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SYNTHESIS OF AN ORGANOPHOSPHORUS ANALOG OF ACETYLCHOLINE

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Thesis for Chemistry 499 and Research Honors: 1996-1997

Illinois Wesleyan University

APPROVAL PAGE

"Synthesis of an Organophosphorus Analog of Acetylcholine"

by Darshan Mehta

A PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR CHEMISTRY 499 AND HONORS IN CHEMISTRY

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ABSTRACT

Acetylcholinesterase (AChE) is an important enzyme in our nervous system. In normal nerve function, AChE catalyzes the hydrolysis of acetylcholine (ACh) into its respective components, choline and acetate. Recent interest has been focused on AChE because of its potential role in the pathology of neurodegenerative diseases such as Alzheimer's disease. Studies have revealed that the active site of AChE contains an esteratic and several hydrophobic/anionic subsites. AChE is inhibited by organophosphorus (OP) compounds like sarin and soman. As a result, OP compounds have been used to study the structure of AChE and the mechanism by which it catalyzes the hydrolysis of ACh. No conclusion has been made as to the stereoselectivity of the phosphorylation of AChE because recent studies have yielded conflicting results. As a result, the synthesis of a conformationally constrained analog of ACh may provide definitive information about the stereoselectivity of the mechanism of AChE phosphorylation. Furthermore, it may increase understanding in the process of aging of the enzyme after phosphorylation. We present our efforts in the synthesis of that OP inhibitor.

INTRODUCTION AND THEORY

Acetylcholine (ACh) (Figure 1, 1) is an important neurotransmitter in many organisms. In the human, it is found at a number of synapses in the peripheral nervous system as well as the central nervous system. Depending on the nature of the post-synaptic receptors, ACh may be excitatory or inhibitory in the brain. ACh is the neurotransmitter released at neuromuscular junctions in skeletal muscle. It is specifically found at the synapse between pre- and post-ganglionic neurons (Figure 2) in the sympathetic nervous system and throughout the parasympathetic nervous system.

ACh is broken down by the enzyme acetylcholinesterase (AChE) into its principle components: acetate and choline. AChE is serine hydrolase.[1] The serine residue in the active site acts as a covalent catalyst and uses water to hydrolyze acetylcholine into acetate and choline. AChE is specifically responsible for the termination of impulse transmission at cholinergic synapses. This enzyme has been a subject of interest because of its possible role in various disorders such as myasthenia gravis, glaucoma, and Alzheimer's disease. With Alzheimer's disease in particular, axons of neurons in the nucleus basalis are destroyed. These neurons are thought to use ACh as a neurotransmitter. Some success in the treatment of neurodegenerative diseases has been seen with drugs that inhibit AChE. Hence, a more complete understanding of the enzyme, and in particular, the active site, could shed light upon the nature of these diseases.

AChE is a homodimer, with the monomers being attached to each other by phosphatidylinositol and an intervening oligosaccharide through hydrophobic interactions. It has

an ellipsoidal shape with dimensions of approximately 45 Å by 60 Å by 65 Å and contains 30 percent α-helical structure and 15 percent β-sheet structure[2].

The active site of AChE is comprised of a deep, narrow gorge that is 20 Å long. It contains esteratic and anionic subsites (Figure 3). The esteratic subsite is believed to resemble catalytic subsites in other serine hydrolases. It is comprised of serine, histidine, and glutamate residues. The three residues form the characteristic catalytic triad similar to that found in other serine proteases. With serine being the residue that is ultimately responsible for the hydrolysis of acetylcholine, other groups stabilize the charge on the putative transition state by hydrogen bonding. The current mechanism for the hydrolysis of ACh involves an acyl-group transfer in which the hydroxyl group of serine is the nucleophile[3] (Scheme 1). The imidazole ring of histidine acts as an acid-base catalyst in the formation and decomposition of tetrahedral intermediates. The hydrogen bonding is further strengthened by the glutamate residue as it interacts with the hydrogen atom bonded to the nitrogen atom on the imidazole ring to stabilize the ring. In some organisms, the glutamate residue is replaced by aspartate. The function of the residue remains unaffected.

The anionic subsite was originally thought to be a site that contained multiple negative charges which would attract the positively charged quaternary ammonium group of the choline moiety. However, it is now believed that this site is, in fact, uncharged and lipophilic. There are a small number of negative charges near the catalytic site, but these are not enough to produce the type of electrostatic field observed.[2] Rather, there are fourteen aromatic residues that line a significant portion of the surface of the gorge. These aromatic residues are thought to be involved in the binding of ACh and guiding the molecule toward the esteratic site.[2] Research has

suggested that there is a preferential interaction of quaternary nitrogens, such as that of the choline moiety, with the π electrons of aromatic residues. Furthermore, the hydrophobicity of the gorge would result in a higher effective local charge because of the low dielectric constant that is produced. This would explain the strong electrostatic field that is generated, greater than what might otherwise be predicted from the nearby anionic residues.[2]

In addition to the so-called anionic subsite, there are also a variety of observed hydrophobic and anionic binding sites which either overlap or are distinct from the active site. These sites are thought to be involved in the inhibition of AChE. Known inhibitors include organophosphate compounds, acridine derivatives, and methylphosphonothioates.[2] Hence, the aromatic surface around the active site is so extensive that it seems to suggest that there are various places for substrate, inhibitors, and agonists to bind.

There are several problems with regard to the current hypothesis of the structure of the gorge. First, the studies on the crystal structure of the enzyme indicate that the active site appears to be too narrow and deep to facilitate the passage of ACh toward the esteratic subsite.[4] However, AChE must somehow be flexible enough to admit quaternary amines toward the active site. Another observation that was made was that the protein generates, as stated earlier, what appears to be a functionally important negative electrostatic field. This appears to be responsible for attracting the positively charge choline moiety of ACh. However, it does not explain the high catalytic rate of the enzyme ($k_{cat} / K_m = 9.6 \times 10^{-3}\ M^{-1}\ min^{-1}$).[2] Such a field would seem to impede the release of the product choline from the mouth of the active site.

There were suggestions that a so-called "back door" existed in the enzyme, as indicated by a molecular dynamics simulation[4]. Around the residue Trp-84, a "thin wall"[4] existed which

offered an alternative route for the passage of substrate and the escape of product. This was in addition to the traditional gorge leading to the active site as revealed by crystallographic studies. When the back door was open, it was observed that the electrostatic field lines exit preferentially through the proposed opening as opposed to the normal opening for the active site.

However, recent studies using site-directed mutagenesis have questioned this hypothesis.[5] By generating mutants at key residues (Val-129, Asp-128, Glu-82) in which the proposed back door should be closed, the study found that catalytic rate of the mutant enzymes were not significantly different from the wild type. Therefore, the proposed back door was not functionally significant to the unusually high catalytic activity of AChE.

We really do not understand the high catalytic activity of ACh because it is not well understood how ACh is brought toward the active site and how its reaction products exit the active site. Furthermore, we have not learned much about the specific geometry of the gorge in solution. To address these issues, we propose the synthesis of a novel organophosphorus (OP) inhibitor and subsequent enzymatic analysis. Several studies in the past have used OP inhibitors to study AChE.[3,5,6,7,8] Organophosphorus esters have been found to be competitive as well as irreversible inhibitors of ACh. This is because OP inhibitors have a stable tetrahedral structure and act as transition-state analogs for the mechanism of the hydrolysis of ACh.

The mechanism behind the phosphorylation of the enzyme is similar to the hydrolysis of ACh. The hydroxyl group of the serine residue attacks the electrophilic phosphorus atom on the OP inhibitor (Scheme 2). The enzyme is then phosphorylated and is unable to hydrolyze acetylcholine due to covalent modifications of the enzyme. There are then two possibilities for the fate of the enzyme. One is that the enzyme is reactivated upon dephosphorylation. The other

possibility is aging, in which the phosphorylated-AChE undergoes dealkylation. This results in an enzyme that cannot be further reactivated. Therefore, the process of aging[6] provides a method for measuring the long-term potency of the inhibitor. The greater the extent of aging implies a more potent inhibitor.

Recent studies on phosphorylation have yielded conflicting results as to the stereoselectivity of the phosphorylation mechanism of AChE. In a study by Eya and Fukuto[7], it was found that that the $(R)_p(+)$ isomer of 2,5-dichlorophenyl methyl phenylphosphonate (Figure 4) was a more potent inhibitor than its $(S)_p(-)$ isomer, using toxicological and absolute configuration studies. However, in a later study by Benschop and De Jong[8], it was reported that the P(-) isomer of the nerve gas soman (Figure 4) was determined to be a more potent inhibitor than its corresponding P(+) isomer. The P(-) isomer was calculated to have an *S* configuration, by absolute configuration studies. This study also looked at the potency of inhibitors with varying conformations at other chiral centers (e.g. soman). They determined that the influence of chirality at the other center on the potency of the inhibitor was not as great. However, the other chiral centers were not conformationally constrained. Therefore, further work must be done to determine the stereoselectivity of phosphorylation in AChE and to determine if another conformationally constrained asymmetric center might exter more influence on this process.

OBJECTIVE

We do not understand how ACh is brought toward the active site of AChE or how its components leave. Moreover, the stereoselectivity of the phosphorylation of AChE has not been established. To address these questions regarding AChE, the first phase in the project was to synthesize a novel OP analog of ACh that closely resembles the tetrahedral transition state in the hydrolysis of ACh by AChE (Scheme 1). To elucidate the stereoselectivity of AChE phosphorylation, we picked an OP compound (Figure 1, 2) that was not only chiral at the phosphorus atom, but also at another site in the molecule, the β–carbon. The pyrrolidine ring in the molecule is somewhat rigid and conformationally constrained. This should allow us to study the effect of chirality at the β-carbon without interference from any other part of the molecule, since rotation about adjacent bonds will be restricted. Hence, the proposed inhibitor should provide better understanding as to the active site geometry than would be possible from an acyclic compound. The proposed synthesis of **2** begins with the protected α-amino acid CBZ-L-proline.

RESULTS AND DISCUSSION

The synthesis of the target compound **2** is not complete (Scheme 3), but preliminary steps have been completed. The synthesis began with (S)-N-(benzyloxycarbonyl) proline (**3**). We attempted to reduce the carboxylic acid with borane-THF. This reduction proved to be extremely difficult. In the course of five trials, a mixture of products appeared which also included unreacted starting material. When the product was separated from the starting material using column chromatography, its ^{13}C and ^{1}H NMR revealed many unexpected peaks when compared to previous syntheses of the same compound.[9] Furthermore, the yields of product for each trial were nowhere near the yields of 82% and 92% obtained in previous work.

