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PHOSPHONIC ACIDS ANALOGUES OF GLYCEROL 3-PHOSPHATE

City University of New York PH.D. 1983

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PHOSPHONIC ACIDS ANALOGUES OF GLYCEROL 3-PHOSPHATE

by

NHORA LALINDE

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1982

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

The main focus of this thesis was the preparation of phosphonic acids analogues of glycerol 3-phosphate, which included nitrogen, epoxy and hydroxyl analogues.

Glycerol 3-phosphate is at a branch point between lipid metabolism and glycolysis; the important role that this material plays in metabolism suggests that it is quite a reasonable target for antimetabolic activity by structurally related phosphonic acids.

For the preparation of the hydroxyl analogue, namely 1,3,4-trihydroxybutylphosphonic acid, advantage was taken of the fact that addition of organoboranes to an unsymmetrical olefin proceeds to place the boron atom on the less substituted of the two carbons forming a double bond. Hydroboration provided a simple, convenient synthetic route for the anti-Markovnikov hydration of Diisopropyl (S)-(E)-3,4-diisopropylidine-3,4-dihydroxybutene-1-yl phosphonate.

The preparation of the nitrogen analogue N-(2,3-dihydroxy-propyl) phosphoramide was accomplished by the Gabriel synthesis of primary amines. This method offers the advantage of the absence of secondary or tertiary amine contamination of the primary amine, the toleration of a wide range of other functional groups in the molecule and the mild conditions needed for accomplishing both stages: condensation and hydrolysis.

Many attempts for the preparation of 1,2-epoxy-3,4-dihydroxy-butane phosphonic acid were made; they included the treatment of halohydrins with base, the direct epoxidation of the vinyl phosphonate by m-chloroperbenzoic acid, and a synthesis similar to the one

reported by Glamkowsky *et al.* for fosfomycin using sodium tungstate as catalyst. All of these efforts were without great success.

To my husband Gerardo and
and my children Gloria
and Nicholas

ACKNOWLEDGEMENT

My special gratitude to Professor Robert Engel for his advice and support which made possible the realization of this work.

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LIST OF SCHEMES

INTRODUCTION

Sn-glycerol 3-phosphate is at the branch point between lipid metabolism and glycolysis. As such it is a very important metabolic intermediate. Glycerol serves as a precursor of glycerol 3-phosphate (1). It is available from glyceride degradation (2).

The metabolism of glycerol and glycerol 3-phosphate by mammalian organisms is regulated mainly by three enzymes: glycerol kinase , the cytosolic NAD^+-dependent glycerol 3-phosphate dehydrogenase, and the mitochondrial FAD-linked glycerol 3-phosphate dehydrogenase (Figure 1) (2A).

Bucher & Klingenberg (3) and Estabrook & Sacktor (4) proposed the "glycerol 3-phosphate shuttle" on the basis of (a) the demonstration by Lehninger (5) of the inability of intact rat liver mitochondria to oxidize external or cytosolic NADH; (b) the ability of mitochondria to oxidize

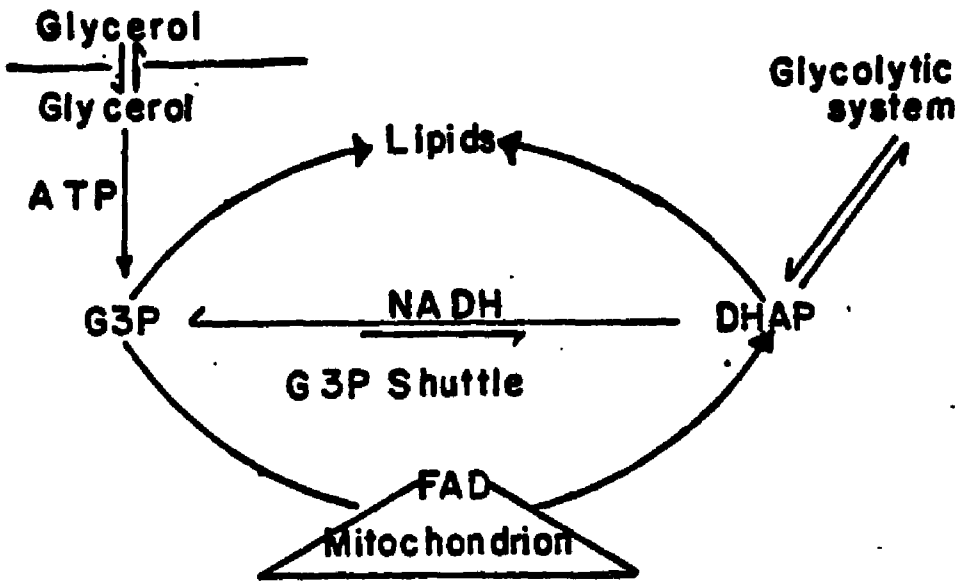


Figure 1

glycerol 3-phosphate but not dihydroxyacetone phosphate; and (c) the existence of a cytosolic NAD^+-linked enzyme that thermodynamically favors the formation of glycerol 3-phosphate and NAD^+. In this process, reducing equivalents in glycolysis are deposited temporarily in dihydroxyacetone phosphate, converting it to glycerol 3-phosphate. Glycerol 3-phosphate then enters the outer membrane of a mitochondrion and is reoxidized to dihydroxyacetone phosphate, which is free to return to the cytosol, ready for another round of hydrogen transport (Figure 1). Such a cycle would serve to dispose of reducing equivalents in the cytosol in exchange for oxidative phosphorylation in mitochondria. Although the importance of

this process for the generation of metabolic energy in muscles of insects is well established (6,7), there is no evidence that the shuttle plays a major role in this respect in vertebrates.

Glycerol kinase, glycerol 3-phosphate oxidoreductase and glycerol 3-phosphate dehydrogenase act in tandem to determine the pool size and turnover rate of the metabolite. As each tissue has a unique mission to fulfill, so the demand for each of the three enzymes varies.

In the world of bacteria there are two ways by which glycerol is dissimilated: one begins with dehydrogenation, which is followed by phosphorylation, and the other begins with phosphorylation, which is followed by dehydrogenation. In both cases the terminal product is dihydroxyacetone phosphate. This simple pattern for the chemical transformations of glycerol, however, belies the complexity of the enzymology that brings forth those changes.

The kinds of proteins employed by one bacterial species, Escherichia coli, for the utilization of glycerol, glycerol 3-phosphate and dihydroxyacetone phosphate are summarized in Figure 2 and briefly discussed below:

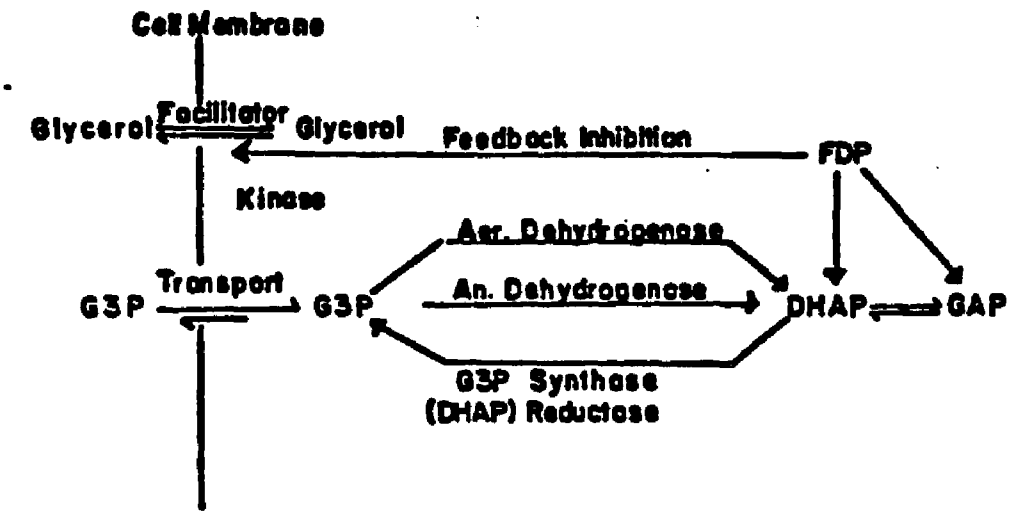


Figure 2

1) Glycerol facilitator (or facilitator protein)- The term facilitator implies that the protein is responsible for the facilitated diffusion of the substrate across the cell membrane rather than transport against a concentration gradient (7A). 2) Glycerol 3-phosphate transport (or permease): This protein catalyzes the active transport of intact sn-glycerol 3-phosphate, i.e. transport against a concentration gradient (7B). 3) Glycerol kinase (ATP:glycerol 3-phosphotransferase): This enzyme catalyzes the phosphorylation of glycerol. It is feedback inhibited by fructose 1,6-diphosphate (7C). 4) Anaerobic glycerol 3-phosphate dehydrogenase: This enzyme catalyzes the

oxidation of glycerol 3-phosphate to dihydroxyacetone phosphate and is coupled to nitrate or fumarate reduction (7D). 5) Aerobic glycerol 3-phosphate dehydrogenase. This enzyme also catalyzes the oxidation of glycerol 3-phosphate to dihydroxyacetone phosphate. In this case either oxygen or nitrate can serve as the ultimate electron acceptor (7E). 6) Glycerol 3-phosphate synthase (or dihydroxyacetone phosphate reductase) (glycerol 3-phosphate:NAD(P)-oxidoreductase) This enzyme catalyzes the conversion of dihydroxyacetone phosphate to glycerol 3-phosphate. As such it serves as the link between glycolysis and phosphoglyceride synthesis. The enzyme is inhibited by high concentration of the product glycerol 3-phosphate (7F). 7) Glycerol 3-phosphate acyl transferase (acyl-CoA:glycerol 3-phosphate O-acyltransferase): This enzyme catalyzes the first step in phosphoglyceride synthesis, the formation of monoacyl glycerol-3-phosphate (7G). It is an important step in metabolic control.

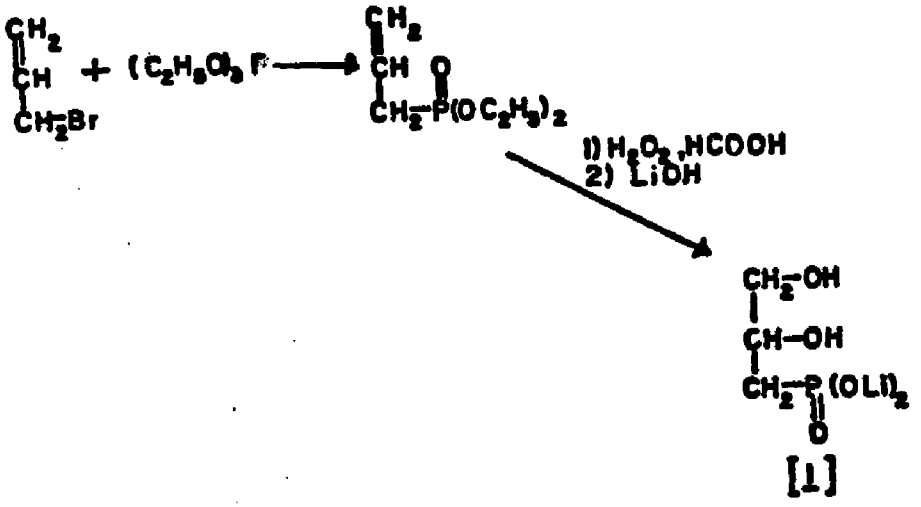
As the backbone of phosphoglycerides glycerol 3-phosphate is ubiquitous. Furthermore, the phosphate ester is readily liberated during biological decomposition, and once set free is chemically stable. Possession of glycerol 3-phosphate permease may well indicate that a

species often inhabits environments rich in cellular material. The ability to utilize dihydroxyacetone, on the other hand, may have a chemical basis, since this compound is unstable in alkaline surroundings. It follows that organisms that tend to live in acid environments may often be equipped to grow on dihydroxyacetone (2B). Alternatively, it is possible that the ability to grow on dihydroxyacetone did not result as a direct consequence of natural selection, but simply accrued as a metabolic bonus for developing a catabolic pathway that is initiated by glycerol dehydrogenase (2B). Dihydroxyacetone and glycerol may be sufficiently similar in structure to share a permeation protein.

Consideration of some of the important roles that glycerol 3- phosphate plays in metabolism suggests that it is quite a reasonable target for antimetabolic activity by structurally related phosphonic acids. The carbon phosphorus bond is incapable of being hydrolyzed by phosphoesterases, Therefore substitution of a natural phosphate metabolite by a phosphonic acid or a phosphonate ester may inhibit or perturb one or more biochemical processes. Several aspects of change must be considered in addition to rendering the phosphorus not liable to

hydrolysis by normal routes(9). A factor of prime importance is the distance between the phosphorus acid site and other functionalities of the molecule when compared to that in the natural phosphate. For example, 2,3-dihydroxypropyl-1- phosphonic acid [1] generated in both racemic (10) and chiral (11) forms is a potential antimetabolite for processes normally utilizing sn-glycerol-3-phosphate,however, it was found to be totally without inhibitory activity toward L-glycerol-3-phosphate:NAD oxidoreductase (49,50). Presumably its compacted structure hindered its interaction with the active sites of the enzymes involved.

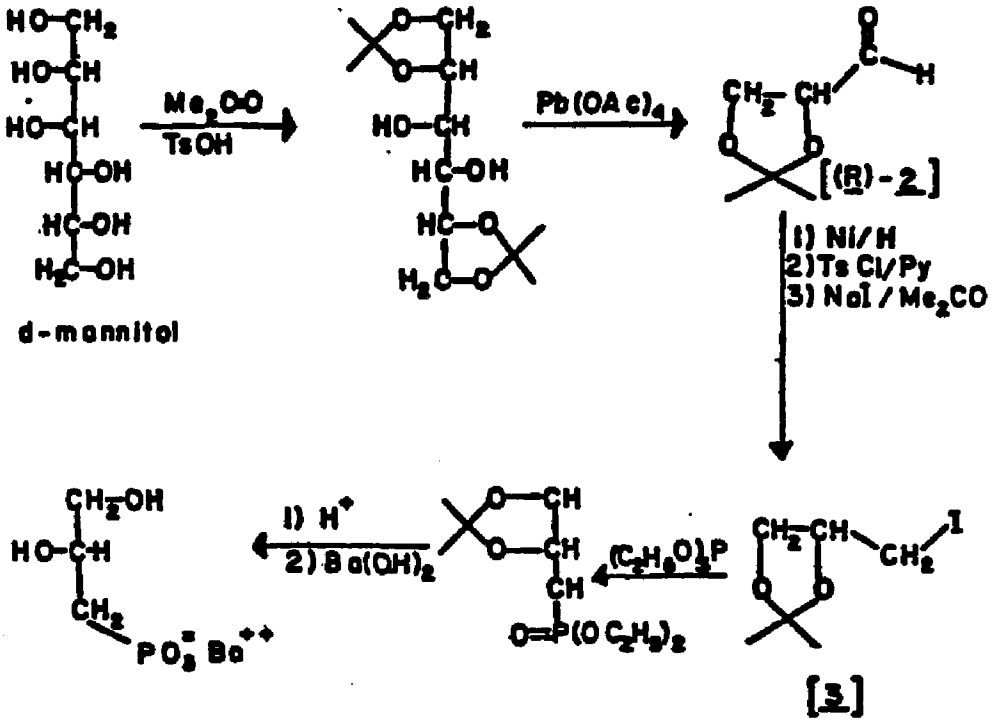
Rosenthal and Geyer (10) first synthesized 2,3-dihydroxypropyl-1- phosphonic acid [1] as a nonisosteric analogue of glycerol 3-phosphate, as shown in Scheme I, by an Arbuzov reaction on allylbromide followed by hydroxylation and ester hydrolysis. The dilithium salts are particularly convenient forms for isolation and purification due to their
unusual solubility properties, such as the precipitation upon heating.



[1]

SCHEME I

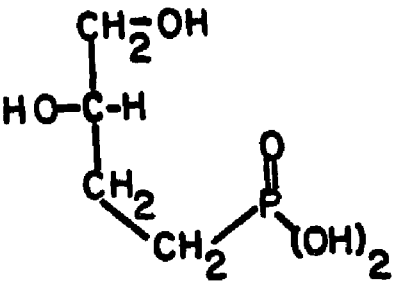
The optically active form, (R)-(-)2,3-dihydroxypropyl-1-phosphonic acid [(R)-1], bearing the same absolute configuration as the natural sn-glycerol-3-phosphate [5] about the internal hydroxyl, was reported by Baer and Basu(11) . This route began with D-mannitol diacetonide and involved an Arbuzov reaction on the iodide derived after cleavage, as shown in Scheme II.



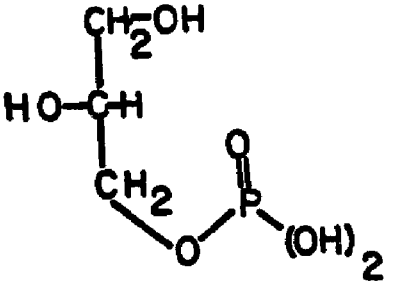
SCHEME II

As noted above the material prepared by either route was found (15) to be inactive as a substitute for sn-glycerol-3-phosphate in reaction involving rabbit muscle L-glycerol-3-phosphate:NAD oxidoreductase. Baer et al. (12) concluded that the ester oxygen was necessary for activity. However, the racemic isosteric analogue, 3,4-dihydroxybutyl-1-phosphonic acid [4], shown in Figure 3, is a substitute for this enzyme (12A). A variety of

synthetic approaches have been developed for generating this compound.



[4]



[5]

Figure 3

3,4-Dihydroxybutyl-1-phosphonate is capable of inhibiting the growth of E. coli at rather low concentration, and has proven to be a useful agent for studying phosphoglyceride metabolism.

The initial synthesis reported by Kabak et al. (13) starting with 4-bromo-1-butene generated the material in racemic form via a route analogous to that of Rosenthal and Geyer(10) for [1], . It was later (15) generated in chiral form using a Wittig phosphonylation of the aldehyde

[(R)-2], Figure 4, derived from D-mannitol diacetonide cleavage followed by hydrogenation and hydrogenolysis.

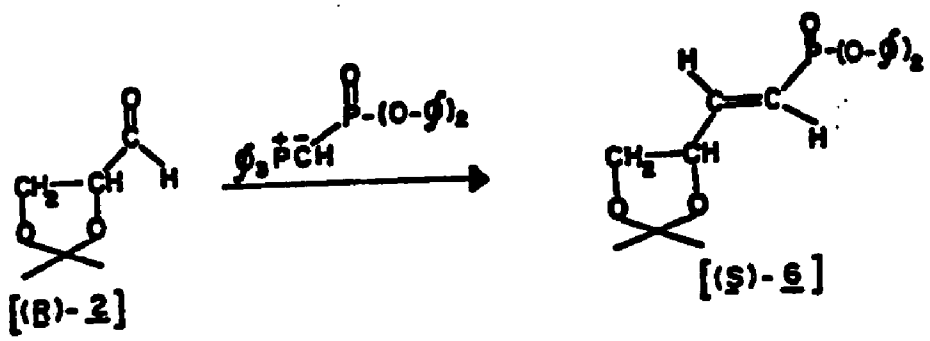
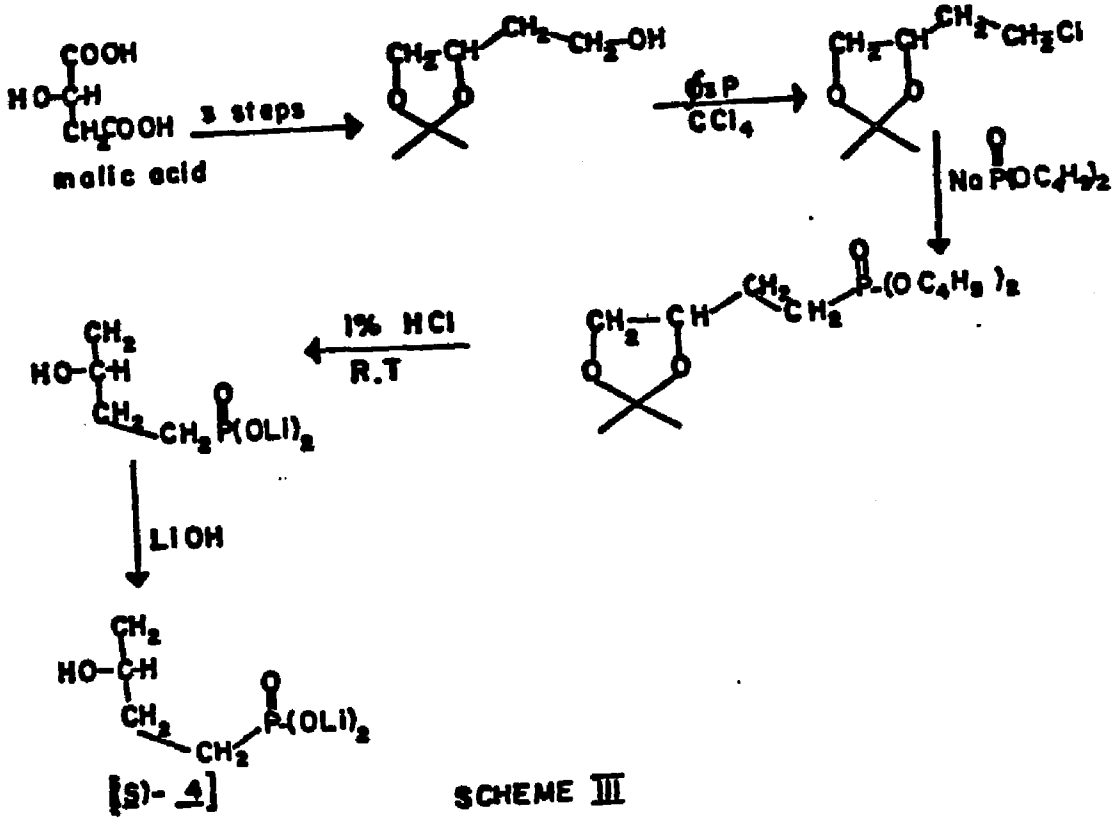


Figure 4

Paulsen and Bartsch (16) reported a similar sequence for the synthesis of racemic [4] starting with a Horner reaction using tetraethyl methylenebisphosphonate on racemic [2]. Phosphonate ester cleavage was accomplished by trimethylchlorosilane treatment.

A further chiral synthesis of [4] has been reported (20) as shown in Scheme III, beginning with optically active malic acid; this route provides both enantiomers.

The compound [4] was of particular interest because many bacterial species actively transport and use the natural phosphate.Thus, there was a high probability that [4] would be used *in vivo*. In fact, the enantiomer of [4]

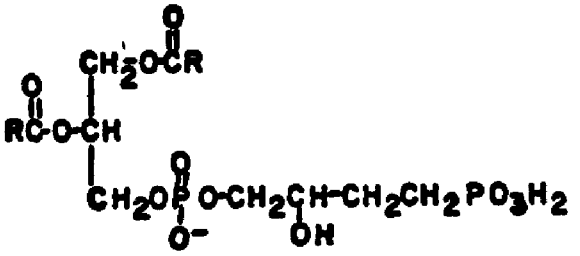


[S]- 4

SCHEME III

corresponding in absolute configuration to sn-glycerol 3-phosphate is transported into a variety of bacterial species and its transport properties have been carefully studied in E. coli (18,19).

Growth inhibition is observed in those species which transport [4]. This characteristic is not shown by the non-isosteric species. Presumably the reason is that the non isosteric species is not transported. The mode of antimetabolic activity of [4] in E. coli has been explored both in vivo and in vitro . Phosphatidylglycerol synthesis is perturbed (20,21). The compound DHBP replaces sn-glycerol-3-phosphate in the reaction catalyzed by CDP-diglyceride:sn-glycerol-3-phosphate phosphatidyltransferase and produces a polar lipid material [5], as shown in Figure 5.

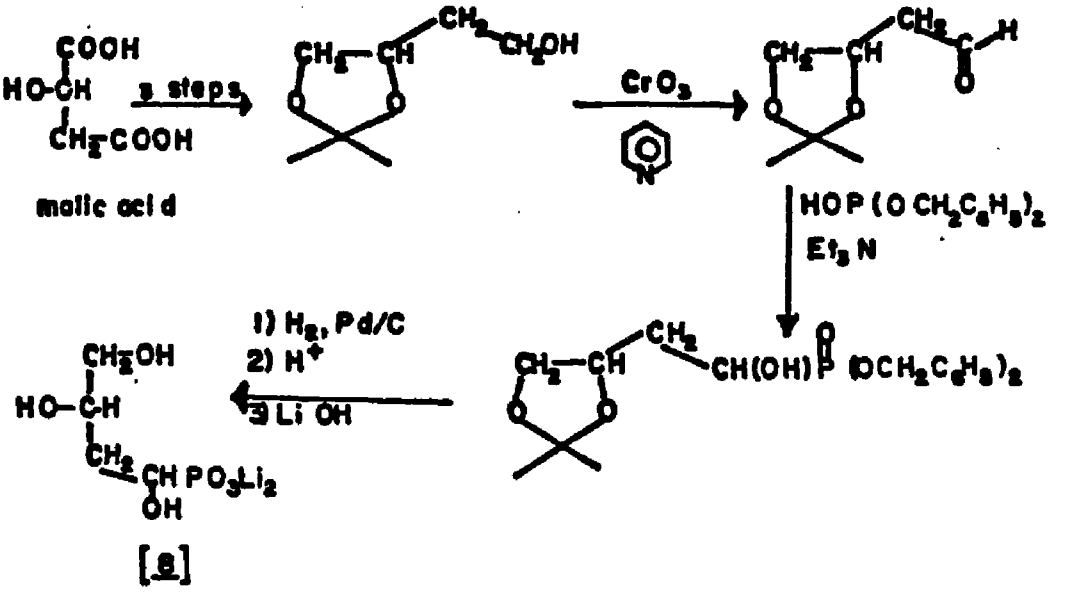


[5]

Figure 5

The material [4] has also been noted to be active in the inhibition of growth of strains of Bacillus subtilis (22,23). Here it is noted, in similarity to E. coli, that phosphatidylglycerol synthesis is inhibited, but also that [4] is incorporated into the cell wall.

