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TITLE: SUICIDE INHIBITORS OF REVERSE TRANSCRIPTASE IN THE THERAPY OF AIDS AND OTHER RETROVIRUSES

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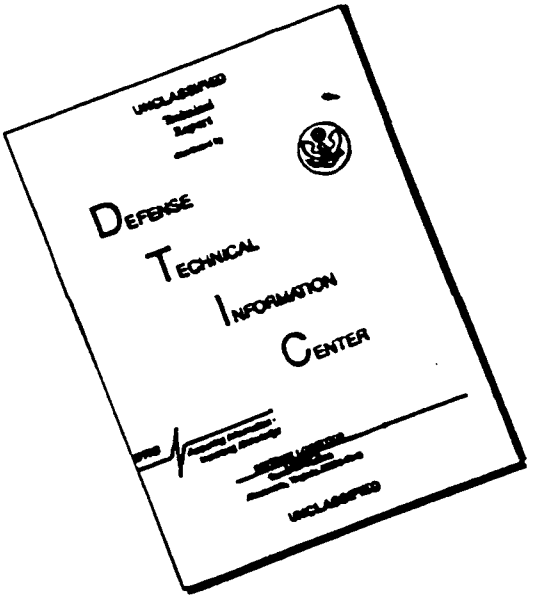


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A comprehensive study of the template and substrate dependence for inhibition of reverse transcriptase by Foscarnet has been completed and published as detailed in this report.

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## PROGRESS REPORT - SUMMARY

### Assay Procedures for Reverse Transcriptase

A rapid assay procedure for the enzyme has been developed based upon dot blotting acid washing using DE filter paper dises. Experimental parameters in the procedure have been carefully defined and controlled so that the assay is of sufficient precision for enzyme kinetics. The procedure is also used as a routine assay of reverse transcriptase in virus supernatants thus providing a quantitative measure of virus replication in tissue culture.

An comprehensive study of the template and substrate dependence for inhibition of reverse transcriptase by Foscarnet has been completed and published as detailed in this report.

### Characterization of HIV Reverse Transcriptase

The Pol gene for the HIV codes for the p11 protease, ribonuclease H and endonuclease (integrase) in addition to the reverse transcriptase so that it had heretofore been difficult to assign highly conserved amino acids to any particular active site. The HIV vaccinia construct VC21, however, grown in cultured cells, produces a 65 KD protein which has only RT activity. The active site appears to contain a lyssine residue present in a highly conserved decapeptide region of the protein. Dr. Lightfoote has purified the HIV-RT from culture fluids and is using it to characterize inhibition of the enzyme by potential antiviral drugs. A particularly interesting recent development has been the production of a variant enzyme which is approximately 600 fold more sensitive to inhibition by the drug Foscarnet than the wild type enzyme. The structural changes underlying this increased sensitivity are being determined.

### Synthesis and Testing of Sterol Phosphonoformates

An Arbuzov type reaction has been developed by Dr. Nelson as a successful route to the cholesterol phosphonoformates. In this reaction, appropriate alkyl and mixed alkyl/aryl triphosphates are condensed with cholesterol chloroformate to yield the desired cholesterol phosphonoformates. These compounds are designed to improve the penetration of the phosphonoformate moiety and thus increase the efficiency of delivery of Foscarnet to cellular sites of viral replication. Dr. Lightfoote has shown that several of these compounds have antiviral activity superior to that of PFA. Further testing to determine if the therapeutic index has been improved are underway using cultured human T lymphocytes.

### Synthesis of Nucleoside Riboxetanes and Riboepoxides

Several routes to the synthesis of the undiscovered thymidine and uridine riboxetanes have been explored. Synthesis of the previously reported adenosine riboepoxide has been accomplished. Several 2, 2' oxtanes have been synthesized and the cytidine derivative has some antiviral activity. Synthesis of the 2', 3' oxetanes has proved more

difficult since the intermediate steps essentially involve the synthesis of undiscovered branched chain sugar derivatives. As detailed in the report, a new procedure for alkylation at C 2' has recently been successful so that cyclization to the 2', 3' oxetanes can now be anticipated. Experiments to synthesize these compounds via 2 + 2 photoaddition of aldehydes and ketones to 2', 3' deoxythymididine have also been initiated by Dr. Mook.

Synthesis of Nucleoside Oxaphospholenes, Dioxaphospholes and Oxaphosphorins

This project has been undertaken by Dr. Robert Mook who joined the group in November 1987. His approach as detailed in the report has focused on a convergent synthesis of the 5 and 6 membered phosphorus heterocycles followed by coupling to the appropriate ribonucleoside moiety. The 6 membered hetrocycles have proved to be more stable than the 5 membered compounds under physiological conditions. Enzyme kinetic analysis against the HIV transcriptase is being initiated and cytotoxicity tests in cultured lymphocytes will be carried out prior to the antiviral screening.

A. Introduction

The most recent evaluation of the AIDS epidemic in the U.S. indicates that the current total of approximately 50,000 AIDS cases in accompanied by a further 1 to 1.5 million infected individuals, the majority of whom will progress to the clinically overt disease states in the next 5-7 years. In the near-term absence of an effective vaccine an increase in this infected/symptomless category is likely to continue for a number of years.

In addition to an antiviral therapy for acute cases of AIDS therefore, there is clearly pressing need for an effective antiviral drug regimen suitable for administration to large numbers of otherwise healthy individuals in order to arrest progression of the disease. By preventing viral replication, such therapy may also reduce contagiousness and thus indirectly fulfill some of the immediate functions of a vaccine.

The drugs being developed in this project are designed with the above requirement for long-term therapy and low toxicity as a primary goal.

Retroviruses are characterized by the anomalous storage of their genetic information in the form of RNA. They are a diverse group of organisms which have been shown to be causative agents in a number of mammalian and avian disease states (1-3). Lentiviruses are a subfamily of retroviruses which have been linked to the induction of arthritis, encephalitis, and slow neurological diseases in certain species. Many of these viruses are characterized by their ability to develop novel antigenic variants that can escape temporarily from host immune surveillance (5,6). Interest in retroviruses in human disease states has increased with the isolation of HTLV I and II as the causative agents of adult human T cell leukemias and of HTLV III/HIV as the causative agent of Acquired Immune Deficiency Syndrome or AIDS (7,8). There is also recent evidence of a related retrovirus as the cause of multiple sclerosis (9).

Replication of the retroviruses is critically dependent upon a single enzyme called reverse transcriptase (RT). This is an RNA-dependent DNA polymerase first found in the purified virions of Rous sarcoma virus by Temin (10) and murine leukemia virus by Baltimore (11). The enzyme transcribes the viral RNA into DNA in the first step of viral replication. Since host cells do not contain reverse transcriptase, two molecules of the enzyme are packaged in each virion and enter the cell together with the viral RNA. It is at this stage that the viral replicative process is likely to be most sensitive to inhibitors of the reverse transcriptase, before amplification of the viral genome and pol gene directed synthesis of endogenous RT has taken place.

The principle that inhibitors of reverse transcriptase will inhibit replication of retroviruses is well established. For example, 3'-azido-3'deoxythymidine, the triphosphate of which inhibits the RT activity of HTLV III/HIV, is a potent inhibitor of virus replication in cultured H-9 cells in the range of 1-10 micromolar (14) and is being used successfully in patients with AIDS. Prolonged administration may cause serious side effects. Another agent which has shown promise is phosphonoformate (PFA) which inhibits the RT activity of HTLV III/HIV with an $I_{50}$ of only 0.1 micromolar. However, much higher concentrations of up to 340 micromolar are required for complete inhibition of HTLV III/HIV replication in H-9 cells (15). Other drugs including the dideoxycytidines

which are also based upon inhibition of RT by chain termination of the viral template are in clinical trial. Since none of these drugs permanently inactivate the reverse transcriptase and since they do not accumulate intracellularly in significant amounts, virus replication will resume when blood levels of the drugs decrease.

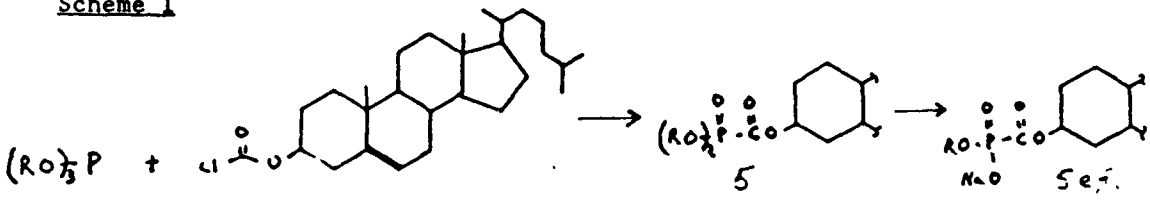
This project represents a collaborative effort between groups of investigators with expertise in virology, cell biology, enzymology, drug design and organic synthesis, to develop new types of antiviral drugs. Novel anti-HIV drugs are being developed based upon the principles of (i) compounds designed to accumulate intracellularly at the sites of viral replication (ii) drugs designed as suicide inhibitors of the viral reverse transcriptase (iii) synergistic combination drugs designed to reduce side effects during long-term therapy.

B. i) Progress in synthesis and Design of Replicationsite Directed Inhibitors: The Sterol Phosphonoformates

I. Cholesteryloxycarbonylphosphonoformates

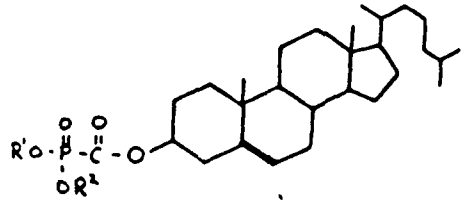
A series of mono- and di-alkyl cholesteryloxycarbonylphosphonoformates have been successfully prepared via an Arbozov reaction[12] (Scheme I) between trialkylphosphites and cholesterylchloroformate. Details of these procedures are given in the appendix.

Scheme I



Triesters of type 5 gave acceptable spectroscopic characteristics (Figure 2) and were selectively hydrolyzed[12] to the mono- (5e) and di-sodium salts (5f).

Table 1

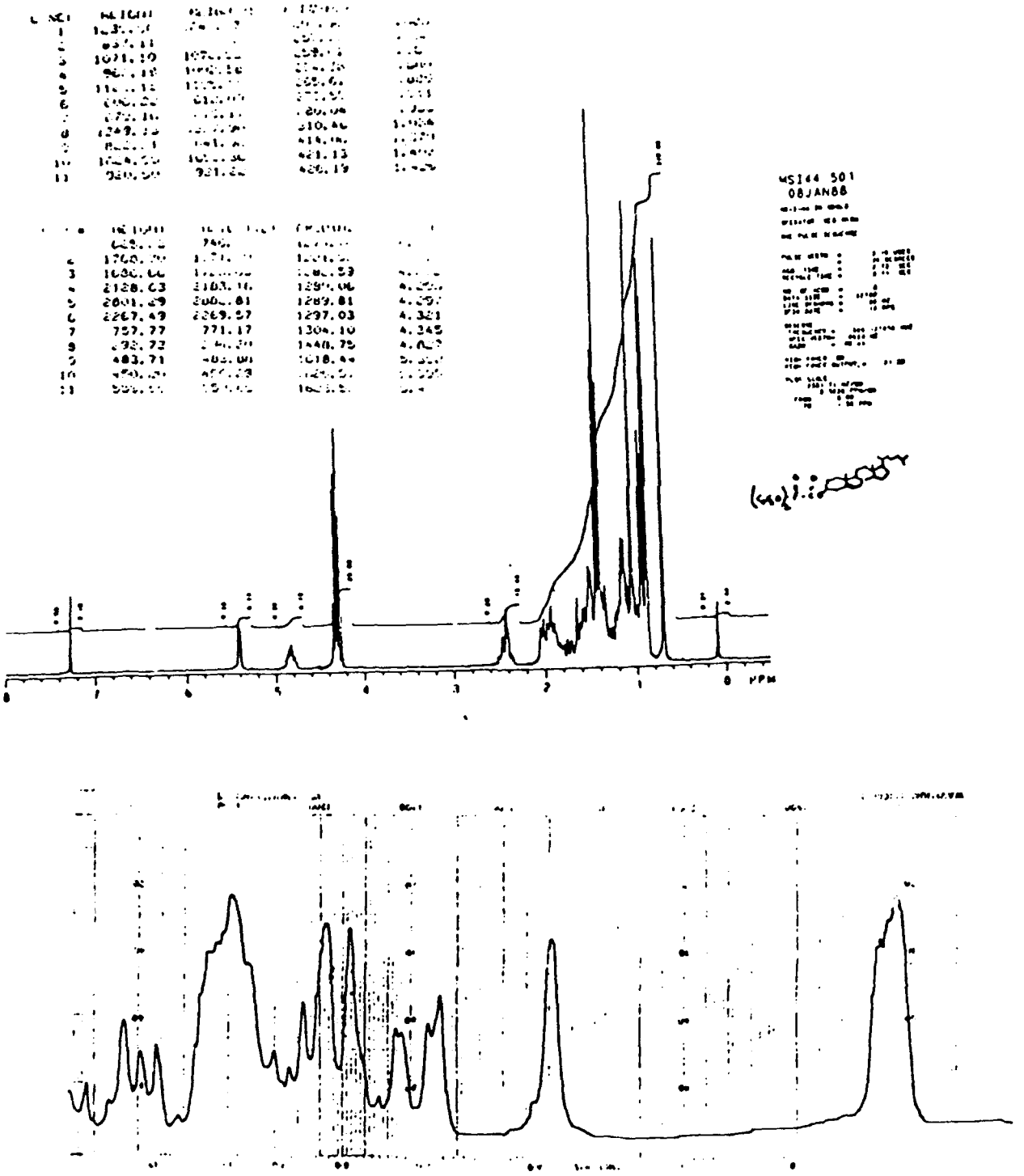


| Cmpd, 5 | $R^1$ | $R^2$ | MP (C°) | $R_f$ |
|---|---|---|---|---|
| a | $C_2H_5$ | $C_2H_5$ | 110-111 | 0.60 (A)[O] |
| b | $CH_2CH_2CH(CH_3)_2$ | $CH_2CH_2CH(CH_3)_2$ | 67-69 | 0.51 (B) |
| c | $CH_2CHO(CH_3)_2OCH_2$ | $CH_2CHO(CH_3)OCH_2$ | 106-108 | 0.30 (A) |
| d | $CH_2CH_2CH_2CO_2C_2H_5$ | $CH_2CH_2CH_2CO_2C_2H_5$ | oil | 0.33 (A) |
| e | $C_2H_5$ | Na | ≥220 | 0.29 (C) |
| f | Na | Na | ≥300 | - |

[O]Solvent systems: A, 50% ethyl acetate/hexanes, B, 20% ethyl acetate/hexanes, C, 52/14/1/1 $CHCl_3/CH_3OH$/con $NH_4OH/H_2O$.

