Synthesis and Antiviral Activity of Some Phosphates of the Broad-Spectrum Antiviral Nucleoside, 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin)

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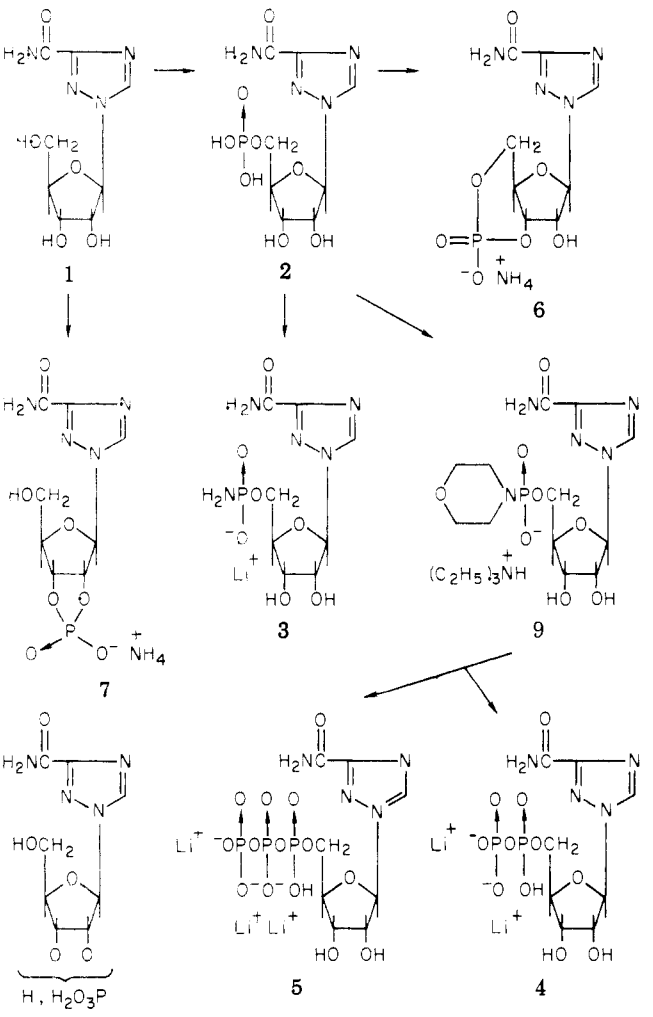
1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate (2) was prepared and converted into the following derivatives: the 5′-phosphoramidate 3, the 5′-diphosphate 4, the 5′-triphosphate 5, and the cyclic 3′,5′-phosphate 6. The cyclic 2′,3′-phosphate 7 was prepared from the parent nucleoside, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1), and was opened to the 2′(3′)-phosphate 8. These compounds were found to exhibit significant antiviral activity against several viruses in cell culture. Ribavirin 5′-phosphate (2) was shown to be effective when tested against lethal infections in mice caused by influenza A_2, influenza B, and murine hepatitis viruses.

Since the first report[1] of the broad-spectrum antiviral activity[1,2] and synthesis[2] of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1, ribavirin), the in vitro and in vivo antiviral activity has been confirmed in many independent laboratories.[3-15] Ribavirin has proved clinically effective against infectious hepatitis[16-19] and has been shown to be active in two double blind clinical trials against influenza A.[20,21] Clinical efficacy has been claimed for ribavirin against herpes zoster[22,23] and herpes stomatitis in a recent double blind clinical trial.[24]

Ribavirin is unique in that as the nucleoside it readily undergoes facilitated transport into cells. It is then phosphorylated to 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate (2) by deoxyadenosine kinase and/or adenosine kinase.[25,26] Ribavirin 5′-diphosphate 4 and the 5′-triphosphate 5 have been identified in tissues from rats given labeled ribavirin orally.[27] The absence of any phosphorylated derivatives in rat serum and urine and the presence of these metabolites in all tissues examined suggest that phosphorylation occurs within the cell and that the 5′-phosphorylated nucleotides of ribavirin remain within the cell until enzymatic dephosphorylation takes place to the nucleoside which is a major urinary product.[27] The present work describes the chemical synthesis of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-diphosphate (4) and 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-triphosphate (5) required for these metabolic studies. The synthesis of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide cyclic 3′,5′-monophosphate (6), 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide cyclic 2′,3′-monophosphate (7), and other related phosphate derivatives was undertaken to investigate the antiviral activity of such derivatives and to provide possible metabolic products which would serve for comparison with potential ribavirin metabolites isolated from animal and human studies.

