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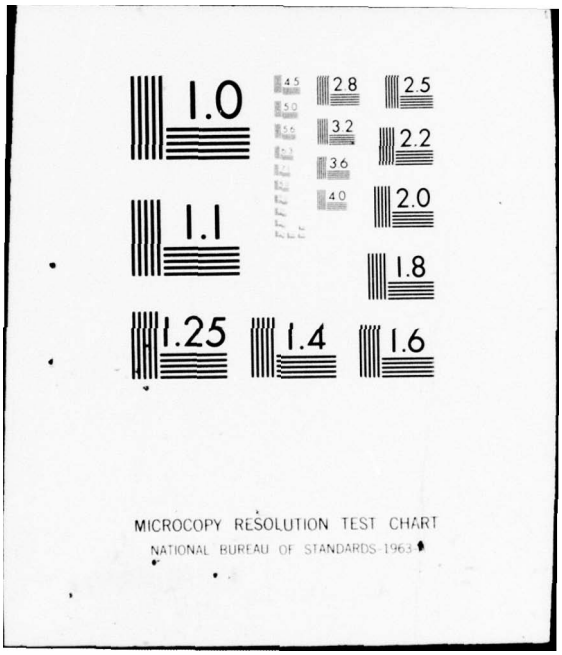
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Synthetic Analogs of Phospholipid Metabolites as Antimalarials

Annual Progress Report

(for the period 1 July 1976 - 30 June 1977)

By

Arthur F. Rosenthal, Ph.D.

September 28, 1977

Supported by

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Long Island Jewish - Hillside Medical Center
New Hyde Park, New York 11040

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
Initial year's progress is described toward the synthesis of analogs of phospholipids and of their biosynthetic intermediates as possible antimalarials of a new type. Emphasis was placed on phosphonic and phosphinic acid analogs of lecithin, phosphatidic acid, cytidine diphosphate diglyceride, and cytidine diphosphate choline. Nine compounds were submitted of which one was a major target and three were secondary target compounds. None of the substances so far tested showed appreciable antimalarial activity.

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CONTRACTOR: LONG ISLAND JEWISH-HILLSIDE
MEDICAL CENTER, NEW HYDE PARK, N.Y. 11040

PRINCIPAL INVESTIGATOR: Dr. Arthur F. Rosenthal,
Chief of Biochemistry, The Long Island
Jewish-Hillside Medical Center

(212) 470-2516

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1. Introduction.

This annual report describes our progress in synthesizing potential antimalarials of a novel type, which are designed to interfere with Plasmodial phospholipid metabolism. Very few prior attempts to produce antimalarial substances with this mode of action have been recorded, although a few previously known inhibitors of certain aspects of lipid metabolism (e.g., clofibrate) have been tested for antimalarial activity.

In part this almost complete neglect of Plasmodial phospholipid metabolism as a point on which to focus the design of new drugs is the result of paucity of substances which are known to interfere in any way, or with any specificity, with phospholipid metabolism in general. In addition, the crucial importance of phospholipid formation to the growth and reproduction of Plasmodia within its host has only begun to be appreciated very recently.

A much more detailed discussion of the biochemical rationale for this approach to the synthesis of new antimalarial drugs may be found in the original contract application. In the present report, the relevant metabolic steps will be mentioned only in passing. Furthermore, only those portions of the overall synthetic program which relate to progress during the first contract year will be discussed.

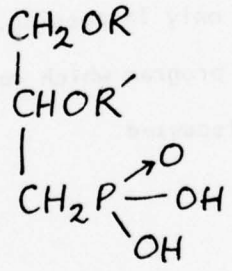
2. Synthetic Targets.

Each of the target compounds is an analog of a biosynthetic intermediate in phospholipid metabolism, either on the biosynthetic or catabolic side. Like their natural counterparts, these analogs can be produced with any of a variety of long-chain (R) groups, resulting in substances which may possess rather widely divergent physicochemical properties. This is particularly true in the case where two homologs differ in degree of unsaturation; this structural

feature is likely to result in more pronounced differences than are found in simple chain length homologs. The choice of R groups in the absence of medicinal activity data a priori must be arbitrary, and therefore other considerations have usually been predominant. Particularly, the question of probable technical simplicity in each synthetic step has generally dictated that in the lipid-containing analogs C_{16} and/or C_{18} saturated alkyl groups have been used. The generally ready crystallizability of such compounds makes the work-up following a synthetic step likely to be much simpler than would be the case with, e.g., polyunsaturated analogs. Should lead data suggest interesting medicinal activity of any of these substances, of course, new syntheses substituting various other R groups can be undertaken.

2.1 Phosphatidic Acid Analogs.

Phosphatidic acids occupy a key role in both the biosynthesis of glycerides and the formation of phospholipids. Diether phosphonate analogs of the following type



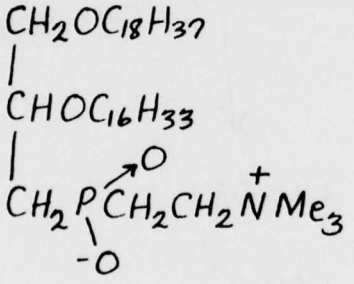
I

have been already shown (1,2) to be inhibitory towards phosphatidic acid phosphatase, a key enzyme in $\alpha\beta$-diglyceride formation. The synthetic scheme had already been worked out sometime previously (3), but for purposes of antimalarial testing, a new homolog, the di-C_{16}, was prepared. The synthetic steps (which were actually completed before the inception of the current contract) are shown

in Figure 1 and are discussed in considerable detail in the first quarterly contract report. Although the phosphonic acid proved to be without appreciable antimalarial activity, it is possible that it may be worthwhile to prepare a homolog with very different physicochemical properties (e.g., the dioleyl), since the activity of both these analogs and the natural substrates of phosphatidic acid phosphatase are highly dependent on physicochemical factors (1,2).

2.2. Lecithin Analogs.

Insofar as phospholipases can be inferred to be necessary for the phospholipid turnover of Plasmodial membranes, it seems reasonable to prepare a lecithin analog which has previously been shown to exert appreciable anti-phospholipase A (venom enzyme) and also anti-phospholipase C (clostridial enzyme) activity. This substance is a completely non-hydrolyzable analog of lecithin containing ether and phosphonate moieties instead of the normally labile carboxylic and phosphoric acid groups.



II

The synthesis of this compound has already been reported (4), so that it was necessary only to prepare the substance again on a scale large enough for extensive testing. In addition, its immediate synthetic precursor, the isopropyl ester chloride salt

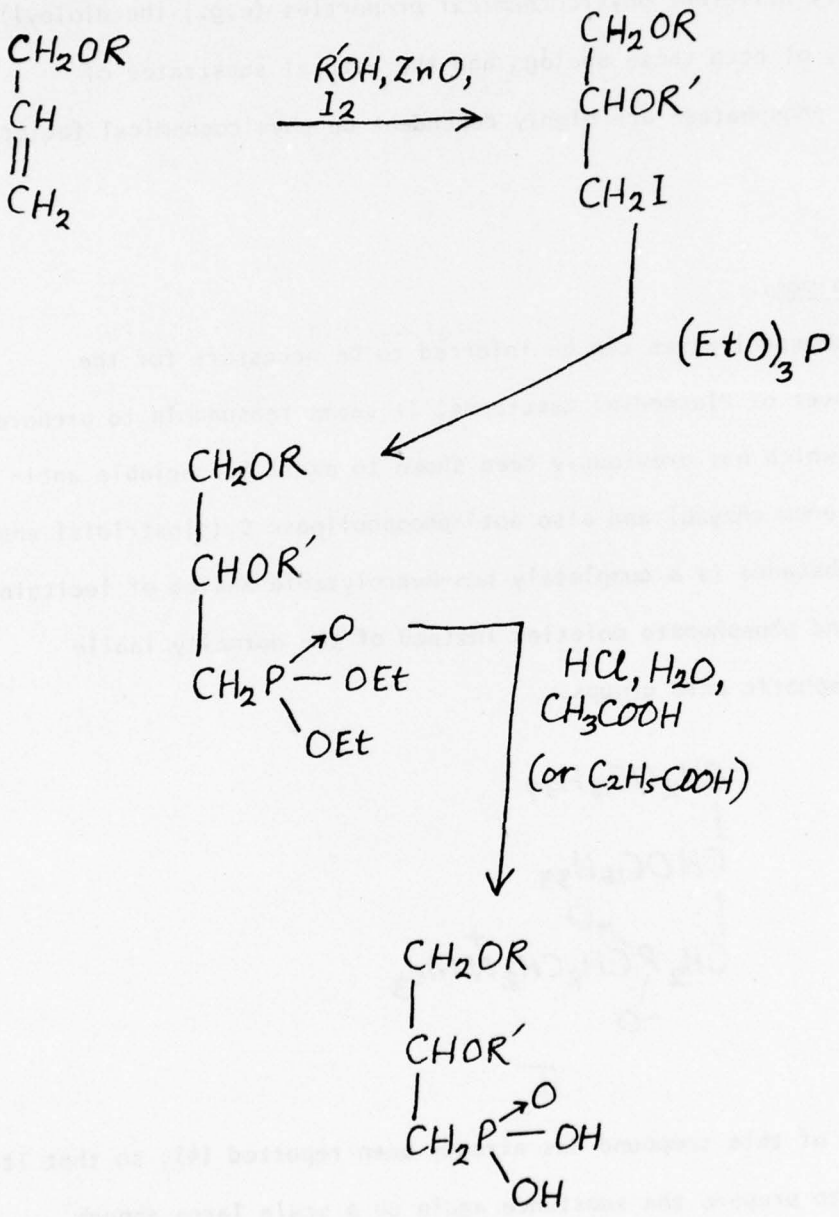
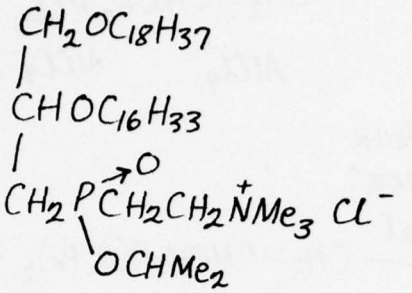

CH2OR
CH
CH2
R'OH, ZnO,
I2
CH2OR
CHOR'
CH2I
(EtO)3P
CH2OR
CHOR'
CH2P
O
OEt
OEt
HCl, H2O,
CH3COOH
(or C2H5COOH)
CH2OR
CHOR'
CH2P
O
OH
OH

FIG 1



III

was taken as a secondary target compound. Its lesser ionic charge was believed to offer some additional hope of increased intracellular penetrability.

