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New strategies for the synthesis of functionalized substituted bisphosphonates: chemistry and biological activity

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New strategies for the synthesis of functionalized substituted bisphosphonates: chemistry and biological activity

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DISSERTATION

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This work is dedicated to my family, close friends, well-wishers and everyone who supported me and believed in my capabilities.

I am eternally grateful.

Declaration

I hereby certify that this thesis has not been submitted before, in whole or in part, to this or any other university for any degree and is, except where otherwise stated, the original work of the author.

Signed: _____ Date: _____

Mulani Iqbal Mubarak

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Abbreviations

aq	aqueous
Ar	aryl (substituted aromatic ring)
ATP	adenosine triphosphate
Bn	benzyl
bp	boiling point
BPs	Bisphosphonates
br	broad (NMR signal)
ca	circa (approximately)
°C	degrees Celcius
cat.	Catalytic
conc.	Concentrated
1,3-DC	1,3-dipolar cycloaddition
DEXA	Dexamethasone
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
E^+	Electrophile
EDG	Electron-donating group
e.e.	Enantiomeric excess
e.g.	exempli gratia (for example)
Equiv	equivalent
Fig.	figure
FPPS	farnesyl pyrophosphate syntheses
ESI	electronspray ionization
Et	ethyl
etc	etcetera

EWG	Electron-withdrawing group
FMO	Frontier Molecular orbital
δ	chemical shift
g	gram
gem	geminal
GC	Gas liquid chromatography
GTP	guanosine triphosphate
h	hour
HA	hydroxyapatite
H-Bonding	Hydrogen bonding
HOMO	Highest occupied molecular orbital
HPLC	High performance liquid chromatography
hν	irradiation with light
J	coupling constant (NMR signal)
i.e.	for example
LAH	lithium aluminum hydride
LA	Lewis acid
LUMO	Lowest unoccupied molecular orbital
m	multiplet (NMR signal)
mg	milligram
MHz	megahertz
min	minutes
mL	milliliter
mmol	millimole
m/z	mass/charge
Me	methyl
MO	Molecular orbital
NBPs	Nitrogen containing Bisphosphonates

MS	mass spectrometry
NMR	nuclear magnetic resonance
Nu	nucleophile (general)
o	ortho
Ph	phenyl
p	para
PEG	Poly-(ethylene glycol)
PPi	pyrophosphate
ppm	parts per million (NMR signal)
Py	pyridine
q	quartet (NMR signal)
Rf	retention factor in chromatography
RT	room temperature
s	singlet (NMR signal)
sec	secondary
t	triplet (NMR signal)
TS	Transition state
THF	Tetrahydrofuran

Contents

Abstract

The ever expanding cutting edge technologies in medicine for the benefit of society, the orthopedic branch is one among those significant branches in medicine pertaining to bone. Bisphosphonates (BP_S) are being increasingly and successfully used to prevent bone fractures and the concerning problems of bone diseases such as Paget's diseases, osteoporosis and tumour bone disease. In view of this specific problem, BP_S are well established in the treatment of osteoclast -mediated resorbtive bone diseases including osteoporosis, Paget's disease and tumor-induced osteolysis. Recent studies suggest that, besides inhibiting bone resorbtion, BP_S may also exert a direct antitumor effect, and this class of drugs has been shown to inhibit proliferation and to induce apoptosis in vitro in different human tumor cell lines. BPs are classified into two groups according to their chemical structure and mechanism of action: (i) non nitrogen containing BP_S such as etidronate and clodronate that are of low potency and inhibit osteoclast function via metabolism into toxic ATP-metabolites and (ii) nitrogen-containing BP_S (NBP_S), such as pamidronate, alendronate, risedronate, ibandronate and zoledronate which is the most potent antiresorptive agent.

Hence in present investigation we synthesized some several bisphosphonates bearing a substituted isoxazolidine ring by direct 1, 3-dipolar cyclization reaction in the absence of solvent and good yield under novel, promising and low cost microwaves catalysis. The method allows the simultaneous incorporation on the geminal position of the bisphosphonate framework, of basic nitrogen and of an oxygen atom, as third hook. The studies on the inhibitory potency of cyclic nitrogen

containing bisphosphonates indicate that the presence of two geminal phosphonate groups is responsible for interaction with the molecular target. In addition, basic nitrogen in the heterocyclic side chain affects potency and its orientation is critical for effective inhibition of bone diseases.

For the synthetic point of view, different aryl and alkyl substituents on the isoxazolidine ring prompt us to investigate the ring opening of these compounds through cleavage of the *N-O* bond. This strategy represents a novel access to new *gem*-hydroxyl bisphosphonates, bearing aryl substituents on the lateral chain. The reductive cleavage of the *N-O* bond in isoxazolidines represents a simple and direct access to *N*-substituted aminoalcohols, valuable intermediates in many synthetic strategies. Moreover, additional reaction path way have been envisaged leading to the formation of non-hydroxyl bisphosphonates.

Abstract in lingua italiana

I derivati bisfosfonati (BPs) sono stati impiegati con successo nella prevenzione delle fratture ossee e nei problemi legati alle malattie ossee come la malattia di Page, l'osteoporosi e il tumore osseo. Più in dettaglio i bisfosfonati sono utili per il riassorbimento mediato degli osteoclasti nelle malattie descritte in precedenza. Secondo studi recenti, i BPs possono esercitare un effetto antitumorale diretto e questa classe di composti inibisce la proliferazione, inducendo apoptosi in vitro in differenti linee cellulari tumorali umane. I BPs sono classificati in due gruppi in base alla struttura chimica e il loro meccanismo d'azione: (i) BPs non contenenti azoto, a bassa potenza inibitoria; (ii) BPs contenenti azoto, più potenti e con una migliore risposta. Di conseguenza, nel seguente lavoro di tesi di dottorato sono stati sintetizzati diversi BPs contenenti un anello isossazolidinico, tramite cicloaddizione 1,3-dipolare e utilizzo di microonde (buone rese in ogni caso). Questo metodo permette

l'incorporazione simultanea di un atomo di azoto e di ossigeno nella struttura dei bifosfonati. Studi sulla potenza inibitoria dell'azoto ciclico presente sui BPs indicano che la presenza di due gruppi fosfonati geminali è responsabile generalmente dell'interazione con il target molecolare. Dal punto di vista sintetico, dopo avere inserito diversi sostituenti arilici e alchilici sull'anello isossazolidinico, ci siamo spinti a investigare l'apertura dell'anello attraverso la rottura del legame *N*-*O*. Questa strategia rappresenta un approccio innovativo alla sintesi di bisfosfonati *gem*-idrossilici con sostituenti arilici sulla catena laterale. La rottura riduttiva del legame *N*-*O* rappresenta, infatti, un diretto accesso all'ottenimento di amminoalcoli *N*-sostituiti, intermedi interessanti in molti processi sintetici. Inoltre, è stato investigato anche il percorso meccanicistico alternativo che porta alla formazione di bisfosfonati non idrossilati.

Chapter 1
Bisphosphonates: structure and biological activity

1.1 Introduction

Recently, the development of organophosphorus chemistry has been characterized by a great interest in bisphosphonates (BPs) and bisphosphonic acids. The discovery and development of the bisphosphonates (BPs) as a major class of drugs for the treatment of bone diseases has been a fascinating story that has extended over three decades. Nowadays, the ultimate goal of many chemists all around the world has become rational or at least semi-rational drug discovery. Furthermore, the development of the medicinal chemistry as both a pure and an applied science is considered to have a significant impact upon it [1]. A boost of medicinal chemistry is based on development of such disciplines like combinatorial chemistry, compounds identification (e.g. NMR), automated synthesis etc. Organo-phosphorus chemistry, which, recently have taken an important effect on the synthesis and design of a wide variety of biologically active compounds. This chemistry, being ignored for many years, recently has achieved important and well-recognized place in the search for new drugs [2]. Organo phosphorus chemistry, as a discrete area of study, is the study of compounds containing a C-P bond [3]. Its present impact on the field of medicinal Chemistry is even difficult to quantify. Among the list of all organo phosphorus compounds the main place is occupied by phosphonates and bisphosphonates, which have found huge

application as pharmaceuticals. For instance, derivatives of phosphonic acid are used in the synthesis of different α-amino phosphonic acids which are considered to be structural analogues of the corresponding α-amino acids. It is noteworthy that, their negligible mammalian toxicity, and the fact that they very efficiently mimic amino carboxylic acids makes them extremely important anti metabolites in the process of designing new drugs [4]. On the other side, bisphosphonates have been found recently as an ideal therapeutic agent for treatment of different bone diseases. Their capability to chelate metal ions and inhibit crystals growth but also a strong affinity to bone was used in synthesizing new drugs by many pharmaceuticals companies[5]. Bone tissue constitutes our bodily scaffold around which our organs are compartmentalized. It is a dynamic tissue that maintains the mineral balance in an organism, as well as providing an environment for cellular machinery involved in different physiological functions[6]. Bone tissue undergoes constant remodelling, where tightly regulated anabolic and catabolic processes enable bone adaptation during the lifespan of an organism. In addition to the cells involved in regulating bone tissue mass, i.e., bone-depositing osteoblasts, bone resorbing osteoclasts and regulatory osteocytes, bone tissue provides a home for a diverse array of cells involved in systemic functions. Immune regulatory cells involved in host defence, mesenchymal stem cells involved in tissue healing repair, and hematopoietic precursors destined for systemic gas transport, are distinct cell populations residing in the bone tissue. Bone tissue is distinguished from the rest of our tissues by the presence of a massive mineral phase, i.e., biological apatite. Approximately 3–4 kg of mineral mass is present in our bodies, and two-thirds of this mineral mass is estimated to be present in the bone tissue[7]. More than 99% of bodily calcium deposits are located in bone. With the exception of dental tissue and pathological calcifications, such as kidney stones and calcified atherosclerotic plaques, no other tissue

systems contain such a concentrated mineral phase. It is the mineral phase in bones that can serve as a unique receptacle for absorption of molecules from the systemic circulation, and molecules in circulation that display a preferential affinity to biological apatite have the potential to seek and concentrate in the bone tissue. This provides a unique opportunity for developing magic bullets for bone diseases, following on from Paul Ehrlich's idea that an ideal drug will act specifically on a disease-causing agent, in this case in bones, without affecting other tissues in an organism. Only a limited number of molecules exhibit a strong affinity to bone. These include heavy metals, such as strontium, rhenium and lead, and the well-known antibacterial agent tetracycline[8]. The conventional therapeutic agents, except one class of molecules (bisphosphonates, the subject of this critical review), do not exhibit any particular affinity to bone. The systemic administration of these molecules accordingly results in non-specific distribution throughout an organism. For developing bone-specific therapeutic agents, the critical challenge becomes the design of molecules that display a preferential affinity to biological apatite with no affinity to other tissues. Systemic administration of such molecules will result in specific deposition to bone tissue with no accumulation at other tissues. This goal is likely to be difficult to achieve, since all therapeutic molecules will display a certain degree of affinity to other tissues, given the diverse array of functional groups (e.g., hydrophobic, polar, charged, etc.) found in biological membranes and surfaces. However, a step towards this goal is to engineer the currently utilized therapeutic agents for an appetite affinity. The benefits of this end ever will be two-fold. First, the molecules that are currently acceptable for treatment of bone diseases (i.e., where the therapeutic action overshadows the undesired activities) will be more effective, since bone targeting will concentrate the pharmacological agents at the desired site of activity. This will allow a more potent activity without

increasing the administered dose, which is not always possible due to undesirable activities of the therapeutic agents at extra-skeletal sites.

Secondly, promising molecules not previously tested for bone diseases due to unacceptable side effects (i.e. where the undesired activities outweigh the therapeutic action) may become effective on bone diseases after being concentrated in the bone tissue. Modifying the therapeutic agents for bone affinity, of course, should not alter the inherent pharmacological activity of the agents. In this way, a given therapeutic agent can be tailored to have a higher specificity by concentrating it to bone sites and, possibly, to display lower toxicity by reducing its exposure to extra-skeletal sites.

Efforts in this direction were set into motion in the early 1960s while probing the physiological function of an endogenous molecule, pyrophosphate **(Fig. 1).**

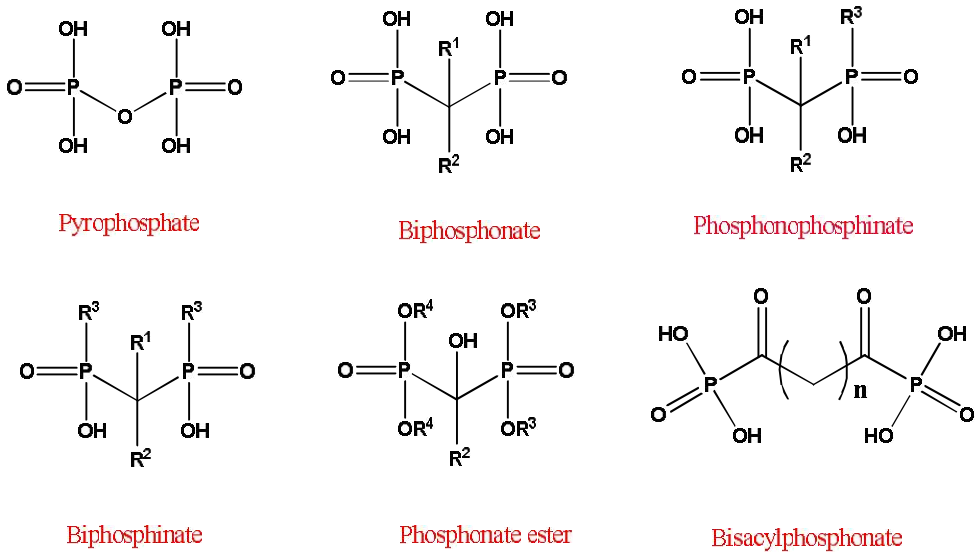

Fig. 1 *Structure of the endogenous pyrophosphonate, and its synthetic analogue, bisphosphonate (BP), which exhibit a strong bone affinity. The geminal (a) carbon in BPs typically contains two separate substituents, R1 and R2, which may significantly affect both the mineral affinity and the pharmacological activity.*

Pyrophosphate is localized throughout an organism, and displays a dual activity on the formation and dissolution of biological apatite, a carbonated form of the tachometric hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$][9,10]. The strong affinity of the pyrophosphate to nucleating HA crystals was considered to be the underlying basis of this dual activity[11]. On one hand, the pyrophosphate appeared to become localized on the growing crystal surfaces, preventing the growth of the HA (i.e., 'poisoning' the fledgling crystal growth). On the other hand, this pyrophosphate coating on HA surfaces provided a protective layer against the dissolution of the already nucleated crystals. The ability of the pyrophosphate to suppress crystal growth is put into constant use in our bodies where preventing aberrant calcification from the supercritical solutions found in the tissues is an enduring process. Indeed, pyrophosphate administration was found early on to be beneficial in an animal model of aberrant calcification, namely the rat aortic calcification model[12]. The ability of the pyrophosphate to suppress apatite dissolution, on the other hand, suggested a means to prevent the loss of tissues already mineralized (i.e. deposited bone). Unlike its beneficial effect in suppressing aortic calcification, pyrophosphate was not beneficial in suppressing bone loss, and this lack of activity in bone resulted in a search for pyrophosphate analogues that displayed superior stability in the bone milieu, the presumed shortcoming of the pyrophosphate in this environment. The search led to identification of phosphonate-based molecules, where the hydrolysis-resistant –C–P (O)–$(OH)_2$ moieties replaced the labile –O–P(O)–$(OH)_2$ moieties in the pyrophosphate[13-16]. Such diphosphonates were shown to be capable of controlling HA dissolution[13, 14], as well as preventing bone loss induced by immobilization[15] and parathyroid extract injection in animal models[14].

The diphosphonates were also active in preventing pathological aorta calcification[16], similar to the first beneficial use of the pyrophosphates. The diphosphonates used in these early studies were dichloromethylene diphosphonate[14, 15], methylene diphosphonate[14] and 1-hydroxyethylene-1, 1-diphosphonate[16]. The two phosphonate moieties in these compounds were located on the same carbon (α-carbon), in fact forming the basis of the bisphosphonate (BP) class of compounds. This promising work spurred intense research activity where the end-goal was to identify pharmacologically active analogues of BPs, i.e. potent compounds where a predictable inhibition of bone loss could be obtained when delivered in a convenient, clinically acceptable fashion without significant side-effects. Human use of the BPs immediately followed with almost no lag time for clinical entry[17]. As with the first generation of BPs, contemporary BPs display an exceptional affinity to HA; once localized to the bone tissue, however, they exert their respective pharmacological activities primarily by modulating local cellular activities, and rather than affecting the physicochemical properties of the apatite. It must be pointed out that Fleisch's early work also recognized the possibility of cellular effects by the early BPs, in addition to their effects on inhibition of HA dissolution[14]. It was within a few years of realization of the pharmacological activities of BPs that their utility as bone carriers was also demonstrated. The initial use of BPs for bone targeting was for delivering the radio nucleotide ^{99m}Tc to skeletal tissues for imaging purposes[18, 19]. Complexes formed between a BP and ^{99m}Tc did not compromise the bone-seeking capability of the compounds, providing a means to visualize skeletal tissues via the c-emitting isotopes. Several critical observations were immediately noted from this collective activity: heterogeneity in bone uptake of the labelled complexes, ability to detect osteolytic metastasis in bones, as well as locating neoplastic tissues extra-skeletally in soft tissues (presumably due

to local spots of calcification) spurred a diagnosis-centred BP research[20]. These studies initially established the existence of a structure–function relationship for BPs, and inspired subsequent studies to further elucidate this relationship. It was not until 1986, however, when the development of a bone targeted therapeutic (i.e. the synthesis of BP-incorporating molecules with pharmacological activities distinctly different from the BP action) was first reported. Two of the earliest examples of bone-seeking therapeutics, which relied on a BP moiety for bone targeting and a distinct moiety for pharmacological activity, were an ^{131}I-containing BP[21], and the anti-neoplastic drug 1,2,4-triglycidylurazol chemically linked to a BP[22]. A wide spectrum of bone-seeking therapeutic agents has subsequently been pursued.

Over the years there were many attempts made to explain how bisphosphonates work on cells, especially via inhibitory effects on enzymes. Various studies suggested possible effects on glycolysis, or direct or indirect inhibition of the osteoclast proton pumping H^+ATPase , phosphatases, or lysosomal enzymes, and even effects on osteoblasts to produce an osteoclast inhibitory factor [23]. The contemporary view is that there are two major but distinct molecular mechanisms by which bisphosphonates affect osteoclasts, and that bisphosphonates can be classified into at least two major groups based on these different modes of action which is shown in **Fig.2**.

The first group comprises the non-nitrogen bisphosphonates, such as clodronate and etidronate that seem able to most closely mimic pyrophosphate. They behave as PPi analogues by being metabolically incorporated into non-hydrolysable analogues of ATP though the reversal of the actions of amino acyl- tRNA synthetases. The resulting metabolites contained the P-C-P moiety in place of the β, γ-phosphate groups of ATP, thus resulting in non-hydrolysable (AppCp) nucleotides [24,25,26,27.] It is likely

that intracellular accumulation of these metabolites within osteoclasts [28, 29] inhibits their function and may cause osteoclast cell death, probably by interference with mitochondrial ATP translocases [30]. This group of non-nitrogen-containing bisphosphonates therefore appears to act essentially as prodrugs, being converted to active drug metabolites following intracellular uptake by osteoclasts in vivo.

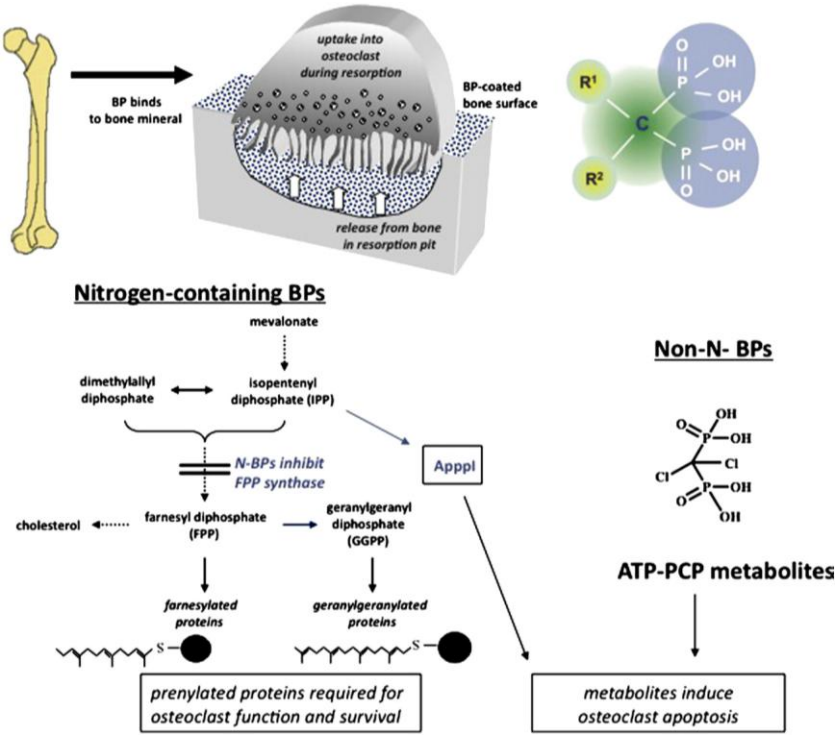

Fig. 2 The *cellular and biochemical mechanisms of action of bisphosphonates*.

In contrast, the second group of bisphosphonates contains all of the more potent, nitrogen-containing compounds (N-BPs), which are not metabolised to AppCp-type metabolites as described above. In contrast, members of this group of N-BPs interfere with specific metabolic reactions, notably in the mevalonate biosynthetic pathway that leads to the synthesis of cholesterol and other sterols. The enzymes in this pathway metabolise pyrophosphate-containing isoprenoid lipids, which are progressively condensed into longer chains. Bisphosphonates are able to inhibit several enzymes in this pathway to varying extents[31, 32], but the major target for the anti-resorptive N-BPs is farnesyl pyrophosphate syntheses (FPPS).

1.2 History

Bisphosphonates have been known to chemists since the middle of the 19 th century, the first synthesis dating back to 1865 in Germany[33]. Due to its ability to chelate metals, the early uses of bisphosphonates were not only for soften water in irrigation systems used in orange groves but also for industrial, mainly for corrosion prevention, and largely used in the textile, fertilizer and oil industries as well as in washing powders. And, because of their property of inhibiting calcium carbonate precipitation, as preventers of scaling.

The study and development of bisphosphonates as a major class of drugs for the treatment of bone diseases began only three decades ago. The first report of the biological characteristics of bisphosphonates was published in 1968[34] **(Fig.3)**. At that time, scientists discovered that bisphosphonates have a marked ability to inhibit bone resorption. The initial rationale for their use in humans was their potential in preventing the dissolution of hydroxyl apatite, the principal bone mineral, thus arresting bone loss. The concept was derived from earlier studies in Prof. H. Fleisch laboratory on inorganic pyrophosphate[35], in which it was found that plasma and urine contained compounds inhibiting calcium phosphate precipitation *in vitro* and it was found that part of this activity was due to inorganic pyrophosphate, a Substance that had not been described previously in these fluids.

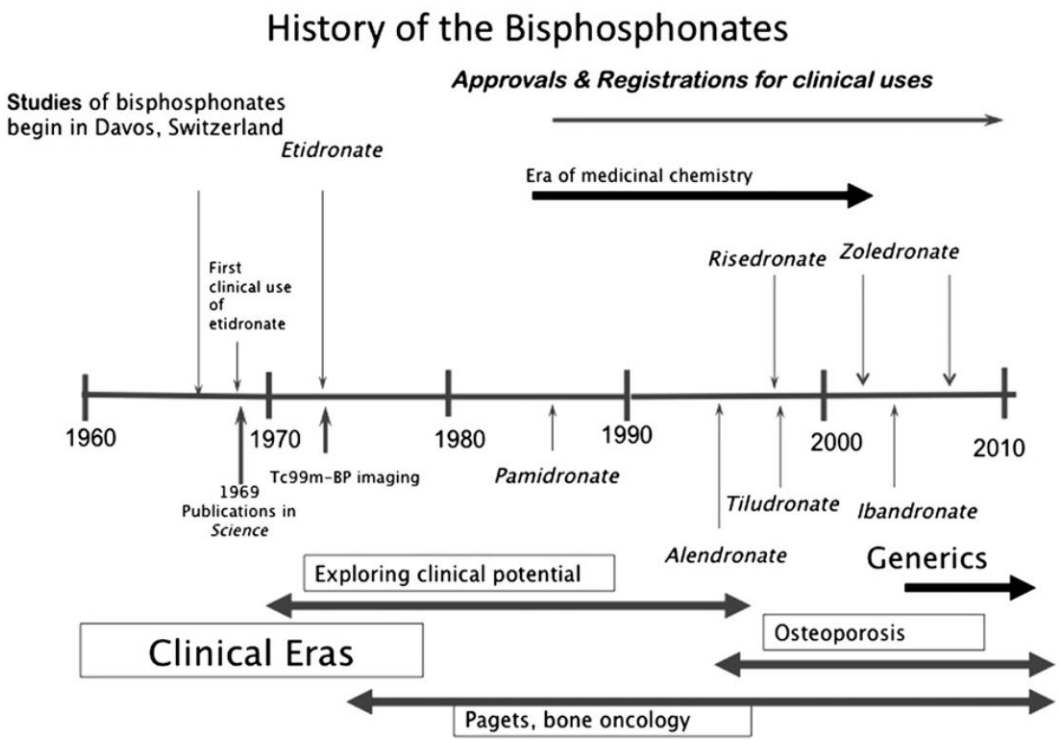

Fig. 3 *The history of bisphosphonates*

The research group of Prof. H. Fleisch then found that pyrophosphate also inhibited calcium phosphate dissolution *in vitro*. *In vivo*, this compound prevented ectopic calcification but had no effect on normal mineralization and on bone resorption, possibly because it was destroyed locally by phosphatises. This prompted scientists to look for analogs of pyrophosphate that were not destroyed enzymatically. The bisphosphonates fulfilled these conditions [36,37]. Only in the 1990s was their actual mechanism of action demonstrated with the initial launch of Fosamax (alendronate) by Merck.

A recent search in PubMed under the term 'bisphosphonates' revealed over 19,000 publications, and even this large list this does not cite abstracts, nor all publications and the many books and review articles available that describe the chemistry, pharmacology, and Clinical applications of bisphosphonates.

1.3 Bisphosphonates

Bisphosphonates (*BPs)* are class of drug that used for over 30 years in treatment of various disorders of mineral metabolism apart from this it serves to regulate calcium, and prevent bone breakdown[38]. The bisphosphonates are powerful, they cause dramatic changes in the bone physiology, and they deserve respect. In women or men whose bone density T-score is lower than -2.5, or who already have a vertebral fracture, these medicines reduce the incidence of fractures and improve the quality of life[39]. In addition to this, a significant number of these compounds are currently being used for the treatment of several bone disorders such as Paget's disease, myeloma, bone metastases and osteoporosis[40], as well as in some childhood diseases[41]. Recently, bisphosphonate drugs have also been found to have activity against the in vitro proliferation of several protozoan parasites, including *Trypanosoma brucei* which causes African trypanosomiasis or sleeping sickness in human and animals[42]. Consequently, the well-proven clinical utility of bisphosphonates has fostered the development of several methodologies for the preparation of novel derivatives, structure -activity studies have actually indicated that bioactivity is highly dependent on the nature of substituents linked to the bisphosphonic skeleton[43]. Some cancers can cause bone pain and weakness. These are most often cancers that have started in another part of the body and have spread to the bone such as myeloma, breast cancer, protest cancer and lung cancer therefore BPs are also of interest in the context of cancer and immunotherapy, as they possess potent Effects against the parasites responsible for sleeping sickness, chagas' disease, malaria and leishmaniasis[44]. Etidronate (Didronel), pamidronate (Aredia, Novartis Pharmaceuticals Corp.; East Hanover, NJ), alendronate (FosamaxR; Merck and Company, Inc.; West Point, PA), risedronate

(Actonel; Proctor and Gamble Pharmaceuticals,Inc.;Cincinnati,OH) zoledronic acid (Zometa or Reclast Novartis Pharmaceuticals Corp.), ibandronate (Bondronat or Boniva; Hoffmann-La Roche Inc.; Nutley, NJ), these are the names of biphosphonate which are available in market. These medicines reduce the incidence of fractures and improve the quality of life. Their common name and uses are mentioned in **Table 1.** A report by Schousboe et.al found that alendronate is not cost-effective in treating women with "osteopenia" who do not already have an osteoporotic fracture. We still don't know the effects of long-term suppression of bone formation[45].

Table 1. List of bisphosphonates used in clinical studies and under clinical development

Bisphosphonates	**R_1**	**R_2**	**Main current uses**
Etidronate	OH	CH_3	Osteoporosis, Paget's disease
Clodronate	Cl	Cl	Metastases, myeloma
Pamidronate	OH	CH_2CH_2 NH_2	Hypercalcaemia, myeloma, Paget's disease
Alendronate	OH	$(CH_2)_3$ NH_2	Osteoporosis and other indications
Residronate	OH	CH_2-3-pyridine	Registration pending for osteoporosis
Tiludronate	H	CH_2-S-phenyl-CI	Paget's disease
Ibandronate	OH	CH_2CH_2 $N(CH_3)$ Pentyl	In development, osteoporosis and several diseases
YH529	OH	CH_2CH_2 $N(CH_3)_2$	
Icadronate	H	N-(cyclo-heptyl)	
Olpadronate	OH	$CH_2CH_2N(CH_3)_2$	
Neridronate	OH	$(CH_2)_5$ NH_2	
EB1053	OH	CH_2-1-pyrrolidinyl	

INDICATIONS (Who can take)

Postmenopausal women with vertebral compression fractures

Postmenopausal women with total hip bone density T-score below -2.5

Elderly men with non-traumatic fractures

Some patients with secondary osteoporosis due to corticosteroids

Paget's disease

Cancer metastatic to bone

Other bone diseases with high bone resorption

CONTRA INDICATIONS (Who can't)

Women who are pregnant or planning pregnancy

Chronic kidney disease stages 4 or 5

Low serum calcium

Osteomalacia

Vitamin D deficiency (until it is corrected)

Oral bisphosphonates should not be used in:

Patients with serious esophageal disease

Patients at bed rest who can't stay upright for an hour

Use with Caution

Patients with abnormal white blood cells
Patients with high PTH
Patients with gastric or intestinal bypass surgery
Children (no long-term safety data) SIDE EFFECTS

Oral or IV forms	Hypocalcaemia	Mild decrease common; severe decease unusual
	Increased PTH	Usually modest
	Skin rash	Rare
	atrial fibrillation	FDA found no association
	Bone pain	Unusual
	Subtrochanteric fractures	Unusual, after long-term use
Oral forms	Upper GI irritation	Common
	Esophageal ulceration	Unusual
	Esophageal cancer	Unusual
Intravenous forms	Fever	Common
	Transient leukopenia	Mild, no symptoms
	Acute-phase reaction	Common, lasts 1-3 days
	Eye inflammation	Rare
	Nephrotic syndrome	Rare
	Jaw osteonecrosis	With very high doses in cancer patients.
Etidronate (Didronel)	Osteomalacia	Common with high doses
	Hyperphosphatemia	Usually mild effect

Dose for osteoporosis

In the large trials the fracture rates with the lower doses were not significantly different from the rates in higher doses, despite greater increases in the DEXA measurements with the higher doses (**Fig 4**). In the risedronate study, with 5445 women between 70 and 79 yrs, the relative risk reduction for a hip fracture with the 2.5mg/d dose was statistically significant (CI 0.3 to 0.9) but for the 5mg/d dose it was not statistically significant (CI 0.4 to 1.1). Nevertheless, the company decided to market the higher dose.The FIT trial of alendronate documented significant fracture reduction at 2 years with the 5mg/day dose. After 2 years the dose was increased to 10mg/day, but the study design precluded actual dose comparisons.Subsequent studies have shown that doses given once a week are as effective as those given daily. Almost all patients prefer this approach. Zoledronic acid continues to suppress bone formation and resorption for at least 2 years. A single dose increased the bone density at two years as well As two annual doses[46].

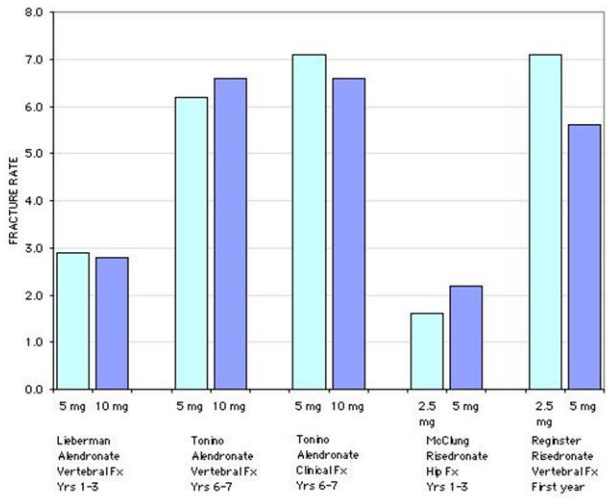

Fig. 4 *Dose for osteoporosis patients*

DOSE FOR OSTEOPOROSIS PATIENT

Everybody needs adequate CALCIUM and VITAMIN D

Alendronate 35 mg once a week (this is the dose approved for prevention, and the official dose for treatment is 70mg a week)

Risedronate 15 mg a week more logical but approved dose is 35mg/week

Ibandronate 150mg/month oral OR 3mg/3 months I.V. push

Zoledronic acid: 5mg I.V. in 100ml NS over 15 minutes.
A single dose lasts at least 2 years. Adjust for kidney function.

Pamidronate 30 to 60mg I.V. in 200-300ml D5W over 2 hrs every 6 months in special cases, with calcium>9, normal creatinine, WBC and vitamin D. This is NOT APPROVED for osteoporosis.

How to take oral doses

This is an important!! These medications are poorly absorbed and any food in the stomach will reduce the amount aborted. They also can cause esophageal erosions so must be "flushed down" with about 4 oz of water. In the FIT trial, a full glass of water caused more reflux and esophageal problems than 1/2 glass of water according to a recent lecture by Dr. David Karpf. Although it has not been carefully studied with newer medicines, calcium supplementation following the doses of etidronate reduced effectiveness,

NOT WITH FOOD IN YOUR STOMACH

NOT WITH TEA, COFFEE or CHOCOLATE MILK

NOT WITH A LITTLE SIP OF WATER (DRINK ABOUT 4 oz)

NOT BEFORE YOU GO TO BED

NOT BEFORE YOUR TOE-TOUCHES

Alendronate

The Fracture Intervention Trial enrolled 6,000 postmenopausal women aged 55-79, with a bone density at the hip lower than 0.68g/cm2 (T-score about -2). There were two arms of the study: 2000 women who already had vertebral compression fractures and 4000 women who did not. The women who had a fracture took alendronate for three years, the others 4.2 years. Dose was 5mg/day for the first 2 years and then 10mg/day. The alendronate group showed increases in bone density of about 8% at the spine and 5% at the hip[47]. Summary of results of the Fracture Intervention Trial: Bone density increased in over 90% of women taking the medication, whether or not they already had a fracture. Notice how the incidence of new fractures depends on presence of baseline fracture shown in **fig 5**.

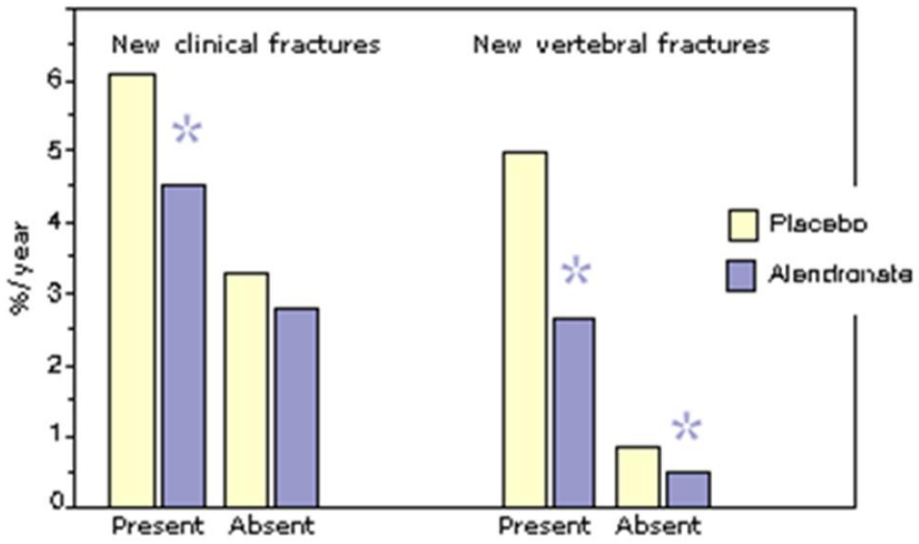

Fig. 5 *Vertebral fractures at baseline*

In women who did not have a vertebral fracture at baseline, the decrease in vertebral fractures was significant but of low magnitude and the decrease in clinical fractures was not significant. In women who already had fractures (established osteoporosis) there was a decrease in fractures that was both statistically significant and clinically important.

Risedronate

The largest risedronate study included 9331 women: 5445 were 70-79 years old with T-score lower than -3 and 3886 were older than 80 with either low T-score or a clinical risk factor. BMD was done on 31% of the women older than 80. **Fig.6** shows the rates of hip fractures.

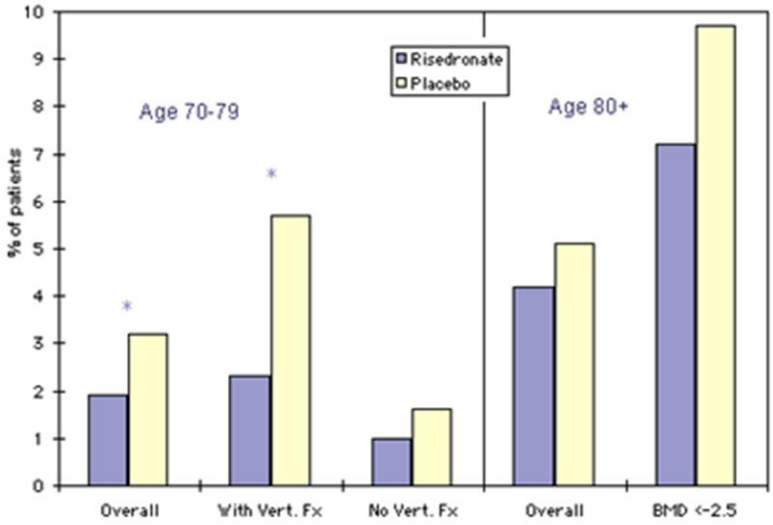

Fig. 6 *Hip fractures in 3 years*

The incidence of hip fractures over time is shown in **Fig.7.** The older women had more fractures but no statistically significant benefit from the risedronate [48].

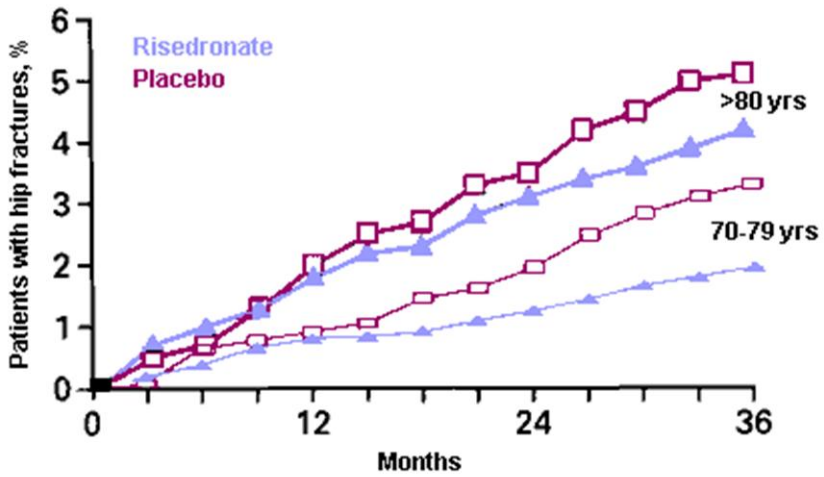

Fig. 7 *Effect of risedronate on the risk of hip fracture in elderly women.*

Long term effects

The **Fig.8** shows change in bone density in 1099 women from the Fracture Intervention Trial who had taken alendronate for 5 years, and then were randomly given either alendronate or placebo for 5 years. The biochemical markers of bone resorption remained suppressed in both groups for 3 years after discontinuation. The serum formation markers were somewhat different; P1NP increased moderately but was still 24% below baseline 5 years after stopping, whereas the BAP gradually increased towards baseline [49].

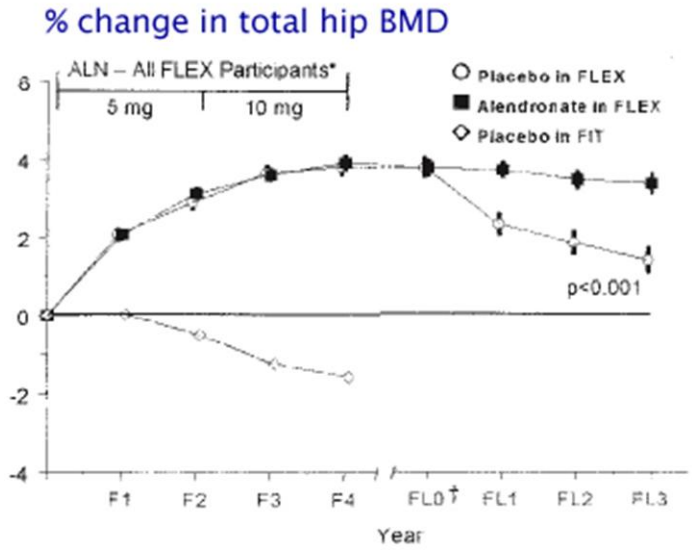

Fig. 8 *change in bone density*

Despite the difference in bone density during the last 5 years, the women who discontinued alendronate had essentially the same number of fractures as the women who kept taking the drug[50]. The alendronate group had fewer "clinical" vertebral fractures (those diagnosed by their physician) but when the xrays were measured the total number of "morphometric" fractures was not different. Also, there was no difference in moderate to severe vertebral fractures. Among the women with fractures there was more height loss in the alendronate group (3.5cm) vs the placebo group (2.1cm, p=.02)[51].

Because there is no benefit to reduce fractures beyond 5 years, it is logical to stop alendronate after 5 years in most patients. Currently there are no practice guidelines about this issue and experts disagree about when to stop biphosphonates[52].

Fracture healing

The clinical trials of bisphosphonates have not reported any increased incidence of fracture non-union in patients treated with active drug. However, Solomon et.al recently found an association between non-union of humerus fractures and bisphoshonates. When bisphosphonates are given to patients after joint replacement surgery, there is less loosening of the prosthesis [53, 54, 55] although after 5 years there was no residual positive effect of a dose of pamidronate given at the time of surgery [56]. When given to patients 2 weeks after a fracture of the lower leg, bisphosphonates prevented the bone loss that was seen in the proximal femur of placebo control patients [57].Animal studies show that callus forms vigourously in animals who had been given bisphosphonates prior to the fracture [58]. However, the callus does not normally remodel. The bisphosphonate-treated fracture callus persists, and woven bone does not get replaced by lamellar bone.

When alendronate was given to pigs after spine fusion with a bone graft, the fusion area had more woven bone and greater amount of fibrous tissue, with no difference in the rate of fusion[59] another study in rats, however, found that despite the higher fusion area, the fusion rate was lower in alendronate-treated groups [60]. After a fragility fracture (for example, a hip fracture) in an untreated patient with osteoporosis, it makes sense to begin a bisphosphonate. The demonstrated risk of a future fracture is greater than the potential risk of non-union or poor callus remodelling. Of course, these patients need an evaluation for other causes, and concomitant treatment

with calcium and vitamin D and physical therapy. It is possible that treatment with anabolic agents will provide even better benefit for the skeleton, but currently bisphosphonates remain the first choice due to their lower costand greater familiarity.

In the recent study of zoledronic acid following a hip fracture, Eriksen EF et.al found that patients dosed later than 6 wk after hip fracture exhibited a greater increase in total hip and femoral neck BMD at month 12 compared with patients dosed earlier than 6 week.The subjects who were treated immediately after the surgery did not respond as well, although it is possible that they were frailer. An animal study by Amanat N. et.al found better fracture healing when zoledronic acid treatment was delayed.

Use in younger patients

Children

Children with severe osteogenesis imperfecta, who have multiple fractures, show reduction in pain and fracture rates with bisphosphonates. The radiographs of the long bones show a unique striped pattern when pamidronate is given intermittently, and this is caused by layers of thick bone alternating with osteopenic bone. There may be some weakness in these areas. Currently it is unclear when to stop giving these medications. The drugs are still excreted in the urine 8 years after stopping. Because of uncertainties about long-term effects, these drugs should be used only in serious cases [61]. Cyclical intravenous pamidronate treatment affects metaphyseal modeling in growing patients with osteogenesis imperfecta[62]. Used with permission from American Society for Bone and Mineral Research.Children with polyostotic fibrous dysplasia or juvinile Paget's disease may also benefit from bisphosphonates. Again, there are uncertainties about howlongtousethemedications[63]. Children with polyostotic fibrous dysplasia or juvinile Paget's disease may also benefit

from bisphosphonates. Again, there are uncertainties about how long to use the medications[63].

Premenopausal women

Bisphosphonates are not aproved for prevention of osteoporosis in premenopausal women. They should not be used in women who got a DEXA out of curiosity and discovered osteopenia. They are beneficial in other situations, such as prolonged high dose steroid use, organ transplantation, fibrous dysplasia, and metastatic carcinoma. Studies in animals show fetal and maternal abnormalities in bones and calcium metabolism, so it is unethical to study this medication in pregnant women or women who might become pregnant while the bisphosphonate is still in the bones.

Recently postmenopausal women

The following graph shows the effect on bone density in women who are recently postmenopausal. Alendronate 5mg/day increases bone density compared to placebo, but not as well as estrogen with norethindrone or estrogen with medroxyprogesterone. These results are from data in the EPIC study[64] (**Fig 9**). There were very few fractures in this study, and no significant difference in fracture rates could be detected.

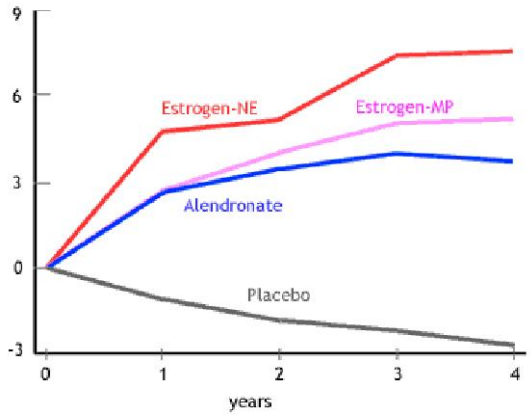

Fig. 9 % *Change in spine bone density*

Many experts say that bisphosphonates could be used instead of estrogen in women with osteopenia, to prevent osteoporotic fractures. This is based on wishful thinking instead of evidence. It takes decades to reach "the age of fracture" and we don't know if any drugs except estrogen will work that long. In my opinion, bisphosphonates should be used only if the risk of fracture within the next ten years is high enough to justify the potential risks. As more evidence accumulates about long-term benefits, my recommendations may change. I also wonder if very small doses might be better for long-term prevention, but I'm not aware of any ongoing studies.

Use in elderly patients

In the Fracture Intervention Trial the women between 75 and 80 who had established osteoporosis showed improvement in bone density and fracture reduction that was similar to the women aged 55-75. One study of alendronate included women until age 85, but mean age was 71 so most women were still younger than 80. In that study there were not enough women to determine fracture rates, but the bone density increased 6% at the spine and 4% at the femoral trochanter.Risedronate studies shown above did not show fracture benefit to women older than 80, even among the 941 women who had osteoporosis by bone densitometry. Maybe this was just bad luck, with a negative finding due to inadequate power. Perhaps in the older women, other factors (such as quality of bones or falls) become relatively more important so it is more difficult to determine a beneficial drug effect. Some women may have lost so much bone mass by age 80 that they will have fractures despite improvement in the bone strength. There is also a possibility that the bisphosphonate does not work as well in women older than 80, because these women may already have low bone formation rate due to inadequate osteoblasts.In the recent report of zoledronate the average age was 73 and women up to age 89 were enrolled. These women

with established osteoporosis had a significant reduction in hip fractures (**Fig 10**).Bisphosphonates are excreted by the kidneys and are not recommended for persons with stage 4 or 5 chronic kidney disease. Often elderly, thin women with low muscle mass have a deceptively normal creatinine. This should be taken into account when prescribing bisphosphonates. Also, it is especially important to insure adequate vitamin D levels in elderly women before starting bisphosphonates, because the skin is less effective at converting vitamin D after sunlight exposure.

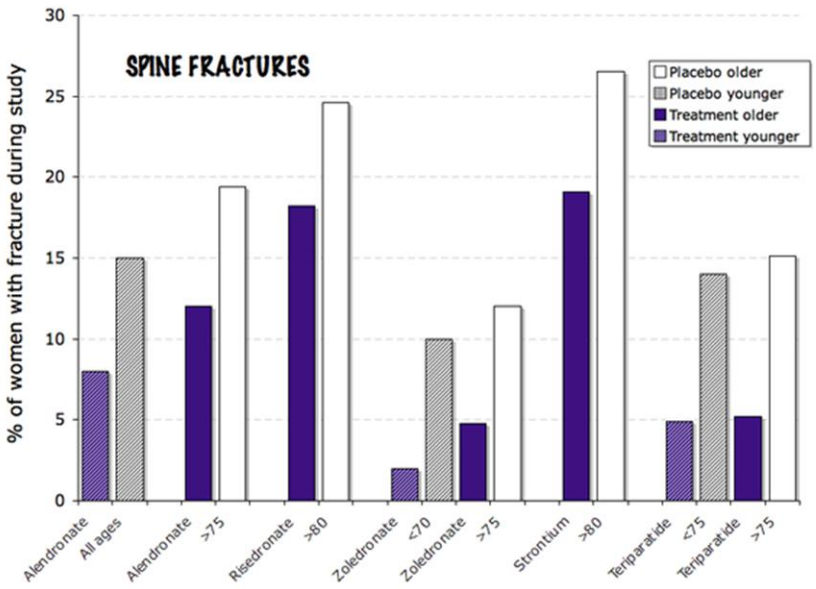

Fig. 10 *This image shows the improvement in rate of spine fractures with various medicines in elderly subjects. Some of the trials compared young to older women, and with alendronate and zoledronate the youn er women had a better respons*

1.3.1 Structure

Biphosphonate (BPs) are hydrolytically stable analogs of a naturally occurring pyrophosphate and constitute an important class of pharmacologically active molecules. In which the labile phosphor anhydride bond (H_20_3P-O-$P0_3H_2$) of pyrophosphate is replaced by a stable methylene group ($H_20_3PCR_1R_2$-$P0_3H_2$) to which two groups (R_1 and R_2) are attached. The long *side-chain* (**R_2** in the diagram) determines the chemical properties, the mode of action and the strength of bisphosphonate drugs.

Biphosphonate **Pyrophosphate**

Fig. 11 *Structure of Biphosphonate and Pyrophosphate*

The short side-chain (**R_1**), often called the 'hook', mainly influences chemical properties and pharmacokinetics. BPs (also called as Diphosphonate) is synthetic organic compounds characterized by a P-C-P backbone structure. They are called bisphosphonates because they have two phosphonate (PO_3) groups and are similar in structure to pyrophosphate. Which is shown in **Fig 11.**

1.3.2 Chemistry

Chemically the bisphosphonates were first synthesized in the 1800s [65], but it is only in the past 40 years that they have been used to treat disorders of calcium metabolism. Diphosphonates and *gem*-diphosphonates are both correct names for bisphosphonates. However, recently and after the wide application of this class of compounds in therapeutic uses, the single term "bis-" is generally used for compounds characterized by the [P-(R_1) C(R_2)-P] structure. This feature allows a great number of possible variations, mostly by changing the two lateral chains (R_1 and R_2) on the carbon. Small changes in the structure in the R_1 or R_2 moiety can lead to extensive alterations in their physicochemical, biological, therapeutic, and toxicological characteristics. For these reasons, it appears there is a need for more BP-compounds with a greater margin between the inhibitions of mineralization with accompanying increase in toxicity, and improved oral bioavailability with side effects.

1.3.3 Biological activity included structure-activity relationship

The evolution of concepts about the structure activity relationships among BPs has been reviewed and some of the key historical aspects summarized in detail by Ebetino et.al [66].

All bisphosphonates have the same generic structure. Even if their chemical structure closely resembles the chemical structure of pyrophosphate (PPi), there are many features, which differs them from each other. First of all, in PPi two phosphate groups are linked by oxygen being hydrolytically unstable, whereas in BPs they are linked to carbon, which render them hydrolytically stable and also to withstand incubation in acids or with

hydrolytic enzymes[67].The P-C-P moiety has been called the "bone hook", namely it is the primary structural feature that endows the molecule its affinity and targets it to the bone [68].The "bone hook" is responsible for giving bisphosphonates high affinity to the bone, which can further be enhanced by the substitution of a hydroxyl group in the side chain R_1. Interestingly, the side chain R_2 plays an important role in determines the potency of bisphosphonates [**Fig.12**] [68, 69.]

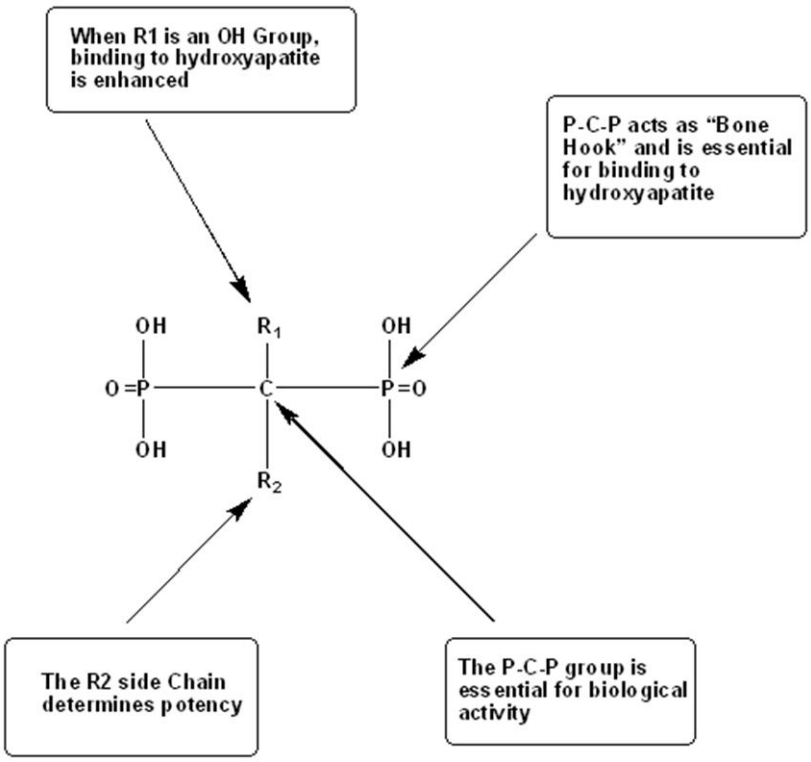

Fig. 12 *Structure of a bone-active bisphosphonate to show functional domains*

It is noteworthy to add, that the presence of a hydroxyl group in the side chain R_1 can increase the ability of BPs to bind to bone mineral by preventing both crystal growth and dissolution[70]. Moreover, its presence enhances the affinity for calcium and thus bone mineral even further, owing to the ability of bisphosphonates to chelate calcium ions.Like PPi, bisphosphonates form a three dimensional structure capable of binding

divalent metal ions such as Ca^{2+}, Mg^{2+}, Fe^{2+} in the bidentate manner [71,72]. Below appears figure **Fig.13** presenting this mechanism.

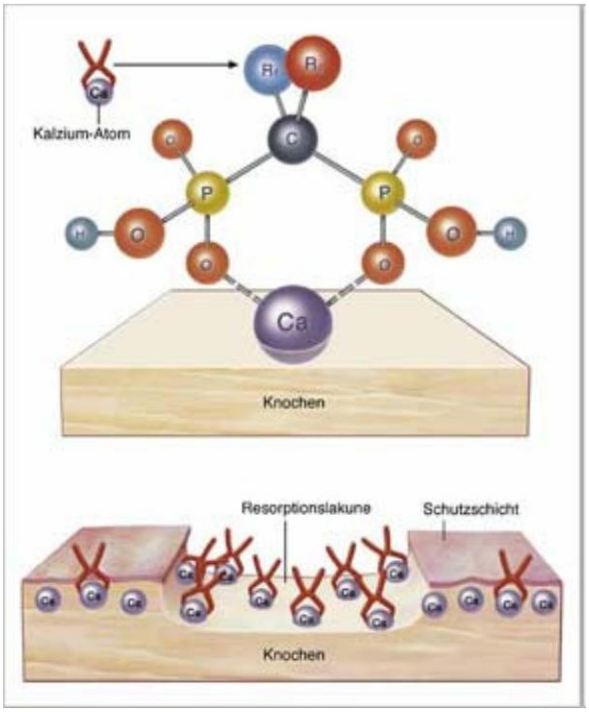

Fig. 13 *Mechanism of bisphosphonates to bind to divalent metal ions such as Ca2+*

More recent studies have explored, that the structure present in R_2 side chains of bisphosphonates is the major determinant of anti resorptive potency. A turning point concerning clinical investigation of bisphosphonates was found in BPs containing an additional moiety a nitrogen atom in the side chain R_2 (N-BPs). In particular, bisphosphonates such as *Pamidronate* or *Alendronate* with a basic primary nitrogen atom were found to be up to 1000-fold (**Table 2**) more potent than non-amino bisphosphonates (*Clodronate* or *Etidronate*). **Fig.14** Moreover, it was determined that bisphosphonates containing a secondary amine group in the side chain R_2 are more potent up to 300-fold than those containing a primary amine (*Incadronate*) **Fig15**. Most importantly, the highest antiresorption potency has been shown by bisphosphonates containing a tertiary nitrogen atom within ring structures [72] in the side chain R_2. An example of this kind of N-BPs is Risendronate and Zolendronate **Fig.15**.