A number of compounds of this type having both aromatic and aliphatic substituents have been tested for antiviral activity in vitro. As shown in Figure 3, incorporation of the sterol moiety in an appropriate triester environment can result in compounds having greater antiviral activity than the parent compound PFA. The mechanism by which this occurs has not yet

Figure 2



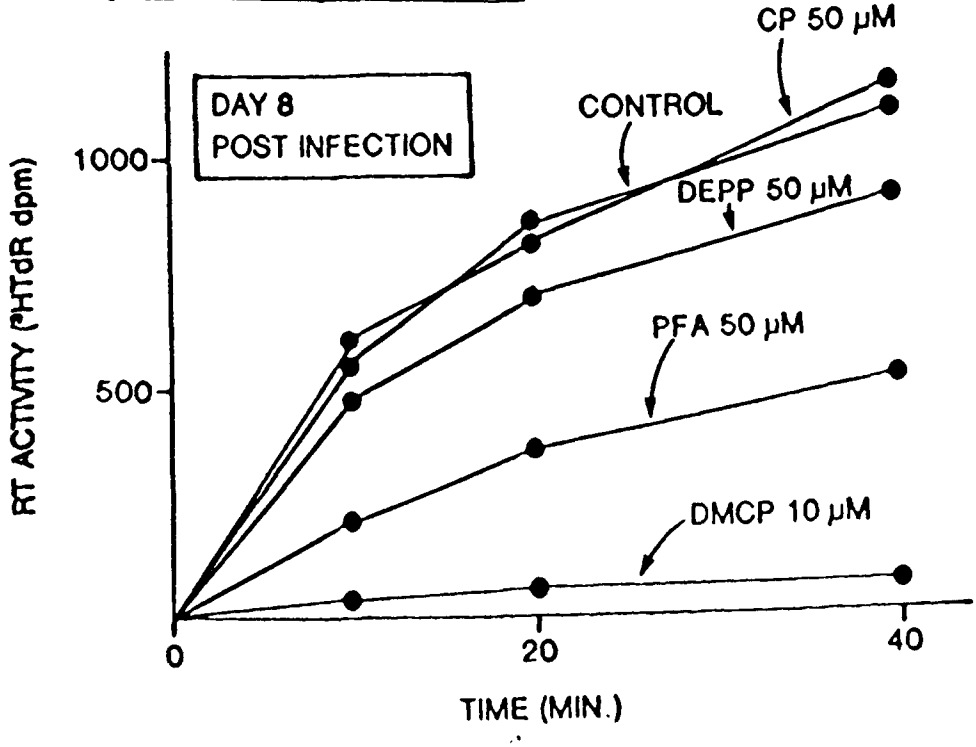
been shown to be due to increased intracellular accumulation, radiolabelled analogs which will be used to determine this will be synthesized in the next project period as described below. The possibility that these analogs prevent viral penetration must also be considered since the $I_{50}$'s appear to vary with the multiplicity of virus/cell infection. Table 1 lists the effect of increasing the number and size of the R groups on the melting point and chromatographic mobilities of some of these derivatives.

ii) Testing Antiviral Activity of Sterol Phosphonoformates

Despite the sensitivity of the HIV-RT to inhibition by PFA, replication of the virus in tissue culture is relatively insensitive, the $I_{50}$ being of the order of 100 $\mu$M.

We have shown in previous studies (21), by use of sterol ester analogs such as the sterically hindered any hydrolysis resistant cholesterol $\alpha\alpha'$ methyl ethyl caproate (CMEC), that cholesterol esters enter cells intact via a specific endocytotic transport process. In an attempt therefore to enhance deliverability of the active PFA moiety to the endosomal sites of viral replication, a series of cholesteryl phosphonoformate ester analogs of PHA were synthesized and characterized as described above. Pending development of the low biohazard CPE assay for HIV infectivity described in Section D, the antiviral potency of the drugs has been evaluated against Equine Infectious Anemia Virus (EIAV) growing in fetal equine fibroblast. The assay consisted of measurements of RT activity on pelletized supernatants of infected cultures 8 days following infection. The sterol esters of phosphonoformate which we have synthesized show and interesting pattern of antiviral activity in this system.

Figure 3. Inhibition of Equine Infectious Anemia Virus (EIAV) Replication by Sterol Phosphonoformates



The approximate $I_{50}$ for inhibition of EIAV replication by PFA is between 40 and 50 $\mu$m. The diethyl-phenyl-phosphonoformate ester (DEPP) is only about 20 % as active as PFA and the doubly charged disodium cholesterol phosphonoformate (CP) is essentially inactive. Substitution of the phenyl group with a cholesterol group, however, results in a enhancement of activity. For example, the closely related DMCP (lower curve, Figure 3) in which cholesterol is esterified to the formate moiety, has several times more anti-viral activity than the parent compound PFA.

The sterol phosphonoformates display another useful property since the antiviral effects persist for a considerable time (up to 8 days) after removal of the drug from the culture medium. It seems possible that this prolonged antiviral protection could be due to intracellular accumulation of the analog followed by slow hydrolysis and sustained release of the active PFA moiety. One of the future objectives in this study is to explore this observation in more depth by measuring rates of intracellular accumulation and hydrolysis of sterol phosphonoformated using $^{14}C$-labelled compounds. In this way ligands which enhance uptake can be distinguished from those which enhance (or reduce) hydrolysis.

iii) Kinetic Analysis of PFA Inhibition of Retroviral Reverse Transcriptases: Significance for Combination Therapy

Because of reports that PFA differentially inhibited incorporation only of certain nucleotide triphosphates into the cDNA (35) and in view of the substantial effort invested in the STEROL PHOSPHONOFORMATES we have carried out an extensive kinetic characterization of the interaction between reverse transcriptase and the inhibitor PFA.

Pending availability of the HIV-RT which we are currently purifying from the a VCF20 vaccinia virus HIV pol-gene construct, the reverse transcriptase from Moloney Murine Leukemia Virus (MMuLV-RT) and Avian Myeloblastosis Virus (AMV-RT) two retroviruses having marked homologies with the HIV-RT (16, 28) were used in these initial studies.

By use of homologous template-primer combinations with the appropriate $^{3}H$ labelled nucleotide triphosphate substrate, it was confirmed that RT is remarkable sensitive to inhibition by phosphonoformate, but only when incorporating thymidine into the primary DNA strand. As shown in Figure 4, the $I_{50}$ for PFA inhibition of AMV-reverse transcriptase incorporating dTTP using a poly dCdG template (corresponding to second strand virus DNA synthesis) is 125 $\mu$M.

A similar insensitivity to PFA was demonstrated for incorporation of both dCTP and dATP. The kinetics of TTP inhibition by PFA were investigated in depth for both the AMV and MMuLV reverse transcriptases. Despite the close similarity in the reaction mechanism for these two enzyme, the kinetics of inhibition were quite different.

Whereas the sensitivity of MMuLV-RT to PFA was markedly increased by lowering the substrate TTP concentration, inhibition of AMV-RT was relatively insensitive to changes in TTP (Figure 5). This could have important therapeutic consequences for combination drug therapy of HIV with kinase inhibitors.

At low TTP concentrations, the sensitivity of MMuLV-RT to PFA was increased about five fold from 8 $\mu$M down to only 1.5 $\mu$M PFA. Thus the effectiveness of PFA as a retrovirus inhibitor is likely to be enhanced at low intracellular TTP concentrations only if the viral reverse

transcriptase is of the MMuLV type. Fortunately, the HIV-RT does appear to be of this latter type since the HIV enzyme shows extreme sensitivity to PFA when transcribing from the viral RNA template in cell lysates (15). The $I_{50}$ for PFA under these conditions is only 0.1 $\mu$M.

Figure 4. Differential Sensitivity of dTTP and dGTP Incorporation to Inhibition by Phosphonoformate (PFA)

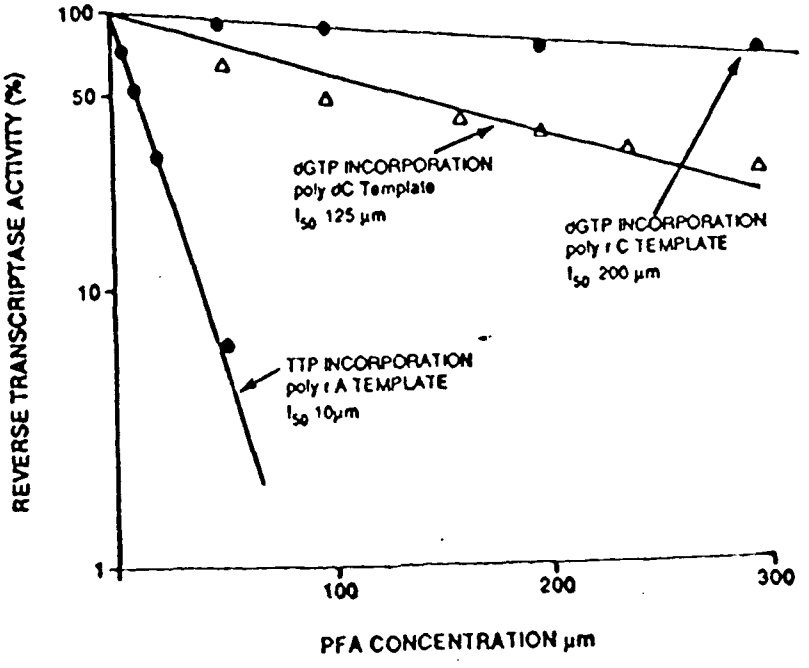
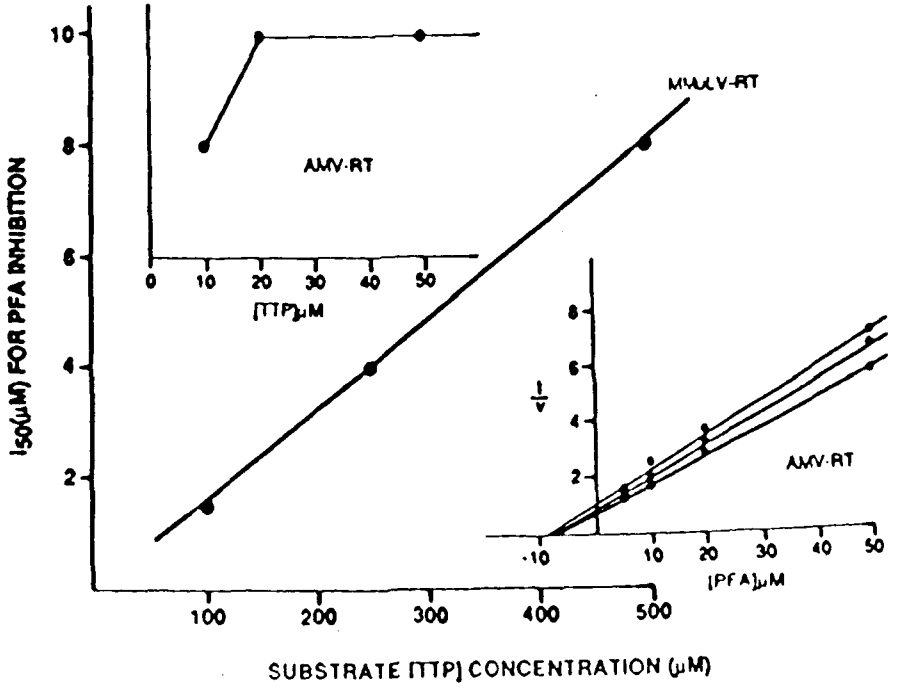



Figure 5. Inhibition of Viral Reverse Transcriptases by PFA; Differential Dependence on Ambient TTP Concentrations (Lower Inset, Dixon Plot for AMV-RT)



(iv) Isolation and Characterization of HIV-1 Reverse Transcriptase from a Vaccinia Virus-Pol gene Construct (VCF-21)

For analysis of compounds synthesized a s potential reverse transcriptase inhibitors, two basic methods of measuring enzyme activity have been employed. One method measures inhibition of activity in an "in vitro" system; a second method involves uptake of the drug in an "in vivo" cell culture system.

For in vitro assays, enzyme was generated from cultured monkey kidney cells which have been infected with a vaccinia virus recombinant construct which contains in its genome, the gene for the polymerase region of HIV virus. Forty-eight hours after infection, cells were harvested and lysed to obtain reverse transcriptase from the cell cytoplasm. Nuclei and cellular debris were removed during a centrifugation at 10,000 rpm for twenty minutes.

