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# A Synthetic Study of an Organophosphorous Compound as an Acetylcholinesterase Inhibitor

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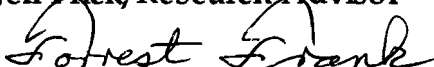May 12,1994

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Approval Page
"A Synthetic Study Of An Organophosphorous Compound As
An Acetylcholinesterase Inhibitor"
by Sulay Jhaveri

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## Abstract

Acetylcholinesterase is the enzyme that catalyzes the hydrolysis of acetylcholine. Based on the transition state of the catalysis, and what is known about the structure of the enzyme, a synthetic study has been conducted on an organophosphorous inhibitor which is capable of probing into the stereospecificity of the enzyme. The asymmetric synthesis was initiated with l-proline, and several methods were employed in phosphorylation in order to get the desired product.

## Introduction

*Target Compound*

The goal of this research is to synthesize an inhibitor of acetylcholinesterase (AChE) that is an organophosphorous (OP) analog of the transition state in the catalysis of acetylcholine(ACh). In the past, OP compounds have been used as AChE inhibitors, but an attempt has been made to synthesize one which would allow the study of the conformation of the catalytic subsite and give insight into the sterospecificity of the enzyme. The more an OP compound resembles the tetrahedral transition state of the catalysis of ACh, the better inhibitor it can be. In ACh, the nitrogen is two carbons away from the oxygen, so the OP compound must also have the and oxygen and nitrogen atoms two carbons away from each other. To account for some special features of the enzyme, it is desired that the nitrogen be represented as a quarternary ion substituted with alkyl groups. This compound would be stable in the environment of the catalytic site, and would have a specific chirality. The use of a compound with a conformationally constrained nitrogen, which would prevent free rotation, will be helpful in studying the stereochemistry of the enzyme. The use of a phosphorylcholine compound chiral at the beta carbon and at the phosphorous will provide insight into the conformation of the active site, as it binds to the molecule.

Current Target Compound



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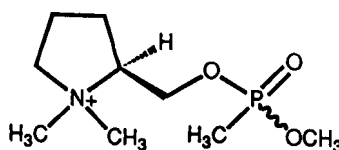
## ACh and AChE

In order to synthesize the inhibitor, it is important to have information about the structure of the enzyme and its substrate. ACh is a neurotransmitter which functions in the central and peripheral nervous systems. It is responsible for the transmission of action potentials across nerve synapses as well as neuro-muscular junctions. The enzyme AChE is responsible for the regulation of ACh. This control is necessary because a build-up of the neurotransmitter would mean a constant firing of neurons, which is extremely dangerous, and could ultimately lead to death. AChE catalyzes the hydrolysis of ACh into choline and acetic acid. AChE is present in the synapses, and choline is reasorbed by the pre-synaptic cell to be re-used. ACh binds to cholinergic receptors located on the the post-synaptic neuron. It is stored in vescicles before release which is triggered by nerve action potential in a fixed quantities. Exocytosis of the ester is initiated by axon terminal depolarization, and mediated by synaptic calcium levels[1]. The binding to the receptors marks the initiation of post-synaptic activity. Since nerve impulses are extremely fast, there has to be a mechanism of destruction of the transmitter. This is the role of AChE.

A Schematic of the Acetylcholinesterase (AChE) Active Site

- AChE is the enzyme responsible for the hydrolysis of acetylcholine (ACh) a neurotransmitter
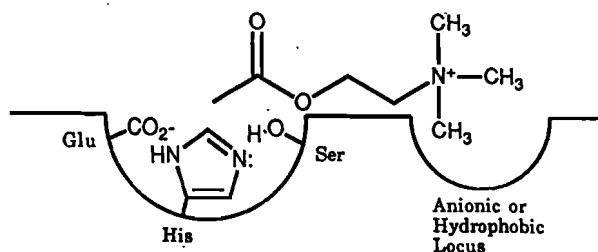


- ACh is hydrolyzed into choline and acetic acid

For a review of AChE see: Quinn, D.M. *Chem. Rev.* 1987, *87*, 955.

AChE is thought to have three major domains: the esteratic site, the anionic binding site, and a hydrophobic region. There is considerable debate over the existence of the anionic binding site. Early kinetic studies indicated that the active site of AChE contained only the esteratic subsite and the anionic subsite[2]. Recently x-ray analysis by Sussman[3] et al. was used to determine the three dimensional structure of the enzyme. It revealed some interesting things. First, it was found that the esteratic site contains glutamate, serine, and histidine as the acid catalytic triad. This is similar to the serine proteases, but Glu takes place of an Asp, and the position of the catalytic portion seems to be a mirror image of what is seen in the serine proteases. It was previously believed hat the anionic subsite binds to the charged quartenary group of the choline moiety, but studies by Cohen and Hasan[4] suggest otherwise. On the basis of studies in which both charged and uncharged homologs were used, it was found that the so called anion binding site is in fact uncharged and hydrophobic. Hasan correlated the partial molal volume of the beta substituent and the rate, and proposed that the enzyme binds the choline moiety with a hydrophobic trimethyl binding site. It was the presence of the three methyl groups not the charge that was important.

Crystallographic analysis reveals the the active site lies at the bottom of a deep narrow gorge which reached halfway into the protein.[3] The active site gorge, a 20Å long channel, penetrates halfway into the enzyme where the active site is contained. 14 aromatic residues line about 40% of the gorge. The aromatic content of the walls and the floor of the gorge may explain why

biochemical studies have revealed a variety of hydrophobic and anionic sites separate from or overlappng the active site. The geometry of the gorge can also give clues as to how certain inhibitors behave by blocking or penetrating the gorge. The aromatic lining could guide the ACh, once trapped on top of the gorge, rapidly down to the active site. There are only a small number of negative charges, but there are many aromatic residues near the catalytic site and on the walls of the gorge which lead down to it. It is thought that the aromatic lining may permit the use of a mechanism involving initial absorption of ACh to low affinity sites, followed by two dimensional diffusion to the esteratic site, a sort of aromatic guidance mechanism. Although this suggests that the anionic site is misnamed, it is important to note that the depth of the gorge and the extent of the aromatic residues lead to the many different ways and places for substrate, agonists, and inhibitors to bind to AChE.

Those who are convinced of the existence of a true anionic locus, have it located 4.7 angstroms away from the esteratic subsite, and it is thought to contain multiple negative charges. Their reply[5] to previous the work previously mentioned is that there is indeed transfer of substrate from solution to a lipid-like environment, but there is no reason to assert that the existence of the hydrophobic regions is the actual binding site for the trimethylammonio moiety of the choline. There is evidence of the anionic site based upon interaction of the enzyme with various types of compounds including aromatic cations, tetraalkylammonium salts, aziridinium covalent modifying reagents, bisquaternary ammonium compounds that span the anionic locus (both peripheral and of the active site), and pyridinium reactivators.