These cyclic nitrogen bisphosphonates has proved to be up to 10.000-fold more active than for example *Editronate* (in experimental systems used for human-beings) [73]. The difference in potencies might arise from the different mechanisms of action exhibited by nitrogen containing bisphosphonates and non-nitrogen containing bisphosphonates (**Table 2**). Non-nitrogen containing bisphosphonates are metabolically incorporated into adenosine triphosphate (ATP) producing non hydrolyzable ATP analogues. Nitrogen-containing bisphosphonates inhibit the enzyme farnesyl diphosphate (FPP) synthase in the melavonate pathway[74].

Table 2: Relative potency of Biphosphonate drugs

Biphosphonate	Common Name	Potency
Etidronate	Didronel	1
Tiludronate	Skelide	10
Pamidronate	Aredia	100
Alendronate	Fosamax	1,000
Risedronate	Actonel	10,000
Ibandronate	Boniva	10,000
Zolendronate	Zometa	>100,000

The results obtained to date indicate that the key features required for high inhibitory potency of N-BPs include:

- The presence of two geminal phosphonate groups responsible for interaction With the molecular target
- The presence of a basic nitrogen in heterocyclic side chain affects potency
- The three-dimensional orientation of those basic nitrogen atom is critical for an effective inhibition
- The geminal hydroxyl group does not influence the ability of the N-BPs to act at the cellular level. The introduction of lipophilic groups into N-BPs backbone can significantly improve their pharmacokinetics increasing the availability for soft tissues.

Noteworthy, even if N-bisphosphonates differ from non-N-bisphosphonates

with the respect to antiresorptive potency, their pharmacokinetics are imilar and characterized by highly elective localization and retention in bone [75].

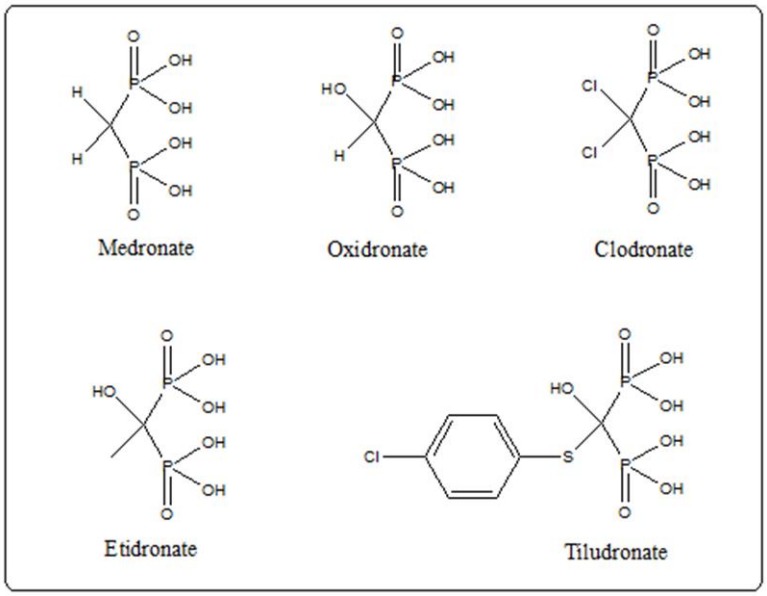

Fig. 14 *Non-nitrogen-BPs currently used in clinical setting*

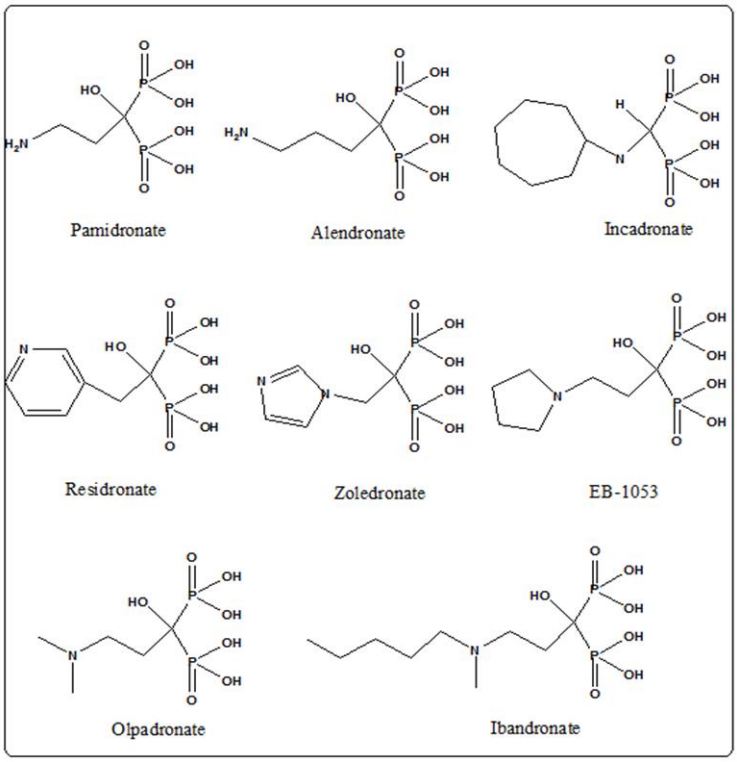

Fig. 15 *Structures of the Nitrogen-BPs currently used in clinical studies classified according to their biochemical mode of action.*

The analysis of structure-activity relationships allowed the spatial features of the active pharmacophore to be defined in considerable detail even

before the molecular mechanism of action was fully elucidated. For maximal potency, the nitrogen atom in the R_2 side chain must be a critical distance away from the P-C-P group and in a specific spatial configuration[76]. This principle was used successfully for predicting the features required in the chemical design of new and more active compounds.

In summary, studies of the relationships between bisphosphonate structure and antiresorptive potency suggested that the ability of bisphosphonates to inhibit bone resorption depend on two separate properties of the bisphosphonate molecule.

1.4 Pharmacological relevant bisphosphonates

As drugs bisphosphonates display a few unusual features. Their remarkable selectivity for their target organ of bone is paramount among these and accounts for much of the efficacy and safety of the drug class, as reviewed by Cremers and Papapoulos[77]. Secondly unlike many drugs, BPs are not metabolized to inactive products, and drug derivatives do not appear in urine. Intracellular conversion of some non-N-BPs to ATP derivatives does occur however, as discussed elsewhere. Thirdly their oral bioavailability is extremely low, characteristically below 1% for many BPs, and rarely above 5% for others. Nonetheless the property of being active by mouth in early animal studies was key to their future use in man. The mechanism of intestinal absorption of BPs has been ascribed to paracellular transport. BPs are highly charged molecules, and no transporters have been identified. Absorption appears to be enhanced by EDTA, an effect attributed to calcium chelation that opens up gap junctions between intestinal mucosal cells[78]. Finally, the overall safety profile of BPs is good, but the issues of safety are much discussed and debated[79-81] as described by Pazianas and Abrahamsen et.al [82].

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Chapter 2
1, 3-dipolar cycloaddition reaction

2.1 Introduction

Cycloaddition reactions have long been considered as the most powerful and promising synthetic tools to construct complex natural products and biologically active materials. Furthermore, these reactions occupy a special place in organic synthesis because of the simultaneous creation of several bonds and generation of new stereogenic centres in often highly stereocontrolled manners. Interest for 1, 3-DC has grown over the past twenty years and in particular 1,3-dipolar cycloaddition reactions of nitrones with alkenes and alkynes have found general applications in organic synthesis.[1,2]

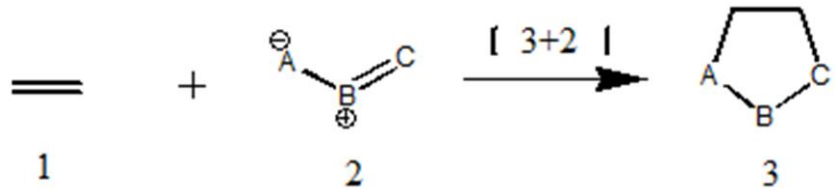
Isoxazolidines are very useful organic compounds because of their application as medicines or insecticides. Besides, it provides a convenient path for the synthesis of natural base and natural compounds containing N and O atoms in the molecules.[3, 4] Thus, they can be used as intermediates to synthesize natural base and multitude of natural products. In particular, they have been used to elaborate various aminoalcohols and β-aminoacid derivatives by reductive cleavage ofthe *N* - *O* bond.[5] Therefore, 1, 3 DC are now utilized in almost every area of chemistry including material chemistry[6], drug discovery[7], and chemical biology[8].

One of the main features of 1, 3-DC reactions of nitrones is their ability to create up to three contiguous stereogenic centres with a stereochemical control. Providing that the regioselectivity of the reaction is totally controlled, the most recent asymmetric developments using chiral Lewis

acids have proved that almost exclusively one stereoisomer can be obtained, from the four possible formed. It is therefore unsurprising that most of the reviews on [3+2] cycloadditions published over the past fifteen years were devoted to its asymmetric version.[2, 9-10] The development of 1,3 - DC reactions has in recent years entered a new stage as control of the stereochemistry in the addition step is now the major challenge. The selectivity challenge is to control the regio-, diastereo-, and enantioselectivity of the 1, 3-DC reaction. The stereochemistry of the 1,3-DC reaction can be controlled by either choosing the appropriate substrates or controlling the reaction by a metal complex acting as a catalyst. Among the large amount of studies published on 1, 3-DC of nitrones, several reviews were dedicated to intramolecular versions[11] or to the cycloaddition of carbohydrate derived nitrones[12]. This thesis will concern the 1,3-DC reactions under microwave conditions, without solvent involving nitrones as dipoles and phosphonate substituted alkenes as dipolarophiles along with reductive cleavage of N-O bond to form 1,3 amino alcohol.

2.1.1 Definition and history of 1, 3-dipolar cycloaddition

1, 3-dipolar cycloaddition is a reaction where two organic compounds, a dipolarophile, **1**, and a 1, 3-dipole (or ylide), **2**, combine to form a five membered heterocycle **3** (**Scheme2.1.1**). The reaction is related to the Diels-Alder reaction where a diene and a dienophile form a six membered ring. From simple starting materials, the 1, 3-dipolar cycloaddition reactions can furnish very complex heterocycles, containing multiple stereogenic centres. Therefore this reaction is often used as a key step in the syntheses of many natural products and pharmaceuticals.

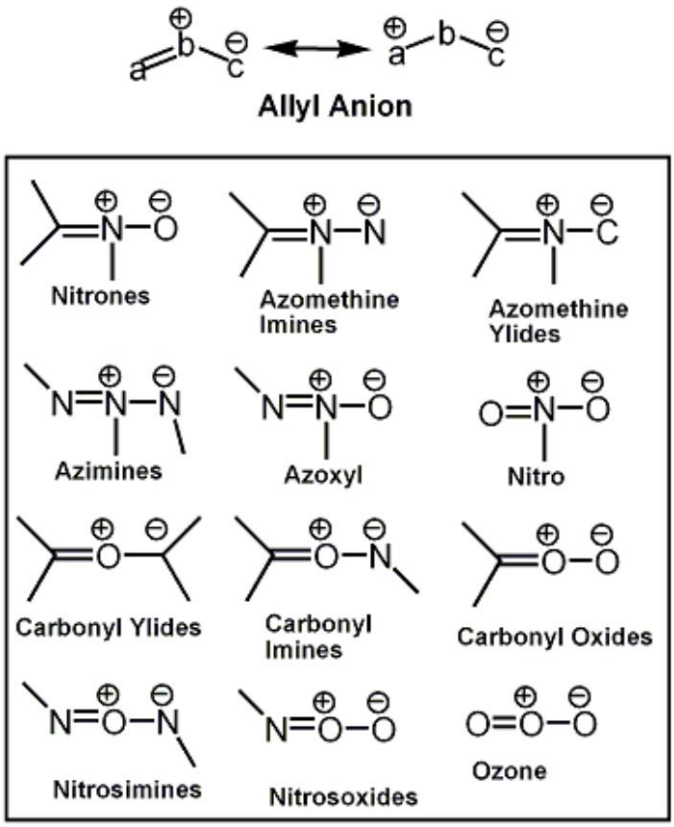


Scheme 2.1.1. *General scheme of 1, 3-DC*

The first dipole and 1, 3-DC were discovered by Curtius[13] who discovered diazoacetic ester in 1883. After five years later Buchner[14] who studied the reaction of diazoacetic ester with α, *β*-unsaturated esters and described the first 1,3-DC reaction. In 1893 he suggested that the product of the reaction of methyl diazoacetate and methyl acrylate was 1-pyrazoline and that the isolated 2-pyrazole was formed after rearrangement of the 1-pyrazole[15]. Five years later nitrones and nitrile oxides were discovered by Beckmann, and Werner and Buss, respectively[16, 17]. The Diels-Alder reaction was found in 1928, [18] and the synthetic value of this reaction soon became obvious. These reactions have been developed into very general and useful methods for the synthesis of five-membered hetereocycles or carbocycles.

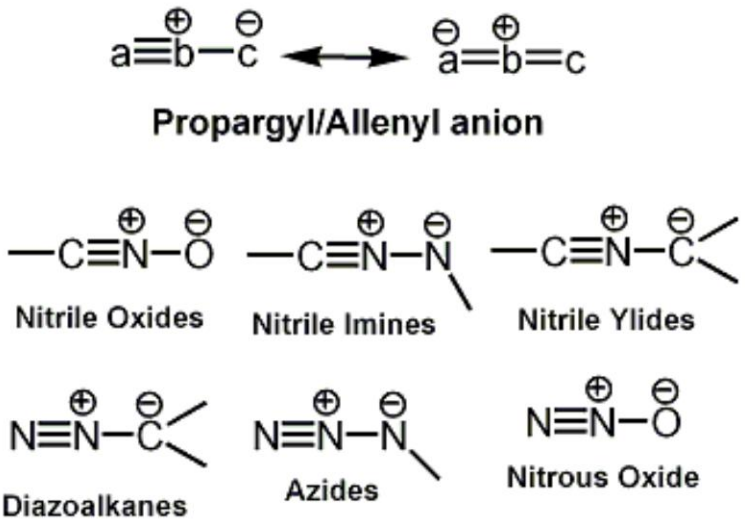
2.1.2 Dipoles or ylides

The chemistry of the 1, 3-DC reaction has thus evolved for more than 100 years and a variety of different 1, 3 dipoles discovered[19]. However, only a few dipoles have found general application in synthesis during the first 70 years after the discovery of the diazoacetic ester. The general application of 1, 3-dipoles in organic chemistry was first established by the systematic studies by Rolf Huisgen and co-workers in the early 1960's[20]. Therefore it is also known as the **Huisgen cycloaddition** or **Huisgen reaction.** Basically, 1, 3-dipoles can be divided into two different types[21]: the allyl anion type and the propargyl (allenyl) anion type. The allyl anion type is bent and has four electrons in three parallel *p* orbitals perpendicular to the plane of the dipole (**Scheme 2.1.2**).



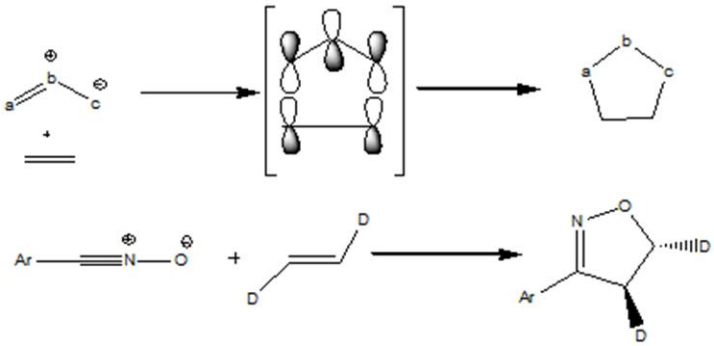
Scheme2.1.2. *Allyl anion 1, 3-dipoles*

The propargyl (allenyl) anion type dipole is normally linear. The central atom is occasionally presented as hypervalent and is limited to nitrogen (**Scheme 2.1.3**). The other dipole atoms can be carbon, oxygen and nitrogen. Other main group IV, V, VI elements like phosphorus and sulfur can also be involved in dipoles.



Scheme 2.1.3 *Propargyl/allenyl anion dipole*

Dipolarophiles display less diversity than dipoles. The most commonly used dipolarophiles are substituted alkenes and alkynes. Double or triple bonds with heteroatoms like carbonyl, iminium and cyano groups can also be dipolarophiles. Two types of mechanisms for 1, 3-DC reactions have been recognized: step-wise and concerted. In the concerted pathway, the 1, 3-DC reaction involves 4 π electrons from the dipole and 2 π electrons from the dipolarophile. According to the Woodward-Hoffmann rules, [24] if the 1,3-DC reaction proceeds via a concerted mechanism, three p_z orbitals of the1,3-dipole and two p_z orbitals of the dipolarophile will combine suprafacially, symbolizedas [π 4s+ π 2s]. The stereochemistry of the reactants could be transferred to the product. For example, the 1,3-DC of benzonitrile oxide with *trans*-di deuterated ethylene gave exclusively the *trans*-isoxazoline (**Scheme 2.1.4**)[25].

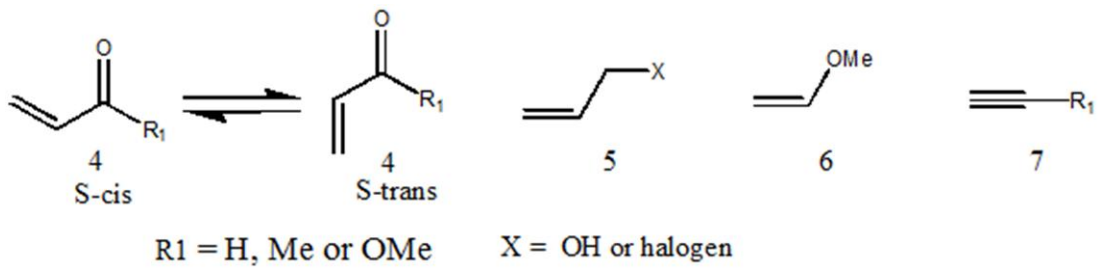


Scheme 2.1.4 *Concerted pathway of 1, 3-DC*

For those step-wise 1, 3-DC involving some intermediates, the stereochemical information will be destroyed during these transformations.

2.1.3 The dipolarophile

The dipolarophile in a 1, 3-dipolar cycloaddition is a reactive alkene moiety containing 2π electrons. Thus, depending on which dipole that is present, α, β-unsaturated aldehydes, ketones, and esters, allylic alcohols, allylic halides, vinylic ethers and alkynes are examples of dipolarophiles that react readily (dipolarophiles **4**-**7**, **Scheme 2.1.5**). It must be noted, however, that other 2π-moieties such as carbonyls and imines also can undergo cycloaddition with dipoles. The alkene moiety can be mono- di-, tri- or even tetra substituted (only mono substituted ones are shown here). However, mostly due to steric factors, tri and tetra substituted ones often display very low reactivity in reactions with dipoles.



Scheme 2.1.5 *Examples of dipolarophiles in 1,3-dipolar cycloaddition reactions*

It must be pointed out that dipolarophiles incorporating two conjugated double bonds such as dipolarophile **4** can exist in two different main conformations, s-*cis* and s-*trans*, respectively (**Scheme 2.1.5**), where the s-*cis*/s-*trans* descriptor refers to the single bond connecting the two double bonds. Suchs-*cis*/s-*trans*-isomerism can have a major impact on the outcome of an asymmetric 1, 3-dipolar cycloaddition reaction.

2.1.4 Frontier Molecular Orbital Interactions

The transition state of the concerted 1, 3-dipolar cycloaddition reaction is controlled by the frontier molecular orbitals (FMO) of the substrates. Based on the relative FMO energies between the dipole and the dipolarophile, these 1, 3-dipolar cycloaddition reactions have been classified into 3 types by Sustman (**Scheme 2.1.6**).[26] Reactions of type I are typical for azomethine ylides and carbonyl ylides and is the case where the HOMO dipole interacts with the LUMO dipolarophile. The reactions of nitrones are normally classified as type II reactions when the similarity of the dipole and dipolarophile FMO energies permits both HOMO-LUMO interactions to be important. In type III reactions the FMO interactions are dominated by the $LUMO_{dipole}$ and the HOMO $_{dipolarophile}$

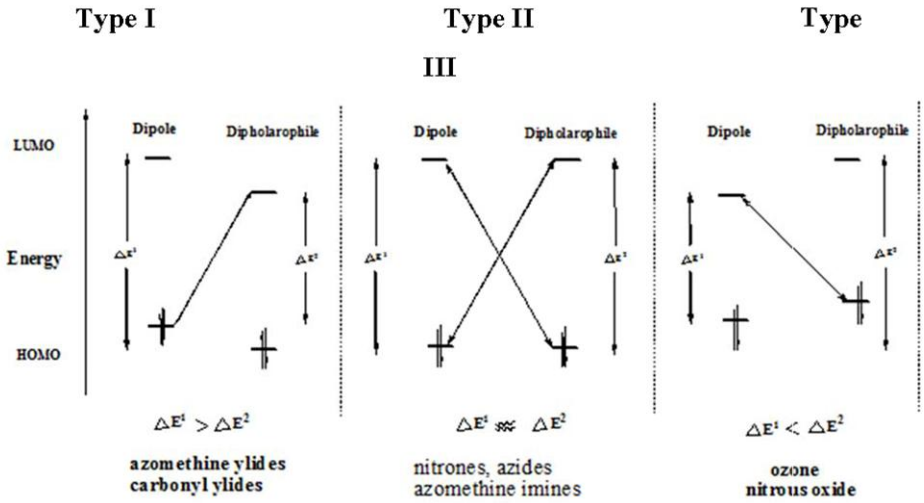
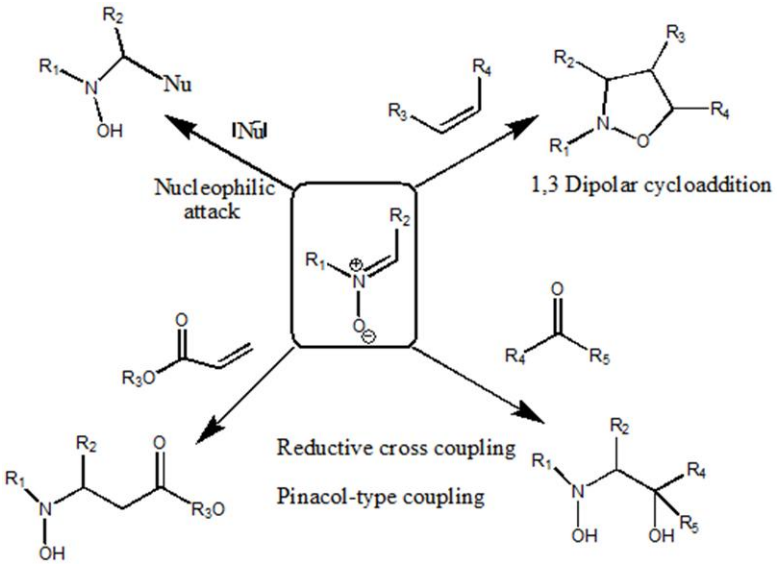


Figure 2.1.6. *Classifications of 1,3-dipolar cycloaddition reaction with representative examples*

For example, reactions of nitrile oxides are better classifieds borderline to the type III than to the type II because nitrile oxides have relatively low LUMO energies of -11 to -10 eV. The catalytic control of reaction is based on the relative FMO energies of the reagents. To be able to control the stereochemistry of a reaction with a sub-stoichiometric amount of a ligand-metal catalyst it is desirable that large reaction rate increases are obtained because then the reaction only takes place in the sphere of the metal and the chiral ligand. The relative energies of the FMO of one of the substrates is changed with catalytic enhancement of the reaction rate when using chiral Lewis acid complexes.[27] As a matter of principle, such activation can be applied to the 1,3- dipolar cycloaddition of nitrones with alkenes in two different ways.

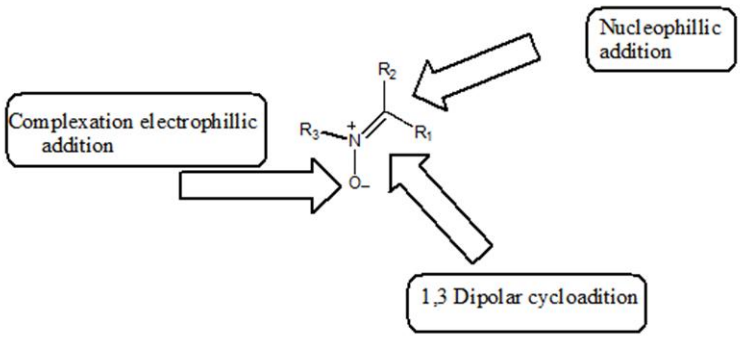
2.2 Overview of the Nitrone Reactivity Profile

Nitrones exhibit a broad reactivity profile and are recognised as versatile synthetic intermediates due to their ability to undergo numerous useful reactions such as 1, 3-dipolar cycloadditions, nucleophilic additions, and pinacol-type coupling reactions (**Scheme 2.2.1**). The chemistry of nitrones have been frequently reviewed, [28-33] but it is ultimately dominated by their use as substrates for 1, 3-dipolar cycloaddition and more recently, nucleophilic attack. The wide breadth of reactivity is due to the structure of the nitrone functionality. Nitrones are isoelectronic with allyl anions and enolates, but the presence of the C=N moiety provides an iminium-type character which is responsible for its reactivity as an electrophile.



Scheme 2.2.1 *Main reaction of nitrones*

Accordingly, in addition to their 1,3-dipolar character, nitrones react with nucleophiles at the carbon atom and with electrophiles at the oxygen atom (**Scheme 2.2.2**). There are increasing reports of nucleophilic additions to nitrones to form α-substituted hydroxylamines. And charged nucleophiles such as H_2O, HO^-, RO^-, RS^-, R_3N, CN^-, (R = alkyl, aryl), [28] and phosphonates.[34-37] Conversely, there are several reports of electrophilic additions to nitrones. The negatively charged oxygen of the nitrone functionality behaves as anucleophile, and reacts at oxygen with a variety of electrophiles including ketenes,[38-40] isocyanates,[41] acetic anhydride,[42] and various acid chlorides.[43-45] Nitrones also serve as excellent scavengers for several types of radical, especially short lived radicals.[28,4]

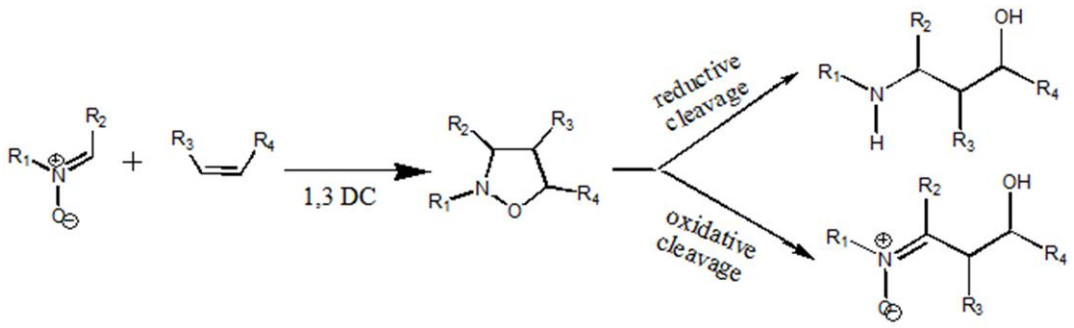


Scheme 2.2.2. *Reaction profile of the nitrone functionality*

Forming more stable radical products, they act as spin traps which can be used in biology for evaluation of both *in vivo* and *in situ* systems for diagnostic purposes.[46,47] They have also found use in chemical analysis, forming stable paramagnetic spin adducts that improve signal to noise ratios in electron spin resonance (ESR) spectroscopy and achieve high efficiency in spin trapping experiments [49-51] as well as allowing the identification of intermediates in the study of radical mechanisms.[52]

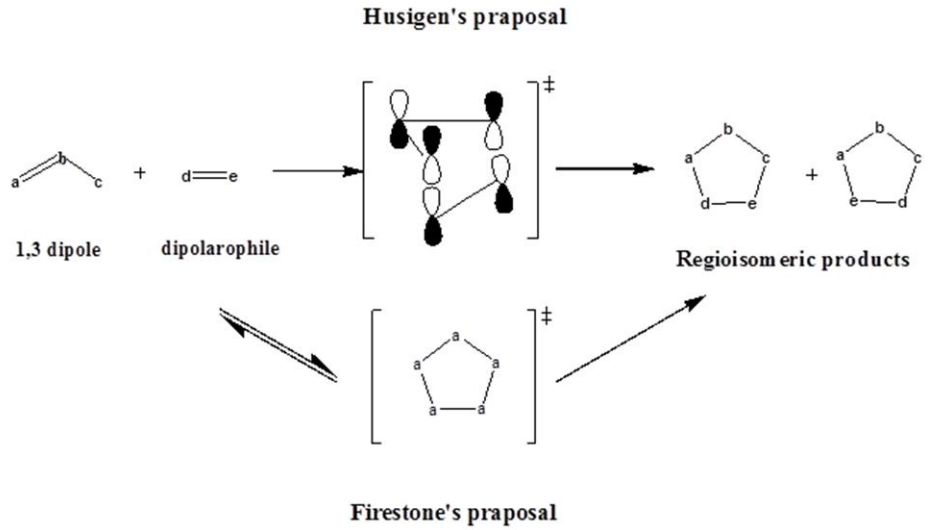
2.2.1 1,3-Dipolar Cycloaddition Reaction to Nitrone

Nitrones undergo 1, 3-dipolar cycloaddition reactions with a wide variety of dipolarophiles including alkenes, alkynes, cumulenes, thiocarbonyls, phosphoranes, isocyanates and nitriles.[53] This powerful reaction can be utilised to create multiple chiral centres in a single step providing excellent synthetic routes to complex systems.The 1, 3-dipolar cycloaddition reaction with substituted alkenes to give isoxazolidines remains the single most studied reaction of nitrones. The isoxazolidine functionality; a saturated five-membered heterocycle containing adjacent nitrogen and oxygen atoms[54] represents an important synthetic intermediate which can be manipulated by reductive cleavage of the relatively weak *N-O* bond to furnish 1, 3-amino alcohols or by oxidation to give the corresponding nitrone (**Scheme 2.2.3**).



Scheme 2.2.3. *Isoxazolidine reaction products*

Huisgen proposed the widely accepted concept of the 1, 3-dipolar cycloaddition reaction proceding *via* a concerted but not simultaneous process.[55-64] An alternate mechanism, proposed by Firestone proceeds *via* a diradical intermediate. However this model does not explain the stereospecificity of the reaction (**Scheme 2.2.4**).[65-70]



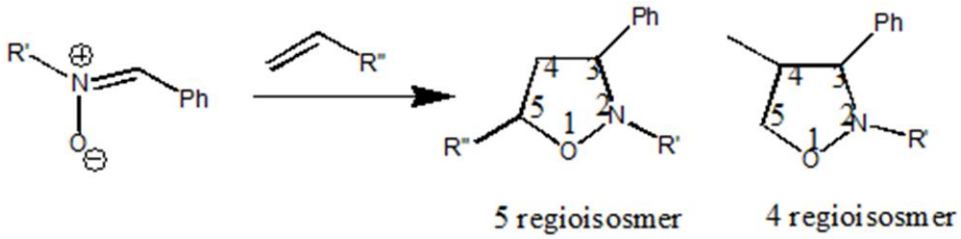
Scheme 2.2.4. *Proposed mechanisms of 1,3-dipolar cycloaddition*

2.2.2 Selectivity of the 1,3-Dipolar Cycloaddition Reaction Between Alkenes and Nitrones

With the ability to form up to three new stereocenters in a single step, it is possible that up to eight isomers may arise from the cycloaddition reaction between the nitrone and alkenes. The preference for a particular product depends on diastereo facial selectivity, regioselectivity and whether the dipolarophile approaches the nitrone in an *endo* or *exo* fashion. This reaction generally proceeds in a predictable fashion, through a highly ordered transition state, allowing the regio- and stereochemical outcomes to be predicted.

2.2.3 Regioselectivity of Alkene Addition

The region selectivities observed for the 1,3-dipolar cycloaddition with alkenes are controlled by a mixture of both steric and electronic effects[28] and have been extensively studied. Steric factors favour the formation of 5-substituted isoxazolidines as the more sterically hindered functionality of the alkene tends to add to the oxygen atom of the1,3-dipole. Electronic factors may favour formation of either the 4- or 5-substituted isoxazolidine depending on the nature of the dipolarophile. The introduction of electron-donating or electron-withdrawing substituents on the dipole or dipolarophile can significantly change the relative FMO energies and hence the HOMO/LUMO interactions employed.[28, 30, 71, 72] For instance, the reaction of *N*-methyl-*C*-phenyl nitrone with methyl acrylate is controlled by the $HOMO_{dipole}$ - $LUMO_{dipolarophile}$ interactions (**Scheme 2.2.5)**, whereas the reaction of the same nitrone with nitroethene is controlled by $HOMO_{dipole}$ $LUMO_{dipolarophile}$ interactions (**Table 1**).[73]

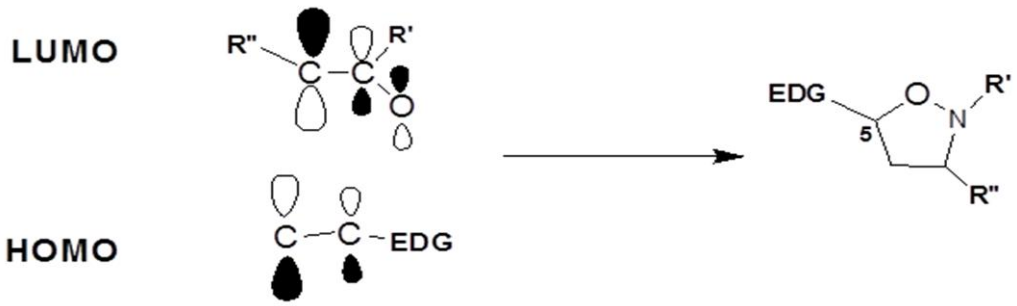


Scheme 2.2.5. *1,3-Dipolar cycloaddition regioselectivity of N-methyl-C-phenyl and N-phenyl-C-phenyl nitrone*

Table 1 . 1,3-Dipolar cycloaddition regioselectivity of N-methyl-C-phenyl and N-phenyl-C-phenyl nitrone

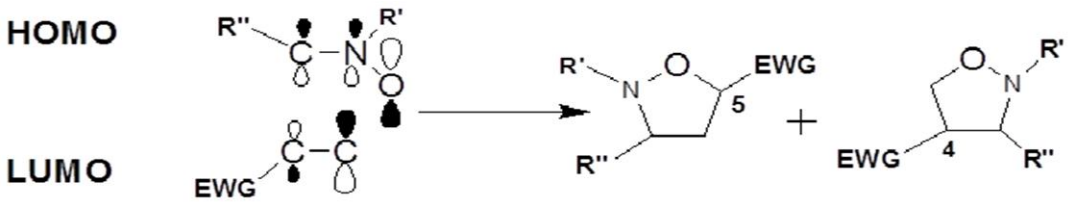
Entry	R'	R''	5-isomer:4-isomer[ab]	Ref
1	Me	NO_2	0:100	81
2	Me	CO_2Me	100:0	81
3	Ph	$PhCO_2Et$	70:30	82

[a] *Stereochemical outcome not shown,*
[b] *Ratio determined by 1H NMR analysis.*



Scheme 2.2.6. *Selectivity of mono-substituted electron-rich or electron-neutral alkenes in 1,3-dipolar cycloadditions*

Generally, the reaction of terminal electron-rich (R = OEt) or electron-neutral alkenes (R = Ph) with nitrones proceeds to give the 5-substituted isomer. Regioselectivity is controlled predominantly by the $LUMO_{nitrone}$ − $HOMO_{alkene}$ interactions where the largest coefficients are at the nitrone α-carbon and the alkene terminal carbon, this reaction closely resembles a Type III process. This selectivity is further enhanced by steric factors. Thus, the nitrone and alkene combine in a regioselective manner to give the 5-substituted isoxazolidine (**Scheme 2.2.6**).[28,74] Conversely, the reaction of terminal alkenes with electron-withdrawing groups (R =NO_2, CO_2Et) resembles a Type I process and is primarily controlled by the $HOMO_{nitrone}$ - $LUMO_{alkene}$ interactions where the largest coefficients are at the nitrone oxygen and the alkene terminal carbon atom. This selectivity favours formation of the 4-substituted isomer. However, since steric factors oppose this selectivity, a mixture of regioisomers is often obtained (**Scheme 2.2.7**).[28,74]



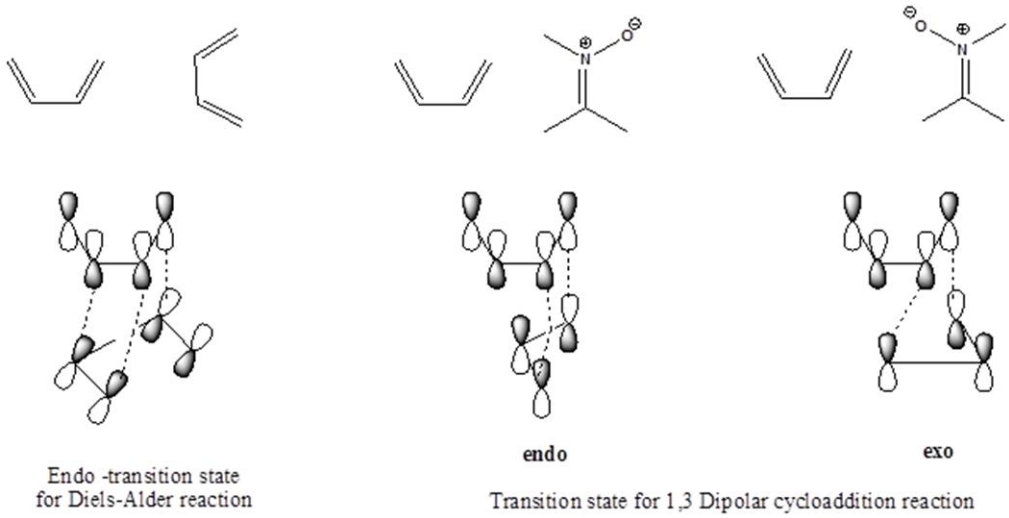
Scheme 2.2.7 *Selectivity of mono-substituted electron-deficient alkenes in 1,3-dipolar cycloadditions*

However, in the reaction of nitrones with 1,2-disubstituted alkenes bearing an electron withdrawing group the steric factor is eliminated, leading to FMO-controlled regioselectivityand the 4-substituted isomer is often obtained as a single product (**Scheme 2.2.8**).[28,74]

Scheme 2.2.8: *Selectivity of 1,2-disubstituted electron-deficient alkenes in 1,3-dipolar cycloadditions*

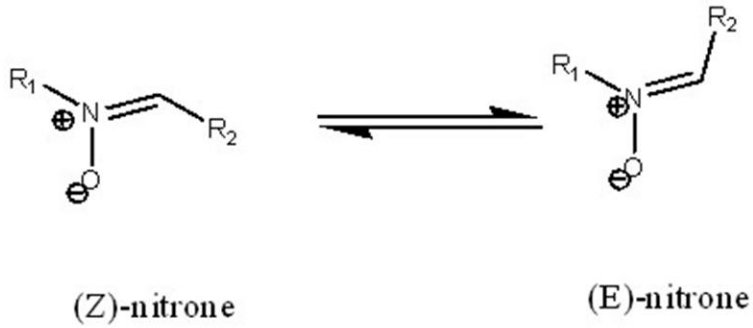
2.2.4 Diastereoselectivity and Enantioselectivity of the 1,3-Dipolar Cycloaddition Reaction with Alkenes

In the 1,3-dipolar cycloaddition reaction with alkenes, the nitrone can be approached in either an *endo* or *exo* fashion, as well as from the α- or β-face.[28] Prediction of the stereoselectivity is complicated by a number of factors including substrate structure, secondary orbital interactions, and nitrone isomerisation.



Scheme 2.2.9: *Comparison of endoselectivity of the Diels-Alder reaction and theendo/exoselectivity of the 1,3-dipolar cycloaddition reaction*

The unflavoured *endo*-isomer of nitrone cycloaddition arises from the transition state in which the nitrogen atom of the dipole points in the same direction as the substituent of the alkene. However, in comparison to the Diels-Alder reaction, in which the *endo*-transition state is favoured due to stabilisation by secondary π-orbital interactions, the interaction is between the *N*-nitrone p_z-orbital and the vicinal p_z-orbital on the alkene and thus the stabilisation is small (**Scheme 2.2.9**).[28, 30] As a result the *endo*/*exo* selectivity in the1,3-dipolar cycloaddition reaction is primarily controlled by the substituents on the alkene or nitrone or by a catalyst.[28, 30]



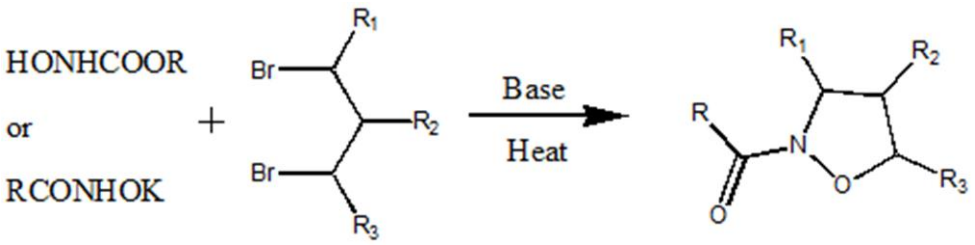
Scheme 2.2.10: *E/Z isomerisation of acyclic nitrones.*

Acyclic nitrones exist in (*E*) - and (*Z*) - forms that may interconvert at high temperature, complicating the stereo chemical prediction of reaction products and resulting in reduced diastereoselectivity (**Scheme 2.2.10**). A number of cyclic nitrones have been developed that avoid the issue of nitrone isomerisation by permitting only a single (*E*)-geometry about the C=N double bond, reducing the number of possible cycloaddition products.[31]

2.3 Methods for Synthesis of Isoxazolidine

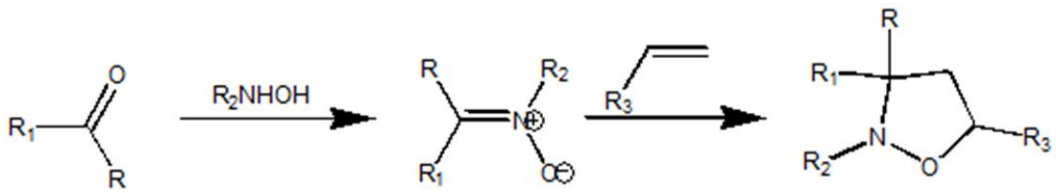
There are three methods for the synthesis of isoxazolidines.

(1) The method for the synthesis of N-unsubstitutedisoxazolidines in very early times involves the reaction of *N*-hydroxyurethanes or hydroxamic acids and their salts with 1,3-dihalo compounds in an alkaline medium (**Scheme 2.3.1**). This reaction is especially valuable for the synthesis of 3- or 5-mono-, and 3,5-disubstituted isoxazolidines, but the yields were not high.[75,76,77]



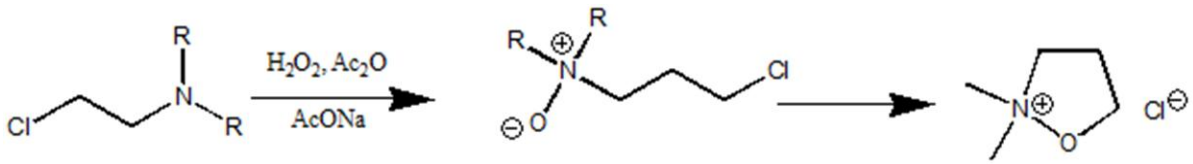
Scheme 2.3.1: *synthetic pathway for isoxazolidine ring in early times*

(2) 1,3-dipolar cycloaddition of 1,3-dipoles with olefins represents the main method of isoxazolidine synthesis of green chemical reactions [78] (**Scheme 2.3.2**). Nitrones are perhaps the most extensively studied 1,3-dipoles and can be prepared by condensation of aldehydes or ketones with N-monosubstitutedhydroxylamines.5-Substituted tereoisomericisoxazolidines were obtained when nitrones reacted with mono-substituted olefins.[79]



Scheme 2.3.2: *1,3 dipolar cycloaddition reaction of nitrone and alkene*

(3) Isoxazolidinium salts can be prepared by intramolecular cyclization of long chain nitrones that are obtained by oxidation of tertiary amines with peracetic acid (**Scheme 2.3.3**), but this method is rarely used.[80, 81]



Scheme 2.2.3: *intermolecular cyclization of long chain nitrone*

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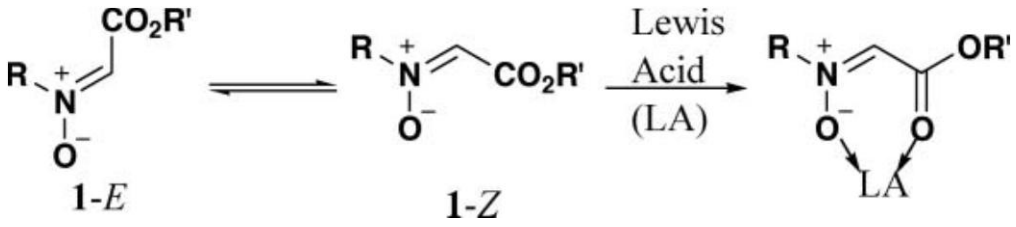
Chapter 3

1,3-DC with hetero substituted alkenes and nitrones.

I. Oxa substituted alkenes

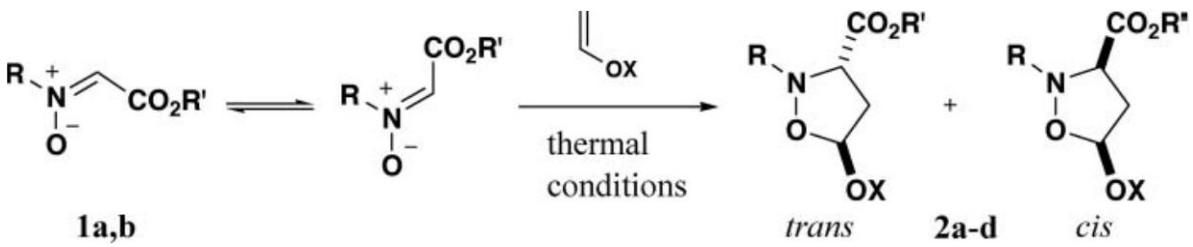
3.1 Nitrones Activated by Electron-withdrawing Groups

Alkoxycarbonyl-substituted nitrones **1** are interesting dipoles. These activated nitrones are thermally stable and are glycine-type electrophilic synthons. However, a difficulty related to their use lies in their configurational unstability (**Scheme 3.1**).



Scheme 3.1: *general reaction between Z/E nitrone and Lewis acid*

Table 1: Data collected of cycloaddition reaction between nitrones 1a, b and vinyl ethers or esters



Nitrone	R	R'	X	Conditions	Yield (%)	Adduct trans: cis	Ref
1a	**Bn**	**Et**	**Et**	CH_2Cl_2, 50 ºC (sealed tube), 66 h	**58**	**2a** (78:22	1
1a	**Bn**	**Et**	**Et**	CH_3CN, 50 ºC, 23 h	**45**	**2a** (65:35)	1
1a	**Bn**	**Et**	**Et**	toluene, 50 ºC, 23 h	**76**	**2a** (88:12)	1
1a	**Bn**	**Et**	**Ac**	AcOCH═ CH_2 (10 eq.), 70 ºC, 24	**87**	**2b** (75:25)	2
1b	**Ph_2CH**	**Me**	**Et**	EtOCH═ CH_2 (20 eq.), rt, 36 h	**89**	**2c** (72:28)	3
1a	**Ph_2CH**	**Me**	***n*-Bu**	*n*-BuOCH═ CH_2 (20 eq.), rt, 36 h	**73**	**2d** (75:25)	3

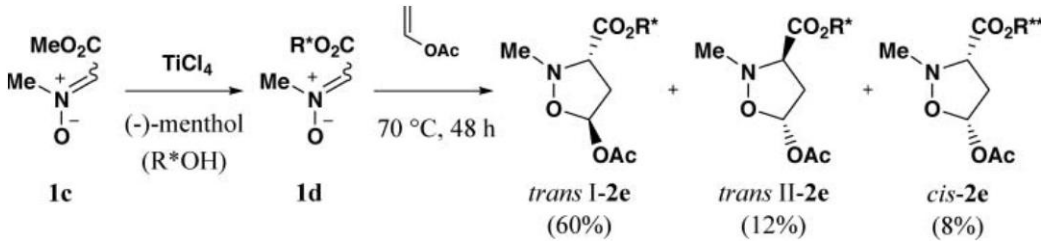
Indeed, although exhibiting a pure *Z* configuration in solid state, such nitrones undergo a rapid *Z*/*E* equilibrium in toluene or chloroform solution, even at room temperature. The *Z*/*E ratio* is dependent on the solvent (dielectric constant), temperature, and steric hindrance of the *N*-substituent and of the ester. Interestingly, this equilibrium can shift from *E* to *Z* geometry in the presence of a chelating Lewis acid.

3.1.1 Thermal conditions

i Acyclic Nitrone

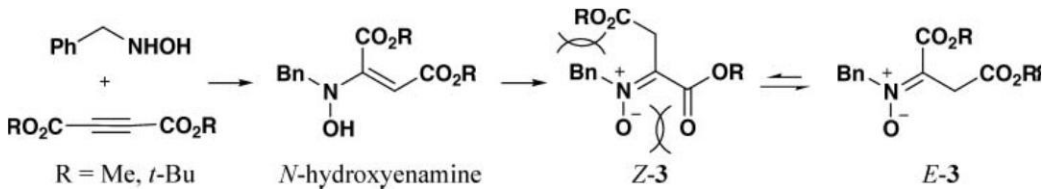
In the absence of a Lewis acid, dipolar cycloadditions of acyclic activated nitrones **1** with vinyl ethers or vinyl acetate proceed with moderate conversions under mild conditions (20–70 ºC, 23–66 h). A moderate to

good *trans* stereocontrol is homogeneously observed, even with nitrones bearing a bulky *N*-substituent (**1b**, R = Ph_2CH, **Table 1**). A favored *exo* approach involving the more reactive *E* nitrone is commonly assumed to explain this (partial) *trans* stereo selectivity. Interestingly, by reaction of a methyl ester-derived chiral nitrone with vinyl acetate, Chiacchio *et al.* observed under the same mild thermal conditions an enhanced *trans* stereoselectivity (9:1) together with a fair facial diastereoselectivity (*trans* I : *trans* II = 5:1) (**Scheme 3.1.1**).[4]



Scheme 3.1.1: *Cycloaddition reactions between acyclic activated nitrones and vinyl ethers or esters*

Very recently, a significant progress in the research of stereo control in the thermal 1,3-DC of acyclic activated nitrones with vinyl ethers was disclosed with the use of aspartic nitrones **3** (**Scheme 3.1.2**).

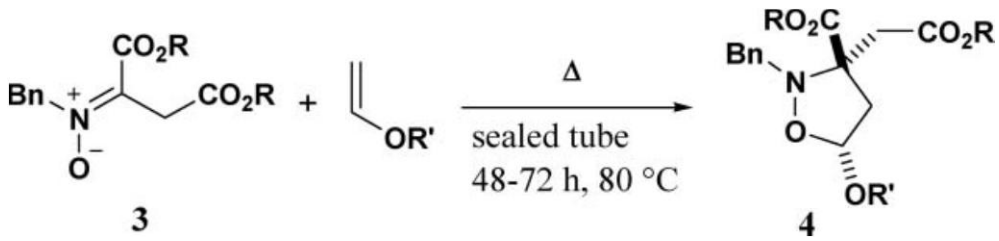


Scheme 3.1.2: *synthesis of acyclic activated nitrones*

This new type of dipole, quantitatively prepared in one step from a *N*-protected hydroxylamine and an dialkyl acetylene dicarboxylate, was found to display a single *E* configuration of the substituted C- N bond in solution in contrast to monosubstituted ones, together with a fair reactivity towards a wide range of dipolarophiles.[5,6] The thermal 1,3-DC of **3** with simple

vinyl ethers afford adducts **4** in high yields and with a remarkable *trans* selectivity, up to 98: 2 with the bulky *t*-butyl vinyl ether (**Table 2**).[6]

Table 2: Thermal cycloaddition of disubstituted nitrones **3** with vinyl ethers

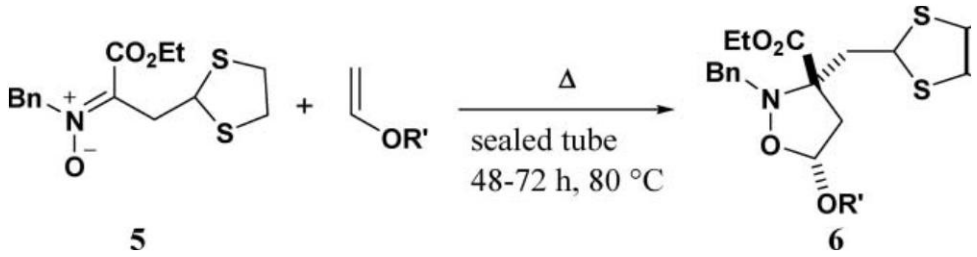


3 4

Entry	Nitrone	R	R'	Adduct	Yield (%)	*trans;cis*
1	**3a**	Me	Ac	**4a**	**89**	80:20
2	**3a**	Me	Et	**4b**	**95**	92:8
3	**3b**	*t*-Bu	Et	**4c**	**99**	95:5
4	**3a**	Me	*t*-Bu	**4d**	**92**	>98:2
5	**3b**	*t*-Bu	*t*-Bu	**4e**	**99**	>98:2

This *trans*-stereocontrolled 1,3-DC access to isoxazolidines containing a quaternarycenter proves equally efficient with nitrones bearing two different functions, such as **5** (**Table 3**).[7]

Table 3: Thermal cycloaddition of disubstituted nitrones **5** with vinyl ethers

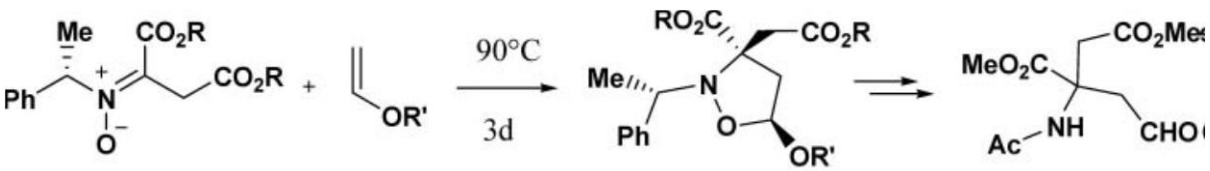


5 6

Entry	R'	Adduct	Yield (%)	*trans;cis*
1	Ac	**6a**	**90**	80:20
2	Et	**6b**	**91**	92:8
3	*t*-Bu	**6c**	**89**	>98:2

Asymmetric extension with either chiral vinyl ethers or chiral aspartic nitrones **7** was studied and afforded, in the latter case, diastereomerically and enantiomerically pure adducts in acceptable yields. These unprecedent isoxazolidines **8** were conveniently converted into enantioenriched new α,α-disubstituted α-amino acids **9** (**Table 4**).[6]

Table 4: Cycloaddition Reactions involving (*S*)-α-Methylbenzylnitrone **7**

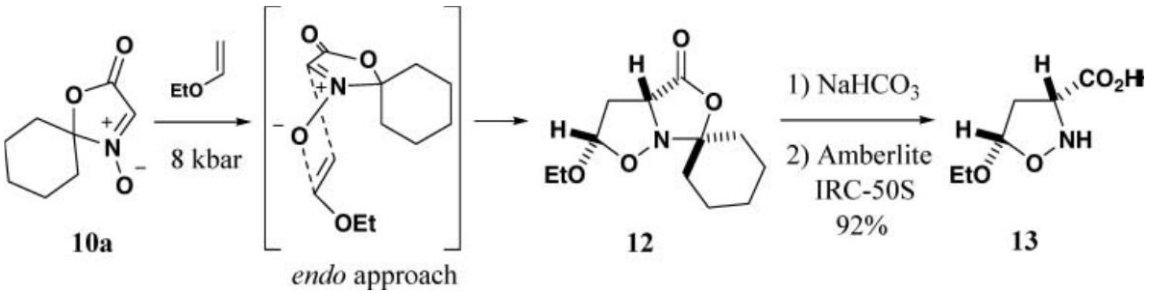


7 8 9

Entry	Nitrone	R	R'	Adduct	Yield (%)	*trans* I:*trans* II *cis* I :*cis* II
1	**7a**	Me	Et	**8a**	**99**	69:31:0:0
2	**7b**	Me	*t*-Bu	**8b**	**95**	72:28:0:0 (major isomer isolable, 50% yield)
3	**7c**	*t*-Bu	Et	**8c**	**99**	67:33:0:0
4	**7d**	*t*-Bu	*t*-Bu	**d8**	**97**	72:28:0:0

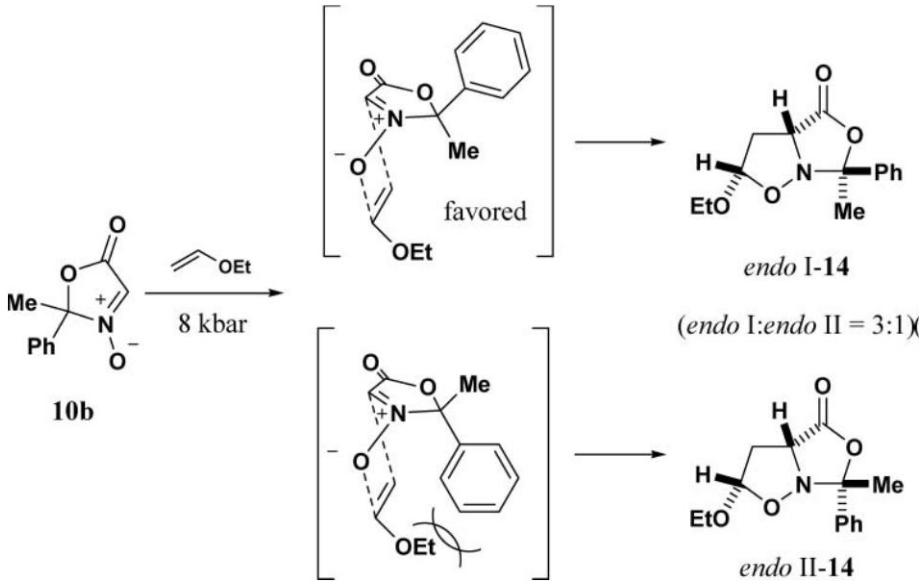
ii Cyclic Nitrones

An obvious way to ensure a pure *E* geometry for the nitrone is to create a 5 or 6 membered lactonic ring between the ester function and the *N*-substituent. The first synthesis of such cyclic activated nitrones **10** was reported by Katagiri's group in 1994.[7] The nitroso ketene intermediate, generated by thermolysis of hydroxyimino Meldrum's acid, was found to react with various ketones to afford the 5-membered ring nitrones **10** after 1,2-rearrangement of the transient imino-lactone. The cycloaddition of cyclic nitrones **10** with ethyl vinyl ether required appropriate conditions.[8] Indeed, with spiro-nitrone **10a**, 1,3-DC was unsuccessful under classical thermal conditions, but proceeded under solvent-free and hyperbaric conditions (8 kbar) with high yields and a total *cis* stereoselectivity, resulting from an *endo* approach (**Scheme 3.1.3**). Adduct **12** was easily transformed into the corresponding acid **13**.



