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4-(Phenylsulfonyl) Butanoic Acid: Preparation, Dianion Generation, and Application to Four Carbon Chain Extension ; Studies of Organophosphorus Compounds Derived from Serine

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I. 4-(PHENYLSULFONYL)BUTANOIC ACID. PREPARATION, DIANION GENERATION, AND APPLICATION TO FOUR CARBON CHAIN EXTENSION

II. STUDIES OF ORGANOPHOSPHORUS COMPOUNDS DERIVED FROM SERINE

by

Jeffrey A. Frick

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VITA

Jeffrey A. Frick is the the son of Allen and June Frick. He was born November 24, 1963, in Arlington Heights, Illinois.

After initial schooling in the Parkway School District, Chesterfield, Missouri, his secondary education was completed in 1982 at Downers Grove South High School, Downers Grove, Illinois. In September 1982, he entered Augustana College, Rock Island, Illinois and received a Bachelor of Arts in Chemistry in May, 1986.

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The author's publications include: 1. "Remote Dianions II. Sythesis of Sulcatole." Synthetic Communications 1988. 2. 4-(Phenylsulfonyl)butanoic Acid. Preparation, Dianion Generation and Application to Four-Carbon Chain Extension." Journal of Organic Chemistry 1989. 3. "Synthesis, Analysis, and Anti-Cholinesterase Properties of 0,0-Dimethylphosphoro-thioate Isomerides." Chemical Research in Toxicology 1989. 4. "Synthesis, Configuration and Chemical Shift Correlations of Chiral 1,3,2-Oxazaphospholidin-2-ones Derived from L-Serine." Journal of Organic Chemistry 1990.

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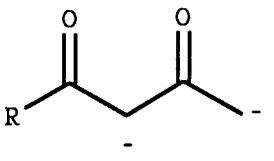
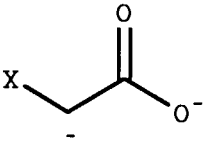
4-(PHENYLSULFONYL)BUTANOIC ACID. PREPARATION, DIANION GENERATION AND APPLICATION TO FOUR CARBON CHAIN EXTENSION

INTRODUCTION

1. Dianions

Dianions have proven extremely useful in a variety of synthetic transformations.[1-3] Two important types of dianions are β-dicarbonyl dianions and α-carboxylic acid dianions.





A. β-Dicarbonyl Dianions

Although several types of β-dicarbonyl dianions have been used in organic syntheses, the dianions of β-keto esters are particularily useful and show the most variability in their synthetic application. For example, they are able to undergo an aldol reaction to yield β-hydroxy ketones which can be further elaborated to

α,β-unsaturated ketones.[4] β,δ-diketo esters result when these dianions undergo a Claisen condensation with another ester.[5] Dianions of this type have also been found useful in the preparation of aromatic compounds when reacted with the monoanions of β-keto esters and in the synthesis of a hydroxypyridone when reacted with nitriles.[6]

B. Carboxylic Acid Dianions

Creger, in 1967, reported that generation of the dianion from aliphatic carboxylic acids was a general phenomenon.[7] In the course of these studies, he focused on the bis-metalation of isobutyric acid and showed that this dianion methodology was useful in the preparation of highly hindered trialkylacetic acid derivatives. This same methodology was also applied to the conversion of spiro-epoxides into spiro-lactones.[8] Other dianions of acetic acid derivatives[9-11] and alkenoic acids[12] have also proven useful. The reaction of β-lithio acrylates with carbonyl compounds has been used in the synthesis of α,β-butenolides.[13] Mulzer et al. have studied the stereoselectivity and regioselectivity of the addition of carboxylic dianions to a variety of carbonyl compounds.[14-16] Belletire and co-workers have shown that carboxylic acid dianions can undergo oxidative coupling to yield diacids.[17,18] The dianion of an aromatic carboxylic acid has even been used in the total synthesis

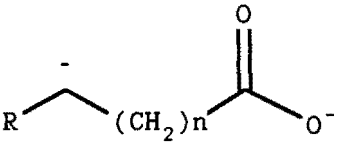
of Milbemycin, a natural product with anti-parasitic properties.[19]

C. Other Dianions

Other dianions of synthetic interest include dianions of β-ketosulfones[20], sulfone-amides[21], carbamates[22], and β-sultams[23].

D. Remote Dianions

Remote dianions can be defined as dianions containing one or more insulating methylene groups between the anionic centers. Despite the synthetic utility of

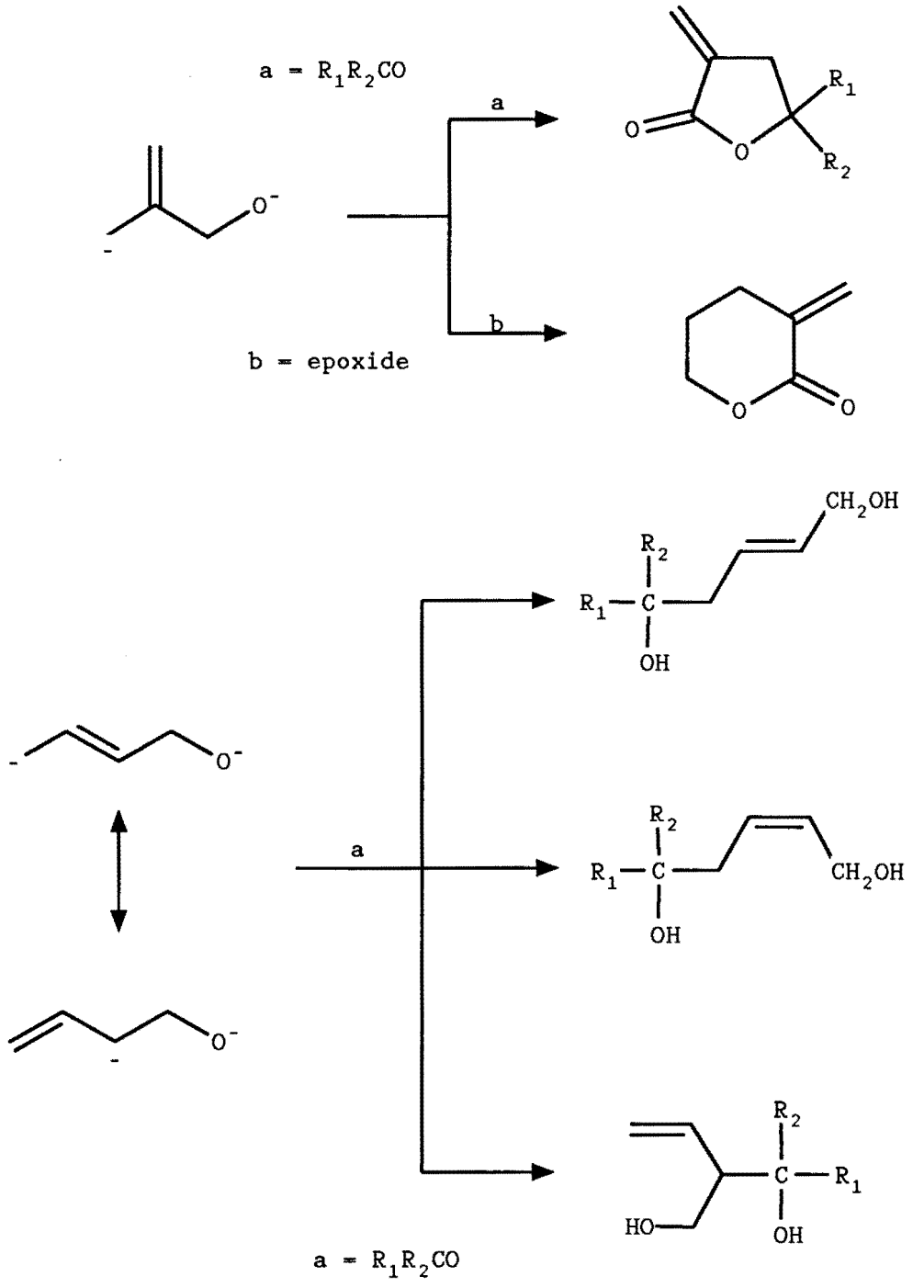


R = EWG

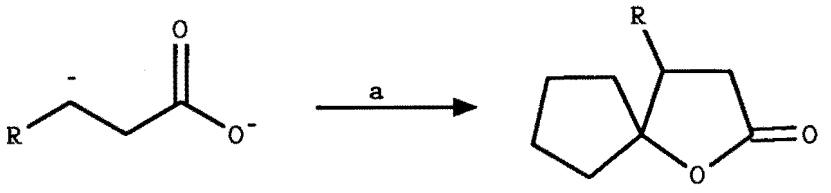
these compounds, the exploration of remote dianions remains in its infancy.

Carlson et al. have explored the utility of some remote alcohol dianions in the preparation of α-methylene-γ-lactones[24] and α-methylene-δ-valerolactones[25] using the dianion of methallyl alcohol. They have also examined the formation of a variety of unsaturated diols[26] with the dianion of crotyl alcohol.

The dianions of 3-phenylsufinyl- and 3-phenyl-sulfonyl-propionic acids have also proven useful in organic syntheses. Condensation of these dianions with cyclopentanone afforded a spirolactone which was also



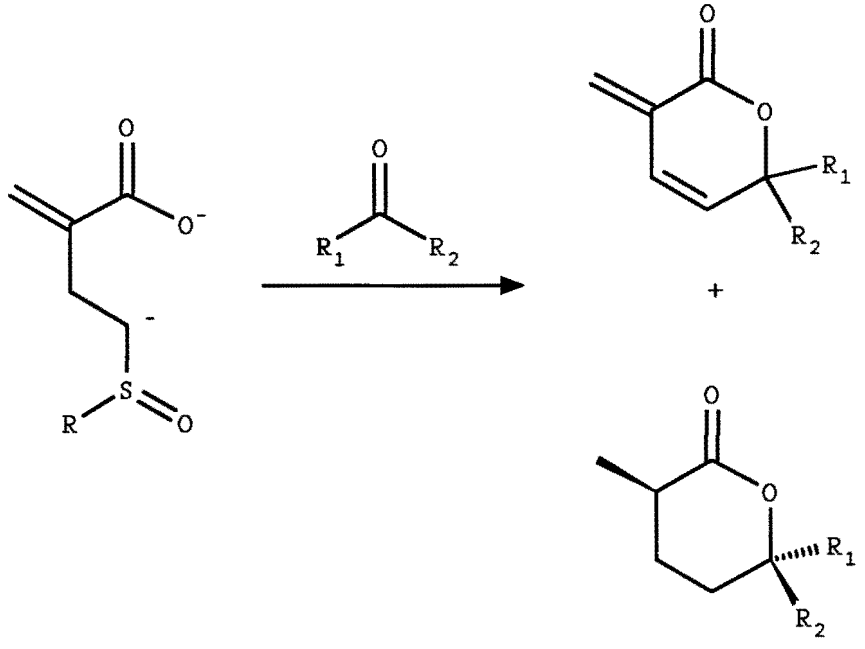
shown to undergo further elaboration to butenolides and/or unsaturated esters.[27,28] Bravo et al. have also made use of remote dianion methodology in the synthesis of



R = PhS(O)
or $PhS(O)_2$

a = cyclopentanone

α-methylene- and α-methyl-δ-lactones.[29] Marc Julia and



co-workers made use of very long chain (up to eleven carbons) sulfone based remote carboxylic acid dianions in the synthesis of a variety of different compounds including the macrocyclic lactone Exaltolide.[30]

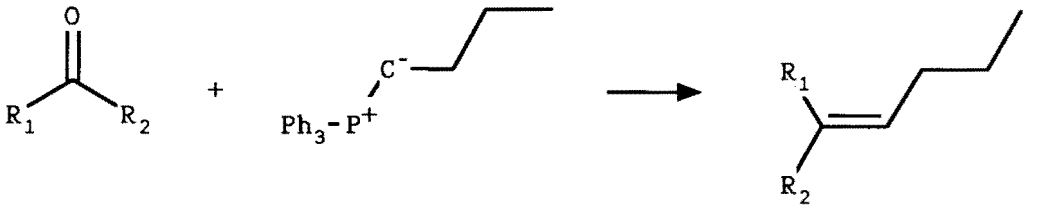
2. Homologations

Homologations or chain extending reactions are important in synthetic chemistry. Martin[31] has reviewed homologations of aldehydes and ketones. A plethora of one

and two carbon homologations exist. Fewer examples of three carbon homologations exist and naturally, four carbon chain extension methodology is quite limited especially where the regio- and stereochemistry is controlled. Two well known reactions capable of extending carbonyl compounds by four carbons are the Wittig and the Reformatsky.

A. Wittig Reaction

In general terms, the Wittig reaction involves the condensation of a phosphorus ylide (generated by treating an alkyl halide with a trialkylphosphine, typically triphenylphosphine, and subsequent treatment with a strong base) and a carbonyl compound resulting in the formation of an olefin.



The Wittig reaction has been shown to be useful for four carbon chain extension.[32] Good examples of the Wittig four-carbon homologation are demonstrated by the synthesis of (RS)-E-nuciferal[33] and as a step in the synthesis of Piperine[34], a known insecticide. Some studies have been conducted in an attempt to control the stereochemistry of the Wittig reaction.[35,36] These studies typically involve the use of lithium salts. In

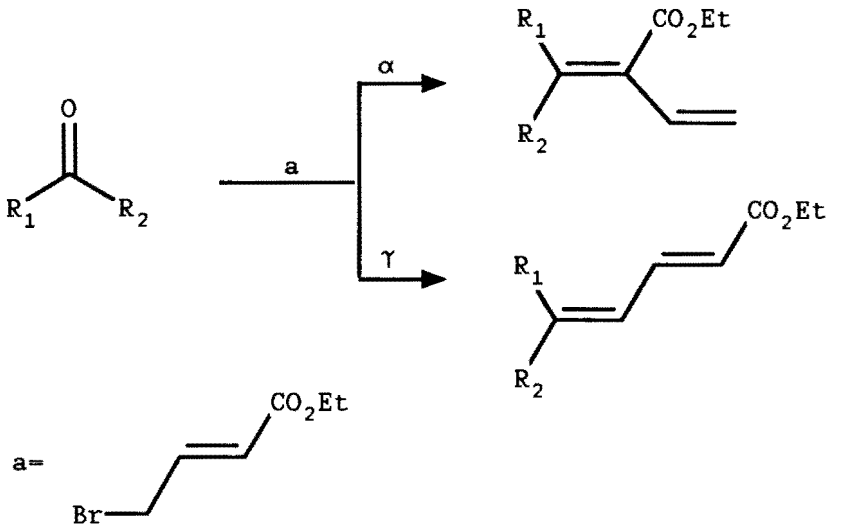
fact, Corey et al.[37] have reported the synthesis of a pheromone in which the double bond created with a Wittig reaction is exclusively trans (normally, the Wittig reaction affords predominantly the cis isomer). Several variations of the Wittig reaction have been reported, and these include reacting carbonyl compounds with sulfinamides[38,39] and reaction of carbonyls with trialkylsilyl-substituted organometallic compounds[40,41](i.e. the Peterson reaction).

Although the Wittig reaction is very general in that it can be used on a variety of carbonyl compounds and the position of the resulting double bond is never in doubt, it has the potential disadvantages that the carbonyl functionality is lost and several substrates are not amenable to generation of the phosphonium salt and/or ylide.

B. Reformatsky Reaction

The Reformatsky reaction involves the reaction of a carbonyl compound with an α-haloester in the presence of zinc to yield a β-hydroxy ester where two carbons have been appended to the original carbonyl carbon. Although the Reformatsky reaction itself is not a method for four carbon chain extension of carbonyl compounds, the vinylogous Reformatsky has been shown to be a useful method of four carbon chain extension.[42-46] This reaction involves the condensation of a carbonyl compound with an

unsaturated γ-halo ester to yield two possible α,β-unsaturated esters.



Like the Wittig, the vinylogous Reformatsky reaction maintains a general scope, however, it may also be problematic. The vinylogs of masked ester enoates have been used, but their ambident nature (α vs. γ attack) must be recognized and the regioselectivity controlled. Studies to examine the regioselectivity of these types of reactions have been conducted.[47-49]

C. Other Homologations

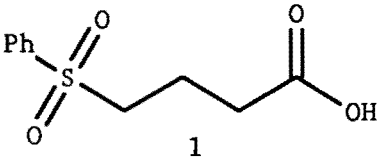
Other homologations have been reported.[50-56] One is useful with aromatic aldehydes[57] and some produce conjugated dienals[58-62]. Some newer methods involve enamidines[63] or have heterocyclic intermediates[64].

3. Purpose

Although there are a few seldom used four-carbon homologation reactions, there still remains a paucity

relative to lower case homologations. Therefore, it would be useful to develop a four-carbon homologation that would be general as far as the starting carbonyl compound and at the same time could be versatile enough to generate a variety of products depending on the reaction sequence employed. For example, it should be noted that most of the methods discussed above result in the formation of α,β-unsaturated carbonyls while the production of γ,δ-unsaturated carbonyls[65] is much less prevalent. In addition, few methods are capable of regio- and stereocontrol of the olefin while maintaining the oxidation state of the appended carbon.

With an interest in furthering the development of four-carbon homologations and an interest in remote dianions, we sought to explore the utility of 4-(phenylsulfonyl)butanoic acid (**1**) as a reagent for four-carbon homologation of carbonyl compounds and begin to address the question of stereo- and regiocontrol of the olefin produced. This reagent was chosen for several



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reasons. First of all, the reagent obviously contains the four carbons necessary for a compound that will be used to extend other compounds by four carbon atoms. The phenyl

sulfone is also a logical choice as the addition of sulfone-stabilized anions to carbonyls is well documented.[66-68] While other electron-withdrawing species may act equally well to stabilize the carbanion, the sulfone moiety can generally be removed while others cannot. Finally, while in principle the tolyl sulfone may also be considered, the methyl group may have been deprotonated by the treatment with *n*-butyllithium necessary to generate the dianion.

RESULTS AND DISCUSSION

1. Preparation and Bis-deprotonation of 4-(Phenylsulfonyl)butanoic Acid.

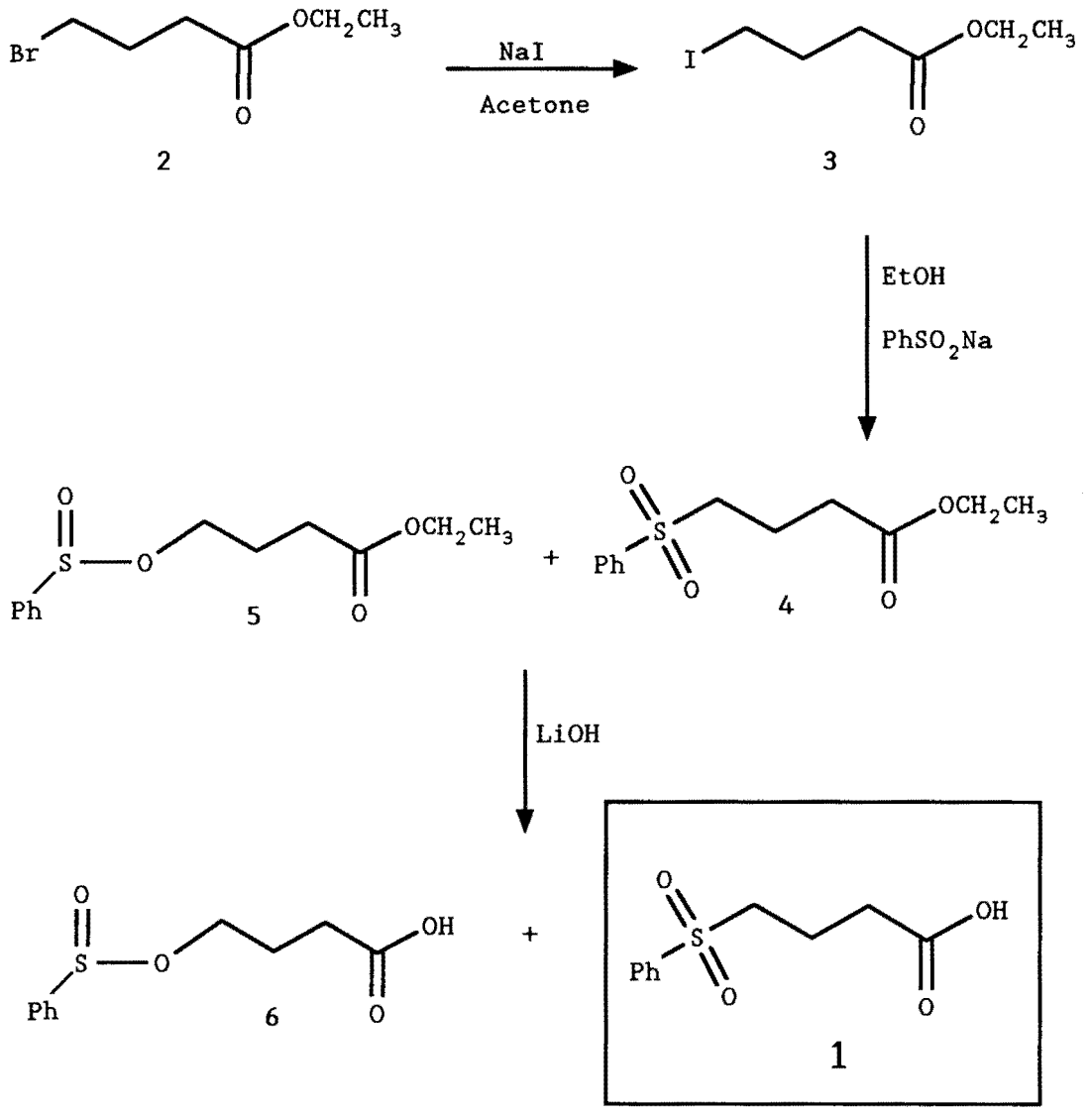
Although 4-PSBA **(1)** appears to be a rather simple molecule with apparent synthetic utility, few literature references[69,70] concerning this compound or its preparation were available. With this in mind, we set about designing a synthetic pathway that would be amenable to scale up. We have succeeded in preparing **1** in three steps (Scheme I) in approximately 60% overall yield from the commercially available ethyl 4-bromobutyrate **(2)**.

In a typical procedure ethyl 4-bromobutyrate **(2)** was treated with sodium iodide in refluxing acetone resulting in conversion to the iodide **(3)** in 96% yield.[71] The crude ethyl-4-iodobutyrate was reacted with sodium benzenesulfinate in ethanol to form sulfone **(4)**. Although the reaction proceeded in greater than 90% yield, TLC of the reaction mixture indicated the formation of two products. The two products were isolated and identified by NMR spectroscopy as the phenylsulfonyl **(4)** and phenylsulfinate **(5)** butyrate esters (Scheme I). While reactions of benzenesulfinates are known to yield mixtures of S- and O-alkylation products,[72,73] we were fortunate in that **4** and **5** were formed in a ratio of 8:1, respectively.

This ester mixture was then saponified with lithium hydroxide to provide the butanoic acids **(1 & 6)** which were isolated following acidification. Recrystallization afforded pure **1**, the sulfinate isomer **(6)** remained in solution, in 60% overall yield without any intermediate purification. 4-(Phenylsulfonyl)butanoic acid may be stored for prolonged periods at room temperature with little decomposition. An interesting olfactory observation is that 4-PSBA has a faint odor of butterscotch.

Generation of dianion **7** was not straightforward, because deprotonation alpha to the carboxylate may have been possible although it was not observed, and several base combinations were tried.[74] It was determined that the use of 200 mole % *n*-butyllithium (*n*-BuLi) resulted in greater than 96% conversion to the dianion) (Figure 1). Precipitation was observed with dianion concentrations greater than 0.05 M using 1.6 M *n*-BuLi; the precipitate is presumably due to the sparing solubility of the dianion in the hexane present. The use of 2.5 M *n*-BuLi was found useful, allowing higher concentrations of the dianion to be achieved. Interestingly, the golden yellow dianion solution is preceded by initial precipitation of the carboxylate salt. This observation may be suggestive of dianion dipole stabilization.[75,76]

Scheme I



2. Preparation of Tetrahydropyran-2-ones (Lactones) from Aldehydes.

The addition of sulfone-stabilized anions to carbonyls is well documented.[66-68] Addition of these anions to carbonyls may be sluggish and complicated by reversible reaction. However, no problems were observed

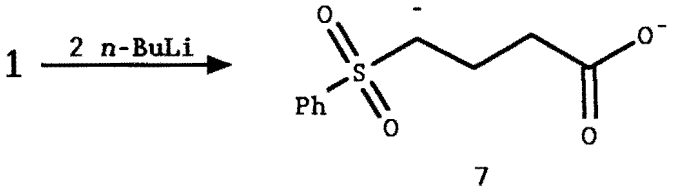


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Figure 1. Generation of the Dianion of 4-PSBA

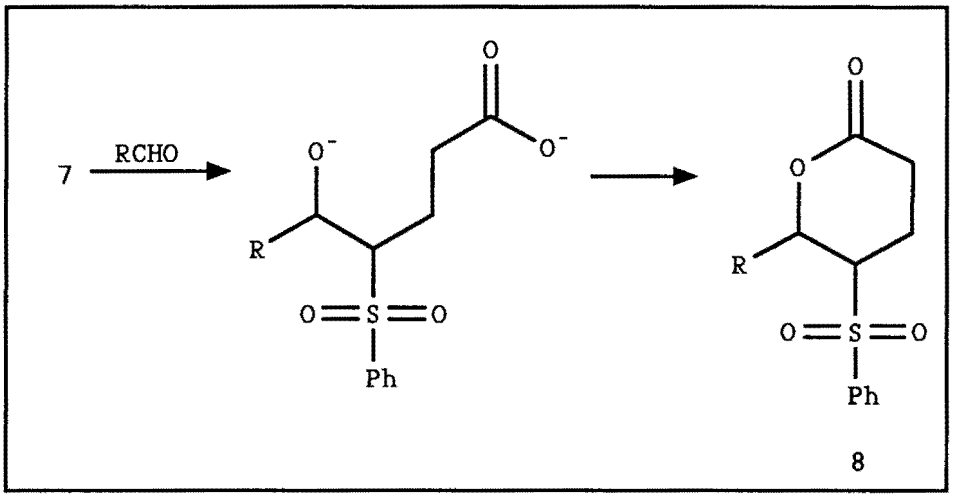
in the addition of **7** to a variety of aldehydes at temperatures ranging from -78 °C to room temperature. Additionally, the dianion was capable of surviving at room temperature without any significant loss in reaction yields. For example, addition of benzaldehyde to **7** even after stirring for four hours at room temperature, resulted in a decrease in yield of only 5%.

In a typical procedure, the aldehyde (4 mmol; neat or in THF) was added to 4.25 mmol of dianion **7** in tetrahydrofuran at -78 °C and stirred for 0.5 h resulting in the formation of an intermediate hydroxy-acid. Cyclization was affected with subsequent addition of trifluoroacetic anhydride[77-79] (TFAA, 8 mmol) and continued stirring for an additional 0.5 h while the reaction was warmed to room temperature. Cyclization presumably arises from formation of the mixed anhydride with subsequent intramolecular displacement by alkoxide. After standard workup, the cyclized products **8a-f** (Table I)[80] were obtained in greater than 90% yield. A nice feature of the workup is that all the reagents are

removed in the sodium bicarbonate wash. Column chromatography afforded isolated yields from 65-85%. In many cases, recrystallization was achieved from chloroform-ethyl ether mixtures.

It is interesting to note that the addition-cyclization sequence affords regiochemical 1,2-addition **8b,d** and is capable of reacting with both aryl and alkyl aldehydes. Furthermore, branching at the α-position of **8f** did not adversely affect the reaction.

Table I. Preparation of 3,4,5,6-Tetrahydropyran-2-ones



entry	R	% yield[a]	J_{H5-H6}, Hz
8a	n-C_3H_7	77	10.8
8b	CH_3CH=CH	75	6.2
8c	Ph	84	6.1
8d	PhCH=CH	72	6.5
8e	n-C_7H_{15}	65	10.7
8f	t-C_4H_9	85	2.6

[a]after chromatography

3. Tetrahydropyran-2-one Stereochemistry.

Since two isomers (diastereomers) of the lactones were formed, an examination of the stereochemistry of the

6-substituted-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-ones produced **(8a-f)** seemed in order. For specific analysis, the 6-phenyl compound **(8c)** was chosen due to its chromatographic characteristics and NMR properties. In particular, the absorbance of the proton alpha to the phenyl ring is shifted downfield. The two isomers of this compound were readily separated on flash silica (with diethyl ether) and will, for the time being, be referred to as the fast band and slow band corresponding to their respective elution from the column. Chromatographic isolation of a 4 mmol reaction afforded a 94:6 fast/slow ratio by weight.

Of particular interest was the H-C5/H-C6 coupling constant which is dependent upon both the phenylsulfonyl group orientation (i.e. Karplus angle) and the slight ring flattening imparted by the lactone function (the conformation of oxacyclohexane ring is best described as a flattened chair)[81-89]. Application of the Karplus correlation[90,91] to the $H\text{-}C_5/H\text{-}C_6$ coupling constants gives an approximation of the dihedral angle between these two vicinal protons. The fast band isomer, J=6.1 Hz, would be predicted to have a dihedral angle of approximately 132°. The slow band isomer, J=3.78 Hz, would be predicted to have a dihedral angle of approximately 47°. However, more recently it has been found that electronegative or electron-withdrawing substituents on the H-C-C-H unit

results in a shift in the Karplus curve to the right.[92,93] This shift in the curve indicates an increase in the dihedral angle for a corresponding J value. For example, a Karplus curve with fluorine as a substituent would predict a dihedral angle of approximately 170° for the fast band isomer and 50° for the slow band isomer. In an effort to determine these angles more precisely, MM2 calculations (Serena Software) were done for both the trans and cis sulfone isomers **(8c)**. Minimized energy conformations suggested that the vicinal hydrogen dihedral angle ($H-C_5-C_6-H$) for the trans and cis isomers were 176.66° and 51.54° respectively. With computational agreement, the fast band isomer was assigned the trans relationship while the slow band corresponded to the cis isomer. Two additional insights can be drawn from the energy minimizations. First, the phenyl ring adopts a periplanar arrangement in the trans case possibly having an effect on the vicinal hydrogen dihedral angle. Secondly, the small dihedral angle in the cis isomer may result from buttressing between the vicinal substituents thereby forcing the protons closer together. All other non-branching derivatives were found to have similar bond angle values when minimized. While limitations may exist for extending molecular mechanics analyses to solution characteristics (i.e. NMR analyses), the results are in good agreement in this study.

Homonuclear decoupling and 2-dimensional NMR (COSY) experiments were done on the trans isomer to further assist in the assignment of the aliphatic protons. The first aliphatic peak appears as a doublet at 5.7 ppm (J=6 Hz). This peak was assigned to the proton alpha to the phenyl ring. It appeared as a doublet due to coupling with the proton on C-5 (3.7 ppm , J=12.5 and 6 Hz). The remaining peaks were somewhat more difficult to assign as they appeared as multiplets in three different positions: a multiplet between 2.87-2.98 ppm integrated one proton, a multiplet between 2.44-2.65 ppm integrated two protons, and a multiplet between 2.19-2.33 ppm integrated one proton. The 2-D NMR indicated that one of the protons in the middle multiplet, 2.44-2.66 ppm, corresponded to one of the protons on C-4. This peak was assigned to the proton in the axial position as Karplus correlation predicted that the equatorial proton would have minimal coupling to the proton on C-5. The assignment of the remaining protons remained ambiguous, but assignments were as follows. The multiplet, 2.87-2.98 ppm, corresponded to the equatorial proton on C-4. The equatorial proton on C-3 corresponded to the remaining proton in the multiplet, 2.44-2.66 ppm, while the axial proton on C-3 gave rise to the signal between 2.19-2.33 ppm. Although not necessary for stereochemical information, other lactone protons may be assigned by analogy.

Introduction of a branched substituent, R group = *tert*-butyl **8f**, led to a dramatic decrease in the coupling constant to 2.7 Hz. Thus, either the ring has adopted a cis conformation where the phenylsulfonyl group occupies the axial position or the trans isomer exists in a ring perturbed form.

Calculations of the trans isomer of **8f** suggested an $H-C_5-C_6-H$ dihedral angle of 132°, which virtually eclipsed the C-5, C-6 substituents and respective vicinal H-6, H-5 protons to avoid crowding. The dihedral angle would be equivalent to the observed coupling constant based on the shifted Karplus curve. Nuclear Overhauser studies were inconclusive.

