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SYNTHESIS AND ANTIVIRAL EVALUATION OF PYRAZOFURIN ANALOGUES

ANNUAL REPORT

STEWART W. SCHNELLER

JUNE 19, 1991

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-89-C-9092

University of South Florida
Tampa, Florida 33620-5250

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION University of South Florida 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Tampa, Florida 33620-5250	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Comman 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-89-C-9092
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63002A PROJECT NO. 3M2-63002D807 TASK NO. AD WORK UNIT ACCESSION NO. WUDA318542

11. TITLE (Include Security Classification)

(U) Synthesis and Antiviral Evaluation of Pyrazofurin Analogues

12. PERSONAL AUTHOR(S)

Stewart W. Schneller

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 6/19/90 TO 6/18/91	1991 June 19	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RA 1, Pyrazofurin, Antiviral, Pyrazoles, Pyrroles, Ribofuranose derivatives
07	03		

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

5'-Deoxypyrazofurin and N-methyl and N,N-dimethyl carboxamido derivatives of pyrazofurin have been prepared and submitted to the Army for antiviral analysis. Antiviral data for 4-deoxy- and 4-homopyrazofurin and their acyclo derivatives (synthesized during the previous year) has been received and the four compounds were found to be non-toxic and lacking any appreciable antiviral properties. Synthetic methods towards a number of target pyrazofurin derivatives have been evaluated. The derivatives sought have been: 5'-homopyrazofurin, pyrazofurin nor-amide, 2-deazapyrazofurin, 1-deazapyrazofurin, 5'-amino-5'-deoxypyrazofurin, two phosphonate pyrazofurins, a phosphoramidite derivative of pyrazofurin, 3'-fluoro-3'-deoxypyrazofurin, and 2'- and 3'-deoxypyrazofurin. These efforts have so far led to one paper having appeared in the professional literature, one paper accepted for publication, and one paper just submitted. During this reporting period, a supply of pyrazofurin was received from Eli Lilly Company that has been useful to this project.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian	22b. TELEPHONE (Include Area Code) 301-663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

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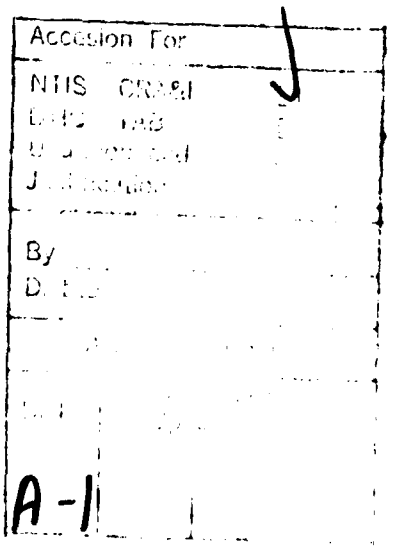

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Introduction

Nucleosides of 5-membered heterocycles are playing a prominent role in the design of antiviral agents.[1a] Included in this group is 4-hydroxy-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (pyrazofurin, **1**), which is a naturally occurring C-nucleoside that shows significant broad spectrum *in vitro* antiviral activity against DNA and RNA viruses.[1b,1c] The extent of its antiviral properties is represented by its activity against pox-, picorna, toga-, myxo-, rhabdo-, arena-, and bunyaviruses[1d-1f] with a high degree of selectivity

Even with its promising activity and broad safety margin in cell cultures, there have been reports[1e,1g] that the toxicity of **1** may[1h] limit its usefulness as an antiviral agent. However, De Clercq and Torrence[1d] have suggested that the toxicity of **1** is unlikely to be associated with the structural components that are responsible for its antiviral properties. To evaluate this suggestion for the proposes of producing non-toxic pyrazofurin-derived agents that are effective against the virus groups mentioned above, a systematic structure-antiviral activity study is being done under this contract. There is no literature precedent for this approach with **1** as an antiviral agent.

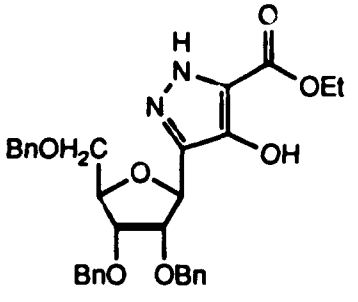
To accomplish the proposed plan, the heterocyclic unit, ring hydroxyl, amide side chain, and ribofuranosyl center of **1** are being sythetically varied. Following the syntheses, the target analogues are being submitted to the USAMRIID for antiviral analyses.

During this reporting period, synthesis of the following analogues has been pursued: (i) two pyrazofurin amides (**2**), (ii) 5'-deoxypyrazofurin (**3**), (iii) 5'-homopyrazofurin (**4**), (iv) pyrazofurin nor-amide (**5**), (v) 2-deazapyrazofurin (**6**), (vi) 1-deazapyrazofurin (**7**), (vii) 5'-amino-5'-deoxypyrazofurin (**8**), (viii) two pyrazofurin phosphonates (**9** and **10**) and a phosphoramidite (**11**), (ix) 3'-fluoro-3'-deoxypyrazofurin (**12**), (x) 2'-deoxypyrazofurin (**13**), and (xi) 3'-deoxypyrazofurin (**14**). The preparation of **2-4** and progress towards **5-14** are reported herein.

Body

1. Pyrazofurin Amides (2)

The synthesis of these analogues has been far from trivial due to the need to protect the 4-hydroxyl substituent in the precursor **15** by benzylation to accomplish amidation; this process also resulted in ring N-benzylation (Scheme 12 of the June 19, 1990 Quarterly Report). The subsequent debenzylations were not easily accomplished. However, both **2a** and **2b** have been prepared (Schemes 1 and 2) and were submitted for antiviral evaluation.



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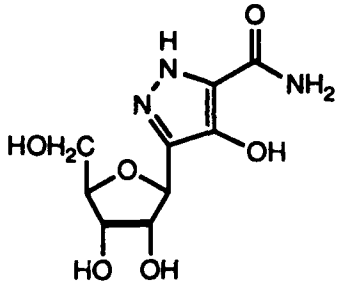

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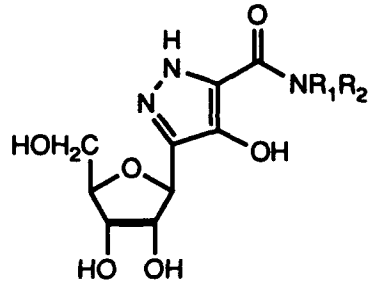
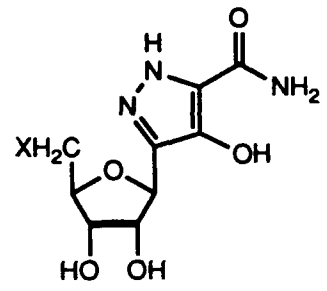
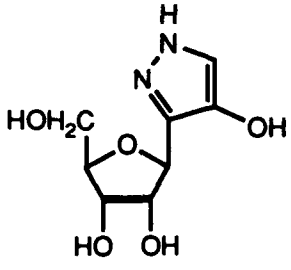


2a; R_1=H, R_2=Me
2b, R_1=R_2=Me



3, X=H
4, X=CH_2OH
8, X=NH_2



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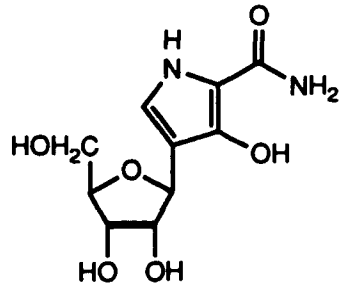

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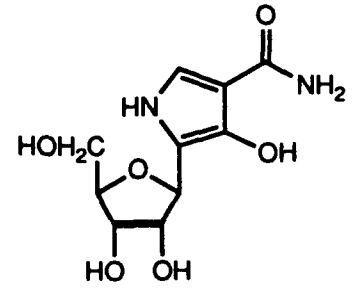

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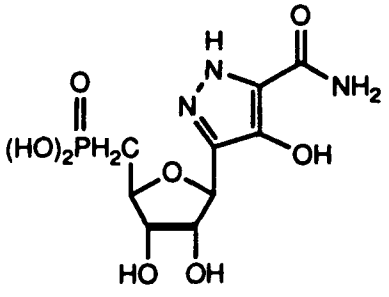

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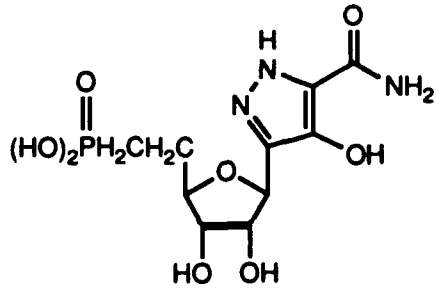

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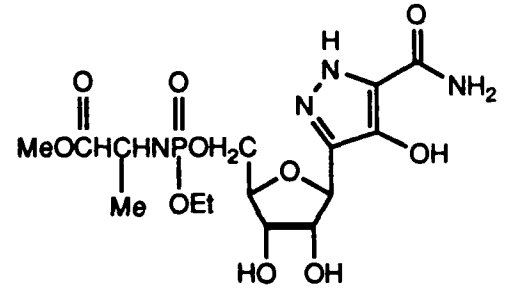

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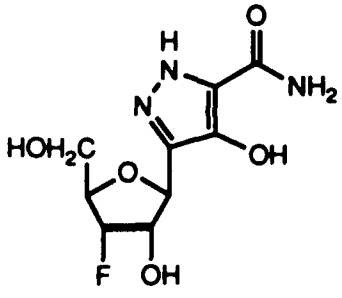

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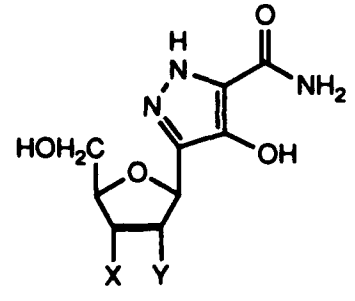
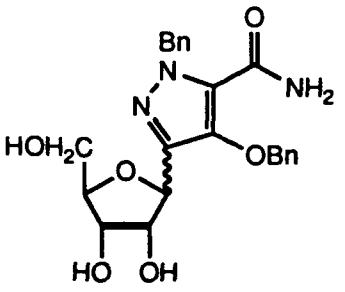


13, X=OH, Y=H
14, X=H, Y=OH

2. 5'-Deoxypyrazofurin (3)

The initial route considered to **3** is shown in Scheme 3 and required[2] protection of the 4-hydroxyl group of pyrazofurin (**1**, but shown as **16** from this point on in the Report). The benzyl group was chosen for this purpose since it could be reductively removed in the same step of the synthesis that was expected to form the methyl substituent from the iodo derivative **17**. After analyzing a variety of conditions, use of benzyl bromide in N,N-dimethylformamide containing potassium carbonate at room temperature was found to be the best for converting **16** into **18** (97% as β/α mixture) with minor contamination by the dibenzyl product **19**. Similar conditions using methyl iodide were found to be the best for preparing the O-methyl derivative **20** and treatment of **16** with two equivalents of benzyl bromide gave a 15:85 ratio of **18**:**19**.



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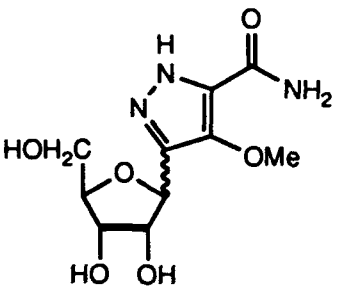
N-2 benzyl derivative also obtained



20

It should be mentioned that the basic reaction conditions used for the monobenzylation (or monomethylation) of pyrazofurin (**16**) led to a β/α mixture of **18** (or **20**). Anomerization of pyrazofurin and its derivatives has been noted previously by us[3] and others[4] when such compounds were subjected to basic conditions and is thought to proceed via a pathway similar to that shown in Scheme 4.

With **18** available attention turned to its iodination. Unfortunately, none of the conditions employed (including carbon tetraiodide/triphenylphosphine,[5] iodine/triphenylphosphine,[6] and methyltriphenoxyphosphonium iodide[7]), which have been used with related nucleosides, led to the desired **17**.

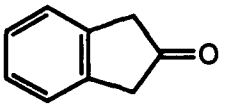
Thus, a different approach (Scheme 5) to **3** was followed. This pathway was based on our previous synthetic route to pyrazofurin amides[3] that was modified from a literature procedure[4] for preparing pyrazofurin. Scheme 5 has yielded anomerically pure **3** that was submitted for antiviral testing.

3. 5'-Homopyrazofurin (4)

The original plan to derivative **4** was to use the iodo compound **17** for homologation via displacement with cyanide followed by conversion of the resultant 5'-nitrile into the desired 5'-hydroxymethyl substituent of **4**.[8] As a consequence of the previously described difficulties associated with obtaining **17**, attention turned to pursuing **4** (Scheme 6) in a manner analogous to that for **3** (Scheme 5). To date, purification of **4** has not been achieved. Once this is accomplished, **4** will be submitted for antiviral testing.

4. Pyrazofurin Nor-amide (5)

The initial approach to **5** (Scheme 7) that has been investigated under this contract foresaw use of the 1,3-dipolar cycloaddition reaction of **34** with benzyloxyacetylene (**35**) to give **36**, which, upon debenzylation, would become **5**. As described in the June 19, 1990 Quarterly Report, **35** had been prepared by the pathway shown in Scheme 8 herein. During the current reporting period, reaction of **35** with **34** was studied. Using a variety of reaction conditions, the only product isolated was 2-indanone (**37**). A similar observation was described in the June 19, 1990 Report if the temperature was not kept sufficiently low in the preparation of **35** via Scheme 8. Compound **37** is believed to arise via a Claisen-type rearrangement[11] of **35** as postulated in Scheme 5 of the June 19, 1990 Report. As a result of the sensitivity of **35** to temperature changes, the Scheme 7 approach to **5** has been abandoned.



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(It should be noted that the use of **35** to prepare pyrazofurin--as proposed in Scheme 6 of the June 19, 1990 Quarterly Report and shown herein in Scheme 9--may still be worth considering since the anion of **35** could be trapped with methyl chloroformate at low temperature to form **38**.)

With the inability to realize **5** by Scheme 7, attention turned to Schemes 10 and 11, which were proposed in the June 19, 1990 Quarterly Report as alternative routes to **5**. In the case of Scheme 10, **39** had been proposed in the aforementioned 1990 Report (Scheme 3) as an intermediate in the formation of benzylamine and benzyl acetate from the dibenzylacetal of chloroacetaldehyde (**40**). It was proposed that **39** could be prepared from **40** by, first, converting **40** into 1,1,2-tribenzyloxyethane and reacting the latter material with methyllithium. Considerable problems were encountered in preparing **39** by this means, with only trace amounts being obtained. Thus, efforts then focused on Scheme 11. However, no conditions (for example, reference 12) could be found for decarboxylation of the benzyl protected pyrazofurin, apparently due to the electron-rich nature of its hydroxy pyrazole ring.

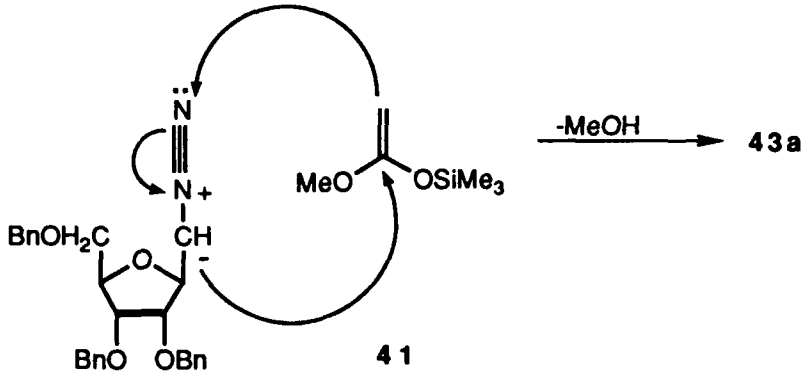
$(BnO)_2CHCH_2Cl$

40

Another approach to **5** is shown in Scheme 12. In this case, the 1,3-dipolar cycloaddition reaction of **34** and **41** led directly to a product that lacked the trimethylsilyl substituent and whose ^1H and ^{13}C data suggest it to be **43b** or its C-5 regioisomer. Electronic considerations (see drawing on the next page) suggest that **43a** is the correct regiochemistry for the cycloadduct. However, steric effects may exist that would lead to the C-5 regioisomer. The rapid decomposition of **43b** prevented spectral or X-ray crystallographic analysis to confirm its structure.

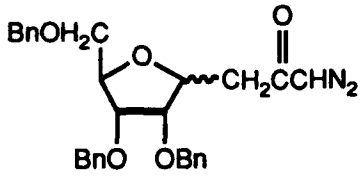
In the final plan to **5** (Scheme 13), which is analogous to the preparation of **3** and **4**, it was desirable to evaluate this method on an analogue of **44** that was more readily available as the β-anomer. Thus, a convenient synthesis of the tribenzoate **45** was developed as shown in Scheme 14. However, under a variety of basic conditions,

treatment of **45** with *p*-toluenesulfonylazide led to unidentifiable products that decomposed on standing and lacked the benzoyl protecting groups.



34 **41**

Considering that the benzoyl groups of **45** may have interfered with the azide addition, a model compound study was abandoned and **44** was treated with *p*-toluenesulfonylazide using triethylamine as the base with the hopes of obtaining **46**. Only starting material was recovered in the latter reaction suggesting that a stronger base was necessary to achieve the **44** to **46** conversion. A variety of bases were considered and only lithium diisopropylamide led to loss of **44** by TLC analysis. However, isomer **47** resulted by this reaction as shown by proton NMR determination, which showed a two proton doublet for $-CH_2COCN_2$ and no $-CH_3$ singlet. Thus, the route proposed by Scheme 13 was not considered further.



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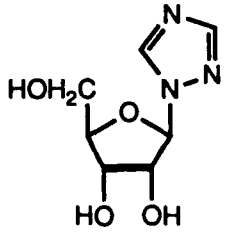

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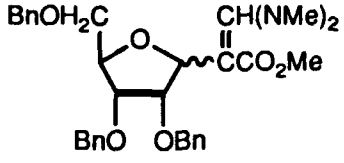
In view of the difficulties described herein and the apparent lack[13] of antiviral activity for the structurally similar ribavirin nor-amide **48**, efforts to prepare **5** for this project have been set aside.[14]

5. 2-Deazapyrazofurin (6)

The previous Annual Report stated (i) that the route to **6** being investigated at that time was that shown herein in Scheme 15, which resembles a literature[17] preparation of the corresponding 4-amino-5-ester (**49**, Scheme 15a) and (ii) that problems were encountered in the attempted ring closure of **50** into **51** with sodium methoxide. It was concluded that the free NH of **50** was interfering with this ring closure as a result of deprotonation under the basic conditions. Thus, during this year, attempts to protect this nitrogen upon reaction with methyl chloroformate (base: triethylamine) or benzyl bromide (base: sodium carbonate) were investigated but were unsuccessful. To achieve the same goal as benzylation of **50**, use of ethyl N-benzylglycinate in place of ethyl glycinate in

reaction with **52** was considered. However, the preparation of ethyl N-benzylglycinate was found to possess numerous problems.

In view of step *c* of Scheme 15a an attempt was made to improve the yield of **50** by preparing **53** for reaction with ethyl glycinate. However, reaction of **54** (Scheme 15) with **55**[18] failed to give **53**. It is unclear why **55** reacts successfully with the nitrile **56** but not with the corresponding ester **54**.



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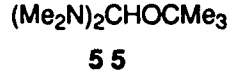


55

Schemes 16 and 17 illustrate two alternative approaches to **6**. In this direction, model studies were done on **57** (R=H) and found to succeed in forming **58** (R=H). When treated with formic acid in hopes of obtaining the formamide **59** (R=H), **58** gave a product that appeared to result from self-condensation of **59** (R=H). Thus, this sequence of reactions was not further evaluated on the less accessible **57** (R=β-D-ribofuranosyl). The reactions in Scheme 16 beginning with bromination of **57** remain to be investigated and no progress on Scheme 17 has been realized.

6. 1-Deazapyrazofurin (7)

At the conclusion of the previous Annual Report, Scheme 18 of this Report was projected as the next route to be pursued towards **7**. Nothing has been done in this direction due to the problems associated with **35**, which is the precursor of **38** (for step *b*), as described previously herein.

By analogy to Scheme 16, model reactions have been carried out on **57** (R=H) in Scheme 19. In this regard, bromination of **57** to **61** succeeded but the subsequent reaction of **61** with silver cyanide to produce **62** could not be accomplished. Thus, this pathway to **7** was not considered further, but the other sequence of reactions of Scheme 19 beginning with the ethyl formate/acetic anhydride treatment should be analyzed.

In view of the inability to prepare **38** for use in Scheme 18, Scheme 20 is projected as an alternative that uses a derivative of Meldrum's acid as the dipolarophile in step *b*. This route should be evaluated as a means to **7**.

38

A final approach to **7** is suggested in Scheme 21 and will be considered as is necessary.

7. 5'-Amino-5'-deoxypyrazofurin (8)

The plan to target compound **8** has been to develop a derivative of pyrazofurin substituted at C-5' with a leaving group that could be displaced by azide to give a product that could then be reduced to the desired C-5' amino moiety. In this direction, as described elsewhere herein, attempts to replace the C-5' hydroxyl of pyrazofurin with an iodo functionality failed. Success has been achieved, however, in realizing the 5'-tosyl

derivative (**63**) from the dibenzyl pyrazofurin **19** as shown in Scheme 22. Treatment of **63** with sodium azide in N,N-dimethylformamide followed by catalytic hydrogenation has yielded the N-1 benzyl derivative of the desired product (that is, **65**). To date, it has not been possible to remove the N-benzyl group from **65** (to realize **8**). This observation is reminiscent of similar problems in obtaining various amides (**2**) from N-benzyl precursors.

8. Phosphonates 9 and 10 and the Phosphoramidite 11

In this case, suitably functionalized C-5' pyrazofurin derivatives were desired as precursors to the side chains of **9-11**. For this purpose, functional group protection was necessary. As a consequence of the difficulty in removal of the N-1 benzyl protecting group from pyrazofurins so protected for synthetic manipulation at C-5' (see Scheme 22 and Section 7 above), attention has turned to using the acetyl moiety for protection during the preparation of **9-11** (and, possibly, useful for obtaining **8**). Thus, Scheme 23 shows that tritylation of pyrazofurin (**16**) yielded the C-5' product (**66**) (with no evidence of anomerization to a β/α mixture). Without full characterization, **66** was acetylated to **67** with only a single acetylation occurring on the ring moiety (unambiguous proof that ring acetylation occurred at the 4-hydroxyl has not been done). To date, attempts to remove the trityl group to provide an unprotected C-5' hydroxyl (**68**) have been unsuccessful (for example, using catalytic hydrogenation or silica gel) but it is anticipated that this can be done using other detritylation conditions (for example, 80% acetic acid or trifluoroacetic acid in 1-butanol). The crucial derivative will become the 5'-iodo compound **69**. In this direction, methyltriphenylphosphonium iodide will be employed to convert **68** into **69**. (It is unlikely that iodination problems similar to those reported previously herein using 4-O-benzylpyrazofurin will occur since the C-2' and C-3' hydroxyls of **68** are protected whereas in the previous studies they were not.) Compound **69** will then serve as the precursor to **9** and **10** as shown in Schemes 23 and 24, respectively. Nothing has been done yet towards target analogue **11** but the planned synthesis is outlined in Scheme 25.

9. 3'-Fluoro-3'-deoxypyrazofurin (12)

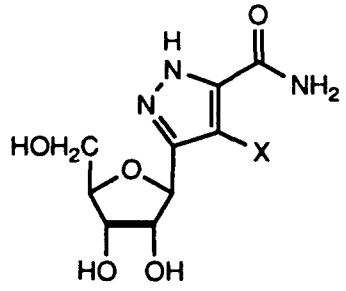
The approach to **12** is following the "de novo" idea used for preparing **3** and **4** (as summarized in Scheme 26) and, consequently, required the synthesis of the fluoro precursor **72**. Since diethylaminosulfur trifluoride (DAST) has found considerable use in the preparation of fluoro derivatives from alcohol precursors (for example, **73** in Scheme 27) with inversion of configuration, this reagent was considered first. For this purpose, **73**[20] was subjected to benzoylation to give **74**. (It should be noted that benzylation of **73** led to C-3,C-5 dibenzylation.) However, treatment of **74** with DAST in methylene chloride at -78 °C[21] or at 0 °C (in the presence of pyridine)[22] gave very low yields of the desired **72** (no reaction occurred at room temperature).[23] On the other hand, conversion of **74** into its triflate derivative **75** followed by reaction with cesium fluoride in N,N-dimethylformamide[22] gave a good yield of **72**. This latter material is now being converted into **12** by the sequence of reactions presented in Scheme 26.

10. 2'- (13) and 3'-Deoxypyrazofurin (14)

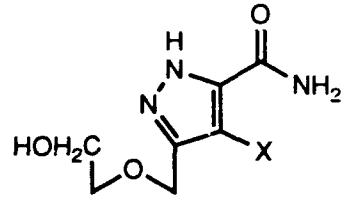
Scheme 28 shows the work to date on achieving **13** and **14**. However, due to the priorities given to other analogues, research in this area, which was begun during this reporting period, is no longer being carried out.

11. Antiviral Data

Antiviral data for the four compounds shown on the next page, which were prepared during the previous annual period, was received this year from the Army (see Appendix). None of the compounds showed any antiviral activity against (i) human immunodeficiency virus (HIV-1), (ii) the RNA containing viruses sandfly fever (bunyavirus), Punta Toro (bunyavirus), Japanese encephalitis (flavivirus), yellow fever (flavivirus), Venezuelan equine encephalomyelitis (alphavirus), and (iii) the DNA containing vaccinia virus (AVS 006973 was mildly active against vaccinia virus). Also, none of the compounds displayed any cytotoxicity.



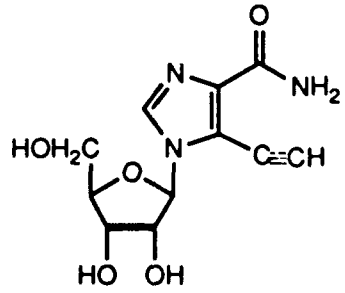
AVS 006973, X=H
AVS 006974, X=CH_2OH



AVS 006441, X=H
AVS 006950, X=CH_2OH

Conclusions

The second year of this contract has seen the successful synthesis of three pyrazofurin derivatives (**2a**, **2b**, and **3**), which have been submitted to the Army for antiviral analysis. Analogue **4** has also been prepared but is pending further purification prior to submission for antiviral analysis. Also, synthetic methods have been investigated towards (i) pyrazofurin nor-amide (**5**), (ii) 2-deazapyrazofurin (**6**), (iii) 1-deazapyrazofurin (**7**), (iv) 5'-amino-5'-deoxypyrazofurin (**8**), (v) two pyrazofurin phosphonates (**9** and **10**) and a phosphoramidite (**11**), (vi) 3'-fluoro-3'-deoxypyrazofurin (**12**), (vii) 2'-deoxypyrazofurin (**13**), and (viii) 3'-deoxypyrazofurin (**14**). Due to synthetic complexities and potential antiviral considerations, derivative **5** will no longer[14] be considered. On the other hand, the coming year will see completion of the synthesis of **8-12** with efforts also continuing towards **6** (Schemes 16 and 17) and **7** (Schemes 19-21). If 5'-amino-5'-deoxypyrazofurin (**8**) cannot be prepared by debenzylation of **65**, an alternative route will be considered (Scheme 29). Finally, it would be interesting to prepare and evaluate the pyrazofurin analogue of 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide[26] (shown below). With the results to date towards to various analogues, success can be anticipated in the work planned for the coming year.



It should be mentioned that a supply of pyrazofurin was provided by Eli Lilly Company this year and it has been used in Schemes 3, 23, and 28 and may be applicable to Scheme 25.

To date, based on the research performed under this contract, one paper has appeared in the professional literature, one has been accepted for publication, and a third has just been submitted for publication consideration.

Experimental

Materials and Methods. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL FX90Q or Bruker AMX-360 spectrometer in $CDCl_3$ or DMSO-d_6 referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm E. Merck Silica gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. The column chromatographic purifications were performed using Davidson Chemical silica gel (60-200 mesh) or Aldrich silica gel (230-400 mesh, 60 Å) eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^1H and ^{13}C NMR) homogeneous materials. The reactions were generally carried out in a N_2 or Ar atmosphere under anhydrous conditions.

4-Hydroxy-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-(N-methyl)carboxamide (2a). 4-Hydroxy-3(5)-(2',3',5'-tri-O-benzyl-β-D-ribofuranosyl)pyrazole-5(3)-(N-methyl)carboxamide (Quarterly Report, June 19, 1990) (1.3 g, 2.4 mmol) was dissolved in MeOH (50 mL) and 5% Pd-C catalyst (40 mg) was added to the mixture. The mixture was subjected to hydrogenation at atmospheric pressure for 13 days or 40 psi for 24 h. Following this period, the catalyst was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residual oil was purified by silica gel column chromatography using MeCN-H_2O (95:5). Compound **2a** was obtained in 78% yield (500 mg) and was recrystallized from MeCN-H_2O: mp 163-164 °C; ^1H NMR (90 MHz, DMSO-d_6) δ 2.52 (s, 3 H, NMe), 3.27 (m, 2 H, H-5'), 3.49-3.96 (m, 3 H, H-2', H-3', H-4'), 4.43 (d, 1 H, H-1'); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 25.5, 61.9, 71.6, 74.2, 76.3, 84.6, 127.1, 132.6, 139.9, 162.0. Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.96; H, 5.53; N, 15.38. Found: C, 44.07; H, 5.70; N, 15.12.

4-Hydroxy-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-(N,N-dimethyl)carboxamide (2b). 1-Benzyl-4-benzyloxy-3(5)-(2',3',5'-tri-*O*-benzyl-β-D-ribofuranosyl)pyrazole-5(3)-(N,N,-dimethyl)carboxamide (Quarterly Report of June 19, 1990) (2.5 g, 3.4 mmol) was dissolved in a solution of AcOH (100 mL) and MeOH (20 mL) and to this was added 10% Pd-C (100 mg). This mixture was subjected to hydrogenation under 90 psi at room temperature for 150 h. Filtration to remove the catalyst and evaporation of the filtrate *in vacuo* gave a residue that was purified by column chromatography (CH_2Cl_2-MeOH, 100:5). A material resulted that recrystallized from MeCN-MeOH (100:5) as white crystals (250 mg, 25.7%) of **2b**: mp 160.5-161 °C; ^1H NMR (90 MHz, DMSO-d_6) δ 3.05 (s, 3 H), 3.15 (s, 3 H), 3.55 (m, 2 H), 3.75 (m, 1 H), 3.95 (d, 1 H), 4.20 (d, 1 H), 4.75 (d, 1 H); ^{13}C NMR (22.5 Hz, DMSO-d_6) δ 36.5, 63.0, 72.3, 74.9, 76.0, 85.6, 126.0, 132.5, 143.5, 165.5. Anal. Calcd. for $C_{11}H_{17}N_3O_6$: C, 45.99; H, 5.97; N, 14.63. Found: C, 46.13; H, 6.10; N, 14.74.

5-Deoxy-2,3-(di-O-benzyl)-D-ribofuranose (22). To a solution of methyl 5-deoxy-2,3-O-isopropylidene-β-D-ribofuranoside (**21**)[10] (40 g, 0.21 mol) in MeOH (500 mL) was added Amberlite IR-120 (H^+) ion exchange resin (400 g) that had been pre-equilibrated several times with absolute MeOH. The mixture was stirred and heated under reflux for 4 h, cooled to room temperature, and filtered. The resin was washed with MeOH. The original filtrate and the washings were combined and evaporated to dryness. The residual syrup (21 g, 0.14 mol) dissolved in dry DMF (150 mL) was added to a suspension of NaH (10 g, 0.33 mol, 80% in oil) in dry DMF (100 mL). To this DMF solution was added benzyl bromide (55 g, 0.32 mol) at 0 °C. The resultant reaction

mixture was stirred for 5 h, which was followed by the careful addition of H_2O at 0 °C. Ethyl ether (300 mL) was added and the ether layer separated and washed with H_2O (5 x 100 mL). The ether layer was then dried (Na_2SO_4) and evaporated. The residual material was dissolved in a mixture of dioxane (300 mL) and 1 N HCl (100 mL) and the solution that resulted was refluxed for 6 h. After removal of the dioxane by rotary evaporation, Et_2O was added and the resultant mixture was washed with H_2O. The ether layer was dried (Na_2SO_4) and evaporated. The residual material was subjected to column chromatography with hexane-AcOEt (5:1) to give **22** (17 g, 38%) as a syrup: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.15 and 1.3 (dd, J = 12 Hz, 3 H, CH_3 of α/β mixture), 3.50-4.40 (m, 3 H, H-2, H-3, and H-4), 4.45-4.70 (4 s, 4 H, $PhC\underline{H}_2$ of α/β mixture), 5.13 (dd, 1 H, H-1 of α/β mixture), 7.32 (m, 10 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 19.5 and 20.5 (CH_3 of α/β mixture), 72.0, 72.3 and 72.5 ($PhCH_2$ of α/β mixture), 77.0, 80.5 and 81.7, 81.7 and 82.5 (C-2, C-3, and C-4 of α/β mixture), 90.5 and 100.0 (C-1 of α/β mixture), 127.0-129.0 and 137.2-138.0 (Ar).

Ethyl 3-Oxo-4-[5'-deoxy-2',3'-(di-O-benzyl)-α- and -β-D-ribofuranosyl]butanoate (23). A solution of **22** (17 g, 54 mmol) and 3-ethoxycarbonyl-2-oxopropylidenetriphenylphosphorane[3] (63 g, 128 mmol) in anhydrous MeCN (100 mL) was refluxed for 48 h. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography purification. Elution with hexane-AcOEt (5:1) gave **23** (16.6 g, 72%) as a viscous oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.05-1.35 (m, 6 H, $CH_2C\underline{H}_3$ and 5'-CH_3), 2.50-3.0 (m, 2 H, H-4), 3.30-4.30 (m, 8 H, $C\underline{H}_2CH_3$, H-2, H-1', H-2', H-3', and H-4'), 4.45-4.65 (m, 4 H, 2 x $PhC\underline{H}_2$), 7.30 (m, 10 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 16.0 ($CH_2\underline{C}H_3$), 21.5 and 21.8 (5'-CH_3 of α/β mixture), 45.5 and 45.8 (C-4 of α/β mixture), 51.5 (C-2), 63.0 ($\underline{C}H_2CH_3$), 73.5, 74.5 and 75.5 (2 x $Ph\underline{C}H_2$ of α/β mixture), 77.2 and 77.8, 79.0 and 79.8, 81.5 and 81.9, 84.6 and 86.2 (C-1', C-2', C-3', and C-4' of α/β mixture), 128.5-131.5 and 139.0-140.5 (Ar), 168.5 (ester carbonyl), 202.5 and 208.0 (ketone carbonyl of α/β mixture).

Ethyl 2-Diazo-3-oxo-4-[5'-deoxy-2',3'-(di-O-benzyl)-α- and -β-D-ribofuranosyl)butanoate (24). Triethylamine (3.9 g, 39 mmol) and *p*-toluenesulfonyl azide (17 mL) were added to a solution of **23** (16.6 g, 39 mmol) in anhydrous MeCN (140 mL). The mixture was kept at 15 °C for 30 min. The solvent was then evaporated under reduced pressure and the residue was subjected to column chromatographic purification. Elution with hexane-AcOEt (5:1) gave **24** as a viscous oil (8.5 g, 48%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.10-1.35 (m, 6 H, $CH_2C\underline{H}_3$ and C-5' CH_3), 3.10-3.30 (m, 2 H, H-4), 3.50-4.50 (m, 5 H, $CH_3C\underline{H}_2$, H-2', H-3', and H-4'), 4.60 (m, 4 H, 2 x $PhC\underline{H}_2$), 4.85 (m, 1 H, H-1'), 7.30 (s, 10 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 15.5 ($CH_2\underline{C}H_3$), 20.2 and 20.7 (C-5' CH_3 of α/β mixture), 45.5 (C-4'), 72.5 and 73.0 (2 x $Ph\underline{C}H_2$), 76.0, 77.7, 79.0, 80.5 and 82.5 (C-1', C-2', C-3', C-2, and C-4), 127.0-130.0 and 137.5-138.5 (Ar), 161.5 (ester carbonyl), 189.5 and 191.0 (ketone carbonyl of α/β mixture).

