#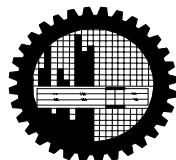 SYNTHESIS AND CHARACTERIZATION OF ORGANOPHOSPHORUS COMPOUNDS COMPOSED OF DIAMINO FUNCTIONALITIES AND STUDY OF THEIR BIOLOGICAL ACTIVITY

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

SUBMITTED BY

SHARMEEN NISHAT

STUDENT NO. 040803107P

SESSION: APRIL, 2008

ORGANIC RESARCH LABORATORY
DEPARTMENT OF CHEMISTRY
BANGLADESH UNIVERSITY OF ENGINERING AND TECHNOLGY (BUET)
DHAKA-1000, BANGLADESH
JULY, 2011

Dedicated

TO

My Parents

CANDIDATE'S DECLARATION

This thesis work has been done by the candidate herself 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.

July, 2011

_____
(Sharmeen Nishat)
M. Phil Student
Roll No.040803107P
Department of Chemistry
BUET, Dhaka
Bangladesh-1000.

Bangladesh University of Engineering and Technology, Dhaka
Department of Chemistry

Certification of Thesis

A thesis on

SYNTHESIS AND CHARACTERIZATION OF ORGANOPHOSPHORUS COMPOUNDS COMPOSED OF DIAMINO FUNCTIONALITIES AND STUDY OF THEIR BIOLOGICAL ACTIVITY

BY

Sharmeen Nishat

Roll No: 040803107P, Session: April/2008 has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Master of Philosophy (M. Phil) in Chemistry on July 13, 2011.

Board of Examiners

1. **Dr. Md. Abdur Rashid**
   Professor
   Department of Chemistry
   BUET, Dhaka.
   (Supervisor)

   _____
   Chairman

2. **Head**
   Department of Chemistry
   BUET, Dhaka.

   _____
   13.7.11
   Member (Ex-officio)

3. **Dr. Shakila Rahman**
   Professor
   Department of Chemistry
   BUET, Dhaka.

   _____
   Member

4. **Dr. Umme Kulsum Rowzatur Romman**
   Professor
   Department of Chemistry
   Dhaka University, Dhaka-1000.

   _____
   Member (External)

ACKNOWLEDGEMENTS

Abbreviations used

UV   : Ultraviolet

IR   : Infrared

FTIR   : Fourier Transformed Infrared

NMR   : Nuclear Magnetic Resonance

S   : Singlet

bs   : Broad Singlet

d   : Doublet

t   : Triplet

m   : Multiplet

J   : Coupling Constant

Hz   : Hertz

TMS   : Tetramethylsilane

δ   : Chemical Shift

DEPT   : Distortionless Enhancement by Polarization Transfer

COSY   : Correlation Spectroscopy

HMBC   : Heteronuclear Multiple Bond Correlation

w/w   : weight/weight

TLC   : Thin Layer Chromatography

R_f : Retardation Factor

CONTENTS

PART –I

ABSTRACT

Organophosphorus compounds have tremendous importance in the field of food technology, animal foodstuffs, pesticides, medicinal compounds, synthetic polymers, fire retardants and natural products. It has great interest to study nucleophilic substitutions reactions at phosphorus in solutions. In view of the extensive use of the chlorophosphates we synthesize organophosphorus compounds composed of diamino functionalities from diamines through the following synthetic scheme
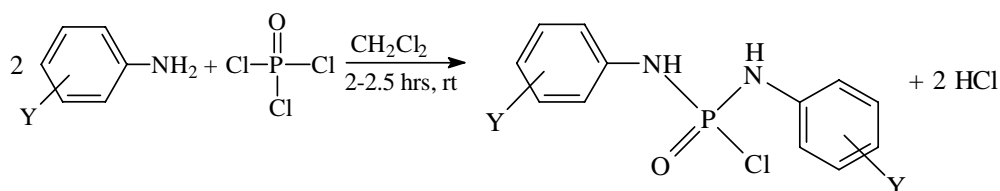
Here, Y= p-NH_2, o-NH_2, m-NH_2
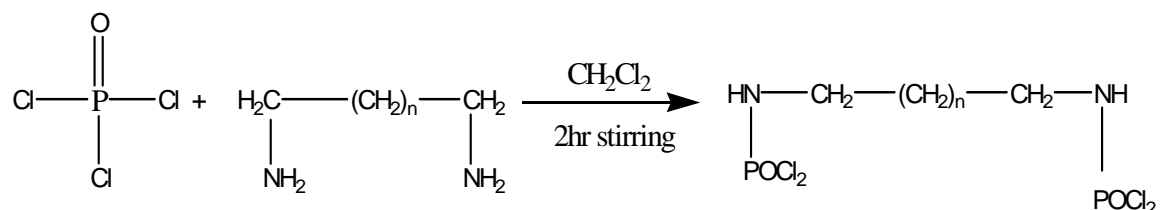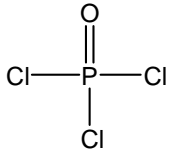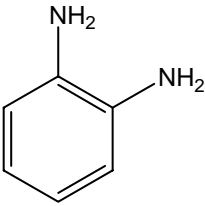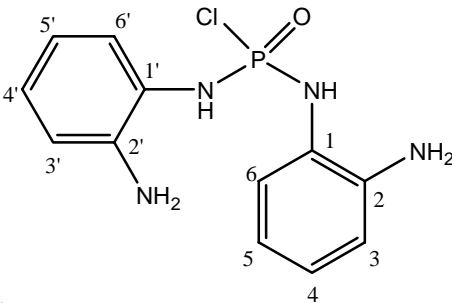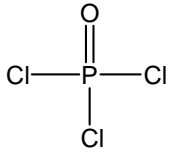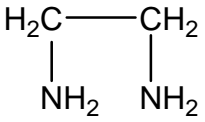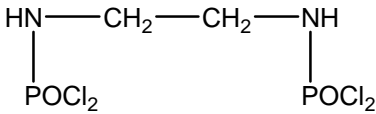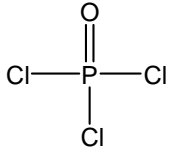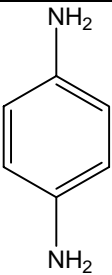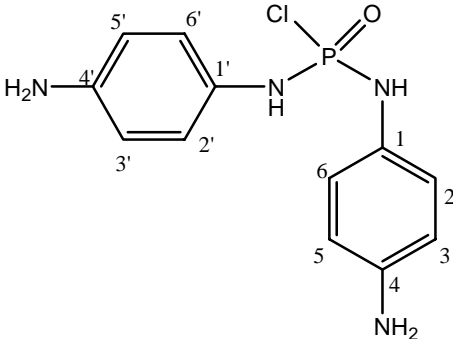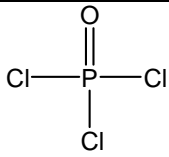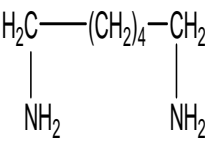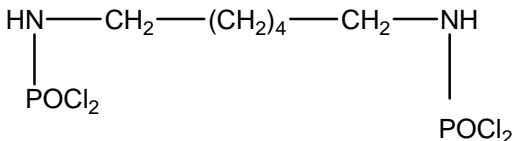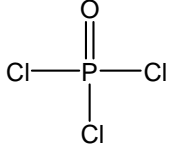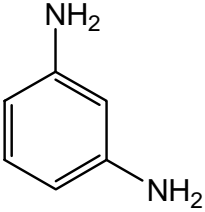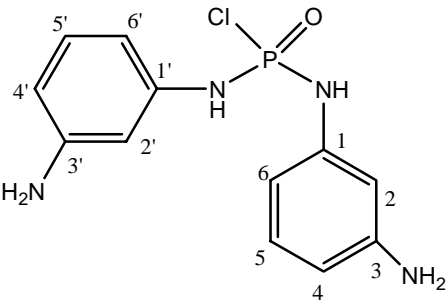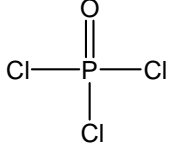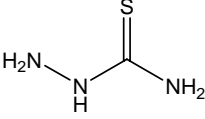
Here n = 0 or 4

All the synthesized compounds were characterized by using analytical data obtained from m.p., IR, ^{1}H NMR, ^{13}C NMR, DEPT 135 NMR, ^{31}P NMR and 2D NMR spectrum.

The mechanism of the synthesis of organophosphorus compounds in this project follows nucleophilic substitution reaction at phosphorus centre of phosphoryl chloride with substituted aromatic amine in presence of methylene chloride. The synthetic scheme is given in this chapter.

The following table shows the synthesized compound in brief.

Sl No.	Starting materials		Product	% Yield
1.	Phosphorylchloride	orthophenylene Diamine	bis–(o–phenylenediamino) chlorophosphine oxide	75
2.	Phosphorylchloride	Ethylene diamine	1,2-Bis-(dichlorophosphamido) ethane	78
3.	Phosphorylchloride	paraphenylene diamine	bis–(p–phenylenediamino) chlorophosphine oxide	62
4.	Phosphorylchloride	Hexamethylene diamine	1,6-Bis-(dichlorophosphamido) hexane	70

5.	Phosphorylchloride	Metaphenylene diamine	bis–(*m*–phenylenediamino) chlorophosphine oxide	69
6.	Phosphorylchloride	Thiosemicarbazide	Not Characterized	--

All the synthesized products were tested for anti-bacterial activities against gram positive and gram negative bacterias. Some of these compounds showed good antibacterial activity and some showed moderate activity. Again some compounds exhibited poor activity against bacteria.

PART- I

Chapter 1

INTRODUCTION

1. Introduction

1.1 Organic Synthesis

Organic synthesis is the complete chemical synthesis of complex organic molecules from simple, commercially available or natural precursors[1]. In a linear synthesis there is a series of steps that are performed one after another until the, molecule is synthesized-this is often adequate for a simple structure. The chemical compounds prepared in each step are usually referred to as "synthetic intermediates". For more complex molecules, a convergent synthesis is often performed. This is where several pieces (key intermediates) of the final product are synthesized separately, then coupled together, often near the end of the synthesis.

Robert Burns Woodward is regarded as the father of modern organic synthesis, who received the Nobel Prize in 1965 in Chemistry for several brilliant examples of total synthesis such as synthesis of strychnine[2].

1.2 Process of Organic Synthesis

Each step of a synthesis involves reagents, conditions and chemical reaction for each of these reactions need to be designed to get a pure product preparing a good yield, with as little work as possible[3]. A method may already exist in the literature for preparing one of the early synthetic intermediates, and this method will usually be used rather than "trying to reinvent the wheel". However most intermediates are compounds that have never been synthesized before, and these will normally be synthesized using general methods developed by Chemists. These methods are useful and need to get high yields and are reliable for a broad range of substrates. Methodology researcher usually targets three main stages- discovery, optimization and studies of scope and limitations. The discovery may be due to serendipity, or may be from a flash of insight. Optimization is where one or two starting compounds are tested in the reaction under a wide variety of conditions of temperature, solvent, reaction, time etc., until the optimum conditions for product yield and purity are found. Then the researcher tries to extend the method to a broad range of different starting materials, to find the scope and limitations. Some

larger research groups may then perform a total synthesis by using the new methodology and demonstrate its value in a real application.

1.3 History of Synthetic Chemistry

In mid-nineteenth century, the development of a radical new direction in chemistry: instead of simply analyzing existing molecules is remarkable. The combination of this new synthetic approach with more traditional analytical approaches revolutionized chemistry, leading to a deep understanding of the fundamental principles of chemical structure and reactivity and to the emergence of the modern pharmaceutical and chemical industries. The history of synthetic chemistry offers a possible roadmap for the development and impact of synthetic biology, a nascent field in which the goal is to build novel biological systems[4]

In 1828, the German chemist Friedrich Wöhler wrote to his former mentor, Jöns Jakob Berzelius with lots of excitation about his new finding[5,6] that he could prepare urea without requiring a kidney of an animal either man or dog. At the beginning of the nineteenth century, the synthesis of this small organic molecule was earth-shattering news. At that time, chemists believed there was a clear distinction between molecules from living beings (referred to as 'organic') and those from nonliving origin (inorganic). It was known that organic substances could be easily converted to inorganic compounds through heating or other treatments; however, chemists could not perform the reverse transformation. Surely, a 'vital force' present only in living organisms was required to convert the inorganic into organic. Wöhler's discovery that ammonium cyanate could be converted to urea in the laboratory was a key nail in the coffin of vitalism, and in the next few decades, chemists began to synthesize hundreds of other organic molecules. In a particularly interesting example in 1854, the French chemist Marcellin Berthelot synthesized the fat molecule tristearin from glycerol and stearic acid, a common naturally occurring fatty acid. Taking this a step further, he realized that he could replace stearic acid with similar acids not found in natural fats, thus generating non-natural molecules that had properties similar to those of natural fats[7]. These and other early syntheses demonstrated that chemists could indeed make 'living' molecules as well

as new compounds that went beyond those that naturally occurred, thus giving birth to synthetic organic chemistry. It was not transparent where this field would lead, and many feared these advances could lead to goals such as the creation of living beings. Today, however, nearly all aspects of our lives are touched by synthetic molecules that mankind has learned to make.

Advances in our ability to build and modify 'organic' molecules on increasingly larger scales have continued to push the frontier of our understanding of the physical principles underlying living systems. For example, chemical synthesis of DNA oligonucleotides (first performed by Gobind Khorana) led directly to the elucidation of the genetic code[8] Bruce Merrifield's complete synthesis of RNase A demonstrated that chemical structure (primary sequence) is sufficient to confer tertiary structure and the seemingly magical activity of enzymes[9]. More recently, complete chemical synthesis of poliovirus complementary DNA was a vivid demonstration that genetic instructions are sufficient to specify an active biological system[10]

Over the last several years, this line of research has culminated in the emergence of a field known as 'synthetic biology.' Whether synthetic biology represents a truly new field is open to debate, but the boldness of the stated goals—to learn how precisely and reliably engineer and build self-organizing systems that both recapitulate biological function and show new functions—is unquestionably novel. These goals hold promise for harnessing the efficiency and precision of living systems for diverse purposes: microbial factories that manufacture drugs, materials or biofuels[11]cells that seek and destroy tumors[12]cells that can carry out rapid tissue repair and regeneration; cells that can direct the assembly of nanomaterials; even living systems that can compute. Synthetic biology, however, is more than a broad set of visionary applications. Much effort is going into developing a common toolkit of well-defined biological parts and devices as well as strategies to link them together into predictable systems[13-16]. These foundational efforts are aimed at one day making engineering cells as reliable and predictable as engineering an electronic device.

The synthetic approach—empirically learning how to systematically manipulate and perturb molecules—directly contributed to developing theories of chemical structure and reactivity. At the simplest level, synthesizing a molecule was often the ultimate proof of the proposed structure. More significantly, the ability to synthesize variants of known molecules allowed a rigorous and systematic exploration of the principles underlying chemical structure and reactivity, thus beginning the field of physical organic chemistry.
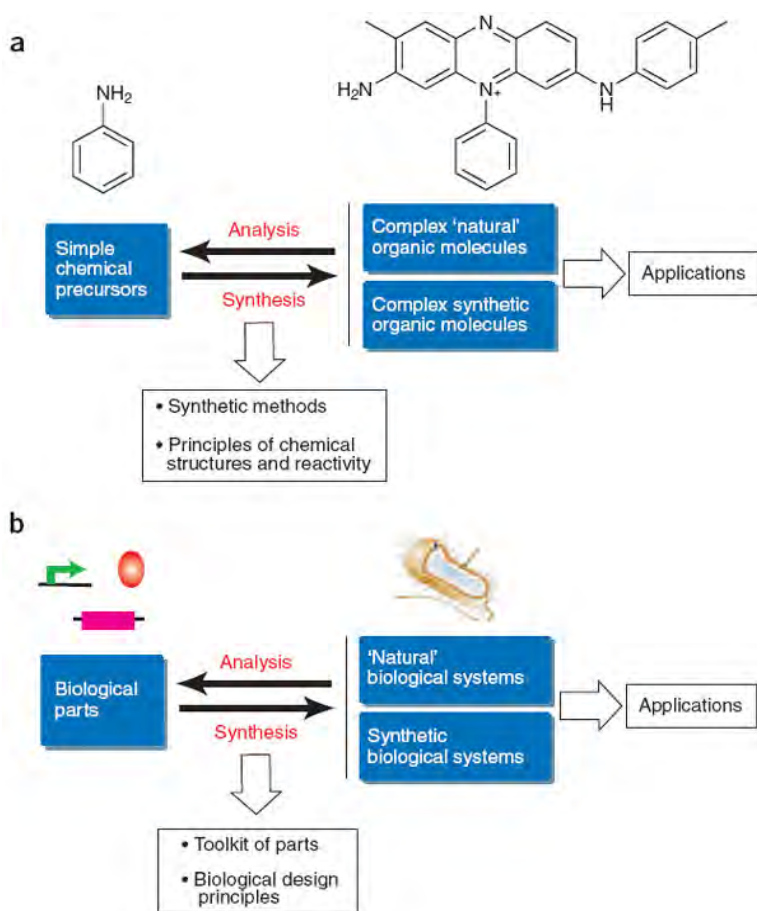
Figure: Synthesis and analysis are complementary

(**a**) In organic chemistry, analysis and synthesis were both critical in determining fundamental principles of chemical structure and reactivity. Synthetic molecules have been used for a wide variety of applications. (**b**) Similarly, synthetic approaches will complement analytical methods in elucidating biological principles, and synthetic cellular systems will prove highly useful.

Like all technologies, synthetic organic chemistry also introduced its own set of problems. In addition to the plethora of beneficial drugs and polymers that have significantly increased our standard of living, harmful or 'dual-use' compounds, including explosives and chemical weapons, have also been created.

In today's world, many tend to link synthetic chemistry with the production of drugs. Indeed, it was abundantly clear to early chemists that synthetic products could improve human health, but their initial efforts actually led to an industrial explosion in an unexpected direction. August von Hofmann and his student William Perkin postulated that it might be possible to synthesize the highly valuable antimalarial agent quinine from aniline, a cheap product of coal tar[7]. However, in attempting to synthesize quinine from aniline in 1856, Perkin unexpectedly produced a brilliant purple compound—a dye. He soon opened a factory to synthesize this molecule, which he called "Aniline Purple," and thus founded the synthetic dye industry. Along with other examples, such as the synthesis of indigo by Adolf von Baeyer in 1867, these advances led to the explosive growth of the German and Swiss dye industry, while simultaneously dismantling the import of indigo and other natural dyes from distant tropical locales. In fact, synthetic indigo remains an important commercial product and is used in today's blue jeans.

Although dyes were the earliest economically important synthetic compounds, the development of the European dye industry would ultimately lead to successes in chemotherapy. Indeed, nearly all of the modern big pharmaceutical companies are in part descended from German or Swiss dye manufacturers. For example, the first effective antibacterials were the sulfa drugs[17]The first of these molecules, sulfanilamide, was synthesized by IG Farbenindustrie in 1908 because of its potential as a dye. In 1932, Gerhard Domagk discovered that sulfanilamide and related compounds have bactericidal activity, and he was aided in his studies by chemists that could make a variety of related compounds.

1.4 The Role of Synthetic Chemistry in Drug Discovery

Synthetic chemistry is considered as a tool for drug discovery because can only carry out biological evaluation of compounds that have been synthesized.Once the evaluation of biological activity and physical properties has been used to design new targets, a suitable synthetic route must be developed. However, considerations of what can be readily prepared factor into design much earlier. Chemists typically recognize familiar structural features for which they know a feasible synthetic route as they analyze data and properties. Design is guided by what can be readily made, especially what can be prepared as a library of compounds, so that work can begin immediately toward initiating the next round of biological testing. Although there will always be limitations to what can be synthesized based on our imperfect knowledge, recent developments in two areas have facilitated the chemist's job: analysis/purification and synthetic methodology. In the first area, routine high-field NMR instruments allow ^{1}H-NMR and ^{13}C-NMR characterization of small amounts (10 mg) of organic compounds.

Liquid-chromatography/mass spectroscopy (LCMS) and other rapid analytical techniques, combined with medium- and high-pressure chromatography, allow for ready separation of reaction mixtures. New technologies such as reactor chips and miniaturization, supercritical fluids and ionic fluid reaction solvents, and chiral separation techniques will continue to improve synthetic capabilities.

In the second area, two recent advances have transformed synthetic methodology: transition-metal catalyzed cross-coupling reactions[19]and olefin metathesis technology[20]. The formation of carbon–carbon bonds is probably the most fundamental reaction in synthetic chemistry. For the first several decades of the 20th century, this reaction depended primarily on displacement of electrophilic leaving groups by enolate anions (or enamines) or addition of organometallic (e.g., Grignard) reagents. The advent of palladium-catalyzed coupling of more stable derivatives, such as olefins and acetylenes, boronic acids/esters, and tin or zinc compounds changed this simple picture. At the same time, the development of air-stable catalysts for producing complex carbon frameworks by metathesis of olefins expanded the chemist's repertoire. These methods

allow much greater flexibility and tolerance for sensitive functional groups, enabling construction of more complicated, highly functionalized carbon frameworks.

Assembling this methodology, along with that developed over the previous century, into library-enabled synthesis allows the preparation of the large numbers of compounds favored for today's search for lead compounds using high-throughput screening (HTS) and in lead compound follow-up.

Combinatorial chemistry was initially facilitated by developments in robotic handling technology and, for solid-phase synthesis, by Merrifield peptide synthesis. Both solution-phase[21] and solid-phase parallel syntheses allow generation of large chemical libraries[22]. The emphasis on these new technologies, combined with the cross-coupling and olefin metathesis synthetic methodologies, facilitates the synthesis of new classes of compounds with complex carbon frameworks. Their emergence as lead series and the ensuing follow-up are largely the result of their preponderance in the collection of compounds screened.

In other words, it can be argued that synthetic methodology creates the chemical space that is available for screening and hence influences in a very profound way the medicines available to mankind. As the synthesis in the succeeding chapters make clear, synthetic chemistry plays a significant role alongside medicinal chemistry in the drug discovery process.

1.5 Organophosphorous compounds

Phosphorous is a ubiquitous element and its chemistry is of great importance. From the very beginning, almost the entire field of phosphorous chemistry was dominated by inorganic phosphorous chemistry and the extent of known organophosphorous compounds was very limited. However since that time, organophosphorous chemistry has become a very broad and existing field, with many possibilities for research and application development.

Compounds containing P-C linkages are usually known as organophosphorus compounds. The term 'Organophosphorus compounds' are reserved for compounds containing phosphorous and carbon. The most important organophosphorus compounds are phosphate esters which are based on P-O-C linkages. Phosphorous chemistry is dominated by oxyphosphorous compounds[23], all of which contain phosphorous-oxygen linkages. Most of these are usually known as phosphates. Almost all naturally occurring phosphorus compounds contain phosphours-oxygen linkages and those of biochemical importance are organic phosphate esters which contain phosphorous-oxygen-carbon linkages. Organophosphorous compounds which are based phosphorous-carbon linkages constitute the second most important group and those containing phosphorous-nitrogen linkages are probably the third.

There are eight major classes of phosphorus compounds:

1. Oxyphosphorus compounds, which contain covalent P- O linkages.
2. Organophosphorus (carbophosphorus) compounds which contain P- C linkages.
3. Azaphosphorus compounds which contain P- N linkages.
4. Metallophosphorus compounds which contain P-metal linkages.
5. Boraphosphorous compounds which contain P-B linkages
6. Silaphosphorous compounds which contain P-Si linkages.
7. Thiaphosphorous compounds which contain P-S linkages.
8. Halophosphorous compounds which contain P-halogen linkages.

Sometimes phosphorous compounds can be classified according to the presence of two characteristics bonds, such as

C-P-O: Organo-oxyphosphorous compound

N-P-O: Aza-oxyphosphorous compound

M-P-O: Metallo-oxyphosphorous compound

N-P-C: Aza-organophosphorous compound

M-P-C: Metallo-organophosphorous compound

M-P-N: Metallo-azaphosphorous compound

Organophosphorous compounds have many fold applications in medicinal chemistry as antifungal[24] antiviral drugs[25] and have also immense importance in agricultural chemistry as insecticidal[26-28] and herbicidal[29] chemicals.

Almost all of the organic compounds with known pharmaceuticals contain phosphorous element[30]. Owing to their high toxicity and availability of alternative drugs, their use was restricted about 50 years back. Nevertheless, the medicinal properties of organo and organic phosphorous compounds are being investigated on an ever increasing scale. Some of these are already in use while others show considerable promises[31, 32].

In spite of the considerable variety of available inorganic, organometallic and other organic fungicides, a number of organophosphorous compounds have achieved significance in this field. Comparatively little is known about the mode of action of these fungicides.

The utilization of phosphorous containing compounds are as follows:

1. In medicinal compounds
2. Pesticides
3. Industrial phosphate esters
4. In food technology
5. Animal foodstuffs
6. Synthetic polymers and fire retardants
7. Natural products
8. In chemical weapon

1.5.1 In medicinal compounds

Organophosphorous compounds have many fold applications in the field of medicinal chemistry. A number of such compounds are known as medicinal compounds. D. Hendrin reported the activity of phosphonoacetic acid against Herpes and Marek's diseases, while phosphonomycin shows anti-bilharziosic and anti-leprosy properties as well as functioning as broad spectrum antibiotic[33]. Again, phosphonoformic acid is also regarded as an anti-viral drug.

Phosphonomycin   Phosphonoformic acid   phosphonoacetic acid
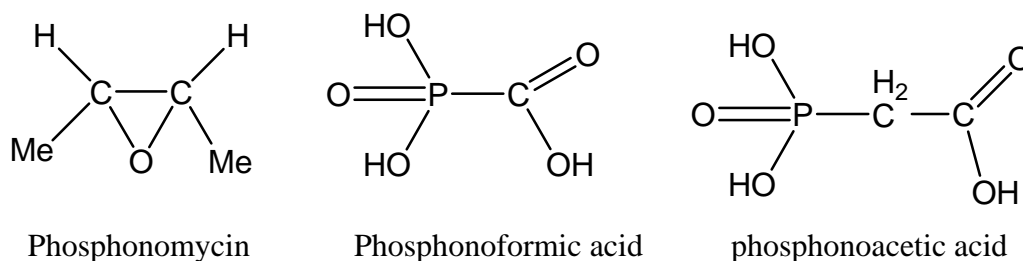
Walker and Thorselt[34-35] reported the broad spectrum antibacterial activity of ribavirin and difficidin. In recent year an important advance have been made in the discovery of the carcinosatic properties of cyclophormamide and it's derivatives[36]. Schaffer[37] reported the potential antibacterial activity of acyclovir, gancidovir and azidothymidine as amino phosphorus compounds. Acyclovir and ganciclovir are outstandingly active against herpes simplex virus (HSV) and azidothymidine is effective in alleviating some of the symptoms caused by HIV. These compounds are metabolized as triphosphates and are believed to act both as viral polymerase inhibitors and as chain terminators.Small difference of structures often corresponding to large difference in antiviral activity.

Ribavirin

(43)
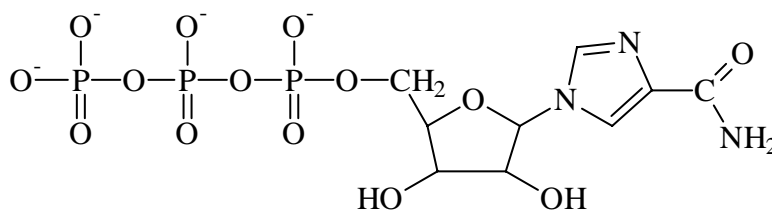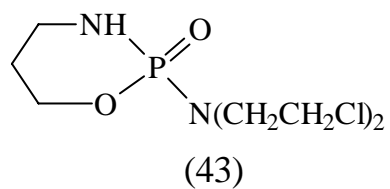
Cyclophosphamide
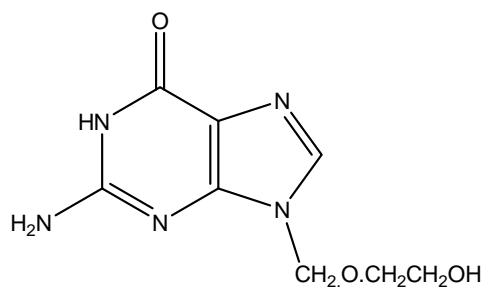
Acyclovir
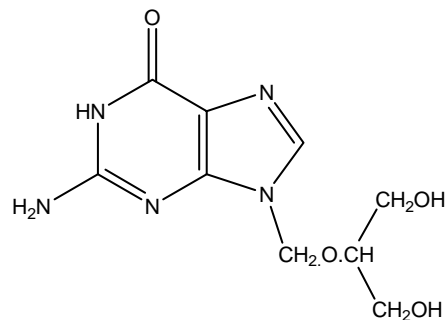
Ganciclovir

Some saccharide derivatives are claimed to ac against meningitis[38]. For example,
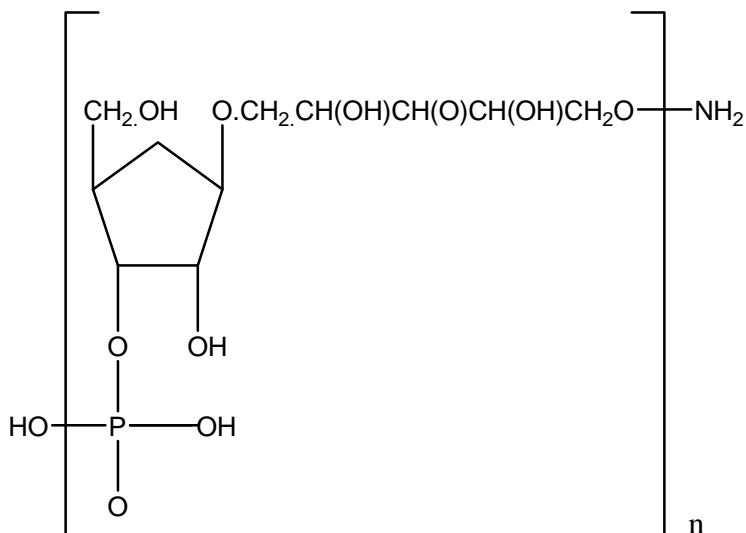
Therapeutic mixtures containing phospholipids have recently been patented as food additives to promote brain functions and to treat dementia[39].Some compounds reduce fatigue by raising the bloodstream choline level and releasing brain acetylcholine, and some are responsible for lowering of blood pressure and relief of hypertension. Following compounds can serve these purposes:
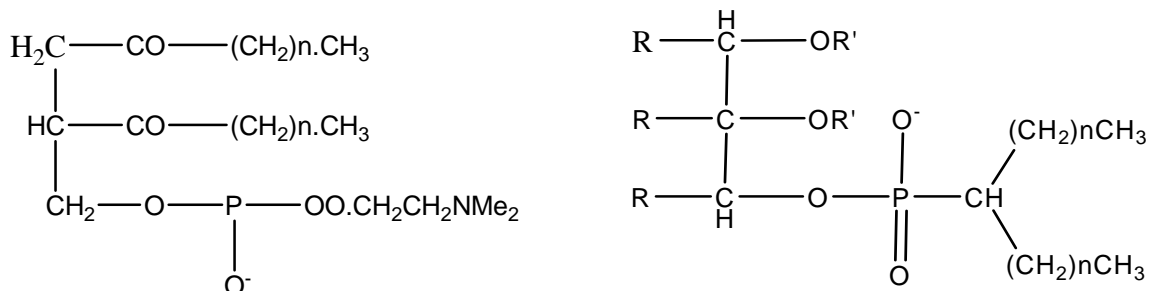
Phosphoglycerides have been prescribed to treat AIDS-related infections and compounds of this type have also been described as artificial lung surfactants.

Therefore, organo phosphorus compounds composed of diamino functionalities have been addressed with importance in organic and bio-organic chemistry. From the literature survey it has drawn a great interest to synthesize diamino organophosphorus compounds which might have potential biological activity.

Some inorganic phosphorus compounds[40, 41] such as inorganic phosphate salts have long been established medicinal uses. These include stomach antacids such as hydrated magnesium phosphate and aqueous suspensions of composition $AlPO_4.XH_2O$ (Phosphagel). Mixtures of Na_2HPO_4/KH_2PO_4 can be used for the treatment of phosphatemia (Phosphorus deficiency). Various calcium phosphates are used in artificial bone formulations in dental practice and in toothpaste formulations. Amorphous zirconium phosphate α-$Zr(HPO_4)_2$ is an excellent sorbent for use in renal dialysis[42].