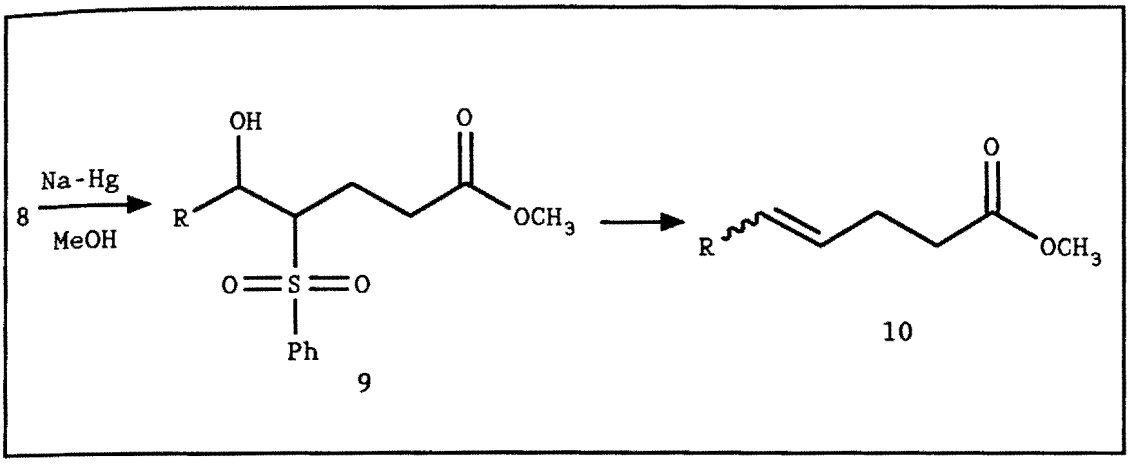
4. Conversion of Tetrahydropyran-2-ones to Methyl 4-Butenoates

With the lactones in hand, we wanted to remove the phenylsulfonyl group as a potential route to lactone containing natural products. Trost et al.[94] have reported that the removal of the phenylsulfonyl group can be affected using sodium-mercury amalgam. The general procedure involved dissolving the lactone in methanol, chilling the solution to 0 °C and adding finely crushed sodium mercury amalgam. The products **(10a-f)** were isolated after filtration, workup, and careful rotary evaporation in 56-85% yield. Analysis of the products **(10a-f**, Table II) indicated that while the phenylsulfonyl

moiety had indeed been removed, further reaction had also occurred. Careful analysis of the products indicated the formation of methyl 4-butenoates (Table II). Upon examination, these results were not that surprising. If the reaction is quenched prior to completion, two products were isolated; the gamma, delta unsaturated-ester **(10)** and the corresponding β-hydroxy sulfone **(9)**. Their isolation provided preliminary evidence that the methoxide formed *in situ* induces lactone ring opening and ester formation followed by reductive elimination to the methyl 4-butenoate **(10)**. Formation of the olefin could be predicted in light of the intermediate β-hydroxy sulfone **(9)**. Julia and Paris[95] have reported examples of the reductive elimination of the phenylsulfonyl group when it is positioned beta to leaving groups such as hydroxy, acetoxy, and methanesulfonate. Since this initial report, much work has been done to examine the specifics of the reductive elimination.[96-98]

The stereochemistry of the resulting olefin was determined by integration of the GC peaks or integration of suitable peaks in the proton NMR spectra of the compounds and was found to be consistent with the the geometry and branching dependence observed (i.e. the 80/20 E/Z mixtures for unbranched chains and the 97/3 E/Z ratio for the *tert*-butyl derivative, Table II) by Lythgoe and co-workers.[97] These values support the radical

Table II. Conversion of 3,4,5,6-Tetrahydropyran-2-ones to Methyl 4-Butenoates



entry	R	yield of 10, %	E/Z[a]
10a	n-C_3H_7	80	4:1
10b	CH_3CH=CH	85	4:1
10c	Ph	70	4:1
10d	PhCH=CH	56	4:1
10e	n-C_7H_{15}	83	4:1
10f	t-C_4H_9	81	97:3

intermediate mechanism proposed by Lythgoe. In this mechanism, an electron from sodium is transferred to the sulfone with subsequent cleavage of the sulfur-carbon bond expelling the benzenesulfinate anion (or benzenesulphinyl radical) and leaving a carbon based radical. A further one electron reduction affords an anion, which is presumably responsible for the elimination. Therefore, the E:Z ratio is independent of the conformation of the β-hydroxy sulfone while being dependent upon the equilibrium mixture of the intermediate anion (or

radical). As with most beta-eliminations, the leaving group, in our case hydroxy, must be trans-periplanar with the anion (radical). Therefore, two different orientations are possible (Figure 2). In one case, the R and R′ groups are anti (i.e. potentially trans) to each other (A) in the other case, the R and R′ are gauche (i.e. potentially cis, B). For steric reasons the anti

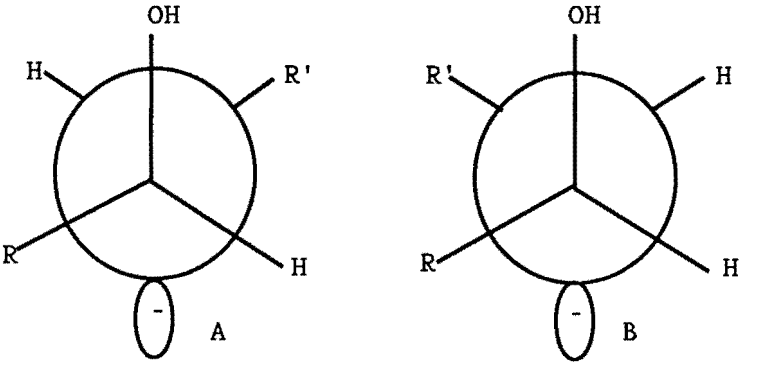


Figure 2. Newman Projections of the Anionic Intermediates During Reductive Elimination.

conformation is much more favorable than the gauche conformation (by 0.85 kcal/mole or E_q=80:20). Thus, we would expect the groups to orient themselves anti to each other to minimize steric repulsion. Therefore, the equilibrium mixture should lie toward the anti orientation resulting in the formation of more trans product. The effect becomes greater as branching of the R group increases naturally increasing the alkyl steric group interaction.[97]

As mentioned above, we suspected that ring opening

precedes elimination as evidenced by isolation of intermediate hydroxy sulfone **9**. This aspect, unfortunately, would work against any possible stereochemical enhancement over acyclic systems due to ring locked precursors. To further examine this aspect, 6-phenyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydro-pyran-2-one **(8c)** (trans:cis = 94:6) was subjected to base-induced methanolysis (CH_3OH, room temperature, 2h, 5 mol % NaH) and subsequently treated with Na-Hg amalgam, which resulted in identical isomer composition of methyl 4-butenoate products (as compared to the one-pot reaction). Elimination to 4-butenoic acid and concomitant esterification apparently does not occur to any noticeable extent. Further evidence for the methoxide ring opening was observed from the stereochemical outcome of the olefin produced. The cis and trans isomer of **8c** were treated separately with Na-Hg amalgam as described. In both cases, an 80:20 E/Z mixture was obtained (Figure 3). If elimination preceded esterification, the geometry about the olefin would be strictly related to the lactone substituent stereochemistry. Therefore, ring opening was the initial event in the reaction sequence followed by elimination to the olefin.

5. Synthesis of Sulcatole

In an effort to apply our methodology towards the synthesis of a natural product, we focused our efforts on

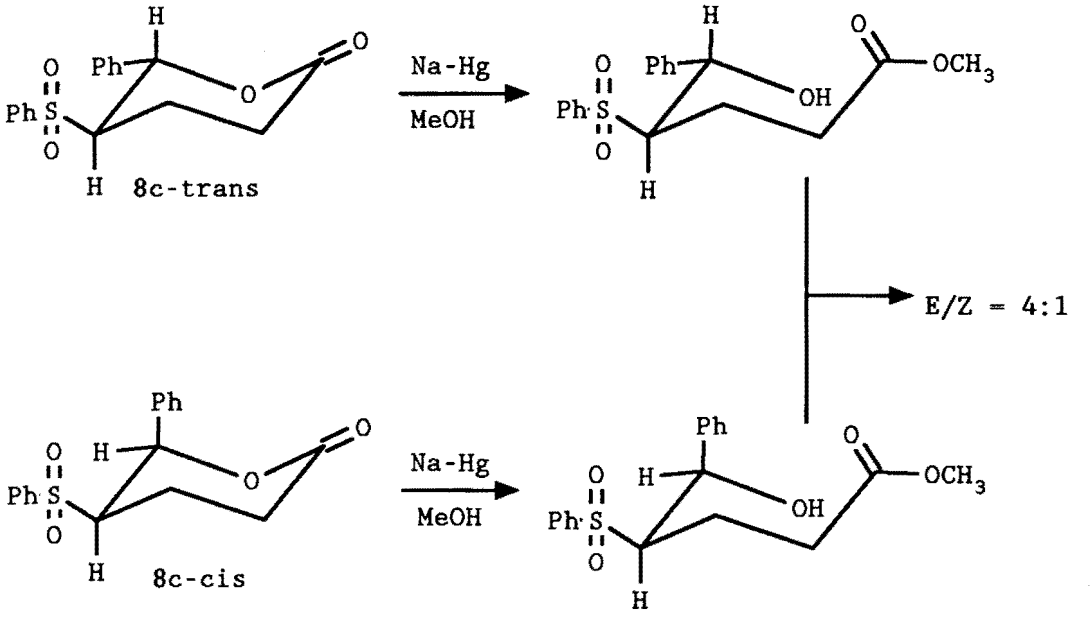


Figure 3. Ring Opening Reaction of the Trans and Cis Phenyl Lactones.

the synthesis of sulcatole[99-101] the aggregation pheromone produced by the males of *Gnathotricus sulcatus*.

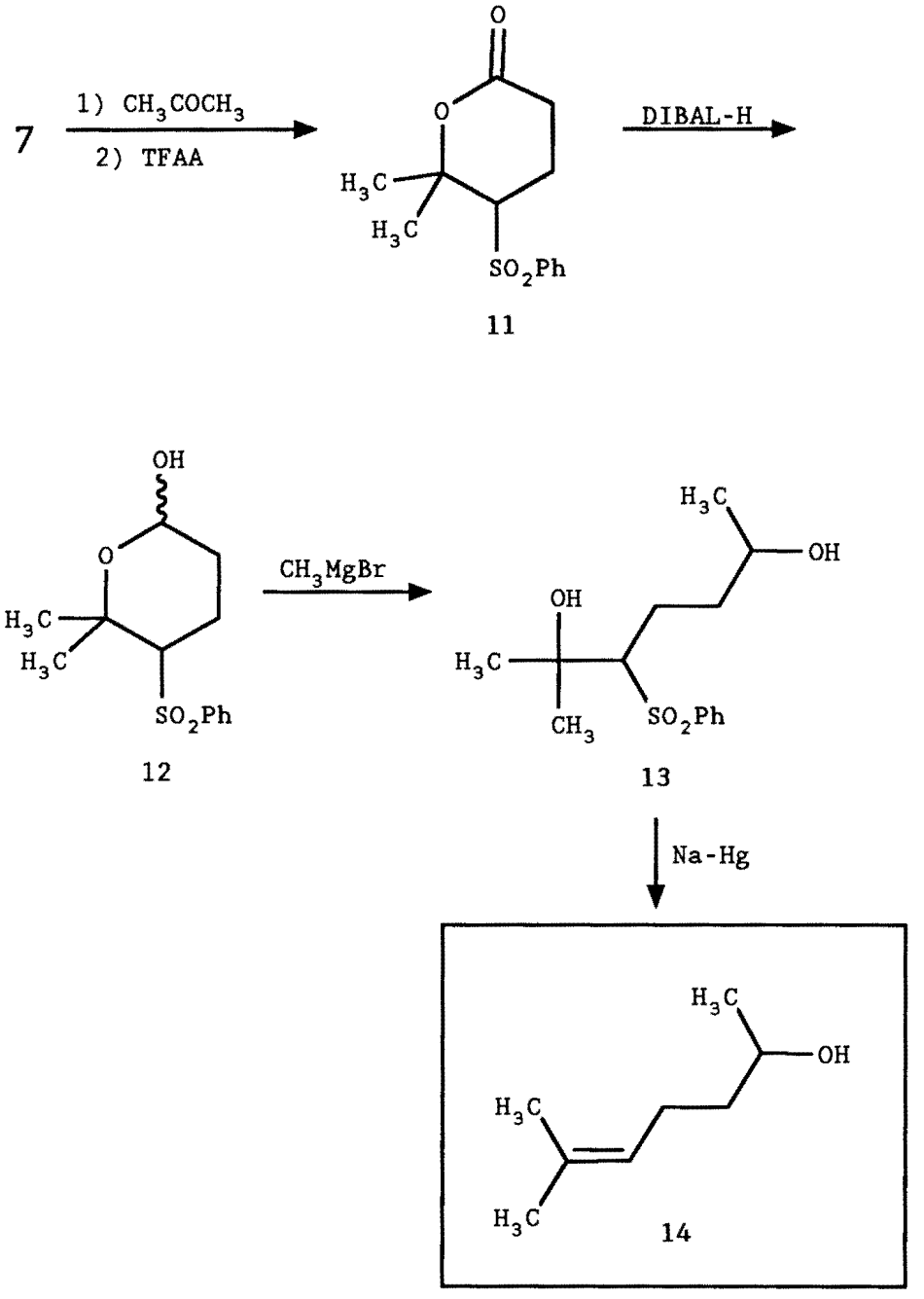
The synthesis is outlined in Scheme II. Acetone was reacted with the dianion of 4-PSBA and cyclized *in situ* with TFAA to yield the lactone **(11)** in 84% yield as described above. The reaction was much more sluggish with acetone than with aldehydes. Warming of the reaction to room temperature was necessary to affect complete addition of the dianion. After recrystallization from chloroform-diethyl ether, **11** was converted to lactol **12** in greater than 95% yield by the dropwise addition of diisobutylaluminum hydride (1.05 equiv., -78 °C, THF) and inverse quench (10% NaOH). The lactol crystallized from

warm chloroform. Lactol **12** was then reacted with three equivalents of methylmagnesium bromide to afford diol **13**, an oil, in 86% yield following chromatography (100% diethyl ether). Other organometallic reagents (i.e. methyllithium) were less successful. Finally, the diol **(13)** was transformed into racemic sulcatole **(14)** with Na-Hg amalgam in 76% yield (52% overall from acetone). This synthesis was especially attractive because no E/Z isomerization was possible since a symmetrical ketone was used in the initial step. The phenylsulfonyl group also served as a molecular anchor to decrease the volatility of the intermediates.

6. Studies Toward the Total Synthesis of Trans-4-cis-7-Tridecadienyl Acetate: The Sex Pheromone of the Potato Tuberworm Moth.

In an effort to further exploit the synthetic utility of 4-PSBA and to pursue a more complex synthesis, we turned our attention to the synthesis of trans-4-cis-7-tridecadienyl acetate **(22)**, the sex pheromone of the potato tuberworm moth.[102-104] Some of the proposed synthetic methodology is outlined in Schemes III and IV. The initial step in this synthesis is the alkylation of 1-heptyne **(15)** with bromoacetaldehyde diethyl acetal. Brattesani and Heathcock[105] have developed a procedure for the alkylation of terminal alkynes. Their procedure makes

Scheme II



use of hexamethylphosphoramide (HMPA) to coordinate the lithium present in the organolithium species that results

when 1-heptyne is treated with butyllithium. Although this procedure worked well for us, we chose to modify the procedure due to the carcinogenic properties of HMPA along with the envisioned need for large scale reactions. We chose to use 1,2-dimethoxyethane (DME) which performs the same function, but has the added benefit of lower toxicity. Reaction of *n*-butyllithium at 0 °C with 1-heptyne followed by the addition of bromoacetaldehyde diethyl acetal and warming to reflux allows the reaction to occur. This procedure yielded a brown liquid even after workup. If necessary, alkyne acetal **(16)** can be purified as a colorless liquid (52%) via bulb-to-bulb distillation.

Once the alkyne was functionalized, we had a few options. Since an aldehyde was needed for reaction with the dianion of 4-PSBA, a logical way to proceed was the hydrolysis of the diethyl acetal to the aldehyde. Traditionally, acetals can be converted to aldehydes in the presence of acid. Several methods were attempted. The use of toluenesulfonic acid or sulfuric acid was unsuccessful. In fact, only starting material was isolated from these reactions. The same proved to be the case when the diethyl acetal **(16)** was stirred with silica gel. The method that worked best, but did not afford pure product, involved treating the diethyl acetal with hydrochloric acid in acetone. While this method afforded some of the product, it was not desirable because in

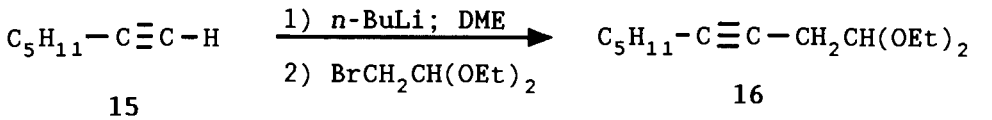
addition to forming the expected 3-nonyn-1-al **(17)**, the acid also converted some of the alkyne to an allenic system **(18)** as evidenced by NMR. A doublet at δ 10.1 and the sextet at δ 5.85 were indicative of the presence of the allenic aldehyde.

In an effort to circumvent this problem and move along in the synthesis, we decided to react the triple bond with hydrogen and palladium on carbon to produce the cis isomer. Reaction of the alkyne diethyl acetal **(16)** with hydrogen in ethanol afforded the desired product. This compound, a colorless liquid, was also isolated by bulb-to-bulb distillation in 80% yield. At this point, it was necessary to attempt the introduction of the aldehyde functionality in the molecule. Therefore, on the basis of the results with the alkyne diethyl acetal, we attempted treating the alkene diethyl acetal with HCl in acetone. The NMR of the product (70% crude), an oil, indicated the presence of two compounds **(19 + 20)**. One of the products **(19)** composed approximately 70% of the mixture based on the relative integration of the aldehydic protons in the NMR. This product was indicated by a triplet at δ 9.85 due to two neighboring protons. The other product **(20)**, which composed the remaining 30% of the reaction mixture, resulted from migration of the double bond into conjugation with the carbonyl moiety. This isomer was indicated by a doublet in the NMR (δ 9.6) due to only one

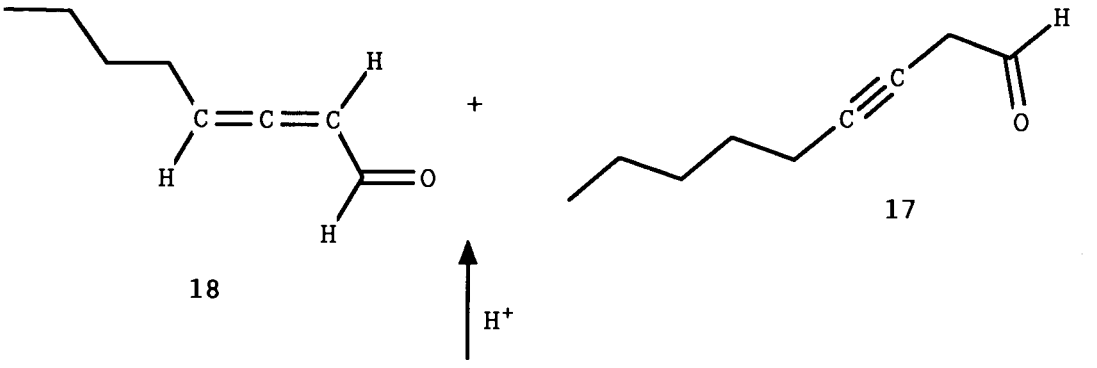
neighboring proton. We were unable to separate **19** from **20**.

It has been shown the boron tribromide (BBr_3) can cleave different types of ethers.[106-110] Therefore, we attempted the hydrolysis of **16** with BBr_3. The reaction was conducted overnight beginning at -78 °C and the reaction mixture was warmed slowly to room temperature as the reaction proceeded. Although the NMR of this compound looked promising, we were unable to purify the compound by chromatography (silica gel, alumina, or celite), bulb-to-bulb distillation, or vacuum transfer. We attempted reacting the crude product with the dianion of 4-PSBA. The results of this reaction were poor. Since we were unable to obtain a suitable aldehyde to react with the dianion, we decided to pursue alternate venues of research.

Scheme III



15 16

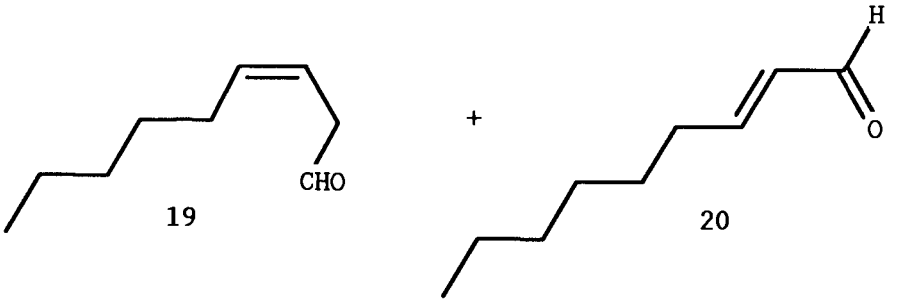


18 17

H^+

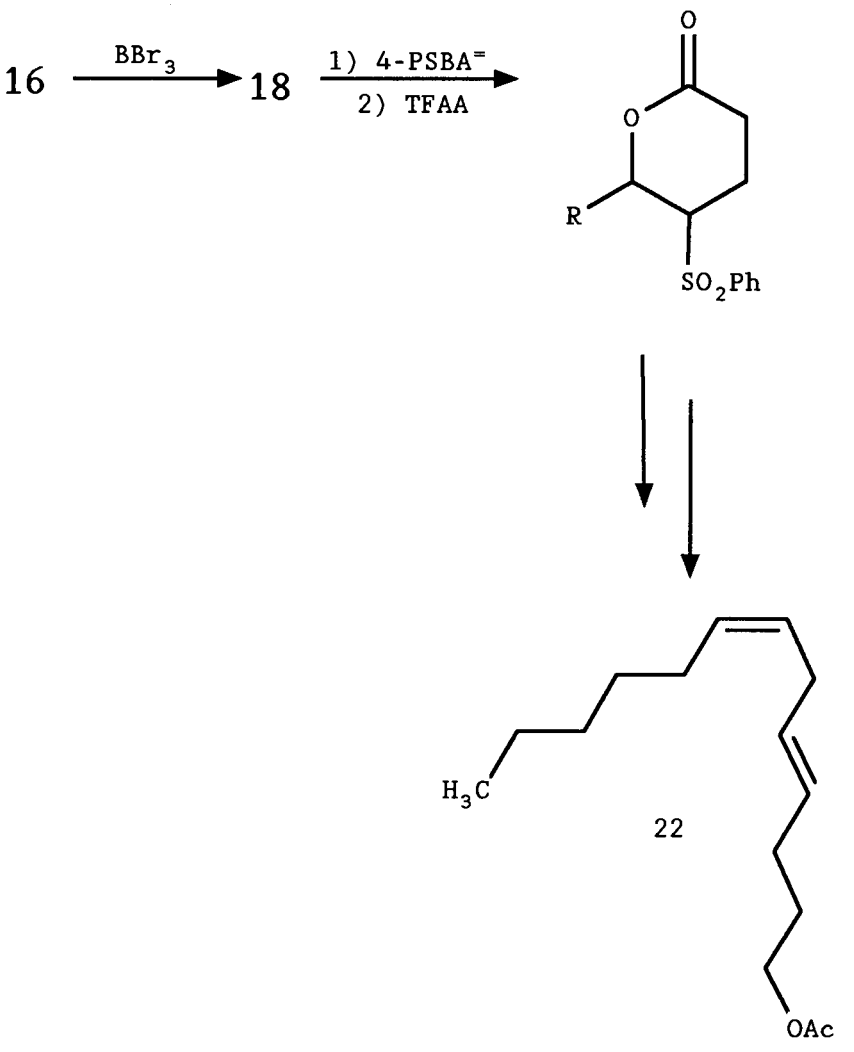
16

1) H_2
2) H^+



19 20

Scheme IV



CONCLUSIONS

4-(Phenylsulfonyl)butanoic acid **(1)** is a synthetically useful reagent that can be prepared in 60% overall yield from commercially available ethyl 4-bromobutyrate. Treatment of **1** with two equivalents of *n*-butyllithium results in conversion to dianion **7**. The dianion of 4-PSBA can add to a variety of aldehydes to yield 6-substituted-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-ones **(8a-f)** following *in situ* cyclization with TFAA. The stereochemical outcome of this reaction results in a 94:6 trans/cis ratio.

The tetrahydropyran-2-ones **(8a-f)** can be converted to methyl 4-butenoates **(10a-f)** upon treatment with 6% Na-Hg amalgam in methanol. Unfortunately, any possible stereochemical enhancement from the ring-locked precursors is lost. Modest E/Z ratios of 4:1 for most unsubstituted butenoates **(10a-e)** were observed with the notable exception of an E/Z ratio of 97:3 for the *t*-butyl case **(10f)**. This result is due to the fact that methoxide induced ring opening preceded elimination allowing the intermediate open chain compound to attain an equilibrium favoring the formation of the trans isomer. It is therefore apparent that regiocontrol of the double bond is unequivocal, but reasonable stereochemical control through

this chain extending process may only be possible for branched carbonyls.

The dianion of 4-PSBA has also been useful in the synthesis of sulcatole **(14)**, the aggregation pheromone of the ambrosid beetle, after condensation with acetone and further elaboration.

Through proper choice of substrates and/or reaction conditions, many other products may be formed through the use of 4-PSBA. For example, the addition of the dianion of 4-PSBA to activated imines results in the corresponding nitrogen heterocyle analogs[111] which may be further elaborated to alkaloids of biological importance.

EXPERIMENTAL SECTION

General Methods. Melting points were determined by using a Mel-Temp melting point apparatus and are uncorrected. Proton NMR spectra were taken in deuterated chloroform ($CDCl_3$) on either a Varian EM 360A or VXR-300-MHz instrument. Carbon NMR was conducted on the VXR-300 instrument and peaks are relative to the deuterated chloroform triplet (δ=77.06). Pertinent ^1H NMR data are tabulated in the following order: chemical shift (ppm in δ), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J in hertz), and number of hydrogens. Infrared data (IR; $CDCl_3$) were obtained on a Perkin-Elmer Model 1310 instrument. Salient IR features are tabulated in decreasing wavenumber (cm^{-1}). Gas chromatography analyses were done on a Hewlett-Packard Model 5890 fitted with a megabore capillary column no. 1251012 from J&W Scientific. Elemental analyses were performed by Micro-Tech Laboratories, Inc.; Skokie, IL. Mass spectral analyses were conducted by the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln.

Analytical thin-layer chromatography (TLC) was conducted with aluminum-backed silica plates (E. Merck) in diethyl ether or diethyl ether-petroleum ether mixtures.

visualization was affected with an ultraviolet lamp and/or anisaldehyde stain (a 2% solution of *o*-anisaldehyde in 95:4:1 absolute ethanol-concentrated sulfuric-glacial acetic acid) with heating and/or iodine. Flash chromatography[112] was conducted with diethyl ether-petroleum ether mixtures.

All solvents were distilled prior to use by literature methods.[113] 4-(Phenylsulfonyl)butanoic acid was recrystallized from chloroform-diethyl ether. Air- or water-sensitive reactions were conducted under a positive argon atmosphere utilizing standard techniques.[114] All carbonyl compounds were distilled from calcium hydride. Ethyl 4-bromobutyrate was prepared by the method of Lavety and Proctor[115] or purchased from Aldrich. Sodium benzenesulfinate and *n*-butyllithium (1.6 and 2.5 M) were purchased from Aldrich. Sodium-mercury amalgam (6%) was prepared by the modified method of Tischler in Fieser and Fieser (Vol. 1).[116] The material thus obtained was pulverized in a mortar and pestle to a fine powder and stored at 10 °C.

4-(Phenylsulfonyl)butanoic Acid (1). Sodium iodide (40 g, 266.6 mmol) was added to 150 mL of dry acetone and brought to gentle reflux, affecting solution. To this stirred solution was added ethyl 4-bromobutyrate (39 g, 200 mmol) dropwise over 1 h. Reflux was continued for 4 h or until no further precipitation was observed. The

mixture was cooled, diluted with an equal volume of diethyl ether, filtered, and rotary evaporated to an oil. The oil was taken up in 250 mL of diethyl ether and washed twice with 100 mL portions of 2% NaOH, water, and brine and dried over sodium sulfate, yielding 46.2 g (95.5%) of crude ethyl 4-iodobutyrate, R_f=0.8 (ethyl ether). If stored, this product must be protected from heat and light. This product was used directly in the next step without purification. ^{1}H NMR: δ 1.3 (t, J = 8, 3 H), 1.95-2.7 (m, 4 H), 3.3 (t, J = 6, 2 H), 4.15 (q, J= 8, 2 H).

Ethyl 4-iodobutyrate (17 g, 70 mmol) was dissolved in 100 mL of absolute ethanol. To this solution was added sodium benzenesulfinate (15.75 g, 96 mmol), and the heterogeneous mixture was refluxed for 6 h or until TLC indicated consumption of the starting material. The mixture was then taken up in 100 mL of water and extracted thrice with 100 mL portions of diethyl ether. The organic layers were combined and washed with 100 mL portions of 5% NaOH, water, and brine, dried over sodium sulfate, filtered and evaporated *in vacuo* to afford 16.24 g (90.6%) of an 8:1 mixture of phenylsulfonyl (**4**) (R_f=0.31, ethyl ether) and phenylsulfinate (**5**) (R_f=0.54; ethyl ether) butyrate esters. This mixture was used without further purification. Isolated (sulfone) ^{1}H NMR: δ 1.25 (t, J = 6.5, 3 H), 1.8-2.65 (m, 4 H), 3.25 (dd, J = 6 and 8, 2

H), 4.13 (q, J = 6.5, 2 H), 7.45-8.15 (m, 5 H).

The butyrate ester mixture (**4** + **5**) (23 g, 90 mmol) was dissolved in 20 mL of 95% ethanol. To this solution was added lithium hydroxide (6.0 g, 250 mmol) predissoved in 120 mL of water. The cloudy solution was stirred for 2 h while being monitored (TLC) for loss of starting ester. The mixture was, at completion, extracted twice with 100 mL portions of ethyl ether. The aqueous layer was acidified to pH = 4 with concentrated phosphoric acid and extracted thrice with 100 mL of diethyl ether. The organic layers were combined, washed with water and brine, dried, and the solvent was removed to afford 16.2 g of a crystalline mass. Alternatively, the ether solution of 4-PSBA may be allowed to stand uncovered, depositing needles. The solid was taken up in a minimum amount of chloroform, and an excess of diethyl ether added to the solution. Needles of 4-(phenylsulfonyl)butanoic acid formed within the hour. The product was filtered, washed with petroleum ether, and dried *in vacuo* to afford 14.38 g (70%), 60.5% overall, from ethyl 4-bromobutyrate, mp 94 °C. Large scale preparation (500 g) affords 56% overall yield. ^{1}H NMR: δ 1.97 (m, 2 H), 2.47 (t, J = 7.1, 2 H), 3.17 (t, J = 7.6, 2 H), 7.5-7.65 (m, 3 H), 7.86 (m, 2 H), 10.6 (s, 1 H). ^{13}C NMR: δ 18.06, 31.97, 54.97, 127.95, 129.39, 133.94, 138.59, 177.74.

General Procedure for the Preparation of Tetrahydropyran-2-ones (Lactones). 4-(Phenylsulfonyl) butanoic acid (0.969 g, 4.25 mmol) was dissolved in 75 mL of anhydrous THF and chilled to -78 °C. *n*-Butyllithium (2.5 M, 8.4 mmol, 3.36 mL) was added dropwise, and the mixture was stirred for 0.5 h. The carbonyl compound (4 mmol) was added either neat or in 3 mL of THF, the solution generally being quenched of color. The dry ice bath was removed, and the solution was stirred for 0.5 h. Trifluoroacetic anhydride (TFAA; 8 mmol, 1.68 g, 1.13 mL) was added to the reaction flask and the progress of the reaction was monitored by TLC (baseline UV activity before TFAA then migrated in 100% ethyl ether; see representative R_f values below), requiring 0.25-0.5 h to complete cyclization.

The solution was poured into 75 mL of saturated sodium carbonate and diluted with 50 mL of ethyl ether. The aqueous layer was washed twice with 50 mL portions of ethyl ether. The organic extracts were combined and washed succesively with 50 mL portions of saturated sodium carbonate and brine, dried over sodium sulfate, and rotary evaporated to a foam. The crude product(s) (yield 85-95%) was crystallized directly from chloroform-ethyl ether or flash chromatographed with ethyl ether-petroleum ether mixtures.

6-*n*-Propyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8a). Procedure yields 0.870 g (77%, mixture of isomers) after chromatography. Mp 101 °C. ^{1}H NMR: δ 0.86 and 0.93 (t, 3 H), 1.38-1.49 (m, 1 H), 1.50-1.91 (m, 3 H), 1.99-2.13 (m, 1 H), 2.29-2.58 (m, 2 H), 2.74 (m, 1 H), 3.56 (m, 1 H), 4.7p (bd, J = 10.8, 1 H), 7.57-7.91 (m, 5 H). IR ($CDCl_3$): 1730, 1215, 1081, 1040, 925 cm $^{-1}$. R_f = 0.6 (100 % Et_2O). Anal Calcd for $C_{14}H_{18}O_4S$: C, 59.55; H, 6.42. Found: C, 59.65; H, 6.46.

6-Crotyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8b). Procedure yields 0.841 g (75%, mixture of isomers) after chromatography. Mp 95-96 °C. ^{1}H NMR: δ 1.58 (d, J = 6.5, 3 H), 2.19-2.55 (m, 3 H), 2.73-2.83 (m, 1 H), 3.35 (q, J = 12.6 and 6.21, 1 H), 5.12 (t, J = 6.53, 1 H), 5.23-5.31 (m, 1 H), 5.70-5.78 (m, 1 H), 7.26-7.89 (m, 5 H). ^{13}C NMR δ 17.68, 18.72, 27.29, 61.13, 76.59, 126.86, 128.89, 129.46, 132.48, 134.40, 137.34, 169.65. IR ($CDCl_3$): 1745, 1309, 1215, 1150, 1085 cm^{-1}. R_f = 0.12 (100% Et_2O). Anal. Calcd for $C_{14}H_{16}O_4S$: C, 59.98; H, 5.75. Found: C, 59.92; H, 5.75.