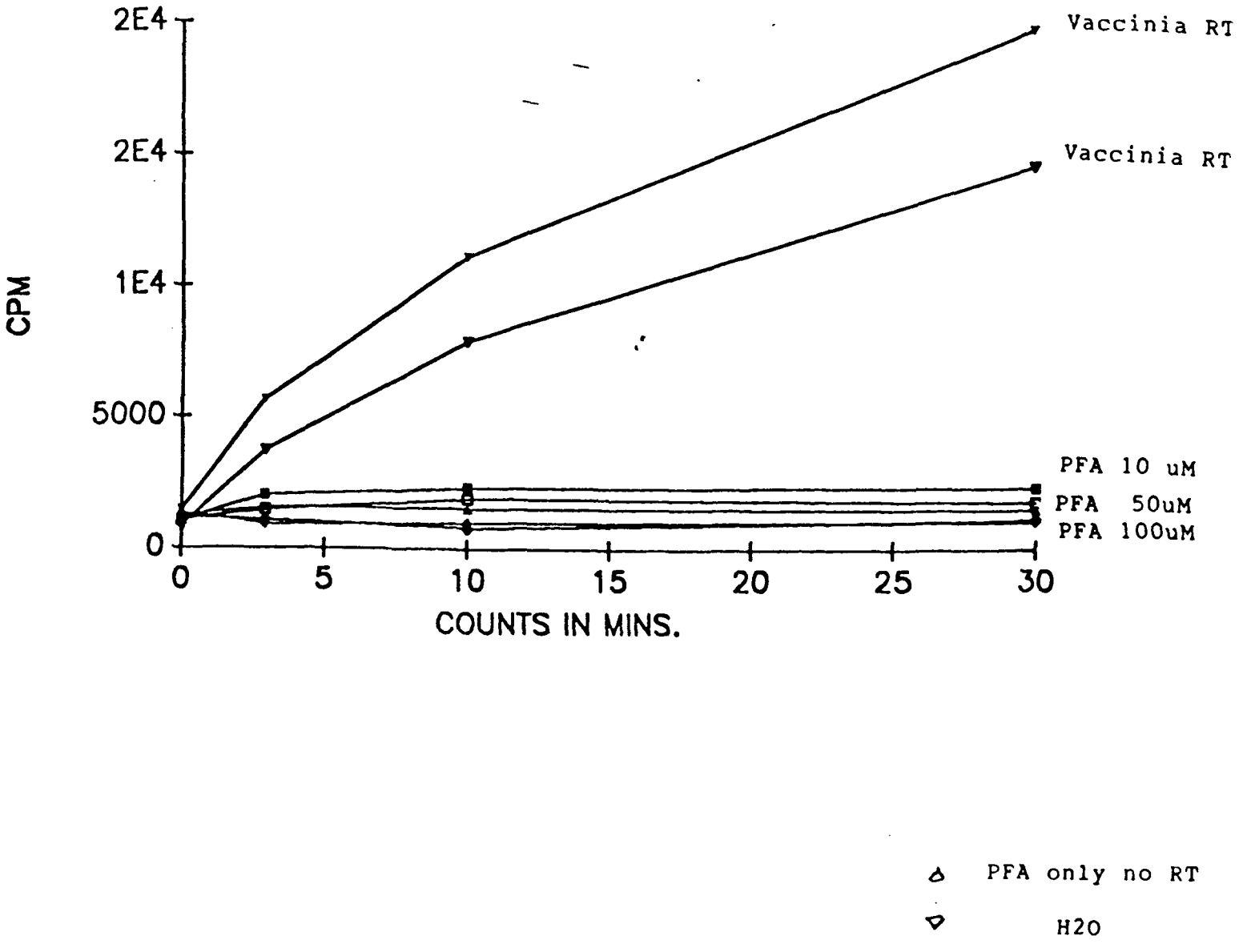
Crude lysate was frozen in the presence of 10% glycerol at -70° C. Reverse transcriptase activity was measured using a standard RT assay (Flexner, Moss) which incorporates $^{3}H$ TTP during DNA synthesis directed by RT enzyme.

Preparation of purified reverse transcriptase was accomplished by (1) DEAE chromatography of the crude extract. Flow though material was collected and chromatographed on (2) phosphocellulose. Fractions from the phosphocellulose column were assayed for RT activity. Active fractions were pooled and frozen in 10% glycerol at -70° C.

In vitro assays of potential inhibitors involved measuring RT activity in the presence of varying concentrations of drugs (usually between 10 and 100 $\mu$M final drug concentration). An example of an in vitro assay of two independent viral extracts and subsequent inhibition by several concentrations of PFA is shown in the accompanying graph (Figure 6). The vaccinia construct and purification procedures were gifts form Drs. Bernard Moss and Charles Flexner at the NIH.

Analysis of potential reverse transcriptase inhibitors in "in vivo" cultures was accomplished using equine dermal cells which were infected with EIAV virus. These monolayer cells at confluence were pre-incubated for -twenty-four hours with drugs at 10 $\mu$M and 100 $\mu$M concentrations. The drug-containing culture media was removed form cells. Inoculation of virus was followed by a one hour incubation at 37° C. Media with drugs was added to the cultures and left for seven days before the initial assaying for RT activity. Cultures were assayed again at 14 days and at 21 days (if cell survival permitted). Each experiment included infected untreated cultures and uninfected cultures as positive and negative controls. Often, a commercial RT was included (1) to determine the relative activity of the tissue culture RT and (2) to monitor the effectiveness of the assay system.

Figure 6. RT Activity of Enzyme Prepared from Cell Lysates of Vaccinia Virus-HIV Pol gene Construct VCF-21; and Inhibition by PFA.

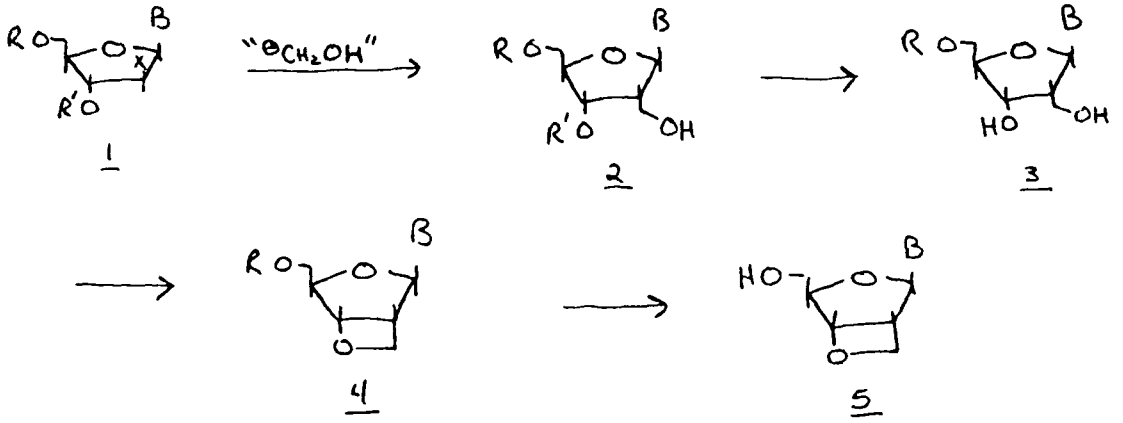


(v) Synthesis and Properties of Riboxetane Nucleosides and Related Derivatives.

This work was carried out by Dr. Keith A. Nelson.

The key step in the synthesis of the riboxetanes, eg. 5

Scheme I




(Scheme I) will be the introduction of the hydroxymethyl moeity, i.e. 1 → 2. The nucleosides themselves were chosen as starting materials in order to take advantage of inherent asymmetry but because of the variety of functional groups present selectivity is a significant problem. This is especially true of the pyrimidine nucleosides which contain an acidic proton that would certainly react with the masked hydroxymethyl anion. Therefore, in addition to protection of the nucleoside base, suitable protecting groups for the hydroxyls at C3' and C5' must be chosen that satisfy three general requirements: a) selective sequential introduction, b) resistance to reaction conditions, and c) selective sequential removal.

The first proposed solution to these requirements is found in compound 8 (Scheme II). The problems of protecting the uracil base and introducing a suitable leaving group X at C2' are simultaneously solved by the use of 2', $O^2$-anhydrouridine. Displacement of $O^2$ by a suitable nucleophile will then regenerate the free base. Protection of the hydroxyl groups at C3' and C5' as a benzyl ether and t-butyldimethylsilyl ether, respectively, satisfies the requirements stated above (vide infra).

Silylation of anhydrouridine 6[1] afforded 7 in good yield. However, benzylation of the hydroxyl group of 7 proved to be quite

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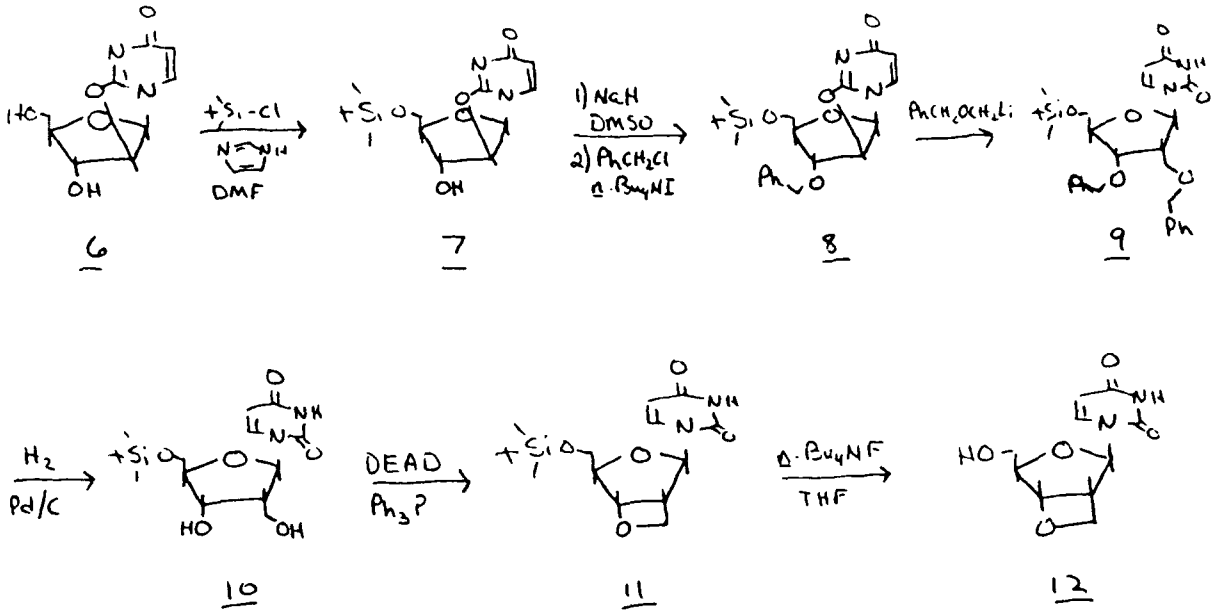
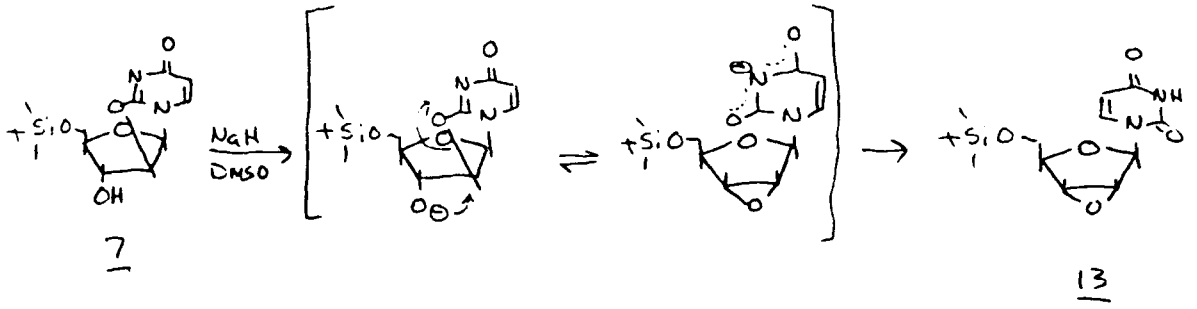
1. Sowa, T.; Tsunoda, K. Bull. Chem. Soc. Jpn. 1975, 48, 505.

Scheme II



difficult. Generation of any negative charge on $O^{3'}$ resulted in rapid displacement of $O^2$ and formation of the epoxide 13 (Scheme III). While

Scheme III



this observation was encouraging in that it showed that the displacement at C2' is possible, it significantly reduced the yield of 8. However, using a large excess of benzyl chloride a satisfactory yield of 8 was obtained.

The masked hydroxymethyl anion of choice is benzyloxymethyllithium.[2] Besides introducing the hydroxymethyl moeity both of the hydroxyl groups of the 1, 3-diol of 10 are revealed in a single deprotection step. Closure of the diol 10 to form the oxetane 11 would be carried out by treatment of 10 with diethylazoclicarboxylate (DEAD)/$Ph_3P$.[3] Desilylation with tetra-n-butylammonium fluoride would then

---

2. Corey, E. I.; Eckrich, T. M. Tetrahedron Lett. 1983, 24, 3163

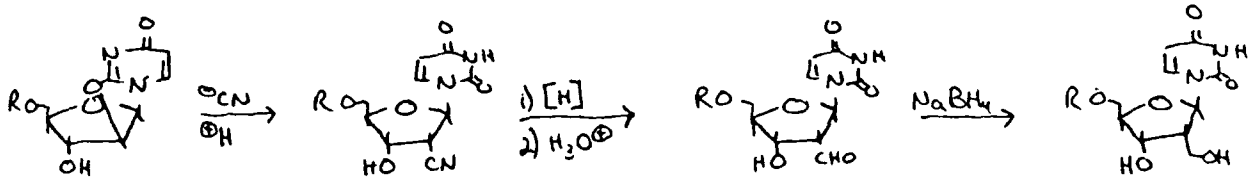
3. Carlock, I. T.; Mack, M. P. Tetrahedron Lett. 1978, 5153

afford the desired oxetane 12.

Unfortunately, treatment of 8 with a variety of organometallic reagents in an attempt to introduce the hydroxymethyl moeity resulted in attack at C2 of the base rather than at C2'. Apparently, approach of an anion to C2' is hindered sterically by the substituent at C3' and electrostatically by the lone pairs at $O^1$ of the sugar.

Displacement of $O^2$ from C2' of anhydrouridine by nucleophiles is known to occur under acid catalysis,[4] a condition that is incompatible with alkyllithiums and other organometallics. Attempts to introduce the hydroxymethyl group via a nitrile (Scheme IV) with acid catalysis failed to give the desired results.

Scheme IV

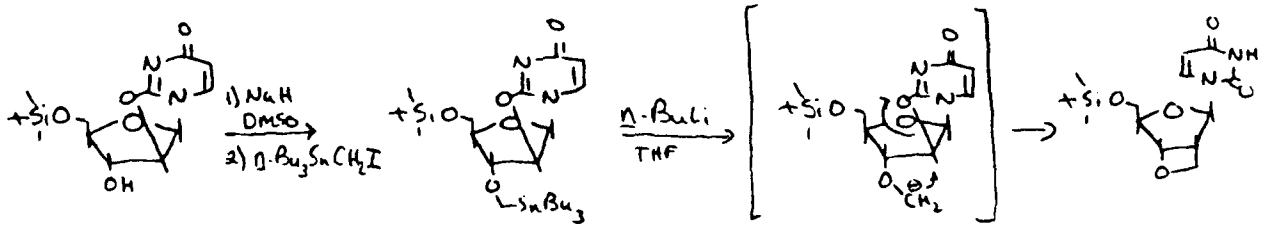


14 15 16 17

Noting the displacement of the base by an alkoxide ion to form the epoxide 13 (Scheme III), attention was directed towards developing a functional group that, when attached to $O^3$', could be converted to an anion that, because of configurational constraints, would be forced to attack C2'.

