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SRI-ORG-92-105-7317-F

CONTRACT NO: DAMD17-91-C-1034

TITLE: DRUG DEVELOPMENT AGAINST VIRAL DISEASES OF MILITARY IMPORTANCE (DIRECTED SYNTHESIS)

PRINCIPAL INVESTIGATOR: John A. Secrist, III
Cecil D. Kwong
Charles A. Krauth
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CONTRACTING ORGANIZATION: SOUTHERN RESEARCH INSTITUTE
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A synthesis laboratory has been established for the preparation of compounds to be evaluated against viruses of interest to U. S. Army Medical Research Institute of Infectious Diseases. The synthesis of known compounds as well as new compounds has been undertaken, and all compounds are being made in sufficient quantity to allow for full evaluation. The compounds prepared under this contract included nucleoside analogs and other heterocyclic compounds including 6-azauridine analogs, ribavirin analogs, and 1-substituted 4-acetyl-4-phenylpiperidines.

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FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

I. Introduction

This report describes the activities supported by Contract No. DAMD17-91-C-1034 entitled, "Drug Development Against Viral Diseases of Military Importance (Directed Synthesis)" from 1 March 1991 through 31 December 1991. The purpose of this contract was to support the synthesis of a wide variety of compounds for evaluation in the USAMRIID antiviral drug testing program. The compounds prepared for this program included: (1) known compounds that were needed in larger quantities for proper evaluation; and (2) new compounds whose structures had been determined by rational processes.

During this project, we pursued the syntheses of a number of compounds which had been specifically requested by Dr. B. J. Gabrielsen, our Contracting Officer's Representative. The following compounds were submitted as a result of these efforts: the *bis*-methylamidine of ribavirin (*N,N'*-dimethyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide); *N*-methyl ribavirin (*N*-methyl-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide); methyl 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidate; the *N*-butylamidine of ribavirin (*N*-butyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide); the asparagine amidine of ribavirin (asparagine, *N*-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl); the glutamine amidine of ribavirin (glutamine, N-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl); two *N*-alkylated 4-acetyl-4-phenylpiperidine derivatives (haloperidol analogs); 2-thio-6-azauridine 2',3',5'-triacetate; 4-thio-6-azauridine 2',3',5'-triacetate; the 5'-(ethyl methoxyalaninyl)phosphate of 6-azauridine; 6-cyanouridine 2',3',5'-triacetate; 6-thiocarboxamidouridine 2',3',5'-triacetate; and 4-thio-6-azauridine. We also obtained a sample of 6-ethynyluridine from Dr. T. Miyasaka of Tokyo University. Another project activity that we performed was the determination of the purities of samples of ribavirin 5-triphosphate (AVS 6753) and adenosine *N*-oxide 5'-triphosphate for Dr. Gabrielsen.

We also pursued the syntheses of the following compounds: 6-carboxamidouridine 2',3',5'-triacetate; the 5'-pivaloylmethylphosphates of 6-azauridine and 2-thio-6-azauridine; 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR), and 6-fluorouridine. Unfortunately, we were unable to complete these syntheses even with the extension. For the same reason, we were not able to pursue the following compounds comprising the remainder of the original requested compound list: the 5'-(ethyl methoxyglycinyl)phosphate and 5'-(2,2,2-trichloroethyl methoxyalaninyl)phosphate of 6-azauridine; the 5'-(ethyl methoxyglycinyl)phosphate, 5'-(ethyl methoxyalaninyl)phosphate, 5'-(2,2,2-trichloroethylmethoxyalaninyl)phosphate of 2-thio-6-azauridine; 6-cyanouridine; 6-thiocarboxamidouridine; 6-carboxamidouridine; 6-cyano-5-azauridine; pyrazofurin 5'-triphosphate; tiazofurin 5'-phosphate; and 4',7-dihydroxy-3-methoxy-5,6-dimethylflavone.

Our other project activities included the preparation of the research plan for shutting down this contract by the end of September, 1991 (submitted during the second quarter) and the preparation

of a plan and projected budget for a project extension that would enable us to continue working on this project during November and December, 1991. The stated objective with these summaries was to complete the syntheses of as many of the most promising compounds as possible, and especially to direct our efforts toward those compounds with the potential to generate publications.

II. Personnel

Name	Hours 1 Dec 88 - 30 Nov 89	Percent of Time
Project Supervision:		
Dr. J. A. Secrist III	53.0	3
Dr. C. D. Kwong	948.0	56
Chemists:		
C. A. Krauth	921.0	55
D. A. Carter	1045.0	62
L. K. Hanna	738.0	44
R. J. Gray	165.0	10
Analytical Services:		
Dr. W. C. Coburn	113.5	7
Dr. J. M. Riordan	114.0	7
M. C. Kirk	130.5	8
C. Richards	64.0	4
S. A. Campbell	6.0	<1
Analytical Services: (cont'd)		
D. M. McCain	10.0	1
J. W. Truss	105.0	6
P. E. Arnold	2.0	<1
S. D. Clayton	3.0	<1
T. D. Stringfellow	2.0	<1
B. F. Meadows	1.5	<1
Glassware Technicians:		
W. Johnson	106.0	6
J. Robinson Bearden	24.5	1
A. Jackson	146.0	9
R. W. Milton	7.0	<1

III. Compounds Submitted

The compounds submitted under this contract are shown on the following pages. We have included their SRI numbers, AVS numbers (if available), and the amounts submitted.

IV. Chemistry

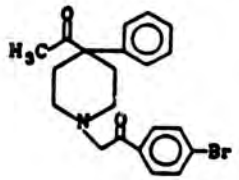
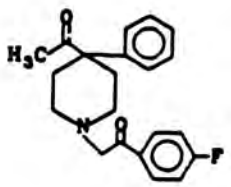
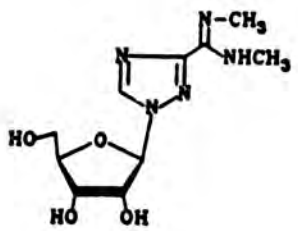
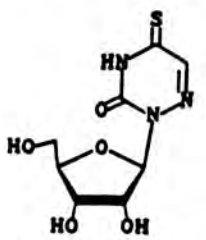
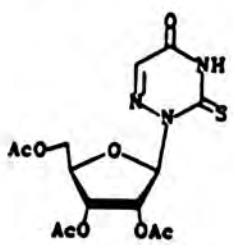
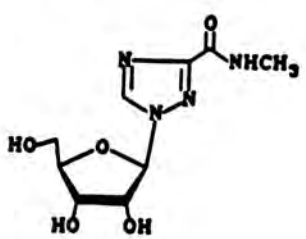
During this contract period, we submitted fourteen compounds for screening: the *bis*-methylamidine of ribavirin (*N,N'*-dimethyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide); *N*-methyl ribavirin (*N*-methyl-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide); methyl 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidate; the *n*-butylamidine of ribavirin (*N*-butyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide); the asparagine amidine of ribavirin (asparagine, *N*-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl); the glutamine amidine of ribavirin (glutamine, N-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl); two *N*-alkylated 4-acetyl-4-phenylpiperidine derivatives (haloperidol analogs); 2-thio-6-azauridine 2',3',5'-triacetate; 4-thio-6-azauridine 2',3',5'-triacetate; the 5'-(ethyl methoxyalaninyl)phosphate of 6-azauridine; 6-cyanouridine 2',3',5'-triacetate; 6-thiocarboxamidouridine 2',3',5'-triacetate; and 4-thio-6-azauridine.

We initially attempted to synthesize the *bis*-methylamidine of ribavirin (*N,N'*-dimethyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide) by a procedure provided by Dr. Gabrielsen.[1] As shown in Scheme I, the desired *bis*-methylamidine 2 should have been obtained by refluxing compound 1, the amidine of ribavirin in a solution of EtOH and aqueous methylamine. Unfortunately, our attempts to repeat this procedure consistently resulted in the isolation of a mixture of products containing *N*-methylamide 3 and a compound that was assumed to be methylamidine 4 instead of the desired product from this procedure. (Compound 4 was indicated by MS only; it was never isolated for characterization.) Compound 3 was then isolated since Dr. Gabrielsen had already indicated that it could be useful in the screening, and since our literature searches had shown that this compound had not been reported previously.

We then evaluated a number of other reaction conditions until we determined that we could cleanly convert 1 to 2 by treatment with anhydrous methylamine in anhydrous ethanol at 60 °C in a stainless steel bomb. The reaction was monitored by TLC (silica gel plates, $CHCl_3$-MeOH-HOAc; 1:1:0.5) until we found that the desired reaction had cleanly gone to completion after 6 days. The solvent was then removed *in vacuo*, and the residue was dried *in vacuo* over P_2O_5 to give 2 as a very hygroscopic, light brown foam.

Next, we performed a hydrolysis study on 2 in a pH 7.4 phosphate buffer solution. We monitored the reaction mixture by TLC (silica gel plates, $CHCl_3$-MeOH-HOAc; 1:1:0.5) and found that after 1, 5, and 8 h, the TLC's showed no evidence of any appreciable change in the composition of reaction mixture. However, the TLC after 24 h showed that the starting carboximidamide (*Rf* 0.45) was no longer present, and instead, a component was present with an *Rf* of 0.69. We the determined that this component was *N*-methylribavirin (*N*-methyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide) (3) after the crude reaction mixture was lyophilized, analyzed by FAB-MS, and later compared with a sample of 3 which had inadvertently been prepared in earlier attempts to prepare 2. This analysis showed an M + 1 = 259 corresponding to the *N*-methyl carboxamide 3 instead of an M + 1 = 272 corresponding to the starting material 1.

