RHODIUM BORON NITRIDE: A RECYCLABLE CATALYST FOR THE SYNTHESIS OF α-AMINOPHOSPHONATES AND DIHYDROPYRIMIDINONES

Thesis submitted in fulfilment of the requirements for the award of the Degree of Master of Applied Science in Chemistry, in the Faculty of Applied Sciences at the Durban University of Technology

By

ABOSEDE OLUWABUKOLA JAIYEOLA

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Supervisor: Prof. R.M. Gengan

Declaration

I, Abosede Oluwabukola Jaiyeola, declare that the thesis submitted for the Master of Applied Science in Chemistry degree at the Durban University of Technology, has not been submitted to any other institution of higher education, and that its only prior publication was in the form of conference papers, book chapters and/or journal articles and the registration of a provisional patent.

I further declare that all the sources cited or quoted are acknowledged and indicated by means of a comprehensive list of references.

Abosede Oluwabukola, Jaiyeola

The final submission of the thesis is hereby approved.

Prof. R.M. Gengan (PhD)

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DEDICATION

This dissertation is dedicated to God Almighty the source of my strength, my help in ages past, my ever present help and my hope of Glory, the loving memory of my late father, Pa Samuel Olusesan Esan and to my beloved family.

ACKNOWLEDGEMENT

First and foremost, I wish to express my sincere appreciation, deep sense of gratitude and indebtedness to my supervisor and mentor **Professor R.M Gengan** for his guidance, constructive criticism, constant encouragement, remarkable patience and motivation throughout my course of study. You are a great source of inspiration, motivating factor and academician per excellence. It was a rare privilege and opportunity to have been a part of your research team and passed through your tutelage.

My special thanks also goes to my senior colleague **Dr Krishnan Anand** for his endless support, valuable suggestions and advice throughout my study. Your dedication, diligence, hard work, creativity and wealth of knowledge inspire me a lot.

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My sincere gratitude also goes to the NMR specialists **Mr Dilip Jagivan** from University of KwaZulu Natal for his assistance with all NMR experiments.

I would also like to articulate my great thanks to Durban University of Technology for provision of fund during my postgraduate studies

I would like to present my special thanks to my family for their blessings, support and encouragement during this work especially my sweet mother who devoted her time taking care of the children while I was in school. Of course none of this work would have been possible without the love, care, understanding and support of my darling husband, I am blessed to have you honey, and you are my hero.

Finally, my biggest thanks goes to God Almighty for giving me strength, support, wisdom and showering his abundant blessings on me all these years, Your grace is indeed sufficient for me Lord. "Not by Power, Not by Might but by my spirit says the lord of Host"; Zechariah 4:6.

ABSTRACT

The α-aminophosphonates (APs) and dihydropyrimidinones (DHPMs) exhibit a wide range of important biological activities. The great potential of these compounds in biological applications prompted an increased interest in the development of efficient synthetic methods for their preparation.

A novel rhodium supported boron nitride (RhBNT) material was synthesized by simply mixing boron nitride in a solution of rhodium acetate, under inert atmosphere for 7 days followed by filtration; the yield was 95 %. It exhibited excellent catalytic properties for the synthesis of 13 novel APs and 5 DHPMs. Characterization of RhBNT was performed by several techniques: the crystalline nature of RhBNT and nano size was confirmed by SEM spectroscopy, EDX pattern for RhBNT showed signals for rhodium metal, the Brumnauer-Emmett-Teller (BET) analysis showed the specific surface area of RhBNT to be 28.12 m^2/g, pore volume 0.23cm^3/g and pore size of 199.8Aº thereby suggesting RhBNT as a potentially effective catalyst for organic reactions; the mesoporous nature of the material was established by a type-IV adsorption isotherm; the DSC-TGA Profile indicates that RhBNT has good thermal stability and can be used adequately for catalysis. The DSC curve showed evidence of a broad exothermic peak.

The RhBNT was subsequently used in the Kabachnik-Fields and Biginelli reaction in order to assess its catalytic potential. Herein Vilsmeier-Haack reagent was used to synthesize 4-oxo-chromene-3-carbaldehyde and 4-oxo-4H-benzo[h]chromene-3-carbaldehyde from 2-hydroxyacetophenone and 1-hydroxy-2-acetonaphthone, respectively. These two carbaldehydes were subsequently used to synthesize thirteen novels APs and five DHMPs using RhBNT as the catalyst

The antimicrobial activities of the synthesized compounds were assessed against *Escherichia coli, Bacillus cereus, Micrococcus luteus, Staphylococcus aureus* and *Candida albicans* using the disc diffusion method. It was found that none of the compounds inhibited growth of bacteria or fungus.

The assessment of toxicity was evaluated by using the brine shrimp lethal test. It was found that six of the novel compounds exhibited more than 50% brine shrimp death and were considered toxic against *Artemia sp.* and hence unsuitable as a potential drug whilst four compounds were found to be less toxic, exhibiting a brine shrimp death of less than 50%.

Molecular docking studies were carried out for 13 APs to estimate their binding interactions with HIV-1 reverse transcriptase. Four APs showed good potential for the inhibition of HIV-1 reverse transcriptase.

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ABBREVIATIONS

AcOH - acetic acid

AEP- 2-aminoethanephosphonic acid

AMC- active methylene compound

APs-aminophosphonates

BN - boron nitride

^{o}C -degree celsius

^{13}C -carbon 13

Cat - catalyst

C_6H_5 – phenyl

$CHCl_3$ -chloroform

CH_3CN - acetonitrile

CO_2 - carbon dioxide

CR - component reaction

CTAB - cetyltrimethylammonium bromide

$CuCl_2$ - copper chloride

DABCO - 1,4-diazabicyclo[2,2,2]octane

DHPM - dihydropyrimidinones

DMF- dimethyl formamide

DMF - dimethyl formamide

DMSO-dimethyl sulfoxide

DSC-TGA - differential scanning calorimetry-thermogravimetric analysis

EDX - energy dispersive X-ray spectroscopy

EtOH – ethanol

$FeCl_3$ – iron (III) chloride

g - grams

^1H - proton

H_2 - di hydrogen

HCl - hydrochloric acid

HIV - human immunodeficiency virus

H_2SO_4 - sulphuric acid

IR -infra-red

K10 clay- montmorillonite

K_2CO_3 - potassium carbonate

KOH - potassium hydroxide

MCRs - multicomponent reactions

MeOH- methanol

Me_2SO_4 - methyl sulphate

min - minute

mL- milli Litre

mol - mole

m.p- melting point

MS-mass spectrometer

MSA - methyl sulphonic acid

MW - microwave radiation

MWI - microwave irradiation

Na_2CO_3 - sodium carbonate

NaH - sodium hydride

Na_2SO_4- sodium sulphate

NMR- nuclear magnetic resonance

OH- hydroxide

$POCl_3$ - phosphorus trichloride oxide

PPA - poly phosphoric acid

ppm - parts per million

PTSA - para toluene sulphonic acid

RhBNT-Rhodium loaded boron nitride

$Rh(OAc)_2$ - rhodium acetate

s –second

SEM - scanning electron microscope

TLC -thin layer chromatography

UV/Vis- ultraviolet visible spectroscopy

VHR - Vilsmeir-Haack reaction

W - watts

%- percentage

GENERAL REMARKS

All the figures pertinent to a chapter are placed after the relevant discussion in the following sequence, IR, NMR and Mass spectra. All experimental procedures are found in chapter 3.

The solvents and reagents used for the synthesis were of reagent grade (unless otherwise mentioned) and were purified by standard methods. Petroleum ether used was of boiling range 60-80^{0}C. Anhydrous sodium sulphate was used to dry the solutions of organic extracts.

Thin layer chromatography (TLC) was performed using TLC plates. Petroleum ether, hexane, ethyl acetate, chloroform and methanol were used as developing solvents. A chamber containing iodine vapour was used to visualize the TLC spots.

Separation or purification of crude products was carried out using chromatographic columns packed with activated neutral alumina or silica gel 60-120 mesh.

Melting points were determined on Mettler FP5 apparatus and were corrected. They were expressed in degree celsius (^{0}C).

The IR spectra were recorded on Perkin Elmer 537 spectrophotometer or Shimadzu-8201 FT instrument, using ATP disc and the absorption frequencies were expressed in reciprocal centimetres (cm^{-1}).

The ^{1}H & ^{13}C NMR spectra were recorded either on Bruker (300 MHz) or

Bruker (400 MHz) spectrophotometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal reference. The chemical shifts were quoted in parts per million (ppm). The following abbreviations were used:

s - singlet

d - triplet

q - quartet

m- multiplet

bs – broad singlet

band

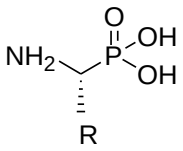
J – spin-spin splitting constant in Hertz (Hz)

Mass spectra were recorded on Waters Micromass LCT Premier TOF-MS Spectrometer. For some of the compounds, especially for its ^1H NMR values, splitting patterns, integral values and the intensity of peaks were ascertained from its expanded version of the spectrum and the copies of the same were produced in this thesis and *J* values are averaged ones.

CHAPTER ONE

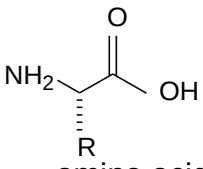
INTRODUCTION

Synthetic chemistry plays a key role in the multidisciplinary development process of new small molecules for pharmaceuticals and allied industries. There are numerous classes and sub-classes of target molecules that can be interrogated for novelty and subsequent application based studies. One such class of compounds are the organophosphorus compounds which are widely used in industrial, agricultural and medicinal chemistry due to their important biological and physical properties (Kaboudin *et al.* 2006). They are also important synthetic intermediates. Amongst this category of compounds are the aminophosphonates also referred to as aminoalkanephosphonic acids **(1)**. The importance of α-aminophosphonates (APs) in biological systems is due to their structural resemblance to the corresponding α-amino acids **(2)** which are building blocks to proteins and enzymes (Jafari *et al.* 2010; Naydenova *et al.* 2010). Also the first naturally occuring APs (2-amino ethyl)-phosphonic acid **(3)** was isolated by Horiguchi and Kandatsu in 1959 (Kandatsu and Horiguchi 1962). The APs are found in bacteria, protozoa, invertebrates and higher organism as constituents of phosphonopeptides, phosphonolipids and other phosphonates (Gallardo-Macias 2009) . They act as enzyme inhibitory neuroactive agents, HIV protease antagonists, collagenease inhibitors, peptide mimics (Karimi-Jaberi and Amiri 2010), antibiotics, herbicides (Kim and Rhie 1997), pharmacological agents (Chandrasekhar *et al.* 2001) , pesticidal (Hudson and Kukhar 2000) and antiviral activity (Minaeva *et al.* 2010) as well as antibody generation (Cristau *et al.* 2000)



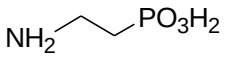
α-aminophosphonic acid

1



α-amino acid

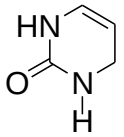
2



(2-aminoethyl)-phosphonic acid

3

Another important type of compounds are dihydropyrimidinones (DHPMs) or Biginelli compounds (**4)** which exhibit a wide range of biological and pharmacological properties, such as antiviral, antitumour, antihypertensive, antibacterial and antinflammatory properties (Paraskar *et al.* 2003) and potential calcium channel blockers (Kappe 2000a). Furthermore these compounds have been found to be potent HIV-gp-120-CD_4 inhibitors (Eshghi and Hassankhani 2006)



4

There are several synthetic methods available to synthesize the APs and DHPMs. Of these, the multicomponent strategy is ideal as it is efficient, conducted in one-pot and produces the target molecule in higher yield than the conventional step-wise strategy. Furthermore, catalysts are used to speed up chemical reaction.

The APs and DHPMs display important biological properties therefore it is essential to develop simple, efficient, quick and general procedure for their synthesis.

Research aim and objectives

The research aim was to investigate a more feasible and efficient recyclable novel catalytic system which could be used for the synthesis of APs and DHPMs.

The objectives of the study are to:

- synthesize a novel metal-based boron nitride catalyst and fully characterize it
- use the novel catalyst to synthesize novel chromone-bearing α-aminophosphonates and dihydropyrimidinones from readily available, simple and cost effective starting compounds.
- purify the novel compounds by chromatographic techniques and characterize them by spectroscopic techniques.
- evaluate the toxicity of the novel compounds using the brine shrimp assay
- determine the antibacterial and antifungal activities of the novel compounds
- Evaluate the possible therapeutic potential of the novel compound against HIV-1 transcriptase via molecular docking studies

The outcome of this research investigation is outlined in four chapters as presented below;

Chapter 2 describes the significance of multicomponent reactions, heterocyclic compound, heterogeneous catalyst, literature review on chromones, aminophosphonates and dihydropyrimidinones which includes previous method of synthesis. The synthesis of aminophosphonates via the Kabachinik-Field reaction and dihydropyrimidinones via the Biginelli reaction is described. Also structure

elucidation (by spectroscopic techniques) is presented. The importance and biological applications of APs and DHPMs such as antimicrobial activity is described. The use of brine shrimp assay and Ames Salmonella mutagenicity test for the evaluation of toxicity of synthetic compound are discussed. The role of molecular modelling studies in the design and development of hits/ leads against several disease targets is described.

Chapter 3, describes the experimental procedures, the purification methods and the spectroscopic data of the catalyst and the novel compounds. Methodology for the evaluation of the antimicrobial activity, safety of the novel compounds and molecular docking studies to explore the biological importance of the novel compounds towards HIV-1 reverse transcriptase is also described.

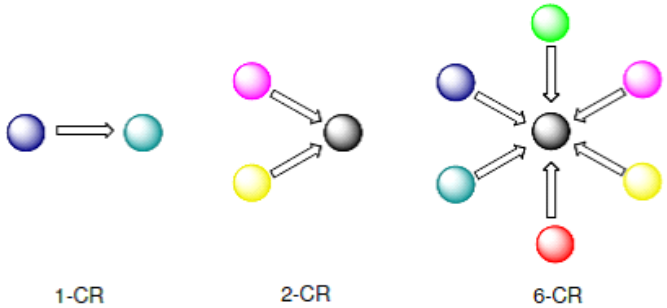
In Chapter 4**,** the results are interpreted and discussed, followed by conclusion and recommendation.

CHAPTER TWO

LITERATURE REVIEW

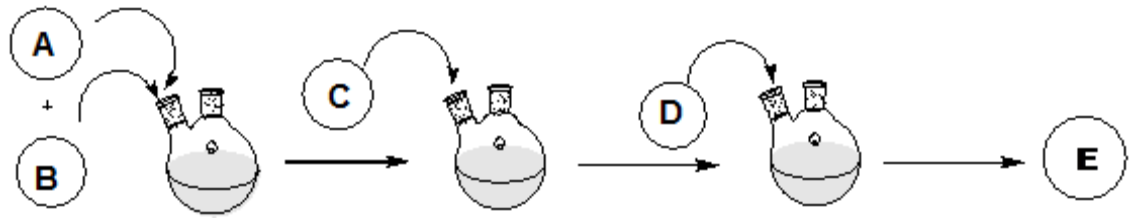
2.1. Multi-Component Reactions

Multi-component reactions (MCRs) are chemical transformations in which three or more reactants form a new product which contains the key parts of all of the starting material (Eckert 2012). They have emerged as an efficient and powerful tool in modern synthesis and medicinal chemistry for different and yet efficient drug discoveries (Mosslemin *et al.* 2010). Furthermore, MCRs offer significant advantages over traditional methods of synthesis such as unparalleled atom economy, straight forward and mild reaction condition, excellent yield and generation of chemical backbones which are particularly useful in the synthesis of libraries of heterocyclic bioactive molecules (Weber 2002). In MCRs, several starting materials assemble to form complex products. Thus, it can be referred to as convergent reactions, in contrast to a divergent multi-step synthesis (Dömling and Ugi 2000) as depicted in **scheme 1** and **2** (Dömling 2006).



Scheme 1: An Illustration of Divergent Reactions

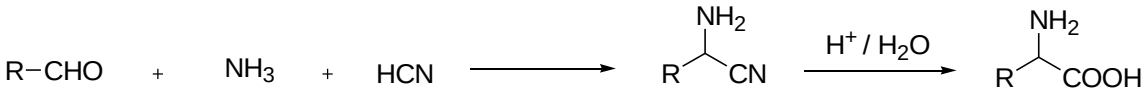
Key: 1-CR, 2-CR and 6-CR are one, two and six component reactions**,**



Scheme 2: All Illustration of Multi-Step Synthesis

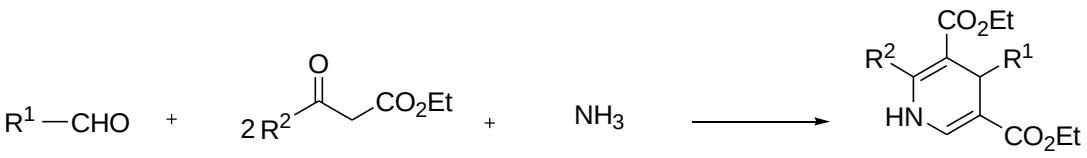
2.1.2. The History of Multi-Component Reactions

Multi-component reactions have been known for over 100 years. The first MCR was described by Strecker in 1850 (Martínez *et al.* 2005) : it involved the reaction of an aldehyde, ammonia and hydrocyanic acid in one-pot which resulted in the formation of an α-amino nitrile which was subsequently hydrolysed to an α-amino acid (**Scheme 3**).



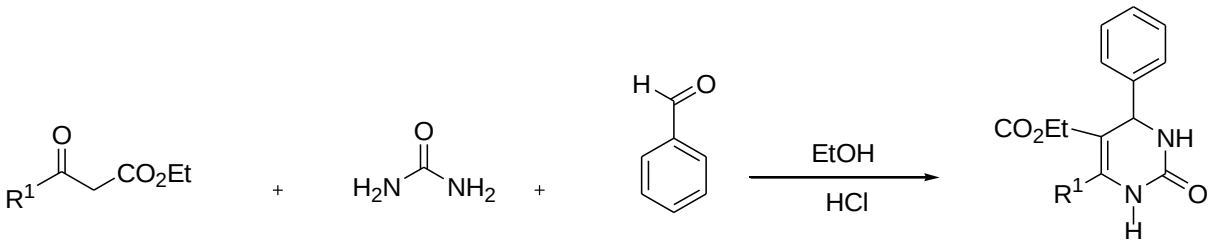
Scheme 3: The Multi-Component Synthesis of α-Amino Nitrile by Streck

Arthur Hantzch, (Hantzsch 1882) in 1881 described the one-pot four component synthesis of dihydropyridine (DHP) from the reaction of an aldehyde, 2 β-ketoesters and ammonia (Faldu *et al.* 2011) (**Scheme 4**).



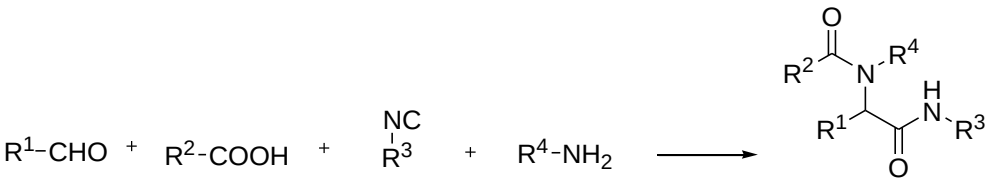
Scheme 4: The Multicomponent Synthesis of Dihydropyridine by Hantzch

The Biginelli three component reaction, which was first described in 1893, represents the synthesis of substituted dihydropyrimidinones by the acid-catalysed cyclo-condensation of β-ketoesters, aromatic aldehydes and urea (Kappe 2000b) **(Scheme 5).**



Scheme 5: The Multicomponent Synthesis of Dihydropyrimidinones by Biginelli

Among all the MCRs, the Ugi four-component synthesis of acylamino amides via the condensation of an amine, aldehyde, carboxylic acid and isocyanide, remains by far, the most accredited and most versatile (Dömling and Ugi 2000) (**Scheme 6**).



Scheme 6: The Multi-Component Synthesis of Acylamino Amides by Ugi

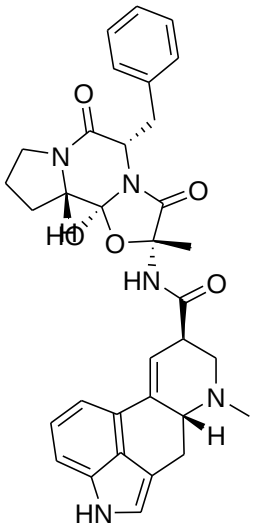
Although in the past decade there have been tremendous developments in the three and four-component reactions and huge efforts have been and still are being made to discover novel MCRs, the development of new synthetic approaches using MCRs remains an active research area (Ugi *et al.* 2003).

2.2. Heterocyclic Compounds and their Biological Importance.

The study of heterocyclic compounds is vital in the discovery of new drug like molecules. The study of the theoretical and practical aspects of these compounds is

a subject of great attention in synthetic organic chemistry (Samanta *et al.* 2005). Heterocyclic compounds are organic compounds whose molecules contain one or more rings of atoms with at least one atom being an element other than carbon; oxygen, nitrogen or sulphur (Cuenca *et al.* 1999) are the usual elements. Heterocycles constitute a large part of organic compounds and are biologically and industrially important. Heterocyclic compounds are categorized as aliphatic and aromatic. The aliphatic heterocycles are the aromatic analogues of amines, ethers, thio ethers, amides whilst the aromatic heterocyclic compounds consist of a heteroatom in the ring and exhibit certain properties similar to benzene. Furthermore they are widely distributed in nature and a variety of pharmacologically active heterocyclic motifs are clinically used as drugs (Kale and Patwardhan 2013). Apart from the diverse distribution of heterocycles in nature, they are a major constituent of important biological molecules such as DNA and RNA, For example the derivatives of pyrimidines and purines form the nucleotides which are the building block of the genes. Heterocycles containing heteroatoms such as nitrogen, sulphur (Dua *et al.* 2011), oxygen (Liu 2001), phosphorus (Liu 2001) and selenium (Abdel-Hafez 2008) are known to possess remarkable biological activity and unique structure that led to several applications in pharmaceuticals, industrial and agrochemical research. Alkaloids are an important group of heterocyclic compounds, occurring in nature, with a variety of biological and pharmacological activity such as Ergotamine (**5),** the indole-based alkaloids displays activity against migraine (Kreilgård 1977), Cinchonine (**6),** a quinolone class of alkaloid which possess anti-malarial activity(Wesełucha-Birczyńska and Nakamoto 1996), Posaconazole **(7),** a triazole anti-fungal drug (Pfaller *et al.* 2002) and Anastrozole **(8),** an aromatase-inhibiting drug widely used for the treatment of post-menopausal breast cancer (Pfaller *et al.*

2002).



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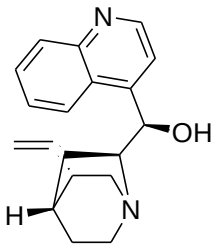

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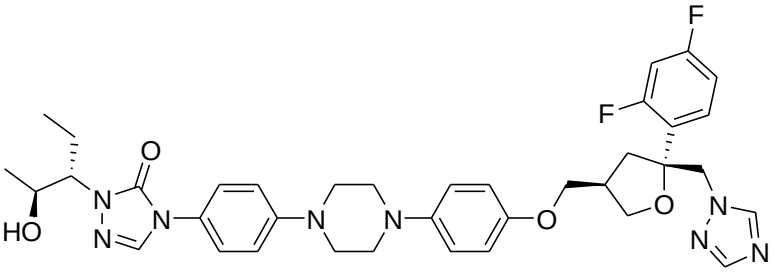

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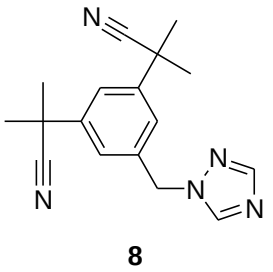

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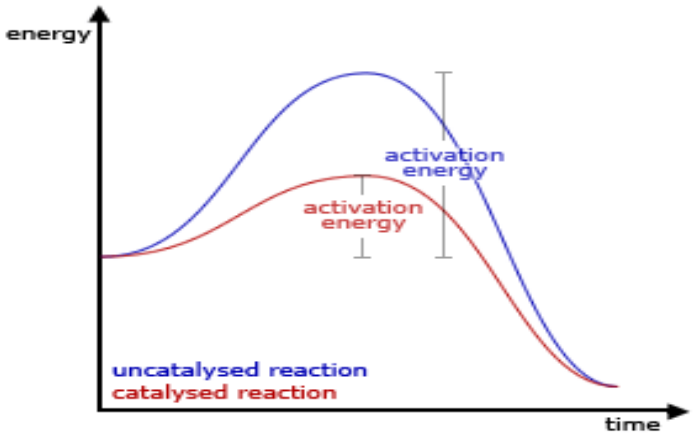
Scheme 7: Some Biologically Active Heterocyclic Compounds

The discovery of effective, selective and bioavailable small molecules that regulate important biological systems continues to be the subject of research interest for modern organic and medicinal chemists (Kale and Patwardhan 2013). Heterocyclic compounds find useful application in the field of biochemistry, polymers, photographic science, dyestuff, adhesives and molecular engineering. Hence, great attention has been directed towards the development of efficient new protocols to synthesize heterocycles.

2.3 Catalyst

A chemical substance that speeds up the reaction rate of a chemical reaction without itself being changed in the process is referred to as a catalyst (Erti and Freund 1999). Catalysis constitutes a new economical and environmental benign way to meet the demands of energy and sustainability for the synthesis of bioactive organic compounds (Daştan *et al.* 2012; Bae *et al.* 2014). Catalysts are generally classified as heterogeneous, homogeneous and biological. A catalyst operates by providing an alternate reaction pathway for the breaking and remaking of bonds; they form temporary bonds with reacting molecules thereby resulting in an intermediate product; the intermediate product in turn reacts with the other reactant molecule to afford the final product. The activation energy for the alternate pathway is often lower than the normal pathway. This effect can be illustrated with the energy profile diagram (**Scheme 8**).



Scheme 8: Energy Profile Diagram Showing Activation Energy of both catalysed and Uncatalysed

2.3.1 Homogeneous Catalysis

A homogeneous catalyst is a catalyst which functions in the same phase where the reaction takes place. Most of the processes using homogeneous catalyst occur in the liquid phase. The key advantage of the homogeneous catalyst is that every single catalytic entity acts as a single active site which makes it more active and selective compared to the heterogeneous catalyst. However it suffers from major drawbacks such as difficulty of its recovery from reaction mixtures, poor thermal stability (Hartley 2012) and high cost.

2.3.2 Heterogeneous catalysis

A heterogeneous catalyst is a type of catalyst in which the catalyst occupies a different phase from the reactant and product. Heterogeneous solid catalysts are mainly based on clay and silica (Sajadi *et al.* 2012) and they play an important role as sustainable alternatives to the classical homogeneous catalyst. Heterogeneous Lewis catalysts such as $InCl_3$ (Ranu *et al.* 1999), DTP/SiO_2 (Mulla *et al.* 2014), $LiClO_4$ (Saidi and Azizi 2002), solid acids such as Amberlite IRC-748 (Shashikumar 2013), Lewis acid ionic liquid system US/[bmim]$AlCl_4$ (Rostamnia and Amini 2014), $TaCl_5$-SiO_2 (Chandrasekhar *et al.* 2001) and bismuth nitrate pentahydrate (Banik *et al.* 2010) have been studied for the Kabachnik-Field reaction for the synthesis of aminophosphonates. The advantages of heterogeneous catalysts include, ease of separation from reaction mixture, good thermal stability, low cost, ease of handling and good selectivity (Nasrollahzadeh *et al.* 2009). The use of heterogeneous catalysts is one of the most vibrant and eco-friendly strategies employed by organic chemists in synthesizing a wide range of bioactive heterocyclic compounds (Ameta and Penoni 2014)

2.3.3 Metal Boron Nitride

Boron nitride (BN) (Figure 1) was first prepared by WH Balmain in 1840 by reacting boric oxide with potassium cyanide (Balmain 1842). BN is isoelectronic to carbon and its hexagonal h-BN form is isostructural to graphite. The graphite like hexagonal BN with covalent bonds in the planes and weak attraction between the planes is the most stable BN isomer under ambient conditions (Alkoy *et al.* 1997). Its strong mechanical and corrosion resistance, superior temperature stability and large thermal conductivity (Paine and Narula 1990) makes BN a promising catalyst support for all applications in which stability is a key point. In general BN is an inert material suitable for catalytic reactions; in a supported metal system such as Pt/BN, BN support has been shown to have negligible interaction with Pt in catalytic oxidation of methanol (Lin *et al.* 2002; Wu *et al.* 2003) and benzene (Lin *et al.* 2002). Furthermore BN have also been used as a support for the hydrogenation of alkynes using Pd/BN catalysts (Yabe *et al.* 2012)

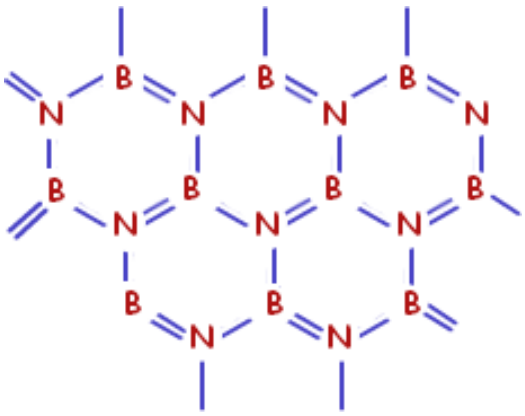
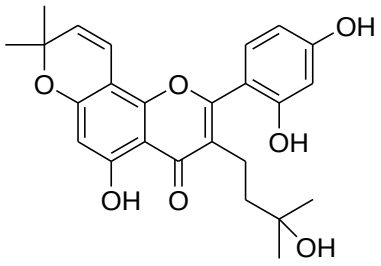


Figure 1: A Section of a Layer of Hexagonal Boron Nitride

2.4 Chromones

2.4.1 Biological Importance of Chromones

Chromones are a group of well-known compounds occurring in nature, especially in plants (Ellis and Lockhart 2007) , and are useful synthetic building blocks in both organic and medicinal chemistry. The chromone moiety is an important component of a large number of bioactive molecules (Joshia *et al.* 2004) which possess several biological activities ranging from antifungal(Prakash *et al.* 2008) anti-oxidant (Kuroda *et al.* 2009), HIV inhibitory (Mahalle and Khaty 2010) to anticancer activities (Musthafa *et al.* 2011). Compounds such as Schumannificine (**9)** exhibits anti-HIV activity (Houghton *et al.* 1994), Leachianone G (**10)** possesses antiviral activity (Houghton *et al.* 1994) and 6-chloro-2-(2-quinoyl) chromone (**11)** is an effective anticancer agent (Gaspar *et al.* 2014).



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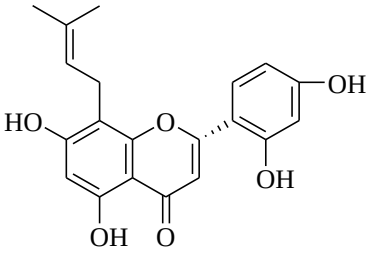

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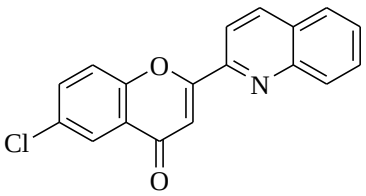

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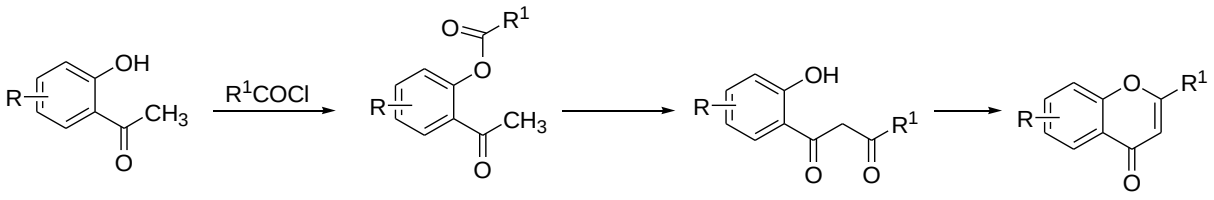
Scheme 9: Some Typical Examples of Biologically Active Chromones

2.4.2. Synthesis of Chromones

Chromones from 2-hydroxyarylalkyl ketones

This involves the acylation of an o-hydroxyacetophenone usually with an acyl chloride in the presence of a base such as potassium carbonate; the acyl intermediate quickly undergoes a Baker-Venkataraman rearrangement which results in the formation of a 1,3-dioxophenoxy intermediate. The resulting intermediate formed is then isolated and undergoes cyclization to afford the desired chromones under strongly acidic condition (**Scheme 10**) Although this is by far the most common method of synthesis of chromones the final step requires harsh acidic conditions (Gaspar *et al.* 2014).

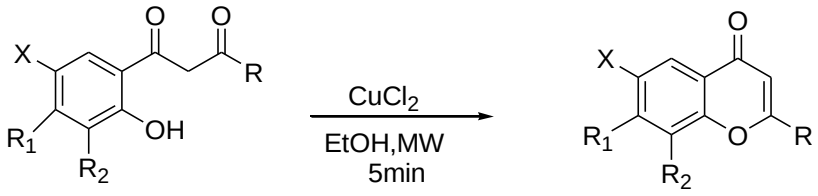


Scheme 10: Synthesis of Chromones via Baker-Venkataraman rearrangement

Synthesis of Chromones via Microwave Irradiation

Microwave irradiation offers considerable advantage over conventional heating such as high yield, short reaction time, cleaner reaction and better selectivity. However many traditional methods of synthesis reported are tiresome and produce low yield products. As a typical example (**Scheme 11**) the cyclization of 1-(2-hydroxyaryl)-3-aryl-1,3-propanedione occurs via the intermediate 1,3-propanediones in 5 minutes through dehydrative cyclization to form the corresponding flavones and chromones in ethanol, in the presence of $CuCl_2$ under microwave irradiation (Kabalka and

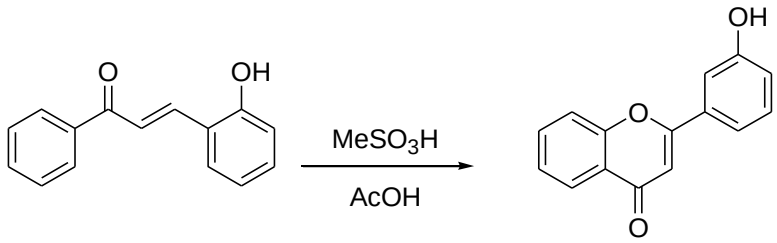
Mereddy 2005).



Scheme 11: Synthesis of Chromones via Microwave Irradiation

Synthesis of Chromone from Chalcone using Methane Sulphonic Acid

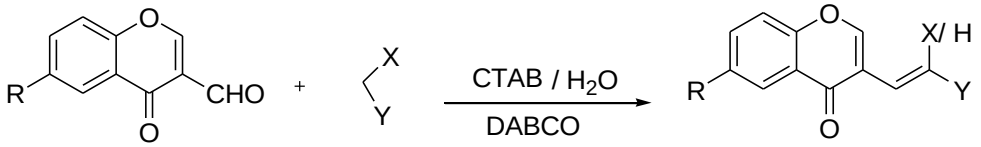
This method involves the addition of methyl sulphonic acid (MSA) to a mixture of 2-hydroxychalcone in acetic acid to afford the corresponding chromone (Kshatriya and Nazeruddin 2014) (**Scheme 12**).The advantages of this protocol is that it is cheap and environmentally friendly.



Scheme 12: Synthesis of Chromone from Chalcone using MSA

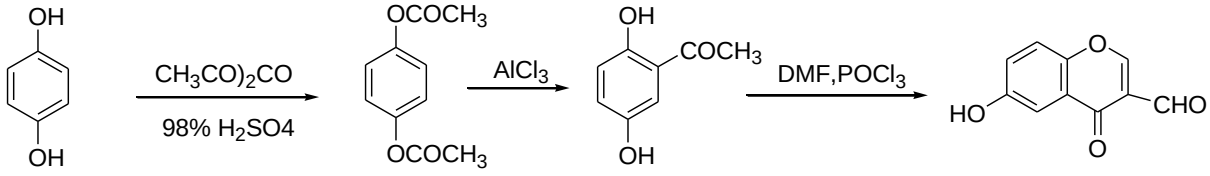
Synthesis of Chromones via Knoevenagel Condensation Reaction

Recently 3-vinylchromone was synthesized from 4-oxo-4H-1-benzopyran-3-carboxaldehyde via a simple Knoevenagel condensation reaction with an active methylene compound (AMC) in a micellar medium in the presence of cetyltrimethylammonium bromide (CTAB) as a catalyst and 1,4-diazabicyclo [2,2,2] octane (DABCO) as a co-catalyst (Kumar *et al.* 2015) (**Scheme 13**).



Scheme 13: Synthesis of Chromone via Knoevenagel Condensation Reaction

Yang et al in 2008 prepared 6-hydroxy chromone-3-carbaldehyde using the Vilsmeier-Haack reaction. The compound was easily synthesized via the reaction of 2,5-dihydroxyacetophenone with DMF in $POCl_3$ solution (Tawfik *et al.* 2014) (**Scheme 14**)



Scheme 14: Synthesis of 6-hydroxy chromone-3-carbaldehyde using Vilsmeier-Haack Reagent

2.4.3 The Use of Vilsmeier-Haack Reaction in the Synthesis of Heterocycles

Recently, the use of Vilsmeier-Haack reagent has attracted attention in organic synthesis of several nitrogen and oxygen heterocycles. The application of the Vilsmeier-Haack reagent as a mild and efficient protocol for the formylation of diverse aromatic and heteroatom substrate has been reported (Pedras and Zaharia 2001; Venkanna *et al.* 2015). Hence it is employed as an efficient synthetic tool for the construction of a library of heterocyclic compounds (Beniwal and Jain 2015) . Vilsmeier reagent acts not only as a formylating agent (Tasneem 2003) but also as an activating reagent for carboxylic acid to give esters (Nagao *et al.* 2008), amides (Zaoral and Arnold 1960) and acid chlorides (Arnold and Holý 1962) and for

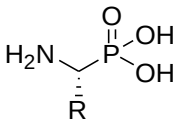
alcohols to give alkyl chlorides (Wong and Huang 2011), esters (Barrett *et al.* 1998), alkyl aryl sulphides (Mukaiyama and Ikegai 2004) and imides (Barrett *et al.* 1997).

In addition to aromatic formylation, a wide variety of carbonyl compounds (Kagan *et al.* 1996) with activated methyl and methylene groups react (Heck *et al.* 1985), efficiently with Vilsmeier-Haack reagent to yield the corresponding iminium salts. The intramolecular cyclisation ability of halomethyleniminium salts formed via Vilsmeier condition and microwave induced Vilsmeier conditions have been studied and reported (Nohara *et al.* 1973; Li *et al.* 2010), with a very recent study being chromonylthiazolidines (Anh *et al.* 2015) and pyrano[2g-c] chromenes.(Ali *et al.* 2012). Based on the above facts, it is clear that the reactive intermediate involved in Vilsmeier-Haack reaction are the halomethyleniminium salts which facilitate easy entry into a large number of novel heterocyclic system (Giles 1994; Chakradhar *et al.* 2009; Rajanna *et al.* 2012)

2.5 α-Amino Phosphonates and Phosphonic acids

α-amino phosphonates **12** (α**-**aminophosphonic acids and their derivatives) occupy a prominent position among the various compound containing the P-C bond and an amino group, because they are structural analogues of corresponding α**-**amino acids (**13)** (Hudson and Kukhar 2000) the building block of peptides and protein (Scheme15). Aminophosphonic acids were unknown until 1959 when Horiguchi and Kandatsu discovered 2-aminoethanephosphonic acid (**14)** (AEP ; “Ciliatine”) in Ciliated sheep rumen Protozoa (Kandatsu and Horiguchi 1962). AEP and aminophosphonates **(15) (**2 amino 3 phosphonopropionic acid) and (**16) (**2-Amino-1-hydroxy-ethyl)-phosphonic acid are found to be present in bacteria, protozoa, insects, vertebrates, and human tissue. Some naturally occurring

aminophosphonates possessing remarkable biological activity include anti-bacterial such as Fosmidomycin **(17),** Fosfomycin **(**1**8)** and related compounds found in Streptomyces (Kandatsu and Horiguchi 1962; Patel *et al.* 1997).



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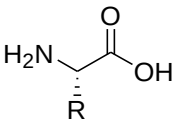

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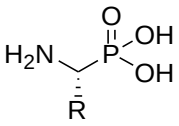

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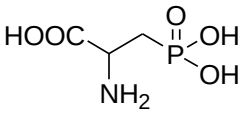

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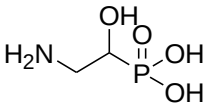

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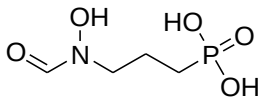
Scheme 15: General Structure of Aminophosphonates

α-amino phosphonates are classified according to the position of the amino group relative to the phosphonates or alkyl side-chain as α-amino phosphonates, β-amino phosphonates, ɣ-amino phosphonates (Troev 2006). Recently the synthesis of α-amino phosphonates has attracted increased attention as they display a variety of intriguing biological and pharmacological activities such as HIV protease antagonists, peptide mimics (Kafarski and Lejczak 1991), antibiotics, pharmacological agent(Abdelkader *et al.* 2015) , enzyme inhibitors (Ranu *et al.* 1999) and anti-tumour agents (Rao *et al.* 2008; Kraicheva *et al.* 2009). Also the α–aminophosphonic acids have been used as anti-cancer drugs and also as a

pesticide (Kafarski and Lejczak 2001). Their potential as peptidomimetics, (Arai *et al.* 1996) enzyme inhibitors including HIV protease (Kim and Rhie 1997), anti-viral agent (Shashikumar 2013) as well as their role in hapten design for anti-body generation has been reported (Chandrasekhar *et al.* 2001). Interest in this class of compounds is not restricted to the field of medicinal chemistry since they are also important in agrochemicals as insecticides, fungicides and herbicides (Yokomatsu *et al.* 1997) and also as plant growth regulators (Heydari *et al.* 2007) , For example glyphosate (amino methyl phosphonic acids) **(19)** is an herbicidal agent, alasphophaline (1-aminoethanephosphonic acids) **(20)** is a broad spectrum antibiotic agent and compounds such as rennin **(21)** and 2-aminoethyl phosphonic acid **(22)** are enzyme inhibitors (Sikora and Gajda 2000; Moonen *et al.* 2004).



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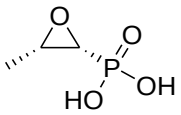

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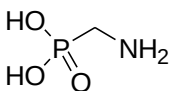

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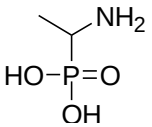

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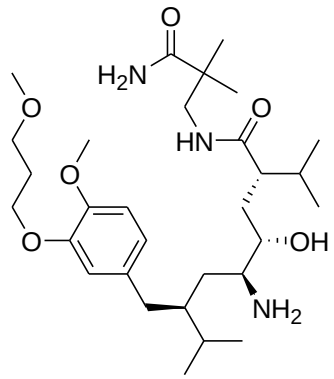

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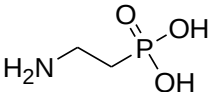

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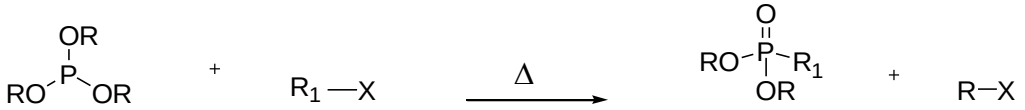
Scheme 16: Some Biologically Active Aminophosphonates

2.5.1. Methods Used for the synthesis of α-Amino phosphonates

Michaelis-Arbuzov reaction

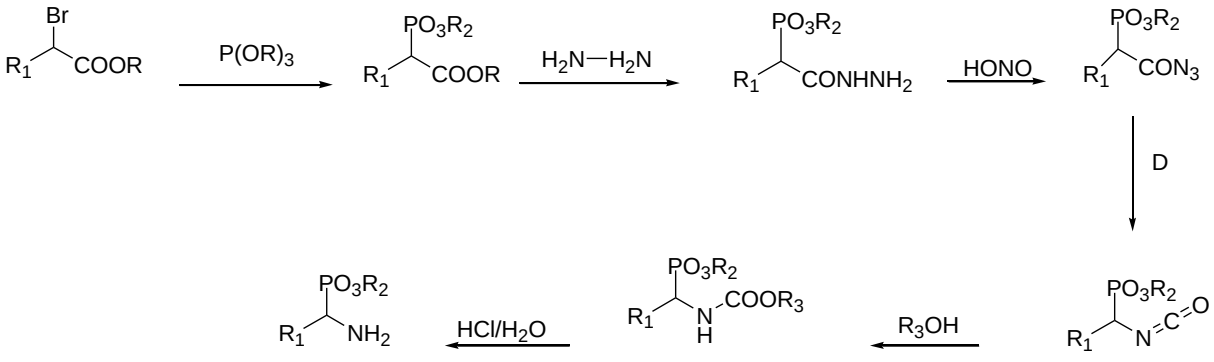
The Michaelis-Arbuzov (Bhattacharya and Thyagarajan 1981) rearrangement is a versatile method to form the P-C bond; it involves the reaction of an alkyl halide with a trialkylphosphite to yield the corresponding phosphonate (**Scheme 17**). This reaction is widely investigated and extensively used to prepare phosphonates, phosphinates and phosphine oxide.



Scheme 17: Synthesis of α-Aminophosphonates via the Michaelis-Arbuzov Reaction

Chambers and Isbell Reaction

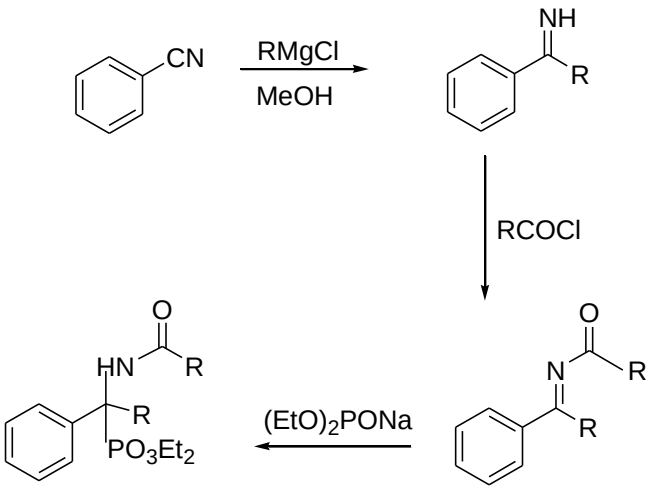
The reaction involves the use of 1-bromoalkanecarboxylates as starting material for the synthesis of APs via the Curtis rearrangement (Chambers and Isbell 1964) (**Scheme 18**).



Scheme 18: Synthesis of α-Aminophosphonates via the Curtis Rearrangement

Synthesis of α-Aminophosphonates by Treatment of Nitriles with Grignard reagent

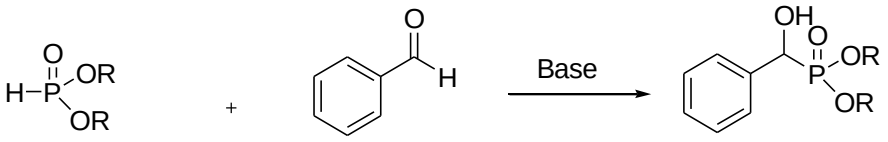
This reaction involves the addition of Grignard reagent with benzonitrile to form an imine, and this is then transformed to the corresponding phosphonate (Cristau *et al.* 1998) (**Scheme19**).



Scheme 19: Synthesis α-Aminophosphonates via Treatment of Nitriles with Grignard Reagents

Pudovik Reaction

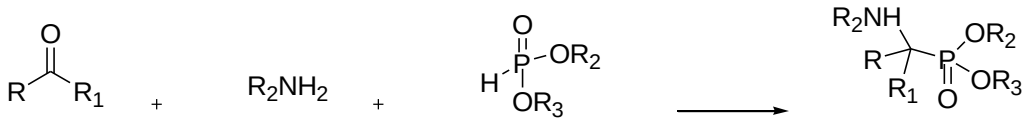
This involves the hydrophosphonylation of aldehydes with dialkyl phosphites under basic condition to obtain the corresponding α-hydroxyl phosphonates (Rai and Namboothiri 2008) (**Scheme 20**). Different Lewis acids such as $SnCl_2$, $SnCl_4$, $BF_3{\cdot}OEt_2$, $ZnCl_2$, $MgBr_2$, and $InCl_3$ (Paraskar and Sudalai 2006; Shashikumar 2013) have been employed for the synthesis of aminophosphonates via the Pudovik approach.



Scheme 20: Synthesis of α-hydroxyphosphonates via the Pudovik Reaction

Kabachnik-Fields Reaction

Kabachnik-Fields reaction is the most widely employed method to synthesize APs (**Scheme 21**).This involves an *in situ* formation of an imine by condensation of amines with an aldehyde or ketone followed by the hydrophosphonylation step (Simoni *et al.* 1998). The one pot Kabachnik-Field is promoted by acidic or basic catalysts. Lewis catalysts such as $ZnCl_2$, $MgBr_2$, $BF_3.OEt_2$ (Disale *et al.* 2012) are known to catalyse this reaction. However the methods suffer from drawbacks such as low yield, long reaction time and the reaction cannot be carried out in a single step.

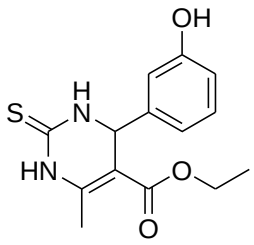


Scheme 21: Synthesis of α–aminophosphonates via the Kabachnik- Field reaction

2.6. Dihydropyrimidinones and their Biological Application

Dihydropyrimidinone (DHPMs) is the product of the multicomponent reaction involving the cyclo-condensation an aldehyde, β-keto-ester and urea. DHPMs also known as “Bignelli compounds” was first identified by Pietro Biginelli in 1893 and correctly characterised as substituted 3,4-dihydropyrimidine-2(1H)-one through a procedure known as the Bignelli reaction (Biginelli and Gazz 1893) . Dihydropyrimidinones belong to an important class of heterocyclic compounds with important pharmacological and biological properties such as antiviral, anti-tumour,

anti-bacterial and anti-hypertensive activities (Rodríguez-Domínguez *et al.* 2007). Dihydropyrimidinones and its derivative have also emerged as potential calcium channel blockers, neuropeptide antagonist, α_{1a}–adrenoceptor antagonists. The 2-oxo-dihydropyrimidine-5-carboxylate core moiety present in marine natural products, including the batzelladine alkaloids, are found to inhibit the binding of HIV-gp-120-CD_4 and could thus be a new development in AIDS therapy (Snider *et al.* 1996). The scope of this pharmacore has been increased by the identification of an anti-cancer agent, a 4-(3-hydroxyphenyl)-pyrimidinone-2-thione derivative known as Monastrol **(23)** (Maliga *et al.* 2002) whilst (R)-SQ 32926 **(24)** (Schnell *et al.* 2000) has been found to have potent anti-hypertensive activity; Felodipine (**25)** and **(26)** (Alajarin *et al.* 1994) is a potent mimic of dihydropyridine calcium channel blockers. The 4-aryl-1,4-dihydropyridines (DHPs) of the nifedipine type 4 (Kleidernigg and Kappe 1997) are still the most effective group of calcium channel modulators available for the treatment of cardiovascular diseases (Kappe 1998).



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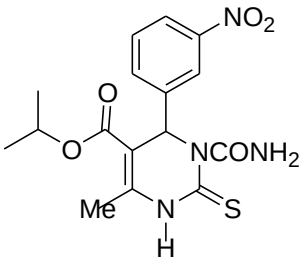

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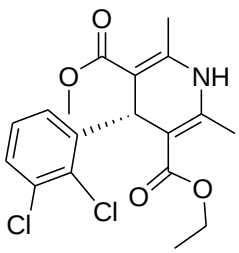

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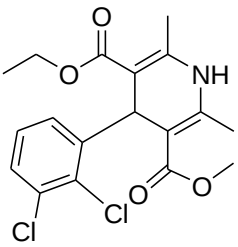

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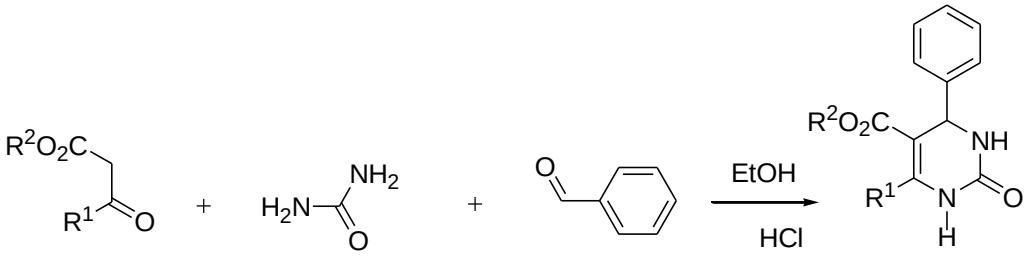
Scheme 22: Some Biologically Active Dihydropyrimidinones

2.6.1. Methods Used for the Synthesis of Dihydropyrimidinones

The most common synthetic approach to 3,4-dihydropyrimidinone is the cyclo-condensation of ethyl acetoacetate, aldehyde and urea in the presence of a strong acid (Kappe 2000b) (**Scheme 23**)



Scheme 23: The Classical Synthesis of Dihydropyrimidinones

Many synthetic methods including classical microwave and ultrasound irradiation and the use of catalyst such as $InCl_3$ (Phucho *et al.* 2009) lathanide triflate (Peng and Deng 2001), $BF_3.OEt_2$ (Kumar *et al.* 2001) has been reported.

2.7 Structure Elucidation

Spectroscopic methods are important requirement in modern chemistry for the identification of molecular structures. They are used in organic chemistry to determine and confirm molecular structures, monitor reactions and control the purity of compounds. The essential spectroscopic methods used in organic chemistry include the Nuclear Magnetic Resonance (NMR) Spectroscopy, Mass Spectrometry (MS), Infrared (IR) and UV/Vis-Spectroscopy (Kemp 1991)

2.7.1. IR Spectroscopy

Infrared Spectroscopy is based on the interaction of infrared light with a molecule (Vollhardt and Schore 2007). Molecules are formed from the combination of atoms which are continually vibrating around average positions. The continuous vibration

leads to changes in bond length and bond angles. An infrared spectrum is obtained by passing Infrared radiation through a sample and determining what part of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of vibration of a part of a molecule. The energy of an infrared photon can be calculated using the Planck energy relation (Colthup 2012);

$E=hv$, where $h= 6.6 \times 10^{-34}$ J.s and v is the frequency (s^{-1}) of the photon. This shows that high energy photons have high frequency. The frequency, *v* and the speed of light, c are related through the relation;

$c = \lambda v$, where $c = 3.0 \times 10^{8}$ m s^{-1} and λ= wavelength of the light (m)

Also a molecule will absorb infrared radiation when the vibration of the atoms in the molecule produces an oscillating electric field with the same frequency as the frequency of incident IR "light". This method measures the vibrations of atoms and hence the functional group in the molecule can be determine. Generally, the stronger the bonds in a molecule the higher the stretching frequency of vibration.(Colthup 2012)

2.7.2. Mass Spectrometry

Mass spectrometry is an analytical method used to identify the quantity and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions (Sparkman 2000).

The spectra are used to determine the elemental or isotopic-signature of a sample, the masses of particles and of molecules, and also to interpret the chemical structures of molecules. Mass Spectrometry technique works by ionizing chemical compounds to produce molecular fragments or charged molecules and measuring

their mass-to-charge ratios. The MS spectrum can be obtained by ionizing a sample of a molecule by bombarding it with electrons. This results in the fragmentation of the charged sample into ions. These ions are then separated according to their mass-to-charge ratio, usually by accelerating them and exposing them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection. The ions are identified by an instrument capable of identifying charged particles, such as an electron multiplier. Results are presented as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by comparing known masses to the identified masses or through a characteristic fragmentation pattern.

2.7.3. Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance Spectroscopy is the use of NMR phenomena to study chemical structure of molecules. NMR is a versatile and preferred analytical tool which offers a thorough analysis and elucidation of entire structure of a molecule. NMR requires only a small amount of samples for analysis which can be recovered hence it is referred to as a non-destructive analytical technique. In a simple NMR experiment information in the form of a spectrum is obtained which provide information about, the types of atoms present in the sample, the relative amounts of atoms present in a sample, the specific environments of atoms within a molecule, the purity and composition of a sample and structural information about a molecule, including constitutional and conformational isomerisation (Fuloria and Fuloria 2013) . Generally NMR spectroscopy can be classified as; one dimension (1D), two-dimension (2D) and three dimension (3D) NMR.

2.7.4 Two Dimensional Nuclear Magnetic Resonance spectroscopy (2D NMR)

In this NMR technique, data are plotted in a space defined by two frequency axes

rather than one. The two-dimensional NMR spectra give more information about a molecule where not available from a one-dimensional NMR spectra. They are particularly useful in interpreting the structure of complex molecule that are difficult to work with using one-dimensional NMR. The most useful and commonly used form of 2D NMR spectroscopy include: Homo-Nuclear Correlated Spectroscopy (COSY) which provides correlation between protons that are coupled to each other (Griesinger *et al.* 1987), Proton Detected Heteronuclear Multi-quantum Coherence (HMQC) used to correlate proton and carbon signals using either one bond or longer range couplings (Bax *et al.* 1990).

Heteronuclear Multi-Bond Connectivity (HMBC); similar to HMQC but optimized to detect proton-carbon correlation over 2 and/or 3 bonds (Lerner and Bax 1986).

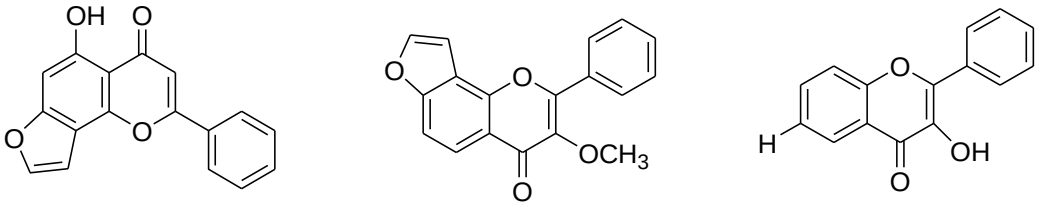
2.8 Biological Activity of Chromones

Chromones have been subjected to extensive studies in medicinal chemistry because of their interesting biological properties as well as other applications such as preparation of fluorescence probes as a result of their photochemical properties (Kim *et al.* 2011). Chromones exhibit diverse biological activities, including the following.

2.8.1 Antimicrobial activity

Over the years, chromones have been recognized as well-known naturally occurring oxygen heterocyclic compounds extracted from various plants. They constitute a core fragment in several flavonoids, such as flavones, flavonols and isoflavones (Rackova *et al.* 2005). Compounds such as Pongaglabol **27** have been reported to exhibit potency against the bacteria S*higella dysenteriae, Streptococcus β-haemolyticus*, and *Staphylococcus aureus* (Simin *et al.* 2002). Compound karangin

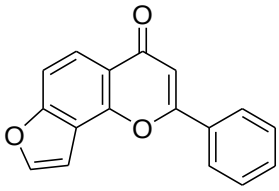
28 in mixture with extracts from the P*ongamia pinnata* plant have also been shown to exhibit antibacterial activity (Magalhães *et al.* 2007). Furthermore extracts of flavonoids from *Lonchocarpus montanus* plants were reported to exhibit activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Cladosporium cladosporioides.* Recently Gharpure, et al (2012) synthesized and evaluated 3-hydroxy-2-phenyl-4H-chromen-4-one **29** as a potent antibacterial agent against *Staphylococcus aureus*, *Bacillus subtilis*, *E. Coli* and *P. Aerugenosa* *(Gharpure et al. 2012)* .



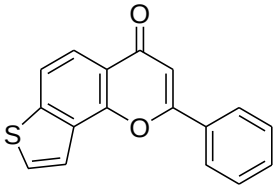
27 **28** **29**

Scheme 24: Some Chromone Compounds with Antimicrobial Activity

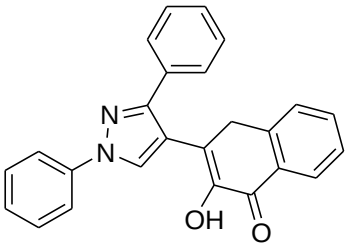
Compound lanceolatin B **30** exhibits antifungal activity against the fungi *Erysiphe polygoni* and *Ustilago tritici (Yadav et al. 2005).* The flavone **31** has been shown to exclusively exhibit potent antifungal activity whereas its chalcone analogue exhibited both fungicidal and antibacterial activity (Rackova *et al.* 2005). 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones **32** have also been synthesized and evaluated for their antifungal activity against three phytopathogenic fungi, namely *Helminthosporium* species, *Fusarium oxysporum* and *Alternaria alternate,* the compounds were reported to exhibit significantly higher anti-fungal activity than the commercial anti-fungal compound Actidione (cycloheximide) against all three phytopathogenic fungi (Prakash *et al.* 2008).