A number of well known phosphate salts of organic drugs[43, 44] are prescribed as medicines. This is because the phosphate generally causes fewer disturbances to physiological pH, it may have a more suitable solubility, or merely because it is the salt most conveniently prepared and purified. Examples are:

$$H_2\overset{+}{N} - CHMeCH_2CH_2CH_2\overset{+}{N}HEt_2$$

HPO_4^{--}

Chloroquinephosphate

$-CH_2CHMe - \overset{+}{N}H_3$

$H_2PO_4^-$

Amphetamine phosphate

$\overset{+}{N}H_2$

$\overset{+}{N}H_2$

HPO_4^{--}

Pipereziae phosphate
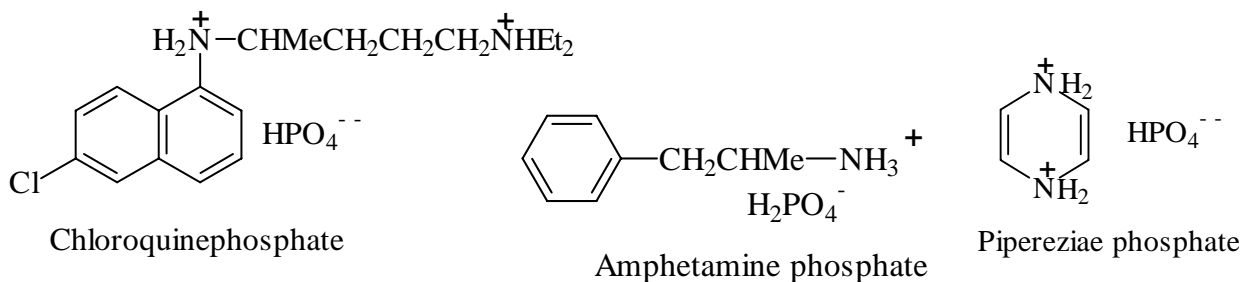
Chloroquinephosphate is used as anti-malarial drug, amphetamine phosphate is used as anti-depressant and piperazine is used as anthelmentic drug.

Ethane-1-hydroxy-1,1 diphosphonate and related compounds inhibit bone resorption and are used in the treatment of bone disease. Complexes of the diphosphonic acid with γ-ray emitting isotopes of technetium are useful for medical diagnostic work since they concentrate in the bone. The use of technetium diphosphonate complexes for bone imaging had revolutionized bone scanning techniques. Technetium pyrophosphate complexes are less satisfactory since the P-O-P linkages are liable to hydrolysis by body enzymes[45,46]. Iron complexes of phosphorylated mono and disaccharides will function as contrast agents in the MRI of the gastrointestinal tract.
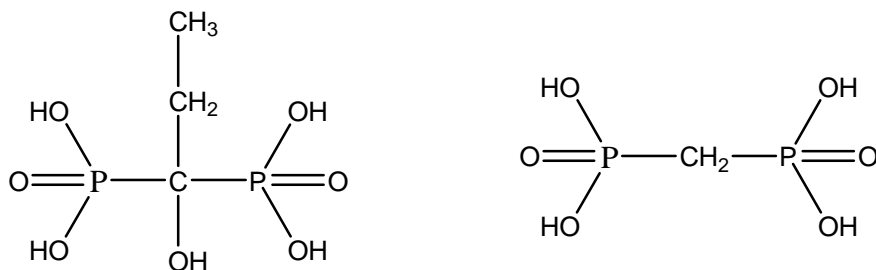
Fig: Ethane-1-hydroxy-1,1 diphosphonate and related compounds

A number of phosphorothioates show anti-radiation activity and are excellent radioprotective agents[47]. Some of these compounds are listed below-

$$H_2N-CH_2CH_2CH_2NHCH_2CH_2$$

$$H_2N \quad SNH_4$$
$$\underset{O}{\overset{}{P}} \quad ONH_4$$
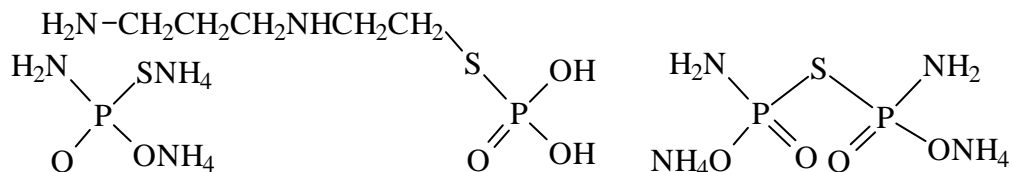
Metrifonate is used for the treatment of urinary tract infections, and the remedy for the prostatic carcinoma is diethylstilbestrol bis phosphate. Casein is used in tonics, dietary supplements, infant foods, special diets, in post operative feeding, in drug carrying capsules, in wound-healing preparations etc[48,49].
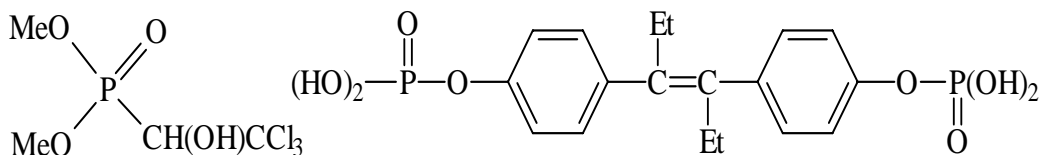
Metrifonate   diethylstilbestrol bis phosphate

1.5.2. In Pesticides

The application of organophosphorous compounds as pesticides is very important and well known. Two main groups of pesticides are insecticides and herbicides. There are also other crop-protection agents such as fungicides, acaricides, rodenticides, bactericides nematicides, molluscides, fumigants, chemosterilants, insect repellants and other specialized products. Some pesticides are very specific in action and may be effective against only one or two species, while other may be broad spectrum and effective against wide range of pests.

The ideal insecticide needs to be highly toxic to the insect pest concerned but at the same time be non-toxic to the operator, the plant and the crop consumer. Persistence in action and cheapness are also necessary. Many insecticides are also classed as acaricides and nematocides. Acaricides deal particularly with mites which attack plants and nematocide deals particularly with leaf, stem and root parasites known as nematodes.

Some typical organphosphorus insecticides are listed below:
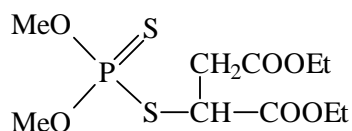
Malathion
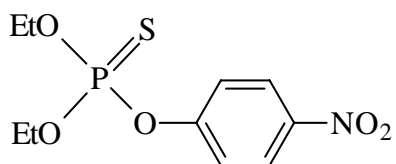
Parathion
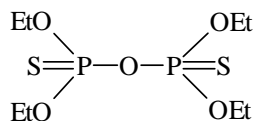
Sulphotepp
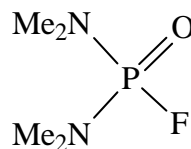
Dimetox
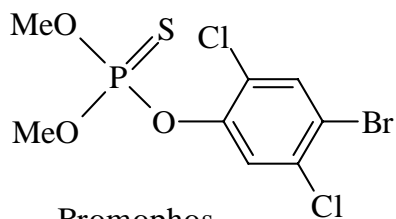
Bromophos
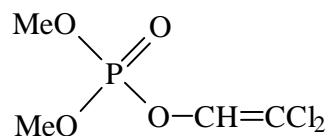
Dichlorovos

Cyanophos

Prothiphos

EtO—P(=S)(OEt)—S—CH₂—CH₂—SEt

$$EtO{-}\underset{EtO}{\overset{S}{P}}{-}S{-}CH_2{-}CH_2{-}SEt$$

Disulphoton

$$(EtO)_2P{-}S{-}CH_2{-}S{-}P(OEt)_2$$
$$\quad\ \|\qquad\qquad\qquad\ \ \|$$
$$\quad\ S\qquad\qquad\qquad\ \ S$$

Ethion

$$EtO{-}\underset{EtO}{\overset{S}{P}}{-}S{-}C_6H_5$$

Fonophos

$$MeO{-}\underset{MeO}{\overset{S}{P}}{-}SCH_2{-}\underset{\|}{C}{-}\underset{H}{N}{-}Me$$
$$\qquad\qquad\qquad\quad O$$
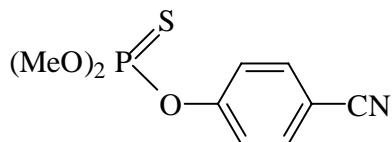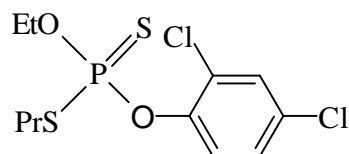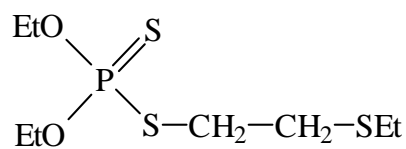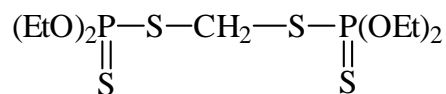
Dimethoate
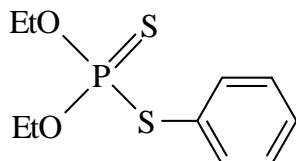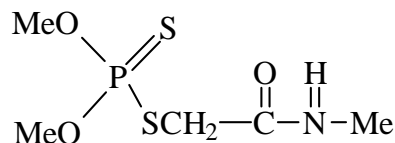
$$MeO{-}\underset{OMe}{\overset{S}{P}}{-}O{-}C_6H_4{-}S{-}C_6H_4{-}O{-}\underset{OMe}{\overset{S}{P}}{-}OMe$$
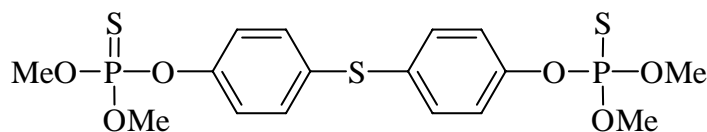
Temephos

Several thousands organophosphorus compounds are known to act as insecticides and about 250 of these are manufactured commercially. New compounds are constantly being patented. Organophosphorus compounds show 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. Others are relatively harmless and almost non-toxic to humans.

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. Persistence of organophosphorus insecticides is related to water solubility, vapour pressure and hydrolytic stability, properties which can vary greatly from one insecticide to another.

Possible undesirable effects of the residual products from pesticides are of great interest and concern to environmentalists. It was observed that continuous use of organophosphorous pesticides will not lead to any serious long-term environmental problem[50, 51].

Most organophosphorous insecticides are based on a single P atom which is linked directly to some combination of S, O, C or N atoms. These include phosphate esters, phosphonates, amides and thioated analogues.

From the very beginning, TEPP, Parathion and Paraoxon are used as pesticides. But these are very toxic to humans. Thus, the toxicity was removed on hydrolysis. Conversion to a phosphoryl derivative is necessary for insecticidal action in order that phosphorylating action can ensure and the compound becomes active. In the case of parathion this may happen by thiono to thiolo isomerisation.

(34)
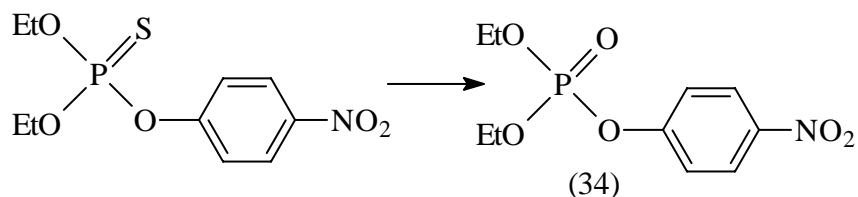
TEPP and Parathion types compounds function as contact insecticides and are effective only in the region of absorption, whereas Dimefox and Schradan are systemic insecticides which after absorption, are disseminated over the whole plant where they become effective.

Malathion and Trichlorophon are particularly suitable for domestic use because of their relatively low mammalian toxicity. Dimethoate has a wide spectrum action against insects, but less toxic to humans.

Some organophosphorous compounds used as nematicides are listed below-
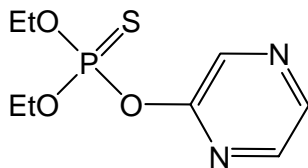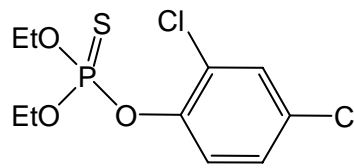
Dichlorfenthion   Zinophos

A wide range of organophosphorous compounds have herbicidal activity which includes Amiprophos, Cremart, DMPA, Glyphosate etc[52].
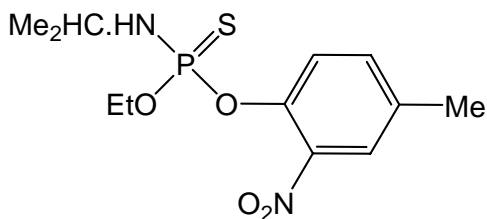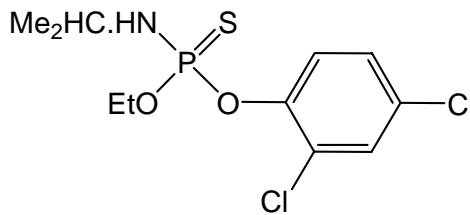
Amiprophos   DMPA

A number of organophosphorous compounds are used used as fungicides. The mechanism of action of these fungicides are different from the insecticides, especially these don't affect on nervous system. They are applied either on foliage, or may be of the soil fumigant type. Some fungicides are listed below-
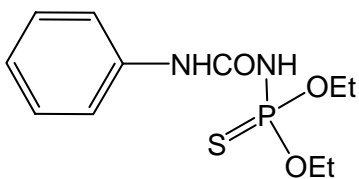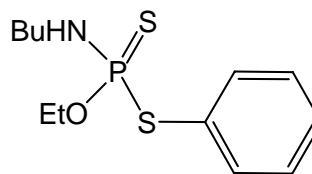
Phosphourea   Phosbutyl

1.5.3. Industrial Phosphate Esters

Phosphate esters have many technological applications[53-55]. Again they are not always pure compounds. Commercial tricresyl phosphate can contain a mixture of meta and para substituted groups. Some of the important phosphate esters are listed below-

$(CH_3—CH_2—O)_3P=O$

Triethyl phosphate

(1)

$\left(CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-O \right)_3 P=O$

Tri-octylphosphate

(3)

$\{(CH_3)_2CHO\}_3P=O$

Tri-isopropylphosphate

(2)

$\left(CH_3-CH_2-CH_2-CH_2-CH (Et)-CH_2-O \right)_3 P=O$

Tris-(2-ethylhexyl)phosphate

(4)

$CH_3-CH_2-CH_2-CH_2-CH (Et)-CH_2-O-P{=}O$ with OPh and OPh

2-ethylhexyldiphynylphosphate

(5)

$CH_3-(CH_2)_3 O$, $CH_3-(CH_2)_3O$, P=O, OH

dibutyl hydrogen phosphate

$CH_3(CH_2)_3CH(Et)CH_2O$, $CH_3(CH_2)_3CH(Et)CH_2O$, P=O, OH

bis-(2-ethylhexyl)hydrogenphosphate

(7)

$CH_3(CH_2)_4O$, $CH_3(CH_2)_4O$, P=O, OH

diamylhydrogenphosphate

(8)

Triethyl phosphate, tripropyl and tributyl phosphates are used as solvent. 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. Esters confer valuable anti-wear and corrosion inhibition properties when used as oil additives.

Esters of pyrophosphoric acid are also efficient complexing agents as well as dialkylamino derivatives such as HMPA and OMPA and diphosphonates. Typical complexes formed by tributyl phosphate (TBP) are:

$M(NO_3)_4.2(BuO)_3PO$ where M= Zr, Th, Np, Pu

$MO_2(NO_3)_2.2(BuO)_3PO$ where M=U, Np, Pu

$M(NO_3)_3.3(BuO)_3PO$ where M=Y, Ce, Eu, Tb, Tm, Lb, Am
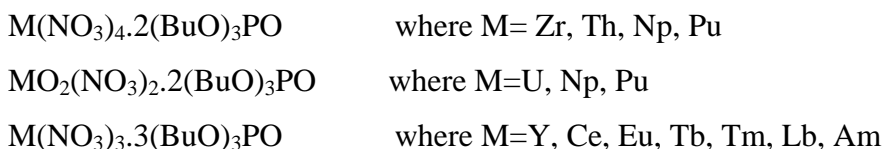
Tributyl phosphate and related esters such as dibutyl phosphate and bis-2-ethylhexyl phosphate (HDEP), find important uses in the extraction of rare earth, actinide and other heavy metals from mineral sources and their recovery from waste products of the atomic energy industry. A solution of TBP in kerosene can be used for solvent extraction of U and Th and other rare earths from their mixtures in a 10% aqueous solution in nitric acid. TBP is widely used for the purification of uranium for nuclear reactors, and in the re-processing of spent nuclear fuels.

HDEP is used commercially for the separation of Co from Ni and for the separation of Zn from impurity Cu and Cd. Mixtures of mono and di octylphenyl phosphates, obtained from octyl alcohol are used for the extraction of U^{4+} from wet process phosphoric acid. Tributyl and tri octyl phosphine oxides are als available commercially as extractants.

Some 5- and 6-membered ring phosphate esters can be polymerized as methyl ethylene phosphate.

$$(11)$$
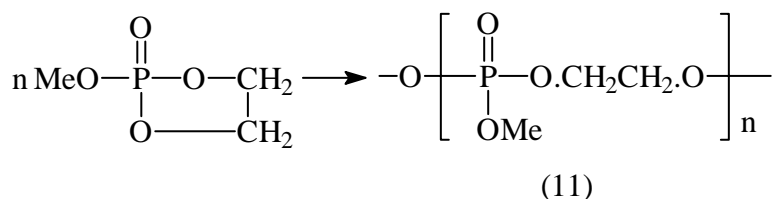
The Friedel-Crafts reaction can be used to prepare some polymeric phosphate esters while others can be prepared by co-polymerization of dialkyl hydrogen phosphates with epoxides.

$$ClCH_2\!-\!\!\bigcirc\!\!-O-\overset{\displaystyle O}{\underset{\displaystyle OAr}{P}}-O-\!\!\bigcirc\!\!-CH_2Cl + Ar\acute{H} \xrightarrow[-HCl]{200°C}$$

$$\left[\!-CH_2\!-\!\!\bigcirc\!\!-O-\overset{\displaystyle O}{\underset{\displaystyle OAr}{P}}-O-\!\!\bigcirc\!\!-CH_2\!-\acute{Ar}\!-\right]_n$$

(14)

$$\acute{R}\overset{}{CH}\!\!-\!\!CH_2 + RO\!-\!\overset{\displaystyle O}{\underset{\displaystyle OH}{P}}\!-\!OR \xrightarrow{-ROH} \left[\overset{\displaystyle O}{\underset{\displaystyle O-CH_2-CH\acute{R}-OH}{P}}\!-\!O\right]_n$$

(15)
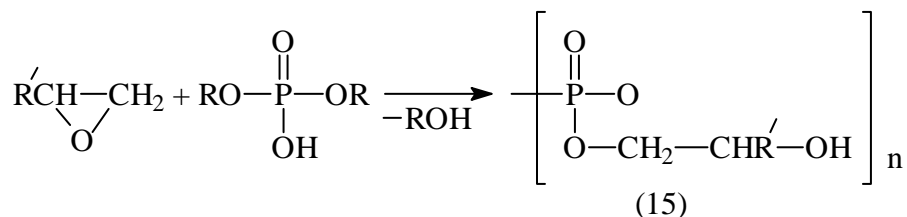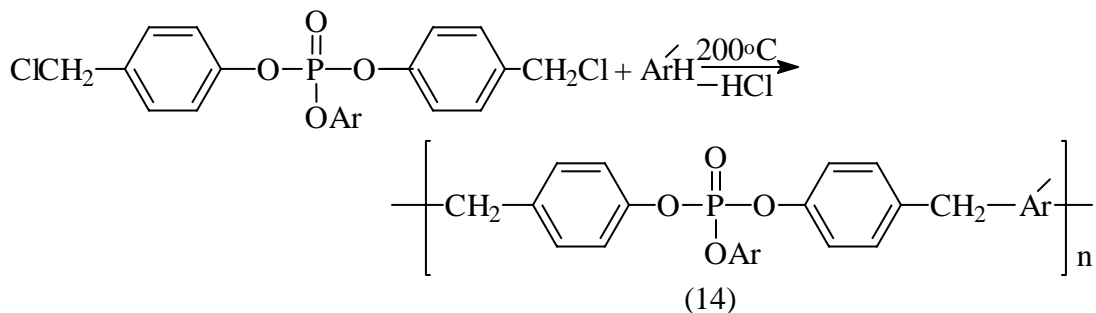
1.5.4 In food Technology

Phosphates[56-61] is presents in most natural foods, particularly meat, milk and dairy products, fruits and cereals. Further addition of phosphates is frequently made in the processing of foods for a variety of purposes which include for increasing nutritive value, for complexing of undesirable metal ions, preservation, prevention of caking, leavening action, color development of stabilization.

The major phosphorous-containing products in current use as food additives may be listed as

(i)   Inorganic salts-ortho, pyro and polyphosphates. Mostly of Na, K or Ca

(ii)   Bipolymer phosphates-casein, lactalbumins phosphates, starch phosphates, lecithin

Medicinal supplementation of phosphorus is usually with casein, orthophosphates of glycophosphates of Na, K, Mg or Ca. The applications of phosphorus compounds are exceedingly numerous in the field of food technology such as in milk and dairy products, meat and fish, fruit and vegetables, beverages, leavening agents, biopolymer phosphates etc.

The chief mineral constituents of milk are phosphorus and calcium together with Na,lk K, Mg and minor quantities are citric acids and great deal of water. The phosphorus content (about 0.95 g of P/liter in cow's milk) is distributed between more than 50 different compounds both organic and inorganic. Most abundant and important of these are the casein phosphoproteins, calcium phosphates and the phospholipids. Other phosphorus compounds present in much smaller quantities are most of the vitamins, various nucleic acids, enzymes, sugarphosphates and proteose peptones (phosphoglycopeptides).

About 2.0% of $H_2PO_4^-$ anions are present in natural citrus fruit juices as well as about 0.02% glucose-6-phosphate and other sugar phosphates. Other phosphorus compounds present in relatively minor quantities are nucleic acids, ATP, phospholipids and B group vitamins. Very useful effects are observed by treatments of fruit and vegetables with added phosphates. These include stabilization against bacteria and rancidity enhancement of color and desirable effects on tenderness and firmness. For example, small addition of $Na_4P_2O_7$ to peas and beans prior to canning lead to a more tender product due to the sequestering of calcium ions.

The addition of sodium polypjosphates stabilizes the colour of strawberries, tomatoes, cherries etc. and the use of such compounds prior to canning or freezing will help to keep vegetables green[62]. Polyphosphates such as $Na_2H_2P_2O_7$ are used to counteract the blackening of raw potato or apple juice which is due to the oxidation of diphenolic compounds in the presence of heavy metal ions. The latter are removed by complexing with the pyrophosphate ions.

Starch phosphates[63, 64] are being increasingly used in manufacturing since they promote thickening without jelly form. Starch phosphates have a fairly low degree of –OPO$_3$ substitution for –OH and are obtained by heating starch with phosphoric acid at about 60^{0}C. Some natural potato starch already contains a few of phosphate ester groups.

$$\text{Starch}-\text{OH} \ + \ \text{NaH}_2\text{PO}_4 \longrightarrow \text{Starch}-\text{O}-\overset{\displaystyle\text{ONa}}{\underset{\displaystyle\text{OH}}{\text{P}}}\!\!=\!\!\text{O} + \text{H}_2\text{O}$$

------------ Starch ------------------
\
O   ONa
\   /
P
/   \\
O   O
---------------- Starch ----------------
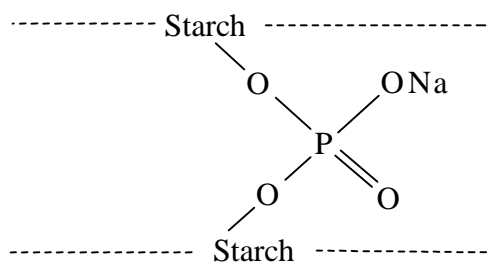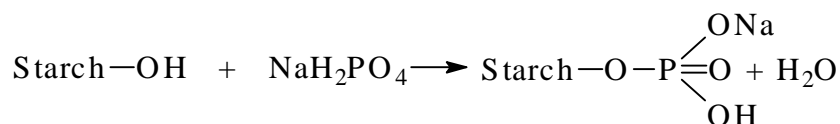
Scheme-1

Sodium Hydrogen phosphate reacts with starch to give a monoester salt, while sodium trimetaphosphate reacts to produce cross linked diester. Cross linked varieties of these kinds are more stable towards heat, agitation, and acidity than monoester salts.

Corn starch processed with cyclic sodium trimetaphosphates is used to make cold water jellies. Phosphorylated varieties of this kind are resistant to hydrolysis and degradation probably due to cross-linking and are used as thickening agents in cooked foods.

Sugar phosphates used in foods are relatively few in number; they include the improvement of the crispness of the breakfast cereals[65] and the flavor of alcoholic beverages[66].

Phospholipids such as lecithin which is available in various grades is widely used in the food industry as a surfactant, an emulsifier and an anti-oxidant.[67-70]. Lecithin is used in baking, where it acts as an emulsifier, a wetting agent to reduce mixing time, a parting

agent to affect cleaner and easier release from moulds and an anti-oxidant to stabilize vegetable and animal fats. Dough-handling properties are improved with lecithin and other improvements are secured I biscuits, pies, cakes, and waffles. Lecithin improves the cheese yield from milk[71]. It is also introduced into foods in the form of egg yolk where it may act as an emulsifier as in mayonnaise and salad dressings.

Synthetic organophophorus compounds are used in food processing, the use of polysubstituted tri-arylphosphione compounds as anti-oxidants and poly (p-diphenylphosphino) styrene retards the formation of peroxides in sunflower oil[72].

1.5.5 In Animal foodstuffs

The phosphorus contents of most animal foodstuffs are not particularly high and the more restricted variety of their diet makes animals much more prone to phosphorus deficiency than humans[73-79]. Phosphorus deficiency is the most widespread and economically important of all mineral deficiencies affecting grazing livestock.

Phosphorous is absorbed as soluble phosphate in the duodenum. The amount of absorption of phosphorus from the dietary input is influenced by many factors. These include the type of food, animal age, internal pH, and the intake of other elements such as Ca, Fe, Al, K, Mg and Zn. Excessive Fe, Mg, Al in the animal diet is known to reduce the absorption of phosphate by forming insoluble phosphates. Mono- and di-calcium phosphates are added to form animal foodstuff to guard against dietary deficiency of phosphorous. Stock feed di-calcium phosphate, $CaHPO_4.2H_2O$ can be made from calcium hydroxide and most wet-process phosphoric acid. Apart from possible reduction of phosphorous absorption, the Fe, Al and Mg salt impurities do not seem to be harmful to animals.

Disodium phosphate, Na_2HPO_4, ammonium phosphate or urea phosphate $CO(NH_2)_2.H_3PO_4$ may also be used as supplements to animal feeding compositions. Pyrophosphates and potassium orthophosphates are sometimes incorporated into pet foods. Ammoniumphosphates are used in cattle foods.

Lecithin and dehydrated casein are also used as animal food supplements. A useful animal food supplement can be obtained by adding phosphoric acid to molasses. The acid reduces the viscosity of the latter as well as increasing its nutrient value.

1.5.6 In synthetic Polymers and Fire Retardants

Application of phosphorous containing synthetic material or synthetic polymer has many considerable advantages. Numerous polymerized products containing P are based on P-C linkages and are generally more difficult to prepare than those based on P-O-C, P-O-Si, P-N, P-B, P-O-P linkages.

Some phosphorus containing monomers can be self condensed to form homo-polymers, while others can be co-polymerized with a non-phosphorous containing monomer. Desirable commercial properties are sought in polymers of the latter type, which employ a minimal amount of the usually more expensive phosphorous compound.

The major application of organophosphorous polymer has so far been in flame proofing and fire retardancy but they have also found an important role in the modification of the properties of established non-phosphorous polymers. In addition, growing applications lie in the areas of ion-exchange materials, surface additives, catalysts and tooth preservation agents

Polymerized phosphates constitute the most studied group of organophosphorus polymers, although in some cases the P-C linkages may be confined to the side chains. Among the methods which have been used for homopolymer formation are the heating of vinyl or allyl phosphonates or vinyl or allyl esters of phosphonic acids.

$$n\ CH_2{=}CH \overset{O}{\diagdown} P(O)R(OR') \longrightarrow \left[CH_2{-}\underset{OP(O)R(OR')}{\overset{|}{CH}} \right]_n$$

$$n\ CH_2{=}CH{-}P(O)(OR)_2 \longrightarrow \left[CH_2{-}\underset{CH}{\overset{P(O)(OR)_2}{\overset{|}{CH}}} \right]_n$$

36
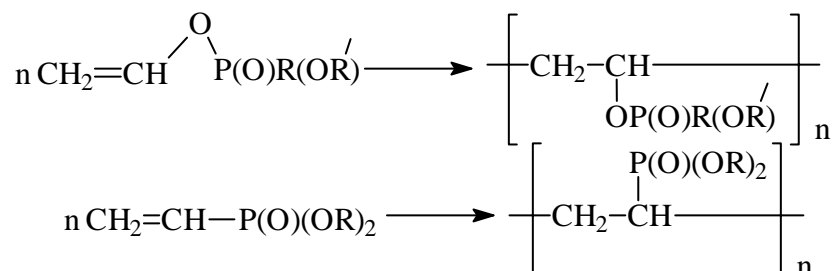
Methods used to obtain phosphonate copolymers include transesterification reactions between suitable diols and phosphonyl dichlorides or phosphonate esters or reaction of the latter with dihalides.

$$nPhPOCl_2 + nHO-\!\!\left\langle\bigcirc\right\rangle\!\!-OH \xrightarrow{-2nHCl} \left[\begin{array}{c} O \\ \| \\ P-O-\!\!\left\langle\bigcirc\right\rangle\!\!-O \\ | \\ Ph \end{array}\right]_n$$

$$nMeP(O)(OPh)_2 + nHO-\!\!\left\langle\bigcirc\bigcirc\right\rangle\!\!-OH \xrightarrow{-2PhOH} \left[\begin{array}{c} O \\ \| \\ P-O-\!\!\left\langle\bigcirc\bigcirc\right\rangle\!\!-O \\ | \\ Me \end{array}\right]_n$$

$$n\ MeP(O)(OMe)_2 + nClCH_2CH_2Cl \xrightarrow{-2nMeCl} \left[\begin{array}{c} O \\ \| \\ P-O-CH_2-CH_2-O \\ | \\ Me \end{array}\right]_n$$
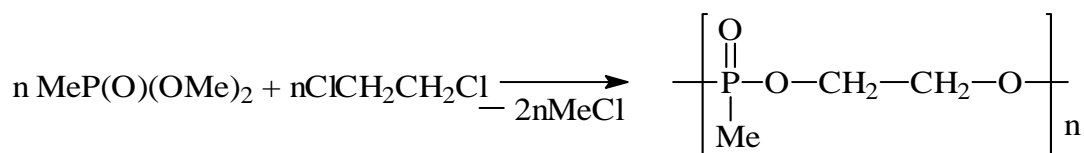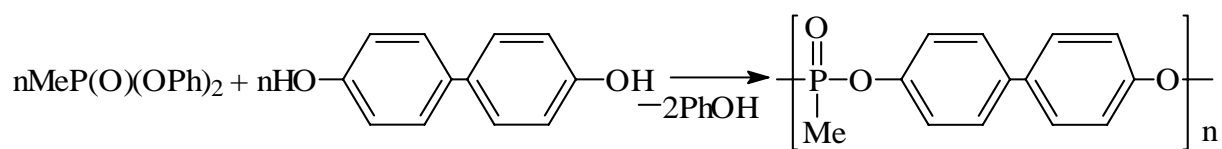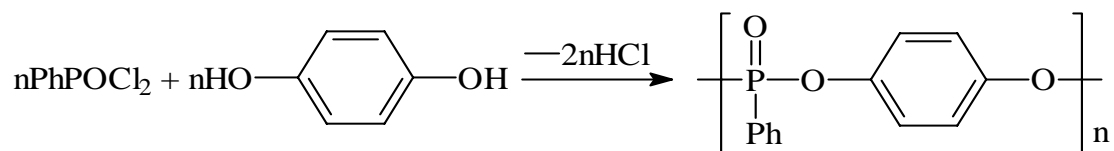
The polymerization properties of allyl esters of arylphosphonic acids were first investigated by Toy[80-82]. If polymerization is effected with a monomer containing only one allyl group, the products obtained is relatively low molecular weight. The presence of more than one allyl group leads to cross-linking and high molecular weight thermosetting polymers.
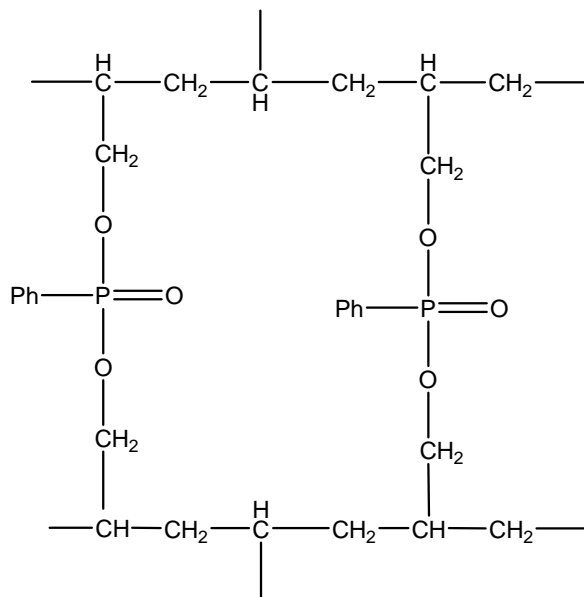
A limited number of polymerised phosphites have been made by reactions between phenyl phosphites and various diols. Typical examples of copolymers are shown below:

$$HO(CH_2)_6OH + (PhO)_3P \xrightarrow[-PhOH]{Na} \left[O-(CH_2)_6-O-\underset{\underset{OPh}{|}}{P} \right]_n$$

$$HO-\langle\rangle-CMe_2-\langle\rangle-OH + (PhO)_3P \xrightarrow{-PhOH}$$

$$\left[\langle\rangle-CMe_2-\langle\rangle-O-\underset{\underset{OPh}{|}}{P}-O \right]_n$$
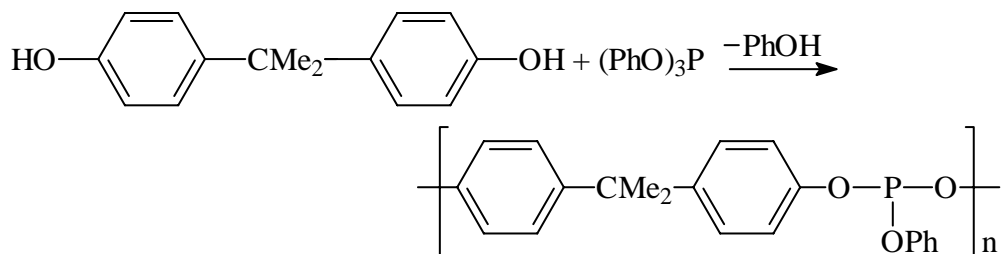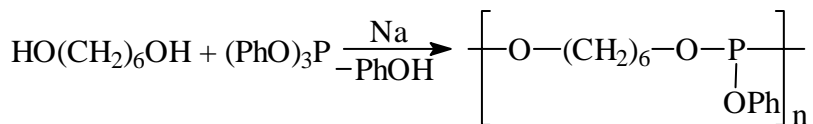
Phosphine copolymers can be obtained by heating primary phosphines with non-conjugated dienes or condensing them with diisocyanates or by reacting aryl phosphonous dihalides with certain hydrocarbons.

$$n\overset{/}{R}PH_2 + nCH_2{=}CHRCH{=}CH_2 \longrightarrow \left[\underset{\underset{P}{|}}{\overset{\overset{R'}{|}}{}}-CH_2CH_2RCH_2CH_2 \right]_n$$

Non-conjugated dienes

$$nRPH_2 + nOCN-\overset{/}{R}-NCO \longrightarrow \left[\underset{\underset{P}{|}}{\overset{\overset{R}{|}}{}}-CO.NH-\overset{/}{R}-NH.CO \right]_n$$

diisocyanates

$$nArPCl_2 + n\langle\rangle-CH_2-CH_2-\langle\rangle \xrightarrow[-HCl]{AlCl_3}$$

$$\left[\langle\rangle-CH_2-CH_2-\langle\rangle-\underset{\underset{Ar}{|}}{P} \right]_n$$

hydrocarbon
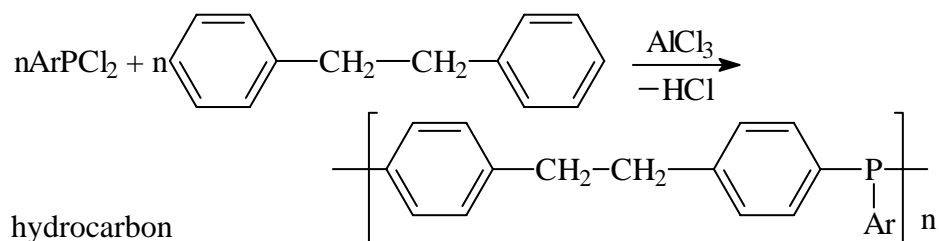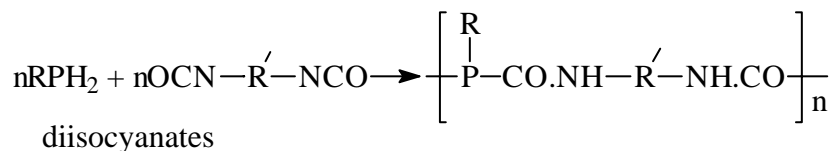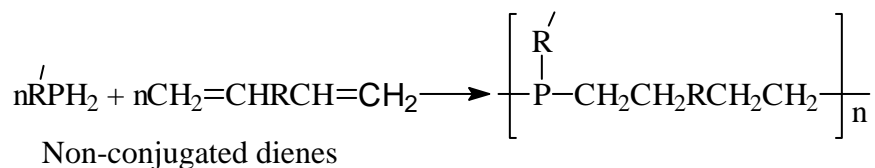
The oxidative polymerization of unsaturated phosphines, the reaction of dibutyl hydrogen phosphate with pentamethylene $MgBr_2$, the condensation of carboxylic acids and the cationic polymerization of cyclic phosphinites all lead to highly polymeric phosphine oxides.

