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Introduction

Acetylcholine (ACh, Scheme 1) is an important neurotransmitter in the human nervous system. Acetylcholine functions as a neurotransmitter in the cholinergic synapses found in smooth and cardiac muscle, autonomic ganglia, and in neuromuscular junctions of skeletal muscle. While the storage site and release mechanism for ACh at the motor end-plates of skeletal muscle have been the most extensively studied, it is likely that the same general principles apply to cholinergic transmission at other sites. ACh is stored and released from motor nerve endings in quanta.[1] The ACh release mechanism involves a multi step process.(Fig. 1) A nerve impulse sent from the previous motor nerve terminal travels through the presynaptic axon to the terminal bulb. This triggers a change in the membrane potential, causing the calcium channel to open allowing calcium ions to migrate into the bulb. Inside the bulb are synaptic vesicles. Each of these vesicles contain10^3~10^4 ACh molecules. The increase in calcium ion concentration causes these vesicles to fuse with the axonal membrane and open, releasing the ACh molecules into the synaptic cleft where they diffuse towards ACh receptors on the postsynaptic membrane. This triggers another nerve impulse that is passed on to the next motor nerve terminal.[2] After ACh has triggered the action potential, it must be rapidly hydrolyzed or the nerve will continue to send impulses and death will eventually result. Acetylcholinesterase (AChE, Fig. 2a), an enzyme present in the synaptic cleft, is responsible for hydrolyzing ACh into choline and acetate (Scheme 1).

The mechanism by which AChE hydrolyzes ACh has been the subject of much debate and is still somewhat unclear. Studies of AChE using analogs of ACh and other compounds that inhibit the enzyme's ability to function have helped clarify some of these questions and have generated additional questions about the stereoselectivity of inhibition. Also, some of these AChE inhibitors (particularly organophosphorus compounds) are used in the treatment of disorders like glaucoma and

myasthenia gravis (a muscular debility). Use of these compounds has even been proposed as a possible therapeutic treatment towards the management of Alzheimer's disease.[3] The major drawback to the use of organophosphorus (OP) inhibitors in the treatment of these diseases is that OP compounds tend to be highly toxic, irreversible inhibitors of AChE. In order to design more effective, nontoxic AChE inhibitor that could better treat these disorders the stereoselectivity and mechanism of inhibition of AChE by OP compounds must be fully elucidated.

Much work has been done using OP compounds to probe AChE stereoselectivity, active site structure, and the mechanism of hydrolysis. Work done by Wilson and his collaborators in the 1950's indicated that the active site of AChE is actually composed of an "anionic site" and an esteratic site (Fig. 3); the latter is known as the truly catalytic site.[4] The catalytic process of AChE is pH dependent which led to the idea that the esteratic site contains an acidic and a basic group. This process was found to operate best when the acid is deprotonated and the base is protonated (the pKa of the imidazole base is ~6.3).[5] It was postulated that when ACh binds to AChE, the ester portion binds to the esteratic site and the quarternary amino group binds to the "anionic site." It was also believed that OP compounds bind to the esteratic site and, depending on the nature of their functional groups, the "anionic site." O'Brien studied the importance of the "anionic site" in ACh binding. He postulated that if binding occurred at an "anionic site" then it would be due mostly to coulombic interactions. He discovered that binding of an OP compound called amiton and several of its carbon analogs occurred through at least 80% hydrophobic interactions (fig.4).[4] This strongly undermined the importance and even the existence of an anionic site on AChE. Ordentlich, based on his research, postulated that a hydrophobic trimethyl binding site bound the quaternary nitrogen of ACh and also bound uncharged substrates and inhibitors. Further research by Ordentlich caused him to postulate that both an anionic and a hydrophobic site may exist as two partially overlapping regions.[6]

Although there is much debate regarding the anionic vs. hydrophobic sites, the structure of the main binding site, the esteratic site, is known more clearly. The active site is located at the bottom of a deep narrow gorge about 20 angstroms long.[3] (Fig. 2b, c) This gorge extends about halfway into the enzyme and is lined with aromatic residues. At the bottom of the gorge is the esteratic site. This site contains the Ser-200 residue and the imidazole group of histidine.[5] The serine hydroxy group acts as a nucleophile and attacks the sp^2 carbonyl carbon of ACh forming a tetrahedral intermediate (Scheme 2). The imidazole group most likely serves as an acid-base catalyst by first deprotonating the hydroxy group during acylation of the Ser residue, then deprotonating water during hydrolysis of the acylenzyme, and finally reprotonating the Ser residue after hydrolysis. Based on the knowledge of other serine proteases, it was believed that a glutamate residue was present in the esteratic site to help stabilize the histidine acid-base catalyst through hydrogen bonding. However, work done by Daniel Quinn challenges the presence of the Glu residue.[5] Other amino acid residues have, through site directed mutagenesis, been found to play important roles in both ACh binding and stabilization of the tetrahedral intermediates. Work done by Ordentlich et. al. has indicated that the Trp-86 residue may constitute the anionic portion of the active site.[6] The indole moiety of Trp-86 may aid in the binding of both the quarternary ammonium groups of the substrate and appropriate functional groups of active site inhibitors through π-cationic interactions.[7] Other studies by Sussman have shown that an oxyanion hole (Gly-121, 122, Ala-204) and an acyl pocket (Phe-295,297; Harel et. al., Vellom et. al., Ordentlich et. al.) are also present in or near the active site.

The composition and size of the active site gorge have also been the subject of much debate. AChE operates at an incredible, almost diffusion-limited, catalytic rate. It has a turnover number near 20,000 s^{-1}.[8] This is an amazingly fast rate considering the location of the active site. The kinetics of the AChE enzyme have raised several questions about the movement of the AChE substrate and products to and from the active

site. The concept of a deep narrow gorge is not consistent with such a high turnover rate because ACh must diffuse into the gorge while products diffuse out. AChE possesses a large dipole moment which may help attract the cationic ACh down the gorge towards the active site but which would also impede the clearance of products.[8] Site directed mutagenesis and molecular modeling studies have been used to explore the possibility of a back-door exit for the hydrolysis products, the contribution of the dipole moment to ACh binding and clearance of products, and the possibility that specific amino acids help guide ACh towards and into the active site gorge. The concept of a back door exit for the products of hydrolysis was proposed to explain AChE's high turnover rate.[7] A molecular dynamics simulation conducted by Gilson in 1994 of *Torpedo californica* AChE revealed a small, thin opening of a short channel through a thin wall of the active site. This back door was thought to potentially function like a shutter, opening to let choline and acetate out and closing immediately afterward. Kronman et. al. studied the effects of mutagenesis on residues flanking the "back door." Several acidic residues line the outer opening of this cavity which would impede outward choline diffusion via an electrostatic field. Mutagenesis would modify this field and possibly change the turnover rate if choline was diffusing out of the enzyme through the back door. Their results suggested that choline did not exit the enzyme through the back door.[7] However, new findings indicated that the diffusion rate of choline in product clearance is not electrostatic field sensitive. This suggests that product clearance from the active site gorge is not as difficult as was first suspected.[7]

Although much research has been done on AChE hydrolysis of ACh, a large part of the process is still unclear. It is generally accepted that hydrolysis of ACh proceeds through a tetrahedral intermediate. The generally accepted mechanism for phosphorylation of AChE is shown in Scheme 3a. It has been postulated that OP compounds function as inhibitors of AChE because they resemble the proposed tetrahedral intermediate of ACh hydrolysis. However, other results refute this hypothesis

and instead suggest that it is actually the phosphorylated AChE enzyme that resembles the transition state.[9] (Scheme 3b) Still other results show that the stability of the complex may not always require tetrahedral geometry. For example, the trigonal carbamoyl-AChE complex (Fig. 5) is hydrolyzed very slowly, indicating apparent stability, with rates that depend on its substituents.[9] Other attempts to clarify the stereospecificity of AChE inhibition by OP compounds have yielded interesting but somewhat contradictory results. It has been shown that stereoisomers of certain nerve agents have different inhibitory potencies.[10] For example, (-)sarin is a much more potent AChE inhibitor (AChE obtained from bovine erythrocytes) than (+)sarin.(Fig. 6) Upon development of better isolation techniques, it was discovered that the P(-) isomers of both sarin and soman had inhibitory rates 3-4 orders of magnitude higher than those of the P(+) isomers.[10] A model of the AChE active site was developed by Jarv to explain this stereospecificity.[11] (Scheme 4) Jarv's model contains, in addition to His and Ser, two hydrophobic groups for binding of the OR"and R groups as well as a third hydrophobic region, ρ_3, for binding of the leaving group X. Structure-activity relationship studies indicated that the size of the ρ_2 pocket can only accommodate acetyl groups while ρ_1 can bind butoxy groups. According to Jarv, during OP inhibition of AChE the leaving group X occupies the axial position of the trigonal bypyramidal transition state and is bound by the ρ_3 subsites. The serine hydroxy group attacks at the axial face of the phosphorus atom opposite X. The phosphoryl oxygen has its position fixed by hydrogen bonding. The R and OR' groups should occupy the ρ_1 and ρ_2 subsites. This explains the preferential binding of one enantiomer over the other when R and OR' differ considerably in size. In the case of sarin and soman (Fig.6) the groups corresponding to R and R' in Jarv's mechanism are considerably different in size. The binding of AChE by taran and VX (other nerve agents, Fig.6) is less stereospecific. The explanation is straightforward: the groups corresponding to the R and R' groups in Jarv's mechanism are more similar in size.[10] Other studies using these compounds have shown

that conflicting data may be obtained regarding which stereoisomer is a more potent inhibitor if AChE is obtained from different sources.

After examining some of the literature regarding studies of AChE inhibition and ACh hydrolysis, it is clearly apparent to me that many of the theories on the mechanism of ACh hydrolysis are still not totally defined. Also, the mechanism and stereoselectivity of AChE inhibition are still quite ambiguous. Therefore, additional work needs to be done with new inhibitors to ascertain the correct information regarding both the stereospecificity and mechanism of the mode of action of the AChE enzyme.

We propose the total synthesis of a conformationally constrained organophosphorus analog of acetylcholine (5, Scheme 5). This compound will contain two chiral centers, one being the phosphorus atom and the other being the pyrrolidine ring carbon β to the phosphorus atom. The chiral β carbon is conformationally constrained because it is part of the pyrrolidine ring. Studies with such a compound should allow us to make substantial progress towards the clarification of the stereospecificity of AChE inhibition by OP compounds and eventually help us understand more about the structure of the AChE active site. The synthesis begins with Cbz-L-proline methyl ester (1) which is commercially available. Reduction of the methyl ester with sodium borohydride in the presence of $CaCl_2$ will yield Cbz-L-prolinol (2). Phosphorylation of the primary alcohol will yield a pair of diastereomers (3). After separation by flash chromatography, each diastereomer will be subjected to hydrogenolysis, to cleave the Cbz-protecting group, followed by methylation of the amine with methyl iodide. By repitition of this process with Cbz-D-proline methyl ester, we should be able to access all four stereoisomers.

Results and Discussion

The first step of the synthesis was to prepare a large amount (4-5g) of Cbz-prolinol (2) by reduction of Cbz-proline methyl ester (1). Previous work on this project had shown that sodium borohydride was not a strong enough reducing agent.[12] A literature procedure indicated that methyl esters could be reduced with calcium borohydride generated *in situ* with sodium borohydride and calcium chloride.[13] Cbz-prolinol was synthesized in 83% yield using this procedure. The ^{1}H spectrum of the pure product was identical to a ^{1}H spectrum of Cbz-prolinol prepared and purified by Darshan Mehta, a former member of Dr. Frick's group, during work on this project in 1996.

Previous work by Darshan Mehta, Sulay Jhaveri, and Jay-James R. Miller on the synthesis of this organophosphorus analog of acetylcholine (5) has indicated that the troublesome step is the phosphorylation of Cbz-prolinol; therefore, much of our work focused on this step (Scheme 6). The first phosphorylating agent we attempted to synthesize was N, N,- diisopropylaminomethylchlorophosphine (6, Table 1). A literature procedure indicated that this reagent had been used to phosphorylate a secondary alcohol.[14] Although we were working with a primary alcohol the procedure for preparing this phosphorylating reagent was not complex so we attempted to phosphorylate our alcohol with it. Following a literature procedure for preparation of this reagent, we attempted a simple displacement of one of the chlorine atoms on dichloromethylphosphine with N,N,- diisopropylamine.[15] However, the ^{1}H spectrum of the product did not show the peaks expected to be present upon introduction of a diisopropylamino group. Communicating with the author of the paper on this synthesis yielded no additional information. This synthesis was attempted again but the ^{1}H spectrum looked quite similar to the first attempt. The ^{1}H spectrum of this compound

did not indicate whether or not the desired product had been synthesized; therefore, phosphorylation was attempted with this product. The ^1H of the crude product of this reaction did not contain the doublet expected from coupling of the methyl protons with phosphorus. It was concluded that this method of phosphorylation was unsuccessful. However, after further analysis of the ^1H spectrum of N, N,-diisopropylaminomethylchlorophosphine it was determined that N, N,-diisopropylamine was quite concentrated in the reaction mixture and may have masked our product. Based on these results, this synthesis and phosphorylation will be attempted again in the future.

Phosphorylation of Cbz-prolinol was also attempted with methylphosphonic dichloride (7, Table 1). Previous work had shown this method to be potentially successful but not a very attractive method because the product reacts with silica gel and is therefore difficult to purify. However, it was attempted because other methods to purify compounds sensitive to silica gel exist e.g.., alumina, fluorisil. Also, methods exist that would allow the phosphoryl oxygen to be converted to sulfur which might make the compound easier to purify. The reaction was run in dichloromethane or benzene using 1*H*-tetrazole as a catalyst. According to the literature, 1*H*-tetrazole enhances both the reactivity of phosphonic dichlorides and the selective displacement of a single chlorine from phosphonic dichlorides.[16] TLC analysis of both reactions showed that all of the prolinol was consumed but only baseline material was present on the plate in the product lane. The product of this reaction should have a greater R_f value than prolinol because it no longer contains an alcoholic proton. The ^1H spectrum of each crude product showed doublets that may have been from coupling of the methoxy and methyl protons with phosphorus at δ 1.4-1.5 ppm; however, the doublets were only separated by about 0.5 ppm. If these doublets corresponded to the methyl and methoxy protons we would have expected the methoxy doublet to be further downfield (more than 0.5 ppm) than the methyl doublet. The ^1H spectrum of the crude products also indicated

that the Cbz protecting group might have been cleaved. Another phosphorylation was attempted with this reagent in THF using triethylamine as a base instead because we suspected the Cbz group may have been cleaved by acid. In organic solvents triethylamine is a stronger base than N,N-diisopropylamine and might prevent Cbz cleavage. TLC analysis of the reaction showed all starting material was consumed; however, only baseline material was present in the product. The ^1H spectrum of the crude product showed that the Cbz group had not been cleaved but did not show the expected doublets for methoxy or methyl proton coupling with phosphorus. Phosphorylation using methylphosphonic dichloride was also attempted in toluene. A slightly modified procedure was used in which the prolinol was added to the reaction mixture last. The Cbz group was not cleaved but other than that, this technique provided results no different than previous attempts with this phosphorylating reagent. The ^1H spectrum did not show the expected doublets.

We also attempted to phosphorylate Cbz-prolinol with thiophosphorylchloride (8, Table 1). A previous paper on this project had indicated that, using this reagent, Cbz-prolinol may have been successfully phosphorylated but the product was quite impure and an attempt at purification had not been successful.[17] The reaction was run in both dichloromethane and THF with triethylamine as a base. TLC analysis showed two spots with R_f values greater than prolinol. This was not expected because the phosphorus atom is not chiral so diastereomers should not have been present. The product of the THF reaction was isolated as the dichloride and purified by chromatography. The ^1H spectrum of the crude product and of the pure product were suprisingly different but neither appeared to verify that the desired product had been synthesized. Methanol and triethylamine were added to the reaction run in dichloromethane, and the product was then isolated as the monomethoxylated chloride compound. The ^1H spectrum (11) of the crude product did not show the expected doublet for methoxy proton coupling with

phosphorus (only one doublet was expected because this phosphorylating reagent did not contain a methyl group).

A literature search yielded a new and promising phosphorylating reagent --methylphosphonothioic dichloride (9, Table1). This compound had several advantages over compounds used previously: 1) it was a more mild phosphorylating reagent, 2) the product would probably be easier to purify because it would contain sulfur, not oxygen. This phosphorylating reagent had one disadvantage: it was not commercially available. However, methylphosphonothioic dichloride was successful prepared according to a literature procedure.[18] The first attempt of the synthesis of methylphosphonothioic dichloride was successful. This was verified by ^{1}H NMR. The literature spectrum[19] for the starting material, dichloromethylphosphine, contained a doublet at around δ 2.2 ppm which corresponded to the methyl protons coupling with phosphorus. Upon addition of sulfur, a moderately electronegative atom, we would expect the methyl protons to be more deshielded and therefore their signal should shift downfield. This was the case as the ^{1}H spectrum of the product showed one doublet at around δ 2.8 ppm corresponding to the methyl protons. The ^{13}C spectrum of the phosphine contained one doublet at about δ 30 ppm. Again we expected this signal to shift downfield in the product for the same reason as we expected the methyl proton signal to shift downfield, which it did, having a value of δ 39.6 ppm. The ^{31}P NMR spectrum of the product showed one phosphorus environment which was expected because the phosphorus atom was achiral. The mass spectrum of the product showed the molecular weight of 148 g/mol. In addition to this peak, fragmentation of both chlorine atoms yielded peaks at 113 and 77 with the appropriate M + 2 and M + 4 peaks corresponding to ^{37}Cl isotopes. However, the yield after short path distillation was extremely low (30 mg, 0.47%). This synthesis was attempted again and the desired product was obtained after distillation in 62% yield (3.93 g). It was determined that the pressure and temperature must be carefully controlled during distillation (see experimental section for details).

Phosphorylation of Cbz-prolinol with methylphosphonothioic dichloride was attempted using a literature procedure.[20] The reaction was run in dichloromethane with pyridine as a base. The ^{1}H spectrum of the crude product contained two doublets at about δ 1.75 ppm (methyl group) and δ 3.70 ppm (methoxy group). However, after purification by flash chromatography neither of the doublets were present and the spectrum in general did not look similar to the spectrum of the crude product. It was concluded that the compound may have decomposed on the silica gel during purification. We attempted this phosphorylation again and hoped to purify the product using fluorisil or alumina but analysis of the product, prior to purification, by mass spectrometry (312 g/mol) showed that the reaction did not yield the desired product. Phosphorylation with this reagent in toluene was also attempted with triethylamine as a base. A slightly modified procedure was followed in which the Cbz-prolinol was added to the reaction last. The mass spectrum of the crude product did not contain the expected molecular weight peak (312 g/mol).

An attempt was made to displace both chloride groups of the methylphosphonothioic dichloride compound with methoxy groups. The phosphorylating agent was stirred in dichloromethane in the presence of an excess of methanol with pyridine as a base. Characterization of the dimethoxylated compound by ^{1}H NMR would give an indication as to where the methoxy protons would show up in the phosphorylation product spectra; however, the mass spectrum of this product did not contain the desired molecular weight peak of 140 g/mol, so no further characterization was performed.

Phosphorylation of benzyl alcohol with methylphosphonothioic dichloride was attempted to see if this reagent could phosphorylate a different primary alcohol. The reactions were both run in dichloromethane with pyridine as a base in one and triethylamine as a base in the other. No methanol was added to displace the remaining chloride with a methoxy group. Neither reaction yielded the desired product. This was

verified by their mass spectra--neither contained the molecular weight peak of 225.05. It was suspected that a substitution reaction may have taken place at the benzylic carbon. Benzylic carbocations are resonance stabilized and methylchlorothiophosphonate is a good leaving group. These conditions would support nucleophilic substitution of by chloride ion at the benzylic carbon. The mass spectra of both reactions contained the appropriate peak for the molecular weight of benzyl chloride (126 g/mol), but they also contained many other peaks. Therefore, benzyl chloride was probably not the only product of the reaction but no further characterization of undesired products was attempted.

To ensure that methylphosphonothioic dichloride was not simply decomposing under the reaction conditions, it was stirred with pyridine in dichloromethane. The molecular weight peak of the sulfur reagent was present at 148 g/mol.

Further attempts to phosphorylate Cbz-prolinol with the monomethoxylated methylphosphonothioic dichloride and methylphosphonic dichloride compounds were unsuccessful. The monomethoxylated reagents were prepared *in situ* by addition of methanol in THF with triethylamine as a base. Triethylamine and prolinol were then added. The ^{1}H spectra of the crude products did not contain the expected methoxy and methyl doublets. This method was attempted again with only the sulfur reagent. Methoxylation was attempted with both methanol and sodium methoxide and completion of this reaction was verified by mass spectrometry. The molecular weight of the methoxylated compound was present (144 g/mol) in the spectra of both products and neither spectra contained a peak at 148 corresponding to the starting material. Addition of Cbz-prolinol to the monomethoxylated product obtained using methanol did not yield the desired product. Neither of the expected doublets were present in the ^{1}H spectrum. However, doublets around δ 2.0 and 3.5 ppm were observed in the spectrum of the product obtained by phosphorylation with the phosphorylating reagent generated via sodium methoxide. The ^{31}P spectrum of this product showed that several phosphorus

made. The ^{31}P spectrum of the starting material showed one environment as expected. Therefore, it was concluded that the desired product was not obtained and the problem did not result from the starting material. Characterization of methylphosphonic dichloride by ^{1}H NMR showed one doublet indicating that this starting material was also sound and pure.

After several unsuccessful attempts at phosphorylating Cbz-prolinol with various reagents (Table 1), one is inclined to think that there is something fundamentally wrong with the reaction arising from the molecule we are trying to phosphorylate. Reactions described herein using these phosphorylating reagents to phosphorylate other alcohols are published in the literature. We have postulated that the Cbz protecting group may be causing an unwanted cyclization reaction with the alcohol oxygen once it is phosphorylated (Fig. 7). This has caused us concern as to the use of the Cbz protecting group.

Future work on this project will include the use of different non-carbamate protecting groups and different phosphorylating reagents. We are currently working on a new method to prepare the monomethoxylated phosphorylating reagent (11, Scheme 7).[21] This procedure began with dimethyl methylphosphonate (12), which was then converted to the phosphonic acid
(13) using t-butylamine and Dowex H^+. The acid converted to the chloridate (11) with oxalylchloride. The chloridate was immediately reacted with Cbz-prolinol in dichloromethane and N,N,-dimethylaminopyridine with triethylamine as a base. Analysis of the crude product by mass spectrometry indicated that the desired product was not obtained (295 g/mol); however, a peak was present at 127 which corresponds to the weight of the cyclization product (14, Fig. 7) mentioned above. This is strong preliminary evidence that the Cbz protecting group is the primary problem with this synthesis. Additional analysis of the product by NMR will be conducted to try and verify the structure of this product. If we are successful in identifying this product then the

verify the structure of this product. If we are successful in identifying this product then the total synthesis of our analog (5) will be reattempted using a Boc protecting group, which is not susceptible to chloride attack, on the Cbz-L-prolinol nitrogen.

Experimental

General Procedures

Dichloromethylphosphine, methylphosphonic dichloride, 1*H*-tetrazole, phosphorylchloride, and dimethyl methylphosphonate were all purchased from Aldrich. All reactions were run under a nitrogen atmosphere. Dichloromethane, t-butylamine, and toluene were distilled from CaH_2. Pyridine was refluxed over NaOH for 0.5 h and then distilled from NaOH. THF was distilled from sodium benzophenone until the still went dry. Then it was obtained from Aldrich. Anhyrous ether was obtained from Fischer Scientific. TLC analyses were carried out using 1:1 ethyl acetate/hexanes as the eluent. All TLC analyses were performed with UV light and 2,6-dibromoquinone-4-chloroimide as a stain (purchased from Lancaster) with the exception of the synthesis of Cbz-prolinol. This was analyzed only by UV light. Column chromatography experiments were performed on silica gel with various ratios of ethyl acetate/hexanes as the eluents. ^1H and ^{13}C NMR spectra were obtained on a JEOL NMR spectrometer in $CDCl_3$ unless otherwise specified. ^1H NMR spectra were obtained at 270 MHz with delta values relative to TMS (0 ppm). ^{13}C spectra were obtained at 63.5 MHz with delta values relative to the $CDCl_3$ triplet (77 ppm). All mass spectra were obtained on an Hewlet Packard HP 6890 GC-MS system in dichloromethane, methanol, or $CDCl_3$ at an oven temperature of 150 ^oC.

