# SYNTHESIS OF OXYPHOSPHORUS DERIVATIVES USING GRIGNARD REAGENT AND STUDY OF THEIR BIOLOGICAL ACTIVITY

**A DISSERTATION SUBLIMITED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PHILOSOPHY (M.PHIL) IN CHEMISTRY**


**SUBMITTED**

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**DHAKA-1000, BANGLADESH**


**MARCH, 2016**

**Dedicated**
**To**
**My Parents**

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This thesis work has been done by the candidate himself and does not contain any material extracted from elsewhere or from a work published by anybody else. The work for this thesis has not been presented elsewhere by the author for any degree or diploma.

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**THESIS ACCEPTANCE LETTER**

A Thesis on

**Synthesis of Oxyphosphorus Derivatives Using Grignard Reagent and study of their Biological Activity**

BY

Sabikun Naher

Roll No. 0411033106F, Registration No. 0411033106, Session- April 2011, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters of philosophy (M.Phil) in chemistry on March 23, 2016.

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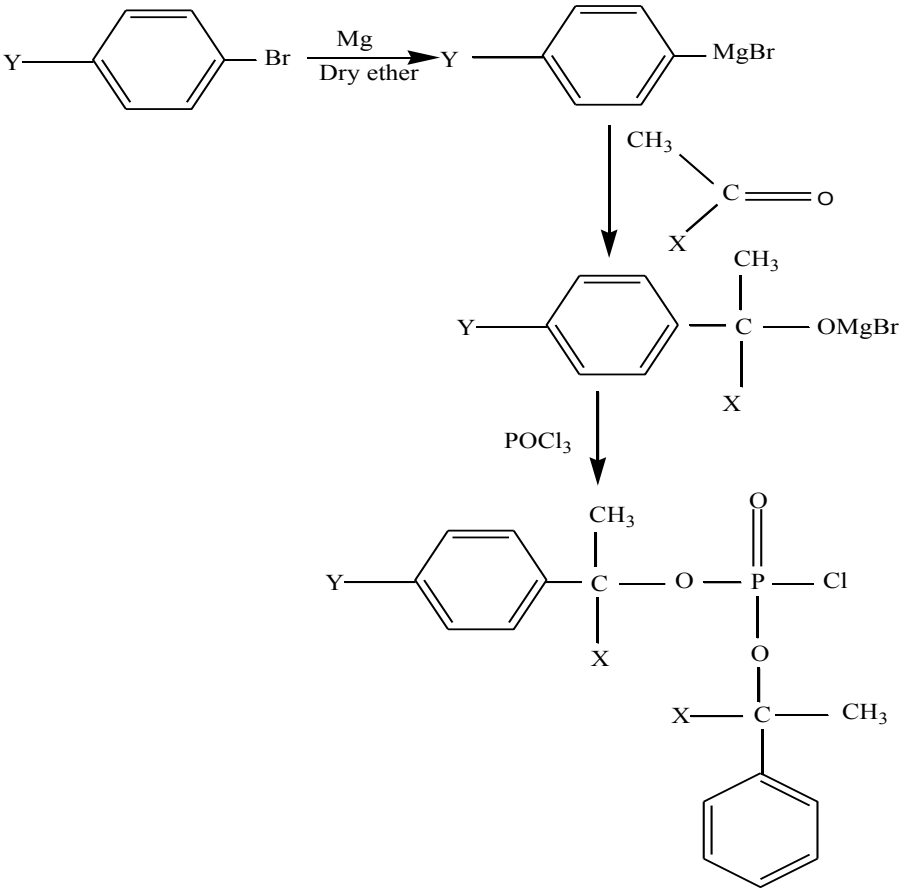
## Abstract

### Thesis Title: Synthesis of oxyphosphorus derivatives using Grignard reagent and study of their biological activity

Organophosphorus compounds have multipurpose uses such as insecticides, harbicides, and fungicides and as antiviral drugs. A convenient and facile method for the Synthesis of bis-(aromatic substituted) chlorophosphine oxide have been developed by the reaction of phosphoryl oxychloride with Grignard reagents in presence of dry ether at room temperature.




Here, X=$CH_3$, H and Y= $CH_3$, H

In vitro antimicrobial activities of bis-(substituted aromatic) chlorophosphine oxide were investigated. The compounds showed mild growth inhibition against antibiotic susceptible standard and clinically isolated strains of Gram positive and Gram negative (aerobic & anaerobic) bacteria as well as human fungal pathogens.

## Table-1 Synthesis of organophosphorus compounds

| SI No | Starting materials | | | | Product | Melting point | % Yield |
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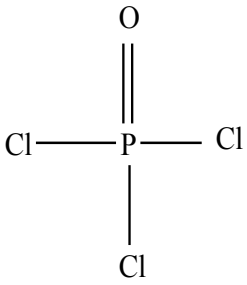
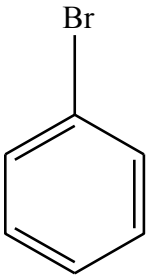
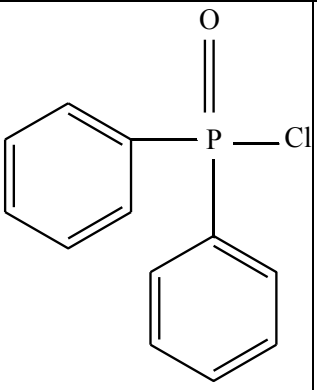
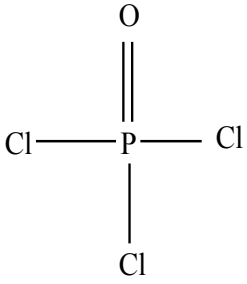
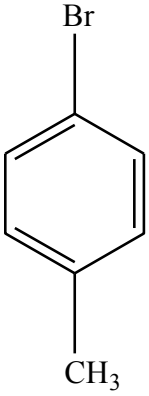
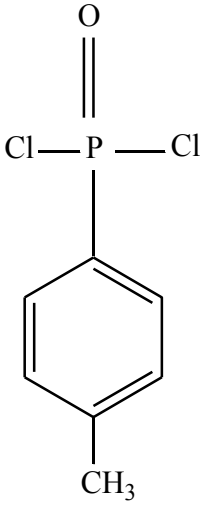
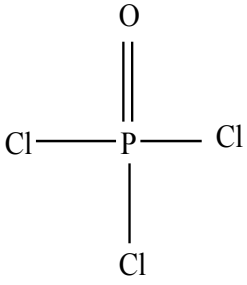
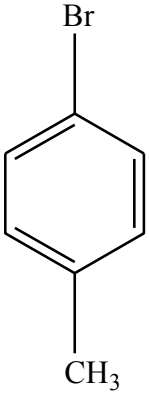
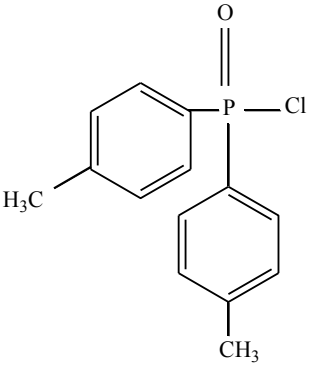
| 1 |  | Mg |  | |  | $120^0$C | 50 |
| 2 |  | Mg |  | |  | $110^0$C | 80 |
| 3 |  | Mg |  | |  | $112^0$C | 80 |

| | | | | | | | |
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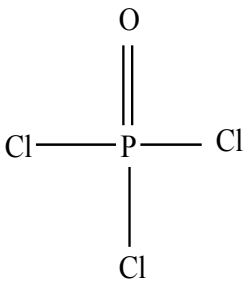
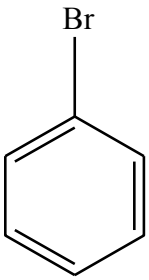
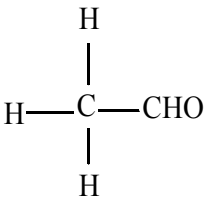
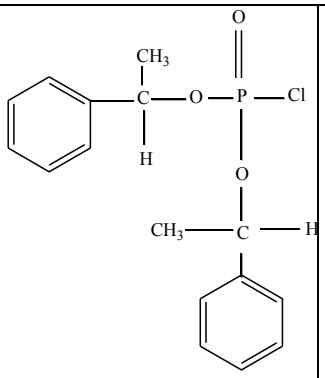
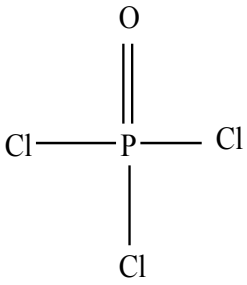
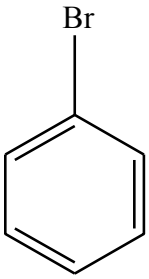
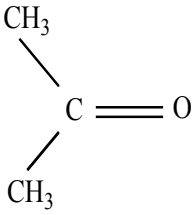
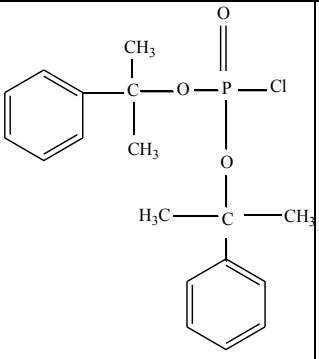
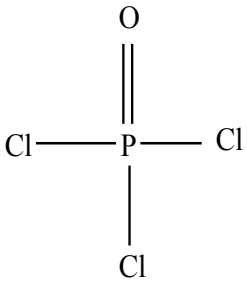
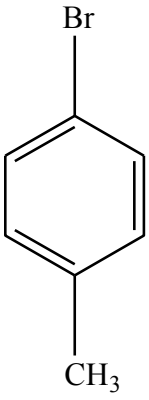
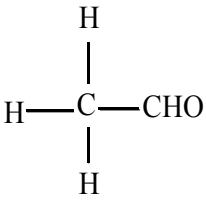
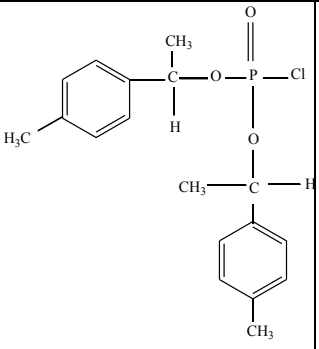
| 4 |  | Mg |  |  |  | $110^0$C | 70 |
| 5 |  | Mg |  |  |  | $99^0$C | 45 |
| 6 |  | Mg |  |  |  | $120^0$C | 75 |

All the synthesized organophosphorus compounds were purified by column chromatography and characterized by using physical and spectral data such as m.p.,% yield, IR and $^1$H NMR.

# PART-1

Chapter-1

# INTRODUCTION

## Introduction

Phosphorus is an element which can be denoted by the symbol P. Its atomic number is 15. It can form bonds with many other elements. It can have different valencies, either 3 or 5. It has an empty d-orbital which readily accept electrons from any good donors. Phosphorus shares group V in the periodic table with nitrogen, and thus phosphorus compounds and nitrogen compounds have many similar properties. As an element, phosphorus exists in two major different forms—white phosphorus and red phosphorus—but due to its high reactivity, phosphorus is never found as a free element on Earth. Instead, phosphorus containing minerals is almost always present in its maximally oxidized state as inorganic phosphate rocks.

Organophosphorus chemistry deals with the properties and reactivities of organophosphorus compounds. [1] Compounds containing P-O-C linkage are usually known as organo-oxy phosphorus compounds. The term organo-oxy phosphorus compounds are reserved for compounds containing phosphorus, oxygen and carbon. The most important organo-oxy phosphorus compounds are organo-phosphorus polymers which are based on P-O-C linkages. Phosphorus chemistry is dominated by organo-oxy phosphorus compounds all of which contain phosphorus-oxygen linkages. Most of these are usually known as phosphates; these compounds are highly effective insecticides, though some are also lethal to humans at minuscule doses (nerve gas) and include some of the most toxic substances ever created by man. [2]

The definition of organophosphorus compounds is variable, which can lead to confusion. In industrial and environmental chemistry, an organophosphorus compound needs to contain only an organic substituent, but need not to have a direct phosphorus-carbon (P-C) bond. Thus a large proportion of pesticides (e.g., malathion), are often included in this class of compounds. Phosphorus can adopt a variety of oxidation states, as phosphorus (V) and phosphorus (III) which are the predominant classes of compounds.

Almost all naturally occurring phosphorus compounds contain phosphorus-oxygen linkages and those of biochemical importance are organic phosphate esters which contain phosphorus-oxygen-carbon linkages. Organophosphorus compounds which are based on phosphorus-carbon linkages constitute the second most important group and those containing phosphorus-nitrogen linkages

are probably the third. Phosphoric acid is the most important industrial commodity based on phosphorus. The organic phosphate ester known as deoxyribonucleic acid (DNA) is present in all life forms and lies all the heart of biochemistry and genetics. It is the most crucial phosphorus compound for the survival and development of the human race concerned. It is now accepted that phosphorus compounds play a vital role in living process and is essential not only for hereditary process but also for growth, development and maintenance of all plants and animals. They are present in soil, bones, teeth, blood and in all cellular organisms. Energy transfer process such as photosynthesis, metabolism, nerve function and muscle action all involve phosphorus compounds.

Although naturally-occurring phosphorus compounds are almost invariably non-toxic but some known synthetic product show a very wide range of toxicity. Most inorganic phosphate based on pentavalent phosphorus are among the safest of all substances known to man.

Although inorganic phosphorus compounds remain by far the most important commercially, the chemistry of organophosphorus compound has evolved rapidly and now represents a sizeable and explosively expanding part of the whole. There are four major classes of phosphorus compounds.

1. Oxyphosphorus compounds which contain covalent P-O linkages.
2. Organophosphorus (carbophosphorus) compounds which contain covalent P-C linkages.
3. Azophosphorus compounds which contain covalent P-N linkages.
4. Metallophosphorus compounds which contain covalent P-Ml linkages.

It will sometimes be useful to classify phosphorus compound in accordance with the presence of five characteristics bonds e.g.

1. C-P-O Organo-oxyphosphorus compound
2. M-P-O Metallo-oxyphosphorus compound
3. N-P-O Azo-oxyphosphorus compound
4. N-P-C Azo-organophosphorus compound
5. N-P-M Metallo-azaphosphorus compound
6. M-P-C Metallo-organophosphorus compound

These compounds vary greatly in their abundance and importance on earth (Table 1.1) Phosphorus chemistry is dominated by compounds with P–O linkages. The three most important types of compounds oxyphosphorus are:

a) Phosphates, which are inorganic salts based on the tetrahedral $PO_4^{3-}$ anion. They exist in many varieties and are commercially the most important P compounds.
b) Phosphate esters, which are organic phosphorus compounds based on P–O–C linkages Phosphorus biochemistry is almost exclusively concerned with such phosphate esters which are vital to all life processes.
c) Phosphoryl compounds, which contain the donor-type phosphoryl linkage, P=O.

The commercial production of orthophosphates and polyphosphates greatly exceeds that of all other compounds of phosphorus. Phosphates esters although produced in smaller quantities have very diverse but important applications. Substituted phosphates particularly phosphonates and thioated derivatives also have a considerable number of current uses organophosphorus compounds, have widespread use throughout the world, mainly in agriculture as insecticides, herbicides, and plant growth regulators. They have also been used as nerve agents in chemical warfare and as therapeutic agents, such as ecothiopate used in the treatment of glaucoma. In academic research organophosphorus compounds find important application in organic synthesis (Wittig, Mitsunobu, Staudinger, organocatalysis etc.). The use of organophosphorus compounds as achiral or chiral ligands for transition metalcatalyzed transformations is also rapidly growing in both laboratory synthesis and industrial production. Industrially produced natural products such as casein and lecithin have a growing number of applications in food products Furthermore, organophosphorus compound, can be used as flame retardants for fabrics and plastic plasticising and stabilising agents in the plastics industry, selective extractants for metal salts from ores, additives for petroleum products, and corrosion inhibitors.

**TABLE 1.1**

**Convenient Major Classification of Phosphorus Compounds in 2010**

| Bond | Oxyphosphorus P---O | | Carbophosphorus P---C | | | Azaphosphorus P---N | | | Metallophosphorus P---M | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P—O | P=O | P—C | P=C | P≡C | P—N | P=N | P≡N | P—M | P=M | P≡M |
| Name | Phosphates | Phosphoryl compounds | Carbophosphines | Carbophosphenes | Carbophosphynes | Azaphosphines | Azaphosphenes | Azaphosphynes | Metallophosphines | Metallophosphenes | Metallophosphynes |
| Natural Occurrence | Almost exclusive | | Rare | Nil | Nil | Rare | Nil | Nil | Rare | Nil | Nil |
| Industrial Importance | Major | | Some | Minor | Possibly some | Minor | Minor | Nil | Some | Possibly some | Nil |
| Biological importance | Major | | Minor | Unknown | | Minor | Unknown | | Some | Unknown | |
| Number of known compounds | Many Thousands | | Thousands | Several hundreds | Handful | Many hundreds | Hundreds | Handful | Thousands | | Handful |

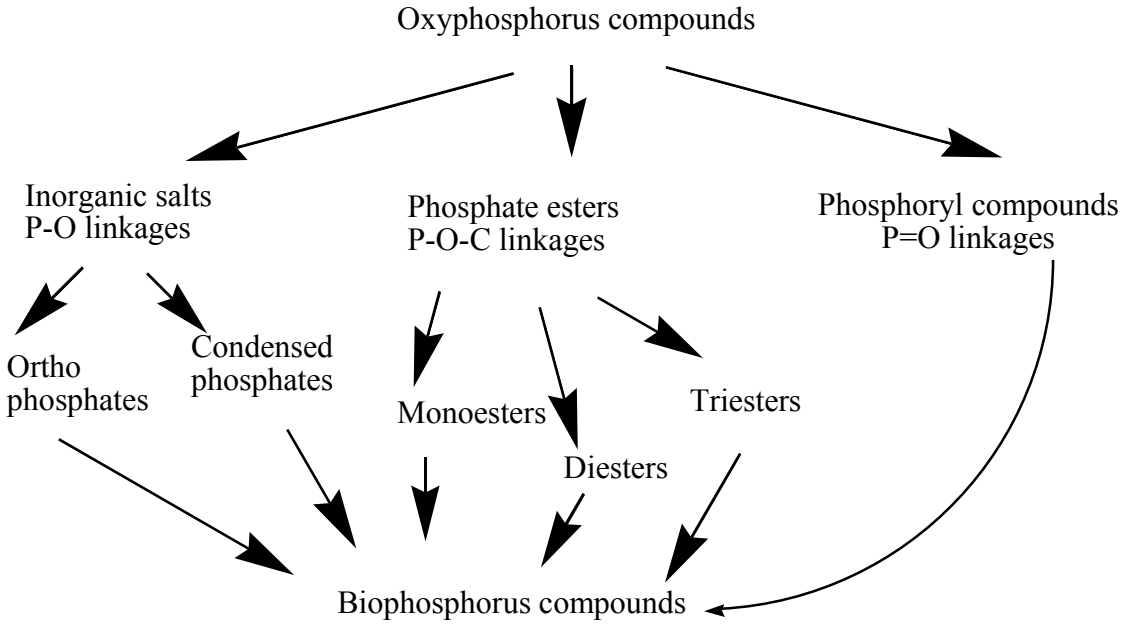



**Fig. 1.1:** Major divisions of oxyphosphorus compounds

Some present fields of use of phosphorus compounds are summarized in Table [3][4][5]

**Table: 1.2 some application areas of Phosphorus compounds in 2010**

| | | |
|---|---|---|
| Animal foodstuffs | Autoradiography | Biochemical research |
| Bacteria culture | Building materials | Catalysts |
| Ceramics | Chemical synthesis | Chromatography |
| Cosmetics | Criminology | Dental materials |
| Desiccants | Detergents | Electroplating |
| Electrical/electronic materials | Fertilizer‘s | Flame retardants |
| Food additives | Genetic engineering | Glass technology |
| Ion exchange | Luminescent phosphors | Matches |
| Medicines | Metal alloys | Metal extraction |
| Metal surfaces | Nerve gases | Oil additives |
| Oil well drilling | Paper manufacture | Pesticides |
| Pigments | Plastics | Refractories |
| Smoke generation | Solvent extraction | Surfactants |
| Textile technology | Toothpaste Water | technology |

## 1. Pesticides

Organophosphate pesticides as well as sarin and VX (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate) nerve agent irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects, humans, and many other animals. Organophosphate pesticides affect this enzyme in various ways, and thus in their potential for poisoning. For instance, parathion, one of the first OPs commercialized is many times more potent than malathion, an insecticide used in combating the Mediterranean fruit fly (Med-fly) and West Nile Virus -transmitting mosquitoes.

Commonly used organophosphates have included parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, fenitrothion,O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate, [6] tetrachlorvinphos, azamethiphos, and azinphos methyl. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. [7] In the US, it is the most commonly used organophosphate insecticide. [8] Forty organophosphate pesticides are registered in the U.S., with at least 73 million pounds used in agricultural and residential settings. [9]

They are of concern to both scientists and regulators because they work by irreversibly blocking an enzyme that's critical to nerve function in both insects and humans. Even at relatively low levels, organophosphates may be most hazardous to the brain development of fetuses and young children. The EPA banned most residential uses of organophosphates in 2001, but they are still sprayed agriculturally on fruits and vegetables. They're also used to control pests like mosquitos in public spaces such as parks. They can be absorbed through the lungs or skin or by eating them on food.[10]

Most commercially important insecticides belong to one of three groups:

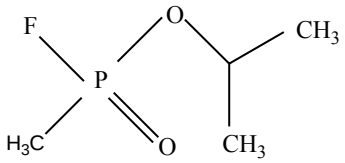
1.Carbamates

2.Organic chlorine compounds

3. Organophosphorus compounds (now the largest group)

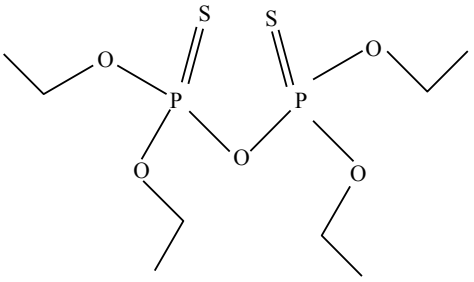
Several thousand organophosphorus compounds are known to act as insecticides, and about 300 of these are, or have been, manufactured commercially. Despite their diverse structures organophosphorus compounds owe their activity to their capacity to phosphorylate and inhibit the action of cholinesterase, although in some instances the inhibition of other vital enzymes is believed to be involved organophosphorus compounds show a wide range of properties, some being highly specific in action while others are effective against a wide range of pests. Some of the compounds are also extremely toxic to humans and are comparable to the nerve gases to which they are chemically related. Others are relatively harmless and almost non-toxic to humans. Thio derivatives are often considerably less toxic to mammals than their oxy-analogues, although their insecticidal activity is not diminished. Some compounds are non-toxic in vitro but are converted to toxic varieties in the course of plan to rinse metabolism .In general, the toxicity of an insecticide depends on both its molecular structure and the species of organism to which it is applied. Selective action is related to the way in which a given insecticide is metabolised by different species. As a class of compounds, organophosphorus insecticides are generally rapid acting, highly effective in small concentrations, and have a low persistence, being easily broken down afterwards to non-toxic materials. Their main drawbacks are relatively high cost and often immediate toxicity to animals. [11-12] Early high-toxicity pesticides such as TEPP and parathion have now fallen out of use. Organophosphorus pesticides (and in some cases other phosphate esters such as tricresyl phosphate) are now believed to produce several responses in humans. Acute symptoms can appear within hours of exposure, intermediate syndromes within days, and a delayed response which may take months or even years to develop. The latter is known as _OP-induced neuropathy‘ (OPIDN) and is difficult to treat and may be irreversible.

Some typical organophosphorus insecticides are listed below:



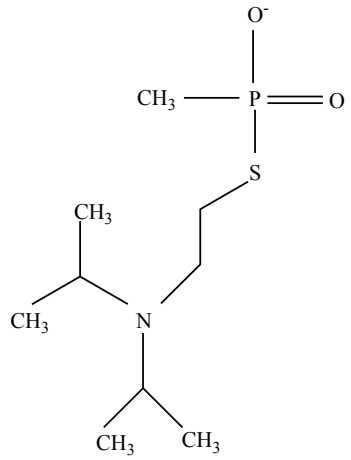
**1**

**sarin**



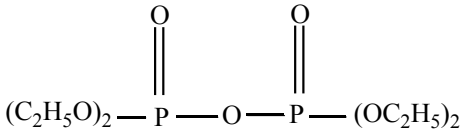
**3**

**Sulphotepp**



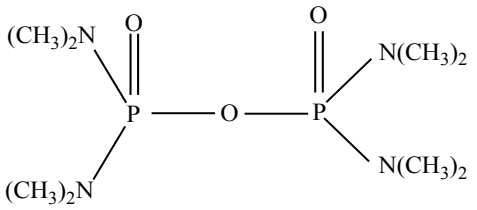
**2**

**O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate**



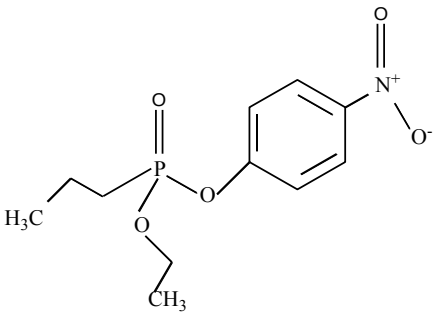
**4**

**TEPP**



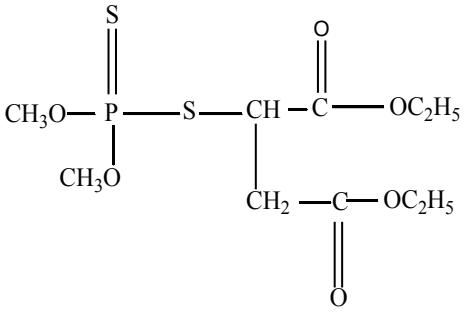
**5**

**Schradan**



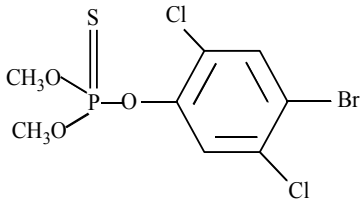
**6**

**Parathion**



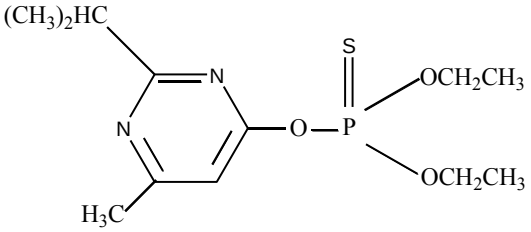
**7**

**Malathion**



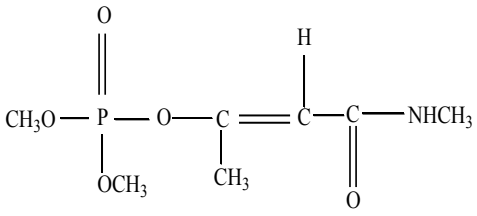
**8**

**Bromophos**



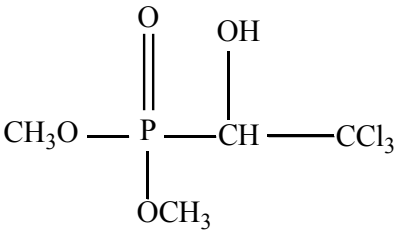
**9**

**Diazinon**



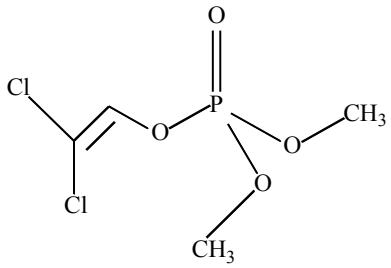
**10**

**Monocrotopho**



**11**

**Dipterex**



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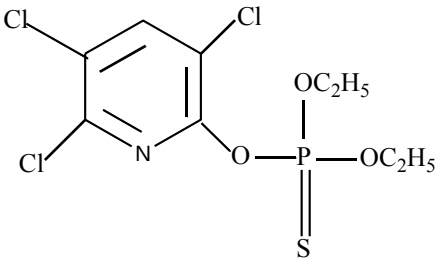
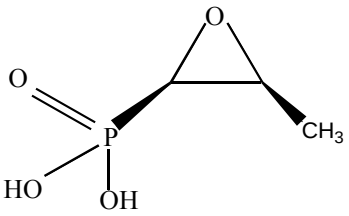
**Dichlorvos**



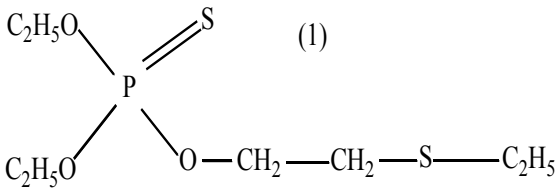
**13**

**Chlorpyrifos**

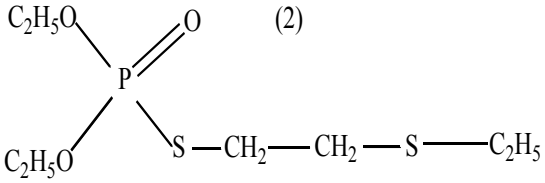


**14**

**Fosfomycin**

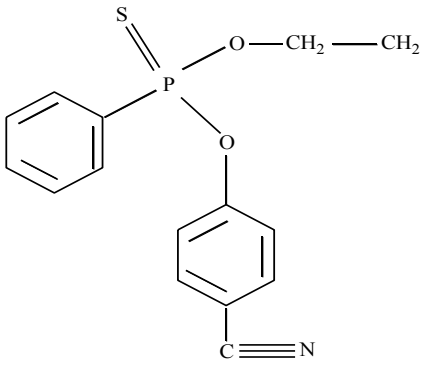


**15(a)**



**15(b)**

**Systox (Dimeton)**



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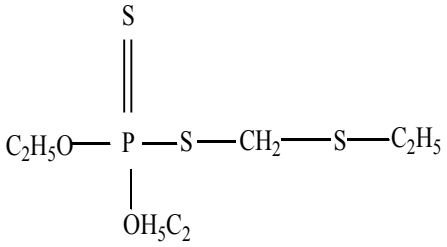
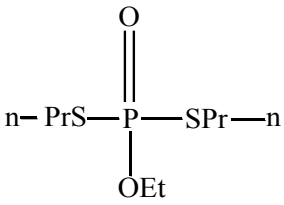
**Cyanofenfos**



**17**

**Phorate**



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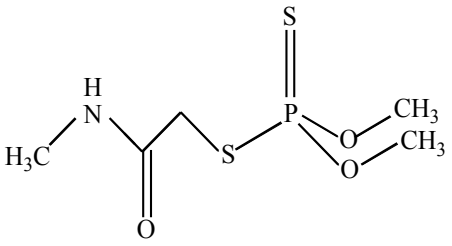
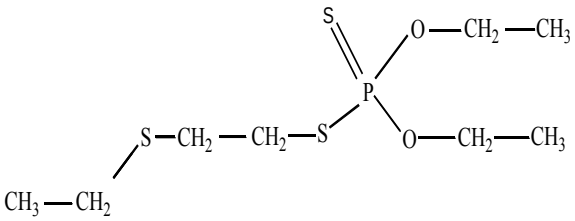
**Ethoprop**



**19**

**Dimethoate**



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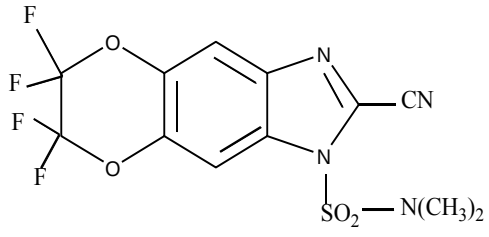
**Disulphoton**



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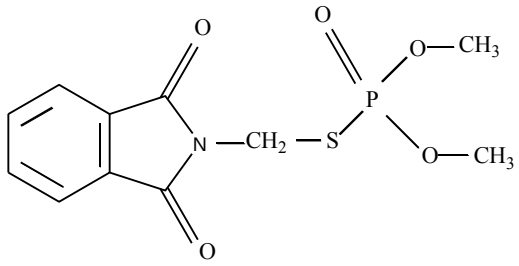
**Fonophos**



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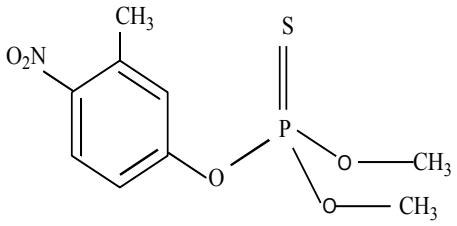
**Phosmet**



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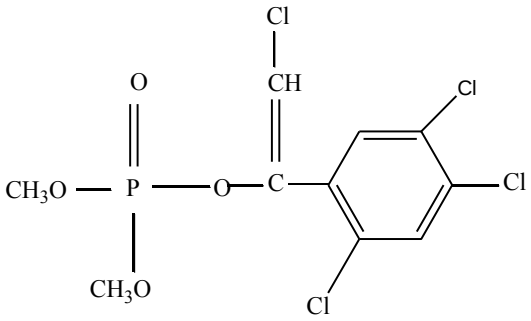
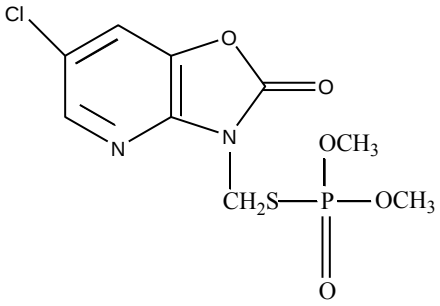
**Fenitrothion**



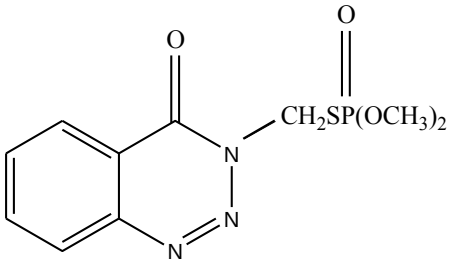
**24**

**Tetrachlorvinphos**



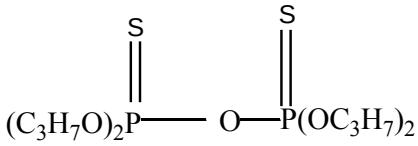
**25**

**Azamethiphos**



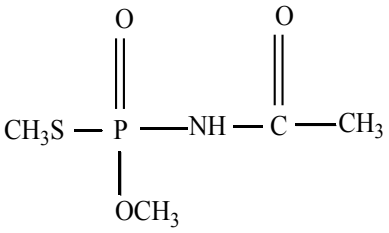
**26**

**Azinphos methyl**



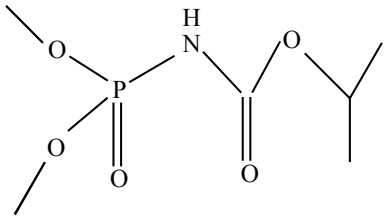
**27**

**Aspon**



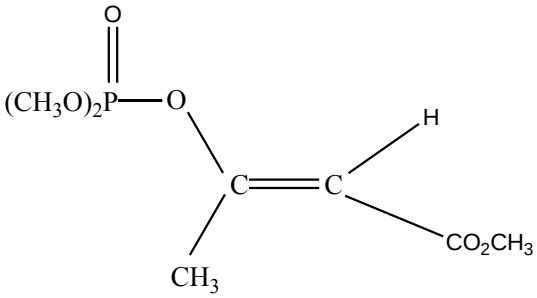
**28**

**Acephate**



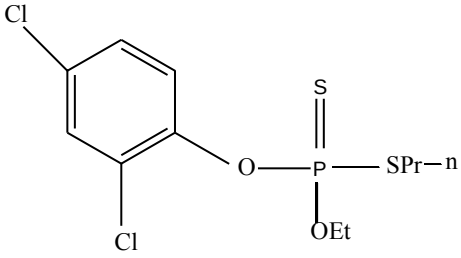
**29**

**Avenin**



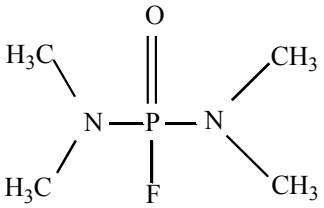
**30**

**Mevinphos**



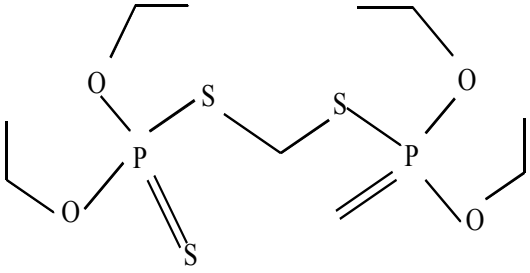
**31**

**Prothiophos**



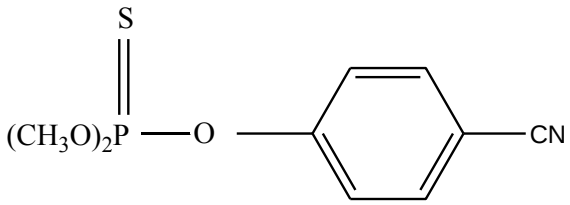
**32**

**Dimefox**



**33**

**Ethion**

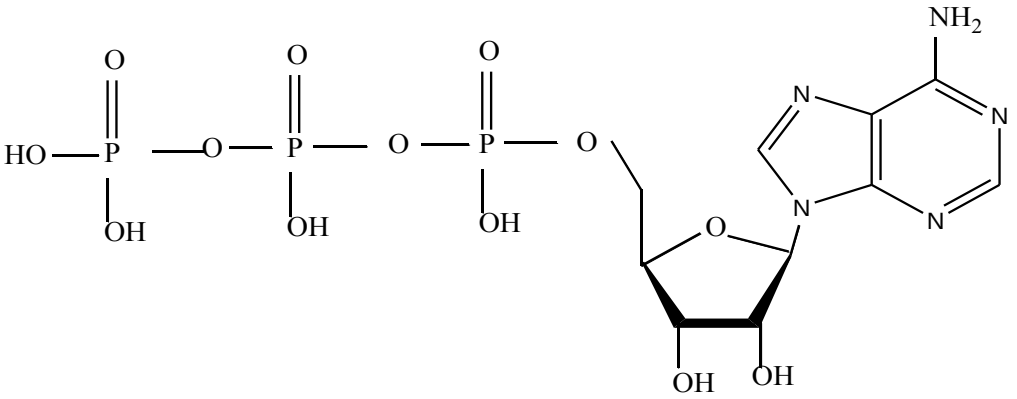



**34**

**Cyanophos**

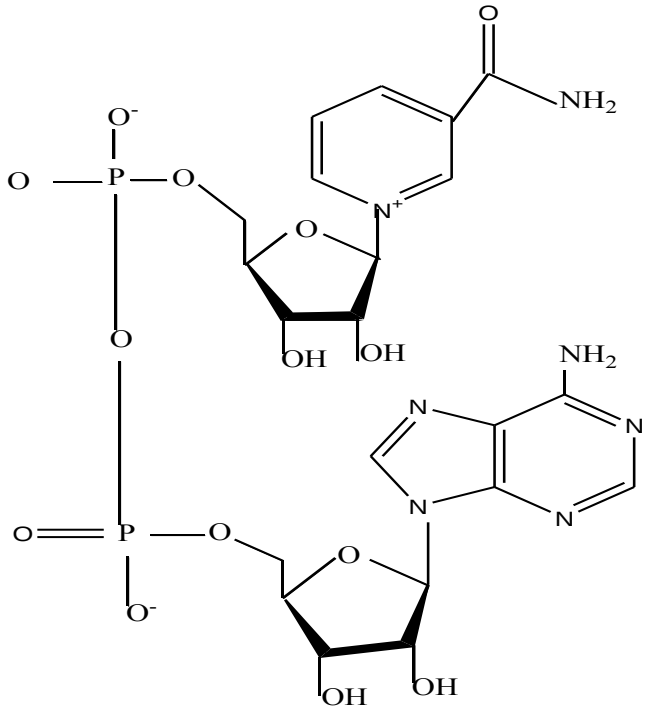
## 2. Phosphorus in Biological Compound

Phosphorus is present in plants and animals. There is over 454 grams of phosphorus in the human body. It is a component of fundamental living compounds. It is found in complex organic compounds in the blood, muscles, and nerves, and in calcium phosphate, the principal material in bones and teeth. Phosphorus compounds are essential in the diet. Organic phosphates, ferric phosphate, and tricalcium phosphate are added to foods. Especially, phosphoric acid is essential in many biological derivatives such as nucleotides, nucleic acids, phospholipids and sugar phosphates. Nucleotides are monomers consisting of a phosphate group, a five carbon sugar (either ribose or deoxyribose) and a one or two ring nitrogen containing base. Nucleotides are the monomers of nucleic acids, with three or more bonding together in order to form a nucleic acid. The genetic material (DNA) is a polymer of four different nucleotides. The genetic information is coded in the sequence of nucleotides in a DNA molecule. Nucleotides and related compounds are also important ~~–e~~nergy carrying" compounds. Among the ones commonly encountered are ATP (35), and NADH (36). [14a]



**35**

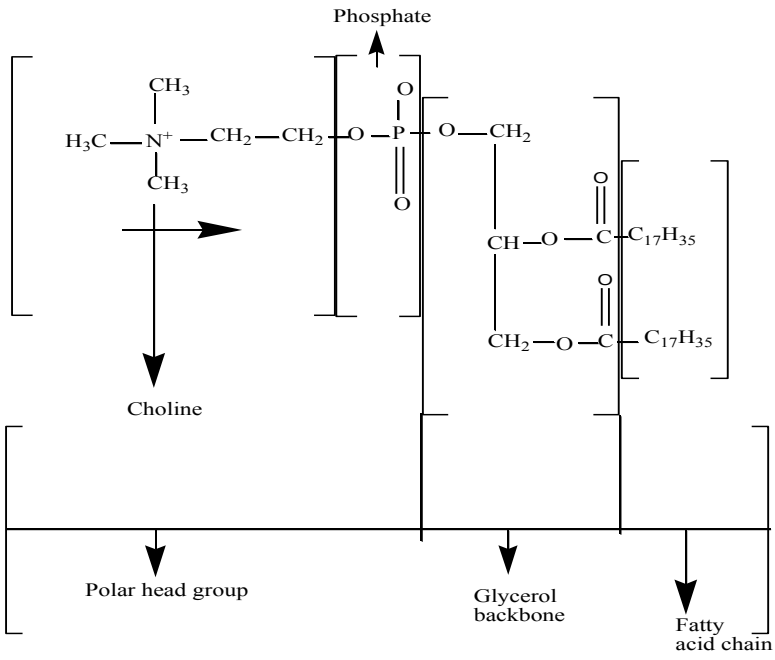
**Adenosine triphosphate (ATP)**



**36**

**Nicotinamide adenine dinucleotide (NADH)**

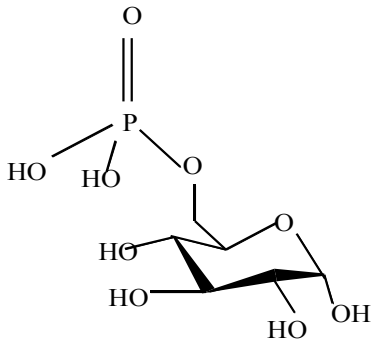
Certain phosphoric acid derivatives play a major role in driving some processes by ~~–e~~nergy release" that accompanies the cleavage of a phosphate group and transfer to a nucleophilic substrate. The best known of the ~~–e~~nergy-rich" phosphates is adenosine triphosphates ATP (35) which can transfer the terminal phosphate group to a substrate with the release of significant energy. [18c] Actually the phosphoryl group transfer mechanism, in ~~–e~~nergy-rich" phosphate substrates, is explained by intervention of pentacoordinated phosphorus in the transition state species. In particular the formation of cyclic pentacoordinated phosphorus species on the reactive phosphate group facilitate the attainment of the required transition state or intermediate allowing to obtain a fast and selective reaction. [13] A phospholipid molecule consists of a hydrophilic polar head group and a hydrophobic tail (37). The polar head group contains one or more phosphate groups. The hydrophobic tail is made up of two fatty acyl chains. When many phospholipid molecules are placed in water, their hydrophilic heads tend to face water and the hydrophobic tails are forced to stick together, forming a bilayer. Phospholipids are a major component of all biological membranes, along with glycolipids and cholesterol. [14b]




**37**

**Phospholipid**

Sugar phosphates are present in the human body as intermediates in the many important processes like glucose metabolism. One example is the glucose 6- phosphate (38). It is glucose sugar phosphorylated on carbon 6. This compound is very common in cells as the vast majority of glucose entering a cell will become phosphorylated in this way. Because of its prominent position in cellular chemistry, glucose 6- phosphate has many possible fates within the cell. It lies at the start of two major metabolic pathways: the Glycolysis and Pentose phosphate pathway. In addition to these metabolic pathways, glucose 6-phosphate may also be converted to glycogen or starch for storage. This storage, in the form of glycogen, is in the liver and muscles for most multicellular animals, and in intracellular starch or glycogen granules for most other organisms. [14c]



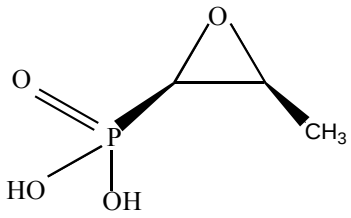

**38**

**Glucose 6-phosphate.**

## 3. Organophosphorus Compounds in Medicine

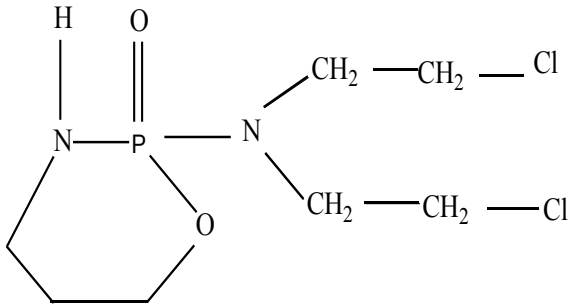
A source of C-P compounds of natural origin was first recognized in 1969. [17] From the products in a fermentation broth of the bacterium Streptomyces fradiae a new phosphoric acid that had the properties of an antibacterial antibiotic was isolated. The compound was named Fosfomycin (39) and its discovery was an extremely important event in phosphorus chemistry. Phosphorus compounds had been largely ignored by medicinal chemists seeking new agents against infectious disease. Fosfomycin (39) is active against both Gram-positive Gram-negative bacteria, and its effectiveness is comparable to that of the well-known antibiotics Tetracycline. [18c] High-level anticancer activity has been found in a large number of phosphorus compounds

of quite different structural types, and there is much current research in this field. Probably the first organophosphorus compound to receive acclaim as a valuable chemotherapeutic agent is the anticancer drug cyclophosphamide (40). [16] Its activity was discovered in 1958, [19] and remains in clinical use to this day.