The chemical synthesis of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate (2) has previously been described[28] from 1. In the present study, the methyl ester of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid[2] was treated with phosphoryl chloride in trimethyl phosphate at 0 °C to give the corresponding 5′-phosphate which was then treated with aqueous ammonia to give the ammonium salt of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate of 2 in 53% yield. A large-scale synthesis of 2, isolated as the dilithium salt, was also developed directly from ribavirin (1) due to the substantial quantities of 2 needed for the present study. The treatment of 2 with 1,1′-carbonyldiimidazole in the presence of imidazole gave 5′-phosphoimidazolate which,

Scheme I



when treated with aqueous ammonia, gave 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphoramidate (3), isolated as the lithium salt in 24% yield. Treatment of the 4-morpholino-*N*,*N*-dicyclohexylcarboxamidine salt of ribavirin 5′-phosphate (2) in pyridine with dicyclohexylcarbodiimide according to the general procedure of Smith et al.[29] gave 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide cyclic 3′,5′-monophosphate (6) (isolated as the ammonium salt) in 23% yield (see Scheme I).

The procedure of Ueda and Kawai[30] employing pyrophosphoric acid in refluxing *N*,*N*-dimethylformamide in

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Table I. In Vitro Antiviral Activity (Virus Rating) against Type 3 Adeno (AV/3), Type 1 Herpes Simplex (HSV/1), Vaccinia Virus (VV), Type 3 Parainfluenza (PIV/3), and Type 13 Rhino (RV/13)

	virus rating				
compd	AV/3	HSV/1	VV	PIV/3	RV/13
1	0.7	1.1	0.8	0.7	0.8
2	0.3	1.0	1.0	0.6	0.6
3	0.1	0.6		0.2	0.2
4	0.0	0.9		0.4	0.3
5	0.0	0.7		0.5	0.4
6	0.0	1.4		0.8	0.6
7	0.0	1.2	0.7	1.2	0.3
8		0.8		1.0	0.7

the presence of tri-*n*-butylamine was utilized to convert ribavirin (1) to the cyclic 2',3'-phosphate **7** which was isolated as the ammonium salt in 19% yield. Treatment of **7** with Dowex 50 (H^+) opened the cyclic 2',3'-monophosphate ring to give 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 2'(3')-phosphate in the usual manner.[30]

For the preparation of ribavirin 5'-di- and -triphosphates **4** and **5**, the general procedure of Moffatt and Khorana[31] was followed which consisted first in the synthesis of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-phosphoromorpholidate (**9**) from ribavirin 5'-phosphate (**2**), followed by reaction of **9** with orthophosphoric acid to give 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-diphosphate (**4**), isolated as the dilithium salt. Similarly, treatment of **9** with pyrophosphate under anhydrous conditions gave a 14% yield of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-triphosphate (**5**), isolated as the trilithium salt. Some ribavirin 5'-diphosphate (**4**) was also isolated as a by-product in this reaction.

Antiviral Evaluation. The nucleotides of ribavirin were all evaluated for antiviral activity in primary in vitro cell culture experiments using type 3 adeno, type 1 herpes simplex, vaccinia virus, type 13 rhino, and type 3 parainfluenza viruses. A 24-h monolayer of human carcinoma of the nasopharynx (KB) cells in disposable plastic microplates was used in these experiments. The cells were exposed to 320 cell culture 50% infectious doses per milliliter of virus, and concentrations of each nucleotide ranging from 1000 to 1 μg/mL were added 15 min later. Inhibition of the viral cytopathogenic effect (CPE) after a 72-h incubation at 37 °C was used for evaluation of antiviral activity. The degree of CPE inhibition was evaluated using a previously described[32] numerical virus rating (VR) which takes into account cytotoxicity and the degree of CPE in treated and untreated cells. In this system, a VR of 0.1–0.4 was considered to indicate only slight antiviral activity; a VR of 0.5 or greater suggested definite antiviral activity. The results of these primary experiments are shown in Table I.