The first proposed route would generate an anion via the tri-n-butylstannylmethyl ether 18 (Scheme V). Unfortunately, the same problem

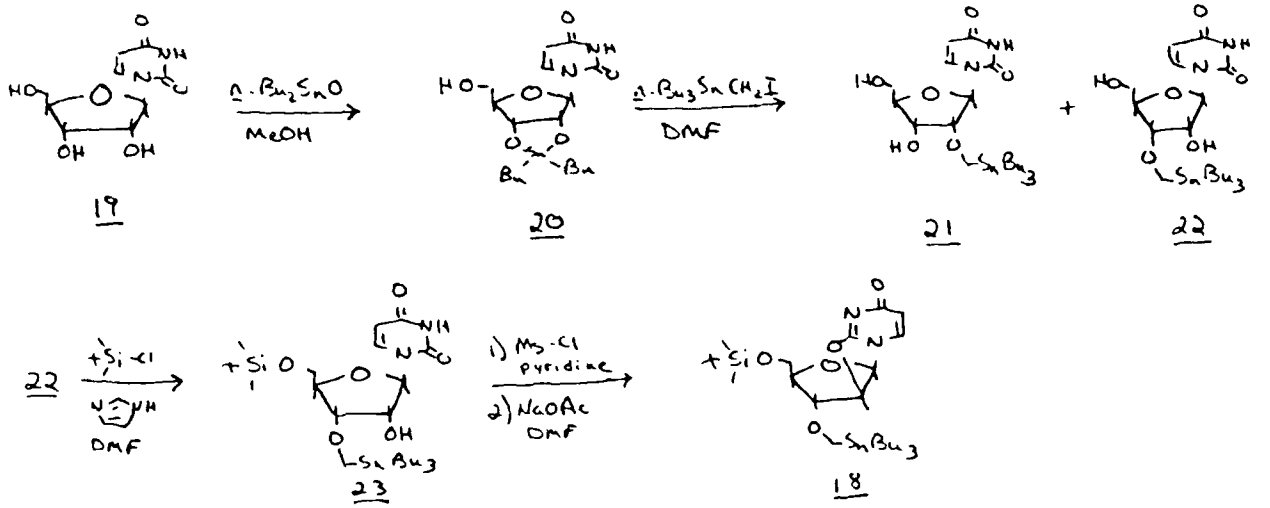
Scheme V



7 18 11

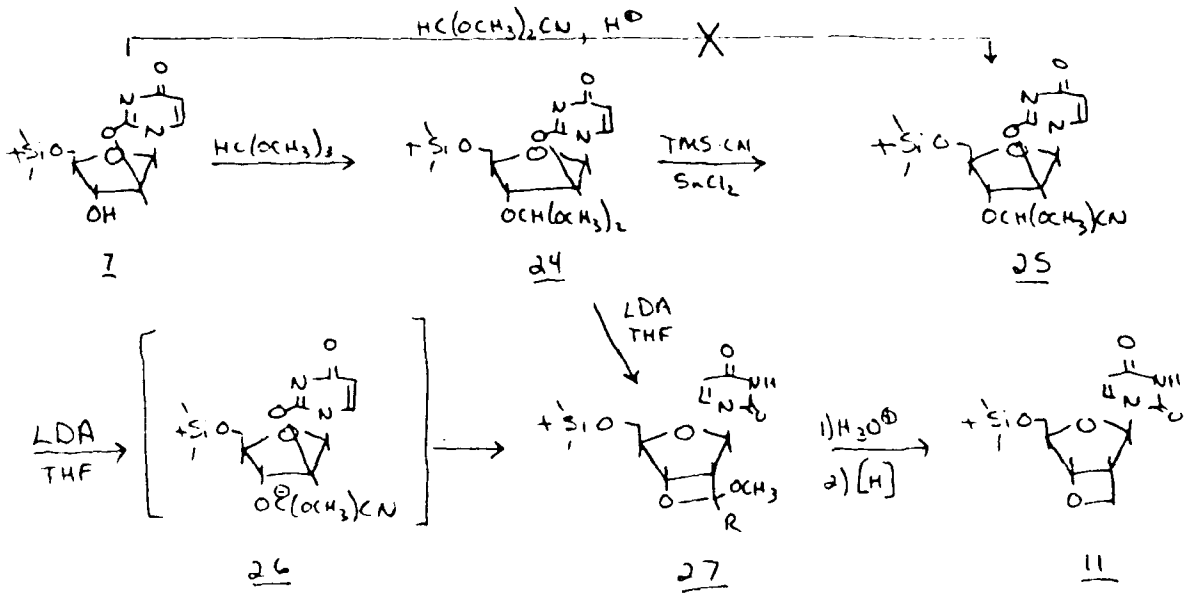
4. a. Fox I. I.; Miller, N. C. J. Org. Chem. 1963, 28, 936
   b. Verheyden, I. P. H.; Wagner, D.; Moffatt, I. G. ibid 1971, 36, 250

encountered in the preparation of 8 was encountered in the preparation 18. This problem is made worse due to the fact that tri-n-butylstannylmethyl iodide is a weaker alkylating agent than benzyl iodide. An alternate route to 18 was devised and is shown in Scheme VI. The key Scheme VI step in this synthesis involve the alkylation of 19 by treatment of the Scheme VI




tin acetal 20[5] with tri-n-butylstannylmethyl iodide. this would most likely result in a mixture of regioisomers 21 and 22. Unfortunately, concomitant alkylation of the base (probably $N^3$) could not be avoided.

When attempts to prepare 21 failed, an alternative synthesis was investigated and is shown in Scheme VII. The key step here involved the deprotonation of the di-alkoxyactonitrile 25 to form 27 (R=CN) via the Scheme VII

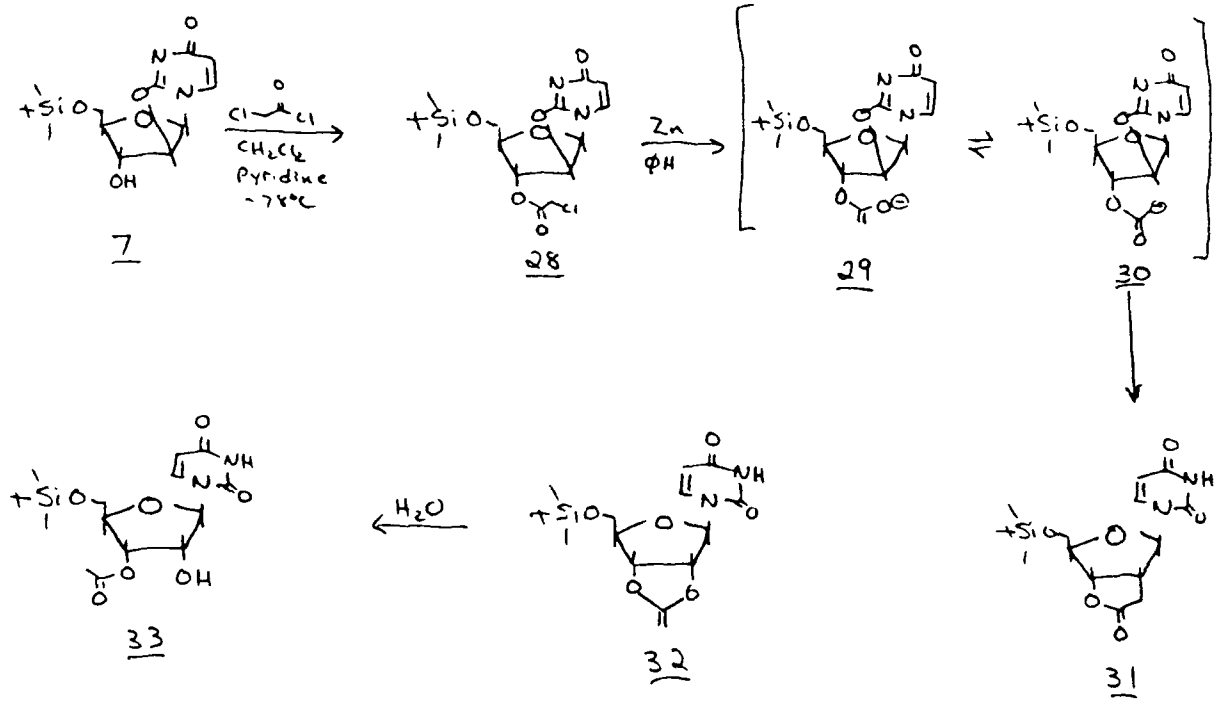



5. Wagner, D.; Verheyden, I. P. H.; Moffatt, I. G. J. Org. Chem. 1974, 39, 24.

stabilized anion 26 which upon hydrolysis and reduction would afford the oxetane 11. Direct transesterification of dimethoxyacetonitrile[6] failed to give 25 and resulted instead in desilylation of 7 to give 2', $O^2$-anhydrouridine 6. Transesterification of trimethylorthoformate with 7 gave 24 nearly quantitatively. However, all attempts convert 24 into the nitrile 25 have failed. It may be possible to metallate 24 to form, via the anion, 27 (R=$OCH_3$) directly. Work is currently underway to determine the optimal conditions for this step.

Although it would result in the attachment of a two carbon unit that would require subsequent trimming, an α-acyl anion generated from an acetate ester group at C3' is also under investigation. A first attempt to generate such an anion was made by use of the Reformatski reaction (Scheme VIII). Treatment of the chloroacetate 28, prepared by treatment

Scheme VIII




of 7 at -78°C with chloroacetyl chloride, with Zn in refluxing benzene, the desired lactone 31 was not obtained. Instead, the compound that was obtained was found, spectroscopically to contain a free uracil base, a hydroxyl group, and an acetate ester group. This is a result of alkylation at acyl oxygen rather than carbon. Stereoelectionic constraints favor, in such intramolecular nucleophilic substitutions to form five-membered rings, alkylation at oxygen.[7] This difficulty was circumvented by the formation of the α-acyl anion in a polar, protic

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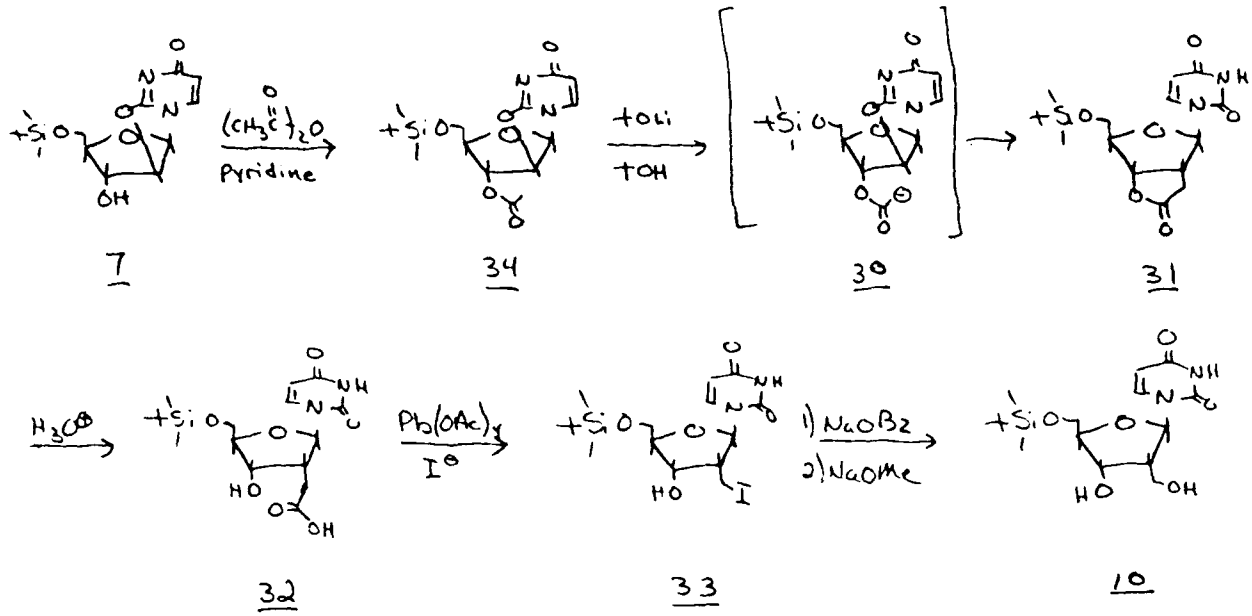
6. Utimoto, K.; Wakabayshi, Y.; Shishiyama, Y.; Inoue, M.; Nozaki, H. Tetrahedron Lett. 1981, 22, 4279

7. a. Baldwin, I. E.; Kruse, T. I. J. Chem. Soc. Chem. Commun. 1977, 233
   b. House, H. O.; Phillips, W. V.; Sayer, T. S. B.; Yau, C. C. J. Org. Chem. 1978, 43, 700

solvent, t-butanol, which promotes alkylation at carbon. Therefore, as shown in Scheme IX, the acetate 34, prepared by treatment of 7 with acetic

Scheme IX

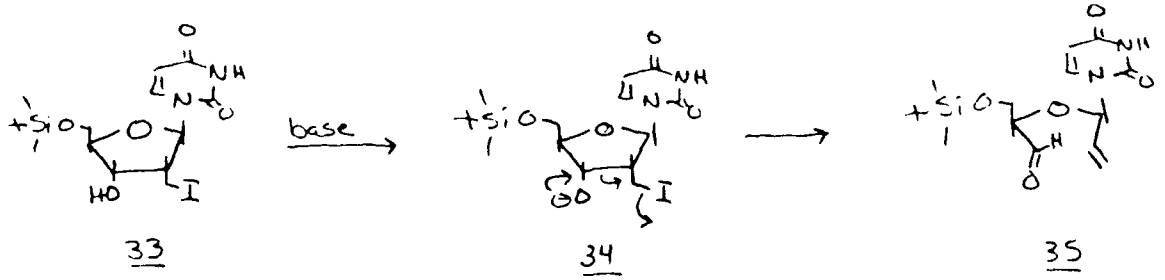



7 34 30 31 32 33 10

anhydride in pyridine, was treated with lithium t-butoxide in t-butanol. The lactone 31, was obtained along with some 7, a result of the deacylation of 30. Although 31 has been obtained in good yield by this procedure (> 50%), work is in progress to optimize this yield.

Trimming of the carboxylic acid 32, obtained by hydrolysis of the lactone, may be accomplished by dicarboxylative iodination. Treatment of 32 with lead tetraacetate should afford the iodide 33. Although the oxetane may be accessible from 33 via a Williamson reaction, the possibility of a Grob reaction taking place to give the olefin-aldehyde 35 (Scheme X) suggests conversion of the iodide to the benzoyl ester by

Scheme X

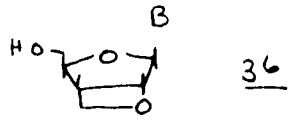



33 34 35

treatment with sodium benzoate followed by hydrolysis to the diol.