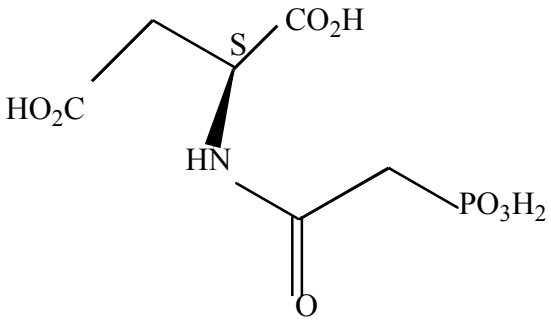

**39**

**Fosfomycin**



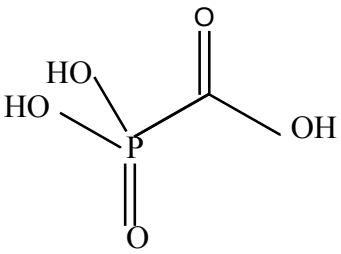

**40**

**Cyclophosphamide**



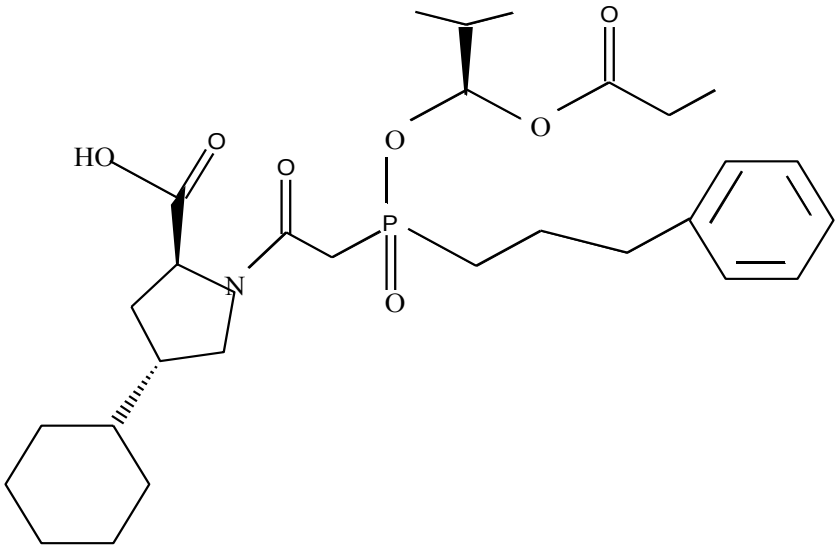

**41**

**N-phosphonoacetyl-Laspartic acid**




**42**

**Foscarnet**



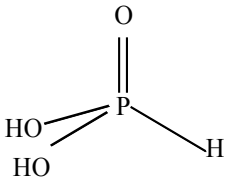

**43**

**Fosinopril**

In the design of anticancer drugs, rationales were done. An obvious one is that an exact phosphonate replica of a known biologically active phosphate could inhibit the process in which the phosphate is involved. The $CH_2$ group attached to P has a very similar size and bond angle with an O atom of a phosphate. [15] The high stability of P-C bond would block any important natural processes involving hydrolysis of a phosphate ester group. A second rationale is that a phosphonic acid designed to be similar to a naturally occurring carboxylic acid might inhibit the biochemical work of acid. [20] Using those concepts a large amount of phosphonic acids has been synthesized and thus had useful chemotherapeutic properties. Some examples of the above rationalization are, the PALA (N-phosphonoacetyl-Laspartic acid (41) which is a potent anticancer drug and the Fosinopril (43) which has an antihypertensive activity.[18c] Phosphorus compounds can also have antiviral activity, the first active compound discovered had the very simple structure of trisodium phosphonoformiate. Its activity was discovered [21] in 1978, and is still in clinical use under the name Foscarnet (42). It inhibits viral DNA polymerase, and it is a useful agent in the treatment of Herpes and is also active against HIV.

## 4. Agricultural

Over the years, many organophoshorus compounds have been made and used in very large quantities in agriculture, not only as insecticides but also later as herbicides and in other applications. Phosphorus compounds have distinct advantages in the pesticides market; they are relatively easy to make, and they biodegrade readily by hydrolysis, so that the problems of residual activity. The active compounds are normally esters, amides, or thiol derivatives of phosphoric or phosphoric acid (44).

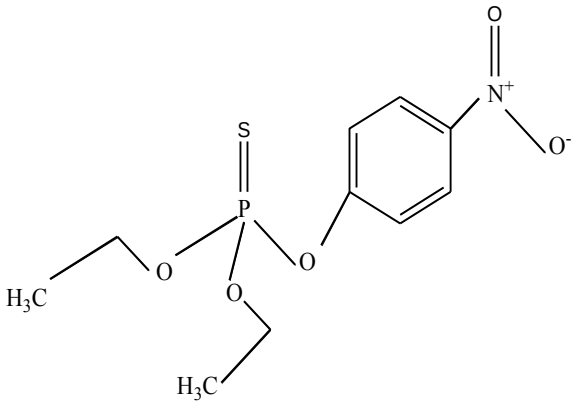



**44**

**Phosphoric acid**

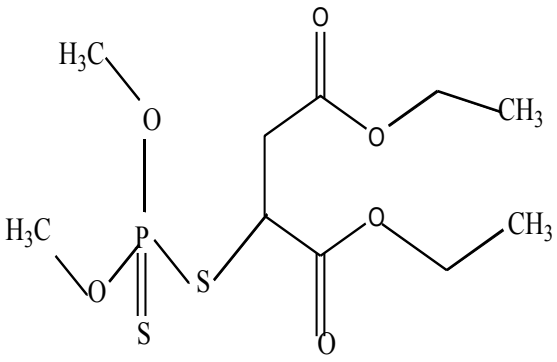
Structure of derivatievs of phosphoric or phosphoric acid where $R_1$ and $R_2$ are usually simple alkyl or aryl groups, both of which may be bonded directly to phosphorus (in phosphinates), or linked via –O-, or –S- (in phosphates). Parathion (45) was one of the first commercially produced insecticides; its toxicity (LD50) is 55 mg/Kg, which is rather low but still requires careful handling and application in the field. It was very popular in 1960s, but after this period the interest in Parathion has greatly declined with the introduction of safer agents. Definitely, many compounds are now produced that are relatively harmless to humans yet with excellent toxicity to insects for example the well-known garden insecticide Malathion (46) and Phosmet (47) with LD50 up 4000 mg/Kg. On the other hand, the phosphorus compounds were late entries in the fields of organic herbicides, and to this date only a few compounds have attained major commercial importance. Glyphosphate (48) was discovered first and still using. It is known to act by the inhibition of the plant enzyme 5- enolpyruvoyl-shikimate-3-phosphate syntheses, which is involved in the biosynthesis of aromatic aminoacids and other aromatic compounds in plants. Many other phosphorus compounds show herbicidal activity and much current research effort is going on in

this area. In addition to the phosphorus-containing amino acid derivatives, other structural types are of interest, and are found in Betasan (49).



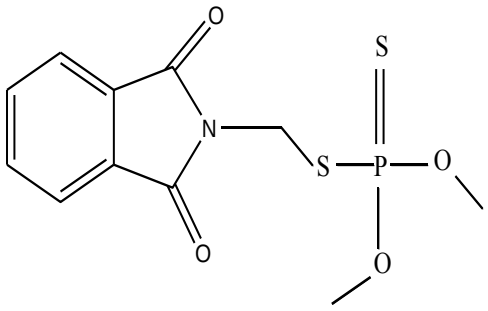
**45**

**Parathion**



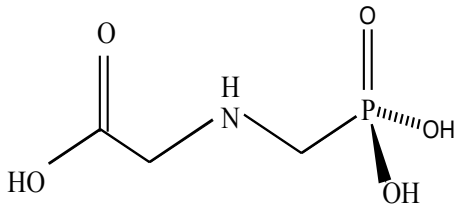
**46**

**Malathion**



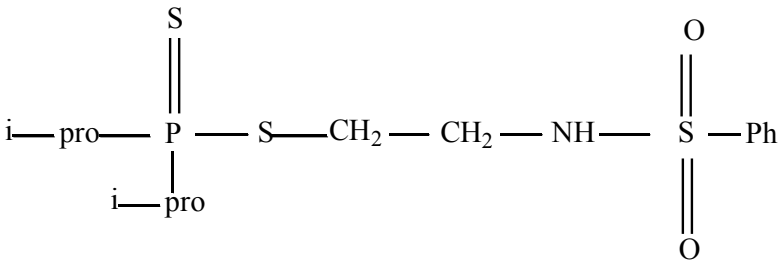
**47**

**Phosmet**



**48**

**Glyphosphate**

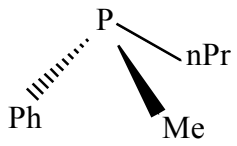


**49**

**Betasan**

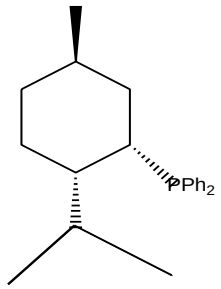
## 5. Catalysis

Between various types of enantiomerically pure ligands used for catalytic asymmetric reactions, chiral tertiary phosphines have established their position as the most effective ligands for most homogeneous transition-metal catalyses. Homogeneous asymmetric hydrogenation started with modest results (ee 15%) in 1968 using chiral monophosphine (MPPP) (50) as ligand.[22] Neomenthyldiphenylphosphine (NMDPP) (51) and menthyldiphenylphosphine (MDPP) (52) were prepared by Morrison [23] giving up to 61% ee in some cases. Knowles also published some interesting results (ee 90%) with chiral phosphines (PAMP) (53) and (CAMP) (54).[24] At the same time alkyldimenthylphosphines (ADMP) (55) were used by Wilke, Bogdanovic as ligands of nickel complexes in the catalysis of alkene codimerization and alkene-1,3-dienes codimerization. [25]` Bogdanovic, demonstrated that a chelating chiral C2- symmetric diphosphine (DIOP) (56) without asymmetric phosphorus atoms was an excellent enantioselective catalyst (ee 88%). [26] A multitude of chelating diphosphines are presently known (of C1 or C2-symmetry), some of them are patented because of industrial applications. [27] One of the most effective chiral biphosphine ligands is BINAP (57), [28] which has exhibited its high enantioselectivity in several asymmetric reactions including rhodium- or ruthenium-catalyzed hydrogenation. Another important class of chelating biphosphine ligand is ferrocenylbiphosphines BPPF-X (58), [29] which had been demonstrated to be effective for palladium-catalyzed allylic substitution reactions, gold- or silver-catalyzed aldol reactions, and so on.



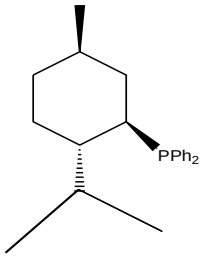

**50**

**MPPP**



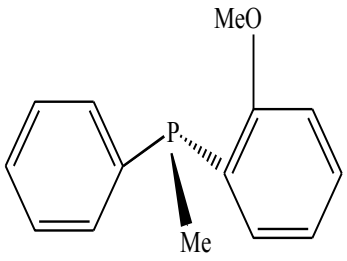

**51**

**NMDPP**



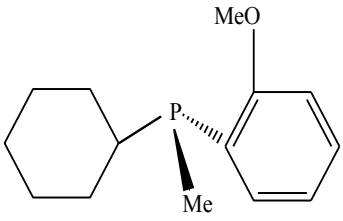

**52**

**MDPP**



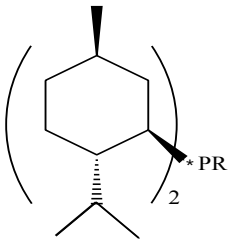

**53**

**PAMP**



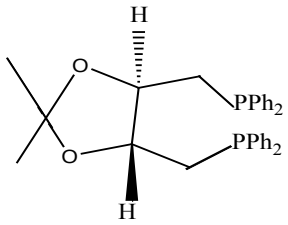

**54**

**CAMP**



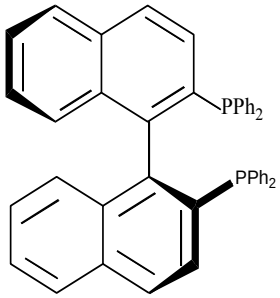

**55**

**ADMP**



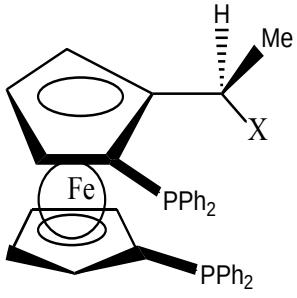

**56**

**(S,S)-DIOP**




**57**

**(S)-BINAP**




**58**

**(S)-(R)-BPPF-X**

## 6. Animal foodstuffs of Organophosphorus compounds

Managing phosphorus waste outputs is a key factor for environmental sustainability of animal production operations. The development of effective nutritional strategies to manage phosphorus waste outputs requires a detailed understanding of phosphorus nutrition (supply, digestion, accretion, excretion) of animals. Phosphorus is a component of several different types of chemical compounds found in ingredients and feeds. These compounds include hydroxyapatite (bone P), *myo*-inositol hexaphosphate (phytate P), phosphorus compounds covalently linked to protein, lipid, and sugar (organic P), and various inorganic phosphate supplements. These compounds are present in various amounts in animal feeds depending on feed formulation and the compositional variability of the ingredients used. Differences in the chemical characteristics and solubility of these compounds are likely to result in different digestion dynamics of phosphorus within the animal gastrointestinal tract, and this, in turn, can significantly affect phosphorus digestibility. It is consequently necessary to quantify the different phosphorus forms in ingredients to better understand and/or predict the digestibility of phosphorus in feeds. Animal protein ingredients (fish meal, poultry byproducts meal, and meat and bone meal) generally have high phosphorus contents and often contribute a significant proportion of the total phosphorus of feeds for fish and, occasionally, other domestic animals. Animal protein ingredients are produced from a wide variety of raw materials and manufacturing techniques and equipment. [30] Consequently, phosphorus content and the proportion of chemical compounds in these ingredients may be highly variable, even for a given type of ingredient. A survey of the literature indicates that there are between 16 and 42 g kg-1 of phosphorus in fish meal, from 25 to 56 g kg-1 of phosphorus in meat and bone meal, and from 17 to 35 g kg-1 of phosphorus in poultry byproducts meal. [31] Very little information on the proportion of phosphorus chemical compounds in these ingredients is available in the literature, although it is well known that in the body of vertebrates, the majority of phosphorus (85- 88%) exists as bone phosphorus, 10-15% is organic phosphorus, and only a small amount is present as free ions or soluble inorganic phosphorus phosphates (Pi). [32] Estimates of the digestibility of phosphorus for animal protein ingredients are highly variable even for similar ingredients. For example, estimates of apparent digestibility of phosphorus in fish meal vary between 17 and 81% for rainbow trout. [32-34] Differences in the levels of different phosphorus chemical forms could explain part of the variability in the estimates of apparent digestibility of

phosphorus.. There have been attempts to estimate bioavailability of phosphorus in ingredients and feeds based on chemical extractions. [35-36] A fractionation method was also used for estimates of composition of animal manures. [35] However, limited work has been carried out to quantify specific chemical compounds in animal protein ingredients. There is also a need for simple methods of estimating total phosphorus and bone phosphorus contents of feed ingredients based on routine chemical analyses (e.g., proximate analysis). The objectives of the study were to (1) quantify bone phosphorus and nonbone phosphorus in animal ingredients and (2) determine the relation among bone phosphorus, total phosphorus, and proximate analysis parameters.

## 7. Microbial degradation of organophosphorus compounds

Use of organochlorine pesticides such as dichloro-diphenyltrichloroethane (DDT), lindane, etc., has been reduced drastically in developed countries due to their long persistence, tendency towards bioaccumulation and potential toxicity towards non-target organisms. This group of compounds has been replaced by the less persistent and more effective organophosphorus compounds. However, most of the organophosphorus compounds possess high mammalian toxicity. Among the organophosphorus compounds, glyphosate, chlorpyrifos, parathion, methyl parathion, diazinon, coumaphos, monocrotophos, fenamiphos and phorate have been used extensively and their efficacy and environmental fate have been studied in detail. The chemical and physical properties of some of these compounds are listed in Table 1.3. The phosphorus is usually present either as a phosphate ester or as a phosphonate.

**Table 1.3: History, toxicity and half-life of some organophosphorus pesticides**

| Name | Type | Year o introduction | Mammalian LD50 (mg kg1 ) | Half-life soi (days) |
|---|---|---|---|---|
| Chlorpyrifos | Insecticide | 1965 | 135–163 | 10–120 |
| Parathion | Insecticide | 1947 | 2–10 | 30–180 |
| Methyl parathion | Insecticide | 1949 | 3–30 | 25–130 |
| Glyphosate | Herbicide | 1971 | 3530–5600 | 30–174 |
| Coumaphos | Acaricide | 1952 | 16–41 | 24–1400 |

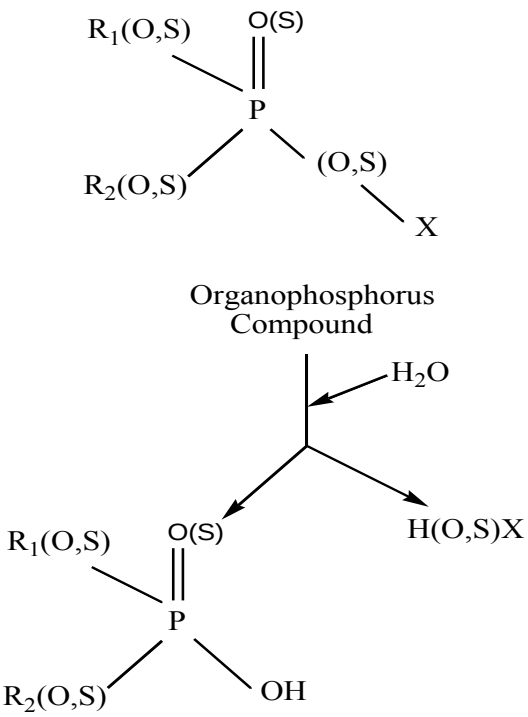
| | | | | |
|---|---|---|---|---|
| Fenamiphos | Nematicide | 1967 | 6–10 | 28–90 |
| Monocrotophos | Insecticide | 1965 | 18–20 | 40–60 |
| Dicrotophos | Insecticide | 1965 | 15–22 | 45–60 |
| Diazinon | Insecticide | 1953 | 80–300 | 11–21 |
| Dimethoate | Insecticide | 1955 | 160–387 | 2–41 |
| Fenitrothion | Insecticide | 1959 | 1700 | 12–28 |
| Ethoprophos | Nematicide | 1966 | 146–170 | 3–30 |

Being esters they have many sites which are vulnerable to hydrolysis. The principal reactions involved are hydrolysis, oxidation, and alkylation and dealkylation. [37] Microbial degradation through hydrolysis of phosphorus-Oxygen-alkyl and phosphorus-Oxygen-aryl bonds is considered the most significant step in detoxification. Both co-metabolic and bio-mineralization of organophosphorus compounds by isolated bacteria have been reported. Hydrolysis of organophosphorus compounds leads to a reduction in their mammalian toxicity by several orders of magnitude. Since most of the research has been directed towards detoxification, studies on the further metabolism of the phosphorus containing products have not been extensive. Hypothetical phospho-ester hydrolysis steps can be postulated, yielding mono-ester and finally inorganic phosphate, but this pathway has not been specifically studied. Analogous phospho-monoesterase and diesterase, which degrade methyl and dimethyl phosphate, respectively, have been reported in Klebsiella aerogenes [38] and are produced only in the absence of inorganic phosphate from the growth medium. The final enzyme in the postulated degradative pathway is bacterial alkaline phosphatase, which can hydrolyze simple monoalkyl phosphates and is also regulated by the level of phosphate available to the cell. [38] A similar mechanism of metabolism has been reported for phosphonates. [39] The way in which metabolism is regulated depends very strongly on what role the organophosphorus compound plays for the particular organisms studied. Most often these compounds are used to supply only a single element (carbon, phosphorus or sulfur) and the relevant gene cannot be expressed as a response to starvation for another of these elements. [39] For example, a strain of Pseudomonas stutzeri isolated to utilize parathion as a carbon source released the diethylphosphorothioanate products quantitatively and could not metabolize them further, even when alternative source of phosphorus or sulfur were removed. [40] Similarly, a variety of isolates that could use phosphorothionate and phosphorodithionate pesticides as a sole source of

phosphorus were unable to utilize these compounds as a source of carbon. [41] Shelton (1988) isolated a consortium that could use diethylthiophosphoric acid as a carbon source but was unable to utilize it as a source of phosphorus or sulfur. [39] Explained possible underlying reasons for this phenomenon. They suggested that the conditions under which environmental isolates enriched were crucial in selecting for strains not only with the desired derivative enzyme systems but also with specific regulation mechanisms for the degradation pathways.

## 8. Toxicology of organophosphorus compounds

Most organophosphorus compounds are ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid. Their general formula is presented in $R_1$ and $R_2$ are mainly the aryl or alkyl group, which can be directly attached to a phosphorus atom (phosphinates) or via oxygen (phosphates) or a sulphur atom (phosphothioates). In some cases, $R_1$ is directly bonded with phosphorus and $R_2$ with an oxygen or sulfur atom (phosphonates or thion phosphonates, respectively). At least one of these two groups is attached with un-, mono- or di-substituted amino groups in phosphoramidates. The X group can be diverse and may belong to a wide range of aliphatic, aromatic or heterocyclic groups. The X group is also known as a leaving group because on hydrolysis of the ester bond it is released from phosphorus. [42] The mode of action of organophosphorus compounds includes inhibition of neurotransmitter acetylcholine breakdown. Acetylcholine is required for the transmission of nerve impulses in the brain, skeletal muscles and other areas. [43] However, after the transmission of the impulse, the acetylcholine must be hydrolyzed to avoid overstimulating or overwhelming the nervous system. This breakdown of the acetylcholine is catalyzed by an enzyme called acetylcholine esterase. Acetylcholine esterase converts acetylcholine into choline and acetyl CoA by binding the substrate at its active site at serine 203 to form an enzyme substrate complex. Further reactions involve release of choline from the complex and then rapid reaction of acylated enzymes with water to produce acetic acid and the regenerated acetylcholine esterase.




**59**

**General formula of organophosphorus compounds and major pathway of degradation**

It has been estimated that one enzyme can hydrolyze 300 000 molecules of acetylcholine every minute. [44] Organophosphorus compounds inhibit the normal activity of the acetylcholine esterase by covalent bonding to the enzyme, thereby changing its structure and function. They bind to the serine 203 amino acid active site of acetylcholine esterase. The leaving group binds to the positive hydrogen of his 447 and breaks off the phosphate, leaving the enzyme phosphorylated. The regeneration of phosphorylated acetylcholine esterase is very slow and may take hours or days, resulting in accumulation of acetylcholine at the synapses. Nerves are then overstimulated and jammed. [45] This inhibition causes convulsion, paralysis and finally death for insects and mammals. [46]

## 9. fertilizer

Phosphorus-containing fertiliser materials were in use for many centuries before their action was identified with the presence of the element. Fish and animal manures were employed several thousand years ago and the Carthaginians were using bird dung in 200 bc. In the twelfth century, guano was used by the Arabs and the Incas. English farms used bones in the seventeenth century, and waste bone and ivory chippings from button and knife manufacture in Sheffield (GB) were used locally around 1750. Bones were used as fertilisers in France and the United States in the early part of the nineteenth century and increasing quantities (often from battlefields) were imported by Great Britain up to about 1850. Animal manure, bones, bone ash, bone meal, guano and dried blood are still used in a relatively minor way, although their phosphate content is lower, or is less readily available than in the manufactured products to be discussed below. In 1842, British patents for the manufacture of ‗superphosphate' by the action of sulphuric acid on bones were taken out independently by J.B. Lawes and J. Murray. [47] This led to the world's first fertilizer factory at Deptford, Kent, England. A few years later superphosphate manufacture commenced in the United States, but it was not until about 1855, however, that the work of Lawes. In practice, not all these factors will be predictable or controllable, and the experience and judgement of the local farmer is of course often vital for success and Gilbert and others, finally established that phosphates were essential for plant development. J. Von Liebig in 1843 proved that ‗phosphate of lime' and not gelatine, as previously believed, was the fertilizing agent present in bones. About this time it also became evident that the phosphate of lime in bones was similar to that present in phosphate rock, and the latter soon began to replace the former as the source of phosphorus in superphosphate manufacture. This replacement was greatly accelerated later in the century when it became evident that abundant supplies of phosphate rock were available in Florida and elsewhere in the world.

## 10. Food Technology [48]-[51]

As long ago as 1827 W. Prout had recognised three types of complex food components, which today are known as carbohydrates, fat (lipids) and proteins. It was not until much later that another component, nucleic acids, was also recognized, and that all these four components were often present in phosphorylated forms. Only in the latter part of the nineteenth century did the beneficial effects of added (often simple) phosphorus compounds start to be appreciated. Only by the middle of the twentieth century was their mass use by the food industries firmly established. Phosphates are present in most natural foods, particularly meat, milk and dairy products, fruit and cereals. Further addition of phosphates is frequently made in the processing of many foods, for a variety of purposes which include

1) Increasing the nutritive value
2) Preservation
3) Influencing flavor
4) Modification of structure or texture
5) Colour development or stabilization
6) pH alteration or control
7) Complexing of undesirable metal ions
8) Increasing water-binding
9) Emulsion stabilization
10) Dispersion
11) Prevention of caking
12) Leavening action

The major phosphorus-containing products in current use as food additives (Table 1.6) may be listed as

1) Inorganic salts – ortho-, pyro- and polyphosphates, mostly of Na, K or Ca.
2) Biopolymer phosphates – casein, lactalbumin phosphate, starch phosphates, lecithin.

Medicinal supplementation of P is usually effected with casein, orthophosphates or glycerophosphates of Na, K, Mg or Ca.

Detailed patent claims and reported applications of P compounds are exceedingly numerous and for present purposes eight divisions will be made:

**Table 1.4**

| | |
|---|---|
| 1) Milk and dairy products | 2) Meat and fish |
| 3) Fruit and vegetables | 4) Beverages |
| 5) Cereals | 6) Leavening agents |
| 7) Biopolymer phosphates | 8) Miscellaneous applications |

**Table 1.5 Some Phosphorus Compounds Used in Food Production**

| | | | |
|---|---|---|---|
| Phosphoric acid | Calcium dihydrogen phosphate | Monosodium hydrogen phosphate | Tricalcium phosphate |
| Disodium monohydrogen phosphate | Hydroxyapatite Trisodium phosphate | Calcium pyrophosphate | Sodium pyrophosphate |
| Calcium dihydrogen pyrophosphate | Disodium dihydrogen phosphate | Calcium polyphosphate | Sodium triphosphate |
| Ammonium polyphosphate | Sodium polyphosphate | Ferric phosphate | Sodium trimetaphosphat |
| Sodium inosine phosphate | Sodium aluminium phosphate | Sodium glycerophosphate | Potassium dihydrogen phosphate |
| Casein (molecular mixture) | Dipotassium hydrogen phosphate | Lecithin (molecular mixture) | Potassium triphosphate |
| Lactalbumin phosphate (molecular mixture) | Potassium polyphosphate | Starch phosphate (molecular mixture) | |

## 11. Detergent

Modern detergent powders combine moderate amounts of polyphosphates (sodium triphosphate in particular) with small amounts of organic surfactants. Introduced over 60 years ago, these powders have made a considerable impact and have replaced traditional cleaning materials such as soap and soda in many applications. [52] The high charge on polyphosphate chains helps to stabilise detergent micelles. The sodium salt, $Na_5P_3O_{10}$, is much used as a detergent builder in this way (it has a more suitable alkalinity than longer-chain derivatives). Builders generally lower the critical micelle concentration, and because many small micelles clean more effectively than one large micelle, less surfactant is needed to achieve the same cleaning power. The triphosphate salt also acts as a sequestering agent, and soluble triphosphate complexes are generally more stable than those formed with pyrophosphate. Sodium triphosphate has not yet been successfully challenged by organic sequestering agents such as nitrilotriacetic acid, $N(CH_2\text{-}COOH)_3$ (NTA), or ethylenediamine tetraacetic acid $[(HOOCCH_2)_2NCH_2]_2$ (EDTA), because the latter are too toxic (possibly carcinogenic), too expensive or have other undesirable properties. Triphosphates are particularly suitable for detergent compositions because

1) They are non-toxic
2) They are safe on colours and fabrics of all types
3) They are non-inflammable and non-corrosive in washing machines
4) They soften water by sequestering $Mg^{2+}$ and $Ca^{2+}$
5) They keep dirt in suspension after it is removed from fibres
6) They keep a P of 10 ~ 11 which does not damage skin on contact
7) They break down satisfactorily in sewage treatment
8) They can be effectively removed by waste processing
9) They are relatively cheap.

There has been a drastic cutback in recent decades, of the quantities of phosphates used in detergents in some countries. This is because of the ecological problems associated (sometimes wrongly) with too high a phosphate content of waste water. [53] Human excreta and fertiliser run-

off also contribute to this, and the fraction contributed by detergents may often only be about a quarter of the total. This fraction is controversial and estimates vary in different parts of the world. However, eutrophication resulting from blue-green algae is getting worse in some places. There is some evidence that this may be due to an increased contribution from phosphate fertiliser run-off rather than the use of detergents. In some countries, second thoughts have been expressed about the wisdom of legally banning detergent phosphates. Other factors include a lack of zoo plankton (which consume blue-green algae) which may have to be taken into account – the issue of phosphates in lakes is not yet clear-cut. [53] Excessive quantities of other elements such as nitrogen are necessary for eutrophication to take place. Inorganic phosphates are only one contributing factor and not always the limiting one. They are not in themselves toxic and should not be classed as first-order pollutants. Any ecological risks or health problems are perhaps more likely to be associated with organophosphate pesticides.

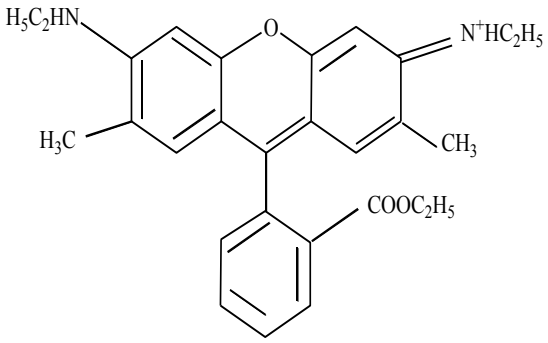
## 12. Pigments and dyestuffs

Important properties usually associated with pigments include

1. Insolubility
2. Light fastness
3. Heat stability
4. Brightness
5. Covering power
6. Tinting strength
7. Dispersibility
8. Toxicity
9. Cost

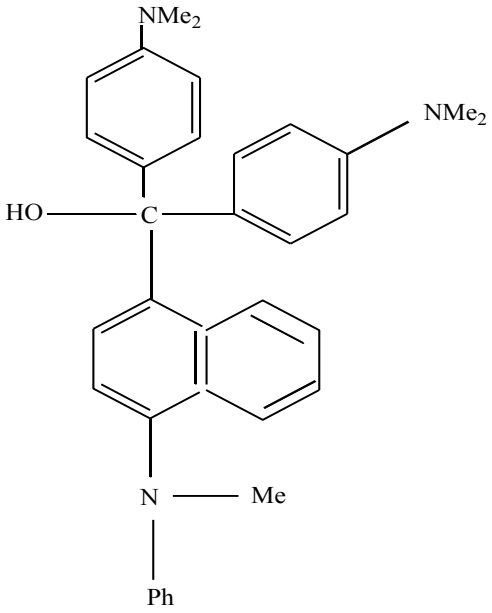
In 1913, Imerheiser [54] discovered insoluble phosphotungstic or phosphomolybdic _lakes‘ by reacting the traditional heteropoly acids with certain cationic dyestuffs. These pigments are noteworthy for their brilliant colours, high tinting strengths and fastness to light. Typical lake-forming dyes are Rhodamine Red (60) and Victoria Blue (61), which have found some application as paper dyes, in printing inks, alkyd resin enamels, and coloured crayons or pencils. Dyes such as Orange G (62) and Aniline Blue (63) are incorporated with phosphomolybdates in formulations for microscopic stains. [54] Essential components of these phosphotungstic _lakes‘ are Al, Ba or Ca cations, phosphotungstic (or phosphomolybdic) anions, and a suitable dyestuff, but their precise structural formulation is not known. [54] Phosphotungstic lakes have some technical drawbacks, however, and are relatively expensive. Competition from other organic pigments has greatly limited their use. In view of the great expansion of heteropoly chemistry in

recent years, it seems not unlikely that entirely new ‗lakes' may be possible with some of the larger cavity structures.



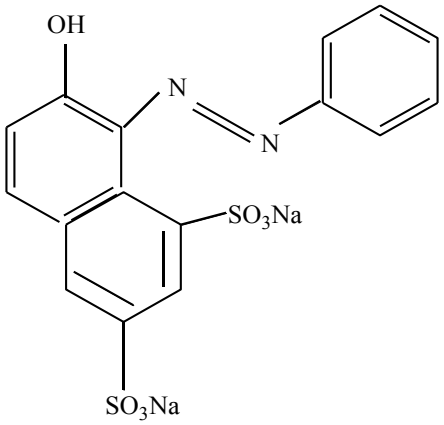

**60**

**Rhodamine red**



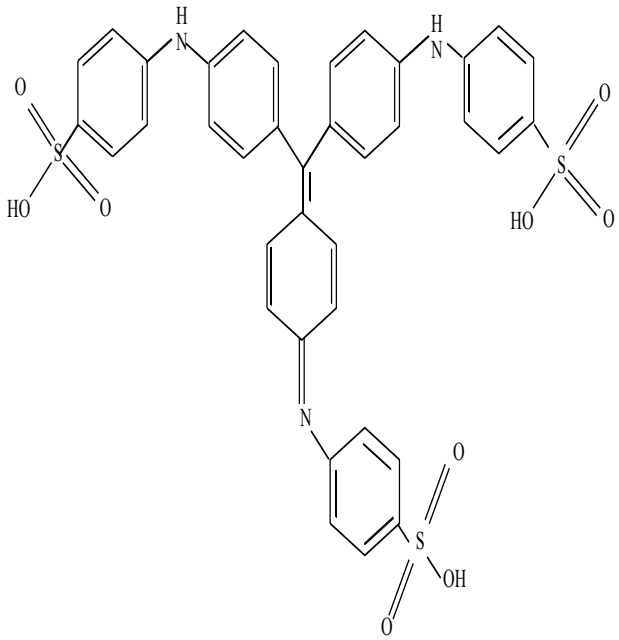

**61**

**Victoria blue**




**62**

**Orange G**




**63**

**Aniline blue**

Azo dyestuffs, which have the general formula Ar–N=NAr', form the largest and most used class of dyestuffs. [55] They can be formed by reactions of the type (64) and (65), which are known as diazotisation and coupling, respectively. The availability of an almost endless number of aromatic derivatives Ar and Ar' for these reactions (benzene and naphthalene derivatives figuring most prominently) has led to the huge range of azo dyestuffs and pigments known (but not necessarily manufactured) today.

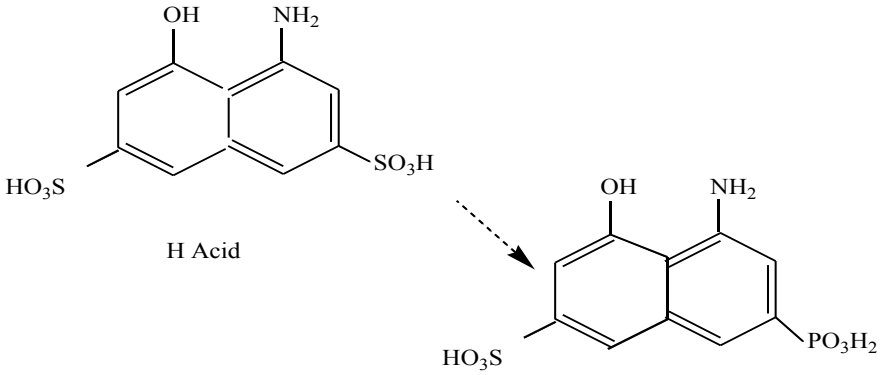
$$Ar.NH_2 + HX + HNO_2 \rightarrow [ArN{\equiv}N]^+X^- + 2H_2O$$

**64**

$$[ArNN]^+X^- + Ar'H \rightarrow Ar{-}N{=}N{-}Ar' + HX$$

**65**

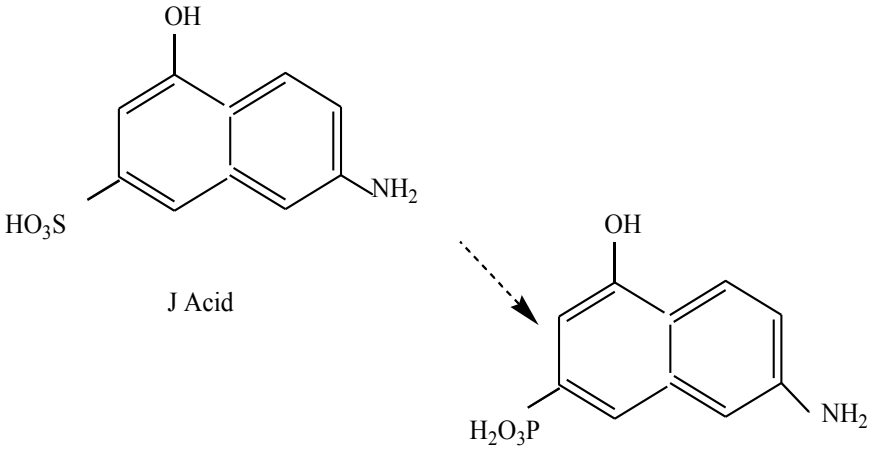
In the most used _direct' method of diazotisation 64, generally X=Cl, but it can be $HSO_4$, $H_2PO_4$, and so on. In special cases, for example, weakly basic amines, $H_3PO_4$ is sometimes more suitable and is used in place of HCl (the nitrous acid is usually generated by the simultaneous action of more acid, HX, on $NaNO_2$). [56] Phosphonic acid analogues (65) of the widely used dyestuff intermediates (Ar orAr') _H Acid' (66) and _J Acid' (67) have been patented for use in this field. The replacement of $SO_3H$ by $PO_3H_2$ in established dyes can be expected to produce at least some modification of colour or other properties. Some azo dyestuffs containing phosphonate groups have been found to be capable of forming exceptionally strong direct dye–fire bonds, particularly in the case of cellulose.




H Acid

**66**

**H Acid**

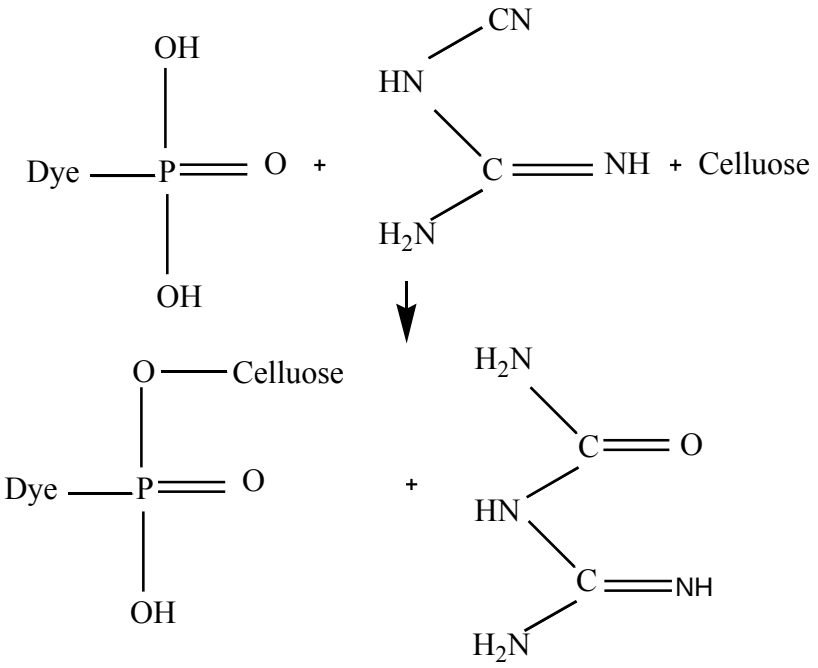



J Acid

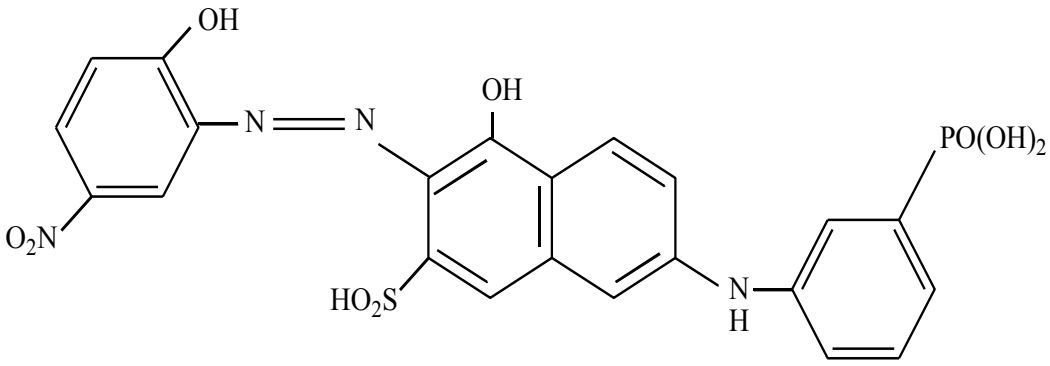
**67**

**J Acid**

These became commercially available in 1977 as Procion dyes, but were later withdrawn because of technical drawbacks experienced during processing. [56] The simple process requires a second agent which is dicyandiamide and it can be summarised in a simple way as (68) Typical dyes used for this process are (69) & (70).