In vitro enzymatic studies with [4] indicate a number of interesting points. While [4] serves as a substrate for CDP-diglyceride:sn- glycerol-3-phosphate phosphatidyltransferase and is an inhibitor of the anaerobic *sn*-glycerol-3-phosphate:NAD(P)oxidoreductase of *E. coli* (14,20), it does not appear to interact with the catabolic membrane - bound *sn*-glycerol 3- phosphate dehydrogenase, CDP-diglyceride:L-serine phosphatidyltransferase or acyl coenzyme A:*sn* -glycerol 3-phosphate acyl transferase (20). The lack of interaction in these latter systems has been postulated to be due to the loss of binding capability resulting from the substitution of a methylene group for the esteratic oxygen of the natural substrate. In an attempt to test this hypothesis further species have been synthesized without the esteratic oxygen but still bearing a potential binding functionality. Tang *et al*. (17) reported the synthesis of 1,3,4-trihydroxybutyl-1-phosphonic acid [8], generated from malic acid according to Scheme IV, with chirality at the 3-position, corresponding to that of *sn*-glycerol-3-phosphate [5]; the pair of diastereoisomers that resulted (due to the hydroxyl at the 1-position) in the Abramov reaction have been partially separated by column chromatography (24).



SCHEME IV

When this material was investigated with acyl coenzyme A:sn-glycerol-3-phosphate acyltransferase it was found to serve as a substrate, supporting the postulate that inactivity of [4] resulted from loss of a binding function. Moreover ,[8] serves as a growth inhibitor of strains of E. coli.

Another system that lacks the esteratic oxygen but still possesses the potential binding functionality is the phosphoramide, wherein the electron pair of the nitrogen could serve in place of those on the esteratic oxygen of the natural substrate. It has been pointed out (25) that as X, the bridging atom, of simple diphosphate compounds ,P-X-P, becomes more electronegative, i.e., CH_2 to N-H or O, the attached phosphate groups become stronger acids. The P-X bond length increases systematically from P-O (1.63 A) to P-N (1.68 A) to P-C (1.79 A), while the nonbonded P-P distance increases only slightly, 2.94, 3.00, 3.05 A, respectively (26). It would appear then that there is a pi component to the P-X bonding when X=N or X=O. The strongest evidence of this pi system is the effect it has on rotation about the P-X bond. A barrier to rotation about P-N bonds has been observed in many pentacoordinate phosphorus compounds. Such a barrier has been suggested to arise from donor 2p(N)$\longrightarrow$3d(P) pi bonding (27) . Alternatively, the barrier may be explained by an interaction between the nitrogen lone pair and that on phosphorus (28). Of most interest are the P-X-P bond angles. The P-O-P and P-N-P bond angles are only slightly (but not significantly) different, 129^{o} and 127^{o}, respectively. This contrasts with the smaller P-C-P angle

117°. The combination of the longer P-C bond distance and the more acute P-C-P bond angle may explain why the methylene analog of ATP (Figure 6), binds so weakly to myosin and actomyoisin

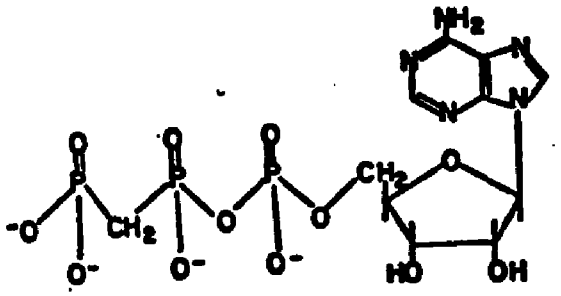


Figure 6

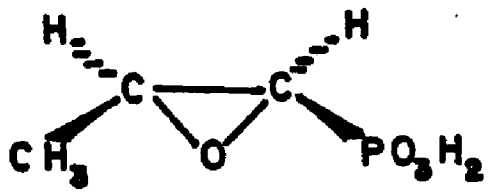
(29), because the methylene group with its two hydrogens protrudes to a much greater degree than it does with either the oxygen or imido linkage. The overall striking similarity of imidodiphosphate P-N-P and pyrophosphate P-O-P structures explains to a large degree the similarity of interaction of ATP and the imidophosphate analog of ATP with myosin and heavy meromyosin (30). Further evidence of the similarity is that the imidodiphosphate ATP analog mimics ATP in relaxing muscle (31). By contrast the methylene analog of ATP is without effect. The usefulness of the substitution of P-N-P bonds for P-O-P linkages in biologically important molecules then is apparent and structurally reasonable.

In relation to the phosphonic acids discussed above and their activity for inhibition of bacterial metabolism it is also of interest to consider a naturally occurring phosphonic acid which is bactericidal. The novel structure of the antibiotic fosfomycin [10] has generated interest in epoxyphosphonates (32,33). Previous studies on epoxyphosphonates have been concerned primarily with either their potential as synthetic intermediates (34) or the mechanism of the reaction of dialkyl phosphonates, with alpha-haloketones (35). The discovery of fosfomycin and its mode of action has given epoxyphosphonates biochemical significance (33). This antibiotic was isolated from fermentation broth in which Streptomyces fradiae was grown.

Fosfomycin is bactericidal in its action. A number of bacterial strains exposed to fosfomycin in growth medium of high osmolarity, are converted to spheroplasts. This strongly implicates fosfomycin in cell wall formation. Moreover, biochemical investigations (36) show that fosfomycin attaches covalently to, and thus inhibits irreversibly, phosphoenolpyruvate:uridine diphospho-N-acetylglucosamine transferase (37) in extracts from several Gram-positive and Gram-negative microorganisms. This enzyme catalyzes the first step in the biosynthetic

pathway of the nucleotide Muramyl peptides that serve as cell-wall precursors in all bacteria.

Fosfomycin, administered orally, is effective in protecting mice against a number of infections caused by Gram-positive and Gram-negative organisms. It is an effective chemotherapeutic agent against a number of systemic infections and compares favorably with tetracycline and chloramphenicol. It has been shown to have the structure (-)-(1R,2S)-1,2-epoxypropylphosphonic acid by synthesis together with a chemical determination of absolute configuration (33), as seen in Figure 7.



[10]

Figure 7

The methods leading to alpha-epoxyphosphonate [11] synthesis have been reviewed (34). These methods include: the reaction of a dialkyl phosphonate halohydrin with base, as seen in Figure 8.

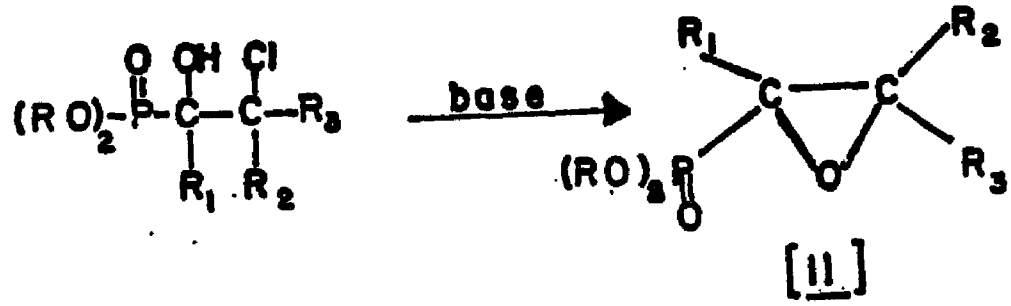


Figure 8

the reaction of sodium dialkylphosphonate with an alpha-haloketone, as in Figure 9.

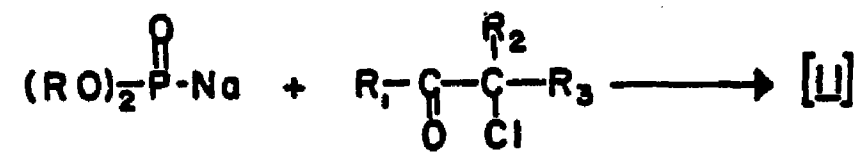


Figure 9

Darzen's (38-40) reactions of dialkyl chloromethylphosphonates with carbonyl compounds, as in Figure 10.

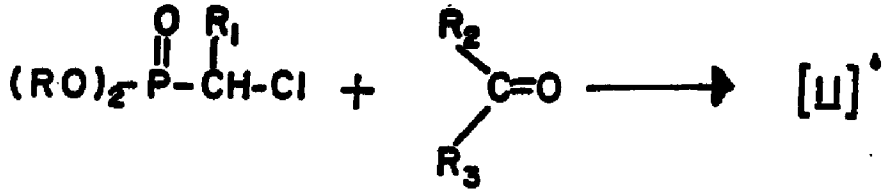


Figure 10

Direct epoxidation of unsaturated phosphonates with a peroxide and catalyst or peracid, as in Figure 11.

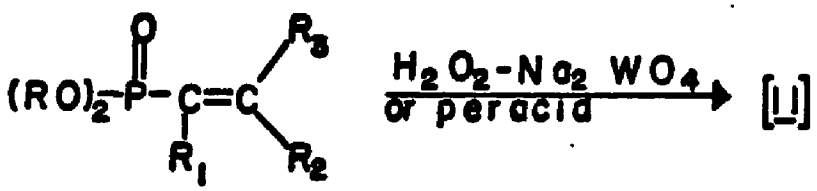
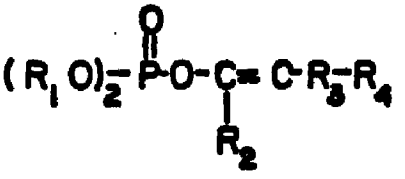


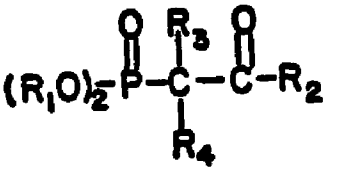
Figure 11

The yields for these reactions mentioned above are at best 60-70% but are also subject to certain structural limitations as indicated below.

The reaction of sodium dialkylphosphonate and alpha-haloketones has been reported to give also the isomeric enol or vinyl phosphonate [12] via Perkov reaction and beta-ketophosphonate [13] via Arbuzov reaction directly (41,42); the halo carbon cannot be tertiary, as shown in Figure 12.



[12]



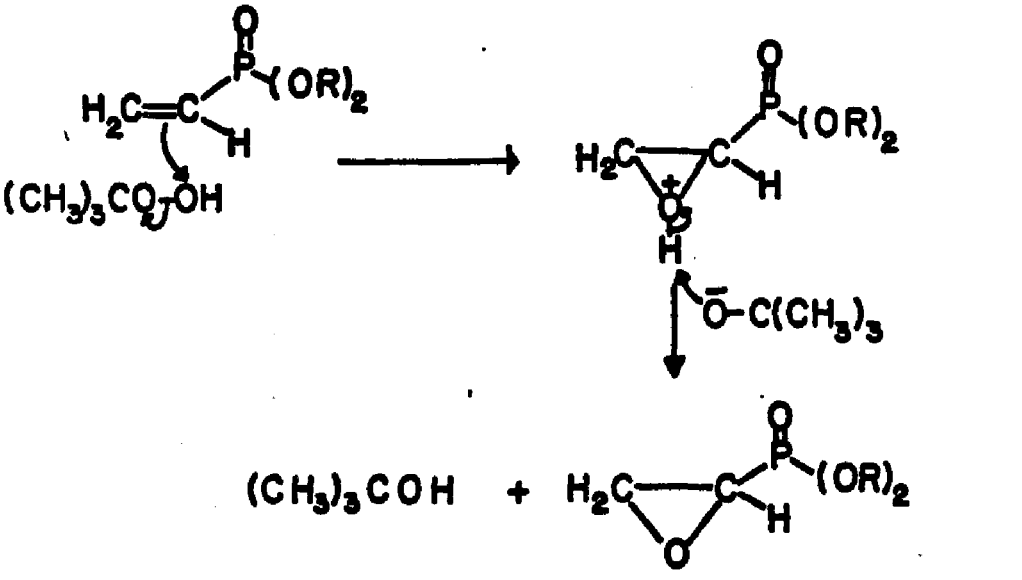
[13]

Figure 12

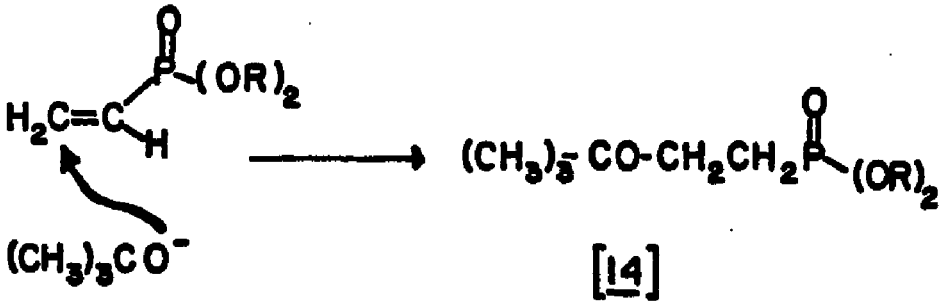
The Darzen's reaction is without side reactions, but it is limited to ketones and aryl aldehydes, i.e., R_2 and R_3=alkyl or R_3=aryl and R_2=H; in addition, this reaction has, to date, been performed only with the methyl and ethyl esters of chloromethylphosphonic acid. (R_1).

Epoxidation of the vinylic phosphonate can result in side reactions; in a buffered solution, trifluoroperacetic acid, when used as the oxidant, may cause ring opening of the epoxide once formed (43), and the use of tertbutyl

peroxide has been shown to result in the Michael addition product [14] of the butoxide to olefin (44), as shown in Scheme V.



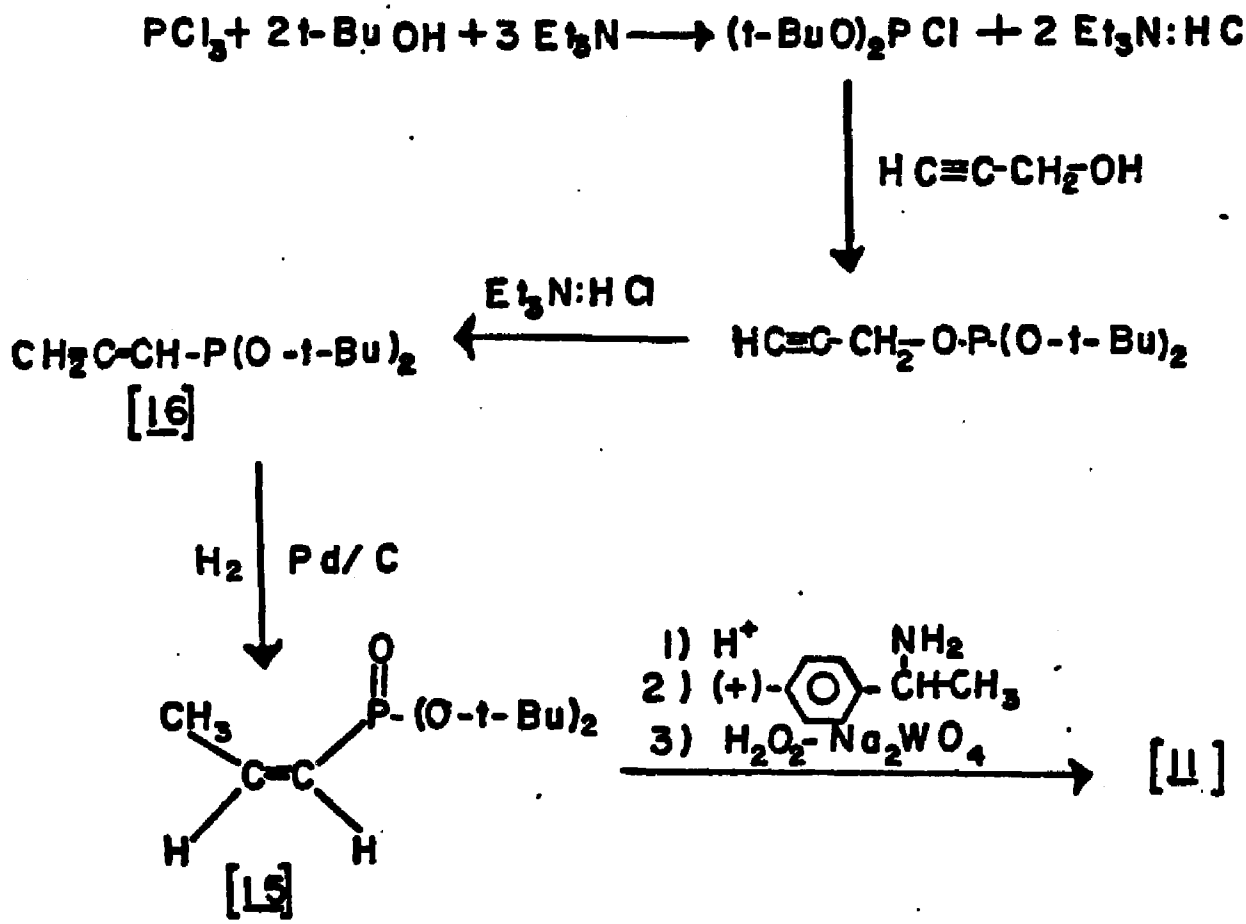
or Michael addition:



[14]

SCHEME V

Epoxidation has two distinct advantages; first, the formation of the intermediate unsaturated phosphonate in the synthetic sequence permits an acid-catalyzed ester hydrolysis prior to epoxidation; or instead, after the epoxide is formed, as in the other reactions discussed, the ester can be removed by hydrogenation if R is a benzyl group (45). Secondly, if R_2 or R_3 is not hydrogen, then there will be two isomeric unsaturated phosphonates formed which may be separated, alternately, the stereospecific unsaturated phosphonate [15] can be obtained *via* catalytic hydrogenation of corresponding allenyl [16] (46) or alkynyl (45) phosphonates . Epoxidation of the appropriate isomer can then take place in the presence of a resolving agent in order to isolate the product with the desired absolute stereochemistry (46), as represented in Scheme VI.



Scheme VI

Haake et al. (47) also reported a facile one-step procedure to alpha-epoxyphosphonates which is a combination

of two reactions previously mentioned (38), as shown in Figure 13.

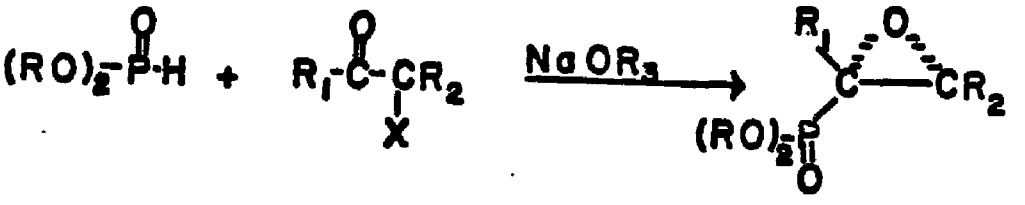
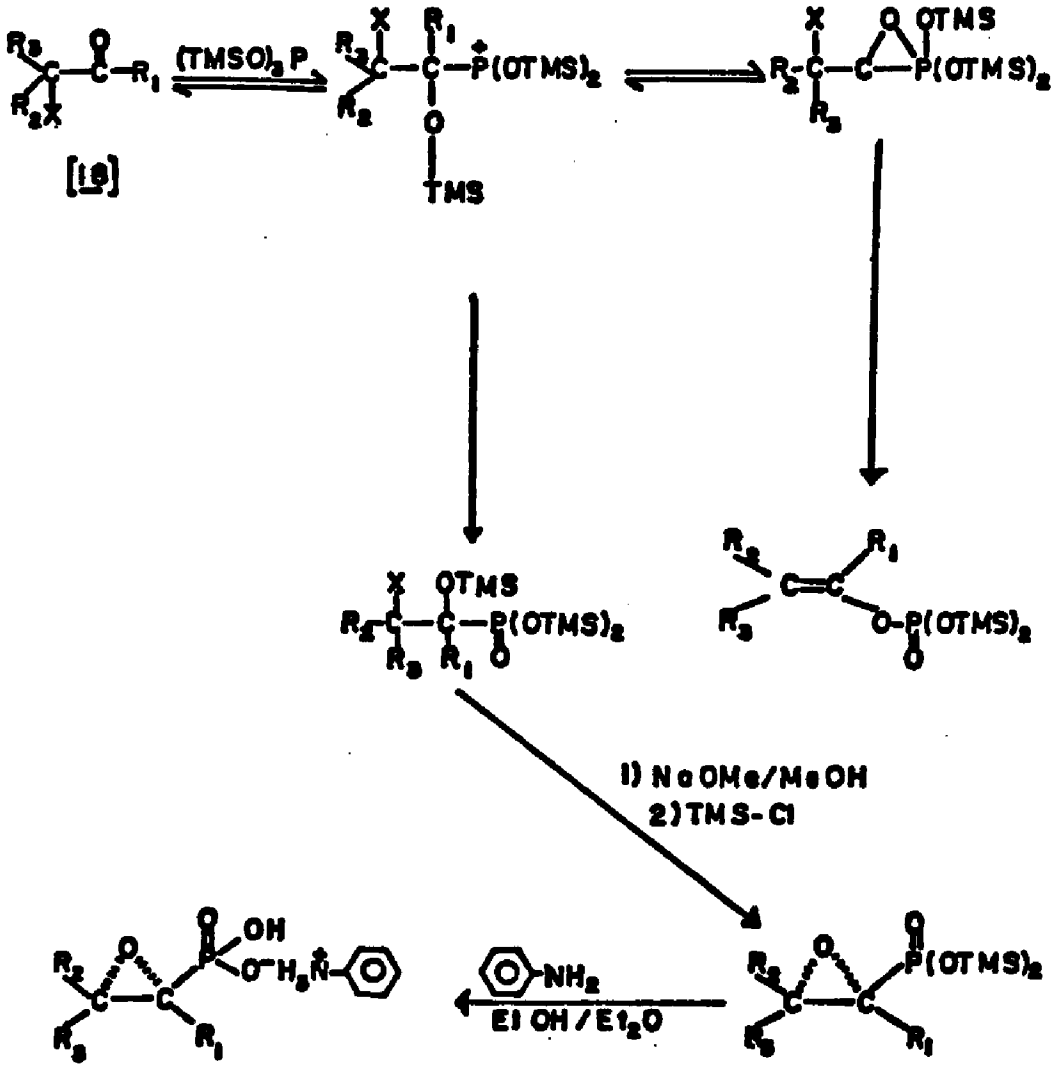


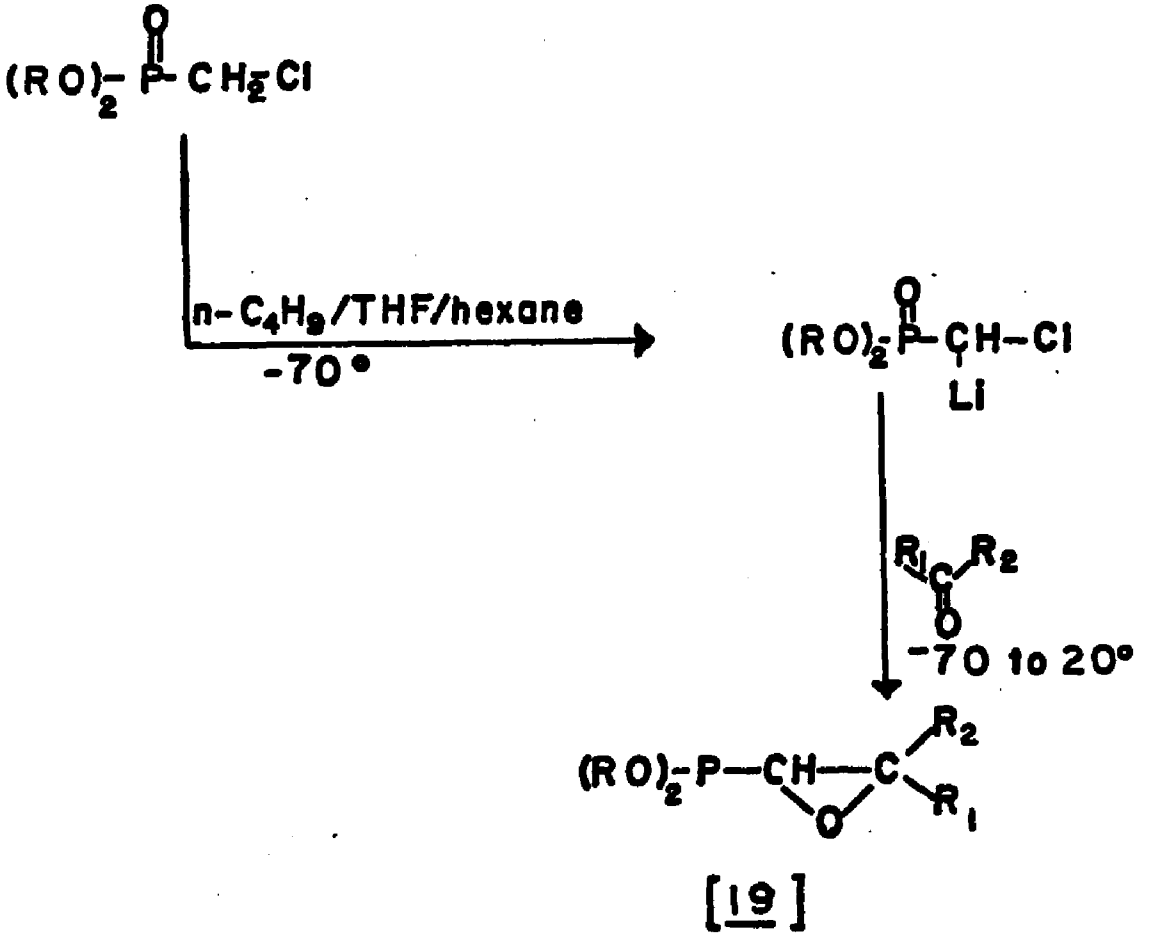
Figure 13

In 1978, a Japanese group (48) reported the facile conversion of bis(trimethylsilyl) esters of phosphonic acids, generated <u>via</u> either Perkov or Arbuzov reaction of a silyl phosphite with halocarbonyl compounds, to the corresponding free acid by simple addition of alcohols. It was also shown that the carbonyl adduct [<u>18</u>] can be converted to the free acid alpha-epoxyphosphonates by a sequence of reactions as shown in Scheme VII.