As a result, we attempted a different synthesis, to reduce the methyl ester of CBZ-proline (**5**) with sodium borohydride. Previous work[10] had demonstrated that methyl ester derivatives could be reduced to alcohols in high yields. In the first several trials, no reaction occurred. Only starting material was recovered. Hence, a stronger reducing agent was needed. Lithium aluminum hydride could not be used since it would cleave the carbamate in the benzyloxycarbonyl (CBZ) protecting group. Previous work[11] showed that the addition of calcium chloride to the solution would be able to reduce the ester to the alcohol. This is because the addition of calcium chloride to the $NaBH_4$ mixture would produce calcium borohydride in solution, which is a stronger reducing agent than sodium borohydride but weaker than lithium aluminum hydride.

This method produced the alcohol (**4**) in 68% yield and the results were reproducible. The production of the alcohol was confirmed by IR and NMR data. In the IR spectrum of the

product, there was the broad peak at 3420 cm^{-1}, which is characteristic of an O-H bond of the alcohol (Spectrum 1). There were two peaks at 1675 cm^{-1} and 1707 cm^{-1}, which appear to correspond to C=O bonds. This seemed to be a bit confusing, since the desired product should contain only one carbonyl peak corresponding to the C=O bond in the CBZ protecting group. The additional peak is probably the result of amide rotamers. In the IR spectrum (Spectrum 2) of the CBZ-proline methyl ester, there were two peaks at 1707 cm^{-1} and 1746 cm^{-1}, which would corresponding to the two carbonyl groups: one with the ester and one with the CBZ protecting group. Hence, since one wavenumber (1707 cm^{-1}) in both compounds was the same, this must correspond to the carbonyl group in the CBZ protecting group. Since the other carbonyl peak in the methyl ester was not present in the product, it seemed to verify that the product did not contain any starting material.

The NMR data was more definitive in verifying that the product was, in fact, the desired alcohol. From ^{1}H NMR data of CBZ-proline methyl ester (Spectrum 3), there was the characteristic peak at δ 3.75 corresponding to the protons on the methoxy group of the ester. This was not present in the ^{1}H NMR of the alcohol (Spectrum 4). There was also a new peak at δ 3.91 that corresponds with the proton on the hydroxyl group. Furthermore, the integration of the peaks of the alcohol revealed the correct number of protons expected. In the ^{13}C NMR spectrum (Spectrum 5) of the methyl ester, there were peaks found at δ 173.71, 173.56, 155.26, and 154.67. Probably due to rotamers, the former two peaks correspond to the carbonyl carbon in the ester linkage to be reduced and the latter two corresponding to the carbonyl carbon of the protecting group. In the ^{13}C NMR of the alcohol (Spectrum 6), there was only one peak found at δ 157.39, corresponding to the carbonyl carbon of the protecting group.

The next step in the synthesis involved phosphorylation (Scheme 3). Methyldichlorophosphine has been shown to produce phosphonochloridates[12] in high yields upon reaction with alcohols. We presumed that phosphonates could also be produced with this method. The reaction (Scheme 4) would proceed by nucleophilic attack of CBZ-prolinol on the phosphine which would displace a chlorine as chloride ion. The resulting compound would react with elemental sulfur and methanol, which would displace the other chlorine atom. Sulfur was chosen since previous work[13] indicated that the P=O bond was fairly reactive with silica. As a result, it would be difficult to separate the diastereomers using column chromatography. On the other hand, the P=S bond is not as reactive. The sulfur could later be exchanged for oxygen when the stereoisomers were isolated. Therefore, it might be much easier to separate the mixture. Moreover, the thiophosphonate compounds (**9**) could provide a basis for comparison with phosphonates in relation to the potency of the inhibitor.

This method of phosphorylation provided inconsistent results. During the five trials that were attempted, we were able to get product in only one trial. In the other trials, unreacted elemental sulfur was recovered along with some starting material. The ^{13}C NMR of the product (Spectrum 7) contained a peak at δ 155.29, which would correspond to the carbonyl peak on the protecting group. There were several peaks between δ 137.08 and 128.24, characteristic of the aromatic carbons on the protecting group. There were several new peaks as well. There were two doublets between δ 56.32 and 56.96. This could potentially correspond to the methoxy group, when compared with related compounds such as dimethyl chlorothiophosphate (Spectrum 8).[14] The methoxy peaks in this compound are found at 55.61 and 55.70. A set of peaks were also found between δ 22.54 and 23.51. When comparing them with the spectrum of

dimethylphosphinic chloride (Spectrum 9)[14], these peaks seem to correspond to the methyl group. For both the methoxy and methyl group, we see doublets. This would be expected since phosphorus has a spin of ½. Furthermore, the expected result would be a mixture of diastereomers. However, there are several anomalies with the spectral data. Although most peaks in the spectra are double in number compared to peaks in CBZ-prolinol, the carbonyl peak at δ 155.29 is a singlet. Moreover, the ^{1}H NMR data (Spectrum 10) does not provide any indication of diastereomers, and the integration of the peaks does not yield the correct number of protons. In fact, based on spectral data, it appears that the isolated material is not pure.

We attempted another method of phosphorylation using DCC coupling (Scheme 5).[15] Using a procedure from previous work[16], this method failed to provide any product. What initially appeared as product (based on TLC analysis) before work-up with 5% HCl and ethyl acetate, disappeared after chromatography. Only unreacted CBZ-prolinol was recovered. While dicyclohexylurea did precipitate out of the solution, indicating that DCC had reacted, no product was found. It is possible that this compound decomposed upon silica gel chromatography. We plan to attempt this reaction again and obtain spectral analysis of the crude product(s).

FUTURE WORK

The continuation of this project must begin with successful phosphorylation of CBZ-prolinol. It is quite possible the first method of phosphorylation yielded the desired mixture of products, but it was not produced in high enough yield. This method should be tried again, trying these or new modifications. Since methyldichlorophosphine is so reactive, it may be necessary to attempt the reaction at extremely low temperatures. Furthermore, the method with DCC-coupling should be tried again, since the potential products may have decomposed. However, instead of using silica gel to pack the column, we should use potentially less reactive adsorbents such as alumina and fluorosil. The next phase in the project would involve separating the mixture of diastereomers and eventual enzymatic analysis.

EXPERIMENTAL

Carbon & proton spectra were taken in deuterated chloroform ($CDCl_3$) on a JEOL Eclipse 270 MHz NMR. Pertinent ^{13}C NMR are listed by chemical shift. Pertinent ^{1}H NMR data are tabulated in the following order: chemical shift (ppm in delta), multiplicity (s, singlet; d, double; t, triplet; q, quartet; m, multiplet), and number of hydrogens. Infrared data (FTIR) were obtained on an ATI Mattson Genesis Series FTIR Model 9423-240-08061 spectrophotometer. Prominent IR features are listed by decreasing wavenumber (cm^{-1}).

Analytical thin layer chromatography (TLC) was conducted on an aluminum backed silica plate (Whatman). The TLC was visualized with an ultraviolet lamp and/or with 2,6-dibromoquinone chlorimide stain (a 0.5% solution of 2,6-dibromoquinone chlorimide in diethyl ether). Column chromatography was conducted on Davisil Grade 643 Type 150A 230-245 mesh silica gel (Fisher).

THF was distilled under nitrogen atmosphere from sodium/benzophenone. Methanol was distilled over calcium hydride. Triethylamine was distilled over potassium hydroxide pellets. Air and/or water sensitive techniques were conducted under a positive nitrogen atmosphere. The borane-THF [14044-65-6] was purchased from Acros Organics; CBZ-L-proline [1148-11-4], CBZ-L-proline methyl ester [5211-23-4], dichloromethylphosphine [676-83-5] were purchased from Aldrich Chemical Company.

(S)-N-(benzyloxycarbonyl)-2-hydroxymethyl-pyrrolidine (4) using Borane.

(S)-N-(benzyloxycarbonyl) proline (1.00 g, 4.0 mmol) was placed in a dry round-bottom flask equipped with a magnetic stirring bar and a rubber septum. Approximately 20 mL of THF was added. The resulting solution was placed under a positive nitrogen atmosphere and cooled to 0 °C. Borane-THF (1M, 11.5 mmol, 11.0 mL) was added to the solution in a dropwise manner. Upon completion of the addition of borane-THF, the reaction mixture was allowed to warm to room temperature. Over the course of 48 hours, the progress of the reaction was monitored using TLC on 1:1 ethyl acetate/hexane. There were two spots, one which corresponded to starting material and the other which corresponded to product. The reaction mixture was partitioned with 50 mL diethyl ether and 50 mL H_2O. The organic layer was dried over sodium sulfate, filtered, and concentrated. The yellow oil was purified by column chromatography to give an undesired mixture of products as an oil.

(S)-N-(benzyloxycarbonyl)-2-hydroxymethyl-pyrrolidine (4) using Sodium Borohydride

(S)-N-(benzyloxycarbonyl) proline methyl ester (2.50 g; 9.5 mmol) was placed in a dry round-bottom flask equipped with a magnetic stirring bar and a rubber septum. Approximately 10 mL THF and 20 mL dry ethanol along with calcium chloride (2.07 g; 19.0 mmol) were added. This was followed by the addition of sodium borohydride (1.45g, 38.0 mmol) to the solution. The mixture was then stirred overnight. The progress of the reaction was monitored using TLC, which revealed two spots. One spot corresponded to the starting material ($R_f = 0.46$). The mixture was then poured into citric acid (1 M, 40 mL). This was followed by separation of the mixture using 20 mL ethyl acetate. The organic layer was removed and the aqueous layer was

washed again with 20 mL ethyl acetate. The combined organic layers were then washed with 20 mL saturated NaCl, and then dried over anhydrous sodium sulfate. The organic layer was filtered and then rotovaped. The yellow oil was purified by column chromatography in 1:1 hexane/ethyl acetate. The fractions with the desired product were rotovaped and left under vacuum overnight to give (S)-N-(benzyloxycarbonyl) prolinol (1.53 g, 68%) as a pale yellow oil (R_f 0.36 with 1:1 ethyl acetate/hexane). ^{1}H NMR: δ 1.53-2.04 (m, 4H), 3.32-3.63 (m, 4H), 3.91 (s, 1H), 4.51 (s, 1H), 5.11 (s, 2H), 7.29-7.34 (m, 5H). ^{13}C NMR: δ 23.66, 28.19, 47.09, 60.45, 66.44, 67.10, 128.12, 128.28, 128.73, 136.81, 157.39. FTIR: 3420, 1707, 1675 cm^{-1}.