Ethyl 4-Hydroxy-3(5)-[5'-deoxy-2',3'-(di-O-benzyl)-α- and -β-D-ribofuranosyl]pyrazole-5(3)-carboxylate (25). A solution of **24** (8.5 g, 18.8 mmol) in dry THF (50 mL) was added dropwise to a stirred, ice cooled suspension of NaH (3 g, 0.1 mol, 80% in oil) in dry THF (50 mL) under N_2. The mixture was stirred at room temperature for 24 h. A solution of AcOH (1.26 g, 21 mmol) in THF was then added dropwise to the stirred, ice cooled reaction mixture. The solvent was evaporated under reduced pressure to give a residue to which were added H_2O and Et_2O. The ethereal layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resultant residue was subjected to column chromatography using hexane-AcOEt (3:1) as the eluting solvent mixture to give **25** (α/β=1:1) as a foam (4.5 g, 53%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.15-1.40 (m, 6 H, 2 x CH_3), 3.80 (q, 1 H, H-4'), 3.90-4.40 (m, 4 H, H-2', H-3', and $C\underline{H}_2CH_3$), 4.35 and 4.60 (m, 4 H, 2 x $PhC\underline{H}_2$), 5.20 and 5.32 (dd, 1 H, H-1' of α/β

mixture), 7.28 (m, 10 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 13.2 ($CH_2\underline{C}H_3$), 18.0 and 18.4 (C-5' of α/β mixture), 60.0 and 60.3 ($\underline{C}H_2CH_3$ of α/β mixture), 71.1 and 71.4, 71.8 and 72.2 (2 x $Ph\underline{C}H_2$ of α/β mixture), 76.0 and 76.5, 76.5 and 76.8, 77.5 and 78.5, 81.8 and 82.5 (C-1', C-2', C-3' and C-4' of α/β mixture), 122.0, 125.5, 132.0, 142.0 and 142.8 (C-3, C-4 and C-5 of α/β mixture), 127.2, 136.2 and 136.8 (Ar), 161.0 and 162.7 (carbonyl of α/β mixture).

4-Hydroxy-3(5)-[5'-deoxy-(2',3'-di-O-benzyl)-β-D-ribofuranosyl]-pyrazole-5(3)-carboxamide (26). A solution of α/β **25** (1.63 g, 3.6 mmol) in dry MeOH (30 mL) was saturated with anhydrous NH_3 at 0 °C. The solution was heated at 90-95 °C in a sealed vessel for 7 h. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography. Elution with hexane-AcOEt (3:1) gave **26** (1.0 g, 66%, only β anomer) as a solid: ^1H NMR (90 MHz, $CDCl_3$) δ 1.35 (dd, 3 H, 5'-CH_3), 3.68 (m, 1 H, H-4'), 4.20-4.50 (m, 2 H, H-2' and H-3'), 4.55 and 4.70 (2 s, 4 H, 2 x $PhC\underline{H}_2$), 5.30 (d, 1 H, H-1'), 7.30 (m, 10 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 18.9 (5'-CH_3), 71.9 (2 x $Ph\underline{C}H_2$), 76.8, 77.4, 79.5 and 82.2 (C-1', C-2', C-3' and C-4'), 123.0, 130.0 and 140.5 (C-3, C-4 and C-5), 126.0-128.5, 137.1, and 137.3 (Ar), 165.8 (carbonyl).

4-Hydroxy-3(5)-(5'-deoxy-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (5'-Deoxypyrazofurin, 3). A suspension of **26** (900 mg, 2.1 mmol) in MeOH (50 mL) containing 10% Pd-C (30 mg) was subjected to a pressure of H_2 (60 psi) for two days. Filtration of the suspension and evaporation of the filtrate gave a residue that was purified by column chromatography using CH_2Cl_2-MeOH (20:1) to give **3** (500 mg, 90%) as a solid: ^1H NMR (90 MHz, DMSO-d_6) δ 1.20 (d, 3 H, CH_3), 3.70 (m, 2 H), 4.25 (t, 1 H), 4.67 (d, 1 H), 7.35 (s, 2 H, NH_2); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 19.0 (C-5'), 73.0 (ribofuranosyl C), 76.0 (2 x ribofuranosyl C), 78.5 (ribofuranosyl C), 127.5, 132.5, and 140.7 (pyrazole C), 163.5 (amide carbonyl). Anal. Calcd. for $C_9H_{13}N_3O_5$: C, 44.44; H, 5.39; N, 17.28. Found: C, 44.57; H, 5.44; N, 17.07.

5-Deoxy-2,3,6-(tri-O-benzyl)-α- and -β-D-allofuranose (29). A solution of methyl 5-deoxy-α- and -β-D-allofuranoside (**27**)[9] (33 g, 0.18 mol) in DMF (100 mL) was added dropwise to a suspension of NaH (23 g, 0.76 mol, 80% oil) in DMF (200 mL) cooled in an ice bath. Benzyl bromide (114 g, 0.66 mol) was then added dropwise. The reaction mixture was stirred for 6 h at room temperature. After ice-H_2O (300 mL) was carefully added, extraction with Et_2O (2 x 300 mL) was carried out and the ethereal layer then washed with H_2O (5 x 200 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue (*ca.* 80 g) was dissolved in a mixture of dioxane (300 mL) and 1 N HCl (150 mL) and this new solution was refluxed for 4 h. After cooling the reaction solution, it was neutralized with $NaHCO_3$ and then evaporated to 200 mL, which was, in turn, washed with CH_2Cl_2 (3 x 200 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The resultant residue was purified by column chromatography using hexane-AcOEt (5:1) as the eluting solvent mixture to give **29** (24 g, 29.8%) as an oil: ^1H NMR (90 MHz, $CDCl_3$) δ 1.78 (q, 2 H, H-5), 3.62 (t, 2 H, H-6), 3.70-4.40 (m, 3 H, H-2, H-3 and H-4), 4.45-4.62 (m, 6 H, 3 x $PhC\underline{H}_2$), 5.30 (d, 1 H, H-1), 7.30 (m, 15 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 34.2 and 35.0 (C-5 of α/β mixture), 66.8 and 67.2 (C-6 of α/β of mixture), 72.0 and 72.3, 72.4 and 72.7, 73.0 and 73.2 (3 x $Ph\underline{C}H_2$ of α/β of mixture), 77.5 and 78.5, 79.2 and 80.2, 80.2 and 81.2 (C-2, C-3 and C-4 of α/β of mixture), 95.7 and 100.0 (C-1 of α/β of mixture), 127.0-129.0 and 138.5-139.5 (Ar).

Ethyl 3-Oxo-4-[5'-deoxy-2',3',6'-(tri-O-benzyl)-α- and -β-D-allofuranosyl]butanoate (30). A solution of **29** (24 g, 55.3 mmol) and 3-ethoxycarbonyl-2-oxopropylidenetriphenylphosphorane[3] (70 g, 143 mmol) in anhydrous MeCN (400 mL) was refluxed for 60 h. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography purification. Elution with hexane-AcOEt (5:1) gave **30** (19 g, 63%, β/α *ca.* 1:1) as a viscous oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.25 (t, 3 H, $CH_2C\underline{H}_3$), 1.80 (m, 2 H, H-5'), 2.45-2.85 (m, 2 H, H-4), 3.40 and 3.60 (2s, 6 H, 3 x $PhCH_2$), 3.50 (t, 3 H, H-6'), 3.60-4.40 (m, 8 H, $C\underline{H}_2CH_3$, H-2, H-1', H-2', H-3', and H-4'), 7.30 (m, 15 H, Ar-H); ^{13}C NMR ($CDCl_3$) was not easily resolved at 22.5 MHz due to the presence of the two anomers.

Ethyl 2-Diazo-3-oxo-4-[5'-deoxy-2',3',6'-(tri-O-benzyl)-α- and -β-D-allofuranosyl]butanoate (31). Triethylamine (3.5 g, 34.7 mmol) and *p*-toluenesulfonyl azide (19 mL) were added to a solution of **30** (19 g, 34.7 mmol) in anhydrous MeCN (155 mL). The mixture was kept at 15 °C for 30 min. The solvent was then evaporated under reduced pressure and the residue was subjected to column chromatographic purification. Elution with hexane-AcOEt (5:1) gave **31** as a viscous oil (13 g, 65%, β/α *ca.* 2:1): ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.30 (t, 3 H, CH_3), 1.85 (m, 2 H, H-5'), 3.10-3.29 (dd, 2 H, H-4 α/β mixture), 3.55 (t, 2 H, H-6'), 3.65-4.35 (m, 6 H, H-4, H-1', H-2', H-3', and H-4'), 4.46, 4.51, and 4.55 (3s, 6 H, 3 x $PhC\underline{H}_2$), 7.30 (s, 15 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 14.38 ($CH_2\underline{C}H_3$), 33.99 and 34.31 (C-5' α/β mixture), 42.03 and 44.34 (C-4 α/β mixture), 61.45 ($\underline{C}H_2CH_3$), 67.14 and 67.30 (C-6' α/β mixture), 71.91 and 72.77 and 72.99 and 73.53 (3 x $Ph\underline{C}H_2$ α/β mixture), 75.65, 77.32, 79.16, and 80.41 (C-1', C-2', C-3', C-4'), 127.0-130.0 and 137-138 (Ar), 167.5 (ester carbonyl), 189.73 and 190.8 (ketone carbonyl and diazo carbon).

Ethyl 4-Hydroxy-3(5)-[5'-deoxy-2',3',6'-(tri-O-benzyl)-α- and -β-D-allofuranosyl]pyrazole-5(3)-carboxylate (32). A solution of **31** (13.0 g, 22.7 mmol) in dry THF (100 mL) was added dropwise to a stirred, ice cooled suspension of NaH (3.5 g, 117 mmol, 80% in oil) in dry THF (100 mL) under N_2. The mixture was stirred at room temperature for 12 h. A solution of AcOH (7.0 g, 117 mmol) in THF (20 mL) was then added dropwise to the stirred (ice cooled) reaction mixture. The solvent was evaporated under reduced pressure to give a residue to which were added H_2O (110 mL) and Et_2O (100 mL). The ethereal layer was dried (Na_2SO_4) and concentrated under reduced pressure. The resultant residue was subjected to column chromatography using hexane-AcOEt (3:1) as the eluting solvent mixture to give **32** (8.0 g, 61.5%, β:α=2:1), mp 111 °C following recrystallization from Et_2O: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.40 (t, 3 H, CH_3), 1.87 (quintet, 2 H, H-5'), 3.60 (m, 2 H, H-6'), 3.75-4.35 (m, 3 H, H-2', H-3', and H-4'), 4.40 (q, 2 H, $C\underline{H}_2CH_3$), 4.45-4.70 (m, 6 H, 3 x $PhC\underline{H}_2$), 5.25 (2d, 1 H, H-1' α/β mixture), 7.30 (s, 15 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 14.4 (CH_3), 33.12 and 34.30 (C-5' α/β mixture), 61.07 and 61.24 ($\underline{C}H_2CH_3$ of α/β mixture), 66.76 (C-6'), 72.07 and 71.4, 72.99 and 73.15 (3 x $Ph\underline{C}H_2$ of α/β mixture), 75.08 and 76.45, 79.33 and 79.55, 79.55 and 79.71, 80.63 and 81.82 (C-1', C-2', C-3' and C-4' of α/β mixture), 124.7 and 126.08, 129.90 and 131.93, 142.77 and 144.18 (C-3, C-4 and C-5 of α/β mixture), 127-129 and 137-139 (Ar), 163.00 (carbonyl of ester). Anal. Calcd. for $C_{33}H_{36}N_2O_7$: C, 69.21; H, 6.34; N. 4.89. Found: C, 69.46; H, 6.32; N, 4.93.

4-Hydroxy-3(5)-[5'-deoxy-2',3',6'-(tri-O-benzyl)-β-D-allofuranosyl]pyrazole-5(3)-carboxamide (33). A solution of α/β **32** (6.5 g, 11.4 mmol) in dry MeOH (30 mL) was saturated with anhydrous NH_3 at 0 °C. The mixture was then heated at 90-95 °C in a sealed vessel for 7 h. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography. Elution with hexane-AcOEt (3:1) gave **33** (4.5 g, 73%, only β anomer) that was used directly in the preparation of **4** given below: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.95 (m, 2 H, H-5'), 3.65 (m, 3 H), 4.29 (m,

2 H), 4.45 (m, 4 H), 4.75 (d, 2 H), 5.29 (d, 1 H, H-1'), 7.28 (s, 15 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 33.25 (C-5'), 66.92 (C-6'), 71.80 and 72.72 (3 x $PhCH_2$), 77.0, 80.68, and 81.05 (C-1', C-2', C-3' and C-4'), 123.1, 131.5 and 140.7 (C-3, C-4 and C-5), 127.5, 127.76, 128.03, 137.51, and 137.68 (Ar), 164.5 (carbonyl).

4-Hydroxy-3(5)-(5'-deoxy-β-D-allofuranosyl)pyrazole-5(3)-carboxamide (5'-Homopyrazofurin, 4). A suspension of **33** (4 g, 7.37 mmol) in MeOH (50 mL) containing 10% Pd-C (30 mg) was subjected to a pressure of H_2 (60 psi) for four days. Filtration of the suspension and evaporation of the filtrate gave a residue that was purified by column chromatography using CH_2Cl_2-MeOH (20:1) to give **4** (1.5 g, 74.55%) as a solid, which was subjected, unsuccessfully, to recrystallization using a number of solvent systems. These efforts caused the product, which was originally only the β-anomer, to become a mixture of β- and α-anomers. This is currently being studied further so that the ^1H NMR and ^{13}C NMR data for β-**4** can be obtained.

2-Indanone (37) [from attempted preparation of 4-benzyloxy-3-(2',3',5'-tri-O-benzyl-β-D-ribofuranosyl)pyrazole, 36]. A mixture composed of 1.2 g (2.5 mmol) of 1-acetamido-2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-D-allitol (Quarterly Report, March 19, 1990) dissolved in 20 mL of a 1:1 mixture of CCl_4-glacial AcOH containing 1.2 g of anhydrous NaOAc was cooled to 3 °C in an ice/H_2O bath. This mixture was then treated with 2 mL of liquid N_2O_4 and stirred for 1.5 h at 3 °C. Following this period, the solution was poured over 120 mL of ice/H_2O with subsequent vigorous stirring of the resultant mixture for 30 min. The organic layer was then separated and the aqueous layer extracted with CH_2Cl_2 (2 x 25 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL), dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo* to yield 2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-(N-nitrosoacetamido)-D-allitol as a light green syrup. This syrup showed no IR absorption at 3311 cm^{-1} (NH) to suggest unreacted 1-acetamido-2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-D-allitol. The N-nitroso amide made in this way was used immediately in the subsequent reaction: IR (neat, cm^{-1}) 1500.

2,5-Anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-(N-nitrosoacetamido)-D-allitol (assumed to be 2.5 mmol) was dissolved in 6 mL of Et_2O and mixed vigorously with an ice cold solution of 1.44 g KOH dissolved in 3 mL of H_2O. The mixture was stirred at 3 °C for 45 min after which the IR spectrum of the Et_2O layer showed the formation of a strong band at 2070 cm^{-1} (CHN_2) and no IR band at 1500 cm^{-1} (NO). The reaction mixture was then diluted with Et_2O (12 mL) and H_2O (25 mL) and the layers separated. The Et_2O layer was washed with H_2O (10 mL) and dried rapidly first by swirling the Et_2O phase over KOH pellets and decantation followed by anhydrous $MgSO_4$. Following filtration, the golden colored filtrate containing 2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-diazo-D-allitol (**34**) was used immediately in the subsequent reaction: IR (neat, cm^{-1}) 2070.

The aforementioned solution of 2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-diazo-D-allitol (**34**) was added to a solution of 0.66 g (3 mmol) of **35** (Scheme 8) in 10 mL of anhydrous Et_2O. The mixture was stirred at 27 °C for 21 h (during this period, the solution color changed from golden yellow to light yellow). The reaction mixture was concentrated *in vacuo* and the residue purified by silica gel column chromatography (hexane-AcOEt, 9:1) to yield 2-indanone (0.33 g, 50%) as white needles: mp 52 °C (lit.[25] 54-56°C); R_f = 0.25 (hexane AcOEt, 9:1); ^1H NMR (90 MHz, $CDCl_3$) δ 3.57 (s, 4 H, 2 x CH_2), 7.29 (s, 4 H, ArH); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 42.98, 123.91, 126.30, 136.70, 212.00.

The Methyl(trimethylsilyl)ketal of Ketene (41). An equimolar amount of 1.6 M butyllithium in hexanes (28.13 mL, 45 mmol) was added dropwise, with stirring, to a solution of distilled diisopropylamine (4.56 g, 45 mmol) in anhydrous THF (30 mL) at 0 °C under N_2. Stirring was continued for 15 min under the same conditions. The flask was then cooled in a dry ice-acetone bath (-78 °C), distilled and dry methyl acetate (3.33 g, 45 mmol) was added dropwise; the mixture was stirred for an additional 30 min to complete the formation of the lithio derivative of methyl acetate. Freshly distilled trimethylsilyl chloride was then added dropwise, with stirring, at -78 °C over a 5 min period and the mixture was stirred for 3 h under the same conditions. Iodomethane (8.4 mL, 135 mmol) and then pentane (30 mL) were added to the mixture. The mixture was kept in a refrigerator overnight and filtered. The filtrate was concentrated *in vacuo* to produce a liquid which was distilled to give **41** in 20% yield by ^{1}H NMR (bp 38-40 °C/2.8 mm Hg) as an azeotropic mixture with its C-silylated counterpart (**42**, 4%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.23 (s, 9 H, $OSiMe_3$), 3.10-3.24 (dd, J = 2.68 Hz, 2 H, $=CH_2$), 3.55 (s, 3 H, OCH_3); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 0.24, 55.33, 60.21, 162.43.

4- or 5-Hydroxy-3-(2',3',5'-tri-O-benzyl-β-D-ribofuranosyl)pyrazole (43b or its C-5 Isomer). 2,5-Anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-diazo-D-allitol (**34**) in Et_2O (prepared as described above) was added dropwise at -78 °C (dry ice-acetone) to a solution of the **41/42** azeotropic mixture (0.55 g, 3.75 mmol) in 10 mL of anhydrous Et_2O. The reaction flask was stoppered at -78 °C and the cooling bath then removed. The reaction mixture was stirred at 27 °C for 16 h (during this time, the solution color changed from golden yellow to light yellow). Following this, it was stirred for an additional period of 24 h and the reaction mixture was then concentrated *in vacuo* and the residue purified by silica gel column chromatography (hexane-AcOEt, 9:1) to yield **43b** or its C-5 Isomer (0.26 g, 21 % from 1-acetamido-2,5-anhydro-3,4,6-tri-O-benzyl-1-deoxy-D-allitol) as a colorless syrup, which discolored on standing for several days: R_f = 0.23 (hexane-AcOEt, 9:1); ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.61-4.85 (m, 12 H), 4.85 (d, 1 H, H-1'), 7.27 (s, 15 H, ArH), 7.32 (s, 1 H, H-4 or H-5); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 69.47, 70.50, 72.34, 76.10, 77.49, 78.69, 82.20, 86.21, 128.00, 128.40, 128.80, 137.95, 138.22, 138.43, 159.72.

1-(2',3',5'-tri-*O*-Benzyl-α- and -β-D-ribofuranosyl)propan-2-one (44).[15] Ethyl 3-oxo-4-(2',3',5'-tri-*O*-benzyl-α- and -β-D-ribofuranosyl)butanoate[4,16] (0.84 g, 1.5 mmol) in 2 M KOH (20 mL) was stirred at 0 °C for 1 h. The reaction mixture was then acidified with conc. H_2SO_4 and then refluxed for 4 h. After this period, the mixture was cooled to room temperature and extracted with Et_2O (3 x 25 mL). The ether phase was dried (Na_2SO_4), filtered, and evaporated to dryness using a rotary evaporator. A heavy syrup remained; this syrup was purified by column chromatography (AcOEt-hexane, 1:1) to afford **44** in 80% yield (R_f = 0.5, AcOEt-hexane, 1:1): ^{1}H NMR same as that in the literature[4]; ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 30.5, 47.5, 70.2, 71.7, 72.0, 72.7, 73.4, 76.0, 76.8, 77.8, 79.5, 79.8, 80.5, 81.6, 127.5, 127.7, 128.0, 128.4, 137.8, 138.1, 206.6, 207.3.

1-Diazo-3-(2',3',5'-tri-*O*-benzyl-α- and -β-D-ribofuranosyl)propan-2-one (47). A mixture of diisopropylamine (1.07 g, 10.6 mmol) in anhydrous THF (10 mL) and 1.6 M butyllithium in hexane (6.63 mL, 10.6 mmol) was stirred at -78 °C for 30 min under N_2. To this cooled mixture was then added **44** (2.45 g, 5.3 mmol) in THF (5 mL) followed by *p*-toluenesulfonylazide (2.91 mL) in one portion. The mixture was

stirred at 0 °C for 2 h and at 25 °C for 20 h. After this period, the reaction mixture was poured into saturated aqueous NH_4Cl (25 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (3 x 25 mL). The combined organic layer and ether phases were washed with saturated aqueous NaCl, dried ($MgSO_4$), filtered, and concentrated using a rotary evaporator to result in a heavy syrup, which was purified by column chromatography. Elution with AcOEt-hexane (1:1 and then rechromatography of this fraction with AcOEt-hexane, 1:9) gave 0.3 g (12%) of **47** as an α/β mixture: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.4 (d, J = 2.7 Hz, 2 H, CH_2CO), 3.4-4.7 (m, 12 H, OCH), 7.3 (s, 15 H, Ar-H), 7.9 (m, 1 H, CHN_2).

When using sodium hydride as the base in the above procedure, a compound (35%) was obtained. This material possessed spectral properties suggestive of a secondary alcohol arising from simple reduction of the keto moiety of **44**.

2-Trimethylsilyloxypropene (Scheme 14). Anhydrous NaI (9.23 g, 62 mmol) in distilled MeCN (62 mL) was placed in a flame dried flask under N_2. Dry acetone (2.9 g, 50 mmol), triethylamine (8.56 g, 62 mmol) and freshly distilled chlorotrimethylsilane (6.74 g, 62 mmol) were each added dropwise with stirring at 0 °C. The reaction mixture was then warmed to room temperature and the stirring continued for 15 min. Following this period, the mixture was poured onto crushed ice (100 g) and the resultant mixture stirred and shaken well. The upper layer was collected to yield 2-trimethylsilyloxypropene (3 g, 46%): ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.17 (s, 9 H, $Si(CH_3)_3$), 1.72 (s, 3 H, CH_3), 4.0 (s, 2 H, $=CH_2$); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 0.51 ($Si(CH_3)_3$), 21.36 (CH_3), 89.94 ($=CH_2$), 154.58 (=C-O).

1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)propan-2-one (45). To a 50 mL round-bottomed flask equipped with a vacuum outlet was added anhydrous zinc iodide (3.2 g, 10 mmol). The flask was heated under vacuum with a flame until a material sublimed and deposited at the inner area of the upper part of the flask (which indicated that all traces of moisture had been removed). The vacuum outlet was replaced with a N_2 inlet and to the zinc iodide remaining after the sublimation process was added, under N_2, CH_2Cl_2 (30 mL) followed by 2-trimethylsilyloxypropene (1.3 g, 10 mmol; prepared above) followed by 1-*O*-acetyl-(2,3,5-tri-*O*-benzoyl)-β-D-ribofuranose (Aldrich). The resulting suspension was stirred at room temperature for 30 h. The mixture was then poured into a saturated aqueous solution of $NaHCO_3$ (300 mL). The aqueous phase was washed with CH_2Cl_2 (3 x 50 mL) and the combined organic phases dried (Na_2SO_4). After filtration and removal of the CH_2Cl_2 filtrate *in vacuo*, the residue was purified by column chromatography (AcOEt-CH_2Cl_2, 1:19) to afford 2.01 g (40%) of **45** as a syrup: R_f=0.3 (AcOEt-CH_2Cl_2, 1:19); ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.08 (s, 3 H, CH_3), 2.97 (brs, 2 H, CH_2), 3.5-3.7 (m, 1 H, H-1), 4.25-4.41 (dd, 2 H, H-5), 4.8-5.0 (dd, 1 H, H-2), 5.23-5.35 (t, 1 H, H-3), 6.2 (d, J = 4 Hz, 1 H, H-4), 7.31-8.04 (m, 15 H, Ar-H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 31.66 (CH_3), 54.68 (CH_2), 63.02, 73.32, 76.51, and 79.33 (C-1, C-2, C-3, C-4, and C-5), 106.25-142.28 (12 Ar-C), 165.52 and 166.02 (ester CO), 203.88 (ketone CO).

N-1(2)-Benzyl-4-benzyloxy-3(5)-(5'-O-tosyl-α-and-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (63). To a solution of N-1(2)-benzyl-4-benzyloxy-3(5)-(α-and-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (**19**) (1.9 g, 4.33 mmol) in dry pyridine (20 mL) at 0 °C was added *p*-toluenesulfonyl chloride (1.00 g, 5.20 mmol). The reaction mixture was stirred at 0 °C for 1 h and then at 4 °C for 36 h. To the clear reaction mixture thus obtained, MeOH (1 mL) was added, and after allowing the mixture to stand

for 30 min, the solvent was evaporated under reduced pressure. The residue was then co-evaporated under high vacuum at least three times with dry MeOH and the new residue was purified by column chromatography using MeOH-CH_2Cl_2 (1:9). The fraction of R_f = 0.68 was collected to afford **63** (1.03 g, 40%, β/α *ca.* 4:1) as a solid: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.36 and 2.40 (s, 3 H, CH_3 of α/β mixture), 3.97 (s, 2 H, NCH_2), 4.05 (m, 2 H, H-2', H-3'), 4.77 (d, J = 4.69, 1 H, H-1'), 5.01 (s, 2 H, $PhCH_2$), 5.17 (dd, 2 H, H-5'), 5.62 (brs, 1 H, H-4'), 7.13-7.76 (m, 16 H, ArH and NH_2); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 21.28 (CH_3), 54,49 (NCH_2Ph), 71.02 (C-5'), 71.34 (C-2'), 73.18 (C-3'), 77.41 (OCH_2Ph), 77.78 (C-1'), 80.76 (C-4'), 126.27, 127.25, 127.73, 128.60, 130.27, 136.29, 137.92, 145.12 (Ar), 132.34 [C-3(5)], 140.14 [C-5(3)], 141.6 (C-4), 159.91 and 164.61 (amide carbonyl of α/β mixture).

N-1(2)-Benzyl-4-benzyloxy-3(5)-(5'-azido-5'-deoxy-α-and-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (64) [N-1(2)-benzyl-4-O-benzyl-5'-deoxy-5'-azidopyrazofurin]. To the α:β mixture of **63** (6.85 g, 11.6 mmol) was added sodium azide (1.50 g, 23 mmol) in dry DMF (60 mL) and this mixture was then heated at 120 °C with stirring for 24 h with monitoring by tlc (MeOH-CH_2Cl_2, 1:9). At this time, the solvent was evaporated under reduced pressure and the residue co-evaporated at least three times with absolute EtOH. The resultant brown syrup thus obtained was purified by column chromatography (MeOH-CH_2Cl_2, 1:9) to afford **64** (R_f = 0.53, 3.00 g, 54 %, β/α *ca.* 5:1): ^{1}H NMR (90 MHz, DMSO-d_6) δ 3.97 (brs, 2 H, NCH_2), 4.40 (m, 2 H, H-2' and H-3'), 4.84 (d, J = 4.40, H-1'), 5.10 (s, 2 H, $PhCH_2$), 5.40 (m, 2 H, H-5'), 5.64 (s, 1 H, H-4'), 7.15-7.60 (m, 12 H, ArH and NH_2); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 52.48 (C-5'), 54.54 (NCH_2Ph), 72.26 (C-2'), 73.45 (C-3'), 77.51 (OCH_2Ph), 77.73 (C-1'), 82.23 (C-4'), 126.11, 127.26, 127.52, 128.60, 136.40, 137.97 (Ar), 131.00 [C-3(5)], 140.30 [C-5(3)], 141.76 (C-4), 159.5 and 159.91 (amide carbonyl of α/β mixture).

N-1(2)-Benzyl-4-hydroxy-3(5)-(5'-amino-5'-deoxy-β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (65) [N-1(2)-benzyl-5'-deoxy-5'-aminopyrazofurin]. To **64** (3 g, 6.25 mmol) in MeOH (100 mL) was added 10% Pd/C (1 g) and this mixture was subjected to 90 psi of H_2 for 5 days using a Parr apparatus. Following this, the reaction mixture was then filtered through a Celite pad and the filtrate evaporated under reduced pressure to result in a thick brown syrup which was purified by column chromatography (MeOH-CH_2Cl_2,1:9). The fraction of R_f = 0.17 was collected to yield **65** (1.50 g, 67%) as a solid, mp 116-117 °C: ^{1}H NMR (90 MHz, CD_3OD) δ 3.1 (brs, 2 H, H-5'), 3.9-4.4 (m, 6 H, H-1', H-2', H-3', H-4', NCH_2), 5.2 (brs, 2 H, 5'-NH_2), 7.0-7.3 (brs, 7 H, ArH and $CONH_2$); ^{13}C NMR (22.5 MHz, CD_3OD) δ 42.39 (C-5'), 55.72 (NCH_2), 73.92 (C-3'), 76.14 (C-2'), 80.96 (C-4'), 81.29 (C-1'), 122.08 [C-3(5)], 128.20, 129.39, 129,72 (Ar), 139.90 [C-5(3)], 150.90 (C-4), 164.72 (amide carbonyl).

2',3',4-Tri-O-acetyl-5'-O-tritylpyrazofurin (67). Pyrazofurin (**16**) (3.5 g, 13.5 mmol) and chlorotriphenylmethane (5.7 g, 20.4 mmol) were dissolved in dry pyridine (50 mL) and the solution was stirred for 48 h at room temperature. After cooling the solution to 0 °C, acetic anhydride (20 mL) was added and the resultant mixture was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the residue was purified by column chromatography using hexane-AcOEt (2:1) to give **67** (3.5 g, 42%) as a syrup: ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.05 (s, 6 H, 2 x CH_3), 2.10 (s, 3 H, CH_3), 3.32 (d, 2 H, H-5'), 4.21 (m, 1 H, H-4'), 5.41 (m, 1 H, H-3'), 5.70 (s, 1 H, H-1'), 5.75 (m, 1 H, H-2'), 7.30 (m, 15 H, Ar-H). ^{13}C NMR (22.5 MHz, DMSO-d_6)

δ 2 x 20.8, 23.0, 63.7, 72.0, 72.3, 74.4, 75.3, 127-129.5 (Ar), 144.5, 161.2, 161.5, 169.5 (note: not all ^{13}C peaks were discernible).

5-O-Benzoyl-1,2-O-isopropylidene-α-D-xylofuranose[24] (74). To a cooled (ice) and stirred solution of 1, 2-O-isopropylidene-α-D-xylofuranose[20] (**73**) (3.6 g, 0.021 moles) in dry pyridine (20 mL) was added, dropwise over a period of 20 min, a solution of benzoyl chloride (2.1 mL, 0.023 moles) in dry $CHCl_3$ (10 mL). The cooling was removed and the mixture allowed to stir overnight at room temperature. The next day H_2O (2 mL) was added and the mixture was stirred for 30 min and then washed with dilute, ice-cold H_2SO_4 until faintly acidic. After two washings with H_2O, the $CHCl_3$ layer was dried (anhydrous Na_2SO_4) and concentrated under reduced pressure to a thick syrup. The crude syrupy mass was purified by flash chromatography (hexane-AcOEt, 4:1) to give **74** as a white solid (4.5 g, 60%), mp 72 °C (lit.[24] 83.5-84.5 °C): IR (neat cm^{-1}) 3500 (OH), 1750 (CO); ^1H NMR (90 MHz, $CDCl_3$) δ 1.3 (s, 3 H, CH_3), 1.5 (s, 3 H, CH_3), 4.1-4.8 (m, 5 H, H-2, H-3, H-4, and H-5), 2.9-6.1 (d, 1 H, H-1), 7.3-7.6 (m, 3 H, ArH), 7.99-8.15 (m, 2 H, ArH); ^{13}C NMR ($CDCl_3$) δ 26.16 and 26.81 (2 x CH_3), 62.51 (C-5), 74.70 (C-2), 78.71 (C-3), 85.32 (C-4), 105.04 (C-1), 111.81 [$(CH_3)_2\underline{C}$], 128.44, 129.85, and 133.32 (Ar), 166.96 (ester carbonyl).

5-O-Benzoyl-3-deoxy-3-fluoro-1,2-O-isopropylidene-α-D-ribofuranose (72). Compound **74** (0 .5 g, 1.7 mmol) was dissolved in dry CH_2Cl_2 (25 mL) and pyridine (0.85 mL) and this solution was cooled to -25 °C with stirring. To this solution, trifluoromethylsulfonic anhydride dissolved in dry CH_2Cl_2 was added dropwise under an Ar atmosphere. After completing the addition, the reaction mixture was allowed to stir at room temperature for 1-2 h. The solution was then washed with saturated aqueous $NaHCO_3$ and the CH_2Cl_2 fraction dried over anhydrous (Na_2SO_4). The CH_2Cl_2 was removed under reduced pressure and the residual yellow solid obtained (assumed to be the triflate **75**) was used directly in the next step without further purification. Thus, this solid was dissolved in dry DMF (20 mL) and to this was added cesium fluoride (0.225 g, 1.4 mmol). After slowly heating the new solution at 150 °C for 30-40 min, it was cooled and poured into H_2O. The aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL) and the extracts were combined and dried (Na_2SO_4). The CH_2Cl_2 was removed under reduced pressure and the residual syrup obtained was purified by flash chromatography (hexane-AcOEt, 3.1:1.5) to yield **72** (0.0712 g, 18%) as a viscous liquid: ^1H NMR (90 MHz, $CDCl_3$) δ 1.3 (s, 3 H, CH_3), 1.5 (s, 3 H, CH_3), 4.3-4.6 (m, 3 H, H-2, H-3, and H-4), 4.8-5.0 (d, 2 H, H-5), 5.86-5.9 (d, 1 H, H-1), 7.3-7.6 (m, 3 H, ArH), 7.9-8.1 (m, 2 H, ArH); ^{13}C NMR ($CDCl_3$) δ 24.1 and 25.0 (2 x CH_3), 57.5 (C-5), 60.1 and 75.5 (C-3, J_{CF} = 343.01), 75.1 and 82.0 (C-4, J_{CF} = 151.37), 103.1 (C-1), 104.81 [$(CH_3)_2\underline{C}$], 100.1 and 110.91 (C-2, J_{CF} = 258.71), 127.51, 128.1, 131.10, and 131.9 (Ar), 164.19 (ester carbonyl).

