THE SYNTHESIS AND STUDY OF NEW RIBAVIRIN DERIVATIVES AND RELATED NUCLEOSIDE AZOLE CARBOXAMIDES AS AGENTS ACTIVE AGAINST RNA VIRUSES

Annual Progress Report

Ganapathi R. Revankar

and

Roland K. Robins

June 1980

(For the period from April 1, 1979 to March 31, 1980)

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-79-C-9046

Department of Chemistry
Cancer Research Center
Brigham Young University
Provo, Utah 84602

DOD Distribution Statement

Distribution limited to U.S. Government agencies only;
studies still in progress

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-B055 800L
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) THE SYNTHESIS AND STUDY OF NEW RIBAVIRIN DERIVATIVES AND RELATED NUCLEOSIDE AZOLE CARBOXAMIDES AS AGENTS ACTIVE AGAINST RNA VIRUSES.	5. TYPE OF REPORT & PERIOD COVERED Annual progress Rept. 1 April 1979 - 31 March 1980
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Ganapathi R. Revankar Roland K. Robins	8. CONTRACT OR GRANT NUMBER(s) DAMD17-79-C-9046
9. PERFORMING ORGANIZATION NAME AND ADDRESS Brigham Young University Provo, Utah 84602 Cancer Research Center	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A803.00.054
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick Frederick, MD 21701	12. REPORT DATE June 1980 13. NUMBER OF PAGES 40
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Distribution limited to U.S. Government agencies only; March 18, 1981. Other requests for this document must be referred to the Commander, U.S. Army Medical Research and Development Command (ATTN: SGRD-RMS) Fort Detrick, Frederick, Maryland 21701.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

In vitro and in vivo testing of certain azole carboxamide nucleosides and nucleotides, such as imidazole, pyrazole and 1,2,4-triazole carboxamide nucleosides related to ribavirin demonstrated that substantial differences in antiviral activity exist depending on the type of viral infection. Improved in vivo antiviral activity was noted for

(see page 2a for continuation)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

To examine the structural parameters necessary for antiviral efficacy of certain azole carboxamide nucleosides and nucleotides, several imidazole, pyrazole and 1,2,4-triazole carboxamide nucleosides have been synthesized. New methods have been developed to prepare ribavirin derivatives. All the compounds prepared during the subject contract have been tested at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick. Ribavirin 3',5'-cyclic phosphate (BJ-58536) exhibited superior in vivo activity against Venezuelan equine encephalitis (VEE) over that of ribavirin. In preliminary

(see page 2a for continuation)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

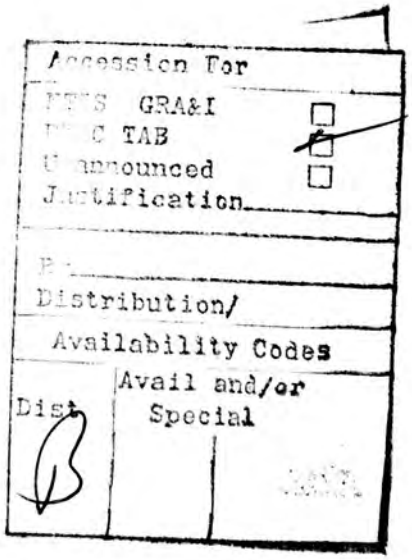
412255

19. continued

ribavirin 3',5'-cyclic phosphate against Venezuelan equine encephalitis over that of ribavirin itself.

20. continued

results on day 20 at 25 mg/kg/day of BJ-58536 there were 20 out of 20 survivors in the VEE-infected mice compared to 8 out of 20 survivors in the control.



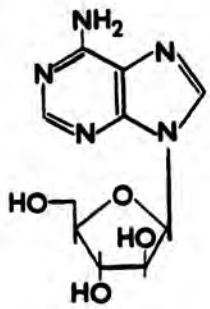
SUMMARY

In the last two decades it has become clear that viruses have programmed certain viral specific enzymes which are responsible for the various stages of viral replication. Several agents are presently available which exhibit specific viral inhibition to a significant degree. Ribavirin is an example of such an agent which has exhibited clinical effectiveness against human RNA viral infection. It is the purpose of the present work to prepare a number of new azole nucleoside and nucleotide derivatives related to ribavirin, based on current knowledge of mechanism of action to gain greater specificity and enhanced potency, especially against RNA viruses which are responsible for virulent viral diseases in tropical parts of the world. Although ribavirin is active in vitro against yellow fever virus, Pichinde virus, Rift Valley fever and Sandfly fever virus, it is least active against Venezuelan equine encephalitis (VEE) virus. Ribavirin triacetate (BJ-29893) has been shown to be highly active (95% Survivors) against Rift Valley fever virus at 100 mg/kg/day in mice. Ribavirin 5'-phosphate (BJ-08456) is more active against VEE than ribavirin in vitro. However, ribavirin 3',5'-cyclic phosphate (BJ-58536), prepared under the subject contract is very potent against VEE in vivo. On day 20 at 25 mg/kg/day of BJ-58536 there were 20 out of 20 survivors in the VEE-infected mice, compared to 8 out of 20 survivors in the control, whereas 19 out of 20 survived at 100 mg/kg/day compared to 8 out of 20 survivals in the control. BJ-58536 also exhibited significant antiviral activity in vitro against parainfluenza, herpes and rhino-13 viruses, indicating ribavirin 3',5'-cyclic phosphate is a very potent antiviral agent.

TABLE OF CONTENTS

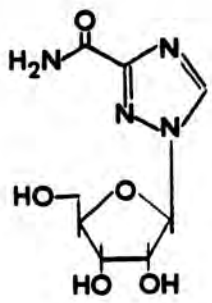
Introduction:

The following is the brief annual progress report on Contract No. DAMD 17-79-C-9046, for the period from April 1, 1979 to March 31, 1980. Progress made in the syntheses and development of antiviral agents has recently been reviewed.[1-4] Substantial progress has been made in recent years. The FDA approval of 9-(β-D-arabinofuranosyl)adenine (ara-A, trade name Vidarabine) for use against herpes infection is indeed a major step forward. The antiviral



ara-A

(Vidarabine)



1

Virazole (Ribavirin)

activity of ara-A has recently been summarized,[5] and even though its use and efficacy would appear to be limited to certain DNA viruses such as herpes, cytomegalo virus and vaccinia virus, and presently must be administered intravenously, it is unquestionably effective against these serious and life-threatening diseases.

The broad spectrum activity of 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (Ribavirin, 1)[6] against influenza, hepatitis, herpes and vaccinia viral infections in vivo have been independently confirmed.[7] The potent in vivo effect of ribavirin against a host of RNA viruses is especially striking.[8-11] The in vivo activity of ribavirin against influenza when administered orally[9-12] or by aerosol spray[13,14] is most remarkable. These animal studies prompted a recent double blind trial of the drug against influenza A (Victoria strain) in humans.[15] Ribavirin was found to be clinically effective in ameliorating influenza symptoms and fever and in diminishing the quantity of influenza virus shed by nasal washing. This beneficial effect is ascribed to its antiviral

activity.[15] Ribavirin is the only known antiviral agent significantly active against a substantial number of both RNA and DNA viruses in vivo.

Ribavirin readily undergoes facilitated transport into cells. It is then phosphorylated enzymatically by adenosine kinase[16,17] within the cell to the 5'-phosphate and then converted enzymatically to the 5'-di- and tri-phosphates.[18,19] Ribavirin inhibits IMP dehydrogenase.[20] Ribavirin is not virucidal and does not induce interferon.[21] It would appear that ribavirin exerts its action by inhibition of a critical step involving viral replication. Ribavirin is very unique in that to date no sensitive rival stain has become resistant to the drug.

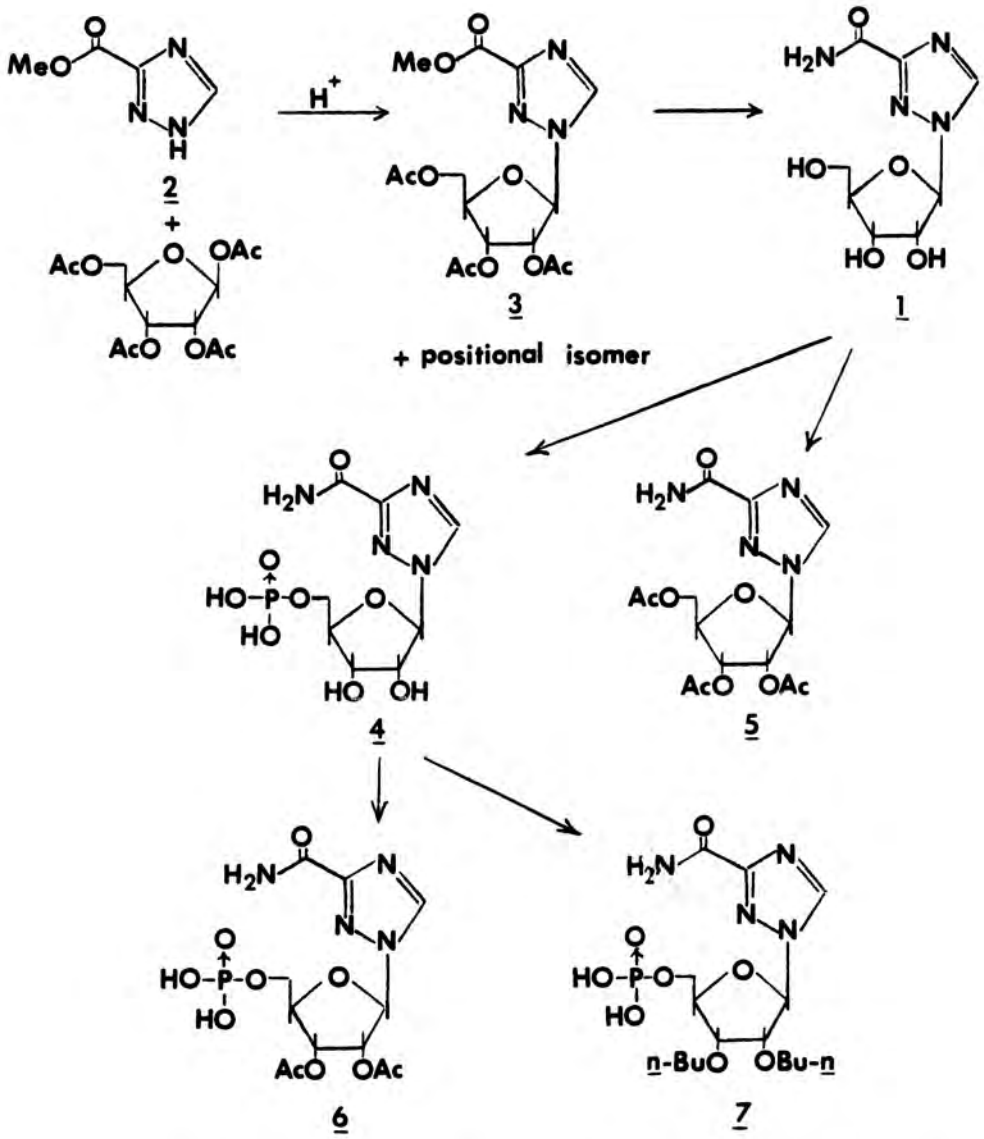
One of the most interesting features of ribavirin is the presence of the carboxamide group which is absolutely essential for its antiviral activity.[22] Presumably the carboxamide group is an essential binding site to the viral RNA polymerase. Ribavirin 5'-phosphate has been shown to be essentially as effective as ribavirin when tested against lethal influenza infections in mice.[23] Also the 2',3'-cyclic phosphate of ribavirin appears, in cell culture, to be superior to ribavirin against herpes 1 and parainfluenza virus.[23] In view of these findings it is quite conceivable that certain ribavirin derivatives may offer significant advantage over the parent nucleoside in drug formulation, drug transport or provide greater organ concentration of the active form of the drug. Therefore, it was proposed that a number of ribavirin derivatives of certain related nucleoside azole carboxamides be prepared and studied for antiviral efficacy.