*The Catalytic Mechanism*

      The general mechanism by which ACh is hydrolyzed is similar to that of a serine protease. There are, however numerous features of AChE that are different from the serine proteases as revealed by x-ray structural analysis. The catalysis involves an acylenzyme mechanism that has the hydroxyl group of a serine residue acting as a nucleophile, and an imidazole ring of a histidine residue acting as a general acid-base catalyst[6]. After the acylenzyme is formed, as ACh attaches to the enzyme, the choline separates from the acetyl group. The deacylation step occurs and the enzyme is reactivated.
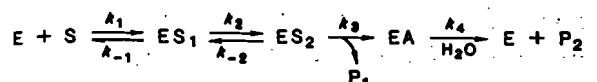
## Mechanism of Acetylcholine Hydrolysis
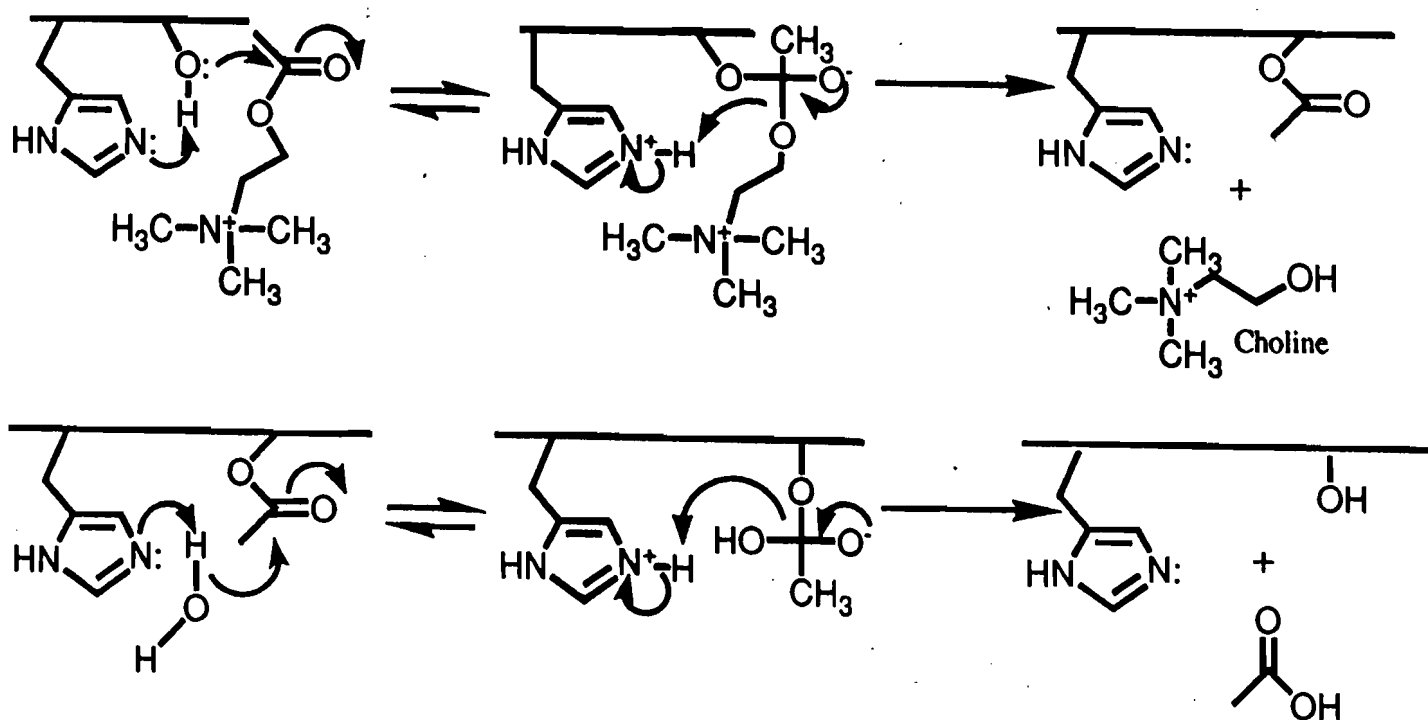
## The Kinetic Mechanism

The kinetics of this process follow an induced fit mechanism, where acylation and deacyltion both contribute to the rate limitation. First, there is an equilibrium in the formation of the Michaelis complex. Then, there is another equilibrium to form the induced-fit complex. The fact that there were two equilibria before the formation of the acylenzyme, led to the belief in the existence of the anionic site. The acylenzyme is formed with the removal of the choline, and in the presence of water, deacylation occurs. An induced-fit mechanism implies that the substrate binds to the enzyme and a conformational change occurs before any catalysis[6].



Acylenzyme Mechanism for AChE Catalysis
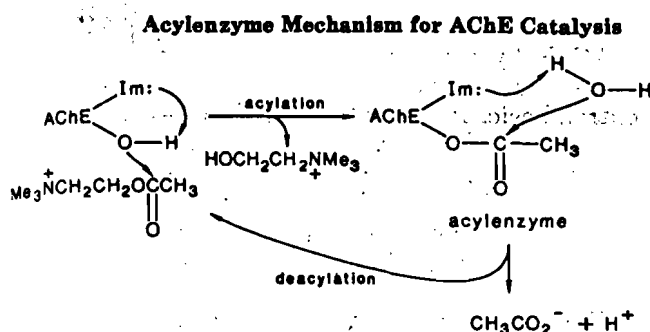
SCHEME II. Kinetic Mechanism for AChE Catalysis that Incorporates an Induced-Fit Step

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2 \overset{k_3}{\underset{P_1}{\longrightarrow}} EA \xrightarrow[H_2O]{k_4} E + P_2$$

E = AChE, S = substrate, EA = acylenzyme, $P_1, P_2$ = products, $ES_1$ = Michaelis complex, $ES_2$ = induced-fit complex
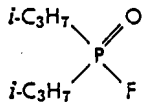
## Other OP Compounds
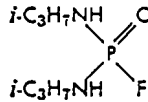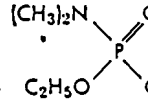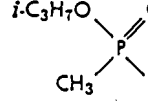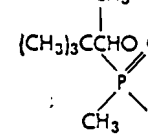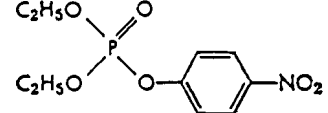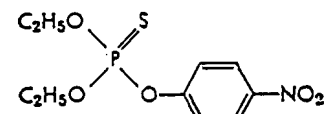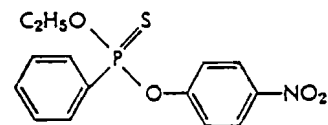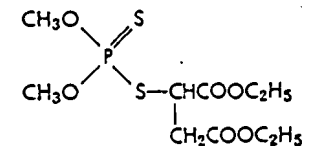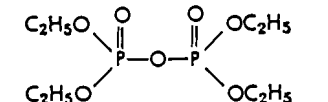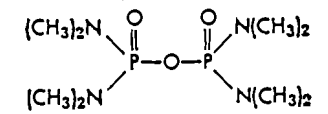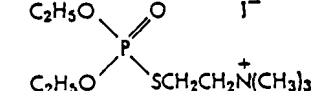
It has been known for a long time that OP compounds are potent nerve poisons[7]. OP compounds inhibit AChE by phosphorylating it. Some are the extremely toxic nerve gases such as Sarin, Soman, and Tabun. Others are used as insecticides, Parathion, Malathion, and OMPA. There is a series of related synthetic compounds which are carbamates that reversibly bind to AChE. These are used clinically, and some examples are: Physostigmine,

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## Table 22-2. CHEMICAL CLASSIFICATION OF REPRESENTATIVE ORGANOPHOS-PHORUS COMPOUNDS OF PARTICULAR PHARMACOLOGICAL OR TOXICOLOGICAL INTEREST

General formula (Schrader, 1952):

$$R_1\diagdown P\diagup\diagup O$$
$$R_2\diagup\diagdown X$$
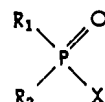
Group A, X = halogen, cyanide, or thiocyanate; group B, X = alkyl, alkoxy, or aryloxy; group C, thiol- and thionophosphorus compounds; group D, pyrophosphates and similar compounds; group E, quaternary ammonium compounds

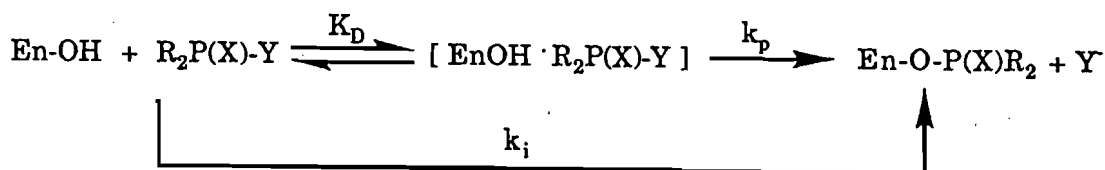| GROUP | STRUCTURAL FORMULA | COMMON, CHEMICAL, AND OTHER NAMES | COMMENTS |
|---|---|---|---|
| A | $i$-$C_3H_7$, $i$-$C_3H_7$, O, F (phosphoryl) | DFP Diisopropyl phosphorofluoridate | Potent, irreversible inactivator; used as miotic in treatment of glaucoma |
| | $i$-$C_3H_7NH$, $i$-$C_3H_7NH$, O, F | Mipafox, Isopestox N,N'-Diisopropyl phosphorodiamidic fluoride | Insecticide; selective inhibitor of nonspecific ChE (BuChE) |
| | $(CH_3)_2N$, $C_2H_5O$, O, CN | Tabun Ethyl-N-dimethyl phosphoramido cyanidate | Extremely toxic "nerve gas" |
| | $i$-$C_3H_7O$, $CH_3$, O, F | Sarin (GB) Isopropyl methyl-phosphonofluoridate | Extremely toxic "nerve gas" |
| | $CH_3$, $(CH_3)_3CCHO$, O, $CH_3$, F | Soman Pinacolyl methyl-phosphonofluoridate | Extremely toxic "nerve gas" |
| B | $C_2H_5O$, $C_2H_5O$, O, O—C$_6$H$_4$—$NO_2$ | Paraoxon, Mintacol, E 600 Diethyl 4-nitrophenyl phosphate | Employed as miotic; active metabolite of parathion |
| C | $C_2H_5O$, $C_2H_5O$, S, O—C$_6$H$_4$—$NO_2$ | Parathion, Thiophos, E 605 (see list of trade names in text) Diethyl O-(4-nitrophenyl) phosphorothioate | Widely employed agricultural insecticide, resulting in numerous cases of accidental poisoning |
| | $C_2H_5O$, C$_6$H$_5$, S, O—C$_6$H$_4$—$NO_2$ | EPN O-Ethyl O-(4-nitrophenyl) phenyl-phosphonothioate | Widely employed agricultural insecticide |
| | $CH_3O$, $CH_3O$, S, S—CHCOOC$_2$H$_5$, CH$_2$COOC$_2$H$_5$ | Malathion O,O-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate | Widely employed insecticide of greater safety than parathion or EPN because of rapid metabolism by higher organisms |
| D | $C_2H_5O$, $C_2H_5O$, O, O, $OC_2H_5$, $OC_2H_5$, P—O—P | TEPP Tetraethyl pyrophosphate | Early insecticide; tested clinically in glaucoma and myasthenia gravis |
| | $(CH_3)_2N$, $(CH_3)_2N$, O, O, $N(CH_3)_2$, $N(CH_3)_2$, P—O—P | OMPA, Schradan Octamethylpyrophosphortetramide | Insecticide; inactive in vitro, but metabolized by animals and plants to potent anti-ChE agent |
| E | $C_2H_5O$, $C_2H_5O$, O, I$^-$, SCH$_2$CH$_2$N(CH$_3$)$_3$$^+$ | Echothiophate, Phospholine, 217MI Diethoxyphosphorylthiocholine iodide | Extremely potent choline derivative; employed in treatment of glaucoma; relatively stable in aqueous solution |