The synthetic routes to these compounds is found in Figure 2. The major point of difficulty in the entire synthesis is at the beginning, in the preparation of diisopropyl allylphosphonite. This reactive, air-sensitive intermediate is prepared through a succession of other labile intermediates and must be used in the Arbuzov reaction at once, since it is not capable of storage for any length of time. Reaction with 2-hexadecoxy-3-octadecoxyiodopropane gave the two diastereomeric forms of isopropyl 2-hexadecoxy-3-octadecoxypropyl(allyl)phosphinate, which were separated from impurities by dry column chromatography on silica gel.

Osmate-periodate oxidation followed by borohydride reduction gave 2-hydroxyethyl phophinate; mesylation yielded the mesylate ester satisfactorily. Reaction with dimethylamine produced the 2-dimethylaminoethyl phosphinate. Quaternization with methyl iodide gave the isopropyl ester of the quaternary ammonium phosphinate as the iodide salt. The insolubility of this substance in cold ether provides a very convenient means of purifying it from ether-soluble contaminants. If direct quaternization of the mesylate by trimethylamine is attempted, only dehydromesylation results.

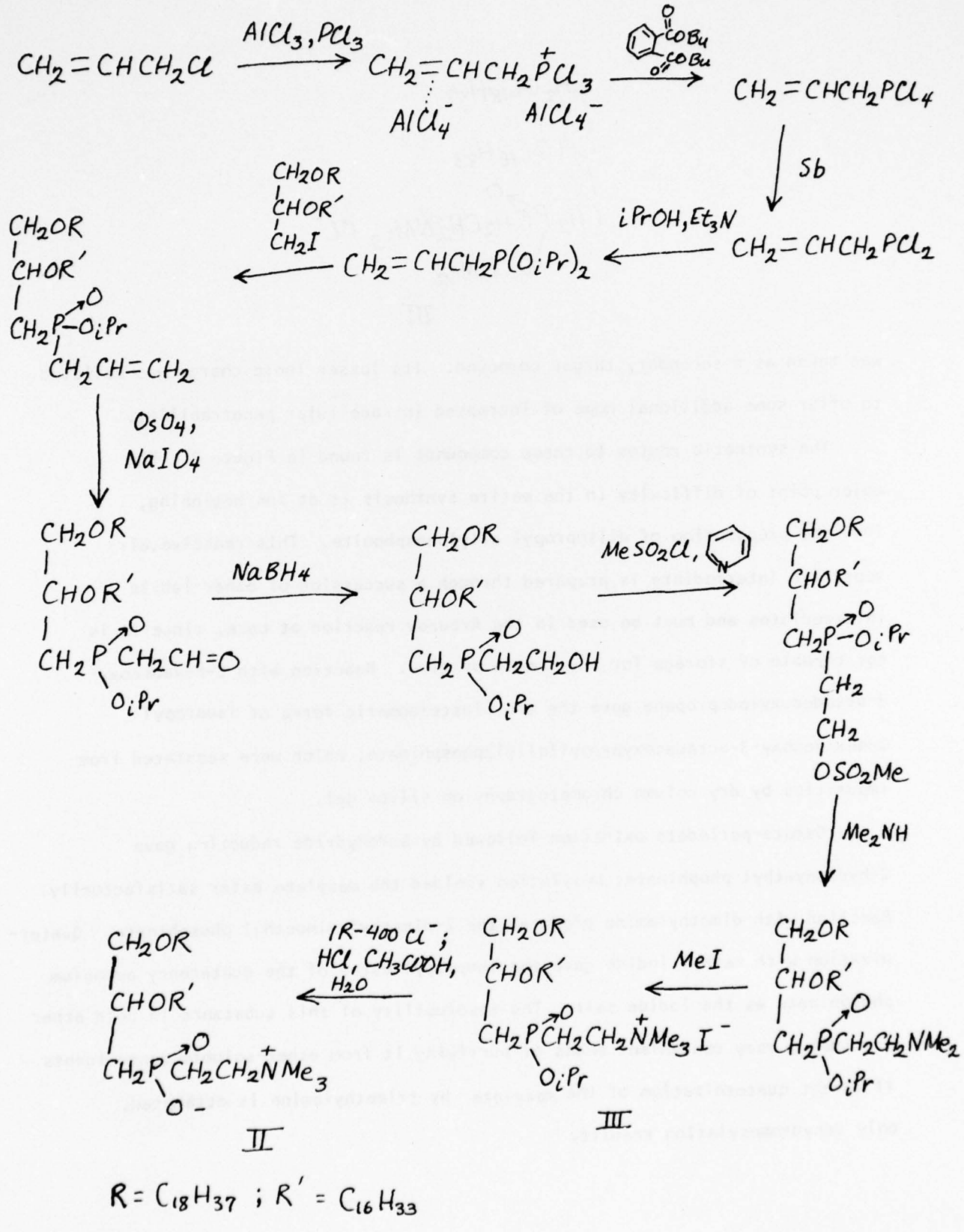

AlCl3, PCl3
Sb
iPrOH, Et3N
OsO4, NaIO4
NaBH4
MeSO2Cl,
Me2NH
MeI
IR-400 Cl⁻; HCl, CH3COOH, H2O
II
III
R = C18H37 ; R' = C16H33

FIG 2

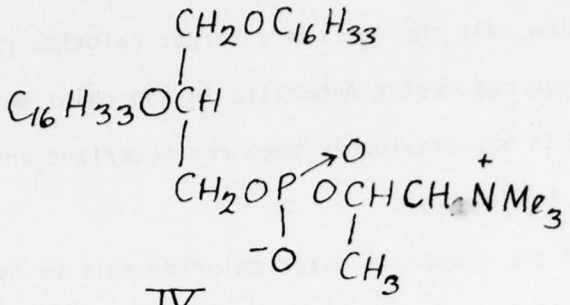
From the iodide salt the secondary target chloride III was readily prepared by ion exchange, using Amberlite IR-400 chloride in 2:1 methanol-chloroform. This salt had not previously been characterized and was purified by recrystallization for analysis.

Hydrolysis of the isopropyl ester chloride salt by hydrochloric acid in acetic acid gave the main target lecithin analog II, which of course had previously been characterized.

Full details of each step of this synthetic sequence are given in the first and second quarterly progress reports, and in reference (4).

Approximately 3.0 g of target compound 2 was submitted to WRAIR for testing. It was found to be inactive as an antimalarial agent. Enough of the compound was available for anti-Leishmanniasis testing
A certain amount of suppression noted, but not of sufficient degree to be considered an active agent. It is possible that other homologs might later be prepared which would show enhanced activity in this respect.

During the fourth quarter, a lecithin analog of rather different type was prepared and sent for antimalarial testing. Although no biochemical information was available on this compound, it is somewhat more similar to a natural lecithin than the phosphinate analog described above. It differs primarily in having an unusual, branched-chain base in place of choline.



IV

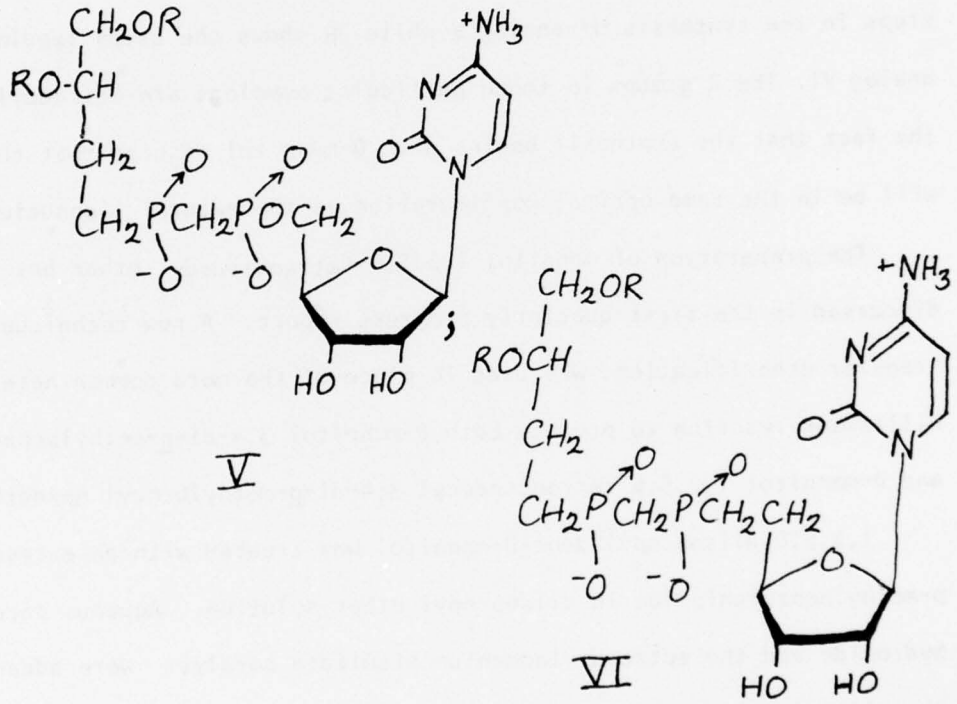
This synthesis was performed in a standard fashion. 1,2-dihexadecyl-sn-glycerol was prepared via D-mannitol, and phosphorylated with diphenylphosphoryl chloride. The protective phenyl ester moieties were removed by hydrogenation at room temperature and atmospheric pressure using a platinum catalyst. After isolation of the phosphatidic acid it was monoesterified by the tosylate salt of 2-hydroxy-1-(trimethylamino)propane, using trichloroacetonitrile in pyridine as the condensing agent. Precipitation by excess acetonitrile of the residue remaining after removal of volatile material gave a dark product. Passage through Amberlite MB-3 in tetrahydrofuran-water decolorized the product, which was finally purified by elution from a silica column. Final removal of silicic acid fines was accomplished by passage through a cellulose membrane filter. The material analyzed satisfactorily for a monohydrate form. More than 500 mg was submitted to WRAIR for antimalarial testing. This was too recent for us to have received a report as yet.