During the past year we initiated the synthetic program designed to provide the selected ribavirin derivatives and related azole nucleosides. Thirty-one of such compounds were prepared and submitted to Medical Research and Development Command, Walter Reed Medical Center, U.S. Army, for antiviral evaluation. The progress made in the synthetic aspect could be divided into three categories.

1. Synthesis of Ribavirin Derivatives

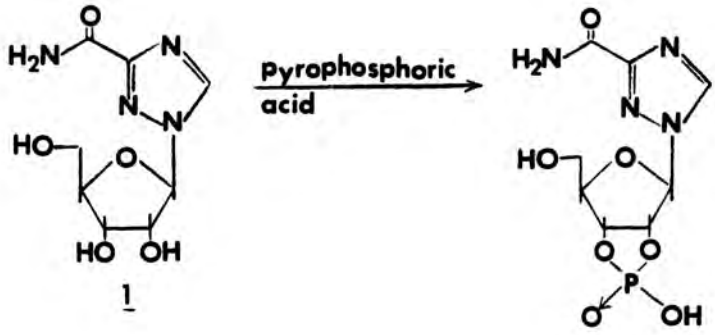
The starting material required for the syntheses of the target compounds in this category was 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (Ribavirin, 1). A relatively large amount (500 g) of ribavirin was prepared by the method developed by Witkowski, et al.[16] The acid-catalyzed [bis(p-nitrophenyl)phosphate] fusion of methyl 1,2,4-triazole-3-carboxylate (2)[24] and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose[25] under reduced pressure (8-10 mm) at 160-165°C provided an 85% yield of methyl 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (3) and trace amount of the positional isomer methyl 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylate which were separated by fractional crystallization. Deacetylation of 3 with concomitant amination of the methyl ester function was achieved in over 90% yield by the treatment of 3 with methanolic ammonia (methanol saturated with ammonia at 0°C) at ambient temperature to obtain ribavirin (1).

In order to obtain ribavirin derivatives which would be more lipid soluble, ribavirin 2',3',5'-tri-O-acetate (5) was prepared by the treatment of 1 with acetic anhydride in pyridine at room temperature. Two crystallizations of the crude product from ethanol gave analytically pure 5. Ribavirin 5'-monophosphate (4) was prepared[20] by phosphorylation of the unprotected ribavirin with phosphoryl chloride at 0°C for 7-8 hr, followed by subsequent column chromatography over activated charcoal (Barnebey Cheney) and cation-exchange resin (Bio-Rad AG50W-X2, H^+ form) provided 4 as the free acid in a 50% yield. By allowing ribavirin 5'-monophosphate to react with acetic anhydride in anhydrous pyridine at ambient temperature for 2 hr, a 65% yield of 2',3'-di-O-acetylribavirin 5'-monophosphate dihydrate (6) was obtained after ion-exchange resin (Bio-Rad, Dowex 50W-X8,H^+ form) chromatography. A similar acylation of 4 with n-butyric anhydride in anhydrous pyridine in the presence of 4-dimethylaminopyridine gave 2',3'-di-



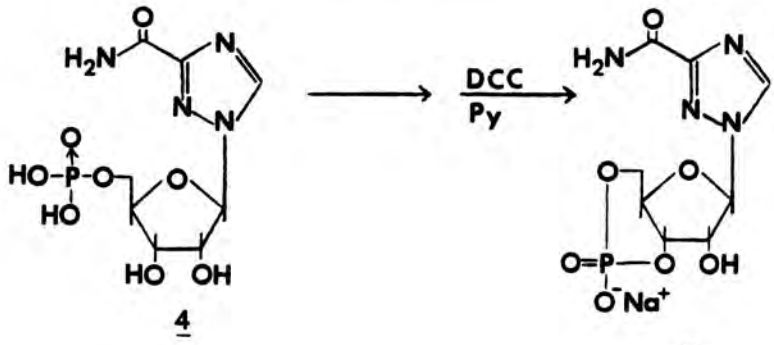
O-n-butyrylribavirin 5'-monophosphate (7), in a rather low yield. Extensive purification by ion-exchange resin (Dowex 1-X2, formate form) and silica gel column chromatography is needed to obtain analytically pure sample. The compounds 5-7 may act as a type of pro-drug form which would be especially effective in reaching certain target organs with liphophilic barriers.

In view of the good in vitro antiviral activity of ribavirin 2',3'-cyclic phosphates (8), more of this compound was prepared for evaluation in animals.



1 8

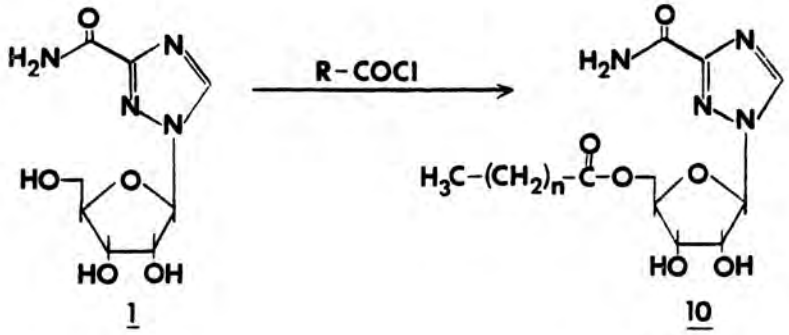
The procedure described by Ueda and Kawai[26] was utilized by using pyrophosphoric acid in boiling N,N-dimethylformamide in the presence of tri-n-butylamine to convert ribavirin to the cyclic 2',3'-phosphate (8),[23] which was isolated as the ammonium salt in 20% yield. Treatment of the 4-morpholino-N,N-dicyclohexylcarboxamidine salt of ribavirin 5'-phosphate (4) in pyridine with dicyclohexylcarbodiimide under high-dilution conditions according to the general procedure of Smith et al.[27] gave 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 3',5'-cyclic monophosphate (9), isolated as the sodium salt, in 25% yield.[23]



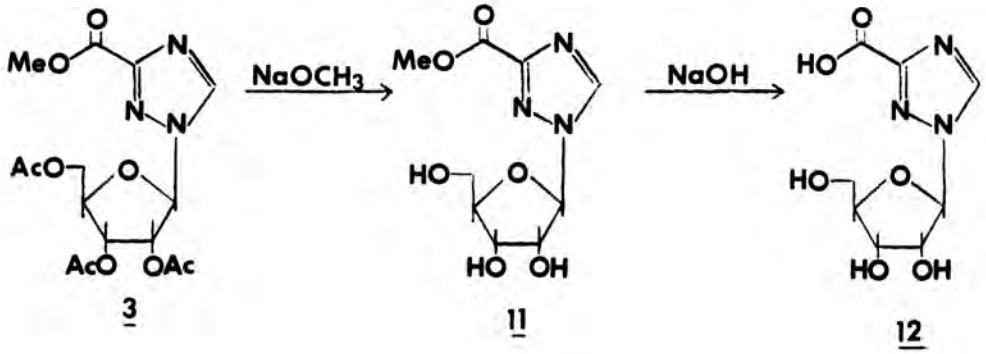
4 9

In view of the recently reported[28] marked increase in antiviral activity of 5'-O-acyl-ara-A (particularly 5'-O-valeryl-ara-A) over that of ara-A itself, the synthesis of certain selected 5'-O-acyl derivatives of ribavirin was undertaken. It is of particular interest to know that 5'-O-butyrylribavirin has

shown superior activity to ribavirin against influenza Japan 305 viral infections in mice.[29] These 5'-O-acyl derivatives of ribavirin (10), designed as prodrugs for ribavirin, should offer a range of solubility, transport characteristics and lipophilic nature differing from ribavirin itself.



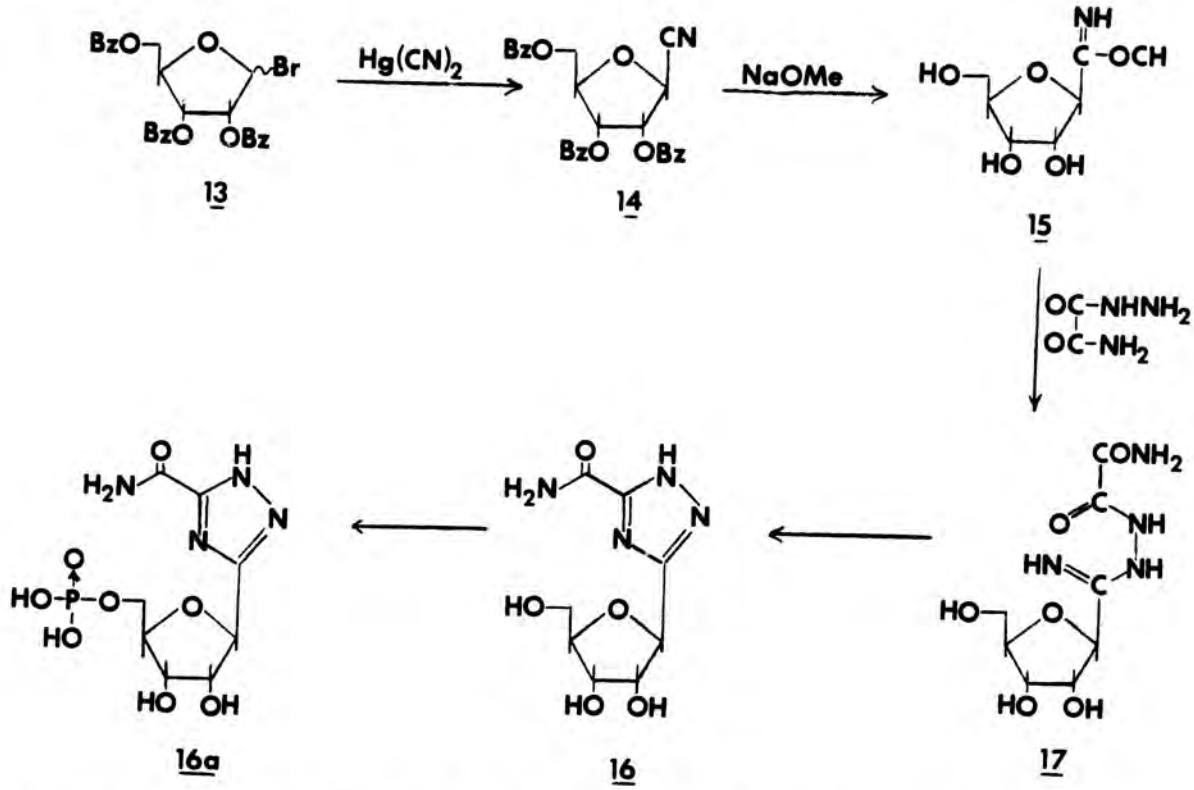
Direct acylation of the free nucleoside ribavirin was accomplished by adding 1.1 equivalent of the appropriate acyl chloride (Butyryl, valeryl or n-caproyl chloride) to a solution of the nucleoside in 1:1 mixture of pyridine-N,N-dimethylformamide. This solvent mixture was found to greatly facilitate the selectivity of the acylation of the primary hydroxyl group over either of the secondary hydroxyl groups. This could be a consequence of having the acylating agent in a charged species (like N-acylpyridinium chloride) in an aprotic, nonpolar solvent such as DMF.[30] The 5'-acylated nucleosides (10) were isolated in over 60% yield as crystalline solids after column chromatography over silica gel to separate minor, peracylated contaminants and unreacted