Compounds Submitted

Compound	SRI Number	AVS Number	Amount Submitted
	7907	11713	1.92 g
	7927	11905	520 mg
	7906	5601	2.63 g
	7717	8355	300 mg
	7924	11717	6 g
	7942	11916	320 mg

Compounds Submitted (Continued)

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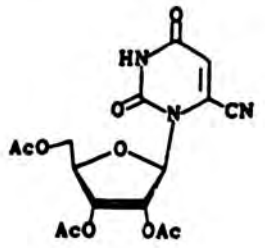
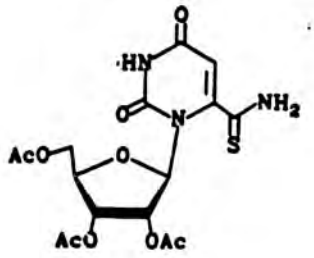
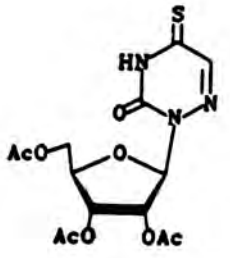
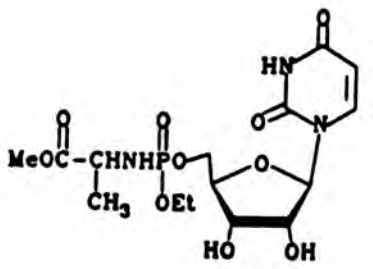
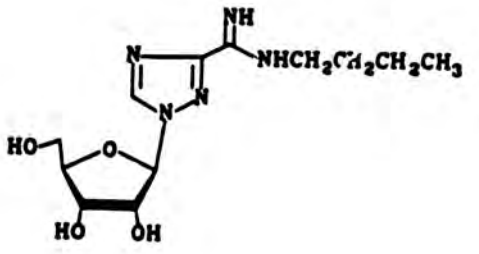
	7946	11915	1 g
	7947	11914	1 g
	7699	8353	300 mg
	7959	11941	2.1 g
	8154		19 mg

Compounds Submitted (Continued)

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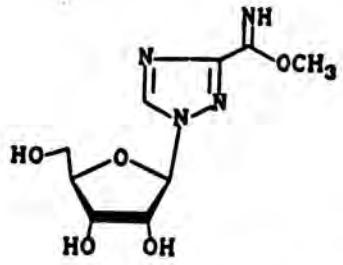
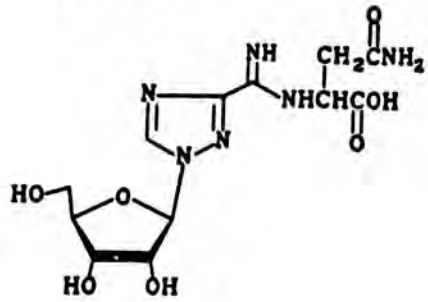
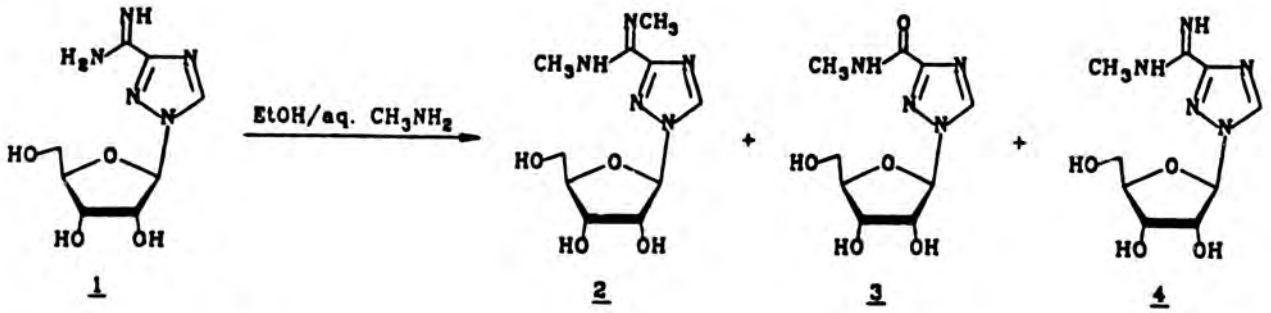
	8153		25 mg
	8155		25 mg

We also began developing an alternate preparation for compound 3 by modifying a preparation for ribavirin.[18] As shown in Scheme II, methyl 1,2,4-triazole-carboxylate 5 and 1,2,3,5-tetra-*O*-acetylribofuranose were fused using *bis*(4-nitrophenyl)phosphate at 160-165 °C, and the resulting adduct 6 was deprotected with methanolic sodium methoxide to give 7. This intermediate was then treated with aqueous methylamine to give the desired 3. Unfortunately, this work was not completed before the termination of this project.

Other ribavirin analogs that we prepared included the *n*-butylamine amidine of ribavirin (*N*-butyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide) (12), the asparagine amidine of ribavirin (asparagine, *N*-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl) (13), and the glutamine amidine of ribavirin (glutamine, N-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl) (14). Scheme III shows that these compounds were prepared from a common intermediate, methyl 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidate (11).[18] Ribavirin 8 was first acetylated to triacetate 9 and then dehydrated to 3-cyano compound 10 by treatment with $POCl_3$. Treatment of 10 with NaOMe-MeOH then gave imidate ester 11. The imidate ester 11 was then reacted with *n*-butylamine, asparagine, or glutamine to give the desired targets 12, 13, and 14, respectively. Compounds 12 and 13 were submitted while 14 was in the final stages of purification and characterization at the termination of this project.

We also performed a hydrolysis study on a sample of the glycine amidine of ribavirin (glycinyl, *N*-[(1-β-D-ribofuranosyl)-1*H*-1,2,4-triazol-3-yl]iminomethyl) (15), another ribavirin congener. Again, we performed this study in a pH 7.4 phosphate buffer solution, and we monitored the reaction mixture by TLC (silica gel plates, $CHCl_3$-MeOH-HOAc; 1:1:0.5) at 1 h intervals over an 8 h period. Our results showed that this compound

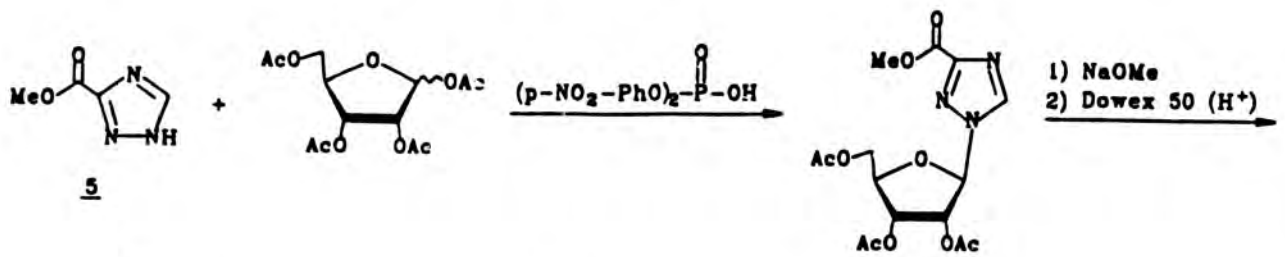
Scheme I



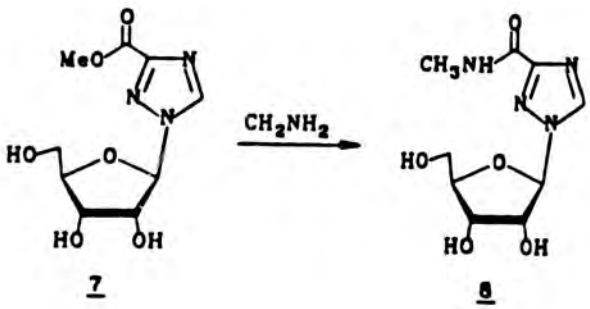
1 2 3 4

1 $\xrightarrow[60\,°C]{CH_3NH_2/EtOH}$ 2

Scheme II

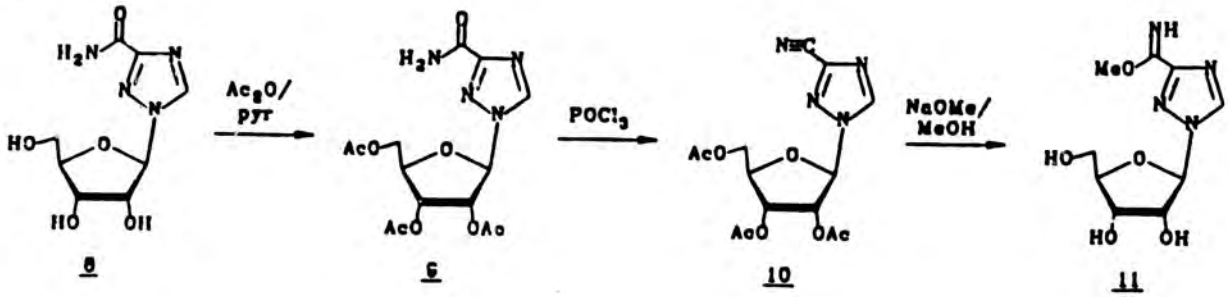


5 6

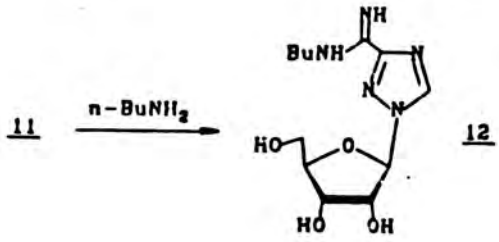


7 8

Scheme ..I



8 6 10 11



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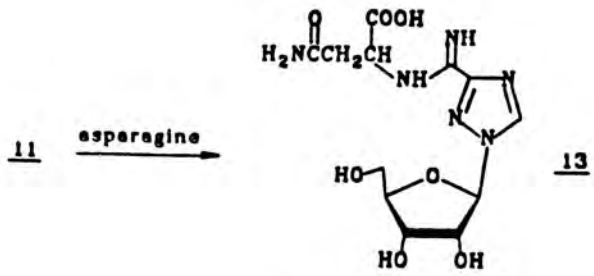

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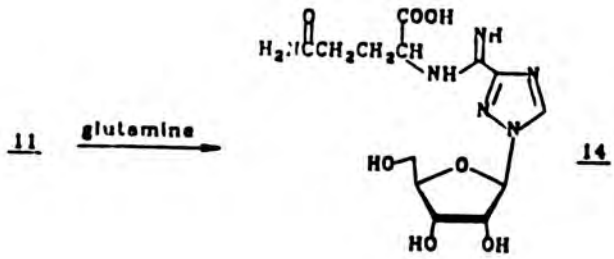


11 14

hydrolyzed quickly since after 1 h, the starting material (*Rf* 0.5) was no longer present in the reaction mixture. The major component in the reaction mixture had an *Rf* of 0.88, which corresponded to ribavirin, the projected hydrolysis product of **15**.