Polymers with either P-N or P-C linkages in the main chain result from reactions involving amides.

$$nRPOCl_2 + nCO(NH_2)_2 \xrightarrow[-HCl]{100^0-160^0C} \left[*\begin{array}{c} O \\ \| \\ P \\ | \\ R \end{array} - NH.CO.NH - * \right]_n$$

$$nRP(O)(CH_2OH)_2 + n\ H_2N.R'.NH_2 \xrightarrow{-H_2O} \left[* - CH_2 - \begin{array}{c} R \\ | \\ P \\ \| \\ O \end{array} - CH_2.NHR' - NH - * \right]_n$$
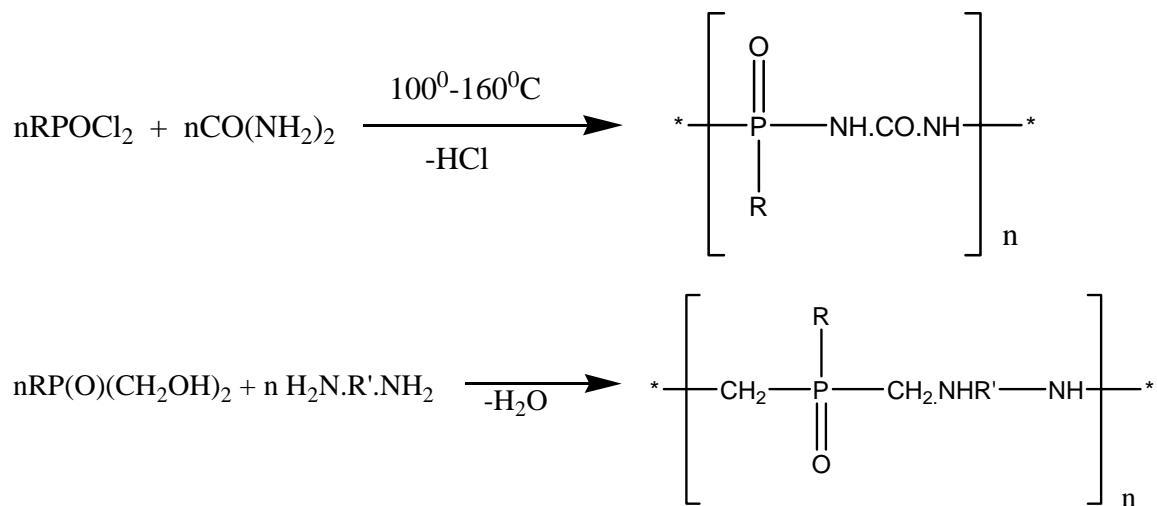
In commerecial practice, the introduction of relatively small quantities of phosphorous into established polymers is at present of much greater impoutance than the synthesis of new polymers with high P content. The properties of conventional polymeric materials such as polyethylene, polystyrene and polyurethane may all be modified with advantage.

Phosphorous modified polymers are obtained by the introduction of P-containing groups into the side chains by a phosphorylation reaction with a pre-formed polymer. Flame retarded varieties of polystyrene can be obtained by co-polymerisation of styrene with propenyl cyclopentafluorophosphazene, when a polymer with the phosphazene in the side chain is produced.

$$xCH\!=\!\!CH_2 \;+\; yP_3N_3F_5\!-\!\!C(CH_3)\!=\!\!CH_2 \longrightarrow \;^*\!-\!\![CH(Ph)\!-\!CH_2]_x\!-\![C(Me)(P_3N_3F_4)\!-\!CH_2]_y\!-\!^*$$
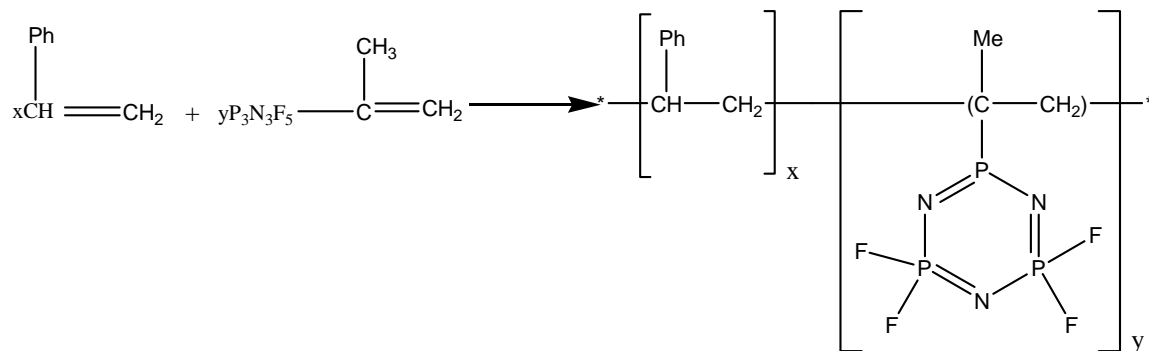
A permanent or semi-permanent fire resistance of paper, wood, plastics, fabrics etc. can be obtained when the fire retardant can be chemically bonded to, or physically incorporated in an insoluble form in these highly polymeric materials. In the case of synthetic materials, the most intimate bonding is usually obtained by copolymerization with a fire retardant monomer or short chain oligomer. Alternatively, it may be possible to attach the phosphorus compound by a suitable reaction with the preformed polymer. There are now several hundred organophosphorus or organic phosphate fire retardants are available for application. Although most of these are considerably more expensive than ammonium phosphate, their use is often commercially justified, particularly with high quality fabrics. Their mode of action in many cases is probably at least partially similar to that of ammonium phosphate. Flame and grease resistance can be imparted to cotton fibers by carrying out reaction in their presence but there is some loss of strength.

Polyvinylphosphonic acid is useful for metal surface treatment where it has an anti-corrosive action and improves the adhesion of subsequently applied coatings. After application of the solution of certain organophosphonic acids to sheet copper or its alloys increase solderability and tarnish resistance. Again, impregnation of wood with phosphoric acid suppresses the formation of carcinogenic materials during pyrolysis[83]. Polyvinyl phosphonic acid and polyethylene phosphonate are adsorbed as monolayers on tooth enamel where they resist decay[84]. Copolymers of vinylphosphonic acid and vinylphosphonyl fluoride are also adsorbed on tooth surface providing extra resistance to decay by slowly releasing fluoride ion which can substitu in the tooth hydroxyapatite[85]. Metals are complexed by various aminophosphonate polymers, vinyl

phosphonate polymers and phosphonated styrene-divinylbenzene polymers and can be used for the selective removal of certain actions from aqueous solutions.

$$\underset{\underset{H_2N-C-NH_2}{\overset{\overset{NH}{|}}{}}}{} + 4HCHO + 2HPO(OMe)_2 \longrightarrow$$

$$(MeO)_2P(O)-CH_2-\underset{}{N}\underset{\underset{NH}{\overset{\overset{CH_2OH}{|}}{}}}{\overset{\overset{CH_2OH}{|}}{C}}-\underset{}{N}-CH_2PO(OMe)_2$$
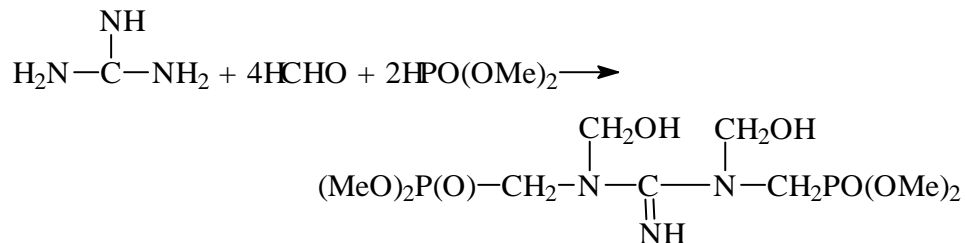
Finely divided red phosphorous is an effective flame retardant which is used in some PVC or moulded nylon products. For fireproofing cotton THPC, Pyrovatex, Fyrol etc. compounds were used in the earliest period.

$$HO.CH_2 \overline{}\underset{\underset{CH_2OH}{|}}{\overset{\overset{CH_2OH}{|}}{P^+}}\overline{}CH_2OH \quad Cl^-$$

THPC

$$\underset{MeO}{\overset{MeO}{}}\overset{\overset{O}{\parallel}}{P}\overset{}{\underset{CH_2CH_2.CO.NH.CH_2OH}{}}$$
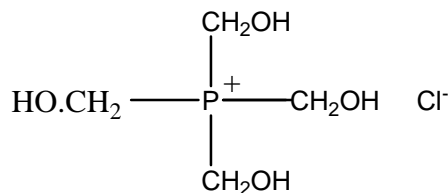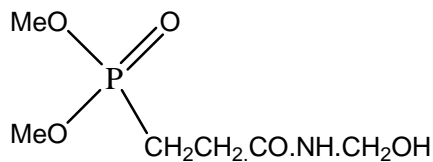
Pyrovatex

Trimeric phenoxycyclophosphazene and certain alkoxy derivatives can be used to obtain fire-retarded polyurethane and polycarbonate plastics, rayon or terylene fibres[86]. Tricresyl phosphate and other triaryl phosphates are used as flame retardants and plasticizers with PVC. Triethyl phosphate, triphenyl phosphate, trioctyl phosphates are used as commercial fire retardants[87].

1.5.7 In natural Products

Natural products in the form of biopolymers are very much important as these are mostly phosphorus containing organic compounds. All nucleic acids are phosphate esters, only some varieties of proteins, lipids, polysaccharides are found in phosphorylated form, and these may be termed phosphoproteins, phospholipids and phosphosaccharides respectively. Phosphorylated forms are intimately involved in the function of the all-important type of proteins known as enzymes.

The four types of biopolymer are frequently encountered in nature as intimately linked of considerable complexity. These associated units are known as lipoproteins, glycoproteins, proteoglycans, glycolipids, nucleoproteins etc. Phosphate groups when present in either biopolymer may also act as bridging groups.

$$-\overset{|}{\underset{|}{C}}-OH + HO-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-OH + HO-\overset{|}{\underset{|}{C}}- \xrightarrow{-2H_2O} -\overset{|}{\underset{|}{C}}-O-\overset{\overset{O}{\|}}{\underset{\underset{O}{|}}{P}}-O-\overset{|}{\underset{|}{C}}-$$
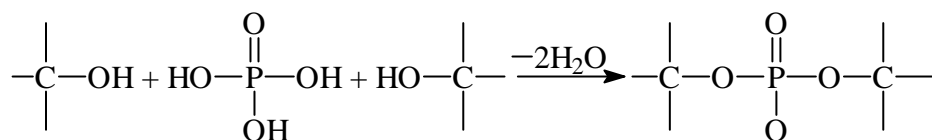
The monosaccharide found in living system is mostly mono and di-phosphate esters. of greatest importance in animal metabolism are the three esters, which also occur in plant life, particularly fruit. They have high water solubilities and high acid strengths. Individual glucose phosphates vary greatly in their hydrolytic behaviour.
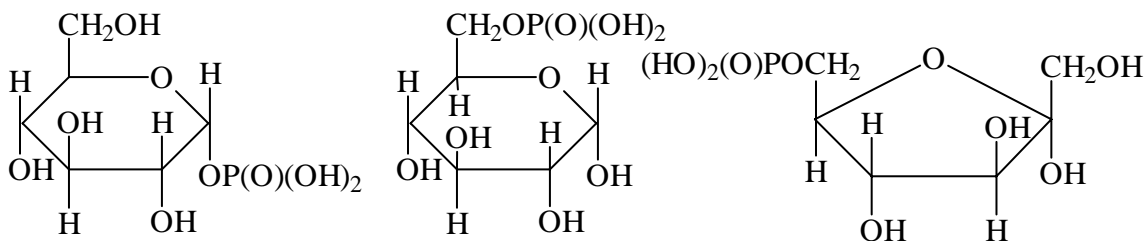
Amongst the ribose phosphates, ribose-5′-phosphate is utilized in forming the all important nucleotides.

R= bose 5′ phosphate
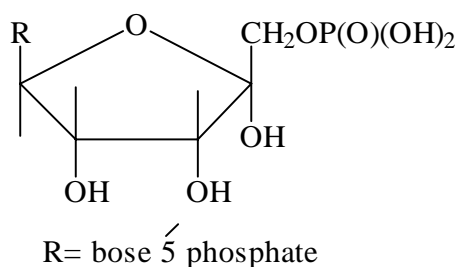
Simple monosaccharides such as D-glucose – 6 – phosphate can be prepared by direct phosphorylation of the unprotected sugar. In general, however, the sugar -OH groups have to be protected while phosphorylation can be carried out at the desired position, and the protective groups afterwards to be removed. Glucose–2–phosphate can be formed according to scheme.
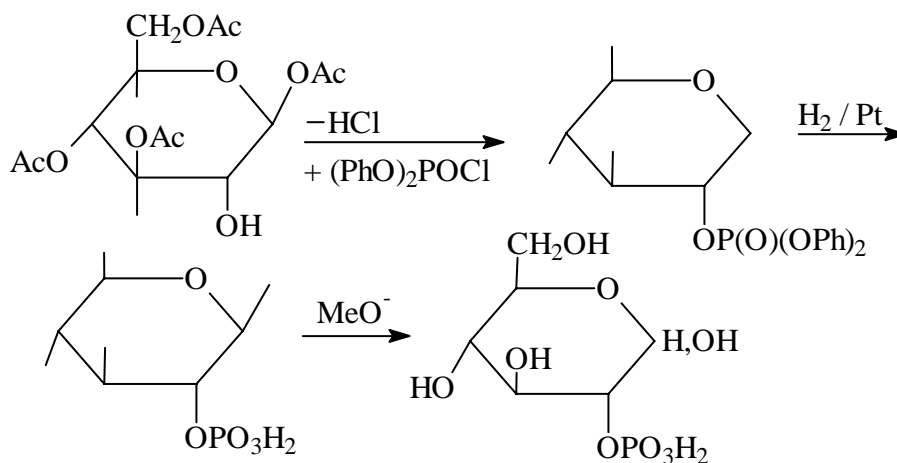
Polysaccharides are widely distributed in plants and animals. They are present both as structural materials as in cellulose and as food storage compound such as starch and glycogen. Phosphorylated polysaccharides, phosphorylation with consequent modification of properties is possible in principle for any polysaccharide. Phosphopolysaccharides (Polysaccharide phosphate esters) of this kind occur frequently in living systems and in a number of important technological products. Many bacterial polysaccharides contain phosphate ester groups, these include the teichoic acids.

Animals generally contain about ten times more protein than plants. All proteins are built from C, H, O, N and usually some S. The pure protein structures are devoid of phosphorus. Phosphoproteins only result when appropriate substitution is made. Proteins are usually of two types conjugated proteins and non-conjugated proteins. Proteins often occur naturally in close association with other biopolymers and such combinations are sometimes known as conjugated proteins. They include nucleoproteins, lipoproteins and glycoproteins. Either or both components of a conjugated protein may be phosphorylated. In the case of nucleoproteins, phosphorus is always present in the nucleic acid component. Some may prefer the prefix 'phospho' to be used to signify which component is phosphorylated e.g. phospholipoprotein or lipophosphoprotein. More than a hundred different phosphoproteins have now been recognized. The best known of these include milk casein, the egg proteins- phosvitin and ovalbumin and the iron-storage protein ferritin.

Casein is a phosphoprotein mixture found in the milk of mammals[88-93]. It constitutes about 80% of the total milk protein. It is used in food products. Again it has many industrial applications such as emulsifier, thickening and gelling agents, in wood glues and plastics, in paper coating compositions. Numerous and diverse products incorporate casein in their formulations. These include dish washing liquids, cosmetics, lightweight concrete, gypsum wallboards, pesticide sprays, water purifiers etc..

Phosphorylation of proteins nearly always occurs on serine residues but threonine, tyrosine, histidine and lysine can also be involved.

$$
\underset{\text{—NH—CH—CO—}}{\overset{\underset{\displaystyle|}{\text{CH}_2\text{—OH}}}{}} \xrightarrow{\text{Phosphoryl action}} \underset{\text{NH—CH—CO———}}{\overset{\underset{\displaystyle|}{\text{CH}_2\text{OP(O)(OH)}_2}}{}}
$$
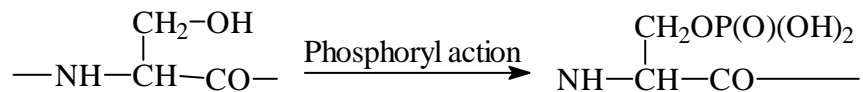
Many enzymes are phosphoproteins and enzyme action is frequently associated with phosphorylation dephosphorylation of the protein residues particularly in serine. Phosphorylation replaces –OH with –OP $(O)(OH)_2$ and places a negative charge on the protein. Interference with the existing hydrogen bonding scheme and the introduction of

a relatively large phosphate group can generally be expected to modify the secondary and tertiary structure of protein.

Protein phosphorylation is involved in numerous biochemical processes. These include the regulation of metabolic pathways, membrane transport, muscle contraction, hormone response, photosynthesis, cell division, gene transcription and translation and brain processes such as learning and memory[94-99].

Phospholipids are major components of cell membranes and occur widely in bacteria, animal and plant tissues. They are involved in enzyme action and transport of tri-glycerides through the liver and they have a role in electron transport and oxidative phosphorylation.

The most important commercial source of phospholipid is lecithin, which has numerous food and nonfood applications. Commercially lecithin is a complex mixture of phosphatides and triglycerides with minor amounts of fatty acids, sterols and other organic compounds. Lecithin can be used in manufactured food products, pharmaceutical preparations, cosmetics, formulations for paints, waxes, polishes, in paper manufacture as dispersing and softening agent etc[100-103]. The properties of starch and bread are modified by their small phospholipid content. The most abundant phospholipids are those with the general formula shown below, where R is a long chain fatty-acid residue and X can be various groups. They are derivatives of glycerophosphoric acid and are sometimes called glycerophospholipids.

$$
\begin{array}{ll}
CH_2{-}O{-}C(O)R & CH_2{-}O{-}C(O){-}R \\
| & | \\
CH{-}O{-}C(O)R' & CH{-}O{-}C(O){-}R' \\
\quad\quad O & \quad\quad O \\
\quad\quad \| & \quad\quad \| \\
CH_2{-}O{-}P{-}O{-}X & CH_2{-}O{-}P{-}OH \\
\quad\quad\quad | & \quad\quad\quad | \\
\quad\quad\quad OH & \quad\quad\quad OH
\end{array}
$$
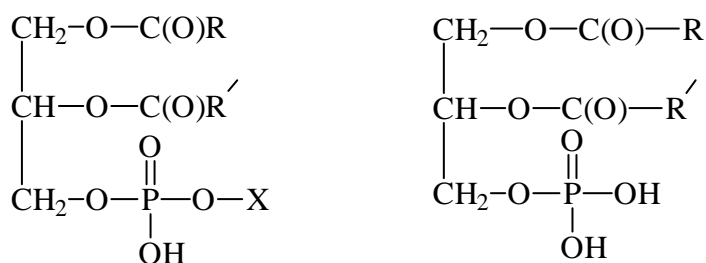
When X=H, these compounds are the parent phosphatidic acids

In naturally occuring phosphoglycerides, X is most frequently choline, ethanolamine, L-serine or inositol and R is a mixture, the principal components of which are palmitic and oleic together with smaller quantities of other long-chain residues. Lecithin is found in egg yolk, brain tissue and in skin. It exists as zwitterions in its physiological environment

$$CH_2.O.CO.R$$
$$|$$
$$CH.O.CO.R$$
$$|\qquad\quad O^-$$
$$CH_2-O-\overset{O^-}{\underset{\underset{O}{||}}{P}}-O-CH_2-CH_2N^+Me_3$$

Lecithin
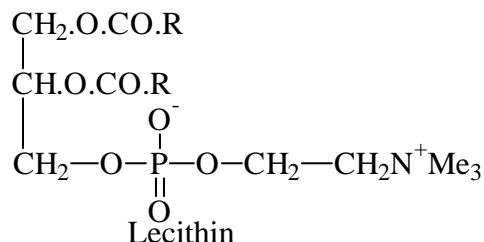
Phospholipids are found in most natural oils. Soyabean products, cottonseed oil and sunflower oil contain sufficient quantities to be important practical sources of phospholipids. Most phospholipids are water soluble as well as fat soluble, because their molecule have hydrophobic as well as hydrophilic regions and are polar in character. So they are called amphiphatic lipids. In general, membrane lipids are amphiphatic phosphoesters, whereas storage lipids are not. Phospholipids are important for their emulsifying properties. In an oil water system the molecules concentrate at the interfaces and lower the surface tension thus enabling droplets to be formed, they act as a barrier at the interfaces and stabilise the emulsion. When heated with acids or bases, most phosphoglycerides are split into their components i.e. fatty acids, glycerol, phosphoric acid and the base head group. Plasmalogens are phosphatidyl derivatives in which the fatty acid in the α-position has been replaced by an unsaturated ester. There are found in brain and nervous tissue.

$$CH_2-O-CH=CHR$$
$$|$$
$$CH-O-COR'$$
$$|\qquad\quad O$$
$$CH_2-O-\overset{O}{\underset{\underset{OH}{|}}{\overset{||}{P}}}-O-X$$

Plasmalogens

$$CH_2(OH)\ .\ CH=CHR$$
$$|$$
$$CH-NH\ .\ CO\ .\ R'$$
$$|\qquad\quad O$$
$$CH_2-O-\overset{O}{\underset{\underset{OH}{|}}{\overset{||}{P}}}-O-X$$
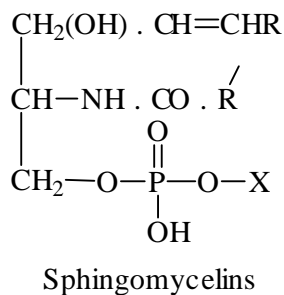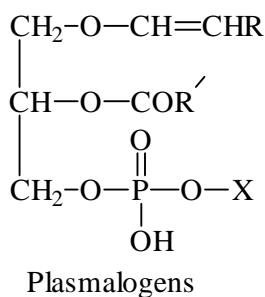
Sphingomycelins

Sphingomycelins are phosphorus containing members of the second large class of membrane lipids known as sphingolipids. The head group X is most commoly choline or ethanolamine, and these compounds resemble the corresponding phosphatidyl compounds in their general properties. They are found in most animal membranes, particularly in the 'myelin sheath' surrounding certain nerve cells.

Some lipids are conjugated with proteins to form lipoproteins. Lipovitellin and lipovitellenin are phospholipoproteins. Blood contains various types of plasma lipoproteins which consist of triglycerides, proteins, phospholipids and cholesterol. These closely associated units may be covalently linked to each other in some cases. Phosphate groups, glycerol, ribitol and saccharide units are the basic components and the simpler derivatives can be represented by the following formulae.

$$
\left[\begin{array}{c} \overset{O}{\overset{\|}{-O-P-O-CH_2-\underset{}{CH}-CH_2-}} \\ \underset{OH}{|} \ \ \ \ \ \ \ \ \ \underset{OR}{|} \end{array} \right]_n
\ \ \ \ \ \
\left[\begin{array}{c} \overset{O}{\overset{\|}{-O-P-O-CH_2-\underset{}{CH}-\underset{}{CH}-\underset{}{CH}-CH_2-}} \\ \underset{OH}{|} \ \ \ \ \ \ \ \ \ \underset{OR}{|} \ \ \underset{OH}{|} \ \ \underset{OR'}{|} \end{array} \right]_n
$$

R=H, sugar or D-alanyl

R= H or D-alanyl
R'= H or sugar
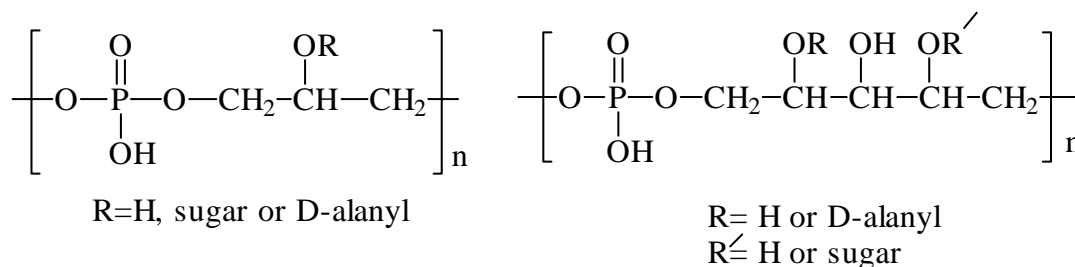
The nucleic acids are not only responsible for the storage and transmission between generations of genetic information[104], but they also pass on this information to direct the synthesis of the proteins characteristic of the cell. Nucleic acids frequently occur in close association with proteins as nucleoproteins. Nucleic acids are closely associated with mononucleotides, modified polynucleotides and nucleotide phosphates. They all contain phosphorous, mononucleotide units are built from three main components, a phosphate group, a sugar-ribose or deoxy-ribose, a nitrogen base, a purine or a pyrimidine.

Adenosine 3′, 5′ mono-phosphate (Base = adenine) is of considerable importance in biochemistry. Hydrolysis of this compound with $Ba(OH)_2$ gives a mixture of adenosine 3′ phosphate and adenosine 5′ phosphate.

Almost all biochemical reactions are catalyzed by enzymes. Enzymes are a special kind of catalyst which are proteins and which are effective in extremely small concentrations. Enzymes are usually proteins, all enzymes contain nitrogen and most of the enzymes contain phosphorous, very high proportions are involved with reactions of phosphate esters and phosphorus is often present in the cofactors.

Enzymes which catalyze hydrolysis are known as hydrolases and if the compounds acted upon (substrate) are esters they are known as esterases. If the action is specific to phosphate esters, these compounds are known as phosphoesterases or phosphatases.

The enzymes which catalyze 'phosphate transfer' or phosphorylation is very important in biochemistry. These have been known variously as phosphotransferases, phosphorylases, phosphokinases, transphosphorylases etc.

There are two phosphorylation processes of fundamental importance of biochemistry. These are photophosphorylation, the process by which green plants convert light energy to chemical energy. And the oxidative phosphorylation, the process by which a large part of the energy in foods is conserved and made available to the cell.

Adenosine tri-phosphate, ATP phosphorylates glucose as it enters the living cell according to reaction which can alternatively be written as below. In this non-reversible reaction in which ATP act as the phosphorylating agent, the enzyme is given a special name hexokinase. Enzymes which catalyze transfers specially to and from ATP are sometimes called phosphokinases.

$$\text{Glucose} \xrightarrow[\text{Mg}^{2+}]{\text{ATP} \quad \text{ADP}} \text{Glucose-6-Phosphate}$$
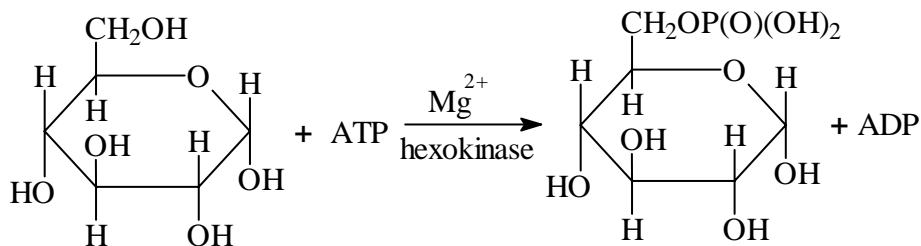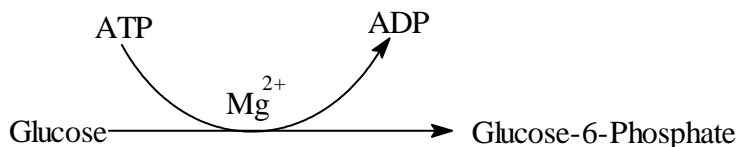
Another example is provided by the phosphorylation of acetic acid (substrate) to form acetyl phosphate, which is catalyzed by the phosphokinase enzyme known as acetate kinase. This reaction can occur in reverse in which case the acetyl phosphate is said to phosphorylate the ADP to ATP. Both di-phosphate and tri-phosphate esters can act as phosphorylating agents.

$$\text{CH}_3\text{COOH} \xrightarrow[\text{acetate kinase}]{\text{ATP} \quad \text{ADP}} \text{CH}_3\text{CO OP(O)(OH)}_2$$
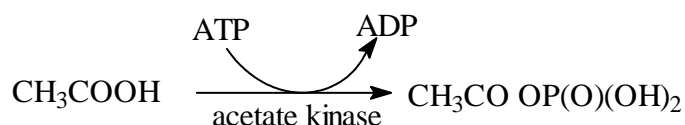
Enzymes which catalyze the transfer of a pyrophosphate group are sometimes known as pyrophosphorylases, although ATP normally functions as a phosphorylating agent, it will sometimes act as a pyrophosphorylating agent, as in the conversion of ribose-5-phosphate to a α-5-phosphoribosyl-1-pyrophosphate.
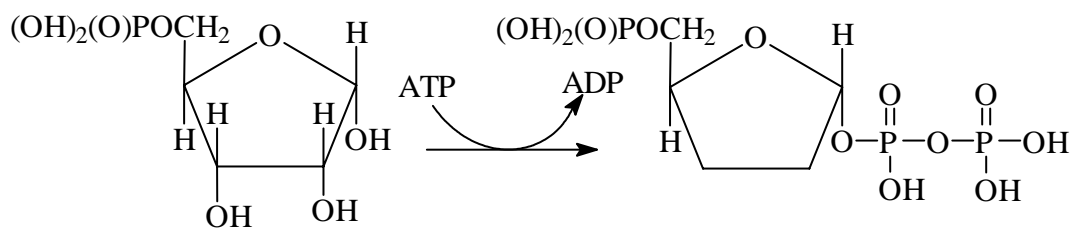
At least a hundred or out of the total of about 30,000 different proteins found in cells are known to be modified by phosphorylation. Even when a protein is phosphorylated, however, only a small proportion of the total -OH groups is generally involved. Whether or not a particular residue is phosphorylated in a given protein is determined by the specific amino-acid sequence around the site of potential phosphorylation.