Synthesis of diisopropylaminochloromethylphosphine (Scheme 8; **6**, Table 1): A 500 mL flask was charged with anhydrous diethyl ether (100 mL). Dichloromethylphosphine (5.0 g, 42.8 mmol) was added and the mixture was cooled while stirring in an ice bath. Diisopropylamine (11.99 mL, 85.6 mmol) was added dropwise. The mixture was stirred

for 1h and then vacuum filtered. The solvent was removed by rotary evaporation. Analysis by ^{1}H NMR showed that the desired product was not obtained

Synthesis of Cbz-L-prolinol (2, Scheme 5)*:* A 100 mL round bottom flask was charged with Cbz-L-proline methyl ester (5.10 g, 19.4 mmol), ethanol (40 mL), THF (20 mL) and $CaCl_2$ (4.32 g, 38.9 mmol). The mixture was cooled in an ice bath and $NaBH_4$ (2.97 g, 78.5 mmol) was added portionwise. The mixture was allowed to stir overnight and come to room temperature, then quenched with 10% citric acid (~90mL). The mixture was then diluted with ethyl acetate (200 mL) and the layers were separated. The aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with citric acid, saturated NaCl, and dried over anhydrous sodium sulfate. The solvent was removed by rotary evaporation to yield a viscous oil which was purified by flash chromatography using 1:1 EtOAc/Hex as the eluent. Purification yielded 4.02 g (84% yield) of pure product. ^{1}H NMR: δ 1.55-2.44 (m, 4H), 3.33-3.67 (m, 4H), 3.92-4.00 (m, 1H), 5.12 (s, 2H), 7.26-7.35 (m, 5H). ^{13}C NMR: δ 24.00, 28.58, 47.70, 60.72, 67.19, 127.88, 128.02, 128.47, 136.53, 157.08.

Phosphorylation of Cbz-L-prolinol (2, Scheme 5) *with methylphosphonic dichloride* (7, Table 1)*:* A 25 mL flask was charged with 1*H*-tetrazole (4 mg, 0.057 mmol), methylphosphonic dichloride (60 mg, 0.45 mmol), dichloromethane (6.5 mL), and diisopropylethylamine (0.25 mL, 1.44 mmol). The mixture was cooled in an ice bath and Cbz-L-prolinol (100 mg, 0.49 mmol), dissolved in dichloromethane (2 mL), was added dropwise. The reaction was stirred overnight and allowed to come to room temperature. Reaction progress was monitored by TLC. Methanol (10 mL) was added, the mixture was stirred for 15 min, and then vacuum filtered. The solvent was removed by rotary. Analysis of the product by ^{1}H NMR indicated that the desired product was not obtained.

Phosphorylation of Cbz-L-prolinol (2) *with diisopropylaminochloromethylphosphine* (6, Table 1)*:* L-prolinol (100 mg, 0.49 mmol) and diisopropylethylamine (0.25 g, 1.95 mmol) were dissolved in toluene (5 mL), and the mixture was cooled in an ice bath. Diisopropylaminochloromethylphosphine (90 mg, 0.50 mmol) was added, and the reaction was stirred for 24h. An additional 45 mg (0.25 mmol) of the phosphine was added after TLC showed that the reaction had not gone to completion. The reaction was stirred for an additional 60 h but was still not complete as indicated by TLC analysis. The mixture was diluted with ethyl acetate, washed twice with 10% sodium bicarbonate, dried over sodium sulfate, and the solvent was removed by rotary evaporation. Analysis of the product by ^{1}H NMR indicated that the product was not the desired one.

Synthesis of methylphosphonothioic dichloride (Scheme 9; 9, Table 1)*:* A 10 mL round-bottom flask was flame-dried and charged with aluminum chloride (342 mg, 2.6 mmol) and dichloromethylphosphine (5 g, 42.76 mmol). The mixture was cooled to -78 ^{o}C, and sulfur (1.36 g, 42.4 mmol) was added portionwise. The mixture was stirred for ~60 h and allowed to rise to room temperature. The mixture was distilled at 9 mmHg and 44 ^{o}C, and the product was analyzed by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and mass spectrometry. ^{1}H NMR: δ 2.2 ppm (d, 3H), ^{13}C NMR: δ 39.6 ppm (d), ^{31}P NMR: δ 80 ppm, GC/MS: 148, (150 and 152 for M+2 and M+4), 113 (115 for M+2), 77.

Phosphorylation of Cbz-L-prolinol (2) *with thiophosphoryl chloride* (8, Table 1)*:* A 25 mL flask was charged with Cbz-L-prolinol (133 mg, 0.65 mmol), triethylamine (0.20 mL, 1.44 mmol), THF (10 mL) and then cooled in an ice bath. Thiophosphoryl chloride (117 mg, 0.69 mmol) was added dropwise and the reaction was stirred overnight and allowed to come to room temperature. TLC analysis indicated that the prolinol had been consumed. The reaction mixture was filtered through celite, the solvent was removed by rotary evaporation, and the product was purified by flash chromatography using 20%

ethyl acetate in hexanes as the eluent. Analysis by ^{1}H NMR indicated that the desired product had not been obtained.

Phosphorylation of Cbz-L-prolinol (2) *with methylphosphonothioic dichloride* (9, Table 1)*:* A 25 mL flask was flame dried and charged with Cbz-L-prolinol (155 mg, 0.763 mmol), dichloromethane (8 mL), and pyridine (0.25 mL, 3.1 mmol), and cooled to 0 ^{o}C. Methylphosphonothioic dichloride (347 mg, 2.33 mmol) was added dropwise and the reaction was allowed to stir overnight while coming to room temperature. The reaction mixture was cooled to 0 ^{o}C. Pyridine (1.75 mL, 21 mmol) and methanol (0.2 mL, 4.93 mmol) were added. The reaction was stirred for 24 h and allowed to come to room temperature. The reaction mixture was diluted with ethyl acetate, and washed with saturated ammonium chloride and water. The aqueous layers were combined and back extracted with ethyl acetate. The organic layers were combined, washed with saturated sodium chloride, and dried over sodium sulfate. The solvent was removed by rotary evaporation and high vacuum yielding 240mg of crude product. The product was purified by silica gel flash chromatography using 20% ethyl acetate in hexanes as the eluent. Analysis of the product by ^{1}H NMR showed that the desired product had not been obtained.

Dimethoxylation of methylphosphonothioic dichloride (Scheme 10)*:*
A solution of methylphosphonothioic dichloride (30 mg, 0.2 mmol), pyridine (0.5 mL, 6.2 mmol), and dichloromethane (3 mL) was cooled in an ice bath and methanol (1 mL, 24.7 mmol) was added. The reaction was stirred overnight and allowed to rise to room temperature. The reaction was filtered, the solvent was removed by rotary evaporation.

Analysis by gas chromatography/mass spectrometry did not show the desired molecular weight peak (140.92 g/mol).

Phosphorylation of benzyl alcohol (Scheme 11) *with methylphosphonothioic dichloride:*
A flask was flame-dried and charged with benzyl alcohol (63 mg, 0.58 mmol), triethylamine (219 mg, 2.16 mmol), and dichloromethane (2 mL). The mixture was cooled in an ice bath and methylphosphonothioic dichloride (280 mg, 1.88 mmol) was added. The reaction was stirred for 60 h and allowed to come to room temperature. TLC analysis indicated that the benzyl alcohol had been consumed. Ether (15 mL) was added and the reaction was filtered twice through celite. The solvent was removed by rotary evaporation. Analysis by gas-chromatography/mass spectrometry indicated that the desired product had not been obtained (225 g/mol).

Decomposition test of methylphosphonothioic dichloride:
A flame-dried flask was charged with a few drops of methylphosphonothioic dichloride. A few drops of pyridine and a few milliliters of dichloromethane were added, and the reaction was stirred overnight, and the solvent was removed by rotary evaporation. Analysis by gas-chromatography/mass spectrometry indicated no decomposition had occurred.

Phosphorylation of Cbz-prolinol (2) *with methylphosphonothioic dichloride* (9) *(procedure 2):*
A flame-dried flask was charged with methylphosphonothioic dichloride (155 mg, 1.04 mmol), toluene (5 mL), and triethylamine (203 mg, 2.0 mmol). Cbz-prolinol (205 mg, 1.01 mmol) was dissolved in toluene (2 mL) and added dropwise to the reaction mixture. Analysis by TLC indicated that the prolinol had been consumed. Methanol (32.4 mg, 1.01 mmol) was added dropwise to the reaction mixture and the mixture was stirred over

the weekend. After analysis by TLC, the mixture was diluted with ether (10 mL), and then filtered through celite. The solvent was removed by rotary evaporation and the product was analyzed by mass spectrometry.

Phosphorylation of Cbz-prolinol (2) *with methylphosphonic dichloride* (7) *(procedure 2):*
A flame-dried flask was charged with methylphosphonic dichloride (106 mg, 0.78 mmol), toluene (5 mL), 1*H*-tetrazole (catalytic amount), and triethylamine (180 mg, 1.78 mmol). Cbz-prolinol (169 mg, 0.831 mmol) dissolved in toluene (2 mL) was added dropwise, and the mixture was stirred overnight. The reaction was analyzed by TLC and ether was added. The mixture was filtered through celite, and the solvent was removed by rotary evaporation and vacuum pumping. The product was analyzed by FT-IR and ^{1}H NMR spectroscopy and then redissolved in toluene (7 mL) and triethylamine (0.25 mL, 1.78 mmol). Methanol (51 μL, 1.25 mmol) was added, and the mixture was stirred overnight. Ether was added, the mixture was filtered through celite, and the solvent was removed by rotary evaporation and high vacuum. Analysis by ^{1}H NMR indicated that the desired product had not been obtained.

Phosphorylation of Cbz-prolinol (2) *with monomethoxylated methylphosphonic dichloride* (11, Table 1)*:*
A flame-dried flask was charged with methylphosphonic dichloride (139 mg, 1.05 mmol), THF (4 mL), and Et_3N (160 mg, 1.58 mmol). Methanol (33.6 mg, 1.05 mmol) was added and the reaction was stirred for 4h. Et_3N (0.15 mL, 1.05 mmol) was added followed by addition of Cbz-prolinol (244 mg, 1.20 mmol) in THF (3 mL). The reaction was stirred overnight, analyzed by TLC, filtered through celite, and the solvent was removed by rotary evaporation. Analysis by ^{1}H NMR indicated that the desired product had not been obtained.

Phosphorylation of Cbz-prolinol (2) *with monomethoxylated methylphosphonothioic dichloride* (10, Table 1)*:*

A flame-dried flask was charged with THF (4 mL), methylphosphonothioic dichloride (108 mg, 0.725 mmol), and Et_3N (109 mg, 1.05 mmol). Methanol (23.7 mg, 0.741 mmol) was added and the reaction was stirred for 3.5 h. An aliquot was removed and analyzed by mass spectrometry. Et_3N (0.15 mL, 1.08 mmol was added followed by addition of Cbz-prolinol (148 mg, 0.728 mmol). The reaction was stirred overnight, filtered through celite, and the solvent was removed by rotary evaporation and high vacuum. Analysis by ^1H NMR indicated that the desired product had not been obtained.

Phosphorylation of Cbz-prolinol (2) *with monomethoxylated methylphosphonothioic dichloride* (10) *(procedure 2):*

A flame-dried flask was charged with sodium methoxide (57 mg, 1.05 mmol), and THF (8 mL). Methylphosphonothioic dichloride (164 mg, 1.10 mmol) was added and the mixture was stirred for 3.5 h. An aliquot was removed and analyzed by mass spectrometry. This analysis indicated that the reaction had not gone to completion. Sodium methoxide (85 mg, 1.57 mmol), Et_3N (0.47 mL, 3.34 mmol), and methanol (33.9 μL, 0.84 mmol) were added. The reaction was stirred for 5 h followed by addition of Cbz-prolinol (340 mg, 1.67 mmol). An additional 5 mL of THF was added. The reaction was stirred overnight, filtered through celite, and the solvent was removed by rotary evaporation and high vacuum. Analysis of the product by ^1H NMR and ^{31}P NMR indicated that the desired product had not been obtained.

Synthesis of methoxymethylphosphonic acid (13, Scheme 7):

A flame-dried flask was charged with dimethyl methylphosphonate (5.13 mL, 47.31 mmol) and tert-butylamine (130 mL, 1.24 mol). The mixture was refluxed for 24 h. An aliquot was remove, diluted with ethyl acetate (2 mL), extracted with citric acid (1 mL), and the organic layer was analyzed by gas-chromatograpy/mass spectrometry. The reaction mixture was refluxed for 24 h and then analyzed by GC/MS again using the same citric acid extraction procedure. The mixture was refluxed for an additional 60 h, and the solvent was removed by rotary evaporation. Methanol (170 mL) and Dowex H^+ (68 g) were added. The mixture was stirred for 45 minutes, vacuum filtered, and the solvent was removed by rotary evaporation yielding 7.5 g of the acid. Analysis of the product by GC/MS indicated that the desired product had been obtained (111 g/mol).

Synthesis of methoxymethylphosphonochloridate (10, Scheme 7):
A flame-dried flask was charged with methoxymethylphosphonic acid (636 mg, 5.78 mmol) and dichloromethane (25 mL). The mixture was cooled to 0 ^{o}C and oxalyl chloride (841 mg, 6.62 mmol) was added dropwise. The reaction was stirred for 6 h, an aliquot was analyzed by GC/MS, and the solvent was removed by rotary evaporation and high vacuum yielding 146 mg of the choridate. The choridate was immediately reacted with Cbz-L-prolinol.

Phosphorylation of Cbz-L-prolinol (2) *with methoxymethylphosphonochloridate* (10):
A flame-dried flask was charged with methoxymethylphosphonochloridate (146 mg, 1.14 mmol), dichloromethane (20 mL), N, N, 4-dimethylaminopyridine (7 mg, 0.057 mmol) in dichloromethane (2 mL), and Et_3N (290 mg, 2.87 mmol). Cbz-L-prolinol (202 mg, 0.99 mmol) was dissolved in dichloromethane (3 mL) and added to the reaction mixture. After analysis by TLC the mixture was concentrated *in vacuo*, diluted with ethyl acetate

(20 mL), and filtered through celite. The mixture was washed with saturated sodium bicarbonate, water, and dried over sodium sulfate. The solvent was removed by rotary evaporation to yield 200 mg of crude product. Analysis of the product by ^{1}H NMR, ^{13}C NMR, and GC/MS indicated that the desired product was not obtained.

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12) *Synthesis of an Organophosphorus Analog of Acetylcholine*, Mehta, D., Illinois Wesleyan University, 1997.

13) Lewis, N., Mckillop, A., Taylor, R.J.K., Watson, R.J., *Synthetic Communications*, **1995**, *25*, 561-568.

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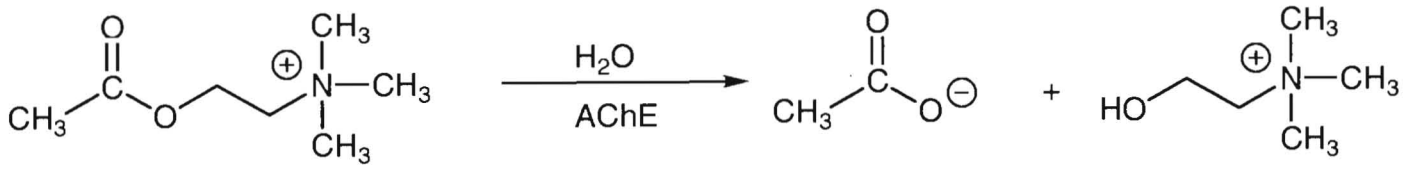
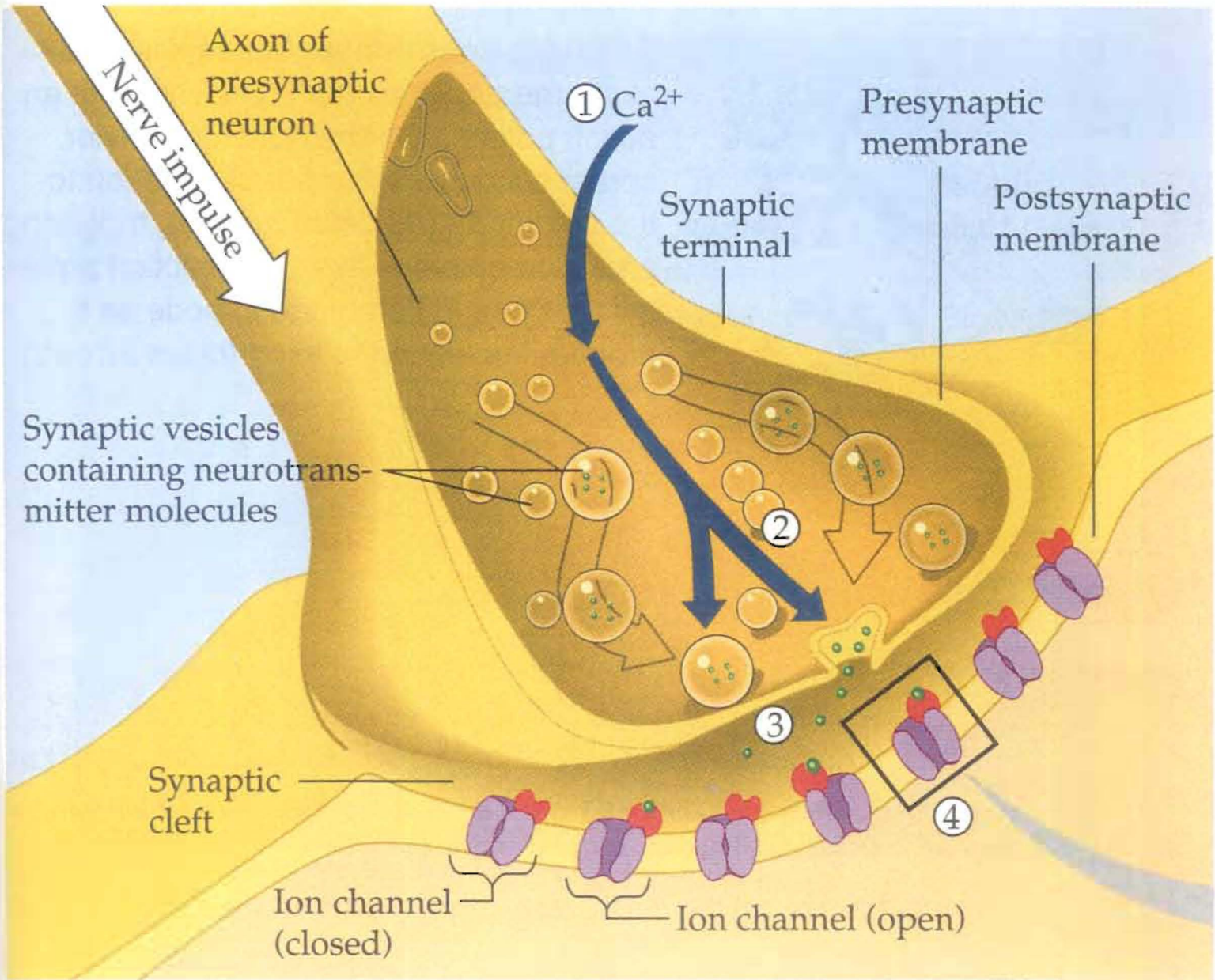
Appendix I: Figures, Schemes, and Tables

Scheme 1
The Hydrolysis of Acetylcholine



Acetylcholine Acetate Choline

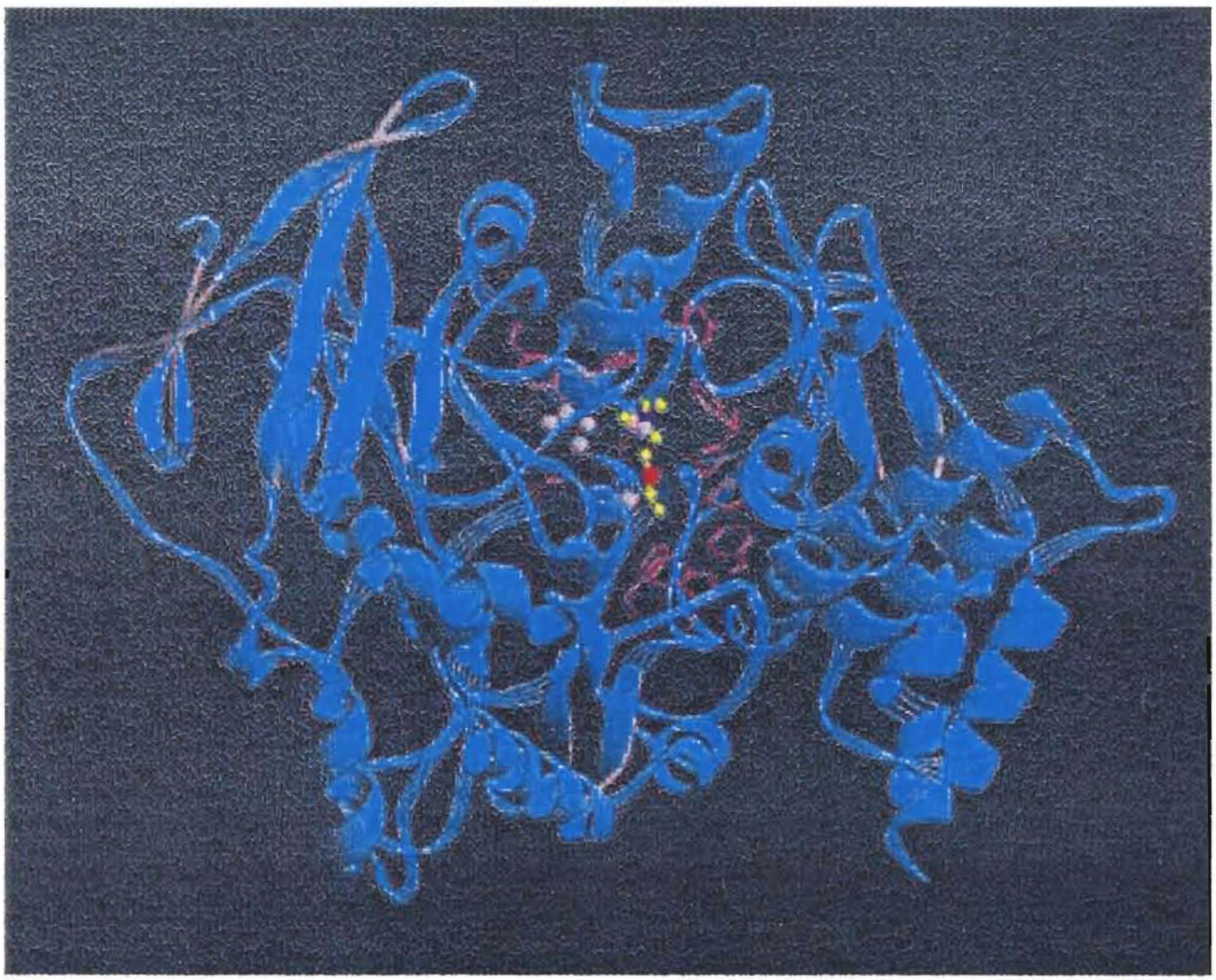
Figure 1



This is a figure of a cholinergic motor nerve terminal showing the acetylcholine release mechanism. The little green spheres are acetylcholine molecules. The red spheres that the acetylcholine molecules bind to are acetylcholine receptors.

Ref. *Biology*, Campbell, N.A., Benjamin/Cummings Publishing Co.: 1993.

Figure 2a



The 3-D structure of *Torpedo californica* acetylcholinesterase looking down the gorge. The gorge is lined with several aromatic residues.