All of the compounds evaluated (Table I) had a significant degree of antiviral activity, particularly against type 1 herpes simplex virus, with efficacy also generally seen against vaccinia virus, type 3 parainfluenza, and type 13 rhino viruses. It is of interest that none of the phosphate derivatives of ribavirin exhibited significant activity against type 3 adenovirus in tissue culture. Ribavirin, on the other hand, does exhibit significant activity against adenoviruses[3,33] in cell culture (Table I). Of the ribavirin nucleotides studied, the 5'-phosphoramidate **3** appears to be the least active. It is interesting that in cell culture the 5'-phosphate **2** appears to be significantly more active than the 5'-diphosphate **4** or the 5'-triphosphate **5**. Ribavirin 5'-phosphate (**2**) was selected for additional in vitro experiments using other viruses to ascertain further its broad-spectrum in vitro antiviral activity. In these tests, efficacy of **2** was also seen against other Picornaviruses (type 1A rhino, VR 0.7; type 2 rhino, VR 0.5; type 56 rhino, VR 0.7), Herpesvirus (type 2 herpes simplex, VR 0.8; infectious bovine rhinotraceitis, VR 1.3; pseudorabies, VR 0.3), and against myxoma virus (VR 1.6). This in vitro antiviral activity was as broad in spectrum and essentially equivalent to ribavirin itself.[1,2,34]

Ribavirin 5'-phosphate, compound **2**, was tested against lethal infections in mice caused by influenza A_2 (strain Japan 305), influenza B (strain Lee), and murine hepatitis (strain Friend–Braunsteiner) viruses (Table II). In the influenza experiments, Swiss mice weighing 14–16 g were exposed intranasally to the virus in concentrations sufficient to kill 65% (influenza A_2) or 95% (influenza B) of the animals. The influenza A_2 virus-infected mice were treated by oral gavage twice daily for 9 days, starting 4 h previrus infection. The influenza B virus-infected mice were treated intraperitoneally (ip) using the same treatment schedule. In the hepatitis virus experiments, Swiss mice weighing 16–18 g were inoculated ip with a 95% lethal dose of the virus and treated ip with ribavirin 5'-phosphate (**2**) twice daily for 8 days beginning 2 h previrus inoculation. In all three experiments, significant increases in survivors occurred as a result of treatment, as compared to placebo (saline)-treated virus control animals. A single dose of ribavirin 5'-phosphate, 75 mg/kg/day, was well tolerated by the animals in the experiments. Other toxicity trials suggest that the maximum tolerated dose of the compound is approximately 150 mg/kg/day. In the hepatitis experiments, the most effective dosage was 37.5 mg/kg/day (Table II).

A comparison of the results of **2** in the treatment of A_2 (Japan 305) influenza in mice (Table II) with ribavirin, as previously reported,[35] shows that ribavirin 5'-phosphate (**2**) is essentially as active as ribavirin at a similar dose level (75 mg/kg/day) in increasing survivors of influenza A_2 infection. Similarly, ribavirin 5'-phosphate (**2**) protects mice against a lethal infection of influenza B (Lee) to about

Table II. Effect of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-Phosphate (**2**) on Influenza A_2, Influenza B, and Hepatitis Virus Infections in Mice

virus	dose, mg/kg/day	toxicity controls, surv/total	surv/total[a]	survival increase, p[b]	mean surv time,[c] days	mean surv time increase, p[d]
influenza A_2	0		7/20		9.0	
	75	5/5	7/10	<0.05	7.6	
influenza B	0		1/20		11.2	
	75	5/5	7/10	<0.001	13.3	*e*
hepatitis	0		1/20		5.2	
(murine)	75	5/5	3/10	<0.1	6.1	>0.05
	37.5	5/5	7/10	<0.001	6.0	*e*
	18.8	5/5	2/10	0.2	5.3	>0.05