Scheme VII

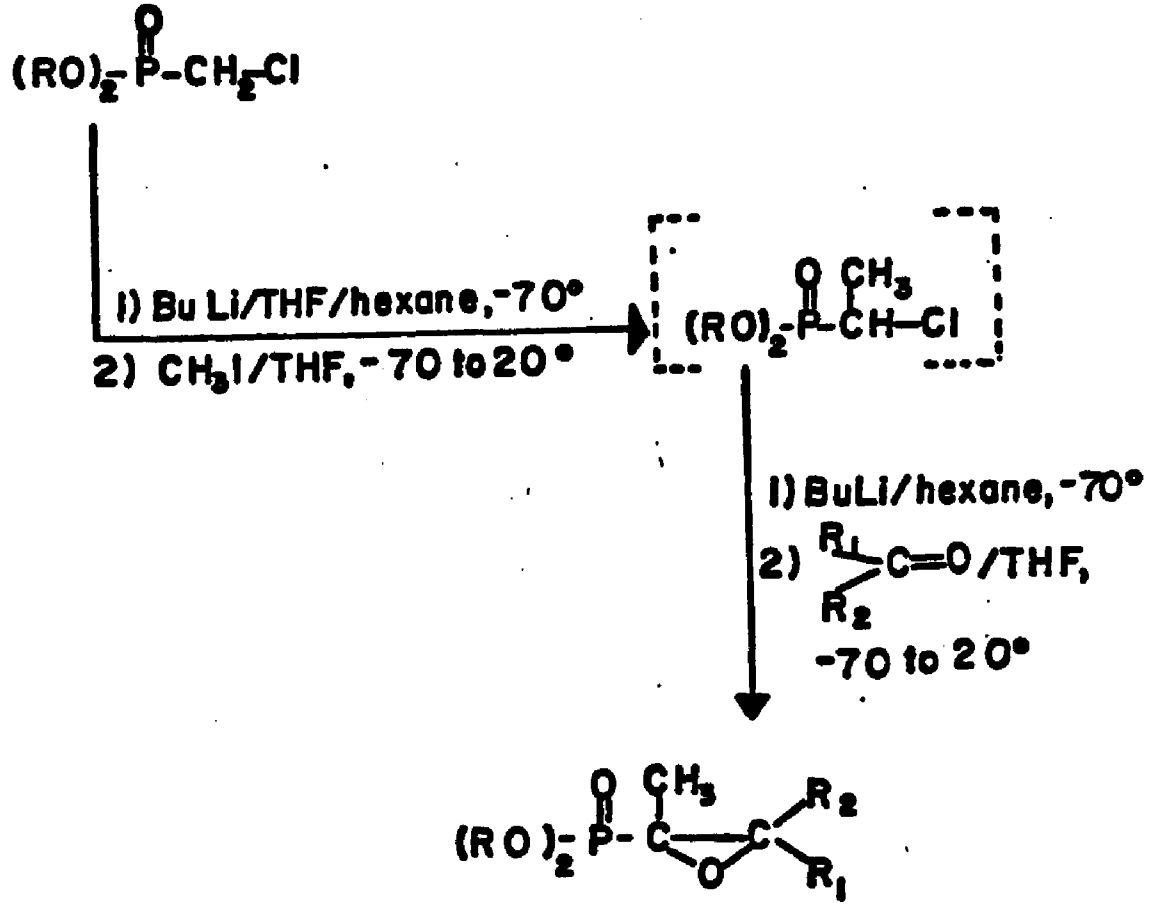
Savinac et al. (49) reported the reaction of dialkyl 1-lithio-1-chloromethanephosphonates with carbonyl compounds to form [19], as shown in Scheme VIII.



[19]

Scheme VIII

Also 1-substitued 1,2-epoxyalkanephosphonates [20] are obtained by this route, as represented in Scheme IX.

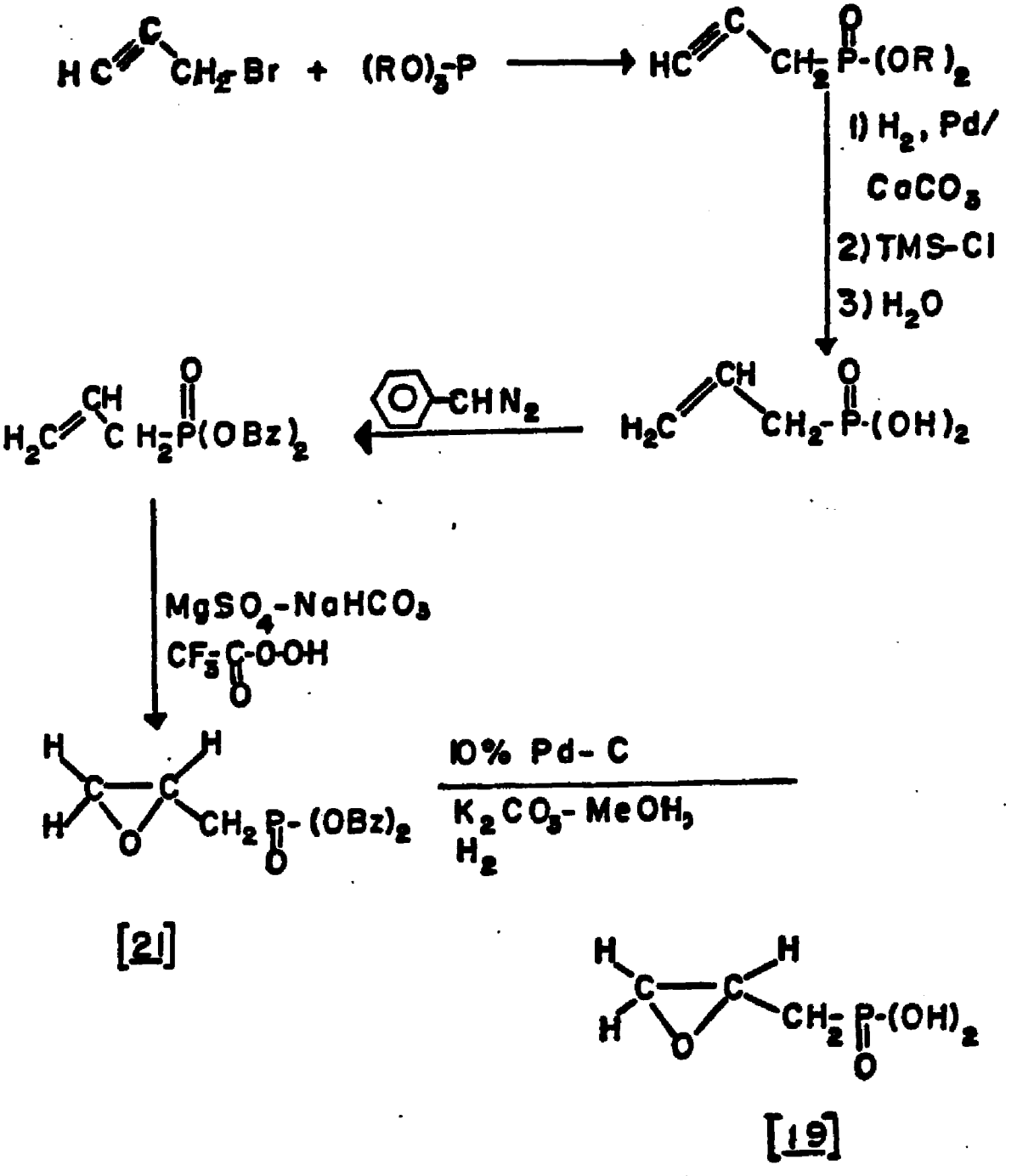


[20]

Scheme IX

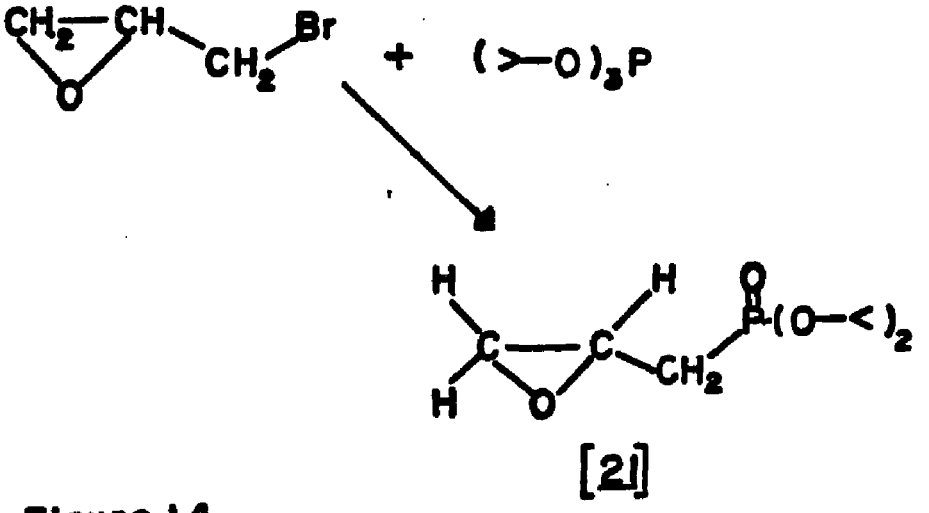
Christensen (45) et al., Scheme X, reported the synthesis of [21] as shown below. The free

beta-epoxypropylphosphonic acid [19] was synthesized from propargyl bromide via an Arbuzov reaction.



SCHEME X

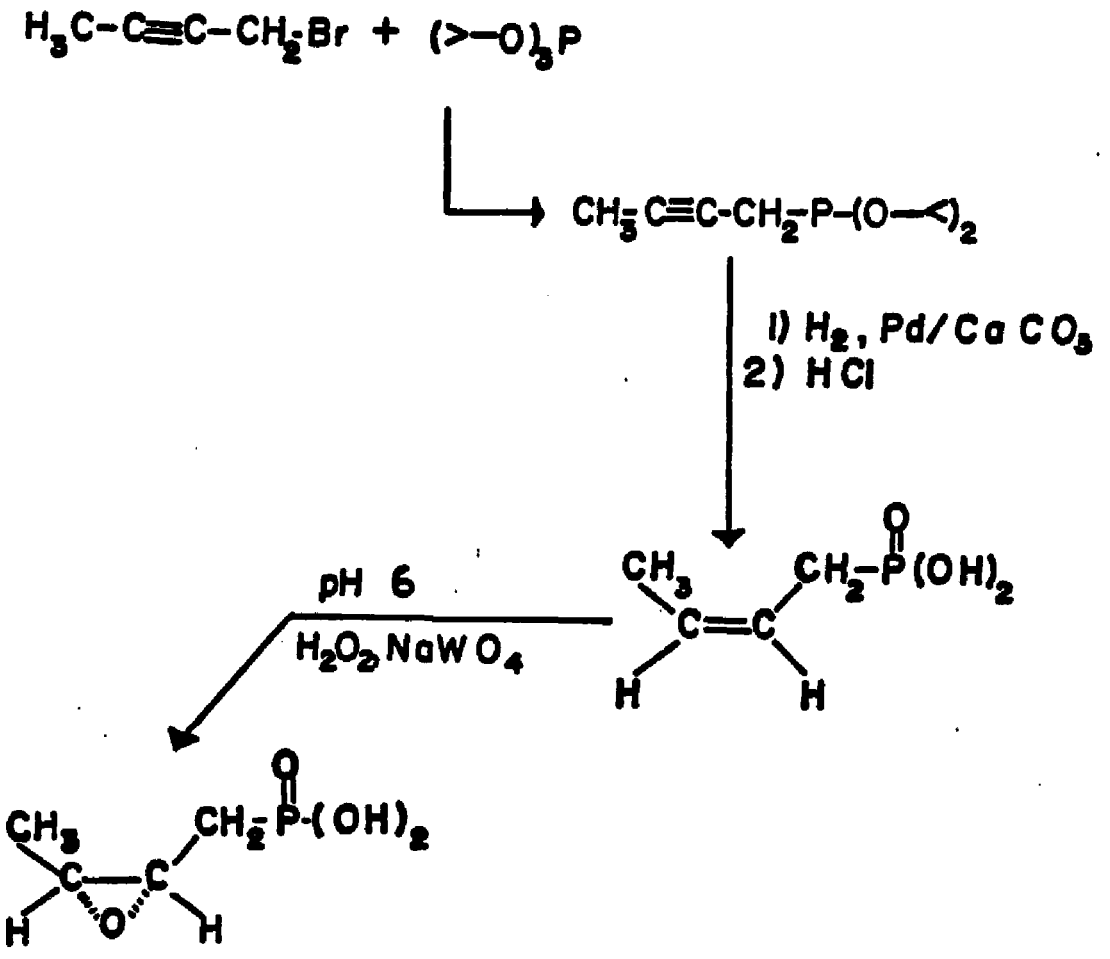
The isopropyl ester of beta-epoxypropylphosphonate [21] was also reported by a one-step reaction (50) as seen in Figure 14.



[21]

Figure 14

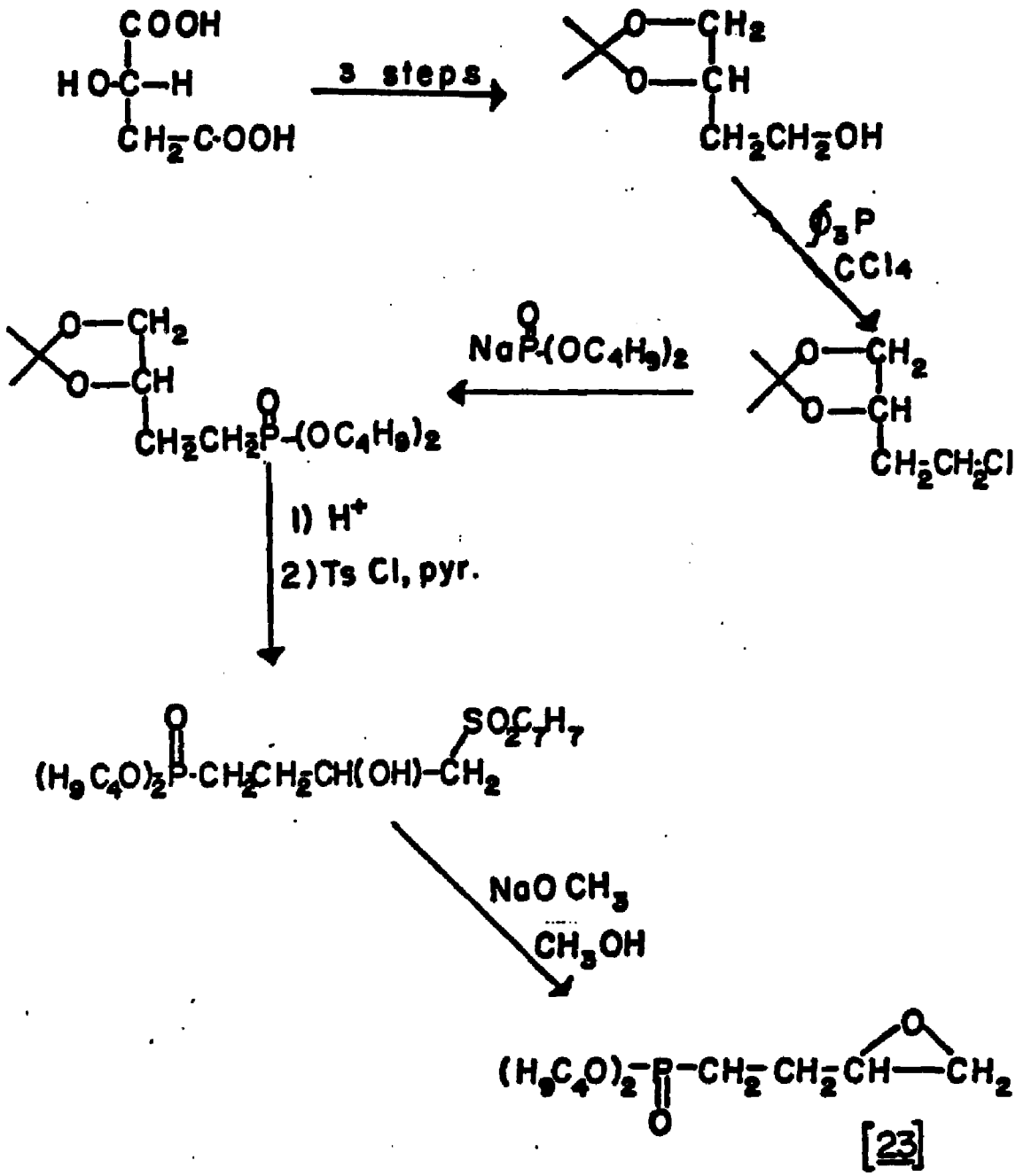
Another beta-epoxyphosphonate, 2,3-epoxybutyl phosphonate [22], was synthesized (44) as shown in Scheme XI.



Scheme XI

Tang et al (17) reported the synthesis of an epoxy analogue of of glycerol-3-phosphate, (RS)-3,4-epoxybutyl-1-phosphonate [23] in which the epoxide

function is in place of the vicinal diol of [4], as seen in Scheme XII.



[23]

Scheme XII

Evaluation of the *in vivo* activity of [23] was performed with *E. coli* strains 8 and 4855 (51), and it was found to be an inhibitor of both strains.

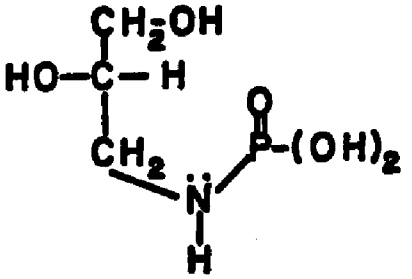
Even though some of the cases mentioned here are of epoxidation of *cis* alkenes, it is well known that epoxidation is a stereospecific reaction,i.e., *cis* alkenes give *cis* epoxides, and *trans* alkenes give *trans* epoxides.

Our interest in this work was inspired by the already mentioned great importance of glycerol 3-phosphate in intermediary metabolism, so the preparation of phosphonic acids having structures related to those of the natural phosphate products was very promising (19,23,24,49,50,51,52)

3,4-Dihydroxybutyl-1-phosphonic acid [4], the isosteric analogue of glycerol 3-phosphate [5],has been of particular value for *in vivo* as well as *in vitro* investigations (51). In the course of biochemical studies in our laboratory it has also been of interest to examine analogues other than the phosphonic acids isosteric with the natural phosphates. In particular, it was deemed desirable to explore the biological properties of analogues related to [4] in which a hydroxyl functional group was placed at C-1 (24). The

route (17) of Tang *et al.* leads to a pair of diastereomers (due to hydroxyl at the 1-position), which have to be separated by column chromatography. A further method was desired which would allow the preparation of the material in high optical purity.

In order to study the attributed loss of binding capability of the isosteres due to the substitution of a methylene group for the esteratic oxygen of the natural substrate, the synthesis of N-(2,3-dihydroxypropyl) [24] phosphoramide appeared to be a good choice , Figure 15,



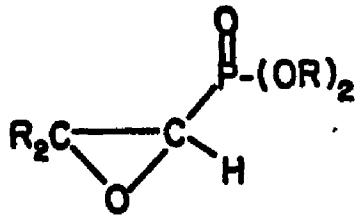
[24]

Figure 15

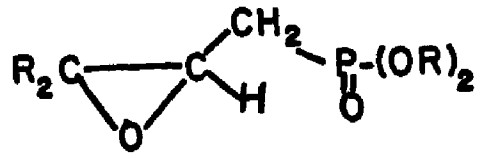
wherein an electron pair on nitrogen could serve in place of those on the esteratic oxygen of the natural substrate.

The discovery of fosfomycin [10] (33), and its mode of action has given epoxyphosphonates great biochemical

significance. There were noted in the literature several methods for the preparation of dialkyl alkylphosphonates possessing an epoxide function in either the alpha, beta [20] (35-37) or beta,gamma [21] positions, as seen in Figure 16.



[20]



[21]

Figure 16

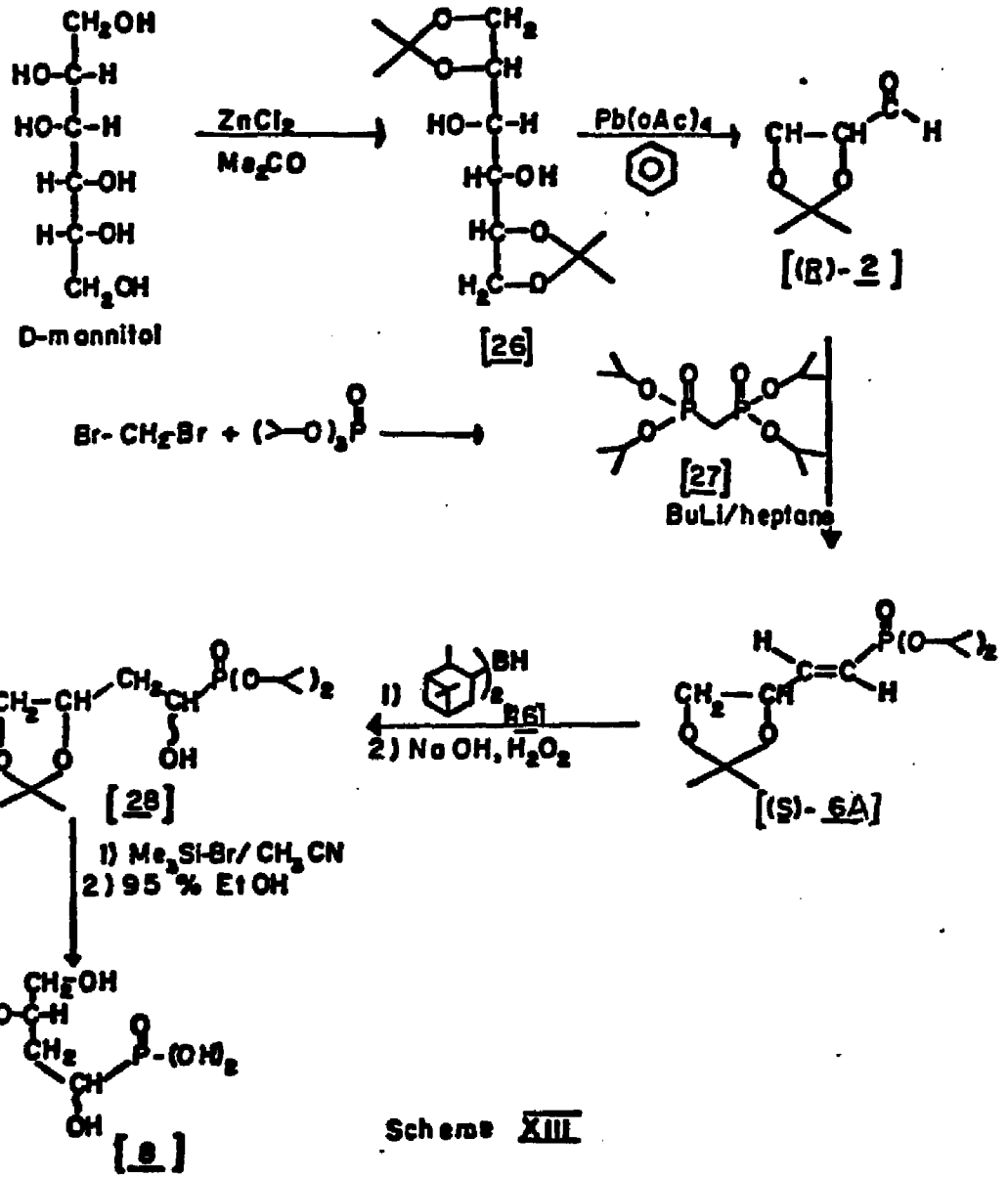
The synthesis of a phosphonic acid with an epoxide function in the alpha,beta position and a diol funtion in C3 and C4 appeared to be a reasonable structural choice for further biological studies.

Results and Discussion

Optically active 1,3,4-trihydroxybutylphosphonic acid [8] was prepared starting with commercially available D-mannitol. D-mannitol was converted to 1,2,5,6, diacetone-D-mannitol [26] by the action of acetone and zinc chloride (56). The preparation of [26] could be considerably shortened if the acetone solution were refluxed for a few minutes. However, the mixture should not boil for more than about 5 minutes, otherwise the yield falls to zero, probably owing to the formation of triacetone D-mannitol. Therefore the entire reaction was worked at room temperature. The melting point, 119°C, could be raised to 122° C by recrystallization from water, but for our purposes the product as obtained was sufficiently pure.