30



31



32

Scheme 25: Some other Chromone Compounds with Antimicrobial Activity

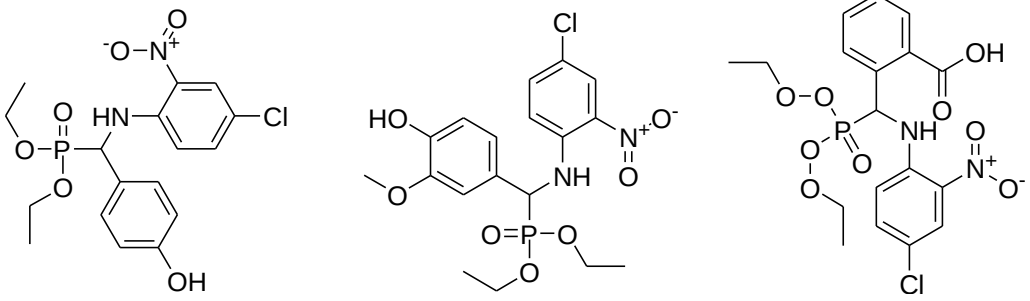
2.9 Biological Activity of α-Aminophosphonates

α-aminophosphonates isolated from various natural sources either as free amino acids or as constituents of more complex molecules have been reported (Seidel *et al.* 1988) . Incorporation of a chromone functionality into the α-aminophosphonate moiety may enhance the biological properties. However only few such compounds are known (Boduszek *et al.* 1998; Khidre *et al.* 1998).

α-aminophosphonates exhibit a variety of biological activity including:

2.9.1 Antimicrobial Activity

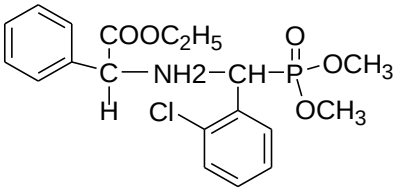
α-aminophosphonates and their derivatives synthesized through the three component condensation involving an aromatic aldehyde, amines and diethyl phosphite in the presence of catalytic amount of ethyl ammonium nitrate ionic liquid have been reported. These compounds were analysed and evaluated for their antibacterial activity against pathogenic bacteria *Escherichia coli*, *Staphylococcus aureus*, Salmonella typhi, Pseudomonas putida and Proteus vulgaris. The synthesized compounds (**33**-**35**) were reported to show high activity against all pathogens and also inhibit the growth of all the bacteria used in the study (Dake *et al.* 2011).



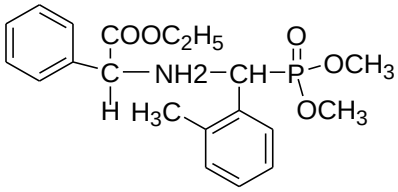
33 **34** **35**

Scheme 26: Some α-Aminophosphonates with Antimicrobial Activity

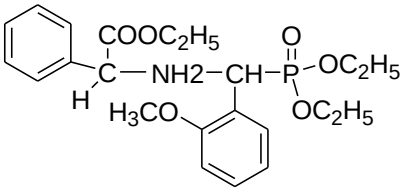
A series of aminophosphonates containing chitosan were synthesized from reactions of chitosan with triphenylphosphite and aromatic aldehyde in the presence catalytic amount of lithium perchlorate. These compounds were assessed for their *invitro* antibacterial and antifungal activity against *Escherichia coli*, *Serratia marcescens*, *Enterobacter cloacae*, *Shigella dysenteriae*, *Salmonella enterica* and *Proteus vulgaris* as Gram-negative bacteria, *Bacillus subtilis* and *Staphylococcus aureus* as Gram-positive bacteria and *Candida albicans* as a fungus. The inhibition zones were measured in triplicates and the results of antimicrobial assays showed that all compounds have high activities against bacteria and fungi (Kenawy *et al.* 2015). The synthesis of aminophosphonates **36-39** have been reported (Reddy *et al.* 2008). The antimicrobial were evaluated and they exhibited significant activity.



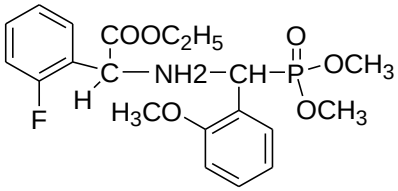
36



37



38



3

Scheme 27: Some other α-Aminophosphonates with Antimicrobial Activity

2.10 Safety of compounds

Although synthetic compounds may exhibit diverse pharmacological properties, they may also be toxic or mutagenic. In order to assess the safety of compounds, there are different toxicity and cytotoxicity tests available. The Ames Salmonella mutagenicity test is a biological test used to evaluate the mutagenic potential of chemical compounds (Mortelmans and Zeiger 2000). The assay serves as a rapid and useful test to evaluate the carcinogenic ability of a compound relative to standard carcinogenic assays on animals which takes time and are costly. A positive test shows that the compound is mutagenic and may be used as carcinogen. However false positive and negative results have been reported and mutagens identified in the Ames test may not be carcinogenic and requires more tests (Swarnalatha *et al.* 2014). Also the brine shrimp lethality assay is assumed to be a useful method for preliminary evaluation of toxicity of heavy metals, pesticides, and medicine especially plant extract (Price *et al.* 1974; Sorgeloos *et al.* 1978) . This Lethality assay was first proposed by Michael *et al* (Michael *et al.* 1956) and later

developed by other scientist (Sleet and Brendel 1983). Additionally it is a functional tool for the throughput cytotoxicity test of bioactive chemicals and screening pharmacological activities in plant extracts. The method is appealing because it is simple, inexpensive and require small amount of sample (Krishnaraju *et al.* 2005). It is based on the ability of compounds to be tested to kill a simple zoological organism-brine shrimp (*Artemia salina*) (Harwig and Scott 1971). The identification of substances capable of investigating mutations has come to be a vital procedure in risk assessment. Carcinogenic and mutagenicity of chemicals could be modulated by supplementary chemicals. It is well understood that ingredients in dietary and supplementary plants, fruits and seeds can exert anti-carcinogenic and anti-mutagenic results (Hayatsu *et al.* 1988; Hartman and Shankel 1990).

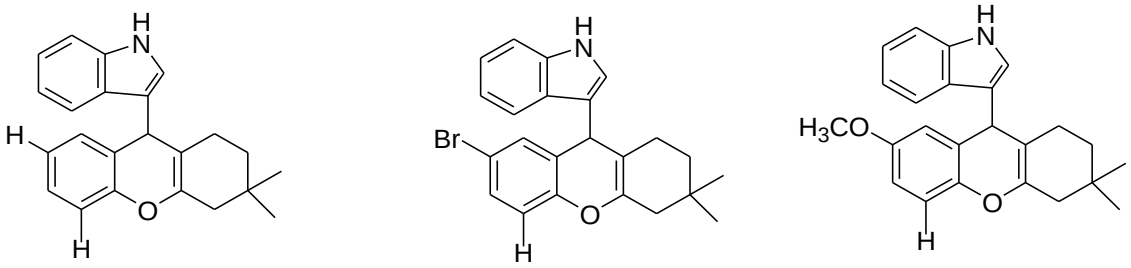
2.11 Molecular docking

Molecular docking has emerged as a key tool for computer assisted drug design against several disease targets. The aim of molecular docking is to give a prediction of a predominant binding modes of a ligand with a protein using computational methods (Meng *et al.* 2011). Molecular docking help to describe the molecular interactions of drug target with biologically important compounds and they are used to perform virtual screening of large libraries of compounds, rank the results and propose the structural hypothesis of how ligands inhibit the target- which is highly important in hit identification and lead optimisation (Klebe 2006; F Sousa *et al.* 2010).

2.11.1 Molecular docking studies on chromones

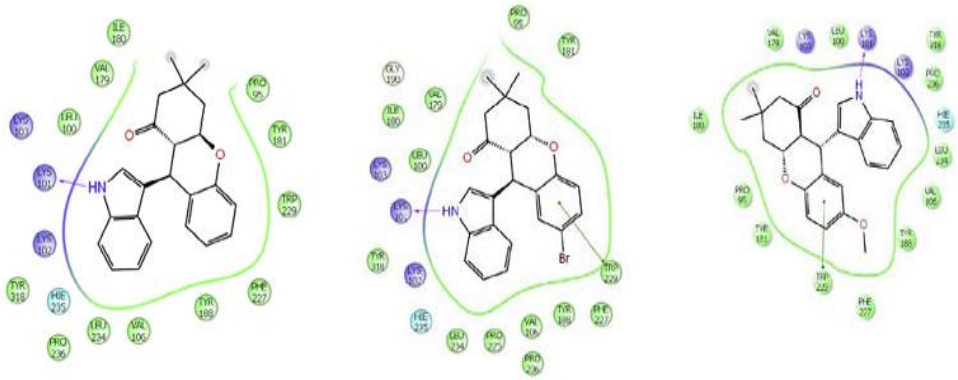
A series of oxochromenyl xanthenone and indolyl xanthenone derivatives synthesized through the three component reaction of substituted salicyaldehyde, 4-

hydroxy coumarin/indole and dimedone at ambient temperature in the presence of ionic liquid [Hmim]HSO_4 in ethanol have been reported (Kasralikar *et al.* 2015). These compounds were studied for their molecular docking as an anti- HIV-1 reverse transcriptase. The synthesized compounds **(40-42)** were reported to show potent reverse transcriptase inhibition activity.



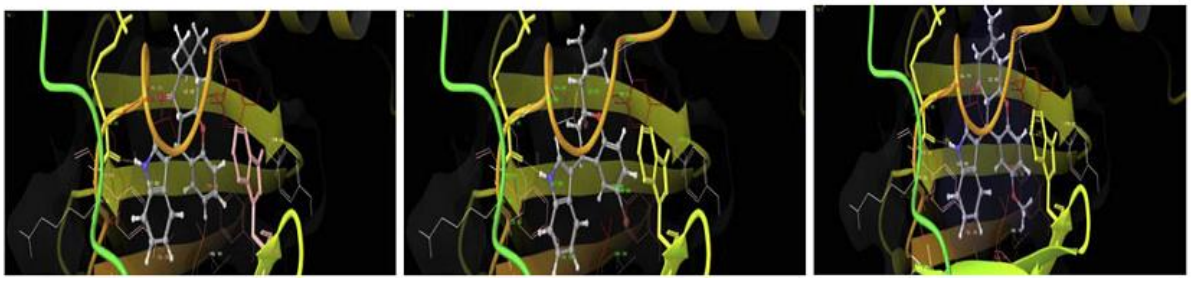
40 **41** **42**

Scheme 28: Some Chromone compounds with anti- HIV-1 reverse transcriptase activity



40 **41** **42**

Figure 2: 2D interaction map of chromone compounds with HIV-1 reverse transcriptase obtained from the pose of molecular docking



40 **41** **42**

Figure 3: 3D interaction map of chromone compounds with HIV-1 reverse transcriptase obtained from the pose of molecular docking

CHAPTER THREE

EXPERIMENTAL

3.1 General

The NMR spectra were recorded on either a BRUKER 400 MHz or 600 MHz spectrometer using TMS as an internal reference. The chemical shifts were expressed in parts per million (ppm). The IR spectra were recorded on Varian Scimitar 1000 FT-IR using KBr pellets and the absorption frequencies are expressed in reciprocal centimeters (cm^{-1}). Melting point was determined by using a Stuart SMP 10 melting point apparatus The DSC-TGA analysis was conducted with a TA Instruments. The X-ray diffraction analysis was conducted with a Philips PW 1050 diffractometer set at 1º per minute with a scanning step size of 0.02º from 40º to 100º 2Θ using monochromated CoK_{α} radiation. Data were captured with a Sietonics 122D automated microprocessor linked to the diffractometer. A Carl Zeiss Ultra Plus scanning electron microscope with EDX detector was used. Mass spectrometry were recorded using Waters Micromass LCT Premier TOF-MS. Biological studies were done using a laminar flow cabinet (Scientific Engineering, INC), Mueller Hinton Agar plates (Fluka, Biochemika), bacterial agar plates and Neubauer counting chamber.

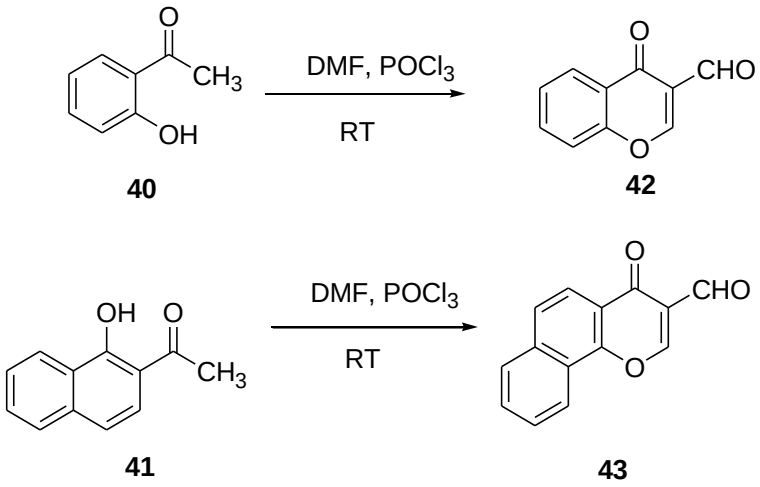
3.2 Preparation of Rhodium Loaded on Boron Nitride Catalyst

To a solution of $Rh(OAc)_2$ (55.24 mg, including 19.28 mg of Rh metal: 0.5wt % Rh metal vs BN) in methanol (50 ml), boron nitride (2.66 g, 0.1 mol) was added and the suspension was stirred at room temperature for 7 days under nitrogen atmosphere. The resulting suspension was filtered and the solid was washed with aqueous methanol and dried under reduced pressure to give 0.3% RhBNT catalyst as a pale

blue powder of mass 2.55 g (92 % yield).

3.3 Typical Procedure for the Synthesis of Starting Substrates

3.3.1 The reaction scheme for the Vilsmeier Haack reaction for the synthesis of starting substrates is presented below



Scheme 29: The Vilsmeier- Haack Reaction for the synthesis of 3-formyl chromone compounds

3.3.2 Synthesis of 4-oxo-chromene-3-carbaldehyde (42)

The Vilsmeier- Haack reagent was prepared by reacting DMF (28 ml) and $POCl_3$ (34 ml) in an ice bath. An accurate mass of 2-hydroxyacetophenone (8.42 g) was added slowly and the mixture was allowed to stir overnight on an oil bath at room temperature. On completion of the reaction, monitored by TLC, a yellowish precipitate was obtained. Compound **(42)** was purified by column chromatography using an eluting solvent system of petroleum ether and ethyl acetate (7:3). The yield was 85%, m.p 142°C.

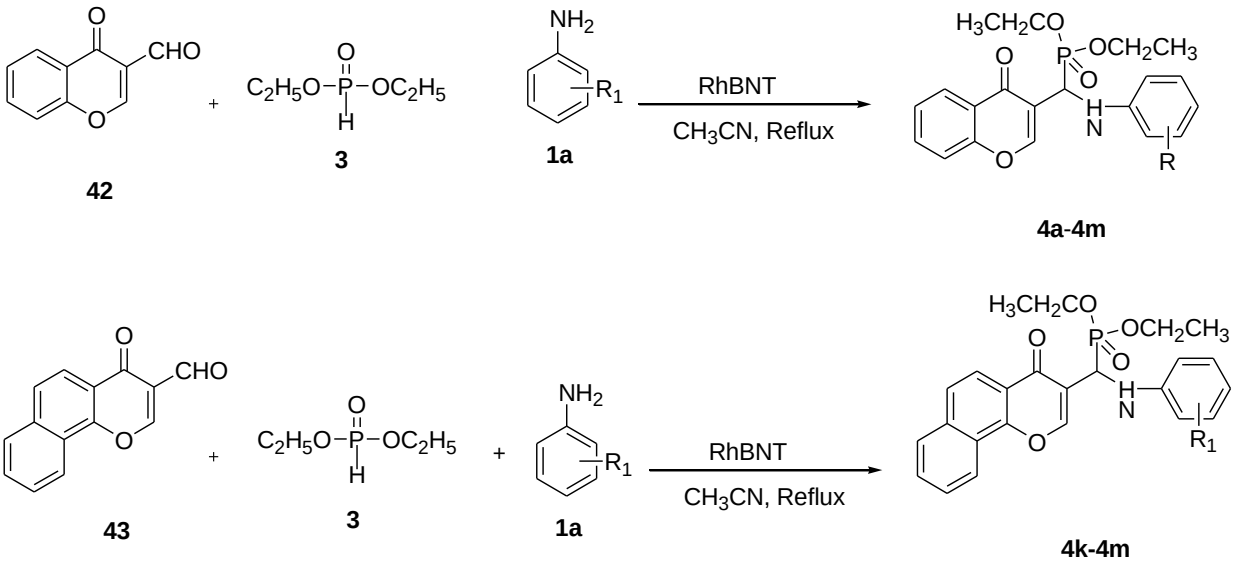
3.3.3 Synthesis of 4-oxo-4H-benzo[h]chromene-3-carbaldehyde (43)

Vilsmeier-Haack reagent was prepared by reacting DMF (28 ml) and $POCl_3$ (34 ml) in an ice bath. An accurate mass 1'-hydroxy-2'-acetonaphthone (13.20 g) was added

and allowed to stir overnight on oil bath at ambient temperature. On completion of the reaction, monitored by TLC, a yellowish precipitate was obtained. Compound (**43)** was purified by column chromatography using an eluting solvent system of petroleum ether and ethyl acetate (8:2). The yield was 82% yield, m.p 148 °C.

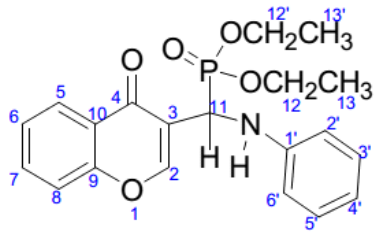
3.4 Synthesis of chromone bearing α-amino phosphonates (4a-4m)

In a 50 ml round bottom flask, a mixture containing the aldehyde **42** or **43** (1.2 mmol), amine **1a-1m** (1.2 mmol), diethyl phosphite **3** (1.2 mmol) and RhBNT (0. 1g) in acetonitrile (6 ml) was refluxed on an oil bath (**Scheme 30**). The progress of the reaction was monitored by TLC. After completion of the reaction, excess water was added and the organic compound was separated with ethyl acetate (3 × 10 ml) and dried over anhydrous Na_2SO_4. The catalyst was recovered by filtration. Evaporation of the solvent followed by purification on silica gel (ethyl acetate: petroleum ether, 90 %) afforded pure α-amino phosphonates (Table 1, entry 4a-4m page 68-70). The products were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR and Mass spectrometry.



Scheme 30: Synthesis of Chromone Bearing α-Aminophosphonates

4a: (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{20}H_{22}NO_5P$ (387.12g mol^{-1})

Yield: 92%

Melting points: 143-145°C

FTIR: 3239.04 (N-H), 2981.86 (C-H), 1644.33 (C=O), 1465.99 (Ar C=C), 1219.97 (P=O), 1021.31 (P-C-O), 746.47 (P-C aliphatic)

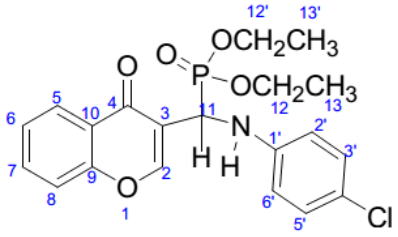
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.22 (d, 1H, J=6.20 H-5), 8.13(s,1H, J=3.36, H-2), 7.64 (dd,1H, J=7.96; 1.28, H-6), 7.40 (dd,1H, J=6.76; 0.72, t, 1H, J=7.20, H-7/8), 7.11 (d, 2H, J=15.04, H-2'/6'), 6.68 (t,1H, J=14.52, H-4'), 6.57 (d, 2H, J=7.76, H-3'/5'), 5.36 (d, 1 H,J=23.68, H-11), 4.22 (dd, 2H, J=3.68, H-12'), 4.07 (dd, 2H, J=0.76, H-12), 1.30 (dd,3H, J=7.08, H-13'), 1.16(dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.31(C-4), 156.26 (C-9), 155.19 (C-2), 145.52 (C-1'), 133.87 (C-7), 129.37 (C-2'/6'), 125.93 (C-5), 125.43 (C-6), 123.42(C-10), 120.23 (C-4') 118.27 (C-8), 113.66 (C-3'/5'), 63.80 (C-12'), 63.45 (C-12), 46.27 (C-11), 16.42 (C-13), 16.27 (C-13')

^{31}P- NMR**:** (400 MHz, $CDCl_3$): δ 22.45

MS-TOF (Na): m/z 410.1141 $[M+Na]^+$

4b: (4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester



Physical description: Pale yellow solid

Molecular formula: $C_{20}H_{21}ClNO_5P$ (421.81 mol^{-1})

Yield: 94%

Melting points: 148-150°C

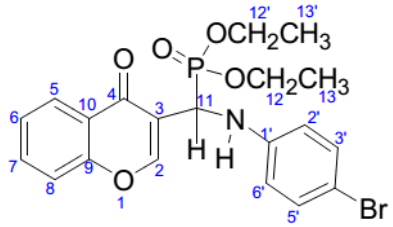
FTIR: 3296.77 (N-H), 2989.06 (C-H), 1638.56 (C=O), 1594.64 (Ar, C=C), 1220.72 (P=O), 1022.46 (P-C-O), 758.89 (P-C aliphatic)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=8.48, H-5), 8.14(s,1H, J=3.52, H-2), 7.63 (dd,1H, J=8.36; 1.36, H-6), 7.43 (dd,1H, J=8.48, t, 1H, J=6.08, H-7/8), 7.06 (d, 2H, J=8.76, H-2'/6') 6.59 (d, 2H, J=8.76, H-3'/5'), 5.30 (d, 1 H,J=23.64, H-11), 4.25 (dd, 2H, J=7.32, H-12'), 4.11 (dd, 2H, J=7.36, H-12), 1.30 (dd,3H, J=7.00, H-13'), 1.16(dt, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.27 (C-4), 156.26 (C-9), 155.21 (C-2), 144.21 (C-1'), 132.17. (C-7), 129.52 (C-2'/6'), 125.74 (C-5), 125.45 (C-6), 122.05 (C-10), 119.94 (C-4') 118.30 (C-8), 117.69 (C-3'/5'), 63.89 (C-12'), 63.53 (C-12), 45.33 (C-11), 16.42 (C-13), 16.26 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.0832.

4c: (4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester



Physical description: Pale yellow solid

Molecular formula: $C_{20}H_{21}BrNO_5P$ (466.26gmol^{-1})

Yield: 93%

Melting points: 146-148°C

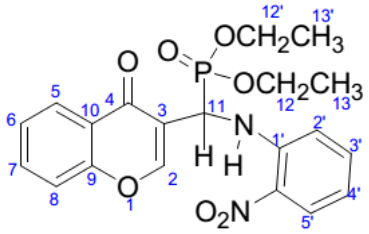
FTIR: 3279.00 (N-H), 2988.10 (C-H), 1634.77(C=O), 1515.26 (Ar, C=C), 1217.55 (P=O), 1011.97 (P-C-O), 755.08 (P-C aliphatic)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=8.48, H-5), 8.09 (s,1H, J=3.36, H-2), 7.64 (dd,1H, J=7.06, H-6), 7.42 (dt,1H, J=7.32; 4.76, t, 1H, J=14.00, H-7/8), 7.02 (d, 2H, J=8.72, H-2'/6'), 6.65 (d, 2H, J=8.76, H-3'/5'), 5.30 (d, 1 H,J=23.68, H-11), 4.23 (dd, 2H, J=3.48, H-12'), 4.09(dd, 2H, J=7.20, H-12), 1.31(dd,3H, J=7.56, H-13'), 1.16(dt, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.26 (C-4), 156.25 (C-9), 155.23 (C-2), 144.69 (C-1'), 133.99 (C-7), 132.08 (C-2'/6'), 125.93 (C-5), 125.55 (C-6), 123.34(C-10), 119.91 (C-4') 118.31 (C-8), 115.31 (C-3'/5'), 63.92 (C-12'), 63.56 (C-12), 45.19 (C-11), 16.42 (C-13), 16.26 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.0249.

4d: (4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{20}H_{21}N_2O_7P$ (432.11gmol^{-1})

Yield: 90%

Melting points: 160-162°C

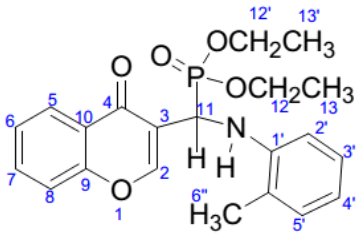
FTIR: 3279.88 (N-H), 2982.45 (C-H), 1636.02 (C=O),1514.21 (Ar, C=C),1220.96 (P=O), 1015.12 (P-C-O), 751.94 (P-C aliphatic)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=7.92, H-5), 8.04(s,1H, J=3.64, H-2), 7.68 (dd,1H, J=8.00; 7.28, H-6), 7.44 (dd,1H, J=11.60; 7.84, t, 1H, J=8.36, H-7/8), 6.66 (d, 2H, J=2.88, H-2'), 6.64 (d, 2H, J=2.84, H-3'/5'), 5.35 (d, 1 H,J=23.12, H-11), 4.21 (dd, 2H, J=4.40, H-12'), 4.09(dd, 2H, J=1.56, H-12), 1.31(dd,3H, J=7..08, H-13'), 1.23 (dd, 3H, J=7.08 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.09 (C-4), 156.26 (C-9), 155.21 (C-2), 151.22 (C-1'), 139.42 (C-7), 134.28 (C-2'/6'), 126.25 (C-5), 125.99 (C-6), 125.81 (C-10), 123.30 (C-4') 119.47 (C-8), 118.35 (C-3'/5'), 63.99 (C-12'), 63.86 (C-12), 45.05 (C-11), 29.69 16.43 (C-13), 16.29 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 21.054

4e: (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester



Physical description: Dark brown solid

Molecular formula: $C_{21}H_{24}NO_5P$ (401.39 $gmol^{-1}$)

Yield: 94%

Melting points: 138-140°C

FTIR: 3282.71(N-H), 2979.29 (C-H), 1637.75 (C=O), 1513.77 (Ar, C=C), 1222.26 (P=O), 1017.18 (P-C-O), 765.47 (P-C aliphatic)

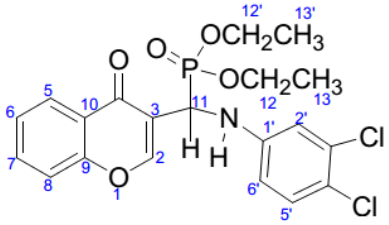
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=8.52, H-5), 8.04(s,1H, J=3.64, H-2), 7.68 (dd,1H, J=7.72; 1.76, H-6), 7.44 (dd,1H, J=8.24; 6.80, t, 1H, J=8.36, H-7/8),7.04 (d,2H,J=8.00,H-2'), 6.68 (d, 2H, J=7.36, H-4'), 6.54(d, 2H, J=8.00, H-3'/5'), 5.35 (d,1 H,J=23.56, H-11), 4.21 (dd, 2H, J=4.52, H-12'), 4.09 (dd, 2H, J=2.00, H-12), 2.19 (s,3H,H-6'') 1.31 (dd,3H, J=7.04, H-13'), 1.29 (dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.34(C-4), 156.28 (C-9), 155.03 (C-2), 143.52 (C-1'), 131.68 (C-7), 128.20 (C-2'/6'), 127.30. (C-5), 125.69 (C-6), 123.45 (C-10), 122.95 (C-4') 120.18(C-8), 118.38 (C-3'/5'), 63.77 (C-12'), 63.49 (C-12), 45.37 (C-11), 23.37 (C-6'') 16.43 (C-13), 16.29 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ22.5997.

MS-TOF(Na): m/z 424.1298 $[M+Na]^+$

4f: (4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{20}H_{20}Cl_2NO_5P$ (456.26 $gmol^{-1}$)

Yield: 90%

Melting points: 154-156°C

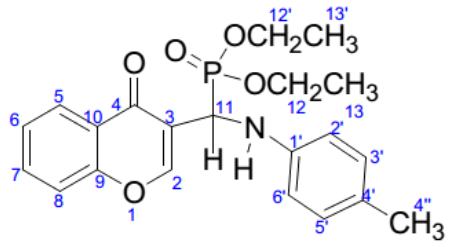
FTIR: 3280.98(N-H), 2987.67 (C-H), 1639.43 (C=O), 1521.08 (Ar, C=C), 1225.38 (P=O), 1046.10 (P-C-O), 760.73 (P-C aliphatic);

^{1}H-NMR: (400 MHz, $CDCl_3$): 8.21 (d, 1H, J=8.28, H-5), 7.65(s,1H, J=3.20, H-2), 7.41 (dd,1H, J=4.84; 3.60, H-6), 7.12 (dd,1H, J=7.20; 2.04, t, 1H, J=14.36, H-7/8), 6.78 (d,2H,J=2.64,H-2'/6'), 6.49(dd, 1H, J=2.44, H-5'), 5.27 (d,1 H,J=23.52, H-11), 4.25 (dd, 2H, J=6.20, H-12'), 4.19 (dd, 2H, J=5.48, H-12),1.31 (ddt,3H, J=7.00, H-13'), 1.29 (dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.30 (C-4), 156.25 (C-9), 155.31 (C-2), 145.48 (C-1'), 134.04 (C-7), 132.87 (C-2'/6'), 125.78 (C-5), 123.34 (C-6), 121.32 (C-10), 119.78 (C-4') 118.30 (C-8), 115.40 (C-3'/5'), 63.94(C-12'), 63.62 (C-12), 45.25 (C-11), 16.43 (C-13), 16.28 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 21.7615.

4g: (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{21}H_{24}NO_5P$ (401.39 $gmol^{-1}$)

Yield: 96%

Melting points: 142-144°C

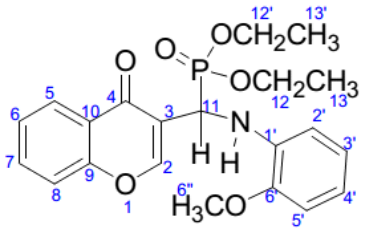
FTIR: 3279.33 (N-H), 2983.17 (C-H), 1637.52(C=O), 1515.01 (Ar C=C), 1221.79 (P=O), 1015.64 (P-C-O), 766.19 (P-C aliphatic)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=8.44, H-5), 8.10(s,1H, J=3.52, H-2), 7.64 (dd,1H, J=8.32; 1.48, H-6), 7.40 (dd,1H, J=8.44; 6.44, t, 1H, J=5.24, H-7/8), 6.92 (d, 2H, J=8.32, H-2'/6'), 6.57 (d, 2H, J=8.32, H-3'/5')5.32 (d, 1 H,J=23.64, H-11), 4.25 (dd, 2H, J=1.96, H-12'), 4.07 (dd, 2H, J=1.96, H-12), 2.17 (s,3H,H-4'') 1.30 (dd,3H, J=7.04, H-13'), 1.16(dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.30(C-4), 156.26 (C-9), 155.18 (C-2), 133.82 (C-1'), 129.86 (C-7), 128.20 (C-2'/6'), 125.94 (C-5), 125.39 (C-6), 123.44 (C-10), 120.22 (C-4') 118.25 (C-8), 113.86 (C-3'/5'), 63.81 (C-12'), 63.40 (C-12), 46.27 (C-11), 20.35(C-4'') 16.40 (C-13), 16.26 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.5708.

4h: (4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester



Physical description: Dark Brown solid

Molecular formula: $C_{21}H_{24}NO_6P$ (417.39 $gmol^{-1}$)

Yield: 95%

Melting points: 153-155°C

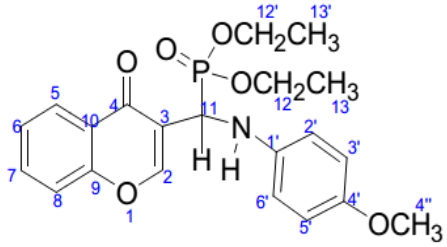
FTIR: 3278.75 (N-H), 2927.19 (C-H), 1626.19 (C=O), 1572.49 (Ar, C=C), 1219.72 (P=O), 1093.89 (P-C-O), 732.72 (P-C aliphatic).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.24 (d, 1H, J=8.52, H-5), 8.10 (s,1H, J=3.52, H-2), 7.63 (dd,1H, J=14.28; 6.92, H-6), 7.41 (dd,1H, J=14.52; 8.12, t, 1H, J=8.72, H-7/8),6.73 (d,2H,J=7.24, H-2'), 6.65 (d, 2H, J=14.08, H-4'), 6.57(d, 2H, J=8.76, H-3'/5'), 5.32 (d,1 H,J=23.43, H-11), 4.22(dd, 2H, J=2.72, H-12'), 4.10 (dd, 2H, J=1.88, H-12), 3.85 (s,3H,H-6'') 1.31 (dd,3H, J=7.00, H-13'), 1.28 (dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.26 (C-4), 156.28 (C-9), 155.28 (C-2), 147.30 (C-1'), 135.35 (C-7), 133.78 (C-2'/6'), 125.86 (C-5), 123.43 (C-6), 120.20 (C-10), 120.48 (C-4') 118.16(C-8), 110.99 (C-3'/5'), 55.46 (C-6''), 63.81 (C-12'), 63.37 (C-12), 45.56 (C-11), 16.40(C-13), 16.27 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.4993.

4i: (4-oxo-4H-chromen-3-yl)-(4-methoxyphenylamino) methanephosphonic acid diethyl ester



Physical description: Dark brown solid

Molecular formula: $C_{21}H_{24}NO_6P$ (417.39gmol^{-1})

Yield: 91%

Melting points: 157-159°C

FTIR: 3278.75 (N-H), 2927.19 (C-H), 1626.19 (C=O), 1572.49 (Ar, C=C), 1219.72 (P=O), 1093.89 (P-C-O), 732.72 (P-C aliphatic).

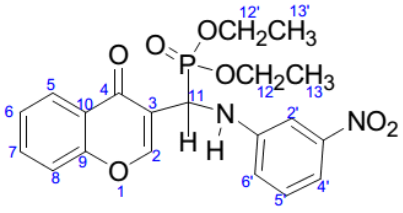
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.20 (d, 1H, J=8.60, H-5), 8.13(s,1H, J=3.40, H-2), 7.62 (dd,1H, J=8.20; 1.20, H-6), 7.39 (dd,1H, J=11.00; 2.36, t, 1H, J=8.48, H-7/8), 6.85 (d, 2H, J=8.88, H-2'/6'), 6.67 (d, 2H, J=8.88, H-3'/5') 5.30 (d, 1 H,J=23.63, H-11), 4.21(dd, 2H, J=3.44, H-12'), 4.19 (dd, 2H, J=8.16, H-12), 3.65 (s,3H,H-4'') 1.31(dd,3H, J=6.72, H-13'),

1.29(dd, 3H, J=7.04,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.34 (C-4), 156.24 (C-9), 155.27 (C-2), 152.98 (C-1'), 139.51 (C-7), 133.83 (C-2'/6'), 125.92 (C-5), 125.39 (C-6), 123.43 (C-10), 120.25 (C-4') 118.21 C-8), 115.04 (C-3'/5'), 55.46 (C-4''), 63.79 (C-12'), 63.33 (C-12), 46.05 (C-11), 16.42(C-13), 16.28(C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.4993.

4j: (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: : $C_{20}H_{21}N_2O_7P$ (432.11gmol^{-1})

Yield: 90%

Melting points: 162-164°C

FTIR: 3285.17 (N-H), 2982.45 (C-H), 1636.02 (C=O), 1541.01 (Ar, C=C), 1220.96 (P=O), 1015.12 (P-C-O), 751.94 (P-C aliphatic)

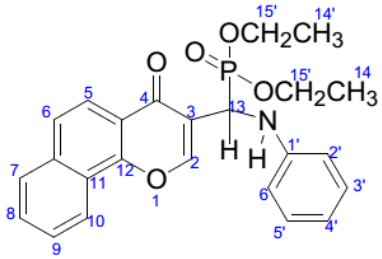
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.23 (d, 1H, J=8.76 H-5), 8.18 (s,1H, J=3.28, H-2), 7.62(dd,1H, J=8.48; 1.40, H-6), 7.45 (dd,1H, J=8.08; 1.40, t,1H, J=1.36, H-7/8), 7.15(d, 2H, J=0.92, H-2'/6'), 6.90 (t,1H, J=1.76, H-4'), 6.88(d, 2H, J=1.80, H-5'), 5.37 (d, 1 H,J=23.48, H-11), 4.21 (dd, 2H, J=1.52, H-12'), 4.19 (dd, 2H, J=2.52, H-12), 1.26 (dd,3H, J=7.04, H-13'), 1.14(dd, 3H, J=7.04 ,H-13)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.14 (C-4), 156.24 (C-9), 155.49 (C-2), 149.24 (C-1'), 134.08 (C-7), 130.05 (C-2'/6'), 126.01 (C-5), 125.65 (C-6), 123.37 (C-10), 119.73 (C-4') 118.39 (C-8), 113.03 (C-3'/5'), 63.98 (C-12'), 63.73 (C-12), 45.17 (C-11), 16.38 (C-13), 16.28 (C-13')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 21.6820

MS-TOF (Na): m/z 455.0994 $[M+Na]^+$

4k: [(4-oxo-4H-benzo[*h*]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester



Physical description: Dark yellow solid

Molecular formula: $C_{24}H_{24}NO_5P$ (437.42gmol^{-1})

Yield: 84%

Melting points: 154-156°C

FTIR: 3253.96 (N-H), 2941.31 (C-H), 1618.51(C=O), 1516.19 (Ar, C=C), 1206.38 (P=O), 109313 (P-C-O), 754.39 (P-C aliphatic)

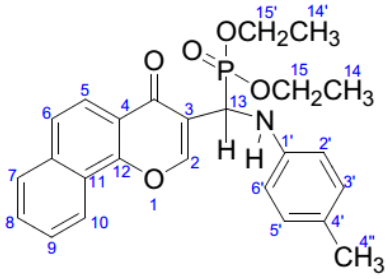
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.38 (d, 1H, J=8.12, H-10), 8.33 (s1H,J=3.52,H-2), 8.15 (d,1H, J=6.72, H-7), 7.86 (d,1H, J=7.96, H-6), 7.76 (d, 1H, J=8.76, H-5), 7.66 (t, 1H, J=14.16, H-9), 7.60 (d, 1H, J=14.64, H-8), 7.12 (t, 1H, J=1.76, H-2'/6') 6.71 (t, 1H,J=6.28,H-4'), 6.70 (d,1H, J=7.92, H-3'/5'), 5.45 (d, J=23.8 H-13), 4.25 (dd2H, J=7.36,H-15'), 4.10 (dd, 2H, J=7.92 ,H-15), 1.32 (dd,3H,J=7.04,H-14'), 1.18(dd,3H,J=14.12,H-14)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.09 (C-4), 154.33 (C-12), 153.83 (C-2), 145.55 (C-1'), 135.8265 (C-11) 129.44 (C-2'/6'), 125.64 (C-8), 123.92 (C-9), 122.25(C-10), 121.71(C-5), 120.72 (C-6), 118.83 (C-7), 119.26 (C-4'), 113.67 (C-3'/5'), 63.86 (C-15'), 63.54 (C-15), 45.27 (C-13), 16.44 (C-14), 16.29 (C-14')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.5069

MS-TOF(Na): m/z 460.1294 $[M+Na]^+$

4l: [(4-oxo-4H-benzo[*h*]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{24}H_{26}NO_5P$ (451.45gmol^{-1})

Yield: 91%

Melting points: 165-167°C

FTIR: 3284.10 (N-H), 2982.17(C-H), 1637.52 (C=O), 1515.01 (Ar, C=C), 1222.62 (P=O), 1099.74 (P-C-O), 764.99 (P-C aliphatic)

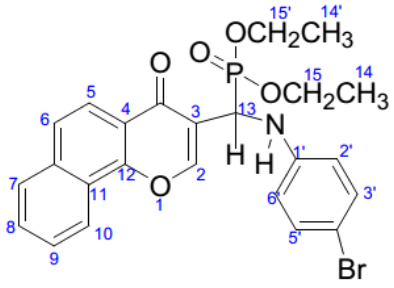
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.40 (d,1H, J= 8.20, H-10), 8.31 (s,1H,J=3.56, H-2), 8.15 (d,1H, J=3.52, H-7), 7.90 (d,1H, J=7.88, H-6), 7.88 (d, 1H, J=6.96, H-5), 7.77 (t, 3H, J=6.72, H-9), 7.63 (d, 1H, J=7.12, H-8), 6.91 (t, 1H, J=8.16, H-2'/6'), 6.61 (d,1H, J=7.92, H-3'/5'), 5.38 (d, J=23.64 H-13), 4.25 (dd,3H, J=6.88,H-15'), 4.10 (dd, 3H, J=8.00,H-15), 2.16 (s,4H, H-4''),1.32(dd, 5H,J=7.04,H-14'), 1.18 (dd,5H,J=7.04,H-14)

^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.15 (C-4), 155.72 (C-12), 154.71 (C-2), 143.15 (C-1'), 135.83 (C-11) 129.46 (C-2'/6'), 125.60 (C-8), 123.95 (C-9), 122.27(C-10), 121.76 (C-5), 120.75 (C-6) 119.70 (C-4'), 118.25 (C-7), 113.75 (C-3'/5'), 63.83 (C-15'), 63.44 (C-15), 45.52 (C-13), 20.34 (C-4''), 16.43 (C-14), 16.28 (C-14')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.5655.

MS-TOF (Na): m/z 474.1455 $[M+Na]^+$

4m: [(4-oxo-4H-benzo[*h*]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester



Physical description: Yellow solid

Molecular formula: $C_{24}H_{23}BrNO_5P$ (516.32g mol^{-1})

Yield: 92%

Melting points: 176-178°C

FTIR: 3283.96 (N-H), 2925.86(C-H), 1639.25 (C=O), 1514.89 (Ar C=C), 1223.38 (P=O), 1018.59 (P-C-O), 763.95 (P-C aliphatic).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.40 (d,1H, J= 8.16, H-10), 8.34 (s, 1H,J=3.48) 8.14 (d,1H, J=8.76, H-7), 7.91 (d,1H, J=8.00, H-6), 7.76 (d, 1H, J=8.76, H-5), 7.78 (t, 3H, J=14.2, H-9), 7.68 (d, 1H, J=14.40, H-8), 7.18 (t, 1H, J=8.72, H-2'/6'), 6.59 (d,1H, J=8.76, H-3'/5'), 5.38 (d, J=23.64 H-13), 4.24 (dd,2H,J=.7.36,H-15'), 4.11 (dd, 2H, J=7.92,H-15), 3.02 1.32(dd, 3H,J=7.08,H-14'), 1.16 (dt,3H,J=7.04,H-14)

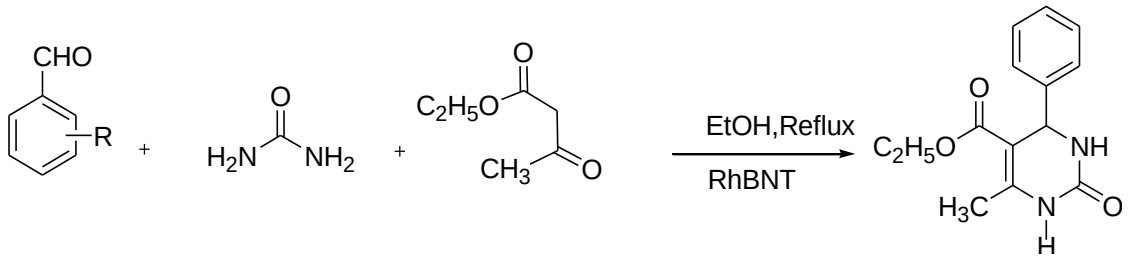
^{13}C-NMR: (400 MHz, $CDCl_3$): δ 176.05 (C-4), 154.32 (C-12), 153.88 (C-2), 144.62 (C-1'), 135.89 (C-11) 130.85 (C-2'/6'), 128.10 (C-8), 127.37 (C-9),125.78 (C-11),123.90 (C-10) 122.26 (C-5), 121.39 (C-6), 120.66 (C-4'), 119.65 (C-7), 115.32 (C-3'/5'), 63.93 (C-15'), 63.62 (C-15), 45.33 (C-13), 16.44 (C-14), 16.28 (C-14')

^{31}P- NMR: (400 MHz, $CDCl_3$): δ 22.0499

MS-TOF (Na): m/z 538.0394 $[M+Na]^+$

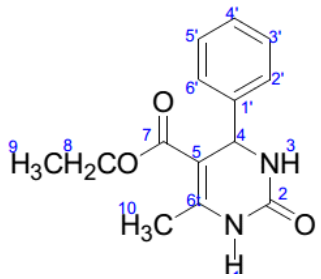
3.5 Synthesis of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester and derivatives (4n-4r)

In a 50 ml round bottom flask, a mixture containing an aldehyde (0.6mmol), β-keto-ester (0.6.mmol), urea (0.6mmol) and RhBNT (0.1g) in ethanol (6 ml) was refluxed on an oil bath. The progress of the reaction was monitored by TLC. After completion of the reaction, excess water was added and the organic layer was separated with ethyl acetate (3 × 10 ml) and dried over anhydrous Na_2SO_4. The catalyst was recovered by filtration. Evaporation of the solvent followed by purification on silica gel (eluent ethyl acetate: petroleum ether, 90 %) afforded pure dihydropyrimidinones (**4n-4r**). The products were characterized by IR, ^{1}H-NMR and Mass spectrometry.



Scheme 31: Synthesis of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

4n: 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester



Physical description: White solid

Molecular formula: $C_{14}H_{16}N_2O_3$ ($260.29 gmol^{-1}$)

Yield: 92%

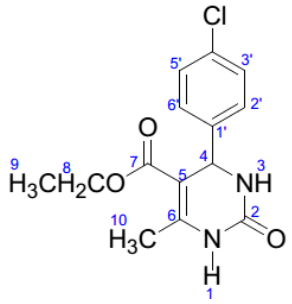
Melting points: 202-203°C

FTIR: 3242.91(N-H), 2981.16(C-H), 1724.05 (C=O).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.02 (s,H-1), 7.39 (d,2H, J=4.28, H-2'/6'), 7.26(dd,2H, J=1.72, H-3'/5'), 7.25 (d, 1H, J=1.92, H-4'), 5.79 (s, H-3), 5.39 (d, 1H, J=1.48, H-4), 4.01 (m, 2H, J=11.88;9.24, H-8), 2.34 (s,H-1), 1.17 (t,3H, J=7.08,H-9)

MS-TOF: m/z 261.1237

4o: 6-methyl-2-oxo-4-(4-chlorophenyl)-1,3,4-trihydro-pyrimidine-5-carboxylic acid ethyl ester



Physical description: Yellow solid

Molecular formula: $C_{14}H_{15}ClN_2O_3$ ($294.73 gmol^{-1}$)

Yield: 92%

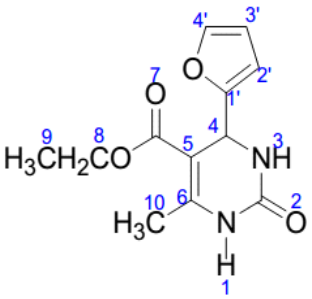
Melting points: 200-201°C

FTIR: 3236.08(N-H), 2956.97(C-H), 1699.66(C=O).

^{1}H-NMR: (400 MHz, $CDCl_3$): 7.96 (s,H-1), 7.22 (d,2H, J=4.16, H-2'/6'), 7.20(t,2H, J=1.32, H-3'/5'), 5.82 (s, H-3), 5.31 (d, 1H, J=1.44, H-4), 4.00 (dd, 2H, J=1.28;1.00, H-8), 2.32 (s,H-10), 1.10 (t,3H, J=7.08,H-9)

MS-TOF: m/z 261.1237

4p: 6-methyl-2-oxo-(4-furan-2-yl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester



Physical description: Yellow solid

Molecular formula: $C_{12}H_{14}N_2O_4$ ($250.25 gmol^{-1}$)

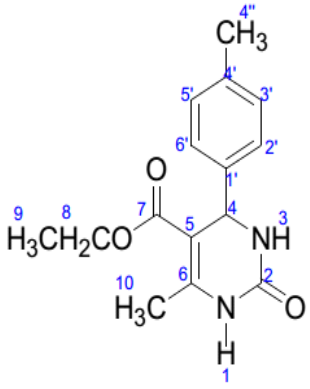
Yield: 92%

Melting points: 197-198°C

FTIR: 3278.20 (N-H), 2983.18(C-H), 1697.58 (C=O).

^{1}H-NMR: (400 MHz, $CDCl_3$): 8.26(s,H-1), 7.29(t,1H, J=8.56, H-3'), 6.81(d,1H, J=8.60, H-2'), 6.09 (d,1H, J=3.08, H-4') 5.95 (s, H-3), 5.46(d, 1H, J=2.52, H-4), 4.10 (dd, 2H, J=4.28;3.76, H-8), 2.27 (s,H-10), 1.18 (t,3H, J=7.12,H-9).

4q: 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester



Physical description: Yellow solid

Molecular formula: $C_{15}H_{18}N_2O_3$ (274.32gmol^{-1})

Yield: 92%

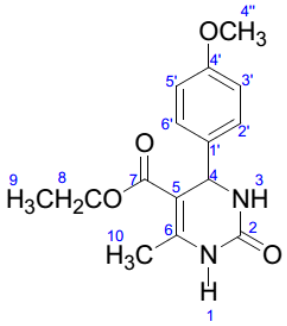
Melting points: 212-213°C

FTIR: 3240.95 (N-H), 2981.26 (C-H), 1701.48(C=O).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.31 (s,H-1), 7.18 (d,2H, J=7.92, H-2'/6'), 7.16(t,2H, J=7.84, H-3'/5'), 5.91 (s, H-3), 5.33(s,H-4), 4.03 (m, 2H, J=7.08;7.12 H-8), 2.30 (d,6H-10/4''), 1.14 (t,3H, J=7.12, H-9).

MS-TOF (Na): m/z 297.1220 $[M+Na]^+$

4r: 6-methyl-2-oxo-4-(4-methoxyphenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester



Physical description: White solid

Molecular formula: $C_{15}H_{18}N_2O_4$ (290.31gmol^{-1})

Yield: 92%

Melting points: 208-209°C

FTIR: 3238.16 (N-H), 2980.85 (C-H), 1699.24(C=O).

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.18 (s,H-1), 7.23 (t, 2H, J=9.16, H-2'/6'), 6.85 (d,2H, J=8.56, H-3'/5'), 5.85 (s, H-3), 5.36 (s,H-4), 4.07 (dd, 2H, J=1.36;1.28, H-8), 3.79 (s,H-4''), 2.34 (s,H-10), 1.18 (t,3H, J=7.12,H-9).

MS-TOF (Na): m/z 313.1169 $[M+Na]^+$

3.5 Antimicrobial Activity

The antibacterial activity was performed using the protocol described by (Ramakrishnan *et al.* 2011) using the well diffusion assay. The bacterial strains used in the study were collected from the culture collection at the Department of Biotechnology and Food Technology, Durban University of Technology, South Africa. The strains include *Staphylococcus aureus, Micrococcus luteus, Esherichia coli*, *Bacillus cereus* and one yeast culture, *Candida albicans.*

Stock cultures were sub-cultured to check their viability and stored using 50% of glycerol in micro bank vials (Davies Diagnostics, South Africa). For the assay, culture were plated on Nutrient Agar (Biolab) and kept in the incubator for 24 h at 37°C, then grown in Nutrient Broth (Biolab) at the same condition. The MacFarland standard of 0.5 absorbance corresponding to 10^8cfu/ml was used to standardize the bacterial and yeast cell concentration. A suspension (100 µl of 10^8 cfu/ml) of the test bacteria was plated on Mueller Hinton Agar plates (Fluka, Biochemika). A well of 6 mm diameter was made using a sterile cork borer. 30 µL of the compounds were added in each well at the concentration of 3 mg/ml and kept at 37°C for 24 h. The assay was carried out in triplicate. Ciprofloxacin (Fluka, Biochemika) (3 mg/ml) was used as the active control while DMSO (100%) as a negative control.

3.6. Brine Shrimp Lethality Assay

The Brine Shrimp toxicity test was carried out using the Brine Shrimp (*Artemia salina*) bioassay. Artificial seawater was filled in a 1 L sterile bottle (33 g/l sea salt adjusted to a pH of 8.5 using 1M Na_2CO_3 solutions), in which the Brine Shrimp eggs were allowed to hatch. This occurred under constant aeration for 48 h so that sufficient time was available for all the active nauplii to become free from their egg

shells. The active nauplii were then collected from the brighter portion of the hatching chamber and used in the bioassay. The protocol involved drawing 10 shrimp through a glass capillary and placing them in a 6-well plate. Each well of the plate contained 5 ml Brine solution. In each experiment, 100 µl prepared DMSO-compound solution (10, 100, 1000 µg/ml) was added to the Brine solution-containing (5 ml) wells. The plate was maintained at room temperature (37^{o}C) under visible light for 1, 2, 3, 4 and 24 h. The dead larvae were counted and percentage death determined (Meyer *et al.* 1982b). Percentage mortality was calculated by following the formula

$$\%Mortality = \frac{Number\ of\ dead\ Artemia\ Nauplii}{Initial\ number\ of\ live\ Artemia\ Nauplii} \times 100$$

Statistical analysis

Mortality was reordered as mean percentage of the death *Artemia* after 24 h exposure. Results are expressed as mean ± standard deviation.

3.7 Molecular docking

Molecular docking was conducted for the compounds (**4a-4m**) to explore its biological importance toward HIV-1 reverse transcriptase. AutoDock 4.0 software packages(Morris *et al.* 1998) was employed to run molecular docking using the crystal structure of HIV-1 reverse transcriptase in complex with inhibitor gsk560 (2YNG). The binding site was defined from the centroid of bound ligand and the top ranked poses of molecules were analyzed to explore the interactions with the therapeutic target.

CHAPTER FOUR

RESULTS AND DISCUSSION

Our aim was to synthesize a novel heterogeneous catalyst, fully characterize it and use it for the synthesis of novel compounds. In this study we selected two important classes of compounds which are biologically active and sought after. These two classes are α -aminophosphonates and the dihydropyrimidinones.

We accomplished the synthesis of RhBNT by using $Rh(OAc)_2$ and BN in a methanolic solution, under inert atmospheric conditions. The reaction was allowed to run for 7 days after which the novel material was collected as a pale blue powder by using simple filtration; the yield was 92 %. Characterization of RhBNT was performed by several techniques: the crystalline nature of RhBNT and nano size was confirmed by SEM spectroscopy and EDX pattern (**Figure 4)**. The particle dimensions of nanomaterial were observed in the 1-10 µm range with plate like particles of boron nitride particle with size 200 nm. EDX pattern for RhBNT showed signals for rhodium metal.

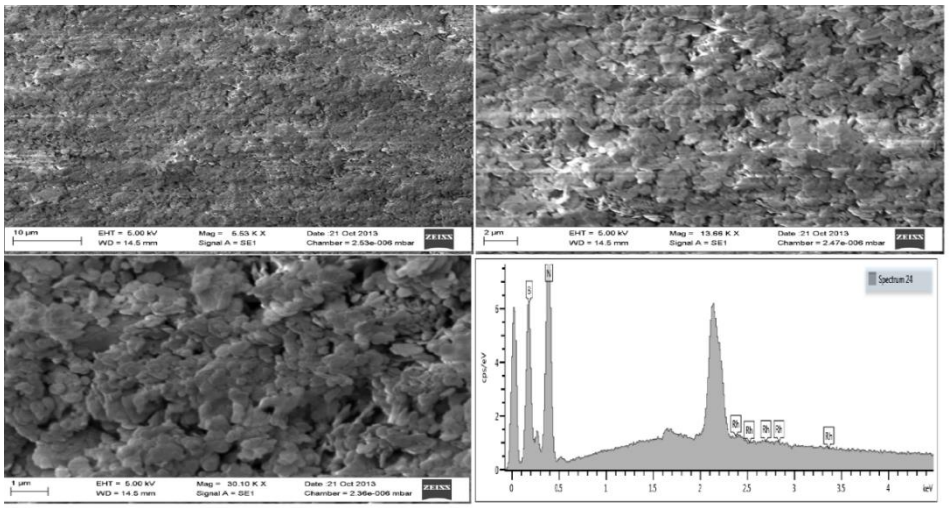


Figure 4: SEM with EDX for Rhodium-loaded Boron nitride

The catalyst was also subjected to X-ray diffraction analyses (**Figure 5)**; the crystalline nature of RhBNT was confirmed and the characteristic Bragg's XRD peaks at 2θ = 26.75˚, 41.58˚, 56.26˚, 75.86˚ are indexed to the (0 0 2), (1 0 0), (2 0 0), (2 2 0) and Rhodium 2θ = 45.58˚, 50.16, 83.58˚ are indexed to the (1 1 1), (2 0 0), (3 1 1) crystallographic planes of the boron nitride and rhodium metal, respectively.

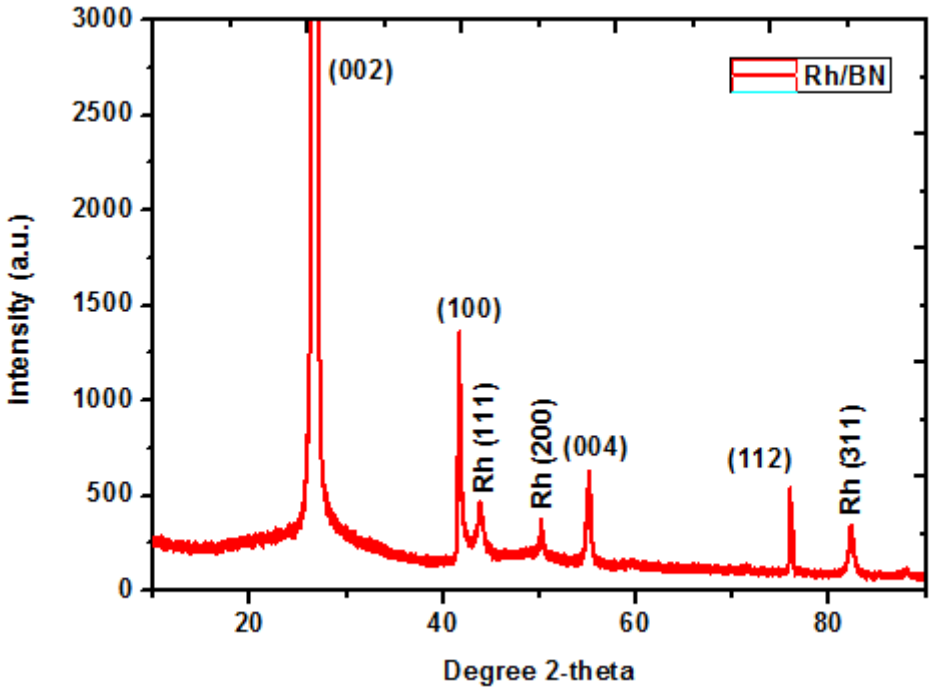


Figure 5: The Powder XRD Pattern for Rhodium-loaded Boron nitride

The Brumnauer-Emmett-Teller (BET) specific surface was calculated from the nitrogen adsorption data at the relative pressure using a multi-point method (**Figure 6**). The BET analysis showed a surface area of 28.12 $m^2 g^{-1}$, pore volume 0.23 $cm^3 g^{-1}$ and pore size of 199.8 Å thereby suggesting RhBNT a potentially effective catalyst for organic reactions; the mesoporous nature of the material was established by a type-IV adsorption isotherm.

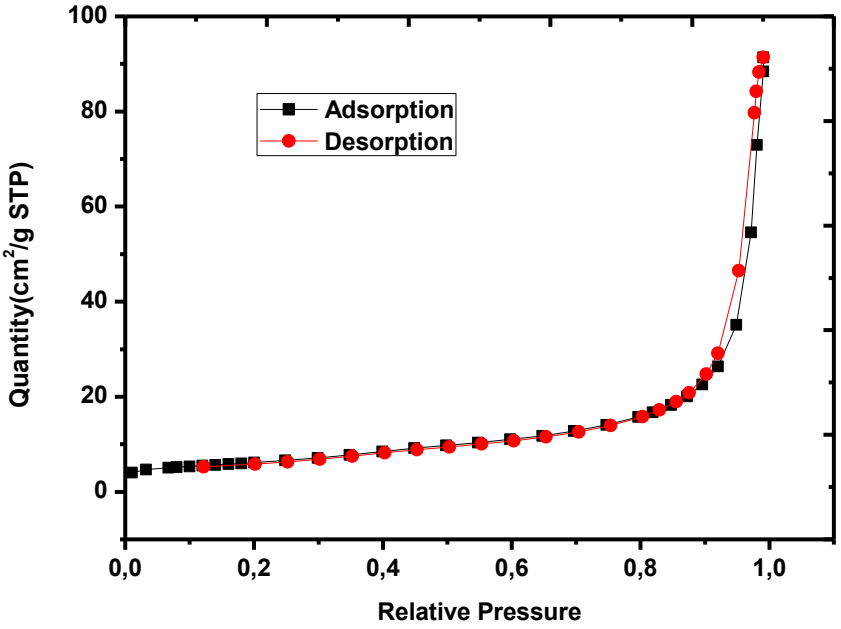


Figure 6: BET Surface Area and Pore Size of Rhodium-loaded Boron nitride

The synthesized RhBNT powder was measured for its thermal properties from room temperature to 800˚C (**Figure 7**). The weight loss of the compound was observed at 100˚C with an endothermic peak at 102˚C.These results indicate that RhBNT has good thermal stability and can be used adequately for catalysis.