Phosphorylation of CBZ-Prolinol using Methyldichlorophosphine (9)

Methyldichlorophosphine (100 mg, 0.86 mmol) along with triethylamine (172 mg, 1.72 mmol) were dissolved in 10 mL dry THF and placed under a positive nitrogen atmosphere in a round-bottom flask with a rubber septum and magnetic stir bar. The solution was cooled externally in solution of dry ice and acetone. (S)-N-(benzyloxycarbonyl) prolinol (100 mg, 0.43 mmol) dissolved in 2 mL dry THF was added dropwise. The solution was allowed to go to room temperature. Then methanol (59 mg, 1.85 mmol) and elemental sulfur (35 mg, 1.08 mmol) were added simultaneously. The reaction was stirred overnight and monitored with TLC. The excess of sulfur was removed by filtration through a pad of Celite, which was washed with hexane and chloroform. The solvents were partially evaporated to a solution of approximately 3 mL. The residue was diluted with chloroform and washed with saturated sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, evaporated, and placed under vacuum overnight. The product (50 mg) was isolated by column chromatography using silica gel and ethyl acetate

(R_f 0.63 in ethyl acetate). ^{1}H NMR: δ 0.79-0.91 (m, 1H), 1.21-1.28 (m, 1H), 1.64-1.95 (m, 6H), 3.42 (s, 2H), 3.96-4.13 (m, 3H), 5.11 (s, 2H), 7.29-7.34 (m, 5H). ^{12}C NMR: δ 22.54, 23.35, 27.25, 28.16, 46.53, 46.90, 56.32, 56.84, 56.96, 65.83, 66.66, 66.93, 128.24, 128.39, 128.73, 137.06, 155.29.

Phosphorylation of CBZ-Prolinol using Dicyclohexylcarbodiimide

CBZ-Prolinol (100 mg, 0.43 mmol) was dissolved in 3 mL of dry pyridine and placed under a positive nitrogen atmosphere in a round-bottom flask with a rubber septum and magnetic stir bar. To the solution was added dicyclohexylcarbodiimide (89 mg, 0.43 mmol) and methylphosphoric acid (41 mg, 0.43 mmol). The reaction was stirred overnight and monitored with TLC. The precipitate was removed by filtration through a pad of Celite, which was washed with ethyl acetate. The solution was worked up with 5% hydrochloric acid, and the organic layer was washed twice with brine. The organic layer was dried over anhydrous sodium sulfate, evaporated, and placed under vacuum overnight. Only starting material was recovered. No product was found.

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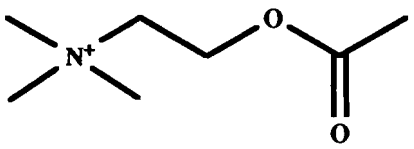
APPENDIX I

FIGURES

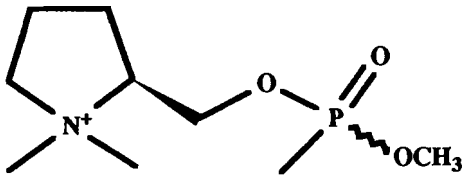
Figure 1

Acetylcholine and Target Compound



1

Acetylcholine

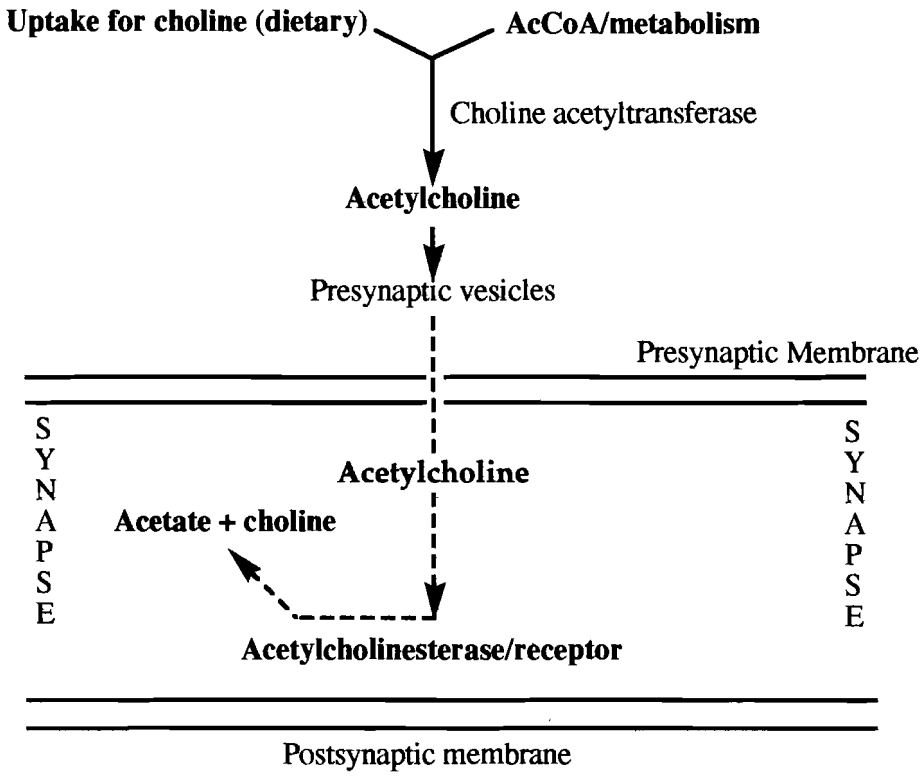


2

Proposed Organophosphorus Inhibitor

Figure 2

Schematic of Synapse



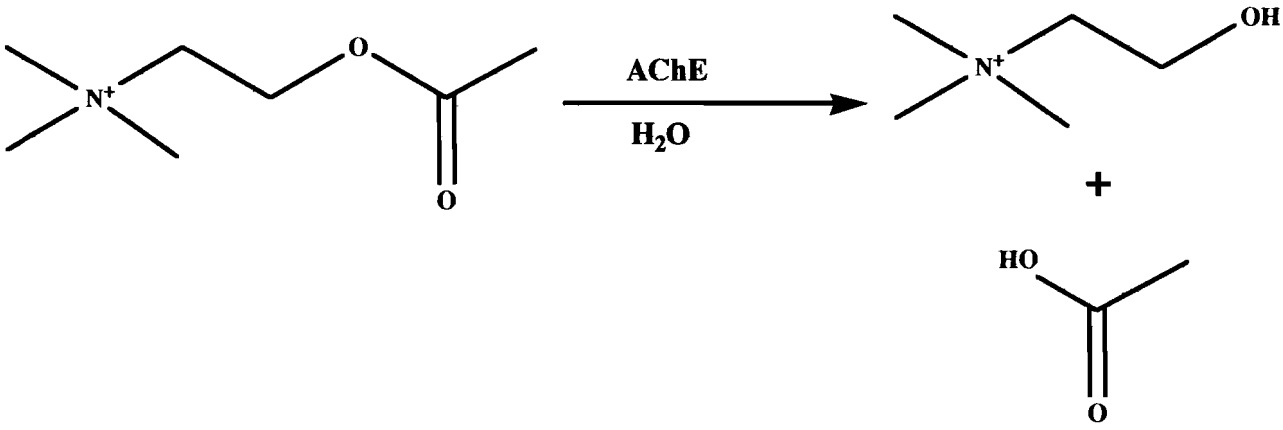


Figure 3

A Schematic of the Acetylcholinesterase Active Site

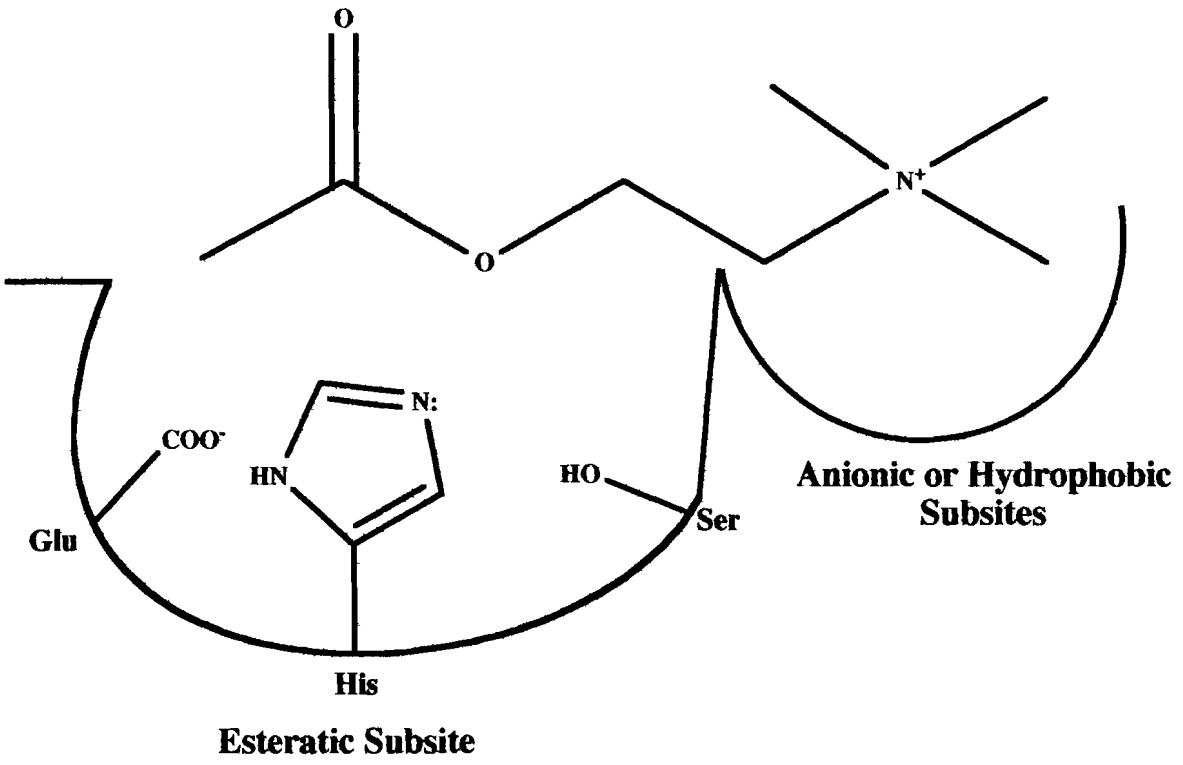
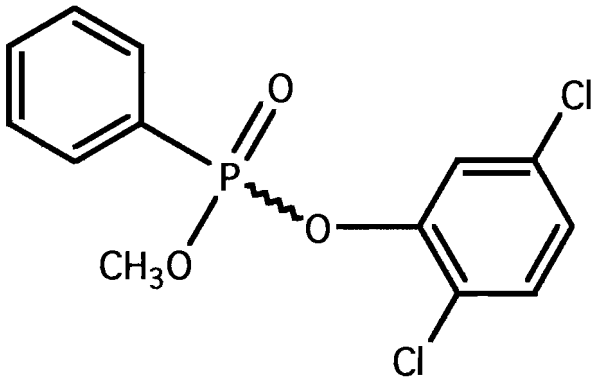
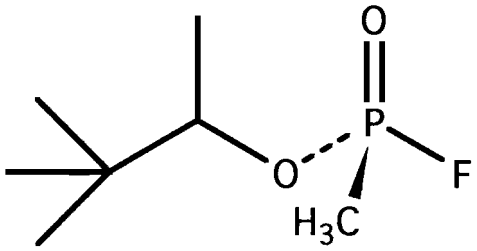