The carbon chain of the diacetone-d-mannitol was split by lead tetraacetate, with the result that two molecules of acetonated D-glyceraldehyde [(R)-2] were formed (57), as

shown in Scheme XIII. The crude [(R)-2] could be distilled under reduced



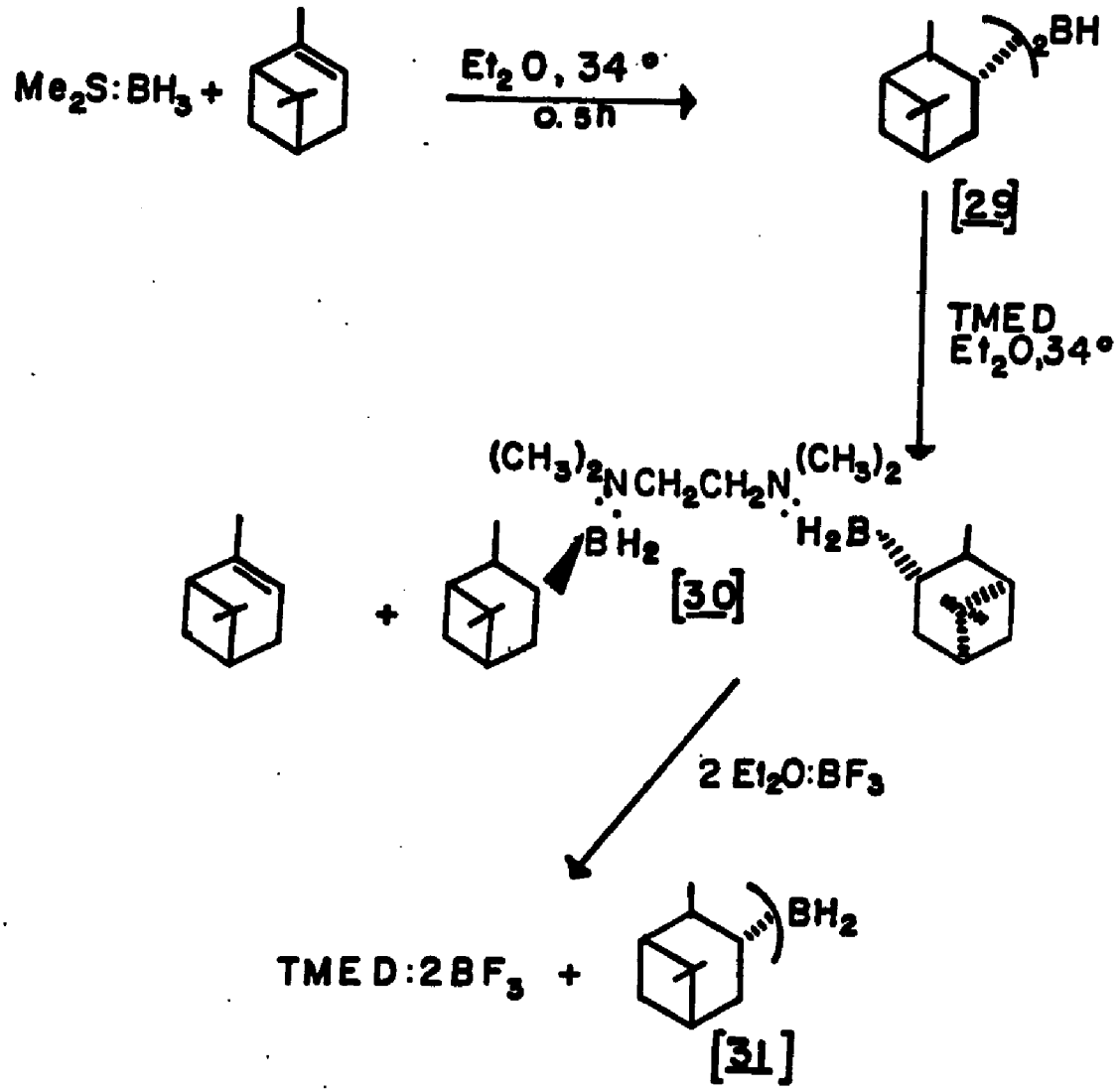
Scheme XIII

pressure but in order to avoid polymerization of the material it was treated immediately with a heptane solution of the anion of tetraisopropylmethylenebisphosphonate using butyl lithium as the base to generate [27] the phosphonate carbanion (58). The product of this modified Wittig reaction is the vinyl phosphonate [(S)-6] which gave satisfactory elemental analysis and spectra. This material is purified by vacuum distillation (b.p. 103-107°C, 0.0075 Torr.), the temperature of the oil never being higher than 165-170°C. Higher temperatures cause pyrolysis of the material.

Since it is well known that addition of organoboranes (59-62) to an unsymmetrical olefin proceeds to place the boron atom on the less substituted of the two carbon atoms forming the double bond, and since the organoborane is readily converted to the corresponding alcohol without rearrangement and with production of stereochemically defined structures by oxidation with alkaline hydrogen peroxide, hydroboration provides a simple,convenient synthetic route for the anti-Markovnikov hydration of the vinylphosphonate [(S)-6]. This particular mode of addition of boranes on phosphonates was also reported by Yamashita et al. (63).

It has been shown that the hydroboration of a number of relatively hindered olefins provides a convenient route for the synthesis of the corresponding mono- and dialkylboranes in high purity. The monoisopinocampheylborane reagent ($IPCBH_2$), which is highly promising for asymmetric hydroboration (6) was first synthesized in high optical purity by a relatively long and time consuming process (64) then Brown *et al*. developed what appeared to be a simpler , and more direct synthesis of optically pure monoisopinocampheylborane, as shown in Scheme XIV. The procedure utilized borane-methyl sulfide (BMS) in Et_2O for the rapid preparation of IPC_2BH (68), a fast displacement of alpha pinene by N,N,N',N',-tetramethylethylene diamine (TMED), and a convenient removal of TMED from the product with $Et_2O{:}BF_3$ to produce a bisadduct [30] of TEMD with monoisopinocampheylborane of very high optical purity (69). This material is reported to be stable to air, which alleviates handling and storing problems.

It is also known that amine boranes react sluggishly with olefins at 25°C (70), thus, the removal of TMED from adduct [30]] was necessary. For this $Et_2O{:}BF_3$ (71) was added to a THF solution of [30], hoping that



Scheme XIV

TEMD;$2BF_3$ would precipitate, leaving $IPCBH_2$ [31 in solution for the ready hydroboration of the

vinylphosphonate. The results were disappointing. This reaction after basic hydrogen peroxide oxidation work-up and distillation did not show the desired product, as indicated by the NMR which exhibited two extra absorption peaks at 0.8-1.2 ppm that could be due to the presence of the amine. In other attempts the white solid TMED:$2BF_3$ was removed by centrifugation (72) and the supernatant was used for hydroboration. Alternatively, the solid was removed using a Millipore filter, but, in either case there was no improvement of the product obtained as seen in the NMR.

In order to test and clarify the hydroboration procedure, some of the vinylphosphonate was allowed to react with BH_3/Me_2S. After basic hydrogen peroxide oxidation and the usual reaction work-up, the NMR analysis of the ester obtained showed the absence of the two extra peaks at low field, what proved that they were due to the presence of the amine in the product. Further hydrolysis ot the free acid was performed and the material exhibited biological activity when assayed with rabbit muscle glycerol 3-phosphate dehydrogenase. On treatment with base this reaction product generated 3,4-dihydroxy butanal, indicating the hydroxyl atom indeed to be attached to the alpha carbon.

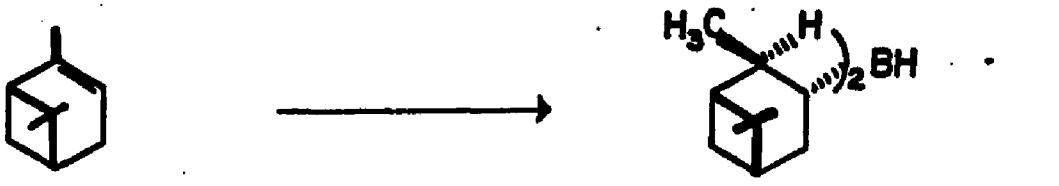
It is presumed from this that difficulties arise from the presence of amine in the product. It was therefore deemed desirable to prepare diisopinocampheylborane (IPC_2BH) instead of the monoisopinocampheylborane ($IPCBH_2$), as Brown *et al.* had used (66) in asymmetric hydroboration without the need of complexation or isolation.

Alpha -Pinene readily undergoes hydroboration at 0^o to form sym-tetraisopinocampheyldiborane. Even in the presence of excess alpha-pinene the reaction does not proceed further. Indeed, in the absence of excess alpha-pinene, there is evidence for a significant dissociation of the tetra alkyl derivative into alpha-pinene and triisopinocampheyldiborane. Oxidation of the reaction product with alkaline hydrogen peroxide yields essentially pure isopinocampheol (66). The hydroboration reaction must, therefore, involve a pure cis addition of the boron hydrogen group to the double bond- from the less hindered side of the molecule- the direction opposite to the gem-dimethyl group followed by oxidation with retention of configuration (73).

Brown *et al.* (66) utilized both dextrorotatory ($+47.6^o$) and levorotatory (-47.9^o) alpha pinene,

indicating optical purities in the 93 to 95% range (74). Hydroboration of (+)-alpha-pinene affords (-)-sym-tetraisopinocampheyldiborane with an optical rotation of -37.1^{o} (75). The following discussion is referred to (-)-and (+)- sym-tetraisopinocampheyldiborane, implying that the reagent was prepared from (+)- and (-)-alpha-pinene, respectively. Finally, although this substance evidently exists in the solid state and in ether solvents as the dimeric diborane derivative, it has proven convenient to refer to it by its more simple monomeric name, diisopinocampheylborane, IPC_2BH , in applications and discussions where the dimeric structure does not appear to be a significant factor. This practice is purely one of convenience and should not be taken to imply that the reaction necessarily involves the monomeric species as an intermediate.

The absolute configuration of alpha-pinene has been assigned (73) and IPC_2BH appears to be formed by a simple cis addition of the borane molecule to the less hindered side (away from the gem-dimethyl group) of the double bond as seen in Figure 17.



(1R:5R)-(+)α pinene

(1R:2S:3R:5R)-(-)-diisopinocampheylborane

Figure 17

The configuration of the optically active alcohol and olefins obtained via hydroboration with (-) diisopinocampheylborane exhibit a definite steric relationship to the configuration of the reagent. Consequently, it appears desirable to consider in detail the precise mechanism of the hydroboration stage in order to establish whether the model arrived at can account for the observed stereospecific syntheses which occur.

Although is not yet certain whether the monomer or the dimer (sym-tetraisopinocampheyldiborane) is the actual hydroborating agent, the following discussion refers to diisopinocampheylborane. However, this is not a decisive factor for the interpretation of results. It should be understood that the correlation rules proposed below are

formal and do not necessarily have mechanistic implications. However, since they serve to correlate the observed configurations in a considerable number of cases in both the alcohol and olefin synthesis, the correlation rules should be quite valuable in predicting the structures of our products.

To minimize possible confusion, the following discussion will be based only upon (-)-diisopinocampheylborane derived from (+)-alpha-pinene.

Inspection of models indicate that the most stable among the possible rotameric conformations for (-)diisopinocampheylborane has a diequatorial arrangement of the borane group and the trans methyl groups in the adjacent position of the pinane moiety and an antiparallel or nearly antiparallel orientation of these two methyl groups in the two different pinane moieties. This leads to a probable description of the model for (-) diisopinocampheylborane as shown in Figure 18.

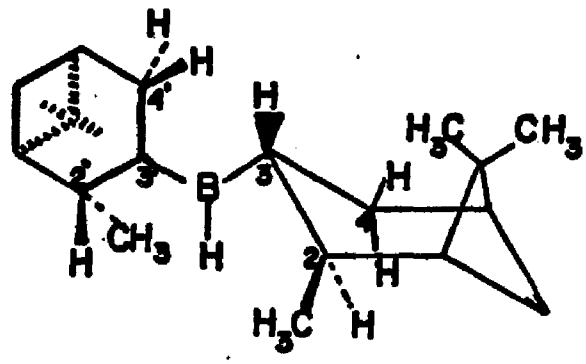


Figure 18

The addition of the boron hydrogen bond to a double bond has been interpreted in terms of a four center transition state (76). The formation of such a highly rigid transition state should be strongly influenced by steric factors of both the reagent and the olefin. Thus the hydroboration of the vinylphosphonate with diisopinocampheylborane can be represented by the two possible transition states, as shown in Figure 19.

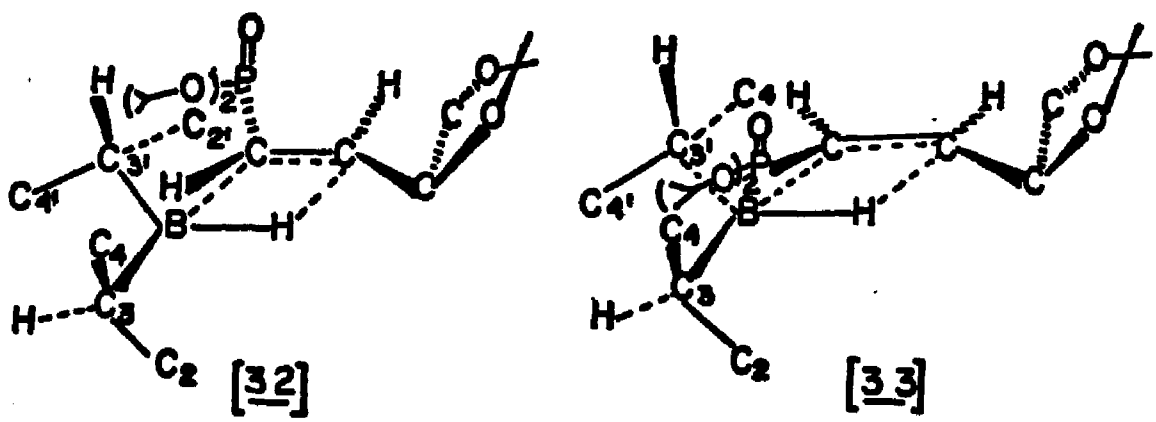


Figure 19

The model I shows that the boron atom is bonded to carbon atoms (C3 and C3') carrying substituents of different size, namely a hydrogen atom at C3' and a larger methylene group at C4. It would, therefore, be anticipated that the preferred transition state would be that in which the phosphorus of the vinylphosphonate is positioned away from the more bulky methylene group at C4 and toward the smaller hydrogen atom at C3' [32]. It is evident that the alternative transition state [33] will be less favorable sterically, with the bulky phosphorus group of the vinylphosphonate and the methylene group at C4 in closer proximity.

In order to simplify the model, the following symbols will be adopted, S (small) for hydrogen, M (medium) for methylene (C4'), and L (large) for the methyl group at C2 and C2'. On this basis, the preferred transition state [32], simplified to [34], as seen in Figure 20A will produce an organoborane which will be oxidized to (1R)-(3S)-diisopropyl,1,3,4 trihydroxy-3,4,-O-isopropylidenebutyl-1-phosphonate [28]

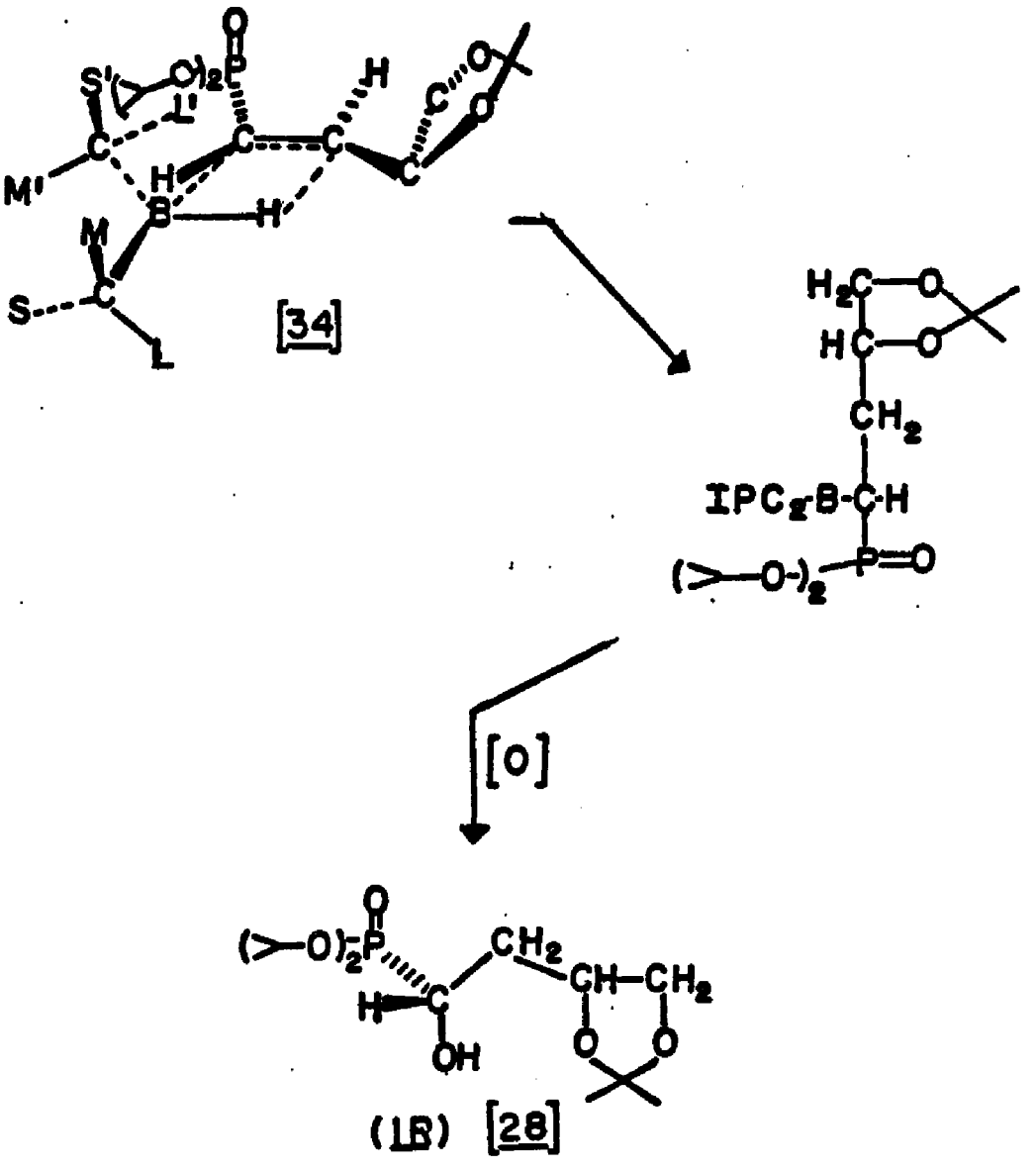


Figure 20 A

In the present work, (+)alpha-pinene in 15% excess was slowly added at 0°C to borane methylsulfide in

sufficient THF to make the final concentration of borane 1.0 M. The reaction mixture was then brought to 25°C and maintained at that temperature for 9 h. and then kept in the refrigerator overnight. The flask and its contents, described above, were cooled to -25°C and treated with the vinylphosphonate. It was required that IPC_2BH be in about 2.5 fold excess for the complete consumption of the starting vinylphosphonate. The reaction was allowed to proceed for 20 hours at R.T. in consideration that the reaction rate of sterically hindered olefins with borane decreases a great deal at the third addition stage of borane, i.e., formation of trialkylborane (75). The product was then oxidized in the usual manner with alkaline hydrogen peroxide. Distillation provided [28] in a yield of 45%. The material exhibited a rotation of -2.42°. NMR and IR data correlate perfectly with the desired ester [28].

For the preparation of the other isomer, namely (+)-isopinocampheylborane, exactly the same procedure was followed but using instead (-)-alpha-pinene. The product of this reaction produced a trihydroxy ester which exhibited an optical rotation of +2.82°. The NMR was identical to that of the material prepared with the other isomer. The product obtained should have a configuration as the one shown in Figure 20B

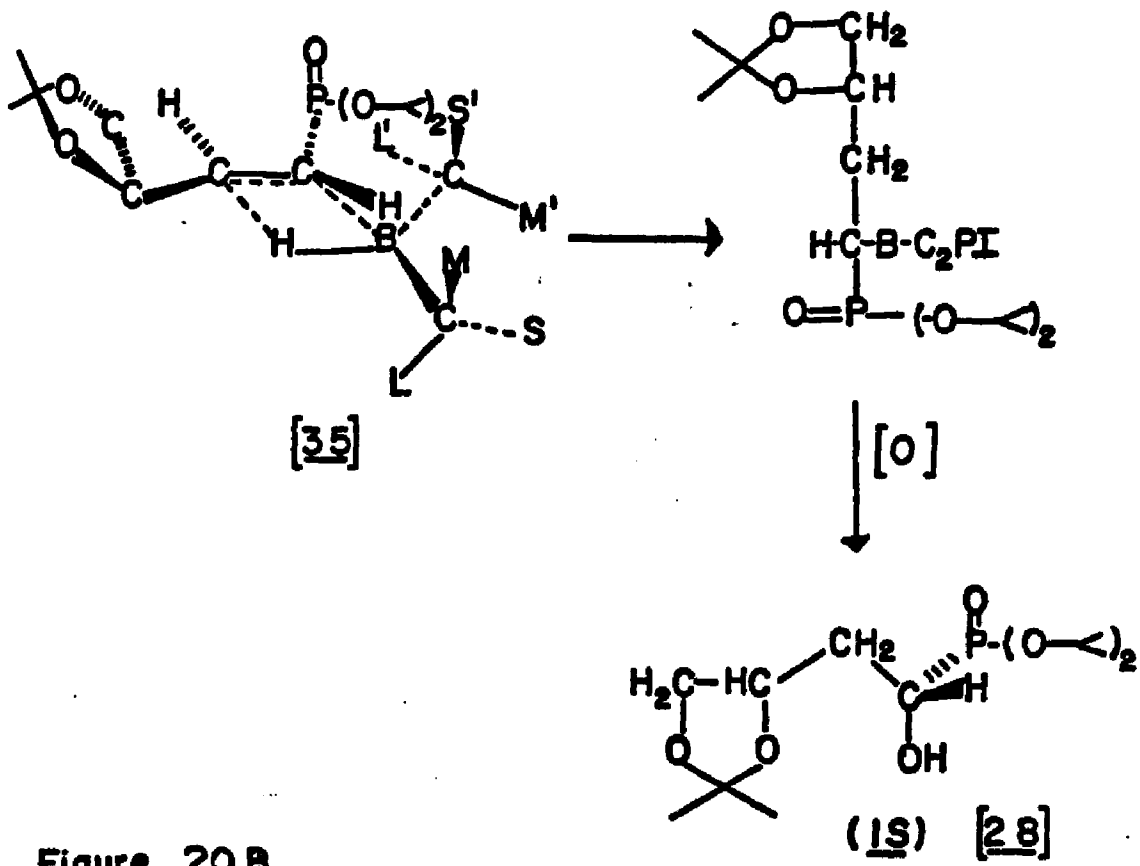
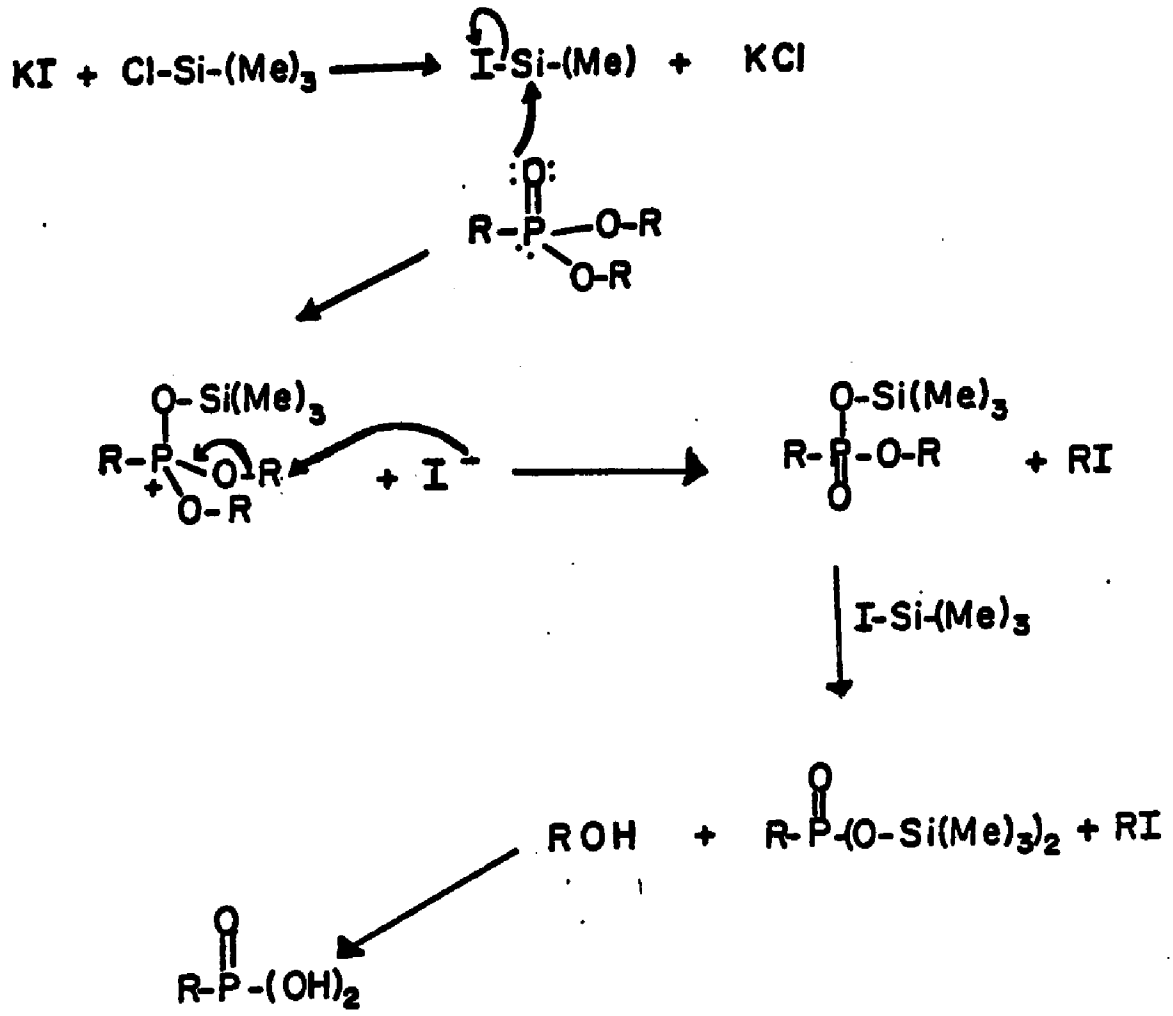


Figure 20 B

For the hydrolysis of the phosphonate esters several methods were tried. In order to overcome the disadvantages inherent in the use of iodotrimethylsilane, the reagent was prepared <u>in situ</u> using chlorotrimethylsilane/KI in acetonitrile (80), Scheme XV

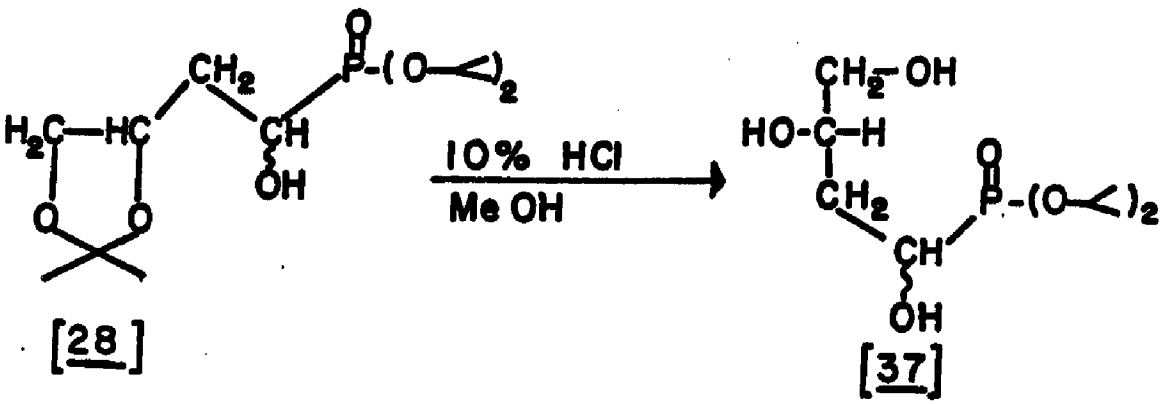


SCHEME XV

The exothermic reaction of the phosphonate [28] with chlorotrimethylsilane/KI occured rapidly at room temperature to give the bis(trimethylsilyl) phosphonates and alkyl

iodide. Treatment of the silyl esters [35] with 95% ethanol at room temperature gave the corresponding phosphonic acid [8]. The acetonide protecting group was also cleaved by the reagent, but the product was greatly contaminated with iodide or some other material which was not analyzed, and gives a very dark brown color to the reaction, then in order to remove the iodide the product was dissolved in 60 mL of 95% EtOH , and the ethanol was removed in the rotary evaporator, this procedure was repeated until the product showed a light yellow color. In order to avoid this problem bromotrimethylsilane can be used instead of the iodotrimethylsilane, either neat or using acetonitrile as a solvent. . Bromotrimethylsilane is reported (81) to be highly selective for P-O silyldealkylation of phosphonates. With this reagent the acetonide ring was also cleaved. Hydrolysis was done with 95% ethanol. Spectral data and elemental analysis were satisfactory.