6-Phenyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8c). Procedure yields 1.062 g (84%, mixture of isomers) after chromatography. Mp 114-115 °C. Trans isomer. ^{1}H NMR: δ 2.21-2.33 (m, 1 H), 2.44-2.65 (m, 2 H), 2.87-2.98 (m, 1 H), 3.73 (q, J = 12.7 and 6, 1 H), 5.77 (d, J = 6, 1 H), 7.11-7.74 (m, 10 H). ^{13}C NMR: δ

18.36, 27.21, 62.17, 78.07, 126.46, 128.50, 128.84, 129.05, 129.37, 134.16, 137.22, 169.60. R_f = 0.16 (100% Et_2O). Cis isomer. ^1H NMR: δ 3.96 (bq, J=10.0 and 3.78, 1 H), 5.95 (d, J=3.78, 1 H). ^{13}C NMR: δ 19.48, 27.62, 61.47, 78.38, 126.55, 128.09, 128.32, 128.62, 129.04, 129.38, 133.53, 134.46, 138.87, 168.73. R_f= 0.12 (100% Et_2O). IR ($CDCl_3$) 1743, 1450, 1380, 1310, 1218, 1148 cm^{-1}. Anal. Calcd for $C_{17}H_{16}O_4S$: C, 64,54; H, 5.10. Found: C, 64.20; H, 5.06.

6-Cinnamyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8d). Procedure yields 0.985 g (72%, mixture of isomers) after chromatography. Mp 128-129 °C. ^1H NMR: δ 2.21-2.44 (m, 2 H), 2.54 (m, 1 H), 2.81 (m, 1 H), 3.53 (q, J = 12.3 and 5.9, 1 H), 5.35 (t, J = 6.5, 1 H), 6.00 (dd, J = 6.3, 1 H), 6.57 (d, J = 15.7, 1 H), 7.19-7.88 (m, 10 H). ^{13}C NMR: δ 17.48, 27.72, 61.28, 122.31, 126.99, 128.68, 128.73, 128.76, 129.64, 134.56, 135.08, 135.42, 127.71, 168.74. IR ($CDCl_3$): 1770, 1465, 1325, 1265, 1170 cm^{-1}. R_f = 0.2 (100% Et_2O). Anal. Calcd for $C_{19}H_{18}O_4S$: C, 66.65; H, 5.30. Found: C, 66.77; H, 5.36.

6-*n*-Heptyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8e). Procedure yields 0.878 g (65%, mixture of isomers) after chromatography. Mp 100-102 °C. ^1H NMR: δ 0.88 (t, 3 H), 1.12-1.43 (m, 9 H), 1.58-1.64 (m, 2 H), 1.77-1.86 (m, 3 H), 2.00-2.17 (m, 2 H), 2.33-2.60 (m, 2 H), 2.71-2.80 (m, 1 H), 3.53-3.60 (m, 1 H), 4.7 (dt,

J = 10.7 and 4.7, 1 H), 7.59-7.75 (m, 5 H). ^{13}C NMR: δ 13.31, 17.50, 18.81, 21.80, 24.05, 25.08, 26.28, 26.44, 28.14, 28.20, 28.28, 31.00, 59.14, 77.02, 127.64, 128.82, 133.65, 137.45, 168.52. IR ($CDCl_3$): 1735, 1305, 1210, 1150, 1080, 1040 cm^{-1}. Anal. Calcd for $C_{18}H_{26}O_4S$: C, 63.74; H, 7.74. Found: C, 63.74; J, 7.72.

6-*tert*-Butyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (8f). Procedure yields 1.01 g (85%, mixture of isomers) after recrystallization. Mp 231 °C. ^1H NMR: δ 1.31 (s, 9 H), 1.99-2.17 (m, 2 H), 2.51-2.61 (m, 1 H), 2.74-2.86 (m, 1 H), 3.59-3.63 (m, 1 H), 4.30 (d, J = 2.55, 1 H), 7.57-7.92 (m, 5 H). ^{13}C NMR: δ 23.59, 25.73, 26.80, 35.08, 56.90, 88.07, 128.01, 129.58, 134.17, 139.09, 169.38. IR ($CDCl_3$): 1735, 1465, 1375, 1318, 1213, 1145 cm^{-1}. R_f = 0.08 (100% Et_2O). MS, *m/z* (M^+ - *tert*-C_4H_9) calcd 239.0383, obsd 239.0378.

General Procedure for the Preparation of Methyl 4-Butenoate Derivatives. The tetrahydropyran-2-one (1 mmol) was dissolved in 10 mL of anhydrous methanol and chilled to 0 °C, 1.5 g of finely crushed Na-Hg amalgam (6%) was added, after which the reaction was monitored by TLC for loss of starting material. The reaction mixture, at completion, was diluted with 15 mL of ethyl ether and filtered into a separatory funnel containing 30 mL of ethyl ether and 30 mL of water. The aqueous layer was extracted twice more with 25 mL portions of ethyl ether.

The organic layers were combined, washed with brine, dried over sodium sulfate, and carefully evaporated to afford the desired butenoate.

4-Octenoic Acid Methyl Ester (10a).[117] Procedure yields 0.125 g (80%, mixture of isomers). ^{1}H NMR: δ 0.90, (t, J = 7.1, 3 H), 1.37 (q, J = 7.3, 2 H), 1.90-2.06 (m, 2 H), 2.26-2.51 (m, 4 H), 3.66 (s, 3 H), 5.35-5.51 (m, 2 H). IR ($CDCl_3$): 1728, 1465, 1380, 1215 cm^{-1}.

4,6-Octadienoic Acid Methyl Ester (10b).[118] Procedure yields 0.131 g (85%, mixture of isomers). ^{1}H NMR: δ 1.72 and 1.77 (d, J = 6.59, 3 H), 2.30-2.58 (m, 4 H), 3.66 (s, 3 H), 5.47-5.73 (m, 2 H). IR ($CDCl_3$): 1730, 1439, 1380, 1215 cm^{-1}.

5-Phenyl-4-pentenoic Acid Methyl Ester (10c).[119] Procedure yields 0.133 (70%, mixture of isomers). ^{1}H NMR: δ 2.40-2.72 (m, 4 H), 3.67 and 3.69 (s, 3 H), 5.59-5.68 and 6.17-6.27 (m, 1 H), 6.42-650 (m, 1 H), 7.21-7.37 (m, 5 H). IR ($CDCl_3$): 1725, 1439, 1365, 1258, 1200 cm^{-1}.

7-Phenyl-4,6-heptadienoic Acid Methyl Ester (10d). Procedure yields 0.121 g (56%, mixture of isomers). ^{1}H NMR: δ 2.30-2.78 (m, 4 H), 3.68 and 3.69 (s, 3 H), 5.76-5.84 (m, 1 H), 6.19-6.50 (m, 1 H), 7.18-7.46 (m, 5 H). ^{13}C NMR: δ 23.51, 28.05, 33.77, 51.55, 123.90, 126.21, 127.29, 128.54, 130.00, 131.03, 131.63, 132.75, 173.29. IR ($CDCl_3$): 1725, 1438, 1365, 1258, 1200. MS, *m/z* (M^+) calcd 216.1150, obsd 216.1129.

4-Dodecenoic Acid Methyl Ester (10e).[120] Procedure yields 0.164 g (83%, mixture of isomers). ^{1}H NMR: δ 0.90 (t, J = 6.6, 3 H), 1.25 (s, 10 H), 1.92-2.16 (m, 4 H), 2.23-2.40 (m, 2 H), 3.66 (s, 3 H), 5.33-5.45 (m, 2 H). IR ($CDCl_3$): 1728, 1465, 1380, 1218 cm^{-1}.

6,6-Dimethyl-4-heptenoic Acid Methyl Ester (10f). Procedure yields 0.138 g (81%, mixture of isomers). ^{1}H NMR: δ 0.97 (s, 9 H), 2.23-2.50 (m, 4 H), 3.66 (s, 3 H), 5.29 (dt, J = 15.6 and 6.1, 1 H), 5.48 (d, J = 15.6, 1 H). ^{13}C NMR: δ 28.02, 29.64, 32.73, 34.38, 51.32, 122.49, 142.83, 173.61. IR ($CDCl_3$): 1730, 1465, 1380, 1215 cm^{-1}. Anal Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 70.65; H, 10.84.

6,6-Dimethyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (11). The general procedure for the preparation of tetrahydropyran-2-ones was followed. Procedure yields 901 mg (84%). Mp 138-139 °C. ^{1}H NMR: δ 1,69 (s, 3 H), 1.75 (s, 3 H), 1.84-1.91 (m, 1 H), 2.24-2.43 (m, 2H), 2.65-2.73 (m, 1 H), 3.29 (dd, J = 11.72 and 4.24, 1 H), 7.57-7.72 (m, 3 H), 7.88-7.91 (m, 2 H). I.R. ($CDCl_3$): 1730, 1465, 1449, 1380, 1310, 1287.

6,6-Dimethyl-2-hydroxy-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran (12). 6,6-Dimethyl-5-(phenylsulfonyl)-3,4,5,6-tetrahydropyran-2-one (536 mg, 2 mmol) was dissolved in 15 mL dry THF and chilled to -78 °C. Diisobutylaluminum hydride (1.5 M, 1.33 mL, 2 mmol) was

added after which the reaction was monitored (TLC) for loss of starting material (approx. 5 min.). The reaction was quenched with 1 mL 10% NaOH and diluted to 50 mL with diethyl ether. The reaction mixture was extracted with 25 mL 10% NaOH and brine, dried over sodium sulfate, and the solvent removed to yield product in greater than 95% yield. The product crystallizes from warm chloroform. ^{1}H NMR ($CDCl_3$): δ 1.15-1.29 (m, 1 H), 1.53 (s, 3 H), 1.62 (s, 3 H), 1.88-2.11 (m, 2 H), 2.99 (dd, J = 8.79 and 3.79, 1 H), 3.44 (bs, 1 H), 4.99 (dd, J = 7.57 and 2.19, 1 H), 7.52-7.66 (m, 1 H), 7.83-7.88 (m, 2 H).

2,6-dihydroxy-2-methyl-3-(phenylsulfonyl)-heptane (13). The lactol (572 mg, 2 mmol) was dissolved in 20 mL THF and chilled to 0 °C. Methyl magnesium bromide (3 M, 2 mL, 6 mmol) was added and the reaction monitored by TLC while warming. The reaction mixture was poured into 100 mL 1% HCl and diluted with 100 mL diethyl ether. The aqueous phase was extracted once more with diethyl ether. The combined ether extracts were back extracted with water, brine, dried with sodium sulfate, and the solvent was removed. The product was isolated after chromatography in 86% yield. ^{1}H NMR ($CDCl_3$): δ 0.94 (dd, J = 6.24 and 2.87, 3 H), 0.99-1.04 (m, 1 H), 1.22-1.29 (m, 1 H), 1.32 (s, 3 H), 1.49 (d, J = 2.49, 3 H), 1.68-2.03 (m, 3 H), 3.92 (q, J = 5.02, 1 H), 3.45-3.50 (m, 1 H), 4.17 (d, J = 6.94, 1 H), 7.51-7.89 (m, 5 H).

6-Methyl-5-hepten-3-ol (Sulcatole) (14). The general procedure for reduction of lactones was followed. The product was isolated in 75% yield (52% overall from acetone). ^{1}H NMR: δ 1.13 (d, J = 6.21, 3 H), 1.39 -1.48 (m, 2 H), 1.57 (s, 3H), 1.64 (s, 3 H), 1.81 (s, 1 H), 2.00-2.07 (m, 2 H), 3.76 (q, J=6.21, 1 H), 5.10 (dt, J=5.91 and 1.39, 1 H).

1,1-Diethoxy-3-nonyne (15). 1-Heptyne (5 g, 52 mmol) was dissolved in 100 mL anhydrous dimethoxy ethane (DME) and chilled to 0 °C. To this stirring solution was added *n*-butyllithium (2.5 M, 21 mL, 52.5 mmol). The resulting red-brown solution was allowed to stir for 0.5 h. Bromoacetaldehyde diethyl acetal (7.8 mL, 52 mmol) was added slowly to the reaction mixture. The ice-water bath was exchanged for a heating mantle and the mixture was brought to reflux. The mixture was allowed to reflux overnight after which the solvent was removed *in vacuo*. The resulting dark brown oil was brought up in 50 mL 1:1 diethyl ether/petroleum ether and extracted with 50 mL water. The aqueous phase was extracted twice more with the ethyl ether/petroleum ether mixture. The organic extracts were combined and back extracted with saturated sodium bisulfite, water, brine, dried with sodium sulfate and the solvent removed. The resulting brown liquid was purified by bulb-to-bulb distillation. 5.69 g (52%) pure product was isolated. ^{1}H NMR: δ 0.85 (t, 3 H), 1.19 (t,

6 H), 1.25-1.35 (m, 4 H), 1.42-1.47 (m, 2 H), 2.08-2.15 (m, 2 H), 2.44-2.47 (m, 2 H), 3.47-3.68 (m, 4 H), 4.58 (t, 1 H).

cis-3-Nonenal (19). cis-1,1-diethoxy-3-nonene (216 mg, 1 mmol) was dissolved in 5 mL acetone. 2 drops of 10% HCl was added and the reaction was allowed to proceed at 40 °C while stirring and monitoring (TLC) for loss of starting material. At completion, the reaction mixture was poured into 25 mL saturated sodium bicarbonate and 25 mL diethyl ether and extracted. The organic layer was extracted with 50 mL portions of water and brine, dried over sodium sulfate and rotary evaporated to afford product in 70% yield as a mixture of isomers (**5** + **6**).

3-Nonynal (17). 1,1-diethoxy-3-nonyne (500 mg, 2.4 mmol) was dissolved in 30 mL of methylene chloride and chilled to -78 °C. In a separate flask, 5 mL of BBr_3 solution (1 M, 5 mmol) was added to 20 mL of methylene chloride and prechilled to -78 °C. The solution of starting material was added slowly to the BBr_3 solution. The reaction was allowed to warm to room temperature and proceeded overnight. TLC of the reaction mixture indicated that the starting material had been consumed. The reaction mixture was diluted with 25 mL water and allowed to stir for approximately 1 h. The entire mixture was poured into a separatory funnel and separated. The aqeuous layer was extracted once more with methylene

chloride after which the combined organic layers were extracted with brine, dried over sodium sulfate, rotary evaporated to an oil, pumped and isolated in quantitative yield.

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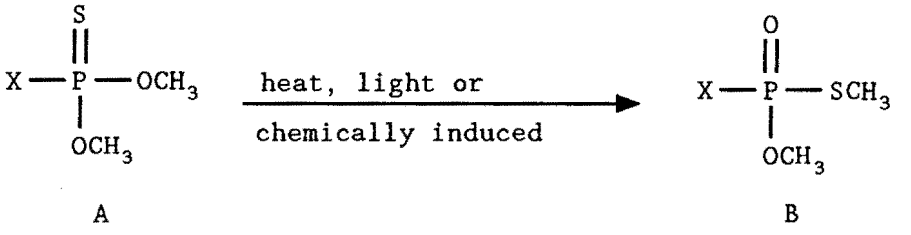
CHAPTER II

STUDIES OF ORGANOPHOSPHORUS COMPOUNDS DERIVED FROM SERINE

INTRODUCTION

1. Organophosphorus Insecticides

With increasing reluctance to make use of chlorinated pesticides, organophosphorus (OP) insecticides have become popularized as safe compounds for the control of agricultural pests. Many commercial pesticides share the dialkyl phosphorothioate formula (A, Fig. 4), which contains a phosphorus-sulfur pi bond, two equivalent alkoxy ligands (usually methoxy or ethoxy) and generally a leaving group (X).



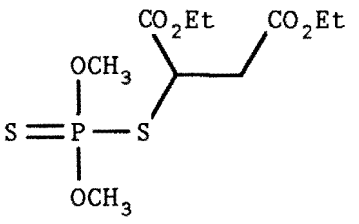
A B

Figure 4. Isomerization of Dialkylphosphorothiotes

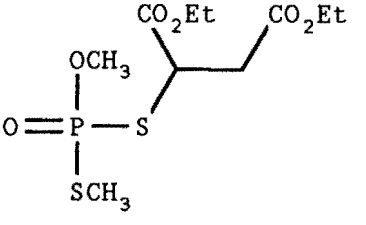
Although the dialkyl phosphorothioates are thought to be generally safe, impurities which may be present in the commercial formulations and may pose a public health

hazard. Although the exact origin of the impurities is unknown, there are ample opportunities for them to form during preparation (i.e. manufacture), storage and environmental exposure.

The thermal "isomerization" or conversion of alkoxy-thiophosphoryl (A) to alkylthio-phosphoryl (B) linkage is one such example of an impurity forming reaction (Fig. 4). This particular rearrangement may occur thermally[1-3], photochemically[1,4], or may be induced chemically[5]. This chemical alteration was brought to public attention when 7500 Pakistani spraymen were exposed to 50% malathion; over 2800 became poisoned and 5 died.[6] The cause of the poisoning was directly related to the corresponding *S*-methyl isomer, isomalathion.[7,8]



malathion



isomalathion

2. Acetylcholinesterase: Its Role in Neurotransmission and Inhibition by Organophosphorus Compounds.

In warm blooded mammals, acetylcholine (ACh) is a neurotransmitter that functions in the central as well as the peripheral nervous system. When a nerve impulse (action potential) arrives at the end of a neuron, serveral molecules of acetylcholine are released and diffuse across the synapse to another neuron which allows

the nerve impulse to propagate. Uncontrolled, this compound can result in a multitude of processes (caused by repetitive firing of the neural signal) including clinical symptoms of respiratory failure and ultimately, death. The agent responsible for the control of ACh concentration in the synaptic cleft is acetylcholinesterase (AChE)[9], an enzyme that is able to hydrolyze ACh to choline and acetic acid (Figure 5). The enzyme binds to ACh before it attaches to the neuron, thus keeping the number of ACh molecules that reach the neuron in control.

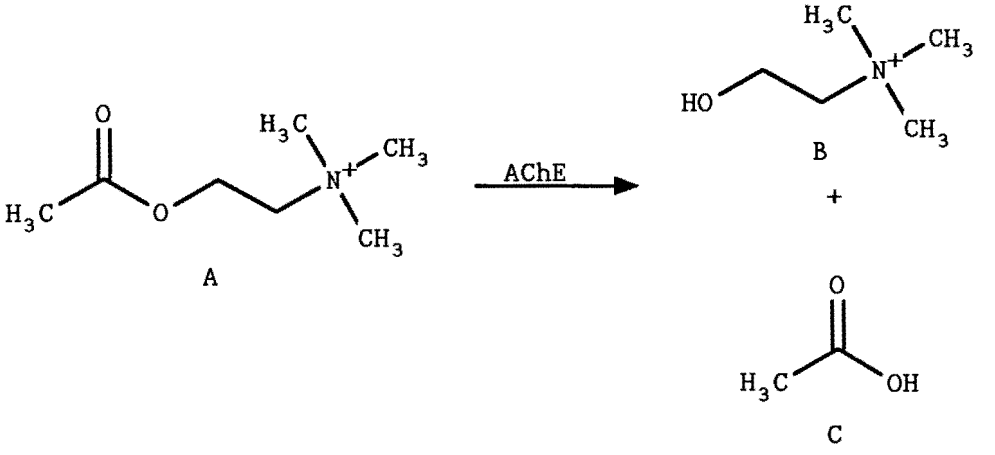


Figure 5. Hydrolysis of Acetylcholine (A) into Choline (B) and Acetic Acid (C).

Five hundred seventy five amino acids comprise the polypeptide chain of AChE with a calculated molecular weight of 65,612. The portion of the enzyme primarily resposible for its catalytic activity is referred to as the active site. For mechanistic interpretation, the active site may be subdivided into three regions: the

esteratic site, the anionic site, and a hydrophobic region (Figure 6).

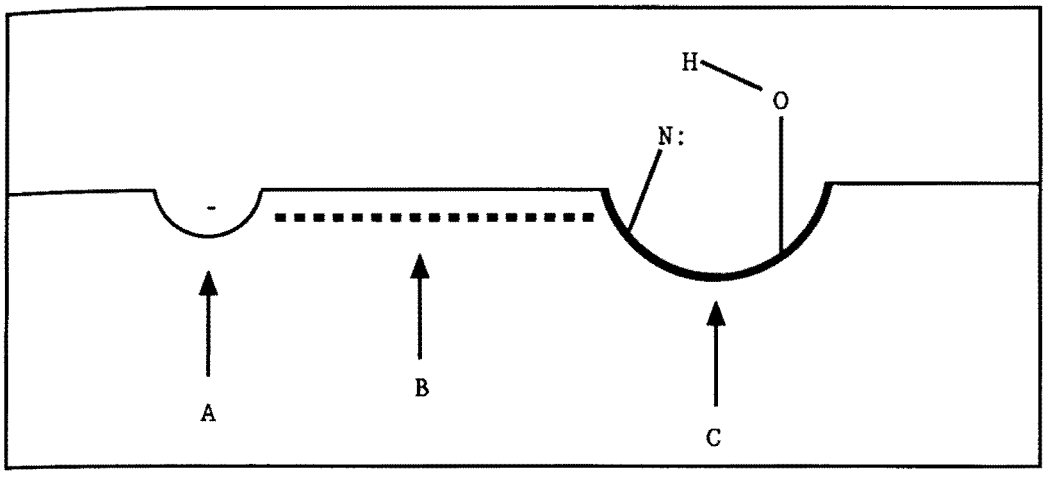


Figure 6. A Schematic of the Active Site of AChE showing the anionc site (A), the hydrophobic region (B), and the esteratic site (C) which contains the serine hydroxyl and an imidazole nitrogen on a histidine residue.

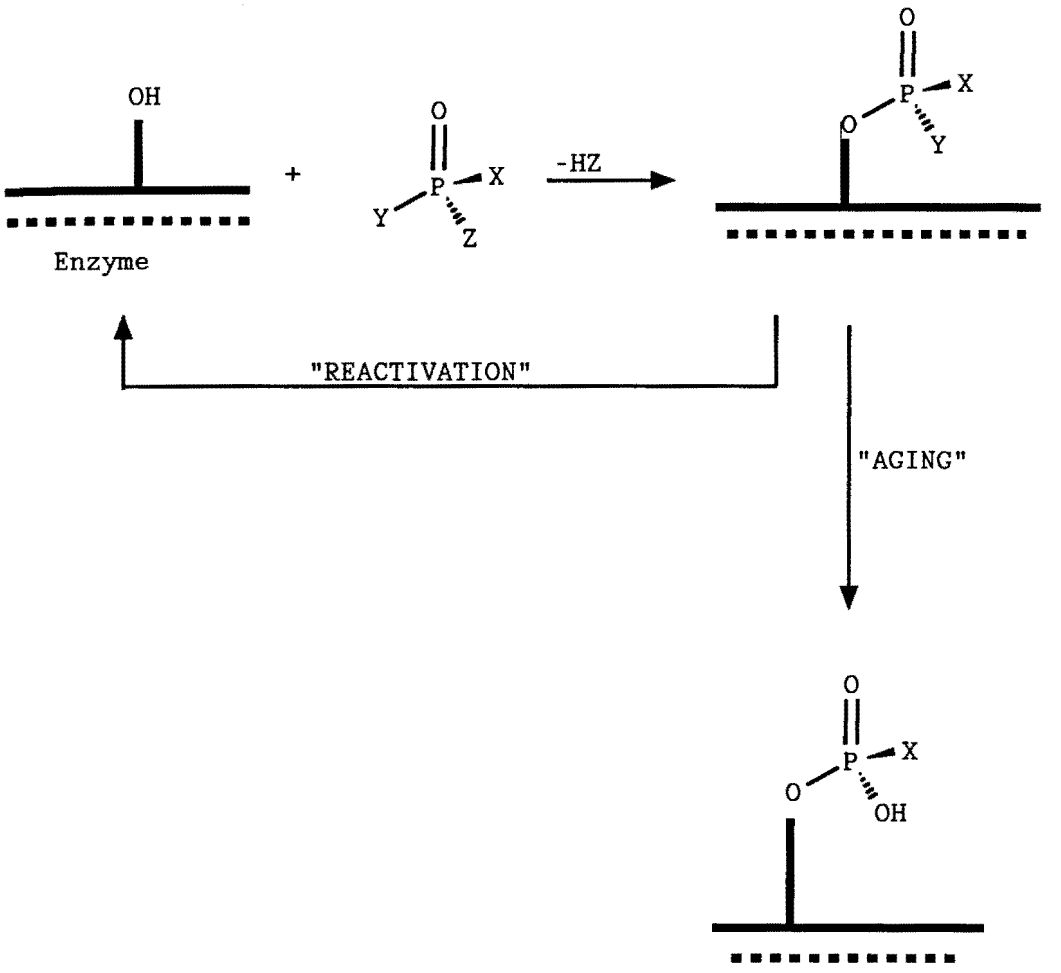
The esteratic site is the portion of the enzyme primarily responsible for its ability to hydrolyze ACh. The major feature of this region is that it contains the amino acid serine. The serine hydroxyl is responsible for nucleophilic attack of the carbonyl carbon of ACh and thus, is the amino acid that is ultimately responsible for the hydrolysis of ACh. A histidine residue, also contained in the esteratic site, plays an important role (by acting as a general acid-base catalyst) in the formation and cleavage of tetrahedral intermediates during ACh hydrolysis. The anionic locus plays a role in binding the quaternary ammonium moiety of ACh. Finally, the

hydrophobic region lies between these two sites of the molecule. These structural features, particularly the nucleophilic nature of the serine hydroxyl, have been used in the design of insecticides.

When AChE comes into contact with an OP compound (Scheme V), several mechanistic stages must be considered. Initial nucleophilic attack at phosphorus by the serine hydroxyl results in the formation of a reversible Michaelis enzyme-inhibitor complex. Once the complex is formed, it may proceed to the stage of a phosphorylated enzyme. After phosphorylation has occurred, three possible fates exist. First, the compound is capable of undergoing reactivation (either spontaneously or induced by chemical means). Secondly, the compound can experience a phenomenon known as aging. Third, the enzyme may remain phosphorylated indefinitely.

Reactivation occurs when the serine hydroxyl-phosphorus bond is cleaved and the active enzyme is regenerated. If reactivation does not occur, the enzyme may remain phosphorylated for extended periods of time with no other chemical transformation taking place. Finally, the complex may undergo replacement of another group (for example the Y group, Scheme V) which is referred to as aging. Aging (first reported in 1955), results in a phosphorylated enzyme that is unable to undergo reactivation.[10-12] Berends et al.[13] were the

Scheme V



first to examine the aging process in detail. Their study, using diisopropyl phosphorofluoridate, pointed toward the loss of isopropanol from the phosphorylated enzyme as the chemical event surrounding the aging process. Studies with the compound Soman have also led to similar conclusions (i.e. dealkylation).[14,15] Dealkylation is characterized by loss of an alkyl group from the *O*-alkylor *S*-alkyl phosphorus ligand. The nature of the dealkylation products characterized suggest that

dealkylation occurs primarily via a carbocation intermediate.[15] These results suggest that during the aging process, at least for the acid-catalyzed process, the oxygen-phosphorus bond remains intact. Research done later in this area demonstrated that, in some cases, the phosphorus-oxygen bond breaks.[16] It is evident that the study of aging is not complete and many questions remain unanswered.

3. Stereochemical Aspects of Cholinesterase Inhibition.

It is important to note that the "isomerization" of *O,O*-dialkylphosphorothioate to *O,S*-dialkylphosphorothioate compounds results in the formation of a chiral center at phosphorus. Thus, an enantiomeric pair of compounds is formed which may have different inhibitory potency and/or mechanisms of intoxication.[17]

Some studies have been done to examine the stereoselectivity of the phosphorylation ac AChE.[18-21] Aaron et al. were first to report a study involving optically resolved OP compounds. Their study took advantage of the resolution of an organophosphonothioic acid to synthesize the optical isomers of *O*-ethyl-*S*-(2-ethylthioethyl) ethylphosphonothiolate. It was found that the *levo*-isomer was more reactive than the *dextro*-isomer in the inhibition of AChE. In another study, it was shown that the $(R)_p(+)$ isomer of 2,5-dichlorophenyl methyl phenylphosphonate was more toxic than the $(S)_p(+)$ isomer.[20] This study took

advantage of the ethyl ester of *l*-proline (a derivative of the naturally occurring amino acid) to resolve the isomeric compounds. A recent study has shown that nerve gases (e.g. Sarin) also exhibit stereoselectivity in the phosphorylation of AChE.[21] The results obtained in this study indicated that compounds with a (-) configuration at phosphorus are better inhibitors than the corresponding (+) isomer. Absolute configuration studies done on one of the compounds in this study indicate that the (+)-isomer of Sarin has the R configuration. By comparing the data of the previous two studies, one can conclude that a general rule relating configuration at phosphorus to toxicity cannot be established.

Since AChE itself is asymmetric it should also be instructive to examine OP compounds that are not only chiral at phosphorus, but also have an asymmetric site elsewhere in the molecule. Perhaps compounds of this type would provide greater understanding of the active site of AChE. A few of these studies have been done using Soman[21] (i.e. *O*-2-butyl-*S*-2-(ethylthio) ethyl ethylphosphonothioate) and related analogs[19]. Results of these studies indicate that phosphorylation of AChE is much more dependent upon the configuration at phosphorus than on another asymmetric center in the molecule. For a variety of AChE preparations, general inhibition trends were found to be consistent, indicating that the stereochemical

interactions of individual OP compounds with AChE remain generally the same regardless of the enzyme source.[19]

4. Purpose.

Despite the evidence that isomalathion was the cause of the severe poisoning experienced by the Pakistani spraymen[7,8], there have been few systematic studies[1,3] directed toward examining the "isomerization" products of phosphorothioates and related materials. Therefore, we initially sought to briefly investigate, characterize, and determine the relative AChE inhibitory potency of *O,O*-dimethylphosphorothioate isomerides.

It would also be beneficial to be able to prepare phosphorylated amino acid sequences of known phosphorus chirality to study how the individual isomers compare to phosphorylated AChE and other phosphorylated peptide fragments. Therefore, we sought to prepare and characterize the stereochemistry of a series of 1,3,2-oxazaphospholidin-2-ones (OAP's) from a suitable substituted serine derivative. Because serine is the amino acid that becomes phosphorylated during AChE poisoning, these compounds could serve as potential intermediates of chirally phosphorylated serine which could then be used to study the stereochemical implication to OP poisoning and toxicity.

Finally, utilizing methodology developed for OAP synthesis, we sought to prepare an O-(*O,S*-dimethyl

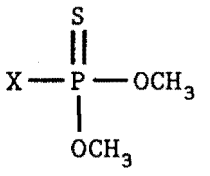
phosphoro)-serine derivative as a potential intermediate in the synthesis of a chirally phosphorylated tripeptide that would resemble a portion of the active site of AChE following phosphorylation by an *O,S*-dimethyl phosphorothioate. The peptide chosen for this study consists of glutamic acid, serine, and alanine (Glu-Ser-Ala), the three amino acids common to the active site sequence of a variety of cholinesterases, particularly that found in human serum[9]. Such a phosphorylated peptide may allow for model studies to help unravel the dynamic molecular events following OP poisoning (i.e. aging and/or reactivation).

RESULTS AND DISCUSSION

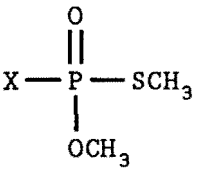
1. Studies on *O,O*-Dimethyl Phosphorothioate Isomerides.

Five *O,O*-dimethyl phosphorothioates **23a-e** and their respective isomerides **24a-e** (Figure 7) were characterized by ^{31}P NMR spectroscopy and examined for their biochemical interaction with AChE. These five compounds were chosen to represent thioate (a-c) and dithioate (d,e) classes of the symmetrical dialkyl phosphorothioate type. As mentioned above, the unwanted conversion of commercial material to *O,S*-dimethyl phosphorothioates is of major concern since enhanced and, perhaps, different mechanisms of toxicity may be elicited by these compounds.

^{31}P NMR has proven useful as an analytical tool for differentiating isomerides from parent material.[22] In all cases examined, the isomerides were shifted approximately 40 ppm upfield from the corresponding parent compound (Table III). This finding was consistent with previous findings.[23] Due to the sensitivity of the isomerides to acids and bases, we chose to employ an external standard (H_3PO_4 in deuterated chloroform) in our experiments. The large difference in chemical shift of the impurity may be useful in the rapid detection of isomeric impurities present in technical material.

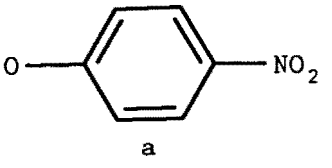


23a-e

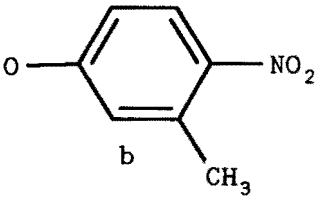


24a-e

X



a



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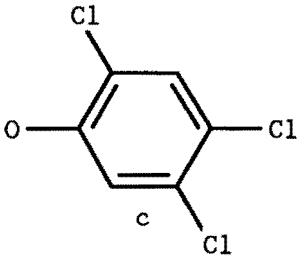
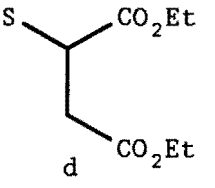
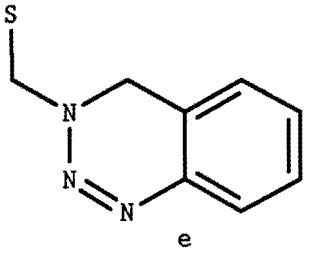


c



d



e

Figure 7. Some Common Insecticides and Their Isomerides.

The parent compounds and isomerides were also examined as inhibitors of AChE. The bimolecular inhibition constants (k_i) for the isomerides and some of

the parent materials are tabulated in Table III. All isomerides displayed pronounced inhibitory potency (10^4-10^5 $M^{-1}min^{-1}$) against rat brain acetylcholinesterase. We were unable to accurately determine k_i values for some of the parent compounds due to poor solubility at the high concentrations needed to induce inhibition. Based on the k_i value obtained for azinphos, these values are probably less than 10 $M^{-1}min^{-1}$. The results of this study indicate that the isomerides are approximately 1000-times more potent as AChE inhibitors than the parent insecticide.