1.5.8 In Chemical Weapon

History of organophosphate as nerve agents

In early 19th century Jean Louis Lassaigne and in 1854 Philip de Clermount, was the pioneer in the field using phosphorous compounds as nerve agents[105]. In 1932, German chemist Willy Lange and his graduate student, Gerde von Krueger, first described the cholinergic nervous system effects of organophosphates, noting a choking sensation and a dimming of vision after exposure. This discovery later inspired German chemist Gerhard Schrader at company IG Farben in the 1930s to experiment with these compounds as insecticides. Their potential use as chemical warfare agents soon became apparent, and the Nazi government put Schrader in charge of developing organophosphate nerve gases. Schrader's laboratory discovered the G series of weapons, which included Sarin, Tabun, and Soman. British scientists experimented with a cholinergic organophosphate of their own, called diisopropylfluorophosphate (DFP), during the war. The British later produced VX nerve agent, which was many times more potent than the G series, in the early 1950s, almost 20 years after the Germans had discovered the G series.

Sarin, or **GB** is an organophosphorus compound with the formula $[(CH_3)_2CHO]CH_3P(O)F$. It is a colorless, odorless liquid[106], which is used as a chemical weapon. It has been classified as a weapon of mass destruction in UN Resolution 687. Sarin is a chiral molecule (typically racemic), with four substituents attached to the tetrahedral phosphorus center[107].

Nerve gases are compounds which block nervous activity and cause death, either quickly or slowly, depending on the condition of exposure[108-113]. 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. The inhibition is associated with a process of phosphorylation whereby the toxic compound becomes linked to the enzyme by a P-O-C linkage.

1.6 Health effects

The signs and symptoms of acute organophosphate poisoning are an expression of the effects caused by excess acetylcholine (cholinergic syndrome); they may occur in various combinations and can be manifest at different times. Signs and symptoms can be divided into three groups: • muscarinic effect • nicotinic effect • central nervous system effect. Those individuals who are exposed to organophosphorus pesticides with pre-existing organic diseases of the central nervous system, mental disorders & epilepsy, pronounced endocrine & vegetative disorders, pulmonary tuberculosis, bronchial asthma, chronic respiratory diseases, cardiovascular diseases & circulatory disorders, gastrointestinal diseases (peptic ulcer), gastroenterocolitis, diseases of liver & kidneys, eye diseases (chronic conjunctivitis & keratitis) are at elevated risk from exposure.

Chronic fatigue is common amongst those who consider their health is affected by pesticides and research from 2003 suggested there was an association between exposure to organophosphates and chronic fatigue symptoms[114]. A 2007 study linked the organophosphate insecticide chlorpyrifos, which is used on some fruits and vegetables, with delays in learning rates, reduced physical coordination, and behavioral problems in children, especially ADHD[115]. A 2010 study has found that organophosphate exposure is associated with an increased risk of Alzheimer's disease[116].

Another 2010 study found that organophosphate exposure is associated with an increased risk of ADHD in children. Researchers analyzed the levels of organophosphate residues in the urine of more than 1,100 children aged 8 to 15 years old, and found that those with the highest levels of dialkyl phosphates, which are the breakdown products of organophosphate pesticides, also had the highest incidence of ADHD. Overall, they found a 35% increase in the odds of developing ADHD with every 10-fold increase in urinary concentration of the pesticide residues. The effect was seen even at the low end of exposure: children who had any detectable, above-average level of pesticide metabolite in their urine were twice as likely as those with undetectable levels to record symptoms ADHD[117].

AIM OF THE PROJECT

Organophosphorus compounds have many fold applications in medicinal chemistry as antifungal and antiviral drugs and have also immense importance in agricultural chemistry as insecticidal and herbicidal chemicals. Almost all of the organic compounds with known pharmaceutical properties contain nitrogen. By contrast, only a handful of presently used pharmaceuticals contain phosphorus element. Owing to their high toxicity and availability of alternative drugs, their uses were restricted about 50 years back. Nevertheless, the medicinal properties of organo and organic phosphorus compounds are being investigated on an ever increasing scale. Some of these are already in use while others show considerable promises.

D. Hendrin reported the activity of phosphonoacetic acid against Harpes and Marek's diseases, while phosphonomycin shows anti-bilharziosic and anti-leprosy properties as well as functioning as broad spectrum antibiotic. Walker and Thorselt reported the broad spectrum antibacterial activity of ribavirin and difficidin. In recent year an important advance have been made in the discovery of the carcinosatic properties of cyclophosphamide and it's derivatives. Schaffer reported the potential antibacterial activity of acyclovir, gancidovir and azidothymidine as amino phosphorus compounds.

Therefore, organo phosphorus compounds composed of diamino functionalities have been addressed with importance in organic and bio-organic chemistry. From the literature survey it has drawn a great interest to synthesize diamino organophosphorus compounds which might have potential biological activity.

The proposed research project is undertaken with the following objectives

     a) To purify the starting materials.

     b) To prepare the unavailable starting materials from phosphoryloxychloride.

     c) To synthesize the different diamino organophosphorus compounds.

     d) To optimize the reaction condition.

     e) To carry out the different physical, chemical and spectroscopic methods to establish the structure of the synthesized products.

     f) To study the biological activity of the synthesized products such as anti-fungal and anti-bacterial properties.

Chapter 2

EXPERIMENTAL

2.1 General methods

2.1.1 Solvents and chemicals

Analytical or laboratory grade solvents and chemicals were used in all experiments. Commercial grade solvents were distilled prior to use for extraction and chromatographic separation.

2.1.2 Evaporation of the solvents

Solvent evaporation was carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 45^0C.

2.1.3 Chromatographic methods

2.1.3.1 Thin layer chromatography (TLC)

Precoated TLC plates; 0.2 mm thin coating of silica-gel 60 PF_{254} on Plastic sheets, were used for this purpose.

2.1.3.2 Application of the samples and Development of the plates

For application of the samples capillary tubes were used. TLC was done by ascending technique in glass jars or tanks.

2.1.3.3 Solvents systems

Solvents used for TLC in this work are given below:

Binary solvent systems were used for less polar fractions and compounds, e.g.

(1) Hexane: dichloromethane   (in different ratio)

(2) Hexane: Ethyl acetate   (in different ratio)

(3) Dichloromethane: Ethyl acetate   (in different ratio)

2.1.3.4 Location of spots

Irrigated plates were developed by one or both of the following methods to detect the positions of the spots.

(1) The plates were kept under UV light for few seconds and then spots were observed.

(2) The spots were located by placing the plates in iodine chamber.

2.1.3.5 Column chromatography

Fractionation of mixture of products were done by silica gel column chromatography

(i) Column

A glass column fitted with a rota-flow was used for chromatographic separation.

(ii) Stationary phase

TLC grade silica gel was used as stationary phase. Column grade silica gel G-60 (230-400 Mesh, Particle size 0.04-0.06 mm, Art. 7734, ASTM, Merck) was used for fractionation.

(iii) Preparation of column

To prepare a particular column, the required amount of silica gel was swelled into a selected solvent (e.g. hexane, dichloromethane, ethyl acetate, methanol or a mixture of different solvents in different ratios) for a while and then poured into the column with continuous flow of the solvent. For homogeneous packing, the column was equilibrated with two or three column volumes of solvent. Separation was performed by gravitational flow with solvents of increasing polarity.

(iv) Application of sample into the column

The sample to be chromatographed was introduced as preadsorbed on the packing material. For preadsorption the sample was dissolved in a particular solvent or a mixture of solvents and silica gel (sample:gel, 1:2-3, w/w) was added to the sample solution. The solvent was then removed by evaporation. The dried material was ground thoroughly in a mortar to make it a fine powder, which was then applied, carefully to the top of the column. Again, some samples were introduced into the column by dissolving them in suitable solvent.

After application of the sample, some cotton or fresh silica gel was placed on the top of the bed so that the surface of the bed was not affected during solvent application.

(v) Fractionation and monitoring procedure

After application of the sample, the column was eluted with the equilibrating solvent and the polarity of the mobile phase was gradually increased by adding dichloromethane or ethyl acetate. The eluted samples were collected in test tubes and the fractions were monitored by using TLC profiles. Similar fractions were combined on the basis of their R_f values.

2.1.4 Determination of melting points

Melting points of different synthesized compounds were determined on Gallenkamp (England) melting point apparatus and paraffin oil bath.

2.1.5 Spectroscopic methods

2.1.5.1 Infrared spectra

The infra-red spectra were recorded on KBr pellet for films with a Shimadzu FTIR spectrophotometer from the department of Chemistry, Bangladesh University of Engineering & Technology, Dhaka, Bangladesh. Major bands (ν_{max}) were recorded in wave number (cm^{-1}).

2.1.5.2 Nuclear magnetic resonance (NMR) spectroscopy

The ^{1}H, ^{13}C and ^{31}P NMR spectra of pure compounds were recorded on a Bruker 400 MHz spectrometer using CDCl$_3$ and CD$_3$OD as solvent. In all cases tetramethyl silane (TMS) was used as the internal reference. Two dimensional NMR (H-H COSY, HMBC) spectra were obtained using standard pulse sequences.

2.2 Synthesis of SN1

2.2.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (1.0g, 6.515 mmol) was taken in a reaction flask equipped with condenser which was then subjected to constant stirring by using magnetic stirrer. A solution of *o*-phenylene diamine (0.35g, 3.241 mmol) in dichloromethane was then added drop-wise to the phosphoryl chloride solution and was stirred for two hours at the room temperature. The progress of the reaction was monitored by TLC. Solvent from the reaction mixture was then evaporated by a rotary vacuum evaporator. The resultant solid mass was then partially dissolved in ethyl acetate and treated with 10% NaOH solution. The reaction mixture was partitioned with ethyl acetate and NaOH solution with the help of a separating flask using brine solution to remove foam arising from the shaking of the solvent in the flask. Ethyl acetate soluble layer was separated and the separation process was carried out three times and the combined solution thus obtained was dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation of the solvent, a yellowish crude product was obtained.

The crude product was then isolated by column chromatography and a white crystalline product **SN1** having a yield of 75% with m.p. 151^{0}C was obtained.
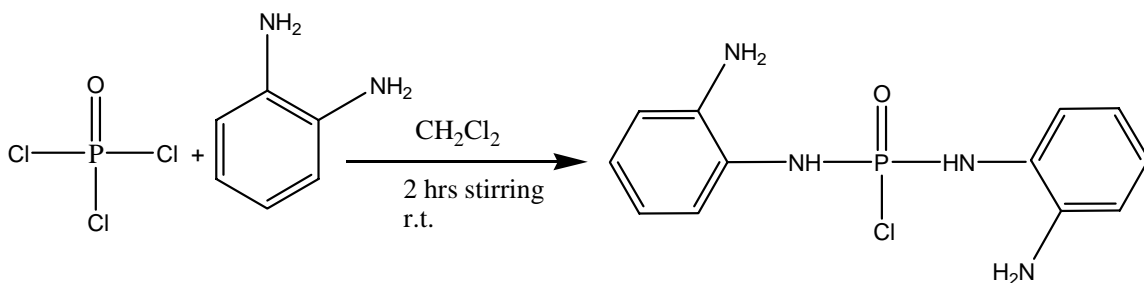
Reaction and Purification Scheme of SN1

$2POCl_3 + o$-phenylene diamine

CH_2Cl_2
Stir for 2 Hours

Reaction mixture

Evaporated to dryness

Solid mass

Partitioned with EtOAc
and NaOH solution

NaOH soluble layer   EtOAc soluble layer

Dried over Anhydrous Na_2SO_4

Filter

Filtrate   Residue

Evaporation

Crude product

Fractionation

Pure compound (SN1)

2.2.2 Spectral evidences of the Compound SN1

IR (KBr) : υ_{max} **(cm^{-1})** 3402.2 (N-H, primary aromatic amine),

3269.1(N-H, 2^0 aromatic amine), 3080.0(C-H, aromatic),

1641.3 (P=0), 1610.1 (C=C, aromatic),

1575.0(C=C, aromatic), 1527.5 (C=C, aromatic),

1510.0 (C=C, aromatic), 1498.6 (C=C, aromatic),

1450.4(C=C, aromatic),1315.4(P-N),1250(C-N), 1170(C-N),

1080.0 (P-Cl), 748.3 (ortho disubstituted benzene ring).

^{1}H NMR : δ_H**(ppm)** 7.934(bs, 4H, C_2 -NH$_2$, C_2' -NH$_2$), 7.537(t, 1H, C_4-H,

(400 MHz, CDCl$_3$)   J=6.8 Hz), 7.875(d, 2H, C_3-H, C_3'-H, J= 7.6 Hz),

7.453(t, 2H, C_5-H,C_4'H, J=7.6 Hz), 7.311(bs, 1H, C_1-NH),

7.292(bs, 1H, C_1'-NH), 7.071(t, 1H, C_5'-H, J= 7.2 Hz),

6.82(d, 2H, C_6-H, C_6'-H, J=7.6 Hz).

^{13}C NMR : δ_C**(ppm)** 118(C-6′), 119(C-5′), 124(C-1′), 125(C-6),

(100 MHz, CDCl$_3$)   127.25(C-5), 127.33(C-3′), 128(C-3 & C-4′), 131(C-4),

134(C-1), 140(C-2′), 165(C-2).

^{31}P NMR : -16.49 (s, P=O, 1P)
(162 MHz, CDCl$_3$)

2D-NMR

The H-H COSY spectral data of **compound SN1** (δ value in ppm)

H		H
7.885(C_3-H)	⟷	7.537(C_4-H)
7.885(C_3'-H)	⟷	7.453(C_4'-H)
6.82(C_6'-H)	⟷	7.071(C_5'-H)
6.82(C_6-H)	⟷	7.31(C_1-NH)

HMBC(Heteronuclear Multiple Bond Correlation) spectral data (δ value in ppm)

^{1}H		^{13}C
7.885(C_3-H, C_3'-H)	⟷	165(C-2), 131(C-4), 128(C4'), 127.25(C-5)
7.537 (C_4-H)	⟷	127.25(C-5)
7.453(C_5-H,C_4'H)	⟷	131(C-4), 128.6(C4'), 127.33(C3')
7.071(C_5'-H)	⟷	140(C-2'), 124(C-1'), 119(C5'), 118(C-6')
6.82(C_6'-H, C_6-H)	⟷	124(C1'), 119(C5')

2.3 Synthesis of SN2

2.3.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (1.0g, 6.516 mmol) was taken in a reaction flask equipped with a condenser which was then subjected to constant stirring by using magnetic stirrer. A solution of ethylene diamine (0.19g, 3.257 mmol) in dichloromethane was then added drop-wise to the phosphoryl chloride solution and was stirred for two hours at the room temperature. The progress of the reaction was monitored by TLC. A solid crude mass was obtained after evaporation of the solvent by a rotary vacuum evaporator. The crude product was then dissolved in ethyl acetate and then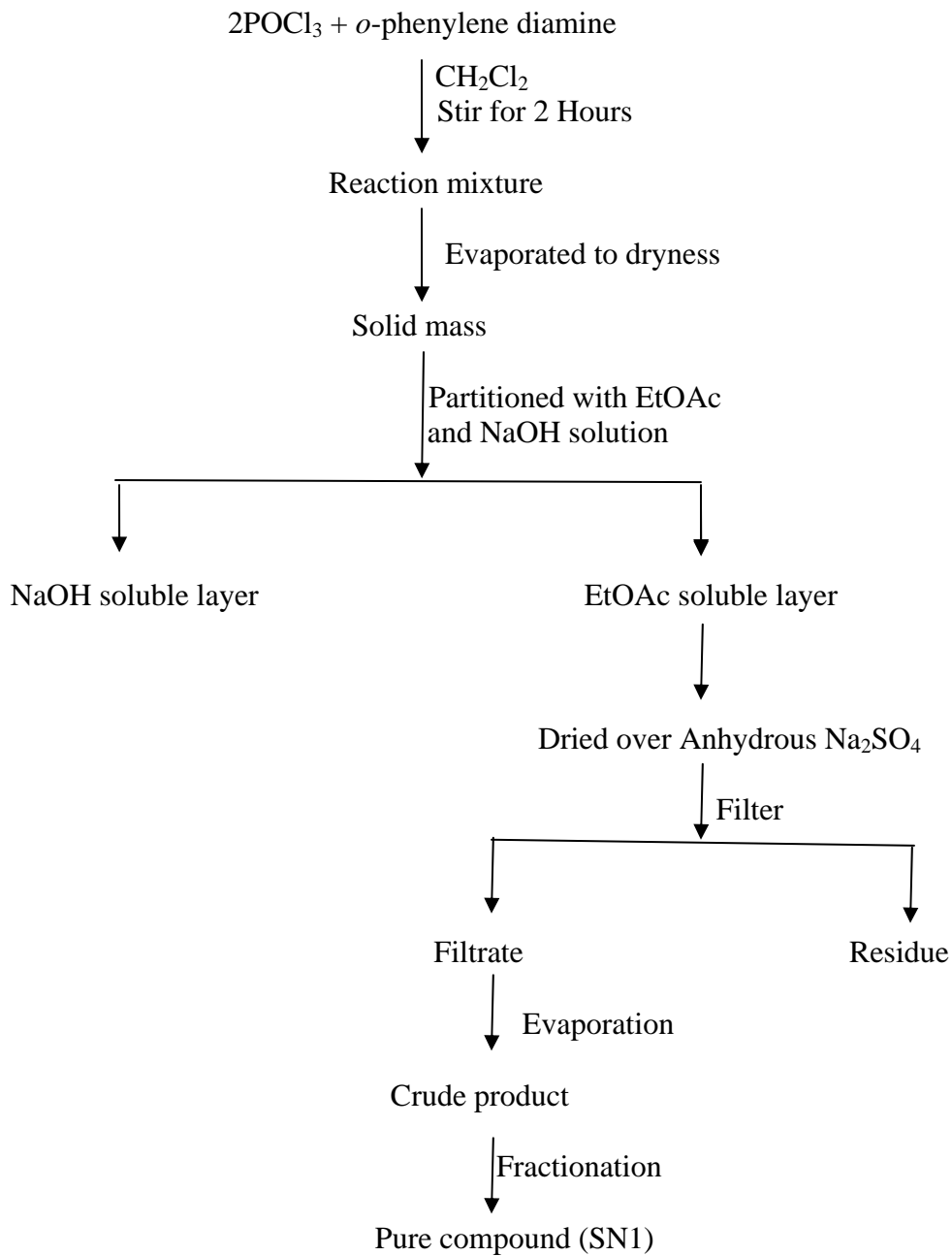 treated with 10% NaOH solution. The reaction mixture was partitioned with ethyl acetate and NaOH solution with the help of a separating flask using brine solution to remove foam arising from the shaking of the solvent in the flask. Ethyl acetate soluble layer was separated. The extraction process was carried out three times and the combined solution thus obtained was dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, a white crude product was obtained.

The crude product was monitored by TLC and was purified by recrystallization. A colourless crystalline product SN2 was obtained, having 78% yield.
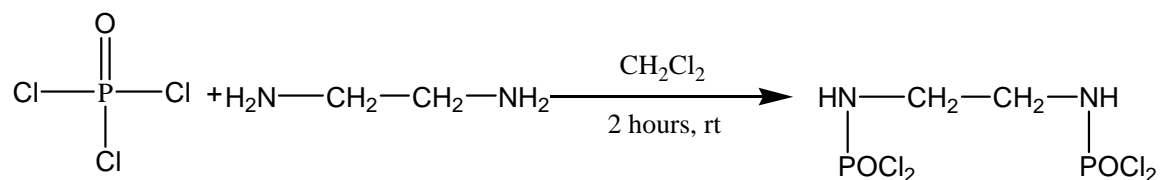
2.3.2 Spectral evidences of the Compound SN2

IR (KBr) : υ_{max} **(cm^{-1})** 3296.1 (N-H, aliphatic amine)

2925.8 (C-H, aliphatic), 1633.6 (P=0), 1332.7 (P-N)

1234.4 & 1294.1 (two C-N), 1182.3 (P-Cl)

^{1}H NMR (400 MHz, CDCl$_3$) : δ_H**(ppm)** 4.96 (bs, 2H, -NH-), 2.1(s, 4H, C$_1$-H, C$_2$-H),

^{13}C NMR (100 MHz, CDCl$_3$) : δ_C**(ppm)** 29.733 (C-1, C-2)

2.4 Synthesis of Compound SN4

2.4.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (1.0g, 6.515 mmol) was taken in a reaction flask equipped with condenser which was then subjected to constant stirring by using magnetic stirrer. A solution of *p*-phenylene diamine (0.35g, 3.241 mmol) in dichloromethane was then added drop-wise to the reaction flask and was stirred for two hours at the room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction dichloromethane was removed by a rotary vacuum evaporator. The resultant solid mass was then partially dissolved in ethyl acetate and then treated with saturated NaHCO$_3$ solution. The entire solution was then subjected to stirring for 10 minutes to complete the proton abstraction procedure. After that the solution was transferred into a separation flask where two layers were obtained. Ethyl acetate soluble layer was collected for further step. The extraction process was carried out three times and the solution thus obtained was dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation of the solvent, a brown crude product was obtained.

The crude product was then subjected to column chromatography and two products were isolated as SN3 and SN4. The solid product SN4 was brown in colour, having a yield of 62%.
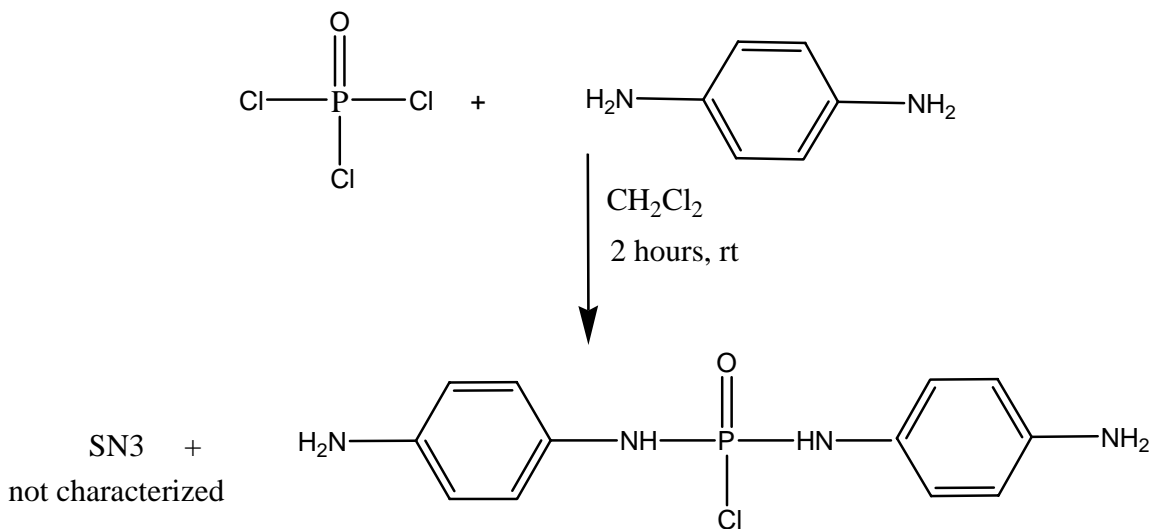
2.4.2 Spectral evidences of the compound SN4

IR (KBr) : υ_{max} **(cm^{-1})** 3446.6 & 3381.0 (N-H, primary aromatic amine),

3330.8 (N-H, secondary aromatic amine),

3033.8(C-H, aromatic), 1645.2(P=0), 1602.7(C = C, aromatic),

1580.1 (C = C, aromatic), 1525.0 (C = C, aromatic),

1517.9 (C = C, aromatic), 1490.0 (C = C, aromatic),

1427.2 (C = C, aromatic), 1319.2 (P-N), 1251.7 (P-Cl),

819.7 (para disubstituted benzene ring)

¹H NMR : δ$_H$ **(ppm)** 7.84(s, 1H, C$_1$′-NH), 7.82(s, 1H, C$_1$-NH),

(400 MHz, CDCl₃) 7.74(bs, 4H, C$_4$-NH$_2$, C$_4$′-NH$_2$),

7.50(d, 2H, C$_2$′-H, C$_5$′-H, J=7.2 Hz)

7.45(d, 2H, C$_2$-H,C$_4$-H, J=8.0 Hz),

7.38(d, 2H, C$_3$′-H, C$_5$′-H, J= 7.6 Hz),

6.67(d, 2H, C$_3$-H, C$_5$-H, J=8.4 Hz).

¹³C NMR : δ$_C$ **(ppm)** 115.59(C-5), 122.38(C-3), 125.98(C-5′), 128.44(C-3′),

(100 MHz, CDCl₃) 128.70(C-2, C-6), 129.34(C-6′), 131.57(C-2′), 135.17(C-4),

143.46(C-4′), 165.67(C-1), 176.19(C-1′).

2.5 Synthesis of SN5

2.5.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (2.0g, 13.029 mmol) was taken in a reaction flask equipped with condenser which was then subjected to constant stirring by using magnetic stirrer. A solution of hexamethylene diamine (0.378g, 3.259 mmol) in dichloromethane was added drop-wise to the reaction flask and was stirred for two hours at the room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction dichloromethane was removed by a rotary vacuum evaporator. The resultant solid mass was then partially dissolved in ethyl acetate and then treated with saturated NaHCO$_3$ solution. The entire solution was then subjected to stirring for 10 minutes to complete the proton abstraction. After that the solution was transferred into a separation flask where two layers were obtained. Ethyl acetate extraction was carried out three times and the combined solution was dried over

anhydrous Na_2SO_4. After filtration and evaporation of the solvent, a white crude product was obtained.

The crude product was then subjected to column chromatography and two products were isolated as SN5 and SN6. Compound SN5 was white in colour, having m.p 157^0C and 70% yield.

.

+

SN6 (not characterized)
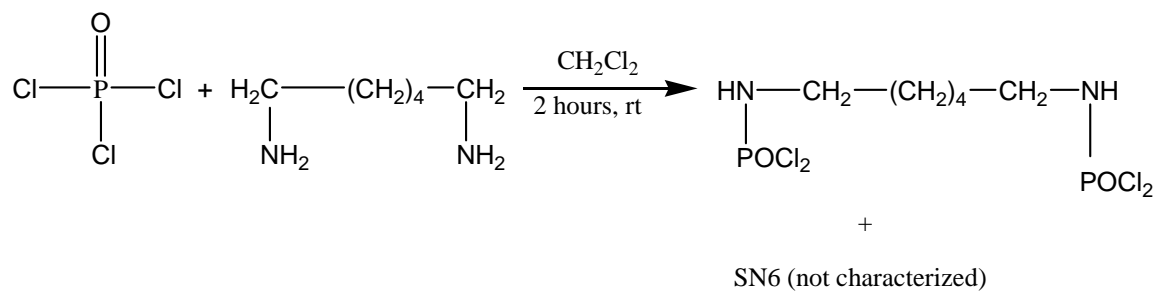
2.5.2 Spectral evidences of the compound SN5

The IR spectrum of Compound SN5 had the following absorption frequencies-

IR (KBr) : υ_{max} **(cm^{-1})** 3321.2 (N-H in secondary aliphatic amine),

2935.5 (C-H, aliphatic), 1629.7 (P=0), 1477.4 (P-N),

1290.3 (C-N), 1100 (P-Cl).

^{1}H NMR : δ_H **(ppm)** 3.45(q, 4H, C_1-H, C_6-H), 1.62(bs, 4H, C_2-H, C_5-H),

(400 MHz, CDCl$_3$) 1.44(s, 4H, C_3-H, C_4-H), 2.03(bs, 2H, C_1-NH, C_6-NH),

^{13}C NMR : δ_C **(ppm)** 26.008(C-3, C-4), 29.577(C-2, C-5),

(100 MHz, CDCl$_3$) 39.486(C-1, C-6)

2.6 Synthesis of SN7

2.6.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (1.0g, 6.515 mmol) was taken in a reaction flask equipped with condenser which was then subjected to constant stirring by using magnetic stirrer. Solid thiosemicarbazide (0.296g, 3.257 mmol) was added periodically to the phosphoryl chloride solution and was stirred for two hours at the room temperature. The progress of the reaction was monitored by TLC. The solid mass obtained after removing the solvent by a rotary vacuum evaporator was dissolved in ethyl acetate and then treated with saturated $NaHCO_3$ solution. The entire mixture was stirred for 10 minutes. Then the mixture was transferred in a separating flask. After adding brine solution to the flask the mixture was shaked vigorously and allowed to stand for one hour to get to separate layer of organic and aqueous solution. Ethyl acetate layer was separated and the extraction process was repeated three times and the combined solution thus obtained was dried over anhydrous Na_2SO_4. After filtration and evaporation of the solvent, a white crude product was obtained.

The crude product was monitored by TLC and three spots were observed. Thus the crude product was washed with dichloromethane and the washed solution was allowed to stand for recrystallization. The resultant white crystals gave a single spot on TLC.
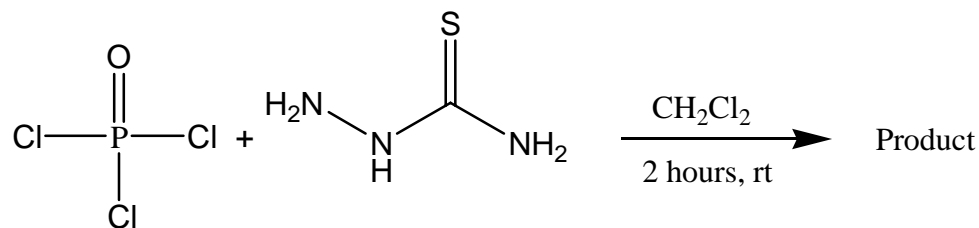
2.6.2 Spectral evidences of compound SN7

IR (KBr) : υ_{max} **(cm^{-1})** 3132.2 (N-H), 1674.1 (P=0), 1315.4 (P-N),

1259.4 (C-N)1250.0 (P-Cl).

2.7 Synthesis of SN8

2.7.1 Procedure

A solution of phosphorous oxychloride in dichloromethane (2.0g, 13.029 mmol) was taken in a reaction flask equipped with condenser which was then subjected to constant stirring by using magnetic stirrer. A solution of metaphenylene diamine (0.35g, 3.241 mmol) in dichloromethane was then added drop-wise to the reaction flask and was stirred for two and half an hours at the room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction dichloromethane was removed by a rotary vacuum evaporator. The resultant solid mass was then partially dissolved in ethyl acetate and then treated with saturated NaHCO$_3$ solution. The entire solution was then subjected to stirring for 10 minutes to complete the proton abstraction procedure. After that the solution was transferred into a separation flask where two layers were obtained. Ethyl acetate soluble layer was collected for further step. The extraction process was carried out three times and the solution thus obtained was dried over anhydrous Na$_2$SO$_4$. After filtration and evaporation of the solvent, a black crude product was obtained.

