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CICLO XXVI

"Design, synthesis and characterization of suitable nitrones for several synthetic applications"

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Declaration

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Abbreviations

δ	Chemical shift
°C	Degree Celsius
1,3-DC	1,3-dipolar cycloaddition
Ac	Acetyl
aq	Aqueous
ATP	Adenosine triphosphate
BBB	Blood brain barrier
Bn	Benzyl
BPs	Bisphosphonates
br	Broad (NMR signal)
calcd.	Calculated
CNS	Central nervous system
DCM	Dichloromethane
DMPO	5,5-dimethyl-1-pyrroline *N*-Oxide
DMSO	Dimethylsulfoxide
Eda	Edavarone
e.g.	*Exempli gratia* (For example)
Equiv.	Equivalent
ESI	Electrospray ionisation
ESR	Electron spin resonance
Et	Ethyl
etc.	Etcetera
EtOH	Ethanol

FRS	Free radical scavenger
g	Gram
gem	Geminal
GC	Gas chromatography
h	Hour
HA	Hydroxyapatite
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
i.e.	*Id est* (For example)
m	Multiplet (NMR signal)
MCA	Middle cerebral artery
mg	Milligram
Me	Methyl
MeOH	Methanol
MHz	Megahertz
min	Minute
mL	Milliliter
mmol	Millimol
MS	Mass spectrometry
MTO	Methyltrioxorhenium
m/z	Mass/charge
NMR	Nuclear magnetic resonance
Nu	Nucleophile
p	Para
PBN	α-phenyl *N*-tert-butyl nitrone
PD	Parkinson's disease
Ph	Phenyl

ppm	Parts per million
Py	Pyridine
q	Quartet
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
r.t.	Room temperature
s	Singlet (NMR signal)
SOD	Superoxide dismutases
t	Temperature
t	Triplet (NMR signals)
TBI	Traumatic brain injury
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
tPA	Tissue-type plasminogen activator
TS	Transition state
UHP	Urea hydrogen peroxide
US FDA	United States Food and Drug administration

Abstract

The present work takes advance of nitrone chemistry flexibility in order to synthesize, on one hand, bisphosphonates containing *N*,*O*-carbocyclic nucleoside units with potential biological activity, starting from nitrones with functionalizable ester or methylen bisphosphonated groups. On the other hand, to synthesize suitable allyl cyclic nitrones undergoing 2-aza-Cope rearrangement in order to study the [3,3]-sigmatropic process that has been rarely detected until now with neutral molecules which are nitrones.

The synthetic strategy that we used for the bisphosphonates compound involves the synthesis of suitable nitrones and subsequently 1,3-dipolar cycloaddition reaction between these substrates and various vinyl nucleobases that carries at formation of isoxazolidinyl nucleosides. We decided to synthesize them for their significant pharmacological properties that make them very appealing: they showed a considerable cytotoxic activity against several human cell lines and therefore they could be successfully employed as anticancer drugs. Furthermore, bisphosphonates can be considered as stable analogs of pyrophosphate, that is implied in the physiological regulation of bone calcification and resorption.

Moreover, during the staying at the University of Zaragoza in Spain, a synthesis of suitable cyclic allyl nitrones was carried out. In general aza-Cope rearrangements have attracted great interest because of the ubiquitous presence of nitrogen-containing structures in natural and biological products as well as synthetic intermediates. These

compounds give rise to 2-aza-Cope rearrangement and we reported a full experimental study based on NMR kinetic experiments of the activation energies required for both neutral and catalyzed 2-aza-Cope rearrangements of nitrones.

Riassunto in lingua italiana: Il presente lavoro di tesi è basato sulla versatilità dei composti nitronici. Sono stati quindi realizzati svariate tipologie di nitroni ed utilizzati, in un primo progetto, per la sintesi di composti a possibile attività farmacologica. In un secondo progetto invece è stata realizzata la sintesi di un nitrone per lo studio cinetico della reazione 2-aza-Cope, un importante strumento per la sintesi stereoselettiva di numerosi composti azotati.

Da un lato, quindi, si sono utilizzate le proprietà chimiche dei nitroni al fine di sintetizzare analoghi nucleosidici bifosfonati, i quali suscitano grande interesse nel campo della chimica farmaceutica. Per la loro sintesi si è partiti dalla formazione di nitroni recanti un gruppo estereo funzionalizzabile o direttamente dalla sintesi di nitroni bifosfonati. Successivamente, essi sono stati utilizzati per reazioni di cicloaddizione 1,3-dipolare con vinil nucleobasi.

Dall'altro lato invece, nel secondo progetto condotto presso l'Università di Saragozza in Spagna, è stato eseguito uno studio cinetico della reazione 2-aza-Cope su allil nitroni ciclici. Essi sono stati ottenuti tramite una sequenza di reazioni di ossidazione seguite da addizioni nucleofile utilizzando i nitroni intermedi come substrati elettrofili. Lo studio cinetico ha poi permesso di mettere in luce svariati fattori che interessano tale reazione.

Contents

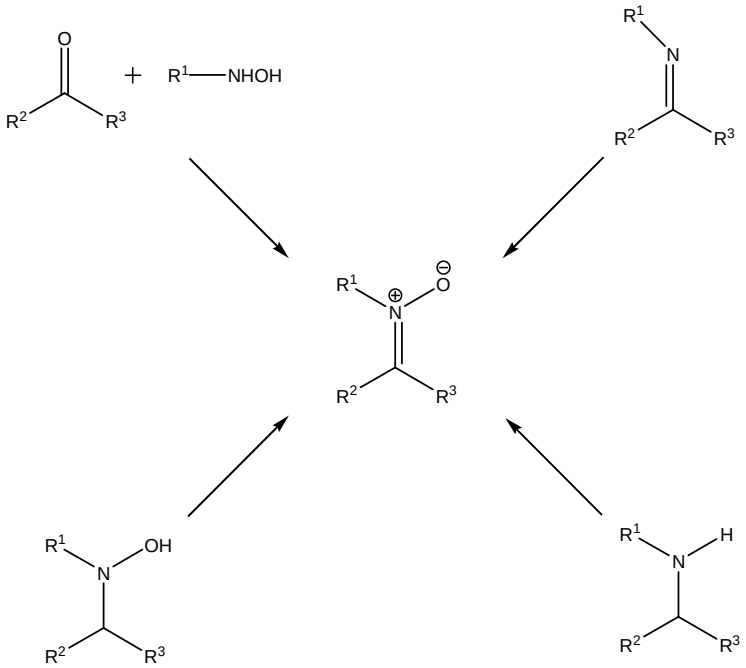
1. Introduction

The huge technologic advances that we have experienced during three decades in the fields of medicinal and materials science are creating growing demand from the chemistry of highly efficient and selective processes that allow to obtain large amounts of chemical compounds for the treatment of illnesses, specialized materials manufacturing or other applications related with quality of our life. The main solution to this demand is organic synthesis that through new methodologies is able to provide a large number of compounds with high levels of functional diversity.[1]

In particular, the synthesis of organic compounds with nitrogen atoms in its structure has aroused considerable interest due to its presence in a large number of natural products as well as wide spectrum of biological functions and different chemical structures that offer. In this context, the regio- and stereoselective synthesis of saturated nitrogen systems, with different oxidation states of nitrogen atom and various functional groups, is a relevant example of substrates oriented to diversity.

Such systems are found in a large number of organic compounds of great importance not only a biological, but also within other areas related to chemistry (pharmaceutical, agrochemical, and food). Nitrogen compounds have also been used in organic synthesis as starting materials for the preparation of chiral auxiliaries and reagents. To the numerous examples that can be found in acyclic systems we have to add those cyclic, certainly of great importance for their presence in nature. From what has been discussed so far follows the need for methodologies that allow the introduction of nitrogen function in a molecule.

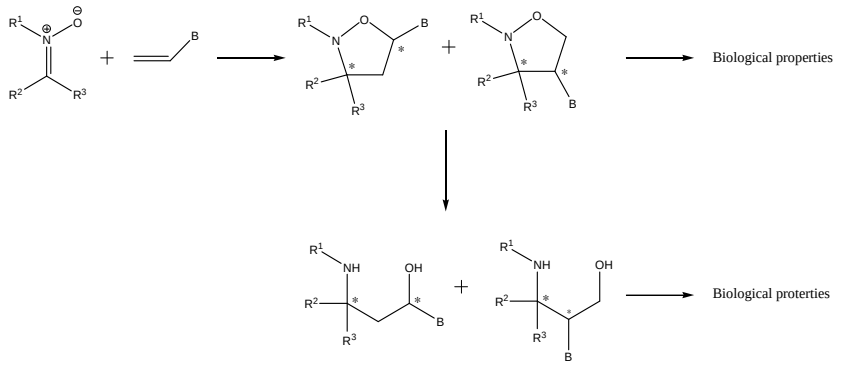
Nitrones were discovered in 1916; the term "nitrone" derives from the fusion of words "nitrogen" and "ketone" as was stated in the first review published in 1938 about these compounds.[2] Since that time nitrone compounds have proven to be of great synthetic utility in the preparation of several nitrogenous systems.[3] From their early chemistry until the present time, nitrone have been largely employed in Organic Chemistry. The importance of the nitrone functionality has been revealed by the growing number of scientific papers which appeared in the literature over the last years concerning nitrones and related compounds. Nitrones can be derived from aldehydes or ketones, so talk about and aldehyde-nitrones (R^2 o R^3 = H) and ketonitrones (R^2 and $R^3 \neq$ H), respectively (Scheme 1.1). In fact, the nitrones can be prepared from these compounds, but also by several methods well-documented in the primary literature as discussed more below. The most used includes condensation reactions between hydroxylamines and carbonyl compounds[4] and oxidation of amines, imines or hydroxylamines.[5] Nitrone functional group is a π-delocalized system formed by near atoms (C, N, O) that share π electrons and is isoelectronic with allyl and enolate anion; however, different atoms present in the functional group confer quite different reactivities.



Scheme 1.1: Methods of nitrones synthesis.

As a general topic, the chemistry of nitrones has been described in the past. It includes a chapter in several books,[6] mainly concerning cycloaddition chemistry, and a more detailed description of the nitrone functionality in various volumes of the Patai series.[7] A major breakthrough in the field of nitrone chemistry came with the use of nitrones as 1,3-dipoles in cycloaddition reactions (Scheme 1.2). Nitrones react with a great variety of dipolarophiles giving rise to a vast array of products. Cycloaddition reactions are divided according to substrates in inter- and intramolecular processes. In both cases regio- and stereoselectivity are important concepts which are treated extensively. Particularly, when chiral substrates are used the asymmetric induction is studied making use of the mechanistic considerations introduced at the start of the section. This kind of

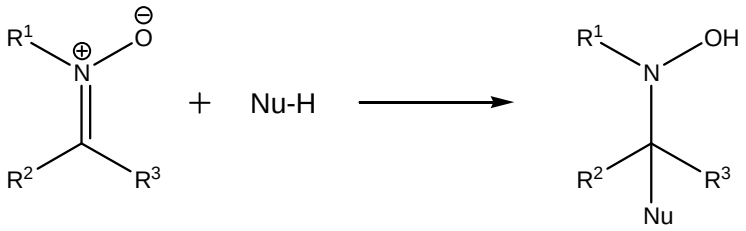
reaction both in the inter-[8] and intramolecular[9] versions has allowed the access to an important class of compounds, isoxazolidines, which are convenient precursors of 1,3-aminoalcohols, a structural fragment present in a number of organic compounds of interest.[10]



Scheme 1.2: 1,3-dipolar cycloaddition: synthetic and biological utilities

However, a second breakthrough followed with the nucleophilic additions to nitrones to afford *N*,*N*-disubstituted hydroxylamines (Scheme 1.3). The possibility of obtaining a nitrogen atom in an intermediate oxidation state (hydroxylamine) makes the nitrone group more attractive in many aspects than the imine group which has been more widely used and, under similar conditions, gives rise to the corresponding amine. The additional higher stability of nitrones vs. imines and the possibility of controlling the stereochemical course of the stereoselective additions in some cases by precomplexation with a Lewis acid, increases the value of nitrones as convenient starting materials for the construction of a vast number of nitrogen-containing compounds. Far more interesting are the

stereoselective reactions which offer the possibility of synthesizing optically active nitrogen-containing compounds.



Scheme 1.3: Nucleophilic addition to nitrones.

Furthermore, the presence of the oxygen atom allows coordination with metals so the nitrone functional group can also act as a nucleophile, serving as ligand. Moreover, nitrones can be undergoing [3,3]-sigmatropic process, that process is an important and powerful tool in organic chemistry with a wide demonstrated synthetic utility.[11] Therefore, we can speak of different nitrone reactivities according to the reactive are facing (Figure.1.1).

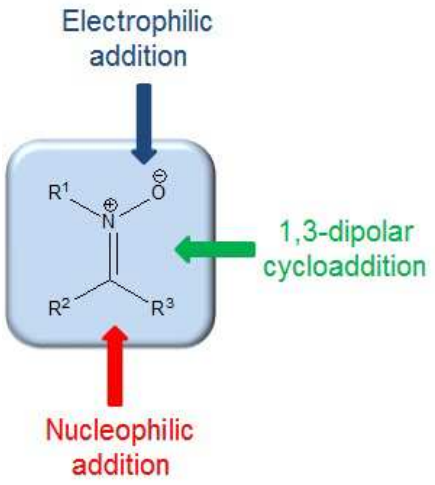


Figure. 1.1: Nitrone reactivity.

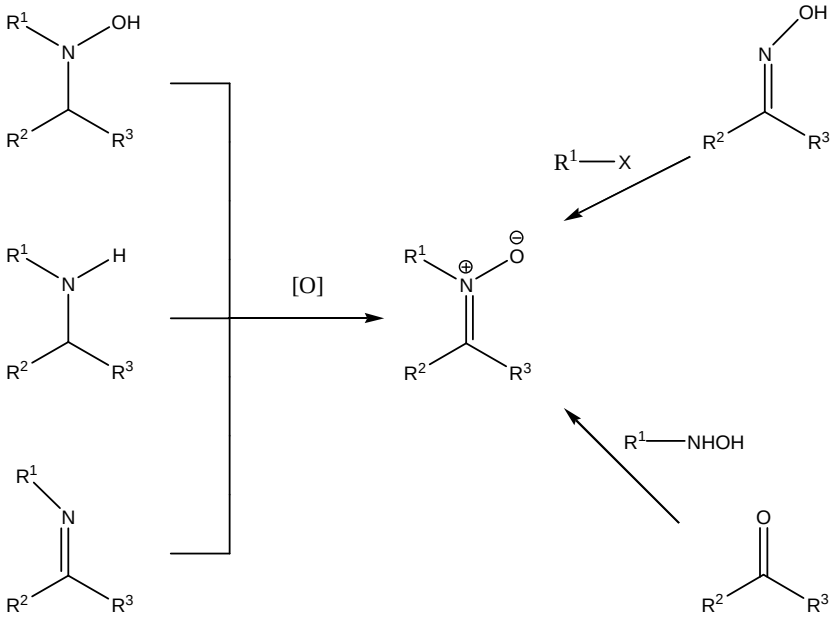
However, the present work take advance of nitrone chemistry versatility in order to synthesize on the one hand bisphosphonates containing *N,O*-carbocyclic nucleoside units with potential biological activity and, on the other hand, to synthesize suitable allyl cyclic nitrones undergoing 2-Aza-Cope rearrangement in order to study the well-known [3,3]-sigmatropic process, but that has been rarely detected until now with this kind of molecules and that can be very useful merged with [3+2] cycloaddition[12] or Mannich type reaction.[13]

2. Nitrones: state of art

2.1 Nitrone synthetic strategies

There are various methods of synthesis of nitrones, which can be grouped into three main categories (Scheme 2.1):

- Condensation of an aldehyde or ketone with *N*-substituted hydroxylamine;
- Oxidation of amines, imines or hydroxylamines;
- Alkylation of oximes.



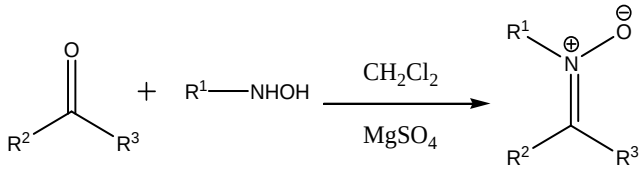
Scheme 2.1: Methods of nitrones synthesis.