3(5)-(2'-O-acetyl-3'-deoxy-3'-bromo-β-D-xylofuranosyl)-4-O-(α-acetoxy*iso*butyryl)pyrazole-5(3)carboxamide (76) and 3(5)-(3'-O-acetyl-2'-deoxy-2'-bromo-β-D-arabinofuranosyl)-4-O-(α-acetoxy*iso*butyryl)pyrazole-5(3)-carboxamide (77). To a suspension of pyrazofurin (**16**) (dried over P_2O_5 overnight at 60 °C under vacuum) (1.5 g, 5.79 mmol) in dry MeCN (85 mL) was added dropwise α-acetoxy*iso*butyryl bromide (4.84 g, 23.16 mmol) over a 5 min period. The mixture was then stirred at room temperature for about 1.25 h with monitoring by tlc (MeOH-CH_2Cl_2, 1:9). The solvent was removed under reduced pressure and the residue was extracted into $CHCl_3$ (100 mL). The $CHCl_3$ phase was washed with saturated

aqueous $NaHCO_3$ (2 x 50 mL) and with H_2O (50 mL) and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a solid (R_f = 0.44) that was purified by column chromatography to yield (2.0 g, 70%) of a mixture of **76** and **77** in a ratio of 1:1.4 (2.0 g, 70%): ^1H NMR (90 MHz, $CDCl_3$) δ 1.56 (s, 12 H, 4 x CH_3), 2.06 (s, 6 H, 2 x CH_3), 2.31 (s, 6 H, 2 x CH_3), 4.47 (m, 6 H, "down" H-2' and H-3' and H-5'), 4.60 (m, 2 H, H-4'), 5.16 (d, *J* = 2.64, 1 H, H-1'), 5.39 (m, 2 H, "up" H-2' and H-3'), 5.71 (d, *J* = 2.05, 1 H, H-1'), 7.00 (brs, 4 H, NH_2); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 20.86, 21.18 (2 x *geminal* CH_3), 24.32, 24.43, 24.65, 24.87 (4 x ester CH_3), 50.87, 53.85, (2 x $\underline{C}Br$), 63.9 [$(H_3C)_2\underline{C}O$], 65.23 and 65.82, (2 x C-5'), 77.63, 78.01, 78.17, 78.39, 78.39, 81.26, 82.45, 83.21 (8 ribofuranose carbons), 127.15, 127.80, 128.29, 129.0 [2 x C-3(5), 2 x C-5(3)], 141.02 and 141.23 (2 x C-4), 165.02 (2 x amide carbonyl), 169.89, 169.89, 170.65, 171.03, 172.60, 172.76, (6 ester carbonyls).

3(5)-(2'-O-Acetyl-3'-deoxy-β-D-ribofuranosyl)-4-O-(α-acetoxy*iso*-butyryl)pyrazole-5(3)-carboxamide (78) and 3(5)-(3'-O-acetyl-2'-deoxy-β-D-ribofuranosyl)-4-O-(α-acetoxy*iso*butyryl)pyrazole-5(3)-carboxamide (79). To the mixture of **76** and **77** (1.73 g, 3.52 mmol) in AcOEt (155 mL) was added triethylamine (0.55 mL) and 10% Pd-C (0.78 g). The mixture was stirred for 48 h on a Parr apparatus at room temperature under H_2 (50 psi). The mixture was then filtered through a Celite pad and the filtrate was removed under reduced pressure. The crude residue was then chromatographed (MeOH-CH_2Cl_2, 1:9) to afford a solid mixture of **78** and **79** (R_f = 0.39) in 81% yield: ^1H NMR (90 MHz, $CDCl_3$) δ 1.54 (s, 12 H, 4 x CH_3), 1.75-2.40 (m, 4 H, H-2' of **79** and H-3' of **78**), 2.04 and 2.11 (2s, 12 H, 4 x CH_3), 4.08-4.46 (m, 6 H, H-4' and H-5'), 5.15-5.25 (2d, *J* = 3.50, *J* = 4.10, 2 H, H-1'), 5.15-5.25 (m, 2 H, H-2' of **78** and H-3' of **79**), 7.29 (brs, 4 H, 2 x NH_2); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 21.24 (2 x *geminal* CH_3), 24.43 and 24.92 (4 x ester CH_3), 27.74 (C-3' of **32**), 31.37 (C-2' of **33**), 65.00 [2 x $(H_3C)_2\underline{C}O$], 66.36 and 67.28 (2 x C-5'), 72.86,74.49, 77.58, 77.90, 79.04, and 82.94 (6 ribofuranose carbons), 128.24, 128.66, 129.15, and 131.43 [(2 x C-3(5) and 2 x C-5(3)], 140.69 and 141.50 (2 x C-4), 165.50 and 166.10 (2 x amide carbonyls), 170.87-173.09 (6 x ester carbonyls).

References and Notes

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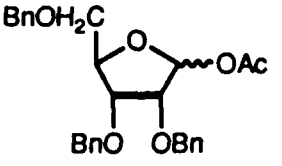
(14) This decision has the concurrence of Dr. Gabrielsen.

(15) It should be noted that attempts to prepare **44** in a manner analogous to the preparation of **45** of Scheme 14 using **a** were unsuccessful.



a

(16) See compound **54** in Scheme 18 of the Annual Report of June 19, 1990.

(17) Lim, M.-I.; Ren, W.-Y.; Otter, B.A.; Klein, R.S. *J. Org. Chem.* **1983**, *48*, 780.

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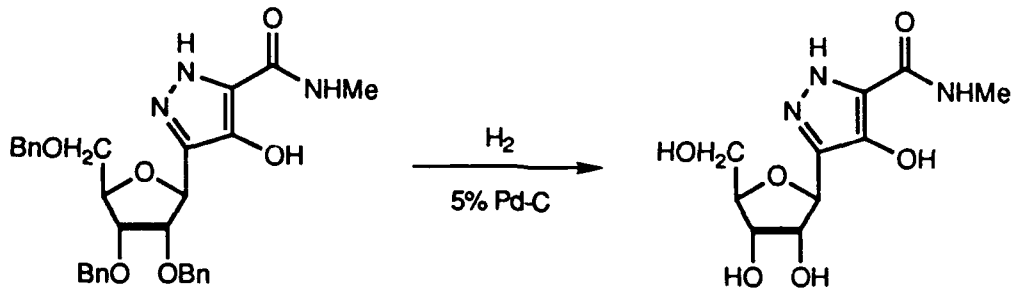
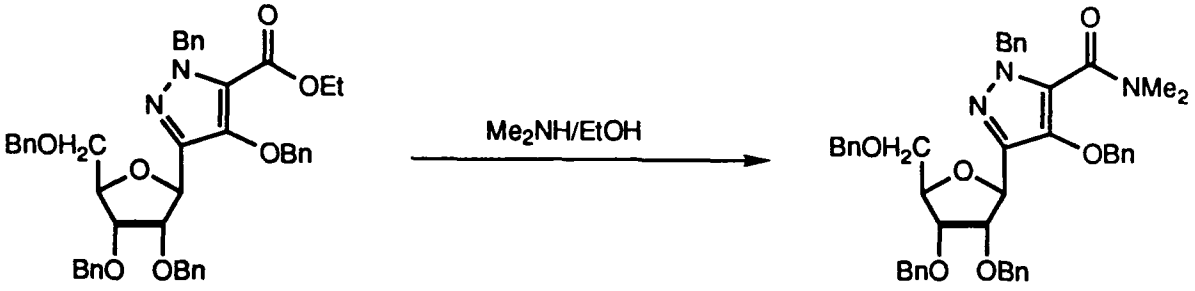
Scheme 1
Synthesis of Amide **2a**



(see Scheme 11 of the Quarterly Report of 6-19-90)

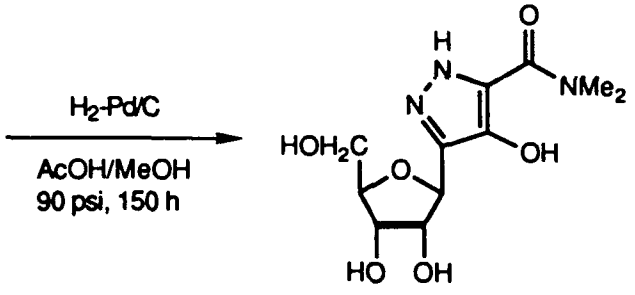
2a (78%)

Scheme 2
Synthesis of Amide **2b**



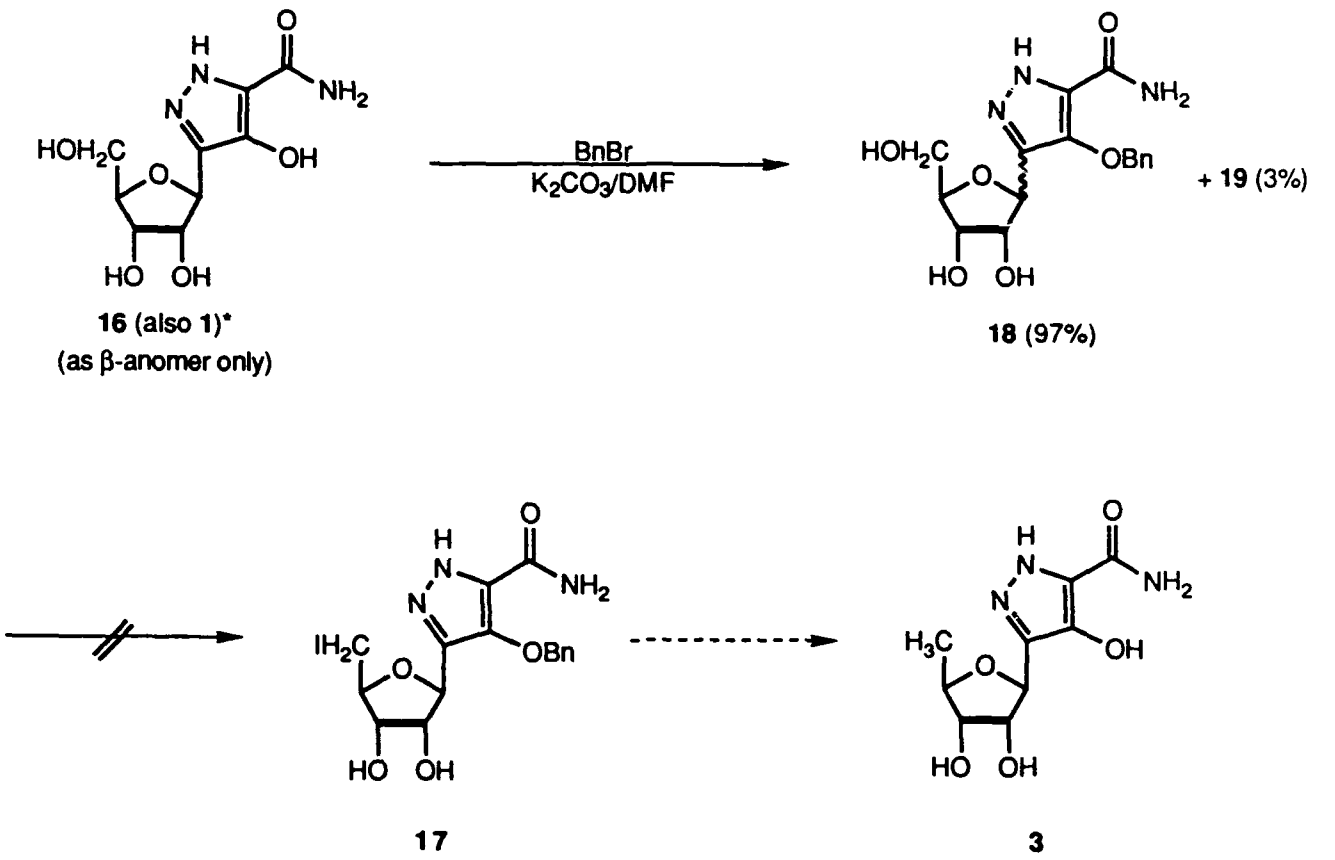
(see Scheme 12 of the Quarterly Report of 6-19-90)

(see Scheme 12 of the Quarterly Report of 6-19-90)



2b (26%)

Scheme 3
Initially Planned Synthesis of 5'-Deoxypyrazofurin (3)



16 (also 1)*
(as β-anomer only)

18 (97%)

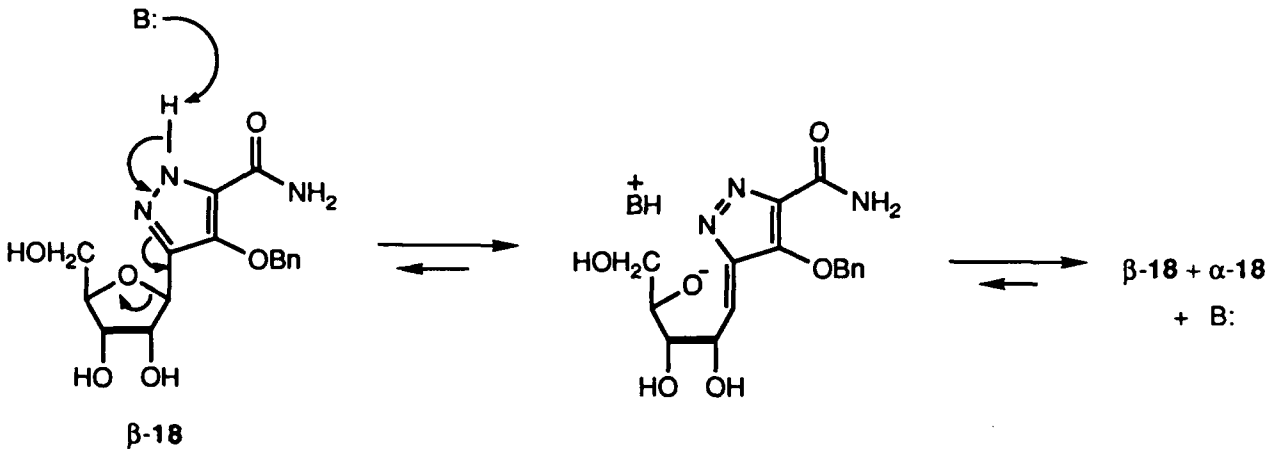
17

3

*supplied by Eli Lilly and Company

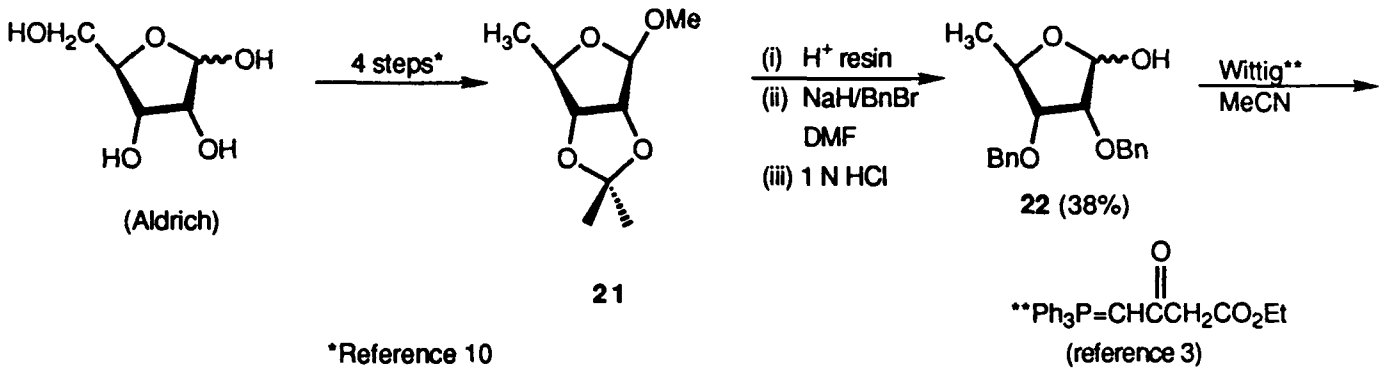
Scheme 4

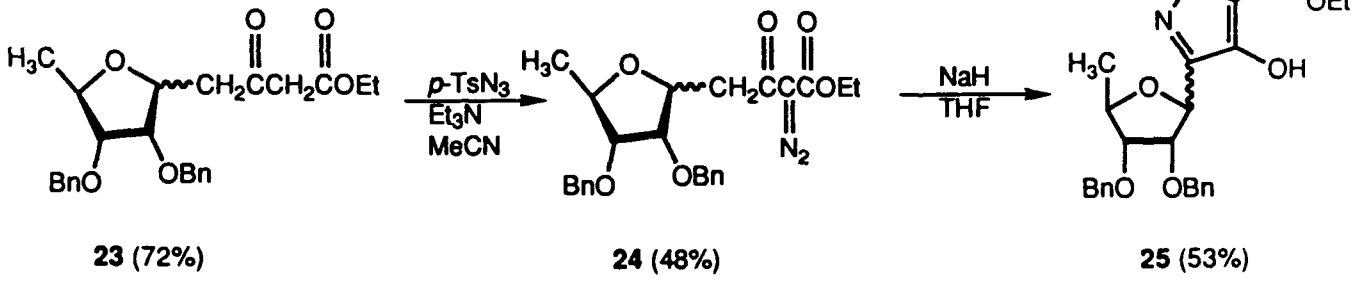
Proposed Pathway for Formation of **18** as Anomeric Mixture*

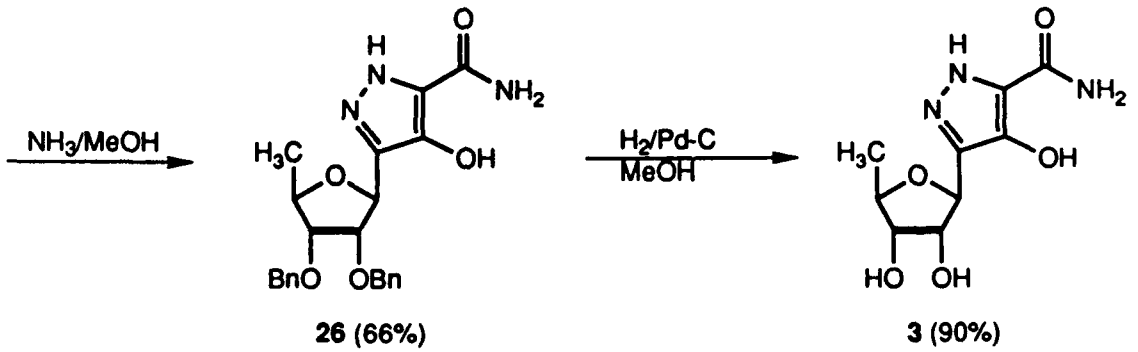


*Anomerization could occur prior to benzylation.

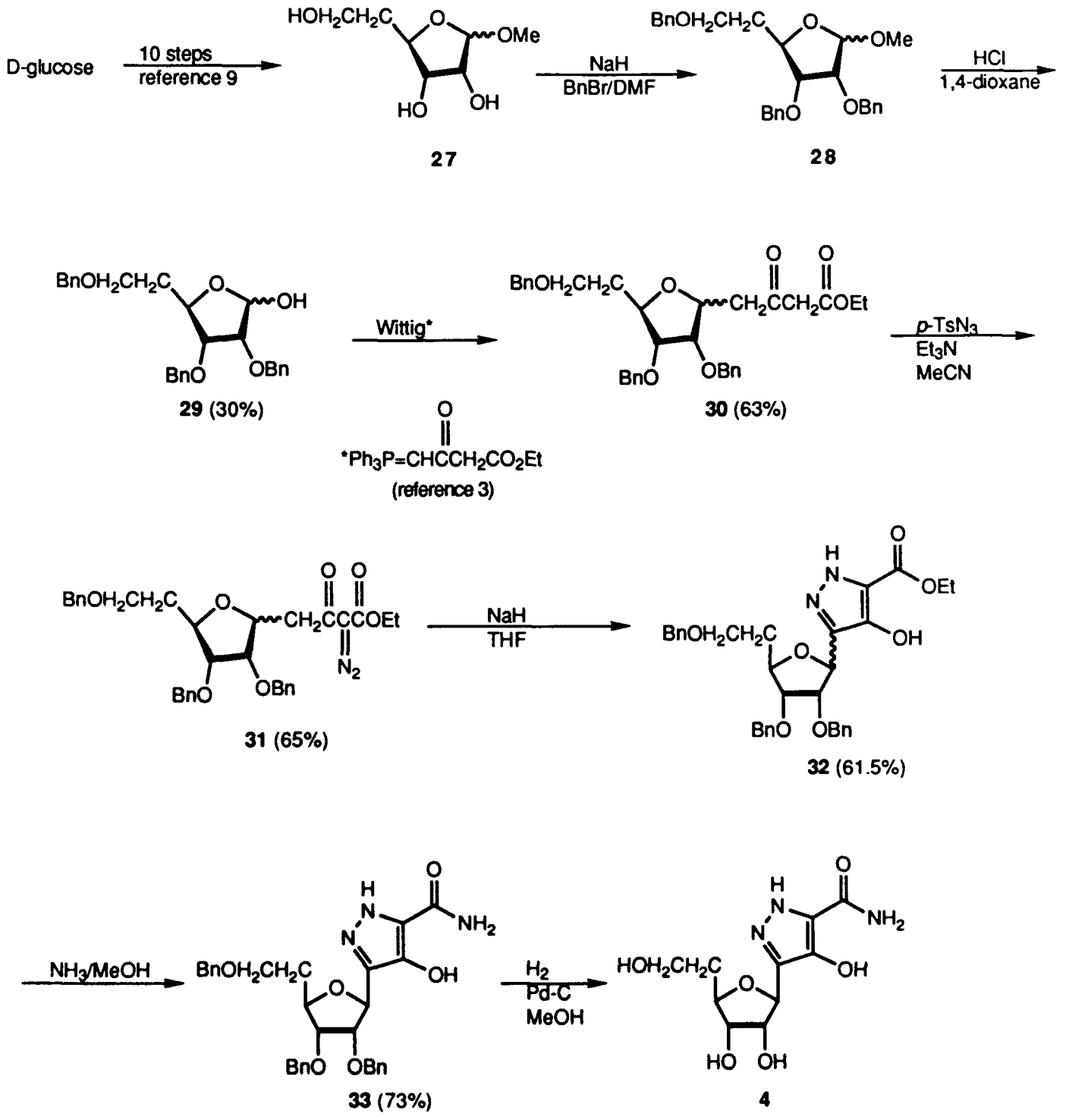
Scheme 5
Synthesis of 5'-Deoxypyrazofurin (3)



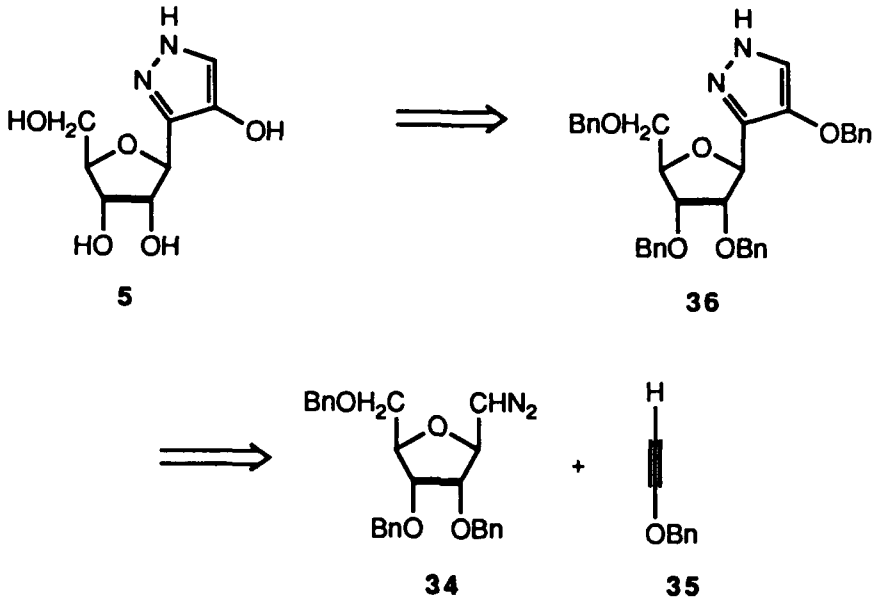




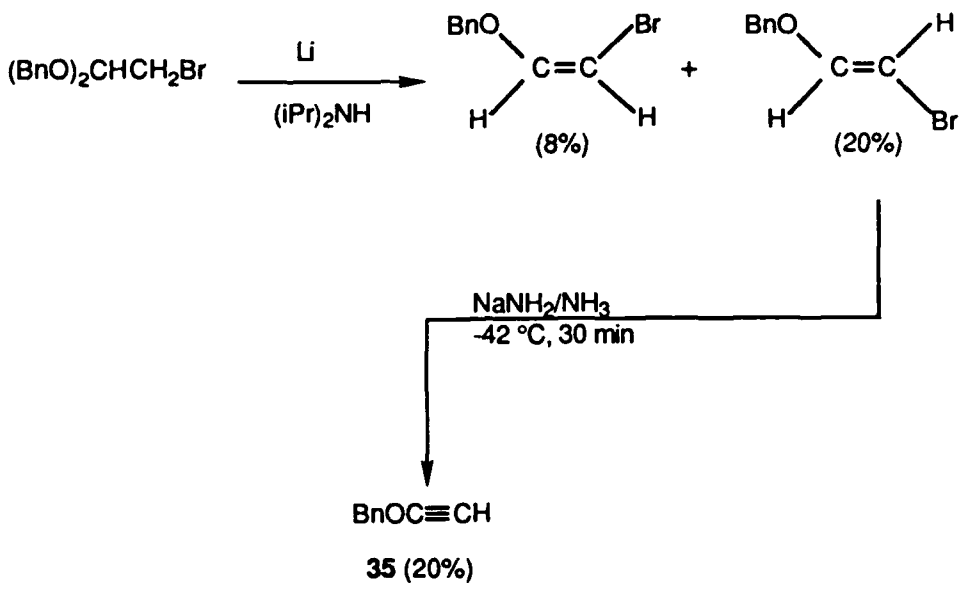
Scheme 6
Synthesis of 5'-Homopyrazofurin (4)



Scheme 7
Retrosynthetic Approach to the Nor-Amide (**5**)

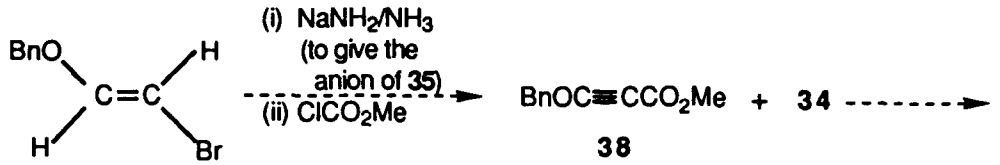


Scheme 8
Synthesis of Benzyloxyacetylene **35***

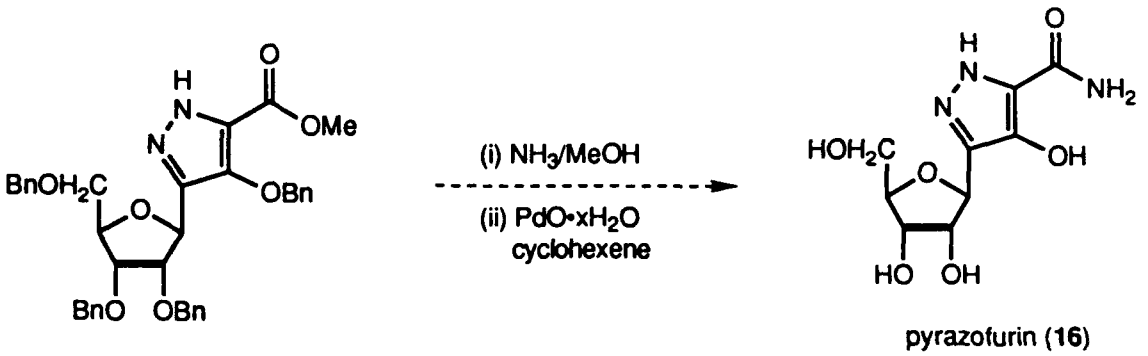


*Refer to the June 19, 1990 Quarterly Report for the experimental details for this Scheme.

Scheme 9
Possible Route to Pyrazofurin

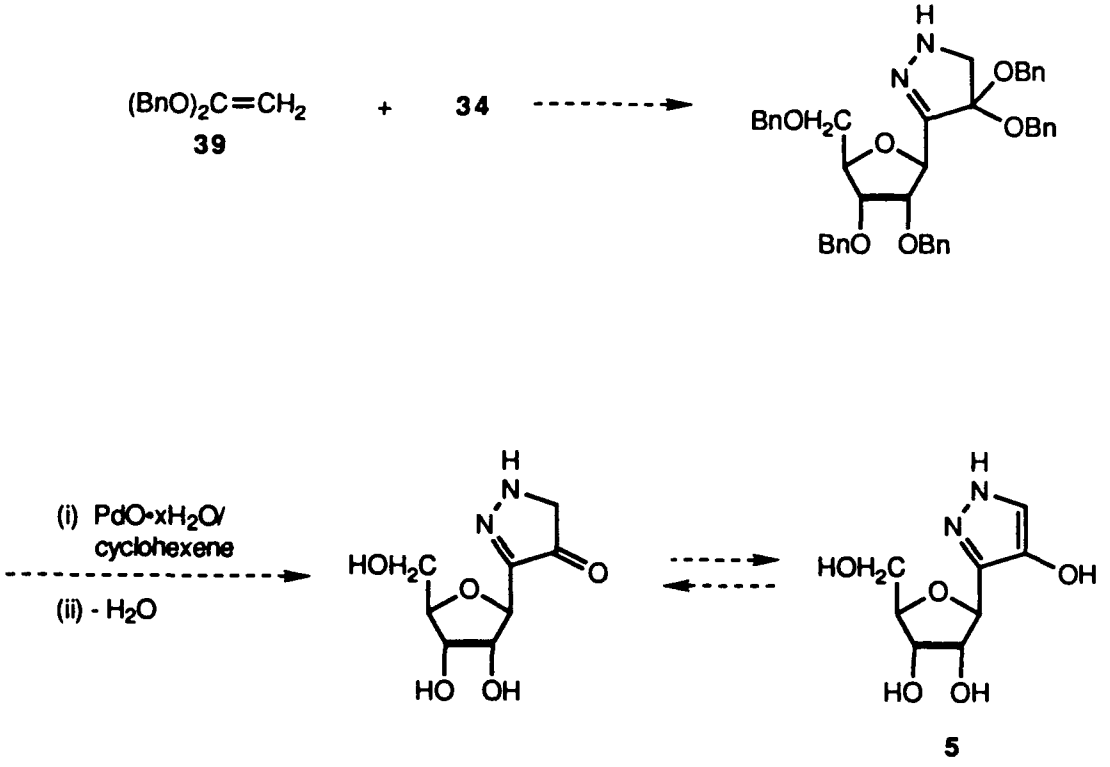


Scheme 8

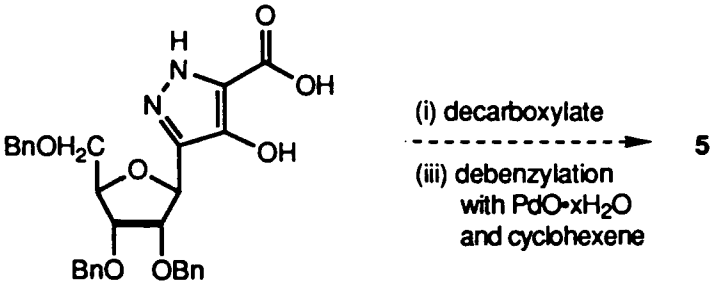


pyrazofurin (16)

Scheme 10
Alternative Synthesis of **5** Evaluated

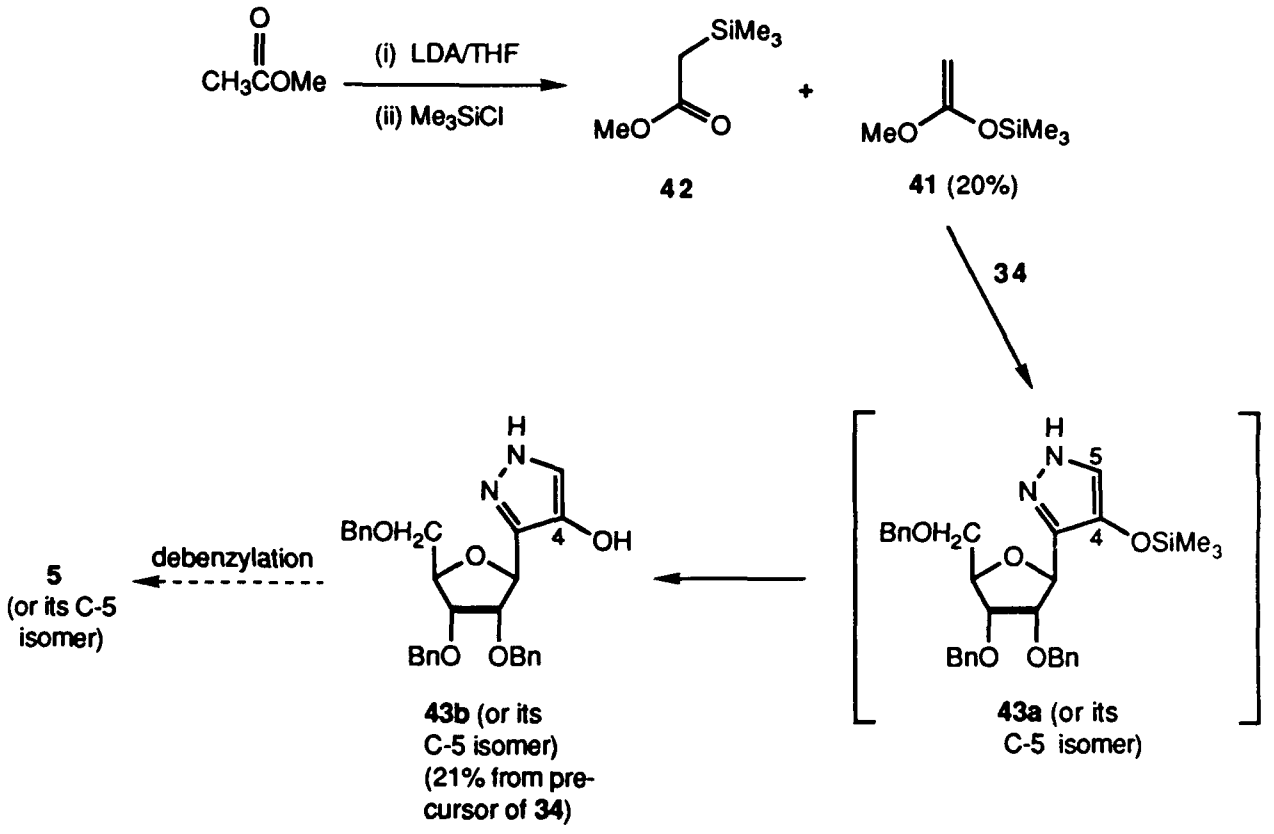


Scheme 11
Attempted Decarboxylative Approach to **5**

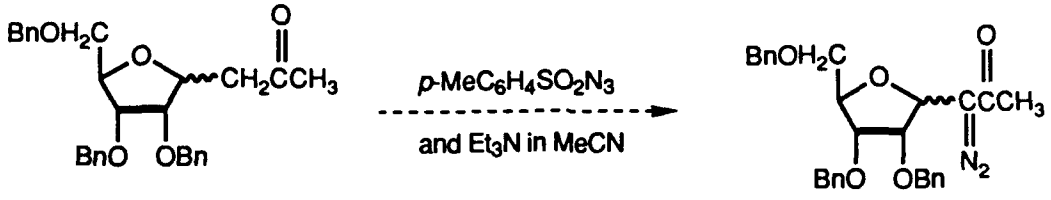


(see Scheme 11, Quarterly Report of 6-19-90)

Scheme 12
Alternative Approach to **5**

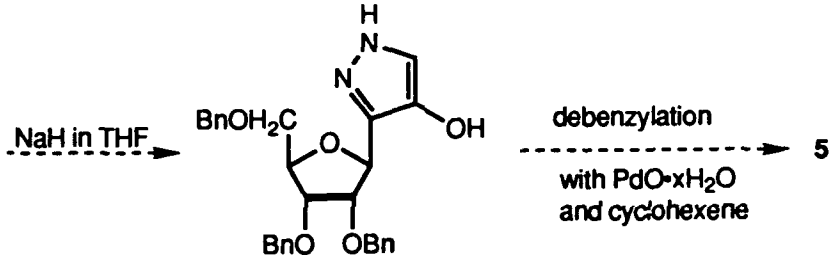


Scheme 13
Final Approach to **5** Considered



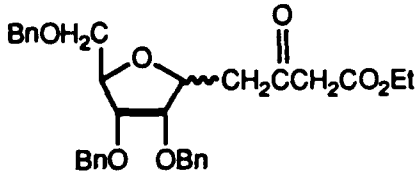
44
For the source of this compound, see*.