Scheme 3.1.3: *Stereoselective synthesis of cycloaddition under solvent free and hyperbaric conditions*

Under the same conditions, 1, 3-DC of ethyl vinyl ether with the nitrone **10b** deriving from acetophenone proceeded again with a total *endo* stereo selectivity, and a 3:1 diastereo facial selectivity (**Scheme 3.1.4**).

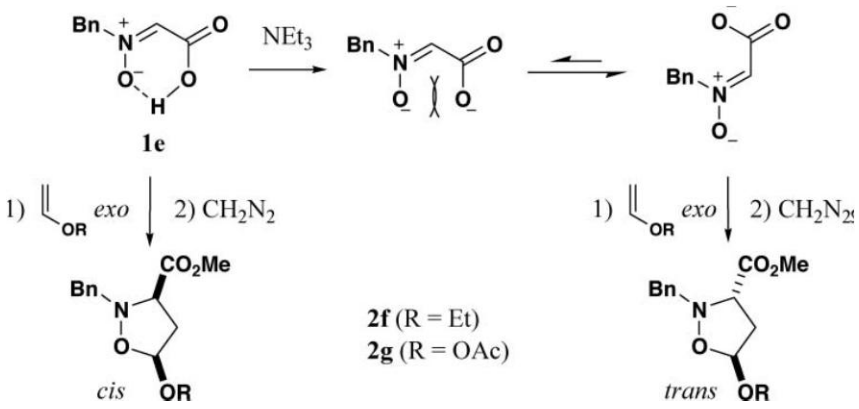


Scheme 3.1.4: *1, 3-DC of vinyl ether and nitrone under solvent free and hyperbaric conditions*

iii brønsted acid-catalyzed conditions

One of the first attempts to fix the configuration of an acyclic nitrone was reported by Fukumoto's group[8] with the use of carboxylic derivatives (**Table 5**). In the absence of a base, nitrone **1e** displays exclusively a *Z* geometry, stabilized by the internal hydrogen bond. 1, 3-DC of this nitrone with ethyl vinyl ether or vinyl acetate led to *cis* adducts with a good to high stereo selectivity (**Table 5, Entries 1 and 2**). With triethylamine, the *Z* geometry is destabilized due to the proximity of the two negatively charged oxygen atoms. As a consequence, the 1, 3-DC takes place with the opposite *trans* selectivity (**Table 5, Entries 3 and 4**).

Table 5: Effect of the presence of a brønsted acid on the configuration of the nitrone



Entry	R	Base	Adduct	Yield (%)	*trans:cis*
1	Et	none	**2f**	20	26:74
2	OAc	none	**2g**	40	7:93
3	Et	NEt_3	**2f**	68	86:14
4	OAc	NEt_3	**2g**	16	68:32

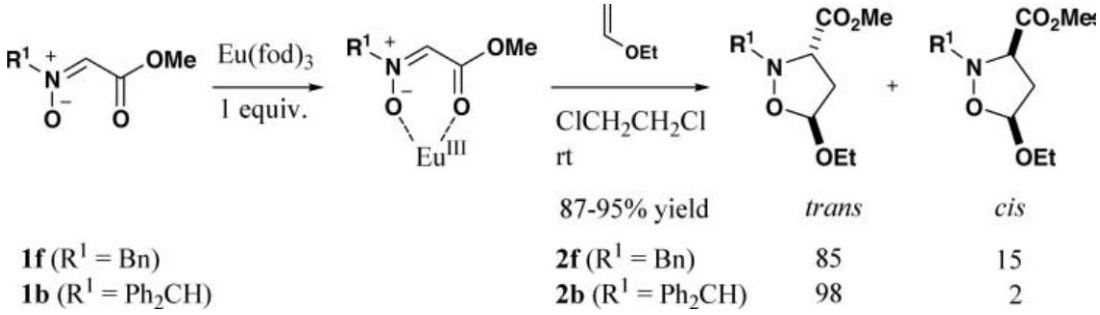
However, yields are restricted by the unstability of nitrone **1e**, in its acidic or salt form.

3.1.2 Lewis Acid-catalyzed Condition

i. Europium (III) Catalyst

Organosoluble lanthanide salts such as $Eu(fod)_3$ (fod = 6,6,7,7,8,8,8-heptafluoro-2,2- dimethyl-3,5-octanedionato) were used by Tamura *et al*. as activating agents,[9] in order to control the stereoselectivity of the 1,3-DC between *N*-alkyl-*α*-carbonyloxyalkylnitrones **1** and vinyl ethers (**Scheme 3.1.5**). The chelation was expected to lower the LUMO of the nitrone in a *Z* configuration and to improve their reactivity towards electron-rich

dipolarophiles such as vinyl ethers. *trans*-adducts **2** were thus selectively obtained under very mild conditions with high yields,

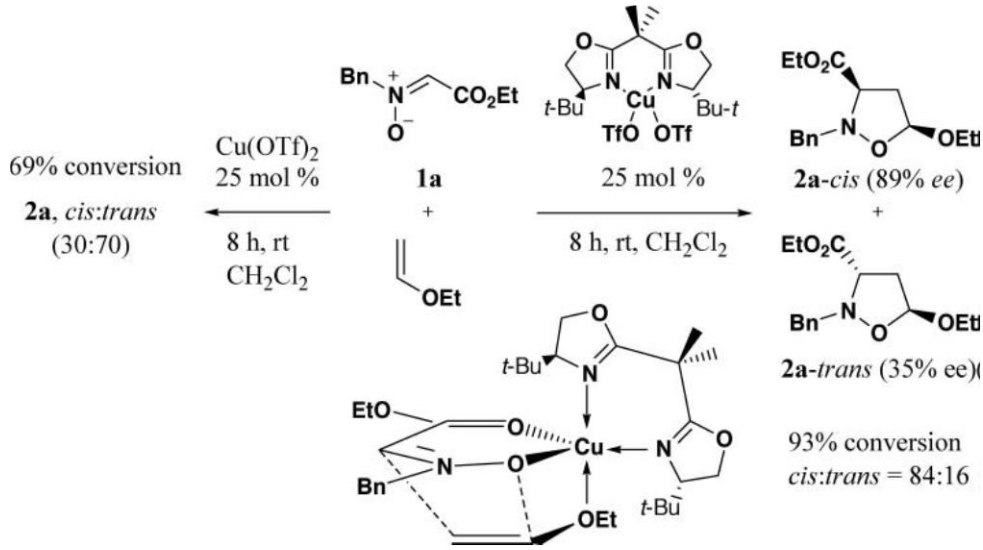


Scheme 3.1.5: *stereoselective 1, 3-DC of vinyl ether and N-alkyl-α-carbonyloxyalkyl nitrones*

Especially when $Eu(fod)_3$ was used in stoichiometric amounts. *trans*-Selectivity was also found to significantly increase with the size of the *N*-substituent of the nitrone **1**.

ii. Copper (II) and Zinc (II) Catalysts

Copper(II)-catalyzed 1,3-DC of activated nitrones and vinyl ethers was studied by Jørgensen's group in 1999.[1] With copper(II) triflate (25 mol%), the reaction of nitrone **1a** and ethyl vinyl ether was moderately accelerated without significant change of the diastereomeric ratio (*trans*:*cis* = 70:30) compared to the thermal process. However, the adducts **2a** result in this case from an *endo* approach on the *Z* nitrone rather than from an *exo* thermal-like process on the *E* nitrone. Interestingly, the chiral complex $Cu(OTf)_2$-BOX deriving from *t*-leucine proved to be more efficient than $Cu(OTf)_2$ to catalyze this reaction (**Scheme 3.1.6**).



Scheme 3.1.6: *Copper (II) catalysed* ***1, 3****-DC of vinyl ether and activated*

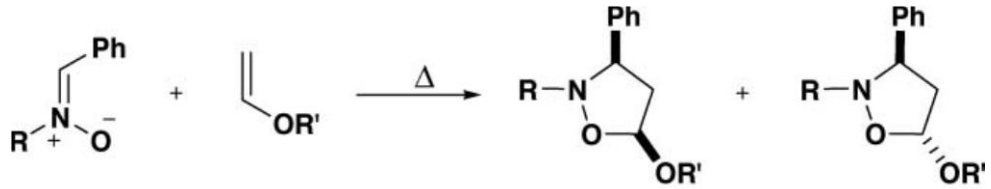
nitrone As another major fact, a significant *cis* stereoselectivity is divergently observed (*cis*:*trans* = 84:16) and the major *cis* diastereoisomer is obtained with a good *ee* (89%), whereas enantioselectivity is poor for the minor *trans* isomer. With the same catalyst, changing the solvent from dichloromethane to toluene increased the *ee* of the major *cis* isomer (up to 93%) but decreased the diastereoselectivity (*cis*:*trans* = 70:30).To explain this stereochemical and asymmetric outcome, an intermediate was postulated, in which the copper (II) atom interacts with both BOX and cycloreactants in a way that minimize steric interactions between *t*-Bu groups and the incoming hetero adduct.

3.2 C-Aryl-substituted Nitrones

3.2.1 Thermal conditions

In contrast to activated acyclic nitrones, C-aryl and C-alkyl acyclic nitrones commonly display a stable *Z* configuration avoiding the steric repulsion assumed in the *E* form. The thermal 1, 3-DC of *Z* *α*-aryl nitrones **17** was reported at 50 ºC with vinyl ethers[10] and at 80 ºC with vinyl acetate[11] and is typically *exo*-controlled, leading mainly to *cis* adducts (**Table 6**). This *cis* stereoselectivity is favoured with a nitrone displaying a high rotation barrier of its C- N bond (*e. g.* **17a**, R = Ph) towards a bulky dipolarophile (R' = *t*-Bu), and can reach up to 97:3 when these two factors are cumulated (**Table 6, Entry 2**). However, in this last case, complete conversion at 50ºC required an extended time of 14 days.

Table 6 Thermal cycloaddition of nitrones **17** with vinyl ethers and vinyl acetate



17 *cis*-**18** *trans*-**18**

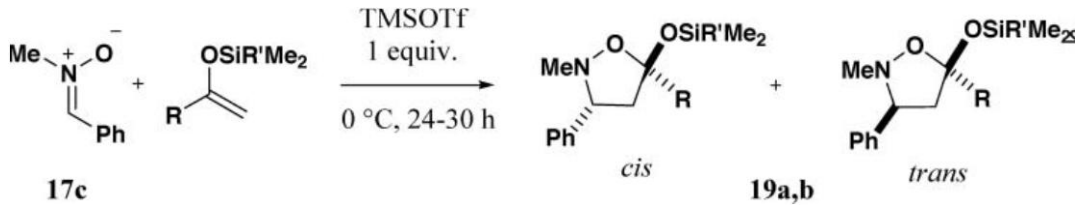
Entry	Nitrone	R	R'	Conditions	Adduct	Yield (%)	cis:trans	Ref
1	**17a**	Ph	Et	50 ºC, 50h	**18a**	72	86:14	10
2	**17a**	Ph	*t*-Bu	50 ºC, 14d	**18b**	70	97:3	10
3	**17b**	Bn	Et	50 ºC, 53h	**18c**	78	67:33	10
4	**17b**	Bn	*t*-Bu	50 ºC, 5d	**18d**	74	80:20	10
5	**17c**	Me	Et	80 ºC, 72h	**18e**	61	50:50	11
6	**17c**	Me	Ac	80 ºC, 72h	**18f**	61	70:30	11

3.2.2 Lewis Acid-catalyzed Conditions

i TMSOTf-promoted Reactions

Tromboni's group demonstrated in 1992 that TMSOTf was a powerful promoter for the 1, 3-DC of *α*-aryl nitrones **17** and silyl enol ethers.[12, 13] When thermal 1, 3-DC of silyl enol ether required harsh conditions (refluxing *p*-xylene), the use of TMSOTf as stoichiometric promoter allowed the reaction to proceed at –10 to 0 ºC with a total conversion after 30 h (**Table 7**) and a moderate *trans* selectivity.

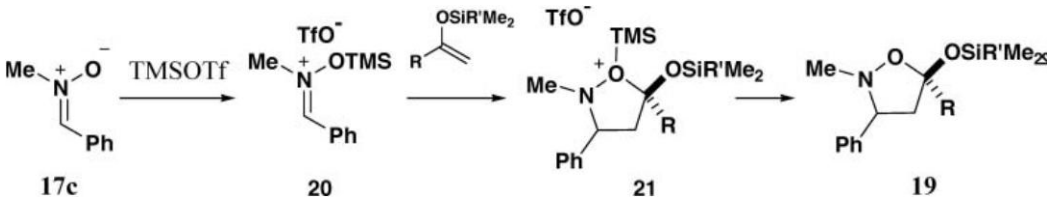
Table 7 Cycloaddition Reactions Promoted by TMSOTf



17c 19a,b

Entry	R	R'	Adduct	Yield (%)	cis:trans
1	H	*t*-Bu	**19a**	87	36:64
2	Ne	Me	**19b**	77	60:40

Concerning the role of TMSOTf, it is assumed that the activation energy required for the formation of the new carbon-carbon bond is reduced by prior silylation of the nitrone **17c** to afford *N*-siloxyiminium ion **20**, which then gives *via* a probable non-concerted mechanism the oxonium ion **21**, an immediate precursor of isoxazolidines **19** (**Scheme 3.2.2**).

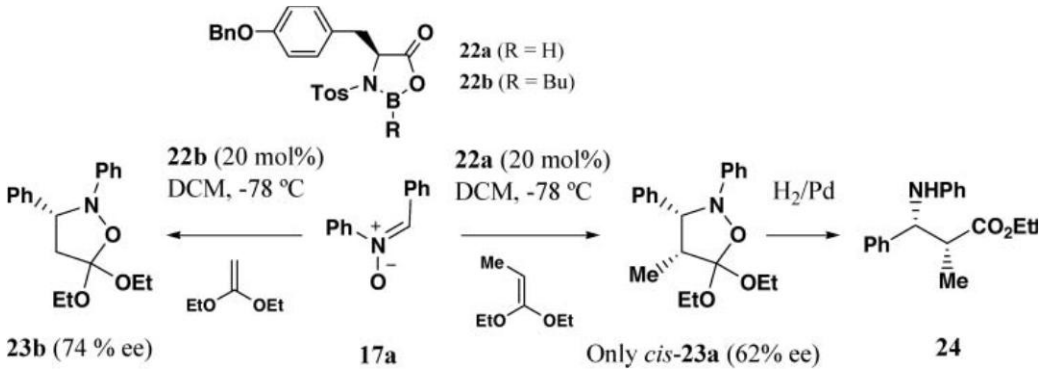


17c 20 21 19

Scheme 3.2.2: *silylation of nitrone followed by 1, 3-DC*

ii Boron(III) Catalyst

In 1994, the use of chiral oxazaborolidinones **22** deriving from *N*-tosyl-L-*α*-aminoacids as catalysts for the 1,3-DC between ketene ketals and the *N*,*α*-diphenylnitrone **17a** was investigated by Scheeren's group.[14] 5,5-Diethoxy-isoxazolidines **23** were thus produced at very low temperature (−78◦C) in high yields (80–98%) and with fair enantioselectivity (up to 74% *ee*) (**Scheme 3.2.3**).[15]

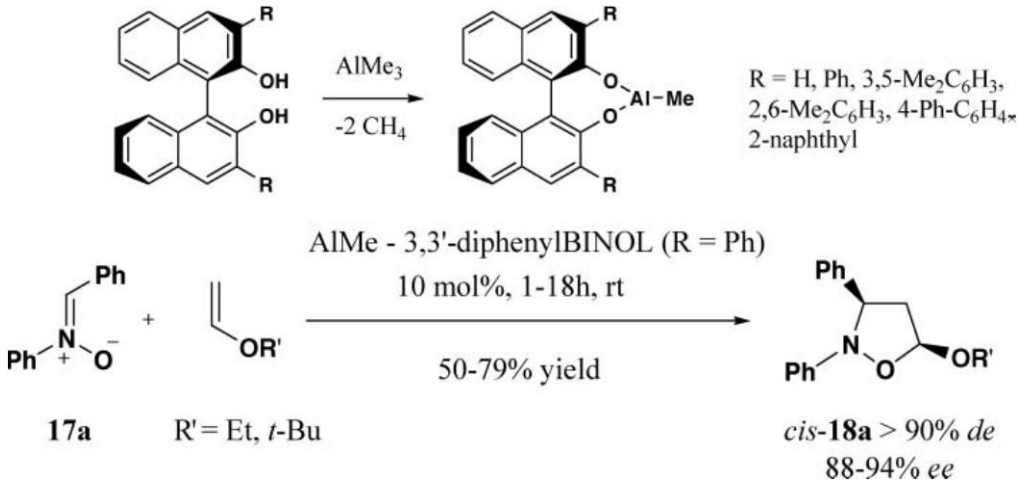


Scheme 3.2.3: *1,3DC between keten ketals and N, α-diphenylnitrone at very low temperature*

This enantioselectivity was found to be highly solvent-dependent and to be reversible by changing the nature of the *α*-side chain substituent of the catalyst, by addition of ligand-like solvents.[16] Hydrogenolysis of the *N-O* bond under mild conditions led quantitatively to enantiopure *β*-amino-esters **24**. Extension of this chiral oxaborolidine methodology to vinyl ethers towards the same nitrone allowed the formation of adducts at room temperature, but only with poor (*cis*) stereoselectivities and low *ee* (<34%).[14,17]

iii Aluminium(III) Catalyst

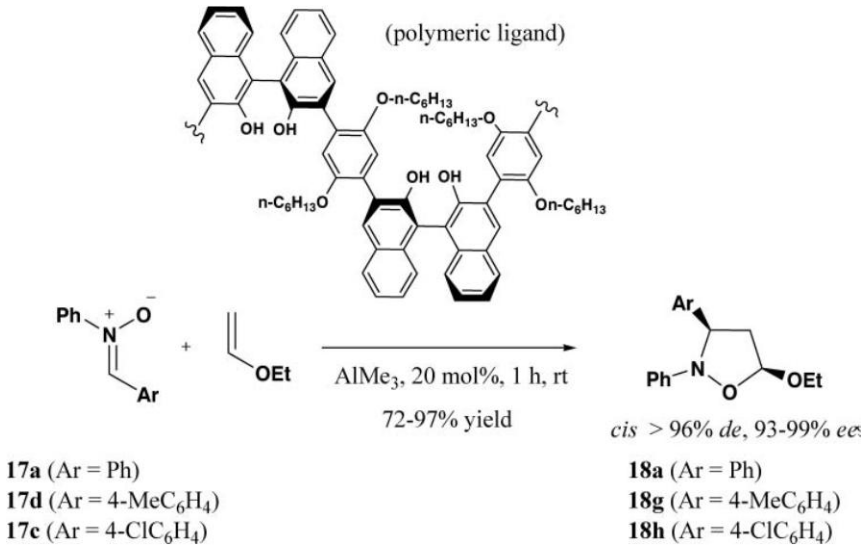
The powerful use of chiral aluminium(III) catalysts was introduced by Jørgensen's group in 1999.[18] The catalysts involved were AlMe-3,3'-diaryl-BINOL complexes obtained by addition of the corresponding ligand to $AlMe_3$ (**Scheme 3.2.4**). The 1, 3-DC reaction between the *N*, *α*-diphenylnitrone **17a** and *t*-butyl vinyl ether was studied extensively, leading to the best results with AlMe-3, 3'-diphenyl-BINOL complex. In this optimal case, the corresponding *cis* isoxazolidine **18a** was obtained as a sole diastereomer in 84% yield and 89% *ee*.



Scheme 3.2.4: *1, 3-DC between vinyl ether and N, α-diphenyl nitrone using AlMe-3, 3'-diaryl-BINOL complexes*

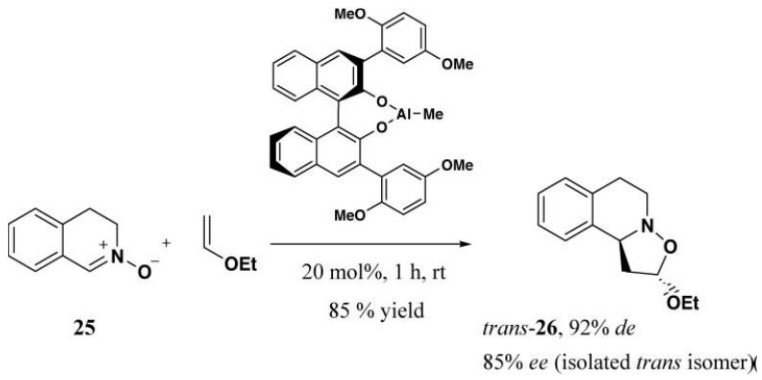
The scope of this powerful enantioselective 1, 3-DC was successfully extended to other *C*-aryl *N*-phenyl nitrones (up to 97% *ee* with C-4-chlorophenyl nitrone), but not evaluated to our knowledge in the critical case of *N*-benzyl-*C*-aryl nitrones which are subject to the Behrend rearrangement. In order to make easier the separation and recycling of the ligand, polymeric 3, 3'- disubstituted BINOL ligands were investigated by the same group (**Scheme 3.2.5**).[19] High yields, *cis* stereoselectivity and enantioselectivity (94–99%) were obtained between *N*-phenyl- *α*-arylnitrones **17** and ethyl vinyl ether. The polymer ligand was efficiently

removed and isolated after final hydrolysis and precipitation in methanol, and was conveniently recycled (without loss of efficiency after 3 cycles).



Scheme 3.2.5: *stereoselective 1, 3-DC using BINOL ligands*

The use of chiral monocomplexing Lewis acids was also investigated in the case of cyclic *C*-aryl nitrones, featuring a *E*-fixed geometry, with the representative 3,4- dihydroisoquinoline-*N*-oxide **25**. As in acyclic series, the best results were reported by Jørgensen's group[20] with Binol derived-aluminium (III) catalysts, affording tricyclic adducts **26** with high *trans* selectivity and good enantioselectivity (up to 85% *ee* with R = 2, 5-diMeO-Ph) (**Scheme 3.2.6**).

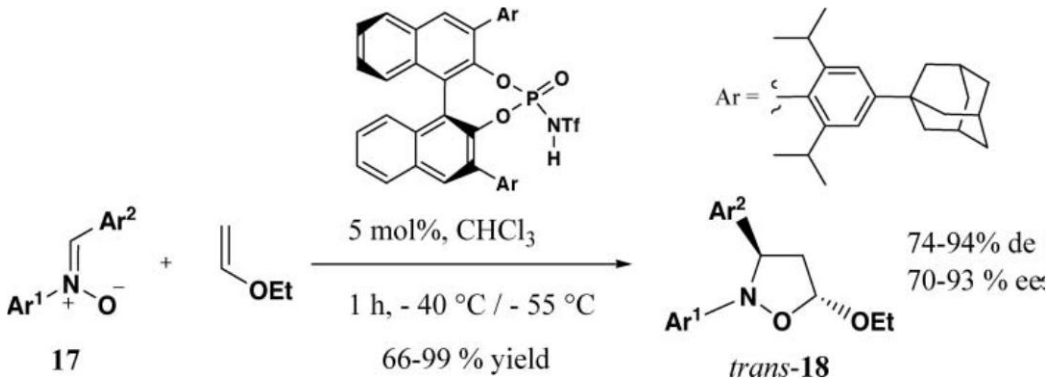


Scheme 3.2.6: *stereoselective 1, 3-DC using BINOL ligands and aluminium (III) catalyst*

Attempts to promote the stereocontrolled and asymmetric 1, 3-DC of 3, 4-dihydroisoquinoline-*N*-oxide **25** towards vinyl ethers or ketene acetals with other chiral Lewis acids (titanocenes, [21] oxazaborolidines[15]) gave only weak results.

iv Brønsted Acid-catalyzed Conditions

Binol-*N*-triflyl-phosphoramides were successfully used as chiral Brønsted acids in the 1,3- DC of aryl and heteroaryl nitrones towards ethyl vinyl ether.[22] From the ligand optimization study performed at −55 ºC in chloroform, Binol-ligand bearing two (4-adamantyl- 2,6-diisopropyl)phenyl groups led to the more efficient catalyst for achieving *endo* and enantiocontrol (*cis* : *trans* = 4:96, 84% *ee*). Interestingly, this reaction that could be extended to a range of *N*,*α*-diaryl and *N*-aryl-*α*-heteroaryl nitrones (**17**) in a 6:1 to 32:1 *trans* selectivity and in 70–93% *ee* (**Scheme 3.2.7**), proved to be usefully complementary to the *exo* selective Binol-AlMe3 method, previously reported.[19]

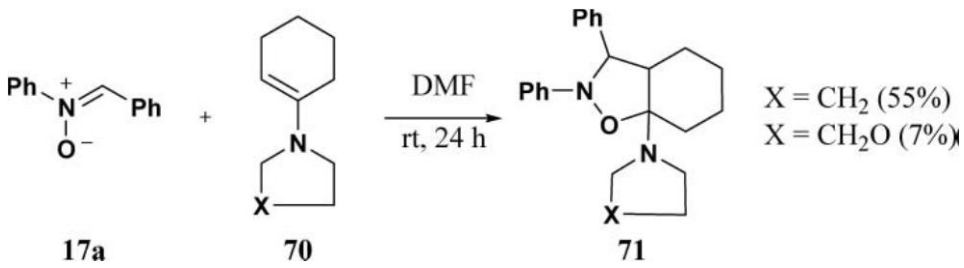


Scheme 3.2.7: *stereoselective 1, 3-DC using Binol-AlMe$_3$ method*

II Aza substituted alkenes

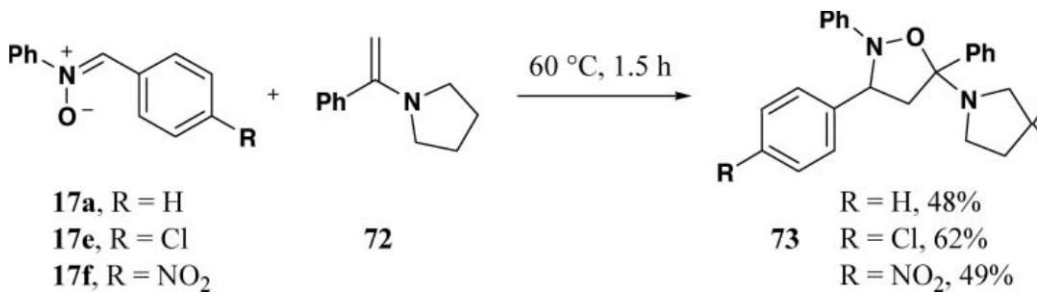
3.3 Enamines as Dipolarophile

Although the cycloaddition of nitrones with enamines has been studied for more than forty years beginning with the pioneering work of Japanese chemists, [23-25] the *cis-trans* geometry of the obtained adducts was not established and remains unknown until now. The high reactivity of such dipolarophiles is illustrated in the cycloaddition reaction of *N*, *α*-diphenylnitrone **17a** with enamines **70** derived from cyclohexanone and pyrrolidine or morpholine which proceeds even at room temperature (**Scheme 3.3**).[23]



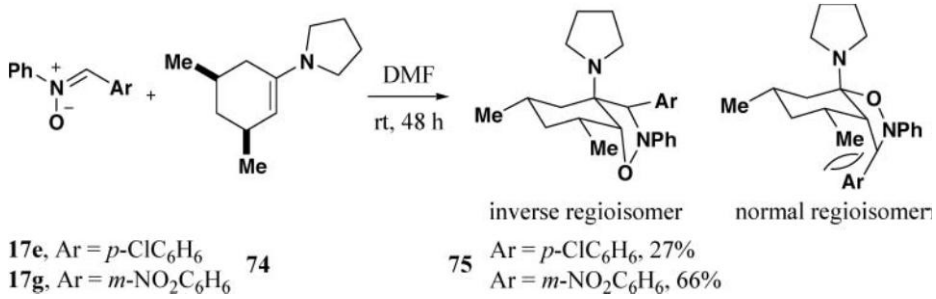
Scheme 3.3: *cycloaddition of nitrone with enamines*

When 1-phenyl-substituted enamines **72** were employed as dipolarophiles, thermal conditions are necessary to achieve good conversions (**Scheme 3.3.1**).[24]



Scheme 3.3.1: *1, 3-DC of nitrone and enamines as dipolarophiles in thermal condition*

The cycloaddition of nitrones **17e, g** with 1-pyrrolidino-*cis*-3, 5-dimethylcyclohexene **74** at room temperature in DMF provided adducts **75** with the inverse regioselectivity (**Scheme 3.3.2**).[25]



Scheme 3.3.2: *regioselective 1, 3-DC of nitrone and enamines in presence of DMF*

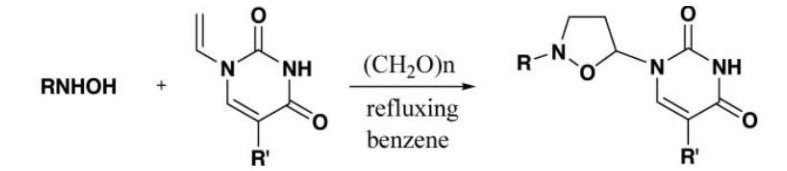
In this case, the regioselectivity is controlled by the 3-methyl group. The transition state leading to the formation of the "normal" regioisomer suffers in this case from severe destabilizing interactions between non-bonded *C*-aryl and methyl group. Moreover, this unfavourable interaction in the corresponding adduct lowers its stability. The formation of reverse-oriented adducts which are free from this type of interaction is kinetically and thermodynamically favoured.

3.4 Enamides as Dipolarophile

3.4.1 N-Vinylnucleobases

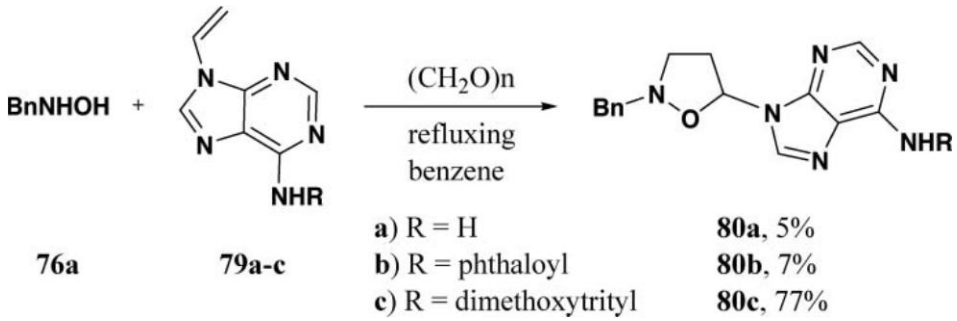
Modified nucleoside analogues inhibit viral polymerases by acting as DNA/RNA chain terminators or as competitive inhibitors.[26] In this context, interesting biological results have been observed by using nucleosides analogues in which the carbohydrate moiety is replaced by an isoxazolidine nucleus. The 1, 3-dipolar cycloaddition methodology between nitrones and vinyl-nucleobases is therefore important to achieve a straight forward

procedure and provide a very useful route to this type of modified nucleosides. As model reaction, the cycloaddition carried out with nitrones (formed *in situ* by the action of hydroxylamines **76** on paraformaldehyde) and vinylthymine **77a** or vinyluracil **77b** (**Scheme 3.4.1**) proceeded under mild thermal conditions in good yields.[27, 28]



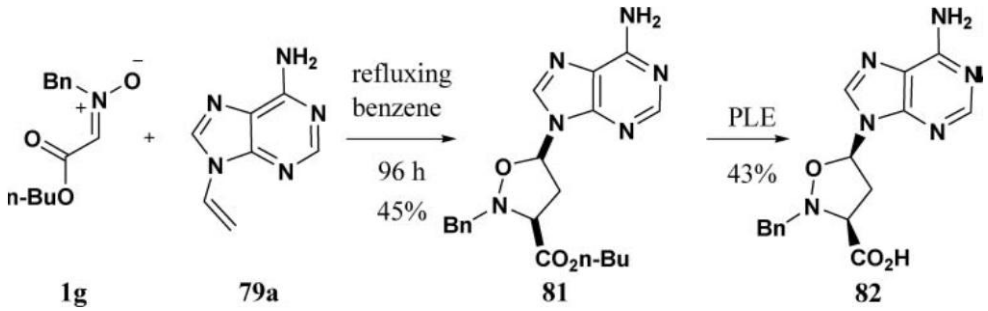
Scheme 3.4.1: *1, 3-DC of nitrone and vinylnucleobase in thermal condition*

The same reaction failed with *N*-9 unprotected adenine derivative **79a**, due to the formation of the side product *N*-9-hydroxymethyl as a result of the interaction of purine bases with formaldehyde. Employing a base labile phthaloyl protecting group (**79b**) was shown to be ineffective because of the side reaction between this protection and the hydroxylamine group. In contrast to these protecting groups, *N*-9 dimethoxytrityl (**79c**) was a more robust protection under these conditions and allowed 1,3-DC reaction to be achieved in good yield (**Scheme 3.4.2**).[29]



Scheme 3.4.2: *1, 3-DC of nitrone and vinylnucleobase in thermal condition using different protecting group*

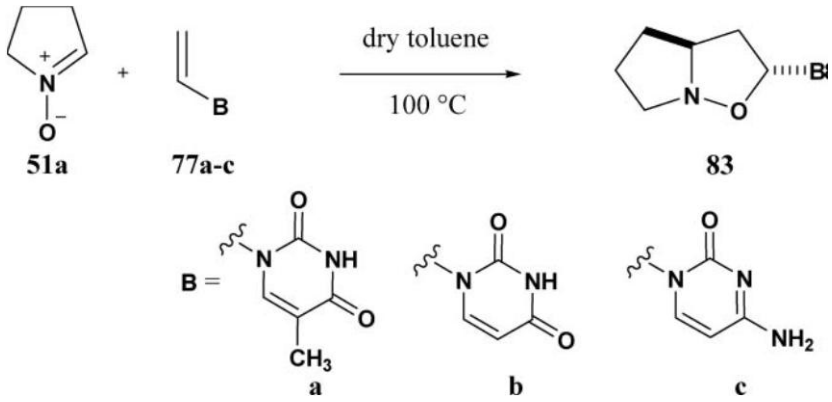
The cycloaddition of ester nitrone **1g** with *N*-9-vinyladenine **79a** is an efficient method to synthesize 4'-aza-2',3'-dideoxyadenosine which could exhibit antiviral activities (**Scheme 3.4.3**).[29] This reaction in refluxing benzene provided *cis*-adduct **81** arising from an *endo* attack of the dipolarophile on the *E*-nitrone. In this approach, the presence of the purine ring provides a high degree of *endo* selectivity, probably arising from strongly favoured secondary orbital overlap between the nitrone phenyl group and purine nucleus. The kinetic resolution of this adduct was achieved by enzymatic hydrolysis of the ester function in the presence of PLE.



Scheme 3.4.3: *An efficient method to synthesize 4-aza-2, 3-dideoxyadenosine*

In order to rigidify the structure of modified nucleosides, the use of a cyclic nitrone such as 3, 4-dihydro-2*H*-pyrrole-1-oxide **51a** has been attempted.[30] Its cycloaddition with vinylnucleobases **77a-c** was found to be highly *exo*-selective and afforded the corresponding *trans* adducts **83** in good to excellent yields (**Table 8**).

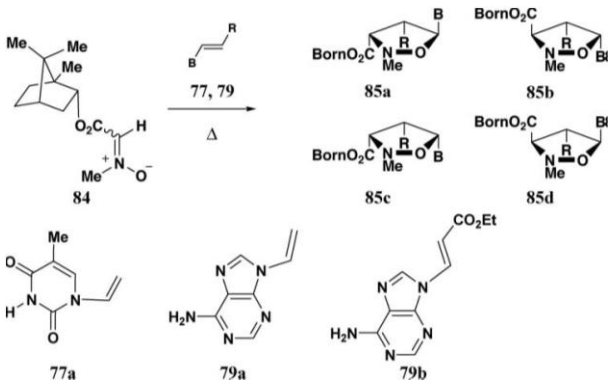
Table 8 Thermal Cycloadditions between Cyclic Nitrone **51a** and Vinylbases **77**



Entry	Nucleobase	Reaction time	Adduct	Yield (%)	*cis:trans*
1	thymine **77a**	6h	**83a**	95	98:02
2	uracil **77b**	17 h	**83b**	65	90:10
3	cytosine **77c**	20 h	**83c**	80	98:02

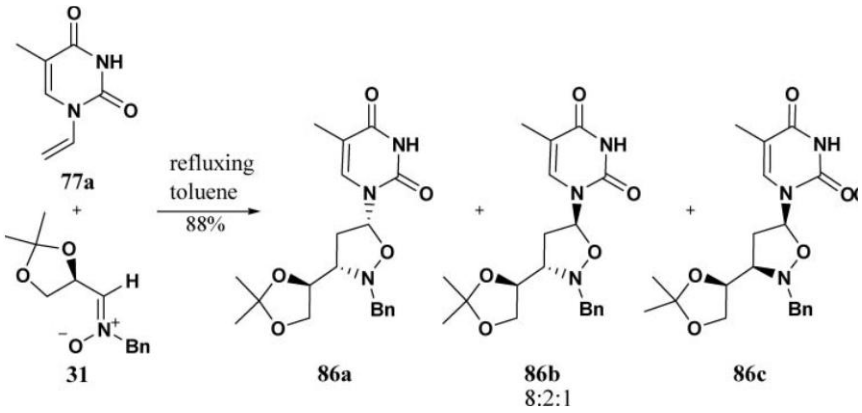
When the chiral nitrone **84** derived from borneol was used as dipole in cycloaddition towards vinylnucleobases a good degree of asymmetric induction was observed (**Table 9**).

Table 9 Thermal Cycloadditions between Chiral Nitrone **84** and Vinylnucleobases



Entry	Nucleobase	Yield (%)	Ratio (a:b:c:d)
1	**77a**	90	78:8:14:0
2	**79a**	88	0:91:0:9
3	**79b**	84	100:0:0:0

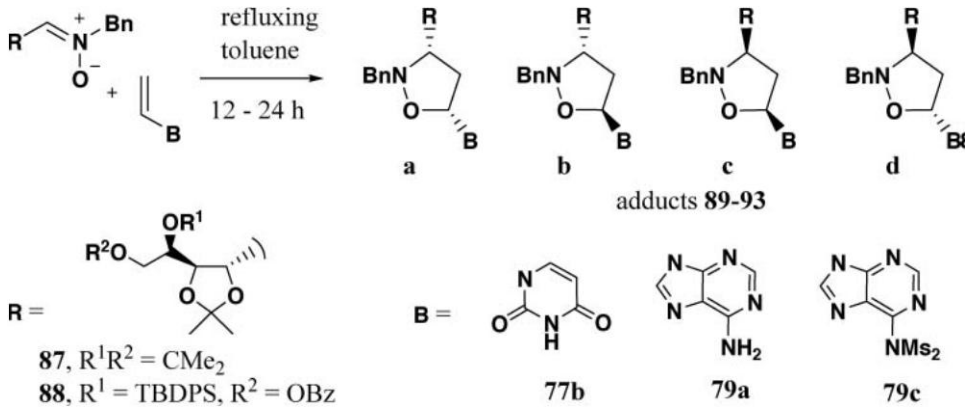
Nitrone **84** (as 3:1 *E*:*Z* mixture) reacted with purine and pyrimidine *N*-vinylnucleobases **77, 79** to furnish the adducts **85** with *trans* (>72% *de*) and facial (>82% *de*) selectivities. Interestingly, in the case of acrylate **79b**, a single diastereoisomer was obtained in good yield (84%). The cycloaddition of nitrone **31** derived from 2,3-*O*-isopropylidene-d-glyceraldehyde and vinylthymine **77a** in refluxing toluene gives a separable 8:2:1 mixture of three adducts **86a-c** in 88% combined yield (**Scheme 3.4.4**).[31] This method provides an alternative approach to modified nucleosides **86** which can be obtained by a two-step sequence: (a) cycloaddition to vinyl acetate, (b) V¨orbruggen nucleosidation using silylated bases.



Scheme 3.4.4: *The cycloaddition of nitrone **31** derived from 2,3-O-isopropylidene-d-glyceraldehyde and vinylthymine in refluxing toluene*

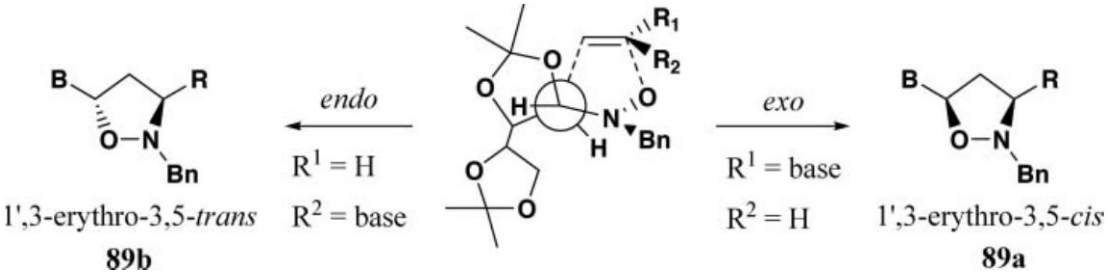
The same strategy applied to two differently protected nitrones **87** and **88** derived from d-xylose resulted in the formation of the four possible adducts with moderate facial (73:27 to 86:14) and *cis*:*trans* (59:41 to 82:18) selectivities (**Table 10**).[32]

Table 10 Cycloadditions of nitrones from d-xylose with vinylnucleobases



	Nitrone	Vinyl base	Yield (%)	Adduct	a:b:c:d	cis:trans	selectivity
1	87	77b	75	**89**	63:17:15:5	78:22	80:20
2	87	79a	86	**90**	39:34:20:7	59:41	73:27
3	87	79c	83	**91**	73:13:9:5	82:18	86:14
4	88	77b	77	**92**	59:24:11:6	70:30	83:17
5	88	79a	95	**93**	45:30:19:6	64:36	75:25

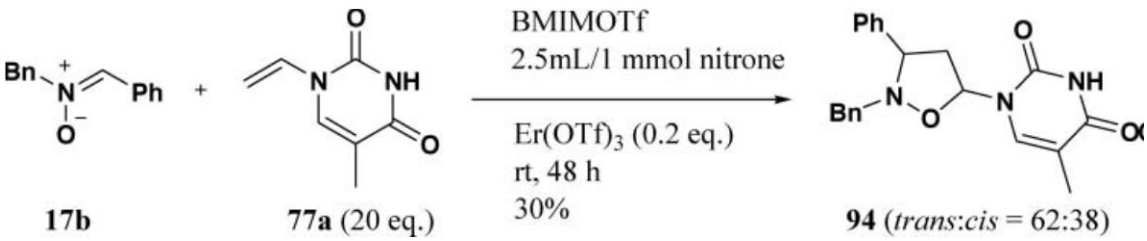
In the case of adenine nucleobases **79**, these selectivities depend not only on C-3 and C-4 protection of the nitrone, but also on *N*-9 protection of the dipolarophile. In the case of the uracil nucleobase **77b**, the two major adducts could arise from an *exo* approach for **89a** and an *endo* approach for **89b** on the less hindered *Si* face of the *Z*-nitrone (**Scheme 3.4.5**)



Scheme 3.4.5: *exo and endo approachon the less hindered Si face of Z- nitrone*

As an effort to avoid drastic thermal cycloaddition conditions, the use of a lanthanide Lewis acid catalyst $Er(OTf)_3$ in an ionic liquid medium

(butylmethylimidazolium triflate BMIM(OTf) proved efficient to promote the reaction, even at room temperature (**Scheme 3.4.6**).[33].

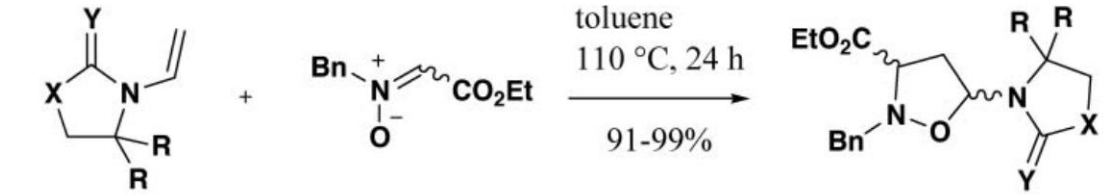


Scheme 3.4.6: *1, 3- DC using Lewis acid in an ionic liquid*

However, some drawbacks of this method (large amounts of dipolarophile, ionic liquid and catalyst required, low conversion) limit its application.

3.4.2 Simple *N*-vinylamides and hetero derivatives

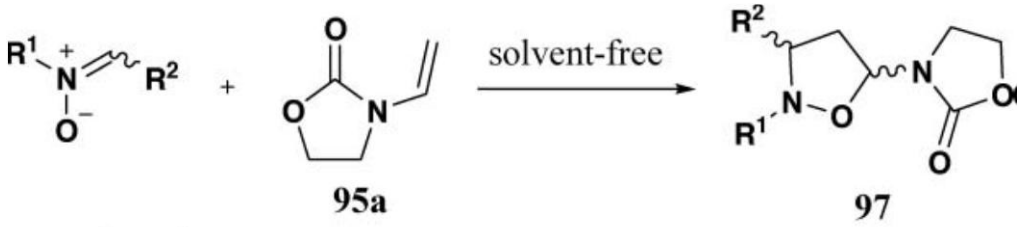
In 2006, the first use of *N*-vinyloxazolidin-2-ones as dipolarophiles in nitrone 1,3-dipolar cycloaddition was disclosed.[34] This type of dipolarophile, readily prepared from the corresponding oxazolidin-2-ones,[35] in one step *via* copper-catalyzed coupling or *via* condensation with aldehydes/ketones,[36,37] exhibits a moderate reactivity under classical conditions compared to alkyl vinyl ethers. Unsuccessful under Lewis-acid catalyzed conditions, the reaction of *N*-vinyloxazolidin-2-one **95a** and analogues with ester nitrone **1a** gave good isolated yields of two adducts **96** but with low-to-moderate *cis-trans* selectivity in refluxing toluene (**Scheme 3.4.7**).[34]



X = O, NMe, CH_2 **1a** **96**
Y = O, S, R = H, Me *trans:cis* 1.4:1 up to 10:1
95a (X = Y = O, R = H)

Scheme 3.4.7: *1, 3 DC refluxing with toluene*

Under these thermal conditions, the *N*-vinyloxazolidinone **95a** exhibited also a good reactivity and a better *cis*-selectivity towards *N*, *α*-diphenylnitrone **17a** (**Scheme 3.4.8**).



95a **97**

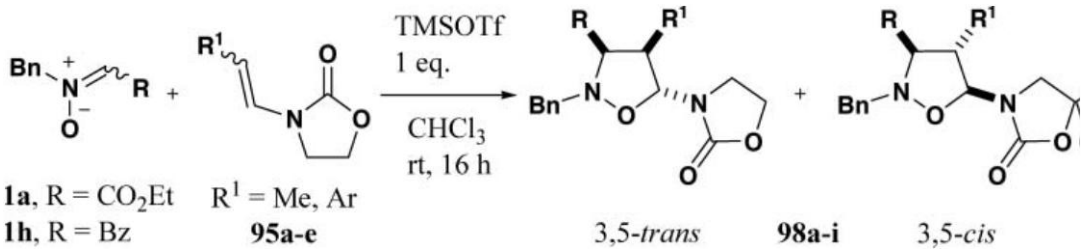
17a, $R^1 = R^2$ = Ph, 30 min at 110 °C, 89%, *cis*:trans = 8:1
17h, R^1 = Bn, R^2 = Ar, 2 h - 72 h at 110-160 °C, *cis:trans* = 1:1 - 4-1

Scheme 3.4.8: *solvent free 1, 3 DC*

However, disappointing results were observed with *N*-benzyl-*α*-arylnitrones **17h**. In fact, due to low reactivity towards these thermally unstable nitrones, prolonged heating promoted decomposition of the starting materials and adducts **97**.The lack of reactivity of this type of dipolarophile was solved by using solvent free conditions. Without solvent, the cycloaddition could be achieved in shorter reaction times and furnished the adducts in higher yield and nearly unchanged *cis*:*trans* ratio. The reactivity of *N*-vinyloxazolidin-2-one is significantly reduced by steric hindrance at the *β*-position of the double bond: *β*-methyl derivative reacted

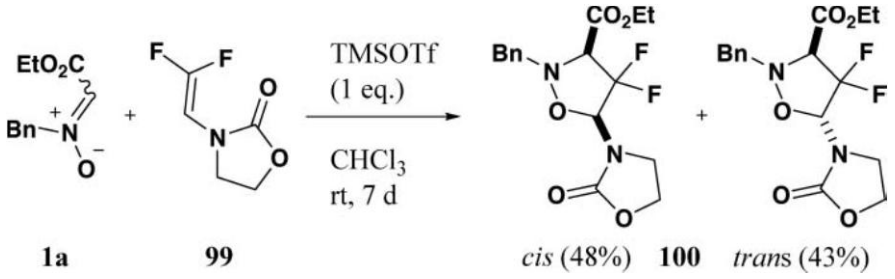
sluggishly and even under more drastic heating conditions, *β*, *β*-disubstituted- or *β*-aryl-*N*-vinyloxazolidin-2-ones failed to react with ester nitrone **1a**. To overcome this drawback, the use of TMSOTf as activating reagent has been proposed.[37] After prior *O*-silylation of the nitrone, which would enhance the electropositivity of the *α*-carbon, the 1, 3-DC reaction is assumed to follow a stepwise Mannich-type mechanism in which the nucleophilic attack of the *β*-substituted-*N*-vinyloxazolidin-2-one **95** on iminium intermediate would be less sensitive to steric hindrance than in a concerted process. Although the reaction could be carried out with a sub-stoichiometric amount of TMSOTf, using one equivalent of this reagent ensured good conversions and provided desired 4-substituted adducts **98** in high to excellent yields (**Table 11**). This modification could be also applied to thermally unstable *α*-benzoylnitrone 1h with success.

Table 11 Cycloaddition Reactions Involving *β*-Substituted Vinyloxazolidinones



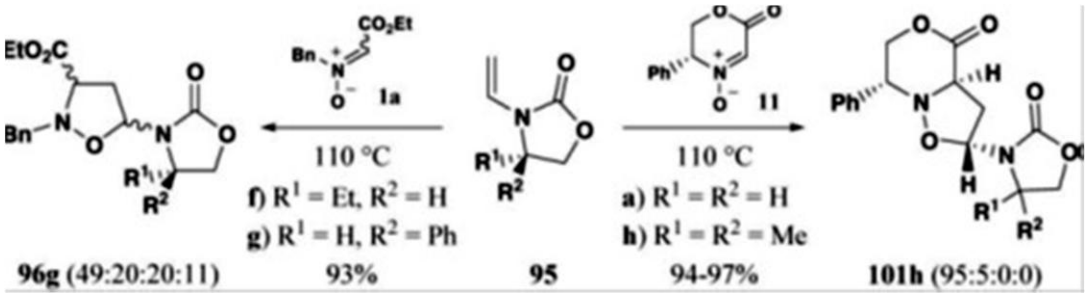
Entry	Nitrone	Enamide	R'	Total yield (%)	Adduct	cis:trans
1	1a	***95b***	Me	90	**98a**	1:4:1
2	1a	***Z-95c***	Ph	93	**98b**	1:2:3
3	1a	***E-95c***	Ph	75	**98c**	2:8:1
4	1a	***Z-95d***	4-$O_2NC_6H_4$	92	**98d**	1:1:5
5	1a	***E-95d***	4-$O_2NC_6H_4$	95	**98e**	2:4:1
6	1a	***E-95e***	4-$MeOC_6H_4$	89	**98f**	1:8:1
7	1h	***95a***	H	88	**98g**	2:5:1
8	1h	***E-95c***	Ph	80	**98h**	*<1:4*
9	1h	***Z-95c***	Ph	76	**98i**	*<1:4*

This study was extended to *N*-(*β*, *β*-difluorovinyl) oxazolidin-2-one **99**. Despite the low reactivity (reaction in seven days at RT in the presence of TMSOTf) and the low selectivity observed with this new dipolarophile, the cycloadduct **100** was obtained with an excellent global yield (**Scheme 3.4.9**).[38]



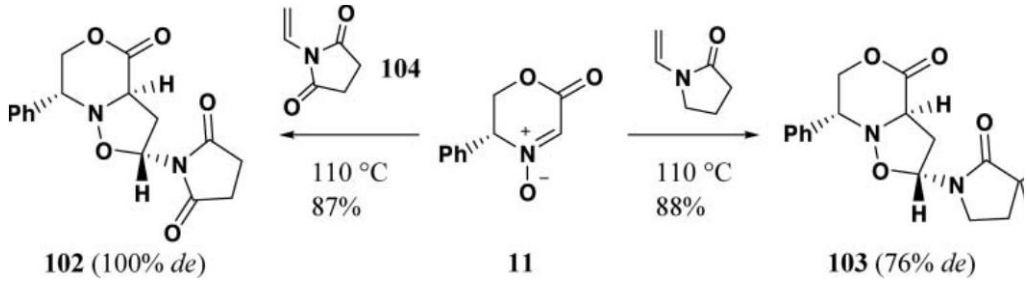
Scheme 3.4.9: *Good yielded 1, 3 DC between nitrone and N-(β, β-difluorovinyl) oxazolidin-2-one in presence of TMSOTf*

An asymmetric version involving *N*-vinyl 4-substituted oxazolidinones **95f, g as** chiral source in the cycloaddition with ester nitrone **1a** was also investigated by the same authors (**Scheme 3.4.10**). This reaction resulted in low *trans*:*cis* (~7:3) and facial selectivities (<7:3), possibly due to the variable configuration of the ester nitrone and the flexible conformation of the dipolarophile.[36,39] Similarly, 1,3-DC between *β*,*β*-difluorinated analog of **95g** with nitrone **1a** led to poor diastereoselectivity (four diastereomers in a 50/30/20/0 ratio).[38]



Scheme 3.4.10: *An asymmetric version involving N-vinyl 4-substituted oxazolidinones as chiral source*

Based upon previous results of Tamura,[40] the use of a geometry-fixed nitrone such as **11** with a more rigid skeleton as dipole proved to be crucial for the stereochemical outcome of the reaction (**Scheme 3.4.10**).[36] The major adducts **101**–**103** obtained in high yields and selectivities arose from an *exo*-approach on the less hindered *β*-face of the nitrone, and the stereoselectivity was culminated with *N*-vinyl succinimide **104** used as dipolarophile (**Scheme 3.4.11**).



Scheme 3.4.11 *stereoselectivity was culminated with N-vinyl succinimide* ***104*** *used as dipolarophile*

3.5 References

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Chapter 4
5-Heterosubstituted Isoxazolidine Ring Opening Methods

4.1 Introduction

Isoxazolidines and 4,5-dihydroisoxazoles are important classes of heterocycles.[1] They possess significant masked functionalities that, on unmasking, give rise to several new functional groups.[2] Most of them are easily prepared by 1,3-dipolar cycloaddition of nitrones and nitrile oxides to olefins.[3,4] Moreover, if stereochemistry can be controlled during the cycloaddition, it can be maintained during the transformation into open-chain compounds.[5] Since common and inexpensive chemicals serve as starting materials and the experimental conditions are simple, these heterocycles can be used as central intermediates in a strategy to prepare complex heteroatom-substituted carbon chains. Optically pure 1,2- and 1,3-aminoalcohols have found wide applications as chiral ligands in asymmetric synthesis.[6] These ligands have primarily been used in enantioselective additions of dialkyl zinc to a,b- unsaturated ketones[7] and for the enantioselective reduction of prochiral ketones.[8,9] Through 1,2-aminoalcohols can be readily obtained by the reduction of commercially available naturally occurring amino acids, 1,3-aminoalcohols usually need to be generated from isoxazolidines by N–O bond cleavage.

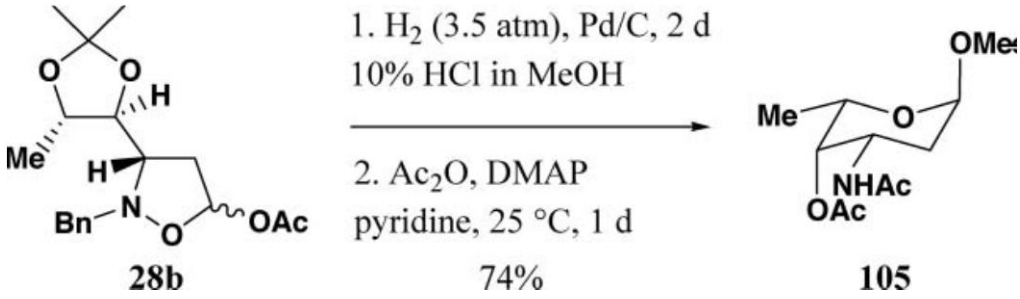
Isoxazolidines are extremely powerful reagents for the construction of nitrogen containing substances.[10] The reductive cleavage of the *N-O* bond releases, in fact, the γ-aminoalcohol functionality which applies to many synthetic strategies. A large number of procedures are found in the

literature to accomplish this fundamental step. The most common include catalytic hydrogenation over Raney-Ni,[11,12] or Pd,[11,13] or reduction by Zn in acetic acid[11,14] or by lithium aluminum hydride.[11,15] As recently transition-metal carbonyls have been used efficiently to reduce closely related isoxazoles,[16] isoxazolines,[17] and 1,2-oxazines,[18] we have now successfully utilized $Mo(CO)_6$ (Molybdenum hexacarbonyl) for the reductive cleavage of isoxazolidines bisphosphonates to afford 1,3- amino alcohol bisphosphonic acids. The *N- O* bond scission of 5-heterosubstituted isoxazolidine can be roughly classified into three categories: reduction, oxidation, and disproportionation methods.