ribavirin. That the carbamoyl function on the aglycon remained unacylated was confirmed by their PMR spectra in DMSO-d_6 that typically showed the carbamoyl protons at δ7.6 and 7.8.

Deacetylation of methyl 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (3) by the treatment of sodium methoxide in methanol afforded the deblocked nucleoside methyl 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (11) in 93% yield. Hydrolysis of the methyl ester 11 with aqueous sodium hydroxide gave a good yield of 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylic acid (12).[6]

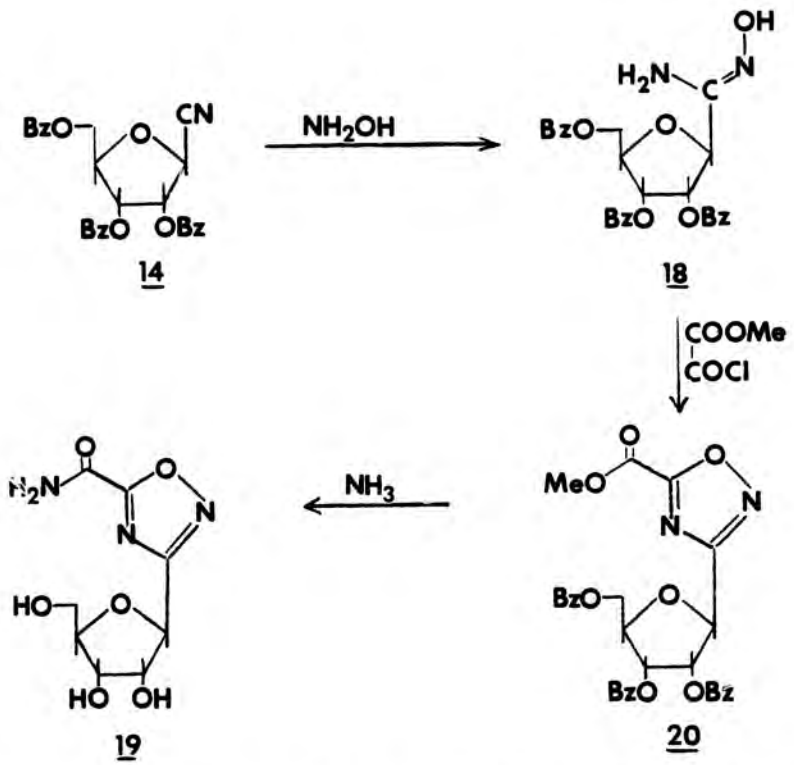
We then undertook the total synthesis of a C-nucleoside analogue of ribavirin (16). The unique structural characteristic which distinguishes this nucleoside is the presence of a stable carbon-to-carbon linkage instead of a carbon to nitrogen bond between C-1 of the carbohydrate moiety and the aglycon of ribavirin. It is of interest that several C-nucleoside antibiotics (e.g. pyrazomycin, formycin, etc.) exhibit significant antiviral activity. The great advantage of such C-nucleosides is that they are stable to enzymatic phosphorolysis whereas most N-nucleosides suffer this type of metabolic degradation. Thus, the C-nucleosides could maintain higher tissue levels for longer periods of time. Of the three independent syntheses reported for the C-nucleoside analogue of ribavirin,[31-33] we elected to use the procedure of Poonian and Nowoswiat.[32] The starting sugar component 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl cyanide (14) was prepared according to the reported procedure,[34] which on treatment with catalytic amounts of $NaOCH_3$ in CH_3OH at room temperature for an hour led to the formation of the debenzoylated imidic ester (15) in 75% yield. Treatment of 15 with stoichiometric amount of oxamidohydrazide in anhydrous dimethyl sulfoxide at room temperature for 18 hours gave almost quantitative yield of 17. When the precursor 17 was heated at 135°C under 0.1 mm vacuum, dehydrative



ring closure occurred to give an 80% yield of 3-(β-D-ribofuranosyl)-1,2,4-triazole-5-carboxamide, the C-nucleoside analogue of ribavirin (16). All the physicochemical properties of 16 are in good agreement with those reported[32] for this compound. Phosphorylation of unprotected 16 with phosphonyl chloride gave 3-(β-D-ribofuranosyl)-1,2,4-triazole-5-carboxamide 5'-monophosphate (16a), isolated as the sodium salt.

The isosteric relationship between 1,2,4-triazole and 1,2,4-oxadiazole strongly suggested the synthesis of the C-nucleoside analogue in the 1,2,4-oxadiazole ring system related to 16. The most frequently encountered route for the synthesis of substituted 1,2,4-oxadiazoles involves the acylation and subsequent cyclization of amidoximes. The most common method for the synthesis of amidoximes involves the coupling of nitriles with hydroxylamine.[35] For our

purposes, the key intermediate would be 2,5-anhydro-3,4,6-tri-O-benzoyl-D-allonamidoxime (18), and this compound was obtained in 85% yield by the reaction

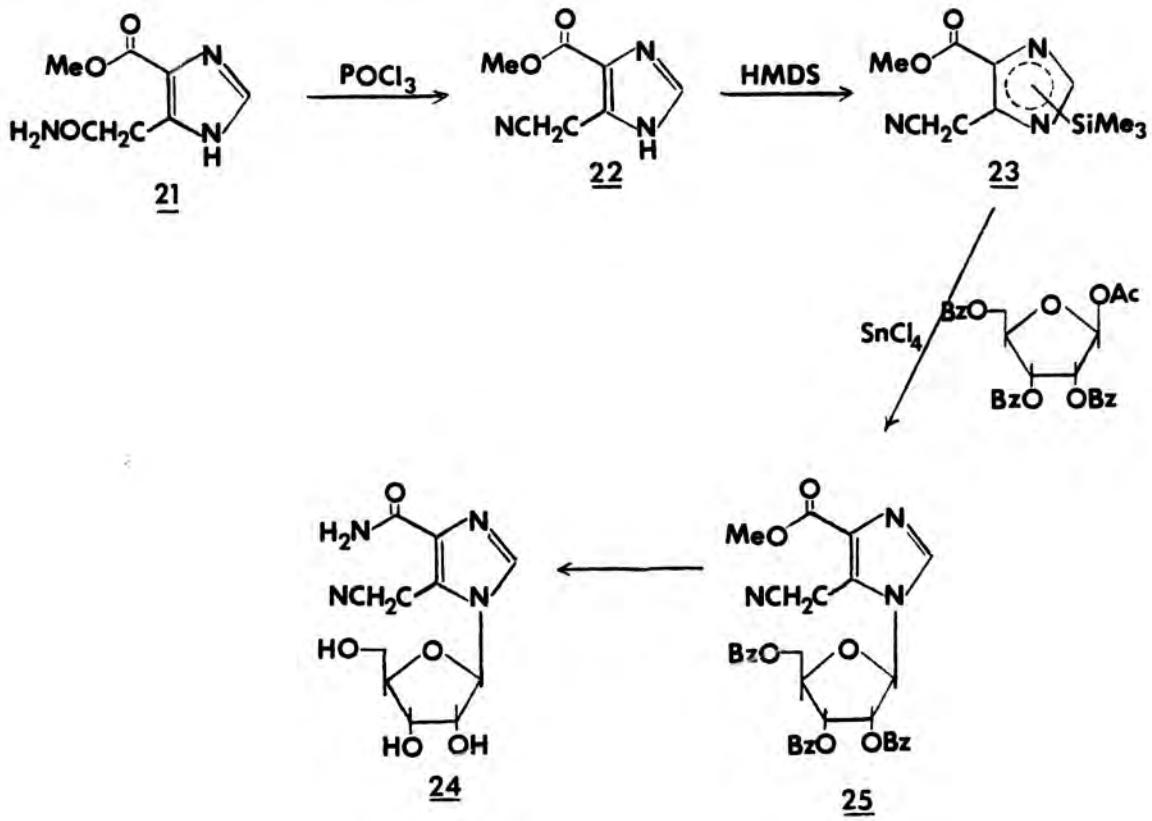


of the readily available 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl cyanide (14)[34] with free hydroxylamine in ethanol according to the procedure of Revankar and Robins.[36] It was necessary to effect purification by column chromatography on silica gel in order to remove several minor polar by-products arising, presumably, from partial debenzoylation. The amidoxime 18 was then subjected to ring closure reaction by the treatment with methyl oxalylchloride in anhydrous dimethoxyethane at reflux temperature to obtain 20. The cyclization occurs, presumably via the O-acyl intermediate, in 70% yield. Compound 20 was obtained as a homogeneous yellow gum following column chromatography on silica gel. Debenzoylation of 20 with concomitant amination of the methyl ester function was readily achieved in over 80% yield using methanolic ammonia (methanol saturated with ammonia at 0°C) at ambient temperature giving 3-(β-D-ribofuranosyl)-1,2,4-oxadiazole-5-

carboxamide (19), the structure of which was assigned by the first-order PMR and elemental analyses.