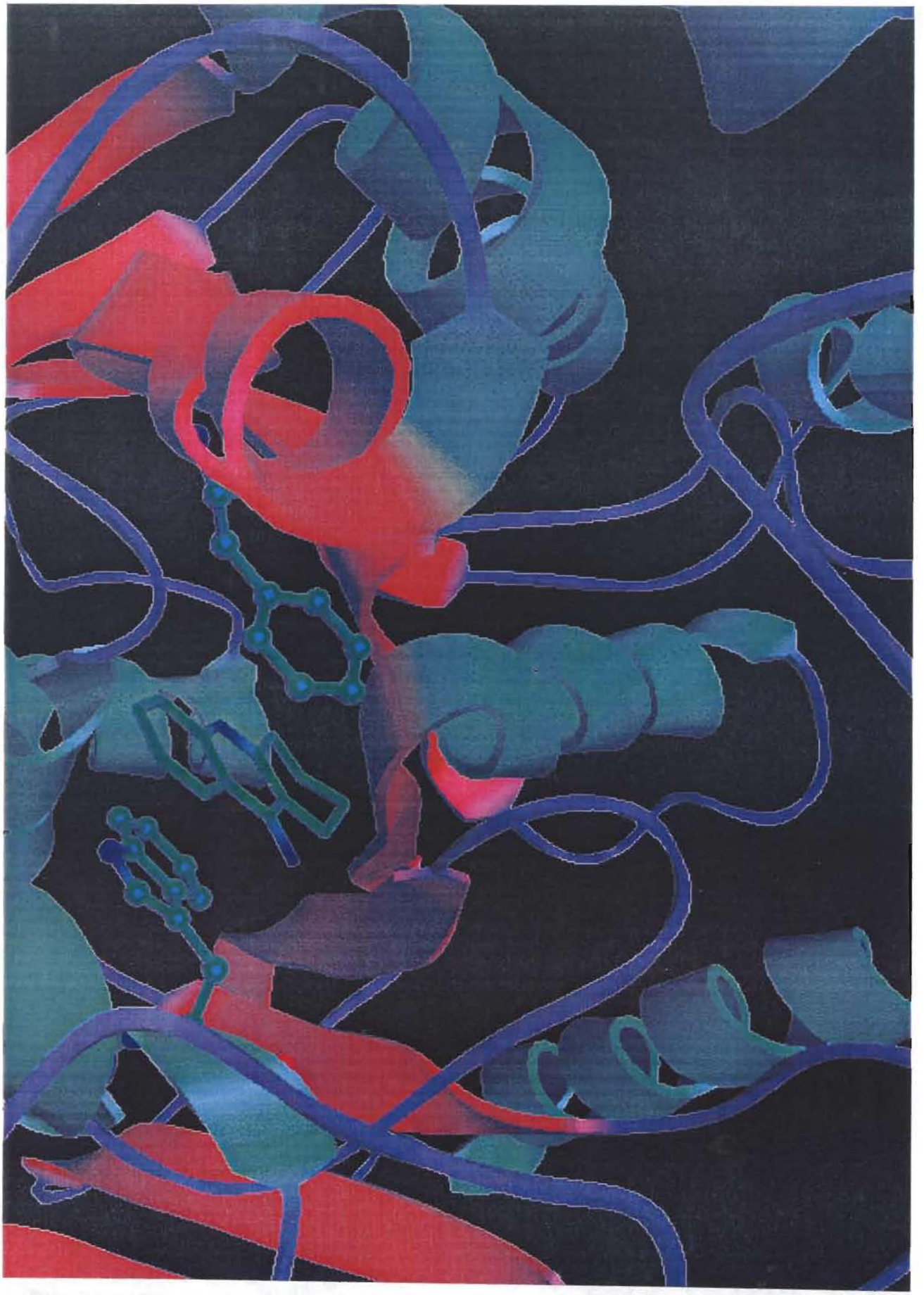
Ref. http://www.weizmann.ac.il/~cskurt/fig3.html

Figure 2b

Once inside the enzyme the acetylcholine molecule is positioned by several amino acid side chains. Serine and histidine side chains (lower right) prepare to catalytically hydrolyze acetylcholine into choline and acetate.

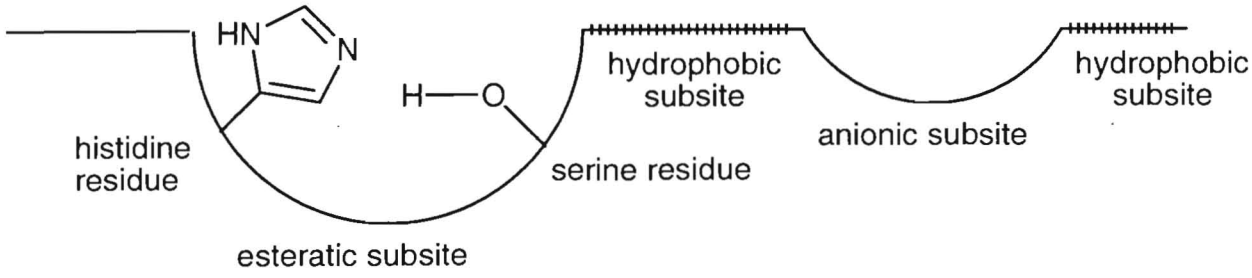
Ref. http://www.tc.cornell.edu/er/sci93/dis28ache/dis28d.html

Figure 2c



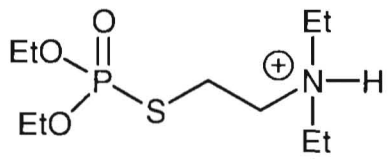
Ref. http://www.ccd.bnl.gov/visualization/gallery/pdb/images/acj_det.gif

Figure 3
AChE Active Site

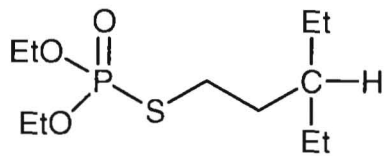


Ref. Quinn, D. M., *Chem. Rev.*, **1987**, 87, 955-979

Figure 4



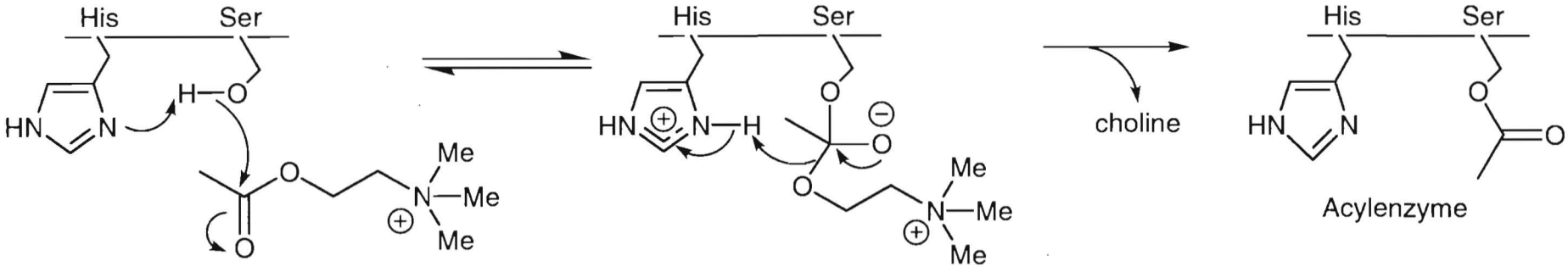
Amiton

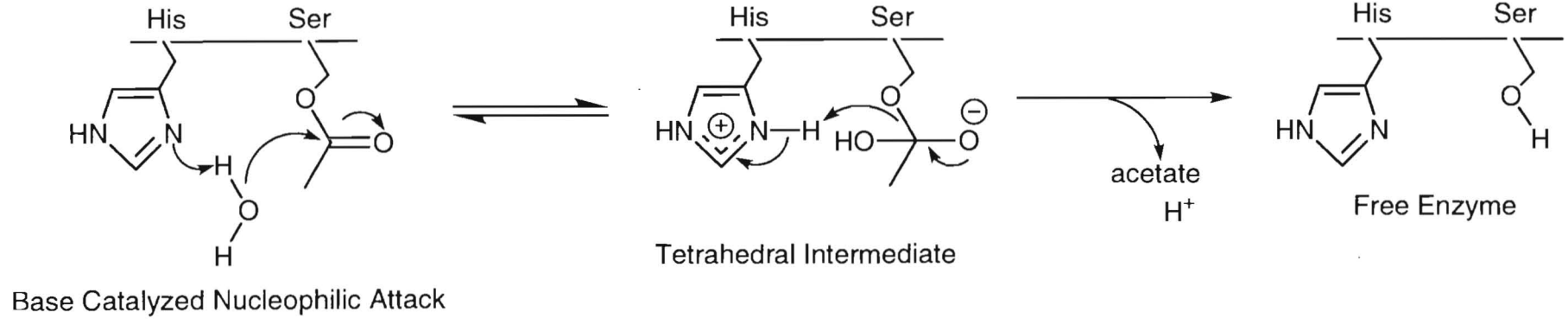


Carbon Amiton

Ref. O'Brien, R.D., Phosphorylation and Carbamylation of Cholinesterase, Annals New York Academy of Sciences, 204-214

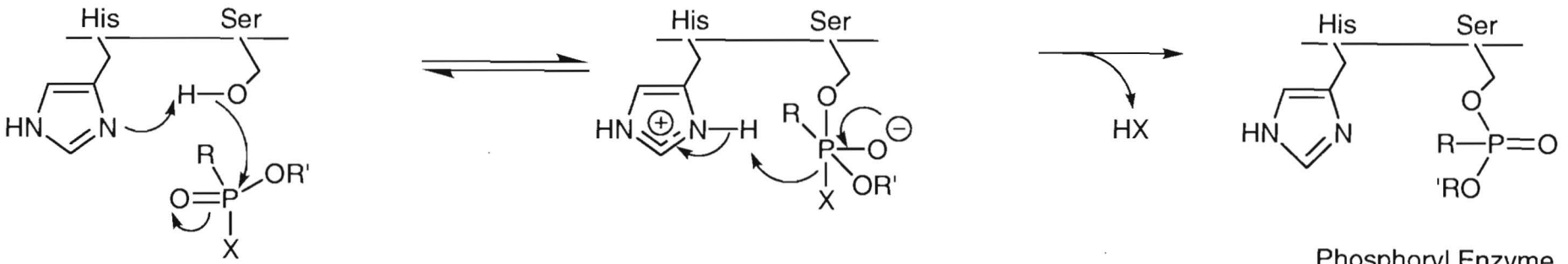
Scheme 2
Mechanism of ACh Hydrolysis





Ref. Quinn, D. M., *Chem. Rev.*, **1987**, 87, 955-979

Scheme 3a
Mechanism of Phosphorylation

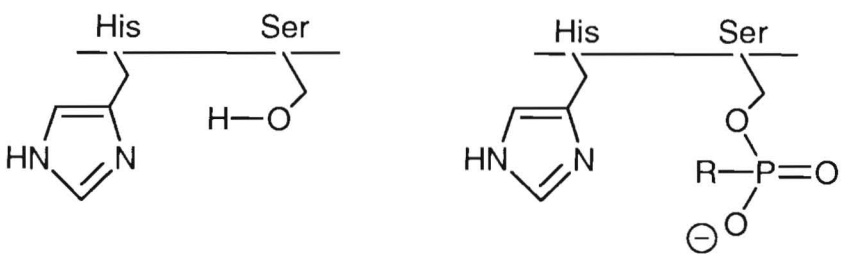


Base Catalyzed Nucleophilic Attack

Trigonal Bypyramidal Intermediate

Phosphoryl Enzyme

A B

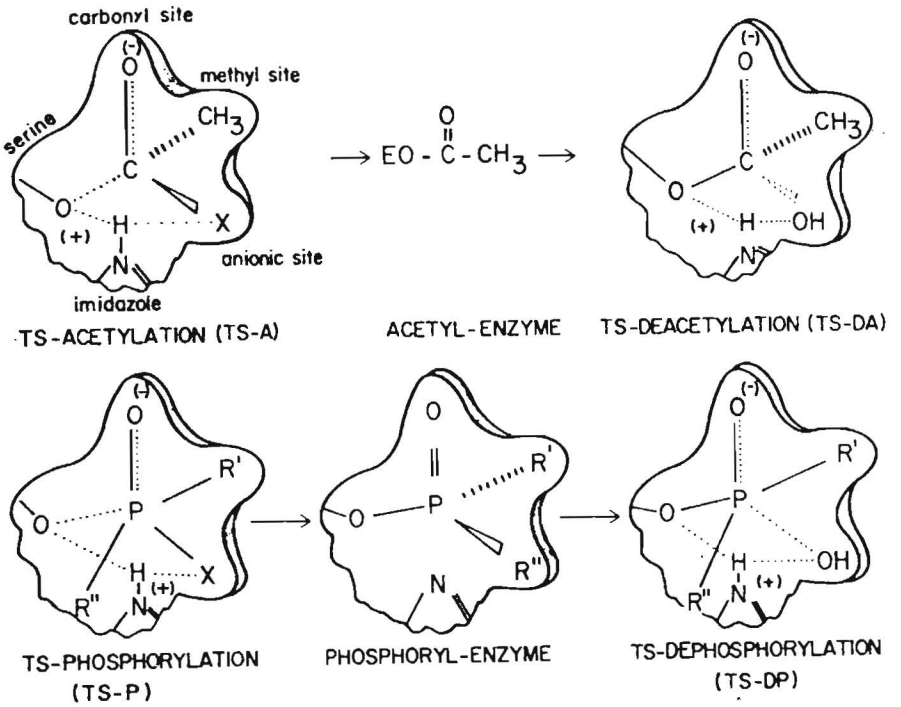


A
Reactivated Enzyme

B
Aged Enzyme

Ref. Ashani, Y. and Green, B.S., Chemical Approaches to Understanding Enzyme Catalysis: Biomimetic Chemistry and Transition State Analogs, **1981**, *10*, 153-180

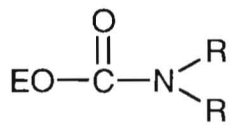
Scheme 3b



This scheme shows the resemblance of the phosphoryl enzyme to the acylation transition state for ACh hydrolysis. Both the phosphoryl enzyme and the acylation transition state have tetrahedral geometries. It is postulated that OP compounds are potent AChE inhibitors because they mimic the geometry of the acylation transition state.

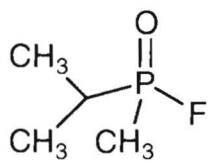
Ref. *Chemical Approaches to Understanding Enzyme Catalysis: Biomimetic Chemistry and Transition State Analogs*, Ashani, Y.,Green, B.S., Elsevier Scientific Publishing Co.: Amsterdam, 1981, Vol. 10.

Figure 5
Carbamoyl-enzyme complex

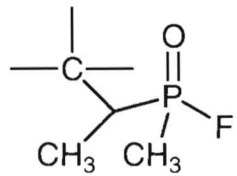


Ref. Ashani, Y. and Green, B.S., Chemical Approaches to Understanding Enzyme Catalysis: Biomimetic Chemistry and Transition State Analogs, *10*, **1981**, 169-188

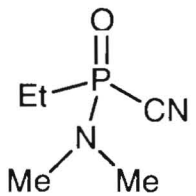
Figure 6
Structures of Nerve Agents



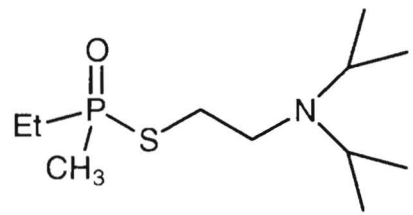
Sarin



Soman



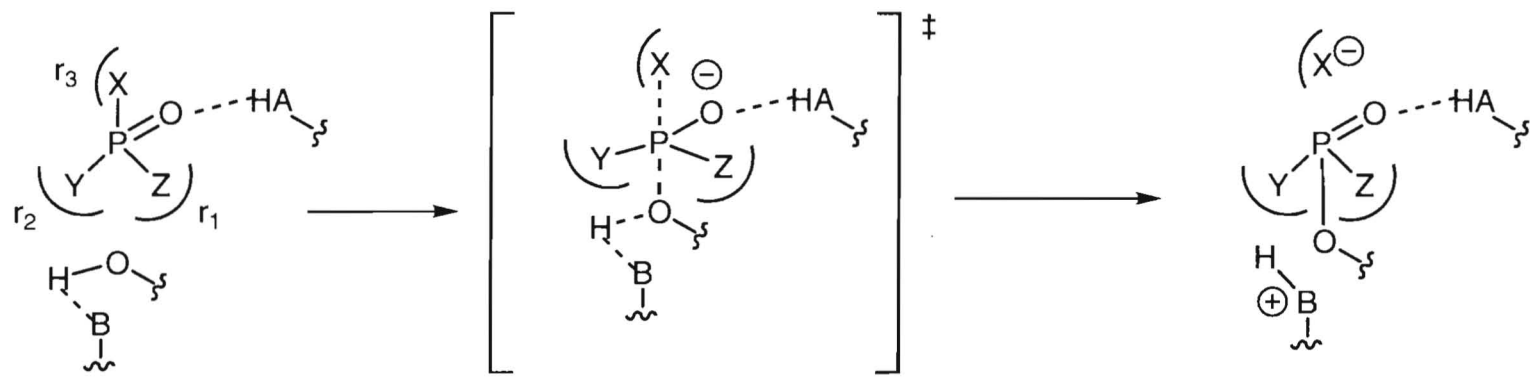
Tabun



VX

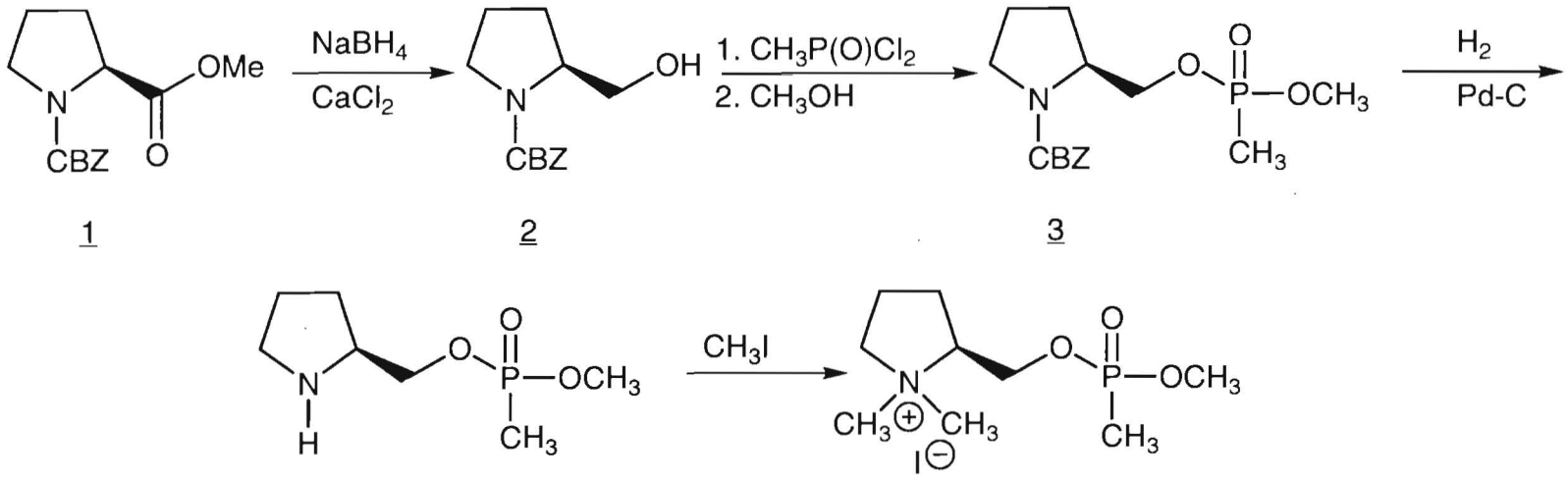
Ref. Benschop, H.P., and L.P.A. De Jong, *Acc. Chem. Res.*, *21*, **1988**, *21*, 368-374

Scheme 4
Jarv's Proposed Mechanism of Phosphorylation



Ref. Jarv, J., *Bioorganic Chemistry*, **1984**, *12*, 259-278

Scheme 5
Proposed Synthesis of The ACh Analog



Scheme 6
The General Method of Phosphorylation

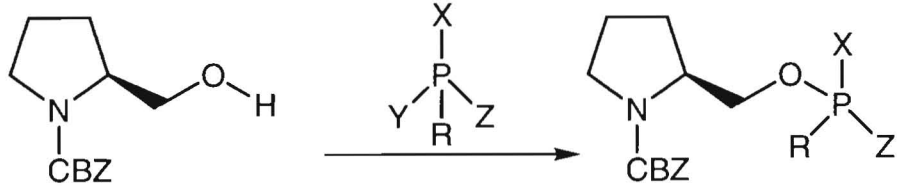
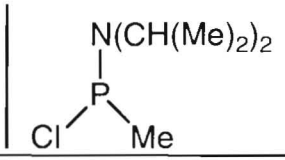
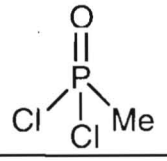
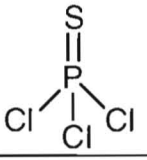
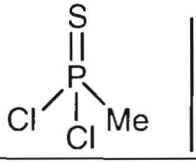
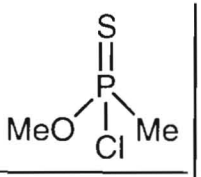
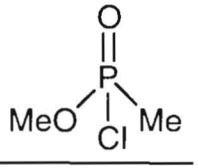
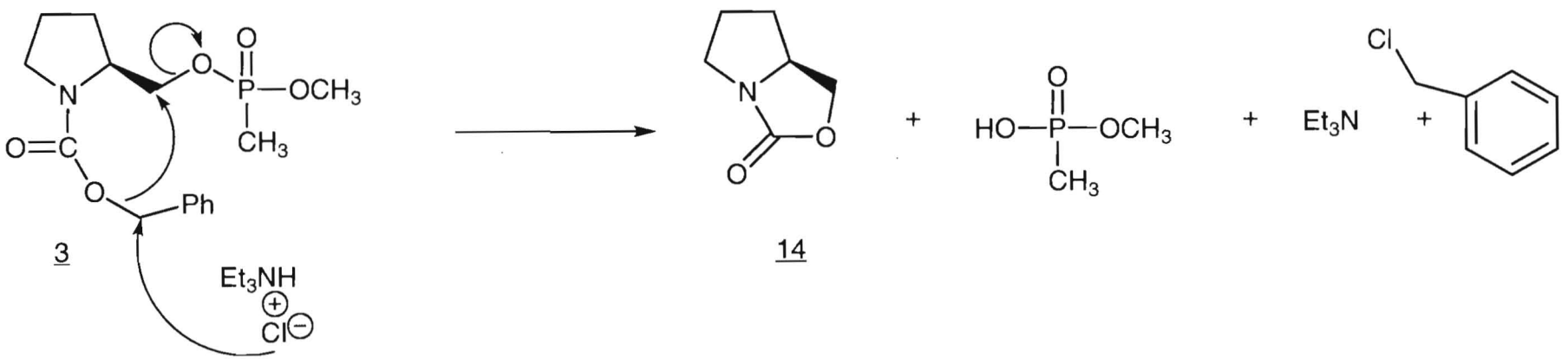


Table 1
Summary of Phosphorylating Reagents and Reaction Conditions

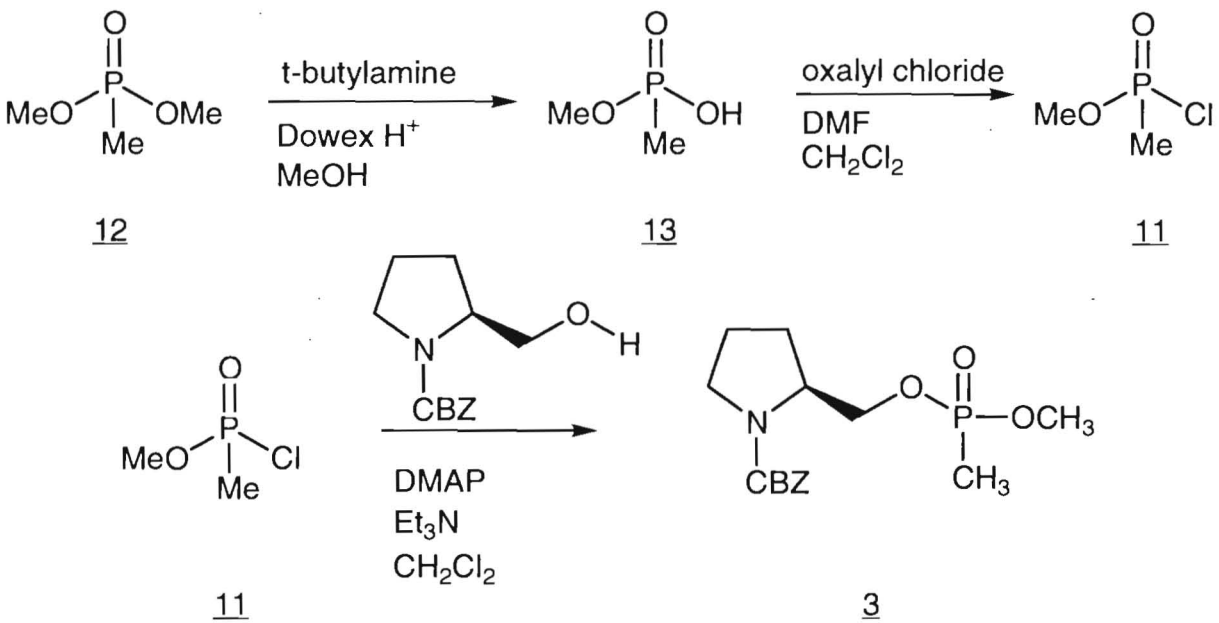
Compound #	6	7	8	9	10	11
						
1*H*-tetrazole, CH_2Cl_2, $(i\text{-Pr})_2NEt$		Undesired Product				
1*H*-tetrazole, Benzene, $(i\text{-Pr})_2NEt$		Undesired Product				
THF, $(i\text{-Pr})_2NEt$	Undesired Product*					
1*H*-tetrazole, THF, Et_3N		Undesired Product				
THF, Et_3N			Undesired Product*		Undesired Product'''	Undesired Product
CH_2Cl_2, Et_3N			Undesired Product	Undesired Product*		
CH_2Cl_2, Pyridine				Desired Prod., dec. on column**		
Toluene, Et_3N		Undesired Product		Undesired Product		

* Product isolated as the chloride prior to addition of MeOH. **Rxn was attempted again but was unsuccessful. '''Methoxy product was obtained using sodium methoxide in one reaction.