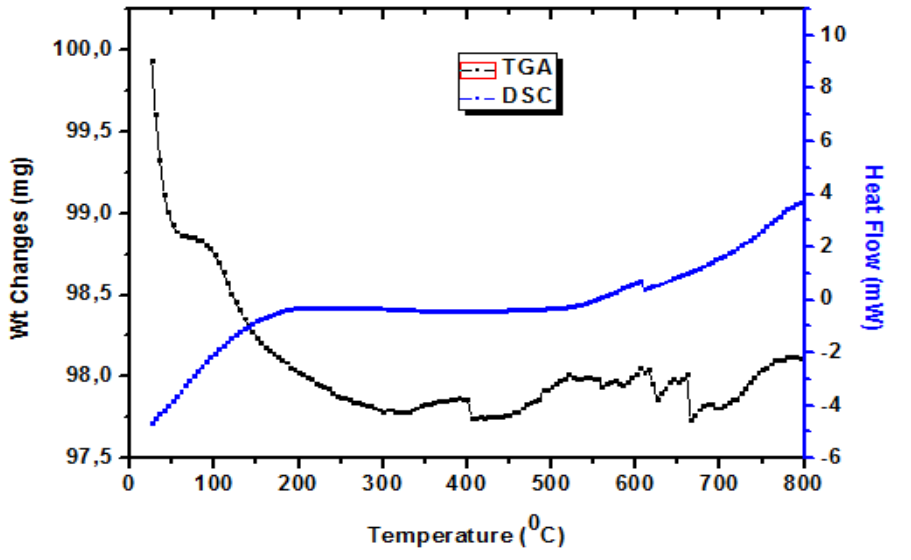


Figure 7: The DSC-TGA Profile of Rhodium-loaded Boron nitride

Raman Spectrum of the RhBNT (**Figure 8)** clearly showed three peaks located at approximately 900 cm^{-1}, 1380 cm^{-1} and 1795 cm^{-1} for hBN with Rh metal. The peak at 1380 cm^{-1} is identified with hBN phonon mode (Kalay *et al.* 2013). The peaks observed at 900 cm^{-1} and 1795 cm^{-1} are related to the component of boron, nitrogen and rhodium.

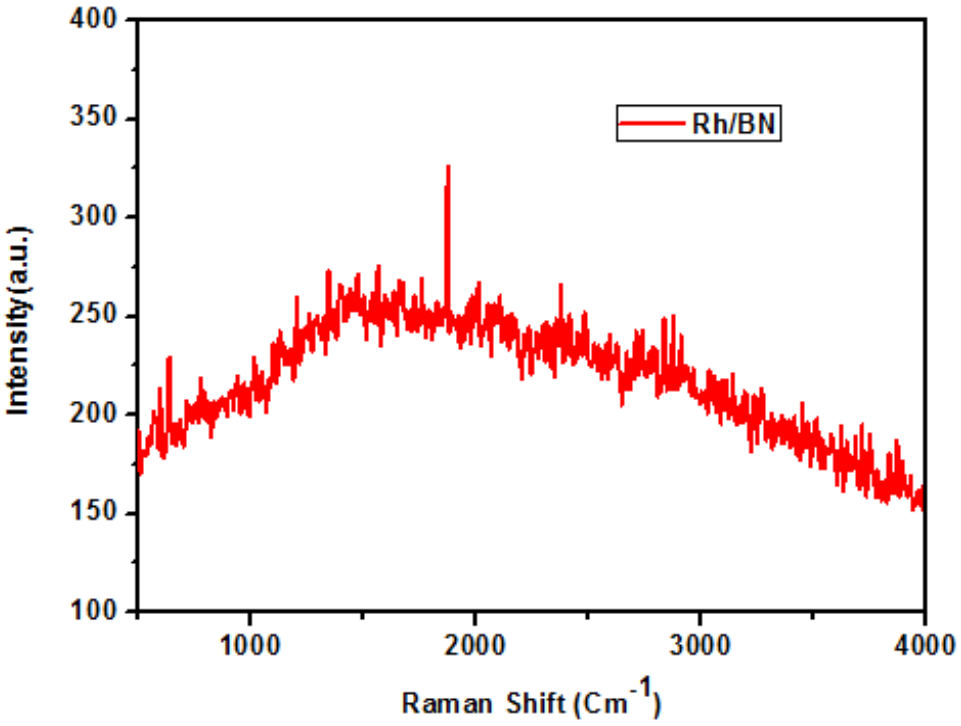
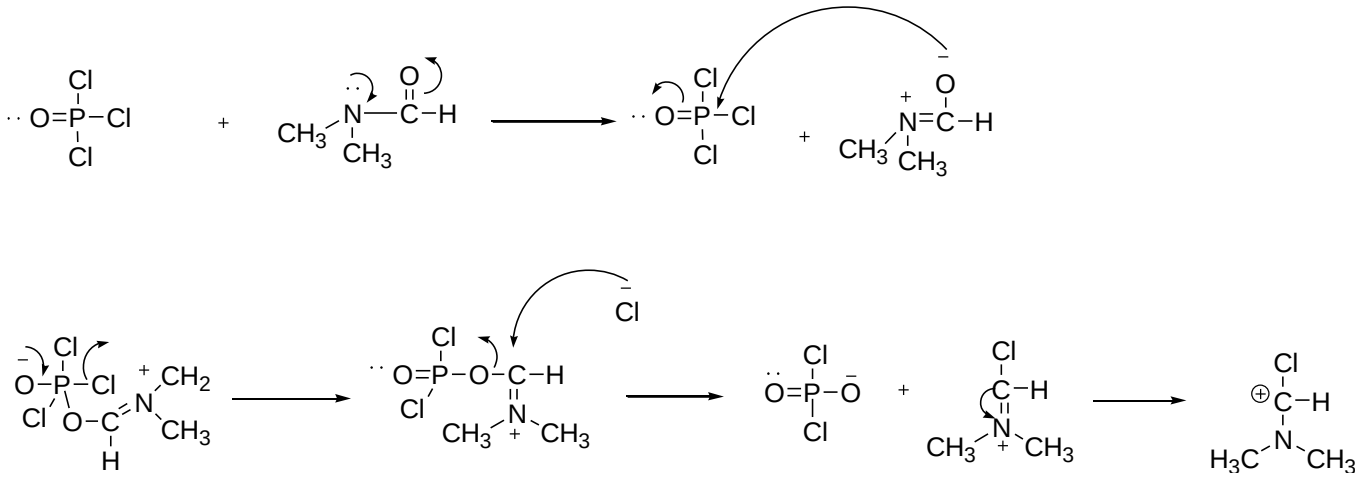


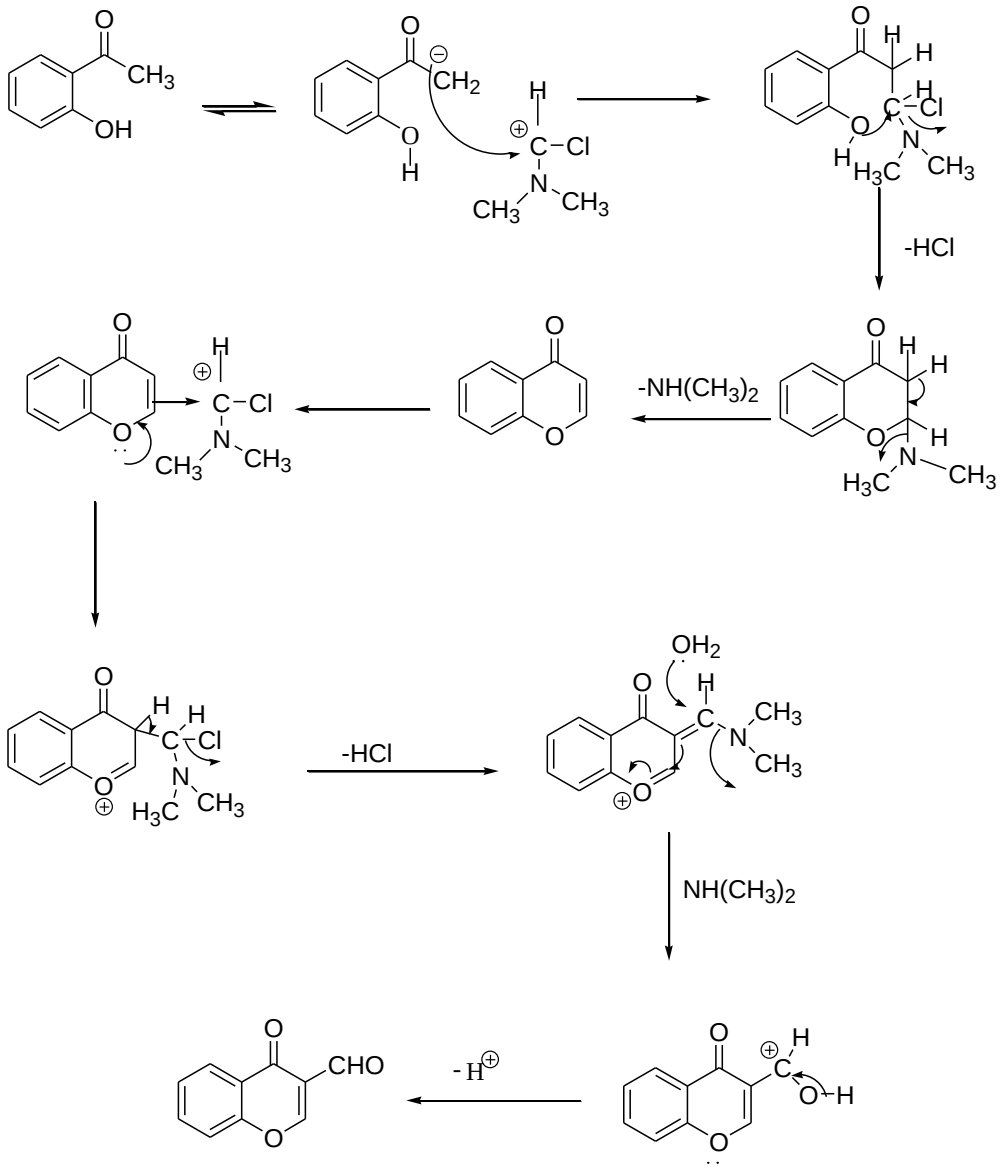
Figure 8: Raman spectrum for Rhodium loaded Boron nitride

The RhBNT was subsequently used in the Kabachnik-Fields reaction in order to assess its catalytic potential. In the first step of synthesis, we prepared the Vilsmeier-Haack reagent by combining DMF and $POCl_3$ at 5 ^0C to form an electrophile in the absence of strong acid or Lewis acid. This type of reaction is a substitute for the Friedel-Crafts acylation reaction and works with aromatic compounds at the more reactive end of the molecule. **Scheme 32** shows the mechanism for the formation of the electrophile.



Scheme 32: The mechanism for the formation of the Electrophile

The prepared Vilsmeier-Haack reagent reacted *in situ* with the substrates 2-hydroxyacetophenone **(40)** and 1'-hydroxy-2'-acetonaphthone **(41)** to obtain 4-oxo-chromene-3-carbaldehyde **(42)** and 4-oxo-4H-benzo[h]chromene-3-carbaldehyde **(43)** respectively (**Scheme 29**, page 36). The yield of **(42)** was 85%, m.p 142°C and that of **(43)** was 82% yield, m.p 148 °C. The mechanism of the reaction is presented in **Scheme 33**



Scheme 33: A general mechanism for the synthesis of 3-formyl chromone compound proposed by (Rajanna et al. 1996)

The spectroscopic data of **42** matched literature (Khan *et al.* 2009) whilst **43** is a novel compound. The compound **42** was characterized by IR and ^{1}H NMR; **Figure 15** (Appendix 1, page 110) shows the ^{1}H NMR spectrum and **Figure16** (Appendix 2, page 111) shows the IR spectrum. The compound **43** was characterized by IR and ^{1}H NMR; **Figure 17** (Appendix 3, page 112) shows the ^{1}H NMR spectrum and **Figure 18** (Appendix 4, page 113) shows the IR spectrum.

The RhBNT was subsequently used in the Kabachnik-Fields reaction in order to assess its catalytic potential. To synthesize novel chromone-bearing aminophosphonates, we set up a model reaction based on a recent research report undertaken in our laboratory (Sureshkumar *et al.* 2016). An equimolar quantity of **(42)**, p-toluidine and diethylphosphite were used and RhBNT was added; toluene was used as the solvent. This mixture was refluxed on an oil bath and the progress of the reaction was monitored hourly for six hours. After working up the mixture, the catalyst was filtered and the organic layer was separated by column chromatography. Pure compound **4g** was obtained in 75 % yield; it was characterized by IR, ^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR and mass spectrometry. Thereafter the reaction was repeated to optimize the solvent system (**Table 1)**

Table 1: Solvent Optimization for the Synthesis of 4g

Entry	Catalyst	Solvent	Temp (°C)	Time (h)	Yield (%)[b]
1	RhBNT	Acetonitrile	82	6	96
2	RhBNT	THF	66	6	90
3	RhBNT	Toluene	110	6	75
4	RhBNT	Ethanol	78	6	70
5	RhBNT	DCM	72	6	65

We found that acetonitrile and 0.1g of RhBNT (**Table 2**) gave the highest yield (96 %). Increasing the quantity of RhBNT did not enhance the yield of **4g** but would rather increase the cost of synthesis. Hence acetonitrile and 0.1g of RhBNT was used in all subsequent reactions.

Table 2: The Optimization of Quantity of Rhodium Supported on Boron Nitride Catalyst (RhBNT) for the Synthesis of 4g by the Kabachnik Field reaction

Entry	RhBNT (g)	Time (h)	Yield(%)
1	0.05	6	82
2	0.08	6	93
3	0.10	6	96
4	0.15	6	96

Furthermore, we compared the % yield of our model reaction for the synthesis of **4g** with literature values (**Table 3**); we found that the % yield was higher than that of literature values. This indicates that RhBNT is more efficient and effective than the traditional catalysts

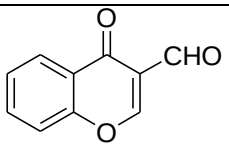
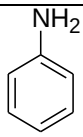
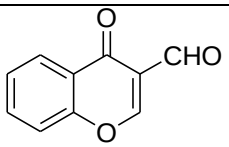
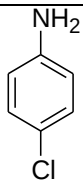
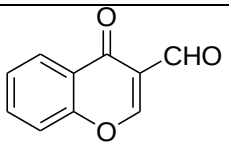
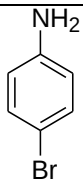
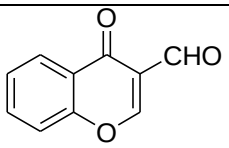
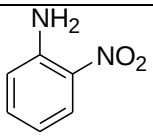
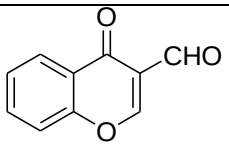
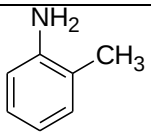
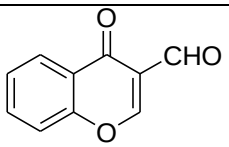
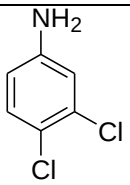
Table 3: A Comparison of Catalyst used for the Synthesis of Aminophosphonates by the Kabachnik Field reaction

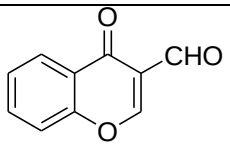
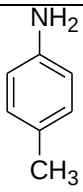
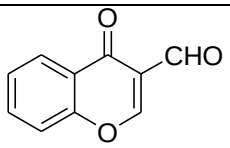
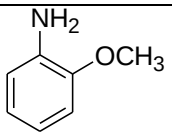
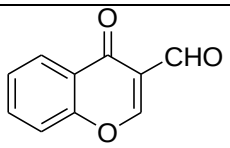
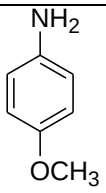
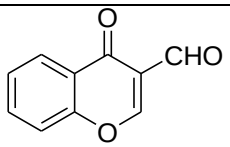
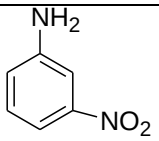
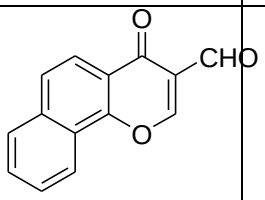
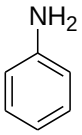
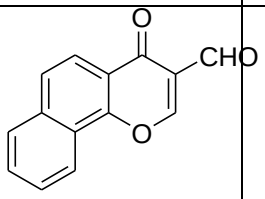
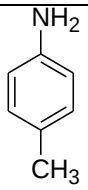
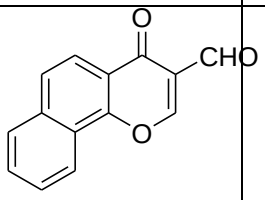
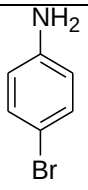
Entry	Catalyst Used	Reaction Condition	Reaction Time	Yield (%)	Reference
1	$TaCl_5$-SiO_2	CH_2Cl_2/RT	22 h	92	(Chandrasekhar *et al.* 2001)
2	$YbCl_3$	CH_3CN/RT	24 h	93	(Xu *et al.* 2006)
3	$In(OTf)_3$	THF/Reflux	21 h	79	(Ghosh *et al.* 2004)
4	ZnO	Solvent-free/RT	9 h	90	(Hou *et al.* 2011)
5	$InCl_3$	THF/RT	11 h	92	(Ranu *et al.* 1999)
6	$BiCl_3$	CH_3CN/reflux	6h	92	(Zhan and Li 2005)
7	$Mg(ClO_4)_2$	Solvent-free/80˚C	5h	95	(Bhagat and Chakraborti 2007)
8	RhBNT	CH_3CN/reflux	6h	96	Present work

Having established an efficient protocol for the synthesis of **4g**, we used various aniline derivative to synthesize novel chromone-bearing α -aminophosphonates

(**4a-4m)** **Table 4**. The general reaction is represented in (**Scheme 30,** page 37**).** In all cases, the corresponding chromone-bearing α-aminophosphonates were obtained in high yield.

Table 4: The Percentage yield and Melting point of chromone-bearing α - Aminophosphonates (4a-m) obtained from the Synthesis using the Kabachnik Field reaction.

Entry	*Aldeyhde*	*Amine*	*Time(h)*	*Product*	*Melting point(°C)*	*Yield (%)*
1			6	4a	143-145	92
2			6	4b	148-150	94
3			6	4c	146-148	93
4			6	4d	160-162	90
5			6	4e	138-140	94
6			6	4f	154-156	90

7			6	4g	142-144	96
8			6	4h	153-155	95
9			6	4i	157-159	91
10			6	4j	162-164	90
11			6	4k	154-156	89
12			6	4l	165-167	91
13			6	4m	176-178	92

A proposed mechanism is presented in **Figure 9**. In this mechanism RhBNT acts as a Lewis acid by pulling the lone pairs of electron on the heteroatom of the carbonyl

compound thereby promoting the reaction. The mechanism involve firstly the condensation of amines with the carbonyl compound **I** to form an activated imine **II.** Secondly is the nucleophilic attack of the dialkyl phosphite on the imino carbon (C=N unit of **I**) to afford the phosphonium intermediate **III**. The phosphonium intermediate **III** reacts with the water molecule generated during imine formation to give the corresponding α-aminophosphonates **IV**.

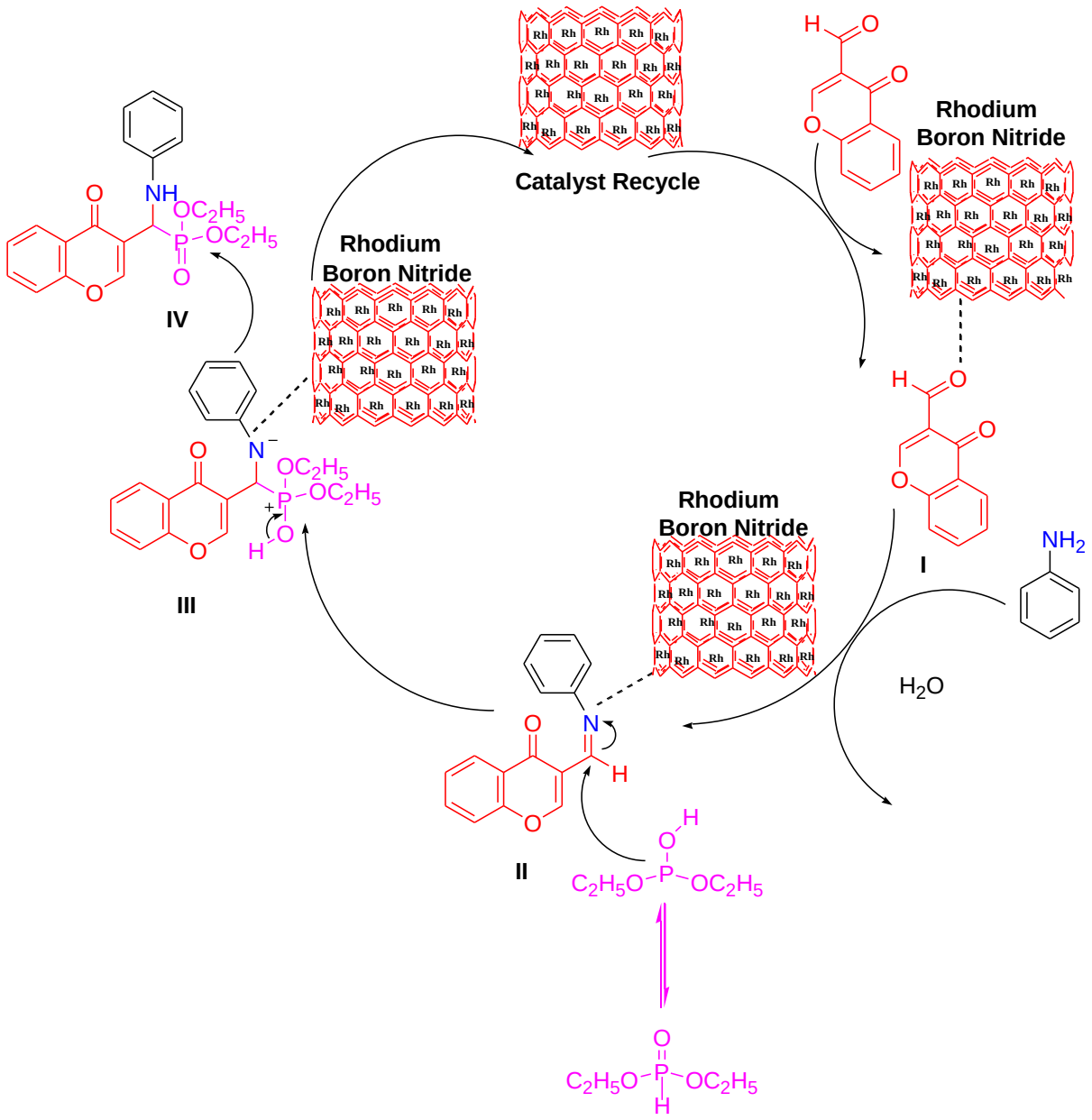


Figure 9: A Proposed Mechanism for the Synthesis of Chromone bearing α-Amino phosphonates

We also investigated the reusability potential of RhBNT; herein we used the filtered solid obtained from the model reaction for the synthesis of **4g**; the solid was rinsed with chloroform and methanol and heated at 100°C. It was found that the catalyst could be re-used five times without any significant loss of its catalytic activity (**Figure 10**). The reusability of the catalyst is an important benefit which makes it useful for commercial applications.

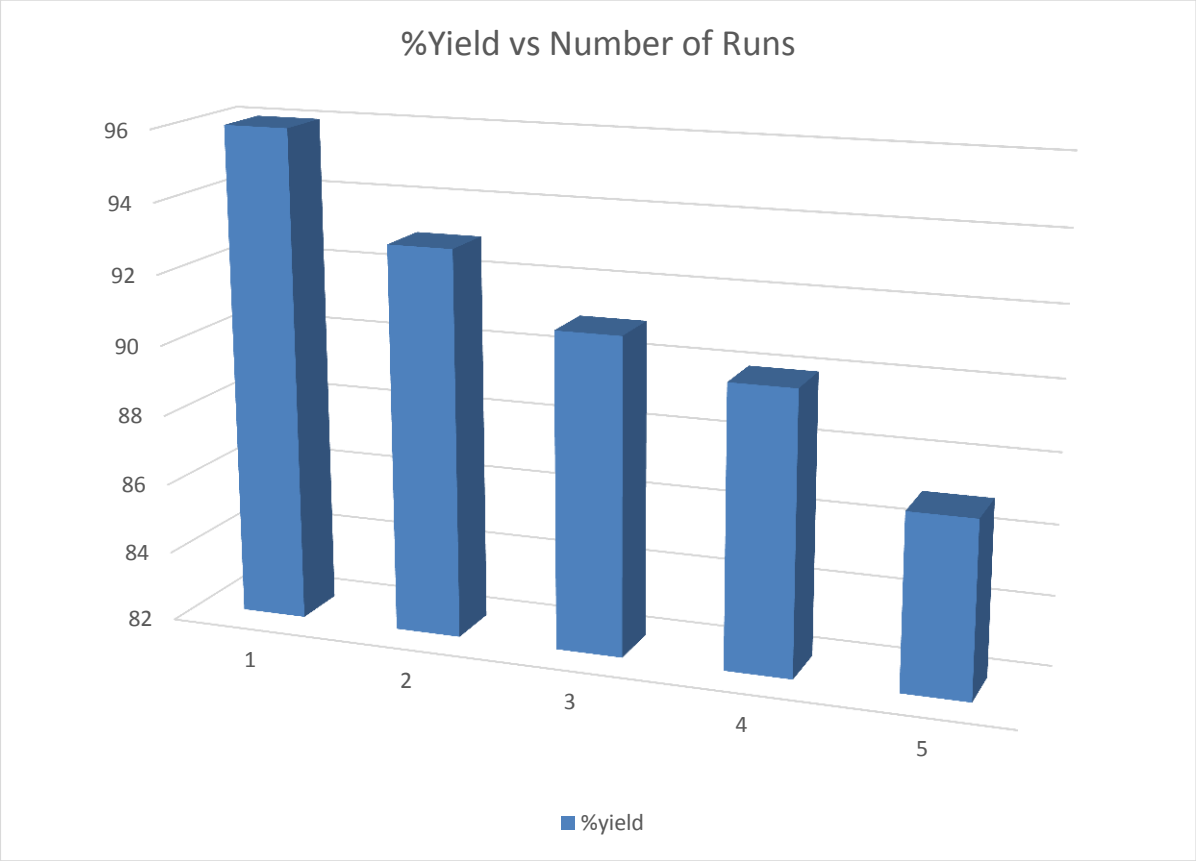


Figure 10: Recycling and Reusability of the Rhodium Supported on Boron nitride catalyst for the Synthesis of **4g**

The identity of all the compounds (**4a-4m)** were confirmed by analyzing the spectral data obtained from IR, ^{1}H NMR, and ^{13}C NMR whilst four compounds were selected for mass spectral analysis. All the spectra were well resolved. We selected **4g** as the

template and characterized the structure fully with the aid of 2D NMR techniques especially **HSQC**, **HMBC and COSY** which led to the unambiguous assigning of all protons, carbons and nitrogen.

The **IR** spectra of **4g**, presented in **Figure 45** (Appendix 31, page140) shows an absorption band at 1637.52 which was assigned to C=O stretching vibrations. The N-H and P=O stretching vibrations are observed in the region 3279.33 and 1221.79 cm^{-1} respectively. The aliphatic C-H stretching is at 2983.17, the P-C-O bending, P-C aliphatic and C=C stretch in ring occurs at 1015.64, 766.19 and 1515.0 cm^{-1} respectively.

The **^{1}H NMR** spectrum of **4g**, presented in **Figure 46** (Appendix 32, page141), shows two triplets at δ 1.16 and 1.30 corresponds to the aliphatic methyl protons and δ 2.16 for the methyl protons attached to the aromatic portion. The methylene protons (H-12, H-12') appeared as multiplets at δ 4.01 and 4.11 whereas the P-C-H proton signal (H-11) appeared as a doublet at δ 5.29 due to its coupling with the neighbouring N-H proton and phosphorus. The N-H proton gave a broad signal at δ 3.50. Eight aromatic protons were observed and unambiguously assigned by using 2 D NMR techniques. The chemical shifts, spin multiplicities and coupling constants (in Hertz) were assigned as: δ 6.57 (d, 2H, H- 3'/5' J=8.20,); δ 6.92 (d, 2H, H-2'/6', J=8.32); δ 7.40 (dd,1H, J=8.44; 6.44, t, 1H, J=5.24, H-7/8); δ 7.65 (dt, 1H, H-6, J=8.32); δ 8.10 (s, 1H, J=3.52, H-2); δ 8.23 (d, 1H, J=8.44, H-5); The expanded **^{1}H NMR** spectrum **is** presented in **Figure 46a-46c** (Appendix 33-35, page142-144).

The **^{13}C NMR** spectrum of **4g**, is presented in **Figure 47**(Appendix 36, page 145). The peaks at δ 16.40 and δ 16.26 were assigned to the two methyl carbons (C-13, C-13'); the peak at δ 20.35 was assigned to methyl carbon of the aromatic ring (C-4''), C-11 was attributed to δ 46.27 whilst C-12 and C-12' appeared at δ 63.40 and

63.81, respectively. The carbon 3'/5' and 2'/6' appeared at δ 113.86 and δ 128.20, whilst C-1', C-4', C-5 and C-6 appeared at 133.82, 120.22, δ 125.94, and δ 125.39. The remaining aromatic carbons appeared as: C-7 at 129.86, C-8 at δ 118.25, C-9 at 156.26, C-10 appeared at 123.44, C-2 at 156.18 whilst C-4 the carbonyl carbon at δ 176.30.

The **COSY** spectrum, presented in **Figure 48** (Appendix 37, page146), shows H-2'/6' has a strong coupling with H-3'/5' whilst H-13 is strongly coupled to H-12 and H-13' with H-12'. H-7 is coupled strongly with H-6 but weakly with H-8 whereas H-6 is strongly coupled with H-7 but weakly with H-8 whilst H-5 is coupled strongly with H-6 but weakly with H-7 and H-8.

The **HSQC** spectrum, presented in **Figure 49** (Appendix 38, page147) gave the corresponding carbon resonances of H-2'/6', H-3'/5', H-6, H-7, H-8, H-5 at δ 129.86, 113.86,133.82, 125.40, 118.25, 125.94 whilst in the aliphatic region resonances of H-13, H-13', aromatic methyl (H-4'') at δ 16.26, 16.40 and 20.35, whereas H-12' and H-12 resonance were at δ 63.81 and 63.40. The methine proton H-11 resonated at δ 46.27.

From the **HMBC** spectrum **Figure 50** (Appendix 39, page 148) H-11 showed correlations to C-2, C-10 and C-4 at δ 155.18, 123.44 and 176.30. The H-2'/6' showed correlations with C-3'/5' and C-4' at δ 118.36, and 120.22 whilst H-7 with C-9 and C-4 at δ 156.26 and 176.30. H-6 showed correlations with C-5 and C-9 at δ 125.94 and 156.26. H-8 showed correlations with C-4 and C-2, C-10, C-11 at δ 176.30, 155.18, 123.44 and 46.27. H-5 was correlated to C-4, C-7 and C-9 at 176.30, 129.86 and 156.26. The HMBC correlation for 4g is presented in **Figure 11**. The ^{31}P-NMR signals **Figure 51** (Appendix 40, page 149) appeared as a singlet at δ 22.5708.

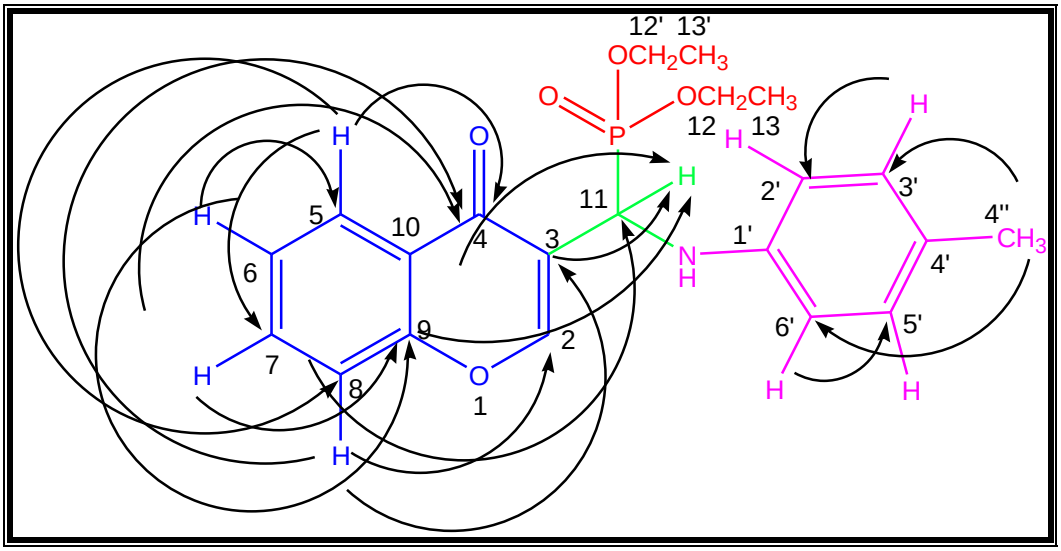


Figure 11: Selected HMBC correlations for 4g

In the NMR spectra of all the related derivatives, the chromone and phosphate were similar. The differences were seen in the phenyl ring attached to the N due to the different substituents in the ring. **Table 5** and **Table 6** presents the chemical shifts and coupling constants of the protons of **4a-4j** and **4k-4m** respectively. The chemical shift of the carbons of **4a-4j** and **4k-4m** is presented in **Table 7** and **Table 8,** respectively.

Table 5: ^{1}H NMR chemical shifts for compounds 4a-4j

H	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**
NH	3.60(s)	3.41(s)	3.41(s)	Not integrated	Not integrated	Not integrated	4.60(s)	Not integrated	Not integrated	Not integrated
CH	5.36 (d,23.68)	5.30 (d,23.64)	5.30 (d,23.68)	5.35 (d,23.12)	5.35 (d,23.56)	5.27 (d,23.52)	5.32 (d,23.64)	5.32 (d,23.43)	5.30 (d,23.63)	5.37 (d,23.48)
2	8.13 (d,3.36)	8.14 (d,3.52)	8.09 (d,3.36)	8.04 (d,3.64)	8.04 (d,3.64)	7.65 (d,3.20)	8.11 (d,3.52)	8.10 (d,3.52)	8.13 (d,3.40)	8.18 (d,3.28)
5	8.22 (dd,6.20)	8.23 (dd,8.48)	8.23 (dd,8.48)	8.23 (dd,7.92)	8.23 (dd,8.52)	8.21 (dd,8.28)	8.23 (dd,8.44)	8.24 (dd,8.52)	8.20 (dd,8.60)	8.23 (dd,8.76)
6	7.64 (dt,7.96)	7.63 (dt,8.36)	7.68 (dt,7.06)	7.64 (dt,8.00)	7.68 (dt,7.72)	7.41 (dt,4.84)	7.64 (dt,8.32)	7.63 (dt, 6.92)	7.62 (dt,8.20)	7.62 (dt,J=8.48)
7/8	7.40-(dt,6.76, 7.20)	7.40 (dt,8.48;6.08)	7.42-(dt,7.32,4, 7.6)	7.44 (dt,7.84,8.36)	7.44 (dt,8.24 ,8.36)	7.12-(dt,7.20, 14.36)	7.40 (dt,8.44;6.4)	7.41(dt, 8.12,8.72)	7.39 (dt,11.00,8.48)	7.45 (dt,8.08;1.40)
2'/6'	7.11 (d,15.04)	7.06 (d,8.36)	7.02 (d, 8.72, H-2'/6	-	-	6.78 (d, 2.64, H-2'/6	6.92 (d,8.32)	-	6.85 (d,8.88)	7.15 (d,0.92)
3'/5'	6.57 (d,J=7.76)	6.59 (d,J=8.76)	6.65 (d,J=8.76)	6.64 (d,J=2.84)	6.54 (d,J=8.00)	-	6.57 (d,J=8.20)	6.57 (d,J=8.76)	6.67 (d,J=8.88)	-
4'	6.68(J-14.52)	-	-	-	6.68(J-7.36)	-	-	6.65 (J=14.08)	-	6.90 (J=1.76)
4''(CH_3)	-	-	-	-	-	-	2.17(s)	-	-	-
12'	4.22 (m,3.68)	4.25 (m,7.32)	4.23 (m, 3.48)	4.21 (m, 4.40)	4.21 (m, 4.52)	4.25 (m, 6.20	4.25 (m,1.96)	4.22 (m,2.72)	4.21 (m,3.44)	4.21 (m,1.52)
12	4.07 (m,0.76)	4.07 (m,7.36)	4.09 (m,7.20)	4.09 (m,1.56)	4.09 (m,2.00)	4.19 (m,5.48)	4.07 (m,1.96)	4.10 (m,1.88)	4.19 (m,8.16)	4.19 (m,2.52)
13'	1.30 (t,7.08)	1.30 (t,7.00)	1.31 (t,7.56)	1.31 (t,7.08)	1.31 (t,7.04)	1.31 (t,7.00)	1.30 (t,7.04)	1.31 (t,7.00)	1.31 (t, 6.75)	1.26 (t, 7.04)
13	1.16 (t,7.04)	1.16 (t,7.04)	1.16 (t,7.04)	1.23 (t,7.08)	1.29 (t,7.04)	1.29 (t,7.04)	1.16 (t,7.04)	1.28 (t,7.04)	1.29 (t,7.04)	1.14 (t,7.04)
4''(OCH_3)	-	-		-	-	-	-	-	3.65(s)	-
6''(OCH_3)		-	-	-	-	-	-	3.85(s)	-	-
6''(CH_3)	-	-		-	2.19,(s)	-	-	-	-	-
2'			-	6.66 (d, 2.88)	7.04 (d,2.88)	-	-	6.73 (d,7.24)	-	-
5'	-		-	-	-	6.49 (dd,2.44)	-	-	-	6.88 (dd,1.80)

Table 6: ^{1}H NMR chemical shifts for compounds 4k-4m δ of 1H (J, Hz)

H	**4k**	**4l**	**4m**
NH	Not integrated	2.29 (s)	3.02 (s)
CH	5.45 (d,23.80)	5.38 (d,23.64)	5.38 (d,23.64)
2	8.33 (d,3.52)	8.31 (d,3.56)	8.34 (d,3.48)
5	7.76 (dd,8.76)	7.88 (dd,6.96)	7.76 (dd,8.76)
6	7.86 (d,J=7.96)	7.90 (d,J=7.88)	7.91(d,J=8.00)
2'/6'	6.70 (d,7.92)	6.91 (d,8.96)	7.18 (d,8.72)
3'/5'	7.12 (d,J=1.76)	6.61 (d,J=7.92)	6.59 (d,J=8.76)
4'	6.71 (J=6.28)	-	-
4''(CH_3)	-	2.16 (s)	-
13	5.45 (d,23.80)	5.38 (d,23.64)	5.38 (d,23.64)
10	8.38 (dd,J=8.12	8.40 (dd,J=8.20)	8.40 (dd,J=8.16)
7	8.15 (d,J=6.7)	8.15 (d,J=3.52)	8.14 (d,J=8.76)
15'	4.25 (dt,J=7.36)	4.25 (dt,J=6.88)	4.24 (dt,J=7.36
15	4.10 (dt, J=7.92	4.10 (dt, J=8.00)	4.11 (dt, J=7.92)
14'	1.32 (dt, J=7.04)	1.32(dt, J=7.04	1.32(dt, J=7.08
14	1.18(dt,J=14.12)	1.18 (dt,J=7.04)	1.18 (dt,J=7.04)
8	7.60 (d, J=14.64)	7.63 (d, J=7.12)	7.68 (d, J=14.40)
9	7.66 (t, J=14.16)	7.77 (d, J=14.40)	7.78 (t, J=14.2)

Table 7: ^{13}C NMR chemical shifts (δ in ppm) for compounds 4a-4j

C	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j
1’	145.12	144.21	144.69	155.22	143.52	145.48	133.82	147.30	152.98	149.24
2	155.19	155.21	155.23	155.21	155.03	155.31	155.18	155.28	155.27	155.49
2’/6’	129.37	129.52	132.08	134.28	128.20	132.87	128.20	133.78	133.83	130.05
3’/5’	113.66	117.69	115.31	118.35	118.38	115.40	113.86	110.99	115..04	113.03
4	176.31	176.27	176.26	176.09	176.34	176.30	176.30	176.26	176.34	176.14
4’	120.23	119.94	119.91	123.30	122.95	119.78	120.22	120.48	120.45	119.73
4’’	-	-	-	-	23.37	-	20.35	55.46	55.63	-
5	125.93	125.74	125.93	126.25	127.30	125.78	125.94	125.86	125.92	126.01
6	125.43	125.45	125.55	125.99	125.69	123.34	125.39	123.43	125.39	125.65
7	133.87	132.17	139.996	139.42	131.68	134.04	129.86	135.35	139.51	134.08
8	118.27	118.30	118.39	119.47	120.18	118.30	118.25	118.16	118.21	118.39
9	156.26	156.26	156.25	156.26	156.28	156.25	156.26	156.28	156.24	156.24
10	123.42	122.05	123.34	125.81	123.45	121.32	123.44	120.20	123.43	123.37
11	46.27	45.33	45.19	45.05	45.37	45.25	46.27	45.56	46.05	45.17
12	63.45	63.53	63.56	63.86	63.49	63.62	63.40	63.37	63.33	63.73
12’	63.80	63.89	63.92	63.99	63.77	63.94	63.84	63.81	63.79	63.98
13	16.42	16.42	16.42	16.43	16.43	16.43	16.40	16.40	16.42	16.38
13’	16.27	16.26	16.26	16.29	16.29	16.28	16.26	16.27	16.28	16.28

Table 8: ^{13}C NMR chemical shifts (δ in ppm) for compounds (4k-4m)

	4k	**4l**	**4m**
1’	145.55	143.15	144.62
2	153.83	154.71	153.88
2’/6’	129.44	129.46	130.85
3’/5’	113.67	113.75	115.32
4	176.09	176.15	176.05
4’	119.26	119.70	120.66
4’’	-	20.34	-
5	121.71	121.76	122.26
6	120.72	120.75	121.39
7	118.83	118.25	119.65
8	125.64	125.60	128.10
9	123.92	123.95	127.37
10	122.25	122.27	123.90
11	135.83	135.83	125.78
12	154.33	155.72	154.32
13	45.27	45.52	45.33
14	16.44	16.43	16.44
14’	16.29	16.28	16.28
15	63.54	63.44	63.62
15’	63.86	63.83	63.92

Comparison of Model compound **4g** with other synthesized APs (**4a-4m**);

4a: The spectroscopic data was similar to that of **4g** except that the H-4’ is present at δ 6.68 as a triplet and absence of the methyl group.

4b: The spectroscopic data was similar to that of **4g** except that the Cl group is substituted in position H-4’ and hence there is no ^{1}H NMR chemical shift value at that position and absence of the methyl group.

4c: The spectroscopic data was similar to that of **4g** except that the Br group is substituted in position H-4’ and hence there is no ^{1}H NMR chemical shift value at that position and absence of the methyl group.

4d: The spectroscopic data was similar to that of **4g** except that the NO_2 group is substituted in position 2’ and hence there is no ^{1}H NMR chemical shift value at that position and absence of the methyl group.

4e: The spectroscopic data was similar to that of **4g** except that the H-4’ is present at δ 6.68 as a doublet and the methyl group in position H-2’ is observed at δ 2.19 as a singlet. Also the C-2’’ is observed by ^{13}C NMR is at δ 23.37.

4f: The spectroscopic data was similar to that of **4g** except that the ^{1}H NMR chemical shift value **at** H-3’ and H-4’ is lacking due to the Cl group present in these positions. Also the methyl signal is absent.

4h: The spectroscopic data was similar to that of **4g** except that the H-4' is present at δ 6.65 as a triplet and the methoxy signal at position 2' is at δ 3.85. Also the methyl signal is absent whilst the ^{13}C NMR signal is at δ 55.46.

4i: The spectroscopic data was similar to the data of **4g** except that the H-4' is present at δ 6.65 as a triplet and the methoxy signal at position 4' is at δ 3.65. Also the methyl signal is absent whilst the ^{13}C NMR signal is at δ 55.46.

4j: The spectroscopic data was similar to that of **4g** except that the NO_2 group is substituted in position 3' and hence there is no ^{1}H NMR chemical shift value at that position and absence of the methyl group.

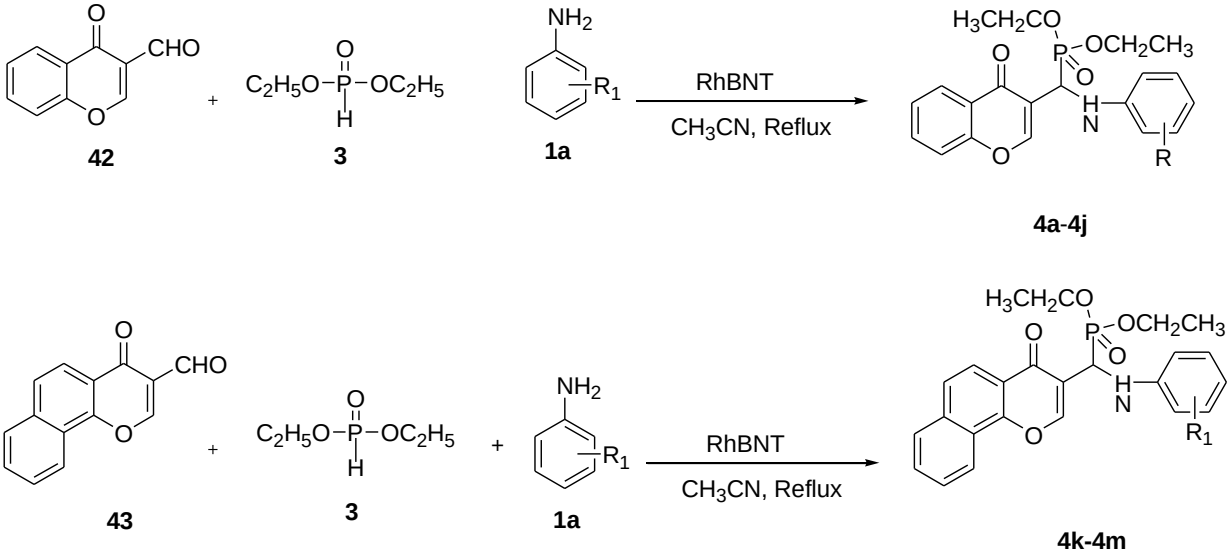
4k: The spectroscopic data was similar to **4g** except for the additional fused benzene ring which shows four additional aromatic protons at δ 8.38, 8.15, 7.66 and 7.60. Also
the H-4' proton is observed at δ 6.71 whilst the ^{13}C NMR is at δ119.26.

4l: The spectroscopic data was similar to **4g** except for the additional fused benzene ring which shows four additional aromatic protons at δ 8.40, 8.15, 7.77 and 7.63. Also
the H-4' proton is missing. The ^{13}C NMR shows δ 20.34 and 119.70 for C-4'' and C-4', respectively.

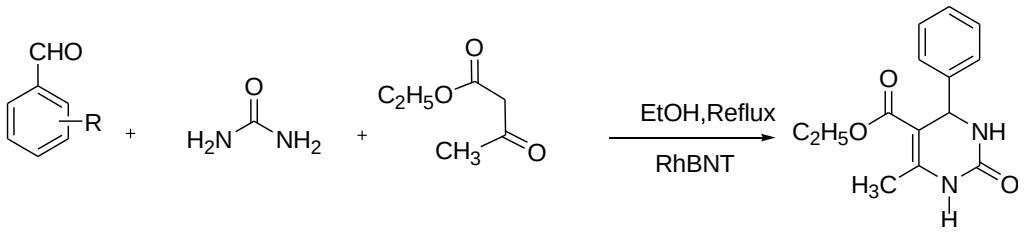
4m: The spectroscopic data was similar to **4g** except for the additional fused benzene ring which shows four additional aromatic protons at δ 8.40, 8.14, 7.78 and 7.68. Also

the H-4' proton is missing due to substitution of Br group at this position hence no chemical shift value at this position. The ^{13}C NMR shows δ 120.66 for C-4'

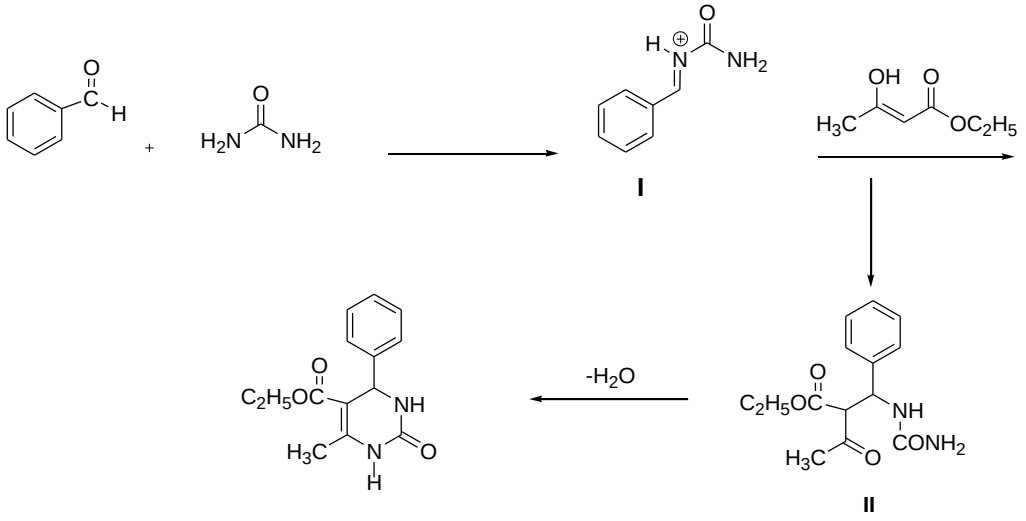
The reaction for the synthesis of **4a**-**4m** is shown below (**Scheme 30,** page 37)



The RhBNT was also used in the Bignelli reaction in order to assess its catalytic potential. To synthesize dihydropyrimidinones (**Scheme 31**, page 51), a mixture containing of an aldehyde, β-keto-ester, urea and RhBNT in ethanol as a solvent was refluxed on an oil bath. The progress of the reaction was monitored by TLC. After working-up the mixture, the catalyst was filtered and the organic compound was separated by column chromatography. Pure compound (**4n**-**4r**) was obtained. The products were characterized by IR, MS and ^{1}H-NMR.



The proposed mechanism for the Biginelli reaction is as proposed by Kappe (Kappe 1997) is shown below. Firstly a rate determining protonated imine formation takes place to produce **I**; then this species react with enol form of β-keto-ester to form the ureside **II.** The final step is the cyclisation with concomitant loss of water.



Scheme 34: A Proposed Mechanism for the Synthesis of Dihydropyrimidinones

Spectroscopic analysis of **4n**-**4r**

4n: The **IR** spectra of **4n**, presented in **Figure 79** (Appendix 68, page 177) shows an absorption band at 1724.05 cm $^{-1}$ which was assigned to C=O stretching vibrations. The N-H and C-H stretching vibrations are observed in the region 3242.91 and 2981.16, respectively. The **^{1}H NMR** spectrum of **4n**, presented in **Figure 80** (Appendix 69, page 178), shows a triplet at δ 1.17 for the aliphatic methyl protons and δ 2.34 for the methyl protons attached to the aromatic portion. The methylene

proton (H-8) appeared as a multiplet at δ 4.01.The N-H protons gave a broad signal at δ 5.79 and 8.02 whilst H-4 is observed at δ 5.39. The aromatic protons are observed at δ 7.39 7.26 and 7.25. Mass spectral analysis of **4n, Figure 81** (Appendix 70, page 179) showed the molecular ion peak at m/z 261.1237 which further confirmed the structure.

4o: The **IR** spectra of **4o**, presented in **Figure 82** (Appendix 71, page 180) shows an absorption band at 1699.66 which was assigned to C=O stretching vibrations. The N-H and C-H stretching vibrations are observed in the region 3236.08 and 2956.97 respectively. The **^{1}H NMR** spectrum of **4o**, presented in **Figure 83** (Appendix 72, page181), shows a triplet at δ 1.10 for the aliphatic methyl protons and δ 2.32 for the methyl protons attached to the aromatic portion. The methylene proton (H-8) appeared as a multiplet at δ 4.00. The N-H protons gave a broad signal at δ 5.82 and 7.96 whilst H-4' is lacking due to the substitution of Cl group in position 4'. H-4 is observed at δ 5.31 whilst the aromatic protons are observed at δ 7.22 and 7.20. Mass spectral analysis of **4o, Figure 84** (Appendix 73, page 182) showed the molecular ion peak at m/z 261.1237 which further confirmed the structure.

4p: The **IR** spectra of **4p**, presented in **Figure 85** (Appendix 74, page 183) shows an absorption band at 1697.58 which was assigned to C=O stretching vibrations. The N-H and C-H stretching vibrations are observed in the region 3278.20 and 2983.18 respectively. The **^{1}H NMR** spectrum of **4p**, presented in **Figure 86** (Appendix 75, page 184), shows a triplet at δ 1.18 for the aliphatic methyl protons and δ 2.27 for the methyl protons attached to the aromatic portion. The methylene proton (H-8) appeared as a multiplet at δ 4.10. The N-H protons gave a broad signal at δ 5.95

and 8.26 whilst H-4 is observed at. H-4 is observed at δ 5.46. The aromatic protons are observed at δ 7.29, 6.81 and 6.09.

4q: The **IR** spectra of **4q**, presented in **Figure 87** (Appendix 76, page 185) shows an absorption band at 1701.48 which was assigned to C=O stretching vibrations. The N-H and C-H stretching vibrations are observed in the region 3240.95 and 2981.26 respectively. The **^{1}H NMR** spectrum of **4q**, presented in **Figure 88** (Appendix 77, page 186), shows a triplet at δ 1.14 for the aliphatic methyl protons and δ 2.30 for the methyl protons attached to the aromatic portion. The methylene proton (H-8) appeared as a multiplet at δ 4.08. The N-H protons gave a broad signal at δ 5.91 and 8.31 whilst H-4 is observed at δ 5.33. The aromatic protons are observed at δ 7.18 and 7.16. Mass spectral analysis of **4q, Figure 89** (Appendix 78, page 187) showed the molecular ion peak at m/z 297.1220 which further confirmed the structure.

4r: The **IR** spectra of **4r**, presented in **Figure 90 (**Appendix 79, page 188) shows an absorption band at 1699.24 which was assigned to C=O stretching vibrations.

The N-H and C-H stretching vibrations are observed in the region 3238.16 and 2980.85, respectively. The **^{1}H NMR** spectrum of **4q**, presented in **Figure 91** (Appendix 80, page 189), shows a triplet at δ 1.18 for the aliphatic methyl protons and δ 2.34 for the methyl protons attached to the aromatic portion. The methylene proton (H-8) appeared as a multiplet at δ 4.07. The N-H protons gave a broad signal at δ 5.85 and 8.81 whilst H-4 is observed at δ 5.36. The aromatic protons are observed at δ 7.23 and 6.85. Mass spectral analysis of **4r,** presented in **Figure 92** (Appendix 81, page 190), showed molecular ion peak at m/z 313.1169 which further confirms product formation. **Table 9** shows ^{1}H NMR chemical shifts for compounds **4n-4r**

Table 9: ^{1}H NMR chemical shifts for compounds 4n-4r δ of 1H (J, Hz)

H	**4n**	**4o**	**4p**	**4q**	**4r**
NH-1	8.02(s)	7.96s)	8.26(s)	8.31(s)	8.18(s)
NH-3	5.79(s)	5.82 (s)	5.95(s)	5.91(s)	5.85 (s)
4	5.39(d,J=1.48)	5.31 (d,J=1.44)	5.46(d,J=2.52)	5.33(s)	5.36(s)
2'/6'	7.39(d,J=4.28)	7.22 (t,J=4.16)	-	7.18(d,J=7.92)	7.23(t,J=9.16)
3'/5'	7.26 (t,J=1.72)	7.20 (t,J=1.32)	6.81 (d,J=8.60)	7.16(t,J=7.84)	6.85 (d,J=7.84)
4'	7.25(J=1.92)	-	6.09 (J=3.08)	-	-
8	4.01 (m,J=11.88;9.24)	4.00 (dd,J=1.28;1.00)	4.10 (dd,J=4.28;3.76)	4.03 (m,J=7.08;7.1 2	4.07 dd,J=1.36;1.2 8
10	2.34 (s)	2.32 (s)	2.27 (s)	**-**	2.34(s)
9	1.17(t,J=7.08)	1.10 (t,J=7.08)	1.18 (t,J=7.12)	1.14(t,J=7.12)	1.18(t,J=7.12)
10/4''(CH_3)	-	-	-	2.30 (d,J=2)	-
4''(OCH_3)	-	-	-	-	3.79 (s)
3'	-	-	7.29	-	-
2'	-	-	6.81	-	-

Antimicrobial activity

The antimicrobial activity of the synthesized compound was investigated using the well diffusion method and the results (**Table 10**) confirms that none of the compounds inhibited the bacteria and fungus organism used. **Figure 12** shows antibacterial screening with zones of inhibition produced by Ciprofloxacin (positive control) (A) and no inhibition zone of compound 4f against *M. Luteus*

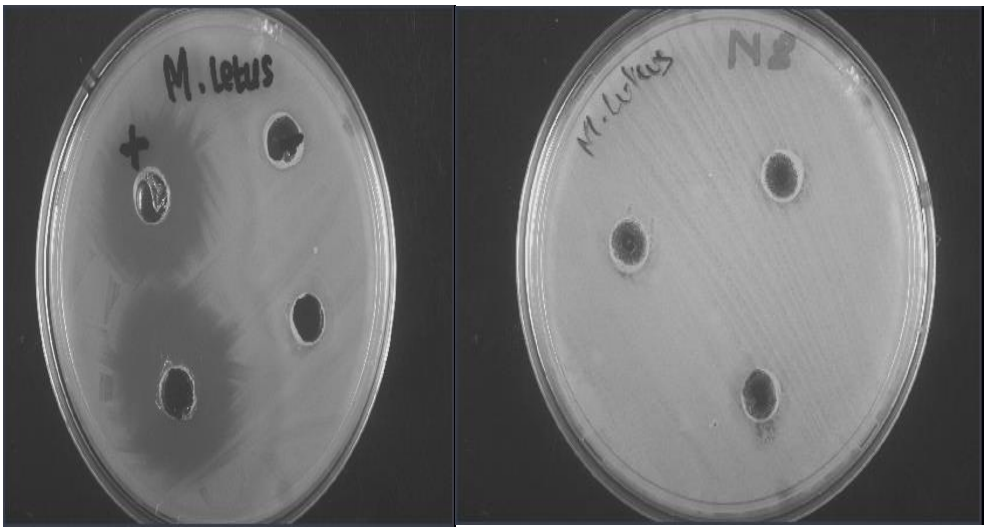


Figure 12: Antibacterial screening showing zones of inhibition produced by Ciprofloxacin (positive control) (A) and no inhibition zone of compound 4f against M. Luteus

Table 10 : Antimicrobial Activity of the compounds

Bacteria	**4a**	**4f**	**4g**	**4j**	**4k**	**4l**	**4m**	**4n**	**4o**	Control Ciprofloxacin
B. cereus	0	0	0	0	0	0	0	0	0	30.0 ± 0.0
E. coli	0	0	0	0	0	0	0	0	0	32.5 ± 0.7
M. luteus	0	0	0	0	0	0	0	0	0	28.5 ±0.7
Fungus										Control Amphotericin B
C. albicans	0	0	0	0	0	0	0	0	0	31.5 ± 0.7

Values are mean ±SD (n=3), 0: no activity

Further investigation needs to be undertaken on other strains of bacteria and fungi in order to assess the profile of the novel synthesized compounds

Safety of compounds

The brine shrimp lethality assay is considered to be a functional instrument for preliminary assessment of toxicity. It is additionally a functional tool for the isolation of bioactive compounds from plant extract. The method is appealing because it is easy, and inexpensive (Krishnaraju *et al.* 2005). In this study, the brine shrimp larvae

(*Artemia salina*) were hatched in sea water for 24-48 h prior to being used in the test. An aliquot of 5 mL sea water and ten brine shrimp were added to each vial and treated with 100 µL of each compounds concentration. The dead ones were counted after 24 h (Meyer *et al.* 1982a). According to Meyer *et al*. (1982), the compound has to reveal brine shrimp death of less than 50% in order for a test compound to be considered not lethal. The cytotoxicity of some selected synthesized compounds were evaluated using organophosphate as a standard; the results were summarized in the **Table 11**. It was observed that the compounds displayed selective toxicity toward the brine shrimp. Compounds **4a**, **4f**, **4g**, **4k**, **4l**, **and 4n** showed brine shrimp death of 62, 53, 71, 55, 65 and 63% and were considered toxic. Hence these results indicate that these compounds may not be suitable as potential new drug type molecules. Compounds **4j**, **4m, 4o and 4r** was found to be less toxic to the brine shrimp and may have more valuable biological application. Therefore, these compounds should be further investigated for several biological activities.