Between these three methods the first two are the most used in the synthesis of cyclic or acyclic nitrones. Besides these three groups

there are other methods of synthesis used to a lesser extent. Depending on the type of nitrone (cyclic/acyclic and aldehyde-nitrones/ketonitrones) and accessibility of the precursors (hydroxylamines, oximes, imines, etc...) may be more appropriate one or another method, as discussed briefly below.[3a, b]

2.1.1 Condensation of aldehydes and ketones with hydroxylamines

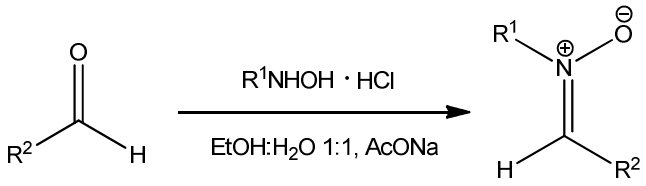
The condensation reaction of *N*-substituted hydroxylamines with an aldehyde or ketone is the main method of synthesis of acyclic nitrones.[2, 3d] In most cases the reaction proceeds with excellent yields in organic solvents as dichloromethane, diethyl ether or methanol and in the presence of a drying agent such as magnesium sulfate, sodium sulfate or molecular sieves (Scheme 2.2).



Scheme 2.2: Condensation reaction between *N*-substituted hydroxylamines and carbonyl compounds.

Recently the same condensation reaction, between hydrochloride *N*-substituted hydroxylamines and carbonyl compounds, has been conducted in a 1:1 solvent mix of water and ethanol (Scheme 2.3).[10d] The only limiting factor of this method is the

availability of the corresponding hydroxylamine that, sometimes, may not be easy to obtain.



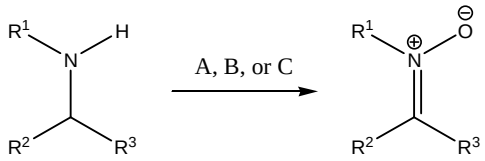
Scheme 2.3: Synthesis of nitrones by condensation of the precursor aldehydes with *N*-methyl or *N*-benzylhydroxylamine in acetate-buffered water/ethanol solution.

This method allows to obtain non-racemic chiral nitrones from carbonyl compounds such as optically active hydroxylamines, including *N*-glycosyl nitrones[14] and *C*-glycosyl nitrones in equilibrium with α-alkoxy hydroxylamines.[15] Furthermore, it is the most commonly employed method in obtaining aromatic and aliphatic nitrones[4b] and can be also used in solid phase conditions.[16] It involves, sometimes, the use of a Lewis acid to activate the carbonyl group of a ketone.[4a] On the other hand, some heterocyclic nitrones can be prepared by directly condensation of hydroxylamine with orthoester.[17]

2.1.2 Oxidation of amines, imines and hydroxylamines

The oxidation of amines and hydroxylamines is the general method used in the synthesis of cyclic nitrones.[18] The conditions used to obtain nitrones from secondary amines are based on treatment with

hydrogen peroxide in presence of a catalyst, usually metal such as sodium molybdate[19] or sodium tungstate[20] or methyltrioxorhenium (MTO) (Scheme 2.4).[21]



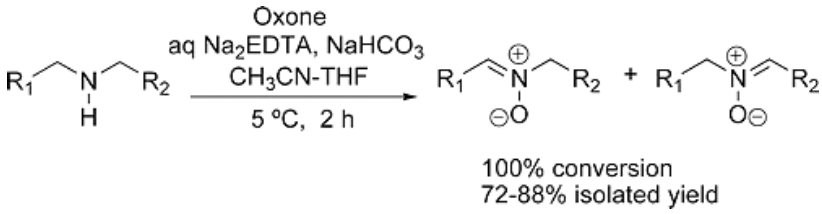
A: H_2O_2, Na_2WO_4 (cat.), Et_4NCl, CH_2Cl_2, H_2O

B: H_2O_2, Na_2MeO_4 (cat.), MeOH

C: UHP, $ReMeO_3$ (cat.), MeOH

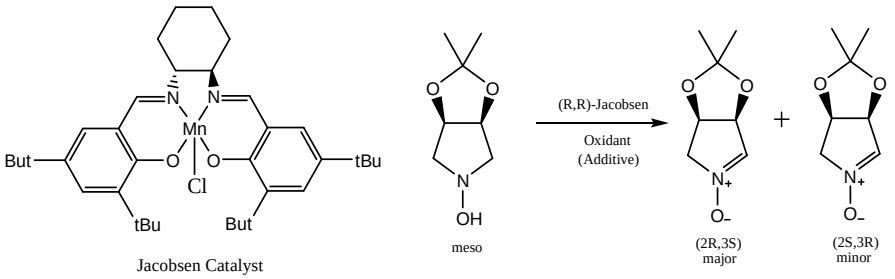
Scheme 2.4: Oxidation of secondary amines.

In several cases, hydrogen peroxide complex can be replaced by urea-hydrogen peroxide (UHP) that is safer than first one.[22] Oxidation with hydrogen peroxide may also be performed in the presence of platinum complex (IV)[23] and titanium (IV);[24] in the latter case the primary oxidizing agent can be replaced by cumyl hydroperoxide.[25] Cyclic and acyclic nitrones can also be obtained from secondary amines by oxidation with hydroperoxy flavins[26] and Oxone® (Scheme 2.5).[27]



Scheme 2.5: Metal-free procedure for oxidation of secondary amines to nitrones.

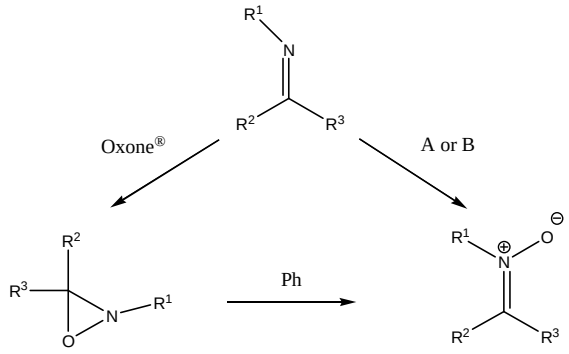
Under similar conditions to those employed with secondary amines, hydroxylamines are also oxidized to nitrones. The reaction can be carried out under milder conditions with reagents such as manganese oxide (IV),[28] sodium hypochlorite,[29] TEMPO[30] or air in the presence of copper acetate (II).[31] In presence of Jacobsen catalyst is possible to carry out asymmetric oxidation of achiral hydroxylamines although with moderate enantioselectivity (Scheme 2.6).[32]



Scheme 2.6: Enantioselective hydroxylamines oxidation.

By contrast, imines do not generally provide nitrones in conditions similar to those employed in the oxidation of secondary amines or hydroxylamines, because they tend to give the corresponding oxaziridines.[33] This one may become nitrones by ultraviolet irradiation,[34] transoximation,[35] treatment with trifluoroacetic acid[36] and in the presence of catalytic amounts of titanium tetrachloride[37] or silver triflate.[38] In the case of *N*-adamantyl oxaziridines it was described his conversion into nitrones in acetonitrile keeping at reflux for two days.[39] Imine oxidation to nitrones can be performed with potassium permanganate in phase transfer catalytic conditions,[40] using metiltrioxorrenio as catalyst[41] or

$MoOCl_4$ supported on Nafion®,[42] in both cases using UHP as primary oxidant (Scheme 2.7).



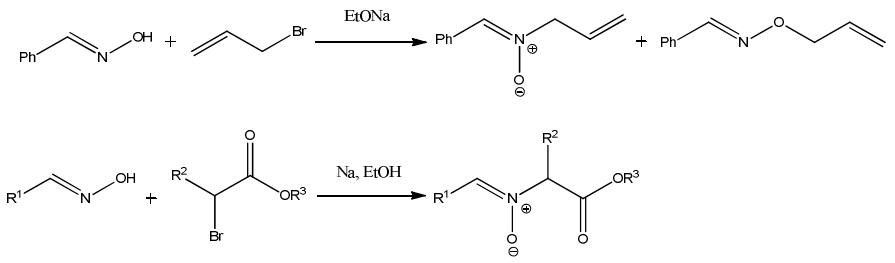
A: $KMnO_4$ (cat.) Bu_4NCl (Cat.), CH_2Cl_2-H_2O

B: $MeReO_4$ (cat.), UHP, MeOH

Scheme 2.7: Imine oxidation.

2.1.3 Alkylation of oximes

N-alkylation of oximes with alkyl halides or with alcohols and with activated olefins is a method that has been described at various times for the preparation of nitrones (Scheme 2.8).[43]



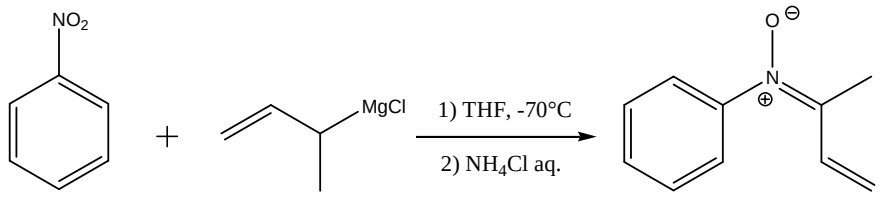
Scheme 2.8: *N*-Alkylation of oximes.

Depending on electrophile and on reaction conditions, there may be competition between the *O*- and *N*-alkylation because oximes have these two reactive nucleophilic centers. Due to this competition, this method is much more suitable for the preparation of cyclic nitrones where is necessary the presence of a leaving group at γ or δ position with respect to the oxime as desired to obtain cycles of 5 or 6 members, respectively.[44] Indeed, the desilylative cyclization of *O*-silyl oxime is one of the methods employed in the preparation of five members polyhydroxylated cyclic nitrones.[45] It also described some examples for the preparation of six-membered cyclic nitrones although to a much lesser extent.[46]

The reaction can also be generating hydroxylamine *in situ* and carrying out the cyclization reaction in one step.[47]

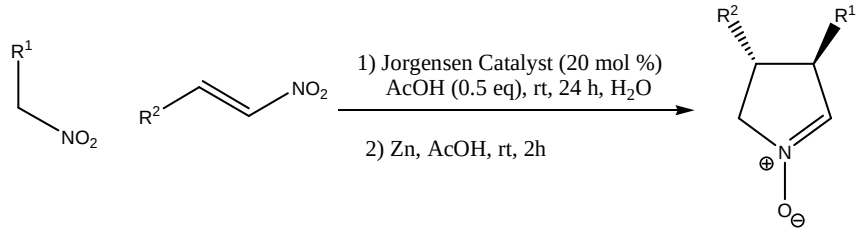
2.1.4 Other methods

The addition of Grignard derivatives of nitro-derivatives is described as a method to obtaining nitrones in good yields and with acceptable regioselectivity (Scheme 2.9).[48] Although the reaction can be considered of general applicability, it have been used only in particular cases.



Scheme 2.9: Addition of Grignard reagents to nitroalkenes.

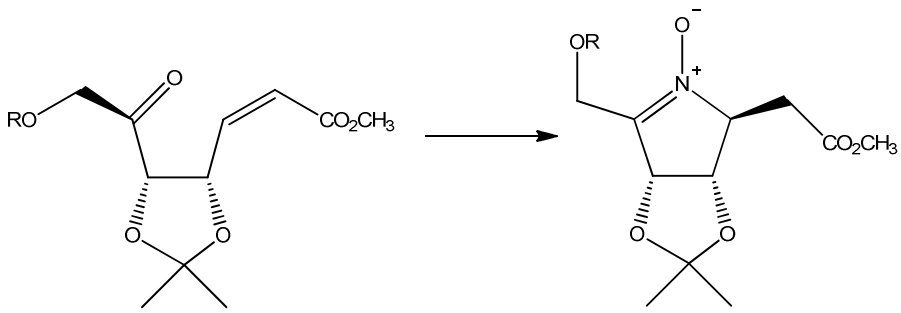
For the synthesis of cyclic nitrones, modifications have also carried out by condensation and alkylation of oximes. Reductive cyclization of the γ-nitro-carbonyl compounds, using various reaction conditions including H_2/Pd-C, Fe/HCl, Zn/NH_4Cl and Zn/AcOH, is an ideal method for synthesizing five members cyclic nitrones. Moreover, since the starting materials are enantiopure available through organocatalytic approximations well described in literature,[49] is a very convenient method for the synthesis of optically active compounds (Scheme 2.10).[50]



Scheme 2.10: Cyclic nitrone synthesis by reductive cyclization.

Intramolecular cyclization reactions may also be of various kinds and so cyclic nitrones were prepared from allenyl oximes,[51] by aza-Michael intramolecular reactions[52] (Scheme 3.11) and ω-epoxy alkanenitriles cyclization.[53] Cyclization between imines and nitroso-alkenes was used in the synthesis of nitrones dihydroimidazole

derived;[54] if the cycloaddition takes place with an enamine will it obtain five member cyclic nitrones.[55] Nitrosoalkenes also be condense with metal salts of nitroalkanes to afford acyclic ketonitrones.[56] In some particular case the preparation of ketonitrones has been possible by the addition of *N*-benzylhydroxylamine to alkynes[57] and allenes.[58]



Scheme 2.11: Aza-Michael intramolecular reactions. Reagent and conditions: $NH_2OH \cdot HCl$, $NaHCO_3$, EtOH, 25 °C, 2 h.

2.2 Nitrone biological properties

Nitrones are widely used organic compounds designed for spin trapping experiments[59] both in chemical and biological systems.[60] Nitrone spin-trapping agents such as α-phenyl *N*-tert-butyl nitrone (PBN, Figure 2.2) have emerged as promising pharmacologic tools because of their robust neuroprotective efficacy and large therapeutic time window in several models of central nervous system injury, such as traumatic brain injury (TBI), stroke, and intracerebral hematoma.[61] The neuroprotective effect of PBN has been attributed to scavenging of reactive oxygen species (ROS), although alternate mechanisms such as influence on inflammatory mediators,[62] transmitter systems,[63] or blocking of Ca^{2+}-channels[64] have been suggested.

On the basis of that, it was designed and synthesed new heteroarylnitrones combining, in their structures, fragments able to show neuroprotection properties, such as nitrone moiety, antioxidant fragments, and heterocyclic groups able to stabilize the generated free radical (Figure 2.1).[65]

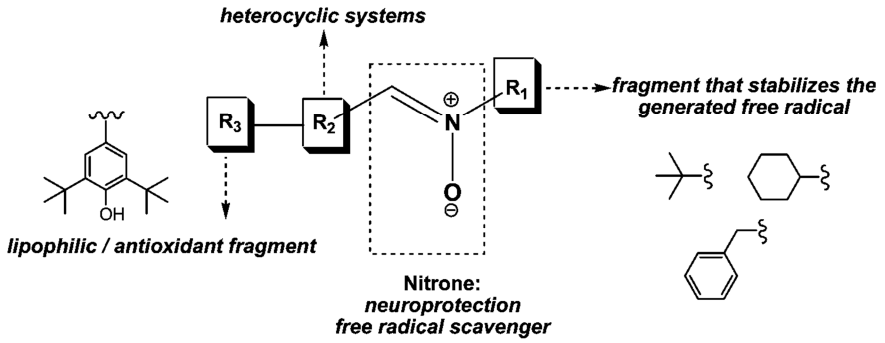


Figure 2.1: Heteroarylnitrones designed as potential drugs for neurodegenerative diseases.

2.2.1 Nitrone neuroprotective ability

ROS and reactive nitrogen species (RNS) produced by oxidative metabolism are capable of damaging cellular components through molecular modifications to a polyunsaturated membrane's lipids, proteins, and nucleic acids.[66] Much evidence suggests that biological oxidation in the human body generates highly pathogenic ROS and RNS such as hydroxyl free radical (•OH), superoxide anion ($O_2^{\bullet -}$), peroxynitrite ($ONOO^-$), and lipid peroxide free radicals ($ROO^{\bullet}$), causing cellular injury.[67] These pathological events have important roles in many degenerative disorders, for example, atherosclerosis, rheumatoid arthritis, and several neurodegenerative diseases such as ischemic conditions, stroke, Parkinson's disease, and Alzheimer's disease.[68] The central nervous system (CNS) is especially sensitive to oxidative damage for reasons such as (a) high use of oxygen during the metabolic process and therefore greater production of ROS, (b) fewer antioxidants defenses than other organs, for example, liver and heart, (c) enrichment in the more easily

peroxidable fatty acids, (d) areas with high levels of the Fe^{3+}/ascorbate pro-oxidant system that reduce H_2O_2 to form •OH, a potent oxidant.[69] Recently, it has been reported that β-amyloid solutions liberate hydrogen peroxide and, subsequently, •OH converted via Fenton's reaction.[70] Consequently, the search of effective treatments that prevent oxidative stress associated with premature aging and neurodegenerative diseases is an important area of neurochemical research.