The crude product was then subjected to column chromatography and two products were isolated as SN8 and SN9. The compound SN8 was white in colour, having m.p. 153^{0}C and 69% yield.
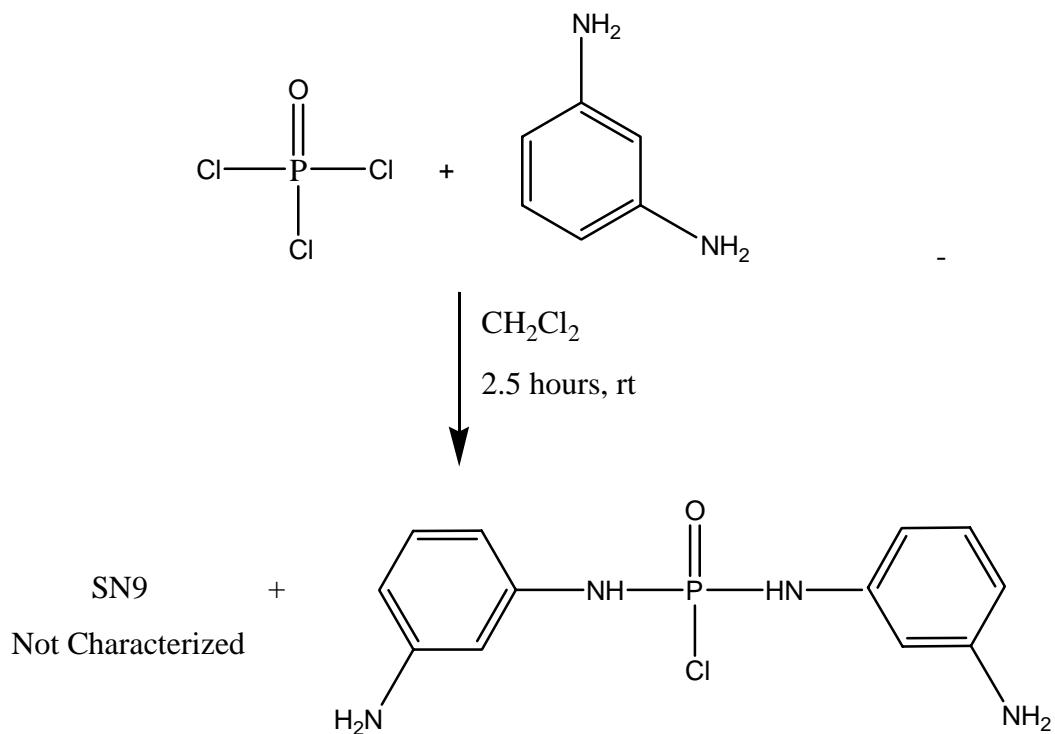
2.7.2 Characterization of compound SN8 by spectroscopic method

IR (KBr) : υ_{max} **(cm^{-1})** 3502.5 (N-H, primary aromatic amine),

3269.1 (N-H, secondary aromatic amine),

3062.7(C-H, aromatic), 1647.1 (P=0), 1600.8 (C=C, aromatic),

1598.0 (C=C, aromatic), 1521.0 (C=C, aromatic),

1519.8 (C=C, aromatic), 1450.0 (C=C, aromatic),

1438.8 (C=C, aromatic), 1323.1 (P-N), 1300.0 (P-Cl).

794.6 & 710.0 (meta disubstituted benzene ring)

¹H NMR : δ_H **(ppm)** 5.492 (bs, 2H, C_3-NH$_2$), 5.472 (bs, 2H, C_3′-NH$_2$),

(400 MHz, CDCl₃) 6.782(bs, 1H, C_2-H),6.762 (bs, 1H, C_2´-H), 6.709(bs, 1H, C_6-H),

6.449(m, 1H, C_5-H), 6.362(bt, 1H, C_4-H, J=7.6 Hz),

6.180(m, 1H, C_5´-H), 6.054(bd, 1H, C_6´-H, J=8.0 Hz),

5.999(t, 1H, C_4´-H, J = 8.0Hz), 5.673(s, 1H, C_1-NH),

5.654(s, 1H, C_1′-NH).

¹³C NMR : δ_C **(ppm)** 147.049 (C-1), 139.084 (C – 1′), 134.562 (C – 3),

(100 MHz, CDCl₃) 134.428 (C – 3´), 131.973 (C – 2), 129.914 (C – 2′),

128.686 (C – 4, C – 4′), 127.068 (C – 6, C – 6´),

124.543 (C – 5), 120.542 (C– 5´).

Chapter 3

RESULT & DISCUSSION

3.1 Characterization of Compound SN1 as bis–(*o*–phenylenediamino) chlorophosphine oxide

The structure of the compound SN1 was established by IR, ^{1}H NMR, ^{13}C NMR, DEPT 135, ^{31}P NMR and 2D NMR spectral evidences.

The IR spectrum (**Fig.1a**) of the compound **SN1** showed sharp absorption band at 3080.0 cm^{-1} revealed the aromatic C-H stretching vibration. The characteristic six absorption peaks at 1610.1 cm^{-1} , 1575.0 cm^{-1} , 1527.5 cm^{-1} , 1510.0 cm^{-1} , 1498.6 cm^{-1} and 1450.4 cm^{-1} were found due to aromatic C=C bond vibration. The presence of the sharp peak at 1641.3 cm^{-1} was ascribed for P=O group of phosphoryl moiety. It showed absorption at comparatively higher frequency due to the attachment of electron withdrawing group. The bands at 3402.2 cm^{-1} and 3269.1 cm^{-1} were indicative for primary aromatic amino and secondary aromatic amino group respectively. The band at 1315.4 cm^{-1} was due to P-N stretching vibration. Two bands at 1250 cm^{-1} and 1170 cm^{-1} were identified for two C-N bond vibration. Again, P-Cl bond can be observed by peak at 1080 cm^{-1}. The appearance of a band at 748.3 cm^{-1} revealed the presence of ortho disubstituted benzene ring.

The ^{1}H NMR spectrum (**Fig.1b**) of the compound **SN1** having broad singlet at δ_H 7.934 ppm was attributable to primary aromatic amino protons. The broad singlet peaks at δ_H 7.311 ppm and δ_H 7.292 ppm were assigned for two different N – H protons attached to two different identical aromatic rings of C-1 and C-1′ respectively. These peaks were observed at down field due to N – H groups attached at aromatic ring. The peak at δ_H 7.537 ppm showed a triplet for one proton at C4 with a coupling constant J= 7.6 Hz. The triplet at δ_H 7.453 ppm with coupling constant J= 7.6 Hz was assigned for the protons at C5 and C4′ respectively. A two proton doublet at δ_H 7.875 ppm with coupling constant J= 7.6 Hz were ascribed for two aromatic protons attached to C-3 and C-3′ position. A triplet at δ_H 7.071 ppm with coupling constant J=7.2 Hz was designated for C_5′ proton. The ring protons at C-6 and C-6′ showed a doublet at δ_H 6.82 ppm with the coupling constant J=7.6 Hz. The two aromatic rings are not in the same plane. So the two rings protons are not equivalent and hence the chemical shift value will not be same

for the two rings. They showed different chemical shift value for their different environment.

The ^{13}C NMR spectrum (**Fig.1d**) of the compound **SN1** displayed 12 carbon resonances, while DEPT 135 (**Fig. 1e**) experimental data indicated that 8 out of 12 carbons were attached to protons. DEPT 135 (100 MHz, $CDCl_3$) spectra revealed the presence of 8 methines and four quaternary carbon atoms.

In the ^{13}C NMR spectrum having peaks at δ_C 134 ppm and at δ_C 165 ppm in conjunction with DEPT experiment proved the presence of two quaternary carbon in aromatic ring which can be assigned as C-1 and C-2 carbon in one ring. Again two quaternary peaks at δ_C 124 ppm and δ_C 140 ppm were assigned for C-1′ and C-2′ carbon of other aromatic ring. The chemical shift with double intensity at δ_C 128 ppm was designated for ring carbon C-3 and C-4′. The peak at δ_C 131 ppm was observed for C-4, δ_C 127.25 ppm for C-5 and δ_C 125 ppm for C-6. Again for another aromatic ring, peaks at δ_C 127.33 ppm, δ_C 119 ppm and δ_C 118 ppm were attributed for C-3′, C-5′ and C-6′ respectively. The two aromatic rings are not in the same plane. So the two rings carbons are not equivalent and hence the chemical shift value will not be same for the two rings.

The ^{31}P NMR spectrum (**Fig. 1f**) showed a peak at δ_P −16.5 ppm was indicative for the 1P of P = O group.

The cross peaks in the COSY spectrum (**Fig. 1g**) between δ_H 7.01 ppm (C_5′-H) and δ_H 6.82 ppm (C_6′-H) indicated that they were vicinal. Again the cross peaks between δ_H 7.875 ppm (C_3-H, C_3′-H) with δ_H 7.53 ppm (C_4-H) & δ_H 7.453 ppm (C_4′-H) indicated that both protons were vicinal with C_3-H and C_3′-H. Study on HMBC spectrum (**Fig. 1h**) showed the correlation of proton δ_H 7.875 ppm (C_3-H, C_3′-H) with C-5 (δ_C 127.25 ppm), C-4 (δ_C 131 ppm), C-4′ (δ_C 128 ppm) and C-2 (δ_C 165 ppm). Similarly several correlations were observed for other protons which were δ_H 7.53 ppm (C_4-H) with C-5 (δ_C 127.25 ppm) and C-3 (δ_C 128 ppm); δ_H 7.45 ppm(C_5-H,C_4′-H) with C-3′ (δ_C 127.33

ppm), C-4 (δ_C 131 ppm) and C-3 (δ_C 128 ppm); δ_H 7.071 ppm (C_5'-H) with C-6′ (δ_C 118 ppm), C-1′ (δ_C 124 ppm), C-2′ (δ_C 140 ppm); δ_H 6.82 ppm (C_6-H, C_6'-H) with C-5′ (δ_C 119 ppm), C-1′ (δ_C 124 ppm).

Analysis of one- and two- dimensional NMR spectra including COSY and HMBC led to the assignment of the structure as shown in figure below-

(a) (b)
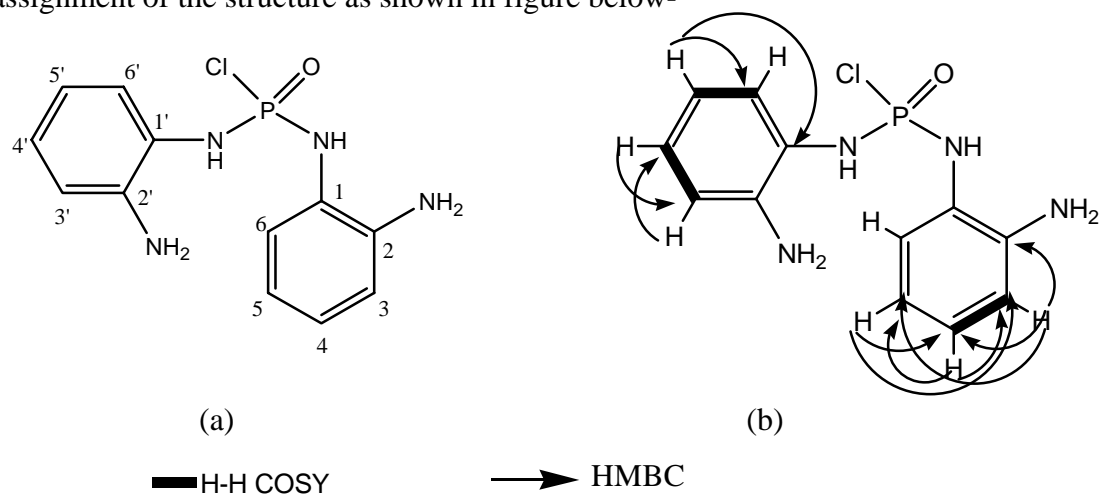
H-H COSY HMBC

Figure: (a) Structure of compound SN1 and (b)Connectivities of Compound SN1

Table: ^{1}H (400 MHz, CDCl$_3$) and ^{13}C (100 MHz, CDCl$_3$) NMR data

no.	δ_H , J in Hz	δ_C	COSY	HMBC
1	7.311(bs, 1H, C_1-NH)	134	...	...
2	7.934(bs, 4H, C_2 -NH$_2$)	165	...	...
3	7.875(d, 2H, C_3-H, J= 7.6 Hz)	128	C_3-H, C_4-H	C-2, C-4, C-5
4	7.537(t, 1H, C_4-H, J=6.8 Hz)	131	...	C-3, C-5
5	7.453(t, 2H, C_5-H, J=7.6 Hz)	127.25	...	C-4, C-3
6	6.82(d, 2H, C_6-H, J=7.6 Hz)	125	...	...
1′	7.292(bs, 1H, C_1'-NH)	124	...	…
2′	7.934(bs, 4H, C_2' -NH$_2$)	140	...	...
3′	7.875(d, 2H, C_3'-H, J= 7.6 Hz)	127.33	...	C-4′
4′	7.453(t, 2H, C_4'-H, J=7.6 Hz)	128	C_3'-H, C_4'-H	C-3′
5′	7.071(t, 1H, C_5'-H, J= 7.2 Hz)	119	…	C-1′, C-2′, C-6′
6′	6.82(d, 2H, C_6'-H, J=7.6 Hz)	118	C_6'-H , C_5'-H	C-1′, C-5′

All the spectral evidences expressed harmony with the given structure of the compound **SN1** as Bis-(*o*-phenylenediamino)chlorophosphine oxide.

.3.2 Characterization of Compound SN2 as 1,2-Bis-(dichlorophosphamido) ethane

The structure of the compound **SN2** was assigned from its spectral data such as, IR, ^{1}H NMR, ^{13}C NMR and DEPT 135 NMR spectral data.

The presence of methylene group was confirmed by the aliphatic-H absorption band at 2925.8 cm^{-1} in IR spectrum (**Fig.2a**) in conjunction with downward peak at δ_C 29.733 ppm in DEPT 135 (**Fig. 2d**) experimental data. Again in ^{1}H NMR spectrum (**Fig. 2b**) a sharp singlet at δ_H 2.1 ppm was ascribed as peak for methylene proton. Here splitting of the peak was not appeared properly due to the presence of electronegative groups. A broad singlet at δ_H 4.96 revealed the presence of secondary amino proton. These amino protons were again confirmed by the absorption band at 3296.1 cm^{-1} in IR spectrum. A sharp peak at 1633.6 cm^{-1} was ascertained the presence of P=O group in the compound. It showed absorption at comparatively higher frequency due to the attachment of electron withdrawing group. Again two bands at 1234.4 cm^{-1} and 1294.1 cm^{-1} were observed for the two C-N bond vibration. In addition, in IR spectrum a band at 1332.7 cm^{-1} was observed for P-N bond vibration and a band at 1182.3 cm^{-1} was for P-Cl bond vibration.

Therefore, all the spectral evidences completely supported the correlation in favour of the given structure of the compound SN2 as 1,2-Bis-(dichlorophosphamido) ethane.
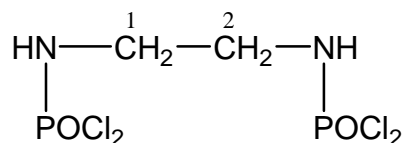
3.3 Characterization of Compound SN4 as bis–(*p*–phenylenediamino) chlorophosphine oxide

The structure of the compound **SN4** was established by IR, ^{1}H NMR and ^{13}C NMR spectral evidences.

The IR spectrum (**Fig.3a**) of the compound **SN4** having the band at 3033.8 cm^{-1} was assigned for the aromatic C – H stretching. The characteristic absorption band at 1602.7, 1580.1, 1525.0, 1517.9, 1490.0 and 1427.2 cm^{-1} were assigned for two aromatic ring C = C bonds of different orientations. The sharp band at 1645.2 cm^{-1} was designated for P = O group of phosphoryl moiety. The peak at 1319.2 cm^{-1} was for P – N and 1251.7 cm^{-1} was indicative for P-Cl bond. The bands at 3446.6 cm^{-1} and 3381.0 cm^{-1} were indicative for two primary aromatic amino groups. Again secondary amino group could be identified by the absorption band at 3330.8 cm^{-1}. The appearance of a band at 819.7 cm^{-1} revealed the presence of para disubstituted benzene ring.

The ^{1}H NMR spectrum (**Fig.3b)** of the compound **SN4** showed broad singlet at δ_H 7.74 ppm was attributable to primary aromatic amino protons designated as C_4-NH$_2$, C_4'-NH$_2$. The of peaks at δ_H 7.82 ppm (s, 1H) and δ_H 7.84 ppm (s, 1H) were assigned for two different N – H protons attached to two different identical aromatic ring linked at C-1 and C-1′ respectively. These proton peaks were observed at down field due to N – H groups attached at aromatic ring. A two proton doublet with a coupling constant J= 8.0 Hz found at δ_H 7.45 ppm was ascertained for the presence of two aromatic proton C_2-H and C_4-H. Again a two proton doublet having resonances at δ_H 6.67 ppm with coupling constant J= 8.4 Hz were ascribed for two aromatic protons attached to C-3 and C-5 position. The doublet at δ_H 7.50 ppm with coupling constant J= 7.2 Hz was assigned for the protons C_2'-H and C_5'-H. The ring protons C_3'-H and C_5'-H were identified as a doublet at δ_H 7.38 ppm with the coupling constant J=7.6 Hz. The two aromatic rings are not in the same plane. So the two rings protons are not equivalent and hence the chemical shift value will not be same for the two rings. They showed different chemical shift value for their different environment.

The ^{13}C NMR spectrum (**Fig.3c**) of the compound **SN4** displayed 12 carbon resonances. The spectrum showed the peaks at δ_C 165.67 ppm for C–1, 176.19 ppm for C–1′; 135.17 ppm for C–4, 143.46 ppm for C–4′. The peak at δ_C 128.70 ppm was observed for C-2, δ_C 122.38 ppm for C-3, δ_C 115.59 ppm for C-5 and δ_C 128.70 ppm for C-6. Again for another aromatic ring, peaks at δ_C 131.57 ppm, δ_C 128.44 ppm, δ_C 125.98 ppm and δ_C 129.34 ppm were attributed for C-2′, C-3′, C-5′ and C-6′ respectively. The two aromatic rings are not in the same plane. So the two rings carbons are not equivalent and hence the chemical shift value will not be same for the two rings.

Therefore, from all the spectral evidences, the structure of the compound SN4 was ascertained as
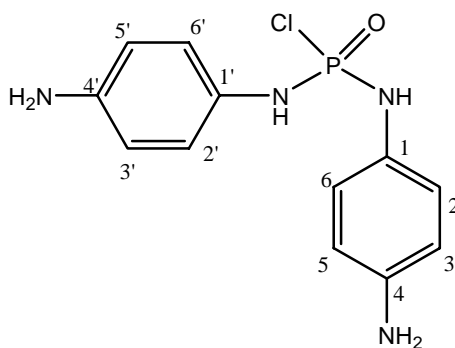
3.4 Characterization of Compound SN5 as 1,6-Bis-(dichlorophosphamido) hexane

The structure of the compound **SN5** was established by IR, ^{1}H NMR, ^{13}C NMR and DEPT 135 experimental data.

In the IR spectrum (**Figure. 4a**), the absorption at the region 2935.5 cm^{-1} was due to aliphatic C-H stretching from methylene group. The appearance of the band at 3321.2 cm^{-1} revealed the presence of secondary amino (-NH-) group in the compound. The sharp and intensified peak at 1629.7 cm^{-1} was assigned for P=O group. It showed absorption at comparatively higher frequency due to the attachment of electron

withdrawing groups. The sharp peak at 1100 cm^{-1} was observed for the P-Cl bond vibration. The absorption band at 1477.4 cm^{-1} was ascribed for the P-N bond vibration and for the presence of C-N bond a peak was observed at 1290.3 cm^{-1}.

The ^{1}H NMR spectrum (**Fig.4b)** of the compound **SN5** having broad singlet at δ_H 2.03 ppm was attributable to two secondary aliphatic amino protons at C-1 and C-6. The 4H singlet at δ_H 1.44 ppm revealed the presence of two methylene group assigned as C_3-H, C_4-H. The relatively upfield value indicates the absence of any electronegative group with them. The resonance at δ_H 1.62 ppm appeared as a broad singlet was ascribed for another methylene protons positioned at C_2-H, C_5-H. Again, the presence of a 4H quartet at δ_H 3.45 ppm ascribed the methylene protons attached with electronegative groups and designated as C_1 -H, C_6 –H.

The ^{13}C NMR spectrum (**Fig.4c)** of the compound **SN5** displayed 3 carbon resonances where each peak was appeared for two carbon atoms. DEPT 135 (**Fig. 4d**) experimental data indicated that all the 6 carbons were attached to protons. In the ^{13}C NMR spectrum having peaks at δ_C 26.008 ppm proved the presence of two methylene carbon which can be assigned as C-3 and C-4. Again the peak at δ_C 29.577 ppm was ascertained for C-2 and C-5 methylene carbon. The presence of a resonance peak at δ_C 39.486 ppm was assigned for   C-1 and C-6 methylene carbon attached with electronegative group. All the peaks were observed at downward direction in DEPT 135 spectrum which was indicative for the presence of all the secondary carbon.

Therefore, from all the spectral evidences, the structure of the compound **SN5** was ascertained as 1,6-Bis-(dichlorophosphamido) hexane.
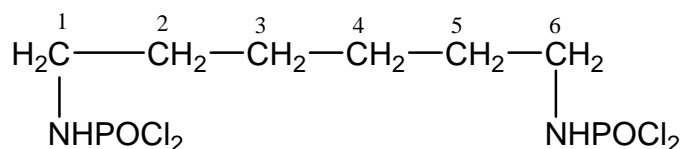
3.5 Characterization of Compound SN7

The structure of the compound **SN7** could not be confirmly elucidated due to the scarcity of the availability of NMR or other spectral data. But from the IR spectral data some functional groups or bonds were identified.

The IR spectrum **(Fig. 5a)** of the compound **SN7** showed band at 1674.1 cm^{-1} was designated for P = O group of phosphoryl moiety. The absorption band at 1315.4 cm^{-1} was indicative for P – N and 1250.0 cm^{-1} was indicative for P-Cl bond vibration. The band at 3132.2 cm^{-1} was ascribed for secondary amino group. The C-N vibration appeared at 1259.4 cm^{-1}.

3.6 Characterization of Compound SN8 as Bis–(*m*–phenylenediamino) chlorophosphine oxide

The structure of the compound **SN8** was established by IR, ^{1}H NMR and ^{13}C NMR experimental data.

The IR spectrum **(Fig. 6a)** of the compound **SN8** showed the broad absorption band at 3502.5 cm^{-1} was assigned for NH$_2$ group. The band at 3269.1 cm^{-1} was indicative for N-H group. The low intensity absorption peak at cm^{-1} was designated as aromatic C-H stretching. The sharp intensified peak at 1647.1 cm^{-1} was for P=O group attached with electronegative groups. The characteristic bands at 1600.8, 1598.0, 1521.0, 1519.8, 1450.0, and 1438.8 cm^{-1} were assigned for aromatic ring C = C bond vibrations. The peak at 1323.1 cm^{-1} was for P – N and 1300 cm^{-1} was indicative for P-Cl bond vibration. The appearance of two bands at 794.6 & 710.0 cm^{-1} revealed the presence of meta disubstituted benzene ring.

The ^{1}H NMR spectrum **(Fig.6b, 6c)** of the compound **SN8** having broad singlet at δ_H 5.492 and 5.472 ppm were attributable to primary aromatic amino protons designated as C$_3$-NH$_2$, C$_3$′-NH$_2$. Almost double intensified signals at δ_H 6.782 ppm and δ_H 6.762 ppm were assigned for the chemical shifts of the ring protons at C$_2$-H and C$_2$′-H

respectively. The chemical shift with almost equal intensity towards more up field at δ_H 6.709 ppm was ascribed for C_6-H ring proton while the resonance at δ_H 6.449 ppm as a multiplet was assigned for the C_5-H ring proton. The triplet at δ_H 6.362 ppm with a coupling constant J=7.6 Hz and the multiplet at δ_H 6.180 ppm were assigned for the chemical shift of the ring protons at C_4-H and $C_5{'}$-H respectively. One doublet at δ_H 6.054 ppm with a coupling constant J=8.0 Hz and one triplet at δ_H 5.999 ppm with a coupling constant J=8.0 Hz were assigned for the protons at $C_6{'}$-H and $C_4{'}$-H respectively. The resonance at δ_H 5.673(C_1-H) ppm and 5.654($C_1{'}$-H) ppm was assigned for the two secondary aromatic amino protons attached to two different aromatic rings. The two aromatic rings are not in the same plane. So the two rings protons are not equivalent and hence the chemical shift value will not be same for the two rings. They showed different chemical shift value for their different environment.

The ^{13}C NMR spectrum (**Fig.6d**) of the compound **SN8** displayed 12 carbon resonances. The spectrum showed the peaks at δ_C139.084 ppm for C–1, 134.542 ppm for C–3, 134.482 ppm for C–3´, 131.973 ppm for C–1´, 129.914 ppm for C–2 carbons. The chemical shift with double intensity at δ_C 128.686 ppm and δ_C 127.068 ppm were assigned for two sets of ring carbon (C–2´, C–4) and (C–6 and C–6´), respectively. The peak at 124.543 ppm was for C – 4, 120.542 ppm for C´– 4, 118.454 ppm for C – 5 and 118.334 ppm for C´–5. The two aromatic rings are not in the same plane. So the two rings carbons are not equivalent and hence the chemical shift value will not be same for the two rings.

Therefore, from all the spectral evidences supported the correlation with the structure of the compound **SN8** was ascertained as
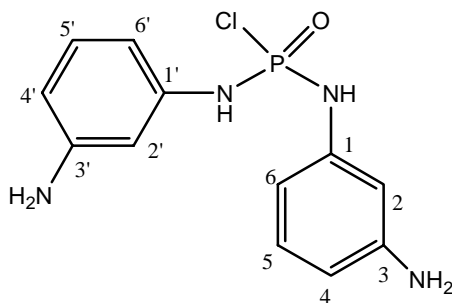
3.11 MECHANISM OF THE SYNTHESIS

Synthesis of bis- substituted phenyl amino chlorophosphine oxide

Synthetic Scheme:
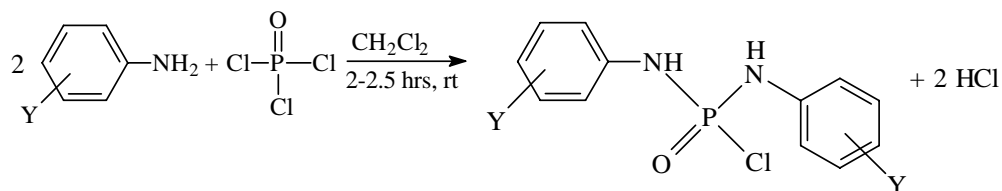
Here, Y= p–NH_2, o–NH_2, m–NH_2

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

The nucleophilic amino compound readily attacks the nucleophilic centre phosphorous atom of the phosphoryl chloride forming a transition state-1. This intermediate TS-1 instantly converted to transition state-2 by removing Cl^- which in terms form chlorophosphine oxide by subsequent removal of HCl.
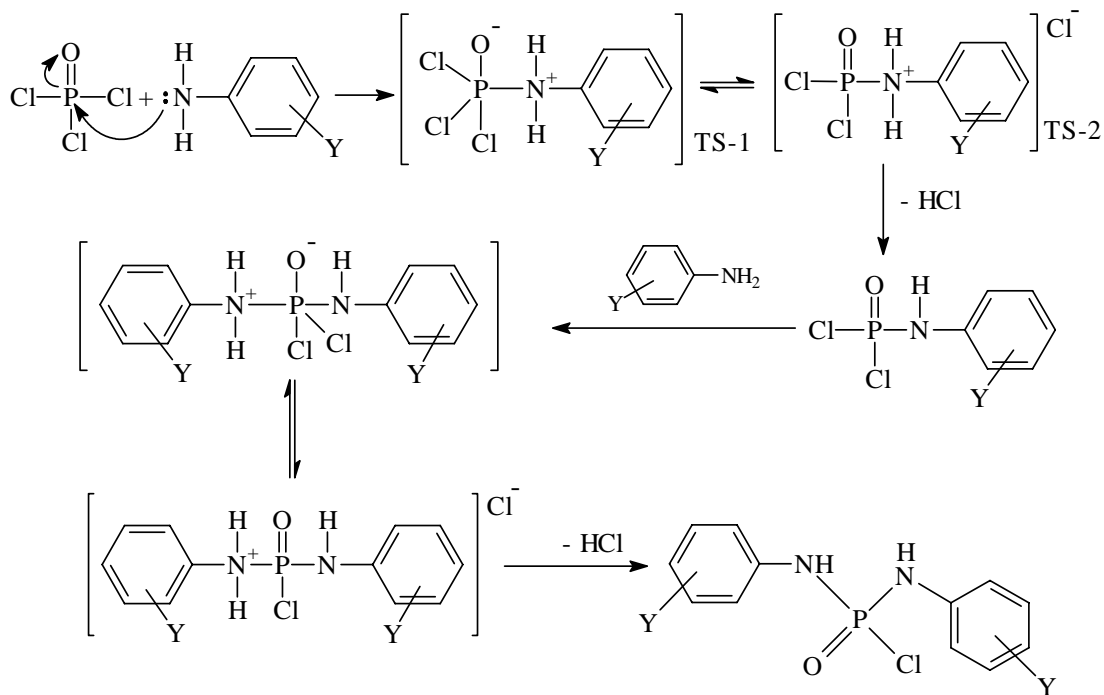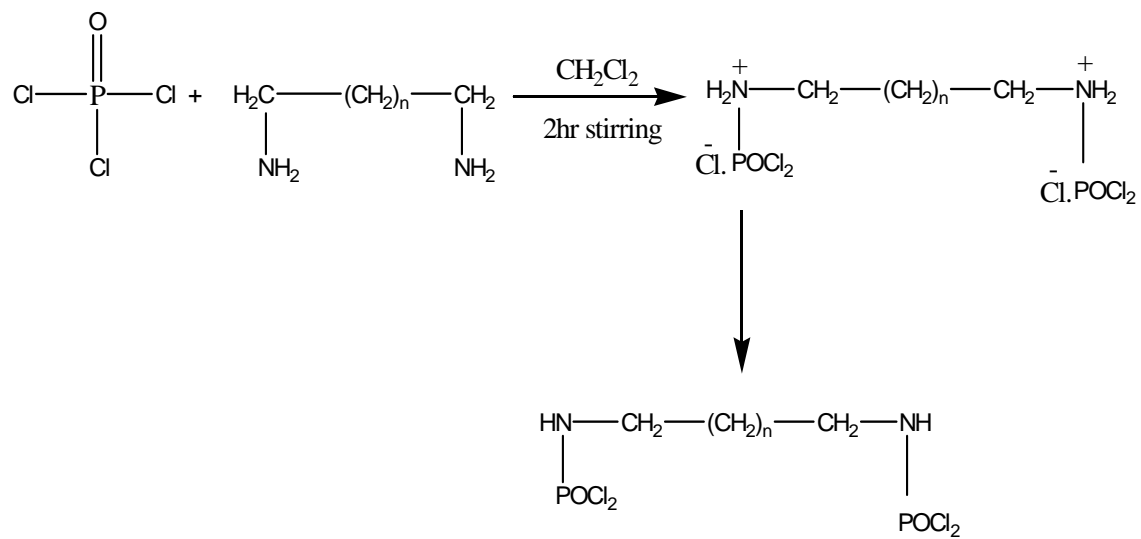
Here n = 0 or 4

PART- II

BIOLOGICAL STUDY

4.1 Introduction

4.1.1 Definition

Antimicrobial

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, or viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). However, the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Currently, bacterial resistance is combated by the discovery of new drugs. However, microorganisms are becoming resistant more quickly than new drugs are being found, thus, future research in antimicrobial therapy may focus on finding how to overcome resistance to antimicrobials, or how to treat infections with alternative means[118].

Antibacterial

Drugs that destroy or inhibit the growth of bacteria in concentrations that is safe for the host and can be used as chemotherapeutic agents to prevent or treat bacterial infections.

4.1.2 Mechanisms of antibacterial action

There are five main mechanisms by which antibacterial agent act[119].

4.1.2.1 Inhibition of cell metabolism

Antibacterial agents which inhibit cell metabolism are called antimetabolites. These Compound inhibit the metabolism of a microorganism, but not the metabolism of the host. They do these by inhibiting an enzyme- catalyzed reaction which is present in the bacterial cell but not in animal cells. The best examples of antibacterial agents acting in this way are the sulphonamides.

4.1.2.2 Inhibition of bacterial cell wall synthesis

Inhibition of cell wall synthesis leads to bacterial cell lysis and death. Agents operating in this way include penicillin and cephalosporin. Some animal cells do not have a cell wall, they are unaffected by such agents.

4.1.2.3 Interactions with plasma membrane

Some antibacterial agents interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fetal results for the call. Polymyxins and tyrothricin operate in this way.

4.1.2.4 Disruption of protein synthesis:

Disruption of protein synthesis means that essential enzymes required for the cells survival can no longer be made. Agents which disrupts protein synthesis include the refamycins, amynoglycosides, tetracyclines and chloramphenicol.

4.1.2.5 Inhibition of nucleic acid transcription and replication

Inhibition of nucleic acid function prevents cell division and/or the synthesis of essential enzymes. Agents in this way include nalidixic acid and proflavine.

4.1.3 Main classes of antibacterials

4.1.3.1 Antimeatabolites

Substance that competes with, replaces, or inhibits a specific compound within a cell, whose functioning is thereby disrupted. Because its structure resembles the compounds, it is taken up by the cell, but it does not react in the same way with the enzyme that acts on the usual compound. It may inhibit the enzyme or be converted into an aberrant chemical. Many antimetabolites are useful in treating disease, including sulfa drugs, which disrupt bacterial but not human metabolism for bacterial diseases, and others (e.g., methotrexate, 5-fluorouracil) for various cancers.

4.1.3.2 Antibiotics

An antibiotic is a chemotherapeutic agent which is generally used to treat bacterial infections. The term "antibiotic" was coined by Selman Waksman in 1942 to describe any substance produced by a micro-organism that is antagonistic to the growth of other micro-organisms in high dilution[120].

At the highest level, antibiotics can be classified as either bactericidal or bacteriostatic. Bacteriostatic antibiotics inhibit growth and reproduction of bacteria without killing those while bactericidal antibiotics work by killig bacteria. Antibiotics target bacterial cell wall such as penicillins, cephalosporins or cell membrane e.g. polymixins, or interfere with essential bacterial enzymes e.g. quinolones, sulfonamides usually are bactericidal in nature. Whilst those which target protein synthesis such as the aminoglycosides, macrolides and tetracyclines are usually bacteriostatic[120].