[a] Determined on day 21 postinfection. [b] p = probability (X^2 analysis). [c] Animals dying on or before day 21. [d] p = probability (t test). [e] Numbers of animals dying insufficient for adequate statistical evaluation.

the same extent at the same dose level as ribavirin[35] (Table II). A study of the comparison of the in vivo activity of ribavirin 5′-phosphate (**2**) against murine hepatitis in mice (Table II) with that reported for ribavirin under a similar test[36] reveals that ribavirin would appear to be slightly superior to **2** with a similar number of survivors at 37.5 mg/kg/day, but ribavirin showed 7/10 and 10/10 survivors at 18.8 mg/kg/day[36] as compared to only 2/10 survivors for **2** (Table II) at the same daily dosage. Scholtissek[37] showed that ribavirin (**1**) inhibits the RNA synthesis of an influenza A (fowl plague) virus. Müller and co-workers[38] studied ribavirin 5′-triphosphate (**5**), supplied from our laboratory, and found that **5** did not inhibit eukaryotic DNA polymerase, α and β eukaryotic RNA polymerase I and II, or eukaryotic poly(A) polymerase. Eriksson and colleagues[39] have recently shown that ribavirin 5′-triphosphate (**5**) (made available by our presently described work) selectively inhibits influenza virus RNA polymerase in a cell-free system. Ribavirin (**1**) and ribavirin 5′-phosphate (**2**) had no effect on this enzyme. This inhibition is competitive with regard to guanosine triphosphate.[39] The RNA polymerases isolated from thymus and *Escherichia coli* were not inhibited by ribavirin 5′-triphosphate (**5**). Eriksson et al.[39] postulate that the prevention of virus multiplication depends on a selective inhibition of the influenza viral RNA polymerase by ribavirin 5′-triphosphate (**5**). Obert and Helgstrand[40] have recently shown that ribavirin 5′-triphosphate (**5**) also significantly inhibits herpes DNA polymerase. DNA polymerases isolated from *Micrococcus luteus* and thymus were not inhibited by **5**. Oberg and Helgstrand[40] have recently shown that the degree of inhibition of cellular DNA, RNA, and protein synthesis exhibited by ribavirin is completely reversible upon removal of the drug.

It is quite possible that certain ribavirin derivatives such as the nucleotides described here may offer a particular advantage of drug administration for a given viral disease. One such example is the superior antiviral activity of 2′,3′,5′-tri-*O*-acetylribavirin over that of ribavirin in aerosol therapy of influenza infected mice.[5]