Table III. Phosphorus NMR Data and Bimolecular Inhibition Constants (k_i)

no.	compound	$\delta^{31}P$	k_i[a,b]
23a	methyl parathion	66.0	0.691(±4.3)
24a	s-methyl methyl parathion	27.76	714
23b	fenitrothion	65.84	<0.1
24b	s-methyl fenitrothion	27.47	698(±5.8)
23c	fenchlorophos	66.67	<0.1
24c	s-methyl fenchlorophos	28.12	80.9(±1.2)
23d	malathion	95.94	<0.1
24d	s-methyl malathion	58.4/57.0[c]	325(±7.7)
25a	azinphos	96.23	0.108(±11.0)
25b	s-methyl azinphos	57.45	1063(±3.6)

a: Calculated by plotting the slope of inhibition (varied concentrations of inhibitor) versus time of inhibition.
b: k_i X $10^{-2}M^{-1}min^{-1}$ Error values in percent c: Diastereomers

The increased potency of the isomerides toward inhibition of AChE may be, at least partially, explained by considering the phosphorylating species. First, conversion of the P=S bond to a P=O bond results in enhanced electrophilicity at phosphorus and, hence,

increased reactivity toward nucleophiles.[24] Second, the alkylthio group is a somewhat better leaving group than the corresponding methoxy.[25] Third, the alkylthio group is susceptible to oxidation[26,27], which may result in the formation of an even more labile leaving group (the sulfoxide). Lastly, alkylthio phosphorothioate compounds may be prone to aging.[28,29]

2. Synthesis of 1,3,2-Oxazaphospholidin-2-ones.

The first portion of the synthesis of these compounds (Scheme VI) centered on the preparation of a derivative of serine that was mono-protected at nitrogen and will also allow for nucleophilic attack by the nitrogen which is necessary for the preparation of the phosphoramide (P-N) bond. With this in mind, we decided that we needed to prepare an N-alkyl serine derivative because the nucleophilicity is greater for amines than for amides or carbamates, which are the traditional nitrogen protecting groups. The preparation of mono-N-alkylated amino acids is somewhat difficult and few known methods are available.[30,31] Most methods rely on protection of nitrogen followed by treatment with base and an alkyl halide. These methods usually rely on protection of the nitrogen as an amide or a carbamate. We briefly investigated alkylation of *L*-N-(phenylsulfonyl) serine, methyl ester[32-34] with methyl iodide. This reaction was not successful. Therefore, we turned our attention to

direct conversion an an amino acid ester into an N-alkyl amino acid ester.

Shaw et al.[35] have reported the preparation of methyl-*D*-N-benzyl threonate from the corresponding amino ester hydrochloride. Application of their procedure to *S*-serine methyl ester hydrochloride **(25)** gave some of the desired product, but in extremely low yield (10%). It was found that conducting the reductive amination under basic conditions[36] (Scheme VI) rather than acidic condition resulted in a much better recovery of the desired methyl-*S*-N-benzyl serinoate **(26)** (66-70%). In a typical procedure, the amine hydrochloride is dissolved in methanol followed by addition of TEA. After stirring for a short time, benzaldehyde is added resulting in the formation of an imine which is reduced by the addition of sodium borohydride. Increased reaction times before the addition of sodium borohydride resulted in modest increases in yield, but not great enough to make longer reaction times necessary. The modified procedure had further benefits other than the vastly improved yield. First of all, the reaction conditions are much milder. Second, the reagents ($NaBH_4$ vs. $NaCNBH_3$) being far less expensive and less toxic. Third, the modified procedure had a simpler workup (extraction) which generally afforded a product which did not require chromatography for further purification. When desired, the product may be purified

by bulb-to-bulb distillation.

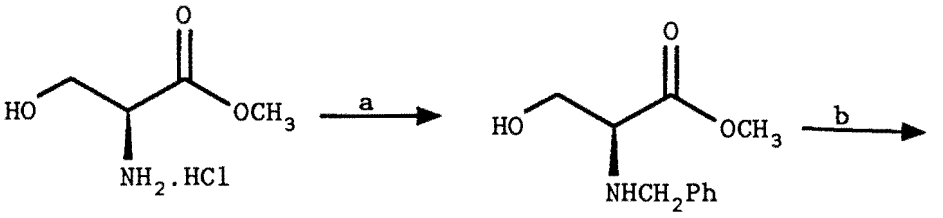
With the amino acid derivative in hand, we were ready to turn our attention toward the synthesis of our target 1,3,2-oxazaphospholidin-2-ones **(28-31)** (Scheme VI). Similar compounds have been prepared by the reaction of a phosphoryl dihalide with a bifunctional chiral auxiliary (e.g. an aminoalcohol) to afford cyclic diastereomers capable of undergoing a series of specific displacement reactions.[37,38] Specifically, Inch and co-workers have extended this methodology to a wide variety of substrates utilizing ephedrine and pseudoephedrine[39-42], as well as sugars[43] and amino sugars[44,45]. We wanted to extend the method of Inch and co-workers to the synthesis of similar derivatives of serine for the obvious biological implications mentioned previously.

Reaction of methyl-*S*-N-benzyl serinoate with phosphorus oxychloride ($POCl_3$) provided the diastereomeric 2-chloro-1,3,2-oxazaphospholidin-2-ones **(27a+b)** in 94% yield. The chloridates were isolated, but found to be quite unstable, decomposing in about 2 days at room temperature. Refrigeration of these intermediates extends the lifetime to about a week. Crude isolation and reaction of the diastereomeric mixture of chloridates with the appropriate alcohol or phenol affords **28-31** (68-96% yield, Table IV) presumably via retention of configuration[37,38,46] although no attempt was made to

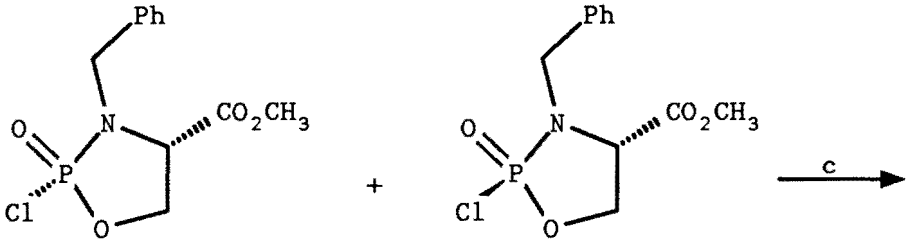
characterize the specific transformation at this time. The alcohols and phenols were chosen to form material that represent a variety of insecticide-based leaving groups. A number of variations were attempted to optimize the overall yield of OAP's **28-31** from **26**. Implementation of a one-pot, two-step synthesis in toluene (TEA, $POCl_3$ followed by the addition of the alcohol of phenol) decreased the yield considerably. Separation of the chloridate stereoisomers and subsequent esterification also led to significant decreases in overall yield primarily due to the loss of chloridate upon purification. The esterification conditions were also examined and it was found that the reaction proceeded in either aromatic solvents with organic base or acetone with inorganic base. No appreciable difference in yield was noted with either method. Although the elevated temperatures required for the acetone-sodium carbonate esterification were of concern for fear of loss of stereochemical integrity no racemization was noted.

The ester OAP's were all isolated in optically active form in a near 1:1 diastereomeric ratio and the purity was confirmed by high performance liquid chromatography and spectral analyses (Table IV). The 1:1 diastereomeric ratio is notable in light of many related studies reporting a clear preference for one of the cyclic isomers.[37-45,47] In fact, a diastereomeric excess of 12:1

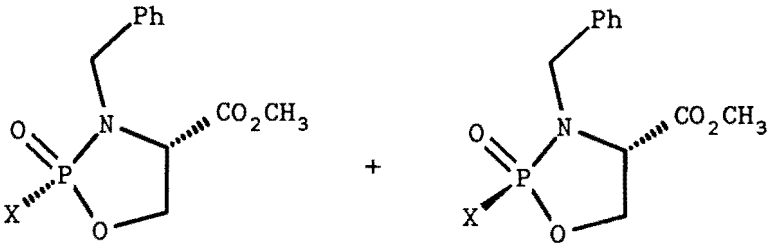
Scheme VI



25 26



27a (cis) + 27b (trans)



28a 28b X = CH_3O
29a 29b X = CH_3CH_2O
30a 30b X = PhO
31a 31b X = p-NO_2PhO

(a) PhCHO, Et_3N, $NaBH_4$; (b) $POCl_3$, 200 mol % Et_3N, toluene, (c) ROH, base, solvent.

was used to advantage in a chiral cyclophosphamide synthesis.[48,49] Perhaps the serine moiety, which contains only one chiral center, is more conformationally

Table IV. 1,3,2-Oxazaphospholidin-2-ones Physical Data

compd.	mp (°C)	$[\alpha]^{23}_{d}$ [a]	^{31}P(ppm)	yield (%)
27a	b	b	23.92	94
27b	b	b	23.68	
28a	77-78	-26.0(1.75)	22.19	68[c]
28b	64-66	-90.2(0.62)	21.14	
29a	oil	-23.9(2.31)	20.83	87[c]
29b	oil	-56.1(1.09)	19.89	
30a	92-94	-29.3(0.96)	16.38	96[c]
30b	83-85	-70.2(1.12)	15.01	
31a	91-92	-4.5(1.18)	15.85	78[c]
31b	101-102	-27.5(0.94)	14.91	

a: (g/100 mL $CHCl_3$) b:Not determined due to instability
c: yield from 27a/27b

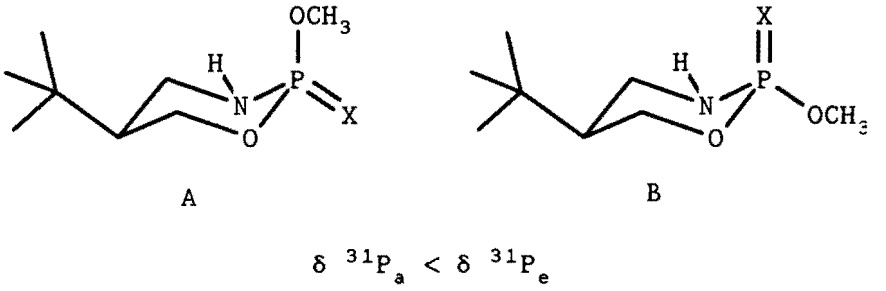
flexible thus showing less stereochemical preference.

3. Stereochemistry of the 1,3,2-Oxazaphospholidin-2-ones.

Nuclear magnetic resonance (NMR) has been used to determine stereochemistry of similar cyclic molecules. For example, Cooper et al.[50] made use of proton NMR to aid in the assignment of the absolute configuration of some 1,3,2-oxazaphospholanes. Recently, Setzer[51] developed an elegant proton NMR analysis (coupling constants) of conformational differences among related ephedrine and pseudoephedrine OAP's. However, the coupling patterns in our spectra are somewhat more complex and therefore more difficult to interpret. Most of the problem lies in overlapping coupling patterns. We used the shift reagent tris-[3-[(heptafluoropropyl) hydroxymethylene]-d-camphorato] europium (III)[52] in an effort to separate and

allow determination of coupling constants. However, the shift reagent, even at varied concentrations, only served to broaden the peaks rather than resolve them; perhaps due to complexation of the reagent with the less encumbered carboxy ester moiety.

A more common method employed to address the question of stereochemistry in phosphorus heterocycles is ^{31}P NMR.[53-63] Bentrude et al.[64] have studied a variety of molecules related to cyclophosphamide by ^{31}P NMR spectroscopy. He took advantage of a *tert*-butyl group to lock the conformation of the six membered ring (Figure 8). They found that when an exocyclic group ($-OCH_3$) resides in the axial position (A), the chemical shift is less that that in the equatorial position (B).



$$\delta\ ^{31}P_a < \delta\ ^{31}P_e$$

Figure 8. Axial and Equatorial Exocyclic Ligands on 1,3,2-Oxazaphosphorinanes.

Perhaps it is more instructive to note the relationship between the exocyclic ligand and the substituent on the six-membered ring. In structure A, these two have a cis relationship while in structure B

they are trans with respect to the *t*-butyl group. Therefore, a generally applicable correlation may be that when an exocyclic group on phosphorus is cis to a ring substituent, the phosphorus chemical shift is less than when the groups are trans. Similar trends have been noted in many 2-oxo- and 2-thioxo-1,3,2,-oxazaphosphorinane systems[65-67] as well as with 1,3,2-oxaza- and -thiazaphospholidines[68].

Although five membered rings are necessarily less defined conformationally, bias can be introduced by substitution at a ring carbon and perhaps by introduction of the N-benzyl moiety (e.g. **27-31**). Perhaps this method may be used to preliminarily assign the relative stereochemistry in our OAP's. Figure 9 shows the possible conformation of both the cis and trans isomers of our compounds. The phosphorus chemical shifts of the slow isomers are consistently 1 ppm upfield from those of the fast band isomer ("slow" and "fast" refer to respective elution during flash chromatography). This relationship is opposite that which is observed for the six-membered ring case. Based upon Bentrude's work[64] and conformational effects, we initially assigned the configurations as follows: the slow eluting isomer corresponded to the exocyclic alkoxy group trans to the carboxy ester while the fast isomer then corresponded to the cis isomer.

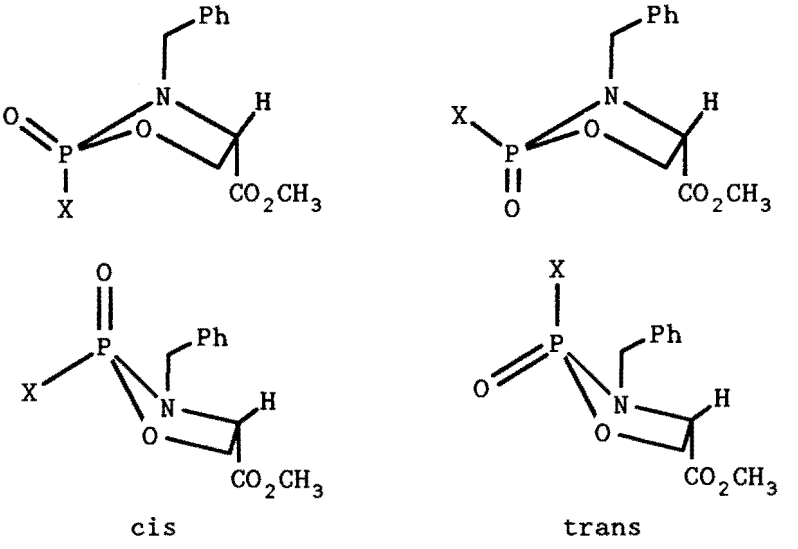


Figure 9. Possible Conformations for Cis and Trans OAP's

Another method that has been used to establish stereochemical relationships in these system is optical activity (i.e. rotational correlation). Cooper[50] has found that in a series of 1,3,2-oxazaphospholanes the "axial" isomers have a larger absolute rotation. The rotations of derivative **27-31** are tabulated in Table IV. In all cases the slow (trans) isomer exhibited a much larger rotation that the corresponding fast (cis) band. Thus, in consideration of our other stereochemical evidence, the larger rotating isomer correlated with the trans orientation of the exocyclic phosphorus ligand and the carboxy ester moiety.

The ultimate determination of stereochemistry derives from the X-ray crystal structure of isomer **27b**. This structure reveals a trans relationship between the

methoxy ligand and the carboxy ester in the slow eluting isomer. Therefore, the fast eluting isomer must have a cis relationship.

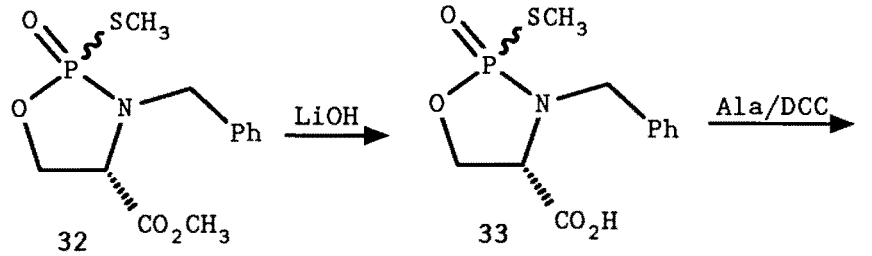
In an effort to determine that all the slow band isomers had the same relative configuration, we attempted a chemical correlation study. As mentioned, it is known that displacement of exocyclic phosphorus ligands occurs with retention of configuration.[37,38,46] Therefore, we treated the *p*-nitrophenoxy slow (trans) isomer **31b** with catalytic sodium methoxide in methanol with the expectation that the slow (trans) isomer of methoxy OAP **27b** would form. Monitoring the reaction by TLC and HPLC determined that **27b** formed exclusively from **31b**. Therefore, we can conclude that the elution properties correspond to configurationally correlated structures.

4. Studies Directed Toward the Synthesis of a Phosphorylated Tripeptide Mimic of Acetylcholinesterase.

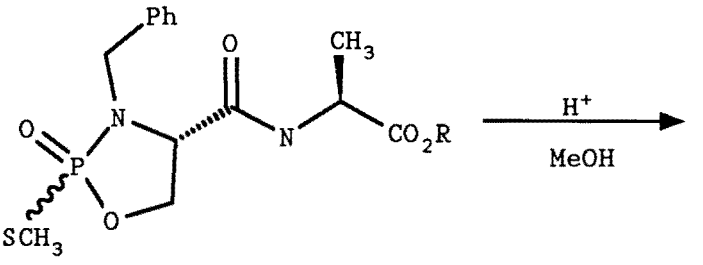
Desiring to make use of the methodology obtained in the preparation of 1,3,2-oxazaphospholidin-2-ones, our initial strategy (Scheme VII) made use of the intermediate 2-thiomethyl OAP **(32)**.

Methyl-(2*S*,4*S*)- and (2*R*,4*S*)-2-thiomethyl-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate **(32)** was prepared by reaction of N-benzyl-*L*-serine, methyl ester and thiomethyl phosphoryl dichloride[69] in 70% yield

Scheme VII



32 33



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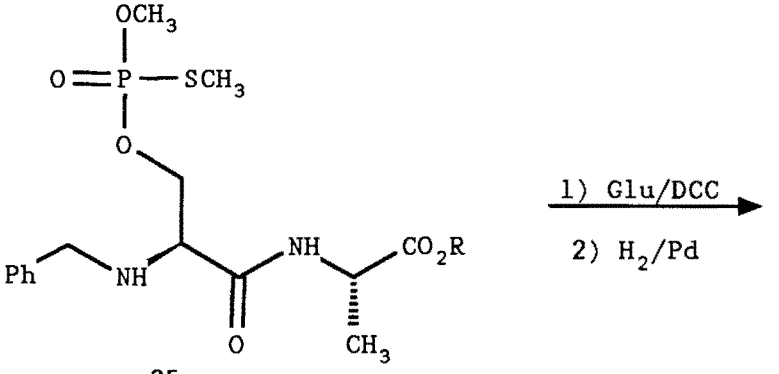

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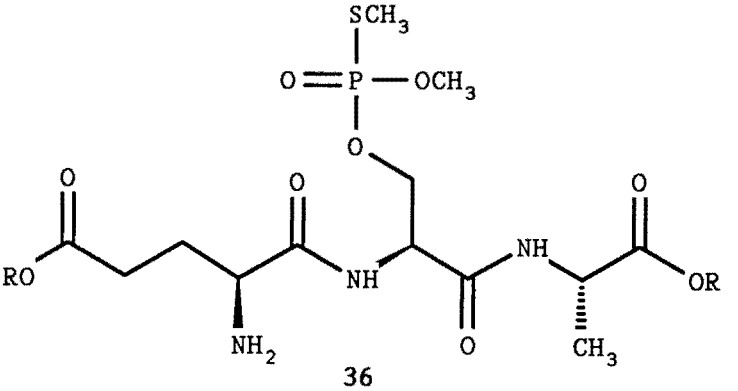


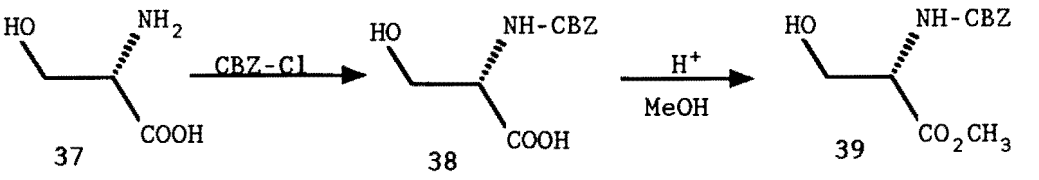
36

R = OH or protected ester/amide

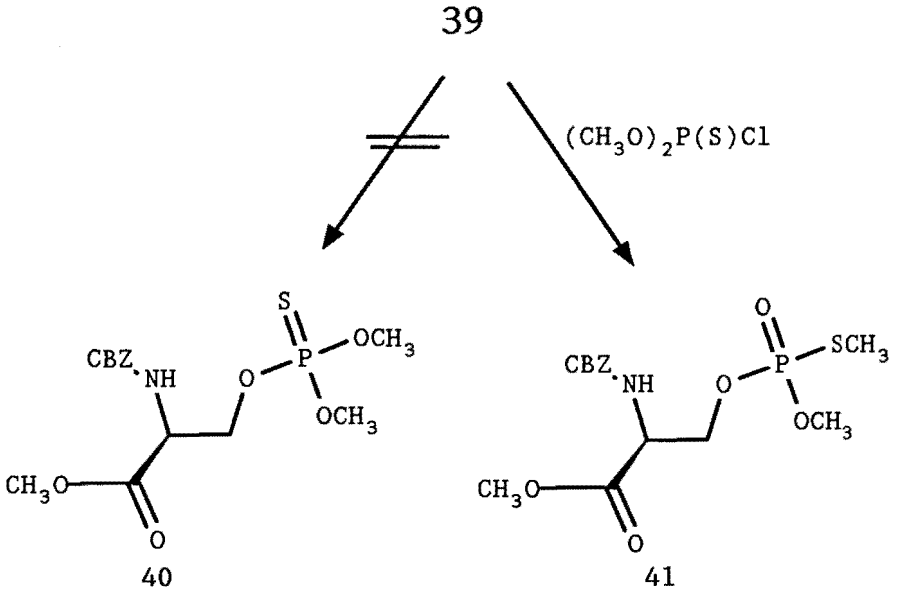
following purification/separation of the diastereomers by flash chromatography. Compound **32** was then transformed into the corresponding carboxylic acid **(33)** upon treatment with lithium hydroxide. Initially, dioxane/water were the co-solvents chosen for this reaction. However, this solvent system resulted in poor conversion of starting material to product with excessively long reaction times (days). Modification to a THF/water mixture resulted in a much better conversion to product (60%) yield along with greatly reduced reaction times (hours). Concurrent studies of the ring opening reaction of **32**, which has been successful when conducted with similar molecules[38,40,41,49,70-73], indicated that exposure to the methanolic hydrogen chloride resulted in complete destruction of the molecule. Attempts at ring opening via catalytic hydrogenolysis[74] were also problematic, presumably due to sulfur poisoning of the catalyst. Therefore, alternate methodology was considered.

We hoped that the three amino acids could be coupled and phosphorylated in such a sequence that, at some point during the synthesis, separation of the diastereomers could be achieved. Since *L*-glutamic acid, 5-methyl ester; *L*-serine; and *L*-alanine, ethyl ester hydrochloride are commercially available, these amino acid derivatives were chosen as logical starting material. *L*-Serine **(37)** was easily converted to the N-CBZ derivative **(38)** upon

treatment with CBZ-Cl. The N-protected derivative **(38)** was then esterified with methanol and gaseous HCl.



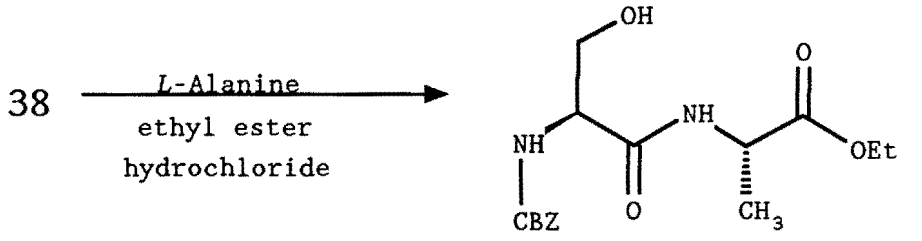
Attempts to phosphorylate **39** with *O,O*-dimethyl thiophosphoryl chloride were somewhat surprising. Rather than the expected *O,O*-dimethyl derivative **(40)** it appeared that some type of isomerization to the *O,S*-dimethyl derivative **(41)** occurred. This isomerization is proposed



based on the appearance of a doublet at approximately 2.5 ppm in the ^{1}H NMR spectrum of the reaction product. This resonance corresponds to an -SCH_3 attached to phosphorus. NMR analysis of the phosphorylating agent confirmed its integrity. While this unexpected result could have actually proven beneficial, attempts to saponify the

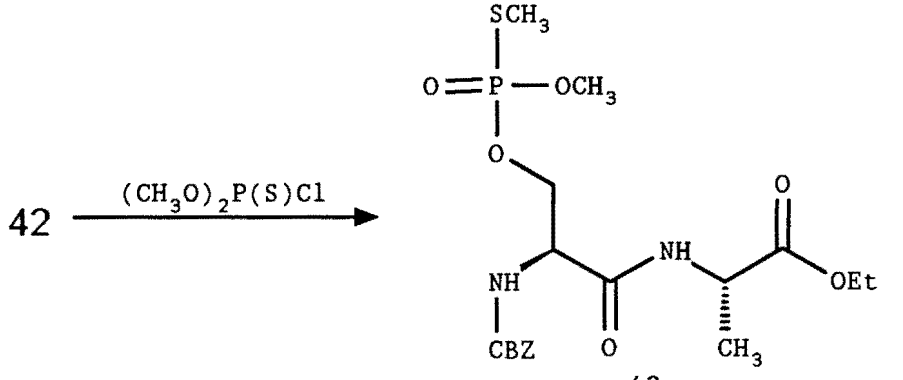
methyl ester for peptide coupling predictably resulted in destruction of the phosphorus ester as well.

In an alternate approach, *L*-alanine, ethyl ester hydrochloride was coupled with **38** (using DCC as a coupling reagent) to provide the protected dipeptide **(42)** in 65% yield after silica gel chromatography.



42

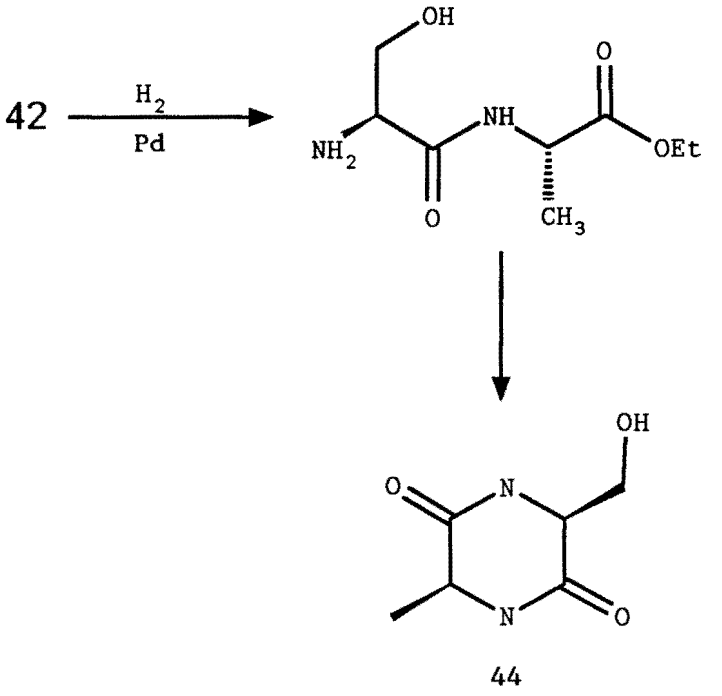
Phosphorylation of **42** with *O,O*-dimethyl thiophosphoryl chloride again proceeded with isomerization and the formation of the *O,S*-dimethyl derivative **(43)**.



43

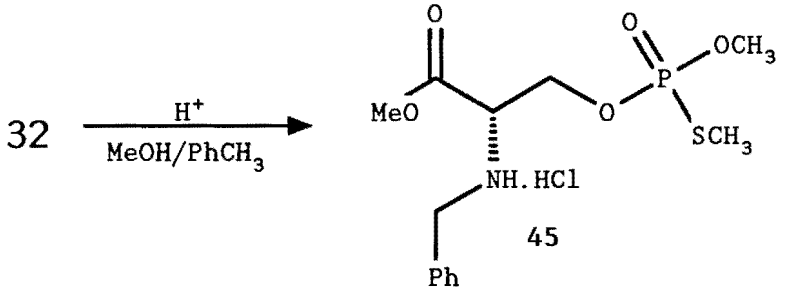
Preliminary studies examining the removal of the CBZ-protecting group from **42** indicated that following removal of the CBZ-protecting group, intramolecular attack upon the ethyl ester by the free amine results in formation of diazine **44** which thwarted this particular

route. Thus, the tripeptide could not be easily constructed prior to phosphorylation.



44

Further exploration of the ring opening reaction led us to the use of a mixed solvent system of acidic methanol and toluene.[75] Treatment of thiomethyl OAP **32**, predissolved in toluene, with acidic methanol presumably resulted in the formation of amine hydrochloride **45**.



45

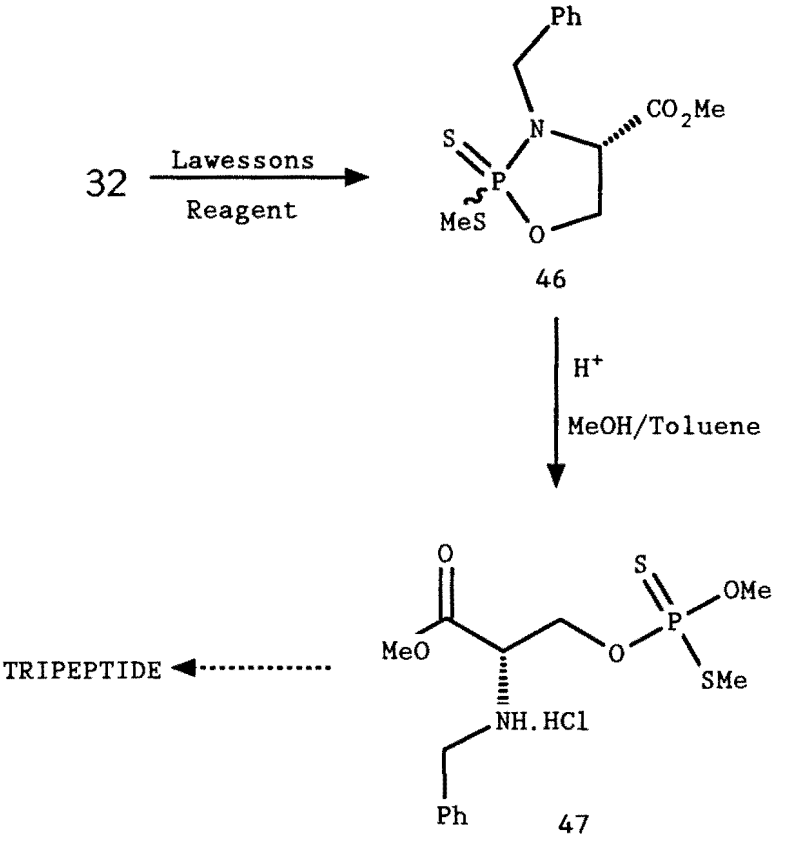
A low field ^{1}H NMR spectra of the reaction product, taken almost immediately after the reaction was complete,

indicated that an $-OCH_3$ group had become attached to phosphorus without loss of the $-SCH_3$. However, attempts to obtain a high field spectrum, after longer exposure to the high vacuum pump revealed apparent decomposition of the product. This decomposition was even more pronounced when we attempted to isolate the free base of **45**. Perhaps the P=O bond is undergoing intramolecular nucleophilic attack by the amine, at least in the case of the free base compound.

The observed decomposition of **45** was not all that surprising in light of our early findings, namely that a P=O bond was much more reactive than a P=S bond. Therefore, we set about modifying our system (Scheme VIII). OAP **32** was converted to the P=S analog **(46)** by treatment with Lawesson's Reagent[76] in refluxing toluene. The product was isolated in 70% yield after purification by flash chromatography. The IR spectra of the product indicated loss of the P=O bond while the carboxy ester carbonyl peak remained intact. This was fortunate because Lawesson's Reagent can convert carbonyl compounds into their corresponding thiocarbonyl analogs. Treatment of **46** with acidic methanol in toluene results in the formation of the ring opened product, **(47)** in quantitative yield. The high field ^1H NMR spectra of these compounds indicated addition of a $-OCH_3$ group. Another good indication was that the ^{31}P NMR spectra contains only one resonance.

This intermediate phosphoserine derivative should serve as a useful precursor to chirally phosphorylated tripeptides.

Scheme VIII



CONCLUSIONS

A series of *O,O*-dimethylphosphorothioates and their *O,S*-isomerides were characterized with ^{31}P NMR and examined for their relative inhibitory power against AChE were determined. In all cases examined, the isomerides were shifted approximately 40 ppm upfield from the parent compound relative to an external phosphoric acid reference. The isomerides were also found to be 1000-times more potent as AChE inhibitors than the parent insecticides.