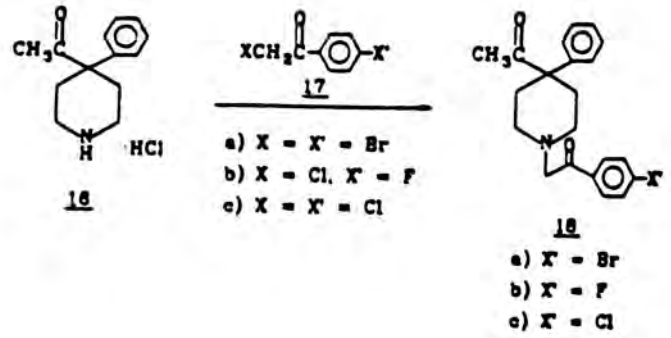
During this quarter, we also worked on the synthesis of a number of *N*-alkylated 4-acetyl-4-phenylpiperidine derivatives, as shown in Scheme IV. Our early efforts attempted to parallel *N*-alkylations of similar piperidine compounds including: heating the reactants (4-acetyl-4-phenylpiperidine (**16**) and commercially available α-haloacetophenones (**17a-c**) in either sodium and potassium carbonate in DMF or *n*-butanol[2] at a number of different temperatures; and heating the reactants with toluene and potassium iodide in a steel bomb at 80 °C.[3] Since these efforts were unsuccessful, we tried the reaction of **16** and **17a** in chloroform-triethylamine at reflux,[4] and we found that we obtained the desired **18a** as the only product. We then reacted **16** with **17b** to obtain product **18b**, the other haloperidol analog originally requested by Dr. Gabrielsen. We also preliminarily reacted **16** with **17c** on a small scale and found that we obtained **18c**. However, this reaction was never scaled up since it was not a specifically requested compound.

The *N*-alkylation of 4-acetyl-4-phenylpiperidine with phenyl-substituted γ-halobutyrophenones such as **19a-c** was also attempted. As shown in Scheme V, we first tried to alkylate **16** with commercially available **19a** using the reaction conditions that had been successful for Scheme IV. Repeated attempts showed that this reaction unexpectedly would not cleanly give **20a**, and therefore, we also tried heating these reactants with toluene and potassium iodide at 80 °C.[3] A mixture of products and unreacted starting materials resulted from this effort, probably due to the relatively low reactivity of the chloride. We then shifted our efforts away from this reaction since the termination of this project was approaching.

We also prepared an additional quantity of 2-thio-6-azauridine 2',3',5'-triacetate (**22**), a compound which had been previously prepared as an intermediate under Contract DAMD17-86-C-6011. Scheme VI shows that this compound was prepared by the treatment of commercially available 2-thio-6-azauridine with acetic anhydride and pyridine, which was a route similar to the preparation of another previously submitted compound, 4-thio-6-azauridine 2',3',5'-triacetate.[5]

We continued our pursuit of the 5'-(ethyl methoxyglycinyl)phosphates and the 5'-(ethyl methoxyalaninyl)phosphates of 6-azauridine and 2-thio-6-azauridine and were successful in submitting the 5'-(ethyl methoxyalaninyl)phosphate of 6-azauridine (**25a**). Our preferred route to all of these compounds was the recently reported synthesis of novel phosphoramidate derivatives of 3'-azido-3'-deoxythymidine.[6] Therefore, as shown in Scheme VII, alanine methyl ester **23a** and glycine methyl ester **23b** were reacted with commercially available ethylphosphorodichloridate to give ethyl methoxyalaninylphosphorochloridate (**24a**) and methoxyglycinylphosphorochloridate (**24b**), respectively. These phosphorochloridates were then reacted with either 6-azauridine or 2-thio-6-azauridine in trimethylphosphate containing *N*-methylimidazole to give the 5'-(ethyl methoxyglycinyl)phosphate and 5'-(ethyl methoxyalaninyl)phosphate of 6-azauridine (**25a-b**) and the 5'-(ethyl methoxyglycinyl)phosphate of 2-thio-6-azauridine (**25c**). Unfortunately, we found the isolation and purification of these compounds was very difficult. The *N*-methylimidazole was very difficult to separate from the product mixture even after repeated column chromatography, and our product recovery after each purification attempt was poor. Because of the time and funding restrictions, we focused our efforts toward

Scheme IV



16

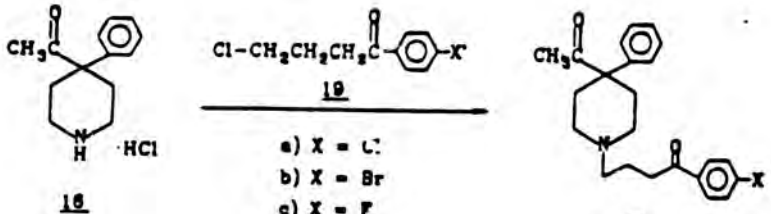
17

a) X = X' = Br
b) X = Cl, X' = F
c) X = X' = Cl

18

a) X' = Br
b) X' = F
c) X' = Cl

Scheme V



16

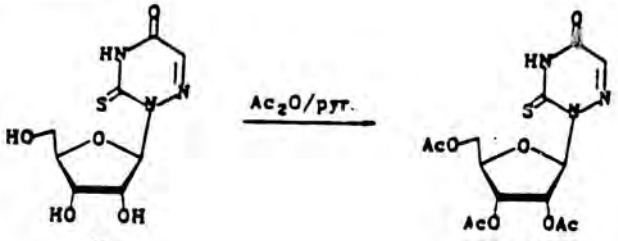
19

a) X = Cl
b) X = Br
c) X = F

20

a) X = Cl
b) X = Br
c) X = F

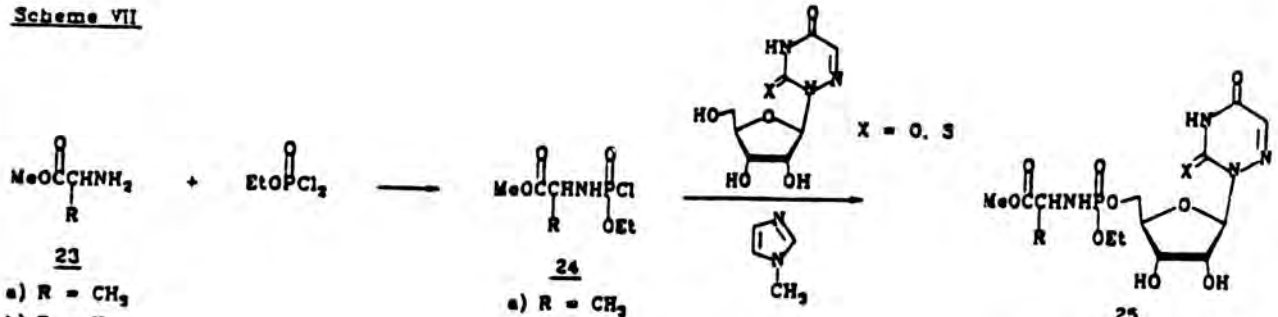
Scheme VI



21

22

Scheme VII



23

a) R = CH_3
b) R = H

24

a) R = CH_3
b) R = H

25

a) R = CH_3, X = O
b) R = H, X = O
c) R = CH_3, X = S

only one of the phosphoroamidate targets, and as a result of this concentrated effort, we were able to prepare and submit **25a** for screening.

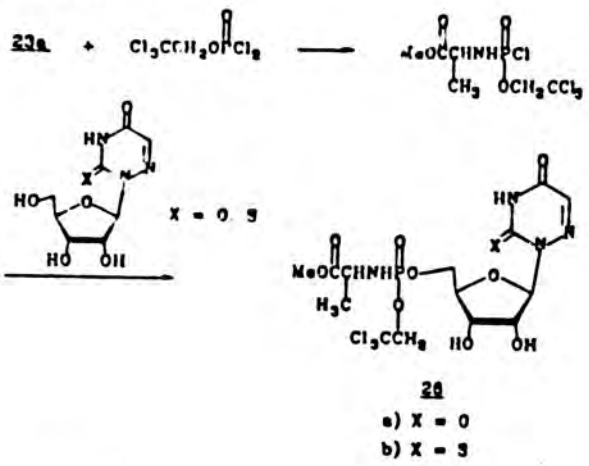
Other phosphoroamidates that we preliminarily pursued were the 5'-(2,2,2-trichloroethyl-methoxyalaninyl)phosphates and 5'-(2,2,2-trichloroethylmethoxyglycinyl)phosphates[7] of 6-azauridine and 2-thio-6-azauridine. As shown in Scheme VIII, we had originally planned to react alanine methyl ester and glycine methyl ester with commercially available 2,2,2-trichloroethylphosphorodichloridate to give 2,2,2-trichloro-ethylmethoxyalaninylphosphorochloridate and 2,2,2-trichloroethylmethoxyglycinylphosphorochloridate, respectively. Next, these adducts were to be reacted with either 6-azauridine or 2 thio-6-azauridine to give the 5'-(2,2,2-trichloroethylmethoxyglycinyl)phosphates or 5'-(2,2,2-trichloroethylmethoxyalaninyl)phosphates of 6-azauridine and 2-thio-6-azauridine (**26a-b**). Our initial effort with 2,2,2-trichloroethylmethoxy glycinylphosphorochloridate (from glycine methyl ester and commercially available 2,2,2-trichloro-ethylphosphorodichloridate) and 6-azauridine resulted in a mixture of products which contained target compound **26a** according to mass spectral data. Therefore, we isolated the desired compound by column chromatography, and then we found that after 12 h, the purified sample decomposed to a mixture that looked identical to the crude reaction mixture, according to TLC. Unfortunately, we were unable to investigate this reaction any further because of the time and budget restraints.