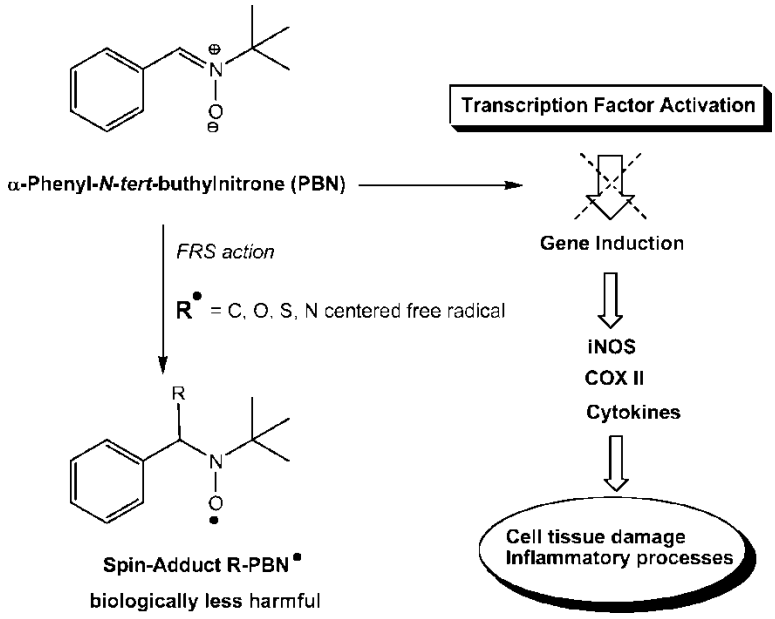


Figure 2.2: Antioxidant and neuroprotective mechanisms of PBN action.

Recently, it has been proved that nitrone-free radical trap, i.e, PBN, could be used in the treatment of neurodegenerative diseases as well as in the prolongation of life span.[71] Initially, the neuroprotective activity was attributed to the nitrone group because of its ability to act as free radical scavenger (FRS, Figure 2.2). Subsequently,

observations that PBN has a good neuroprotective activity at lower doses than those necessary to act as an effective FRS demonstrated the ability of PBN to inhibit signal transduction processes such as suppression of proinflammatory cytokines, genes, and mediators associated with enhanced neuroinflammatory processes (Figure 2.2).[72] The neuroprotective activity of the nitrone pharmacophore depends in great part on the connectivity and the nature of substituents on the nitrone group. In this sense, the chemical and pharmacological aspects of different heteroarylnitrones have been reviewed,[73] describing some patented nitrone containing furans (I-III, Figure 2.3) with good activity against the neuronal cell damage induced by β-amyloid.[74] On the other hand, imidazolylnitrones (IV and V, Figure 2.3) has been developed and biologically evaluated, showing *in vivo* neuroprotective properties.[75] Additionally several nitrones has been widely used as spin trap for the specific detection of transient radicals (e.g., •OH, $O_2^{\bullet-}$, $ROO^{\bullet}$) or relatively stable radicals (e.g., NO) that are undetectable under normal conditions,[76] in the electron spin resonance (ESR) spectroscopy. With this aim two nitrones have been commonly used in the spin trapping technique: the linear nitrone PBN (Figure 2.2) and the cyclic nitrone DMPO (5,5-dimethyl-1-pyrroline *N*-oxide VI, Figure 2.3).

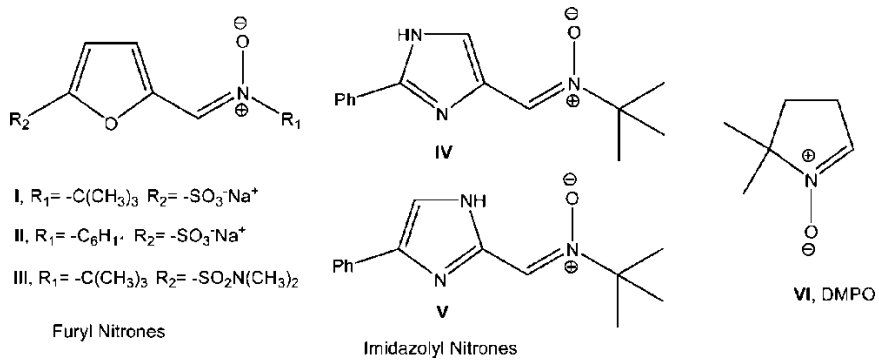


Figure 2.3: Heteroarylnitrones with neuroprotective and spin trapping properties.

Moreover, early assessment of compound availability in the central nervous system (CNS) is essential for CNS drugs and useful for optimizing the toxicity profile of non CNS drugs. Drug's penetration through the blood brain barrier (BBB) is one of the major obstacles for the treatment of diseases in the CNS but several nitrones was evaluated as able to cross the BBB by passive permeation.

Although Parkinson's disease (PD)[ϕ] has been heavily researched in the last decades, the precise etiology of the disease is still unknown. However, research in recent years has provided substantial evidence supporting the hypothesis that oxidative stress plays a major role in disease pathogenesis.[77] Studies in Parkinson proposed a possible role for ROS and/or RNS in selective loss of SN dopaminergic neurons in the disease.[78] Enhancing antioxidant and radical scavenger capabilities in the SN therefore constitutes a rational approach to prevent or slow ongoing damage of dopaminergic neurons.

[ϕ] Parkinson's disease (PD) is a neurodegenerative disorder involving the progressive degeneration of dopamine neurons arising in the substantia nigra (SN).

In the last decade, nitrone spin traps, α-phenyl *N*-tert-butylnitrone and structurally related compounds (Figure 2.4), have shown their utility in the treatment of neurodegenerative diseases as well as in the prolongation of life span due to their neuroprotective effects.[79] In an effort to optimize the biological profile of PBN, a wide structural diversity of PBN-like nitrones has been designed and synthesized.[80] In particular, a phenolic nitrone-TDZ (Figure 2.4) showed excellent free radical scavenger ability and good neuroprotective effects without cellular toxicity.[81] Furthermore, recently experimental *in vitro* antioxidant properties, as well as preliminary in vivo pharmacological activities of nitrone derivatives of Trolox (α-tocoferol derivative) were reported, showing that nitrones bearing free phenol groups exerted the best antioxidant values.[82]

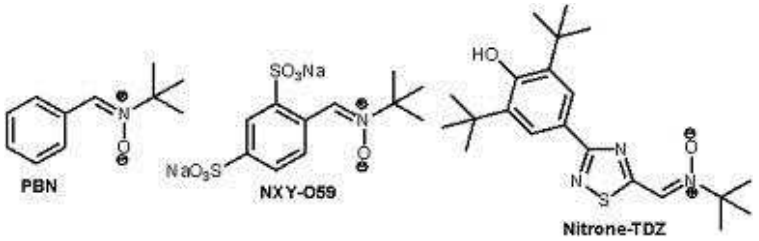


Figure 2.4: Chemical structure of PBN, NXY-059 and Nitrone-TDZ.

2.2.2 Nitrone for treatment of ischemic stroke

Stroke is one of the most devastating diseases after heart disease and cancer in developed countries. Despite the remarkable

progress achieved in the last two decades in understanding the pathophysiology of stroke, tissue-type plasminogen activator (tPA) remains the only therapy approved by the US FDA for acute ischemic stroke, which accounts for 70–85% of all stroke patients and carries a 15–33% mortality rate.

Brain tissues rely on circulating blood to deliver oxygen and other nutrients, as well as remove metabolic wastes. When a blood clot (thrombus) forms in a brain blood vessel, the delivery system to the brain may be compromised, resulting in an ischemic stroke. Therefore, the first task of ischemic stroke therapy is to remove/dissolve the blood clot, that is, thrombolytic therapy. tPA activates zymogen plasminogen into plasmin, resulting in thrombolysis. However, tPA has a narrow therapeutic window of three hours within the occurrence of a stroke, limiting its clinical use.

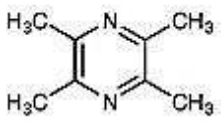
An ischemic stroke results in a cascade of biochemical events producing profound cellular changes. These include a rapid decrease in ATP, calcium overload, disruption of various ion pumps, and excitotoxic changes resulting from glutamate release, acidosis, and edema.[83] Many of these changes are associated with increased free radical production, occurring both during ischemia and during the subsequent reperfusion stage. ROS, which have short half-lives, can be extremely detrimental to the surrounding tissue. Normal tissues have a defense system against these toxic ROS; however, ischemia either interrupts or overwhelms the protective mechanisms and allows increased ROS production in the surrounding tissues, leading to neuronal cell damage/death. For this reason, thrombolytic therapy

alone is not enough to cure an ischemic stroke. Therefore, therapeutics which reduce the damage caused by ROS are needed.

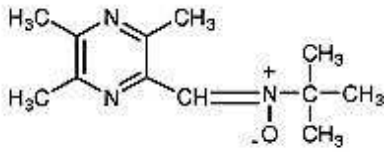
Emerging treatments for acute ischemic stroke include use of thrombolytic and neuroprotective agents.[84] While thrombolytic treatments lyse blood clots to restore blood flow, neuroprotective treatments prevent cell death during and after ischemia and reperfusion. One of the most extensively studied classes of neuroprotective agents is the free radical-scavenging nitrone. Nitrones react with free radicals to form nitroxides, which act as superoxide dismutases (SOD), mimicking and catalyzing the dismutation of superoxide anions,[85] thereby protecting cells from free radicalmediated cell damage.

Later experiments demonstrated that PBN markedly reduced infarct volumes in rats subjected to long periods of focal ischemia induced by middle cerebral artery (MCA) occlusion.[61a] PBN was found to be effective when administered 5 h after onset of ischemia. The nitrone NXY-059 (disodium 4-[(tert-butylimino)methyl] benzene-1,3-disulfonate *N*-oxide, Figure 2.4) was shown to significantly reduce infarct volumes in animal stroke models.[86] Based on the impressive preclinical data, NXY-059 was evaluated in two phase III clinical trials.[87] Unfortunately, the SAINT II trial conducted in about 350 centers worldwide across approximately thirty countries failed to reveal any positive effects in ischemic stroke patients.[87b] Although the clinical results of NXY-059 are disappointing, the concept of using neuroprotective agents for stroke therapy remains viable. For example, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, Eda) such as TMP (Figure 2.5), also a free radical scavenger, has

been approved for treatment of patients with acute ischemic stroke in Japan[88] and China. For these reasons, it was synthesized some nitrones, i.e. TBN (derived from TMP, Figure 2.5), possessing both the thrombolytic and free radical-scavenging activities necessary for effective stroke treatment. Some of these compounds showed good activity both *in vitro* and *in vivo* models.[82a]



TMP



TBN

Figure 2.5: Chemical structure of TMP and TBN.

3. Bisphosphonates containing *N,O*-carbocyclic nucleoside units

3.1. Bisphosphonates in medicinal chemistry

In last three decades, the significant pharmacological properties of bisphosphonates (BPs) and bisphosphonic acids have made them very appealing. The development of organophosphorus chemistry has been characterized by a great interest in these compounds. They showed a considerable cytotoxic activity against several human cell lines and therefore they could be successfully employed as anticancer drugs. Moreover, bisphosphonates can be considered as stable analogs of pyrophosphate, that is implied in the physiological regulation of bone calcification and resorption. So the discovery and development of the bisphosphonates were become them the major class of drugs for the treatment of bone diseases. Indeed, these compounds are actually in use for treatment of Paget's disease, myeloma, bone metastases and osteoporosis.[89]

Therefore, organophosphorus chemistry has been an important branch of medicinal chemistry and recently has taken an important effect on the synthesis and design of a wide variety of biologically active compounds. This chemistry, being ignored for many years, nowadays has achieved important and well-recognized place in the search for new drugs.[90] Organophosphorus chemistry, as a discrete area of study, is the study of compounds containing a C-P bond.[91] Its present impact on the field of medicinal Chemistry is even difficult to quantify. Among the list of all organophosphorus compounds the main place is occupied by phosphonates and bisphosphonates, which

have found huge application as pharmaceuticals. For example, 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. They are very efficiently mimic amino carboxylic acids for that they are extremely important anti metabolites. On the other hand, bisphosphonates, for their high affinity to biological apatite, have been found recently as an ideal therapeutic agent for treatment of different bone diseases because they are efficiently bone-seeking compounds able to act mainly on bone tissue with limited pharmacological activity at other anatomical sites. This ability is explained by the fact that bone tissue is distinguished from the rest of our tissues by the presence of a massive mineral phase, with few exceptions no other tissue systems contain such a concentrated mineral phase: more than 99% of bodily calcium deposits are located in bone. Therefore, the bisphosphonates capability to chelate metal ions and inhibit crystals growth so also a strong affinity to bone is the reason they began to be synthesized as new important drugs with the potential to seek and concentrate in the bone tissue. This allowed 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.

Efforts in this direction were set into motion in the early 1960s while probing the physiological function of an endogenous molecule, pyrophosphate (Figure 3.1). Pyrophosphate P-O-P is localized throughout an organism, and displays a dual activity on the formation and dissolution of biological apatite, a carbonated form of the stoichiometric hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$.[92] The strong

affinity of the pyrophosphate to nucleating hydroxyapatite (HA) crystals was considered to be the underlying basis of this dual activity.[93] 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.[94]

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.[95-98] Such diphosphonates P-C-P were shown to be capable of controlling HA dissolution,[95,96] as well as preventing bone loss induced by immobilization[97] and parathyroid extract injection in animal models.[96]

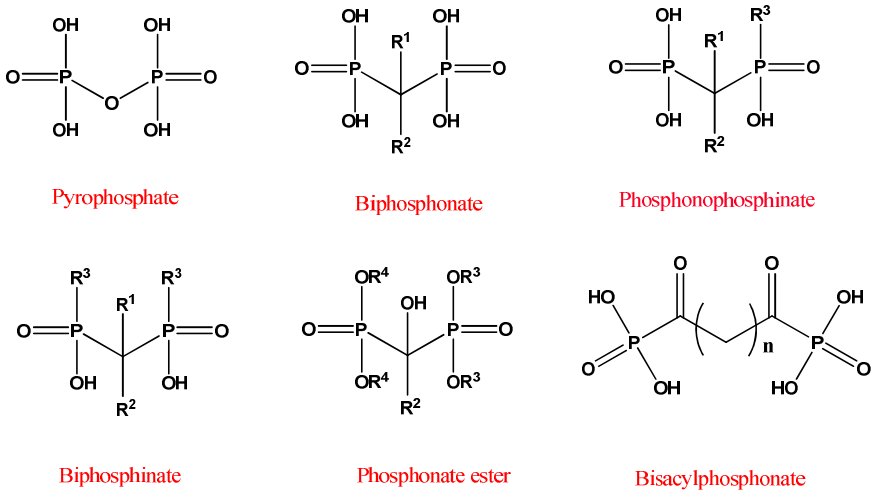


Figure 3.1: Structure of the endogenous pyrophosphonate and its synthetic analogue, bisphosphonate (BP), which exhibit a strong bone affinity. The geminal (α) carbon in BPs typically contains two separate substituents, R^1 and R^2, which may significantly affect both the mineral affinity and the pharmacological activity.