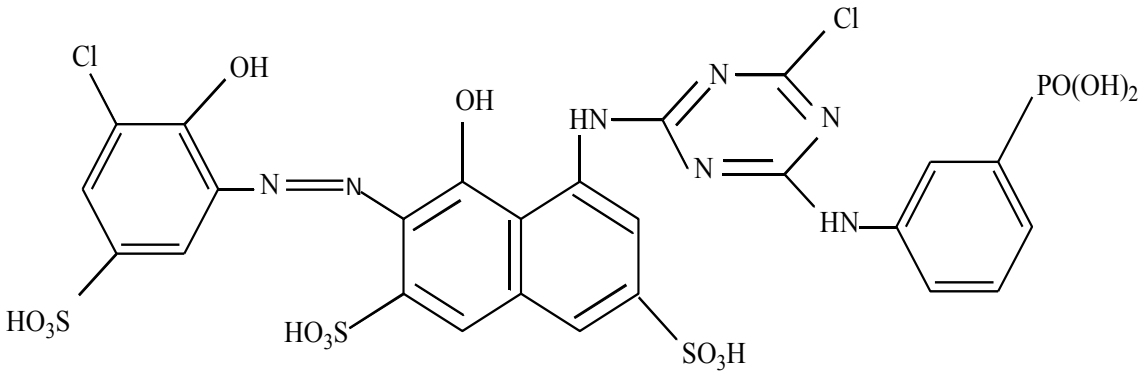



**68**




**69**

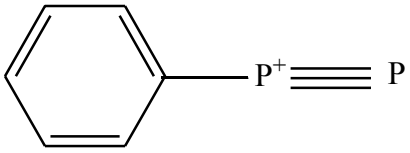
**Reactive red**




**70**

**Reactive violet**

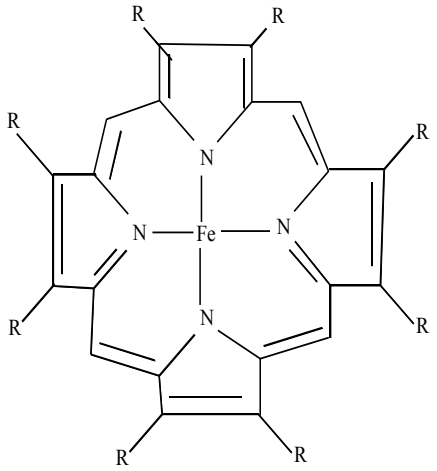
Polyphosphonates will bind to both azo dyestuffs and cotton fires, thus increasing fixation. [57] Azo polymer dyestuffs may be possible since aromatic azo groups can be introduced into the side chains of phosphazene polymers. [58] Not only might preformed azo dyestuffs be attached to the polymer, but side chain aromatic amines might be diazotised and coupled as in (64) and (65). Knowledge of the chromophoric properties of the –P=P– group is somewhat limited and the effect of substituting this group for –N=N– in established dyestuffs, for example, (69) & (70) (assuming it can be done) remains unknown Phosphorus analogues of diazonium salts, that is, $[Ar–P\equiv P]^{+}X^{-}$ have not been well characterised and a coupling reaction analogous to (65) remains unknown at present. [59] The benzene diphosphonium cation (71) should be capable of existence.




**71**

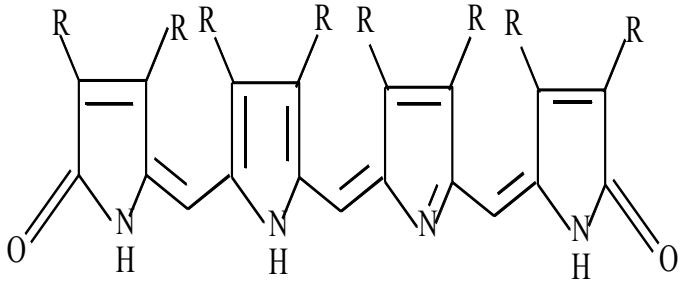
**Benzene diphosphonium cation**

Possible P analogues of hemi (72) and the bile pigments (73) (R=Various combinations of Me, Et, $CH_3CH_2COOH$,$CH=CH_2$etc) have not been established as practical pigments.



**72**

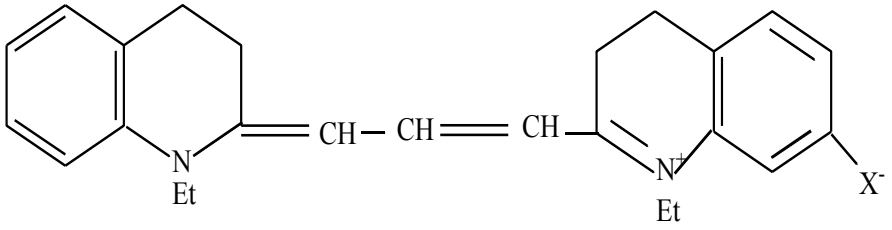
**Hemi**



**73**

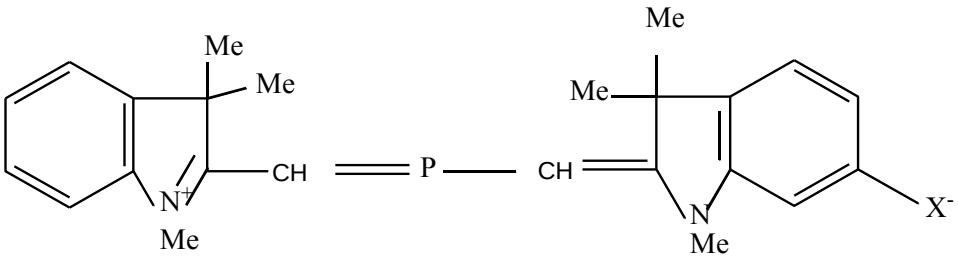
**Bile pigments**

Phosphacyanins of type R–P=R or R–CH=P–CH=R are the phosphorus analogues of the aza cyanins, R–N=R or R–CH=N–CH=R and the important photographic dyes of the type R–CH=R or R–CH=CH=R, where R is usually a quinoline or indole derivative (74).



**74**

**Indole derivative (a)**



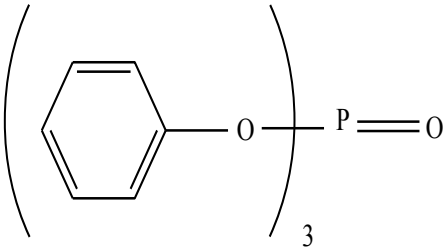

**75**

**Indole derivative (b)**

Compound (75) is a dye, but investigations of this type of phosphorus compound are at present very limited A full assessment of their potential as photographic sensitisers or as ordinary textile dyestuffs will be premature Commercially, any increased costs compared to existing products would have to be justified Miscellaneous compounds such as (74) & (75) continue to be reported as potentially useful dyestuffs. [57]

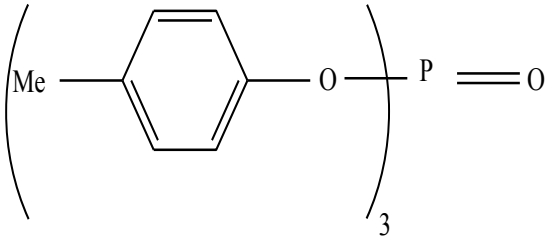
## 13. Industrial Phosphate Esters

Apart from their enormous importance in biochemistry, phosphate esters have many technological applications. Industrial production began in the 1920s and had become significant by the middle of the twentieth century. They are not always pure compounds – commercial tricresyl phosphate, for example, may contain a mixture of meta- and para-substituted groups. Commercial phosphate esters are frequently very toxic. Commercial applications often utilise mono- and diester mixtures, usually obtained by the method in. Triphenyl phosphate (76), $(C_6H_5O)_3PO$, has a mp =51°C, bp = 260°C and is reported to be stable up to at least 340°C. Density ρ = 1.2033 g/cc and water solubility is 0.002% at 54°C. It is soluble in many organic solvents such as EtOH, $C_6H_6$, $CCl_4$, $CHCl_3$, EtOH and $Et_2O$. The trialkyl phosphates and lower dialkyl phosphates are low-viscosity liquids at room temperatures and show a regular progression in their physical properties. Most aromatic di- and triesters are solids at room temperatures and are generally more stable than the alkyl esters.



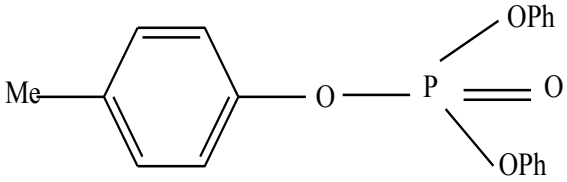
**76**

**Triphenyl phosphate**



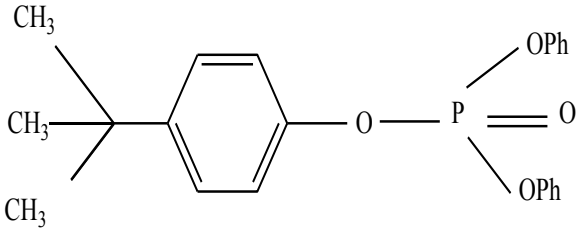
**77**

**Tricresyl phosphate**



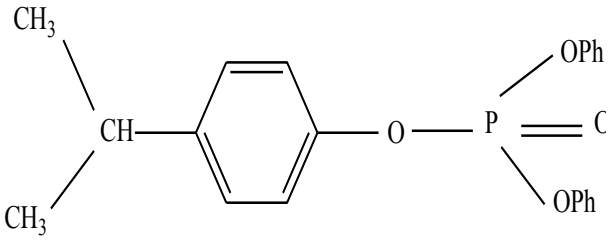
**78**

**Cresyldiphenyl phosphate**



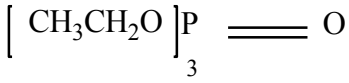
**79**

**t-butylphenyl phosphate**



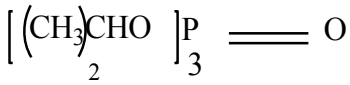
**80**

**Isopropyldiphenyl phosphate**



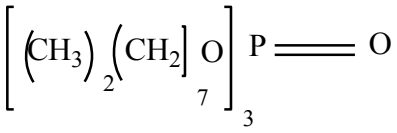
**81**

**Triethyl phosphate**



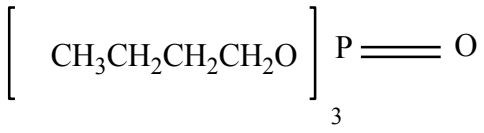
**82**

**Tri-isopropyl phosphate**



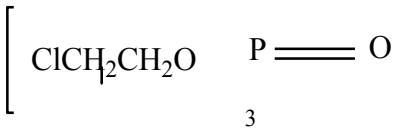
**83**

**Trioctyl phosphate**



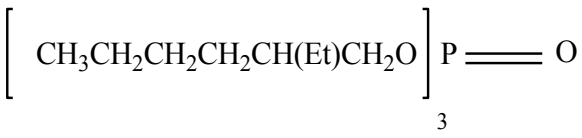
**84**

**Tributyl phosphate**



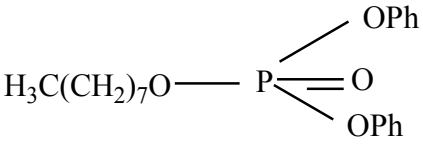
**85**

**Tris(2-chloroethyl) phosphate**



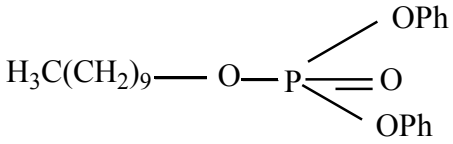
**86**

**Tris(2-ethylhexyl) phosphate**



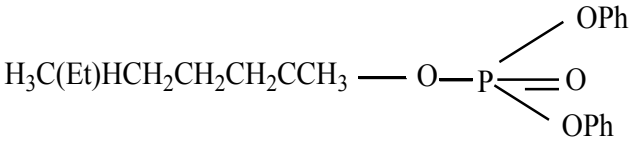
**87**

**Octyldiphenyl phosphate**



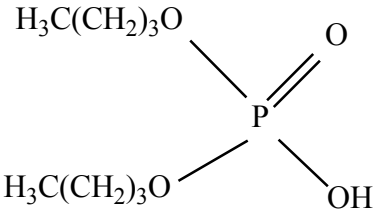
**88**

**Decyldiphenyl phosphate**



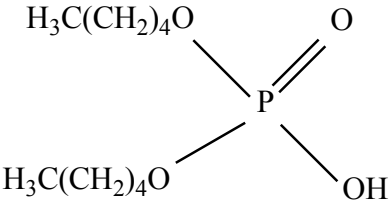
**89**

**2-ethylhexyldiphenyl phosphate**



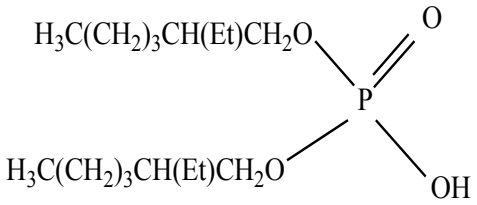
**90**

**Dibutyl hydrogen phosphate**



**91**

**Diamyl hydrogen phosphate**




**92**

**Bis(2-ethylhexyl) hydrogen phosphate**

Triethyl phosphate (81) is miscible with water and like tripropyl and tributyl phosphates, it fids use as a solvent. Surfactant long (carbon)-chain phosphates such as cetyl phosphate $C_{16}H_{33}OP(O)(ONa)_2$ (83) can replace the corresponding sulphates in detergent compositions . The use of esters such as tributyl, tricresyl or cresyl diphenyl phosphate results in smoother combustion and improved engine performance when incorporated as petroleum additives. One function of the esters is to combine with lead from lead tetraethyl and expel it as relatively harmless lead orthophosphate. Esters (and thioesters,) confer valuable anti-wear and corrosion inhibition properties when used as oil additives Dialkyldithiophosphates can be used in vulcanization processes to reduce staining of latex products. They remove metallic cations which otherwise can form intensely coloured complexes with the thiazole accelerators which are used. Many commercial phosphate esters are exceedingly toxic. Mass poisoning occurred in the United States in the early 1930s when some Jamaican ginger supplies became contaminated with triorthocresyl phosphate. Nearly 20,000 people were affected, some with paralysis, tremors and worse symptoms. Various instances of poisoning from accidental contamination with industrial organophosphate esters have since been reported from other parts of the world. [60] The matter has been intensively studied and is related to effects (which can be long term) experienced with certain pesticides and also nerve gases.

## 14. Dental and medical materials

**Toothpastes:** Dicalcium phosphate dihydrate, $CaHPO_4 \cdot 2H_2O$, was introduced into toothpaste over 70 years ago as a mild abrasive and polishing agent to replace calcium carbonate. Another advance in toothpaste formulation occurred about 30 years ago with the introduction of sodium floride and stannous floride as anti-caries agents. These compounds provide F− ions which

exchange with the $OH^-$ in the hydroxyapatite of tooth enamel, making it harder and more resistant to decay. Sodium and stannous florides, however, are not very compatible with $CaHPO_4 \cdot 2H_2O$ or $CaCO_3$, since some reaction occurs to precipitate insoluble $CaF_2$. The latter does not provide $F^-$ for substitution in tooth hydroxyapatite. If dicalcium phosphate is replaced with calcium or stannous pyrophosphate the situation is improved, but the best answer is provided by sodium monoflorophosphate, $NaPO_3F$, which is now widely used in toothpaste formulations [61]. Ingestion of $Na_2PO_3F$ causes an accumulation of $F^-$ in teeth (and bones) comparable with that produced by NaF. It has been shown to reduce the incidence of caries in children. Modern toothpaste formulations include a variety of substances, each of which fulfis a specific purpose. The humectant prevents dehydration of the dicalcium phosphate dihydrate to the anhydrous form which is too abrasive. A representative formulation is

**Table 1.6**

| | | |
|---|---|---|
| Dicalcium phosphate dehydrate | 45% | Primary abrasive |
| Dicalcium phosphate anhydrous | 5% | Secondary abrasive |
| Sodium lauryl sulphate | 2% | Detergent |
| Glycerol | 25% | Humectant |
| Sodium carboxymethylcellulose | 1% | Builder |
| Sodium monoflorophosphate | 1% | Anti-caries |
| Sodium benzoate | 0.5% | Preservative |
| Peppermint | 1% | Flavour |
| Saccharin | 0.2% | Sweetener |
| Water | 100 | |

Insoluble forms of sodium polyphosphates are compatible with florides and have been used as toothpaste abrasives. Peroxy-diphosphates have also been used. [61] Some currently available floride-containing toothpaste also includes $Na_5P_3O_{10}$ in their formulations. Sodium acid pyrophosphate and potassium pyrophosphate as well as some organophosphonic acids are included in some formulations as anti-tartar agents. [61] Tooth powders and denture cleaners incorporate phosphates:

**Table 1.7**

| **denture cleaner** | **%** | **tooth Powder** | **%** |
|---|---|---|---|
| Sodium perborate | 40.0 | $CaHPO_4 \cdot 2H_2O$ | 75.0 |
| Sodium chloride | 30.0 | $CaCO_3$ | 23.0 |
| Trisodium phosphate | 30.0 | Na lauryl sulphate | 1.0 |
| | | $NaPO_3F$ | 0.8 |
| | | Flavour | 0.2 |

Phosphopeptides have potential uses in toothpastes as anti-cariostatic agents . Phosphate esters have been patented as dental adhesives.

**2. Tooth fillings** [62]

The requirements of an ideal tooth filing are many:

1. Adequate strength and hardness
2. Rapid setting
3. Chemical resistance to mouth flids
4. Compatibility with dentine and pulp
5. Slight expansion on setting
6. Adhesion to tooth
7. Anti-bacterial properties
8. Thermal insulator value
9. Suitable colour

For almost a century zinc phosphate was the most successful dental cementing medium, but other materials are now offering strong competition Particular advantages of zinc phosphate are its high strength, opacity and whiteness combined with insolubility and resistance to dimensional changes Zinc phosphate cements are rather brittle, however, and do not adhere directly to tooth apatite, but they are still used for _luting‘ procedures (Table 1.8).

**Table 1.8**

**Compressive Strengths of Dental Materials**

| | **Psi** |
|---|---|
| Tooth enamel | 37,000 |
| Dentin | 44,000 |
| Amalgam | 57,000 |
| Zinc phosphate cement | 12,000 |
| Portland cement concrete | 6000 |
| Magnesium phosphate concrete | 5000 |

Immediately prior to use, phosphoric acid is mixed with an excess of zinc oxideThis sets within less than 5 min producing a core of ZnO particles embedded in a matrix of crystalline and amorphous zinc phosphates Subsequent hardening processes are associated with changes of crystallinity and the formation of more basic and insoluble salts 93.

$$H_3PO_4 \xrightarrow[\text{Fast}]{ZnO} Zn(H_2PO_4)_2 \xrightarrow[\text{Slow}]{ZnO} ZnHPO_4.3H_2O \xrightarrow[\text{Slow}]{ZnO} Zn_3(PO_4)_2.4H_2O$$

**93**

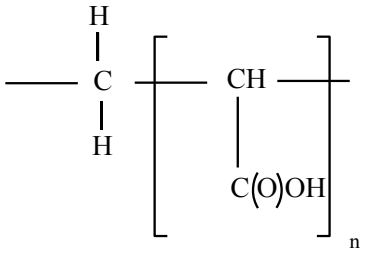
The setting rates of these dental cements are controlled by prior sintering of the oxide powder and by the addition of buffering aluminium salts to the phosphoric acid Small quantities of MgO, $SiO_2$, $Al_2O_3$, and so on are usually included in the formulationAnti-bacterial action can be achieved by the addition of some CuO which produces small quantities of non-white copper phosphates Zinc silicophosphate cements are made by mixing $H3PO_4$ with ZnO and ground silica glass Various high-strength dental silicate cements are obtained by using ground calcined mixtures of $Al_2O_3$, $SiO_2$, $Na_3AlF_6$ (cryolite), $AlPO_4$, $Ca_3(PO4)_2$, and so on, which are mixed with $H_3PO_4$ The resulting product is a mixture of unreacted $SiO_2$, $Al_2O_3$, and so on embedded in a complex matrix of aluminophosphosilicate gels, crystalline $CaF_2$ and $AlPO_4$. [63] One of these products is obtained from mixture A which is fied at 1350°C, then ground and mixed with boiling solution B. [64]

(Table 1.9).

**Table 1.9**

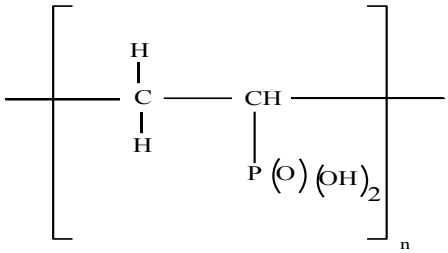
| **mixture A** | **%** | **solution B** | **%** |
|---|---|---|---|
| $Al_2O_3$ | 23.2 | $Al_2O_3$ | 8 |
| $SiO_2$ | 33.3 | ZnO | 9 |
| Cryolite | 36.0 | $H_3PO_4$ (85%) | 83 |
| $CaHPO_4$ | 7.5 | | |

Glass monomer cements have now largely replaced zinc phosphate cements. [65] They are based on ground glasses made with similar components to the above, which are mixed with polycarboxylic acids such as polyacrylic acid, immediately prior to useSuperior adhesion to both dentine and tooth enamel are claimed The aluminosilicate glass formulations may sometimes include a phosphate component In the more recent polyphosphonate ionomer cements, the polyacrylic acid (94) is replaced by polyvinylphosphonic acid (95)High compressive strengths with setting times ~3 min can be achieved.



**94**

**Polyacrylic acid**



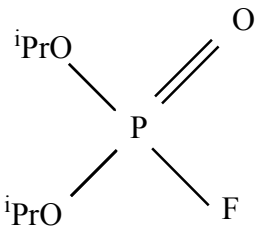
**95**

**Polyvinylphosphonic acid**

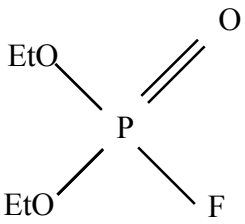
## 15. Nerve Gases

The phosphorus-containing nerve gases constitute the most deadly poisons at present known to man. Nerve gases are compounds which block nervous activity and cause death, either extremely quickly or agonisingly and very slowly, depending on the conditions of exposure. They act by inhibiting the action of cholinesterase, the enzyme which controls the hydrolysis of acetylcholine,

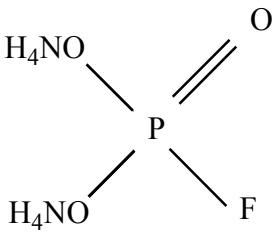
the substance immediately involved in the conduction and transmission of nerve impulses in the body. This inhibition is associated with a process of phosphorylation whereby the toxic compound becomes linked to the enzyme by a P–O–C linkage. The early development of nerve gases proceeded in parallel with the development of organophosphorus insecticides to which they are related. Serious work on the synthesis of these compounds began early in World War II, led by Saunders in Great Britain and Schrader in Germany. During the past 70 years, various highly effective nerve gases have been evolved by the great powers, but much of the work has remained secret. [66-67] Many of these compounds are volatile, colourless, odourless and effective in extremely small concentrations. They can be absorbed through the skin, the eyes, and by inhalation or ingestion. In non-lethal amounts, these nerve gas compounds generally produce myosis (constriction of the eye pupils), tightness of the chest, headache, nausea and vomiting. Somewhat greater concentrations lead to death after causing dizziness, anxiety, mental impairment, muscle twitching, convulsions, paralysis of breathing and many other symptoms. Effects from non-lethal concentrations can be prolonged and cumulative.



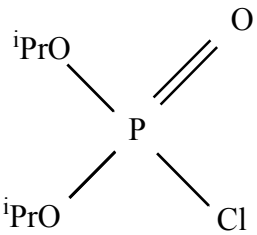

**96**




**97**




**98**




**99**

Although originally discovered by Lange [68] in 1932, one of the earliest compounds found by Saunders in 1941 to be effective was DFP, mp = −82°C, bp = 183°C (96). The gas is colourless and odourless, and inhalation will produce symptoms (at non-lethal level) when the concentration reaches ~1 ppm. The high toxicity of this compound is associated with the presence of both isopropoxy groups and florine bound to phosphorus. Compounds with other alkoxy groups (97) are much less toxic, and the presence of other groups renders the compound virtually non-toxic (98, 99). The florine derivative is considerably more resistant to hydrolysis than the chloro or other alkoxy floro derivatives. A 1% solution of DFP takes 72 h at 25°C to hydrolyse to $(PrO)_2P(O)OH$ and HF. Hydrolysis is speeded up under acidic or alkaline conditions. In the absence of moisture, DFP can be stored for considerable periods without decomposition. Comparatively small

differences in chemical constitution sometimes determine whether a compound is toxic or non-toxic. The preparation of DFP can be carried out by heating the relatively non-toxic chlorine derivative with sodium floride (100), or alternatively
by (101).

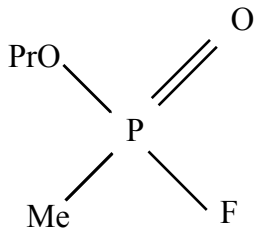
$$(^{i}PrO)_2POCl + NaF \rightarrow (^{i}PrO)_2POF + NaCl$$

**100**

$$Cl_2POF + 2\,^{i}PrOH \rightarrow (^{i}PrO)_2POF + 2HCl$$

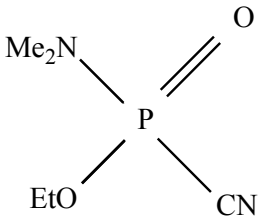
**101**

Mixtures of DFP with mustard gas, $(ClCH_2CH_2)_2S$, mp = 11.5°C, have been suggested as particularly lethal combinations for war use. A composition of 87% DFP with 13% mustard gas has a melting point of −36°C and is more suitable than pure DFP, for application in most climates. There is some evidence that injections of DFP (dissolved in peanut oil) might be useful in the treatment of schizophrenia and manic depression! [69] The three extremely toxic nerve gases evolved in Germany before and during World War II were sarin (102), tabun (103) and soman (104).



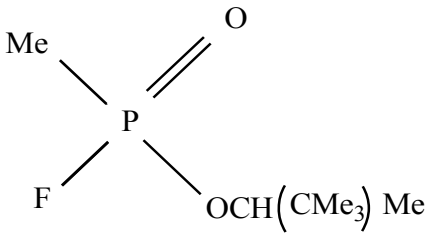

**102**

**Sarin**




**103**

**Tabun**

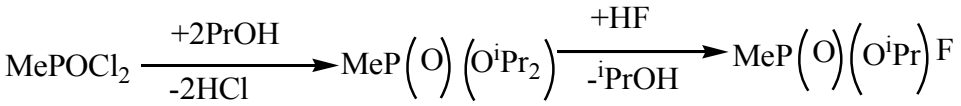



**104**

**Soman**

Various amidohalophosphates (RR'N)(RO)P(O)X, particularly florine derivatives such as $(Me_2N)_2P(O)F$, are also highly toxic. In some cases, enough of the compound can be absorbed through minor cuts and scratches to cause death. Sarin (‗GB') can be made by heating

methylphosphonic dichloride with isopropyl alcohol, followed by treatment of the resulting ester with HF (105).

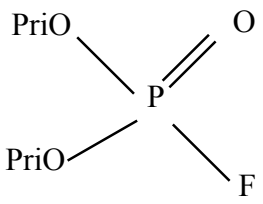


**105**

**Table 2.0**

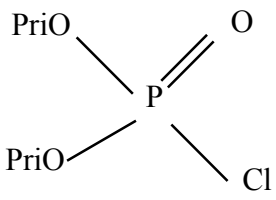
Neurotoxicity of related compounds

**Toxic**

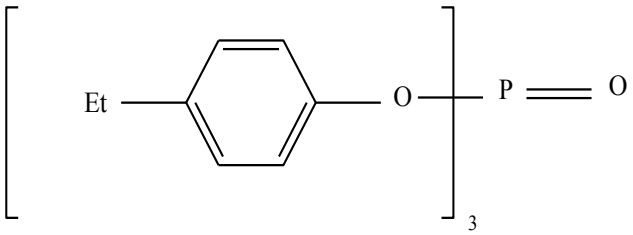


**106**

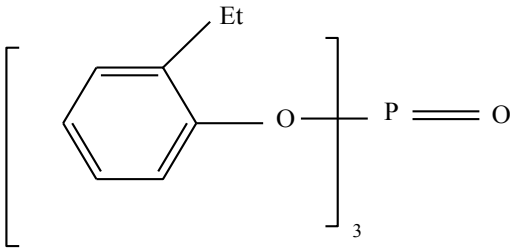
**Non- Toxic**



**107**



**108**



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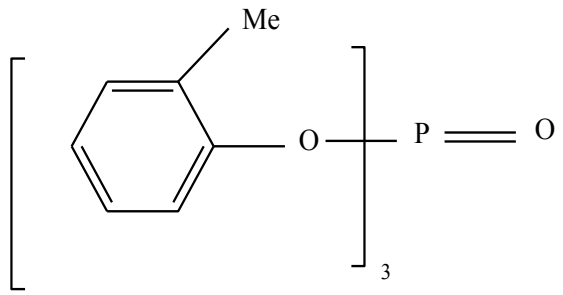
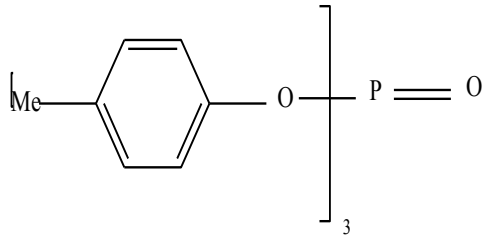
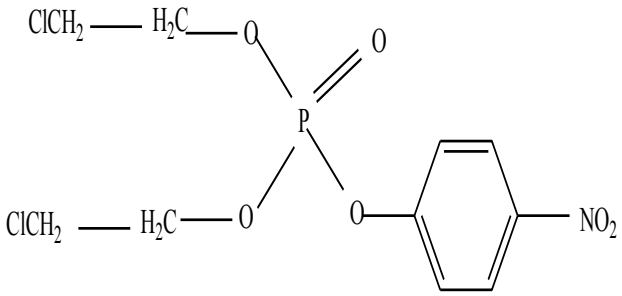


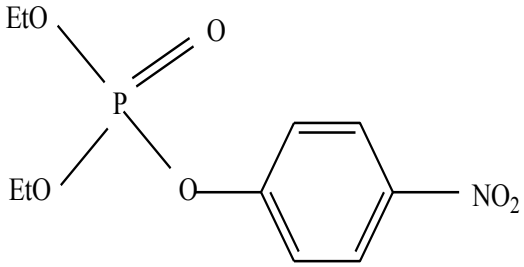
**110**



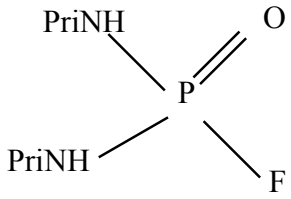
**111**



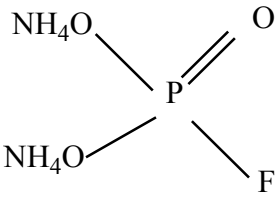
**112**



**113**



**114**



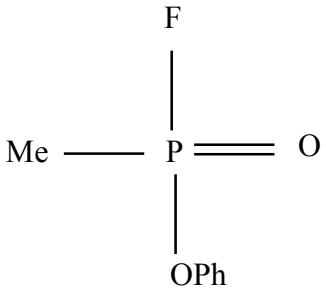
**115**

Tabun (‗GA') is a colourless liquid with a faint fruity odour, soluble in organic solvents and slightly soluble in water. It is hydrolysed by the latter with splitting of the P–CN linkage. Tabun can be prepared by (116).

$$Me_2N.POCl + EtOH + NaCN \rightarrow (Me_2N)(EtO)P(O)CN + 2NaCl + HCN$$

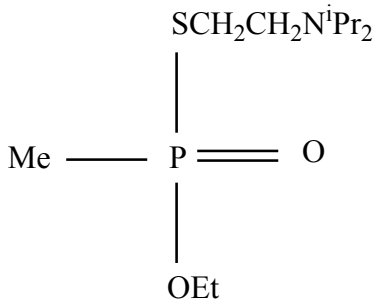
**116**

Two other nerve gases are ‗GF' (117) and ‗VX' (118). The latter, together with soman are probably the most toxic of all these compounds. One process for making VX is (119). (Tables 2.1 and 2.2)



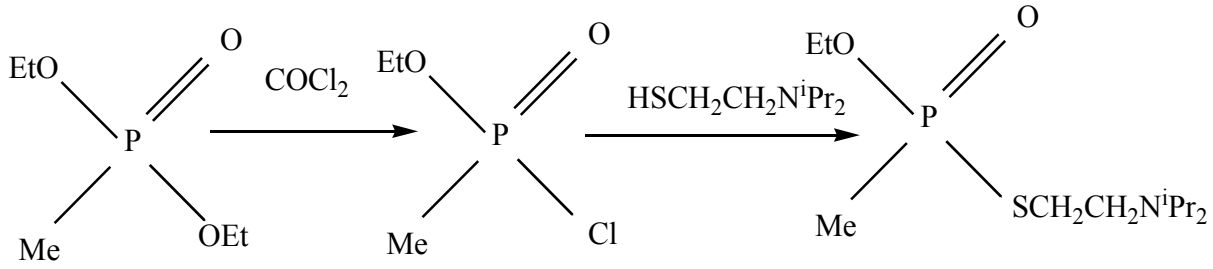

**117**

**GF**




**118**

**VX**




**119**

Tabun, sarin, soman and VX have emerged as the major nerve gases known to have been produced and weaponised in large quantities since the beginning of World War II. Initial war production in Germany was followed by Great Britain and the United States, the latter amassing large

**Table 2.1**

**Physical data for nerve Gases**

| | bp (°c) | fp (°c) | density (g/cc) 20°c | solubility in ( $H_2O$) |
|---|---|---|---|---|
| DFP | 180 | −80 | 1.07 | 1.5% |

| | | | | |
|---|---|---|---|---|
| Tabun | 240 | −50 | 1.08 | Miscible |
| Sarin | 147 | −57 | 1.10 | Miscible |

**Table 2.2**

**Relative toxicities—insecticides and nerve Gases**

| | ld 50 (mg/kg) |
|---|---|
| Malathion | 1200 |
| Parathion | 6 |
| Sarin | 0.17 |
| VX | 0.01 |

Stocks in the 1950s. This has unfortunately been followed later by Israel, and some Islamic countries. Comparatively little seems to be generally known about the stocks of China and Russia. There remains a fear that P compounds even more toxic than those already mentioned may one day be produced (and used) in some part of the world. International agreement has led to some disposal of nerve gas stocks, but this has so far resulted in only about a 25% reduction of known stocks. [70] According to some sources [2], _VX‘ is considerably more toxic than tabun, sarin or soman, but reliable reports of its being manufactured and tested are not available.

## 16. OPC Poisoning Situation in Bangladesh and other Countries

A study performed at Rajshahi Medical College [71] from January 1991 to December 1994 showed that among 405 cases of poisoning, OPC poisoning was the commonest one (38.8%), followed by poisoning with sedative (29.1%). Out of those 405 cases; 310 were suicidal (76.54%) and 95 were homicidal (23.45%)Similar study performed in Urban area like Dhaka Medical College showed that for suicidal purpose, sedative poisoning is the commonest followed by OPC poisoning. [72] Study performed at Dhaka Medical College from January 2004 to December 2004 showed that out of

4378 admitted patients 40% were male an 60% were female. 44% patient came from urban area, while 56% from rural area. Incidence was high among students (35%), followed by house wives (30%). Most common reason for poisoning was suicide (93.3%) and sudden anger was the commonest drive (53.3%). However a study done in paediatric ward at Sir Salimullah Medical College showed that in case of children, all the cases of poisoning were accidental in nature. [73] Another study at Dhamrai Thana Health Complex performed from January 1993 to December 1997 showed that males (61.30%) were predominant than females (38.70%) in poisoning cases. Acute poisoning was observed more in married group (68.64%) than unmarried group (31.36%).Commonest poisoning agent was insecticides OPC. [74] Epidemiological work from Spain supports link between chronic OPC exposure and increased suicidal rate. [75] Chronic exposure to OPC also gives rise to a condition called COPIND- Chronic Organo Phosphate Induced Neuro-psychiatric Disorder. [76-78] Genetic differences also play important role in Chronic OPC poisoning cases. [79] Present scenario of OPC poisoning in Bangladesh is alarming. Farmers of this country use pesticides without knowing their harmful side effects. 15,376 M Ton of pesticides were sold in this country during 2001, which increased to 37,712 M Ton in 2007; a rise of 145.26%. According to Bangladesh Crop Protection Association (BCPA), 61 member companies import, distribute and sell pesticides in this country. According to Plant protection wing of Department of Agriculture, the number is 153. Organo phosphate, ogano carbamate and synthetic pyrithroid are used as most popular pesticides in Bangladesh. After application in the field, there is a residual period for this insecticide, during which crops should not be consumed. This period varies from 4- 5 days for synthetic pyrithroids, 13- 35 days for organo carbamate and 11- 27 days for organophosphates. But farmers harvest and sell vegetables from the field just after next day of spraying insecticides. This causes serious harmful effects to human body giving rise to complications like cancer, mental disturbance, infertility, GIT disturbance, liver and kidney failure etc. In western countries every vegetable is checked for maximum residual limit (MRL) of insecticides, but it is not followed in Bangladesh. Due to indiscriminate use of insecticides, useful insects, birds, animals and fishes are killed. Even good microorganisms from upper 6-8 inches of soil are destroyed, making land infertile and prone to various diseases for crops. Any person from age 15 to 55 years can obtain pesticide sales permission by paying only taka 75 as government fees. No educational back ground is required for this. However the Government has amended the pesticide rule in mid-2007 allowing bio technological pest control process, which includes control by sex hormone feromone. It has

been seen by using chemical pesticides in one hector of land at a cost of taka 65,000/-, only 50% good brinjal are harvested, whereas by using environment friendly sex hormone feromone trap in the same land at a cost of 12,000 taka, 90 % healthy brinjal can be harvested. Recently 4 ecofriendly steps have also been taken to solve insecticides related problems, preservation of useful insects and animals, cultivation of insect resistant high breed variety crops, modern cultivation method by using good seeds, fertilizer, irrigation etc and control of insects by using integrated pest management, light trap, hand net, etc. [80] Study from Chandigarh Medical College, India (1970-1979) showed that out of 312 cases of poisoning 30.12 were barbiturate poisoning, 19.23% organo chemicals and 17.95% metallic irritants and corrosive. [81] During 1980- 1989, another 555 cases of poisoning were reported from the same region and 31.35% fatalities were attributed to aluminium-phosphide, 27.03% to organophosphates and carbamates, 8.83% to barbiturates and 9.36% to metallic irritants and corrosives. [82] A total of 1035 cases of acute poisoning were studied during 1983 to 1996 at All India Institute of Medical Sciences, New Delhi and the trends showed the increasing use of agro-chemicals. [83] Yet another study from Rohtak, India in 1993-1994 analyzed 559 cases of poisoning [84] and Aluminium Phosphide was found to be the most common poison. The studied scenario was not different from these reports and agrochemicals continue to be the most common agents responsible for suicidal and/or accidental poisoning. [85] According to National Crime Records Bureau India, every 5 minutes a person commits suicide and 7 attempts to kill themselves, forming about 1,00,000 death per year. [86] Suicide rate was highest in the state of Kerala. [87] Majority of the victims belonged to the group 14- 34 years [88] and OPC was the most common agent used for suicide purpose. [89] In Sri Lanka, many thousands of hospitals admissions each year are for agrochemical poisoning, (16,649 in 1983) with over a thousand death annually (1521 in 1983). Of these, about three quarter are self-administered, the remainder being occupational and accidental. [90-92] In Sri Lanka, another study showed, incidence of suicide due to poisoning was more than 80 %, followed by hanging, which constituted 10.7%. [93] However changing use from the most toxic pesticides to less toxic pesticides has had a remarkable effect in Sri Lanka and the suicide rate has fallen by 50% over ten years since such legislation was passed. [94-96] In USA, during 1980, out of total fatalities 49.7 % were suicides and 39.5% were accidental. The overall suicide rate changed little between 1970 and 1980 as the rate among young persons increased and women preferred firearms than poisoning to commit suicide.

# Aim of the project

Organophosphorus compounds have tremendous importance in the field of food technology, animal foodstuffs, pesticides, medicinal compounds, synthetic polymers, fire retardants and natural products. These compounds can be used as flame retardants for fabrics and plastics, plasticizing and stabilizing agents in the plastic industries, additives in the petroleum products and corrosion inhibitors. The intimate involvement of organophosphate in living process is now well recognized and modern biochemistry is dominated by it such as ATP and DNA. Nucleophilic substitutions at the carbon centre are very important topic in organic chemistry. In many aspects phosphorus rivals carbon in its structural versatility, the general variety of its compounds and its biochemical importance. The mechanism of nucleophilic substitutions at the carbon centre is very well known. Considerable amount of work have been carried out on nucleophilic substitutions at the carbon centre but much less is known about nucleophilic substitutions at the phosphorus centre. Nucleophilic substitutions at the phosphorus centre are very important topic in organophosphorus chemistry. The nucleophilic substitutions at the carbon centre are well established but the mechanism of nucleophilic substitution reactions at phosphorus is not well established.It has great interest to study nucleophilic substitutions reactions at phosphorus in solutions. The nucleophilic substitutions reactions at neutral phosphoryl species such as in phosphoryl chloride have been considered to proceed either stepwise through a pentacoordinate intermediate of trigonal bipyramidal shape or concertedly through a single transition state.

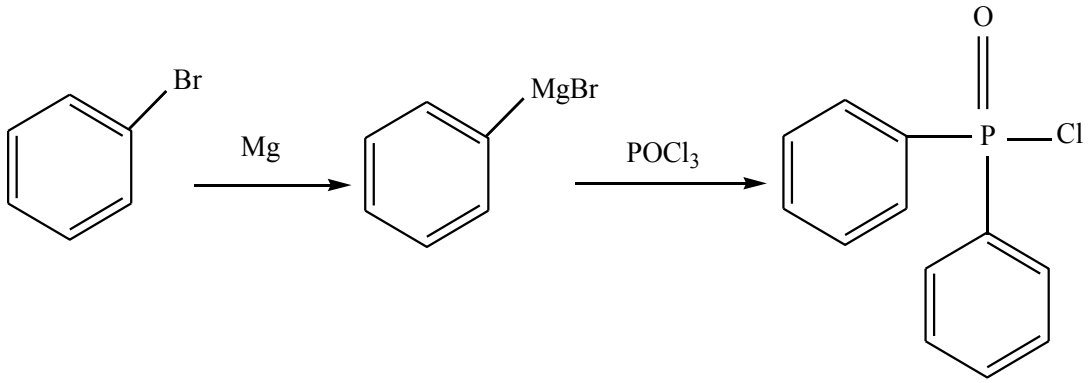
Therefore, the proposed research project is undertaken with the following objectives:

- To prepare the intermediate precursors to synthesis the oxy phosphorus compound.
- To synthesize the organophosphorus compounds.
- To optimize the reaction condition.
- Characterization of the synthesized product by physical and chemical methods also by spectroscopic analysis.
- Investigation of the biological activity of the derivative products.