During this quarter, we also prepared and submitted 6-cyanouridine 2',3',5'-triacetate and 6-thiocarboxamidouridine 2',3',5'-triacetate. Scheme IX shows that we prepared these compounds as intermediates leading to 6-cyanouridine, 6-carboxamidouridine, and 6-thiocarboxamidouridine.[8] Both 5-bromouridine 2',3'-isopropylidene-5'-acetate (**27a**) and 2',3',5'-triacetate (**27b**) were prepared and treated with sodium cyanide in DMF at room temperature to give **28a** and **28b**. Preliminary experiments then showed that the 6-cyanouridine 2',3'-isopropylidene-5'-acetate (**28a**) could then be deprotected by sequential treatment with MeOH-NH_3 and formic acid to give 6-cyanouridine (**29**). Compound **28a** could also be treated with NaOH and converted to amide **30a** and then deprotected to give **31**. Compound **28b** could be treated with H_2S and pyridine to give **32** and then deprotected to **33** by treatment with methanolic ammonia. All of these reactions were successful only during our preliminary attempts. When these reactions were repeated on larger scales, the deprotection reactions resulted in mixtures of products for all reactions. Since time and funding were limited, we decided to concentrate our efforts on preparing two of these compounds that we could obtain in the necessary quantities, 6-cyanouridine 2',3',5'-triacetate (**28b**) and 2-thiocarboxamidouridine 2',3',5'-triacetate (**32**). These were prepared as previously described.

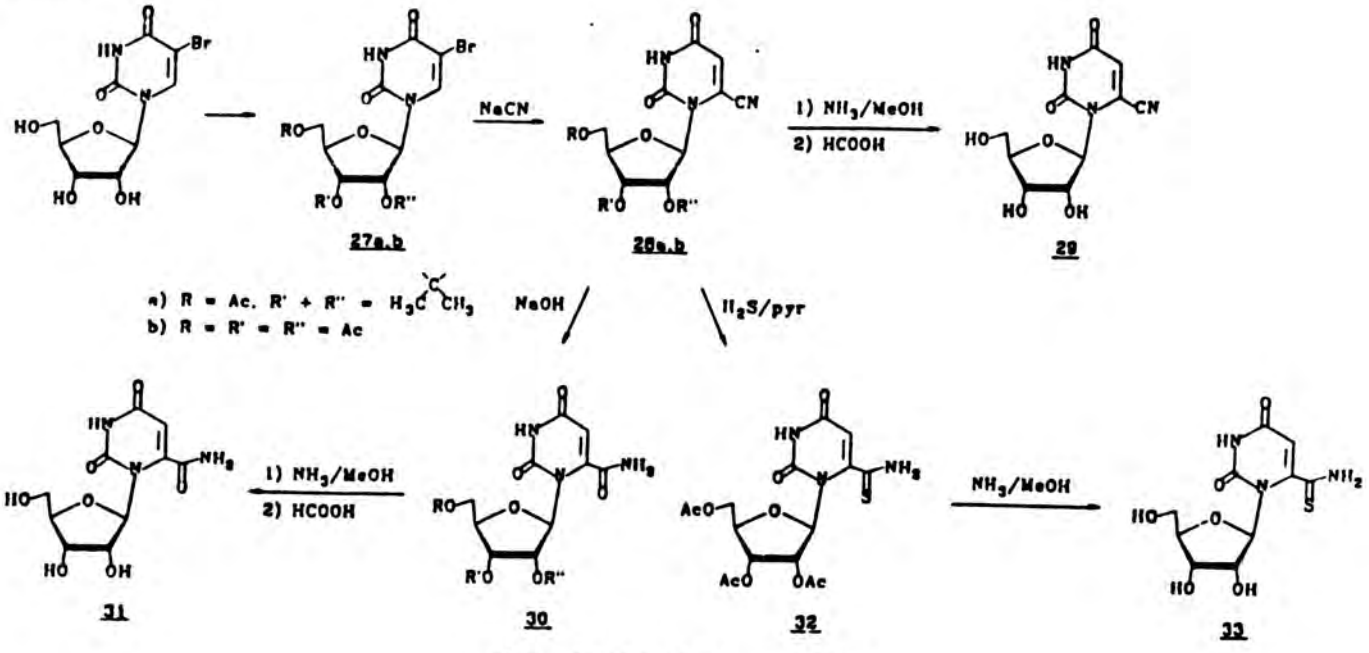
We also prepared another batch of 4-thio-6-azauridine[5] (**36**), a compound that we had previously prepared and submitted under Contract DAMD86-17-C-6011. As shown in Scheme X, 4-thio-6-azauridine[5] (**36**) was prepared by treating 6-azauridine 2',3',5'-triacetate (**34**) with P_2S_5 to obtain 4-thio-6-azauridine 2',3',5'-triacetate (**35**) followed by deprotection to **36** with Dowex 1 resin (OH^-form) in MeOH.

Other compounds that we preliminarily pursued included: 1-(3-hydroxy-2-phosphonylmeth-oxypropyl)triazole-3-carboxamide; the pivaloylmethylphosphates of 6-azauridine and 2-thio-6-azauridine; and 6-fluorouridine.

Scheme VIII



Scheme IX

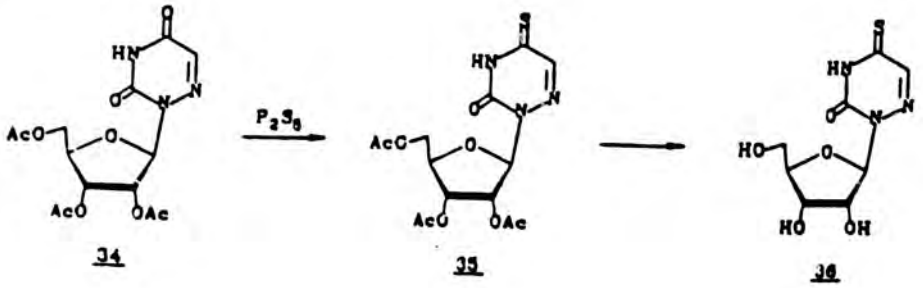


As shown in Scheme XI, our approach to 1-(3-hydroxy-2-phosphonylmethoxypropyl)triazole-3-carboxamide (44) followed a synthetic route to 1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine.[9] We reacted commercially available methyl triazole-3-carboxylate (5) with 1-tosyl-2,3-isopropylidene glycerol (37) in DMF with potassium carbonate and isolated both 38 and 39 from the product mixture. Compound 38 was then treated with ammonia to give amide 40 and then hydrolyzed to dihydroxypropyl compound 41. We had originally planned to react 41 with chloromethylphosphonic dichloride to obtain a mixture of separable products containing 42 and 43. Compound 42 would then be isolated and rearranged to give the target compound 44. Unfortunately, we were not able to complete the synthesis of this compound due to lack of time and funding.

Our approach to the pivaloylmethylphosphates of 6-azauridine and 2-thio-6-azauridine, as shown in Scheme XII, was to follow the synthesis of similar nucleotide diesters.[10,11] According to this procedure, 3'-*O*-acetylthymidine- and 3'-*O*-acetyl-5-fluorouridine 5'-monophosphates could be pivaloylmethylated by treatment of their respective disilver phosphate salts with iodomethylpivalate in anhydrous benzene. Our approach similarly reacted the disilver phosphate salts of 6-azauridine (46a) or 2-thio-6-azauridine (46b) with iodomethylpivalate (47) to obtain our pivaloylmethylphosphates (48a and 48b). Since iodomethylpivalate (47) was not commercially available, we also needed to synthesize this starting material. This requirement was complicated by our inability to find a clear, well-defined preparation for iodomethylpivalate in the literature. We were able to find boiling point/distillation data and a somewhat vague reference suggesting that this compound could be prepared from chloromethylpivalate (49) by treatment with KI,[12] and therefore, we tried the following sequence: refluxing chloromethylpivalate (49) in acetone with KI; evaporating the acetone; dissolving the residue in with EtOAc; extracting with aqueous sodium bisulfite and water; evaporating the EtOAc; and distilling the residue. These efforts resulted in a reasonable yield of a yellow liquid product which was shown to be predominantly the desired product by MS. However, according to the literature, the desired product should have been clear and colorless, and therefore the distillation of the product was repeated. Unfortunately, the second distillation also was not effective in removing the color. Furthermore, ^{1}H NMR spectra of the product after each distillation indicated that the product also contained 5-10 % of an undetermined impurity that was not significantly reduced by the second distillation. Also, when we repeated the overall synthesis with some minor variations in the work-up procedure, we again were not successful in completely removing the impurity. Our plans for this synthesis had been to try the KI reflux in a higher boiling solvent system such as MEK. However, we were unable to pursue this sequence any further due to the termination of this project.