2. Synthesis of Imidazole Carboxamide Nucleosides Related to Ribavirin

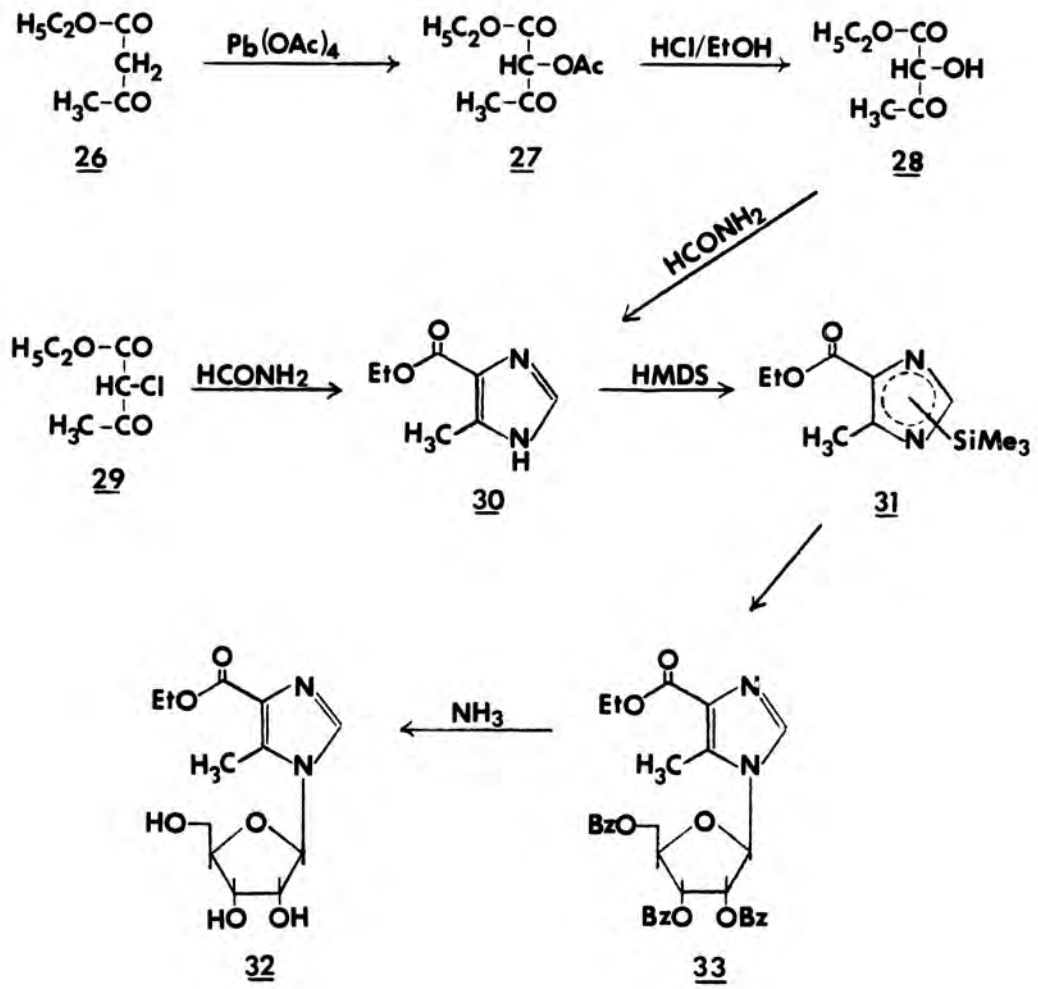
In the imidazole carboxamide nucleoside series, the preparation of 5-cyanomethyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (24)[37] was accomplished



via a repetitive four-step synthetic sequence starting with methyl 5(4)-carbamoylmethylimidazole-4(5)-carboxylate (21).[38] The key intermediate in the synthesis of 24 is methyl 5(4)-cyanomethylimidazole-4(5)-carboxylate (22), and was obtained in good yield by dehydration of 21 with boiling phosphoryl chloride. The conventional trimethylsilylation[39] of 22, which involved heating the heterocyclic base with hexamethyldisilazane (HMDS) in the presence of the catalyst $(NH_4)_2SO_4$ gave the oily trimethylsilyl derivative (23). Without extensive purification 23 was condensed with one equivalent of 1-O-acetyl-

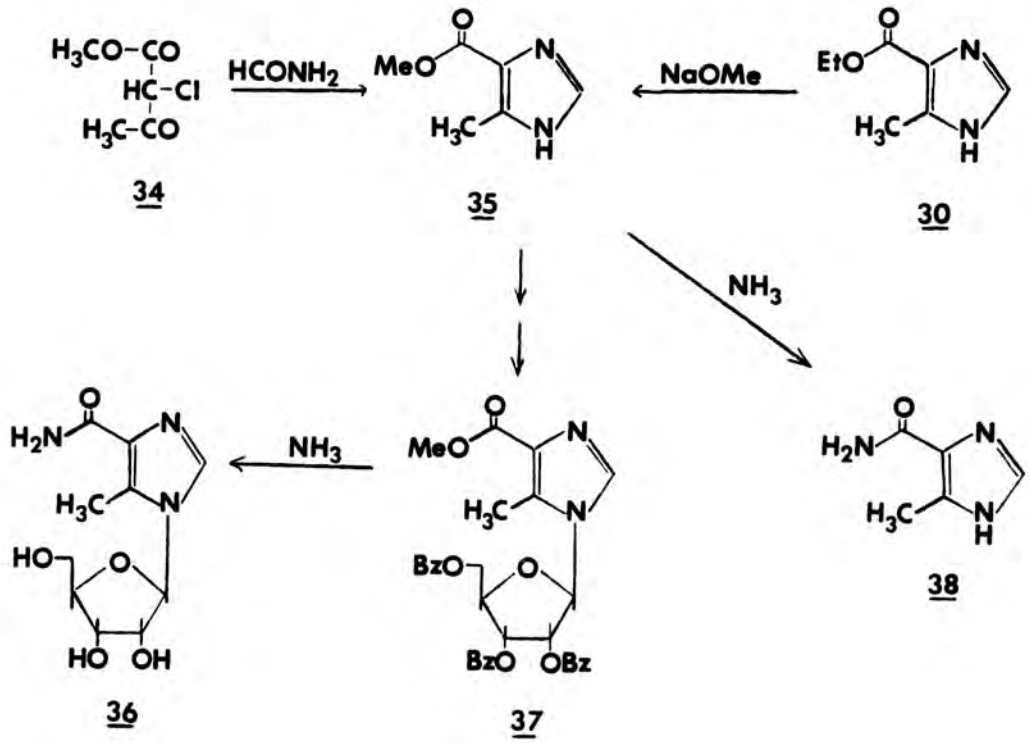
2,3,5-tri-O-benzoyl-β-D-ribofuranose[25] in the presence of 1.44 molar equivalent of anhydrous $SnCl_4$ in dry 1,2-dichloroethane at ambient temperature for 25 hr. Under these conditions and after silica gel column chromatography using benzene-ethyl acetate (1:1) as solvent, afforded almost quantitative yield of methyl 5-cyanomethyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (25). Debenzoylation of 25 with concomitant amination of the methyl ester function was achieved by the treatment of 25 with liquid ammonia (3 hr , 100°C), followed by silica gel column chromatography of the reaction residue using chloroform-methanol (4:1) as eluent, provided 5-cyanomethyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (24) in 80% yield. All the physicochemical properties of 24 are similar to those described for this compound by Robins and co-workers.[37]

The next target was the synthesis of 5-methyl-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (36). On the basis of our previous experience, we elected to use ethyl 5(4)-methylimidazole-4(5)-carboxylate (30) for the glycosylation studies. The synthesis of 30 involved the cyclization of ethyl α-hydroxyacetoacetate (28) with a one carbon unit.[40] Reaction of freshly prepared lead tetraacetate with acetoacetic ester in anhydrous benzene gave α-acetoxyacetoacetic ester (27) which was subsequently converted to the intermediate 28 with ethanolic hydrogen chloride. The intermediate 28 was immediately cyclized with formamide at relux temperature for 3 hr to obtain ethyl 5(4)-methylimidazole-4(5)-carboxylate (30) in rather low yield (~20% overall). In order to obtain larger quantities of 30, which was needed for the detailed glycosylation studies, an alternate route[41] was followed. Treatment of commercially available[42] ethyl 2-chloroacetoacetate with formamide in the presence of a molar equivalent of water at 155°C for 5 hr gave 30, in over 60% yield. After several recrystallizations from water, compound 30 was isolated in pure form.



In recent years, the more stable 1-acyloxy derivatives of suitably protected furanoses in place of 1-halo sugars have been widely employed to couple with trimethylsilylated heterocyclic bases in the presence of Lewis-acid catalyst (particularly $SnCl_4$ and at room temperature) to obtain the blocked nucleosides, exclusively with β-configuration.[43] We employed this elegant procedure for the preparation of imidazole nucleosides. The trimethylsilylation of 30 by heating the base with HMDS in presence of the catalyst $(NH_4)_2SO_4$ gave the syrupy trimethylsilyl derivative, which was distilled under reduced pressure to obtain 31 as pure, colorless liquid of b.p. 98-100°C/0.1 mm, in a 90% yield. As in the case of the synthesis of 25, the trimethylsilyl

derivative 31 was condensed with one equivalent of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in presence of 1.44 molar equivalent of anhydrous $SnCl_4$ in dry 1,2-dichloroethane at ambient temperature for 24 hr. After the standard workup, a quantitative yield of crystalline ethyl 5-methyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (33) was obtained. Several of our conventional attempts (with liquid NH_3 or methanolic NH_3 at room as well as elevated temperatures) to effect debenzoylation with concomitant ammonolysis of the ethyl ester function of 33 have not been successful, and only ethyl 5-methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxylate (32) was isolated in good yield. The strategy for the successful synthesis of the required 5-methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (36), however, involved the ammonolysis of the corresponding methyl ester (37). The starting aglycon methyl 5-methylimidazole-4-carboxylate (35) was analogously prepared