Statistical analysis

Mortality was reordered as mean percentage of the death *Artemia* after 24 h exposure. Results are expressed as mean ± standard deviation

Table 11: Brine shrimp assay

Compounds	Concentrations µg/mL		
	1000	**100**	**10**
	Percentage death of brine shrimp (Mean ±SD)		
4k	65±0,70	40±0,70	30±0.0
4l	55±0,70	50±0,00	25±0.0
4m	30±0,00	25±0.0	0
4j	45±2,12	35±0,70	0
4r	40±1,41	20±0,70	0
4g	71±2.08	36±0.57	30±0.0
4a	62±1.52	33±0.57	0
4f	53±0.5	30±0.7	0
4o	44±1.52	33±0.57	0
4n	63±0.55	30±0.55	0
DMSO	0	0	0
Organophosphate	0	0	0

Values are expressed as mean (±SD), n=3

Molecular docking prediction

Molecular docking studies were carried out for all the synthesized APs compound of (R/S) isomer to estimate the binding interactions between HIV-1 reverse transcriptase and APs. The (R)-isomer of **4i**, **4j**, **4k** and **4m** were found to have strong interaction with HIV-1 reverse transcriptase with the docking score of (-8.5, -8.8, -7.0 and -7.7) (Table 12). In case of **4i**, **4j**, Tyr 181 formed a pi-pi interaction with chromone ring leading to higher docking score whereas this Pi-pi interaction was lost in **4k** leading to lower docking score. In **4m**, cation-pi interaction was observed lys73 (**Figure 13 and Figure 14**). In addition, the (S)-isomer of **4f**, **4k**, **4l** and **4m** also exhibited strong interaction with HIV-1 reverse transcriptase (-7.1, -7.9, -8.1, -7.4). The molecular docking studies reveal that **4f**, **4i**, **4j**, **4k**, **4l** and **4m** as potential candidates for the inhibition of HIV-1 reverse transcriptase.

Table 12: Molecular docking scores of APs inside the binding site of HIV-1

No.	Code Number	Name of compound	Binding energies of of (R/S) in kcal/mol
1.	BCOXO-A (**4a**)	(4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester	-6.4 / -6.7
2.	BCOXO-4CL (**4b**)	(4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester	-6.5 / -6.8
3.	BCOXO-4Br (**4c**)	(4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester	-6.5 / -6.7
4.	BCOXO-2NA (**4d**)	(4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester	-6.5 / -6.7
5.	BCOXO-OT (**4e**)	(4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester	-6.6 / -6.7
6.	BCOXO-3,4DCLA (**4f**)	(4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester	-6.8 / -7.1
7.	BCOXO-PT (**4g**)	(4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester	-6.6 / -6.7
8.	BCOXO-OAN (4h)	(4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester	-6.2 / -6.1
9.	BCOXO-PA (**4i**)	(4-oxo-4H-chromen-3-yl)-(4-methoxyphenylamino) methanephosphonic acid diethyl ester	-8.5 / -6.6
10.	BCOXO-3NA (**4j**)	(4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester	-8.8 / -6.8
11.	BCD-OXO-A (**4k**)	[(4-oxo-4H-benzo[*h*]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester	-7.0 / -7.9
12.	BCD-OXO-PT (**4l**)	[(4-oxo-4H-benzo[*h*]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester	-6.9 / -8.1
13.	BCD-OXO-4BR (**4m**)	[(4-oxo-4H-benzo[*h*]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester	-7.7 / -7.4

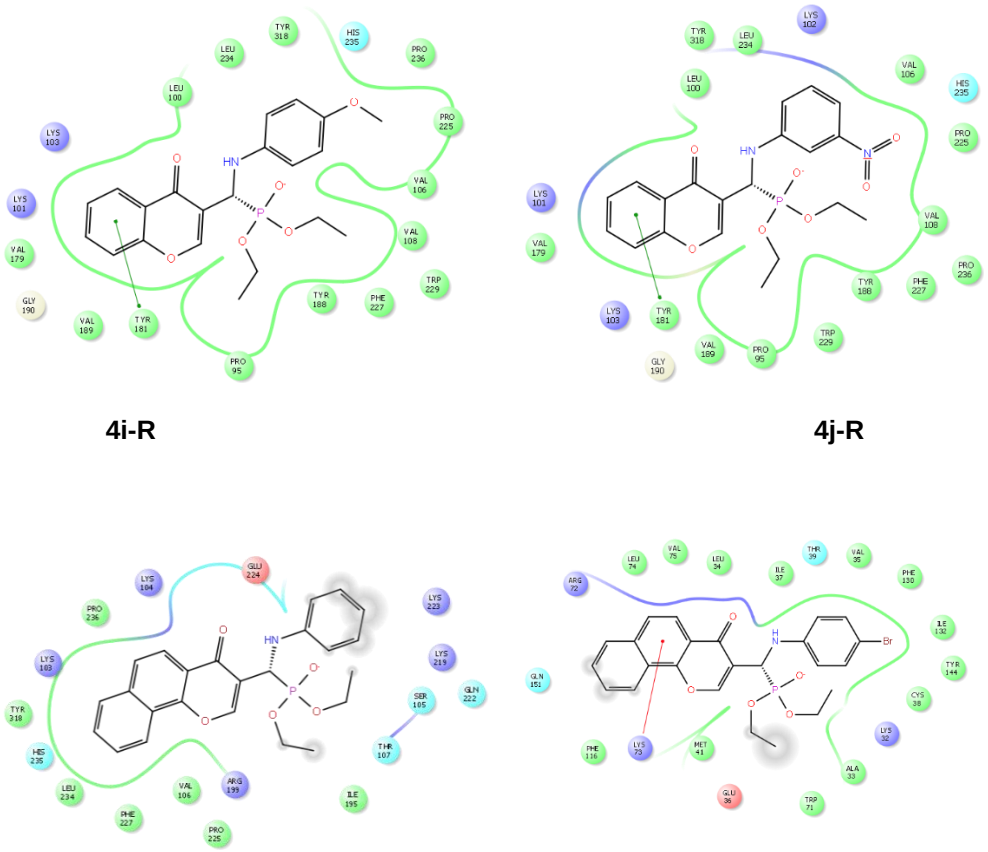


Figure 13: 2D interaction map of APs with HIV-1 reverse transcriptase obtained from molecular docking

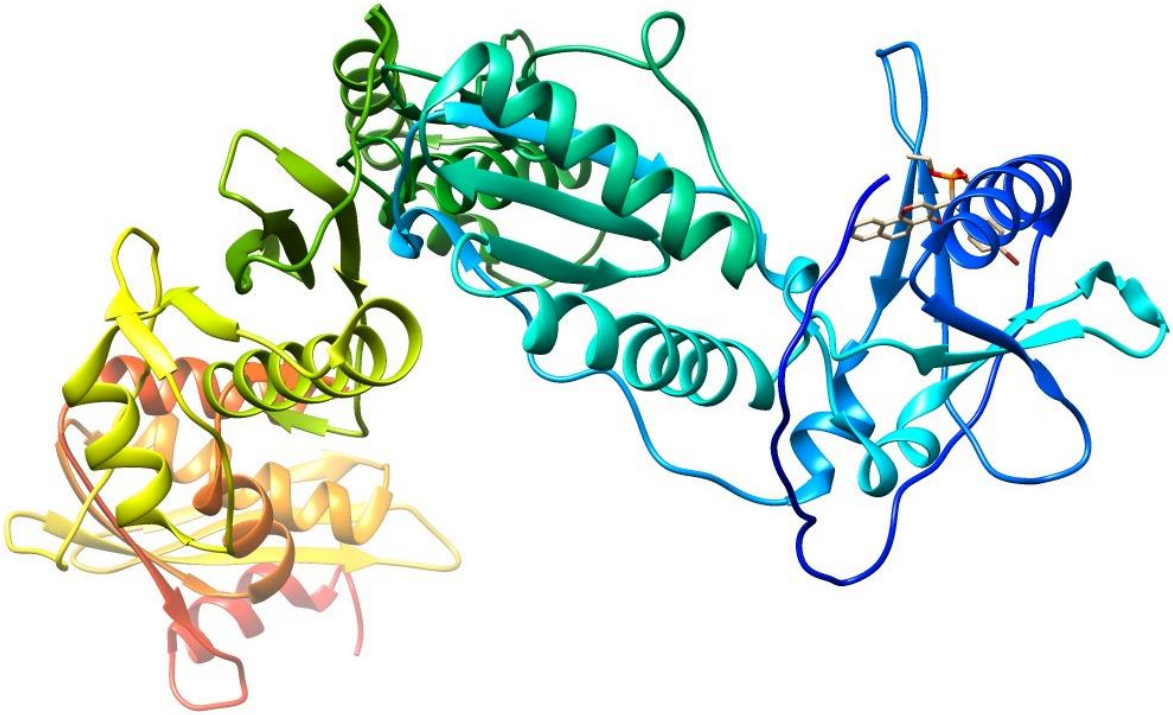


Figure 14: 3D interaction map of 4m with HIV-1 reverse transcriptase obtained from molecular docking

CONCLUSION

A novel heterogeneous rhodium loaded on boron nitride catalyst was prepared and characterized by several instrumental techniques. Furthermore, this material was used to prepare novel chromone-bearing α-aminophosphonates via the Kabachnik-Fields reaction and dihydropyrimidinones via the Biginelli reaction. Eighteen compounds were synthesized and fully characterized. The catalyst is stable and does not show any decrease in catalytic activity hence it can be used more than four times. The yields of the products are good. The reaction proceeds with high efficiency to give the corresponding aminophosphonates and dihydropyrimidinones which are extremely useful in the construction of biologically important compounds. This methodology offers significant advantages in terms of cleaner conversion, higher yield, and simplicity of operation, cost effectiveness and excellent reusability of the catalyst.

Antimicrobial activities of the synthesized compounds were investigated using the well diffusion method. It was found that none of the compounds inhibited growth of both fungus and bacteria. Hence they cannot be used against any of these organisms. It is recommended that further investigation be undertaken on other organism to assess the biological profile of the novel synthesized compounds.

The toxicity of selected ten compounds were evaluated using the brine shrimp assay. It was found that the compounds exhibited selective toxicity towards the brine shrimp which means six were found to be toxic to the brine shrimp and may not be suitable as potential new drug type molecules whilst four of the evaluated compounds were found to be less toxic to the brine shrimp and may have more

valuable biological applications. Therefore, these compounds should be investigated for other biological activities.

Molecular docking was conducted for the synthesized α-aminophosphonates derivatives since most aminophosphonates are known inhibitors of HIV-1 reverse transcriptase. Molecular docking studies suggested four compounds (**4a**, **4i**, **4j** and **4k**) as potential candidates for the inhibition of HIV-1 reverse transcriptase. The study may help in the discovery of novel molecules against acquired immune deficiency syndrome (AIDS).

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Appendix 1: ^{1}H-NMR spectrum of 4 oxo-chromene-3-carbaldehyde

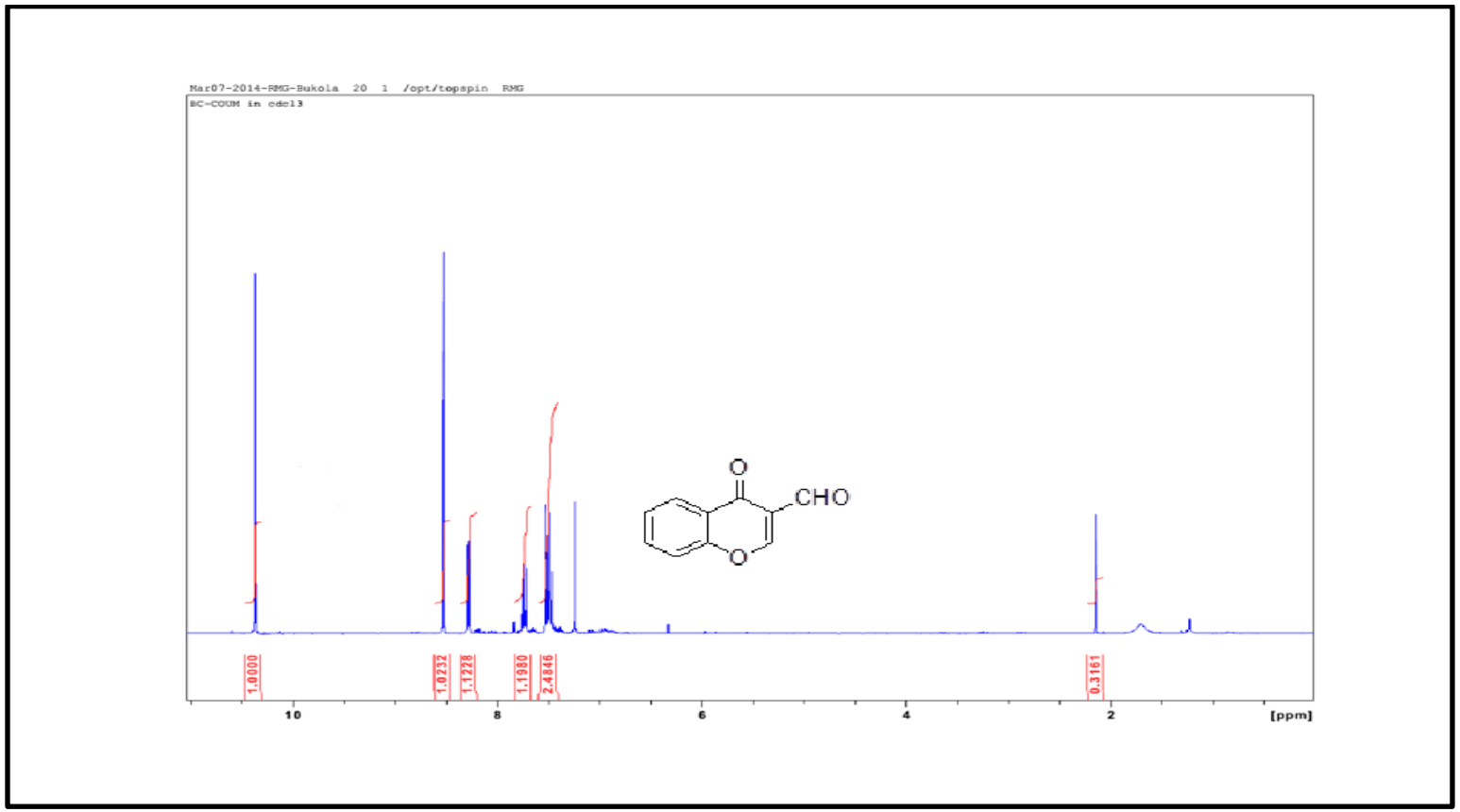


Figure 15

Appendix 2: IR Spectrum of 4 oxo-chromene-3-carbaldehyde

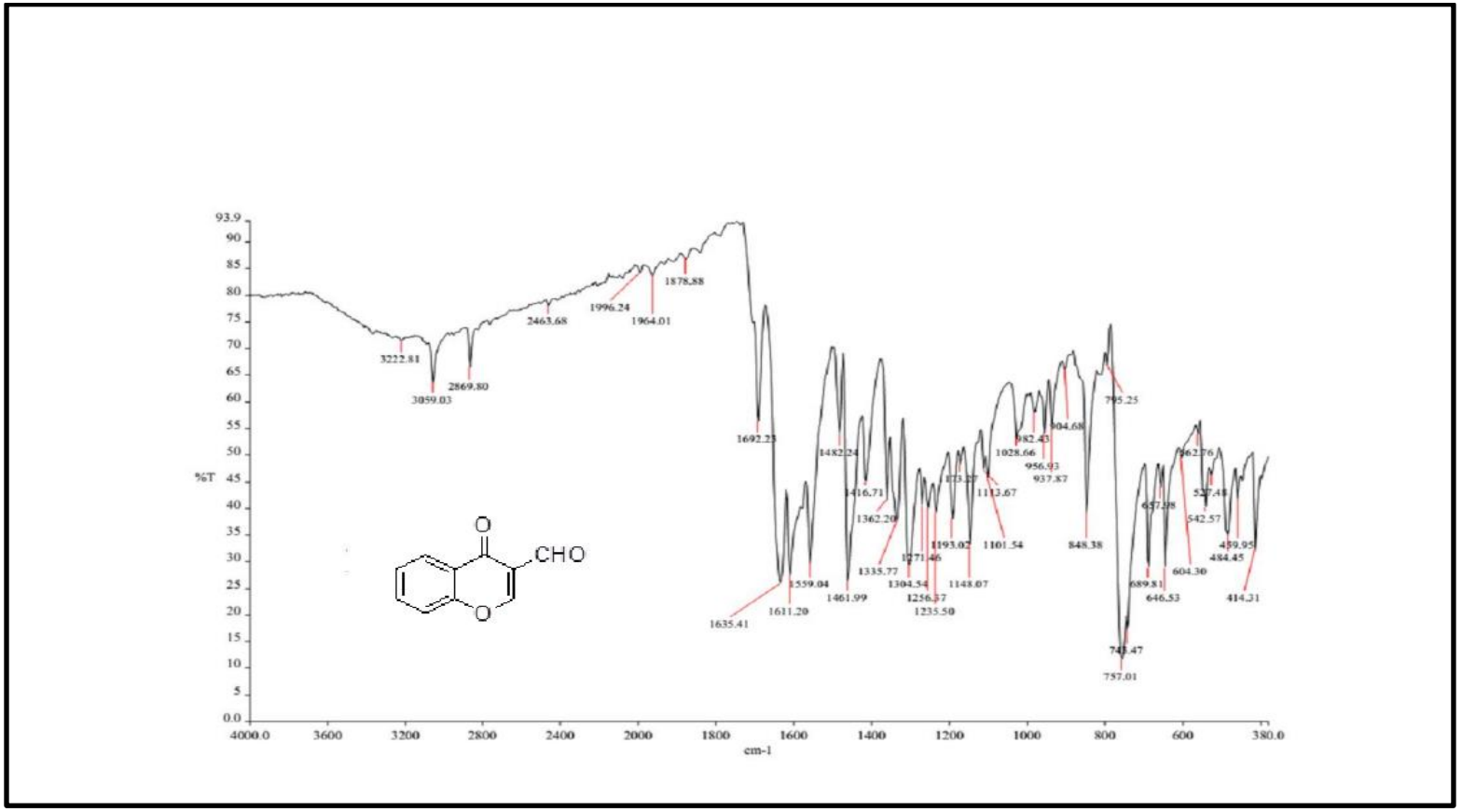


Figure 16

Appendix 3: ^{1}H-NMR spectrum of 4-oxo-4 H-benzo[h] chromene-3-carbaldehyde

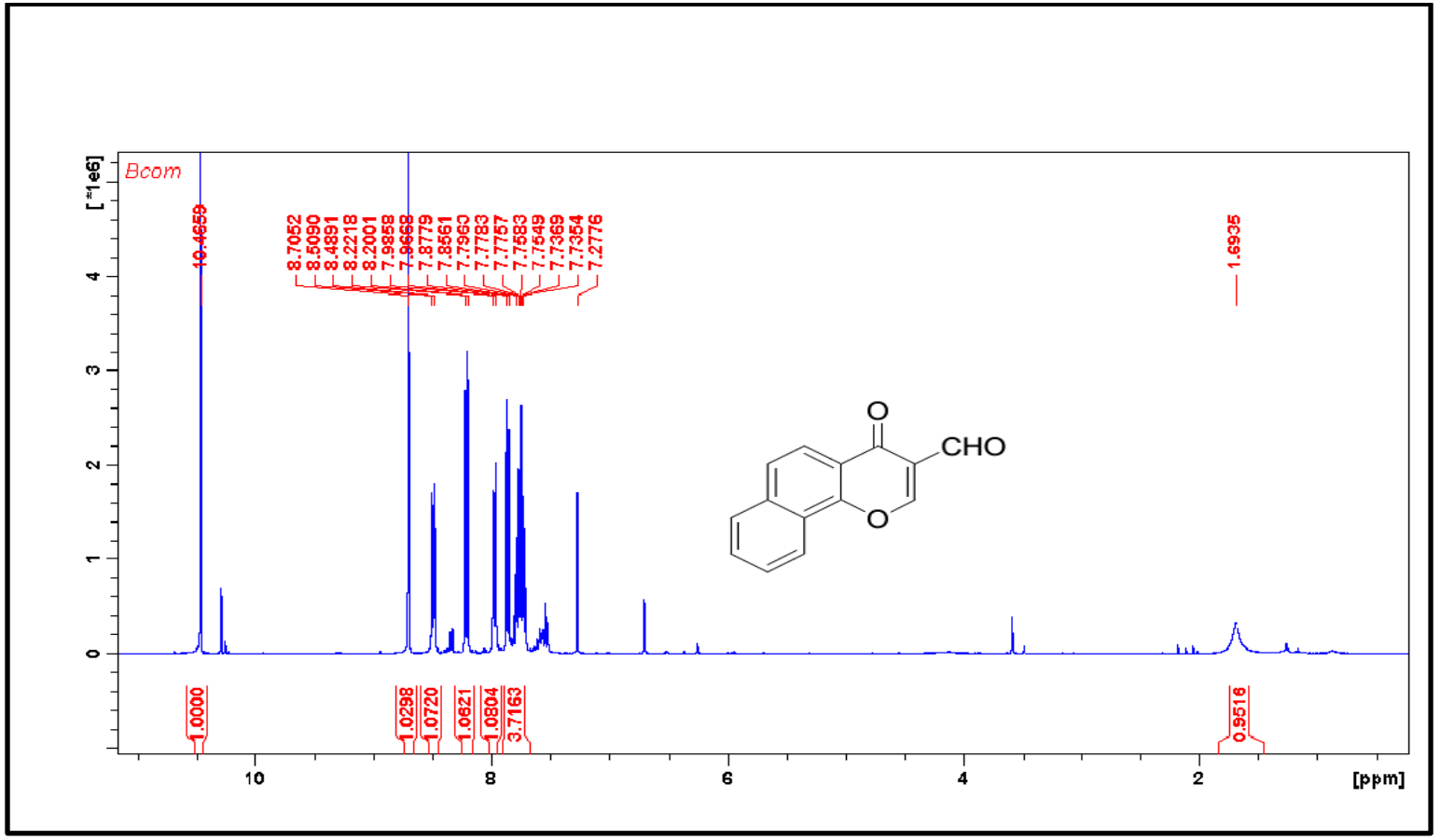


Figure 17

Appendix 4: IR spectrum of 4-oxo-4 H-benzo[h] chromene-3-carbalde**hyde**

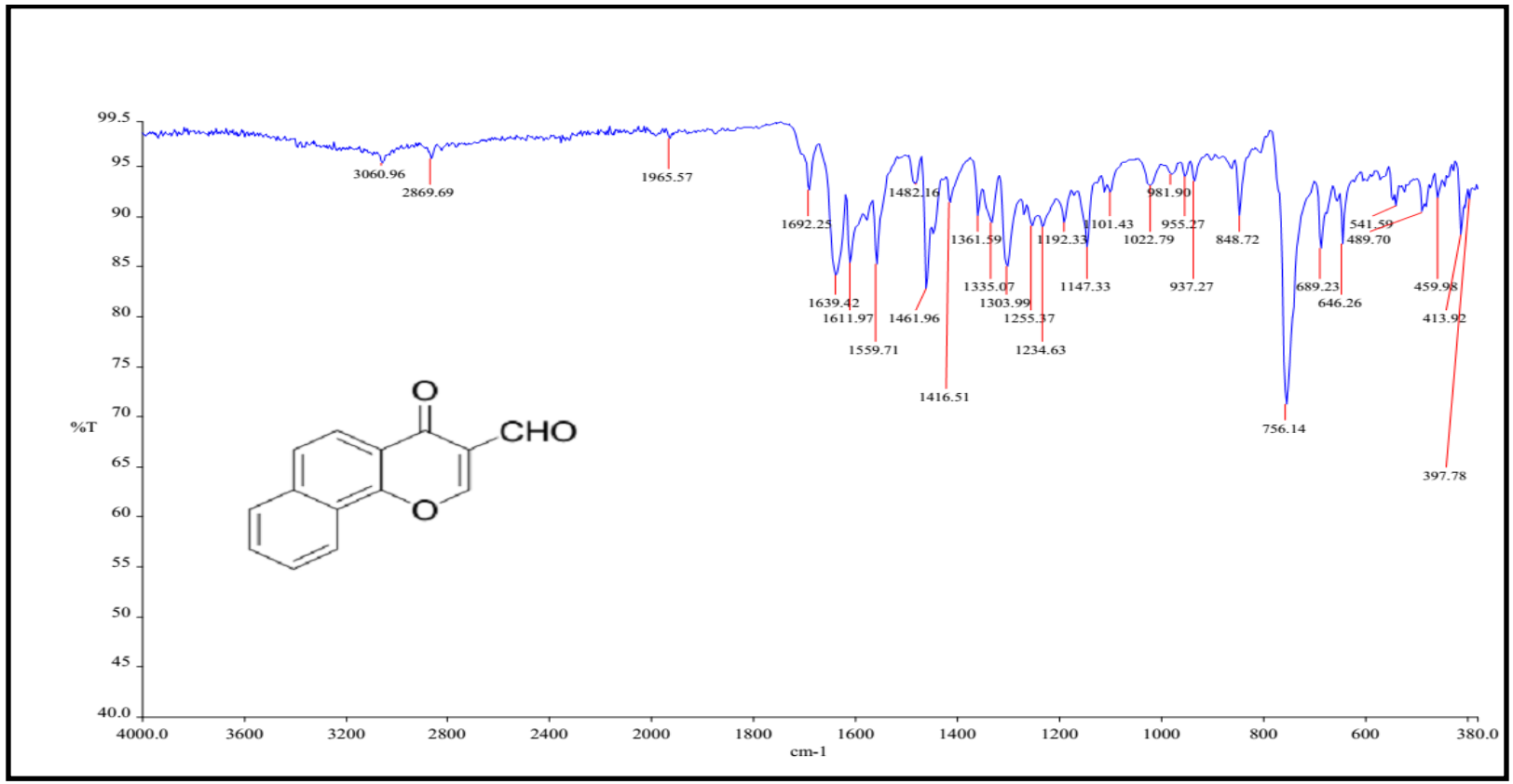


Figure 18

Appendix 5: IR Spectrum of (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester

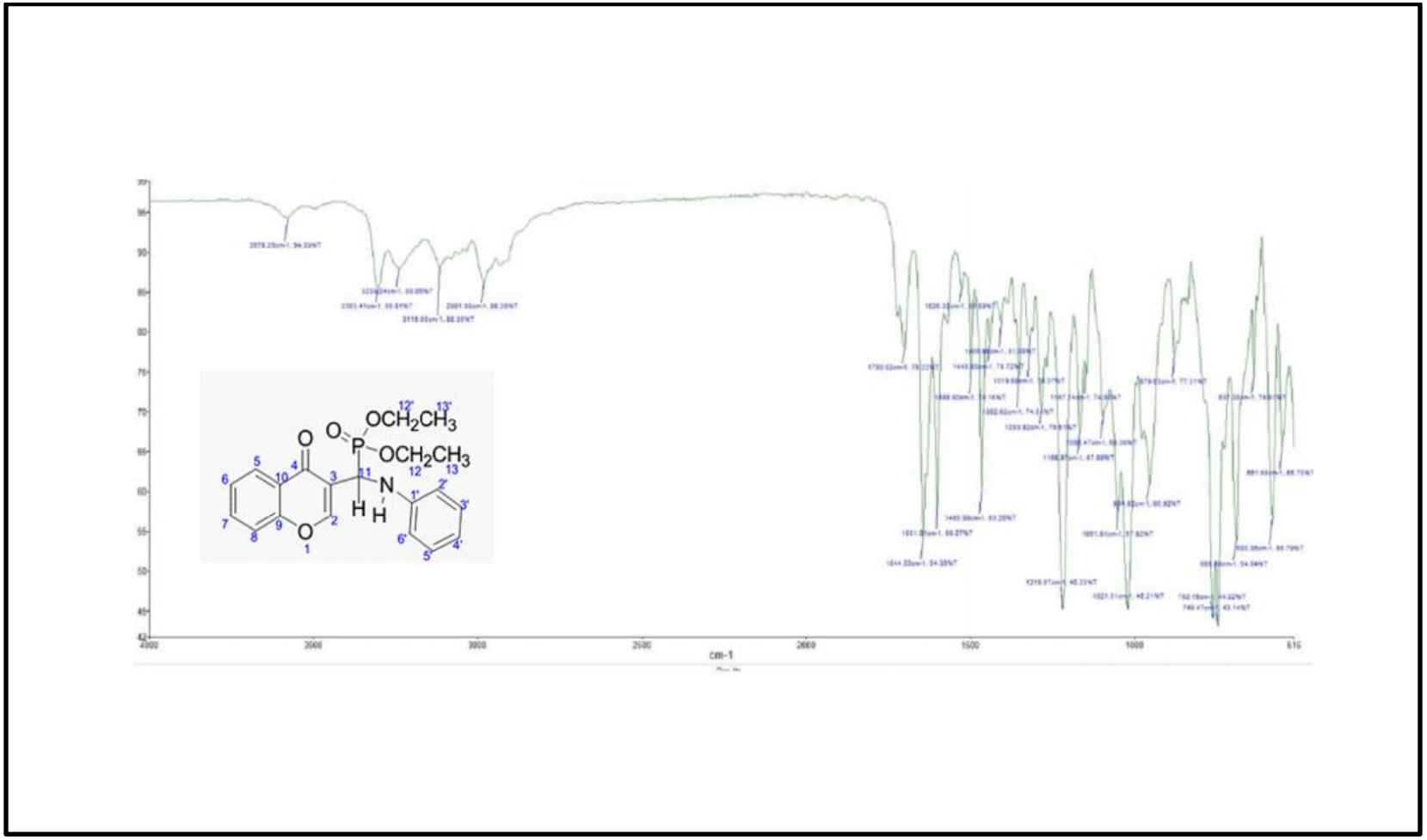


Figure 19

Appendix 6: ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester

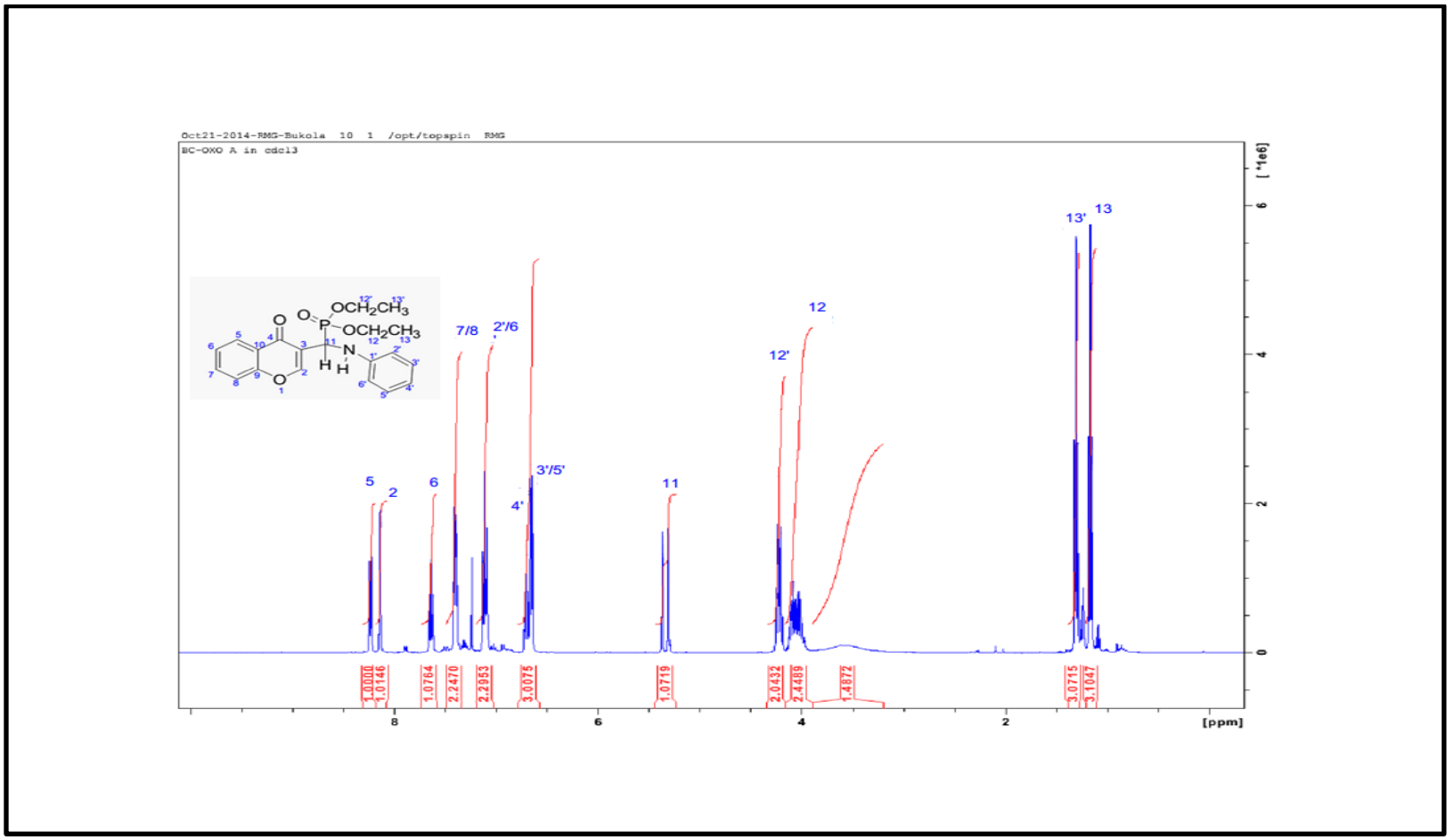


Figure 20

Appendix 7: ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester

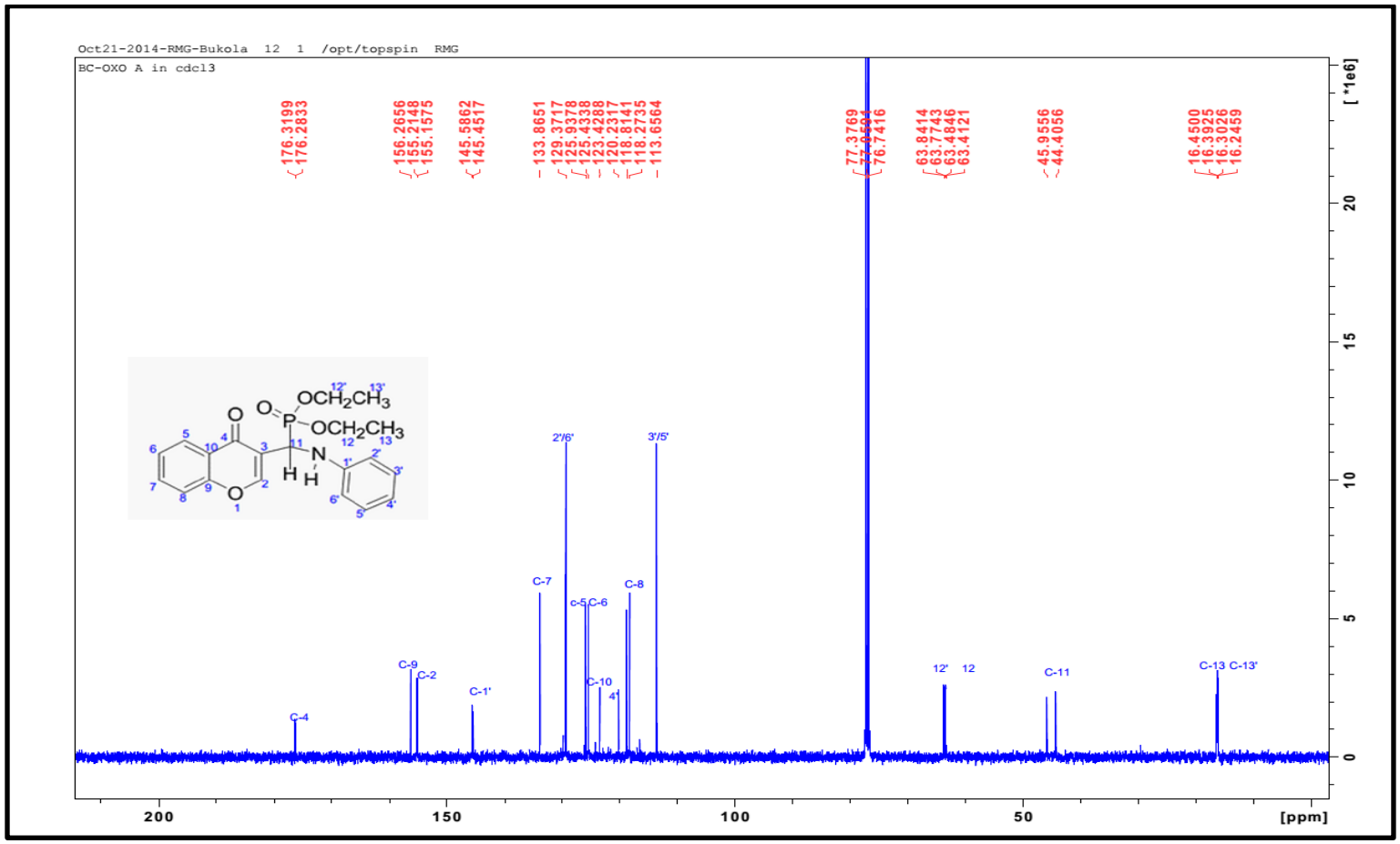


Figure 21

Appendix 8: ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester

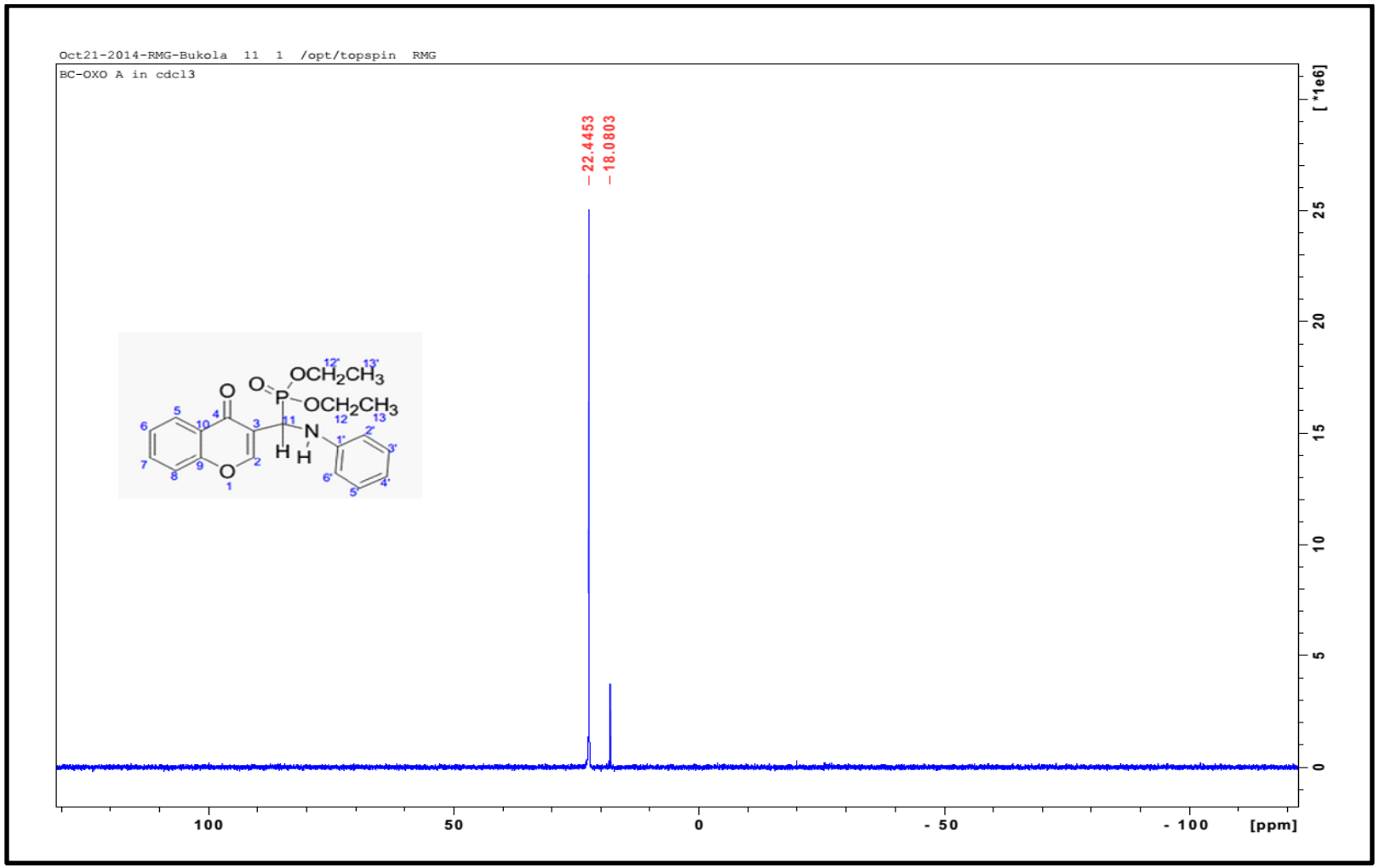


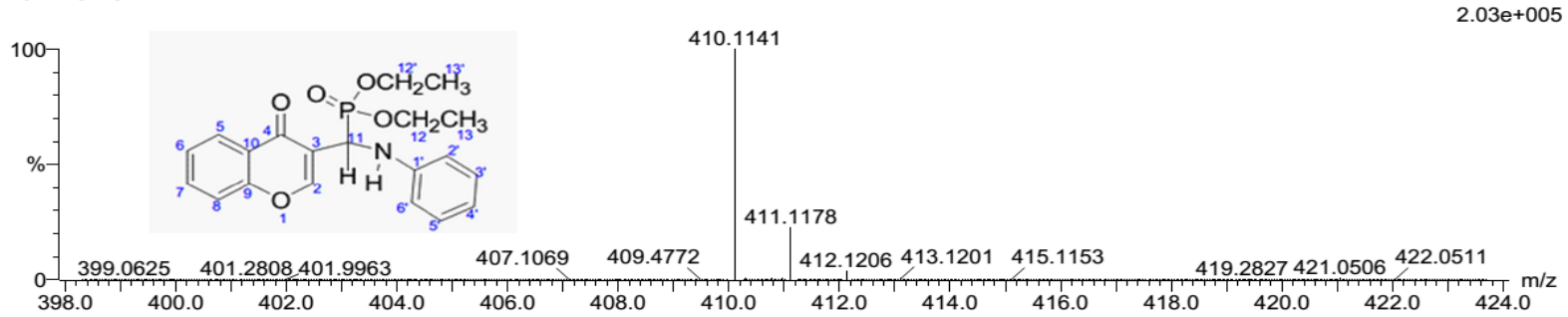
Figure 22

Appendix 9: Mass Spectrum of (4-oxo-4H-chromen-3-yl)-1-(phenylamino) methanephosphonic acid diethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
15 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 0-5 Na: 1-1 P: 1-1
BC-OXO-A 34 (1.114) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
410.1141	410.1133	0.8	2.0	10.5	566.0	0.0	C20 H22 N O5 Na P

Figure 23

Appendix 10: IR Spectrum of (4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester

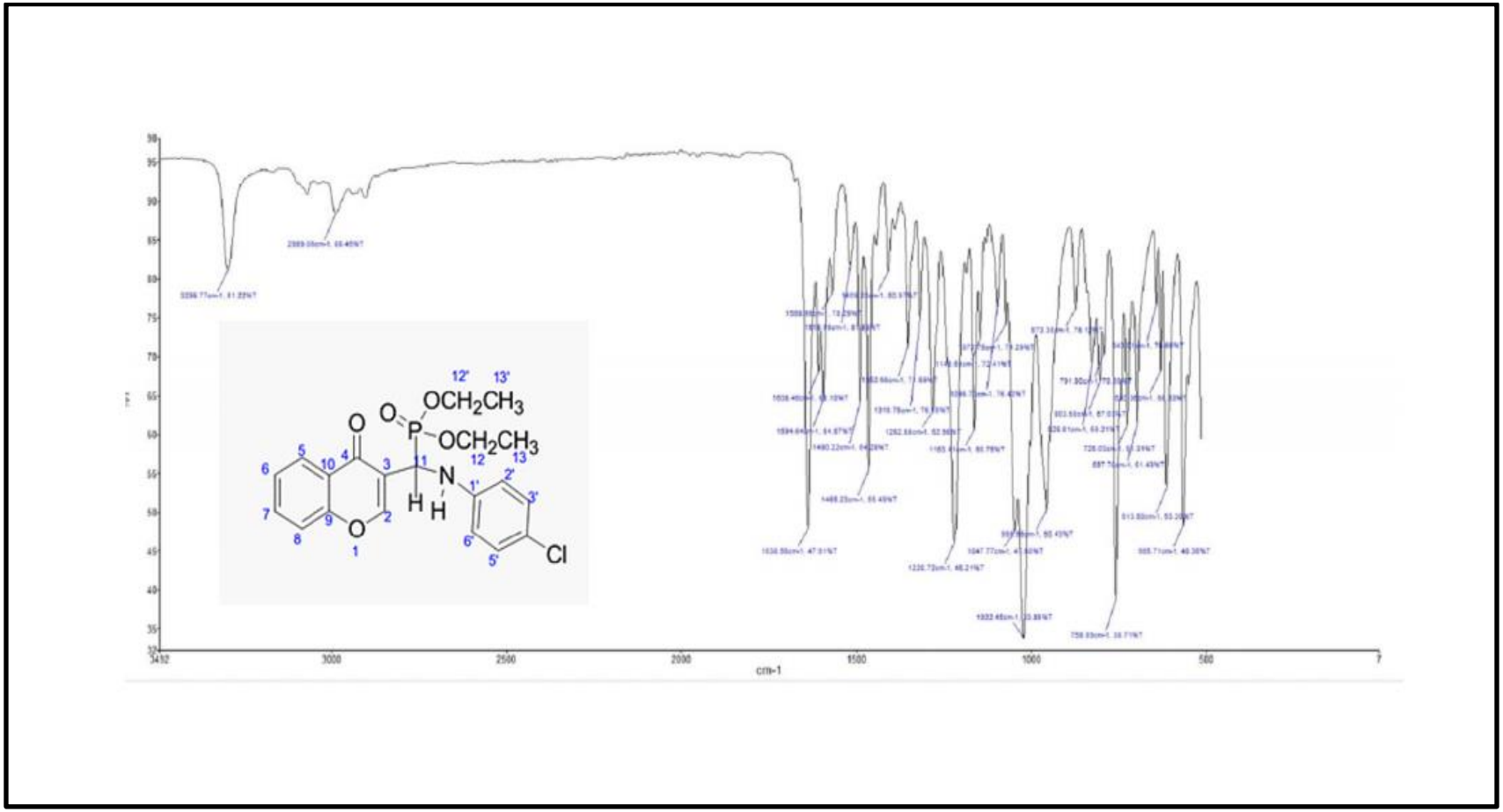


Figure 24

Appendix 11: ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester

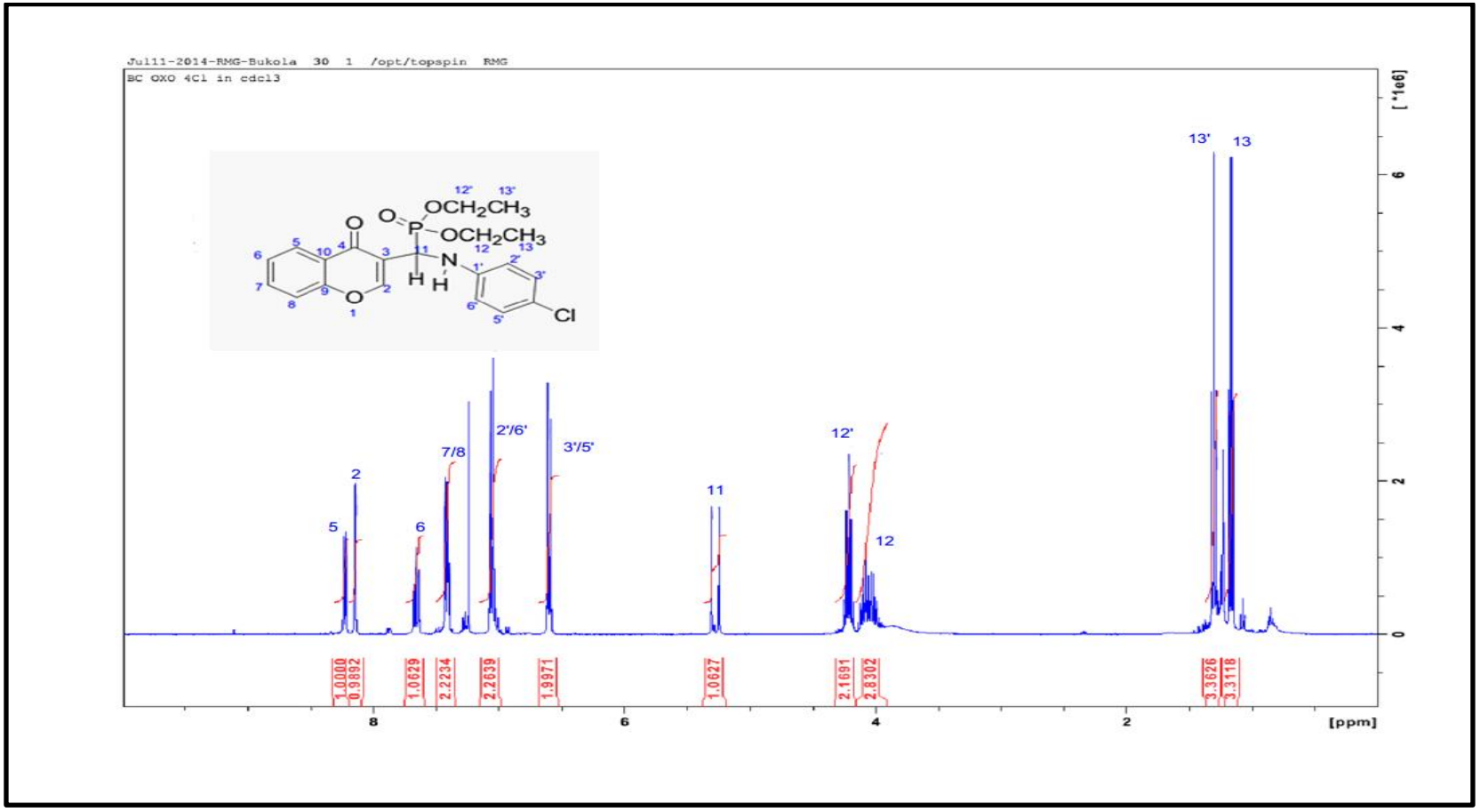


Figure 25

Appendix 12: ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester

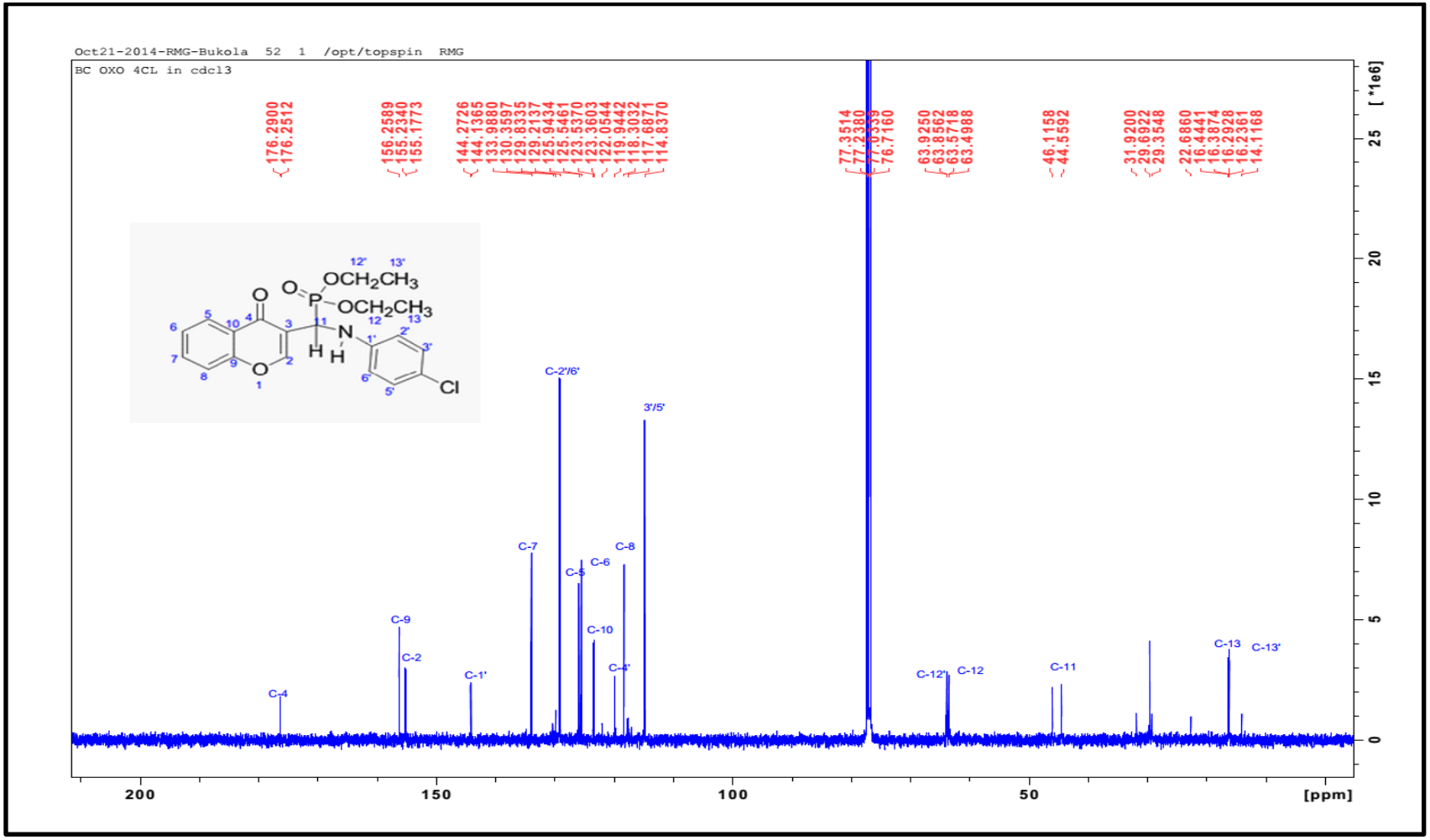


Figure 26

Appendix 13: ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(4-chlorophenylamino) methanephosphonic acid diethyl ester

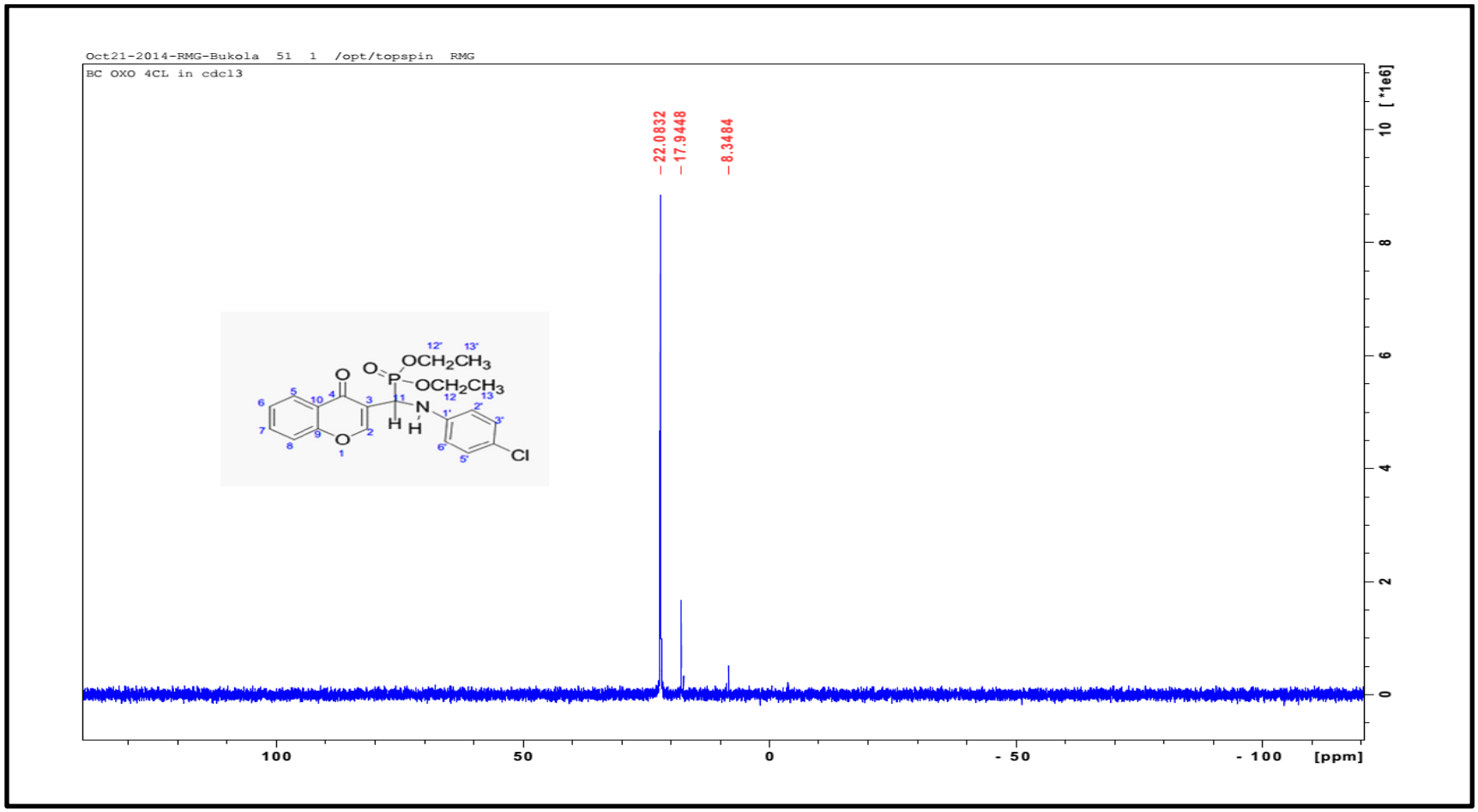


Figure 27

Appendix 14 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester

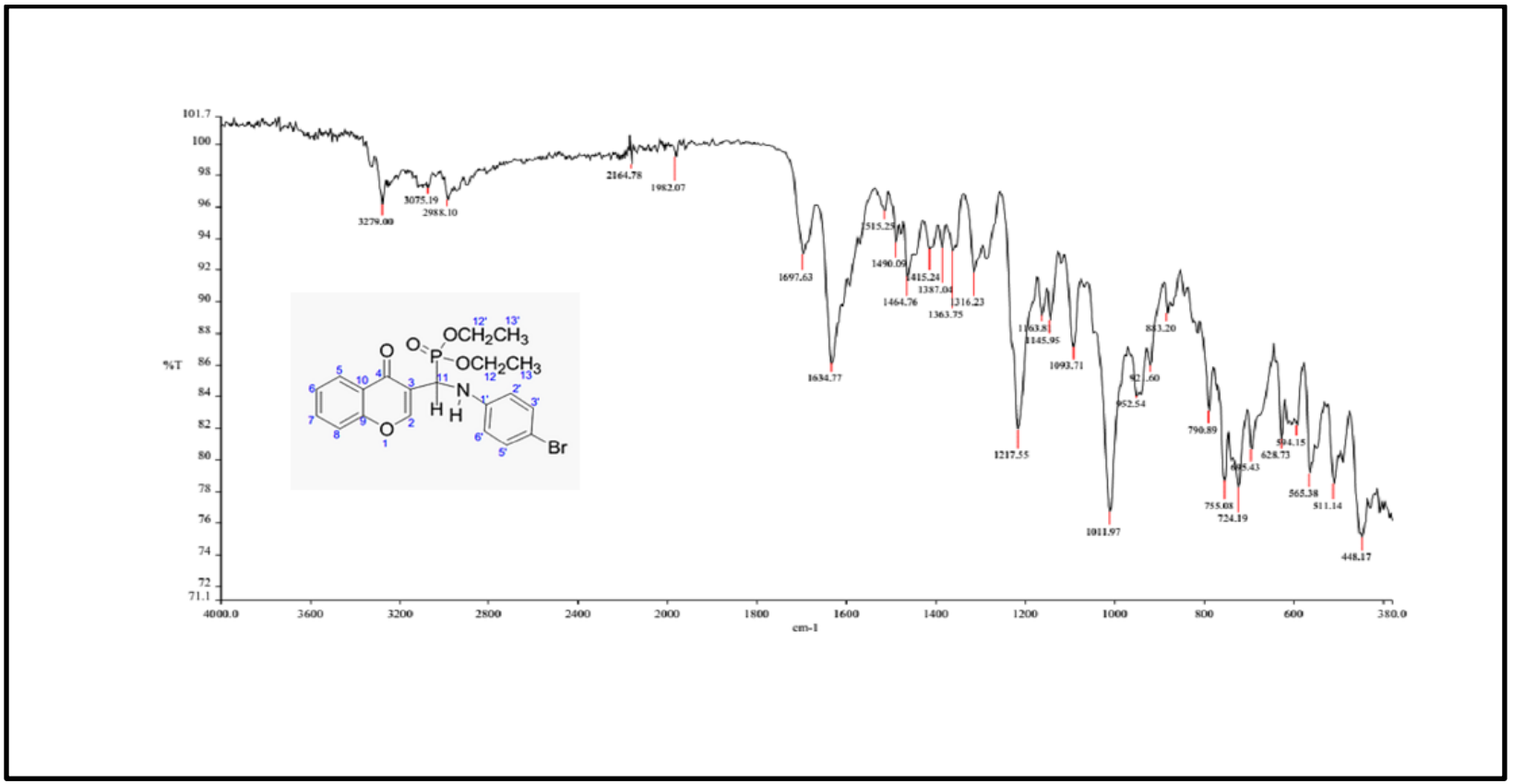


Figure 28

Appendix 15 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester

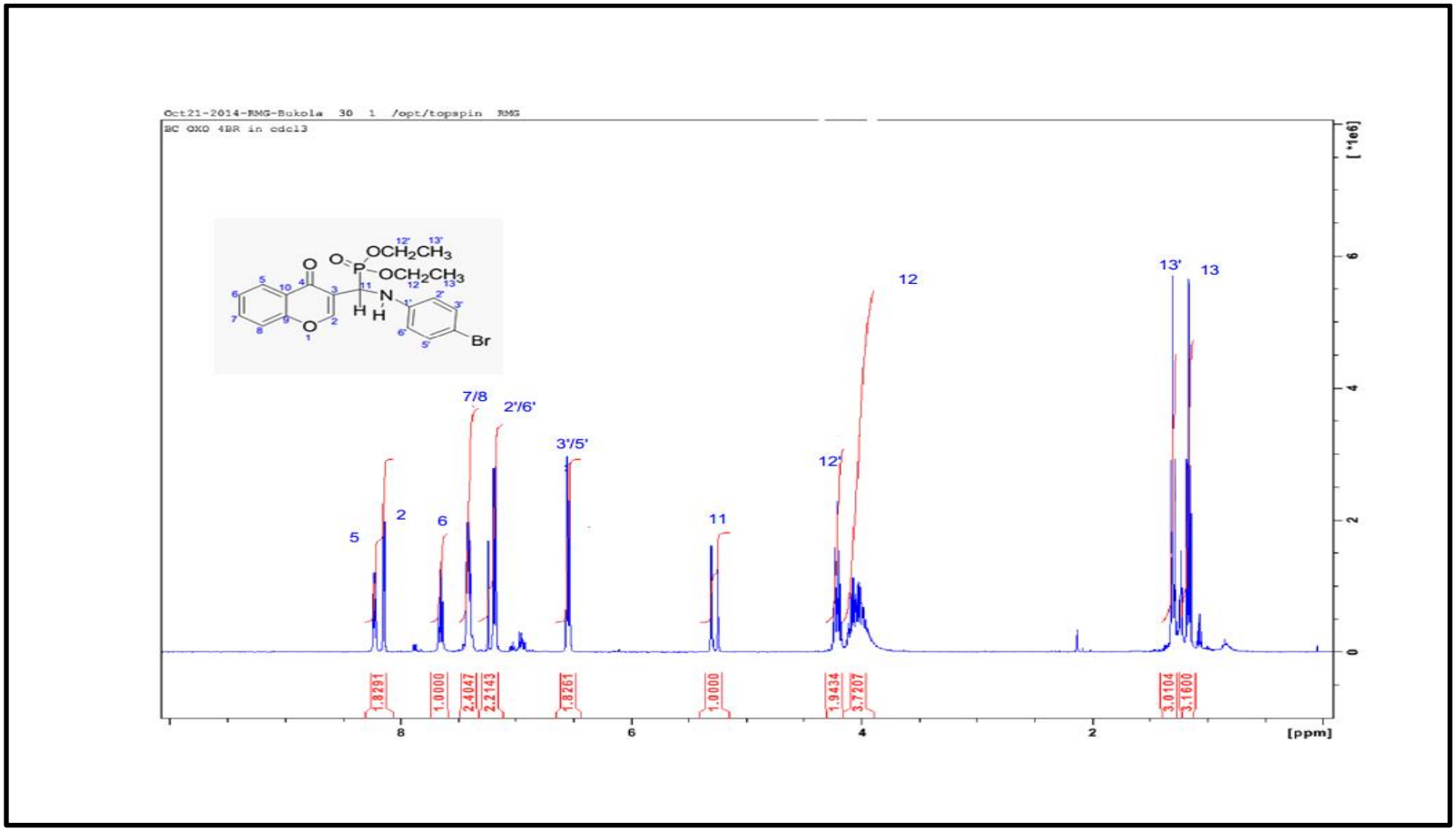


Figure 29

Appendix 16 : ^{13}C-NMR Spectrum of (4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester

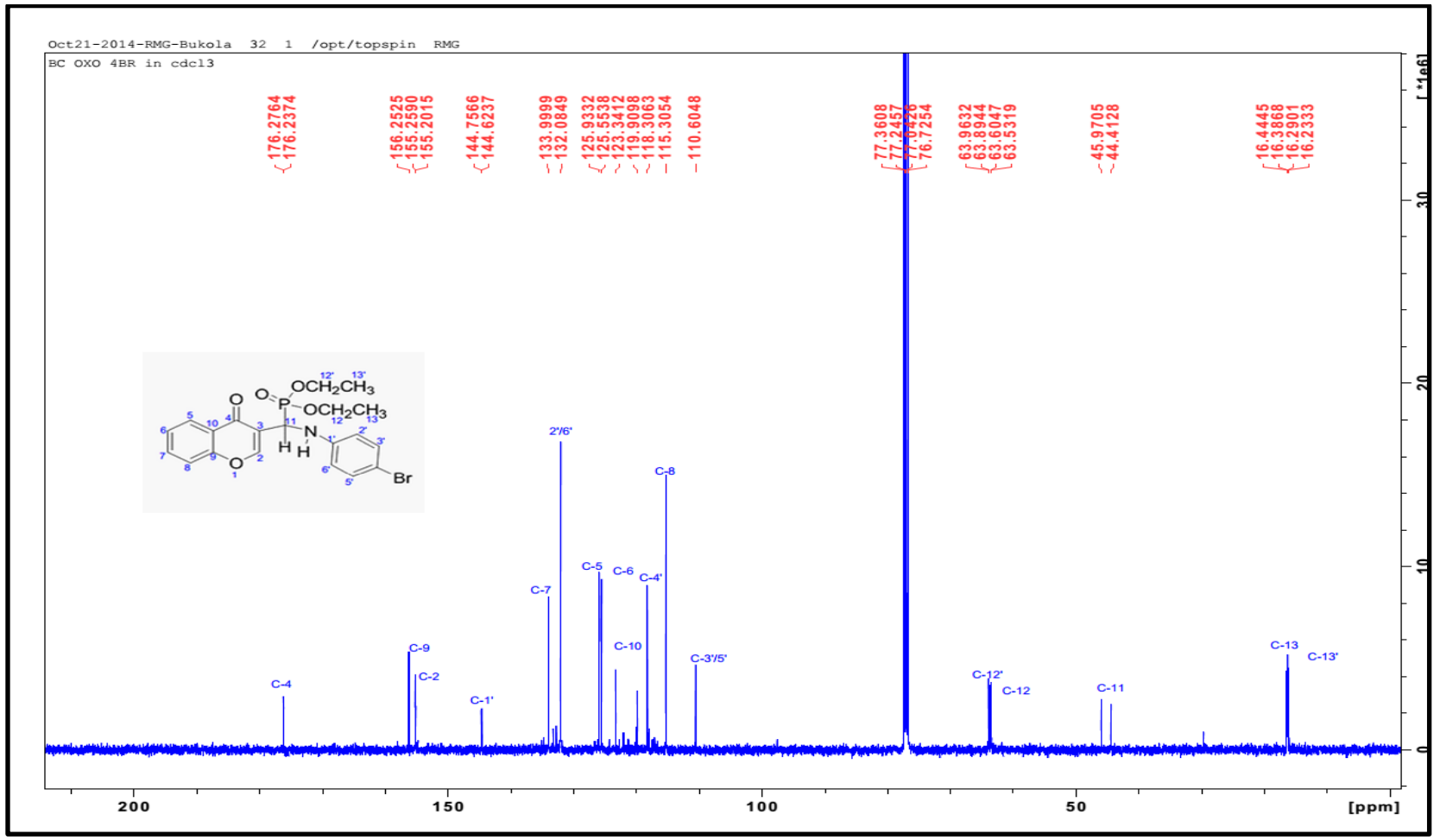


Figure 30

Appendix 17: ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(4-bromophenylamino) methanephosphonic acid diethyl ester

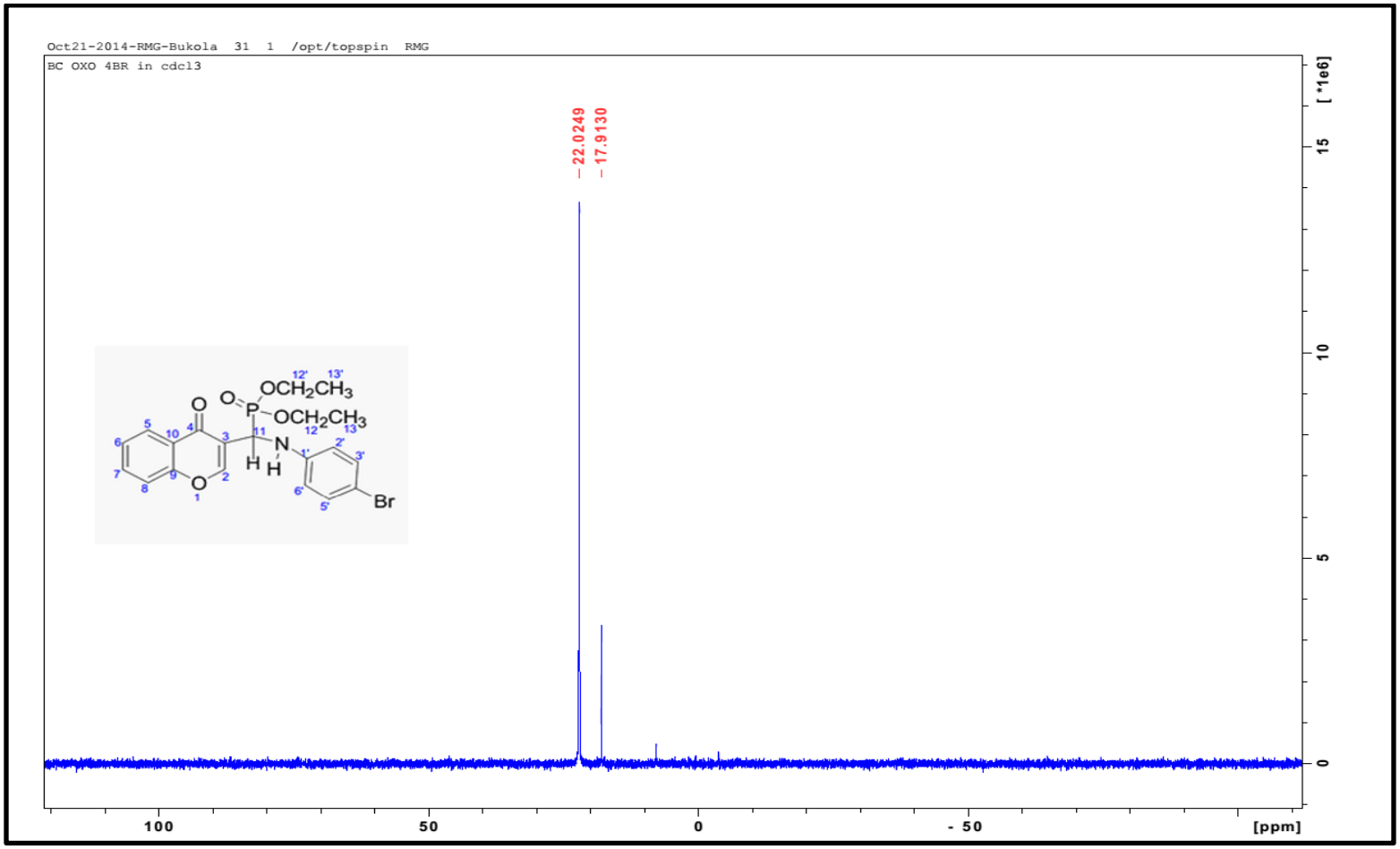


Figure 31

Appendix 18 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester

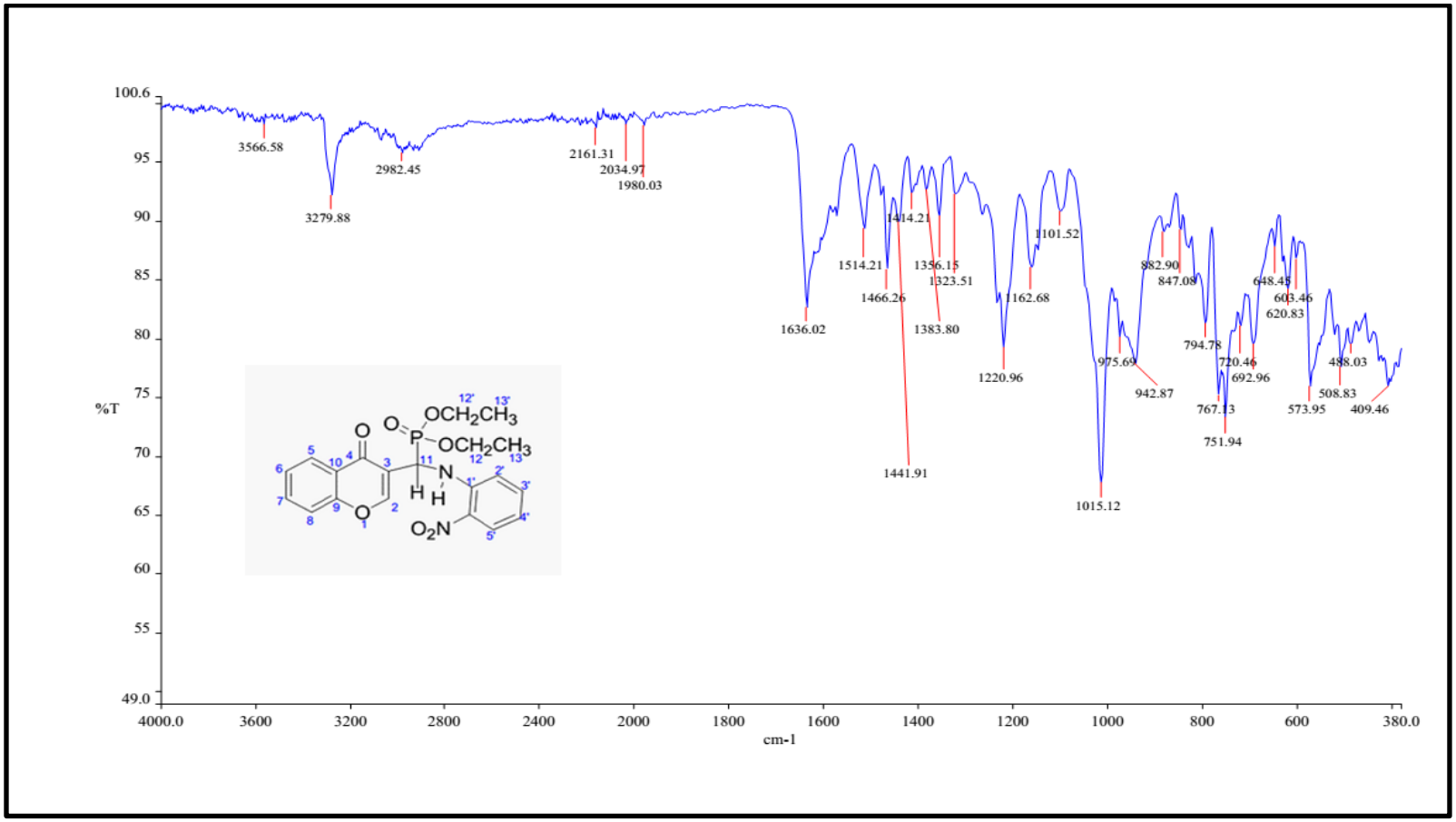


Figure 32

Appendix 19 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester

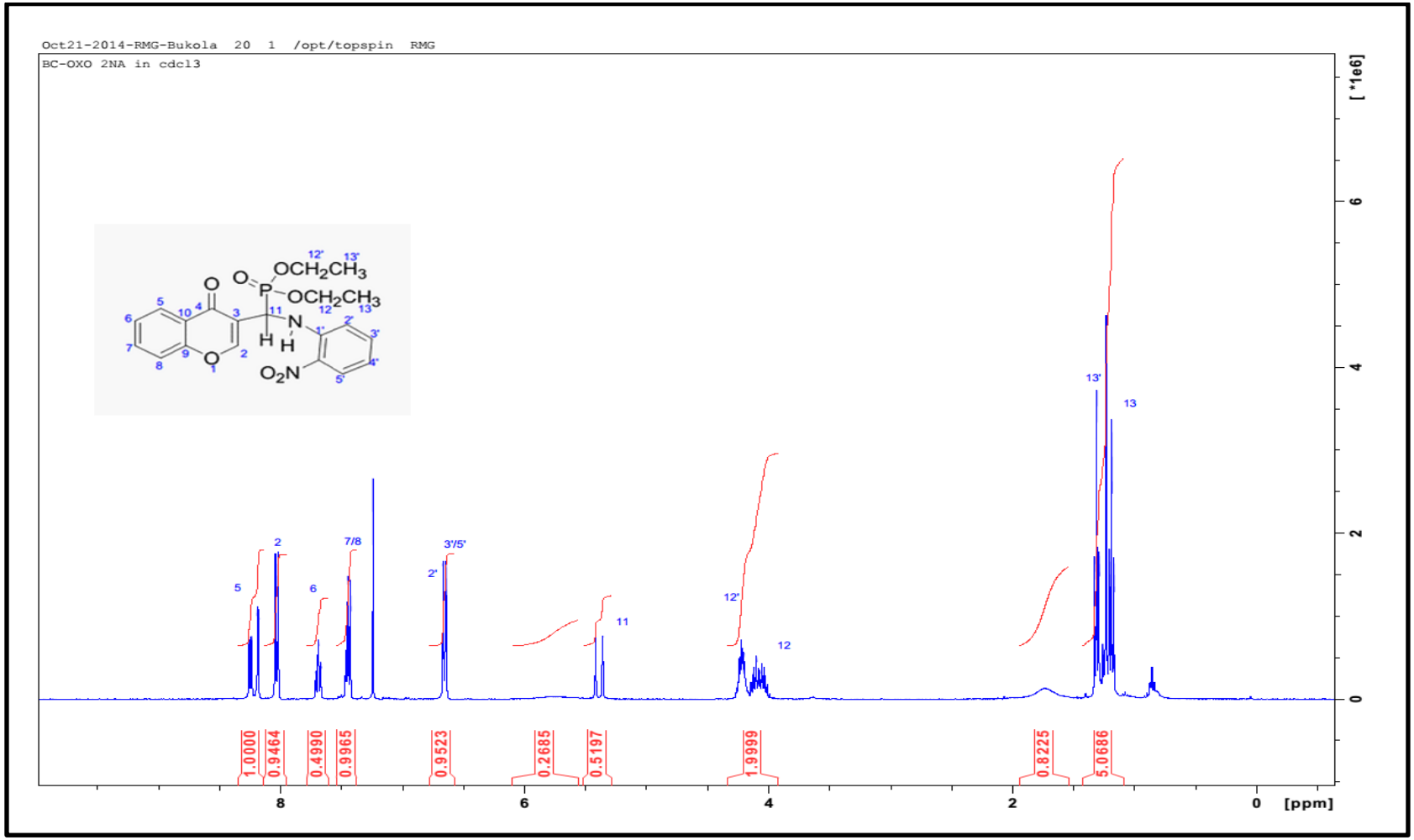


Figure 33

Appendix 20 : ^{13}C-NMR Spectrum of (4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester

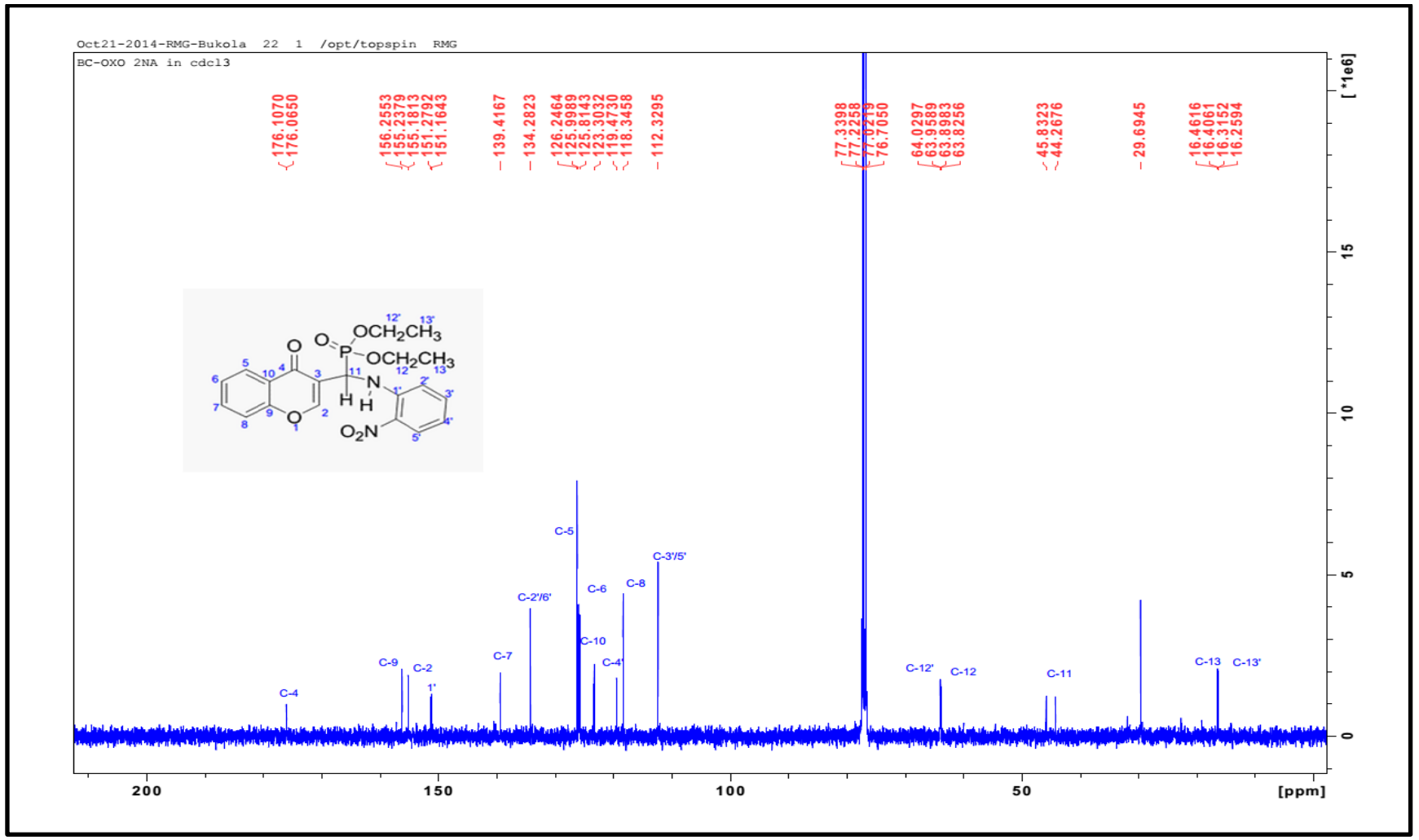


Figure 34

Appendix 21 : ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(2-Nitrophenylamino) methanephosphonic acid diethyl ester

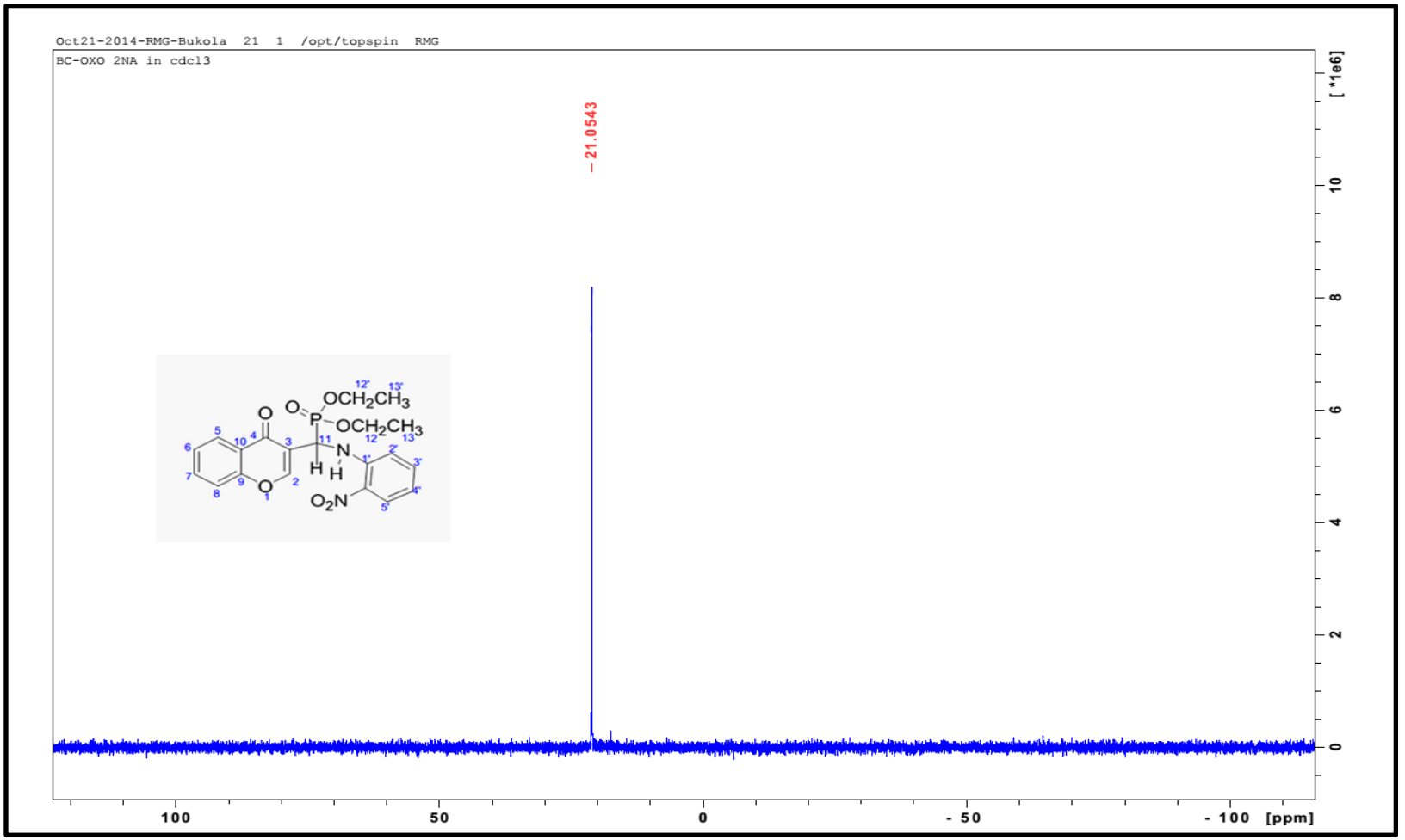


Figure 35

Appendix 22 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester

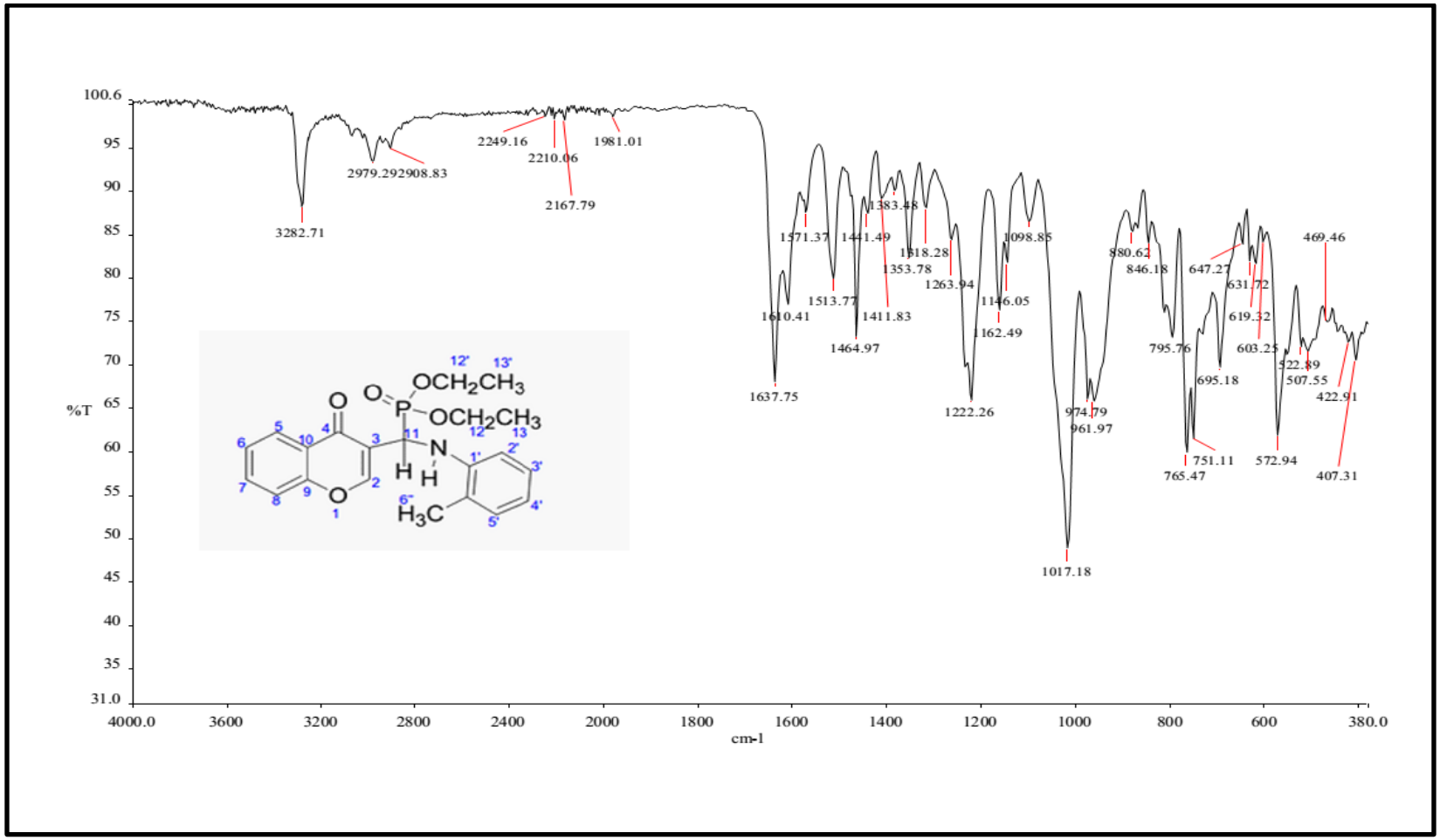


Figure 36

Appendix 23 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester

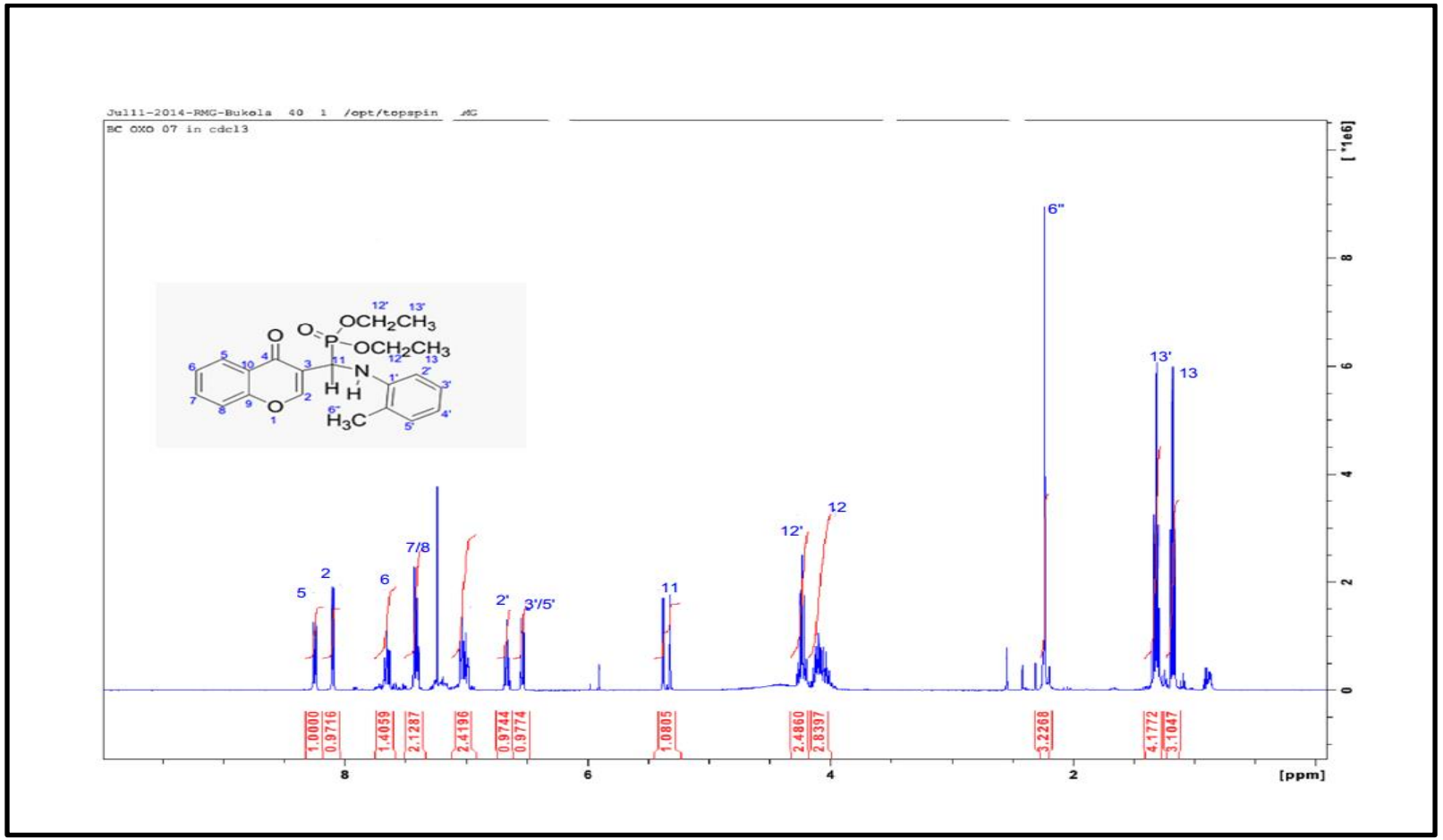


Figure 37

Appendix 24 : ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester

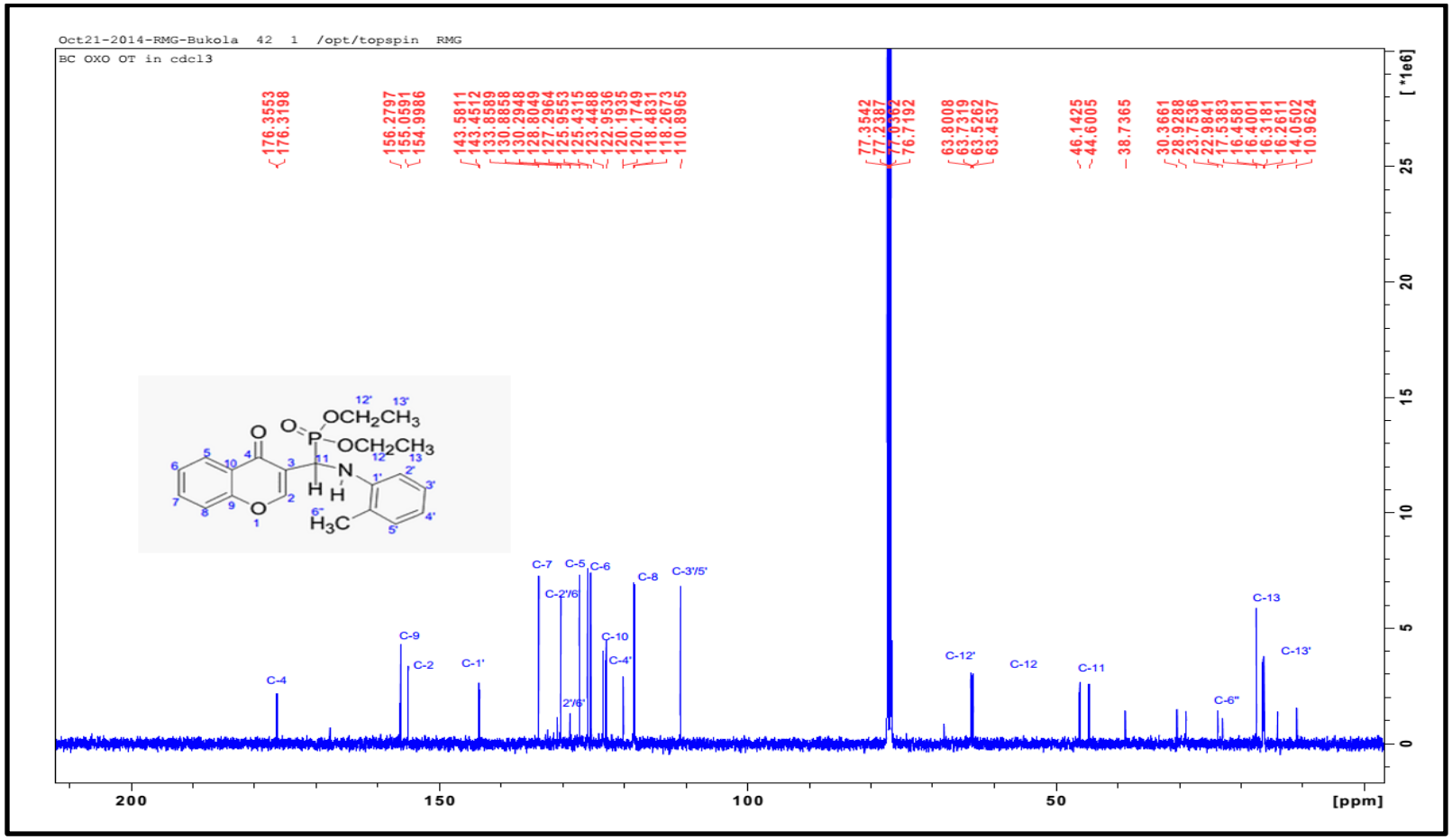


Figure 38

Appendix 25 : ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester

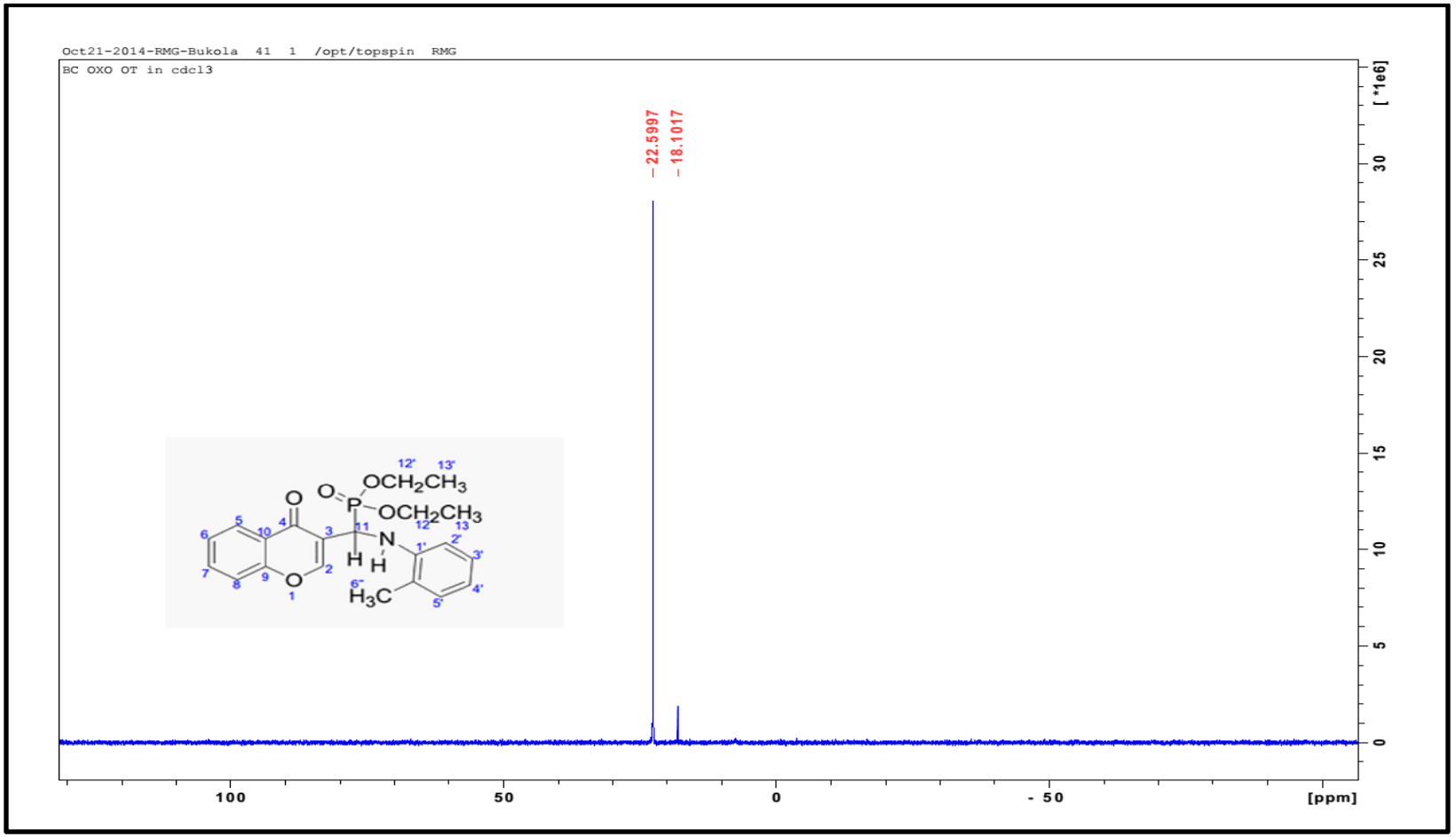


Figure 39

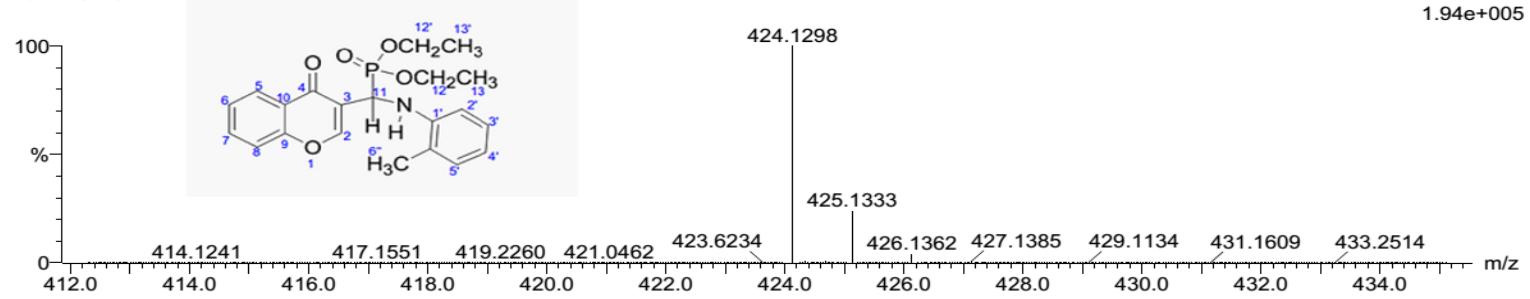
Appendix 26 : Mass spectrum of (4-oxo-4H-chromen-3-yl)-(o-tolylamino) methanephosphonic acid diethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-3 O: 0-5 Na: 1-1 P: 1-1

BC-OXO-OT 38 (1.248) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
424.1298	424.1290	0.8	1.9	10.5	559.4	0.0	C21 H24 N O5 Na P

Figure 40

Appendix 27 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester

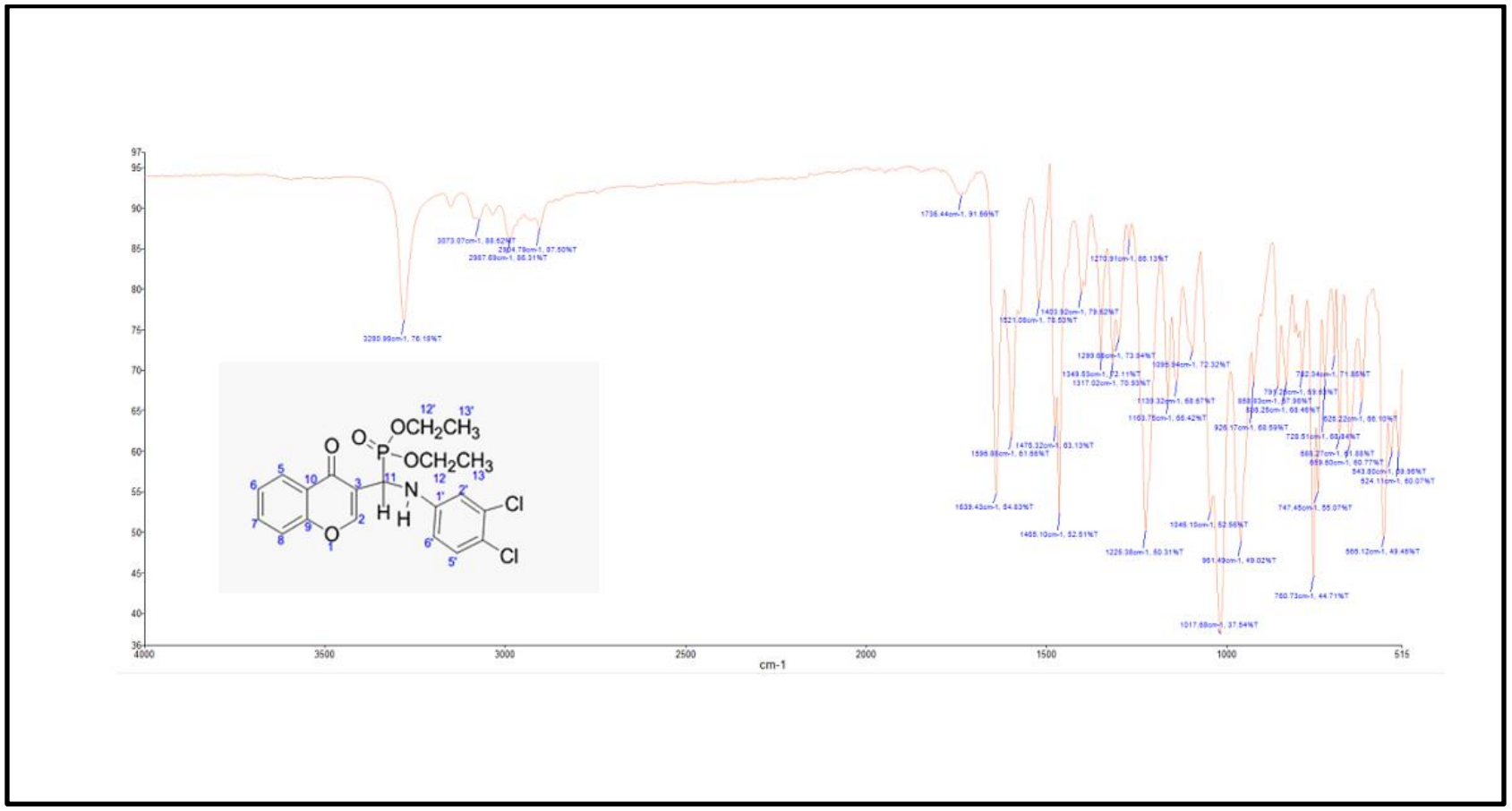


Figure 41

Appendix 28 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester

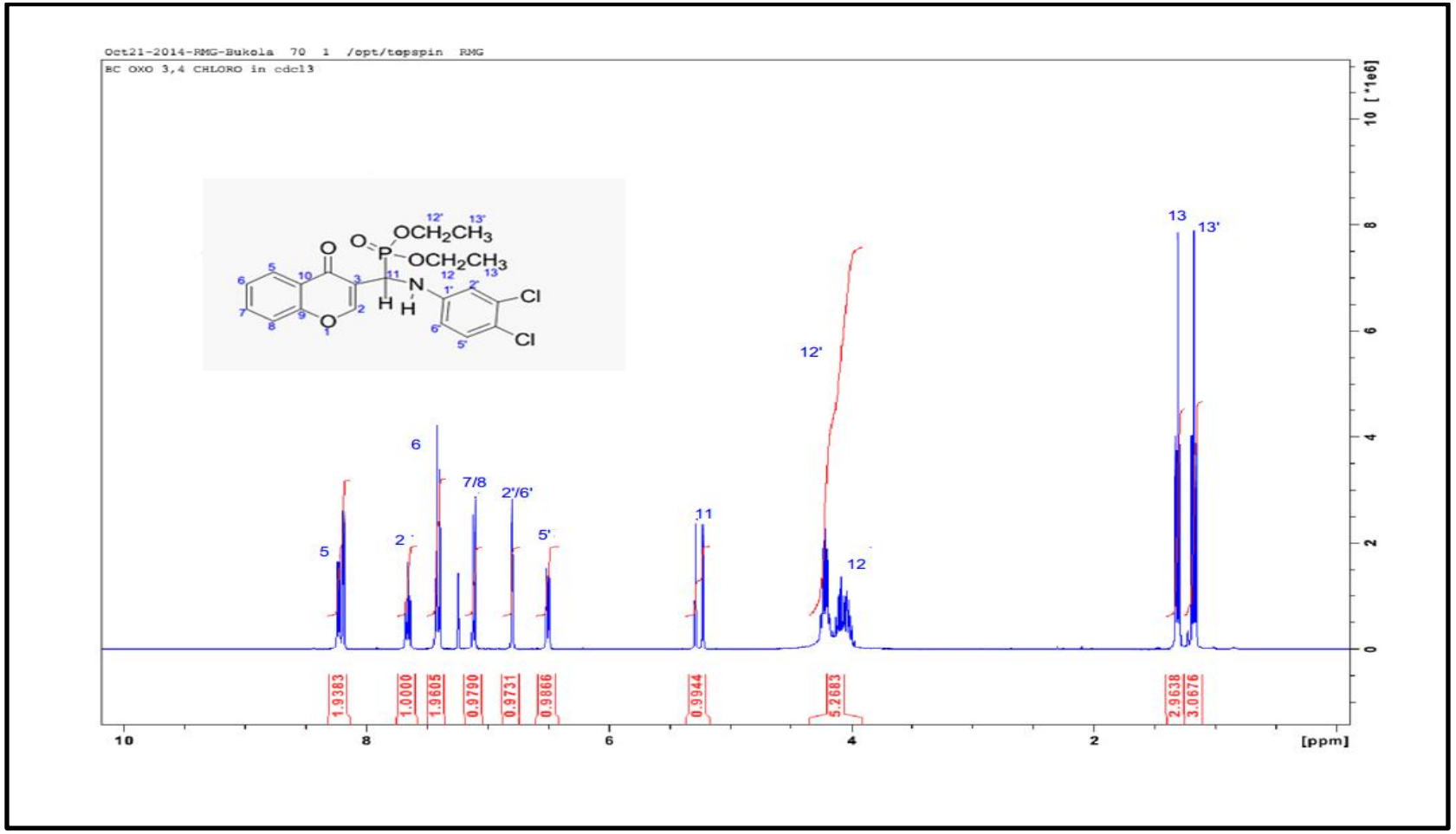


Figure 42

Appendix 29: ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester

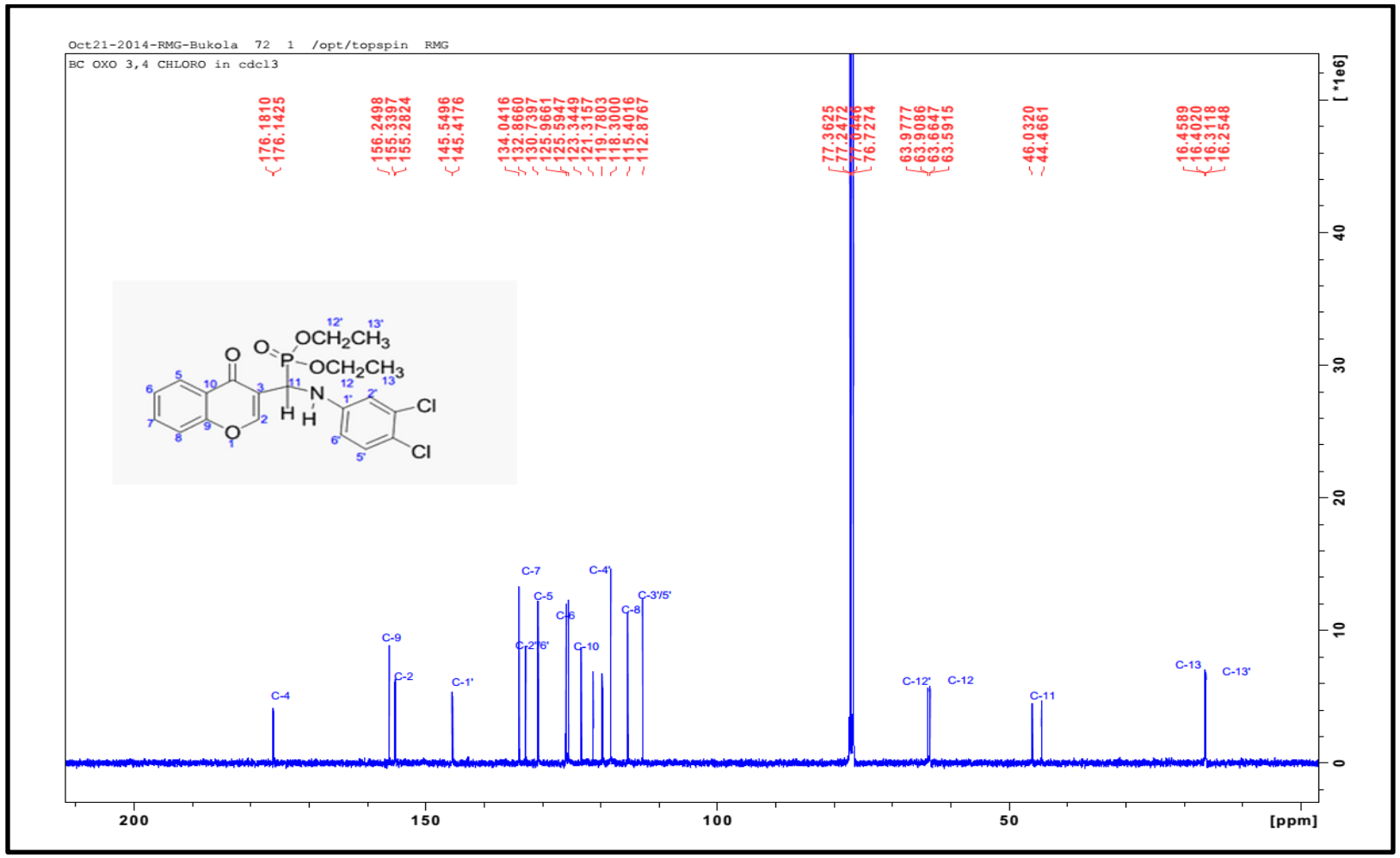


Figure 43

Appendix 30 : ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3,4-dichlorophenylamino) methanephosphonic acid diethyl ester