An alternate approach involves cleaveage first of the acetonide ring of [28] by treating it with 10% HCl in methanol, followed by neutralization and extraction in ethylacetate, as shown in Figure 21.



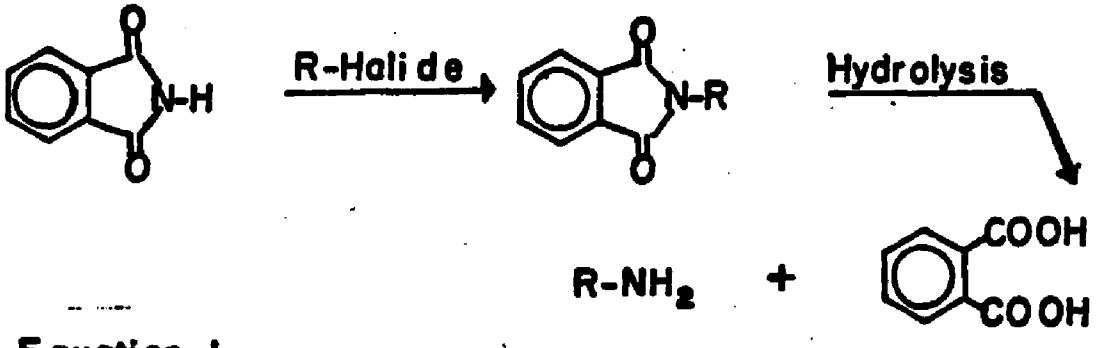
[28] [37]

Figure 21

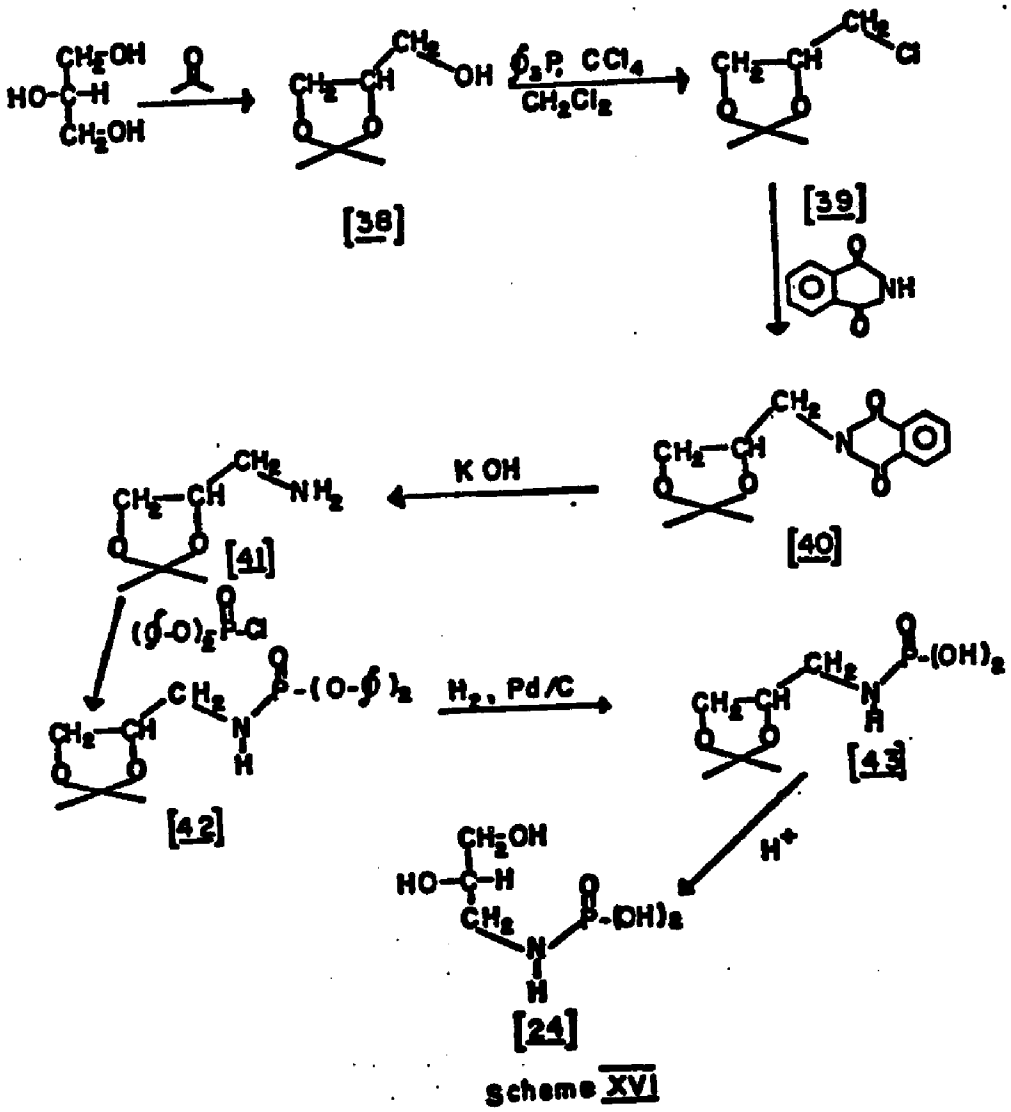
In this way one would avoid any possibility of racemization at carbon 3. The triol obtained [37] was then subjected to the already mentioned deprotection by bromotrimethylsilane.

The synthesis of N-(2,3-dihydroxypropyl) phosphoramide [24] started with commercially available glycerol which was treated with acetone and acid (81) to give glycerol acetonide [38], as shown in Scheme XVI. Subsequent halogenation of [38] to obtain 3-chloro-1,2-O-isopropylidene-1,2-propanediol [39] was accomplished by the use of triphenylphosphine and carbontetrachloride in dichloro methane solution. The precipitation of the by-product triphenylphosphine oxide was promoted by the addition of pentane. On several occasions it was very difficult to precipitate completely the triphenylphosphine oxide. For this reason, in subsequent preparations, commercial 3-chloro-1,2-propanediol was used instead of glycerol.

The primary amine [41] was prepared by the Gabriel synthesis, as shown in equation 1.



Equation I



Scheme XVI

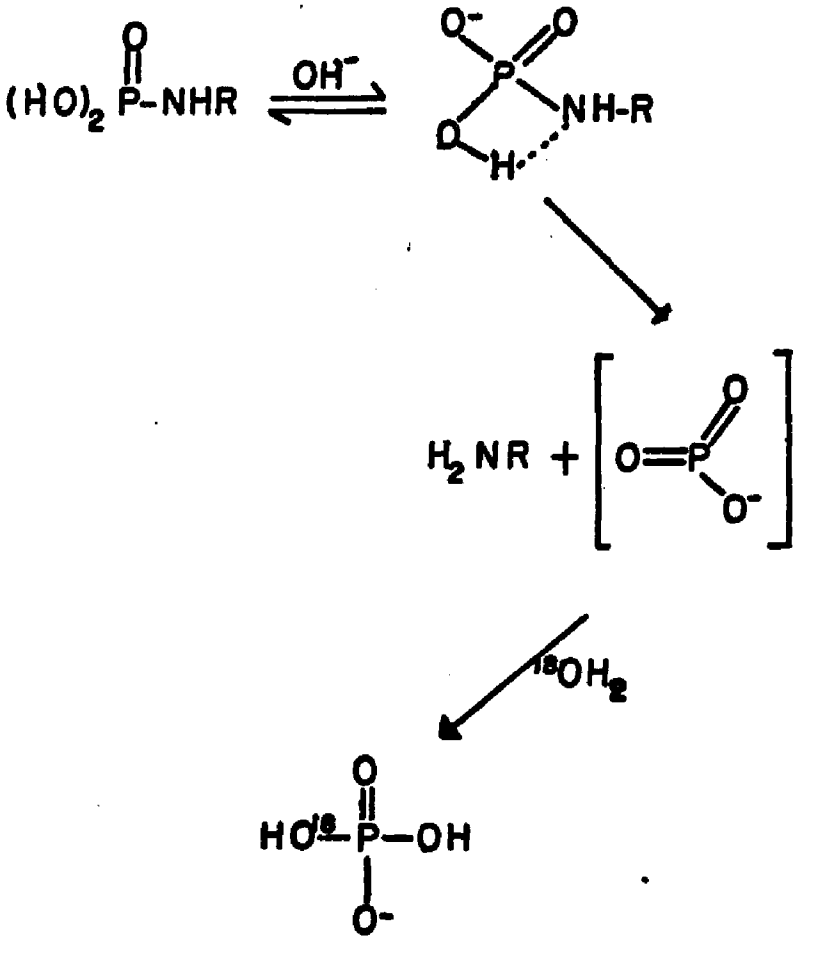
This method offers the advantage of the absence of secondary or tertiary amine contamination (82) of the primary amine, the toleration of a wide range of other functional groups in the molecule, and the mild conditions needed for accomplishing both stages: condensation and hydrolysis. The condensation to form [40] was performed in dimethylformamide, in which potassium phthalimide is appreciably soluble (83). A mild exothermic reaction starts spontaneously at room temperature. The reaction was stirred for an additional 18 hours at 90°C to ensure complete displacement of the halide. A condensation product [42] of high purity, as shown by spectral data and elemental analysis, was obtained in 25% yield by chloroform extraction of the reaction mixture after dilution with water. Dimethylformamide remains in the aqueous phase. Product crystallization resulted from storing the oily residue in the freezer.

Hydrolysis of N-substituted phthalimides by hydrochloric acid is widely used in the Gabriel synthesis, but due to the acetonide (acid sensitive) part of the molecule [40] the hydrolysis had to be accomplished under alkaline conditions (84). Phenylhydrazinolysis as described by Shumann *et al.* (85) using phthalyl-glycine was tried but separation of the

free primary amine from the by products was a very difficult task. The commonly used hydrazine hydrolysis could not be used here because it requires acid in order to precipitate the phthalyl hydrazide from the reaction mixture. Hydrolysis was then atempted by refluxing the phthalimide adduct in 15% aqueous KOH and the free primary amine [41] was recovered by means of a continuous extraction system using pentane as the solvent. Preparation of the phosphoramide [42] was accomplished by treatment of the primary amine with diphenylphosphorochloridate in ether, in the presence of Et_3N. The salt Et_3N:HCl was filtered and the solvent removed. Spectral analysis confirmed the presence of [42] , the phenyl groups of which were hydrogenolyzed over PtO_2 at 46 psi. After filtration of the catalyst through celite, pure [43] was obtained. This oily material was then treated with Dowex 50 in the acid form obtaining then the final product [24] N-2,3 dihydroxypropylphosphoramide.

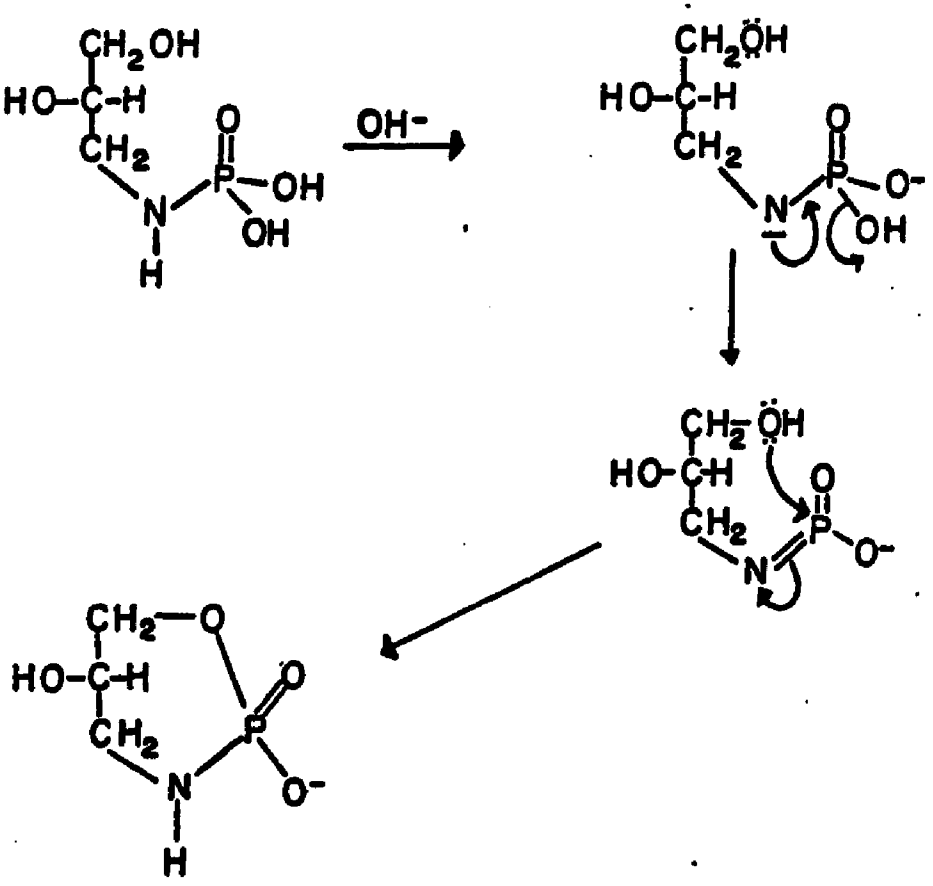
This material did not inhibit E. Coli growth. One possible reason for this inactivity of [24] is possible hydrolysis of the molecule similar to that proposed by Halmann (87) et al. They studied the hydrolysis of N-substituted phosphoramiates, $(HO)_2$ P(O) NHR, and they

concluded that this hydrolysis goes by an $SN_1(P)$ mechanism , where one atom of O^{18} is incorporated in the phosphate product on hydrolysis with H_2O^{18} and $R=CO_2Et$, they rationalized their findings as shown in Scheme XVII



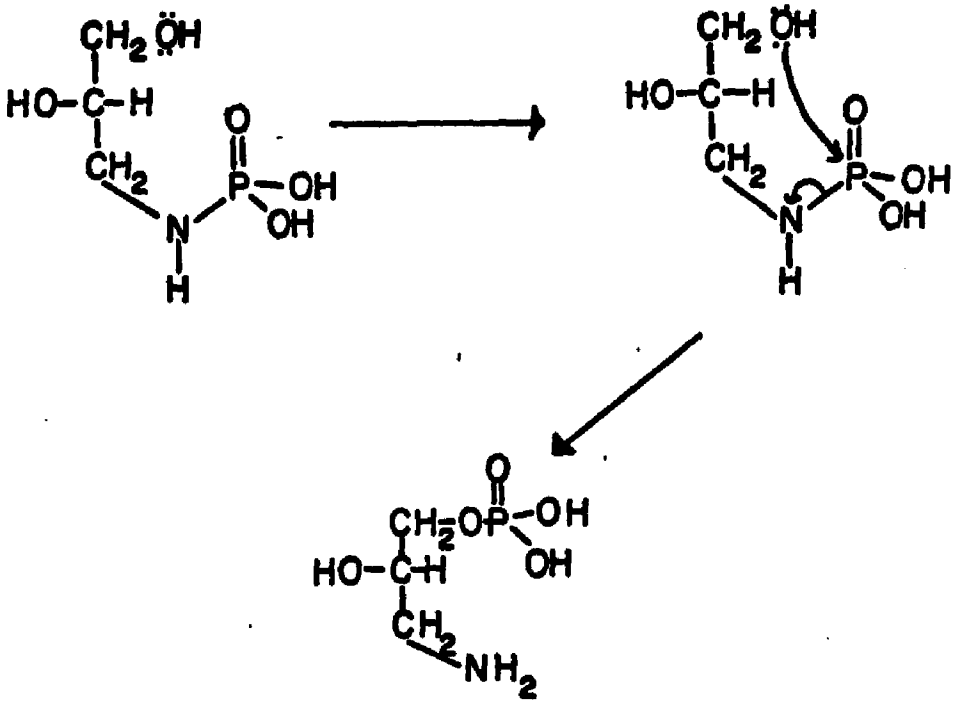
SCHEME XVII

In view of the mechanism of amidate hydrolysis proposed by Halmann one can think that the lack of biological activity of the phosphoramidate [47] is probably due to a hydrolysis similar to the ones mentioned above, and shown in Scheme XVIII.



SCHEME XVIII

Another rearrangement that may be considered , is shown in Scheme XIX,



SCHEME XIX

This rearrangement is similar to the one occuring during acid hydrolysis of dilute solutions of some phosphorus-nitrogen compounds (89), where the ultimate

products are oxylinked phosphoramidates, as seen in Figure 23.

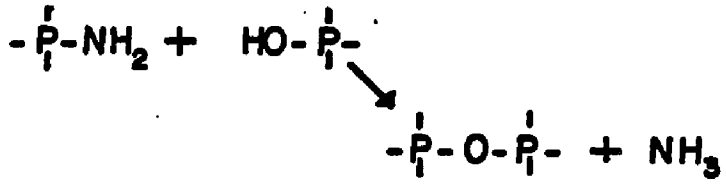
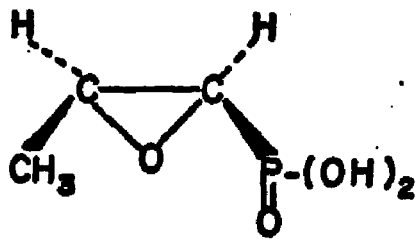


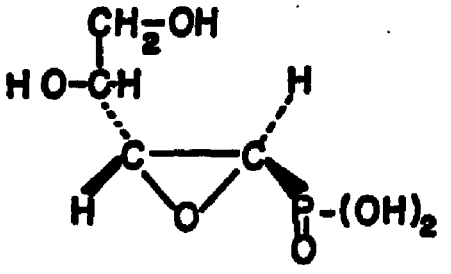
Figure 23

The synthesis of oxirane phosphorus derivatives has been greatly stimulated by the discovery of the antibiotic fosfomycin, (1R,2S)-1,2-epoxyphosphonic acid [10]



[10]

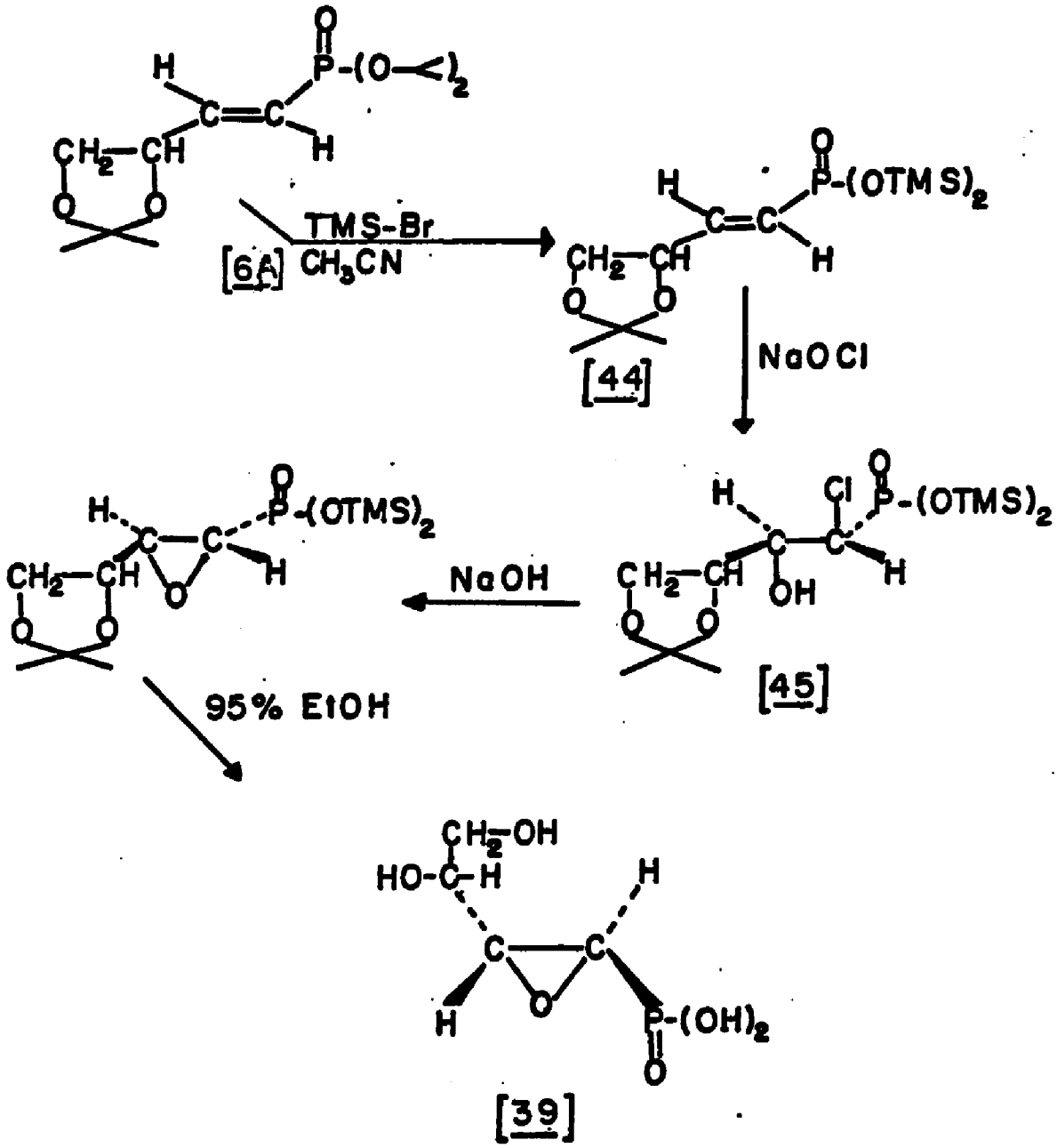
I was interested in the preparation of an alpha,beta-epoxy phosphonic acid analogue of glycerol 3-phosphate, namely: 1,2-epoxy-3,4-dihydroxybutane phosphonic acid [39].



[39]

We tried numerous methods, always starting with the vinylphosphonate [(S)-6] but all our efforts were without great success. The treatment of halohydrins with base is a well-known method for the formation of epoxides (90) and has been exploited in the synthesis of epoxyphosphonates. The halohydrin derived from the treatment of [45] with sodium hypochlorite (chlorox) and base (93) as shown in Scheme XXI, after reaction work-up did not give the expected alpha, beta epoxyphosphonic acid [39].

Deprotection of acetonide and ester groups was always accomplished with trimethylsilylbromide before epoxidation, to avoid possible nucleophilic attack on the fragile epoxide ring.