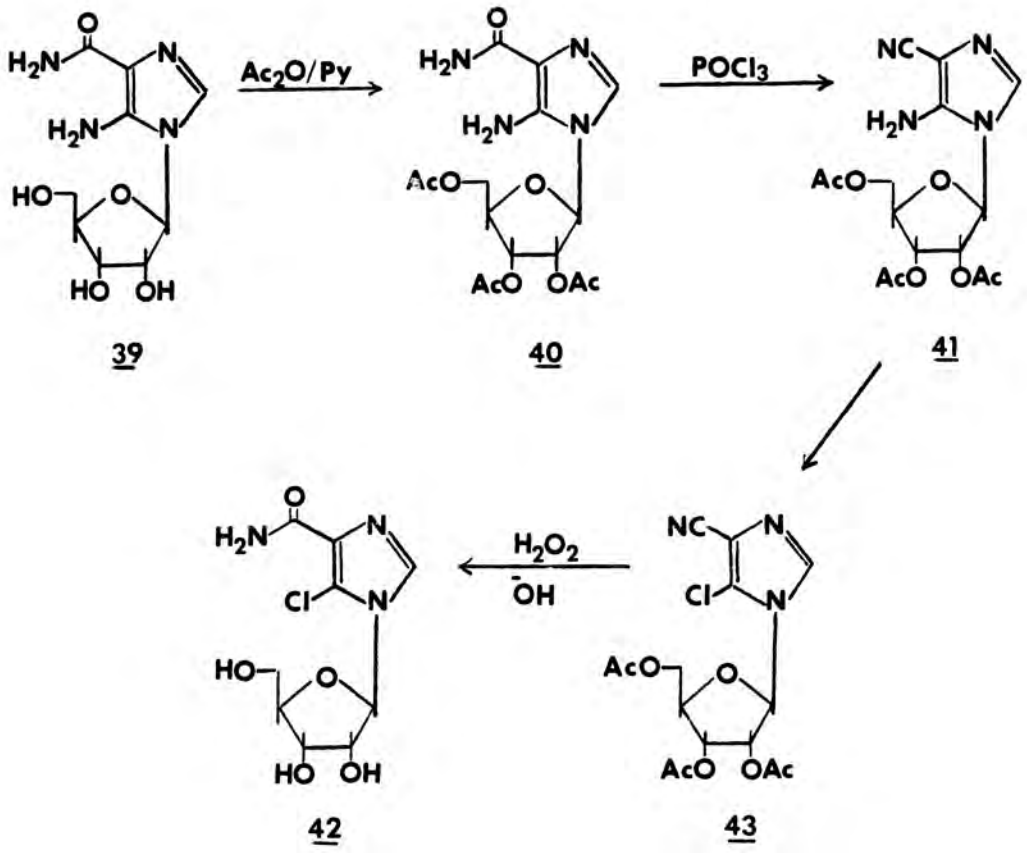


34 35 30 38 36 37

by the treatment of methyl 2-chloroacetoacetate (34) with formamide in the presence of one molar equivalent of water at 140°C for 5 hrs. However, the yield of 35 was rather low (~22%). The aglycon 35 was also obtained by the transesterification of 30 with sodium methoxide in methanol at reflux temperature for 24 hr. The compound 35 prepared by both the routes is found to be identical in all respects. Treatment of 35 with liquid ammonia at 100-110°C for 15 hr provided 5(4)-methylimidazole-4(5)-carboxamide (38). The ir spectrum of 38 showed the amide carbonyl band at 1660 cm^{-1}, in place of the ester carbonyl band of 35 at 1720 cm^{-1}. Trimethylsilylation of 35 with HMDS in presence of $(NH_4)_2SO_4$ gave, after vacuum distillation, a 90% yield of colorless, oily trimethylsilyl derivative. The $SnCl_4$ catalyzed glycosylation of this TMS-imidazole with 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in 1,2-dichloroethane gave, after silica gel column chromatography, syrupy blocked nucleoside methyl 5-methyl-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazole-4-carboxylate (37). When 37 was treated with methanolic ammonia at 100-110°C for 3 days, a 37% yield of 5-methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide(36) was obtained. The TLC of the reaction mixture indicated a complex mixture, thus the isolation and purification of 36 was much more difficult and cumbersome. A combination of silica gel column chromatography and repeated recrystallization, however, resulted in the isolation of analytically pure 36.

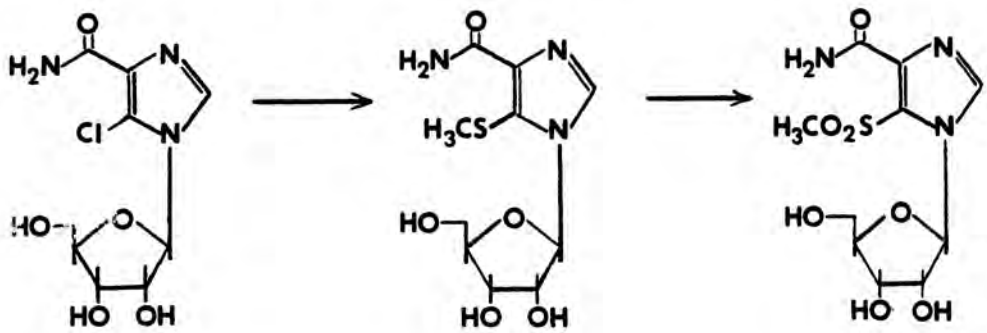
In view of the recent disclosure by DeClercq and Luczak[44] that 5-fluoro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide exhibited potent and broad spectrum antiviral activity against RNA viruses in vitro, it was proposed to prepare certain 5-halogen and mercapto substituted 4-carbamoylimidazole nucleosides. In this regard, the versatile nucleoside, 5-chloro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (42) was prepared by the method of Srivastava, et al.[45] Acetylation of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (39, AICAR) with acetic anhydride in pyridine at low temperatures (0-5°C) provided a quantitative yield

of tri-O-acetyl-AICAR (40) as crystalline solid. Dehydration of 40 with phosphorous oxychloride in the presence of triethylamine at reflux temperature followed by silica gel column chromatography using chloroform-acetone (8:2,v/v) as the solvent gave pure, syrupy 5-amino-4-cyano-1-(2,3,5-tri-O-acetyl-β-D-



ribofuranosyl)imidazole (41).[46] When a concentrated solution of 41 in methanol was treated with sodium nitrite solution at -25 ± 3°C in the presence of 6 N hydrochloric acid, followed by cuprous chloride, a 42% yield of crystalline 5-chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile (43) was obtained. In this reaction, the formation of a significant amount (10-15%) of dehalogenated product 1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile was also observed. This hydrogenodediazoniation probably arises via the formation of an aryl (imidazolyl) radical which can abstract a proton from the solvent. Hydrolysis of 43 with hydrogen peroxide in ammonium hydroxide provided the required 5-chloro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (42).

Compound 42 served as a versatile intermediate for the synthesis of a variety of 5-substituted-1-(β-D-ribofuranosyl)imidazole-4-carboxamides. Thus, treatment of 42 with $KSCH_3$ in alkaline media gave good yield of 5-methylthio-1-



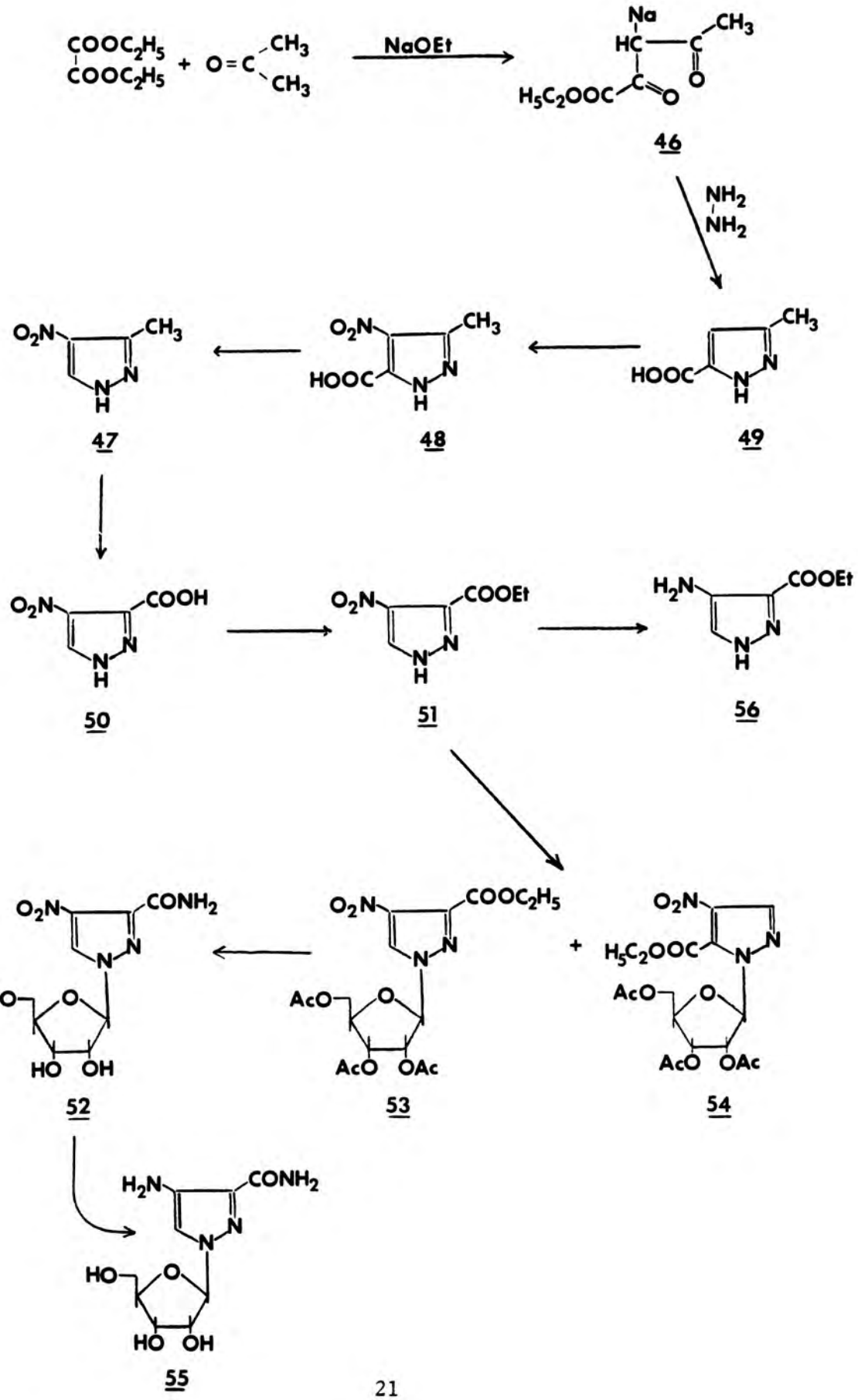
42 44 45

(β-D-ribofuranosyl)imidazole-4-carboxamide (44) which, on subsequent oxidation with m-chloroperbenzoic acid gave 5-methylsulfonyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (45). Since the methylsulfonyl group is an excellent leaving group, the chemical and physical properties of 45 will be fully explored.

3. Synthesis of Pyrazole Carboxamide Nucleosides Related to Ribavirin

The synthesis of pyrazole carboxamide nucleosides related to ribavirin was achieved using ethyl 4-nitro-pyrazole-3-carboxylate (51), first synthesized by Robins, et al.[47] in 1956. About 200 g. of this useful intermediate has been prepared by the sequence of reactions as shown below.