Experimental Section

All compounds gave correct elemental analyses (Galbraith Laboratories, Knoxville, Tenn.). Structures were supported by single-spot TLC mobility; spots were detected by spraying the silica plates (Woelm silica gel 254F) with 5% methanolic H_2SO_4 and charring or by spraying with 1% anisaldehyde in methanol containing 10% H_2SO_4 followed by charring.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-Phosphoramidate (3). A solution of **2** (360 mg, 1.1 mmol) in 20 mL of DMF was cooled to 0 °C, 1,1-carbonyldiimidazole (810 mg, 5.0 mmol) and imidazole (160 mg, 23 mmol) were added, and solution was stirred for 20 min at 0 °C. The solvent was evaporated and the residue dissolved in EtOH. Addition of 10 vol of Et_2O precipitated a solid which was collected by centrifugation and washed with Et_2O. After desiccation, the solid was dissolved in 10 mL of 2 N NH_4OH and heated in a bomb at 65 °C overnight. The solution was evaporated and the residue dissolved in a small volume of H_2O and put onto a column of Dowex 1-X8 (Cl^-, 15 mL, 2 cm i.d.). The column was washed with H_2O and then eluted with 400 mL of 0–0.2 N LiCl gradient. The fractions corresponding to the first major peak were pooled in a minimum volume of H_2O and 20 vol of EtOH was added. The solid was collected by centrifugation, washed twice with EtOH and twice with Et_2O, and dried to yield 85 mg (24%) of **3**. Anal. Calcd for $C_8H_{13}LiN_5O_7P$: C, 29.19; H, 3.98; N, 21.28; Li, 2.11. Found: C, 29.10; H, 4.30; N, 21.43; Li, 1.96.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-Phosphate (2). (1) Ammonium Salt. A solution of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxylic acid methyl ester[2] (259 mg, 1.00 mmol), trimethyl phosphate (3.0 mL), and phosphoryl chloride (0.20 mL) was stirred at 0 °C for 1.5 h. Ice water was added and the solution was neutralized with aqueous sodium hydrogen carbonate. The solution was extracted with chloroform. The aqueous phase was cooled to 0 °C and saturated with ammonia. The solution was kept at 25 °C for 16 h and then filtered, and the filtrate was concentrated to a small volume. Addition of ethanol gave a precipitate which was dissolved in water and passed through a Bio-Rad AG50W-X2 (NH_4^+) column (20 mL). Concentration of the fractions containing the nucleotide provided the ammonium salt of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate (190 mg, 53.0%). Anal. Calcd for $C_8H_{16}N_5O_8P\cdot H_2O$: C, 26.74; H, 5.05; N, 19.50. Found: C, 26.78; H, 5.23; N, 19.68.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-Phosphate (2). (2) Dilithium Salt. To a precooled (−5 °C) solution of 250 mL of trimethyl phosphate (redistilled), 37 g (22 mL, 0.240 mol) of phosphoryl chloride, and 1.08 mL (0.060 mol) of H_2O was added 14.6 g (0.060 mol) of finely ground 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide, and the mixture was stirred 5 h at exactly 0 °C. The resulting solution was poured into 500 g of ice, and 2 N NaOH was added portionwise with stirring to bring the pH to 2.5. This hydrolysis was slow and the solution was allowed to stand overnight at ambient temperature after initial neutralization before making the final adjustment of pH. The solution was extracted with 2 × 500 mL of $CHCl_3$, then filtered (to remove traces of suspended $CHCl_3$), and passed slowly twice through a 4 × 23 cm column containing about 250 mL of Barnebey-Cheney UU activated charcoal. This salt solution was saved for additional product recovery. The charcoal was washed with 2 L of H_2O; then the product was eluted with 2 L of Et_3N–EtOH–H_2O (3:10:7 v/v). After evaporation of the solvent, the residue was taken up in 250 mL of H_2O and the pH was adjusted to 9.0 with sodium hydroxide. This solution was applied to a 4 × 27 cm column of Dowex 1-X8 (Cl^- form, 100–200 mesh) and the column was washed with water and then eluted with a gradient of 2 L of H_2O in the mixing chamber and 2 L of 0.3 N LiCl in the reservoir. The product appeared in the fractions from 1.8 to 2.75 L of eluate. Evaporation of these fractions gave a residue which was taken up in 50 mL of hot H_2O and diluted with 400 mL of hot EtOH and then cooled. The product was collected and washed (EtOH), then was redissolved in 50 mL of H_2O, and reprecipitated in an identical manner. The thoroughly dried product weighed 6.2 g.

To the original salt solution from the charcoal column was added 100 mL of active charcoal, the pH was adjusted to 2.0 with HCl, and the mixture was stirred overnight. The charcoal was filtered, washed with water, and heated 1 h with 100 mL of pyridine, 300 mL of MeOH, and 200 mL of H_2O. The filtered solution was evaporated and the residue was subjected to ion-exchange chromatography and workup exactly as described above, giving 3.3 g of additional product: total yield 47%. Anal. Calcd for $C_8H_{11}Li_2N_4O_8P\cdot 0.5H_2O$: C, 27.84; H, 3.51; Li, 4.02; N, 16.24. Found: C, 27.83; H, 3.63; Li, 3.88; N, 15.96.