4.1.4 Methods of determining the antimicrobial activity

Bacteria and fungi are responsible for many infectious diseases. The increasing clinical implications of drug resistant fungal and bacterial pathogens have lent additional urgency to antimicrobial drug research. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the *in vitro* fungal and bacterial growth. This ability may be estimated by any of the following three methods.

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

But there is no standardized method for expressing the results of antimicrobial screening. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extract to inhibit the growth of microorganisms. However, a great

number of factors viz., the extraction methods, inoculums volume, culture medium composition, p^H, and incubation temperature can influence the results.

Among the above mentioned techniques the disc diffusion[121] is a widely accepted *in vitro* investigation for preliminary screening of test agents which may possess antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method[122].

4.1.5 Determination of Antibacterial Activity

4.1.5.1 Disc diffusion methods

The disc diffusion technique[123] is a widely accepted in vitro investigation for primary screening of agents which may possess any antibacterial activity. It is essentially a qualitative or quantitative test indicating the sensitivity or resistance of the microorganism to the test materials. However, no distinction between bacteriostatic or bactericidal activity can be made by this method.

The Kirby-Bauer and Stokes' methods are usually used for anti-microbial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here. NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate anti-microbial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results

NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

4.1.5.2 Principle of disc diffusion method:

Solution of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in definite volume of solvents. Sterilized and dried filter paper discs (4 mm in diameter) were then impregnated with known amounts of test substances by using micropipette. Discs containing the test materials are placed on nutrient agar medium uniformly seeded with the test organisms. Discs soaked in respective solvent are used as positive control. These plates are then kept at low temperature (4ºC) for two to four hours to allow maximum diffusion of compound. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the media. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result, there is a gradual change of test materials concentration in the media surrounding the discs .The plates were then incubated at 37ºC for 24 hours to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called "Zone of Inhibition". The antibacterial activity of the test agent is determined by measuring the zone of inhibition expressed in millimeter, the antibacterial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

The experiment is carried out more than once and mean of the reading is required.

4.2 EXPERIMENTAL

4.2.1. Apparatus and Reagents

Filter paper discs	Petridishes	Inoculating loop
Sterile cotton	sterile forceps	Spirit burner
Micropipette	Screw cap test tubes	Nosemask & Hand gloves
Laminar air flow hood	Autoclave	Incubator
Refrigerator	Nutrient Agar Medium	Ethanol, Chloroform

4.2.2. Preparation of test Solution

A measured amount of test samples were dissolved in definite volumes of solvent (chloroform or methanol) to give solution of known concentration (e.g. 250 µg/ 500µg/1000 µg /10µl).

4.2.3. Test Organisms

In the present study, to screen the anti-microbial activity of plant extracts 6 bacteria and 1 fungi were used as test organisms. Among 6 bacteria, 5 were gram positive and 1 was gram negative. Test experiments were conducted in Industrial Microbiology Research Division, BCSIR Laboratories Chittagong, Bangladesh.

Table: List of test Organism (Bacteria)

Bacterial type	Test organism	Source	ID No.
Gram (+)	*Bacillus subtilis*	BTCC	17
	Staphylococcus aureus	BTCC	43
	Bacillus cereus	BTCC	19
	B. polymyxa.	BTCC	16
	Klebsiella sp.	ICDDR'B	-
Gram (-)	*Proteus sp.*	ICDDR'B	-

4.2.4 Reagents for the Disk Diffusion Test

4.2.4.1 Müeller-Hinton Agar Medium:

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
- It gives satisfactory growth of most non-fastidious pathogens.
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

4.2.4.2 Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar prepared from a commercially available dehydrated base (Hi-media, India) according to the manufacturer's instructions.

2. Immediately after autoclaving, allowed it to cool in a 45 to 50°C water bath.

3. Freshly prepared and cooled medium poured into glass, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. (This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.)

4. The agar medium allowed cooling to room temperature.

4.2.4.3 Turbidity standard for inoculum preparation

To standardize the inoculums density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A BaSO4 0.5 McFarland standards prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

2. The correct density of the turbidity standard verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standards.

3. The Barium Sulfate suspension transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculums.

4. These tubes were tightly sealed and stored in the dark at room temperature.

5. The barium sulfate turbidity standard vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance.

4.2.5 Procedure for Performing the Disc Diffusion Test

4.2.5.1 Inoculums Preparation

The growth method is performed as follows:
At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as Mueller Hington broth. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually 2 to 6 hours). The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to

obtain turbidity optically comparable to that of the 0.5 McFarland standards. (This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E. coli* ATCC 25922). To perform this step properly, either a photometric device is used.

4.2.5.2 Preparation of discs

Whatman-1 filter papers were used for disc preparation. The discs of about 4 mm in diameter were prepared by using punching machine. The discs were taken in a petridish and sterilized by autoclave, dried in oven at 100°C. And then impregnated with required concentration of the sample.

4.2.5.3 Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculums from the swab.
2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar is swabbed.
3. The lid was left ajar for 3 to 5 minutes (but no more than 15 minutes), to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

4.2.5.4 Application of Discs to Inoculated Agar Plates

1. The predetermined battery of anti-microbial discs is dispensed onto the surface of the inoculated agar plate. Each disc pressed down to ensure complete contact with the agar surface. Discs are distributed evenly so that they are no closer than 24 mm from center to center.
2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied.

4.2.5.5 Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc.
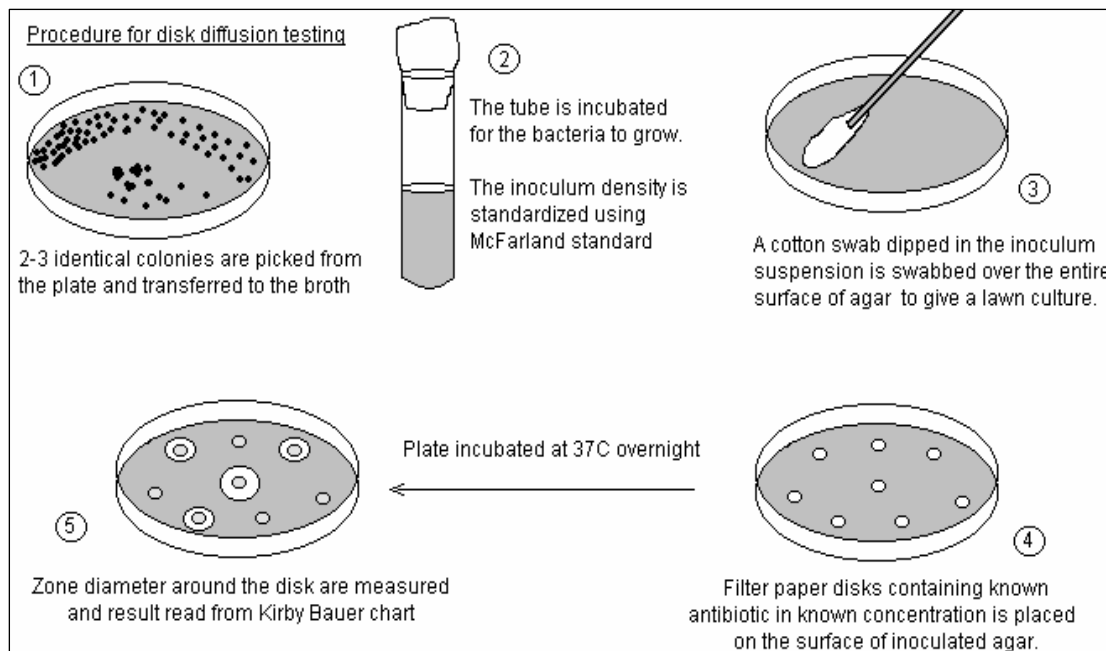
Fig: 4.6. Procedure of disc diffusion method

4.3 Result and discussion

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

Antibacterial activity of the compounds were studied on five Gram positive and one Gram-negative bacteria by disc diffusion method and compared with the standard

antibiotic disc- Tetracycline (30 µg/disc). Antibacterial activity of the compounds was measured at 250, 500 and 1000 µg/disc concentration.

4.3.1 Antibacterial activities of SN1

Compound SN1 showed no zone of inhibition at 250 µg/disc and 500 µg/disc, but had poor zone of inhibition at 1000 µg/disc against all the gram positive bacteria. But SN1 has no activity against the gram negative bacteria *Proteus sp.*

Table: *in vitro* antibacterial activity of SN1

Bacterial type	Test organism	Diameter of zone of inhibition (mm)			
		SN1			Tetracycline (30 µg/disc)
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	-	-	7	21
	Staphylococcus aureus	-	-	6	24
	Bacillus cereus	-	-	6	12
	B. polymyxa.	-	-	7	21
	Klebsiella sp.	-	-	6	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity'

4.3.2 Antibacterial activities of SN2

Compound SN2 showed no zone of inhibition at 250 µg/disc. It had poor activity against *Staphylococcus aureus* at 500 µg/disc concentration. At 1000 µg/disc concentration it exhibited poor to activities against *Klebsiella sp.*,*Staphylococcus aureus*,*B. polymyxa* and*Bacillus subtilis* but no activities against other two test bacteria.

Table: *in vitro* **antibacterial activity of SN2**

Bacterial type	Test organism	Diameter of zone of inhibition (mm)			
		SN2			Tetracycline (30 µg/disc)
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	-	-	8	21
	Staphylococcus aureus	-	7	7	24
	Bacillus cereus	-	-	-	12
	B. polymyxa.	-	-	6	21
	Klebsiella sp.	-	-	6	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity'

4.3.3 Antibacterial activities of SN4

Compound SN4 exhibited poor activity againt *Bacillus subtilis* bacteria at 250 µg/disc concentration. At 500 µg/disc concentration it showed poor activity against *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*, but no activity against other test bacterias. Again at 1000 µg/disc concentration it exhibited moderate activity against *Bacillus subtilis*, *Bacillus cereus* & *Staphylococcus aureus* bacteria, and poor activity against *B. polymyxa* & *Klebsiella sp.*, but no activity against *Proteus sp*

Table: *in vitro* **antibacterial activity of SN4**

| Bacterial type | Test organism | Diameter of zone of inhibition (mm) | | | |
| | | SN4 | | | Tetracycline (30 µg/disc) |
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	8	8	9	21
	Staphylococcus aureus	-	6	10	24
	Bacillus cereus	-	8	11	12
	B. polymyxa.	-	-	8	21
	Klebsiella sp.	-	-	6	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity'

4.3.4 Antibacterial activities of SN5

Compound SN5 showed no zone of inhibition to any of the test bacterias.

Table: *in vitro* **antibacterial activity of SN5**

| Bacterial type | Test organism | Diameter of zone of inhibition (mm) | | | |
| | | SN5 | | | Tetracycline (30 µg/disc) |
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	-	-	-	21
	Staphylococcus aureus	-	-	-	24
	Bacillus cereus	-	-	-	12
	B. polymyxa.	-	-	-	21
	Klebsiella sp.	-	-	-	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity

4.3.5 Antibacterial activities of SN7

Compound SN7 had no activity at 250 µg/disc and 500 µg/disc concentrations against all the bacterias except *Bacillus subtilis*, in which case the compound had poor activity. At 1000 µg/disc concentration the compound showed moderate activity againt *Bacillus subtilis*., poor activity against *Bacillus cereus* & *Klebsiella sp.,* and no activity againt others.

Table: *in vitro* **antibacterial activity of SN7**

Bacterial type	Test organism	Diameter of zone of inhibition (mm)			
		SN7			Tetracycline (30 µg/disc)
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	7	7	9	21
	Staphylococcus aureus	-	-	-	24
	Bacillus cereus	-	-	6	12
	B. polymyxa.	-	-	-	21
	Klebsiella sp.	-	-	6	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity'

4.3.6 Antibacterial activities of SN8

Amongs all the compounds, SN8 exhibited the greatest antibacterial activity. At a concentration of 250 µg/disc, it showed good activities against *Bacillus subtilis*, moderate activity against *B. polymyxa.*, poor activity against *Bacillus cereus* & *Klebsiella sp.,* and no activity against *Staphylococcus aureus* & *Proteus sp.*. At other concentrations it showed good activities against all the bacteria except *Proteus sp.*, in which case it had no activity

Table: *in vitro* antibacterial activity of SN8

Bacterial type	Test organism	Diameter of zone of inhibition (mm)			
		SN8			Tetracycline (30 µg/disc)
		250 (µg/disc)	500 (µg/disc)	1000 (µg/disc)	
Gram (+)	*Bacillus subtilis*	11	13	14	21
	Staphylococcus aureus	-	8	11	24
	Bacillus cereus	7	12	15	12
	B. polymyxa.	10	13	13	21
	Klebsiella sp.	8	8	9	12
Gram (-)	*Proteus sp.*	-	-	-	-

"--" Indicates 'No activity'
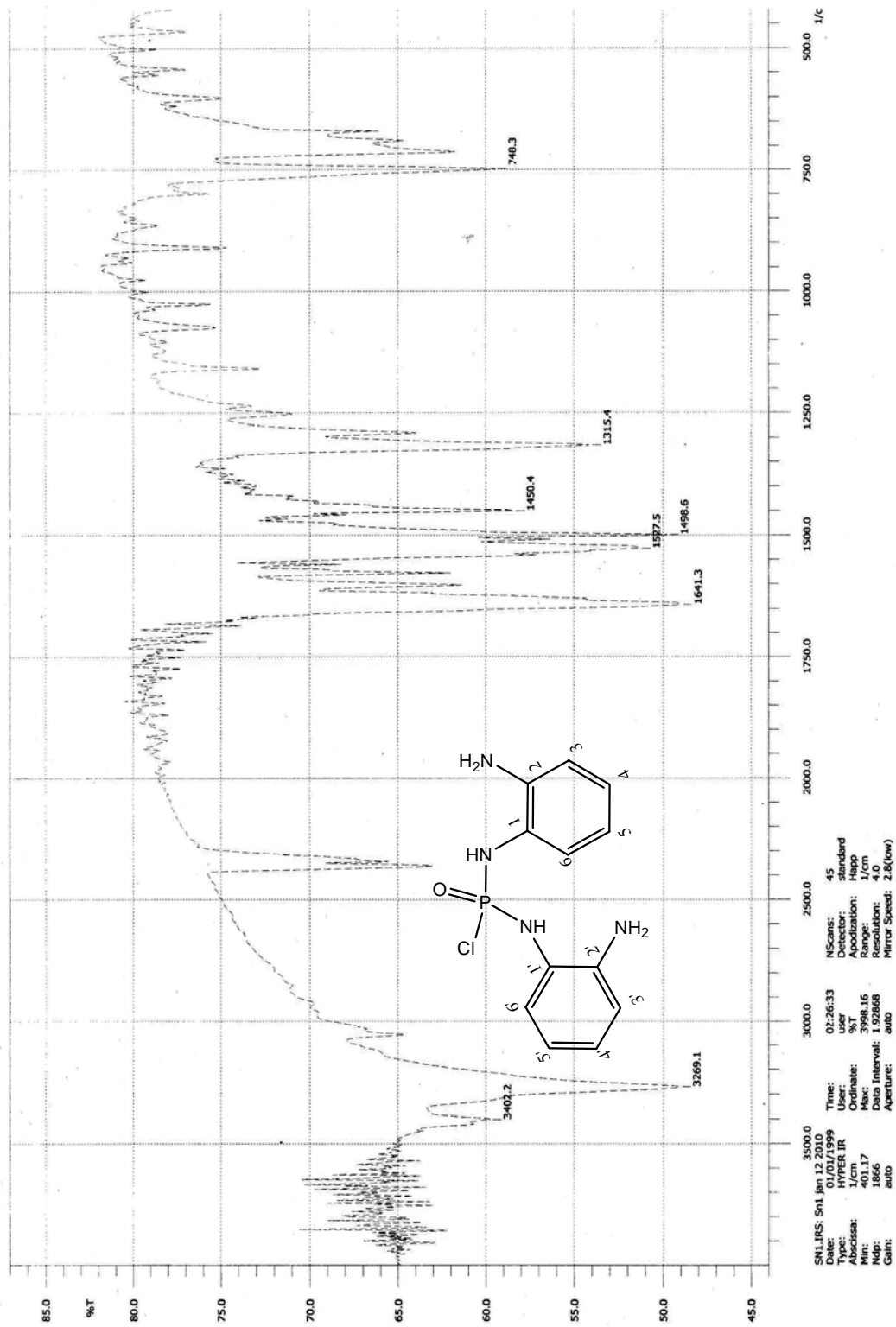
Figure-1(a): IR spectrum of Compound SN1

Figure 1(b): ^{1}H NMR spectrum of Compound SN1

ARD,BCSIR, H! spectrum, SN-1 in CDCl3, Shamim Nupur, BUET

0.875
1.255
1.690

5.279
6.801
6.820
6.833
7.051
7.071
7.089
7.159
7.250
7.292
7.311
7.435
7.453
7.472
7.518
7.537
7.554
7.658
7.866
7.885
7.934
9.092

0.115
0.413

1.993
1.000
0.960
2.006
1.071
3.103

0.031
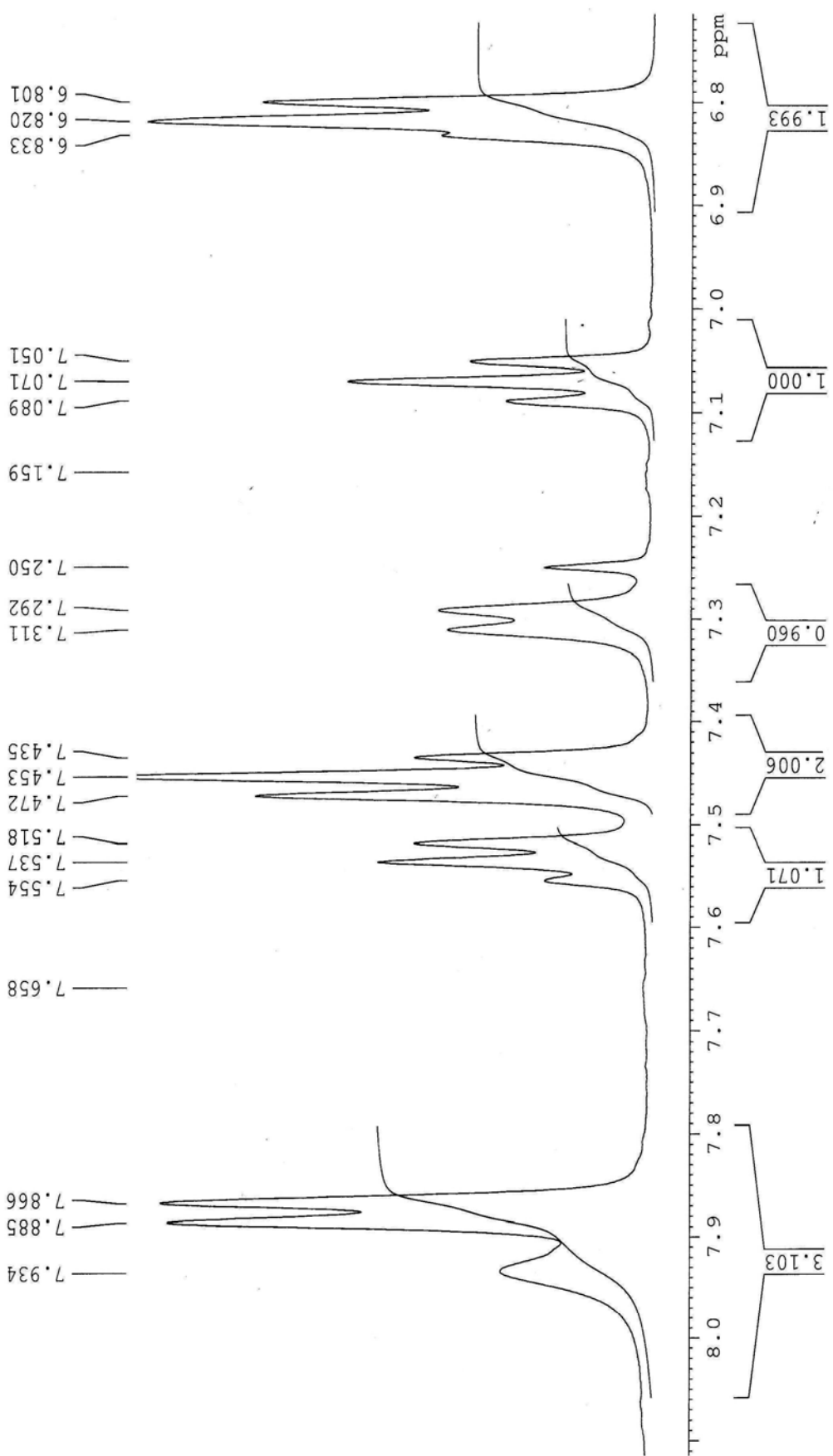
Figure-1(c): ^{1}H spectrum of Compound SN1 (expanded)

ARD, BCSIR, !H spctrum, SN-1 in CDC13, SHamim Nupur, BUET

6.801
6.820
6.833

7.051
7.071
7.089

7.159

7.250
7.292
7.311

7.435
7.453
7.472
7.518
7.537
7.554

7.658

7.866
7.885
7.934

1.993
1.000
0.960
2.006
1.071
3.103
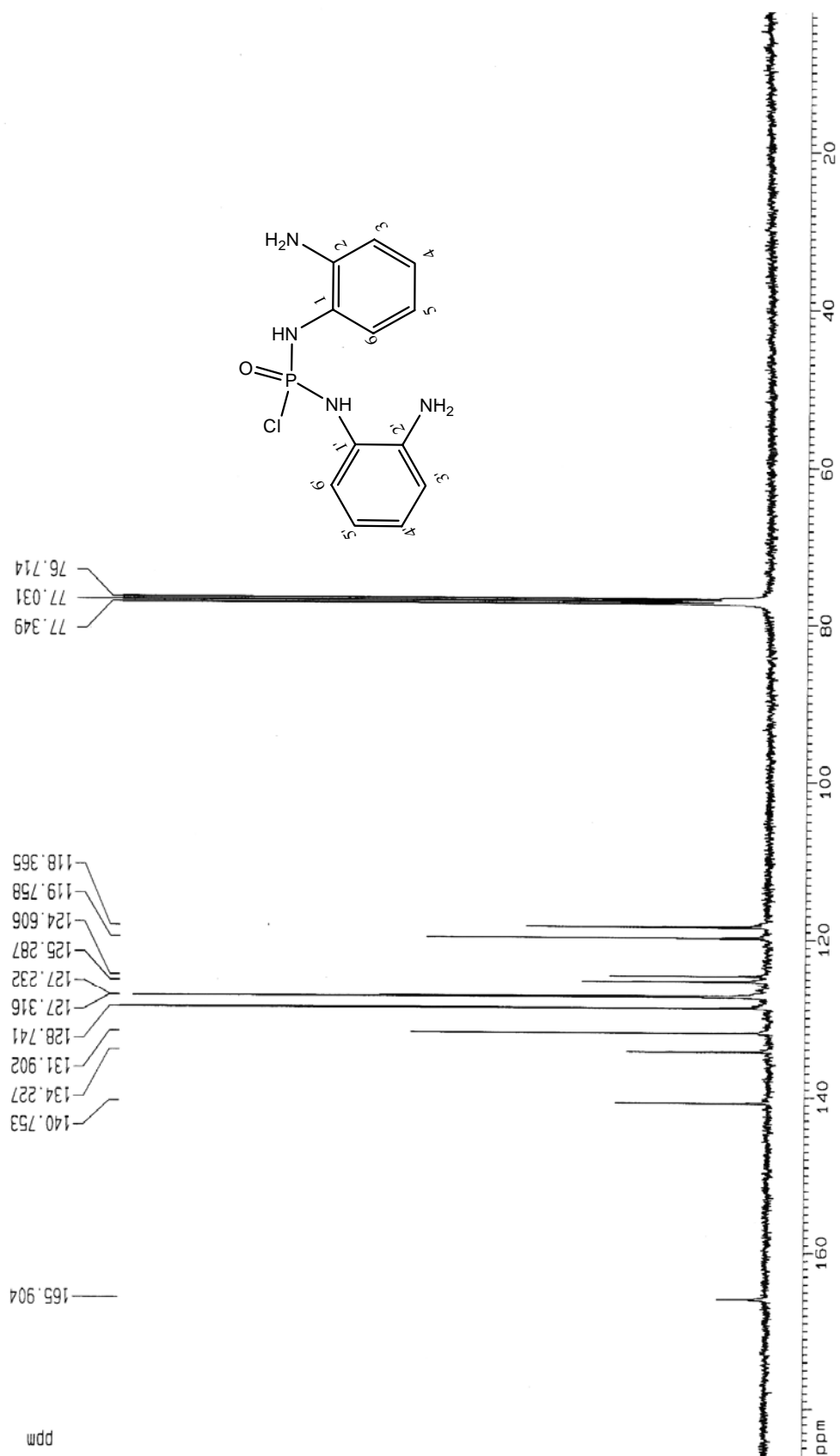
Figure-1(d): ^{13}C spectrum of Compound SN1

ARD, BCSIR, 13C spectrum, SN-1 in CDCl3, Nupur (Shamim)

76.714
77.031
77.349

118.365
119.758
124.606
126.287
127.232
127.316
128.741
131.902
134.227
140.753

165.904

ppm

ARD, BCSIR, Dept 135, SN-1 in CDCl3, Nupur (Shamim)

Current Data Parameters
NAME   A5817
EXPNO   6
PROCNO   1

F2 - Acquisition Parameters
Date_   20110405
Time   22.30
INSTRUM   dpx400
PROBHD   5 mm Multinuc
PULPROG   dept135
TD   32768
SOLVENT   CDCl3
NS   2000
DS   8
SWH   24154.590 Hz
FIDRES   0.737140 Hz
AQ   0.6783476 sec
RG   13004
DW   20.700 usec
DE   6.00 usec
TE   300.0 K
CNST2   145.0000000
D1   4.00000000 sec
d2   0.00344828 sec
d12   0.00002000 sec
DELTA   0.00000764 sec

======== CHANNEL f1 ========
NUC1   13C
P1   6.00 usec
p2   12.00 usec
PL1   -6.00 dB
SFO1   100.6253045 MHz

======== CHANNEL f2 ========
CPDPRG2   waltz16
NUC2   1H
P3   8.30 usec
p4   16.60 usec
PCPD2   80.00 usec
PL2   -6.00 dB
PL12   16.00 dB
SFO2   400.1420007 MHz

F2 - Processing parameters
SI   32768
SF   100.6152804 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

1D NMR plot parameters
CX   20.00 cm
F1P   169.774 ppm
F1   17081.91 Hz
F2P   -6.732 ppm
F2   -677.32 Hz
PPMCM   8.82531 ppm/cm
HZCM   887.96143 Hz/cm

0.021

77.255

118.419
119.812
125.277
127.258
127.337
127.489
128.784
131.944

ppm
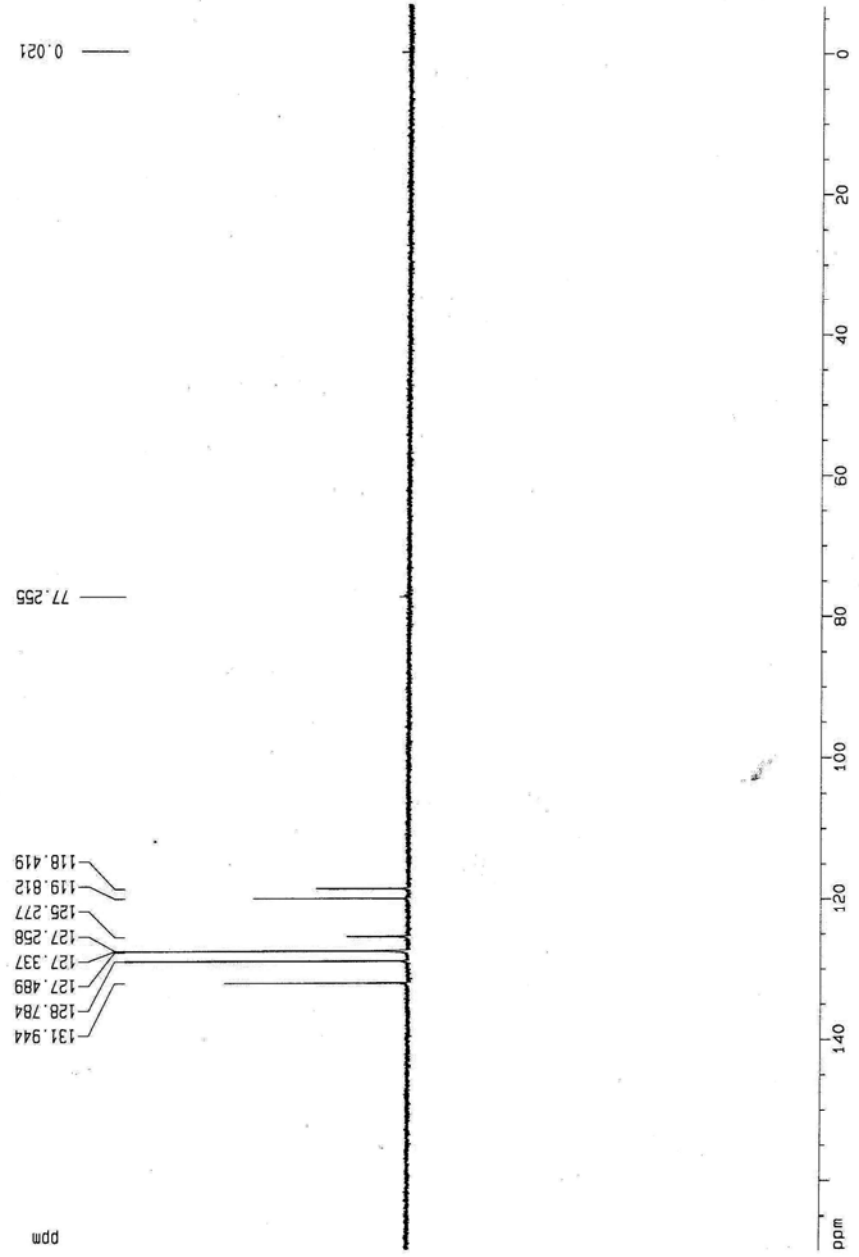
Figure-1(e): DEPT 135 spectrum of Compound SN1

102

ARO. BCSIR. 31P spectrum, SN-1 in CDCl3. Nupur (Shamim)