Figure 7
Possible Cyclization Reaction After Phosphorylation

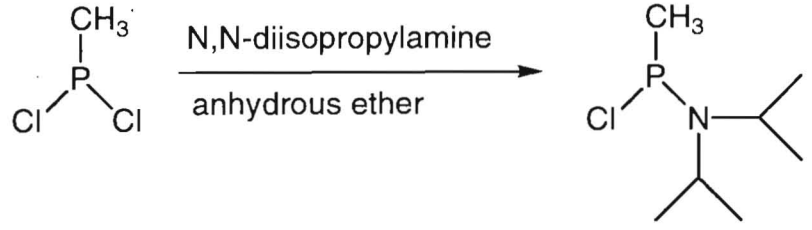


Scheme 7
Current Work



Ref. Malachowski, W.P., and Coward, J.K., *J.Org.Chem*, **1994**, *59*, 7616-7624

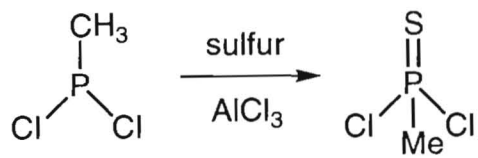
Scheme 8



6

Ref. Agrawal, S., and Goodchild, J., *Tet. Lett.*, **1987**, *28*, 3539-3542

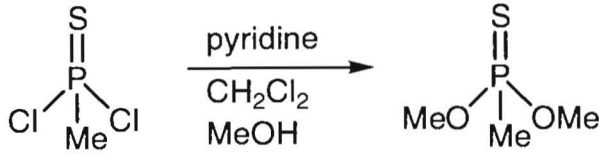
Scheme 9



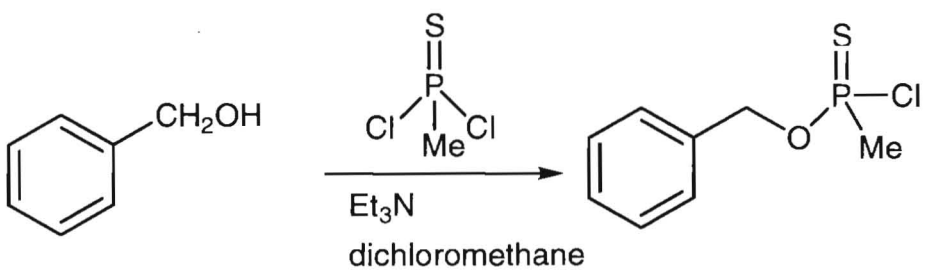
9

Ref. Wolfgang, K.D., Brill, W.K.D., Caruthers, M.H., *Tet. Lett*, **1987**, *28*, 3205-3208

Scheme 10

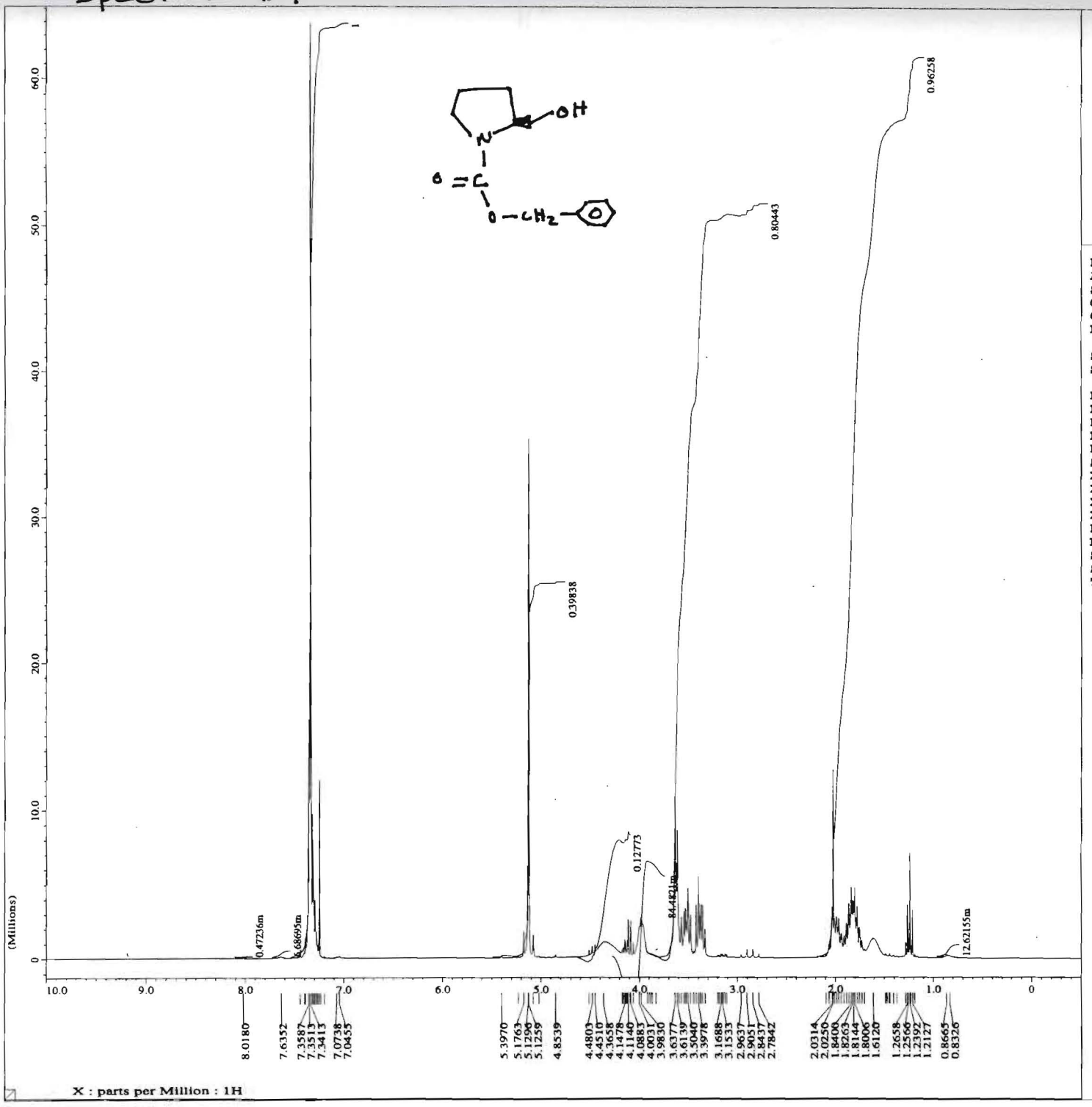


Scheme 11



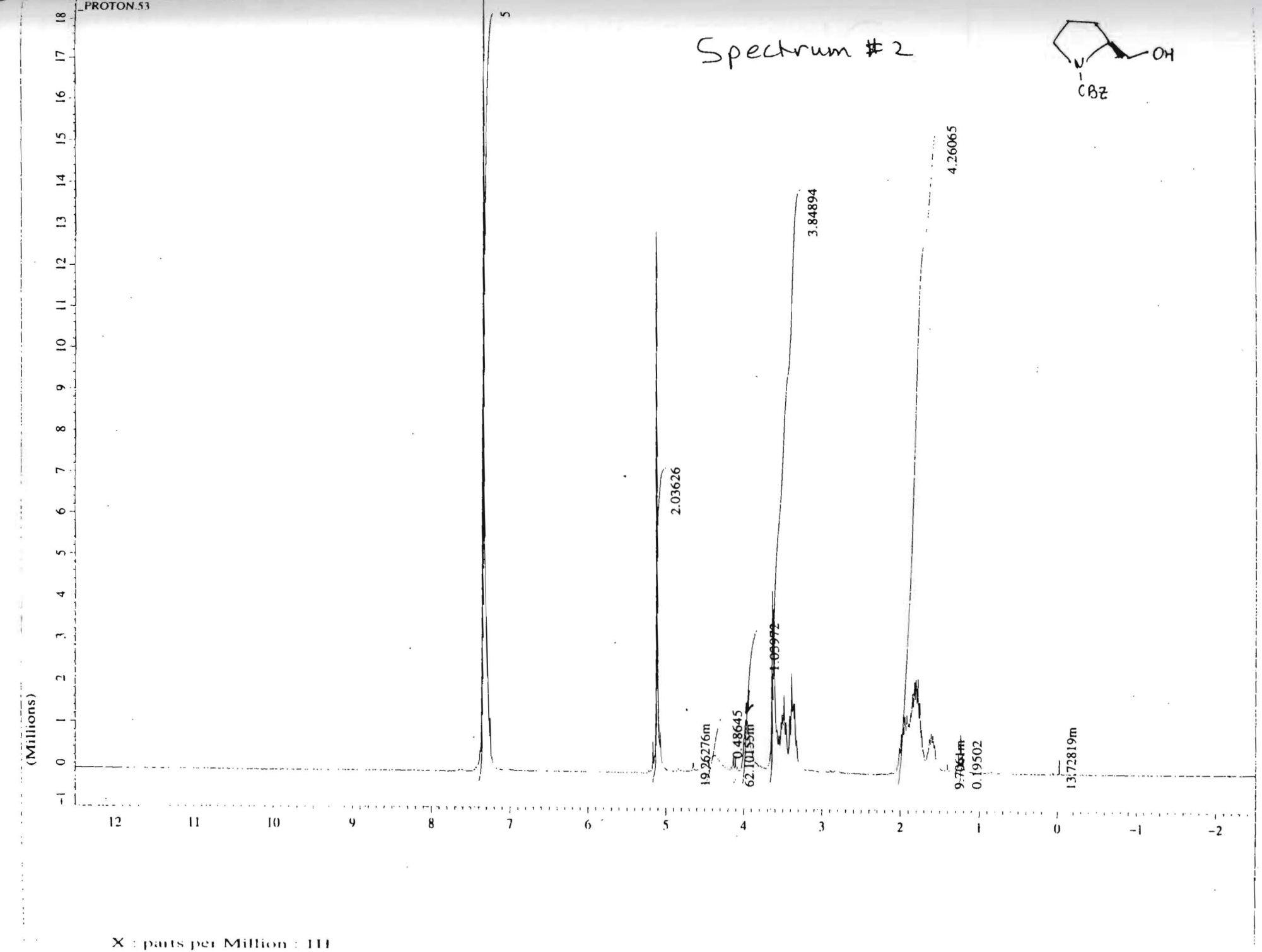
Appendix II: Spectral Data

Spectrum #1

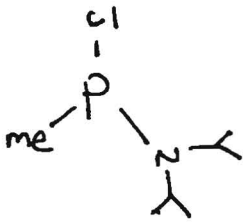


DJM1-15

File Name	= ~~_PROTON.5~~
Author	=
Sample ID	= S#155279
Content	=
Creation Date	= 2-OCT-1997 07:25:58
Revision Date	= 2-OCT-1997 04:26:39
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 13
Solvent	= CHLOROFORM-D
Spin_get	= 14[Hz]
Temp_get	= 20.9[dC]


_PROTON.53
Spectrum # 2
OH
CBZ
4.26065
3.84894
2.03626
19.26276m
0.48645
62.10155m
1.00000
0.19502
13.72819m
(Millions)
X : parts per Million : 111

Spectrum #3

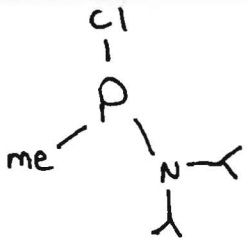


JEOL

-13 in lab book

File Name	= DJM1-11cr.PROTON.2
Author	=
Sample ID	= DJM1-11cr
Content	=
Creation Date	= 23-SEP-1997 01:59:48
Revision Date	= 22-SEP-1997 23:00:22
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 7
Solvent	= CHLOROFORM-D
Spin_get	= 13[Hz]
Temp_get	= 19.5[dC]

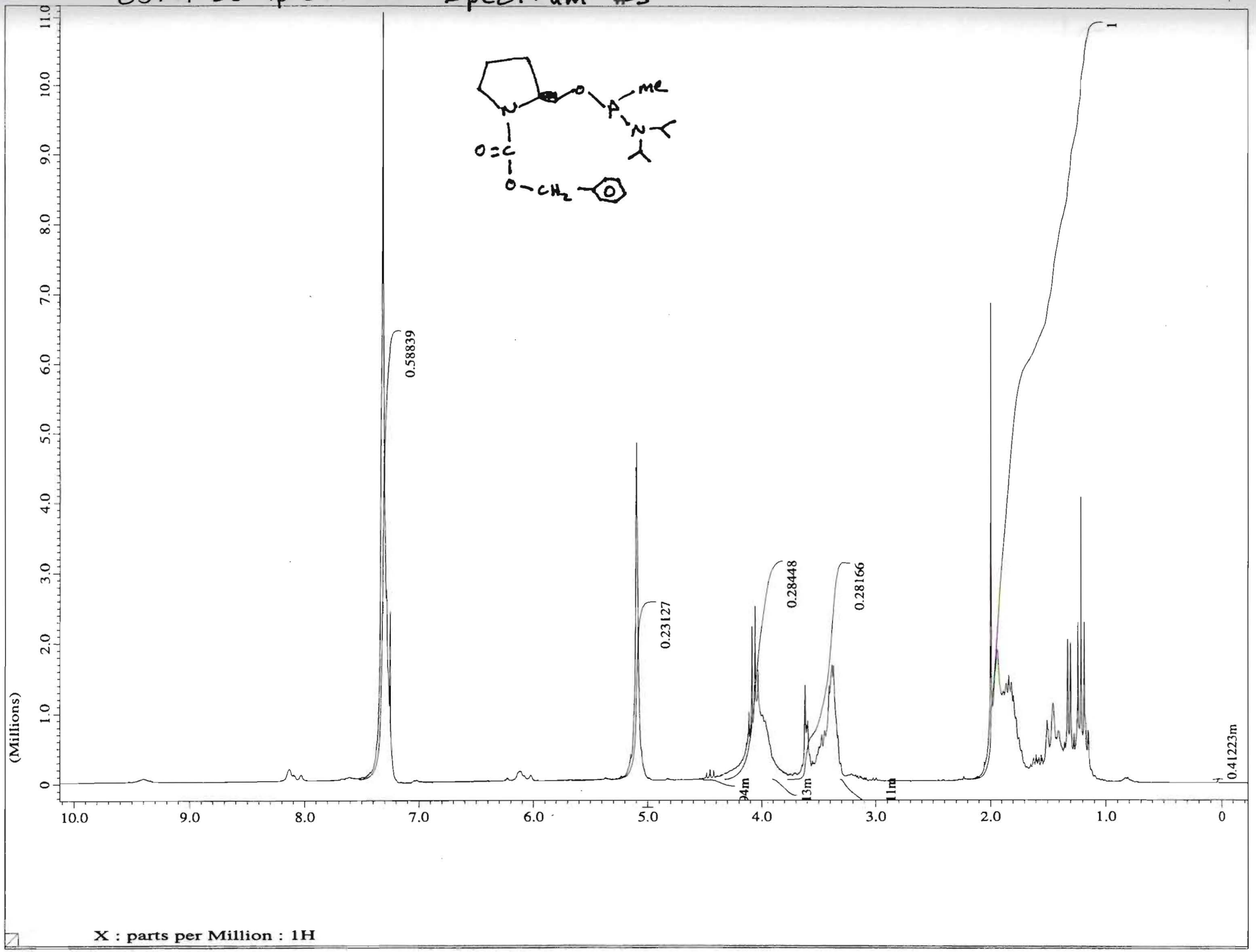
Spectrum #4



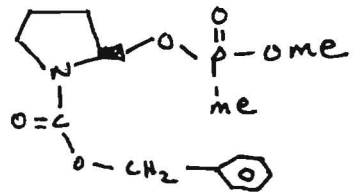
JEOL

File Name	= DJM1-21-1_PROTON.2
Author	=
Sample ID	= DJM1-21-1
Content	=
Creation Date	= 29-OCT-1997 07:40:54
Revision Date	= 29-OCT-1997 04:41:38
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 14
Solvent	= CHLOROFORM-D
Spin_get	= 17[Hz]
Temp_get	= 20.3[dC]

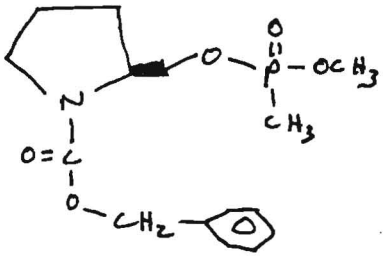
DJM1-23-1proton Spectrum #5

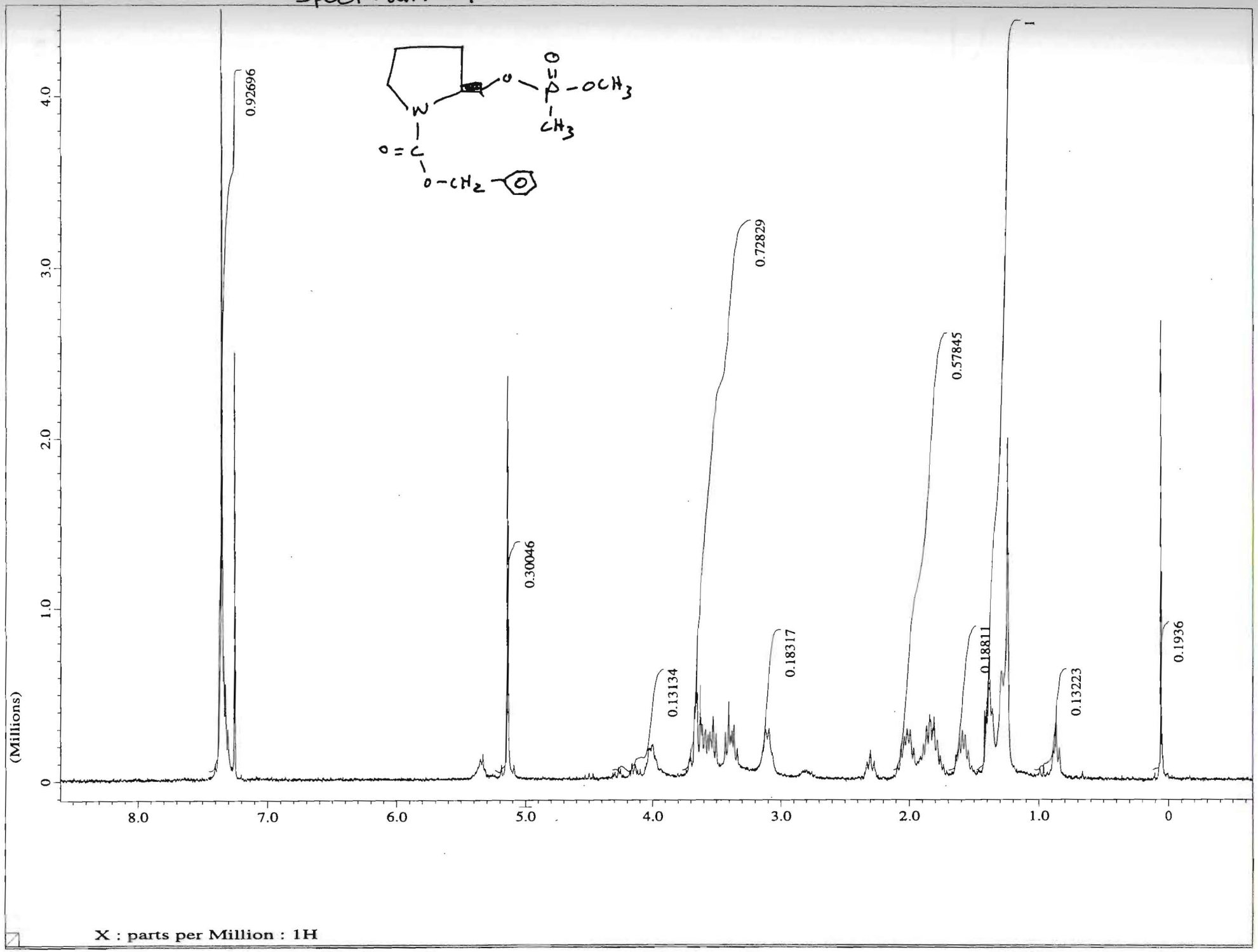


DJM1-77 Spectrum *6a

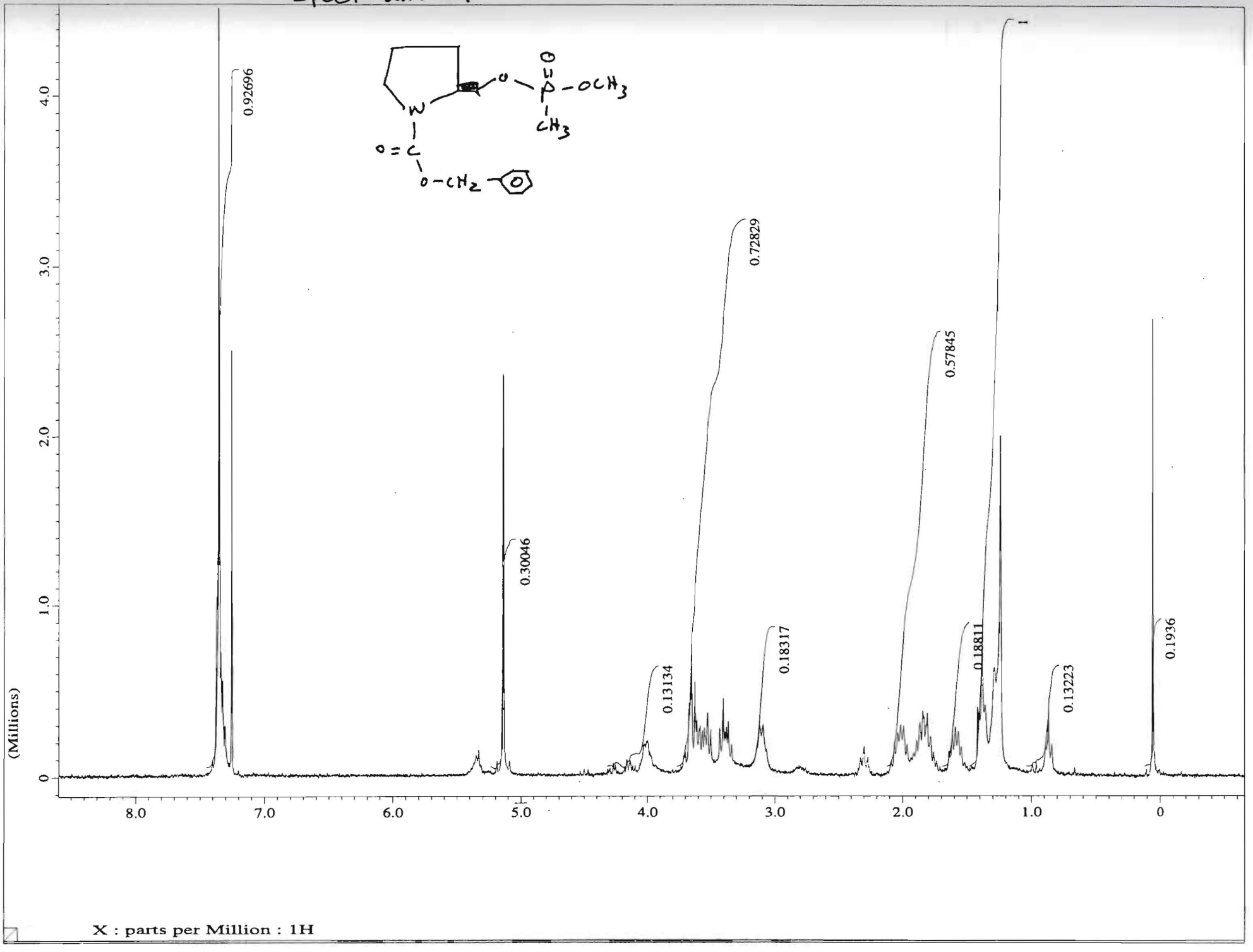


DJm1-19 Spectrum #6b

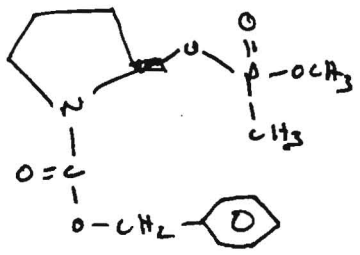



Spectrum #7
0.92696
0.30046
0.13134
0.72829
0.18317
0.57845
0.18811
0.13223
0.1936
(Millions)
X : parts per Million : 1H

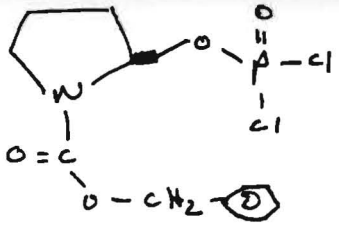
DSM1-25 Spectrum #7



DJM1-49A Spectrum #8



Spectrum #9



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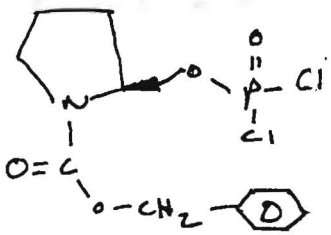
Author	=
Sample ID	= DJM1-29
Content	=
Creation Date	= 16-FEB-1998 05:33:16
Revision Date	= 16-FEB-1998 02:34:06
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 11
Solvent	= CHLOROFORM-D
Spin_gat	= 17[Hz]
Temp_gat	= 20.5[dC]