Chapter-2

# EXPERIMENTAL

## 2.1 Synthesis of diphenyl chlorophosphine oxide

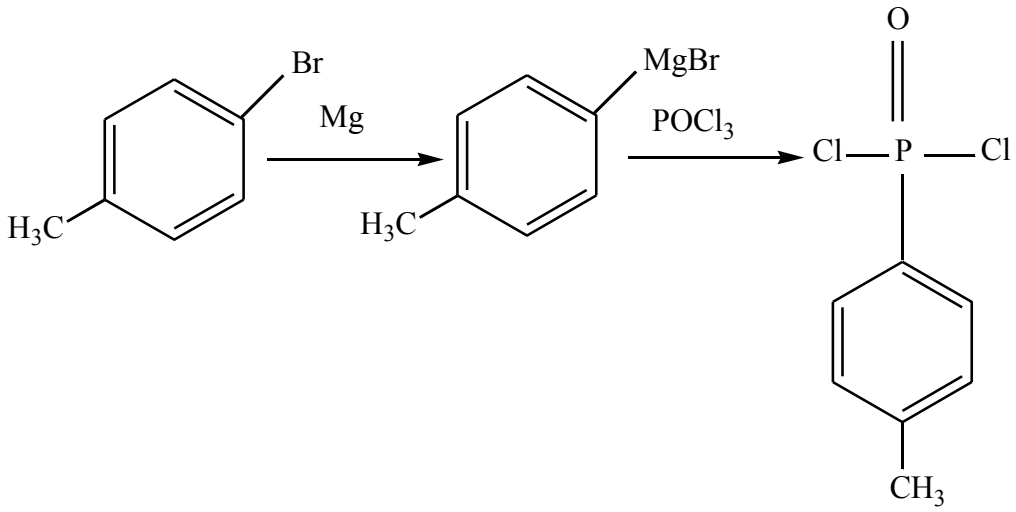



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

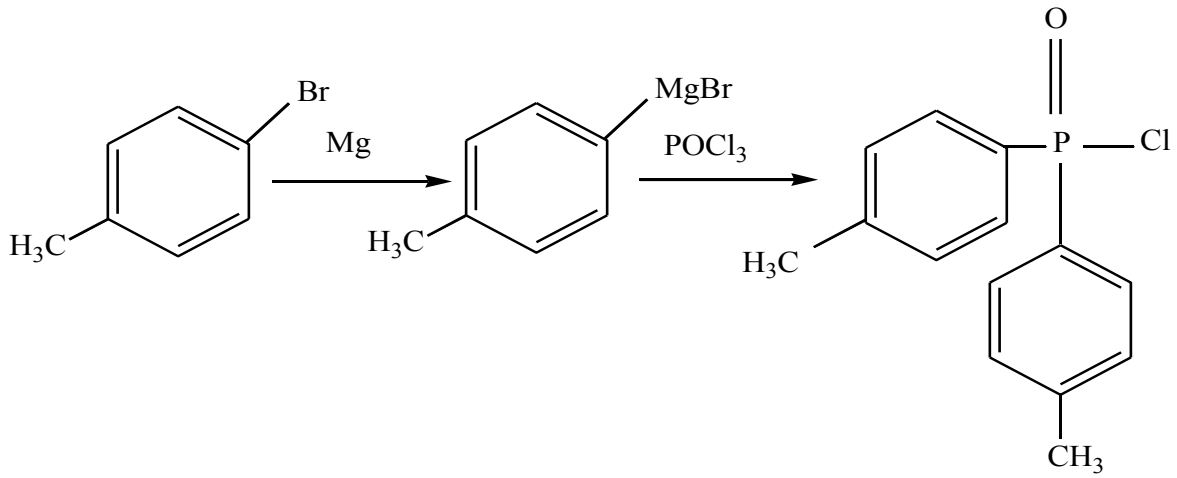



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

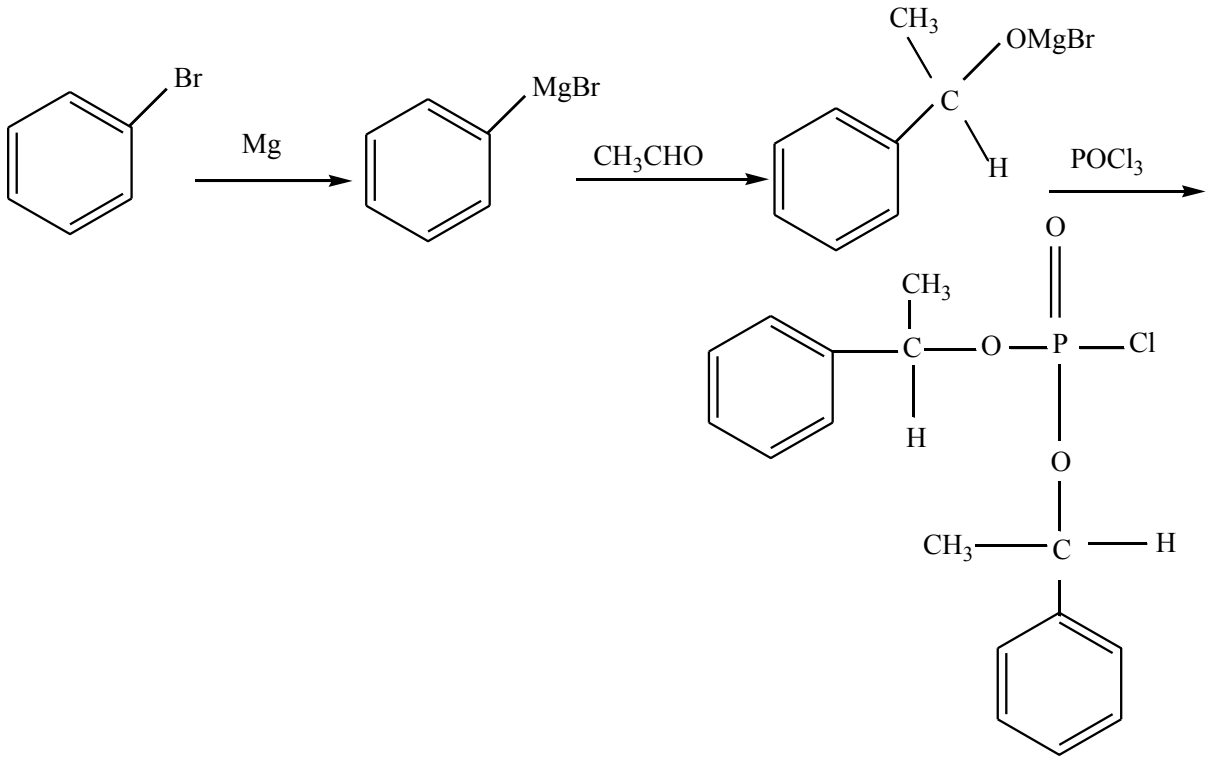



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

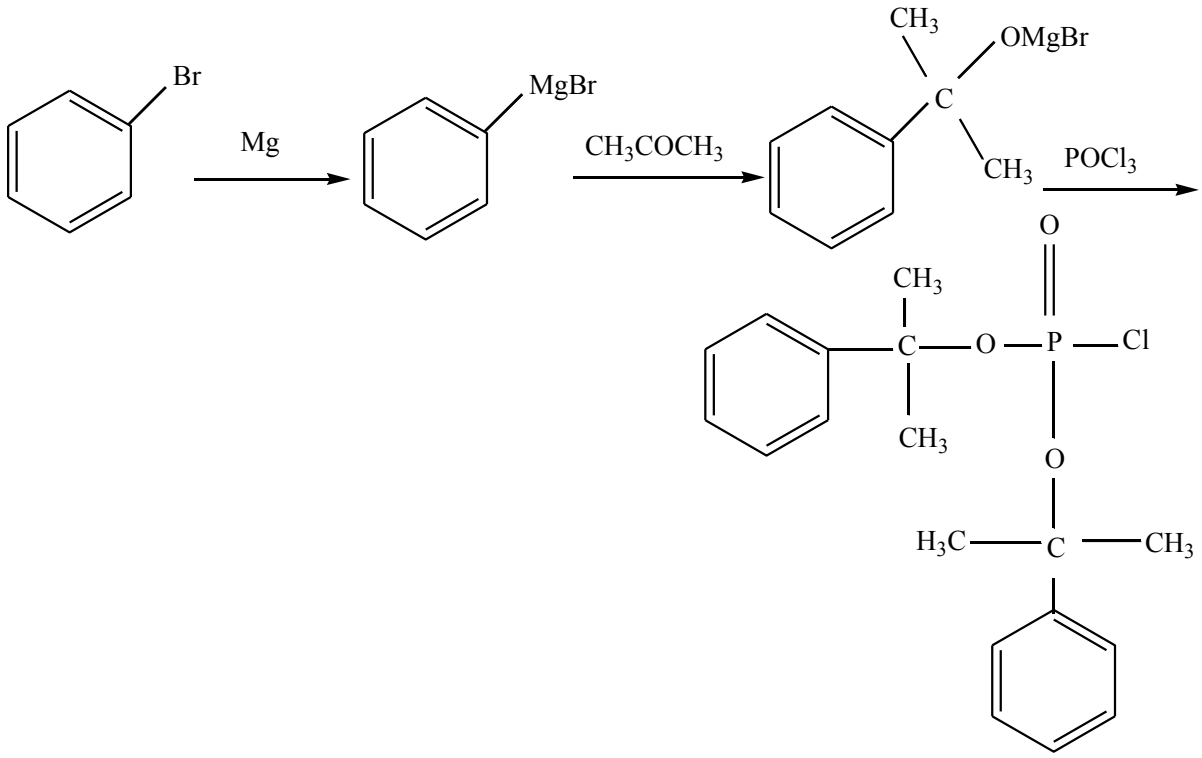



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

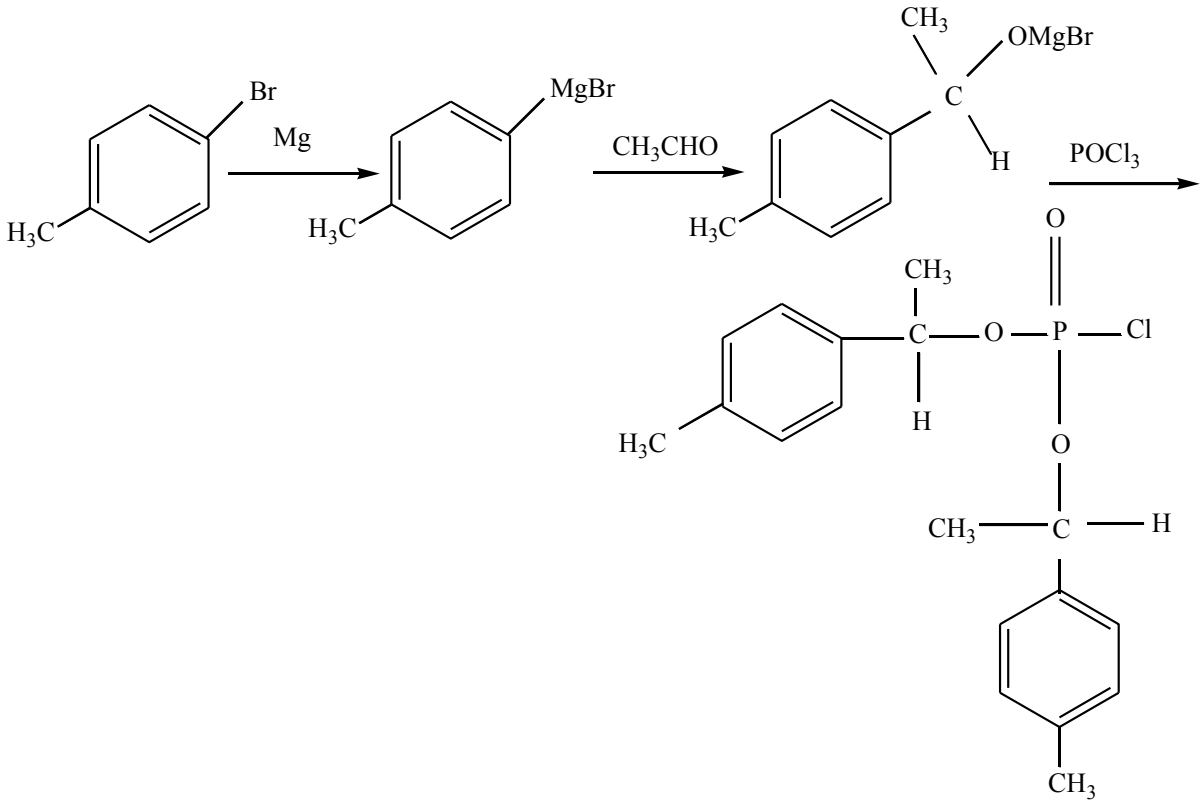



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^{1}$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

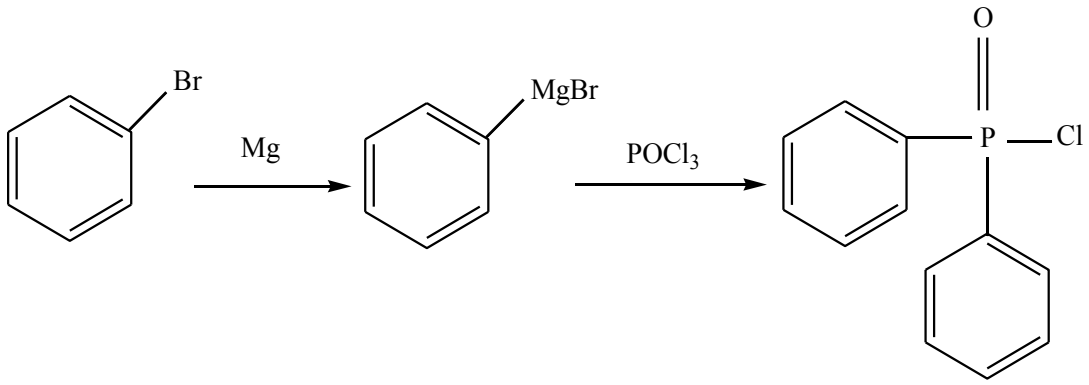



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_3$), 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

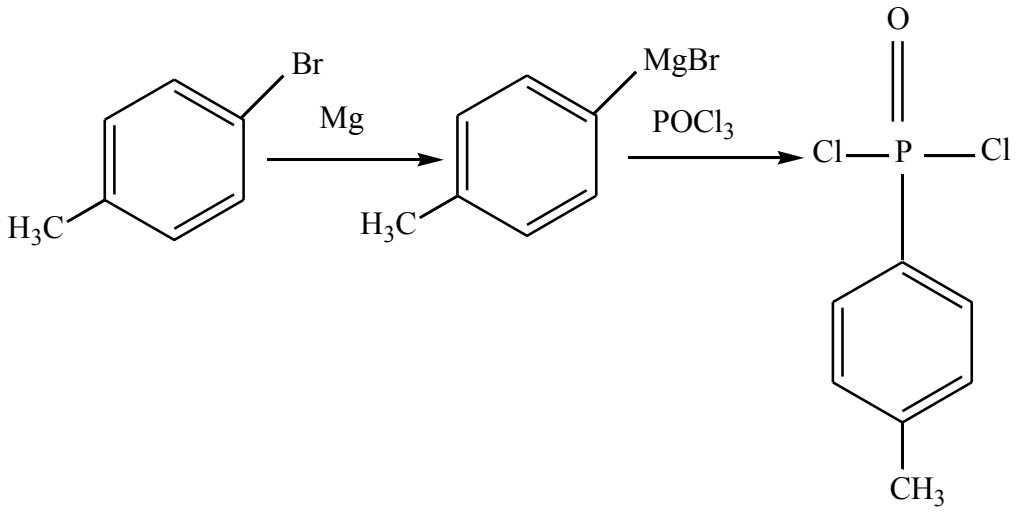



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

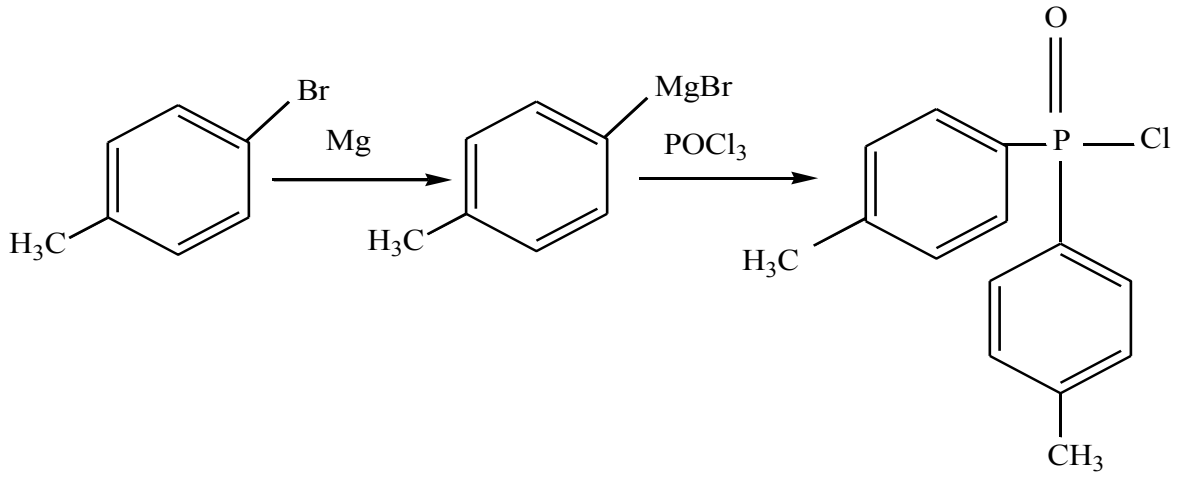



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

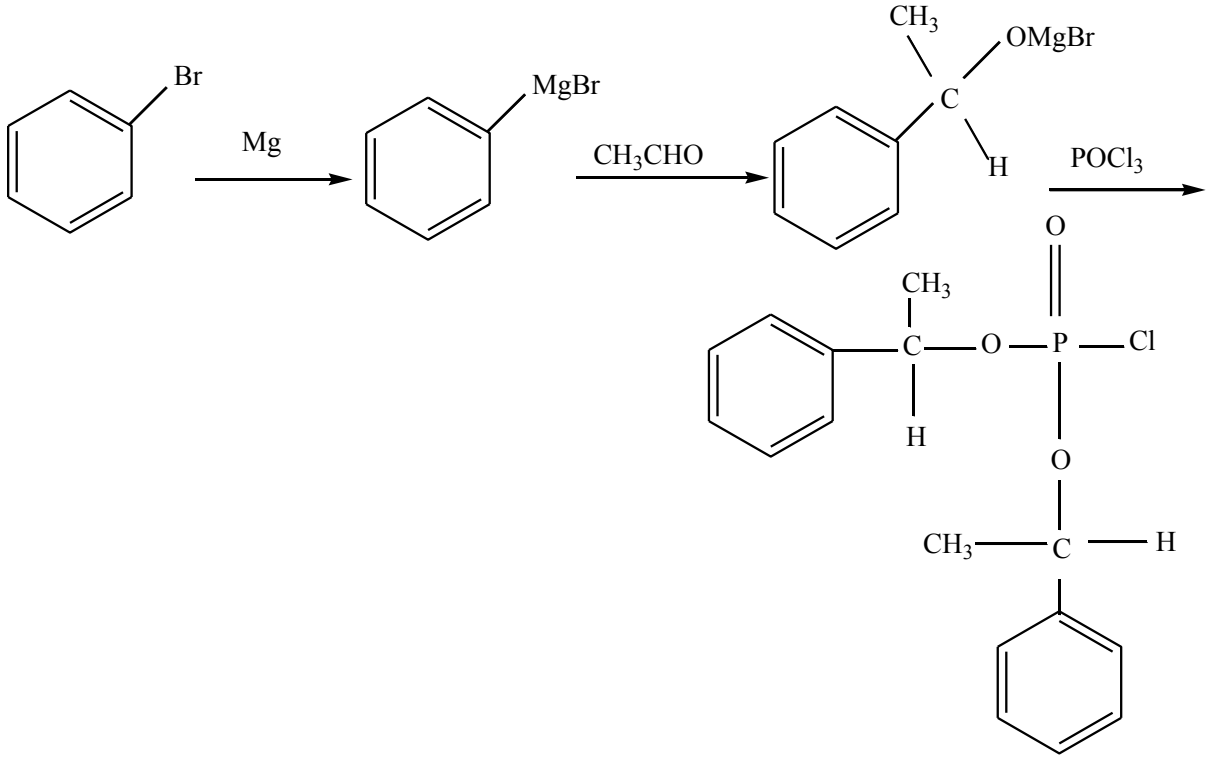



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

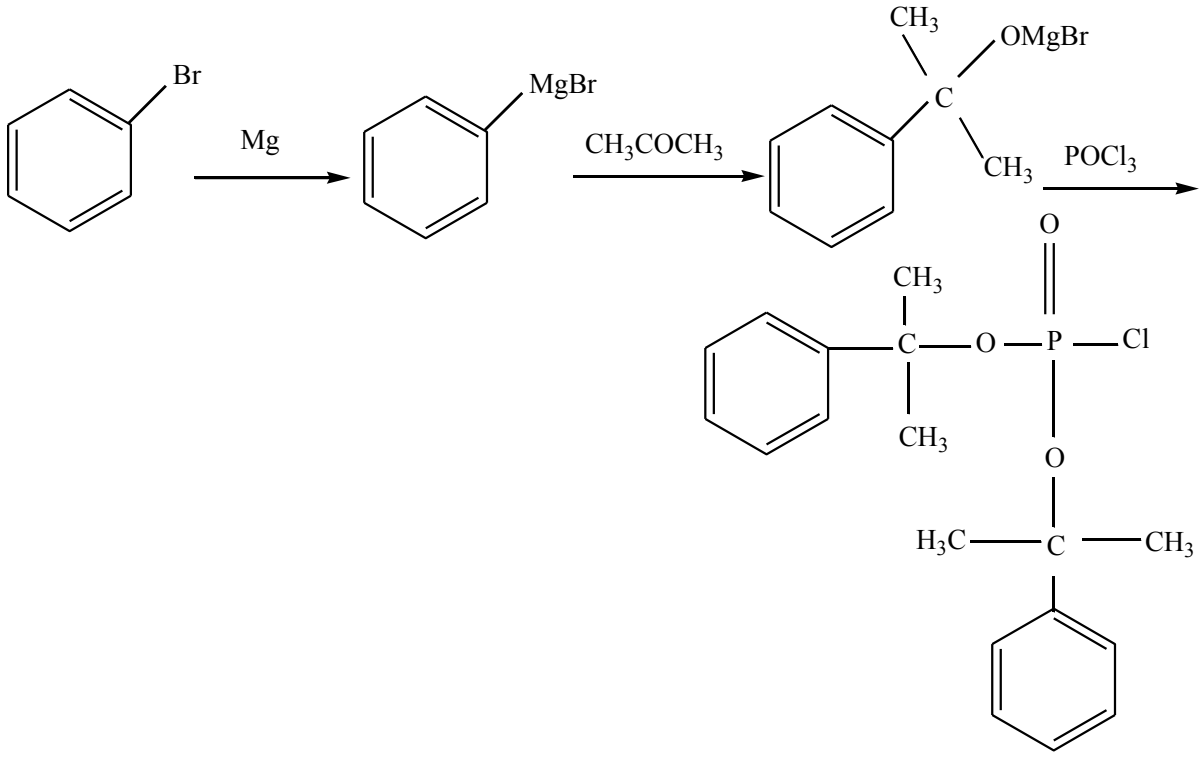



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

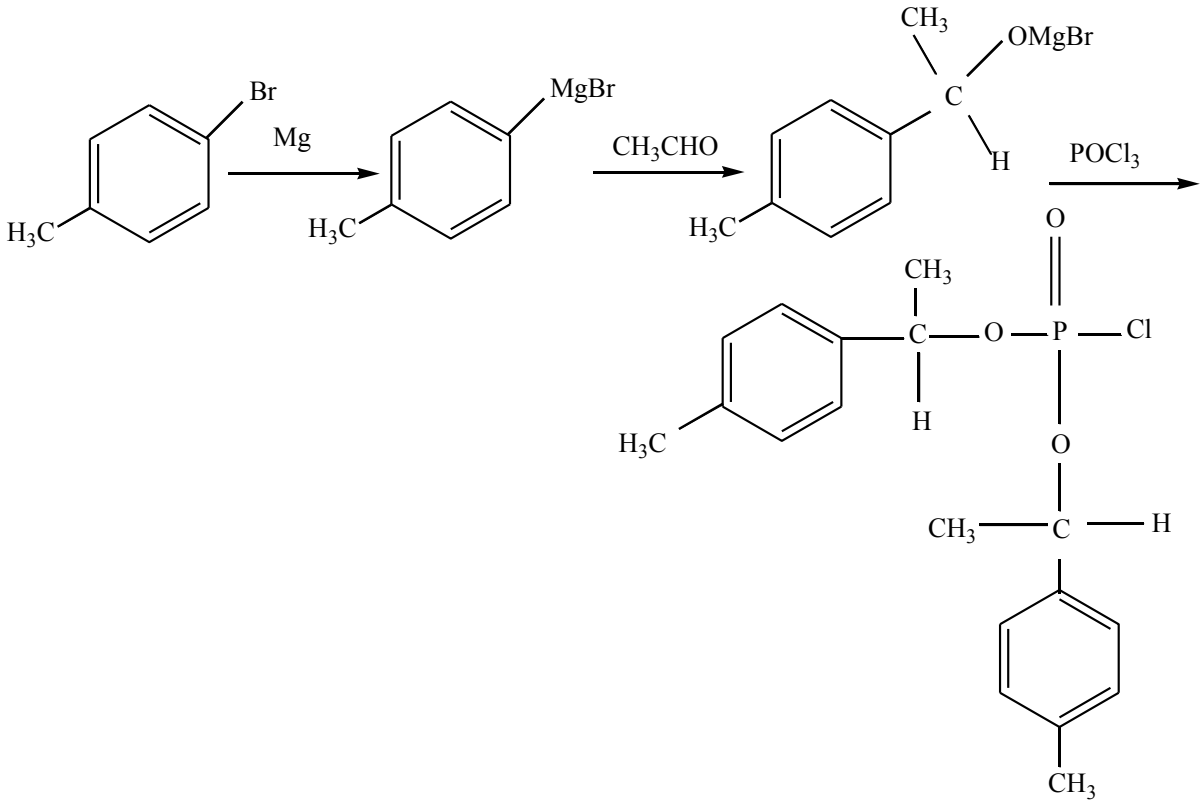



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

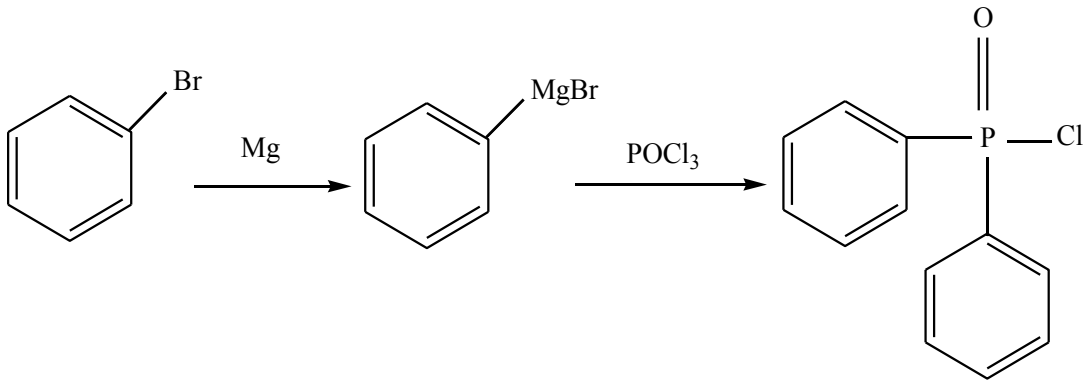



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_3$), 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

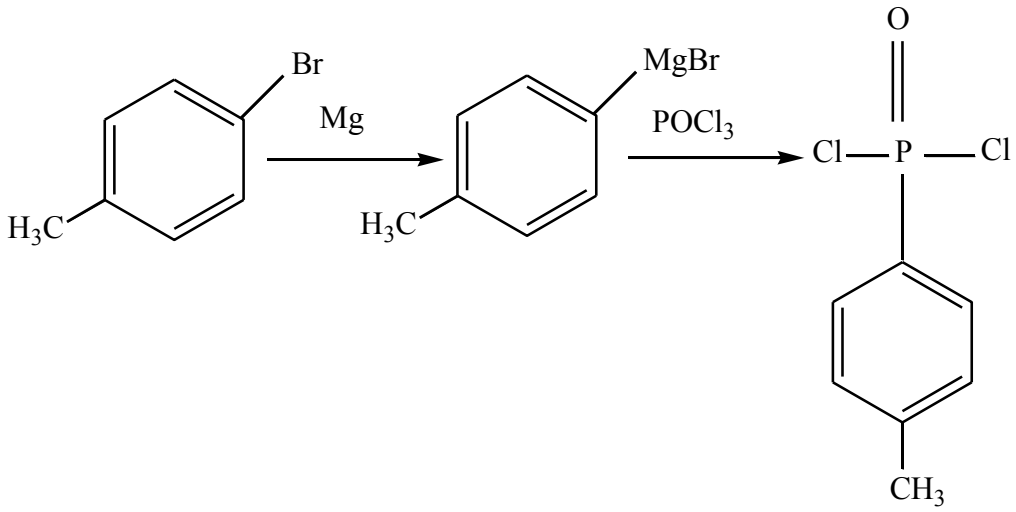



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

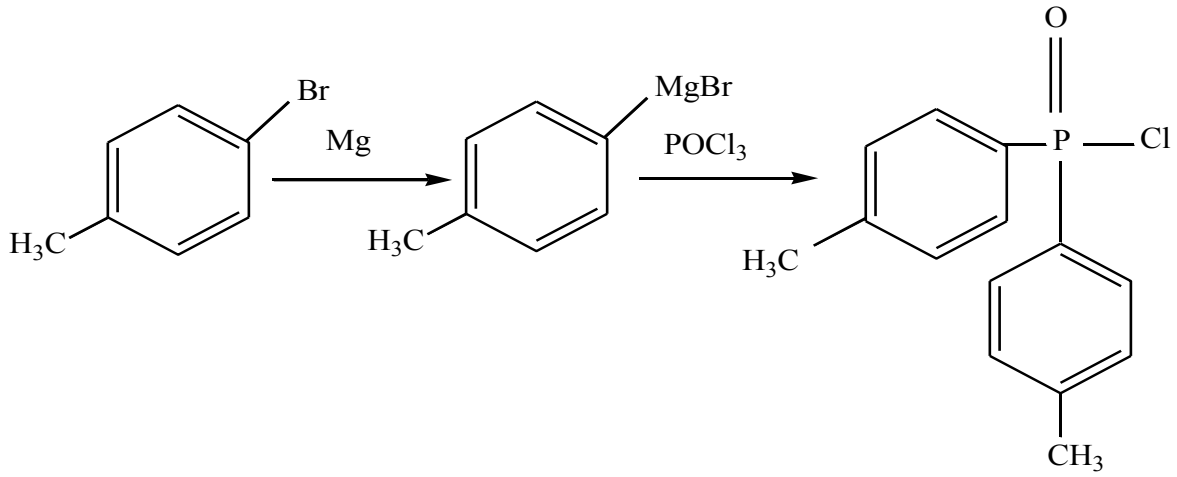



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

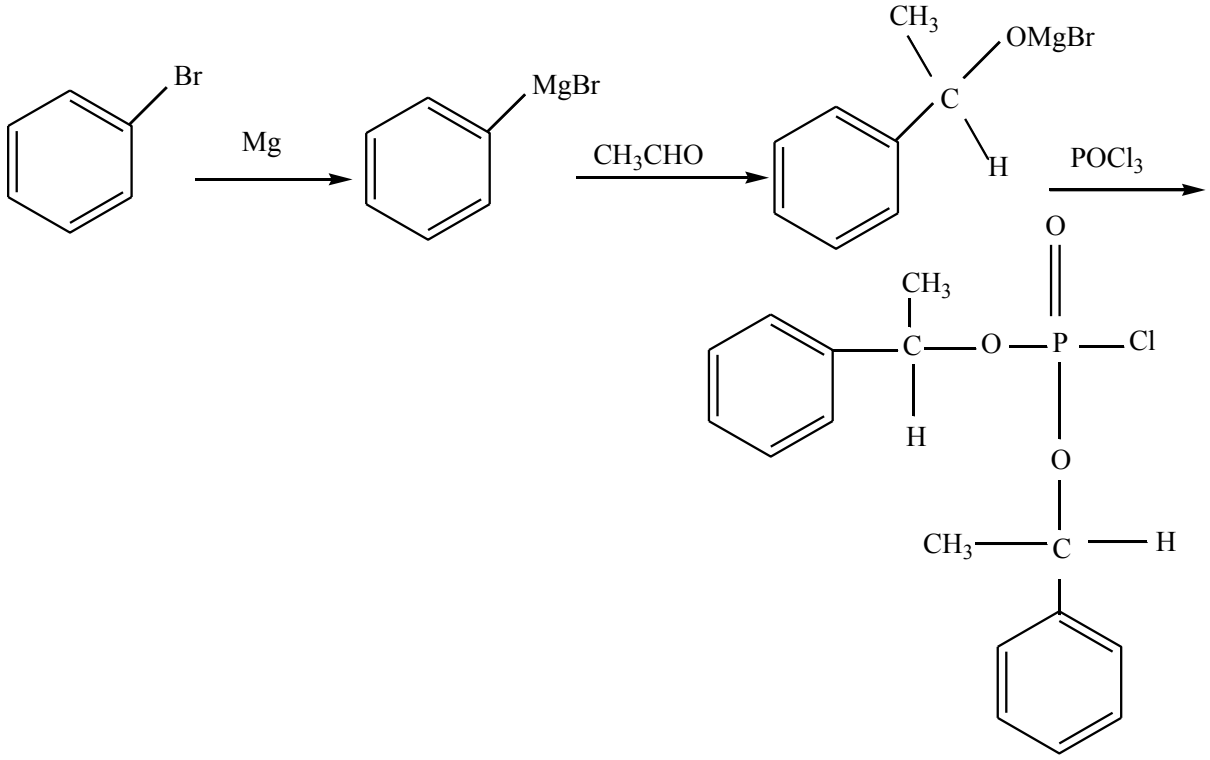



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

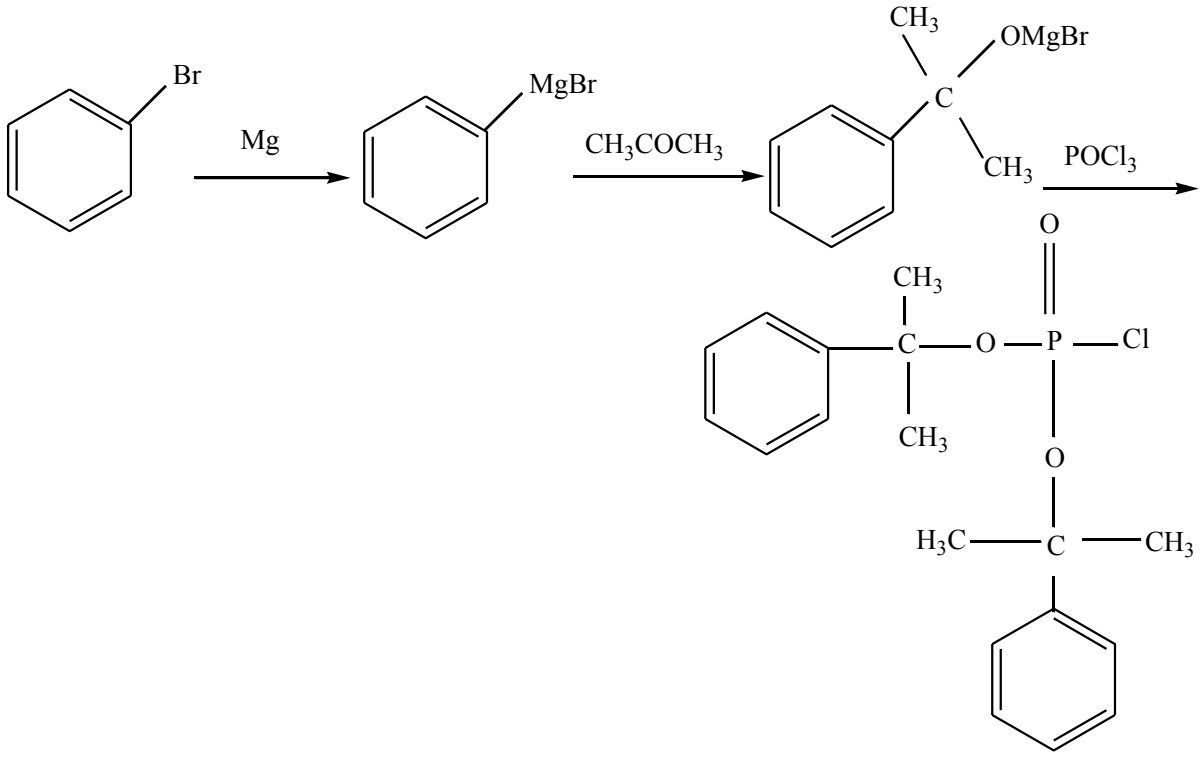



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

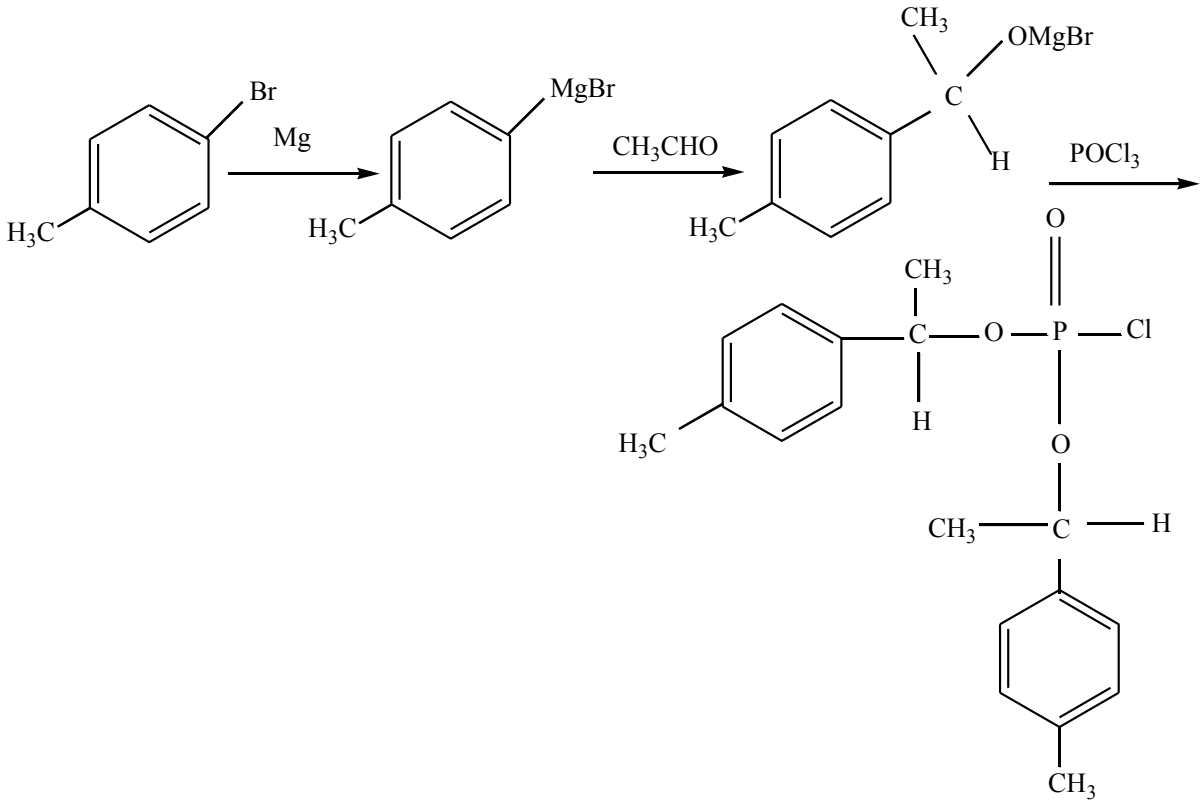



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

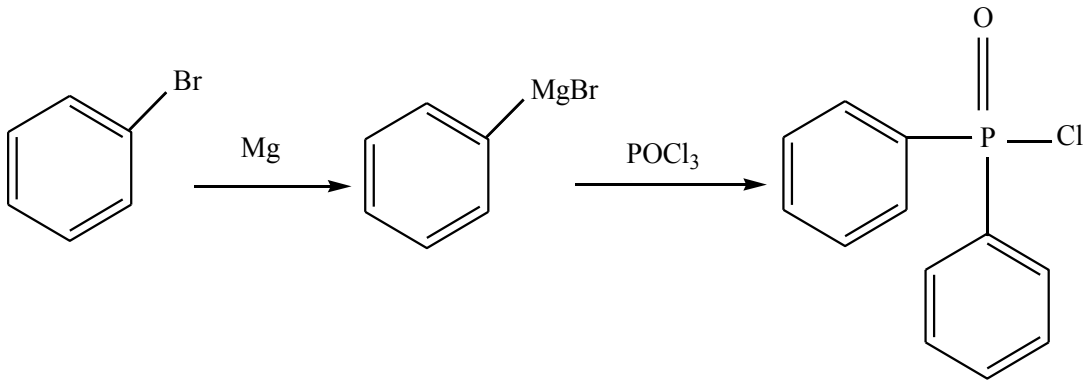



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_{3)}$, 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