The diphosphonates used in these early studies were dichloromethylene diphosphonate,[96, 97] methylene diphosphonate[96] and 1-hydroxyethylene-1,1-diphosphonate[98]. 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. BPs immediately became to use for clinical entry by virtue of his inhibition capability of bone loss without significant side-effects.[99] The promising results led to a considerable increase of the research concerning these compounds and early was discovered more potent BP drugs that including a nitrogen atom in the molecule (second-generation) but the most of highly potent one include an additional moiety in the molecule, namely a nitrogen heterocycle, as in zoledronate (third-generation).[100-103]

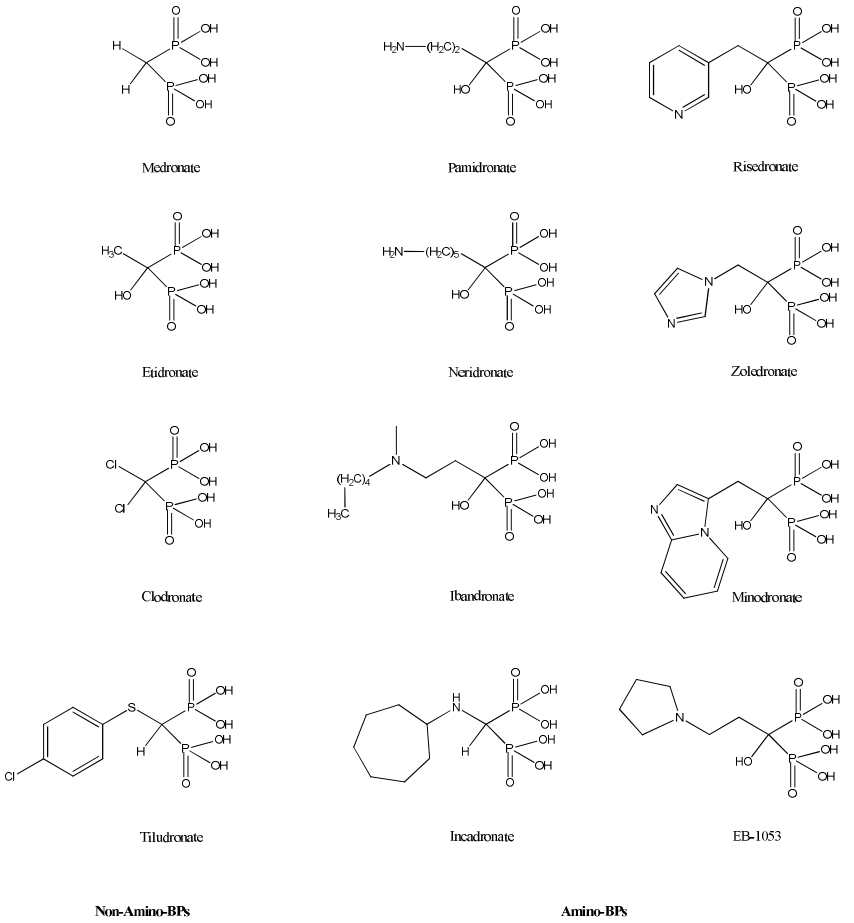
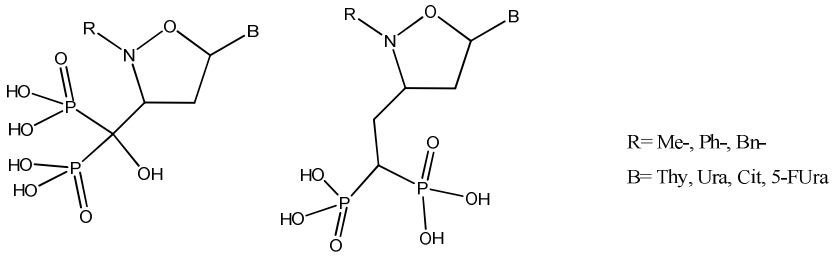


Figure 3.2: Examples of BP class of compounds currently used in a clinical setting. The amino-BPs typically exhibit a higher potency in antiresorptive effects, the primary clinical utility of BPs. Most of the BPs contain a geminal –OH group that enhances the mineral affinity of the compound.

It should be noted 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.[96] It was within a few years of realization of the pharmacological activities of BPs that their utility as bone carriers was also demonstrated. Indeed, when the development of a bone targeted therapeutic was first reported the synthesis of BP-incorporating molecules with pharmacological activities distinctly

different from the BP action was became. Nowadays, BPs are beginning to be utilized as the building blocks of generic carriers that can transport a spectrum of molecule to bone, rather than linking them directly to a given molecule for its delivery to bone. Combining anticancer agents with BPs is intended to concentrate the antineoplastic activity of therapeutic agents in bones after systemic administration.

Towards this end, in this work we considered the synthesis of a new class of bisphosphonates having an isoxazolidinyl nucleoside unit that should makes it particularly attractive from the rapidly dividing cells such as cancer cells, for the purpose of obtain two different types of nucleotides analogues (Figure 3.3): type **1** having a further additional moiety named a geminal hydroxyl group and other one (type **2**) only containing a *N,O*-carbocyclic unit but without the geminal hydroxyl group.



1 **2**

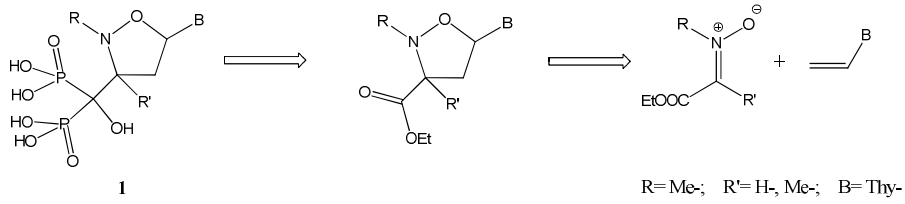
Figure 3.3: Structure of target compounds (type **1** and **2**).

Accordingly, in order to find high potent anticancer drugs, we synthesized various compounds with this characteristic changing some isoxazolidinic ring substituent groups; in particular we used

various nitrogenous bases and three different *N*-substituted nitrones as shown in the following part of this chapter.

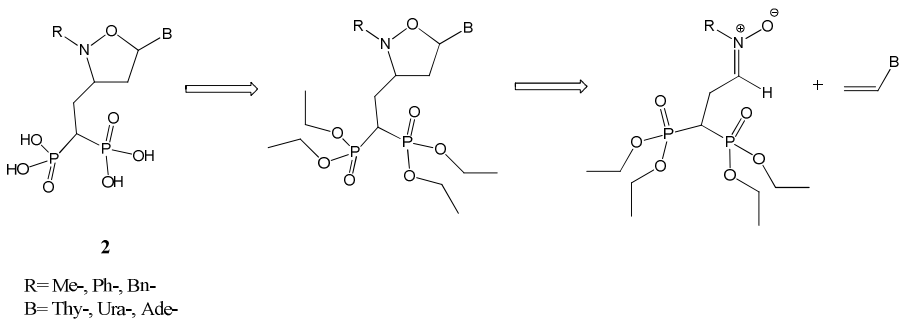
3.2. Results and discussion

In order to obtain nucleotides analogs having a geminal hydroxyl group (type **1**) as zoledronate, which increases the affinity for calcium even further owing to the ability of such derivatives to act as tridentate ligands, we synthesized *C*-ester nitrones and unprotected *N*-vinyl nucleobases that, via 1,3-dipolar cycloaddition, conduct to 3-ester isoxazolidinyl nucleosides. These compounds should represent the substrates that can be functionalized with the bispfosphonate group (Scheme 3.1). Unfortunately, so far, we couldn't obtain this kind of molecules for the reasons that will be explained below.



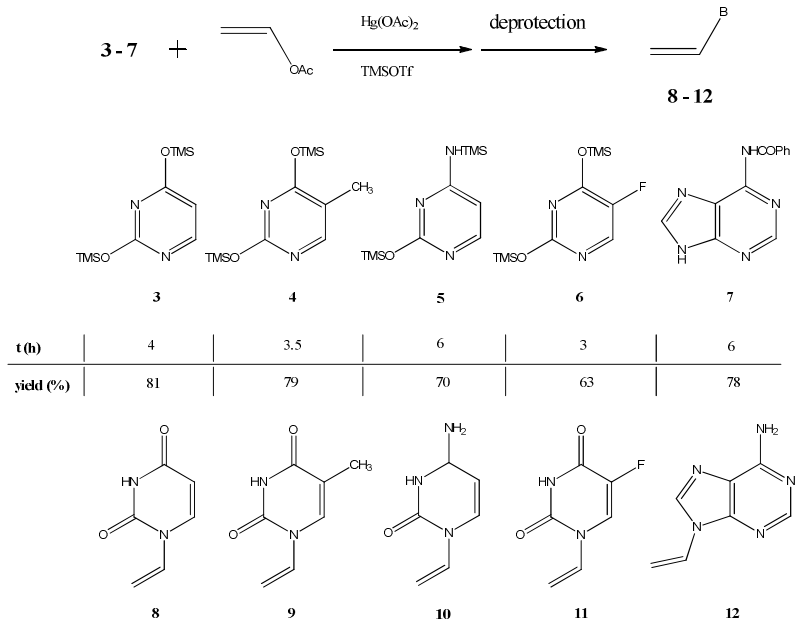
Scheme 3.1: Synthetic strategy to obtain product **1**.

Instead we obtained bisphosphonates type **2** starting from the synthesis of bisphosphonate nitrones and unprotected *N*-vinyl nucleobases, via 1,3-dipolar cycloaddition (Scheme 3.2). Therefore, in order to find the most active molecule of this compound class, we synthesized various isoxazolidines varying the substituent groups: in particular we used various nucleobases and three different *N*-substituents (compounds **2**, Figure 3.3).



Scheme 3.2: Synthetic strategy to obtain product **2**.

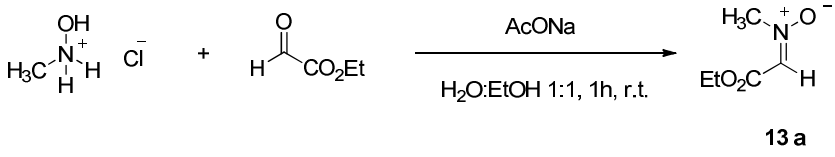
Furthermore, *N*-vinyl derivates of nucleobases were synthesized accordingly to method of Procopio *et al.*[104] and were used without protection for the cycloaddition reactions. The synthesis involves a simple one pot-pot procedure to prepare 1-vinyluracil (**8**), 1-vinylthymine (**9**), 1-vinylcitosine (**10**), 1-vinyl-5-fluorouracil (**11**) and 9-vinyladenine (**12**) using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst in direct exchange of the acetate group of vinyl acetate with pyrimidine and purine bases (Scheme 3.3).



Scheme 3.3: Synthesis of *N*-vinyl nucleobases.

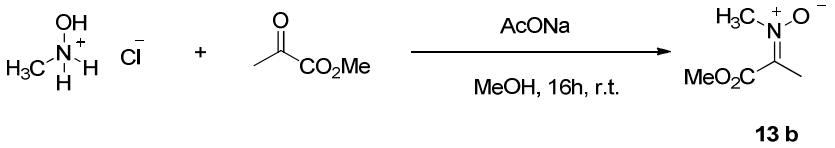
3.2.1. Preparation of bisphosphonates type 1

With the set of vinyl nucleobases **8-12** in our hands the first step of the project **1** was the synthesis of the nitrones (**13a** and **13b**), starting by *N*-methyl hydroxylamine hydrochloride and ethyl glyoxylate. The synthesis was carried out at room temperature for 1 hour and gave a yield of 76% (*E*:*Z* = 3.5:1) as shown in Scheme 3.4.



Scheme 3.4: Synthesis of nitrone **13a**.

On the other hand the same reaction was carried out using *N*-methyl hydroxylamine hydrochloride and methyl pyruvate in methanol and in presence of sodium acetate (Scheme 3.5), giving a yield of 82%.[105]

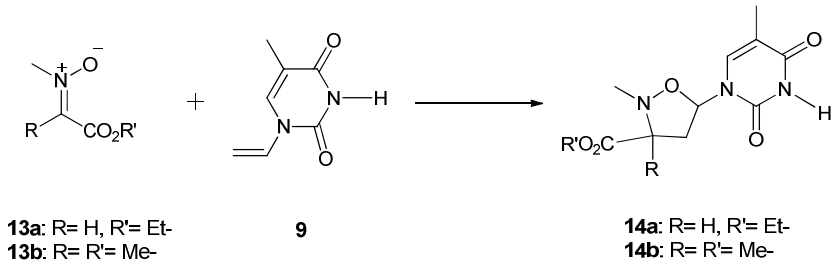


13 b

Scheme 3.5: Synthesis of nitrone **13b**.

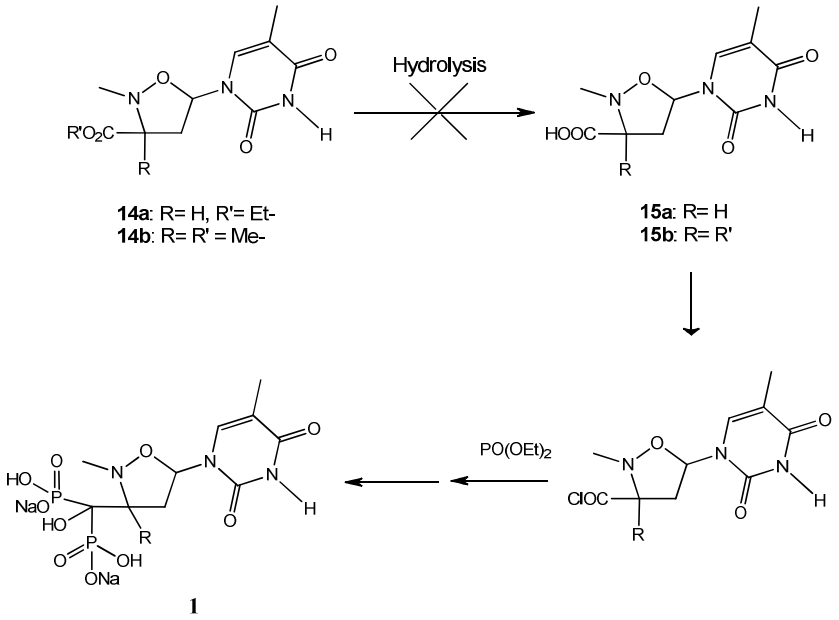
The second step was the direct 1,3-dipolar cycloaddition between the obtained nitrones and the *N*-1-vinylthymine (**9**). The choice of *N*-1-vinyl thymine was due to its excellent reactivity and to the fact that it does not require any protection.

In the case of product **14a**, the reaction was conducted both under microwave irradiation giving a yield of 62% (without solvent), and in classic conditions giving a yield of 93% (in toluene at 60 °C). Both reactions give a similar diastereomeric ratio, but the reaction carried out under microwave irradiation, in the absence of solvent, takes only 10 minutes (yield 62%, *cis*:*trans*=17:83), while the one in toluene takes 6 days (yield 93%, *cis*:*trans*=13:87). In the case of product **14b**, the reaction carried out under microwave irradiation giving a yield of 51% (Scheme 3.6).



Scheme 3.6: Synthesis of cycloadducts **14a** and **14b**.

Unfortunately, as shown in Scheme 3.7, the synthesis was stopped at the ester hydrolysis because we retrieved only a product of beta-elimination in all the several attempts made so far (Table 3.1). Actually the work is in progress to resolve these problems.



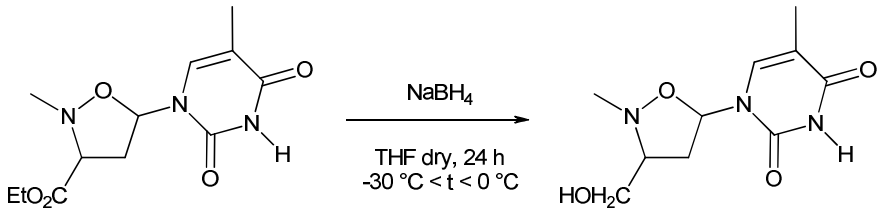
Scheme 3.7. Missing steps for synthesis of compounds type **1**.

	Molar ratio	Condition
Base = NaOH	1:5	MeOH, r.t.
Base = NaOH	1:3	MeOH, r.t.
Base = NaOH	1:2	MeOH, r.t.
Base = NaOH	1:1	MeOH, r.t.
Base = LiOH	1:3	H_2O/MeOH, 0-5 °C
Base = LiOH	1:1	H_2O/MeOH, 0-5 °C
Acid = NH_4Cl	-	H_2O/Dioxane, pH =5
Enzyme = Lipase A	3600 U/g	pH = 6.8 - 7.4

Table 3.1: Hydrolysis reaction condition.