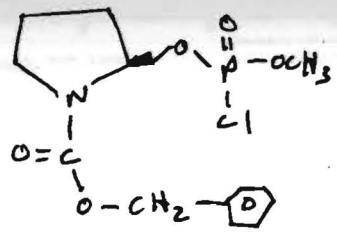
Spectrum # 10



JEOL

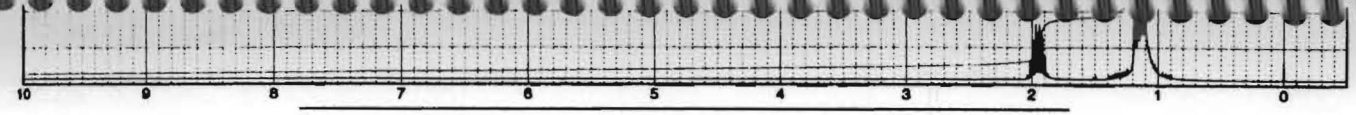
File Name	= DJM1-29-2_PROTON.2
Author	=
Sample ID	= DJM1-29-2
Content	=
Creation Date	= 15-JAN-1998 07:25:34
Revision Date	= 15-JAN-1998 04:26:23
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 16
Solvent	= CHLOROFORM-D
Spin_get	= 13[Hz]
Temp_get	= 20.3[dC]

Spectrum #11



JEOL

File Name	= DJM1-31-2_PROTON.2
Author	=
Sample ID	= DJM1-31-2
Content	=
Creation Date	= 15-JAN-1998 07:49:01
Revision Date	= 15-JAN-1998 04:49:44
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 15
Solvent	= CHLOROFORM-D
Spin_get	= 17[Hz]
Temp_get	= 20.2[dC]



Aldrich 30,155-8 *CAS [13716-10-4]*

Di-*tert*-butylchlorophosphine, 96%

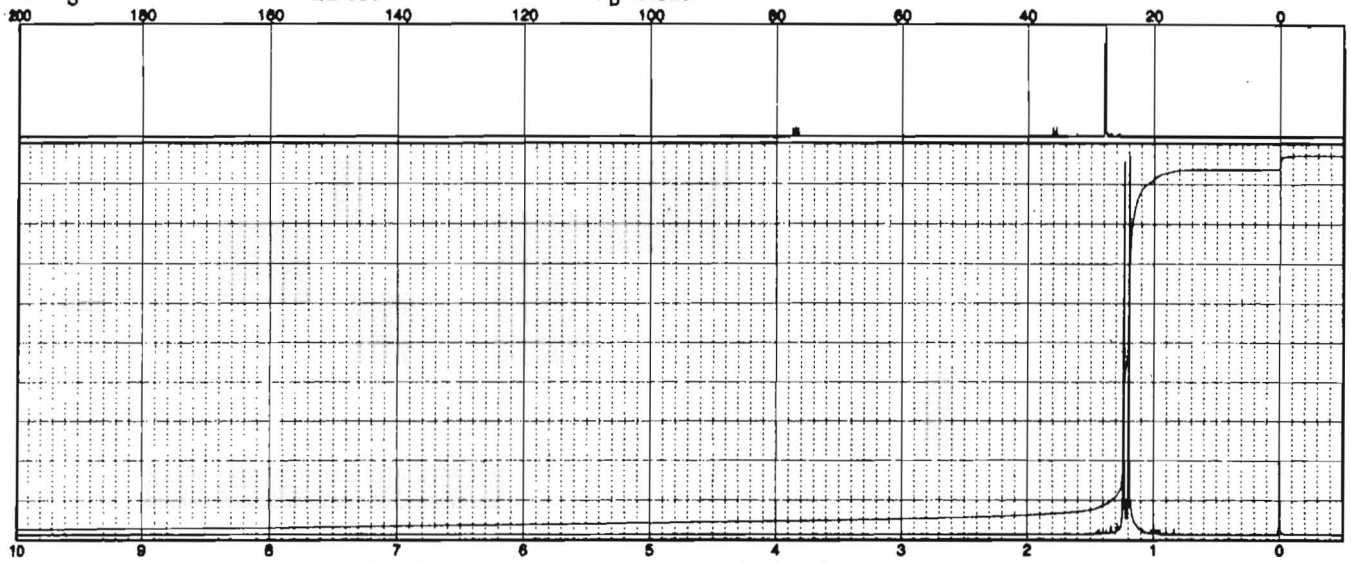
$C_8H_{18}ClP$
FW 180.66
bp 48°C (3 mm)
d 0.951
n_D^{20} 1.4820

Fp 142°F

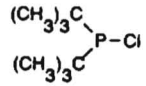
VP-FT-IR: 3, 837D

36.53
36.07
27.90*
27.66*

$CDCl_3$ QE-300



B



Aldrich 33,686-6 *CAS [676-83-5]*

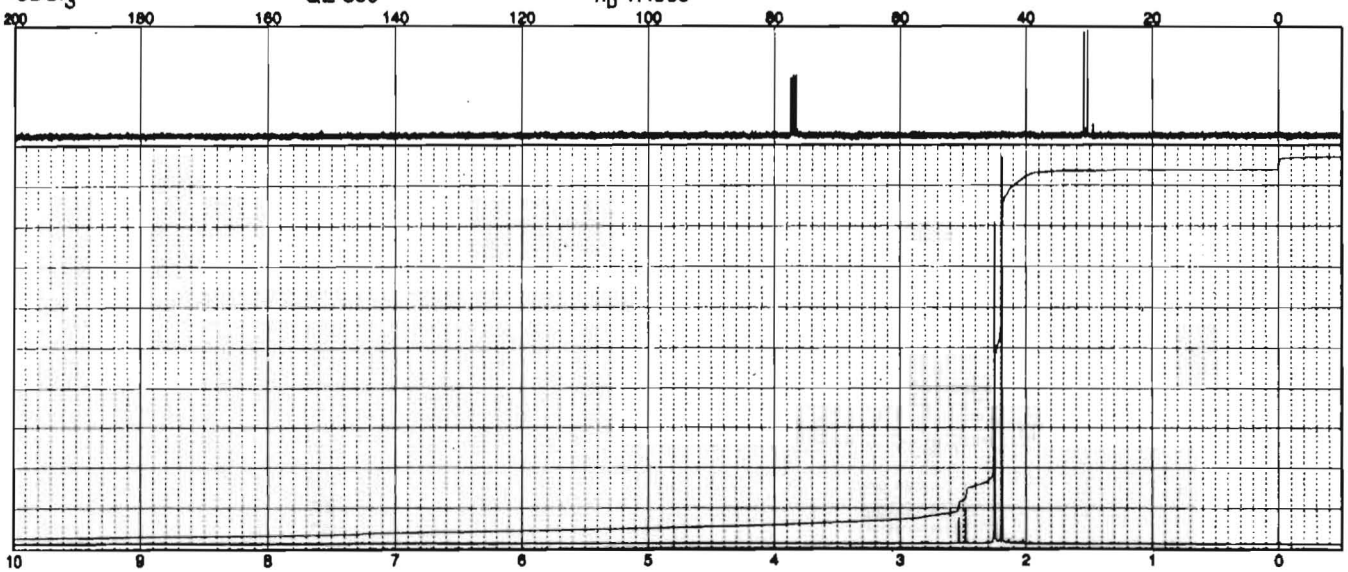
Dichloromethylphosphine, tech., 90%

CH_3Cl_2P
FW 116.92
bp 82°C
d 1.304
n_D^{20} 1.4960

Fp 120°F

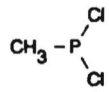
30.67*
30.28*

$CDCl_3$ QE-300



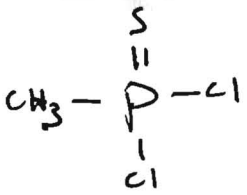
C

Spectrum #12b



Spectrum #12a

Spectrum #13



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Author =
Sample ID = DJM1-33-1
Content =
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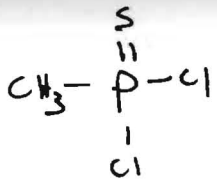
Revision Date = 22-JAN-1998 04:02:19

Spec Site = Eclipse 270
Spec Type = DELTA_NMR

Data Format = 1D COMPLEX
Dimensions = X
Dim Title = 1H
Dim Size = 16384
Dim Units = [ppm]
Scans = 16
X_domain = 1H
X_offset = 5.0[ppm]
X_freq = 270.16743928[MHz]
X_sweep = 4.05350628[kHz]
Field_strength = 6.345446[T]
Recvr_gain = 15
Solvent = CHLOROFORM-D
Spin_get = 15[Hz]
Temp_get = 20.3[dC]

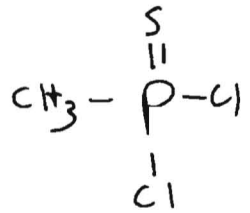
Spectrum #14



JEOL

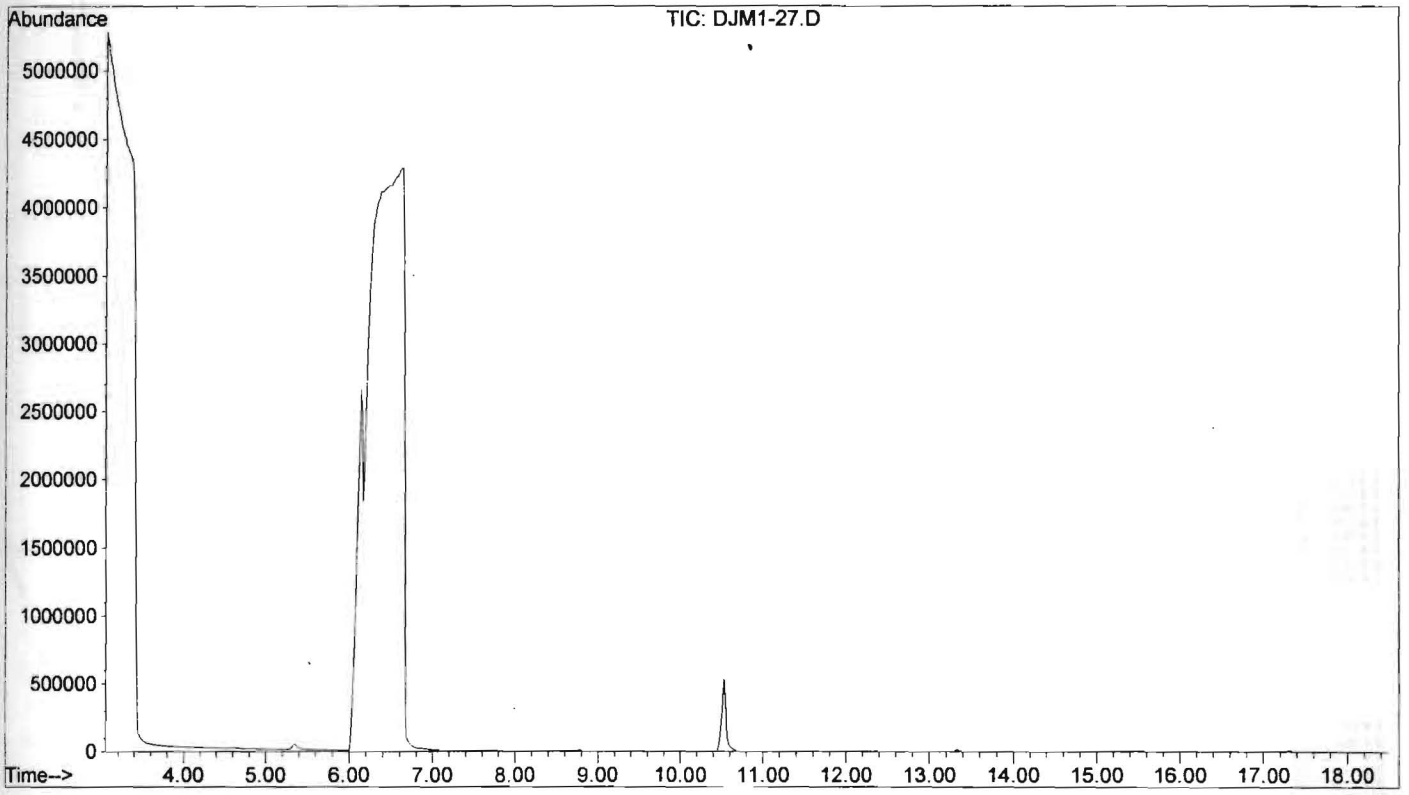
File Name	= DJM1-33-1_CARBON.2
Author	=
Sample ID	= DJM1-33-1
Content	=
Creation Date	= 22-JAN-1998 07:29:46
Revision Date	= 22-JAN-1998 04:30:40
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 13C
Dim Size	= 32768
Dim Units	= [ppm]
Scans	= 545
X_domain	= 13C
X_offset	= 100.0[ppm]
X_freq	= 67.94010394[MHz]
X_sweep	= 17.00680272[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 27
Solvent	= CHLOROFORM-D
Spin_get	= 15[Hz]
Temp_get	= 21.5[dC]

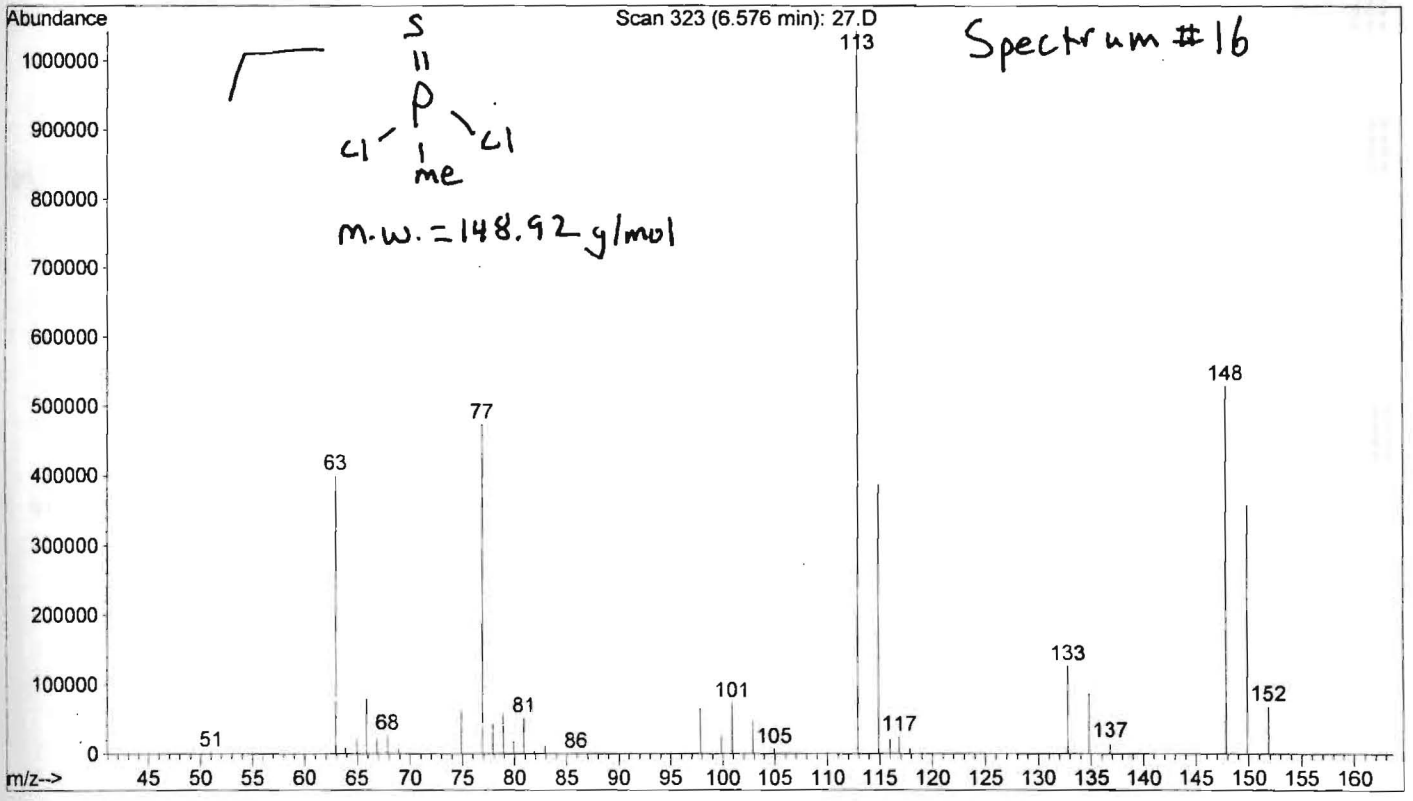
Spectrum # 15



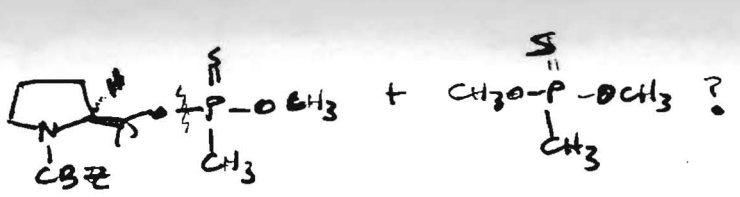
400.0
300.0
200.0
100.0
0
(Millions)
88.0
87.0
86.0
85.0
84.0
83.0
82.0
81.0
80.0
79.0
78.0
77.0
76.0
75.0
74.0
73.0
X : parts per Million : 31P

File : C:\HPCHEM\1\DATA\DJM1-27.D
Operator : D. Megott
Acquired : 14 Jan 98 2:49 pm using AcqMethod DEFAULT
Instrument : GC/MS Ins
Sample Name: Phosphine + Sulfur
Misc Info :
Vial Number: 1





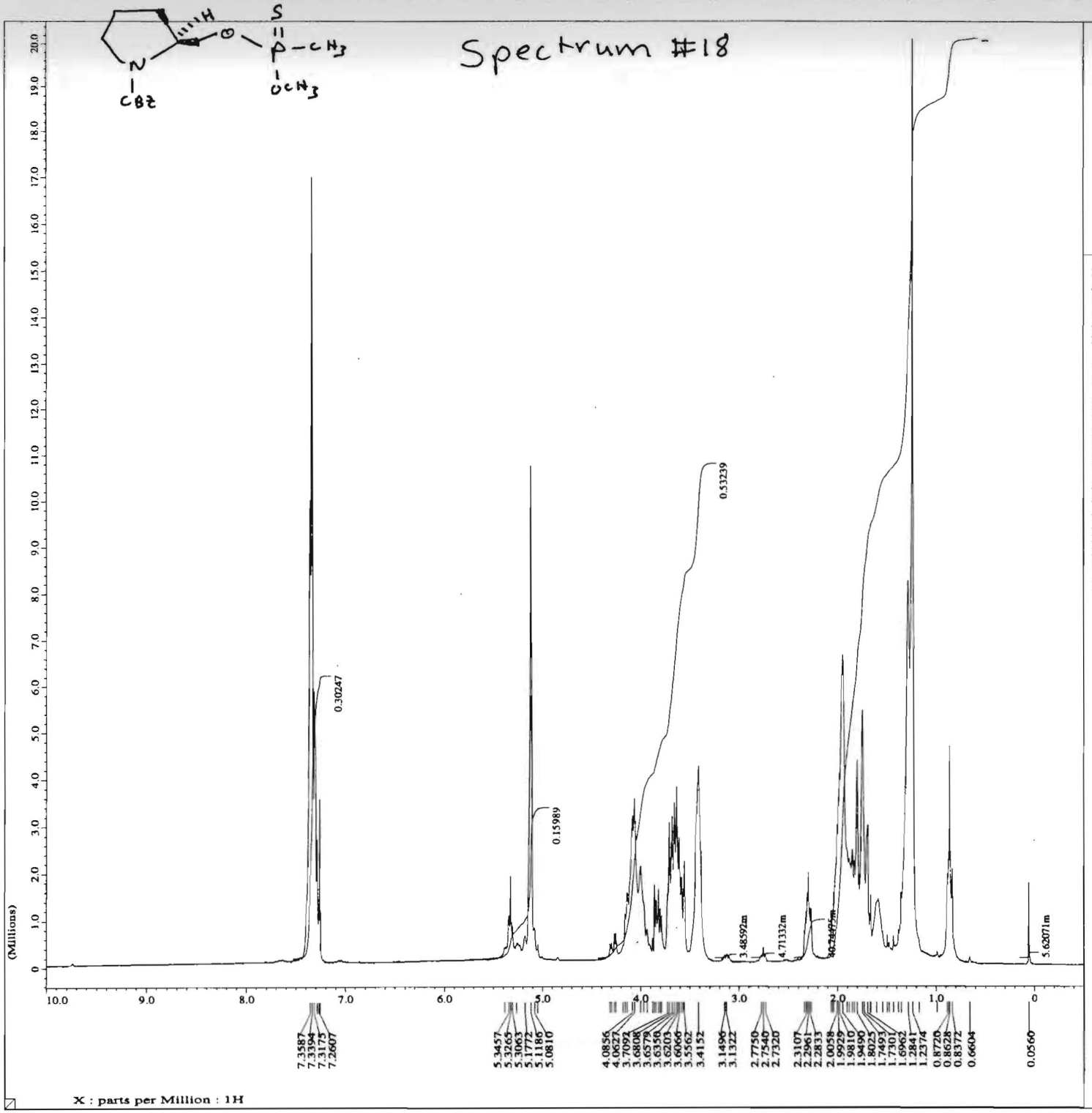
Spectrum #17



CRUDE

JEOL

File Name	= DJM1-35-1_PROTON.2
Author	=
Sample ID	= DJM1-35-1
Content	=
Creation Date	= 28-JAN-1998 11:53:41
Revision Date	= 28-JAN-1998 08:54:23
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 21
Solvent	= CHLOROFORM-D
Spin_set	= 16[Hz]
Temp_set	= 20.3[dC]