46



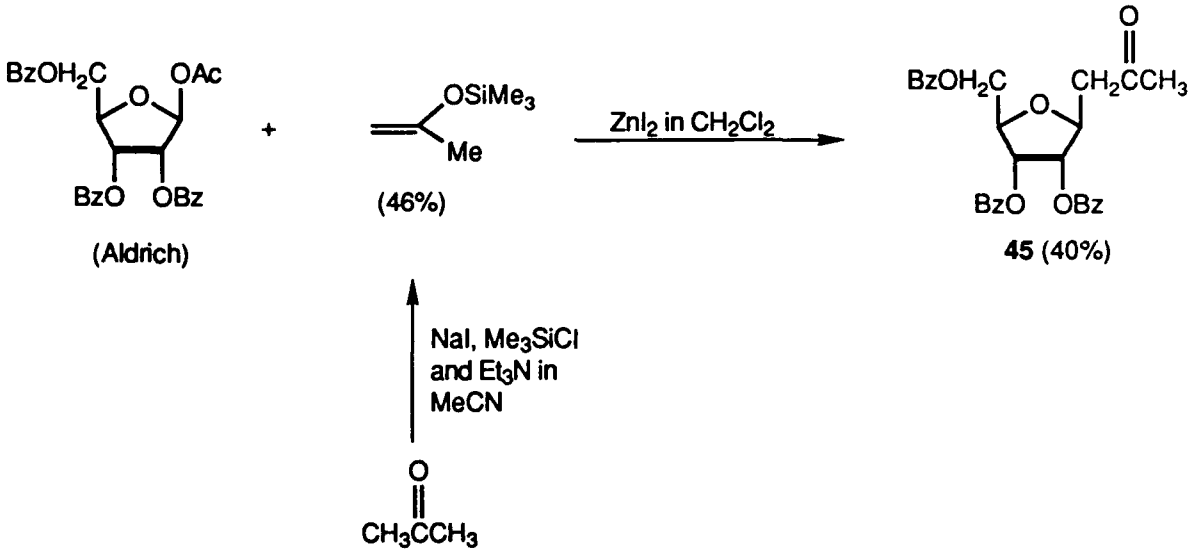
Reference 4 suggests that only the β-anomer would form

* This ketone is a by-product in the synthesis of the compound shown below, which is a precursor to pyrazofurin.

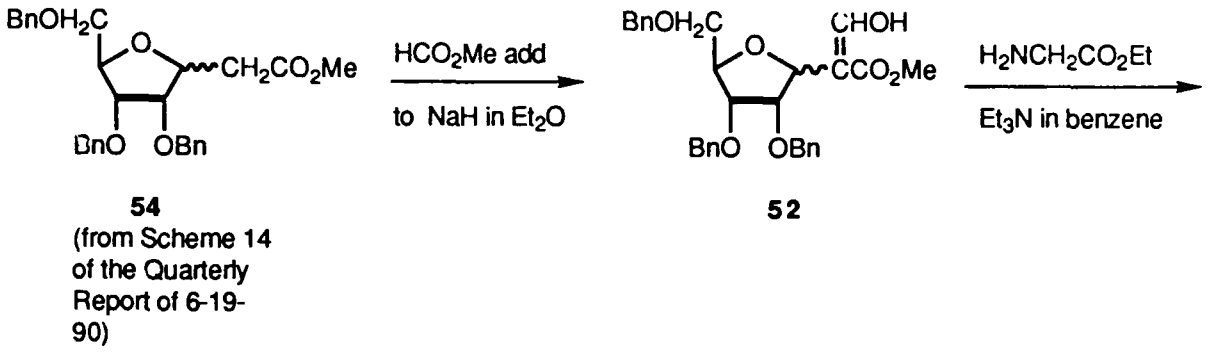


(compound **25** of Scheme 9 of Quarterly Report of 6-19-90)

Scheme 14
Preparation of Tribenzoate **45**

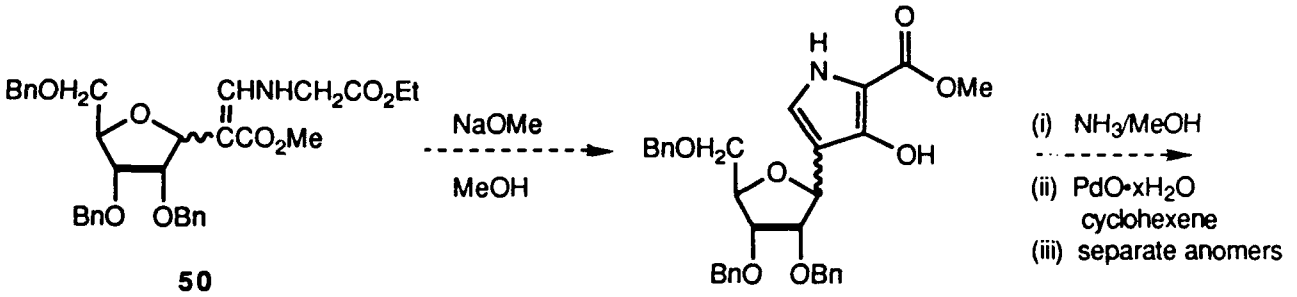


Scheme 15
Approach to 2-Deazapyrazofurin (6)



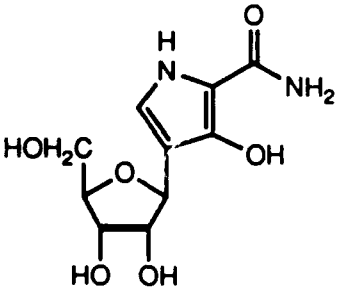
54
(from Scheme 14 of the Quarterly Report of 6-19-90)

52



50

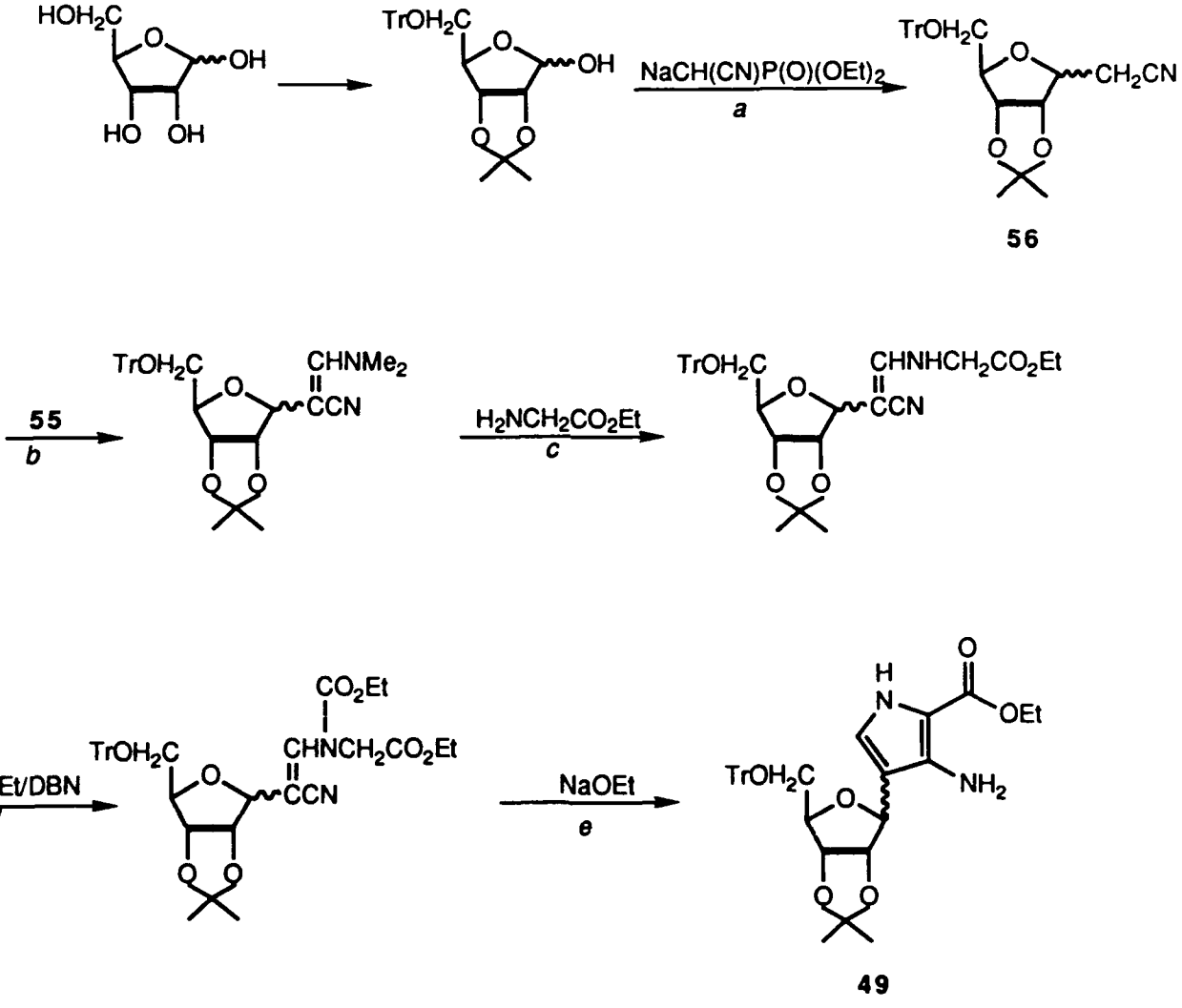
51



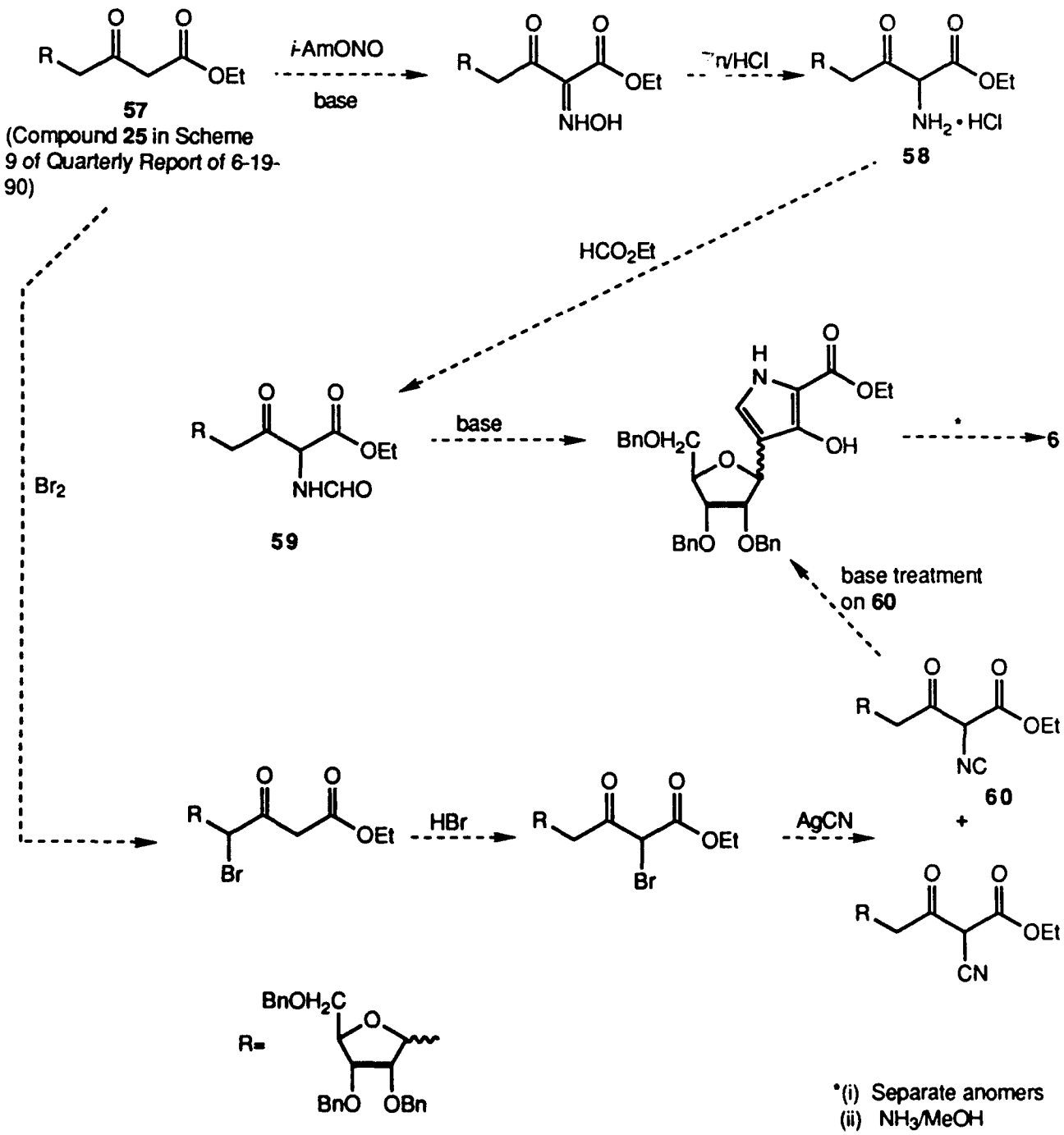
6

Scheme 15a

Overview of Literature Approach[17] to Aminoester **49**

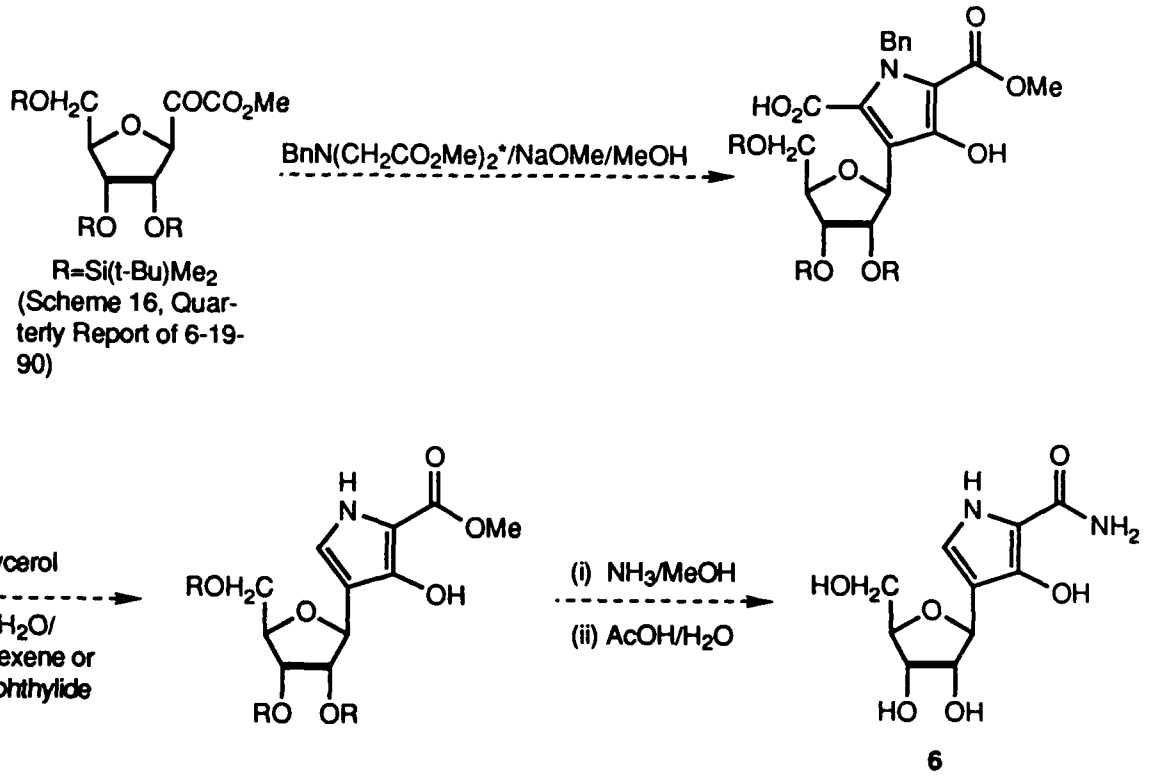


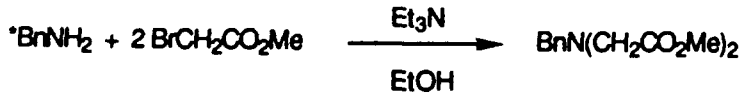
Scheme 16
Approaches to 2-Deazapyrazofurin (6)



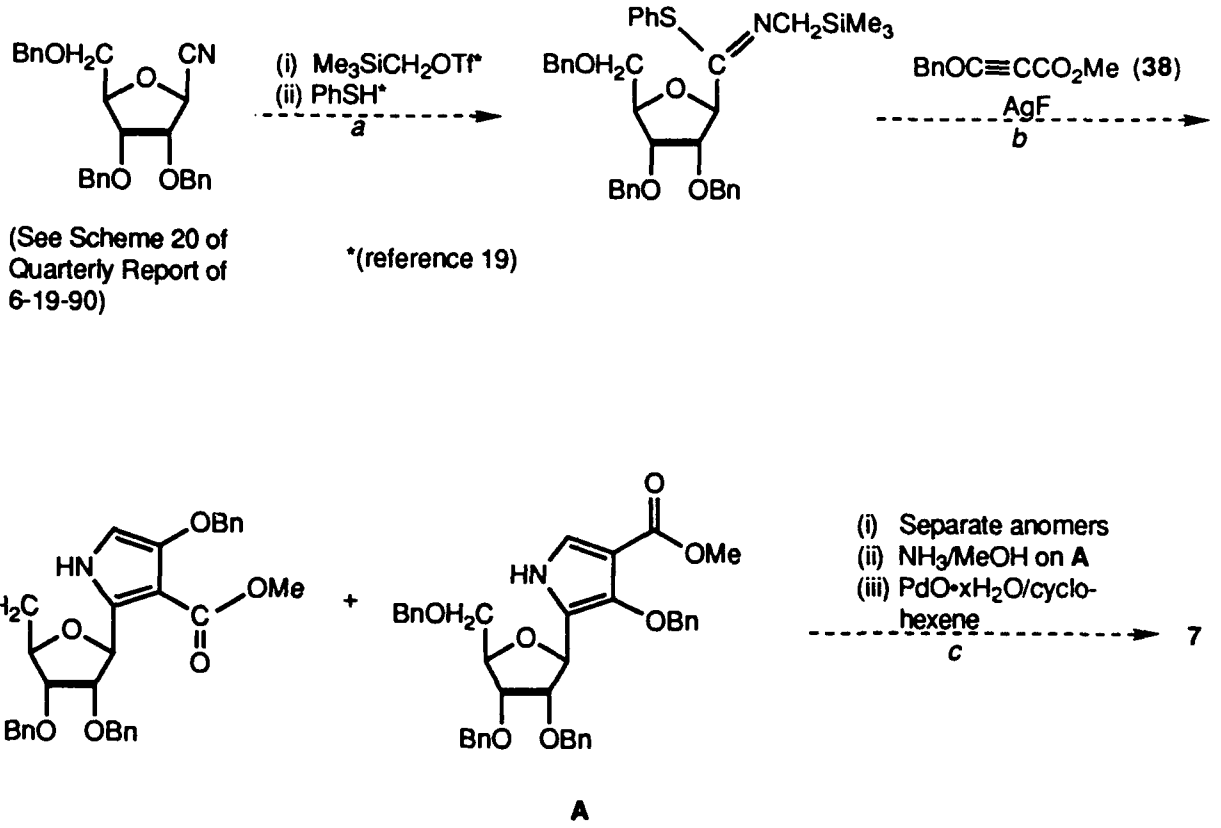
*(i) Separate anomers
(ii) NH_3/MeOH
(iii) $PdO \cdot xH_2O$/cyclo-hexene

Scheme 17
Another Approach to 2-Deazapyrazofurin (6)

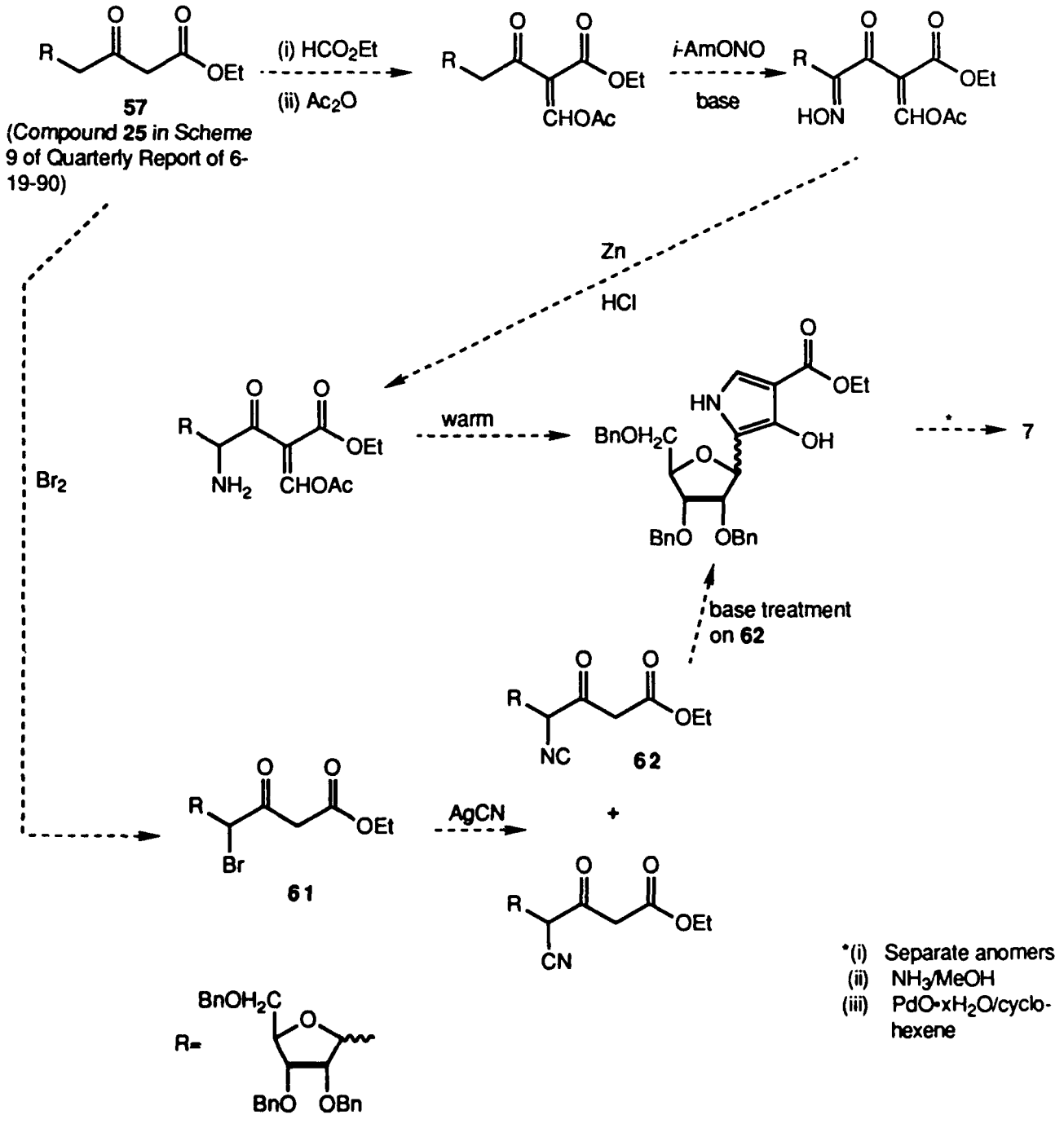




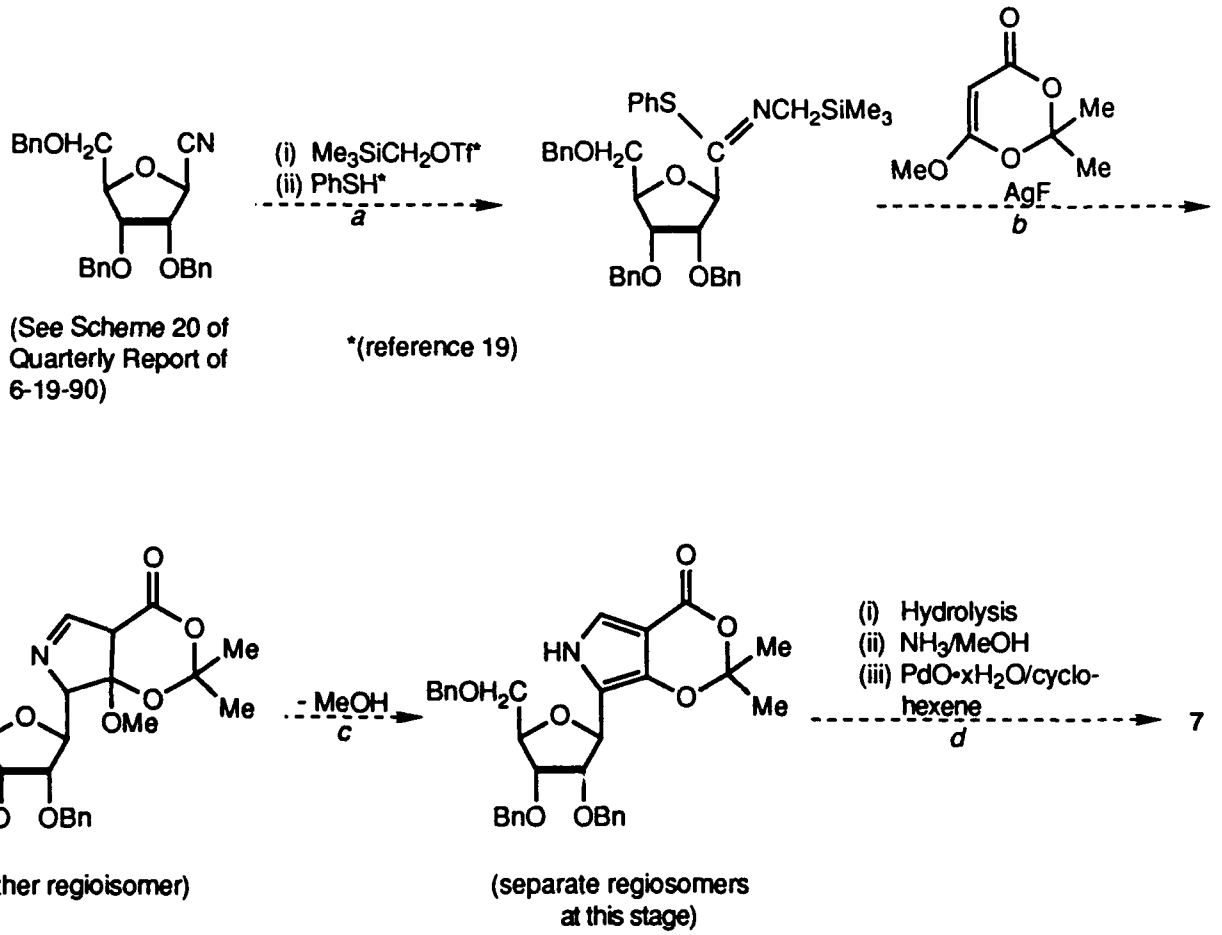
Scheme 18
Planned Approach to the Synthesis of 1-Deazapyrazofurin (7)



Scheme 19
Possible Approaches to 1-Deazapyrazofurin (**7**)



Scheme 20
Another Approach to the Synthesis of 1-Deazapyrazofurin (7)



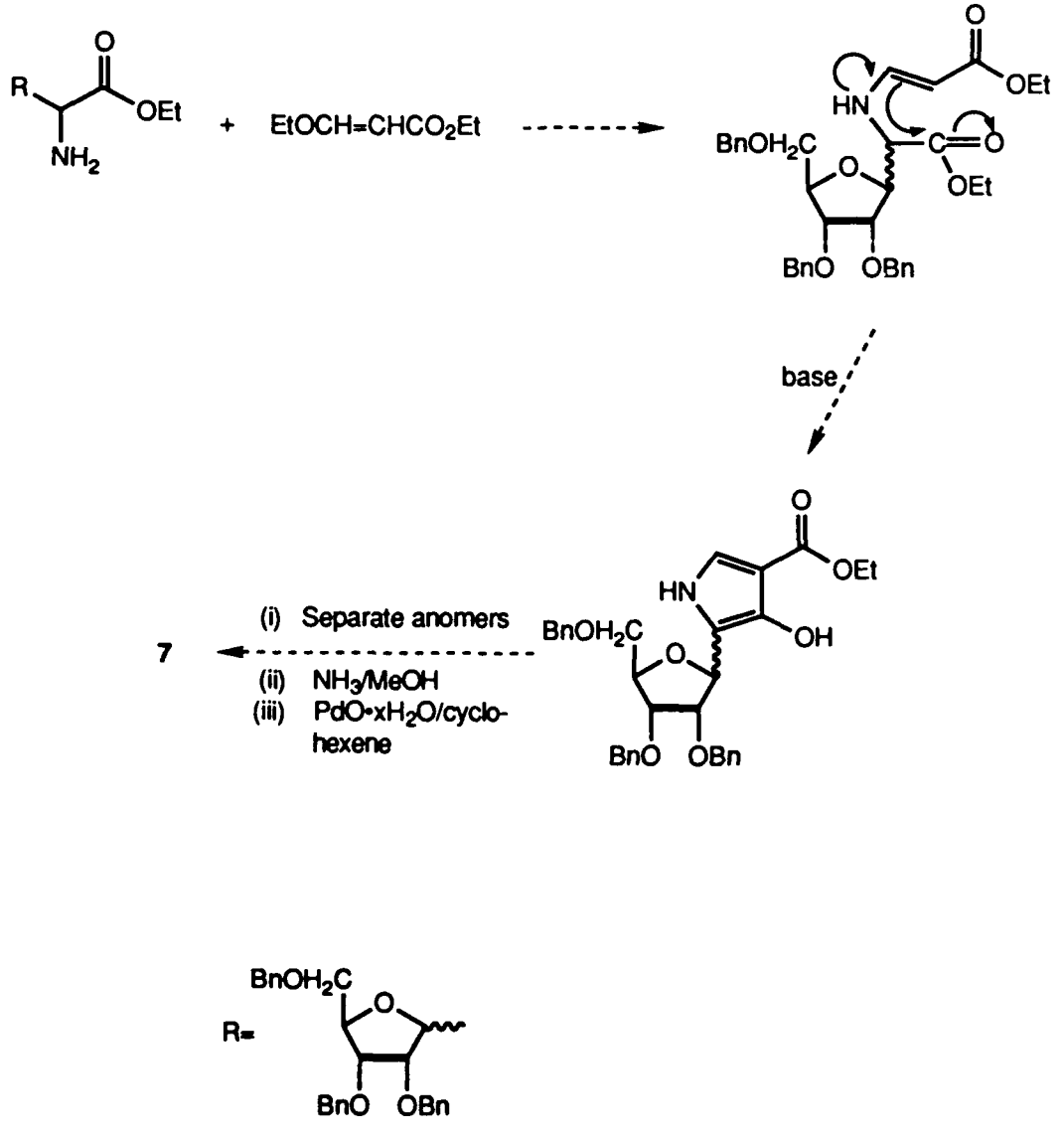
(See Scheme 20 of Quarterly Report of 6-19-90)

*(reference 19)

(+ other regioisomer)

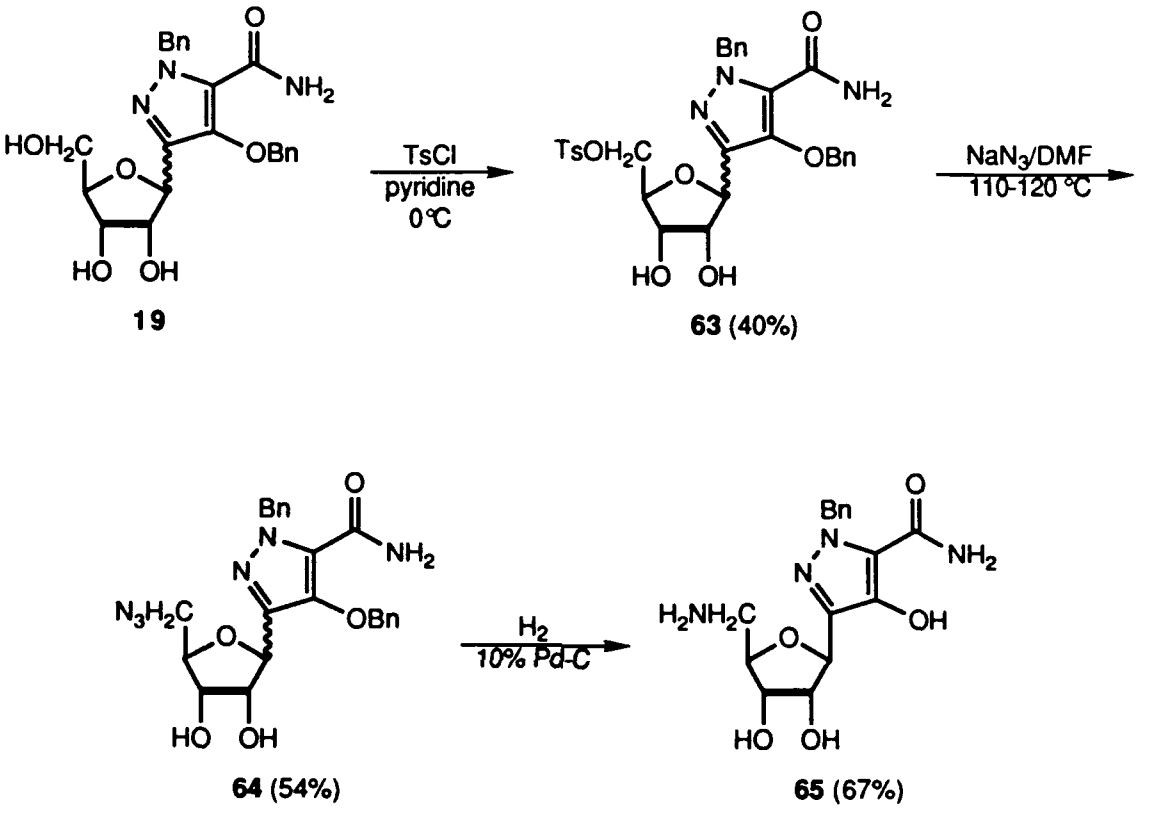
(separate regiosomers at this stage)

Scheme 21
Additional Approach to 1-Deazapyrazofurin (**7**)



Scheme 22

Initial Synthetic Progress Towards 5'-Amino-5'-deoxypyrazofurin (8)



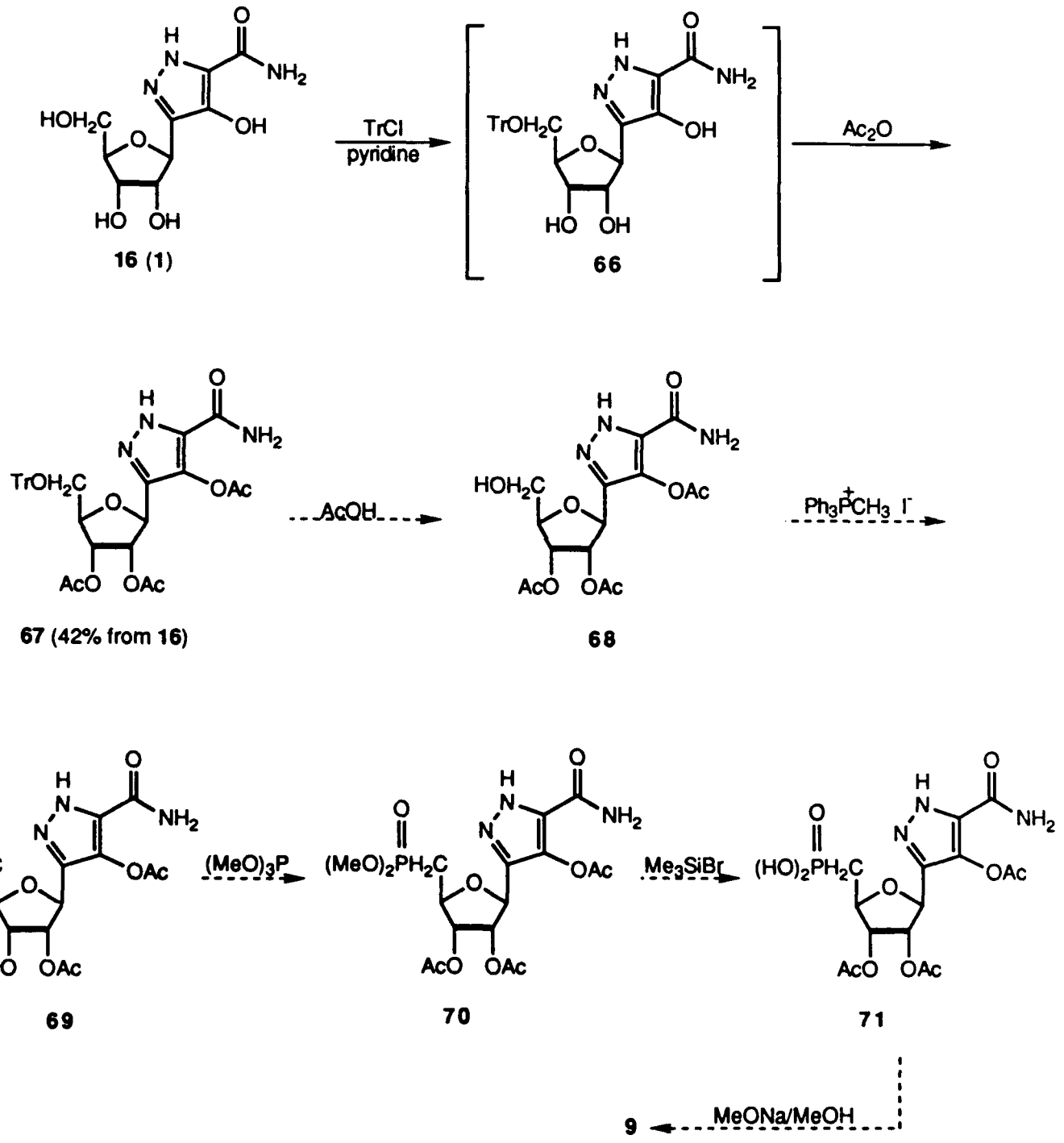
19

63 (40%)