13.5889
13.8070

ppm

35.901
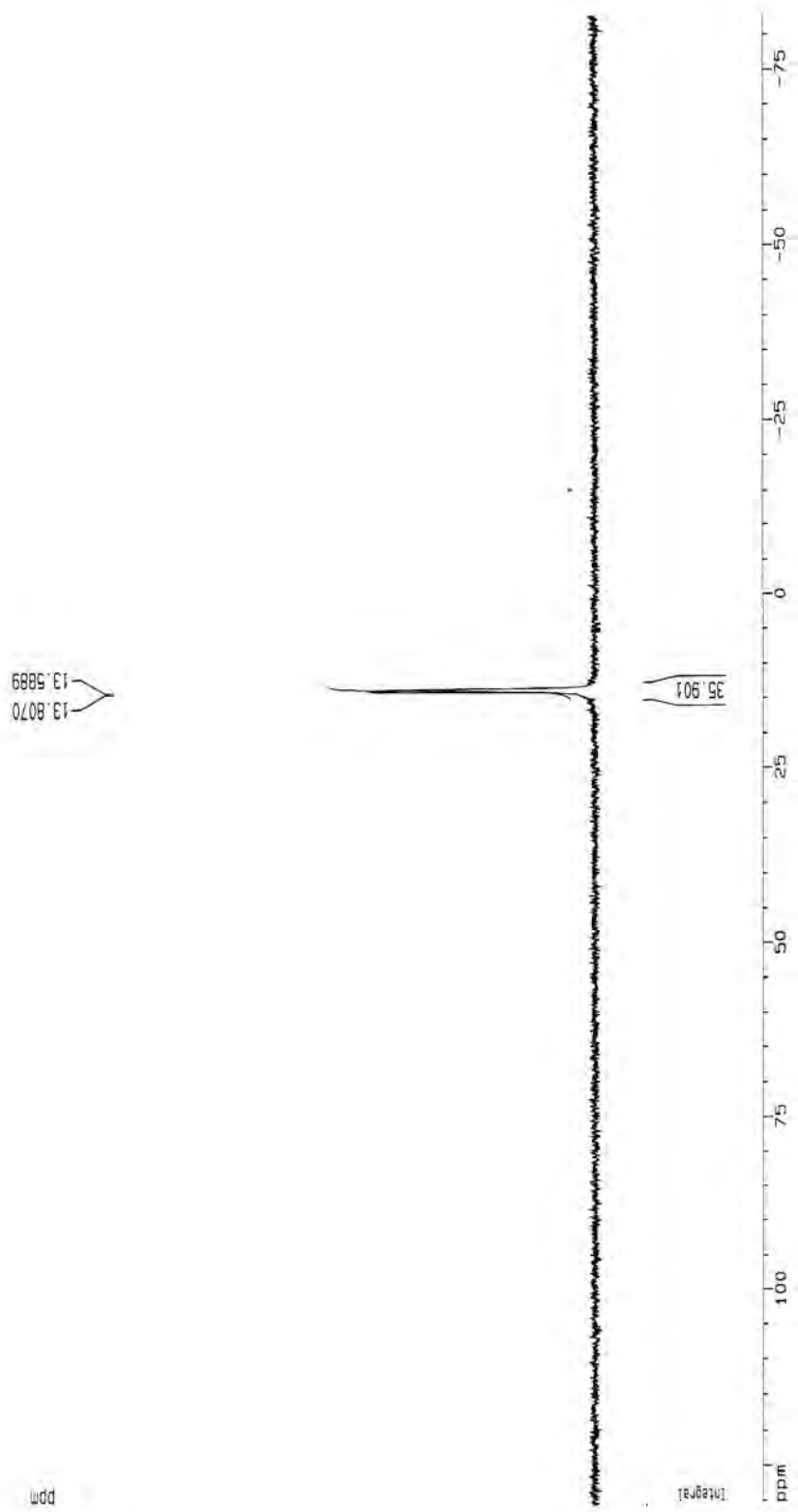
Figure-1(f): ^{31}P NMR spectrum of Compound SN1
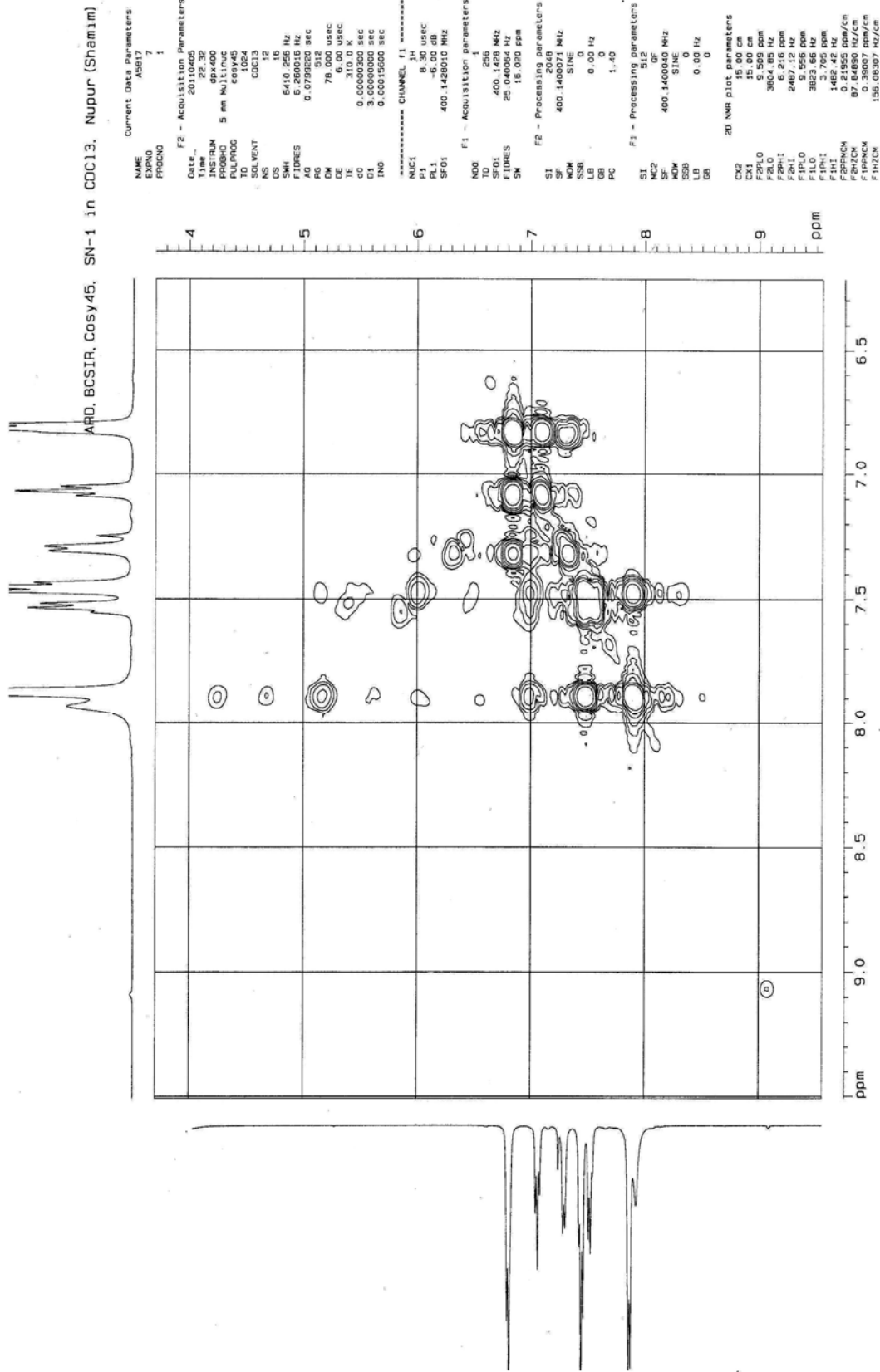
Figure-1(g): H-H COSY spectrum of Compound SN1

Figure-1(h): HMBC spectrum of Compound SN1
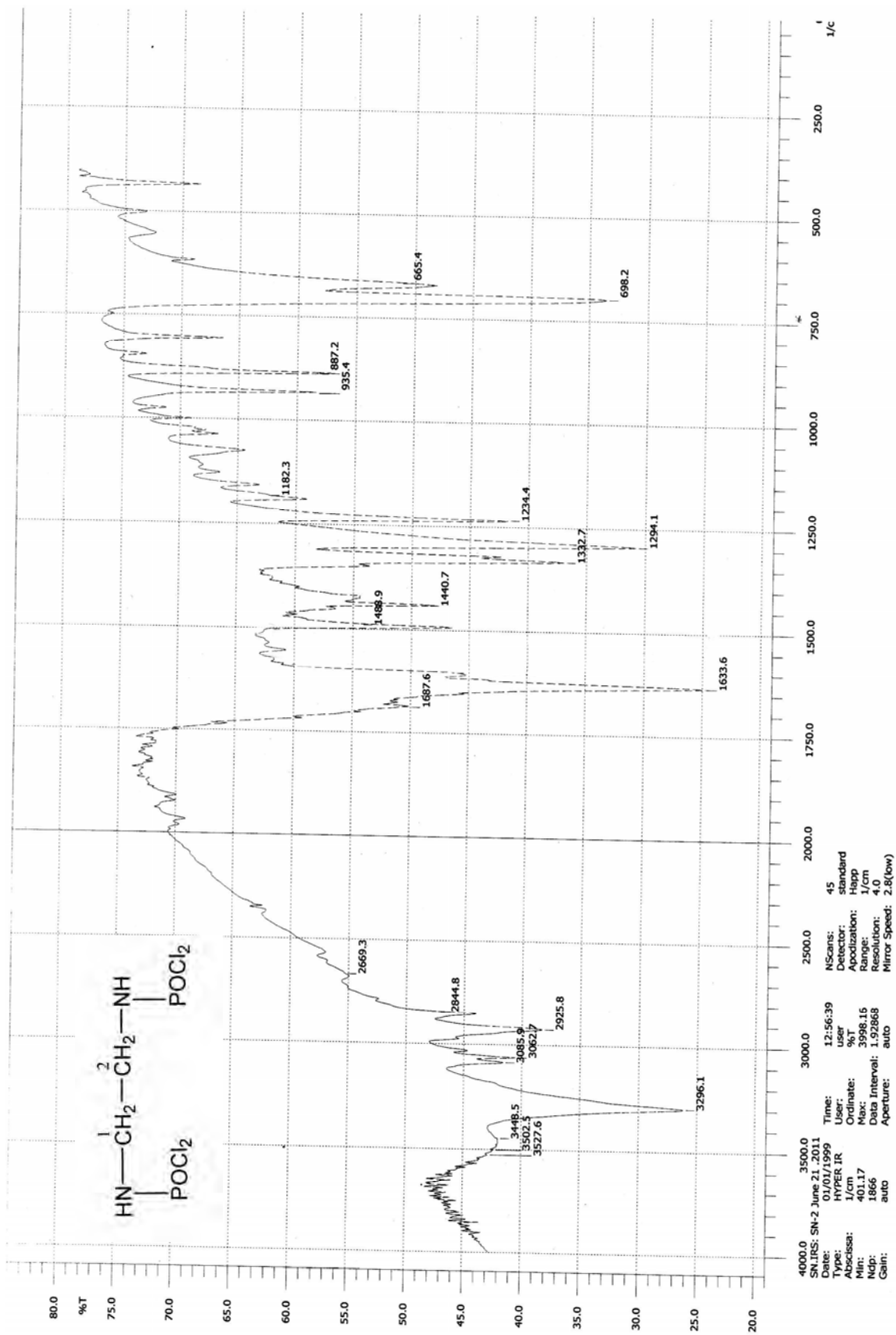
Figure-2(a): IR spectrum of Compound SN2
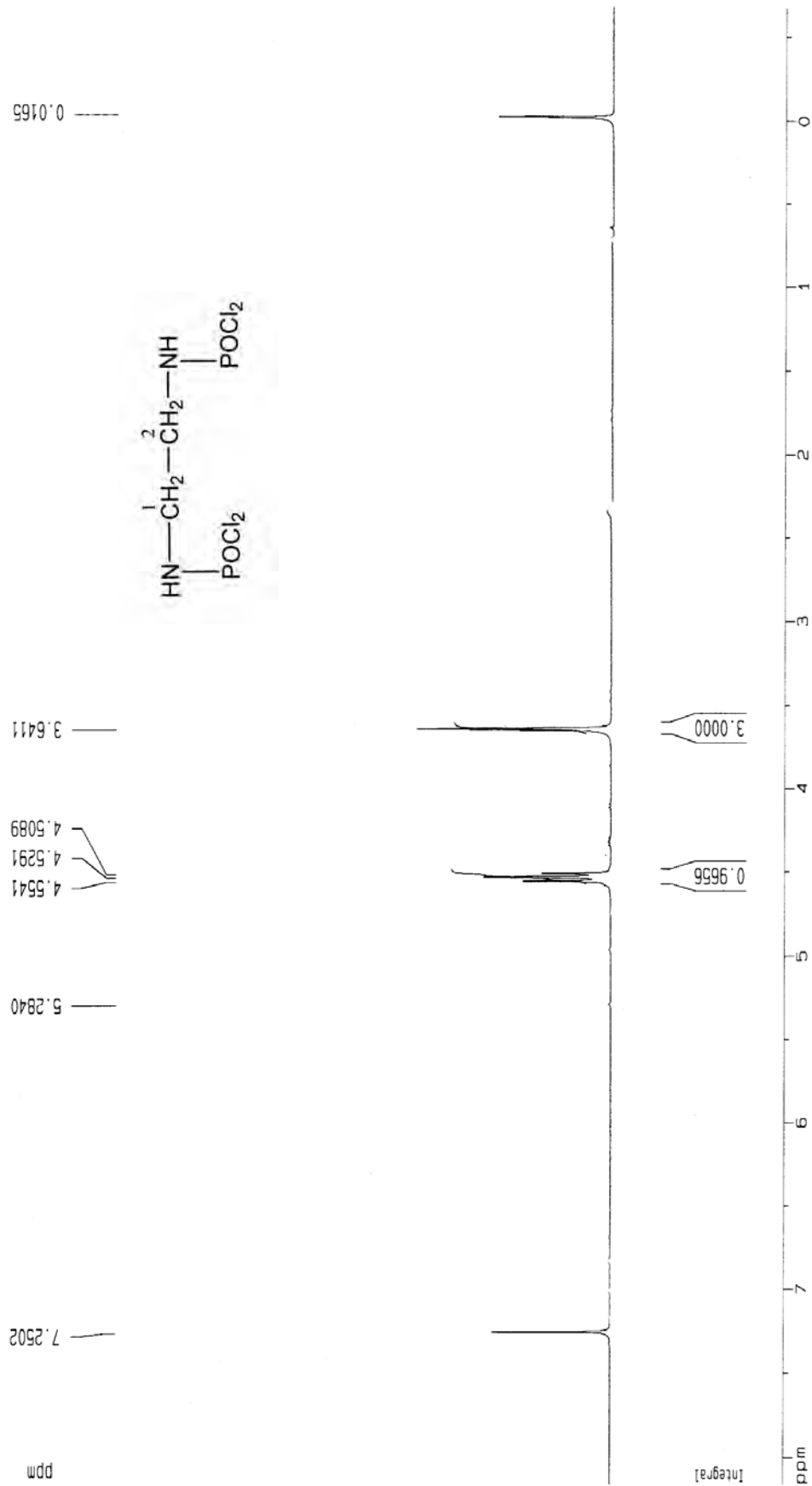
Figure-2(b): ^{1}H NMR spectrum of Compound SN2

107

ARD, BCSIR, 13C spectrum, SN-. in CDCL3, NUPUR (Shamim)

22.711
24.756
28.980
29.183
29.381
29.533
29.723
31.951
33.839

76.712
77.030
77.347

Figure-2(c): ^{13}C spectrum of Compound SN2

108

ARD. BCSIR. dept 135. SN-3 in CDCl3. Nupur (ShamimO

Current Data Parameters
NAME   A5868
EXPNO   3
PROCNO   1

F2 - Acquisition Parameters
Date_   20110419
Time   16.07
INSTRUM   dpx400
PROBHD   5 mm Multinuc
PULPROG   dept135
TD   32768
SOLVENT   CDCl3
NS   498
DS   8
SWH   24154.590 Hz
FIDRES   0.737140 Hz
AQ   0.6783476 sec
RG   13004
DW   20.700 usec
DE   6.00 usec
TE   300.0 K
CNST2   145.0000000
D1   4.00000000 sec
d2   0.00344828 sec
d12   0.00002000 sec
DELTA   0.00000764 sec

======== CHANNEL f1 ========
NUC1   13C
P1   6.00 usec
p2   12.00 usec
PL1   -6.00 dB
SF01   100.6253045 MHz

======== CHANNEL f2 ========
CPDPRG2   waltz16
NUC2   1H
P3   8.30 usec
p4   16.60 usec
PCPD2   80.00 usec
PL2   -6.00 dB
PL12   16.00 dB
SF02   400.1420007 MHz

F2 - Processing parameters
SI   32768
SF   100.6152804 MHz
WDW   EM
SSB   0
LB   1.00 Hz
GB   0
PC   1.40

1D NMR plot parameters
CX   20.00 cm
F1P   170.028 ppm
F1   17107.39 Hz
F2P   -9.011 ppm
F2   -906.64 Hz
PPMCM   8.95193 ppm/cm
HZCM   900.70129 Hz/cm

1.044

22.718
29.390
29.733
30.427
31.958
33.846

ppm

Figure-2(d): DEPT 135 NMR spectrum of Compound SN2

109
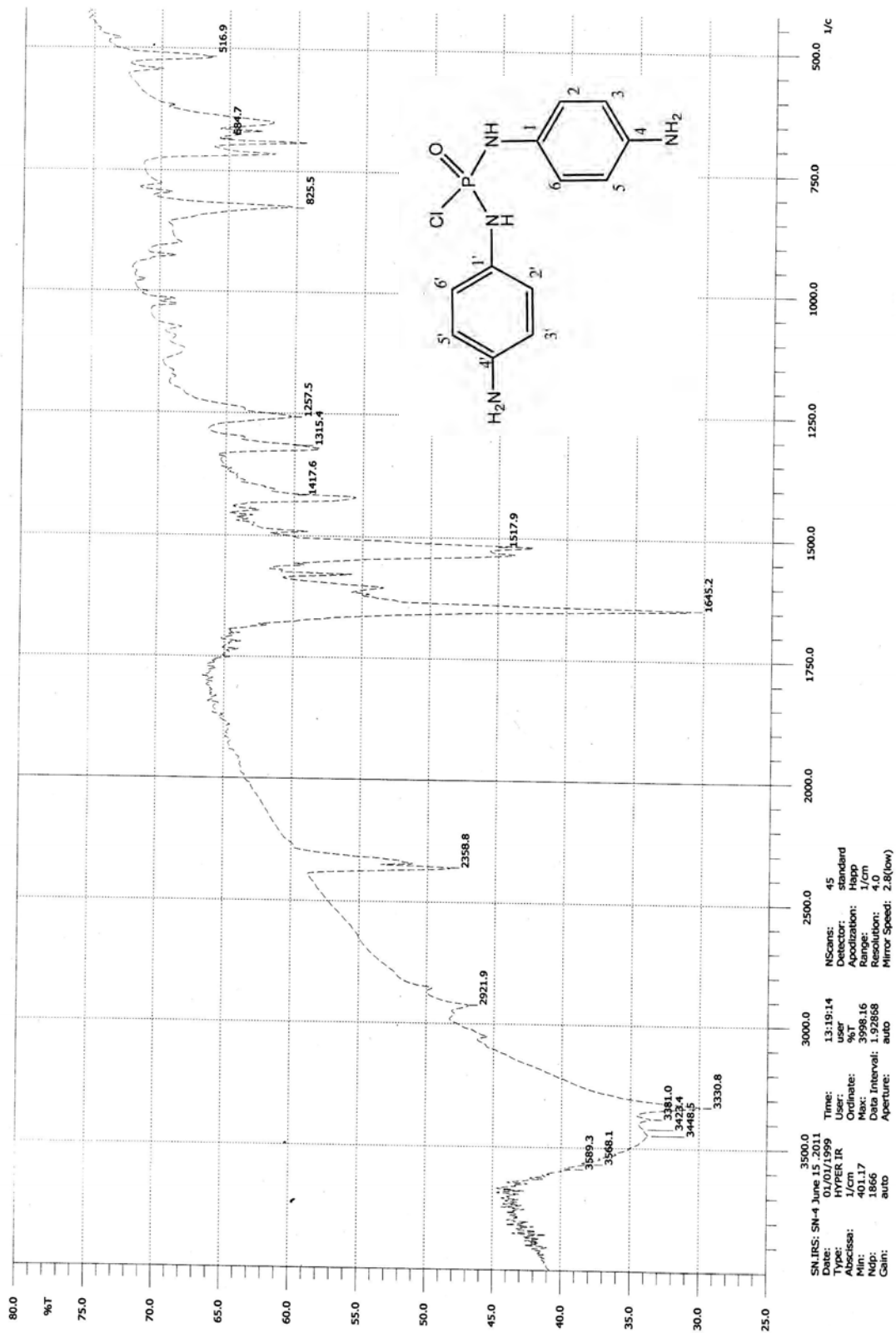
Figure-3(a): IR spectrum of Compound SN4

ARD, BCSIR, 1H spectrum, SN-4 in CDCl3, Nupur (Shamim)

6.66646
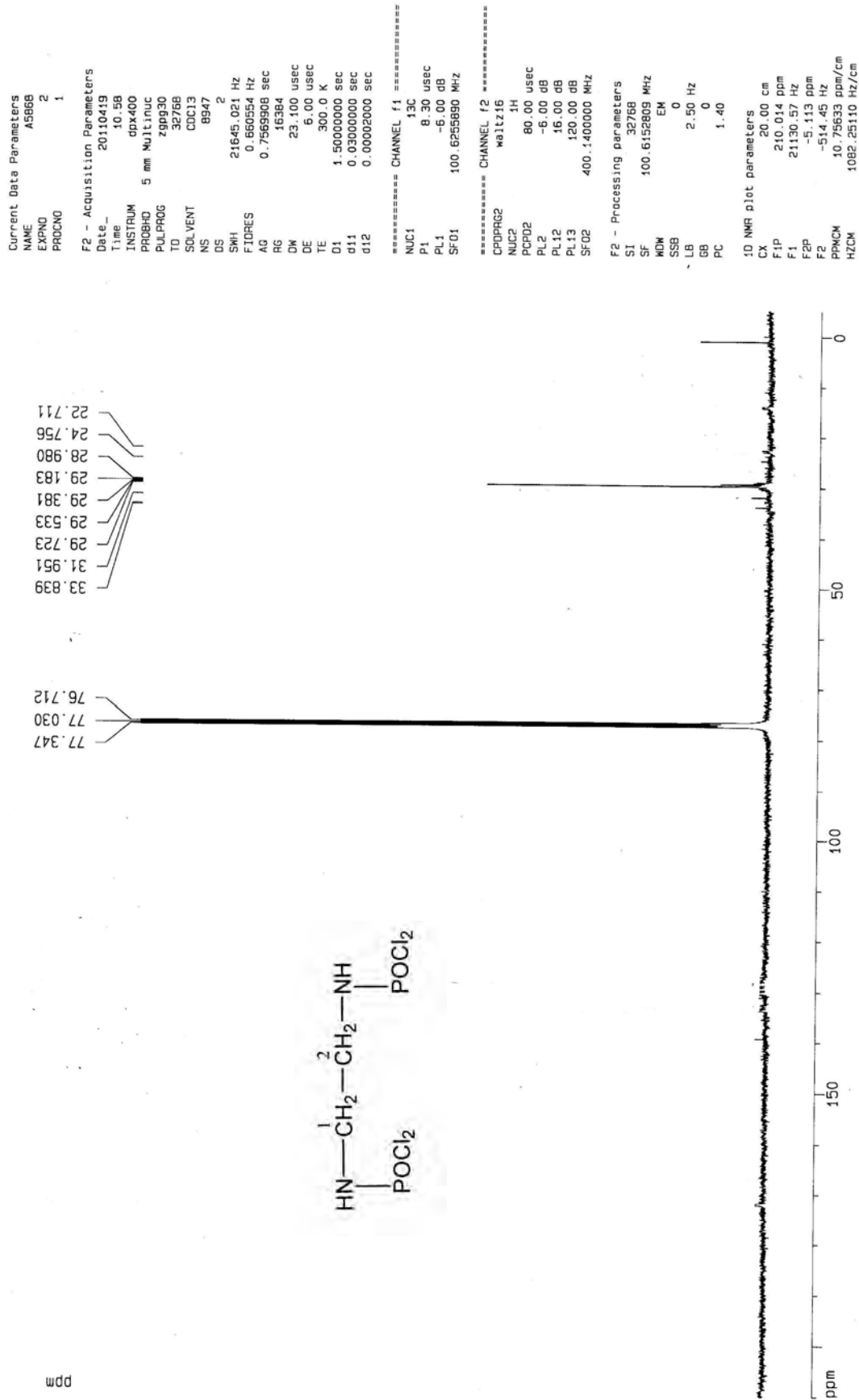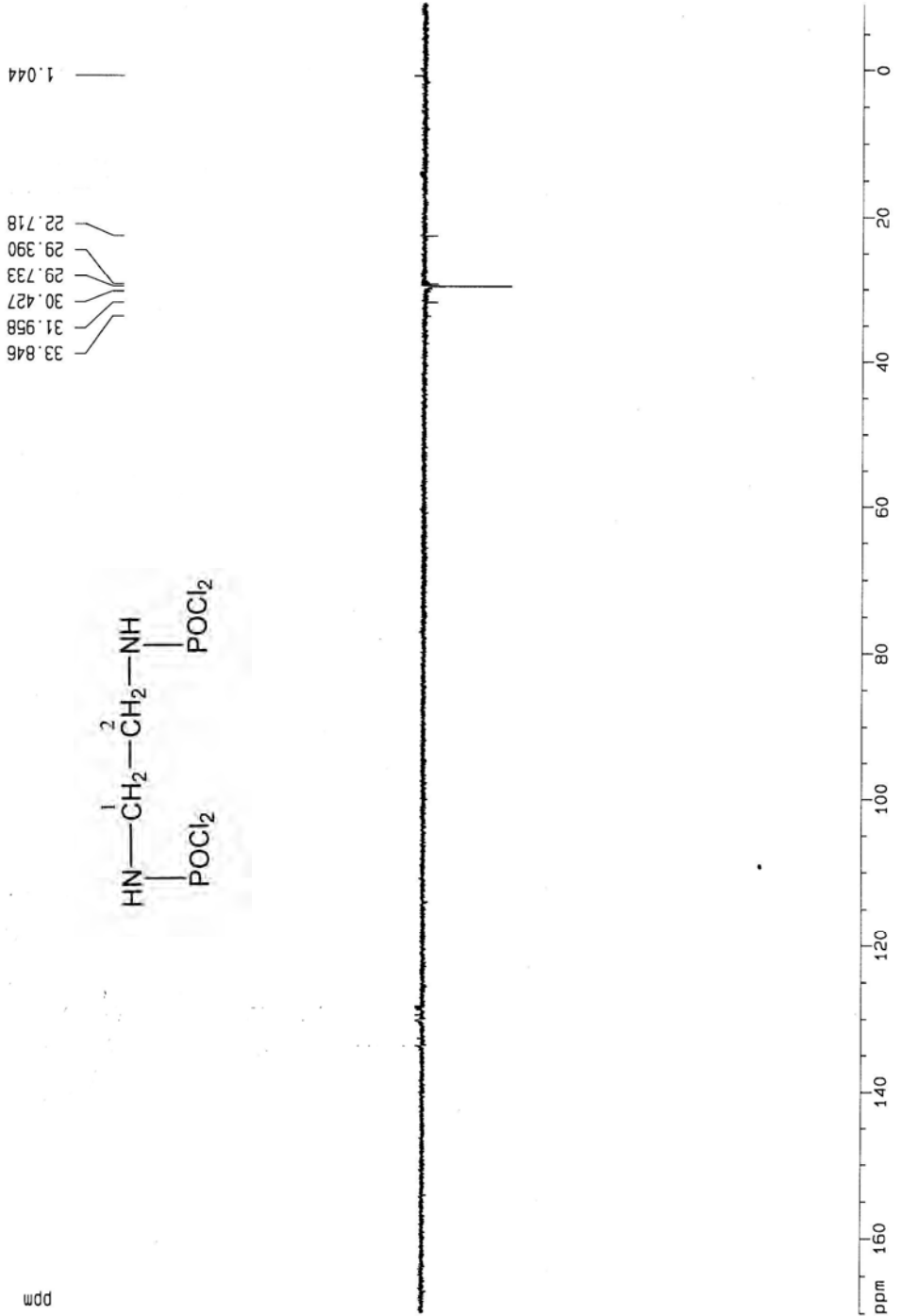6.68715

7.25105

7.37239
7.39179
7.42791
7.44593
7.46440
7.49331
7.51117
7.52793
7.56526

7.62912

7.73643

7.77668

7.82266
7.84088

ppm

1.0000

2.7480

1.0773

Integral

6.6   6.8   7.0   7.2   7.4   7.6   7.8   ppm
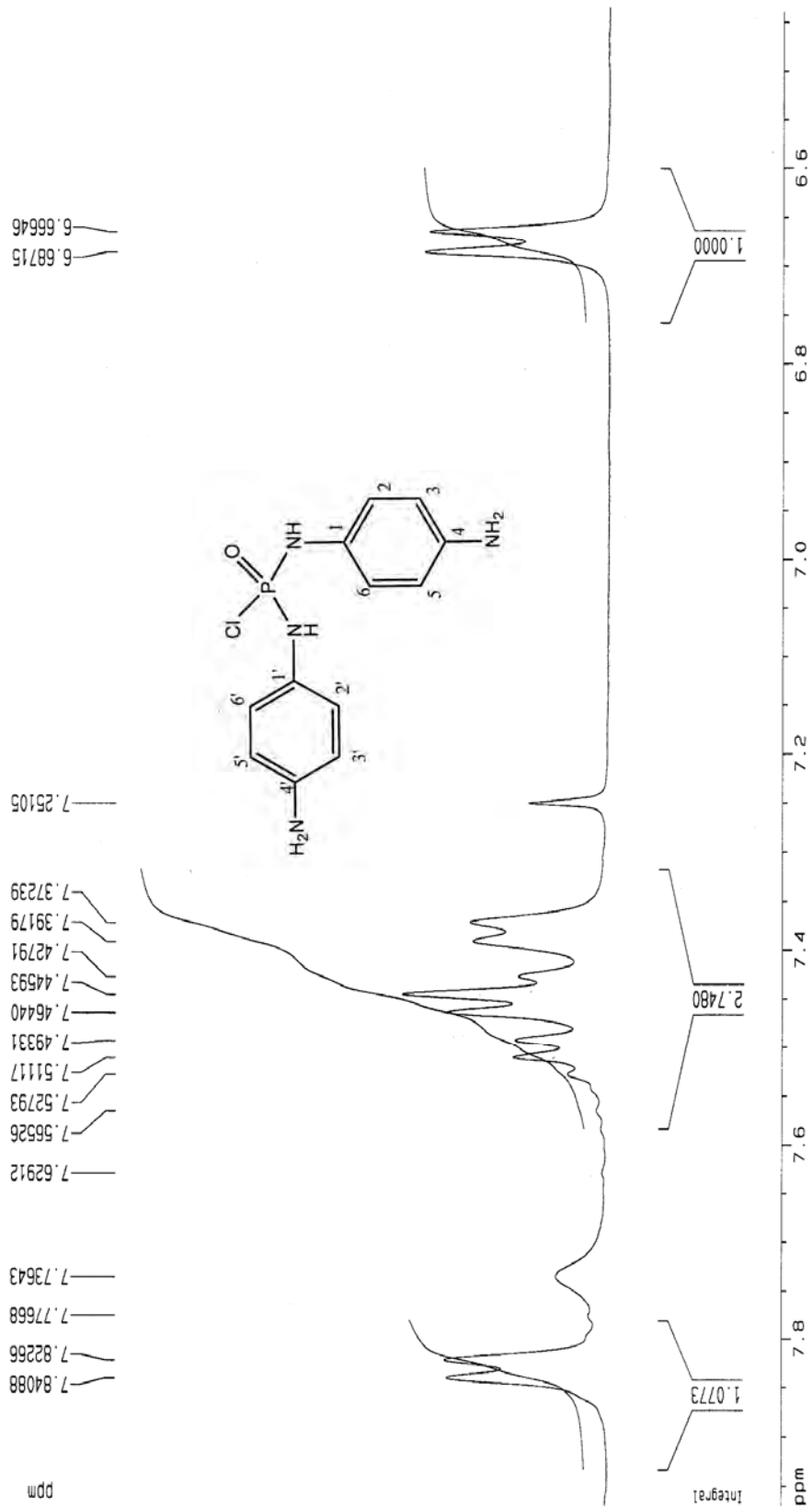
Figure-3(b): ¹H NMR spectrum of Compound SN4 (expanded)

111
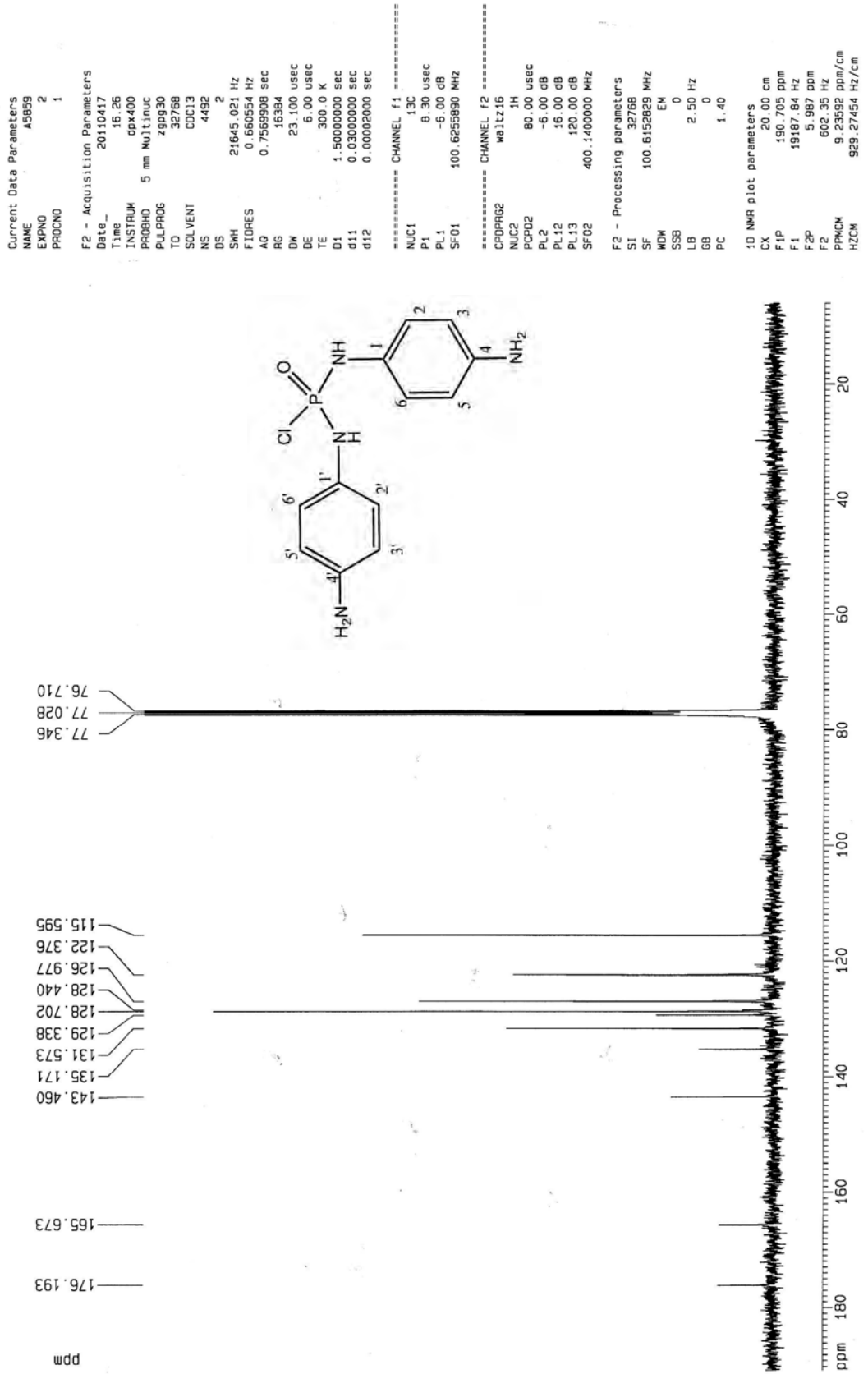
Figure-3(c): ^{13}C NMR spectrum of Compound SN4