JEOL

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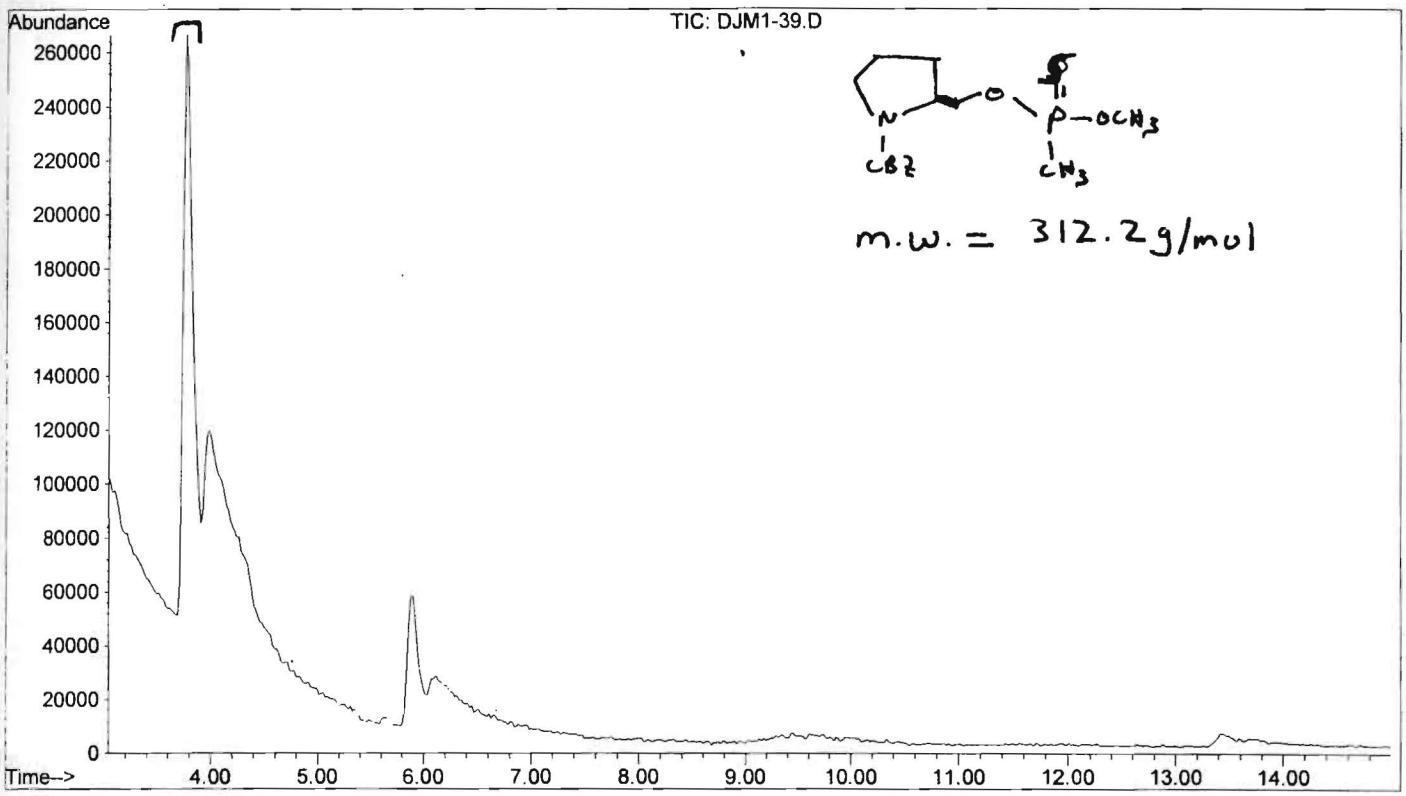
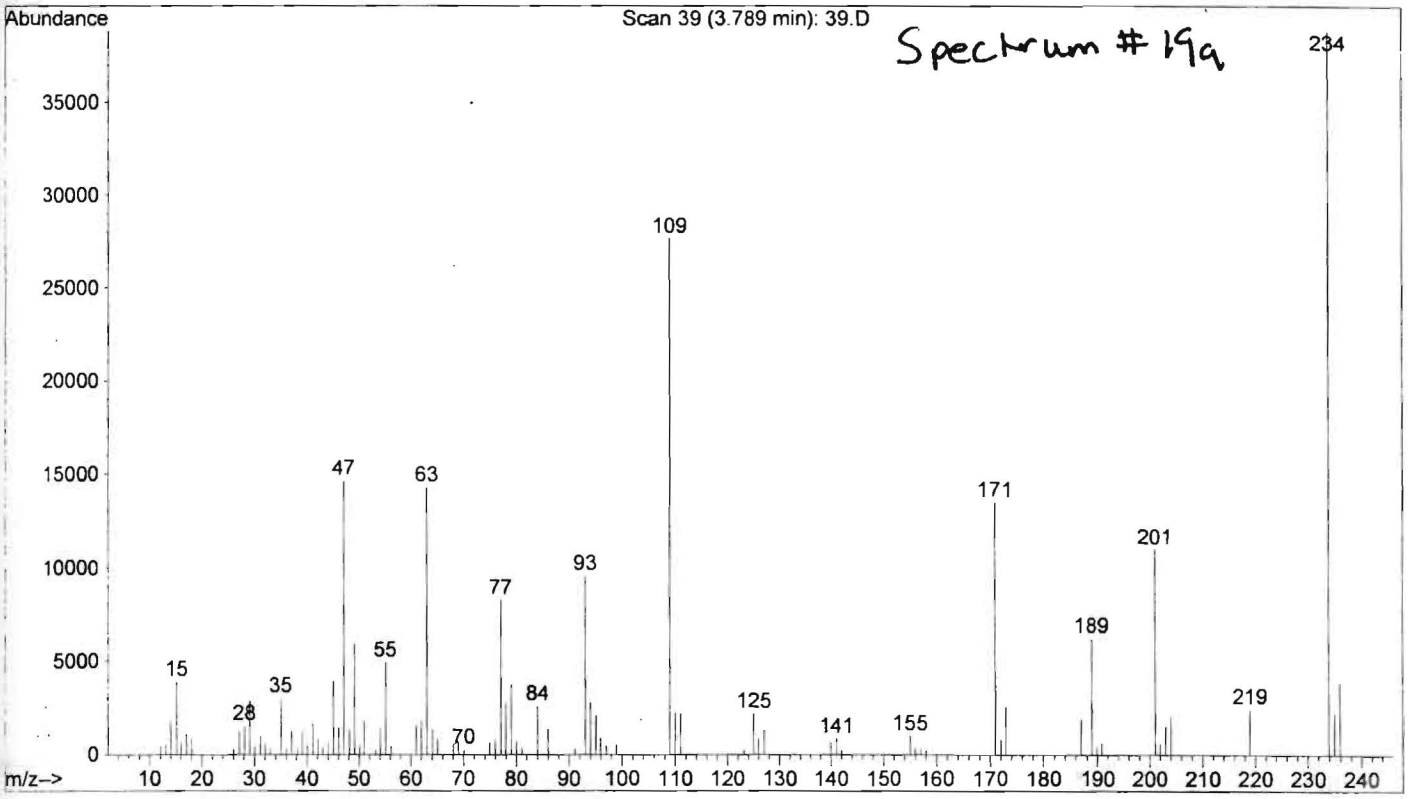
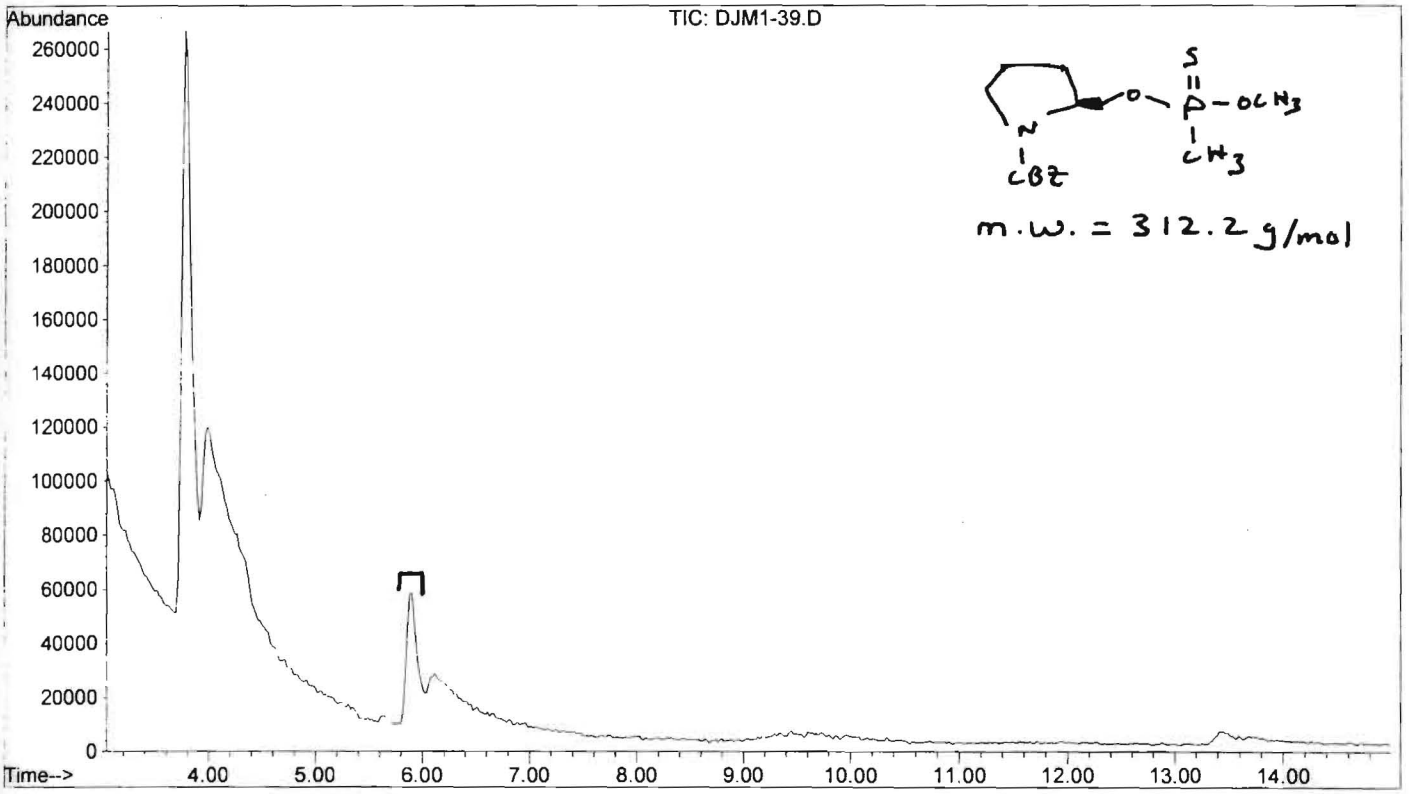
Author	=
Sample ID	= DJM1-35-F1-3
Content	=
Creation Date	= 4-FEB-1998 06:13:56
Revision Date	= 4-FEB-1998 03:14:34
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 16
Solvent	= CHLOROFORM-D
Spin_get	= 15[Hz]
Temp_get	= 20.6[dC]

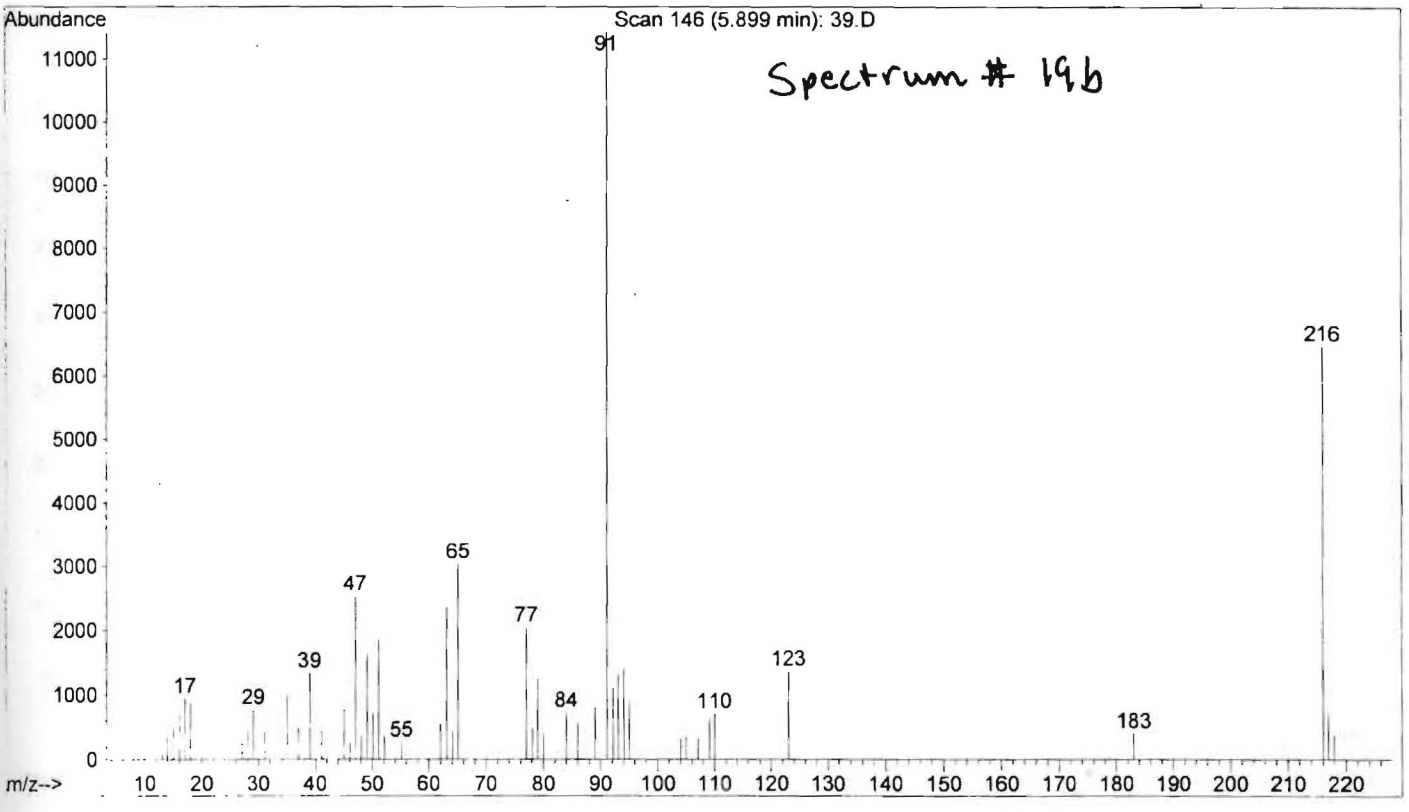
File : C:\HPCHEM\1\DATA\DJM1-39.D
Operator : dustin mergott
Acquired : 11 Feb 98 2:38 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



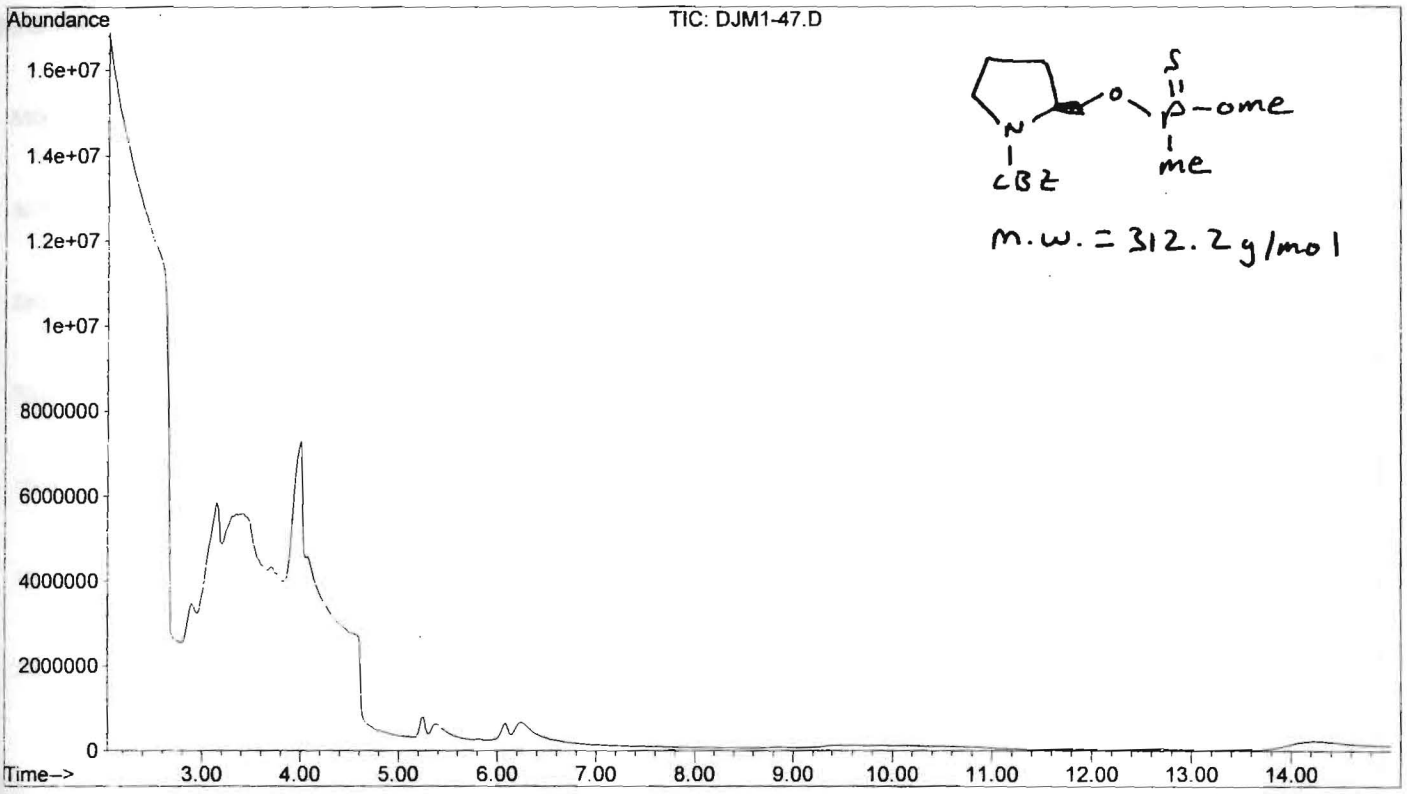


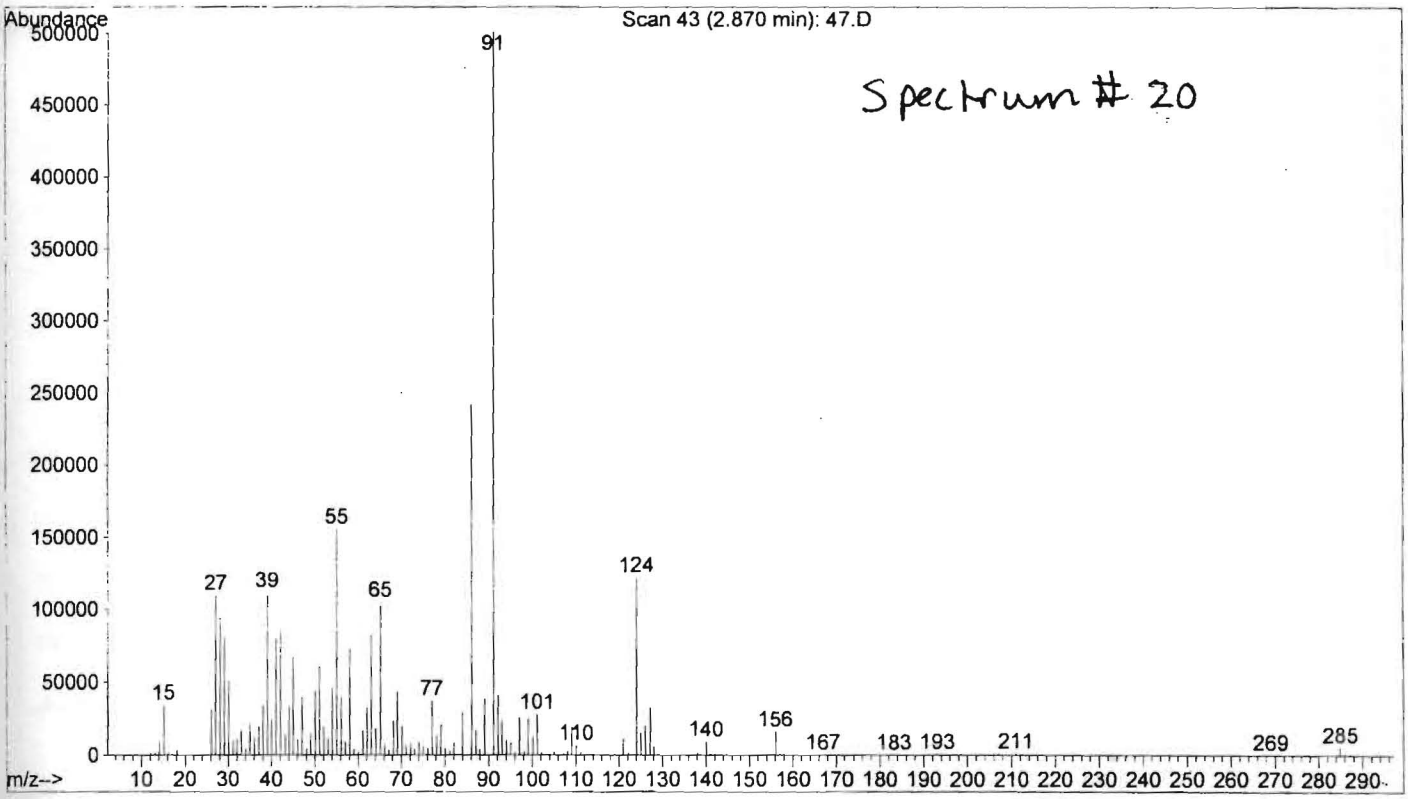
File : C:\HPCHEM\1\DATA\DJM1-39.D
Operator : dustin mergott
Acquired : 11 Feb 98 2:38 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



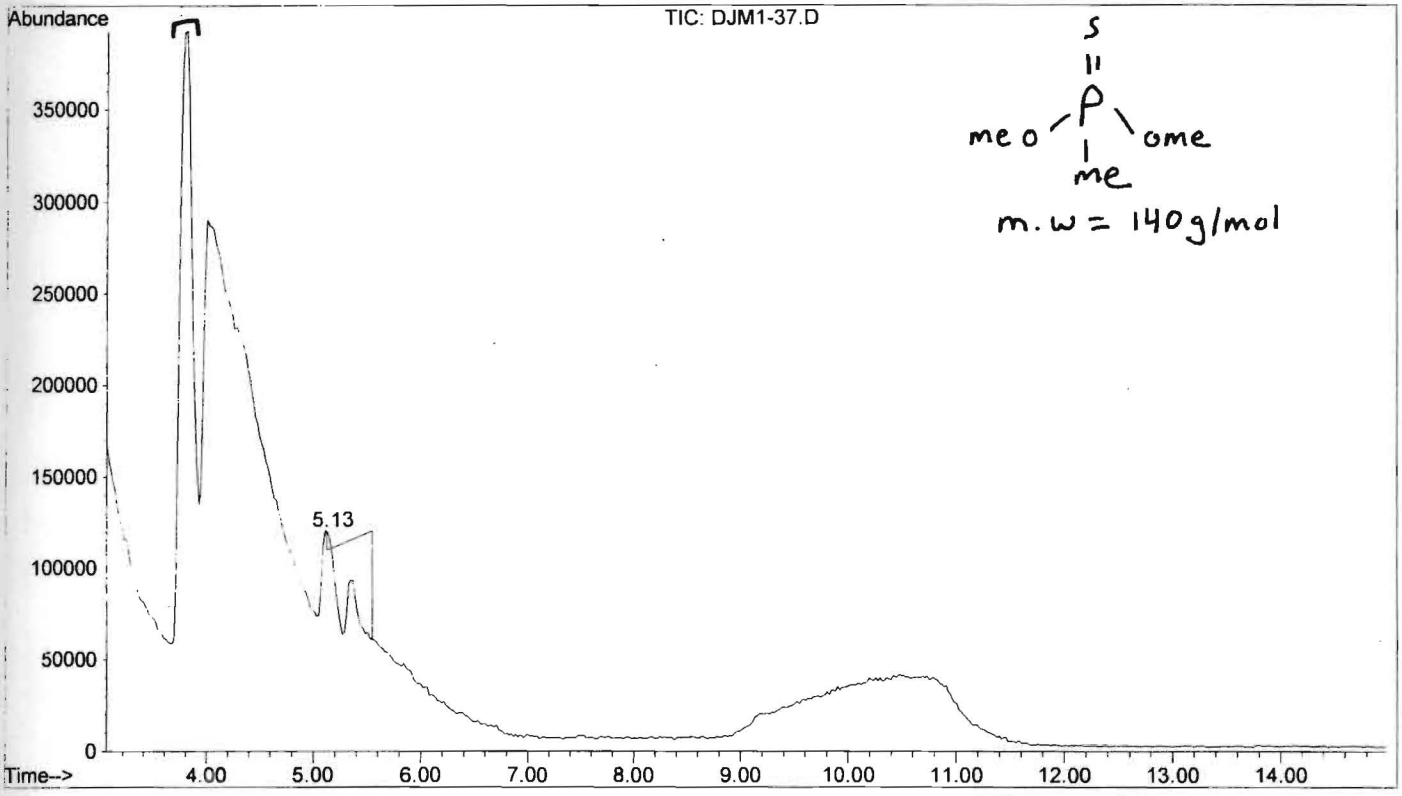


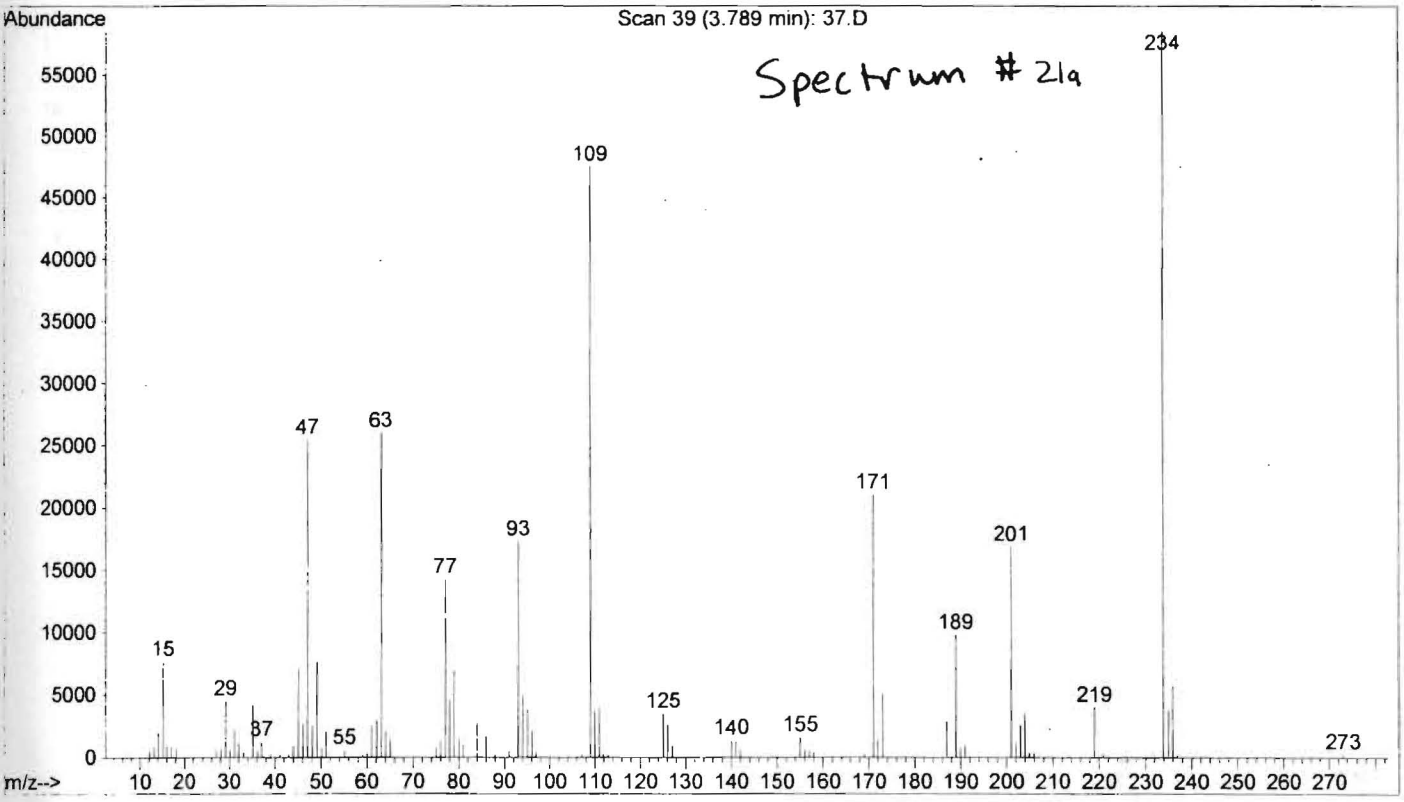
File : C:\HPCHEM\1\DATA\DJM1-47.D
Operator : ofv
Acquired : 25 Feb 98 4:40 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