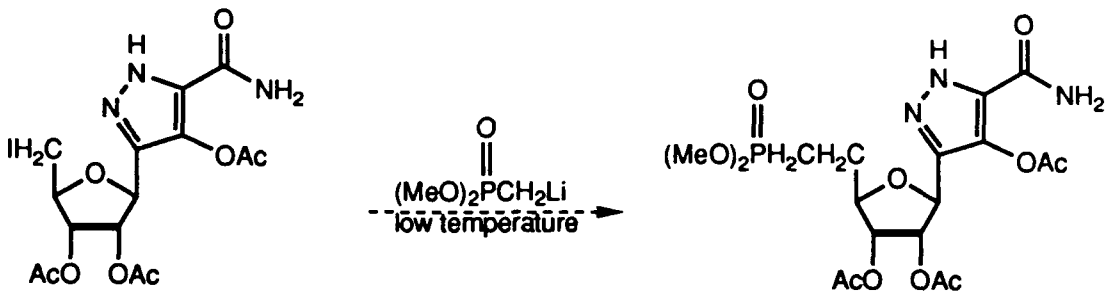
64 (54%)

65 (67%)

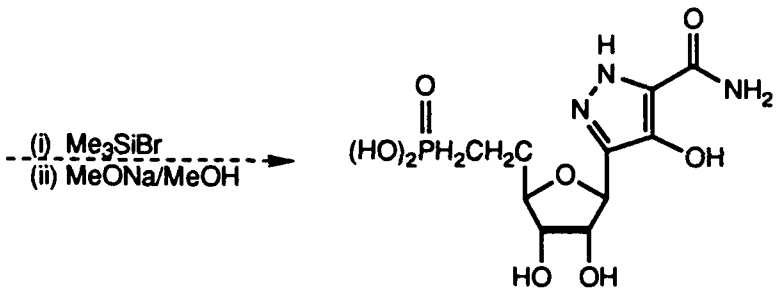
Scheme 23
Completed and Planned Steps Towards Phosphonate 9



Scheme 24
Planned Steps Towards Phosphonate **10**

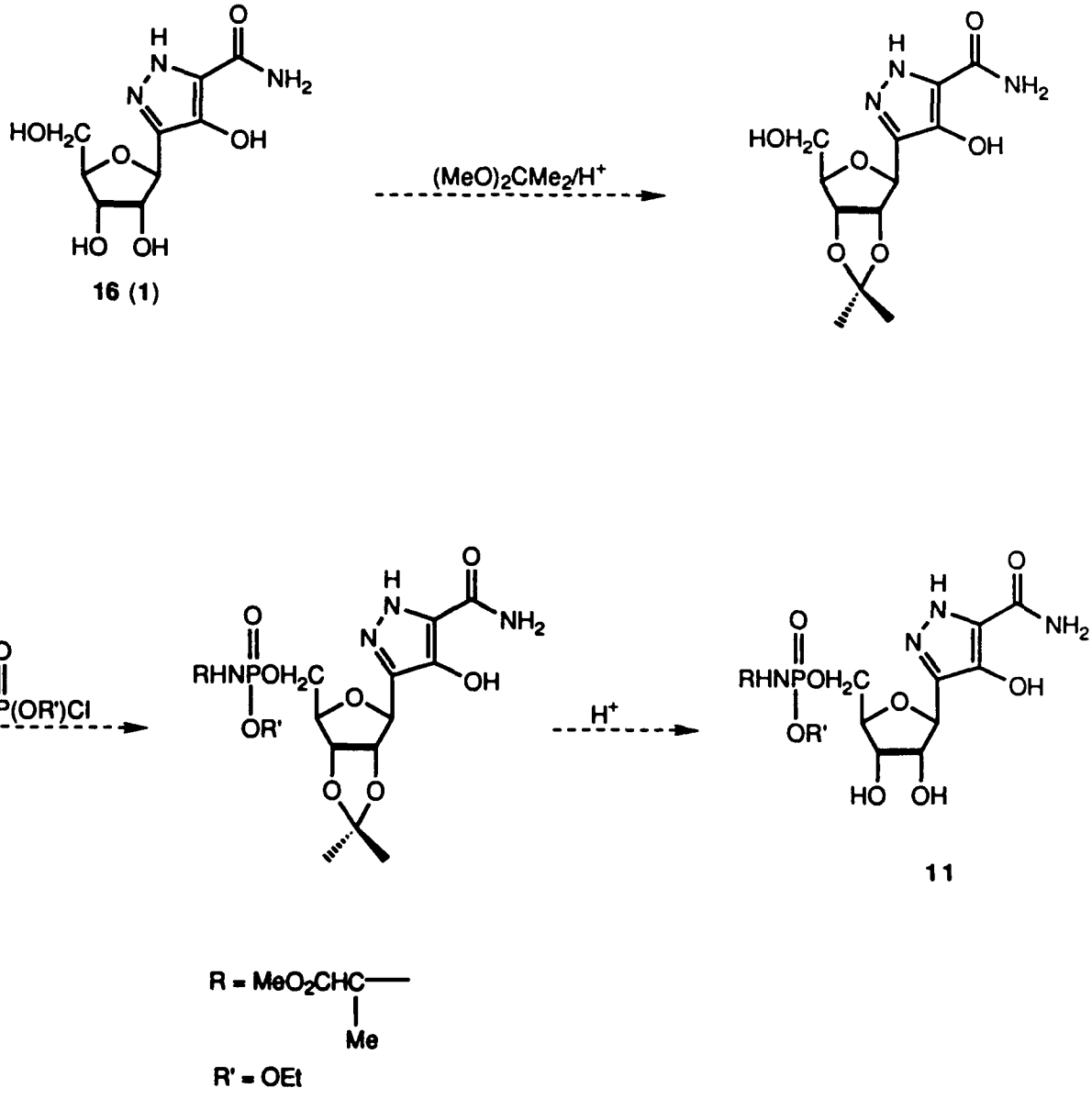


69
(Scheme 23)

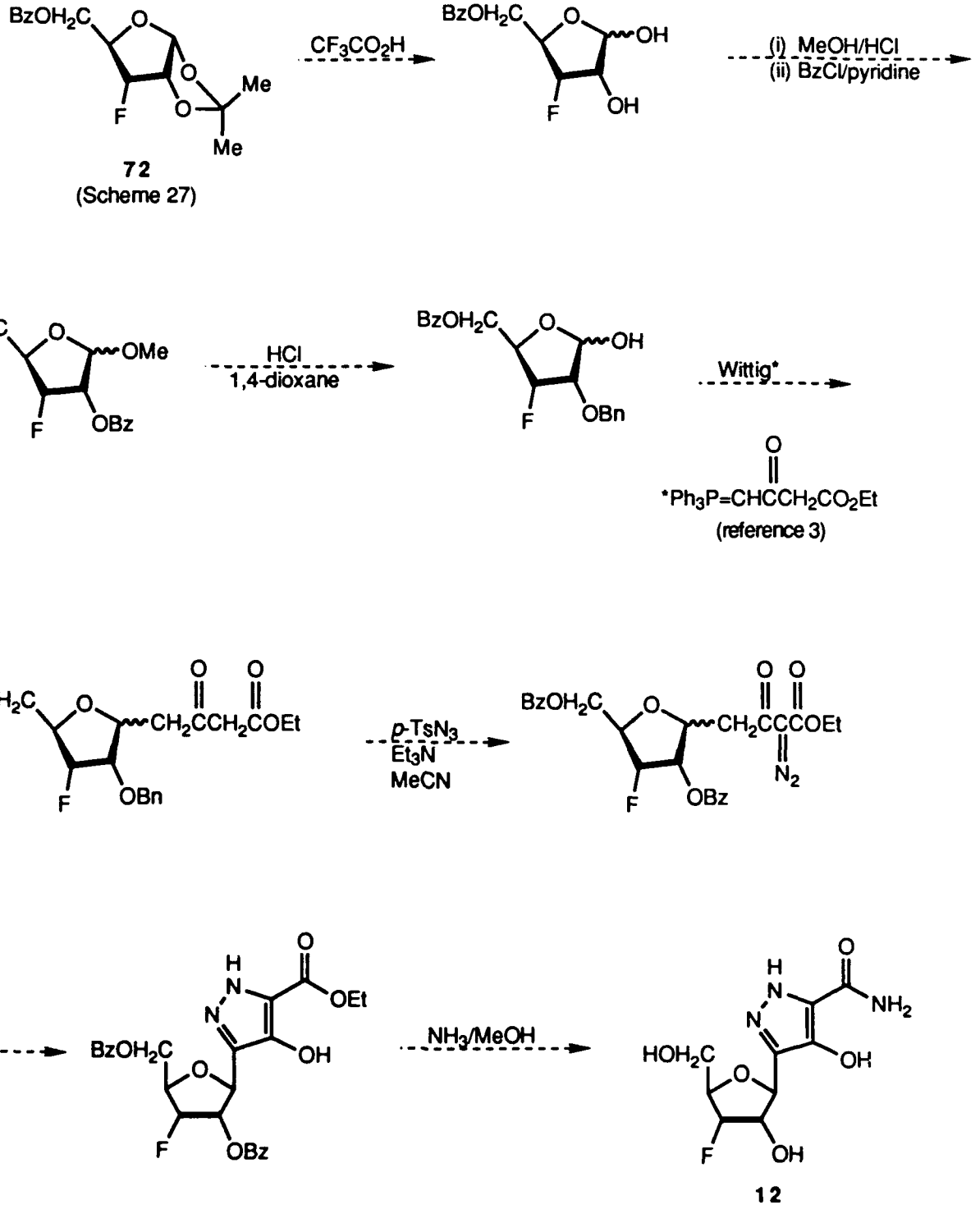


10

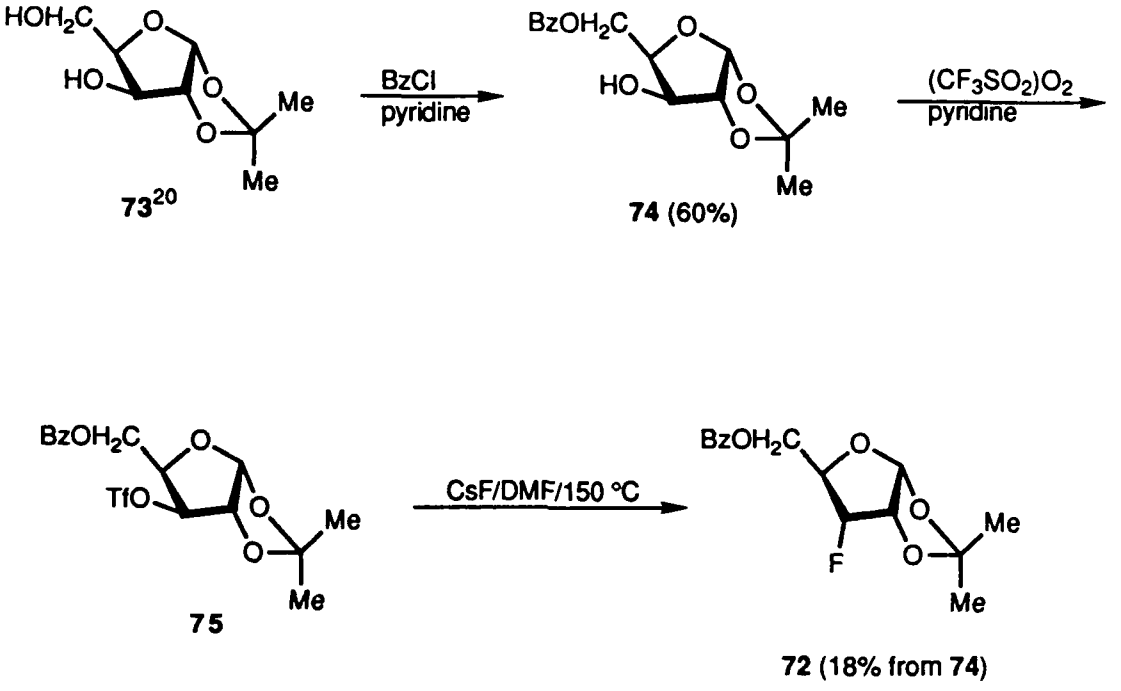
Scheme 25
Planned Steps Towards Phosphoramidite **11**



Scheme 26
Planned Synthesis of Target Compound **12**



Scheme 27
Synthesis of Precursor Fluoro Compound **72**



73[20]

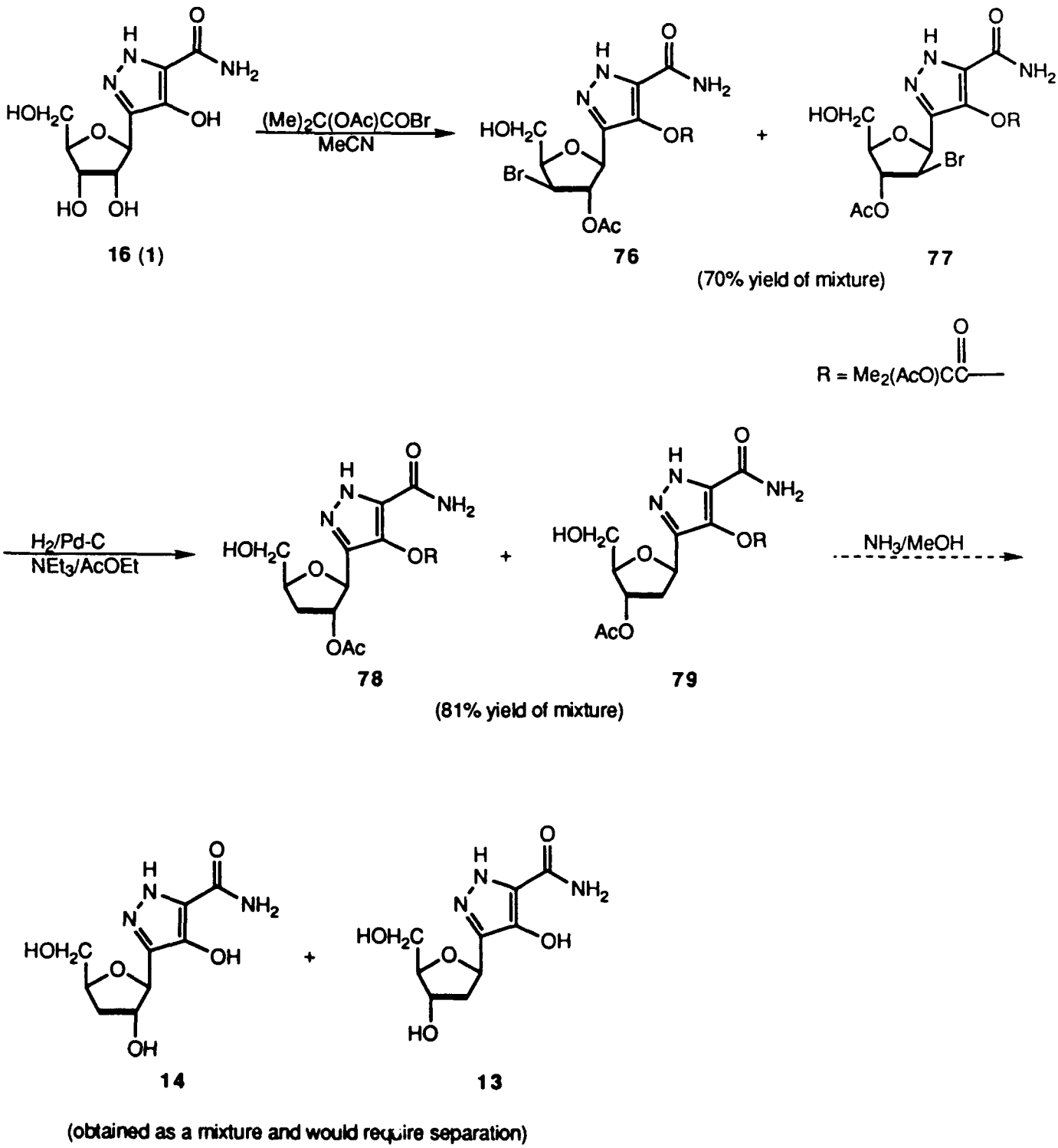
74 (60%)

75

72 (18% from **74**)

Scheme 28

Progress Towards 2'-Deoxypyrazofurin (**13**) and 3'-Deoxypyrazofurin (**14**)



16 (1) **76** **77**

(70% yield of mixture)

78 **79**

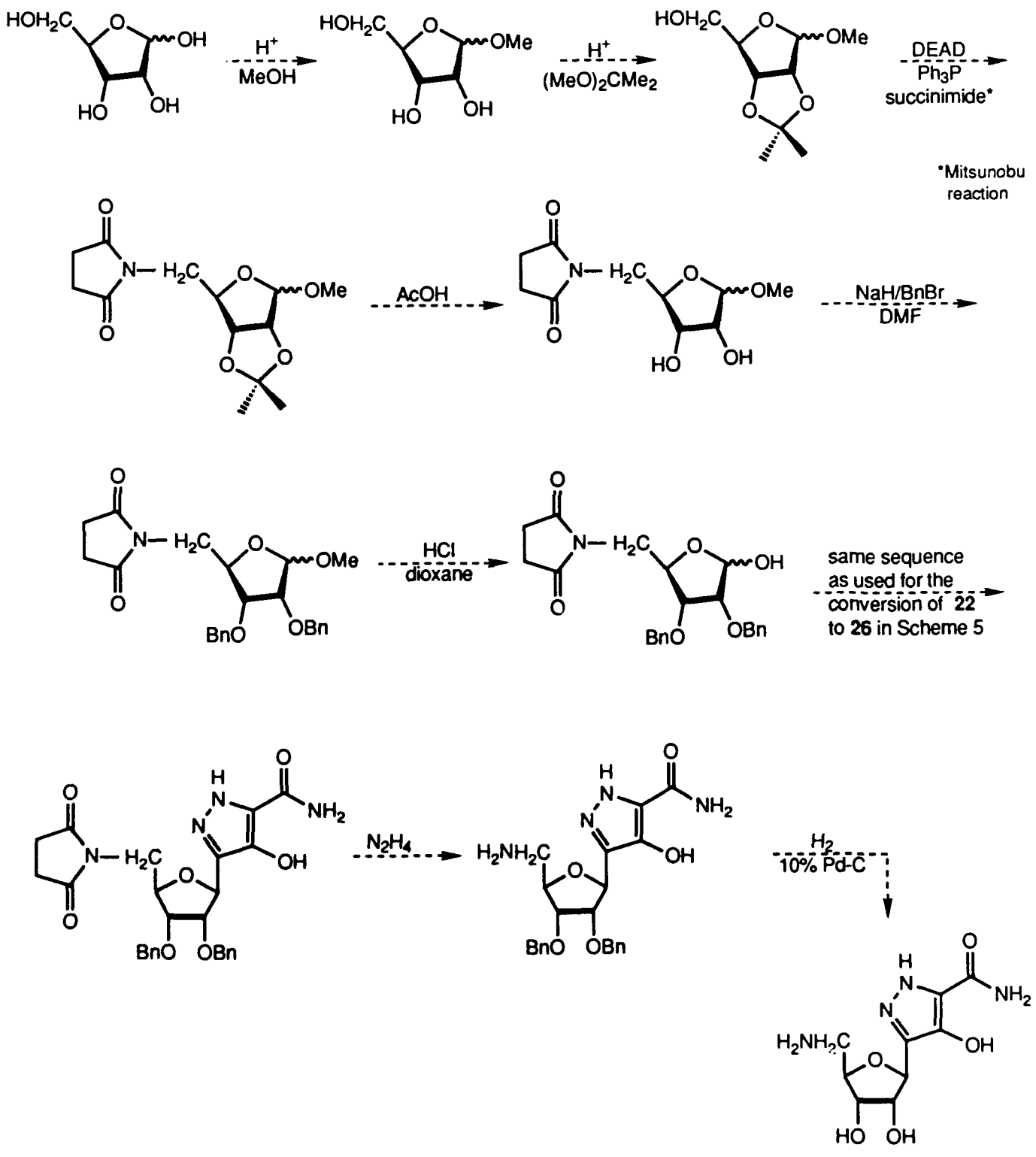
(81% yield of mixture)

14 **13**

(obtained as a mixture and would require separation)

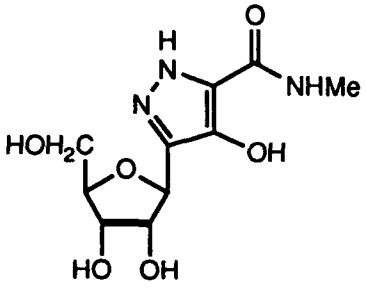
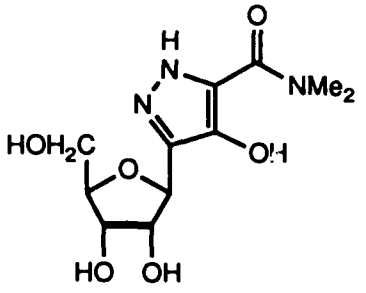
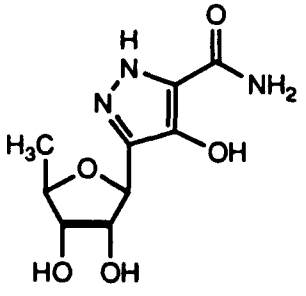
Scheme 29

Alternative Synthetic Approach to 5'-Amino-5'-deoxypyrazofurin (**8**)



8

COMPOUNDS SUBMITTED TO THE ARMY DURING THE REPORTING PERIOD

Structure	AVS Number	Contractor's Number	Reference to Synthesis*	Amount Submitted
	009437	PF-5 (A-CX)	**2a**	120 mg
	009438	PF-6 (B-CX)	**2b**	120 mg
	none assigned yet	PF-7 (E-CX)	**3**	100 mg**

*All syntheses are presented in this report; numbers in this column refer to the compound number for this analogue in the report to aid in locating the experimental details for its preparation.

**30 mg of this compound was also submitted to Eli Lilly Company by the Principal Investigator.

PUBLICATIONS SUPPORTED BY THE CONTRACT

1. Sauer, D.R.; Schneller, S.W. "The Synthesis of 3(5)-[(2-Hydroxyethoxy)-methyl]pyrazole-5(3)-carboxamide, An Acyclic Analogue of 4-Deoxypyrazofurin," *J. Org. Chem.* **1990**, *55*, 5535.

2. Sauer, D.R.; Schneller, S.W. "A Convenient Synthesis of 4-Deoxypyrazofurin," accepted for publication in *Synthesis*.

3. Sauer, D.R.; Schneller, S.W. ; Gabrielsen, B. "4-Homopyrazofurin and Its Acyclic Analogue," submitted to *J. Med. Chem.*

PROFESSIONAL PRESENTATIONS SUPPORTED BY THE CONTRACT

None

PERSONNNEL RECEIVING CONTRACT SUPPORT

Name	Category	Degree Received
Stewart W. Schneller	Principal Investigator	Not applicable
Linda Morgan	Technician	Not applicable
Purna Pradhan	Postdoctoral	Not applicable
Xing Chen	Postdoctoral	Not applicable
Samalo Rao	Postdoctoral	Not applicable

Appendix

PLATE 1YJ
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: >3.17 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.134	0.128	0.127	0.129	0.130	0.132	0.042	0.037	0.040	0.040	0.039	0.035
		cc/vc					tox	drug 6973 experimental			cc/vc	tox
B		1.567					1.623	0.365	0.359	0.344	1.572	1.734
C		1.570					1.565	0.351	0.342	0.358	1.515	1.634
D		1.558					1.661	0.352	0.344	0.377	1.441	1.596
E		0.328					1.654	0.360	0.385	0.334	0.305	1.615
F		0.313					1.681	0.359	0.337	0.371	0.315	1.627
G		0.305					1.212	0.346	0.340	0.341	0.290	1.105
									drug 6973 colorimetric background			
H							0.121	0.122	0.126	0.125	0.124	0.142

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		
CELLS	MT2	Satisfactory	
SHIPMENT NUMBER	68		
STRN	2.5		
REAGENT	0.130		
VIRUS CONTROL	0.179		
CELL CONTROL	1.407		
DIFFERENTIAL	1.228		

PROJECT #	6520		
SPONSOR	USAMRIID		
TEST DATE	08/08/90		
DATE READ	08/16/90		

DRUG 6973	25%	50%	95%
TC (uG/mL)	97.40	> 100.00	> 100.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.035	3%	1.537	100%	0.012
C	1	0.047	4%	1.476	100%	-.006
D	3.2	0.053	4%	1.504	100%	-.005
E	10	0.054	4%	1.509	100%	-.004
F	32	0.054	4%	1.532	100%	-.008
high G *	100	0.042	3%	1.038	74%	-.009

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

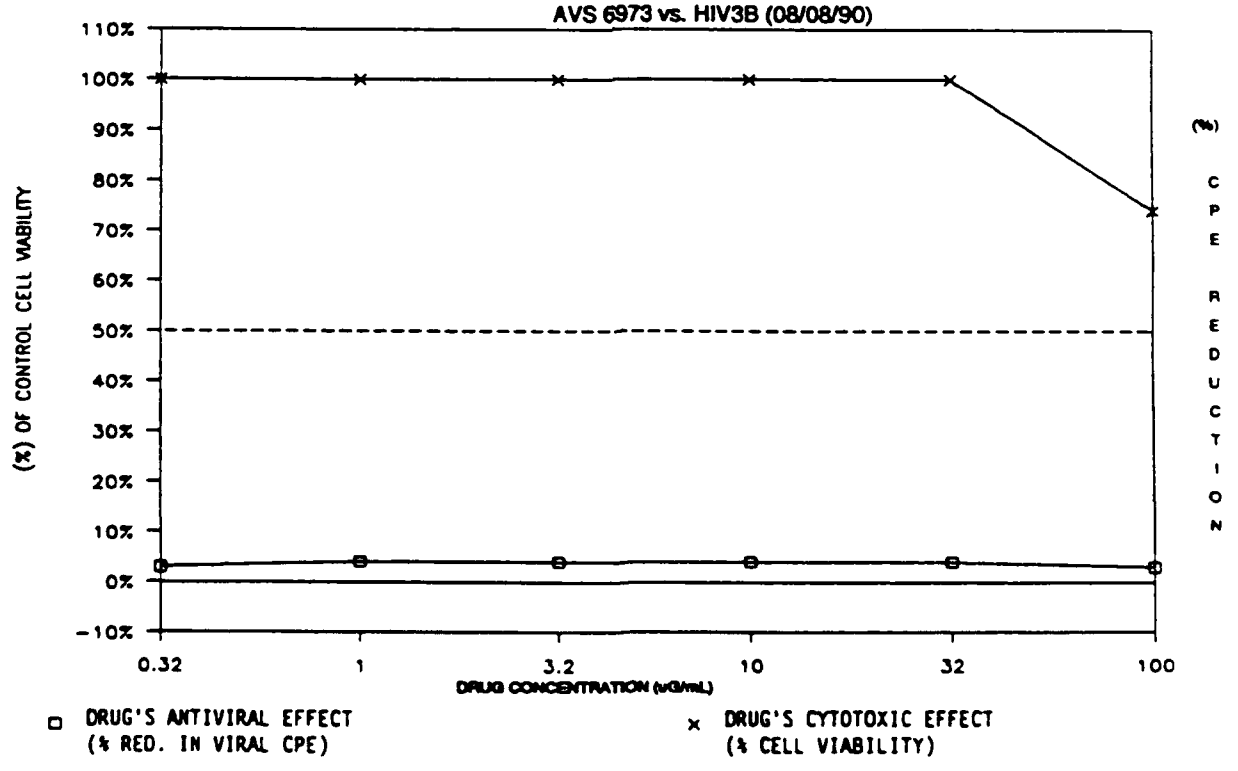


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PLATE WYC
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.062	0.060	0.056	0.056	0.063	0.053	0.002	0.001	0.001	0.001	0.002	0.002
		co/vc					tox	drug 6973 experimental			co/vc	tox
B		1.310					1.231	0.353	0.342	0.344	1.255	1.073
C		1.241					1.304	0.361	0.356	0.350	1.280	1.230
D		1.241					1.274	0.350	0.369	0.356	1.206	0.980
E		0.404					1.402	0.355	0.380	0.360	0.343	1.075
F		0.417					1.311	0.379	0.409	0.384	0.368	0.945
G		0.412					1.440	0.362	0.373	0.351	0.384	1.369
							drug 6973 colorimetric background					
H							0.048	0.056	0.057	0.054	0.056	0.061

tox=cell toxicity co=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	JE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/21/90
STRN	NAKAYAMA	DATE READ	06/27/90
REAGENT	0.058		
VIRUS CONTROL	0.330		
CELL CONTROL	1.197		
DIFFERENTIAL	0.868		

DRUG 6973	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.045	100%	1.091	91%	0.003
C	3.2	-.030	100%	1.211	100%	-.002
D	10	-.026	100%	1.077	90%	-.004
E	32	-.022	100%	1.181	99%	-.001
F	100	0.005	99%	1.072	90%	-.002
high G *	320	-.016	100%	1.356	100%	-.010

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

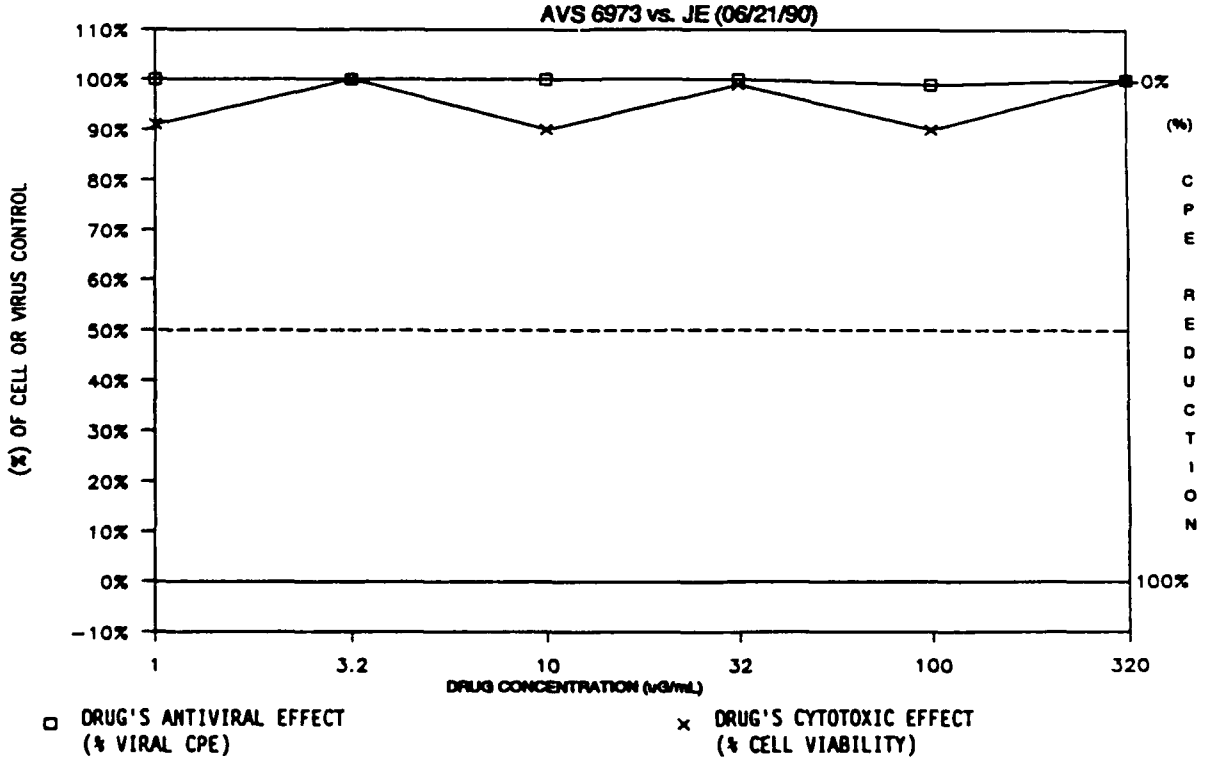

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PLATE X1L
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: >1.03 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.065	0.062	0.060	0.060	0.063	0.065	0.002	0.001	0.002	0.002	0.001	0.001
		cc/vc					tox	drug 6973 experimental			cc/vc	tox
B		1.233					1.221	0.496	0.597	0.556	1.234	1.251
C		1.234					1.215	0.479	0.455	0.499	1.225	1.220
D		1.248					1.222	0.474	0.427	0.464	1.236	1.238
E		0.453					1.209	0.484	0.470	0.450	0.491	1.222
F		0.509					1.099	0.579	0.544	0.643	0.493	1.109
G		0.468					0.668	0.498	0.504	0.482	0.465	0.794
							drug 6973 colorimetric background					
H							0.051	0.067	0.063	0.062	0.062	0.060

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ADAMES		
REAGENT	0.063		
VIRUS CONTROL	0.417		
CELL CONTROL	1.173		
DIFFERENTIAL	0.755		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/21/90		
DATE READ	06/29/90		