As can be seen from this summary, the most difficult step in the synthesis of the riboxetanes has been the introduction of the hydroxymethyl moeity to C2'. With the latest attempt via an intramolecular α-acyl alkylation, the difficulty appears to have been

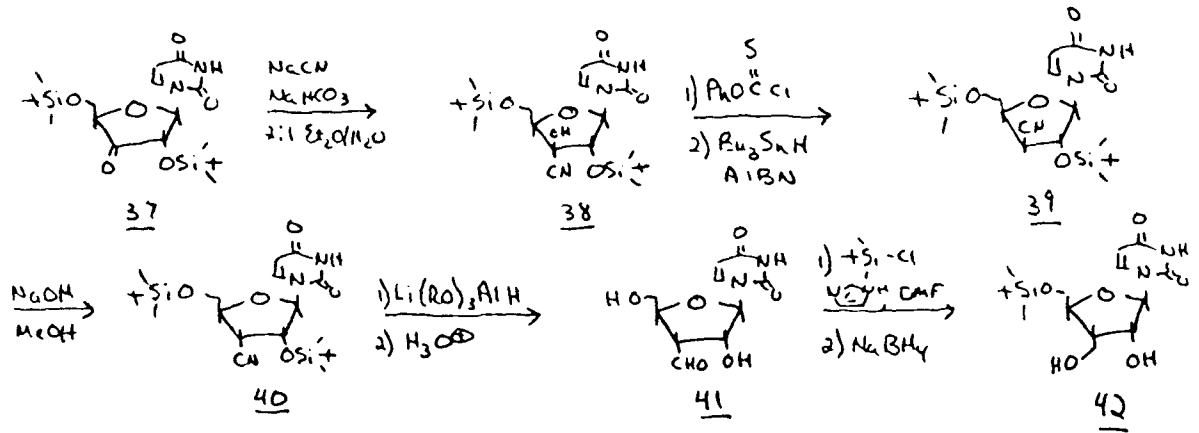
overcome. An advantage of this method is that the remaining ribo-isomer 36 should be easily prepared via 3', $O^2$-anhydrouridine.



36

An alternate route to 36 is currently under investigation, and is shown in Scheme XI. This proposed route introduces the hydroxymethyl moeity as a nitrile. Compound 39 will be prepared by the method of Mateo

Scheme XI



et al[8] who prepared the thymidine analogue. Equilibration will most likely give a mixture of 39 and 40 that will be separated. Reduction of the nitrile 40 to the aldehyde 41 represents a significant hurdle that is now being addressed.

Experimental

5'-O-t-Butyldimethylsilyl-2'-$O^2$-anhydrouridine, 7.

To a stirred suspension of 2' $O^2$-anhydrouridine (184 mg, 0.81 mmol) in anhydrous DMF (4 ml) at room temperature was added imidazole (125 mg, 1.84 mmol). After stirring for ≈ 5 minutes to dissolve the imidazole, t-butyldimethylsilyl chloride (140 mg, 0.93 mmol) was added and the resulting solution stirred at room temperature for 20 hours. Removal of the solvent in vacuo (0.5 mm Hg, < 30°C) gave the crude product as a colorless oil which was then dissolved in ethyl acetate (20 mL) and washed with 1% aqueous HCl (1 x 5 mL) and saturated aqueous sodium bicarbonate (1 x 5 mL). The aqueous washings were back-extracted with ethyl acetate (2 x 5 mL) and then the combined organic phases were dried ($MgSO_4$) filtered, and concentrated in vacuo. Column chromatography on silica gel 60 (25g) eluted with 10% $CH_3OH/CH_2Cl_2$ afforded the product as a white, brittle foam homogeneous by TLC (Rf 0.33, 10% $CH_3OH/CH_2Cl_2$). Yield: 213 mg, 0.63 mmol, 77%. IR (neat) 3270 (br), 2960, 2930, 2900, 2860, 1655, 1530, 1475, 1250, 1125, 1075, 1035, 950, 830, and 775 $cm^{-1}$.

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8. Mateo, A. C.; Camarasa, M. I.; Diaz-Ortiz, A.; De las Heras, F. G. Tetrahedron Lett. 1988, 29, 941

stirring at room temperature for four hours, the incomplete reaction (large excesses of NaH and/or longer reaction times severely decrease the yield) was quenched by the addition of saturated aqueous sodium bicarbonate (0.1 mL). The resulting mixture was diluted with $CH_2Cl_2$ (15 mL), washed with water (2 x 5 mL) and brine (2 x 5 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography on silica gel 60 (45 g) eluted with 10% $CH_3OH/CH_2Cl_2$) gave a product as a yellow oil that solidified on standing, homogeneous by TLC (Rf 0.58, 10% $CH_3OH/CH_2Cl_2$). Yield: 88 mg, 0.20 mmol, 71% (based on unrecovered starting material). IR (neat) 3060, 3030, 2950, 2925, 2860, 1645, 1540, 1465, 1245, 1080, 1035, 945, 830, 780, and 700$cm^{-1}$. NMR ($CDCl_3$) $\delta$-0.03 (3, S), 0.01 (3, S), 0.83 (9, S), 3.40 (1, dd), 3.57 (1, dd), 4.34-4.39 (1, m), 4.42 (1, br s), 4.64 (1, d), 4.69 (1, d), 5.29 (1, d), 6.10 (1, d), 6.14 (1, d) and 7.32-7.42 (6, m).

5'-O-t-Butyldimethylsilyl-3'-O-dimethoxymethyl-2'-$O^2$-anhydrouridine, 24.

A solution of 7 in freshly distilled (from $CaH_2$) trimethyl orthoformate (2 mL) was heated at reflux over molecular sieves. After 17 hours the clear solution was cooled to room temperature, poured into dilute aqueous sodium bicarbonate (10 mL), and the product extracted with $CH_2Cl_2$ (3 x 5 mL). The combined organic extracts were dried ($Na_2SO_4$), filtered, and concentrated in vacuo to afford the product as a white solid homogeneous by TLC (Rf 0.50, 10% $CH_3OH/CH_2Cl_2$). Yield: 131 mg, 0.32 mmol, 94%. IR (neat) 2950, 2930, 2890, 2855, 1645, 1540, 1465, 1250, 1195, 1080 (br), 1030, 950, 835, and 780 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ 0.02 (3, 5), 0.04 (3, 5), 0.86 (9, 5), 3.39 (6, 5), 3.45 (1, dd), 3.62 (1, dd), 4.32-4.38 (1, m), 4.72 (1, br s), 5.16 (1, 5), 5.39 (1, d), 6.10 (1, d), 6.13 (1, d) and 7.34 (1, d).

5'-O-t-Butyl-3'-O-chloroacetyl-2', $O^2$-anhydrouridine 28.

To a solution of 7 (212 mg, 0.62 mmol) in dry dichloromethane (3.1 mL) was added pyridine (59 mg, 0.75 mmol). The resulting solution was cooled to -75°C and chloroacetyl chloride (78 mg, 0.69 mmol) added dropwise. The yellow solution was stirred for ≈10 minutes at -75°C then allowed to warm to room temperature. The color was lost as the solution warmed. After 2 hours at room temperature the reaction was quenched with saturated aqueous sodium bicarbonate (1 mL), and diluted with dichloromethane (5 mL). The mixture was washed with water (2 x 3 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography on silica gel 60 (60 g) eluted with 10% $CH_3OH/CH_2Cl_2$ afforded the product as an amorphous white powder homogeneous by TLC (Rf 0.49, 10% $CH_3OH/CH_2Cl_2$). Yield: 193 mg, 0.46 mmole, 74%. IR (KBr) 3110, 3005, 2930, 2880, 2860, 1765, 1655, 1635, 1535, 1470, 1310, 1260, 1245, 1190, 1150, 1135, 1075, 1045, 1000, 950, 925, 885, 835, 775, and 665 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ 0.10 (6, S), 0.90 (9, S), 3.57-3.79 (2, m), 4.16 (2, S), 4.25-4.50 (1, m), 5.44 (1, d), 5.54 (1, br d), 6.00 (1, d), 6.32 (1, d), and 7.35 (1, d).

3'-O-Acetyl-5'-t-butyldimethylsilyl-2'-$O^2$-Anhydrouridine 34.

To a solution of 7 (194 mg, 0.57 mmol) in anhydrous pyridine (1.5 mL) at room temperature was added acetic anhydride (70 mg, 0.7 mmol).

After 2.5 hour absolute ethanol was added (1.5 mL) and the solvents removed in vacuo. The white solid residue was co-evaporated with absolute ethnol (2 x 1.5 ml) then purified by column chromatography on silica gel 60 (25 g, 10% $CH_3OH/CH_2Cl_2$) to afford the product as a white solid homogeneous by TLC (Rf 0.44, 10% $CH_3OH/CH_2Cl_2$). Yield: 196 mg, 0.51 mmol, 90%. IR (neat) 3080, 3040, 2995. 2950, 2925, 2860, 1750, 1655, 1615, 1530, 1475, 1240, 1215, 1100, 1075, 1065, 1025, 965, 945, 840, and 775 $cm^{-1}$. NMR ($CDCl_3$) $\delta$.

(1S, 5R)-8-(t-butyldimethylsilyloxymethyl)-3-oxo-6-(pyrimidin-1-yl)-2, 5-dioxabicyclo [3, 3, 0] octane 31

To a solution of 34 (31 mg, 81 $\mu$mol) in anhydrous t-butanol (0.40 mL) at 80°C was added lithium t-butoxide (10 mg, 120 $\mu$mol, 1 M solution in THF). After 10 minutes the clear, yellow solution was cooled to room temperature and quenched with 3-4 drops of saturated aqueous ammonium chloride. The resulting mixture was dilated with ethyl acetate (4 mL), washed with water (2 x 2 mL) and brine (1 x 2 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatograp y on silica gel 60 (2 g, 230-400 mesh) eluted with 10% $C_2H_5OH/CHCl_3$ gave the product as brittle white foam homogeneous by TLC (Rf 0.73, 10% $CH_3OH/CH_2Cl_2$). Yield: 16 mg, 42 $\mu$mol, 52%. IR (neat) 3200, 3060, 2955, 2930, 2860, 1690 (br), 1405, 1380, 1310, 1275, 1255, 1215, 1180, 1125, 1060, 1030, 980, 935, 835 (br), 780, 715, 665, and 625 $cm^{-1}$. NMR ($CDCl_3$) $\delta$.

Also recovered was 5'-0-t-butyldimethylsilyl-2'-$0^2$-anhydrouridine. Yield: 12 mg, 35 $\mu$mol, 43%.

2'-5'-0-Bis(t-butyldimethylsilyl)-3'-cyanouridine 38.

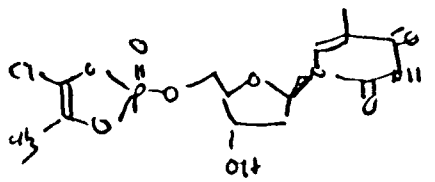
A solution of 2'-5'-0-bis(t-butyldimethylsilyl)-3'-kitouridine 37 (102 mg, 0.21 mmol), sodium cyanide (104 mg, 2.12 mmol), and sodium bicarbonate (89 mg, 1.1 mmol) in 2;1 $Et_2O/H_2O$ (2 mL) was heated at reflux. After 3 h the solution was cooled to room temperature and diluted with $Et_2O$. The organic phase was removed, dried ($MgSO_4$), filtered, and concentrated in vacuo. Column chromatography on silica gel 60 (50 g) eluted with 50% EtOAc/hexanes afforded the product as a brittle, white foam homogeneous by TLC (Rf 0.47, 5% $CH_3OH/CH_2Cl_2$). Yield: 60 mg, 0.12 mmol, 57%. IR (neat) 3200 (br), 2950, 2930, 2880, 2860, 1700 (sh); 1680, 1460, 1385, 1255, 1120, 835, and 785 $cm^{-1}$. NMR ($CDCl_3$) $\delta$ 0.00 (12, S), 0.95 (18, S), 4.18-4.45 (4, m), 5.61 (1, d), 5.81 (1, S), 6.14 (1, br S), ad 7.75 (1, d).

(VI) Synthesis of 5' Nucleoside Dioxaphosphole and 5' Nucleoside Oxaphospholene Derivatives

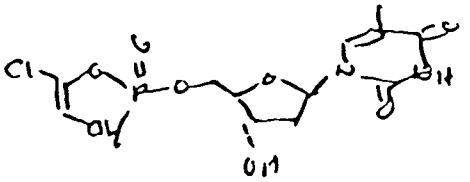
This work was performed by Dr. Robert A. Mook, Jr. who joined the group on October 1, 1987.

The goal of this research was to prepare 5'- nucleoside dioxaphosphole and 5'-nucleoside oxaphospholene derivatives as potential suicide inhibitors of reverse transcriptase and to test these compounds for antiviral activity (Figure 1). Toward this end we prepared the following phosphorus heterocyclic derivatives (Figure 2).

Figure 1

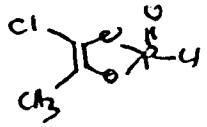


1

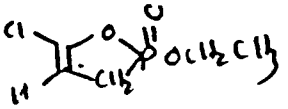


2

Figure 2



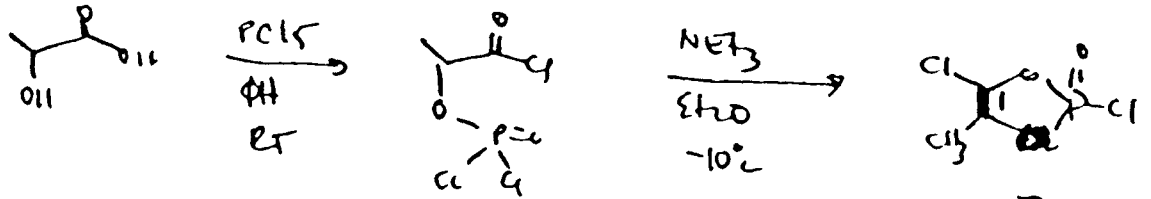
3



4

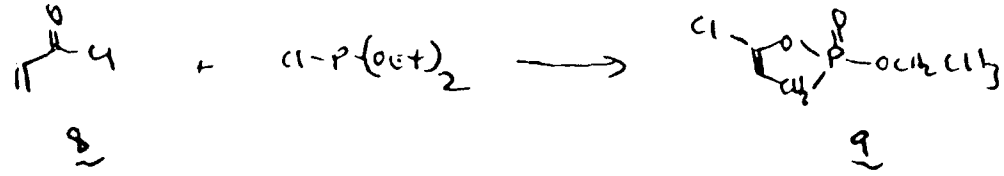
The synthesis of both dioxaphosphole 3 and oxaphospholene 4 were attempted simultaneously[1,2]. Although the syntheses of both these derivatives were reported in the literature, little experimental detail was provided. Our attempts to prepare these compounds according to the experimental detail given were unsuccessful. Thus, it became necessary to perform a number of experiments in order to repeat the work reported in the literature (see experimental given in the appendix). The syntheses of both dioxaphosphole 3 and oxaphospholene 4 is shown below (Scheme 1). Freshly distilled lactic acid was treated with phosphorus pentachloride in dry benzene and gave acid chloride 6 after distillation. Subsequent treatment of 6 with triethylamine gave the cyclic derivative 7 after distillation. Oxaphospholene 9 was prepared by treatment of acryloyl chloride 8 with diethyl chlorophosphite (Scheme 2).