After drying at 100 °C for 16 h, the following analysis was obtained. Anal. Calcd for $C_8H_{11}Li_2N_4O_8P$: C, 28.59; H, 3.30. Found: C, 28.48; H, 3.48.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide Cyclic 3′,5′-Monophosphate (6). To 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 5′-phosphate ammonium salt (**2**) (5.7 g, 15.9 mmol) in pyridine was added 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine (4.65 g, 15.9 mmol), and the resulting solution was evaporated in vacuo several times with pyridine to an anhydrous syrup. The syrup was dissolved in 1 L of pyridine and added dropwise (over a 1-h period), through a reflux condenser, into a refluxing anhydrous solution of dicyclohexylcarbodiimide (16.4 g, 79.6 mmol) in 3 L of pyridine. The solution was refluxed for a further 2 h and 200 mL of water was added slowly. After 12 h the solution was evaporated in vacuo and to the residue was added 200 mL of water and 100 mL of ether. The suspension was stirred vigorously and then filtered. The aqueous layer was separated and extracted with 2 × 100 mL ether. The aqueous layer was passed through a Dowex 50-X8 (NH_4^+ form, 100–200 mesh) column and the eluent evaporated to a syrup. Ethanol (100 mL) was added to the syrup and the resulting mixture set at room temperature for 12 h. A small amount of impurity was filtered and discarded. The filtrate was left at room temperature for 2 weeks. The resulting precipitate (1.2 g) was dissolved in 5 mL

of warm water and then 40 mL of ethanol was added. The resulting crystals were filtered and dried 12 h at 78 °C over P_2O_5 in vacuo to give 1.05 g (22.6%) of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide cyclic 3',5'-phosphate ammonium salt (6): $[\alpha]^{25}_D$ 67° (c 1, H_2O); IR (KBr) 1688 cm^{-1} [-C(=O)-]; mp 245 °C dec. Anal. Calcd for $C_8H_{11}N_4O_7P \cdot NH_3$: C, 29.73; H, 4.36; N, 21.66. Found: C, 29.86; H, 4.67; N, 21.47.

Preparation of 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide Cyclic 2',3'-Phosphate (7). A solution of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (1.22 g, 5.0 mmol), pyrophosphoric acid (10.0 mmol), and tri-*n*-butylamine (40.0 mmol) in dimethylformamide was refluxed for 2.5 h. The solvent was removed in vacuo and the residue was dissolved in 1 N ammonium hydroxide (100 mL). The solution was extracted with ether and the aqueous phase was concentrated to a small volume. The solution was diluted with water to 60 mL and the pH was adjusted to 8.5 with ammonium hydroxide. This solution was applied to a Whatman DE52 DEAE cellulose (130 g) column in the bicarbonate form. Elution was with a linear gradient of water (1000 mL) to 0.05 M triethylammonium bicarbonate (1000 mL) and fractions of 20 mL were collected. The product was contained in fractions 35–50 which were combined and evaporated to dryness. The residue was dissolved in water (20 mL) and passed through a Dowex 50-X8 (20 mL) column in the ammonium form. The column was eluted with water and the solution containing the product was evaporated to dryness. The residue was dissolved in water. The solution was lyophilized to afford 320 mg (19%) of the ammonium salt of the cyclic 2',3'-phosphate 7. Anal. Calcd for $C_8H_{14}N_5O_7P \cdot H_2O$: C, 27.93; H, 4.58; N, 21.13. Found: C, 28.16; H, 4.73; N, 20.53.

When an aqueous solution of 7 was passed through a column containing a large excess of Dowex 50-X8 (H^+), 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide 2'(3')-phosphate (8) was obtained after lyophilization. Anal. Calcd for $C_8H_{13}N_4O_8P$: C, 29.62; H, 4.04; N, 17.28. Found: C, 29.39; H, 4.08; N, 17.10.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-Phosphoromorpholidate Triethylamine Salt Dihydrate (9). Ribavirin 5'-phosphate[28] free acid (8 mmol) was dissolved in water (80 mL) and *tert*-butyl alcohol (80 mL). This solution was brought to reflux and freshly distilled morpholine (2.72 mL, 32 mmol) was added. Then dicyclohexylcarbodiimide (6.592 g, 32 mmol) dissolved in 120 mL of *tert*-butyl alcohol was added dropwise over 2.5 h and the solution refluxed an additional 2 h. The solution was evaporated in vacuo. The residue was diluted with water (200 mL) and extracted three times with ether. The aqueous solution was concentrated to a small volume and applied to a column (50 mL) of Dowex 1-X2 (bicarbonate, 100–200 mesh) resin. Elution with a 1-L gradient from 0 to 0.5 N triethylammonium bicarbonate gave the product after about one-third of the gradient. This fraction was evaporated, then repeatedly dissolved in methanol, and evaporated. It was finally dissolved in water and freeze-dried to give a greater than 80% yield of the triethylamine salt.