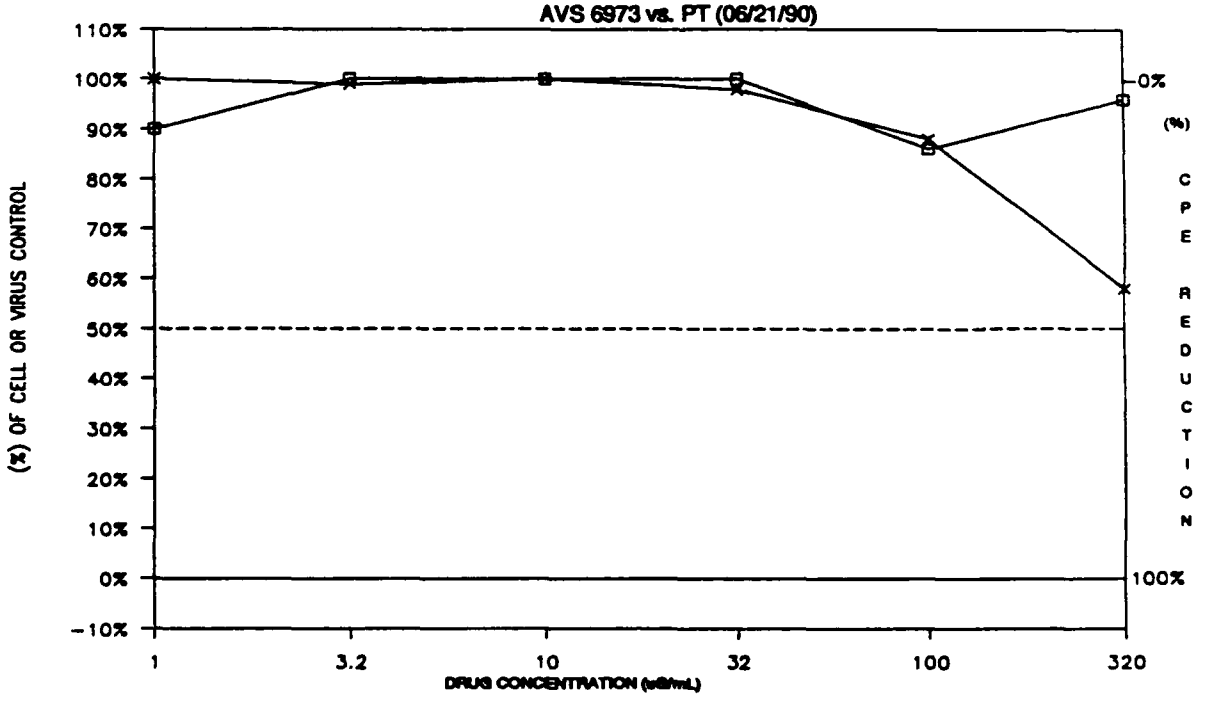
DRUG 6973	25%	50%	95%
TC (uG/mL)	195.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.073	90%	1.177	100%	-.003
C	3.2	-.001	100%	1.156	99%	-.001
D	10	-.024	100%	1.169	100%	-.001
E	32	-.013	100%	1.152	98%	0.001
F	100	0.104	86%	1.037	88%	0.005
high G *	320	0.027	96%	0.681	58%	-.012

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6973 vs. PT (06/21/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 07/06/90

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PLATE X20
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plasma background					
A	0.057	0.056	0.058	0.056	0.054	0.054	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6973 experimental			cc/vc	tox
B		1.110					0.963	0.368	0.465	0.394	1.275	1.019
C		1.086					1.058	0.319	0.344	0.359	1.262	0.927
D		1.170					1.082	0.351	0.323	0.325	1.302	0.896
E		0.373					1.009	0.299	0.269	0.293	0.338	1.006
F		0.403					1.146	0.268	0.253	0.285	0.373	0.964
G		0.376					0.920	0.104	0.102	0.100	0.348	0.860
							drug 6973 colorimetric background					
H							0.045	0.049	0.054	0.051	0.053	0.050

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	SF	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/21/90
STRN	SICILIAN	DATE READ	06/28/90
REAGENT	0.056		
VIRUS CONTROL	0.313		
CELL CONTROL	1.145		
DIFFERENTIAL	0.832		

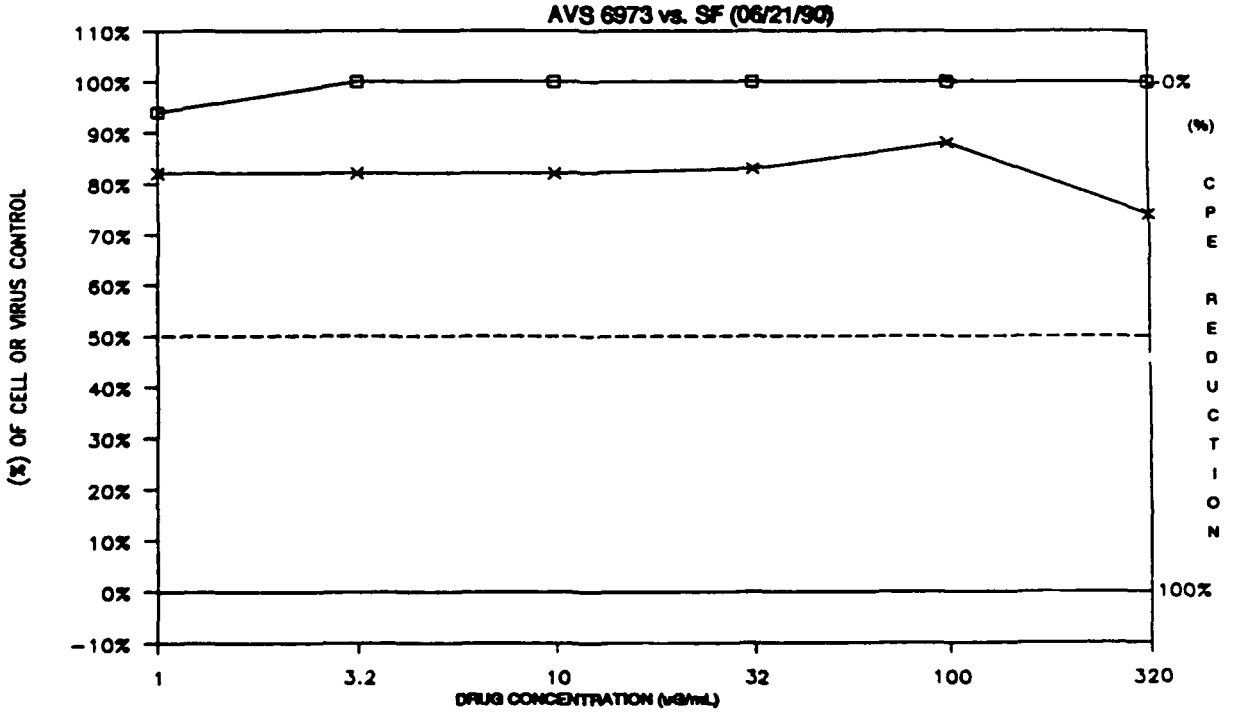
DRUG 6973	25%	50%	95%
TC (uG/mL)	384.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.047	94%	0.941	82%	-.006
C	3.2	-.025	[illegible]	0.940	82%	-.003
D	10	-.030	[illegible]	0.938	82%	-.005
E	32	-.080	[illegible]	0.954	83%	-.002
F	100	-.093	[illegible]	1.006	88%	-.007
high G *	320	-.256	[illegible]	0.845	74%	-.011

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6973 vs. SF (06/21/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

SOUTHERN RESEARCH INSTITUTE

PLATE WZK
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: >0.80 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.070	0.072	0.069	0.068	0.072	0.072	0.001	0.002	0.002	0.001	0.001	0.001
		cc/vc					tox	drug 6973 experimental			cc/vc	tox
B		1.219					1.247	0.109	0.115	0.096	1.186	1.355
C		1.267					1.474	0.107	0.112	0.105	1.259	1.293
D		1.241					1.342	0.109	0.127	0.093	1.377	1.562
E		0.127					1.406	0.108	0.124	0.106	0.097	1.545
F		0.124					1.431	0.112	0.126	0.097	0.121	1.520
G		0.106					1.491	0.116	0.119	0.112	0.110	1.545
							drug 6973 colorimetric background					
H							0.050	0.054	0.056	0.058	0.061	0.064

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/22/90
STRN	TRINIDAD	DATE READ	06/26/90
REAGENT	0.071		
VIRUS CONTROL	0.044		
CELL CONTROL	1.188		
DIFFERENTIAL	1.144		

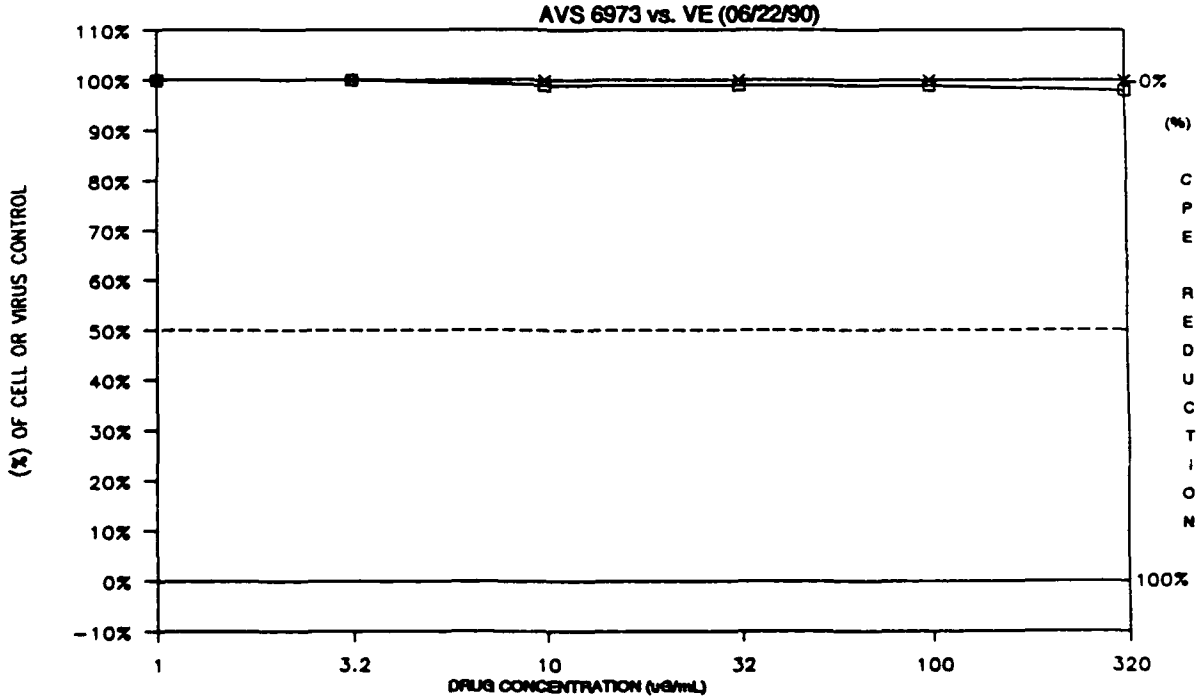
DRUG 6973	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.001	100%	1.238	100%	-.006
C	3.2	0.004	100%	1.323	100%	-.010
D	10	0.008	99%	1.394	100%	-.012
E	32	0.014	99%	1.420	100%	-.015
F	100	0.015	99%	1.422	100%	-.017
high G *	320	0.023	98%	1.469	100%	-.021

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6973 vs. VE (06/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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PLATE 0ZB
DRUG 6973

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6973
TAI: >15.73 SI: >2.40

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.123	0.123	0.123	0.118	0.120	0.128	0.000	0.000	0.000	0.000	0.000	0.000
	tox	cc/vc	drug 6973 experimental			tox					cc/vc	
B	1.268	1.409	0.463	0.414	0.486	1.545					1.393	
C	1.340	1.480	0.348	0.285	0.495	1.494					1.488	
D	1.299	1.418	0.449	0.448	0.449	1.547					1.479	
E	1.236	0.507	0.594	0.489	0.421	1.488					0.333	
F	1.299	0.664	0.626	0.686	1.135	1.536					0.464	
G	1.399	0.507	1.285	1.620	1.222	1.439					0.455	
	drug 6973 colorimetric background											
H	0.120	0.104	0.124	0.113	0.117	0.130						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV
CELLS	VERO
SHIPMENT NUMBER	68
STRN	LEDCA
REAGENT	0.123
VIRUS CONTROL	0.366
CELL CONTROL	1.322
DIFFERENTIAL	0.956

Satisfactory; Active; Retest
RETEST AT 320 UG/ML

PROJECT #	5975-4
SPONSOR	USAMRIID
TEST DATE	06/27/90
DATE READ	07/03/90

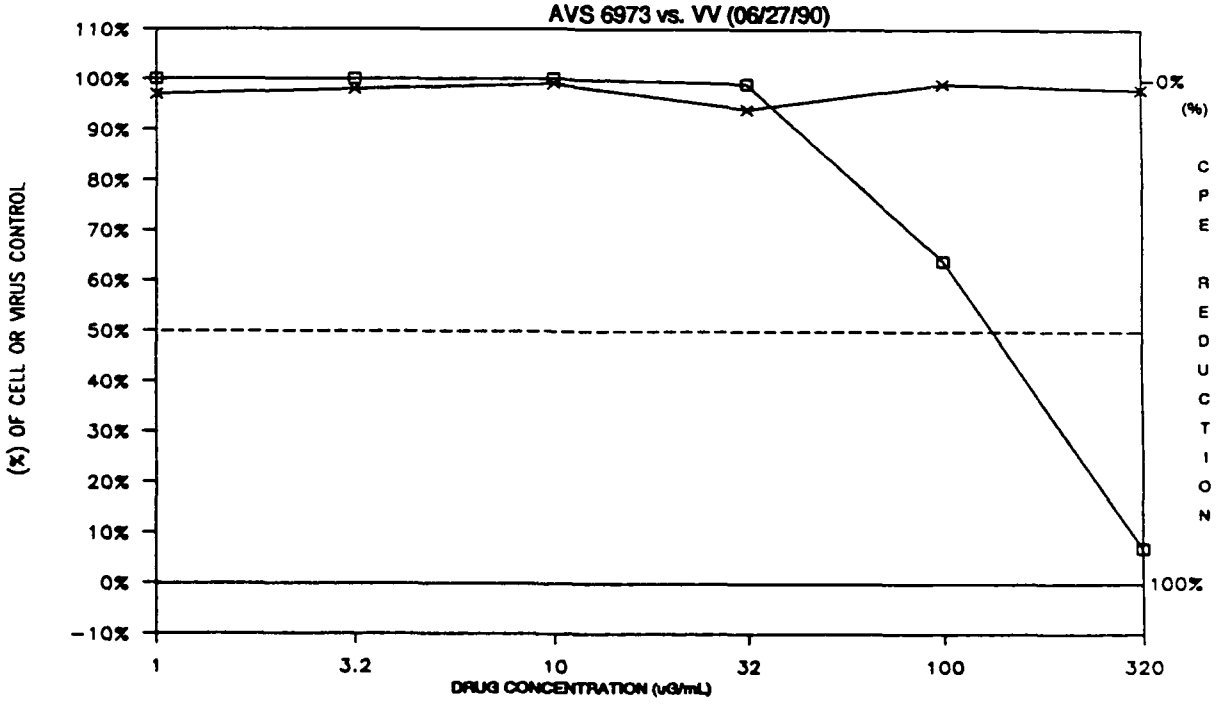
DRUG 6973	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	69.90	133.00	-----
ANTIVIRAL INDEX (AI)	> 4.58	> 2.40	-----

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.042	100%	1.276	97%	0.008
C	3.2	-.107	100%	1.300	98%	-.005
D	10	-.031	100%	1.310	99%	-.009
E	32	0.011	99%	1.238	94%	0.002
F	100	0.346	64%	1.314	99%	-.019
high G *	320	0.890	7%	1.300	98%	-.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6973 vs. VV (06/27/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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PLATE X16 — IN VITRO ANTIVIRAL RESULTS — DRUG: AVS 6973

DRUG 6973 — MTT ASSAY — TAI: 2.96 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.065	0.065	0.066	0.067	0.073	0.069	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6973 experimental			cc/vc	tox
B		1.028					0.933	0.432	0.375	0.395	1.220	1.243
C		1.383					1.427	0.493	0.451	0.465	1.110	1.300
D		1.366					1.349	0.467	0.467	0.501	1.189	1.164
E		0.501					1.391	0.471	0.510	0.511	0.437	1.237
F		0.465					1.647	0.421	0.433	0.479	0.456	1.291
G		0.417					1.287	0.273	0.265	0.260	0.426	1.315
							drug 6973 colorimetric background					
H							0.047	0.058	0.059	0.058	0.064	0.060

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ASIBI		
REAGENT	0.068		
VIRUS CONTROL	0.383		
CELL CONTROL	1.149		
DIFFERENTIAL	0.766		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/21/90		
DATE READ	06/27/90		

DRUG 6973	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6973		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.042	100%	1.029	90%	-.008
C	3.2	0.023	97%	1.300	100%	-.004
D	10	0.038	95%	1.199	100%	-.010
E	32	0.056	93%	1.256	100%	-.009
F	100	0.004	99%	1.412	100%	-.010
high G *	320	-.163	100%	1.255	100%	-.021

* highest drug concentration tested — values shown are final adjusted numbers

SUMMARY GRAPH

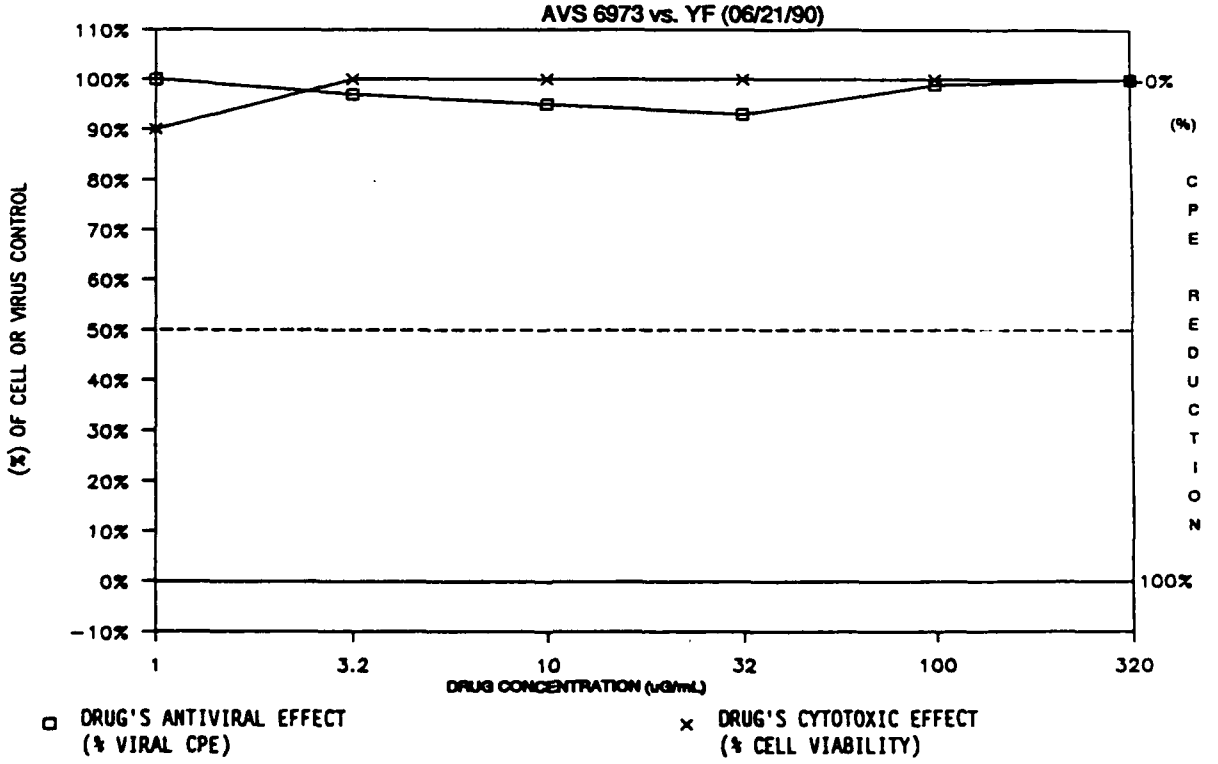

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PLATE 1YK
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: >3.60 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.129	0.127	0.126	0.129	0.127	0.129	0.036	0.036	0.036	0.036	0.036	0.039
	tox	cc/vc	drug 6974 experimental			tox					cc/vc	
B	1.673	1.509	0.380	0.351	0.358	1.639					1.592	
C	1.630	1.557	0.359	0.350	0.363	1.653					1.525	
D	1.615	1.563	0.369	0.378	0.348	1.612					1.591	
E	1.648	0.326	0.364	0.384	0.395	1.659					0.337	
F	1.585	0.319	0.370	0.365	0.382	1.623					0.328	
G	1.667	0.324	0.373	0.366	0.351	1.602					0.308	
	drug 6974 colorimetric background											
H	0.127	0.127	0.128	0.127	0.127	0.128						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

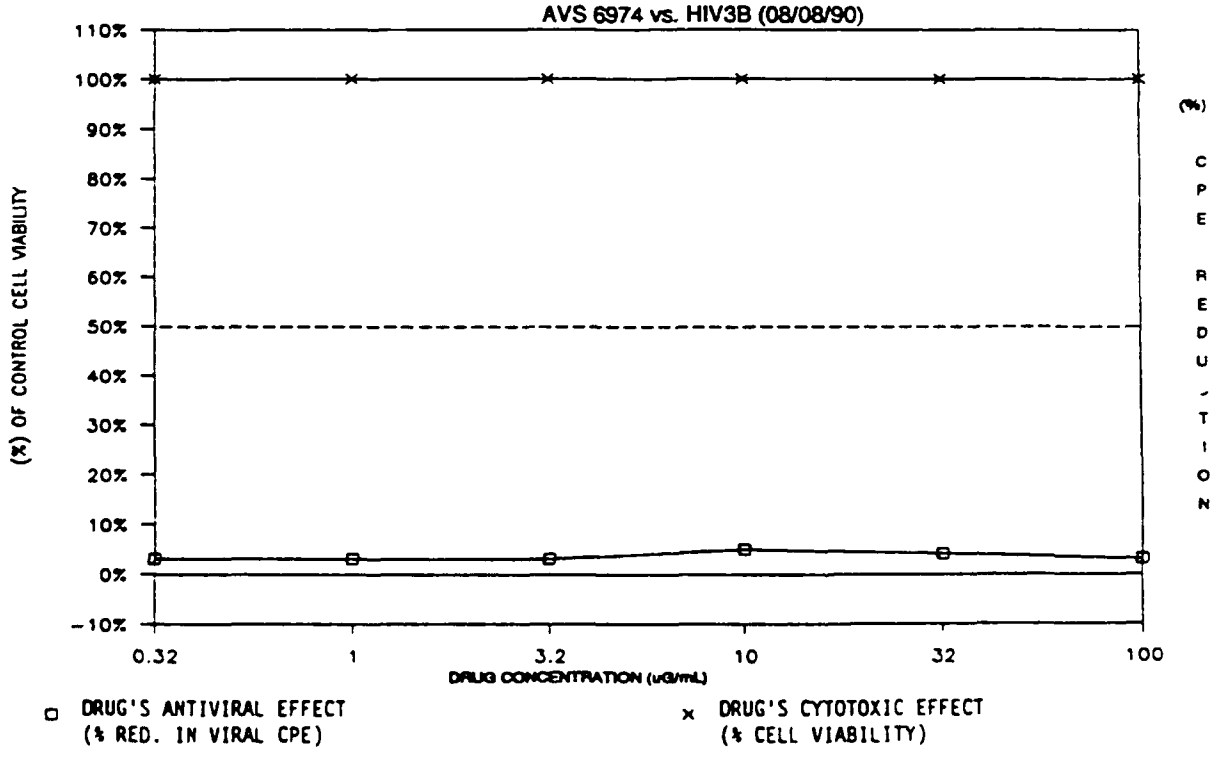
VIRUS	HIV3B		PROJECT #	6520
CELLS	MT2	Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68		TEST DATE	08/08/90
STRN	2.5		DATE READ	08/16/90
REAGENT	0.128			
VIRUS CONTROL	0.196			
CELL CONTROL	1.428			
DIFFERENTIAL	1.233			

DRUG 6974	25%	50%	95%
TC (uG/mL)	> 100.00	> 100.00	> 100.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.039	3%	1.528	100%	0.000
C	1	0.035	3%	1.515	100%	-.001
D	3.2	0.042	3%	1.487	100%	-.001
E	10	0.057	5%	1.526	100%	0.000
F	32	0.050	4%	1.477	100%	-.001
high G *	100	0.041	3%	1.508	100%	-.001

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



PRINTED 08/23/90

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PLATE WYD
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.058	0.058	0.063	0.068	0.060	0.059	0.002	0.001	0.001	0.002	0.002	0.001
	tox	cc/vc	drug 6974 experimental			tox					cc/vc	
B	0.991	1.328	0.357	0.344	0.328	0.850					1.225	
C	1.090	1.358	0.384	0.371	0.359	1.370					1.208	
D	1.056	1.280	0.360	0.352	0.339	1.151					1.187	
E	1.110	0.410	0.358	0.343	0.339	1.216					0.355	
F	1.037	0.405	0.333	0.335	0.339	1.216					0.363	
G	1.041	0.403	0.337	0.315	0.317	1.498					0.360	
	drug 6974 colorimetric background											
H	0.049	0.057	0.055	0.057	0.053	0.054						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	JE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/21/90
STRN	NAKAYAMA	DATE READ	06/27/90
REAGENT	0.061		
VIRUS CONTROL	0.322		
CELL CONTROL	1.203		
DIFFERENTIAL	0.882		

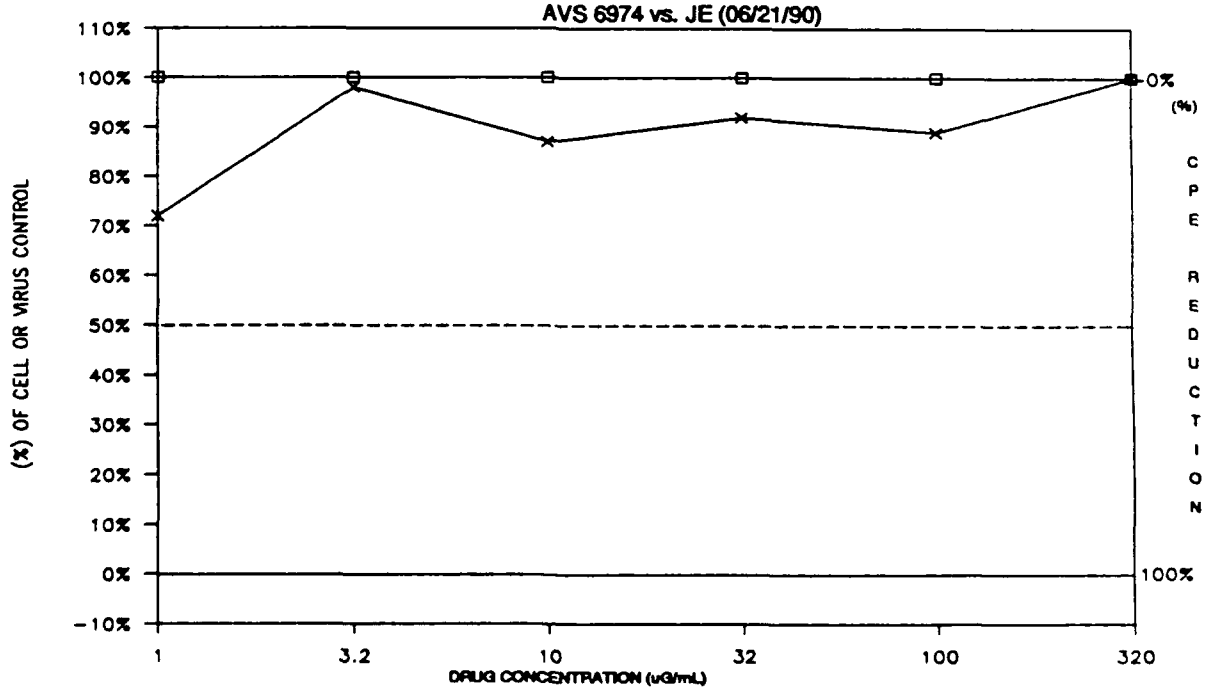
DRUG 6974	25%	50%	95%
TC (uG/mL)	0.89	> 320.00	> 320.00
IC (uG/mL)	------	------	------
ANTIVIRAL INDEX (AI)	------	------	------

	DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
	ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low	B	1	-.033	100%	0.867	72%	-.007
	C	3.2	-.003	100%	1.177	98%	-.008
	D	10	-.028	100%	1.047	87%	-.004
	E	32	-.030	100%	1.108	92%	-.006
	F	*100*	*-.043*	*100%*	*1.070*	89%	-.004
high	G *	320	-.048	100%	1.221	100%	-.012

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6974 vs. JE (06/21/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE X1M
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: >0.87 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.066	0.064	0.063	0.068	0.074	0.064	0.001	0.001	0.002	0.001	0.001	0.001
	tox	cc/vc	drug 6974 experimental			tox					cc/vc	
B	1.322	1.297	0.612	0.589	0.527	1.339					1.263	
C	1.325	1.299	0.489	0.471	0.445	1.161					1.295	
D	1.331	1.300	0.450	0.463	0.437	1.202					1.290	
E	1.187	0.454	0.436	0.416	0.396	1.137					0.513	
F	1.105	0.518	0.430	0.415	0.416	1.196					0.526	
G	0.967	0.478	0.400	0.361	0.354	0.806					0.489	
	drug 6974 colorimetric background											
H	0.052	0.061	0.061	0.062	0.060	0.063						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ADAMES		
REAGENT	0.067		
VIRUS CONTROL	0.430		
CELL CONTROL	1.224		
DIFFERENTIAL	0.794		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/21/90		
DATE READ	06/29/90		

DRUG 6874	25%	50%	95%
TC (uG/mL)	247.00	> 320.00	> 320.00
IC (uG/mL)	----	----	----
ANTIVIRAL INDEX (AI)	----	----	----

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.084	89%	1.268	100%	-.004
C	3.2	-.021	100%	1.184	97%	-.007
D	10	-.041	100%	1.205	98%	-.005
E	32	-.074	100%	1.102	90%	-.006
F	100	-.070	100%	1.090	89%	-.006
high G *	320	-.110	100%	0.835	68%	-.015

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

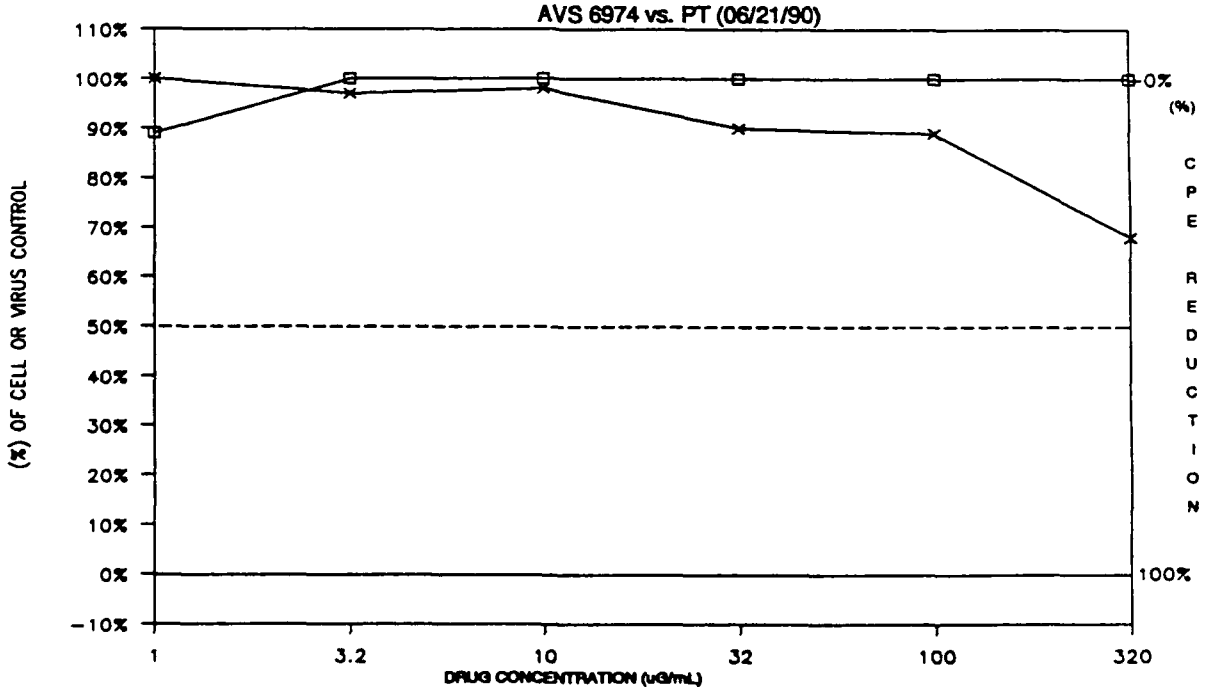


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PLATE X21
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: >0.26 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.057	0.057	0.056	0.058	0.053	0.055	0.001	0.001	0.001	0.001	0.001	0.002
	tox	co/vo	drug 6974 experimental			tox					co/vo	
B	1.223	1.184	0.481	0.440	0.476	1.043					1.247	
C	1.003	1.121	0.381	0.333	0.337	1.030					1.142	
D	0.956	1.100	0.330	0.366	0.352	1.032					1.099	
E	1.037	0.396	0.310	0.329	0.268	1.044					0.367	
F	0.926	0.433	0.276	0.257	0.261	1.008					0.434	
G	0.974	0.390	0.105	0.095	0.086	0.874					0.420	
	drug 6974 colorimetric background											
H	0.046	0.053	0.053	0.055	0.054	0.055						

tox=cell toxicity co=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	SF	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/21/90
STRN	SICILIAN	DATE READ	06/28/90
REAGENT	0.056		
VIRUS CONTROL	0.351		
CELL CONTROL	1.093		
DIFFERENTIAL	0.742		

DRUG 6974	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	------	------	------
ANTIVIRAL INDEX (AI)	------	------	------

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.060	92%	1.078	99%	-.001
C	3.2	-.054	100%	0.963	88%	-.002
D	10	-.056	100%	0.939	86%	-.001
E	32	-.101	100%	0.988	90%	-.003
F	100	-.139	100%	0.914	84%	-.003
high G *	320	-.301	100%	0.878	80%	-.010

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

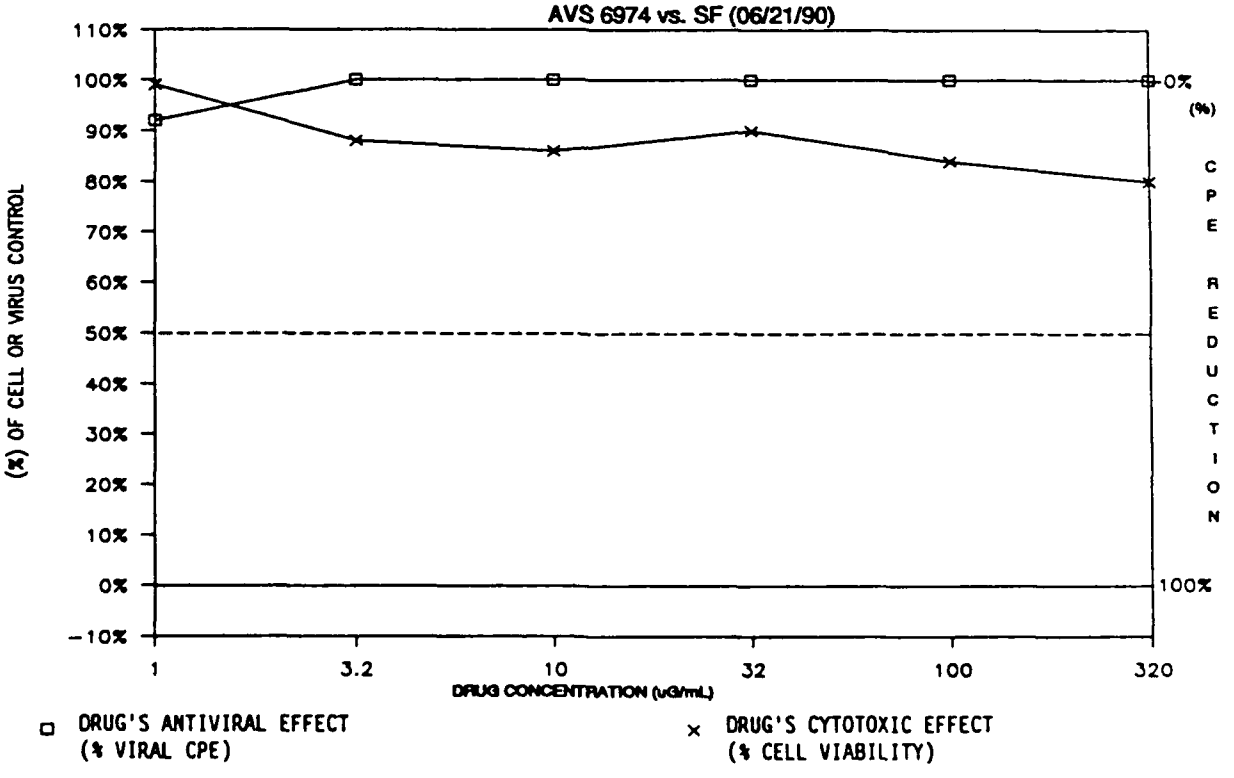


PLATE WZL
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: 0.40 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.065	0.069	0.062	0.064	0.063	0.064	0.002	0.001	0.001	0.001	0.001	0.001
	tox	cc/vc	drug 6974 experimental			tox					cc/vc	
B	1.366	1.276	0.114	0.128	0.117	1.121					1.081	
C	1.376	1.185	0.108	0.127	0.103	1.411					1.506	
D	1.358	1.168	0.095	0.122	0.123	1.383					1.430	
E	1.295	0.110	0.111	0.149	0.116	1.416					0.122	
F	1.323	0.120	0.096	0.104	0.098	1.282					0.119	
G	1.358	0.114	0.072	0.079	0.075	1.420					0.131	
	drug 6974 colorimetric background											
H	0.061	0.064	0.054	0.051	0.053	0.053						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/22/90
STRN	TRINIDAD	DATE READ	06/26/90
REAGENT	0.065		
VIRUS CONTROL	0.055		
CELL CONTROL	1.210		
DIFFERENTIAL	1.155		

