-chloro-2,3-*O*-isopropylidene-5-*O*-(*tert*-butyldimethylsilyl)-α-D-ribofuranose[63] (**23**, 10 mmol, in 40 mL of dry THF) was added and stirring was continued for 12 h at room temperature. The reaction mixture was adsorbed onto silica gel (20 g) and purified by loading the adsorbed material onto the top of a prepacked silica gel column (5 × 50 cm, 100 g, hexanes/EtOAc, 7:3, v/v). On elution with the same solvent system, homogeneous fractions were collected, pooled, and evaporated to furnish 2.6 g (52%) of **24** as a syrup: ^1H NMR ($CDCl_3$) δ 0.10 and 0.11 (2 s, 6 H, Si(CH_3)$_2$), 0.91 (s, 9 H, C(CH_3)$_3$, 1.33 (t, 6 H, 2 CH_2CH_3), 1.38 and 1.61 (2 s, 6 H, C(CH_3)$_2$), 3.83 (m, 2 H, $C_{5'}CH_2$), 4.32 (q, 4 H, CH_2CH_3), 4.38 (m, 1 H, $C_{4'}H$), 4.69 and 4.83 (2 m, 2 H, $C_{3',2'}H$), 5.62 (d, 1 H, $J_{1',2'}$ = 3.7 Hz, $C_{1'}H$), 7.44 (s, 2 H, C_2, C_5H). Anal. ($C_{24}H_{39}NO_8Si$) C, H, N.

1-[2,3-O-Isopropylidene-5-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]pyrrole-3,4-dicarboxamide (25). A mixture of **24** (1.5 g, 3 mmol) and MeOH/NH_3 (MeOH saturated with NH_3 at 0 °C, 60 mL) was heated in a steel reaction vessel at 130 °C for 24 h. After cooling, the solvent was evaporated; the residue was dissolved in MeOH, adsorbed onto silica gel (10 g), and placed onto the top of a silica gel column (4 × 20 cm). The column was eluted with EtOAc; homogeneous fractions were combined and evaporated to dryness, and the residue was crystallized from EtOAc/hexanes (1:1, v/v) to furnish 0.66 g (50%) of **25**: mp 212 °C; ^1H NMR ($CDCl_3$) δ 0.10 and 0.11 (2 s, 6 H, Si(CH_3)$_2$), 0.90 (s, 9 H, C(CH_3)$_3$), 1.39 and 1.61 (2 s, 6 H, C(CH_3)$_2$), 3.82 (m, 2 H, $C_{5'}CH_2$), 4.40 (m, 1 H, $C_{4'}H$), 4.73 and 4.82 (2 m, 2 H, $C_{3',2'}H$), 5.64 (d, 1 H, $J_{1',2'}$ = 3.5 Hz, $C_{1'}H$), 7.54 (s, 2 H, C_2, C_5H), 5.5–6.0 and 7.0–8.0 (2 br s, 4 H, 2 $CONH_2$). Anal. ($C_{20}H_{33}N_3O_6Si$) C, H, N, Si.

1-β-D-Ribofuranosylpyrrole-3,4-dicarboxamide (4). A solution of **25** (0.43 g, 1 mmol) in TFA/water (2 mL, 9:1, v/v) was stirred at room temperature for 30 min. The excess TFA was coevaporated with EtOH (3 × 50 mL) and the residue was dried under reduced pressure for 12 h. The residual white foam was crystallized from aqueous EtOH to furnish 0.14 g (50%) of **4**: mp 199–200 °C; UV λ_{max} nm ($\epsilon \times 10^{-3}$) (pH 1) 253 (13.2); (pH 7) 248 (12.1); (pH 11) 248 (12.0); ^1H NMR (Me_2SO-d_6) δ 3.55 (m, 2 H, $C_{5'}CH_2$), 3.87 (m, 1 H, $C_{4'}H$), 3.98 and 4.10 (2 m, 2 H, $C_{3',2'}H$), 4.97 (t, 1 H, $C_{5'}OH$), 5.0 and 5.43 (2 d, 2 H, $C_{2',3'}OH$), 5.41 (d, 1 H, $J_{1',2'}$ = 6.5 Hz, $C_{1'}H$), 7.13 and 8.88 (2 br s, 4 H, 2 $CONH_2$), 7.63 (s, 2 H, C_2, C_5H). Anal. ($C_{11}H_{15}N_3O_6$) C, H, N.

Cell Growth and Differentiation. The following cell lines were grown in vitro in RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in an atmosphere of 5% CO_2 in air. A murine leukemia cell line, L1210, was started from an ascites sample of a cell line passaged in vivo by Dr. Thomas Avery of the Institute. A human B-lymphoblast cell line, WI-L2, was provided by Dr. Randall Willis of the Institute. The human myeloid leukemia cell lines K562 and HL-60 were obtained from the American Type Culture Collection (Rockville, MD). Cell growth inhibition was determined after 6 days of incubation with compounds by measurement of cell densities using a Coulter Counter (Hialeah, FL). The differentiation of HL-60 cells was assessed by the cells' ability to produce superoxide following a stimulus.[73] HL-60 cells were incubated with the test compounds for 4 days as described for the growth studies, and then 1 × 10^5 cells were suspended in 1 mL of a solution containing 0.137 M NaCl, 5.7 mM glucose, 5.4 mM KCl, 4.2 mM $NaHCO_3$, 0.44 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 5% (v/v) FBS, and 0.2 mg/mL luminol. In some experiments 5 μL of 162 μM tetradecanoylphorbol acetate was added to stimulate superoxide production. In other experiments the HL-60 cells were suspended in 1 mL of the solution described above plus 5% (v/v) conditioned medium from GCT cells (American Type Culture Collection), incubation was carried out for 2 h at 37 °C and 10 μL of 500 μM formylmethionylleucylphenylalanine (fMLP, ICN Biomedicals, Costa Mesa, CA) was added to stimulate superoxide production.[70] A

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Beckman scintillation counter equipped with a single photon monitor was used to detect the chemiluminescence produced by the reaction of superoxide and luminol.