Scheme 1




5 6 7

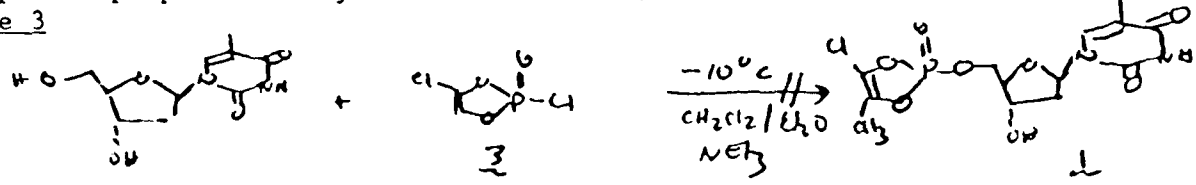
Scheme 2



8 9

Attempts to prepare the thymidine derivative 1 were unsuccessful (Scheme 3).

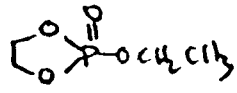
Scheme 3



3 1

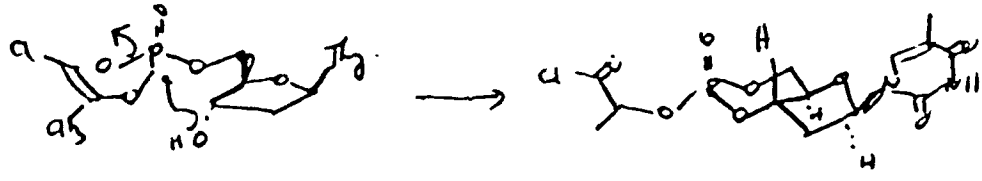
Kinetic data reported in the literature for the closely related dioxaphospholanes derivatives (Figure 3) indicate that the rate of hydrolysis of these derivatives is highly sensitive to the pH[3]. Since intramolecular reaction are known to occur

Figure 3



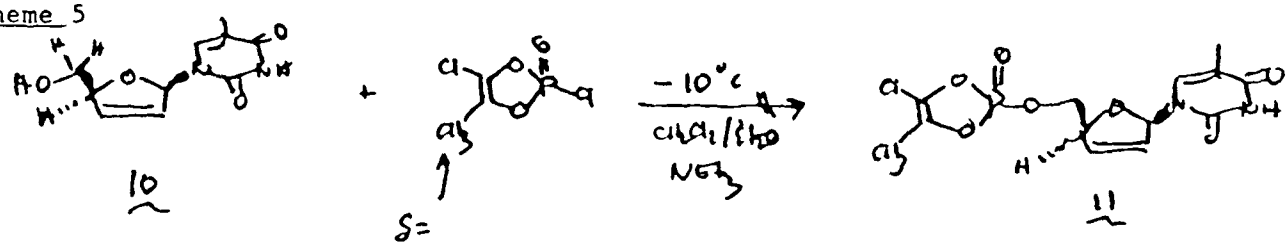
more readily than intermolecular reactions, we surmised that intramolecular cyclization of the 3'- hydroxy group with the 5'- phosphorate ester could be problematic (Scheme 4). Therefore, thymidinene[4] 10 was prepared and used in the following experiments.

Scheme 4



Attempts to synthesize the thymidinene dioxaphosphole derivative 11 also appeared to be unsuccessful (Scheme 5). Upon inspection of the crude reaction products in

Scheme 5

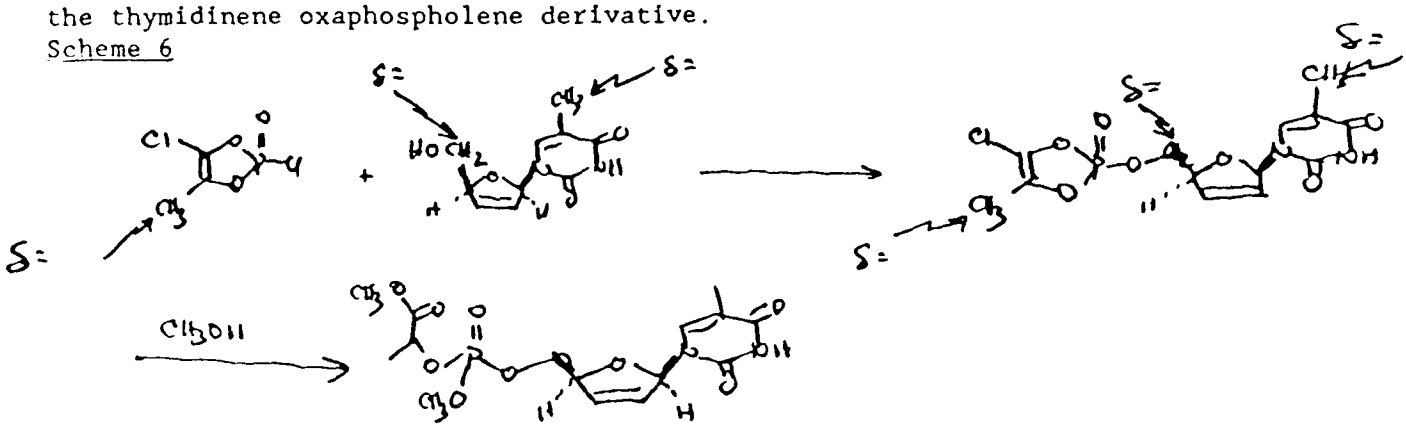


10 S= 11

a NMR spectrum, however, the presence of the desired product was observed. Thus, the attempts to isolate the desired nucleoside dioxaphosphole derivative cause it to decompose. We reasoned that the by-products of the reaction, triethylamine and triethylamine hydrochloride, might catalyze the hydrolysis and decomposition of the desired material during the work-up procedure. This was shown to be the

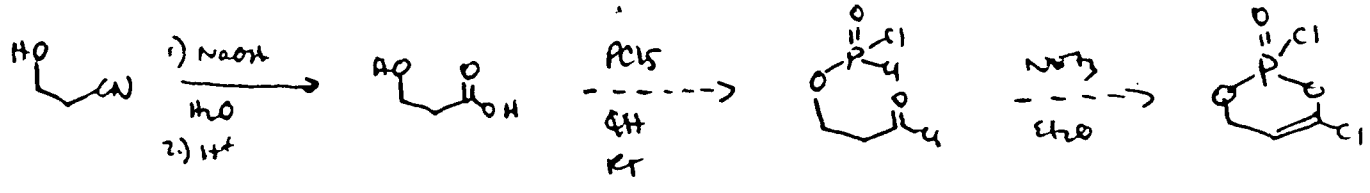
case as determined by the results of a simple NMR experiment. In the event, addition of two drops of methanol to a NMR tube containing the crude reaction products resulted in the complete disappearance of the desired material in less than three minutes (Scheme 6). Since oxaphospholanes are reported to be as reactive as their dioxaphopholane counterparts[3], we anticipated a similar resultwith the oxaphospholene derivatives. Thus, we did not attempt to prepare the thymidinene oxaphospholene derivative.

Scheme 6



Based on reports which indicate the reactivity of six - membered ring phosphorus derivatives are less sensitive to pH, we have begun to synthesize the analogous six-membered ring derivative. These molecules could also act as suicide substrates reacting analogously to the five - membered ring derivatives. The information gained in the the synthesis and isolation of the six - membered ring system could be useful in further attempts to synthesize the five - membered ring derivative. Work toward this end has just begun. The synthesis proposed to prepare these derivatives is shown below (Scheme 7).

Scheme 7



C. Appendix

1. Synthetic Procedures for Sterol Phosphonoformates.

Diethyl (cholesteryloxycarbonyl) phosphonate

Cholesteryl chloroformate (964 mg, 2.15 mmol) was suspended in triethyl phosphite (1.5 g, 8.7 mmol) at room temperature. The mixture solidified slowly over a period of 18 hours. Purification of the crude mixture by column chromatography on silica gel 60 (60 g) eluted with 30% ethyl acetate/hexanes afforded the product as a white solid homogeneous by TLC (Rf 0.70, 50% ethyl acetate/hexanes). Yield: 1.10 g, 2.00 mmol, 93%.

IR (KBr) 2920, 2840, 1695, 1455, 1430, 1370, 1360, 1255, 1200, 1155, 995 (br), and 655 $cm^{-1}$. MP 110-111° C.

Bis (3-methylbutyl) (cholesteryloxycarbonyl) phosphonate

A suspension of cholesteryl chloroformate (113 mg, 0.25 mmol) in tri-i-amylphosphite (220 mg, 0.75 mmol) was stirred under $N_2$ at room temperature. After 18.5 hours more tri-i-amylphosphite (220 mg, 0.75 mmol) was added. After 24 hours the reaction was judged completed by TLC analysis (silica gel 60, 20% ethyl acetate/hexanes). Purification of the crude mixture by column chromatography on silica gel 60 (30 g) eluted wity 20% ethyl acetate/hexanes gave the product as a colorless oil, homogeneous by TLC (Rf 0.51, 20% ethyl acetate/hexanes), that solidified on standing. Yield: 153 mg, 0.24 mmol, 96%.

IR (mineral oil) 2900 (br), 1715, 1275, 1210, 1040, and 985 $cm^{-1}$. MP 67-69° C.

Disodium (cholesteryloxycarnonyl) phosphonate

Diethyl (cholesteryloxycarbonyl) phosphonate (176 mg, 0.32 mmol) was suspended in trimethylgromosilane (290 mg, 1.9 mmol) at RT. The solid slowly dissolved voer a period of 2 hours. After 3 hours all volatiles were removed in vacuo. To the residual pale yellow gum were added $H_2O$ (1.5 mL) and Amberlite IRC 50 ion exchange resin (1 g, $Na^+$ form). After stirring for 1.5 hours the mixture was placed in a column and eluted with $H_2O$ (10 mL). The eluent was washed with diethyl ether (3 x 5 mL) and concentrated in vacuo (<40°C) to afford the product as a white solid that was found by TLC (65:17.5:1.5:1.5 $CHCl_3/CH_3OH$/con. aq. $NH_4OH/H_2O$) to be contaminated with a small amount of sodium ethyl (cholesteryloxycarbonyl) phosphonate. Yield: 71 mg, 0.13 mmol, 41%.

IR (mineral oil) 3340 (br), 2890 (br), 1670, 1220 (br), 1060, 1030, and 915 $cm^{-1}$.

Bis (2, 3-dimethyl-1, 3-dioxalanomethyl) (cholesteryloxycarbonyl)-phosphonate

To a suspension of cholesterylchloroformate (120 mg, 0.27 mmol) in tris (2, 2-dimethyl-1, 3-diosalanomethyl) phosphite (350 mg, 0.82 mmol) was added N, N-dimethylaniline (27 mg, 0.2 mmol) and the resulting mixture

warmed to 50°C. After 7 h the now clear solution was xooled to RT, placed on a column of silica gel 60 (30 g), and eluted with 50% ethyl acetate/hexanes. The product was obtained as a pale yellow wax, homoteneous by TLC (Rf 0.30, 50 % ethyl acetate/hexanes). Yield: 110 mg, 0.15 mmol, 57&.

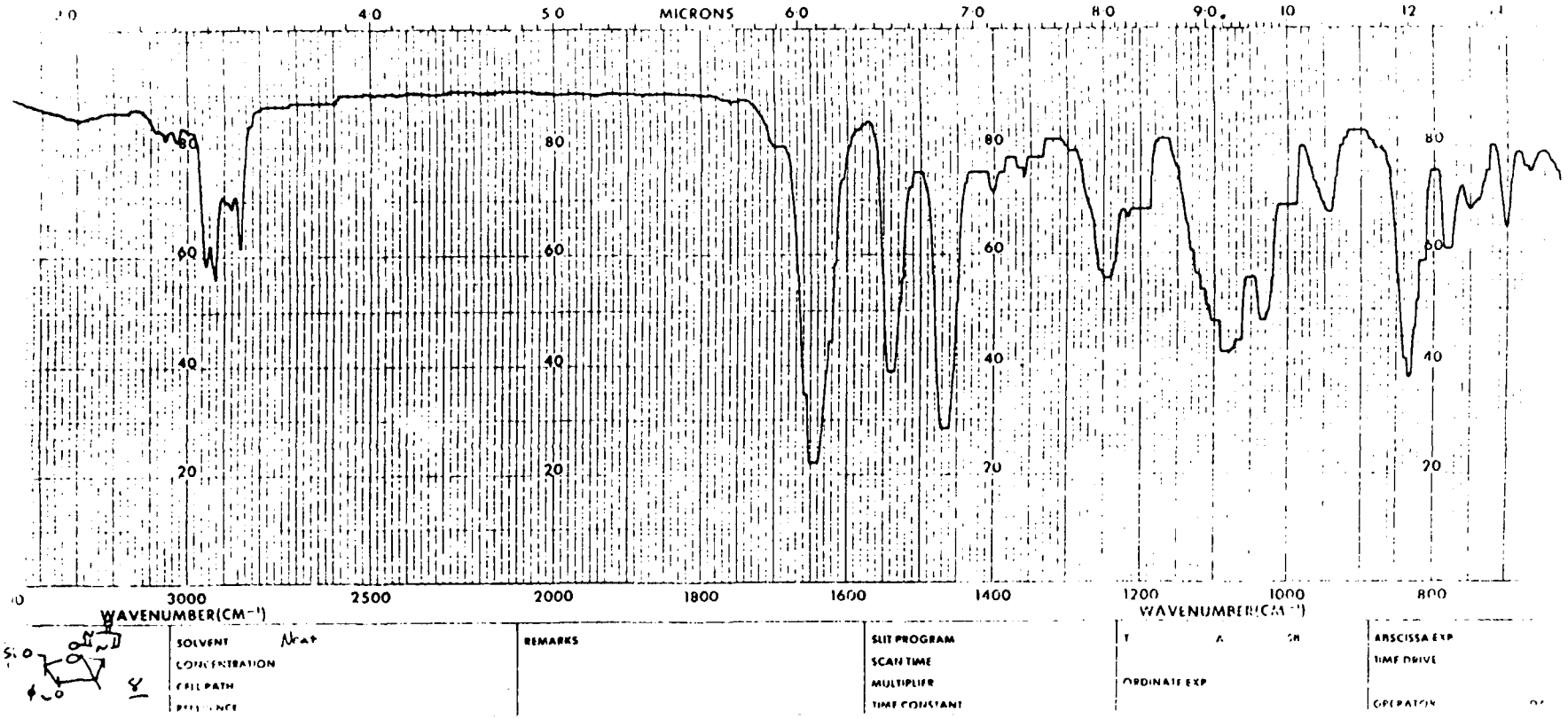
IR (mineral oil) 2980-2840 (br), 2730, 1715, 1280, 1220, 1155, 1050, 970, 935, 840, and 725 $cm^{-1}$.