112
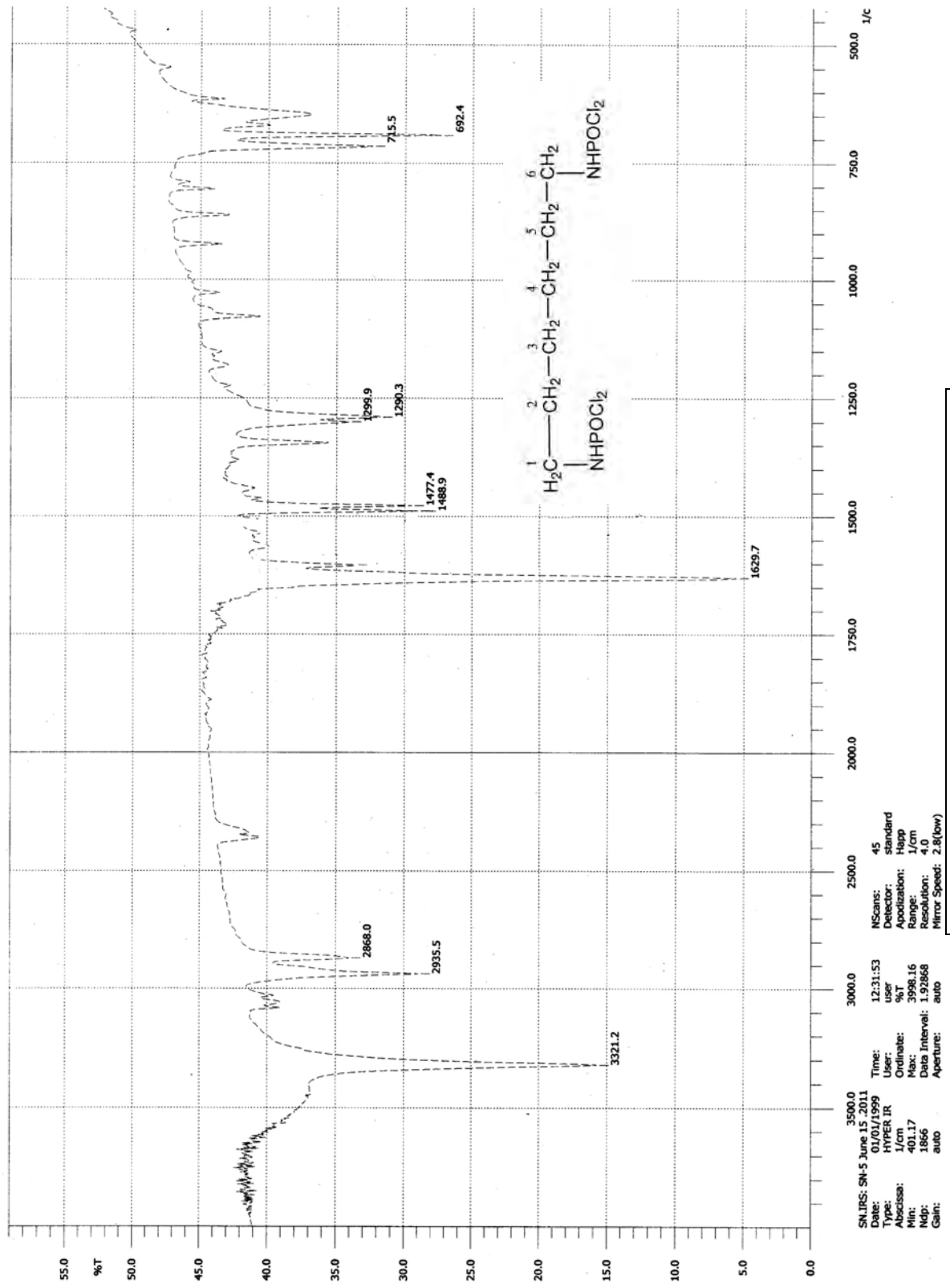
Figure-4(a): IR spectrum of Compound SN5

ARD, BCSIR, 1H spectrum, SN-5 in CDCl3. Nupur (Shamim)

Current Data Parameters
NAME   A5911
EXPNO   1
PROCNO   1

F2 - Acquisition Parameters
Date_   20110509
Time   10.43
INSTRUM   dpx400
PROBHD   5 mm Multinuc
PULPROG   zg30
TD   32768
SOLVENT   CDCl3
NS   128
DS   2
SWH   6410.256 Hz
FIDRES   0.195625 Hz
AQ   2.5559540 sec
RG   574.7
DW   78.000 usec
DE   6.00 usec
TE   310.0 K
D1   1.00000000 sec

========= CHANNEL f1 =========
NUC1   1H
P1   8.30 usec
PL1   -6.00 dB
SFO1   400.1428010 MHz

F2 - Processing parameters
SI   32768
SF   400.1400128 MHz
WDW   EM
SSB   0
LB   0.30 Hz
GB   0
PC   1.40

1D NMR plot parameters
CX   20.00 cm
F1P   3.584 ppm
F1   1434.27 Hz
F2P   1.136 ppm
F2   454.44 Hz
PPMCM   0.12244 ppm/cm
HZCM   48.99148 Hz/cm

Figure-4(b): ^{1}H NMR spectrum of Compound SN5 (expanded)

114

Figure-4(c): ^{13}C NMR spectrum of Compound SN5

ARD, BCSIR, 13C spectrum, SN-5 in CDCl3, Nupur (Shamim)

Figure-4(d): DEPT 135 spectrum of Compound SN5

116

Figure-5(a): IR spectrum of Compound SN7
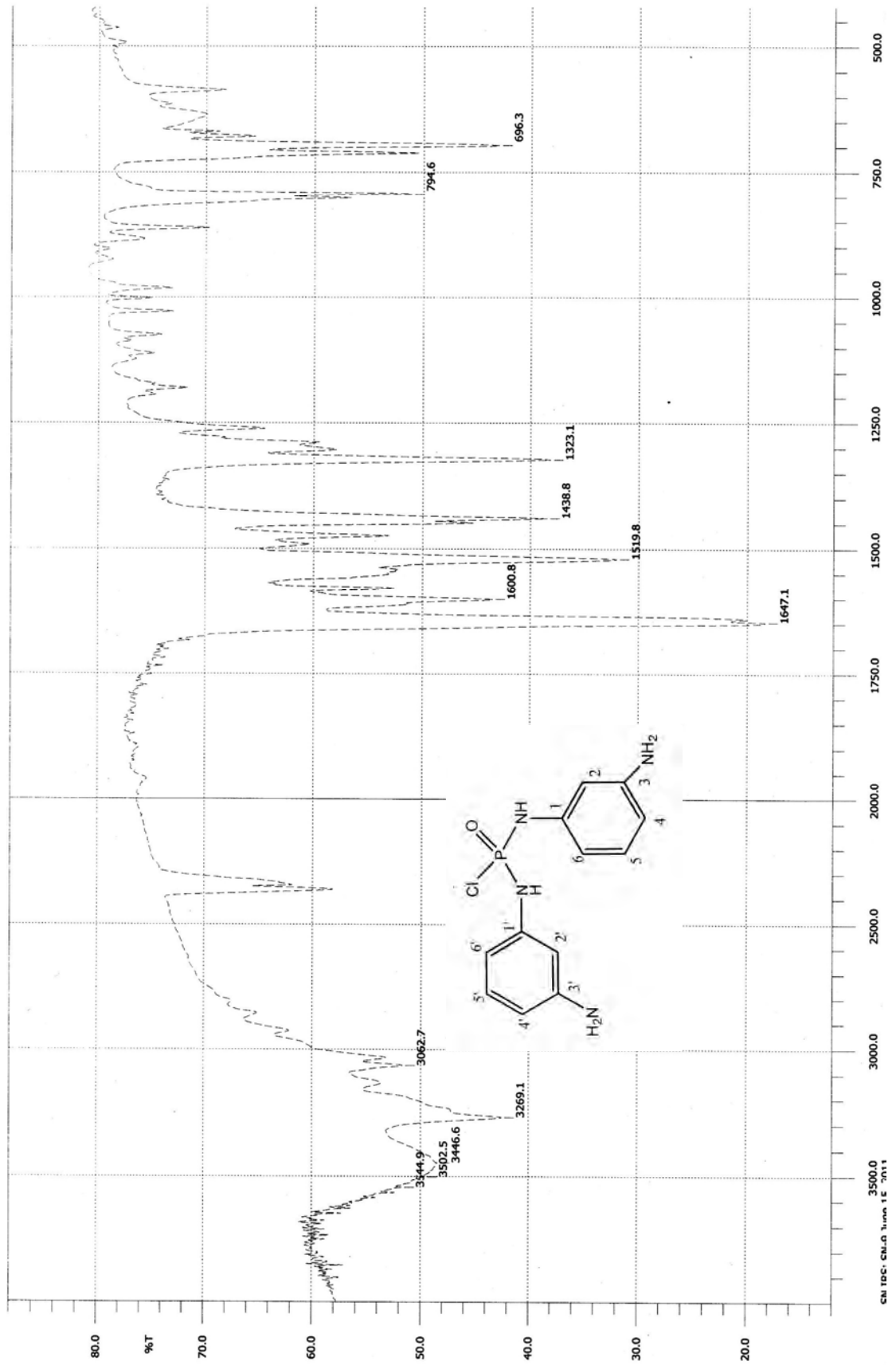
Figure-6(a): IR spectrum of Compound SN8

Figure-6(b): ^{1}H NMR spectrum of Compound SN8

Figure-6(c): ¹H NMR spectrum of Compound SN8 (expanded)

ARD, BCSIR, 13C spectrum, SN-8 in CDCl3, Nupur (Shamim), BUET

29.658

76.715
77.033
77.351

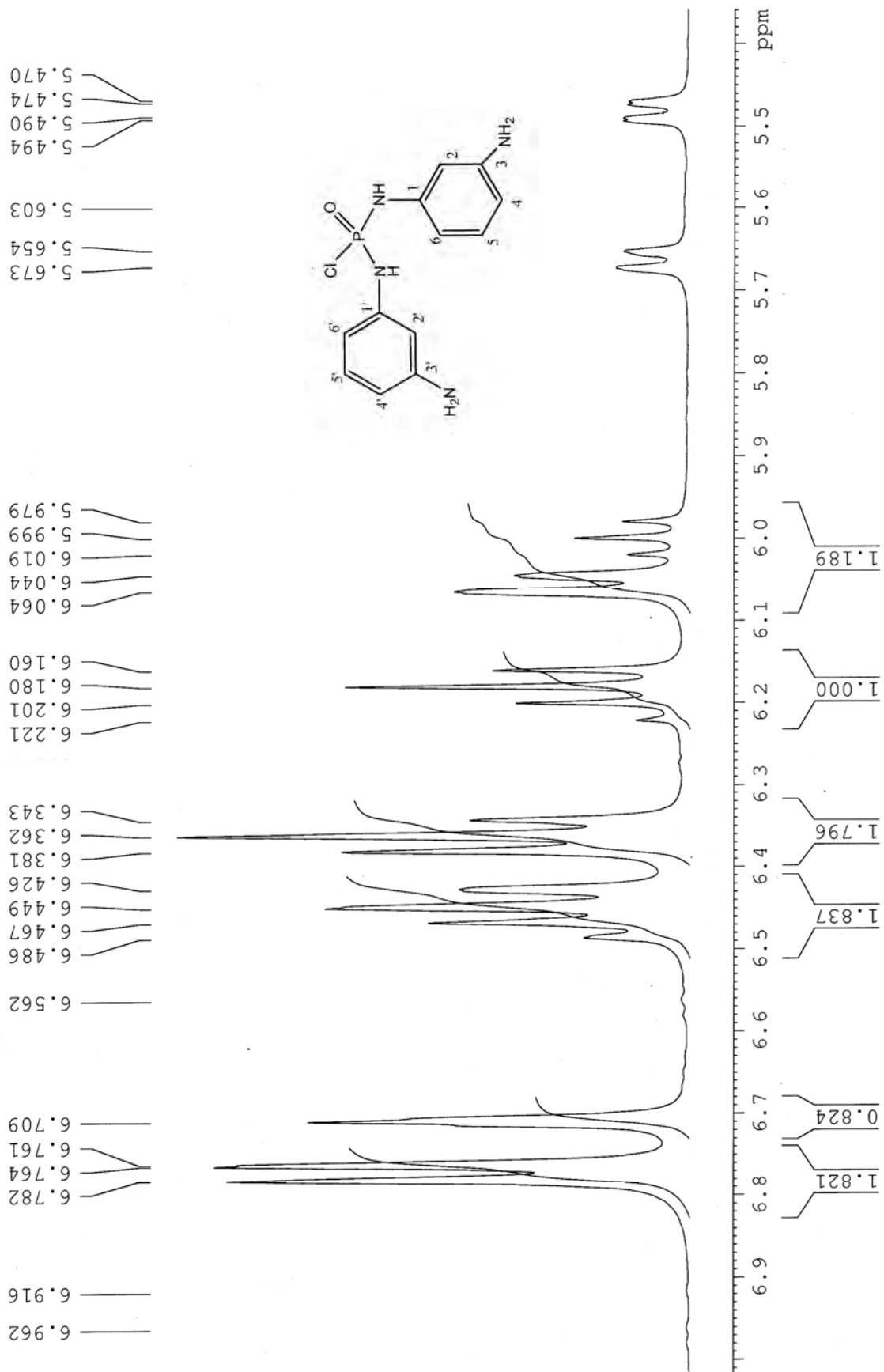113.164
114.875
118.334
118.454
120.542
124.543
127.068
128.686
129.914
130.239
131.973
134.428
134.562
134.754
139.084
147.649
166.148

ppm
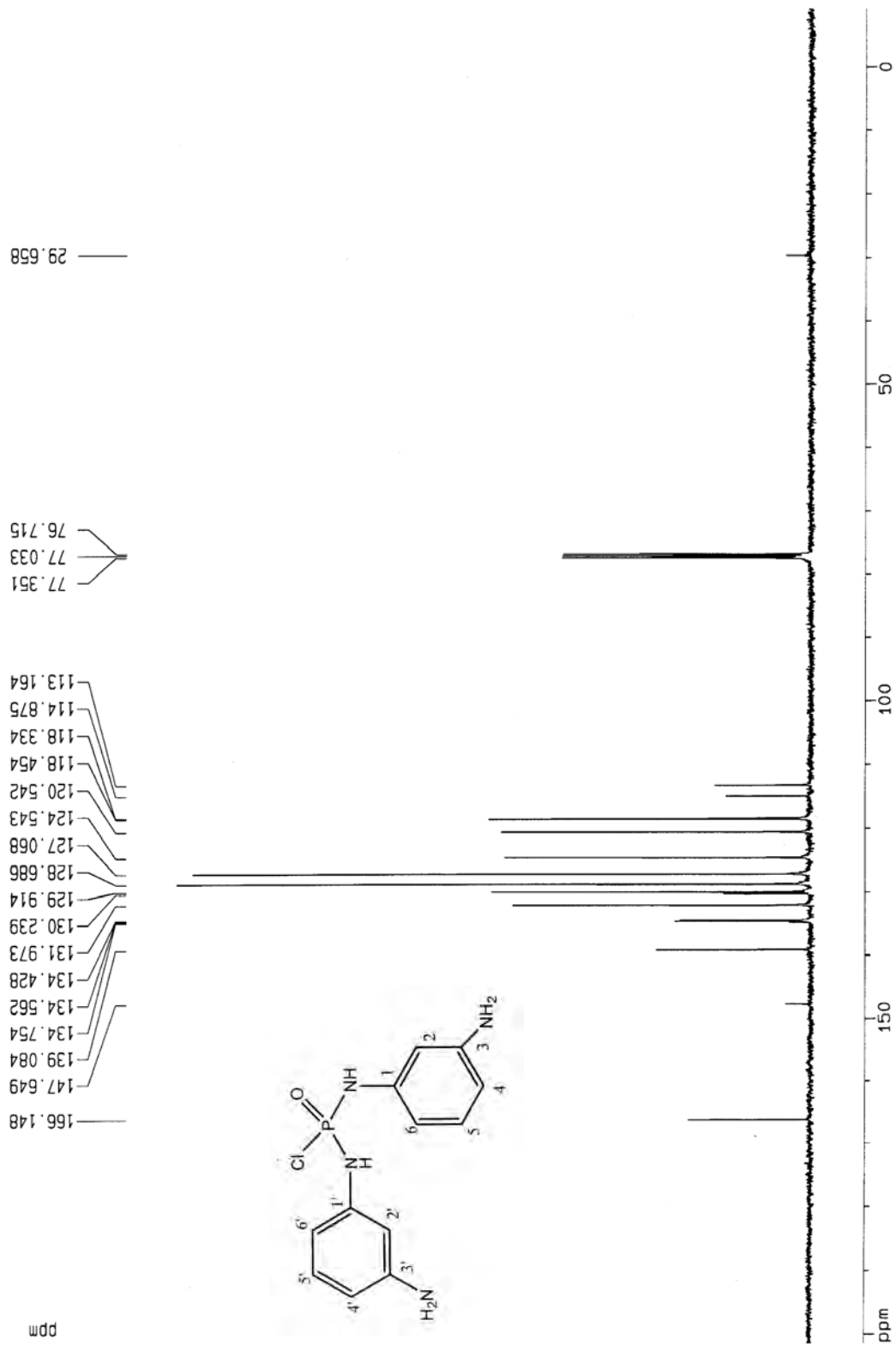
Figure-6(d): ^{13}C NMR spectrum of Compound SN8

0   50   100   150   ppm

121

REFERENCES

References

1. Kabas G. and Rutz H.C., *Tetrahedron*, 22, p-1219, 1966

2. Golodsmith D.J. and Hartman J.A., *J. Org. Chem.*, 29, 3520-3524, 1964

3. Sevarajan R., John J.P., Narayanan K.V., Swaminathan S., *Tetrahedron*, 22, 949, 1966.

4. Brian J Yeh; Wendell A Lim.; "Synthetic biology: lessons from the history of synthetic organic chemistry", *Nature Chemical Biology* 3, 521 - 525 (2007) , doi:10.1038/nchembio0907-521

5. Jaffe, B. Crucibles: The Story of Chemistry from Ancient Alchemy to Nuclear Fission (Dover Publications, New York, 1976).

6. Wöhler, F. Poggendorff's, *Ann. Phys.* 12, 253–256 (1828).

7. Asimov, I. A Short History of Chemistry (Anchor Books, Garden City, New York, USA, 1965).

8. Khorana, H.G. Fed. Proc. 24, 1473–1487 (1965). PubMed, ISI, ChemPort

9. Andrianantoandro, E., Basu, S., Karig, D.K. & Weiss, R. Mol. Syst. Biol. 2, 2006.0028 (2006).

10. Benner, S.A. & Sismour, A.M. Nat. Rev. Genet. 6, 533–543 (2005). Article, PubMed, ISI,ChemPort

11. Khosla, C. & Keasling, J.D. Nat. Rev. Drug Discov. 2, 1019–1025 (2003). Article, PubMed, ISI, ChemPort.

12. Anderson, J.C., Clarke, E.J., Arkin, A.P. & Voigt, C.A. J. Mol. Biol. 355, 619–627 (2006). Article, PubMed, ISI, ChemPort.

13. Andrianantoandro, E., Basu, S., Karig, D.K. & Weiss, R. Mol. Syst. Biol. 2, 2006.0028 (2006).

14. Benner, S.A. & Sismour, A.M. Nat. Rev. Genet. 6, 533–543 (2005). Article, PubMed, ISI, ChemPort.

15. Endy, D. Nature 438, 449–453 (2005). Article, PubMed, ISI, ChemPort,

16. Voigt, C.A. Curr. Opin. Biotechnol. 17, 548–557 (2006). Article, PubMed, ISI, ChemPort.

17. Hoffmann, R. The Same and Not the Same (Columbia University Press, New York, 1995).

18. Rastogi S., "Medicinal chemistry, like synthetic chemistry, comprises both art and science", 2008.

19. Stenberg, P., Bergstroem, C. A. *Clin. Pharmacokinet*. 2002, 41(11): 877–899.

20. Stoner, C. L., Cleton, A., Johnson, K. *Int. J. Pharmaceutics*, 2004, 269(1): 241–249.

21. Tickle, I. J., Vonrhein, C (Astex Technology Ltd., UK), 2005, US patent 2005159901.

22. Waterbeemd, H. and Gifford, E. *Nat. Rev. Drug Discovery*, 2003, 2(3): 192–204.

23. D. E. C. Corbridge, *''An outline of Phosphorus chemistry, Biochemistry and Uses''*, ELSEVIER SCIENCE B. V., p-1, 1995.

24. Schrader, G., "Insecticidal phosphorous ester", Mc. Millan, London, 1982.

25. Call, F; "Biological aspects of phosphorus", Long man's, London, vol-8, 1971.

26. Toy, A.D.F and Walsh, I.N; "Phosphorus Chemistry every day in living" 2nd Ed. *ACS*, Washington DC, 1987.

27. Fest, C; Schmidi, K. J; "The Chemistry of organophosphorus pesticides", Springer Verlag, Berlin, 1982.

28. Hassal, K. A; "The Chemistry of pesticides", Mc Millan, London, 1982.

29. Cremlyn, R.J.W; "Organophosphorus Herbicides and Fungicides", Proceeding 1st International Congress of phosphorus compounds; Rabat, 1978.

30. Taylor, J.B; Kennewell, P.D; " Introduction to Medicinal Chemistry", 1981.

31. Luzio, J.P; Thomson, R.J; "Molecular Medical Biology", *Cambridge University Press*, 1990.

32. Atherton, F.R; Hassal, C.S; *J.Med.Chem.*, 29, 29, 1986

33. Hendrin, D; "Science" 166, 122, 1969.

34. Walker, R.I; Gait, M.J; "Nucleic acid in Chemistry and Biology", *IRL Press*, 1990.

35. Thorselt, E.D; "193rd meeting, ACS Denver, MEDI, 65, 1987.

36. Gebelein, C.G; *ACS Symposium*, 186, 193, 2002.

37. Schaffer, H.J; "*Nature*", 272, 583, 2008.

38. Hoogerhout P., T. Lett., **28**, 1553, 1987.

39. *Chem. Abs*. **120**, 253418.

40. Cramer F., *AWC,* 1960, **72,** 236.

41. Lapiens G., Pretula J., Penczek S., *Macromols.,* 1983, **16,** 153.

42. Shultz G. E. & Schirmer R. H., *''Principles of Protein Structure''*, *Springler*, New York,1979.

43. Dickerson R. E. & I. Gets, *''Proteins :Structure-''*, 2nd Ed, Benjamin-Cumins, Menlo Park, California, 1983.

44. Barret G. C. , (Ed)*''Chemistry & Biochemistry of the Amino Acids''*Chapman & Hall, London, 1985.

45. Armas R., Neumann R., Goldsmith S.J., *J. Nucl. Med.*, **24**, 799, 1983.

46. Brill D.R., *Semi. Nucl. Med.*, **11**, 277, 1981.

47. Klayman D.L., Copeland E.S., Kirk-Othmer Encycl. *Chem. Tech.* Vol 19, p 801, 1982.

48. Dymsza H.A. et al., *Food. Technol*. Chicago., **20**, 1349, 1966.

49. GB 2,014,424 App US 4,251,550 USSR 731,949

50. Wilkinson C.F., "Insecticide Interactions" in (16)

51. Mel'nikov N. N.,"Pesticides in the Environment", *Russ. Chem. Revs*., **61**, 1058, 1992.

52. Grossbard E., Atkinson D.,"The Herbicide Glyphosate", *Butterworth*, 1985.

53. *The Agrochemical Handbook*, 2nd Ed, Roy. Soc. Chem. Nottingham, 1987.

54. Martin H. & Worthing C. R., *Pesticide Manual,* Brit. Corp. Prot. Council, 5th Ed, 1977.

55. Cremlyn R. J. W.,*''Organophosphorus Herbicides & Fungicides''*, Proc. 1st Internat. Congr. Phos.Compounds., Rabat, 1978.

56. Ellinger R. H.,*''Phosphates in food processing''* Hdbk Food Additives, *CRC press*, Cleveland, 1972.

57. VanWager J. R., *''Phosphates-Food & Dentifrice Applications ''* in *Phosphorus & its Compounds*: Vol 2, Wiley, New York, 1960.

58. Machlin L. J.*,''Phosphates in Human Nutrition''* in *Environmental Phosphorus* Hbk, Ed E. J. *Griffith, Wiley*, New York, 1973.

59. Paul A. A. & Southgate D. A. T., ''Composition of Foods '' (McCance & Widdowson) HMSO, London, 1978.

60. Coultate T. P.,''Food : Chemistry of its Components '' *Roy. Soc. Chem.* London., 1984.

61. Toy A.D.F. & Walsh E. N.,''Phosphorus Chemistry in Everyday Living'', 2nd Ed, *ACS*, Washington, 1987.

62. Zapsalts C., Beck & R. A.,''Food Chemistry & Nutritional Biochemistry '', *Wiley*, New York, 1987.

63. Solarek D. B., *''Phosphated Starches''* in Modified Starches, O. B. Wurzburg Ed, CRC 1986.

64. Radley J. A., ''Industrial Uses of Starch'', *Applied Science Pub*. London,1976.

65. Bondi A. A.,''Animal Nutrition'', *Wiley,* New York, 1987.

66. Taylor T. G., ''Availability of P in Animal Feeds'' in Recent Advances in Animal Nutrition, *Butterworth*, 1979.

67. Cullison A. E. & Lowery R. S. (Eds), ''Feeds & Feeding'',4th Ed, Prentice-Hall, 1987.

68. ''The Nutritional Requirements of Ruminant Livestock'', Agric. Bureau, Slough, UK ARC 1980.

69. Underwood E. J.,''The Mineral Nutrition of Livestock'', *Adv. Agron.*1978, **30,** 1.

70. McDowell L. R., ''Minerals in Animal & Human Nutrition''*Acad. Press*.,1992.

71. McCay C. M., ''Notes on the History of Nutrition Research''*,* H. Huber, Berne, 1973.

72. Marschner H.,''Mineral Nutrition of Higher Plants'', *Academic Press*, London, 1986.

73. Carreras E. et al, *Int. J. Cosmet. Sci.,*1984, **6,** 159. US 4, 857, 308.

74. Sander. S. R. & Karo W.,''Organophosphorus Polymers'' *in Polymer Synthesis* Vol 1, Acad. Press, 1974.

75. FEST C. & SCHMIDT K-J ,''The Chemistry of Organophosphorus Pesticides'', *Springer-Verlag*, Berlin, 1982.

76. HASSAL K. A., ''The Chemistry of Pesticides'', McMillan, London, 1982.

77. SCHRADER G., ''Insecticidal Phosphorus Esters'', *AWC,* 1957, **69**, 86.

78. GRAPOV A. F. & MELNIKOV N. N.,''Organophosphorus Fungicides'', *Russ. Chem. Revs.,* 1973, **42**, 772.

79. European Directory of Agricultural Products, Parts, 1, 2, 3 *Roy. Soc. Chem.* London, 1984.

80. Toy A.D.F., Cooper R. S., *JACS*, **76**, 2191, 1954.

81. Toy A.D.F., *Chem. Eng. News.*, **25**, 2030, 1947.

82. Toy A.D.F., Modern Plastics, **24**, 226, 1947.

83. *Chem. Abs.*, **118**, 8428.

84. Anbar M., Farley E.P., *J. Dent. Res.*, **53**, 867, 1974.

85. Anbar M. et al., *J. Dent. Res.*, **58**, 1134, 1979.

86. Godfrey L. E. A., Schappel J. W., *Ind. Eng. Chem. Prod. Res. Dev.*, **9**, 426, 1970.

87. Krauskopf L.G., Nass L.I., *Encyclopedia of PVC*, p 529, Dekker, New York,1976.

88. Hall C. W., "Milk & Milk Products" in Kirk-Othmer, *Encl. Chem. Tech.*, **15**, 3rd Ed, Wiley, New York, 1981.

89. Swaisgood H. E., Fennema's Food Chemistry, 1985.

90. Muller L. L.,"Manufacture of Casein & Caseinates", *Devel. Dairy. Chem.*, **1**, Ed P. Fox, App. Sci. Pub., Lond,1982.

91. Southwood C. R., "Uses of Casein & Caseinates", *Devel. Dairy. Chem.*, **4**, 173, 1989.

92. Sutermeister E., Browne F. L. , (Eds), "Casein and its Industrial Applications", 2nd Ed Rheinhold, 1939.

93. Muller . L. L., *Dev. Dairy. Chem.*, **1**, 315, 1982.

94. PIZER C., Vol. 6, Ed P. D. Boyer, *Acad. Press.*, 1973.

95. DAHL J. L. & HOKIN L. E., Ann. Revs. Biochem., 1974, **43,** 327.

96. Koh H. J., Kim O. S., Lee H. W., *J. Phys. Org. Chem.,* 1997, **10,** 725-730.

97. Koh H. J., Shin C. H., Lee H. W. and Lee, I.,*J. Chem. Soc., Perkin Trans.,* 1998, **2,** 1329-1332.

98. Hoque M. E. U., Dey S., Guha A. K., Kim C. K., Lee B. S. and Lee H. W., *J. Org. Chem.,* 2007, **72**, 5493-5499.

99. Adhikary K. K., Lee H. W. and Lee I., *Bull. Korean Chem Soc.,* 2003, **24,** 1135-1139.

100. Eichberg J.,"Lecithin" Kirk-Othmer, *Encl. Chem. Tech*., **14**, 3rd Ed. Wiley, New York, 1981.

101. Schmidt, J. C., Orthoefer F. T., "Non-food uses of Lecithins", in Lecithins, 1985.

102. Tanno H., "Lecithin"in *Ullmans Encyclopedia of Industrial Chemistry*, A**15**, 1990.

103. Freeman I. P., Margarine, A**16**, 1990.

104. GRAVES D. J. & WANG J. H., Vol. 7, Ed P. D. Boyer, *Acad. Press*., 1973.

105. Wikipedia, the free encyclopedia.mht

106. Sarin (GB). Emergency Response Safety and Health Database. National Institute for Occupational Safety and Health. Accessed April 20, 2009.

107. Corbridge D. E. C., "Phosphorus: An Outline of its Chemistry, Biochemistry, and Technology" 5th Edition *Elsevier: Amsterdam* 1995. ISBN 0-444-89307-5.

108. Saunders B.C., "Recent Aspects of Chemistry & Toxicity of Compounds Containing P & F", *Camb. Univ. Press*, England, 1957.

109. Schrader G., "Die Entwirklung nuer Insectizider Phosphorester", Verlag Chemie GmbH Weinheim, Germany, 1963.

110. Heath D. F., "Organophosphorous Poisons", *Pergamon, Oxford*, 1961.

111. O'Brien R. D., "Toxic Phosphorous Esters", *Acad. Press*, New York, 1960.

112. Sartori M. F., "New Chemical Warfare Agents", *Chem.Revs*., **48**, 225, 1951.

113. Saunders B. C. & Stacey M., *JCS*, 695, 1948.

114. Tahmaz, S. and Cherrie. Chronic fatigue and organophosphate pesticides in sheep farming: a retrospective study amongst people reporting to a UK pharmacovigilance scheme. The Annals of Occupational Hygiene (2003) vol. 47 (4) pp. 261-7. http://annhyg.oxfordjournals.org/content/47/4/261.full

115. Study Links Organophosphate Insecticide Used on Corn With ADHD. *Beyond Pesticides.* 5 January 2007.

116. Hayden, K.; Norton, M.; Darcey, D.; Ostbye, T.; Zandi, P.; Breitner, J.; Welsh-Bohmer, K.; Cache County Study, I. (2010). "Occupational exposure to pesticides increases the risk of incident AD: the Cache County study". *Neurology* **74** (19): 1524–1530. doi:10.1212/WNL.0b013e3181dd4423. PMC 2875926.PMID 20458069. http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pmcentrez&artid=2 875926

117. Klein, S.. Study: ADHD linked to pesticide exposure. *CNN.* 17 May 2010.

   Costa LG. *Current issues in organophosphate toxicology*. Clin Chim Acta. 2006 Apr;366(1-2):1-13. Epub 2005 December 6. Review. PMID: 16337171.

118. Ellof, J.N., 1998: Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol*. 60, 1-6.

119. Graham L. Patrick, Medicinal Chemistry, 2 ed. P 379-380.Oxford.

120. Pelczar, M.J., Chan, E.C.S. and Krieg, N.R., 1999: "Host-Parasite Interaction; Nonspecific Host Resistance", In: Microbiology Conceptsand Applications, 6th ed., McGraw-Hill Inc., New York, U.S.A. pp. 478-479.

121. Bauer, A. W., Kirby., V.M.M., Sherries, J.C., and Tuck, M. F966. *Am. J. din. Pathol*. 45, 493-496.

122. Roland, R. Antibiotics, An Introduction . F Hoffmann La Roche and Co., Basle, Switaerland, 1982, p.70-71.s

123. Bauer, A.W.; Kirby, E.; Sherris, E.M.; Turk, M., 1966: Antibiotic by standarized single disk method. *Am. J. Clin. Path*. 45, 493-496.