Neostigmine, and Pyridostigmine. Besides that there are OP compounds which are employed clinically to treat such maladies as glaucoma such as Echothiophate, and DFP, and it is believed that anti-cholinesterase agents may be therapeutically useful in treating symptoms of Alzheimer's Disease.

*Inhibition By OP Compounds*

When an OP compound is presented to AChE, the serine will attack the carbonyl-analog phosphorous, and instead of acylation, phosphorylation will occur. From here, several things can happen. It could stay as a phospho-enzyme for an indefinite period of time. It could irreversibly inactivate it in a process called aging, where a specific group is lost and the enzyme is unable to be reactivated. Or it could undergo reactivation. The inhibition of AChE by OP compounds is very stereoselective, but previous studies regarding toxicity and stereospecificity show no trend. Therefore, individual compounds have to be studied.

## Kinetics of Enzyme Inhibition

$$En\text{-}OH + R_2P(X)\text{-}Y \underset{\longleftarrow}{\overset{K_D}{\longrightarrow}} [\, EnOH \cdot R_2P(X)\text{-}Y \,] \xrightarrow{k_p} En\text{-}O\text{-}P(X)R_2 + Y$$
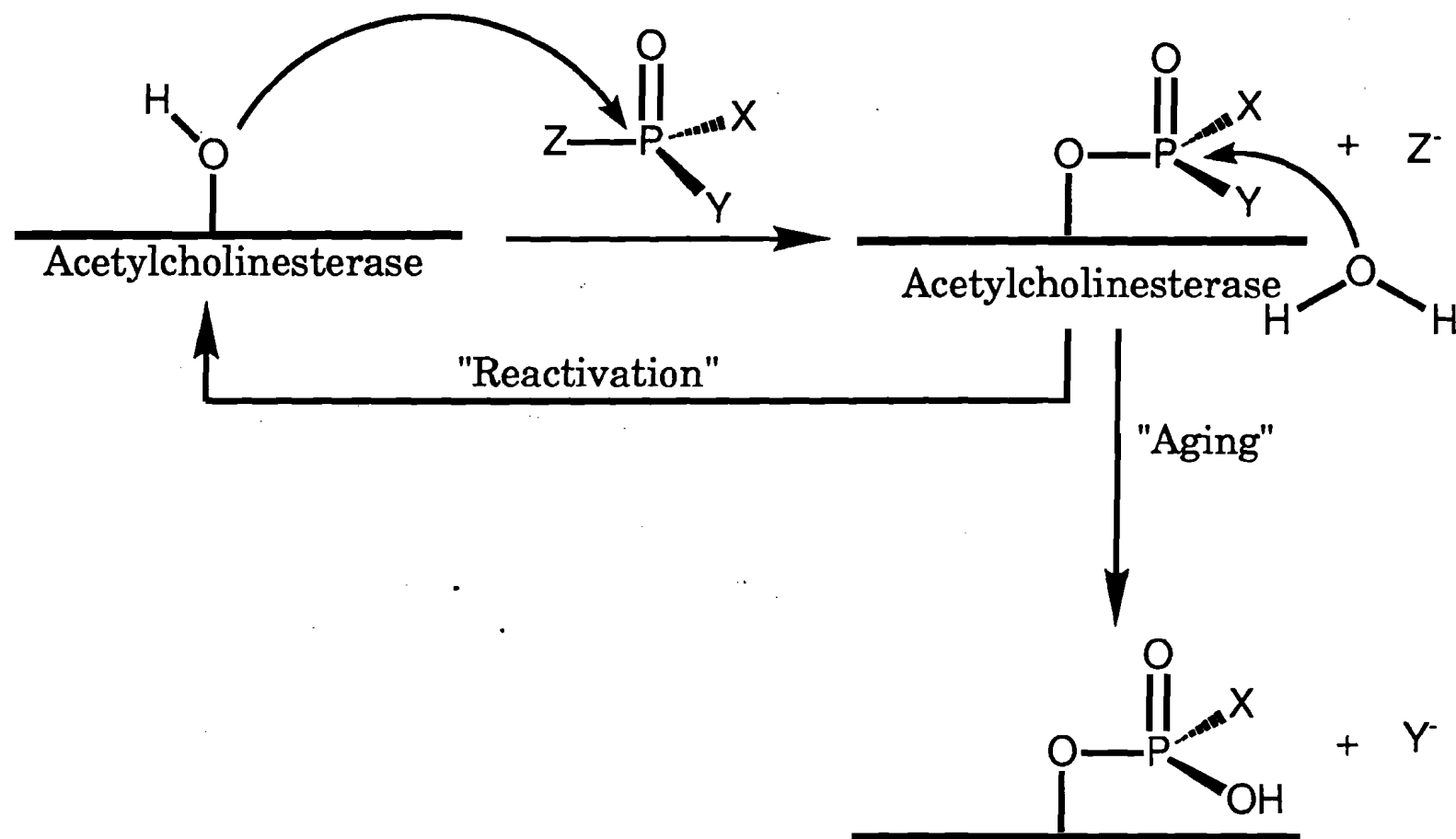
$$k_i$$

$K_D$: dissociation constant (how much do the enzyme and inhibitor like each other)

$k_p$: phosphorylation constant (how quickly does phosphorylation occur after enzyme/inhibitor complex forms)

$k_i$: bimolecular inhibition constant (potency of the inhibitor)

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# Phosphorylation of Acetylcholinesterase

## Synthetic Schemes

A synthetic scheme of the target compound would have to involve several things: the chirality of the pentavalent phosphorous, the groups attached to the nitrogen, and how the OP compound binds to the enzyme so that the phospho-enzyme can form. To ensure chirality at the beta carbon and constraint of conformation at the nitrogen, the chosen starting product is l-proline. Initial steps involve protecting the nitrogen, and then converting the carboxylic group to an alcohol. At this point, there were two possible approaches in the second stage of the synthesis which involve the attachment of the phosphorous moiety.

One was in connection with the automated synthesis of oligonucleotides[8]. This scheme started out with methoxydichlorophosphine, and used methoxybis(diisopropylamino)phosphine as an intermediate to get the desired product. The dialkyamino groups would be replaced by deprotonated alcohols in the presence of a strong base, and oxidation, and treatment with methyl Grignard would follow.



Model study using BzOH
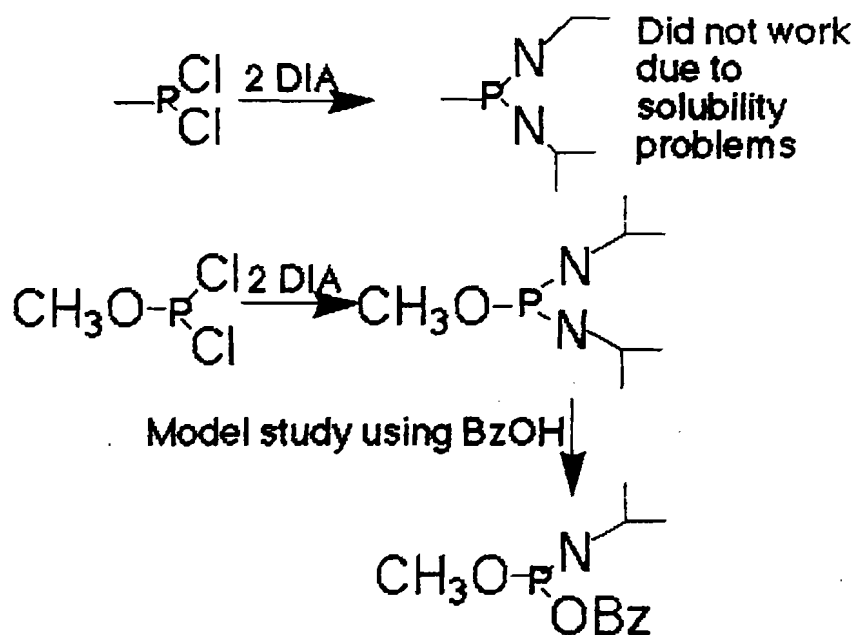
DIA = diisopropylamine
BzOH = benzyl alcohol