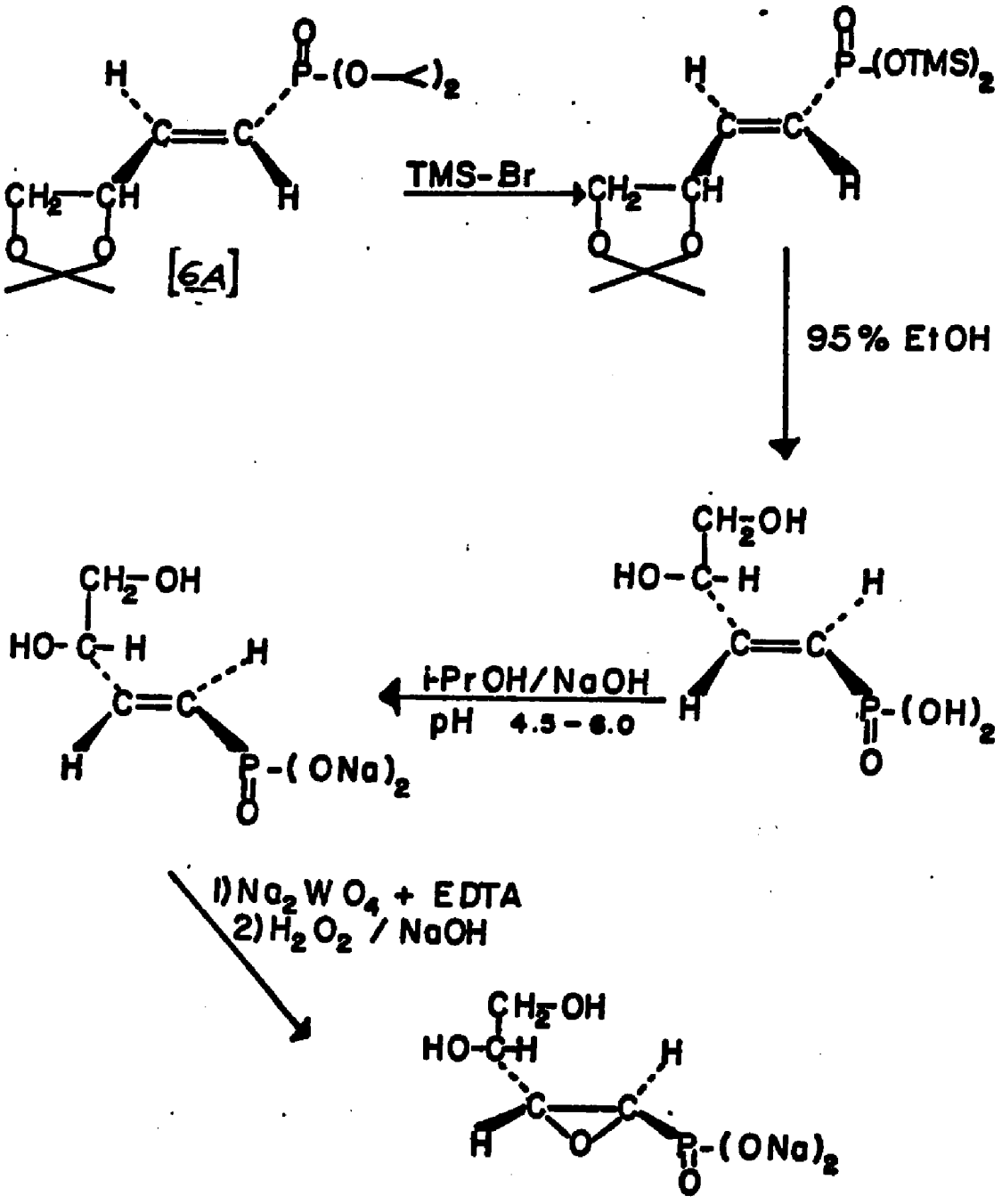
Scheme XX

Direct epoxidation of [(S)-6] by m-chloroperbenzoic acid in methelene chloride (92) at room temperature gave very low yield (approx. 1% of theoretical) and a complex mixture of products very difficult to identify. Similar findings are mentioned by Swern (94) who concluded that the reaction of an organic peracid with an olefin is slowed down considerably or completely suppressed by the presence of a carboxyl or carboalkoxy group (electron withdrawing groups) in close proximity to the double bond.

In view of the relative electrophilicity of the double bond of the vinylphosphonate and considering the tendency of this compound to undergo nucleophilic addition (95), the epoxidation was tried with methanolic hydrogen peroxide maintaining the pH between 9.5-10.0. After extraction of the product in methylene chloride, the material was dried over $MgSO_4$ and the solvent removed under reduced pressure. Analysis of the NMR showed large amounts of impurities plus a discrete amount of possible epoxide. In order to purify the material the dicyclohexylamonium salt was formed by dissolving the crude product in toluene, acetone and dicyclohexylamine. The fine precipitate was filtered and recrystallized from methanol. Elemental analysis showed a mixture of 80% mono-salt and 20% di-salt.

This material exhibited biological activity and was a very promising compound for biological investigations. However a more reliable route was sought.

A similar synthesis to the one reported by Glamkowsky et al. (96) for fosfomycin using sodium tungstate as catalyst was proposed. This synthesis which is simple and elegant as seen in Scheme XXI, was reported to afford fosfomycin in much higher yield than the previous synthesis reported. Deprotection of the vinyl phosphonate [6] was accomplished by the use of trimethylsilylbromide. The TMS esters were cleaved to the free acid by the action of 95% ethanol. After removal of the solvent, the vinylic acid was dissolved in isopropyl alcohol and treated with 10% NaOH solution. For this $Na_2WO_4:2H_2O$ + $Na_2EDTA:2H_2O$ were used as the catalyst. H_2O_2 , 30%, was added dropwise being extremely careful to maintain the reaction pH between 4.5-6.0 during the whole reaction time. The product was extracted and after removal of the solvent the oily product was treated with Dowex 50 in the acid form. Unfortunately none of the expected epoxide was obtained,but rather a complex mixture of various compounds which were not analyzed any further.



[6A]

Scheme XXI

A possible explanation for the lack of success in the preparation of 1,2-epoxy,3,4-dihydroxybutane phosphonic acid involves rearrangement of the molecule. The migration of a dialkoxyphosphono group to an electron deficient terminus has been demonstrated by two classes of rearrangements (97,98). A number of alpha,beta-epoxyvinylphosphonates of type I have been converted by Churi and Griffin to alpha-formylalkylphosphonates as seen in Figure 24

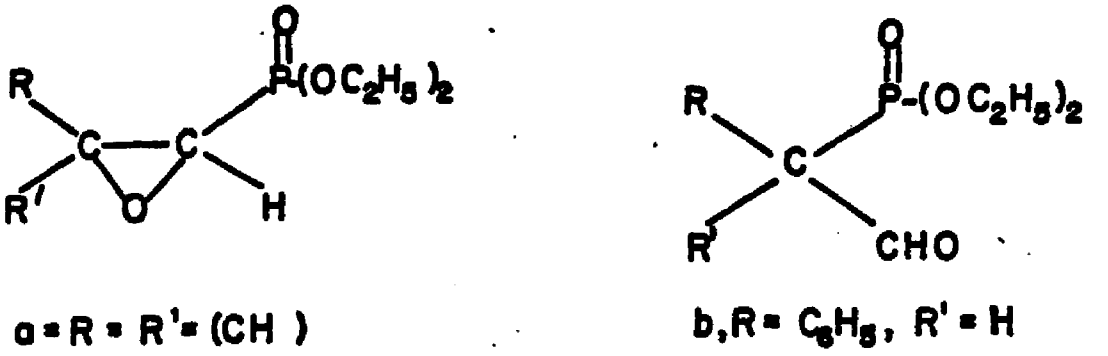
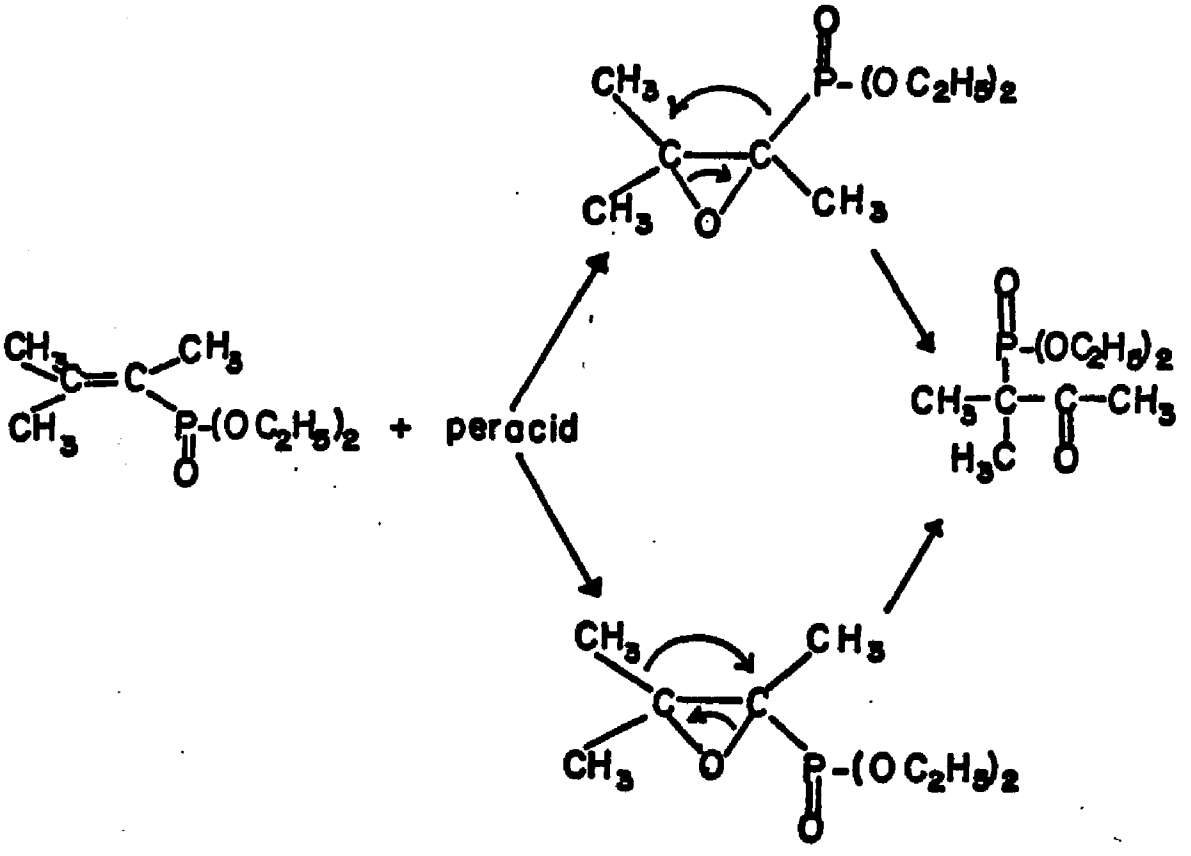


Figure 24

some by heating, others (Ia,Ib) by treatment with boron trifluoride etherate. The later reactions are undoubtedly "carbonium ion" type rearrangements involving cleavage of the tertiary C-O bond and 1,2 migation of the diethoxyphosphono substituent in preference to hydrogen. Hunger (92) also explains the lack of success in the

epoxidation of vinylphosphonates by peracids by the formation of carbonyl compounds either by dialkylphosphono group or alkyl migration, as represented in Scheme XXII.



Scheme XXII

A further point of consideration in our system is the existence of the diol function on the beta carbon, which produces an electron withdrawing effect in the already destabilized double bond.

EXPERIMENTAL

General

All chemicals were reagent quality and used without further purification with the following exeptions: benzene, pentane and hexanes were dried over sodium ribbon, acetonitrile was distilled over calcium hydride, tetrahydrofuran was distilled over lithium aluminum hydride, dimethylformamide (DMF), and methylene chloride were distilled immediately prior to use and stored over molecular sieves.

Thin-layer chromatography was performed using polygram Sil-N-HR silica gel sheets which were purchased from Brinkmann Instruments, Inc. Silica gel for preparative chromatography was from Baker (60-200 mesh).

Infrared spectra were measured using Perkin Elmer 237-B and 598 spectrophotometers, NMR spectra were measured using

a Varian EM-360 spectrometer. The elemental analyses of the compounds were performed by Galbraith Laboratory, Knoxville, Tennessee. Butyllithium, borane methyl sulfide (BMS) 10 M and (+) and (-) alpha pinene were bought from Aldrich Chemicals. The optical rotation of the compounds were measured on a Perkin Elmer 141 polarimeter.

1,2,5,6-Diacetone-D-mannitol [26]

To a solution 180g $ZnCl_2$ in 900 mL of acetone, 30g (0.16 mole) of finely powdered D-mannitol was added. The mixture was stirred at room temperature until the mannitol dissolved (approx. 35 min.). Then the reaction was allowed to stand at room temperature for 18h, and after this time was poured into a solution of 210g potassium carbonate in 210 mL water and 900 mL Et_2O. This mixture was then vigorously shaken for 0.5 h The acetone-ether solution was separated from the agglommerated $ZnCO_3$ pellets. The pellets were washed twice with 300 mL of 1:1 acetone-ether solution and the combined filtrates were concentrated in a vacuum pump at a bath temperature between 60-70°C. The residue was refluxed 5 times for 20 min. in 250 mL portions of petroleum ether, the resulting solutions were rapidly

filtered through a steam heated funnel. On slow cooling long crystals appeared; they were dried on the vacuum pump. Yield:22g, 55% of theoretical, M.P.: 99-103°C

Acetone-D-glyceraldehyde [2]

A suspension of 15.6 g. (0.0065 mole) of 1,2,5,6-diacetone-D- mannitol [40] in 550 mL of benzene, was quickly mixed by gentle stirring. Then lead tetraacetate 26.4g (0.06 mole) was added and the mixture was stirred for 2h. The sticky precipitate of plumbous salt was triturated to a fine and colorless powder. It was tested with potassium iodide-starch paper, and if lead tetraacetate was shown still to be present (test paper turns dark-brown), more diacetone-D- mannitol was carefully added until the oxidizing agent was used up. The preparation was filtered and all the solvent removed from the filtrate by flash evaporation. This material is unstable and must be used immediately after preparation. Yield: 12g (96%), N.M.R. shows the absorption peak at about 9ppm characteristic of aldehydes. IR: 1630 C=C, 1248 P=O

Tetraisopropylmethylenebisphosphonate [27]

Triisopropyl phosphite 312.35g (1.5 mole) and dibromomethane 86.95g (0.5 mole) were combined in a 500 mL round bottom flask connected to a condenser in which the temperature of the circulating water was maintained at 65C° during the entire reaction period because at this temperature the side product, isopropylbromide (B.P. 59°C) can be collected. The reaction commences at 140°C. The temperature of the mixture was gradually raised to 185°C and was kept at this temperature for an additional 2h. The excess of triisopropylphosphite was removed under vacuum at 50°C/0.007Torr. Yield 90% The tetraisopropylmethylenebisphosphonate was distilled at 118°C/0.007Torr. Yield 90% N.M.R. ($CDCl_3$): 1.2-1.6 (d, 24H); 1.9-2.7 (t, 2H); 4.4-5.0 (m, 4H)

Diisopropyl (S)-(E)-3,4-diisopropylidene-3,4-dihydroxybutene-1-ylphosphonate [6]

To 17.94g of tetraisopropylmethylenebisphosphonate in 200 mL of dry heptane under an inert (N_2) atmosphere, 32.5 mL of butyllithium (1.5 M in hexane) were added at room

temperature and stirred for 2h, after which time the reaction medium was cooled to 0°C and 6.78g (0.052 mole) of acetone-D-glyceraldehyde [2] in 25 mL of dry heptane were added dropwise by means of an addition funnel. The reaction mixture was allowed to warm to room temperature and was heated at reflux for 2 h. The reaction mixture was diluted with 500 mL H_2O, the heptane layer separated and the aqueous layer was extracted 2 times with 100 mL of heptane. The combined heptane extracts were dried over $MgSO_4$. After filtration and evaporation of the solvent at reduced pressure the product was vacuum distilled B.P. 105-115°C/ 0.025 Torr, $[\alpha]_D^{25}$ = +15.48° (c 0.32 EtOH) N.M.R.($CDCl_3$): 1.1-1.3 (m, 18H); 4.3-5.0 (m, 2H); 3.3-4.3 (m, 2H); 5.5-7.0 (m, 2H) Elemental analysis for $C_{13}H_{25}O_5P$, Calculated: C,53.42; H,8.56. Found: C,51.58; H,8.19

Preparation of (-)-diisopinocampheylborane [46 A]

Borane methyl sulfide (BMS) 8.0 mL (0.008 mole) in 40 mL Et_2O were cooled at 0°C; to this 21.8g (0.16 mole) of (+)-alpha-pinene was added dropwise over a period of 20 min. After this time the ice bath was replaced by a water bath at 25°C and the reaction mixture stirred for

another two hours. It was then allowed to stand at this temperature for 9h; before being placed in the refrigerator overnight.

(1R,3S)-Diisopropyl-1,3,4-trihydroxy-3,4-O-isopropyledene butyl-1-phosphonate [28 A]

The reaction flask containing the (-)-diisopinocampheylborane, from the above reaction was cooled to -25°C (dry ice/CCl_4 bath) and 6g (20 mmol) of [6] was added dropwise. The reaction was stirred overnight. Then 10 mL of H_2O were added very slowly to the cooled reaction mixture at 0°C (ice bath), and 9 mL 30% NaOH was added dropwise. This was followed by the dropwise addition of 18 mL 30% aqueous H_2O_2 , care being taken to maintain the temperature of the solution below 40°C at all times. The stirring was continued for 2h at this temperature. The reaction mixture was then cooled, a further 9 mL of 30% NaOH were added, and the aqueous layer was saturated with anhydrous K_2CO_3. The organic layer was separated and the aqueous layer extracted 4 times with 50 mL Et_2O. The combined ether extracts were washed twice with 20 mL of saturated NaCl solution and finally dried over $MgSO_4$. The product was

vacuum distilled B.P. 110-120°C/0.0025 Torr. Yield 2g (48%). N.M.R. ($CDCl_3$) : 1.3-1.5 (d,18H); 1.7-2.2 (m, 3H); 3.6-3.8 (s, 1H); 4.0-4.3 (m, 2H); 4.5-5.0 (m, 2H).

Elemental analysis for $C_{13}H_{27}O_6P$ calculated: C, 50.32; H, 8.77 Found: C,50.92; H,9.07. $[\alpha]_D^{25}$ = -2.43° (c 0.32, EtOH)

(+)-diisopinocampheylborane [46B]

Borane methyl sulfide (BMS) 8.0 mL (0.008 mole) in 40 mLof Et_2O were cooled at 0°C, to this 21.8g (0.16 mole) of (-)-alpha-pinene were added dropwise over a period of 20 min, after this time the ice bath was replaced by water bath 25°C and then allowed to stand at this temperature for 9h, then the reaction was kept in the refrigerator overnight.

(1S,3S)-Diisopropyl-1,3,4-trihydroxy-3,4-O-isopropylidene butyl-1-phosphonate [28 B]

The reaction flask containing the (+)-diisopinocampheylborane, from the above, was cooled to

-25°C (dry ice/CCl_4 bath) and 6g (20 mmol) of [6] was added dropwise. The reaction was stirred overnight . Then 10 mL of H_2O were added very slowly to the cooled reaction mixture at 0°C (ice bath), and 9mL of 30% NaOH, followed by 18mL of 30% H_2O_2 were added dropwise maintaining the temperature of the solution below 40°C. Then it was further stirred for 2h at this temperature. The reaction mixture was then cooled, 9 mL of 30% NaOH was added, and the aqueous layer was saturated with anhydrous K_2CO_3. The organic layer was separated and the combined organic extracts were washed twice with 20 mL of saturated NaCl solution and finally dried over $MgSO_4$. The product was vacuum distilled B.P. 110-120°C/0.025Torr. Yield 2.5g (52%). NMR ($CDCl_3$): is exactly as the one obtained for the preparation of the other isomer [42A]. $[\alpha]_D^{25}$ = +2.82° (c 0.32, EtOH)

(+)-(1R,3S)-1,3,4-trihydroxybutylphosphonic acid [8A]

Under an inert atmosphere (N_2) were dissolved 2g of (-)-[28] a 10 fold excess of $(CH_3)_3$-Si-Br were added by means of a syringe. The reaction was stirred overnight. The solvent and the excess of $(CH_3)_3$-Si-Br were

removed in the rotary evaporator. The silyl ester was obtained as an oil which was then treated with 95% EtOH, the solvent was removed under reduced pressure and the free acid was obtained as a pink-brown oil. N.M.R.(D_2O) shows the disappearance of the signals due to the methyls and isopropylidene ring at about 1.0 ppm. The signals due to the hydrogens of the isopropul group at about 5.00ppm also disappear. Calculated elemental analysis: C,25.80; H,5.91, Found C,24.69; H,5.29. $[alpha]_D^{25}$ = +2.65° (c 0.32, EtOH)

(-)-(1S,3S)-1,3,4-trihydroxybutylphosphonic acid [8B]

Under an inert atmosphere (N_2) were dissolved 2g of (+)-[28] (6.45 mmol) in dry acetonitrile and a 10 fold excess of $(CH_3)_3$-Si-Br were added by means a syringe. The reaction was stirred overnight. The solvent and the excess of trimethylsilylbromide were removed in the rotary evaporator. The silyl ester was obtained as an oil which was then treated with 95% ethanol. The solvent was removed under reduced pressure and the free acid was obtained as a pink-brown oil. The N.M.R. (D_2O) was exactly as the other isomer. Elemental analysis for

$C_4H_{11}O_6P$, Calculated: C,25.80; H,5.91; Found: C,25.80; H,5.87 $[\alpha]_D^{25} = -10.23^\circ$ (c 0.24, EtOH)

D-Glycerol acetonide [38]

A mixture of 100g (0.75 mole) of glycerol, 300 mL of acetone, 50g of anhydrous sodium sulfate, and 5 mL conc. sulfuric acid was stirred for 16 h. The acid was neutralized by stirring the reaction mixture about 1.5h with 70g of lead carbonate. A second treatment of lead carbonate was used if the solution was still acidic. The salts were filtered, and the filtrate dried by stirring it 1h with 40g of anhydrous postassium carbonate. The filtered solution was concentrated under reduced pressure at a bath temperature of 40°C to remove acetone. 60g (62% yield) of crude product were obtained. N.M.R. ($CDCl_3$): 1.1-1.4 (d, 6H); 3.4-4.2 (m, 6H)

3-Chloro-1,2-O-isopropylidene-1,2-propane diol [39]

Triphenylphosphine 23.84g (0.0909 mole) in 35 mL CH_2Cl_2 (dried) was added dropwise to a solution of acetonide 12g (0.0909 mole) and CCl_4 34.5g (0.22 mole)

at room temperature. The reaction was stirred for 48h. Triphenylphosphine oxide was precipitated by the addition of 200 mL of pentane, the solid was removed by filtration and washed with 100 mL of pentane. The combined pentane solutions were washed with saturated $NaHCO_3$, water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and a further 100 mL of pentane were added to precipitate the last traces of triphenylphosphine oxide. After removal of the solvent in the rotary evaporator, a yield of 4.66g (32%) was obtained. N.M.R. ($CDCl_3$): 1.3-1.7 (d, 6H); 3.3-3.6 (t, 2H); 3.8-4.4 (m, 3H)

3-Phthalimido-1,2-O-isopropyledene-1,2-propane diol [40]

Phthalimide 26g (0.176 mole) and NaH 4.224 g (0.176 mole) in 180 mL DMF (dried) were shaken for 1h, then refluxed for another hour. To this solution was added dropwise 3-chloro-1,2-O-isopropylidene- 1,2-propanediol [39] 22.54g (0.15 mole) which was refluxed for 18h. The cooled reaction mixture was diluted with 250 mL $CHCl_3$ and poured into 1000 mL H_2O. The water layer was extracted 3 times with 100 mL $CHCl_3$. The combined $CHCl_3$ solutions were washed with 500 mL of NaOH, 0.1 N,

and dried over $MgSO_4$. The solvent was removed under reduced pressure, the oily material was left overnight in the refrigerator to promote crystallization. Yield 8g, 25%, M.P. 80-85°C N.M.R. ($CDCl_3$): 1.3-1.7 (d, 6H); 3.7-5.0 (m, 5H); 7.8-8.3 (d, 4H) Elemental analysis for $C_{14}H_{15}O_4N$, Calculated: C,64.60; H,5.80 Found: C,64.35; H,5.79

3-Amino-1,2-O-isopropylidene-1,2-propane diol [41]

3-Phthalimido-1,2-O-isopropylidene-1,2-propanediol 10g (0.0383 mole) was refluxed in 300 mL of pentane and 50 mL KOH (25%). The water was removed by means of Dean Stark condenser and the salt was filtered from the pentane solution and washed with 100 mL of pentane. The solvent was removed under reduced pressure and a yield of 3.2g (62%) was obtained N.M.R. ($CDCl_3$): 1.3-1.7 (t, 8H); 2.6-2.9 (d, 2H); 3.6-4.3 (m, 3H)

N-Diphenyl(2,3-isopropylidene-2,3-propanediol)phosphoramidate [42]

In a 250 mL round bottom flask 3-amino-1,2-O-isopropylidene-1,2- propanediol 3.2 g

(0.244mole) was mixed with Et_3N 3.5 mL (2.5g) and 30 mL Et_2O. To this solution diphenyl phosphoroclhoridate 6.55g (0.0244 mole) dissolved in 20 mL Et_2O was added dropwise, the mixture was stirred overnight. The Et_3N:HCl salt was filtered and the solvent removed under reduced pressure. A yield of 3.9g (72%) was obtained. NMR ($CDCl_3$) 1.0-1.4 (q, 7H), 2.9-4.3 (m, 5H), 7.0-7.3 (d, 10H) Elemental analysis for $C_6H_{22}NO_5P$, Calculated: C, 59.50; H, 6.10; N, 3.85 Found: C, 59.26; H, 6.03; N, 4.11

N-(2,3-O-isopropylidene-2,3-propanediol)phosphoramide [43]

A solution of 3g. (0.014 mole) of [46] in a 100 mL absolute EtOH with 0.3g of Pd/C in a Parr apparatus was hydrogenated at 46 psi of hydrogen until no more hydrogen was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure. and a yield of 2.1g (68%) of product was obtained. N.M.R. shows disappearance of the aromatic signals. Elemental analysis for $C_6H_{14}NO_5P$, Calculated: C,34.12; H,6.68; N,6.63 Found: C, 33.97; H, 7.08; N, 6.32

N-(2,3-dihydroxypropyl)phosphoramide [24]

In 30 mL of 95% ethanol 2.2g of [49] were dissolved and this was treated with 15mL 10% HCl. The mixture was stirred for 1h. After this time reaction was neutralized with 0.2 M NaOH to pH. 7 and the material was extracted with 3 times 100 mL of Et_2O. The solvent was removed under reduced pressure and residue dried overnight in the high vacuum pump. 1.3g of product were obtained (60% yield). Elemental analysis for $C_3H_{10}NO_5P$, Calculated: C, 21.05; H, 5.8; N, 8.18 Found: C, 20.87; H, 5.93; N, 8.29

Attempted preparations of:

(1RS,2RS,3R)3,4-dihydroxy-1,2-epoxybutyl,1-phosphonic acid [44]

A)

To a 500mL flask equipped with stirrer, thermometer and a dropping funnel, was added 4g (0.0125 mole) of [6A] under an inert (N_2) atmosphere dissolved in 35mL CH_2Cl_2, to this 4.6g (0.025 mole) of m-chloroperbenzoic acid (mCPBA) dissolved in 55 mL

CH_2Cl_2 were added by means of the dropping funnel during a 10 min period at room temperature. The reaction mixture was stirred overnight. The excess of peracid was destroyed by the addition of 10% sodium sulfite solution, enough so that a test with starch-iodide paper was negative, a positive test shows a black spot in the test paper. The organic layer was then separated and washed two times with 5% Na_2CO_3 solution. Then it was washed successively with H_2O and NaCl solution. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The product of this reaction was very difficult to identify, very little yield.