4.2 5-Oxa-substituted Isoxazolidines

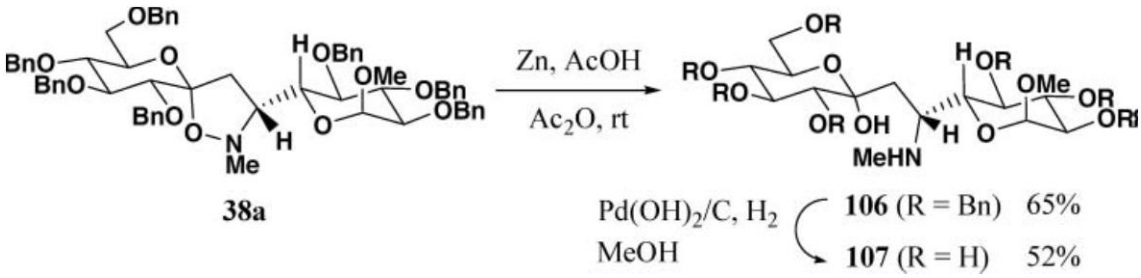
4.2.1 via reduction

Reduction of the *N- O* bond of a 5-oxa-isoxazolidine adduct releases in principle a secondary amine function and a masked aldehyde function (hemiacetal) which could react further together in a hardly controllable way. With the exception of some cases in which one of these functions could be trapped by a reagent in the reaction medium or by other functions of the same molecule, this method was rarely applied to such adducts. De Shong *et al.* disclosed a concise synthesis of amino sugars *via* dipolar cycloaddition (**Scheme 4.2.1**).[19] Adduct **28b** was cleaved by hydrogenolysis in acidic medium. The cyclic product was obtained as a result of the reaction between the hemiacetal function released by *N- O* bond scission and the terminal alcohol function resulting from acetonide removal in methanol solution. Peracetylation of this cyclic product afforded amino sugar **105** as an isolable product in high yield.



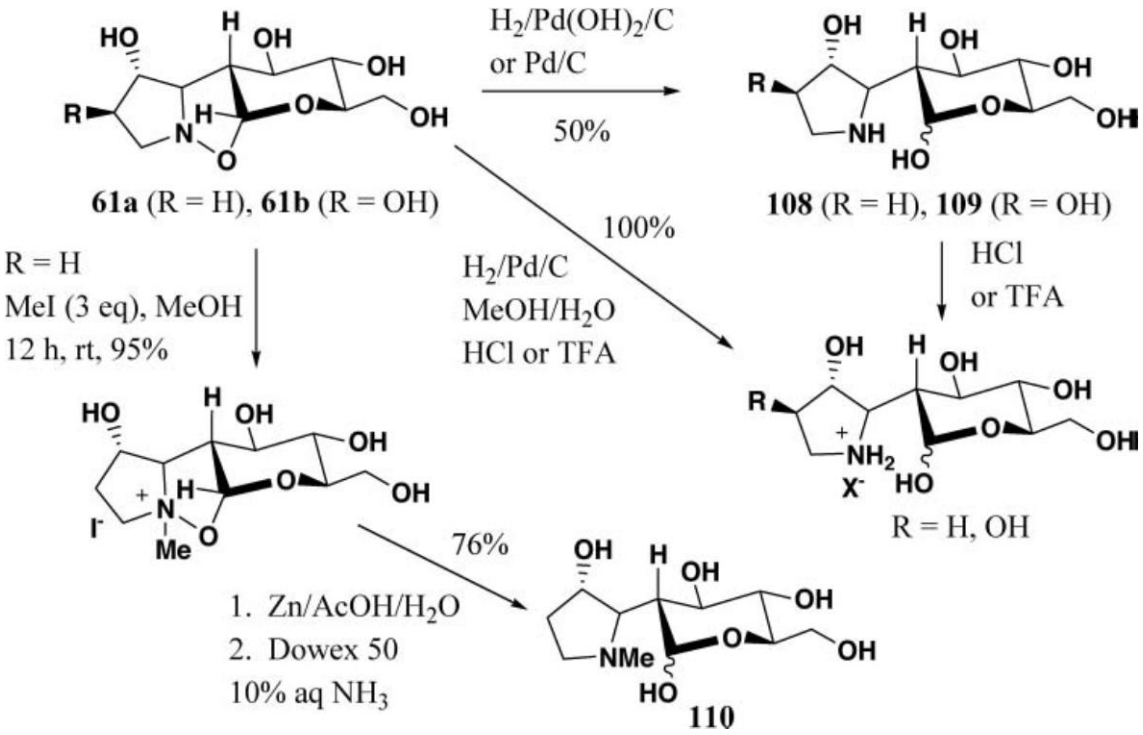
Scheme 4.2.1: *N-O bond cleaved by hydrogenolysis in acidic medium*

Ikegami *et al.* explored the reductive cleavage of the *N- O* bond of adduct **38a** under different conditions (**Scheme 4.2.2**).[20] By exposure to activated zinc powder in AcOH/Ac_2O, **38a** could be converted in the 1, 3-aminoalcohol **106** in 65% yield. The reductive ring opening could be accompanied with debenzylation by catalytic hydrogenolysis conditions. However, the stability of product **107** thus obtained was not mentioned.



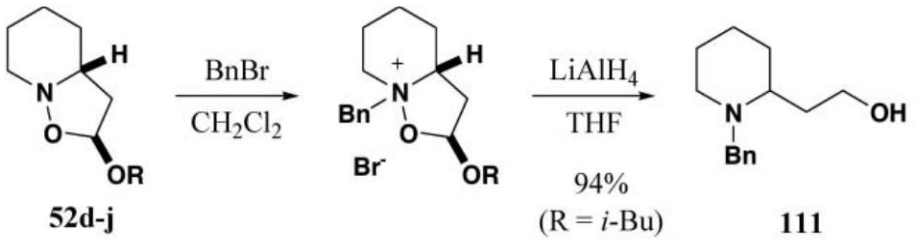
Scheme 4.2.2: *Reductive cleavage of N-O bond in different conditions*

Brandi *et al.* also reported the direct reductive ring-opening of **61a** and **61b** under palladium catalyzed hydrogenolysis conditions, which produced pseudo azasaccharides **108** and **109** in anomeric mixture and moderate yields (**Scheme 4.2.3**).[21,22] This limitation due to the low stability of **108** and **109** was solved by *N*-protonation or by *N*-alkylation before the *N- O* bond scission. The



Scheme 4.2.3: *reductive ring-opening under palladium catalysed hydrogenolysis conditions*

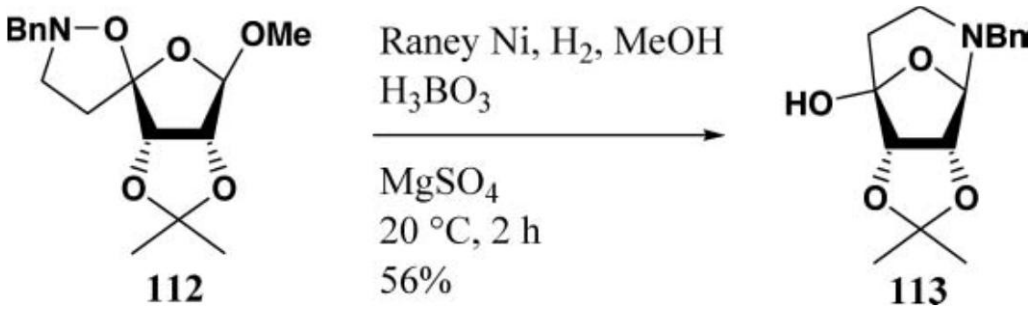
latter provides a more efficient protection as the *N*-methyl group could not react further with the hemiacetal and the product **110** could be obtained in high overall yield. This sequence of alkylation-reduction was also successfully applied to the bicyclic adduct **52** by Carruthers[23, 24] (**Scheme 4.2.4**) by using BnBr as alkylating agent. In this case, the hemiacetal intermediate was *in situ* reduced by $LiAlH_4$ to yield alcohol **111** in excellent yield without debenzylation.



d) R = *i*-Bu; **e)** R = (-)-menthyl; **f)** R = 8-phenylmenthyl; **g)** R = isopinocamphenyll
h) R = (*S*)-MeCHPh; **i)** R = (S)-*i*-Pr-CHPh; **j)** R = (*R*)-*t*-Bu-CHPh

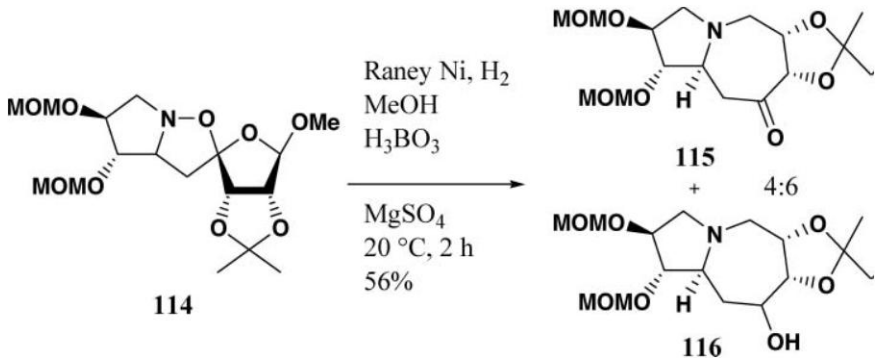
Scheme 4.2.4: *alkylation-reduction sequence in bicyclic compounds*

It has been shown by Gallos *et al*. that the reductive scission of *N- O* bond of **112** could lead to **113** in good yield by utilizing H_2/Ni Raney.[25] *N-O* bond cleavage was accompanied by methanol elimination to form transiently an aminoketoaldehyde, which spontaneously cyclized to yield *N*,*O*-acetal-*O*,*O*-hemiketal **113** (**Scheme 4.2.5**). The same conditions applied to related tricyclic adduct **114** afforded a mixture of ketone **115** and alcohol **116** as a result of two over-reductions: reductive amination of the



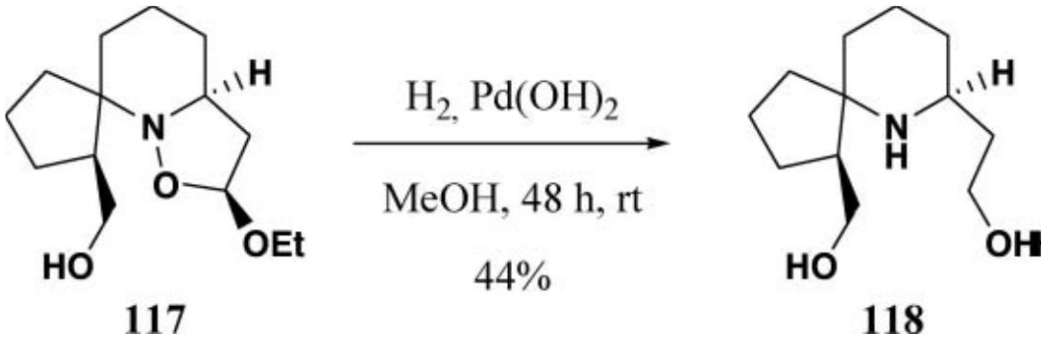
Scheme 4.2.5: *Reductive cleavage of N-O bond using Raney/Ni*

aldehyde-derived carbon center and partial reduction of the ketone-derived carbon center (**Scheme 4.2.6**).*In situ* over-reduction of aldehyde-derived carbon center into alcohol has also been used to avoid undesirable interaction between the amino function and the hemiacetal

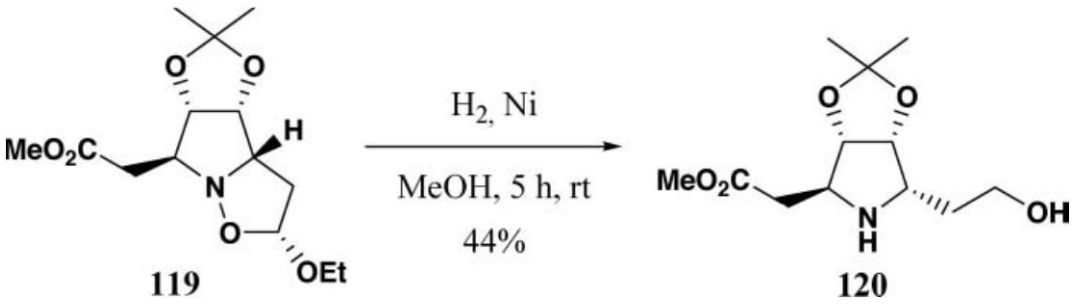


Scheme 4.2.6: *Partially reductive cleavage of keto-derived carbon center*

function as described in the preparation of 1, 3-aminoalcohols **118**[26] (**Scheme 4.2.7**) and **120**[27] (**Scheme 4.2.8**).



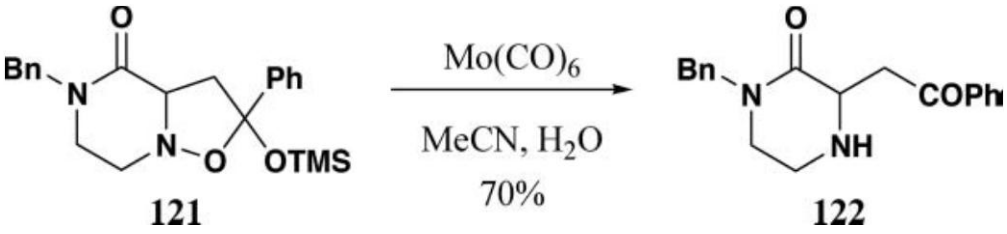
Scheme 4.2.7: *Preparation of 1, 3 aminoalcohol using $Pd(OH)_2$*



Scheme 4.2.8: *Preparation of 1, 3 aminoalcohol using Ni*

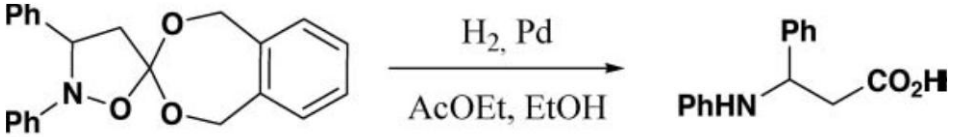
When the masked function of the isoxazolidine is a ketone (5-carba-5-oxa substituted) or an ester/acid (5, 5-dioxa substituted), the *N- O* bond cleavage can readily occur, leading to a stable amino ketone or amino acid. Amino ketone **122**[28] and amino acid derivatives **24**, **124** and **126**[29, 30] could thus be obtained in high yields as exemplified (**Scheme 4.2.9, Scheme 4.2.10 and Scheme 4.2.11**). In the former case, the use of $Mo(CO)_6$ in wet acetonitrile is highly efficient as this reagent could selectively reduce the *N-O* bond under very mild conditions with respect to multifunctionality of

molecules.



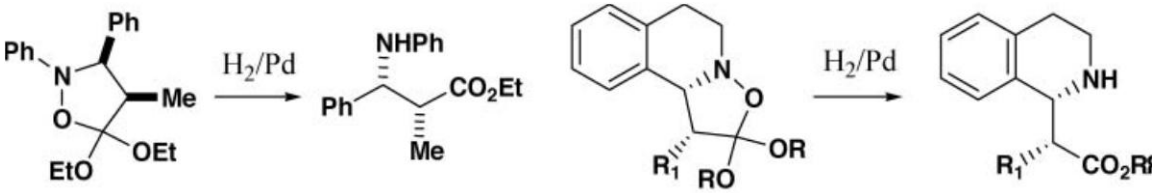
121 122

Scheme 4.2.9: *reduction of N-O bond using $Mo(CO)_6$*



123 124a

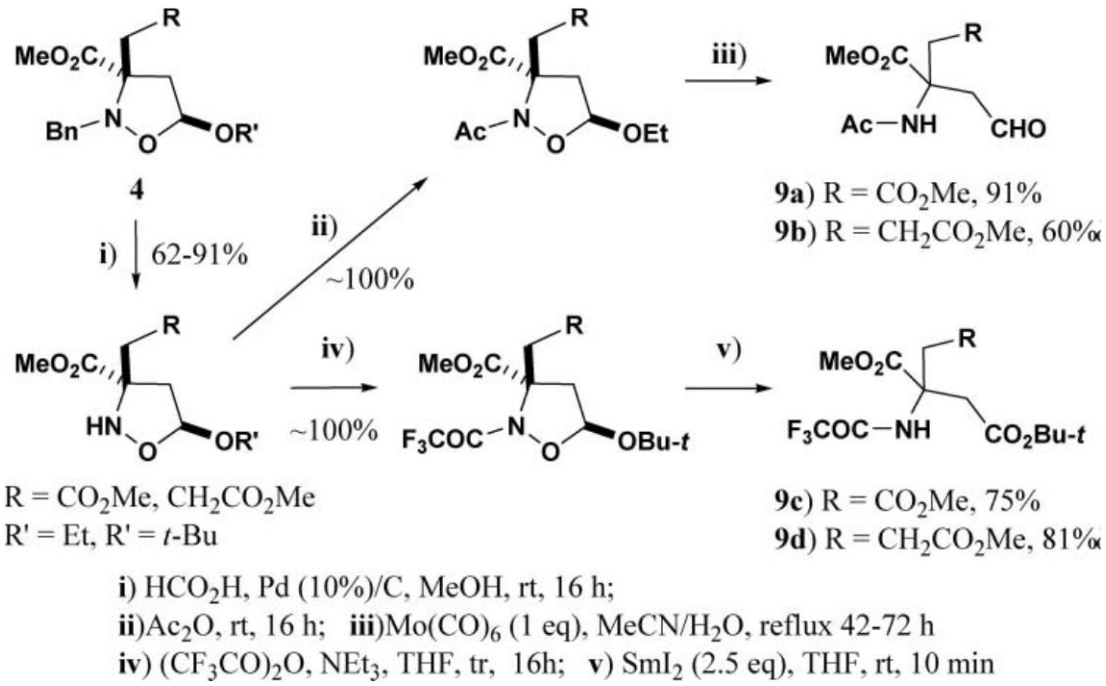
Scheme 4.2.10: *reduction of N-O and deprotection of acetyl in presence of H_2/Pd*



23a 24 125 126

Scheme 4.2.11: *cleavage of N-O bond radially occurs in easter isoxazolidine*

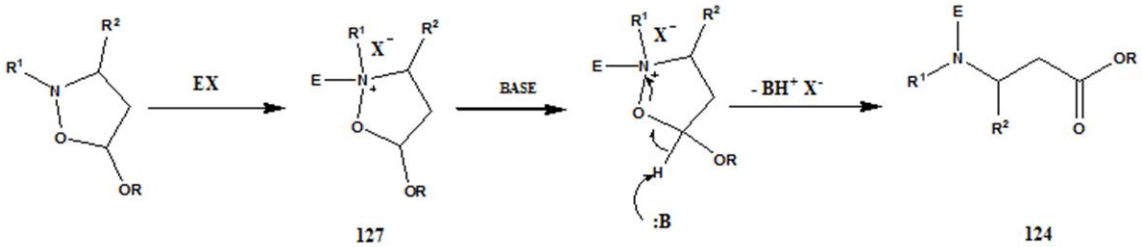
Selective reduction of *N-O* bond with $Mo(CO)_6$ was used to prepare new aldehydic *α*,*α*-disubstituted *α*-aminoacids *N*-acetyl derivatives **9a**,**b** while SmI_2 in THF was efficient to obtain *N*-trifluoroacetyl triester derivatives **9c**,**d** (**Scheme 4.2.12**).[31]



Scheme 4.2.12: *Selective reduction of N-O bond with $Mo(CO)_6$ and SmI_2*

4.2.2 Via disproportionation

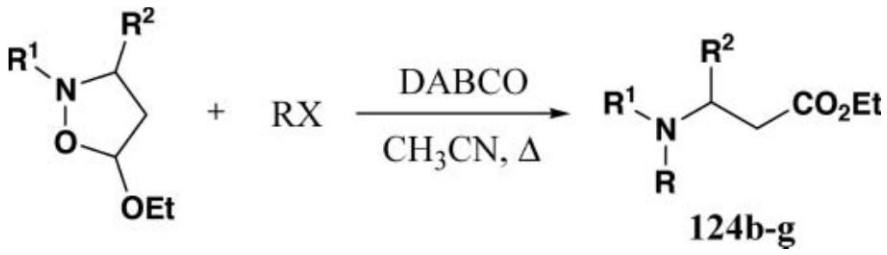
Quarternization of 5-oxa-isoxazolidine adducts with strong alkylating agents (E^+) such as alkyl halides followed by the thermal treatment with an appropriate base were first reported by Murahashi *et al.* to give the corresponding *β*-amino acid esters efficiently[24]. The mechanism of this transformationwas outlined in (**Scheme 4.2.13)**. The reaction of isoxazolidines with electrophiles gives ammonium salts **127**. Deprotonation at the C-5 position of **127** would lead to the ring-opening to give *β*-amino acid esters **124**



Scheme 4.2.13: *Quarternization of 5-oxa-isoxazolidine adducts with strong alkylating agents (E^+)*

As a base, triethylamine and DABCO (**Table 1**) were shown to be highly efficient, in contrast to sodium ethoxide, potassium *t*-butoxide or sodium hydride which could not afford ring-opening products.

Table 1 Disproportionation of Isoxazolidines by *N*-Alkylation in the presence of DABCO



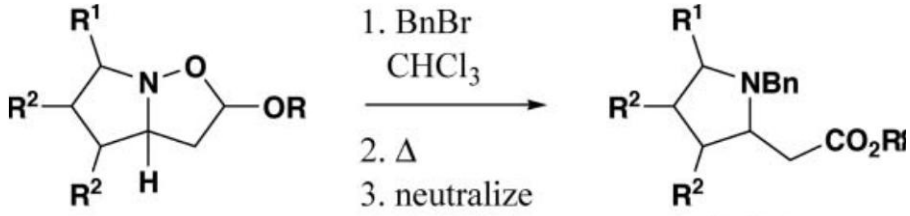
124b-g

Entry	Adducts	R^1	R^2	RX	Amino ester	Yield (%)
1	18i	Et	Me	BnBr	124b	39
2	18c	Bn	Ph	MeI	124c	20
3	52a	$(CH_2)_3$		MeI	124d	40
4	52a	$(CH_2)_3$		CH_2=$CHCH_2Br$	124e	41
5	52c	$(CH_2)_4$		BnBr	124f	42
6	52c	$(CH_2)_4$		(*E*)-PhCH= $CHCH_2Br$	124g	43

More recently, another version of this method was applied successfully on different substrates without using a base as demonstrated in themodification of Bayon,[32] Defoin,[33,34] Meske[35] (**Table 2 and Table 3**), Fisera[36] (**Scheme 4.2.14**) and Jørgensen[37] (**Scheme 4.2.15**). As an alternative for heat sensitive substrates, using alkyl triflates as alkylating agents could avoid prolonged heating and the quaternarization step could

therefore be carried out at low temperature (**Scheme 4.2.16**).[38] Subsequent base-induced ring opening of triflate salts furnished the expected *β*-amino esters **133** in moderate yields.

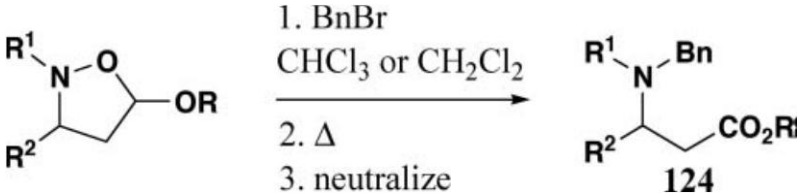
Table 2: Disproportionation of bicyclic Isoxazolidines by *N*-Alkylation promoted by Benzyl Bromide



124h-k

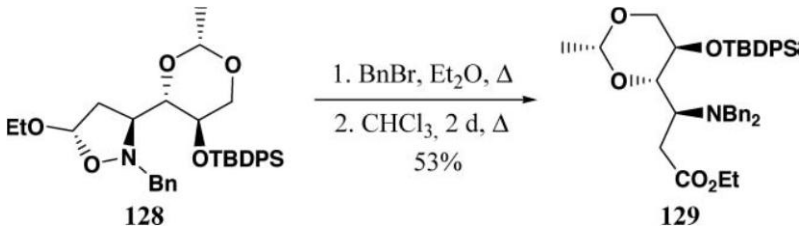
Entry	Adducts	R^1	R^2	R	Aminoester	Yield (%)	Ref
1	52a	H	H	Et	124h	57	32
2	52b	H	H	Ph	124i	78	33
3	56	H	OMOM	Ph	124j	quant.	33
4	58	Me	acetonid	Ph	124k	quant.	34

Table 3: Disproportionation of Isoxazolidines by *N*-Alkylation promoted by Benzyl Bromide

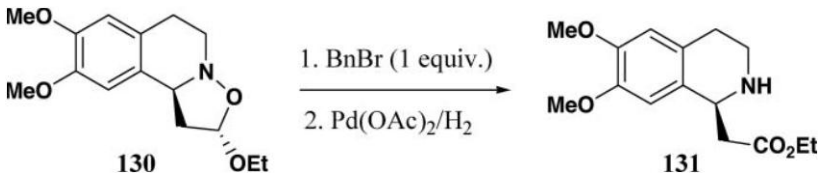


124

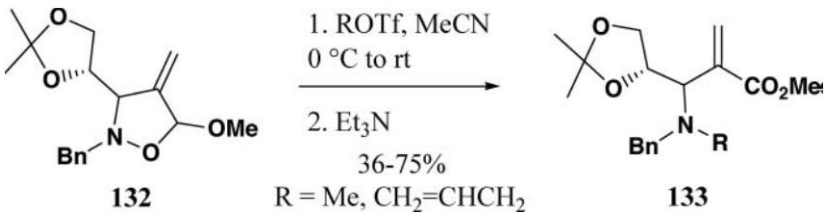
Entry	Adducts	R^1	R^2	R	Aminoester	Yield (%)	Ref
1	18j	*n*-Bu	*n*-Pr	Ph	124l	73	33
2	52k	$(CH_2)_4$		Ph	124m	65	33
3	18k	Me	Ph	Et	124c	91	35



Scheme 4.2.14: *N-dibenzylation and then reduction of N-O bond*



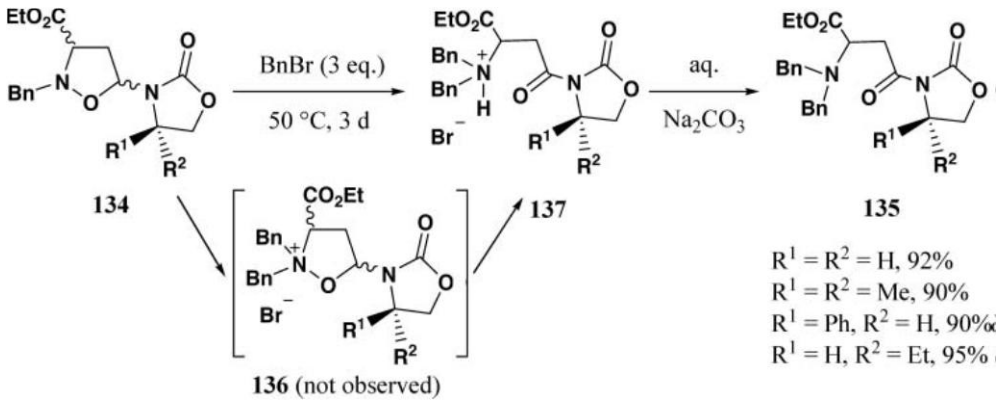
Scheme 4.2.15: *N- reduction of N-O bond using palladium acetate in presence of hydrogen*



Scheme 4.2.16: *reduction of N-O bond in presence of different substituents*

4.3 5-Aza-substituted Isoxazolidines

Similar limitations encountered with 5-oxa isoxazolidines could be envisioned with 5-aza adducts. The only example to date of such a recently reported transformation, relies on disproportionation pathway (**Scheme 4.3**).[39]



Scheme 4.3: *disproportionation pathway In the presence of benzylbromide in excess without solvent*

5-oxazolidinyl adducts **134** were converted cleanly into the aspartimides **135** which are valuable precursors for carboxy-differentiated aspartates. The reaction proceeds *via* intermediate quaternary isoxazolidiniums **136** of low thermal stability, which transformed spontaneously into the ring-opened products **137**. Subsequent treatment with a slightly basic aqueous solution provided the desired products **135** in excellent yields.

4.4 References

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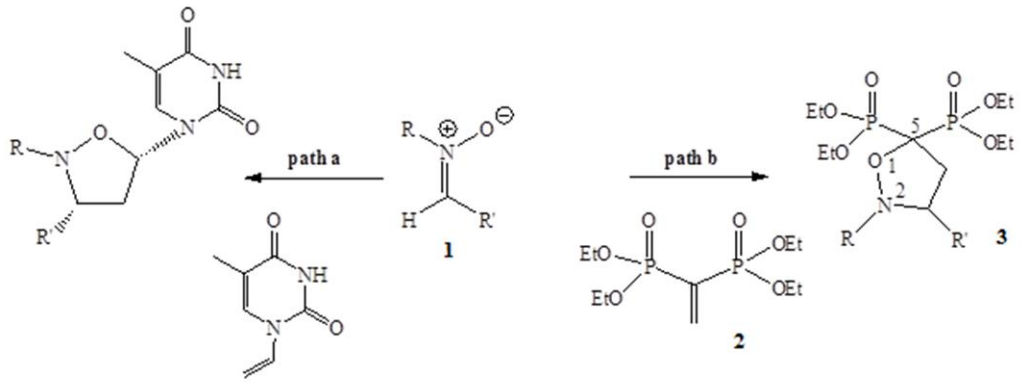
Chapter 5
Result and discussion

5.1 INTRODUCTION

Different synthetic strategies have been proposed to insert heterocyclic rings directly on the germinal position, including the reaction of phosphorus electrophiles with enolates,[1] the reaction with diazo compounds,[2] and the condensation of oxaphospholenes with isocyanates.[3] During last year's we have developed an efficient method for the preparation of substituted isoxazolidines using the classical 1,3-dipolar cycloaddition of an alkene as dipolarophile with a suitable nitrone, under microwave irradiation, in the absence of solvent, **Scheme 5.1**.[4,5] Following this strategy it was possible to prepare several *N,O*-nucleosides by reaction of nitrones with unprotected vinylated nucleobases, as shown in path ***a*** of the **Scheme 5.1**. In my PhD credentials, we have performed 1,3-dipolar cycloaddition using vinyl nucleobases and vinyl bisphosphonates as dipolarophile for the preparation of isoxazolidine-substituted nucleobases path ***a*** and bisphosphonates, path ***b*** of **Scheme 1**, maintaining in the same time the microwaves catalysis in the absence of solvent successfully tested in previous studies.

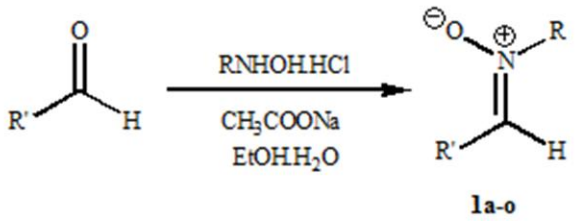


Scheme 5.1. *Synthesis of substituted isoxazolidines via 1, 3-dipolar cycloaddition of nitrones and substituted alkenes: vinylnucleobases to N, O-nucleosides,* ***path a****, vinylbisphosphonates to isoxazolidine bisphosphonates,* ***path b****.*

A laboratory scale preparation of new class 1, 3 amino alcohol bisphosphonic acids described in present work. Which compounds are resembled on currently available biological active bisphosphonates (Aldronate, pamidronate, residronate and zolodranate). This synthesis carried out in three stages.

5.1.1 Synthesis of Nitrones

In the first part of my curriculum, synthesis of several nitrones and a tetraethylvinylidene-1, 1-bisphosphonate was argument. According to aim, although, there are different methods for synthesis of compound **1 (scheme 5.1.1)**, described in the literature by several authors. We have partially modified the procedures with the result of a substantial increase in the yields of both derivatives.



1a-o

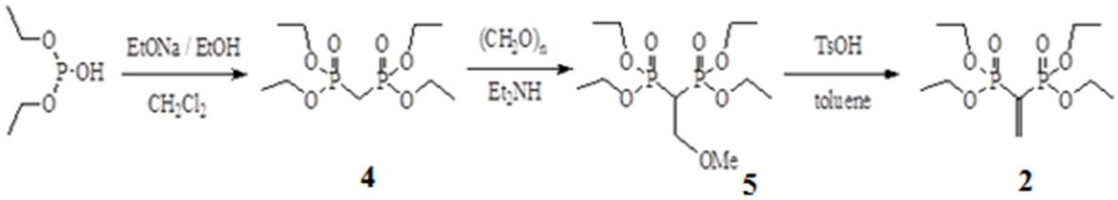
R=Me, R'=Ph	**1a**
R=Me, R'=*o*-Cl-C_6H_4	**1b**
R=Me, R'=3-pyridyl	**1c**
R=Me, R'=2-furyl	**1d**
R=Bn, R'=*p*-OH-C_6H_4	**1e**
R=Bn, R'=Ph	**1f**
R=Bn, R'=*o*-Cl-C_6H_4	**1g**
R=Bn, R'=*o*-F-C_6H_4	**1h**
R=Me, R'=*p*-Cl-C_6H_4	**1i**
R=Me, R'=naphtyl	**1j**
R=Bn, R'=*p*-Cl-C_6H_4	**1k**
R=Bn, R'=naphtyl	**1l**
R=Bn, R'=3-pyridyl	**1m**
R=Bn, R'=2-furyl	**1n**
R=Bn, R'=*p*-OMe-C_6H_4	**1o**

Scheme 5.1.1. *Synthesis of alkyl and aryl substituted nitrones* ***1a-o***

Nitrones **1a-o** were obtained in excellent yields and high purity by condensation of the precursor aldehydes with *N*-methyl- or *N*-benzylhydroxyl amine in presence of sodium acetate and sodium bicarbonate, water-ethanol solution. This synthetic conversation took place in approx. 5 min, without further purification we can isolate high yielded nitrone.

5.1.2 Synthesis of tetraethylvinylidene-1, 1-bisphosphonate

In second part, we started to synthesis commercially available but economically very costly tetraethylvinylidene-1,1-bisphosphonate **2** and was successfully prepared by direct reaction of diethylphosphite in the presence of freshly prepared sodium ethoxide and dry dichloromethane at ambient temperature (**scheme 5.1.2)**.



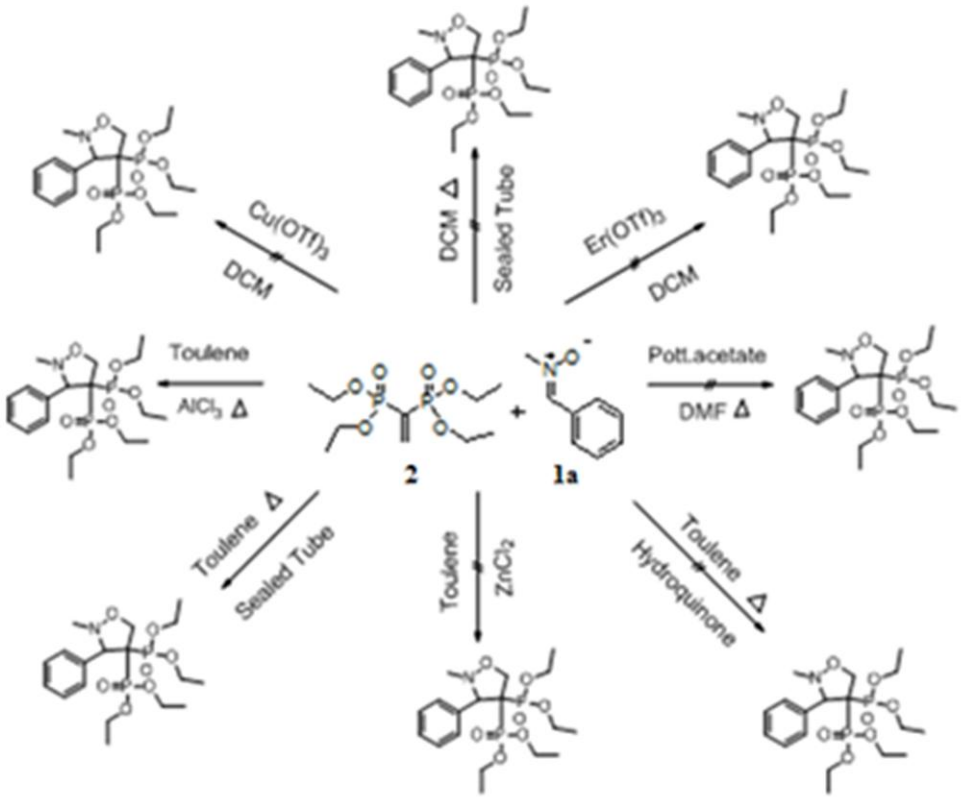
Scheme 5.1.2. *Synthesis of tetraethylvinylidene-1,1-bisphosphonate*

This method is simple and convenient in comparison with other methods reported in literatures. The long reaction times (2 months) are balanced by the low cost of the reagents and work-up. Furthermore, derivative **5** was obtained from **4** by reaction with paraformaldehyde and diethyl amine at refluxed condition for 24 h. In particular, for this reaction you should maintain basic condition up to compilation of reaction. Finally, compound **5** was productively converted to tetraethylvinylidene-1, 1-bisphosphonate **2**

by reaction with catalytic amount of *para*-toluensulfonic acid in toluene at refluxed temperature**.**

5.1.3 Performed reactions to obtain 1, 3 dipolar cycloadduct

With the set of nitrones **1a-l** in our hands we initially examined the 1,3-dipolar cycloadditions with **2** in conventional conditions, that is in organic solvent (toluene, dichloromethane), by heating (from 0°C up to refluxing toluene) and in the presence of a Lewis acid ($ZnCl_2$, $AlCl_3$, $Er(OTf)_3$, $Cu(OTf)_3$) as catalyst, **Scheme 5.1.3**.



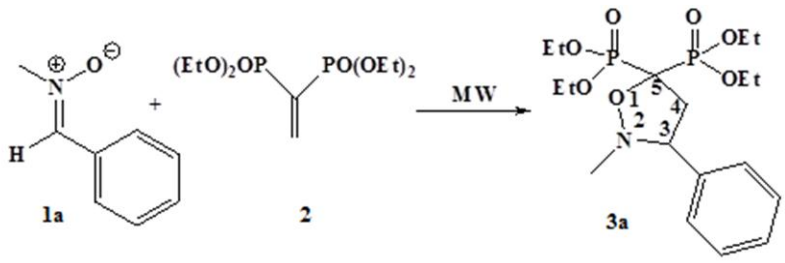
Scheme 5.1.3. *Synthesis of cycloadduct by various classical conditions*

What we observed that, the reaction with lewis acid in heating conditions, we could not able to get high yield. Besides, there are more chances to get mixture of rgeioisomers. Moreover, this method is time consuming and very difficult to purify product through column chromatography. Apart

from that, we could not isolate cycloadduct product in Lewis acid catalyzed reaction. In other study, refluxing the reactants in toluene (or heating in toluene solution in a sealed tube at 110ºC–115ºC) for 48 h was not going to completion and yielded two regioisomeric compounds. Much of our subsequent efforts have been devoted to the detailed studies of the cycloaddition of *N*-methyl-*C*-phenyl nitrone **1a** and compound **2.** In the best cases, a conversion of 65-75% is obtained in 14 hours reaction time for a 35% of isolated yield.

5.1.4 Synthesis and regeiochemistry of 1, 3 dipolar cycloadduct

The poor results of earlier reactions prompted us to test different experimental conditions as the use of microwaves catalysis, successfully applied in similar reactions.[4,5] **Table 1** collects the optimization of the reaction parameters as the MW power, the time and the stoichiometric ratio of the diene-dienophile couple, using the *N*-methyl-*C*-phenyl nitrone **1a** as model compound **scheme 5.1.4**.

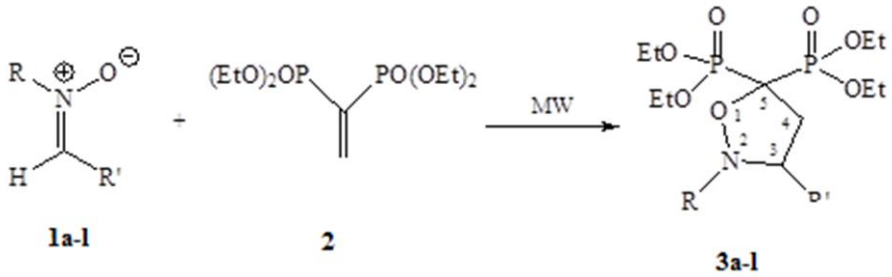


Scheme 5.1.4. 1, 3 *dipolar cycloaddition reaction performed using N-methyl-C-phenyl nitrone as model compound*

Table 1 collects the optimization of the reaction parameters as the MW power

Entry	MW power (W)	Time (min)	Equiv. of **1a**	Conversion (%)	Yield (%)
1	750	5	2	85	10
2	750	8	1.6	90	13
3	600	10	2	84	24
4	400	10	2	88	37
5	400	15	1.5	83	40
6	200	10	1.2	99	75

A low MW power and a slight excess of nitrone are required to complete the cycloaddition with formation of the tetraethyl-2-methyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate in 75% isolated yield, **entry 6 of Table 1**. These conditions were next applied to the entire collection of available nitrones to perform 1,3 dipolar cycloaddition for path ***a*** and ***b*** of **scheme 5.1**. For complete conversion of these reactions, short time, maximum 20 mins were required. In some cases, we observed that isolated yield was lowered by formation of other minor side products. Pertinent result of this reaction with various nitrones in our hand and compound **2 (scheme 5.1.5)**, illustrated in **table 2.**



Scheme 5.1.5. *1,3 dipolar cycloaddition reaction performed using various nitrones1a-l and compound* ***2***

Table 2. Pertinent results of the reaction between various nitrones **1a-l** and compound **2**

Entry	R	R'	Time min	Product	Yield
1	Me	Ph	10	**3a**	75
2	Me	*o*-Cl-C_6H_4	12	**3b**	77
3	Me	3-pyridyl	13	**3c**	73
4	Me	2-furyl	15	**3d**	68
5	Bn	*p*-OH-C_6H_4	14	**3e**	74
6	Bn	Ph	18	**3f**	83
7	Bn	*o*-Cl-C_6H_4	18	**3g**	85
8	Bn	*o*-F-C_6H_4	20	**3h**	86
9	Me	*p*--Cl-C_6H_4	16	**3i**	78
10	Me	naphtyl	14	**3j**	76
11	Bn	*p*--Cl-C_6H_4	18	**3k**	85
12	Bn	naphtyl	16	**3l**	88

The regiochemistry of the reaction followed the usual pattern with exclusive formation of the 3,5-isomer over the 3,4-derivative [6]. Accordingly, the cycloaddition takes place between the nitrone in the more reactive (Z) configuration and the vinylidene bisphosphonate, with attack of the N-oxygen atom on the germinal carbon of the vinylidene group. The regiochemistry has been further confirmed by decoupling ^{1}H NMR experiments using **3g** as model compound. **Fig. 1** shows the expansion of the NMR spectrum in the region 2.5-5.0 ppm where the signals of protons H_{C4} (2.84 ppm), H_{C4}' (3.28 ppm), and H_{C3} (4.66 ppm) are located. **Fig. 1B** refers to absence of irradiation, whereas **Fig. 1A** and **Fig. 1C** describe the

^{1}HNMR spectra upon decoupling at 2.84 and 3.28 ppm, respectively. In the latter cases the signal of H_{C3}, originally a doublet of doublets, is modified to a doublet thus confirming the proximity of H_{C3} to both H_{C4} and H_{C4}'. On the other hand, the multiplicity of the signals pertaining to H_{C4} and H_{C4}', although simplified by this decoupling, necessarily accounts for coupling with phosphorous nuclei and H_{C3}.

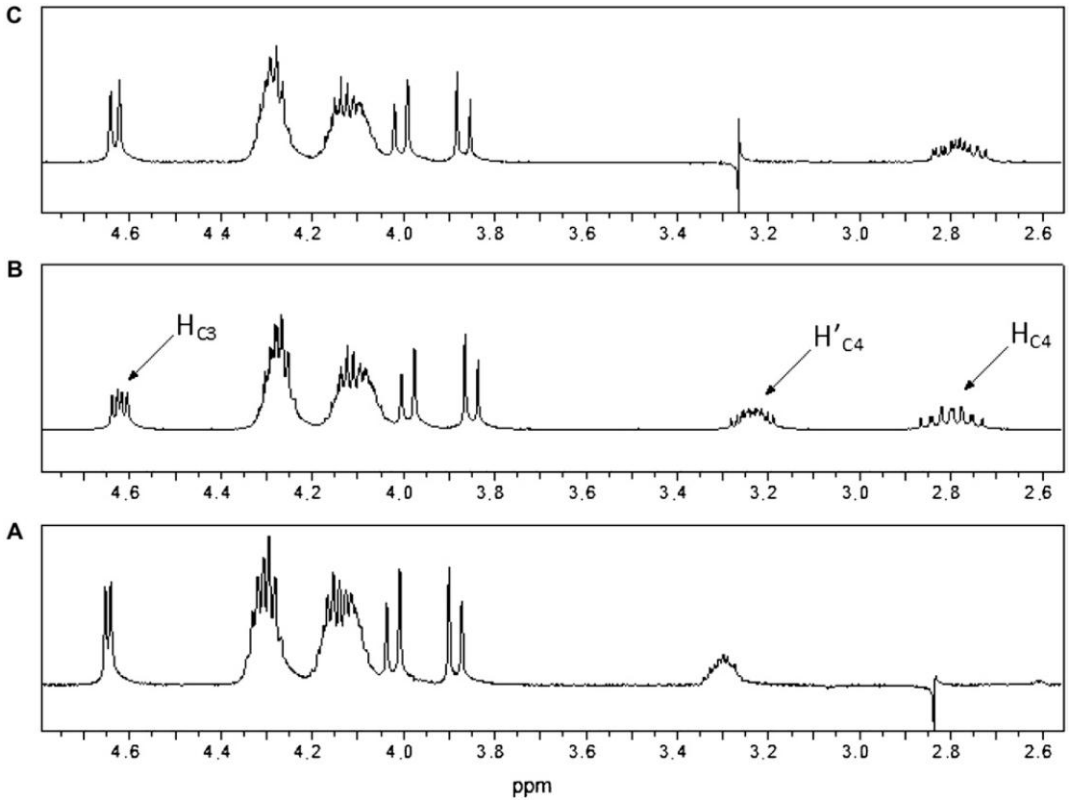
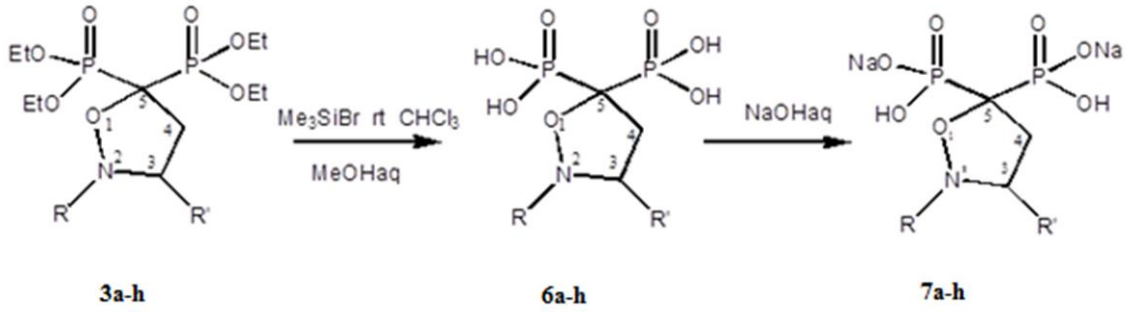


Fig. 1. *Expansion of the ^{1}H NMR spectra of compound 3g in: (B) absence of decoupling, (A) decoupling at 2.84 ppm, and (C) decoupling at 3.28 ppm.*

5.1.5 Hydrolysis of isoxazolidines bisphosphonates and salt formation

In the third part of synthesis, we successfully hydrolysed obtained cycloadduct **(3a-h)** to afford corresponding bisphosphonic acid **(6a-h)** in presence of excess trimethylsilyl bromide in dry dichloromethane followed

by treatment with methanol. The conversion of bisphosphonate esters to their acids is quite efficient in yields in ranging from 82% to 92%. The isoxazolidinyl-substituted bisphosphonic acids transformed into the corresponding disodium salts **(7a-h)** by reaction with exact 2 eq of sodium hydroxide in water (**scheme 5.1.6**). All results during hydrolysis and salt formation reactions collectively mentioned in **table 3**.



Scheme 5.1.6. *Hydrolysis of cycloadducts followed by disodium salt formation*

Table 3 collected results during hydrolysis and salt formation reaction

Entry	R	R′	Product	Yield	Product	Yield
1	Me	Ph	**6a**	89	**7a**	94
2	Me	*o*-Cl-C_6H_4	**6b**	90	**7b**	96
3	Me	3-pyridyl	**6c**	82	**7c**	89
4	Me	2-furyl	**6d**	85	**7d**	92
5	Bn	*p*-OH-C_6H_4	**6e**	83	**7e**	96
6	Bn	Ph	**6f**	90	**7f**	94
7	Bn	*o*-Cl-C_6H_4	**6g**	92	**7g**	95
8	Bn	*o*-F-C_6H_4	**6h**	91	**7h**	96

5.1.6 Biological Activity results

Compound **7a** (Iqbal 53) and **7b** (Iqbal 54) were subjected to carry out biological activity at Rizzoli, Bologna, and more interestingly, these compounds showed similar activity towards hydroxyapatite compare to Neridronate as reference bisphosphonate. Practically, the biological activity of the new compounds has been evaluated in two steps. Firstly, a cytotoxicity screening has been performed in order to define the concentration of compounds, which passed to the following step. The toxicity of compounds was measured by using the neutral-red assay on the L929 cell line exposed to a wide range of drug concentrations.[7] the cytotoxicity screening allowed to establish which compounds and concentrations are suitable for the biological activity evaluation. The neutral-red (NR) assay is a colorimetric test for the quantification of the membrane permeability and lysosomal activity of cells in response to drugs, chemical and environmental compounds, and nutrients **(Fig.2)**.[8]

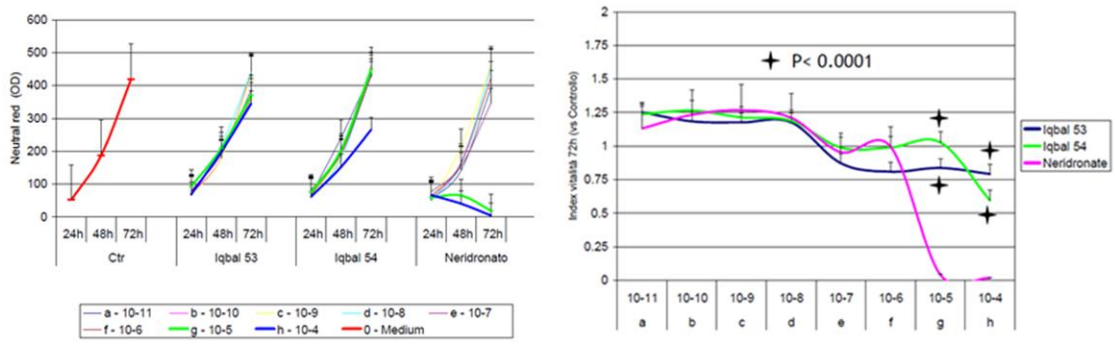


Fig.2 cytotoxicity screening of bisphosbhonate **7a, 7b** (iqbal53, 54) respectively

Next, primary cultures of osteoclasts have been used to test the bioactivity of the new compounds in comparison with bisphosphonates of established activity. In spite of the lower cytotoxicity, the bisphosphonate **7a** (iqbal 53) **(Fig.3)** was not less active than conventional bisphosphonate in inhibiting the osteoclastogenisis. Since, nitrogen-containing bisphosphonates can

cause apoptosis in a variety of cell types in vitro, by inhibiting the mevalonate pathway; we hypothesized that the effect of these agents on the gastrointestinal (GI) tract may be due to apoptosis or inhibition of growth of gut epithelial cells.

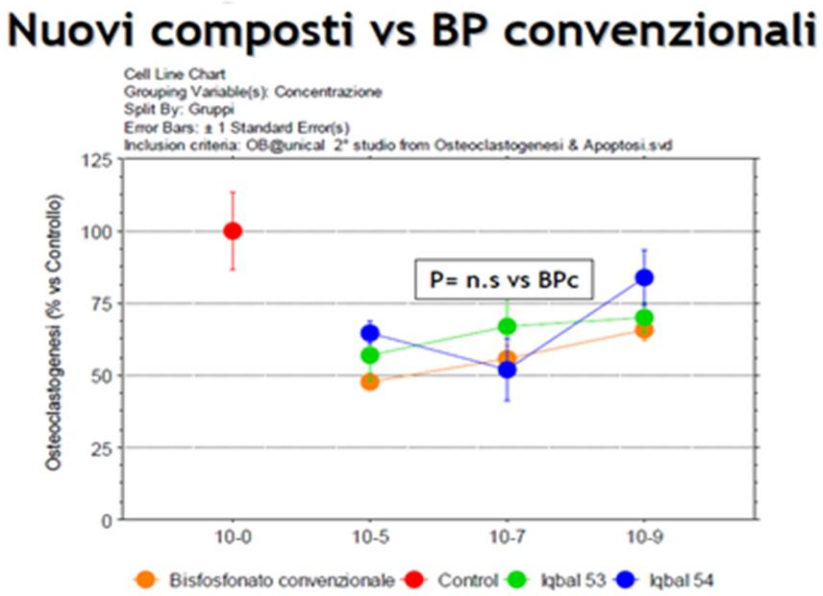


Fig.3 Osteoclastogenisis screening of bisphosphonates **7a, 7b** (iqbal53, 54) with conventional bisphosphonates

A comparison between neridronate, pamidronate or conventional bisphosphonates and our subjected compounds demonstrated that only the nitrogen-containing bisphosphonates were effective at inducing apoptosis. In **fig 4,** one can observe that our compounds having quite similar inducing apoptosis with conventional bisphosphonates.

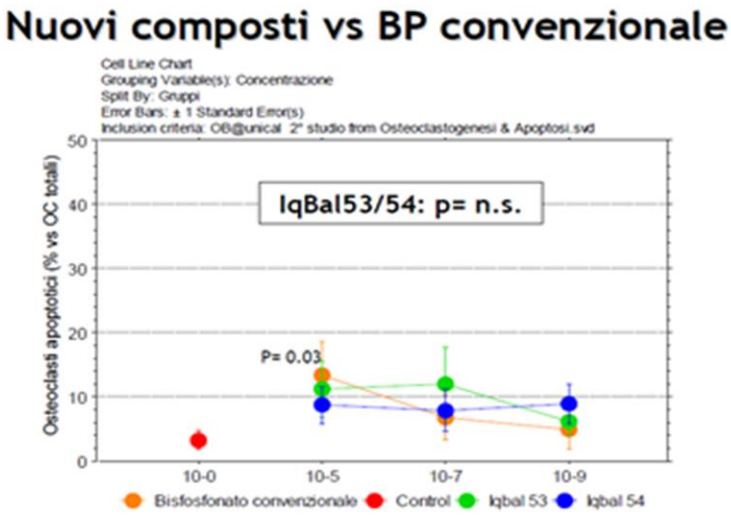
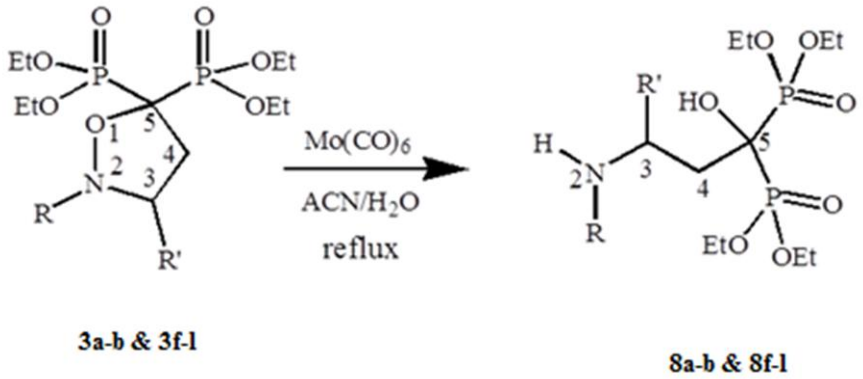


Fig.4 Inducing apoptosis of bisphosphonates **7a, 7b** (iqbal53, 54) with conventional bisphosphonate

5.2 Isoxazolidiene ring reduction using transition metal carbonyls

For synthetic point of view, our next target was to reduce isoxazolidinyl-substituted bisphosphonates in such way that will get derivatives of gem-hydroxyl bisphosphonates. The simultaneous presence of an oxygen and nitrogen atoms on the basic structure of cycloadduct **3a-l, except 3c, 3d and 3e)** and the possibility to modulate different aryl and alkyl substituents on the isoxazolidine ring prompt us to investigate the ring opening of these compounds through cleavage of the *N-O* bond. This strategy represents a novel access to new *gem*-hydroxyl bisphosphonates, bearing aryl substituents on the lateral chain. The reductive cleavage of the *N-O* bond in isoxazolidines represents a simple and direct access to *N*-substituted aminoalcohols, valuable intermediates in many synthetic strategies. Over the years, many methods have been described for the hydrogenolytic cleavage of the *N-O* bond in different isoxazoline ring systems that include

Zn/acetic acid,[9] catalytic hydrogenation with palladium on charcoal,[10] $LiAlH_4$ or $NaBH_4$,[11] SmI_2,[12] and Raney nickel.[13] In our case all these reagents invariably failed to afford the expected ring opening product. Nevertheless, we treat cycloadduct intermediate with methyl iodide to form quaternary ammonium salt and the tried to reduced *N-O* bond [14]. In our case quaternary salt was formed which was identified by ^{1}HNMR but further reduction was failed. Only the use of Zn/AcOH, under reflux for 24 h afforded the *gem*-hydroxyl bisphosphonates in acceptable yields of c.a. 50%, depending on R and R' substituents. We decided, then, to verify the use of transition-metal carbonyls as reducing agents, since these compounds have been efficiently employed to reduce closely related isoxazolidines[15]. The reaction proceeds in very mild conditions by gentile refluxing of the bisphosphonates (BPs) **3a-b and 3f-l** and $Mo(CO)_6$ in wet acetonitrile, **scheme 5.2.** The cycloadducts are almost quantitatively converted into the expected hydroxyl bisphosphonates **8a-b and 8f-l**, which are recovered in rather good yield upon purification by column chromatography, **Table 4.**



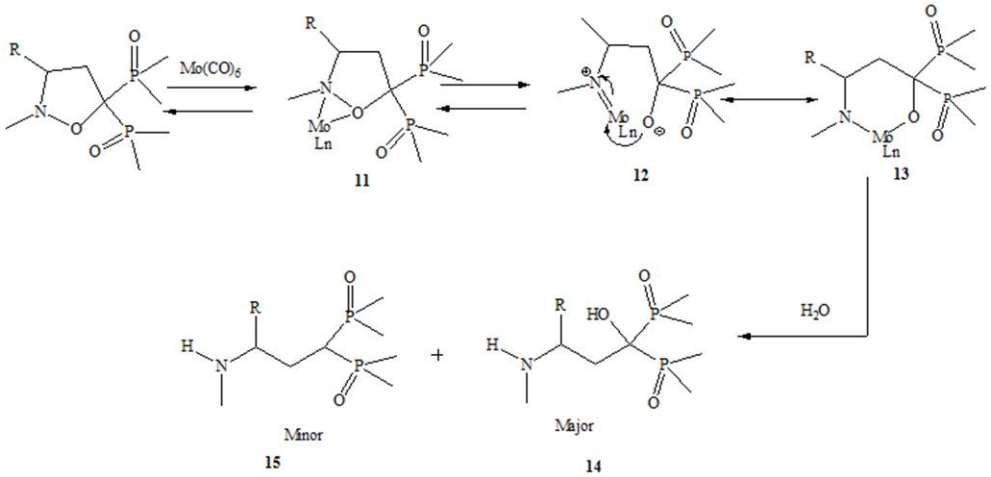
Scheme 5.2. *Reductive cleavage of N-O bond using transition metal carbonyls*

Table 4. Reductive cleavage of isoxazolidine rings of bisphosphonate derivatives with $Mo(CO)_6$.