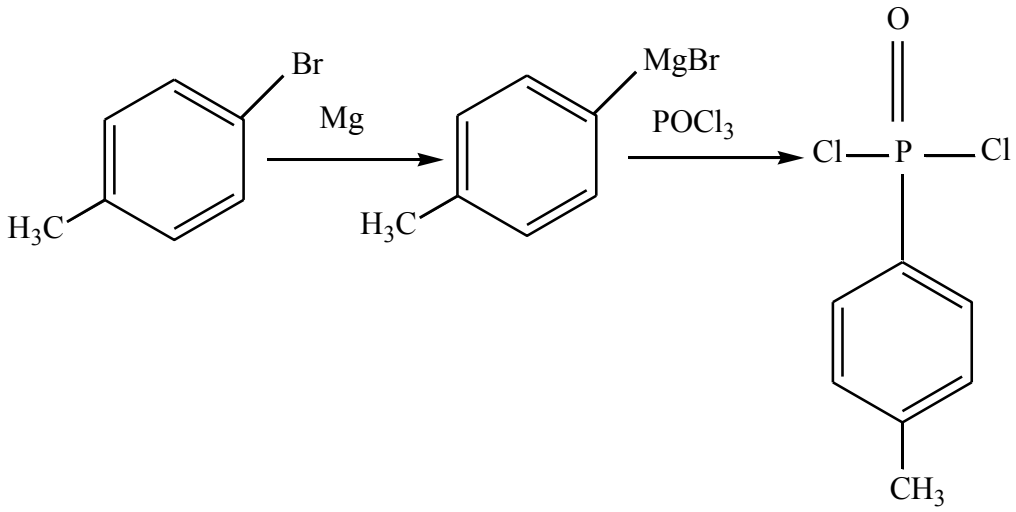



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

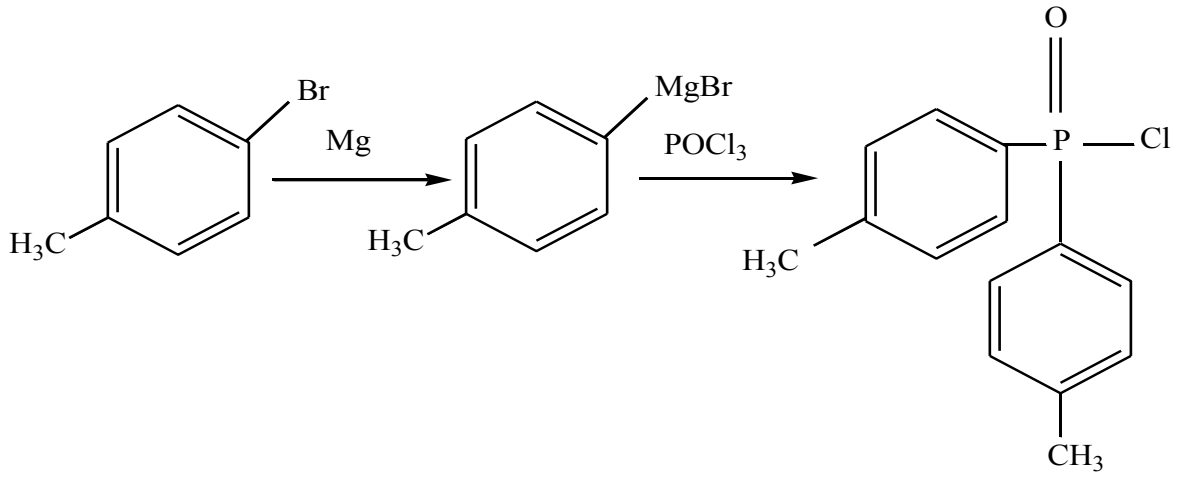



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

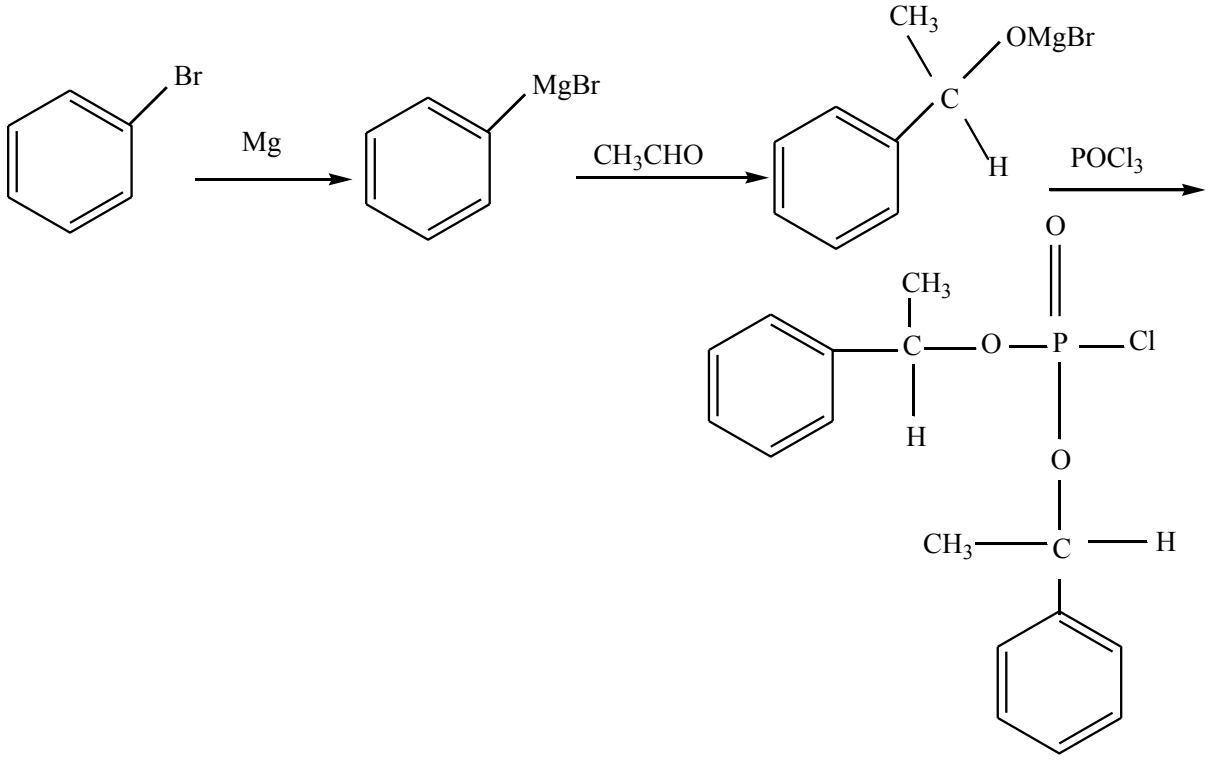



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

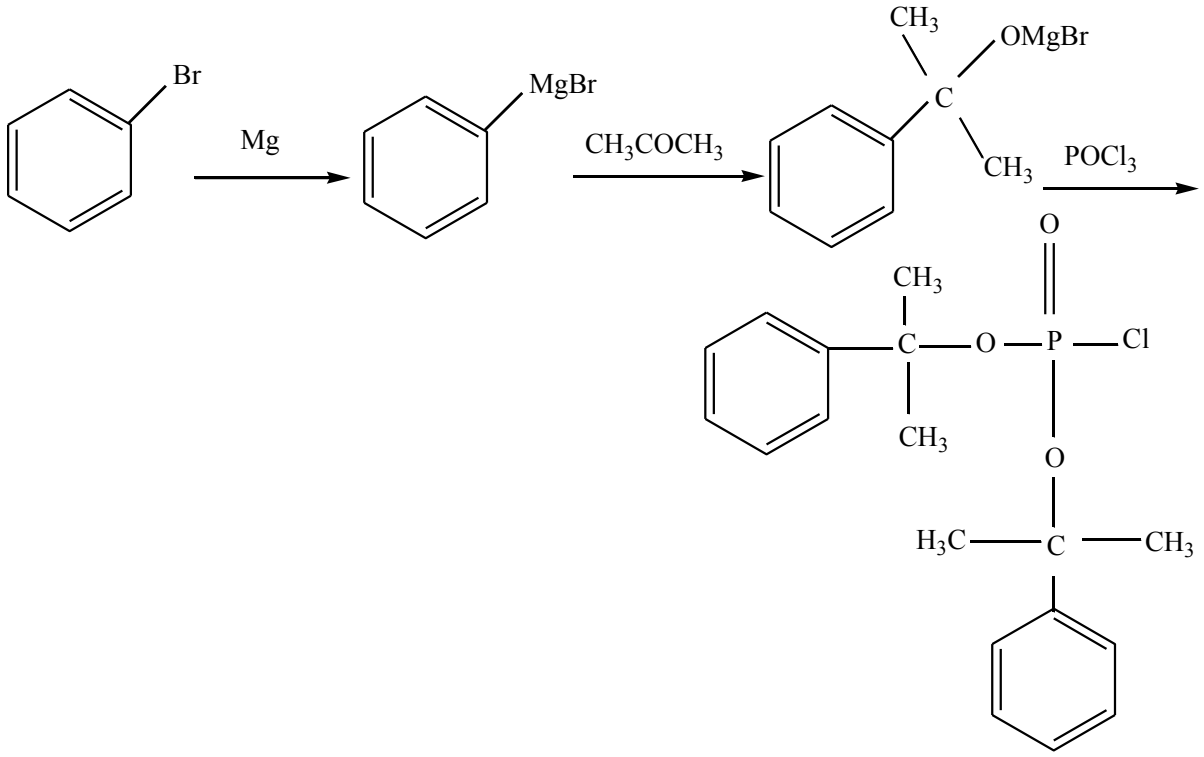



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

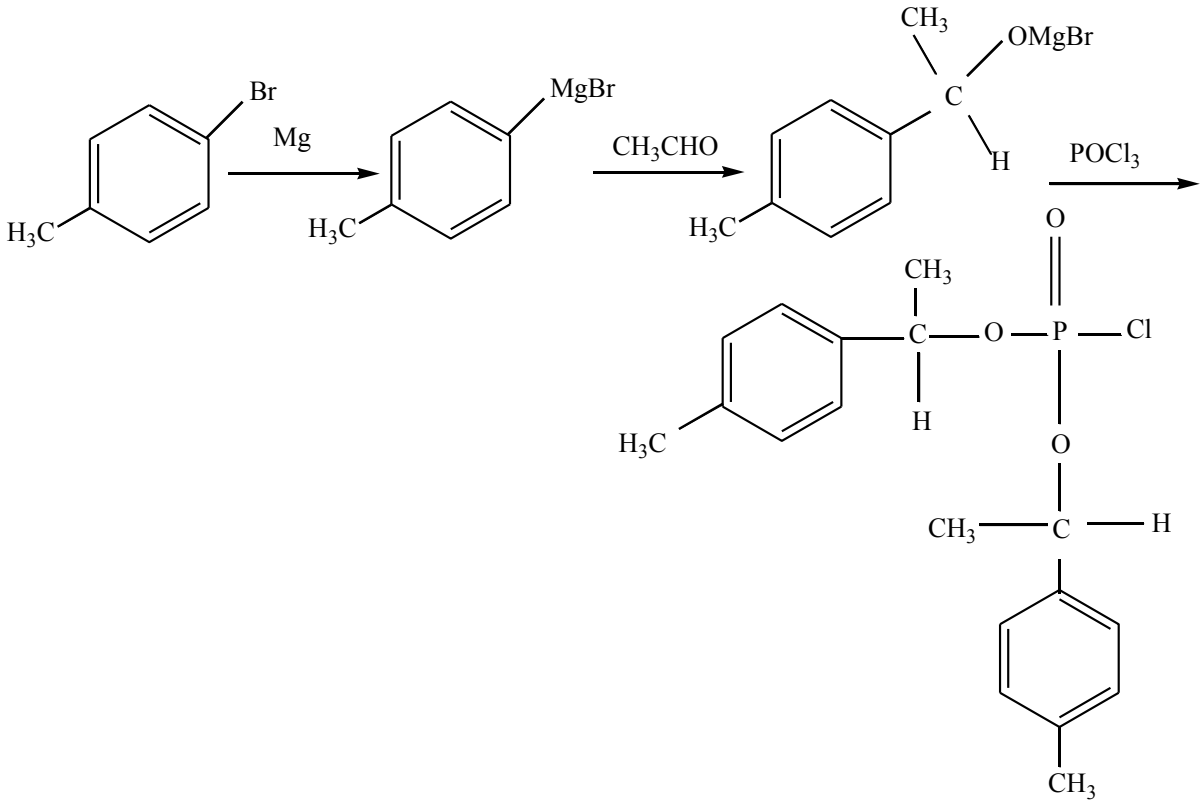



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

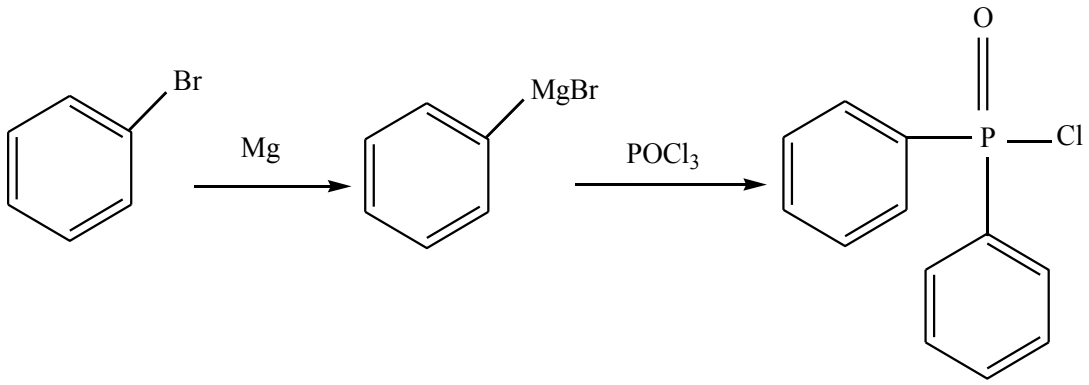



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_3$), 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

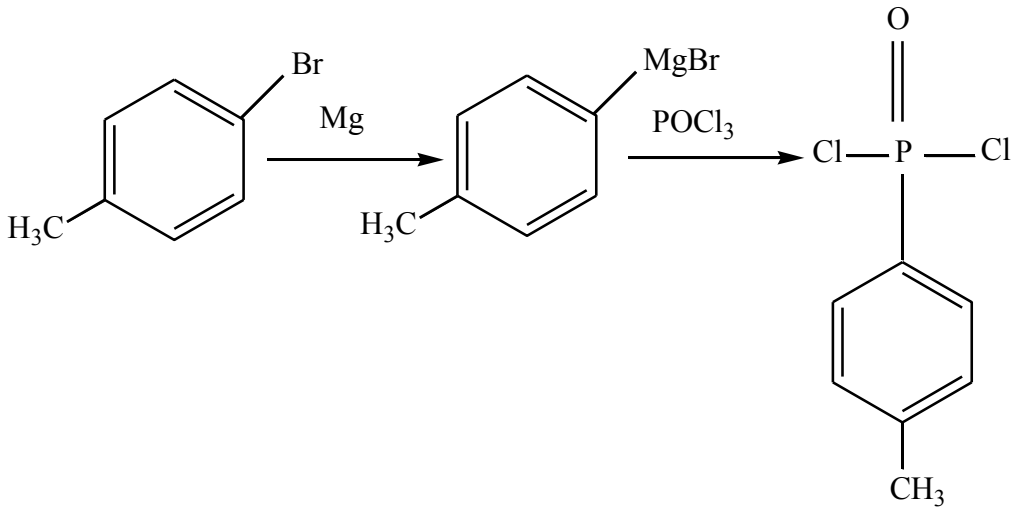



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

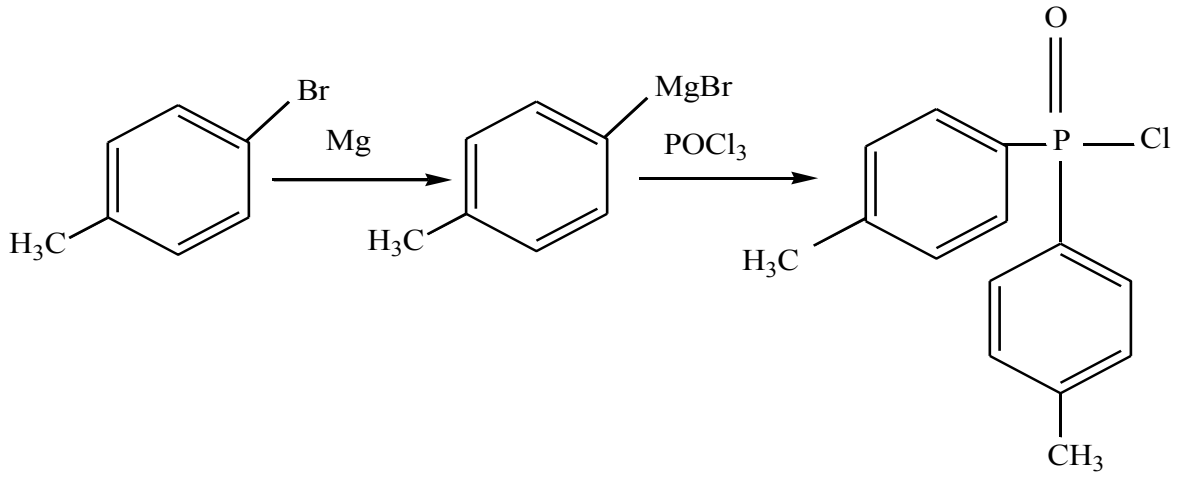



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

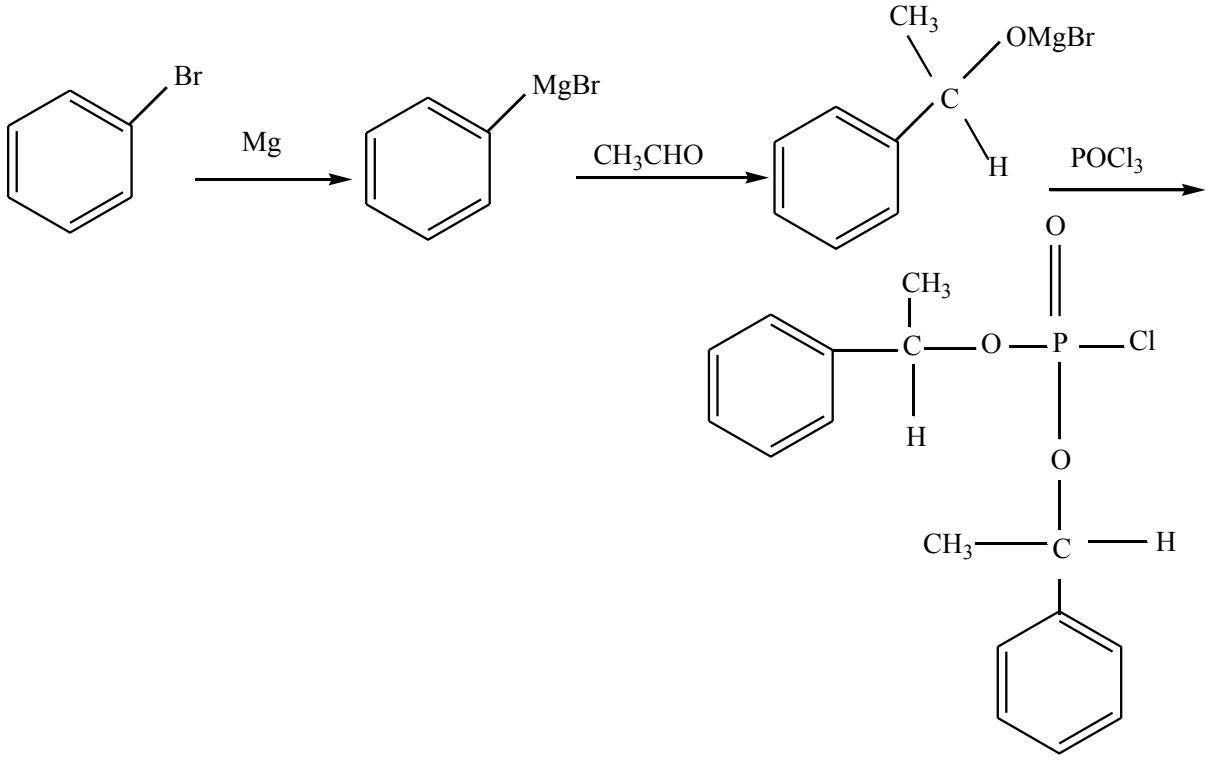



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

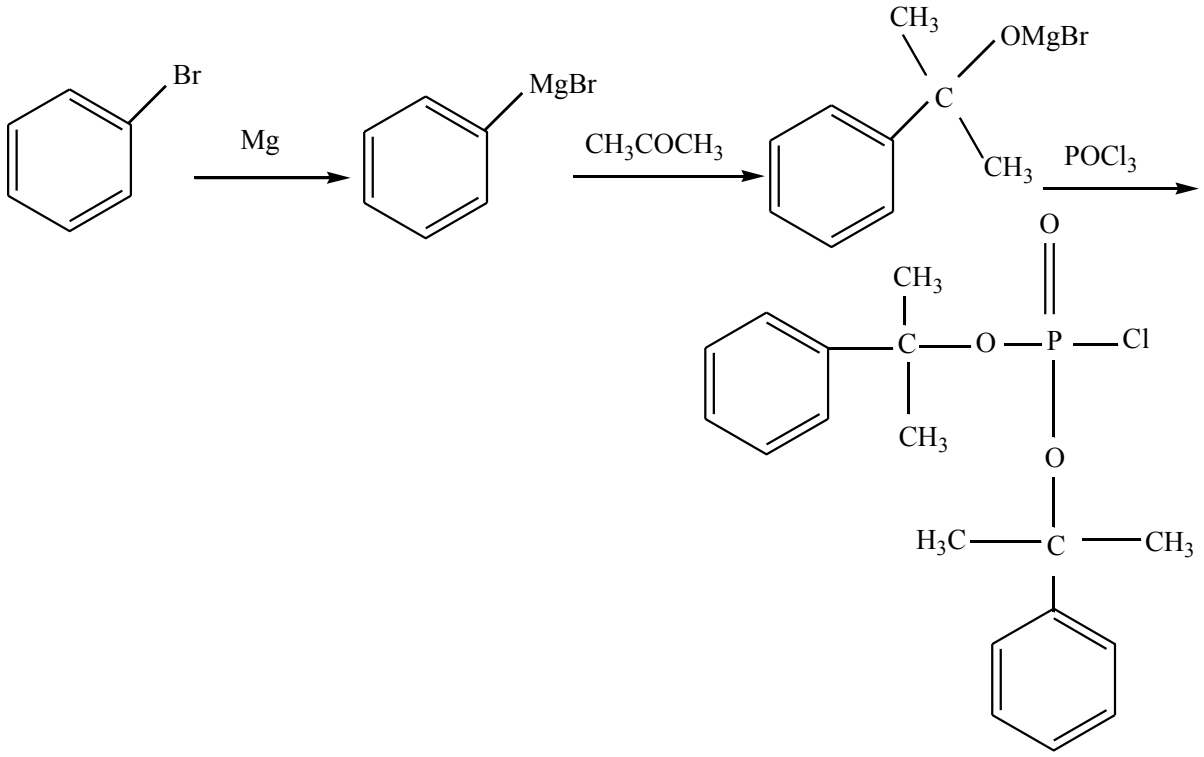



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

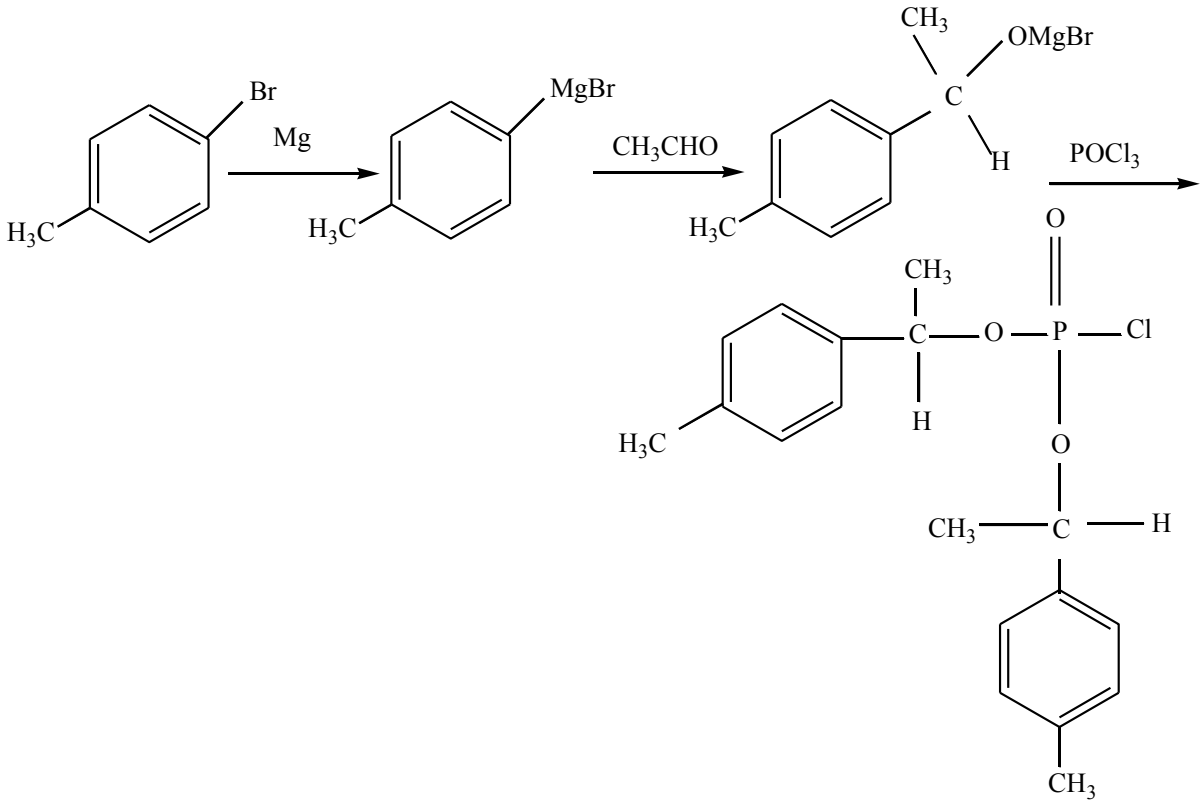



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

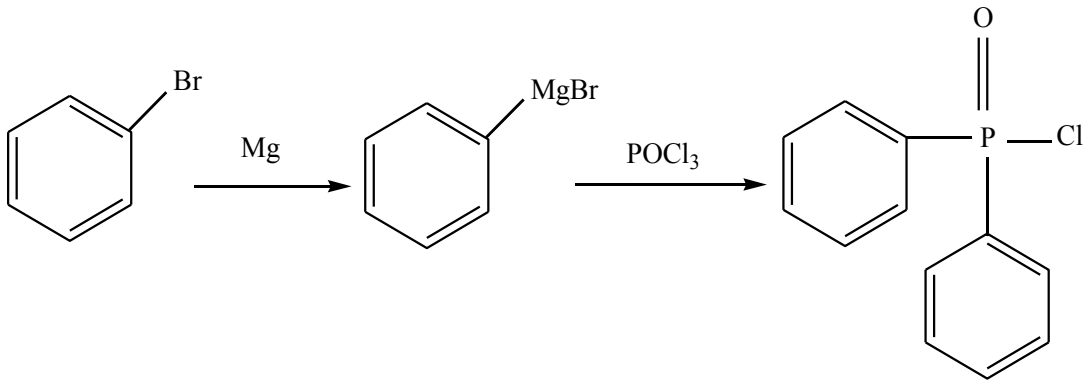



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_{3)}$, 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

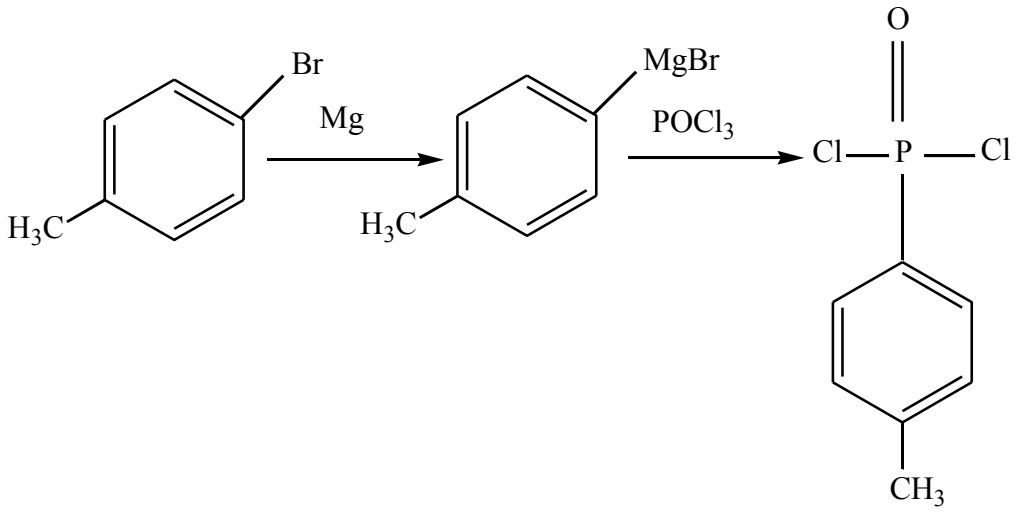



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

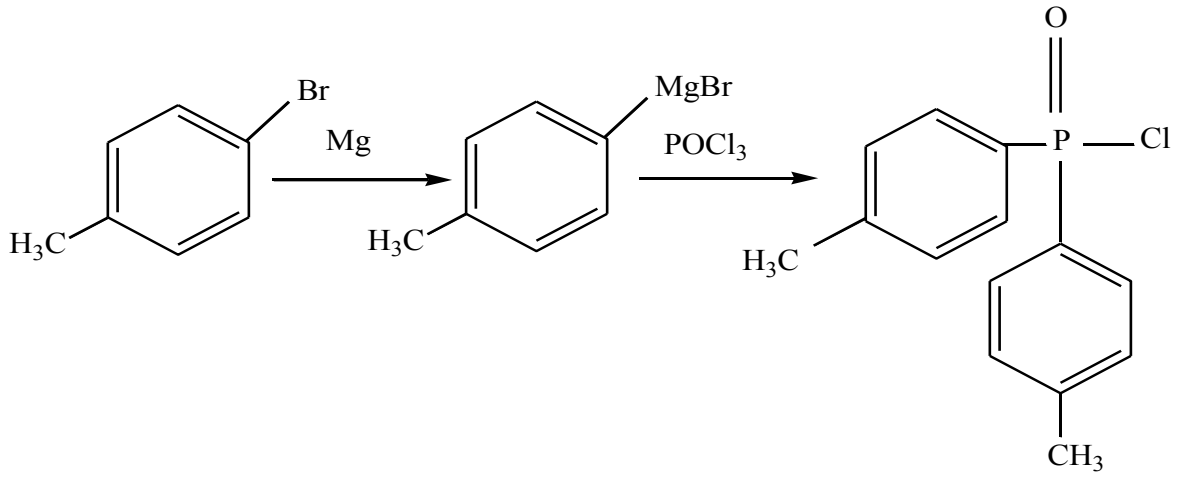



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

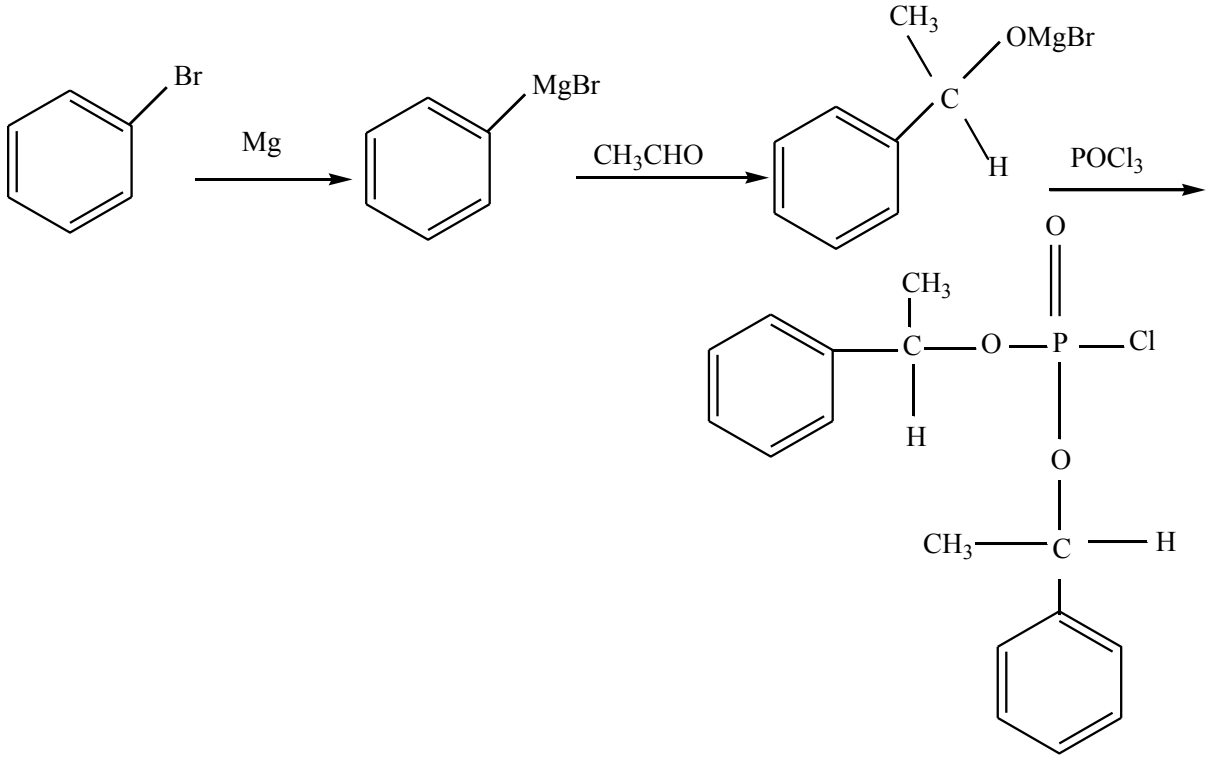



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

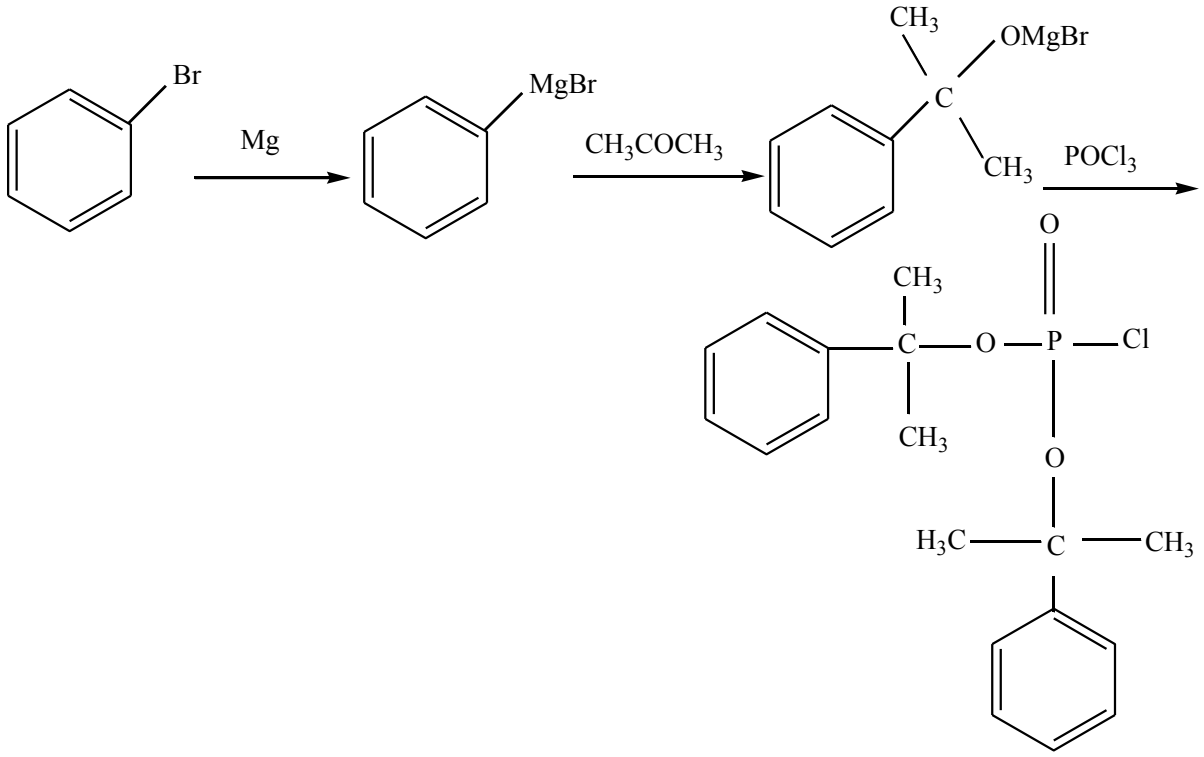



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

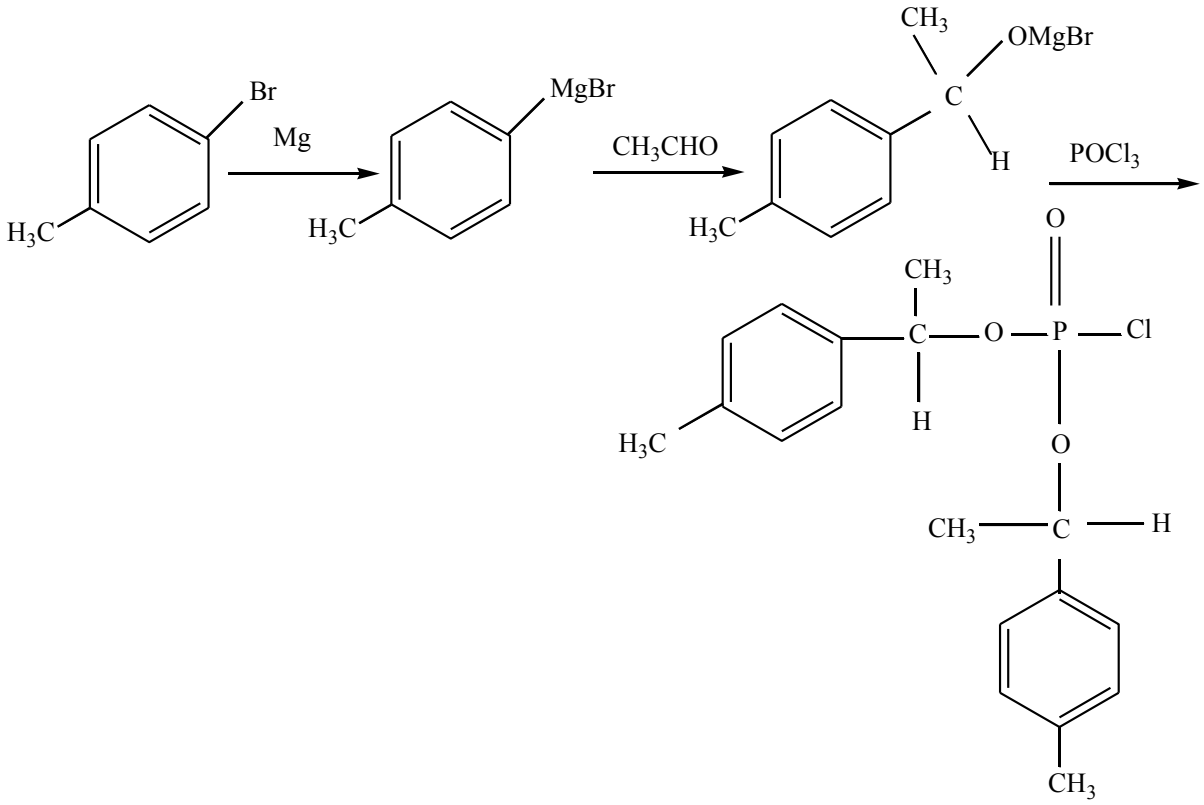



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

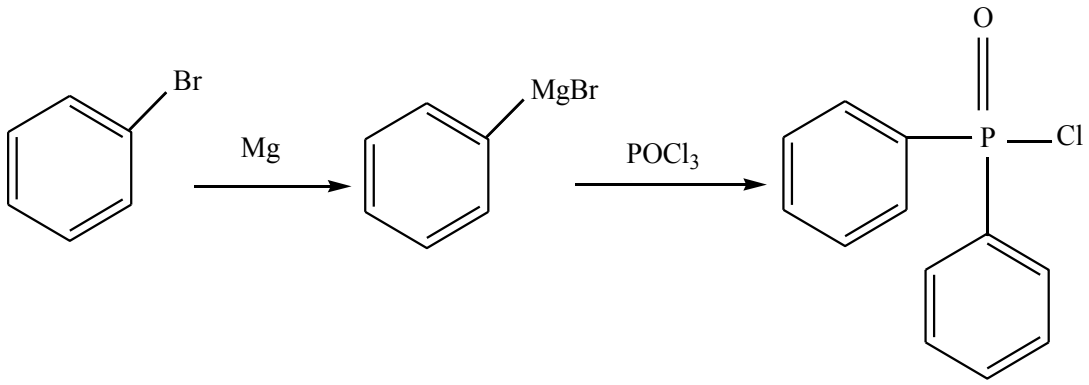



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_3$), 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

## 2.1 Synthesis of diphenyl chlorophosphine oxide

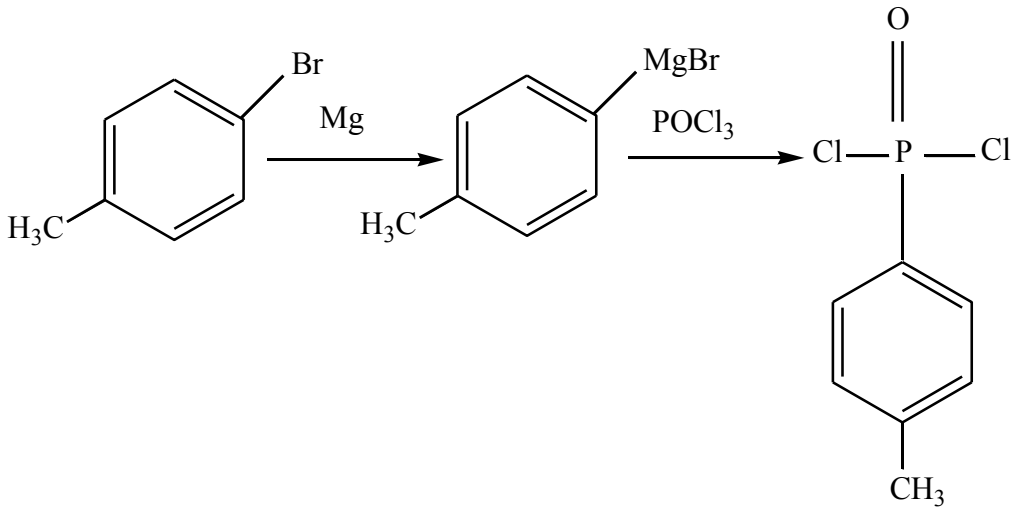



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (60%) and the melting point was recorded as $120^0$C. The product was found to be homogenous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

**IR (KBr)**: $\nu_{max}$; 3050 (C-H, Aromatic), 2950 (C-H, Aliphatic), 1691(P=O), 1456.2, 1421.4, 1315.4 (C= C, Aromatic), 1128.3 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 7.466-7.502 (br, S, 4H, C-H, Aromatic), 7.603-7.639 (br, S, 2H, C-H, Aromatic), 8.123-8.1433(br, S, 4H, C-H, Aromatic).

## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

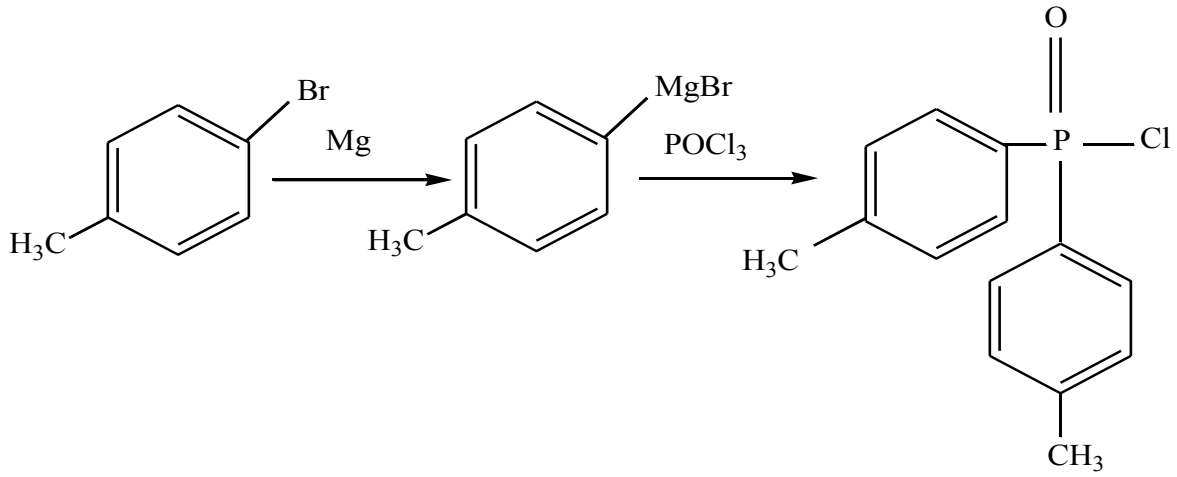



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

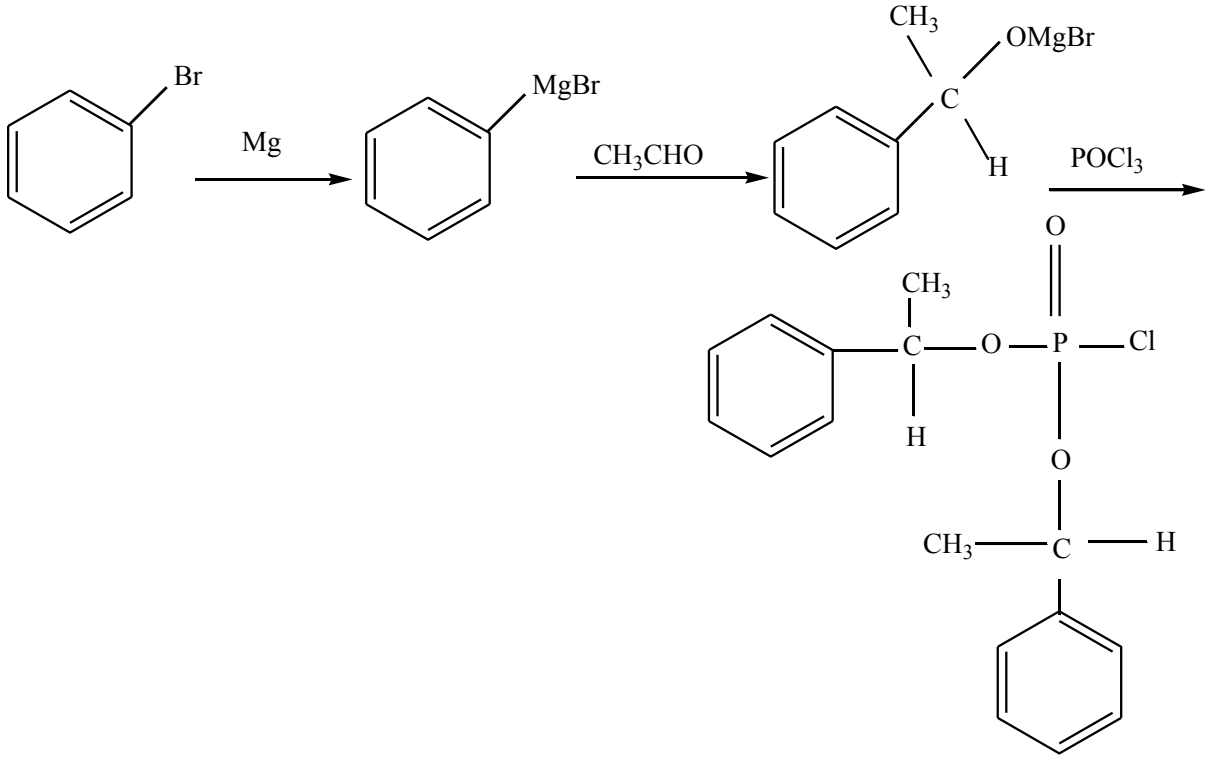



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

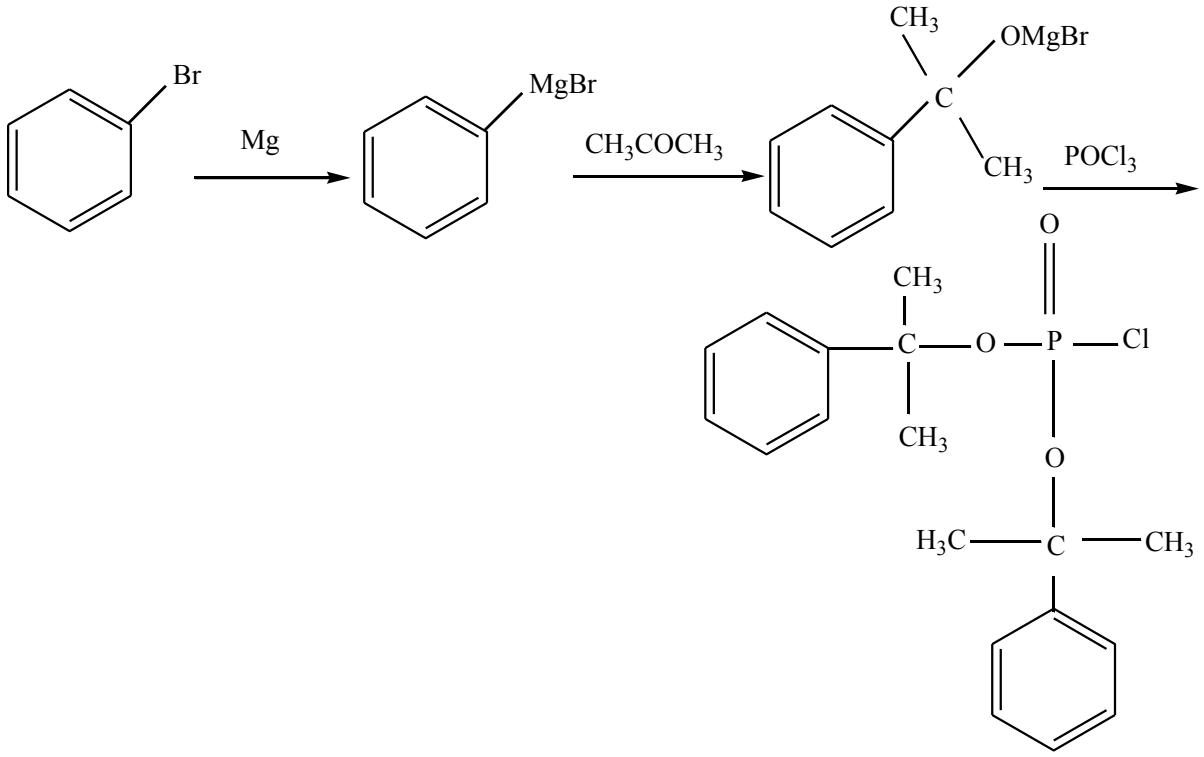



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

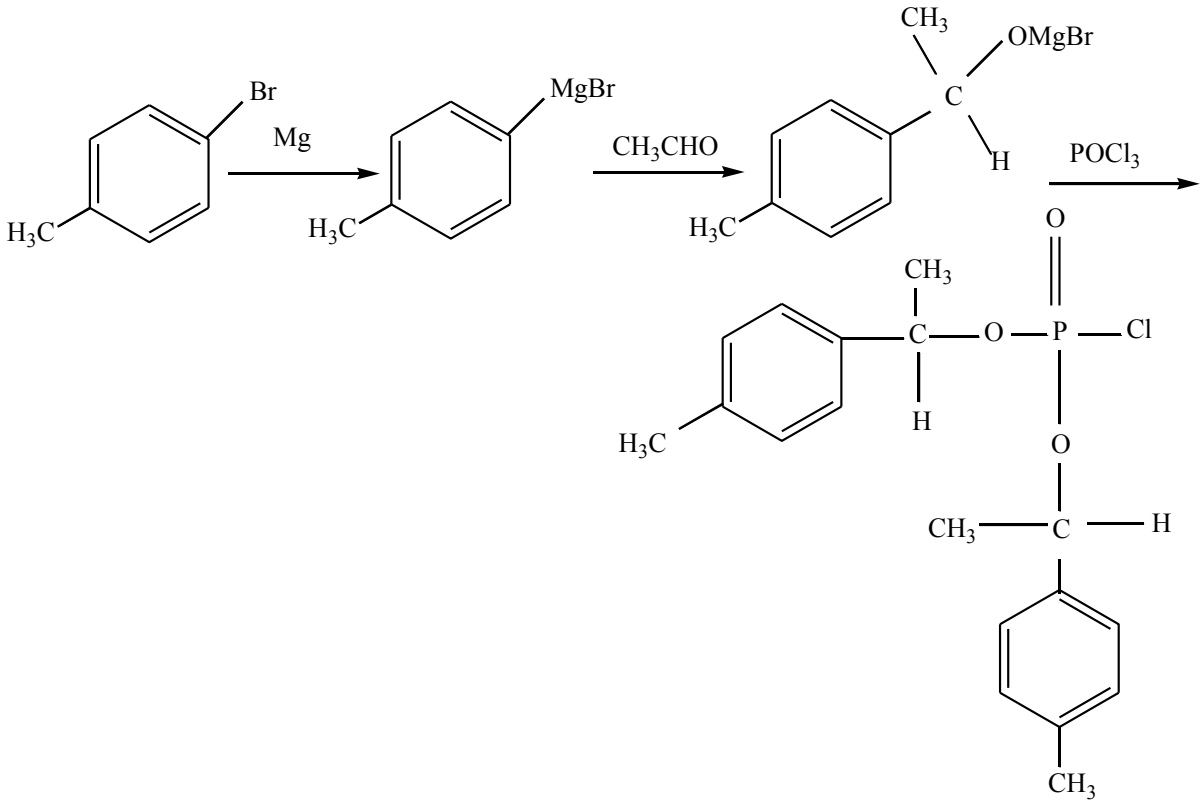



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

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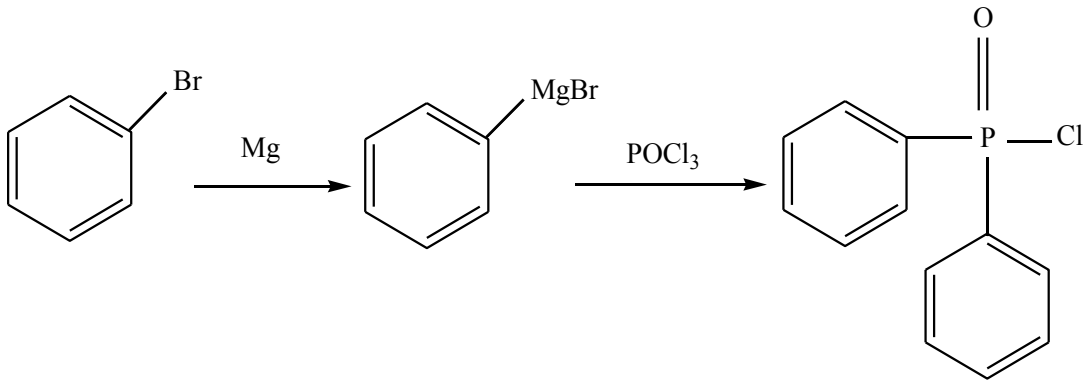
## 2.1 Synthesis of diphenyl chlorophosphine oxide