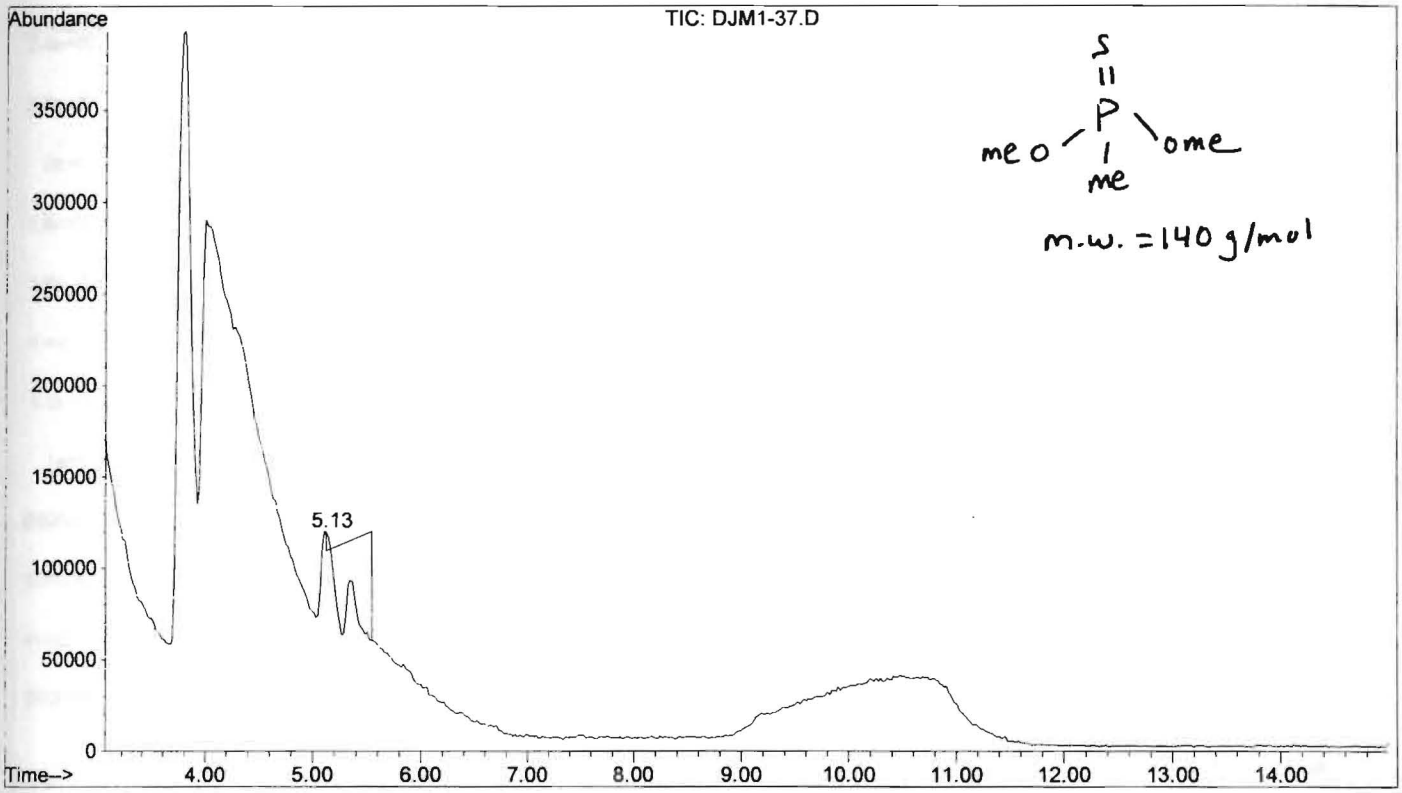


File : C:\HPCHEM\1\DATA\DJM1-37.D
Operator : dustin mergott
Acquired : 11 Feb 98 3:58 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1

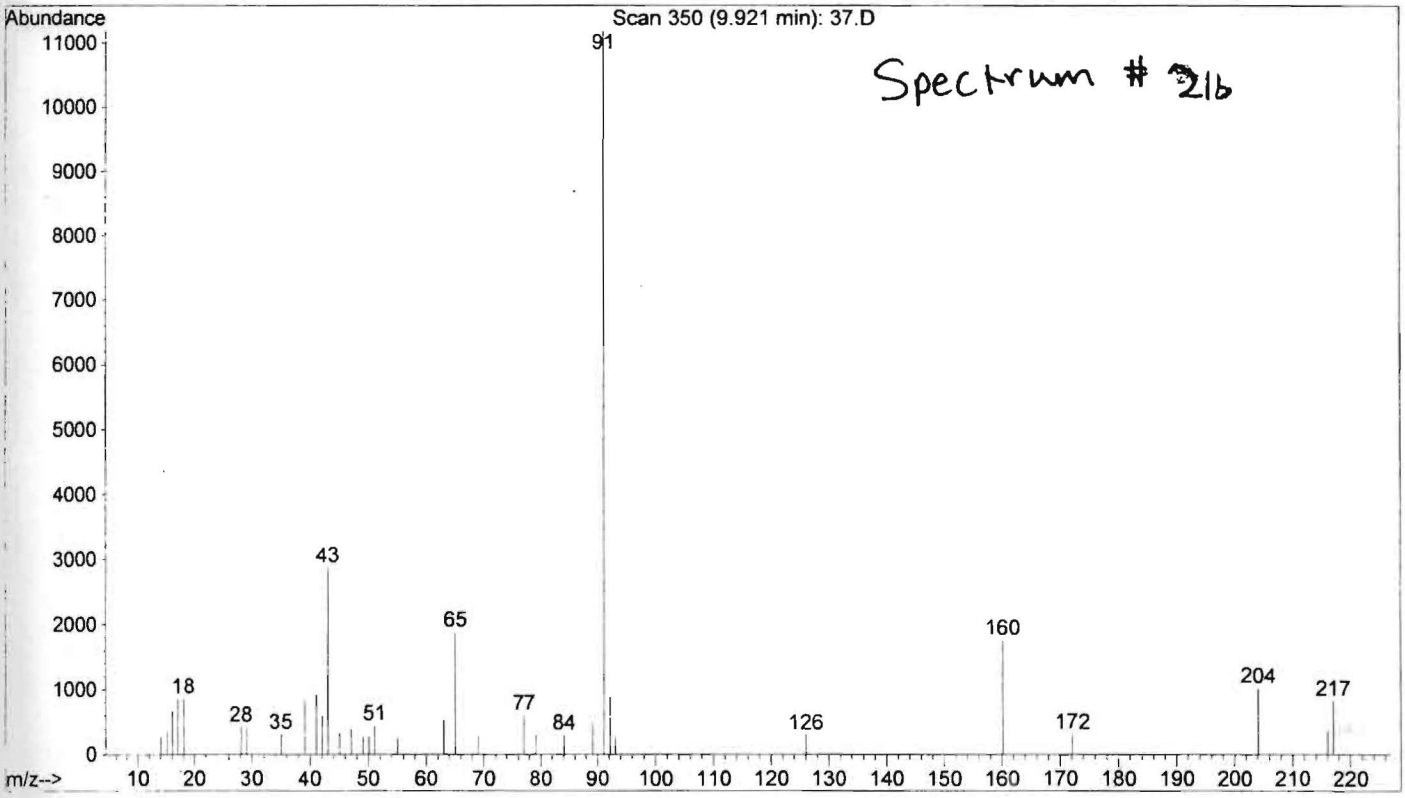




File : C:\HPCHEM\1\DATA\DJM1-37.D
Operator : dustin mergott
Acquired : 11 Feb 98 3:58 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1

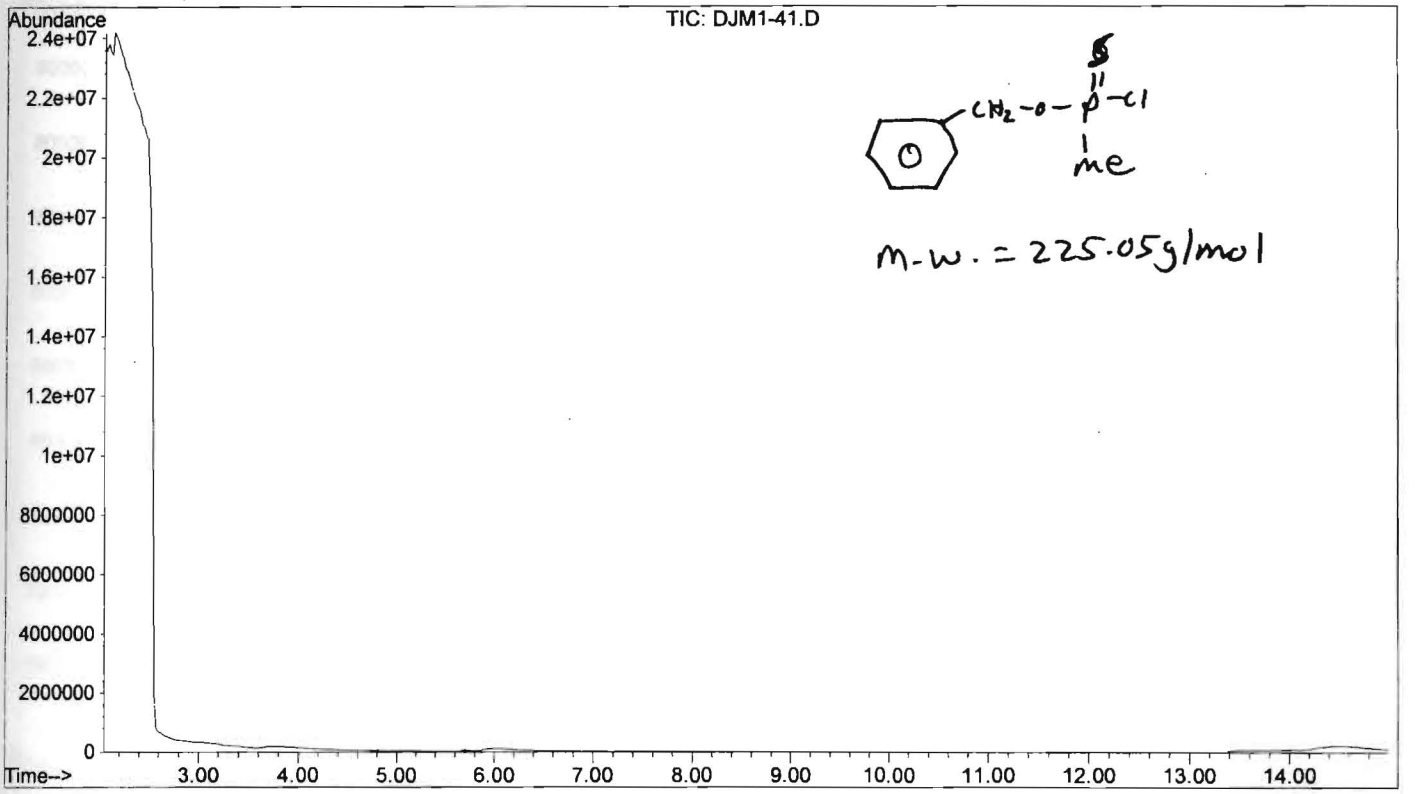


m.w. = 140 g/mol

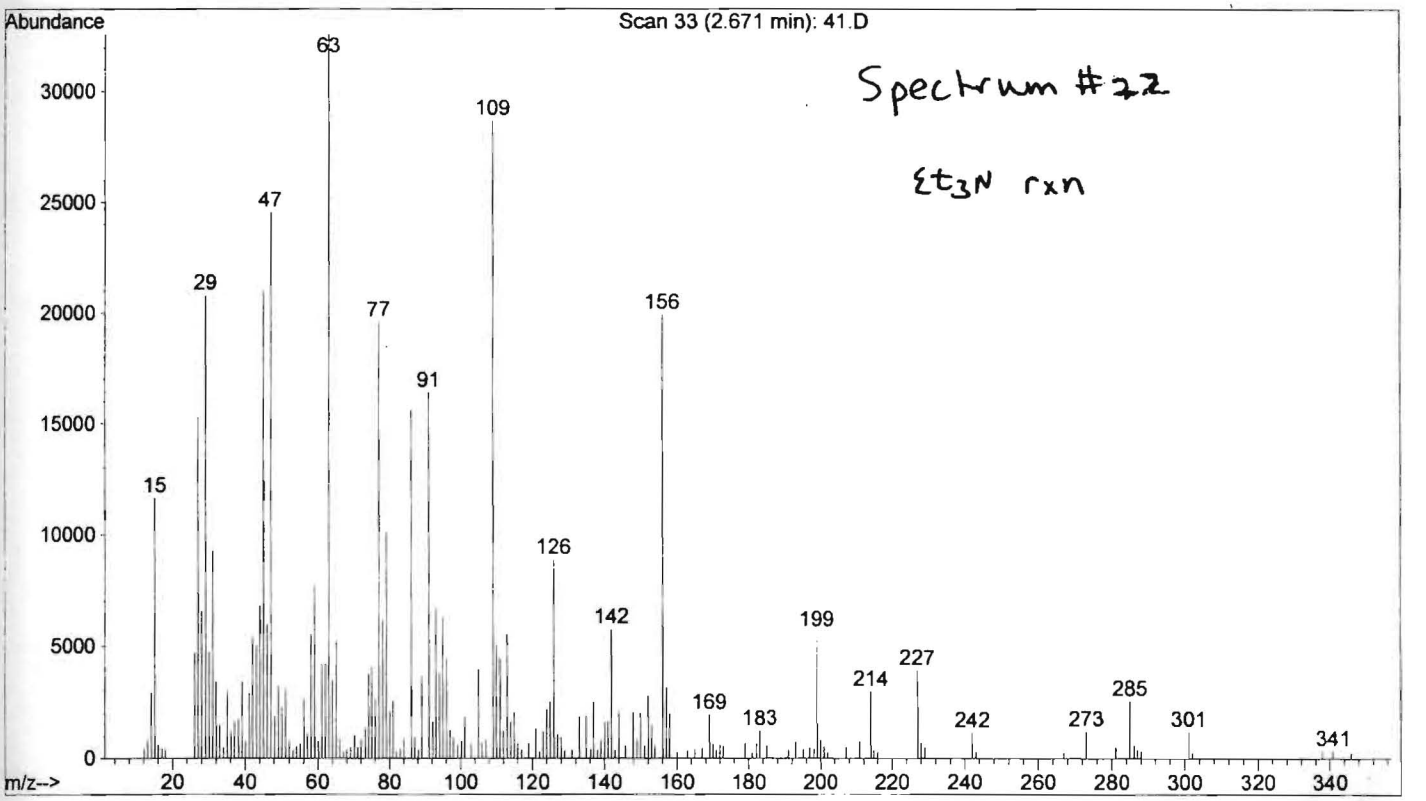


Spectrum # 216

File : C:\HPCHEM\1\DATA\DJM1-41.D
Operator : Dustin Mergott
Acquired : 16 Feb 98 11:47 am using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



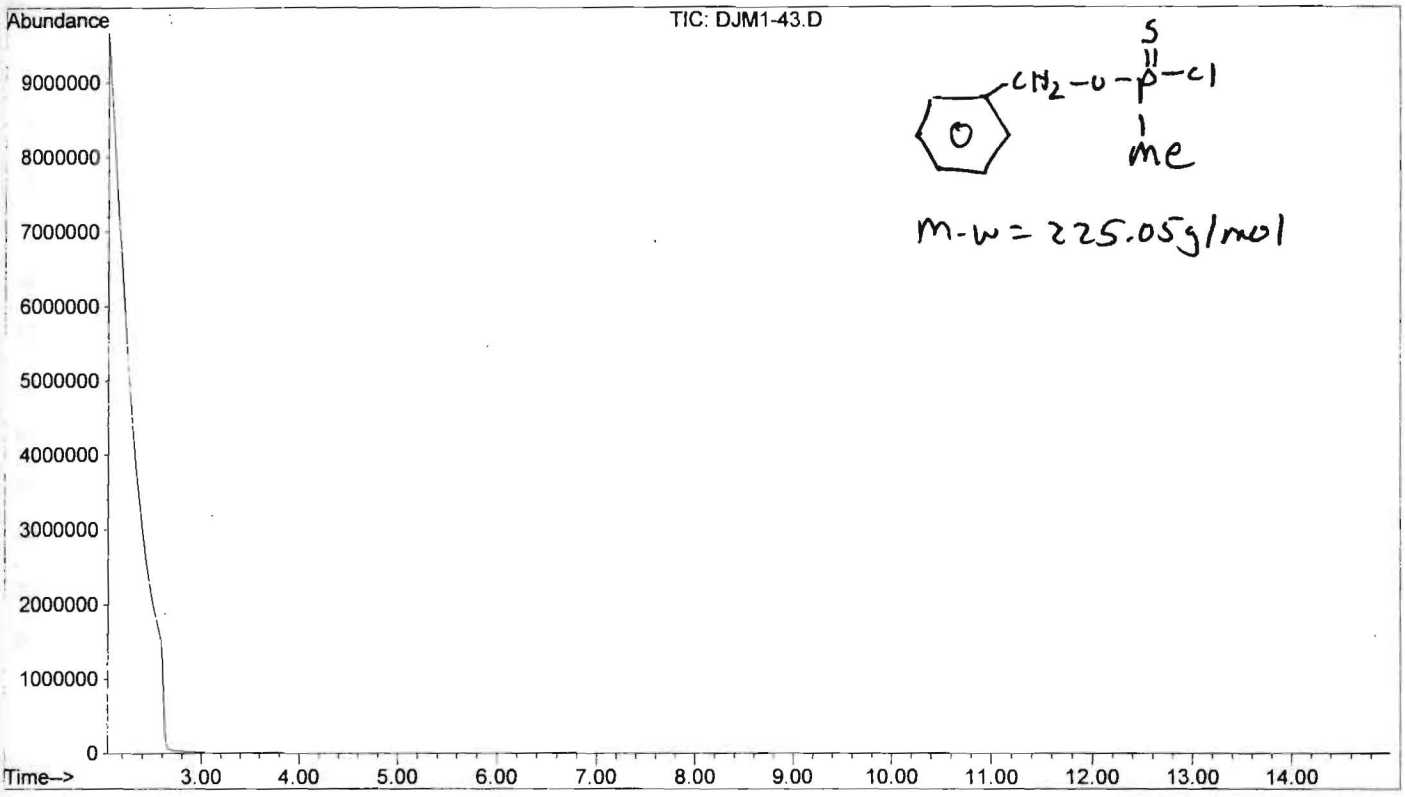
m.w. = 225.05 g/mol



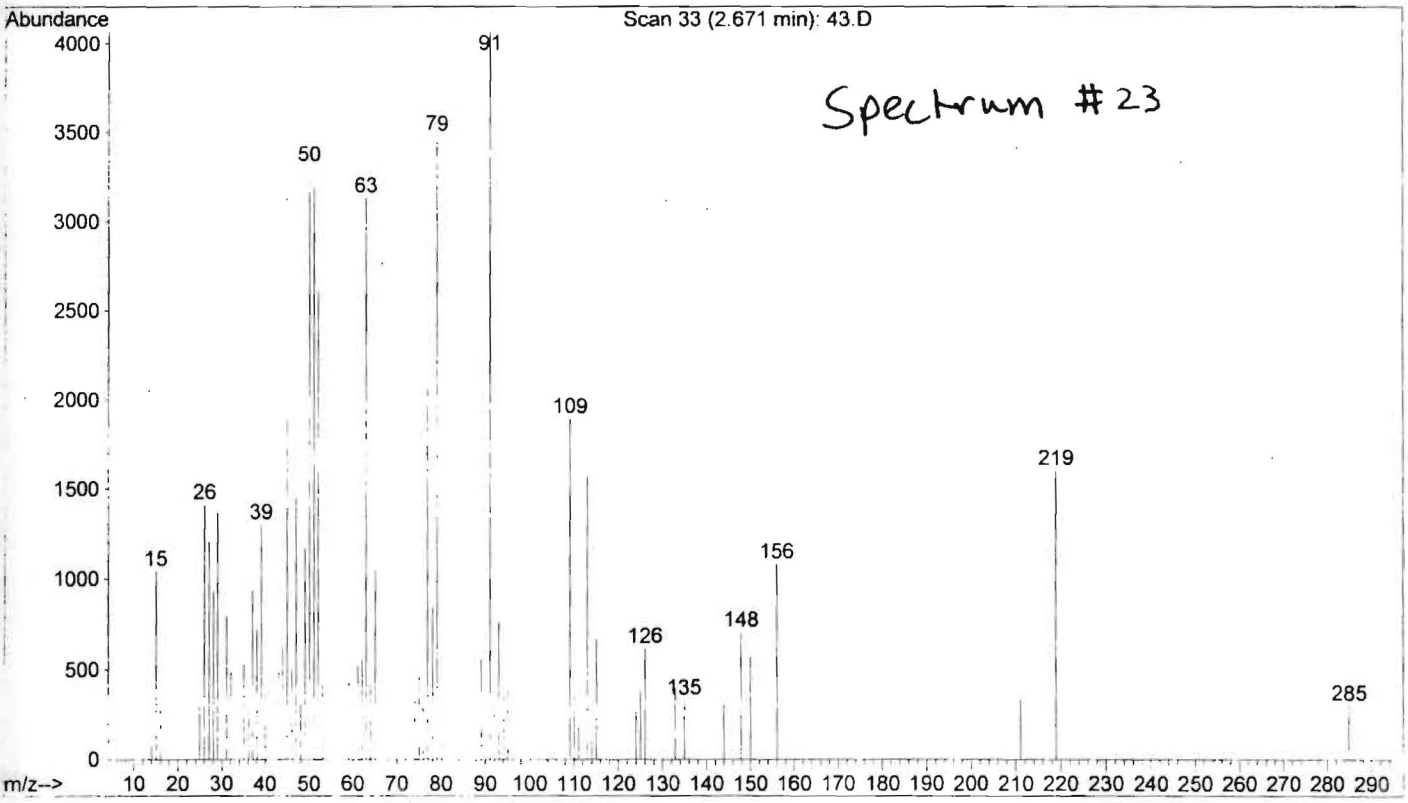
Spectrum #22

Et_3N rxn

File : C:\HPCHEM\1\DATA\DJM1-43.D
Operator : Dustin Mergott
Acquired : 16 Feb 98 12:29 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1