Entry	BP	R	R′	Product	Yield (%)
1	**3a**	Me	C_6H_5	**8a**	80
2	**3b**	Me	*o*-Cl-C_6H_4	**8b**	75
3	**3f**	Me	*p*-Cl-C_6H_4	**8f**	78
4	**3g**	Me	Naphthyl	**8g**	80
5	**3h**	Bn	C_6H_5	**8h**	75
6	**3i**	Bn	*o*-Cl-C_6H_4	**8i**	81
7	**3j**	Bn	*p*-Cl-C_6H_4	**8j**	76
8	**3k**	Bn	*o*-F-C_6H_4	**8k**	87
9	**3l**	Bn	Naphthyl	**8l**	90

5.2.1 Reaction mechanism of *N-O* bond cleavage

Noteworthy, the same reaction performed in the presence of metal carbonyls other than Mo, as $Cr(CO)_6$ or $W(CO)_6$ was unsuccessful, affording the nitrone derivative through a retro-cycloaddition process. The general accepted mechanism of this reaction is reported to occur via the formation of a Mo(CO)x heterocycle intermediate **11**,[15,16] responsible of the weakening of the *N-O* bond and its consequent opening **(scheme 5.2.1)**.

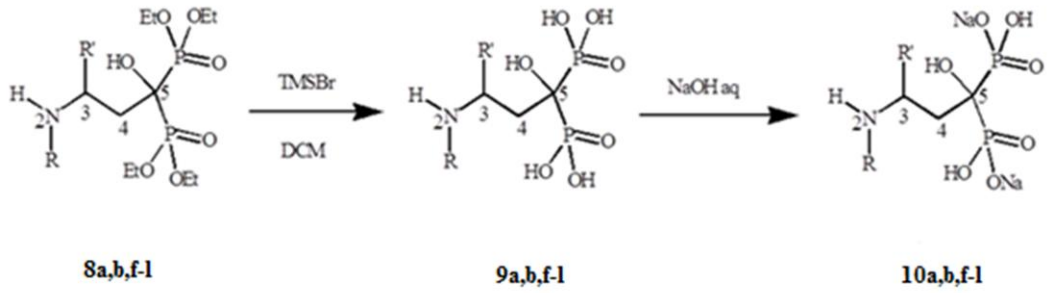


Scheme 5.2.1. *General accepted mechanism for reductive cleavage of N-O bond using $Mo(CO)_6$*

Together with this bond activation, however, additional reaction pathways are possible, [17] likely involving an oxidation of the metal that would explain the formation of **15**. This alternative reaction pattern is directly proportional to the amount of $Mo(CO)_6$ used to carry out the reaction, as demonstrated by the fact that increasing quantities of **15** are formed by doubling the carbonyl derivative[18].

5.2.2 Hydrolysis of ring opened products and their salt formation

Furthermore, The achieved bisphosphonate esters **8a,b,f-l** subjected for hydrolysis to have corresponding bisphosphonic acids **9a,b,f-l** by reaction with trimethylsilyl bromide in dichloromethane, followed by treatment with MeOH, (**Scheme 5.2.2)**.[16]



Scheme 5.2.2. *Hydrolysis of ring opened adducts followed by disodium salt formation*

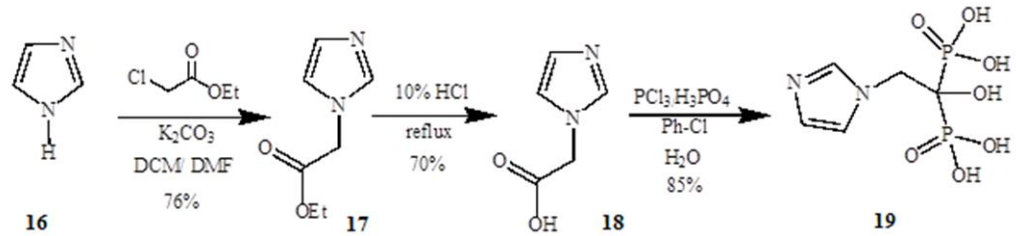
The acids are obtained as white solids by precipitation using a mixture of methanol/diethyl ether. Finally and as requested by biological activity assays, bisphosphonic acids treated with exact 2 eq of sodium hydroxide with water at an ambient temperature to get disodium salt salts of corresponding acids. The results of hydrolysis and salt formation collectively screened in **Table 5.**

Table 5 A screened results during hydrolysis of ring opened adducts and salt formation reaction

Entry	BP	R	R′	Product	Yield (%)	Product	Yield (%)
1	**8a**	Me	C_6H_5	**9a**	80	**10a**	88
2	**8b**	Me	*o*-Cl-C_6H_4	**9b**	75	**10b**	81
3	**8f**	Me	*p*-Cl-C_6H_4	**9f**	78	**10f**	87
4	**8g**	Me	Naphthyl	**9g**	80	**10g**	77
5	**8h**	Bn	C_6H_5	**9h**	75	**10h**	79
6	**8i**	Bn	*o*-Cl-C_6H_4	**9i**	81	**10i**	81
7	**8j**	Bn	*p*-Cl-C_6H_4	**9j**	76	**10j**	78
9	**8k**	Bn	*o*-F-C_6H_4	**9k**	87	**10k**	72
10	**8l**	Bn	Naphthyl	**9l**	90	**10l**	75

5.3 Synthesis of Zoledronic acid

In additional work, we were engaged with total synthesis of Zoledronic acid. This work was carried out by following synthetic scheme **(scheme 5.3)**. This work was started with preparation of compound **17** using imidazole as starting material. Several authors demonstrated synthetic pathway to carry out zoledronic acid **(19).**



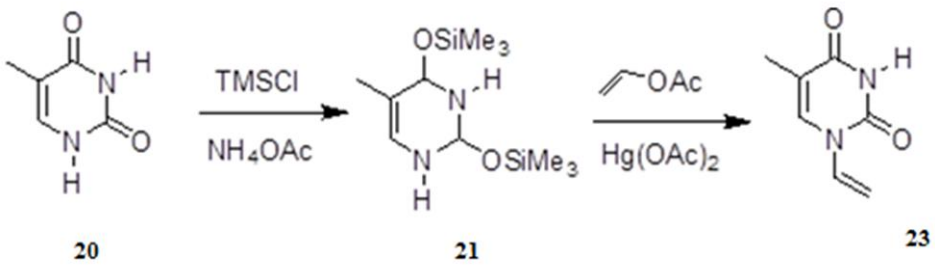
Scheme 5.3. *Total synthesis of Zoledronic acid*

Imidazol-1-yl-acetic acid (**18**), a key precursor of zoledronic acid (**19**), has been reported. Thus, imidazol-1-yl-acetic acid ethyl ester (**17**) was synthesized in 76% yield via the reaction of 1 equiv of imidazole (**16**) with

1.0 equiv of 1-chloro ethyl acetate in the presence of 1.5 equiv. K_2CO_3 in dichloromethane and small amount of DMF followed by ester hydrolysis in 10% aqHCl. Obtained compound **18** was treated with phosphoric acid and phosphorus trichloride in presence of chlorobenzene, yielded compound **19** as zoledronic acid.

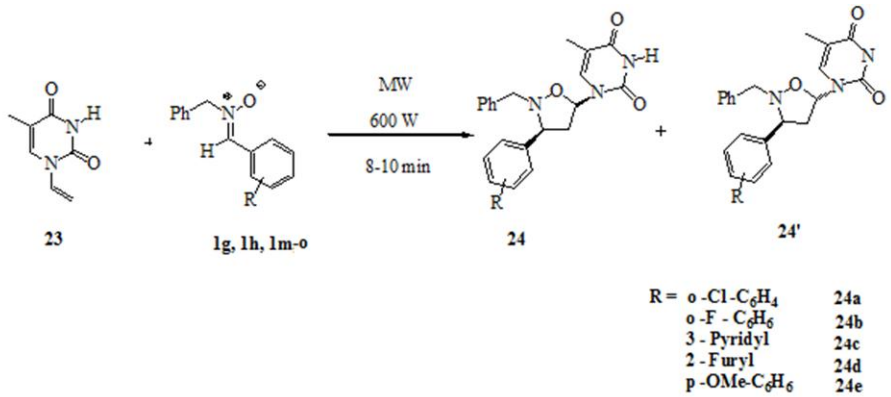
5.4 Synthesis of vinyl thymine and substituted isoxazolidiene

Besides from above novel work, we were associated with synthesis of vinyl nucleobase. Several methods have been developed for the preparation of vinyl derivatives of nucleobases, some consisting of relatively low yielding multistep procedures, [19, 20] and some others involving the direct exchange of the acetyl group of vinyl acetate[21, 22], but, earlier in our research group discovered a very simple and easy to handle experimental procedure for synthesis of Vinyl nucleobase[23]. With the help of these references, we were able to resynthesize *N*-1-vinyl thymine (**scheme 5.4**).



Scheme 5.4. *Synthetic scheme for synthesis of N-1-vinyl thymine*

Furthermore, potently, we did 1, 3 dipolar cycloaddition of vinyl nucleobase **23** and some nitrones which were in our hand **4g, 4h, 4m, 4n** and **4o** using microwave irradiation reaction. The strategy of the synthetic approach is fast and simple consisting in the direct reaction of the selected nitrone and the unprotected vinyl thymine in the absence of solvent and/or catalyst (**Scheme 5.4.1).**



Scheme 5.4.1. *1, 3 dipolar cycloaddition reaction between N-1-vinyl thymine and nitrones*

The cycloadducts are formed in good yield and with remarkable *cis–trans* selectivity, in some cases higher than 91:9. The stereoselectivity of the reaction may be predicted taking into account the possible geometries of approach of the two reacting species. The obtainment of the *cis* adduct is explained by invoking either an *exo* approach of the alkene to the (Z) nitrone isomer or an *endo* approach to the (E) nitrone isomer. The corresponding opposite parallel may be done in the case of *trans*-cycloadducts. The stereochemical outcome of the reaction showed a certain degree of control that appeared to be very highly regioselective and good yield, result shown in **Table 6.**

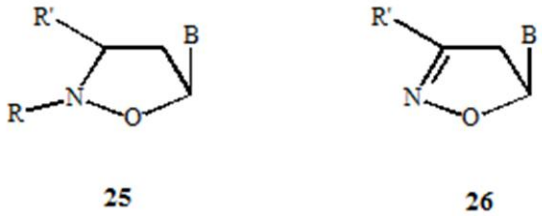
Table 6 results obtained during 1, 3 dipolar cycloadditon between Vinyl thymine and Nitrones

Sr No	**Dipolarophile**	**Nitrone**	**Reaction conditions**		**Total yields of adducts**	**Ratio of adducts 24 : 24'**
			MW(w)	**Time (min)**		
1	Vinyl thymine **23**	**1g**	600	10	86	91:9
2		**1h**	600	12	92	87.13
3		**1m**	600	15	83	85:15
4		**1n**	600	16	85	83:17
5		**1o**	600	14	87	88:12

The *cis–trans* ratio of the *N, O*-nucleosides has been further con- firmed by HPLC analysis. It is interesting to note that for all the mixtures of stereoisomers examined the minor diastereoisomer, possessing *trans* stereochemistry, elutes earlier than the major *cis* one, on a Jupiter 10μ -18 column. Although these findings should not lead to a direct diastereoisomer configuration assignment, [24] they may help in the definition of the stereochemical course of the reaction and in the one-step separation/purification of the isomers, especially for biological assays.

5.5 Biological activity result of substituted thymine isoxazolidiene

In recent years a large number of nucleoside analogues with antiviral and/or antitumor properties have been designed and synthesised.[25, 26] Nucleosides, in fact, comprise the largest class of clinically useful antiviral agents and they continue to be excellent candidates as anticancer drugs.[27] In search for effective, selective and nontoxic agents a variety of modifications of the naturally occurring structure have been devised on both the sugar and the nucleobase. Of particular interest are the carbocyclic nucleoside analogues in which the furanose ring has been replaced by a *N, O*-heterocyclic systems as in isoxazolidine **25** and isoxazoline **26** derivatives, being B pyrimidine or purine nucleobases, **Fig**.5.[28]

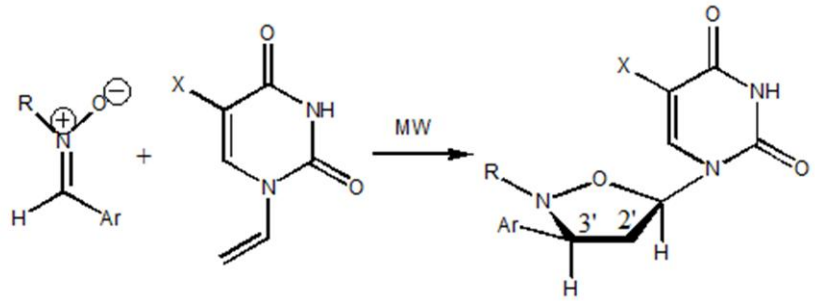


25 **26**

Fig. 5 N, O-heterocyclic systems as in isoxazolidine **25** and isoxazoline **26** derivatives

Most of the *N,O*-containing nucleoside analogs described in the literature possess a remarkable antiviral activity[29] but a modest potential toward human cancer cell lines,[30] contrary to what observed with substituted nucleosides[27,31] and thionucleosides in particular 4,-Thio-Ara-C.[27,32]

In a previous study we have reported the efficient synthesis of a number of *N, O*-nucleosides by direct 1,3-dipolar cyclization methodology.[33,34] The cyclization was carried out on selected nitrones and a set of unprotected vinyl nucleobase, under microwave (MW) irradiation, in the absence of solvent or catalyst, **Scheme 5.5**. The cycloadducts are formed in good yield, with a complete regioselectivity and notable *cis*-diastereoselectivity, up to 98%, resulting from a dominant (*Z*)-exo nitrone-alkene approach.[34]

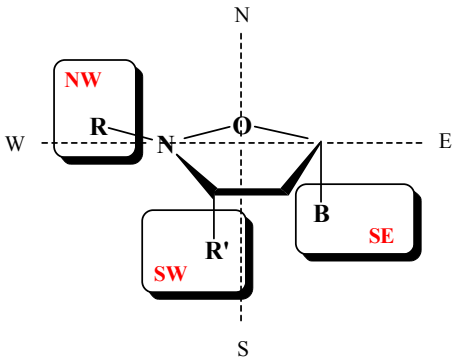


Scheme 5.5. *Synthesis of N, O-nucleosides via 1, 3-dipolar cycloaddition of nitrones with vinylnucleobases*

The *N, O*-nucleosides of the previous study were evaluated by *in vitro* assays for their antiproliferative activity, that was found particularly promising.[33] In analogy to SAR tests designed to prove the minimal structural requirements for anti-proliferative activity in the NCI 60 panel of human cancers, we investigated the variations in the four canonical quadrants of our compounds on LCLs, JiJoye and Jurkat cell lines. The most interesting compounds were then submitted to growth inhibition assays on SKOV3 (cisplatin-resistant) and SW480 cell lines established for ovarian and colon cancers, respectively, **Table 7**. Indeed compound **30** exhibited greater potency (25-50 μM) than either compounds **27** or **31** on these tumoral lines, comparable to that of cisplatin *cis*-$[PtCl_2(NH_3)_2]$, used

as model reference. Taking into account these findings we prepared a new set of *N, O*-nucleosides bearing a thymine or fluoro-uracil as nucleobases, a benzyl group as R substituent and varying the SW-quadrant moiety. Pyrimidine nucleoside analogs are essential components of hematological malignancy therapy and are also used in the treatment of solid tumors.[35] The procedure for the preparation of the new nucleosides closely follows the synthetic strategy described in detail in the previous paper, based on the direct 1,3-dipolar cycloaddition of the nitrone (2 equivalents) with the vinyl nucleobase (1 equivalent) in the absence of solvent, under microwave irradiation.[34] Among the new derivatives, the *ortho*-cloro **32** and *ortho*-fluoro **33** compounds displayed a biological activity similar or even enhanced than that shown by **30**, thus confirming that thymine, *N*-benzyl substituent and aromatic rings are the optimal combination for biological activity.

Table 7. Estimated IC_{50} on cisplatin-resistant SKOV3 and SW480 cell lines



Compounds **27-34**

R	R'	Nucleobase (B)	compound	SKOV3	SW480
Bu^t	Ph	Thy	**27**	>100	>100
Me	Ph	Thy	**28**	>100	>100
Bu^t	Ph	F-Ura	**29**	>100	>100
Bn	Ph	Thy	**30**	25-50	50-100
Me	*Cl*-Ph[a]	Thy	**31**	>100	>100
			cisplatin	>50	>50
Bn	*Cl*-Ph[a]	Thy	**32**	25-50	25-50
Bn	*F*-Ph[b]	Thy	**33**	25-50	25-50
Bn	3-Py	Thy	**34**	>100	>100

[a] 2-chloro phenyl; [b] 2-fluoro phenyl

This small library of *N,O*-nucleosides was analyzed taking into consideration the physico-chemical data related to solubility and permeability.[36] These properties are easily obtained by research databases as SciFinder® or may be calculated.[37] **Table 8** lists the liphophilicity expressed as log P, the molecular weight and the number of hydrogen donor/acceptor sites of our *N,O*-nucleosides, that in all cases is in accordance with the "rule of five", a number of requirements used for the development of orally bioavailable drug candidates.[36,38] These rules have been enhanced by other authors with additional parameters as number of rotable bonds, however the original proposal still maintains its emphasis.[39] In the present case, all the compounds display similar parameters with Respect to MW and H donor/acceptor numbers. The only exception is represented by lipophilicity that is higher for the most biologically active compounds **30, 32, 33**. Respect to MW and H donor/acceptor numbers. The only exception is represented by lipophilicity that is higher for the most biologically active compounds **30, 32, 33**. Following this line of reasoning *N, O*-nucleosides substituted with *N*-benzyl groups and aromatic rings, preferably with thymine as nucleobase, displaying values of lipophilicity in the range 3.5-4.5 should represent the optimal combination for an increased biological activity. To support this hypothesis we planned the synthesis of three new *N,O*-nucleosides **35-37** possessing log P values higher than 3.5 and we extended the number of human cancer cell lines for biological evaluation to A2780 (cisplatin sensitive human ovarian cancer), HCT116 (colon cancer), MDA (breast cancer) and K562 (human caucasian chronic myelogenous leukaemia). The related results are collected in the lower part of **Table 8** that includes the biological activities of **30**, **32** and **33** with the new cancer lines, as well. The presence of nucleobases other than Thymine is detrimental for cytotoxicity, as demonstrated by the results found with compound **32** (B=Thy) IC_{50} in the range 25-50 μM and **11**(B=F-Ura) $IC_{50} > 100$ μM. On the other hand, within the set of nucleosides bearing *N*-benzyl, Thymine and aromatic substituents, increasing biological activity is observed for compounds at increased values of log P, likely approaching 5 the upper limit of the "rule of five".[36]

Table 8. Estimated IC50 (μM) on Cisplatin-resistant SKOV3, SKOV480, K562, MDA, HCT116 and A2780 cancer cell lines.

R	R'	B	Comp.	SKOV3	SW480	K562	MDA	HCT116	A2780	Log P	m. w.	H_{acc}	H_{don}
Bu^t	Ph	Thy	**27**	>100	>100					2.906	329	6	1
Me	Ph	Thy	**28**	>100	>100					1.656	287	6	1
Bu^t	Ph	F-Ura	**29**	>100	>100					2.586	333	6	1
Bn	Ph	Thy	**30**	25-50	50-100	25-50	50-100	25-50	50-100	3.579	363	6	1
Me	*2-Cl*-Ph[a]	Thy	**31**	>100	>100					2.381	321	6	1
			cisplatin	>50	>50								
Bn	*2-Cl*-Ph[a]	Thy	**32**	25-50	25-50	25-50	25-50	25-50	25-50	4.066	397	6	1
Bn	*2-F*-Ph[b]	Thy	**33**	25-50	25-50	25-50	25-50	50-100	25-50	3.555	381	6	1
Bn	3-Py	Thy	**34**	>100	>100					2.165	364	7	1
Bn	*2-Cl*-Ph[a]	F-Ura	**35**	>100	>100	50-100	>100	>100	>100	3.850	401	6	1
Bn	*4-Cl*-Ph[c]	Thy	**36**	10-25	10-25	25-50	25-50	10-25	10-25	4.070	397	6	1
Bn	naptyl	Thy	37							4.397	413	6	1

5.6 Referances

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Conclusion

In summary, in first part of thesis, we were able to synthesis new class of bisphosphonates having in *gem* position an isoxazolidine ring, which simultaneously holds the required basic nitrogen and an oxygen atom in place of the hydroxy group, acting as third hook. For the synthesis of these new classes of drugs, we have shown an expeditious, easy-to-handle, and environmentally friendlier approach, microwave irradiation. Microwave irradiation (MW) has been used for the rapid synthesis of a variety of compounds and this technique, under solvent- free conditions, is regarded as an environmentally acceptable practice for a number of reasons, including the fact that the reactions are quite often cleaner, faster, and higher yielding than conventional synthesis.

Although the syntheses of the different nitrones are described in the literature by several authors, we have partially modified the procedures with the result of a substantial increase in the yields of nitrones. By this method, nitrones were obtained in excellent yields and high purity. Furthermore, the commercially available tetraethyl methylene-1, 1- bisphosphonate was prepared by direct reaction of commercially very cheap and easily available diethyl phosphite in the presence of sodium ethoxide. These 3 steps synthesis has long reaction times (2 months) are balanced by the low cost of the reagents and work-up.

With the set of different nitrones and tetraethyl methylene-1, 1- bisphosphonate we successfully did 1, 3 dipolar cycloadditon using microwave irradiation. Microwave irradiation (MW) has been used for the rapid synthesis of a variety of compounds and this technique, under solvent-free conditions, is regarded as an environmentally acceptable practice for a number of reasons, including the fact that the reactions are quite often cleaner, faster, and higher yielding than conventional synthesis.

Besides, obtained isoxazolidine bisphosphonate easters are hydrolysed using teramethylsilylbromide. In addition hydrolysed products converted into their di sodium salts using exact 2 mole of sodium hydroxide. Some of our bisphosphonic derivatives were examined preliminary biological activity tests mainly cytotoxicity, apoptosis and oestoclasogenices. Rest of biological tests are under the way.

In the second Part of thesis, we reduced isoxazolidine ring in such way that we have 1, 3 amino alcohol bisphosphonic easter derivatives. We carried out ring opening synthesis using transition metal complex, in presence of acetonitrile and water. Obtained bisbhosphonic compounds are hydrolysed with trimethylsilyl bromide followed by formation of disodium salt of bisphosphonic acids. Studies on the biological potential of the different salts and comparison with bisphosphonate salts of well established activity are currently under way.

For biological point of view we resynthesized zoledronic acid in bulk quantity, since its unreachability and cost.

In case of nucleoside substituted isoxazolidine, we have shown an expeditious, easy-to-handle, and environmentally friendlier approach to the synthesis of a variety of non-easily-available *N*-1 vinylthymine isoxazolidiene prepared for the first time from unprotected vinyl thymine. We were tested a preliminary library of *N,O*-nucleosides that demonstrate the impact of substitution in all the four canonical quadrants of lead antiproliferative agent compounds, with a cytotoxic activity in the range 10-25 μM. To our knowledge this is the first example of modified *N, O*-nucleosides showing such promising inhibitory activity against different lines of ovarian and colon carcinoma.

Chapter 6
Experimental

Methods and Instruments

Unless otherwise stated, reactions were performed using freshly purified solvents. Solvents were purified using solvent purification methods by Vogel et.al. All chemicals and reagents were used as received from Sigma-Aldrich, Acros chemical, and Alfa Aesar.

Flash column chromatography was performed using silica gel 60 (Merck, 0.040-0.063 mm) on a Buchi Automated Flash system comprising of a fraction collector C-660 Buchi UV Monitor C-630, Buchi Pump Module C-601 and Buchi Pump Manager C- 615. Analytical thin layer chromatography was carried out on aluminium sheets precoated with Merck TLC Silica gel 60 F_{254}, developed sheets were visualised using a portable UVItec CV-006 lamp ($\lambda = 254$).

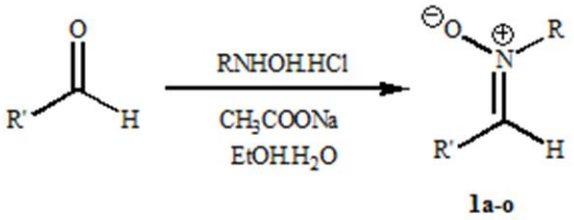
Analytical chiral gas-phase chromatography (GC) was performed on a Perkin-Elmer Clarus 500 equipped with a flame ionization detector using a CHIRALDEX B-DA β- Dex 110 fused silica capillary column (30 m x 0.25 mm x 0.25 μm). High Performance Liquid Chromatography (HPLC) was performed on a Perkin-Elmer Totalchrom v.6.2.0.0.1 or Gilson analytical HPLC column using a CHIRALCEL OD analytical column in both cases (250 x 4.6 mm).

Reactions at 25 °C, Low and high temperature reactions were carried out on heldolph stirrer in water and oil bath respectively, and the temperature was controlled by a Julabo FT920 cooler. All microwave irradiation reactions were carried out in Whirlpool household microwave without solvents. Solvents were evaporated under Heidolph Rotary Evaporators.

All NMR spectra were recorded on a Bruker Avance spectrometer at a probe temperature of 25 °C, unless otherwise stated, operating at 300 and 500 MHz for the ^{1}H nucleus and 75.5 and 126 MHz for the ^{13}C nucleus. Low temperature NMR spectroscopy experiments were carried out by cooling the probe with liquid nitrogen blow off. Spectra were recorded in deuteriochloroform ($CDCl_3$) and deuterated dimethylsulfoxide unless otherwise stated. Tetramethylsilane (TMS) was used as internal standard in all cases. Chemical shifts are given in ppm downfield from the internal standard and coupling constants are given in Hz. Data were reported as follows: chemical shift, integration, multiplicity, coupling constants and assignment (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, dt = doublet of triplets, td = triplet of doublets and br = broad). ^{13}C NMR spectra were recorded with complete proton decoupling.The regiochemistry was established by decoupling experiments of selected signals. The mass spectrometric data were acquired on a Finnigan LCQ Deca, equipped with an electrospray ionization source. Standard experimental conditions are as follows: sample concentration10^{-6} M; elution solvent MeOH; flow rate 8 µL min^{-1}; nebulizing gas 40 units flow rate; spray voltage 4 kV; capillary voltage 14 V; capillary temperature 270 °C. Melting points were obtained on a Kofler apparatus. GC MS spectra were carried out on a Shimadzu QP 5000.

Infrared (IR) Spectra were recorded on a Perkin Elmer System 2000 FT spectrometer. Solid samples were finely ground with an excess of dry potassium bromide and liquid/oil samples were added to an excess of Nujol.

General synthetic scheme and procedure for the synthesis of nitrones 1a-o.



1a-o

R=Me, R'=Ph	**1a**
R=Me, R'=*o*-Cl-C_6H_4	**1b**
R=Me, R'=3-pyridyl	**1c**
R=Me, R'=2-furyl	**1d**
R=Bn, R'=*p*-OH-C_6H_4	**1e**
R=Bn, R'=Ph	**1f**
R=Bn, R'=*o*-Cl-C_6H_4	**1g**
R=Bn, R'=*o*-F-C_6H_4	**1h**
R=Me, R'=*p*-Cl-C_6H_4	**1i**
R=Me, R'=naphtyl	**1j**
R=Bn, R'=*p*-Cl-C_6H_4	**1k**
R=Bn, R'=naphtyl	**1l**
R=Bn, R'=3-pyridyl	**1m**
R=Bn, R'=2-furyl	**1n**
R=Bn, R'=*p*-OMe-C_6H_4	**1o**

Experimental procedure

Most of the substituted nitrones were synthesized under stirring and in short reaction times, 10–15 min, via condensation of the suitable aldehyde (1 mol) with *N*-methyl- or *N*-benzylhydroxylamine hydrochloride (1 mol) in the presence of sodium acetate (1.2 mol) dissolved in EtOH-H_2O 1:1 (10 mL) at room temperature. The bulk of the solvent was removed under reduced pressure and the residue was extracted with dichloromethane (3x20 mL). The combined organic layers were dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure.

N-Methyl-C-phenyl nitrone **1a**. White solid (yield 92 %), mp 83-84 ^{o}C, GC/MS m/z 135, 'H NMR (500MHz, $CDCl_3$) δ: 3.89 (s, 3H, *N*-CH_3), 7.36 (s, 1H, =CH), 7.40—7.43 (m, 3H, Ar), 8.19—8.22 (m, 2H, Ar), ^{13}C NMR (500 MHz, $CDCl_3$) δ: 54.40, 128.54, 128.68, 128.87, 129.34, 129.78, 130.40, 132.2.

N-Methyl-C-o-chloro phenyl nitrone **1b**. White solid (yield 96 %), mp 73-74 ^{o}C, GC/MS m/z 169, 'H NMR (500MHz, $CDCl_3$) δ: 3.93 (s, 3H, *N*-CH_3) 7.29-7.41 (m, 3H, Ar), 7.86 (s, 1H, =CH), 9.30 (dd, 1H, *J*=2.1, 7.7 Hz, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 55.13, 126.92, 128.13, 128.80, 129.28, 130.68, 130.90, 132.5.

N-Methyl-C-3-pyridyl nitrone **1c.** White solid (yield 83 %), mp 75-77 ^{o}C, GC/MS m/z 136, 'H NMR (500MHz, $CDCl_3$) δ: 3.93 (s, 3H, *N*-CH_3), 7.38 (dd, *J*= 7.9 & 4.9 Hz, 1H, Pyr–H), 7.46 (s,1H, =CH), 8.60 (d, *J*= 3.5 Hz,1H, Pyr–H), 8.97 (s,1H, Pyr–H),9.01 (d, *J*= 8.09 Hz,1H, Pyr–H).^{13}C NMR (500 MHz, $CDCl_3$) δ: 54.6, 123.6, 127.0, 132.3, 134.6, 149.5, 150.5.

N-Methyl-C-2-furyl nitrone **1d.** White solid (yield 86 %), mp 90-92 ^{o}C, GC/MS m/z 109, 'H NMR (500MHz, $CDCl_3$) δ: d 3.84 (s, 3H, *N*-CH_3), 6.56 (d, *J*= 1.3 Hz, 1H, Fur–H),7.48 (br s, 1H, Fur–H), 7.55 (s, 1H, =CH), 7.76 (d, *J*= 3.3 Hz, 1H, Fur–H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 52.6, 112.1, 115.1,126.1, 143.5, 146.5.

N-Benzyl-C-p-hydroxy phenyl nitrone **1e**. White solid (yield 93%), mp 206-207 ^{o}C, GC/MS m/z 227, ^{1}H NMR (500 MHz, DMSO-d_6) δ: 4.99 (s, 2H, *N*-CH_2) 6.79-6.83 (m, 2H, Ar), 7.34-7.49 (m, 5H, Ar), 7.91 (s, 1H, =CH), 8.10-8.15 (m, 2H, Ar), 10.04 (bs, 1H, OH), ^{13}CNMR (500 MHz, DMSO-d_6) δ: 69.33, 115.06, 122.37, 128.05, 128.24, 128.76, 130.03, 133.05, 134.98, 158.87.

N-Benzyl-C-phenyl nitrone **1f**. White solid (yield 94 %), mp 85-87 ^{o}C, GC/MS m/z 211, 'H NMR (300MHz, $CDCl_3$) δ: δ 8.42-8.37 (s, 2 H, *N*-CH_2), 7.92 (s, 1 H, =CH), 7.80-7.75 (m, 2 H, Ar), 7.54-7.46 (m, 6 H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 70.96, 126.25, 128.19, 128.63, 128.74, 128.96, 129.04, 129.18, 129.62, 130.16, 130.71, 132.23.

N-Benzyl-C-o-chloro phenyl nitrone **1g**. White solid (yield 96%), mp 86-87 ^{o}C, GC/MS m/z 245, ^{1}H NMR (300MHz, $CDCl_3$) δ: 5.09 (s, 2H, *N*-CH_2) 7.28-7.52 (m, 8H, Ar), 7.93 (s, 1H, =CH), 9.30 (m, 1H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 71.90, 126.90, 128.09, 128.76, 128.84, 128.91, 129.12, 129.20, 129.77, 130.86, 132.71, 133.23.

N-Benzyl-C-o-fluoro phenyl nitrone **1h**. White solid (yield 97 %), mp 84-85 ^{o}C. GC/MS m/z 229, 'H NMR (300MHz, $CDCl_3$) δ: 5.07 (s, 2H, CH_2), 7.02-7.51 (m, 8H, Ar), 7.75 (s, 1H, =CH), 9.24 (td, 1H, *J*=1.8, 7.8 Hz, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 71.68, 114.47 (d, *J*=21.3 Hz), 119.00 (d, *J*=8.5 Hz), 124.31 (d, *J*=3.2 Hz), 126.37 (d, *J*=9.6 Hz), 128.63, 128.84, 128.90, 129.11, 131.64 (d, *J*=8.5 Hz), 133.26, 159.01 (d, *J*=252.9 Hz).

N-Methyl-C-p-chloro phenyl nitrone **1i**. White solid (yield 96 %), mp 126-128 ^{o}C, GC/MS m/z 169, 'H NMR (300MHz, $CDCl_3$) δ: 3.88 (s, 3H, *N*-CH_3), 7.36 (s, 1H, =CH), 8.27 (d, *J*=8.8 Hz, 2H, Ar), 8.38 (d, *J*=8.8 Hz, 2H, Ar). ^{13}C NMR (300 MHz, $CDCl_3$) δ:54.40, 128.71, 128.95, 129.52, 134.00, 135.81,

N-Methyl-C-naphthyl nitrone **1j.** White solid (yield 94%), mp 106-108 ^{o}C, GC/MS m/z 185, 'H NMR (300MHz, $CDCl_3$) δ: 3.49 (s, 3H, *N*-CH_3), 7.51 (dd, 2H, *J*= 2.4 & 9.6 Hz), 7.51 (s, 1H, Ar), 7.80—7.83 (m,1H, Ar), 7.84—7.90 (m, 2H, Ar), 7.91—7.95 (m, 1H, Ar), 9.20 (s, 1H, =CH). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 56.32, 125,30 126.48, 127.68, 128.37, 128.43, 129.78, 130.40, 132.43. 133.87.

N-Benzyl-C-p-chloro phenyl nitrone **1k**. White solid (yield 96%), mp 130-132 ^{o}C, GC/MS m/z 245, ^{1}H NMR (300MHz, $CDCl_3$)δ: 5.19 (s, 2H, *N*-CH_2) 7.28-7.52 (m, 5H, Ar), 7.93 (s, 1H, =CH),), 8.13 (d, *J*=8.8 Hz, 2H, Ar), 8.32 (d, *J*=8.8 Hz, 2H, Ar). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 71.46, 126.78, 128.09, 128.80, 129.00, 129.28, 129.67, 130.89, 132.73, 133.48.

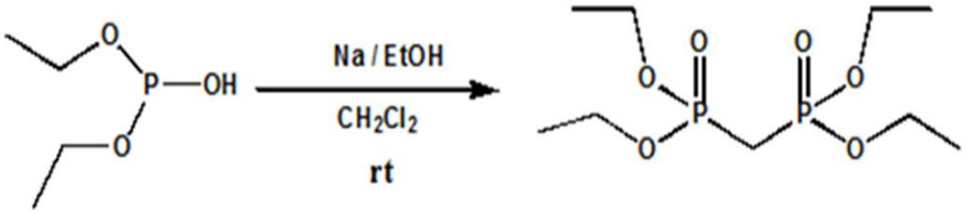
N-Benzyl-C-naphthyl nitrone **1l .** White solid (yield 92%), mp 107-109 ^{o}C, GC/MS m/z 261, 'H NMR (300MHz, $CDCl_3$) δ: 5.09 (s, 2 H, *N*-CH_2), 7.55-7.39 (m, 8 H),7.92-7.77 (m, 4 H), 9.25 (s, 1 H, =CH), ^{13}C-NMR (50MHz, CDCl3) δ: 71.12, 125.64, 126.23, 127.16, 127.26, 127.44, 127.63, 128.35, 128.76, 128.91, 129.09, 132.81 , 133.07 , 133.82 , 134.24.

N- Benzyl-C-3-pyridyl nitrone **1m.** White solid (yield 89 %), mp 95-97 ^{o}C, GC/MS m/z 212, 'H NMR (500MHz, $CDCl_3$) δ: 5.10 (s, 2H, *N*-CH_2), 7.28 (dd, *J*= 7.9 & 4.9 Hz, 1H, Pyr–H), 7.46-7.52 (m, 6H, Ar & =CH), 8.60 (d, *J*= 3.5 Hz,1H, Pyr–H), 8.97 (s,1H, Pyr–H),9.01 (d, *J*= 8.09 Hz,1H, Pyr–H).^{13}C NMR (500 MHz, $CDCl_3$) δ: 72.23, 123.86, 127.08, 132.13, 134.65, 149.15, 150.57.

N- Benzyl-C-2-furyl nitrone **1n. ;** White solid (yield 86 %), mp 90-92 ^{o}C, GC/MS m/z 224, 'H NMR (500MHz, $CDCl_3$) δ: 5.06 (s, 2H), 7.62 (br s, 1H), 7.65–7.32 (br s, 7H), 7.79 (br s, 1H), ^{13}C NMR (500 MHz, $CDCl_3$) δ: 69.57; 112.34, 115.37, 125.24, 128.44, 129.04, 129.41, 132.84, 143.72, 146.83,

N-Benzyl-C-p-methoxy phenyl nitrone **1o**. White solid (yield 96 %), mp 91-93 ^{o}C, GC/MS m/z 241, 'H NMR (300MHz, $CDCl_3$) δ: 3.82 (s, 3H, *O*-CH_3)5.38 (s, 2H, *N*-CH_2), 7.92 (s, 1H, =CH), 7.48-7.54 (m, 7H, Ar), 7.71-7.78 (m, 2H, Ar), ^{13}C NMR (500 MHz, $CDCl_3$) δ: 54.36, 70.94, 126.15, 128.22, 128.65, 128.64, 128.94, 129.16, 129.21, 129.58, 130.09, 130.67, 132.25.

Tetraethyl methylene-1,1-bisphosphonate (4)



4

Compound	Quantity	Density(g/lit)	MW (g/mol)	mmol	Eq
Diethyl phosphite	100mL	1.08	138.04	782	1
Na metal	17.99g		23	782	1
Ethanol	250mL				
Dichloromethane	300ml				

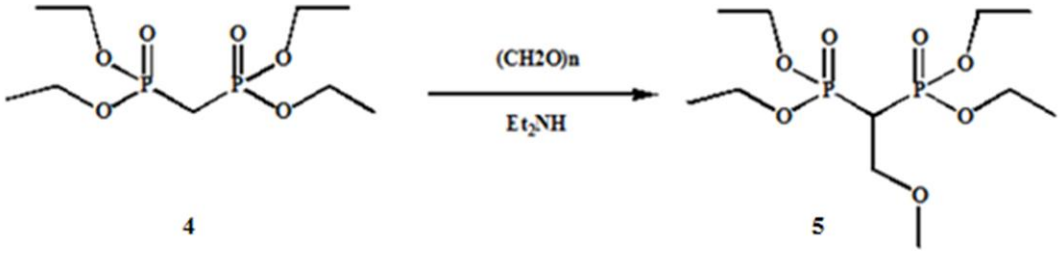
Experimental procedure

A solution of sodium ethoxide was prepared by adding sodium (17.99g, 782 mmol) in portions to absolute ethanol (250 ml). Then diethyl phosphite (100 ml, 782 mmol) was added and the mixture was stirred for 1h at room temperature. The excess of alcohol was evaporated using a rotary evaporator. Dry dichloromethane (300mL) was added and the mixture was stirred for 2 months in a round bottom flask. The reaction mixture was washed with water (2x500 ml) and the organic phase was dried over anidrous sodium sulfate. The solvent was evaporated and the product was purified by distillation. Colourless liquid, (114.7g, yield 55%), Colourless liquid, (114.7g, yield 55%), bp: 138°C 0.03mm/Hg, as compound **4**.

Spectral Analysis

^{1}H NMR (300MHz, $CDCl_3$) δ: 1.35 (t, *J*=7 Hz, 12H, CH_3), 2.52 (t, *J*=21.0 Hz, 2H, PCH_2P), 4.15 (m, 8H, CH_2), ^{13}C NMR (300MHz, $CDCl_3$) δ: 13.50-19.00 (m), 25.44 (t, J_{PCP}=136.21Hz), 60.89-64.98 (m), ^{31}P NMR (500MHz, $CDC1_3$) δ: 19.10.

Tetraethyl 2-methoxyethylene-1,1-bisphosphonate (5)



4 5

Compound	Quantity	Density (g/lit)	MW (g/mol)	mmol	Eq
Comp.4	5.0g		273.06	17.1	1
p-formaldehyde	2.60g		30.03	86.7	5
Diethyl amine	1.26g	0.707	73.14	17.3	1
Methanol	50mL				

Experimental procedure

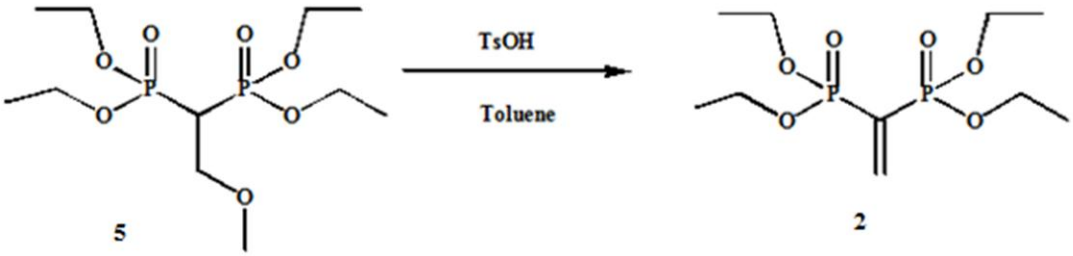
Paraformaldehyde (2.6g, 86.7 mmol) and diethylamine (1.26 g, 17.3 mmol) were combined in methanol (50 mL). The mixture was warmed until clearness, bring back to room temperature and added of **4** (5.0 g, 17.3 mmol). The resulting mixture was refluxed for 24 h, additional methanol

was added (50 mL) and the solution was concentrated under vacuum. A portion of toluene was added (25 mL) and the solution was concentrated. This last step was repeated for two times to ensure the complete removal of methanol. Compound **5** (4.20 g) was obtained in 85% yield, Colourless liquid.

Spectral Analysis

GC/MS m/z 332, ^{1}HNMR (300 MHz, $CDCl_3$) δ: 1.34 (t, *J*=7.02 Hz, 12H, CH_3), 2.70 (tt, *J*=5.5, J_{HP}=23.8 Hz, 1H, PCHP), 3.37 (s, 3H, CH_3O), 3.63 (td, *J*=5.5, J_{HP}=16.1 Hz, 2H, CH_2O), 4.04-4.27 (m, 8H, OCH_2CH_3), ^{13}CNMR (300 MHz, $CDC1_3$) δ: 6.38 (d, *J*=6.6 Hz), 38.83 (t, J_{PCP}=132.6 Hz), 58.74, 62.52-62.74 (m), 68.04-68.10 (m); ^{31}PNMR (500 MHz, $CDC1_3$,) δ: 21.05.

Tetraethylvinylidene-1,1-bisphosponate (2)



Compound	Quantity	Density (g/lit)	MW (g/mol)	mmol	Eq
Comp.5	5.04g		332.11	15.1	1
TsOH	0.006g		172.20	0.03	0.002
Toluene	50mL				

Experimental procedure

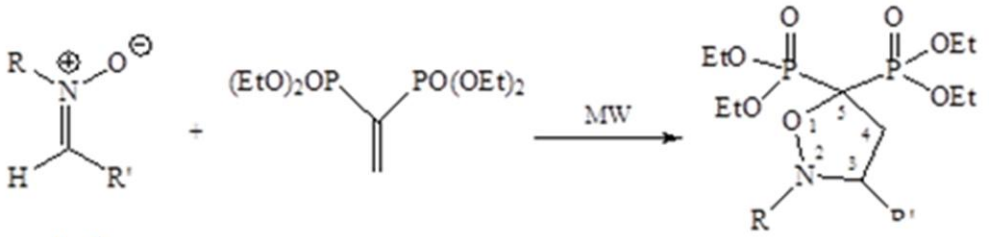
A solution of **7** (5.04 g, 15.1 mmol) in 50 mL of toluene was added of *p*-toluenesulfonic acid monohydrate (0.006 g, 0.03 mmol)under stirring. The reaction mixture was refluxed for 24 h and methanol was removed by collection in a Dean-Stark trap. The solution was concentrated up to dryness. The crude product was diluted with 50 mL of chloroform and washed with water (2 x 100 mL). Combined organic layers were dried over anhydrous sodium sulphate, concentrated and the product was purified by high vacuum distillation, afforded compound **2** as, Clear liquid, (158.3 g, yield 79%), bp 125-126 $^{\circ}$C (0.03 mm/Hg).

Spectral Analysis

GC/MS m/z 300, ^{1}HNMR (300MHz, $CDCl_3$) δ: 1.35(t, J = 7.1Hz, 12H, CH_3), 4.11-4.19 (m, 8 H, CH_2), 6.99 (distorted dd, J = 33.75, 37.8 Hz, 2 H, =CH_2), ^{13}CNMR (300MHz, $CDCl_3$) δ: 16.30 (t, J=2.67 Hz), 62.66 (d, J=2.68 Hz), 132.80 (t, J_{PCP}=166.78 Hz), 149.21, ^{31}P NMR (500MHz, $CDC1_3$) δ: 18.29.

Typical experimental procedure for 1,3-cycloaddition

A mixture of 5 (0.1 mmol) and the selected nitrone **4a-l** (0.12 mmol) was placed in a 10 mL Pyrex container and irradiated at 200 W power using an unmodified household microwave oven. After the appropriate time the reaction mixture is submitted to flash chromatography, using variable mixtures of chloroform and acetone or acetonitrile. Pure compounds **4a-l** were collected as colorless thick mass. For further information. The nitrone in excess is recovered and may be re-used.



1a-l **2** **3a-l**

R=Me	R'= Ph	t=10 min	**3a**
R=Me	R'= *o*-Cl-C_6H_4	t=12 min	**3b**
R=Me	R'= 3-pyridyl	t=13 min	**3c**
R=Me	R'= 2-furyl	t=15 min	**3d**
R=Bn	R'= *p*-OH-C_6H_4	t=14 min	**3e**
R=Bn	R'= Ph	t=18 min	**3f**
R=Bn	R'= *o*-Cl-C_6H_4	t=18 min	**3g**
R=Bn	R'= *o*-F-C_6H_4	t=20 min	**3h**
R=Me	R'= *p*-Cl-C_6H_4	t=16 min	**3i**
R=Me	R'= naphtyl	t=14 min	**3j**
R=Bn	R'= *p*-Cl-C_6H_4	t=18min	**3k**
R=Bn	R'= naphtyl	t=16min	**3l**

Spectral Analysis of Cycloadducts.

Tetraethyl-2-methyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate **(3a)**. Flash chromatography eluent chloroform/acetone (93:7 v/v) Rf 0.48, yield 75% (0.326 g). IR (film, cm^{-1}) 974, 1021, 1254, 1444, 1456, 2931, 2985, 3479; ESI-MS positive ion-mode $[M+Na]^+$ m/z 458; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.30-1.48 (m, 12H, CH_3), 2.63 (s, 3H, *N*-CH_3), 2.88-3.14 (m, 2H, H_{C4}, H'_{C4}), 3.81 (dd, 1H, J = 6.5, 9.8 Hz, H_{C3}), 4.29-4.42 (m, 8H, OCH_2), 7.30-7.49 (m, 5H, Ar); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.54 (d, J = 3.46 Hz), 43.20, 45.57, 63.80, 72.77, 128.01, 128.32, 128.74, 136.91; ^{31}P NMR (500 MHz, $CDC1_3$) δ: 20.43. Anal. Calcd (%) for C18H31NO7P2: C, 49.66; H, 7.18; N,3.22. Found (%) C, 49.75; H, 7.21; N, 3.18.

Tetraethyl-2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonate (3b). Flash chromatography eluent chloroform/acetone (93:7 v/v) Rf 0.55, yield 77% (0.361 g). IR (film, cm^{-1}) 975, 1042, 1255, 1441, 1454, 2930, 2983, 3468; ESI-MS positive ion-mode $[M+Na]^+$ m/z 492; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.35-1.43 (m, 12H, CH_3), 2.68 (s, 3H, *N*-CH3), 2.72-2.86 (m, 1H, H_{C4}), 3.19-3.27 (m, 1H, H'_{C4}), 4.28-4.40 (m, 9H, OCH_2, H_{C3}), 7.20 (td, 1H, J = 1.4, 7.7 Hz, Ar-H_5), 7.28 (t, 1H, J = 7.8 Hz, Ar-H_4), 7.36 (dd, 1H, J = 0.7, 7.8 Hz, Ar-H_6), 7.61 (dd, 1H, J = 1.4, 7.7 Hz, Ar-H_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.57, 43.39, 43.80, 63.85, 67.98, 127.47, 128.26, 128.92, 129.54, 133.96, 135.00; ^{31}P NMR (500 MHz, $CDC1_3$) δ: 19.26 (d, J = 55.8 Hz), 19.35 (d, J = 55.8 Hz). Anal. Calcd (%) for $C_{18}H_{30}ClNO_7P_2$: C, 46.01; H, 6.44; N, 2.98. Found (%) C, 45.96; H, 6.40; N, 3.04.

Tetraethyl-2-methyl-3-(30-pyridyl)-isoxazolidinyl-5,5-bisphosphonate (3c). Flash chromatography eluent chloroform/acetone (93:7 v/v) Rf 0.49, yield 73% (0.318 g). IR (film, cm^{-1}) 970, 1024, 1251, 1443, 1476, 2927, 2983, 3449; ESI-MS positive ion-mode $[M+Na]^+$ m/z 459; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.33-141 (m, 12H, CH_3), 2.64 (s, 3H, *N*-CH_3), 2.89-3.17 (m, 2H, H_{C4}, H'_{C4}), 3.84-3.91 (m, 1H, H_{C3}), 4.14-4.32 (m, 8H, OCH_2), 7.29-8.59 (m, 4H, Ar); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.55, 43.21, 45.53, 62.68, 63.88, 70.26, 123.84, 132.90, 135.55, 149.73, 149.94; ^{31}P NMR (500 MHz, $CDC1_3$) δ: 18.77. Anal. Calcd (%) for $C_{17}H_{30}N_2O_7P_2$: C, 46.79; H, 6.93; N, 6.42. Found (%) C, 46.85; H, 6.97; N, 6.38.

Tetraethyl-2-methyl-3-(20-furyl)-isoxazolidinyl-5,5-bisphosphonate (3d). Flash chromatography eluent chloroform/acetone (93:7 v/v) Rf 0.62, yield 68% (0.289 g). IR (film, cm^{-1}) 975, 1035, 1253, 1444, 1479, 2910, 2984, 3486; ESI-MS positive ion-mode $[M+Na]^+$ m/z 448; ^1H NMR (300 MHz,

$CDCl_3$) δ: 1.29-1.34 (m, 12H, CH_3), 2.68 (s, 3H, *N*-CH_3), 2.90-3.03 (m, 1H, H_{C4}), 3.18-3.26 (m, 1H, H'_{C4}), 3.88-3.96 (m, 1H, H_{C3}), 4.22-4.27 (m, 8H, OCH_2), 6.33-7.40 (m, 3H, Ar); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.53, 40.92, 43.72, 62.68, 64.01, 65.74, 108.85, 110.44, 142.92, 149.10; ^{31}P NMR (500 MHz, CDC13) δ: 21.87 (d, *J* = 52.4 Hz), 22.35 (d, *J* = 52.4 Hz). Anal. Calcd (%) for $C_{16}H_{29}NO_8P_2$: C, 45.18; H, 6.87; N, 3.29. Found (%) C, 45.10; H, 6.91; N, 3.32.

Tetraethyl-2-benzyl-3-p-hydroxyphenyl-isoxazolidinyl-5,5-bisphosphonate (3e). Flash chromatography eluent chloroform/acetonitrile (98:2 v/v) Rf 0.51, yield 74% (0.390 g). IR (film, cm^{-1}) 974, 1026, 1252, 1444, 1478, 2909, 2984, 3480; ESI-MS positive ion-mode $[M+Na]^+$ m/z 550; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.30-1.38 (m, 12H, CH_3), 2.71-2.91 (m, 1H, H_{4C}), 3.16-3.28 (m, 2H, H'_{4C}, OH), 3.84 (s, 2H, H_{Bn}), 4.11-4.19 (m, 9H, OCH_2, H_{C3}), 7.26-7.38 (m, 9H, Ar); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.29 (d, *J* = 3.4 Hz), 43.95, 54.58, 63.75, 68.47, 127.37, 127.90, 128.06, 128.39, 128.71, 128.83, 129.73, 133.12, 136.94; ^{31}P NMR (500 MHz, $CDC1_3$) δ: 19.48. Anal. Calcd (%) for $C_{24}H_{35}NO_8P_2$: C, 54.65; H, 6.69; N, 2.66. Found (%) C, 54.75; H, 6.64; N, 2.63.

Tetraethyl-2-benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate (3f). Flash chromatography eluent chloroform/acetonitrile (98:2 v/v) Rf 0.53, yield 83% (0.424 g), IR (film, cm^{-1}) 977, 1022, 1252, 1440, 1476, 2920, 2973, 3475; ESI-MS positive ion mode $[M+Na]^+$ m/z 534; ^1H NMR (300 MHz, $CDCl_3$) δ: 1.21-1.28 (m, 6H, CH_3), 1.33-1.38 (m, 6H, CH_3) 2.85-3.18 (m, 2H, H_{C4}, H'_{C4}), 3.75 (d, 1H, *J* = 14.2 Hz, H_{Bn}), 3.97 (d, 1H, *J* = 14.2 Hz, H_{Bn}), 4.01-4.16 (m, 5H, OCH_2, HC_3), 4.21-4.33 (m, 4H, OCH_2), 7.19-7.50 (m, 10H, Ar); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.49 (d, *J* = 3.9 Hz), 45.27, 60.08, 64.05, 70.32, 127.11, 127.87, 128.09, 128.31,

128.82, 129.12, 137.19, 137.42; ^{31}P NMR (500 MHz, CDC1$_3$) δ: 18.74 (d, J = 52.1 Hz), 18.79 (d, J = 52.1 Hz). Anal. Calcd (%) for $C_{24}H_{35}NO_7P_2$: C, 56.36; H, 6.90; N, 2.74. Found (%) C, 56.43; H, 6.87; N, 2.78.