Condensation of diethyl oxalate with anhydrous acetone in presence of freshly prepared sodium ethoxide in ethanol gave an 85% yield of sodium ethyl acetopyruvate (46).[48] Treatment of 46 with 85% hydrazine hydrate at 25-30°C followed by acidification of the reaction mixture with H_2SO_4 gave 3-methylpyrazole-5-carboxylic acid (49) in a 63% yield. Nitration of 49 with a mixture of 90% HNO_3 and fuming H_2SO_4 at 100°C for 3 hr gave a 43% yield of 3-methyl-4-nitro-

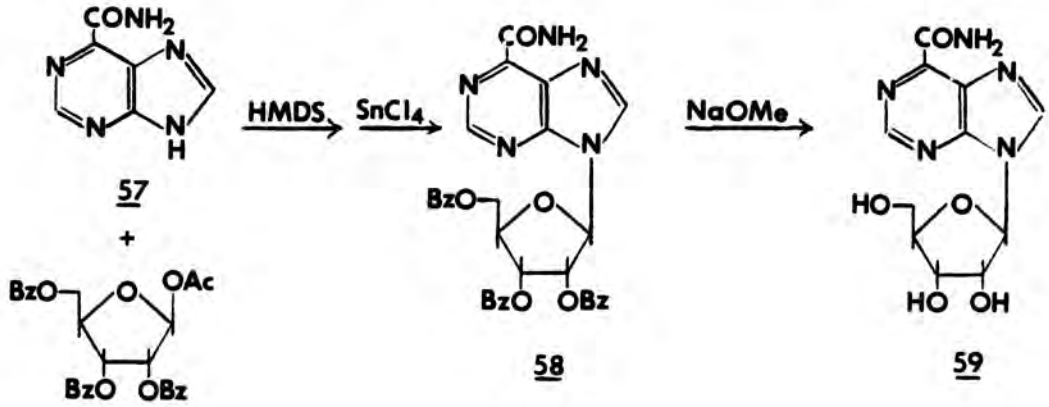

NaOEt
46
47
48
49
50
51
56
52
53
54
55

pyrazole-5-carboxylic acid (48).[49] Decarboxylation of 48 by heating at 130°C followed by crystallization from water gave 4-nitro-3-methylpyrazole (47).[47] $KMnO_4$ oxidation of 47 in a large excess of water furnished 4-nitropyrazole-3-carboxylic acid (50), which on esterification with absolute ethanol in the presence of H_2SO_4 gave the key intermediate ethyl 4-nitropyrazole-3-carboxylate (51).[47]

Glycosylation of ethyl 4-nitropyrazole-3-carboxylate (51) was accomplished following the elegant procedure described by Preobrazhenskaya et al.[50] Non-catalytic fusion of 51 with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose at 150-155°C for 3 hr gave a mixture of ethyl 4-nitro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-pyrazole-3-carboxylate (53) and the positional isomer ethyl 4-nitro-2-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-3-carboxylate (54). The mixture of these two isomers were separated on a silica gel column using chloroform-methanol (19:1) as the solvent. The N-2 glycosyl isomer (54) was eluted first. Deacetylation of 53 with concomitant amination of the ethyl ester function was achieved in good yield by the treatment of 53 with methanolic ammonia (methanol saturated with ammonia at 0°C) at room temperature for five days, to obtain crystalline 4-nitro-1-(β-D-ribofuranosyl)pyrazole-3-carboxamide (52). Catalytic reduction of 52 in presence of Pd/C readily gave 4-amino-1-(β-D-ribofuranosyl)-pyrazole-3-carboxamide (55) in over 82% yield. All the spectroscopic and analytical data is in support of this structure. A similar reduction of 51 in presence of Pd/C in methanol gave a good yield of ethyl 4-aminopyrazole-3-carboxylate (56). Compound 56 should prove to be a versatile starting material for the preparation of 4-fluoro-1-(β-D-ribofuranosyl)pyrazole-3-carboxamide.

We also initiated a synthetic program to prepare certain novel purine nucleosides containing a carboxamide function. The formation of the carbon-carbon bond at the 6-position of purine nucleoside through the nucleophilic

displacement of the corresponding halogenopurine nucleosides has been generally unsuccessful. Although the synthesis of C_6-substituted purine nucleosides could be approached by several routes, we elected to glycosylate directly the appropriate purine base. Treatment of purine-6-carboxamide (57) with hexa-



57 + 58 59

methyldisilazane in presence of ammonium sulfate gave the trimethylsilyl derivative which, without further purification, was treated with one equivalent of 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose in presence of 1.4 molar equivalent of anhydrous $SnCl_4$ in 1,2-dichloroethane at ambient temperature for 24 hr. Under these conditions and after silica gel column chromatography, an 83% yield of crystalline nucleoside material was obtained which was identified as 9-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)purine-6-carboxamide (58). Careful investigation furnished chromatographic evidence of the formation of other nucleoside material in very minor amount, presumably the positional isomer. Debenzoylation of 58 with methanolic sodium methoxide at ambient temperature gave a good yield of 9-(β-D-ribofuranosyl)purine-6-carboxamide (59). The purity of 59 was assured by elemental analysis and by PMR spectroscopy.

Antiviral Evaluation:

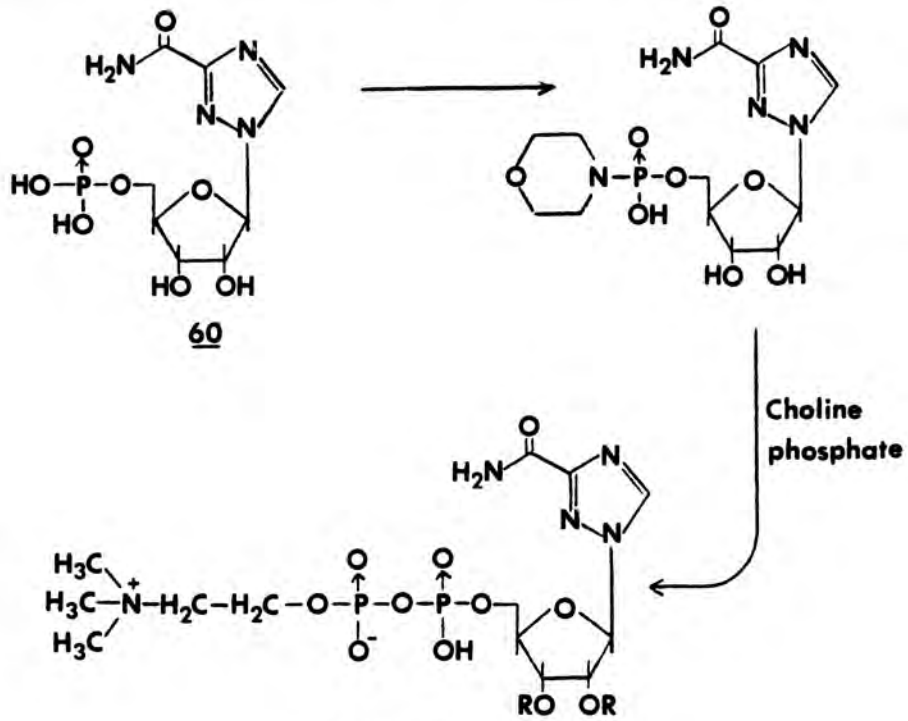
Most of the compounds synthesized during the report period were tested against a variety of viruses in tissue culture as well as in mice. Of the

compounds tested, in parallel with ribavirin, ribavirin 5'-monophosphate (RA-84) and 2',3'-di-O-acetylribavirin 5'-monophosphate (RA-83) were found to be more potent than 2',3',5'-tri-O-acetylribavirin against Venezuelan Equine Encephalitis (VEE), Rift Valley Fever (RVF), Pichinde (PICH) and Yellow Fever (YF) viruses in cell culture. The antiviral activity of RA-83 and RA-84 is comparable to that of ribivirin. While ribavirin 5'-monophosphate is highly active against PICH, VEE and RVF in relatively low concentrations, 2',3'-di-O-butyryl-ribavirin 5'-monophosphate (RA-90) and 5-chloro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (RA-105) are moderately active against PICH and RVF. 5'-O-Valeryl- (RA-121) and 5'-O-caproyl- (RA-119) derivatives of ribavirin are more potent than RA-105 against PICH and VF viruses. Ribavirin 2',3'-cyclic phosphate (RA-114) is moderately active against PICH. The C-nucleoside analog of ribavirin (RP-14) and the rest of the compounds, ethyl 5(4)-methylimidazole-4(5)-carboxylate (RB-122), ethyl 5-methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxylate (RB-124), 5-cyanomethyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (RB-125), 4-nitropyrazole-3-carboxylic acid (RK-15), 3-(β-D-ribofuranosyl)-1,2,4-oxadiazole-5-carboxamide (SB-111) and 5-chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-imidazole-4-carbonitrile (RA-125) are essentially inactive against the viruses used. It is of considerable interest that 9-(β-D-ribofuranosyl)purine-6-carboxamide (RI-51A) showed significant antiviral activity against PICH and RVF viruses. In view of this significant antiviral activity of RI-51A, more of this compound will be made for detailed antiviral evaluation.

Work in Progress:

Since ribavirin is known to have difficulty penetrating the blood brain barrier, the synthesis of certain nucleotide diphosphate sugar analog of ribavirin is undertaken. As cytidine diphosphate choline readily cross the blood brain barrier, the logical analog to be prepared is ribavirin diphosphate

choline (61). At the present time the preparation of large amounts of ribavirin 5'-monophosphate, the key intermediate for the synthesis of 61, is under progress. Ribavirin 5'-monophosphate will then be converted to more active 1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-phosphoromorpholidate by the procedure recently described by Robins et al.[23] The morpholidate deriv-



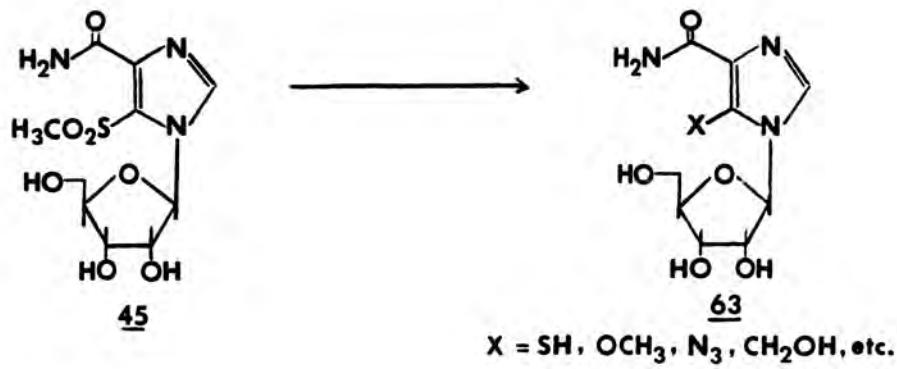
60

61, R = H

62, R = Ac

tive will then be condensed with choline phosphate in the presence of a suitable condensing agent (like DCC). The corresponding 2',3'-di-O-acetyl derivative (62) will also be prepared. These compounds should provide a form of the drug which should penetrate the brain and eventually be enzymatically converted to ribavirin 5'-diphosphate and further kinased to ribavirin 5'-triphosphate, the presumed active form of the drug. Such a nucleoside diphosphate sugar would provide an ideal form of the drug for i.v. treatment. Should such compounds prove useful or superior to ribavirin, different sugars (like 2-deoxy and 2-amino-D-glucose) will be employed in the synthetic scheme.