2.3. Analogs of Cytidine Diphosphate Diglyceride

Cytidine diphosphate diglycerides are a family of unique liponucleotides which are obligatory intermediates in the biosynthesis of phosphatidylserine,

with possible decarboxylation to phosphatidylethanolamine, of phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol and several other less common phospholipids. (The only exception to the previous statement is the fact that in some organisms deoxycytidine diphosphate diglyceride can substitute for the corresponding ribose liponucleotides; The requirement for cytidine molety is essentially absolute, however.)

The liponucleotide analogs which are the ultimate targets of these synthetic efforts are the following:



V

VI

The much greater complexity of these substances requires that by far the greatest portion of our synthetic efforts go into this portion of the work, and that directed toward the synthesis of the

cytidine diphosphate choline and cytidine diphosphate ethanolamine analogs discussed below (2.4). Moreover, these synthesis involve a number of relatively unexplored areas of organic phosphorus synthetic chemistry, in which analogous known cases are non-existent. Therefore, a significant effort must be expended to find the most suitable way to perform a particular synthetic step. Thus, progress in this area is always somewhat slower than it might appear a priori.

Figure 3 shows the portion of the synthetic scheme which is common to both types of liponucleotide analog. Figure 3a illustrates the remaining steps in the synthesis of analog V while 3b shows the steps leading to analog VI. The R groups in these particular homologs are octadecyl; the fact that the synthesis begins with D-mannitol assures that the final product will be in the same optical configuration as the natural liponucleotides.

The preparation of mannitol 1,2,5,6-tetraoctadecyl ether has been discussed in the first quarterly progress report. A new technique, phase-transfer etherification, was used in place of the more common heterogeneous Williamson reaction to produce both D-mannitol 3,4-di-p-methylbenzyl ether and D-mannitol 1,2,5,6 tetraoctadecyl 3,4-di-p-methylbenzyl hexaether.

1,2,5,6 Diisopropylidene-D-mannitol was treated with an excess of p-methylbenzylchloride in diisopropyl ether solution. Aqueous potassium hydroxide and the tetrabutylammonium bisulfate catalyst were added and the mixture stirred below the boiling point of the solvent. Work-up afforded the diether in good yield after hydrolysis of isopropylidene groups. Treatment of this product with an excess of octadecyl bromide in a mixture of isopropyl ether and tetrahydropyran afforded the hexaether, which after preliminary purification was isolated by dry column chromatography

on a hybrid silica-alumina column.

Hydrogenation of the hexaether was readily accomplished, using a borohydride-reduced paladium catalyst. Full experimental details of this portion of the synthesis are given in the first quarterly report. Purified samples of the tetraether and hexaether intermediates were submitted to WRAIR for testing. To date no reports have been received for either substance.

Reaction with periodic acid in tetrahydrofuran gave D-glyceraldehyde dioctadecyl ether, which was immediately reacted with phenyl chloromethyl-(triphenylphosphinemethylene)phosphinate to give phenyl R-3,4-dioctadecoxybut-1-enyl(chloromethyl)phosphinate. Some considerable difficulty was found in purifying this chloromethyllipid by silicic acid chromatography, so that ultimately it was used in succeeding steps without complete purification. Experimental details are given in the second quarterly report, along with additional discussion of the problems of purification. Further reaction necessitated the preparation of additional amounts of certain intermediates. In fact, a large portion of the second and third quarters was concerned with building up stocks of particular intermediates which were required for further synthetic steps.

Tris(trimethylsilyl)phosphite had previously been prepared from trimethylsilyl chloride, triethylamine, and phosphorous acid; however, it became evident that the filtration step which was necessary in this preparation to remove triethylamine hydrochloride would make the procedure very cumbersome for large scale preparations. Thus, it was found that warming phosphorous acid in acetonitrile solution with trimethylsilyldiethylamine was a superior means of preparing the silylphosphite, and in fact produced higher yields. The silylamine, in turn, had to be prepared on a large scale in order to prepare the phosphite, and also bis(trimethylsilyl)trimethylsilyoxymethylphosphonite.

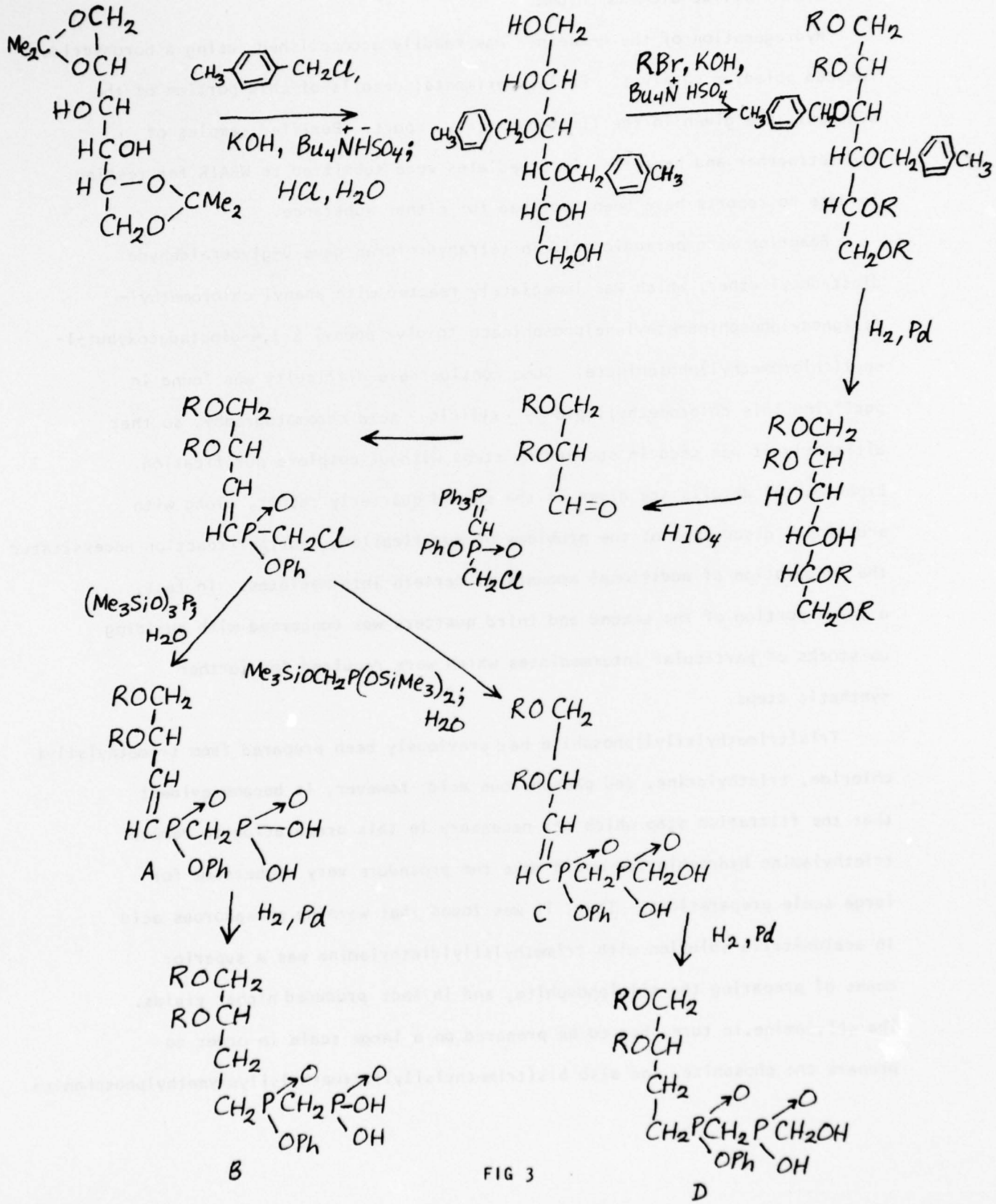
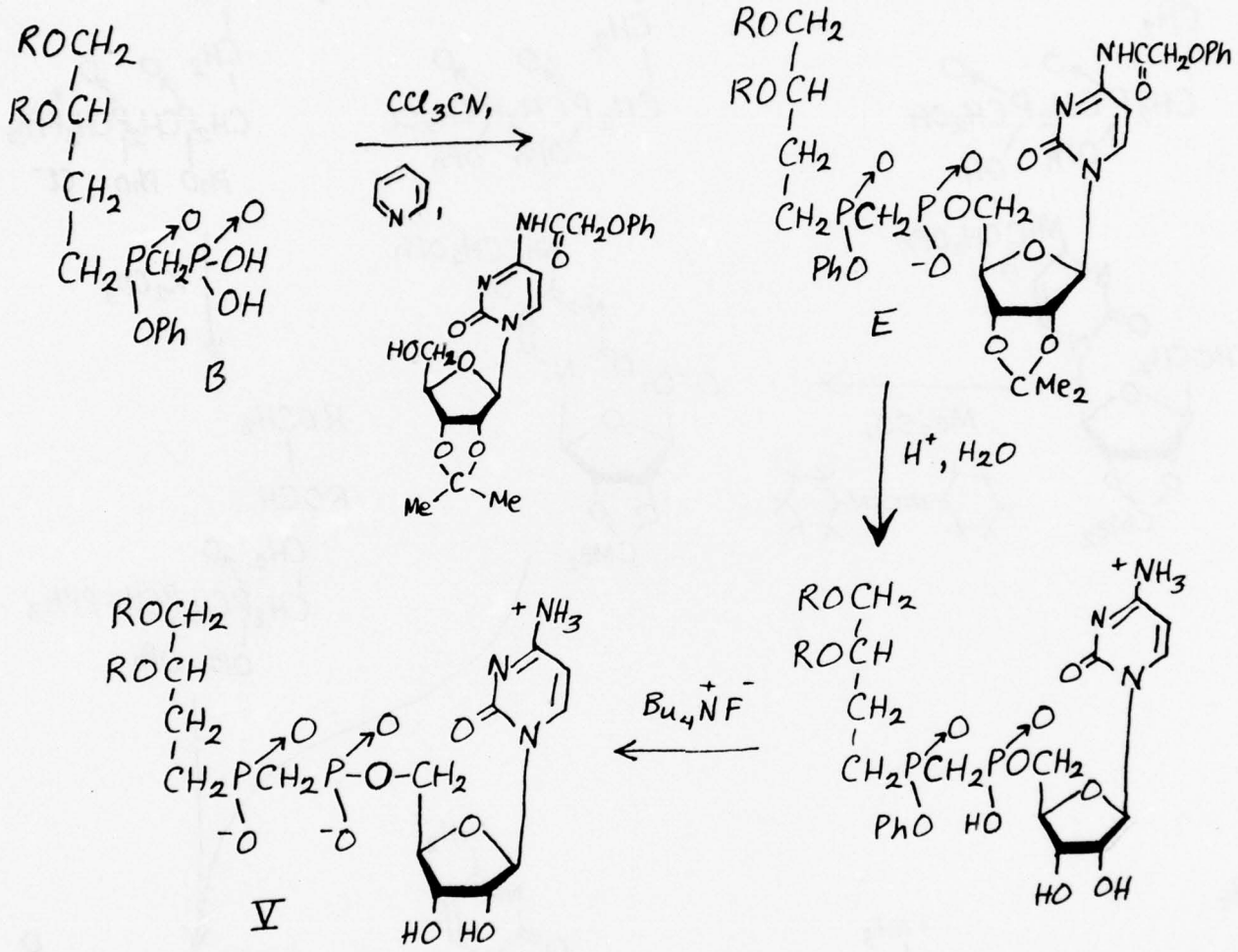