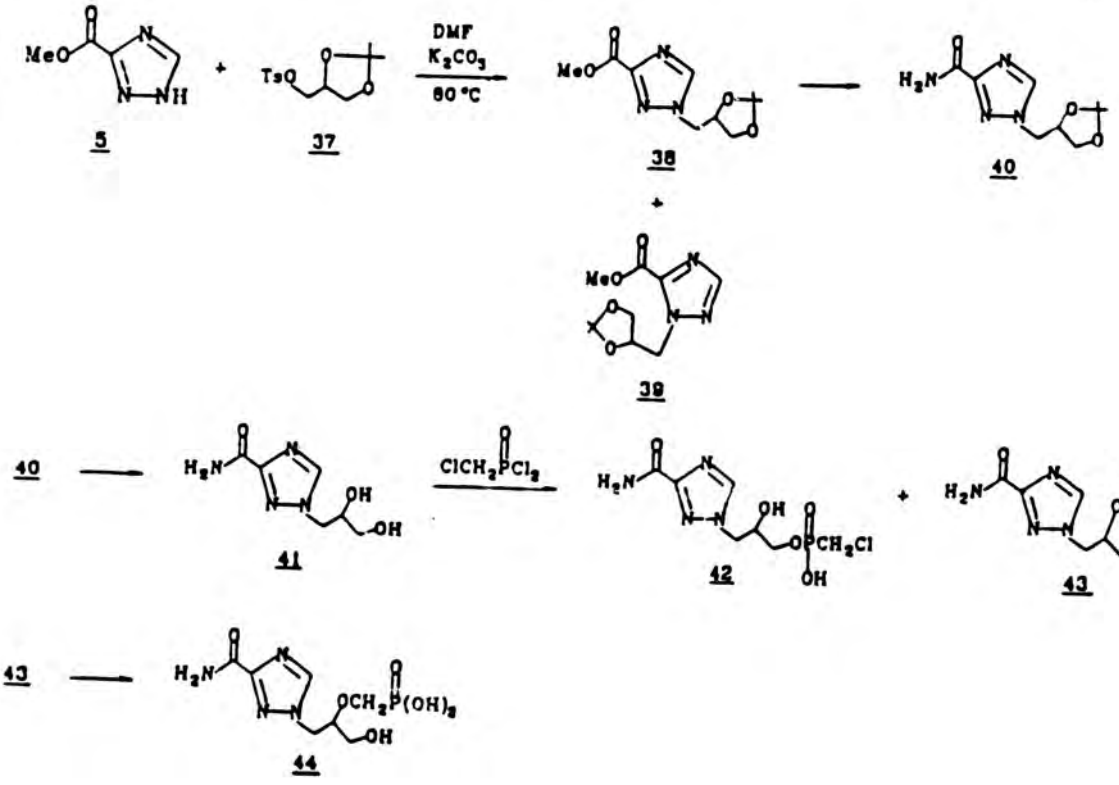
Scheme XIII shows that we had originally planned to synthesize 6-ethynyluridine (55) by following a literature preparation for this compound.[13] According to this preparation, 6-ethynyluridine (55) could be prepared from 6-iodo-2',3'-*O*-isopropylidene-5'-*O*-methoxymethyluridine (52) by treatment with trimethylsilylacetylene, $Pd(Ph_3P)_2Cl_2$, and CuI in Et_3N under argon to give 53, followed by removal of the trimethylsilyl protecting group by treatment with NH_3-MeOH and removal of the sugar protecting groups with 50% TFA. Therefore, our synthesis of 6-ethynyluridine first required our preparation of 6-iodo-2',3'-*O*-isopropylidene-5'-*O*-methoxymethyluridine (52). According to the literature, this compound could be prepared from 2',3'-*O*-isopropylideneuridine (50) by first 5'-*O*-methoxymethylating to 51, followed by iodination to

Scheme X



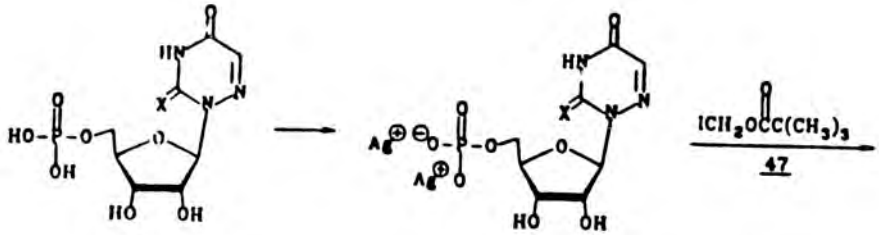
34 35 36

Scheme XI

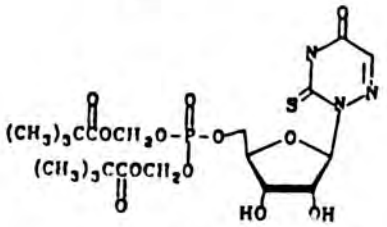


5 37 38 39 40 41 42 43 44

Scheme XII



45 46 47

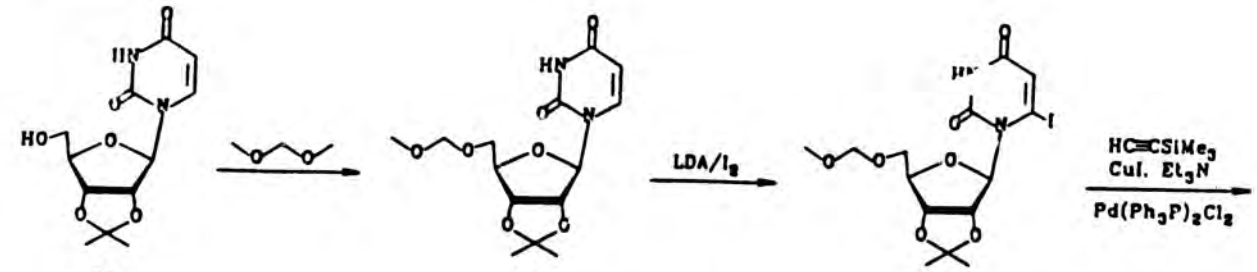


a) X = O
b) X = S

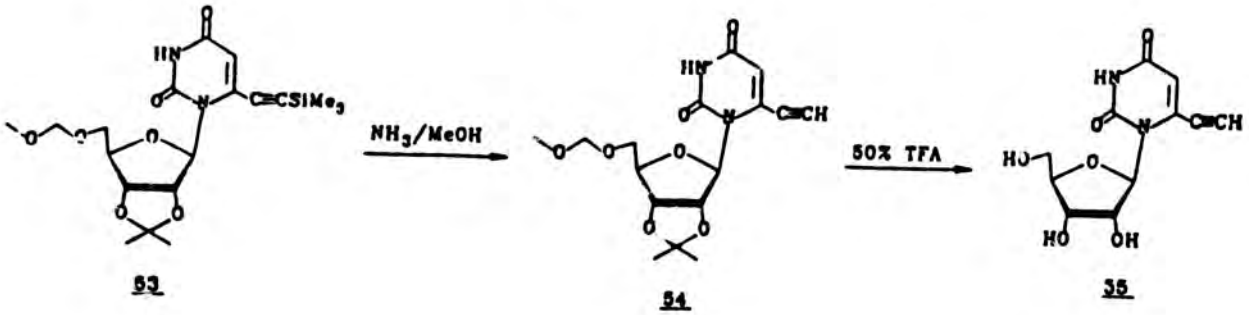
48

49 47

Scheme XIII



50 51 52



53 54 55

52 by treatment with iodine and lithium diisopropylamide.[14] In our laboratories, we were able to prepare the 2',3'-*O*-isopropylidene-5'-*O*-methoxymethyluridine (51) in reasonable yields. However, our attempts at the 6-iodination were not successful, and therefore, we chose to divert our efforts toward other compounds. We later contacted one of the authors for our literature reference, Dr. T. Miyasaka, and he indicated that he would provide some of this compound to us for screening.

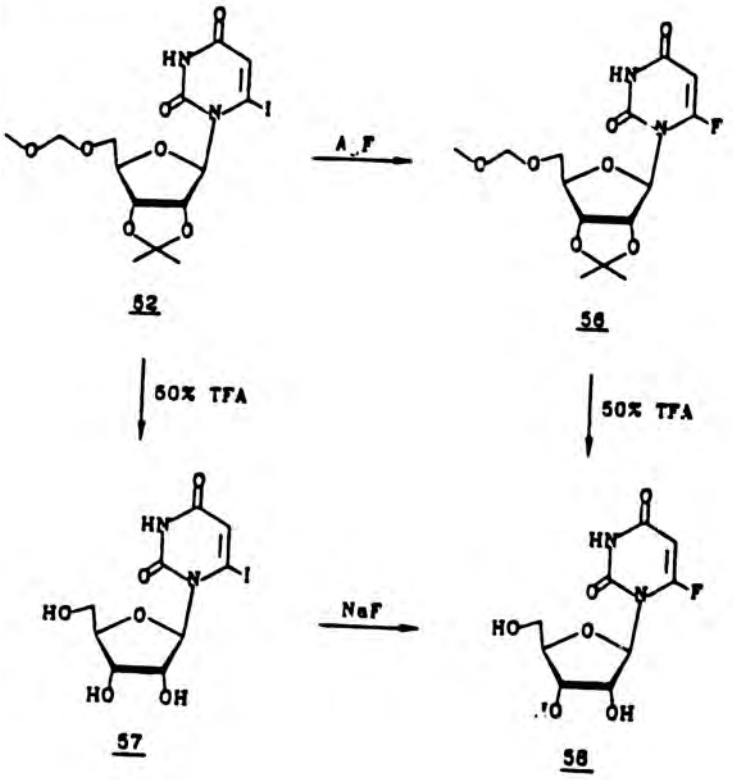
Scheme XIV shows that we had also wanted to try to synthesize 6-fluorouridine (58) from either 6-iodo-2',3'-*O*-isopropylidene-5'-*O*-methoxymethyluridine (52) or 6-iodouridine (57). Initially, we were surprised to find that 6-fluorouridine was not reported in the literature, but a close inspection of the literature for the preparation of 6-fluorouracil may have offered a partial explanation in the consistently reported hydrolytic instability under acidic conditions of the 6-fluoro group.[15-17] We still felt that our approach was reasonable. Unfortunately, the difficulties that we encountered in our previously discussed attempts to synthesize 6-iodo-2',3'-*O*-isopropylidene-5'-*O*-methoxymethyluridine (52) and 6-iodouridine (57) (needed for our approach to 6-ethynyluridine) also prevented us from pursuing the synthesis of 6-fluorouridine by this route. We feel that our proposed route was reasonable since we had found references showing that treatment of 6-chlorinated pyrimidine with silver fluoride would give 6-fluoropyrimidines. We felt that analogously, our 6-iodo group could also be displaced with fluoride.

The final compound that we pursued was 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamido riboside (EICAR). As shown in Scheme XV, we planned to prepare this compound by following a recent literature preparation for this compound.[19] According to this procedure, commercially available 5-amino-4-imidazolecarboxamide riboside (59) (AICAR) would first be converted to 2',3',5'-tri-*O*-acetylate **60** with triethylamine, acetic anhydride, and DMAP in acetonitrile. Compound **60** would then be reacted with isoamyl nitrite in diiodomethane at 100 °C to give the corresponding 5-iodo compound **61**, and this intermediate would be 5-alkynylated to **62** with trimethylsilylacetylene, *bis*(benzonitrile)palladium dichloride, and triethylamine in a sealed tube at 100 °C. Compound **62** would then be deprotected to **63** by treatment with methanolic ammonia. When we tried to reproduce this work, we were able to prepare 5-iodo compound **61** in reasonable yield. However, our attempts to 5-alkynylate resulted in a complex product mixture which was shown to contain the expected compound **62** by MS. Unfortunately, we were unable to isolate the desired component from the product mixture, and therefore, we were unable to complete the synthesis of this compound.