Anal. Calcd for $C_{13}H_{20}N_5O_8P \cdot C_5H_{15}N \cdot 2H_2O$: C, 40.75; H, 7.41; N, 15.84. Found: C, 40.45; H, 7.47; N, 16.05.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-Diphosphate Dilithium Salt (4). The triethylammonium salt of ribavirin 5'-phosphoromorpholidate dihydrate (9) (530 mg, 1 mmol) was dried by two repetitions of dissolution in pyridine (10 mL) and evaporation in vacuo. Separately, 85% orthophosphoric acid (0.205 mL, 3 mmol) was dissolved in pyridine (10 mL) containing tri-*n*-butylamine (0.715 mL, 3 mmol) and then the mixture was rendered anhydrous by three evaporations of pyridine (10-mL portions). The two pyridine solutions were combined and the evaporation procedure was repeated two more times. The flask was opened to dry air each time. The final residue was dissolved in dry pyridine (10 mL). TLC evaluation after 3 days showed little of the morpholidate remaining. The solvent was removed in vacuo. The residue was dissolved in water which was then evaporated, and this procedure was repeated several times. The pH of aqueous solution was adjusted to 6.5 with 0.5 M lithium hydroxide and the water was washed twice with ether. The pH of the aqueous phase was adjusted to 12 with LiOH, total volume about 30 mL. The suspension was cooled at 0 °C for 0.5 h. The lithium phosphate was removed by filtration and washed with a small amount of 0.01 N LiOH. The pH of the aqueous solution was adjusted to about 8 with Dowex 50 (H^+) resin and then applied to a column of Dowex 2-X8 (Cl^-) resin (2.5 × 12 cm). The column was washed with water and then eluted with a gradient from 0.003 M HCl to 0.5 M LiCl in 0.003 M HCl over 2 L. The product eluted as a rather broad band about two-thirds of the way through the gradient. The column effluent was monitored by UV at 210 nm. The corresponding fractions were evaporated. The residue was treated with methanol, diluted with acetone, and centrifuged, and the liquid was decanted to remove the lithium chloride. This was repeated several times. The residue was then dissolved in water and freeze-dried to give the nucleoside diphosphate dilithium salt, 186 mg (36% yield). Anal. Calcd for $C_8H_{12}N_4O_{11}P_2Li_2 \cdot 4H_2O \cdot 0.5LiCl$: C, 18.91; H, 3.97; N, 11.03; P, 12.19; Li, 3.18; Cl, 3.49. Found: C, 19.06; H, 3.29; N, 10.73; P, 11.77; Li, 3.15; Cl, 3.81.