In the second portion of this work, we sucessfully prepared a series of diastereomerically pure 1,3,2-oxazaphospholidine-2-ones (OAP's). Reaction of N-benzyl serine methyl ester with phosphorus oxychloride yielded the intermediate chloridate OAP which was then further reacted with an alcohol or phenol to yield the 2-alkoxy (or aryloxy) OAP's. The diastereomers were separated by flash chromatography on silica gel and then analyzed to determine the relative configuration of the ligand on phosphorus and the carboxy ester moiety. Initial assignments were made based on ^{31}P NMR. The definitive assignment was made by an X-ray crystal

structure of one of the 2-Methoxy OAP's. It was revealed that the isomer that eluted from a silica gel column more slowly is the trans isomer while the fast band is the cis isomer.

In studies directed toward the synthesis of a phosphorylated tripeptide mimic of AChE, we determined that the 2-thiomethyl OAP could be prepared from reaction of N-benzyl serine methyl ester with thiomethyl phosphoryl dichloride. Attempts to cleave the exocyclic phosphorus-oxygen bond for further elaboration to the tripeptide were thwarted by decomposition of the ring opened product. However, conversion to the thioxo analog and subsequent ring opening afforded phopshorylated serine derivatives which should serve as a useful precursor to chirally phosphorylated tripeptides. Studies are currently being conducted in our laboratory to explore this synthetic approach in more detail. If this route proves viable, this peptide may serve as a model to examine some of the dynamic molecuar events that occur following OP poisoning such as aging and/or reactivation.

The methodology developed on the synthesis of the OAP's is also currently being explored as a possible route to a serine derived analog of cyclophosphamide, a potent chemotherapuetic agent.

EXPERIMENTAL SECTION

General Methods. Melting points were determined using a Mel-Temp melting point apparatus and are uncorrected. Proton NMR spectra were taken is deuterated chloroform ($CDCl_3$) on either a Varian EM-360A of VXR-300 instrument. Carbon and phosphorus NMR were also conducted on the VXR-300 and the chemical shifts are relative to the deuterated chloroform triplet (δ=77.06) and external phosphoric acid (H_3PO_4 in $CDCl_3$, δ=0) respectively. Pertinent proton NMR data are tabulated in the following order: chemical shift (ppm in delta), multiplicity, coulpling constants (J in Hertz) and number of hydrogens. Prominent infrared data are obtained in $CDCl_3$ and are expressed in cm^{-1}.

Analytical thin layer chromatography (TLC) was conducted with aluminum backed silica plates (E. Merck). Visualization was affected with an ultraviolet lamp and/or anisaldehyde stain (a 2% solution of o-anisaldehyde in 95:4:1 absolute ethanol-concentrated sulfuric acid-glacial acetic acid) with heating and/or DBQ (5% 2,4-dibromo-quinone-4-chloroimide in diethyl ether) and/or ninhydrin (5% in ethanol) and/or ammonium molybdate (2.5% in 9:1 water-concentrated sulfuric acid with 1% ceric sulfate).

Flash chromatography[77] was conducted on Kieselgel 60 0.04-0.06 mm (E. Merck).

High performance liquid chromatography (HPLC) was conducted with a variable wavelength ultraviolet detector. Reverse phase chromatography was conducted on a Regis (Morton Grove, IL) 10 μm ODS (30 cm) column utilizing a 55:45 CH_3OH/H_2O solvent system at a flow rate of 1 mL/min for the OAP's or where otherwise specified.

All solvents and reagents were purified (when necessary) prior to use by literature methods.[78] Air or water sensitive reactions were conducted under a positive argon atmosphere utilizing standard techniques.[79]

Acetylcholine iodide (ATCh-I) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical C. (St. Louis, MO). *L*-Serine was purchased from Chemical Dynamics Corp. *L*-Alanine, ethyl ester hydrochloride; *L*-Glutamic acid, 5-methyl ester; and phosphorus oxychloride were purchased from Aldrich. The N-CBZ-amino acids were prepared via the method of Asami et al.[80] The *S*-methyl phosphoryl dichloride was prepared by previously reported methods.[69]

Cholinesterase Determinations. Rat brain acetylcholinesterase activity determination were accomplished by a previously reported method.[81] The procedure, with some minor modifications, is presented here. Freshly excised brains (stored no more than 6 days)

were homogenized in phosphate buffer (0.1 M, pH = 7.6) to give a final volume of 20 mL. This stock solution was kept at 0 °C up to 7 days. For analysis a 1:4 dilution of stock solution to buffer was employed (approximating a hydrolysis rate of 0.04-0.07 *A* unit/min). This solution was vortexed gently, and 1.09 mL of this sample was added to a test tube and placed in a 25 °C Forma Scientific constant-temperature shaker bath. A modified Ellman method[82] was used to determined AChE activity as follows.

To each of six cuvettes was added 2.5 mL of DTNB solution (3.33 X 10^{-4} M DTNB, 5.9 X 10^{-4} M sodium bicarbonate; phosphate buffer) and 0.020 mL ATCh-I solution (7.5 X 10^{1} M ATCh-I; phosphate buffer). These cuvettes were then placed in a Beckman DU-40 spectrophotometer equipped with a kinetic Soft-Pac module. From the test tube of brain homogenate 0.1 mL was withdrawn and added to cuvette 1 to serve as control. To the remaining 0.990 mL in the the test tube 0.01 mL of the inhibitor (varied concentration) was added and the solution gently vortexed. At 3, 6, 9, 12, and 15 min, 0.1 mL of the homogenate-inhibitor solution was added to cuvettes 2, 3, 4, 5, and 6, resepectively. The rate of hydrolysis of acetylthiocholine was monitored at 412 mn at 60-second intervals for 30 min from the addition of enzyme. The bimolecular inhibition constants (k_i) were determined in triplicate by plotting the slopes

(hydrolysis rate) against time, and the resulting slope was analyzed by linear regression.

(*S*)-N-Benzylserine, Methyl Ester (26). (*S*)-Serine methyl ester hydrochloride (10 g, 0.065 mol) was dissolved in 50 mL of anhydrous methanol and cooled to 0 °C. Triethylamine (9.0 mL, 0.065 mol) was added, the reaction was stirred for 10 min, and 6.6 mL benzaldehyde (0.065 mol) was added. The reaction mixture was stirred for 2 h, at which time sodium borohydride (4.8 g, 0.13 mol) was added portionwise to the reaction mixture over a period of 0.5 h. The solution was partitioned between 50 mL of 20% HCl and 50 mL of diethyl ether. The organic phase was extracted twice more with 20 mL portions of 20% HCL. The combined aqueous layers were washed with an additional 20 mL portion of diethyl ether and the organic layer were discarded. The aqueous layers were carefully neutralized with solid sodium carbonate and extracted three times with 20 mL portions of diethyl ether. After extraction with brine, the combined ether extracts were dried over sodium sulfate and evaporated to afford 8.76 g (66-70%) of a slightly yellow oil. The product may be further purified by bulb-to-bulb distillation (110-120 °C, 0.1 mm Hg), yielding a waxy solid upon cooling. R_f = 0.28 (100% diethyl ether). $[\alpha]^{23}_D$ +36.5° (c 0.395, $CHCl_3$). ^1H NMR: δ 2.42 (bs, 2 H), 3.41 (bt, J = 4.8, 1 H), 3.60 (dd, J=

10.3 and 6.7, 1 H), 3.72 (s, 3 H), 3.75 (dd, J = 10.8 and 6.0, 1 H), 3.78 (q, 12.4, 2 H), 7.25-7.32 (m, 5 H). ^{13}C NMR: δ 52.08, 61.82, 62.41, 76.59, 127.32, 128.24, 128.51, 139.26, 173.43.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Chloro-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (27). (*S*)-N-benzyl-serine, methyl ester (1 g, 4.78 mmol) was dissolved in 20 mL of distilled toluene and chilled to 0 °C. Triethylamine (TEA; 1.4 mL, 10 mmol) was added, followed by phosphorus oxychloride ($POCl_3$; 0.45 mL, 4.78 mmol). The progress of the reaction was monitored by TLC for loss of starting material. At completion, the reaction mixture was diluted with 50 mL of THF, filtered through Celite, and evaporated to yield the crude product as a mixture of diastereomers (94%). The diastereomers were separated by flash chromatography with 100% diethyl ether. [Note: this material is relatively unstable to storage and it is suggested that this compound be immediately converted to the corresponding ester]. Anal. Calcd for $C_{11}H_{13}NO_4ClP$: C, 45.61; H, 4.52; N, 4.83. Found: C, 45.72; H, 4.66; N, 4.79.

Fast band (27a, cis): R_f = 0.38 (diethyl ether). ^1H NMR: δ 3.75, (s, 3 H), 3.89 (ddd, J = 16.5, 7.4, 4.4, 1 H), 4.25 (dd, J = 14.4, 7.5, 1 H), 4.41-4.48 (m, 2 H), 4.59 (dd, J = 14.4, 9.0, 1 H), 7.26-7.33 (m, 5 H). ^{13}C NMR: δ 46.98, 52.71, 55.57, 66.60, 128.44, 128.90,

134.52, 169.38. ^{31}P NMR: δ 23.92.

Slow band (27b, trans): R_f = 0.32 (diethyl ether). ^{1}H NMR: δ 3.64 (s, 3 H), 3.79 (ddd, J = 10.9, 7.2, 2.7, 1 H), 4.25 (dd, J = 14.9, 10.3, 1 H), 4.44 (dd, J = 14.5, 10.3, 1 H), 4.44 (dd, J = 9.5, 2.9, 1 H), 4.56 (dd, J = 9.7, 2.4 1 H), 7.26-7.32 (m, 5 H). ^{13}C NMR: δ 47.50, 52.75, 56.78, 66.85, 128.31, 128.72, 130.79, 134.27, 169.46. ^{31}P NMR: δ 23.68.

General Procedure for the Preparation of Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Alkoxy(aryloxy)-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylates (28-31). Method A. The alcohol (or phenol) (150 mol %) was added to a room temperature solution of methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-chloro-2-oxo-1,3,2-oxazaphospholidine-4-carboxylate **(27)** in toluene (15 mL/g of starting material) followed by the addition of TEA (100 mol %). The reaction was allowed to proceed until TLC indicated consumption of starting material (generally 1-2 h). The reaction mixture was then partitioned between ethyl ether and saturated sodium carbonate and the organic phase was washed twice with sodium carbonate, water, and brine and finally dried over sodium sulfate. The solvent was removed in vacuo to afford the crude product.

Method B. A 5% solution of the chloridate in anhydrous acetone was treated sequentially with 100 mol % of anhydrous sodium carbonate and 200 mol % of alcohol

(phenol). The reaction was warmed to reflux until TLC indicated complete loss of starting material. The reaction mixture was then cooled, filtered through Celite, and rotary evaporated to afford the crude product, which was purified by flash chromatography. See representative examples below.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Methoxy-2-ox-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (28). Yield 68%. The diastereomers were separated via flash chromatography using diethyl ether. Anal. Calcd for $C_{12}H_{16}NO_4P$: C, 50.53; H, 5.65; N, 4.91. Found: C, 50.54; H, 5.86; N, 4.84. IR: 1745, 1260.

Fast Band (28a, cis): R_f = 0.13 (diethyl ether). Off white crystals developed very slowly from the oil obtained. White crystals were obtained by trituration with diethyl ether; mp = 77-78 °C; $[\alpha]^{23}_D$ -26.0° (c 1.75, $CHCl_3$). ^1H NMR: δ 3.68 (s, 3 H), 3.71 (d, J = 12.4, 3 H), 3.83 (dt, J = 8.5, 4.5, 1 H), 4.15 (dd, J = 14.5, 9.4, 1 H), 4.22-4.40 (m, 2 H), 4.42 (dd, J = 14.9, 9.6, 1 H), 7.25-7.39 (m, 5 H). ^{13}C NMR: δ 47.78, 52.32, 54.50, 56.70, 65.38, 128.08, 128.51, 128.73, 135.58, 170.62. ^{31}P NMR: δ 22.19. HPLC: t_R = 9.5 min.

Slow band (28b, trans): R_f = 0.08 (diethyl ether). This diastereomer was recrystallized from methylene chloride/hexane; mp = 64-66 °C; $[\alpha]^{23}_D$ -90.16° (c, 0.62, $CHCl_3$). ^1H NMR: δ 3.72 (s, 3 H), 3.77 (d, J = 11.4, 3

H), 3.74-3.78 (m, 1 H), 4.16 (dd, J = 15.3, 8.6, 1 H), 4.26-4.42 (m, 2 H), 4.31 (dd, J = 15.7, 8.4, 1 H), 7.28-7.32 (m, 5 H). ^{13}C NMR: δ 47.19, 52.57, 54.75, 57.26, 65.86, 127.97, 128.25, 135.94, 170.67. ^{31}P NMR: δ 21.14. HPLC: t_R = 8.8 min.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Ethoxy-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (29). Yield 87%. The diastereomers were separated via flash chromatography using diethyl ether. Anal. Calcd for $C_{13}H_{18}NO_5P$: C, 52.18; H, 6.06; N, 4.68. Found: C, 51.99; H, 6.29; N, 4.90. IR: 1735, 1260.

Fast band (29a cis): R_f = 0.13 (diethyl ether), $[\alpha]^{23}_D$ -23.90° (c 2.31, $CHCl_3$). ^{1}H NMR: δ 1.28 (t, J = 1.7, 3 H), 3.66 (s, 3 H), 3.82 (dt, J = 8.3, 4.6, 1 H), 4.01-4.42 (m, 6 H), 7.26-7.39 (m, 5 H). ^{13}C NMR: 16.04, 47.56, 52.27, 56.67, 63.94, 67.27, 127.83, 128.47, 128.79, 135.63, 170.66. ^{31}P NMR: δ 20.83. HPLC: t_R = 12.4 min.

Slow band (29b, trans): R_f = 0.09 (diethyl ether), $[\alpha]^{23}_D$ 56.06° (c 1.09, $CHCl_3$). ^{1}H NMR: δ 1.28 (t, J = 7.1, 3 H), 3.64 (s, 3 H), 3.72 (ddd, J = 10.8, 6.8, 2.8, 2 H), 4.02-4.14 (m, 2 H), 4.06 (q, J = 7.0, 2 H), 4.21-4.36 (m, 2 H), 7.25-7.31 (m, 5 H). ^{13}C NMR: δ 16.37, 47.04, 52.55, 57.67, 64.25, 66.12, 127.89, 128.44, 128.62, 135.98, 170.78. ^{31}P NMR: δ 19.89. HPLC: t_R = 11.7 min.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Phenoxy-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (30). Yield

96%. The diastereomers were separated by flash chromatography utilizing ethyl acetate/petroleum ether as the eluent. Anal Calcd for $C_{17}H_{18}NO_4P$: C, 58.79; H, 5.22; N, 4.03. Found: C, 58.75; H, 5.29; N, 4.01. I.R.: 1745, 1275.

Fast band (30a, cis): R_f = 0.29 (diethyl ether). This diastereomer was crystallized as white plates from ethyl acetate petroleum ether; mp = 92-94 °C, $[\alpha]^{23}_D$ -29.27° (c, 0.96, $CHCl_3$). ^1H NMR: δ 3.58 (s, 3 H), 3.80 (ddd, J = 14.5, 8.9, 1 H), 4.21-4.40 (m, 3 H), 4.56 (dd, J = 14.5, 8.9, 1 H), 7.31-7.41 (m, 10 H). ^{13}C NMR: δ 47.83, 52.49, 55.92, 65.63, 120.92, 125.12, 128.20, 128.72, 128.91, 129.38, 135.29, 150.68, 170.06. ^{31}P NMR: δ 16.38. HPLC: t_R = 24.4 min.

Slow band (30b, trans): R_f = 0.22 (diethyl ether). This diastereomer recrystallized as needles from methylene chloride/ether/petroleum ether; mp 83-85 °C, $[\alpha]^{23}_D$ -70.22° (c 1.12, $CHCl_3$). ^1H NMR: δ 3.70 (s, 3 H), 3.82 (ddd, J = 12.1, 7.4, 2.0, 1 H), 4.30 (dd, J = 15.6, 8.1, 1 H), 4.33-4.49 (m, 2 H), 4.52 (dd, J = 15.1, 7.9, 1 H), 7.14-7.33 (m, 10 H). ^{13}C NMR: δ 47.36, 52.69, 57.15, 66.66, 120.27, 125.05, 128.08, 128.58, 128.74, 129.69, 135.55, 151.06, 170.43. ^{31} P NMR: δ 15.01. HPLC: t_R = 23.6 min.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-(4-Nitrophenoxy)-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (31).

Yield 78%. The diastereomers were separated via flash chromatography using a gradient of diethyl ether/ petroleum ether (70:30) to diethyl ether (100%). Recrystallization was accomplished with methylene chloride/diethyl ether/petroleum ether. Anal. Calcd for $C_{17}H_{17}N_2O_7P$: C, 52.05; H, 4.37; N, 7.10. Found: C, 51.94; H, 4.34; N, 7.10. IR: 1750, 1280.

Fast band (31a, cis): R_f = 0.35 (diethyl ether), $[\alpha]^{23}_D$ -4.49° (c, 1.18, $CHCl_3$), mp = 91-92 °C. ^1H NMR: δ 3.70 (s, 3 H), 3.91 (ddd, J = 11.0, 8.1, 4.6, 1 H), 4.23 (dd, J = 14.4, 9.0, 1 H), 4.30-4.49 (m, 2 H), 4.51 (dd, J = 14.5, 9.8, 1 H), 7.27-7.36 (m, 7 H), 8.20 (d, J = 7.4, 2 H). ^{13}C NMR: δ 47.99, 52.72, 56.26, 66.12, 121.41, 125.49, 128.41, 128.93, 134.91, 155.74, 169.93. ^{31}P NMR: δ 14.91. HPLC: t_R = 24.6 min.

Slow band (31b, trans): R_f = 0.20 (diethyl ether), $[\alpha]^{23}_D$ -27.45° (c, 0.94, $CHCl_3$), mp = 101-102 °C. ^1H NMR: δ 3.76 (s, 3 H), 3.92 (ddd, J = 12.5, 6.5, 2.9, 1 H), 4.28 (dd, J = 15.1, 9.9, 1 H), 4.43-4.53 (m, 2 H), 4.52 (dd, J = 14.6, 7.9, 1 H), 7.26-7.38 (m, 7 H), 8.19 (d, J = 9.2, 2 H). ^{13}C NMR: δ 47.46, 52.93, 57.31, 66.97, 120.76, 120.83, 125.57, 128.53, 128.90, 135.18, 155.99, 170.12. ^{31}P NMR: δ 14.91. HPLC: t_R = 24.6 min.

Conversion of 2-(4-Nitrophenoxy)- (31b) into Methyl (2*S*,4*S*)-2-Methoxy-2-oxo-3-benyl-1,3,2-oxazaphospholidine-4-carboxylate (28b). The starting phosphorus ester (31b;

0.196 g, 0.5 mmol) was dissolved in 10 mL of anhydrous methanol and chilled to 0 °C. A catalytic amount (5 m) of sodium methoxide was added and the reaction was warmed to room temperature, stirred, and monitored by TLC and HPLC for loss of starting material and appearance of product.

Methyl (2S,4S)- and (2R,4S)-2-Thiomethyl-2-oxo-3-benzyl-1,3,2-oxazphopholidine-4-carboxylate (32). (S)-N-benzyl methyl serinoate (3.22 g, 15.4 mmol) was dissolved in 50 mL toluene. To this solution was added TEA (4.7 mL, 33.9 mmol) and thiomethyl phosphoryl dichloride (2.53 g, 15.4 mmol). The reaction was stirred while monitoring (TLC) for consumption of starting material. Upon completion, the reaction was partitioned between 75 mL ethyl ether and 50 mL saturated sodium carbonate. The organic phase was extracted twice with sodium carbonate, water and brine and dried over sodium sulfate. The solvent was removed *in vacuo* and the resulting semi-solid was purified by chromatography. Procedure yields 2.93 g (70%).

Fast Band: R_f=0.23 (ethyl ether). ^{1}H NMR: δ 2.31 (d, J = 16.22, 3 H), 3.68 (s, 3 H), 3.89 (dt, J = 8.67, 3.90, 1 H), 4.18 (dd, J = 14.77, 10.13, 1 H), 4.25-4.35 (m, 1 H), 4.45-4.48 (m, 1 H), 4.54 (dd, J = 14.43, 9.06, 1 H), 7.27-7.43 (m, 5 H). ^{13}C NMR: δ 13.15, 13.22, 46.81, 46.89, 52.44, 56.36, 66.61, 66.64, 127.94, 128.59, 128.66,

135.29, 170.46, 170.52. ^{31}P NMR: δ 44.67.

Slow Band: R_f=0.13 (ethyl ether). ^{1}H NMR: δ 2.31, (d, J = 15.47, 3 H), 3.71 (s, 3 H), 4.19-4.32 (m, 3 H), 4.43-4.55 (m, 2 H), 7.27-7.39 (m, 5 H). ^{13}C NMR: δ 13.51, 13.57, 46.66, 46.75, 52.75, 56.94, 67.49, 67.53, 128.11, 128.55, 128.76, 135.40, 170.45. ^{31}P NMR: δ 44.05.

(2S, 4S) and (2R, 4S)-2-Thiomethyl-2-oxo-3-benzyl-1,3,2-oxazaphosphlidine-4-carboxylic acid (33). Ester 17 (540 mg, 2 mmol) was dissolved in 10 mL THF. 2 mL of LiOH solution (240 mg LiOH in 10 mL water) was added. The reaction mixture was allowed to stir until the starting material was consumed. The reaction generally stirred overnight. Upon completion, the solvent was evaporated yielding an off-white oil. The oil was partitioned between 50 mL $CHCl_3$/*i*-PrOH (9:1) and 20 mL dilute oxalic acid (100 mg in 40 mL water). The organic layer was then washed with water and brine, dried over sodium sulfate and evaporated. Procedure yields 60%.

Fast Band. ^{1}H NMR: δ 2.30 (d, J = 16.71, 3 H), 3.88
(dq, J = 11.67, 8.09, 3.60, 1 H), 4.19 (dd, J = 14.76, 10.26, 1 H), 4.35 (ddd, J = 16.65, 9.32, 3.63, 1 H), 4.49-4.55 (m, 1 H), 4.57 (dd, J = 14.05, 9.38, 1 H), 5.65, (bs, 1 H), 7.28-7.40 (m, 5 H). ^{13}C NMR: δ 13.21, 46.68, 56.00, 67.17, 128.18, 128.81, 128.88, 135.28, 172.38. ^{31}P

NMR: δ 48.19. mp 153 °C (dec.).

Slow Band. ^{1}H NMR: δ 2.32, (d, J = 16.11, 3 H), 3.68, (dd, J = 19.75, 6.35, 1 H), 4.23-4.29 (m, 1 H), 4.36 (dd, J = 14.65, 4.29, 1 H), 4.58, (dd, J = 14.65, 8.69, 1 H), 4.71 (dd, J = 20.27, 9.53, 1 H), 7.29-7.36 (m, 5 H), 9.50 (bs, 1 H). ^{13}C NMR: δ 13.57, 46.36, 56.40, 69.24, 128.18, 128.84, 128.87, 135.63, 171.61. ^{31}P NMR: δ48.13. mp 145 °C (dec.).

N-Benzyl-*L*-O-(*O,S*-dimethyl phosphoro)-serine, methyl ester hydrochloride (45). Methyl (2S,4S)- or (2R,4S)-2-thiomethyl-2-oxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (270 mg, 1 mmol) was dissolved in 5 mL toluene and chilled to 0 °C. 1 mL acidic methanol (1L saturated for 45 min., with HCl gas) was added dropwise to the reaction mixture. The reaction was allowed to stir while monitoring for loss of starting material. Upon completion (about 15 min.) the solvent was removed *in vacuo* to yield the product.

Methyl (2*S*,4*S*)- and (2*R*,4*S*)-2-Thiomethyl-2-thioxo-3-benzyl-1,3,2-oxazaphospholidine-4-carboxylate (46). 1.2 g (4 mmol) of OAP **32** was dissolved in 40 mL of toluene. To this solution was added Lawessons Reagent (1.6 g, 4 mmol) and the mixture was brought to reflux. After being maintained at reflux for 1 h, the mixture was cooled and the toluene was removed by rotary evaporation. The resulting semi-solid was partitioned between 50 mL of

saturated sodium bicarbonate and 50 mL of diethyl ether. The aqueous layer was washed once more with a 50 mL portion of diethyl ether. The organic layers were combined and washed sucessively with 50 mL portions of saturated sodium bicarbonate, water and brine. After drying over sodium sulfate the ether was removed and the product was purified by flash chromatography (silica gel 3:2 petroleum ether/diethyl ether).

Fast band. ^{1}H NMR: δ 2.37 (d, J = 17.96, 3 H), 3.67 (s, 3 H), 3.81-3.88 (m, 1 H), 4.25 (dd, J = 14.57, 9.42, 1 H), 4.36-4.52 (m, 2 H), 4.59 (dd, J = 14.65, 11.12, 1 H), 7.27-7.43 (m, 5 H). ^{31}P NMR: δ 107.46.

Slow band. ^{1} NMR: δ 2.37 (d, J = 17.74, 3 H), 3.73 (s, 3 H), 3.78-3.81 (m, 1 H), 4.24 (dd, J = 14.87, 5.43, 1 H), 4.32-4.55 (m, 3 H), 7.28-7.35 (m, 5 H). ^{31}P NMR: δ 105.27.

Ring Opened Products (47). OAP **46** (175 mg, 0.55 mmol) was dissolved in 4 mL toluene. 2 mL of methanol previously saturated with HCl gas (approximately 3 M) was added and the reactions were monitored by TLC for consumption of starting material. Upon completion the solvent was removed resulting in an off-white to white solid.

From fast band of 46. ^{1}H NMR: δ 2.32 (d, J = 15.66, 3 H), 3.76 (d, J = 15.14, 3 H), 3.82 (s, 3 H), 4.06 (bs, 1 H), 4.32, 4.45 (AB_q, J = 13.16, 2 H), 4.66-4.71

(m, 1H), 4.79-4.81 (m, 1 H), 7.35-7.66 (m, 5 H). ^{31}P NMR: δ 99.84.

From slow band of 46. ^{1}H NMR: δ 2.33 (d, J = 17.78, 3 H), 3.77 (d, J = 15.14, 3 H), 3.92 (s, 3 H), 4.06 (bs, 1 H), 4.35, 4.47 (AB_q, J = 12.62, 2 H), 4.65 (m, 1 H), 4.76-4.82 (m, 1 H), 7.36-7.66 (m, 5 H). ^{31}P NMR: δ 99.81.

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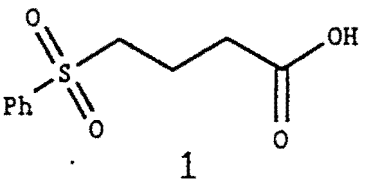
APPENDIX



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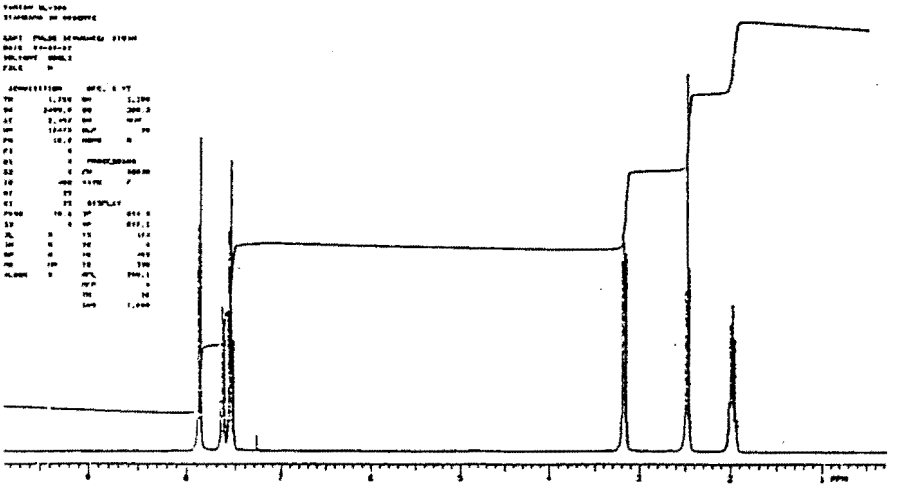

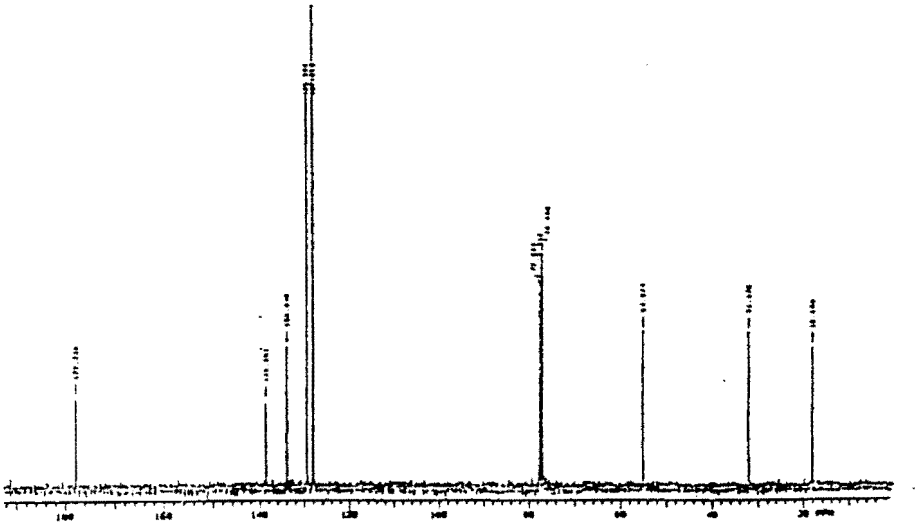

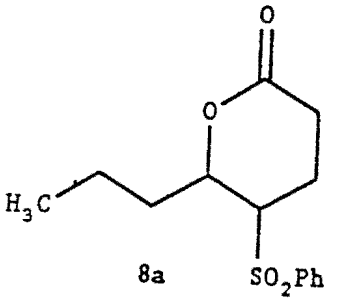

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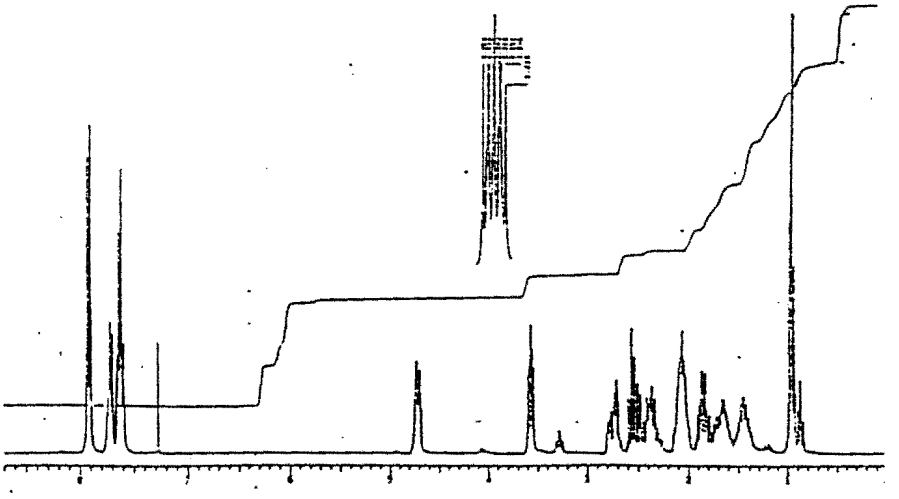

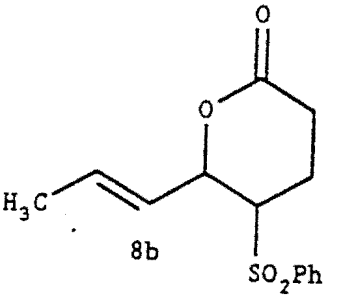

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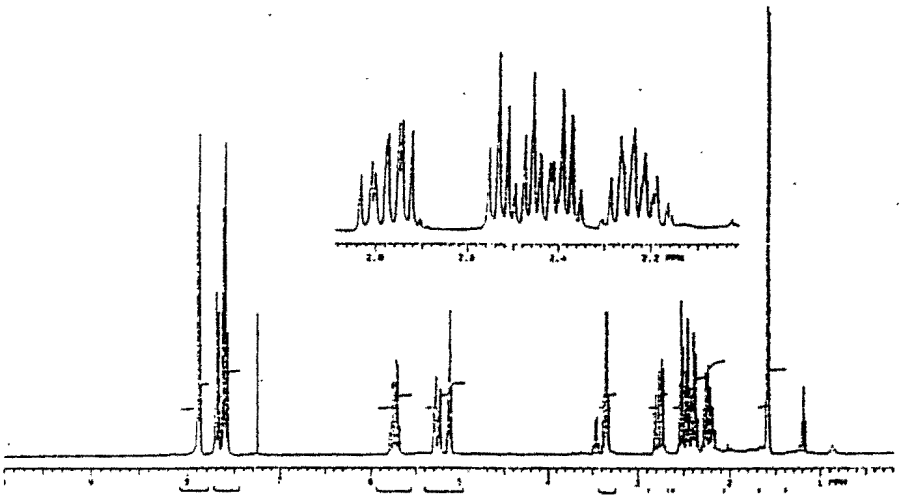