Tetraethyl-2-benzyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonate (3g). Flash chromatography eluent chloroform/acetonitrile (98:2 v/v) Rf 0.58, yield 85% (0.463g). IR (film, cm^{-1}) 974, 1022, 1255, 1441, 1475, 2929, 2981, 3468; ESI-MS positive ion mode $[M+Na]^+$ m/z 568; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.20-1.29 (m, 6H, CH_3), 1.34-1.40 (m, 6H, CH_3), 2.67-2.90 (m, 1H, H_{C4}), 3.15-3.31 (m, 1H, H'_{C4}), 3.84 (d, 1H, J = 14.2 Hz, H_{Bn}), 3.99 (d, 1H, J = 14.2 Hz, H_{Bn}), 4.06-4.17 (m, 4H, OCH_2), 4.22-4.34 (m, 4H, OCH_2), 4.66 (dd, 1H, J = 6.6, 10.4 Hz, H_{C3}), 7.19-7.76 (m, 9H, Ar); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.44 (d, J = 2.7 Hz), 16.53 (d, J = 2.7 Hz), 43.45, 60.22, 63.57, 63.88, 65.59, 127.20, 127.53, 127.94, 128.44, 128.99, 129.16, 129.55, 133.92, 135.23, 137.03; ^{31}P NMR (500 MHz, CDC1$_3$) δ: 18.73 (d, J = 54.1 Hz), 18.77 (d, J = 54.1 Hz). Anal. Calcd (%) for $C_{24}H_{34}ClNO_7P_2$: C, 52.80; H, 6.28; N, 2.57. Found (%) C, 52.88; H, 6.24; N, 2.53.

Tetraethyl-2-benzyl-3-o-fluoro-phenyl-isoxazolidinyl-5,5-bisphosphonate (3h). Flash chromatography eluent chloroform/acetonitrile (98:2 v/v) Rf 0.56, yield 86% (0.455 g). IR (film, cm^{-1}) 975, 1024, 1254, 1455, 1493, 2910, 2983, 3468; ESI-MS positive ion mode $[M+Na]^+$ m/z 552; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.24-1.28 (m, 6H, CH_3), 1.34-1.38 (m, 6H, CH_3), 2.86-3.00 (m, 1H, H_{C4}), 3.09-3.18 (m, 1H, H_{C4}), 3.83 (d, 1H, J =14.3 Hz, H_{Bn}), 3.98 (d, 1H, J =14.3 Hz, H_{Bn}), 4.06-4.16 (m, 4H, OCH_2), 4.25-4.29 (m, 4H, OCH_2), 4.52 (dd, 1H, J = 6.4, 10.6Hz, H_{C3}), 7.02-7.69 (m, 9H, Ar); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 14.98-17.04 (m), 42.80-45.13 (m), 59.44-65.04 (m), 114.88-116.22 (m), 124.17-137.12 (m), 137.16, 161.10 (d, J_{CF}

= 258.26 Hz); ^{31}P NMR (500 MHz, $CDC1_3$) δ: 18.75. Anal. Calcd (%) for $C_{24}H_{34}FNO_7P_2$: C, 54.44; H, 6.47; N, 2.65. Found (%) C, 54.50; H, 6.51; N, 2.59.

Tetraethyl-2-methyl-3-(para-chloro)-phenyl-isoxazolidinyl-5,5-bisphosphonate (3i). Flash chromatography eluent chloroform: acetone (93:7 v:v),) Rf 0.56, yield 78%. IR (film, cm^{-1}) 973, 1045, 1245,1440, 1434, 2932, 2981, 3465; ESI-MS positive ion mode $[M+Na]^+$ m/z 470; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.34-1.42 (m, 12H, CH_3), 2.61 (s, 3H, *N*-CH_3), 2.81-3.16 (m, 2H, H_{4C}, H'_{4C}), 3.77-3.82 (m, 1H, H_{3C}), 4.26-4.36 (m, 8H, OCH_2), 7.28-7.32 (m, 4H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.50-16.65 (m, OCH_2CH_3), 43.16, 45.60, 63.55-64.16 (m, OCH_2CH_3, CP_2), 128.99, 129.36, 134.11, 135.65. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 22.16.

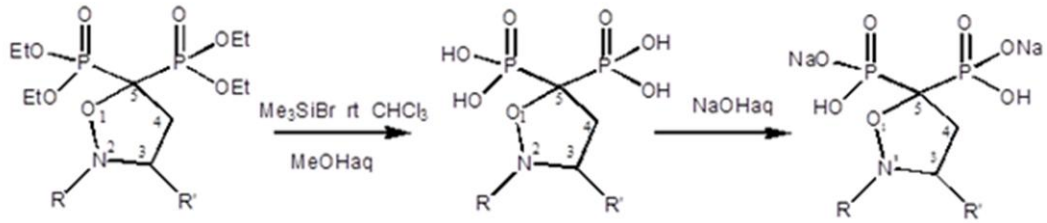
Tetraethyl-2-methyl – 3 –naphthyl - isoxazolidinyl -5, 5 bis phosphonate (3j). Flash chromatography eluent chloroform: acetone (93:7 v:v), Rf 0.50, yield 76%, IR (film, cm^{-1}) 972, 1023, 1258, 1440, 1459, 2933, 2980, 3478; ESI-MS positive ion mode $[M+Na]^+$ m/z 486; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.32-1.47 (m, 12H, CH_3), 2.73 (s, 3H, *N*-CH_3), 2.92-3.05 (m, 1H, H_{4C}), 3.21-3.36 (m, 1H, H'_{4C}), 4.27-4.41 (m, 8H, OCH_2), 4.62-4.73 (m, 1H, H_{3C}), 7.42-7.55 (m, 4H, Ar), 7.70 (d, *J* = 7.02 Hz, 1H, Ar), 7.79 (d, *J* = 8.22 Hz, 1H, Ar), 7.87 (d, *J* = 9.36 Hz, 1H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.49-16.65(m, OCH_2CH_3), 43.66, 44.68, 62.67-64.16 (m, OCH_2CH_3, PCP'), 121.85, 123.45, 125.75, 126.35, 128.32, 128.85, 131.67, 133.83. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 22.09 (d, *J* = 53.33 Hz), 22.64 (d, *J* = 53.33Hz).

Tetraethyl-2-benzyl-3-(para-chloro)-phenyl-isoxazolidinyl -5, 5-bisphosphonate (3k). Flash chromatography eluent chloroform : methanol (98:2 v:v), Rf 0.55, yield, 85%, IR (film, cm^{-1}) 974, 1021, 1250, 1442, 1473, 2932, 2901, 3470; ESI-MS positive ion mode $[M+Na]^+$ m/z 546; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.21-1.28 (m, 6H, CH3), 1.30-1.41 (m, 6H, CH3), 2.82-2.98 (m, 1H, H4C), 3.04-3.13 (m, 1H,H'4C), 3.75 (d, 1H, *J* = 14.1 Hz, HBn), 3.92 (d, 1H, *J*=14.1 Hz, HBn), 3.98-4.13 (m, 4H, OCH2), 4.19-4.33 (m, 5H, OCH2, H3C), 7.18-7.29 (m, 5H, Ar), 7.34 (dd, *J* = 8.44 Hz, 2H, Ar), 7.42 (dd, *J* = 8.44 Hz, 2H, Ar). ^{13}C NMR (500MHz, $CDCl_3$) δ: 16.39-16.57(m, OCH_2CH_3), 45.24, 60.09, 63.44-64.20 (m, OCH_2CH_3), 69-60 (t, *J* = 182.42 Hz, P*C*P'), 127.20, 127.93, 129.11, 129.45, 134.05, 135.89, 137.05. ^{31}P NMR (500MHz, $CDC1_3$) δ: 21.68 (d, *J* = 50.86 Hz), 22.01(d, *J* = 50.86 Hz).

Tetraethyl -2 -benzyl- 3-naphthyl -isoxazolidinyl -5, 5-bis phosphonate (3l). Flash chromatography eluent chloroform : methanol (98:2 v:v), Rf 0.52, yield, 88%, IR (film, cm^{-1}) 975, 1021, 1250, 1442, 1479, 2923, 2970, 3479; ESI-MS positive ion mode $[M+Na]^+$ m/z 562; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.21-1.32 (m, 6H, CH_3), 1.35-1.42 (m, 6H, CH_3), 2.90-3.14 (m, 1H, H_{4C}), 3.21-3.34 (m, 1H, H'_{4C}), 3.83 (d, 1H, *J* = 13.6 Hz, H_{Bn}), 4.01-4.20 (m, 5H, OCH_2, H_{Bn}), 4.26-4.41 (m, 4H, OCH_2), 4.86-5.01 (m, 1H, H_{3C}), 7.19-7.28 (m, 4H, Ar), 7.32 (d, *J* = 7.15 Hz, 2H, Ar), 7.47-7.60 (m, 3H, Ar), 7.78-7.91 (m, 3H, Ar).^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.38-16.65 (m, OCH_2CH_3), 44.12, 60.48, 63.27-64.19 (m, OCH_2CH_3, P*C*P'), 125.81, 126.37, 127.11, 127.45, 127.92, 128.45, 128.66, 128.86, 129.11, 131.68, 133.91, 137.21. ^{31}P NMR (500MHz, $CDC1_3$) δ: 21.67 (d, *J* = 52.07 Hz), 22.45 (d, *J* = 52.07 Hz).

General procedure for hydrolysis of tetraethyl isoxazolidinyl bisphosphonate

A solution of trimethylsilyl bromide (3.7 mmol) dissolved in dichloromethane (1 mL) was added drop wise to a solution of **3** (0.230 g, 0.52 mmol) in dichloromethane (2 mL). The reaction mixturewas stirred at room temperature for 3 days, monitoring the reaction with 1H NMR. The solvent was removed under reduced pressure and the residue dissolved in methanol (2 mL). After stirring for 1 h, the methanol was evaporated in vacuo. The crude was precipitated in methanol/diethyl ether mixture (2:8), filtered and the residue was dried under vacuum to give a white solid. Bisphosphonates are calcium regulating agent used in the form of the sodium salt for a number of reasons that include the specific delivery and absorption of the Drug. For these advantageous and further more investigations, We productively transformed The isoxazolidinyl-substituted bisphosphonic acids into their corresponding disodium salts by reaction of isoxazolidine derivatives stirred at room temperature with 2 equiv of aqueous NaOH. After appropriate time, solvent was removed under reduced pressure and obtained residue was crystallised by treatment of methanol.



3a-h **6a-h** **7a-h**

R=Me	R'= Ph	**6a**	**7a**
R=Me	R'= *o*-Cl-C_6H_4	**6b**	**7b**
R=Me	R'= 3-pyridyl	**6c**	**7c**
R=Me	R'= 2-furyl	**6d**	**7d**
R=Bn	R'= *p*-OH-C_6H_4	**6e**	**7e**
R=Bn	R'= Ph	**6f**	**7f**
R=Bn	R'= *o*-Cl-C_6H_4	**6g**	**7g**
R=Bn	R'= *o*-F-C_6H_4	**6h**	**7h**

2-Methyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6a). Yield 89% (0.151 g), mp=165-166 °C. IR (KBr, cm^{-1}) 939, 1011, 1224, 1463, 1497, 2654; ESI-MS negative ion-mode $[M-H]^-$ m/z 322; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.51 (s, 3H, *N*-CH_3), 2.72-2.85 (m, 1H, H_{C4}), 2.89-2.98 (m, 1H, H'_{C4}), 3.76-3.84 (m, 1H, H_{C3}), 7.30 (br s, 4H, OH), 7.33-7.41 (m, 5H, Ar); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 43.38, 44.76, 72.70, 79.18 (t, J_{PCP} = 152.0 Hz),128.49, 128.90, 129.19, 137.5; ^{31}P NMR (500 MHz, DMSO-d_6) δ: 18.50 (d, *J* = 53.2 Hz), 18.67 (d, J¼53.2 Hz). Anal. Calcd (%) for $C_{10}H_{15}NO_7P_2$: C, 37.16; H, 4.68; N, 4.33. Found (%) C, 37.25; H, 4.64; N, 4.29.

2-Methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6b). Yield 90% (0.191 g), mp = 160-161 °C. IR (KBr, cm^{-1}) 936, 1009, 1223, 1465, 1496, 2663; ESI-MS negative ion-mode $[M-H]^-$ m/z 356; ^1H NMR (500 MHz, DMSO-d_6) δ: 2.58 (s, 3H, *N*-CH_3), 2.60-2.64 (m, 1H, H_{C4}), 2.99-3.02 (m, 1H, H'_{C4}), 4.19-4.29 (m, 1H, H_{C3}), 7.34 (t, 1H, *J* = 7.5 Hz, Ar.H_5), 7.40 (t, 1H, *J* = 7.5 Hz, Ar-H_4), 7.47 (d, 1H, *J* = 7.5 Hz, Ar-H_6), 7.61 (d, 1H, *J* = 7.5 Hz, Ar-H_3), 8.36 (br s, 4H, OH); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 42.89, 42.98, 67.35, 78.41 (t, J_{PCP} = 151.0 Hz), 127.62, 128.47, 129.26, 132.79, 135.25; ^{31}P NMR (500 MHz, DMSO-d_6) δ: 19.04 (d, *J* = 50.2 Hz), 19.14 (d, *J* = 50.2 Hz). Anal. Calcd (%) for $C_{10}H_{14}ClNO_7P_2$: C, 33.58; H, 3.95; N, 3.92. Found (%) C, 33.52; H, 3.99; N, 3.86.

2-Methyl-3-(30-pyridyl)-isoxazolidinyl-5,5-bisphosphonic acid (6c). Colorless oil, yield 82% (0.121 g). IR (film, cm^{-1}) 938, 1011, 1220, 1467, 1491, 2878; ESI-MS negative ion-mode $[M-H]^-$ m/z 323; ^1H NMR (300 MHz, D_2O) δ: 2.88 (s, 3H, *N*-CH3), 3.02-3.07 (m, 1H, H_{C4}), 3.78-3.91 (m, 1H, H'_{C4}), 4.55-4.65 (m, 1H, H_{C3}), 8.20-9.03 (m, 4H, Ar); ^{13}C NMR (300 MHz, D_2O) δ: 43.20, 49.65, 59.09, 70.14, 128.71, 142.24, 142.71, 147.06,

147.83; ^{31}P NMR (500 MHz, D_2O) δ: 18.52 (d, *J* = 50.5 Hz), 18.69 (d, *J* = 50.5 Hz). Anal. Calcd (%) for $C_9H_{14}N_2O_7P_2$: C, 33.35; H, 4.35; N, 8.64. Found (%) C, 33.28; H, 4.38; N, 8.59.

2-Methyl-3-(20-furyl)-isoxazolidinyl-5,5-bisphosphonic acid (6d). Colorless oil, yield 85% (0.147 g). IR (film, cm^{-1}) 929, 1021, 1223, 1465, 1493, 2886; ESI-MS negative ion-mode $[M-H]^-$ m/z 312; ^{1}H NMR (300 MHz, D_2O) δ: 2.90 (s, 3H, *N*-CH3), 3.76-3.89 (m, 1H, H_{C4}), 4.10-4.22 (m, 1H, H'_{C4}), 4.31-4.43 (m, 1H, H_{C3}), 6.57-6.62 (m,1H, Ar), 6.90-6.98 (m,1H, Ar), 7.51-7.78 (m, 1H, Ar), ^{13}C NMR (300 MHz, D_2O) δ: 44.51, 48.75, 60.15, 71.24, 140.33, 143.81, 148.13, 148.72; ^{31}P NMR (500 MHz, D_2O) δ: 18.90. Anal. Calcd (%) for $C_8H_{13}NO_8P_2$: C, 30.68; H, 4.18; N, 4.47. Found (%) C, 30.62; H, 4.22; N, 4.52.

2-Benzyl-3-p-hydroxy-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6e). Colorless oil, yield 83% (0.182 g). IR (film, cm^{-1}) 936, 1015, 1232, 1448, 1496, 2888; ESI-MS negative ion-mode $[M-H]^-$ m/z 414; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.63-2.75 (m, 1H, H_{C4}), 3.25-3.33 (m, 1H, H'_{C4}) 3.82-3.89 (m, 2H, H_{Bn}), 4.40-4.51 (m, 1H, H_{C3}), 7.25-7.90 (m, 14H, Ar, OH); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 45.86, 49.23, 59.23, 71.61, 126.12, 126.80, 127.72, 127.93, 128.33, 128.59, 129.00, 135.16; ^{31}P NMR (500 MHz, DMSO-d_6) δ: 18.66. Anal. Calcd (%) for $C_{16}H_{19}NO_8P_2$: C, 46.28; H, 4.61; N, 3.37. Found (%) C, 46.32; H, 4.56; N, 3.41.

2-Benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6f). Yield 90% (0.224 g), mp = 143-144 °C. IR (KBr, cm^{-1}) 919, 1003, 1230, 1457, 1498, 2885; ESI-MS negative ion-mode $[M-H]^-$ m/z 398; ^{1}H NMR (500 MHz, DMSO-d6) δ: 2.68-2.95 (m, 2H, H_{C4}, H'_{C4}), 3.82 (d, 1H, *J* =15.7 Hz, H_{Bn}), 3.90 (d, 1H, *J* = 15.7 Hz, H_{Bn}), 3.96-4.21 (m, 1H, H_{C3}), 6.37 (br s, 4H, OH),

7.18e7.64 (m, 10H, Ar); ^{13}C NMR (300 MHz, DMSO-d6) δ: 43.51, 59.34, 65.35, 79.69, 128.28, 129.11, 129.41, 129.62, 129.92, 131.20, 135.02, 138.52; ^{31}P NMR (500 MHz, DMSO-d6) δ: 18.54 (d, *J* =52.3 Hz), 18.72 (d, *J* =52.3 Hz). Anal. Calcd (%) for $C_{16}H_{19}NO_7P_2$: C, 48.13; H, 4.80; N, 3.51. Found (%) C, 48.20; H, 4.74; N, 3.48.

2-Benzyl-3-o-chloro-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6g). Yield 92% (0.255 g), mp = 152-153 °C. IR (KBr, cm^{-1}) 938, 1087, 1227, 1443, 1486, 2889; ESI-MS negative ion-mode [M-H]$^-$ m/z 432; ^{1}H NMR (300 MHz, DMSO-d6) δ: 2.61-2.72 (m, 1H, H_{C4}),3.04-3.11 (m, 1H, H'_{C4}), 3.82 (d, 1H, *J* = 15.5 Hz, H_{Bn}), 3.95 (d, 1H, *J* = 15.5 Hz, H_{Bn}), 4.56 (dd, 1H, *J* = 6.3, 9.6 Hz, H_{C3}), 7.16-7.85 (m, 13H,OH, Ar); ^{13}C NMR (300 MHz, DMSO-d6) δ: 43.15 (d, J¼38.7 Hz), 59.85 (t, J¼101.7 Hz), 66.10 (d, *J* = 145.5 Hz), 79.16 (t, J_{PCP} = 151.7 Hz), 125.90, 127.07, 127.18, 127.36, 128.28, 128.77, 129.72, 132.73, 135.90, 137.95; ^{31}P NMR (500 MHz, DMSO-d6) δ: 18.53 (d, *J* = 54.8 Hz), 18.70 (d, *J* = 54.8 Hz). Anal. Calcd (%) for $C_{16}H_{18}ClNO_7P_2$: C, 44.31; H, 4.18; N, 3.23. Found (%) C, 44.37; H, 4.21; N, 3.17. HRMS: calculated for $C_{16}H_{17}NO_7P_2Cl$ 432.0168 and found 432.0163.

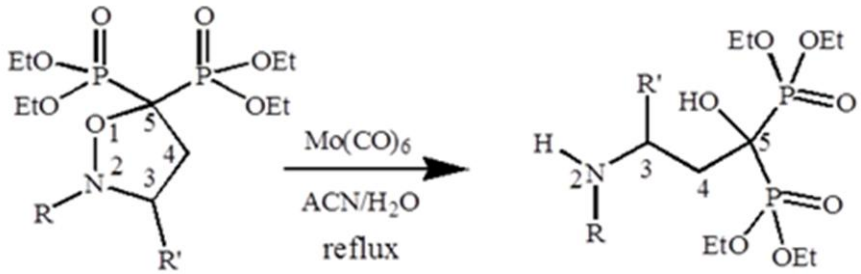
2-Benzyl-3-o-fluoro-phenyl-isoxazolidinyl-5,5-bisphosphonic acid (6h). Yield 91% (0.272 g), mp = 155-156 °C. IR (KBr, cm^{-1}) 948, 1008, 1234, 1458, 1498, 2881; ESI-MS negative ion-mode [M-H]$^-$ m/z 416; 1H NMR (500 MHz, DMSO-d_6) δ: 2.07-2.89 (m, 1H, H_{C4}), 2.96-3.07 (m, 1H, H'_{C4}), 3.84-3.98 (m, 2H, H_{Bn}), 4.42-4.53 (m, 1H, H_{C3}), 7.14-7.68 (m, 9H, Ar), 8.43 (br, s, 4H, OH); ^{13}C NMR (500 MHz, DMSO-d_6) δ: 42.10-43.20 (m), 58.70-59.49 (m), 62.11-63.01(m), 77.40-80.41(m), 124.51-124.96(m), 126.39-126.76(m), 127.23-127.31(m), 127.70-128.55 (m), 129.44-129.69 (m), 137.53-137.75 (m), 160.36 (d, *J* = 245.3 Hz); ^{31}P NMR (500 MHz,

DMSO-d_6) δ: 18.52 (d, J = 54.3 Hz), 18.68 (d, J = 54.3 Hz). Anal. Calcd (%) for $C_{16}H_{18}FNO_7P_2$: C, 46.06; H, 4.35; N, 3.36. Found (%) C, 46.16; H, 4.31; N, 3.40. HRMS: calculated for $C_{16}H_{17}NO_7P_2F$ 416.0464 and found 416.0471.

Ring opening general procedure

A mixture of 1 mmol of isoxazolidine bisphosphonate **2a-i** and 0.7 mmols of $Mo(CO)_6$ were dissolved in 15mL of acetonitrile and 1mL of water. The reaction mixture was refluxed for 4

hours. The reaction color turned from brown to black, indicating completion. The reaction mixture was cooled, filtered through celite and concentrated under reduced pressure. The crude compound was purified by flash column chromatography, finally appearing as colorless thick mass.



3a-b & 3f-l **8a-b & 8f-l**

R=Me	R'= Ph	**8a**
R=Me	R'= *o*-Cl-C_6H_4	**8b**
R=Me	R'= *p*-Cl-C_6H_4	**8f**
R=Me	R'= naphtyl	**8g**
R=Bn	R'= Ph	**8h**
R=Bn	R'= *o*-Cl-C_6H_4	**8i**
R=Bn	R'= *p*-Cl-C_6H_4	**8j**
R=Bn	R'= *o*-F-C_6H_4	**8k**
R=Bn	R'= naphtyl	**8l**

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-phenyl-3-(methylamino)] **(8a).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28-1.41 (m, 12H, CH_3), 2.12- 2.38 (m, 6H, *N*-CH_3, *N*H, H_{4C}, H'_{4C}), 3.83 (m, 1H, H_{3C}), 4.05-4.31 (m, 8H, OCH_2), 4.86-4.99 (m, 1H, *O*H), 7.22-7.40 (m, 5H, Ar), ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.04-16.61 (m,), 33.28, 38.60 , 59.82 (d, J = 11.47 Hz), 63.08-64.51 (m), 70.75 (t, J_{PCP} = 170.94 Hz), 127.31, 127.76, 128.77. ^{31}P NMR (500 MHz, CDC13) δ: 25.04 HRMS: *m/z* calculated for $[C_{18}H_{33}O_7P_2N+H]+$ 438.181055 found 438.18173

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(methylamino)] **(8b).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.28-1.43 (m, 12H, CH_3), 1.89 (bs, 1H, *N*-H), 2.02-2.20 (m, 1H, H_{4C}), 2.23 (s, 3H, *N*-CH_3), 2.31-2.47 (m, 1H, H'_{4C}), 4.08-4.26 (m, 9H, OCH_2, H_3), 5.03- 5.14 (m, 1H, OH), 7.16-7.46 (m, 5H, Ar), ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.03-16.50 (m), 33.72 , 37.62, 56.15 (d, J = 13.22), 62.86- 64.20 (m), 71.80 (t, J_{PCP} = 170.61), 127.11, 127.86, 128.17, 129.84, 132.74, 140.35. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 24.33 (d, J = 22.30 Hz), 25.11 (d, J = 22.30 Hz) HRMS: *m/z* calculated for $[C_{18}H_{32}O_7P_2NCl+H]+$ 472.142083 found 472.14285

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(methylamino)] **(8f).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 78%. 1H NMR (300 MHz, $CDCl_3$) δ: 1.28-1.41 (m, 12H, CH_3), 1.94 (bs, 1H, *N*H) 2.02-2.16 (m, 2H, H_{4C}, H'_{4C}), 2.22 (s, 1H, *N*-CH_3), 3.78 (dd, 1H, J = 4.38, 9.54 Hz, H_{3C}), 4.10-4.26 (m, 8H, OCH_2), 4.83-4.96 (m, 1H, OH), 7.20-7.27 (m, 2H, Ar), 7.29- 7.35 (m, 2H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.05-16.51(m), 33.83, 39.24 , 59.38 (d, J = 11.47 Hz), 63.01 (dd, J = 6.88, 12.62 Hz), 64.20-64.28 (m), 70.50 (t, J_{PCP}

= 170.95 Hz), 128.42, 128.72, 132.84, 141.75. ^{31}P NMR (500 MHz, CDC1$_3$) δ: 24.63. HRMS: *m/z* calculated for [$C_{18}H_{32}O_7P_2NCl$+H]+ 472.142083 found 472.141530.

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(naphtyl) -3-(methylamino)] **(8g).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 80%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18-1.44 (m, 12H, CH_3), 2.14 (bs, 1H, *N*H), 2.18-2.35 (m, 5H, *N*-CH_3, H_{4C}, H'$_{4C}$), 3.90-4.32, 4.10-4.30 (m, 8H, OCH_2), 4.79 (dd, 1H, *J* = 2.74, 9.87 Hz, H_{3C}), 5.12-5.26 (m, 1H, OH), 7.42-7.61 (m, 4H, Ar), 7.50 (d, 1H, *J* = 7.89 Hz, Ar), 7.53 (d, 1H, *J* = 7.24 Hz, Ar), 7.59 (d, 1H, *J* = 7.90 Hz, Ar). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 16.19-16.42 (m), 33.85, 39.10 , 55.31-55.53 (m), 62.69-64.36 (m), 71.25 (t, J_{PCP} = 173.62 Hz), 123.23, 125.51, 125.63, 126.01, 127.64, 129.02, 131.99, 132.02, 134.34, 139.07. ^{31}P NMR (500 MHz, CDC1$_3$) δ: 25.64, HRMS: *m/z* calculated for [C22H35O7P2N+H]+ 488.196152 found 488.19822.

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(phenyl) - 3-(benzylamino)] **(8h).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 75%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23-1.42 (m, 12H, CH_3), 2.08- 2.32 (m, 2H, H_{4C}, *N*H), 2.34-2.61 (m, 1H, H'$_{4C}$), 3.55 (d, 1H, *J* = 12.93 Hz, H_{Bn}), 3.67 (d, 1H, *J* = 12.93 Hz, H_{Bn}), 3.97-4.37 (m, 9H, OCH_2, H_{3C}), 4.62-4.71 (m, 1H, OH), 7.14-7.44 (m, 10H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.21-16.69 (m), 37.67 , 50.93 , 60.69 (d, *J* = 14.72 Hz), 62.82-64.12 (m), 71.74 (t, J_{PCP} = 171.33 Hz), 126.27, 126.91, 127.44, 127.80, 128.37, 129.06, 138.40, 141.80. ^{31}P NMR (500 MHz, CDC1$_3$) δ: 24.58 (d, *J* = 39.67 Hz), 25.78 (d, *J* = 39.67 Hz). HRMS: *m/z* calculated for [$C_{24}H_{37}O_7P_2N$+H]+ 514.211802 found 514.21317.

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(benzylamino)] **(8i).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 81%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27-1.41 (m, 12H, CH_3), 1.73 (bs, 1H, *N*H), 2.22-2.30 (m, 1H, H_{4C}), 2.45-2.64 (m, 1H, H'_{4C}), 3.50 (d, 1H, J = 12.47 Hz, H_{Bn}), 3.68 (d, 1H, J = 12.47 Hz, H_{Bn}), 4.09-4.39 (m, 9H, OCH_2, H_{3C}), 5.19-5.28 (m, 1H, OH), 7.16-7.36 (m, 8H, Ar), 7.41 (d, 1H, J = 7.89 Hz,). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.51-16.69 (m), 35.61, 50.86, 63.23-63.88 (m), 72.73 (t, J_{PCP} = 169.41 Hz), 127.47, 127.56, 128.36, 128.45, 128.56, 128.90, 129.26, 130.26, 136.73, 138.36. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 20.94 (d, J = 38.45 Hz), 21.81 (d, J = 38.45 Hz). HRMS: *m/z* calculated for $[C_{24}H_{36}O_7P_2NCl+H]+$ 548.172830 found 548.17352.

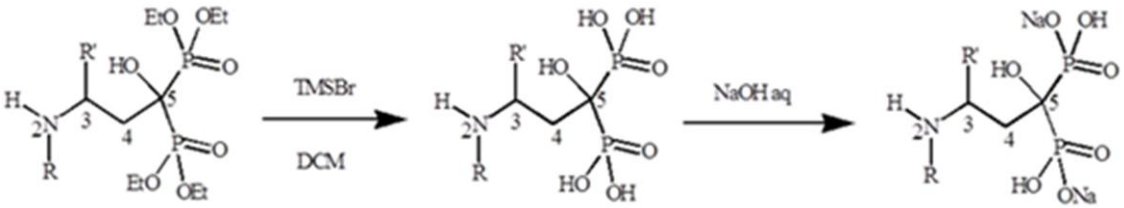
Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(benzylamino)] **(8j).** Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 76%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.19-1.41 (m, 12H, CH_3), 2.01- 2.28 (m, 2H, H_{4C}, *N*H), 2.30-2.65 (m, 1H, H'_{4C}), 3.52 (d, 1H, J = 12.27 Hz, H_{Bn}), 3.66 (d, 1H, J = 12.27 Hz, H_{Bn}), 3.91-4.35 (m, 9H, OCH_2, H_{3C}), 4.62-4.71 (m, 1H, OH), 7.15-7.42 (m, 9H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.11-16.57 (m), 37.95, 51.03, 59.93 (d, J = 13.22 Hz), 62.95-64.20 (m, P*C*P), 126.92, 127.78, 128.37, 128.67, 128.87, 129.26, 138.51, 140.63. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 20.90 (d, J = 38.46 Hz), 22.08 (d, J = 38.46 Hz). HRMS: *m/z* calculated for $[C_{24}H_{36}O_7P_2NCl+H]+$ 548.172830 found 548.17380.

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-fluoro phenyl)-3-(benzylamino)] **(8k).**Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 87%. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.22-1.43 (m, 12H, CH_3), 1.85 (bs, 1H, *N*H), 2.21-2.35 (m, 1H, H_{4C}), 2.52-2.70 (m, 1H, H'_{4C}),

3.55 (d, 1H, J = 12.45 Hz, H_{Bn}), 3.72 (d, 1H, J = 12.45 Hz, H_{Bn}), 4.07-4.38 (m, 9H, OCH_2, H_{3C}), 4.91-5.01 (m, 1H, OH), 7.14-7.32 (m, 9H, Ar). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 15.99-16.56 (m), 36.23, 51.30, 56.15 (d, J = 13.82 Hz), 62.17-64.74 (m, PCP), 116.13, 125.90 (d, J_F = 208.45 Hz), 127.46, 127.92, 128.26, 128.37, 128.57, 129.15, 129.42, 138.47. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 20.83 (d, J = 38.43 Hz), 22.10 (d, J = 38.43 Hz). HRMS: *m/z* calculated for $[C_{24}H_{36}O_7P_2NF+H]+$ 532.202933 found 532.20384.

Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(naphtyl) -3-(benzylamino)] **(8l).**Flash chromatography eluent chloroform: methanol (98:2 v:v), yield 90%. ^1H NMR (300 MHz, $CDCl_3$) δ: 1.17-1.44 (m, 12H, CH_3), 1.84 (bs, 1H, *N*H), 2.15-2.75 (m, 2H, H_{4C}, H'_{4C}), 3.54 (d, 1H, J = 12.42 Hz, H_{Bn}), 3.72 (d, 1H, J = 12.42 Hz, H_{Bn}), 4.05-4.51 (m, 9H, OCH_2, OH), 4.94-5.06 (m, 1H, H_{3C}), 7.18-7.95 (m, 11H, Ar), 8.42 (d, 1H, J = 8.19 Hz).^{13}C NMR (500 MHz, $CDCl_3$) δ: 16.00-16.69 (m), 37.83, 51.14, 63.07-64.22 (m, P*C*P'), 123.33, 125.54, 126.04, 126.57, 127.49, 128.17, 128.29, 128.37, 128.44, 128.64, 128.92, 131.90, 139.17, 141.12. ^{31}P NMR (500 MHz, $CDC1_3$) δ: 21.10 (d, J = 37.20 Hz), 22.25 (d, J = 37.20 Hz). HRMS: *m/z* calculated for $[C_{28}H_{39}O_7P_2N+H]+$ 564.227452 found 564.22850.

Ester hydrolysis followed by disodium salt formation general procedure



8a,b,f-l **9a,b,f-l** **10a,b,f-l**

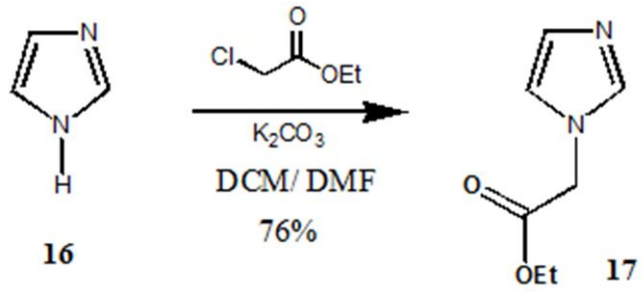
R=Me	R'= Ph	**9a**	**10a**
R=Me	R'= *o*-Cl-C_6H_4	**9b**	**10b**
R=Me	R'= *p*-Cl-C_6H_4	**9f**	**10f**
R=Me	R'= naphtyl	**9g**	**10g**
R=Bn	R'= Ph	**9h**	**10h**
R=Bn	R'= *o*-Cl-C_6H_4	**9i**	**10i**
R=Bn	R'= *p*-Cl-C_6H_4	**9j**	**10j**
R=Bn	R'= *o*-F-C_6H_4	**9k**	**10k**
R=Bn	R'= naphtyl	**9l**	**10l**

A solution of trimethylsilyl bromide (3.7 mmol) dissolved in dichloromethane (1 mL) was added drop wise to a solution of the bisphosphonate **3a-i** (0.52 mmol) in dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 3 days, monitoring the reaction with 1H NMR. The solvent was removed under reduced pressure and the residue dissolved in methanol (2 mL). After stirring for 1 h, the methanol was evaporated in vacuo. The crude was precipitated in methanol/diethyl ether mixture (2:8), filtered and the residue was dried under vacuum to give a white solid corresponding to the acid **4a-i**. As requested by the biological activity assays, We productively transformed The 1,1 hydroxy bisphosphonic acids into their corresponding disodium salts by reaction of ring opened derivatives stirred at room temperature with 2 equiv of aqueous NaOH. After appropriate time,

solvent was removed under reduced pressure and obtained residue was crystallised by treatment of methanol.

Synthesis of Zoledronic Acid

Preparation of imidazol-1-yl-acetic acid ethyl ester (**17**)



Compound	Quantity	Density(g/lit)	MW (g/mol)	mmol	Eq
Imidazole	5.0 g	1.23	68.07	73.453	1
K_2CO_3	15.22 g		138.20	110.180	1.5
ethyl chloroacetate	7.89 mL	1.14	122.55	73.453	1
TBAB	Catalytic amount		322.36		

Experimental procedure

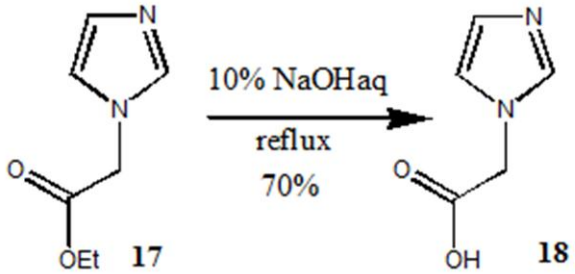
To a solution of imidazole (5.0 g, 73.4537 mmol) in dichlomethane (50 mL) and 5 mL of DMF was added powdered K_2CO_3 (29.0 g, 0.21 mol), followed ethyl chloroacetate (25.7 mL, 0.18 mol) and pinch of tetrabutyl ammonium bromide at room temperature. The mixture was refluxed for 10.0 h. After completion of reaction , the reaction mass was quenched with cold water (50 mL) and the DCM layer was separated. The aqueous layer was extracted with DCM (2 × 25 mL) and the combined DCM layers were washed with brine, dried with anhydrous sodium sulfate and then

concentrated under vacuum. The resulting solid was stirred with hexane (50 mL) at RT, filtered and washed with hexane (2 × 20 mL) to afford the title compound as an off-white solid.

Spectral Analysis

Imidazol-1-yl-acetic acid ethyl ester **(17)**. White solid (yield 76 %), mp 111-113 ^{o}C, GC/MS m/z 154, IR (cm−1): 3458, 3132, 3115, 2999, 2981, 2884, 1740, 1508, 1380, 1288, 1236, 1154, 1079, 908, 855, 819, 745, 662, 583; 'H NMR (300MHz, $CDCl_3$) δ: 1.28 (t, *J*= 7.08 Hz, 3H), 4.22 (q, *J*= 7.05 Hz, 2H), 4.69 (s, 2H), 6.95 (d, *J*= 1.32 Hz, 1H), 7.07 (d, *J*= 1.11 Hz, 1H), 7.49 (s, 1H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ: 14.23, 56.42. 61.89, 119.8, 129.2, 137.7, 166.3

Preparation of imidazol-1-yl-acetic acid (18)



Compound	Quantity	MW (g/mol)	mmol	Eq
Comp.**17**	2.0 g	154.07	12.981	1
10% NaOH		5mL		
Ethanol		15mL		
1N HCl		15 mL		

Experimental procedure

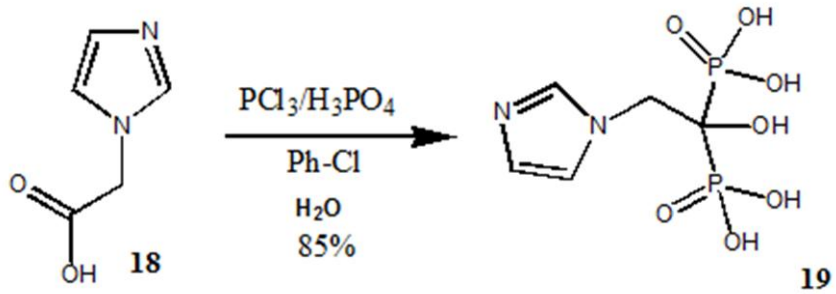
To a solution of imidazol-1-yl-acetic acid ethyl ester **(17)** (2.0 g, 12.981 mmol) in ethanol (15 mL) was added 10% aq sodium hydroxide (5 mL) dropwise slowly at 0 °C for 1 h and the mixture was stirred at room

temperature for 2 h. Additional 10% aq sodium hydroxide (2-3 mL) was added dropwise at room temperature and the mixture was stirred for 1 h. After complitation of reaction mixture, ethnol was evaporated under reduced pressure and residue was neutralised using 1N HCl solution. Resulting crystalline solid precipitated was filtered to afford the title compound as an off-white crystalline solid.

Spectral Analysis

imidazol-1-yl-acetic acid **(18)**. White solid (yield 76 %), mp 200-2002 ^{o}C, GC/MS m/z 126, IR (cm−1): 3175, 3125, 3064, 2945, 2869, 2524, 2510, 1732, 1581, 1547, 1403, 1223, 1193, 1081, 780, 650; 'H NMR (300MHz, $CDCl_3$) δ: 4.36 (s, 2H), 6.85 (d, *J*= 1.02 Hz, 1H), 7.04 (d, *J*= 1.17 Hz, 1H), 7.54 (s, 1H) ; ^{13}C NMR (300 MHz, $CDCl_3$) δ: 57.28, 120.80, 129.2, 136.99, 175.36.

Preparation of zoledronic acid (19)

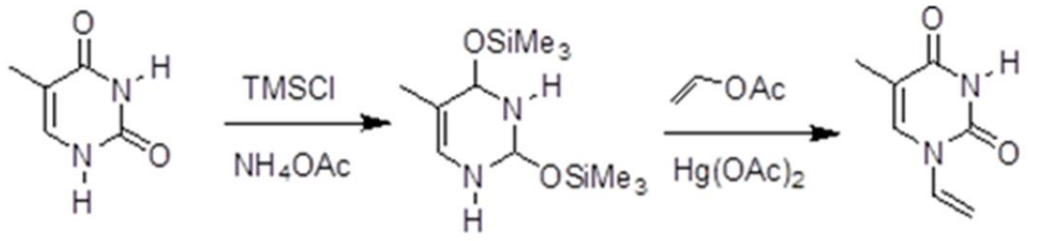


Compound	Quantity	Density (g/lit)	MW (g/mol)	mmol	Eq
Comp.**18**	0.411 g		126.04	3.260	1
H_3PO_4 (85%)	0.570 mL	1.68	97.99	9.782	3
PCl_3	0.570 mL	1.57	137.33	6.521	2
Cholorobenzene		3mL			

Experimental procedure

To a suspension of imidazol-1-yl-acetic acid **(3)** 0.411 g, 3.260 mmol) and phosphorous acid (0.570 mL, 9.782 mmol) in chlorobenzene (3 mL) was added phosphorous trichloride (0.570 mL, 6.521 mmol) at 80–85 °C over a period of 2 h then heated to 90–95 °C for 2.5 h. The reaction mass was cooled to 60–65 °C and water (10 mL) was added at the same temperature. The aqueous layer was separated, collected and refluxed for 18 h. It was then cooled to room temperature and diluted with methanol (15 mL). The mixture was cooled to 0–5 °C and stirred for 3 h. The precipitated solid was filtered, washed with cold water followed by methanol and then dried under vacuum at 60 °C for 12 h to afford the title compound (85% yield) as a white solid; mp 237–239 °C with decomposition).

Experimental procedure for Synthesis of vinyl thymine.



20 **21** **23**

Compound	Quantity	Density (g/lit)	MW (g/mol)	mmol	Eq
Thymine	0.55 g		126.11	4.36	1
hexamethyldisilazane	3.27 g	0.774	161.39	15.70	3.6
trimethylsilyl chloride	0.25 g	0.856	108.64	1.96	0.45
$(NH_4)_2SO_4$		Trace amount			
vinyl acetate	25 mL	0.934	86.09	271.22	62.20
$Hg(OAc)_2$	0.096 g	3.27	318.07	0.300	0.068
trimethylsilyl trifluoromethane	0.2 mL	1.228	222.26	1.1	0.25

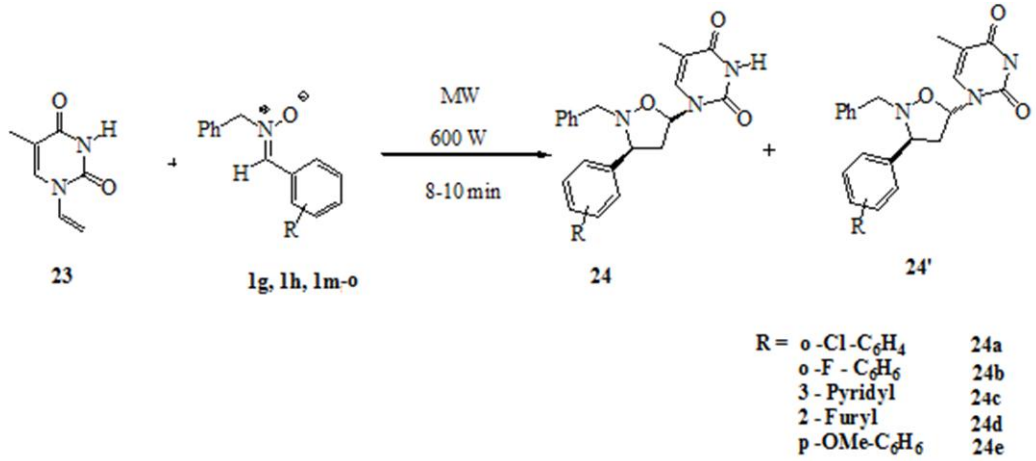
sulfonate					
hydroquinone	0.1 g	1.3	110.11	0.908	0.2

Thymine (0.55 g, 4.4 mmol) was heated at 140–150 °C with hexamethyldisilazane (3.27 g, 15.70 mmol), trimethylsilyl chloride (0.25 g, 1.96 mmol) and a trace of $(NH_4)_2SO_4$ until a clear solution was formed. Then, the solution was concentrated in vacuo. The residue was suspended in vinyl acetate (25.0 mL) and $Hg(OAc)_2$ (0.096 g, 0.3 mmol), trimethylsilyl trifluoromethane sulfonate (0.2 mL, 1.1 mmol), and hydroquinone (0.1 g, 0.908 mmol), the latter as as a polymerisation inhibitor, were added under N_2. The mixture was refluxed for the appropriate time given in the Scheme. When the reaction was finished, the mixture was filtered through neutral activated alumina and was washed with EtOAc. The solvents were removed at reduced pressure and the crude product was purified by flash chromatography with $CHCl_3$–MeOH (92.5:7.5) as eluent.

Spectral Analysis

Flash chromatography eluent chloroform: methanol (92.5:7.5 v:v), yield 79%. white solid; mp 205–207 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.84 (d, *J* = 1.44Hz, 3H, CH_3,), 4.84 (dd, *J*gem = 1.95, *Jcis* = 9.27 Hz 1H, 2'-CH), 5.33 (dd, *J*gem = 1.95, *Jtrans* = 16.09 Hz, 1H, 2'-CH), 7.10 (dd, *Jcis* = 9.27,*Jtrans* = 16.09 Hz, 1H, 1'-CH), 7.90 (d, *J* = 1.44 Hz, 1H, CH,), 11.51 (br s, 1H, NH). ^{13}C NMR (500 MHz, DMSO-d_6) δ: 11.9, 99.7, 110.61, 129.1, 134.9, 149.3, 163.6. MS: *m*/*z* = 153 [M + H]+, 110, 82. HRMS: *m*/*z* calcd for $C_7H_8N_2O_2$: 152.15070; found: 152.15073. Anal. Calcd for $C_7H_8N_2O_2$: C 55.26, H 5.30, N 18.41, O 21.03.Found: C 55.59, H 5.72, N 17.93.

General procedure for 1,3 dipolar cycloadditon of vinyl thymine and nitrones.



The vinyl nucleobase (0.1 mmol) and the nitrone (0.13 mmol) were placed in a 10 mL Pyrex container and irradiated at 600 W power using an unmodified ousehold microwave oven.After the appropriate time the reaction mixture is submitted to flash chromatography, using mixtures of chloroform and methnol (98.75:1.25). The nitrone in excess is recovered and may be re-used. The cycloadducts were analyzed by HPLC and H NMR to establish the diastereo isomeric cis–trans ratio. Yields were calculated on isolated compounds.

***cis-4-Aza-4-(N-Benzyl)-3-o-chlorophenyl-2,3-dideoxythymidine* (24a)**

White solid, Flash chromatography eluent chloroform/methanol (98.75:1.25 v/v) Rf 0.65, (yield, 75%), mp 148-150^{o}C, ESI-MS positive mode$[M+H]^{-}$ m/z 398.12; IR (KBr, cm^{-1}) 3165, 3035, 1693, 1461, 1272, 1088, 903, 768; ^{1}H NMR (500 MHz, $CDCl_3$) $\partial_{\mathbf{H}}$ 1.80 (d, *J*= 1.13 Hz, 3H, *Thy*-CH_3), 2.20-2.25 (ddd, *J*= 3.78, 9.44 & 13.60 Hz, 1H, H_C), 3.48-3.54 (ddd, *J*= 1.13, 7.93, & 14.36 Hz, 1H, H_b), 3.82(d, *J*= 14.35 Hz, 1H, *N* - CH), 4.08 (d, *J*= 13.98 Hz, 1H, *N* – CH'), 4.46-4.49 (dd, *J*= 7.93 & 9.44 Hz, 1H, H_d), 6.05-6.07 (dd, *J*= 3.78 & 6.80 Hz, 1H, H_a), 7.32-7.42 (m, 9H,

Ar), 7.57- 7.59 (dd, *J*= 1.51 & 7.55 Hz, 1H, Ar), 8.75 (bs, 1H, NH), ^{13}C NMR (500MHz, $CDCl_3$) ∂_C 12.54, 46.37, 60.03, 66.08, 83.56, 109.86, 127.41, 127.68, 127.87, 128.54, 128.84, 129.25, 130.10, 133.93, 135.09, 135.76, 136.81, 150.35, 163.96.

***cis-4-Aza-4-(N- Benzyl)-3-o-fluorophenyl-2,3-dideoxythymidine* (24b)**

White solid, Flash chromatography eluent chloroform/methanol (98.75:1.25 v/v) Rf 0.68, (yield, 71%), mp 176-178^{o}C, ESI-MS positive mode$[M+H]^-$ m/z 382.39; IR (KBr, cm^{-1}); ^{1}H NMR (500 MHz, $CDCl_3$) ∂_H 1.81 (d, *J*= 0.76 Hz, 3H, *Thy*-CH_3), 2.37-2.42 (ddd, *J*= 3.78, 9.82 & 13.97 Hz, 1H, H_C), 3.34-3.76 (ddd, *J*= 1.13, 7.95, & 14.35 Hz, 1H, H_b), 4.05(d, *J*= 14.36 Hz, 1H, *N* - CH), 4.25 (d, *J*= 14.35 Hz, 1H, *N* – CH'), 4.26-4.28 (dd, *J*= 7.93 & 9.44 Hz, 1H, H_d), 6.07-6.09 (dd, *J*= 3.77 & 7.55 Hz, 1H, H_a), 7.08-7.19 (t, *J*= 9.44 & 17.75 Hz, 1H, Ar), 7.20- 7.29 (t, *J*= 7.55 & 15.11 Hz, 1H, Ar), 7.30-7.48 (m, 8H, Ar), 8.94(bs, 1H, NH); ^{13}C NMR (500MHz, $CDCl_3$) ∂_C 12.51, 46.25, 59.96, 63.46, 83.45, 109.93, 116.09 (d, *J*= 22.41Hz), 123.88 (d, *J*= 11.74Hz), 124.87 (d, *J*= 3.2 Hz), 127.81, 128.27(d, *J*= 3.2 Hz), 128.48, 128.80, 129.96(d, *J*= 8.54 Hz), 135.99,136.81, 150.47, 160.12, 162.09, 164.11.

***cis-4-Aza-4-(N- Benzyl)-3-pyridyl-2,3-dideoxythymidine* (24c)**

White solid, Flash chromatography eluent chloroform/methanol (98.75:1.25 v/v) Rf 0.63, (yield, 83%), mp 160-162^{o}C, ESI-MS positive mode$[M+H]^-$ m/z 365.40; IR (KBr, cm^{-1}) 3175, 3032, 1689, 1469, 1274, 966, 713; ^{1}H NMR (500 MHz, $CDCl_3$) ∂_H 1.86 (d, *J*= 0.76 Hz, 3H, *Thy*-CH_3), 2.24-2.39 (ddd, *J*= 3.78, 9.82 & 13.60 Hz, 1H, H_C), 3.48-3.54 (t, *J*= 7.56, & 14.35 Hz, 1H, H_b), 3.96 (d, *J*= 13.98 Hz, 1H, *N* - CH), 3.97 (t, *J*= 7.93 & 9.82 Hz, 1H, H_d), 4.01 (d, *J*= 13.98 Hz, 1H, *N* – CH'), 6.03-6.05 (dd, *J*= 3.78 & 7.56 Hz, 1H, H_a), 7.29-7.71 (m, 8H, Ar), 8.60 (d, *J*= 1.89

Hz, 1H, Ar), 8.62 (dd, *J*= 1.51 & 4.91 Hz, 1H, Ar), 8.66 (d, *J*= 7.94 Hz, 1H, Ar), 9.25 (bs, 1H, NH); ^{13}C NMR (300MHz, $CDCl_3$) ∂_C 12.56, 48.36, 59.66, 67.95, 83.44, 110.09, 124.04, 127.98, 128.54, 128.93, 132.68, 135.09, 135.41, 136.31, 149.43, 150.15, 150.41, 164.08.

cis-4-Aza-4-(N- Benzyl)-3-furyl-2,3-dideoxythymidine **(24d)**

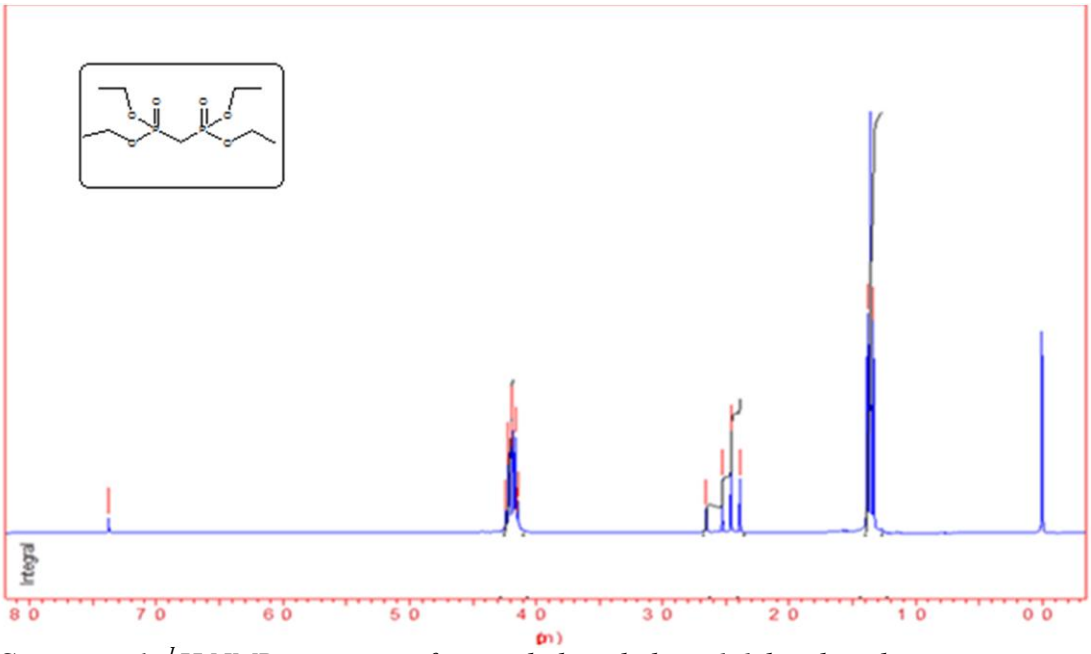
White solid, Flash chromatography eluent chloroform/methanol (98.75:1.25 v/v) Rf 0.62, (yield, 85%), mp 158-160^{o}C, ESI-MS positive mode[M+H]$^-$ m/z 354.38; IR (KBr, cm^{-1}); ^{1}H NMR (500 MHz, $CDCl_3$) ∂_H 1.81 (d, *J*= 1.08 Hz, 3H, *Thy*-CH_3), 2.63-2.72 (ddd, *J*= 3.69, 9.56 & 13.69 Hz, 1H, H_C), 3.17-3.26 (t, *J*= 7.60, & 13.91 Hz, 1H, H_b), 3.79 (d, *J*= 14.13 Hz, 1H, *N* - CH), 4.02 (dd, *J*= 7.83 & 9.78 Hz, 1H, H_d), 4.25 (d, *J*= 14.35 Hz, 1H, *N* – CH'), 6.11-6.15 (dd, *J*= 3.91 & 7.60 Hz, 1H, H_a), 6.28 (d, *J*= 1.31 Hz 1H, Ar), 7.30-7.52 (m, 7H, Ar), 9.07 (bs, 1H, NH) ; ^{13}C NMR (300MHz, $CDCl_3$) ∂_C

cis-4-Aza-4-(N- Benzyl)-4-p-Methoxyphenyl-2,3-dideoxythymidine **(24e)**

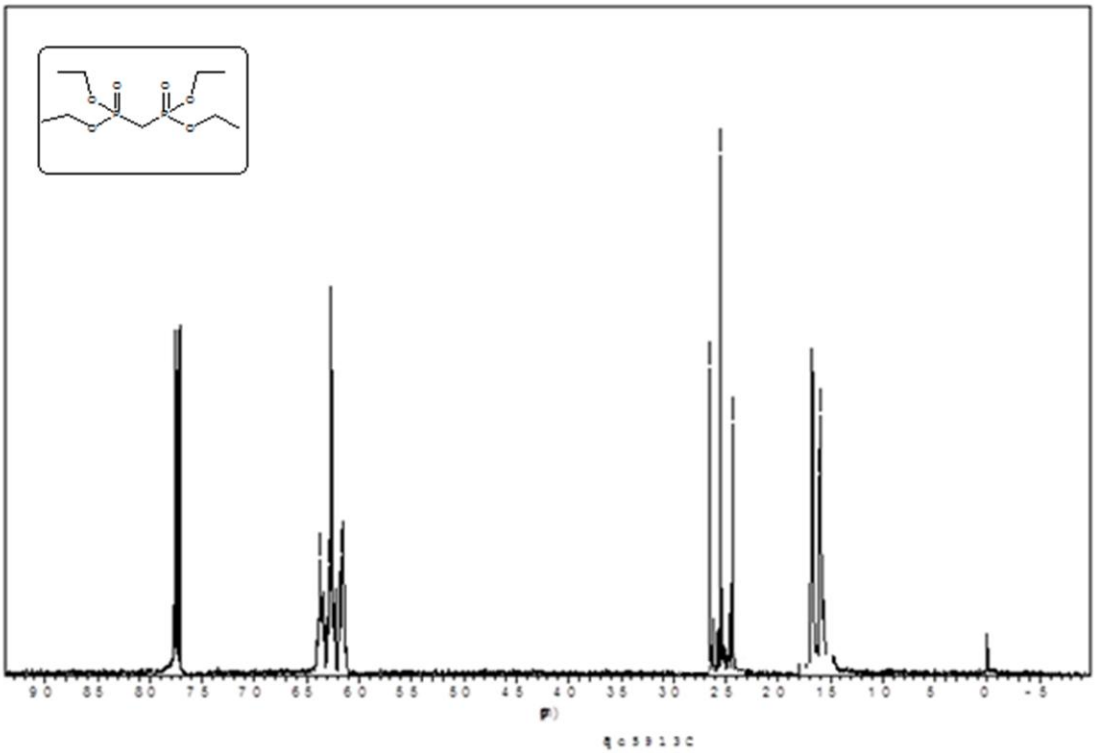
White solid, Flash chromatography eluent chloroform/methanol (98.75:1.25 v/v) Rf 0.65, (yield, 80%), mp 148-150^{o}C, ESI-MS positive mode[M+H]$^-$ m/z 394.42; IR (KBr, cm^{-1}) 3154, 3038, 1689, 1468, 1276, 1080, 910, 765; ^{1}H NMR (500 MHz, $CDCl_3$) ∂_H 1.82 (d, *J*= 1.11 Hz, 3H, *Thy*-CH_3), 2.28-2.34 (ddd, *J*= 3.69, 9.28 & 13.46 Hz, 1H, H_C), 3.52-3.61 (ddd, *J*= 1.12, 7.91, & 14.34 Hz, 1H, H_b), 3.70 (s, 3H, *O*-CH_3), 3.90 (d, *J*= 14.35 Hz, 1H, *N* - CH), 4.18 (d, *J*= 14.42 Hz, 1H, *N* – CH'), 4.50-4.53 (dd, *J*= 7.93 & 9.44 Hz, 1H, H_d), 6.15-6.17 (dd, *J*= 3.76 & 6.78 Hz, 1H, H_a), 7.30-7.40 (m, 9H, Ar), 7.58- 7.61 (dd, *J*= 1.50 & 7.55 Hz, 1H, Ar), 8.73 (bs, 1H, NH), ^{13}C NMR (500MHz, $CDCl_3$) ∂_C 12.51, 46.27, 54.32, 61.03, 66.28, 83.46, 110.06, 127.56, 127.78, 127.93, 128.44, 128.63, 129.18, 130.21, 134.02, 134.98, 135.63, 136.78, 150.45, 164.09.