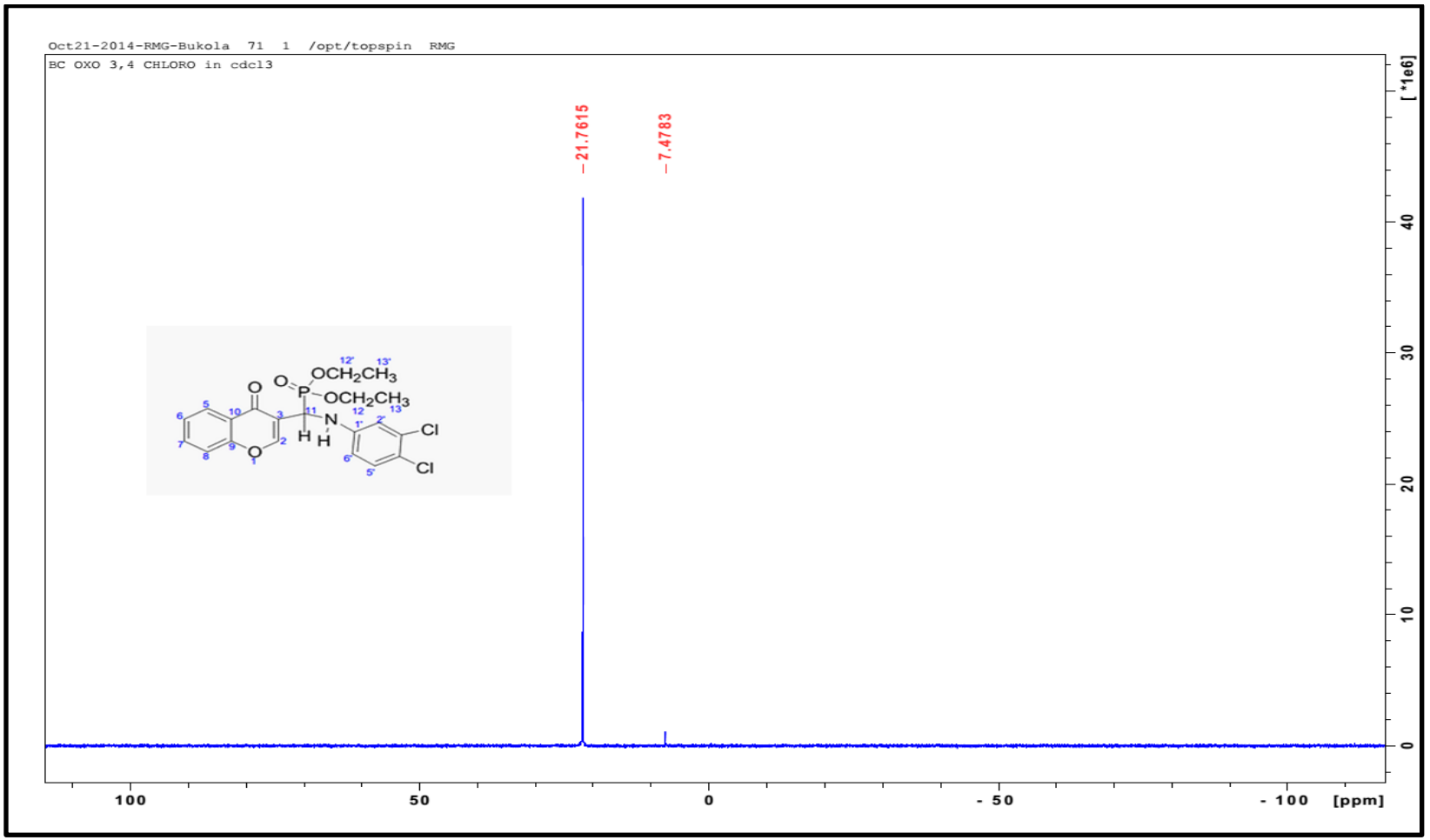


Figure 44

Appendix 31 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

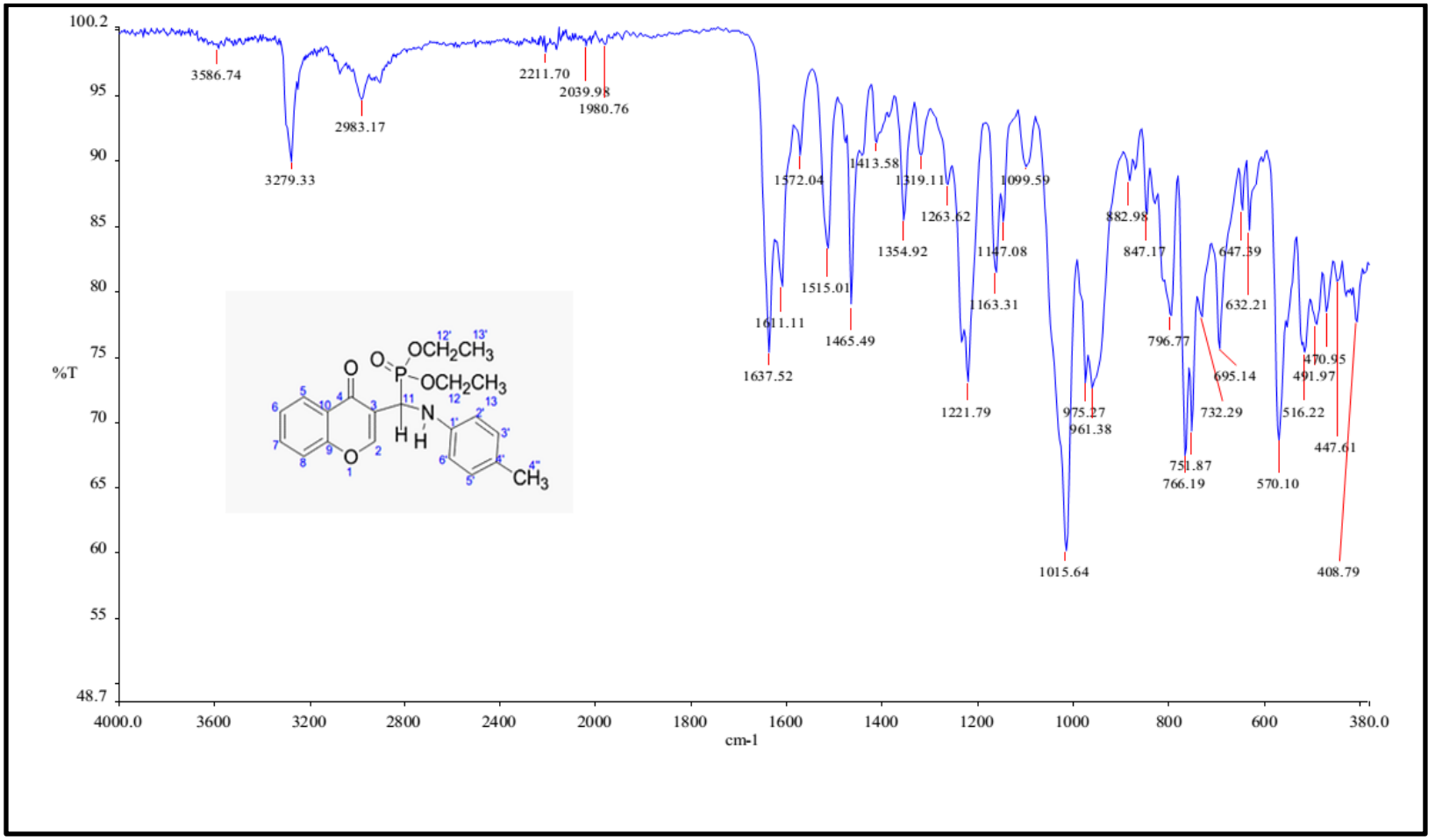


Figure 45

Appendix 32 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

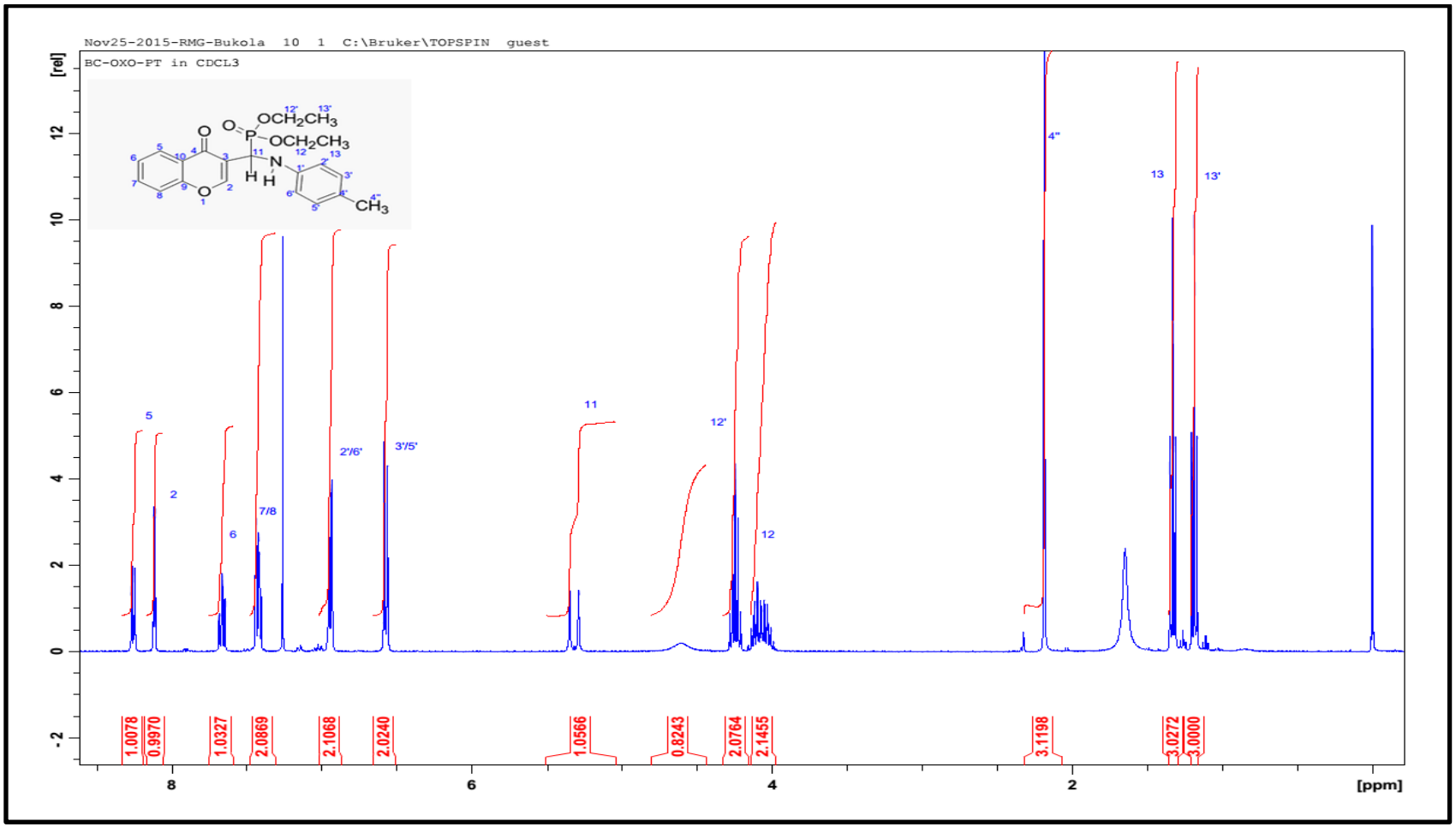


Figure 46

Appendix 33 : The expanded ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

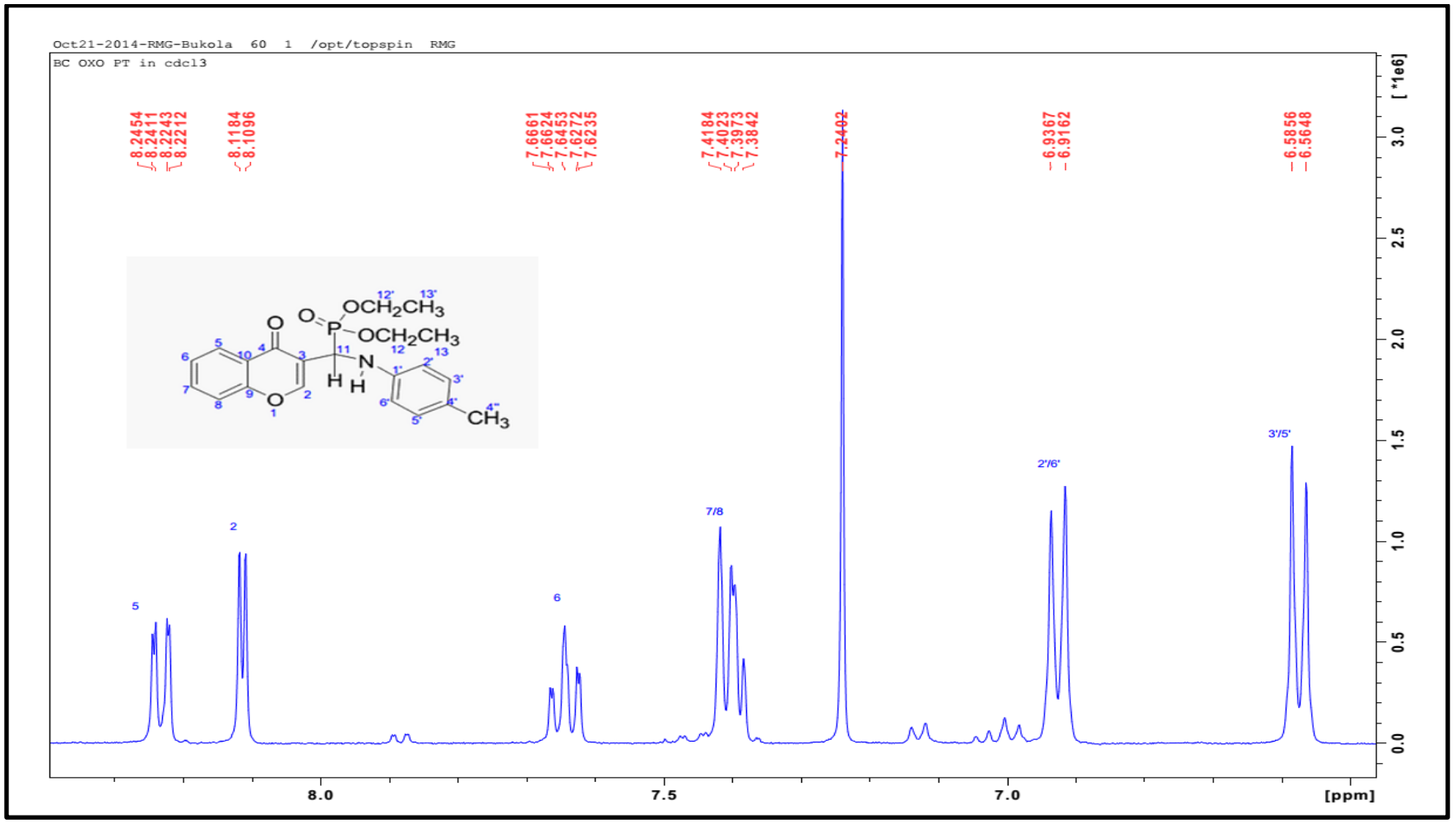


Figure 46a

Appendix 34 : The expanded ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

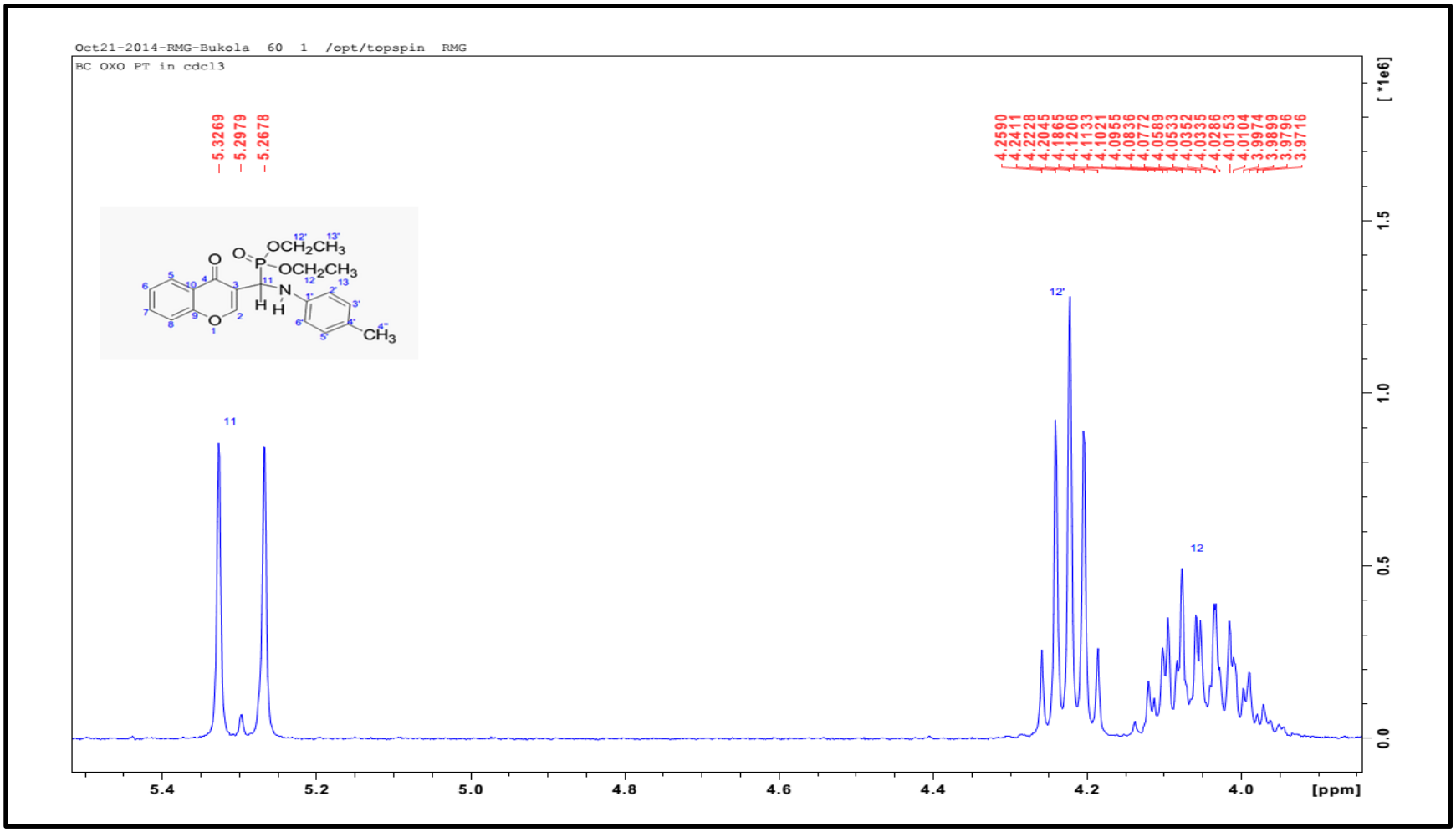


Figure 46b

Appendix 35: The expanded ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

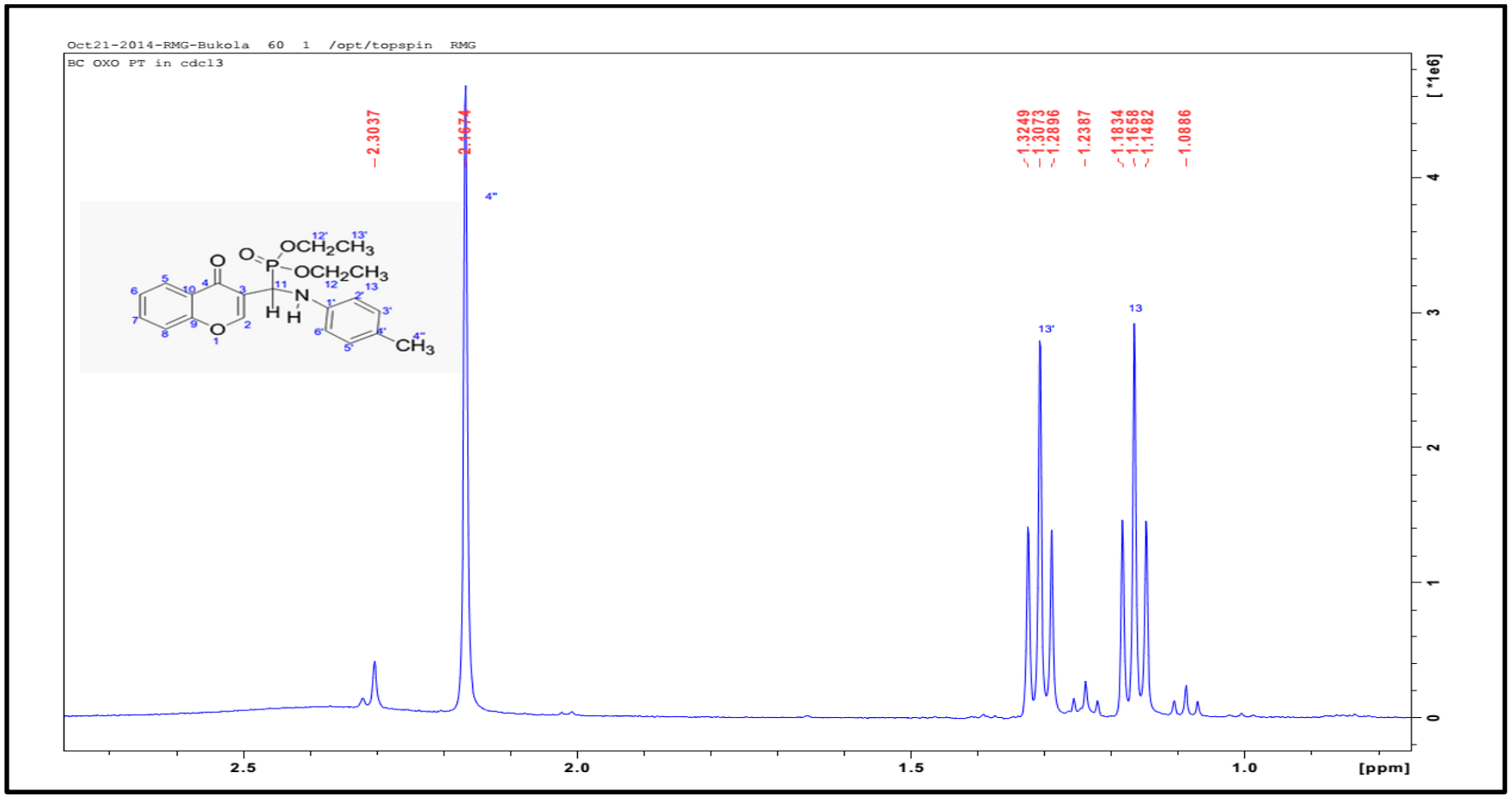


Figure 46c

Appendix 36 : ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

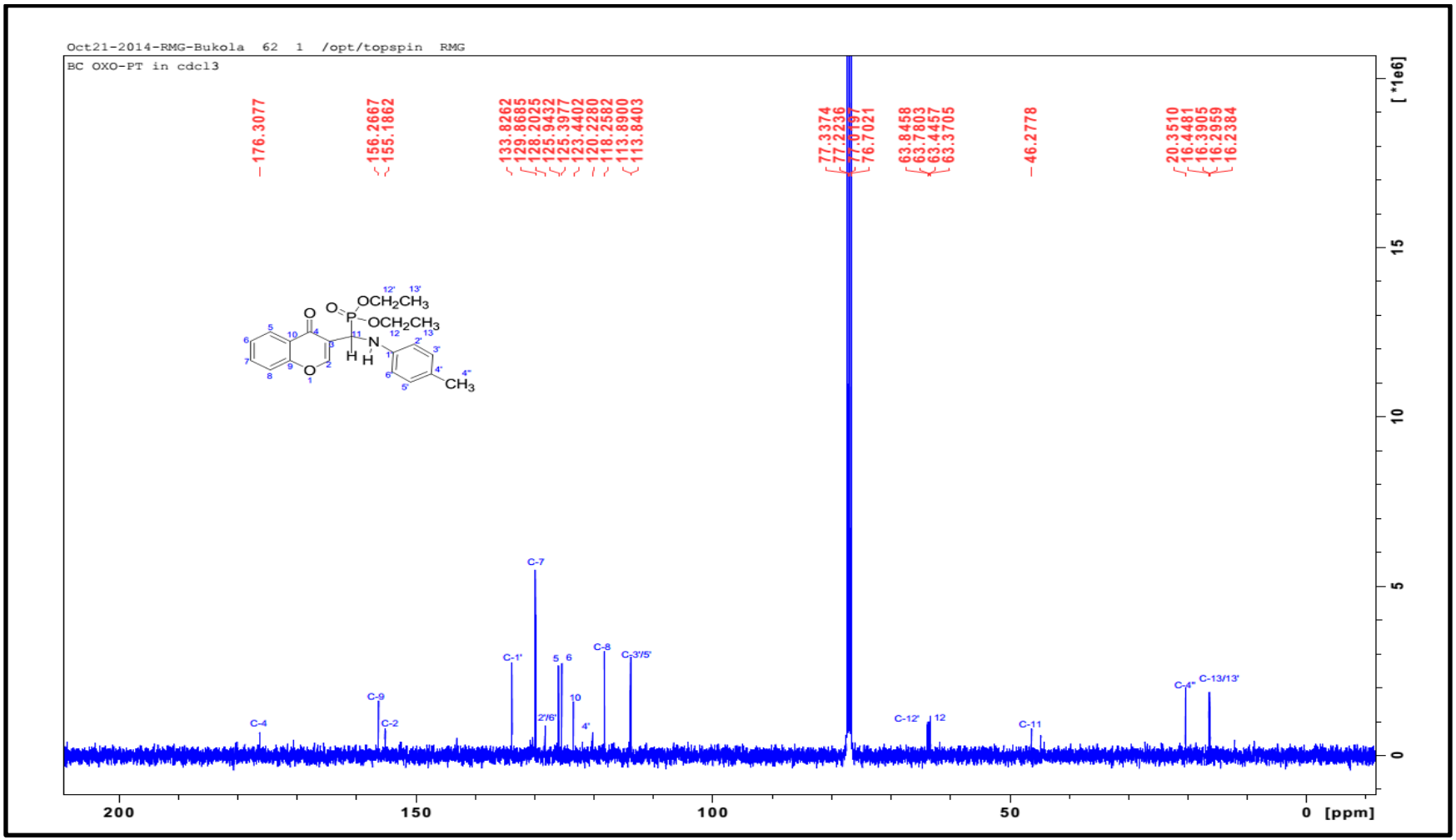


Figure 47

Appendix 37 : Cosy spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

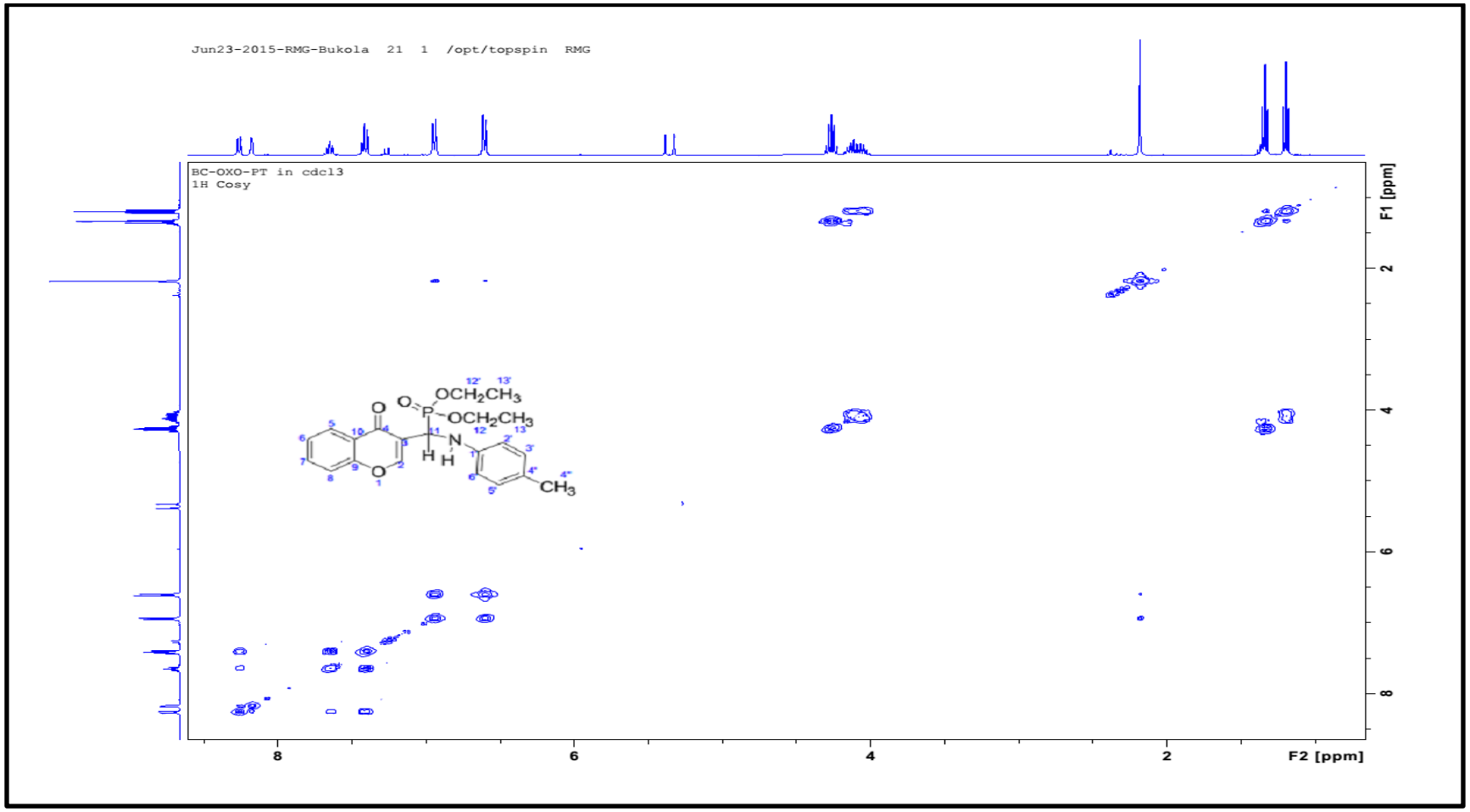


Figure 48

Appendix 38 : HSQC spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

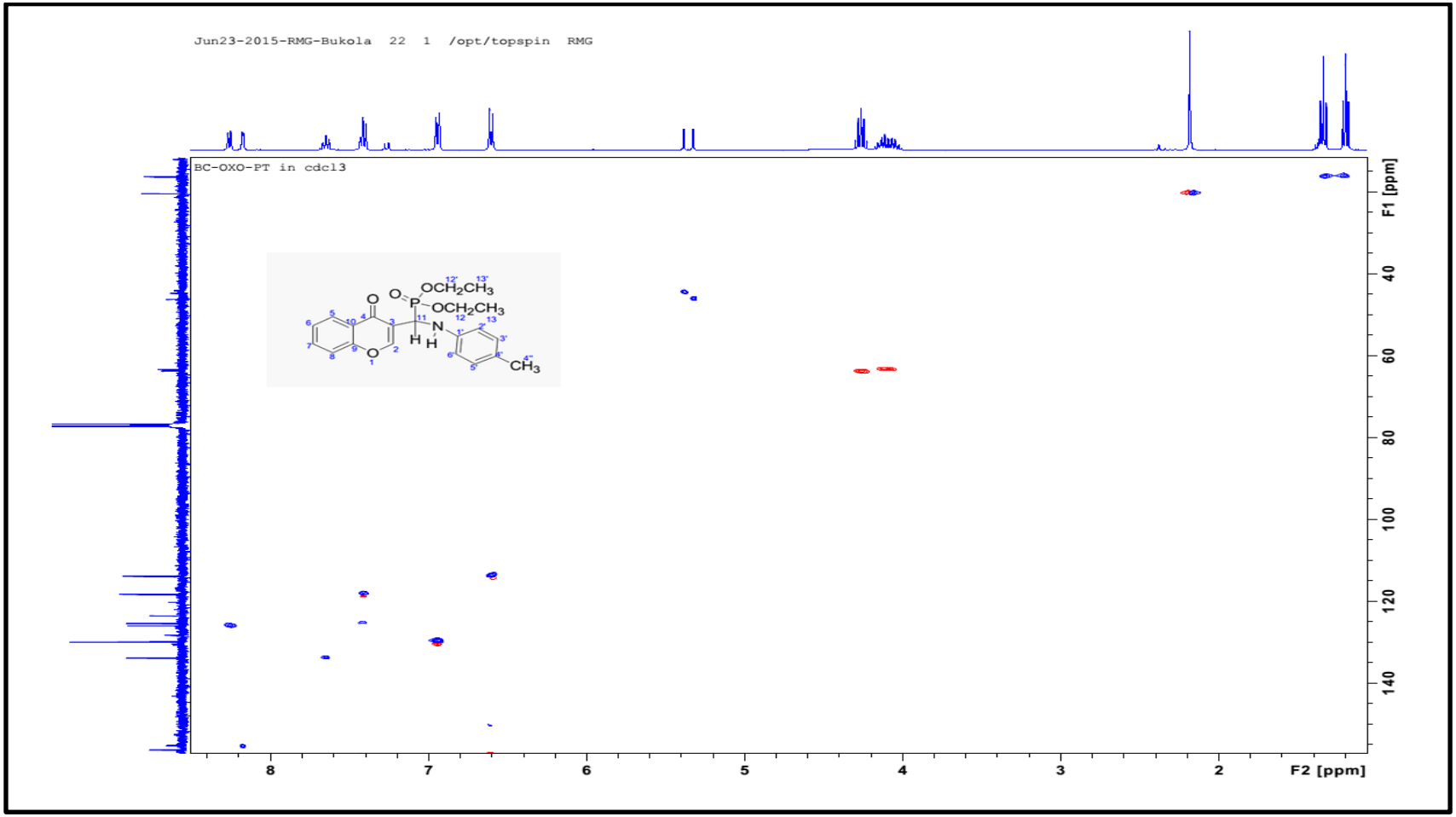


Figure 49

Appendix 39 : HMBC spectrum of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl este**r**

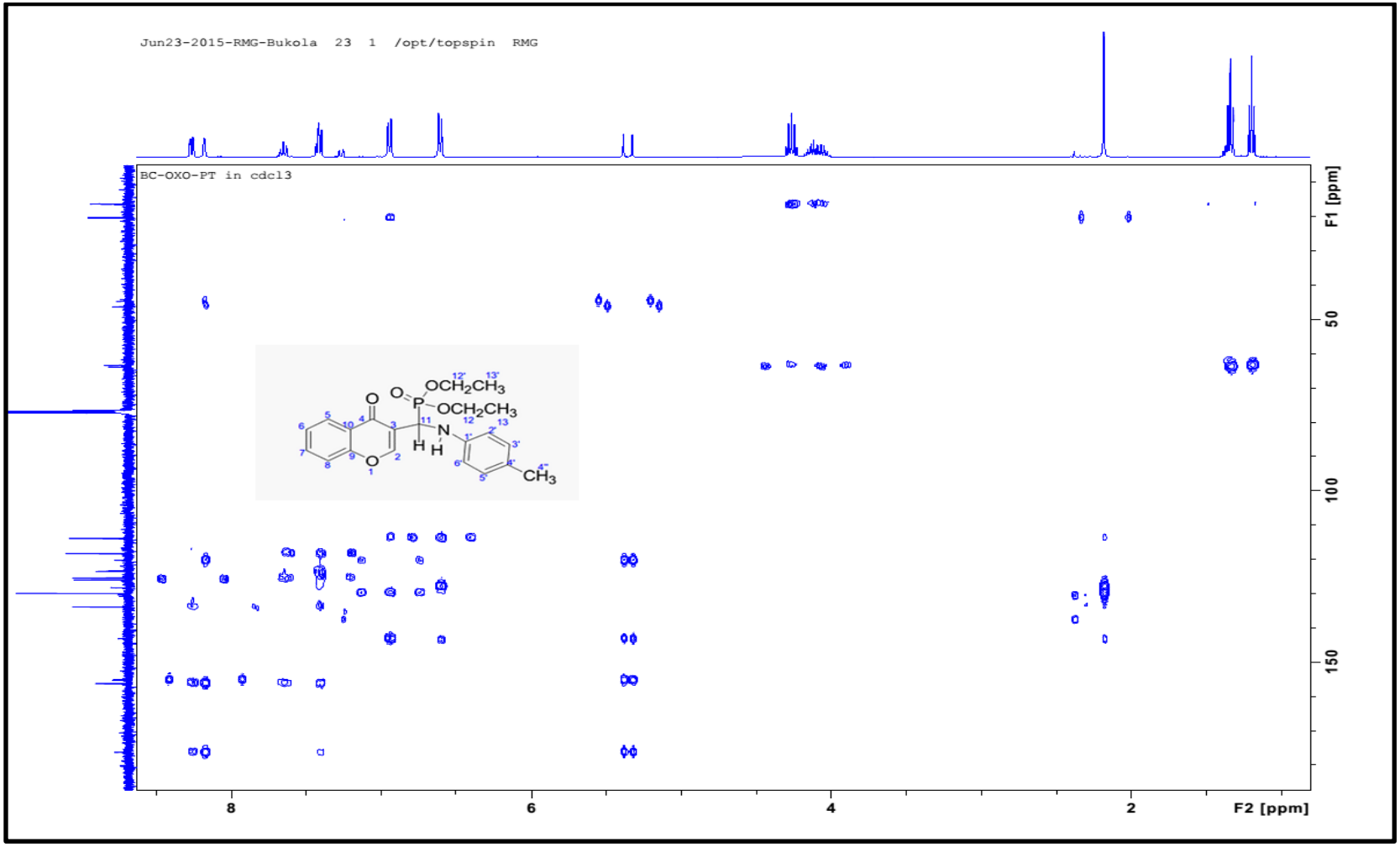


Figure 50

Appendix 40 : ^{31}P-NMR of (4-oxo-4H-chromen-3-yl)-(p-tolylamino) methanephosphonic acid diethyl ester

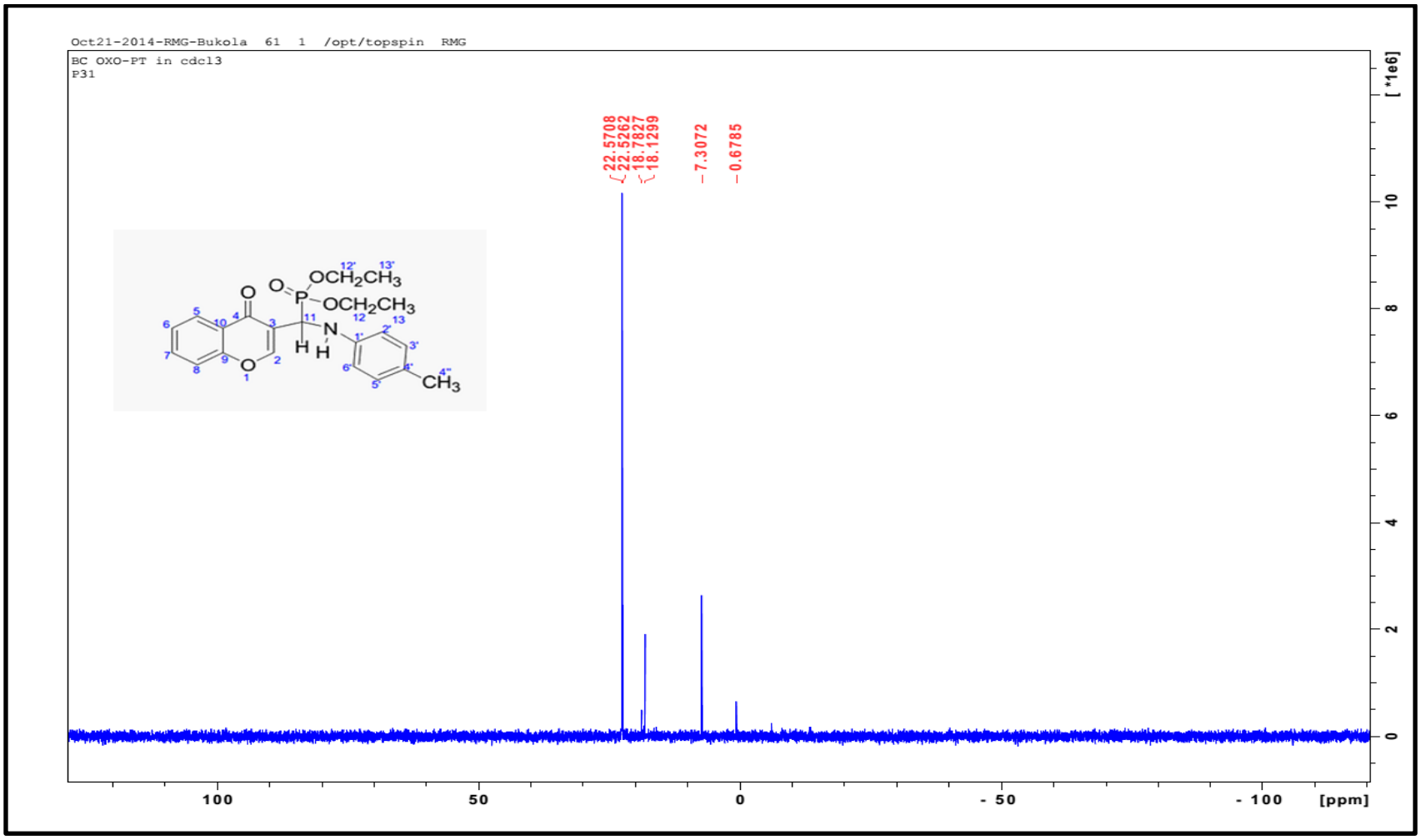


Figure 51

Appendix 41 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester

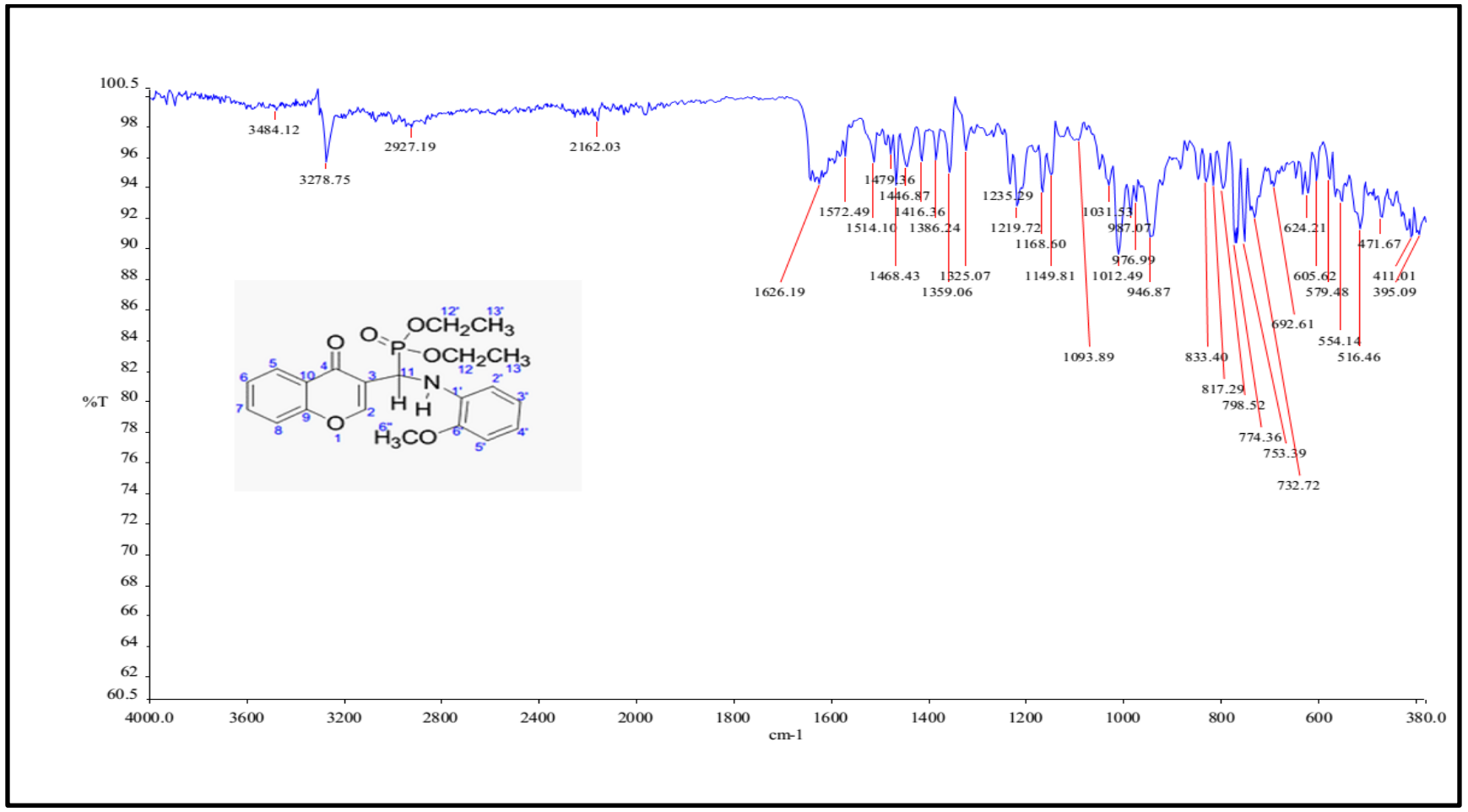


Figure 52

Appendix 42 : ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester

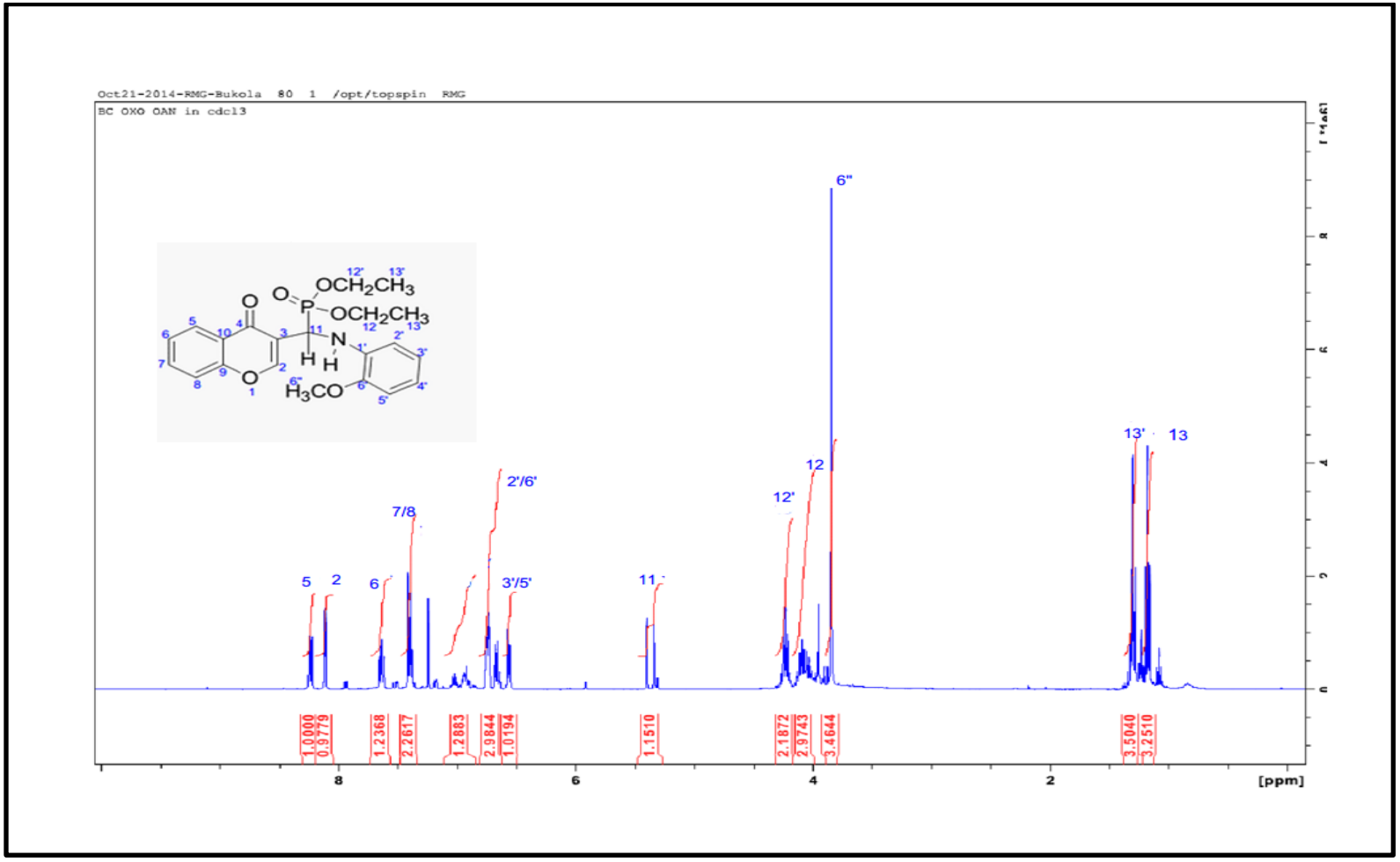


Figure 53

Appendix 43: ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester

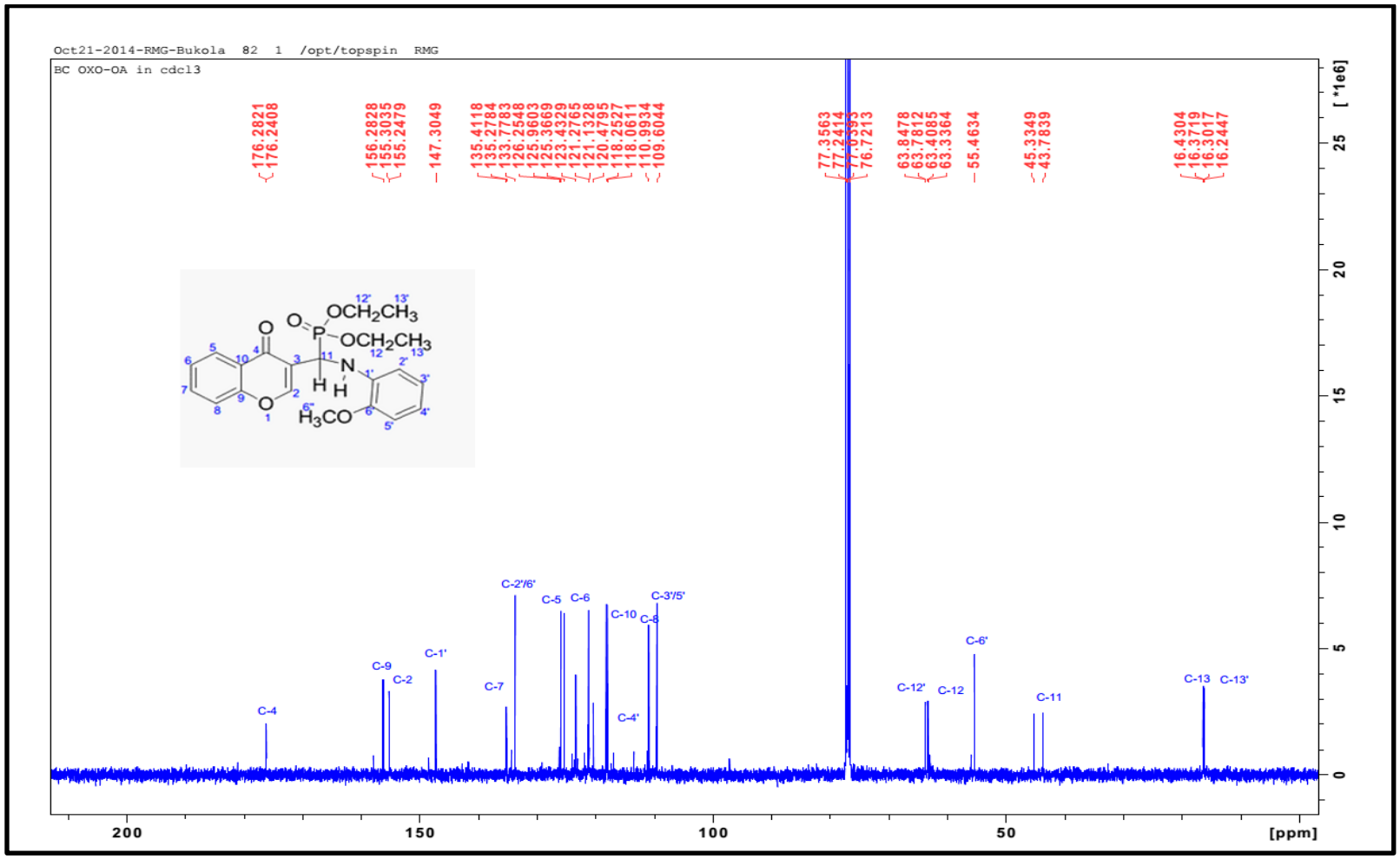


Figure 54

Appendix 44 : ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(2-methoxyphenylamino) methanephosphonic acid diethyl ester

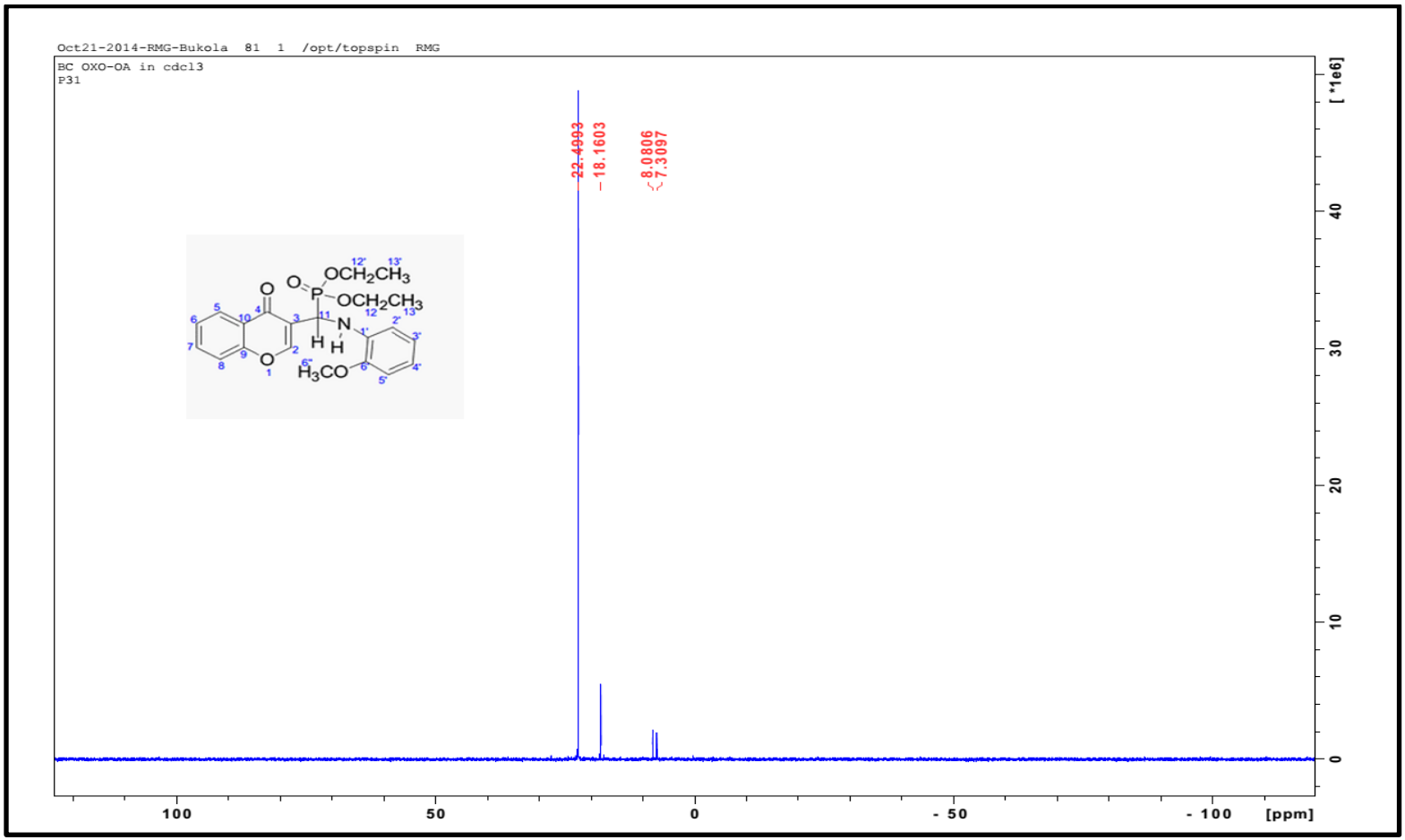


Figure 55

Appendix 45 : ^{1}H-NMR spectrum of 4-oxo-4H-chromen-3-yl)-(4-methoxyphenylamino) methanephosphonic acid diethyl ester

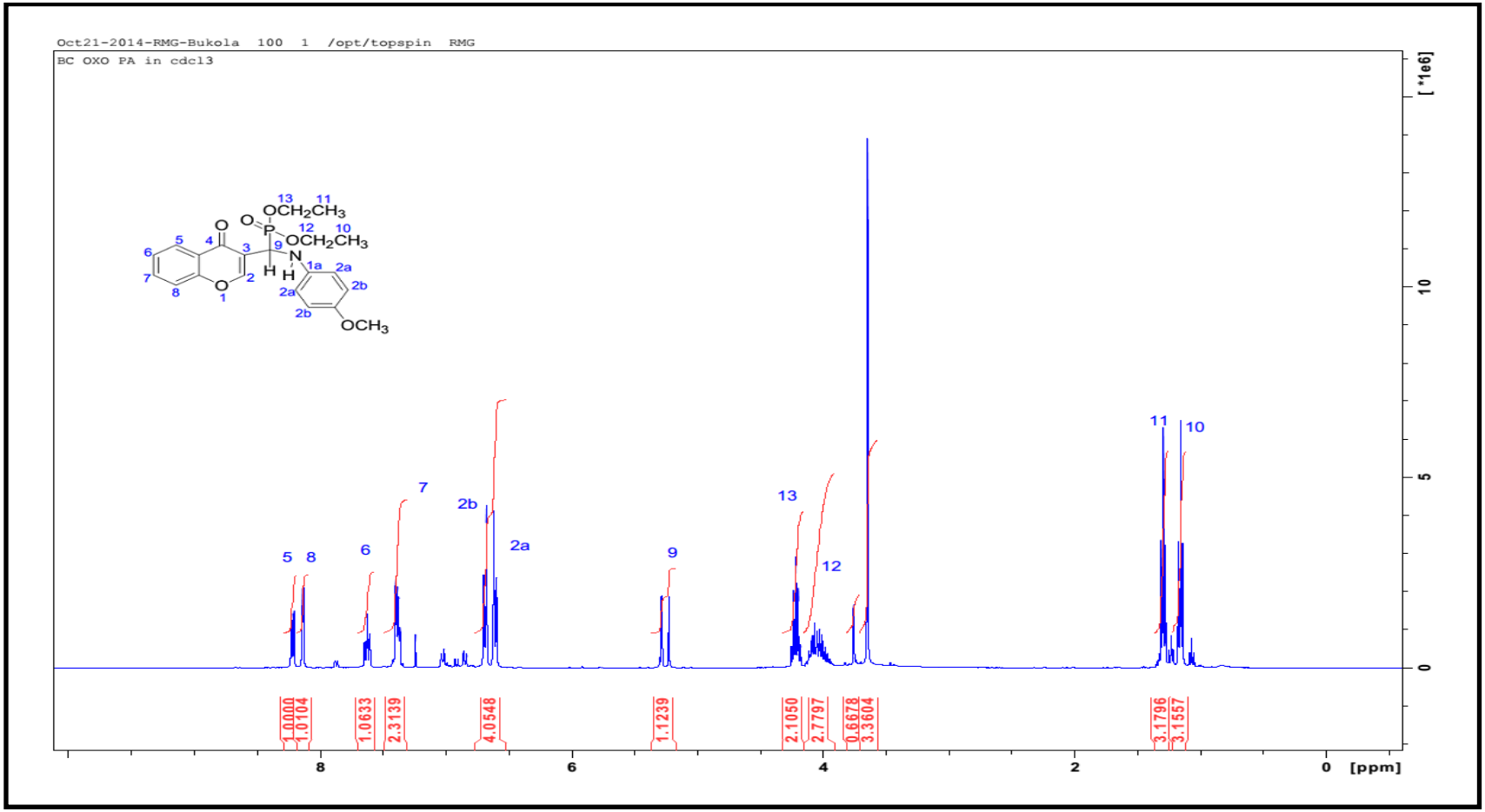


Figure 56

Appendix 46 : ^{13}C-NMR spectrum of 4-oxo-4H-chromen-3-yl)-(4-methoxyphenylamino) methanephosphonic acid diethyl ester

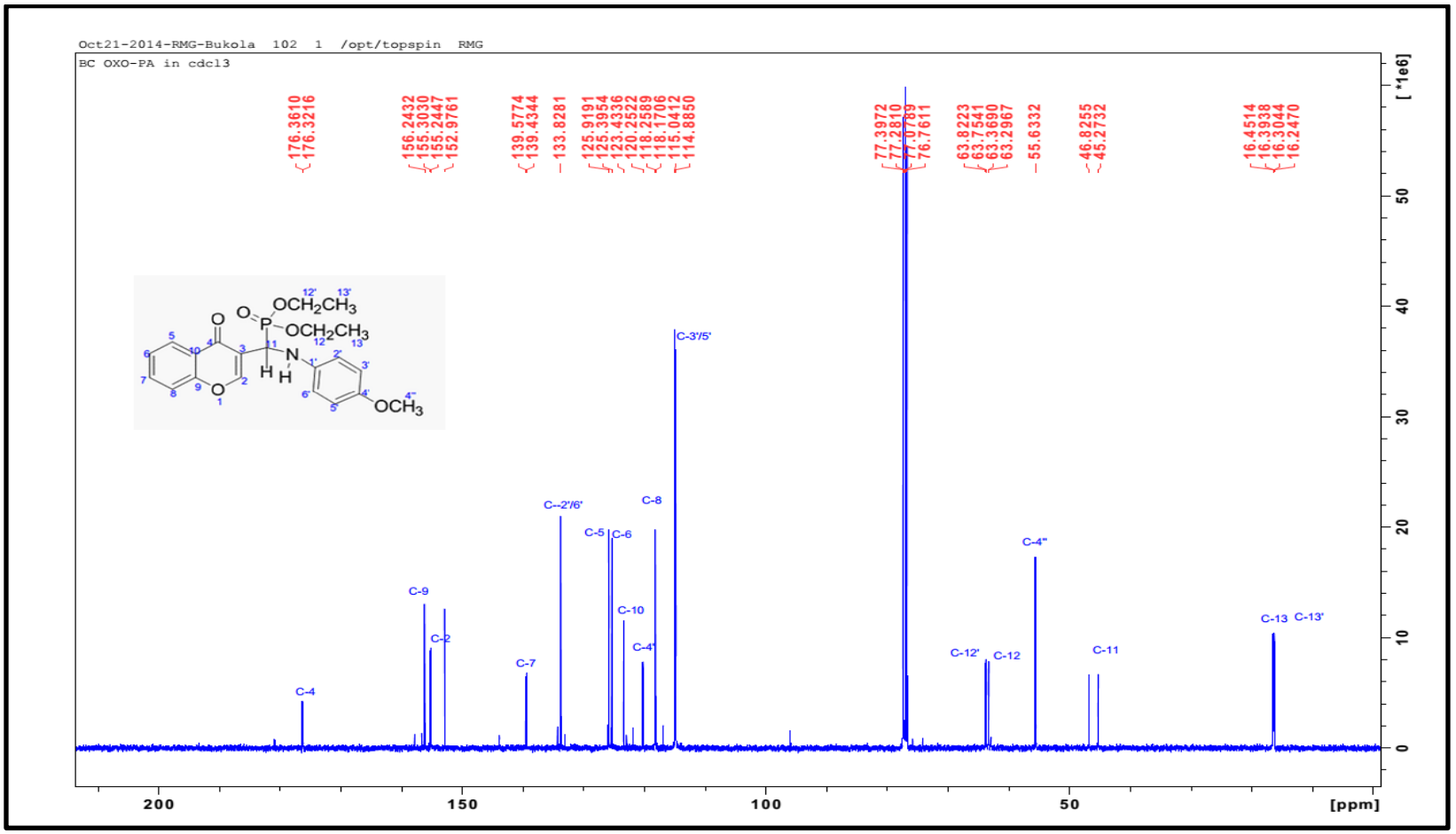


Figure 57

Appendix 47: ^{13}C-NMR spectrum of 4-oxo-4H-chromen-3-yl)-(4-methoxyphenylamino) methanephosphonic acid diethyl ester