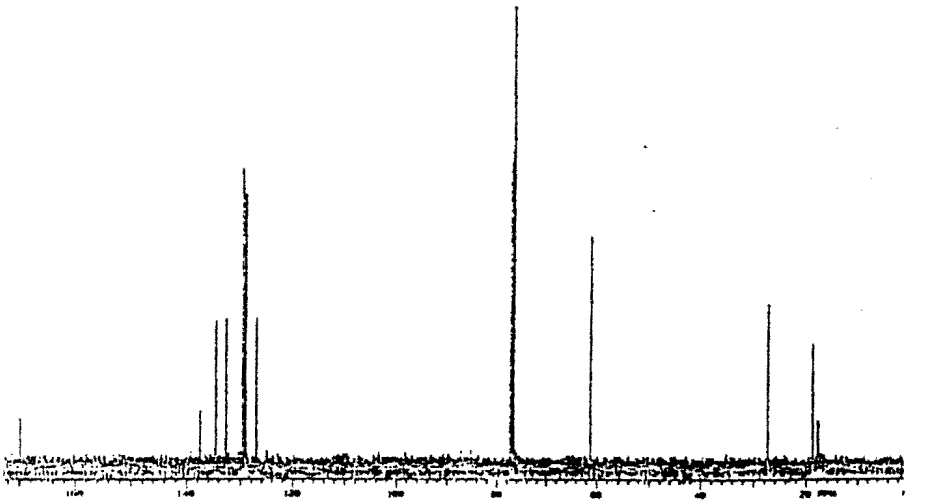

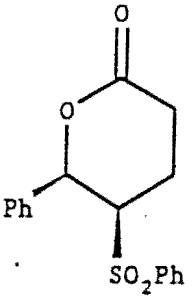

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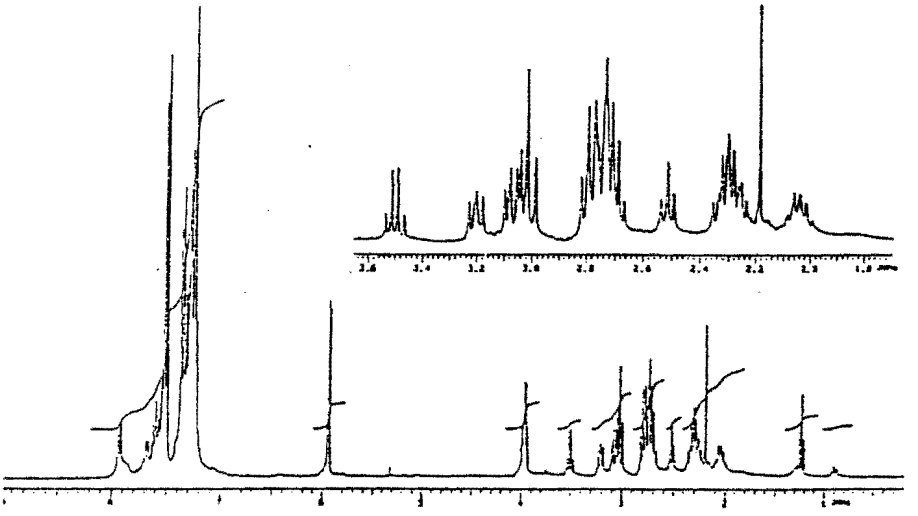

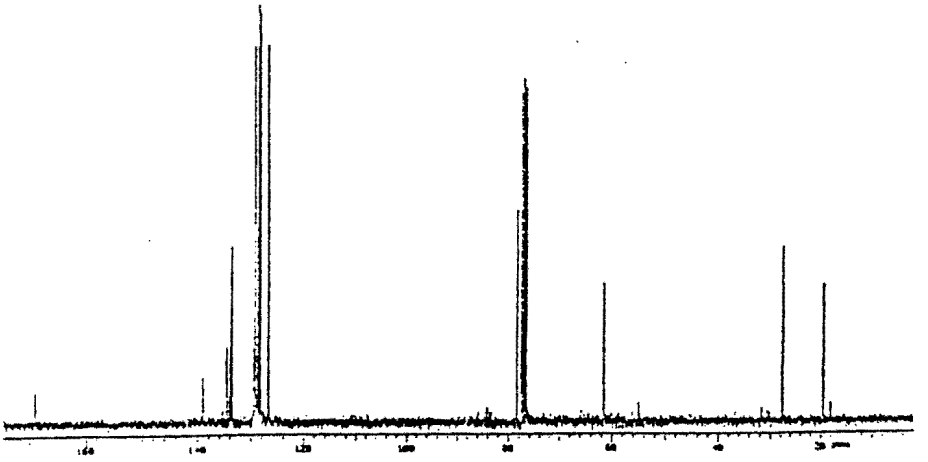

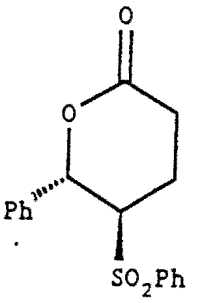

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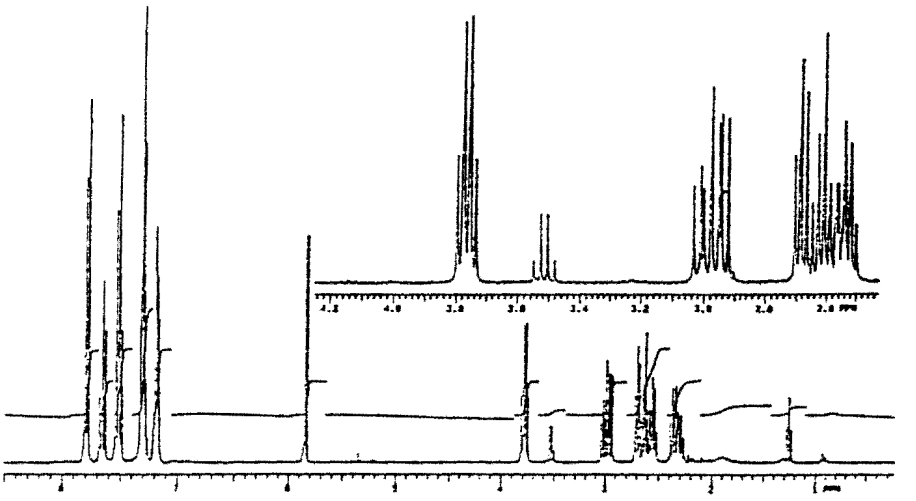

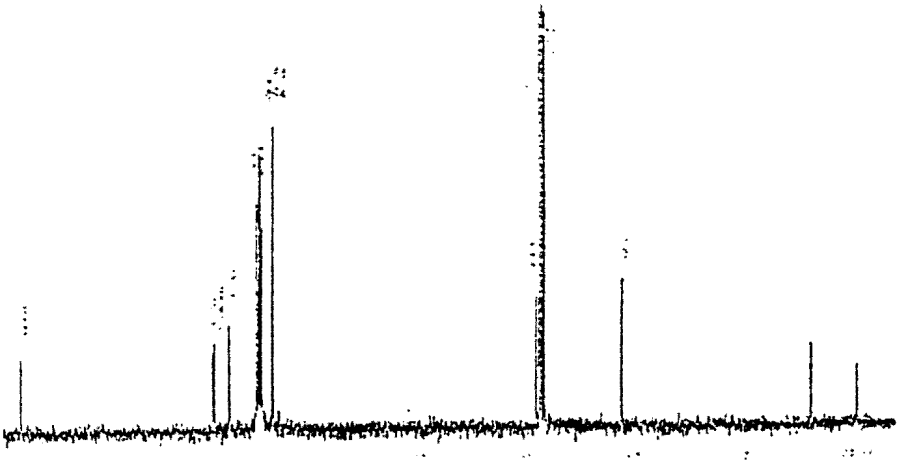

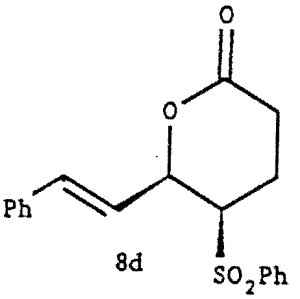

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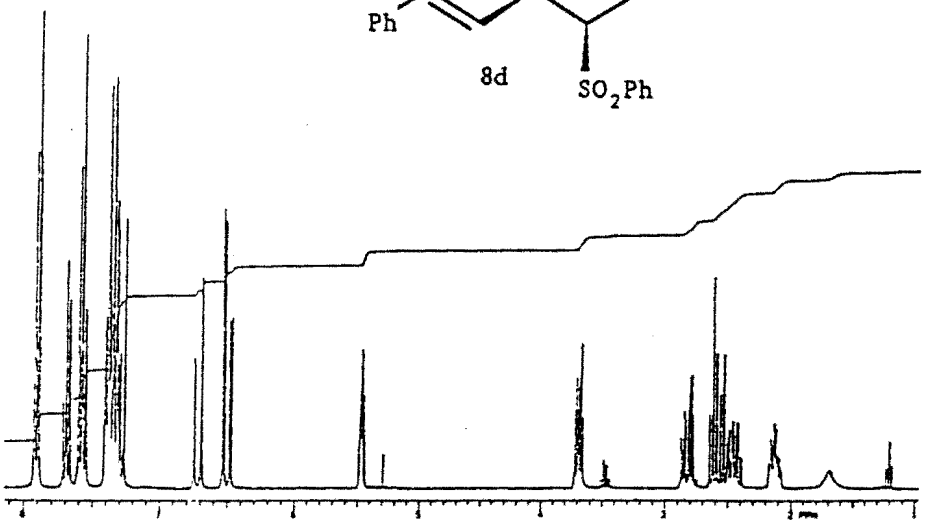

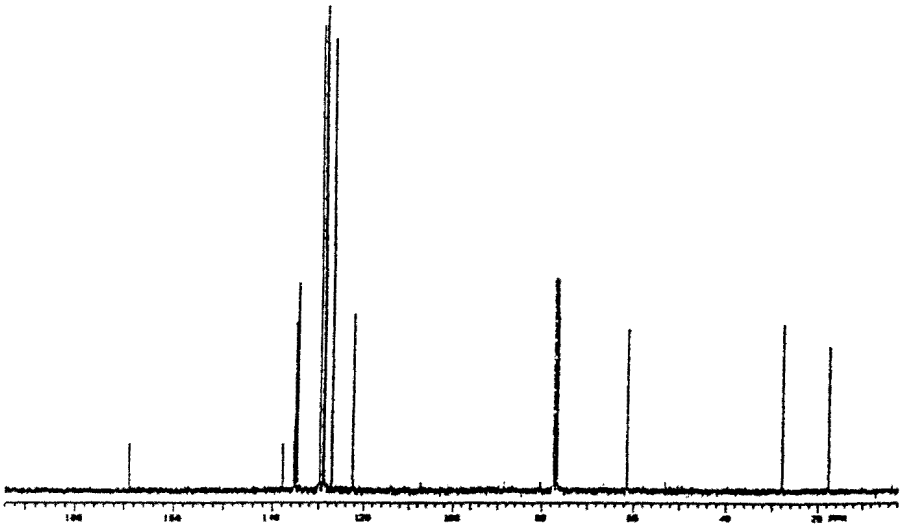

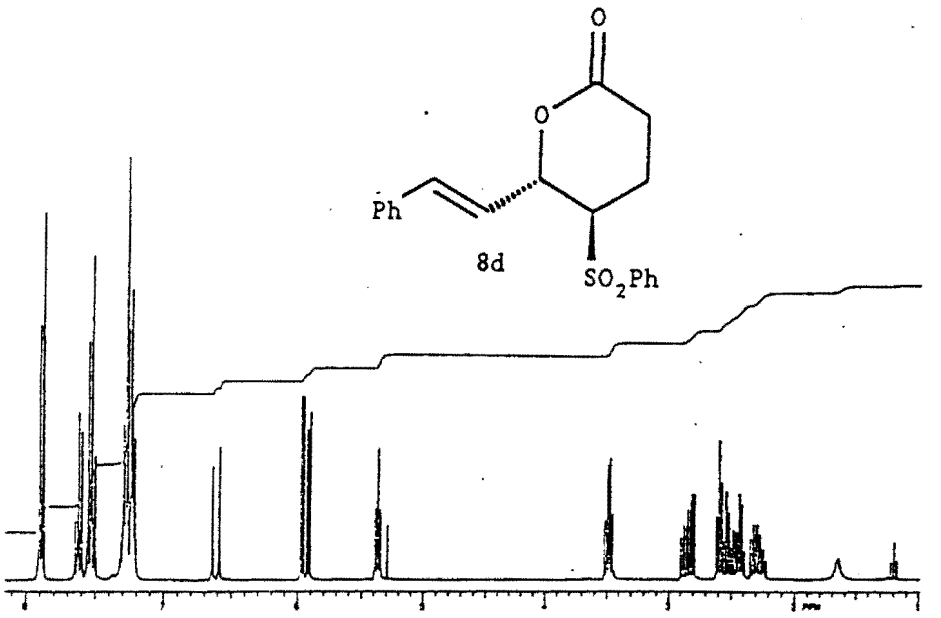
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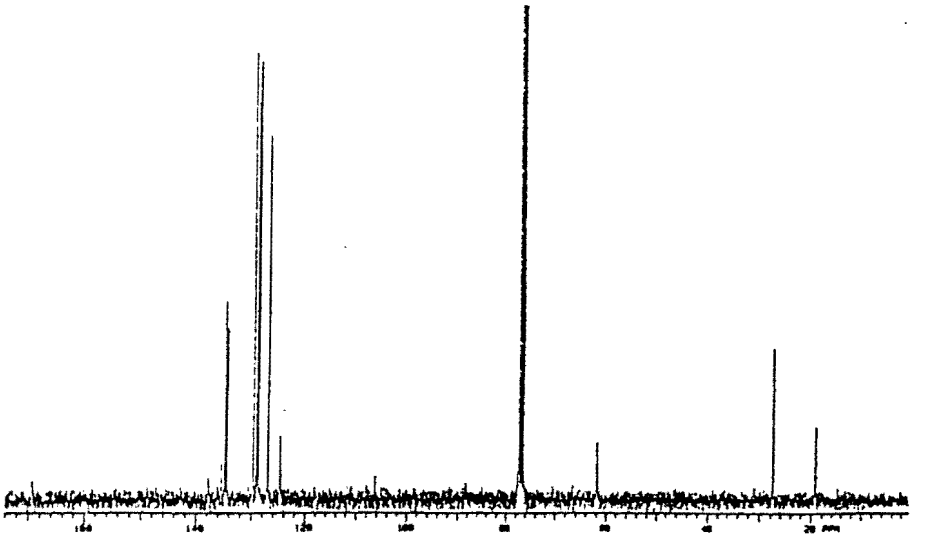

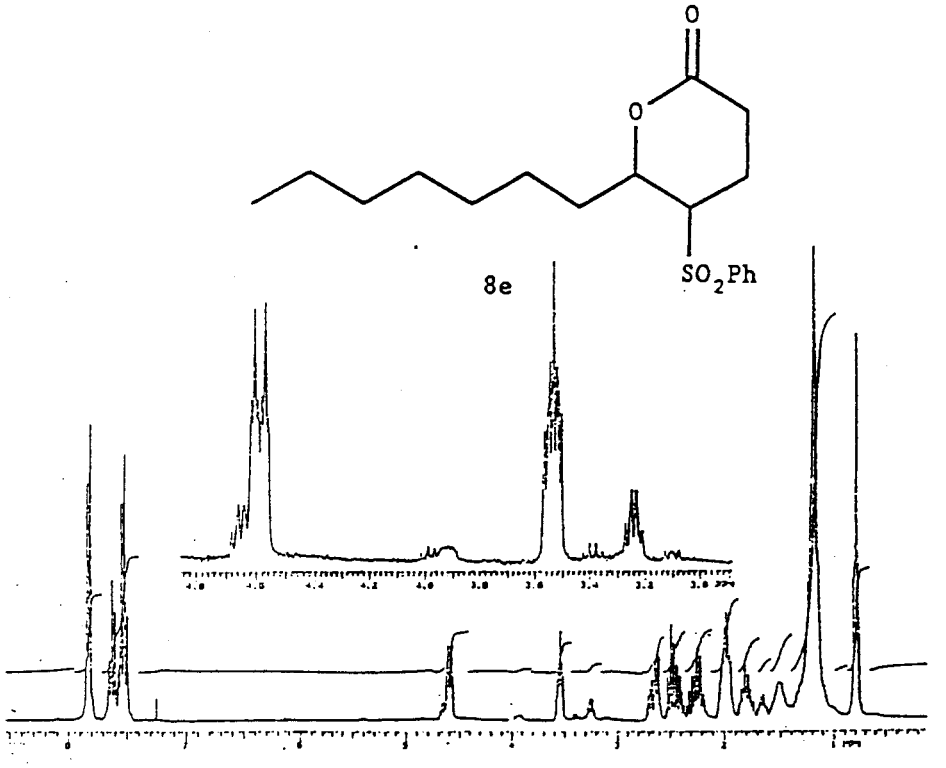
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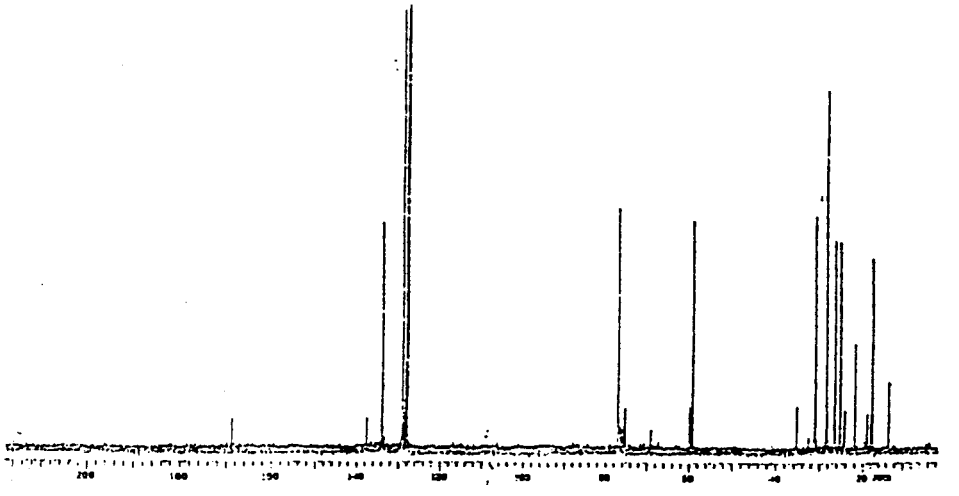

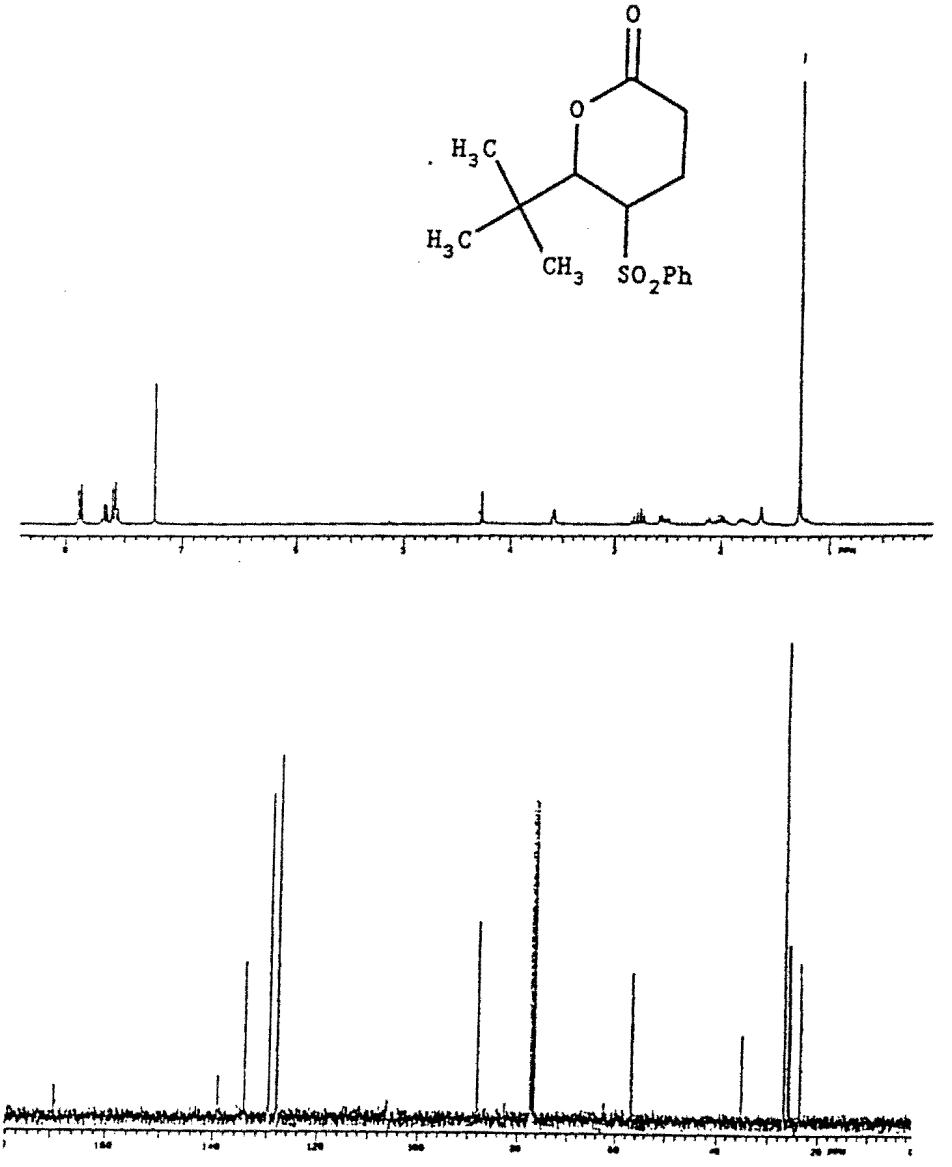
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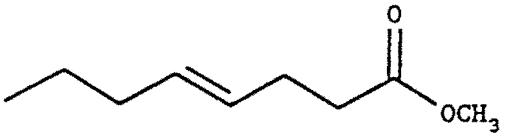
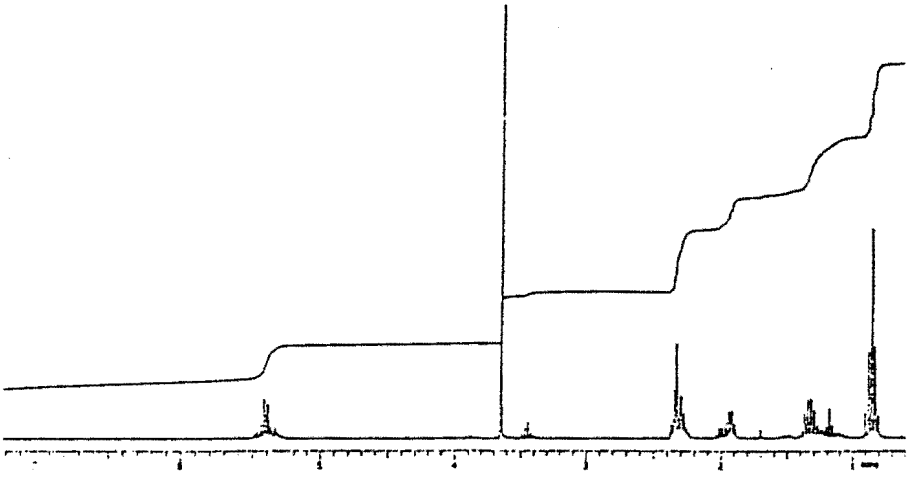
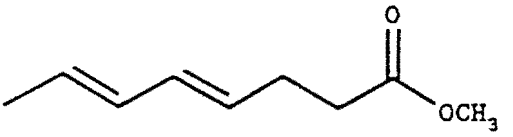


10a





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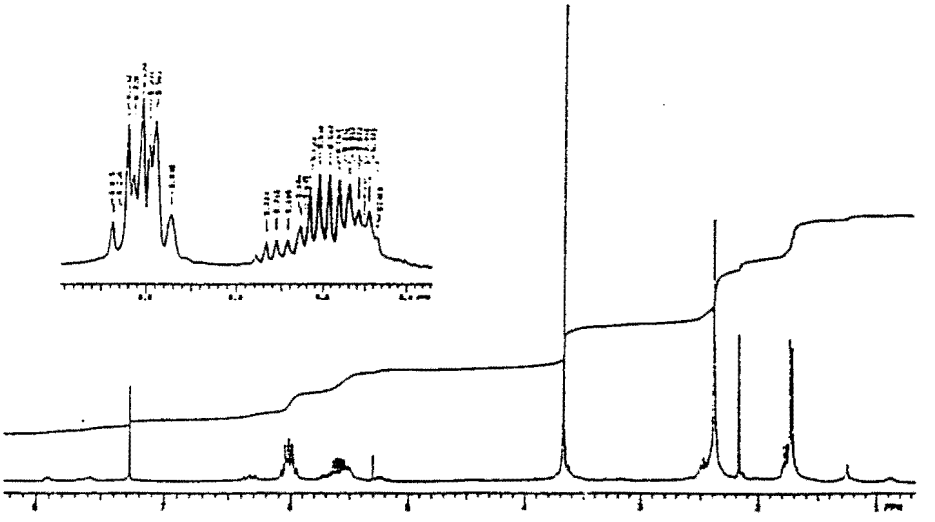

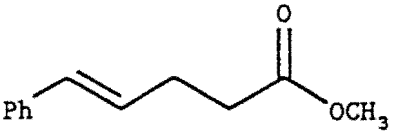

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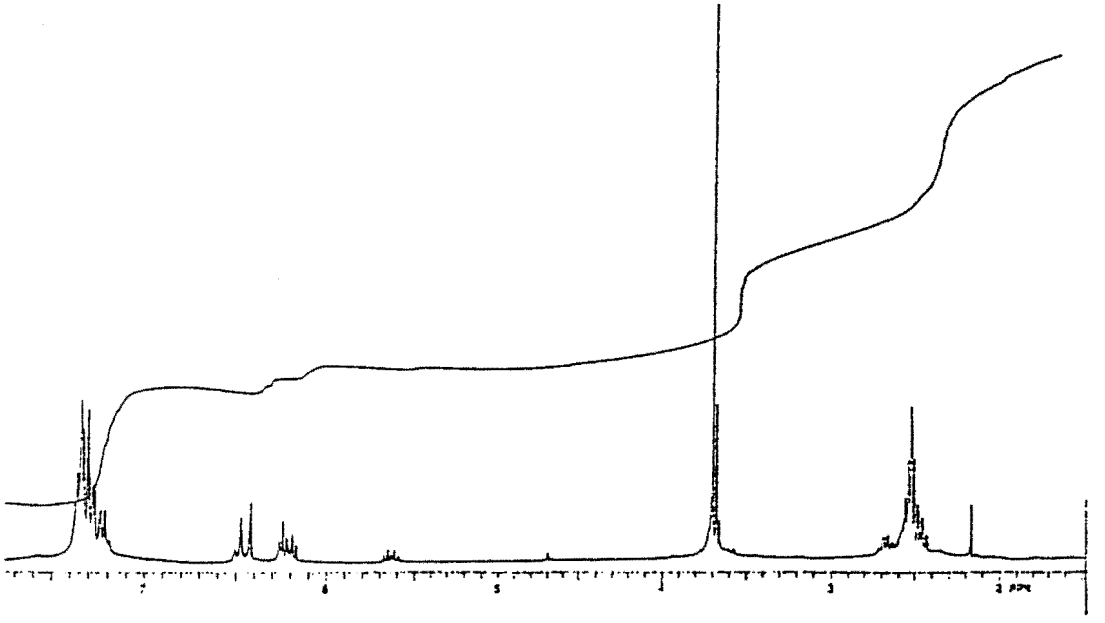

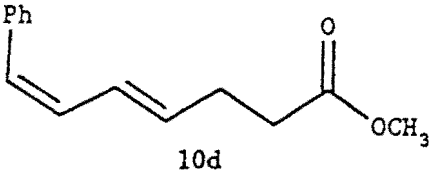

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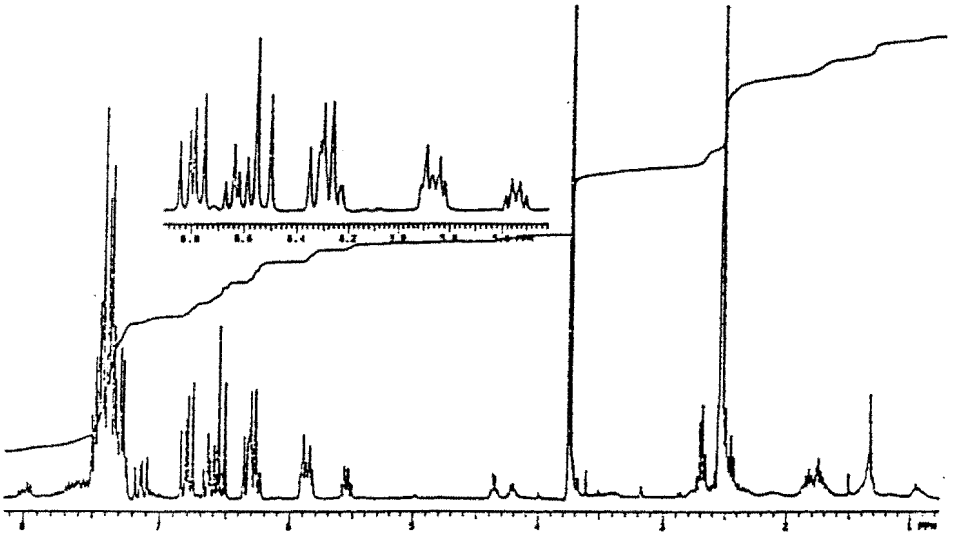

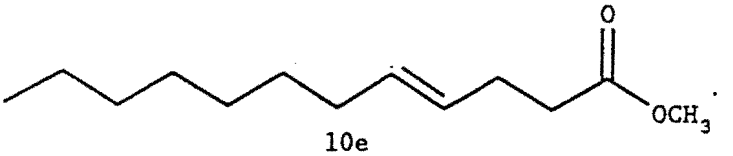

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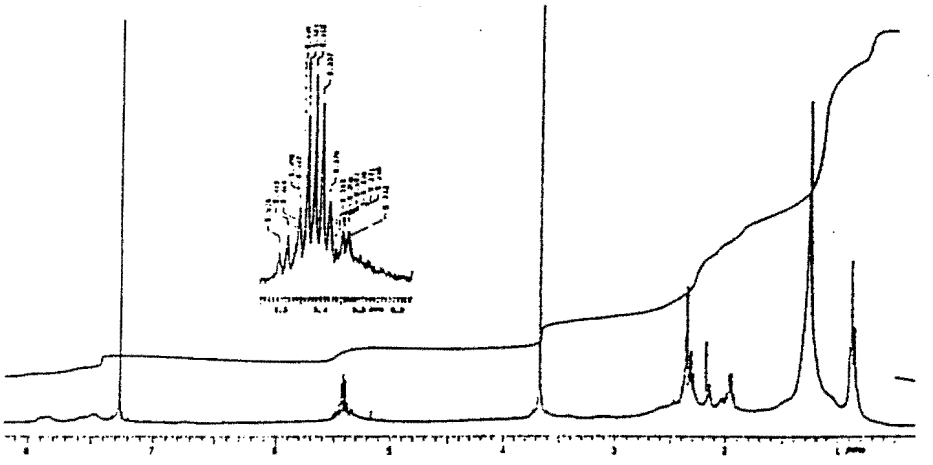

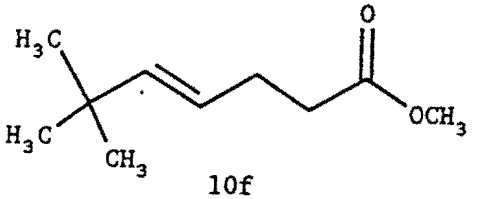

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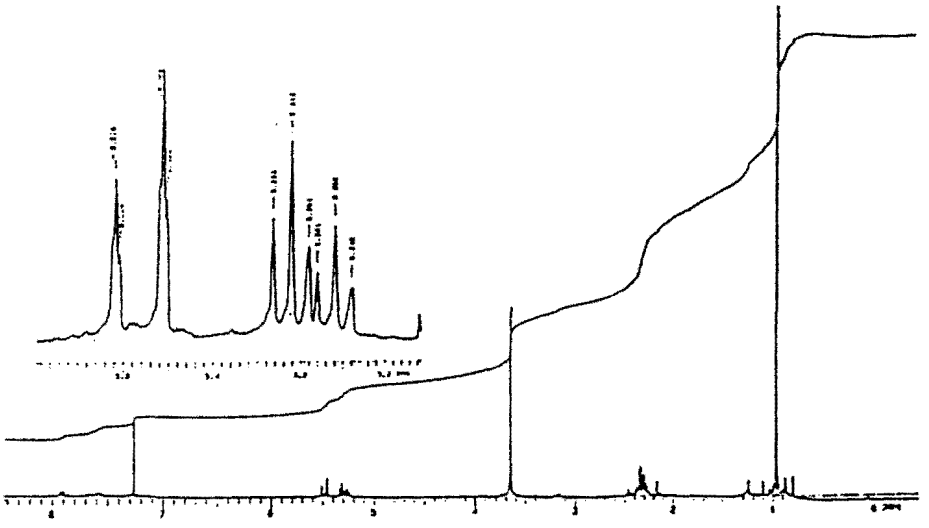