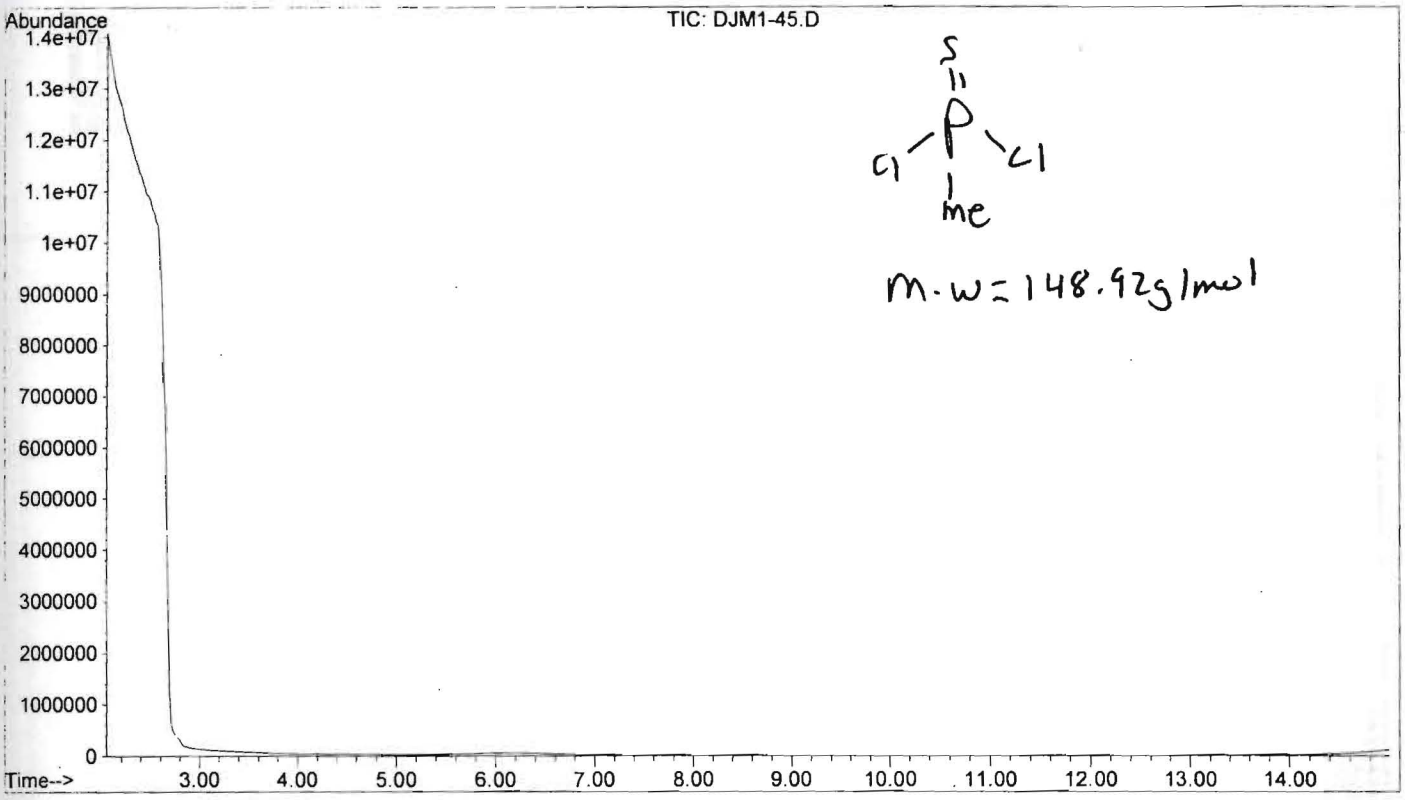


m-w = 225.05 g/mol

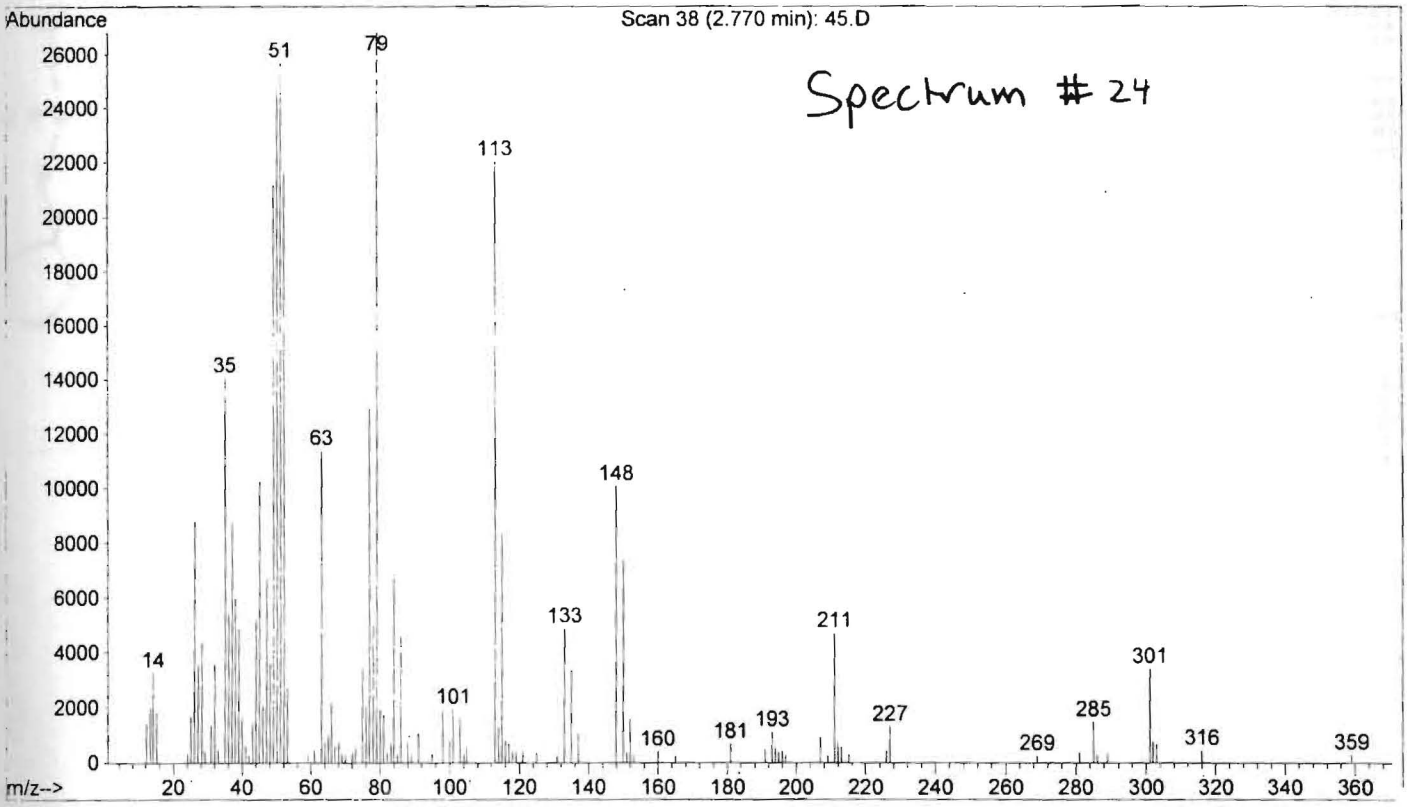


Spectrum #23

File : C:\HPCHEM\1\DATA\DJM1-45.D
Operator : Dustin Mergott
Acquired : 17 Feb 98 11:53 am using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1

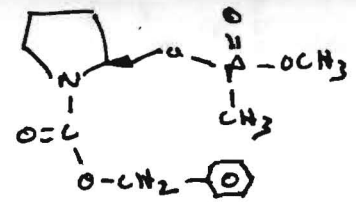


m.w = 148.92 g/mol



Spectrum # 24

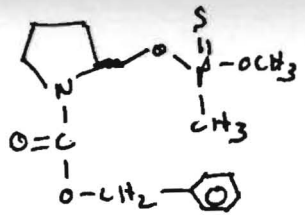
Spectrum #25



JEOL

File Name	= DJM1-51_PROTON.2
Author	=
Sample ID	= DJM1-51
Content	=
Creation Date	= 11-MAR-1998 03:01:30
Revision Date	= 11-MAR-1998 00:02:21
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 8
Solvent	= CHLOROFORM-D
Spin_set	= 16[Hz]
Temp_set	= 20.7[dC]

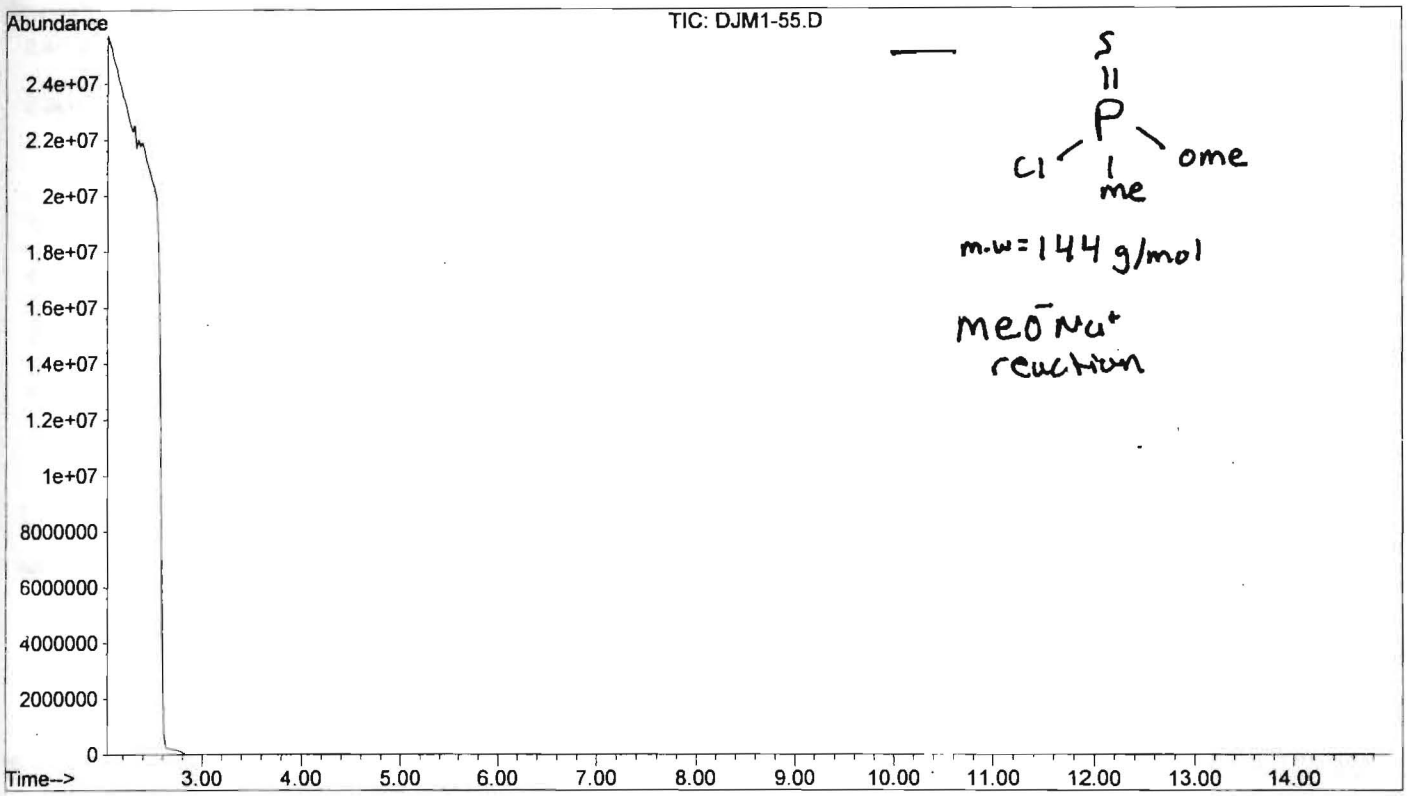
Spectrum # 26

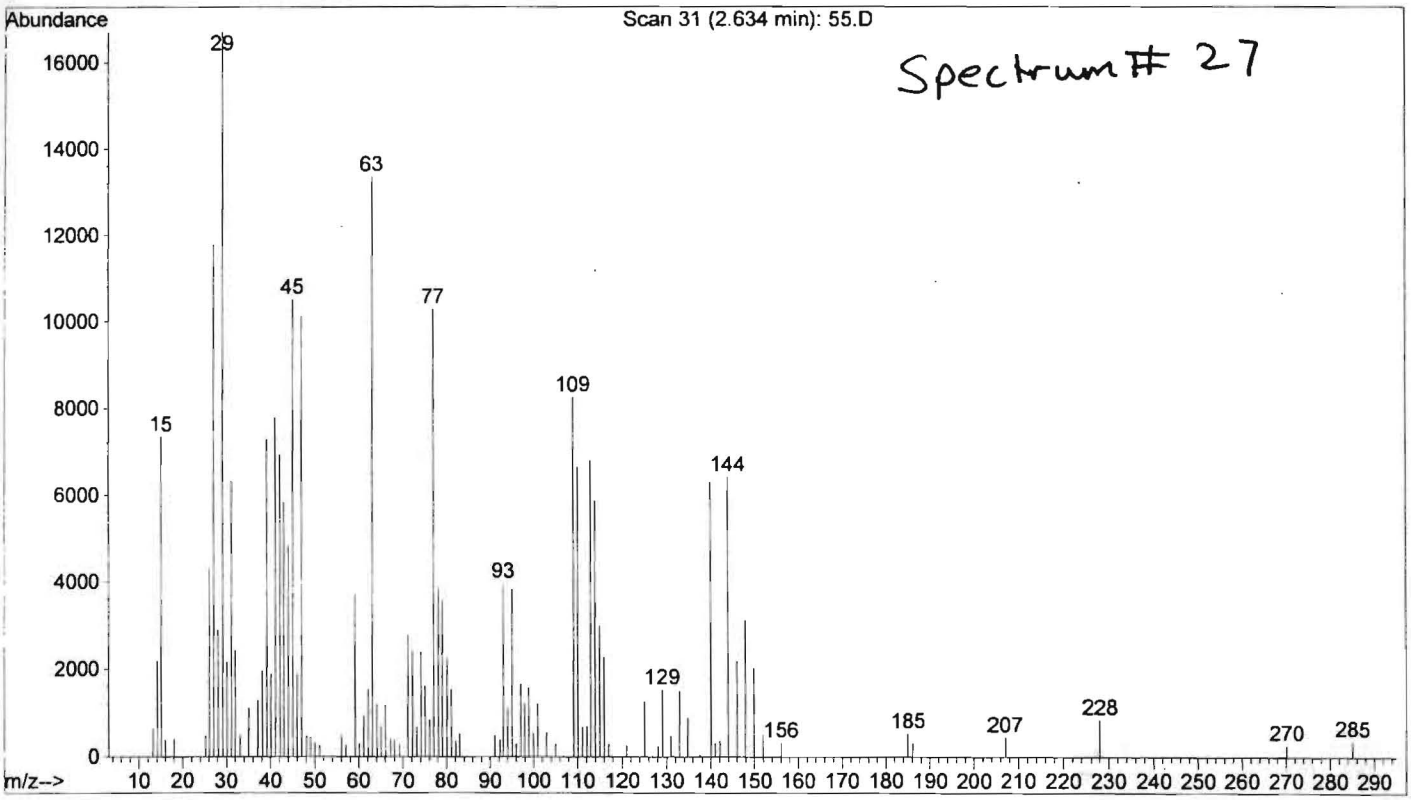


JEOL

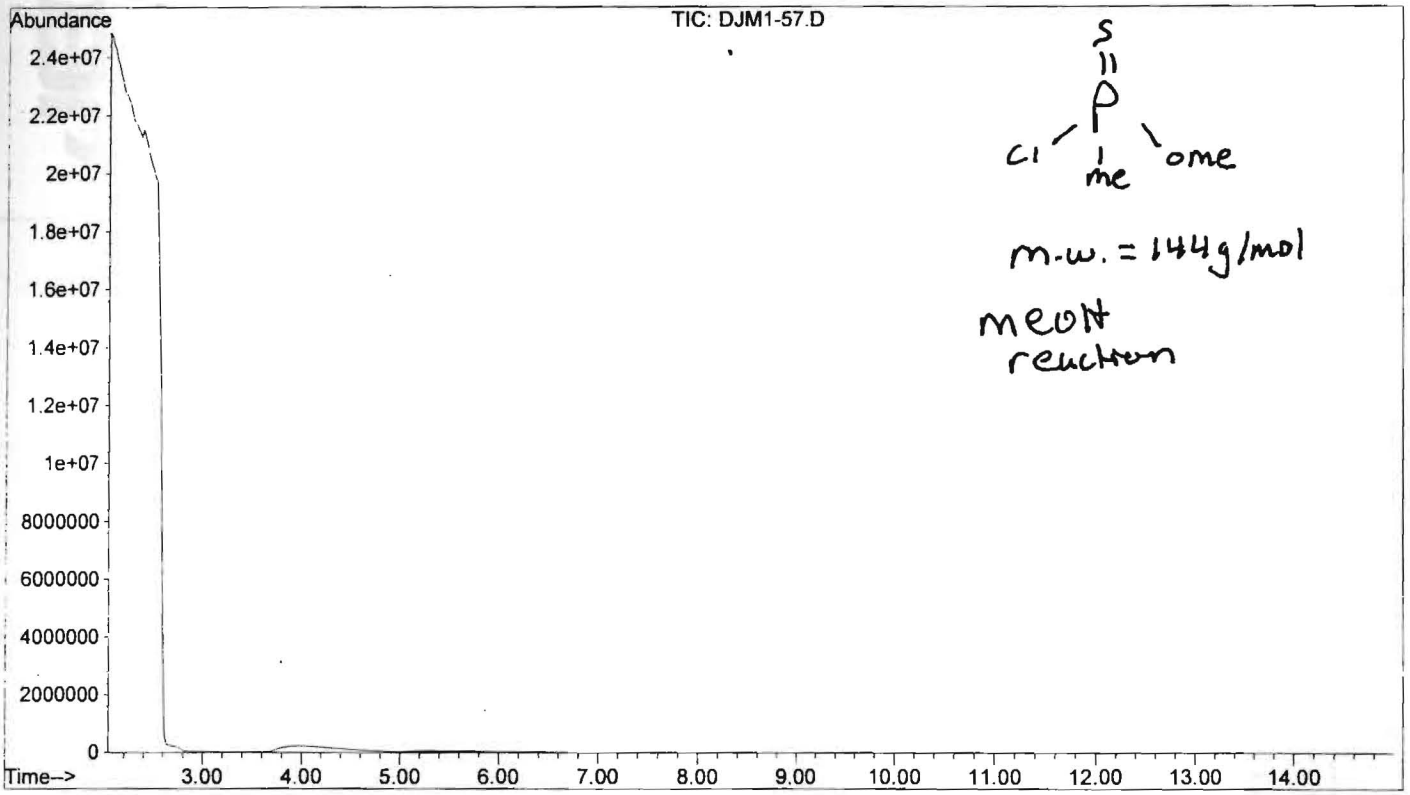
File Name	= DJM1-53_PROTON.2
Author	=
Sample ID	= DJM1-53
Content	=
Creation Date	= 11-MAR-1998 01:49:28
Revision Date	= 10-MAR-1998 22:50:09
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 9
Solvent	= CHLOROFORM-D
Spin_gat	= 16[Hz]
Temp_gat	= 20.6[dC]

File : C:\HPCHEM\1\DATA\DJM1-55.D
Operator : Dustin Mergott
Acquired : 12 Mar 98 4:43 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



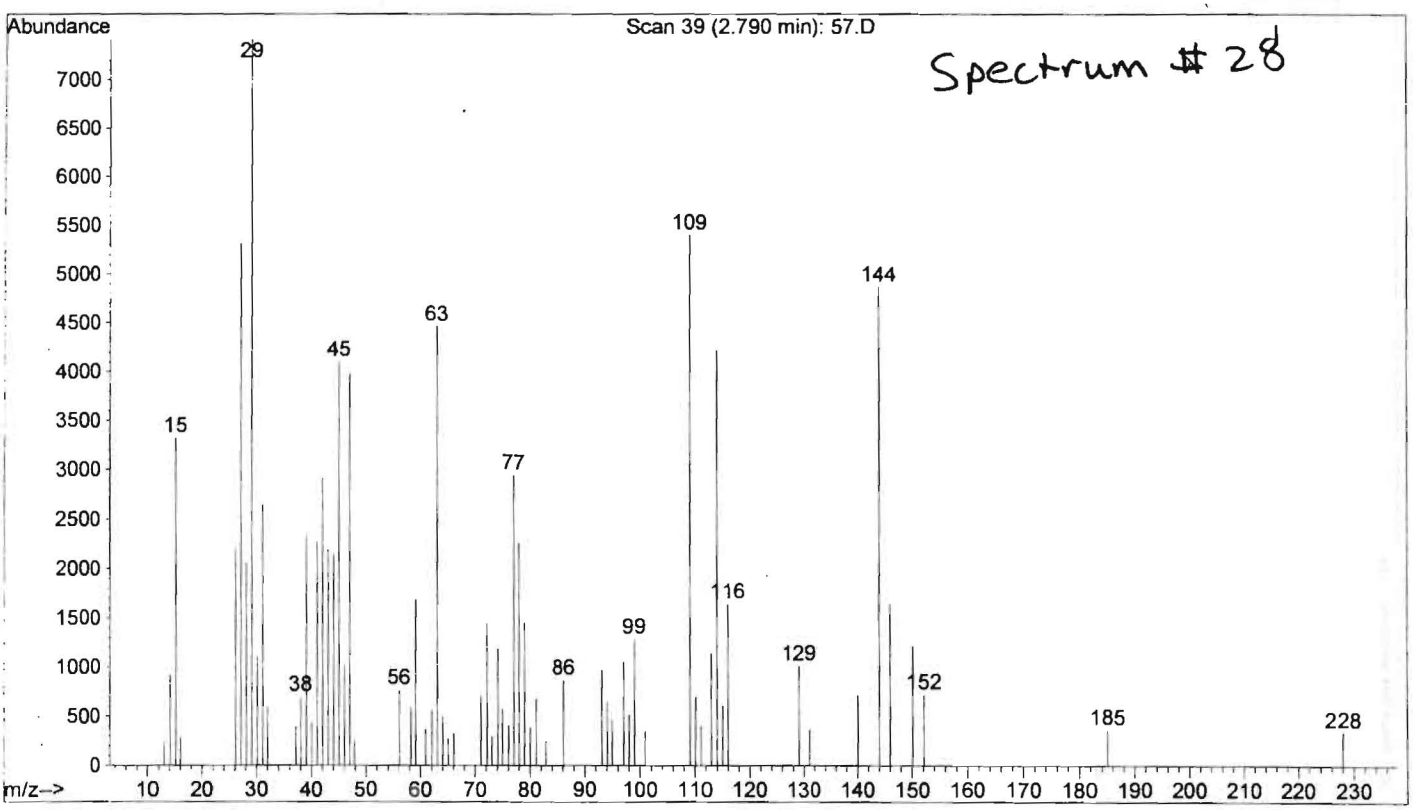


File : C:\HPCHEM\1\DATA\DJM1-57.D
Operator : Dustin Mergott
Acquired : 12 Mar 98 5:04 pm using AcqMethod DUSTIN
Instrument : GC/MS Ins
Sample Name:
Misc Info :
Vial Number: 1



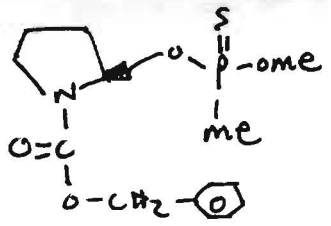
m.w. = 144 g/mol

meOH reaction



Spectrum # 28

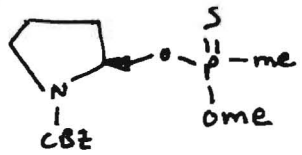
Spectrum# 29



JEOL

File Name	= DJM1-57_PROTON.2
Author	=
Sample ID	= DJM1-57
Content	=
Creation Date	= 13-MAR-1998 06:10:54
Revision Date	= 13-MAR-1998 03:11:34
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 9
Solvent	= CHLOROFORM-D
Spin_get	= 17[Hz]
Temp_get	= 20.6[dC]

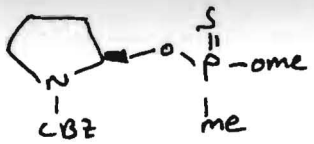
Spectrum #30



JEOL

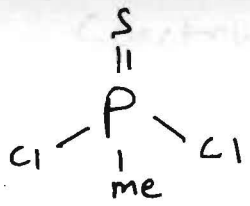
File Name	= DJM1-55_PROTON.2
Author	=
Sample ID	= DJM1-55
Content	=
Creation Date	= 13-MAR-1998 05:57:38
Revision Date	= 13-MAR-1998 02:58:23
Spec Site	= Eclipse 270
Spec Type	= DELTA_NMR
Data Format	= 1D COMPLEX
Dimensions	= X
Dim Title	= 1H
Dim Size	= 16384
Dim Units	= [ppm]
Scans	= 16
X_domain	= 1H
X_offset	= 5.0[ppm]
X_freq	= 270.16743928[MHz]
X_sweep	= 4.05350628[kHz]
Field_strength	= 6.345446[T]
Recvr_gain	= 6
Solvent	= CHLOROFORM-D
Spin_get	= 14[Hz]
Temp_get	= 21[dC]

spectrum #31


S
O
P
ome
N
CBZ
me

(Millions)
33.0
31.0
29.0
27.0
25.0
23.0
21.0
19.0
17.0
15.0
13.0
11.0
9.0
7.0
5.0
3.0
1.0
105.0 104.0 103.0 102.0 101.0 100.0 99.0 98.0 97.0 96.0 95.0 94.0 93.0 92.0 91.0 90.0 89.0 88.0 87.0 86.0 85.0 84.0 83.0 82.0 81.0 80.0 79.0 78.0 77.0 76.0
X : parts per Million : 31P

Spectrum #32


S
P
Cl
Cl
me

400.0
300.0
200.0
100.0
0
(Millions)
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
X : parts per Million : 31P

methyl phosphonic dichloride