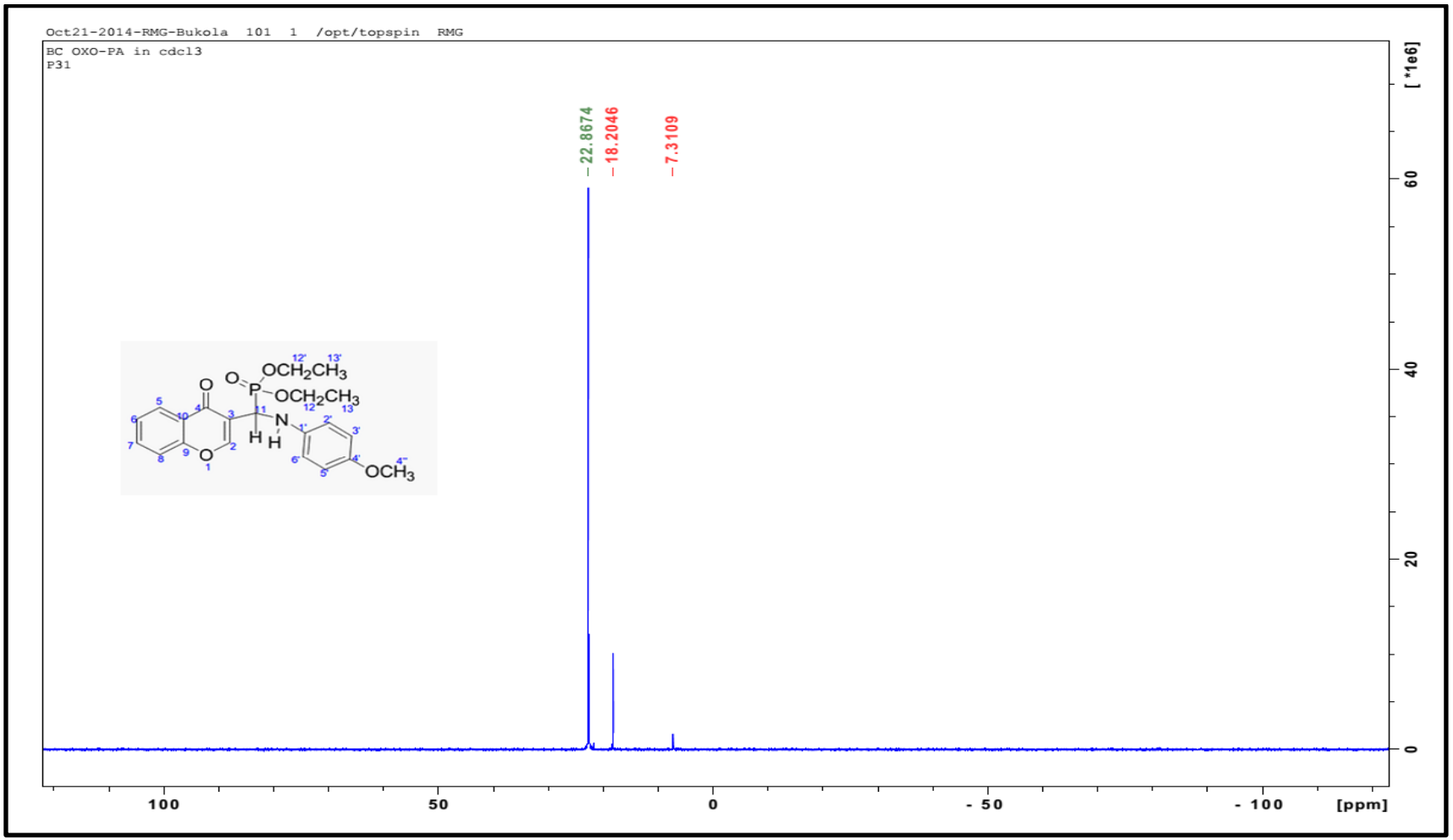


Figure 58

Appendix 48 : IR spectrum of (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester

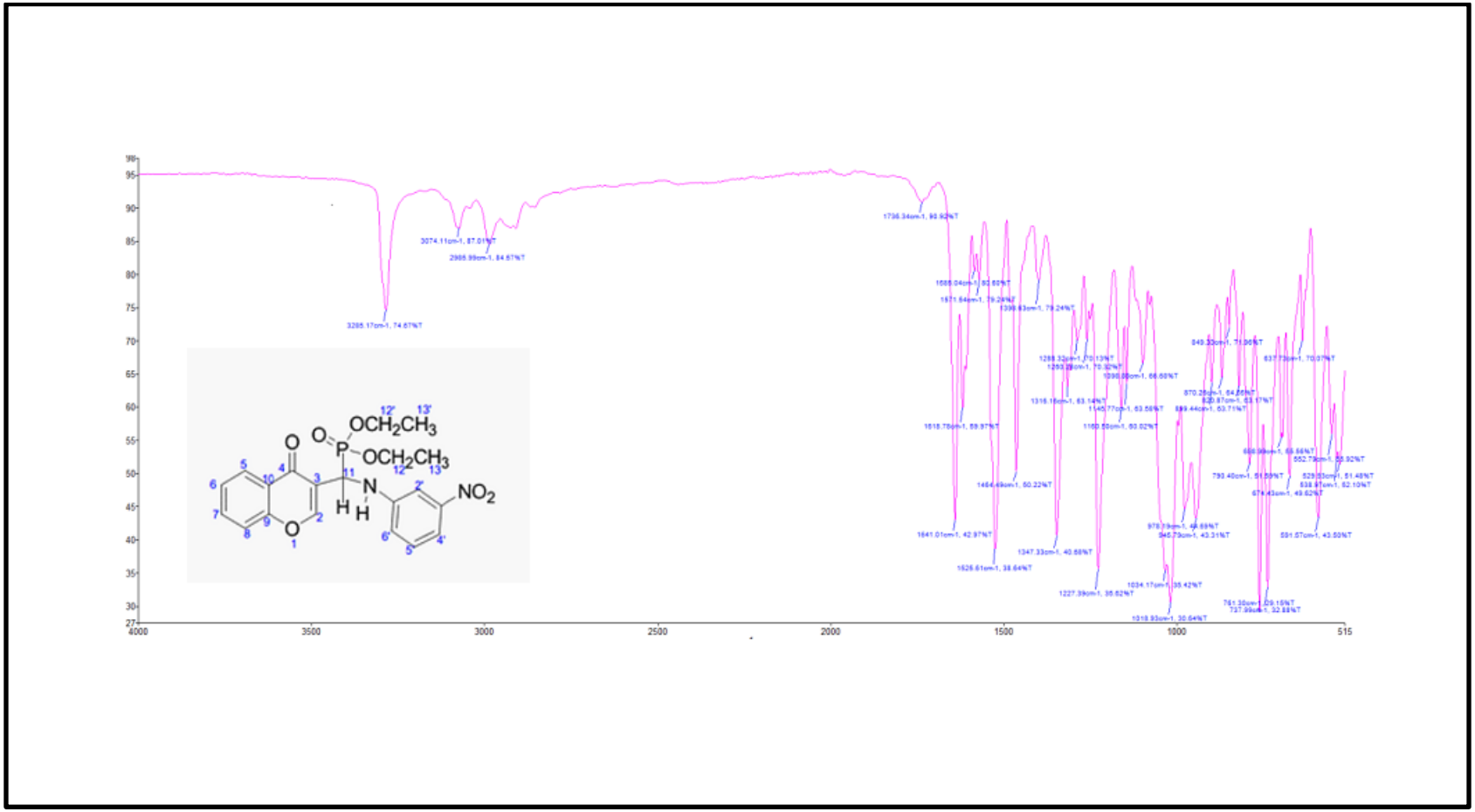


Figure 59

Appendix 49: ^{1}H-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester

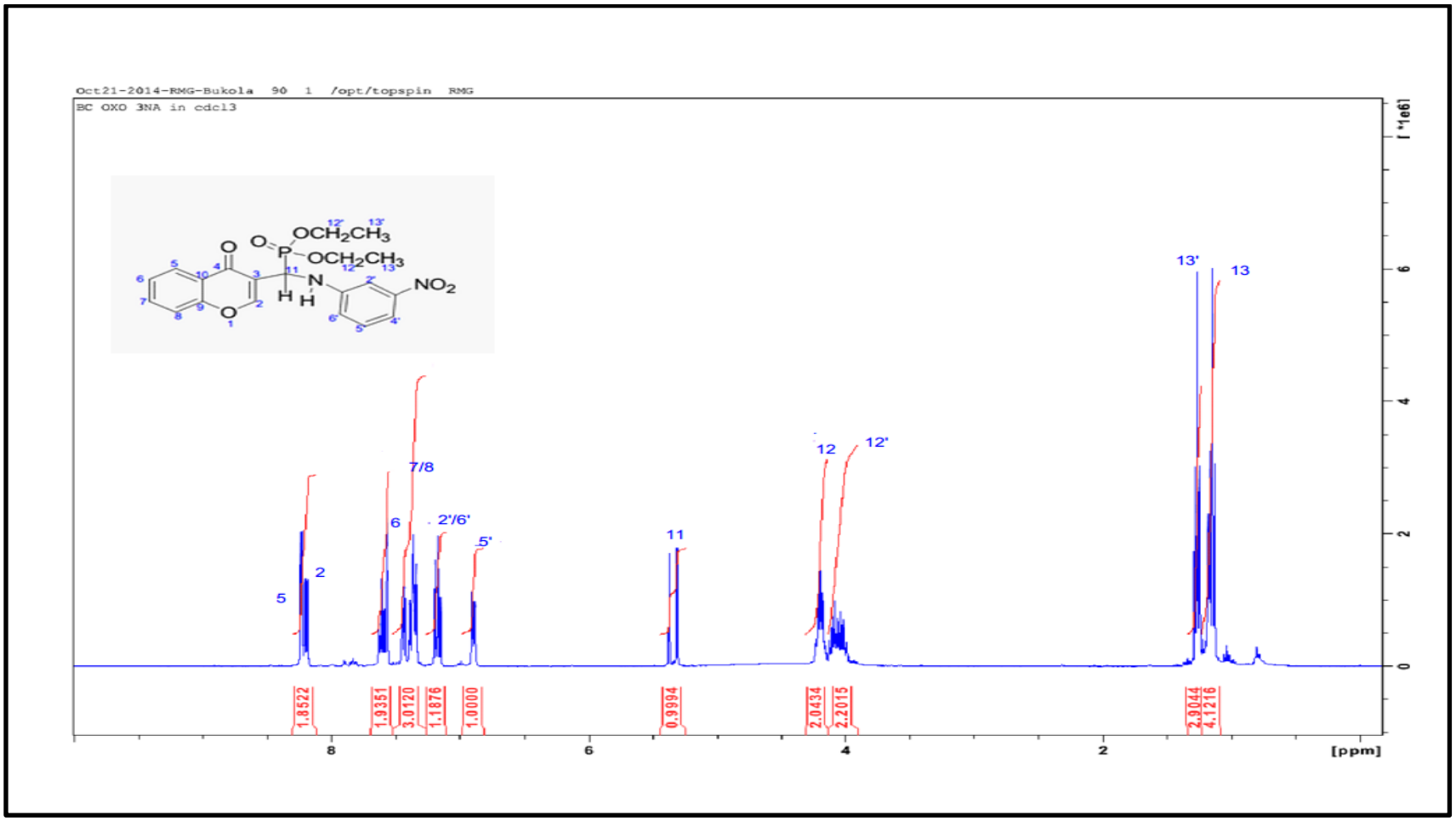


Figure 60

Appendix 50 : ^{13}C-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic **acid diethyl ester**

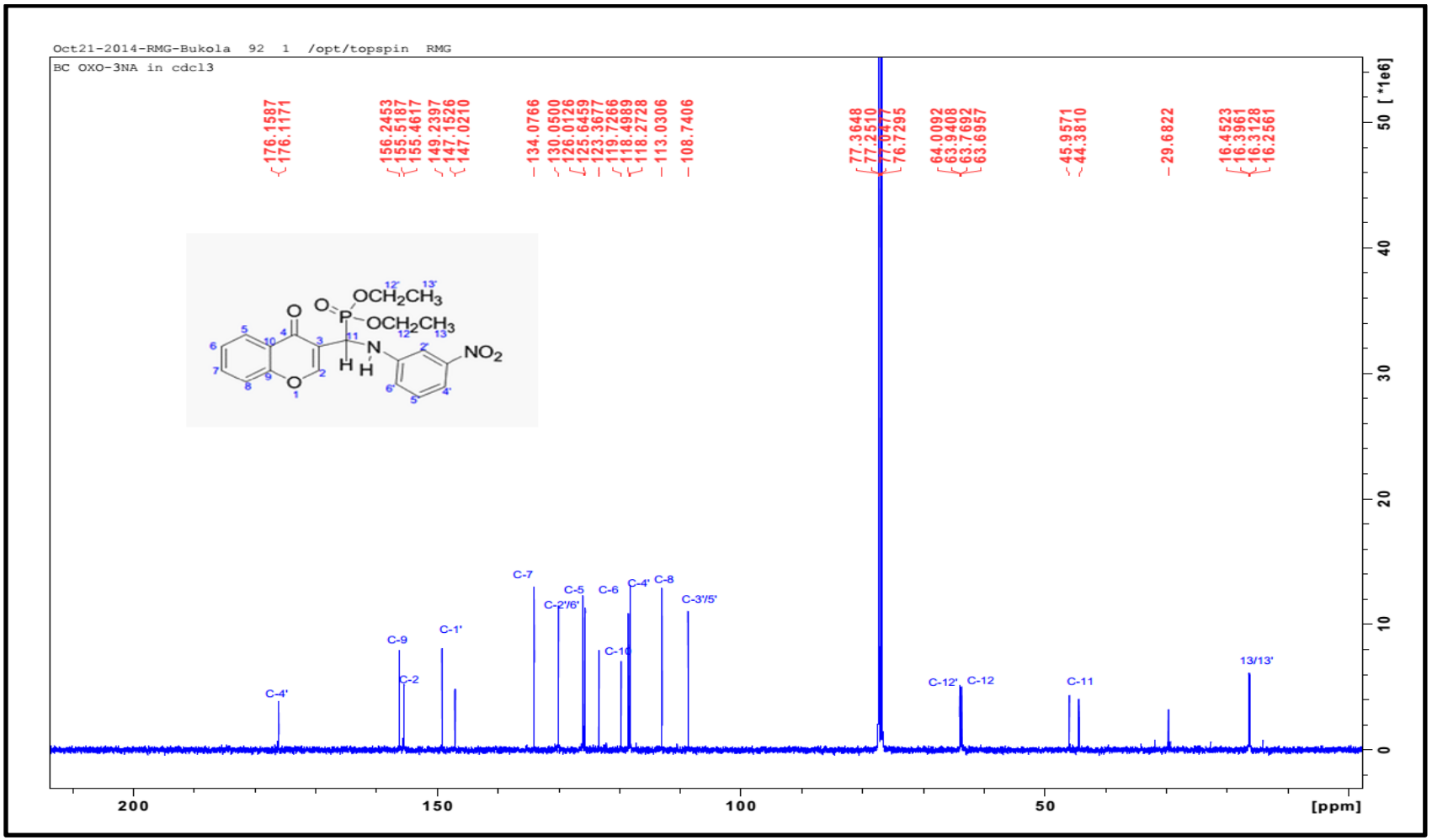


Figure 61

Appendix 51: ^{31}P-NMR spectrum of (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester

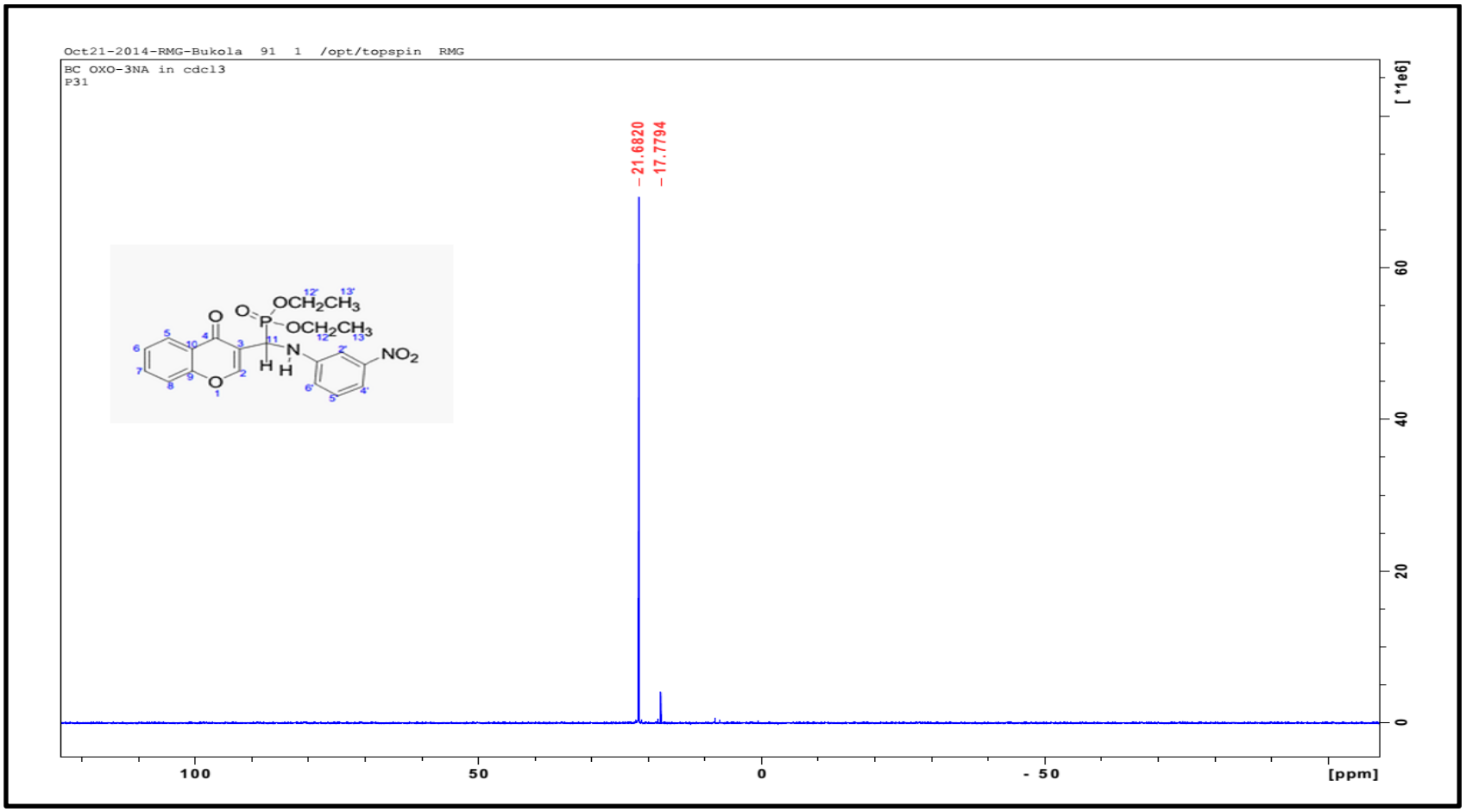


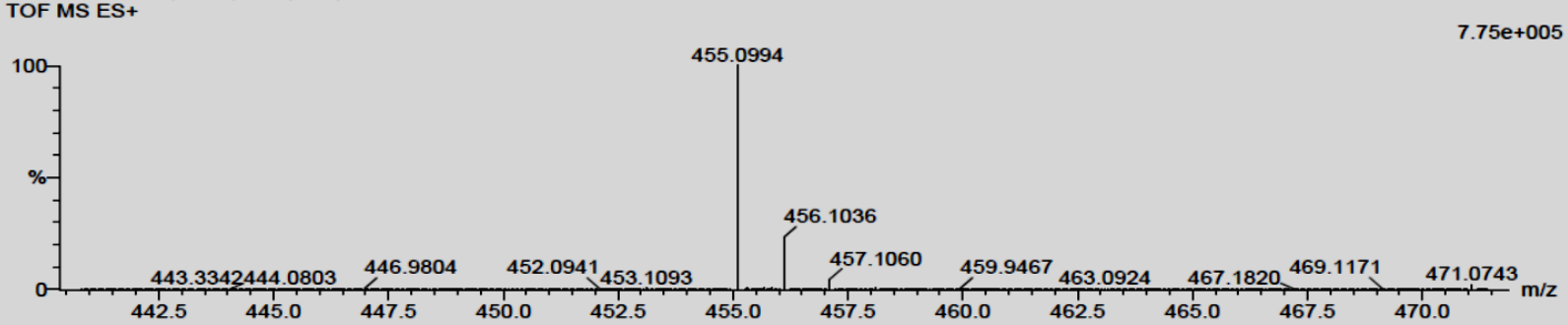
Figure 62

Appendix 52: Mass-spectrum of (4-oxo-4H-chromen-3-yl)-(3-Nitrophenylamino) methanephosphonic acid diethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 5-10 Na: 1-1 P: 0-1
BC-oxo-3NA 12 (0.371) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
455.0994	455.0984	1.0	2.2	11.5	644.6	0.0	C20 H21 N2 O7 Na P

Figure 63

Appendix 53 : IR-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester

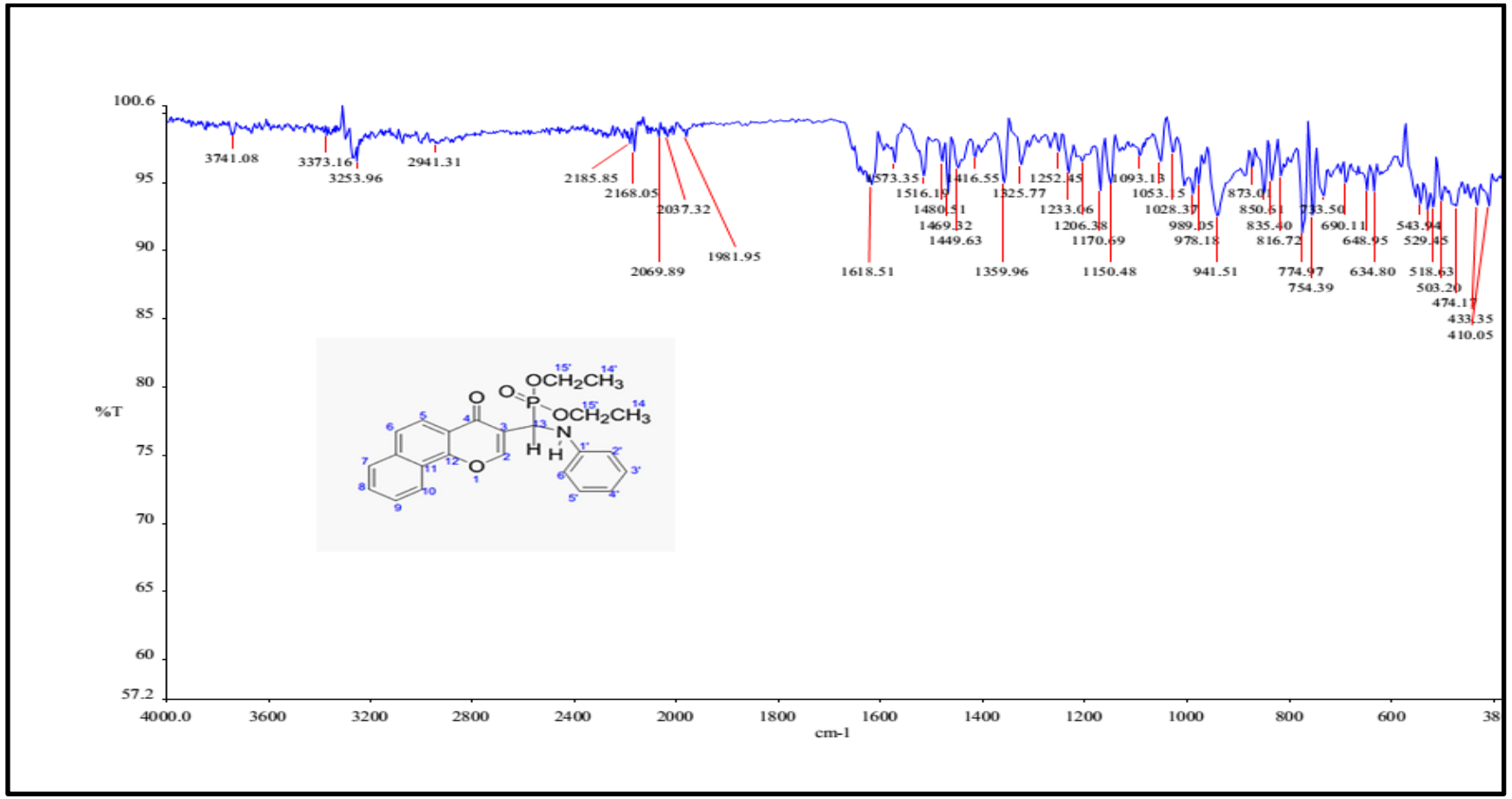


Figure 64

Appendix 54: ^{1}H-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester

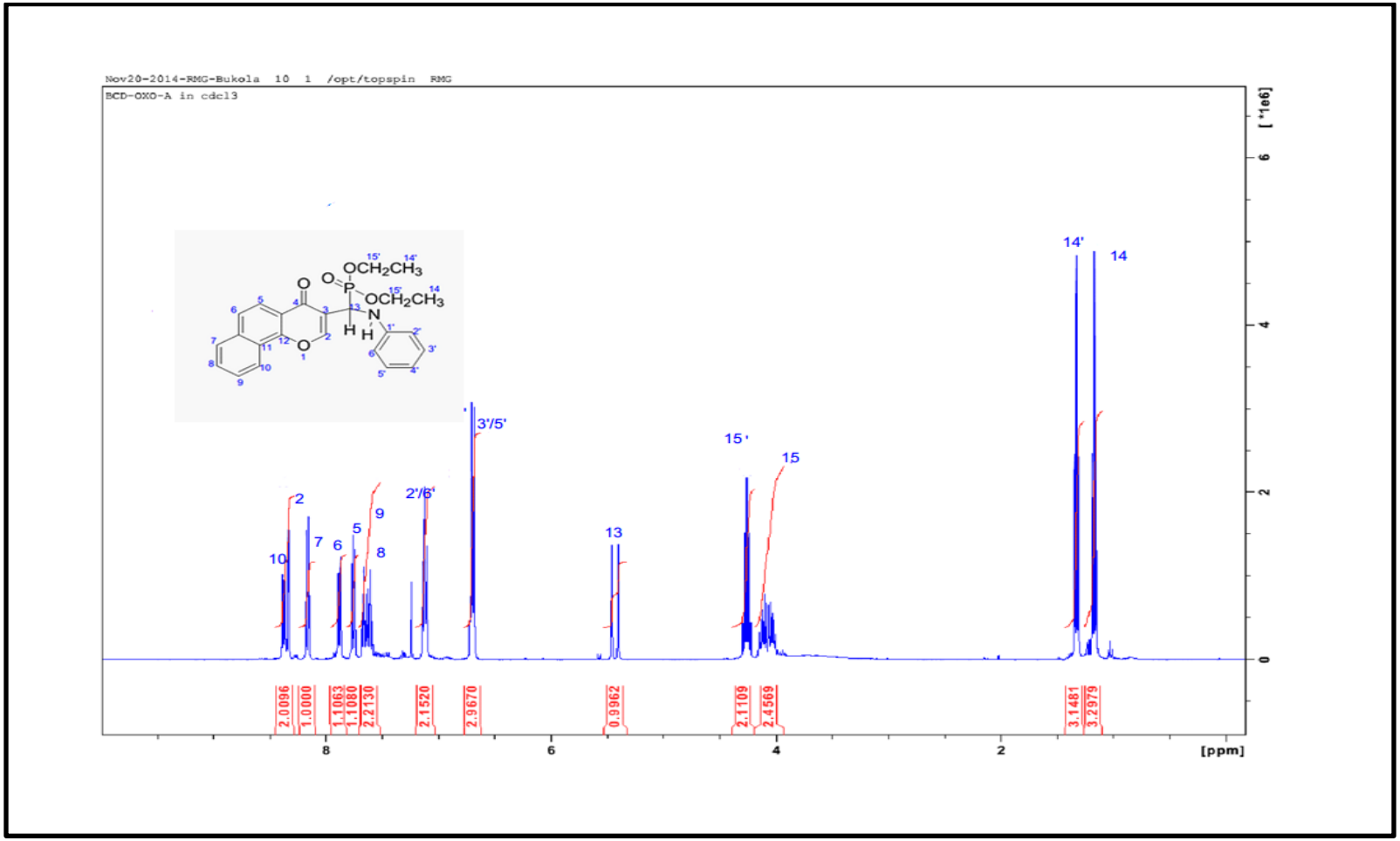


Figure 65

Appendix 55 : ^{13}C-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester

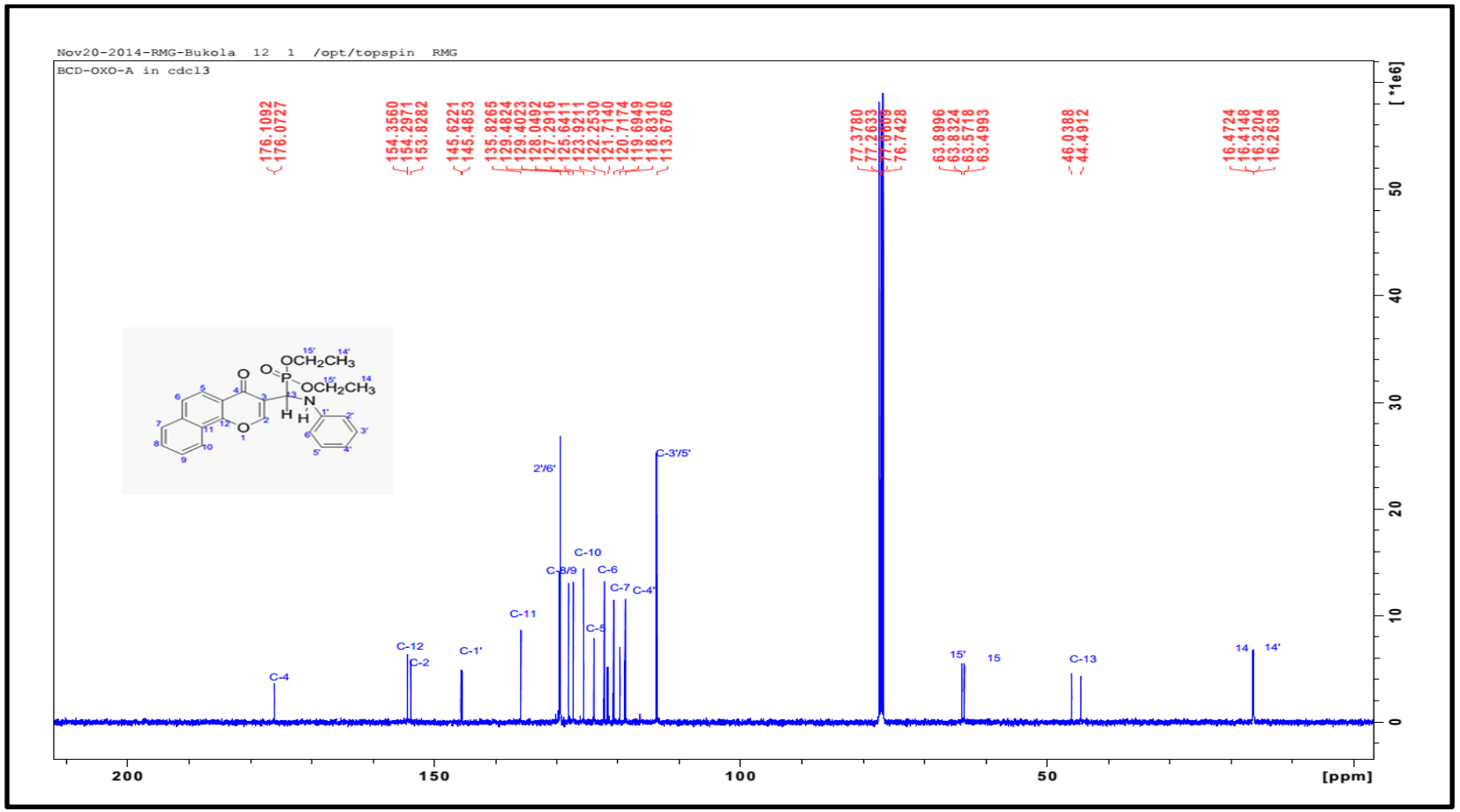


Figure 66

Appendix 56 : ^{31}P-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(phenylamino)]-methane phosphonic acid diethyl ester

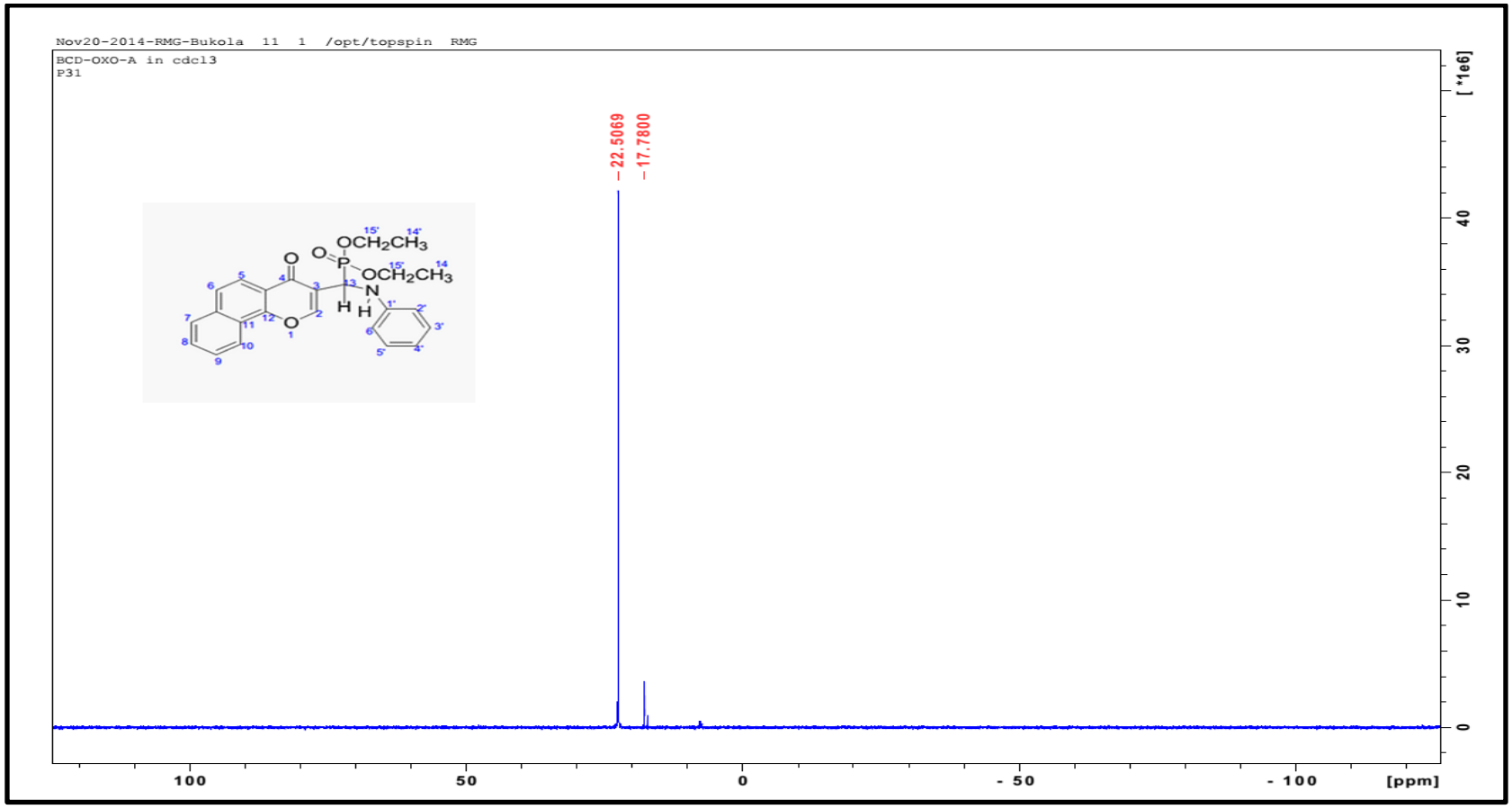


Figure 67

Appendix 57: Mass-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

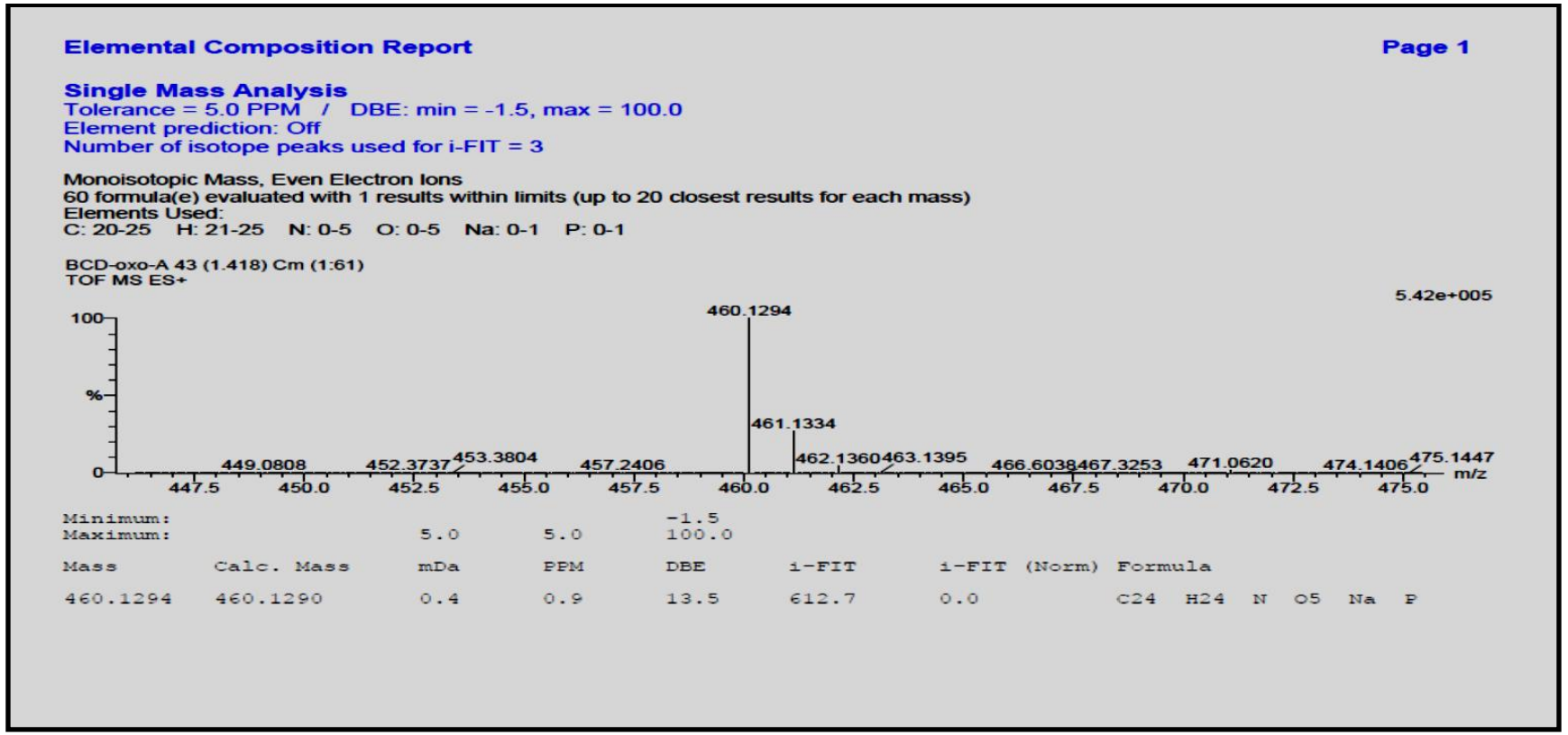


Figure 68

Appendix 58 : IR-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

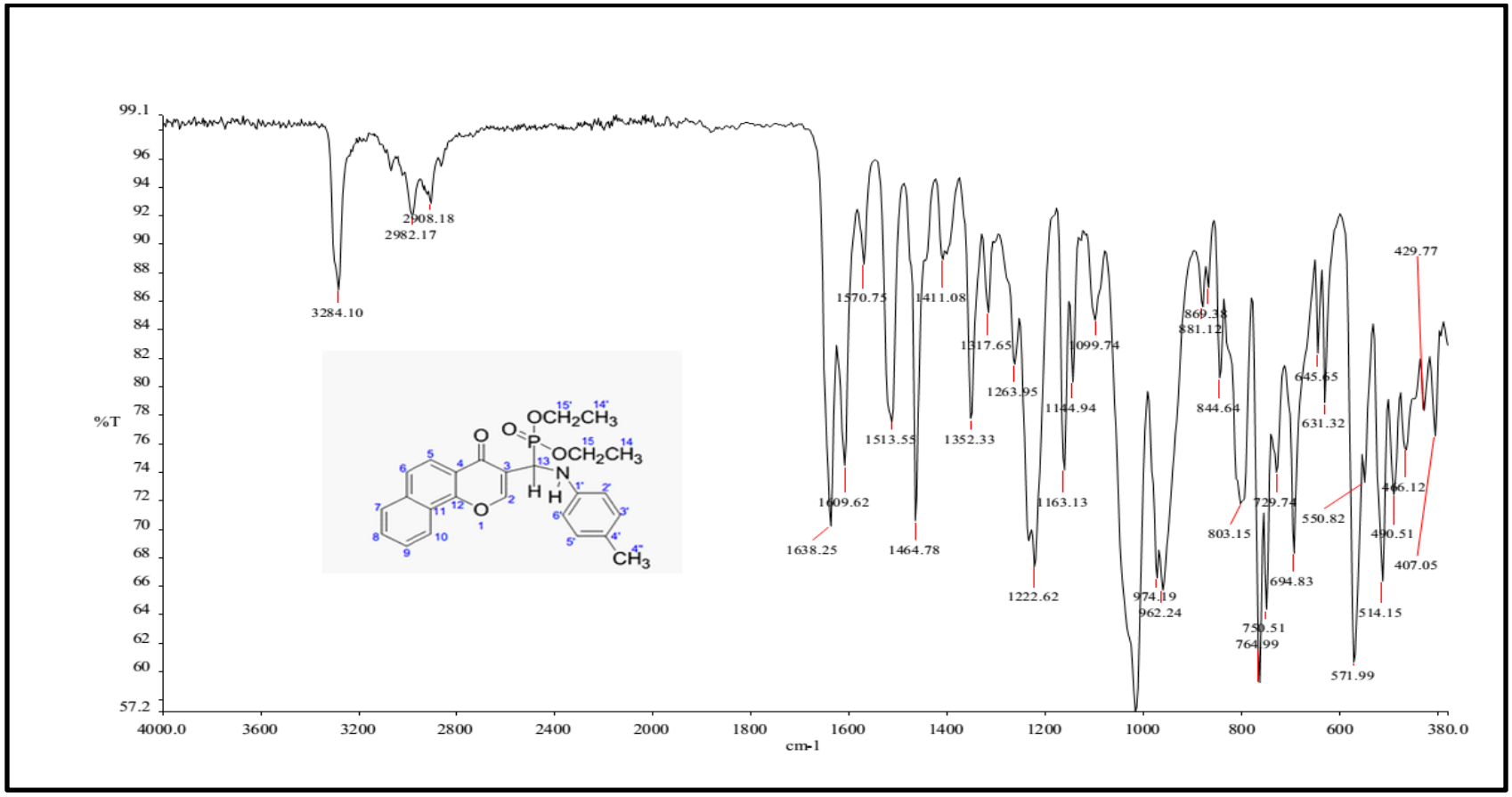


Figure 69

Appendix 59 : ^{1}H-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

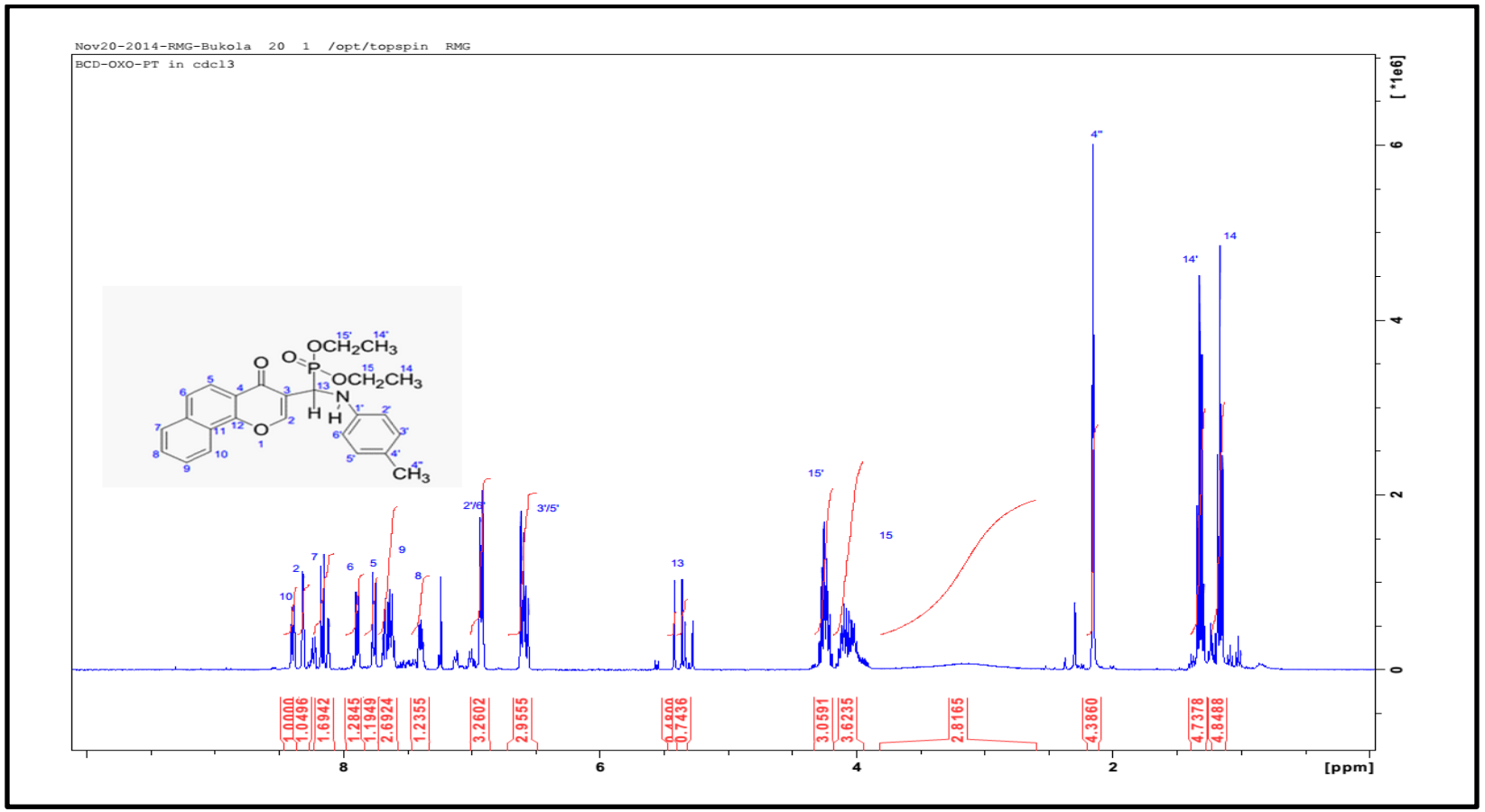


Figure 70

Appendix 60: ^{13}C-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

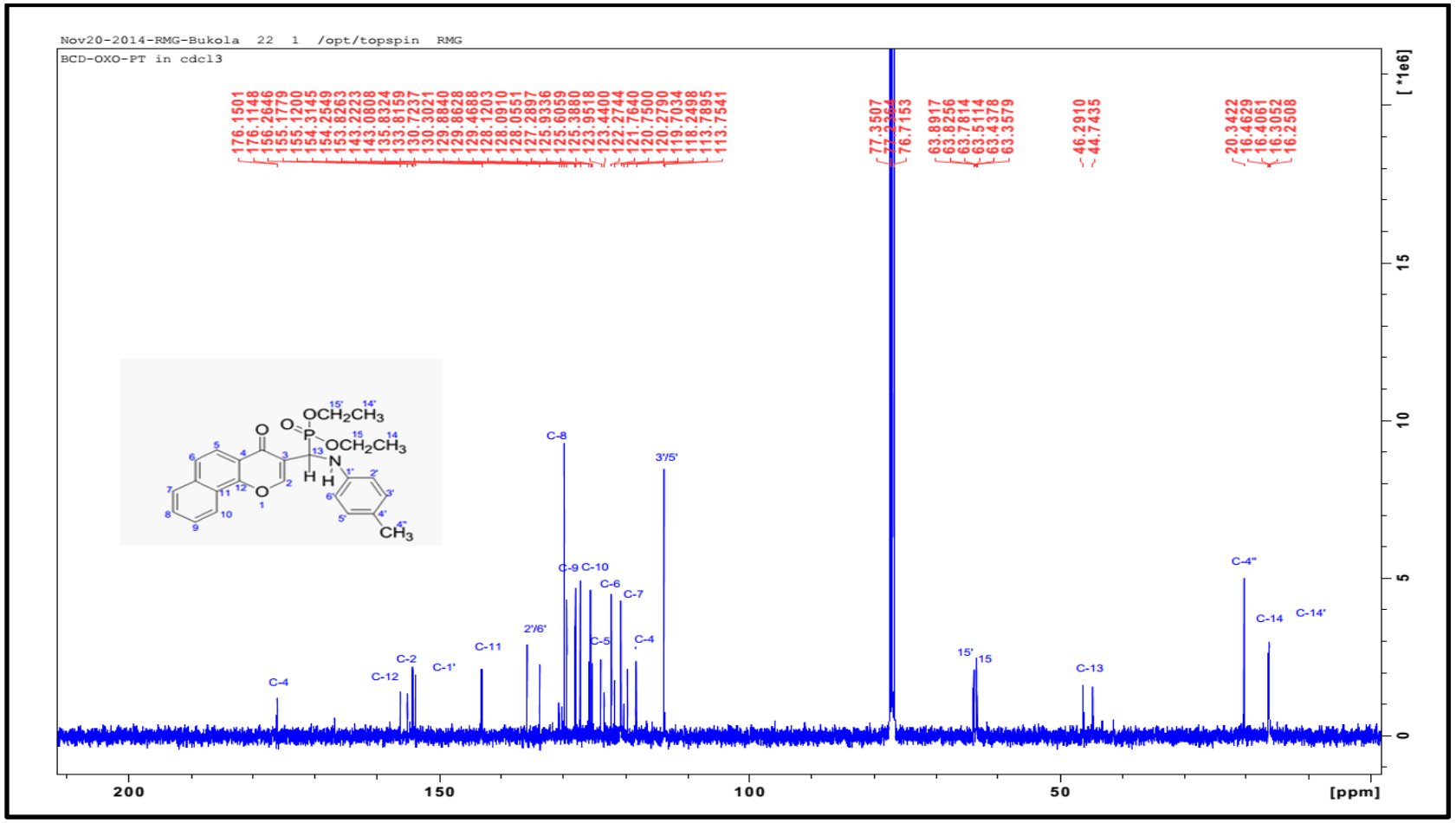


Figure 71

Appendix 61: ^{31}P-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

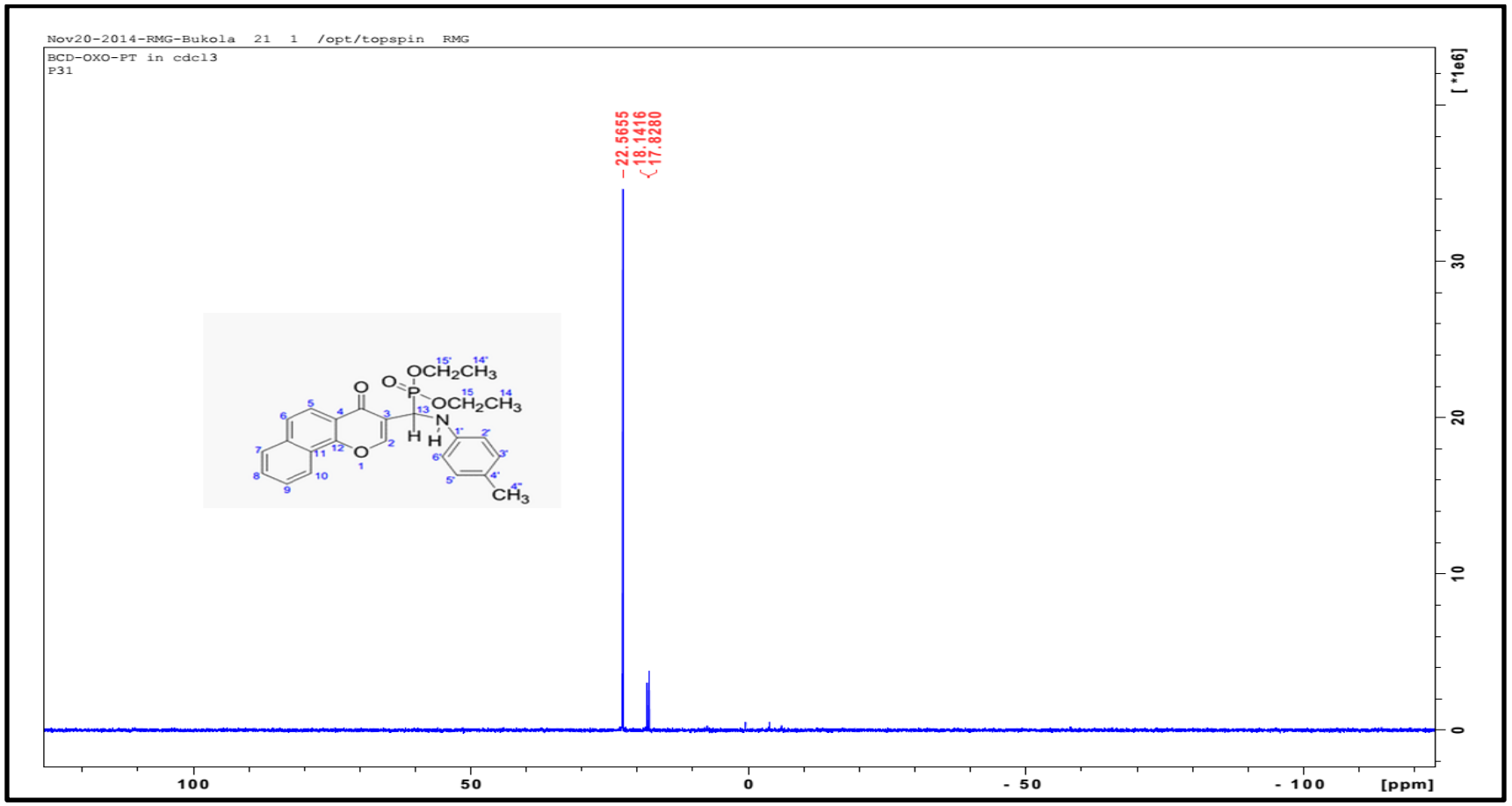


Figure 72

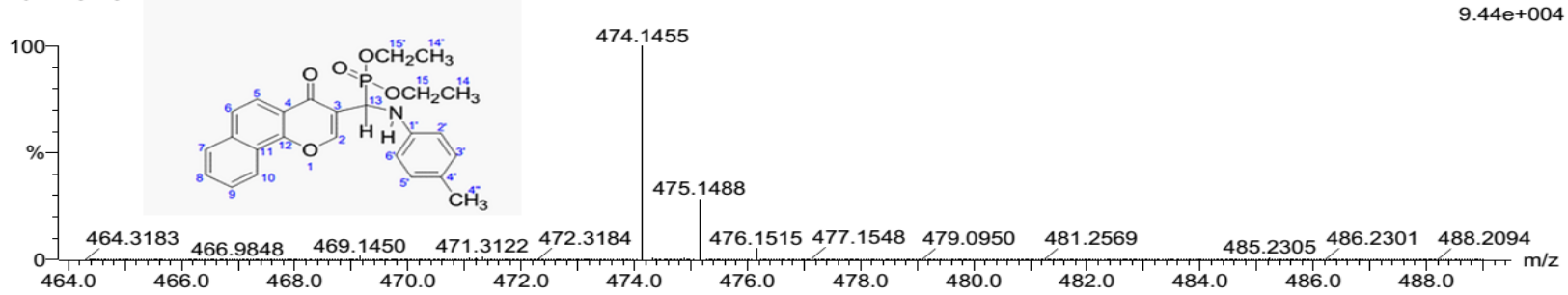
Appendix 62 : Mass-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(p-tolylamino)]-methane phosphonic acid diethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
14 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 25-30 N: 0-5 O: 0-5 Na: 1-1 P: 1-1

BCD-OXO-PT 45 (1.485) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
474.1455	474.1446	0.9	1.9	13.5	502.3	0.0	C25 H26 N O5 Na P