KOH, Bu4NHSO4; HCl, H2O
RBr, KOH, Bu4N HSO4
H2, Pd
HIO4
(Me3SiO)3P; H2O
Me3SiOCH2P(OSiMe3)2; H2O
A
B
C
D

FIG 3



$R = C_{18}H_{37}$

FIG 3a

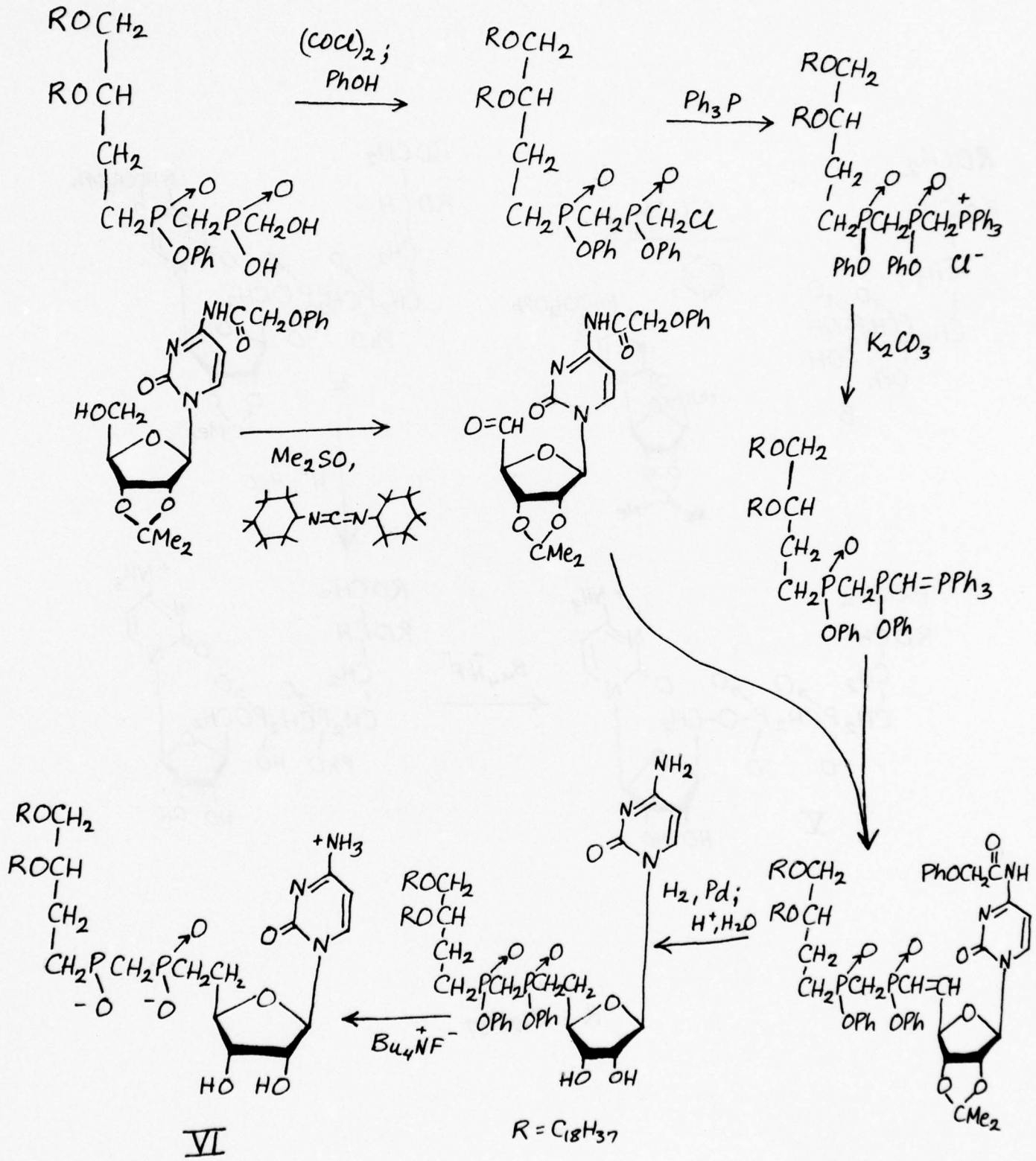

ROCH2
ROCH
CH2
CH2P CH2P CH2OH
OPh OH
(COCl)2;
PhOH
CH2P CH2P CH2Cl
OPh OPh
Ph3P
CH2P CH2P CH2PPh3
PhO PhO Cl-
K2CO3
NHCCH2OPh
HOCH2
Me2SO,
N=C=N
CMe2
O=CH
CH2P CH2PCH=PPh3
NH2
+NH3
H2, Pd;
H+,H2O
Bu4N+F-
HO HO
HO OH
PhOCH2CNH
R = C18H37
VI

FIG 3b

A large-scale preparation of the silylamine was therefore undertaken; the reactants are trimethylsilylchloride and diethylamine. The large amount of diethylamine hydrochloride which is produced must also be filtered off, which is quite tedious on a very large scale, but is preferable at this step since the product is not readily oxidizable in air as are the phosphite or phosphonite.

From the silylamine was prepared tris(trimethylsilyl)phosphite and bis(trimethylsilyl)trimethylsilyoxymethylphosphonite, and both were used to carry out further steps. An additional quantity of phosphinic-phosphonic acid phenylester was prepared for condensation with protected cytidine derivatives to explore the synthesis of analog V.

This analog and the corresponding analog of cytidine diphosphatecholine required a suitable quantity of a protected cytidine derivative. For this purpose N-phenoxyacetyl isopropylidene cytidine was prepared from 2,4-dinitrophenyl phenoxyacetate (in turn prepared from phenoxyacetyl chloride and 2,4-dinitrophenol) and isopropylidene cytidine. N-acetyl isopropylidenecytidine was also prepared from acetic anhydride and isopropylidenecytidine. These preparations are detailed in the second quarterly report.

In order to work out conditions for the condensation of the phosphonic acid with the cytidine derivatives to produce analog V, a thin-layer chromatographic study was first undertaken. Since the phosphinic-phosphonic acid was, of course,difficult to prepare, the conditions were worked out using a model long-chain phosphonic acid, 2-hexadecoxy-3-octadecoxypropylphosphonic acid. As was evident from the TLC studies, the condensation would occur under relatively mild conditions in the presence of trichloroacetonitrile. Deprotection of the resulting condensed product was more difficult, but ultimately was achieved.