B)

2.9g (0.1 mole) of [6A] , under N2, was treated with 5 moles of TMS-Br neat, and stirred at room temperature during 16h. The excess of TMS-Br was removed in the rotary evaporator and after this 15mL of isopropanol were added to the reaction, followed by the addition 10% NaOH solution until the reaction pH was 5.8-6.0 (pH meter). 0.12g (0.3 mmole) $Na_2WO_4:2H_2O$ and 0.02g of EDTA

Na_2:$2H_2O$ dissolved together in 5mL of warm water were added dropwise to the reaction, followed by 2.6mL 30% H_2O_2 ,added also dropwise. The temperature of the reaction was maintained between 40-50°C and the pH 5.5-6.0, and the reaction was stirred at this temperature overnight. Then the solution was cooled to -5°C to initiate crystallization. After stirring for two hours the solid product was filtered and dried over the high vacuum. The NMR shows only the disappearance of the vinylic signals and is otherwise very complex. Elemental analysis of the monosodium salt,$C_4H_8O_6PNa$: Calculated: C,23.3; H,3.90 Found: C, 7.3; H,4.13 This material did not show any biological activity.

C)

To 2g (6.5 mmole) of [6] was added dropwise TMS-Br, in a 4 fold excess, this reaction mixture was stirred overnight at room temperature under inert (N_2) atmosphere. The excess of TMS-Br was removed under vacuum. The product, the TMS-esters, was dissolved in 10 mL CH_2Cl_2 and 5 mL of MeOH. This solution was cooled at 0°C (ice bath) and then 4 mL of 30% H_2O_2 was added dropwise. This was

followed by the dropwise addition of 0.2N NaOH to maintain the pH of the reaction between 9.5-10 (pH paper). The resulting two phase system was stirred overnight at R.T . The layers were separated and the aqueous layer was extracted 3 times with 80 mL of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvent was removed in the rotary evaporator. The NMR shows the disappearance of the vinylic protons signals but also many impurities.

To purify the material the dicyclohexylamonium salt was prepared as follows: The product of the previous reaction was dissolved in a solution made up of 10 mL toluene and 5mL acetone, to this was added dropwise (0.115 mole) of dicyclohexylamine that had been previously dissolved in 5mL toluene. The reaction was stirred at room temperature for 3h. The fine precipitate was filtered and washed 3 times with 20mL Et_2O. The product was recrystallyzed from MeOH. Elemental analysis for $C_{16}C_{32}O_6NP$, Calculated: C,54.70; H,9.12; Found: C,54.39;H,8.77

The elemental analysis correlates very well for 80% mono- and 20% di-salt compound. This material exhibited biological activity.

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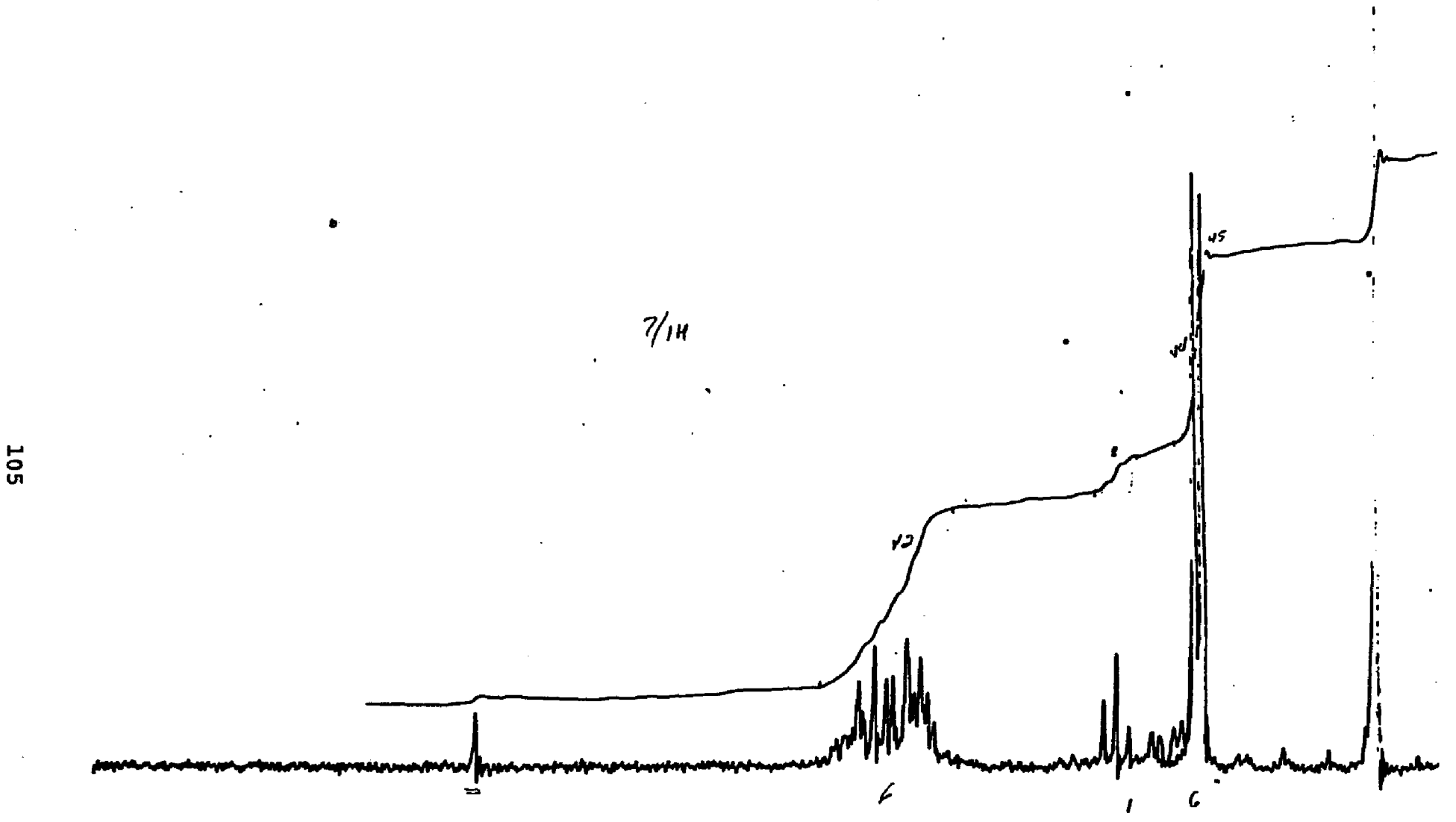
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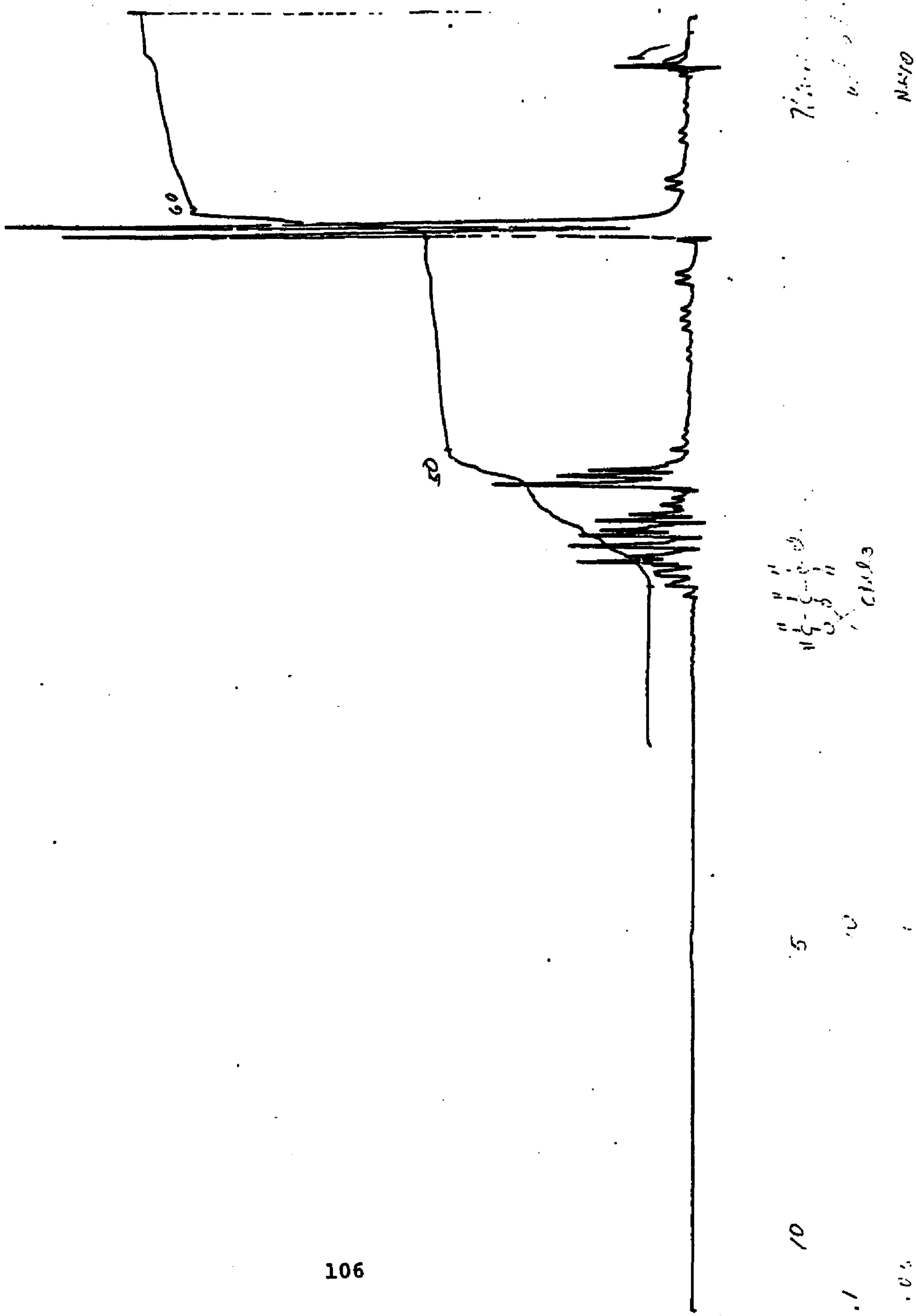
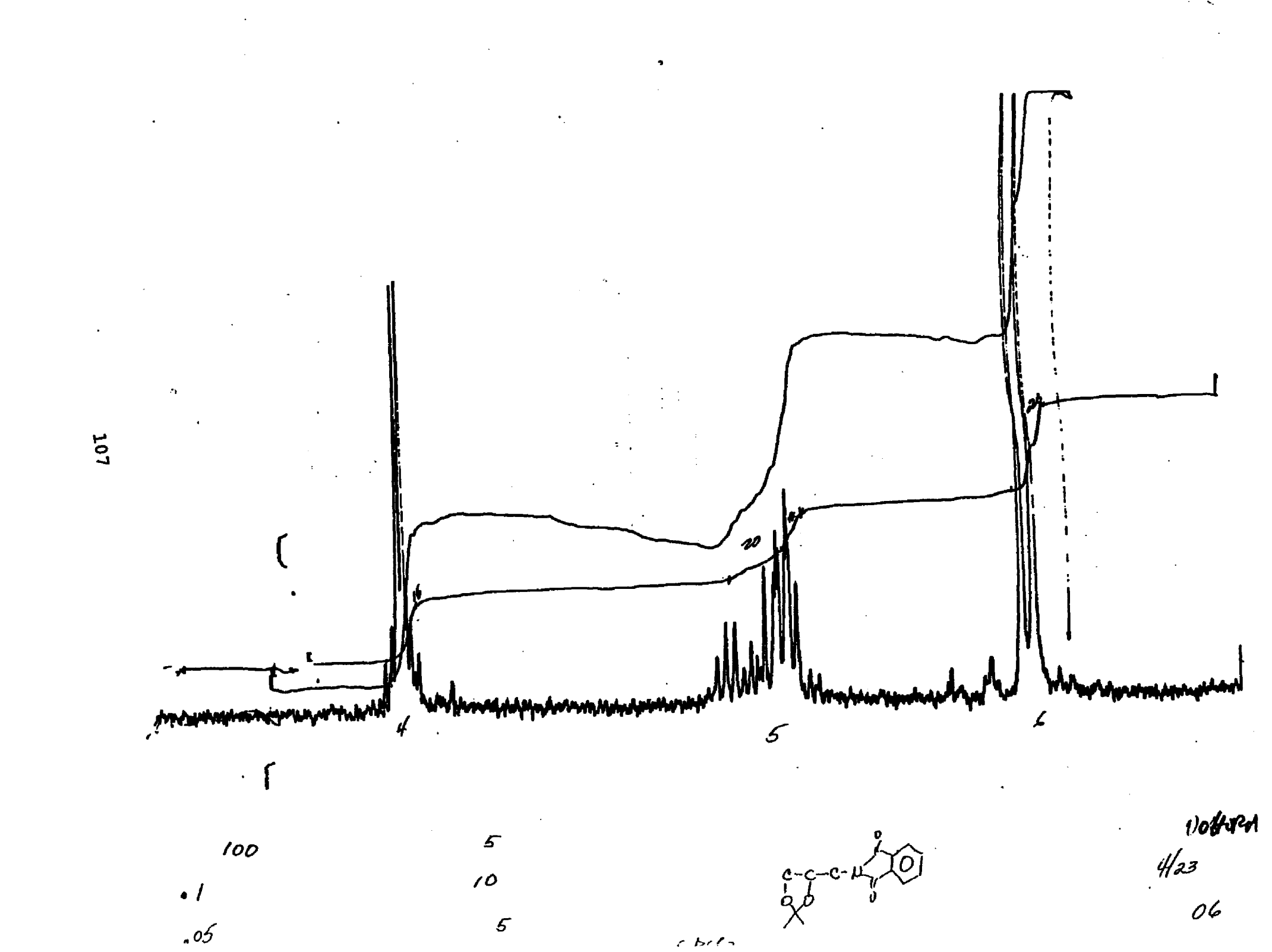
CDCl3

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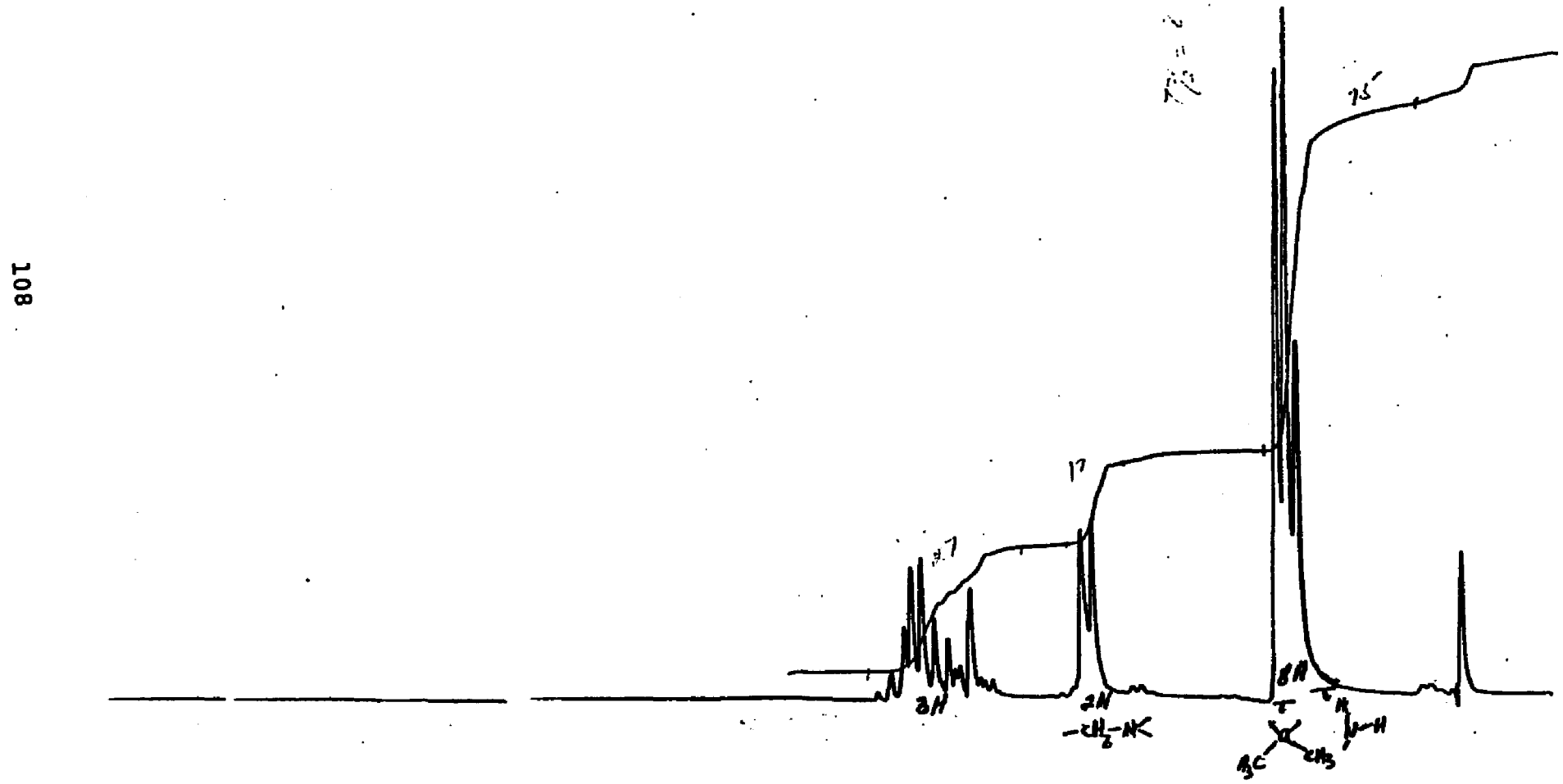

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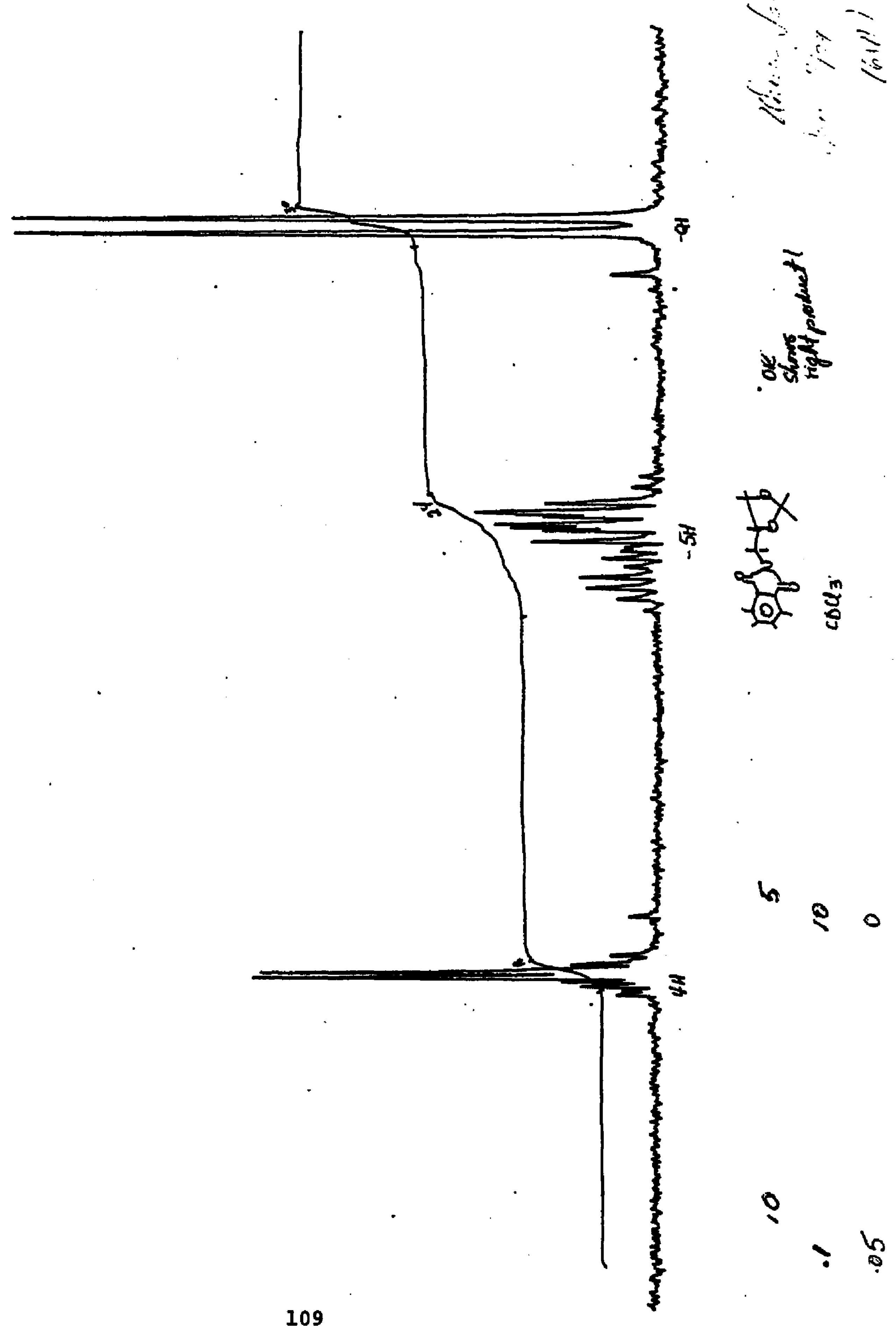
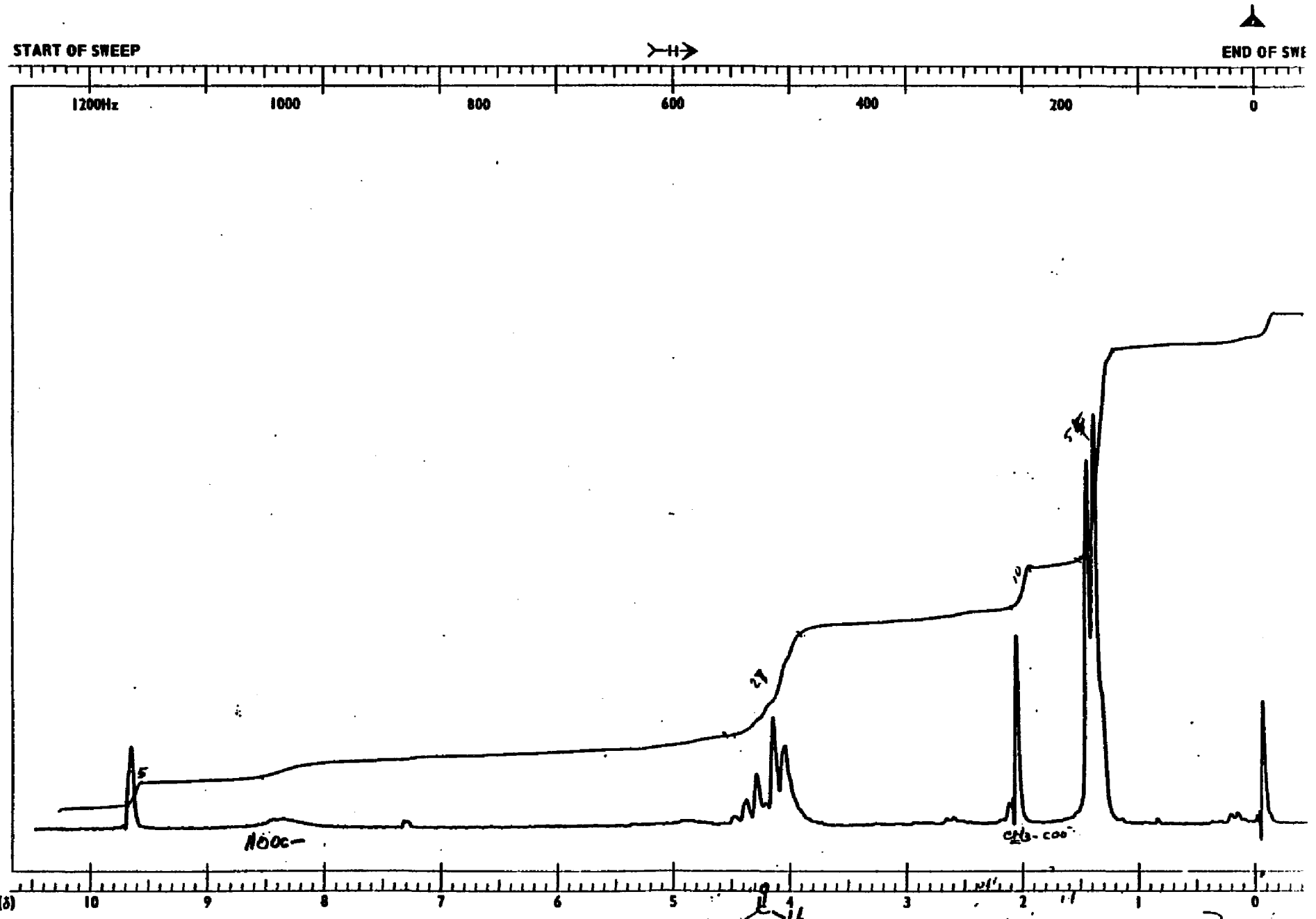