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The other approach was to start out with the pentavalent phosphorous in the form of thiophosphoryltrichloride. Direct addition of alkoxides would result in their being substituted in the place of the chlorines, so the CBZ-l-prolinol would have to be converted to the alkoxide. Again, methyl Grignard would have to be added to alkylate the phosphorous.
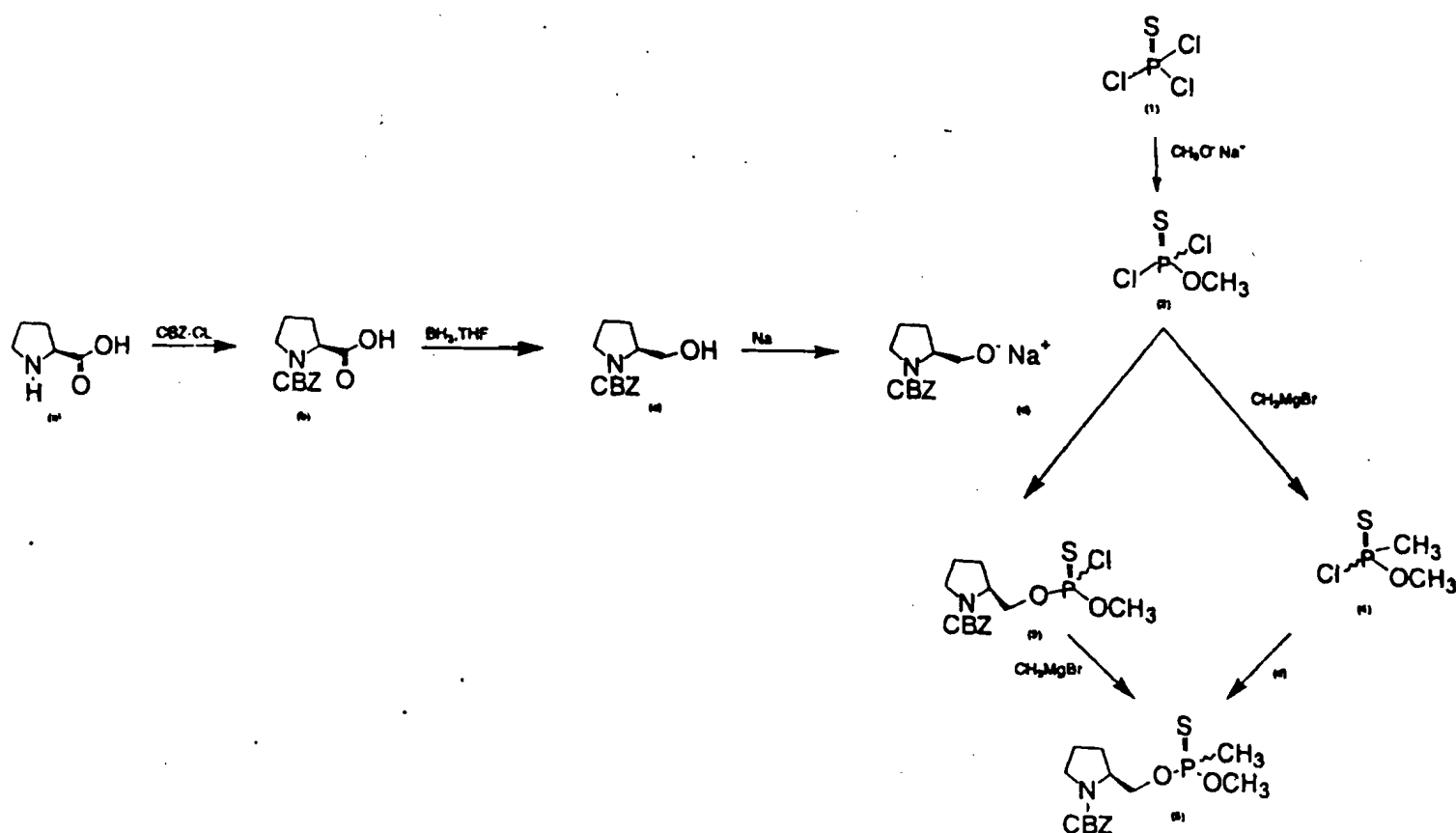
Once the protected methoxymethylthiophosphoryl-l-prolinoxide has been synthesized,the protecting group is then taken off, and various alkyl groups or hydrogen can be placed on the nitrogen.

## Results and Discussion

The goal of this research was to synthesize a reversible anti-cholinesterase agent. The implications of this effort lie in studying the stereospecificity of the enzyme, and the possible pharmaceutical benefits from the completion of this quest. So far, the synthesis is not complete, so at best we can only comment on the presence of what we have so far and why we think we have the desired product.

*Transformation of l-proline*

The synthesis started out with the protection of the amino acid at the nitrogen. Benzyloxycarbonyl was used as a protecting group was very appropriate, since it forms a carbamate with the nitrogen and the big group sterically prevents reaction at that site until it is removed by hydrogenolysis. This reaction was special in that there were two phases, and the reaction actually occurred at the boundries between the aqueous and the organic layers. There were problems in obtaining crystals and finally the use of seed crystals obtained from previous work were used to promote recrystallization. Interestingly, in other batches the problem was not evident and pure solid crystals were available for use, so it was probably a matter of improved lab technique.

The next step was the conversion of the carboxylic acid to the alcohol. The mechanism of this reaction is thought to involve a boroester. The use of the borane adduct was especially appropriate, since it is known that it is capable of reducing carboxylic acids to the corresponding alcohols in the presence of other functional groups such as the carbamate[9]. The next step was the addition of the phosphoryl group to the alcohol.

*The scheme that didn't seem to work*

1 2

The synthesis had many detours along the way in the effort to attach the phosphoryl group to the l-prolinol. First, a procedure was tried which was similar to that involving the synthesis of oligonucleotides[8]. It required a dialkylamino intermediate. Using methyldichlorophosphine as the starting product, it was thought that substitution with two alkoxy groups followed by the addition of a Grignard reagent and oxidation would give the desired product. But there were difficulties in separating the product after the first substitution resulting in the bis(dialkylamine) product due to solubility problems. Both the Hunigs base and the product after treating with diisopropylamine, the nucleophile, were soluble in methylene chloride, but not in ether. Exposure to water was thought to decompose the product, since the literature[8] called for the procedure to be conducted in an inert atmosphere. So, we tried to use the second reaction and the results were better. This procedure worked, as it verified by examining the NMR spectrum, and those of the reactants. So, instead of wasting precious l-prolinol, we attempted to do a model study using benzyl alcohol. It would be a similar sort of nucleophile and it would displace one of the diisopropylamines. NMR spectra showed some disparities as to whether or not the reaction actually proceeded.

*The scheme that seemed to work*

At this time we encountered an article[10] which described the nucleophilic displacement upon the thiophosphoryl rather than the trivalent phosphine. The procedure didn't involve using any amines which would pose problems in isolating the product, but called for the use of the alkoxide. So, starting with thiophosphoryltrichloride, we performed the substitution with sodium methoxide, making sure that the addition of methoxide was very slow, so as to produce only mono-substituted product. Nevertheless, some dimethoxylted product did form.

This substitution is somewhat analogous to the reaction of a carboxyllic acid to form acid derivatives and vice versa where the oxygen becomes negatively charged and then donates the extra electrons to the carbon to from the product. In this case, with the phosphorous however, there is a 5-coordinated transition state. In order to understand the mechanism of the reaction, it is necessary to invoke some aspects of molecular orbital theory and the structure of phophorous.

The displacement of the chlorine by the methoxy is thought to proceed with an inversion of configuration. This is based on some investigation on the displacement reaction of tetrahedral optically active phosphorous compounds. They suggest that there exists a bipyramidal structure with weak axial bonds with p-d bonding and the radial bonds in an $sp^2$-hybridized state. This transition state, as the positive charge on the phophorous increases, changes into another transiton state in which 2 d-orbitals are excited and the equatorial bonds become stronger than the radial bonds, so diplacement of groups on the basal plane are preferred in a substitution reaction. The assumption made is that the increase in charge reduced the energy gap between the d-orbital and the low-lying s-orbital. Hence, the substitution, should theoretically proceed with complete inversion of configuration, for thiophosphates and phosphoryl compounds, as well as phosphonium salts[11].