Figure 73

Appendix 63: IR-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester

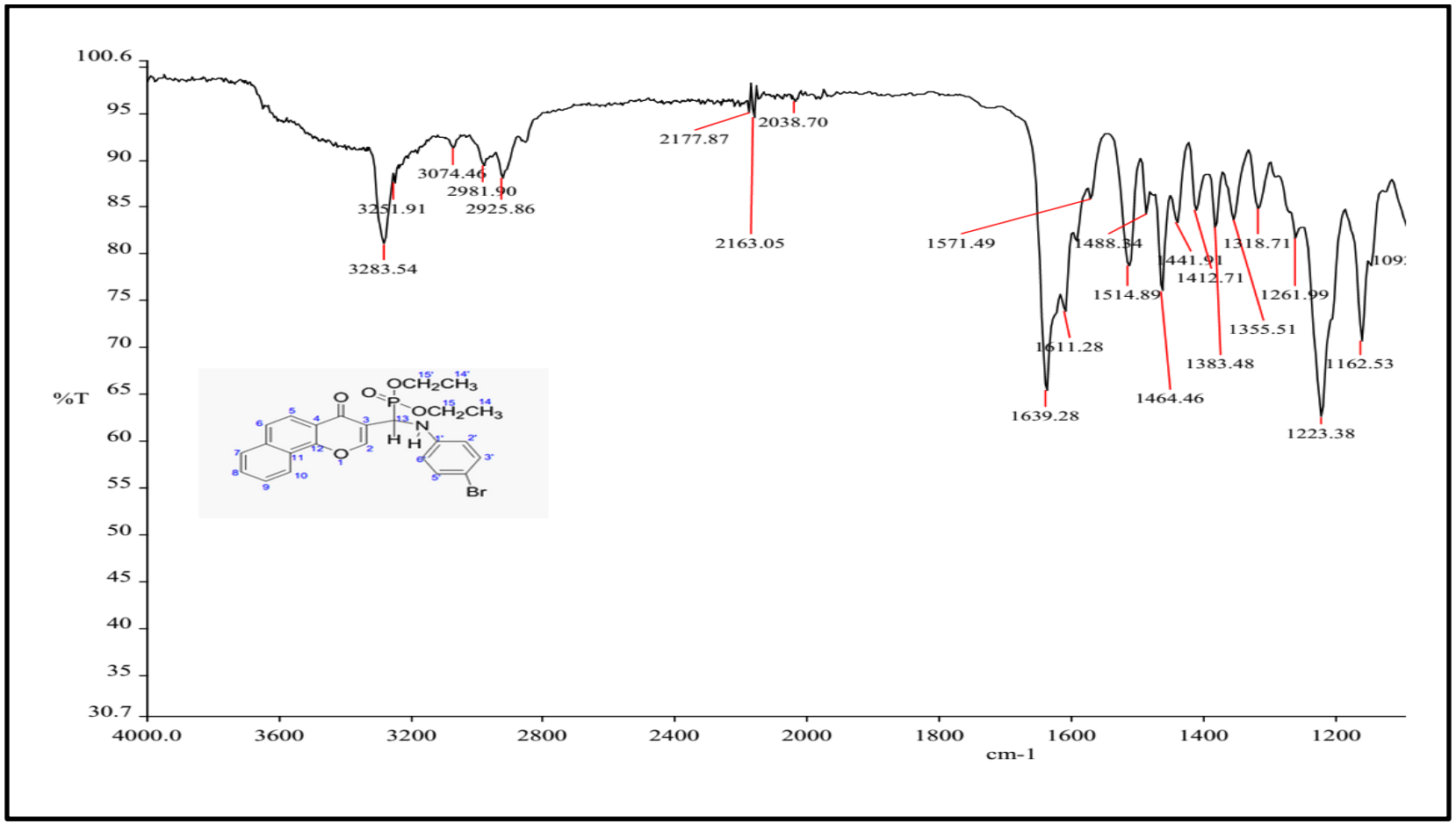


Figure 74

Appendix 64: ^{1}H-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester

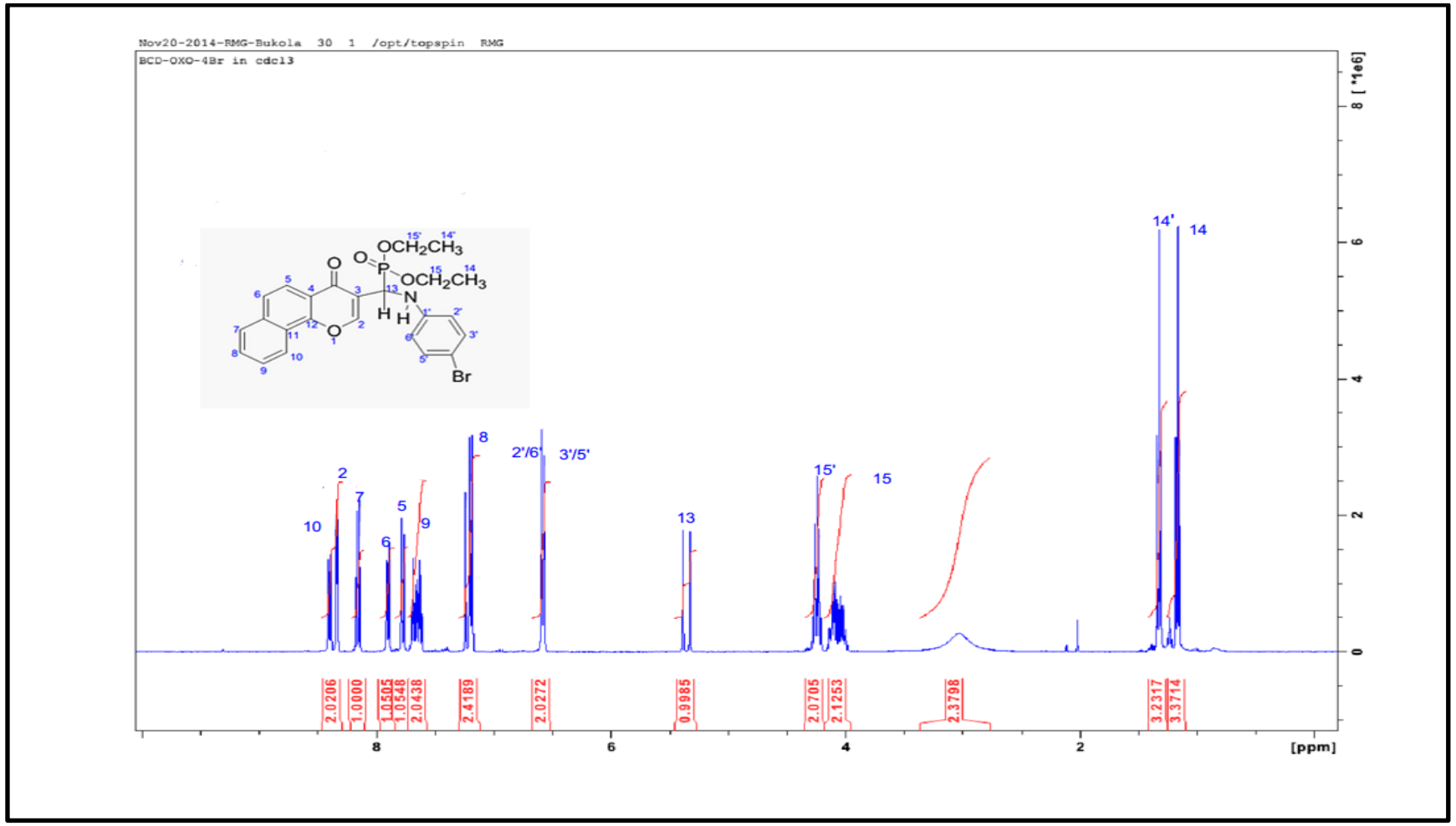


Figure 75

Appendix 65: ^{13}C-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester

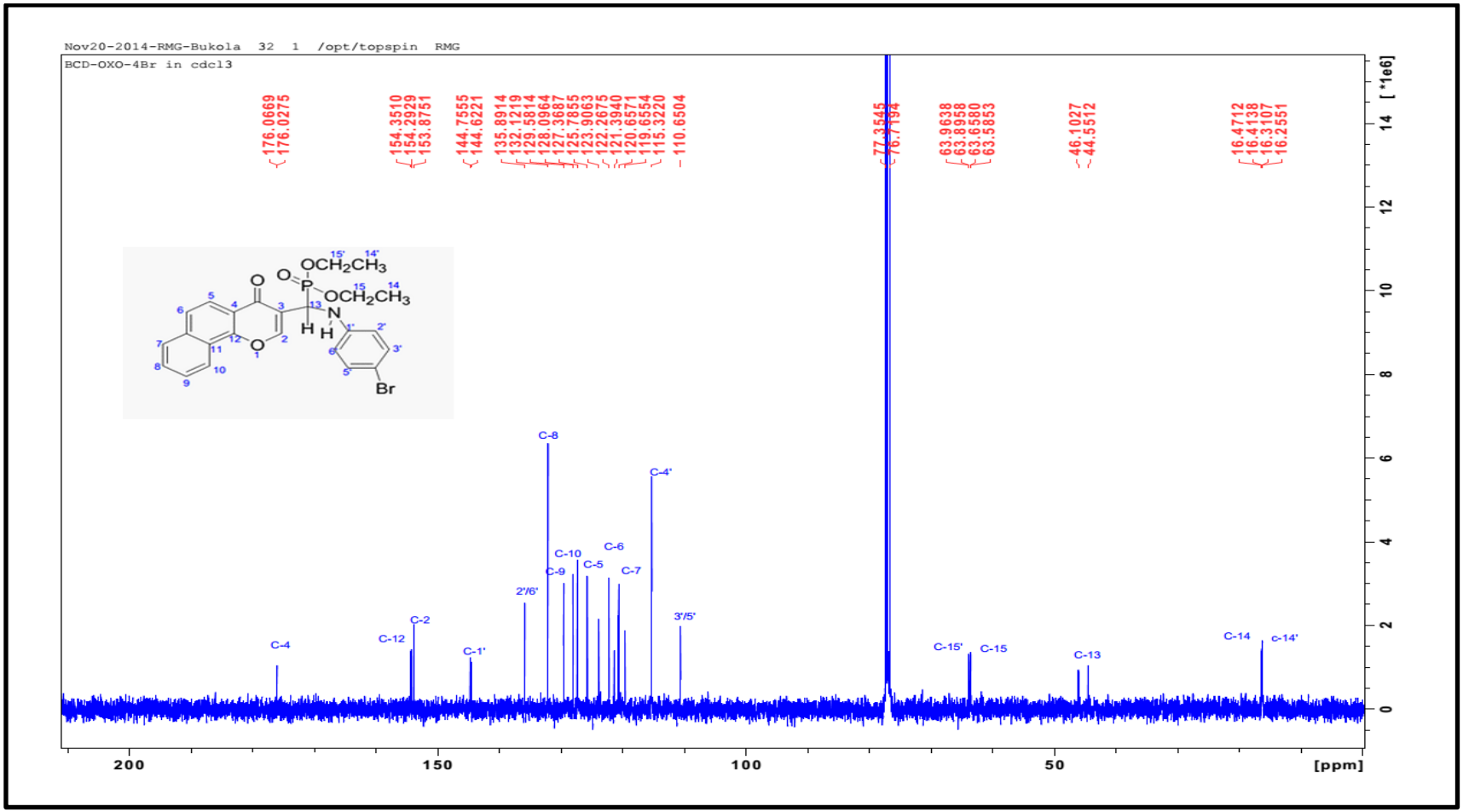


Figure 76

Appendix 66 : ^{13}C-NMR spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester

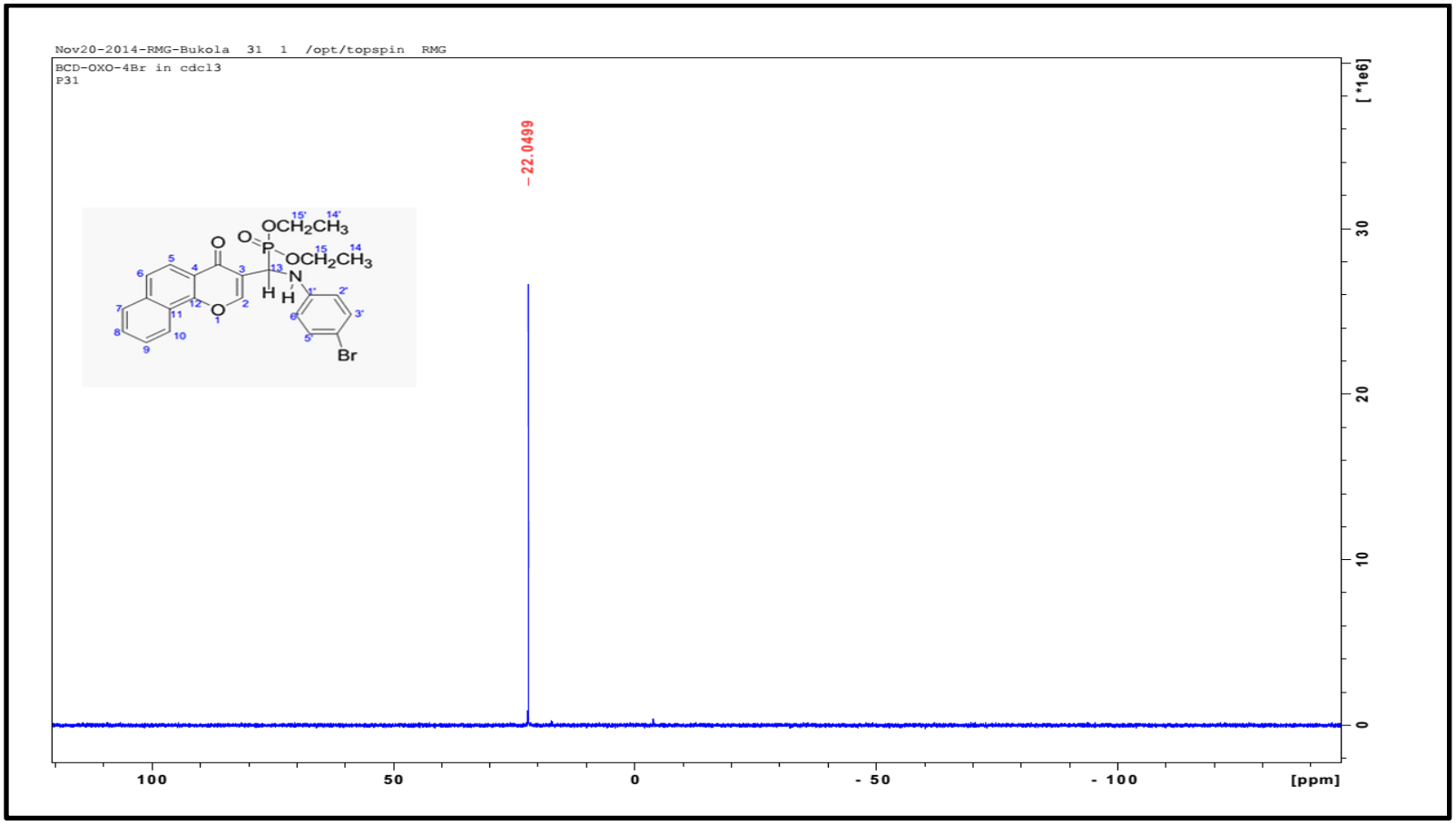


Figure 77

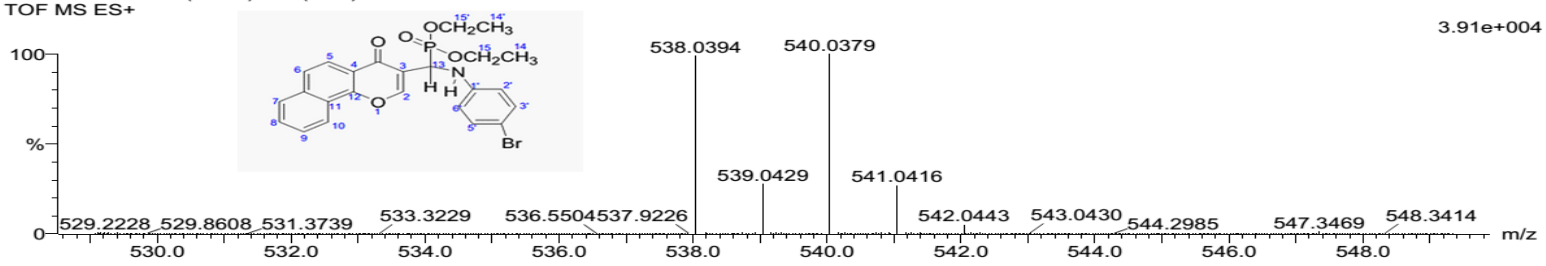
Appendix 67: Mass-spectrum of [(4-oxo-4H-benzo[h]chromen-3-yl)-(4-bromophenylamino)]-methane phosphonic acid diethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
40 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 20-25 N: 0-5 O: 0-5 Na: 1-1 P: 1-1 Br: 0-1

BCD-OXO-4Br 47 (1.552) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
538.0394	538.0395	-0.1	-0.2	13.5	429.3	0.0	C24 H23 N O5 Na P Br

Figure 78

Appendix 68: IR spectrum of [(6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

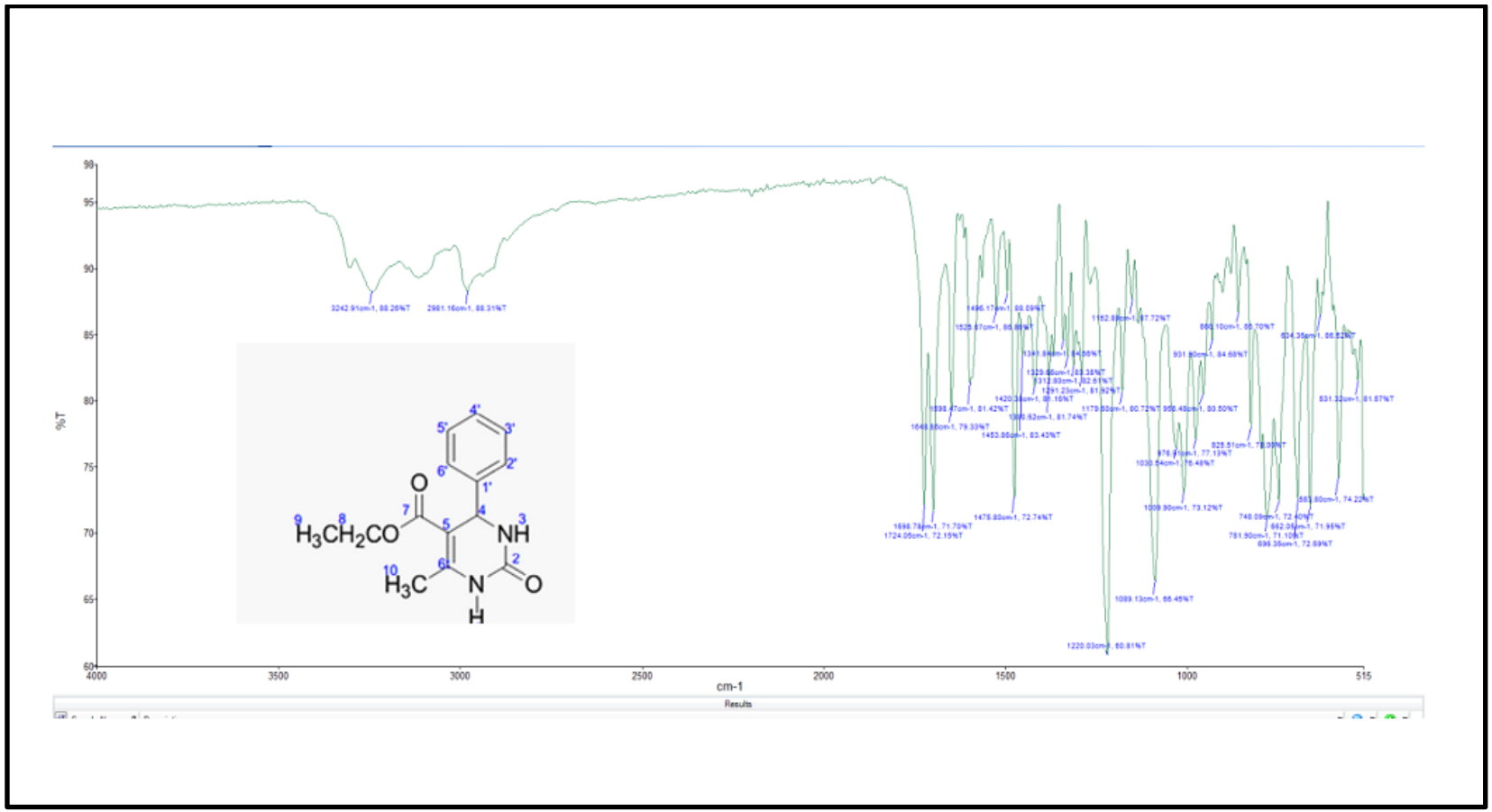


Figure 79

Appendix 69 : ^{1}H-NMR of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

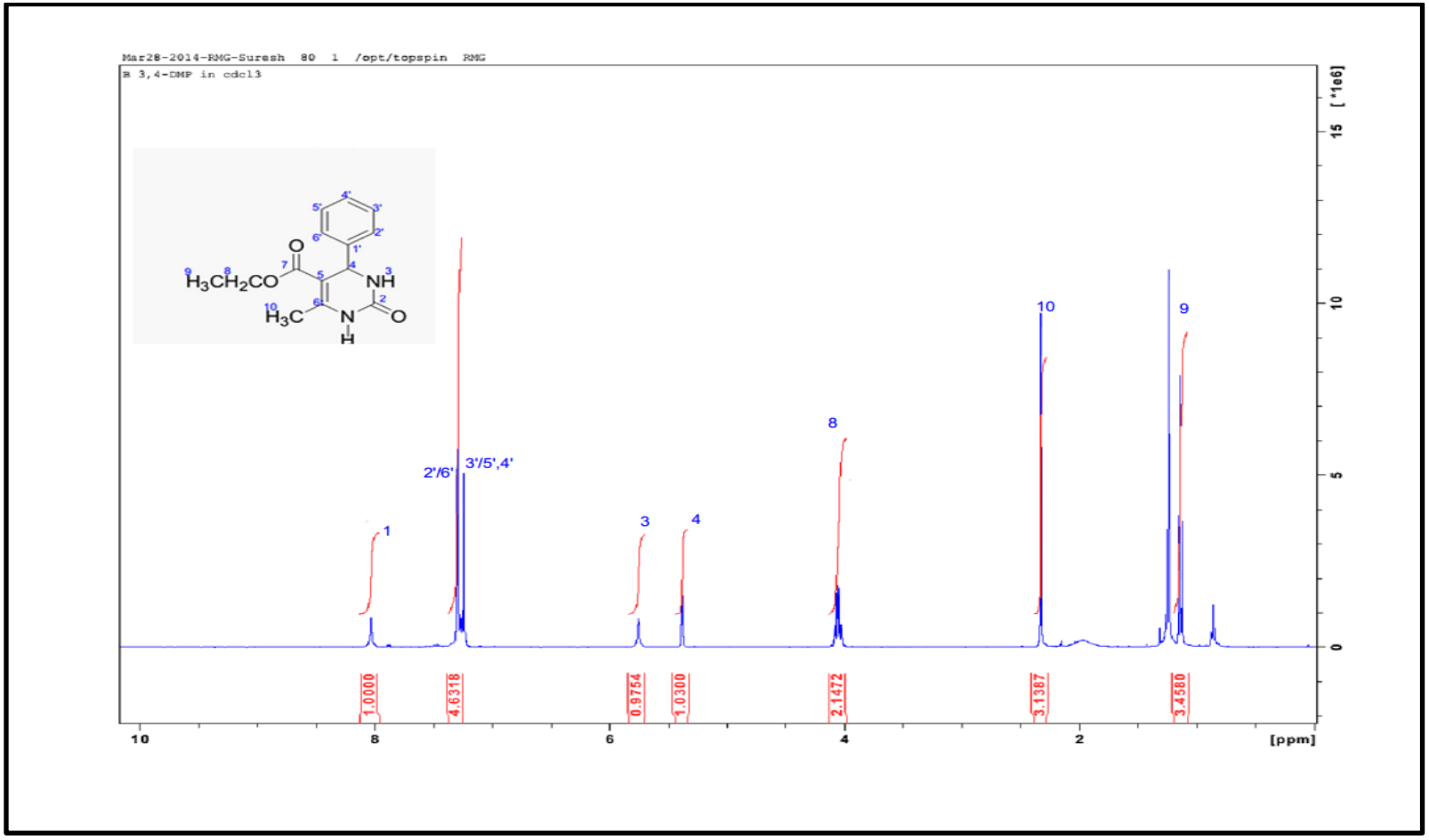


Figure 80

Appendix 70: Mass spectrum of 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

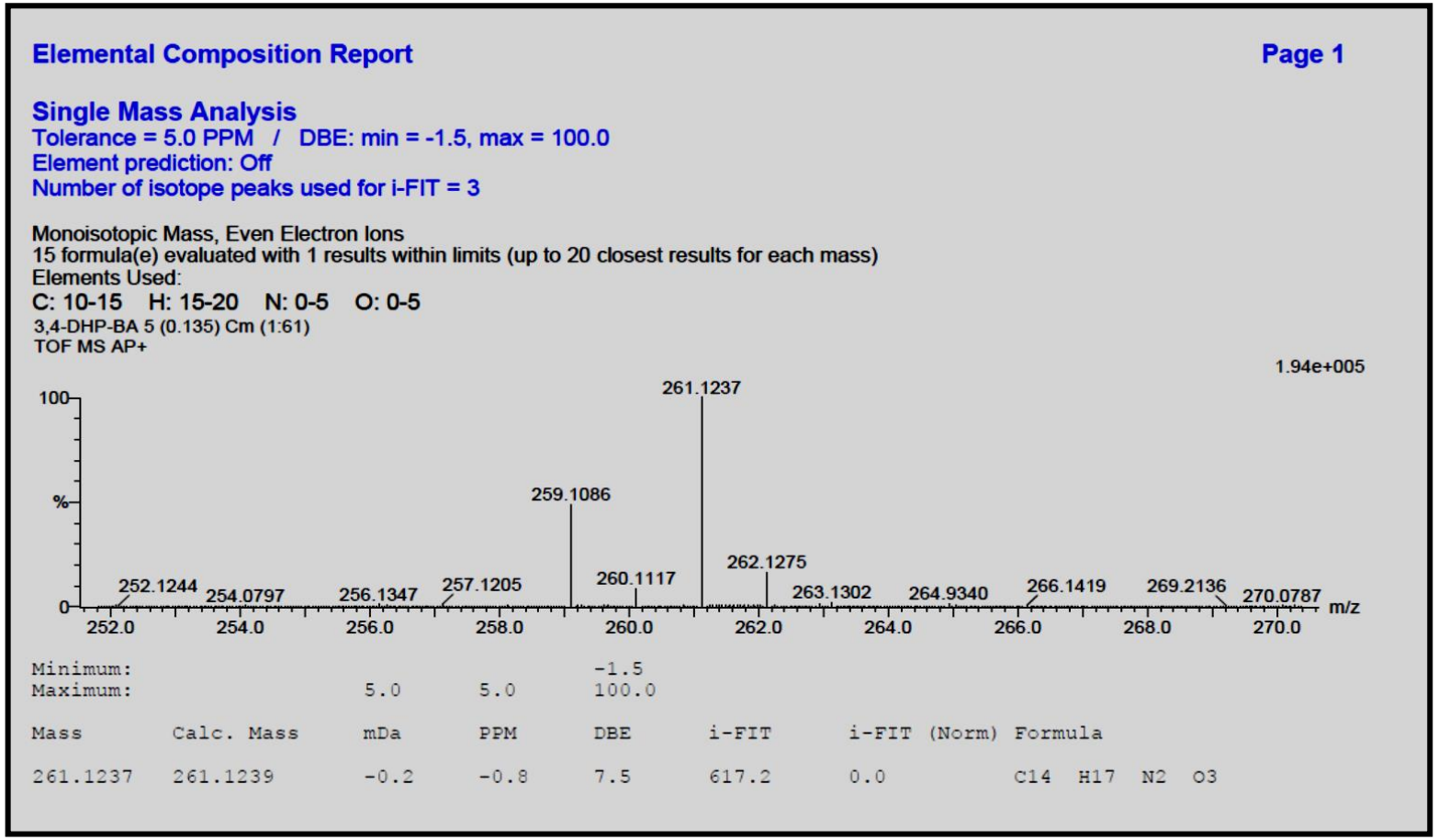


Figure 81

Appendix 71: IR Spectrum of 6-methyl-2-oxo-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

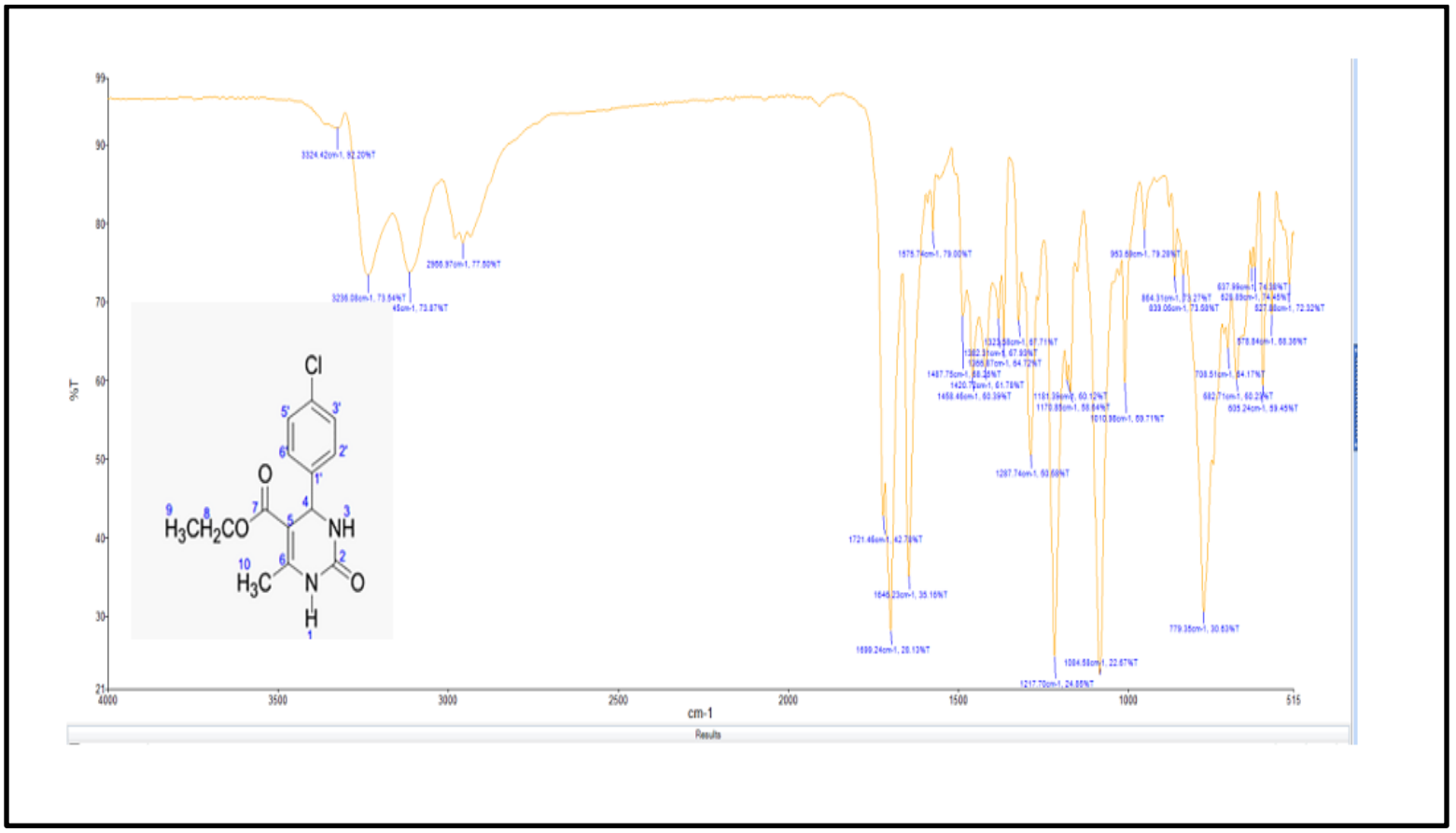


Figure 82

Appendix 72: ^{1}H-NMR of 6-methyl-2-oxo-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

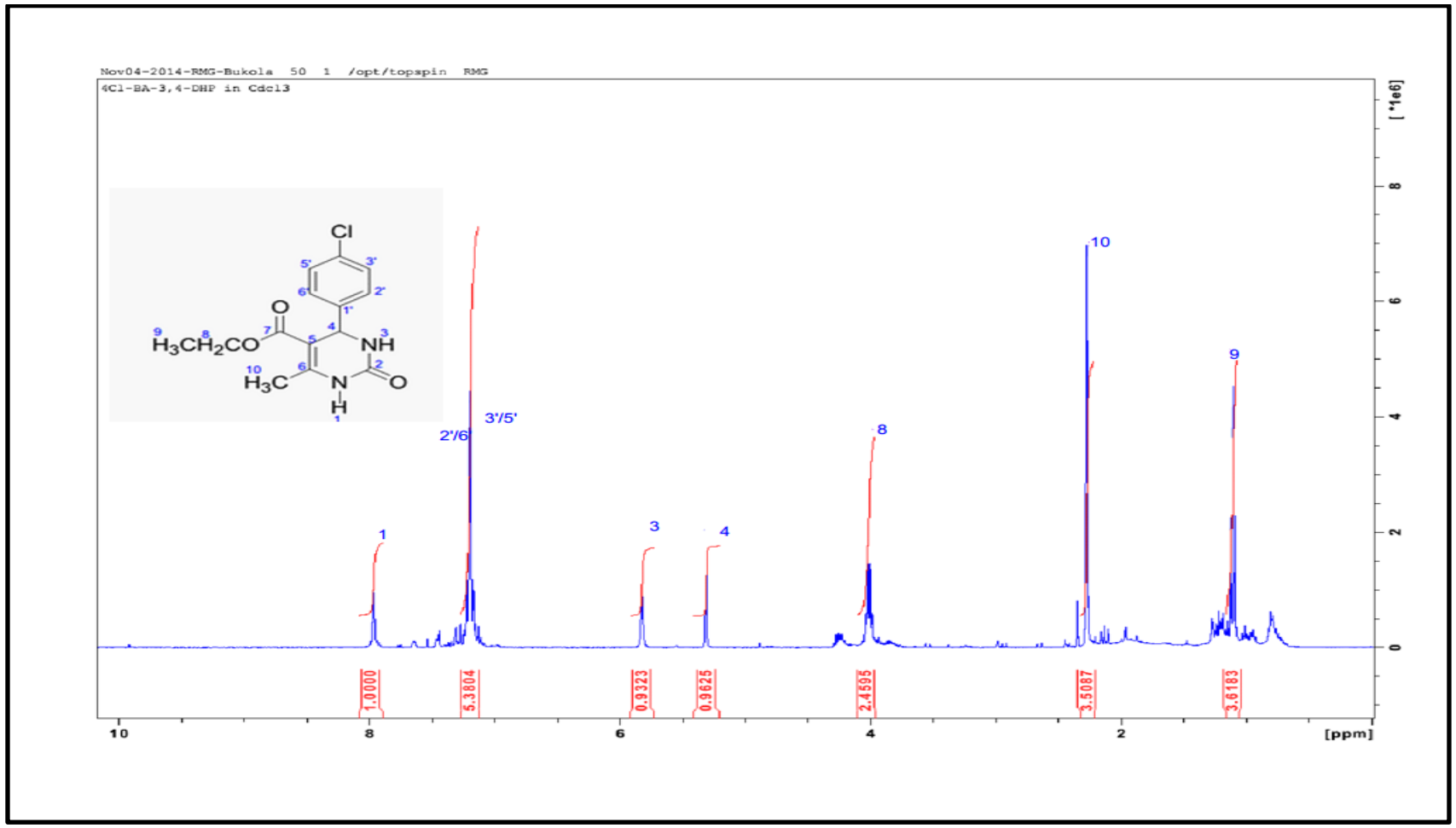


Figure 83

Appendix 73: Mass Spectrum of 6-methyl-2-oxo-4-(4-chlorophenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

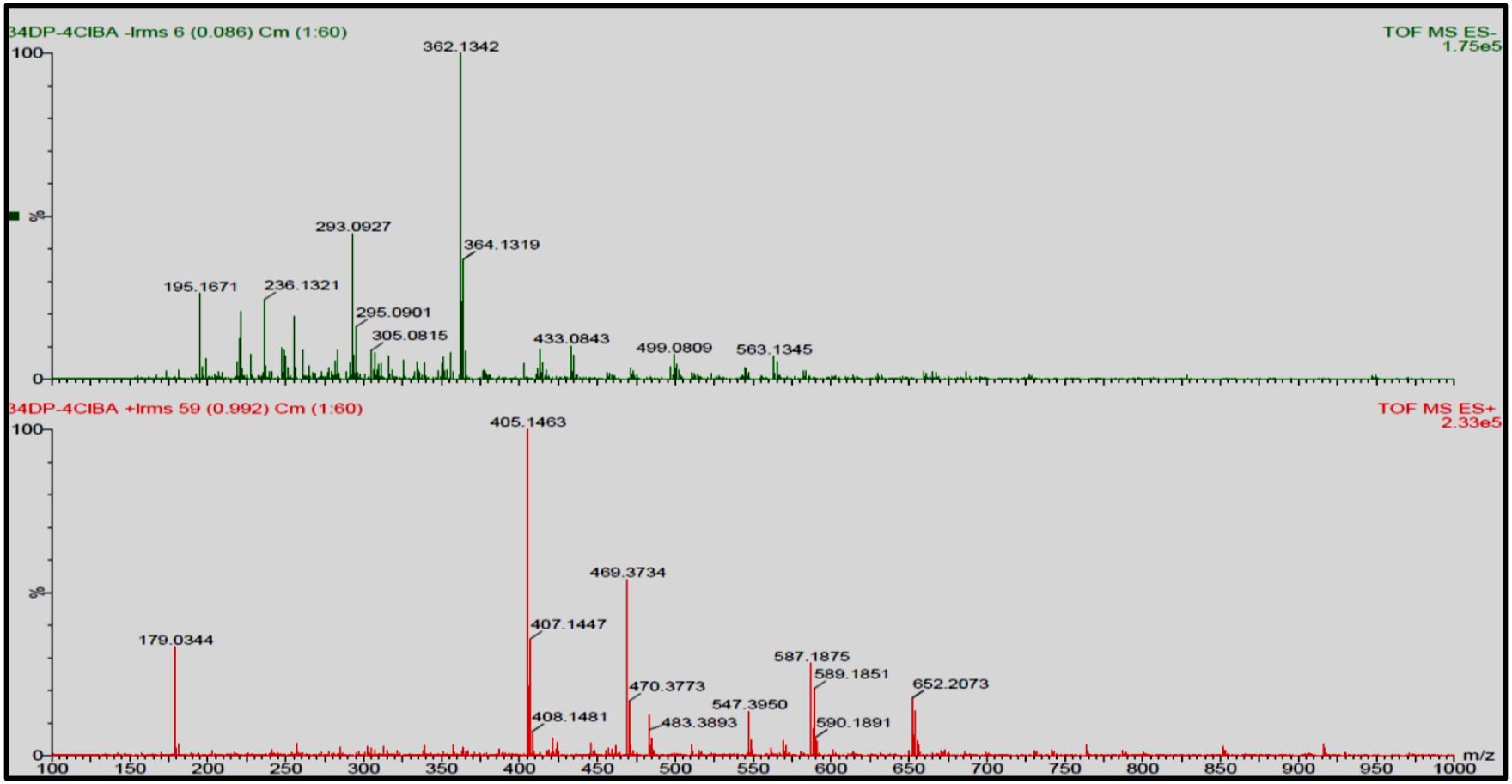


Figure 84

Appendix 74:IR spectrum of 6-methyl-2-oxo-(4-furan-2-yl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

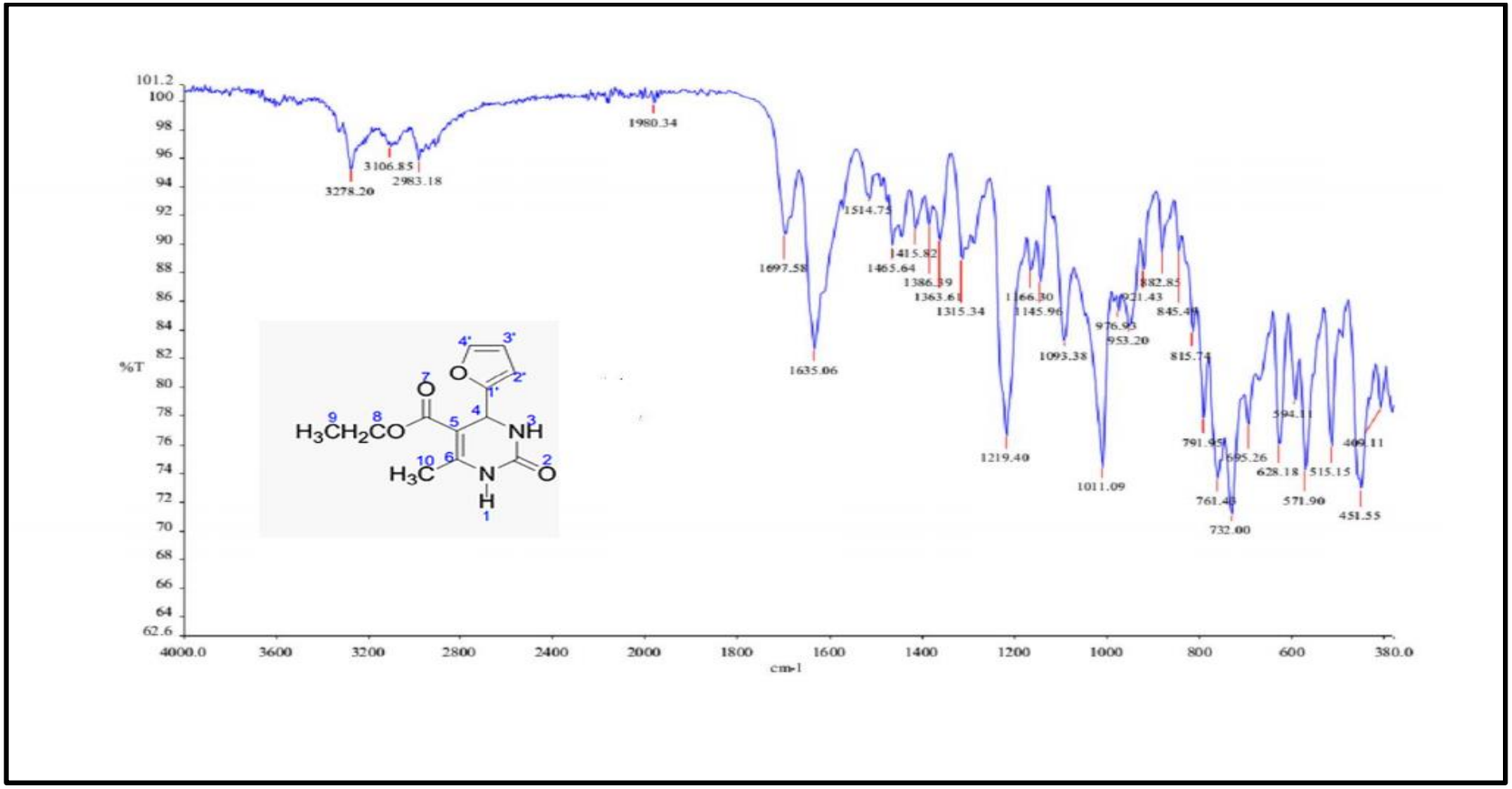


Figure 85

Appendix 75: ^{1}H-NMR of 6-methyl-2-oxo-(4-furan-2-yl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

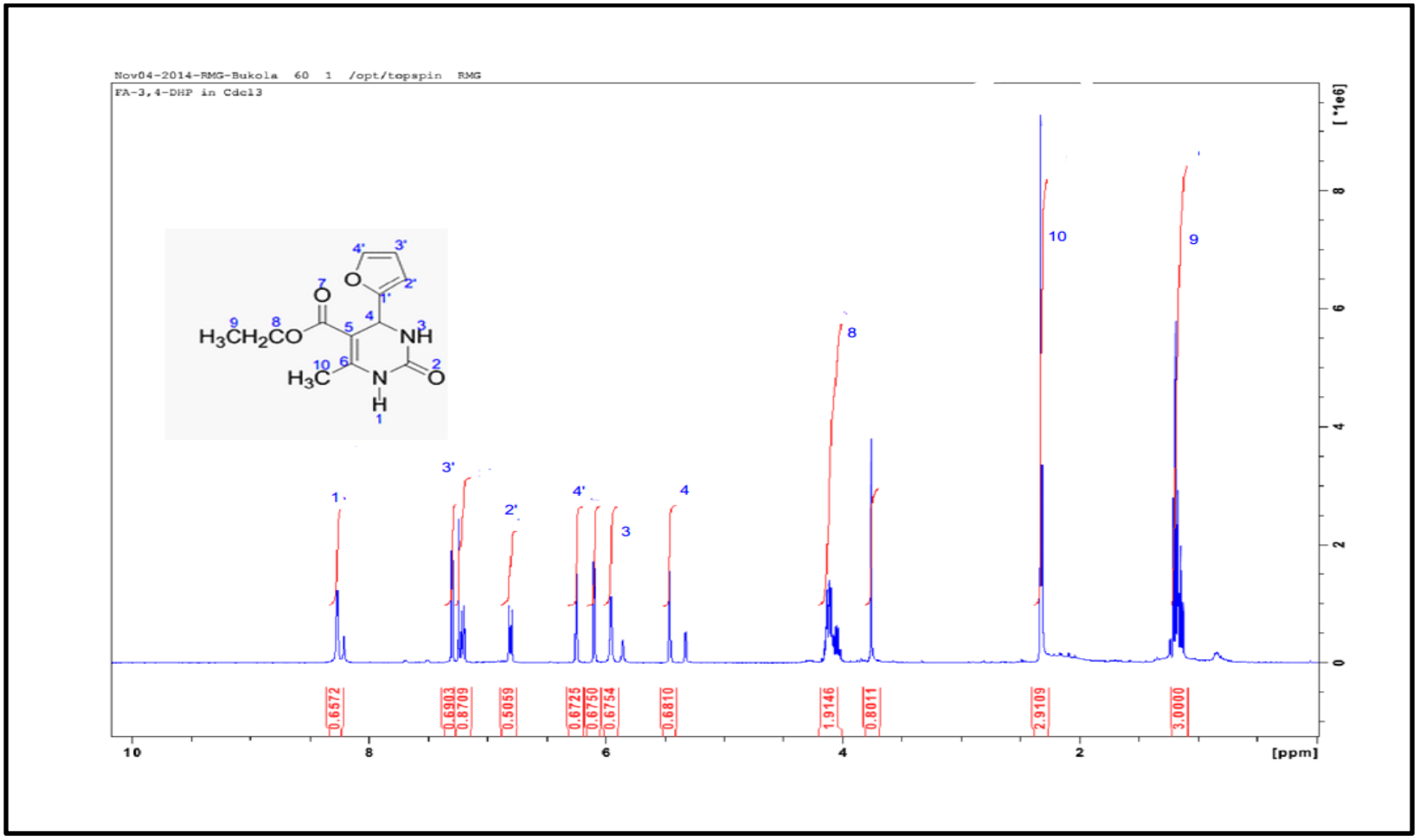


Figure 86

Appendix 76 : IR spectrum of 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

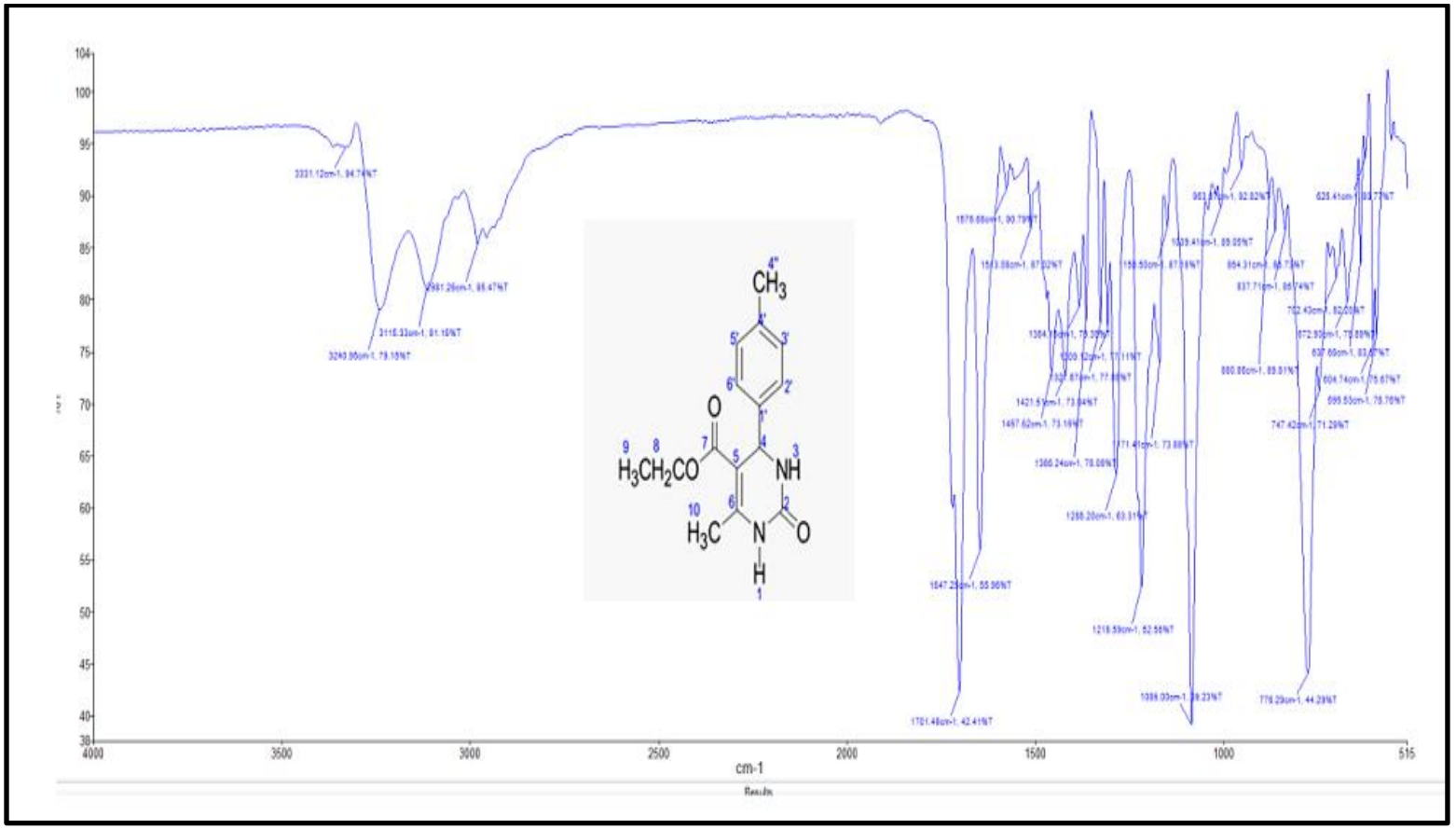


Figure 87

Appendix 77 : ^{1}H -NMR of 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

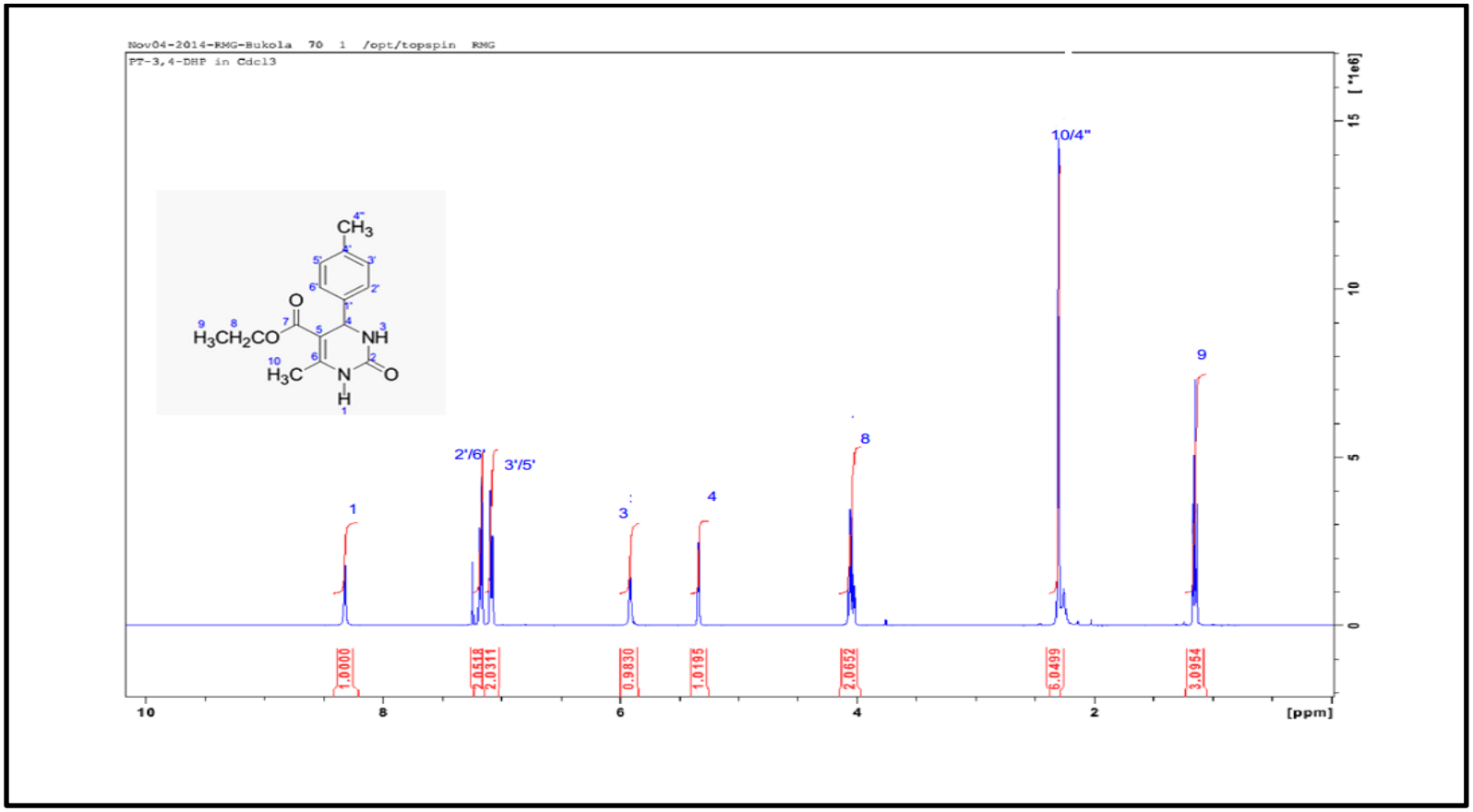


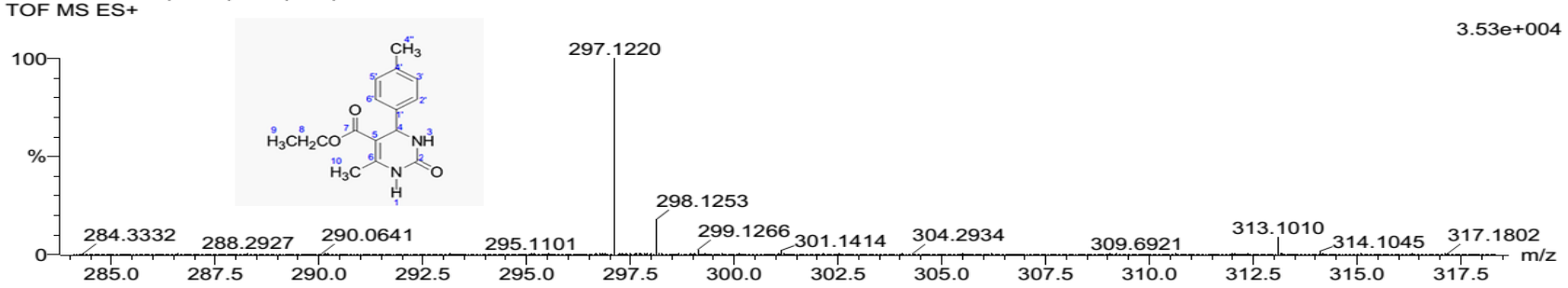
Figure 88

Appendix 78 : Mass spectrum of 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-5 Na: 1-1
3,4-DHP-PT 48 (1.586) Cm (1:61)
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
297.1220	297.1215	0.5	1.7	7.5	510.8	0.0	C15 H18 N2 O3 Na

Figure 89

Appendix 79 : IR spectrum of 6-methyl-2-oxo-4-(4-methoxyphenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

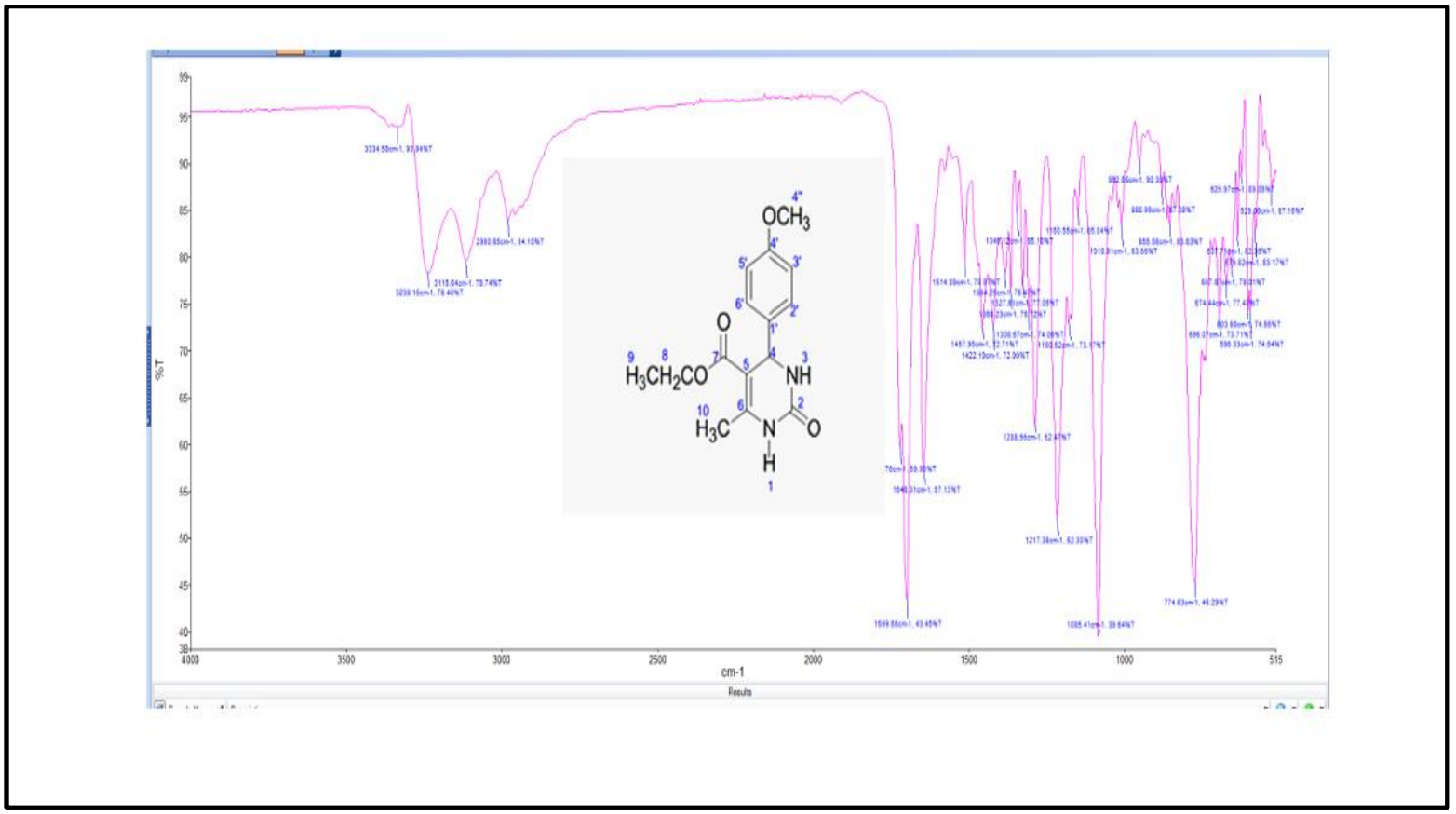


Figure 90

Appendix 80 : ^{1}H-NMR of 6-methyl-2-oxo-4-(4-methoxyphenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

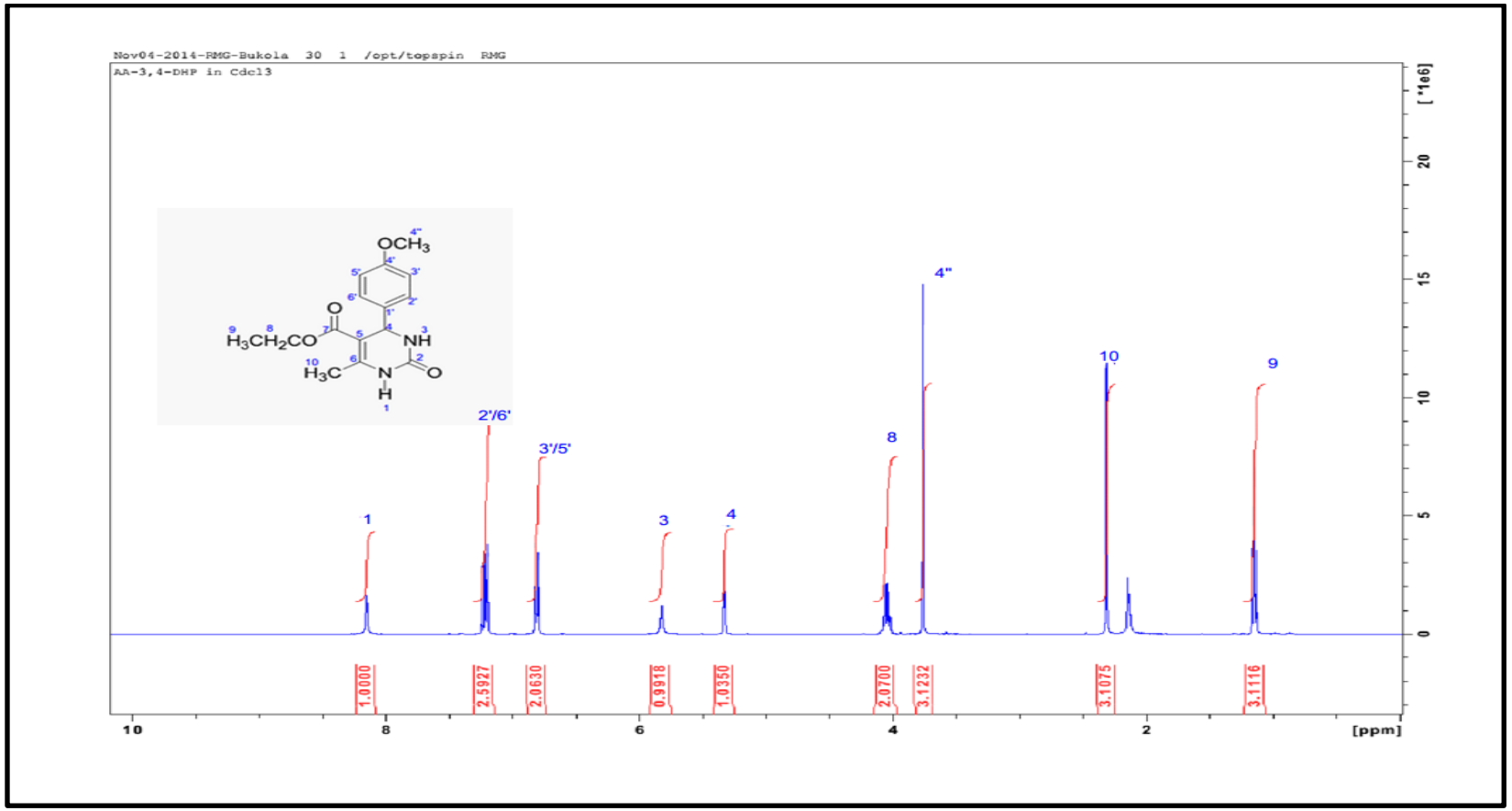


Figure 91

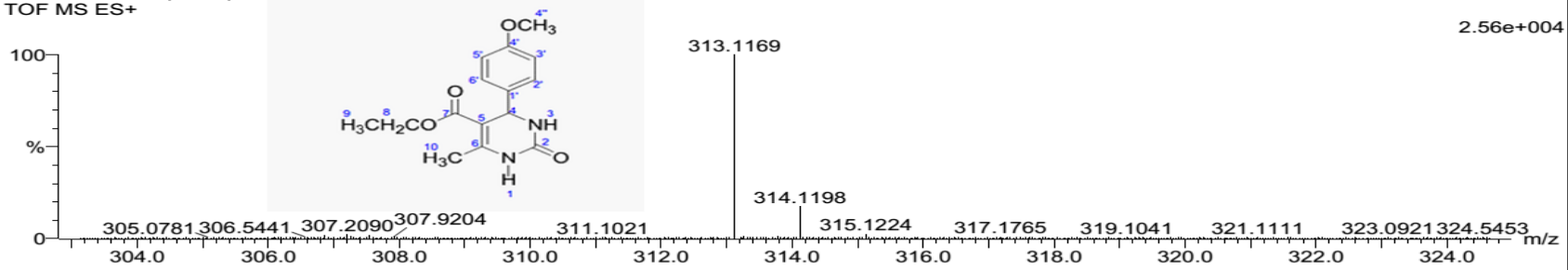
Appendix 81: Mass spectrum of 6-methyl-2-oxo-4-(4-methoxyphenyl)-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 15-20 N: 0-5 O: 0-5 Na: 1-1

3,4-DHP-AA 54 (1.787) Cn
TOF MS ES+



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
313.1169	313.1164	0.5	1.6	7.5	512.3	0.0	C15 H18 N2 O4 Na

Figure 92