3.2.2. Preparation of bisphosphonates type 2

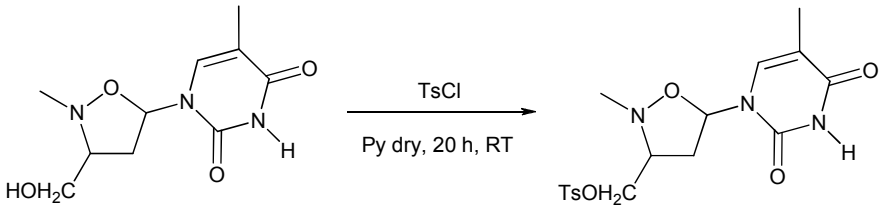
In order to obtain the bisphospohonates type **2** a first attempt of synthesis involved, firstly, the reduction of the ester group of compound **14a** reducing to alcohol (**16**) by sodium boron hydride under anhydrous conditions. The reaction gives a yield of 70% (Scheme 3.8).



14a **16**

Scheme 3.8: Synthesis of compound **16**.

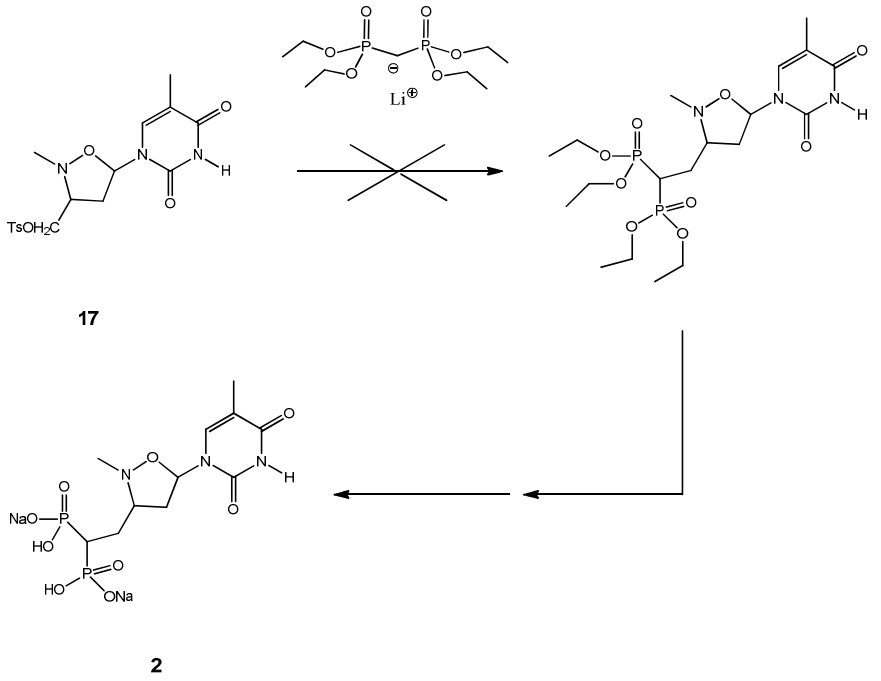
The subsequent step involved the tosylation of the alcohol group in order to allow the nucleophilic attack of a bisphosphate anion. It was carried out in classic condition and gives a yield of 63% (Scheme 3.9).



16 **17**

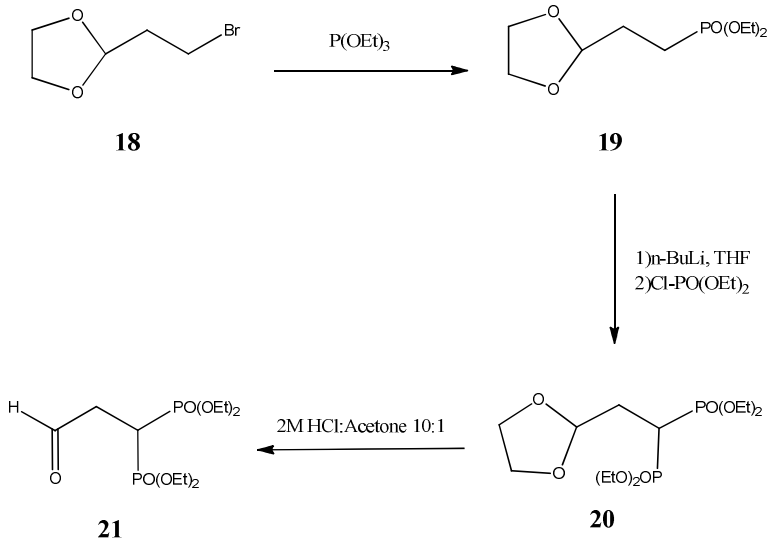
Scheme 3.9: Synthesis of compound **17**.

One of the last steps planned to complete the synthesis involved the nucleophilic substitution with a bisphosphonate anion (Scheme 3.10), but unfortunately it was impossible to complete probably for steric hindrance.



Scheme 3.10: Unsuccessful steps to functionalize compound **17**.

For these reasons, we totally changed the synthetic strategy and we decided to synthesize some nitrones having a methylene bisphosphonate group in β-position (**22 a - c**, Scheme 3.12). The new synthesis involved, firstly, the formation of the bisphosphonate moiety (**21**). The aldehyde **21** was obtained in three steps from 2-bromoethyl-1,3-dioxolane, as shown in Scheme 3.11.[106]



Scheme 3.11. Synthesis of precursor's bisphosphonate nitrones.

The Michaelis-Arbuzov reaction of compound **18** with triethyl phosphite afforded the phosphonate **19** in 62% yield. To carry out the synthesis we used triethyl phosphite (2 equivalents), added dropwise to neat 2-(2-bromoethyl)-1,3-dioxolane (1 equivalent) at room temperature under nitrogen and heated to reflux for 4 h. Pure product was purified by distillation *in vacuo*.

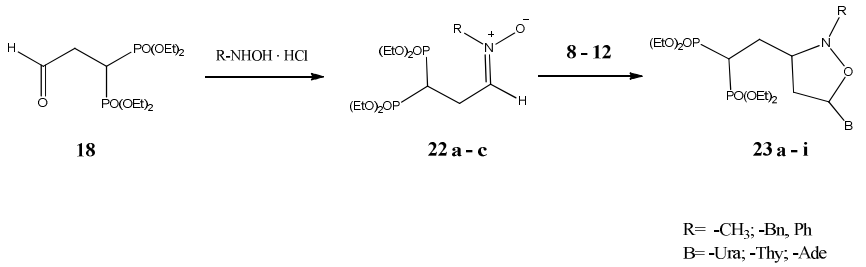
The second step involved the synthesis of tetraethyl 2-(1,3-dioxolan-2-yl) ethane-1,1-diyldiphosphonate (**20**). It was synthesized adding *n*-BuLi to a solution of phosphonate **19** in dry THF, cooled down to -78 °C, to generate a carbanion. Therefore was added diethyl chlorophosphate and, after purification by distillation under vacuum, bisphosphonate **20** was obtained with a yield of 48%.

To complete the formation of *gem*-bisphosphonate moiety, we synthesized tethraethyl 3-oxopropane-1,1-diyldiphosponate (**21**). Deprotection of cyclic acetal was carried out under acidic conditions:

dioxolane **20** was added to a solution 10:1 2M HCl_{aq} : acetone and heated to 50 °C for 3 h. The pure aldehyde **21** was obtained without chromatographic purification with a yield of 92%.

The second part of the synthesis involved, before, the formation of nitrone by condensation of aldehyde **21** with suitable hydroxylamines and, after, a cycloaddition of bisphosphonate nitrones with unprotected *N*-vinyl nucleobases.

In order to obtain nitrones **22 a - c**, aldehyde **21** was added to *N*-methyl hydroxylamine hydrochloride, *N*-benzyl hydroxylamine hydrochloride, or *N*-phenyl hydroxylamine hydrochloride respectively. The synthesis (Scheme 3.12) was carried out at room temperature for 1 hour in a mixture of H_2O:EtOH 1:1, in presence of sodium acetate (1.2 equivalents), extracted in DCM and dried over $NaSO_4$, to give, without further purification, pure nitrones as shown in Table 3.2.



Scheme 3.12. Final steps of the synthesis.

Nitrones	R	Conditions	Yield
a	-CH_3	H_2O:EtOH, r.t., 1h	93%
b	-Bn	H_2O:EtOH, r.t., 1h	84%
c	-Ph	H_2O:EtOH, r.t., 1h	89%

Table 3.2: Synthetic results of compounds **22 a – c**.

Last step, also showed in Scheme 3.12, involved 1,3 dipolar cycloaddition between nitrones (**22 a – c**) and three of more reactive and biological active *N*-vinyl nucleobases (**8**, **9**, **12**). The reactions were carried out under microwave irradiations, without solvent, for few minutes and crude products were purified by flash chromatography ($CHCl_3$:MeOH 97:3) giving pure isoxazolidines (**23 a – i**) as shown in Table 3.3.

Nucleosides	R	B	Conditions	Yield
a	-CH_3	-Ura	850W, 2,5 min.	62%
b	-CH_3	-Thy	850W, 3 min.	66%
c	-CH_3	-Ade	850W, 4 min.	59%
d	-Bn	-Ura	700W, 4 min.	72%
e	-Bn	-Thy	700W, 3 min.	73%
f	-Bn	-Ade	850W, 5 min.	65%
g	-Ph	-Ura	700W, 2,5 min.	77%
h	-Ph	-Thy	700W, 2 min.	80%
i	-Ph	-Ade	850W, 3 min.	71%

Table 3.3: Synthetic results of compounds **23 a – i**.

Actually the same procedure is extending to other *N*-vinyl nucleobases in order to find the most active molecules of this compound class on the scheduled biological evaluations.

3.3. Conclusions

We have synthesized various bisphosphonate containing *N,O*-carbocyclic units with significant pharmacological interest, as bisphosphonate nucleoside analogs. These compound can began important biological study in order to find drugs for treatment of many bone disease or tumor disease. For these reasons, in the near future, will be carry out suitable biological evaluations.

Furthermore, during the several steps of the synthetic process we observed numerous problems relating to the high complexity of compounds. We exceeded this hurdles developing a new synthetic strategy that involved the synthesis of innovative bisphosphonate nitrones and using it for microwave irradiated 1,3-dipolar cycloadditions with unprotected *N*-vinyl nucleobases.

3.4. Experimental section

3.4.1. General methods

Commercial starting materials were used without further purification. Solvents were distilled prior to use.

Reactions were monitored by TLC on silica gel 60 F254 (Merck) with detection by UV or by exposing the TCL to iodine vapor; flash column chromatography was performed on silica gel 60 (Merck Kieselg 60H).

^{1}H and ^{13}C NMR spectra were recorded at 300 and 500 MHz and 75.5 and 125.7 MHz, respectively, in $CDCl_3$ using tetramethylsilane (TMS) as internal standard (Bruker ACP 300 MHz and 500 MHz), whereas for ^{1}H- decoupled ^{31}P NMR (202.4 MHz) an external standard was used. Chemical shifts (δ) are given in parts per million and coupling constants (J) in Hertz.

GC/MS analysis were carried out on a Shimadzu GCMS-QP using diluted samples (1:1000).

The ESI mass spectrometric data were acquired on a Finnigan LCQ Deca, equipped with an electrospray ionization source. Standard experimental conditions are as follows: sample concentration 10^{-6} M; elution solvent MeOH; flow rate 8 mL min^{-1}; nebulizing gas 40 units flow rate; spray voltage 4 kV; capillary voltage 14 V; capillary temperature 270 °C.

High resolution mass spectra (HRMS) were acquired on a Q-star pulsar-i (MDS Sciex Applied Biosystems, Toronto, Canada) equipped with an ion-spray source at 10,000 resolution.

Solvent free cycloaddition reactions were carried out in a household microwave oven (Whirpool AVM119/1/WP/WH) at suitable irradiation power.

3.4.2. Experimental procedures

General procedure for *N*-vinylation of pyridine nucleobases (8-11): the appropriate pyrimidine nucleobase (4.4 mmol) was heated at 140-150°C with hexamethyldisilazane (2.62 g, 16.2 mmol), trimethylsilyl chloride (217 mg, 2.0 mmol) and trace of $(NH_4)_2SO_4$ until a clear solution was formed. Then, the solution was concentrated *in vacuo*. The residue was suspended under nitrogen in vinyl acetate (25.0 ml, 271 mmol) and $Hg(OAc)_2$ (96 mg, 0.3 mmol), trimethylsilyl trifluoromethanesulfonate (245 mg, 1.1 mmol), and hydroquinone (0.1 g), the latter as a polymerization inhibitor. The mixture was refluxed for the appropriate time when the reaction was finished, the mixture was filtered through neutral activated alumina and was washed with EtOAc. The solvents were removed at reduce pressure and the crude product was purified by flash chromatography with $CHCl_3$:MeOH (92.5:7.5) as eluent.

NMR data for substrates **8-10** are comparable with those of reference 104, while for compound **11** ^{1}H NMR spectrum is reported on end:

^{1}H NMR (500 MHz, DMSO-d_6): δ = 4.90 (dd, 1H, 2'-CH_{cis}, J_{gem}=2.03, J_{cis}=9.15); 5.38 (dd, 1H, 2'-CH_{trans}, J_{gem}=2.03, J_{trans}=16.02); 7.00-7.20 (m, 1H, 1'-CH); 8.46 (d, 1H, 6-CH, J=7.13); 12.01 (br, 1H, N-H).

General procedure for *N*-vinylation of purine nucleobase (12): the appropriate protected purine nucleobase (4.4 mmol) was added under nitroger to a suspension of $Hg(OAc)_2$ (96 mg, 0.3 mmol) in vinyl acetate (25mL, 271 mmol). Than trifluoromethane sulfonate (245 mg, 1.1 mmol) and hydroquinone (0.1 g), the latter as a polymerization inhibitor, were added. The mixture was refluxed for the appropriate time. When the reaction was over, the mixture was filtered through neutral activated alumina and was washed wit EtOAc. The solvents were removed at reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$:MeOH, 97.5:2.5 v/v) after suitable deprotection with gaseous ammonia.

NMR data obtained for compound **12** are comparable with those of reference 104.

***α*-Carbethoxy-*N*-methylnitrone (13a)**: A mixture of Methyl hydroxylamine hydrochloride (0.76 g, 9.2 mmol), NaOAc (0.90 g, 11 mmmol), ethyl glyoxylate (1.13 g, 11 mmol), water (13 mL) and EtOH (13 mL) was stirred at r.t. for 1 h and than concentrated under vacuum. The white semisolid residue was dissolved in a mixture of CH_2Cl_2 and H_2O. The organic phase was separated, dried ($MgSO_4$), filtered and concentrated under vacuum to afford, without further purifications, a colourless oil (yield 76%). (*E*:*Z* = 3.5:1) ^{1}H NMR

(300 MHz,$CDCl_3$): δ = 1.3 (t, 3H), 3.9 (s, 3H, *Z* isomer), 4.2 (s, 3H, *E* isomer), 4.2 (q, 2H, both isomer), 7.1 (s, 1H, *Z* isomer), 7.2 (s, 1 H, *E* isomer). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.1; 53.7; 67.0; 137.5; 162.7.

(*E*)-*N*-(1-Methoxy-1-oxopropan-2-ylidene) (methyl) methanamine Oxide (13b): A mixture of Methyl hydroxylamine hydrochloride (0.4 g, 4.8 mmol), NaOAc (0.43 g, 5.22 mmmol), methyl piruvate (0.44 g, 4.35 mmol), and MeOH (10 mL) was stirred at r.t. for 15 h and than concentrated under vacuum. The white semisolid residue was dissolved in a mixture of CH_2Cl_2 and H_2O. The organic phase was separated, dried ($MgSO_4$), filtered and concentrated under vacuum to afford, without further purifications, a pail oil (yield 82%). ^1H NMR (300 MHz,$CDCl_3$): δ = 2.25 (s, 3H), 3.85 (s, 3H), 4.2 (s, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 15.7; 52.6; 66.9; 137.8; 161.8.