For this project, we also preliminarily investigated the following target compounds. They were not pursued beyond the acquisition of literature references and other pertinent data: 6-cyano-5-azauridine; pyrazofurin 5'-triphosphate; tiazofurin 5'-phosphate; and 4',7-dihydroxy-3-methoxy-5,6-dimethylflavone.

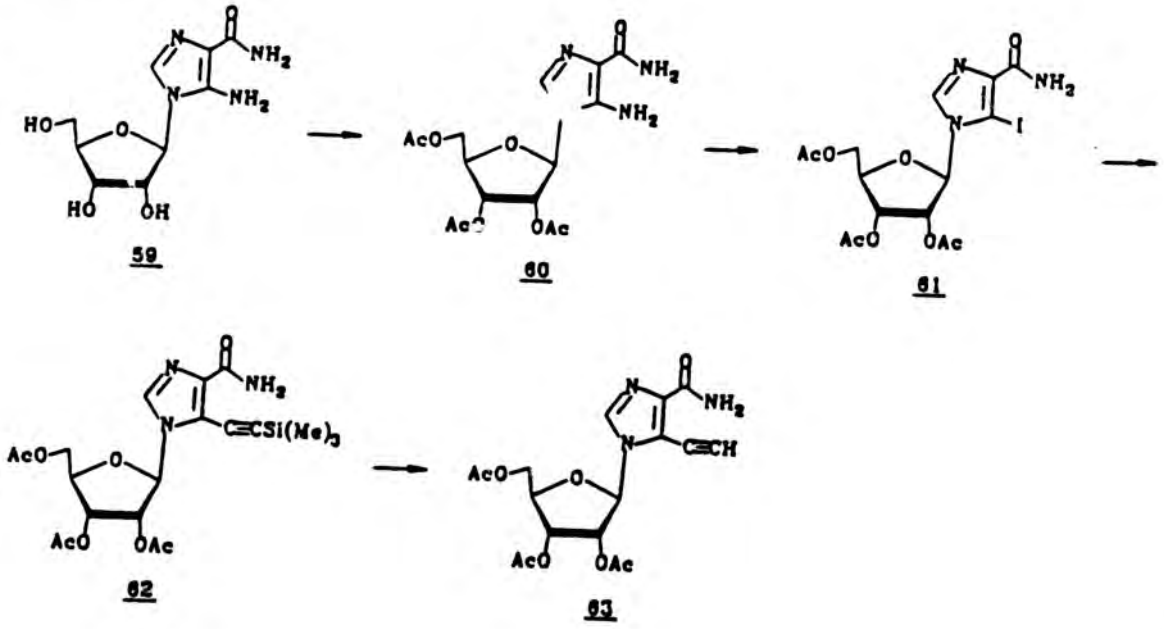
Finally, we also checked the purities of samples of ribavirin 5'-triphosphate (AVS 6753) and adenosine *N*-oxide 5'-triphosphate for Dr. Gabrielsen. We determined that the ribavirin 5'-triphosphate (AVS 6753) was essentially pure by TLC and elemental analysis. On the other hand, HPLC showed that the adenosine *N*-oxide 5'-triphosphate was contaminated with pyrophosphate and with adenosine *N*-oxide 5'-mono- and diphosphate as decomposition products. Originally, we were to purify these samples if necessary. However, no further action was pursued since the project was cancelled.

Scheme XIV



52 56 57 58

Scheme XV



59 60 61 62 63

Experimental

All solvents and materials were reagent grade and were either used as received or purified as required. ^{1}H NMR and ^{13}C NMR spectra were run with a Nicolet NMC NT-300 NB spectrometer operating at 300.65 MHz with tetramethylsilane as an internal reference. Chemical shifts (δ) for multiplets were measured from the appropriate centers. The mass spectral data were obtained from a Varian MAT 311A mass spectrometer in fast atom bombardment (FAB) or electron-impact (EI) mode (direct probe temperature 20 °C), as indicated. Infrared data were obtained with a Nicolet 10-MX spectrometer. In most cases, only strong or medium peaks in the 1800-600 cm^{-1} range were reported. UV absorption spectra were determined in the appropriate pH 1 (0.1 *N* HCl), pH 7 buffer, and pH 13 (0.1 *N* NaOH) solutions with either a Cary 17 spectrometer or a Perkin Elmer Model Lambda 9 UV/VIS/NIR spectrophotometer. M :ting point data was obtained with a Mel-Temp Capillary Melting Point apparatus, and all melting points were uncorrected. Elemental analysis data were obtained from an in-house Perkin Elmer Model 240 Elemental Analyzer.

***Bis*-Methylamidine of Ribavirin (or *N,N'*-Dimethyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide Hydrochloride) (2).** Approximately 40 mL of anhydrous ethanol was cooled to -20 °C in the glass liner of a stainless steel bomb. Liquid methylamine was then bubbled into the cooled solution until a total volume of ~90 mL was obtained. To this solution was added in portions, 1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide (1) (3.0 g, 12 mmol). The reaction mixture/glass liner was transferred to the reaction bomb, the bomb was sealed, and the mixture was heated to 60 °C and stirred for 6 days. After thin-layer chromatography (silica gel plates, $CHCl_3$-MeOH-HOAc; 1:1:0.5) showed that the reaction had gone to completion, giving a single component, the solvent was then removed *in vacuo* and the residue was dried *in vacuo* over P_2O_5 to give a very hygroscopic, light brown foam (3.0 g, 92.3% yield). MS (FAB) *m/z* 272 (M + 1); IR (KBr) 3320 (broad), 3100, 2945, 1660, 1600, 1500 cm^{-1}; ^{13}C NMR (Me_2SO-d_6 + TFA-*d*) δ 29.61 (d, $NHCH_3$), 32.18 (d, $=NCH_3$), 61.01 (C-5'), 69.98 (C-3'), 74.93 (C-2'), 85.91 (C-4'), 92.55 (C-1'), 145.82 (C-5), 152.02 and 153.70 (C-3, N-C=N); ^{1}H NMR (Me_2SO-d_6 + TFA-*d*) δ 3.02 (s, 3 H, $NHCH_3$), 3.28 (s, 3 H, $=NCH_3$), 3.54 (d, 2 H, CH_2OH), 3.59 (d, 1 H, OH-5'), 4.02 (m, 1 H, H-4'), 4.20 (t, 1 H, H-3'), 4.42 (t, 1 H, H-2'), 5.97 (d, 1 H, H-1'), 7.90 (br s, 2 H, OH-2',3'), 9.92 (br s, 1 H, $NHCH_3$). *Anal.* Calcd for $C_{10}H_{17}N_5O_4 \cdot 1.2H_2O \cdot 0.5EtOH \cdot 1.0HCl$: C, 37.51; H, 6.31; N, 19.56. Found: C, 37.49; H, 6.69; N, 19.87. Our attempts to obtain a melting point for this material were complicated by its extreme hygroscopicity. However, using a N_2-filled glove box, we observed that the sample became gummy at 60 °C, and its melting point range was from 95-115 °C.

Hydrolysis Study of *Bis*-Methylamidine of Ribavirin. The *bis*-methylamidine of ribavirin (2, 200 mg) was added to 5 mL of a pH 7.4 phosphate buffer solution at room temperature. The solution was then stirred and monitored by thin-layer chromatography (silica gel plates, $CHCl_3$-MeOH-HOAc, 1:1:0.5) at various times over a 24-h period. After 1, 5, and 8 h, there was no evidence of any appreciable change in the composition of reaction mixture. However, after 24 h, the starting carboximidamide (*Rf* 0.45) was no longer present; instead, the TLC showed a component at *Rf* 0.69 which was later shown to correspond to *N*-methyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide. This conversion was confirmed by FAB-MS analysis of the lyophilized crude reaction mixture which showed an M + 1 = 259 corresponding to the methylcarboxamide,

instead of an M + 1 = 272 corresponding to the starting material, *N,N'*-dimethyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide.

***N*-Methyl Ribavirin (or *N*-Methyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide) (3).** Compound 1 (250 mg, 0.001 mol) was added to a round-bottomed flask and treated with 40% aqueous methylamine (8 mL, 0.229 mol). The mixture was refluxed for 36 h before it was evaporated *in vacuo*. A TLC of the residue showed that the starting material had been completely converted to a new product (silica gel plates, $CHCl_3$-MeOH-HOAc, 1:1:0.5, *Rf* 0.8), and therefore, the reaction mixture residue was crystallized from ethanol and dried over P_2O_5. (Yield, 320 mg, essentially quantitative.) MS (FAB) *m/z* 259 (M + 1); ^{1}H NMR (Me_2SO-d_6) δ 2.78 (d, 3 H, $NHCH_3$), 3.49, 3.64 (m, 2 H, H-5'), 3.96 (m, 1 H, H-4'), 4.14 (m, 1 H, H-3'), 4.36 (m, 1 H, H-2'), 5.00 (t, 1 H, OH-5'), 5.36 (d, 1 H, OH-3'), 5.65 (d, 1 H, OH-2'), 5.82 (d, 1 H, H-1'), 7.5 (br s, 1 H, NH, H^+), 8.50 (d, 1 H, NH), 8.90 (s, 1 H, H-5); IR (KBr) 3320, 1668, 1530, 1505, 1460, 1350, 1230, 1100 cm^{-1}. *Anal.* Calcd for $C_9H_{11}N_4O_5 \cdot 0.3H_2O \cdot 0.5EtOH$: C, 41.82; H, 6.35; N, 19.51. Found: C, 41.61; H, 6.11; N, 19.67.