Registry No. 2, 123027-68-9; 3, 123027-69-0; 4, 123027-70-3; 5, 123027-71-4; 6, 707-94-8; 7, 37687-26-6; 8, 14215-97-5; 9, 123027-72-5; 10, 123027-73-6; 11, 70042-31-8; 12, 123027-74-7; 13, 123027-75-8; 14, 123027-76-9; 15, 670-54-2; 16, 55781-00-5; 17, 123027-77-0; 18, 123027-78-1; 19, 123027-79-2; 20, 123027-80-5; 21, 123027-81-6; 22, 41969-71-5; 23, 102690-94-8; 24, 123027-82-7; 25, 123027-83-8.

Supplementary Material Available: Hydrogen bonding in 2 and 3 as determined by X-ray crystallographic studies (1 page). Ordering information is given on any current masthead page.

Orally Effective Acid Prodrugs of the β-Lactamase Inhibitor Sulbactam

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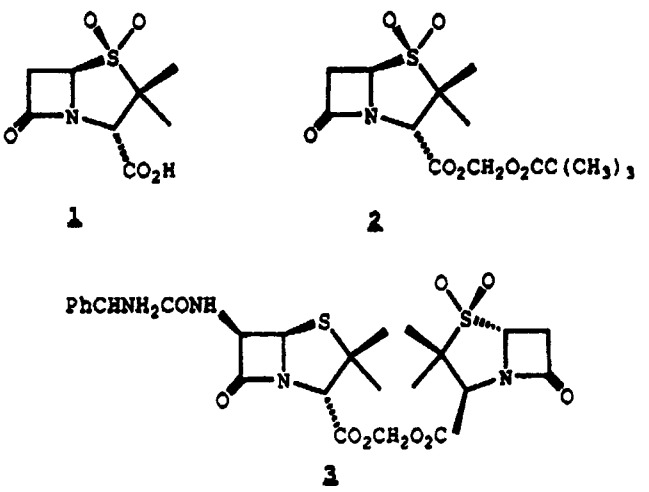
Sulbactam (1) is a β-lactamase inhibitor with limited oral bioavailability. Lipophilic double-ester prodrug sulbactam pivoxil (2) significantly improves the oral absorption of sulbactam, as does the mutual prodrug double ester sultamicillin (3). We have found that double-ester prodrugs of sulbactam terminating in a carboxyl group (8) also were effective oral-delivery vehicles in rats. Carboxyl-terminated double esters have several potential advantages over their nonionizable lipophilic counterparts, including water solubility, crystallinity, choice of salts for dosage forms, and formation of innocuous byproducts on hydrolysis.

The penicillins remain among the safest and most effective β-lactam antibiotics available for the treatment of bacterial infections. Their effectiveness has, however, been eroded over time through extensive use and natural selection for resistant strains. A majority of these strains inactivate penicillins through the action of β-lactamase enzymes. Effective inhibitors of these defensive enzymes have been developed and proved clinically useful in restoring and expanding the antibacterial spectrum of semisynthetic penicillins.[1] Sulbactam (1) is a β-lactamase inhibitor derived from 6-aminopenicillanic acid,[2] and like other penicillins, it has limited bioavailability after oral administration because of poor absorption from the gastrointestinal tract. This problem was alleviated by the synthesis of double-ester prodrugs,[3] such as sulbactam pivoxil (2), in analogy to the ampicillin prodrug esters,[4] and culminated in the discovery of the mutual prodrug 3, sultamicillin[5] (Chart I).

Double-ester prodrugs of these β-lactam antibiotics are very well absorbed and biolabile, effectively delivering the desired drugs to serum upon rapid enzymatic hydrolysis.[3-5] There are, however, limiting aspects of the double ester prodrug concept in its current embodiment. Bundgaard and Nielsen have recently enumerated these limitations as poor water solubility, limited stability in vitro, and the propensity of many of these lipophilic esters to exist as oils, creating formulation problems.[6] We report here the results of our efforts to address these limitations through synthesis and evaluation of a series of double-ester prodrugs of sulbactam terminating in a free carboxylic acid moiety. While we presumed that a carboxylic acid terminus could provide greatly improved water solubility at neutral pH and crystalline salts with good formulation characteristics, it was not obvious to us that intestinal absorption would equal that observed with lipophilic esters 2 or 3.

Chart I



1 2 3

Table I. Physical Constants, Synthetic Data

compd	X	% yield	mp,[a] °C	formula	anal.
8a	CH_2	88		$C_{12}H_{14}NO_9SNa$	C,H,N
8b	$C(CH_3)_2$	90	120-122	$C_{14}H_{18}NO_9SNa$	C,H,N
8c	$(CH_2)_3$	85	71-72	$C_{14}H_{18}NO_9SNa$	C,H,N,Na
8d	$(CH_2)_4$	84	100-102	$C_{15}H_{21}NO_9S \cdot H_2O$	C,H,N

[a] Melting points are of crystalline free carboxylic acids.

Synthesis

Synthesis of the desired novel double esters started from the benzyl half-ester of selected diacids 4. Formation of

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