This material was pure as determined by TLC. Development with *i*-PrOH–NH_4OH–H_2O (7:1:2) and development with isobutyric acid–1 M NH_4OH—0.1 M EDTA (100:60:1.6) each showed a single spot. The latter system distinguishes clearly between the mono-, di-, and triphosphates of ribavirin.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide 5'-Triphosphate Trilithium Salt (5). Tetrasodium pyrophosphate decahydrate ($Na_4P_2O_7 \cdot 10H_2O$, 5 mmol, 2.23 g) was converted to the free acid by passage through a Dowex 50 (H^+) column. The solution was evaporated, below 30 °C, and to the free acid residue was added excess pyridine and tri-*n*-butylamine (10 mmol, 2.38 mL) and solution was effected. The material was dried by evaporation with several portions of pyridine. Ribavirin phosphoromorpholidate triethylammonium salt (9) (530 mg, 1 mmol) in an anhydrous solution of pyridine was combined with the above pyrophosphate. The pyridine was evaporated and the mixture was dissolved in 50 mL of fresh, dry pyridine. The container was sealed and the mixture stirred at room temperature for 4 h. The pyridine was evaporated, followed by addition and evaporation of several portions of water. The residue was dissolved in 100 mL of water and the pH was adjusted to 7.5 with 2 N NaOH. This solution was applied to a column (125 mL, 2.5 × 25 cm) of Dowex 2-X8 (Cl^-, 50–100 mesh) resin. Elution was performed with a 3-L gradient going from 0.003 M HCl to 0.5 M LiCl in 0.003 M HCl. Twenty-milliliter fractions were collected and monitored by UV absorption at 220 nm. Ribavirin 5'-diphosphate eluted in fractions 75–110. These fractions were evaporated, and the residue was treated with 50 mL of methanol, then 200 mL of acetone added, and the solution stirred overnight. This process was repeated and the solid filtered to give ribavirin 5'-diphosphate dilithium salt (4) (830 mg). Fractions 125–150 were combined and the pH was adjusted to 6.5 with 0.5 N LiOH. The solvent was removed in vacuo. The residue was dissolved in 100 mL of methanol and diluted with 300 mL of acetone. The solid was collected by centrifugation. The solid was washed several times with 2 mL of MeOH and 10 mL of acetone. It was then dissolved in water and freeze-dried to give 80 mg (14% yield) of ribavirin 5'-triphosphate trilithium salt (5). Anal. Calcd for $C_8H_{12}N_4O_{14}P_3Li_3 \cdot 1.5H_2O \cdot 0.5LiCl$: C, 17.47; H, 2.75; N, 10.18; P, 16.89; Li, 4.44; Cl, 3.22. Found: C, 17.50; H, 2.93; N, 9.90; P, 16.63; Li, 4.44; Cl, 3.11.

TLC showed that the material moved as a single spot with *i*-PrOH–NH_4OH–H_2O (7:1:2) and with isobutyric acid–1 M NH_4OH–0.1 M EDTA (100:60:1.6). The latter solvent system clearly demonstrated the absence of the monophosphate 2 and the diphosphate 4.

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Synthesis and Antihypertensive Activity of Some Ester Progenitors of Methyldopa

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A variety of esters of methyldopa was synthesized with the objective of obtaining derivatives that would be more efficiently absorbed from the gastrointestinal tract than the free amino acid and would undergo conversion to methyldopa readily in the blood or target tissues. Two of the esters, α-pivaloyloxyethyl (**4u**) and α-succinimidoethyl (**4w**), were found to be more potent antihypertensive agents than methyldopa in animal models and were selected for further study in man. The amino esters were prepared by three different methods, including direct esterification of methyldopa without the use of N- or O-protecting groups.

Methyldopa (**1**) is an effective, orally active antihypertensive agent which has found wide use for the reduction of blood pressure in hypertensive subjects. In an attempt to enhance oral absorption and thereby improve antihypertensive potency, a variety of methyldopa derivatives was synthesized with the objective of obtaining compounds that would be more efficiently absorbed from the gastrointestinal tract than the free amino acid and would undergo conversion to methyldopa readily in the blood or target tissues. Of these derivatives, several novel esters of methyldopa were found to have antihypertensive activity in the spontaneously hypertensive (SH) rat. Two esters, **4u** and **4w**, which exhibited good oral absorption and were particularly potent in hypertensive animal models, were selected for further study in man. This report describes the synthesis of some methyldopa esters and their evaluation as antihypertensive agents in the SH rat.

Chemistry. The acid **2b**, in which the catechol and amino functions of methyldopa are protected as the diphenyl ketal[1] and the benzylcarbamate, respectively, could be prepared easily from **1** in 52% overall yield. Initial attempts to esterify **2b** through carboxyl activation, e.g., with dicyclohexylcarbodiimide, and subsequent reaction with alcohols were generally unsuccessful. This is probably

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