General procedure for solvent free synthesis of isoxazolidines 14a and 14b: *N*-vinyl thymine (**9**) (0.1 mmol) and the nitrone (**13a** or **13b**) (0.2 mmol) are co-grinded in a mortar and further mixed in a vortex. The mixture of the two compunds is transferred in a 50 mL Pyrex container that is placed within an unmodified household microwave oven, at 750W irradiation power. After the appropriate time the reaction mixture is submitted to flash chromatographic separation, using $CHCl_3$:MeOH (98:2). The nitrone in excess is recovered and may be re-used.

Compound **14a** was also synthesized in classical condition to compare results: *N*-vinyl thymine (**9**) (0.1 mmol), nitrone **13a** (0.2 mmol) and a catalytic amount of hydroquinone were dissolved in toluene (5 mL) and heated at 60 °C for 6 days. Compound **14a** was finally purified by flash chromatography ($CHCl_3$:MeOH 98:2).

(*cis*) Ethyl 4-aza-4-methyl-2,3-dideoxythymidine-3-carboxylate (14a): ^{1}H NMR (500 MHz,$CDCl_3$): δ = 1.31 (t, 3H), 1.95 (s, 3H), 2.60-2.67 (m, 1H), 2.87 (s, 3H), 3.15-3.25 (m, 1H), 3.38-3.42 (m, 1H), 4.25 (m, 2H), 6.30 (dd, 1H), 7.78 (d, 1H), 9.33 (br, 1H).

(*trans*) Ethyl 4-aza-4-methyl-2,3-dideoxythymidine-3-carboxylate (14a): ^{1}H NMR (500 MHz,$CDCl_3$): δ = 1.33 (t, 3H), 1.95 (s, 3H), 2.52-2.55 (m, 1H), 2.86 (s, 3H), 3.12-3.18 (m, 1H), 3.87-3.89 (m, 1H), 4.25 (m, 2H), 6.27 (dd, 1H), 7.45 (d, 1H), 9.51 (br, 1H). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 12.5; 14.2; 61.6; 83.4; 111.2; 135.9; 150.6; 163.8; 168.7.

Methyl 4-aza-3,4-dimethyl-2,3-dideoxythymidine-3-carboxylate (14b): ^{1}H NMR (300 MHz; $CDCl_3$) δ = 1.42 (s, 3H), 1.96 (s, 3H), 2.76-2.94 (m, 2H), 2.74 (s, 3H), 3.78 (s, 3H), 6.24 (dd, 1H), 7.74 (s, 1H), 9.35 (br, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 12.1; 12.8; 31.2; 35.7; 41.7; 60.4; 81.3; 112.1; 133.9; 152.0; 166.3; 169.6

(*trans*) 4-aza-4-methyl-3-hydroxymethyl-2,3-dideoxythymidine (16): Isoxazolidine **14a** (1.14 mmol) was dissolved in dry THF (50 mL) at -78 °C under nitrogen atmosphere, than $NaBH_4$ (5.11 mmol) was added portionwise. The reaction mixture was allowed to warm to r.t. overnight. Reaction was worked up adding saturated aqueous solution of NH_4Cl, extracted by $CHCl_3$ and than dried. Pure product was obtained (yield 70%) by flash chromatography ($CHCl_3$:MeOH 98:2). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.75 (d, 3H), 2.30-2.58 (m, 2H), 3.20-3.42 (m, 1H), 3.45-3.66 (m, 2H), 4.01 (d, 1H), 4.27 (d, 1H), 4.94 (t, 1H), 5.98 (dd, 1H), 11.27 (br, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 11.5; 36.5; 43.7; 60.6; 61.5; 81.3; 111.0; 138.9; 151.9; 166.8.

(*trans*) 4-aza-4-methyl-3-methyltosyl-2,3-dideoxythymidine (17): Isoxazolidine **16** was dissolved in dry pyridine under nitrogen and cooled in an ice bath (0 °C). Than tosyl chloride was added and mixture was stirred for 20 h leaving temperature natural increasing at rt. Reaction was worked up adding water, extracted by Et_2O and than dried ($MgSO_4$). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.80 (d, 3H), 2.39-2.45 (m, 3H), 2.44 (s, 3H), 2.63 (s, 3H), 4.12 (m, 2H), 5.97 (dd, 1H), 7.48-7.53 (m, 3H), 7.55-7.84 (m, 2H), 11.33 (br, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 14.7; 22.1; 38.9; 44.7; 69.8; 74.8; 111.4; 122.2; 130.9; 131.6; 136.7; 150.8; 155.1; 164.2.

Diethyl 2-(1,3-dioxolan-2-yl)ethylphosphonate (**19**): Triethyl phosphite (3.43 mL, 20 mmol, 1 equiv.) was added dropwise to neat 2-(2-bromoethyl)-1,3-dioxolane (4.70 mL, 40 mmol, 2 equiv.)

at r.t. under nitrogen. The reaction mixture was heated to 110 °C until the complete disappearance of triethyl phosphite was confirmed by ^{31}P NMR analysis of the reaction mixture. Pure product **19** (2.93 g, 62%) was obtained as a pale oil after vacuum distillation of diethyl ethylphosphonate (60 °C at 1 mmHg). ^{31}P NMR (121 MHz; $CDCl_3$) δ = +33.2; ^{1}H NMR (300 MHz; $CDCl_3$) δ = 1.32 (t, 6H), 1.79-2.00 (m, 4H), 3.84-3.99 (m, 4H), 4.06-4.15 (m, 4H), 4.95 (t, 1H); ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 16.8, 19.9, 27.3, 61.9, 65.5, 103.7; MS *m/z* (positive, ES) calcd. for $C_9H_{19}O_5NaP$ $[M + Na]^+$ 261.0875 found 261.0868.

Tetraethyl 2-(1,3-dioxolan-2-yl)ethane-1,1-diyldiphosphonate (20): *n*-BuLi (3.44 mL of a 1.6 M solution in hexane, 5.5 mmol, 1.1 equiv.) was added dropwise to a solution of phosphonate **19** (1.19 g, 5.0 mmol, 1 equiv.) in dry THF (30 mL) under argon, cooled down to -78 °C. After stirring for 1 h at -78 °C, diethyl chlorophosphate (759 mL, 5.25 mmol, 1.05 equiv.) was added to the reaction mixture. The reaction mixture was allowed to warm to r.t. overnight. A saturated aqueous NH_4Cl solution (30 mL) was used to quench the reaction mixture. The aqueous layer was extracted with diethyl ether (3 x 40 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to give 1.59 g of crude product. Purification by vacuum distillation (162 °C, 0.1 mmHg) afforded the pure bisphosphonate **20** (907 mg, 48%) as a yellow oil. ^{31}P NMR (121 MHz; $CDCl_3$) δ = +24.4; ^{1}H NMR (300 MHz; $CDCl_3$) δ = 1.34 (t, 12H), 2.18 (tt, 2H), 2.63 (tt, 1H), 3.85-3.99 (m, 4H), 4.13-4.24 (m, 8H), 5.25 (t, 1H); ^{13}C NMR (75.5 MHz; $CDCl_3$) δ =

16.8, 30.5, 32.8, 63.0, 63.1, 65.2, 102.8; MS *m/z* (positive, ES) calcd. for $C_{13}H_{28}O_8NaP_2$ $[M + Na]^+$ 397.1157 found 397.1171.

Tetraethyl 3-oxopropane-1,1-diyldiphosphonate (21): A solution of dioxolane **20** (2.68 g, 7.15 mmol) in a 10:1 2M HCl_{aq} : acetone mixture (55 mL) was heated to 50 °C for 3 h. The reaction mixture was cooled down to r.t.. Volatiles were evaporated *in vacuo*. The reaction mixture was then extracted with DCM (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated under vacuum to give the pure aldehyde **21** (1.85 g, 78%) as a pale oil. ^{31}P NMR (121 MHz; $CDCl_3$) δ = +23.3; ^1H NMR (300 MHz; $CDCl_3$) δ = 1.32 (t, 6H), 1.32 (t, 6H), 2.93-3.25 (m, 3H), 4.11-4.22 (m, 8H), 9.72 (t, 1H); ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 16.6, 30.2, 39.5, 63.2, 63.4, 197.7; MS *m/z* (positive, ES) calcd. for $C_{11}H_{25}O_7P_2$ $[M + H]^+$ 331.1076 found 331.1086; $[M + Na]^+$ 353.0895 found 353.0911.

General procedure for synthesis of bisphosphonate nitrones (22 a – c): A mixture of Methyl, Phenyl or Benzyl hydroxylamine hydrochloride (1 mmol), NaOAc (1.2 mmmol), aldehyde **21** (1.1 mmol), water (5 mL) and EtOH (5 mL) was stirred at r.t. for 1 h and than concentrated under vacuum. The aqueous layer was extracted with DCM (3 x 20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated under vacuum. Concentrated organic layer was washed with *n*-exane to give pure nitrones as in Table 3.2.

***N*-(3,3-bis(diethoxyphosphoryl)propylidene) methanamine oxide (22a)**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.15-1.45 (m, 12H), 2.69-3.12 (m, 3H), 3.70 (s, 3H), 4.00-4.32 (m, 8H), 7.08 (t, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 16.33-16.43, 23.78, 32.50 ($J_{P\text{-}C\text{-}P}$ = 134.56) 52.21, 62.88-63.15, 138.12; MS m/z (positive, ES) calcd. for $C_{12}H_{27}NO_7P_2$ $[M + H]^+$ 360.1336 found 360.1315; $[M + Na]^+$ 382.1155 found 382.1491.

***N*-(3,3-bis(diethoxyphosphoryl)propylidene)-1-phenyl methanamine oxide (22b)**: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.23-1.39 (m, 12H), 2.87-3.08 (m, 3H), 4.07-4.26 (m, 8H), 4.91 (s, 2H), 7.15 (t, 1H), 7.28-7.39 (m, 3H), 7.40-7.45 (m, 2H). ^{13}C NMR (125.7 MHz; $CDCl_3$): δ = 16.22-16.40, 25.25, 37.22 (t, $J_{P\text{-}C\text{-}P}$ = 136.22), 55.20, 65.20-66.18, 125.20, 125.57, 127.76, 128.77, 137.15.

***N*-(3,3-bis(diethoxyphosphoryl)propylidene)aniline oxide (22c)**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28-1.39 (m, 12H), 2.75-2.86 (m, 3H), 4.05-4.35 (m, 8H), 7.01 (t, 1H), 7.29-7.46 (m, 5H) ; ^{13}C NMR (75.5 MHz; $CDCl_3$): δ = 16.18-16.27, 27.92, 36.25 (t, $J_{P\text{-}C\text{-}P}$ = 135.41), 68.25, 69.87, 127.22, 127.49, 128.55, 129.01, 136.87.

General procedure for synthesis of bisphosphonate isoxazolidines (23 a – i): The selected vinyl nucleobase (**8, 9** and **12**) (0.2 mmol) and the nitrone (**21 a – c**) (0.1 mmol) are mixed in a vortex. The mixture of the two solids is transferred in a 50 mL Pyrex container that is placed within an unmodified household microwave oven, at suitable irradiation power (Table 3.3). After the appropriate

time the reaction mixture is submitted to flash chromatographic separation, using variable mixtures of chloroform and methanol. The vinyl nucleobase in excess is recovered and may be re-used.

4-aza-4-methyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyuridine (23a): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26-1.36 (m, 12H), 2.23-2.42 (m, 3H), 2.74 (s, 3H), 2.96-3.13 (m, 3H), 4.11-4.31 (m, 8H), 5.85 (dd, 1H), 7.99 (d, 1H), 8,22 (d, 1H), 8.83 (br, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 16.21, 29.63, 31.01, 33.96 (t, J_{P-C-P} = 134.98), 41.02, 62.89, 63.00, 84.06, 110.95, 135.33, 150.10, 162.94.

4-aza-4-methyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxythymidine (23b): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.31-1.42 (m, 12H), 1.95 (s, 3H), 2.11-2.37 (m, 3H), 2.77 (s, 3H), 3.00-3.18 (m, 3H), 4.03-4.28 (m, 8H), 6.13 (dd, 1H), 7.63 (s, 1H), 8.70 (br, 1H). ^{13}C NMR (125.7 MHz; $CDCl_3$) δ = 12.67, 16.41, 29.71, 31.26, 34.01, 34.20 (t, J_{P-C-P} = 134.36), 40.82, 63.01, 83.62, 111.03, 135.41, 150.21, 163.48.

4-aza-4-methyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyadenosine (23c): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38-1.47 (m, 12H), 2.32-2.45 (m, 3H), 2.81 (s, 3H), 3.22-3.32 (m, 3H), 4.14-4.36 (m, 8H), 6.29 (dd, 1H),7.48 (br, 2H), 8.22 (s, 1H), 8.57 (s, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 17.22, 29.82, 31.57, 34.87 (t, J_{P-C-P} = 134.55), 41.22, 62.98, 63.17, 84.64, 127.12, 139.01, 147.10, 153.00, 156.40.

4-aza-4-benzyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyuridine (23d): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36-1.44 (m, 12H), 2.12-2.29 (m, 3H), 3.05-3.23 (m, 3H), 4.18-4.36 (m, 8H), 4.41 (s, 2H), 6.05 (dd, 1H), 7.18-7.39 (m, 5H), 8.03 (d, 1H), 8.33 (d, 1H), 8.96 (br, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 15.88, 29.57, 30.76, 34.05 (t, J_{P-C-P} = 135.43), 54.67, 63.28, 63.10, 84.31, 111.26, 124.80, 125.26, 127.01, 128.11, 135.87, 150.27, 163.89.

4-aza-4-benzyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxythymidine (23e): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22-1.36 (m, 12H), 2.08 (s, 3H), 2.21-2.34 (m, 3H), 3.04-3.18 (m, 3H), 4.10-4.22 (m, 8H), 4.34 (s, 2H), 6.21 (dd, 1H), 7.22-7.43 (m, 5H), 7.74 (s, 1H), 8.95 (br, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 11.84, 15.69, 29.44, 30.21, 33.88 (t, J_{P-C-P} = 134.76), 54.26, 62.84, 63.25, 84.16, 111.03, 124.78, 125.18, 126.87, 128.03, 135.74, 150.31, 163.67.

4-aza-4-benzyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyadenosine (23f): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.41-1.51 (m, 12H), 2.33-2.45 (m, 3H), 3.20-3.35 (m, 3H), 4.16-4.39 (m, 8H), 4.65 (s, 2H), 6.56 (dd, 1H), 7.19-7.38 (m, 5H), 6.53 (br, 2H), 8.21 (s, 1H), 8.49 (s, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 17.31, 29.92, 31.62, 34.95 (t, J_{P-C-P} = 135.32), 54.77, 63.03, 63.28, 85.60, 124.86, 125.26, 126.90, 127.25, 128.22, 139.10, 147.34, 153.69, 156.58.

4-aza-4-phenyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyuridine (23g): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.29-1.38 (m, 12H), 2.20-2.42 (m, 3H), 2.89-3.07 (m, 3H), 4.09-4.22 (m, 8H), 5.98 (dd, 1H),.6.77-6.86 (m, 3H), 7.02-34 (m, 2H), 7.98 (d, 1H), 8.25 (d, 1H), 8.87 (br, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 15.88, 29.57, 30.76, 34.05 (t, J_{P-C-P} = 134.78), 63.28, 63.10, 84.31, 111.26, 127.26, 127.89, 128.37, 129.56, 135.87, 150.27, 163.89.

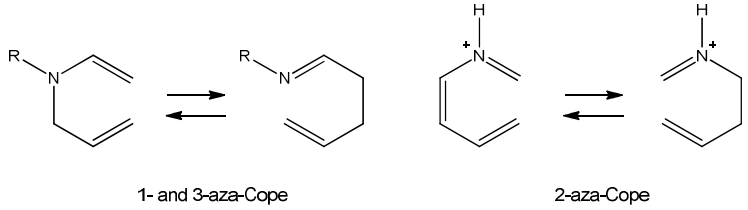
4-aza-4-phenyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxythymidine (23h): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33-1.46 (m, 12H), 2.12 (s, 3H), 2.19-2.28 (m, 3H), 2.98-3.20 (m, 3H), 4.13-4.27 (m, 8H), 6.01 (dd, 1H), 6.84-7.01 (m, 3H), 7.24-7.38 (m, 2H), 7.55 (s, 1H), 8.31 (br, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 12.13, 15.47, 29.69, 30.79, 34.07 (t, J_{P-C-P} = 134.04), 63.00, 63.68, 84.53, 111.36, 127.41, 127.70, 128.34, 129.50, 135.11, 150.64, 163.61.