Figure 4

Organophosphorus Inhibitors of Acetylcholine



2,5-dichlorophenyl methyl phenylphosphonate



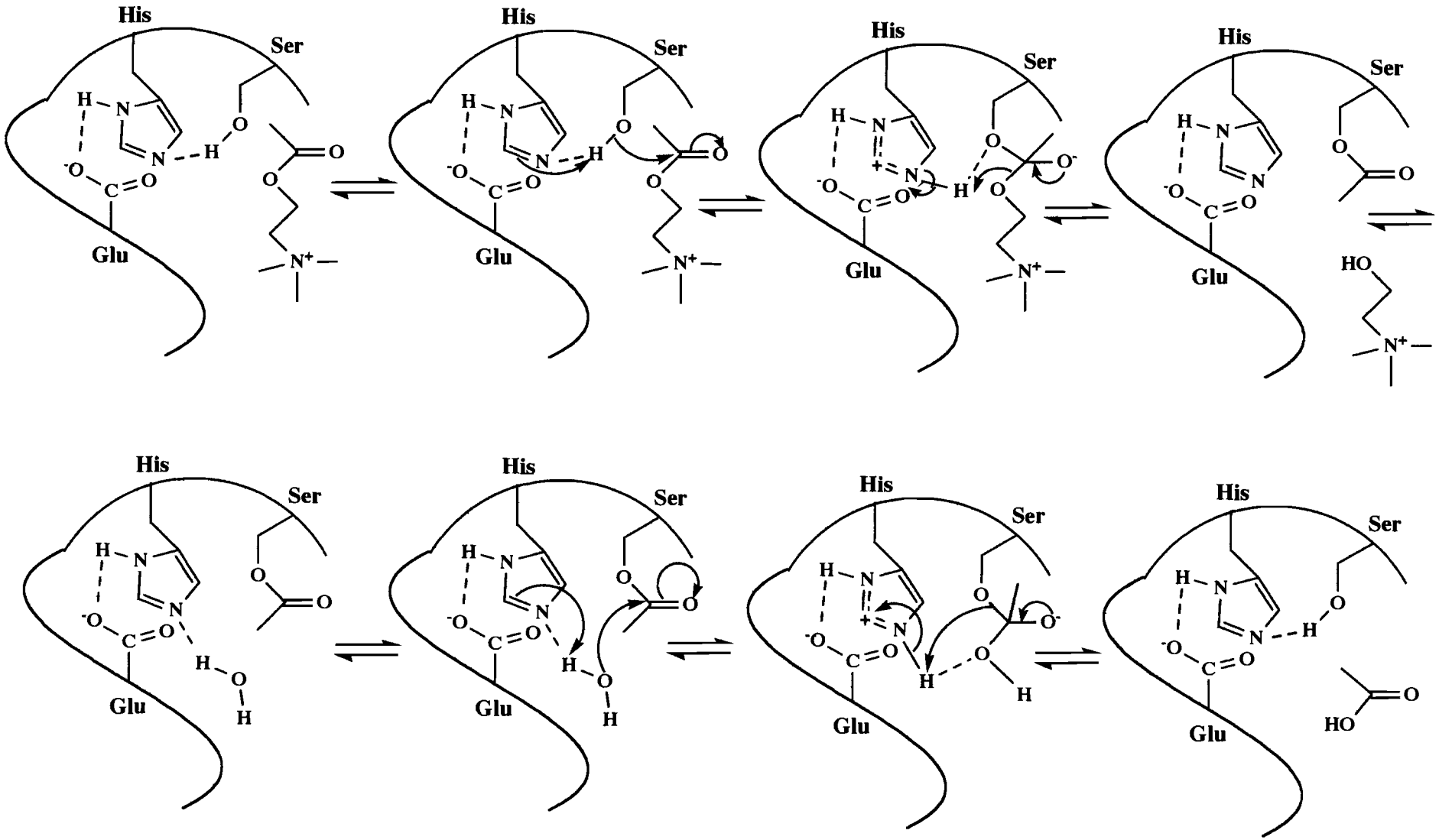
***O*-(1,2,2-trimethylpropyl) methylphosphonofluoridate (soman)**