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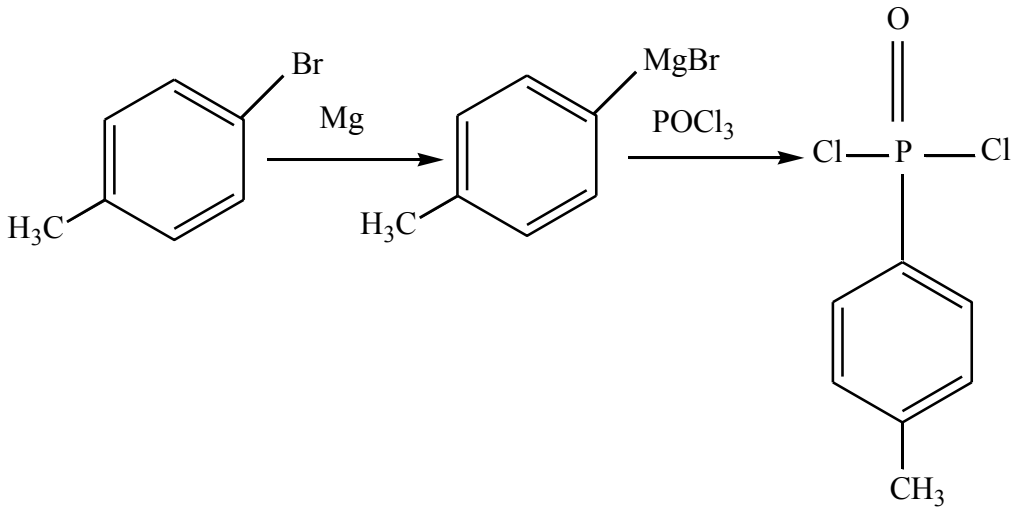
## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide




A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

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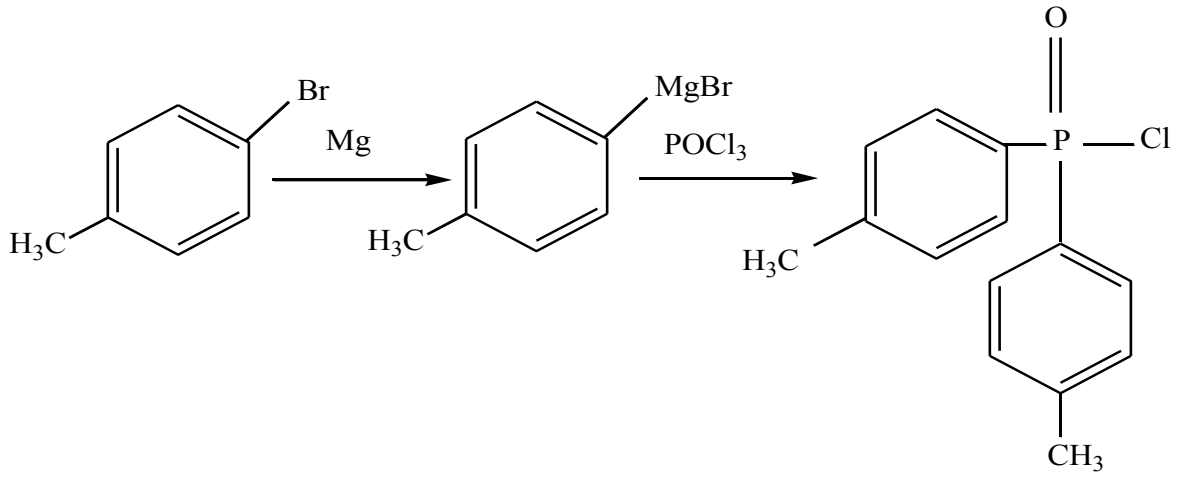
## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

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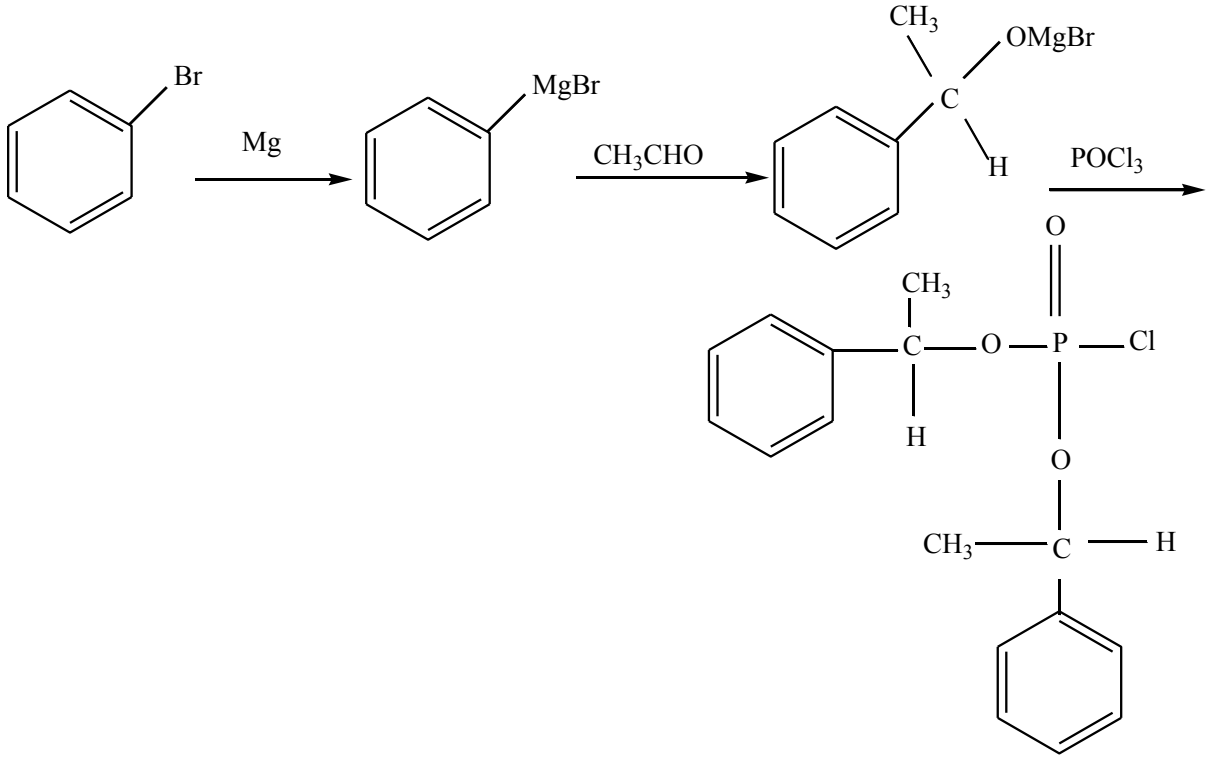
## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

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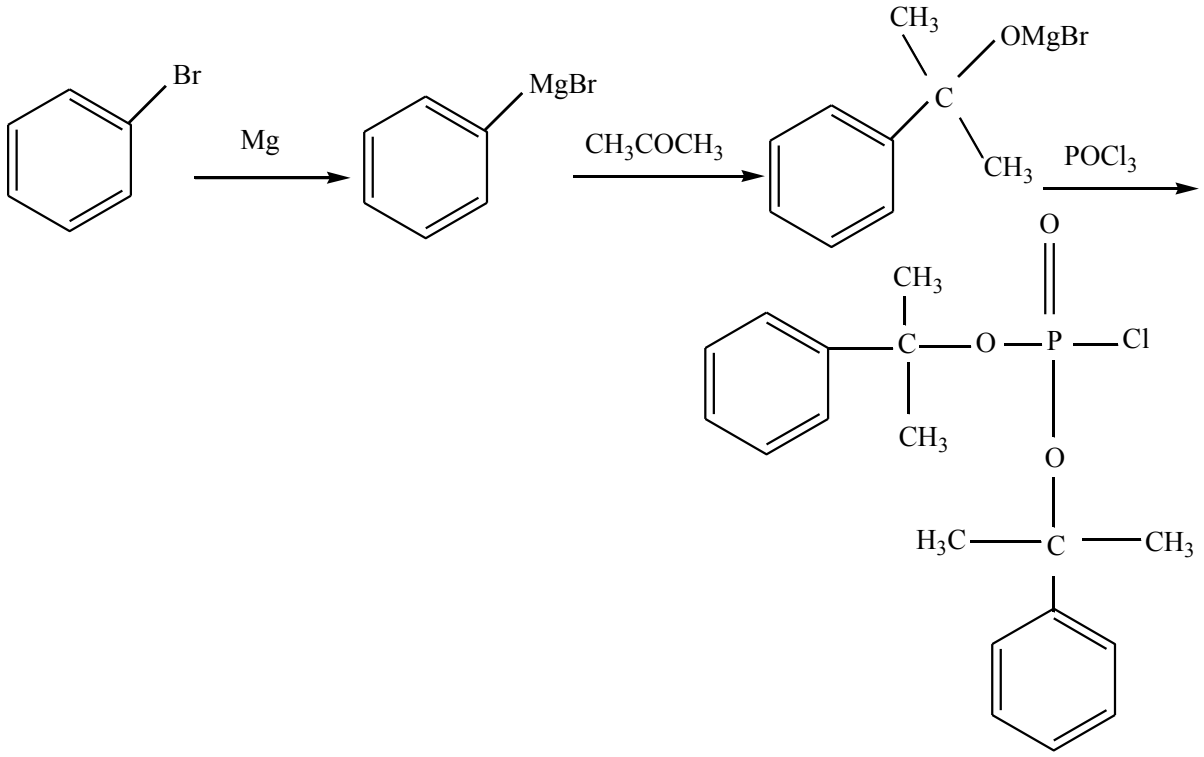
## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

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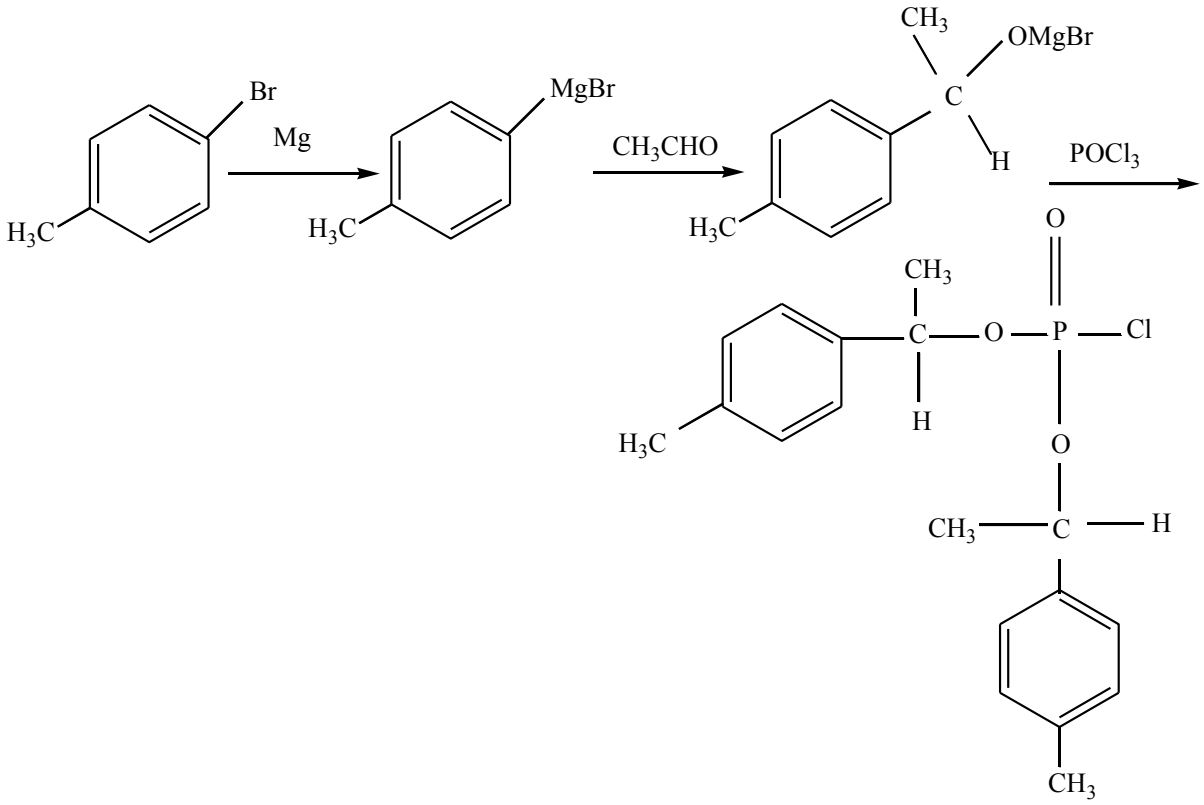
## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide

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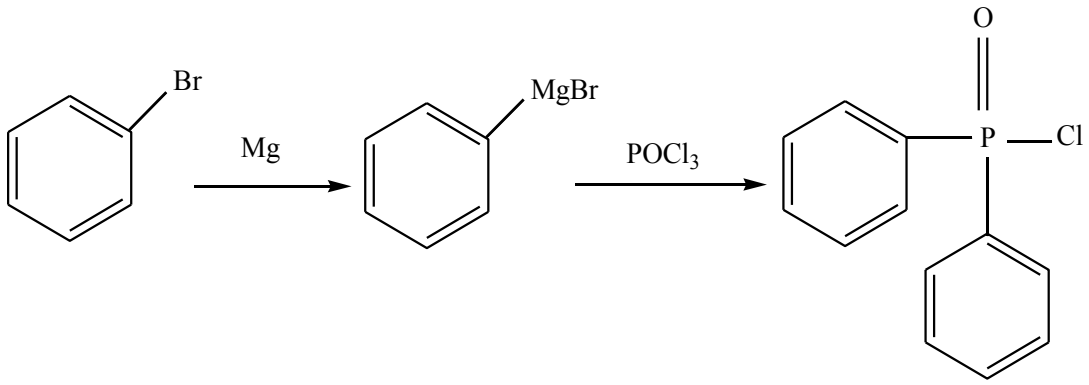


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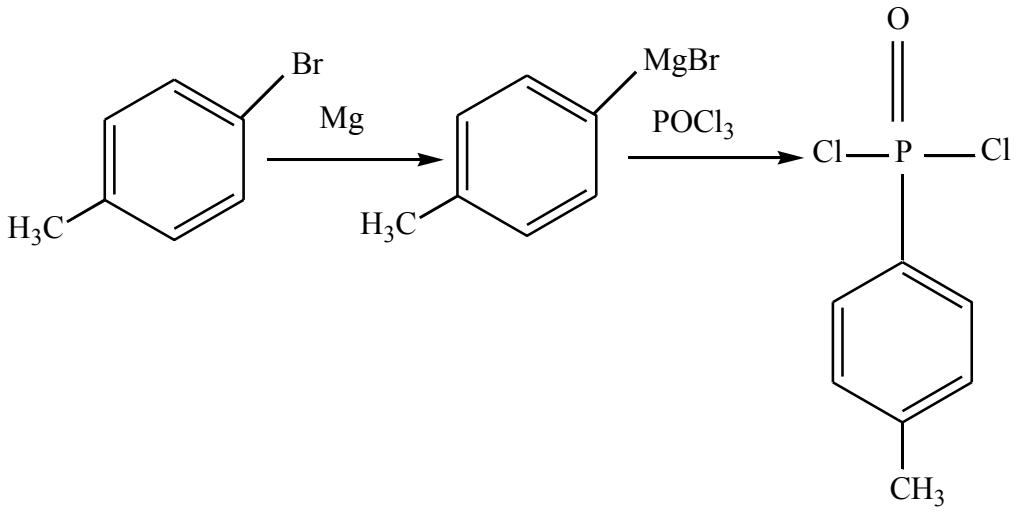
## 2.2 Synthesis of (*p*-tolyl) dichlorophosphine oxide

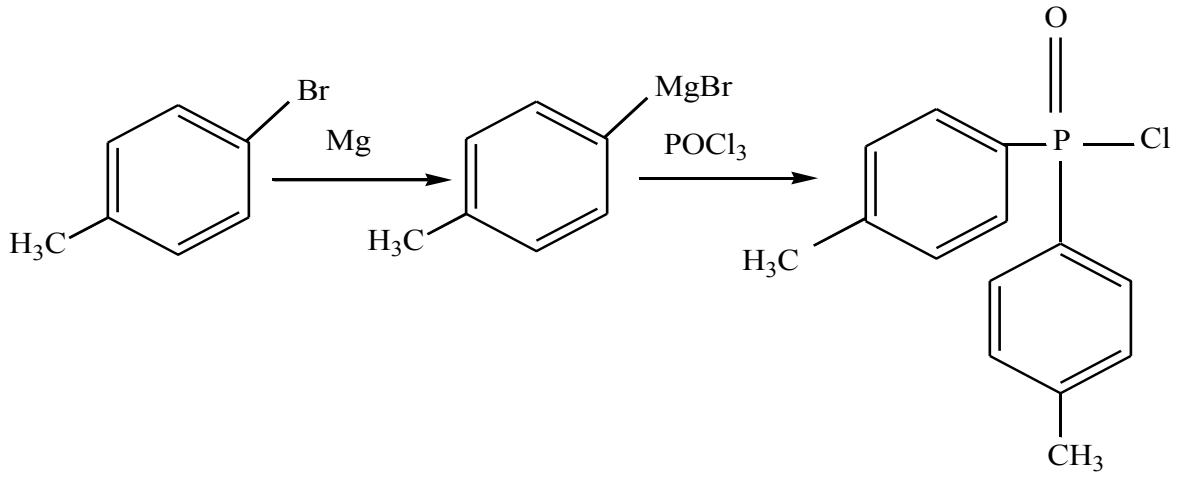



A mixture of ethereal solution of 4-methylbromobenzene (1.334mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour at room temperature, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further two hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (80%) the melting point was recorded as $110^0$C. The product was found to be homogenous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3076.2 (C-H, Aromatic), 2901 (C-H, Aliphatic), 1685.7(P=O hydrate), 1494, 1456.2, 1421.2, 1325.0 (C=C, Aromatic), 1292.2 (P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.250 (S, 3H, $CH_3$), 7.231-7.244 (br, S, 2H, C-H, Aromatic), 7.597-7.609 (br, S, 2H, C-H, Aromatic).

## 2.3 Synthesis of bis (*p*-tolyl) chlorophosphine oxide

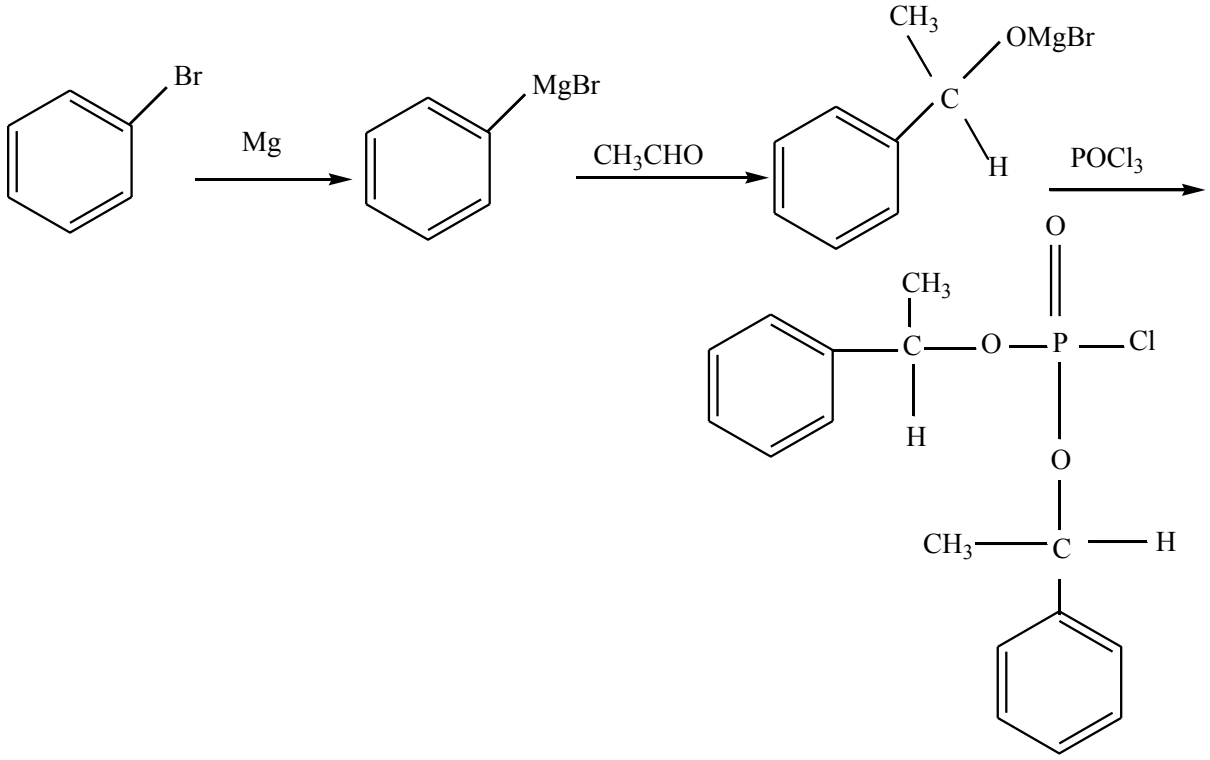



A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for 1.5 hours, then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (80%) the melting point was recorded as $112^0$C. The product was found to be homogenous on TLC plate, Rf = 0.44(Ethyl acetate: n-hexan = 3:7).

**IR (KBr)**: $\nu_{max}$; 3010 (C-H, Aromatic), 2958.6 (C-H, Aliphatic), 2937.4(C-H, Aliphatic), 1668.3 (P=O hydrate), 1556.4, 1460.0, 1379.0 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.241-1.384 (br, S, 6H, $2CH_3$, Aliphatic), 7.424-7.487 (br, S, 4H, C-H, Aromatic), 8.105-8.123 (br, S, 4H, C-H, Aromatic).

## 2.4 Synthesis of bis (phenyl acetoxy) chlorophosphine oxide

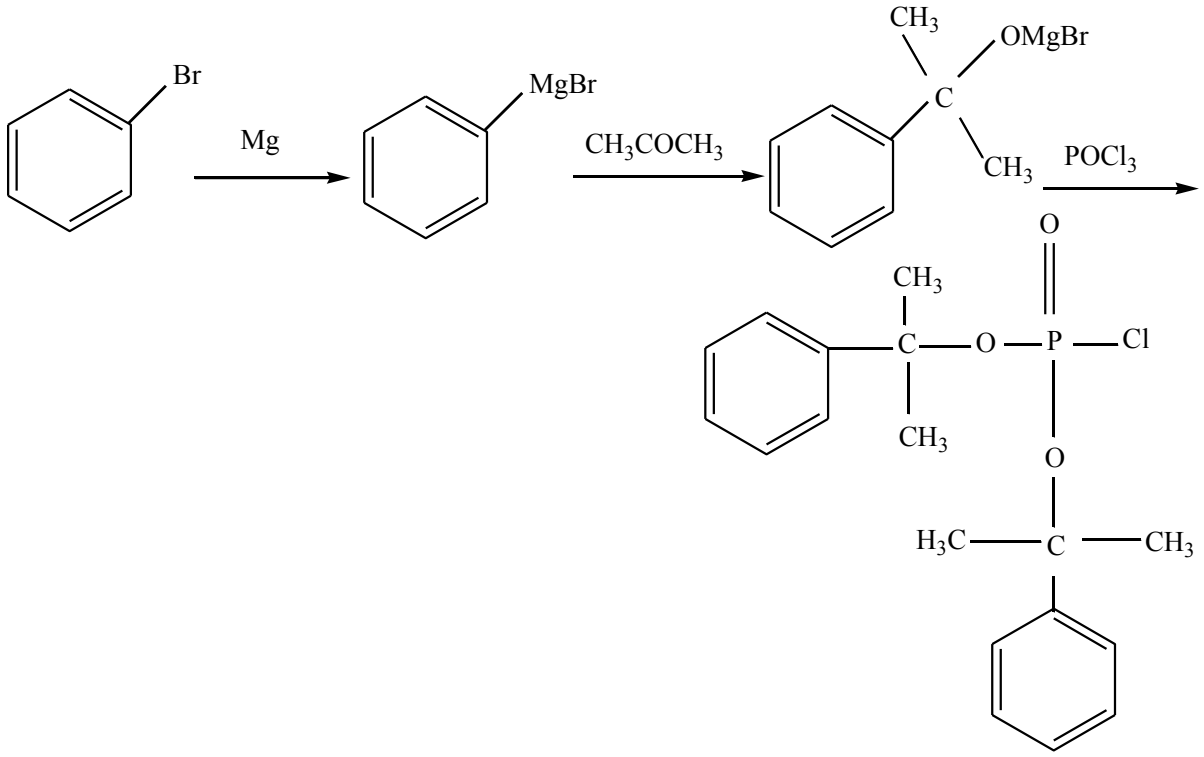



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for one hour at room temperature, to this mixture, acetaldehyde (0.7322mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further three hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a grayish crude product was obtained. The crude was then purified by column chromatography. A grayish solid product afforded yield (70 %) the melting point was recorded as 110 $^0$C. The product was found to be homogenous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 1:4).

**IR (KBr)**: $\nu_{max}$; 3066.6 (C-H, Aromatic), 2923.9 (C-H, Aliphatic), 1595.0 (P=O), 1485.1, 1446.5, 1430 (C=C, Aromatic), 1263(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 1.265 (S, 6H, $2CH_3$), 1.552 (S, 1H, $CH_3$), 2.817 (S, 1H, $CH_3$), 7.151-7.358 (br, S, 8H, Aromatic), 7.426-7.442 (S, 1H, Aromatic) 7.507-7.641(S, 1H, Aromatic).

## 2.5 Synthesis of bis (dimethyl phenyl methoxy) chlorophosphine oxide

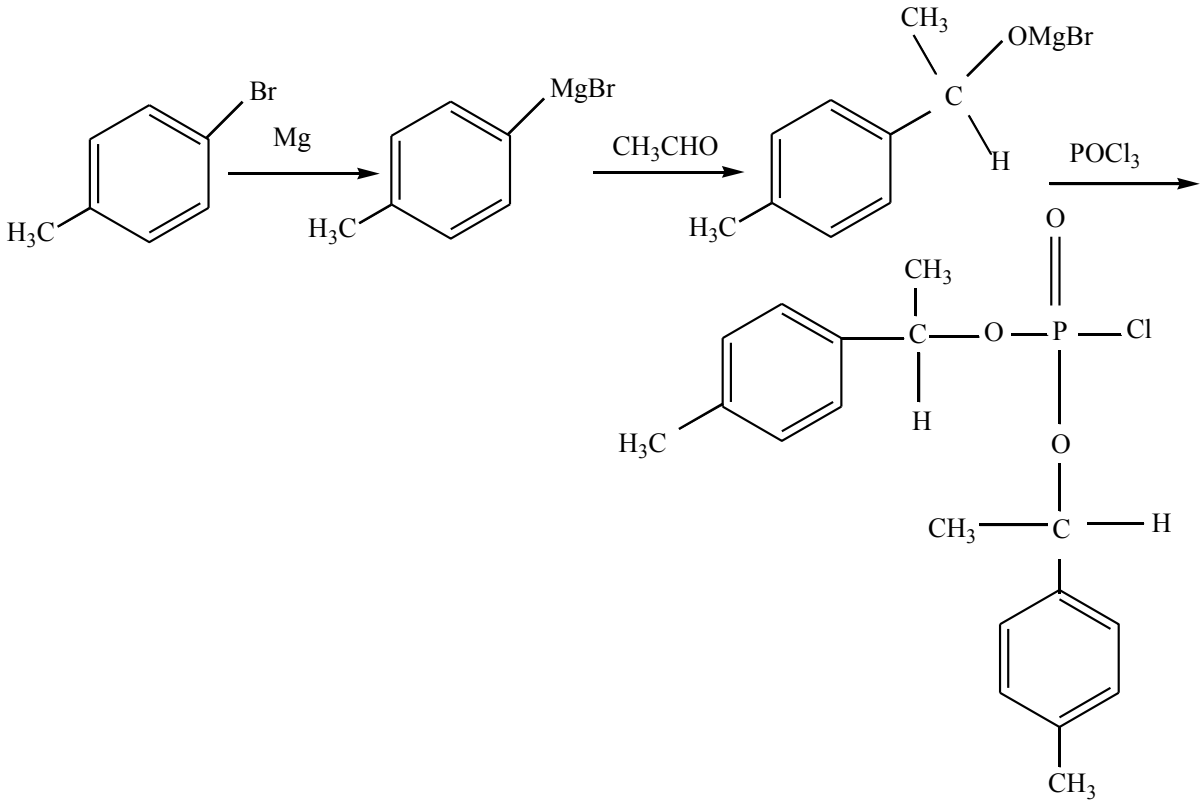



A mixture of ethereal solution of bromobenzene (2.552mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetone (0.9599mol) was added drop-wise and stirred for further one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, an off white crude product was obtained. The crude was then purified by column chromatography. An off white solid product afforded yield (55%) the melting point was recorded as $99^0$C. The product was found to be homogenous on TLC plate, Rf = 0.294 (Ethyl acetate: n-hexan = 2:8).

**IR (KBr)**: $\nu_{max}$; 3070.5 (C-H, Aromatic), 2962.5 2925.8, 2856.4 (C-H, Aliphatic), 1695.3 (P=O hydrate), 1585.4, 1456.2, 1427.2 (C=C, Aromatic), 1263.3(P-Cl) $cm^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)** : $\delta_{H;}$ 0.752-0.853 (br, S, 6H, $2CH_3$), 1.143-1.249 (br, S, 3H, $CH_3$), 2.024-2.206 (br, S, 3H, $CH_3$), 6.976-7.452 (m, 10H, Aromatic).

## 2.6 Synthesis of bis (*p*-tolyl methyl methoxy) chlorophosphine oxide




A mixture of ethereal solution of 4-methylbromobenzene (2.779mol) and magnesium (0.398mol) was taken in a round bottom flask and stirred for about one hour, to this mixture; acetaldehyde (0.732mol) was added drop-wise and stirred for one hour. Then phosphoryl chloride (0.0065mol) was added drop-wise and the stirring was continued for further four hours at room temperature. The progress of the reaction was monitored by TLC. The reaction mixture was then diluted with dichloromethane and was treated with distilled water. The organic layer was separated, washed with brine and dried over anhydrous $Na_2SO_4$. After filtration and evaporation of the solvent, a white crude product was obtained. The crude was then purified by column chromatography. A white solid product afforded yield (65%) the melting point was recorded as 119-121$^0$C. The product was not found homogenous on TLC plate.

**IR (KBr)**: $\nu_{max}$; 3095.5, 3072 (C-H, Aromatic), 2966.3 2925.8 (C-H, Aliphatic), 1695.3 (w, P=O), 1581.5, 1456.2, 1421.2 (C=C, Aromatic), 1325 (P-Cl) cm$^{-1}$.

**$^1$H NMR (400 MHz, $CDCl_3$)**: $\delta$; 0.885-1.066 (br, S, 6H, $2CH_3$), 1.361-1.548 (m, 2H, CH-$CH_{3)}$, 7.257-7.261 (S, 4H, Aromatic), 7.453-7.489 (br, S, 2H, Aromatic), 8.093-8.112 (br, S, 4H, Aromatic).

Chapter-3

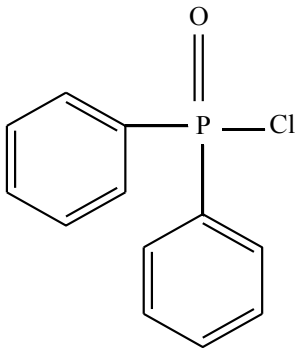
# RESULT & DISCUSSION

## 3.1 Characterization of diphenyl chlorophosphine oxide

The compound diphenyl chlorophosphine oxide was synthesized from the reaction of phenyl magnesium bromide (Grignard reagent) and phosphonyl oxychloride. The pure reaction product was white solid, afforded 60% yield and the melting point was recorded as $120^0$C. The compound was found to be homogeneous on TLC plate, Rf = 0.31 (Ethyl acetate: n-hexan = 1:9).

The IR spectrum (Fig.1) of the expected compound showed aromatic C-H stretching at 3050 $cm^{-1}$ and aliphatic C-H stretching at 2950 $cm^{-1}$. An intensified wide band at 1691 $cm^{-1}$ was assigned for hydrated P=O group. The band at 1456.2, 1421.4 and 1315.4 $cm^{-1}$ were for aromatic C=C. The band at 1128.3 was designated for P-Cl bond.

The $^1$H NMR spectrum (Fig. 2) showed broad singlet at $\delta$ value 7.466-7.502 was assigned for aromatic protons at C-2, C$^/$-2, C-6 and C$^/$-6 carbon. Another board singlet at 7.603-7.639 was designated for two aromatic protons at C-4 and C$^/$-4 carbon. The third broad singlet at 8.123-8.143 was indicative for from aromatic protons at C-3, C$^/$-3, C-5 and C$^/$-5 carbon. The above physical and spectral expressed harmony to evidences the structure of the compound diphenyl chlorophosphine oxide as



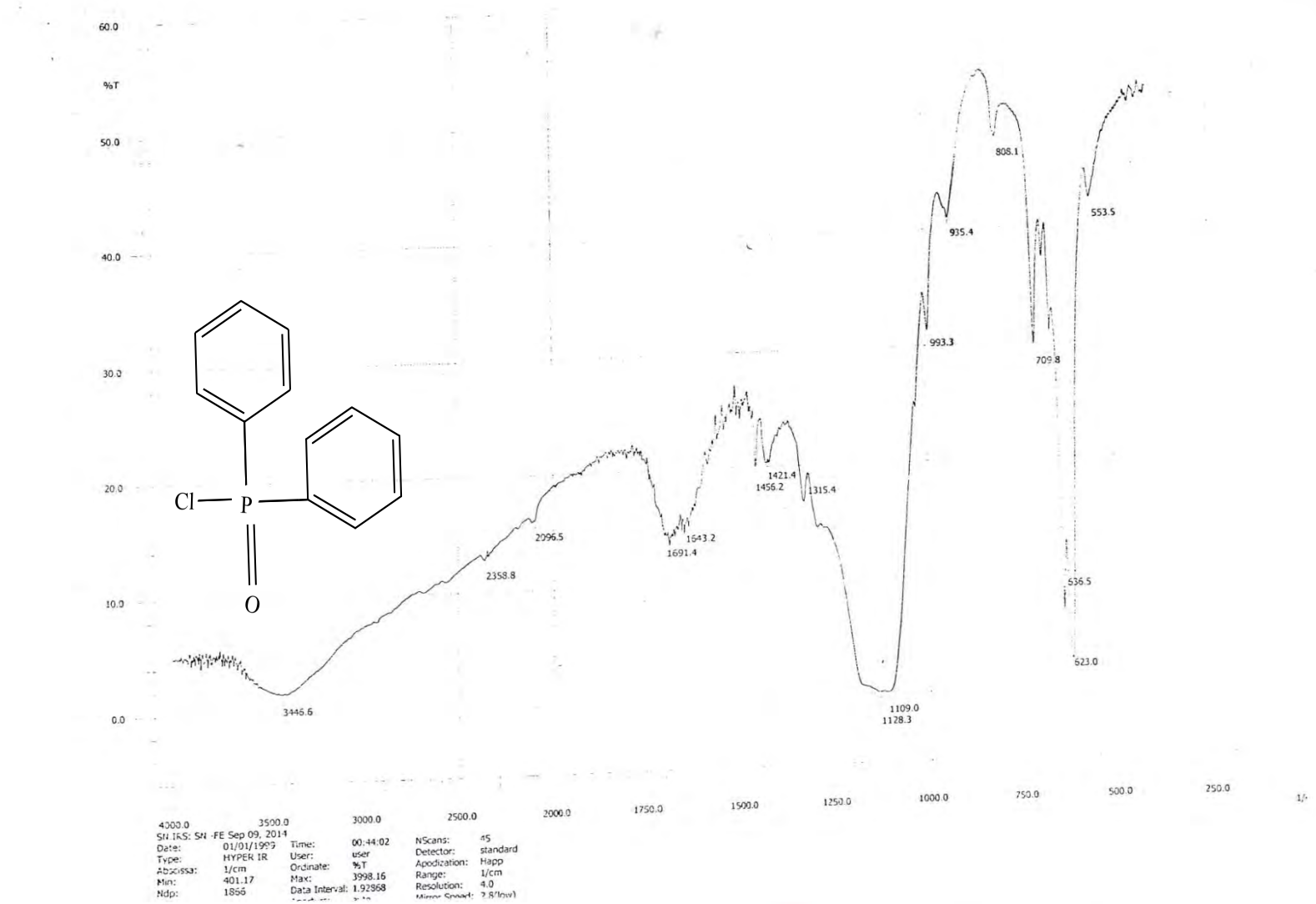
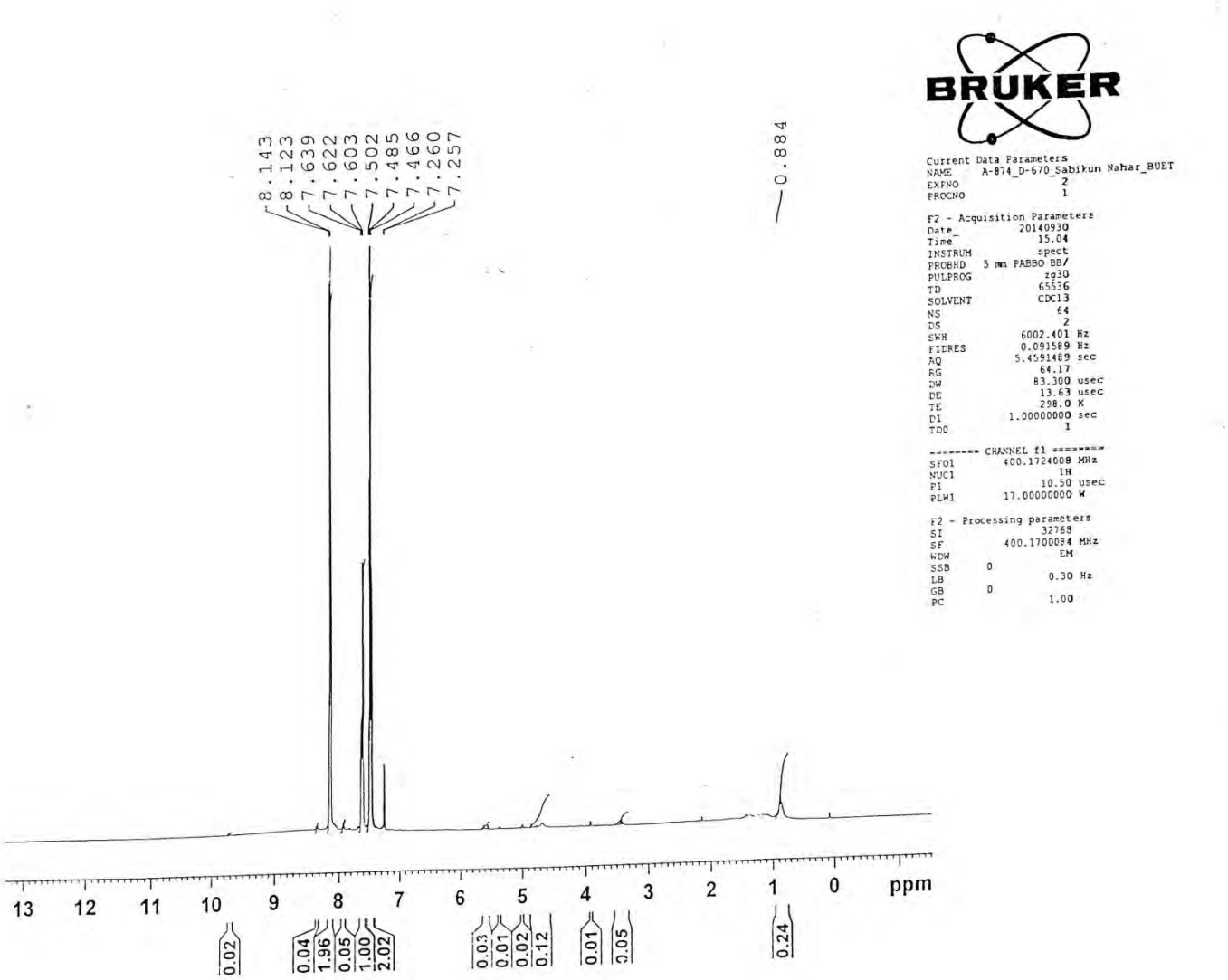