Chapter 7

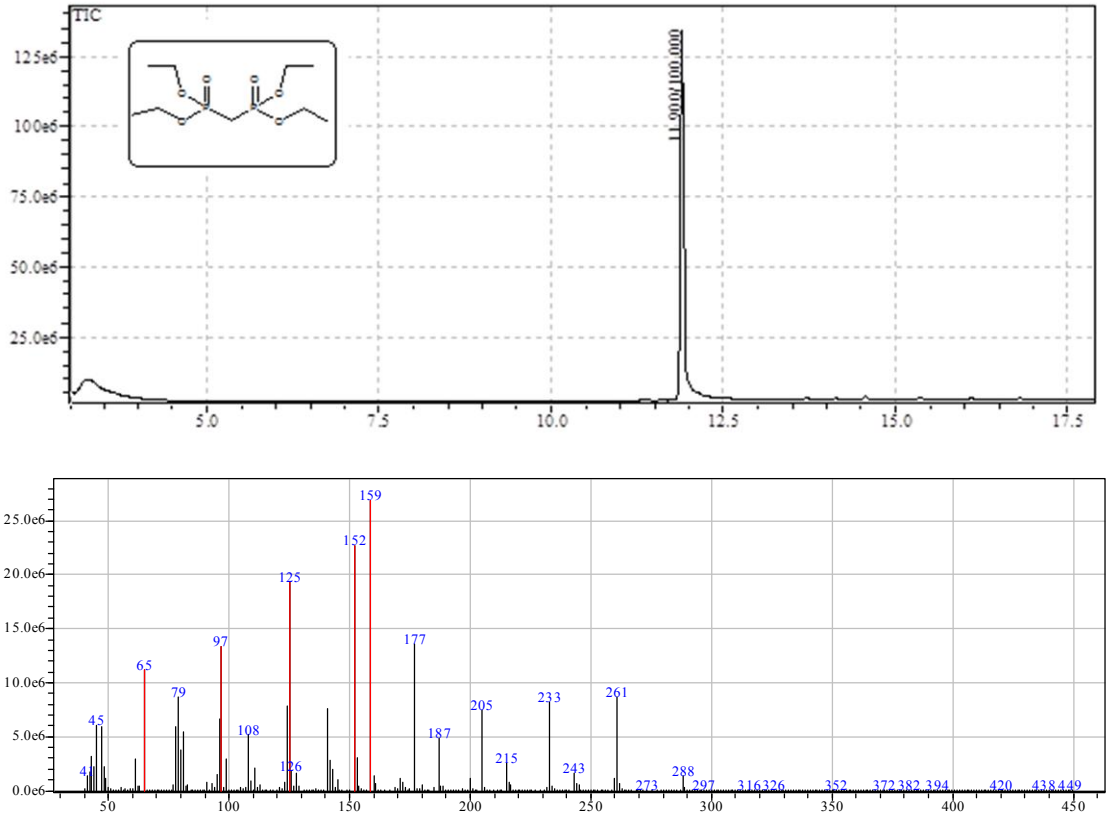
Spectral Data



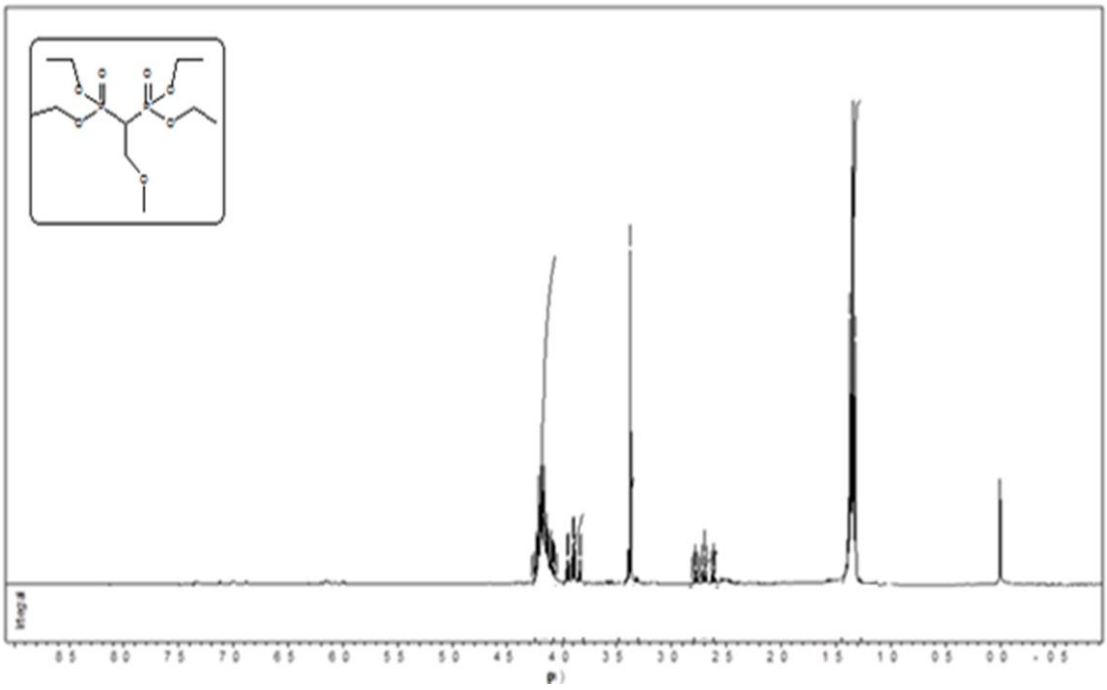
***Spectrum 1**: [1]H NMR spectrum of tetraethyl methylene-1,1-bisphosphonate*



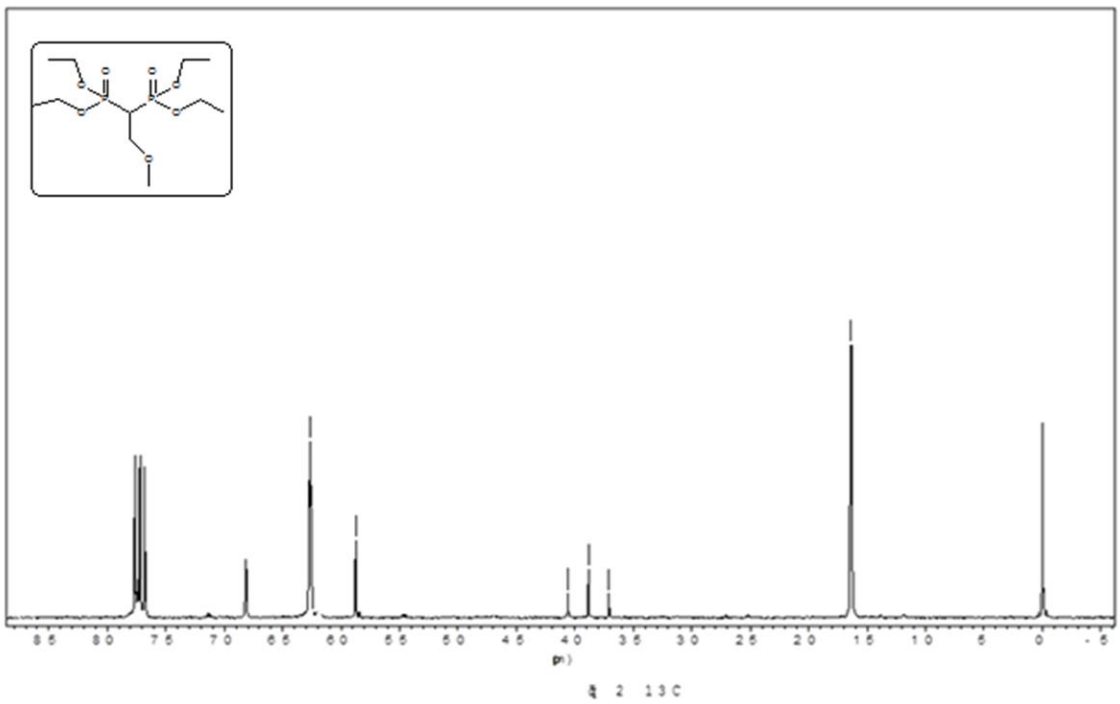
***Spectrum 2:** [13]C NMR spectrum of tetraethyl methylene-1,1-bisphosphonate*



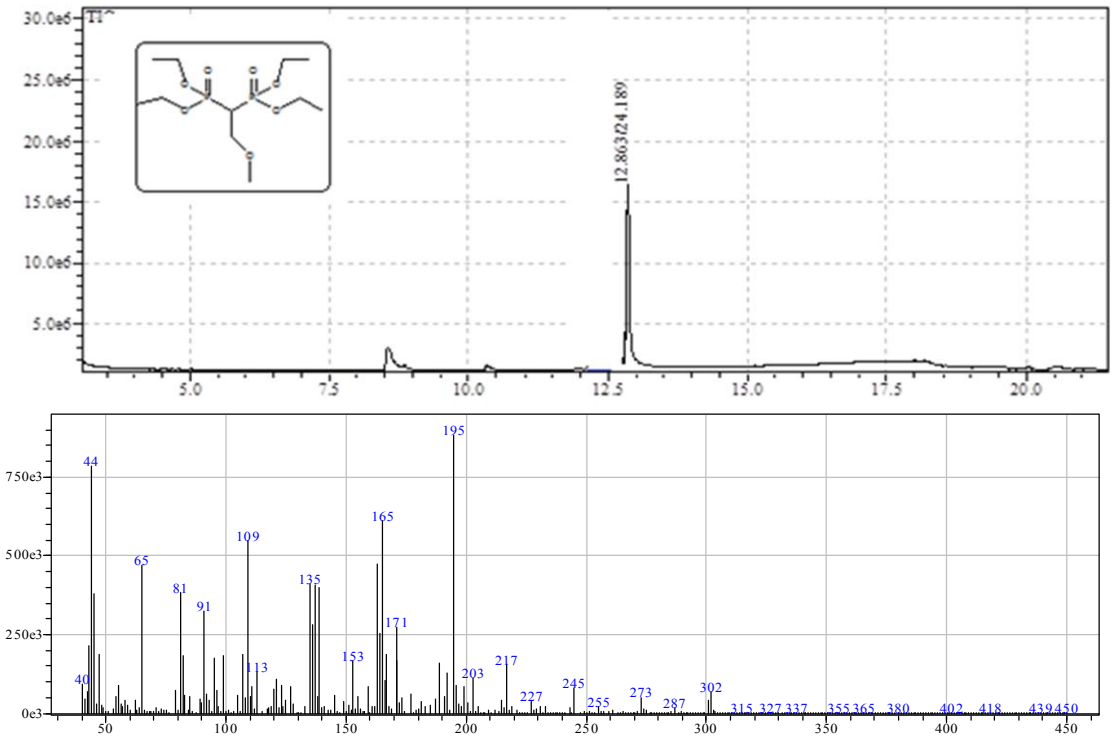
Spectrum 3: *GC spectrum of tetraethyl methylene-1,1-bisphosphonate*



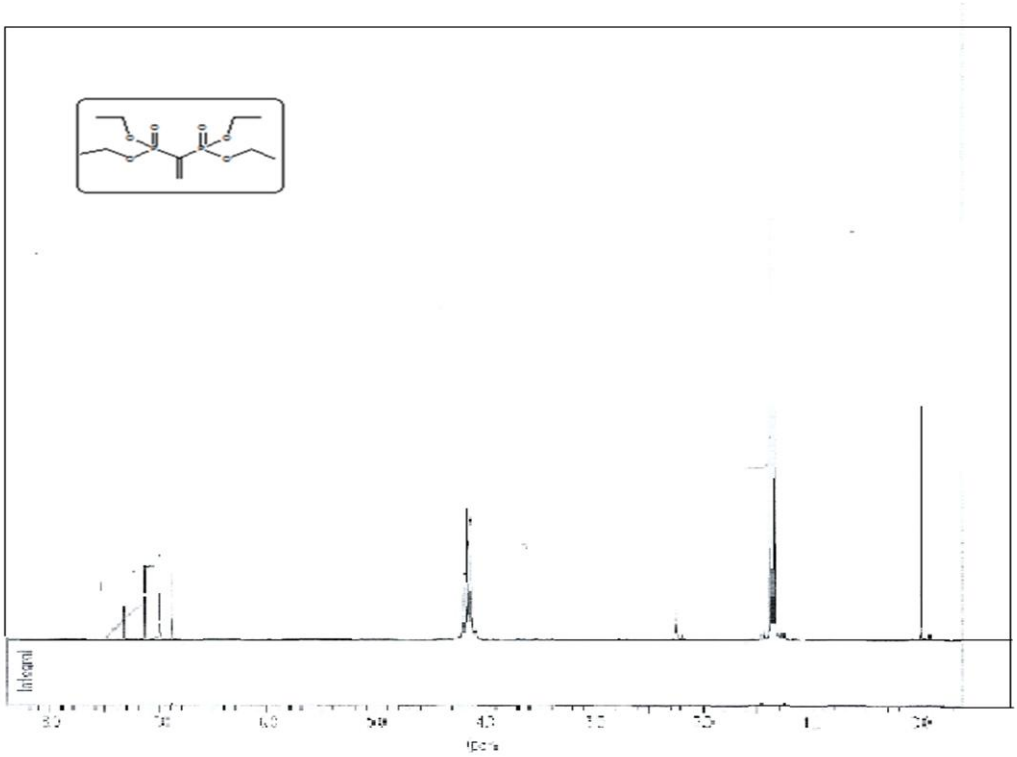
Spectrum 4: *^{1}HNMR spectrum of tetraethyl 2-methoxyethylene-1,1-bisphosphonate*



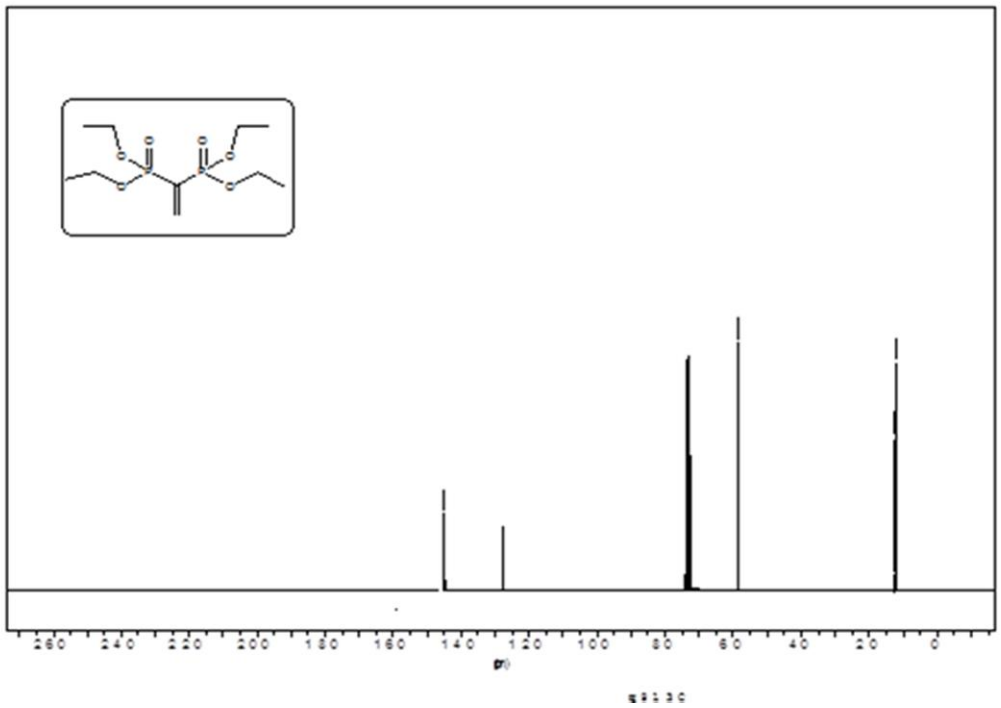
Spectrum 5: *^{13}CNMR spectrum of tetraethyl 2-methoxyethylene-1,1-bisphosphonate*



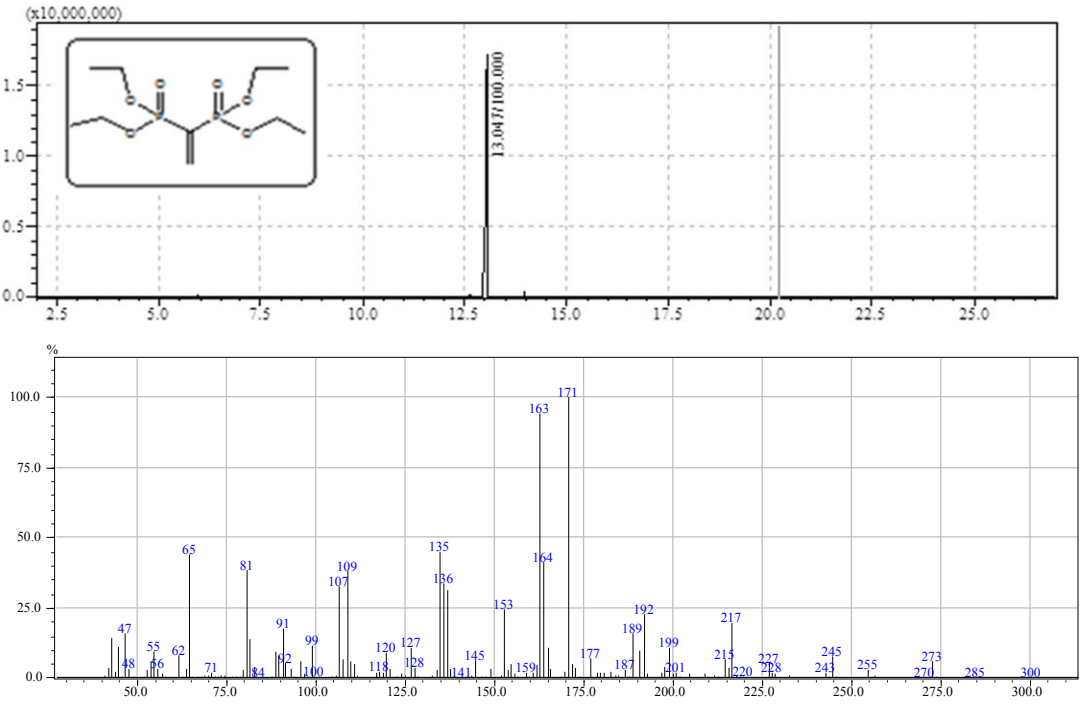
Spectrum 6: *GC spectrum of tetraethyl 2-methoxyethylene-1,1-bisphosphonate*



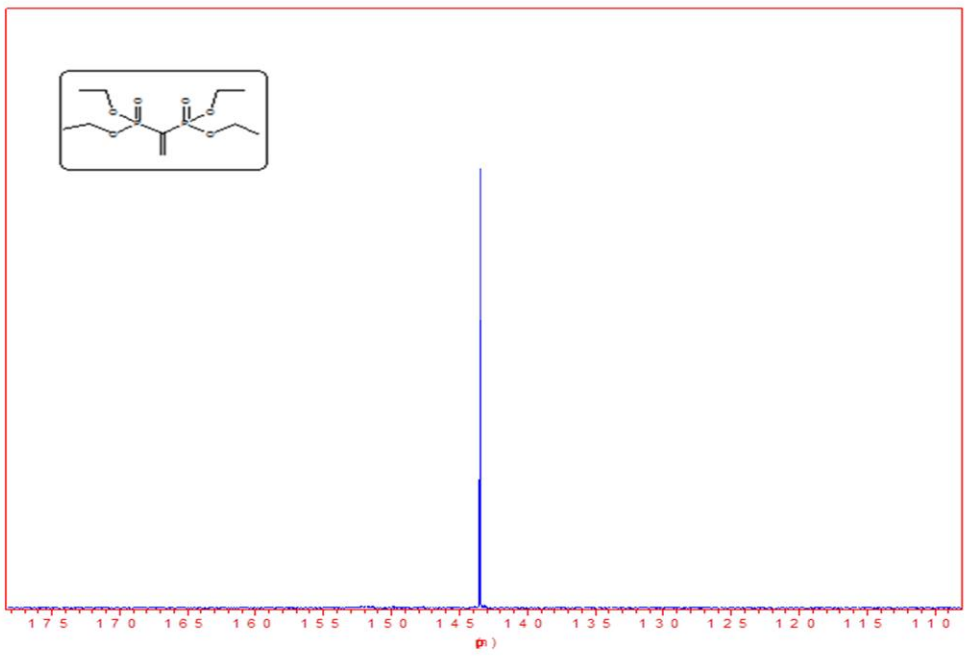
***Spectrum 7**: [1]HNMR spectrum of tetraethylvinylidene-1,1-bisphosponate*



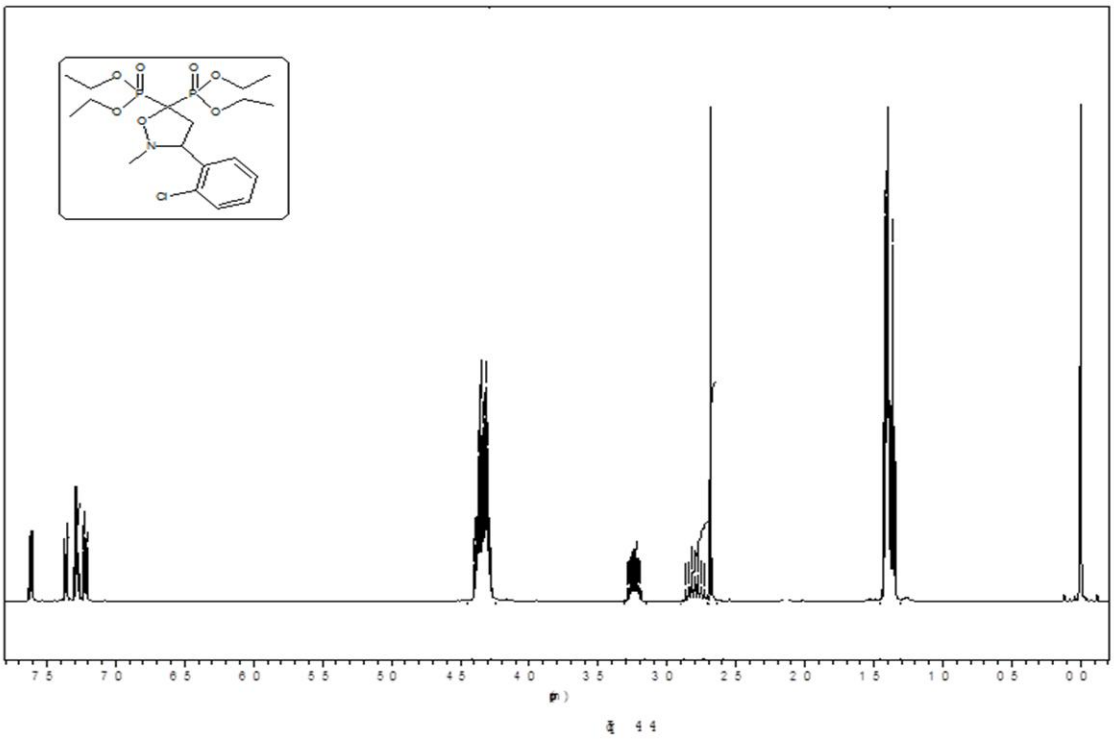
***Spectrum 8:** [13]CNMR spectrum of tetraethylvinylidene-1,1-bisphosponate*



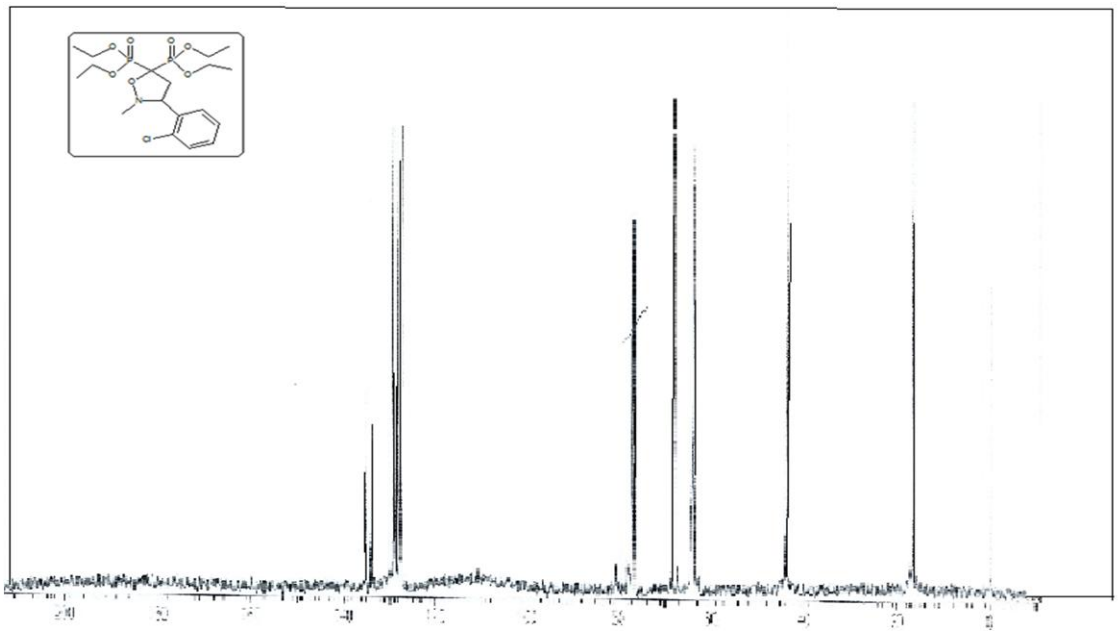
Spectrum 9: *GC spectrum of tetraethylvinylidene-1,1-bisphosponate*



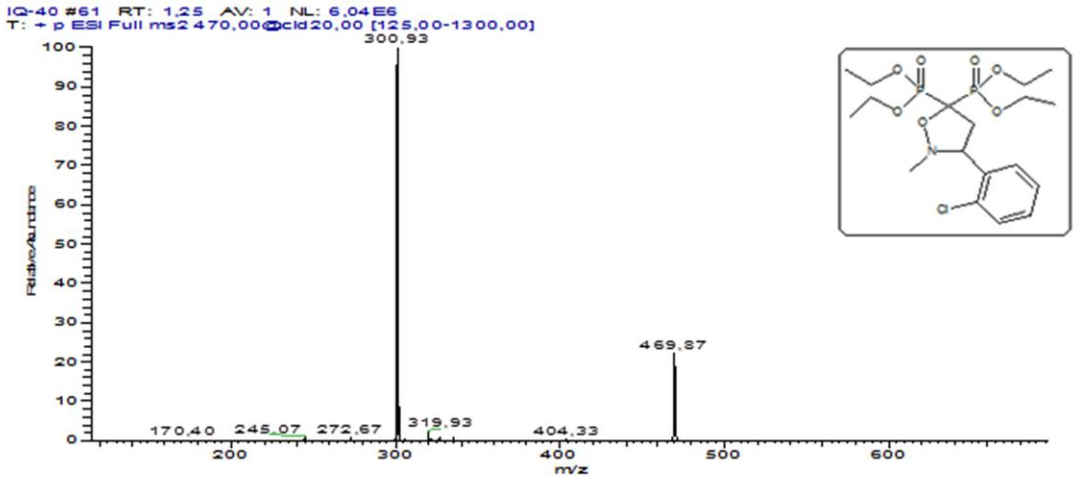
Spectrum 10: *^{31}PNMR spectrum of tetraethylvinylidene-1,1-bisphosponate*



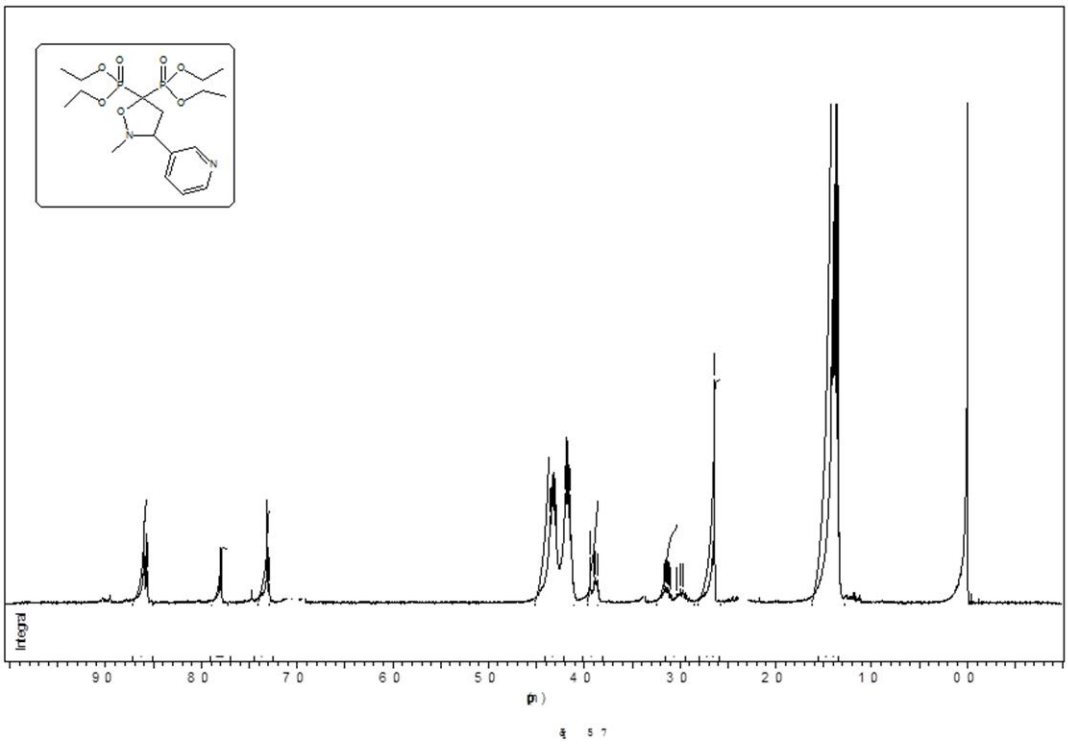
Spectrum 11: *^{1}HNMR spectrum of tetraethyl-2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5 bisphosphonate*



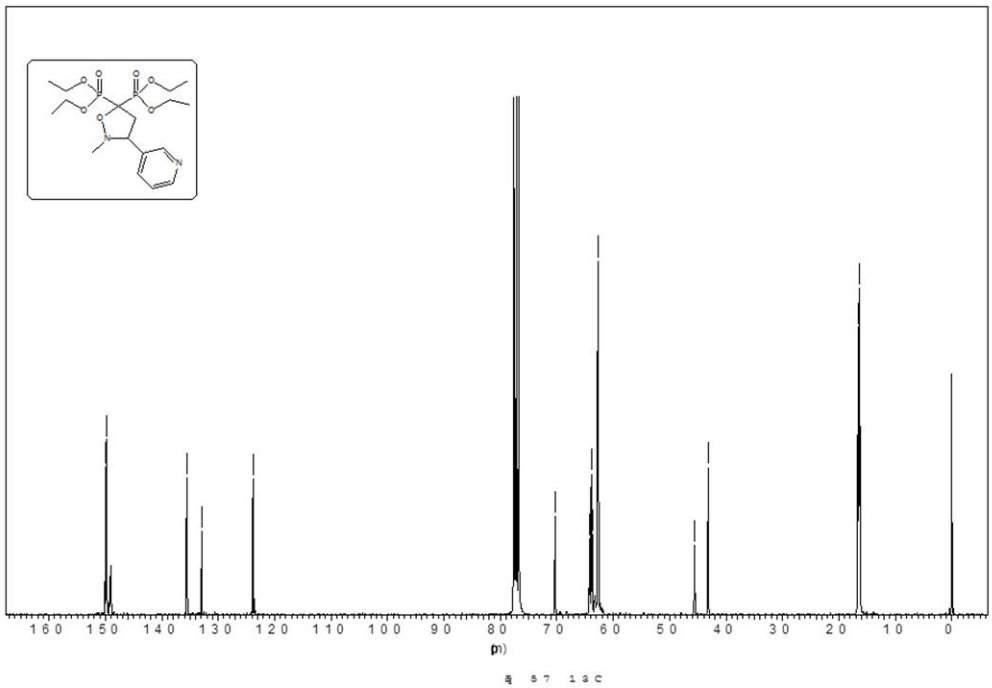
Spectrum 12: *^{13}CNMR spectrum of tetraethyl-2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5 bisphosphonate*



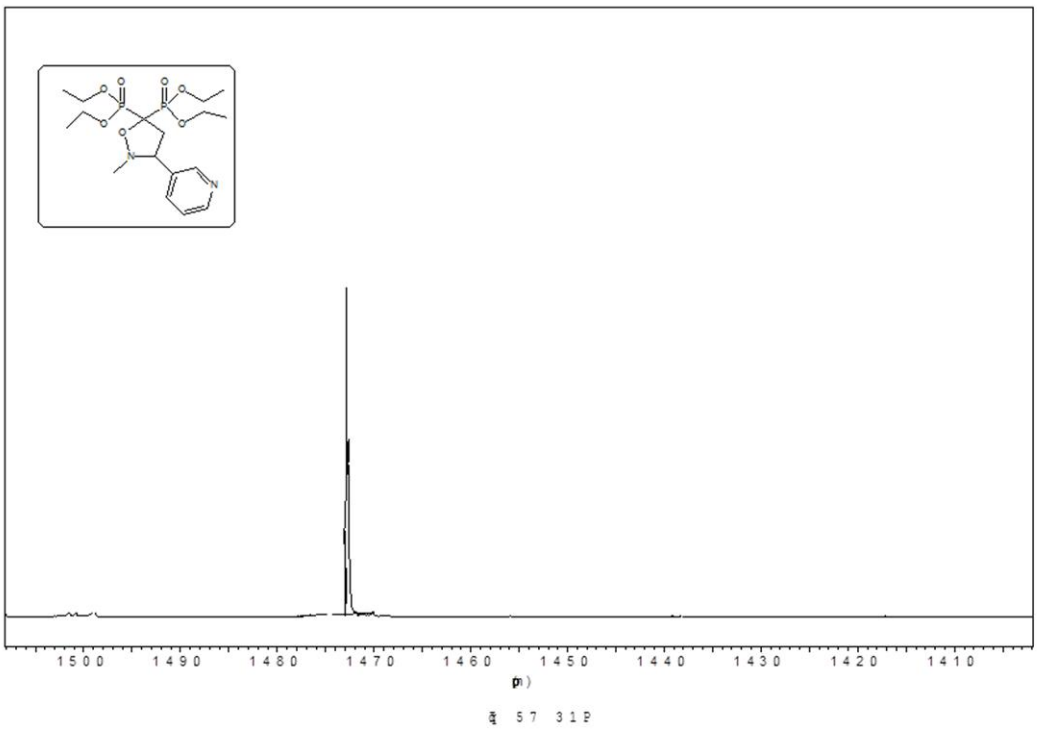
Spectrum 13: *ESI-MS spectrum of tetraethyl-2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5 bisphosphonate*



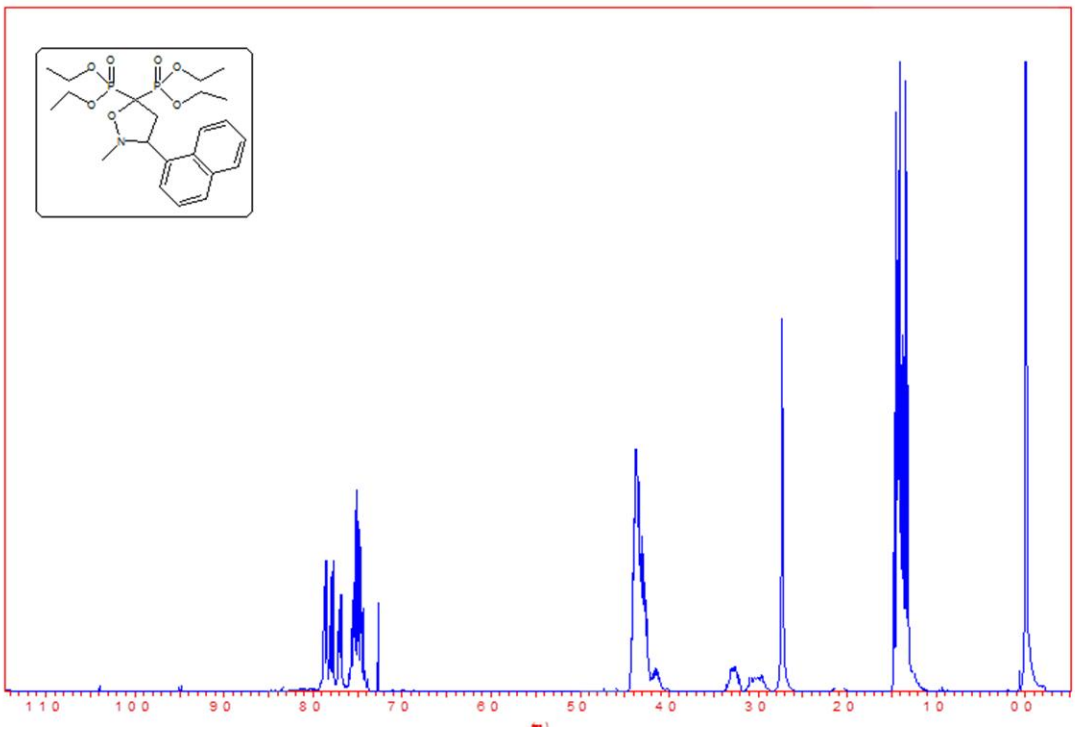
Spectrum 14: *[1]HNMR spectrum of tetraethyl-2-methyl-3-(30-pyridyl)-isoxazolidinyl-5,5-bisphosphonate*



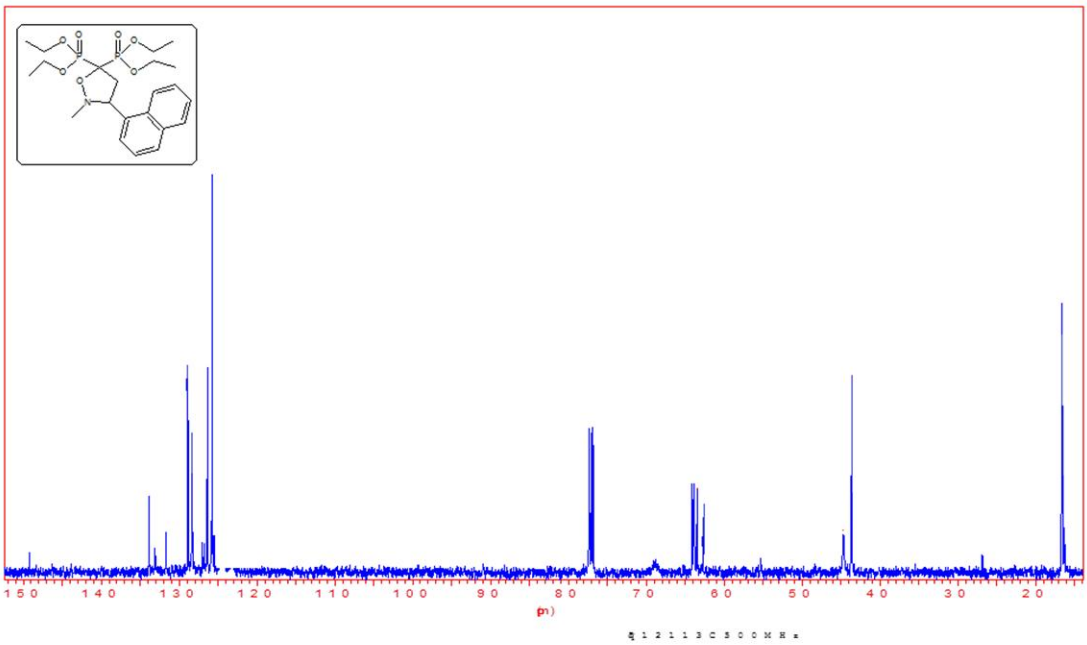
Spectrum 15: *^{13}CNMR spectrum of tetraethyl-2-methyl-3-(30-pyridyl)-isoxazolidinyl-5,5-bisphosphonate*



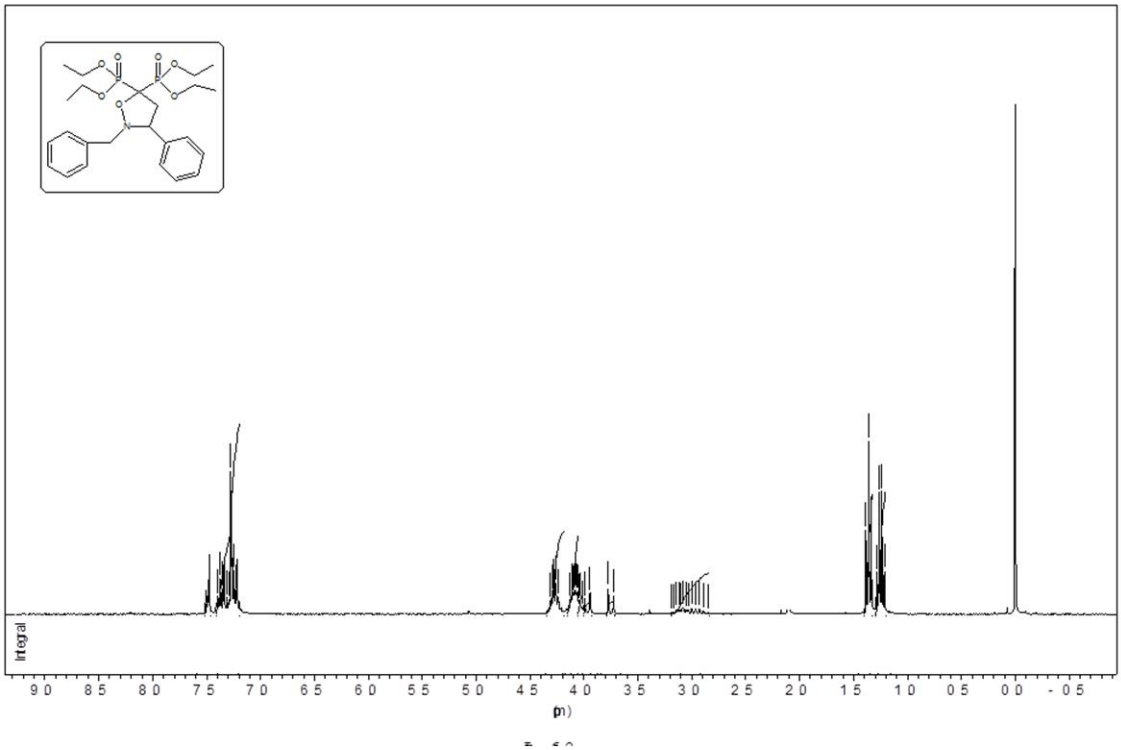
Spectrum 16: *^{31}PNMR spectrum of tetraethyl-2-methyl-3-(30-pyridyl)-isoxazolidinyl-5,5-bisphosphonate*



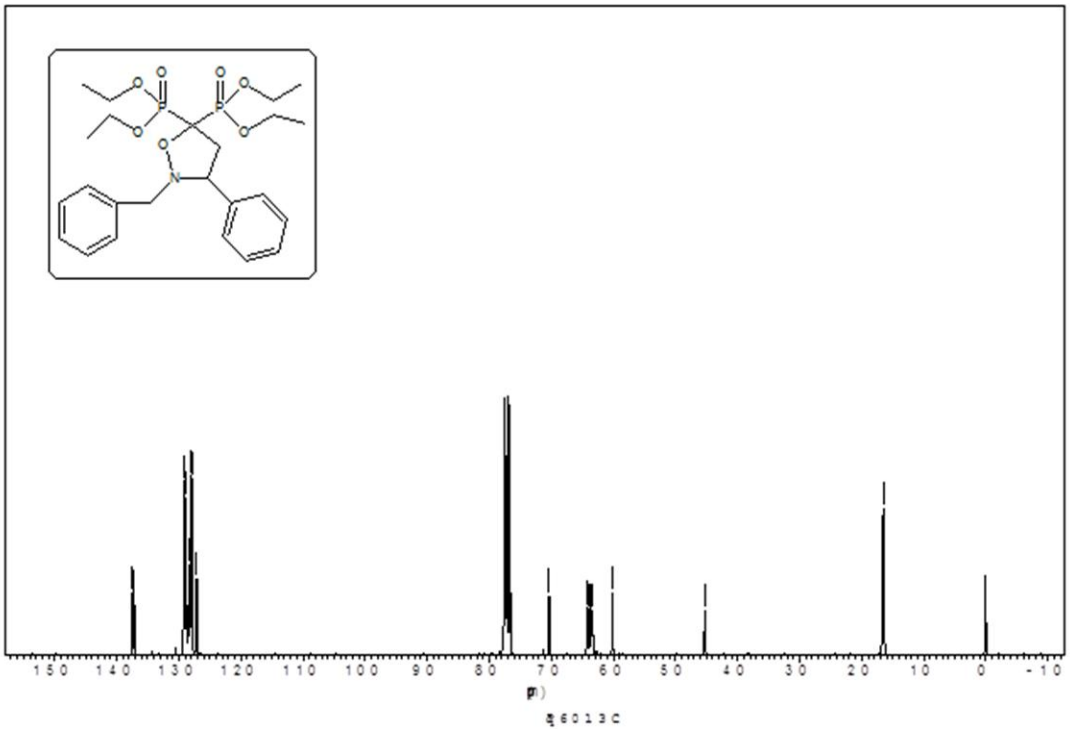
Spectrum 17: *^{1}HNMR spectrum of tetraethyl-2-methyl – 3 –naphthyl - isoxazolidinyl -5, 5 bis phosphonate*



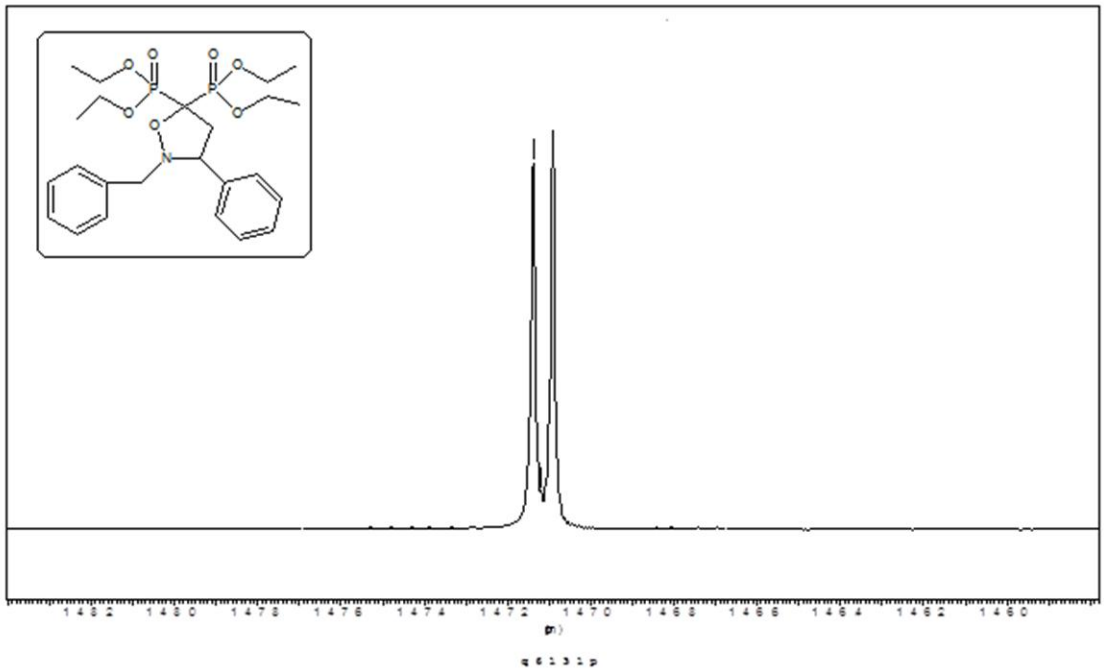
Spectrum 18: *^{13}CNMR spectrum of tetraethyl-2-methyl – 3 –naphthyl - isoxazolidinyl - 5, 5 bis phosphonate*



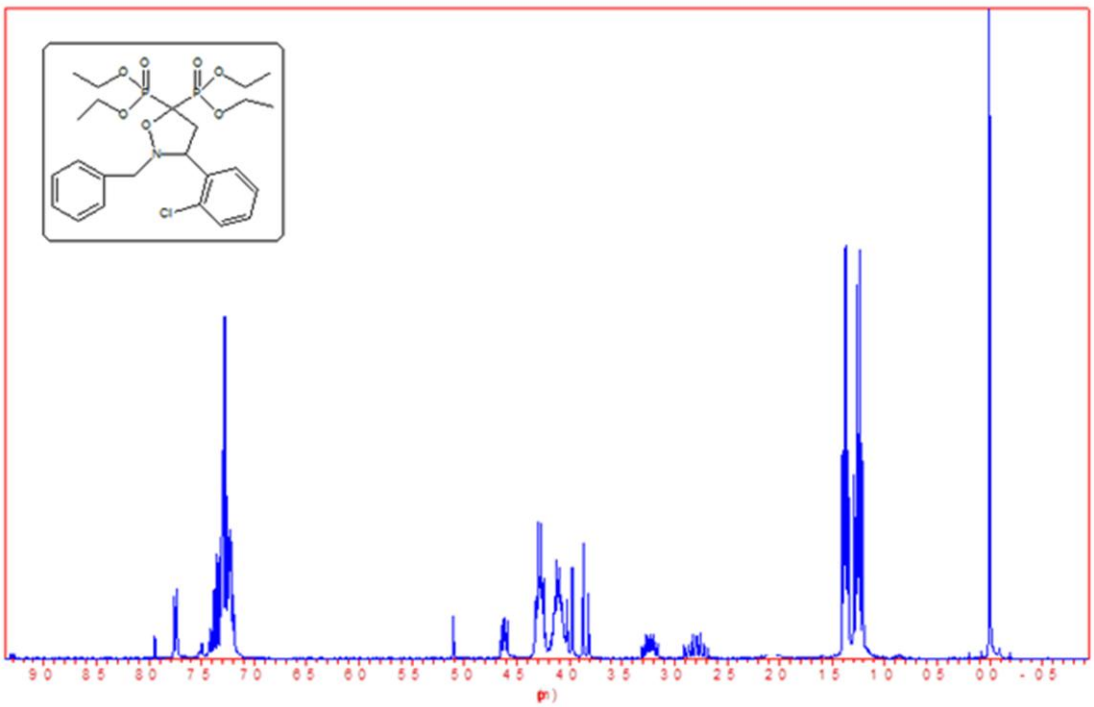
Spectrum 19: *[1]HNMR spectrum of tetraethyl-2-benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate*



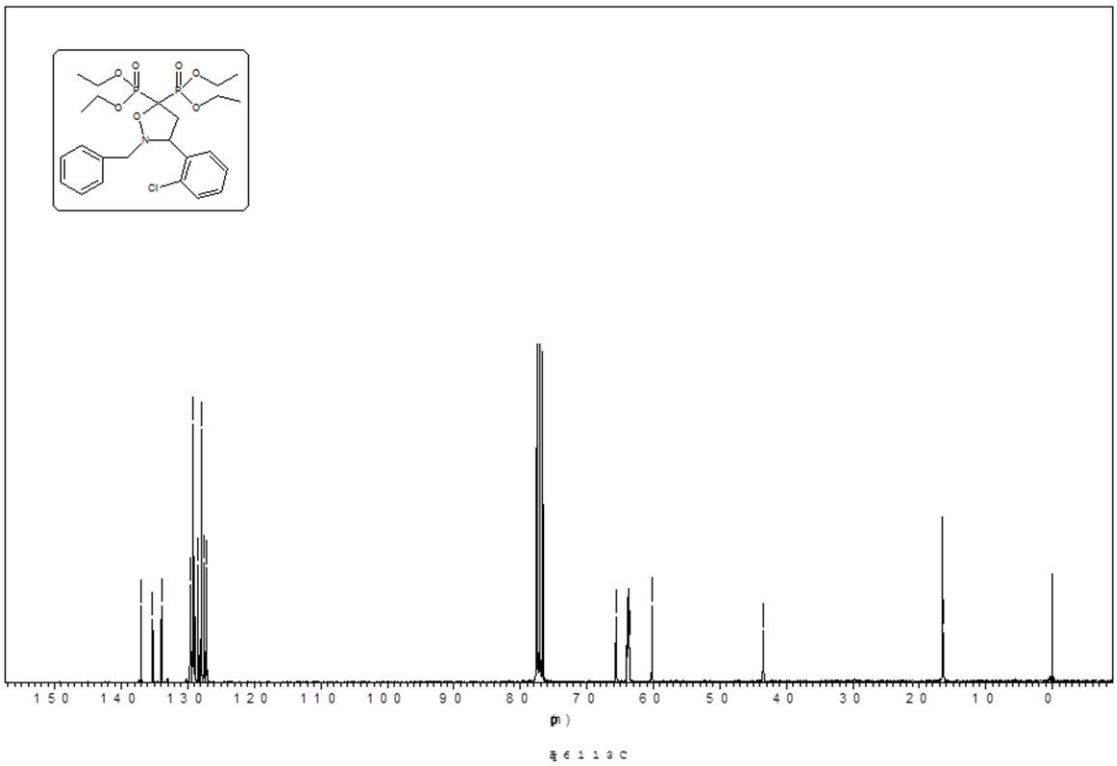
Spectrum 20: *[13]CNMR spectrum of tetraethyl-2-benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate*



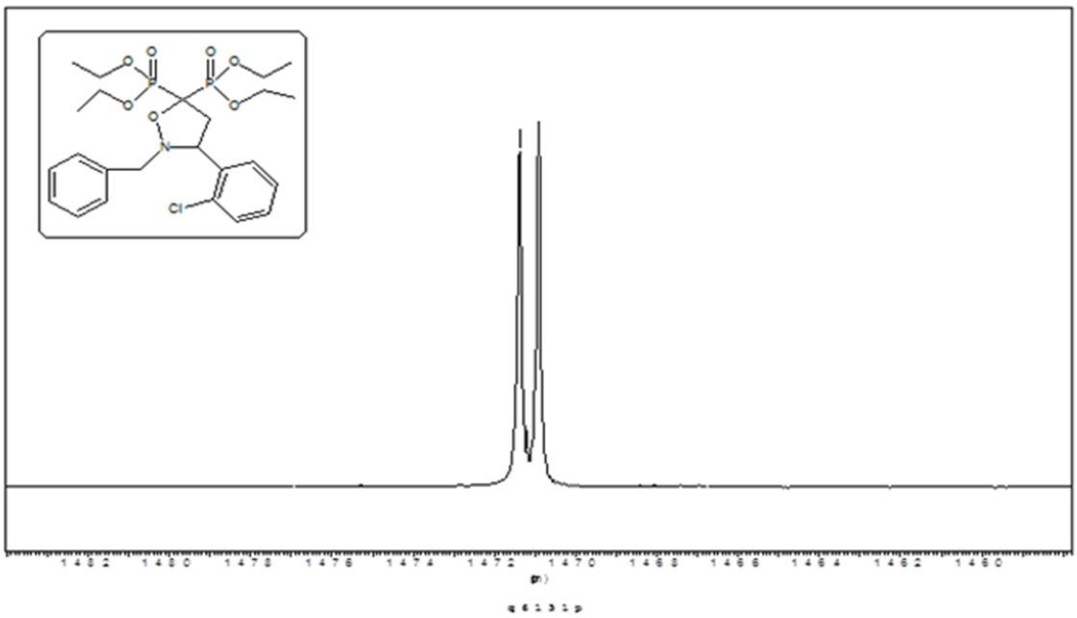
Spectrum 21: *^{31}PNMR spectrum of tetraethyl-2-benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonate*