APPENDIX II

SCHEMES

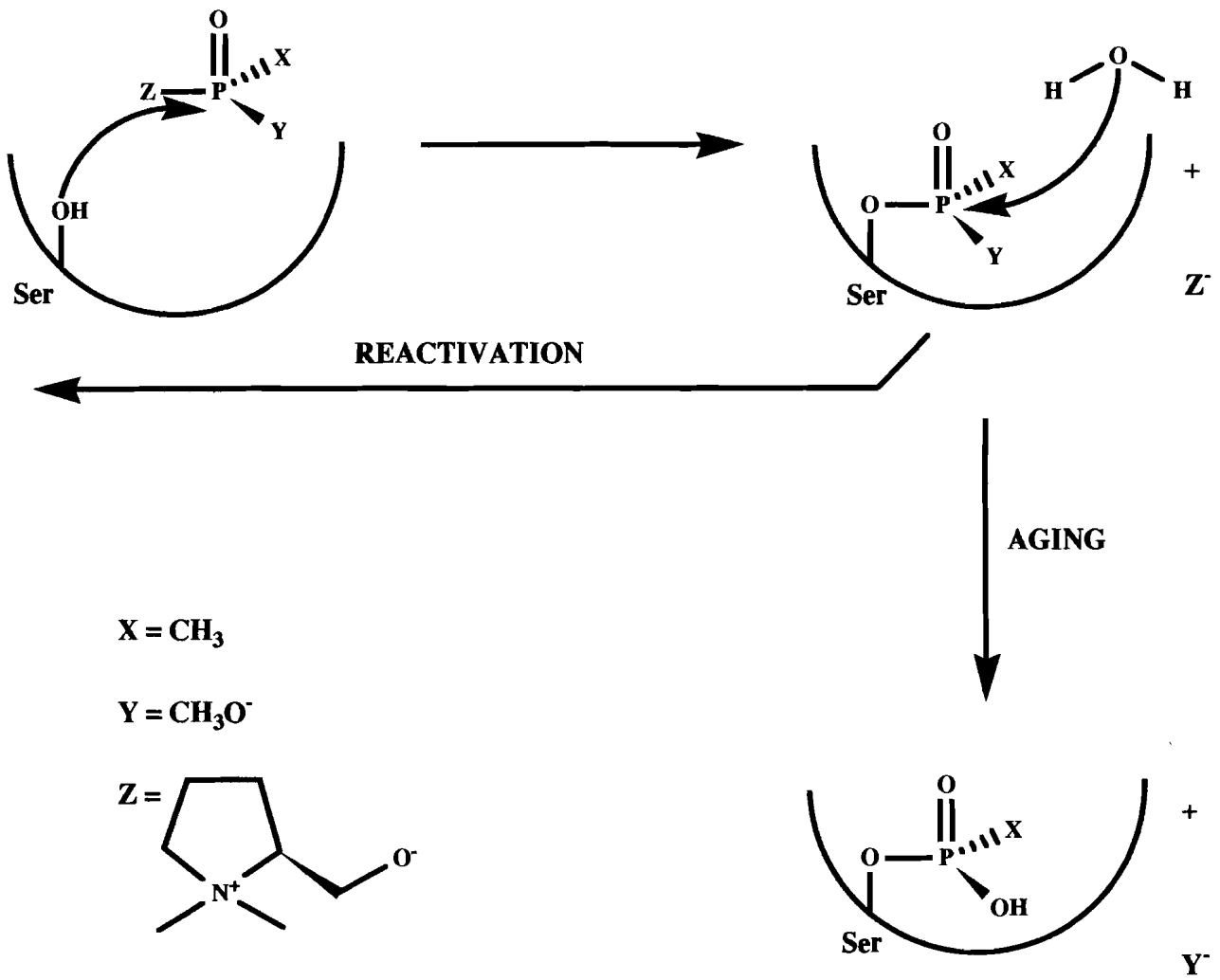
Scheme 1

Mechanism for the Hydrolysis of Acetylcholine

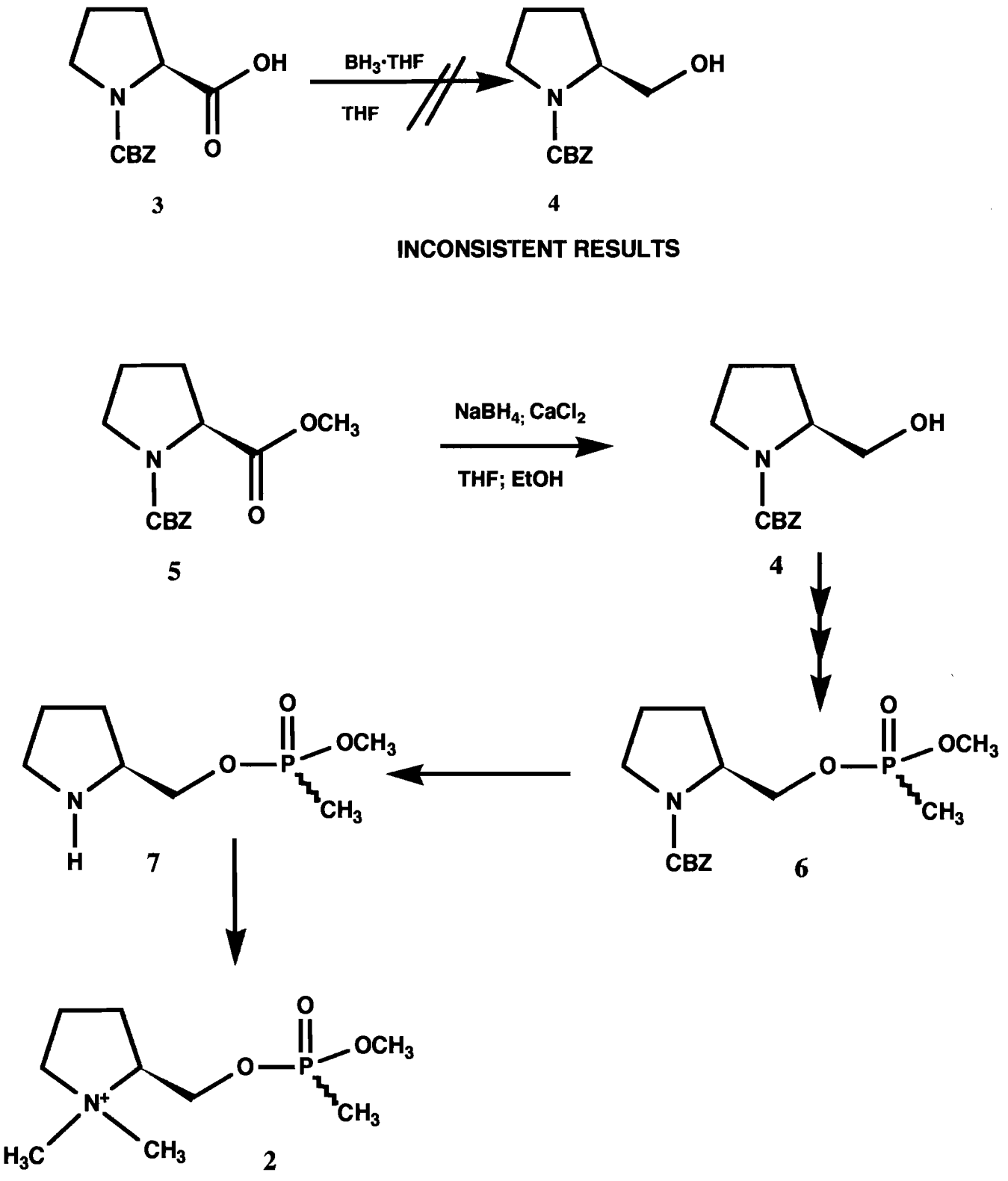


Scheme 2

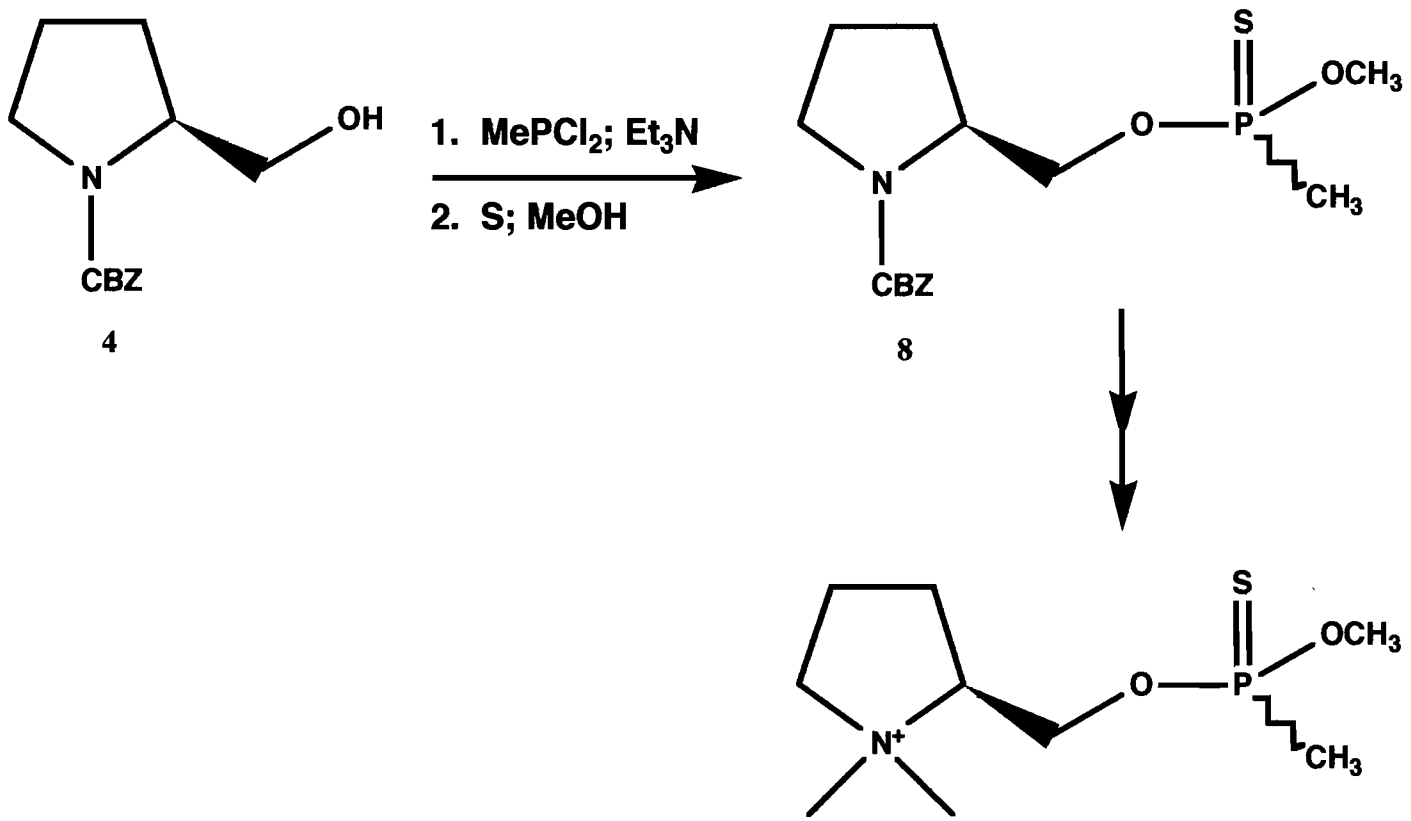
Phosphorylation of Acetylcholinesterase



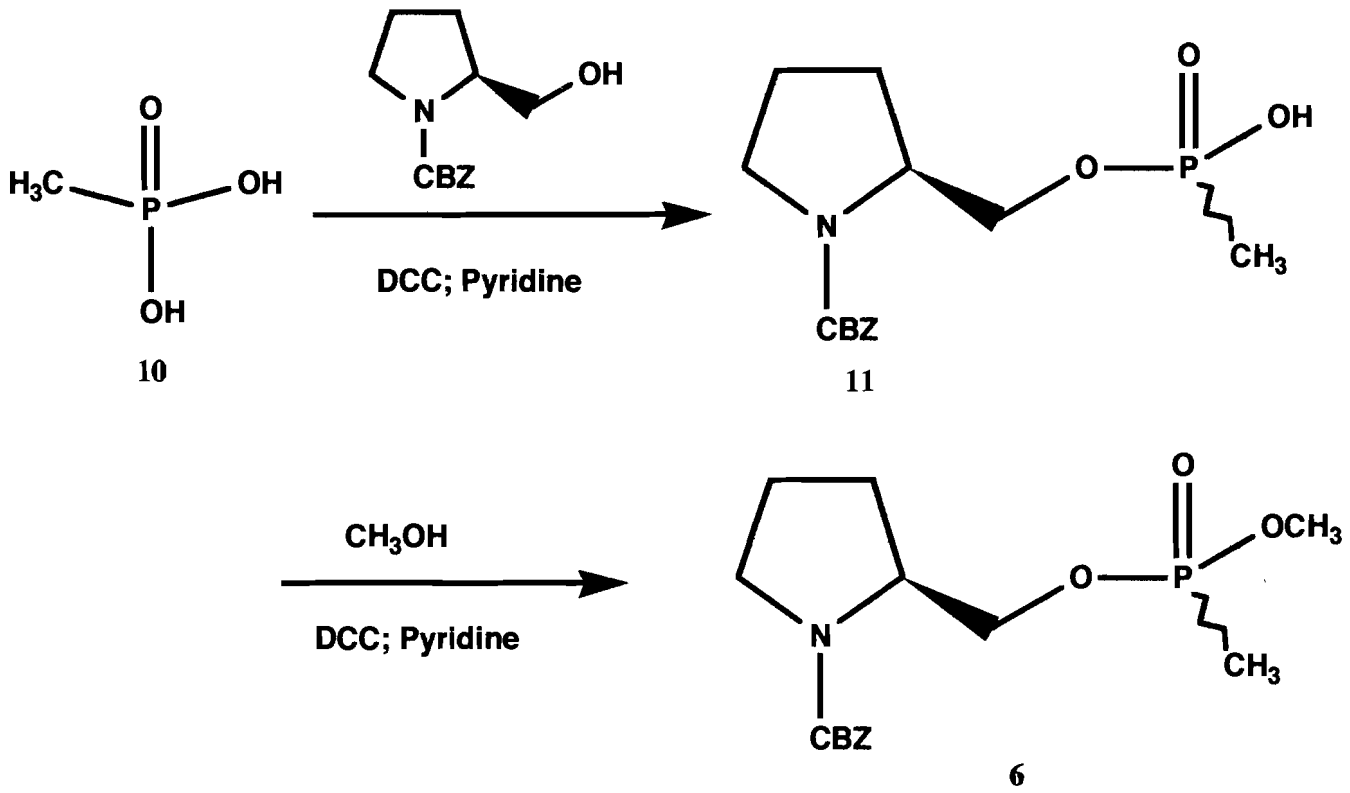
SCHEME 3
Schematic Overview



SCHEME 4
Phosphorylation using Methyldichlorophosphine



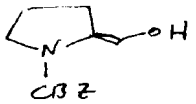
SCHEME 5
Phosphorylation by DCC-coupling