Bis [1-(3-carboethoxypropyl)] (cholesteryloxycarbonyl) phosphonate

A suspension of cholesterylchloroformate (2.55 g, 5.68 mmol) in tris [1-(3-carboethyxypropyl)] phosphite (2.55 g, 6.01 mmol) was stirred at RT for 120 h. The resulting sloutio was then placed on a column of silica gel 60 (60 g) and eluted with 50% ethyl acetate/hexanes. The product was obtained as a viscous, cololess oil homogeneous by TLC (Rf 0.41, 50% ethyl acetate/hexanes). Yield: 1.28 g, 1.83 mmol, 32%.

IR (neat) 2940, 2860, 1725, 1460, 1375, 1270, 1215, 1175, and 1010 $cm^{-1}$.

2. NMR and IR Spectra of Riboxetane Nucleoside Derivatives.




SOLVENT Neat

CONCENTRATION

CELL PATH

REMARKS

SLIT PROGRAM

SCAN TIME

MULTIPLIER

TIME CONSTANT

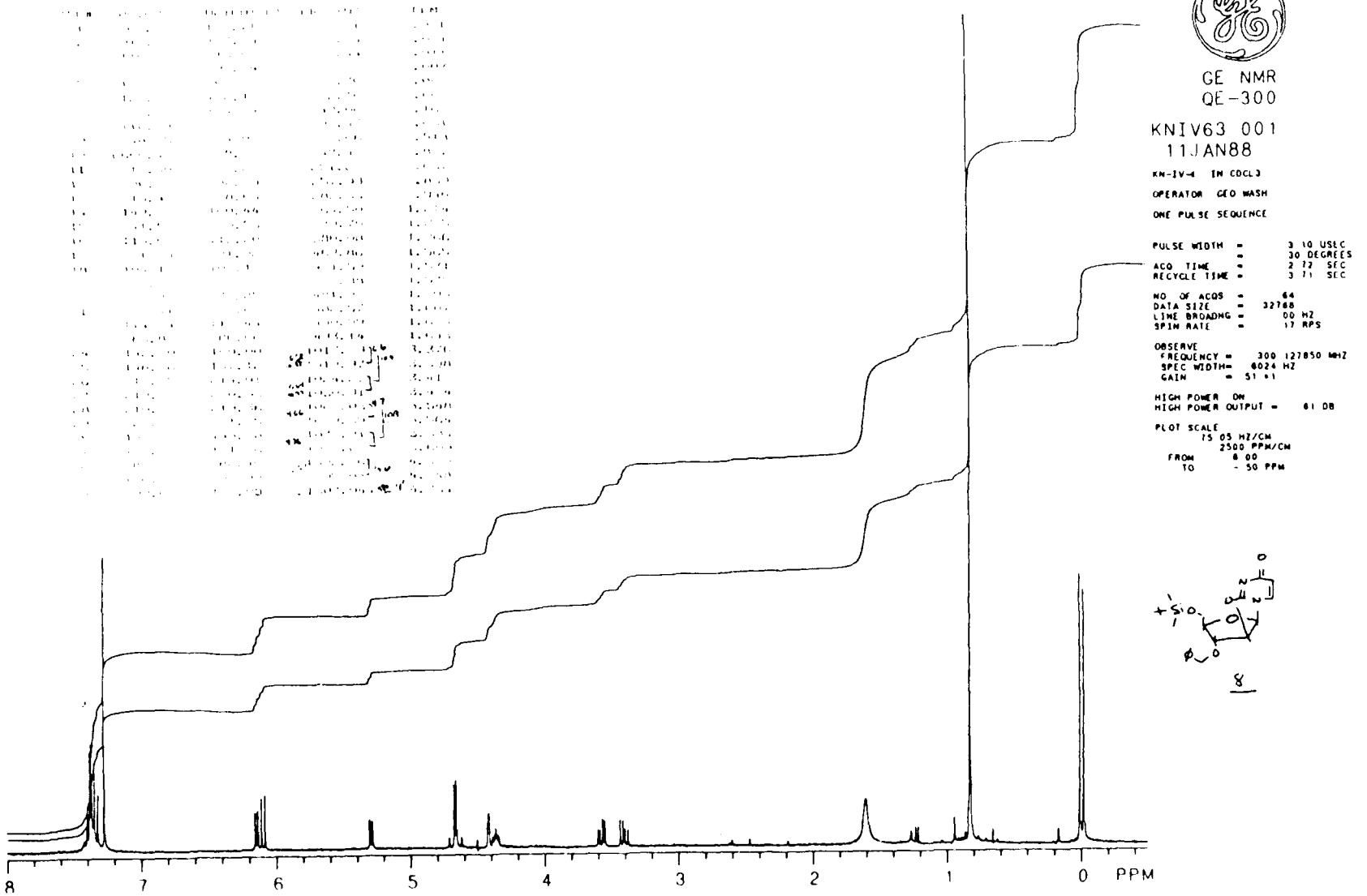
ORDINATE EXP

ABSCISSA EXP

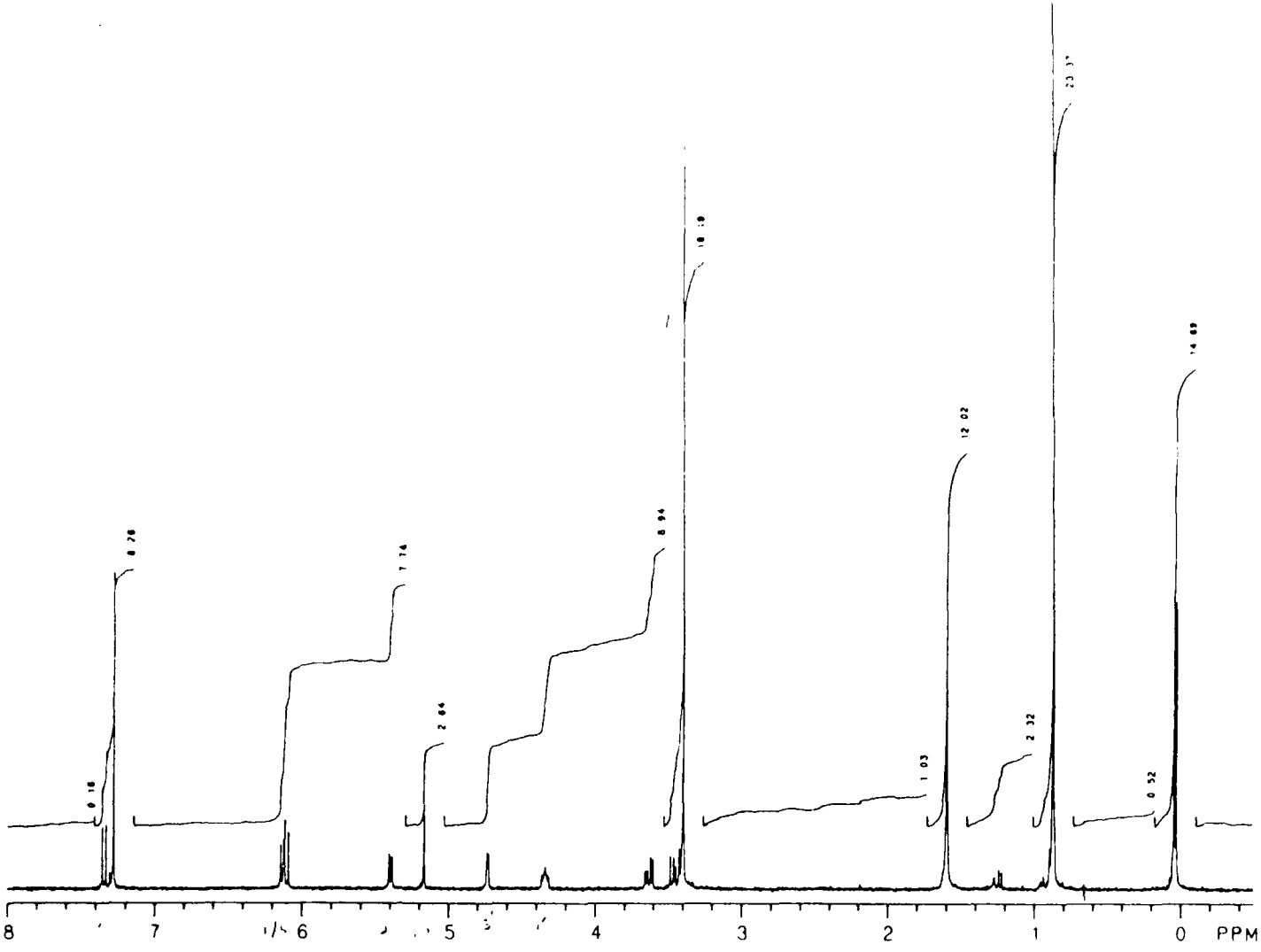
TIME DRIVE

OPERATOR

D


GE NMR
QE-300
KNIV63 001
11JAN88
KN-IV-4 IN CDCL3
OPERATOR GEO WASH
ONE PULSE SEQUENCE
PULSE WIDTH = 3 10 USEC
= 30 DEGREES
ACQ TIME = 2 72 SEC
RECYCLE TIME = 3 71 SEC
NO OF ACQS = 64
DATA SIZE = 32768
LINE BROADNG = 00 HZ
SPIN RATE = 17 RPS
OBSERVE
FREQUENCY = 300 127850 MHZ
SPEC WIDTH= 6024 HZ
GAIN = 51 +1
HIGH POWER ON
HIGH POWER OUTPUT = 61 DB
PLOT SCALE
75 05 HZ/CM
2500 PPM/CM
FROM 8 00
TO - 50 PPM
8
PPM

27a




GE NMR
QE-300

KNV49 501
08JAN88

KN-V-49 IN CDCL3

OPERATOR GEO WASH

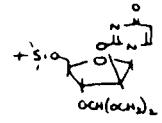
ONE PULSE SEQUENCE

| | | |
|---|---|---|
| PULSE WIDTH | = | 3.10 USEC |
| | = | 30 DEGREES |
| ACQ. TIME | = | 2.72 SEC |
| RECYCLE TIME | = | 3.71 SEC |
| NO. OF ACQS | = | 8 |
| DATA SIZE | = | 32768 |
| LINE BROADNG | = | .00 HZ |
| SPIN RATE | = | 18 RPS |