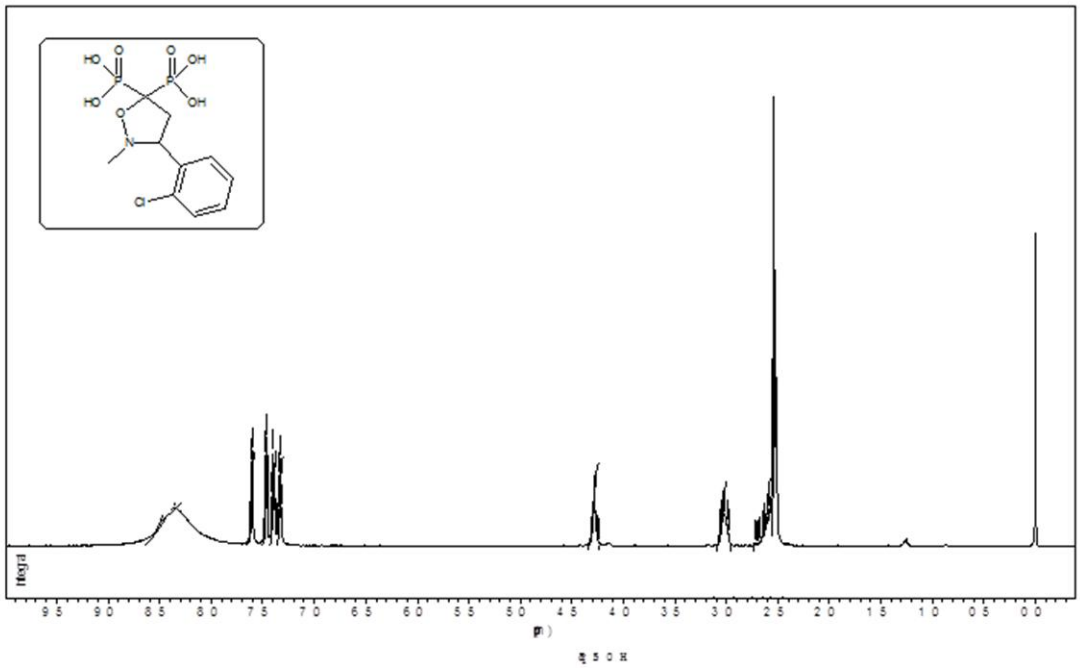
Spectrum 22: *^{1}HNMR spectrum of tetraethyl-2-benzyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonate*



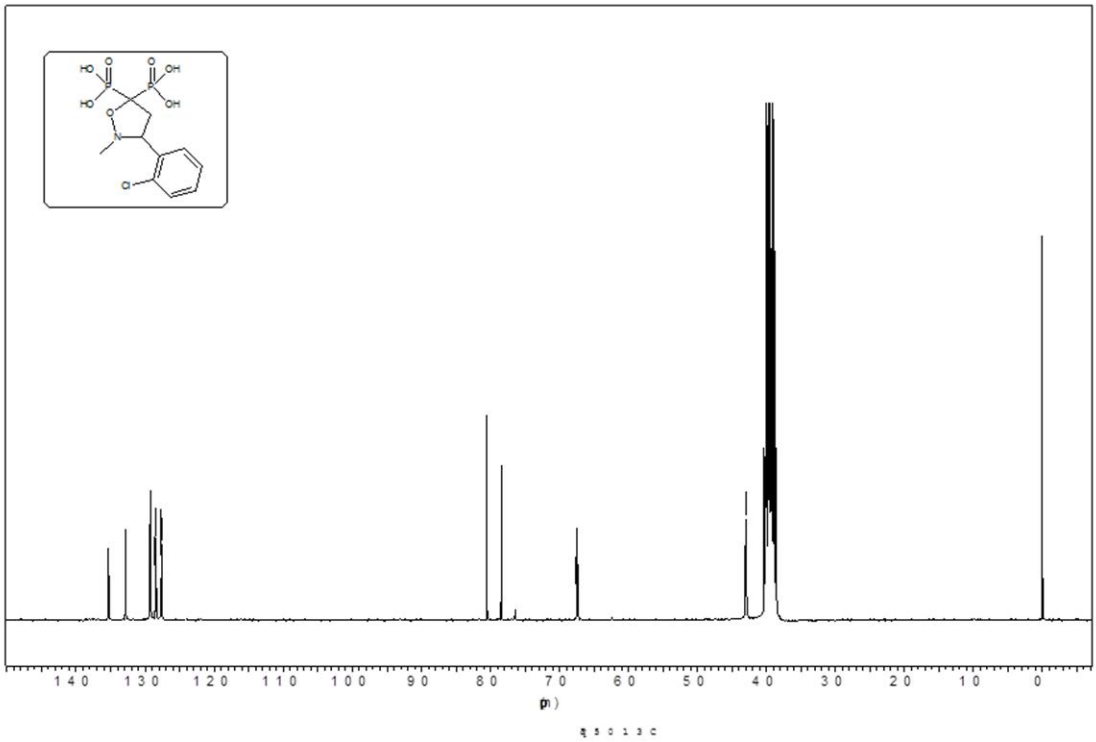
Spectrum 23: *[13]CNMR spectrum of tetraethyl-2-benzyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonate*



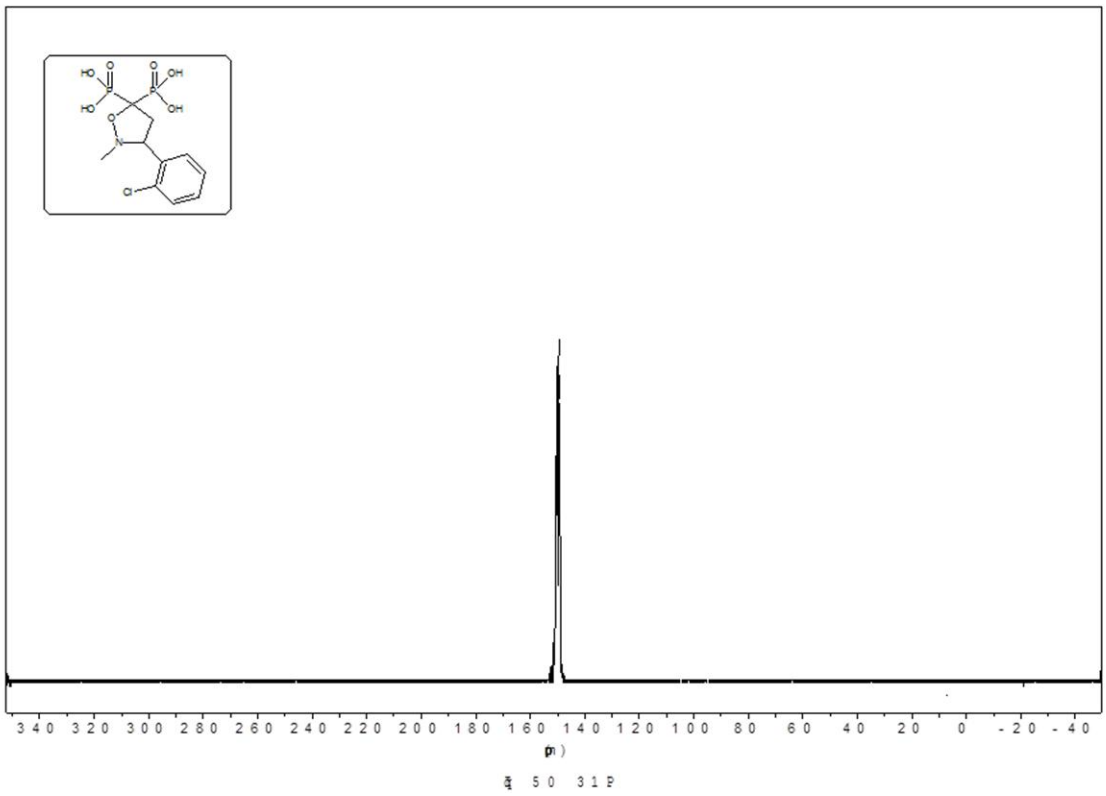
Spectrum 24: *[31]PNMR spectrum of tetraethyl-2-benzyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonate*



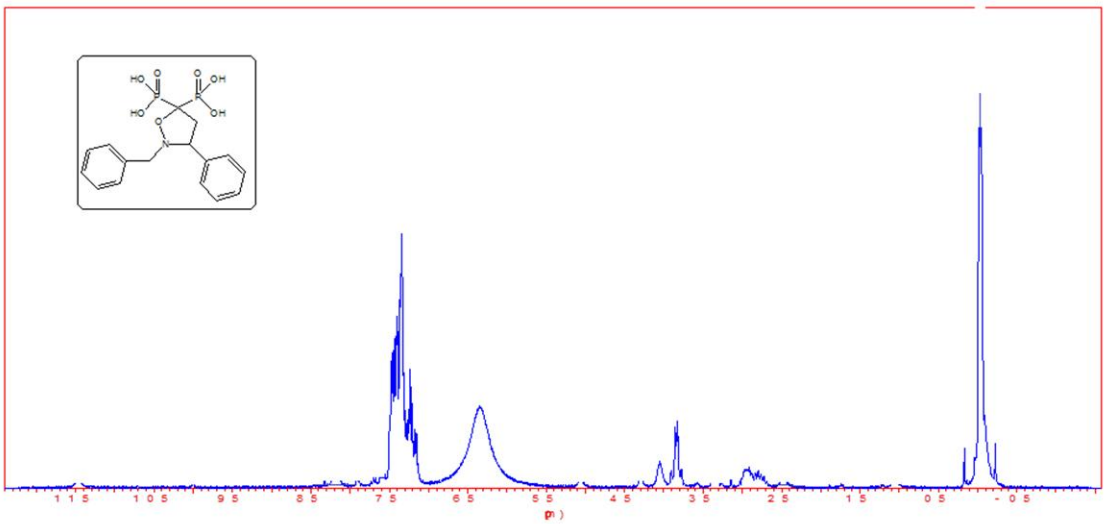
Spectrum 25: *^{1}H NMR spectrum of 2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



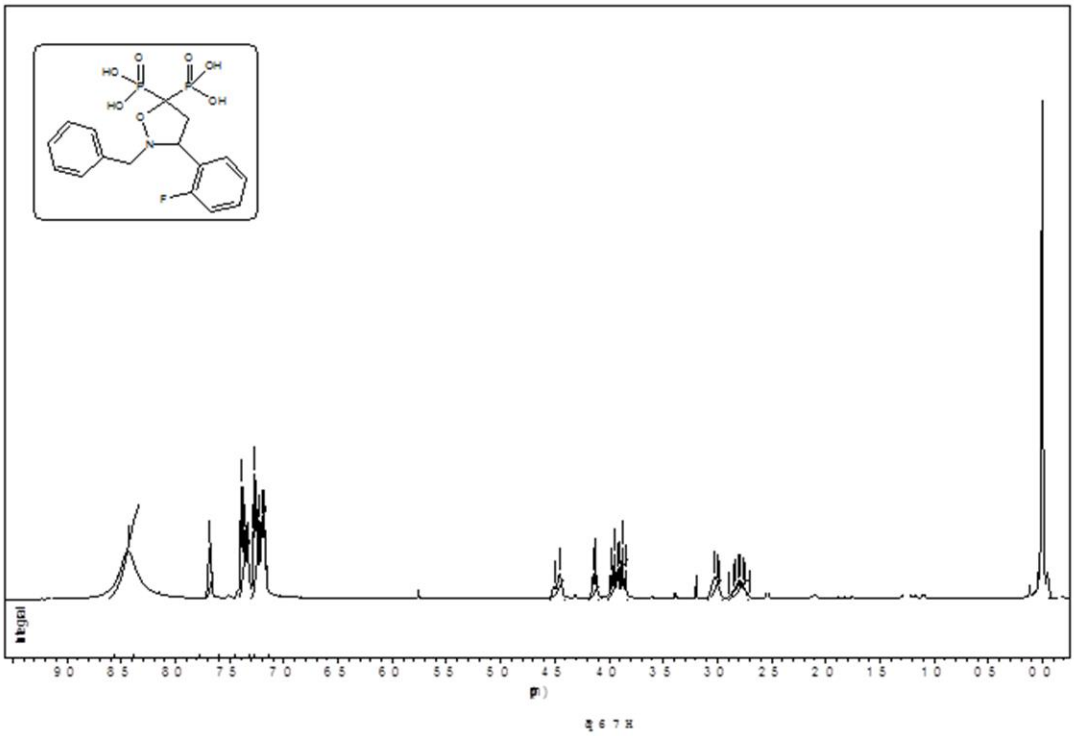
Spectrum 26: *^{13}CNMR spectrum of 2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



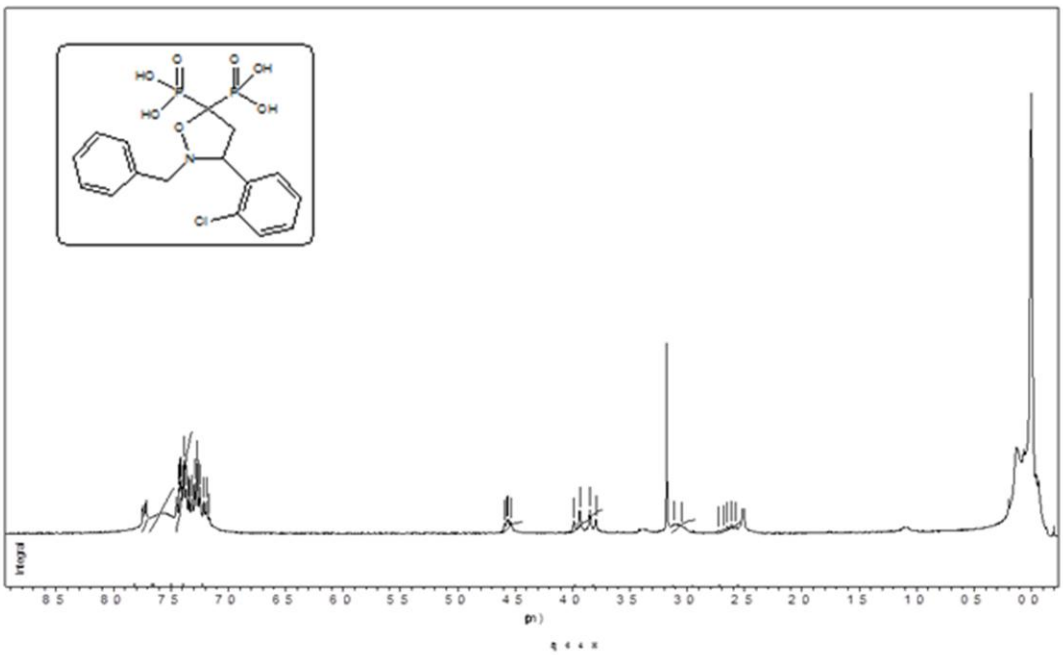
Spectrum 27: *^{31}PNMR spectrum of 2-methyl-3-o-Cl-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



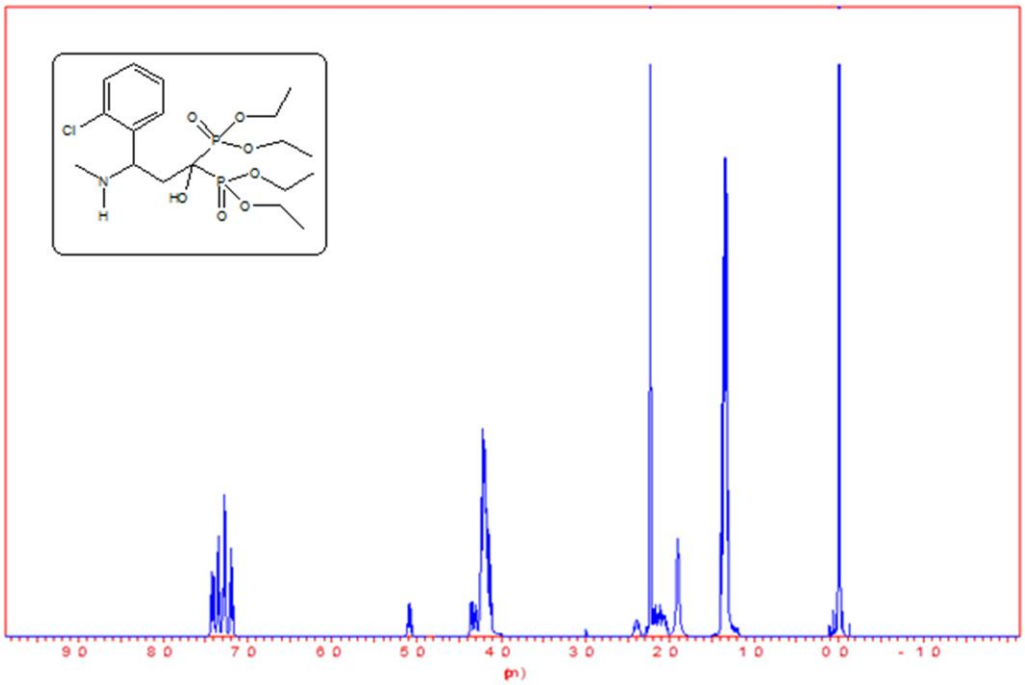
Spectrum 28: *^{1}HNMR spectrum of 2-benzyl-3-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



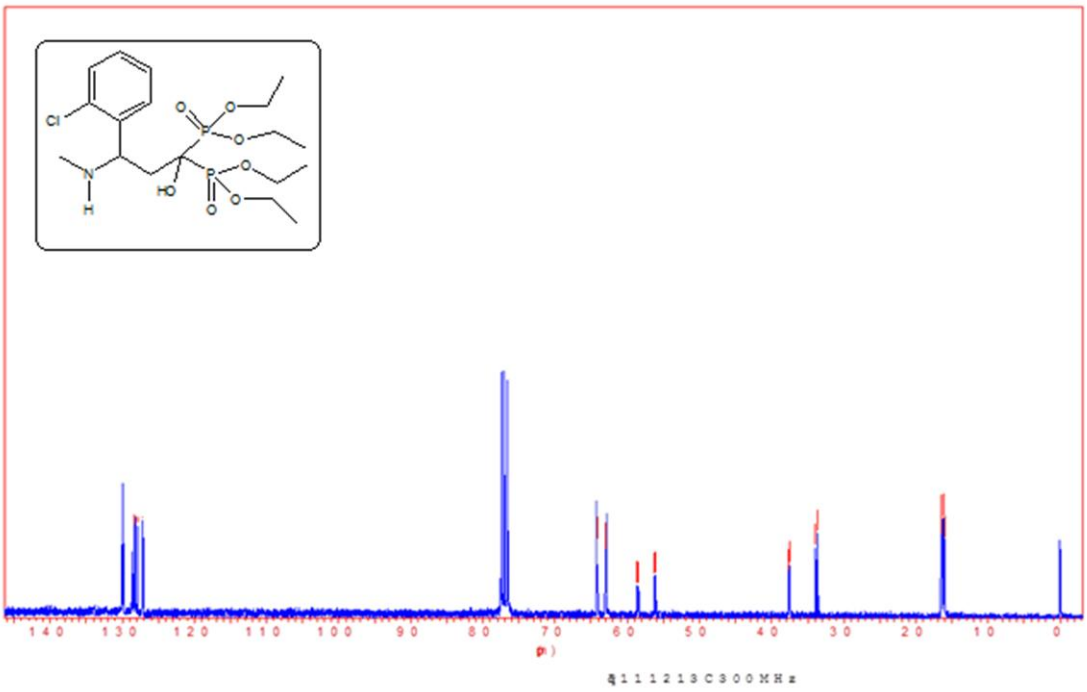
Spectrum 29: *[1]HNMR spectrum of 2-benzyl-3-o-fluoro-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



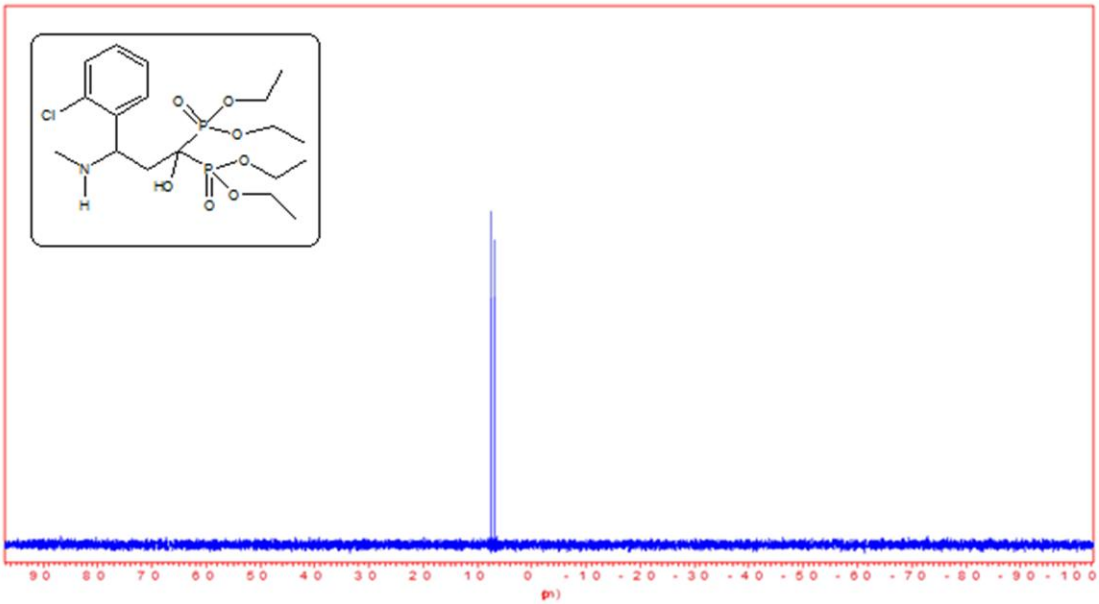
Spectrum 30: *[1]HNMR spectrum of 2-benzyl-3-o-chloro-phenyl-isoxazolidinyl-5,5-bisphosphonic acid*



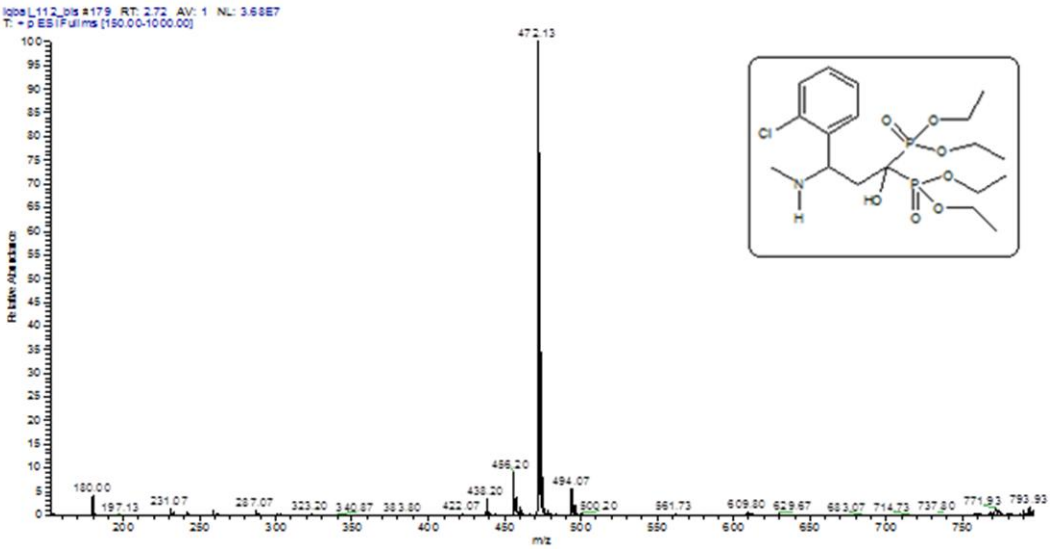
Spectrum 31: *^{1}H NMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(methylamino)]*



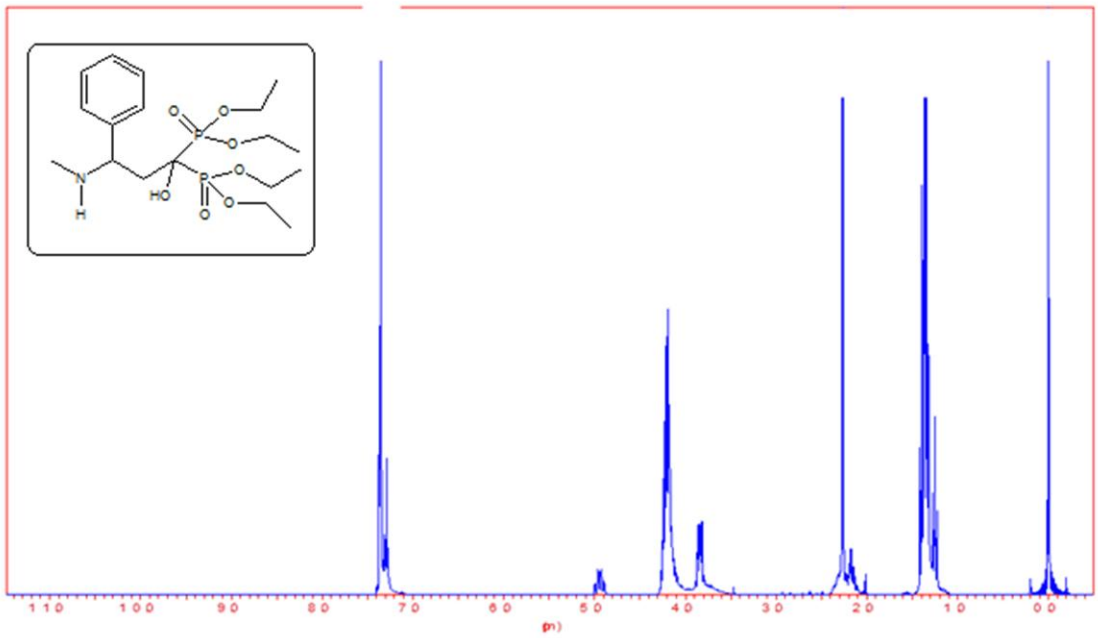
Spectrum 32*: ^{13}CNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(methylamino)]*



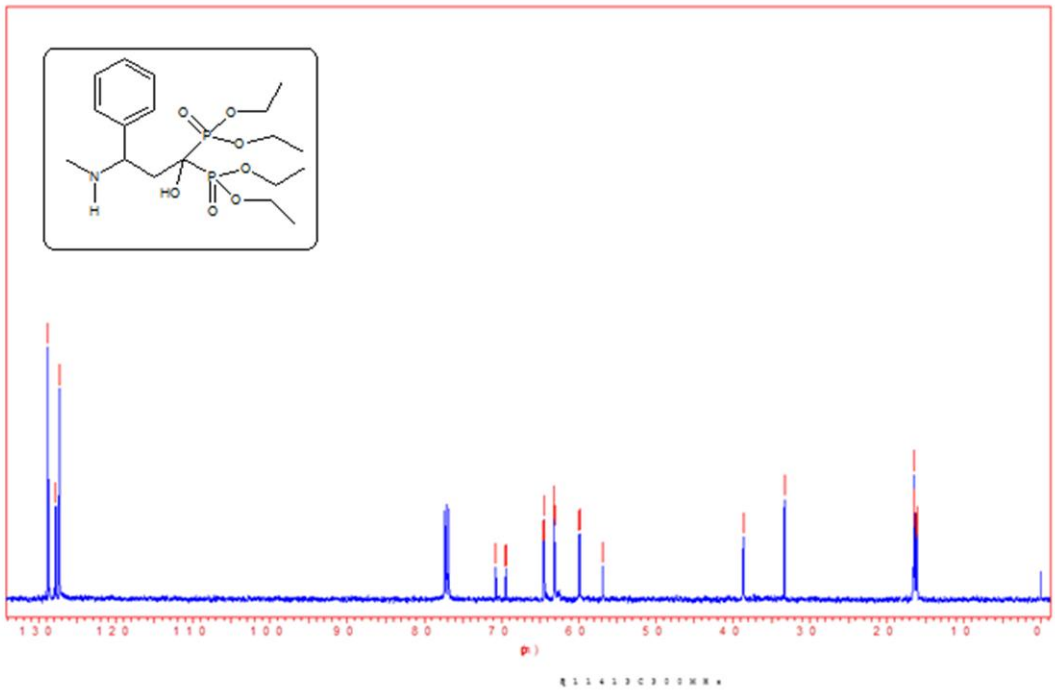
Spectrum 33: *^{31}PNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(methylamino)]*



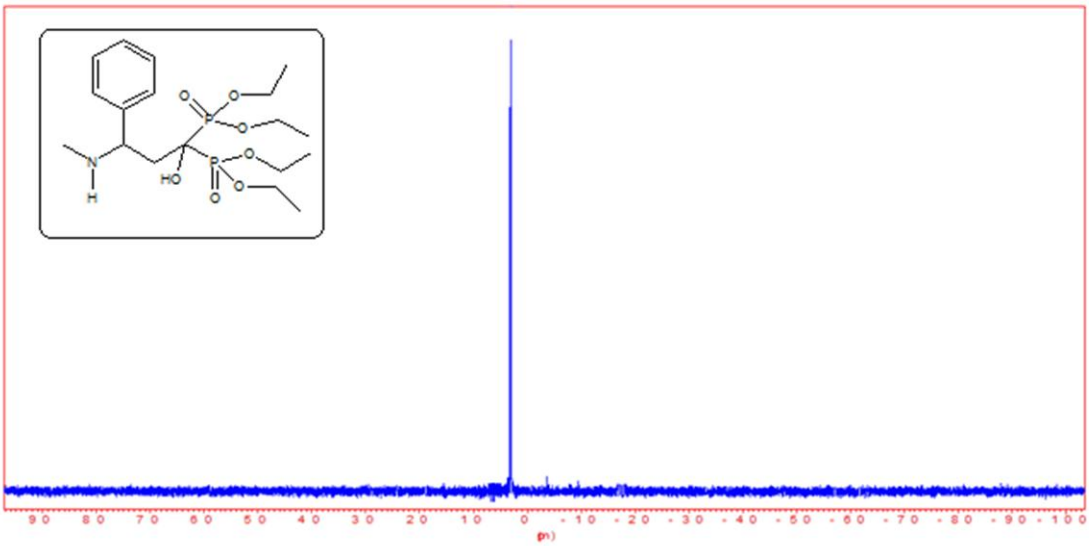
Spectrum 34: *ESI-MS spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(methylamino)]*



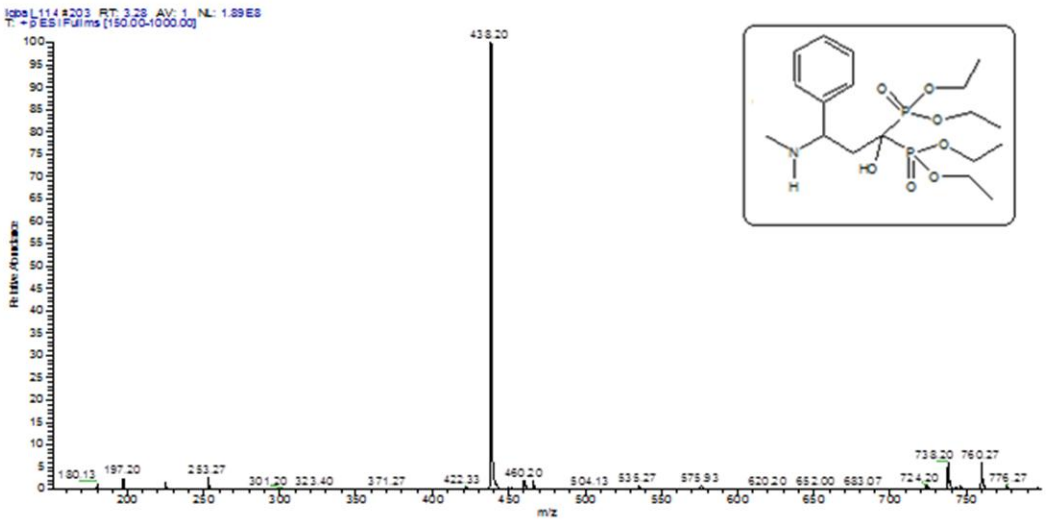
Spectrum 35: *^{1}H NMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-phenyl-3- (methylamino)]*



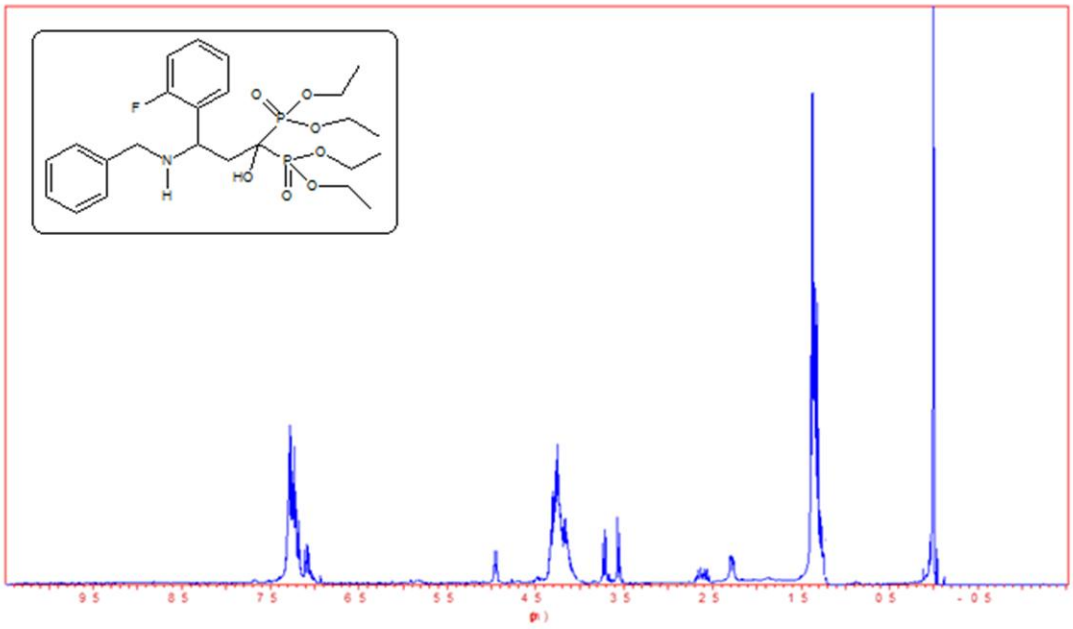
Spectrum 36: *^{13}CNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-phenyl-3- (methylamino)]*



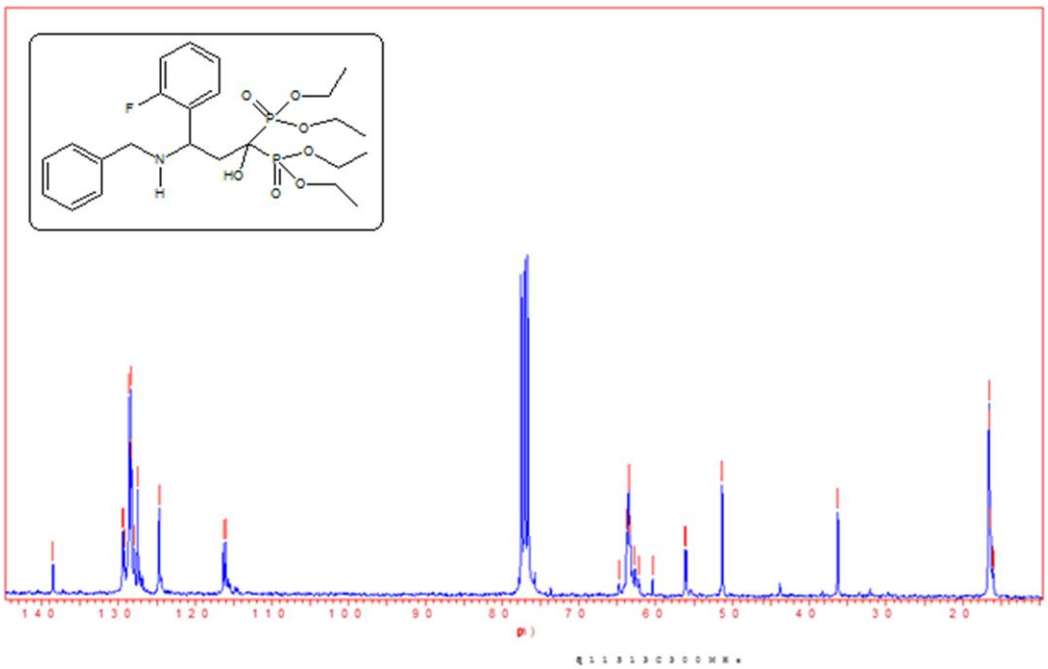
Spectrum 37: *^{31}PNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-phenyl-3- (methylamino)]*



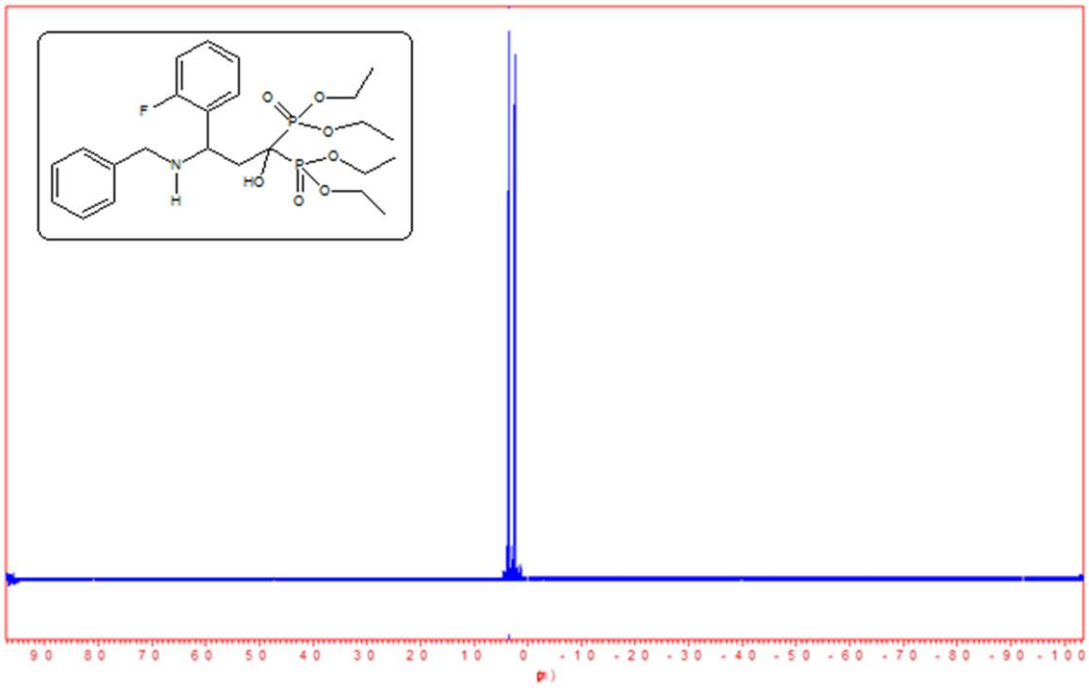
Spectrum 38: *ESI-MS spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-phenyl-3- (methylamino)]*



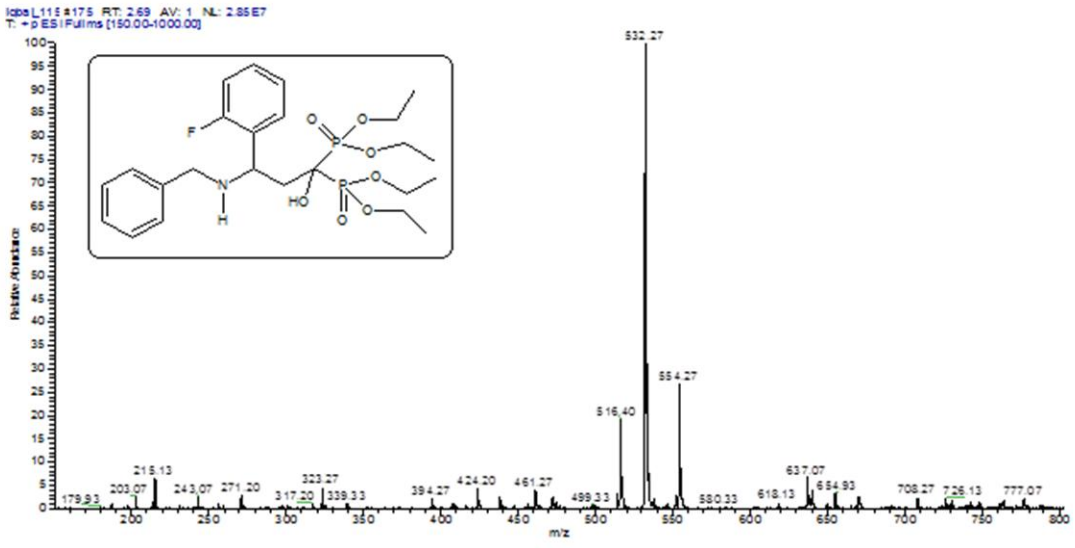
Spectrum 39: *^{1}HNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-fluoro phenyl)-3-(benzylamino)]*



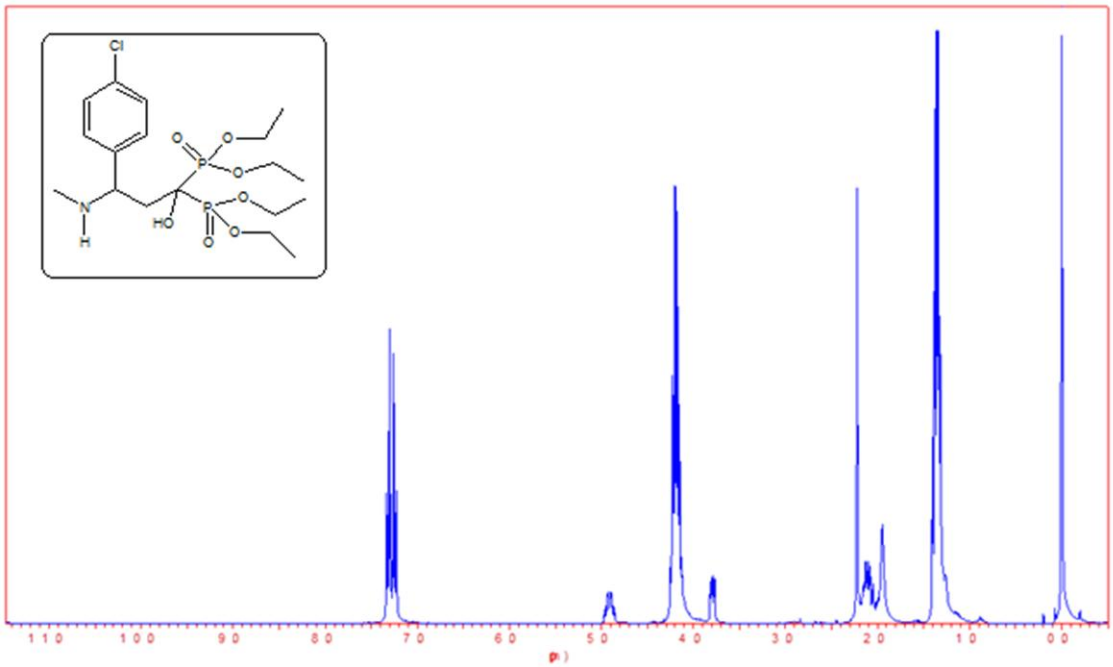
Spectrum 40: *^{13}CNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-fluoro phenyl)-3-(benzylamino)]*



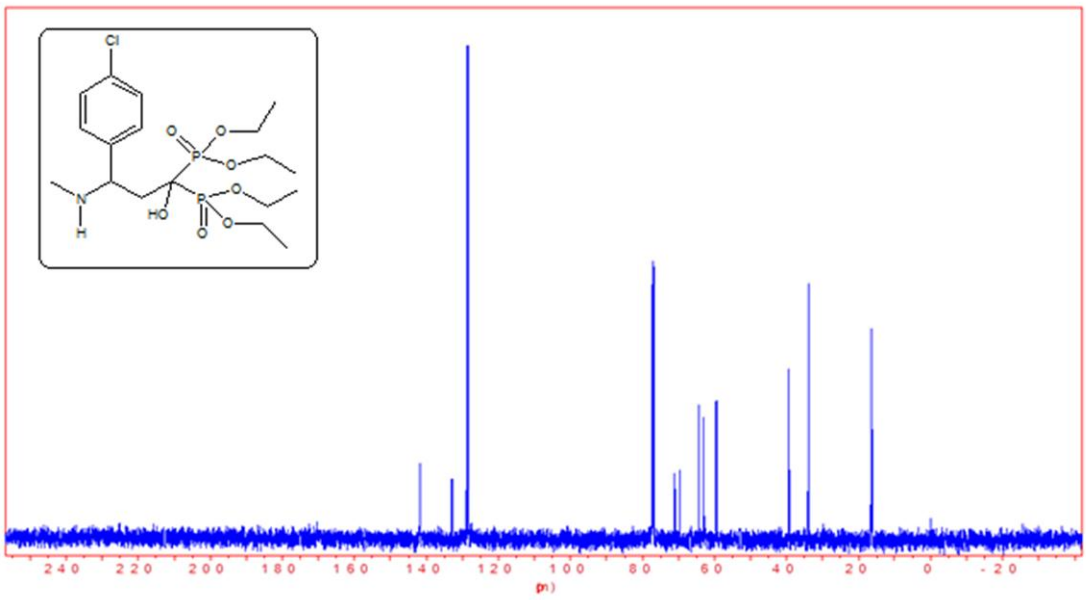
Spectrum 41: *^{31}PNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-fluoro phenyl)-3-(benzylamino)]*



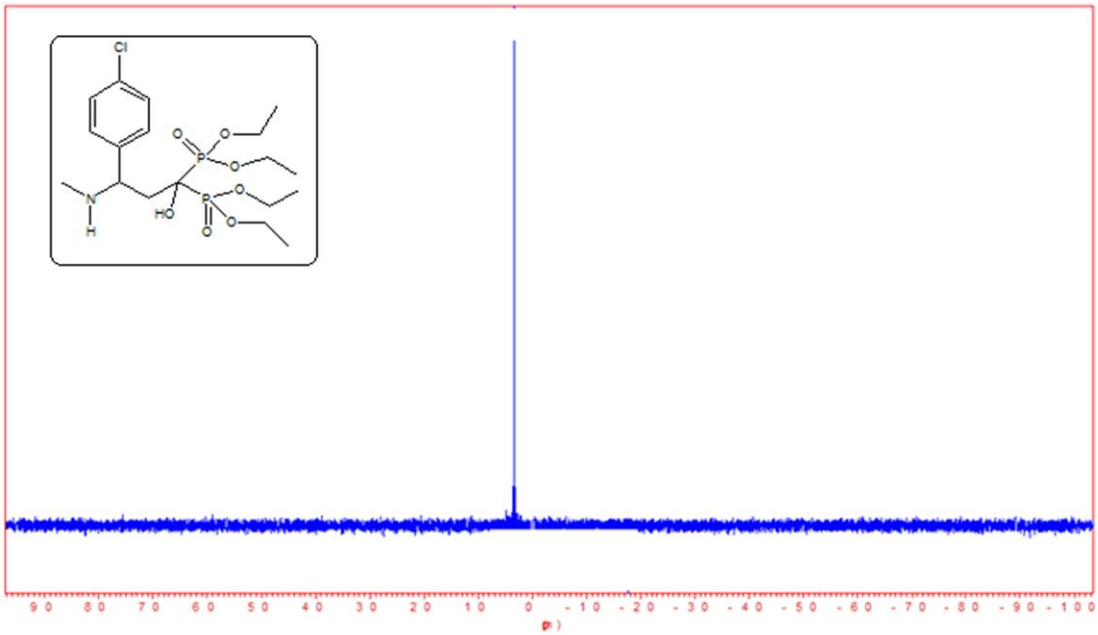
Spectrum 42: *ESI.MS Spectrum of Bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-fluoro phenyl)-3-(benzylamino)]*



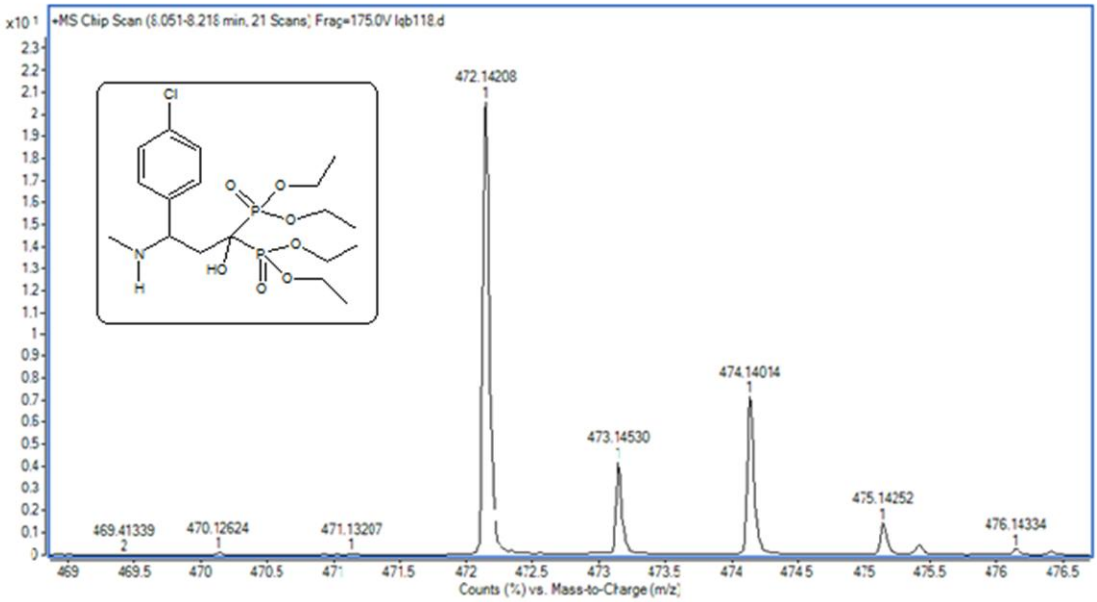
Spectrum 43: *^{1}HNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(methylamino)]*



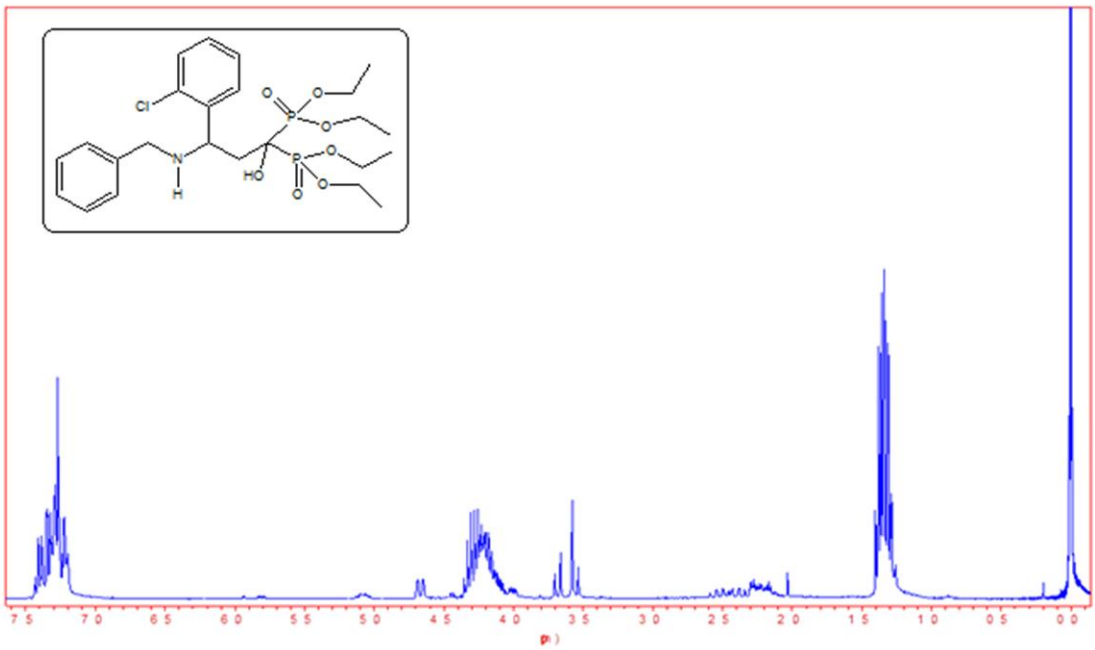
Spectrum 44: *^{13}CNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(methylamino)]*



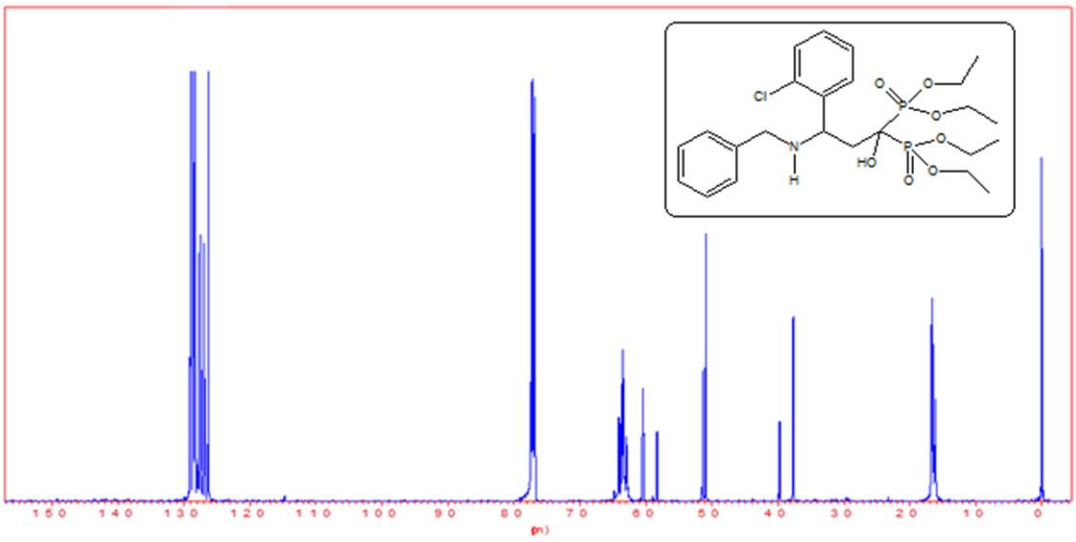
Spectrum 45: *^{31}PNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(methylamino)]*



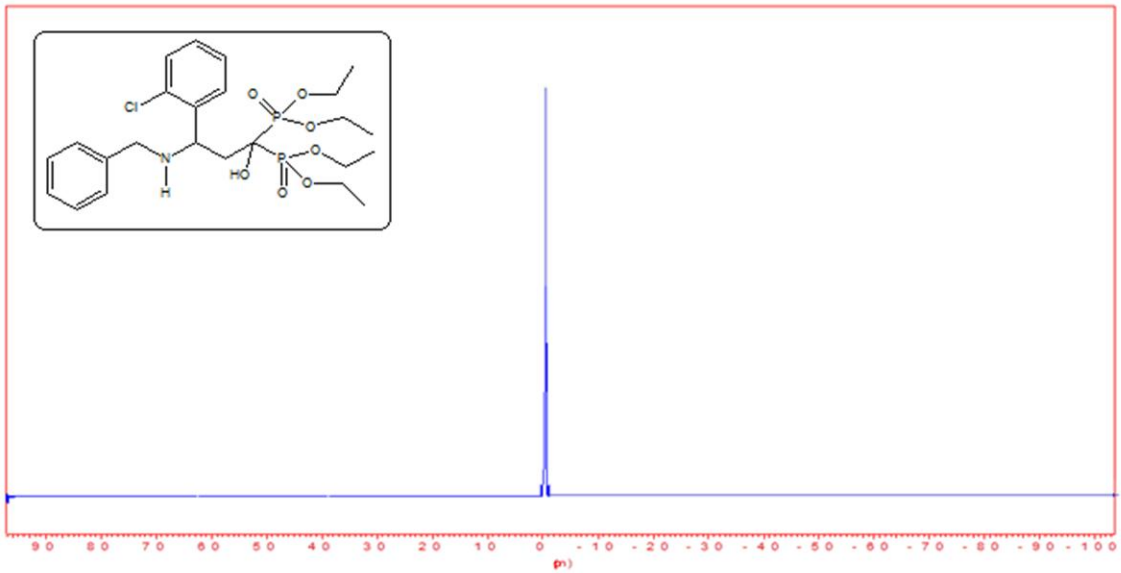
Spectrum 46: *ESI-MS spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(p-chloro phenyl)-3-(methylamino)]*



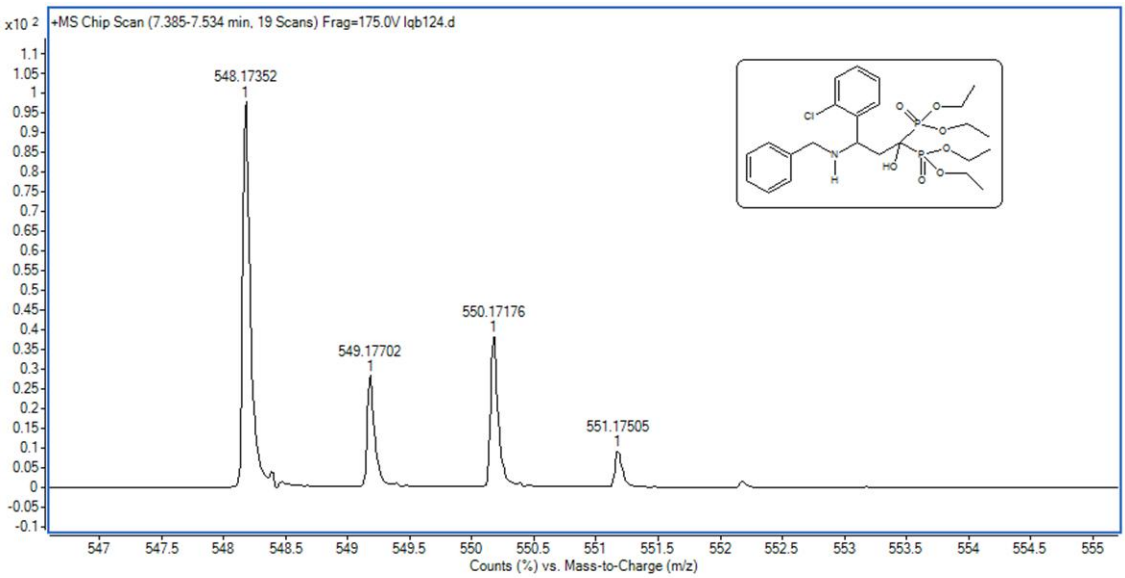
Spectrum 47: *[1]HNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(benzylamino)]*



Spectrum 48: *[13]CNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(benzylamino)]*



Spectrum 49: *^{31}PNMR spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(benzylamino)]*



Spectrum 49: *ESI-MS spectrum of bis-phosphonic acid tetraethyl ester [1-hydroxy-3-(o-chloro phenyl)-3-(benzylamino)]*