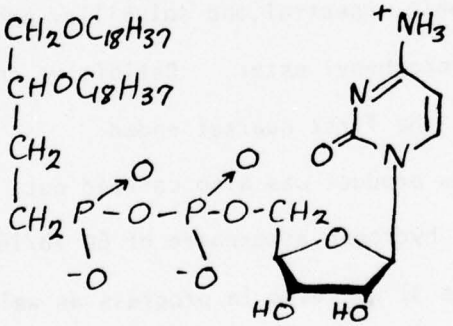
It then became possible to prepare a much larger quantity of the condensed model compound, and to purify it and send it to WRAIR for testing. More than 500 mg of cytidine 5'-2"-hexadecoxy-3"-octadecoxypropylphosphonate was sent; antimalarial testing data indicated that it was inactive. This may not be a surprising finding in view of the fact that, unlike the natural coenzyme, it contains no central P-O-P or its isostere.

The successful preparation of the model liponucleotide naturally indicated that the actual phosphinic-phosphonic acid compound B (figure 3) should be employed. Unfortunately, only a small amount of this substance was available and a thin-layer chromatographic study appeared the only feasible method of investigating this condensation reaction for the present. The preparation of much larger amounts of this acid, which should allow isolation and direct characterization of intermediates, must await the preparation of more tris-(trimethylsilyl)phosphite, which in turn is awaiting the large-scale preparation of trimethylsilyl diethylamine. The preparation of additional N-phenoxyacetyl isopropylidenecytidine is also being awaited for preparation of the final compound on sufficient scale.

In any event, it was quickly found that reaction of the acid B with the protected cytidine required more vigorous conditions than was the case of the model compound. It is possible that steric or other factors retard the condensation; when it was carried out at 50° overnight, very little condensed product (presumably E, figure 3a) was formed. However, raising the temperature to 70° while maintaining the reaction period and other conditions (pyridine solution, trichloroacetonitrile as condensing agent) showed that a reasonable yield of E was formed. A preliminary purification by precipitation with cold acetonitrile gave a product which retained a small amount of the protected cytidine, plus some polar, presumably acidic material,

but was otherwise mainly the desired product. Identification, as in the previous TLC studies with the model condensation, were made according to the spots which were UV absorbing (at about 260 nm) and contained phosphorus (Dittmer-Lester reagent). Only the condensation product should fulfill both criteria. The Rf values in chloroform-methanol-water-acetic acid (80:13:8:0.3) and in chloroform-methanol-formic acid (44.5:5:0.5) approximated those which would be expected from a protected liponucleotide of the expected degree of polarity.

Hydrolysis of the acid-labile protecting group was carried out in trifluoroacetic acid containing a small amount of water for 24 hours at 45°. Evaporation of volatile material, followed by solution of the residue in chloroform, filtration, re-evaporation, and finally precipitation by cold ether from a concentrated chloroform solution, yielded a product which appeared to be almost homogeneous by TLC. Since its Rf in the former solvent (above) was 0.6 compared to 0.58 for the following liponucleotide analog:



, it is at present uncertain whether the trifluoroacetic acid treatment removed the protective phosphinic phenyl ester group. It was anticipated that a fluoride treatment would be necessary to remove this moiety, and it seems doubtful that the acid treatment would hydrolyze the phenyl ester without removing the cytidine ester group as well. Definitive characterization has not as yet been

possible. Infrared indicates no clear phenyl-type absorption, but these would occur in regions where other expected absorptions are found. The milligram quantities of materials so far available have not allowed nmr, which would have been definitive, to be performed. The large molecular weight also has militated against the possibility of obtained useful mass spectra, even if the compounds were derivatized. Therefore, a final answer must await the preparation of larger samples, for which we have some of the intermediates, but not others, on a sufficient scale as yet.

The synthesis and characterization of the bis-phosphinic acid derivative C and its dihydro derivative D were also the subjects of considerable effort during the fourth quarterly period. Reaction of crude phenyl 3,4-dioctadecoxybut-3-enyl(chloromethyl)phosphinate with bis(trimethylsily)trimethylsilyoxymethyl phosphonite on a small scale gave, after removal of excess phosphonite and other volatile material, followed by hydrolysis, a compound in reasonably homogeneous form which showed chromatographic, spectral, and solubility properties expected of the bis-phosphinic acid monophenyl ester. Definitive characterization of this substance was underway as the first quarter ended.

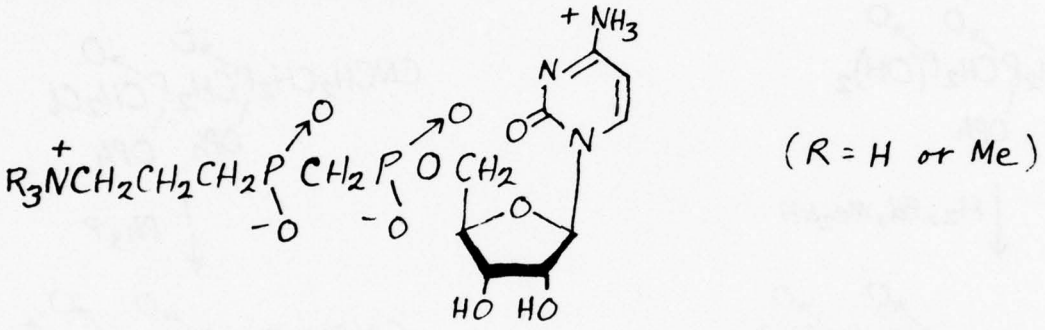
Hydrogenation of this product was also carried out, using 10 percent paladium on charcoal in a hydrogen atmosphere of 60 lb/in^2. Characterization of this product (D, figure 3) was also in progress as well.

2.4. Analogs of Cytidine Diphosphate Choline and Cytidine Diphosphate Ethanolamine.

The non-liponucleotide coenzymes cytidine diphosphate choline and -ethanolamine utilized 1,2-diglyceride rather than phosphatidic acid as the co-reactant, to produce lecithin and phosphatidyl ethanolamine directly. The coenzyme thus acts as a phosphorylated base donor rather than as a phosphatidyl donor,

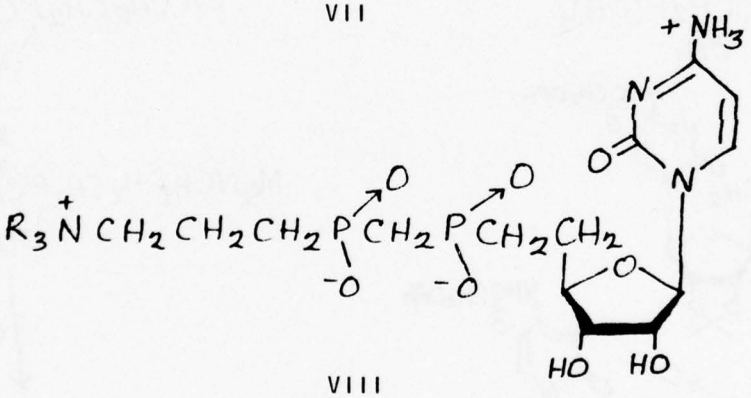
as in the case of CDP-diglyceride. This route is probably the major one for the formation of lecithin at least, and interference with the reaction should exert a significant effect on membrane phospholipid formation.

The target analogs of the base-dinucleotides have the same relationship to their natural coenzymes as do the liponucleotide analogs discussed above, and have the following structure:



(R = H or Me)

VII



VIII

The synthetic route to these compounds, however, is rather different from that discussed in 2.3 above. This is occasioned by the obvious difference between the base moiety and the diglyceride analog moiety. However, it should be carefully noted that certain features of the synthesis of compounds VII and VIII will serve as model reactions for analogous synthetic steps in the liponucleotide analog syntheses. The reverse, of course, is also the case. The synthetic schemes are shown in figures 4a and 4b. The preparation of the initial intermediate F of figure 4 has been the object of a great deal of investigation during the latter phases of this first annual project. Three separate and distinct synthetic routes were considered; these are shown in figure 5. Figure 5a