Figure 1: IR Spectrum of the compound **1A**

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PLW1                    17.00000000 W

F2 - Processing parameters
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GB                                     0
PC                                 1.00
```

Figure 2: $^{1}$H NMR Spectrum of the compound **1A**

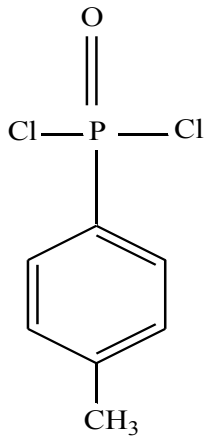
Figur

## 3.2 Characterization of (*p*-tolyl) dichlorophosphine oxide

The compound *p*-tolyl dichlorophosphine oxide was synthesized from the reaction between the Grignard reagent *p*-tolyl magnesium bromide and phosphonyl oxychloride. The pure reaction product was grayish solid, afforded 80% yield and the melting point was recorded as $110^0$C. The compound was found to be homogeneous on TLC plate, Rf = 0.49 (Ethyl acetate: n-hexan = 3:7).

The IR spectrum (Fig: 3) showed aromatic C-H stretching band at 3076.2 $cm^{-1}$ and aliphatic C-H stretching at 2901 $cm^{-1}$ . The band at 1685.7 $cm^{-1}$ was assigned for P=O bond. The intensified band at 1494, 1456.2, 1421.2 and 1325.0 $cm^{-1}$ were designated for aromatic unsaturated C=C bonds. The peak at 1292.2 $cm^{-1}$ was distinctive for P-Cl bond.

The $^1$H NMR spectrum (Fig: 4) showed a sharp singlet at 1.250 for three aliphatic protons of methyl group. A broad singlet at 7.231-7.244 was assigned for two aromatic protons of C-2 at C-6.. The singlet at 7.597-7.609 was distinctive for two protons at C-3 and C-5. The above evidence expresses harmony to structure of the compound as *p*-tolyl dichlorophosphine oxide as



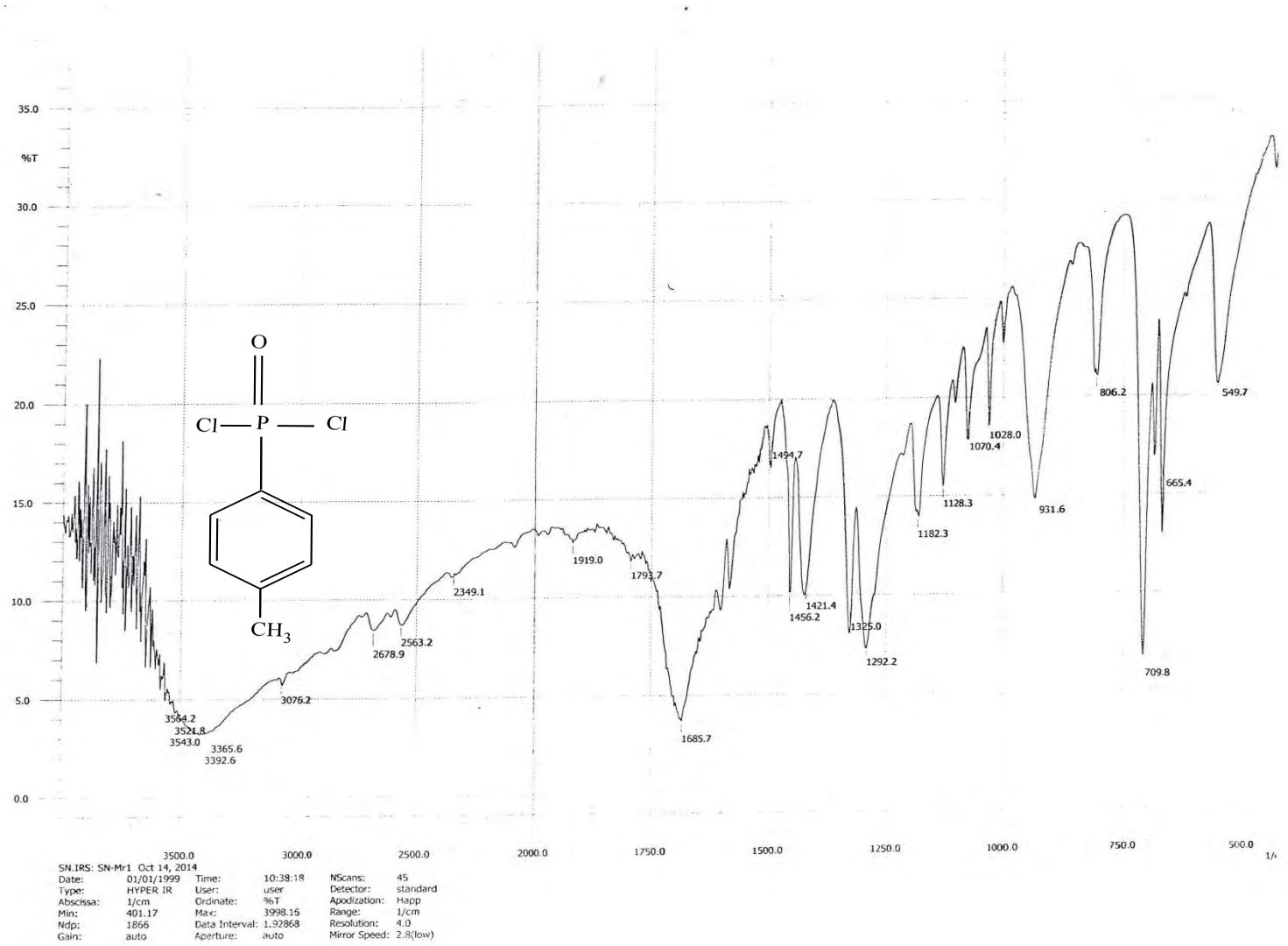



Figure 3: IR Spectrum of the compound **2A**

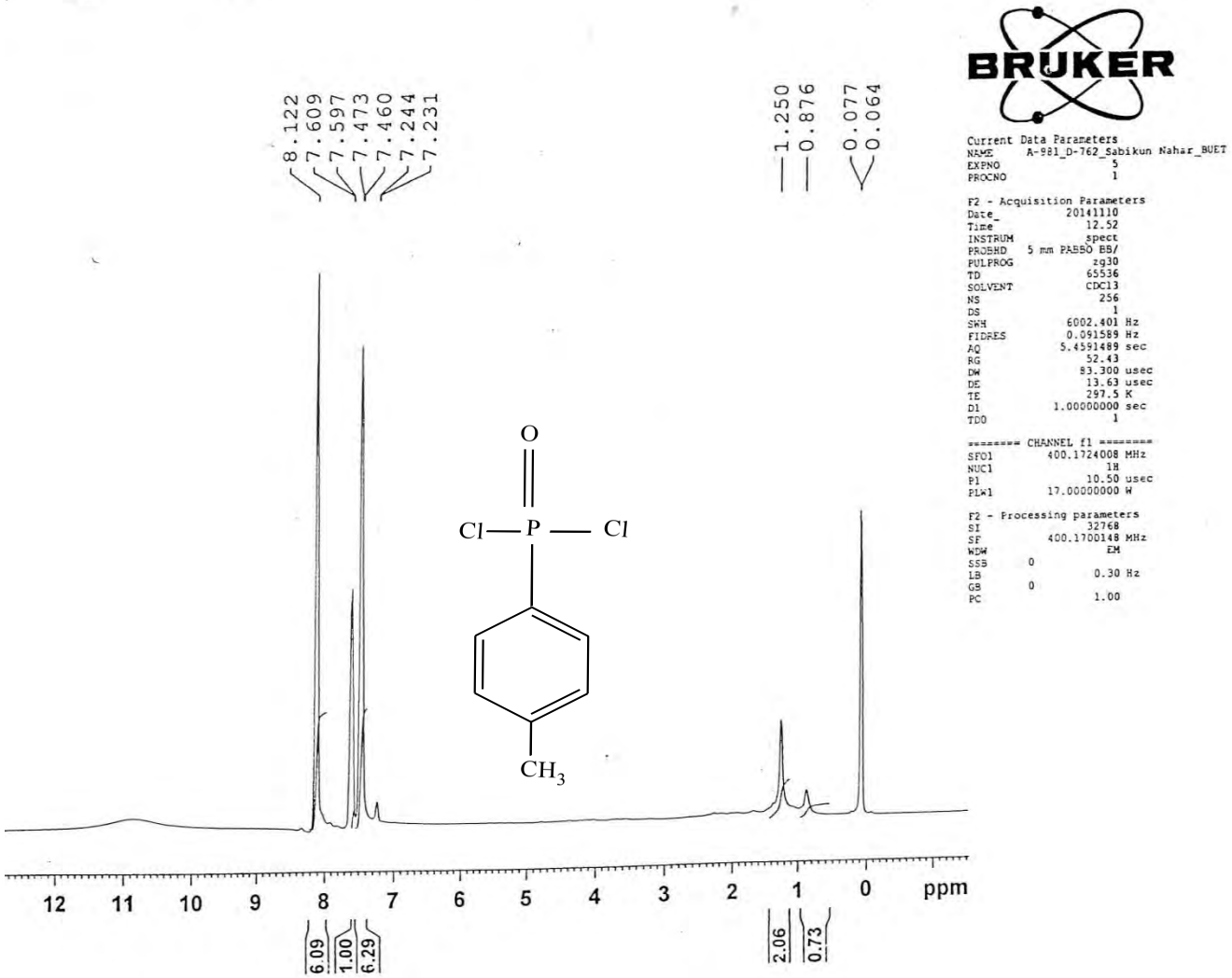



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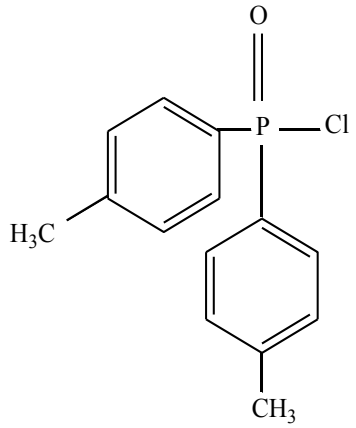
Figure 4: $^{1}$H NMR Spectrum of the compound **2A**

## 3.3 Characterization of bis (*p*-tolyl) chlorophosphine oxide

The expected compound bis *p*-tolyl chlorophosphine oxide was synthesized from the Grignard reagent *p*-tolyl magnesium bromide and phosphonyl oxychloride. The pure product was white solid afforded 80% yield and the melting point was recorded as $112^0$C. The product was found to be homogeneous on TLC plate, Rf = 0.44 (Ethyl acetate: n-hexan = 3:7)

The IR spectrum (Fig: 5) showed C-H aromatic stretching at 3010 $cm^{-1}$ and aliphatic C-H stretching at 2958.6 $cm^{-1}$ and 2937.4 $cm^{-1}$. The intensified band at 1668.3 was distinctive for P=O bond. The bands at 1556.4, 1460.0, 1379.0 $cm^{-1}$ were assigned for aromatic unsaturated C=C bonds. The peak at 1263 $cm^{-1}$ was designated for P-Cl bond.

The $^1$H NMR spectrum (Fig: 6) showed a broad singlet at 1.241-1.384 for six proton of two methyl groups. The broad singlet at 7.424-7.487 was assigned for four protons of C-2, C-6, C$^{/}$-2 and C$^{/}$-6 carbons. The third broad singlet at 8.105-8.123 for four protons were designated for C-3, C-5, C$^{/}$-3 and C$^{/}$-5 carbons. The spectral evidences support the structure of the compound bis *p*-tolylchlorophosphine oxide as



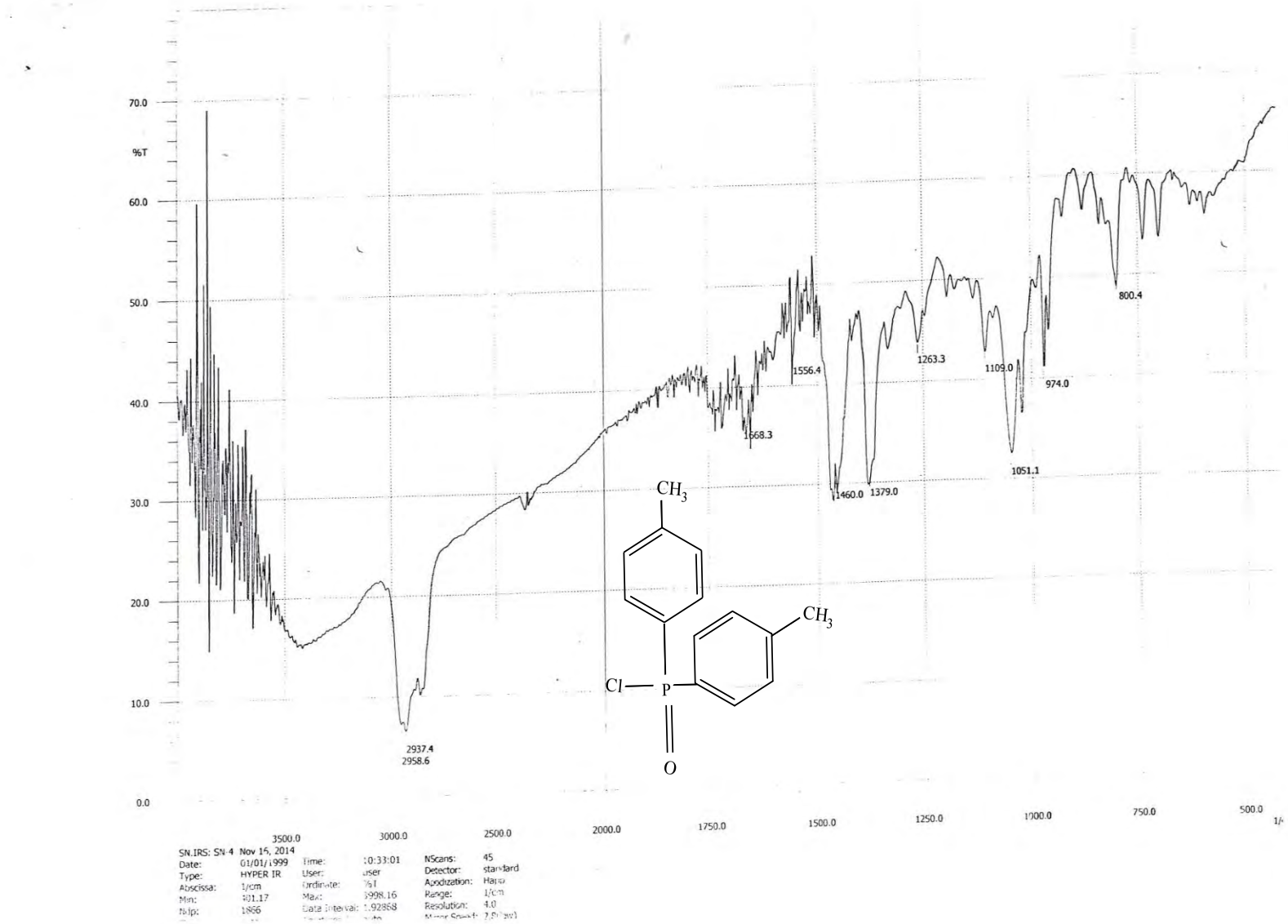



Figure 5: IR Spectrum of the compound **3A**

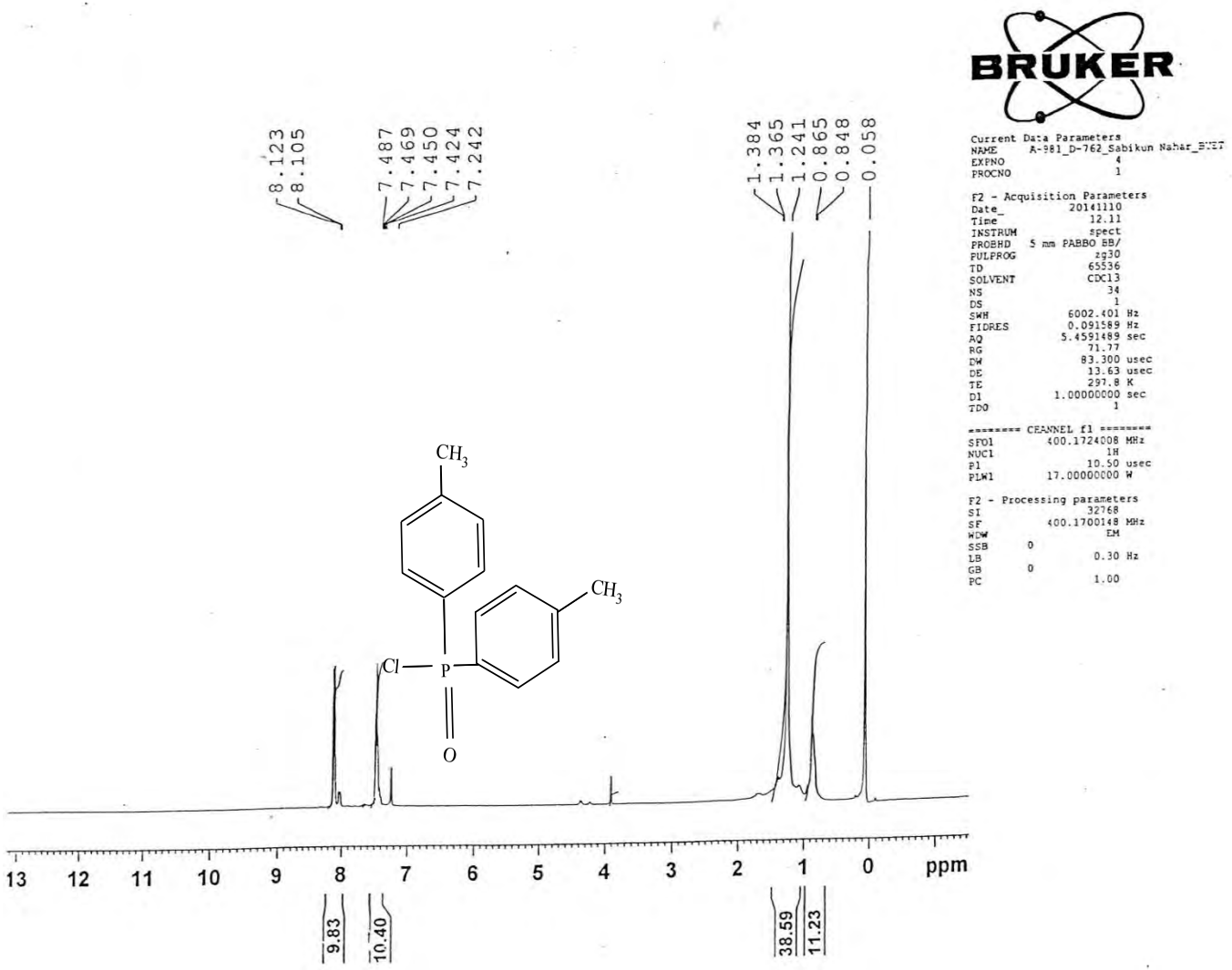






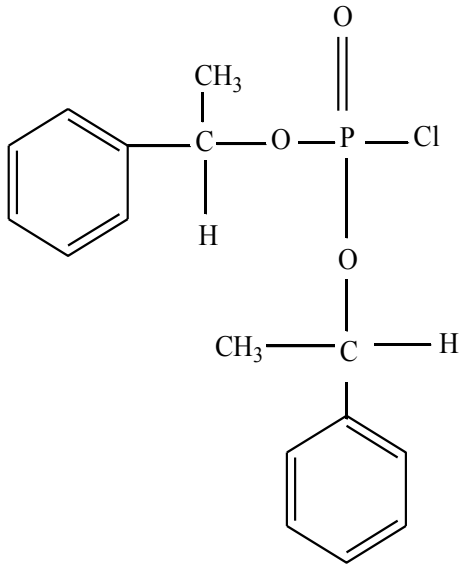
Figure 6: $^{1}$H NMR Spectrum of the compound **3A**

## 3.4 Characterization of bis (phenyl acetoxy) chlorophosphine oxide

The desired compound was synthesized from the reaction between Grignard reagent phenyl methyl methoxy magnesiumbromide and phosphorus oxychloride. The reaction product was isolated as white solid, afforded 70% yield and the melting point was recorded as $110^0$C. The product was found to be homogeneous on TLC plate, Rf = 0.54 (Ethyl acetate: n-hexan = 2:8).

The IR spectrum (Fig: 7) showed aromatic C-H stretching at 3066.6 $cm^{-1}$ and aliphatic C-H stretching at 2923.9 $cm^{-1}$. The wide intensified peak at 1595.0 was assigned for P=O group. The band at 1485.1, 1446.5 and 1430 $cm^{-1}$ were indicative for aromatic unsaturated C=C bonds. The sharp peak at 1263 $cm^{-1}$ was for P-Cl bond.

The $^1$H NMR spectrum (Fig: 8) showed singlet at 1.265 for six proton of two methyl groups. The singlet at 1.552 and 2.817 were assigned for two H-$CH_3$ protons. The wide band at 7.157-7.358 was assigned for eight aromatic protons. The broad singlet at 7.426-7.442 and 7.507-7.641 were distinctive for one proton of C-4 and other one proton of C$^/$-4 respectively. All the spectral evidences express harmony with the structure of the compound bis (phenyl acetoxy) chlorophosphine oxide as



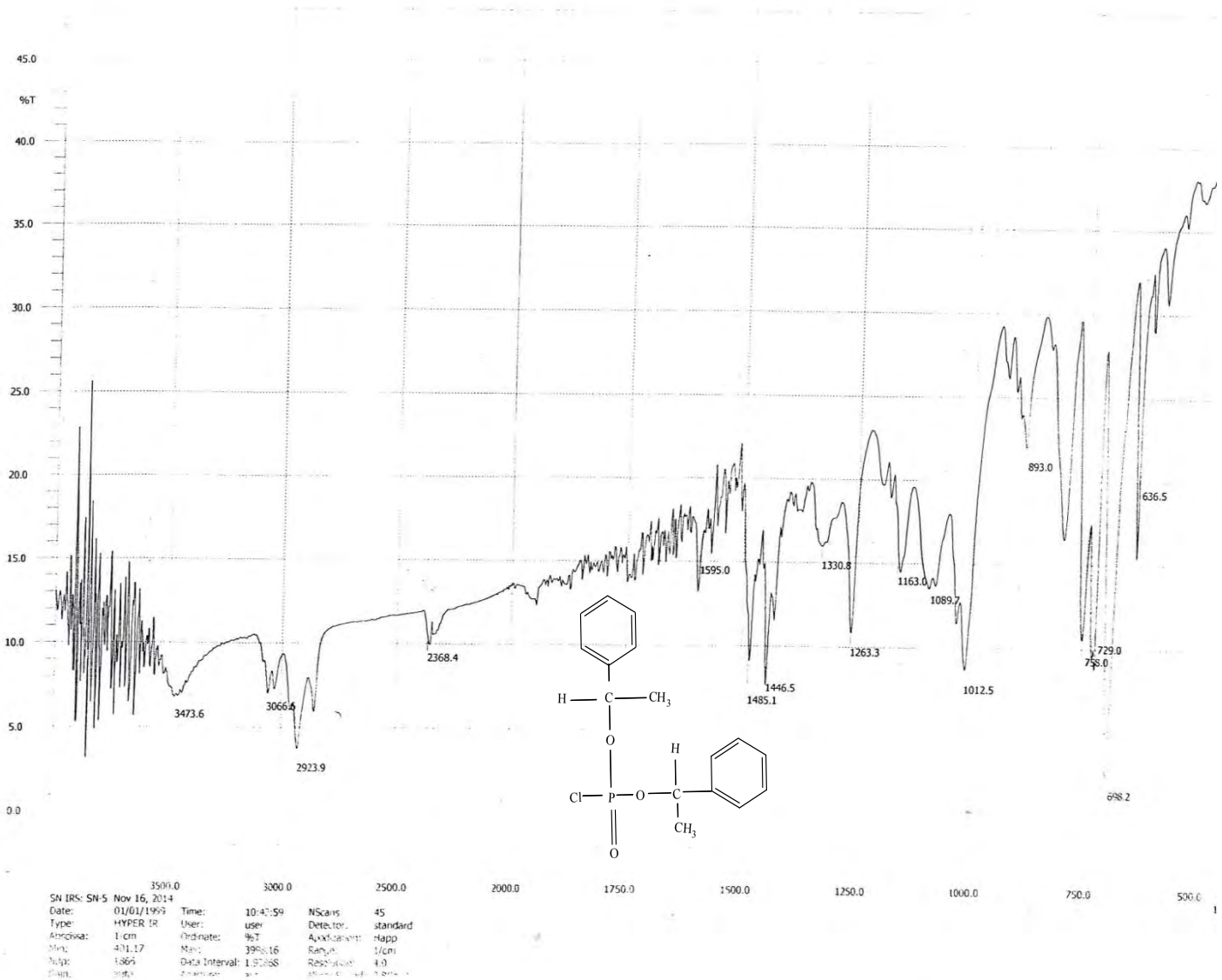



Figure 7: IR Spectrum of the compound **4A**

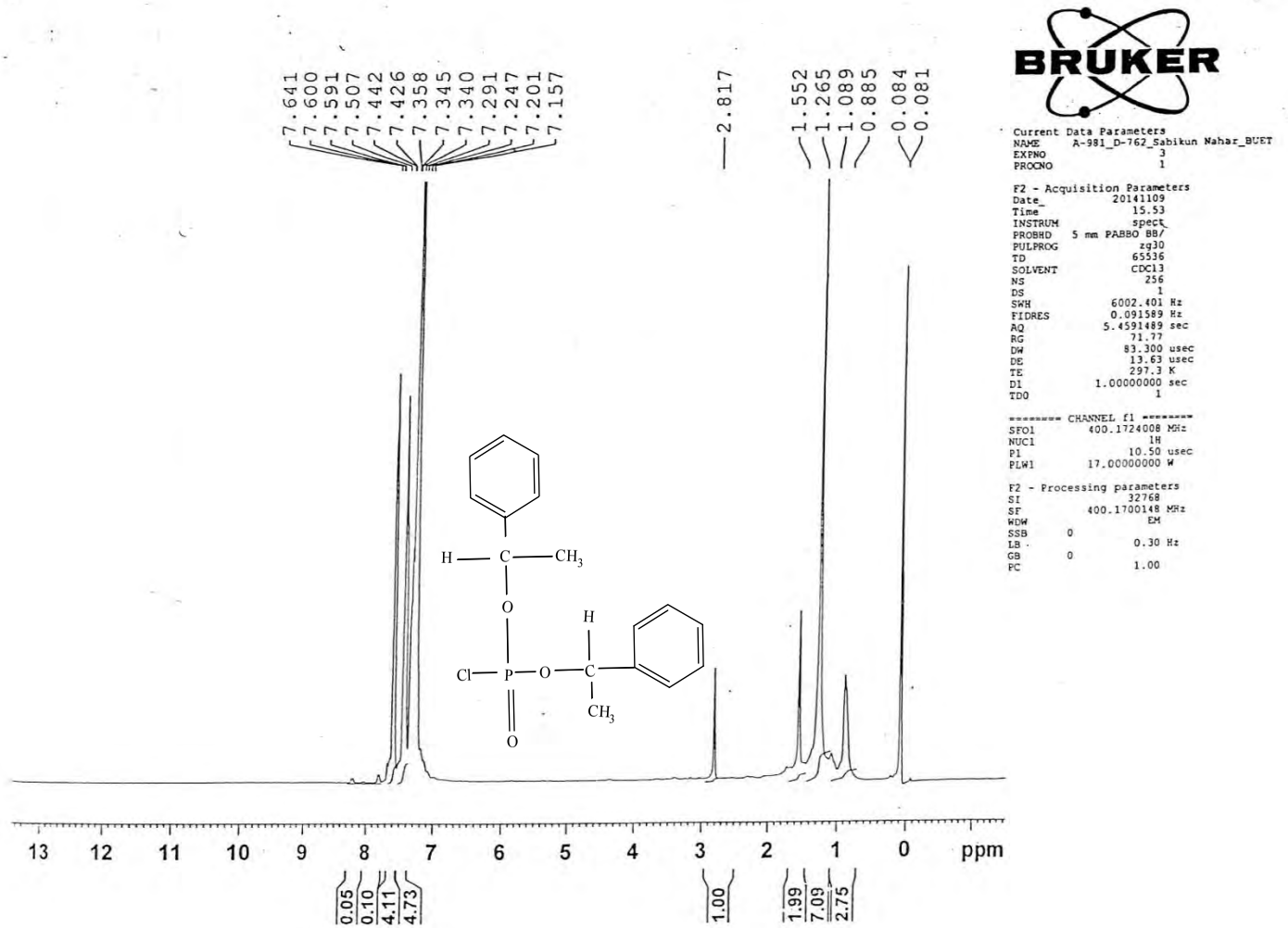


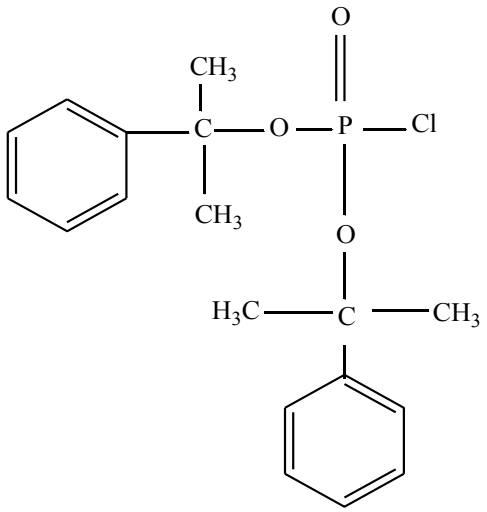

Figure 8: $^{1}$H NMR Spectrum of the compound **4A**

## 3.5 Characterization of bis (dimethyl phenyl methoxy) chlorophosphinc oxide

The desired compound was synthesized by the addition of Grignard reagent dimethyl phenyl methoxy magnesiumbromide to the phosphorus oxychloride. The pure product was off white solid, afforded 45% yield and the melting point was recorded as $99^0$C. The product was homogeneous on TLC plate, Rf = 0.29 (Ethyl acetate: n-hexan= 2:8).

The IR spectrum (Fig: 9) showed aromatic C-H stretching at 3070.5 $cm^{-1}$ and aliphatic C-H stretching at 2962.5, 2925.8, 2856.4 $cm^{-1}$. The intensified broad band at 1695.3 was assigned for P=O group and a group of bands at 1585.4, 1456.2, 1427.2 $cm^{-1}$ were distinctive for aromatic unsaturated C=C bonds. The peak 1263.3 was ascribed for P-Cl bond.

The $^1$H NMR spectrum (Fig: 10) showed a broad singlet at 0.752-0.853 for six protons of $2CH_3$ groups and a second broad singlet at 1.143-1.249 was designated for three protons of $CH_3$ group and another broad singlet was ascribable for three proton of $CH_3$ group . The wide multiplet at 6.976-7.452 was designated for ten aromatic protons. The above evidences support the structure of bis (dimethyl phenyl methoxy) chlorophosphine oxide as



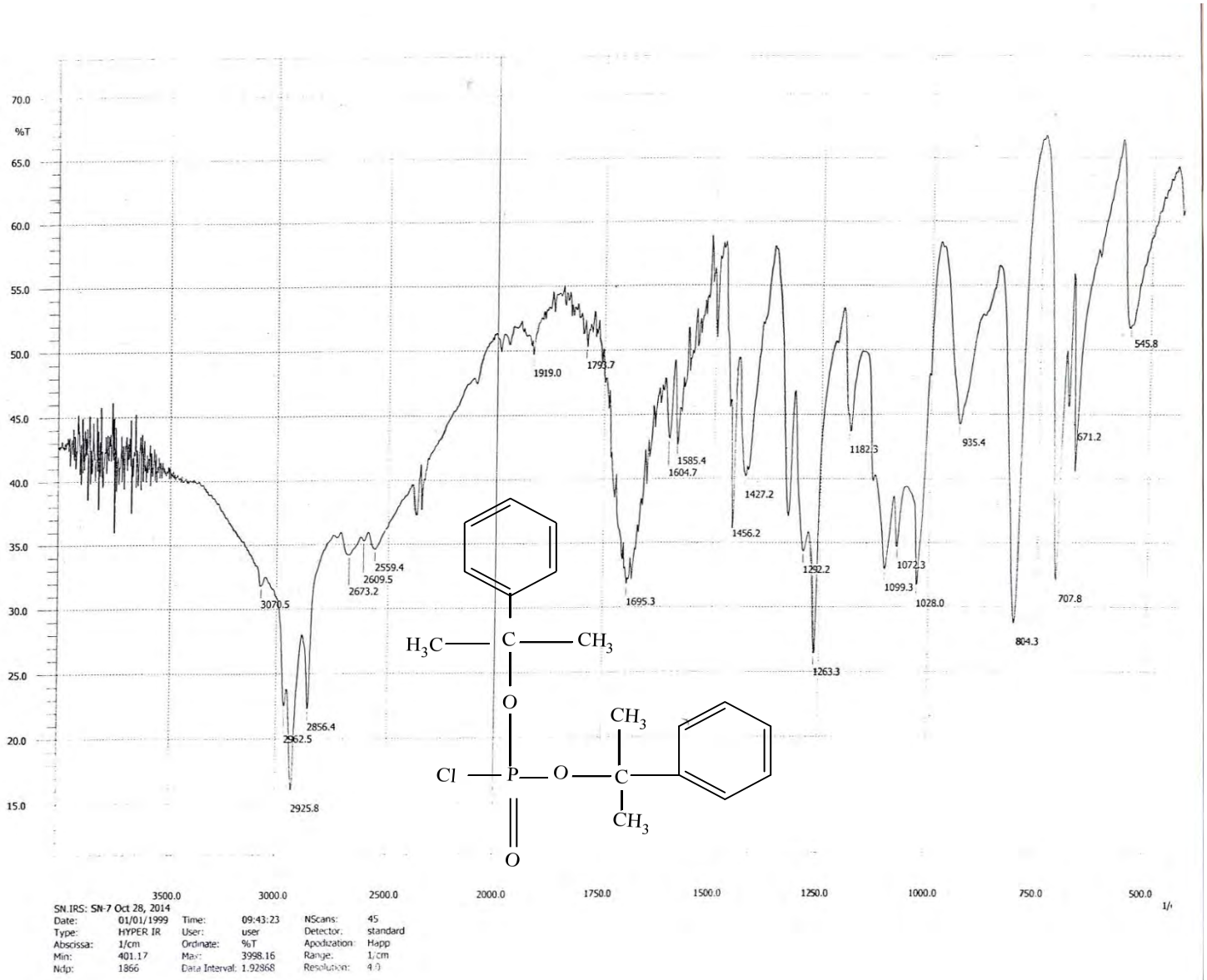



Figure 9: IR Spectrum of the compound **5A**

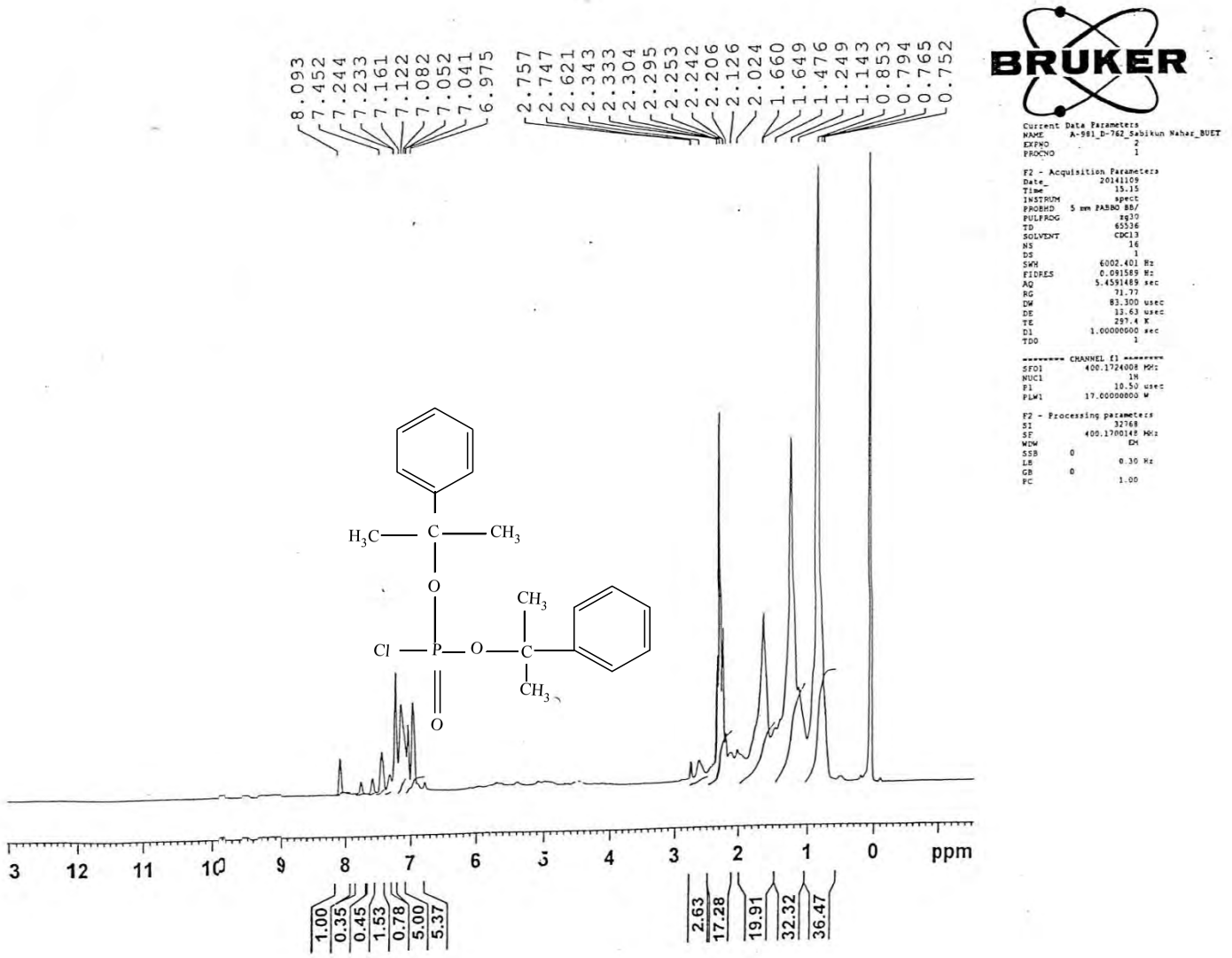



Figure 10: $^{1}$H NMR Spectrum of the compound **5A**

## 3.6 Characterization of bis (*p*-tolyl methyl methoxy)) chlorophosphine oxide

The target compound was synthesized by the addition of *p*-tolyl methyl methoxy magnesium bromide Grignard reagent to phosphonyl oxychloride. The crude compound was white color, afforded 65% yield and the melting point was recorded as 119-121$^0$C. The product was not found homogeneous on TLC plate.

The IR spectrum (Fig:11) showed peak at 3095.5 and 3072 $cm^{-1}$ for aromatic C-H stretching and 2966.3, 2925.8 $cm^{-1}$ were distinctive for aliphatic C-H stretching. The wide intensified peak at 1695.3 $cm^{-1}$ was for P= O group. The several bands at 1581.5, 1456.2 and 1421.2 $cm^{-1}$ were assigned for aromatic C=C bonds. The band at 1325 was ascribed for P-Cl bond.

The $^1$H NMR spectrum (Fig: 12) showed a broad singlet for six protons of $CH_3$ group but it was not clear. The multiplet at 1.361-1.548 for two proton of -CH-$CH_3$ was not fairly distinctive. The singlet at 7.257-7.261 for four aromatic protons. The other peaks at 7.453-7.489 and 8.093-8.112 were not detectable. So, it was not possible to confirm completely the compound as bis (*p*-tolyl methyl methoxy) chlorophosphine oxide.

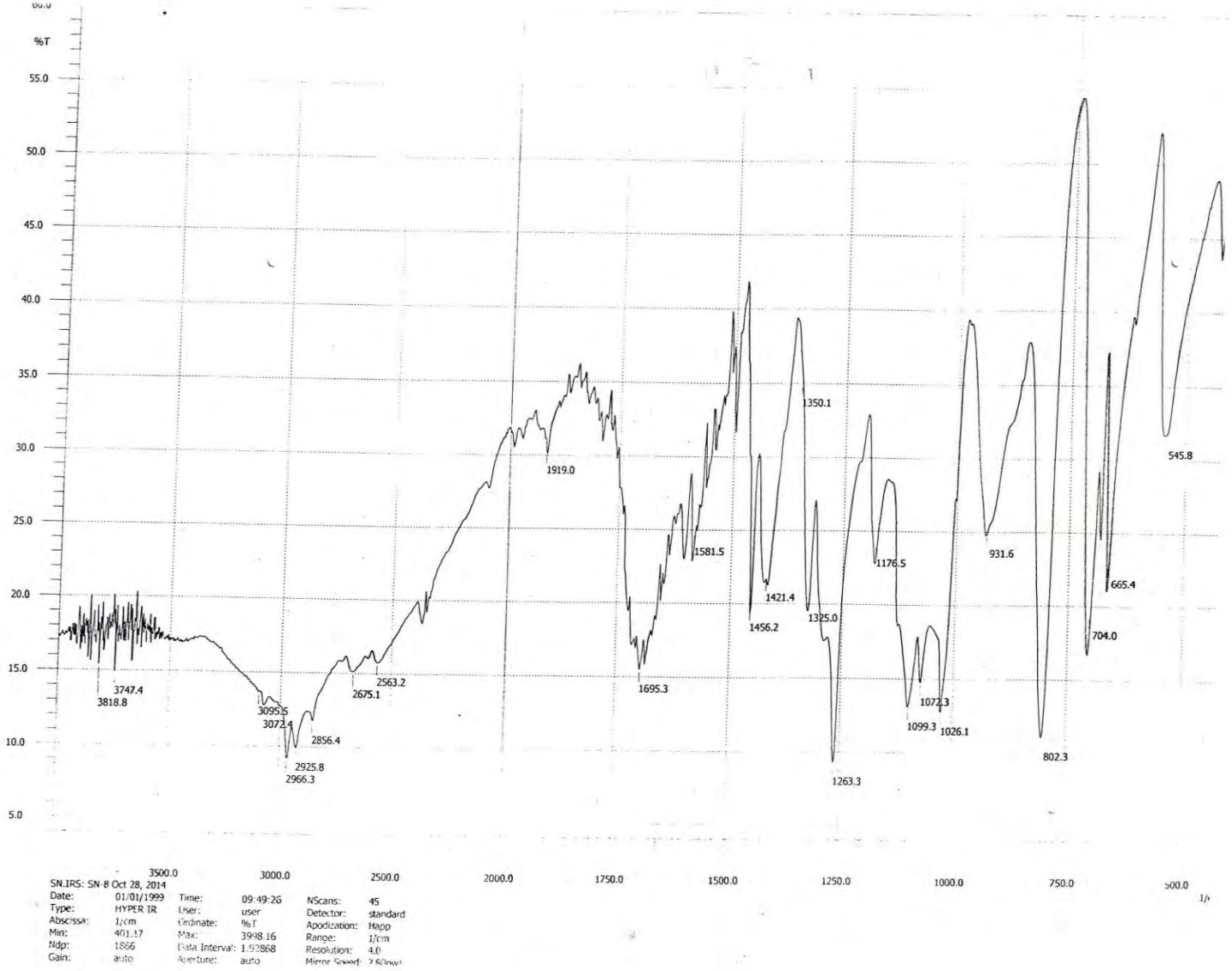



Figure 11: IR Spectrum of the compound **6A**

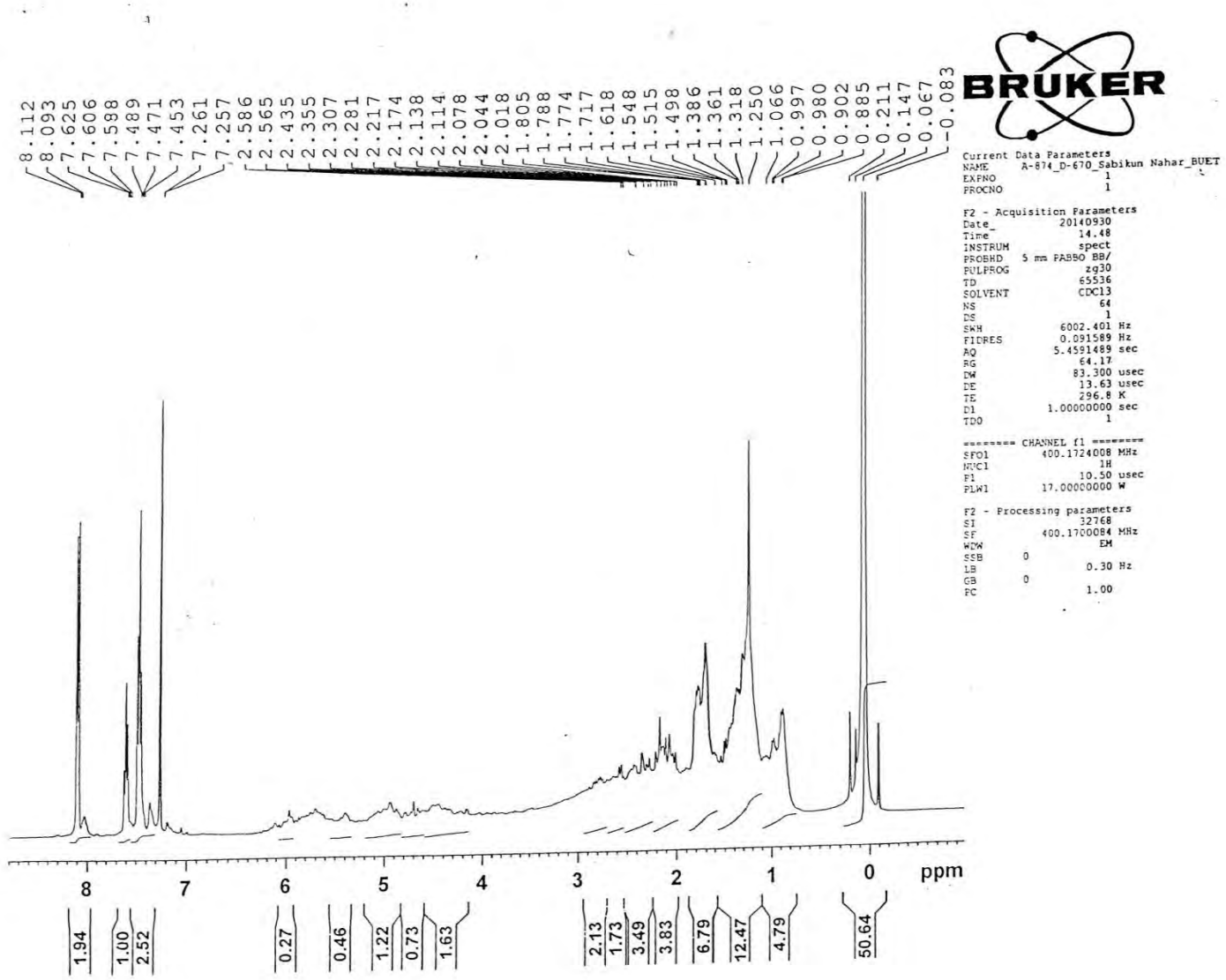
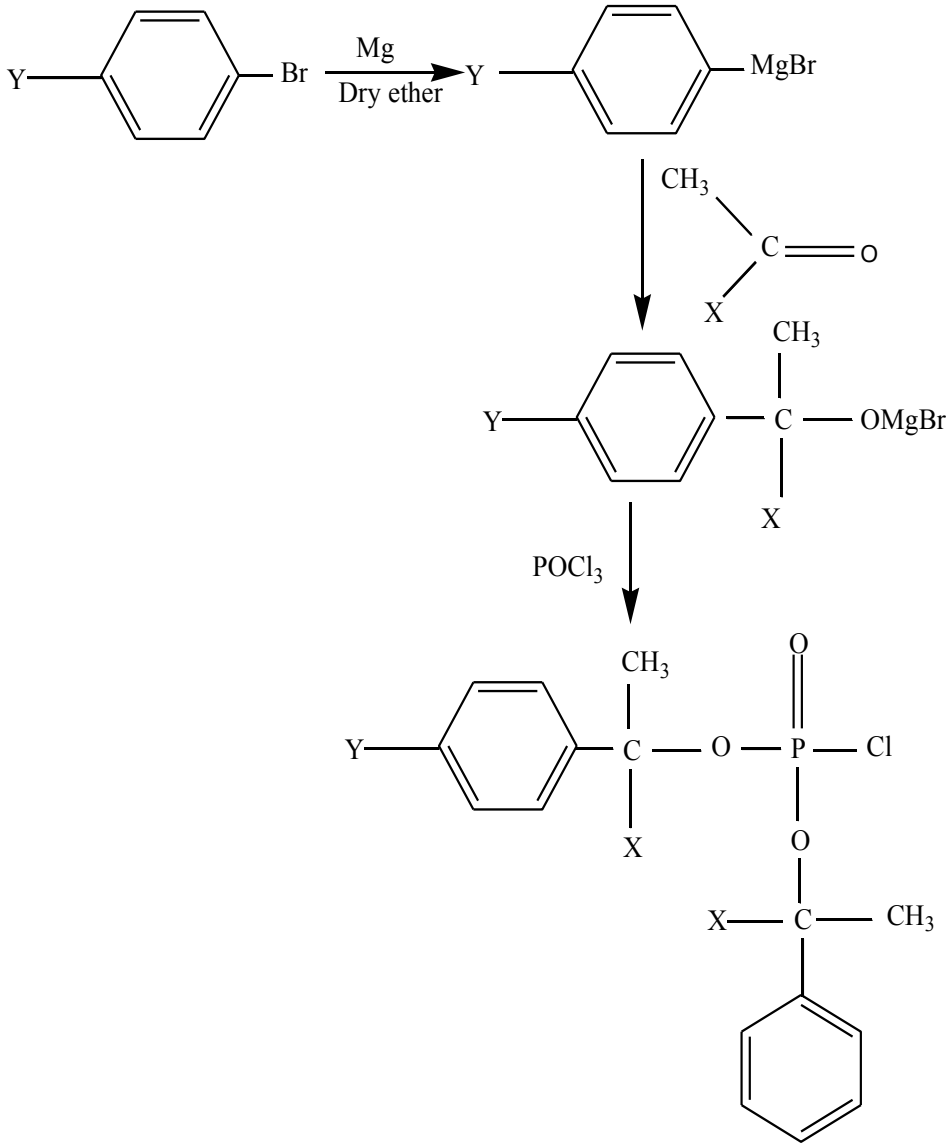



Figure 12: $^{1}$H NMR Spectrum of the compound **6A**

## 3.7 MECHANISM OF THE SYNTHESIS

**Synthesis of oxyphosphorus derivatives using Grignard reagent**

**Synthetic Scheme:**

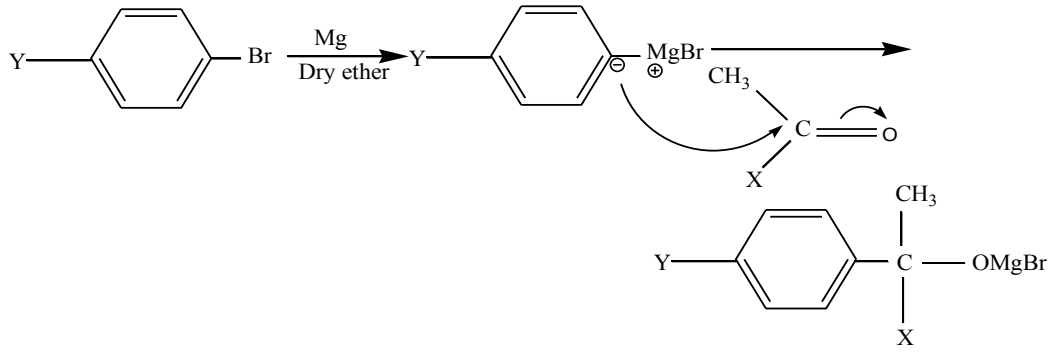



Here, X=$CH_3$, H and Y= $CH_3$, H

The mechanism of the reaction has been considering by the following scheme.

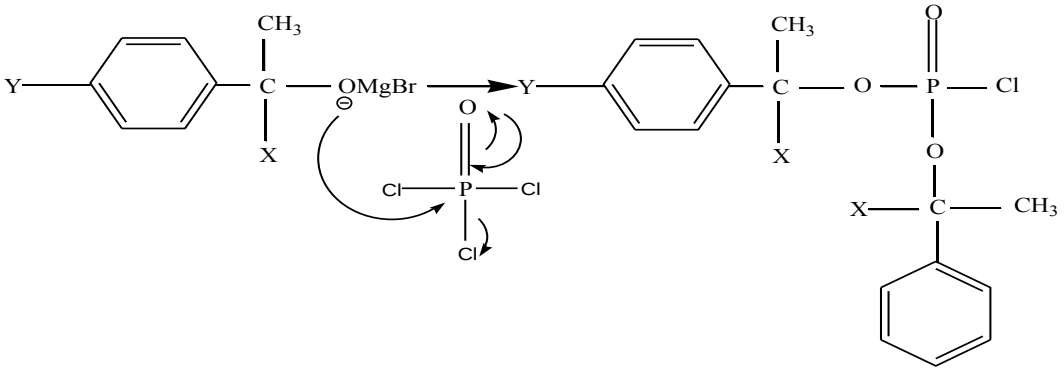
- Grignard reagent mechanism
- Reaction with phosphorus oxy chloride
- Trigonal bipyramidal pentacoordinate intermediate

**Grignard reagent mechanism**:



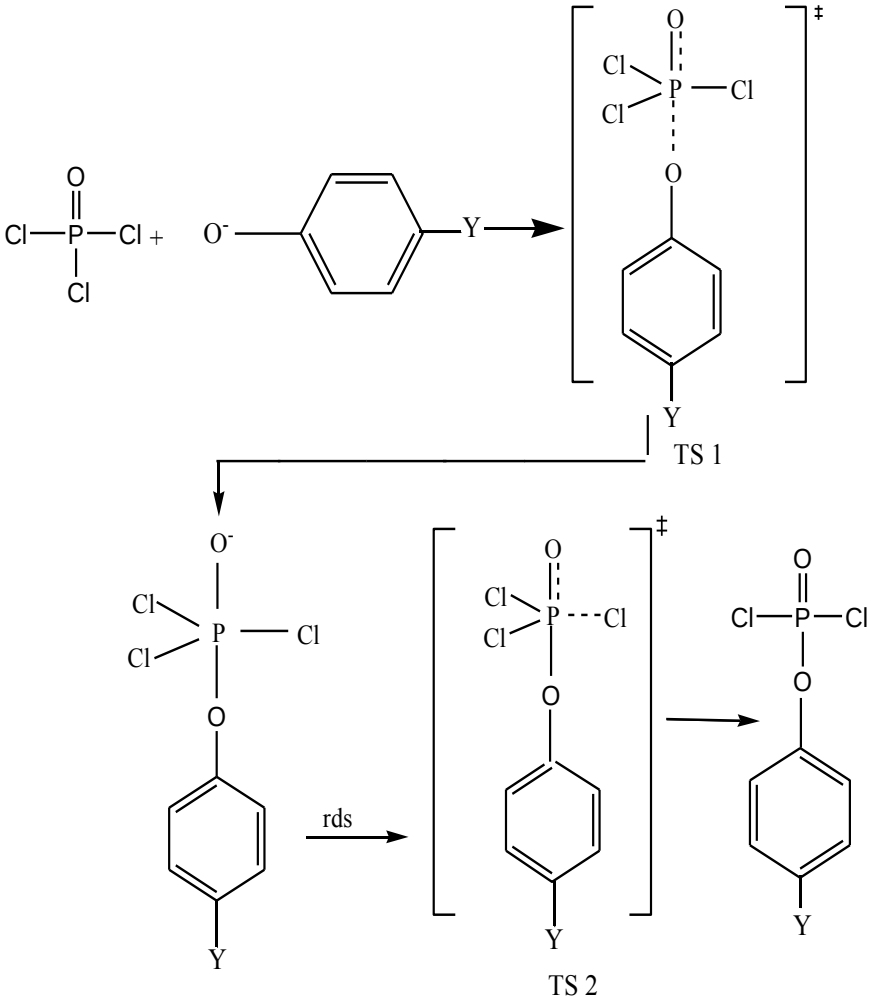

Grignard reagents form via the reaction of an aryl halide with magnesium metal. The reaction is conducted by adding the organic halide to a suspension of magnesium in an etherical solvent, which provides ligands required to stabilize the organomagnesium compound. Empirical evidence suggests that the reaction takes place on the surface of the metal. The reaction proceeds through single electron transfer. In the Grignard formation reaction, radicals may be converted into carbanions through a second electron transfer. Grignard reagents are extremly strong nucleophiles, the electrons in the C-Mg bond are heavily polarized toward carbon.

**Reaction with phosphorus oxy chloride:**



**Trigonal bipyramidal pentacoordinate intermediate:**

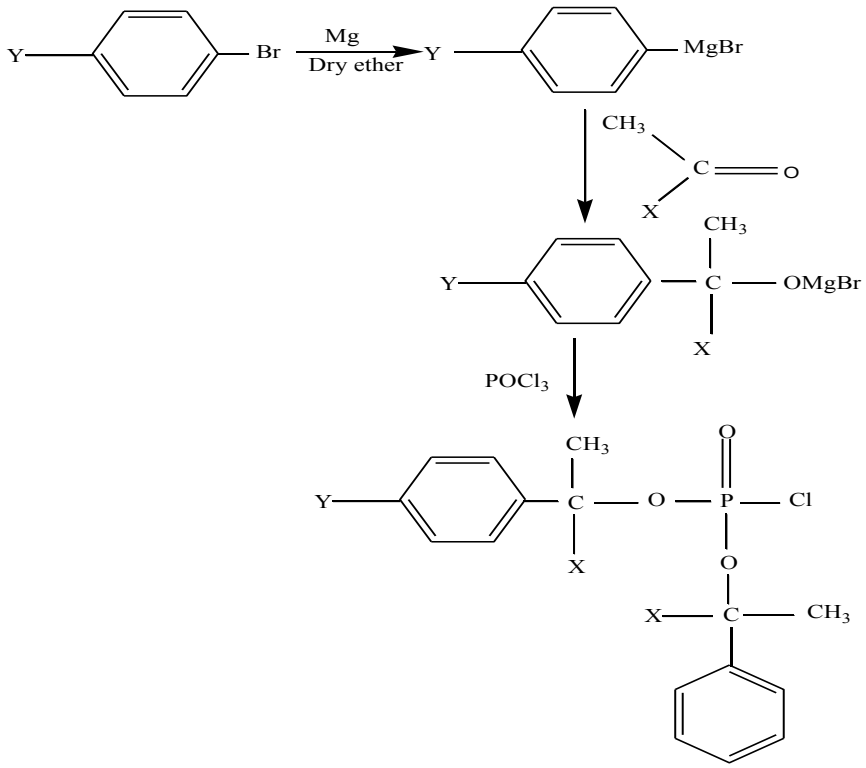
In this step produced nucleophile readily attacks the positive center of phosphorus of the phosphoryl chloride forming a transition state-1. As this state had very short life Period it readily rearranged and formed a trigonal bipyramidal pentacoordinate (TBP-5C) intermediate. This intermediate stage instantly converted to transition State-2 and then it decomposed rapidly to produce the phenyl substituted Chlorophosphate.



# SUMMARY

## SUMMARY

Organophosphorus compounds have tremendous importance in the field of pesticide, medical compounds, food technology, catalysis, animal foodstuffs, synthetic polymer, fire retardents and natural products. Nucleophilic substitutions at the carbon centre are very important topic in organic chemistry. Considerable amount of work have been carried out on nucleophilic substitutions at the carbon centre but much less is known about nucleophilic substitutions at the phosphorus centre. Nucleophilic substitutions at the phosphorus centre are very important topic in organophosphorus chemistry. The Nucleophilic substitutions at the carbon centre is well established but the mechanism of the nucleophilic substitution reaction at phosphorus is not well established. It has great interest to study nucleophilic substitution reaction at phosphorus in solutions. In view of the extensive use of the chlorophosphates we synthesize substituted aromatic chlorophosphine oxides from Grignard reagents through the following synthetic scheme



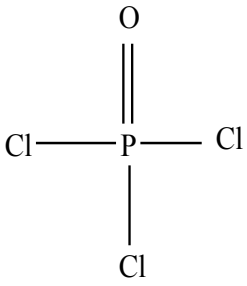
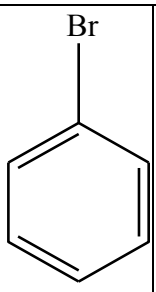
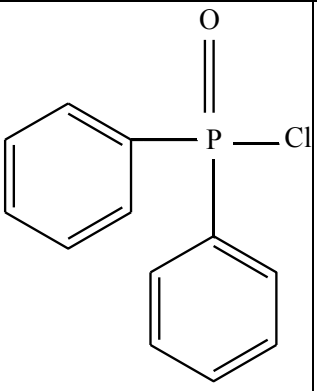
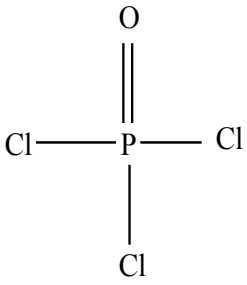
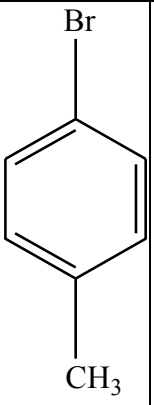
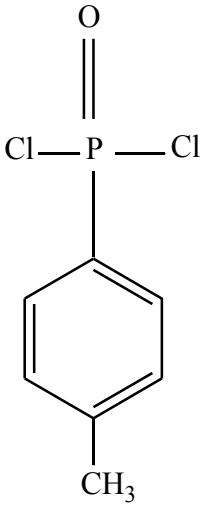
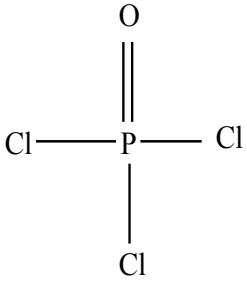
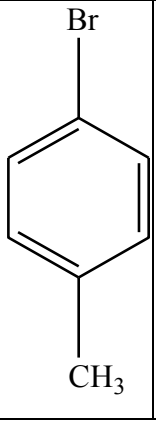
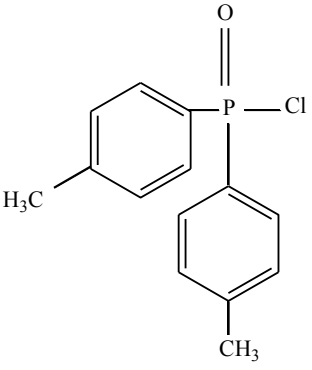

Here, X=$CH_3$, H and Y= $CH_3$, H

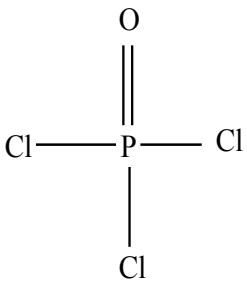
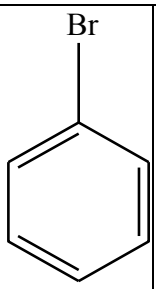
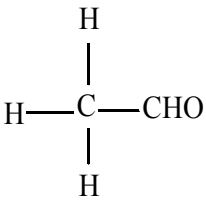
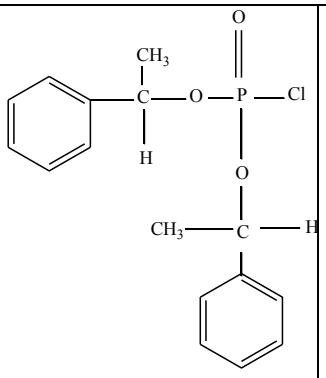
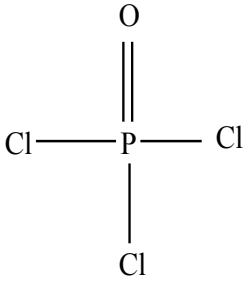
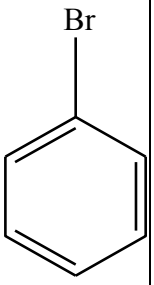
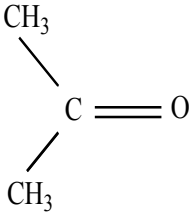
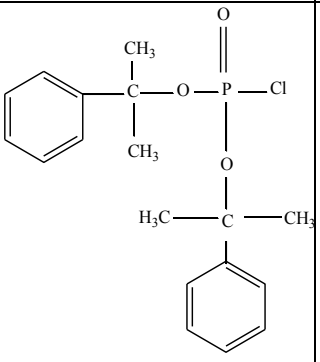
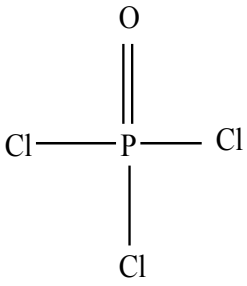
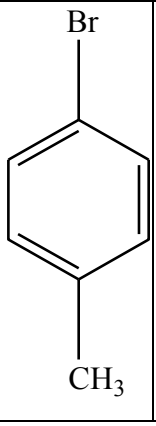
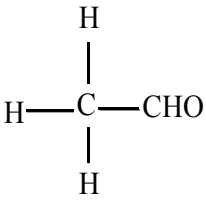
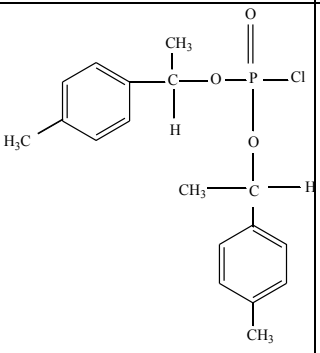
All the synthesized organophosphorus compounds were characterized by using physical and spectroscopic data such as m.p., % yield, IR, $^{1}H$ NMR spectrum.

Some of these compounds showed mild antibacterial activity.

The mechanism of the synthesis of organophosphorus compounds in this project follows nucleophilic substitution reaction at phosphorus centre of phosphorus oxy chloride with Grignard reagents in presence of dry ether at room temperature. The synthetic scheme is given in this chapter. The following table shows the synthesized compound in brief.

## Table-1 Synthesis of organophosphorus compound

| SI No | Starting materials | | | | Product | Melting point | % Yield |
|---|---|---|---|---|---|---|---|
| 1 |  | Mg |  | |  | $120^0$C | 50 |
| 2 |  | Mg |  | |  | $110^0$C | 80 |
| 3 |  | Mg |  | |  | $112^0$C | 80 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 4 |  | Mg |  |  |  | $110^0$C | 70 |
| 5 |  | Mg |  |  |  | $99^0$C | 45 |
| 6 |  | Mg |  |  |  | $120^0$C | 75 |

All the synthesized organophosphorus compounds were purified by column chromatography and characterized by using physical and spectral data such as m.p.,% yield, IR and $^1$H NMR.

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## 3.10 REFERENCES


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# PART – II

Chapter-1

# INTRODUCTION

## Introduction

From the time immemorial when the man felt necessity of medicine most probably when the man realized about the cause of disease, they have been trying to discover any preventive agent against disease. It is Universal truth that disease, decay and death always co-exist with human life. The study of disease and their treatment come together when the man achieved sufficient knowledge of chemistry.

Most of the people in rural and urban areas of the world were depended on the medicinal plants for the treatment of infectious diseases. The Ayurvedic and Unani systems of medicines are widely used by the people of Bangladesh subcontinent. Various plant species have been serving as the best natural source of drugs and medicines since the beginning of civilization. Most of the plant constituents, particularly the secondary metabolites possess potent antibacterial and antifungal activity. Among the different plant derived secondary metabolites, alkaloids proved to be the most important group of compounds that showed wide range of antimicrobial activity. [97-98] So, there is a continuing need to search for new antimicrobial agents since none of the available drugs is free from adverse effects and limitation. Medicinal plants possess various remedial properties along with worthless materials and it is important to separate the worthless materials from the good ones. So intensive antimicrobial and physiochemical investigation is needed in this field.

Infectious diseases are major health problems in Bangladesh requiring frequent use of antimicrobials. Diagnosis and treatment of most of the bacterial diseases are empirical. Microbial sensitivity patterns of common infections like respiratory tract infection, urinary tract infection, enteric fever, wound infection are not routinely available for decision making in drug selection. Many infectious diseases do not respond to conventional antimicrobial agents. Standard treatment guidelines of different microbes are not sufficient for the purpose; moreover the community awareness programme is imperfect. There is no routine antimicrobial surveillance or quality assurance in place. Multidrug resistant (MDR) TB in primary infection is ~3%, and MDR enteric fever is an emerging threat in Bangladesh. Due to drug resistant falciparum malaria, artemisinin-based combination therapy is used; visceral leishmaniasis is treated with oral miltefosine although sodium stibugluconate is still sensitive but found to be at

times toxic and difficult to deliver. Available evidence does not support the optimal diagnosis and treatment of bacterial infections in Bangladesh. Antibiotics are available as non-prescription drugs in medicine shops and irrational use is not uncommon. Adherence to treatment protocol and compliance with treatment course of antimicrobials need to be emphasized at different levels. Measures for prevention and containment of antimicrobial resistance are necessary in Bangladesh. It should be taken as a national priority and the establishment of a national alliance or regulation governing the use of antimicrobials should be considered.

Bangladesh is a developing country of South Asia. Rural population of this country is mostly dependant on agricultural cultivations. Plant diseases caused by different microorganism play a different role on the agrological condition. Various chemicals are used to kill the pathogenic microorganism. Some chemicals do not kill the microorganism. They simply inhibit the microbial growth. Microorganisms are very small living or non-living organisms, such as fungi, bacteria, and viruses. Most raw agricultural commodities, other unprocessed foods, water, and all decaying matter contain some types of microorganisms. Microorganisms occur on the skin and in intestinal tracts of people and animals. Under normal conditions, many microorganisms are beneficial and perform useful and necessary functions. In industry, for example, fungi and bacteria have important uses in manufacturing processes such as the production of organic acids. Some fungi have indispensable roles in the fermentation processes required for making wine, bread baking, cheese making, and brewing beer and other alcoholic beverages. Some types of intestinal bacteria help people and animals digest particular foods into usable nutrients. Certain other microorganisms, however, are harmful or dangerous. Should conditions become favorable for these microorganisms to grow, they may cause illnesses or diseases in people and animals, produce odors or stains, clog or damage equipment, or contaminate food and beverage products. As part of your duties where you work, you may need to apply substances known as antimicrobial pesticides to suppress or destroy troublesome microorganisms in order to protect people from illness, prevent contamination of food or beverages, or prevent damage to equipment and other items.

Many organophosphorus compounds have significant antimicrobial activity and have been developed into fungicides. Some of these are in commercial use. Examples of this group of fungicide are phosphonomycin, phosphonoformic acid (PFA) etc. Over the years, considerable

works have been done in the field of antimicrobial screening studies of chemical compounds. [99] Different classes of chemical compounds have been screened for in vitro antimicrobial activities all over the world. Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity. [100] Literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nuclei. [101] It was also revealed that if an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity. Results of our previous works on synthesis and antimicrobial evaluation of monosaccharide derivatives, it was noticed that in many cases the combination of two or more acyl substituent enhanced the antimicrobial activity considerably as compared to the parent molecule [111]. Encouraged by our past findings and in continuation of the project, we synthesized a series of D-mannose derivatives containing a wide variety of acyl substituents and evaluated their antimicrobial functionalities.