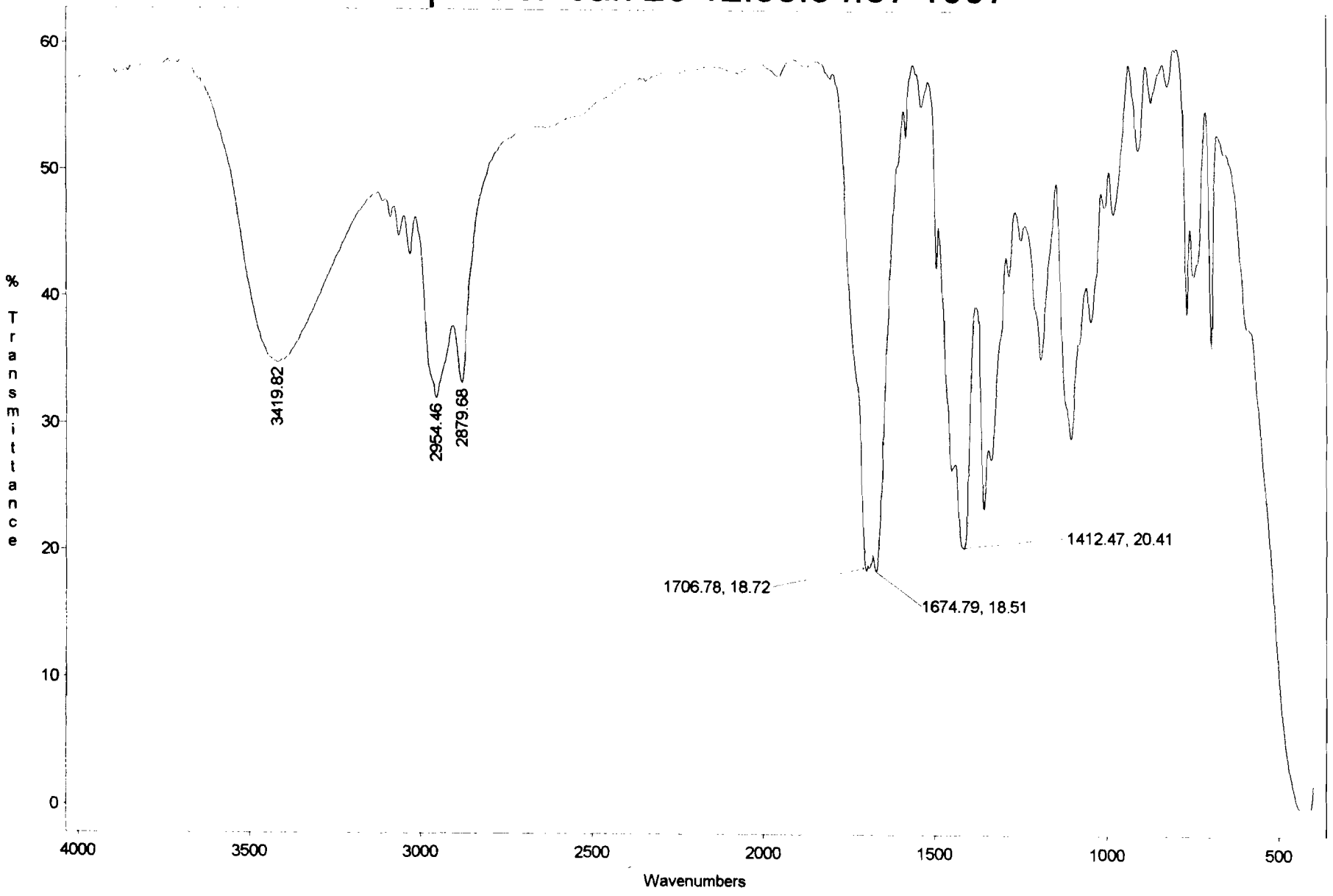
APPENDIX III

IR AND NMR SPECTRA

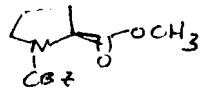
SPECTRUM 1



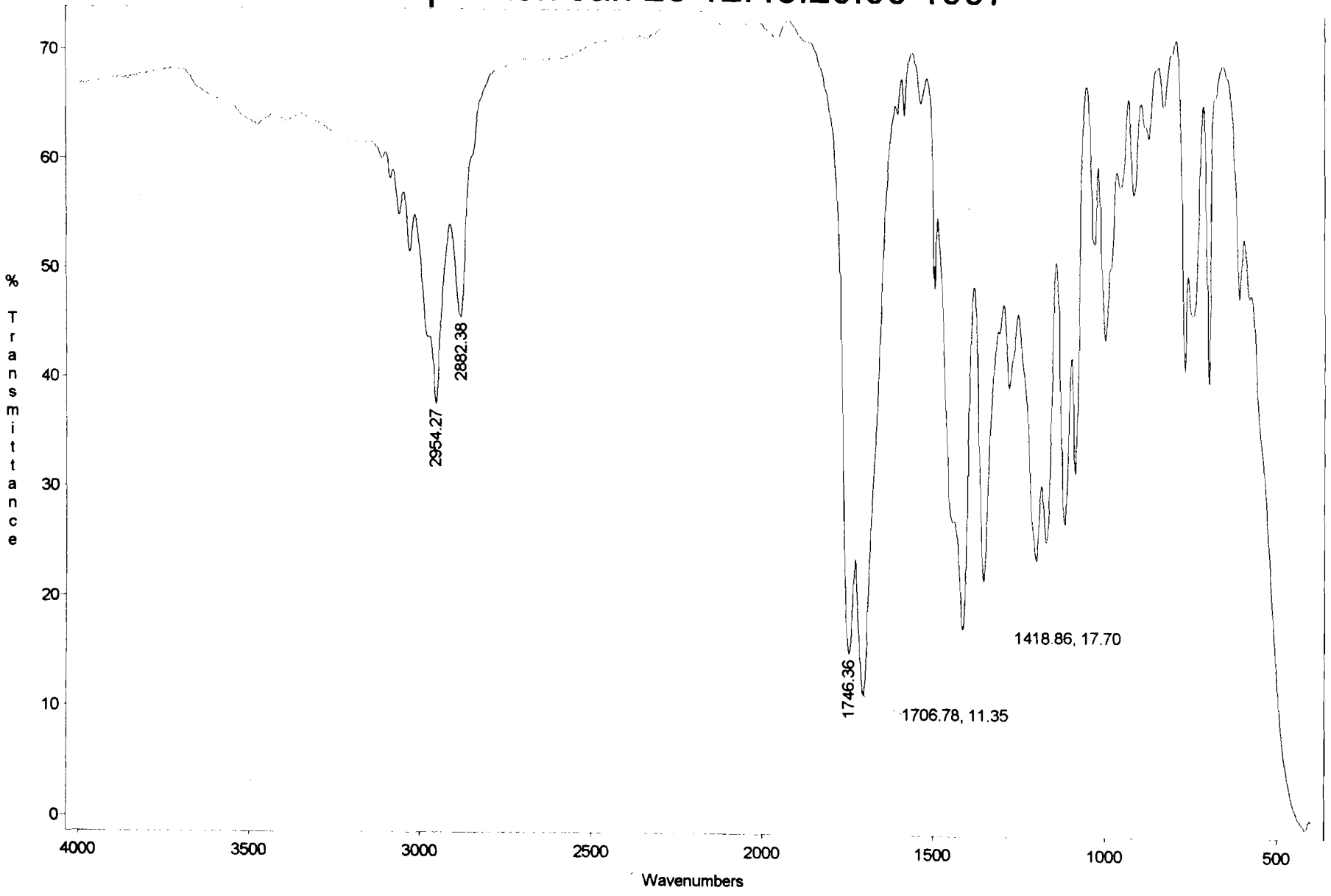
tmp Mon Jan 20 12:38:34:87 1997



SPECTRUM 2

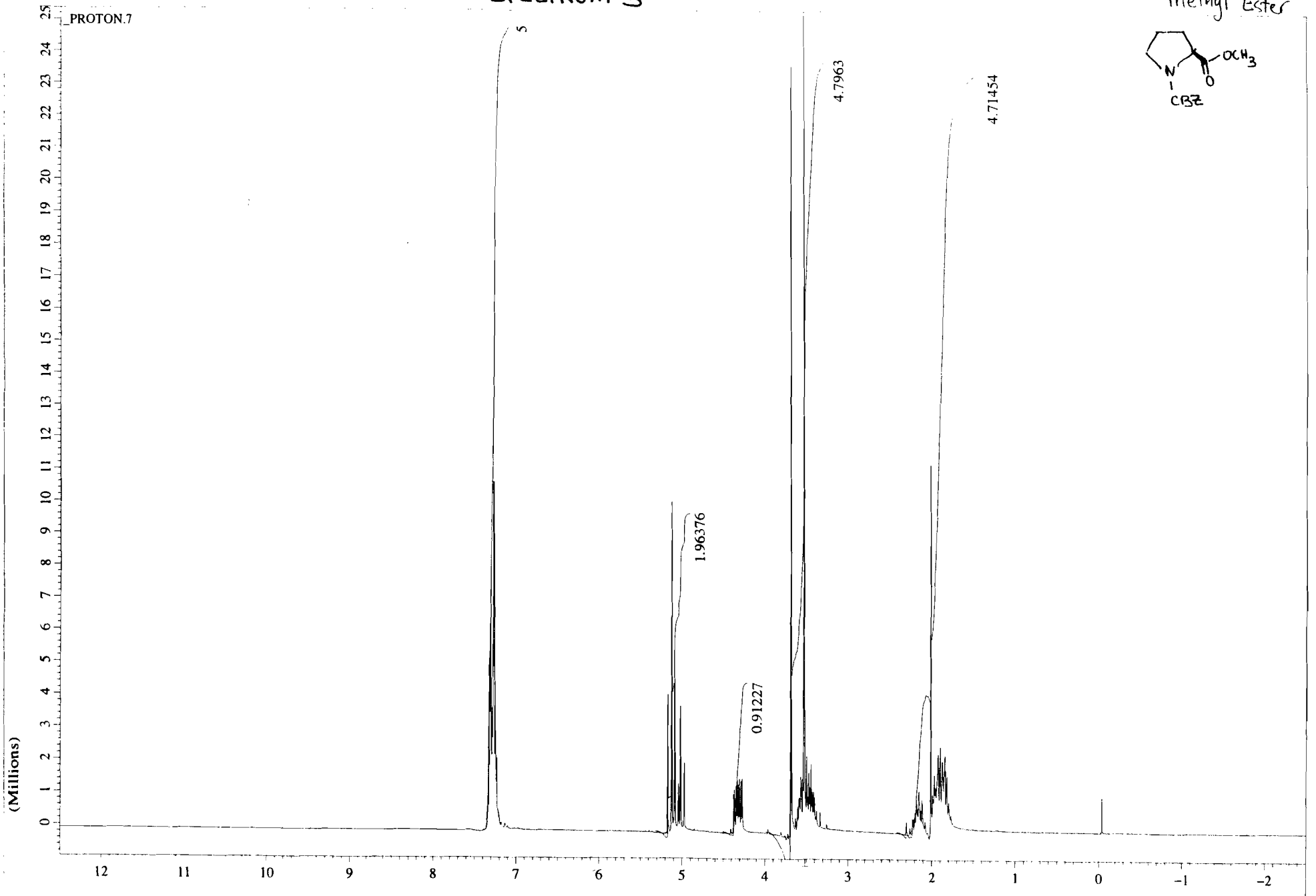


tmp Mon Jan 20 12:45:29:99 1997



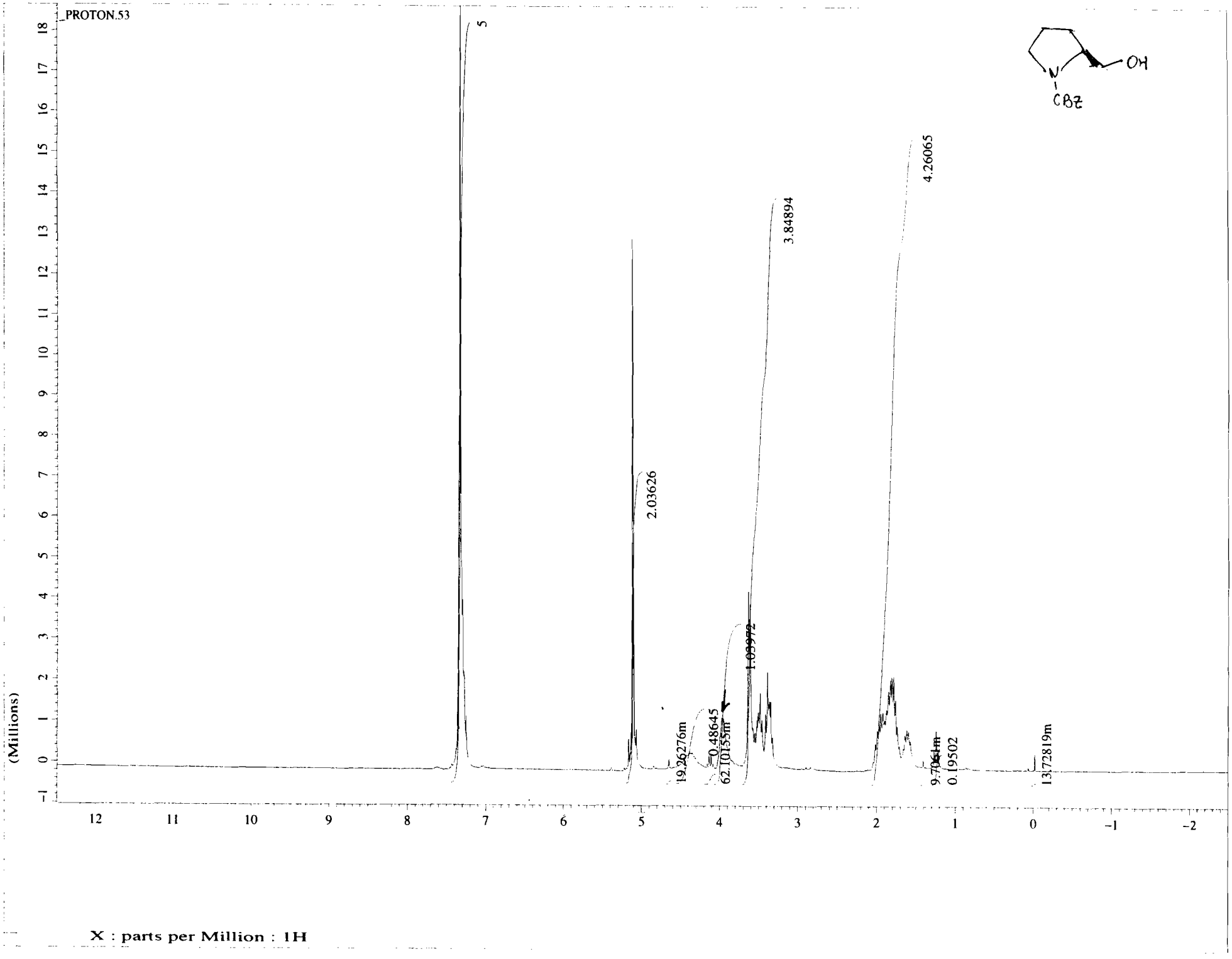
SPECTRUM 3

Methyl Ester



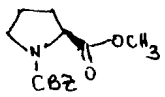
X : parts per Million : 1H

SPECTRUM 4


PROTON.53
(Millions)
X : parts per Million : 1H
5
2.03626
19.26276m
0.48645
62.10155m
1.03972
3.84894
4.26065
9.70641m
0.19502
13.72819m