Once the substitution was made, there were two possibilities, the attachment of a methyl group, or of the l-prolinoxy group. A methyl group on the phosphorous was desired because although many potent AChE inhibitors contained thio-alkyl groups as well as alkoxy groups around the phosphorous, and adding a methyl group resembled ACh even more. The choice of procedure was obvious. Using an organometallic compound, methylmagnesium bromide, the alkyl group acts as a nucleophile, and magnesium chloride salt is precipitated out. As of yet, no

analysis of the product has been performed. Although it has been reported that the alkyl group will replace the alkoxide, the presence of the Cl, an even weaker base than OMe, indicates that mono-substitution will occur if the Grignard reagent is added slowly enough.

The attachment of the l-prolinoxide to the phosphoryl group has been accomplished, and that is evident by NMR. By comparing the spectra of l-prolinol and methoxythiophosphoryldichloride, we see that there are many similarities which indicate that there may be reactants present, but TLC analysis revealed that there were two spots different from those of the reactants. The aromatic ring shows a singlet at 7.3 ppm. There is a singlet at 5.1pm which accounts for the presence of the alcohol, but the relative intensity is less that it was in the spectrum of just the CBZ-prolinol. The peak resulting from the hydrogen attached to the oxygen in methanol at 2.3ppm for compound {2}'s spectra is no longer present in the one for the product. The pair of doublets between 3.5 and 4 ppm are still present and are a reminder of the presence of the dimethoxylated product. It is of interest to note the coupling pattern around 2ppm. The presence of the five member ring is still there, but the intensities of the peaks in the octet seem to be very similar. They range from 17.6 to 20.2 and that is not expected for the protons on the different positions on the l-proline ring.

It is expected that the sample is not very pure. It was obtained after combining all the eleunts off a flash column, since no individual eluent had anything detectable when spotted on a TLC plate and examined under UV light. In this mixture, we expect that there exist 2 diastereomers of {4} because there is chirality at the phosphorous and the chirality at the beta carbon is chosen to have only one form. Also there are present some reactants, either as a result of

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decomposition, or due incomplete reaction. At first, a 1:1 mixture of ethyl acetate to hexane was used, and the product eluted was detectable by the UV lamp. But it would be prudent to say that was probably some decomposition product, since its NMR spectrum showed no indications of the presence of l-proline. That product was 30mg. The other 80mg of impure product had a spectrum that indicated the presence of some of the desired product. No further analysis is practically feasible for it since purification would be required and there is not enough compound for a feasible purification. Compound {4} has not been analyzed, because it needs the addition of the l-prolinoxide.

## Conclusion

A penultimate precursor to an AChE inhibitor has been synthesized based on the transition state analog of the tetrahedral intermediate structure of ACh when bound to the enzyme. There are only two steps remaining in the completion of the synthesis of the target compound. Removal of the protecting group, and addition of alkyl groups to the nitrogen. After that, further purification and identification of isomers is necessary in order to study the kinetics of inhibition. The kinetics study would involve purification of the enzyme and the use of thiocholine, a substrate detectable by UV-Vis spectrophotometry.

## Experimental

General: Proton NMR were taken on a high field 300MHz NMR spectrometer courtesy of ISU, and a 60 MHz Varian EM360L spectrometer. The solvents used were deuterated chloroform and trimethylsilane. Analytical thin layer chromatography was conducted with aluminum plates backing 0.25mm silica. Visualizations were performed under an ultraviolet lamp. Air sensitive techniques were conducted under a nitrogen atmosphere.

Preparation of CBZ-l-proline{a}: To a suspension of 8.77g sodium bicarbonate in 50ml water, 5.02g of l-proline (Aldrich 99+%) were added. Over a period of .5 hrs, a solution of 28g of benzyloxycarbonylchloride(Aldrich) in ether was added to the solution, while maintaining a basic environment (monitored wit pH paper with the dropwise addition of 3M NaOH. After letting stir for 1 hr, the aqueous layer was carefully neutralized with the dropwise addition of 6M HCl. Extractions were performed 3 times with 50 ml portions of ethyl acetate. The organic layer was dried with sodium sulfate and the solvent was evaporated resulting in a 2.45g yield after recrystallization from ether and purification via column chromatography using ethyl acetate. Further trials had improved yields upto 55% of the white crystals. Progress of the reaction was monitored by TLC. An NMR spectrum of the product was taken. ppm 7.4 (5H); 5.1-5.3 (3H); 4.1 (2H); 3.4-3.7 (4H); 2.1(1H)

Preparation of CBZ-prolinol{b}: In 20ml of THF, freshly distilled, 1.0g of CBZ-l-proline was added and stirred. To that, a solution of 5.5 ml of BH3-THF (Aldrich) was slowly added dropwise under a nitrogen atmosphere. The mixture was allowed to reach room temperature overnight while stirring. After 2 extractions with ether, drying, and removal of solvent, a 74% recovery was observed when the

product was purified by column chromatography. Presence of product was verified by NMR. ppm 7.4 (5H); 5.2(3H); 4.0 (2H); 3.7 (1H); 3.6-3.4(3H); 1.9(4H); 1.2(2H)

Attempted preparation of methylbis(diisopropylamino)phosphine: Via syringe, 0.64ml of diisopropylamine (Aldrich 99%) was added to a 0.97 ml of peptide synthesis grade Hunigs base and 0.20ml of methyldichlorophosphine (Alpha 98%) in 5ml of methylene chloride. After white solid appeared, it was filtered out after stirring overnight. No appreciable yield was present after removal of solvent.

Preparation of methoxybis(diisopropylamino)phosphine{2}: In 10ml anhydrous ether, .7ml of methoxydichlorophosphine (Aldrich 98%) was dissolved and to it, 7.25ml of diisopropylamine was added at -10C. 0.746g were recovered after filtration to remove the amine salt and evaporation of solvent using a Rotovap, a yield of 47%. Presenceof product was verified by NMR.

Preparation of benzoxymethoxydiisopropylaminophosphine: In 4ml dichloromethane 0.208g of {2} were dissolved . To it .06ml of benzylalcohol was added and the solution was stirred for 1.5 hrs. Following usual workup, a NMR spectrum was taken. Purification was done via thin layer planar chromatography on a glass plate, using ethyl acetate and hexane, 3:1 as solvent.

Preparation of methoxythiophosphoryldichloride{2}: To 16.55g of thiophosphoryltrichloride (TPTC) (Aldrich 98%) dissolved in 45 ml of toluene, 10.8g of sodium methoxide in 120ml methanol were added slowly over a period of 3 hrs. using an addition funnel. After two extractions with benzene, washing with water, another extraction in benzene, and removal of solvent, there was an observed yield of 17.3g. NMR spectrum was taken. ppm 4.0(1H); 3.9(1H); 3.7(1H); 3.6(1H)