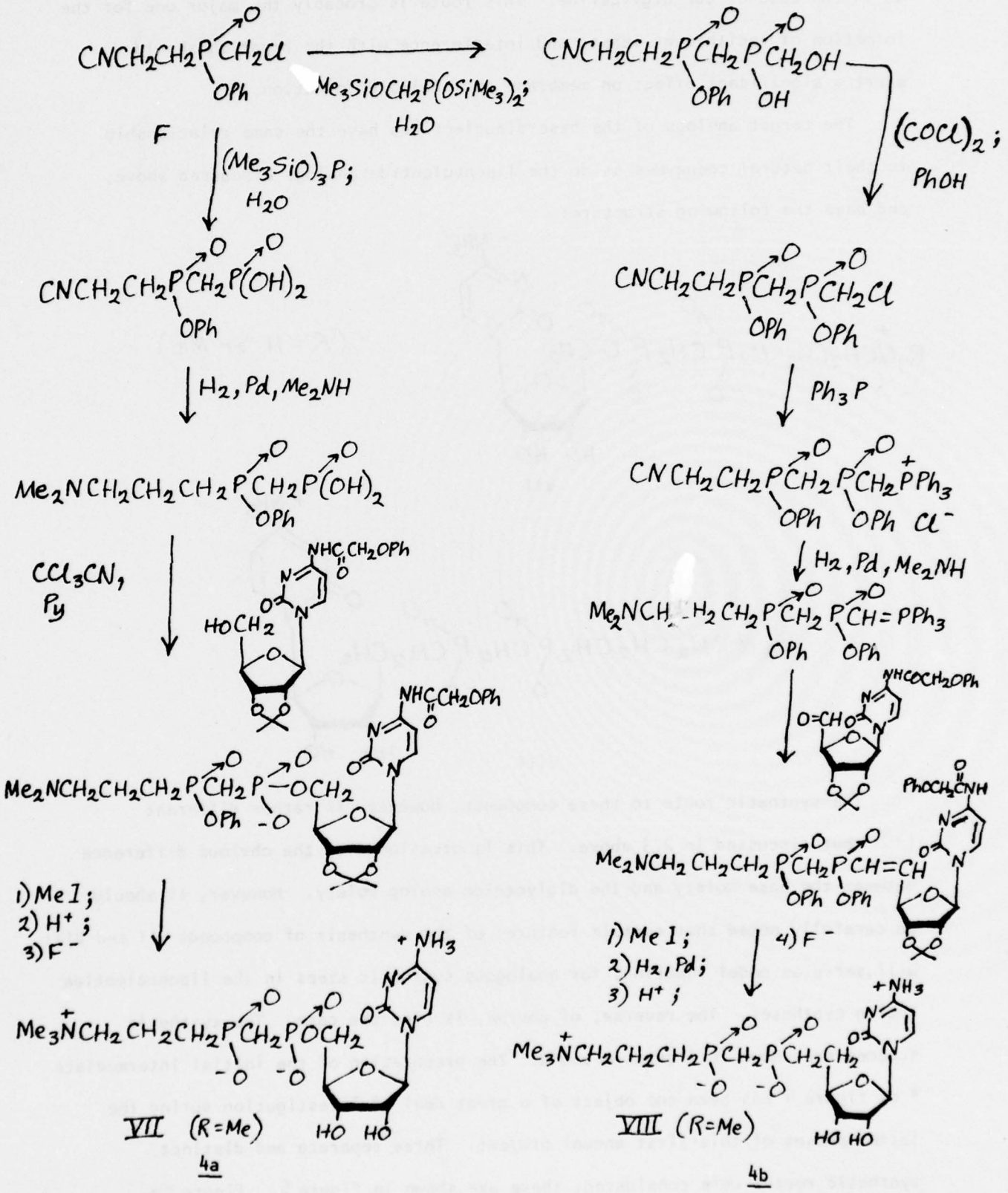

CNCH2CH2P(O)(OPh)CH2Cl
Me3SiOCH2P(OSiMe3)2; H2O
F
(Me3SiO)3P; H2O
CNCH2CH2P(O)(OPh)CH2P(O)(OH)2
H2, Pd, Me2NH
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OH)2
CCl3CN, Py
NHC(O)CH2OPh
HOCH2
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(O-)OCH2
1) MeI; 2) H+; 3) F-
+NH3
Me3N+CH2CH2CH2P(O)(O-)CH2P(O)(O-)OCH2
HO HO
VII (R=Me)
4a
CNCH2CH2P(O)(OPh)CH2P(O)(OH)CH2OH
(COCl)2; PhOH
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH2Cl
Ph3P
CNCH2CH2P(O)(OPh)CH2P(O)(OPh)CH2P+Ph3 Cl-
H2, Pd, Me2NH
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OPh)CH=PPh3
NHCOCH2OPh
O=CHO
PhOCH2CNH
Me2NCH2CH2CH2P(O)(OPh)CH2P(O)(OPh)CH=CH
1) MeI; 2) H2, Pd; 3) H+; 4) F-
Me3N+CH2CH2CH2P(O)(O-)CH2P(O)(O-)CH2CH2
VIII (R=Me)
4b

Fig. 4

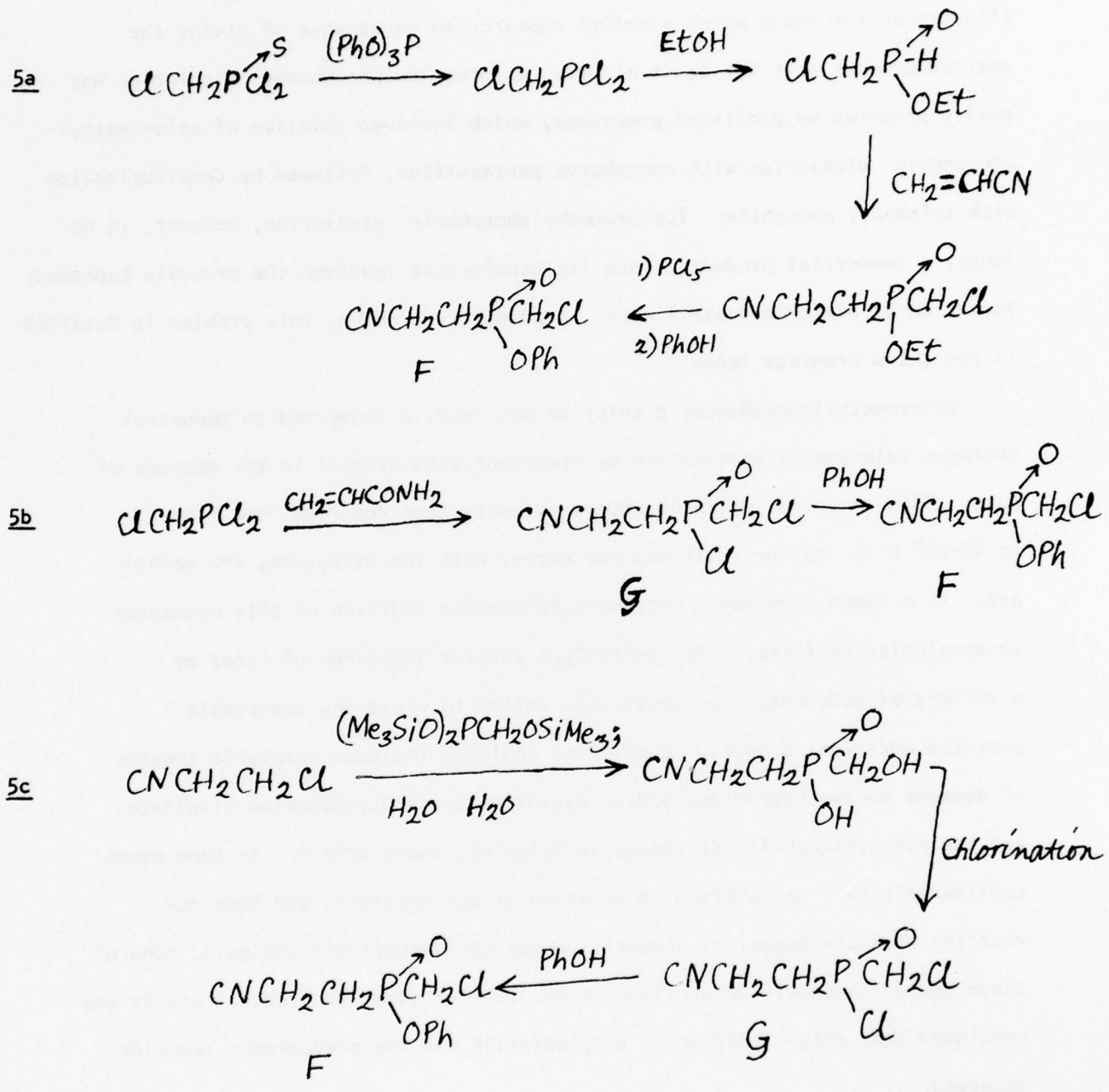

5a
ClCH2PCl2
(PhO)3P
ClCH2PCl2
EtOH
ClCH2P(O)(H)OEt
CH2=CHCN
CNCH2CH2P(O)(OEt)CH2Cl
1) PCl5
2) PhOH
CNCH2CH2P(O)(OPh)CH2Cl
F
5b
ClCH2PCl2
CH2=CHCONH2
CNCH2CH2P(O)(Cl)CH2Cl
G
PhOH
CNCH2CH2P(O)(OPh)CH2Cl
F
5c
CNCH2CH2Cl
(Me3SiO)2PCH2OSiMe3;
H2O H2O
CNCH2CH2P(O)(OH)CH2OH
Chlorination
CNCH2CH2P(O)(Cl)CH2Cl
G
PhOH
CNCH2CH2P(O)(OPh)CH2Cl
F

Fig. 5

illustrates the route which a priori appeared to be capable of giving the desired product with the least effort. Chloromethylphosphonous dichloride was easily prepared by published procedure, which involved thiation of chloromethylphosphonic dichloride with phosphorus pentasulfide, followed by desulfurization with triphenyl phosphite. (Chloromethylphosphonic dichloride, however, is no longer a commercial product, since its manufacture involves the probable byproduct formation of bis(chloromethyl)ether, a potent carcinogen; this problem is detailed in the third progress report).

Chloromethylphosphonous dichloride was readily converted to monoethyl hydrogen chloromethylphosphonite by treatment with ethanol in the absence of base. This compound, which has not previously been reported, had a bp at 48-50° C at .05 torr. IR and nmr agreed with the structure; the methyl ester is a known compound. Attempts to produce addition of this monoester to acrylonitrile (itself, incidentally, a suspect substance of late) by a variety of base-catalytic conditions failed to yield any detectable expected addition product. Conditions employed included catalytic amounts of aqueous sodium hydroxide; sodium hydroxide-tetrabutylammonium bisulfate; and the non-nucleophilic diisopropylethylamine, among others. In some cases considerable heat was evolved on addition of the catalyst, and some new reaction products appeared; however, on gas chromatographic analysis, none of these could reasonably be ascribed to the desired addition product, and it was concluded that polymerization of acrylonitrile was the predominant reaction observed.