OH
N
CBZ

SPECTRUM 5

Methyl Ester



1d_13c_spectrum.5

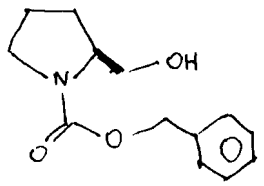
(Millions)

175.0785
173.7133
173.5608
155.2574
154.6701
136.9463
136.8548
128.6563
128.5953
128.0615
77.6355
77.1626
76.6898
66.9051
66.8441
59.0193
58.6914
52.0106
51.8352
46.6950
46.2069
30.5880
29.5813
23.9530
23.1751
20.2923

X : parts per Million : 13C

SPECTRUM 6

Reaction Product



1d_13c_spectrum.9

(Billions)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1 1.1

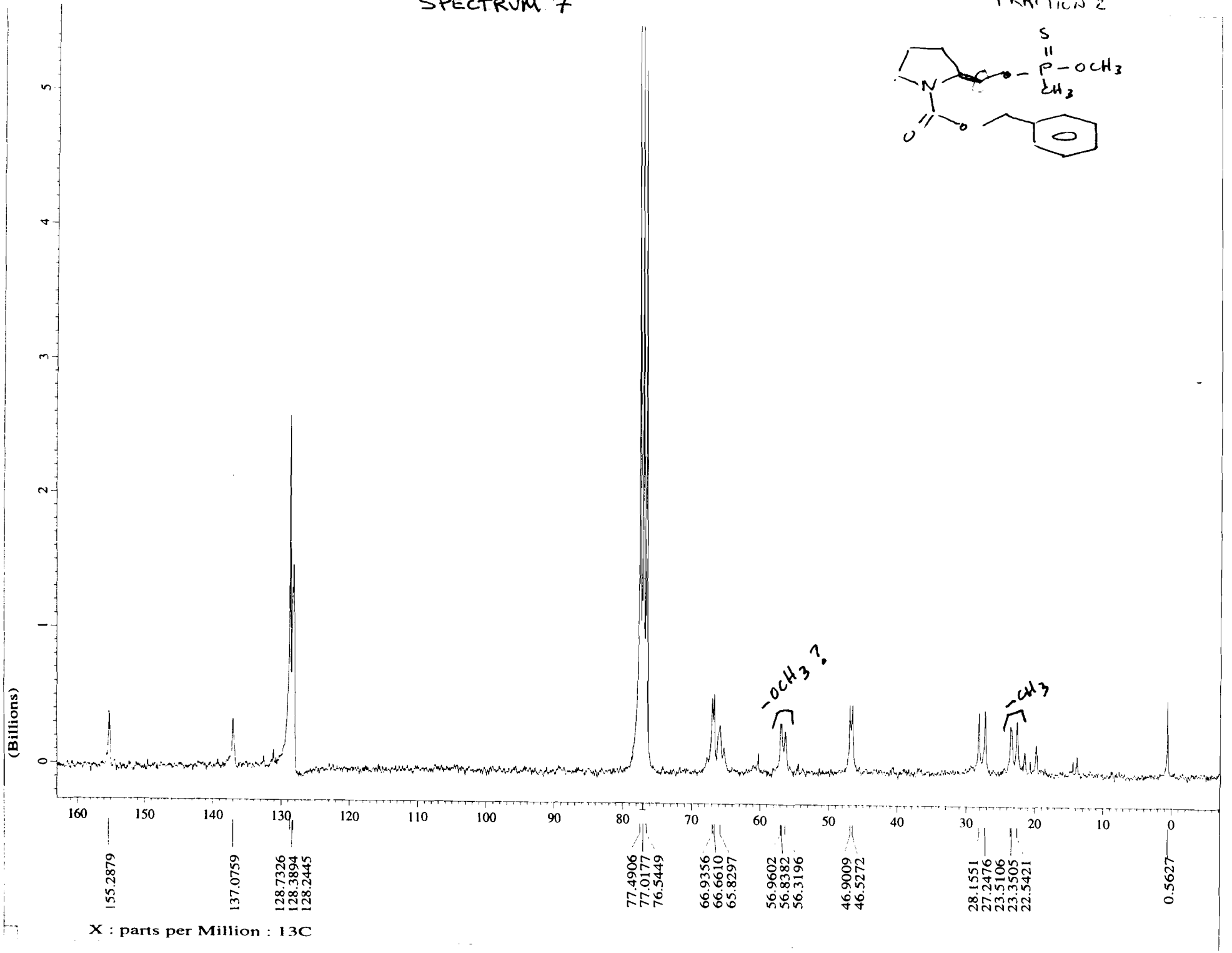
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10 −20