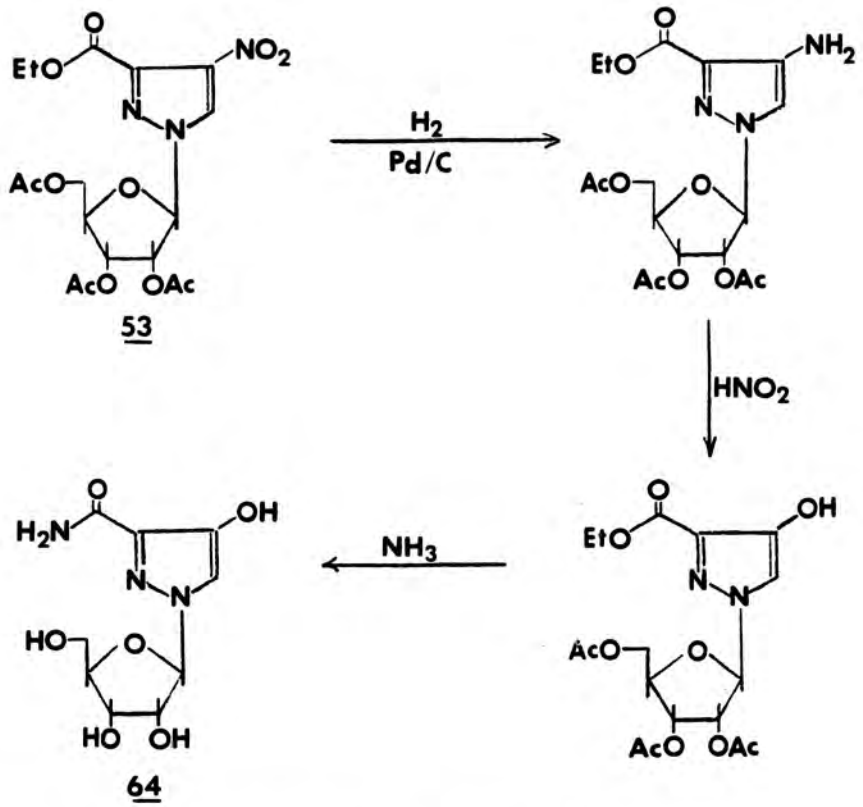
The synthesis of a number of 5-substituted-1-(β-D-ribofuranosyl)imidazole-4-carboxamides of the general formula 63 is also under progress. 5-Methylsulfonyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (45) is proved to be an excellent starting material and larger amounts of 45 will be prepared in order



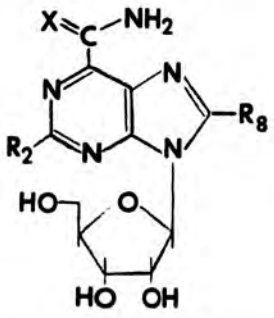
45 63

to study various nucleophilic displacement reactions to provide the appropriately fimctopma;ozed imidazole carboxamide nucleosides, including thiobredinin.

In the area of pyrazole-carboxamide nucleosides, the conversion of ethyl 4-nitro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-3-carboxylate (53) to the corresponding N-glycosyl analogue (64) of broad spectrum antiviral nucleoside antibiotic pyrazomycin is under progress. The protected nucleoside 53 should prove to be an excellent starting material. Reduction of 53 with Pd/C in an hydrogen atmosphere should give the corresponding 4-amino derivative which could be further deaminated with aqueous nitrous acid to provide ethyl 4-hydroxy-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)pyrazole-3-carboxylate. Deacetylation with concomitant amination of the ester function with methanolic ammonia should give the N-glycosyl analogue of pyrazomycin (64). Reduction of 53 followed by diazotization and displacement of the diazo group to the corresponding 4-halo (particularly F) analogue will also be explored.



To examine the structural parameters necessary for antiviral potency of 59, the synthesis of several 2- and 8-substituted-9-(β-D-ribofuranosyl)purine-6-carboxamides (and thiocarboxamides) (65) is also under progress. The unambiguous



65

X = O, S

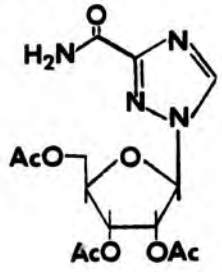
R_2 = H, NH_2, CH_3

R_8 = H, $CONH_2$

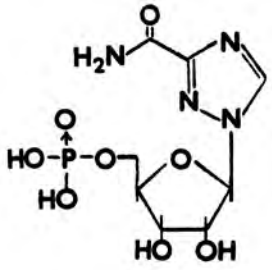
assignment of anomeric configuration and proof of site of ribosylation of these purine nucleosides will present a real challenge.

The following thirty-one compounds have been prepared and submitted to Medical Research and Development Command, Walter Reed Medical Center, U.S. Army, for antiviral evaluation, each in pure form. The chemical structure of each of these compounds is shown below:

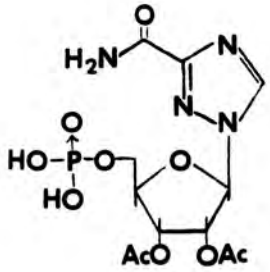
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
1.	1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	RA-98	BJ-29893	p.7



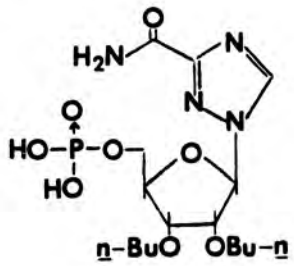
2.	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-monophosphate	RA-84	BJ-08456	Ref.20



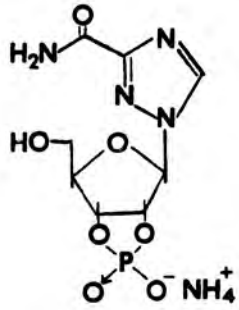
3.	1-(2,3-Di-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-monophosphate	RA-83	BJ-22483	p.7



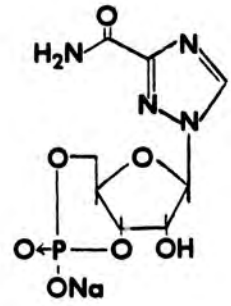
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
4.	1-(2,3-Di-O-butyryl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide 5'-phosphate	RA-90	BH-22492	p.7



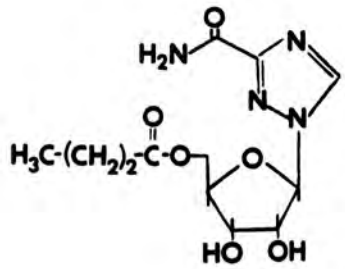
5.	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide 2',3'-cyclic phosphate ammonium salt	RA-114	BJ-45511	Ref.23



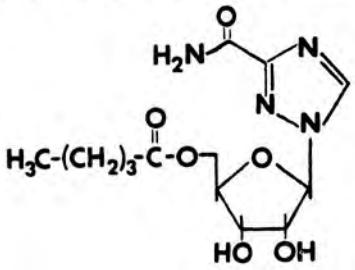
6.	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxamide 5',5'-cyclic phosphate sodium salt	RV-33	BJ-58536	Ref.23



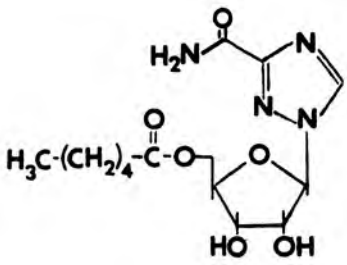
7.	1-(5-O-Butyryl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	RA-116	BJ-45529	p.10



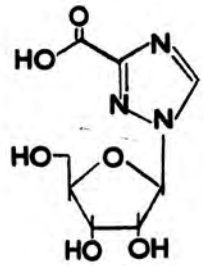
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
8	1-(5-O-Valeryl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	RA-121	BJ-45548	p.10



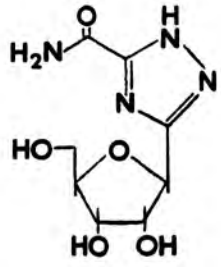
9.	1-(5-O-Caproyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide	RA-119	BJ-45539	p.10



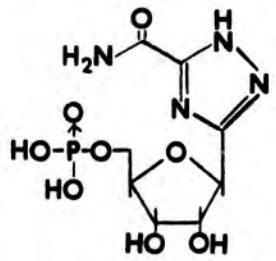
10.	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-carboxylic acid	RA-136	BJ-58518	Ref.6



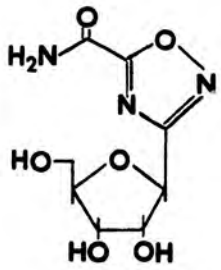
11.	3-(β-D-Ribofuranosyl)-1,2,4-triazole-5-carboxamide	RP-14	BJ-45502	Ref.32



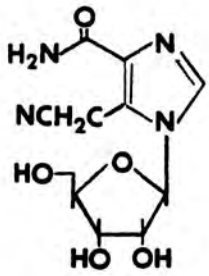
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
12.	3-(β-D-Ribofuranosyl)-1,2,4-triazole-5-carboxamide 5'-phosphate	RV-22	BJ-58527	p.12



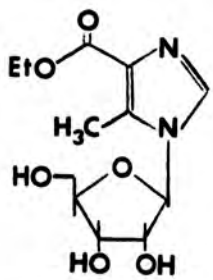
13.	3-(β-D-Ribofuranosyl)-1,2,4-oxadiazole-5-carboxamide	SB-111	BJ-42332	p.13



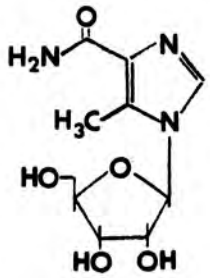
14.	5-Cyanomethyl-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide	RB-125	BJ-08465	Ref.37



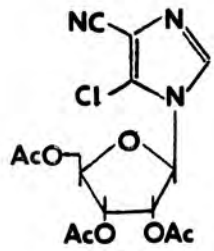
15.	Ethyl 5-methyl-1-(β-D-ribofuranosyl)-imidazole-4-carboxylate	RB-124	BJ-29919	p.17



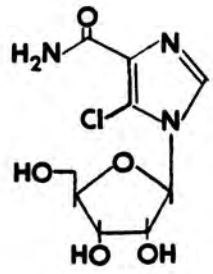
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
16.	5-Methyl-1-(β-D-ribofuranosyl)imidazole-4-carboxamide	RH-49	BJ-29946	p.18



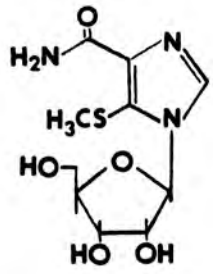
17.	5-Chloro-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)imidazole-4-carbonitrile	RA-125	BJ-45557	Ref.45

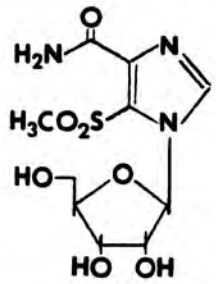
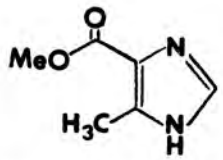
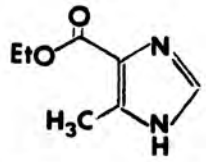
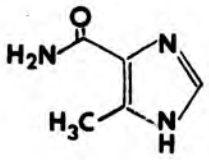
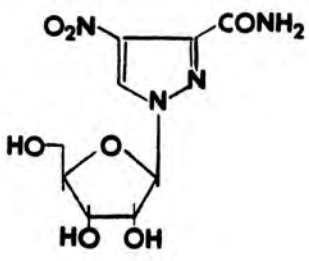