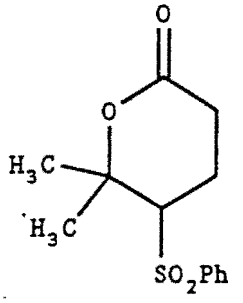

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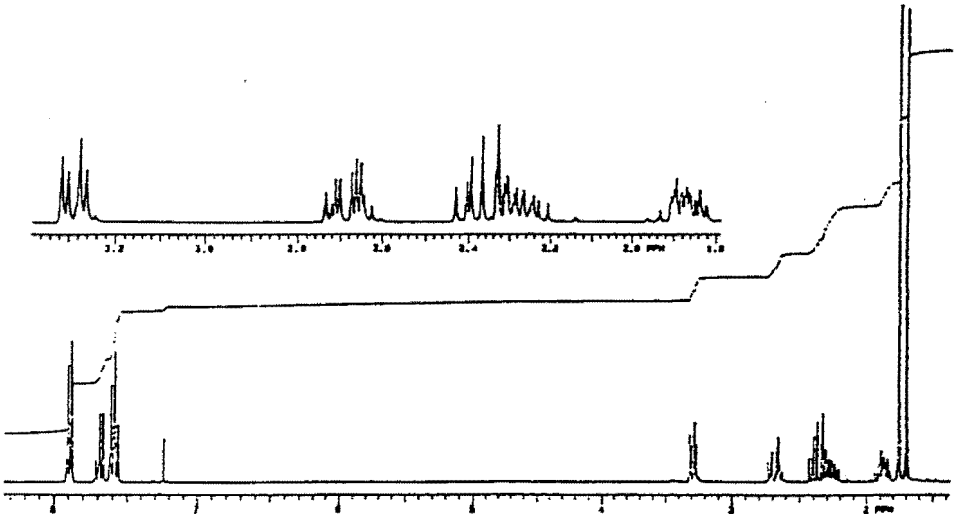

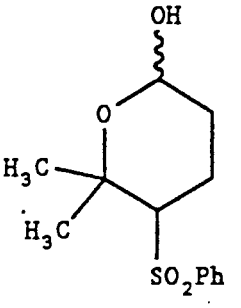

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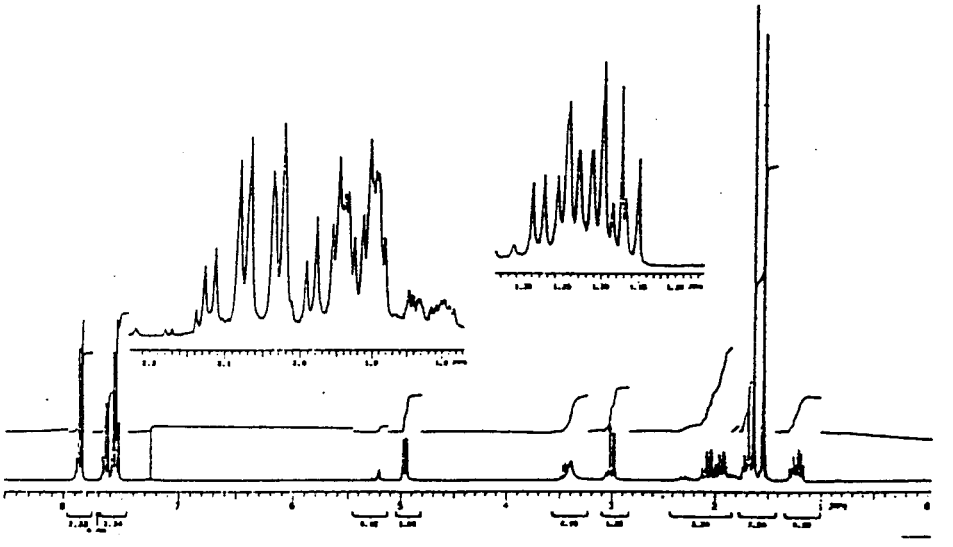

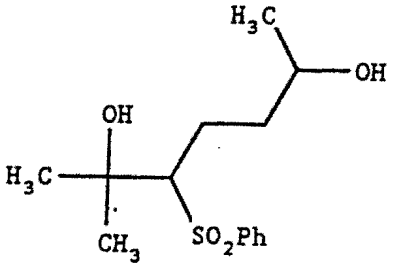

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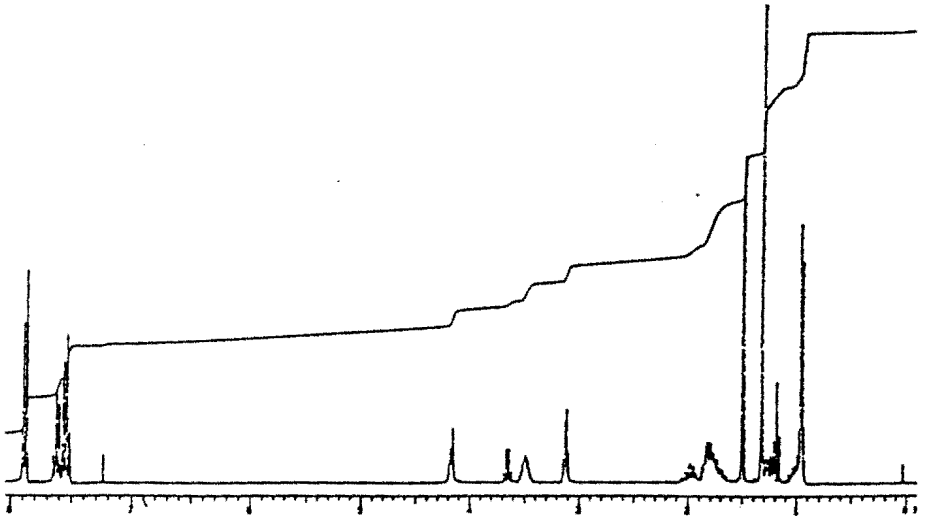

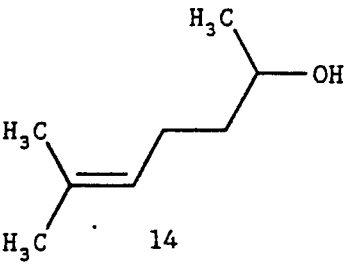

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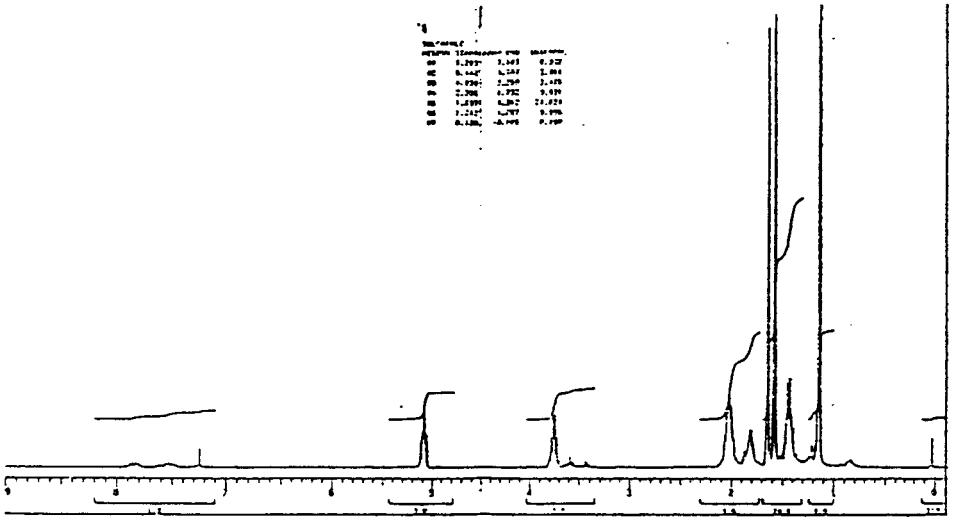

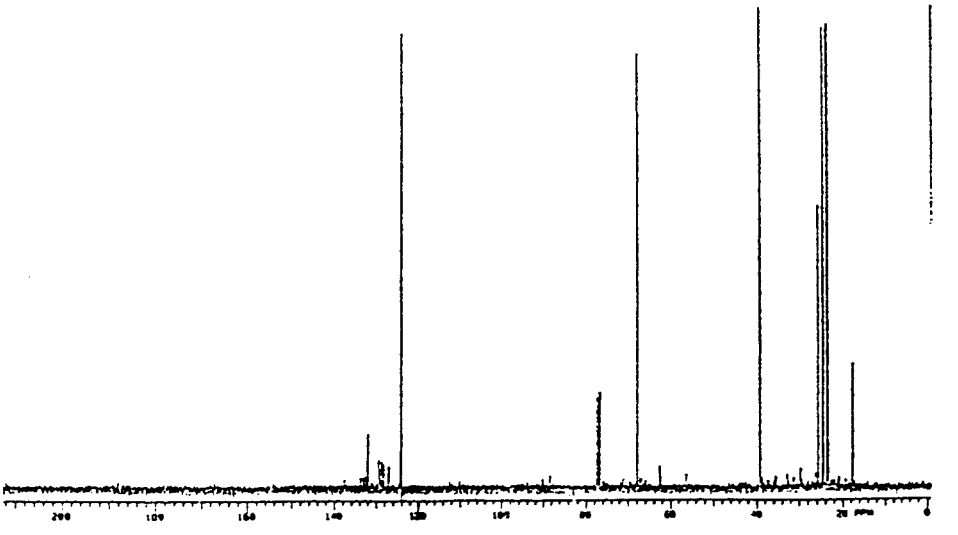

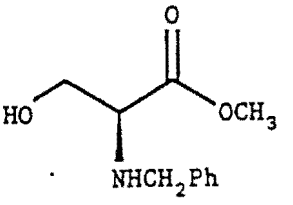

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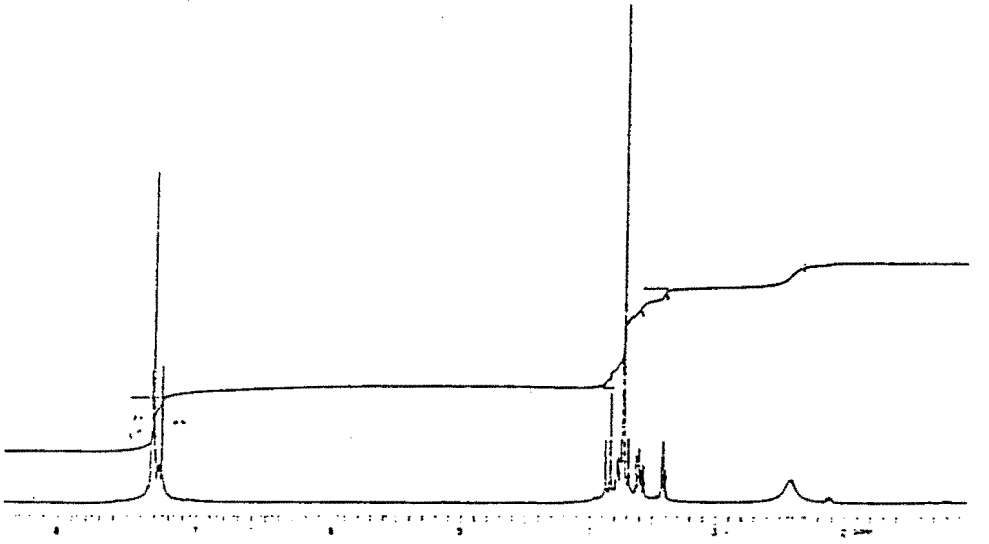

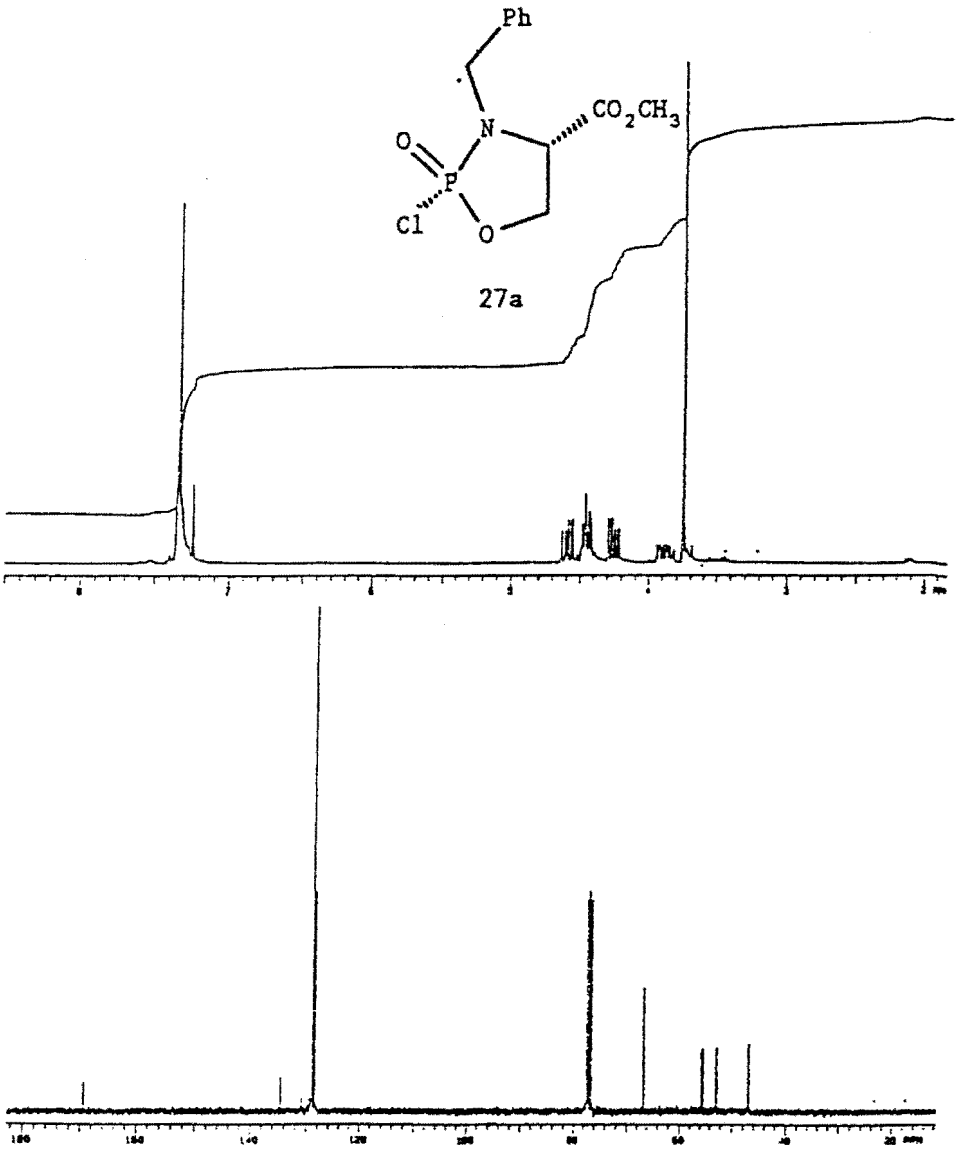

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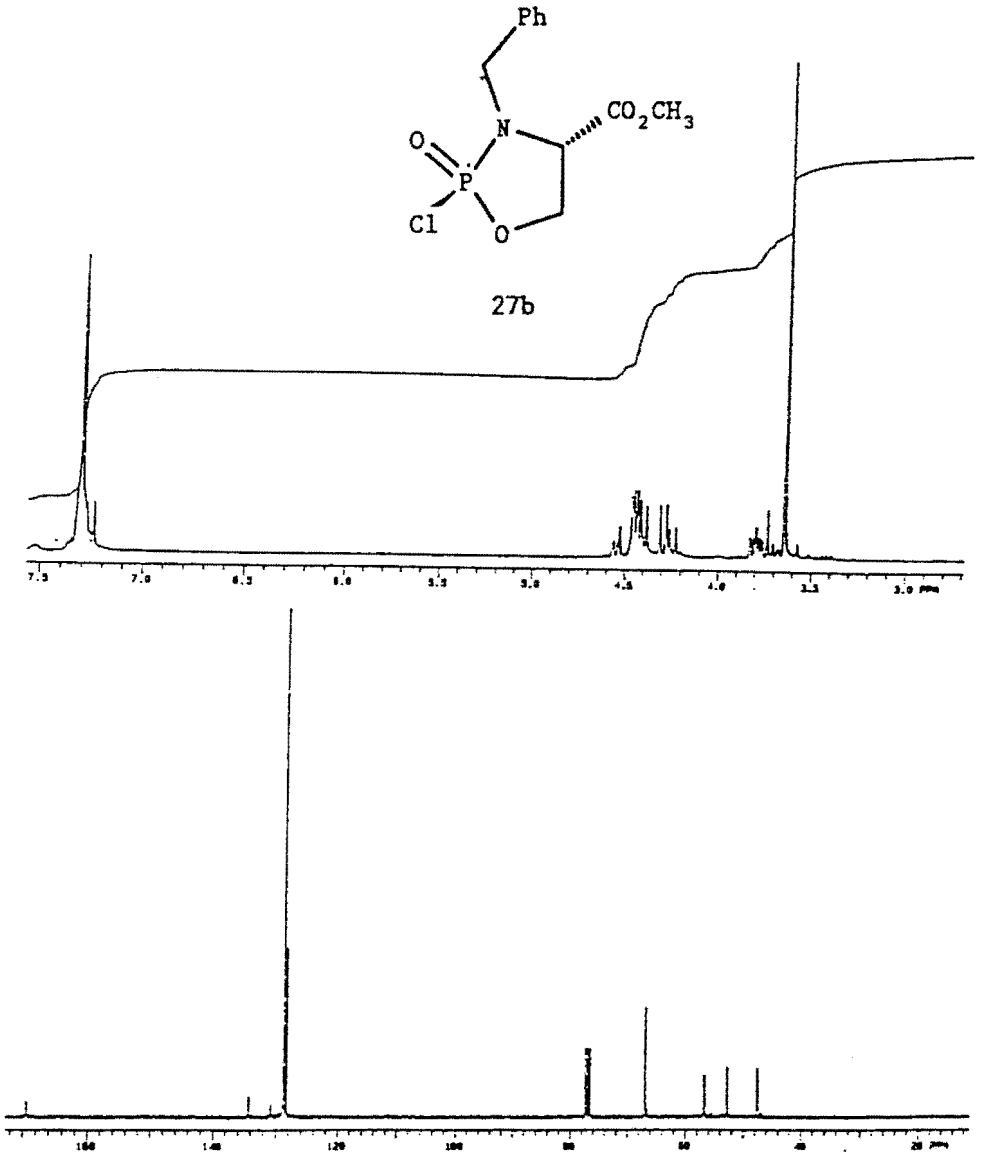

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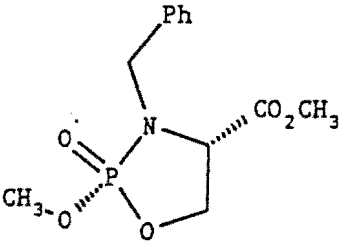

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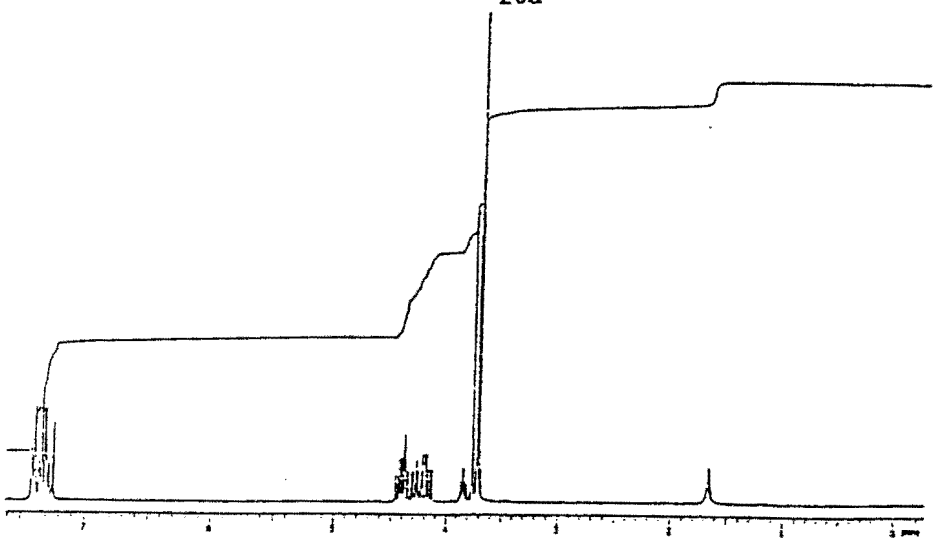

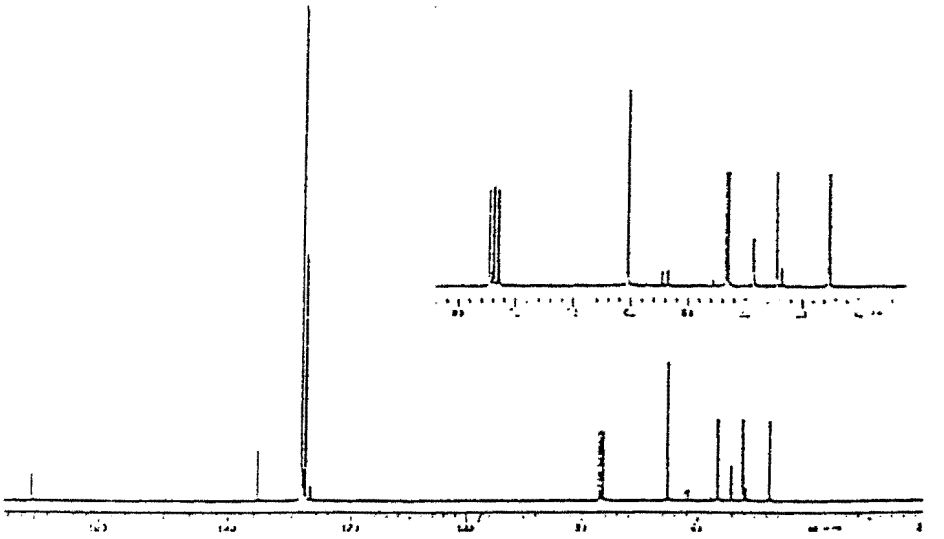

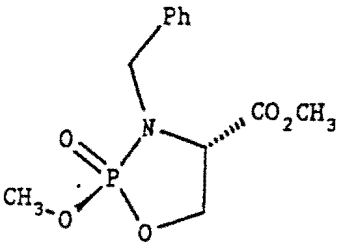

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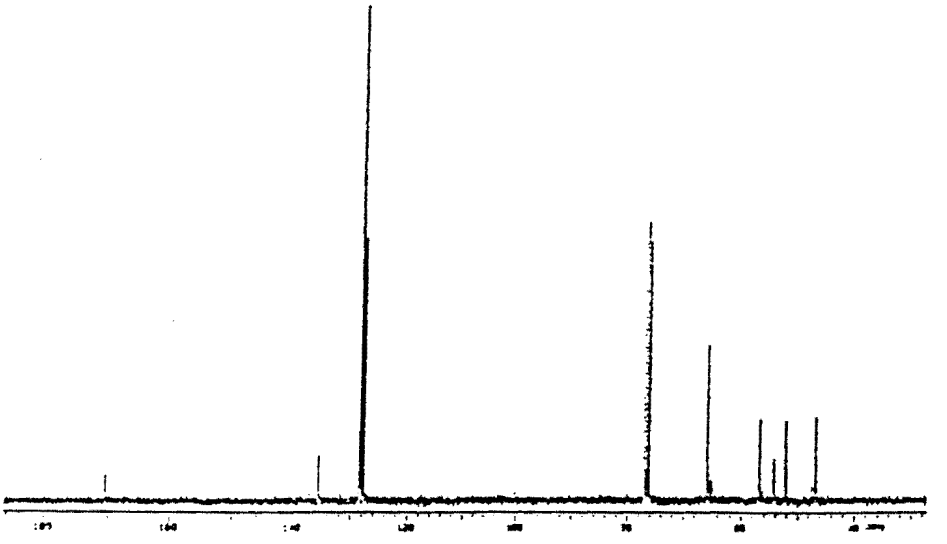

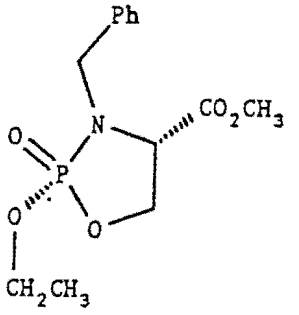

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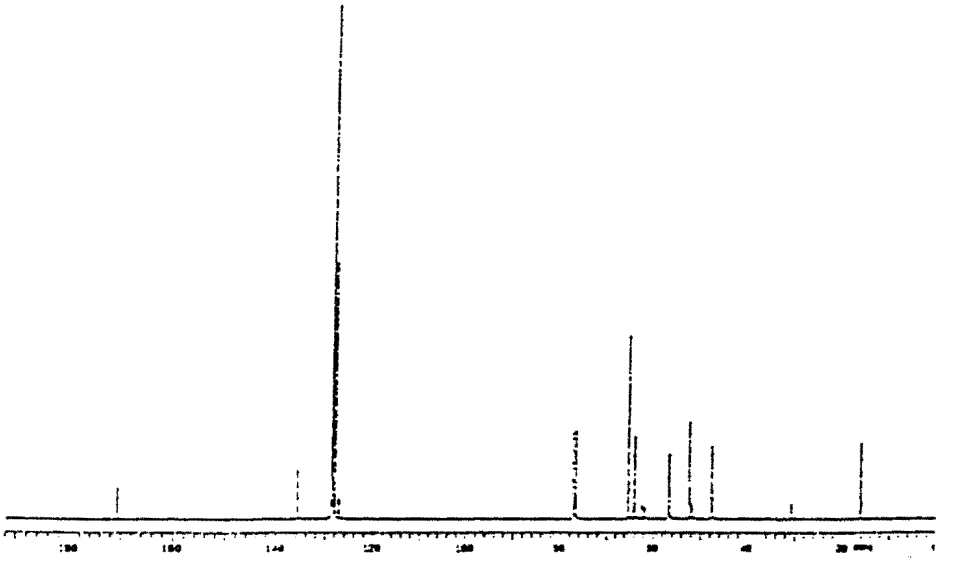

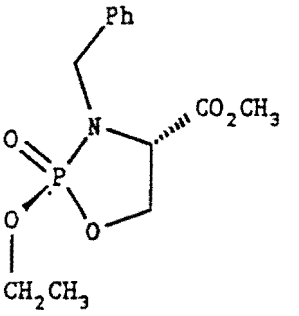

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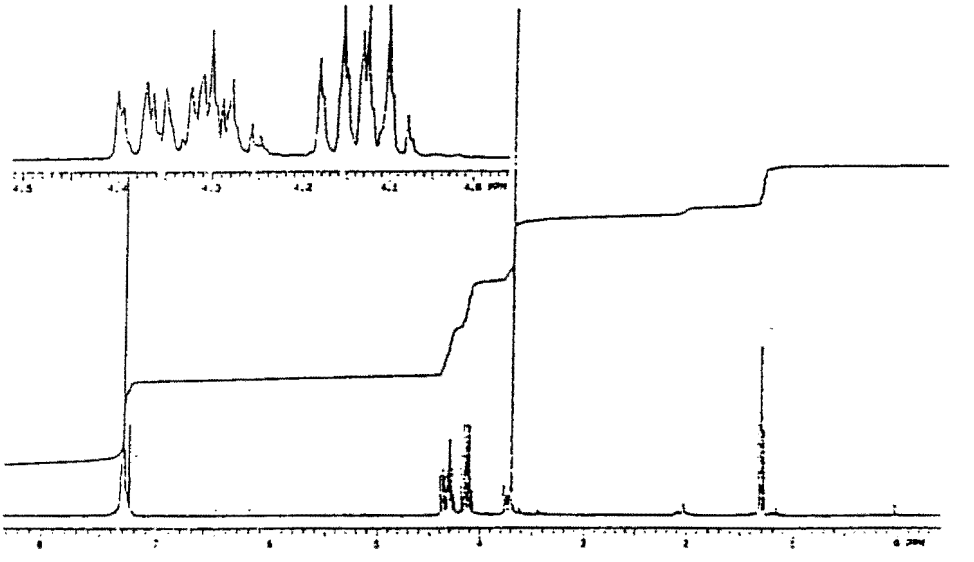

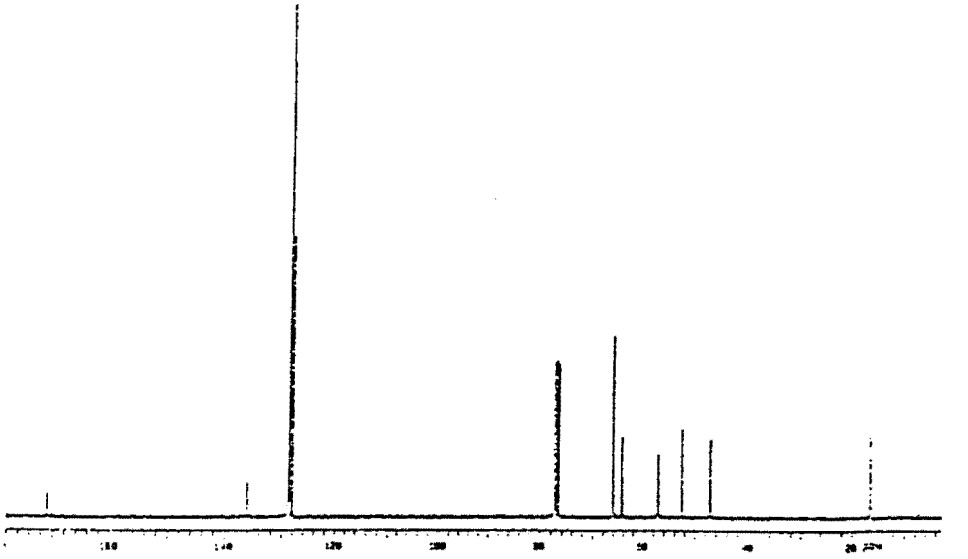

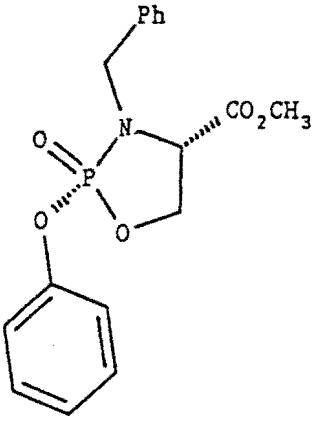
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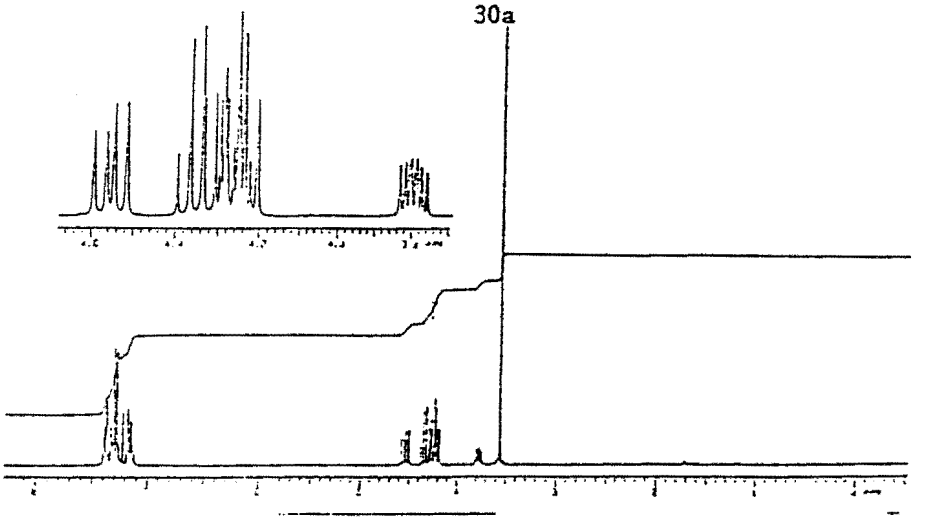
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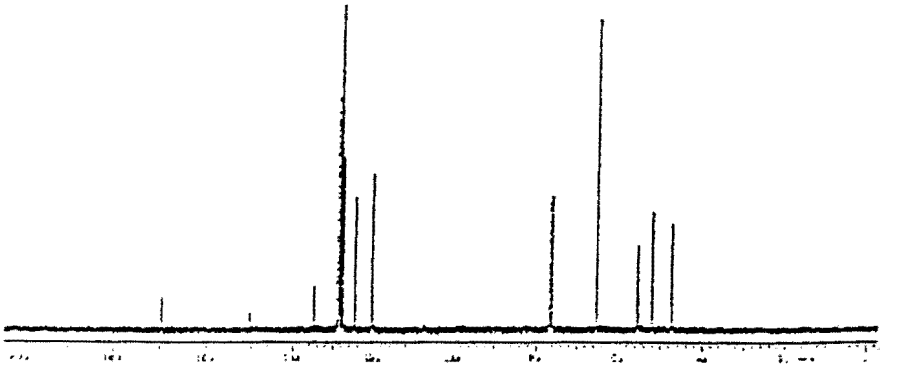

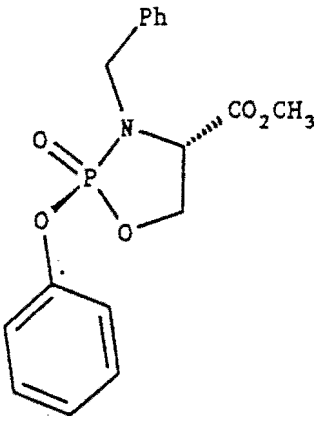

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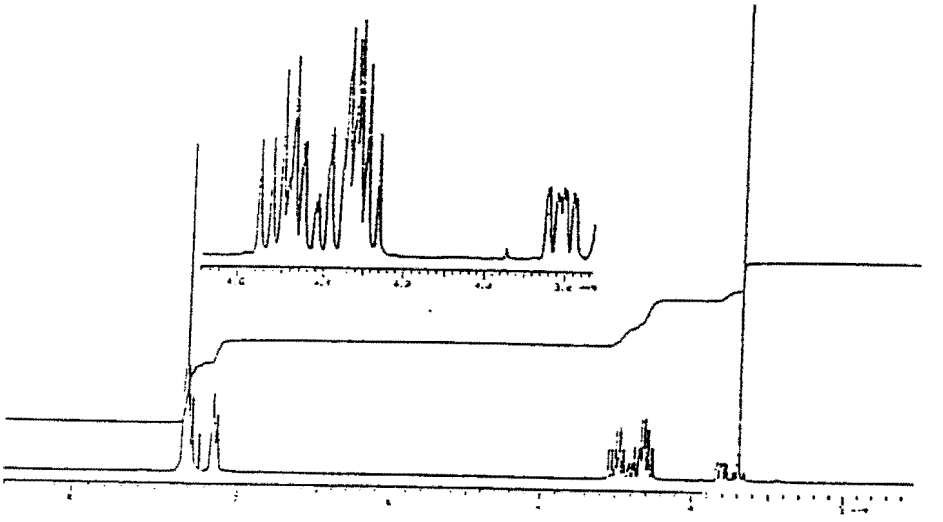