Preparation of methylmethoxythiophosphorylchloride{4}: 2.11 g of {2} were dissolved in 25ml toluene. 4ml of 3.0M methyl Grignard in ether was added over .5hrs at 0C. After washing with water and benzene, 3 extractions and removal of solvent, 1.8g recovered.
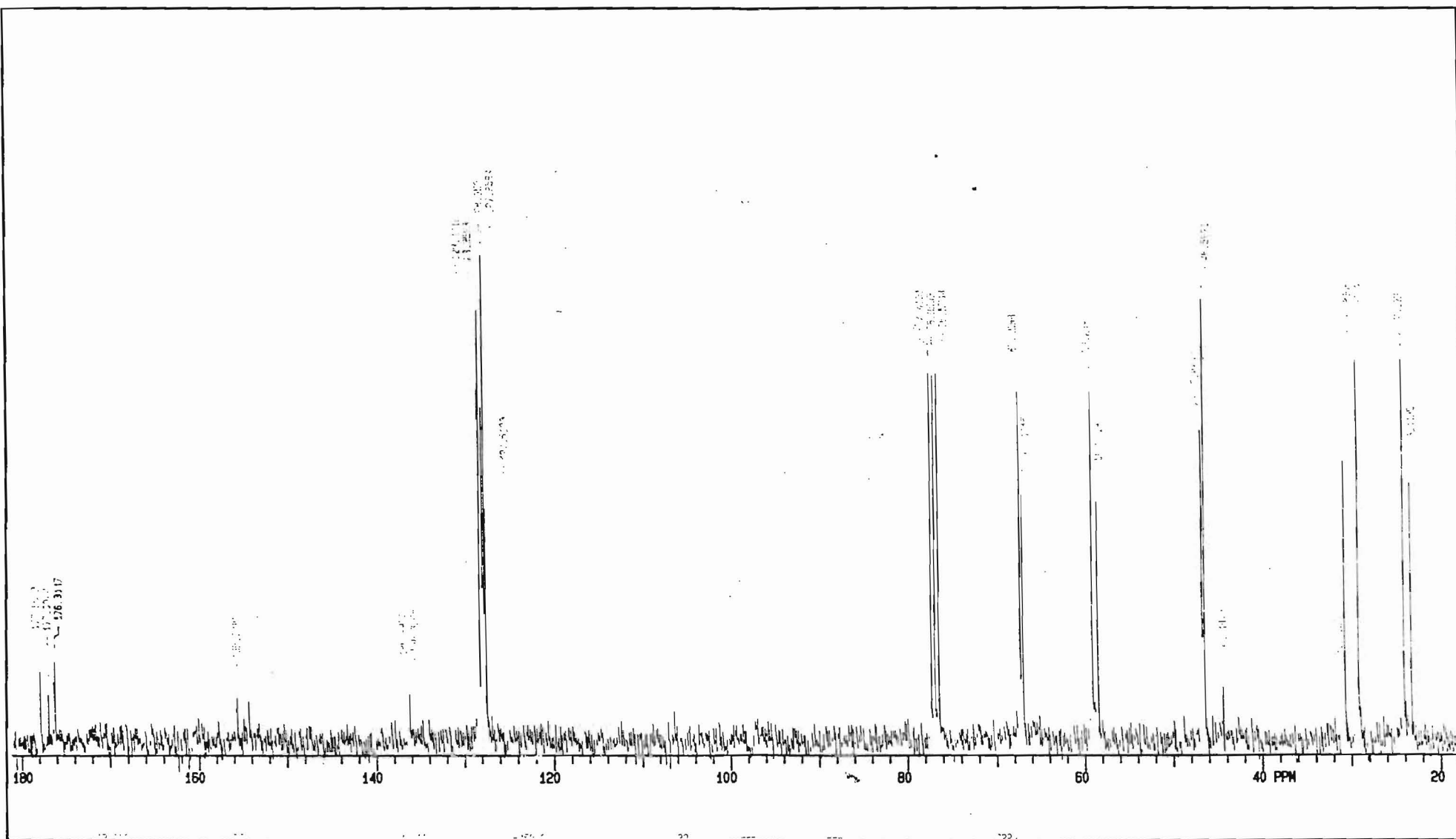
Preparation of methylprolinoxythiophosphorylchloride{5}: Sodium prolinoxide was prepared in situ by adding .05 g of Na metal to .52g of {b} in hexane. This was added slowly via syringe in an inert atmosphere to .42g of {2} dissolved in toluene. After letting the reaction run overnight, TLC was taken to detect progress of the reaction. Rf values were .7 and .5, and comparison with starting products revealed that they were probably isomers. Attempt to separate by flash chromatography (using ethyl acetate and hexane, 1:1, at first, and then ethyl acetate alone) failed, an NMR specrtum was taken. ppm 7.4 (5H); 5.2(4H); 4.5(2H); 3.6, 3.7(1H); 1.8-2.2(7H), 1.3(1H).

References

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SAMPLE

SULAY CBZ-PROLINE OIL

| 20ppm | 1200Hz | 1000 | 800 | 600 | 400 | 200 | 0 |
| 10ppm | 600 | 500 | 400 | 300 | 200 | 100 | 0 |
| 5ppm | 300 | 250 | 200 | 150 | 100 | 50 | 0 |
| 2ppm | 120 | 100 | 80 | 60 | 40 | 20 | 0 |
| 1ppm | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| 0.5ppm | 30 | 25 | 20 | 15 | 10 | 5 | 0 |

ppm (δ)   10   9   8   7   6   5   4   3   2   1   0

EM-360 60 MHz NMR SPECTROMETER

| LOCK POS. _____ ppm | SPECTRUM AMPL. _____ | SWEEP TIME _____ min | NUCLEUS _____ | SAMPLE: | OPERATOR _____ |
| LOCK POWER _____ mG | | | | | |
| DECOUPLE POS. _____ ppm | FILTER _____ sec | SWEEP WIDTH _____ ppm | ZERO REF. _____ | | DATE _____ |
| DECOUPLING POWER _____ mG | RF POWER .05 mG | END OF SWEEP _____ ppm | SAMPLE TEMP. _____ °C | SOLVENT: | SPECTRUM NO. _____ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20ppm | 1200Hz | 1000 | 800 | 600 | 400 | 200 | 0 |
| 10ppm | 600 | 500 | 400 | 300 | 200 | 100 | 0 |
| 5ppm | 300 | 250 | 200 | 150 | 100 | 50 | 0 |
| 2ppm | 120 | 100 | 80 | 60 | 40 | 20 | 0 |
| 1ppm | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| 0.5ppm | 30 | 25 | 20 | 15 | 10 | 5 | 0 |

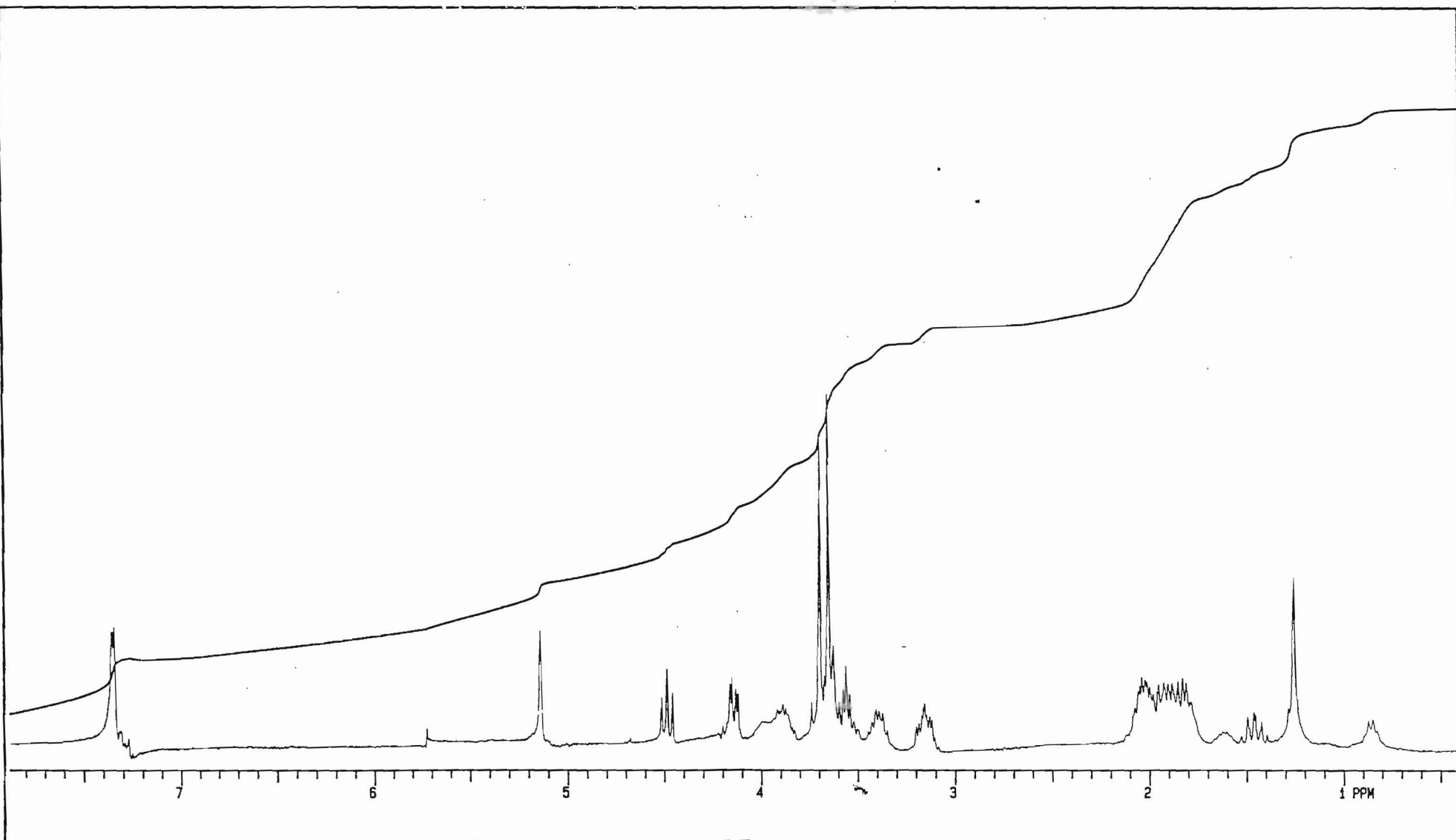EM-360 60 MHz NMR SPECTROMETER

ppm (δ)    10    9    8    7    6    5    4    3    2    1    0

LOCK POS. _____ ppm    SPECTRUM AMPL. _____    SWEEP TIME _____ min    NUCLEUS _____    SAMPLE:    OPERATOR _____

LOCK POWER _____ mG

DECOUPLE POS. _____ ppm    FILTER _____ sec    SWEEP WIDTH _____ ppm    ZERO REF. _____    DATE _____

DECOUPLING POWER _____ mG    RF POWER _____ mG    END OF SWEEP _____ ppm    SAMPLE TEMP. _____ °C    SOLVENT:    SPECTRUM NO. _____