OBSERVE
FREQUENCY = 300.127850 MHZ
SPEC WIDTH= 8024 HZ
GAIN = 58

HIGH POWER ON
HIGH POWER OUTPUT = 61 DB

PLOT SCALE
75.05 HZ/CM
2500 PPM/CM
FROM 8.00
TO -.50 PPM




24

27b

# DIFFERENTIAL INHIBITION OF FIRST AND SECOND STRAND INCORPORATION OF dTTP BY PHOSPHONOFORMATE

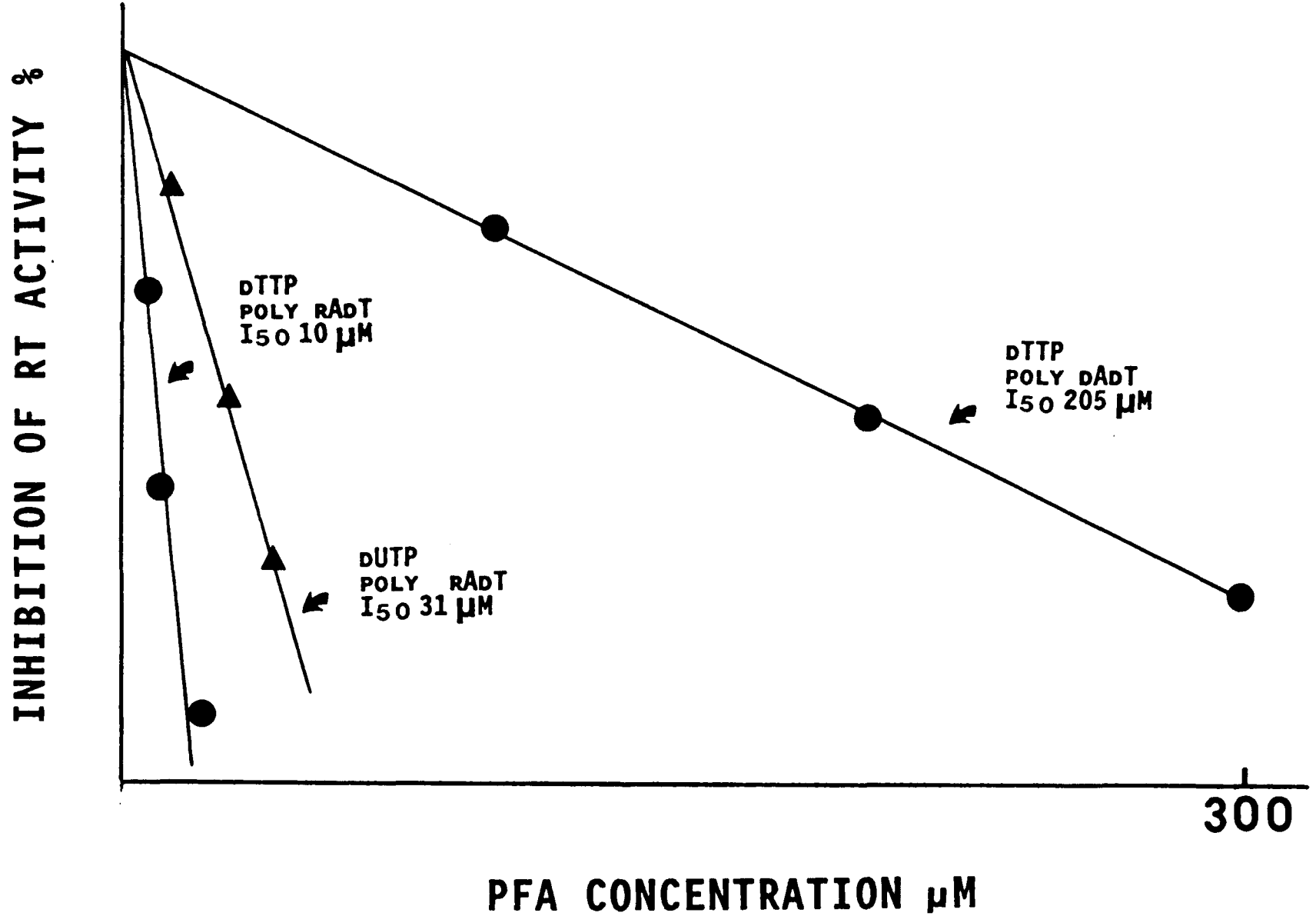



FIGURE 9

FIGURE 8

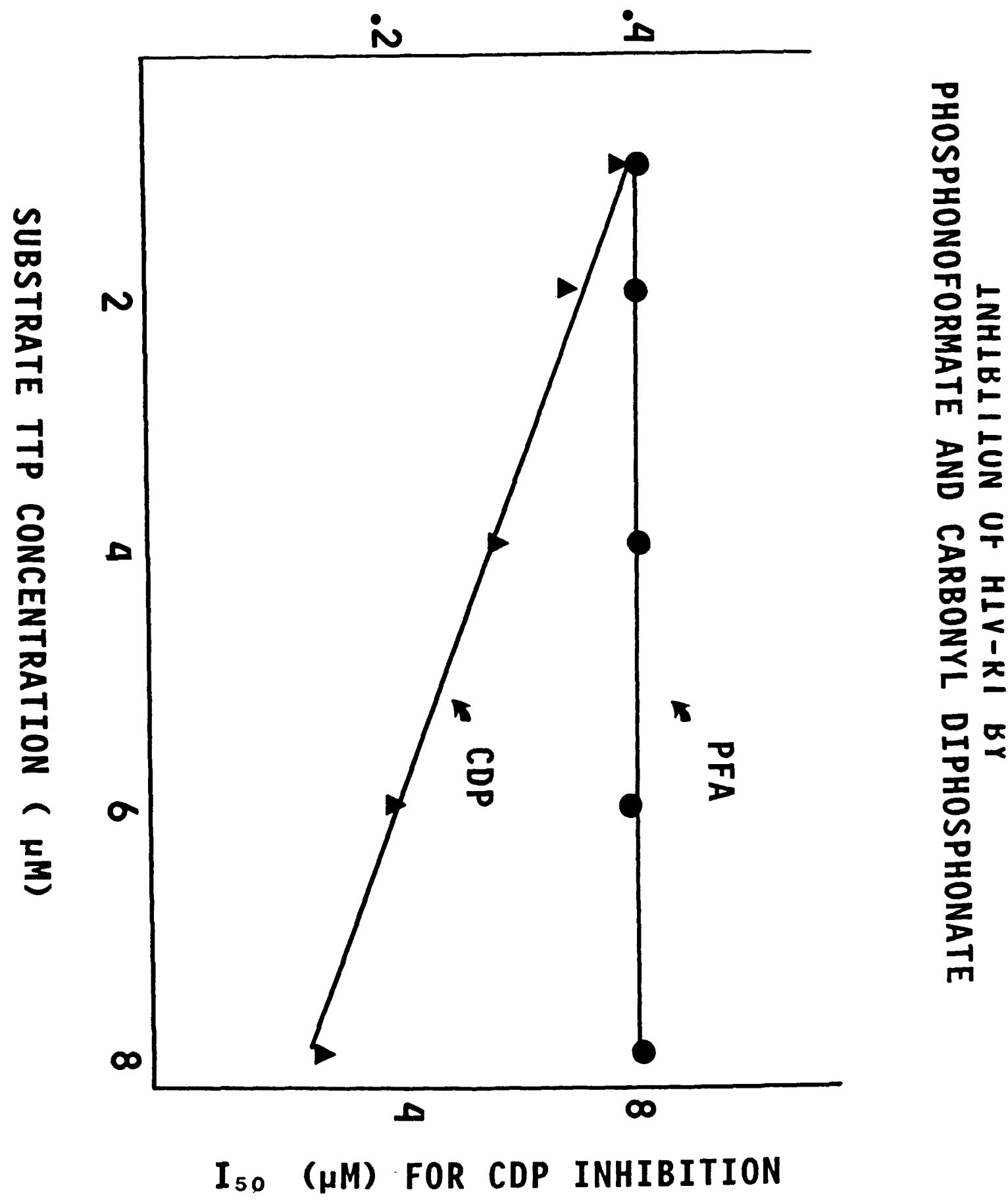

INHIBITION OF HIV-RT BY
PHOSPHONOFORMATE AND CARBONYL DIPHOSPHONATE
I50 (μM) FOR PFA INHIBITION
.2
.4
SUBSTRATE TTP CONCENTRATION ( μM)
2
4
6
8
CDP
PFA
4
8
I50 (μM) FOR CDP INHIBITION

FIGURE 13

# SENSITIVITY OF VIRAL REVERSE TRANSCRIPTASES TO INHIBITION BY PHOSPHONOFORMATE

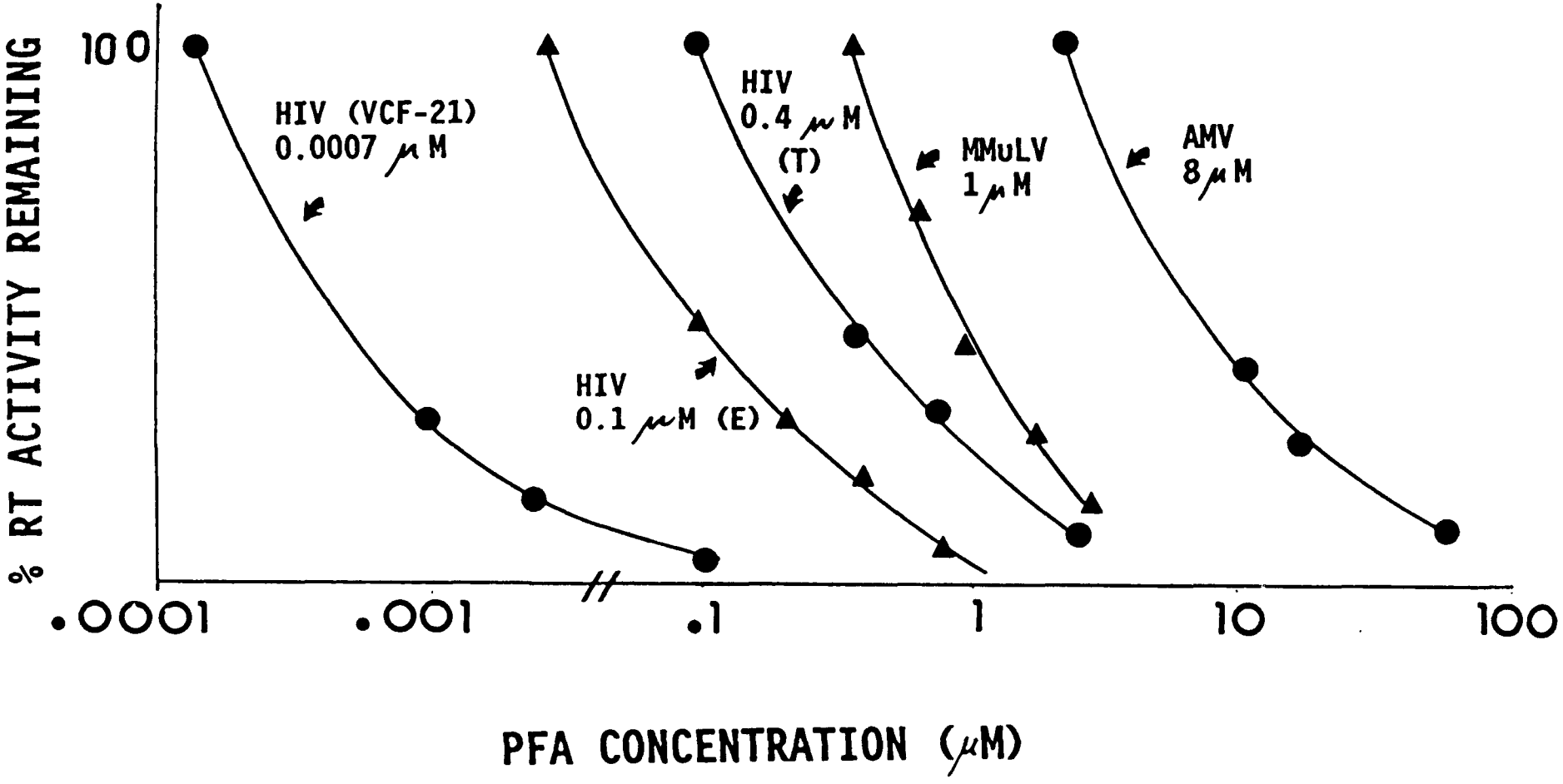


TABLE 2

# INFLUENCE OF PFA ON PROCESSIVE vs DISTRIBUTIVE INCORPORATION OF dTTP vs dGTP IN TEMPLATE COMPETITION ASSAYS

| TEMPLATE MIXTURE | NUCLEOTIDE INCORPORATION (DPM/10 MIN) | |
|---|---|---|
| | $^{32}$P-dGTP | $^{3}$H-dTTP |
| POLY rAdT ALONE | 0 | 6721 |
| POLY rCdG ALONE | 6865 | 0 |
| POLY rCdG + POLY rAdT | 0 | 6533 |
| POLY rCdG + POLY rAdT + PFA (10 $\mu$M) | 0 | 3142 |

MIXTURES OF $^{32}$P-LABELLED dGTP AND $^{3}$H-LABELLED dTTP WERE INCUBATED WITH AMV-REVERSE TRANSCRIPTASE PLUS THE INDICATED TEMPLATE MIXTURE. NOTE THAT DUE TO THE PROCESSIVE NATURE OF dTTP INCORPORATION, dGTP INCORPORATION IS COMPLETELY COMPETED OUT IN THE MIXED TEMPLATE SYSTEM BOTH IN THE PRESENCE AND ABSENCE OF PFA.

Table 3. List of Compounds Synthesized and Available for Screening.

| Compounds | Ref. # |
|---|---|
| 2',$O^2$-Anhydrouridine | KN-III-19 |
| 2',$O^2$-Anhydrocytidine hydrochloride | KN-II-95 |
| 3',5'-Di-O-benzoyl-2'-$O^2$-anhydrouridine | MS-II-5 |
| 5'-O-t-Butyldimethylsilyl-3'-O-benzoyl-2',O'-anahydrouridine | MS-I-45 |
| 2',3'-Anhydro-5'-O-trityluridine | MS-I-23 |
| 3'-Deoxy-2'-thymidinene | KN-II-55 |
| $N^3$-Benzyl-2',5'-di-O-trityluridine | KN-V-109 |
| 5'-O-t-Butyldimethylsilylanhydrouridine | MS-I-41 |
| $N^4$-Benzoyleytidine | KN-III-99 |
| 2',3'-Di-O-mesyl-5'-O-trityluridine | MS-I-53 |
| 5'-O-t-Butyldimethylsilyl-2',3'-isopropylideneuridine | KN-III-27 |
| 2',3'-Isopropylideneuridine | KN-II-53 |
| 2',3'-O-Sulfinyluridine | KN-V-99 |
| 2',3'-Benzylideneuridine | KN-IV-83 |
| $N^4$-Benzoyl-2',3'-O-Sulfinylcytidine | KN-III-109 |
| 2',3'-O-Sulfinylcytidine | KN-II-71 |
| 3',5'-Di-O-trityl-2'deoxy-2'-oxouridine | KN-VII-21 |
| 3',5'-Di-O-t-butyldimethylsilyl-2'-deoxy-2'-oxouridine | MS-III-5 |
| 2',5'-Di-O-t-butyldimethylsilyl-3'-deoxy-3'-owouridine | MS-III-8 |
| Diethyl (cholesteryloxycarbonyl) phosphonate | MS-I-44 |
| Disodium (cholesteryloxycarbonyl) phosphonate | MS-I-48 |
| Di-[1-(3-carboethoxypropyl)] cholexteryloxycarbonyl phosphonate | EB-I-9 |
| Di-(2,3-isopropylideneglyceryl) cholesteryloxycarbonyl phosphonate | EB-I-77 |

| | |
|---|---|
| Di-[1-(3-methylbutyl)] cholesteryloxycarbonyl phosphonate | KN-I-105 |
| Di-[1-(lithium 3-carboxypropyl)] cholesteryloxycarbonyl phosphonate | EB-I-14 |
| Sodium ethyl (cholesteryloxycarbonyl) phosphonate | MS-I-47 |
| Sodium 1-(3-carboxypropyl) 1-(30carboethoxypropyl) [cholesteryloxycarbonyl] phosphonate | EB-I-11 |

Adenosine 2',3'-Riboepoxide

Thymidine 5'-(1,3,2-dioxaphosphorin-2-oxide)

Thymidinene 5'-(1,3,2-dioxaphosphorin-2-oxide)

Thymidinene

2-Ethoxy-5-chloro-6-methyl-1,3,2-dioxaphosphorin-5-ene-2-oxide

2 Ethoxy-5-chloro-1,2-oxaphosphol-4-ene-2-oxide

2,4-dichloro-5-methyl-1,3,2-dioxaphosphole-2-oxide

2-methoxy-4,5-dimethyl-1,3,2-dioxaphole-2-oxide

Thymidine 3',5'-oxetane