18.	5-Chloro-1-(β-D-ribofuranosyl)imidazole-4-carboxamide	RA-105	BJ-42350	Ref.45

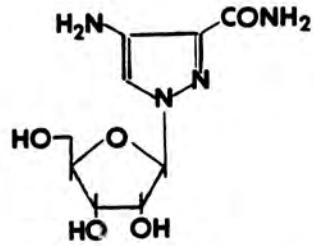


19.	5-Methylthio-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide	RP-52		p.20

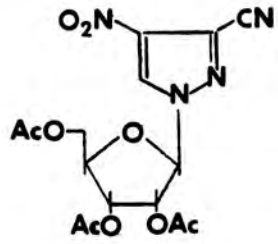


No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
20.	5-Methylsulfonyl-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide	RP-67(III)	BJ-58554	p.20
				
21.	Methyl 5(4)-methylimidazole-4(5)-carboxylate	RH-30	BJ-29937	p.17
				
22.	Ethyl 5(4)-methylimidazole-4(5)-carboxylate	RB-122	BJ-29900	Ref.41
				
23.	5(4)-Methylimidazole-4(5)-carboxamide	RH-26	BJ-29928	p.18
				
24.	4-Nitro-1-(β-D-ribofuranosyl)pyrazole-3-carboxamide	RA-101	BJ-33860	Ref.50
				

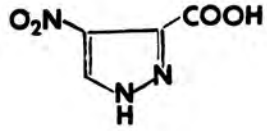
No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
25.	4-Amino-1-(β-D-ribofuranosyl)pyrazole-3-carboxamide	RA-102	BJ-33879	Ref.50



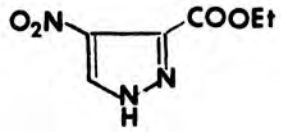
26.	3-Cyano-1-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)-4-nitropyrazole	RV-8		p.22



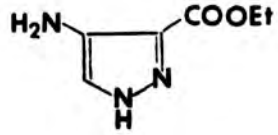
27.	4-Nitropyrazole-3-carboxylic acid	RK-15	BJ-33888	Ref.49



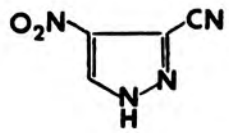
27,	Ethyl 4-nitropyrazole-3-carboxylate	RA-99	BJ-33851	Ref.47



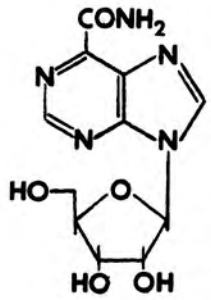
29.	Ethyl 4-aminopyrazole-3-carboxylate	RV-39	BJ-58545	p.22



No.	Compound	BYU-CRC No.	WRAIR No.	Ref.
30.	3-Cyano-4-nitropyrazole	RA-111		p.22



31.	9-(β-D-Ribofuranosyl)purine-6-carboxamide	RI-51A	BJ-42341	p.23



References:

1. G. D. Diana and F. Pancic, Angew. Chem. Int. Ed. (English), 15, 410 (1976).

2. A. S. Galabov, Arzneim. Forsch., 26, 169 (1976).

3. T. H. Maugh, Science, 192, 128 (1976).

4. W. H. Prusoff and D. C. Ward, Biochem. Pharmacol., 25, 1233 (1976).

5. D. Pavan-Langston and F. Hess, Infect. Dis., Subject of the month, 42-48 (1977).

6. J. T. Witkowski, R. K. Robins, R. W. Sidwell and L. N. Simon, J. Med. Chem., 15, 1150 (1972).

7. P. Jacobi and K. Hoffman, 10th Int. Congr. Chemother., Zurich, Sept. 18-23, Abstract #401 (1977).

8. R. W. Sidwell, G. P. Khare, L. B. Allen, J. H. Huffman, J. T. Witkowski, L. N. Simon and R. K. Robins, Chemotherapy, 21, 205 (1975).

9. G. P. Khare, R. W. Sidwell, J. T. Witkowski, L. N. Simon and R. K. Robins, Antimicrob. Agents Chemother., 3, 517 (1973).

10. F. E. Durr and H. F. Lindh, Ann. N.Y. Acad. Sci., 255, 367 (1975).

11. F. E. Durr, H. F. Lindh and M. Forbes, Antimicrob. Agents Chemother., 7, 582 (1975).

12. For a review of the antiviral activity of Ribavirin, see Ann. N.Y. Acad. Sci., 284, 201-293 (1977).

13. R. F. Berendt, J. S. Walker, J. W. Dominik and E. L. Stephen, Antimicrob. Agents Chemother., 11, 1069 (1977).

14. J. B. Arensman, J. W. Dominik and D. E. Hilmas, Antimicrob. Agents Chemother., 12, 40 (1977).

15. C. R. Magnussen, R. G. Douglas, Jr., R. F. Betts, F. K. Roth and M. P. Meagher, 10th Int. Congr. Chemother., Zurich, Sept. 18-23, Abstract #400 (1977).

16. D. G. Streeter, L. N. Simon, R. K. Robins and J. P. Miller, Biochemistry, 13, 4543 (1974).

17. R. C. Willis, D. A. Carson and J. E. Seegmiller, Proc. Nat'l Acad. Sci., USA, 75, 3042 (1978).

18. J. P. Miller, L. J. Kigwana, D. G. Streeter, R. K. Robins, L. N. Simon and J. Roboz, Ann. N.Y. Acad. Sci., 284, 211 (1977).

19. T. P. Zimmerman and R. D. Deeprose, Biochem. Pharmacol., 27, 709 (1978).

20. D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins and L. N. Simon, Proc. Nat'l Acad. Sci., USA, 70, 1174 (1973).

21. L. B. Allen, J. H. Huffman and R. W. Sidwell, Antimicrob. Agents Chemother., 3, 534 (1973).

22. R. W. Sidwell, L. N. Simon, J. T. Witkowski and R. K. Robins, Progr in Chemother. (Proc. 8th Int. Congr. Chemother.), Vol. 2, 889 (1974).

23. L. B. Allen, K. H. Boswell, T. A. Khwaja, R. B. Meyer, Jr., R. W. Sidwell, J. T. Witkowski, L. F. Christensen and R. K. Robins, J. Med. Chem., 21, 742 (1978).

24. G. I. Chipen and B. Ya. Grinshtein, Chem. Heterocycl. Compds. USSR, 1, 420 (1965).

25. Purchased from Pfanstiehl Laboratories, Inc., Waukegan, Illinois.

26. T. Ueda and I. Kawai, Chem. Pharm. Bull. (Tokyo), 18, 2303 (1970).

27. M. Smith, G. I. Drummond and H. G. Khorana, J. Am. Chem. Soc., 83, 698 (1961).

28. D. C. Baker, T. H. Haskell and S. R. Putt, J. Med. Chem., 21, 1218 (1978).

29. L. B. Allen et al., unpublished results.

30. A. H. Haines, Adv. Carbohydr. Chem. Biochem., 33, 11 (1976).

31. T. Huynh-Dinh, J. Igolen, E. Bisagni, J. P. Marquet and A. Civier, J. Chem. Soc., Perkin Trans. I, 761 (1977).

32. M. S. Poonian and E. F. Nowoswiat, J. Org. Chem., 45, 203 (1980).

33. T. Vaněk, J. Farkaš and J. Gut, Collect. Czech. Chem. Commun., 44, 1334 (1979).

34. M. Bobek and J. Farkaš, Collect. Czech. Chem. Commun., 34, 247 (1968).

35. F. Eloy and R. Lenaers, Chem. Reviews, 62, 155 (1962).

36. G. R. Revankar and R. K. Robins, Nucleic Acid Chem., Improved and New Synthetic Procedures, ed. Townsend and Tipson, Pt. 1, p. 465 (1978).

37. P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr. and R. K. Robins, J. Am. Chem. Soc., 98, 1492 (1976).

38. R. K. Robins, J. K. Horner, C. B. Greco, C. W. Noell and C. G. Beames, Jr., J. Org. Chem., 28, 3041 (1963).

39. E. Wittenburg, Z. Chem., 4, 303 (1964).

40. H. Bohme and H. Schneider, Chem. Ber., 91, 1001 (1958).

41. H. Bohme and H. Schneider, Chem. Ber., 91, 988 (1958).

42. Aldrich Chemical Co., Inc., Milwaukee, Wisconsin.

43. U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3654, 3660, 3672 (1974) and references cited therein.

44. E. DeClercq and M. Luczak, Life Sciences, 17, 187 (1975).

45. P. C. Srivastava, D. G. Streeter, T. R. Matthews, L. B. Allen, R. W. Sidwell and R. K. Robins, J. Med. Chem., 19, 1020 (1976).

46. K. Suzuki and I. Kumashiro, U.S. Patent 3,450,693 (1969); Chem. Abstr., 71, 816982 (1969).

47. R. K. Robins, F. W. Furcht, A. D. Grauer and H. W. Jones, J. Am. Chem. Soc., 78, 2418 (1956).

48. C. S. Marvel and E. E. Dreger, Org. Syn., Coll. Vol. 1, 238 (1941).

49. C. Musante, Gazz. Chim. Ital., 75, 121 (1945).

50. I. A. Korbukh, O. V. Budanova, N. G. Yakunina, V. I. Seraya and M. N. Preobrazhenskaya, Zh. Org. Khim. (USSR), 12, 1560 (1976).

51. L. B. Mackay and G. H. Hitchings, J. Am. Chem. Soc., 78, 3511 (1956).

Staffing

Contract No. DAMD 17-79-C-9046

During the report period the following personnel have been engaged in the work on the contract:

Name	Effort
Roland K. Robins, Ph.D. Principal investigator	10% - No charge, April 1-April 30, 1979 100% - May 1, 1979 to March 31, 1980
Ganapathi R. Revankar, Ph.D. Member	50% - April 1, 1979 to March 31, 1980
Steven G. Wood Graduate Research Assistant	25% - Sept. 1, 1979 to March 31, 1980
David Adams Technician	100% - January 1 to March 31, 1980
Coreen Smith Lab Technician	50% - Dec. 13, 1979-March 31, 1980
Stephen E. Palmer*	50% - April 1-May 31, 1979
Kelly Ence*	50% - May 2-July 29, 1979
Steven Harris*	25% - Sept. 1-Oct. 31, 1979

* Student involved in receiving university credit for organic synthesis.

DISTRIBUTION LIST

5 copies	Director Walter Reed Army Institute of Research ATTN: SGRD-UWZ-AC Walter Reed Army Medical Center Washington, DC 20012
4 copies	USAMRDC (SGRD-SI) Fort Detrick Frederick, MD 21701
2 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, TX 78234