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EM-360 60 MHz NMR SPECTROMETER

| 20ppm | 1200HZ | 1000 | 800 | 600 | 400 | 200 | 0 |
| 10ppm | 600 | 500 | 400 | 300 | 200 | 100 | 0 |
| 5ppm | 300 | 250 | 200 | 150 | 100 | 50 | 0 |
| 2ppm | 120 | 100 | 80 | 60 | 40 | 20 | 0 |
| 1ppm | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| 0.5ppm | 30 | 25 | 20 | 15 | 10 | 5 | 0 |

ppm (δ)    10      9      8      7      6      5      4      3      2      1      0

LOCK POS._____ppm    SPECTRUM AMPL._____    SWEEP TIME_____5_____min    NUCLEUS_____    SAMPLE:    OPERATOR_____

LOCK POWER_____mG

DECOUPLE POS._____ppm    FILTER_____sec    SWEEP WIDTH_____10_____ppm    ZERO REF._____    DATE__2/16/74__

DECOUPLING POWER_____mG    RF POWER____05____mG    END OF SWEEP____0____ppm    SAMPLE TEMP._____°C    SOLVENT:    SPECTRUM NO. 1/2

EM-360 60 MHz NMR SPECTROMETER

| | | | | | | |
|---|---|---|---|---|---|---|
| 20ppm | 1200HZ | 1000 | 800 | 600 | 400 | 200 | 0 |
| 10ppm | 600 | 500 | 400 | 300 | 200 | 100 | 0 |
| 5ppm | 300 | 250 | 200 | 150 | 100 | 50 | 0 |
| 2ppm | 120 | 100 | 80 | 60 | 40 | 20 | 0 |
| 1ppm | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| 0.5ppm | 30 | 25 | 20 | 15 | 10 | 5 | 0 |

START OF SWEEP    ⊢H→    END OF SWEEP
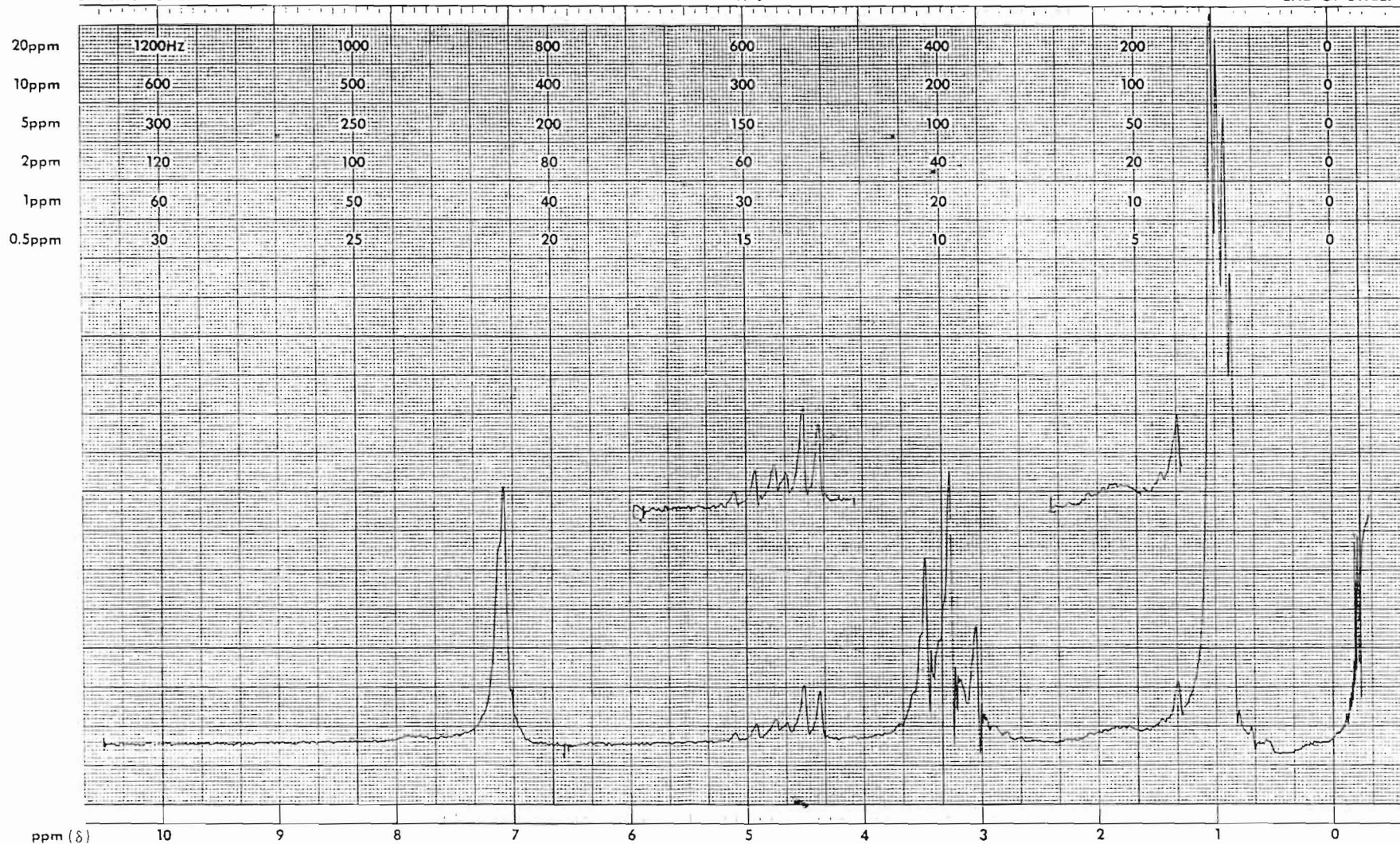
ppm (δ)   10   9   8   7   6   5   4   3   2   1   0

LOCK POS._____ppm      SPECTRUM AMPL. _800_      SWEEP TIME_____min      NUCLEUS_____      SAMPLE      OPERATOR _AJ_

LOCK POWER_____mG      FILTER_____sec      SWEEP WIDTH_____ppm      ZERO REF._____      DATE _2/24_

DECOUPLE POS._____ppm

DECOUPLING POWER __01__ mG      RF POWER __.04__ mG      END OF SWEEP_____ppm      SAMPLE TEMP._____°C      SOLVENT:      SPECTRUM NO. _4_

EM-360 60 MHz NMR SPECTROMETER

| 20ppm | 1200Hz | 1000 | 800 | 600 | 400 | 200 | 0 |
| 10ppm | 600 | 500 | 400 | 300 | 200 | 100 | 0 |
| 5ppm | 300 | 250 | 200 | 150 | 100 | 50 | 0 |
| 2ppm | 120 | 100 | 80 | 60 | 40 | 20 | 0 |
| 1ppm | 60 | 50 | 40 | 30 | 20 | 10 | 0 |
| 0.5ppm | 30 | 25 | 20 | 15 | 10 | 5 | 0 |

ppm (δ)    10    9    8    7    6    5    4    3    2    1    0

LOCK POS. _____ ppm

LOCK POWER _____ mG

DECOUPLE POS. _____ ppm

DECOUPLING POWER _____ mG

SPECTRUM AMPL. _____

FILTER _____ sec

RF POWER _____ mG

SWEEP TIME _____ min

SWEEP WIDTH _____ ppm

END OF SWEEP _____ ppm

NUCLEUS _____

ZERO REF. _____

SAMPLE TEMP. _____ °C

SAMPLE:

$CH_3-O-P$

OPERATOR _____

DATE _____

SOLVENT:

SPECTRUM NO. _____

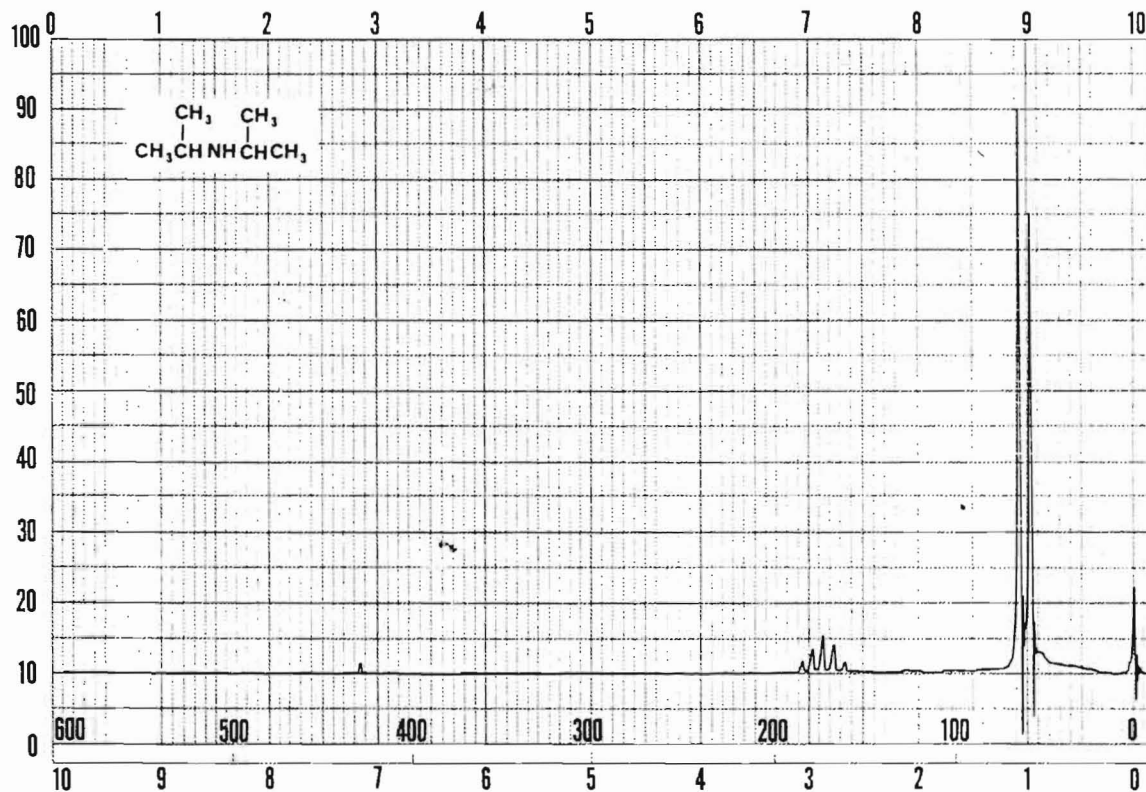**11,001-9**
**Diisopropylamine, 99%**
$[(CH_3)_2CH]_2NH$
M.W. 101.19   m.p. -70°   b.p. 84°   $n_D^{20}$ 1.3915
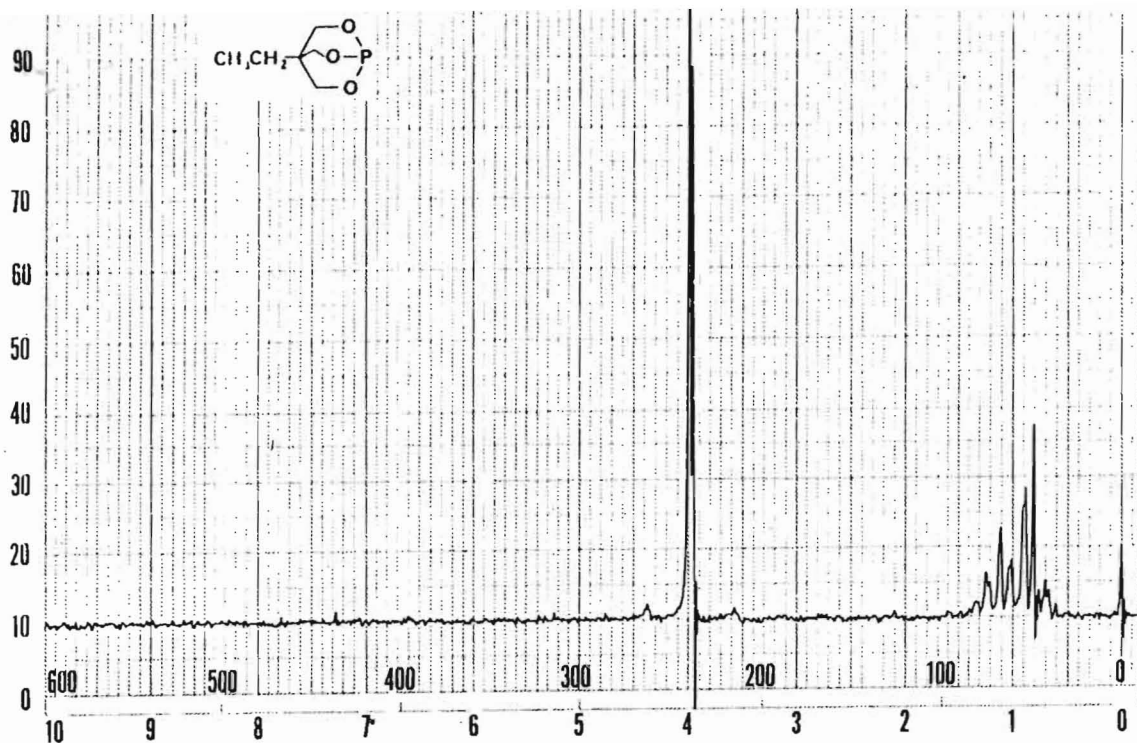d 0.722   Beil. 4,154   **IR** 136F   *LACHRYMATOR*

A



$CH_3CH NH CHCH_3$ with CH$_3$ groups

**16,212-4**
**N-Methylbutylamine**
$CH_3(CH_2)_3NHCH_3$
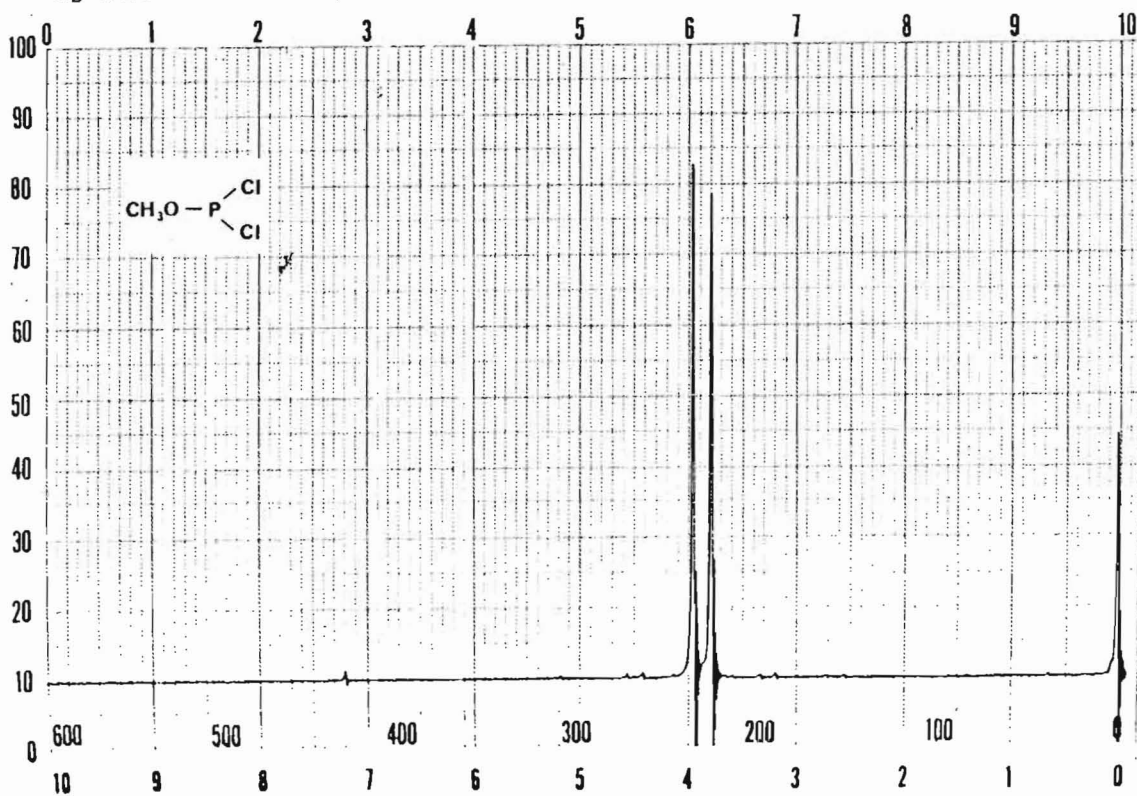
CH₃CH₂ structure with cyclic phosphite (O-P-O)

23,522-9
**Methyl dichlorophosphite,** 98% (methyl phosphoro-
dichloridite)
$CH_3OPCl_2$   FW 132.91   mp -91°   bp 93°
$n_D$ 1.4736   d 1.406   Disp. C



CH₃O—P structure with two Cl groups

D12,580-6
**N,N-Diisopropylethylamine,** 97%
(N-ethyldiisopropylamine)
$[(CH_3)_2CH]_2NC_2H_5$
M.W. 129.25   b.p. 127°   $n_D^{20}$ 1.4133   Fieser **1**,371
**IR** 138E   *IRRITANT*

CH₃  CH₃
|    |
CH₃CHNCHCH₃
|
CH₂CH₃

# ATIC AMINES