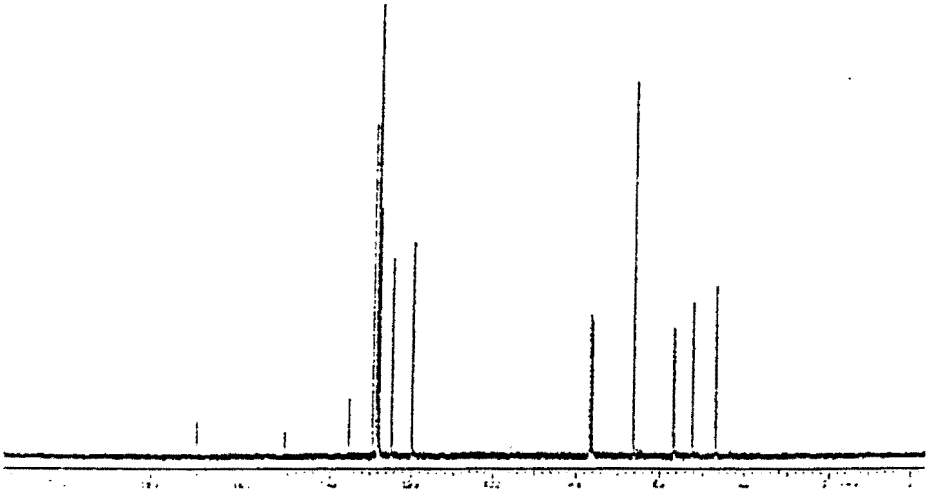

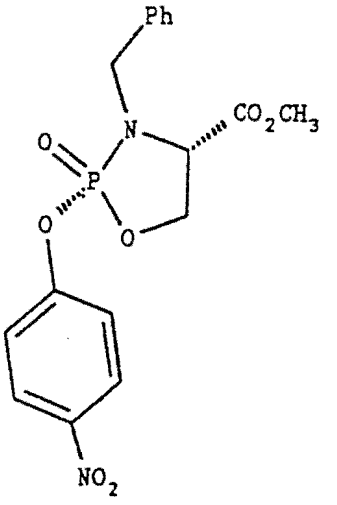

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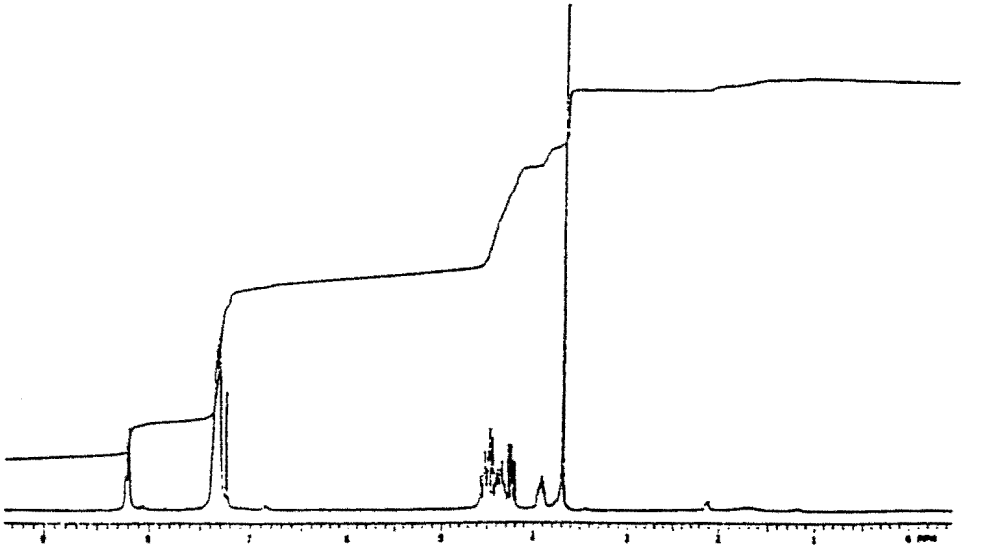

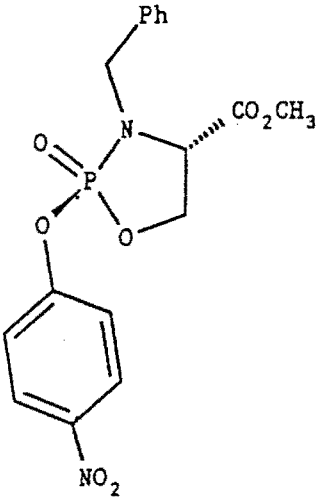

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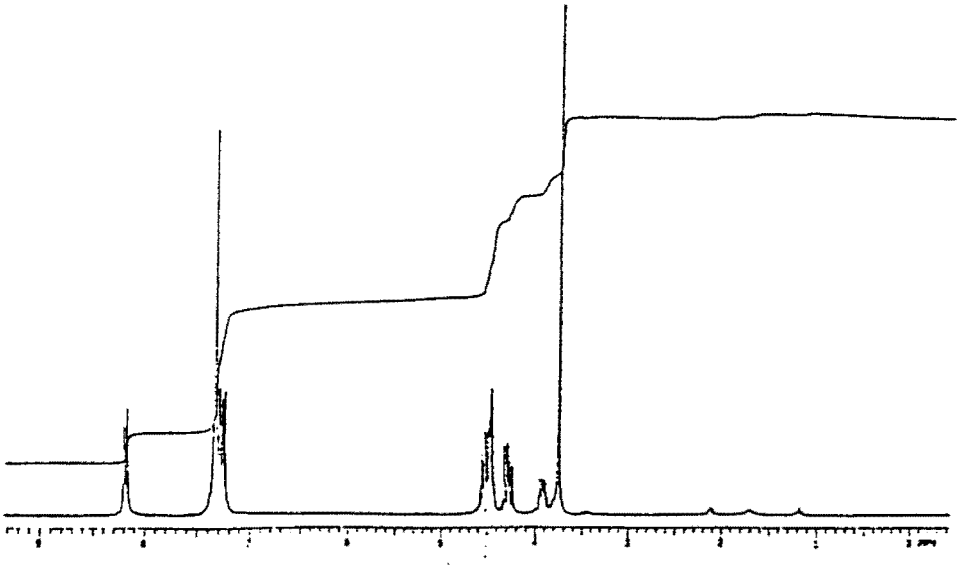

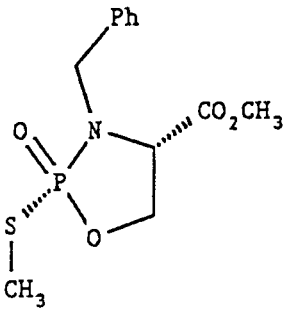

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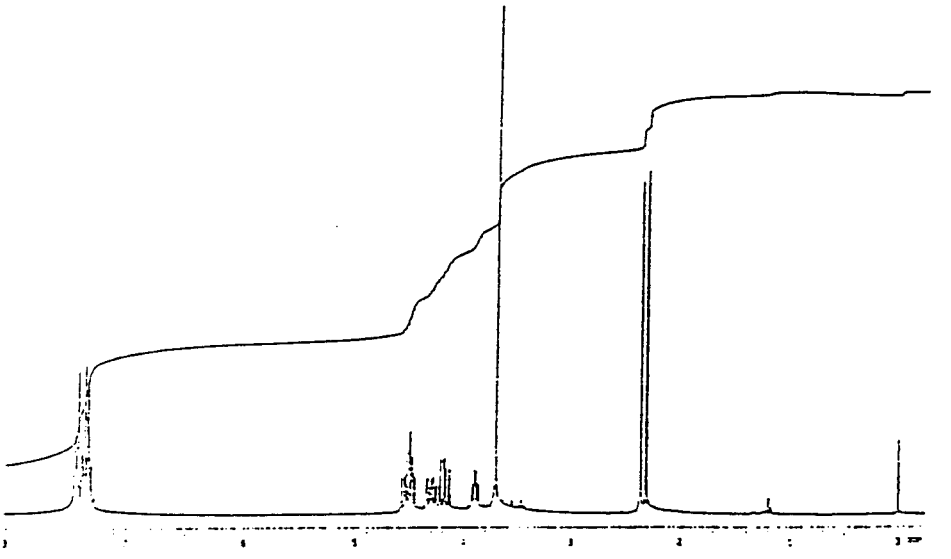

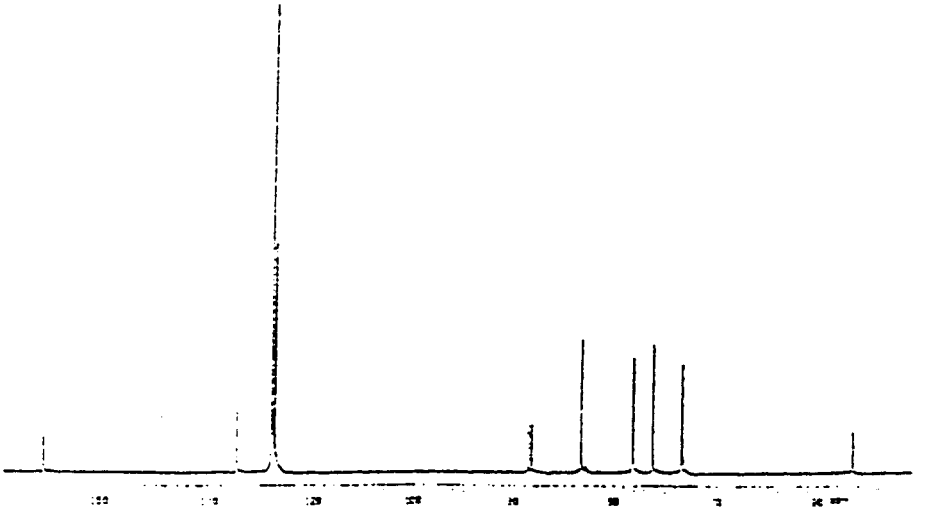

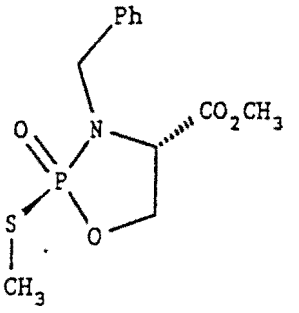

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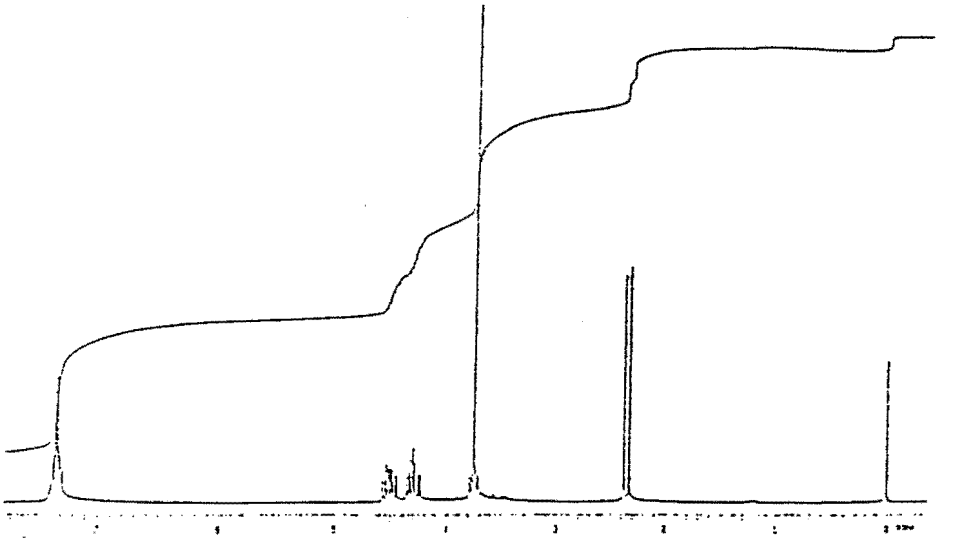

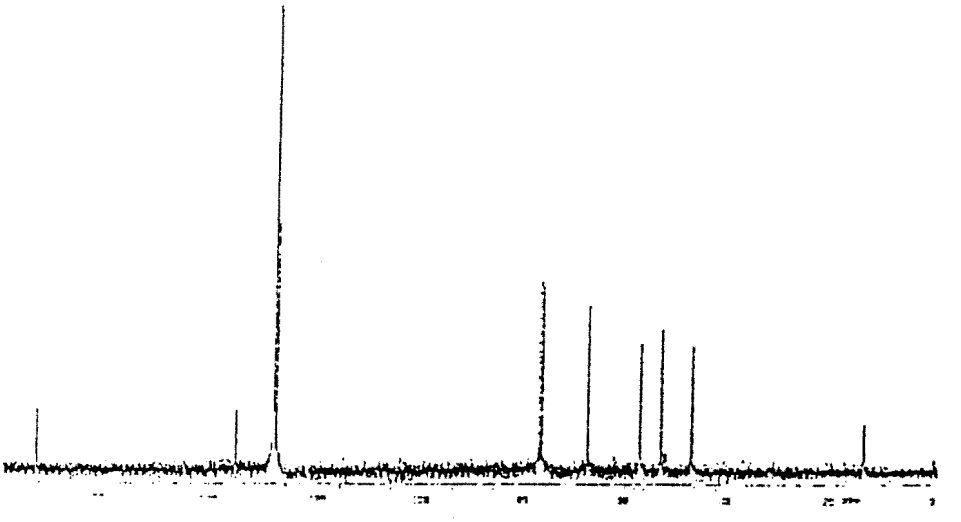

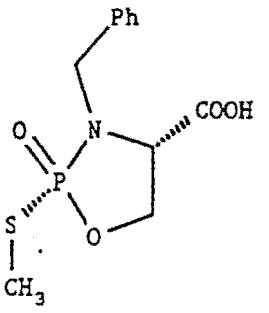

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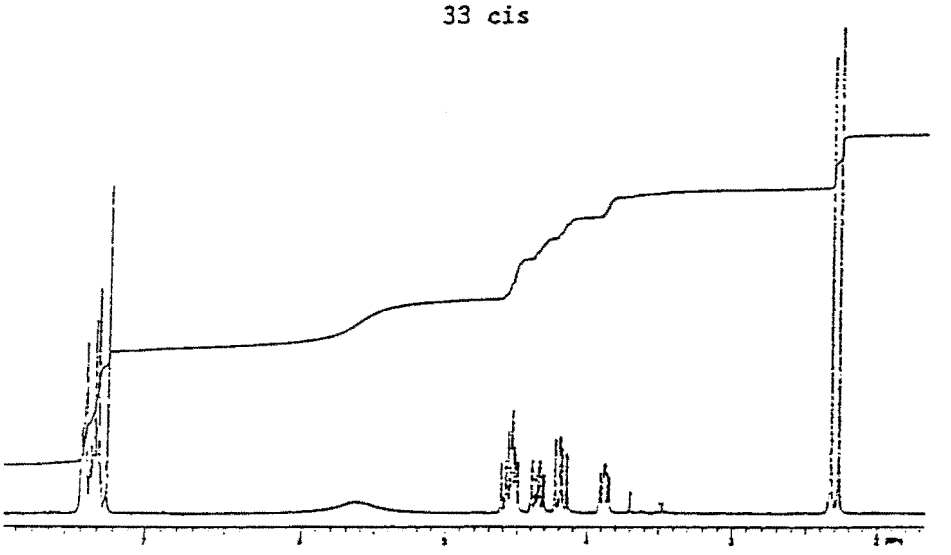

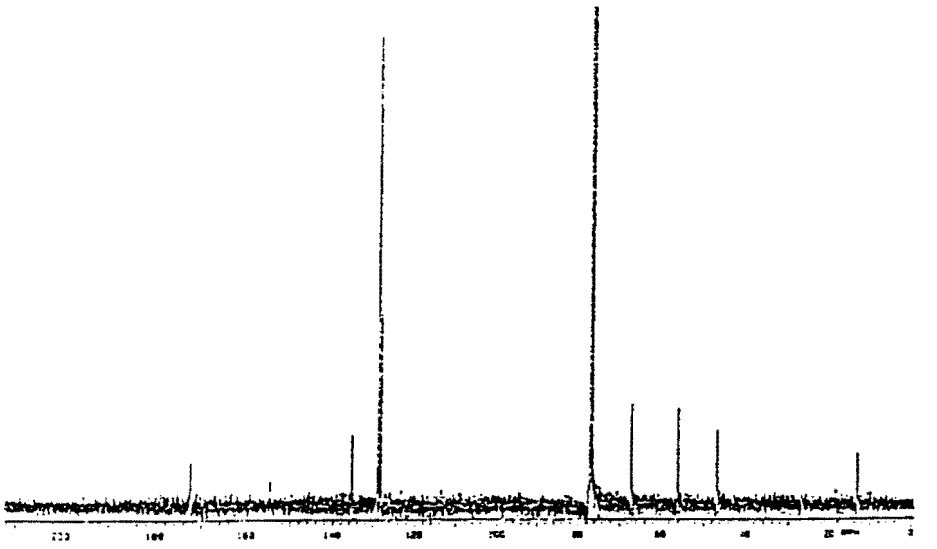

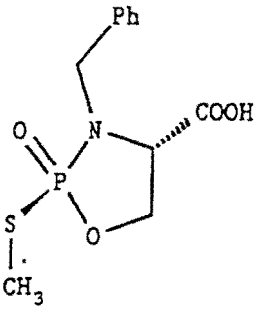

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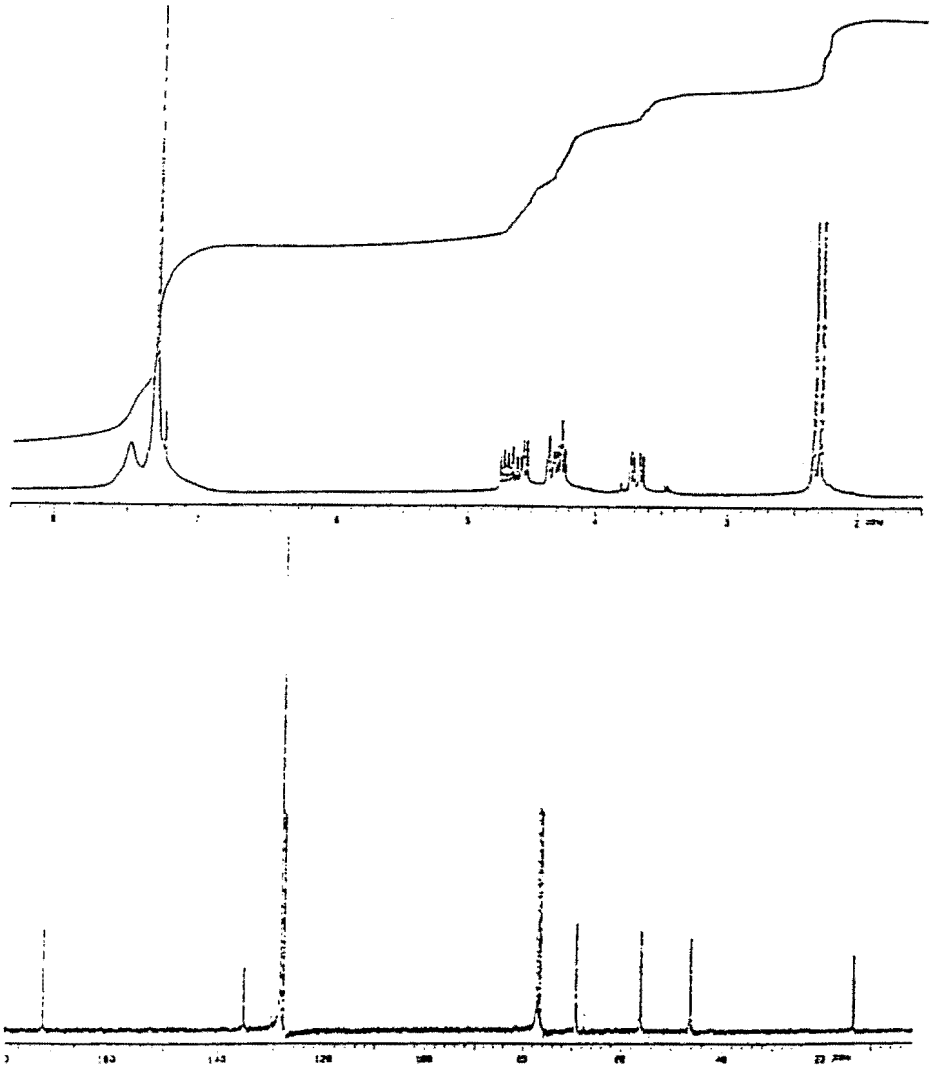

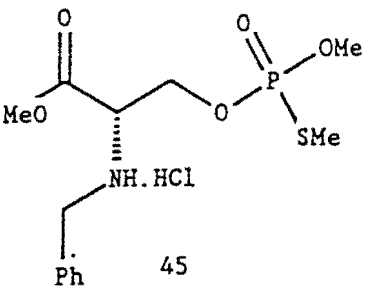

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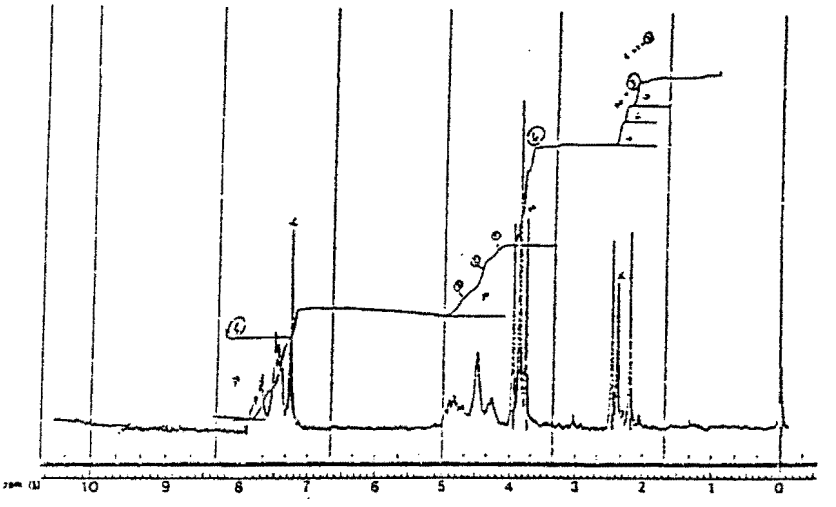

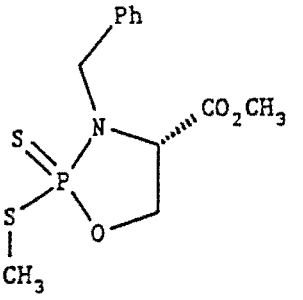

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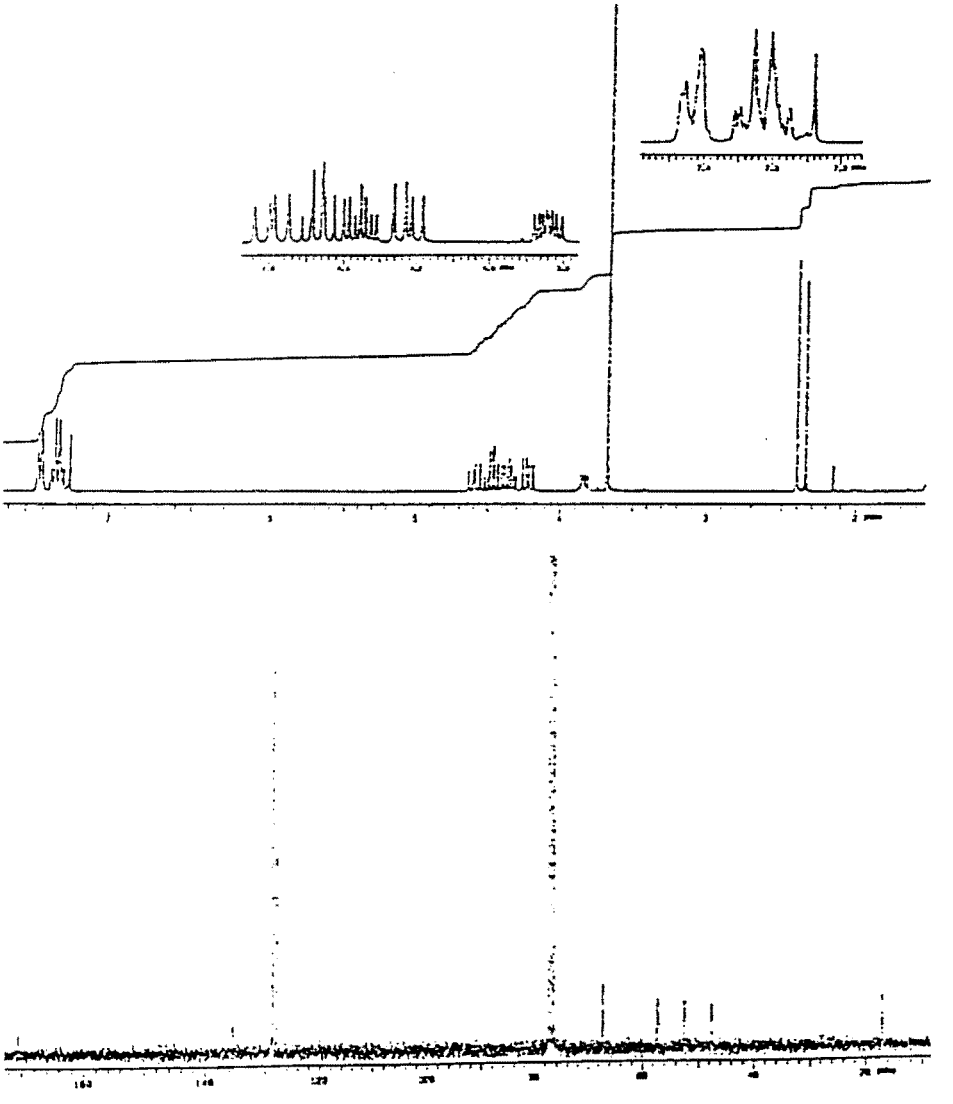
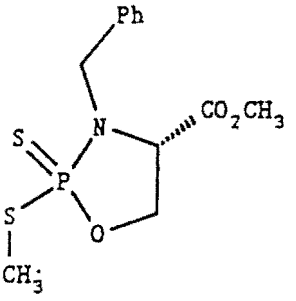
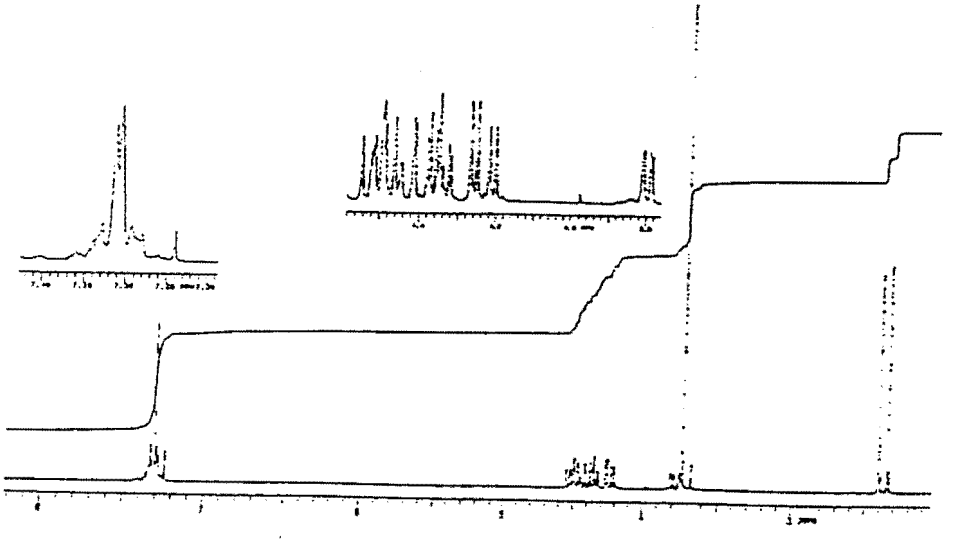
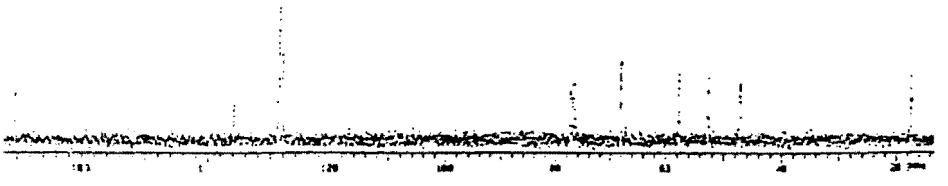
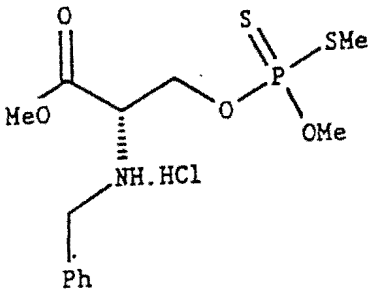




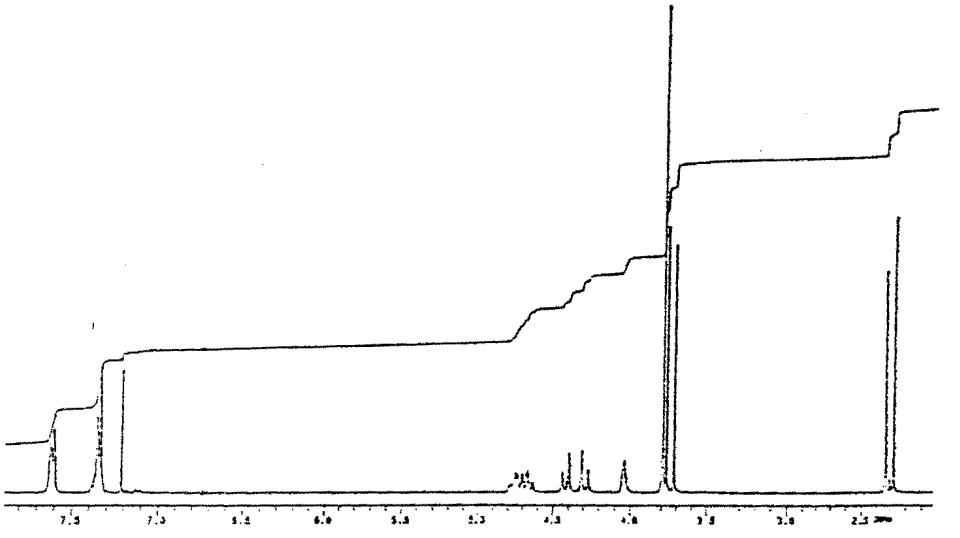
46 Slow

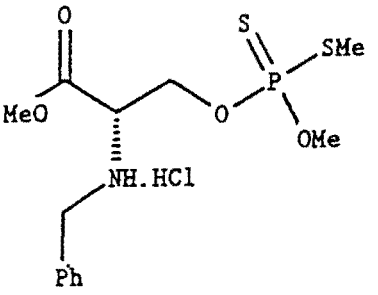




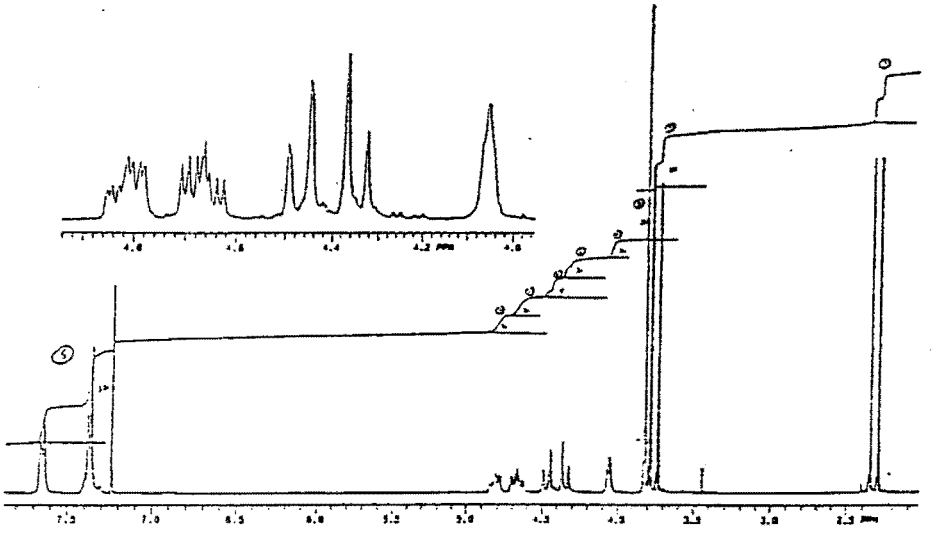


47 "fast"





47 "slow"



APPROVAL SHEET

The dissertation submitted by Jeffrey A. Frick has been read and approved by the following committee

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

This dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

6/25/1990
Date

Chuck Thompson
Directors Signature