The reported reaction of chloromethylphosphonous dichloride (5) with acrylamide to give the dichloride G was next tried. The reaction mixture became very viscous, black, and completely failed to yield any significant amount of the desired product on attempted high vacuum distillation. It was thereby concluded, by a process of elimination, that route 5c would have to serve

to produce the important intermediate. A satisfactory reaction of 3-chloropropionitrile with bis(trimethylsily)trimethylsilyoxymethylphosphonite had, of course, previously been observed, yielding on aqueous hydrolysis cyanoethyl(hydroxymethyl)phosphinic acid (9). Careful subsequent investigation showed that the acid is usually contaminated with byproducts which may be difficult to remove at a later stage and which may even interfere with subsequent reactions, however. Thus, it became important to purify the acid as much as possible before further synthetic steps were undertaken.

Purification was accomplished by recrystallization several times from acetone-hexane mixtures from room temperature to 5°. The procedure was accompanied by appreciable losses of material, so that it was not entirely optimal,but was the most satisfactory found. Fortunately, purity of the acid could be checked quite easily by gas chromatography after silylation with bis(trimethylsily)trifluoroacetamide. None of the samples of the acid were grossly contaminated, and all could be purified to a satisfactory state of homogeneity by recrystallization. The product appeared at a retention time of 4.9 minutes (3% OV-17, 170°) while all the impurities appeared at shorter retention times. Evidence cited below indicates strongly that the two major and most pertinent impurities were 2-cyanoethylphosphonic acid and hydroxymethylphosphonic acid; the latter almost certainly arises from oxidation and hydrolysis of the starting silyl reagent.

Conditions under which cyanoethyl(hydroxymethyl)phosphinic acid could be chlorinated and converted to its phenylester to produce the intermediate F were the subject of a great deal of investigation as well. Treatment with thionyl chloride followed by phenol in pyridine completely failed to give the desired material in any appreciable yield. Milder conditions using triphenylphosphine and carbon tetrachloride, followed by phenol, gave a complex mixture which also concluded not to be preparatively useful. It seems to be clear

after considerable study that the presence of a base, at least for the phenylation,was undesirable. Besides pyridine and triethylamine, the non-nucleophilic bases diisopropylethylamine and 2,6 di-tert-butylpyridine were tried, all without success.

Finally, a very simple procedure was found to give the product quite satisfactorily, by the use of oxalyl chloride for chlorination; and following removal of excess reagents in vacuo, phenol was added and the mixture warmed for some hours under reduced pressure to remove the liberated hydrogen chloride. Excess phenol was removed from the reaction product after cooling by repeated extraction with sodium bicarbonate solution, followed by high vacuum sublimation of any residual phenol.

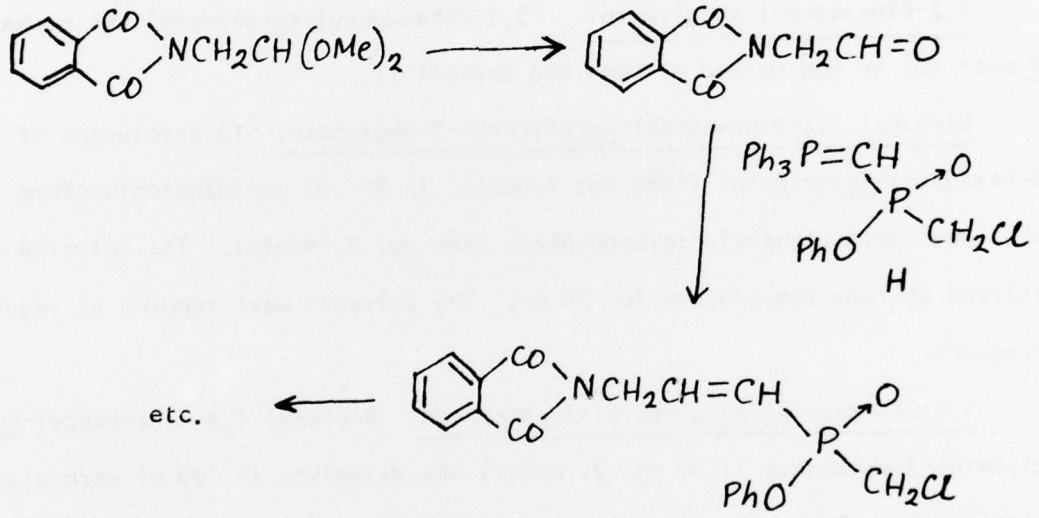
The composition of the crude reaction product could be followed readily by gas chromatography, using both a flame ionization detector and a phosphorus-sensitive detector. On 3% OV-17 at 200°, the product eluted at 12.0 min. In some samples, one or two contaminants were also noted; the earlier one eluting at 13.5 min. and the later one eluting at this temperature at 33.1 min. Combined gas chromatography-mass spectroscopy confirmed the main peak as the desired product and showed that the 13.5 min. peak was diphenyl chloromethyl-phosphonate,which apparently arises from an excess of the original silyl reagent remaining in the reaction mixture prior to hydrolysis.

The 33.1 min. impurity was more interesting and was found to be diphenyl cyanoethylphosphonate. This substance almost certainly arose at the stage of Arbuzov reaction; it must have arisen by contamination of the silyl reagent with tris(trimethylsilyl)phosphite. Except for the fact that the silyl reagent was twice distilled before use, and tris(trimethylsilyl)phosphite has a distinctly lower boiling point, the presence of the latter is most logically explained by contamination of the hypophosphorous acid at the first step

with phosphorous acid. Possibly on storage, some sort of disproportionation leading to tris(trimethylsilyl)phosphite formation may have occurred as well.

Methods to completely purify the main product fraction had just begun to be studied at the conclusion of the first year's work. In any event, the impurities were largely minimized by the recrystallization procedure described above for the phosphinic acid.

A secondary access route into the cytidine diphosphate choline analog was investigated for a time during the fourth quarter. This begins with the commercially available phthalimidoacetaldehyde dimethylacetal:



The acetal was satisfactorily hydrolyzed to the corresponding aldehyde, but reaction with the ylid H gave a number of products in addition to what was apparently the desired compound. Triphenylphosphine oxide (revealed by gas chromatography) contaminated the reaction mixture and, like the other contaminants,

was very difficult to remove. No satisfactory purification procedure could be found; and since in addition the reaction did not seem to go in satisfactory yield, this route was at least temporarily set aside.

EXPERIMENTAL

Only the portion of the annual report which deals with the fourth quarter's work is discussed in this section. The preceding three quarterly reports give full experimental details for the work done during those respective periods.

1,2-Dihexadecyl-sn-glycerol-3-phosphoryl-1'-methylcholine, IV.

1,2-Dihexadecyl-sn-glycerol. 1,2-Dihexadecyl-sn-glycerol was prepared from D-mannitol by the method of Chen and Barton(6).

Diphenyl 1,2-Dihexadecyl-sn-glycerol-3-phosphate. To a solution of 1,2-dihexadecyl-sn-gylcerol (1080 mg; 2.mmols) in 25 ml pyridine-chloroform (4:1; v/v) was added diphenylchlorophosphate (540 mg; 2. mmols). The solution was stirred at room temperature for 24 hr. The solvents were removed at reduced pressure.

1,2-Dihexadecyl-sn-glycerol-3-phosphate. Diphenyl 1,2-dihexadecyl-sn-glycerol-3-phosphate (1540 mg; 2. mmols) was dissolved in 100 ml warm glacial acetic acid. This solution was slowly added to a vigorously stirred suspension of reduced platinum in glacial acetic acid (100 ml) and reduced with hydrogen at room temperature and atmospheric pressure. When hydrogen was no loger taken up, chloroform (200 ml) was added and the mixture filtered through a sintered glass filter. The platinum was washed with another 25 ml of chloroform. The filtrate and washings were combined and the solvents removed at reduced pressure. The remaining white solid was dissolved in 2-propanol and again taken to dryness at reduced pressure.

1,2-Dihexadecyl-sn-glycerol-3-phosphoryl-1'-methylcholine. To a solution of 1,2-dihexadecyl-sn-glycerol-3-phosphate (1240 mg; 2 mmols) in pyridine (50 ml) was added a 10-fold excess (5.8 g) of 2-hydroxy-1-(trimethyl amino) propane tosylate salt (7). Trichloroacetonitrile (20 ml) was added and the suspension was stirred at 50° C for 48 hr, during which time the mixture turned dark brown. Removal of the solvents at reduced pressure yielded a dark brown viscous oil. Acetonitrile (100 ml) was added and the mixture allowed to stand at 4° overnight. The tan colored precipitate was removed by filtration on a sintered glass filter and dried in vacuo overnight. The waxy solid was dissolved in tetrahydrofuran-water (9:1, v/v) and applied to a column (1.5 cm x 40 cm) of Amberlite MB-3 resin previously washed with the same solvent. The column was eluted with 500 ml THF-water (9:1, v/v) at a flow rate of 2 ml/min. The colorless eluate was concentrated under reduced pressure and dried by repeated azeotrophic distillation with 2-propanol at reduced pressure. The waxy white solid was dissolved in chloroform and applied to a column of silic AR CC-7. The column was first eluted with 500 ml chloroform-methanol (4:1, v/v) to remove unreacted 1,2-dihexadecyl-sn-glycerol-3-phosphate. Elution of the column with chloroform-methanol (2:3, v/v) removed the desired product. After removal of the solvent, the residue was redissolved in chloroform and passed through a Metricel™ Alpha-6 (.45μm) filter to remove suspended silicic acid. The solvent was removed, leaving a chromatographically pure (TLC on silica gel G, chloroform-methanol-water, 65:25:4, v/v/v) white solid. Yield 1123 mg (78% from 1,2-dihexadecyl-sn-glycerol). M.p. 206-208° C (dec.).