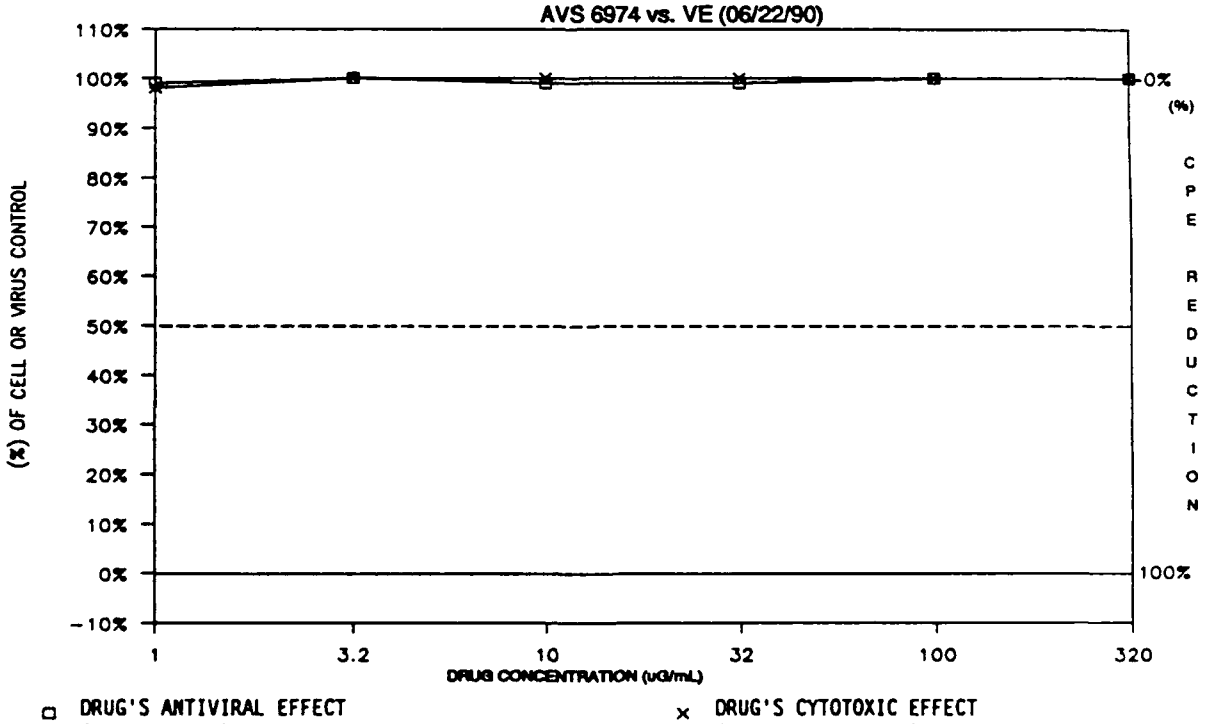
DRUG 6974	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	------	------	------
ANTIVIRAL INDEX (AI)	------	------	------

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.012	99%	1.191	98%	-.012
C	3.2	0.005	100%	1.341	100%	-.012
D	10	0.008	99%	1.320	100%	-.014
E	32	0.017	99%	1.302	100%	-.011
F	100	-.019	100%	1.239	100%	-.001
high G *	320	-.040	100%	1.329	100%	-.004

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6974 vs. VE (06/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

SOUTHERN RESEARCH INSTITUTE

PLATE 10L
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: >0.50 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.112	0.119	0.118	0.120	0.118	0.115	0.000	0.000	0.000	0.000	0.000	0.000
		cc/vc					tox	drug 6974 experimental			cc/vc	tox
B		1.694					1.753	0.308	0.166	0.405	1.584	1.462
C		1.694					1.786	0.219	0.339	0.301	1.673	1.585
D		1.654					1.839	0.242	0.282	0.372	1.766	1.650
E		0.461					1.905	0.276	0.327	0.338	0.363	1.564
F		0.299					1.850	0.244	0.549	0.372	0.354	1.538
G		0.326					1.881	0.449	0.427	0.487	0.529	1.581
							drug 6974 colorimetric background					
H							0.118	0.119	0.116	0.115	0.121	0.125

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV
CELLS	VERO Satisfactory
SHIPMENT NUMBER	68
STRN	LEDCA
REAGENT	0.117
VIRUS CONTROL	0.272
CELL CONTROL	1.561
DIFFERENTIAL	1.289

PROJECT #	5975-4
SPONSOR	USAMRIID
TEST DATE	07/19/90
DATE READ	07/25/90

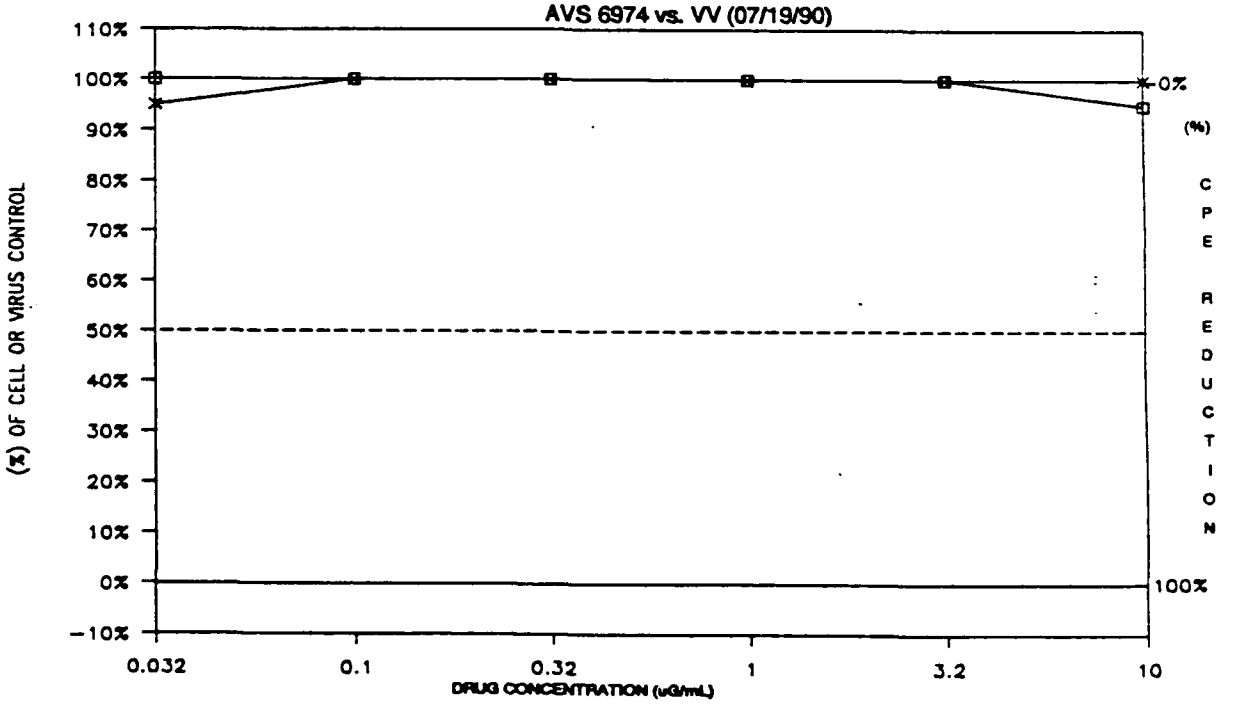
DRUG 6974	25%	50%	95%
TC (uG/mL)	> 10.00	> 10.00	> 10.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE		CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low	B	0.032	-.104	100%	1.483	95%	0.008
	C	0.1	-.106	100%	1.565	100%	0.004
	D	0.32	-.088	100%	1.630	100%	-.002
	E	1	-.074	100%	1.619	100%	-.001
	F	3.2	-.002	100%	1.575	100%	0.002
high	G *	10	0.065	95%	1.613	100%	0.001

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6974 vs. VV (07/19/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE X17
DRUG 6974

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6974
TAI: 0.00 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.065	0.068	0.066	0.066	0.069	0.068	0.001	0.001	0.002	0.001	0.001	0.001
	tox	cc/vc	drug 6974 experimental			tox					cc/vc	
B	1.110	1.012	0.426	0.402	0.396	1.125					1.007	
C	1.052	1.459	0.454	0.436	0.417	1.435					1.086	
D	1.006	1.446	0.486	0.454	0.450	1.332					1.143	
E	1.042	0.490	0.439	0.449	0.419	1.405					0.419	
F	1.186	0.506	0.396	0.359	0.391	1.340					0.386	
G	1.297	0.460	0.185	0.190	0.192	1.186					0.530	
	drug 6974 colorimetric background											
H	0.054	0.061	0.061	0.064	0.066	0.064						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ASIBI		
REAGENT	0.067		
VIRUS CONTROL	0.398		
CELL CONTROL	1.125		
DIFFERENTIAL	0.727		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/21/90		
DATE READ	06/27/90		

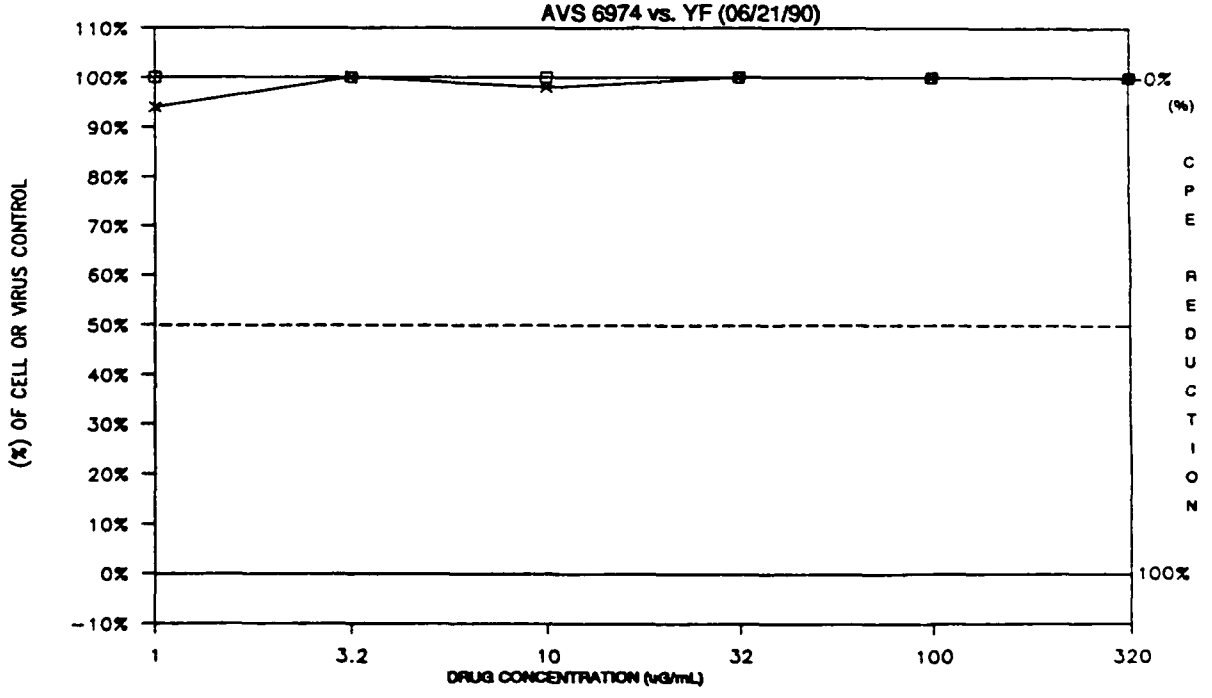
DRUG 6974	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	----	----	----
ANTIVIRAL INDEX (AI)	----	----	----

DRUG 6974		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.054	100%	1.054	94%	-.003
C	3.2	-.029	100%	1.178	100%	-.001
D	10	0.001	100%	1.105	98%	-.003
E	32	-.024	100%	1.163	100%	-.006
F	100	-.077	100%	1.202	100%	-.006
high G *	320	-.263	100%	1.188	100%	-.013

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6974 vs. YF (06/21/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE 1GX
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: >0.43 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.115	0.115	0.118	0.125	0.122	0.128	0.035	0.036	0.036	0.036	0.034	0.034
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		1.576					1.628	0.321	0.326	0.342	1.651	1.560
C		1.520					1.534	0.326	0.321	0.302	1.560	1.497
D		1.563					1.578	0.318	0.306	0.340	1.584	1.457
E		0.333					1.601	0.324	0.325	0.333	0.283	1.532
F		0.314					1.669	0.350	0.325	0.343	0.320	1.487
G		0.329					1.659	0.323	0.315	0.322	0.288	1.608
								drug 6441 colorimetric background				
H							0.124	0.126	0.126	0.123	0.119	0.125

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV3B		
CELLS	MT2	Satisfactory	
SHIPMENT NUMBER	63		
STRN	2.5		
REAGENT	0.121		
VIRUS CONTROL	0.191		
CELL CONTROL	1.455		
DIFFERENTIAL	1.265		

PROJECT #	6520-2		
SPONSOR	USAMRIID		
TEST DATE	03/28/90		
DATE READ	04/05/90		

DRUG 6441	25%	50%	95%
TC (uG/mL)	> 100.00	> 100.00	> 100.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.014	1%	1.469	100%	0.005
C	1	0.007	1%	1.397	96%	-.002
D	3.2	0.008	1%	1.395	96%	0.002
E	10	0.010	1%	1.440	99%	0.006
F	32	0.022	2%	1.452	100%	0.006
high G *	100	0.006	0%	1.510	100%	0.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

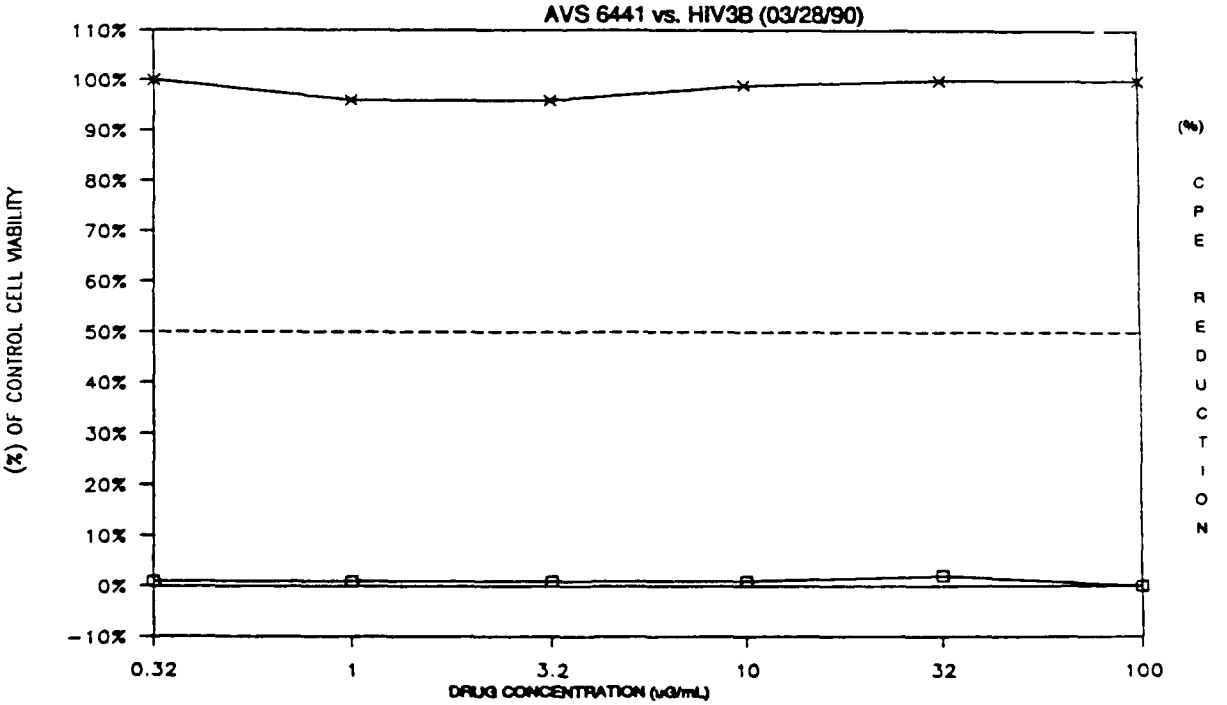


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PLATE U9M
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: 0.00 SI: 0.00

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.052	0.050	0.050	0.049	0.047	0.049	0.001	0.001	0.001	0.001	0.000	0.001
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		1.352					1.106	0.128	0.132	0.136	1.091	1.018
C		1.072					0.948	0.163	0.168	0.195	0.976	1.050
D		1.336					1.105	0.150	0.172	0.163	1.013	0.956
E		0.142					1.055	0.171	0.175	0.166	0.131	0.884
F		0.137					0.856	0.194	0.208	0.183	0.136	0.746
G		0.130					0.767	0.214	0.241	0.217	0.130	0.720
							drug 6441 colorimetric background					
H							0.039	0.048	0.050	0.051	0.048	0.047

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	JE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TEST DATE	03/01/90
STRN	NAKAYAMA	DATE READ	03/09/90
REAGENT	0.050		
VIRUS CONTROL	0.085		
CELL CONTROL	1.091		
DIFFERENTIAL	1.006		

DRUG 6441	25%	50%	95%
TC (uG/mL)	72.80	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	0.00	0.00	0.00

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.001	100%	1.016	93%	-.003
C	3.2	0.043	96%	0.952	87%	-.002
D	10	0.026	97%	0.980	90%	0.001
E	32	0.035	97%	0.919	84%	0.001
F	100	0.063	94%	0.754	69%	-.002
high G *	320	0.101	90%	0.705	65%	-.011

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

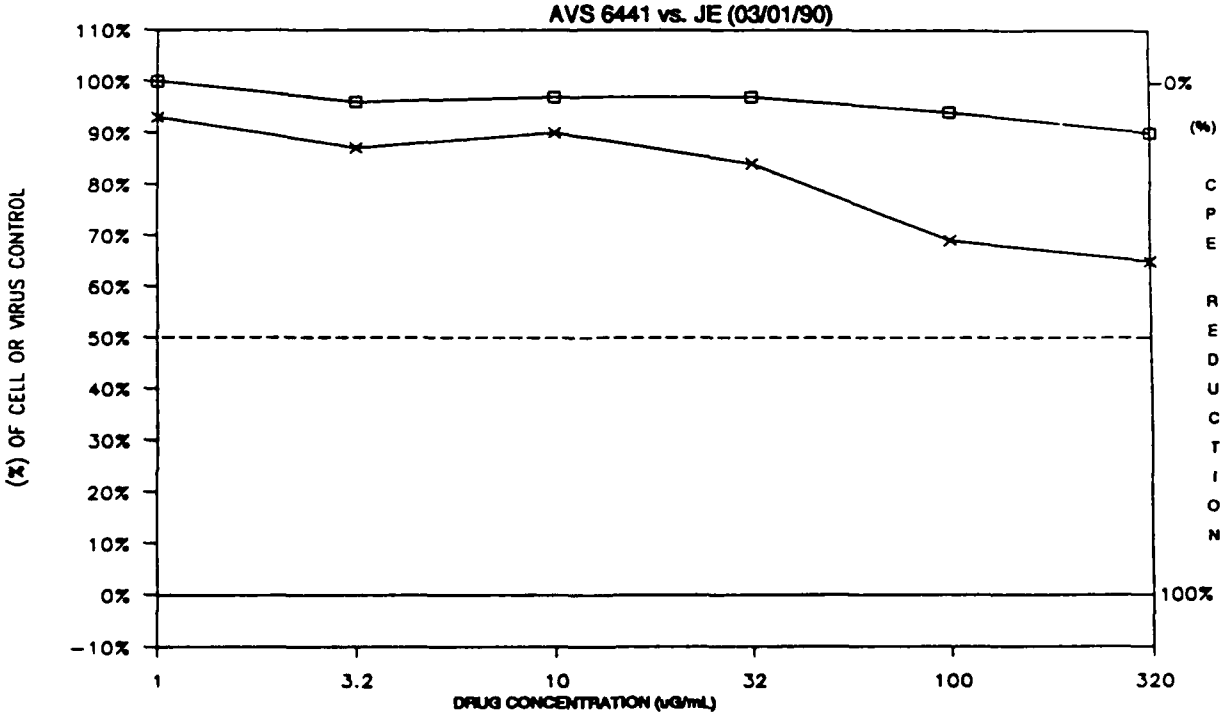

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PLATE U99
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: >1.30 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.044	0.044	0.043	0.044	0.040	0.041	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		0.946					0.878	0.296	0.385	0.379	0.469	0.890
C		0.924					0.928	0.297	0.337	0.364	1.033	1.030
D		1.006					0.956	0.361	0.344	0.344	0.884	0.931
E		0.259					0.920	0.362	0.345	0.358	0.363	0.854
F		0.302					0.835	0.373	0.365	0.362	0.360	0.870
G		0.435					0.747	0.375	0.390	0.381	0.382	0.879
							drug 6441 colorimetric background					
H							0.031	0.037	0.037	0.038	0.039	0.039

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	63		
STRN	ADAMES		
REAGENT	0.043		
VIRUS CONTROL	0.308		
CELL CONTROL	0.834		
DIFFERENTIAL	0.527		

PROJECT #	5975-1			
SPONSOR	USAMRIID			
TEST DATE	03/01/90			
DATE READ	03/09/90			

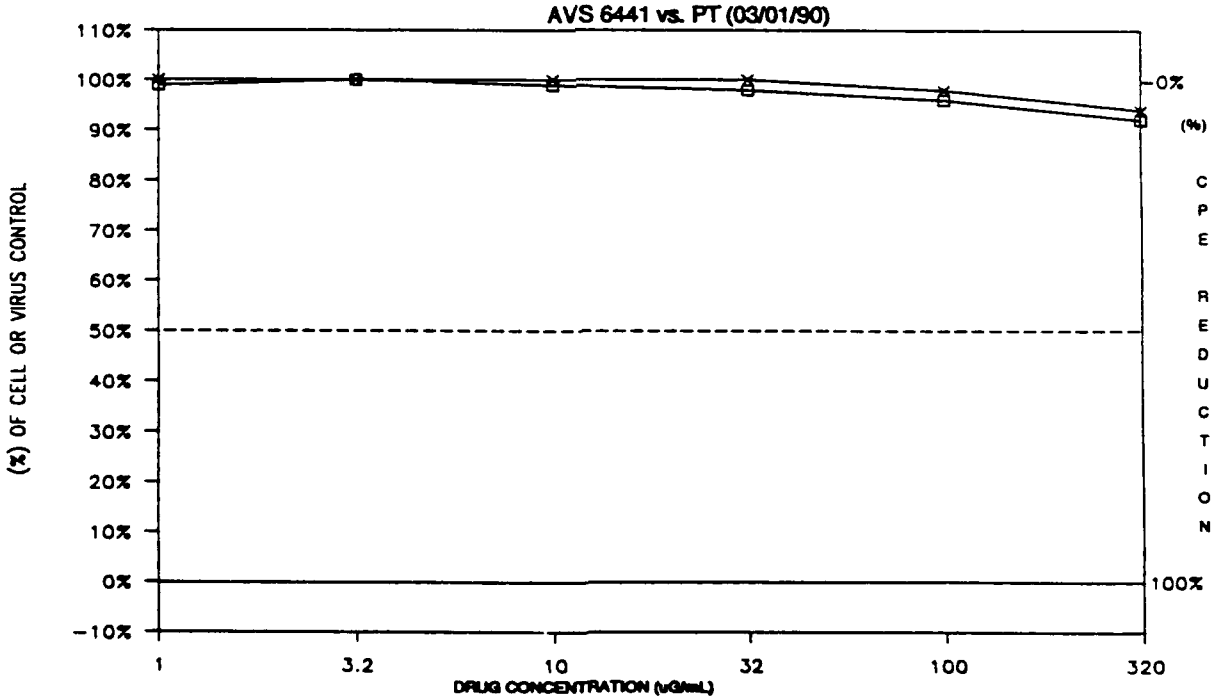
DRUG 6441	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.007	99%	0.845	100%	-.004
C	3.2	-.014	100%	0.940	100%	-.004
D	10	0.004	99%	0.906	100%	-.005
E	32	0.011	98%	0.850	100%	-.006
F	100	0.022	96%	0.816	98%	-.006
high G *	320	0.044	92%	0.782	94%	-.012

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6441 vs. PT (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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PLATE U9Z
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: 0.22 SI: ------

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.051	0.048	0.047	0.043	0.046	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		1.003					0.904	0.226	0.233	0.249	0.985	0.874
C		1.054					1.039	0.261	0.265	0.277	1.032	0.915
D		0.914					1.000	0.243	0.254	0.268	0.989	0.858
E		0.228					0.940	0.242	0.266	0.263	0.247	0.837
F		0.235					0.919	0.248	0.229	0.237	0.243	0.828
G		0.246					0.745	0.136	0.136	0.133	0.260	0.676
							drug 6441 colorimetric background					
H							0.032	0.035	0.036	0.035	0.039	0.038

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	SF		
CELLS	VERO Satisfactory	PROJECT #	5975-1
SHIPMENT NUMBER	63	SPONSOR	USAMRIID
STRN	SICILIAN	TEST DATE	03/01/90
REAGENT	0.046	DATE READ	03/09/90
VIRUS CONTROL	0.197		
CELL CONTROL	0.950		
DIFFERENTIAL	0.753		

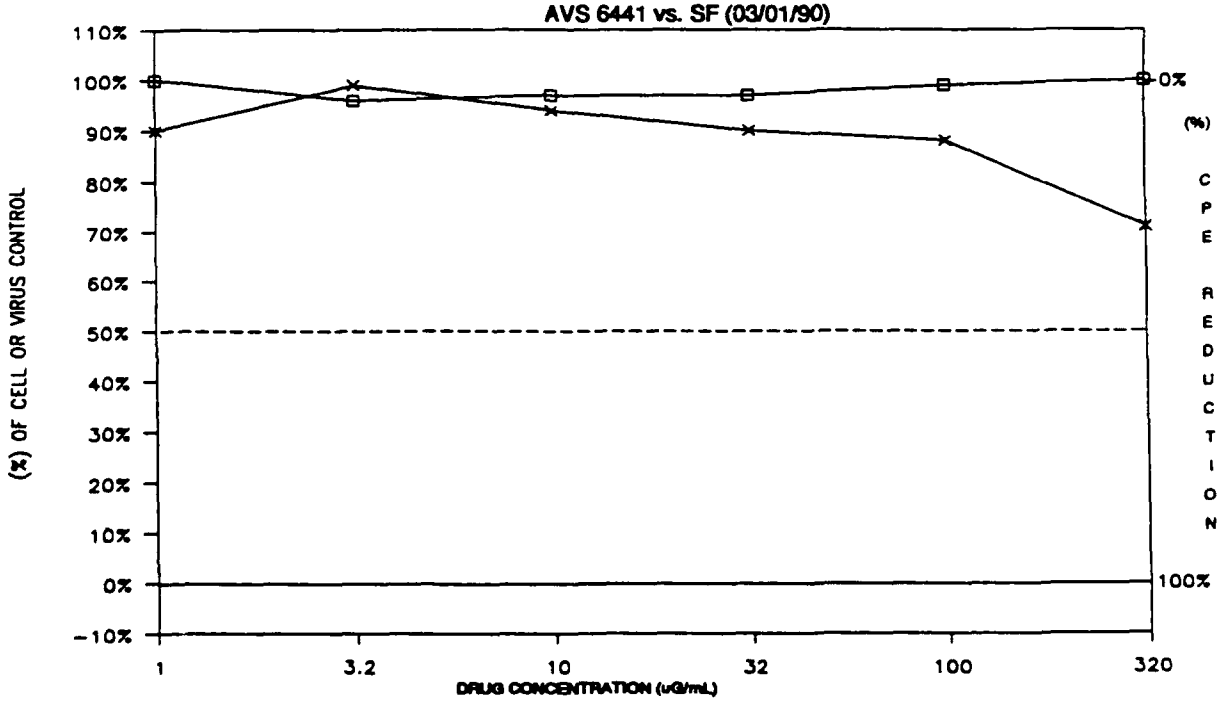
DRUG 6441	25%	50%	95%
TC (uG/mL)	268.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.001	100%	0.851	90%	-.008
C	3.2	0.032	96%	0.938	99%	-.007
D	10	0.023	97%	0.894	94%	-.011
E	32	0.024	97%	0.852	90%	-.010
F	100	0.006	99%	0.838	88%	-.011
high G *	320	-.094	100%	0.678	71%	-.014

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6441 vs. SF (03/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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PLATE UAC
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: 0.00 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.031	0.031	0.029	0.036	0.027	0.030	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		1.331					1.376	0.054	0.058	0.052	1.282	1.002
C		1.005					1.271	0.063	0.065	0.061	1.003	0.938
D		1.025					1.111	0.075	0.072	0.054	1.077	1.069
E		0.058					1.212	0.102	0.125	0.130	0.061	0.835
F		0.071					1.217	0.146	0.175	0.152	0.062	0.844
G		0.074					0.777	0.166	0.177	0.162	0.058	0.828
							drug 6441 colorimetric background					
H							0.033	0.035	0.036	0.036	0.036	0.037

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TEST DATE	03/02/90
STRN	TRINIDAD	DATE READ	03/06/90
REAGENT	0.031		
VIRUS CONTROL	0.033		
CELL CONTROL	1.090		
DIFFERENTIAL	1.057		

DRUG 6441	25%	50%	95%
TC (uG/mL)	276.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.015	100%	1.152	100%	0.006
C	3.2	-.006	100%	1.069	98%	0.005
D	10	-.002	100%	1.054	97%	0.005
E	32	0.050	95%	0.988	91%	0.005
F	100	0.090	91%	0.996	91%	0.004
high G *	320	0.102	90%	0.770	71%	0.002

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

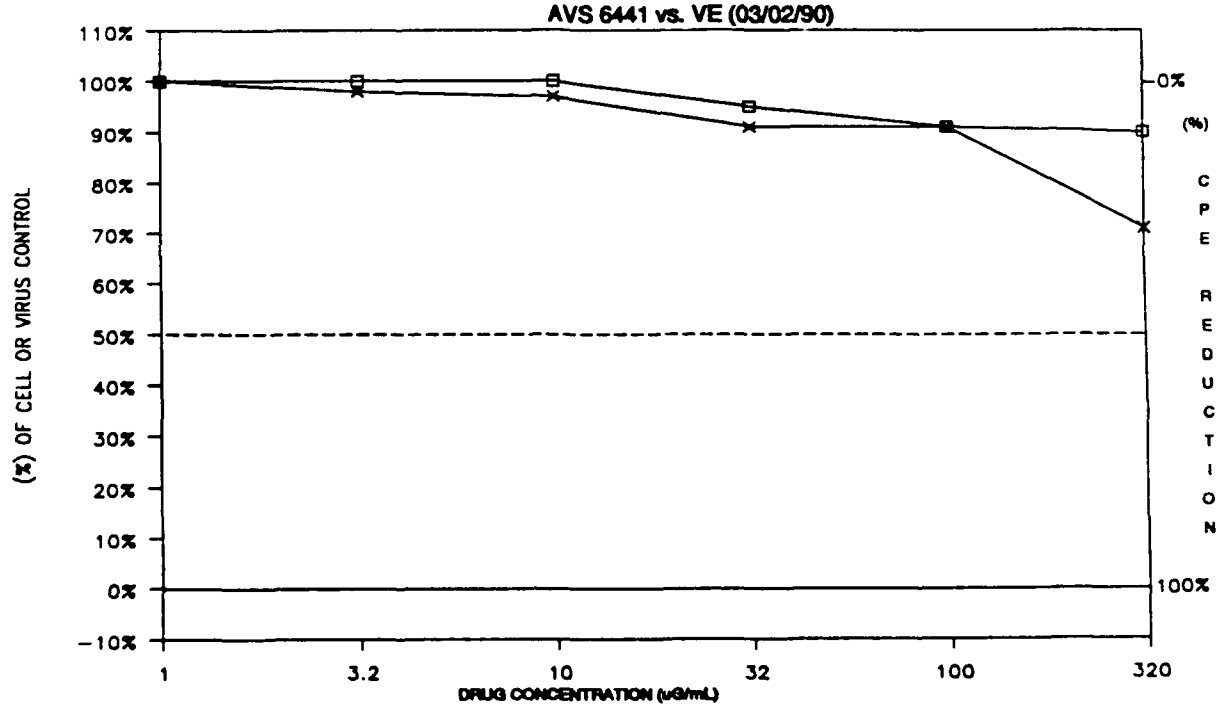

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PLATE 0SD
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: 0.00 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.103	0.104	0.106	0.106	0.107	0.117	0.000	0.000	0.000	0.000	0.000	0.000
	tox	cc/vc	drug 6441 experimental			tox					cc/vc	
B	1.438	1.668	0.274	0.185	0.271	1.655					1.646	
C	1.538	1.508	0.219	0.265	0.260	1.646					1.524	
D	1.430	1.443	0.287	0.209	0.240	1.683					1.462	
E	1.386	0.207	0.199	0.229	0.225	1.553					0.295	
F	1.459	0.242	0.325	0.260	0.336	1.491					0.277	
G	1.551	0.265	0.280	0.259	0.314	1.496					0.261	
	drug 6441 colorimetric background											
H	0.110	0.113	0.110	0.108	0.107	0.123						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV	PROJECT #	5975-4
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	63	TEST DATE	03/22/90
STRN	LEDCA	DATE READ	03/28/90
REAGENT	0.107		
VIRUS CONTROL	0.151		
CELL CONTROL	1.435		
DIFFERENTIAL	1.284		

DRUG 6441	25%	50%	95%
TC (uG/mL,	> 320.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.030	100%	1.423	99%	0.016
C	3.2	-.010	100%	1.485	100%	0.000
D	10	-.014	100%	1.448	100%	0.001
E	32	-.043	100%	1.359	95%	0.003
F	100	0.043	97%	1.362	95%	0.006
high G *	320	0.024	98%	1.413	98%	0.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