10H
OK shows right product!
5H
CDCl3
4H

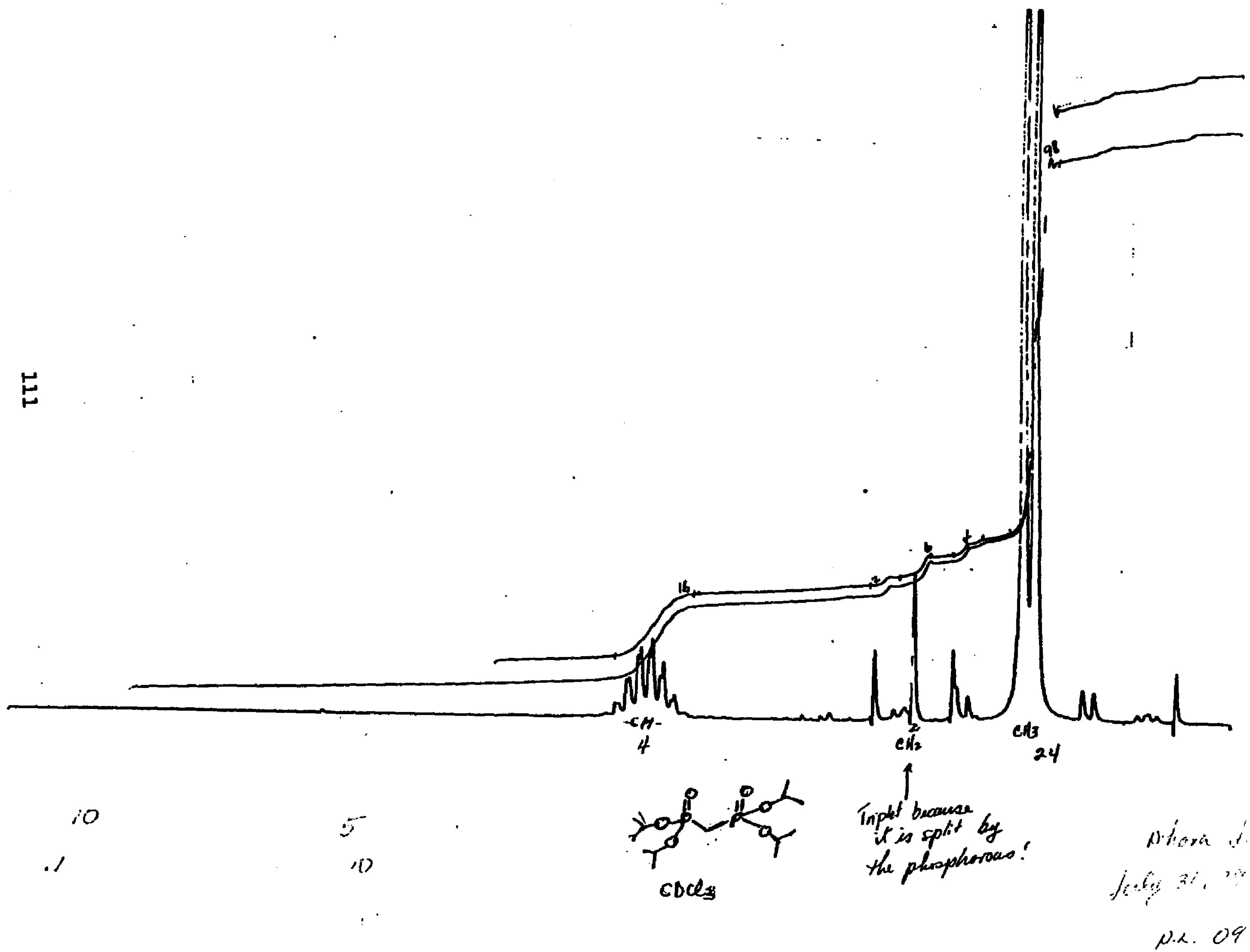


314 ARBOR AVENUE
LANDISVILLE, N.J. 08326
Phone: (609) 697-0020

SPECTRUM AMPL. 10 | SWEEP TIME 5 min min. | SAMPLE: | REMARKS: | OPERATOR M. Ferl

FILTER .1 sec | SWEEP WIDTH 10 ppm or Hz | DATE 12/10/8

RF POWER 0.5 mG | END OF SWEEP 0 ppm or Hz | SOLVENT: $CDCl_3$ | SPECTRUM NO. 34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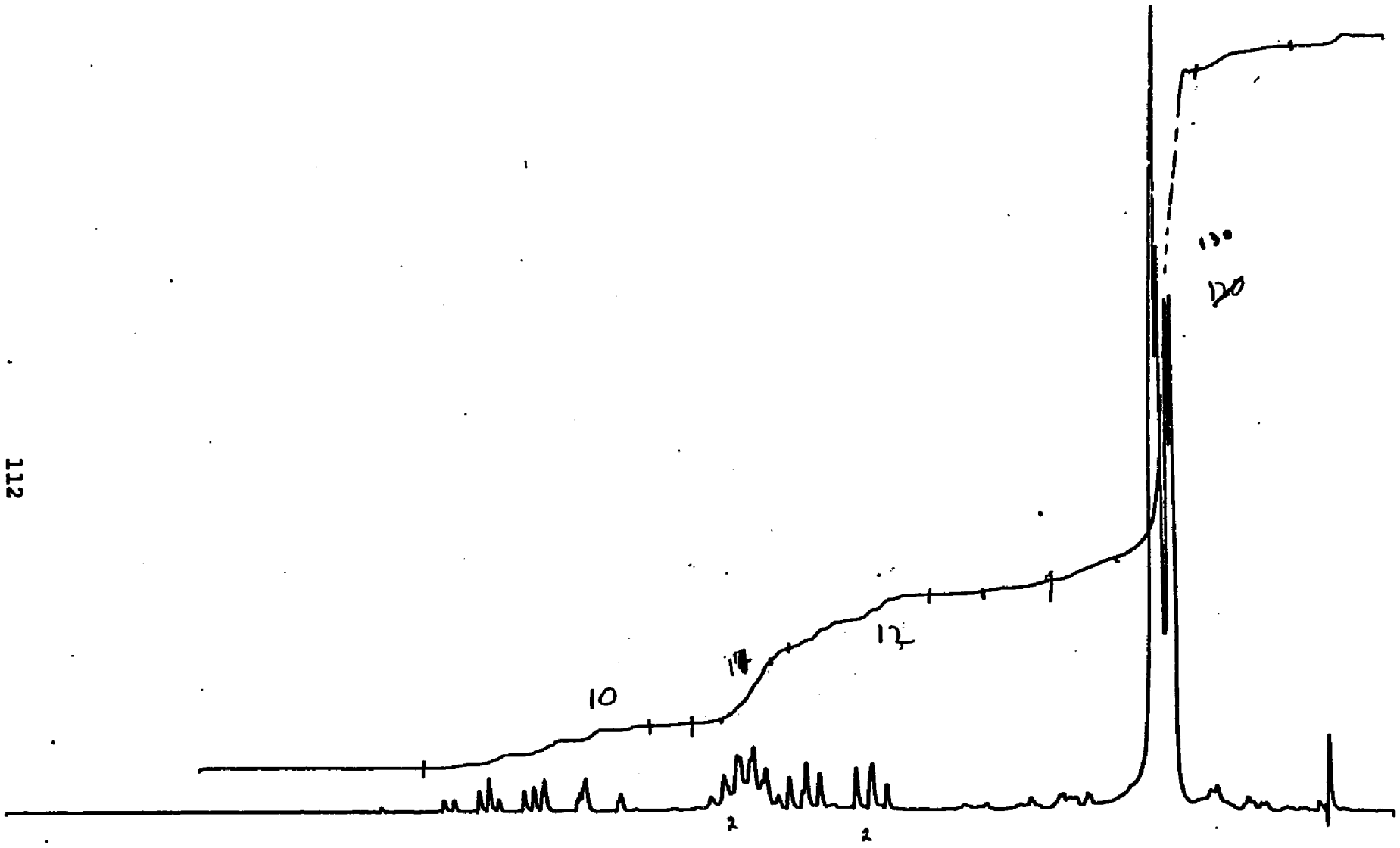
CDCl3
Triplet because it is split by the phosphorous!
D.L. 09



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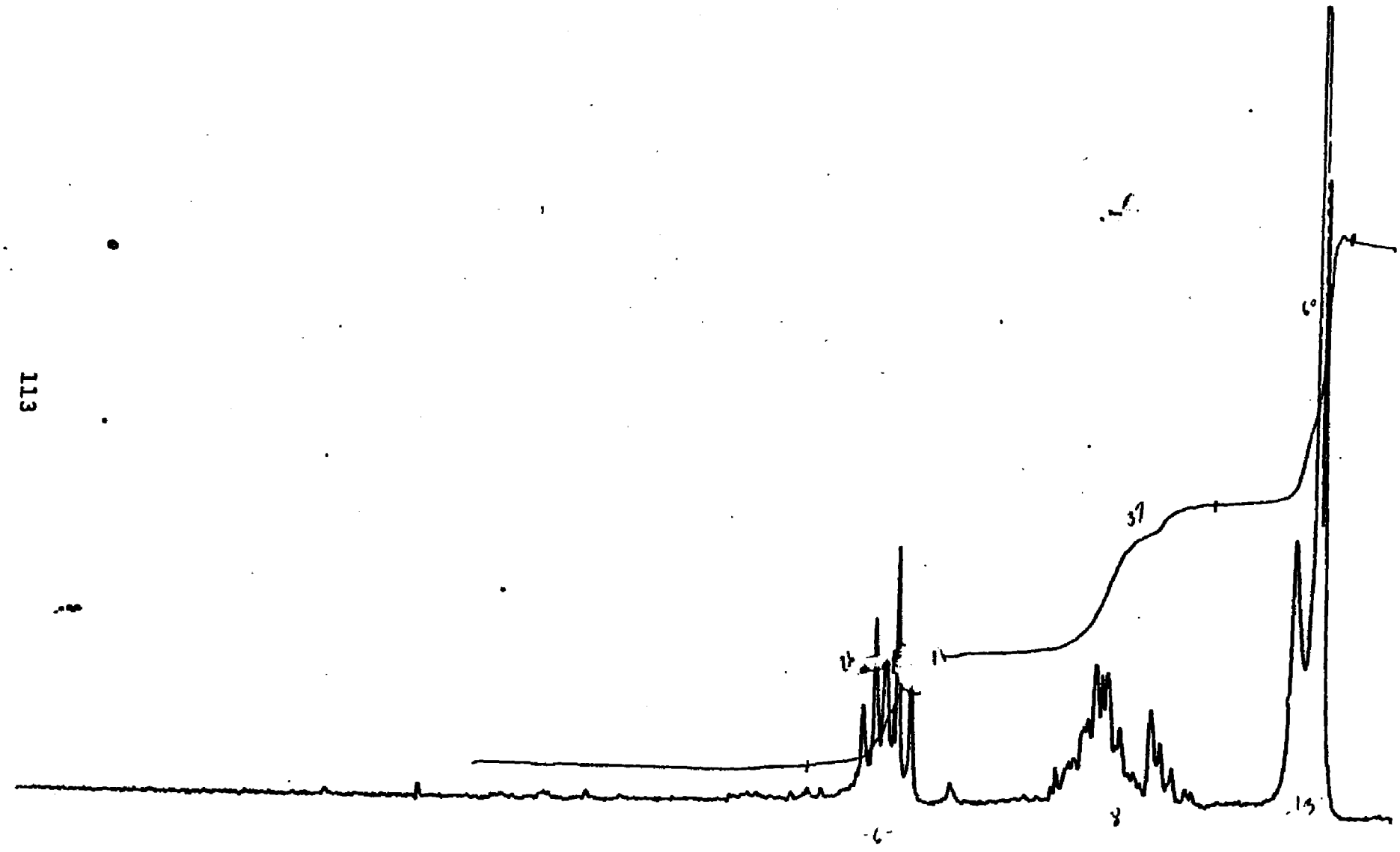
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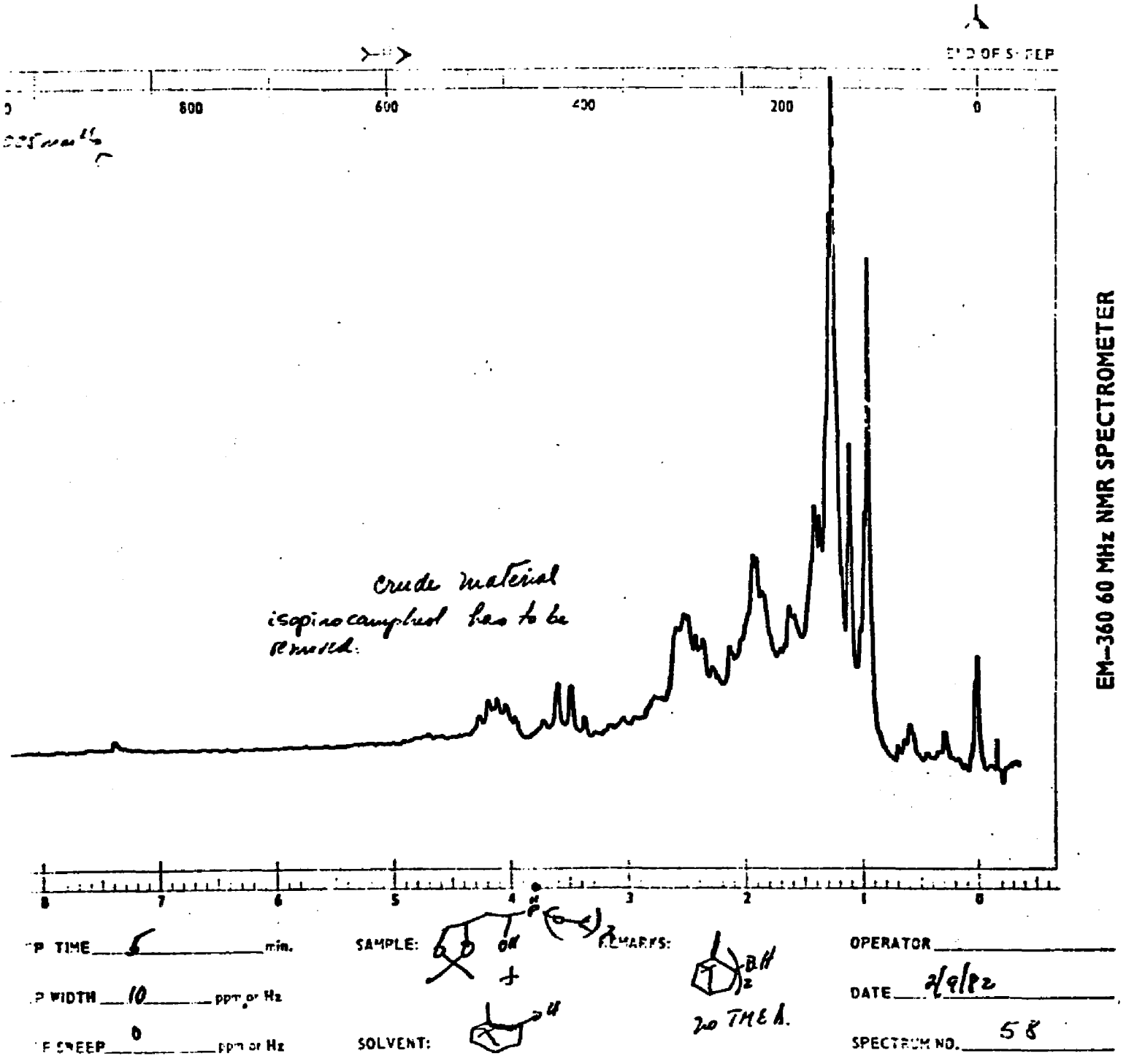
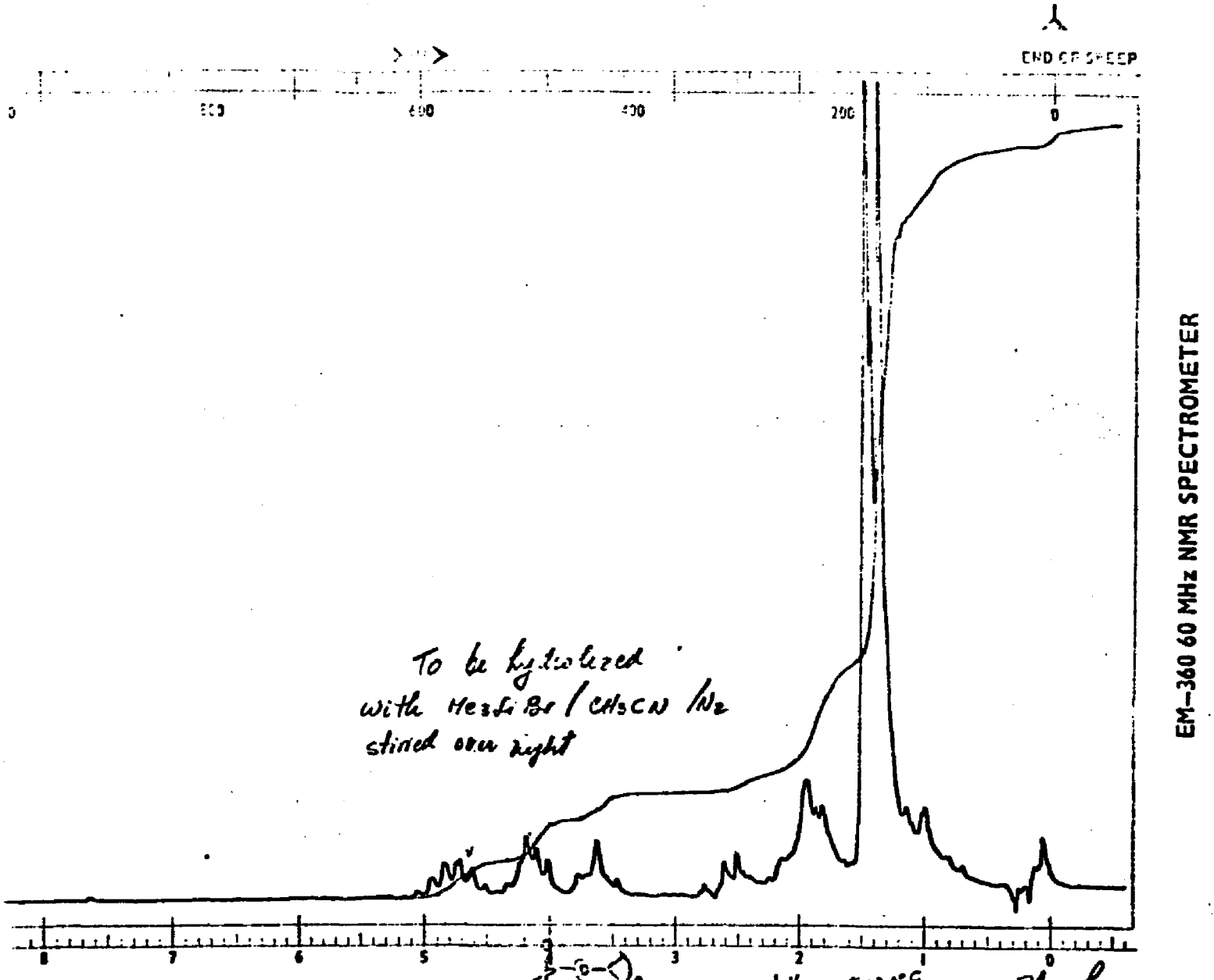
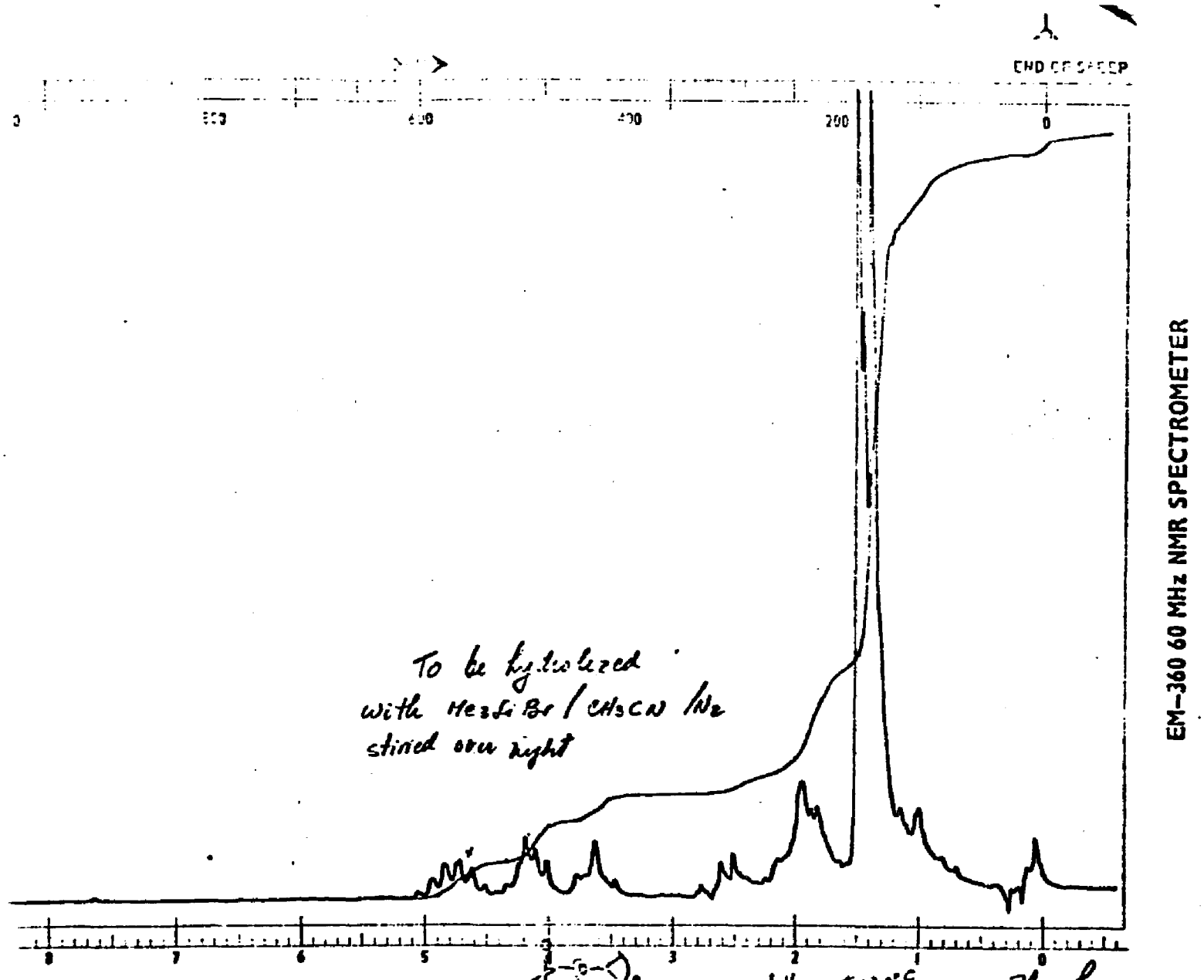

END OF SWEEP
800
600
400
200
0
Crude material
isopinocamphenol has to be
removed.
8
7
6
5
4
3
2
1
0
EM-360 60 MHz NMR SPECTROMETER
P TIME 6 min.
P WIDTH 10 ppm or Hz
P SWEEP 0 ppm or Hz
SAMPLE:
SOLVENT:
REMARKS:
2% TMS in
OPERATOR
DATE 2/9/82
SPECTRUM NO. 58


END OF SWEEP
EM-360 60 MHz NMR SPECTROMETER
To be hydrolyzed
with Me3SiBr / CH3CN N2
stirred over night
REMARKS: Distilled at 120°C
0.025 mm
OPERATOR N. L.
DATE Feb 10/82
SPECTRUM NO. 58 A
SAMPLE:
SOLVENT:
CDCl3
EEP TIME min.
EEP WIDTH ppm or Hz
OF SWEEP ppm or Hz



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OPERATOR N. L

DATE Feb 10/82

SOLVENT: CDCl3

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