157.3928
136.8090
128.7326
128.2827
128.1225
77.5821
77.1092
76.6364
67.1034
66.4399
60.4531
47.0916
28.1933
23.6555

X : parts per Million : 13C

SPECTRUM 7

FRACTION 2



SPECTRUM 8

Aldrich D15,295-1 CAS [2524-03-0]

Dimethyl chlorothiophosphate, 97%

$C_2H_6ClO_2PS$
FW 160.56
bp 67 °C (16 mm)
d 1.322
n_D^{20} 1.4820

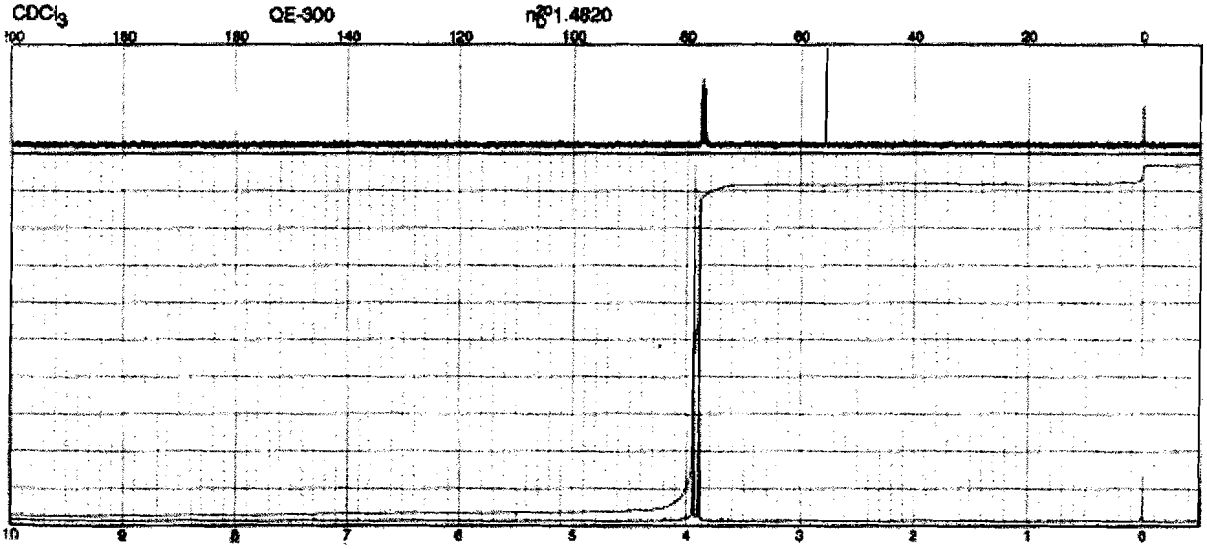
Fp 221 °F

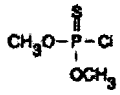
60 MHz: 2, 874D
FT-IR: 1, 924D
VP-FT-IR: 3, 846A

55.70°
55.61°

B

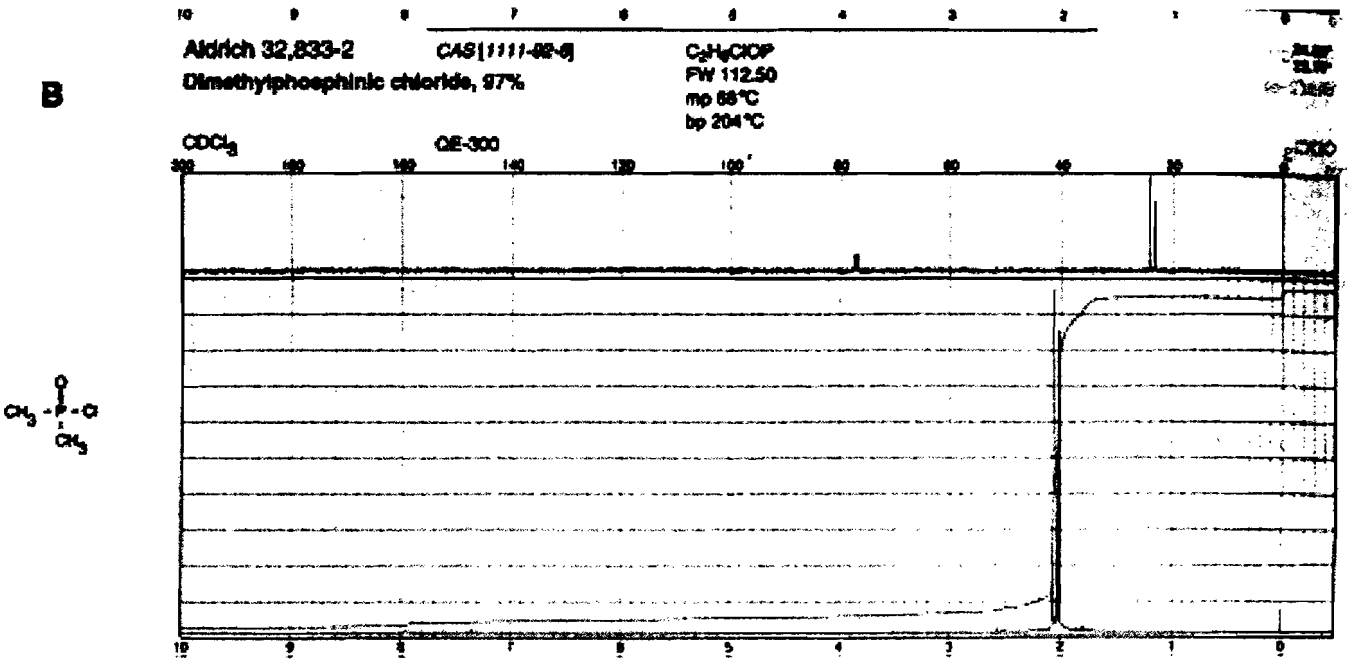
$CDCl_3$ QE-300



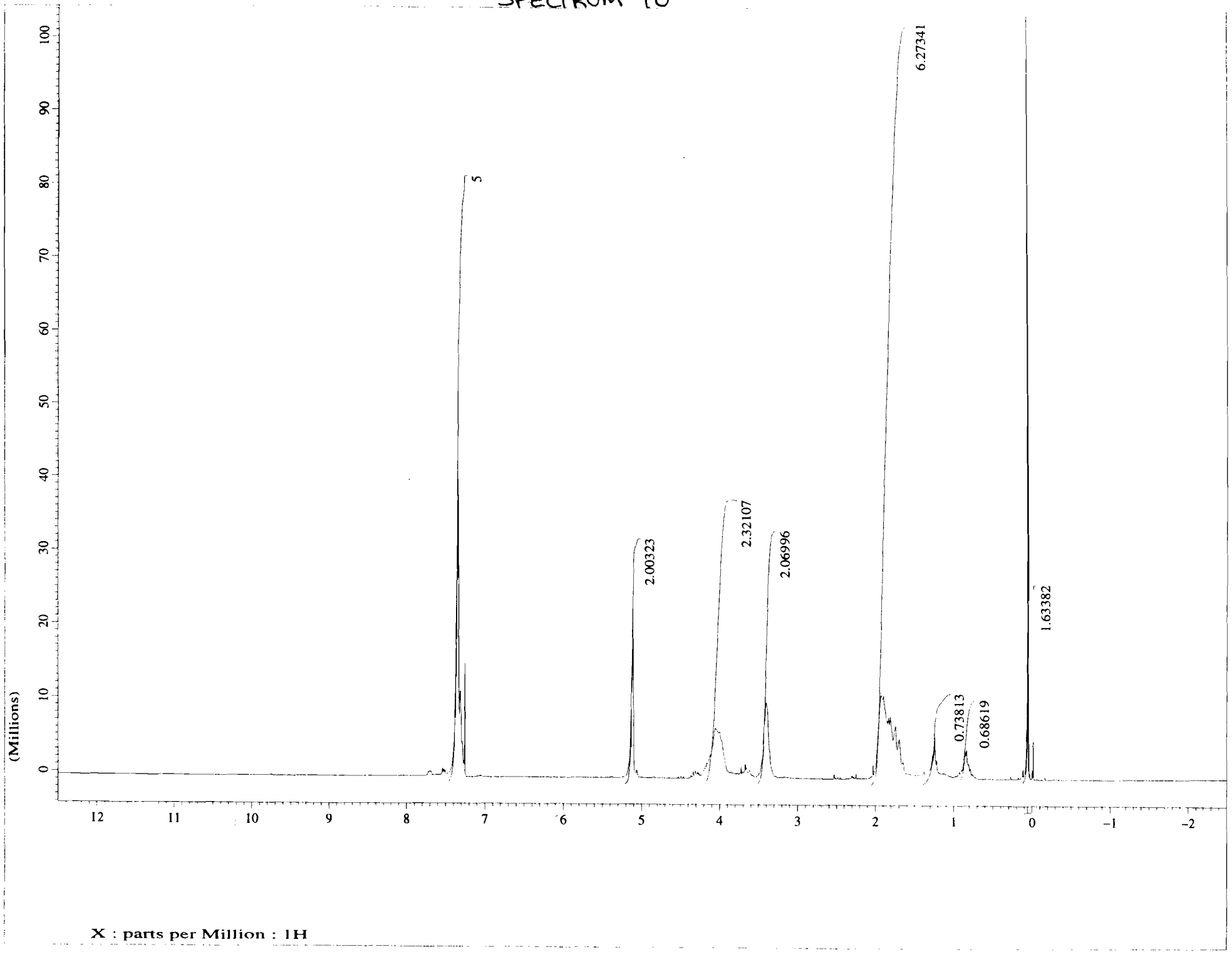


SPECTRUM 9

B


Aldrich 32,833-2
CAS[1111-92-6]
Dimethylphosphinic chloride, 97%
C_2H_6ClOP
FW 112.50
mp 68°C
bp 204°C
$CDCl_3$
QE-300
CH_3
O
P
Cl
CH_3

SPECTRUM 10


(Millions)
100
90
80
70
60
50
40
30
20
10
0
5
2.00323
2.32107
2.06996
6.27341
0.73813
0.68619
1.63382
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
X : parts per Million : 1H