Butylamine Amidine of Ribavirin (or *N*-Butyl-1-β-D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboximidamide) (12). A solution of methyl imidate 11 (300 mg, 116 mmol), *n*-butylamine (100 mg, 1.37 mmol) in anhydrous methanol (40 mL) was flushed with argon, sealed, and stirred at room temperature for 5 days. The solvent was then removed *in vacuo* and the residue was stirred overnight with anhydrous ether (50 mL). The ether was decanted, and the procedure was repeated with a fresh portion of anhydrous ether. Removal of the residual ether *in vacuo* gave 12 as a glassy solid, 316 mg, (87%), mp 63-66 °C. ^{1}H NMR (Me_2SO-d_6) δ 8.86 (s, 1 H, H-5), 5.80 (d, 1 H, *J* = 3.9 Hz, H-1'), 5.8 and 5.0 (br s, 5 H, OH, NH), 4.356 (dd, 1 H, *J* = 3.8, 4.9 Hz, H-2'), 4.147 (dd, 1 H, *J* = 4.9, 3.8 Hz, H-3'), 3.955 (dt, 1 H, *J* = 4.0, 5.1 Hz, H-4'), 3.63 (dab, 1 H, *J* = 4.1, 12.0 Hz, H-5'a), 3.51 (dab, 1 H, *J* = 5.0, 12.0 Hz, H-5'b), 3.27 (dab, 1 H, *J* = 7.0, 13.0 Hz, H-1''a), 3.23 (dab, 1 H, *J* = 7.2 Hz, 13.0 Hz, H-1'b); 1.55 (pentuplet, 2 H, *J* = 7.3 Hz, H-2''), 1.32 (sextet, 2 H, *J* = 7.4 Hz, H-3''), 0.90 (t, 3 H, *J* = 7.2 Hz, CH_3); ^{13}C NMR (Me_2SO-d_6) δ 157.35 (C=N), 153.4 (C-3 triazole), 144.94 (C-5 triazole), 91.85 (C-1'), 85.54 (C-4'), 74.55 (C-2'), 69.99 (C-3'), 61.31 (C-5'), 40.78 (C-1''), 30.99 (C-2''), 19.83 (C-3''), 13.79 (CH_3). MS (FAB) *m/z* 300 (M + 1). *Anal.* Calcd for $C_{12}H_{21}N_5O_4 \cdot 0.9H_2O \cdot 0.25Et_2O$): C, 46.74; H, 7.63; N, 20.96. Found: C, 46.64; H, 7.28; N, 20.71.

Asparagine Amidine of Ribavirin (or Asparagine, *N*-[[(1-β-D-ribofuranosyl)-1,2,4-triazole-3-yl]-iminomethyl]- (13). The procedure for synthesizing 12 was followed. A solution of imidate ester 11 (300 mg, 1.2 mmol) and asparagine (159 mg, 1.2 mmol) was flushed with argon, sealed, and stirred at room temperature for 5 days. The solvent was removed *in vacuo*, and the residue was stirred overnight with ether (50 mL). The ether was decanted, and the procedure was repeated 5 times with fresh portions of anhydrous ether. Removal of residual solvent *in vacuo* gave 13 (424 mg, >95%) as a white solid, mp 151-153 °C; ^{1}H NMR (Me_2SO-d_6) δ 9.14 (s, 1 H, H-5), 9.10 (br s, 3 H, NH), 7.77 and 7.10 (d, *J* = 1.7 Hz, $CONH_2$), 5.90 (2 d, 1 H, *J* = 3.5 Hz, H-1'), 5.9, 5.5, 5.0 (3 br s, 3 H, OH's), 4.39 (d d, 1 H, *J* = 3.5, 4.8 Hz, H-2'), 4.21 (dd, 1 H, *J* = 3.8, 7.1 Hz, H-1'), 4.17 (d d, 1 H, *J* = 5.4, 4.7 Hz, H-3', 3.99 (d d d, 1 H, *J* = 3.8, 4.8, 5.4 Hz, H-4'), 3.66 (dab, 1 H, *J* = 3.8, 12.1 Hz, H-5'a), 3.54 (dab, 1 H, *J* = 4.7, 12.1 Hz, H-5'b), 2.86 (dab, 1 H, *J* = 3.7 16.4 Hz, H-2''a), 2.67 (dab, 1 H, *J* = 7.2 Hz, 16.5 Hz, H-2''b); ^{13}C NMR (Me_2SO-d_6) δ

174.00 ($CONH_2$), 169.16 (COOH), 152.72 and 151.49 (C=N and C-3 triazole, unassigned), 146.14 (C-5 triazole), 92.57 (C-1'), 85.78 (C-4'), 74.73 (C-2'), 69.70 (C-3'), 61.00 (C-5'), 53.93 (C-1''), 37.50 (C-2''); MS (FAB) *m/z* 359 (M + 1) *Anal.* Calcd for $C_{12}H_{18}N_6O_7 \cdot 0.1H_2O \cdot 0.9MeOH$: C, 38.44; H, 5.71; N, 20.51. Found: C, 38.24; H, 5.87; H, 20.74.

Hydrolysis Study of Glycine Amidine of Ribavirin. The glycine amidine of ribavirin (triazole-3-carboximidamide (15) (6 mg) was added to 3 mL of a pH 7.4 phosphate buffer solution at room temperature, and the solution was then stirred. Monitoring by thin-layer chromatography (silica gel plates; $CHCl_3$-MeOH-HOAc, 1:1:0.5; originally planned to occur at 1 h intervals over an 8-h period) after 1 h showed an appreciable change in the composition of the reaction mixture. The starting material (*Rf* 0.5) was no longer present, and instead, the TLC showed a component at *Rf* 0.88 which corresponded to a reference sample of ribavirin.

2-(4-Acetyl-4-phenyl-piperidinyl)-1-(4-bromophenyl)ethanone (18a). Acetylphenylpiperidine 16 (3 g, 12 mmol) and 2,4'-dibromoacetophenone (17a) (1.7 g, 6 mmol) were added to $CHCl_3$ (30 mL) to give a beige suspension. Triethylamine (2.5 g, 25 mmol) was then added dropwise over 10 min, resulting in a clear solution. The reaction was allowed to stir at 60 °C for 48 h until TLC monitoring showed that all of the dibromoacetophenone had been consumed. The reaction mixture was then evaporated to dryness and the crude residue was purified by column chromatography (silica gel, $CHCl_3$). Collection and evaporation of the appropriate fractions gave dark orange crystals. The product was then recrystallized from ethanol. Yield 1.92 g, 80%; mp 103-105 °C; IR (KBr) 1697, 1683, 1677, 1583, 1400, 1356, 1298, 1292, 1262, 1109, 1012, 968, 794, 760, 725, 699 cm^{-1}; MS (FAB) *m/z* 400 (M + 1); ^1H NMR (Me_2SO-d_6) δ 1.86 (s, 3 H, CH_3), 1.96 (m, 2 H, -CH_2-), 2.32 (m, 2 H, -CH_2-), 2.38 (m, 2 H, -CH_2-), 2.66 (m, 2 H, -CH_2-), 7.32 (m, 5 H, Ar-H), 7.20 (d, 2 H, Ar-H, 7.92 (d, 2 H, Ar-H). *Anal.* Calcd for $C_{21}H_{22}BrNO_2$: C, 62.98; H, 5.53; N, 3.49. Found: C, 62.63; H, 5.49; N, 3.29.

2-(4-Acetyl-4-phenyl-piperidinyl)-1-(4-fluorophenyl)ethanone (18b). Acetylphenylpiperidine 16 (2 g, 8 mmol) and 2-chloro-4'-fluoroacetophenone (17b) (0.69 g, 4 mmol) were added to $CHCl_3$ (30 mL) to give a beige suspension. Triethylamine (1.67 g, 16 mmol) was then added dropwise over 10 min, resulting in a clear solution. The reaction was allowed to stir at 60 °C for 72 h until TLC monitoring showed that all of the chlorofluoroacetophenone had been consumed. The reaction mixture was then evaporated to dryness and the crude residue was purified by column chromatography (silica gel, $CHCl_3$). Collection and evaporation of the appropriate fractions gave dark orange crystals. The product was then recrystallized from ethanol. Yield 0.58 g, 42.7%; mp 93-94 °C; IR (KBr) 2879, 1692, 1687, 1595, 1351, 1222, 1210, 1157, 1150, 1112, 963, 959, 853, 815, 759, 699, 567, 559 cm^{-1}; MS (FAB) *m/z* 368 (M + 1); ^1H NMR (Me_2SO-d_6) δ 1.89 (s, 3 H, CH_3), 1.98 (m, 2 H, -CH_2-), 2.32 (m, 2 H, -CH_2-), 2.40 (m, 2 H, -CH_2-), 2.66 (m, 2 H, -CH_2-), 4.75 (s, 2 H, $NCH_2C{=}O$), 7.60 (m, 7 H, Ar-H), 8.18 (m, 2 H, Ar-H). *Anal.* Calcd for $C_{21}H_{22}FNO_2$: C, 74.30; H, 6.53; N, 4.13. Found: C, 74.10; H, 6.47; N, 4.08.

2-Thio-6-azauridine, 2',3',5'-Triacetate (22). The 2-thio-6-azauridine (8.7 g, 33.3 mmol) was dissolved in dry pyridine (100 mL) with stirring. Acetic anhydride (10.9 mL) was added, the system was flushed with argon, and the reaction solution was stirred for 3 h at room temperature and then stored overnight in the

refrigerator. The reaction mixture was concentrated to a light yellow oil *in vacuo*. The residue was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$ solution, H_2O, dilute HCl, and finally with brine. The $CHCl_3$ was dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in $CHCl_3$-MeOH (99:1) and purified by flash chromatography (silica gel). The appropriate fractions were combined and evaporated. Since pyridine was still present, the combined fractions were dissolved in $CHCl_3$ and washed well again with dilute HCl, saturated $NaHCO_3$ solution, and brine. The solution was again dried with $MgSO_4$, filtered, and evaporated, and then it was further dried under vacuum to remove all traces of solvent; yield 7.0 g, 54%; UV λ_{max} 271 nm (13,200, 218 (10,425) at pH 1; 268 (14,550), 236 (10,150) at pH 7; 267 (15,200), 236 (10,230) at pH 13; MS (FAB) *m/z* 388 (M + 1); IR (KBr) 1750, 1718, 1476, 1375, 1237 (broad), 1185, 1047 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.07 (m, 1 H, H-4'), 4.32 (m, 2 H, H-5'), 5.35 (t, 1 H, H-3'), 5.51 (m, 1 H, H-2'), 7.06 (d, 1 H, H-1'), 7.97 (s, 1 H, H-5). *Anal.* Calcd for $C_{14}H_{17}N_3O_8S$: C, 43.41; H, 4.42; N, 10.85. Found: C, 43.33; H, 4.56; N, 10.48.