4-aza-4-phenyl-3-(2'-(2',2'-bis-diethoxyphosphoryl)ethyl)-2,3-dideoxyadenosine (23i): ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32-1.41 (m, 12H), 2.24-2.37 (m, 3H), 3.12-3.31 (m, 3H), 4.08-4.37 (m, 8H), 6.22 (dd, 1H), 6.93-7.07 (m, 3H), 7.15-7.38 (m, 2H), 7.56 (br, 2H), 8.30 (s, 1H), 8.51 (s, 1H). ^{13}C NMR (75.5 MHz; $CDCl_3$) δ = 16.24, 29.20, 31.32, 34.74 (t, J_{P-C-P} = 134.89), 62.91, 63.20, 85.44, 127.37, 127.75, 127.86, 128.42, 129.96, 139.32, 147.84, 153.50, 156.65.

4. Kinetic studies of 2-aza-Cope rearrangements with cyclic nitrones

4.1. Aza-Cope rearrangements

Stereoselective [3,3]-sigmatropic processes are wellknown and powerful tools in organic synthesis.[107] Among them, Claisen[108] and Cope[109] rearrangements have developed exceptionally well in the past 50 years. In particular, aza-Cope rearrangements have attracted great interest because of the ubiquitous presence of nitrogen-containing structures in natural and biological products as well as synthetic intermediates. Moreover, the merging of aza-Cope rearrangements with other reactions such as [3+2] cycloaddition[110] or Mannich type reaction[13] provide direct access to a variety of complex structures. Depending on the position of the nitrogen atom, different aza-Cope rearrangements can be identified (Scheme 4.1).



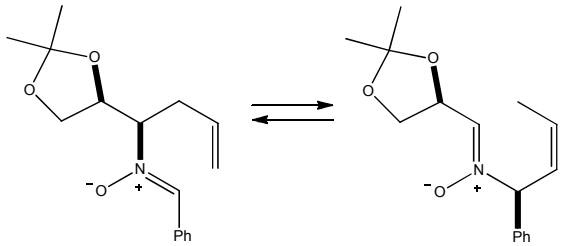
Scheme 4.1: Aza-Cope rearrangements.

The 1-aza-Cope rearrangement, developed by Fowler and co-workers from aza-diene precursors,[111] has been used in the synthesis of nitrogen heterocycles.[112] In 1992, Stille and co-workers reported the inverse process as a 3-aza-Cope rearrangement starting from *N*-

alkyl-*N*-allylenamines.[113] This reaction has also been studied for quaternary *N*-allyl enammonium salts.[114]

Both 1- and 3-aza-Cope rearrangements are thermal processes that are catalyzed by Lewis acids. The 2-aza-Cope rearrangement had only been described as an equilibrium system for cationic substrates,[115] and several protocols were developed to drive the process to a single product.[116] These protocols included trapping the iminium ion with a nucleophile incorporated into the structure[117] and a Mannich type reaction.[118] The synthetic utility of the reaction has been extensively demonstrated[119] and, more recently, a catalytic asymmetric version has been reported for protonated imines.[120]

Wuts and Jung reported the 2-aza-Cope rearrangement of nitrones under catalysis with trimethylsilyl triflate,[121] which actually involves hydroxyiminium cations. A similar process was described twenty years later by Loh and coworkers using 10-camphorsulfonic acid (CSA) as a catalyst.[122] In both cases, the rearrangement involved cationic species, as in other 2-aza-Cope rearrangements. On the other hand, the possibility of inducing a thermal 2-aza-Cope rearrangement with neutral substrates had only been suggested,[123] and in 2007 Merino *et al.* reported for the first time experimental and theoretical evidence of a neutral thermal 2-aza-Cope rearrangement of nitrones (Scheme 4.2).[11]



Scheme 4.2: First evidence (2007) of neutral and thermal 2-aza-Cope. Reagent and conditions: Toluene, 100 °C, 72 h.

As far as we are aware there are no examples in the literature concerning thermal 2-aza-Cope rearrangements with neutral substrates other than those reported from this work.[124] In this chapter, we report a full experimental study based on NMR kinetic experiments of the activation energies required for both neutral and catalyzed 2-aza-Cope rearrangements of nitrone **24** (Figure 4.1).

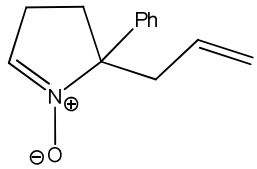


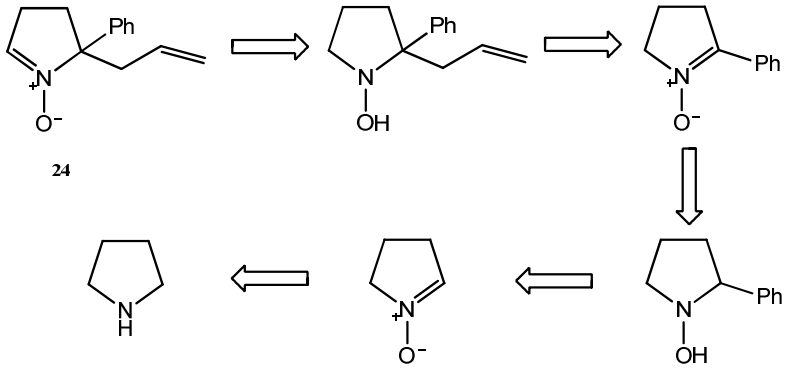
Figure 4.1: Nitrone **24.**

We designed nitrone **24** with suitable substituent that stabilize the rearranged form in order to easily direct the reaction and we synthesized it with a sequence of nucleophilic addition and hydroxylamine oxidation as shown in the follower section of this chapter.

4.2. Results and discussion

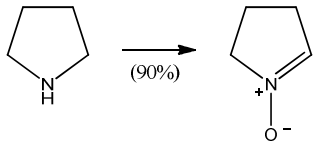
4.2.1. Preparation of nitrones

The synthesis of nitrone **24** was carried out starting from oxidation of pyrrolidine and subsequent sequence of four steps of hydroxylamine oxidation and nucleophilic addition to intermediate nitrones (Scheme 4.3).



Scheme 4.3: Retrosynthesis of nitrone **24**.

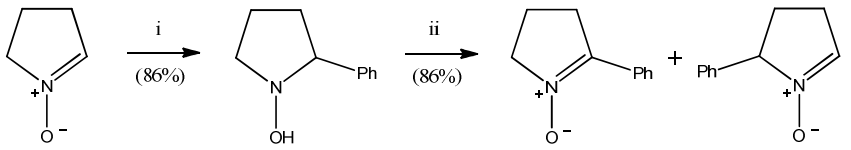
Nitrone **24** was prepared from nitrone **26**, which was obtained by oxidation of pyrrolidine **25** with methyltrioxorhenium as described in Scheme 4.4.[125]



25 **26**

Scheme 4.4: Synthesis of nitrone **26**. Reagents and conditions: $MeReO_3$ (2 mol-%), UHP, MeOH, r.t.., 24 h;

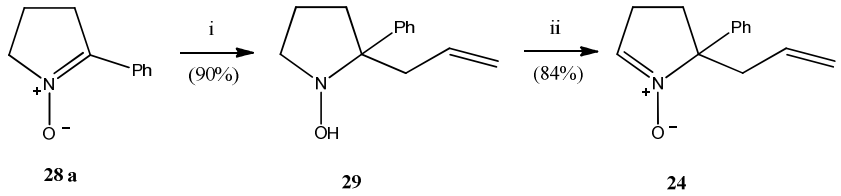
After nucleophilic addition of phenylmagnesium bromide and oxidation with manganese(IV) oxide,[126] a 9:1 mixture of nitrones **28a** and **28b** was obtained (Scheme 4.5).



26 **27** **28 a** **28 b**

Scheme 4.5: Synthesis of nitrones **27** and **28**. Reagents and conditions: (i) phenylmagnesium bromide, THF 0 °C, 2 h; (ii) MnO_2, CH_2Cl_2, 0 °C, 7 h.

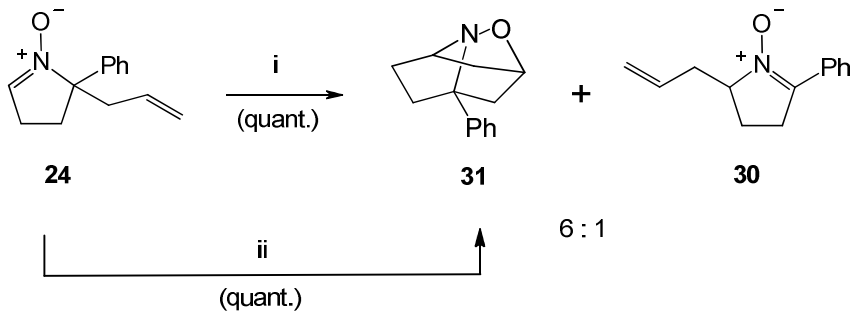
The major isomer **28a** was separated by column chromatography and allylated following our standard procedure[127] to give hyroxylamine **29**. Finally, oxidation of **29** (carried out another time with MnO_2) furnished nitrone **24** in 48% overall yield (five steps).



Scheme 4.6: Synthesis of nitrones **29** and **24**. Reagents and conditions: (i) allylmagnesium bromide, THF 0 °C, 8 h; (ii) MnO_2, CH_2Cl_2, 0 °C, 7 h.

4.2.2. Kinetic studies

Heating nitrone **24** in dimethyl sulfoxide (DMSO) in a sealed tube at 70 °C for 6 hours afforded mixtures of rearranged nitrone and the corresponding cycloadducts formed through an intramolecular 1,3-dipolar cycloaddition (Scheme 4.7). Prolonged heating for 36 hours only provided cycloadducts **31**, in quantitative yield, from nitrone **24** indicating that nitrone **30** is intermediate of the reaction.



Scheme 4.7: Rearrangement of nitrones **24**. *Reagents and conditions:* (i) DMSO, 70 °C, 6 h, sealed tube; (ii) toluene 120 °C, 36 h, sealed tube.

To obtain detailed information on the factors and activation energy of the process, we investigated the rearrangement of nitrone

24 by ^{1}H NMR spectroscopic analysis at a range of temperatures between 40–90 °C, in two different deuterated solvents (toluene and dimethyl sulfoxide), in catalyzed and uncatalyzed conditions, and determined the activation parameters. The disappearance of the NMR signal of nitrone **24** at δ = 7.03 ppm, as shown in Figure 4.2, with shifting of other signals is evidence that the process monitored is that illustrated in Scheme 4.3. The changes in concentration of nitrone **24** were determined from the corresponding ^{1}H NMR spectra by measuring integral data.

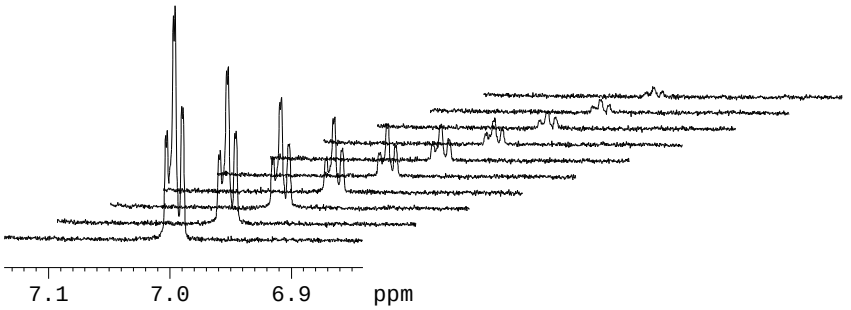


Figure 4.2: Stack plot illustrating decreasing of ^{1}H NMR signal (proton in position 2) for starting nitrone **24** at 343 K, at 30 min intervals.

In the case of nitrones **24**, we ascertained that the relationship between the concentration of the rearranged nitrones **30** and the corresponding starting nitrone is best described by a straight line, which is a mathematical proof that the thermal 2-aza-Cope rearrangement takes place prior to the cycloaddition reaction. Indeed, a plot of the natural logarithm of concentration, ln {χ}, against time, was a straight line, confirming that the process is first-order in all cases. The reaction rate follows the equation kt = - ln {χ}. From the reaction conducted at various temperatures (40–90 °C), the

corresponding kinetic constants were calculated for each temperature (Figure 4.3 and Table 4.1).

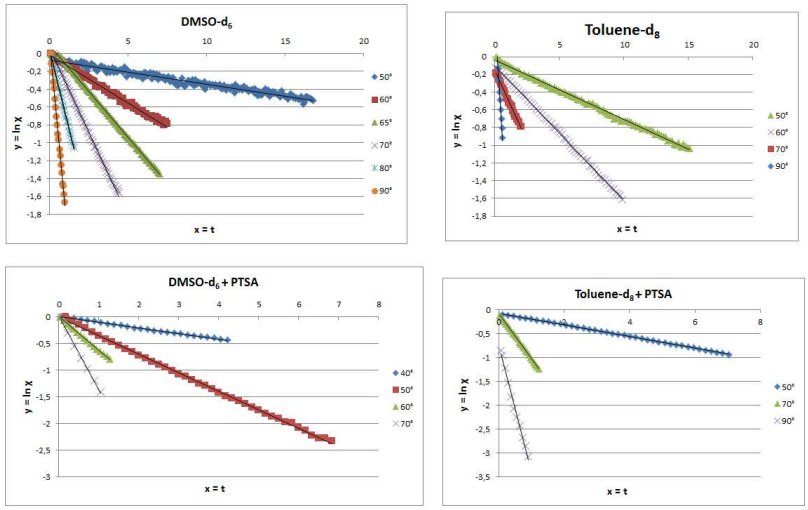
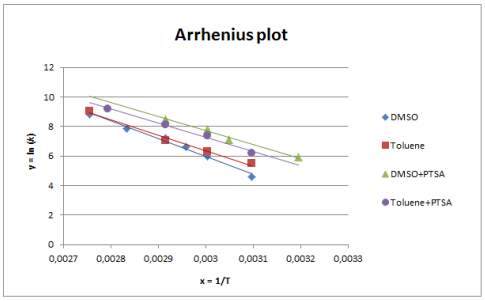


Figure 4.3: Fitting of first-order exponential decay equation. y = - ln (χ), x = t

T(ºC)	DMSO	Toluene	DMSO catalyzed	Toluene catalyzed
40	$2.79\cdot10^{-6}$	$5.99\cdot10^{-6}$	$2.83\cdot10^{-5}$	$2.06\cdot10^{-5}$
50	$7.44\cdot10^{-6}$	$1.86\cdot10^{-5}$	$6.99\cdot10^{-5}$	$3.85\cdot10^{-5}$
60	$2.98\cdot10^{-5}$	$4.39\cdot10^{-5}$	$1.84\cdot10^{-4}$	$1.24\cdot10^{-4}$
65	$5.78\cdot10^{-5}$	n.d	n.d.	n.d.
70	$1.05\cdot10^{-5}$	$8.72\cdot10^{-5}$	$3.83\cdot10^{-4}$	$2.61\cdot10^{-4}$
80	$2.00\cdot10^{-4}$	$2.56\cdot10^{-4}$	$8.34\cdot10^{-4}$	$4.39\cdot10^{-4}$
90	$5.14\cdot10^{-4}$	$6.49\cdot10^{-4}$	$1.74\cdot10^{-3}$	$7.39\cdot10^{-4}$

Table 4.1: Kinetic constants (k_{obs} s^{-1}) at different temperatures. n.d.: not determined.

The Arrhenius plot (see Figure 4.4) permitted evaluation of the respective activation energies and Eyring plots (Figure 4.5), a direct evaluation of the activation parameters $\Delta H^{\neq}$ and $\Delta S^{\neq}$ and, in consequence $\Delta G^{\neq}$ at 298.15 K (Table 4.2).



Series	$\Delta E^{\ddagger}$ (Kcal/mol)	R^2
DMSO-d_6	24,06	0,9883
Toluene-d_8	20,61	0,9891
DMSO+PTSA	18,57	0,9933
Toluene+PTSA	16,79	0,9895

Figure 4.4: Arrhenius plots [ln (*k*) vs. 1/T] for the rearrangement of nitrone **24**.