Anal. Calcd. for $C_{41}H_{86}O_6NP \cdot H_2O$ (738.129):

C, 66.72; H, 12.15; N, 1.90;, P, 4.20

Found:

C, 66,35; H, 11.95; N, 1.98; P, 4.20.

Condensation of L-3,4-dioctadecoxybutyl(phenoxyphosphino)methylphosphonic acid with N-phenoxyacetyl-2',3'-isopropylidenecytidine. The purified phosphonic acid B (35 mg, 0.042 mmol) and 4 N-phenoxyacetyl-2',3'-isopropylidenecytidine (51 mg, 0.122 mmol; 3 X excess) were dissolved in 40 ml of anhydrous pyridine and to this solution was added trichloroacetonitrile (1.5 ml). The solution was stirred at 53° overnight.

The solvent was removed in vacuo and the residue precipitated with acetonitrile. Little or no condensation product was evident on TLC in chloroform-methanol-water-acetic acid, 80:13:8:0.3. The precipitate (28 mg) consisted of the starting phosphonic acid as a salt. The acetonitrile solution contained the cytidine derivative,which was recovered as well.

The acid salt (28 mg, 3.4×10^{-5} mol) was dissolved with a fresh portion of the cytidine derivative (52 mg, 12.6×10^{-5} mol) and to the mixture was added 2 ml of trichloroacetonitrile. This time the reaction was carried out overnight at 70°. TLC of the residue remaining after removal of volatile materials showed the condensed product with an Rf similar to that of the model compound (see text above). The product was precipitated again by addition of acetonitrile; the cytidine derivative was again recovered from the solution. The precipitated product (11 mg) gave essentially one spot which was phosphorus-positive and UV-absorbing at Rf 0.75 in the solvent mixture employed above.

Hydrolysis of the Condensation Product E. The crude condensation product obtained by acetonitrile precipitation (10 mg) was dissolved in 1 ml of trifluoro-acetic acid containing 2 drops of water and the mixture heated at 45° for 24 hrs. Evaportion of the acid, followed by dehydration twice with isopropyl alcohol gave a residue which was dissolved in a minimum amount of chloroform. Addition of ether gave a solid which was filtered off and dried in vacuo after thorough washing with cold ether. This material gave a single spot which was UV- and phosphorus-positive by TLC in the above solvent mixture; Rf 0.6.

This value is similar to that of authentic phosphonic acid analogs of CPD-diglyceride (8) and chraacterization of the product was underway as the quarter ended.

Reaction between L-3,4-dioctadecoxybut-3-enyl(chloromethyl)phosphinic acid phenyl ester and bis(trimethylsily)trimethylsilyoxymethylphosphonite. The crude chloromethyl compound (2.3 g, 2.9 mmol) was reacted with 10 ml (10X excess) of the freshly distilled silyl reagent under a nitrogen atmosphere at 130°. After 1 hr, a few drops of trimethylsilyl chloride had distilled. The temperature of the bath was increased to 135° and the reaction mixture left overnight. After cooling, the excess reagent was removed by distillation in high vacuum, and the insoluble residue dissolved in tetrahydrofuran (9 ml). To the solution was added water (5 ml) and the clear solution left overnight. The solvent was evaporated and water was removed by repeated re-evaportion with isopropyl alcohol. The residual white solid was dissolved in a minimum quantity of chloroform and the product precipitated with acetonitrile. Approximately 1.7 g of material was obtained in this way; its thin-layer chromatographic behavior was similar to that of 1-octadecoxy-2-hexadecoxypropyl phosphonic acid. Final characterization of this important intermediate had not yet been completed at the end of the fourth quarter.

The crude product was hydrogenated in a warm (50°) mixture of tetrahydrofuran-acetic acid (1:1) with 0.5 g of 10% paladium on charcoal at 50 lb hydrogen pressure overnight.

After filtration and washing of the catalyst, the solvents were thoroughly evaporated and the residue reprecipitated with acetonitrile. This experiment was carried out just at the very end of the fourth quarter, and therefore further steps in the characterization of the hydrogenated product extended beyond the period covered by this report.

Chloromethyl ethyl hydrogen chloromethylphosphonite.

Chloromethyl phosphonous dichloride was obtained in about 60% yield starting with the procedure published in Organic Syntheses (10). To 8 ml of the dichloride cooled in an ice bath was added, drop wise under a nitrogen atmosphere, 50 ml of absolute ethanol freshly distilled from calcium hydride. After stirring for a few minutes further, excess ethanol was distilled off in vacuo at water pump pressure and the product distilled in high vacuum; $b_{0.05}$ 48-50°. Infrared and nmr data were consistent with the supposed structure, although gas chromatography indicated that the product was impure.

This crude material, on attempted reaction with acrylonitrile in the presence of diisopropylethylamine, or of sodium hydroxide in catalytic amount with or without tetrabutylammonium bisulfate, failed to give any product with the expected properties although some type of reaction had obviously occurred in the latter cases.

An attempt to prepare chloromethyl cyanoethyl phosphinic chloride by heating chloromethylphosphonous dichloride with acrylamide according to the procedure of Pudovik et al (5) failed to give the desired product from the black tarry reaction mixture.

Thus, the desired phenyl cyanoethyl(chloromethyl)phosphonate was prepared according to the procedure given in the third quarterly report, starting from cyanoethyl(hydroxymethyl)phosphinic acid synthesized as reported in the same place. The acid failed to form a crystalline cyclohexylamine salt, so that purification of the free acid had to be accomplished by repeated crystallization from acetone-hexane.

Attempted reaction between phthalimidoacetaldehyde and phenyl chloromethyl-(triphenylphosphinemethylide)phosphinate.

The aldehyde was prepared from its dimethylacetal by hydrolysis with hydrochloric acid in dioxane solution. The ylid was prepared correspondingly from its phosphonium

chloride. Reaction between the aldehyde and ylid at 110° for 24 hrs gave a mixture of products, of which none was predominent. Triphenylphosphine oxide was clearly visible by TLC and gas chromatography. Various chromatographic separation procedures were tried without any clear success in isolating the desired product.

COMPOUNDS SUBMITTED FOR ANTIMALARIAL TESTING

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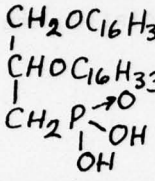
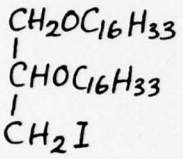
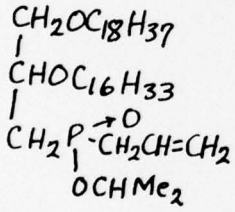
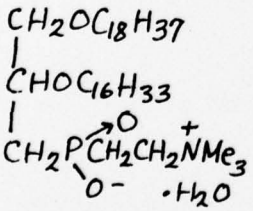
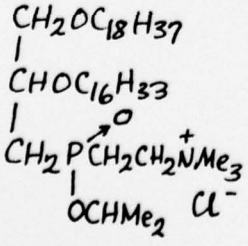
1.		BG 37644	6-4-76	Inactive. Non-toxic.
2.		BG 79188	12-16-76	Inactive. Non-toxic.
3.		BG 84317	2-23-77	Inactive. Non-toxic.
4.		BG 82182	2-10-77	Inactive. Non-toxic.
5.		BG 81777	2-17-77	Inactive. Non-toxic.

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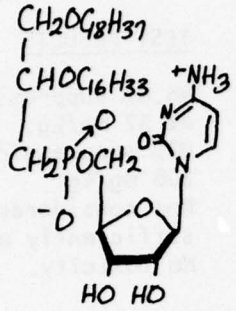
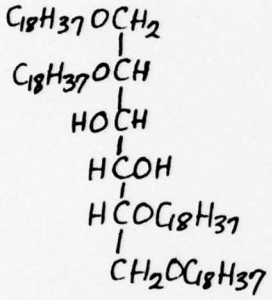
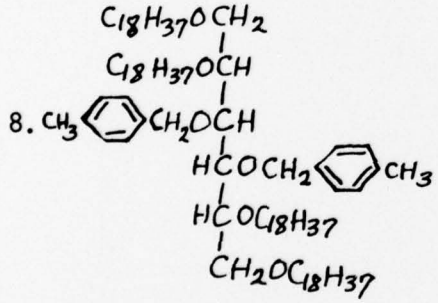
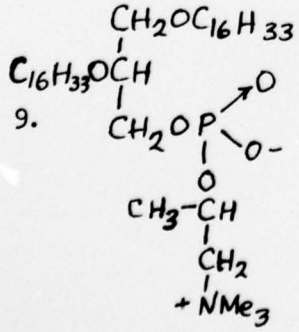
6.		BH 08611	6-7-77	Inactive. Non-Toxic.
7.			Submitted 1-77. Not tested yet.	
8.			Submitted 5-77. Not tested yet.	
9.		BH 16597	Submitted 6-1-77 Not tested yet.	

COMPOUND SUBMITTED FOR LEISHMANIASIS TESTING

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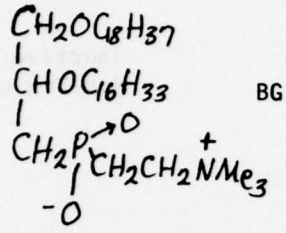
1.		BG 83182	3-11-77	48.8% suppression at 52 mg/kg; 93% suppression at 208 mg/kg Not considered sufficiently active. No toxicity.

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