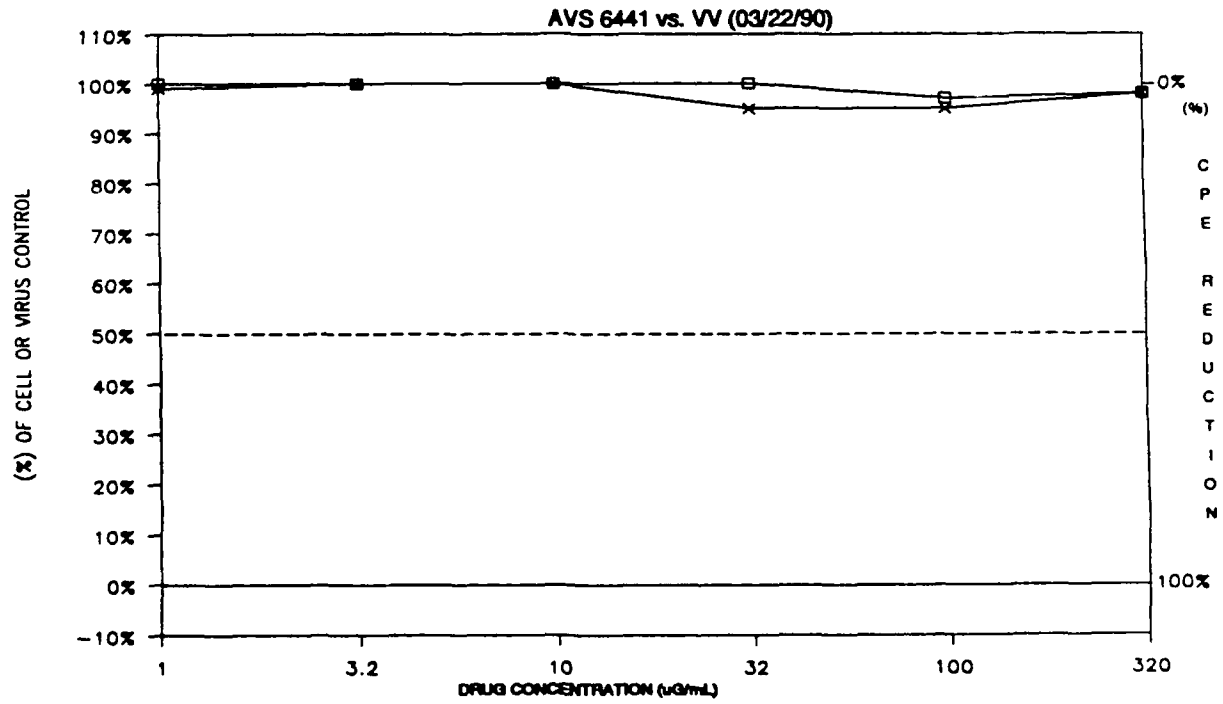

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PLATE UAP
DRUG 6441

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6441
TAI: >0.66 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.041	0.040	0.038	0.038	0.037	0.039	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6441 experimental			cc/vc	tox
B		0.949					0.875	0.295	0.205	0.355	0.888	0.899
C		0.928					0.831	0.201	0.258	0.191	0.940	0.955
D		0.811					0.933	0.294	0.202	0.228	0.971	0.970
E		0.189					1.085	0.189	0.240	0.239	0.215	0.875
F		0.214					0.824	0.289	0.207	0.311	0.310	0.862
G		0.230					0.662	0.137	0.166	0.161	0.216	0.836
							drug 6441 colorimetric background					
H							0.031	0.036	0.038	0.036	0.036	0.037

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	YF		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	63		
STRN	ASIBI		
REAGENT	0.039		
VIRUS CONTROL	0.190		
CELL CONTROL	0.876		
DIFFERENTIAL	0.686		

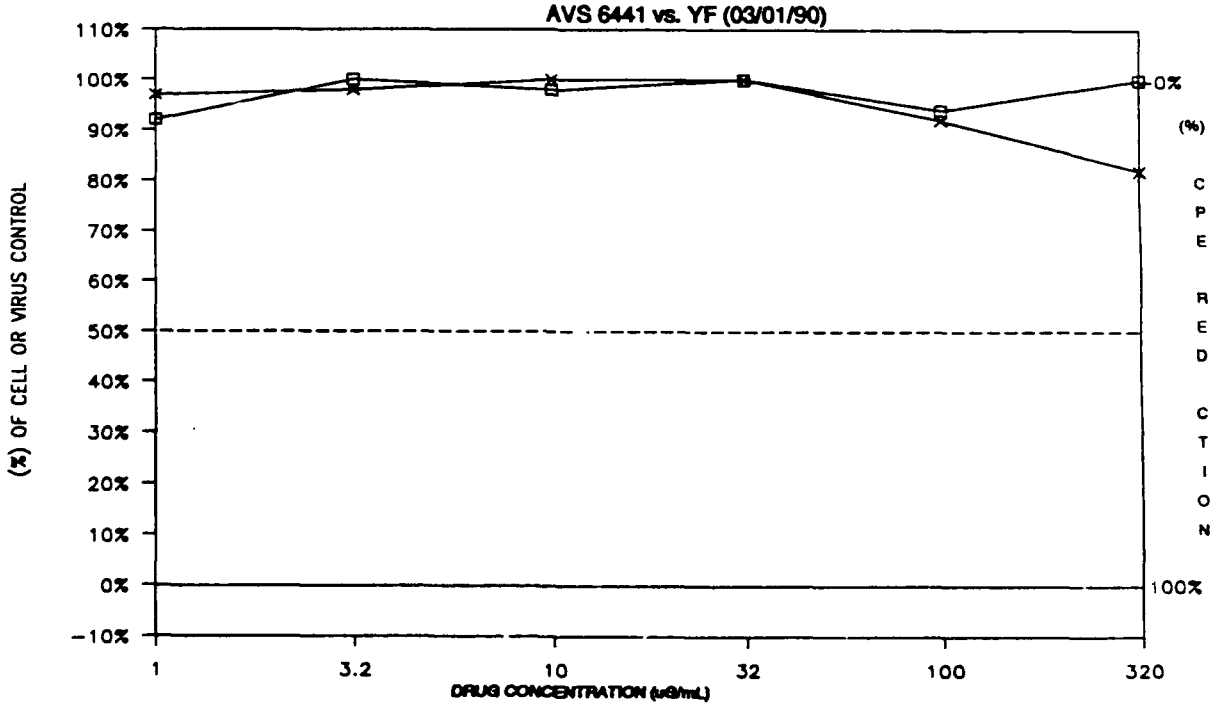
PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	03/01/90		
DATE READ	03/09/90		

DRUG 6441	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6441		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.058	92%	0.850	97%	-.002
C	3.2	-.009	100%	0.857	98%	-.003
D	10	0.015	98%	0.916	100%	-.003
E	32	-.005	100%	0.942	100%	-.001
F	100	0.043	94%	0.807	92%	-.003
high G *	320	-.066	100%	0.718	82%	-.008

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



PRINTED 03/15/90

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PLATE 1ZN
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: >2.30 SI: -----

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.129	0.124	0.124	0.125	0.126	0.127	0.037	0.036	0.036	0.036	0.038	0.035
		cc/vc					tox	drug 6950 experimental			cc/vc	tox
B		1.611					1.642	0.417	0.409	0.390	1.565	1.552
C		1.526					1.575	0.434	0.418	0.463	1.564	1.410
D		1.577					1.576	0.428	0.410	0.501	1.555	1.414
E		0.345					1.621	0.410	0.419	0.432	0.374	1.392
F		0.381					1.565	0.425	0.431	0.427	0.365	1.454
G		0.355					1.562	0.436	0.448	0.478	0.345	1.478
							drug 6950 colorimetric background					
H							0.122	0.124	0.125	0.127	0.124	0.126

tox=cell toxicity cc=cell control vc=virus control **BOLD** = highest drug conc values shown are optical densities

VIRUS	HIV3B		
CELLS	MT2	Satisfactory	
SHIPMENT NUMBER	68		
STRN	2.5		
REAGENT	0.126		
VIRUS CONTROL	0.235		
CELL CONTROL	1.441		
DIFFERENTIAL	1.206		

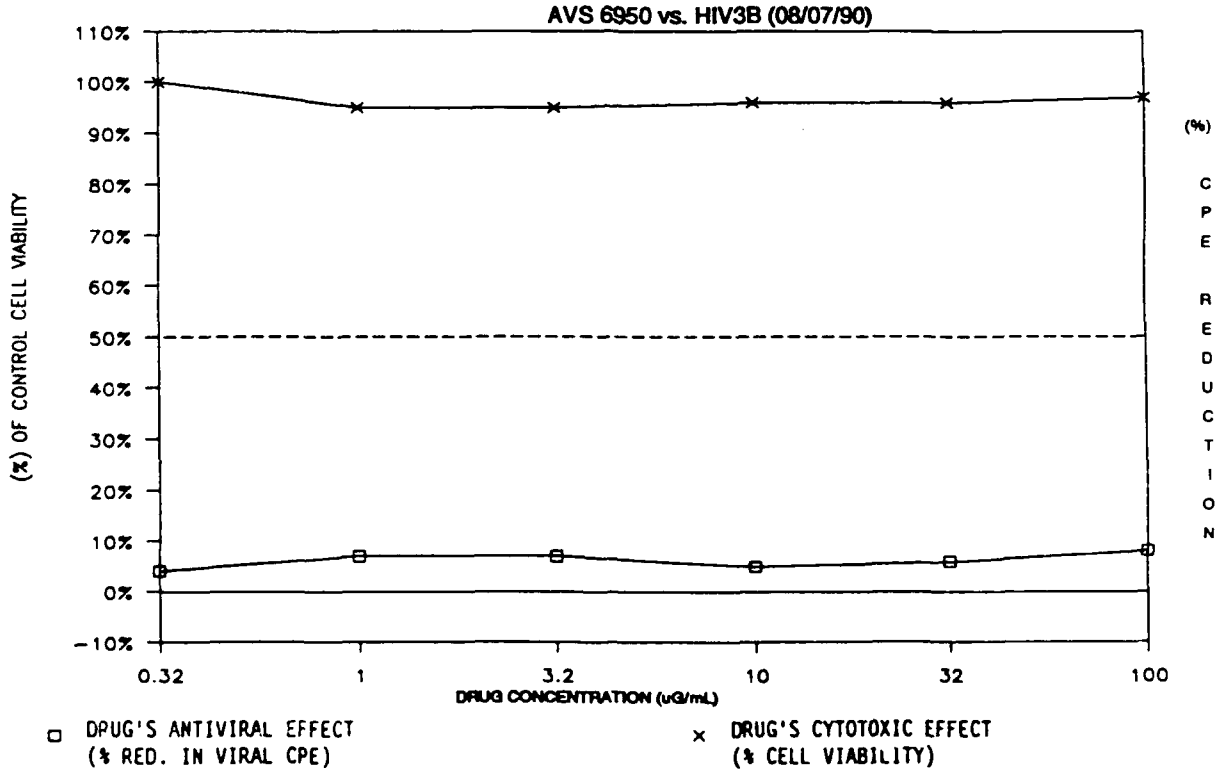
PROJECT #	6520-2		
SPONSOR	USAMRIID		
TEST DATE	08/07/90		
DATE READ	08/15/90		

DRUG 6950	25%	50%	95%
TC (uG/mL)	> 100.00	> 100.00	> 100.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	0.044	4%	1.471	100%	0.000
C	1	0.080	7%	1.369	95%	-.002
D	3.2	0.084	7%	1.368	95%	0.001
E	10	0.061	5%	1.382	96%	-.001
F	32	0.069	6%	1.386	96%	-.002
high G *	100	0.097	8%	1.398	97%	-.004

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



PRINTED 08/17/90

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PLATE XVV
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.076	0.094	0.098	0.100	0.095	0.070	0.001	0.002	0.001	0.002	0.001	0.002
		cc/vc					tox	drug 6950 experimental			cc/vc	tox
B		1.274					0.939	0.347	0.365	0.353	1.170	1.169
C		1.227					1.159	0.344	0.347	0.358	1.133	1.197
D		1.195					1.181	0.345	0.341	0.335	1.112	1.153
E		0.380					1.112	0.339	0.341	0.353	0.377	1.143
F		0.375					1.045	0.344	0.352	0.362	0.394	1.053
G		0.355					0.971	0.304	0.306	0.310	0.375	1.087
							drug 6950 colorimetric background					
H							0.069	0.079	0.077	0.087	0.087	0.074

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	JE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	08/01/90
STRN	NAKAYAMA	DATE READ	08/07/90
REAGENT	0.089		
VIRUS CONTROL	0.287		
CELL CONTROL	1.096		
DIFFERENTIAL	0.809		

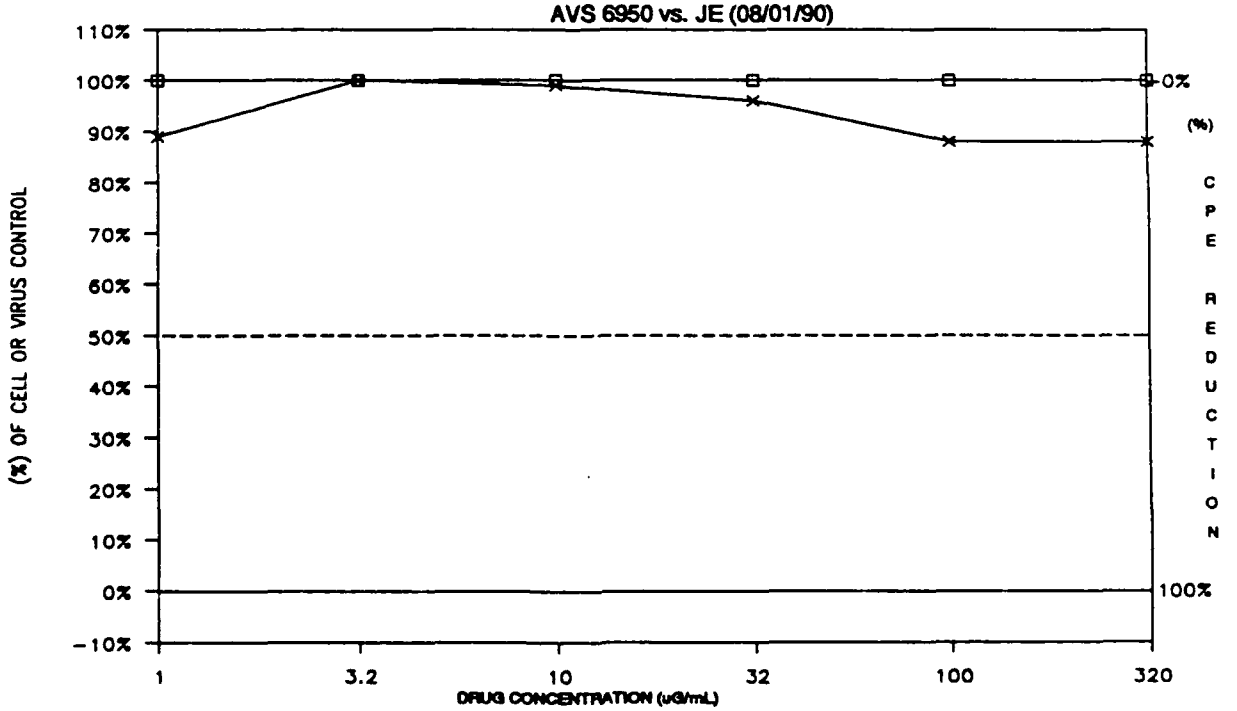
DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.006	100%	0.980	89%	-.015
C	3.2	-.024	100%	1.091	100%	-.002
D	10	-.034	100%	1.080	99%	-.002
E	32	-.020	100%	1.051	96%	-.012
F	100	-.013	100%	0.970	88%	-.010
high G *	320	-.049	100%	0.960	88%	-.020

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6950 vs. JE (08/01/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE X08
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: 0.00 SI: ——

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.057	0.056	0.051	0.050	0.048	0.046	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vc					tox	drug 6950 experimental			cc/vc	tox
B		1.330					1.293	0.690	0.675	0.720	1.107	1.398
C		1.337					1.417	0.740	0.692	0.707	1.273	1.262
D		1.326					1.446	0.786	0.706	0.714	1.388	1.424
E		0.795					1.471	0.685	0.689	0.660	0.729	1.408
F		0.852					1.447	0.639	0.613	0.654	0.807	1.271
G		0.973					1.231	0.447	0.412	0.428	0.825	1.405
							drug 6950 colorimetric background					
H							0.048	0.057	0.057	0.059	0.061	0.059

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	PT		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ADAMES		
REAGENT	0.051		
VIRUS CONTROL	0.779		
CELL CONTROL	1.242		
DIFFERENTIAL	0.463		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/19/90		
DATE READ	06/26/90		

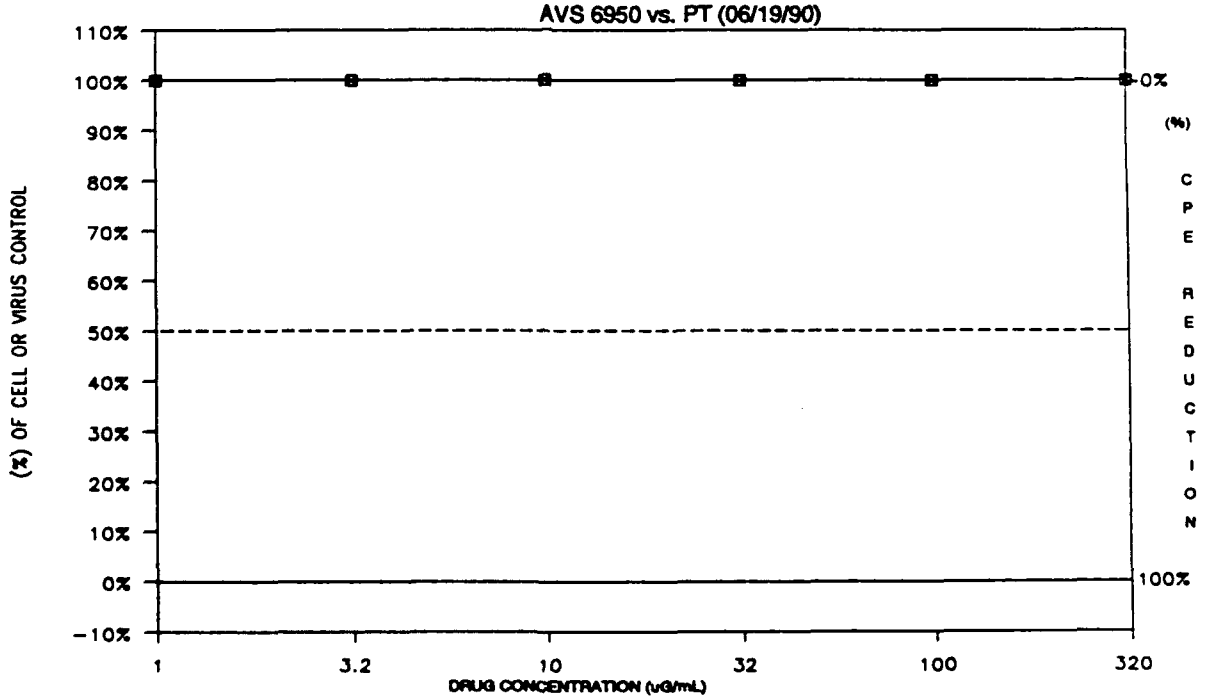
DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	——	——	——
ANTIVIRAL INDEX (AI)	——	——	——

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.143	100%	1.286	100%	0.008
C	3.2	-.127	100%	1.278	100%	0.010
D	10	-.103	100%	1.376	100%	0.008
E	32	-.158	100%	1.382	100%	0.006
F	100	-.201	100%	1.302	100%	0.006
high G *	320	-.398	100%	1.270	100%	-.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6950 vs. PT (06/19/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PRINTED 07/05/90

SOUTHERN RESEARCH INSTITUTE

PLATE XON
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.042	0.047	0.044	0.044	0.044	0.045	0.001	0.001	0.001	0.001	0.001	0.000
		co/vc					tox	drug 6950 experimental			co/vc	tox
B		1.171					1.177	0.396	0.479	0.500	1.240	1.186
C		1.372					1.348	0.391	0.344	0.448	1.314	1.202
D		1.288					1.269	0.368	0.349	0.388	1.285	1.107
E		0.480					1.277	0.325	0.350	0.333	0.431	1.125
F		0.485					1.218	0.285	0.262	0.344	0.440	1.089
G		0.434					0.979	0.217	0.261	0.194	0.419	0.970
							drug 6950 colorimetric background					
H							0.037	0.042	0.044	0.043	0.043	0.044

tox=cell toxicity co=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	SF		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	SCILIAN		
REAGENT	0.044		
VIRUS CONTROL	0.404		
CELL CONTROL	1.234		
DIFFERENTIAL	0.830		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/19/90		
DATE READ	06/26/90		

DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	------	------	------
ANTIVIRAL INDEX (AI)	------	------	------

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.010	99%	1.137	92%	0.000
C	3.2	-.053	100%	1.232	100%	-.001
D	10	-.079	100%	1.145	93%	-.001
E	32	-.112	100%	1.157	94%	0.000
F	100	-.149	100%	1.111	90%	-.002
high G *	320	-.217	100%	0.937	76%	-.007

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

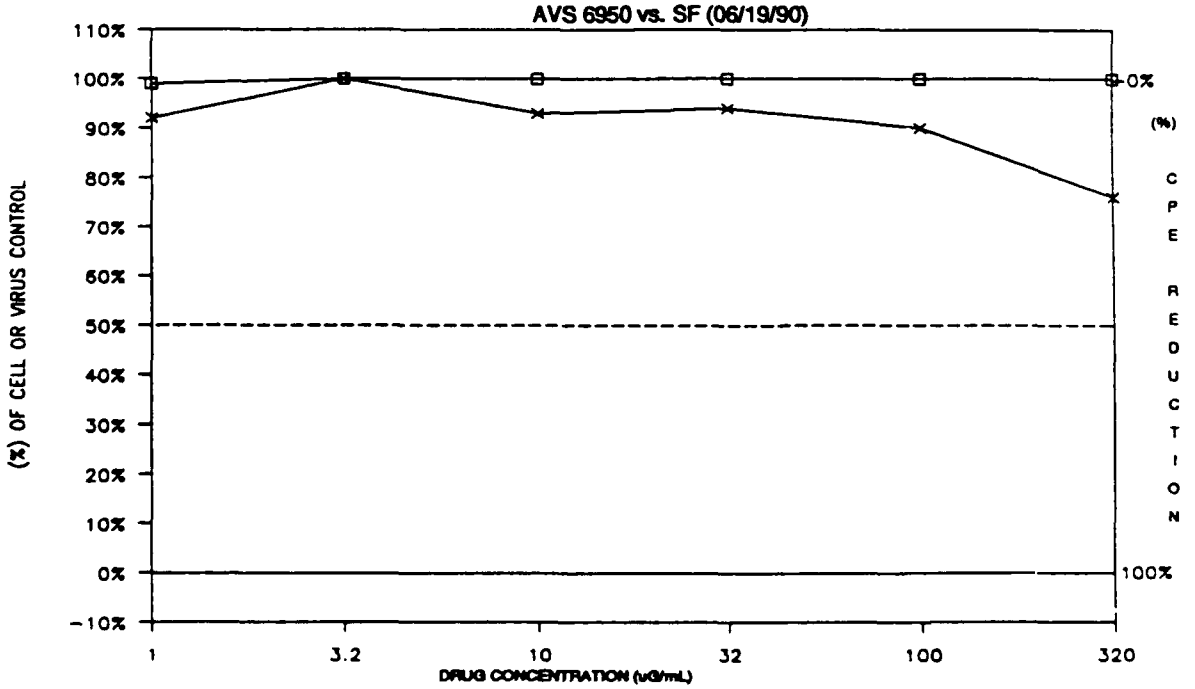


PLATE WX5
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.067	0.063	0.060	0.060	0.059	0.060	0.001	0.001	0.001	0.001	0.001	0.001
		cc/vo					tox	drug 6950 experimental			cc/vo	tox
B		1.565					1.385	0.102	0.094	0.095	1.231	1.380
C		1.539					1.390	0.112	0.115	0.120	1.109	1.287
D		1.457					1.412	0.108	0.096	0.117	1.414	1.146
E		0.113					1.336	0.101	0.108	0.154	0.125	1.360
F		0.130					1.319	0.098	0.123	0.170	0.120	1.390
G		0.122					1.399	0.076	0.079	0.076	0.141	1.351
							drug 6950 colorimetric background					
H							0.052	0.054	0.057	0.062	0.062	0.063

tox=cell toxicity cc=cell control vo=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VE	PROJECT #	5975-1
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	06/22/90
STRN	TRINIDAD	DATE READ	06/26/90
REAGENT	0.062		
VIRUS CONTROL	0.064		
CELL CONTROL	1.324		
DIFFERENTIAL	1.261		

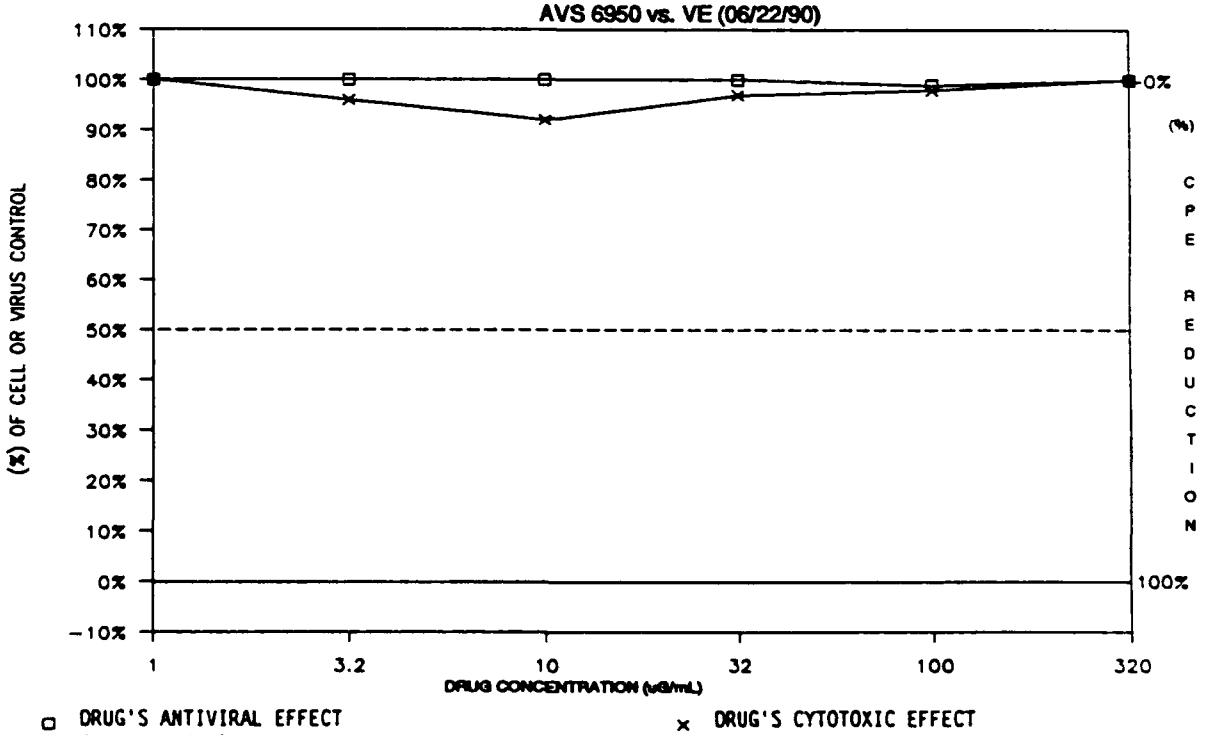
DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.030	100%	1.319	100%	0.002
C	3.2	-.010	100%	1.276	96%	0.001
D	10	-.019	100%	1.217	92%	0.001
E	32	0.000	100%	1.291	97%	-.004
F	100	0.013	99%	1.301	98%	-.008
high G *	320	-.038	100%	1.324	100%	-.010

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6950 vs. VE (06/22/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE 0ZM
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: 0.00 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.107	0.108	0.099	0.110	0.105	0.110	0.000	0.000	0.000	0.000	0.000	0.000
	tox	cc/vc	drug 6950 experimental			tox					cc/vc	
B	1.488	1.577	0.311	0.209	0.272	1.756					1.580	
C	1.504	1.583	0.263	0.277	0.303	1.865					1.685	
D	1.524	1.647	0.292	0.252	0.315	1.799					1.636	
E	1.459	0.283	0.280	0.263	0.259	1.769					0.488	
F	1.436	0.391	0.271	0.261	0.257	1.774					0.283	
G	1.365	0.375	0.376	0.264	0.302	1.494					0.291	
	drug 6950 colorimetric background											
H	0.107	0.113	0.110	0.108	0.111	0.113						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	VV	PROJECT #	5975-4
CELLS	VERO Satisfactory	SPONSOR	USAMRIID
SHIPMENT NUMBER	68	TEST DATE	07/12/90
STRN	LEDCA	DATE READ	07/18/90
REAGENT	0.107		
VIRUS CONTROL	0.245		
CELL CONTROL	1.512		
DIFFERENTIAL	1.266		

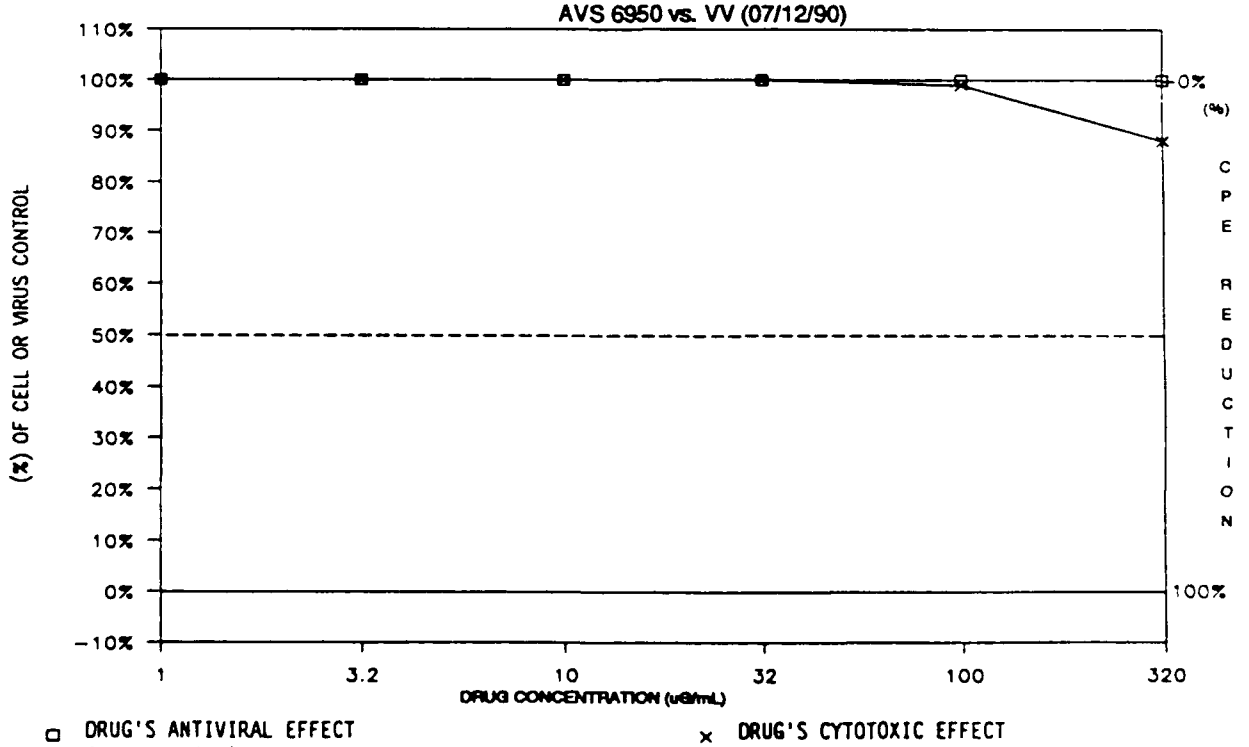
DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	-----	-----	-----
ANTIVIRAL INDEX (AI)	-----	-----	-----

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	-.095	100%	1.509	100%	0.007
C	3.2	-.076	100%	1.573	100%	0.005
D	10	-.067	100%	1.554	100%	0.001
E	32	-.089	100%	1.504	100%	0.004
F	100	-.096	100%	1.492	99%	0.007
high G *	320	-.038	100%	1.323	88%	0.000

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6950 vs. VV (07/12/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

PLATE WXZ
DRUG 6950

IN VITRO ANTIVIRAL RESULTS
MTT ASSAY

DRUG: AVS 6950
TAI: >0.20 SI: ———

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.083	0.072	0.084	0.073	0.070	0.073	0.002	0.002	0.002	0.002	0.002	0.002
		cc/vc					tox	drug 6950 experimental			cc/vc	tox
B		1.327					1.271	0.217	0.212	0.205	1.251	1.293
C		1.269					1.370	0.201	0.190	0.215	1.121	1.221
D		1.136					1.277	0.204	0.208	0.195	1.048	1.141
E		0.214					1.336	0.187	0.188	0.196	0.190	1.183
F		0.204					1.203	0.194	0.189	0.166	0.229	1.143
G		0.221					1.356	0.211	0.207	0.185	0.207	1.270
							drug 6950 colorimetric background					
H							0.055	0.059	0.061	0.064	0.071	0.063

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	YF		
CELLS	VERO	Satisfactory	
SHIPMENT NUMBER	68		
STRN	ASIBI		
REAGENT	0.076		
VIRUS CONTROL	0.135		
CELL CONTROL	1.116		
DIFFERENTIAL	0.981		

PROJECT #	5975-1		
SPONSOR	USAMRIID		
TEST DATE	06/20/90		
DATE READ	06/26/90		

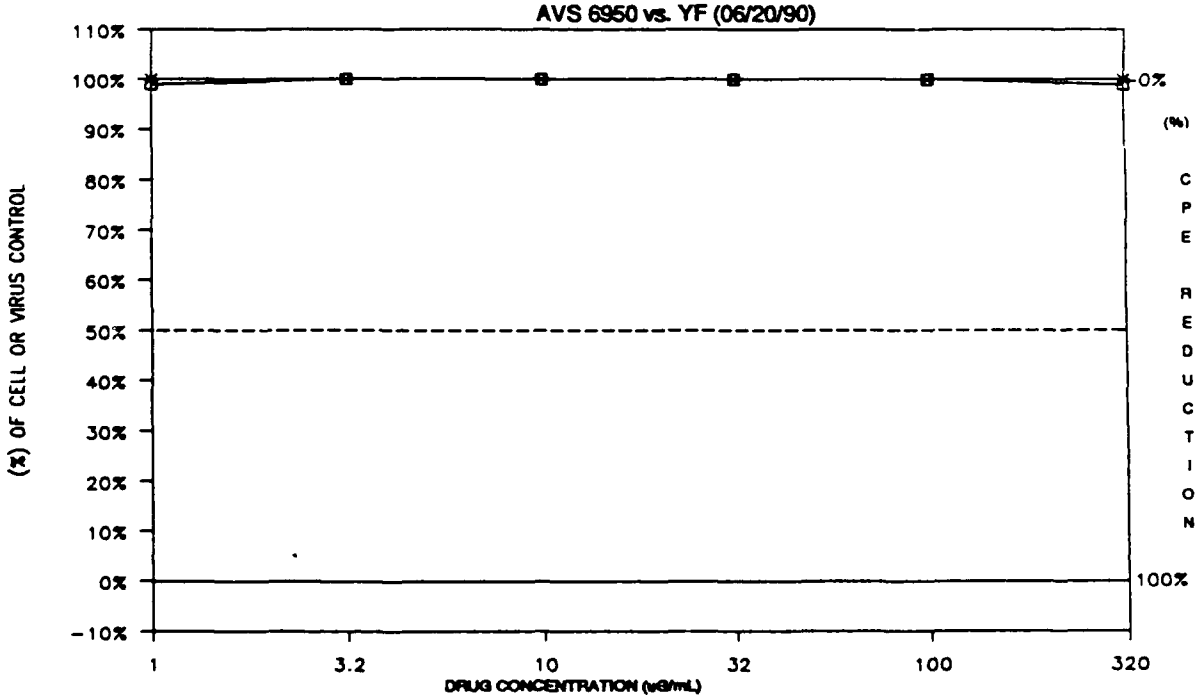
DRUG 6950	25%	50%	95%
TC (uG/mL)	> 320.00	> 320.00	> 320.00
IC (uG/mL)	———	———	———
ANTIVIRAL INDEX (AI)	———	———	———

DRUG 6950		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	1	0.013	99%	1.219	100%	-.013
C	3.2	-.004	100%	1.225	100%	-.005
D	10	0.003	100%	1.145	100%	-.012
E	32	-.006	100%	1.199	100%	-.015
F	100	-.011	100%	1.114	100%	-.017
high G *	320	0.011	99%	1.258	100%	-.021

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH

AVS 6950 vs. YF (06/20/90)



□ DRUG'S ANTIVIRAL EFFECT (% VIRAL CPE)

× DRUG'S CYTOTOXIC EFFECT (% CELL VIABILITY)

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