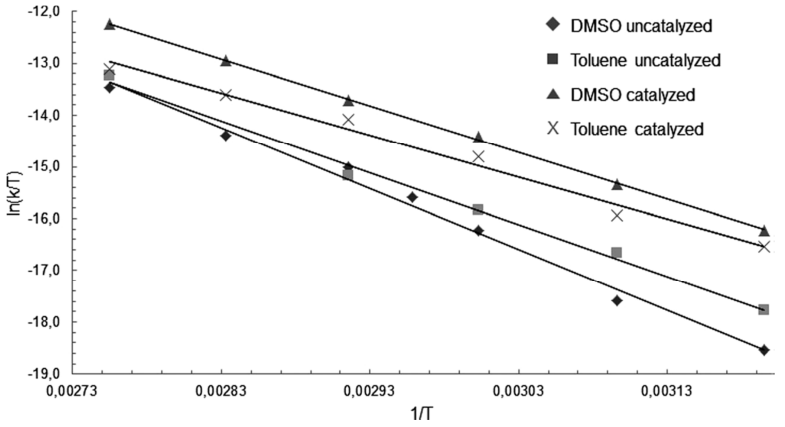
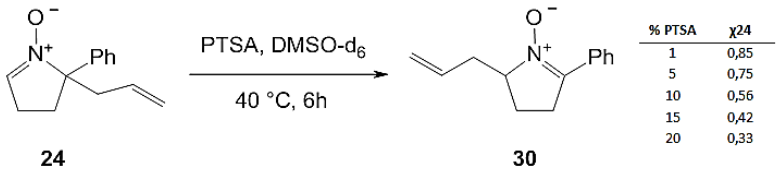


Figure 4.5: Eyring plots [ln (*K*/*T*) vs. 1/*T*] for rearrangement of nitrone **24** carried out in DMSO, toluene, and in the presence of 20 mol-% PTSA.

Thus, the results show that the same reactivity illustrated in Scheme 4.3 was observed both in DMSO-d_6 and in toluene-d_8, although in last one solvent the reaction was completed in 4 h, in agreement with a neutral pericyclic transition state,[11] which should be stabilized in aromatic solvents. Three acid catalysts, Camphorsulfonic acid (CSA), *p*-toluensulphonic acid (PTSA) and phosphoric acid, were tested for the rearrangement of nitrone **24**, with the best results being obtained for CSA and PTSA. In particular, the optimization study carried out for the PTSA-catalyzed process showed that 20

mol-% catalyst was necessary to achieve good conversion (Scheme 4.8). In the case of acid-catalyzed reactions, nitrone **30** was obtained as the only product of the reaction and no cycloaddition was observed.



Scheme 4.8: Optimization study for the PTSA-catalyzed rearrangement of nitrone **24**.

The rearrangement of **24** in toluene exhibited also a first-order rate constant k (70 °C) = 8.72 x 10^{-5} s^{-1} from which the activation energy (E_a) was calculated to be 20.61 kcal/mol (Table 4.2). This value is clearly lower than that found for the rearrangement in DMSO (E_a = 24.06 kcal/mol), in agreement with a neutral rearrangement proceeding through a pericyclic transition state. The rate constant for the rearrangement of **24** in DMSO and in the presence of 20 mol-% PTSA was higher [k (70 °C) = 3.83 x 10^{-4} s^{-1}] than in the absence of acid [k (70 °C) = 1.05 x 10^{-5} s^{-1}]. Accordingly, the activation energy was lower (E_a = 18.57 kcal/mol). When the acid-catalyzed process was carried out in toluene a rate constant k (70 °C) = 2.61 x 10^{-4} s^{-1} was found. In this case, the activation energy was E_a = 16.79 kcal/mol. The Eyring plots for the acid-catalyzed rearrangement of nitrone **24** are illustrated in Figure 4.8 and the corresponding thermodynamic parameters are given in Table 4.2.

Thermodynamic parameters	**DMSO**		**Toluene**	
	uncatalyzed	**catalyzed**	**uncatalyzed**	**catalyzed**
k (343 K, s^{-1})	1.05×10^{-5}	3.83×10^{-4}	8.72×10^{-5}	2.61×10^{-4}
E_a (kcal/mol)	24.06	18.57	20.61	16.79
$\Delta H^{\neq}$ (kcal/mol)	23.40	17.90	19.94	16.12
$\Delta S^{\neq}$ (cal/K mol)	–9.25	–21.79	–18.76	–28.52
$\Delta G^{\neq}$ (298.15 K, kcal/mol)	26.15	24.40	25.54	24.62

Table 4.2: Kinetic parameters for the rearrangements of nitrone **24**.

These studies reveal that the rearrangement performed in the presence of 20 mol-% PTSA have lower transition state energies than those of the uncatalyzed reaction. The catalyzed process in toluene is faster than the corresponding process in DMSO but with a minor difference (1.78 kcal/mol) in the values of E_a with respect to the uncatalyzed rearrangements (3.45 kcal/mol). This observation is in agreement with a typical cationic 2-aza-Cope rearrangement for the catalyzed processes in which the stability of the transition structures could be increased, to some extent, in polar solvents such as DMSO.

4.3. Conclusions

We have carried out the synthesis of vinyl cyclic nitrone **24** undergoing 2-aza-Cope rearrangement, by a multistep process involving a sequence of nucleophilic addition and hydroxylamine oxidation. Subsequently we have experimentally studied the first reported neutral 2-aza-Cope rearrangement of nitrones to determine the main factors affecting the process. Kinetic studies reveal that the rearrangement is favored in aromatic solvents, in agreement with an aromatic transition state. Acid catalysis accelerates the process and diminishes the difference between aromatic and polar solvents. These results clearly demonstrate that whereas the uncatalyzed process occurs through a neutral transition state, the catalyzed process takes place through cationic species. In spite of these observations, the aromaticity of the transition state favors in all cases the reaction conducted in an aromatic solvent (toluene).

4.4. Experimental section

4.4.1. General methods

The reaction flasks and other glass equipment were heated in an oven at 130 °C overnight and assembled in a stream of Ar. All reactions were monitored by TLC on silica gel 60 F254; the position of the spots were detected with 254 nm UV light or by spraying with one of the following staining systems: 50% methanolic sulfuric acid, 5% ethanolic phosphomolybdic acid or iodine. Preparative chromatography was performed with a Combiflash RF apparatus and with solvents that were distilled prior to use; the cartridges were filled with silica gel 60 microns. Melting points were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 or 500 instruments or on a Varian Mercury 400 in $CDCl_3$. Chemical shifts are reported in ppm (δ) relative to $CHCl_3$ (δ = 7.26) in $CDCl_3$. Elemental analysis were performed on a Perkin Elmer 240B microanalyzer or with a Perkin-Elmer 2400 instrument.

4.4.2. Experimental procedures

3,4-Dihydro-2*H*-pyrrole 1-Oxide, (26): A solution of pyrrolidine **25** (0.427 g, 6 mmol) in methanol (15 mL) was treated sequentially with $MgSO_4$ (0.720 g), methyl trioxorhenium (30 mg,

0.112 mmol) and urea-hydrogen peroxide complex (1.7 g, 18 mmol). The resulting mixture was stirred for 24 hours at ambient temperature, filtered and evaporated under reduced pressure. The crude product was purified by column chromatography (hexane-EtOAc, 1:1) to give the pure nitrone **26** (0.460 g, 90%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.18-2.26 (m, 2H), 2.69-2.73 (m, 2H), 3.91-3.97 (m, 2H), 6.87 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 19.1, 28.7, 62.0, 135.7. Anal Calcd. for C_4H_7NO: C, 56.45; H, 8.29; N, 16.46. Found: C, 56.67; H, 8.34; N, 16.25.

2-Phenylpyrrolidin-1-ol, (27): To a well-stirred solution of nitrone **26** (0.3 g, 3.5 mmol) in THF (25 mL) was added dropwise under argon atmosphere a 3.0 M ethereal solution of PhMgBr (2.91 mL, 8.73 mmol). When the addition is complete the reaction mixture is stirred at ambient temperature for an additional 1 hour at which time the reaction is quenched with a saturated aqueous solution of $NaHCO_3$. The organic layer is separated and the aqueous layer is extracted twice with Et_2O. The combined organic extracts were dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to give the crude product. The NMR analysis of the crude product showed the presence of only one diastereomer to the limit of detectability. Purification by column chromatography (Hexane/EtOAc, 4:1) gave pure **27** (0.491 g, 86%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.85-1.77 (m, 1H), 1.95-1.87 (m, 2H), 2.27-2.18 (m, 1H), 2.93-2.86 (m, 1H), 3.35-3.30 (m, 1H), 3.82-3.77 (m, 1H), 5.32 (br s, 1H), 7.65-7.22 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 19.1, 30.3, 57.3, 72.6, 127.2, 127.8, 128.1, 141.4. Anal Calcd. for

$C_{10}H_{13}NO$: C, 73.59; H, 8.03; N 8.58. Found: C, 70.48; H, 8.21; N, 8.70.

5-Phenyl-3,4-dihydro-2*H*-pyrrole 1-Oxide, (28a): Manganese (IV) oxide (0.325 g, 3.52 mmol) was added in portions to an ice-cooled solution of **27** (0.478 g, 2.93 mmol) in CH_2Cl_2 (25 mL). The reaction was stirred for an additional 4 hours at 0 °C. The solution was then filtered through Na_2SO_4 and concentrated under reduced pressure to give a 9:1 (^{1}H NMR) mixture of nitrones **28a** and **28b**. Column chromatography (CH_2Cl_2/MeOH, 9:1) of this mixture afforded pure **28a** (0.364 g, 77%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.25-2.17 (m, 2H), 3.21-3.16 (m, 2H), 4.26-4.21 (m, 2H), 7.47-7.42 (m, 3H), 8.35-8.32 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.7, 31.1, 64.9, 127.4, 128.5, 130.5, 139.0, 157.1. Anal Calcd. for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.39; H, 6.95; N, 8.80

2-Allyl-2-phenylpyrrolidin-1-ol, (29): To a well-stirred solution of nitrone **28a** (0.564 g, 3.5 mmol) in THF (25 mL) was added dropwise under argon atmosphere a 1.0 M ethereal solution of allylmagnesium bromide (8 mL, 8 mmol). When the addition is complete the reaction mixture is stirred at ambient temperature for an additional 1 hour at which time the reaction is quenched with a saturated aqueous solution of $NaHCO_3$. The organic layer is separated and the aqueous layer is extracted twice with Et_2O. The combined organic extracts were dried ($MgSO_4$), filtered and the solvent evaporated under reduced pressure to give the crude product. The

NMR analysis of the crude product showed the presence of only one diastereomer to the limit of detectability. Purification by column chromatography (Hexane/EtOAc, 4:1) gave pure **29** (0.640 g, 90%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.91-1.80 (m, 1H); δ: 2.02-1.93 (m, 1H); δ = 2.14-2.06 (m, 1H), 2.38-2.31 (m, 1H), 2.51-2.46 (m, 1H), 2.73-2.68 (m, 1H), 3.12-3.05 (m, 1H), 3.28-3.22 (m, 1H), 4.96-4.89 (m, 2H), 5.50-5.40 (m, 1H), 6.32 (br s, 1H), 7.43-7.21 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 21.4, 30.4, 43.2, 56.1, 117.3, 120.2, 126.8, 127.1, 128.1, 134.5, 137.1. Anal Calcd. for $C_{13}H_{17}NO$: C, 76.81; H, 8.43; N, 6.89. Found: C, 76.99; H, 8.31; N, 6.76.

2-Allyl-2-phenyl-3,4-dihydro-2*H*-pyrrole 1-Oxide, (24): Manganese (IV) oxide (0.325 g, 3.52 mmol) was added in portions to an ice-cooled solution of **29** (0.596 g, 2.93 mmol) in CH_2Cl_2 (25 mL). The reaction was stirred for an additional 4 hours at 0 °C. The solution was then filtered through Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 10:1) to give pure nitrone **24** (0.495 g, 84%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 2.46-2.40 (m, 1H), 2.57-2.52 (m, 3H), 2.79-2.74 (dd, 1H), 3.15-3.10 (m, 1H), 5.29-5.21 (m, 2H), 5.86-5.75 (m, 1H), 7.03-7.05 (m, 1H), 7.46-7.27 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 25.1, 32.2, 41.4, 118.9, 120.3, 125.5, 127.8, 128.7, 132.5, 134.6, 139.1. Anal Calcd. for $C_{13}H_{15}NO$: C, 77.58; H, 7.51; N, 6.96. Found: C, 77.43; H, 7.39; N, 6.83.

2-Allyl-5-phenyl-3,4-dihydro-2*H*-pyrrole 1-Oxide, (30): A solution of the nitrone **24** (1 mmol) was dissolved in anhydrous

DMSO (25 mL), placed in a sealed tube and heated at 70 °C under an argon atmosphere for 6 h, at which time the solvent was partially evaporated and the resulting solution was filtered through a pad of silica gel. After washing the silica with diethyl ether, the resulting solution was evaporated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc,4:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.90 (ddd, *J* = 6.8, 9.1, 13.3 Hz, 1 H), 2.25 (ddd, *J* = 7.0, 8.1, 13.3 Hz, 1 H), 2.50–2.57 (m, 1 H), 2.77–2.84 (m, 1 H), 2.98–3.04 (m, 2 H), 4.18–4.26 (m, 1 H), 5.05–5.15 (m, 2 H), 5.7 (ddt, *J* = 7.1, 10.2, 17.2 Hz, 1 H), 7.32–7.40 (m, 3 H), 8.25–8.31 (m, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.1, 39.7, 48.1, 64.1, 119.9, 125.5, 126.7, 128.4, 133.6, 134.6, 135.3 ppm. $C_{13}H_{15}NO$ (201.27): calcd. C 77.58, H 7.51, N 6.96; found C 77.64, H 7.41, N 7.06.

3-phenyl-8-oxa-7-azatricyclo [5.9.1.0^{6,7}] nonane, (31): Nitrone **24** (63 mg, 0.31 mmol) was heated at reflux in toluene (25 mL) for 3 days. The solvent was removed and the brown residue was purified, before, by column chromatography (SiO_2, 4:1 hexane/ethyl acetate), and, after, by preparative HPLC to give **31**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42-1.35 (m, 1H), 1.93-1.85 (m, 2H), 2.10-2.03 (m, 1H), 2.21-2.14 (m, 1H), 2.35-2.24 (m, 2H), 2.46-2.39 (m, 1H), 3.79-3.74 (app. t, 1H), 4.80-4.78 (app. t, 1H), 7.21-7.16 (app. tt, 1H), 7.33-7.29 (m, 2H), 7.50-7.47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.66; 35.11; 42.69; 50.33 ; 63.38 ; 67.53; 79.87; 125.15, 126.15, 128.37; 134.11. $C_{13}H_{15}NO$ (201.27): calcd. C 77.58, H 7.51, N 6.96; found C 77.62, H 7.43, N 7.09.

General procedure for the catalyzed rearrangement of nitrone 24: A solution of nitrone **2** (0.201 g, 1 mmol) was dissolved in anhydrous DMSO (25 mL), treated with *p*-toluensulfonic acid (34 mg, 0.2 mmol), placed in a sealed tube, and heated at 40 °C under an argon atmosphere for 6 h, at which time the solvent was partially evaporated and the resulting solution was filtered through a pad of silica gel. After washing the silica with diethyl ether, the resulting solution was evaporated under reduced pressure and the residue was purified by column chromatography (hexane/EtOAc, 4:1).

4.4.3. Kinetic supplementary data

General procedure for ^{1}H NMR kinetic investigations: Nitrone (0.05 mmol) was added to a NMR tube containing toluene-d_8 or DMSO-d_6 (650 μL) and the resulting mixture was allowed to equilibrate for 15 min before spectra were recorder at the stated temperature. ^{1}H NMR spectra were recorded at 30 min intervals on a Bruker 400 ARX spectrometer, using 5 mm quartz tubes at 400 MHz. The rearrangements were monitored by following the intensity of a signal area of representative signals (7.03-7.05 ppm for nitrone **24**).

All kinetic runs were performed under first-order conditions with respect to the nitrone. The observed first order rate constants k_{obs} were obtained by fitting a first-order exponential decay equation through the data points using statistical software (Table 4.1). Activation parameters, ΔH and ΔS, were determined from temperature dependence studies over the temperature range of 40-90º C at 10º C intervals.

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