There are two types of organisms and tiny single-celled bacteria called aerobic and anaerobic bacteria in the human body. Aerobics are able to use oxygen, whereas anaerobic bacteria can sustain itself without the presence of oxygen. Aerobic bacteria use the oxygen present in the air for energy metabolism, versus anaerobic bacteria that does not need oxygen from the air for energy metabolism.

1. Aerobic bacteria inhale oxygen to remain alive.
2. Anaerobic bacteria die in the presence of oxygen, and therefore avoid $O_2$.
3. Aerobic respiration produces energy with the help of a complex process in the cells.
4. Anaerobic respiration produces crystals, and causes pain in muscled areas.
5. Humans and animals, and most fungi, etc are all obligate aerobes that need to breathe and inhale oxygen to survive.

Shahed [110] performed antifungal activities of heterocylic nitrogen compounds. They used four plan pathogenic fungi such as Fusarium equisetai, Macrophomia phaseolina, Alternaria alternata and Curvularia lunata. They found good inhibition against these tested organisms.

Rahman [106] Showed that antimicrobial activities of that antimicrobial activity of the alkaloids of three plants levels. The alkaloid fractions were screened against eight pathogenic bacteria. viz.

*Salmonella typhi, Sheigella dysenteri, Sheigella sonnei, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphyococcus aurreus, Pscudomonas aeruginosa.* The highest zone of inhibition was recorded against *Salmonella typhi.*

Abe Kawasar [111] carried out vitro antimicrobial activities of a series of acylated uridine derivatives. They used ten bacteria such as *Salmonella typhi, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphyococcus aurreus, Pscudomonas aeruginosa, Sheigella dysenteri, Sheigella dysenterial* INABA-ET (vibrio) and *Sarcina lutea*. It was observed that most of the acylated compounds exhibit moderate to good antibacterial activity. Amongst the acylated compounds habit moderate to good antibacterial activity.

Fakruddin [109] carried out antifungal activities of fused pyrimidine. They used pathogenic five human pathogenic bacteria, viz. *Salmonella typhi, Bacillus megaterium, Bacillus subtilis, Staphyococcus aurreus, Esherichia coli* and four phytopathogenic fungi, viz. *Verticillum SP, Fusarium solanae, Aspergilius SP, Penicillum SP*. They found that some of the tested chemicals showed very effective antibacterial and antifungal activity.

Chapter-2

# METHODOLOGY OF THE BIOLOGICAL WORK

## 2.2.1 Materials and Method

Bacteria and fungi are responsible for many diseases. The clinical importance of drug resistant microbial pathogens has lent additional urgency antimicrobial research. The antimicrobial screening which is the first stage of antimicrobial research is performed to ascertain the susceptibility of various microbes to any agent. This test measures the ability of each antimicrobial agent to inhibit the vitro microbial growth.

This ability may be estimated by any of the following three methods

i. Disc diffusion method
ii. Serial dilution method
iii. Bioautographic method

The disc diffusion technique (Bauer [102]) is a widely accepted in Vitro investigation for preliminary screening agents which may possess any antibacterial activity. It is essentiality a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between baceriostatic and bactericidal activity between then methods (Roland [103]).

## 2.2.2 Principle of Disc Diffusion Method

Solution of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in definite volume of solvents. Dried and sterilizer filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette . Discs containing the test materials are placed on nutrient agar medium uniformly seeded with the test microorganism. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature ($4^0$C) for 2h to allow maximum diffusion. During the time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffusion of molecules through agar gel. As a result there is gradual change of test materials concentration in the media surrounding the discs. The plates are then incubated at $37^0$C for 24h to allow maximum growth of the organisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganism

giving a clear, distinct zone call –Zone of Inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter.

The experimental is carried out more than once and the mean of the readings is calculated (Bauer [102]). In the present study some pure compounds were tested for antibacterial activity by discs diffusion method.

## 2.2.3Experimental

### 2.2.3. A Apparatus and Reagents

| | |
|---|---|
| Filter paper discs | Screw cap test tubes |
| Sterile cotton | Autoclave |
| Micropipette | Nutrient Agar Medium |
| Laminar air flow hood | Inoculating loop |
| Refrigerator | Spirit burner |
| Chloroform | Nose mask and Hand gloves |
| Petri dishes | Incubator |
| Sterile forceps | Ethanol |

### 2.2.3. B Test of Organisms

Gram positive and Gram negative (aerobic & anaerobic) organisms and fungi were taken for the test and they are listed in the table-6 and table-7.

**Table-6: List of Test Bacteria**

| **Gram positive** | **Gram negative** |
|---|---|
| *Bacillus cereus* (aerobic) | *Aeromonus hydrophilia* (anaerobic) |
| *Bacillus megaterium* (aerobic) | *Esherichia coli* (aerobic) |
| *Bacillus subtilis* (aerobic) | *Pscudomonas aeruginosa* (aerobic) |
| *Staphyococcus aurreus* ( aerobic) | *Salmonella parratyphi A*( aerobic) |
| *Streptococcus agalactiae* ( aerobic) | *Salmonella parratyphi C*( aerobic) |
| *Mycobacterium tuberculosis* (aerobic) | *Salmonella parratyphi SPP*( aerobic) |
| *Nocardia asteroids* (anaerobic) | *Sheigella boydii* (anaerobic) |
| *Nocardia brasiliensis* (anaerobic) | *Sheigella dysenteri* (anaerobic) |
| *Nocardia farcinica* (anaerobic) | *Sheigella flexneriae* (anaerobic) |
| | *Sheigella sonnei* (anaerobic) |
| | *Vibrio mimicus* (aerobic) |
| | *Vibrio parahemolyticus* (aerobic) |

**Table-7: List of Fungi**

| **Fungi** |
|---|
| *Aspergillus niger* |
| *Candida albicans* |
| *Rhizopus oryzae* |
| *Saccharo myces cerevisiae* |

## 2.2.4 Test of Materials

**Table-8: List of Test Materials**

| **SL No** | **Compounds No.** | **Name of Test Materials** |
|---|---|---|
| **1** | **1A** | Diphenyl chlorophosphine oxide |
| **2** | **2A** | (*p*-tolyl) dichlorophosphine oxide |
| **3** | **3A** | Bis (*p*-tolyl) chlorophosphine oxide |
| **4** | **4A** | Bis (phenyl acetoxy) chlorophosphine oxide |
| **5** | **5A** | Bis (dimethyl phenyl methoxy) chlorophosphine oxide |
| **6** | **6A** | Bis (*p*-tolyl methyl methoxy)) chlorophosphine oxide |

## 2.2.5 Culture Medium

Muller-Hinton (MH) medium and potato Dextrose Agar (PDA) were used for making plates on which antibacterial and antifungal sensitivity tests were carried out respectively. The antibacterial activities of the materials were detected by diffusion method (Bauer [102]) and antifungal activities of the materials were assessed by food poison technique (Miah [1104] and Grover [105]). This media were also used to prepare fresh cultures.

## 2.2.6 Medium Used

Nutrient Agar (NA) and Dextrose Agar (PDA) were used thoroughly out the work. The composition and preparation procedure of NA and PDA are described blow.

**Composition of Nutrient Agar Medium**

| **Ingredients** | **Amounts (gm/lit)** |
|---|---|
| Peptone | 5.0 |
| Sodium chloride | 5.0 |
| Beef extract | 1.5 |
| Yeast extract | 1.5 |
| Agar | 14.0 |
| pH (at $250^0$C) | 7.2-7.6 |

**Procedure**

To prepare required volume of this medium, calculated amount o each of the constituents was taken in a conical flask and distilled water was added to it make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at $250^0$C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by auto calving at 15-1bs/sq, pressure at $121^0$C for 20 minutes. The slants were used for making fresh culture of bacteria that were in turn used for sensitivity study.

## 2.2.7 Composition of potato Dextrose Agar

| **Ingredients** | **Amounts (gm/lit)** |
|---|---|
| | |
| Potato | 200.0 |
| Dextrose | 20.0 |
| Agar | 15.0g |

**Procedure**

200g of sliced potato was boiled in 500ml distilled water and extract decanted after paper boiling. The extract was taken in a 1000 ml beaker and solution was made up to the mark with distilled water. This solution was taken in suspense and 20g dextrose was added slowly in the solution. Then 15g of agar powder was added in the solution and they were mixed thoroughly with a glass rod. After 10 minutes of boiling the medium was transferred in 250 ml conical flask was closed with the cotton plug and rapping with aluminium foil. The medium was autoclaved for 15 minutes at $121^0$C and 15-1bs/sq pressure. After autoclaving the medium was used for culture of different microorganisms.

## 2.2.8 Sterilization Procedure

In order to avoid any type of contamination by the test organisms the antibacterial screening was done in laminar hood and all types of precautions were highly maintained. UV light was switched on an hour before working in the laminar hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of $121^0$C and pressure of 15-1bs/sq for 20 minutes. Micropipette tips, cotton, forceps dishes etc. were also sterilized.

## 2.2.9 Preparation of Subculture

In an aseptic under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24h at $37^0$C for their optimum growth. These fresh cultures were used for the sensitivity test.

## 2.2.10 Preparation of the Test Plates

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anti clockwise to assure homogenous distribution of the best organisms in the media.

## 2.2.11 Preparation of Dishes

Three types of dishes were used for antibacterial screening.

**Standered discs**

These were used as positive control to ensure the activity of slandered antibiotic against the test organisms as well as comparison of the response produced by the known antibacterial agent with that of produced by the test sample. In this investigation, kanamycin (30µg/dishes) standerd disc was used as the reference.

**Blank discs**

These were used as negative control which ensures that the residual solvents (left over the discs even after air-drying) and filter paper were not active themselves.

**Preparation of Sample Discs with Test Sample**

Measured amount of each test sample dissolved in Specific volume of solvent to obtain the desired concentrations in an aseptic condition. Then discs were soaked with solution of test samples and dried.

### 2.2.12 Diffusion and Incubation

The test sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at $4^0$C for about 24h to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at $37^0$C for 24h.

### 2.2.13 Determination of Antibacterial Activity by Measuring the Zone of Inhibition

After incubation, the antibacterial actives of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

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Chapter-3

# RESULT & DISCUSSION

## 2.3.1 Result and Discussion

A result of six (**1A, 2A, 3A, 4A, 5A, 6A**) organophosphorus derivatives have been tested for in vitro antimicrobial activity against Gram positive and Gram negative bacteria as for human fungal pathogens. The selected microbes were collected as fresh cultures from the Beximco pharmaceuticals Limited, Charagali, Tongi, Dhaka. No clinically isolated resistant strains were used for the present study. The antimicrobial activities were measured in terms of diameter zone of inhibition (mm).All the experiment was performed thrice to minimize the experiment plus individual errors. The mean values of the diameters of zone of inhibition (M.DIZ) were taken as in discs for determining antimicrobial spectra. Sensitivity test results are interpreted in (Tables-9) and were compared with standard antibiotic kanamycin (30µg/disc).

The Gram positive as well as Gram negative (Aerobic) bacteria used in the present investigation was found to be completely resistant four Organo-oxy phosphorus compounds (**1A, 2A, 3A, 6A**) at a dose level of 200µg /disc (table-9). But aerobic (*Staphylococcus aurreus, Esherichia coli, Pscudomonas aeruginosa, Salmonella parratyphi A Salmonella parratyphi C, Salmonella parratyphi SPP*), anaerobic bacteria and Fungi were not found any Organo-oxy phosphorus compounds. The compounds **3A, 6A** showed mild in vitro antimicrobial activity especially against the Gram positive bacteria *Bacillus cereus* **3A** (M.DIZ 12), **6A** (M.DIZ 12) *Bacillus megaterium* **3A** (M.DIZ 11), **6A** (M.DIZ 13), *Bacillus subtilis* **3A** (M.DIZ 10), **6A** (M.DIZ 10), *Streptococcus agalactiae* **3A** (M.DIZ 12), **6A** (M.DIZ 11) and *Mycobacterium tuberculosis* (aerobic) **3A** (M.DIZ 13), **6A** (M.DIZ 10) and Gram negative bacteria *Vibrio mimicus* **3A** (M.DIZ 12), **6A** (M.DIZ 11) and *Vibrio parahemolyticus* **3A** (M.DIZ 13), **6A** (M.DIZ 11). The compounds **1A, 2A** showed mild in vitro antimicrobial activity especially against the Gram positive bacteria *Bacillus cereus* **1A** (M.DIZ 10), **2A** (M.DIZ 12) *Bacillus megaterium* **1A** (M.DIZ 10), **2A** (M.DIZ 10), *Bacillus subtilis* **2A** (M.DIZ 11), *Streptococcus agalactiae* **1A** (M.DIZ 11), **2A** (M.DIZ 11) and *Mycobacterium tuberculosis* **1A** (M.DIZ 10), **2A** (M.DIZ 13) and Gram negative bacteria *Vibrio mimicus* **1A** (M.DIZ 10), **2A** (M.DIZ 12) and *Vibrio parahemolyticus* 1**A** (M.DIZ 11), **6A** (M.DIZ 13). This study can therefore, confer that substitution at the oxyphosphorus oxygen increase antimicrobial activity through it concludes that oxyphosphorus group is essential for such microbial group inhibition.

The two Organo-oxy phosphorus compounds **4A** and **5A** exhibited mild to moderate degrees of bacteria group inhibition at 200µg /disc dose level (table-9). Among the selected microbes, *Bacillus cereus* **4A** (M.DIZ 10), *Vibrio mimicus* **4A** (M.DIZ 10), **5A** (M.DIZ 10) and *Vibrio parahemolyticus* **5A** (M.DIZ 10) were found to be sensitive these size phosphates.

It appears that oxygen phosphorus linkage bondate compounds are necessary for antimicrobial action since the corresponding **1A, 2A, 3A** and **6A** table 9 and **4A** and **5A** table 9 were completely resistant to these bacteria. Therefore, it is possible to conclude that the oxyzen phosphorus linked group is required for antimicrobial action. From the above discussion it is said that oxygen is necessary for these compounds.

## 2.3.2 Conclusion

A total of six (**1A, 2A, 3A, 4A, 5A,6A**) organophosphorus derivatives have been tasted for in vitro antimicrobial activity against Gram positive and Gram negative bacteria as for human fungal pathogens. Most of this compound demonstrated mild to moderate antimicrobial activity against most of the test organism. Among tested compounds **1A, 2A, 3A** and **6A** exhibited relatively greater inhibition of growth of the microorganism as comparative to the **4A** and **5A** analogous. The higher activity of the compounds **1A, 2A, 3A, 6A** cold probably is due to their greater solubility in non-polar medium, which subsequently facilitated the diffusion of the chemical entities through the microbial cell wall.

Substitution of Chlorine atom of phosphorus oxy trichlorides with bulkier aromatic oxy group increases the antimicrobial activity of the compounds **1A, 2A, 3A, 6A.**

However, substitution at para-position the bulky group of Bis (*p*-tolyl) chlorophosphine oxide (*p*-tolyl) **3A** or **6A** Bis (*p*-tolyl methyl methoxy) chlorophosphine oxide revealed better microbial growth inhibition than other substitution.

**Table9: In Vitro Antimicrobial activity of Synthetic organophosphorus compounds**

| **Diameter of Zone of inhibition(mm)** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Done | 200µg /disc | 200µg /disc | 200µg /disc | 200µg /disc | 200µg /disc | 200µg /disc | 30µg/disc |
| **Microorganisms** | **1A** | **2A** | **3A** | **4A** | **5A** | **6A** | **kanamycin** |
| **Gram positive** | | | | | | | |
| *Bacillus cereus* (aerobic) | 10 | 12 | 12 | 10 | - | 12 | 23 |
| *Bacillus megaterium* (aerobic) | 11 | 10 | 11 | - | - | 13 | 24 |
| *Bacillus subtilis* (aerobic) | - | 11 | 10 | - | - | 10 | 23 |
| *Staphylococcus aurreus* (aerobic) | - | - | - | - | - | - | 22 |
| *Streptococcus agalactiae* (aerobic | 11 | 11 | 12 | - | - | 11 | 20 |
| *Mycobacterium tuberculosi* (aerobic) | 10 | 13 | 13 | - | - | 10 | 22 |
| *Nocardia asteroids* (anaerobic) | NT | - | - | - | - | - | 24 |
| *Nocardia brasiliensis* (anaerobic) | - | - | NT | NT | NT | NT | 22 |
| *Nocardia farcinica* (anaerobic) | - | - | - | - | - | - | 24 |
| *Nocardia farcinica* (anaerobic | NT | - | NT | - | - | NT | 26 |
| **Gram Negative** | | | | | | | |
| *Aeromonus hydrophili* (anaerobic) | - | - | NT | NT | NT | NT | 23 |
| *Esherichia coli* (aerobic) | - | - | - | - | - | - | 22 |
| *Pscudomonas aeruginosa* (aerobic | - | - | - | - | - | - | 20 |
| *Salmonella parratyphi A* (aerobic) | NT | NT | - | - | - | - | 24 |
| *Salmonella parratyphi C*(aerobic) | - | - | - | - | - | - | 22 |
| *Salmonella parratyphi SPI* (aerobic) | - | - | - | - | - | - | 23 |
| *Sheigella boydii* (anaerobic) | - | - | NT | NT | NT | NT | 25 |
| *Sheigella dysenteri* (anaerobic) | - | - | - | - | - | - | 22 |
| *Sheigella flexneriae* (anaerobic) | NT | NT | - | - | - | - | 23 |
| *Sheigella sonnei* (anaerobic) | NT | NT | NT | NT | NT | NT | 25 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Vibrio mimicus* (aerobic) | 10 | 12 | 12 | 10 | 11 | 11 | 22 |
| *Vibrio parahemolyticus* (aerobic) | 11 | 13 | 13 | - | 10 | 11 | 22 |
| **Fungi** | | | | | | | |
| *Aspergillus niger* | NT | NT | - | - | - | - | 22 |
| *Candida albicans* | - | - | NT | NT | NT | NT | 23 |
| *Rhizopus oryzae* | - | - | - | - | - | - | 24 |
| *Saccharo myces cerevisiae* | - | - | NT | - | - | - | 25 |

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