5'-(Ethyl Methoxyalaninyl)phosphoramidyl-6-azauridine (25a). Into a 500 mL round-bottomed flask equipped with a stirring bar, additional funnel, and a drying tube was placed 6-azauridine (5 g, 20.4 mmol), trimethylphosphate (200 g), and 1-methylimidazole (16.7 g, 0.204 mol). The resulting mixture was well-stirred, and a solution of the ethyl methoxyalaninylphosphorochloridate (**24a**, 23.4 g, 0.102 mol, resulting from the addition of commercially available ethylphosphorodichloridate with alanine methyl ester) in trimethylphosphate (20-30 mL) was added dropwise over 45 min at room temperature. Three additional unweighed portions of the **24a** (the first two dissolved in trimethylphosphate) were then added with good stirring until the TLC of the reaction solution no longer showed any unreacted 6-azauridine. The reaction mixture was poured into ethyl acetate (2 L) and stored in a freezer for 2 h. The solution was decanted from the gum which had collected on the bottom of the flask. The gum was dissolved in CH_2Cl_2-MeOH (95:5) + 2 mL/L HOAc and passed through a 1 kg flash chromatography column. The product fractions were combined into three portions totalling 3.8 g. Since all three portions were contaminated with 1-methylimidazole, they were combined and rechromatographed (silica gel activated at 140 °C for 4 h). This provided one fraction of slightly more than 1 g of purified product. This sample was combined with a similar product obtained from an earlier synthesis by dissolving the samples in EtOH and evaporating. In an effort to reduce the amount of HOAc in the sample, two portions of benzene and then three portions of EtOH were added and evaporated. The resulting foam was dried *in vacuo* for several hours, covered with argon, and stored in a freezer; yield 2.4 g; MS (FAB) *m/z* 439 (M + 1); IR (KBr) 1730, 1700, 1216, 1060, 1052, 973 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.20 (m, 6 H, $CHCH_3$, CH_2CH_3), 3.61 (s, 3 H, OCH_3), 3.90 (br m, 8 H, H-5',4',3', $NCHC{=}O$, OCH_2CH_3), 4.20 (br s, 1 H, H-2'), 5.45 (app. q, 1 H, $CHNHP$), 5.90 (d, 1 H, H-1'), 7.52 (d, 1 H, H-5), 12.25 (br s, 1 H, H-3). *Anal.* Calcd for $C_{14}H_{23}N_4O_{10}P \cdot 0.5H_2O \cdot 0.4EtOH$: C, 38.13; H, 5.80; N, 12.02. Found: C, 38.26; H, 5.74; N, 11.99.

6-Cyanouridine, 2',3',5'-Triacetate (28b). 5-Bromouridine 2',3',5'-triacetate (27b) (8.77 g, 0.02 mol) and potassium cyanide (1.95 g, 0.03 mol) were added to DMF (100 mL) and stirred overnight at room temperature. Ethyl acetate (250 mL) and water (500 mL) were then added and the pH was then adjusted to 6-7 with 1*N* HCl. The organic layer was separated, dried with $MgSO_4$, filtered, and evaporated. The

resulting pale yellow, viscous oil (8.7 g) was purified by column chromatography (silica gel, $CHCl_3$-MeOH, 15:1) to give 4.2 g of a white solid; mp 71-75 °C; MS (FAB) *m/z* 396 (M + 1); IR (KBr) 3103, 1747, 1714, 1642, 1618, 1459, 1437, 1373, 1236, 1096, 1048 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 12.05 (br s, 1 H, H-3), 6.66 (s, 1 H, H-5), 5.83 (d, 1 H, H-1'), 5.62 (d d, 1 H, H-2'), 5.46 (t, 1 H, H-3'), 4.25 (m, 1 H, H-4'), 4.11 (d d, 1 H, H-5'), 2.10, 2.06, 2.02 (s, 9, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.699, 170.341, 169.572 (C=O), 160.334 (C-4), 148.365 (C-2), 127.264 (C-6), 113.307 (C-5), 110.604 (C≡N), 92.768 (C-1'), 79.767 (C-4'), 72.957 (C-2'), 69.652 (C-3'), 20.736, 20.467, 20.354 (CH_3). *Anal.* Calcd for $C_{16}H_{17}N_3O_9 \cdot 0.19NaBr$: C, 46.32; H, 4.13; N, 10.13. Found: C, 46.58; H, 4.14; N, 9.74.

6-Thiocarboxamidouridine, 2',3',5'-Triacetate (30b). Hydrogen sulfide was bubbled for 20 min into a stirred mixture of 6-cyanouridine 2',3',5'-triacetate (28b) (6.3 g, 0.05 mol) and pyridine (200 mL). The mixture was allowed to stand for one hour before the excess H_2S was removed by passing a stream of nitrogen through the flask and the reaction mixture was evaporated under reduced pressure. The residue (6.8 g) was passed through a 1 kg flash column chromatography (silica gel, $CHCl_3$-MeOH, 12:1) to give 5.9 g of a yellow, fluffy solid: mp 110-115 °C (became a glass); MS (FAB) *m/z* 430 (M + 1); IR (KBr) 1744, 1699, 1627, 1458, 1424, 1372, 1239, 1047 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.77 (s, 1 H, NH), 9.02 (s, 1 H, NH), 8.31 (s, 1 H, NH), 5.88 (m, 1 H, H-2'), 5.72 (d, 1 H, H-1'), 5.68 (s, 1 H, H-5), 5.54 (m, 1 H, H-3'), 4.46 (m, 1 H, *J*-5', 4.25 (m, 2 H, H-5', H-4', 2.10, 2.09, 2.08 (s, 9, CH_3); ^{13}C NMR ($CDCl_3$) δ 169.837, 169.451, 169.096 (C=O), 162.550 (C-4), 149.888 (C-2), 125.498 (C-6), 114.084 (C-5), 111.601 (C≡N), 90.718 (C-1'), 78.552 (C-4'), 72.171 (C-2'), 68.711 (C-3'), 62.367 (C-5'), 20.388, 20.200, 20.139 (CH_3). *Anal.* Calcd for $C_{16}H_{19}N_3O_9 \cdot 0.5$ H_2O: C, 43.84; H, 4.60; N, 9.56. Found: C, 43.59; H, 4.59; N, 9.28.

4-Thio-6-Azauridine, 2',3',5'-Triacetate (35).[5] Into a 100-mL round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and drying tube was added with stirring, 5 g (13.5 mmol) of 6-uridine, 2',3',5'-triacetate (34), and dry pyridine (50 mL). Phosphorus pentasulfide (2.1 g, 4.7 mmol) was then added quickly, the system was flushed with argon, and the reaction was heated at gentle reflux for 3 h. The reaction was then cooled, and the solution was decanted from the dark gum and evaporated at reduced pressure. The residue was dried *in vacuo* over P_2O_5 to remove pyridine. The residue was taken up in EtOH, treated with charcoal, filtered, and evaporated. The dark residue was dissolved in $CHCl_3$, washed with water, dried, filtered, and evaporated. A portion of the product was purified by flash chromatography on 100 g of silica gel, using $CHCl_3$ as the eluate. The product was isolated as an orange foam; yield, 380 mg; UV λ_{max} 325 nm (14,400), 242 (6,10) at pH 1; 335 (13,000), 247 (7,700) at pH 7; 335 (13,300), 247 (7,300) at pH 13; MS (FAB) *m/e* 388 (M + 1); IR (KBr) 1748, 1729, 1580, 1375, 1200 (broad), 1132, 1100, 1074, 1047 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.11 (m, 9, $COCH_3$), 4.17, 4.39 (2 m, 2, H-5'), 4.40 (m, 1, H-4'), 5.43 (t, 1, H-3'), 5.66 (q, 1, H-2'), 6.25 (d, 1, H-1'); 7.67 (s, 1, H-5), 10.40 (s, 1, NH). *Anal.* Calcd. For $C_{14}H_{17}N_3O_8S \cdot 0.1EtOH \cdot 0.1H_2O$: C, 43.31; H, 4.56; N, 10.67. Found: C, 43.26; H, 4.80; N, 10.66.

4-Thio-6-Azauridine (36).[5] A solution of 4-thio-6-azauridine, 2',3',5'-triacetate (35) (2.3 g, 5.94 mmol) in 150 mL of MeOH was slowly passed through a column of Dowex 1 resin (OH^-form). The column was washed with about 800 mL of MeOH, then washed with 1600 mL 5% HOAc in MeOH, and the resulting product was eluted with 5% HCOOH in MeOH. The appropriate fractions were combined, evaporated, the

residual HOAc and HCOOH were removed by azeotroping off several portions (2 X 50 mL) of EtOH-benzene followed by 2 portions of EtOH. The product was purified by chromatography through a flash column of 200 g of silica gel with $CHCl_3$-MeOH (7:1). The appropriate fractions were combined, evaporated, and the residue was dissolved in EtOH, evaporated, and dried at 56 °C over phosphorus pentoxide; yield, 400 mg; mp 104-115 °C $_{dec.}$; UV λ_{max} 328 nm (13,700), 243 (5,600) at pH 1; 334 (12,600), 247 (7,000) at pH 7; 336 (12,700), 247 (7,100) at pH 13; MS (FAB) *m/e* 262 (M + 1); IR (KBr) 1704, 1575, 1283, 1050 (broad), 990, 590 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.39, 3.50 (2 m, 2, H-5'), 3.80 (app. q, 1, H-4'), 4.02 (app. q, 1, H-3'), 4.24 (app. q, 1, H-2'), 4.65 (t, 1, OH-5'), 5.04 (d, 1, OH-3'), 5.30 (d, 1, OH-2'), 5.84 (d, 1, H-1'), 7.75 (s, 1, H-5), 13.7 (br s, 1, NH). *Anal.* Calcd. For $C_8H_{11}N_3O_5S \cdot 0.1HOAc \cdot 0.3EtOH$: C, 37.60; H, 4.73; N, 14.95. Found: C, 37.52; H, 4.98; N, 14.88.

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